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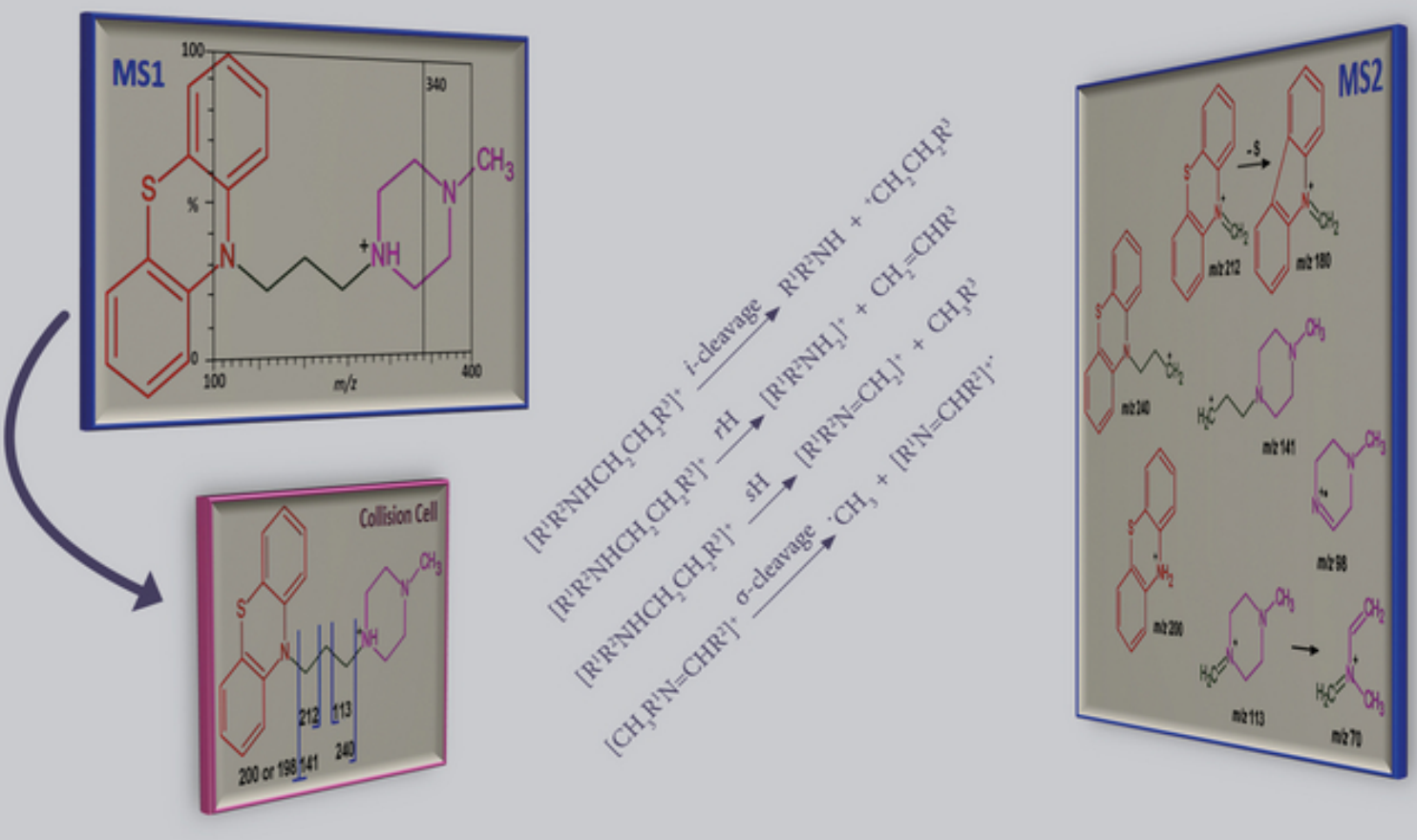

Interpretation of MS–MS Mass Spectra of Drugs and Pesticides

Wilfried M. A. Niessen • Ricardo A. Correa C.

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INTERPRETATION OF MS–MS MASS SPECTRA OF DRUGS AND PESTICIDES

WILEY SERIES ON MASS SPECTROMETRY

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PREFACE

In the 1980s, tandem mass spectrometry was introduced for the structural elucidation of even-electron ions (protonated or deprotonated molecules) generated by soft ionization techniques such as fast-atom bombardment, thermospray, and electrospray. When compared to the fragmentation of odd-electron ions generated by electron ionization, scientists were well aware of the fact that different rules apply to the fragmentation of even-electron ions. Surprisingly, no major fundamental research was carried out on trying to understand and describe these differences. More effort was placed on the development of improved instrumentation and advanced applications for the emerging technologies. This particular effort paid off, as exemplified by tandem mass spectrometry which, often in combination with gas or liquid chromatography, has been a major contributor to the progress of many scientific disciplines, for example, pharmaceutical, biochemical, and environmental sciences; food safety; sports doping analysis; clinical diagnostics; forensics; and toxicology.

This work is an attempt to add to the understanding of the fragmentation of even-electron ions. This has been done by studying the fragmentation of a wide variety of compounds, with a special focus on chemical structure similarities, that is, from the same class. The basic data set used comprises a number of mass spectral libraries developed for general unknown screening in toxicology. In this respect, we need to thank Dr Wolfgang Weinmann (originally at the Institute of Legal Medicine, University of Freiburg, Germany, and currently at the Institute of Forensic Medicine, University of Bern, Switzerland) for providing public access to his toxicology library and the library of designer drugs via the Internet (http://www.chemicalsoft.de/index.html); Dr Pierre Marquet (of the Faculty of Medicine, Department of Pharmacology, Toxicology, and Pharmacovigilance at the University Hospital of Limoges, France) for providing his mass spectral library of negative-ion mass spectra; and Dr Bernhard Wüst of Agilent Technologies for his help with using the Agilent Broecker, Herre & Pragst PCDL for forensic toxicology. The information from these libraries and other data sets is complemented by data from the scientific literature.

The origins of this book can be found in two publications describing the fragmentation of toxicologically relevant drugs in both positive-ion tandem mass spectrometry (Niessen, 2011) and negative-ion tandem mass spectrometry (Niessen, 2012). Soon after, the authors decided to develop the project further by extending the number of compounds covered and the detail of the information provided. The fragmentation of some 1300 compounds and the product-ion mass spectra of even more are studied and interpreted in this book.

This volume consists of five chapters. Chapters 3 and 4 are the main chapters, where proposed fragmentation rules for the "Fragmentation of Even-Electron Ions" (Chapter 3) are derived from the behavior of the "Fragmentation of Drugs and Pesticides" (Chapter 4) pertaining to many different classes of compounds. Chapter 1, "Introduction to LC–MS–MS Technology", provides a concise introduction to mass spectrometry technology. Chapter 2, "Interpretation of Mass Spectra" gives the basic concepts and definitions related to the information that can be extracted from mass spectra. Finally, Chapter 5, "Identification Strategies" gives an overview of the different classes of unknowns and identification strategies that exist as well as how they relate to multiple areas of application.

Last but not least, special thanks go to our families, and the many people who have inspired us to continue working on this project. We hope that you, as our reader, find this material

useful and inspirational to further extend our understanding of the fragmentation of even-electron ions in tandem mass spectrometry.

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Niessen WMA. 2012. Fragmentation of toxicologically relevant drugs in negative-ion liquid chromatography–tandem mass spectrometry. Mass Spectrom Rev, 31: 626–665.

ABBREVIATIONS

AC	alternating current potential
ADC	analog-to-digital converter
APCI	atmospheric-pressure chemical ionization
API	atmospheric-pressure ionization
APPI	atmospheric-pressure photoionization
BPC	base-peak chromatogram
CE	charge exchange
CECI	charge-exchange chemical ionization
CI	chemical ionization
CID	collision-induced dissociation
CIS	coordination electrospray ionization
CLND	chemiluminescence nitrogen detector
CNS	central nervous system
CPA	chlorinated phenoxy acid
CRF	charge-remote fragmentation
CRM	charge-residue model
DC	direct current potential
DDA	data-dependent acquisition
DESI	desorption electrospray ionization
DIA	data-independent acquisition
EA	electron affinity
ECD	electron-capture dissociation
ECNI	electron-capture negative ionization
EE^+ and EE^-	even-electron ion
EI	electron ionization
EM	electron multiplier
EPA	environmental protection agency
EPI	enhanced product-ion
ESI	electrospray ionization
ETD	electron-transfer dissociation
EU	European Union
FAB	fast-atom bombardment ionization
FAIMS	high-field asymmetric waveform ion mobility spectrometry
FDI	field-desorption ionization
FT-ICR	Fourier-transform ion cyclotron resonance
FWHM	full peak width at half maximum height
GC	gas chromatography
GC–MS	gas chromatography–mass spectrometry
G_R	reagent gas
H/D exchange	hydrogen/deuterium exchange
HCD	higher-energy collision-induced dissociation
HFBPC	(*S*)-(−)-*N*-(heptafluorobutanoyl)prolyl chloride
HILIC	hydrophilic interaction (liquid) chromatography
HIV	human immunodeficiency virus
HRAM-MS	high-resolution accurate-mass mass spectrometry
IE	ionization energy
IEM	ion-evaporation model
IMS	ion-mobility spectrometry
IMS–MS	ion-mobility spectrometry–mass spectrometry
IRMPD	infrared multiphoton photodissociation

IUPAC	International Union for Pure and Applied Chemistry
LC	liquid chromatography
LC–MS	liquid chromatography–mass spectrometry
LIFDI	liquid injection field desorption ionization
LINAC	linear-acceleration high-pressure collision cell
LIT	linear ion trap
LOD	limit of detection
LOQ	limit of quantification
m/z	mass-to-charge ratio
MALDI	matrix-assisted laser desorption ionization
MAOI	monoamine oxidase inhibitor
MCP	microchannel plate
MDA	3,4-methylenedioxy-amphetamine
MDEA	3,4-methylenedioxy-ethylamphetamine
MDF	mass-defect filtering
MDMA	3,4-methylenedioxy-methamphetamine
Met-ID	metabolite identification
MS	mass spectrometry
MS–MS; MS^n	tandem mass spectrometry
NCE	new chemical entity
NICI	negative-ion chemical ionization
NLA	neutral-loss analysis
NMR	nuclear magnetic resonance spectroscopy
NPLC	normal-phase liquid chromatography
nNRTI	non-nucleoside reverse transcriptase inhibitors
NRTI	nucleoside reverse transcriptase inhibitors
NSAIDs	non-steroidal anti-inflammatory drugs
$OE^{+\bullet}$ and $OE^{-\bullet}$	odd-electron ion
PA	proton affinity
PDA	photodiode array spectrometry
PDI	^{252}Cf plasma desorption ionization
PIA	precursor-ion analysis
PICI	positive-ion chemical ionization
Q–LIT	quadrupole–linear ion-trap hybrid
Q–TOF	quadrupole–time-of-flight hybrid
QuEChERS	quick, easy, cheap, effective, rugged, and safe
RDA	retro-Diels–Alder
RDBE	ring double-bond equivalent
RF	radiofrequency alternating current potential
RPLC	reversed-phase liquid chromatography
RSD	relative standard deviation
S/N	signal-to-noise ratio
SIL-IS	stable-isotope-labeled internal standard
SNRI	selective non-serotonin reuptake inhibitor
SPE	solid-phase extraction
SQ	single-quadrupole
SRM	selected-reaction monitoring
SSRI	selective serotonin reuptake inhibitor
STA	systematic toxicological analysis
SWATH	sequential windowed acquisition of all theoretical fragment ion mass spectra
TCA	tricyclic antidepressant
TDC	time-to-digital converter
TeCA	tetracyclic antidepressant
TIC	total-ion chromatogram
TOF	time-of-flight
TQ	tandem-quadrupole
TSI	thermospray ionization
UHPLC	ultra-high-performance liquid chromatography
UV	ultraviolet spectroscopy
WADA	world anti-doping agency
XIC	extracted-ion chromatogram

1

INTRODUCTION TO LC–MS TECHNOLOGY

Interpretation of MS–MS Mass Spectra of Drugs and Pesticides, First Edition. Wilfried M. A. Niessen and Ricardo A. Correa C.

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1.1 INTRODUCTION

In order to separate and quantify ions using mass spectrometry (MS), one must first generate and then send them to the mass analyzer, which is no easy task by any means. This process takes place in the ion source, where the introduced neutral atoms or molecules (the sample) are rendered ionized and in the gas phase. From there, they are sent into the mass analyzer and separated according to their m/z (mass-to-charge ratio (Section 2.2), where m is the mass number of an ion and z is the number of elementary charges regardless of sign). The order in which ionization and vaporization happen depends on the chosen technique, but ultimately the ions will have to find themselves under vacuum so that the mean free path between them is long enough to avoid random collisions, for example, fragment–fragment reactions. This is essential for the tenet of unimolecular reactions in MS to hold, whereby all the ions seen in the mass spectrum arise from the initially ionized sample in question. The ions generated can be odd-electron ions ($OE^{+\bullet}$ or $OE^{-\bullet}$) or even-electron ions (EE^+ or EE^-). Providing the m/z for all ions and especially for the ions related to the intact molecule, for example, molecule ion or (de)protonated molecule, is the main reason of MS success as an analytical technique. In general, one can say that there are two main types of ionization techniques: hard and soft ionization techniques. In the former case, the molecular ion undergoes significant fragmentation (even with no molecular ion detection), whereas in the latter case ions do not undergo extensive (or any) fragmentation and an ion related to the intact molecule is readily detected.

In practice, chemical analysis begins with two critical steps that determine the ultimate quality of the experiments: sample collection and preparation, which should always strive at getting the highest purity specimen possible. The ion source contribution to the overall instrumental sensitivity arises from the two main events taking place within: sample ionization and ion transmission to the mass analyzer. Ionization efficiency is defined as the ratio of the number of ions generated to the number of molecules consumed in the ion source of a mass spectrometer: the method for determining the number of molecules consumed has to be clearly stated. The transmission efficiency is defined as the ratio of the number of ions leaving a region of a mass spectrometer to the number of ions entering that region. Since the performance

of a source is tightly related to its actual components and their operating principles, sensitivity optimization depends on the kind and model of instrument used.

Sample introduction to the source is done by several methods: the most common being directly via a direct vapor inlet, or a direct insertion or exposure probe; indirectly via hyphenated techniques such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS), or surface-related desorption techniques such as thermally or laser-assisted techniques. Hyphenated techniques refer to the coupling of two (or more) separate analytical techniques by means of an appropriate hardware interface. In such cases, the instruments used in the hyphenated techniques work together in an automated manner as a single integrated unit (Hirschfeld, 1980). Particularly interesting is the coupling of powerful separation techniques, for example, GC, LC, thin-layer chromatography, electrophoresis, with spectrometry-related methods, for example, MS, infrared, ultraviolet–visible, atomic absorption, fluorescence, light scattering, Raman, nuclear magnetic resonance, for the analysis and characterization of all kinds of known matter.

1.2 ANALYTE IONIZATION: ION SOURCES

1.2.1 Electron Ionization

Electron ionization (EI) is a hard ionization technique and one of the oldest ionization methods in existence, yet still one the most widely used (Märk & Dunn, 1985). Vaporization of sample molecules must take place before their ionization, and therefore this limits the scope of the technique to volatile and thermostable compounds. EI furnishes ions by extracting one (or more) electron (e^-) out of the neutral sample molecule (M), according to Eq. 1.1. This process is carried out with high-energy electrons produced by means of thermionic emission from a heated (tungsten or rhenium) filament inside the source. Typically, the electrons are accelerated with a potential difference of 70 V. The energetic electrons interact with the analyte molecules, transfer part of their energy to the molecules, and render them ionic. The result is the production of a radical cation $M^{+\bullet}$ (molecular ion) and two electrons: the electron ejected from the neutral molecule and the ionizing electron after transferring part of its energy to M.

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

The fate of the radical cation ($M^{+\bullet}$) produced depends on its internal energy at the moment of formation, which is determined by the kind and number of chemical bonds present in the sample molecule. It is $M^{+\bullet}$ and its fragmentation products (when present) that constitute the EI mass spectrum of the sample, and in principle for a given set of experimental conditions, each individual compound analyzed gives a unique mass spectrum (except for enantiomers).

1.2.1.1 Ionization Using Electrons The general operating components of an EI source are illustrated in Figure 1.1. These are contained within a heated (to avoid condensation of sample and ions) metal housing called the source block. EI uses thermionic emission as the main working principle for the production of high-energy (usually 70 eV, $1\,\text{eV} = 1.602177 \times 10^{-19}$ J) electrons under vacuum (0.1–1 Pa; 10^{-3}–10^{-2} mbar) in order to disrupt the nonbonding and bonding electrons of molecules.

An appropriately housed (coiled) tungsten or rhenium filament (cathode) is heated by passing a current through it (2–5 A). Once it reaches a certain temperature, the thermal energy of the electrons (greater than the work function of the metal) at the metal surface is sufficient to allow them to leave the metal thereby creating a flow of electrons. This is the thermionic emission of electrons from the filament. Concurrently, a negative potential (−70 V) is applied to the filament (e^- energy), and the electrons are thus accelerated

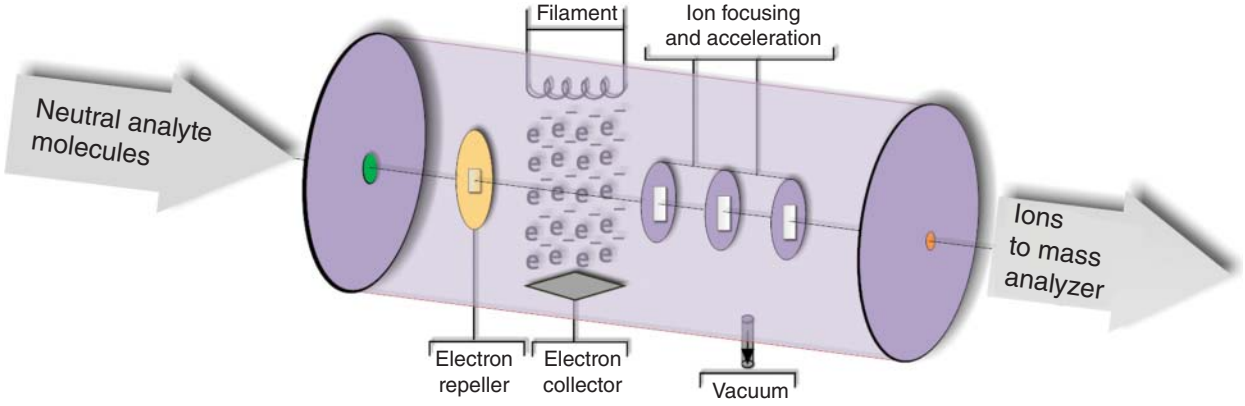


FIGURE 1.1 Schematic diagram of an electron ionization (EI) source.

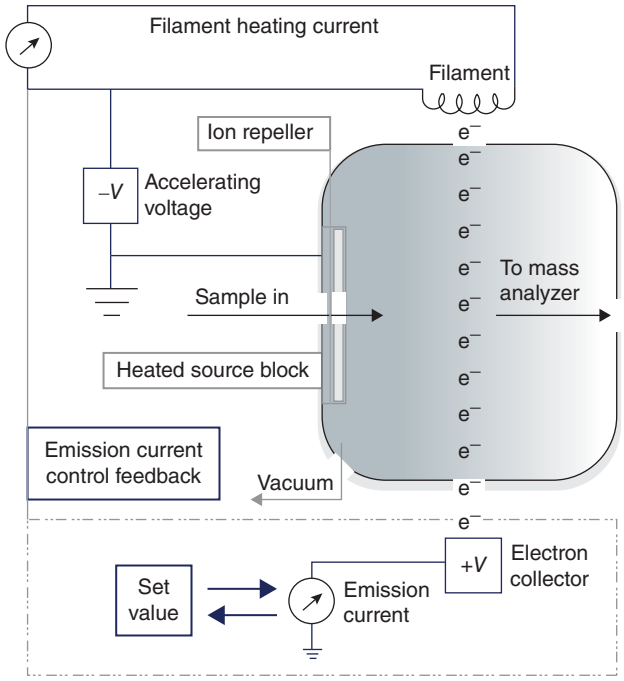


FIGURE 1.2 Scheme for the generation of ionizing electrons in an EI source.

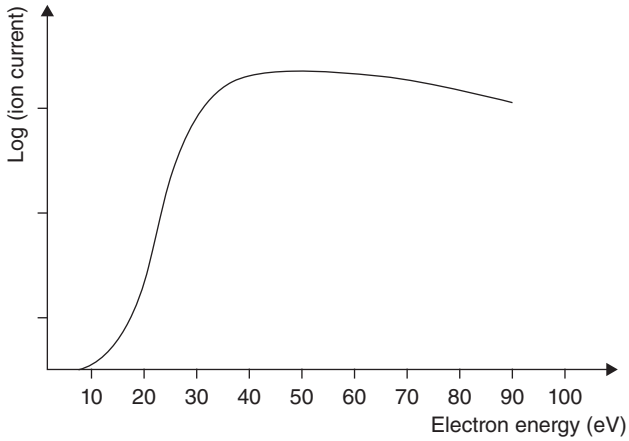


FIGURE 1.3 Relationship between ion current and electron energy.

and travel across from the surface of the metal filament to within the volume of the ion source. These electrons are attracted (by a positive voltage) to the e^- collector (anode) located opposite and on-axis to the filament. This filament current (emission current) is measured and kept constant (150 µA) via a feedback mechanism with the heating current driven through the filament. This ensures constant ionization conditions (the number of electrons emitted by the filament is constant). Effectively, this setup places a shower of electrons that analyte molecules must cross as they are transmitted from the inlet (sample in) to the outlet (to mass analyzer) of the EI source (Figure 1.2). Often, by using a magnet, the flight path of the electrons is made helical; since the electrons must travel a longer path, their interaction with analyte molecules is enhanced.

Fortunately, the value of 70 eV has been used for the electron energy (and to less extent 150 µA for the emission current) throughout the years, and this has allowed for the creation of searchable EI mass spectral libraries that are of critical importance to the analytical applications of MS. By controlling the energy of the electrons, one can achieve different ionizing conditions for a given sample. The plot of the ion current versus the electron energy for most atoms and molecules shows the general behavior illustrated in Figure 1.3. A rise in the ion current is observed once the analyte ionization energy (IE, minimum energy required to eject an e^- out of a neutral atom or molecule in its ground state) is reached. As the electron energy increases (≈20 eV), so does the ion current, mostly due to the formation of molecular ions. Further increase in energy (>30 eV) promotes fragmentation until a plateau is reached (around 70 eV); higher electronic energies actually cause a decrease in the ion current (Hübschmann, 2015). Operating the source at 70 eV for the electron energy, that is, at the plateau in Figure 1.3, ensures stable performance of the EI source. The EI efficiency is evaluated by the ratio of the number of ions formed to the number of electrons used in an ionization process.

Considering that helium has the highest ionization energy of any element (24.6 eV), along with the fact that the IE for most organic compounds lies between 5 and 12 eV, electrons with 70 eV will have more energy than the IE required to ionize incoming neutral species (Montalti et al., 2006). In chemistry, eV (non-SI unit) is expressed in molar terms and thus $70\,\text{eV} = 6{,}754\,\text{kJ mol}^{-1}$. The amount of excess energy transferred from the electron to the molecule, typically a few eV (≈5 eV), and the structure of the molecule will determine the degree of fragmentation. The general trend of atomic IE is the same as the one for electronegativity, for example, F > Cl > Br > I. For molecules, nonbonding (nb) electrons are easier to ionize than bonding electrons, for example, IE of F-nb > N-nb > O-nb > S-nb. The greater the s character of a covalent bond, the more the electronegative it is; thus, the IE of a sigma sp bond (alkynes) > sp^2 sigma bond (alkenes) > sp^3 sigma bond (alkanes) > nb electrons. Special molecular features, for example, conjugation, which can help stabilize the resulting radical cation, greatly influence the IE value of a molecule.

1.2.1.2 Ionization and Fragmentation As the sample is introduced into the source (perpendicular to the electron axis), electrons and neutral molecules interact. When the rapprochement of sample molecules and electrons is within the ionization cross-sectional area (area the electron must cross to lead to an effective ionization) of the analyte molecule and

the energy transferred is at least equal to the ionization energy, the loss of one (or more) electron is observed, along with the eventual fragmentation of the molecular ion thus produced. In the vacuum of the EI source, a random collision between an e^- and a sample molecule is extremely unlikely. Furthermore, the electrostatic repulsion of valence electrons makes it even more improbable. It is the electric field of the fast-moving charge (e^-) that causes a distortion in the orbits of the valence electrons. This interaction leads to a kinetic energy transfer from the e^- to the analyte cloud of electrons. If enough energy is transferred (IE) during this process, a valence electron is ejected from the analyte molecule, thereby forming an $M^{+\bullet}$. It is worthwhile noting that the de Broglie wavelength (λ) of the ionizing electrons must be of the same order as the bond length of the sample molecule, otherwise the energy transfer from the electrons to the analyte molecule will not happen effectively, for example, a 70 eV electron has a λ of 150 pm, an sp^2 hybridized C—C double bond has a bond length of ≈130 pm (Allen et al., 2006).

Approximately speaking, molecules have a diameter ranging from 0.1 nm for the smallest molecule (H_2), through macromolecules and supramolecular assemblies with diameters between 10 and 90 nm, for example, polymers, ATP synthase, to viruses and complex biological structures with >100 nm in diameter, for example, influenza virus, phages, chromosomes (Goodsell, 2009). Considering that the reaction in Eq. 1.1 is happening between two classical particles, an e^- with an energy of 70 eV travels approximately at a speed of 5000 km s^{-1} (0.017c, where c is the speed of light), which means that for a molecule like sucrose (nominal mass of 342 Da) with a 1 nm molecular diameter (Ramm et al., 1985), the electron will pass by the molecule in 2×10^{-16} s. In this timescale, the interaction between the electron and the molecule occurs much faster than that of an sp^3 O—H bond stretching vibration (10^{-14} s). As this electronic transition happens before any change occurs in the position of the nuclei involved (Franck–Condon principle), it can happen vertically from the electronic ground state of M to a (meta)stable excited electronic state of $M^{+\bullet}$ (or higher energy states) as illustrated in Figure 1.4. Taking a homodiatomic molecule as an example (Demtröder, 2010), its electronic ground state can be represented as shown in Figure 1.4a: the potential energy well is defined by the bond dissociation energy and the bond length. When the high-energy electrons match an electronic transition *i* (Figure 1.4b), the energy transfer leads to a stable excited electronic state (molecular ion), plus an e^- ejected off from the neutral sample. It is important to notice that electronic states higher than the ground state have potential energy wells with shallower minima and longer internuclear separations. Therefore, the bond is both weaker and elongated as a result of the ionization process (Figure 1.4b). Equally, if the energy of the electrons matches an electronic transition like *j* in Figure 1.4b, the formation of the radical cation will lead to an unstable excited state and fragmentation ensues.

What happens to the newly formed ions depends on their total energy and the ease with which they dissipate the excess energy among their other modes of motion, namely translational, vibrational, and rotational. Generally, the ions can be stable and last long enough to be detected, they can rapidly decompose producing fragment ions, or they can be

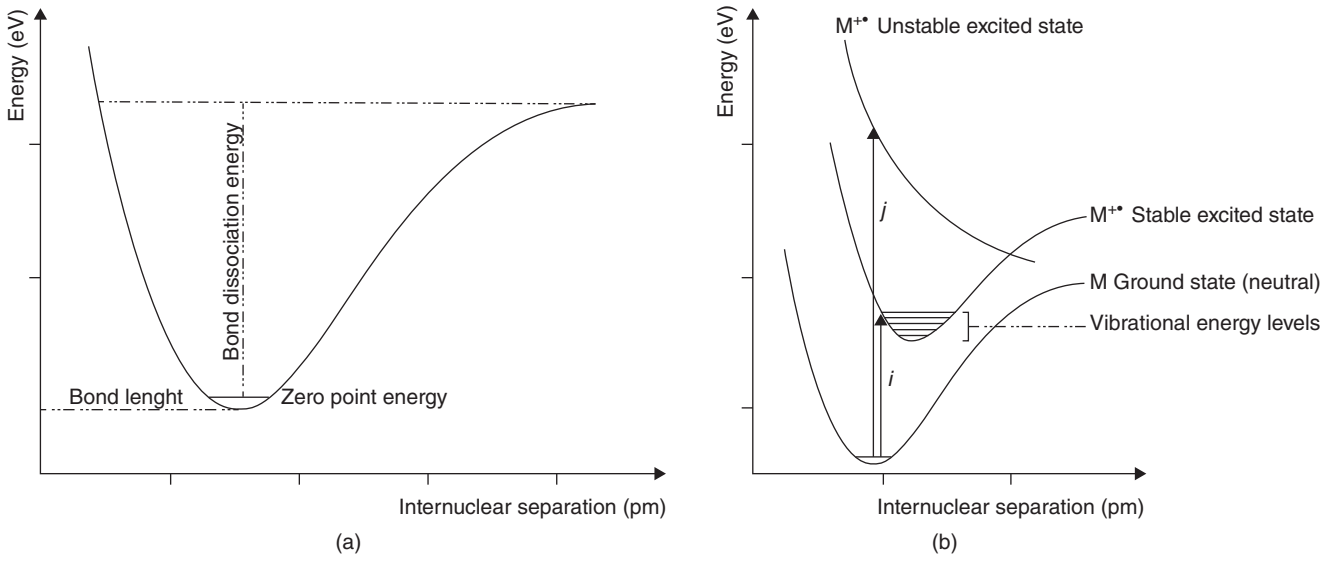


FIGURE 1.4 Ground electronic state of a neutral homodiatomic molecule (a). Vertical transitions depicting the ionization process in an EI source (b).

metastable and decompose in their flight to the detector. It is a process that is tightly related to the exact chemical structure of a molecule (Blanksby & Ellison, 2003).

1.2.1.3 Ion Transmission Ion transmission refers to the process of moving ions from one section to another within the mass spectrometer, for example, from the source through the analyzer and furthest to the detector. This process is not always necessarily accompanied by an *m*/*z* separation. In fact, in an EI source when transferring the ions produced into the analyzer, the goal is to do so with highest efficiency and lowest *m*/*z* spreading. Two complementary and simultaneous devices are applied (Figure 1.5). First, as the ions are being produced, a potential difference of the same sign is applied to the ion repeller, which is a plate placed before and perpendicular to the electron flux. This ion repeller pushes the ions toward the mass analyzer.

Second, three parallel (exact design changes depending on manufacturer) electrostatic lenses of equal sign are placed opposite and on-axis to the ion repeller, between the e^- flux and the mass analyzer. A potential difference of opposite sign to the ion repeller is applied in order to extract the ions out of the source, followed by a lower potential difference in order to focus the ions to finally reaccelerate them as they are sent into the mass analyzer, where separation according to their *m*/*z* takes place. Typical fragmentation characteristics under EI conditions are briefly discussed in Section 3.3.

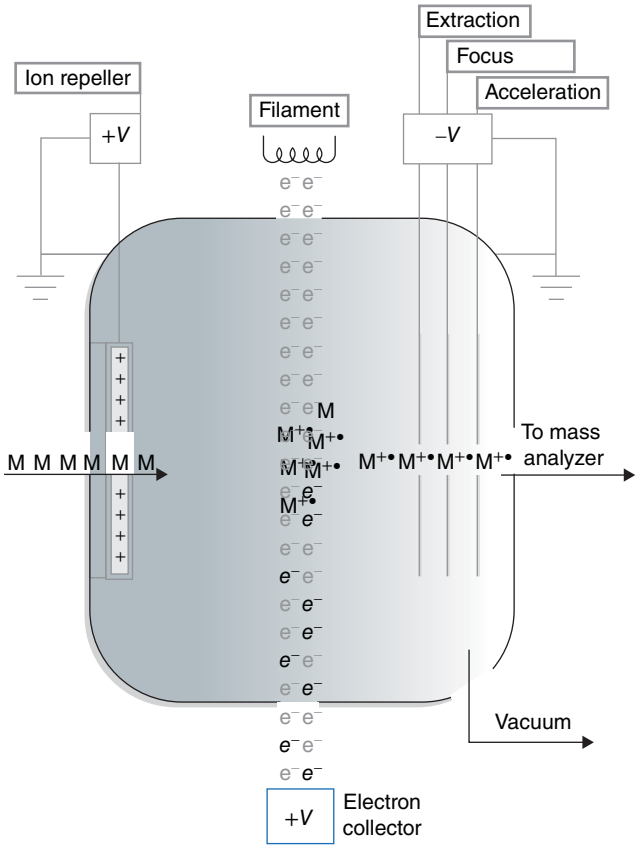


FIGURE 1.5 Devices for ion transmission from the EI ion source to the mass analyzer.

1.2.1.4 Analytical Applications of Electron Ionization EI is probably the most widely applied ionization technique in MS. It is extensively used in GC–MS, where it provides good sensitivity for most compounds and structure-informative fragmentation in highly reproducible mass spectra. Besides, after basic tuning of the ion source, which can be performed automatically under software control, there are essentially no experimental parameters to set or optimize. In terms of qualitative analysis, interpretation of the EI mass spectra can be performed based on a solid understanding of the fragmentation behavior of $M^{+\bullet}$ (Section 3.3) (McLafferty & Tureček, 1993; Smith, 2004). In addition, elaborate and searchable mass spectral libraries have been compiled to assist in the identification of compounds (Atwater et al., 1985; Stein & Scott, 1994; Ausloos et al., 1999; Koo et al., 2013). The results of these library searching routines can be quite powerful. If a mass spectrum of the unknown compound is present in the library, expert comparison of library and experimental mass spectra can lead to compound identification. If the compound is not present in the library, the computer library search often provides insight about the presence of substructures or other structural features of the unknown compound, which facilitates further spectrum interpretation. Although many researchers take the result of the library search for granted, a thorough and critical evaluation of the agreement between experimental and library spectrum is recommended. In addition, GC–MS with EI is also frequently used in quantitative analysis using either extracted-ion chromatograms (Section 1.3.1.1) or selected-ion monitoring (Section 1.5.2) before peak area determination. More recently, gas chromatography tandem mass spectrometry (GC–MS–MS) in selected-reaction monitoring (SRM) (Section 1.5.2) mode has become the method of choice in routine quantitative analysis of compounds present at very low levels in complex biological matrices.

As EI is limited to the analysis of volatile and thermostable analytes, analyte derivatization strategies have been developed to enhance the volatility and stability of more polar analytes. Derivatization obviously changes the fragmentation behavior of the analyte because the fragmentation may be directed from a different site in the molecule (Zaikin & Halket, 2009; Sparkman et al., 2011). Silylation and oximation reactions are most frequently carried out. Characteristic fragment ions derived from the derivatizing agent are readily seen, thereby improving analysis selectivity. For instance, the trimethylsilyl ether derivative ($(CH_3)_3SiOR$) of hydroxy group (OH) containing molecules show the trimethylsilyl group ion with *m*/*z* 73 ($[(CH_3)_3Si]^+$) and an ion with *m*/*z* 75 corresponding to protonated dimethylsilanone

($[(CH_3)_2SiO+H]^+$). When the target compound has several trimethylsilyl ether moieties, the formation of the pentamethyldisiloxane cation ($[(CH_3)_2SiOSi(CH_3)_3]^+$) with *m/z* 147 is observed (a commonly seen ion from GC column bleeding). These ions may undergo ion–neutral reactions with analyte molecules (M), one of these reactions is the adduct formation of an ion with *m/z* (M+73) (Carles et al., 2007).

After seeing the power of EI in GC–MS, the implementation of EI in LC–MS has been pursued as well. However, given the gas load of the mostly aqueous mobile-phase vapor admitted into the ion source and the MS vacuum system in LC–MS, it is more complicated to achieve the high-vacuum ion source conditions required for successful EI. The most successful approaches to EI in LC–MS (which were also commercialized) were the moving-belt interface (Arpino, 1989) and the particle-beam interface (Creaser & Stygall, 1993), both quite complex instrumental solutions. Unfortunately, these solutions did not provide the reliability, user-friendliness, and sensitivity required. More recently, the so-called direct-EI interface has been described, which provides nebulization of the effluent of a nano-LC column (flow rates < 100 nL min^{-1}), directly into the EI source (Cappiello et al., 2011).

1.2.2 Chemical Ionization

Chemical ionization (CI) is a soft ionization technique used to study chemical structure and reactivity. A CI source uses a reagent gas (G_R) inside a modified EI source to create conditions of high source pressure, such that G_R ions–molecule and molecule–e^- reactions can occur in high yield (Harrison, 1992; Munson, 2000). In fact, most instruments are equipped with a source that can be switched between EI and CI conditions. As seen so far, an EI source is an environment where neutral molecules (or atoms) and radicals, radical cations, cations, and electrons coexist. Intuitively, the presence of electrons in the source begs the question of whether or not positive ions are the only ions present in the source. As expected, negative-ion formation is an inherent process in EI and formation of radical anions is also observed (Bowie, 1984).

Thus, there can be a simultaneous presence of positive and negative ions inside an EI/CI source. Their transmission and detection are a matter of choice and depend on the voltage polarities chosen to carry out the experiments, for example, when analyzing negative ions except for the e^- collector voltage in Figure 1.5, all other voltages must be switched in polarity. CI creates conditions that favor the production of EE^+ and EE^-, and as a result, CI can be carried out in two different modes: positive mode as in positive-ion chemical ionization (PICI) and negative mode as in negative-ion chemical ionization (NICI) and electron-capture negative ionization (ECNI). Both modes can use the same source and often but not necessarily use the same G_R. Nevertheless, the function of the G_R serves a different purpose on each mode, and experimental conditions must be optimized for each type of analyte in relation to the mode of CI chosen. Ionization in CI happens without the transfer of large excess of energy from a G_R (and ions thereof) or from a secondary e^-; thus, the initially generated ions do not undergo extensive fragmentation. CI is a technique that offers both high sensitivity and selectivity. Nevertheless, it is not suitable to all kinds of molecules as the analytes must be volatile and thermostable and must present special structural features in order to be responsive to the technique.

1.2.2.1 Electron Ionization of the Reagent Gas, G_R

For particles of similar shape and at a given temperature, the mean free path between them is inversely proportional to the pressure. Usually, in EI the mean free path is ≥1 m, and caution must be taken as mean free paths of ≤0.5 m lead to ion–ion reactions, generating an atypical mass spectrum. As the G_R flows into the CI source, it establishes conditions of high pressure (1–100 Pa; 10^{-2}–1 mbar; while the pressure in the vacuum manifold is ≤10^{-3} Pa; 10^{-5} mbar) and its ionization by primary 70 eV electrons readily yields molecular ions ($G_R^{+\bullet}$). In many CI sources, higher electron energies (up to 400 eV) are applied in order to ensure that the electrons penetrate well the high-pressure environment of the ion source. Ensuing fragmentation of $G_R^{+\bullet}$ occurs by forming cations (G_{EE}^+), other radical cations ($G_{OE}^{+\bullet}$), neutral species (R, $R^\bullet$), and secondary electrons (e^-) (Eqs 1.2 and 1.3).

$$G_R + e^- \rightarrow G_R^{+\bullet} + 2e^- \quad (1.2)$$

$$G_R^{+\bullet} \rightarrow G_{EE}^+ + R^\bullet \quad (1.3a)$$

$$G_R^{+\bullet} \rightarrow G_{OE}^{+\bullet} + R \quad (1.3b)$$

Given a controlled flow of G_R into the source, it is the most abundant species and reacts (ion–molecule reactions) with the newly formed $G_R^{+\bullet}$, G_{EE}^+, $G_{OE}^{+\bullet}$ yielding reactive electrophilic cations that can undergo further reactions with analytes of interest. While EI is a unimolecular process, in CI bimolecular and even termolecular reactions generate a steady-state plasma inside the source as shown in Figure 1.6; methane is used as an example to illustrate the reactions observed.

When the sample is introduced into the source, it encounters a plasma of both positive and negative (low-energy electrons) reactive species. The most common reactions taking place involve proton transfer, electron capture, or adduct formation between the analyte of interest and charged species of the reactants. In this technique, the presence of the (de)protonated molecule is characteristic, which serves as a complementary tool to other types of MS methods. The ions generated in PICI, NICI, and ECNI happen via different mechanisms; nevertheless, all three can happen concurrently.

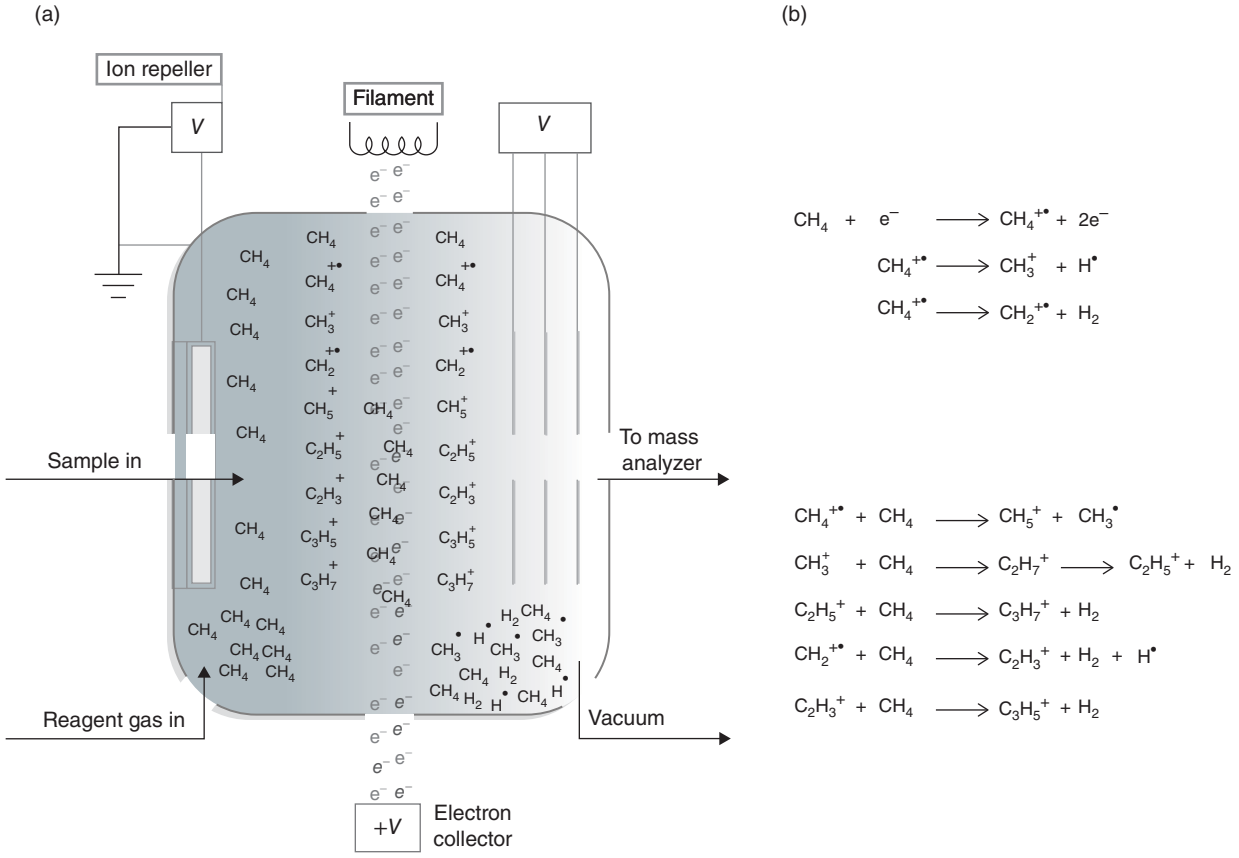


FIGURE 1.6 Ongoing processes inside a chemical ionization (CI) source during reagent gas ionization (methane) in a CI experiment (a). Main chemical reactions involved in the ionization of methane reagent gas in an EI source during a CI experiment (b).

1.2.2.2 Positive-Ion Chemical Ionization The main pathways that explain the experimental observations regarding ion formation in PICI between analyte molecules and G_R plasma are as follows: (i) proton transfer, (ii) electrophilic addition, (iii) anion abstraction, and (iv) charge exchange (CE).

Proton transfer Proton transfer is the most commonly observed reaction and serves as the basis for PICI measurements. These Brønsted–Lowry acid–base reactions afford protonated analyte molecules as long as their gas-phase basicity is greater than that of the reactive species present in the source. However, hydride (H^-) abstraction from the analyte molecules can also occur. The former case yields a cation $[M+H]^+$ with m/z (M+1), where M is the (monoisotopic) mass of the analyte molecule (Eq. 1.4a), whereas the latter case yields a cation $[M–H]^+$ with m/z (M−1) (Eq. 1.4b).

$$[G_R+H]^+ + M \rightarrow [M+H]^+ + G_R \quad (1.4a)$$

$$G_{EE}{}^+ + M \rightarrow [M–H]^+ + G_RH \quad (1.4b)$$

In addition to methane (Section 1.2.2.1), several other gases including propane, butane, isobutane, and ammonia can form cations that serve as G_R in Brønsted–Lowry acid–base reactions. If the reaction is exothermic, these cations will readily transfer protons to analyte molecules (M) forming $[M+H]^+$ cations. The exothermicity of the reaction is determined by the proton affinity (PA) difference between the reacting species (Table 1.1). In general, the more exothermic the reaction is, the more fragmentation is observed (more energy transferred to analyte molecule).

Careful choice of acid–base pairs allows control of the extent of the ionization and fragmentation process, thus either inducing or eliminating ionization and/or fragmentation. Eq. 1.5 shows the protonation and hydride abstraction reactions of an analyte molecule (M) when using methane as G_R.

$$[CH_5]^+ + M \rightarrow [M+H]^+ + CH_4 \quad (1.5a)$$

$$[C_2H_5]^+ + M \rightarrow [M–H]^+ + CH_3CH_3 \quad (1.5b)$$

TABLE 1.1 Proton affinities of compounds commonly used in GC–MS and LC–MS.

Compound	PA (kJ mol^{-1})	Compound	PA (kJ mol^{-1})
Methane (CH_4)	552	Methyl acetate (CH_3COOCH_3)	828
Ethyne ($HC{\equiv}CH$)	641	Ethenone ($H_2C{=}C{=}O$)	830
Ethene ($H_2C{=}CH_2$)	680	Diethyl ether ($C_2H_5OC_2H_5$)	838
Water (H_2O)	697	Ammonia (NH_3)	854
Hydrogen sulfide (H_2S)	712	Aniline ($C_6H_5NH_2$)	877
Formaldehyde ($H_2C{=}O$)	718	Methylamine (CH_3NH_2)	896
Propene ($CH_3CH{=}CH_2$)	752	Alanine ($(CH_3CHNH_2)COOH$)	899
Benzene (C_6H_6)	759	Ethyl amine ($CH_3CH_2NH_2$)	908
Methanol (CH_3OH)	761	Dimethylamine ($(CH_3)_2NH$)	923
Ethanol (C_2H_5OH)	788	Pyridine (C_5H_5N)	924
Acetonitrile ($CH_3C{\equiv}N$)	788	Dimethyl aniline ($C_6H_5N(CH_3)_2$)	935
Toluene ($C_6H_5CH_3$)	794	Trimethylamine ($(CH_3)_3N$)	942
Ethyl formate ($HCOOC_2H_5$)	808	Piperidine ($C_5H_{11}N$)	947
iso-Butene ($(CH_3)_2C{=}CH_2$)	820	Quinoline (C_9H_7N)	948
Acetone (CH_3COCH_3)	823	Triethylamine ($(C_2H_5)_3N$)	972

Source: Adapted from Lias, 1984 and Hunter, 1998. Reproduced with permission of the American Institute of Physics.

The methanium ion ($[CH_5]^+$) with *m/z* 17 is a good example of a G_R ionic species reacting in both protonation (Eq. 1.5a) and hydride abstraction reactions with analyte molecules (Eq. 1.5c).

$$[CH_5]^+ + M \rightarrow [M–H]^+ + CH_4 + H_2 \quad (1.5c)$$

Electrophilic addition Electrophilic addition (adduct formation, e.g., alkylation) is another type of acid–base reaction that occurs when analyte molecules have Lewis base character, for example, presence of heteroatoms with nonbonding electrons or π-electrons, allowing their reaction with electrophiles (even-electron cations, $G_{EE}{}^+$) present in the G_R plasma (Eq. 1.6).

$$G_{EE}{}^+ + M \rightarrow [M{+}G_{EE}]^+ \quad (1.6)$$

Some examples of adduct formation when using methane as G_R are shown in Eq. 1.7. Knowing the mass of the alkylating cation allows one to find the molecular mass of the target compound. For methane, these ions are found with *m/z* (M+15), (M+29), and (M+41).

$$[CH_3]^+ + M \rightarrow [M{+}CH_3]^+ \quad m/z \text{ (M+15)} \quad (1.7a)$$

$$[C_2H_5]^+ + M \rightarrow [M{+}C_2H_5]^+ \quad m/z \text{ (M+29)} \quad (1.7b)$$

$$[C_3H_5]^+ + M \rightarrow [M{+}C_3H_5]^+ \quad m/z \text{ (M+41)} \quad (1.7c)$$

Conditions within the source can be changed in order to promote or inhibit a given type of acid–base reaction from happening. This can be achieved by establishing physical conditions, for example, e^- energy and G_R pressure, in the source that will favor the formation of the G_R ions needed for either proton transfer or adduct formation. Table 1.2 shows the most common CI reagent gases used in MS, along with the adducts formed from analyte molecules–G_R plasma reactions.

Anion abstraction Anion abstraction happens when $G_{EE}{}^+$ ions react with sample molecules to form an analyte-derived cation and a neutral species as shown in Eq. 1.8. Proton abstraction is a good example (exothermic reaction with the nitrosonium cation (NO^+) for most alkanes) leading to $[M{-}H]^-$ ions with *m/z* (M−1). Alcohols (1° and 2°), aldehydes, and ketones undergo this kind of reaction. Tertiary alcohols undergo abstraction of hydroxy group (OH) leading to a stable tertiary carbocation $[M{-}OH]^+$ with *m/z* (M−17).

$$G_{EE}{}^+ + M \rightarrow [M–A]^+ + G_{EE}A \quad (1.8)$$

Hydride abstraction from alkanes when using cations such as $[C_2H_5]^+$ (Eq. 1.5b) and $[CF_3]^+$ is a good example as well; group electronegativity is useful in this respect (Wells, 1968). There is no reagent gas system exclusively developed for this mode of CI; the nitrosyl radical ($^{\bullet}NO$) or a mixture of nitrogen/nitrous oxide (N_2/NO_2) are reagent gases used to produce NO^+, which acts as hydrogen abstractor, and can also participate in adduct formation and charge-transfer reactions.

Charge exchange (CE) CE is the outcome of the interaction between a $G_R{}^{+\bullet}$ and a neutral analyte molecule. Ionization takes place when there is a transfer of charge to the analyte molecule producing an $M^{+\bullet}$ and a neutral G_R. The reaction

TABLE 1.2 Common reagent gases used in positive-ion CI and adducts formed thereof.

Reagent Gas (G_R)	G_{EE}^+ Plasma Ions	Adducts Formed	*m/z*
Methane (CH_4)	$[CH_3]^+$	$[M+CH_3]^+$	M+15
	$[CH_5]^+$	$[M+H]^+$	M+1
		$[M-H]^+$	M−1
	$[C_2H_3]^+$	$[M-H]^+$	M−1
	$[CH_2CH_3]^+$	$[M+C_2H_5]^+$	M+29
	$[CH_2CHCH_2]^+$	$[M+C_3H_5]^+$	M+41
Isobutane ($(CH_3)_2CHCH_3$)	$[(CH_3)_3C]^+$	$[M+(CH_3)_3C]^+$	M+57
	$[CH_3CHCH_3]^+$	$[M+H]^+$	M+1
		$[M+C_3H_7]^+$	M+43
	$[C_3H_3]^+$	$[M+C_3H_3]^+$	M+39
Ammonia (NH_3)	$[NH_4]^+$	$[M+H]^+$	M+1
		$[M+NH_4]^+$	M+18
	$[NH_4+NH_3]^+$	$[M+[NH_4+NH_3]]^+$	M+35

is observed when the recombination energy (exothermicity of the reaction $G_R^{+\bullet} + e^- \rightarrow G_R$) of G_R is higher than the IE of M (Eq. 1.9). The degree of fragmentation of $M^{+\bullet}$ depends on the exothermicity of the reaction. However, the molecular ions produced are usually of low internal energy. The presence of protonating species must be kept at a minimum in order to avoid formation of G_RH. Pure compounds are usually used as G_R for charge-exchange chemical ionization (CECI), nonetheless, mixtures with an inert buffer gas such as N_2 find application. Despite the fact that alkanes, for example, CH_4, and aromatic compounds, for example, benzene, chlorobenzene, can be used as G_R for CECI, aprotic solvents are preferred: rare gases, for example, Ne, Ar, Xe, methanedithione (S═C═S), sulfanylidenemethanone (S═C═O), nitrosyl ($^\bullet$NO).

$$G_R^{+\bullet} + M \rightarrow M^{+\bullet} + G_R \tag{1.9}$$

In addition to its routine application as an analytical tool, CI has also been used in mechanistic studies, such as the study of gas-phase ion–molecule reactions (organic chemistry in the high-vacuum gas phase), *regio-* and *stereo-*selectivity questions, conformational analysis, and the measurement of relative reaction rate constants.

1.2.2.3 Negative-Ion Chemical Ionization The study of reactions between negative ions of G_R and neutral sample molecules has not been carried out as thoroughly as it has been done for their positive counterparts. This mode of ionization happens in two different methods: NICI and ECNI. In the former case, it is the result from reactions of G_R anions present in the source and neutral analyte molecules (M). This occurs readily when stable anions of the G_R can be formed. ECNI, in contrast, is the process by which thermal electrons present in the source (e^-) react with neutral analyte molecules generating radical anions ($OE^{-\bullet}$) and anions (EE^-).

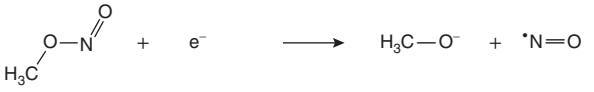
The main reactions in NICI can be grouped as (i) proton transfer, (ii) nucleophilic addition, (iii) nucleophilic displacement, and (iv) CE.

Proton transfer Proton transfer occurs when an anion (G_R^-) derived from a G_R or a G_R mixture reacts with a neutral analyte molecule containing a removable proton. This happens when the PA (or gas-phase basicity) of G_R^- is greater than the PA of the conjugate base of the analyte ($[M-H]^-$), according to Eq. 1.10.

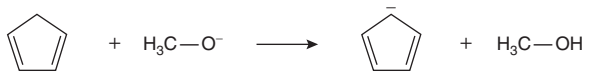
$$G_R^- + M \rightarrow [M-H]^- + G_RH \tag{1.10}$$

Molecules with acidic H-atoms (removable) such as carboxylic acids and phenols are common examples of functional groups undergoing proton-transfer reactions. Therefore, the PA of typical anions can be used to predict the outcome of NICI proton-transfer reactions. Some examples of G_R^- are as follows: Cl^-, $[CN]^-$, $[O_2]^{-\bullet}$, F^-, $[CH_2CN]^-$, $[CH_3O]^-$, $O^{-\bullet}$, $[OH]^-$, H^-, $[NH_2]^-$, and $[C_5F_5]^-$ (Table 1.3).

There exist many gas mixtures to generate the anions of interest, for example, the use of fluorocarbons (trifluoromethane, CHF_3) and chlorofluorocarbons (CF_2Cl_2) to generate F^- and Cl^-, respectively, and the use of ammonia (NH_3) to generate $[NH_2]^-$ (Dougherty, 1981). Most of these anionic reactive species themselves are produced by associative electron-capture reactions, for example, formation of $[O_2]^{-\bullet}$. The reaction between methoxide ion ($[CH_3O]^-$, PA ≈ 1580 kJ mol^{-1}) and cyclopentadiene producing the cyclopentadiene anion ($[C_5H_5]^-$) (ΔPA ≈ −100 kJ mol^{-1}) serves as an example (Eq. 1.11).

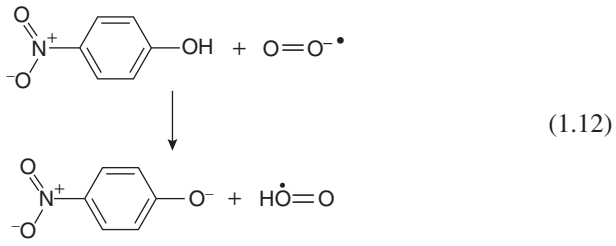


(1.11a)



(1.11b)

Methyl nitrite (CH_3ONO) undergoes dissociative electron capture to produce the reactive species of interest CH_3O^- (Eq. 1.11a), which deprotonates cyclopentadiene producing the $[C_5H_5]^-$ (Eq. 1.11b). Superoxide ($O_2^{-\bullet}$, $PA \approx 1465\,kJ\,mol^{-1}$), formed by electron capture of nitrous oxide (NO_2) or a molecular oxygen/argon gas mixture, can behave as a basic species and deprotonates acidic compounds such as 4-nitrophenol producing the corresponding phenoxide ion ($PA_{calc} \approx 1350\,kJ\,mol^{-1}$) (Chandra & Uchimaru, 2002) and hydroperoxyl radical ($HOO^{\bullet}$), as illustrated in Eq. 1.12.



(1.12)

Hydroxide ions (HO^-, $PA \approx 1635\,kJ\,mol^{-1}$) are frequently used for their ability to produce NICI mass spectra of a diversity of functional groups: alcohols, ethers, neutral lipids, and hydrocarbons.

TABLE 1.3 Anions used for neutral analyte negative ionization in GC–MS and LC–MS.

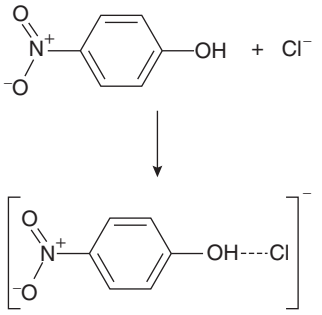
Anion	PA ($kJ\,mol^{-1}$)
NH_2^- (amide)	1689
H^- (hydride)	1676
OH^- (hydroxide)	1636
$O^{-\bullet}$ (atomic oxygen radical anion)	1595
CH_3O^- (methoxide)	1583
$(CH_3)_2CHO^-$ (isopropoxide)	1565
$^-CH_2CN$ (cyanomethide)	1556
F^- (fluoride)	1554
$C_5H_5^-$ (cyclopentadiene anion)	1480
$O_2^{-\bullet}$ (molecular oxygen radical anion)	1465
CN^- (cyanide)	1462
Cl^- (chloride)	1395
$HCOO^-$ (formate)	1444*
CH_3COO^- (acetate)	1458*
CF_3COO^- (trifluoroacetate)	1350*

Source: Bruno & Svoronos, 2010; *Harrison, 1992. Reproduced with permission of American Chemical Society.

Nucleophilic addition Nucleophilic addition can occur when anions do not have very high proton affinities (e.g., $O_2^{-\bullet}$, $[CN]^-$ ($PA \approx 1460\,kJ\,mol^{-1}$), Cl^- ($PA \approx 1395\,kJ\,mol^{-1}$). Instead of undergoing acid–base reactions leading to deprotonated products, they form adducts by nucleophilic addition to analyte molecules (Eq. 1.11a).

$$G_R^- + M \rightarrow [M+G_R]^- \tag{1.13}$$

Examples of this reaction are hydrogen-bonded adducts formed by chloride ions (Cl^-) with analyte molecules containing functional groups with electrophilic H-atom, such as carboxylic acids, amides, aromatic amines, phenols, and organophosphorus pesticides. This leads to the production of $[M+Cl]^-$ ions with *m/z* (M+35) and *m/z* (M+37) in a ≈3:1 ratio of relative intensities. For instance, 4-nitrophenol reacts with Cl^- as shown in Eq. 1.14.



(1.14)

Nucleophilic addition is also observed with $O_2^{-\bullet}$ and compounds of low acidity such as aliphatic compounds forming the corresponding $[M+O_2]^{-\bullet}$ radical ion. Alcohols also undergo nucleophilic addition adduct formation. For instance, it was found that 11 different anionic species form adducts with neutral oligosaccharides (Jiang & Cole, 2005).

Nucleophilic displacement Nucleophilic displacement is a substitution reaction where an electrophilic center of an analyte molecule undergoes nucleophilic attack (e.g., S_N2). The leaving group thus produced can be a neutral radical or a new anionic species as illustrated in Eq. 1.15.

$$G_R^{-\bullet} + M \rightarrow [MG_R\text{–}H]^- + H^{\bullet} \tag{1.15a}$$

$$G_R^- + MA \rightarrow MG_R + A^- \tag{1.15b}$$

Many strongly basic anions such as atomic oxygen radical anion ($O^{-\bullet}$, $PA \approx 1595\,kJ\,mol^{-1}$) and HO^- usually react in proton-transfer reactions. Nonetheless, with certain analytes, they participate in gas-phase nucleophilic reactions. Both of these ions can be produced by using N_2O as G_R (e.g., N_2O, N_2O/CH_4). Examples of this mechanism are the gas-phase reactions of $O^{-\bullet}$ with phthalic acid alkyl esters (Stemmeler et al., 1994; Lépine et al., 1999) and the analysis of steroids with HO^- where both proton abstraction and nucleophilic displacement are observed (Roy et al., 1979).

Charge exchange (CE) CE occurs when a G_R (Lewis base) with lower electron affinity (EA) than that of the neutral analyte (Lewis acid) is allowed to react in the CI ion source and an electron transfer is effected as shown in Eq. 1.16. The degree of fragmentation depends on the exothermicity of the reaction. An important characteristic of this type of reaction is the possibility of obtaining single peak mass spectra, consisting of the anionized analyte molecule.

$$G_R{}^{-\bullet} + M \rightarrow M^{-\bullet} + G_R \tag{1.16}$$

As an example, the analysis of dibenzothiophene using $[O_2]^{-\bullet}$ as G_R delivered $M^{-\bullet}$, while the G_R was oxidized to molecular oxygen (O_2) (Hunt et al., 1976). Care must be taken to avoid the presence of competing species that would react with $M^{-\bullet}$, thereby lowering the sensitivity of the analysis. For instance, the presence of fluorine radicals ($F^{\bullet}$) would lead to the formation of fluoride ions (F^-) and neutral analyte M.

Despite the successes of NICI as an analytical tool, the most common technique used for the generation of negative ions is ECNI. Strictly speaking, these electron–molecule reactions are not chemical ionization processes. If at a given temperature there is an equilibrium between the generation and recombination of electrons, the electrons are said to be in thermal equilibrium. Thermal electrons have a kinetic energy $\leq$2 eV. Under these conditions, they can be captured by electronegative atoms present in analyte molecules, thereby forming radical anions ($OE^{-\bullet}$). The thermionic emission of electrons from heated filaments is the usual way of producing high-energy primary electrons in EI. The main source of secondary (thermal) electrons is the deceleration of primary electrons by collisional energy transfer with gases inside the source, such as G_R ionization as shown in Eq. 1.17.

$$2G_R + e^-_{70\,eV} \rightarrow G_R^* + G_R{}^{+\bullet} + 2e^-_{2\,eV} \tag{1.17}$$

Polyatomic gases are more efficient collisional energy sinks than diatomic and monoatomic gases, and therefore their rate of e^- thermalization is higher (e.g. $NH_3 > CO_2 > i\text{-}C_4H_{10} > CH_4 > N_2 > Ar$). After the reaction of the secondary electrons with the analyte molecules, the presence of a G_R (or a buffer gas) is essential for collisional stabilization of the newly formed excited radical anion $OE^{-\bullet}$. Otherwise, e^- detachment can happen and no analyte anion is observed.

Neutral analyte molecules undergo EC to form radical anions ($OE^{-\bullet}$). The ease, with which this process happens, depends on the EA of the neutral analyte and its ability to dissipate the excess internal energy after its formation (Eq. 1.18).

$$M + e^- \rightarrow M^{-\bullet} \tag{1.18}$$

Since charge density leads to instability, for example, HO^- is less stable than H_2O, charge dissipation must be effective. Therefore, analyte molecules must have electronic features that promote electron capture. Factors that contribute most prominently in the stabilization of a negative charge are as follows: orbital hybridization of the atom bearing the charge, for example, for carbanions the stability follows $sp > sp^2 > sp^3$, the presence of geminal or vicinal electronegative elements ($F > O > Cl > N > Br > I > S > C > P$) and/or electron-withdrawing functional groups or substituents ($—CF_3 > —CCl_3 > —CH_3$; $—CN \approx —CCH > —CHCH_2 \approx —C_6H_5$; $—OH > —NO_2 > —NH_2$), charge delocalization by resonance or aromaticity, and molecular polarizability whereby small atoms and molecules dissipate a charge less effectively than large ones, for example, the I-atom is more polarizable than an F-atom, thus I^- is a much better leaving group than F^- in substitution reactions. Usually, the most electronegative element present in the molecule determines its EA. For this reason, molecules with electronegative elements or groups, for example, nitro (NO_2), acyl (RCO), and cyano (CN), are attractive targets of ECNI. The main processes that explain the formation of negative species in ECNI are as follows: (i) associative electron capture, (ii) dissociative electron capture, and (iii) ion-pair formation reactions (Hiraoka, 2003; Stemmeler & Hites, 1988).

Associative electron capture Associative electron capture as shown in Eq. 1.18 gives the molecular radical anion $M^{-\bullet}$ after reaction of M with a low energy e^- (<2 eV). The molecular anion is formed without great excess energy, and additional collisional stabilization with (buffer) gases present in the source explains the high relative intensity of $M^{-\bullet}$ observed.

Dissociative electron capture Dissociative electron capture happens when electrons inside the ion source with a kinetic energy of up to $\approx$15 eV react with analyte molecules containing electronegative atoms or substituent groups that can form good leaving groups, for example, halogens, benzyl ($C_6H_5CH_2{}^-$), and methoxy (CH_3O^-), according to Eq. 1.19. The formation of a stable anion $[M–X]^-$ or X^- is the basis for this sensitive and selective type or CI analysis.

$$MX + e^- \rightarrow [M–X]^- + X^{\bullet} \tag{1.19a}$$

$$MX + e^- \rightarrow M^{\bullet} + X^- \tag{1.19b}$$

As expected, all these reactions are exothermic, and the outcome depends on the difference between the bond energy of the X group in the analyte and the EA of the analyte [M−X] and X fragments.

Ion-pair formation Ion-pair formation happens with electrons of $\approx$10–15 eV. The initially formed $OE^{-\bullet}$ has enough internal energy to dissociate into positive and negative ions

(Eq. 1.20). This process is not very common and does not find widespread use as an analytical method.

$$MX + e^- \rightarrow [M\text{–}X]^- + X^+ + e^- \quad (1.20a)$$

$$MX + e^- \rightarrow [M\text{–}X]^+ + X^- + e^- \quad (1.20b)$$

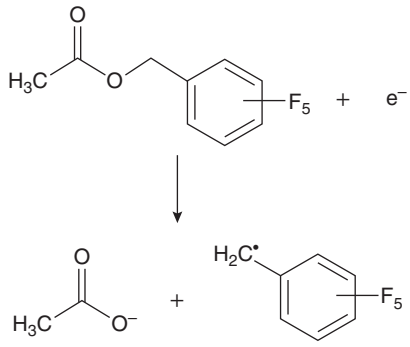
Attention must be given when choosing the buffer gases in such a way that they do not form stable negative ions or reactive species, in order to avoid competition reactions or reactions with neutral or charged analyte molecules, which inevitably lower the sensitivity of the analysis. Equally important is keeping matrix effects and impurities to a minimum. In addition, the vacuum pump speed must also be adequate to fulfill the pressure requirements of CI experiments.

1.2.2.4 Analytical Applications of Chemical Ionization

CI is not applied in combination with GC–MS as widely as is EI. In terms of analytical applications, the various modes of performing CI have different application areas. PICI is mainly used to determine or confirm the mass of the intact analyte molecule, for example, in cases where $M^{+\bullet}$ is not observed or is present with a very low relative intensity under EI conditions. In this context, PICI may become more important in GC–MS in the future, given the increasing use of SRM in tandem-quadrupole (TQ) instruments. The introduction of atmospheric-pressure chemical ionization (Section 1.2.5) for GC–MS is also highly interesting (van Bavel et al., 2015; Li et al., 2015). Different CI reactions can be achieved under those conditions, which are largely dependent on the reagent gas used and the instrumental parameters for attaining the sought-after results.

GC–MS with ECNI has found a wide range of applications in targeted quantitative analysis, for instance in forensic toxicology and pharmacology for the analysis of polar compounds. For such applications, pentafluoropropyl or pentafluorobenzyl ester derivatives are produced. As such, GC–ECNI-MS is routinely applied in forensic toxicology to determine illicit drugs, for instance for the presence of tetrahydrocannabinol (THC) in hair (Foltz, 1992; Moore et al., 2006). Enantioselective analysis of amphetamines has been reported after derivatization with (*S*)-(−)-*N*-(heptafluorobutanoyl)prolyl chloride (HFBPC) (Lim et al., 1993). HFBPC and its related compounds are very efficient chiral derivatizing reagent of amino groups (Leis & Windischhofer, 2012). GC–ECNI-MS also plays an important role in the analysis of environmental pollutants such as polybrominated compounds of both synthetic (polybrominated diphenyl ethers as fire retardants) and natural (polybrominated hexahydroxanthene derivatives) origins. In such cases, bromide ions (Br^-) are produced during dissociative ECNI (Eq. 1.19b). The high selectivity of the analysis lies in the production of ions with *m*/*z* 79 and 81 ($^{79}Br^-$ and $^{81}Br^-$ with ≈1:1 relative intensity) (Rosenfelder & Vetter, 2009).

Another possibility of dissociative electron capture leads to retention of charge by the analyte molecule, to effectively produce $[M-H]^-$ of the underivatized analyte, in combination with the production of a neutral radical ($X^\bullet$) leaving group (Eq. 1.19a). This behavior is applied in the GC–ECNI-MS analysis of fatty acids (RCOOH) such as arachidonic acid analogs after derivatization to their pentafluorobenzyl esters. In this case, the dissociative ECNI process leads to an ion corresponding to the deprotonated acid with *m*/*z* (M−1) and pentafluorobenzyl radical, as shown in Eq. 1.21 (Hadley et al., 1988).



(1.21)

When comparing modes of ionization in CI, sensitivity is a parameter often employed to quantitatively gauge them. Inherently, neither NICI nor PICI is a more sensitive technique than the other. What determines the sensitivity is the number of extractable and detectable analyte ions present in the source at any time. For that reason and when possible, the relative second-order reaction rates in ECNI versus proton transfer and adduct formation in PICI are used to determine the sensitivity of a particular method. Generally speaking, electron-capture rate constants can be up to 1000 times larger or smaller than proton transfer, for example, methanol gas-phase H/D-exchange rate constant is $\approx 10^{-11}$ cm^3 molecule^{-1} s^{-1}, Green & Lebrilla, 1997). Therefore, CI experiments must be carefully planned to use G_R-analyte partners that will offer optimum sensitivity and selectivity.

1.2.3 Atmospheric-Pressure Ionization

GC enjoys the advantage of being able to deliver the analyte molecules inside the source in the gas phase, and that makes it suitable when using an EI source. Notwithstanding the technological challenges, precedents exist shortly after its development of GC coupling to MS (Holmes & Morrell, 1957). LC coupling to MS presents a greater challenge: analytes elute out of the LC column dissolved in liquid solvents of varying volumes and polarities (volatilities). The *conditio sine qua non* for MS is to have ions under vacuum and in the gas phase. Therefore, in order to couple LC to MS, devising a way to desolvate sample molecules, ionize, and transmit them to the high-vacuum environment of

the mass analyzer was indispensable. Atmospheric-pressure ionization (API) sources were developed to achieve that task, and three kinds of API are routinely used: electrospray ionization (ESI), atmospheric-pressure chemical ionization (APCI), and atmospheric-pressure photoionization (APPI). API techniques provide soft-ionization processes where the post-ionization energy of analyte molecules is not large enough to cause extensive fragmentation (if any), with an ion related to the intact molecule (as a cationized or anionized molecule) usually present. Equally important, API techniques offer an alternative ionization way apt for polar, low volatility (high molecular mass), and thermolabile compounds. Figure 1.7 is an approximate chart showing the molecular mass and polarity ranges of application for the most common ionization techniques in MS.

The three techniques accomplish the same task in different but related ways, the main difference being the process of analyte ionization itself. Desolvation and ion transmission share the same electromechanical principles in all three techniques: sample nebulization in an atmospheric-pressure chamber, inert gasses and thermal energy for desolvation, and reduced pressure. The source is also designed to keep neutral molecules from reaching the detector (lower background noise).

Since the analyte is dissolved in the mobile phase, one must make sure that prior to mass analysis the removal of unwanted material is as complete as possible, for example, remnants of solvents, buffers, and additives used to guarantee the ionization of neutral compounds while avoiding signal suppression by interfering chemicals. Therefore, the use of volatile solvents and additives is indicated. In this respect, gradient elution must be carefully planned not to adversely affect the mass spectrum. A flow reduction of the eluting mobile phase leads to more efficient analyte desolvation and analyte ionization. Several techniques exist to reduce the flow rate to the ESI source such as pre-source flow split (for concentrated samples as well) or the use of nL min^{-1} flow rates with LC columns of 10–100 μm internal diameter (Chervet et al., 1996).

In an API source, the coupling to an LC system column effluent or any other liquid flow is done via the sample inlet, where the liquid is nebulized into a fine aerosol of small droplets. The nebulization process in ESI (Section 1.2.4.1) differs from the one used for APCI and APPI (Section 1.2.5.1). In the course of droplet solvent evaporation mediated by heated desolvation gas, for example, nitrogen (N_2), analyte ionization is achieved by different processes in ESI (Section 1.2.4.2), APCI (Section 1.2.5.2), and APPI

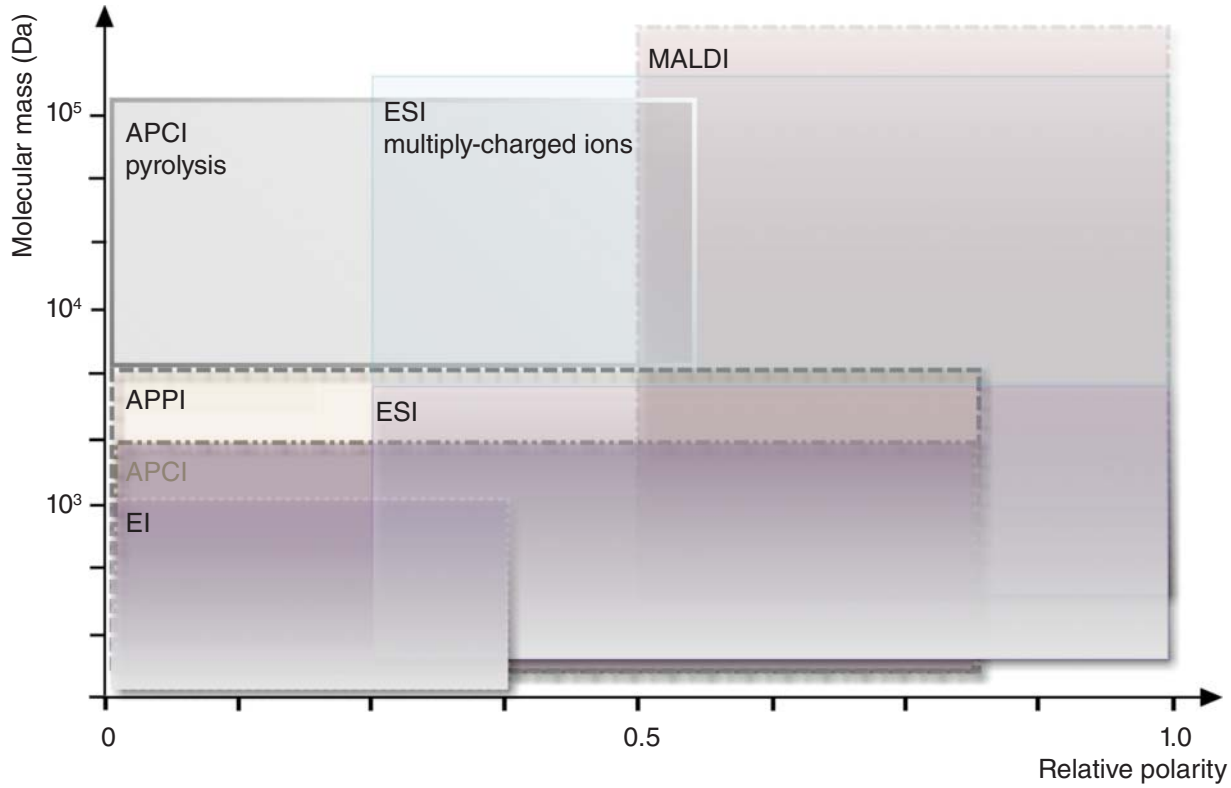


FIGURE 1.7 Approximate range of molecular mass and polarity for the most common ionization sources in MS.