Unit 6.4 Mass spectroscopy (Marks 10)

Mass spectroscopy - principle – idea of mass spectrometer – fragmentation pattern –nitrogen rule - simple applications in structure elucidation (butane, ethane, acetone) –McLafferty rearrangement (hexanoic acid, pentanal).
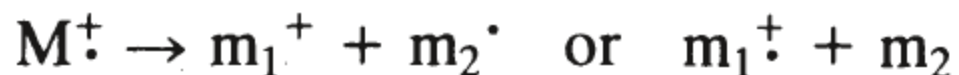
What is Mass Spectroscopy ?

Mass spectrometry is an analytical tool useful for measuring the **mass-to-charge ratio** (m/z) of one or more molecules present in a sample. These measurements can often be used to calculate the **exact molecular weight** of the sample components as well.

Applications of mass spectrometry:
(1) to measure *relative molecular masses (molecular weights) with very high* accuracy ; from these can be deduced exact molecular formulae
(2) to detect within a molecule the places at which it *prefers to fragment ; from this* can be deduced the presence of recognizable groupings within the molecule and
(3) as a method for identifying analytes by comparison of their mass spectra with libraries of digitized mass spectra of known compounds.

Basic Principles of Mass Spectrometry

In the simplest mass spectrometer (figure 5.1) , organic molecules are bombarded with electrons and converted to highly energetic positively charged ions *(molecular ions or parent ions), which can break up into* smaller ions *(fragment ions, or daughter ions) ; the loss of an electron from* a molecule leads to a radical cation, and we can represent this process as $M \rightarrow M^{+\cdot}$. The molecular ion $M^{+\cdot}$ commonly decomposes to a pair of fragments, which may be either a radical plus an ion, or a small molecule plus a radical cation. Thus,

$$M^{+\cdot} \rightarrow m_1{}^+ + m_2{}^\cdot \quad or \quad m_1{}^{+\cdot} + m_2$$

The molecular ions, the fragment ions and the fragment radical ions are separated by deflection in a variable magnetic field according to their mass and charge, and generate a current (the *ion current) at the collector in* proportion to their *relative abundances.*

Basic Principles of Mass Spectrometry

A mass spectrum is a plot of relative abundance against the ratio mass/charge (the *m/z value) . For* singly charged ions, the lower the mass the more easily is the ion deflected in the magnetic field. Doubly charged ions are occasionally formed: these are deflected twice as much as singly charged ions of the same mass, and they appear in the mass spectrum at the same value as do singly charged ions of half the mass, since *2m/2z = m/z.*

Neutral particles produced in the fragmentation, whether uncharged molecules (m2) or radicals $(m_2^{\cdot})$, cannot be detected directly in the mass spectrometer.

Figure 5.1 shows a block layout for a simple mass spectrometer. Since the ions must travel a considerable distance through the magnetic field to the collector, very low pressures (= 10^{-6} to 10^{-7} mmHg = 10^{-4} Nm^{-2}) must be maintained by the use of diffusion pumps.

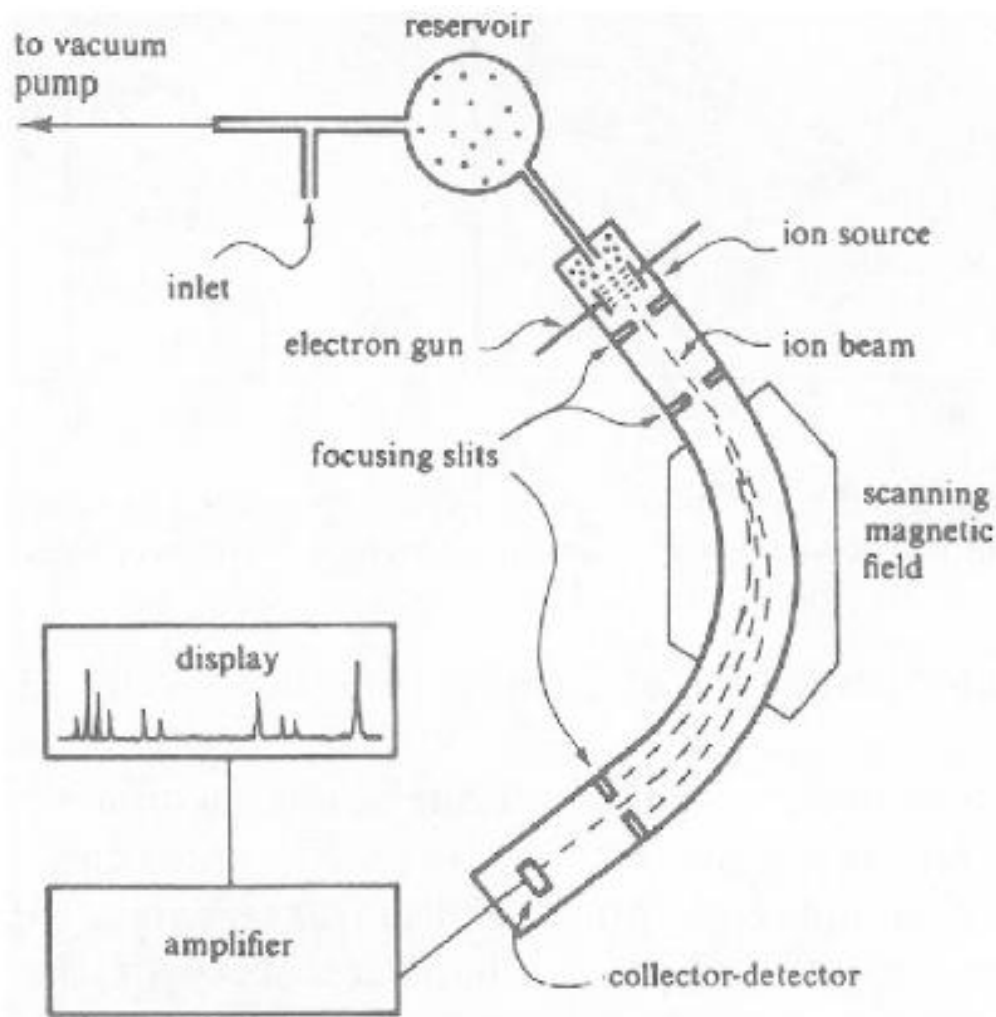# Basic Principles of Mass Spectrometry

Figure 5.1   *A simple mass spectrometer. Molecules drift from the reservoir into the ion source, where they are ionized by electron bombardment. The resulting ion beam consists of molecular ions, fragment ions and neutral fragments; the ions are deflected by the magnetic field onto the collector-detector.*

Mass Spectrum of 2-methylpentane

Figure 5.2 shows a simplified line-diagram representation of the mass spectrum of 2-methylpentane (C_6H_{14}) . The most abundant ion has an *m/z* value of 43 (corresponding to $C_3H_7^+$), showing that the most favored point of rupture occurs between C_x, and C_y: *this most abundant ion (the base peak) is given an arbitrary abundance of 100, and all other intensities are* expressed as a percentage of this *(relative abundances). The small peak at m/z 86 is obviously the molecular ion. The peaks at m/z 15, 29 and 71* correspond to CH_3^+, $C_2H_5^+$ and $C_5H_{11}^+$, respectively, etc.: the fragment ions arise from the rupture of the molecular ion, either directly or indirectly, and the analysis of many thousands of organic mass spectra has led to comprehensive semiempirical rules about the preferred *fragmentation modes of every kind of organic molecule.*
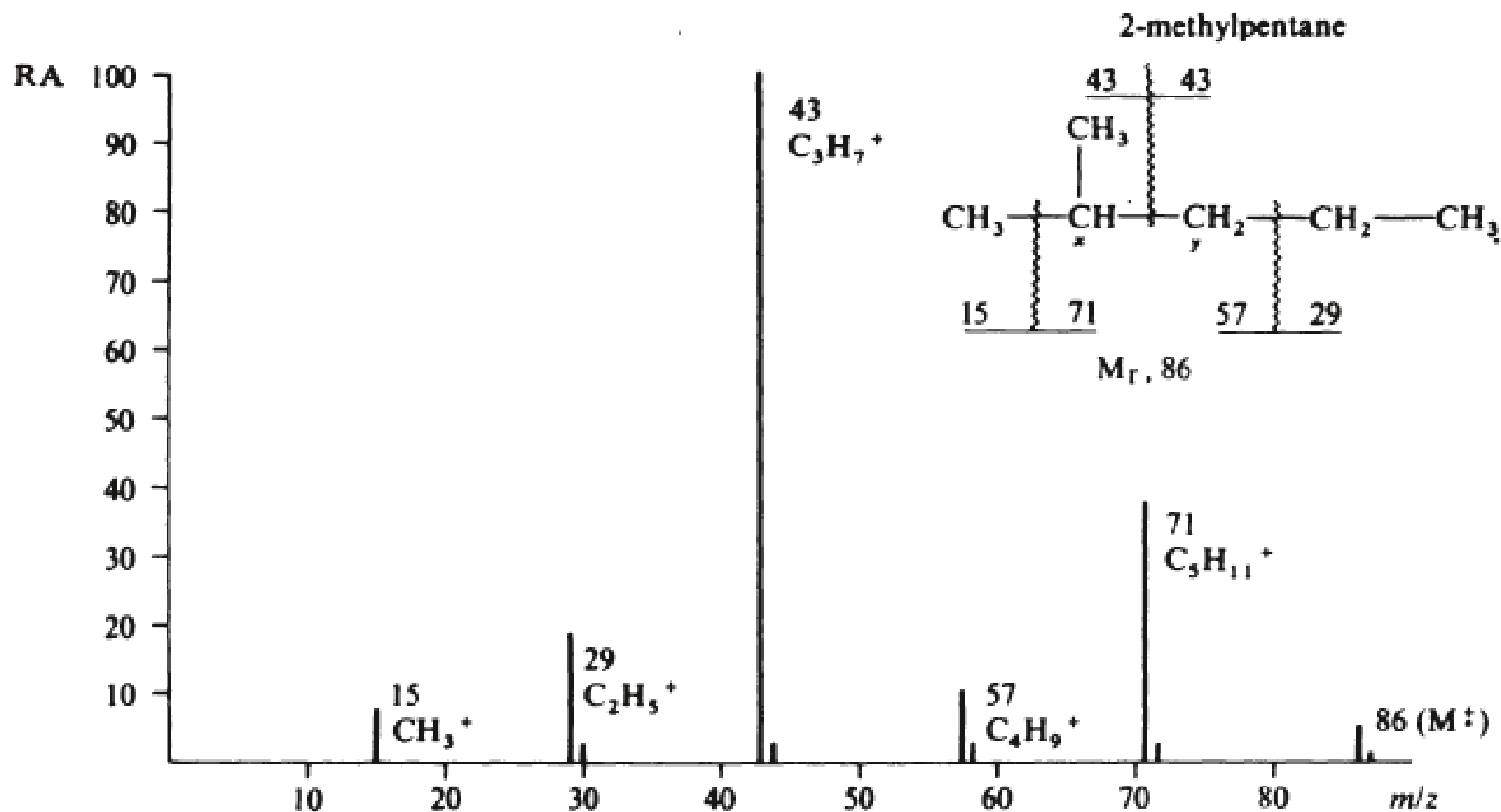
Mass Spectrum of 2-methylpentane

Figure 5.2 *Simplified mass spectrum of 2-methylpentane showing how the chain can break in several places; but breaks occur preferentially at the branching point, the most abundant ion being m/z 43 ($C_3H_7^+$).*

Disadvantages of Mass Spectrometry Technique

The mass spectrum of a compound can be obtained on a smaller sample size *(in extremis down to 10^{-12} g) than for any other of the main* spectroscopic techniques , the principal disadvantages being the destructive nature of the process, which precludes recovery of the sample, the difficulty of introducing small enough samples into the high-vacuum system needed to handle the ionic species involved and the high cost of the instruments. Mass spectrometry is unlike the other spectroscopic techniques does not measure the interaction of molecules with the spectrum of energies found in the electromagnetic spectrum, but the output from the instrument has all other spectroscopic characteristics, in showing an array of signals corresponding to a spectrum of energies; to highlight this distinction, the name *mass spectrometry is* preferred.

Instrumentation of Mass Spectrometer

1. SAMPLE INSERTION-INLET SYSTEMS: Organic compounds that have moderate vapor pressures at temperatures up to around 300°C (including gases) can be placed in an ampoule connected via a reservoir to the ionization chamber. Depending on volatility, it is possible to cool or heat the ampoule, etc ., to control the rate at which the sample volatilizes into the reservoir, from which it will diffuse slowly through the sinter into the ionization chamber. Samples with lower vapor pressures (for example, solids) are inserted directly into the ionization chamber on the end of a probe, and their volatilization is controlled by heating the probe tip .
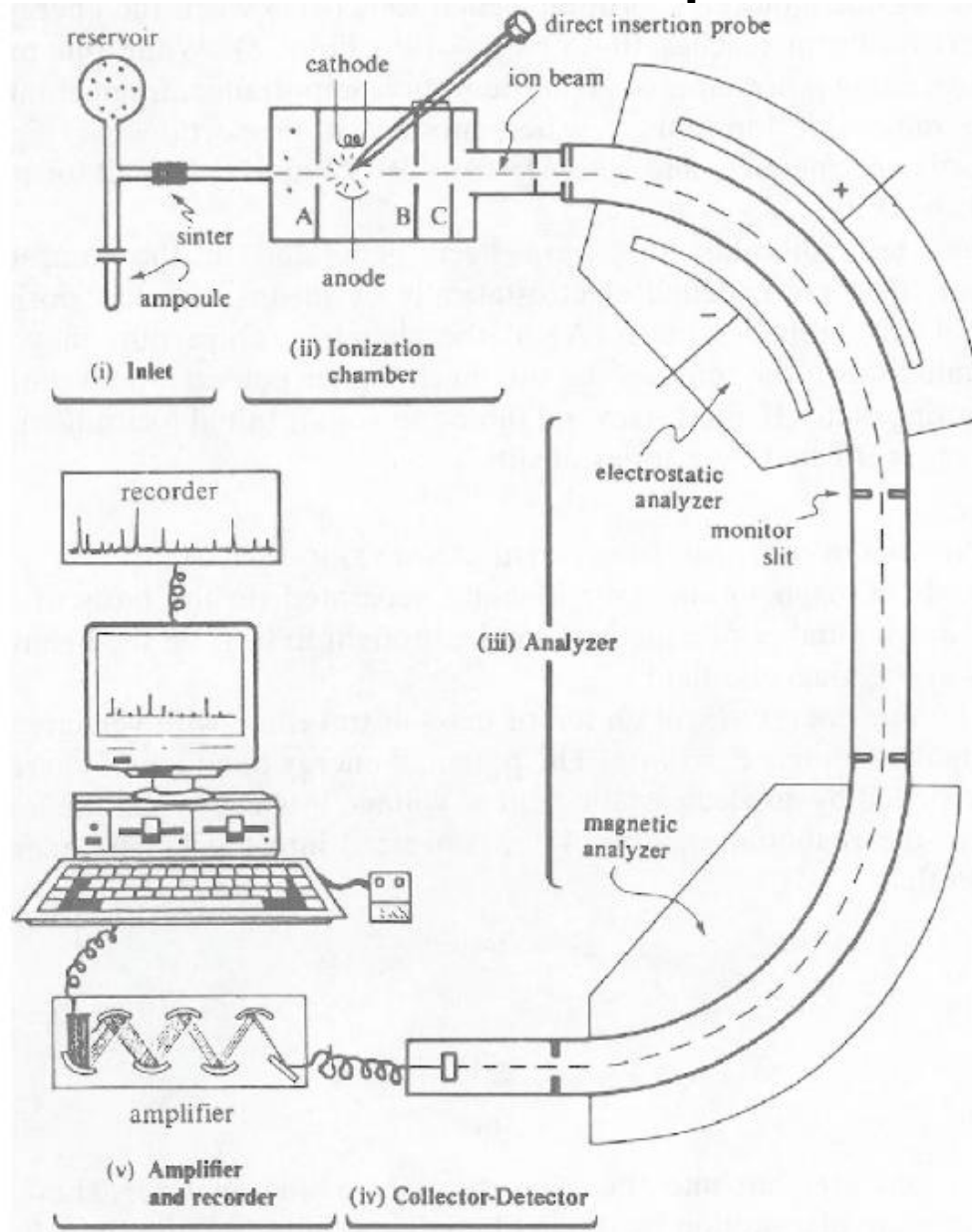
Instrumentation of Mass Spectrometer

Figure 5.3 *High-resolution, double-focusing mass spectrometer. (Evacuation system not shown.)*

Instrumentation of Mass Spectrometer

2. **ION PRODUCTION IN THE IONIZATION CHAMBER:** The several methods available for inducing the ionization of organic compounds are discussed in section 5S.1, but electron bombardment isroutinely used . Organic molecules react on electron bombardment in two ways: either an electron is captured by the molecule, giving a radical anion, or an electron is removed from the molecule, giving a radical cation :

$$M \xrightarrow{\text{e}} M^{\underline{\cdot}} \text{ or } M \xrightarrow{-\text{e}} M^{\underline{+}} + 2e$$

The latter is more probable by a factor of 10^2, and positive-ion mass spectrometry is the result. Most organic molecules form molecular ions (M") when the energy of the electron beam reaches 10-15 eV ($\sim 10^3$ kJmol^{-1}) . While this minimum *ionization potential is of great theoretical importance, fragmentation* of the molecular ion only reaches substantial proportions at higher bombardment energies, and 70 eV ($\sim$ 6 X 10^3 kJmol^{-1}) is used for most organic work. When the molecular ions have been generated in the ionization chamber, they are expelled electrostatically by means of a low positive potential on a repeller plate (A) in the chamber.

Instrumentation of Mass Spectrometer

Once out, they are accelerated down the ion tube by the much higher potential between the accelerating plates Band C (several thousand volts) . Initial focusing of the ion beam is effected by a series of slits.

3. SEPARATION OF THE IONS IN THE ANALYZER:

Theory. In a magnetic analyzer ions are separated on the basis of m/z values, and a number of equations can be brought to bear on the behavior of ions in the magnetic field. The kinetic energy, E, of an ion of mass m travelling with velocity v is given by the familiar *E = ½ mv² .The potential energy of an ion of charge z being repelled by an electrostatic field of voltage V is zV. When the ion is* repelled, the potential energy, *zV, is converted into the kinetic energy, ½ mv², so that*

$$zV = \tfrac{1}{2}mv^2$$

Therefore,

$$v^2 = \frac{2zV}{m} \qquad\qquad (5.1)$$

Instrumentation of Mass Spectrometer

When ions are shot into the magnetic field of the analyzer, they are drawn into circular motion by the field , and at equilibrium the centrifugal force of the ion *(mv²/r) is equalled by the centripetal force exerted on it by* the magnet *(zBv), where r is the radius of the circular motion and B is the* field strength. Thus,

$$\frac{mv^2}{r} = zBv$$

Therefore,

$$v = \frac{zBr}{m} \qquad\qquad (5.2)$$

Combining equations (5.1) and (5.2),

$$\frac{2zV}{m} = \left[\frac{zBr}{m}\right]^2$$

Therefore,

$$\frac{m}{z} = \frac{B^2r^2}{2V} \qquad\qquad (5.3)$$

Instrumentation of Mass Spectrometer

It is from equation (5.3) that we can see the inability of a mass spectrometer to distinguish between an ion m^+ and an ion $2m^{2+}$, since the ratio, *m/z, between them has the same value of ($B^2r^2/2V$), and these three parameters B, r and V dictate the path of the ions.* To change the path of the ions so that they will focus on the collector and be recorded, we can vary either *V (the accelerating voltage) or B (the* strength of the focusing magnet) . *Voltage scans (the former) can be* effected much more rapidly than *magnetic scans and are used where fast* scan speed is desirable .

4. Resolution: The ability of a mass spectrometer to separate two ions (the spectrometer resolution) is acceptably defined by measuring the depth of the valleys between the peaks produced by the ions . If two ions of *m/z 999* and 1000, respectively, can just be resolved into two peaks such that the recorder trace almost reaches back down to the baseline between them, leaving a valley which is 10 per cent of the peak height, we say the resolution of the spectrometer is '1 part in 1000 (10 per cent valley resolution)' . Simple magnetic-focusing instruments have resolving powers of around 1 in 7500 on this basis.

Isotope Abundances

Few elements are monoisotopic, and table 5.1 gives the natural isotope abundance of the elements we might expect to encounter in organic compounds. Ions containing different isotopes appear at different *m/z* values . For an ion containing *n carbon atoms, there is a probability that* approximately 1.1n per cent of these atoms will be 13C , and this will give rise to an ion of mass one higher than the ion that contains only 12C atoms. The molecular ion for 2-methylpentane (figure 5.2) has an associated M + 1 ion, whose intensity is approximately 6.6 per cent of that of the molecular ion ; while the molecular ion has only 12C atoms, the M + 1 ion contains 13C atoms. (The contribution of 2H atoms should not be over-looked, even though the probability of their presence is not as high as for 13C, and in exact work the statistics of both 13C and 2H abundances must be calculated.)

Isotope Abundances

Table 5.1 Natural isotope abundances and isotopic masses
($^{12}C = 12.000\ 000$) for common elements

Isotope	Natural abundance (%)	Isotopic mass/m_u
^1H	99.985	1.007 825
^2H	0.015	2.014 102
^{12}C	98.9	12.000 000
^{13}C	1.1	13.003 354
^{14}N	99.64	14.003 074
^{15}N	0.36	15.000 108
^{16}O	99.8	15.994 915
^{17}O	0.04	16.999 133
^{18}O	0.2	17.999 160
^{19}F	100	18.998 405
^{28}Si	92.2	27.976 927
^{29}Si	4.7	28.976 491
^{30}Si	3.1	29.973 761
^{31}P	100	30.973 763
^{32}S	95.0	31.972 074
^{33}S	0.76	32.971 461
^{34}S	4.2	33.967 865
^{35}Cl	75.8	34.968 855
^{37}Cl	24.2	36.965 896
^{79}Br	50.5	78.918 348
^{81}Br	49.5	80.916 344
^{127}I	100	126.904 352

Isotope Abundances

A second associated peak can arise at *mlz M + 2 if two 13C atoms are* present in the same ion (or if two 2H atoms, or one 13C and one 2H, are pre sent) ; these probabilities can be calculated and may be a help in deciding the formula for an ion in the absence of exact mass measurement. For example , the two ions C8H12N3+ and C9H10O2+ have the same unit mass *(m/z 150), and the M + 1 relative abundances are similar (9.98 per*cent and 9.96 per cent , respectively); however, the M + 2 abundances are sufficiently different to enable differentiation of the structures (0.45 per cent and 0.84 per cent, respectively) . The ability to see M + 2 peaks of such low abundance depends on there being a large $M^{\ddagger}$ peak. Ions containing one bromine atom create a dramatic effect in the mass spectrum because of the almost equal abundance of the two isotopes; pairs of peaks of roughly equal intensity appear, separated by two mass units.

Isotope Abundances

Equally characteristic are the ions from chlorine compounds engendered by the 35Cl and 37Cl isotopes; for ions containing one Cl atom, the relative intensities of the lines, separated by two mass units, is 3:1. Ions containing one sulfur atom also have associated m + 2 peaks. The picture becomes much more complicated when one considers the relative abundances of ions containing several polyisotopic elements; the presence of two bromine atoms in an ion gives rise to three peaks at m, m + 2 and m + 4, the relative intensities being 1:2:1, while for three bromines the peaks arise at m, m + 2, m + 4, m + 6, with relative intensities 1:3:3: 1. These figures ignore any contribution from 13C that may be present.
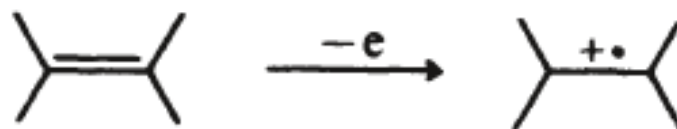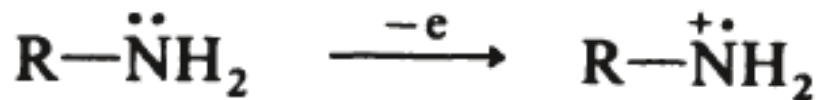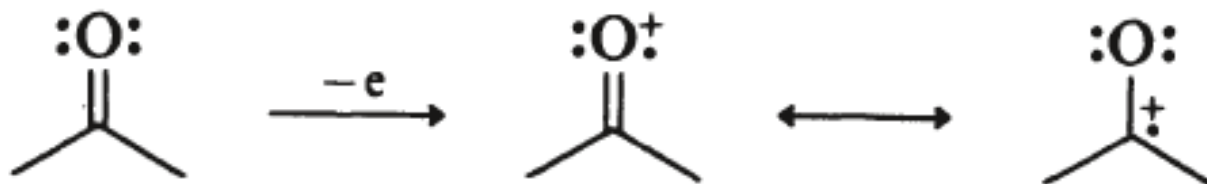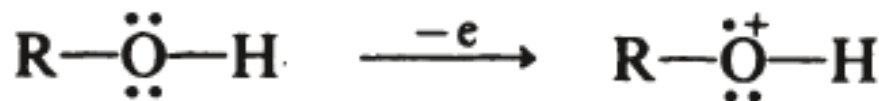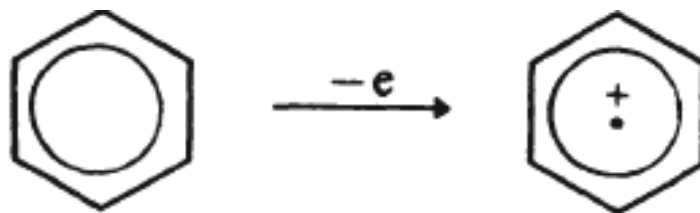
Isotope Abundances

For each element in a given ion, the relative contributions to m + 1, m + 2 peaks, etc .. can be calculated from the binomial expansion of *(a + b)n, where a and b are the relative abundances of the isotopes and n is* the number of these atoms present in the ion. Thus, for three chlorine atoms in an ion, expansion gives *a3 + 3a2b + 3ab2 + b3. Four peaks arise;* the first contains three 35Cl atoms and each successive peak has 35Cl replaced by 37Cl until the last peak contains three 37Cl atoms. The *m/z* values are separated by two mass units , at m, m + 2, m + 4, m + 6. Since the relative abundances of 35Cl and 37Cl are 3:1 (that is, *a = 3, b = 1), the* intensities of the four peaks (ignoring contributions from other elements) are *a3 = 27, 3a2b = 27, 3ab2 = 9, b3 = 1 (that is, 27:27:9 :1)* .

Molecular Ion

STRUCTURE OF THE MOLECULAR ION: Radical cation $M^{\ddagger}$ is produced when a neutral molecule is bombarded with an electron. *Where does the electron come from?*

For electron bombardment around their ionization potentials (10-15 eV = 10^3 kJ/mol), it is meaningfully possible in organic molecules to say which are the likeliest orbitals to lose an electron . The highest occupied orbitals of aromatic systems and nonbonding orbitals on oxygen and nitrogen atoms readily lose an electron; the π electrons of double and triple bonds are also vulnerable . At the instant of ionization in a Franck-Condon process, before any structural rearrangement can occur, these ionizations can then be represented as shown below. In alkanes, all we can say is that the ionization of C-C **σ** bonds is easier than that of C-H bonds.

Molecular Ion

$$\text{R}-\overset{..}{\underset{..}{\text{O}}}-\text{H} \xrightarrow{-e} \text{R}-\overset{.+}{\underset{..}{\text{O}}}-\text{H}$$

I   II   m/z 45

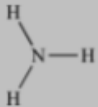# Molecular Ion
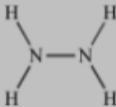
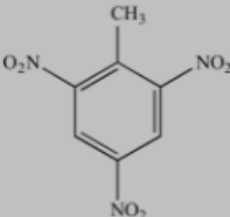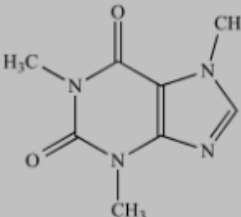## RECOGNITION OF THE MOLECULAR ION:

The molecular ions of roughly 20 per cent of organic compounds decompose so rapidly ($<10^{-5}$ s) that they may be very weak or undetected in a routine 70 eV spectrum. For most unknown compounds the ion cluster appearing at highest *m/z value is likely to represent the molecular ion with* its attendant M + 1 peaks, etc., but we must apply a number of tests to ensure that this is so.

Abundant molecular ions are given by aryl amines, nitriles, fluorides and chlorides. Aromatic hydrocarbons and heteroaromatic compounds give strong $M^{+\cdot}$ peaks, provided that no side-chain of C2 or longer is present: indeed the $M^{+\cdot}$ peak is often the base peak, and doubly charged ions may often be observed in the mass spectra of these compounds appearing at *m/2z values. Aryl bromides and iodides lose halogen too readily to give* strong molecular ion peaks. Other classes with weakened $M^{+\cdot}$ peaks are aryl ketones (which fragment easily to ArCO+) and benzyl compounds such as side-chain hydrocarbons (ArCH2R) or ArCH2X (both of which fragment at the benzylic carbon) .

Molecular Ion

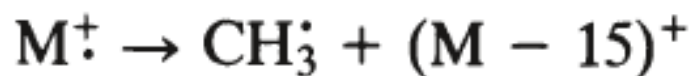*Absence of molecular ions (or an extremely weak $M^{+\cdot}$ peak) is characteristic* of highly branched molecules whatever the functional class. Alcohols and molecules with long alkyl chains also fragment easily and lead to very weak $M^{+\cdot}$ peaks. *Isotope abundances should correlate with the appearance of the purported* molecular-ion cluster. The intensities of M + 1, M + 2 peaks, etc., are obviously most easily measured and of greatest value when the $M^{+\cdot}$ peak is fairly abundant.

Nitrogen Rule: *Nitrogen-containing compounds with an odd number of nitrogen atoms* in the molecule must have an *odd molecular weight (relative molecular* mass) . An *even number of nitrogen atoms, or no nitrogen at all, leads to an even* molecular weight (relative molecular mass).

Molecule:	Ammonia	Hydrazine	TNT	Caffeine
Formula:	NH_3	N_2H_4	$C_7H_5N_3O_6$	$C_8H_{10}N_4O_2$
m/z (M):	17	32	227	194
	One nitrogen	Two nitrogens	Three nitrogens	Four nitrogens
	m/z (M) is odd	m/z (M) is even	m/z (M) is odd	m/z (M) is even

Molecular Ion

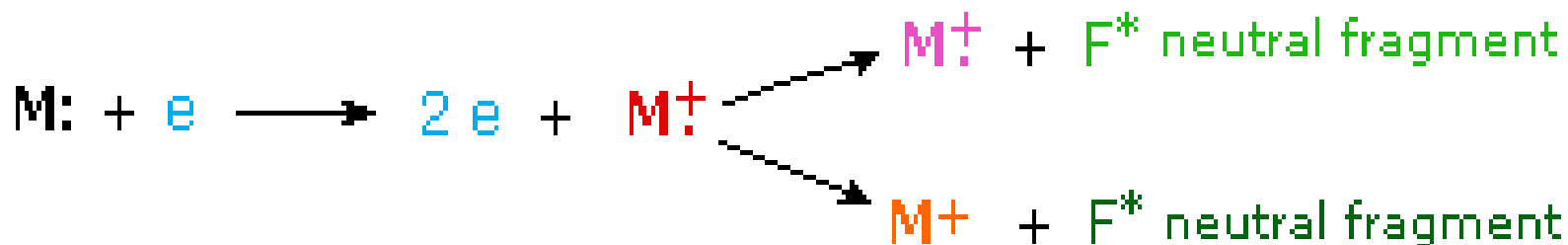**Representation of Fragmentation Process:** If a molecular ion loses a methyl radical (CH3·) , the mass spectrum will show an ion 15 mass units below the molecular ion: we can write this process as

$$M^{+}_{.} \rightarrow CH^{+}_{3} + (M - 15)^{+}$$

An alternative is to write the fragment ion as M - CH3 . We shall use this convention frequently, referring to ions as M - 18, M - 24, M - CO, M – H2S, etc., it being understood that these may in fact be even-electron ions-for example, (M - 15)$^{+}$ or odd electron radical ions-for example, $(M - 18)^{+}_{.}$ The same convention can be used to represent fragment ions as m+ (or $m^{+}_{.}$}, and these may, in turn, fragment to m - 1, m - CH3, m – C2H5, etc.

The Nature of Mass Spectra

When a high energy electron collides with a molecule it often ionizes it by knocking away one of the molecular electrons (either bonding or non-bonding). This leaves behind a **molecular ion** (colored red in the following diagram). Residual energy from the collision may cause the molecular ion to fragment into neutral pieces (colored green) and smaller **fragment ions** (colored pink and orange). The molecular ion is a radical cation, but the fragment ions may either be radical cations (pink) or carbocations (orange), depending on the nature of the neutral fragment.

$$M: + e \longrightarrow 2e + M^{\pm} \underset{\nearrow}{\searrow} \begin{array}{l} M^{\pm} + F^* \text{ neutral fragment} \\ M^+ + F^* \text{ neutral fragment} \end{array}$$
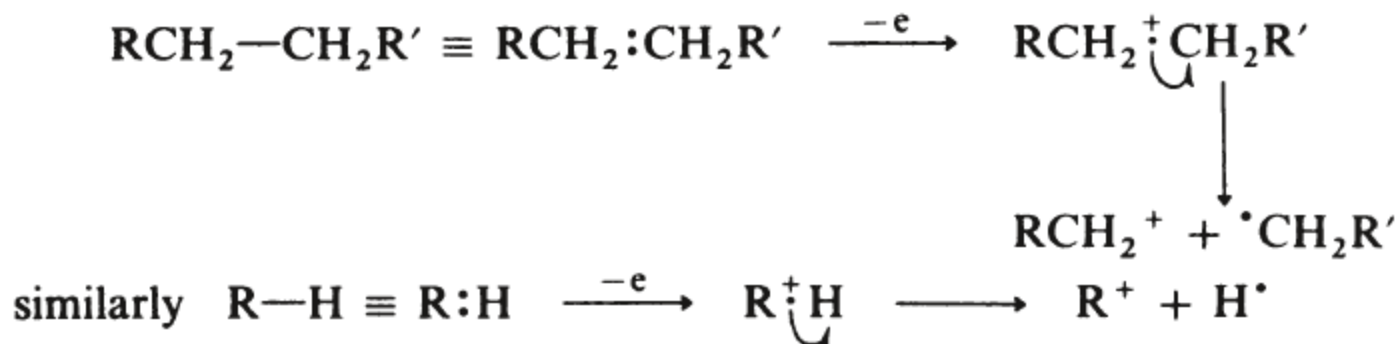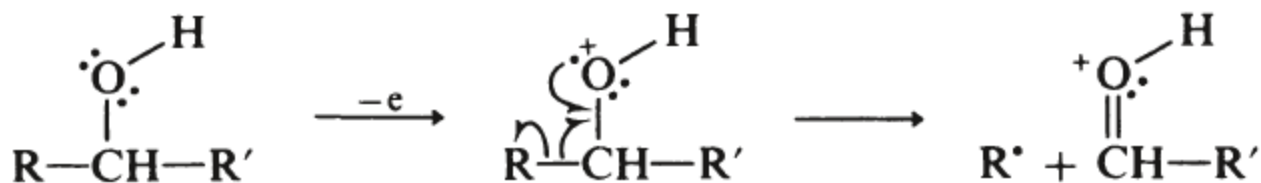
The Nature of Mass Spectra

A mass spectrum will usually be presented as a vertical bar graph, in which each bar represents an ion having a specific mass-to-charge ratio (m/z) and the length of the bar indicates the relative abundance of the ion. The most intense ion is assigned an abundance of 100, and it is referred to as the **base peak**. Most of the ions formed in a mass spectrometer have a single charge, so the m/z value is equivalent to mass itself. Modern mass spectrometers easily distinguish (resolve) ions differing by only a single atomic mass unit (amu), and thus provide completely accurate values for the molecular mass of a compound. The highest-mass ion in a spectrum is normally considered to be the molecular ion, and lower-mass ions are fragments from the molecular ion, assuming the sample is a single pure compound.

Fragmentation Rules and Types

1. **σ -*Bond rupture in alkane groups:*** This can really only be represented by assuming that , at the instant of ionization, sufficient excitation energy is concentrated on the rupturing bond to ionize it.
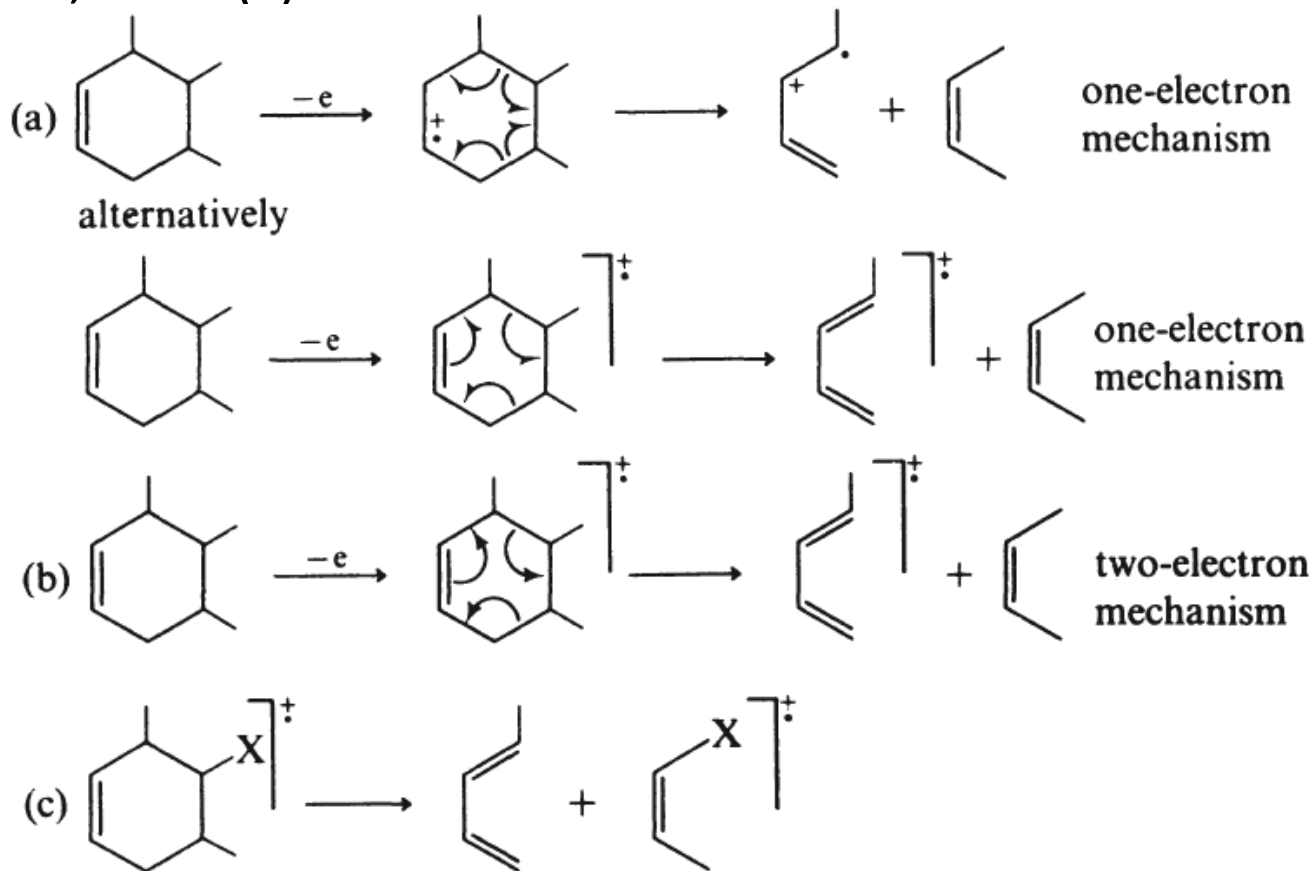
$$RCH_2—CH_2R' \equiv RCH_2:CH_2R' \xrightarrow{-e} RCH_2 \overset{+}{\underset{\cup}{C}}H_2R'$$

$$\downarrow$$

$$RCH_2^+ + \,^\cdot CH_2R'$$

similarly $\quad R—H \equiv R:H \xrightarrow{-e} R\overset{+}{\underset{\cup}{H}} \longrightarrow R^+ + H^\cdot$

2. **σ -*Bond rupture near functional groups:*** This may be facilitated by the easier ionization of that group's orbitals, as in alcohols, where the nonbonding orbitals of oxygen are more easily ionized than the **σ-**orbitals. Other groups in this category are ethers, carbonyl groups and compounds containing halogen, nitrogen, double bonds, phenyl groups , etc .

Fragmentation Rules and Types

3. Elimination by multiple σ -bond rupture: Elimination by multiple **σ –** bond rupture may occur , leading to the extrusion of a neutral molecule such as CO, C2H4, C2H2 etc . A well-known example is the retro-Diels-Alder reaction of cyclohexenes, which can be represented as in (a) or (b). Highly stabilized ene fragments may cause charge retention to be in part reversed, as in (c).

Mass Spectrometry Ionization Methods

1. **Electron impact (EI):** An EI source uses an electron beam, usually generated from a tungsten filament, to ionize gas-phase atoms or molecules. An electron from the beam knocks an electron off of analyte atoms or molecules to create ions.

2. **Electrospray ionization (ESI):** The ESI source consists of a very fine needle and a series of skimmers. A sample solution is sprayed into the source chamber to form droplets. The droplets carry charge when the exit the capillary and, as the solvent evaporates, the droplets disappear leaving highly charged analyte molecules. ESI is particularly useful for large biological molecules that are difficult to vaporize or ionize.

3. **Plasma and glow discharge:** A plasma is a hot, partially-ionized gas that effectively excites and ionizes atoms. A glow discharge is a low-pressure plasma maintained between two electrodes. It is particularly effective at sputtering and ionizing material from solid surfaces.
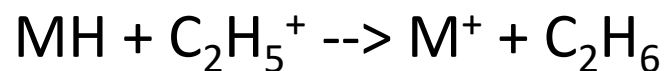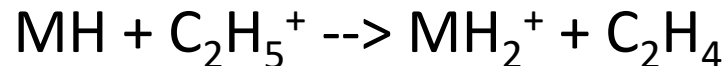
Mass Spectrometry Ionization Methods

4. Fast-atom bombardment (FAB): In FAB a high-energy beam of netural atoms, typically Xe or Ar, strikes a solid sample causing desorption and ionization. It is used for large biological molecules that are difficult to get into the gas phase. FAB causes little fragmentation and usually gives a large molecular ion peak, making it useful for molecular weight determination.T he atomic beam is produced by accelerating ions from an ion source though a charge-exchange cell. The ions pick up an electron in collisions with netural atoms to form a beam of high energy atoms.

5. Matrix-assisted laser desorption ionization (MALDI): MALDI is a LIMS method of vaporizing and ionizing large biological molecules such as proteins or DNA fragments. The biological molecules are dispersed in a solid matrix such as nicotinic acid. A UV laser pulse ablates the matrix which carries some of the large molecules into the gas phase in an ionized form so they can be extracted into a mass spectrometer.

Mass Spectrometry Ionization Methods

6. **Chemical ionization (CI)**: CI uses a reagent ion to react with the analyte molecules to form ions by either a proton or hydride transfer:

$$MH + C_2H_5^+ \rightarrow MH_2^+ + C_2H_4$$
$$MH + C_2H_5^+ \rightarrow M^+ + C_2H_6$$

The reagent ions are produced by introducing a large excess of methane (relative to the analyte) into an electron impact (EI) ion source. Electron collisions produce CH_4^+ and CH_3^+ which further react with methane to form CH_5^+ and $C_2H_5^+$:

$$CH_4^+ + CH_4 \rightarrow CH_5^+ + CH_3$$
$$CH_3^+ + CH_4 \rightarrow C_2H_5^+ + H_2$$