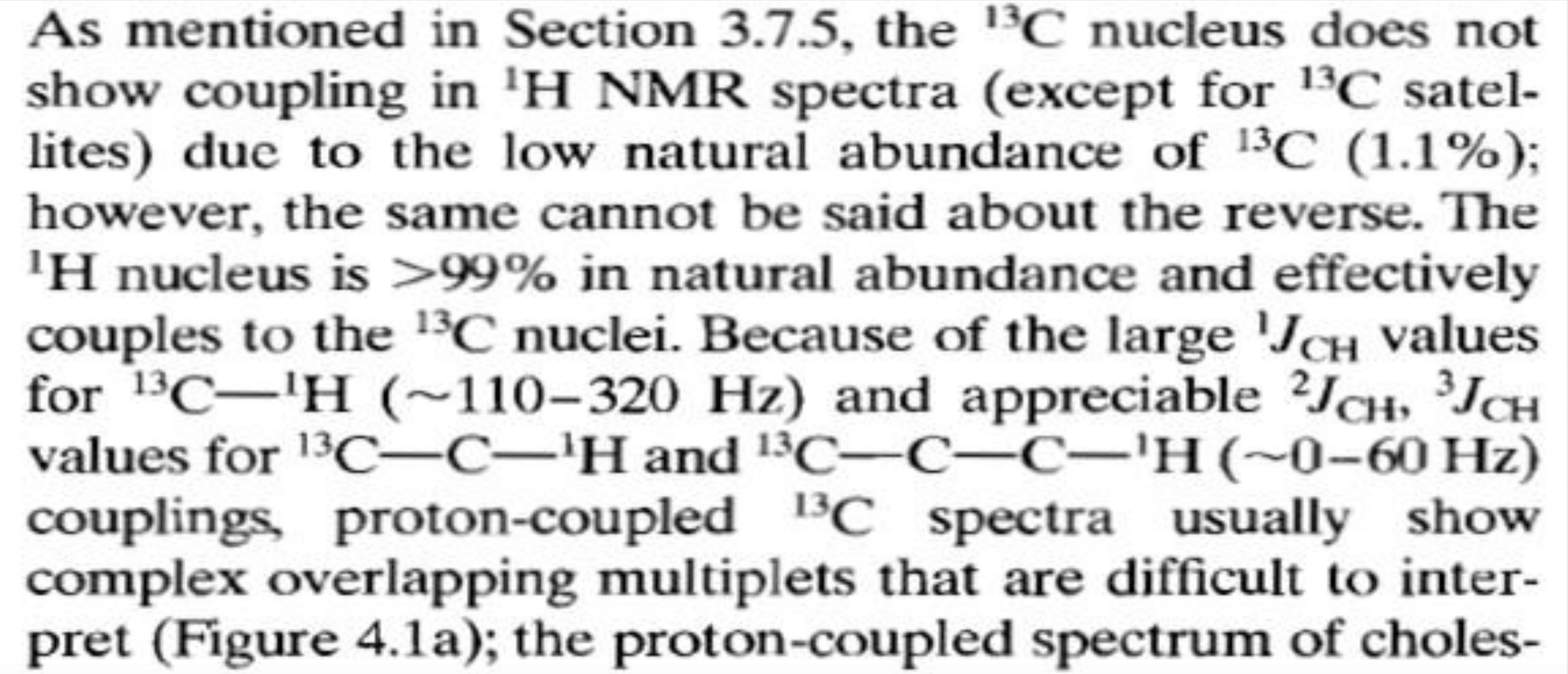
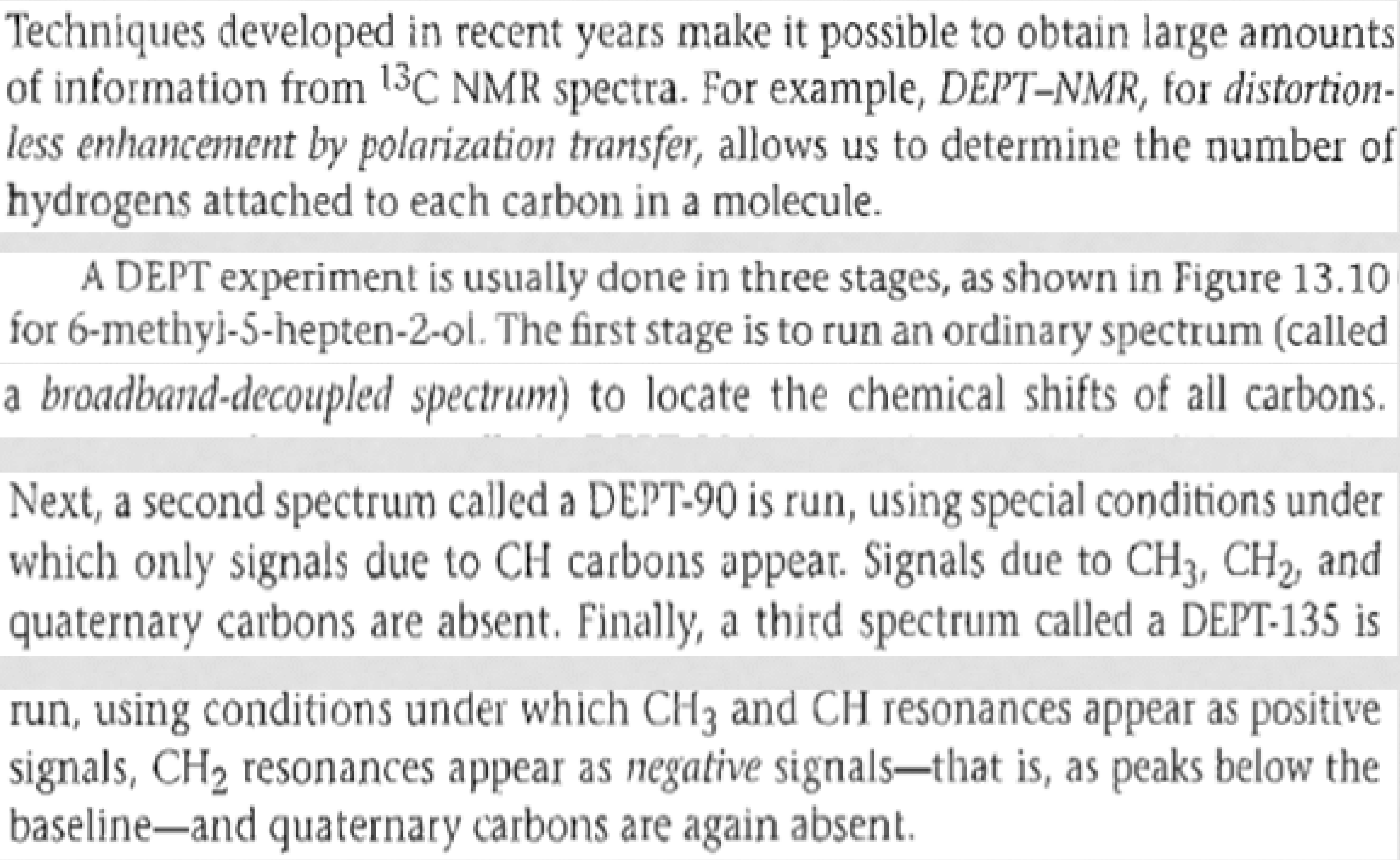
^{1}H Decoupling Techniques



As mentioned in Section 3.7.5, the ^{13}C nucleus does not show coupling in ^{1}H NMR spectra (except for ^{13}C satellites) due to the low natural abundance of ^{13}C (1.1%); however, the same cannot be said about the reverse. The ^{1}H nucleus is >99% in natural abundance and effectively couples to the ^{13}C nuclei. Because of the large $^{1}J_{CH}$ values for ^{13}C—^{1}H (~110–320 Hz) and appreciable $^{2}J_{CH}$, $^{3}J_{CH}$ values for ^{13}C—C—^{1}H and ^{13}C—C—C—^{1}H (~0–60 Hz) couplings, proton-coupled ^{13}C spectra usually show complex overlapping multiplets that are difficult to interpret (Figure 4.1a); the proton-coupled spectrum of choles-

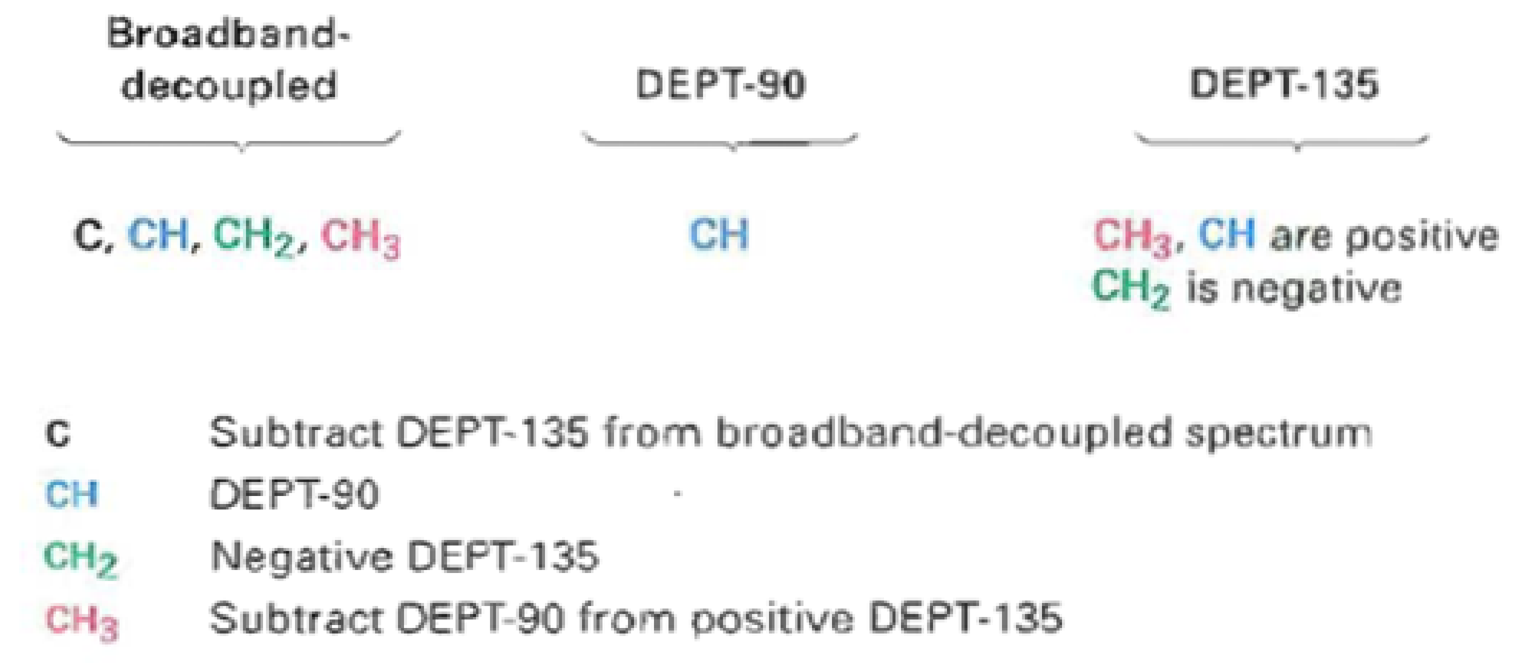
DEPT ^{13}C NMR Spectroscopy



Techniques developed in recent years make it possible to obtain large amounts of information from ^{13}C NMR spectra. For example, *DEPT–NMR,* for *distortionless enhancement by polarization transfer,* allows us to determine the number of hydrogens attached to each carbon in a molecule.

A DEPT experiment is usually done in three stages, as shown in Figure 13.10 for 6-methyl-5-hepten-2-ol. The first stage is to run an ordinary spectrum (called a *broadband-decoupled spectrum*) to locate the chemical shifts of all carbons.

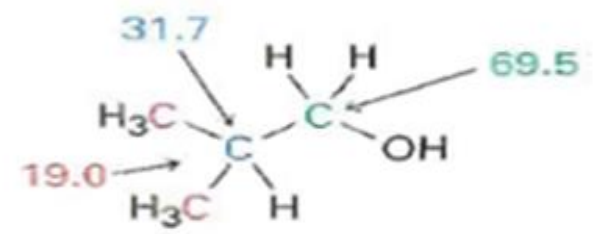
Next, a second spectrum called a DEPT-90 is run, using special conditions under which only signals due to CH carbons appear. Signals due to CH_3, CH_2, and quaternary carbons are absent. Finally, a third spectrum called a DEPT-135 is run, using conditions under which CH_3 and CH resonances appear as positive signals, CH_2 resonances appear as *negative* signals—that is, as peaks below the baseline—and quaternary carbons are again absent.



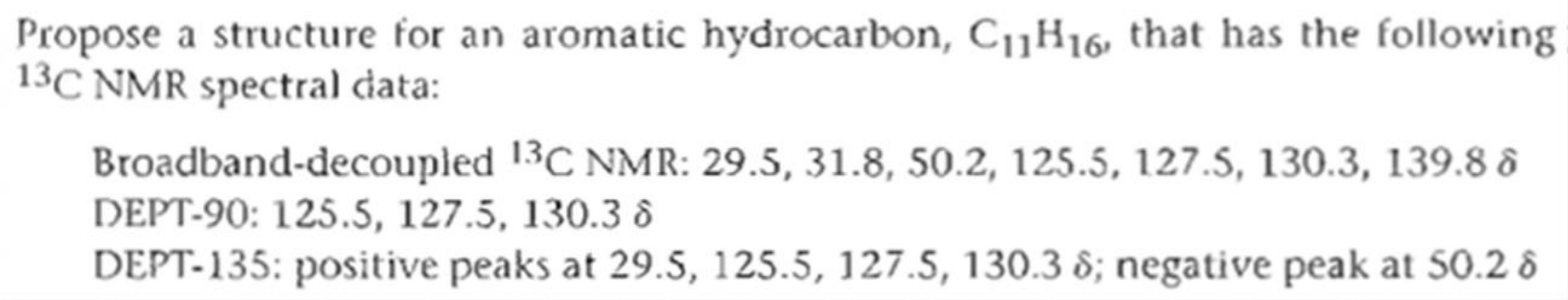
Broadband-decoupled	DEPT-90	DEPT-135
C, CH, CH_2, CH_3	CH	CH_3, CH are positive CH_2 is negative

C	Subtract DEPT-135 from broadband-decoupled spectrum
CH	DEPT-90
CH_2	Negative DEPT-135
CH_3	Subtract DEPT-90 from positive DEPT-135

Propose a structure for an alcohol, $C_4H_{10}O$, that has the following ^{13}CNMR spectral data:
Broadband _ decoupled ^{13}CNMR: 19.0, 31.7, 69.5 б
DEPT _90: 31.7 б
DEPT _ 135: positive peak at 19.0 & 31.7 б, negative peak at 69.5 б



2-Methyl-1-propanol



Propose a structure for an aromatic hydrocarbon, $C_{11}H_{16}$, that has the following ^{13}C NMR spectral data:

Broadband-decoupled ^{13}C NMR: 29.5, 31.8, 50.2, 125.5, 127.5, 130.3, 139.8 δ
DEPT-90: 125.5, 127.5, 130.3 δ
DEPT-135: positive peaks at 29.5, 125.5, 127.5, 130.3 δ; negative peak at 50.2 δ

Mass Spectrometry

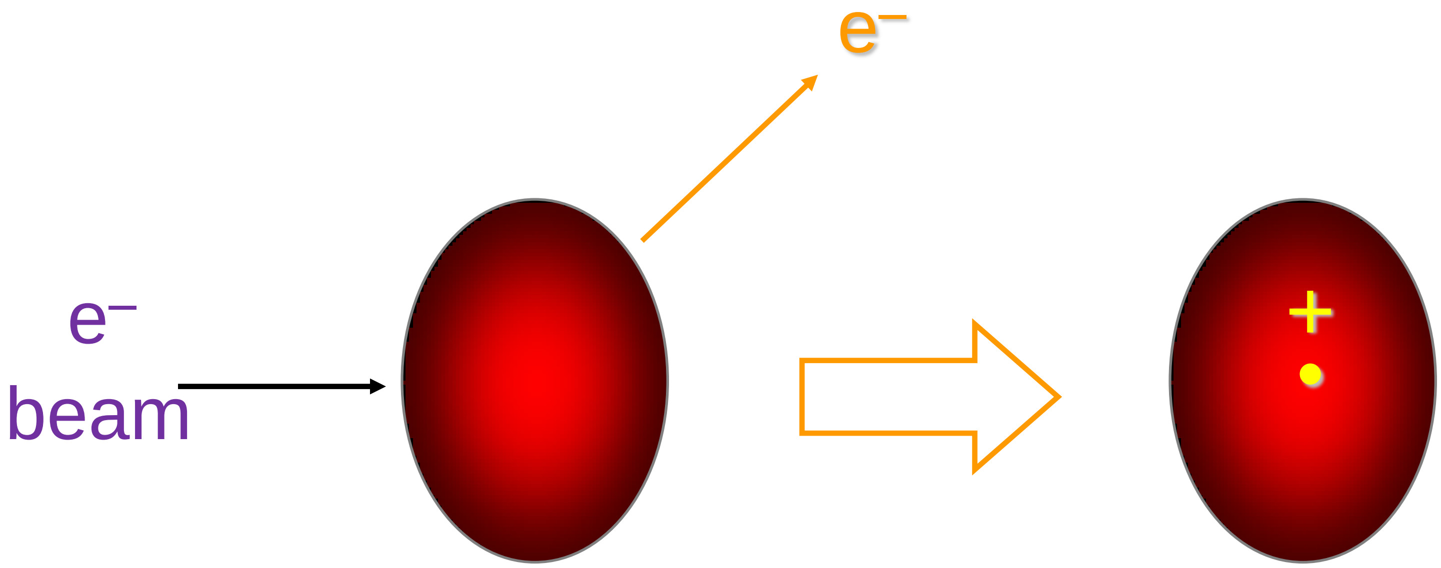
- Mass Spectrometry is an analytic technique that utilizes the degree of deflection of charged particles by a magnetic field to find the relative masses of molecular ions and fragments. It is a powerful method because it provides a great deal of information.

- Though organic mass spectrometry is routinely used along with IR, NMR and UV for structure determination, its basic theory is different from the others.

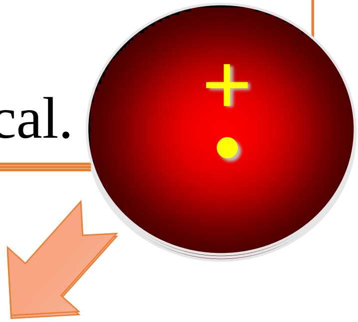
- In mass spectrometry no characteristic selective absorption of radiation is involved as in the case of the other three methods, also, in the mass spectrometry, the compound undergoes irreversible chemical changes unlike in the others, where the changes are reversible physical changes.

Principles of Electron-Impact Mass Spectrometry

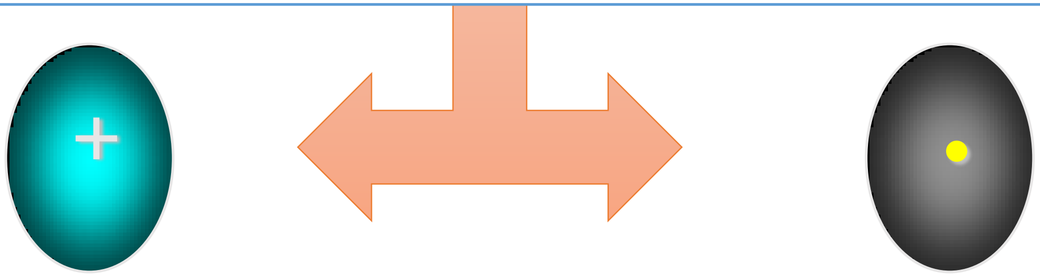
Atom or molecule is hit by high-energy electron from an electron beam forming a positively charged, odd-electron species called the molecular ion.



- However, the molecular ion often fragments to a mixture of species of lower m/z.
- The molecular ion dissociates to a cation and a radical.



The molecular ion dissociates to a cation and a radical.

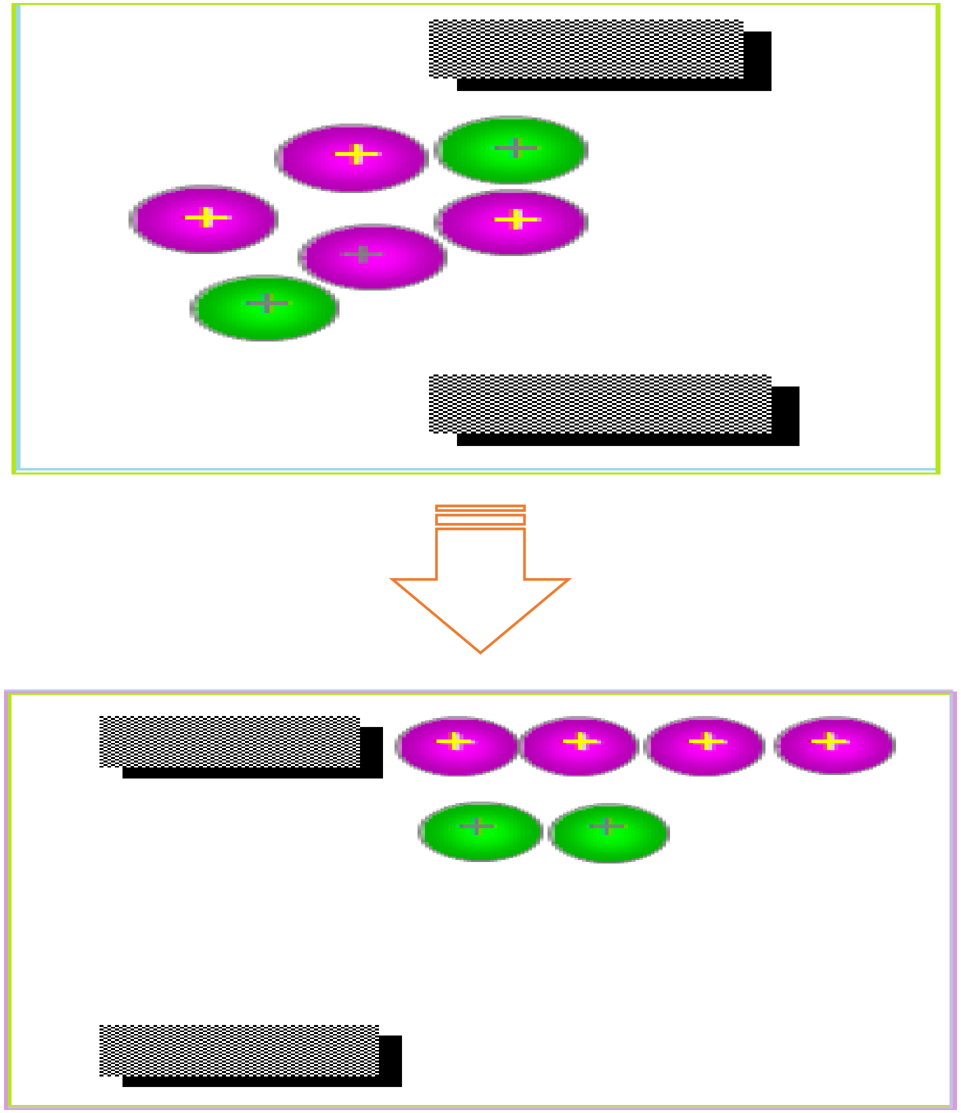


Usually several fragmentation pathways are available and a mixture of ions is produced.

mixture of ions of different mass gives separate peak for each m/z

intensity of peak proportional to percentage of each ion of different mass in mixture

separation of peaks depends on relative mass



In mass spectrometry, a substance is bombarded with an electron beam having sufficient energy to fragment the molecule.

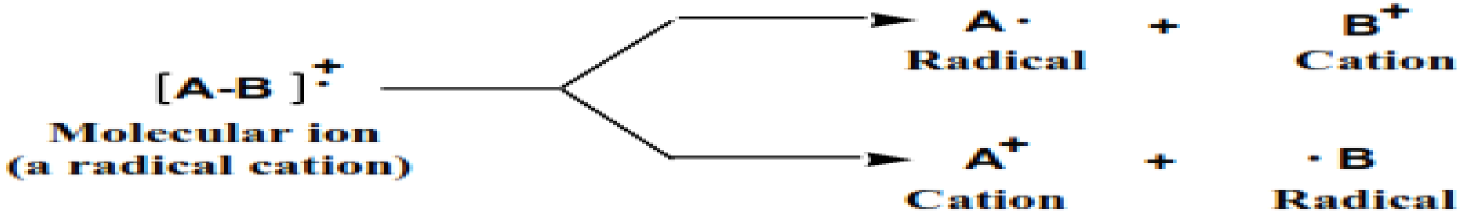
The positive fragments which are produced (cations and radical cations) are accelerated in a vacuum through a magnetic field and are sorted on the basis of mass-to-charge ratio.

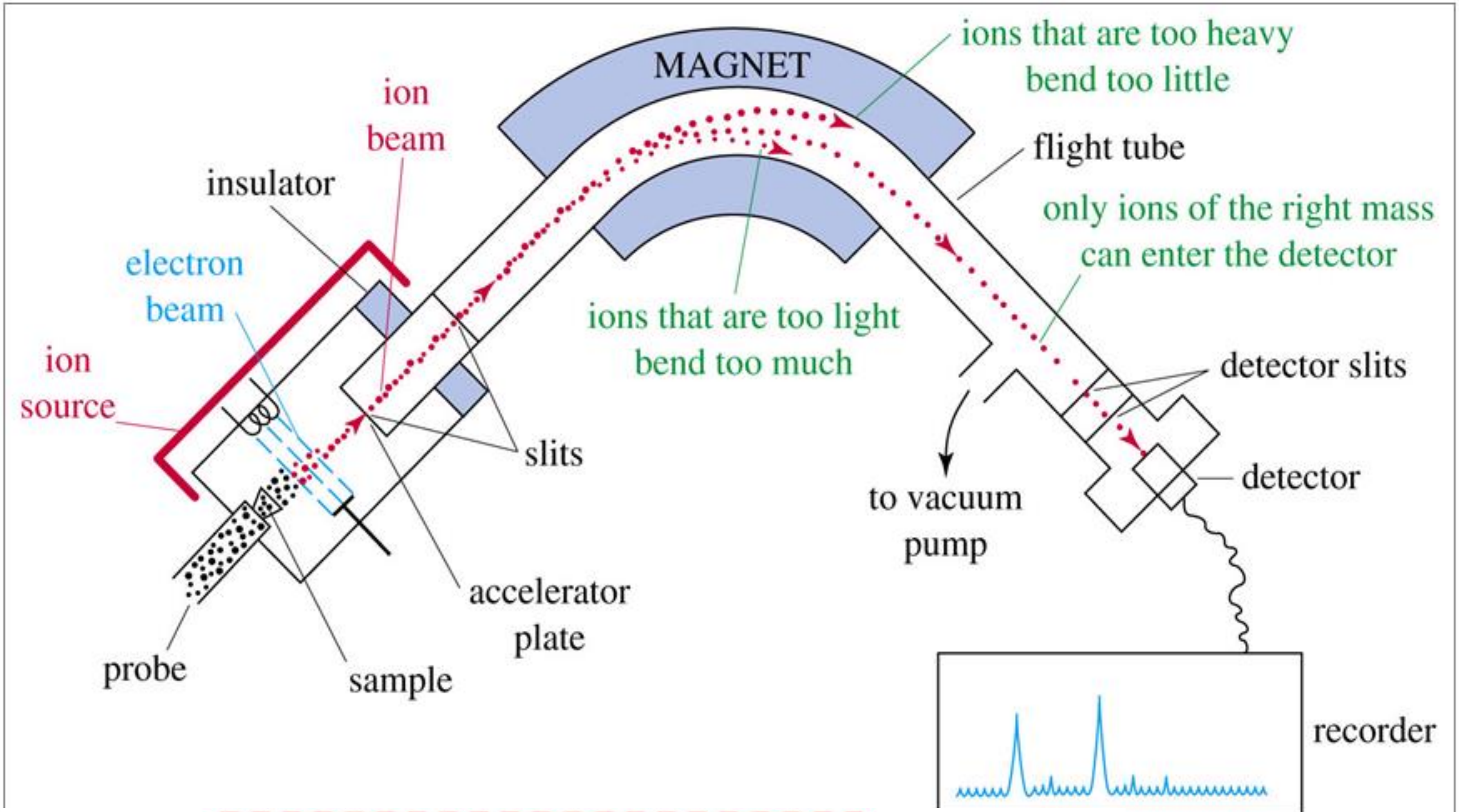
Since the bulk of the ions produced in the mass spectrometer carry a unit positive charge, the value m/e is equivalent to the molecular weight of the fragment.

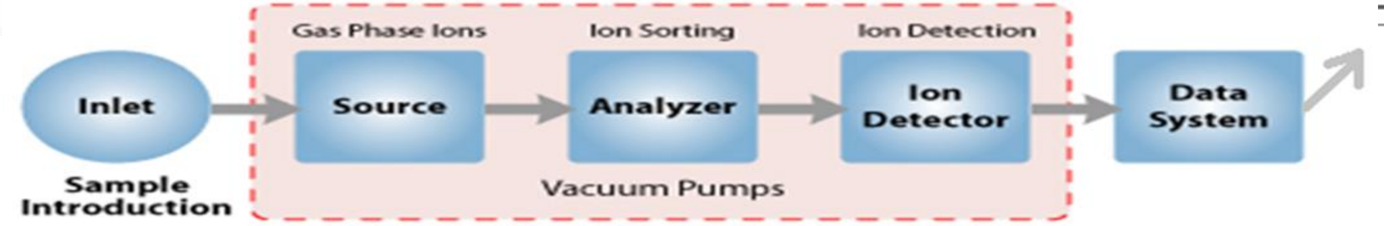
$$M\,(\text{vapor}) + e^- \rightarrow M^{+\cdot} + 2e^-$$

- The analysis of mass spectroscopy information involves the re-assembling of fragments, working backwards to generate the original molecule. A schematic representation of a mass spectrometer is shown below:

- **Fragmentation of a molecular ion, M, produces a radical and a cation. Only the cation is detected by MS**




MAGNET
ions that are too heavy bend too little
ion beam
flight tube
insulator
only ions of the right mass can enter the detector
electron beam
ions that are too light bend too much
ion source
detector slits
slits
detector
to vacuum pump
accelerator plate
probe
sample
recorder


Gas Phase Ions
Ion Sorting
Ion Detection
Inlet
Source
Analyzer
Ion Detector
Data System
Sample Introduction
Vacuum Pumps

- A very low concentration of sample molecules is allowed to leak into the ionization chamber (which is under a very high vacuum) where they are bombarded by a high-energy electron beam.

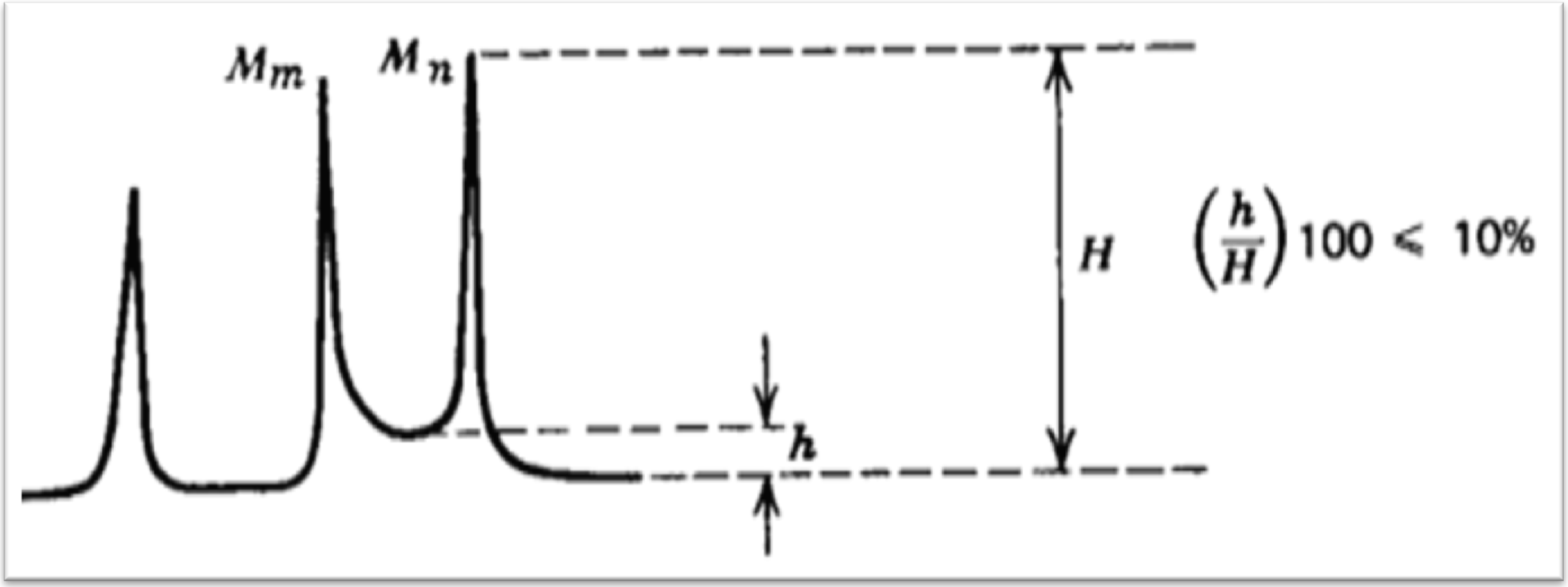
- The path of the charged molecules is bent by an applied magnetic field.

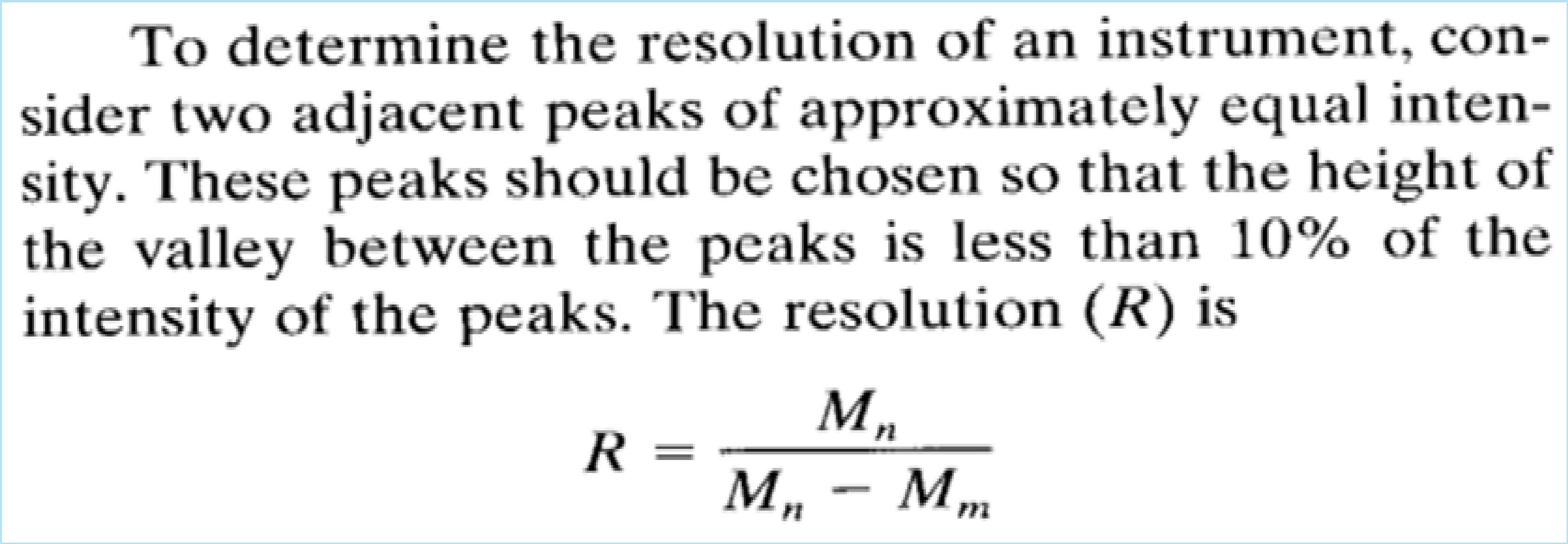
- Ions having low mass (low momentum) will be deflected most by this field and will collide with the walls of the analyzer.

- Likewise, high momentum ions will not be deflected enough and will also collide with the analyzer wall.

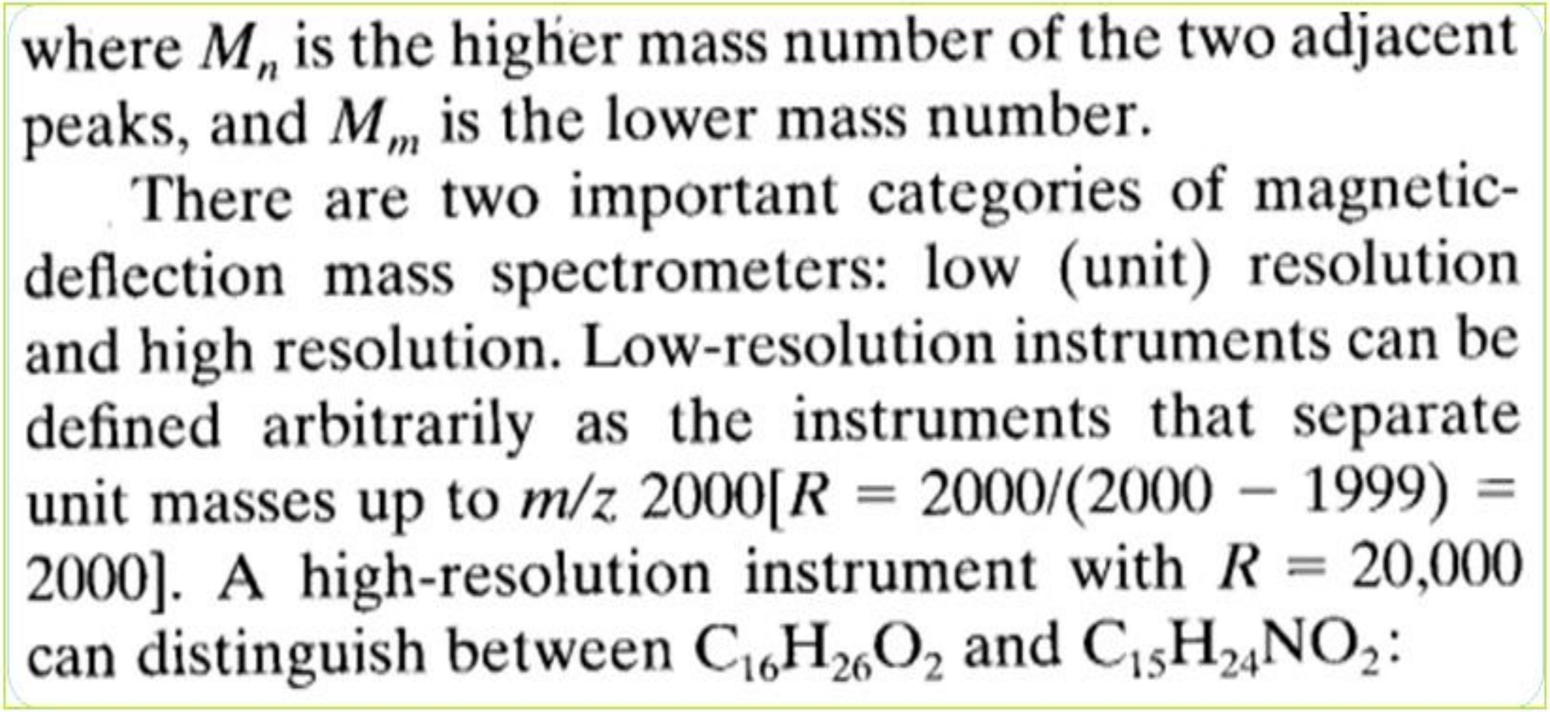
- Ions having the proper mass-to-charge ratio, however, will follow the path of the analyzer, exit through the slit and collide with the Collector.

- This generates an electric current, which is then amplified and detected. By varying the strength of the magnetic field, the mass-to-charge ratio which is analyzed can be continuously varied.




To determine the resolution of an instrument, consider two adjacent peaks of approximately equal intensity. These peaks should be chosen so that the height of the valley between the peaks is less than 10% of the intensity of the peaks. The resolution (R) is

$$R = \frac{M_n}{M_n - M_m}$$



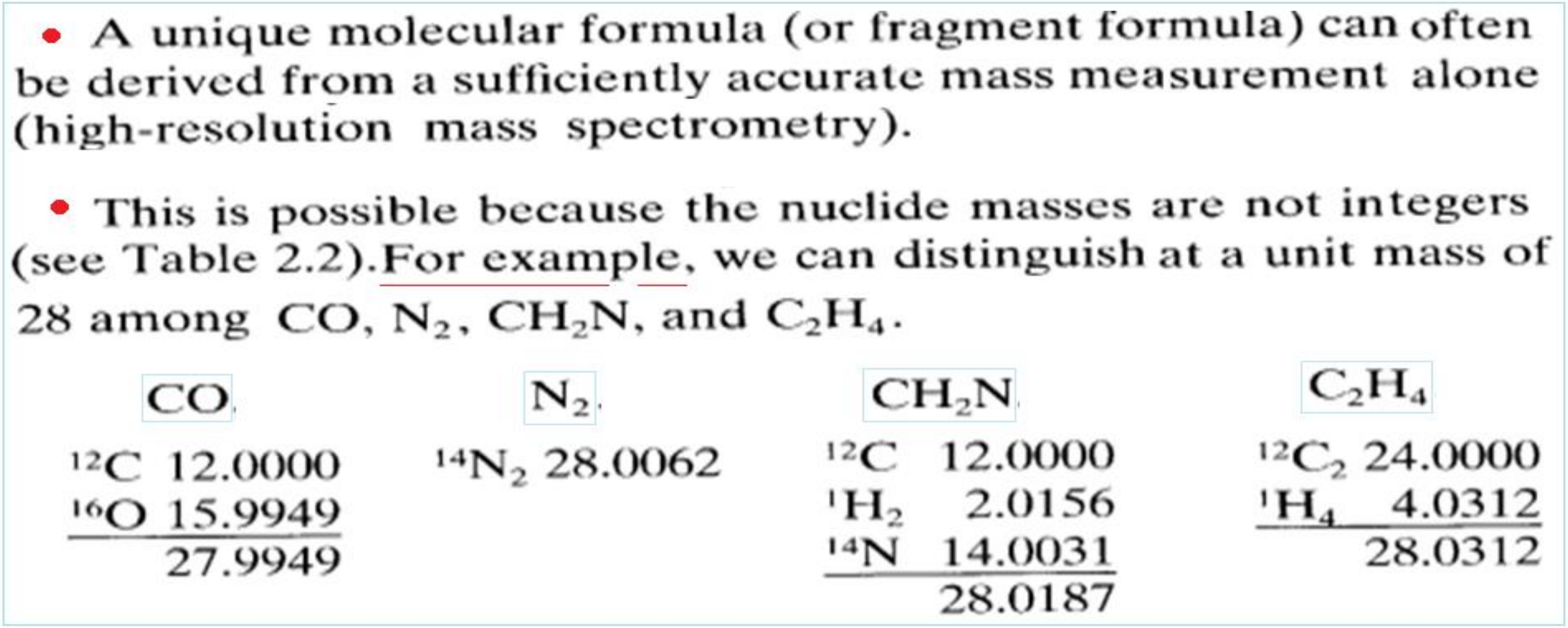
where M_n is the higher mass number of the two adjacent peaks, and M_m is the lower mass number.

There are two important categories of magnetic-deflection mass spectrometers: low (unit) resolution and high resolution. Low-resolution instruments can be defined arbitrarily as the instruments that separate unit masses up to m/z 2000[$R = 2000/(2000 - 1999) = 2000$]. A high-resolution instrument with $R = 20{,}000$ can distinguish between $C_{16}H_{26}O_2$ and $C_{15}H_{24}NO_2$:

$$R = \frac{250.1933}{250.1933 - 250.1807} \simeq 20{,}000$$

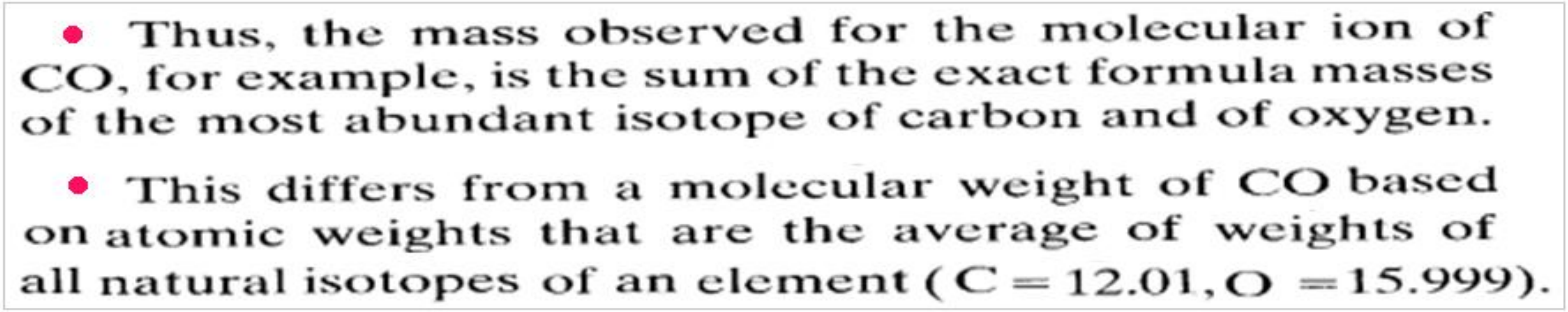
This important class of mass spectrometers can measure the mass of an ion with sufficient accuracy to determine its atomic composition.

High-Resolution Molecular Ion



- A unique molecular formula (or fragment formula) can often be derived from a sufficiently accurate mass measurement alone (high-resolution mass spectrometry).
- This is possible because the nuclide masses are not integers (see Table 2.2). For example, we can distinguish at a unit mass of 28 among CO, N_2, CH_2N, and C_2H_4.

CO		N_2		CH_2N		C_2H_4	
^{12}C	12.0000	$^{14}N_2$	28.0062	^{12}C	12.0000	$^{12}C_2$	24.0000
^{16}O	15.9949			$^{1}H_2$	2.0156	$^{1}H_4$	4.0312
	27.9949			^{14}N	14.0031		28.0312
					28.0187		



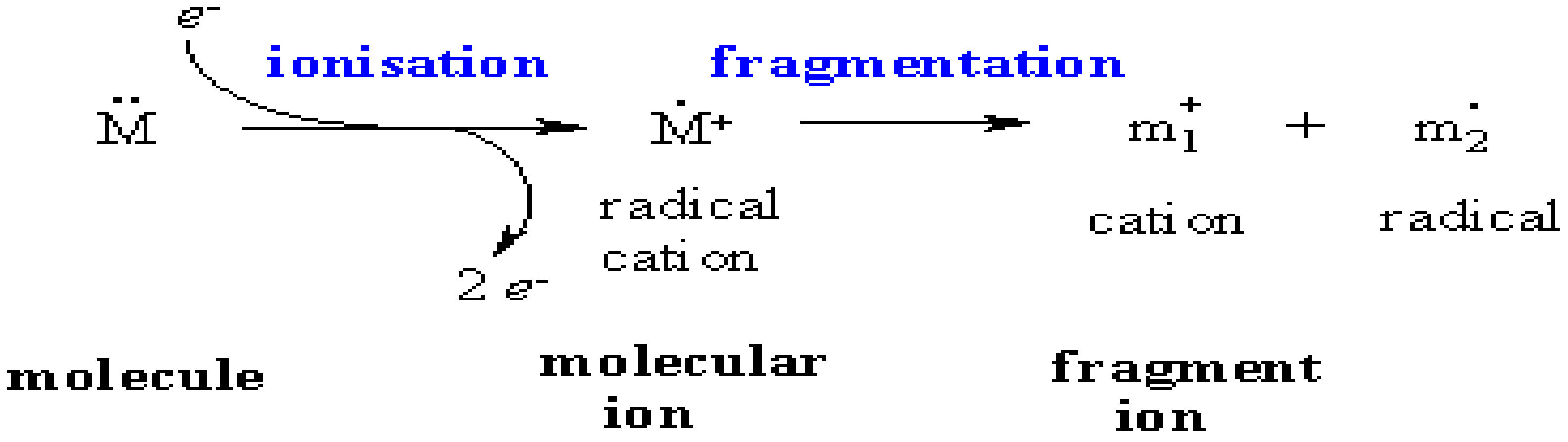
- Thus, the mass observed for the molecular ion of CO, for example, is the sum of the exact formula masses of the most abundant isotope of carbon and of oxygen.
- This differs from a molecular weight of CO based on atomic weights that are the average of weights of all natural isotopes of an element (C = 12.01, O = 15.999).

Table 2.2 gives the masses to four or five decimal places for the common nuclides; it also gives the familiar atomic weights (average weights for the elements).

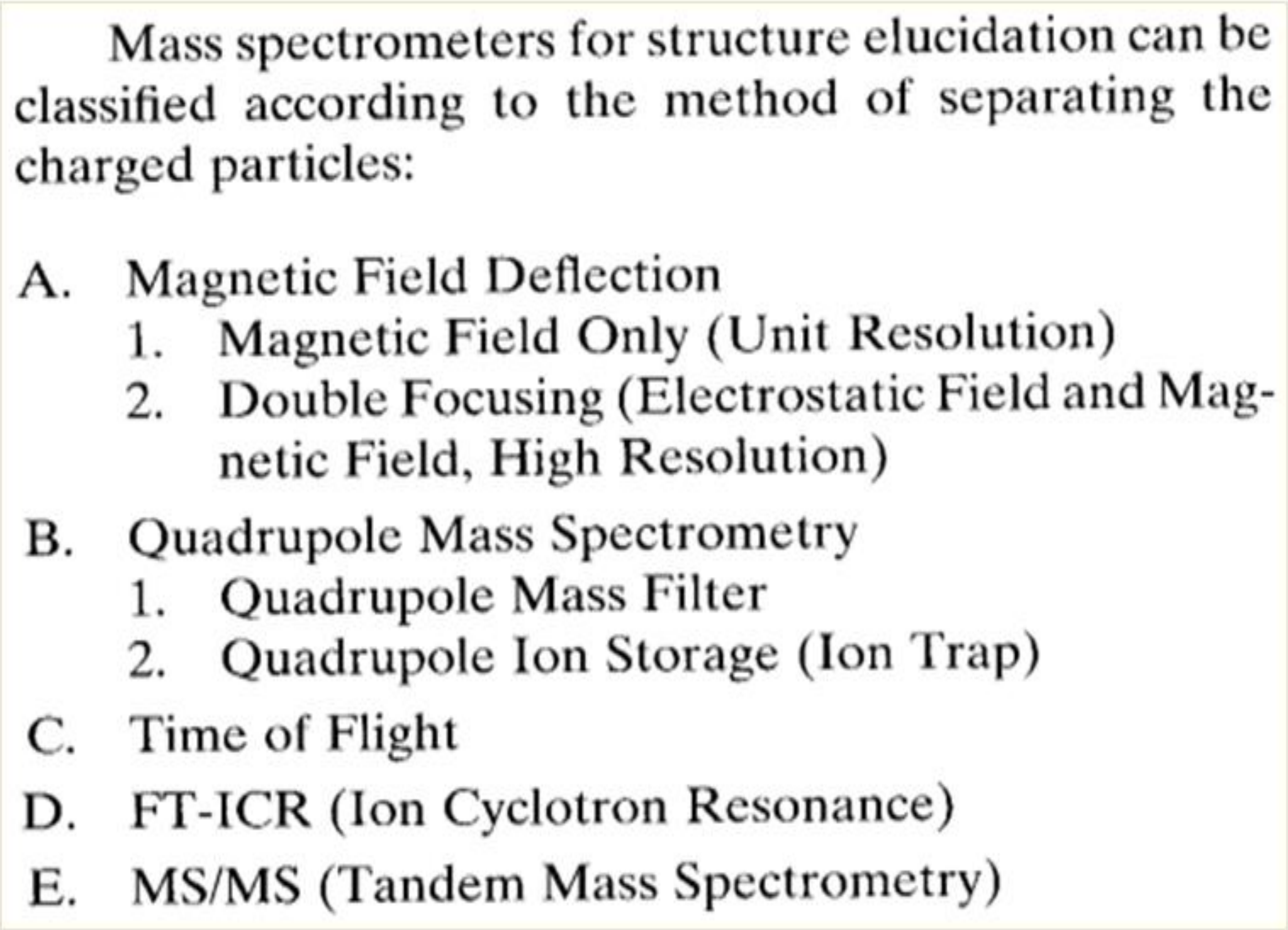
Table 2.2 Exact Masses of Isotopes

Element	Atomic Weight	Nuclide	Mass
Hydrogen	1.00794	^{1}H	1.00783
		D(^{2}H)	2.01410
Carbon	12.01115	^{12}C	12.00000 (std)
		^{13}C	13.00336
Nitrogen	14.0067	^{14}N	14.0031
		^{15}N	15.0001
Oxygen	15.9994	^{16}O	15.9949
		^{17}O	16.9991
		^{18}O	17.9992
Fluorine	18.9984	^{19}F	18.9984
Silicon	28.0855	^{28}Si	27.9769
		^{29}Si	28.9765
		^{30}Si	29.9738
Phosphorus	30.9738	^{31}P	30.9738
Sulfur	32.066	^{32}S	31.9721
		^{33}S	32.9715
		^{34}S	33.9679
Chlorine	35.4527	^{35}Cl	34.9689
		^{37}Cl	36.9659
Bromine	79.9094	^{79}Br	78.9183
		^{81}Br	80.9163
Iodine	126.9045	^{127}I	126.9045

Ionization to Radical Cation Molecular Ion (m^+)



Molecular ion	The ion obtained by the loss of an electron from the molecule also called parent ion
Base peak	The most intense peak in the MS, assigned 100% intensity
M+	Symbol often given to the molecular ion. Mol. With an unpaired e-
Radical cation	+ve charged species with an odd number of electrons
Fragment ions	Lighter cations formed by the decomposition of the molecular ion. also called daughter ion



Mass spectrometers for structure elucidation can be classified according to the method of separating the charged particles:

A. Magnetic Field Deflection
    1. Magnetic Field Only (Unit Resolution)
    2. Double Focusing (Electrostatic Field and Magnetic Field, High Resolution)

B. Quadrupole Mass Spectrometry
    1. Quadrupole Mass Filter
    2. Quadrupole Ion Storage (Ion Trap)

C. Time of Flight

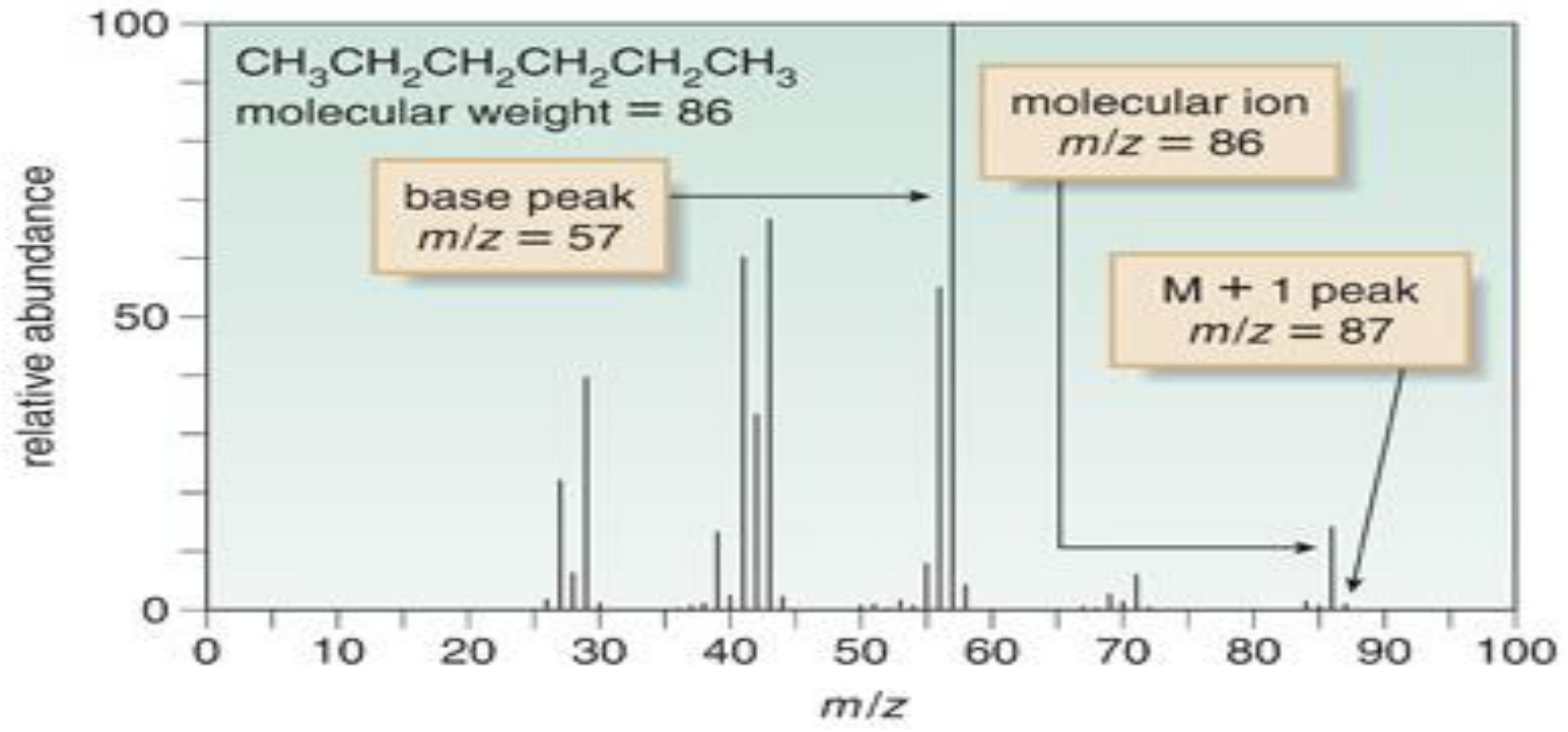
D. FT-ICR (Ion Cyclotron Resonance)

E. MS/MS (Tandem Mass Spectrometry)

The mass spectrum:

Mass spectra (EI) are routinely obtained at electron beam energy of 70 eV. The simplest event that occurs is the removal of a single electron from the molecule in the gas phase by an electron of the electron beam to form the molecular ion, which is a radicalcation ($M^{\cdot +}$) .

- The output of the mass spectrometer shows a plot of relative intensity vs the mass-to-charge ratio (m/e).



The output of the mass spectrometer shows a plot of relative intensity vs the mass-to-charge ratio (m/e).

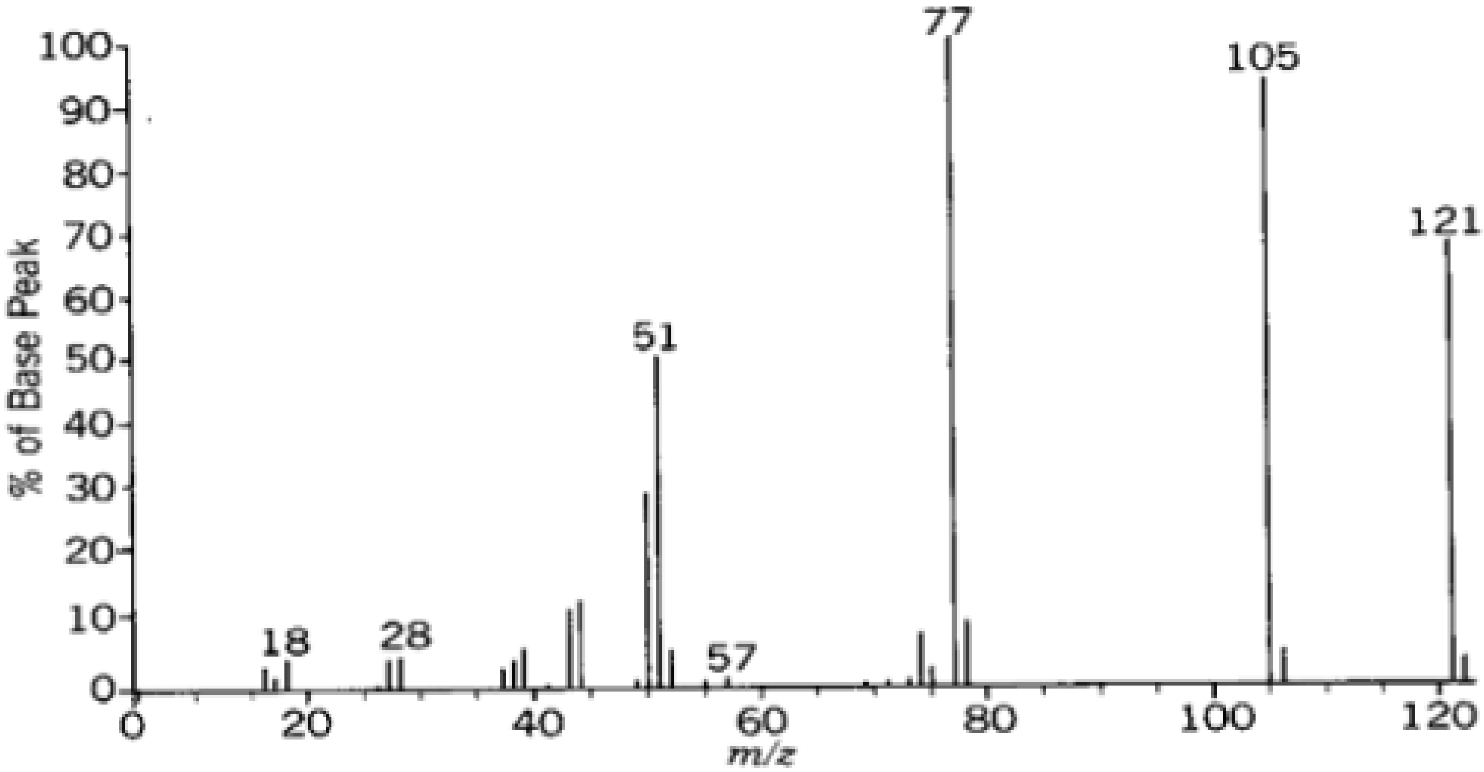
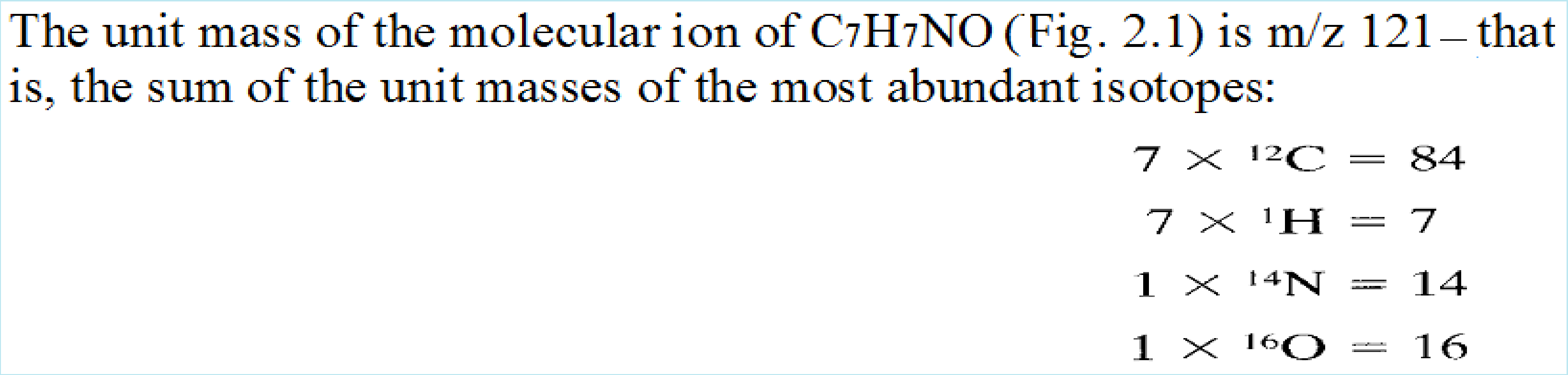


Figure 2.1: Computer-generated, electron-impact (EI) mass spectrum of benzamide $\left(C_6H_5-\overset{\overset{\displaystyle O}{\|}}{C}-NH_2\right)$ in bar graph form.

- The most intense the spectrum is termed the base peak and all others are reported peak in relative to its intensity.
- Because M +• is unstable, some ions decompose to form fragments of radicals and cations that have a lower molecular weight than M+•.

- **Electron is first removed from site with lowest ionization potential**
  - **non-bonding electrons > pi bond electrons > sigma bond electrons** **NB > π > σ**
- **Only CHARGED species are detected**

Determination of molecular formula:



The unit mass of the molecular ion of C_7H_7NO (Fig. 2.1) is m/z 121 – that is, the sum of the unit masses of the most abundant isotopes:

$$7 \times {}^{12}C = 84$$
$$7 \times {}^{1}H = 7$$
$$1 \times {}^{14}N = 14$$
$$1 \times {}^{16}O = 16$$

Recognition of the Molecular Ion Peak:

- The process of fragmentation follows simple and predictable chemical pathways and the ions which are formed will reflect the most stable cations and radical cations which that molecule can form.
- molecular ion (M+): is the highest molecular weight peak observed in a spectrum will typically represent the parent molecule, minus an electron.

What information can be determined?

- Molecular weight
- Molecular formula (HRMS)
- Structure (from fragmentation fingerprint)
- Isotopic incorporation / distribution
- Protein sequence (MS-MS)

Problems in recognition of the molecular ion peak:

1- Generally, small peaks are also observed above the calculated molecular weight due to the natural isotopic abundance of 13C, 2H, etc.

2- under electron impact (EI) recognition of the molecular ion peak (M) poses a problem:

a/ the peak may be very weak or may not appear at all

b/ we cannot be sure that is the molecular ion peak and not a fragment peak or an impurity.

- The best solution is to obtain a chemical ionization spectrum.

The usual result is an intense peak at M + 1 and little fragmentation.

3- Many molecules with especially labile protons do not display molecular ions; an example of this is alcohols, where the highest molecular weight peak occurs at m/e one less than the molecular ion (m - 1).

The intensity of the molecular ion peak depends on the stability of the molecular ion. The most stable molecular ions are those of purely aromatic systems.

If substituents that have favorable modes of cleavage are present, the molecular ion peak will be less intense, and the fragment peaks relatively more intense

In general:

the following group of compounds will, in order of decreasing ability, give prominent molecular ion peaks:

aromatic compounds > conjugated alkenes > cyclic compounds > organic sulfides > short, normal alkanes

Recognizable molecular ions are usually produced for these compounds in order of decreasing ability:

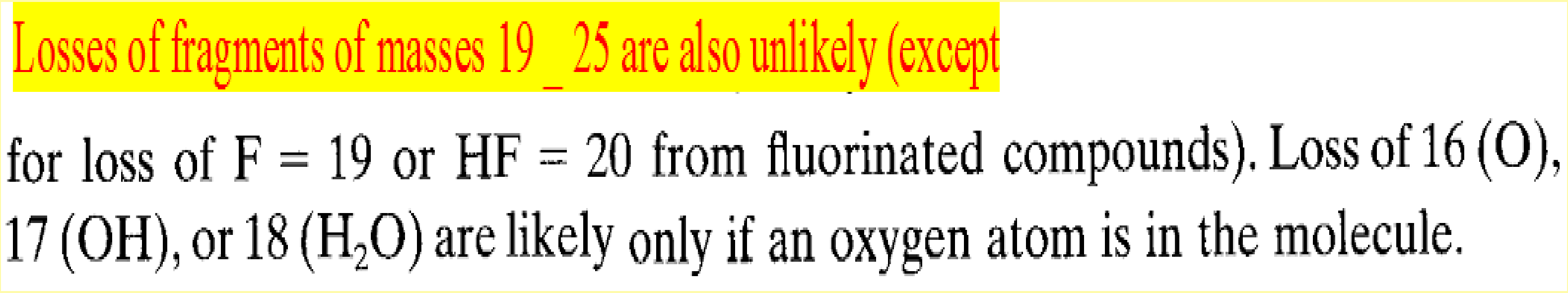
ketones > amines > esters > ethers > carboxylic acids ~ aldehydes ~ amides ~ halides.

The molecular ion is frequently not detectable in aliphatic alcohols, nitrites, nitrates, nitro compounds, nitriles, and in highly branched compounds.

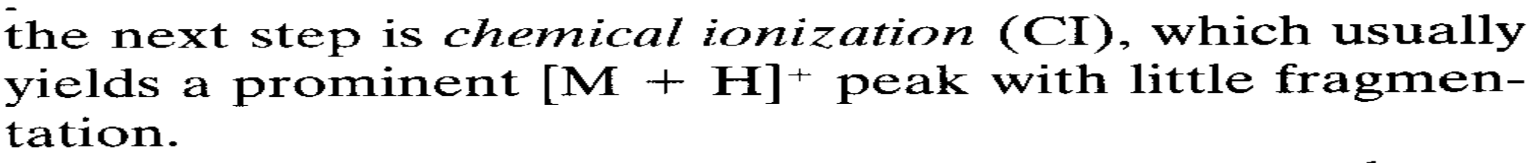
Fragments can be identified by their mass-to-charge ratio, but it is often more informative to identify them by the mass which has been lost. That is:

The presence of an M − 15 peak (loss of CH_3), or an M − 18 peak (loss of H_2O), or an M − 31 peak (loss of OCH_3 from methyl esters), and so on, is taken as confirmation of a molecular ion peak. An M − 1 peak is common, and occasionally an M − 2 peak (loss of H_2 by either fragmentation or thermolysis), or even a rare M − 3 peak (from alcohols) is reasonable.

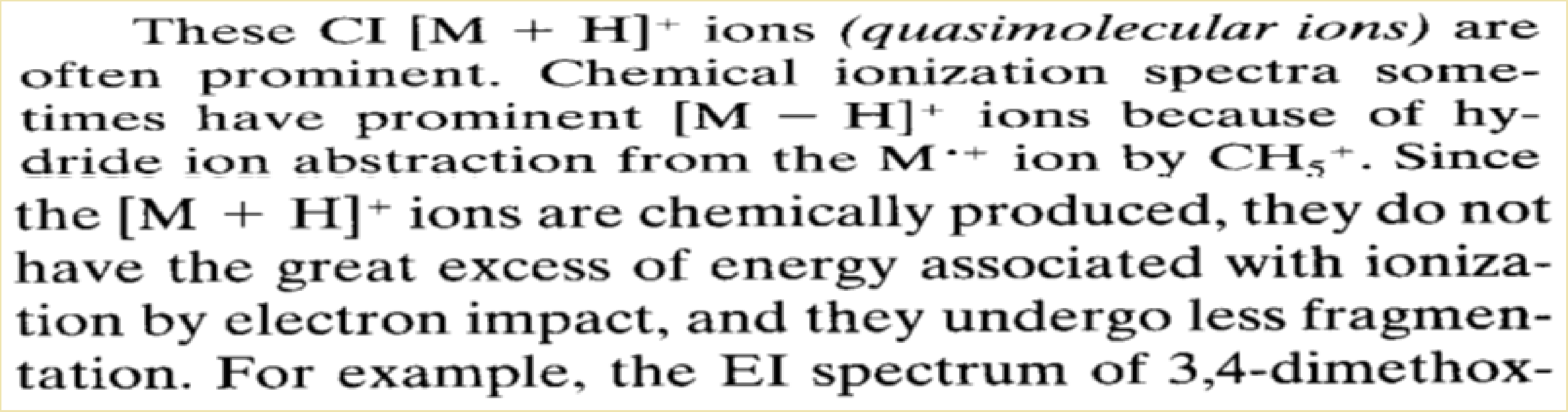
- Peaks in the range of M_ 3 to M_14, however, indicate that:
- 1. Contaminants may be present.
- 2. The presumed molecular ion peak is actually a fragment ion peak.



Losses of fragments of masses 19 _ 25 are also unlikely (except for loss of F = 19 or HF = 20 from fluorinated compounds). Loss of 16 (O), 17 (OH), or 18 (H_2O) are likely only if an oxygen atom is in the molecule.



the next step is *chemical ionization* (CI), which usually yields a prominent $[M + H]^+$ peak with little fragmentation.



These CI $[M + H]^+$ ions *(quasimolecular ions)* are often prominent. Chemical ionization spectra sometimes have prominent $[M - H]^+$ ions because of hydride ion abstraction from the $M^{\cdot+}$ ion by CH_5^+. Since the $[M + H]^+$ ions are chemically produced, they do not have the great excess of energy associated with ionization by electron impact, and they undergo less fragmentation. For example, the EI spectrum of 3,4-dimethox-

The "Nitrogen Rule"

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

  (If m/z is odd, then the number of nitrogens is odd)

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

  (If m/z number is even, the number of nitrogens in the compound is even.)

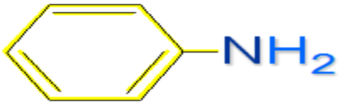
  (Note: 0 is even)

For example, ethylamine, $C_2H_5NH_2$ has one nitrogen atom, and its mass is an odd number (45).

Ethylenediamine, $H_2N\text{-}CH_2\text{-}CH_2\text{-}NH_2$, has two nitrogen atoms, and its mass is an even number (60)

m / z

93



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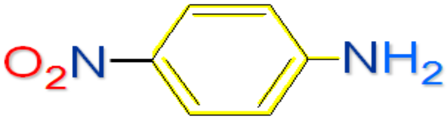

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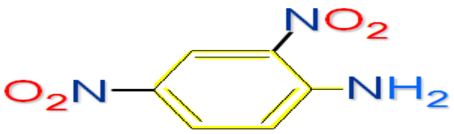


Information from hydrogen deficiency index

- Compound with an Index of one must have one double bond or one ring, but not both.

  If no double bond is present, the compound is cyclic and saturated.

- Compound with an index of two could have a triple bond, or it could have two double bonds, or two rings, or one of each.

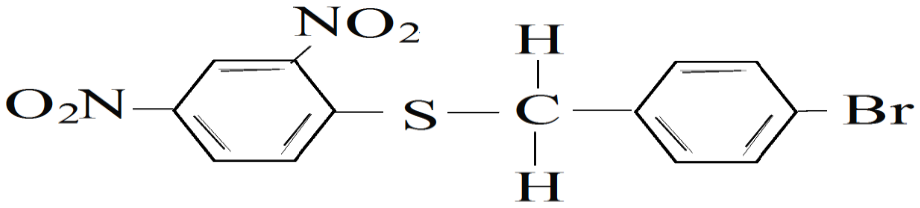
- Benzene contains one ring and three double bonds and thus an index of four.

  Any substance with an index of four or more may contain a benzenoid ring: a substance with an index less than four cannot contain such a ring.

i.e. for $C_{13}H_9N_2O_4BrS$

$HDI = IV - 1/2\ (I) + \frac{1}{2}\ (III) + 1$ ⇨ $HDI = 13 - \overset{(9\ H\ +\ 1\ Br)}{10} / 2 + \frac{1}{2}\ (2) + 1$

$HDI = 10$ the consist structure would be : $HDI = 4$ for each benzene rings (two rings) + 1 per each NO_2



The rule of thirteen

The “Rule of Thirteen” can be used to identify possible molecular formulas for an unknown hydrocarbon, C_nH_m.

Step 1 : n = M^+ / 13 [integer only, use remainder (**R**) in step2]

Step 2 : m = n + remainder from step 1

Example: The formula for a hydrocarbon with M^+ =106 can be found :

Step 1: n = 106 / 13 = 8 (r = 0.153 * 13 = 2)

Step 2: m = 8 + 2 = 10

Formula: C_8H_{10}

106 / 13 = 8.153

- If a heteroatom is present,
  - Subtract the mass of each heteroatom from the MW
  - Calculate the formula for the corresponding hydrocarbon
  - Add the heteroatoms to the formula.

Example: A compound with a molecular ion peak at m/z=102 has a strong peak at 1739 cm^{-1} in its IR spectrum. Determine its molecular formula.

Or It Contain 2 heteroatom of O

Step 1: Due to the compound has a strong peak at 1739 cm-1 in its IR spectrum therefore it contain ester group (COO-), so it Contain 2 heteroatom O. (2 * 16 = 32)

102-32 =70 MW of corresponding hydrocarbon (C & H).

Step 2: 70/13 = 5.384 n = 5 → (R= 0.384 X 13 = 5)

Step 3: m = n + remainder from step 1 → m = 5+5 =10

Step 4: Chemical formula = CnHm + heteroatom

$C_5H_{10}O_2$ $CH_3CH_2CH_2COOCH_3$