



# Pharmaceutical Instrumental Analysis

الأستاذ الدكتور جمعة الزهوري ( دكتوراه صيدلة-ألمانيا 1991 )

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# Molecular Spectroscopy

- *UV/Vis Molecular Absorption Spectrometry*
- *Molecular Mass Spectrometry.*
- *Molecular Infrared Spectrometry.*
- *Nuclear Magnetic Resonance Spectroscopy.*



# Molecular Mass Spectroscopy

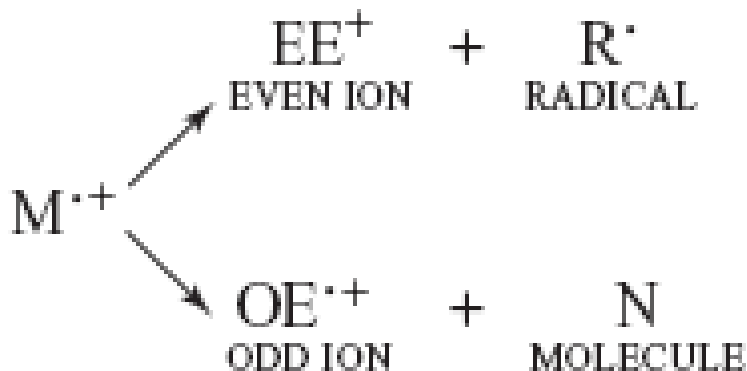
(spectrometry)

## Principles

The first step in the mass spectrometric analysis of compounds is the production of gasphase ions of the compound, for example by electron ionization:



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 and an ion with an even number of electrons, or a molecule and a new radical cation. We stress the important difference between these two types of ions and the need to write them correctly:

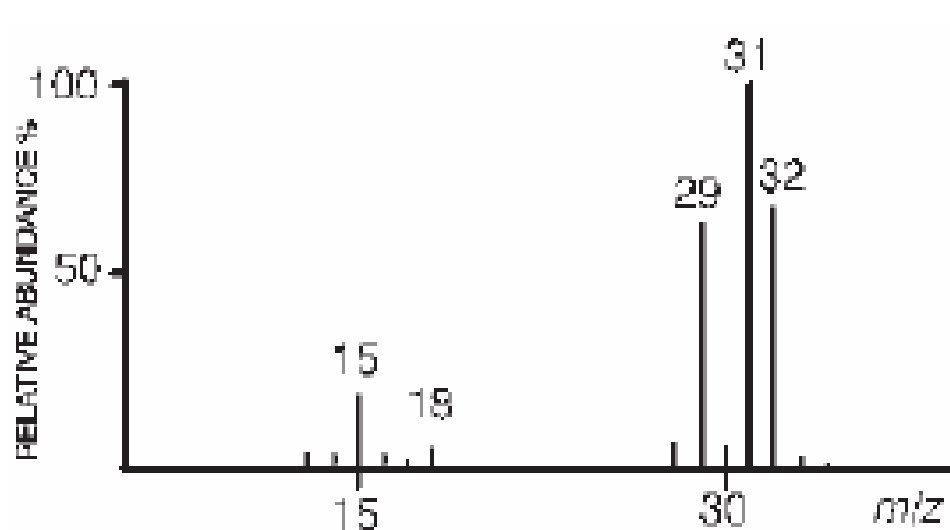


زوجي = even

فردی = Odd

4 Prof

These two types of ions have different chemical properties. Each primary product ion derived from the molecular ion can, in turn, undergo fragmentation, and so on. All these ions are separated in the mass spectrometer according to their mass-to-charge ratio, and are detected in proportion to their abundance



$m/z$	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

Mass spectrum of methanol by electron ionization, presented as a graph and as a table.



## ***Molecular Mass Spectrometry.***

The definition of MS according to Ph. Eur ( text 2.2.43) is :

***Mass Spectrometry is based on the direct measurement of the ratio of the mass to the number of Positive elementary charges of ions (  $m/z$  ) in the gas phase obtained from the substance being analyzed.***

***(  $m/z = m/e$  )***



# Development of Mass Spectrometry

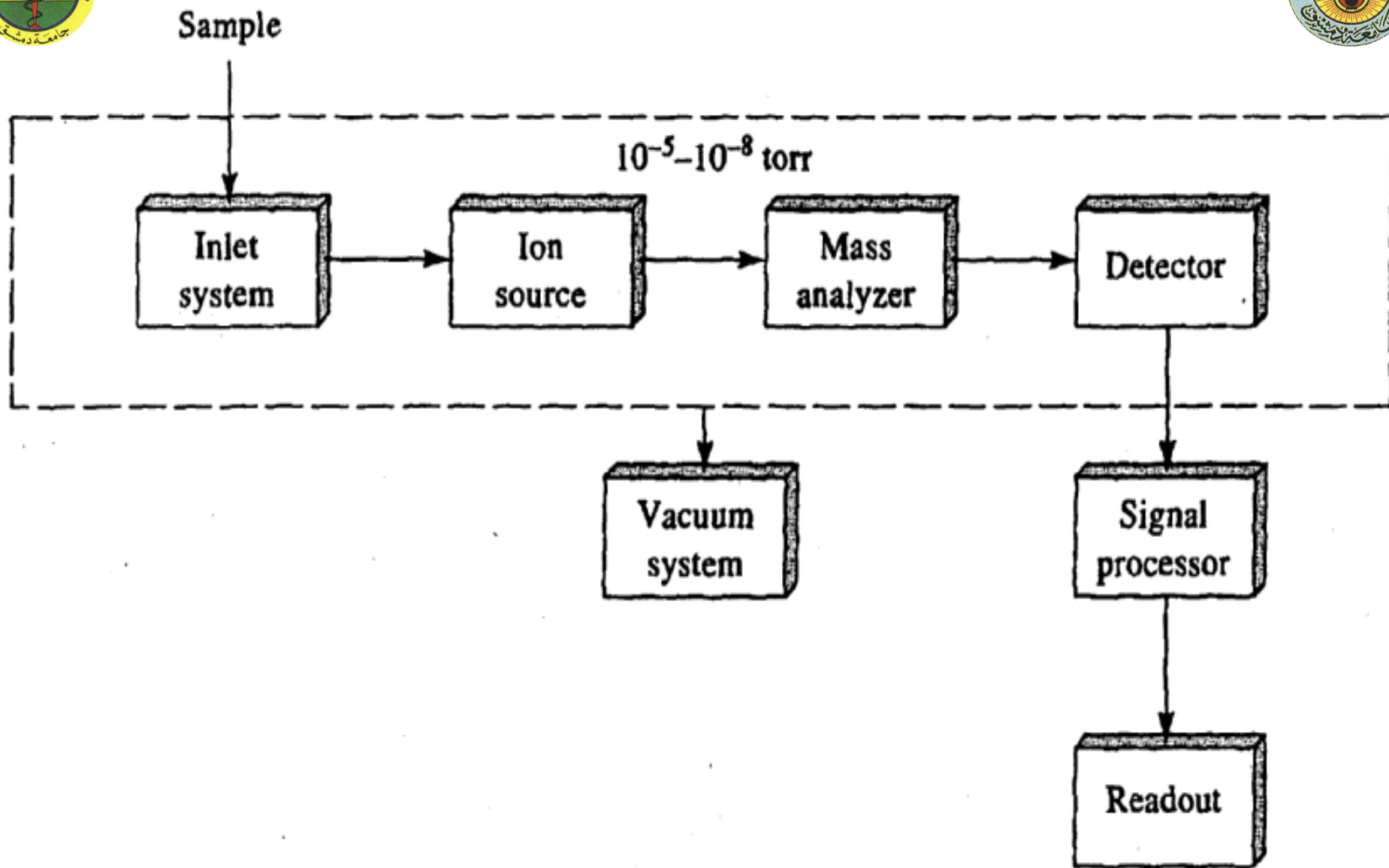
- 1886 Goldstein ( obtain cathodes radiation under Vacuum low pressure )
- 1898 Wien ( Deviations of radiation contain positive ions in electromagnetic field ).
- 1912 Thompson ( proved 2 isotopic for Ne)
- 1918 Aston and Dempster ( isotopic separation).
- 1940 (First application in petroleum industry for the quantitative analysis of hydrocarbon mixtures)
- 1950 Commercial instrument produced.



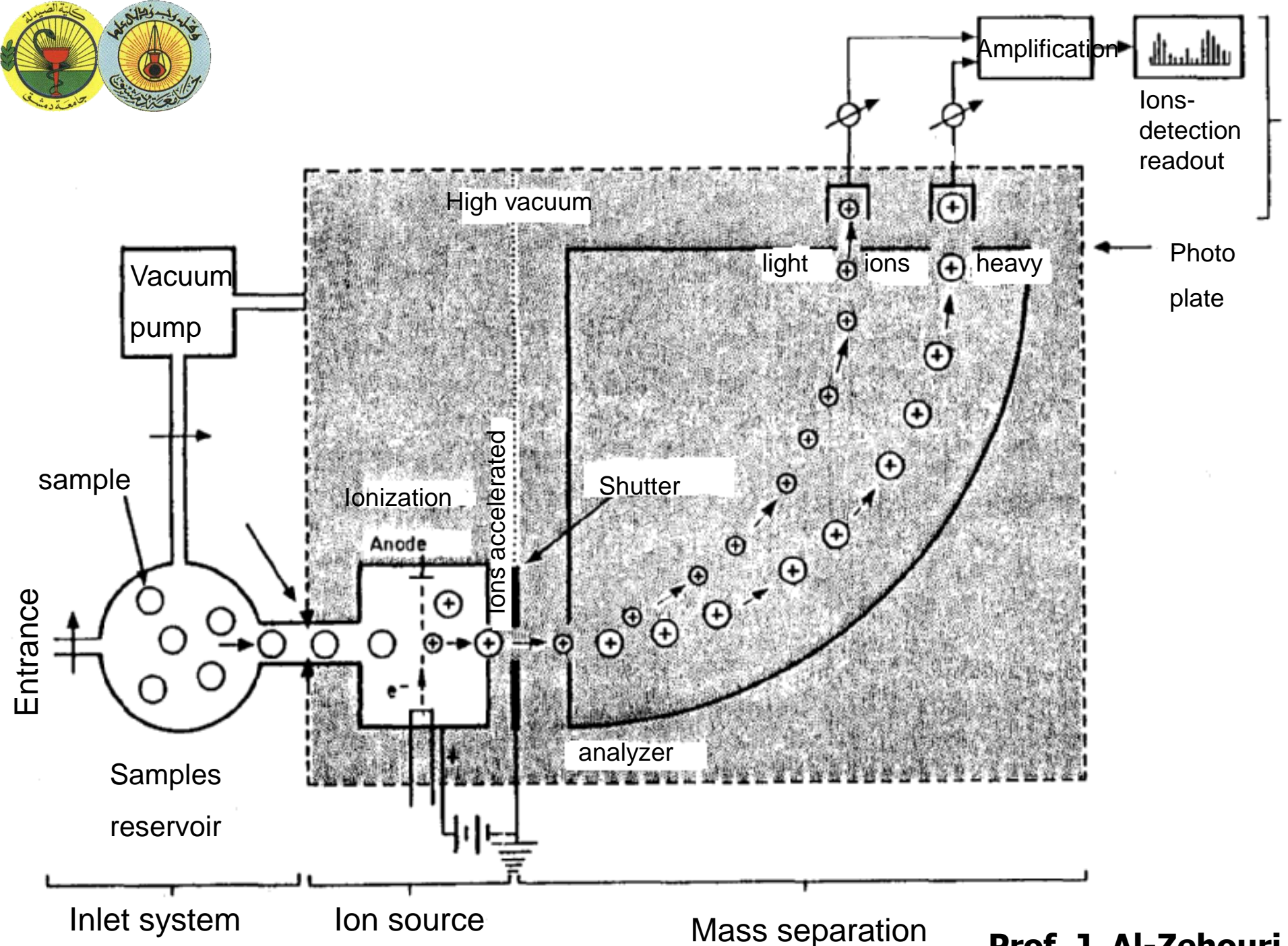


## *Molecular Mass Spectrometry.*

- Mass spectrometry is capable of providing information about:
  - 1-  $M_w$
  - 2- the element composition of samples of mater.
  - 3- the structures of inorganic, organic and biological molecules.
  - 4- the qualitative and quantitative composition of complex mixtures.
  - 5- Isotopic ratio of atoms in samples.

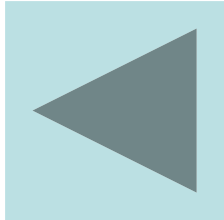
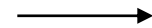
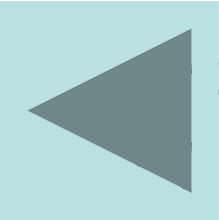


Components of a mass spectrometer.



# ***MASS SPECTROSCOPY***

prof. Dr. Joumaâ AL-Zehouri





مدخل / مقبض

# Sample Inlet System

- The purpose of the inlet system is permit introduction of a representative sample into the ion source with minimal loss of vacuum.
- Most modern mass spectrometers are equipped with several types of inlets to accommodate various kinds of sample ,these include:
  - 1- Batch Inlet System ( Gas+ some liquid)
  - 2- The Direct Probe Inlet ( Solid+ Liquid)
  - 3- Chromatography and Capillary Electrophoretic Inlet System.



الشامل

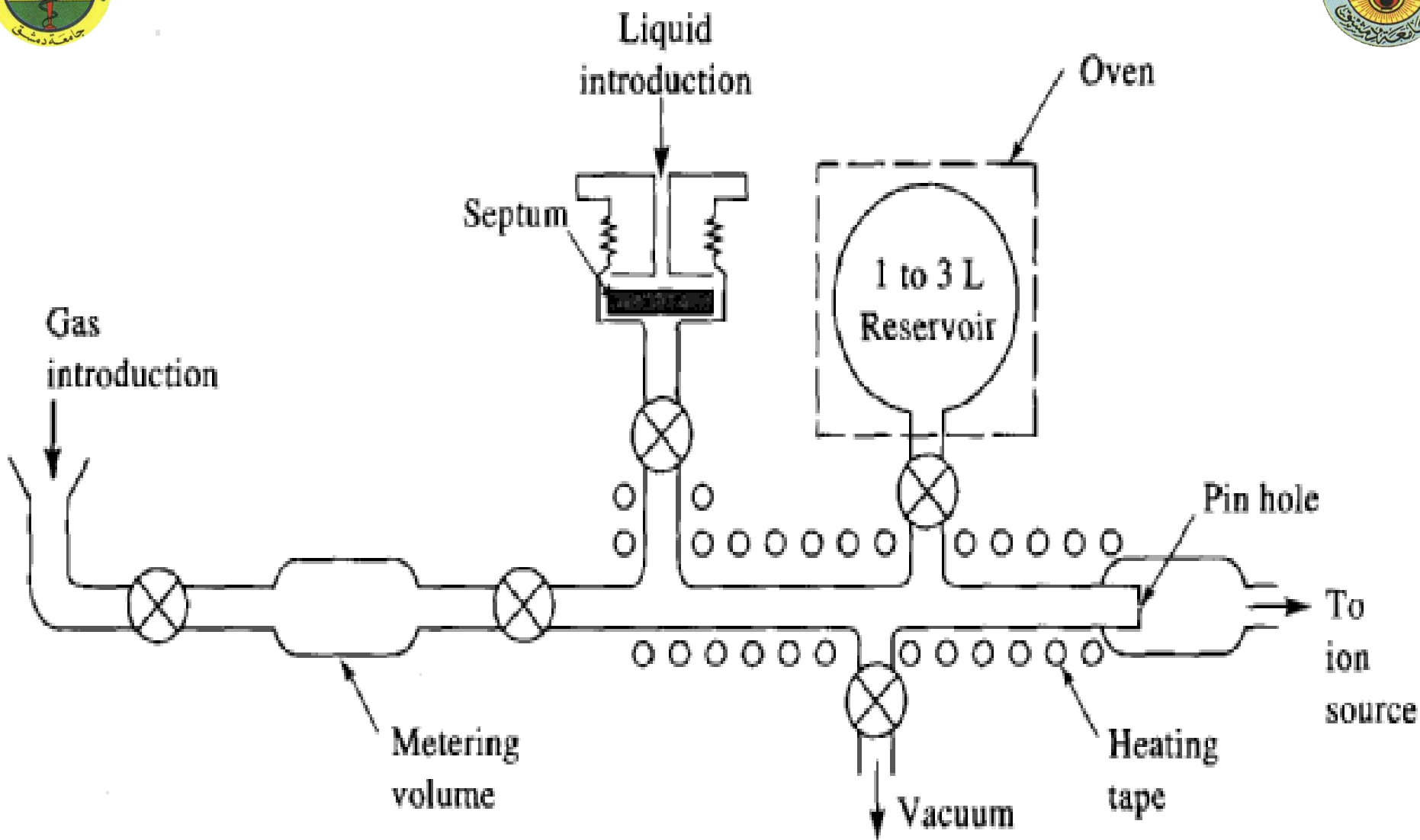
# Batch Inlet System



- The classical and simplest method.
- The sample is volatilized externally and then allowed to leak into the evacuated ionization region.
- Gaseous sample, a small measured volume is trapped between the two valves enclosing the metering area and is then expended into the reservoir flask.
- Liquids introduced with microliter syringe, but the vacuum system is used to achieve a sample pressure of  $10^{-5}$  torr.
- The entrance temperature increases up to  $500\text{ C}^\circ$ .
- The sample, which is now in gas phase, is leaked in to ionization area via a metal (gold) or glass diaphragm containing one or more pinholes.

الثقوب

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15D PRO

Schematic of an external sample introduction system.

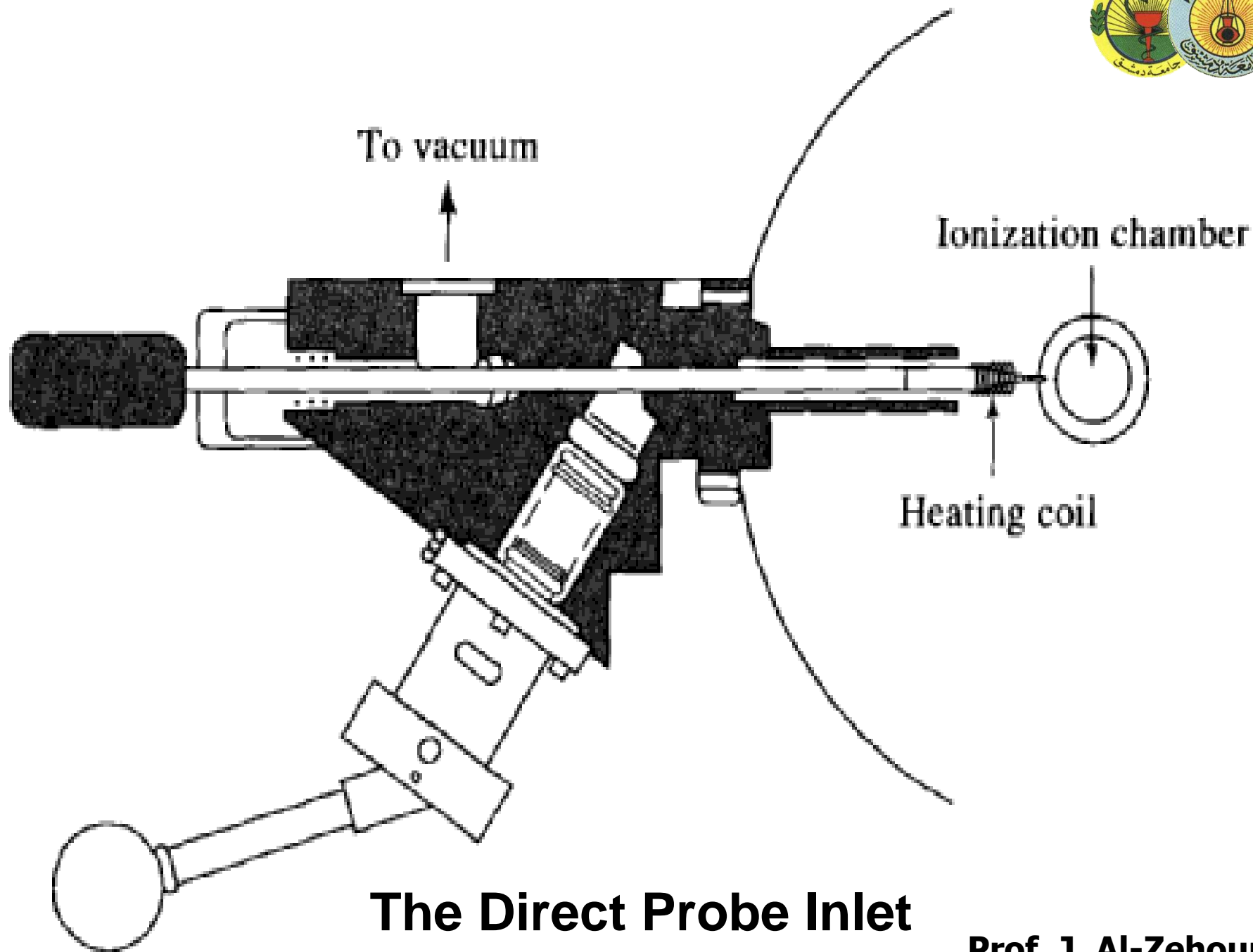
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# The Direct Probe Inlet

- Solid and nonvolatile liquids( such carbohydrates ,steroids, low MW polymeric) can be introduced in to the ionization region by means of sample holder, or probe ,which is inserted through a vacuum lock. مخلية مقفلة
- The lock system is designed to limit the volume of air that must be pumped from the system after insertion of the probe into the ionization region. إدخال
- The low pressure also leads to elevated concentration of relatively nonvolatile compounds in the ionization area. رفع





**The Direct Probe Inlet**



# Chromatography and Capillary Electrophoretic Inlet System

- Mass spectrometers are often coupled with gas or high – performance liquid chromatographic systems or capillary electrophoresis columns to permit the separation and determination of the components of complex mixtures.

ربط

رحلان

- Linking a chromatographic or electrophoretic column to a mass spectrometer requires the use of specialized inlet systems.



# The vacuum system

In order for the MS process to work, it must be conducted under vacuum conditions -  $10^{-5}$  -  $10^{-8}$  torr.

Major reason - increase the **mean-free path**.

“The average distance that our ions and molecules will travel before colliding with another ion or molecule.”

A high mean-free path insures predictable and reproducible fragmentation, high sensitivity and reliable mass analysis.



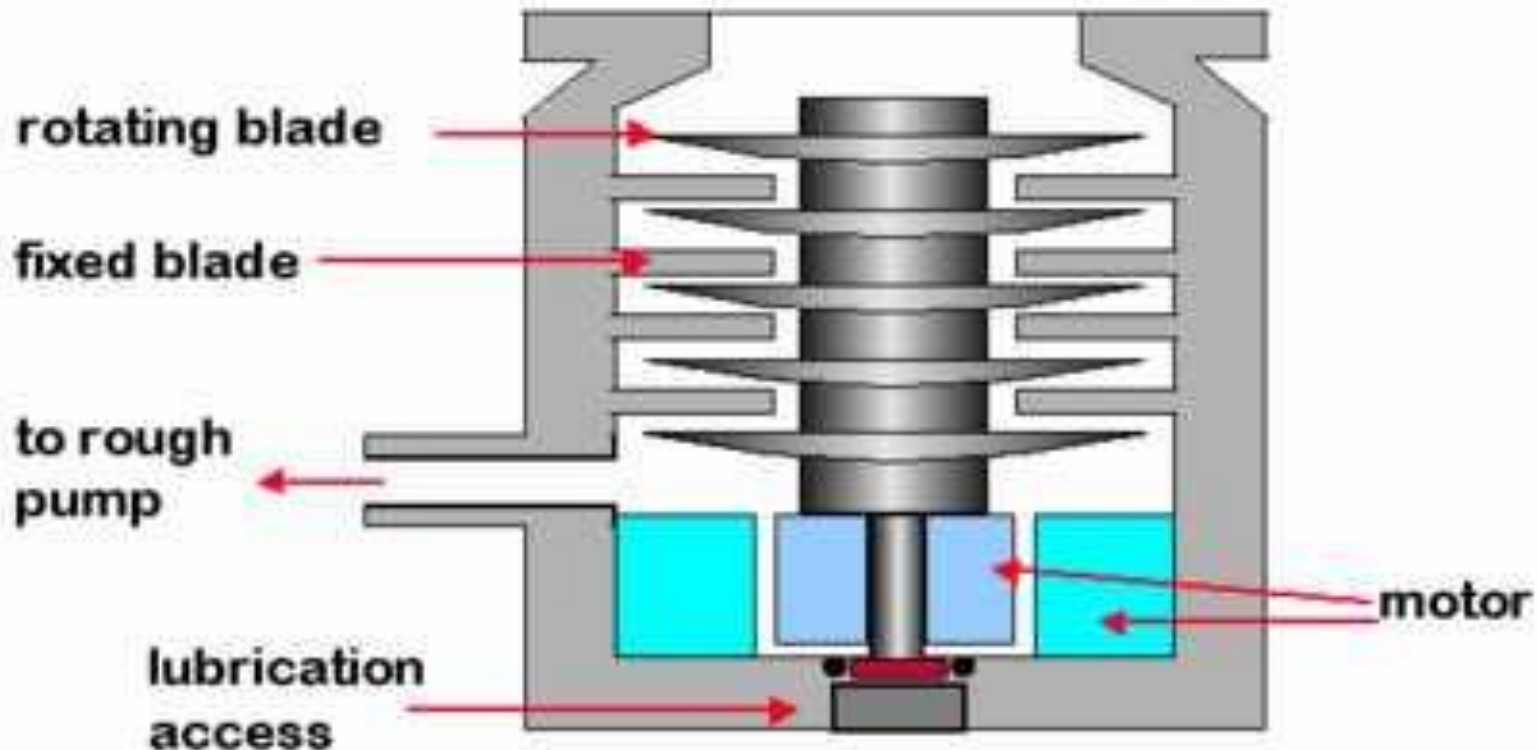
# The vacuum system

A vacuum is produced using a combination of two pumps - **two stage vacuum system**.

- A rotary pump serves as our rough or fore pump. It can produce a vacuum of  $10^{-2}$  -  $10^{-4}$  torr.
- Either a turbomolecular or diffusion pump is used to achieve vacuums in the  $10^{-5}$  torr range.
- These pumps actually act like compressors.

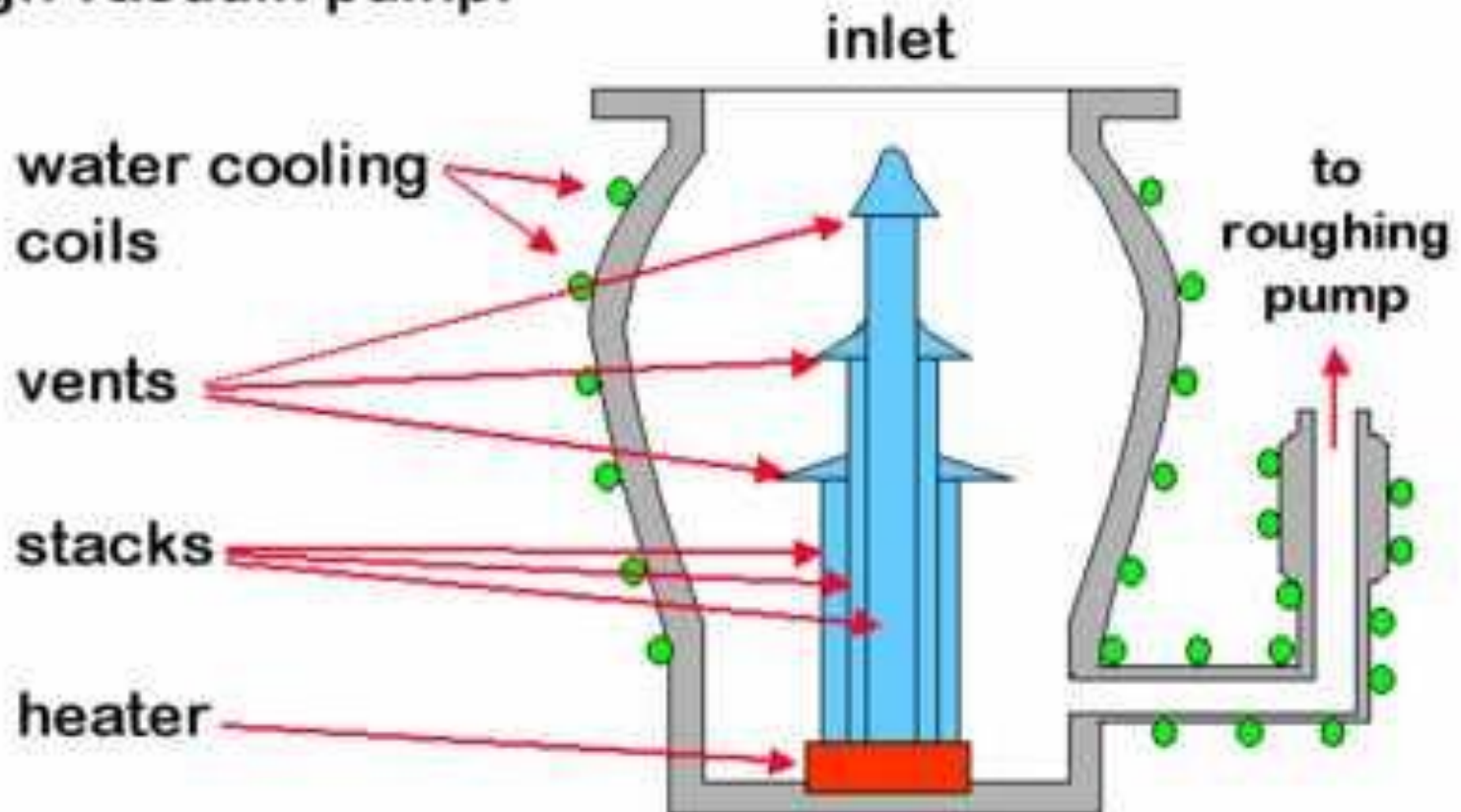
# Turbomolecular pump

A turbomolecular pump relies on a series of blades or airfoils that spin at 30,000 - 90,000 RPM. This tends to deflect gas molecules down and out the outlet.



# Oil diffusion pump

A diffusion pump is another commonly used type of high vacuum pump.





# Ion sources

- ***The starting point for a mass spectrometric analysis is the formation of gaseous analyte ions, and the scope and the utility of a mass spectrometric method is dictated by the ionization process.***
- ***The appearance of mass spectra for a given molecular species is highly dependent upon the method used for ion formation.***



# Molecular Mass Spectra

e.g. Ethyl benzene :

- Ethyl benzene vapor was bombarded with a stream of electrons that led to the loss of an electron by the analyte and formation of the molecular ion  $M^+$  :



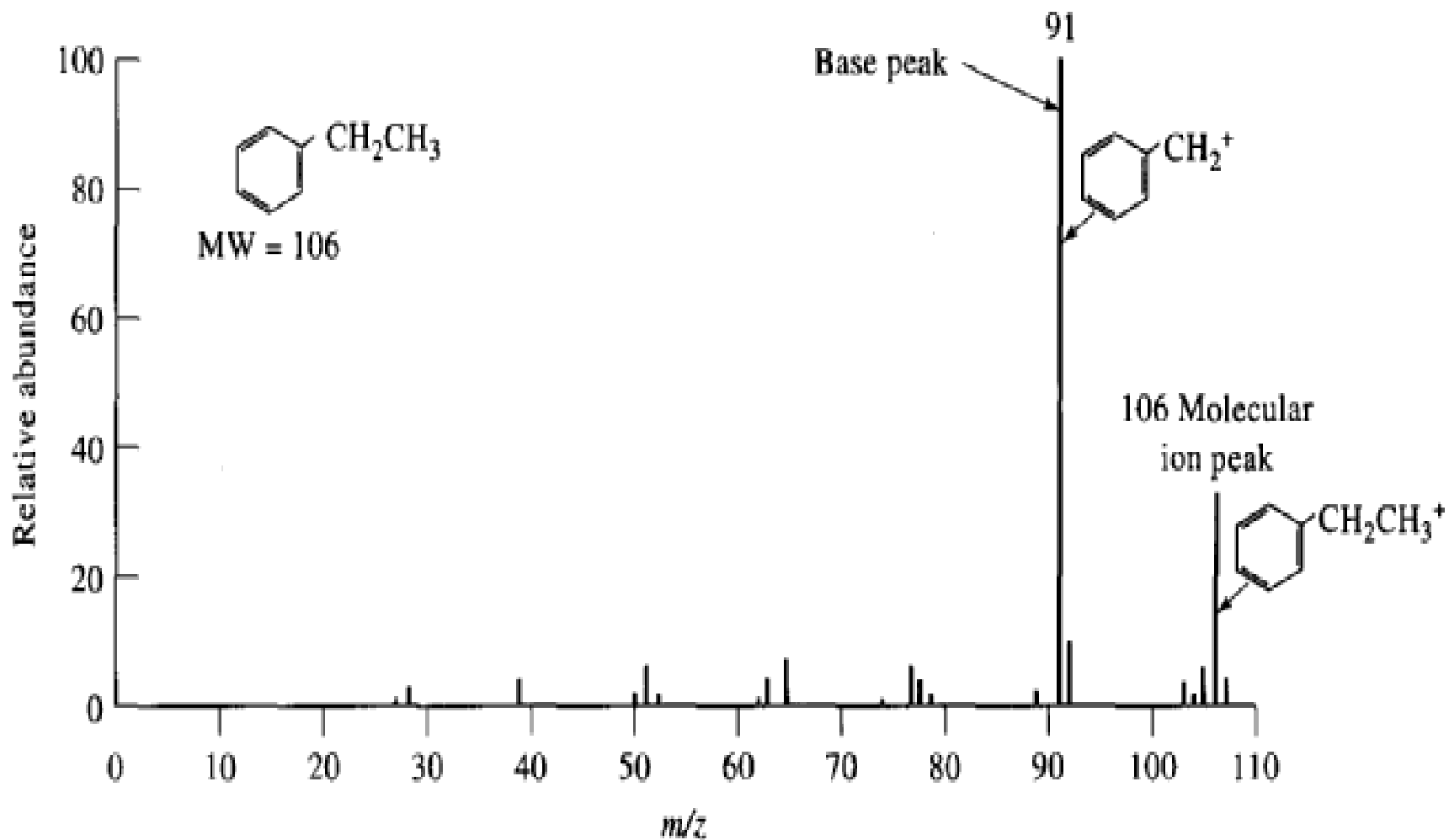
- The charged species  $C_6H_5CH_2CH_3^+$  is the molecular ion.
- The molecular ion is a radical ion that has the same MW of the molecule.





# Molecular Mass Spectra

- The collision between energetic electrons and analyte molecules are usually imparts<sup>تنفل</sup> enough energy to the molecules to leave them in an excited stat. Relaxation then often occurs by fragmentation of part of the molecular ions to produce ions of lower masses.***



Mass spectrum of ethyl benzene.





- ***The ionization methods fall into two major categories:***

***the sample is first vaporized and then ionized.***

***a- Electron impact (EI)***

***b- Chemical ionization (CI)***

***c- Field ionization (FI)***



***the sample in solid or liquid state is converted directly in to gaseous ions.***

***a- Field desorption (FD)***

***b- Electrospray ionization (ESI)***

***c- Matrix-assisted Laser desorption/ionization (MALDI)***

***d- Plasma desorption (PD)***

***e- Fast atom bombardment (FAB)***

***f- Secondary ion mass spectrometry (SIMS)***

***h- Thermospray ionization (TI)***



## Ion Sources for Molecular Mass Spectrometry

Basic Type	Name and Acronym	Ionizing Agent
Gas phase	Electron impact (EI)	Energetic electrons
	Chemical ionization (CI)	Reagent gaseous ions
	Field ionization (FI)	High-potential electrode
Desorption	Field desorption (FD)	High-potential electrode
	Electrospray ionization (ESI)	High electrical field
	Matrix-assisted desorption/ionization (MALDI)	Laser beam
	Plasma desorption (PD)	Fission fragments from $^{252}\text{Cf}$
	Fast atom bombardment (FAB)	Energetic atomic beam
	Secondary ion mass spectrometry (SIMS)	Energetic beam of ions
	Thermospray ionization (TS)	High temperature

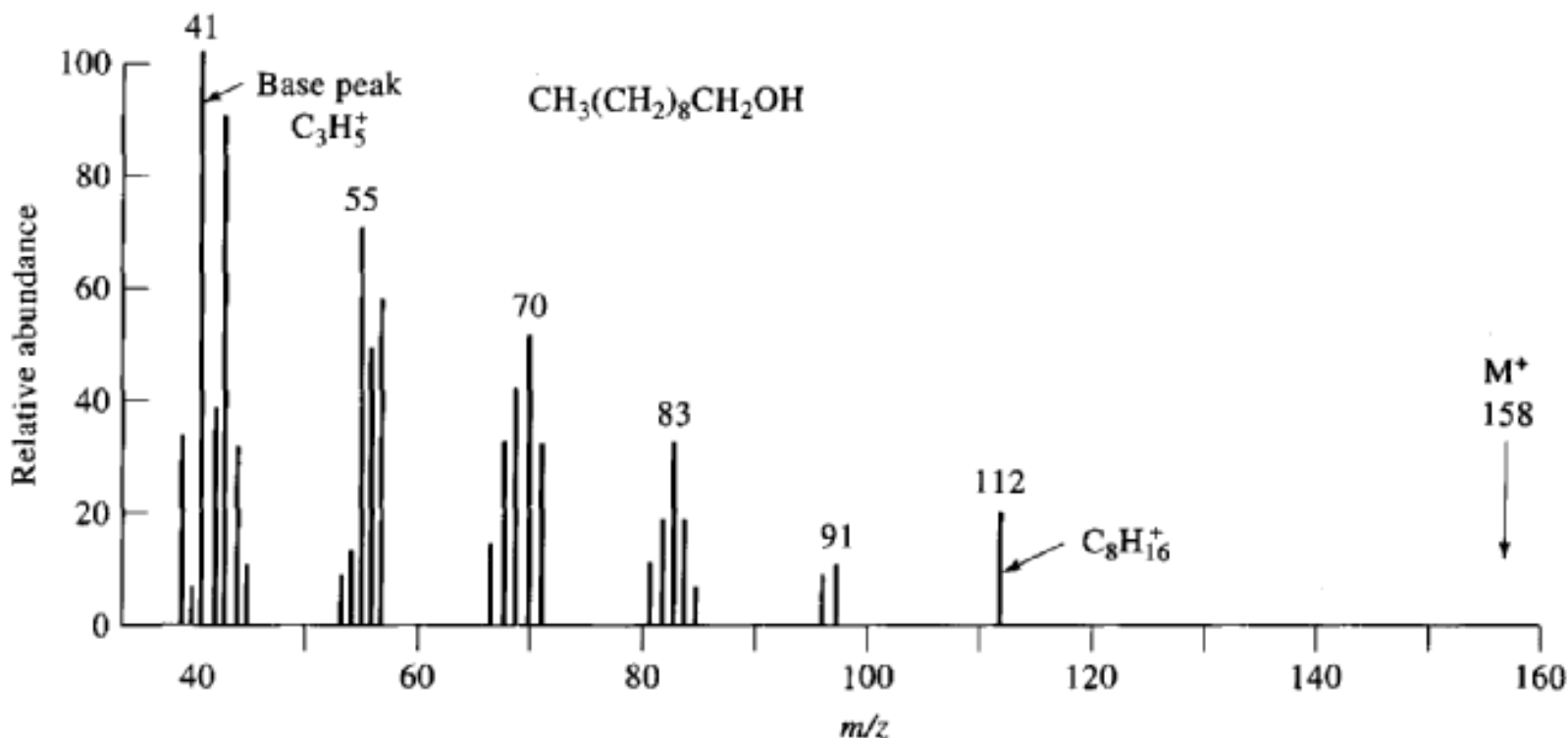


- ***An advantage of desorption sources is that they are applicable to nonvolatile and thermally unstable samples.***
- ***Gas-phases are restricted to thermally stable compounds that have boiling points less than about 500C°, and molecular weights less than roughly 10<sup>3</sup>.***
- ***Desorption source for MW 10<sup>5</sup>.***
- ***Ion sources are also classified as being hard sources and soft sources.***



Zehouri

- Hard source → several fragmentation peaks.

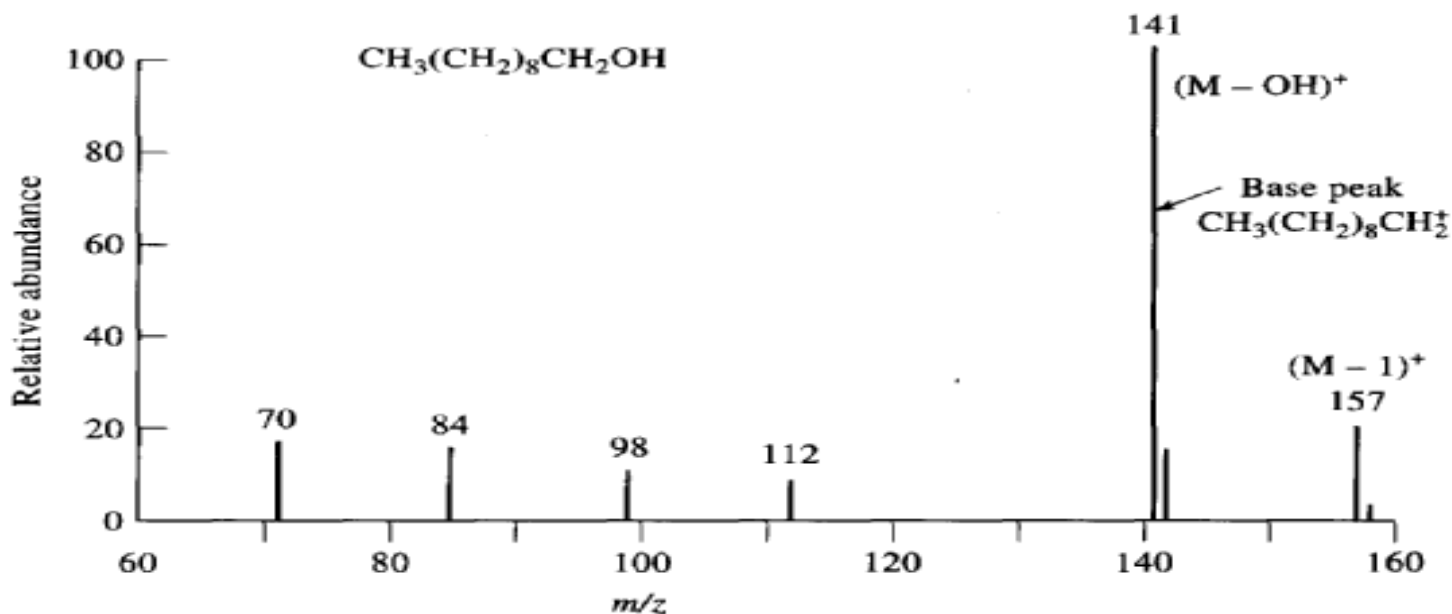


MS of 1- decanol in hard source

37D  
Pro



Soft source  $\longrightarrow$  Little fragmentation peaks.



MS of 1- decanol in soft source

**Note that the both spectrum (soft +hard) consists of molecular ion peak.**



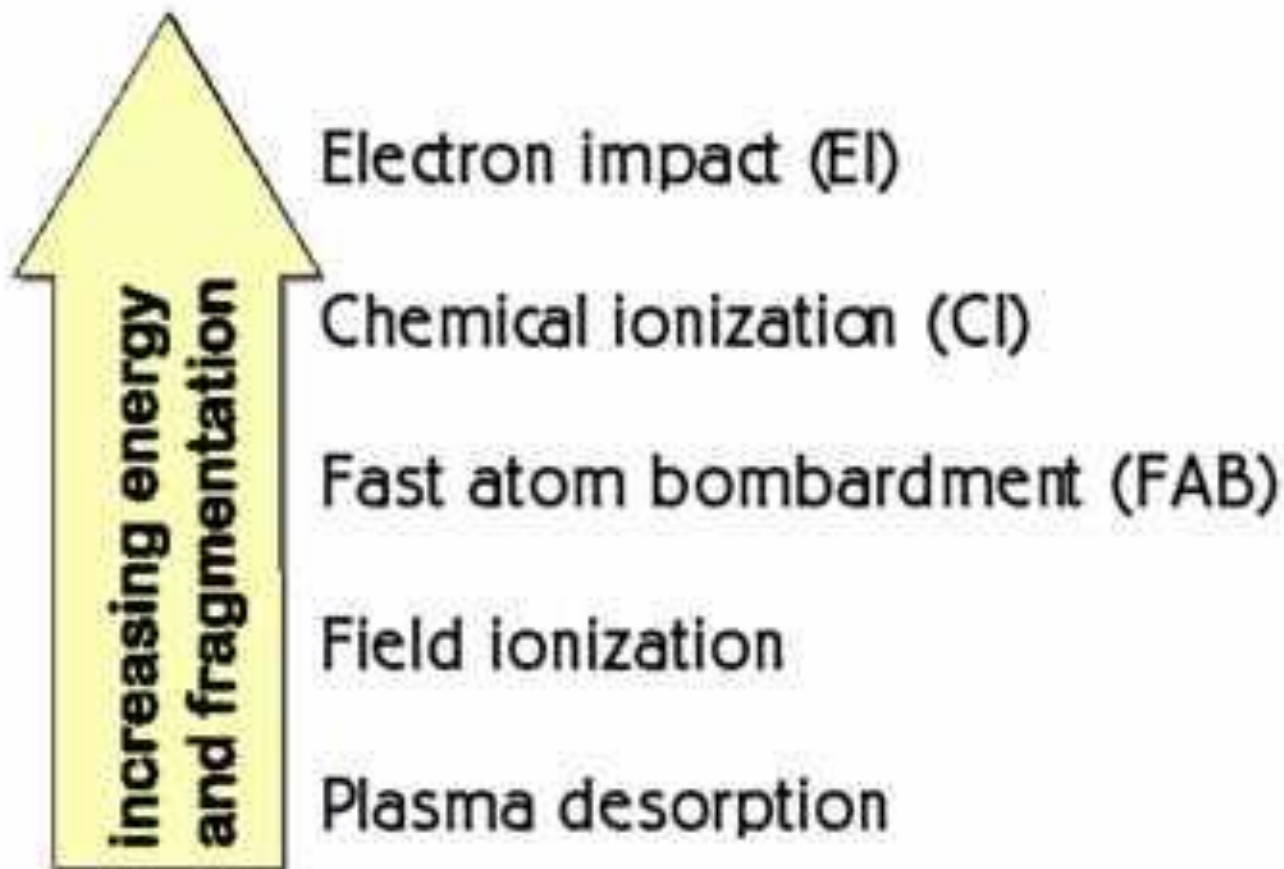


- ***Both hard and soft source spectra are useful for analysis.***
- ***The many peaks in a hard source spectrum provide useful information about the kinds of functional groups and thus structural information about analyte.***
- ***Soft source spectra are useful because they supply accurate information about molecular weight of the molecule.***



# Ionization

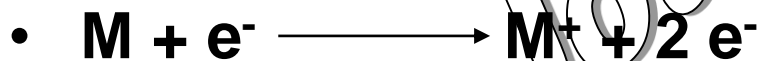
A number of ionization techniques exist.





# 1-The Electron-Impact Source

- The sample is brought to a temperature high enough to produce a molecular vapor, which is then ionized by bombarding the resulting molecules with a beam of energetic electrons.
- Electrons are emitted from a heated tungsten or rhenium filament and accelerated by a potential of approximately 70 V that is impressed between the filament and the anode.



- The kinetic energy (KE) given by the equation :

$$\text{KE} = \frac{1}{2} m v^2$$

$v =$  velocity ,  $M =$  mass



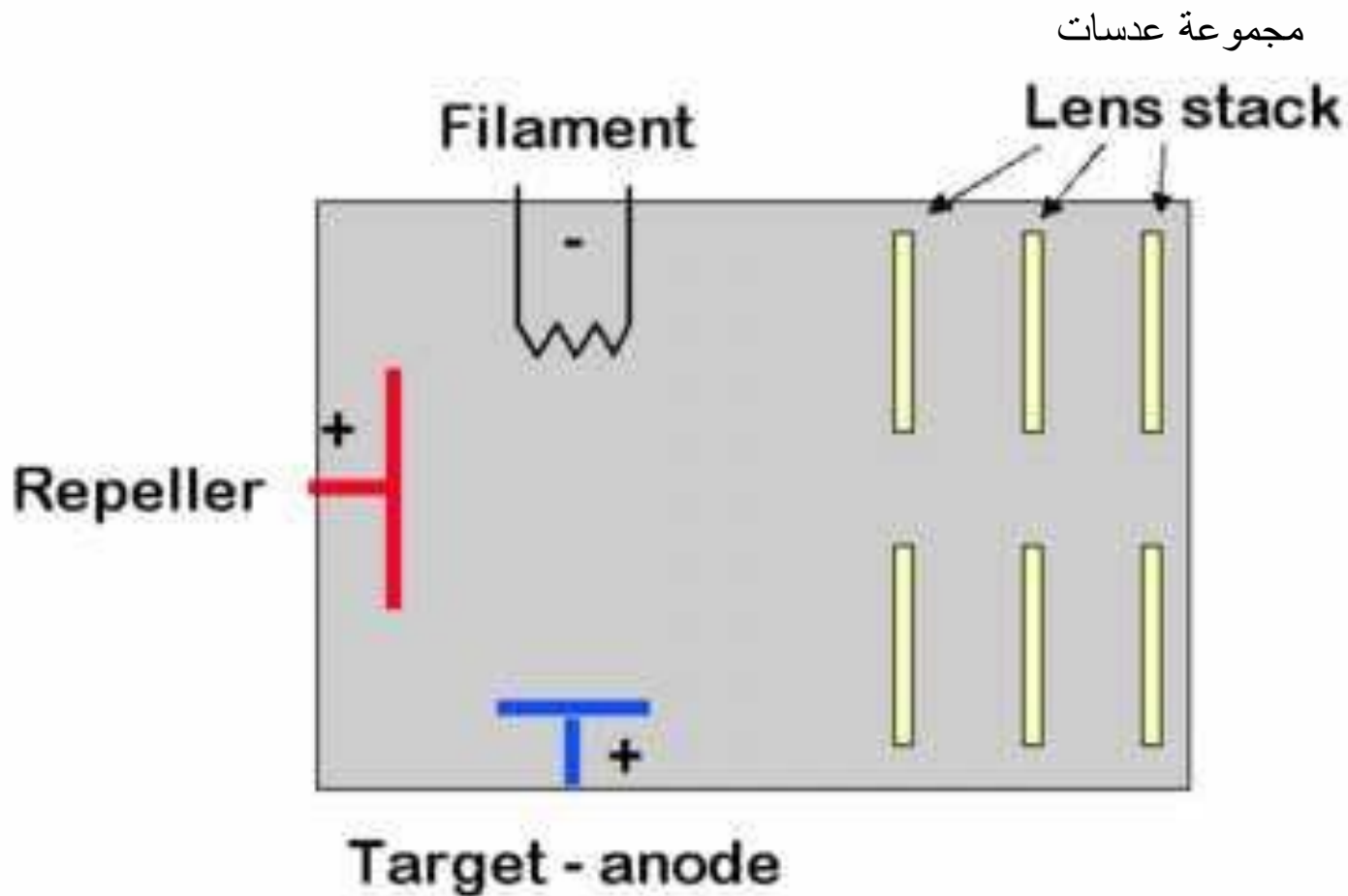
# The Electron-Impact Source

$$KE = (1/2)mv^2 \text{ or } v = (2 KE/m)^{1/2}$$

Thus, if all ions acquire the same amount of kinetic energy, those ions with largest mass must have the smallest velocity.



# Electron impact source





# Electron impact source

السلك الكهربائي

**Filament** - Typically made of Re. Our source of 70 eV electrons.

المصعد

**Target** - anode used in association with the filament to produce electrons.

**Repeller** - positively charged electrode used to 'push' positive ions out of the ionization source.

**Lens stack** - series of increasingly more negative electrodes used to accelerate our ions to constant kinetic energy.

المسرعات : اقطاب سالبة الشحنة تزداد شحنتها كلما اقتربنا من المحل ، مهمتها المساعدة على تسريع الأيونات الموجبة وإيصالها لطاقة حركية ثابتة .

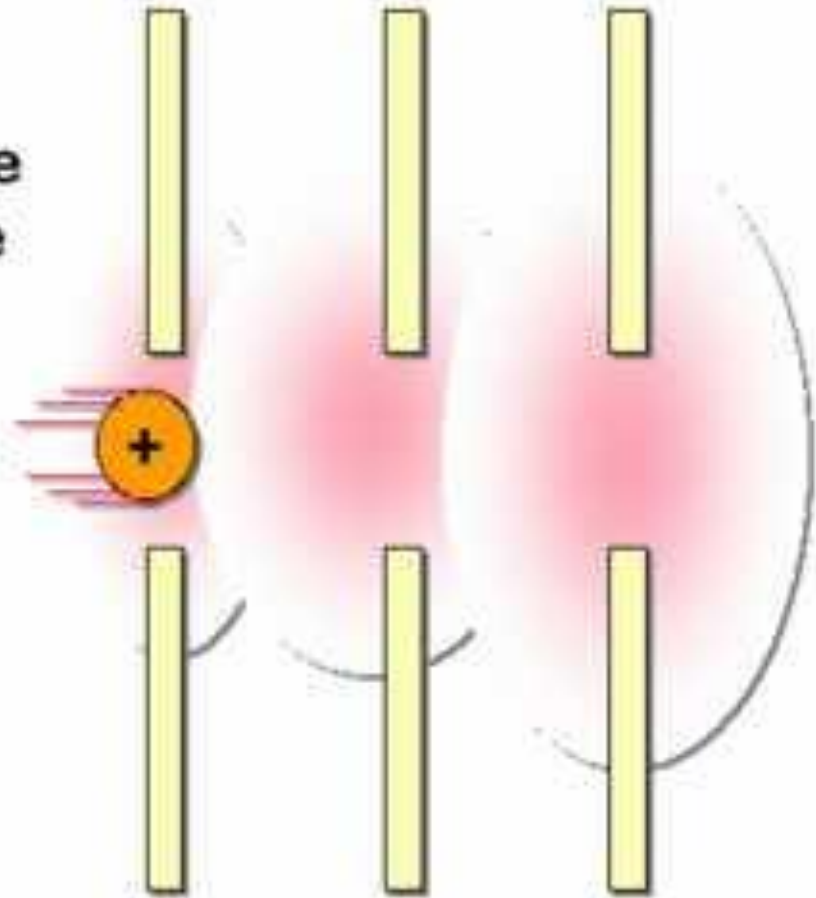


# Electron impact source

As an ion accelerates towards the first lens, it comes under the influence of the next, more negative lens. It passes the first lens and accelerates towards the next.

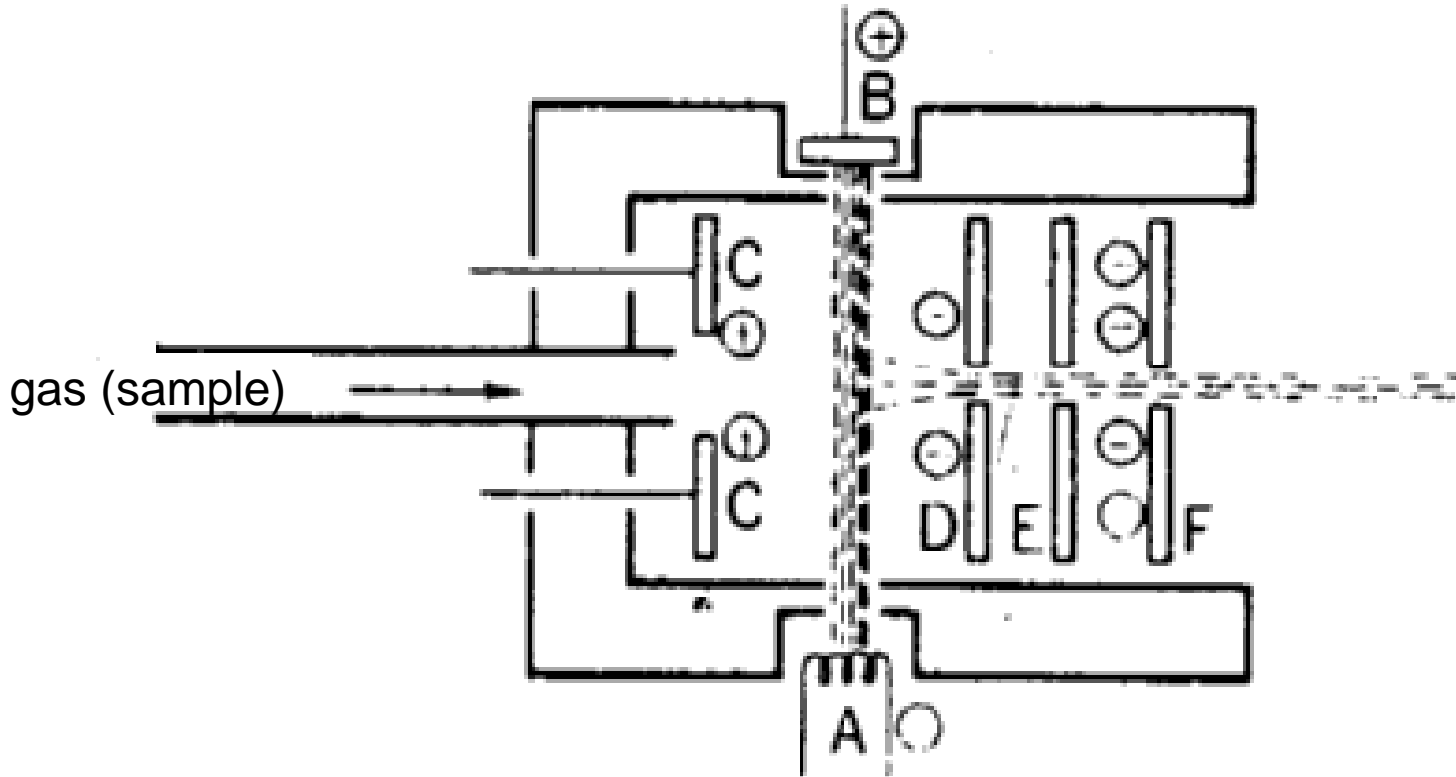
By the final lens, it is traveling so fast that it simply passes directly into the analyzer.

- constant KE



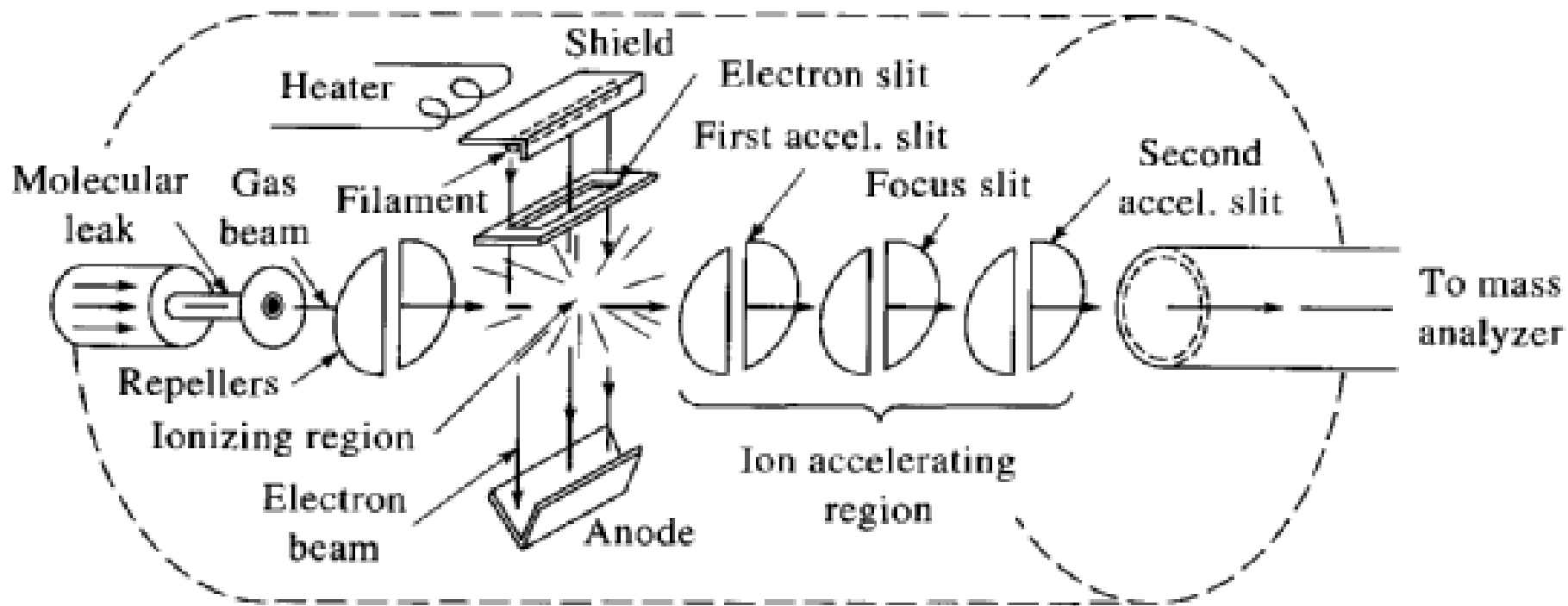


hour



40  
PROU





An electron-impact ion source.



# Fragmentation

- The low mass and high kinetic energy of the resulting electrons cause little increase in the translational energy of impacted molecules.
- Instead, the molecules are left in highly excited vibrational and rotational states. Subsequent relaxation then usually takes place by extensive fragmentation, giving a large number of positive ions of various masses that are less than that of the molecular ion.



+•

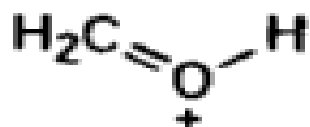
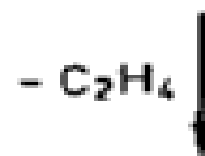
( $m/z = 102$ )



$m/z = 87$



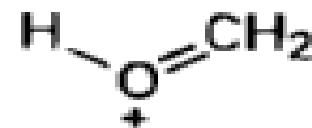
$m/z = 59$



$m/z = 31$



$m/z = 45$



$m/z = 31$



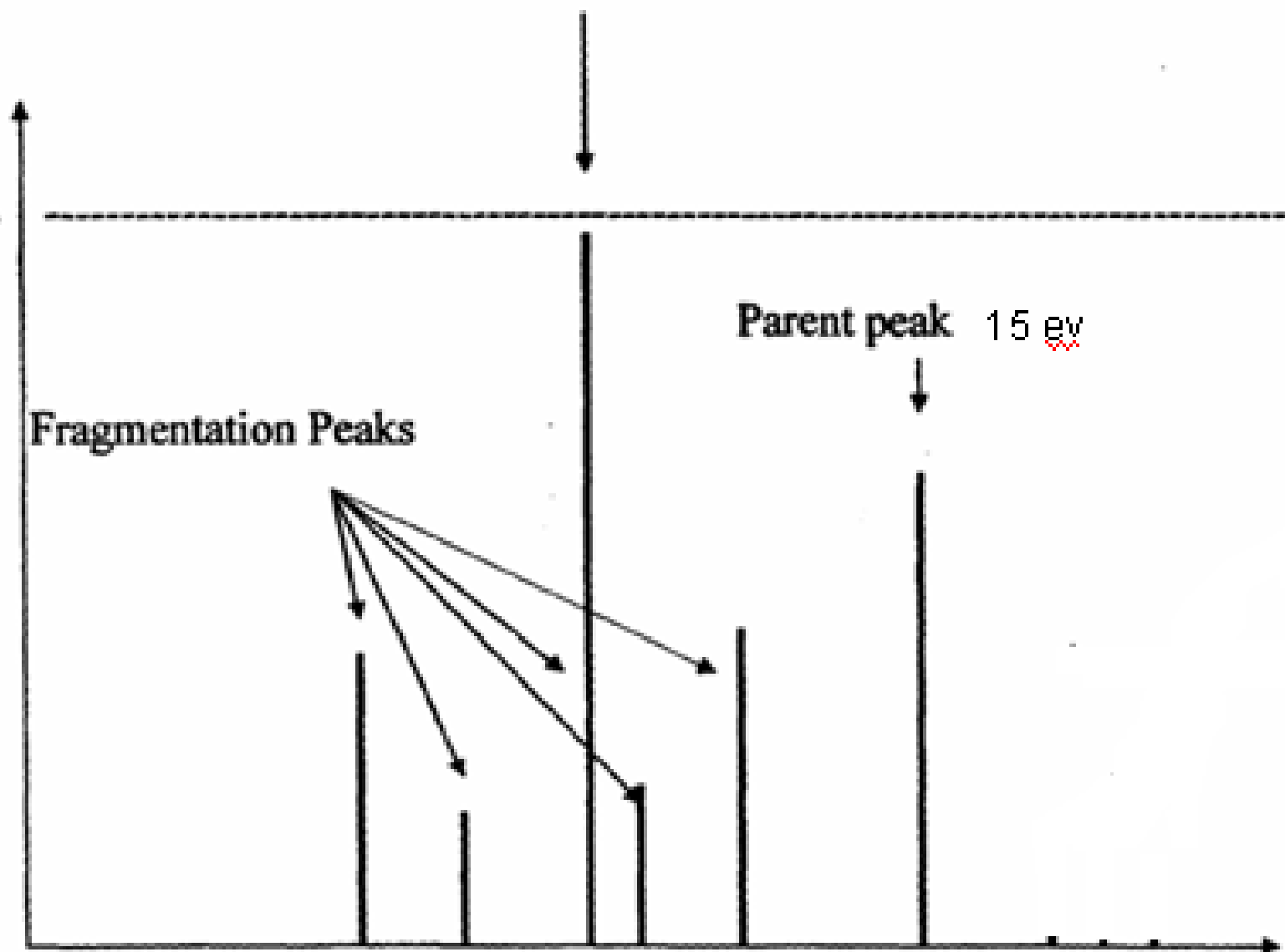
Base peak 70 eV

Parent peak 15 eV

100%

Fragmentation Peaks

M/e





# The Mass Spectrum

- 1- Parent Peak = molecular ion Peak( 15 ev)
- 2-Base Peak ( The highest peak = 100%)(70ev)
- 3- Fragmentation Peaks ( Peaks of Positive ions )
- 4- The isotope distribution Peaks.



# Advantages and Disadvantages of Electron-Impact Sources

- **Advantages :**
  - high ion current ,thus giving good sensitivities.
  - Fragmentation unambiguous identification of analyte possible.
- **Disadvantages :**
  - disappearance of molecular ion peak (sometime) الكلوروفورم  $CCl_4$
  - the need to volatilize the sample (degradation before ionization can occur )
  - limited to analyte having MW smaller than  $10^3$  Daltons.

غير غامض

# الأيون الجزيئي والقمة (الذروة) الأم

- تزداد استقرارية الأيون الجزيئي إذا احتوى على منظومة الكترون  $\pi$  والتي يمكن أن تتحكم بسهولة أكبر بفقدان الأليكترون مقارنة بـ  $\sigma$ .
- يتناقص الارتفاع للذوة الأم حسب مايلي :
  - < الحلقات العطرية < الهيدروكربونات غير المتفرعة < الكيتونات < الأمينات < الأستيرات < الأثيرات < الحموض الكاربوكسيلية < الهيدروكربونات المتفرعة والأغوال .

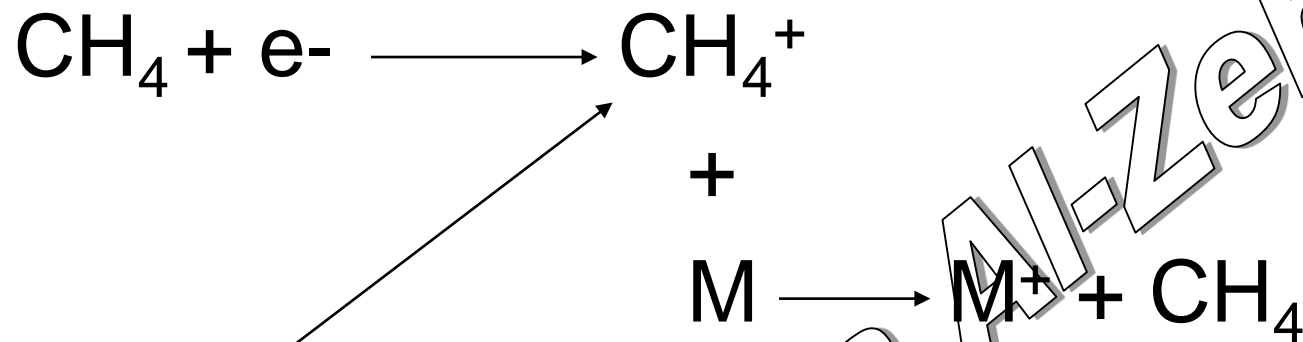


## 2- Chemical Ionization Source

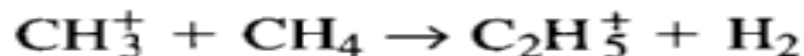
- Most modern MS are designed so that electron-impact ionization and chemical ionization can be carried out interchangeably.
- In Chemical ionization, gaseous atoms of the sample are ionized by collision with ions produced by electron bombardment of an excess of a reagent gas.
- One of the most common reagent is methane which react with high-energy electrons to give several ions such as  $\text{CH}_4^+$ ,  $\text{CH}_3^+$  and  $\text{CH}_2^+$ . The first two predominate and represent about 90%. This ions react rapidly with additional methane molecules.



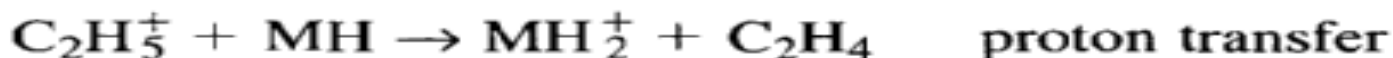
- Chemical ionization Source :



These ions react rapidly with additional methane molecules as follows:



Generally, collisions between the sample molecule MH and  $\text{CH}_5^+$  or  $\text{C}_2\text{H}_5^+$  are highly reactive and involve proton or hydride transfer. For example,



انتقال للبروتون أو سحبه





- Chemical ionization Source :

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Note that proton transfer reactions give the  $(M + 1)^+$  ion whereas the hydride transfer produces an ion with a mass one less than the analyte, or the  $(M - 1)^+$  ion.

القمة الأم ، إما أن تشير للوزن الجزيئي + 1 او للوزن الجزيئي - 1

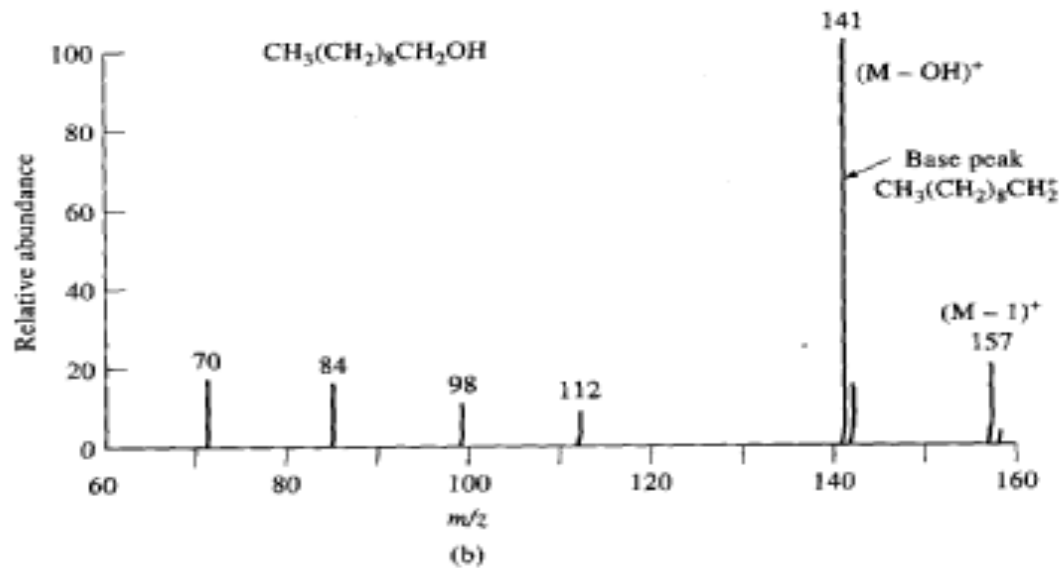
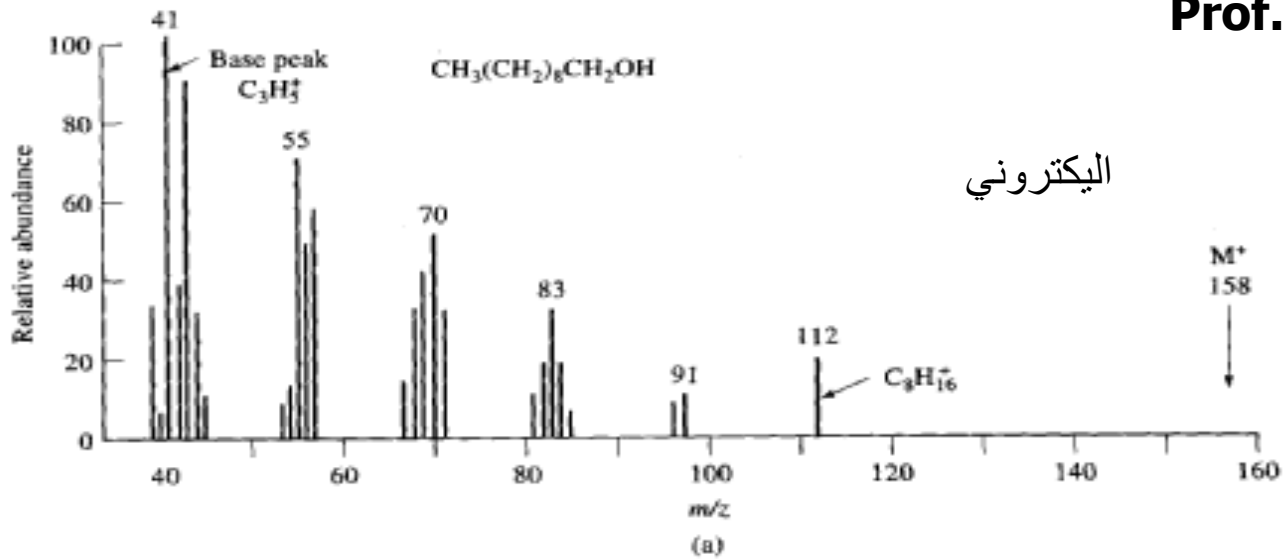
الطيف الكتلي هنا أبسط منه بحالة التأين الأليكتروني





المكتبة

اليكتروني



كيميائي

51D PR

Mass spectrum of 1-decanol from (a) a hard source and (b) a soft source.



# 3- Field Ionization Source and spectra

(only for dipole Substances)

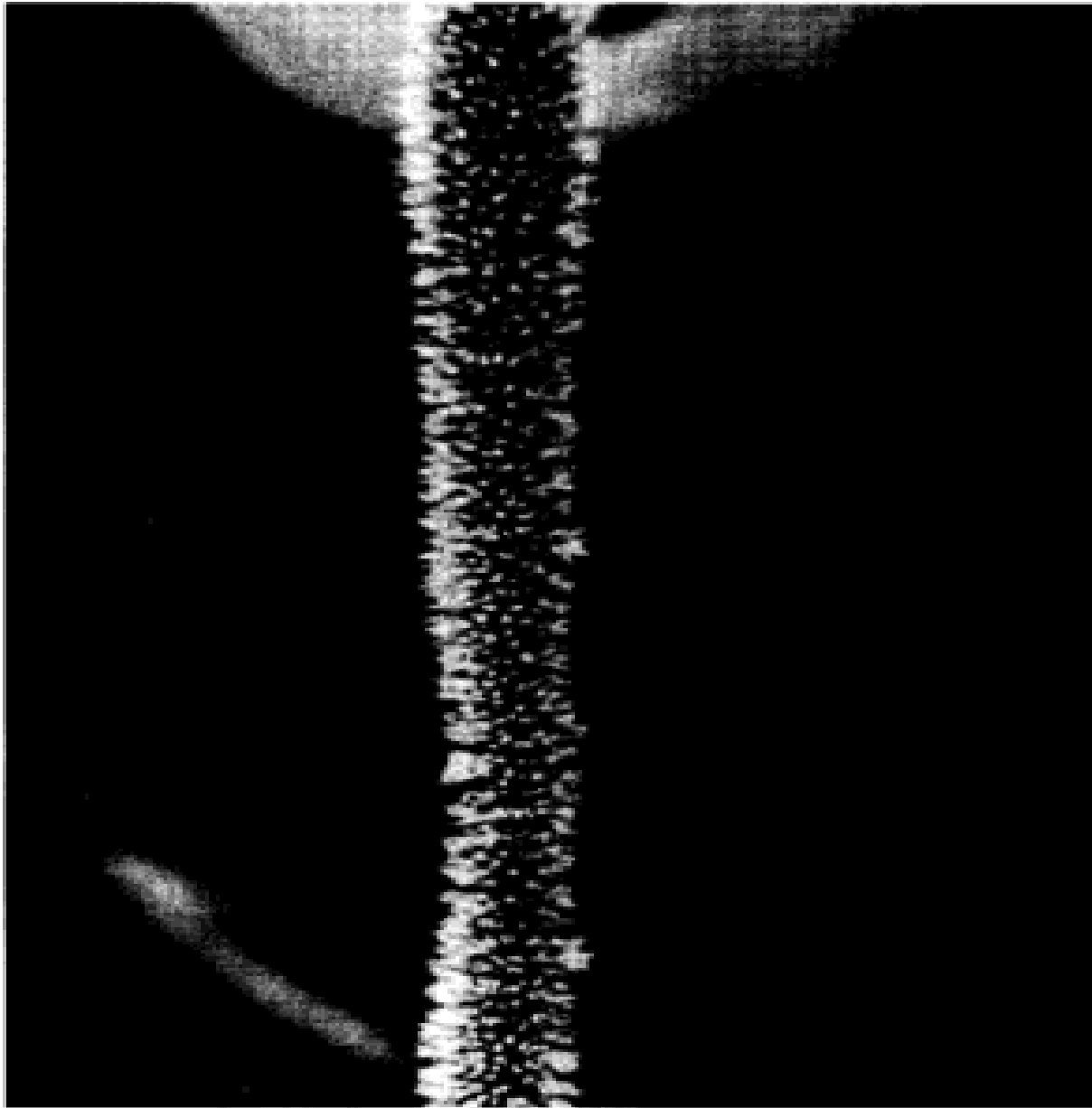
- In field ionization sources, ions are formed under the influence of **a large electric field** ( $10^8\text{V/cm}$ ). Such fields are produced by applying high voltages (10-20kV) to specially formed emitters consisting of numerous fine tips having diameters of less than  $1\ \mu\text{m}$ . The emitter often take the form of **fine tungsten wire** ( $\approx 10\ \mu\text{m}$  diameter) **on which microscopic carbon dendrites, or whiskers**, have been grown by the pyrolysis of benzonitrile in a high electric field.

خاص بالمواد ثنائية القطب حيث توضع في حقل كهربائي عالي يؤدي لأعطاء أيون موجب منها حيث يستخدم مهبط مدبب لهذه الغاية .



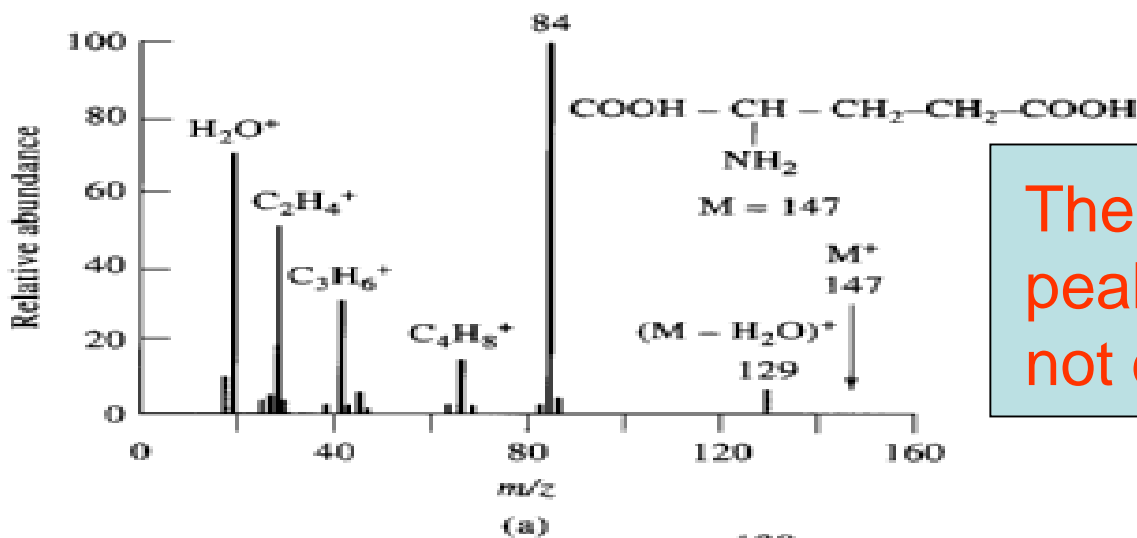
## Field Ionization Source and spectra

- The result of this treatment is a growth of many hundreds of carbon microtips projecting from the surface of the wire.
- Field ionization emitters are mounted 0.5 to 2 mm from the cathode, which often also serves as a slit.
- The gaseous sample from a batch inlet system is allowed to diffuse into the high-field area around the microtips of the anode.
- The electrons from the analyte are extracted by the microtips of the anode.
- Little fragmentation.

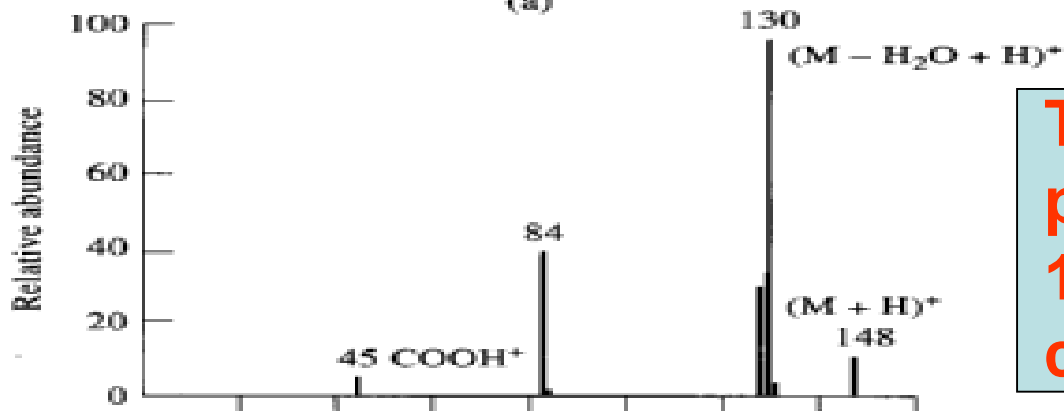


54 Prof

Photomicrograph of a carbon microneedle



The parent ion peak at 147 is not detectable



The parent ion peak at mass 148 (M+1)+ is clear.

Mass spectra for glutamic acid: (a) electron-impact ionization, (b) field ionization,

**Field ionization is limited for dipole substances.**

التأين المجال ( الحقل )

من محاسن هذه الطريقة انتاج أيونات جزيئية  
مستقرة لا تخضع لعملية التفتت وهذا مفيد  
جداً في دراسة المنتجات الطبيعية  
والجزيئات الكبيرة الأخرى التي يصعب  
الحصول على أيون أم لها باستخدام مصادر  
الصدمة الأليكتروني ..



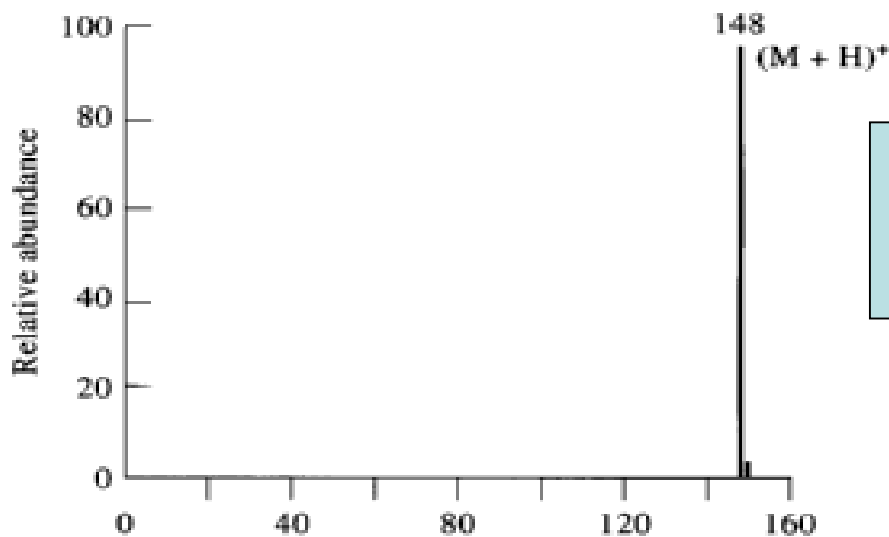


# Desorption Sources

للمواد التي تتخرب بالحرارة ووزنها أقل من 1000

## 1- Field Desorption Sources

A multitipped emitter similar to that described in FI. the spectrum is very simple.



Field Desorption of glutamic acid

Mass spectra for glutamic acid



## 2- Matrix-Assisted Laser Desorption/Ionization (MALDI)

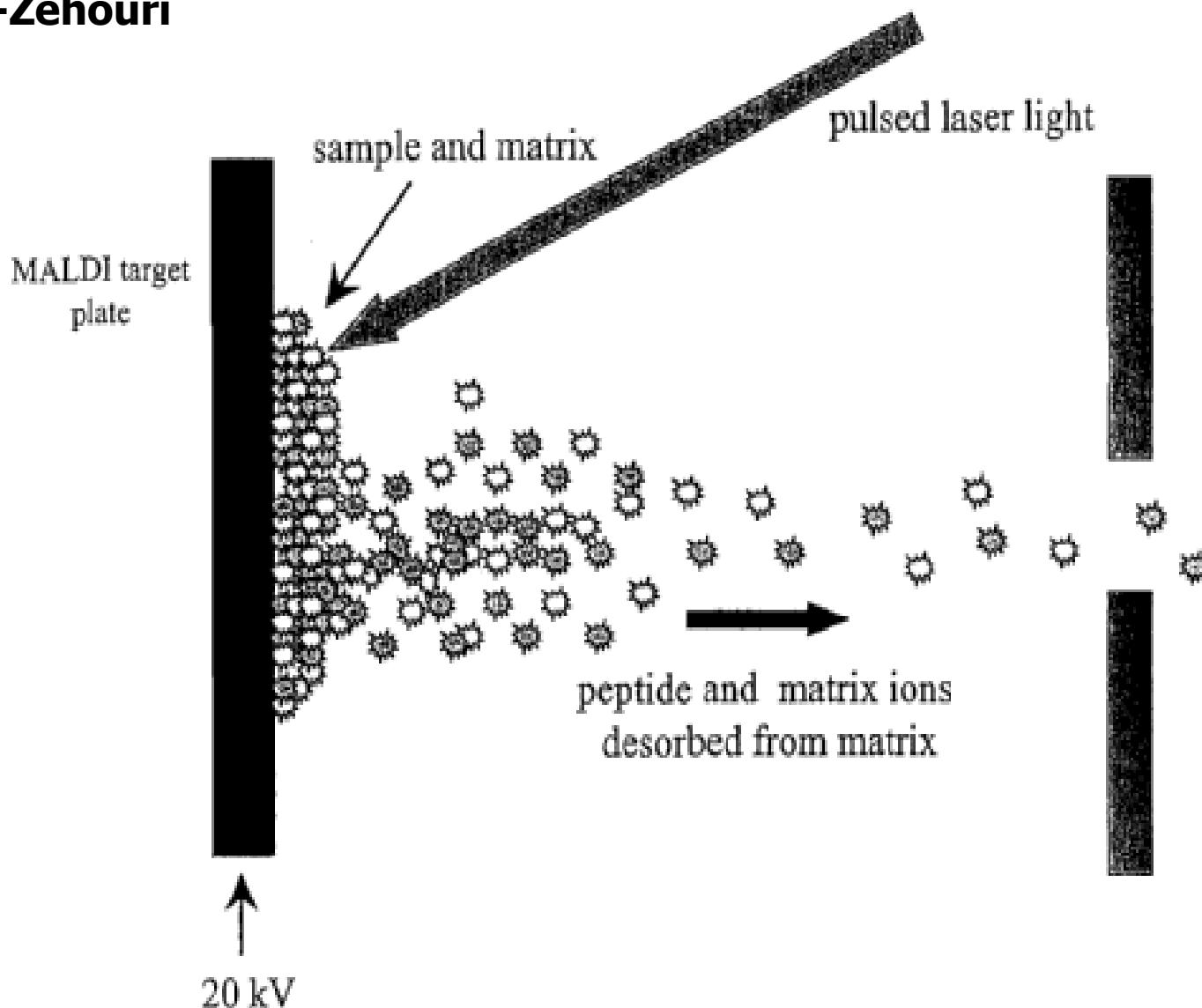
- In this technique the sample is mixed with an equal volume of a saturated solution of matrix (excess of a radiation-absorbing matrix material) prepared in a solvent such as water, acetonitrile, acetone or tetrahydrofuran .
- The matrix is a small, highly conjugated organic molecule that strongly absorbs energy in the UV region.

الماتركس : + العينة (نسب متساوية) ثم تجفف على سطح معدني خاص يستخدم لأغفال العينة للمطياف وبعد الجفاف تعرض لأشعة ليزر فتؤدي لأصدار الأيونات .



## (Matrix-Assisted Laser Desorption /Ionization MALDI)

- ***The resulting solution was evaporated on the surface of metallic probe that was used for introduction of the sample into the mass spectrometer.***
- ***The solid mixture was then exposed to a pulsed laser beam ,which resulting the ions.***



Principles of MALDI. The sample is cocrystallized with a large excess of matrix, and short pulses of laser light are focused on to the sample spot. The matrix absorbs the laser energy and dissipates it into the sample, causing part of the illuminated substrate to vaporize. The rapidly expanding plume of matrix and sample ions are then drawn into the mass analyzer via a pressure differential between the analyzer and the source region.



## Matrices Most Frequently Used for MALDI Together with the Usable Wavelengths

Matrix	Wavelength (nm)
Nicotinic acid	266, 220–290
Benzoic acid derivatives:	
2,5-Dihydroxybenzoic acid <b>DHB</b>	266, 337, 355
Vanillic acid	266
2-Amino-benzoic acid	266, 337, 355
Pyrazine-carboxylic acid	266
3-Aminopyrazine-2-carboxylic acid	337
Cinnamic acid derivatives: <b><math>\alpha</math>-cyano-4-hydroxycinnamic acid (CHCA)</b>	
Ferulic acid	266, 377, 355
Sinapinic acid ←	266, 337, 355
Caffeic acid	266, 337, 355
3-Nitrobenzylalcohol	266



## Matrix-Assisted Laser Desorption/Ionization (MALDI)

- *Could be used to analyze **proteins** with mass **of >10.000 Da**.*
- *MALDI generates high mass ions by using a pulsed **laser beam** to irradiate a solid mixture of an analyte dissolved in a suitable matrix compound.*
- *The most commonly used matrices for proteins and peptides include (**CHCA, DHB and sinapinic acid.**)*



# 3- Electrospray Ionization

التأين بالأرذاذ الأليكتروني

- One of the most important techniques for analyzing biomolecules, such as polypeptides, proteins, and oligonucleotides having molecular weight of **100,000** Da or more.
- A solution of the sample is pumped through a stainless steel capillary needle at a rate of a few microliters per minute.
- The Ions Produced with help of Electrostatic lenses.



المحلل الطيفي ( الأنبوب المحلل أو الفاصل )



## Mass analyzers

A mass analyzer or filter is the portion of a mass spectrometer that is responsible for resolving different mass fragments.

القسم المسؤول عن فصل الأيونات الموجبة ، ولهذا يجب ان يتمتع بقوة فصل كبيرة.

Typically, all ions are accelerated to the same kinetic energy ( $1/2 mv^2$ ).

تدخل الأيونات بطاقة حركية واحدة  
تساوي نصف كتلته مضروباً بسرعه

Some aspect of these accelerated ions is then exploited as the basis for resolving them.

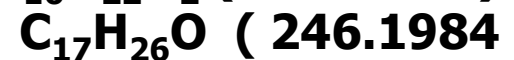
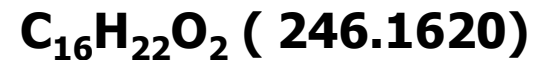




# Mass Analyzers

- ***Ideally , the mass analyzer should be capable of distinguishing between minute mass differences.***
- ***In addition ,the analyzer should allow passage of a sufficient number of ions to yield readily measurable ion currents.***
- ***As with optical monochromator ,to which the analyzer is analogous.***

يجب أن يملك إمكانية عالية بالفصل ( مثال ) :





## Resolution of Mass Spectrometers :

- The capability of a mass spectrometer to differentiate between masses is usually stated in terms of its resolution , $R$ , which is defined as :

$$R = m/\Delta m$$

Where  $\Delta m$  is the mass difference between two adjacent peaks that are just resolved, and  $m$  is the nominal mass of the first peak.

- The resolution needed in a mass spectrometer depends greatly upon its application.
- Commercial spectrometers are available with resolution ranging from about 500 to 500,000.



## ***Resolution of Mass Spectrometers :***

- Example :***

**What resolution is needed to separate the two ions  $C_2H_4^+$  (28.0313) and  $CH_2N^+$  (28.0187) in MS ?**

$$\Delta m = 28.0313 - 28.0187 = 0.0126$$

$$R = m / \Delta m = 28.025 / 0.0126 = 2.22 \times 10^3$$

where 28.025 is the mean mass for the two species.



# Mass Analyzer

Type of mass analyzer :

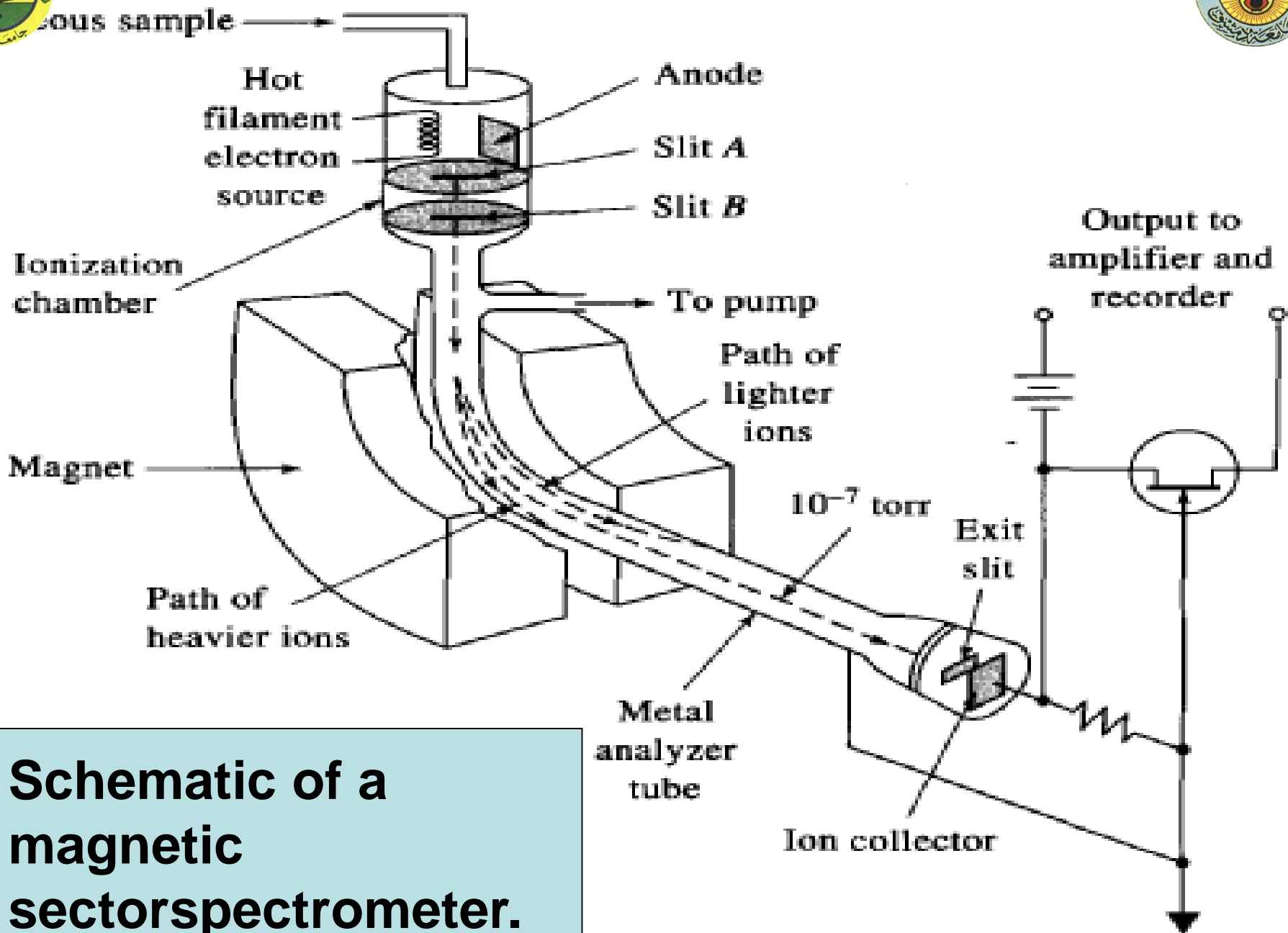
1. **Magnetic sector Analyzer. (single-focusing Spectrometer)** الأنحراف المغناطيسي أحادي التركيز البؤري
2. **Double-Focusing Spectrometers.** ثنائي التركيز البؤري
3. **Quadrupole Mass Spectrometers.** رباعي الأقطاب
4. **Time-of-Flight Mass Analyzers.** بزمان الطيران
5. **Ion Trap Analyzers ( Quadrupole ion storage)** الحاجز للأليكترونات



# 1- Magnetic Sector Analyzers

يستخدم هنا مغناطيس ثابت أو مغناطيس كهربائي يتسبب بانحراف تيار الأيونات بطريق دائري

- It is employ a permanent **magnet** or an **electromagnet** to cause the beam from the ion source to travel in a circular path, most commonly of 180,90 or 60 deg.
- Will  $KE = z e V = \frac{1}{2}mv^2$  ( $V =$  voltage between A and B , and  $e =$  electronic charge  $= 1.60 \times 10^{-19}$ ) all ions have the same number of charges  $z$  are assumed to have the same kinetic energy after acceleration regardless of their mass.
- The heavier ions must travel through the magnetic sector at lower velocities.



**Schematic of a magnetic sectors spectrometer.**



# Magnetic Sector Analyzers

الذي يحدد مسار الأيونات في القطاع المغناطيسي هو محصلة قوتين هما :

- The path in the sector described by ions of a given mass and charge represents a balance between two forces acting upon them.

قوة مغناطيسي

1- The **magnetic force**  $F_M = Bzev$

Where B the magnetic field strength.

2- The balancing **centripetal force**  $F_c = \frac{mv^2}{r}$

where r is the radius of curvature of the magnetic sector.

قوة نابذة : وتمثل r نصف قطر المسار بالقطاع المغناطيسي



## Magnetic Sector Analyzers



- In order for an ion to traverse the circular path to the collector,  $F_M$  and  $F_c$  must be equal, so:

$$Bzev = mv^2/r \quad \longrightarrow \quad v = Bzer/m$$

Substituting in  $KE = z eV = 1/2mv^2 \quad \longrightarrow$

$$m/z = B^2 r^2 e / 2V$$





$$m/z = B^2 r^2 e / 2V$$

The equation reveals that mass spectra can be acquired by varying one of three variables (  $B$ ,  $V$ , or  $r$  ) while holding the other two constant. Most modern sector mass spectrometers contain an electromagnet in which ions are sorted by holding  $V$  and  $r$  constant while varying the current in the magnet and thus  $B$ .

الذي يتحكم بالنهاية هو قوة الحقل المغناطيسي



## 2- Double-Focusing Spectrometers (Electrostatic)

- In order to measure atomic and molecular masses with a precision of a few parts per million, it is necessary to design instruments that correct for both the directional distribution and energy distribution of ion leaving the source.

اضافة للقطاع المغناطيسي ، يستخدم أيضاً قطاع من الكهرباء الساكنة لأختزال انتشار الطاقة قبل دخول الأيونات للحقل المغناطيسي مما يساعد على تمايزها



## Double-Focusing Spectrometers (Electrostatic)



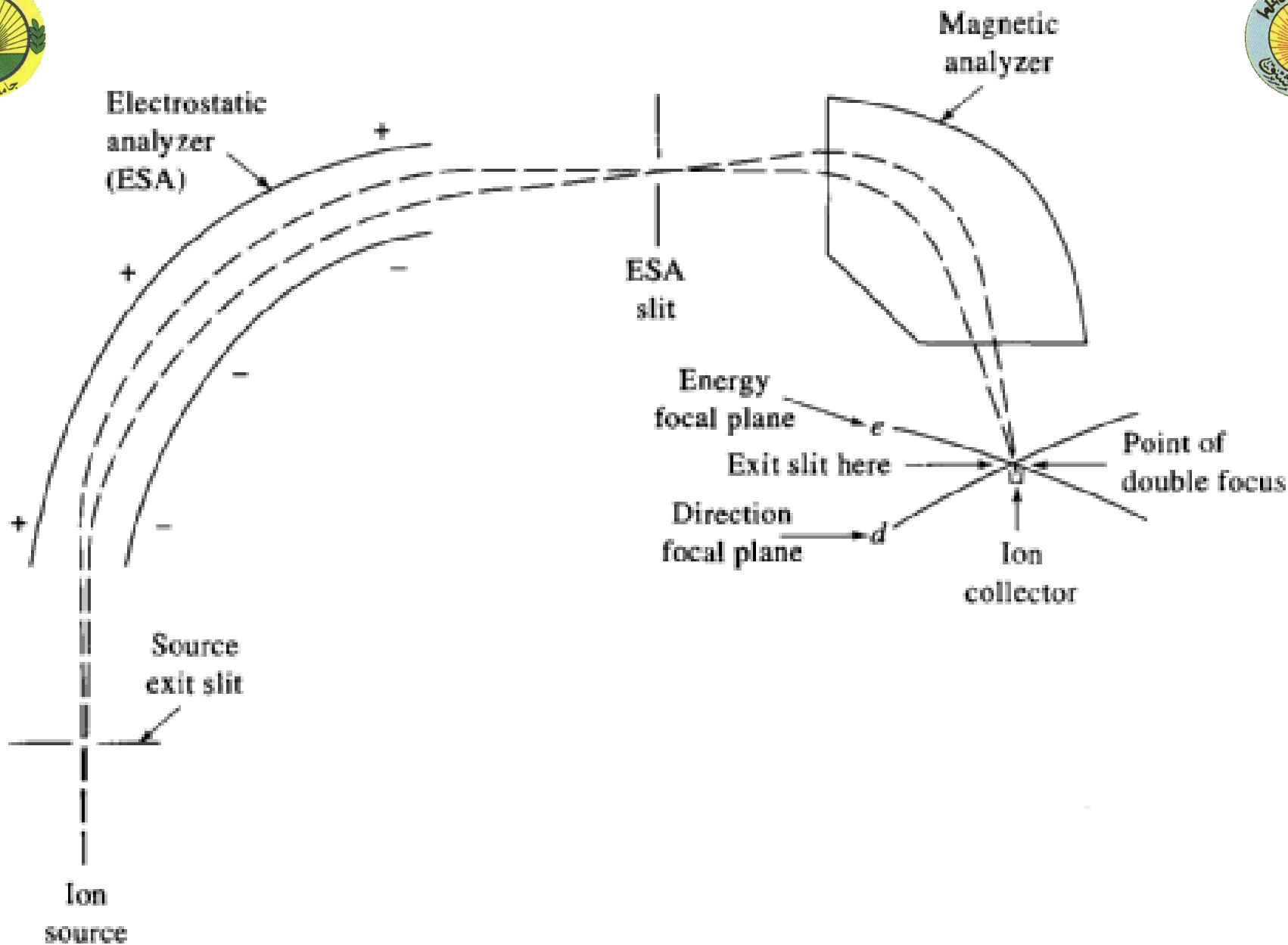
- Double focusing is usually achieved by the use of carefully selected combinations of electrostatic and magnetic fields.
- In this technique the ion beam is first passed through **an electrostatic analyzer (ESA)** consisting of two smooth curved metallic plates across which a dc potential is applied.
- This potential has the effect of limiting the kinetic energy of the ions reaching the magnetic sector to a closely defined range.



## Double-Focusing Spectrometers (Electrostatic)



- Ions with energies greater than average strike the upper side of the ESA slit and are lost to ground.
- Ions with energies less than average strike the lower side of the ESA slit and are thus removed.
- Directional focusing in the magnetic sector occurs along the focal plane (d). (**Nier-Johnson design**)
- Energy focusing takes place along the plane labeled (e).
- Thus only ion of one  $m/z$  are double focused at the intersection of d and e for any given accelerating voltage and magnetic field strength. Therefore, the collector slit is located at this locus of double focus.



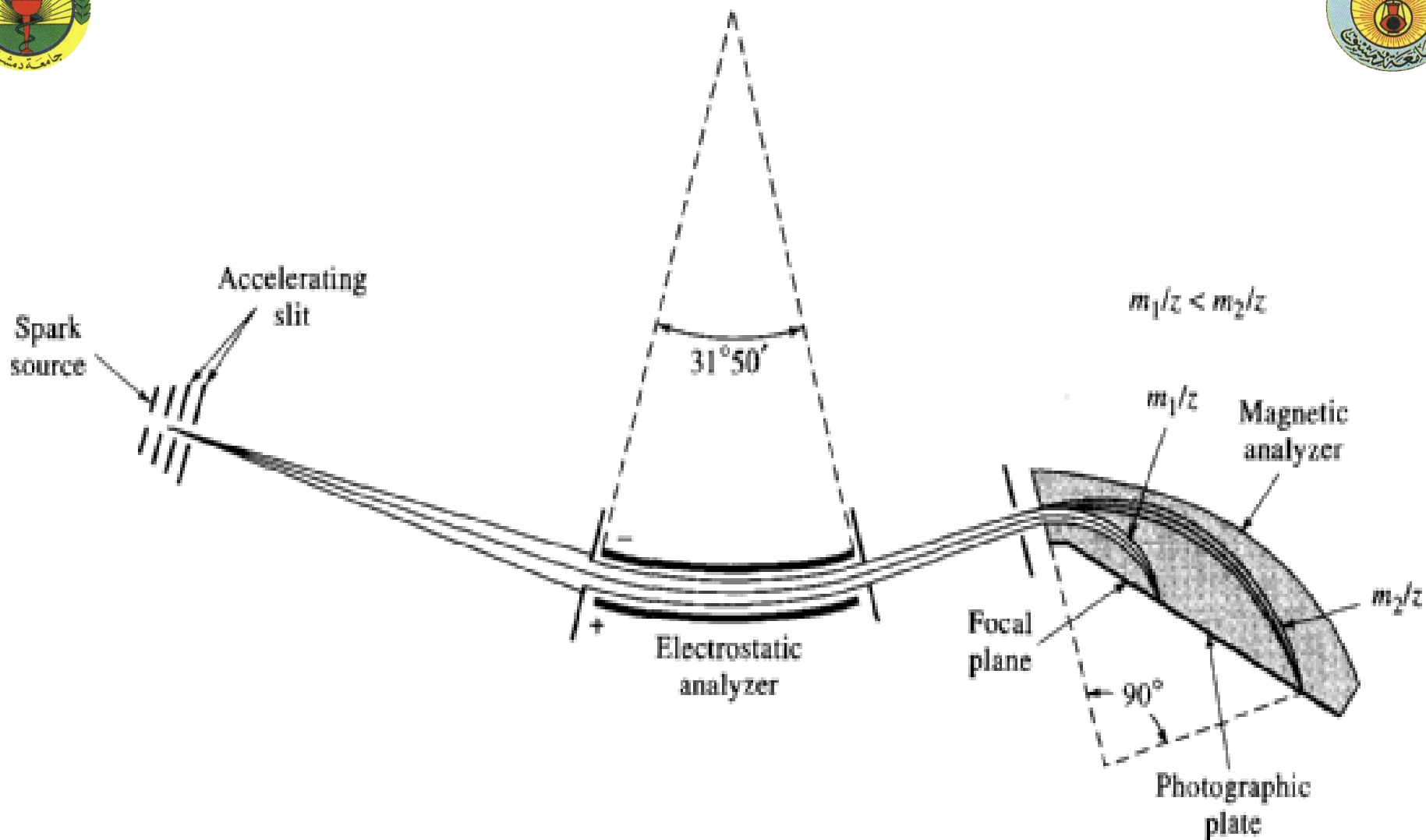
Nier-Johnson design of a double-focusing mass spectrometer.



## Double-Focusing Spectrometers (Electrostatic)



- Another double focusing design is **Mattauch-Herzog geometry**.
- The geometry of this type of instrument is unique in that energy and direction focal planes coincide, for this reason the mattauch –Herzog design often uses a photographic plate for recording the spectrum.
- The photographic plate is located along the focal plane, where all of the ions are in focus ,regardless of mass-to-charge ratio.



Mattauch-Herzog-type double-focusing mass spectrometer. Resolution  $> 10^5$  has been achieved with instruments based on this design.



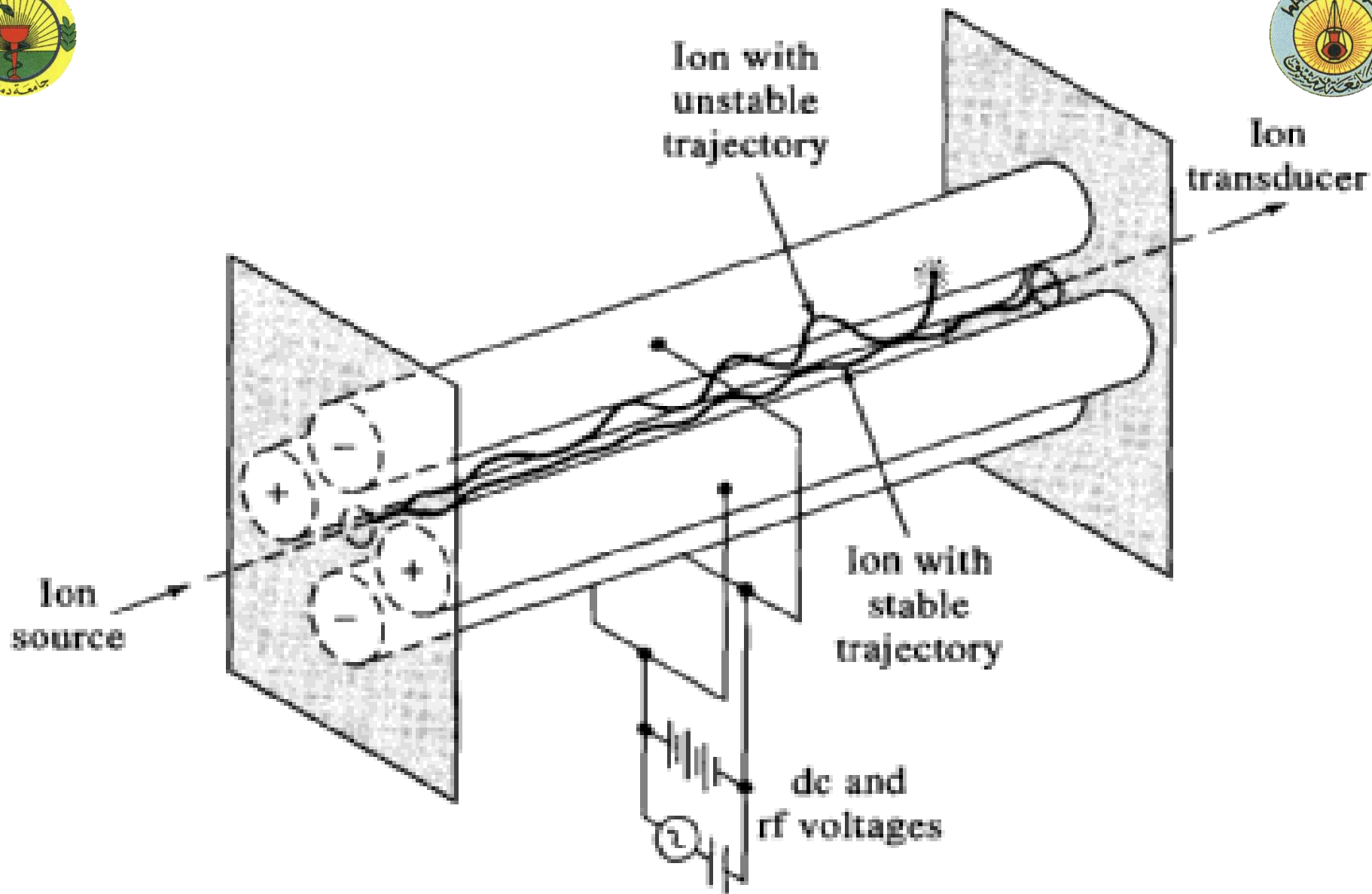
### 3- Quadrupole Mass Spectrometers

- The most common mass analyzers in use today.
- More compact , less expensive, and more rugged than most other type of MS.
- It offers the advantage of low scan times, which is particularly useful for real-time scanning of chromatographic peaks.





- The heart of a quadrupole instrument is **the four parallel cylindrical rods** that serve as electrodes.
- Opposite rods are connected electrically, one pair being attached to the **positive side** of a variable dc source and the other pair to the **negative** terminal.
- At any given moment, all of the ions except those having a certain  $m/z$  values strike the rods and are converted to neutral molecules. Thus only ions having limited range of  $m/z$  value reach the transducer.



A quadrupole mass spectrometer.



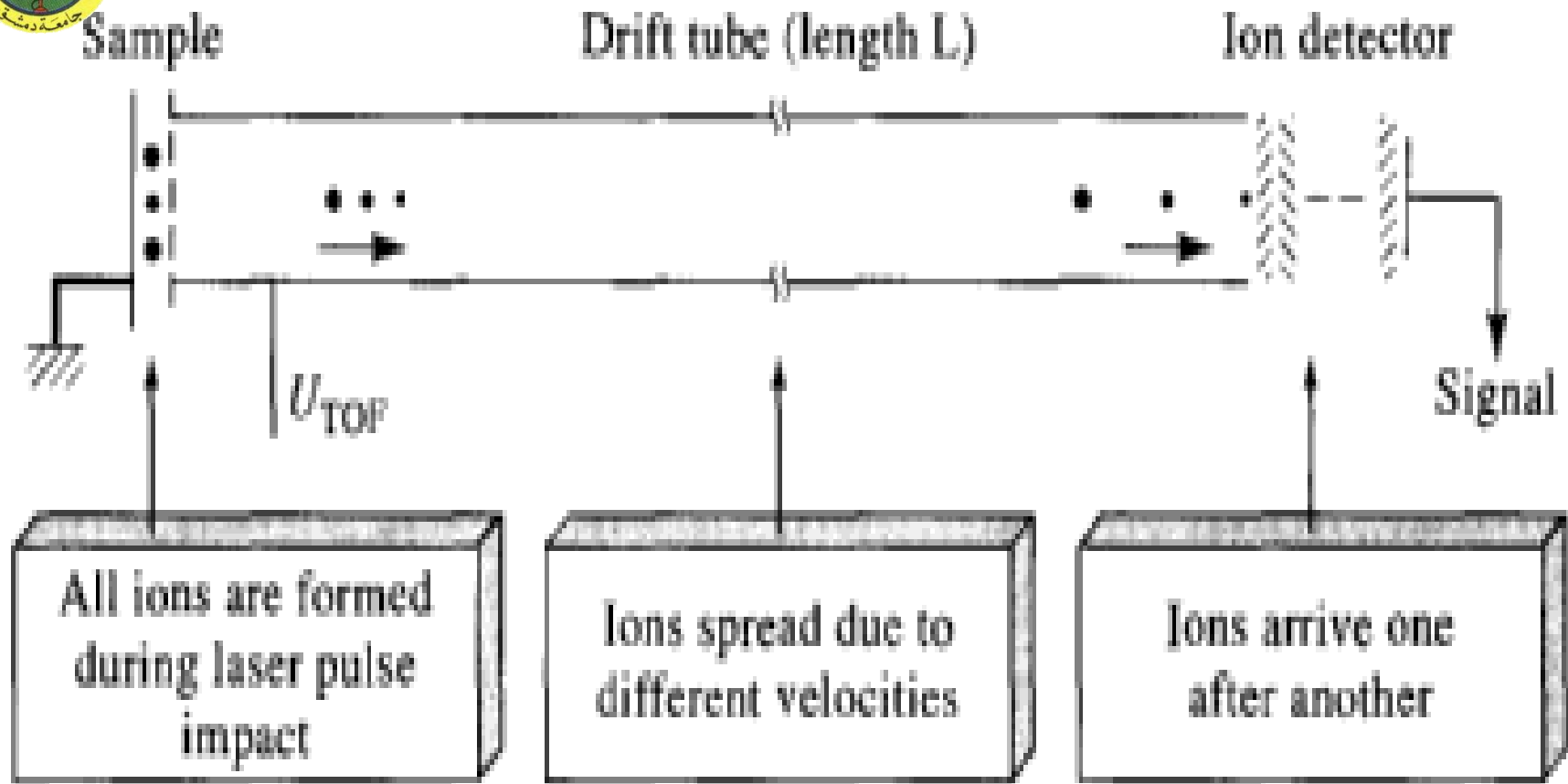
# 4- Time-of-Flight Mass Analyzers (TOF)

كل ما سبق اعتمد على فصل الأيونات بالقطاع المغناطيسي ، هنا يستخدم أنبوب فصل طوله 1 م حيث تتناسب سرعتها عكساً مع كتلتها مؤدية لفصلها .

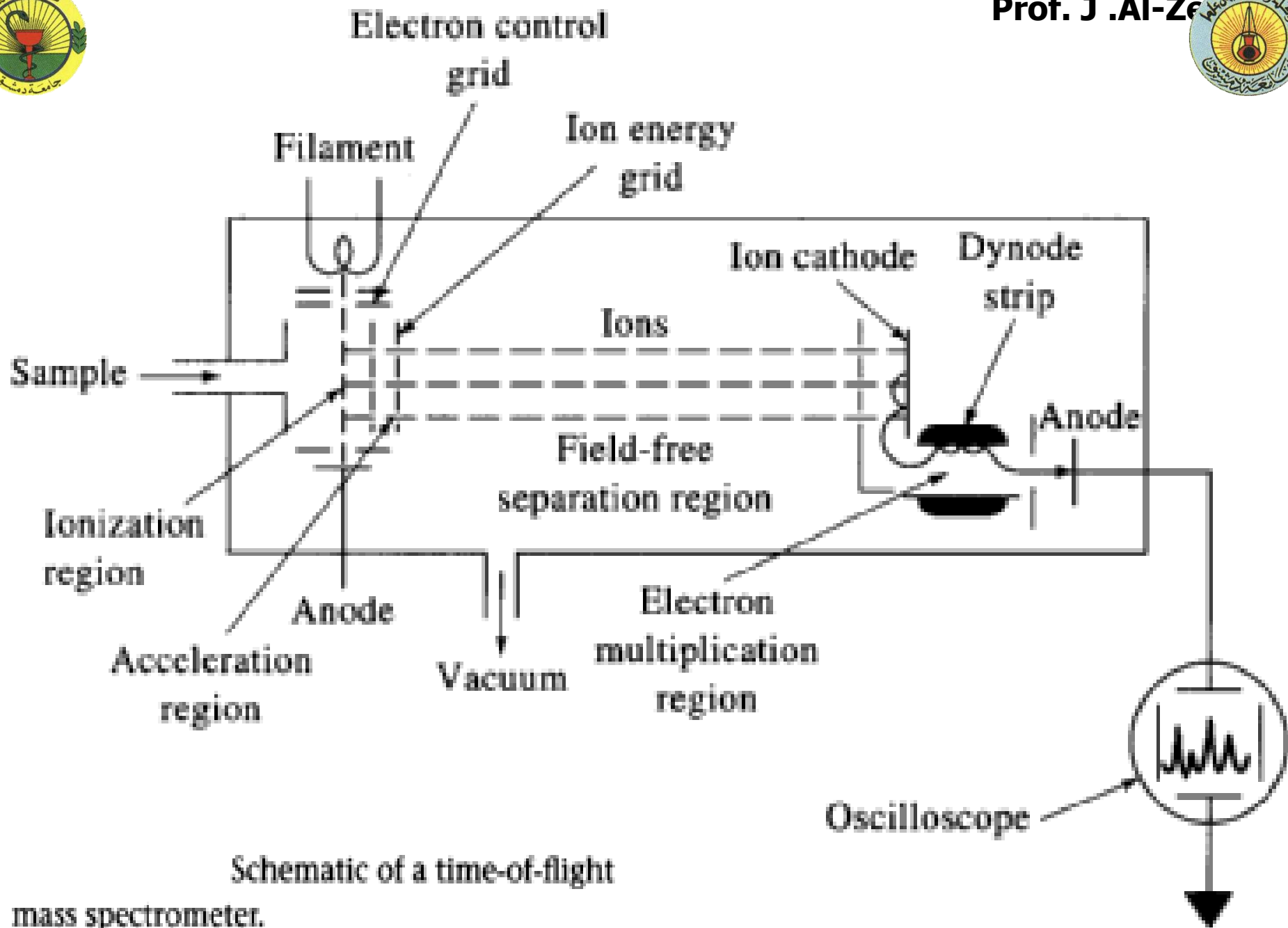
- Positive ions are produced periodically by bombardment of the sample with brief pulses of electrons, secondary ions, or laser generated photons.
- These pulses typically have a frequency of 10 to 50 kHz and lifetime of 0.25  $\mu$ s.
- The ions produced in this way are then accelerated by an electric field pulse of  $10^3$  to  $10^4$  V that has the same frequency as, but lags behind, the ionization pulse.



- The accelerated particles pass into a field-free drift **tube about a meter in length**.
- Because all ions entering the tube ideally have the same kinetic energy, their velocities in the tube must vary inversely with their masses, with the lighter particles arriving at the detector earlier than the heavier ones. Typically flight times are 1 to 30  $\mu\text{s}$ .
- The transducer in TOF is electron multiplier.



Principle of a time-of-flight mass spectrometer. A bunch of ions produced by a laser probe is accelerated into the drift tube where separation occurs.



Schematic of a time-of-flight mass spectrometer.



- Advantages of TOF over other type of MS:

1. Simplicity
2. Ruggedness
3. Easy of accessibility of the ion source
4. Virtually unlimited mass range.

- Disadvantages :

They suffer, however from limited resolution and sensitivity.

يعاني من انخفاض الميز  
والحساسية



## 5- Ion Trap Analyzers

- An ion trap is a device in which gaseous anions or cations can be formed and confined for extended periods by electric and/or magnetic fields.
- Several types of ion traps have been developed, and two are currently used in commercial MS.
  - 1- The ion cyclotron resonance trap.
  - 2- The Simple ion trap.





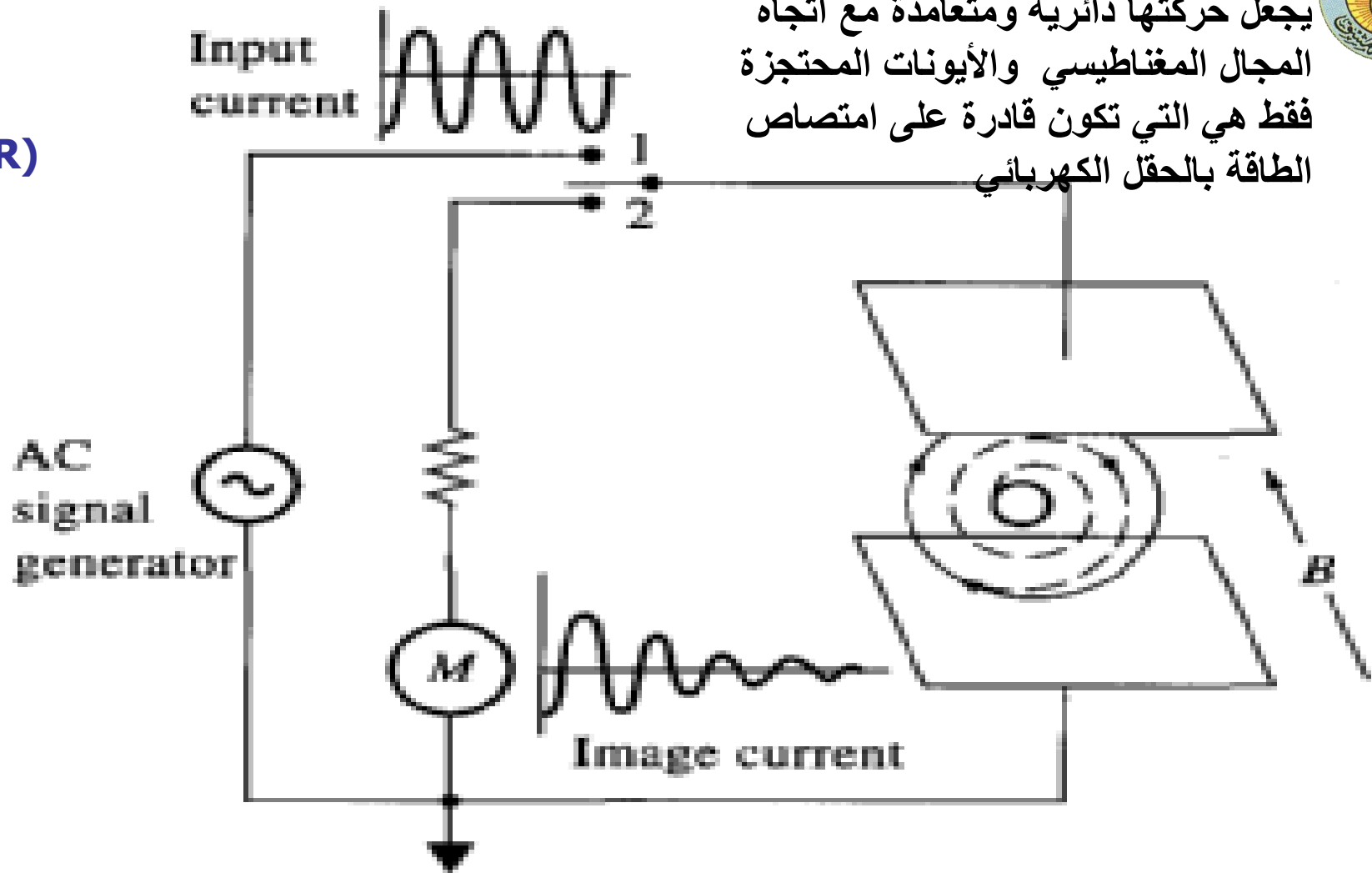
# a- The Ion Cyclotron Resonance (ICR)

- When a gaseous ion drifts into or is formed in a strong magnetic field, its motion becomes circular in a plane that is perpendicular to the direction of the field.
- The angular frequency of this motion is called the cyclotron frequency  $\omega_c$
- An ion trapped in a circular path in a magnetic field is capable of absorbing energy from an electric field, provided the frequency of the field matches the cyclotron frequency.



توضع الأيونات بمجال مغناطيسي عالي يجعل حركتها دائرية ومتعامدة مع اتجاه المجال المغناطيسي والأيونات المحتجزة فقط هي التي تكون قادرة على امتصاص الطاقة بالحقل الكهربائي

(ICR)

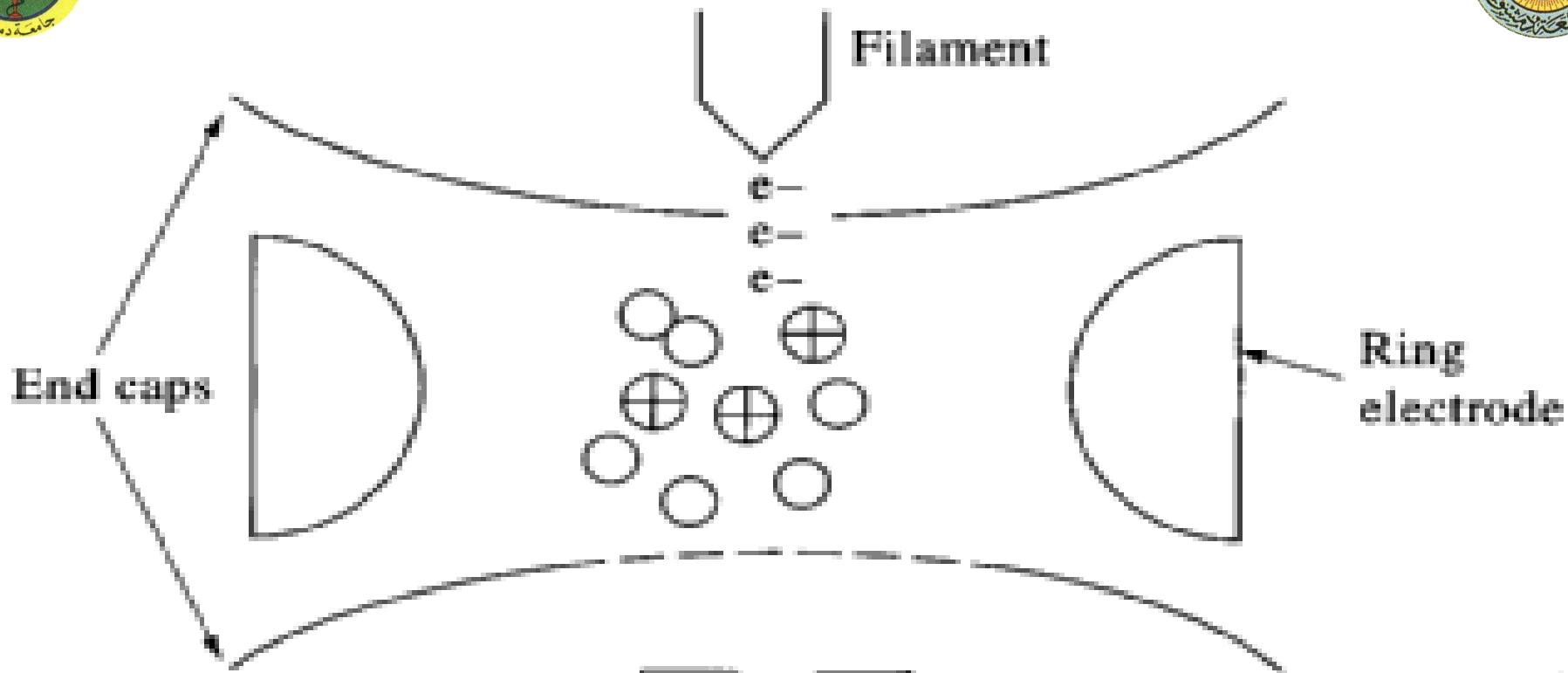


Path of an ion in a strong magnetic field. Inner solid line represents the original circular path of the ion.



## b- Simple type of ion trap`

- It consists of a central **doughnut-shaped** ring electrode and a pair of endcap electrodes.
- A variable **radio-frequency** voltage is applied to the ring electrode while the two end-cap electrodes are grounded.
- Ions with an appropriate  $m/z$  value circulate in a stable orbit within the cavity surrounded by the ring.
- As the frequency increased ,the orbits of heavier ions become stabilized, while those for lighter ions become destabilized ,causing them to collide with the wall of the ring electrode.



Electron multiplier transducer

Ion signal

يطبق تردد راديوي متغير على القطب الدائري حيث تدور الأيونات بمدار ثابت خلال الفجوة حول الحلقة حيث تصمد فقط الأيونات الثقيلة

Ion trap mass spectrometer.



# ***Transducers for Mass Spectrometry***

محولات الطاقة إلى طيف

**1- Electron Multipliers.**

مضاعف اليكتروني

**2- The Faraday Cup.**

فنجان فارادي

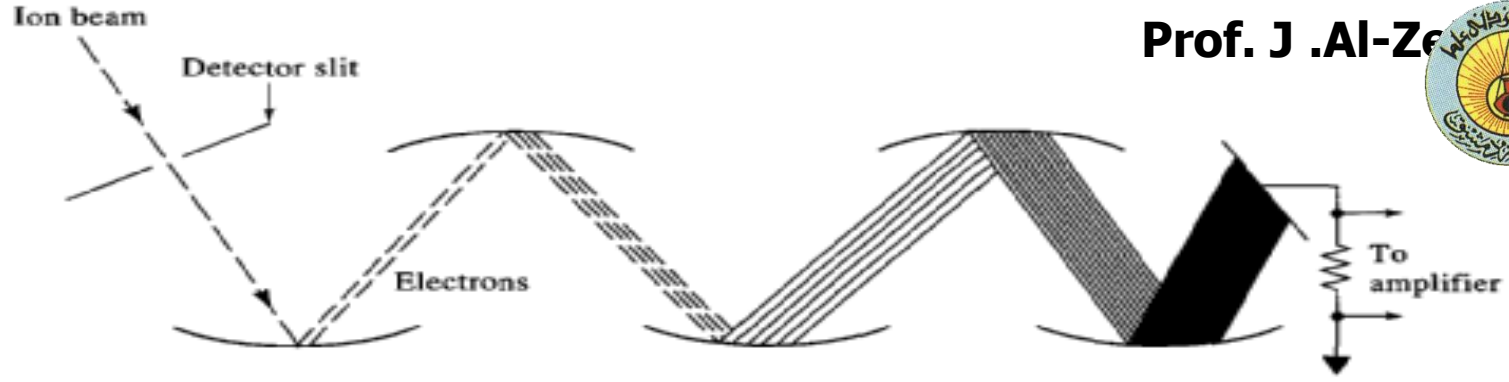
**3- Other Types of Detection Systems.**



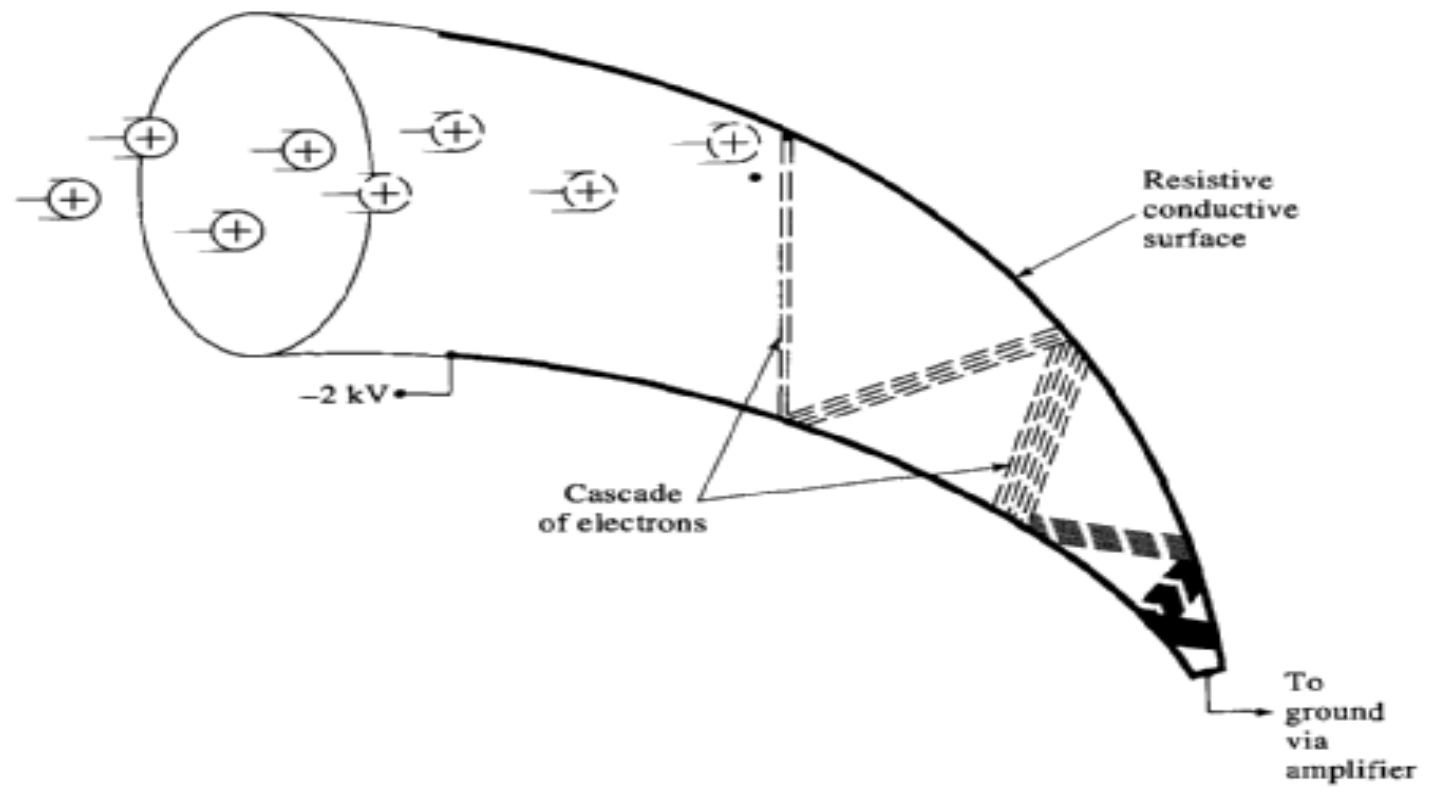
# Electron Multipliers

- Ion beam exiting the analyzer is accelerated to several thousand electron volts prior to striking the first stage.
- We have a discrete-dynode electron multiplier and a continuous – **dynode electron multiplier**.

تسرع الحزمة الأيونية بشكل كبير جداً قبل اصطدامها بالسطح الداخلي للمتجري الذي يخضع عدد الأليكترونات حيث تصدم بالحافة الثانية وتاعف ثانية وهكذا للحصول على اشارة واضحة.



(a)



(b)

(a) Discrete dynode electron multiplier. Dynodes are kept at successively higher potentials via a multistage voltage divider. (b) Continuous dynode electron multiplier.



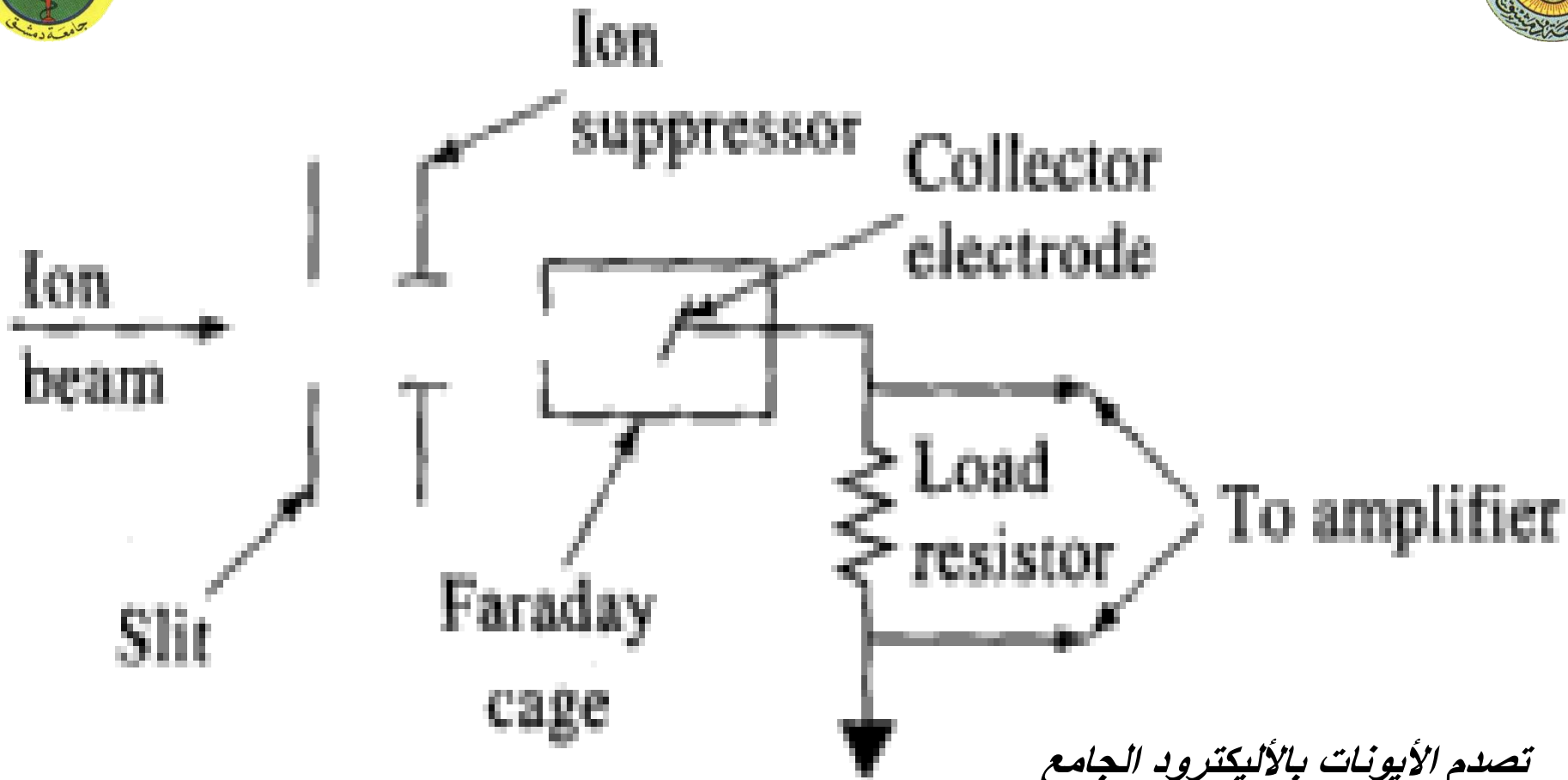
# The Faraday Cup

- The transducer is aligned so that ions exiting the analyzer strike the collector electrode.
- This electrode is surrounded by a cage that prevents the escape of reflected ions and ejected secondary electrons.
- The collector electrode is inclined with respect to the path of the entering ions so that particles striking or leaving the electrode are reflected a way from the entrance to the cup.





- The collector electrode and cage are connected to ground potential through a large resistor.
- The charge of the positive ions striking the plate is neutralized by a flow of electrons from ground through the resistor.
- The resulting potential drop across the resistor is amplified by a high-impedance amplifier.
- The response is independent of the energy, the mass, and the chemical nature of the ion.



Faraday cup detector.

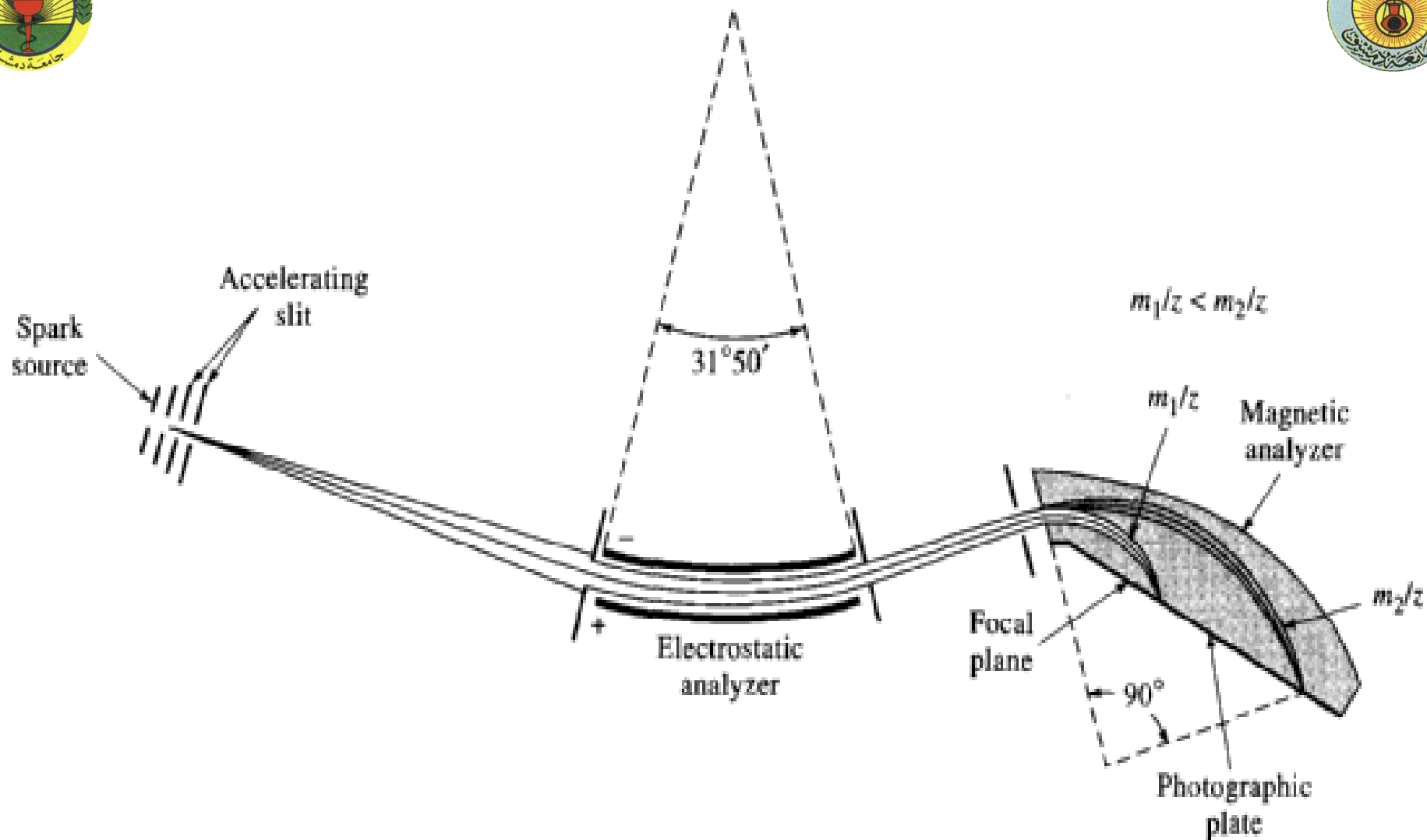
تصدم الأيونات بالأليكترود الجامع  
 (شكل الفنجان) المتصل بفرق  
 كمون أرضي حيث تغدو الأيونات  
 الموجبة معتدلة في حين إن فرق  
 الكمون الناتج ينسحب خلال  
 المقاومة ويضخم .



# Other Types of Detection System

## 1- Photographic :

- Photographic plates coated with a **silver bromide emulsion** are sensitive to energetic ions.
- Photographic detection is most frequently encountered in **spark source** instruments because this type of detection is well suited to the simultaneous observation of a wide range of  $m/z$  values in instruments that focus ions along a plane.



Mattauch-Herzog-type double-focusing mass spectrometer. Resolution  $> 10^5$  has been achieved with instruments based on this design.



# Other Types of Detection System

## 2- Scintillation-type :

النمط الوميضي (التأقني)

- Scintillation-type transducers also find some use.
- These transducers consist of a crystalline phosphor dispersed on a thin aluminum sheet that is mounted on the window of a photomultiplier tube.
- When ions impinge upon the phosphor ,they produce flashes of light, which are detect by the photomultiplier.

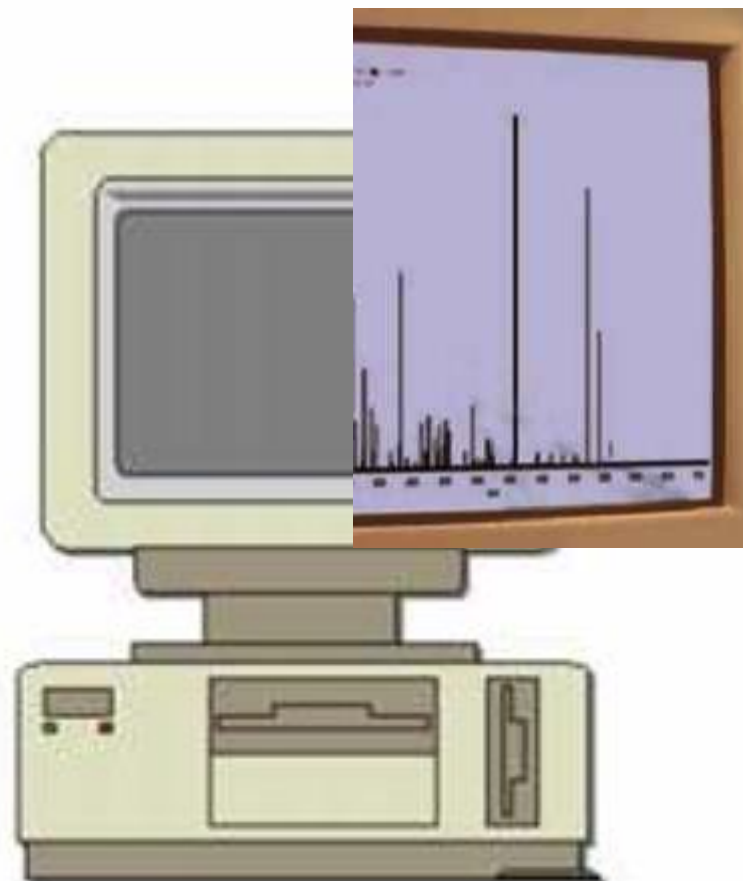
تصطدم الأيونات بالفوسفور فتصدر وميض ضوئي يمكن كشفه ومضاعفته ضوئياً وقياسه



# Data system

**Microcomputers and microprocessors are an integral part of modern MS**

Modern MS systems rely on computers for rapid data collection, processing and identification of mass spectra.





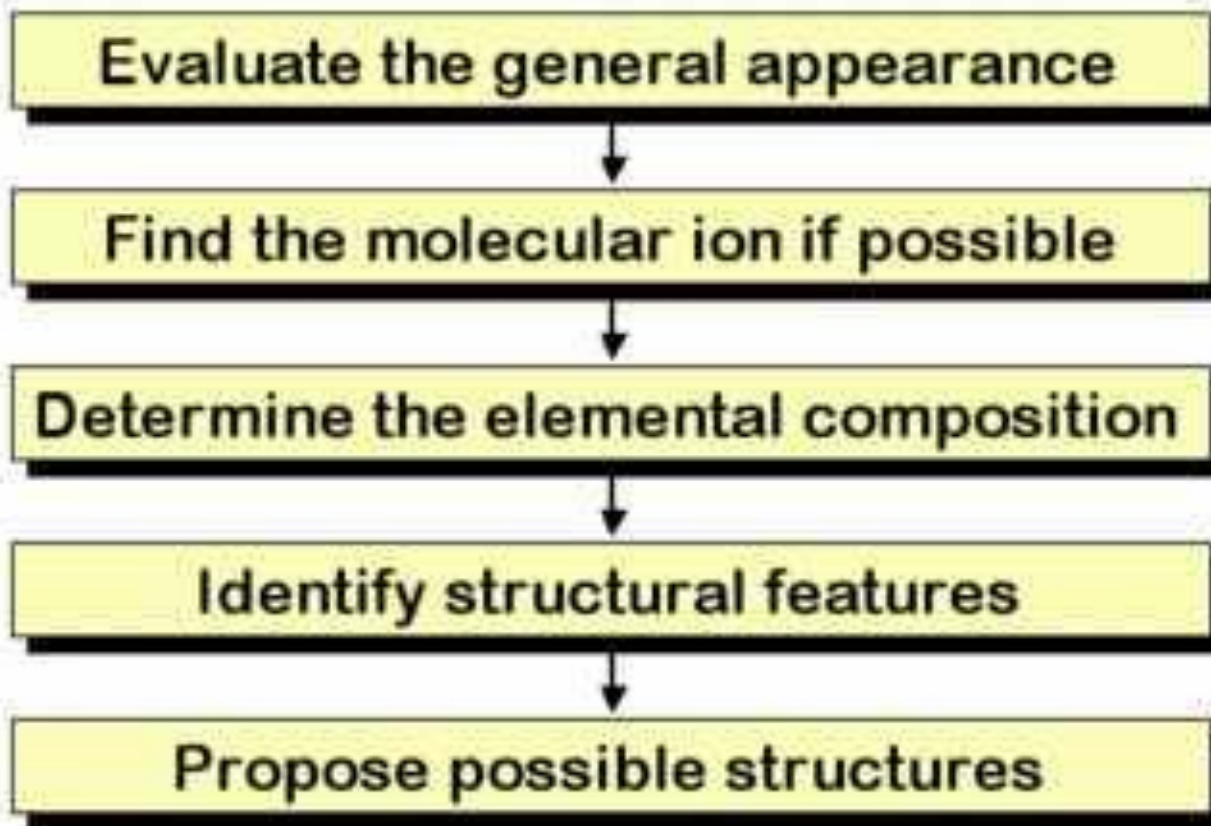
# Application of Molecular Mass Spectrometry

## 1- Identification of pure compounds:

- Molecular weight
- Molecular formulas
- Structural information from Fragmentation Pattern
- Compound Identification from Comparison Spectra.



# Interpretation of mass spectra







# Application of Molecular Mass Spectrometry

## 2- Quantitative Determination of Molecular Species.

- Using Hyphenated Mass spectral Methods ( GC-MS , LC-MS, CE-MS .)
- Determine the concentration directly from the heights of the mass spectral peaks.( under this circumstances , calibration curves of peak heights vs concentration can be prepared and use for analysis of unknown.



## Applications of Molecular Mass Spectrometry

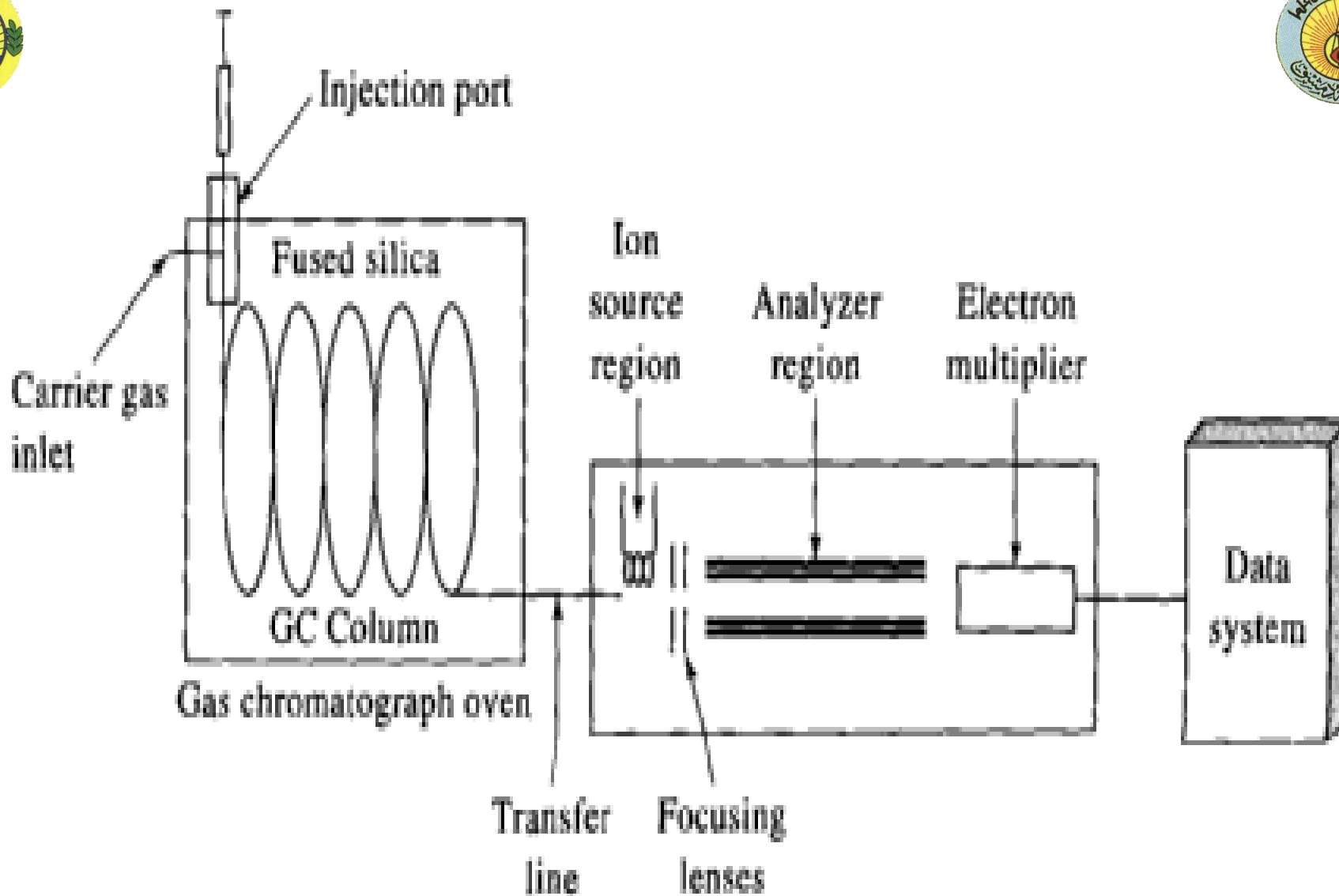
1. Elucidation of the structure of organic and biological molecules
2. Determination of the molecular weight of peptides, proteins, and oligonucleotides
3. Identification of components in thin-layer and paper chromatograms
4. Determination of amino acid sequences in sample of polypeptides and proteins
5. Detection and identification of species separated by chromatography and capillary electrophoresis
6. Identification of drugs of abuse and metabolites of drugs of abuse in blood, urine, and saliva
7. Monitoring gases in patient's breath during surgery
8. Testing for the presence of drugs in blood in thoroughbred racing horses and in Olympic athletes
9. Dating archaeological specimens
10. Analyses of aerosol particles
11. Determination of pesticide residues in food
12. Monitoring volatile organic species in water supplies



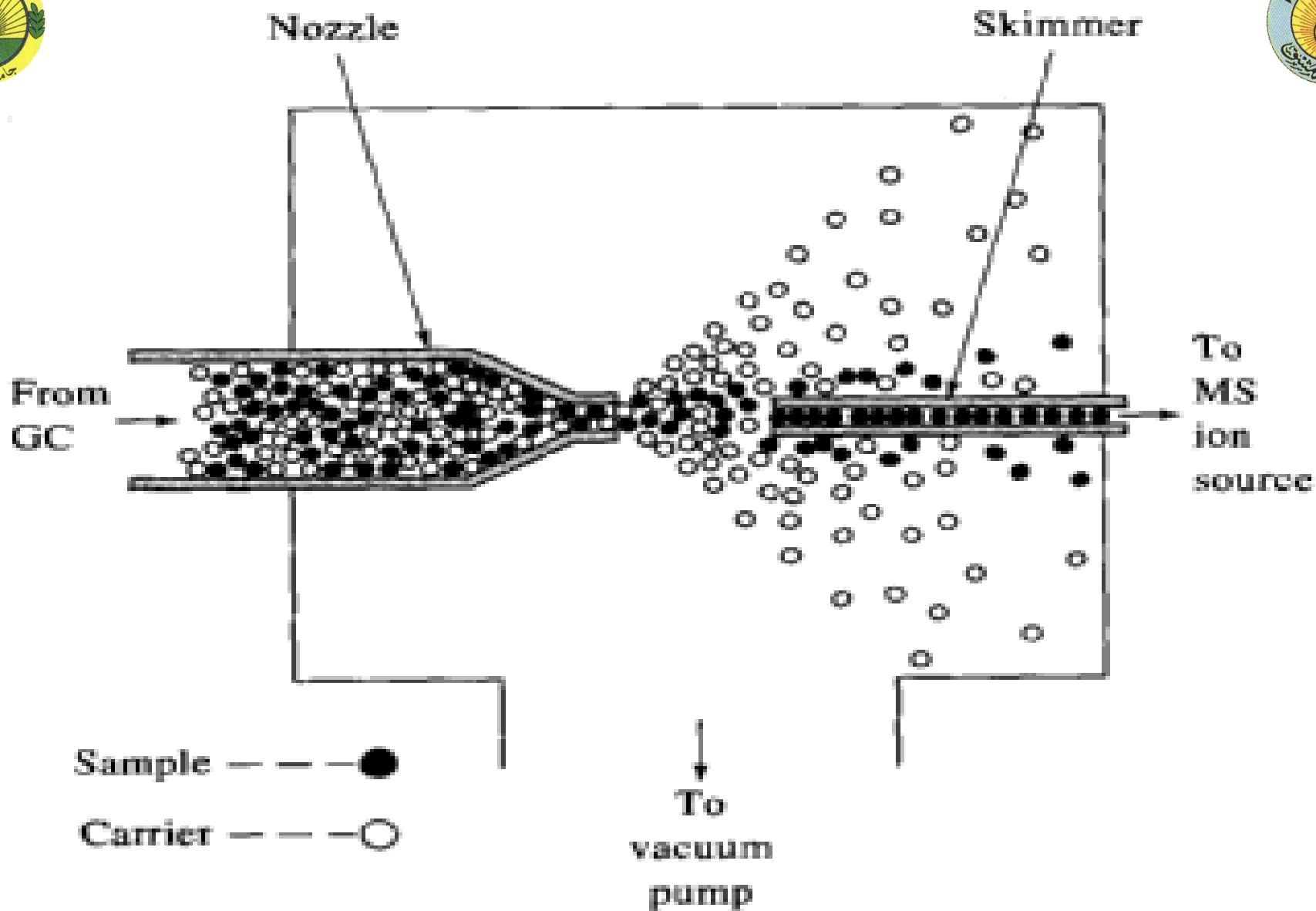
# Gas Chromatography/Mass Spectrometry (GC/MS)



- *One type of the hyphenated method.*
- *( low pressure and high sample concentration is necessary before coupling)*
- *The flow rate from CC is generally low enough that the column output can be fed directly into the ionization chamber of the MS.*
- *For packed columns ,a jet separator ,most be employed.*



Schematic of a typical capillary gas chromatography/mass spectro-



Schematic of a jet separator.

***Gas chromatography/mass spectrometry***  
**The use of suitable columns -**  
**(capillary or semi-capillary) allows -**  
**the end of the column to be introduced -**  
**directly into the source of the-**  
**apparatus without using a separator**

hourri

# Total Ion Chromatogram

So exactly how *is* the chromatogram generated in GC/MS?

117  
Proble



# Total Ion Chromatogram

**The answer involves a method of generating a signal that varies, like an FID or ECD detector, with the amount of analyte eluting from the GC column.**







# Total Ion Chromatogram

**Each MS scan involves a tabulated list of the mass detector's signal for each  $m/z$  fragment. All of these intensities of each scan are added together and they yield...**



# Total Ion Chromatogram

**the total ion current (TIC), a detector signal that varies over time, the typical components of a chromatographic plot: signal versus time. (Note that the total ion chromatogram is also often abbreviates as TIC.)**

# Total Ion Chromatogram

Surri

Data from one MS scan

m/z	Intensity
30	3005
29	2385
28	10050
27	4856
26	2993
25	1534
15	1723
14	1543
13	1195
12	0805

The ratios of these are constant for a given ionization process (EI, CI, etc.) and, **most important**, for a given compound.

30089

Total ion current (TIC) for a particular scan (a single datum)

Prof. Dr. ...

# Total Ion Chromatogram

Data from one MS scan

m/z	Intensity	Normalized Abundance
30	3005	29.9%
29	2385	23.7%
<b>28</b>	<b>10050</b>	<b>100.0%</b>
27	4856	48.3%
26	2993	29.8%
25	1534	15.3%
15	1723	17.1%
14	1543	15.4%
13	1195	11.9%
12	0805	8.0%

Most mass spectra are normalized. All peaks are ratioed to the **base peak**, the most intense peak, and expressed as a % of the base peak.

30089

← Total ion current (TIC) for a particular scan (a single datum)

# **Total Ion Chromatogram & Mass Spectra**

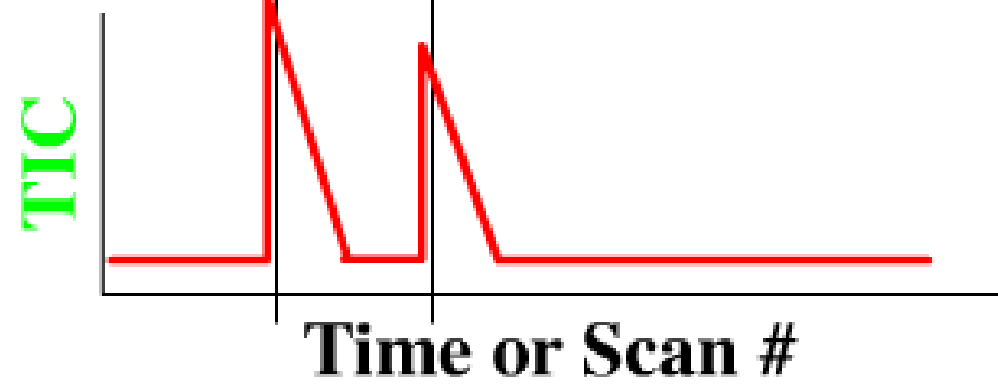
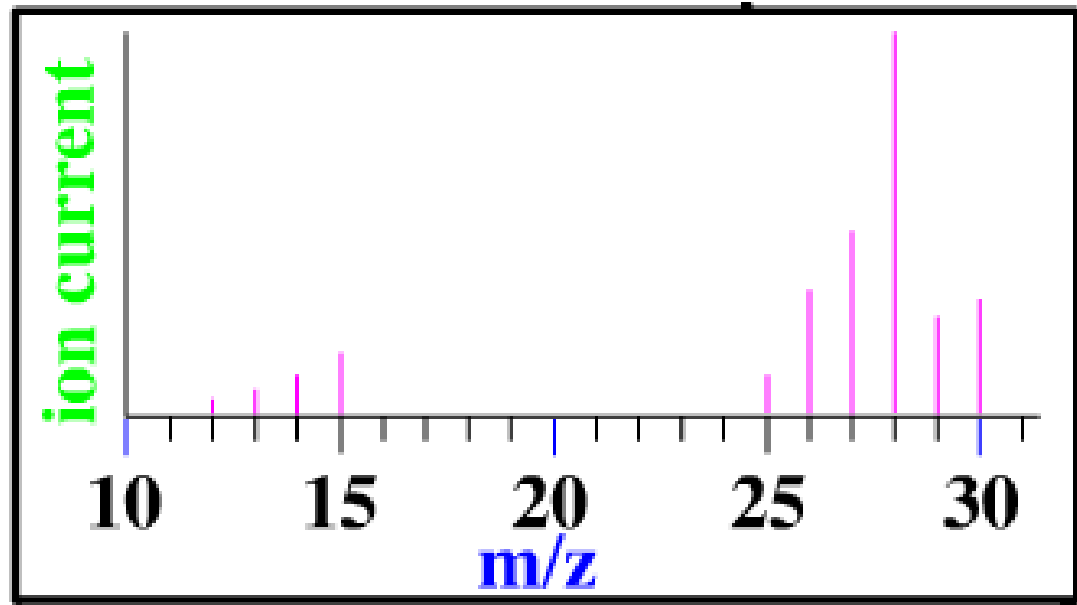
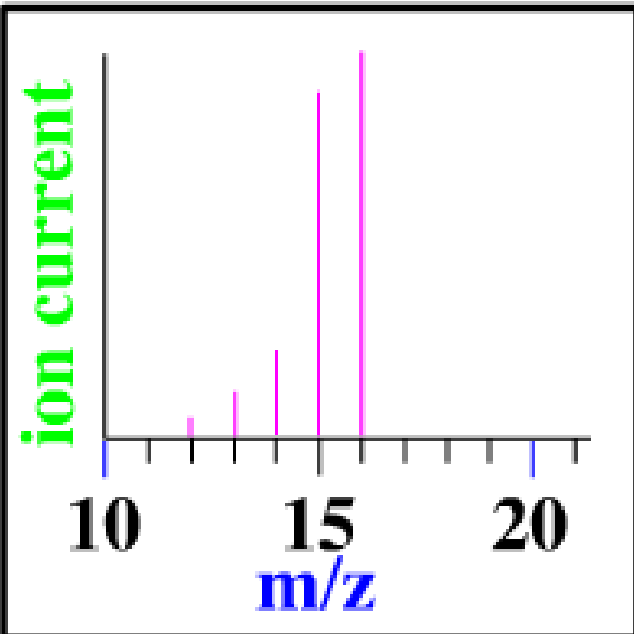
hourri

**Each GC/MS run generates a chromatogram (TIC versus time or scan number) like other GC chromatography but also a mass spectrum for *each* scan--often literally hundreds of mass spectra in a normal run. The chromatographic signal via the MS scanning start when the ionization source is turned on.**

## **Total Ion Chromatogram & Mass Spectra**

auri

**To reiterate: GC/MS chromatography yields a familiar chromatogram and the success of separation — the first job of a chromatographer — can be confirmed. But there is another dimension of data: each MS scan can be examined individually using the MS software.**





## LANSOPRAZOLE

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# ***Analytical Profiles of Drugs Substances and Excipients – Volume 28 /2001 P( 117-153)***



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## 6. Pharmacology, Metabolism, and Pharmacokinetics

### Acknowledgment

### References



## 1. Description

### 1.1 Nomenclature

#### 1.1.1 Systematic Chemical Names [1-3]

2-(2-Benzimidazolylsulfanylmethyl)-3-methyl-4-(2,2,2-trifluoroethoxy)pyridine.

2-[[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridinyl]methyl]-sulfanyl]-1H benzimidazole.

2-[4-(2,2,2-Trifluoroethoxy)-3-methyl-2-pyridinylmethylsulfanyl] 1H benzimidazole.

3-[[3-Methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]-sulfanyl benzimidazole.

#### 1.1.2 Nonproprietary Name

Lansoprazole

#### 1.1.3 Proprietary Names [1-3]

Agepton, Esamite, Dakar, Lansox, Lansor, Limpidex, Ognol, Opiren, Prevacid, Prezal, Takepron, Zoton

## 1.2 Formulae

### 1.2.1 Empirical Formula, Molecular Weight, CAS Number

C<sub>16</sub>H<sub>14</sub>F<sub>3</sub>N<sub>3</sub>O<sub>2</sub>S [MW = 369.363]

CAS number = 103577-45-3

### 1.2.2 Structural Formula

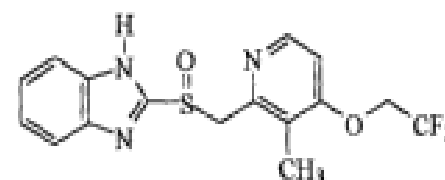


Figure 9. The mass spectrum of Lansoprazole.

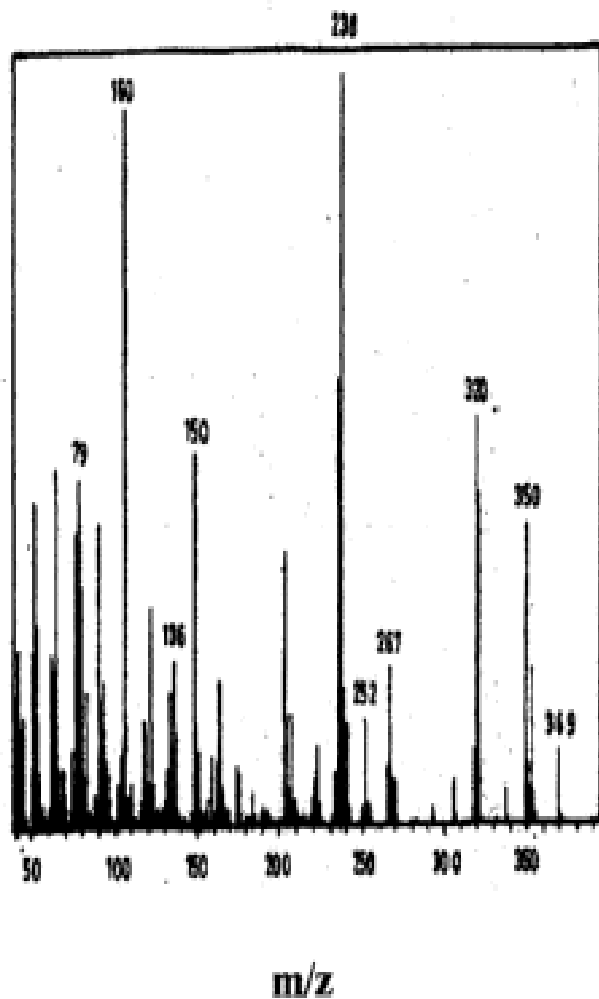


Table 4

Summary of Assignments for the Fragmentation Ions Observed in the Mass Spectrum of Lansoprazole

m/z	Relative intensity	Fragment
369	9%	
350	39%	
320	52%	
319	9%	
293	2%	
268	6%	

**Q&A**

**MS**



***Thank you***

# Q&A

Prof. Dr. Joumana Al-Zehouri



*Gas chromatography/mass spectrometry* - The use of suitable columns (capillary or semi-capillary) allows the end of the column to be introduced directly into the source of the apparatus without using a separator.

*Liquid chromatography/mass spectrometry* - This combination is particularly useful for the analysis of polar compounds, which are insufficiently volatile or too heat-labile to be analyzed by gas chromatography coupled with mass spectrometry. This method is complicated by the difficulty of obtaining ions in the gas phase from a liquid phase, which requires very special interfaces such as:



## *Molecular Mass Spectrometry.*

• Mass spectrometry is capable of providing information about :

- 1-  $M_w$
- 2- the element composition of samples of mater.
- 3- the structures of inorganic, organic and biological molecules.
- 4- the qualitative and quantitative composition



# Mass Spectroscopy

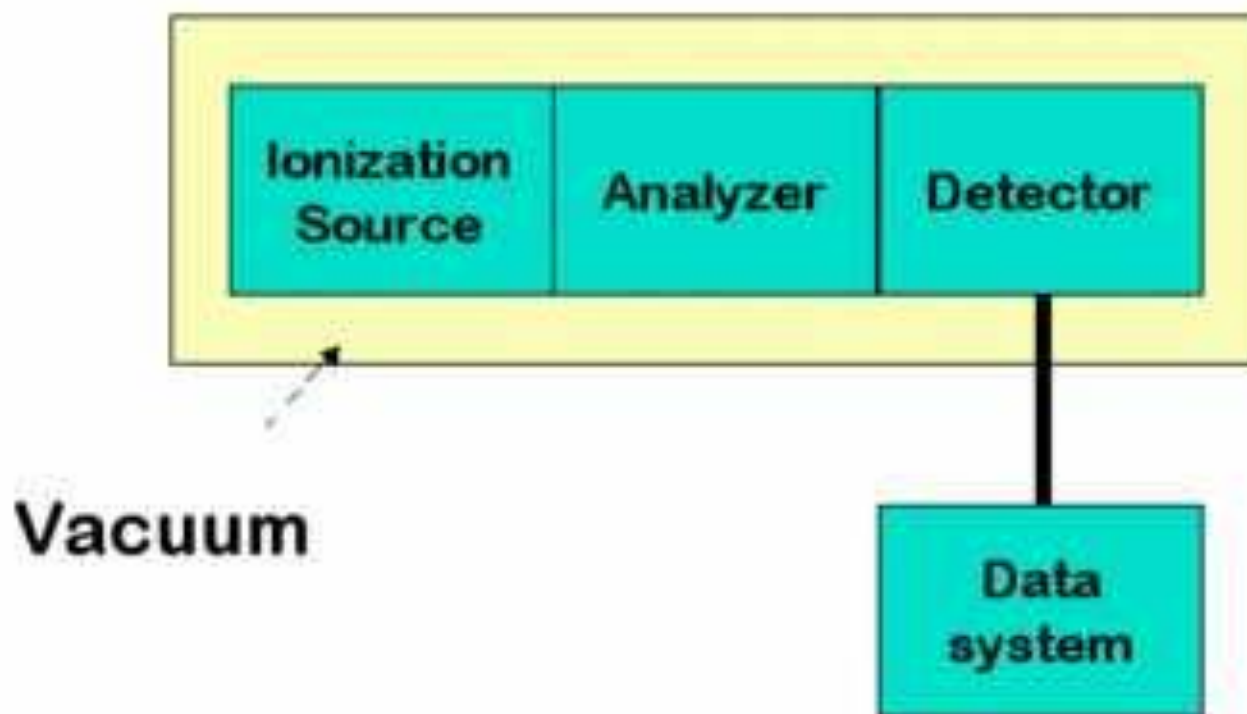
This is a powerful tool for the identification of materials.

- It relies on the production of ions from a parent compound and the subsequent characterization of the patterns that are produced.
- While it is a qualitative tool, it can be coupled with other methods.
- We'll look at some of the basic equipment and types of data obtained.



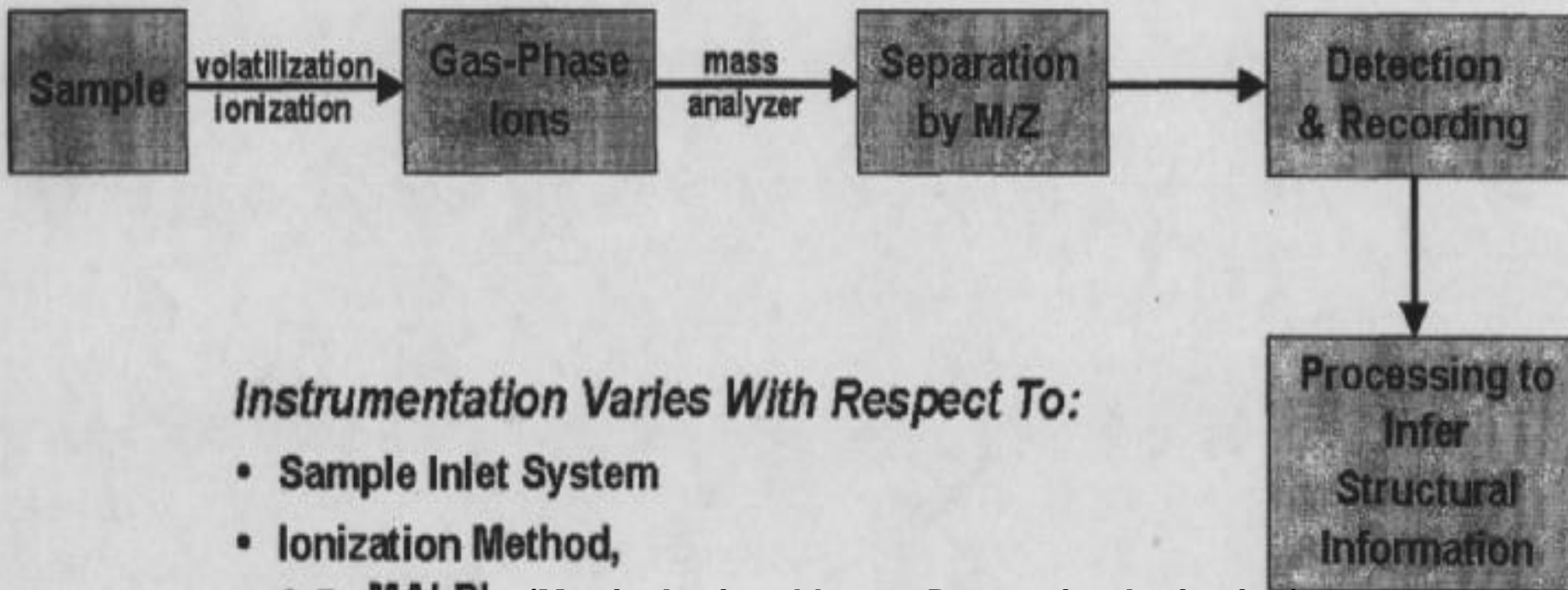


# MS block diagram





# MASS SPECTROMETRY

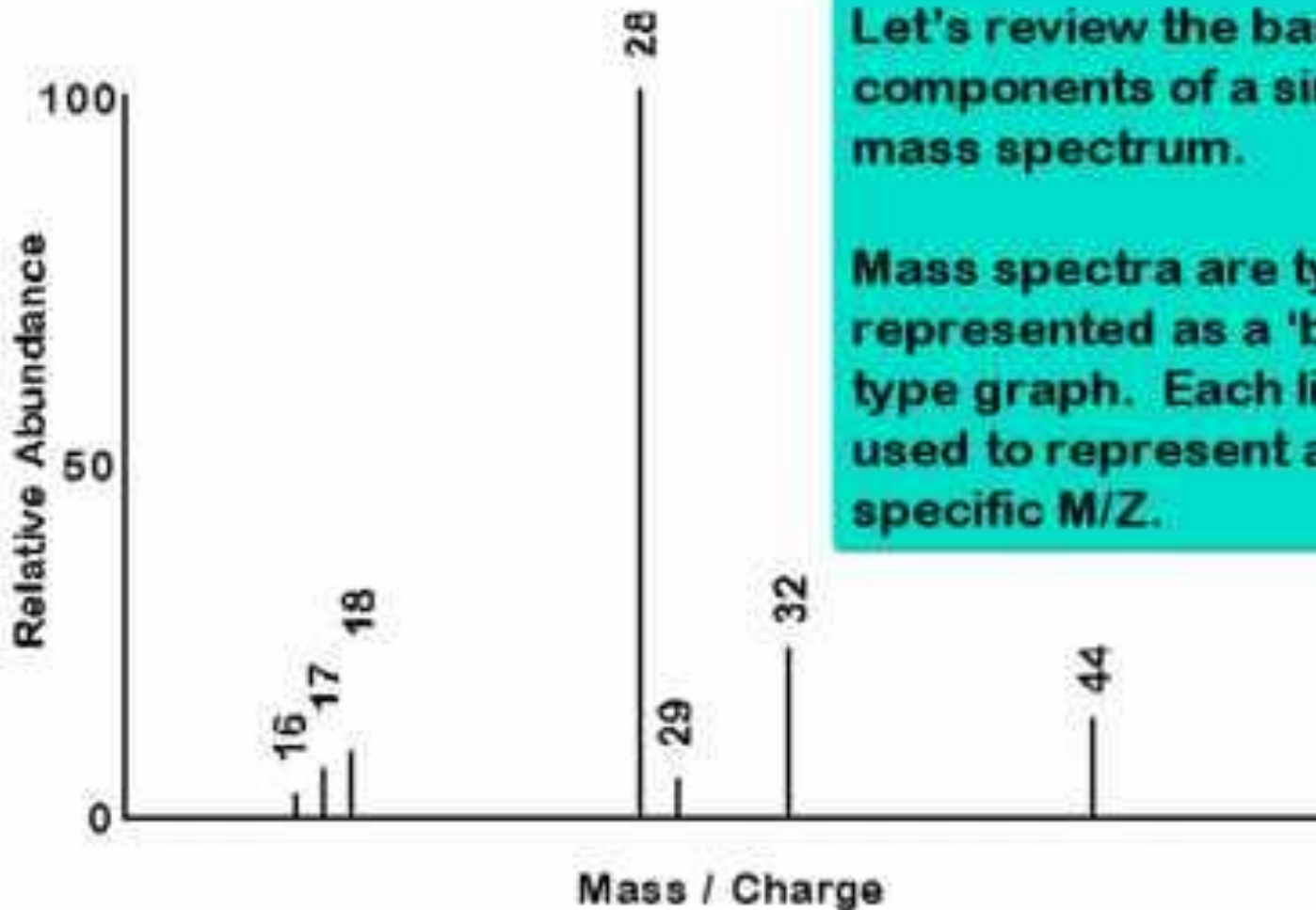


*Instrumentation Varies With Respect To:*

- Sample Inlet System
- Ionization Method, e.g., MALDI (Matrix-Assisted laser Desorption Ionization)
- Mass Analyzer, e.g., Quadrupole



# What is a mass spectrum

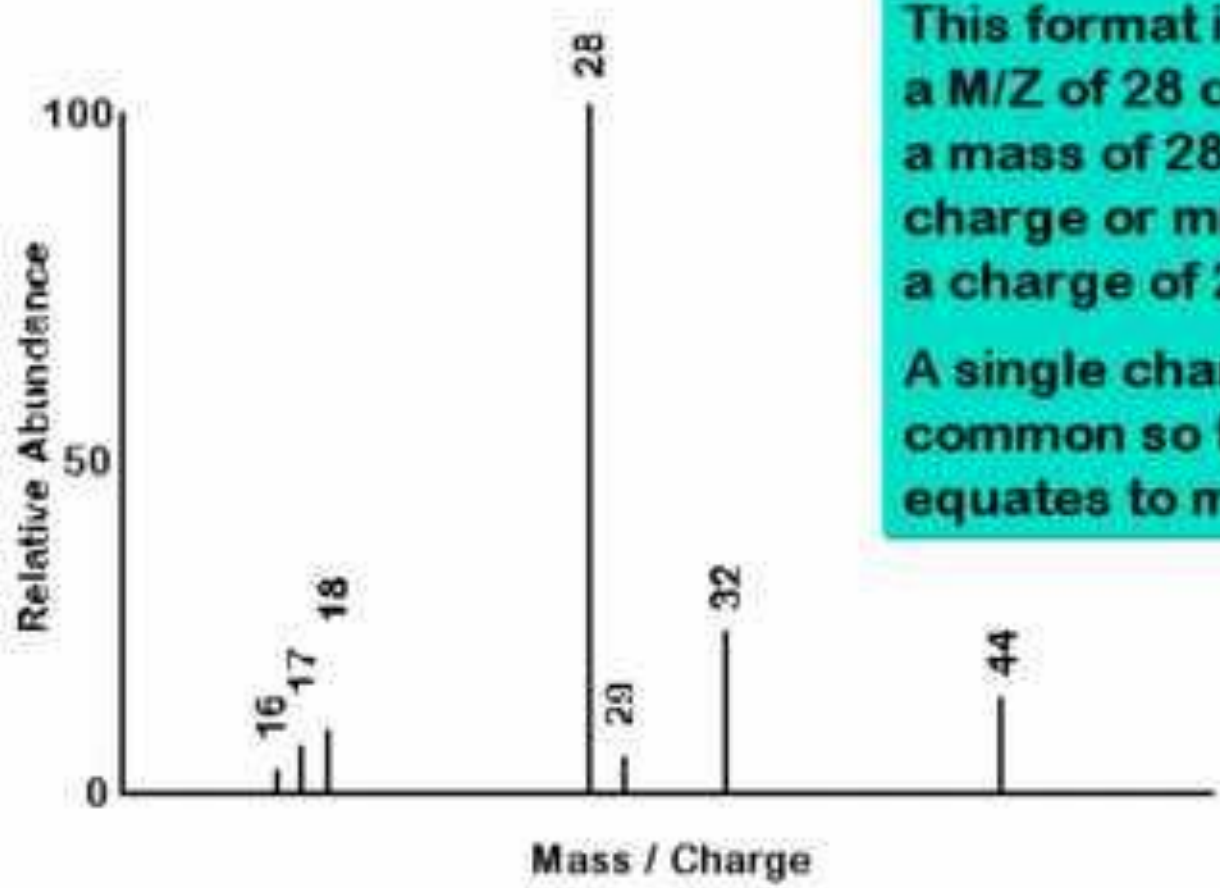


Let's review the basic components of a simple mass spectrum.

Mass spectra are typically represented as a 'bar' type graph. Each line is used to represent a specific M/Z.



# A simple mass spectrum



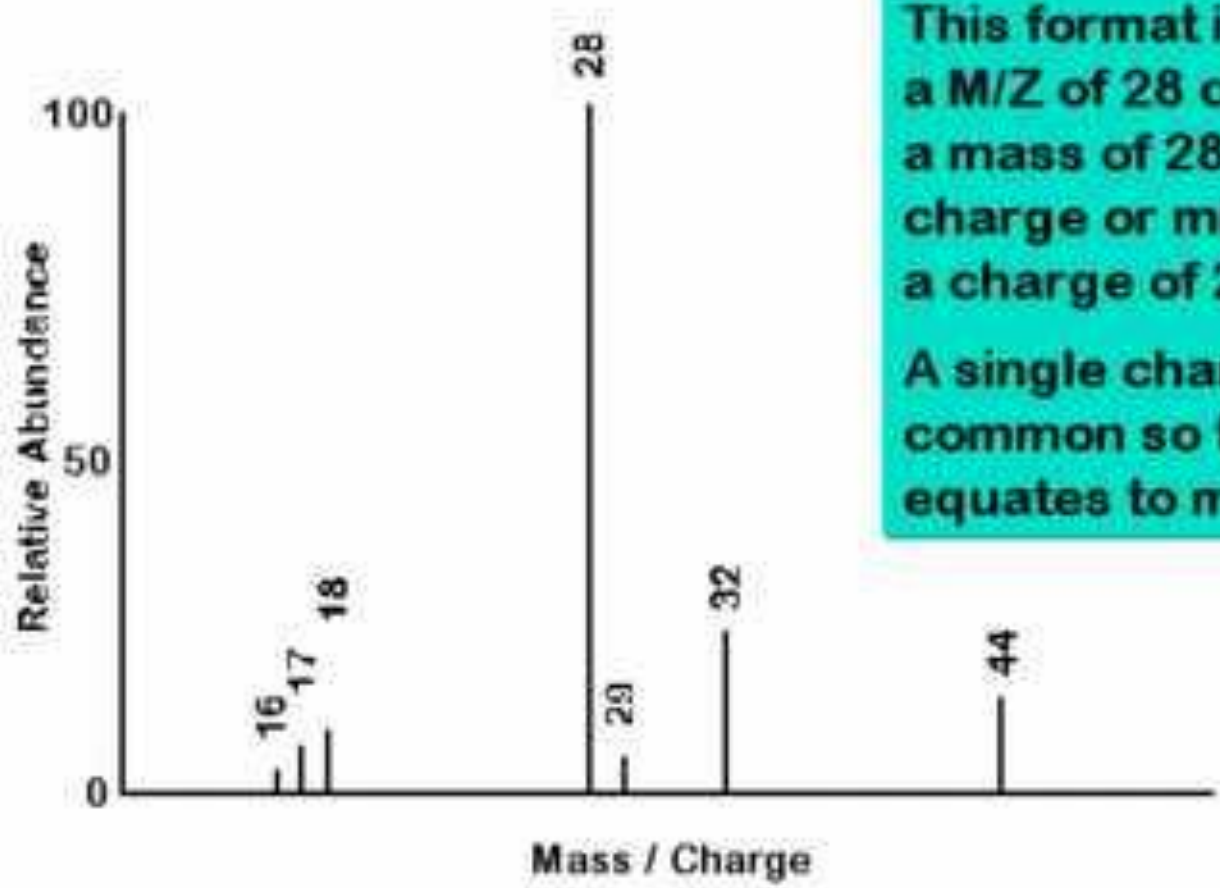
**X axis - Mass / Charge - (M/Z)**

This format is used because a M/Z of 28 can represent a mass of 28 with a single charge or might be 56 with a charge of 2, .....

A single charge is the most common so the X axis best equates to mass.



# A simple mass spectrum



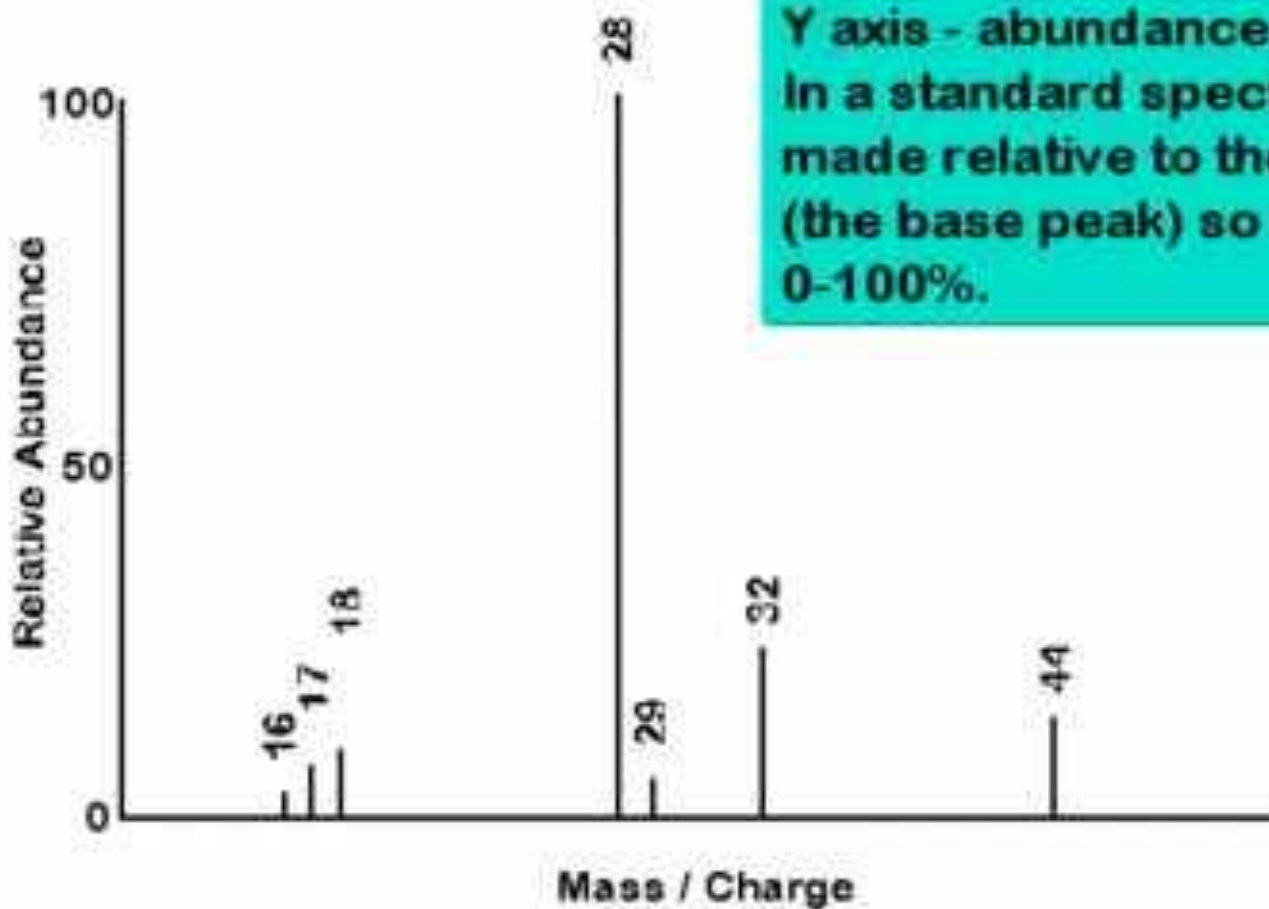
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A single charge is the most common so the X axis best equates to mass.



# A simple mass spectrum



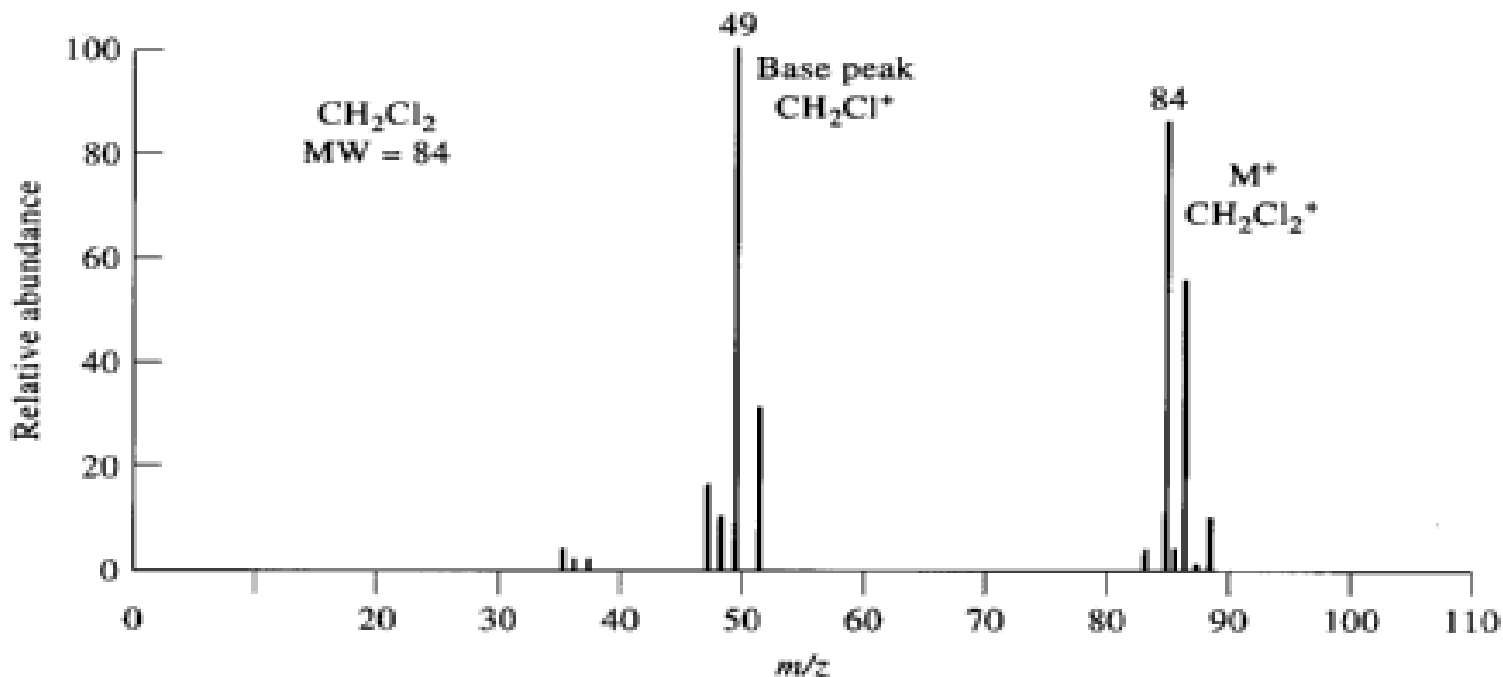
Y axis - abundance.  
In a standard spectrum, the is made relative to the largest line (the base peak) so it will run from 0-100%.



534

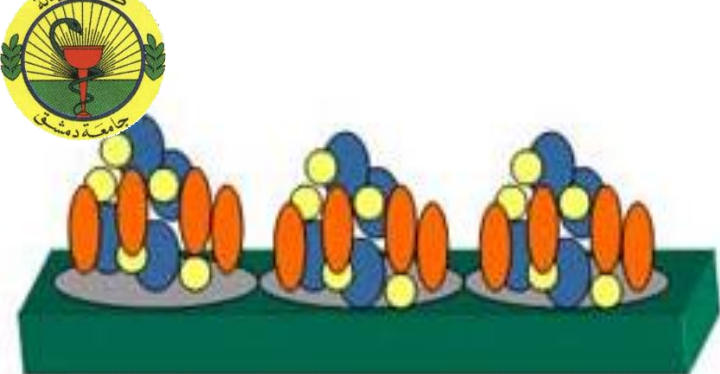


Identify the ions responsible for the four peaks having greater masses than the  $M^+$  peak in the following Figure :

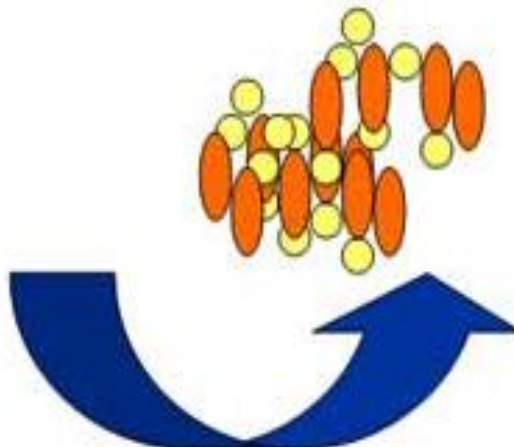


A

$m = 84$  due to  $^{35}\text{Cl}_2^{12}\text{CH}_2^+$ ,  $m = 85$  due to  $^{35}\text{Cl}_2^{13}\text{CH}_2^+$ ,  
 $m = 86$  due to  $^{37}\text{Cl}^{35}\text{Cl}^{12}\text{CH}_2^+$ ,  $m = 87$  due to  $^{37}\text{Cl}^{35}\text{Cl}^{13}\text{CH}_2^+$ ,  
 $m = 88$  due to  $^{37}\text{Cl}_2^{12}\text{CH}_2^+$



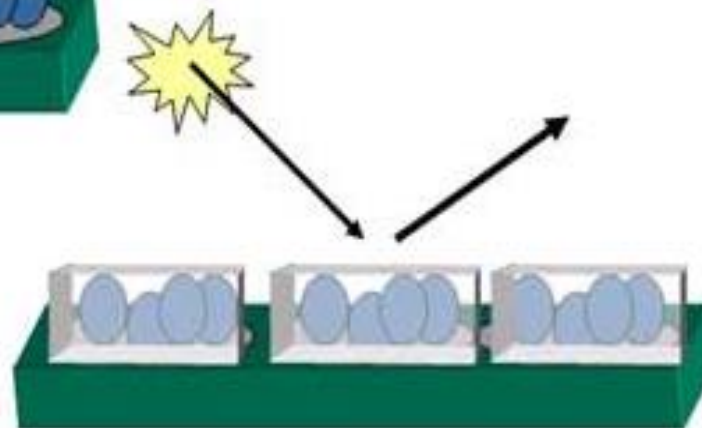
1) Sample applied to ProteinChip.



2) Nonspecifically bound proteins washed away.

EAM = sinapinnic acid

3) EAM is applied to each sample. A laser is fired that desorbs and ionizes the proteins in the EAM.



4) Ions are detected and mass of each protein calculated by time-of-flight mass spectroscopy.







Q

At what  $m/e$  values will the molecular ions of  $C^{12}H_4$  and  $C^{13}H_4$  appear?

A-

$m/e$  16 (called M)

$m/e$  17 (called M + 1)

The symbol M is used to denote the molecular ion containing the most abundant isotopes of the elements present.