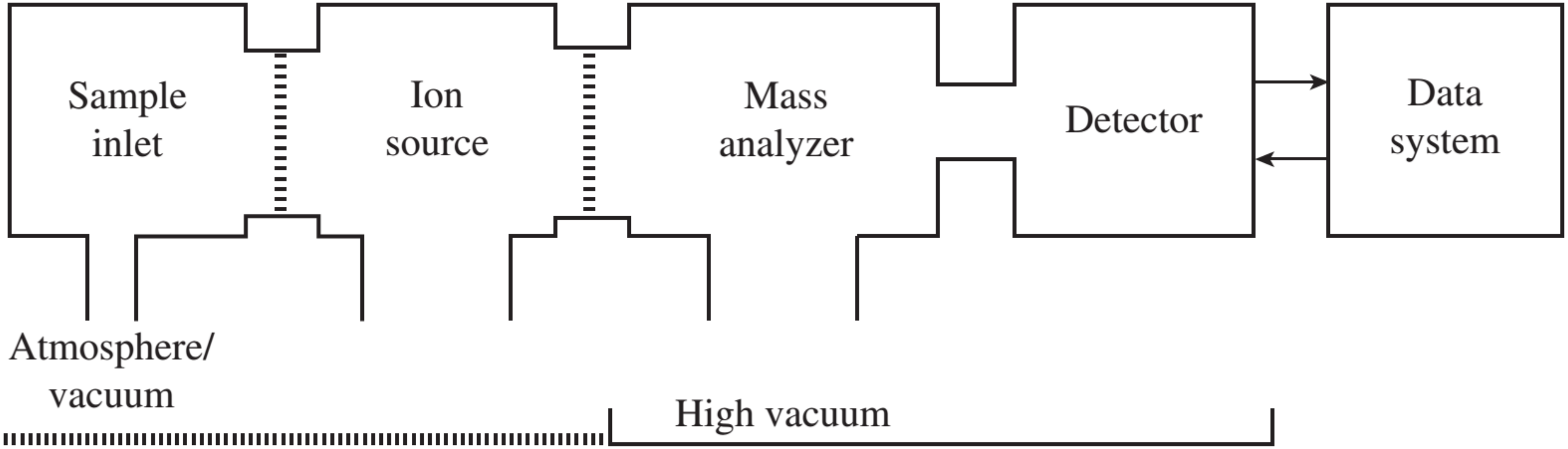
Chapter 6

Part IV

Mass Spectroscopy

The components of a mass spectrometer



- Regardless of the method of sample introduction, once the stream of sample molecules has entered the mass spectrometer, the sample molecules must be converted to **charged particles** by the **ion source** before they can be analyzed and detected.

Classification of Mass spectroscopic Methods

- **IONIZATION METHODS:**

+ **Electron Ionization (EI)**

+ **Chemical Ionization (CI)**

- **DESORPTION IONIZATION TECHNIQUES:**

+ **Fast atom bombardment (FAB)**

+ **Matrix-assisted laser desorption ionization (MALDI)**

+ **Secondary ion mass spectrometry (SIMS).**

- **ELECTROSPRAY IONIZATION (ESI)**

IONIZATION METHODS: Electron Ionization (EI)

- The simplest and most common method for converting the sample to ions is **electron ionization** (**EI**).

+ In EI-MS, a beam of **high-energy electrons** strike the stream of molecules.

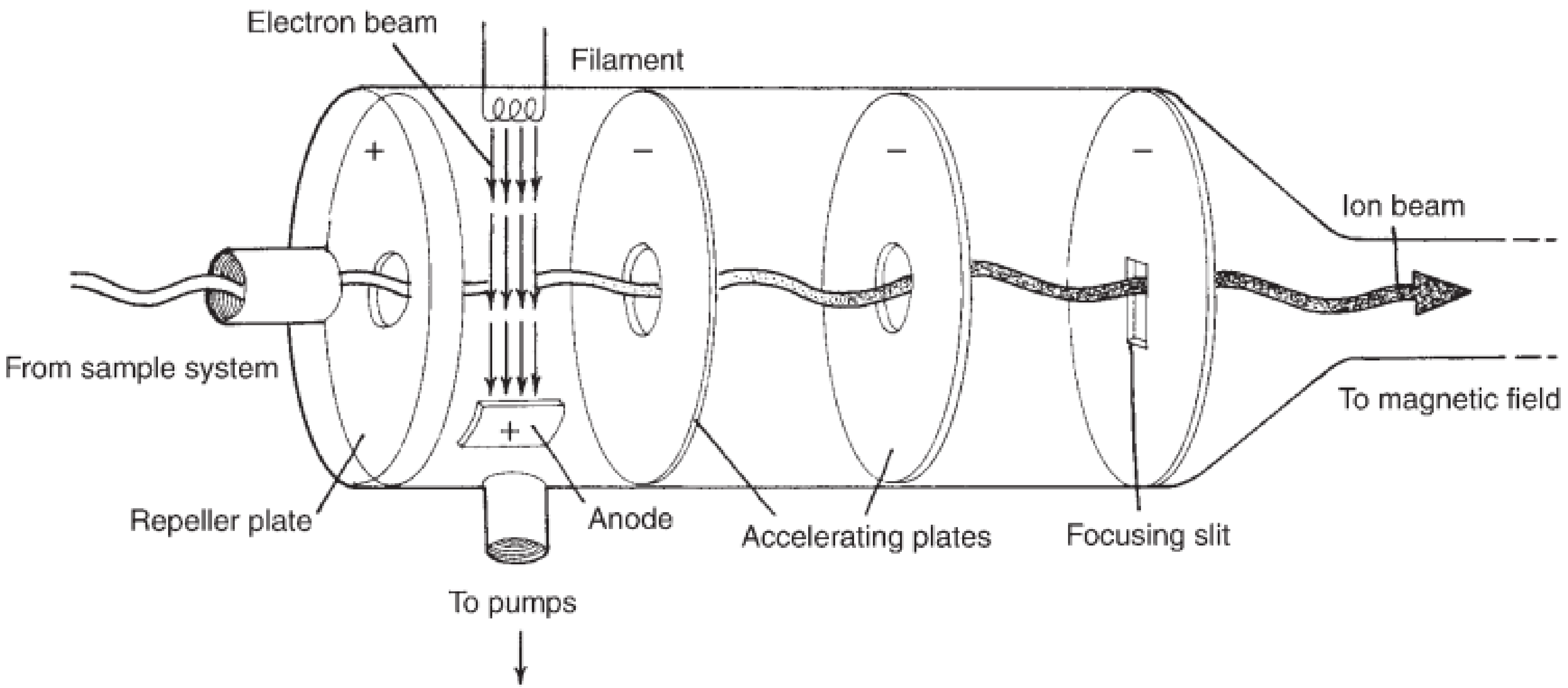
+ The electron–molecule collision strips an electron from the molecule, creating a radical cation.

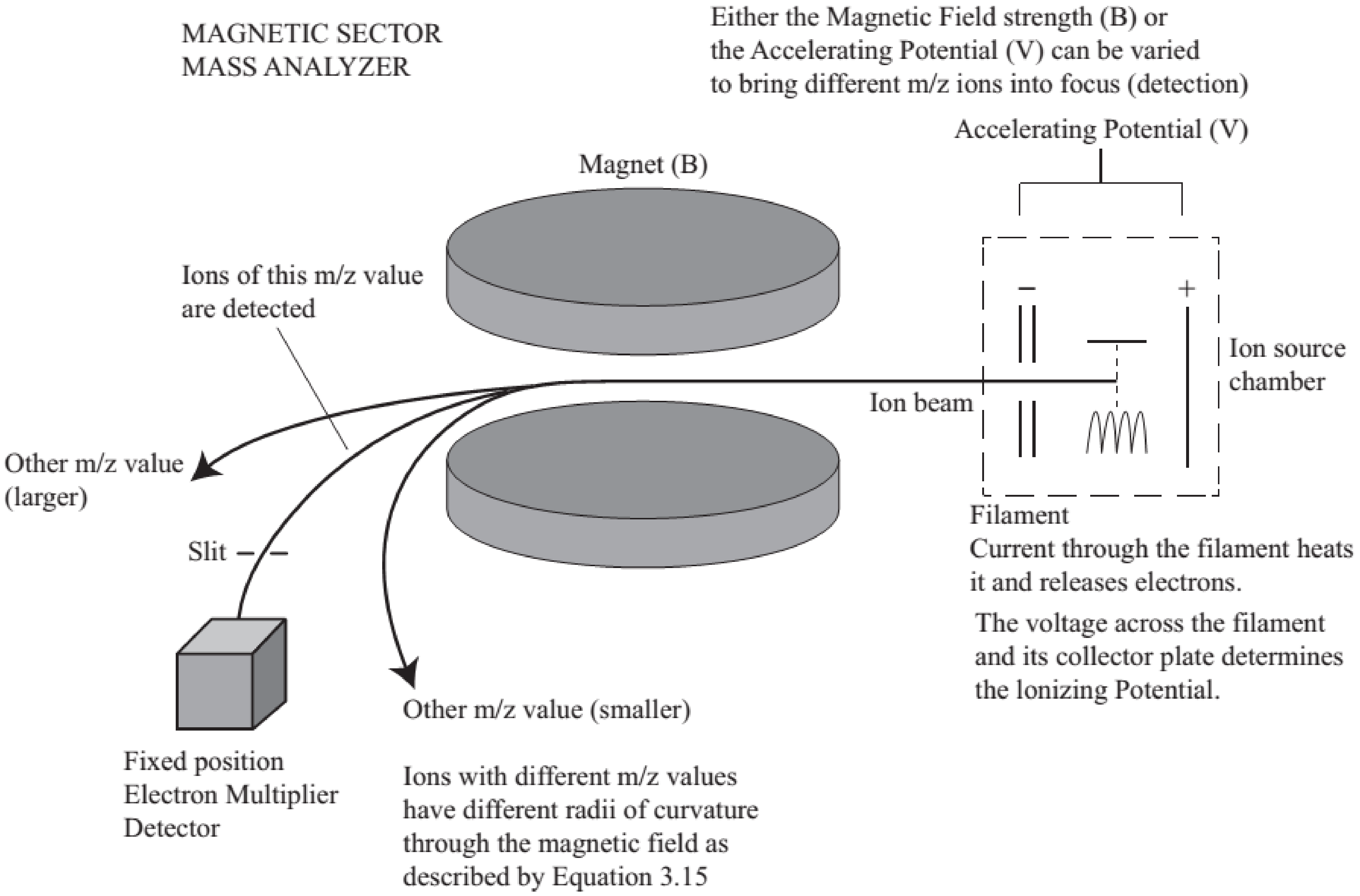
+ A **repeller plate,** which carries a positive electrical potential, directs the newly created ions toward a series of **accelerating plates.**

+ A large potential difference, ranging from 1 to 10 kilovolts (kV), applied across these accelerating plates produces a beam of rapidly traveling positive ions. One or more **focusing slits** direct the ions into a uniform beam.

IONIZATION METHODS: Electron Ionization (EI)

- The energy required to remove an electron from an atom or molecule is its **ionization potential** or **ionization energy.**
- Most organic compounds have ionization potentials ranging between 8 and 15 electron volts (eV).





Either the Magnetic Field strength (B) or the Accelerating Potential (V) can be varied to bring different m/z ions into focus (detection)

Current through the filament heats it and releases electrons.

The voltage across the filament and its collector plate determines the Ionizing Potential.

Ions with different m/z values have different radii of curvature through the magnetic field as described by Equation 3.15

FIGURE 3.11 Schematic of a magnetic sector mass analyzer.

Chemical Ionization (CI)

- In **chemical ionization–mass spectrometry** (**CI-MS**), the sample molecules are combined with a stream of ionized reagent gas that is present in great excess relative to the sample.

- When the sample molecules collide with the preionized reagent gas, some of the sample molecules are ionized by various mechanisms, including proton transfer, electron transfer, and adduct formation.

- Almost any readily available gas or highly volatile liquid can be used as a reagent gas for CI-MS. Common ionizing reagents for CI-MS include methane, ammonia, isobutane, and methanol.

Desorption Ionization Techniques (SIMS, FAB, and MALDI)

- EI and CI methods require a relatively volatile (low molecular weight) sample.

- Ionization techniques allow the analysis of large, nonvolatile molecules by mass spectrometry are all **desorption ionization** (**DI**) techniques. For example:
  - **Fast atom bombardment (FAB)**,
  - **Matrix-assisted laser desorption ionization (MALDI)**
  - **Secondary ion mass spectrometry (SIMS)**.

Desorption Ionization Techniques (SIMS, FAB, and MALDI)

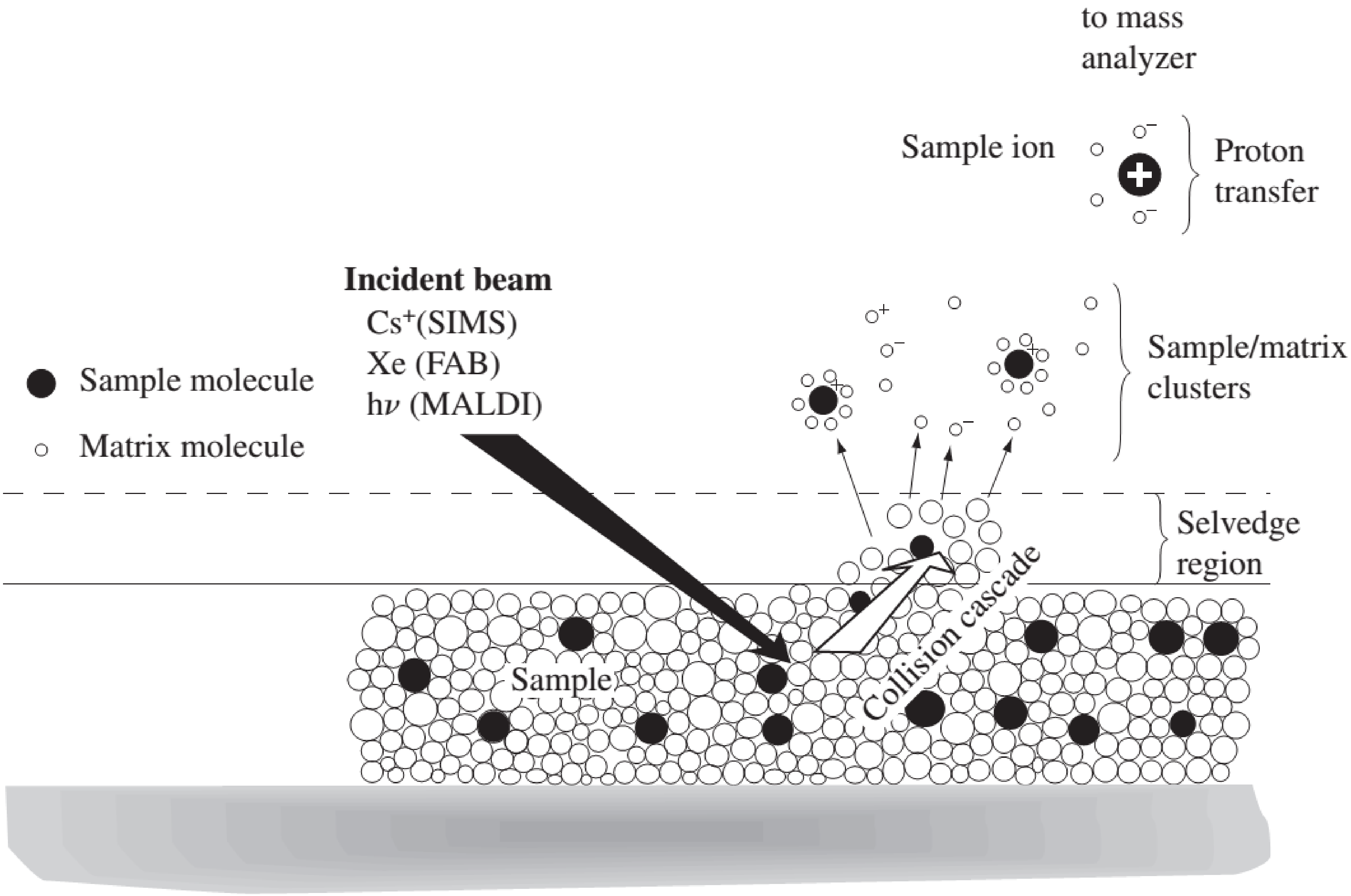
- In desorption ionization, the sample to be analyzed is dissolved or dispersed in a matrix and placed in the path of a high-energy (1- to 10-keV) beam of ions (SIMS), neutral atoms (FAB), or high-intensity photons (MALDI).

- Beams of Ar^+ or Cs^+ are often used in SIMS, and beams of neutral Ar or Xe atoms are common in FAB. Most MALDI spectrometers use a nitrogen laser that emits at 337 nm, but some applications use an infrared (IR) laser for direct analysis of samples contained in gels or thin-layer chromatography (TLC) plates.

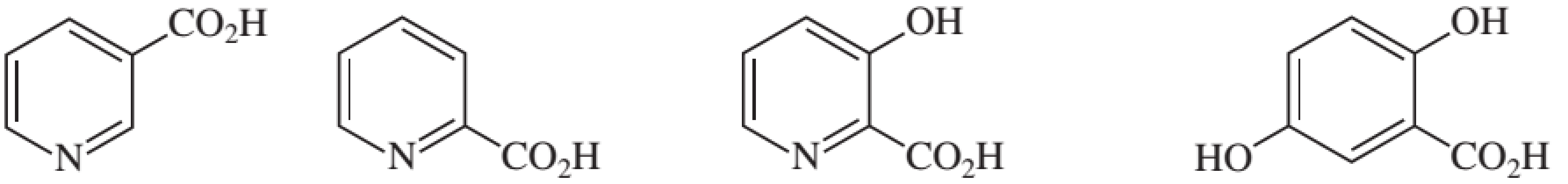
Desorption Ionization Techniques (SIMS, FAB, and MALDI)

- The collision of these ions/atoms/photons with the sample ionizes some of the sample molecules and ejects them from the surface.
- The ejected ions are then accelerated toward the mass analyzer as with other ionization methods.
- Since FAB uses neutral atoms to ionize the sample, both positive-ion and negative-ion detection are possible.
- Molecular ions in SIMS and FAB are typically $(M + H)^+$ or $(M - H)^-$, but adventitious alkali metals can create $(M + Na)^+$ and $(M + K)^+$ ions also.
- SIMS and FAB ionization methods may be used on sample compounds with molecular weights up to about 20.000, such as polypeptides and oligonucleotides.

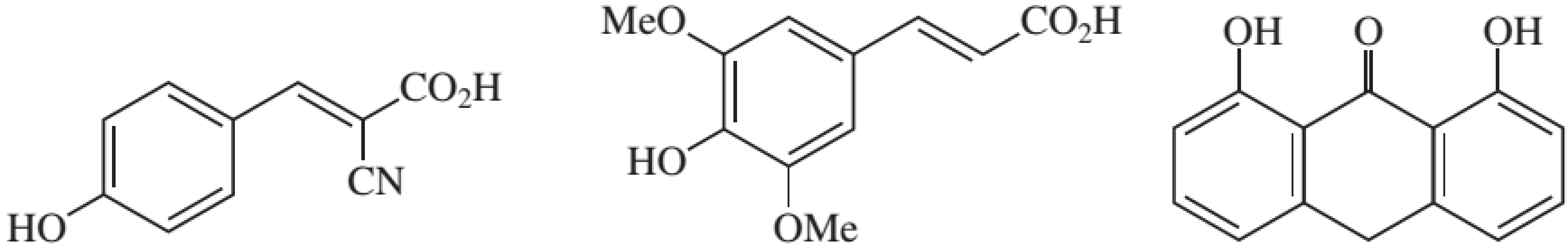
Desorption Ionization Techniques (SIMS, FAB, and MALDI)



Desorption Ionization Techniques (SIMS, FAB, and MALDI)



Nicotinic acid Picolinic acid 3-Hydroxypicolinic acid 2,5-Dihydroxybenzoic acid



α-Cyano-4-Hydroxycinnamic acid Sinapinic acid Dithranol

FIGURE 3.8 Common matrices for MALDI applications.

Electrospray Ionization (ESI)

- An even more useful technique for studying high molecular weight biomolecules and other labile or nonvolatile compounds is **electrospray ionization (ESI)**.

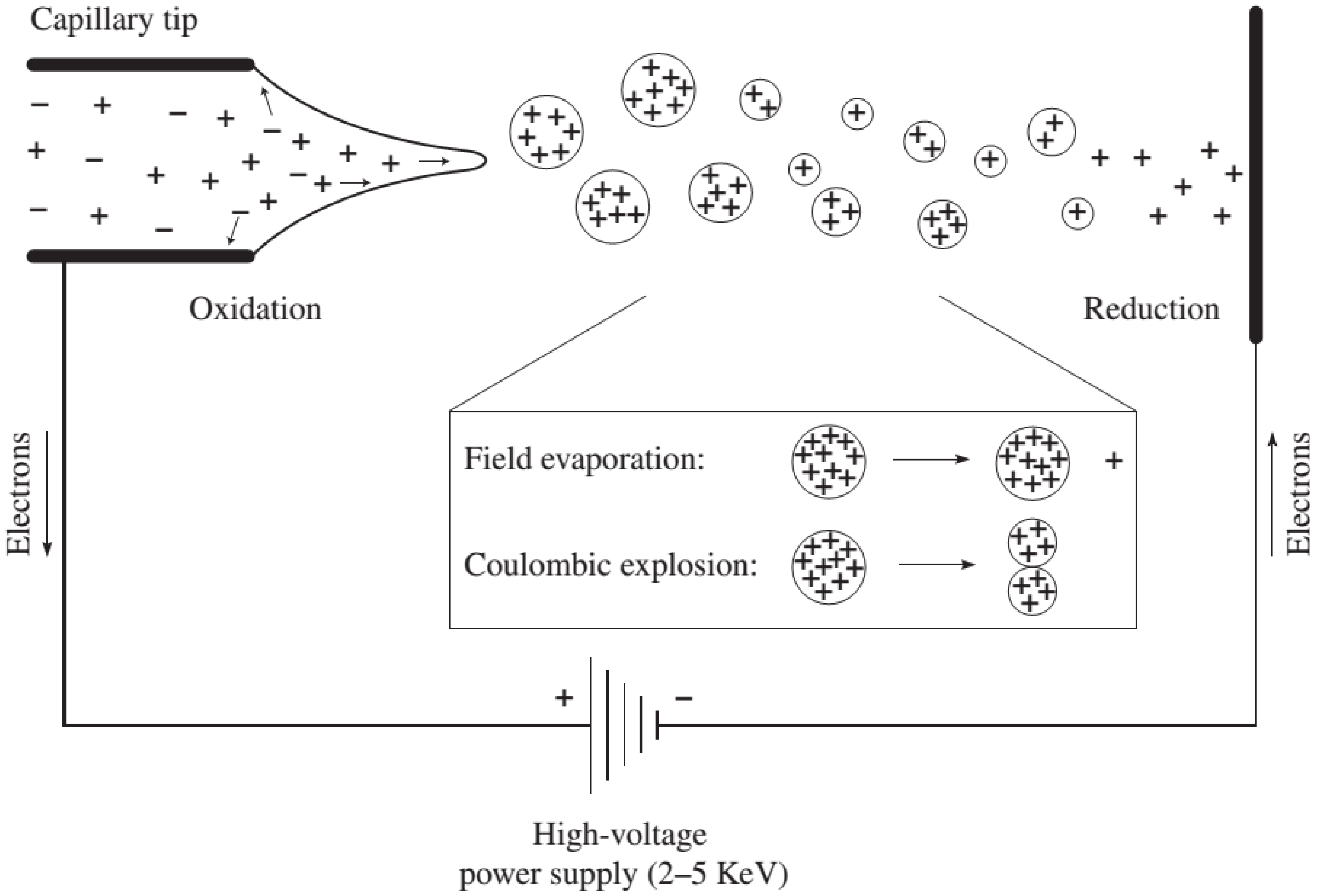
+ In ESI, a solution containing the sample molecules is sprayed out the end of a fine capillary into a heated chamber that is at nearly atmospheric pressure.

+ The capillary through which the sample solution passes has a high voltage potential across its surface, and small, charged droplets are expelled into the ionization chamber.

Electrospray Ionization (ESI)

- The charged droplets are subjected to a counterflow of a drying gas (usually nitrogen) that evaporates solvent molecules from the droplets.
- Thus, the charge density of each droplet increases until the electrostatic repulsive forces exceed the surface tension of the droplet (the Rayleigh limit), at which point the droplets break apart into smaller droplets.
- This process continues until solvent-free sample ions are left in the gas phase.
- Negative ions may also be formed in ESI by loss of protons from the sample to basic species in solution.
- Because **ESI relies on a sample in solution, ESI is the most logical method to be employed in LC-MS systems**.

Electrospray Ionization (ESI)



ESI-MS is not limited to the study of large biomolecules. Many small molecules with molecular weight in the 100–1500 range can be studied by ESI-MS.

Resolution

- An important consideration in mass spectrometry is **resolution,** defined according to the relationship.

$$R = \frac{M}{\Delta M}$$

where:

R is the resolution;

M is the mass of the particle;

ΔM is the difference in mass between a particle of mass M and the particle of next higher mass that can be resolved by the instrument.

- A magnetic sector analyzer can have R values approaching 10,000, depending on the radius of curvature and slit widths.

THE RULE OF THIRTEEN

(1) Generate a **base formula**:

\+ **A base formula** contains only carbon and hydrogen.

\+ The base formula is found by dividing the molecular mass M by 13 (*the mass of one carbon plus one hydrogen*).

\+ This calculation provides a numerator n and a remainder.

$$\frac{M}{13} = n + \frac{r}{13}$$

\+ The base formula thus becomes:

$$C_nH_{n+r}$$

\+ The **index of hydrogen deficiency** (*unsaturation index)* U that corresponds to the preceding formula:

$$U = \frac{(n - r + 2)}{2}$$

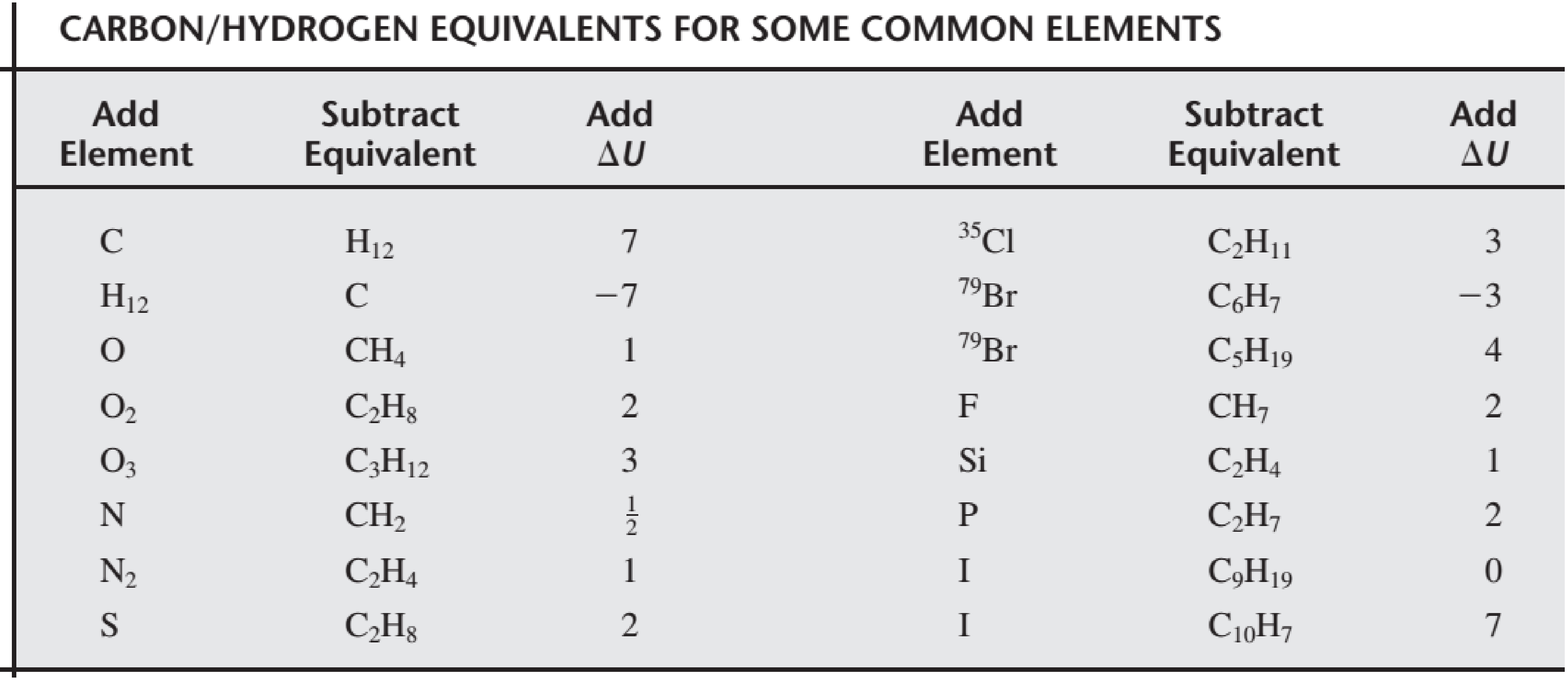
THE RULE OF THIRTEEN

(2) Derive a molecular formula that includes other atoms besides carbon and hydrogen

+ Subtract the mass of a combination of carbons and hydrogens that equals the masses of the other atoms being included in the formula.

+ For example, if we convert the base formula to a new formula containing one oxygen atom, then we subtract one carbon and four hydrogens at the same time that we add one oxygen atom. Both changes involve a molecular mass equivalent of 16 (O = CH_4 = 16).

THE RULE OF THIRTEEN



CARBON/HYDROGEN EQUIVALENTS FOR SOME COMMON ELEMENTS

Add Element	Subtract Equivalent	Add ΔU	Add Element	Subtract Equivalent	Add ΔU
C	H_{12}	7	^{35}Cl	C_2H_{11}	3
H_{12}	C	−7	^{79}Br	C_6H_7	−3
O	CH_4	1	^{79}Br	C_5H_{19}	4
O_2	C_2H_8	2	F	CH_7	2
O_3	C_3H_{12}	3	Si	C_2H_4	1
N	CH_2	$\frac{1}{2}$	P	C_2H_7	2
N_2	C_2H_4	1	I	C_9H_{19}	0
S	C_2H_8	2	I	$C_{10}H_7$	7

- Consider an unknown substance with a molecular mass of 94 amu (atomic unit, also writen as au).

(1) Application of the formula provides:

$$\frac{94}{13} = 7 + \frac{3}{13}$$

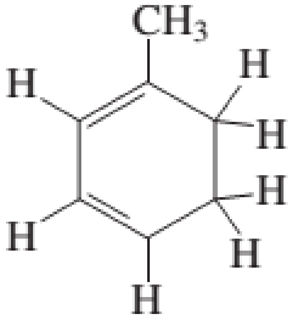
According to the formula, $n = 7$ and $r = 3$. The base formula must be:

$$C_7H_{10}$$

(2) The index of hydrogen deficiency is:

$$U = \frac{(7 - 3 + 2)}{2} = 3$$

A substance that fits this formula must contain some combination of three rings or multiple bonds. A possible structure might be:



C_7H_{10}

$U = 3$

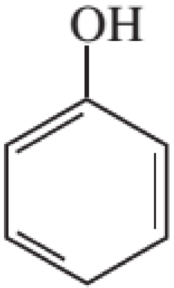
THE RULE OF THIRTEEN

(3) Other substance that had the same molecular mass but that contained one oxygen atom would be C_6H_6O.

+ This formula is determined according to the following scheme:

1. Base formula = C_7H_{10}, $U = 3$
2. Add: + O
3. Subtract: - CH_4
4. Change the value of U: $\Delta U = 1$
5. New formula = C_6H_6O
6. New index of hydrogen deficiency: $U = 4$.

A possible substance that fits these data is:



C_6H_6O

$U = 4$

THE RULE OF THIRTEEN

- There are additional possible molecular formulas that conform to a molecular mass of 94 amu.

$C_5H_2O_2 \quad U = 5$

$C_5H_2S \quad U = 5$

$C_6H_8N \quad U = 3\frac{1}{2}$

$CH_3Br \quad U = 0$

THE NITROGEN RULE

- When the number of nitrogen atoms present in the molecule is **odd**, the molecular mass will be an **odd** number;

- When the number of nitrogen atoms present in the molecule is **even** (or zero), the molecular mass will be an **even** number.

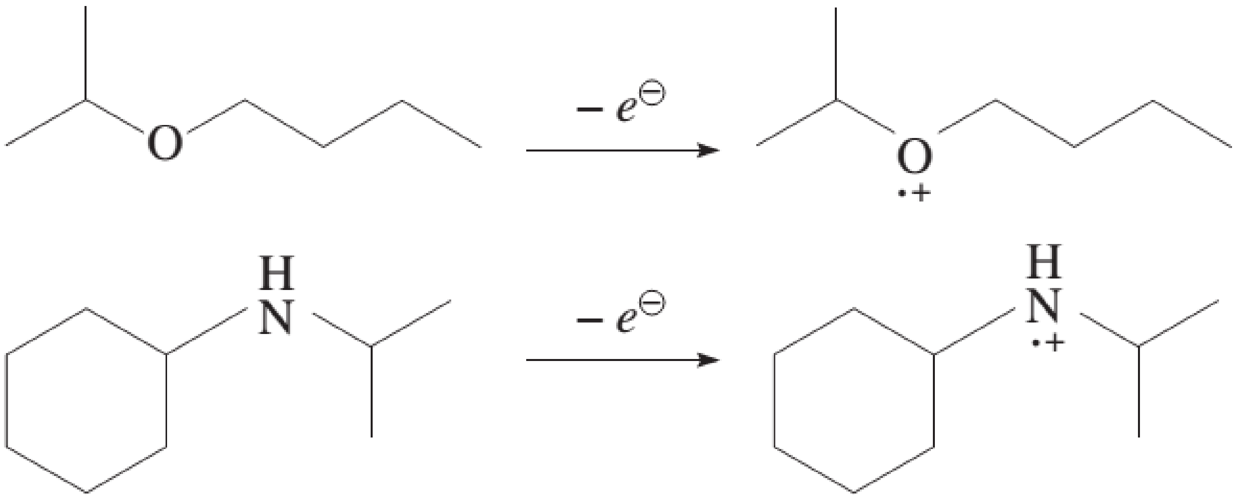
THE INITIAL IONIZATION EVENT

- Electron ionization results in highly energetic radical cations, many of which have sufficient energy to undergo one or more bond cleavage events leading to fragment ions.

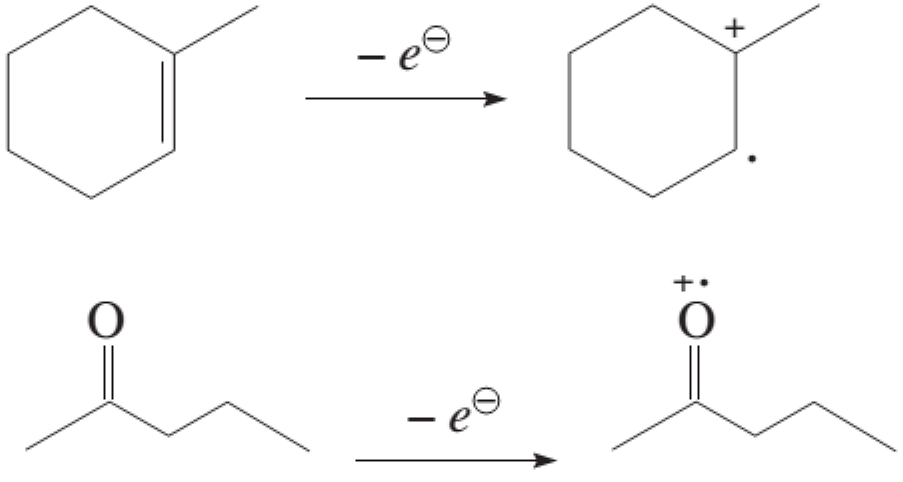
- The electrons most likely to be ejected during the ionization event are the ones that are in the highest potential energy molecular orbitals, that is the electrons held least tightly by the molecule.

+ Thus, it is easier to remove an electron from a nonbonding orbital, *n*, than it is to strip an electron from a π orbital.
+ Similarly it is much easier to eject an electron from a π orbital in comparison to a σ orbital.

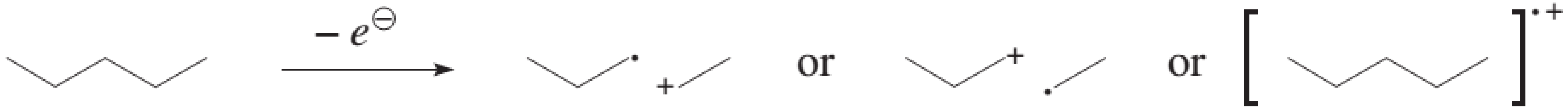
Loss of an electron from a non-bonding orbital:



Loss of an electron from a π orbital:



Loss of an electron from a σ orbital:



FUNDAMENTAL FRAGMENTATION PROCESSES

- **Stevenson's Rule**:

"Fragmentation processes that lead to the **formation of more stable carbocations are favored** over processes that lead to less stable carbocations".

$$H_3C^+ < RCH_2^+ < R_2CH^+ < R_3C^+ < H_2C{=}CHCH_2^+ \sim HC{\equiv}CCH_2^+ < C_6H_5CH_2^+$$

difficult *easy*

- Often, fragmentation involves the loss of an electrically neutral fragment (radicals).

\+ This fragment does NOT appear in the mass spectrum.

\+ Processes that lead to the formation of a more stable neutral fragment are favored over those that lead to less stable neutral fragments.

FUNDAMENTAL FRAGMENTATION PROCESSES

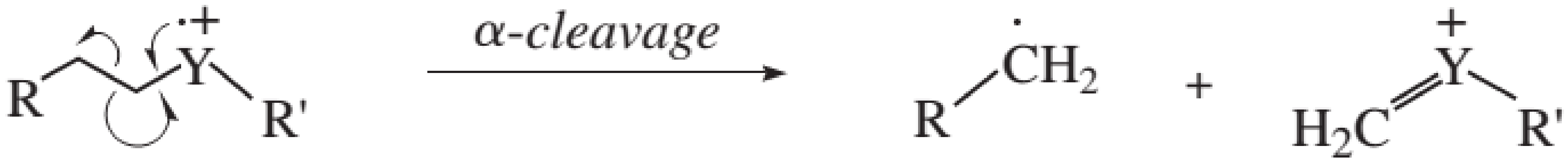
- “**Even-electron rule**”

+ Fragmentation of a radical cation or a cation always leads to a cation and a neutral species.

Radical-Site-Initiated Cleavage: α-Cleavage

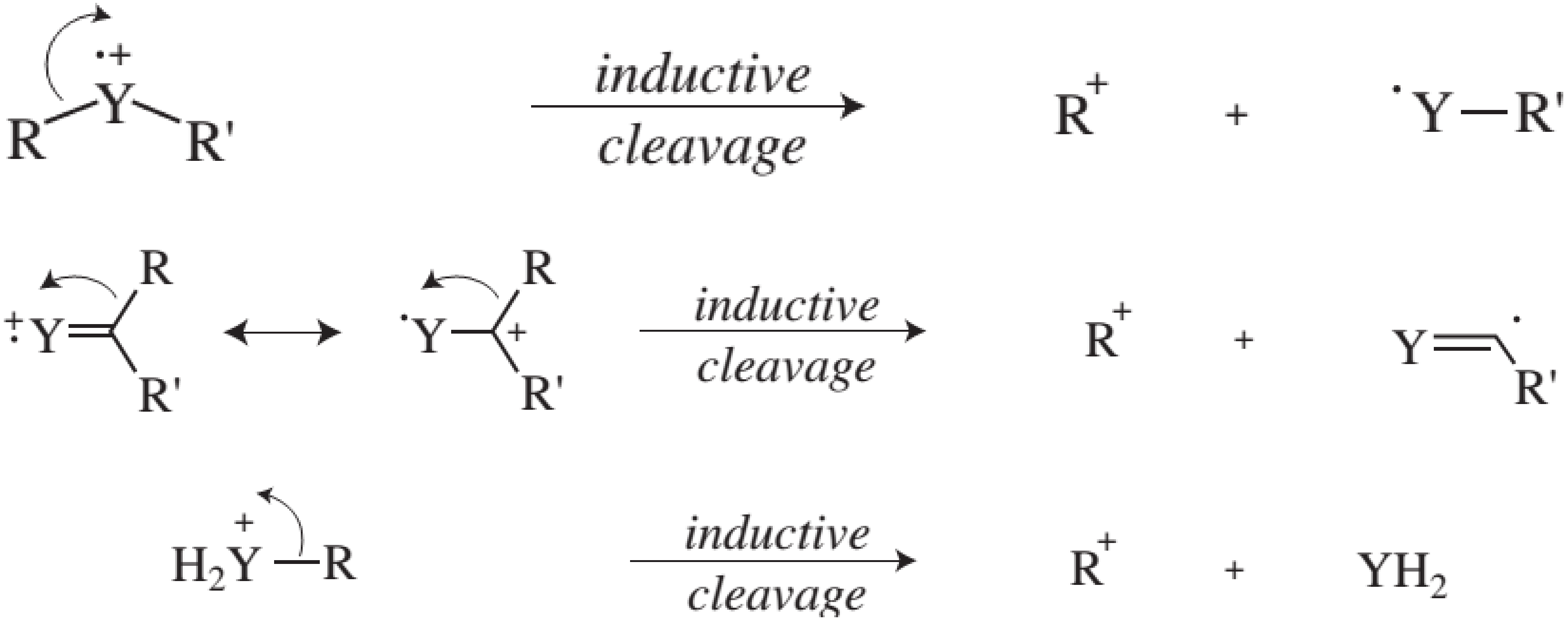
- The bond that is broken is not directly attached to the radical site but is rather the bond to the next neighboring atom (the α-position).

- α-Cleavages may occur at saturated or unsaturated sites that may or may not involve a heteroatom.



Charge-Site-Initiated Cleavage: Inductive Cleavage

- Inductive cleavage involves the attraction of an electron pair by an electronegative heteroatom that ends up as a radical or as a closed-shell neutral molecule.
- While α-cleavage is a fragmentation of OE^{+} only, inductive cleavage can operate on either an OE^{+} or an EE^{+}.



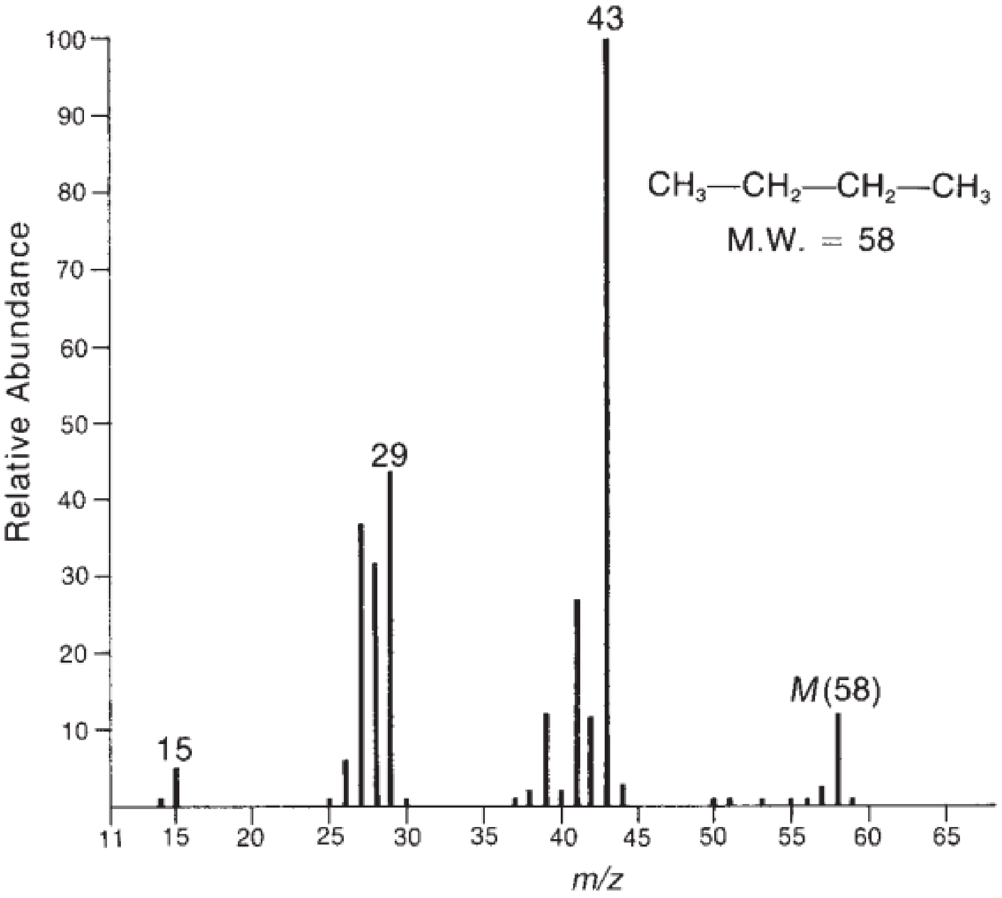
FRAGMENTATION PATTERN OF ALKANES

- Straight-chain hydrocarbons undergo fragmentation by breaking carbon–carbon bonds, resulting in a homologous series of fragmentation products.

- In the case of butane:

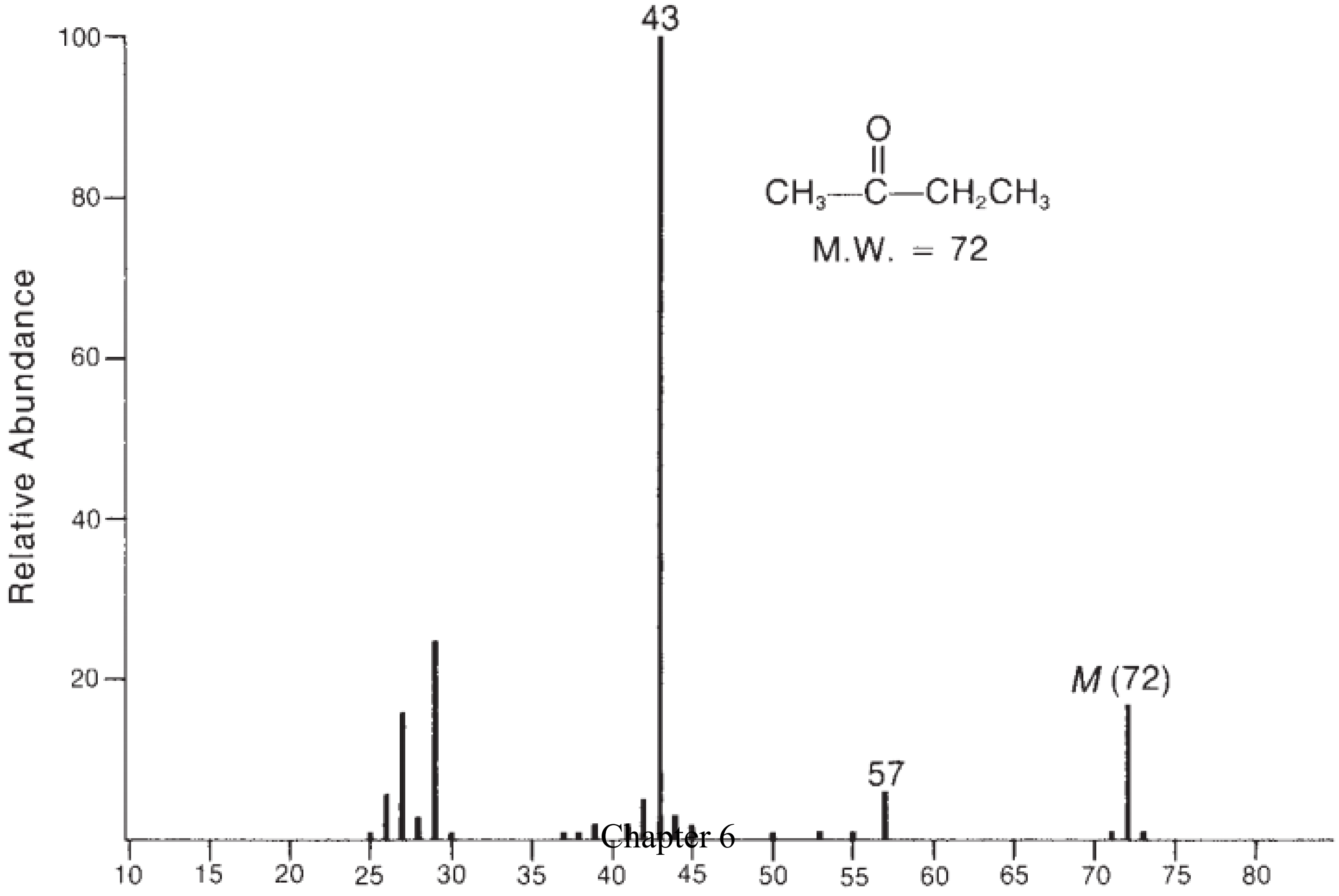
\+ Cleavage of the C1-C2 bond results in the loss of a methyl radical and the formation of the propyl carbocation (*m*/*z* = 43).

\+ Cleavage of the C2-C3 bond results in the loss of a ethyl radical and the formation of the ethyl carbocation (*m*/*z* = 29).



FRAGMENTATION PATTERN OF KETONES

- The mass spectra of ketones show an intense molecular ion peak.
- Loss of the alkyl groups attached to the carbonyl group by means of α-cleavage is one of the most important fragmentation processes.



STRATEGIC APPROACH TO ANALYZING MASS SPECTRA AND SOLVING PROBLEMS

(1) Identifying the molecular ion

+ Note its nominal mass and examine the isotope cluster (if the formula is not already known) for the presence of Cl, Br, and other $M + 2$ elements.

+ Depending on whether the *m*/*z* value of the molecular ion is odd or even, the nitrogen rule will tell you how many nitrogens, if any, to incorporate into your analysis.

+ If the molecular ion is not visible, consider running the sample under CI conditions to determine the molecular mass of the sample.

+ Consider what logical losses could have created the high mass peaks in the spectrum you have (loss of water from an alcohol, for example).

(2) **Use the fragmentation information to piece together a proposed structure**.

\+ More than one potential structure may be reasonable pending further analysis.

\+ In some cases, it may only be possible to come up with a partial structure.

An unknown compound has the mass spectrum shown. The infrared spectrum of the unknown shows significant peaks at

3102 cm^{-1} 3087 3062 3030 1688
1598 1583 1460 1449 1353
1221 952 746 691

There is also a band from aliphatic C—H stretching from 2879 to 2979 cm^{-1}.

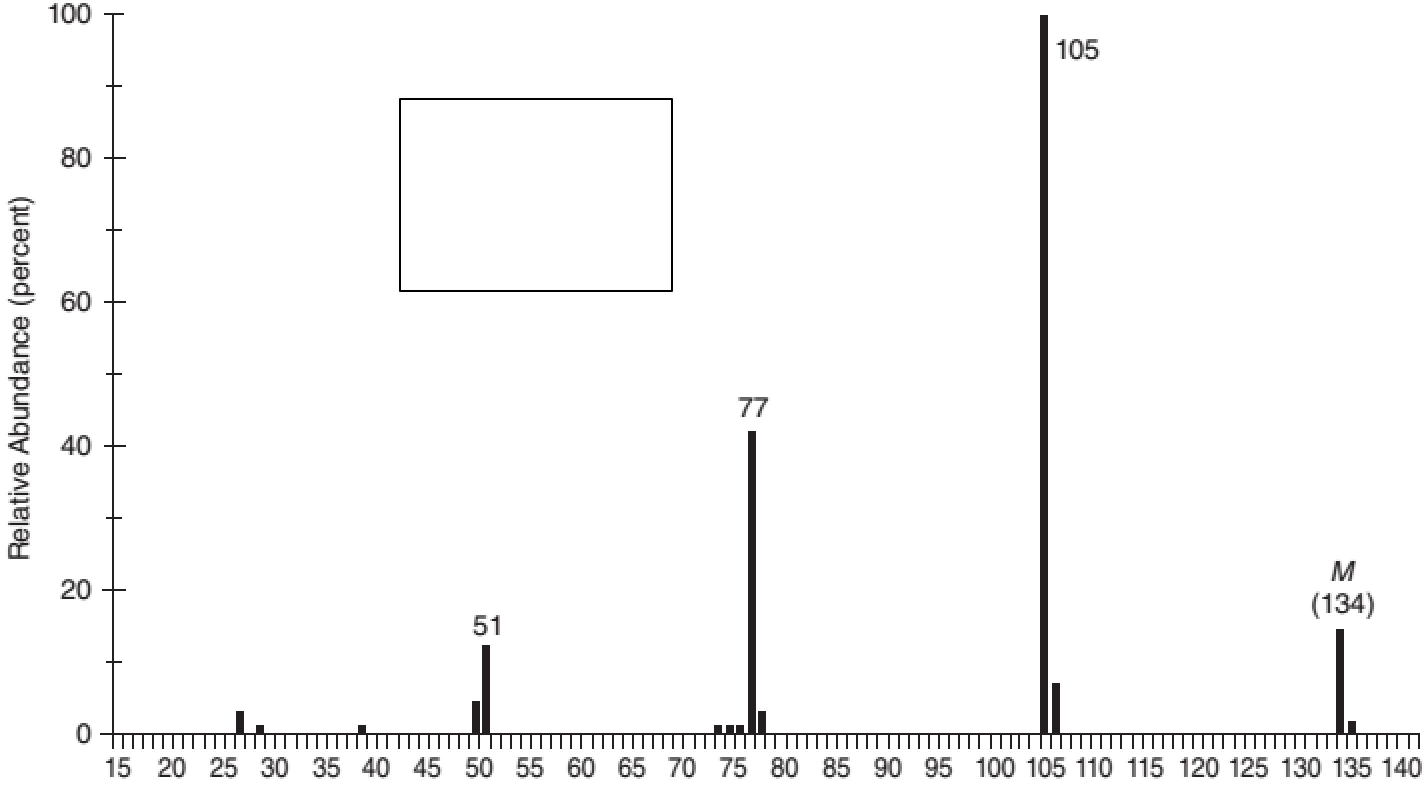


TABLE 3.5

NATURAL ABUNDANCES OF COMMON ELEMENTS AND THEIR ISOTOPES

Element	Relative Abundance					
Hydrogen	^{1}H	100	^{2}H	0.016		
Carbon	^{12}C	100	^{13}C	1.08		
Nitrogen	^{14}N	100	^{15}N	0.38		
Oxygen	^{16}O	100	^{17}O	0.04	^{18}O	0.20
Fluorine	^{19}F	100				
Silicon	^{28}Si	100	^{29}Si	5.10	^{30}Si	3.35
Phosphorus	^{31}P	100				
Sulfur	^{32}S	100	^{33}S	0.78	^{34}S	4.40
Chlorine	^{35}Cl	100			^{37}Cl	32.5
Bromine	^{79}Br	100			^{81}Br	98.0
Iodine	^{127}I	100				

- Which molecule below would exhibit *m/z* peaks at 43, 57, 87, 101, and 116?

(1) Propyl chloride

(2) Isopropyl bromide

(3) Propyl chloride

(4) s-Butyl isopropyl ether