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Lectures of Molecular Spectroscopy
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Prof. Dr. Abbas A-Ali Drea

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# Lecture No. 9: Mass Spectroscopy

## 1-Introduction:

- Mass spectrometry is a powerful analytical technique used to quantify known materials and identify unknown compounds within a sample.
- The complete process involves the sample conversion into gaseous ions, with or without fragmentation, which is characterized by their mass to charge ratios (m/z) and relative abundances.
- Study the effect of ionizing energy on molecules.
- It depends upon chemical reactions in the gas phase in which sample molecules are consumed during the formation of ionic and neutral species.

## 2-Basic Principle:

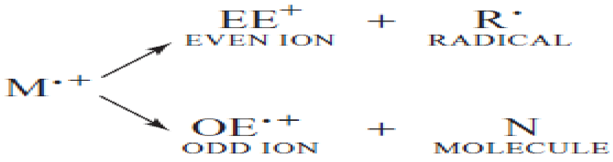
**In general**, a mass spectrometer generates multiple ions from the sample under investigation; it then separates them according to their specific mass-to-charge ratio (m/z), and then records the relative abundance of each ion type. (What is the analysis process steps of mass spectroscopy?)

There are several steps for analysis process:-

**First step** in the mass spectrometric, involve analysis of compounds it is the production of gas phase ions of the compound, by electron ionization.

$$M + {}_{fast}e^{-} \rightarrow M^{\cdot +} + 2e^{-} \quad ---1$$

**Second step**, this molecular ion normally undergoes fragmentations. Because it is a radical cation with an odd number of electrons, it can fragment to give either a radical or an ion with an even number of electrons, or a molecule and a new radical cation. The important of difference between these two types of ions and the need to write them correctly:



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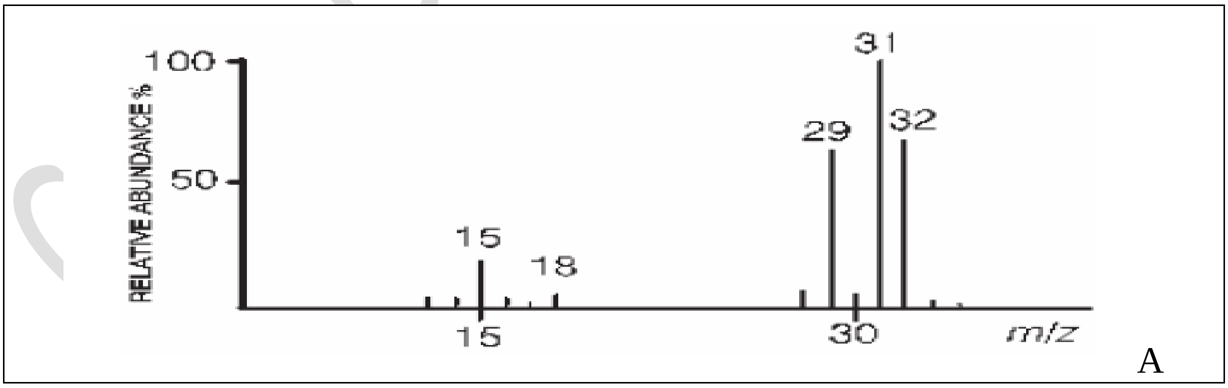
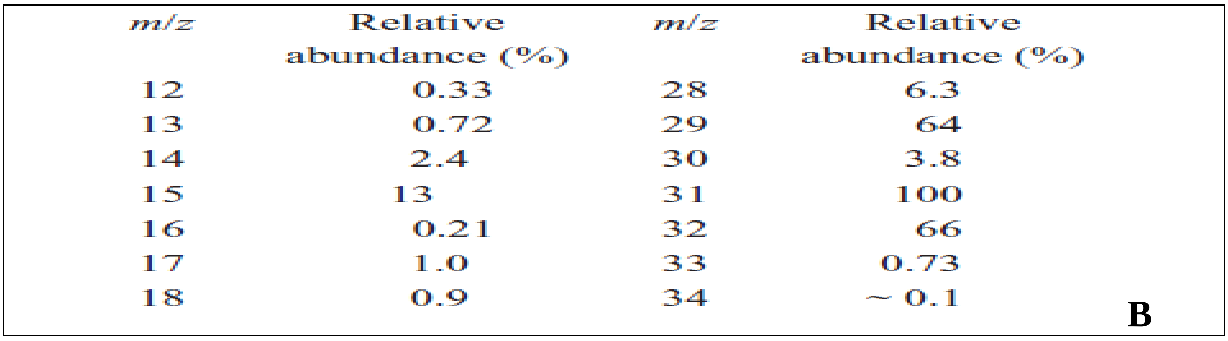
These two types of ions have different chemical properties. Each primary product ion derived from the molecular ion can undergo fragmentation, and so on.

**<u>Third step</u>,** all these ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance.

**<u>Fourth step</u>**, a mass spectrum of the molecule is produced. It provides this result as a plot of ion abundance versus mass-to-charge ratio.

As illustrated in Figure 1, mass spectra is represented as a barograph or as a table. In either presentation, the most intense peak is called the base peak and is arbitrarily assigned the relative abundance of 100 %. The abundances of all the other peaks are given their proportionate values, as percentages of the base peak. Many existing publications label the y-axis of the mass spectrum as number of ions, ion counts or relative intensity.





| m/z | Relative abundance (%) | m/z | Relative abundance (%) |
|---|---|---|---|
| 12 | 0.33 | 28 | 6.3 |
| 13 | 0.72 | 29 | 64 |
| 14 | 2.4 | 30 | 3.8 |
| 15 | 13 | 31 | 100 |
| 16 | 0.21 | 32 | 66 |
| 17 | 1.0 | 33 | 0.73 |
| 18 | 0.9 | 34 | ~ 0.1 |

**Figure 1:** Mass spectrum of methanol by electron ionization, presented as a graph and as a table.

Relative abundance is better used to refer to the number of ions in the mass spectra.

Most of the positive ions have a charge corresponding to the loss of only one electron.

For large molecules, multiply charged ions also can be obtained.

Ions are separated and detected according to the mass-to-charge ratio.

The total charge of the ions will be represented as q.

the electron charge by e and the number of charges of the ions by **z**:

$$q = \mathbf{z}e \quad \text{and } e = 1.6 \times 10^{-19}\ \text{C} \quad \text{------}3$$

The x-axis of the mass spectrum that represents the mass-to-charge ratio is commonly labelled m/z. When **m** is given as the relative mass and **z** as the charge number, m/z is used to denote a dimensionless quantity.

Generally in mass spectrometry, the charge is indicated in multiples of the elementary charge or charge of one electron in absolute value (1 e=$1.602177\times10^{-19}$ C) and the mass is indicated in atomic mass units (**1 AMU=$1.660540\times10^{-27}$ kg).** As already mentioned, the physical property that is measured in mass spectrometry is the mass-to-charge ratio. When the mass is expressed in atomic mass units **(AMU)** and the charge in elementary charge units (e) then the mass-to-charge ratio has **AMU/e as** dimensions. For simplicity, a new unit, the Thomson, with symbol Th, has been proposed. The fundamental definition for this unit is

$$1\text{Th} = 1\text{AMU/e} = 1.036\,426 \times 10^{-8}\ \text{kg C}^{-1} \quad \text{----}4$$

## 3-Components:

The instrument consists of three major components:

1. **Ion Source:** For producing gaseous ions from the substance (analyst).
2. **Analyzer:** For resolving the ions into their characteristics mass components according to their mass-to-charge ratio.
3. **Detector System:** For detecting the ions and recording the relative abundance of each of the resolved ionic species.

In addition, a sample introduction system is necessary to admit the samples to study to the ion source while maintaining the high vacuum requirements (~$10^{-6}$ to $10^{-8}$ mm of mercury) of the technique; and a computer is required to control the instrument, acquire and manipulate data, and compare spectra to reference libraries.

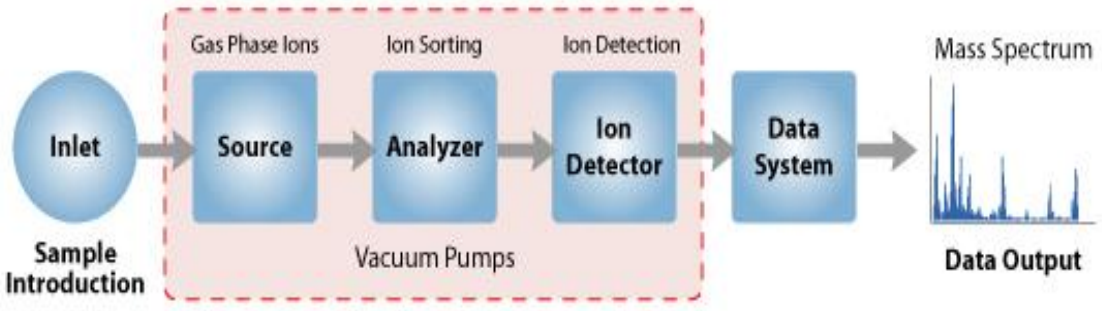


Figure.2: Components of a Mass Spectrometer.

Q//What are the process, that's should be preform in mass spectrometer?

With all the above components, a mass spectrometer should always perform the following processes:

1. Produce ions from the sample in the ionization source.

2. Separate these ions according to their mass-to-charge ratio in the mass analyzer.
3. Eventually, fragment the selected ions and analyze the fragments in a second analyzer.
4. Detect the ions emerging from the last analyzer and measure their abundance with the detector that converts the ions into electrical signals.
5. Process the signals from the detector that are transmitted to the computer and control the instrument using feedback.

### 4-Ion Free Path:

All mass spectrometers must function under high vacuum (low pressure). This is necessary to allow ions to reach the detector without undergoing collisions with other gaseous molecules. Indeed, collisions would produce a deviation of the trajectory and the ion would lose its charge against the walls of the instrument. On the other hand, ion–molecule collisions could produce unwanted reactions and hence increase the complexity of the spectrum. Nevertheless, we will see later that useful techniques use controlled collisions in specific regions of a spectrometer. According to the kinetic theory of gases, the mean free path L (in m) is given by:

$$L = \frac{kT}{\sqrt{2}P\sigma}\text{-------}5$$

Where k is the Boltzmann constant, T is the temperature (Kalvin unit), p is the pressure (Pascal unit) and σ is the collision cross-section (square meter $m^2$); $\sigma = \pi d^2$ where d is the sum of the radii of the stationary molecule and the colliding ion (in m). In fact, one can approximate the mean free path of an ion under normal conditions in a mass spectrometer ($k=1.38\times10^{-21}$ $JK^{-1}$, $T \approx 300$ K, $\sigma \approx 45\times10^{-20}$ $m^2$) using either of the following equations where L is in centimeters and pressure p is, respectively, in Pascal or millitorr.

Large differences in pressure is controlled with the help of an efficient pumping system using mechanical pumps in conjunction with turbo molecular, diffusion or cryogenic pumps. The mechanical pumps allowed vacuum of about $10^{-3}$ Torr to be obtained. Once this vacuum is achieved, the operation of the other pumping systems allows a vacuum as high as $10^{-10}$ Torr to be reached.

### 5-Ion source of mass spectrometer:

There are several types of ionization methods in mass spectrometry.  Most common methods are:

A-Matrix-assisted laser desorption/ionization (MALDI).

B- Electrospray Ionization (ESI).

C- Electron ionization (EI).

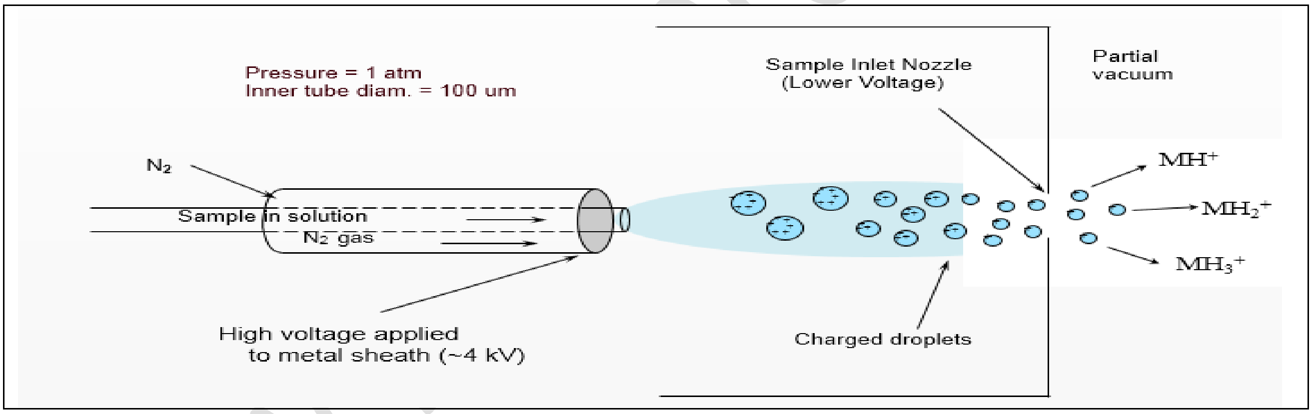


Figure 3: General diagram of electrospray ionization mechanism.

### A-Matrix-assisted laser desorption/ionization (MALDI)

- Method of ionization is a soft ionization method and results in minimum fragmentation of sample.
- Method is used for non-volatile, and thermally labile compounds such as proteins, oligonucleotides, synthetic polymers.
- Sample is mixed with 1000 times molar excess of sample and spotted onto a metal plate and dried.

- Matrix plays a key role in this technique by absorbing the laser light energy and causing a small part of the target substrate to vaporize.
- Although, the process of forming analyte ions is unclear, it is believed that matrix which has labile protons, such as carboxylic acids, protonates neutral analyte molecules after absorbing laser light energy.

**B- Electrospray Ionization (ESI):**

- Electrospray Ionization (ESI) is a preferred method of ionization when the sample is in liquid form.
- A soft method of ionization and results in less fragmentation.
- ESI is a very valuable method for analysis of biological samples.
- The analyte is introduced either from a syringe pump or as the eluent flow from liquid chromatography with a flow rate 1µl min$^{-1}$ .
- The analyte solution passes through the electrospray needle (Stainless steel capillary with 75-150 µm internal diameters) that has a high potential difference (with respect to the counter electrode) applied to it (typically in the range from 2.5 to 4 kV).
- This forces the spraying of charged droplets from the needle with a surface charge of the same polarity to the charge on the needle.
- As droplet moves towards counter electrode cone (which passes it to analyzer), solvent evaporation occurs and droplet shrinks until it reaches the point that the surface tension can no longer sustain the charge (the Rayleigh limit) and at that point droplets break.

**C-Electron ionization:**

- Method is work well for many gas phase molecules, but it results in extensive fragmentation and molecular ions are not observed for many compounds.
- Fragmentation mass spectra are sometime useful because it provides structural information of a molecule.

- A filament of rhenium or tungsten wire produces the electron beam by thermionic emission.
- When cathode filament of rhenium or tungsten is heated at temperature over 1000 K, electrons are emitted.
- The generated electrons are accelerated to 70 eV which results in electron beam.
- The volatile sample or sample in gaseous phase containing neutral molecules is introduced to the ion source in a perpendicular direction to the electron beam.
- Electron impact on the analyte results in either loss of electron (to produce cation) or gain of electron (to produce anion).
- Chemical bonds in organic molecules are formed by pairing of electrons.
- Electron impact may knock out one of the electron.
- This leaves the bond with a single unpaired electron.
- Radical as well as being cation written as $M^{+\cdot}$, where (+) indicates ionic state while (. ) indicates radical.
- Electron impact may result in electron capture (extra unpaired electron).
- This generates a radical as well as being anion written as $M^{-\cdot}$ , where (-) indicates ionic state while (·) indicates radical.

**6-Chemical Ionization:**

They found several steps for analyst to be chemical ionization, as follow:

- A reagent gas like $CH_4$ is injected in the ion chamber.
- Due to electron impact, the reagent gas in the chemical ionization source is ionized.
- Injection of analyst molecule is followed up.
- Analyst molecules undergo many collisions with the reagent gas.
- The reagent gas ions in this cloud react and produce adduct ions, which are excellent proton donors for analyst.

1-Electron impact on reagent gas $CH_4 +_{fast} e^- \text{-------------}\rightarrow CH\cdot_4^+ + 2e^-$

2- reaction of reagent gas to form 1 on$CH_4^+ + CH_4 \text{---------}\rightarrow CH_3^{\cdot} + CH^+_5$

3- reaction of reagent gas 1 on with analyst. $CH^+_5{=}M\text{--}\rightarrow CH_4+CH_4+MH^+$

**Applications:-**

Q1//Calculate the units of Thomson for the following molecular ions:-

$CH_3OH^{\cdot+}$ , $C_2H_5SO_3CH_4^{\cdot+}$ , $C_5H_{11}NO_2{}^{\cdot+}$ if you know the atomic mass weights.

(C=12.011, H=1.00794 , N=14.00674 , S=32.066 ,O=15.9994 ).

**Solution:**

1- Find the molecular Wight for radical ions as follow:-

$CH_3OH^{\cdot+}$ =12.011+(3*1.00794+1.00794)+15.9994 =32.04216 g/mole

2- Find the molecular Wight for radical ions in atomic mass units as follow:-

AMU= M. Wt * AMU **($1.660540\times10^{-27}$mol. kg)**

32.04216 g/mol× $1.6605\times10^{-30}$mol= $53.2060067\times10^{-30}$=$53.2060067\times10^{-27}$ Kg

3- Find the mass-to-charge ratio has **AMU/e** , since 1 e=$1.602177\times10^{-19}$ C as follow:-

$53.2060067\times10^{-27}$ Kg /$1.602177\times10^{-19}$ C= M/Z=$33.2085698\times10^{-8}$ Kg/C.

4- Find the units of Thomson, with symbol Th, . The fundamental definition for this unit is1Th = 1AMU/e = 1.036 426 × $10^{-8}$ kg $C^{-1}$ as follow:-

$33.2085698\times10^{-8}$ Kg/C /1.036 426 × $10^{-8}$ kg $C^{-1}$ =32.0414287Th . This process are done for all radical

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Q2/ The following signals of mass chart spectroscopy in Th units, find the respected molecular weighs for each fragments in g/mol:- 1-32.03Th 2- 48.56 Th 3- 108.45Th

**Solution:**

1-Find the ratio of AMU/C by multiplying each signal by 1Th as :

108.45Th× 1.036 426 × $10^{-8}$ AMU $C^{-1}$ .$Th^{-1}$ =112.4004*$10^{-8}$ AMU/ C.

2-Find the atomic mass unit of signal by multiplying each signal by charge of electron1 e=$1.602177\times10^{-19}$ C as follow:-

$112.4004\times10^{-8}$ AMU/ C ×$1.602177\times10^{-19}$ C =$180.085335\times10^{-27}$AMU.

3- Find the molecular Wight for radical ions in Kg/mol as follow:

$180.085335\times10^{-27}$AMU/**1AMU($1.660540\times10^{-27}$mol. Kg)=108.449863g/mol** This process are done for all signals.