برنامج المسار الوظيفي للعاملين بقطاع مياه الشرب والصرف الصحي

دليل المتدرب

البرنامج التدريبي كيميائي مياه

Basic Courses In Organic Analysis - الدرجة الثالثة

تم اعداد المادة بواسطة الشركة القابضة لمياه الشرب والصرف الصحي
قطاع تنمية الموارد البشرية - الادارة العامة لتخطيط المسار الوظيفي V1 1-7-2015

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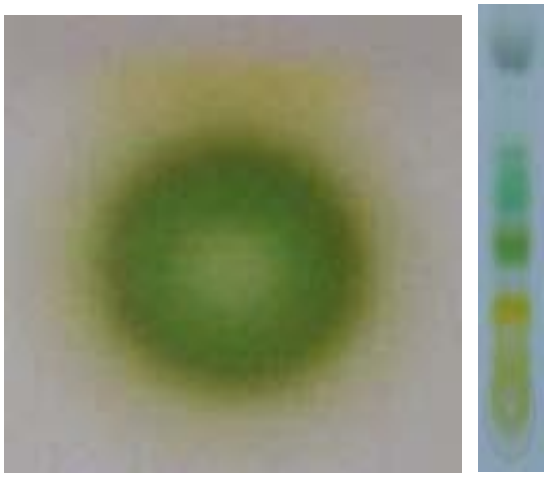
Gas Chromatography
1-What is Chromatography?

It is technique used for separation a mixture of different compounds using certain stationary phase (column) carrying by certain mobile phase.

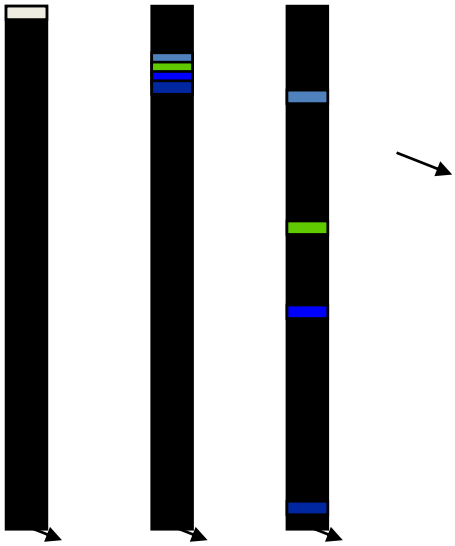
Or it is the process (Adsorption) in which the mix of components to be separated by distributed between two phase one called mobile phase and other called stationary phase.

SO the Chromatography in its simplest form is like putting ink on blotting paper and watching the colors separate.



2-What is the History of Chromatography?

First classic (LC) was carried out by scientific Russian called Tswett in 1903 by separation plant pigments using glass packed column consist of powdered calcium carbonate and alumina as stationary phase calling (Precipitated Chalk) and Hexane as mobile phase and the flow was gravity so the pigment separate in different colors in the column which occur due to some pigments move faster than other due to related to different compounds, then he take from Greeks words two word!! First **Chroma** mean color and **graph** mean writing (Color writing)



3-How can we decide using GC or LC?

First to answer this question we should know the different between them.

The first different is the mobile phase

Which is in GC is Gas and in LC is Liquid and it appear Cleary from the name of two instruments!!

Second different is the column

In GC it is capillary column and the mixture need to separate carrying by gas and the separation depends on the different in temperature called (thermal program)

In LC is packed column is made from bare or bonded silica; it separates a mixture of compounds by how polar they are. We can use a gradient of different solvents for different polarity.

"**Column**" which is made from bare or bonded silica, it separates a mixture of compounds by how polar they are. We can use a gradient of different solvents. So if the column in non-polar, the compound to elute first will be the most polar.

So our decision will be:

For GC analysis, a compound must have sufficient volatility and thermal stability.

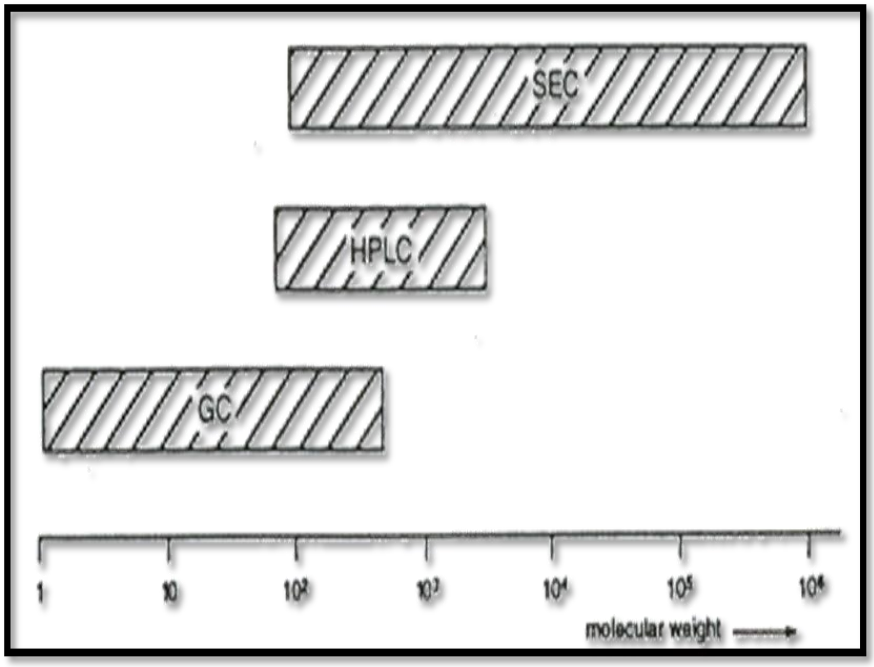
- If the species in a mixture are in the gas or vapor phase at 400 – 450 °C or below, and they do not decompose at these temperatures, the mixture can probably be analyzed by GC.(more volatile).

For LC a compound must has polarity to can be separated

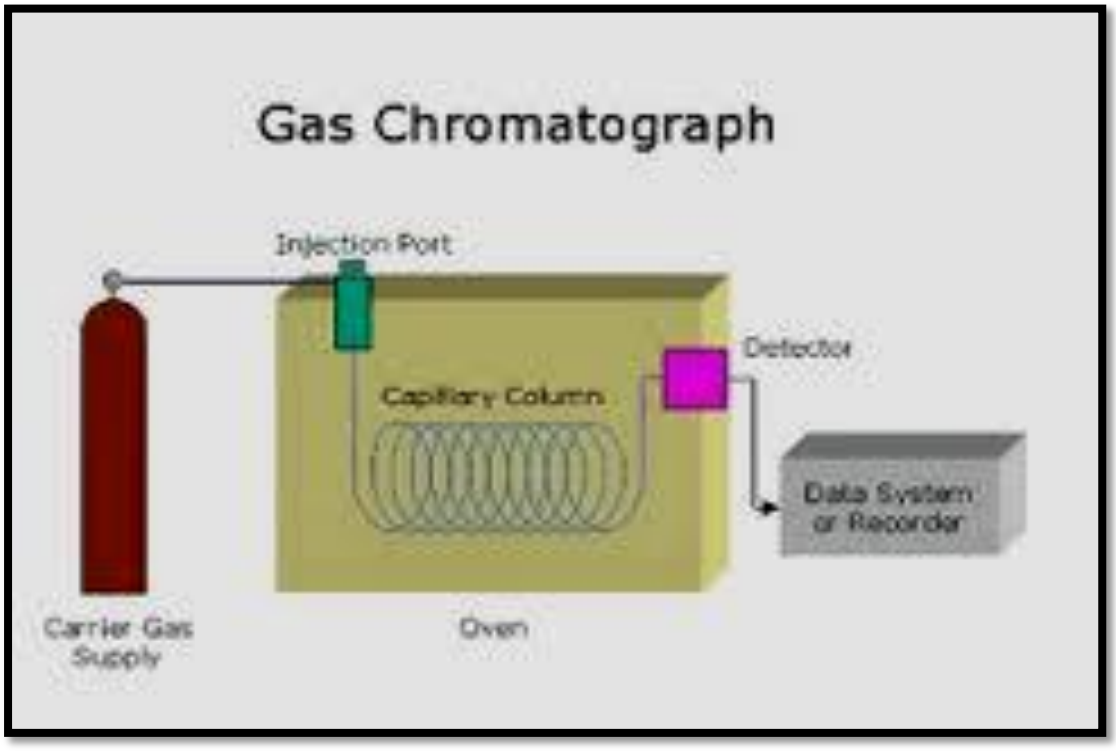
- If species in a mixture are not easily transformed into the gaseous phase or not thermally stable LC is likely to be appropriate(less volatile).

Chromatographic Areas
4-What the GC consist of?

1- Sample introduction
2- Column Oven and column
3- Detector



Schematic GC System



What the sample introduction consist of

1-Auto sampler
2-Injection port

1- Auto sampler

The auto-sampler consists of:

A-Tray

It is multi vials position in which the vials are arranged.

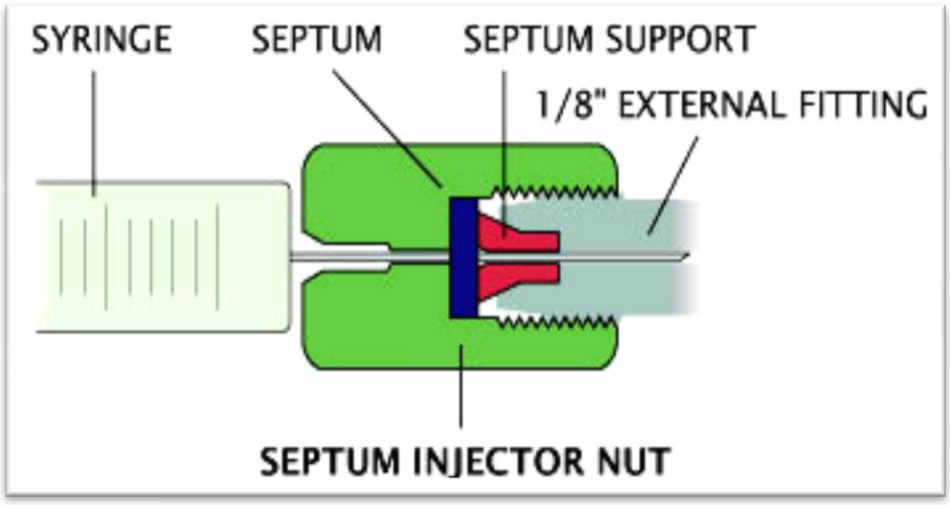
B-Injector tower

It is mechanical motion by which the sample introduction into the injection port and it consist of (Syringe for drawing sampling & Motor for mechanical motion).

2- Injection port

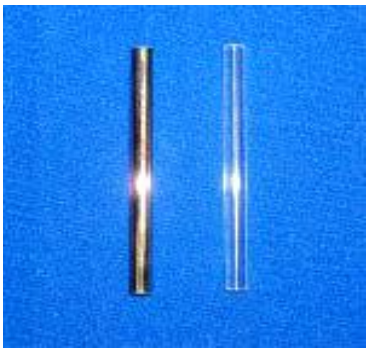
It is a port that transferee the sample to the column which consists of:

1- Septum which is from silica rubber and chemically inert prevent the sample get out from the glass liner.



2- Glass liner

Which coated to be inert and it had different shape according to the application like (line with goose nick, line with two goose nick, straight line and line with or without glass wall).



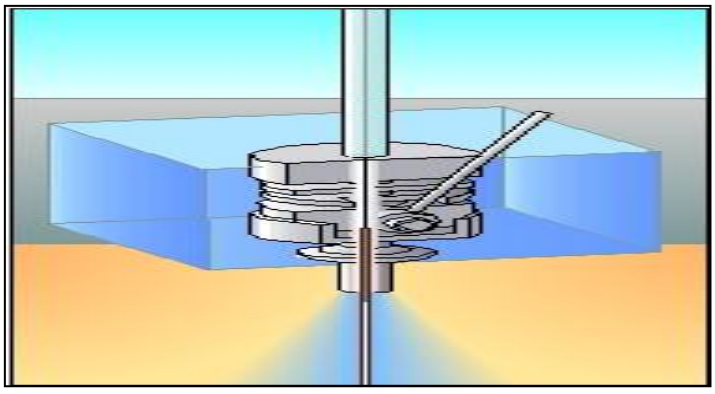
3- Heater

Very impotent to evaporate the sample before going to column it temperature from 50°C to 300°C).

- **Types of injection on GC**

1- On column

1- Were the sample inject directly on column without any effecting of injection port.
2- Excellent Quantitative Results.
3- No Discrimination.
4- Suited for trace Analysis.
5- No Septum.
6- No Thermal Decomposition.
7- Narrow injection Band by Solvent Focussing.
8- Not Suitable for “dirty” samples.



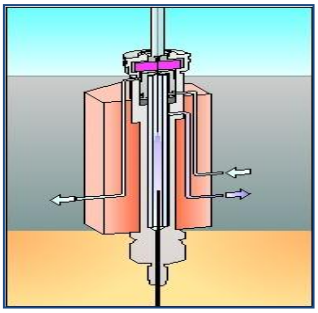
On-column

2- Split/Splitless Injector

Which had two mode:

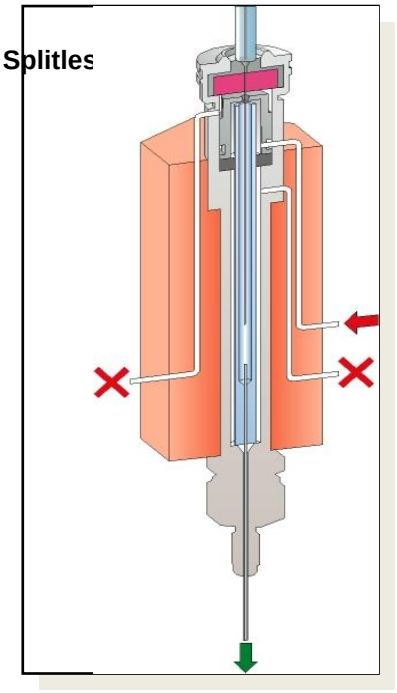
Frist Split injection

1- Narrow Injection Band
2- Elimination of Pollution
3- Uniform
4- Not for trace analysis
5- Critical Design
6- Discrimination of high boiling compounds

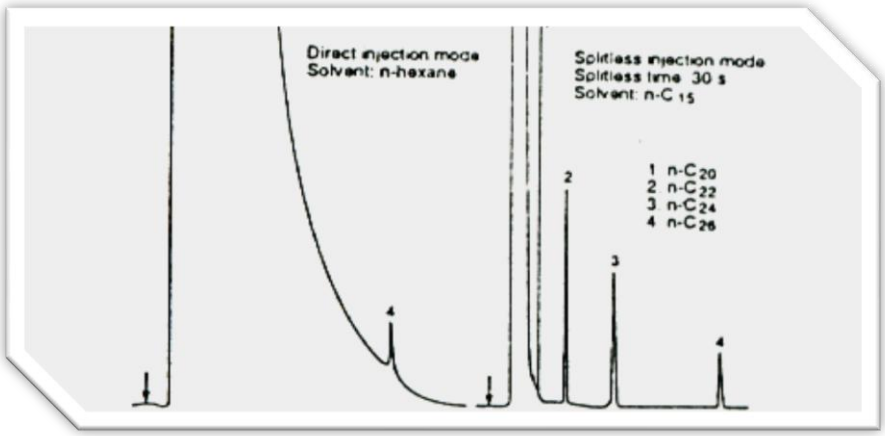


Second Splitless Injection

1- Suited for trace analysis
2- Narrow injection band by solvent trapping and cold trapping
3- Difficult to employ: trial and error
4- Discrimination of high boiling compounds
5- Only suitable with chemically bonded phases



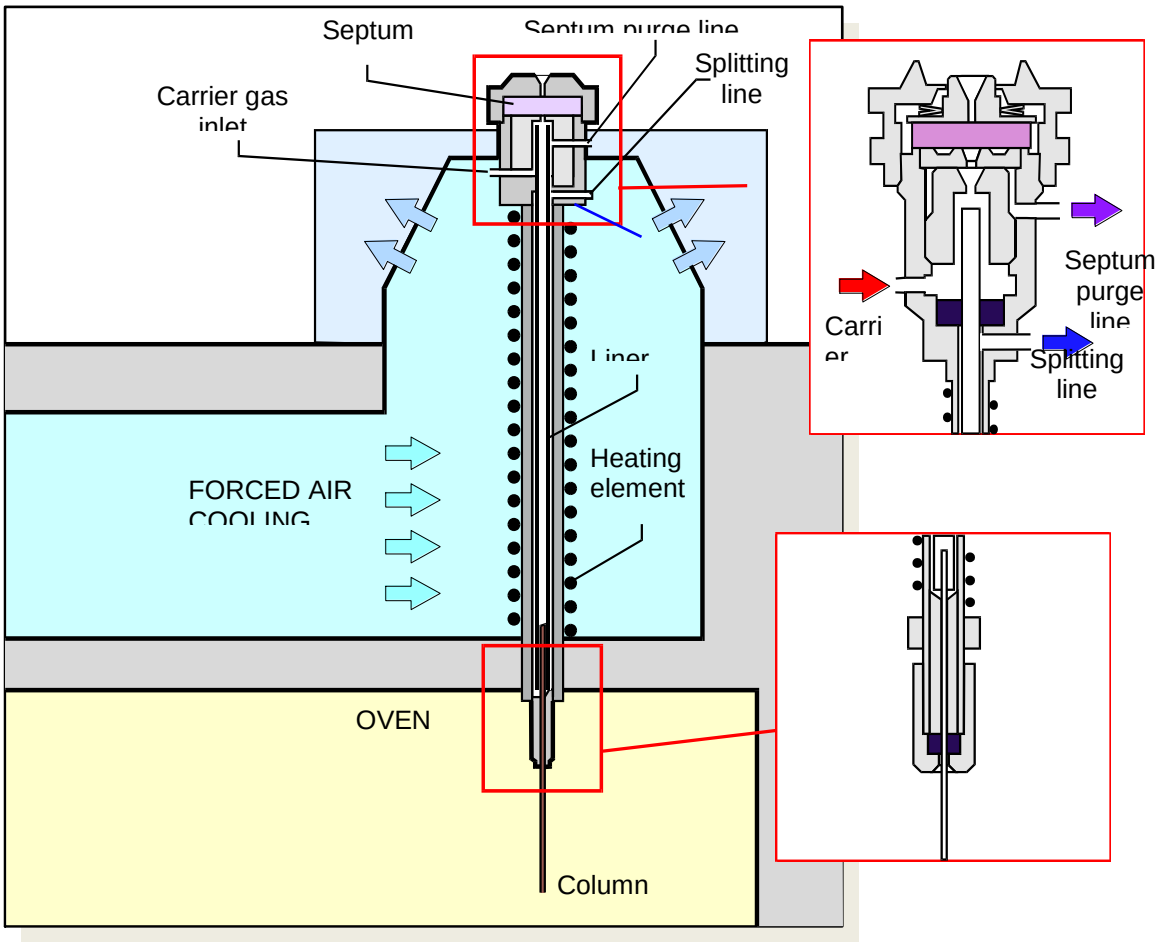
Chromatogram showing the difference between the two types of injection (Direct vs. Split less Injection).



A- Programmed Temperature Vapourisation (PTV)

This mode is universal it can use in hot or cold and Spilt or spiltless.

The most advantage it can progrmmed Temperature for sample injection before the column thermal program and also it help for injaction large volume for more sensitvty



Column and Column Oven

The GC column is the heart of GC and it consist of coil tube packed of stationary phase.

General specs of column

1- Low Bleed
2- Lower Detection Limits
3- Improved Quantitation
4- Improved Compound Identification
5- Less System Maintenance
6- Stable in high temp.

To get will know about column we need Define tow term in any column must be consist of:

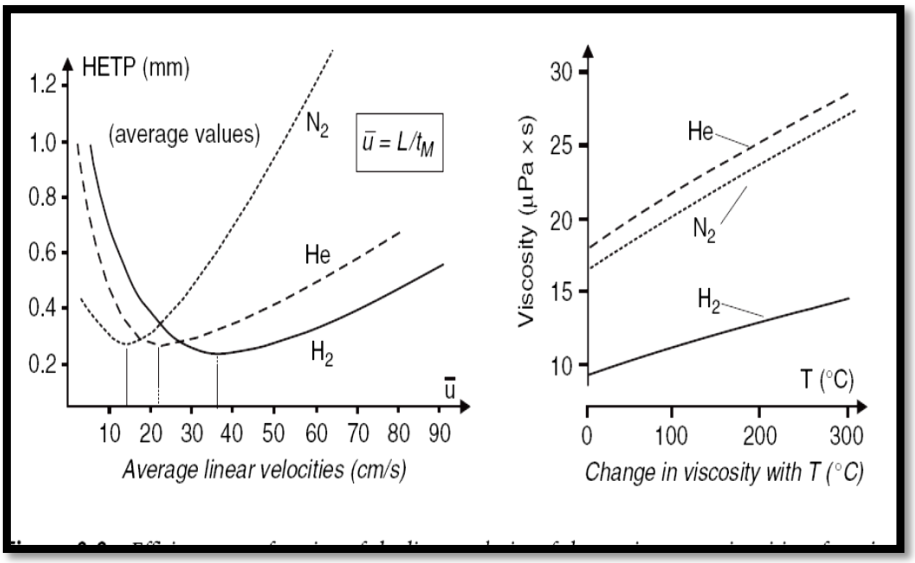
1- **Mobile phase**: The fluid which carry out the components along the stationary phase to the detector and t may be Gas(Helium or Hydrogen & methane or Nitrogen)or Liquid.
2- **The Stationary**: The bed in which happened adsorption for the components on it and it may be solid or gel or liquid an if liquid must be distributed on solid.

- **The GC Mobile phase**

The mobile phase in GC is Gases!! It may be (Helium, Hydrogen, Nitrogen and Argon/methane) it can be drawn from gas cylinder or obtained in Hydrogen or Nitrogen from onsite generators.
The carries gas must be free from hydrocarbon, water and oxygen because all of them may damage the stationary phase and decrease the sensitivity of the detectors.
For this reason the line of carriers' gases and all gases in GC like make up gases or gases using in detectors supplying by filter contains a molecular sieve for removing the impurities.

The relationship between the viscosity and Linearity

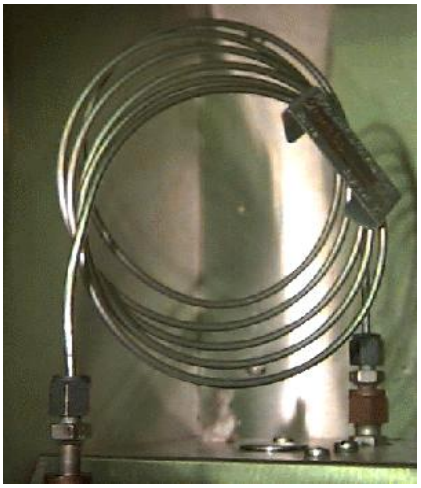
The viscosity of gas and its flow affecting on the analyte dispersion in the stationary phase and their diffusion on the mobile phase.
The best gas for separation is hydrogen, but due to safety considerations, it is preferable to use Helium.
By increasing the temperature, the viscosity of gas will increase, these will effect on the flow rate of carrying gas (Increase) and also it will effect on the pressure, so we need to maintain the flow rate constant during the change of Temperature which called **Electronic Pressure Control (EPC)**



Types of Columns in Gas Chromatography:

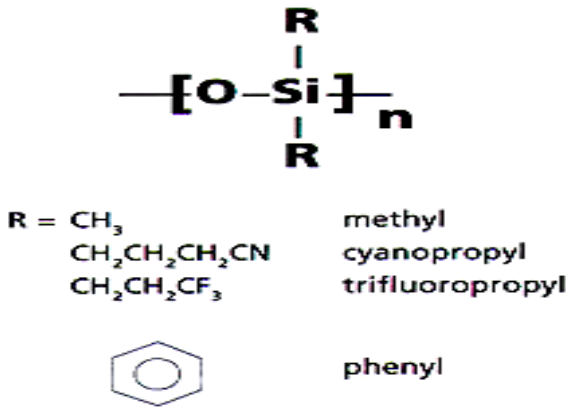
1. **Conventional packed column**

- The former are usually 2 to 4 mm I.D. and 1 to 4 meters long and, packed with a suitable adsorbent, are mostly used for gas analysis.
- Packed columns are usually constructed from stainless steel or Pyrex glass.
- Pyrex glass is favored when thermally labile materials are being separated such as essential oils and flavor components. However, glass has pressure limitations and for long packed columns, stainless steel columns are used as they can easily tolerate the necessary elevated pressures.



- The sample must, of course, be amenable to contact with hot metal surfaces. Short columns can be straight, and installed vertically in the chromatograph. Longer columns can be U-shaped but columns more than a meter long are usually coiled. Such columns can be constructed of any practical length and relatively easily installed.
- Pyrex glass columns are formed to the desired shape by coiling at about 700°C and metal columns by bending at room temperature.
- Glass columns are sometimes treated with an appropriate silanizing reagent to eliminate the surface hydroxyl groups which can be catalytically active or produce asymmetric peaks. Stainless steel columns are usually washed with dilute hydrochloric acid, then extensively with water
- Followed by methanol, acetone, methylene dichloride and n-hexane. This washing procedure removes any corrosion products and traces of

lubricating agents used in the tube drawing process. The columns are then ready for packing.



2. Capillary Column

The capillary column is the type which we use in trace analysis in organic compound in water

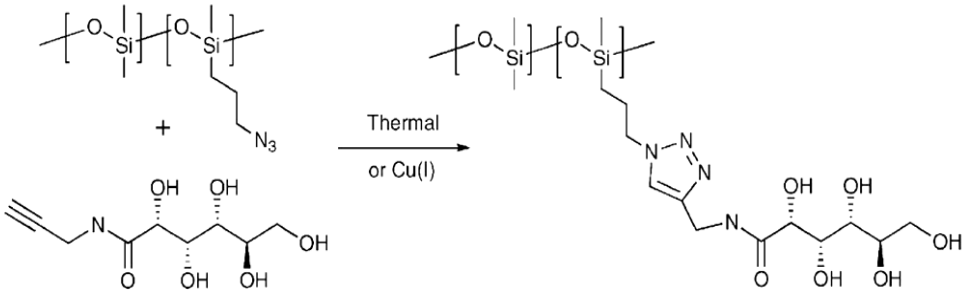
- The stationary phase in capillary column is limited because the generation of the film at the surface of column required a different principle of impregnation.

There are different type of stationary phase

1- Polysiloxames

- Based on repetitive back bone that consists of two hydrocarbon chain per silicon atom.
- There are about 20 different composition of alkyl & aryl chain (Methyl or Phenyl) to which can be incorporated further functional groups (e.g. Cyanopropyl ,trifluropropyl).

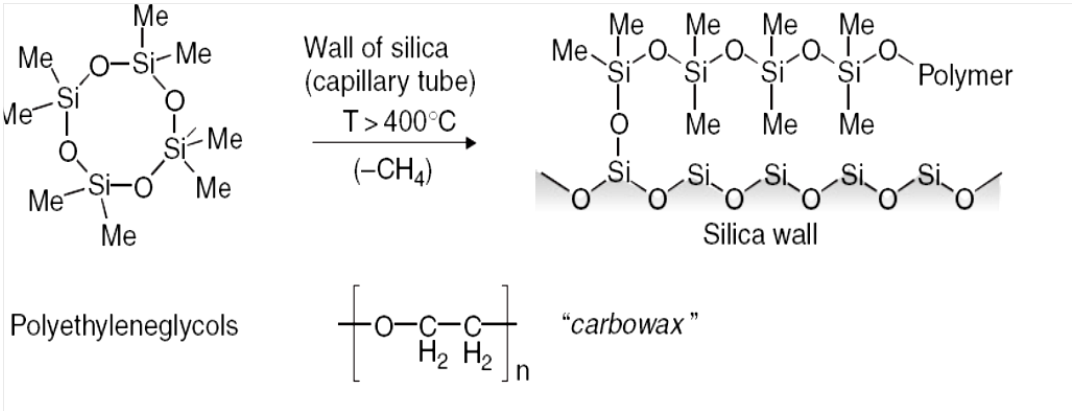
- Monomer Combined in variable proportions also convey change in the properties of stationary phase e.g. (Polarity & Temperature) from 50C to 300/350C for the dimethyl polysiloxanes.



2- Poly Ethylene Glycols

The best know representative at this family is Carbowax these is polar polymer

3- Chiral stationary Phase



These generally polysiloxane basic phase mixed with 10 to 20 percent by weight of B-cydodextrine (polysaccharide) this column suitable for racemic mixture.

4- Solid Stationary phase

These phase are constituted from variety of adsorbent materials as Silica or alumina which deactivated by minerals salts or molecular sieves or porous glass or graphite.

This column is made by the deposition of these materials in the form of porous layer are called (Plot) this column used in the separation of gases and volatile organic compounds and light hydrocarbon.

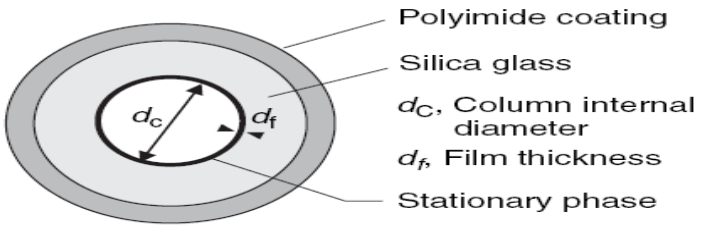


Figure 2.13 *Capillary column section*

How we can predict the behavior of column?

- We can predict by this equation
- The phase ratio (B)= dc/4df
- Where dc is the internal diameter of column
- And the df is the film thickness

So if the B < 100 this column suitable for volatile organic compounds

Types of GC Detectors

Classification of GC Detectors

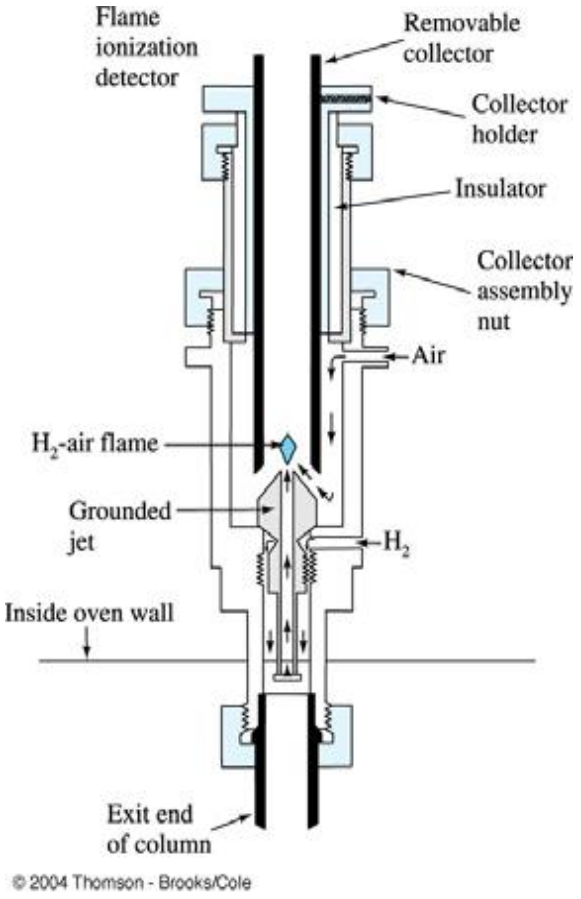
Gas Chromatographic Detectors

Type	Applicable Samples	Typical Detection Limit
Flame ionization	Hydrocarbons	0.2 pg/s
Thermal conductivity	Universal detector	500 pg/mL
Electron capture	Halogenated compounds	5 fg/s
Mass spectrometer	Tunable for any species	0.25–100 pg
Thermionic	Nitrogen and phosphorous compounds	0.1 pg/s (P) 1 pg/s (N)
Electrolytic conductivity (Hall)	Compounds containing halogens, sulfur, or nitrogen	0.5 pg Cl/s 2 pg S/s 4 pg N/s
Photoionization	Compounds ionized by UV radiation	2 pg C/s
Fourier transform IR	Organic compounds	0.2 to 40 ng

1. The Flame Ionization Detector

- The Flame Ionization Detector responds to any molecule with a carbon-hydrogen bond, but its response is either poor or nonexistent to compounds such as H_2S, CCl_4, or NH_3.
- Since the FID is mass sensitive, not Concentration sensitive, changes in carrier gas flow rate have little effect on the detector response.
- It is preferred for general hydrocarbon analysis, with a detection range from 0.1ppm to almost 100%.
- The FID's response is stable from day to day, and is not susceptible to contamination from dirty samples or column bleed.
- It is generally robust and easy to operate, but because it uses a hydrogen diffusion flame to ionize compounds for analysis, it destroys the sample in the process.
- This ignitor is positioned perpendicular to the stainless steel detector jet and does not penetrate the flame.
- Opposite this flame is the collector electrode.

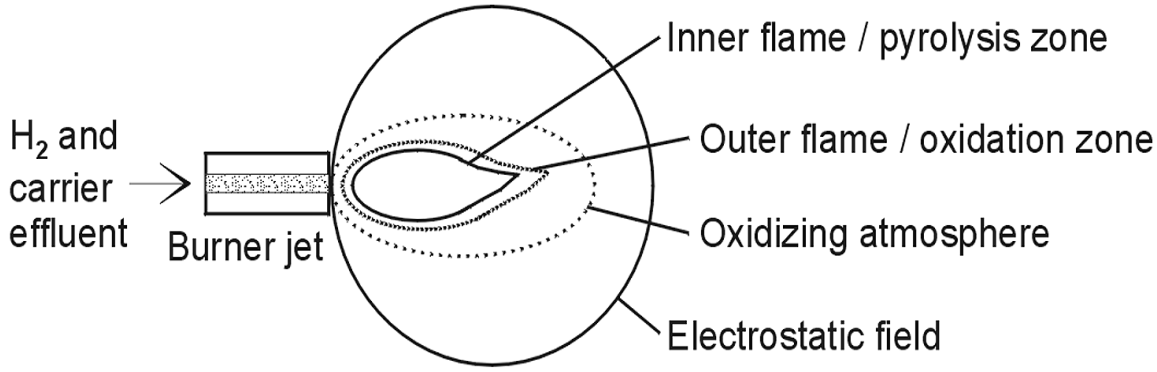
- A thermostatic aluminum heater block maintains stable detector temperature which is ushered just able up to 375°C.



Theory of Operation

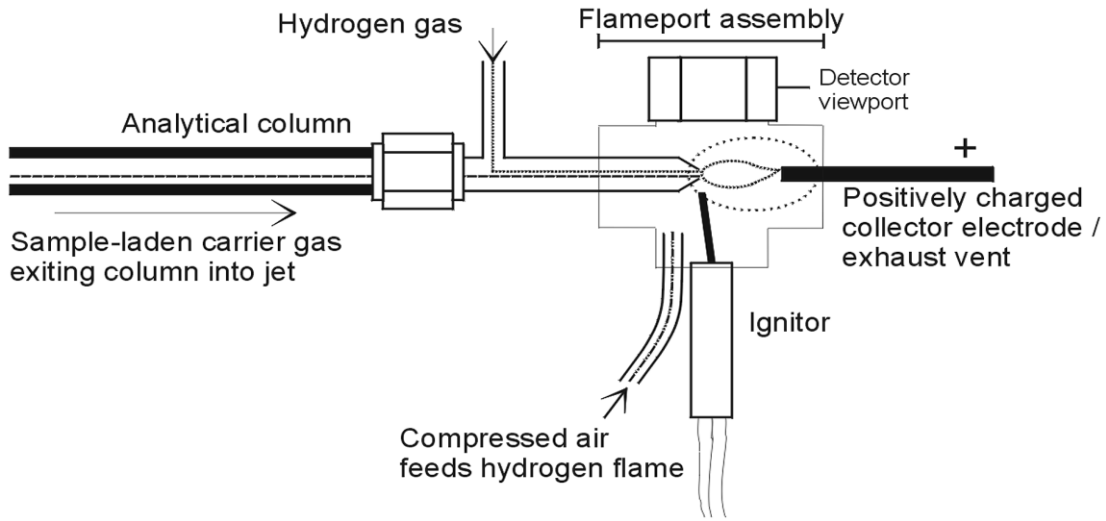
- In the FID, the carrier gas effluent from the GC column is mixed with hydrogen, and then routed through an unbreakable stainless steel jet.
- The hydrogen mix supports a diffusion flame at the jet's tip which ionizes the analyte molecules.
- Positive and negative ions are produced as each sample component is eluted into the flame.
- A collector electrode attracts the negative ions to the electrometer amplifier, producing signal for the data system input.
- An electrostatic field is generated by the difference in potential between the positively charged collector electrode and the grounded FID jet.
- Because of the electrostatic field, the negative ions have to flow in the direction of the collector electrode.

The FID hydrogen diffusion flame



- The ratio of air to hydrogen in the combustion mixture should be approximately 10:1.
- If the carrier flow is higher than normal, the combustion ratio may need to be adjusted.
- Flow is user adjusted through the Electronic Pressure Controllers (EPC)

FID detector schematic



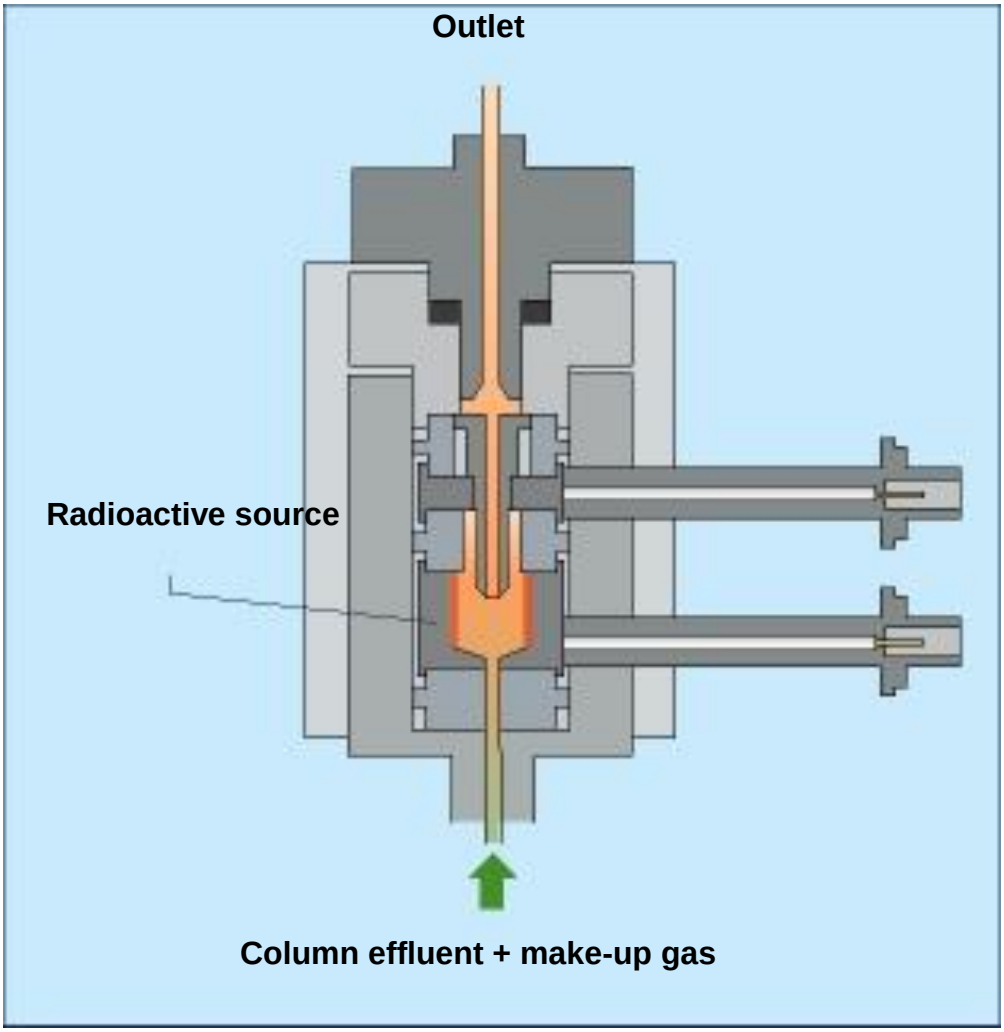
So we can summarize the FID

- Universal response
- Ionization detection
- Mass detector
- Destructive
- Very liner Dinamic range up to 8 magntiued
- Not sensitive in commen impruities in carries gas.
- Operating parameter
    - **Air flow**
- Supplied in stoichiometric excess
- The working value is in the range 300 - 600 ml/min
    - **Hydrogen**

- Adjusted to optimize the S/N ratio
- The working value is in the range 30 - 50 ml/min
    - **Makeup gas**
- Supplied with hydrogen when capillary columns are used
- Nitrogen provides a better sensitivity than Helium
- The working value is in the range 10 - 60 ml/min
    - **Standing current**
- Current generated in absence of sample
- High values at standard conditions indicate contaminations or column bleeding
- Good standing current must be in the range 5-15 pa

2. Electron Capture Detector

- The Electron Capture Detector (ECD) is selective to electronegative compounds, especially chlorinated, fluorinated, or brominated molecules. It is sensitive to some of these compounds in the parts per trillion (ppt)
- The ECD detector requires nitrogen or argon / 5% methane (P5) to operate. The ECD detector is mounted immediately adjacent to the right rear column oven wall on GC chassis. Two BNC cables connect the anode and cathode, respectively, to the ECD amplifier. The ECD detector consists of a stainless steel cylinder containing 5 millicuries of radioactive Nickel 63 in an oven enclosure that is thermostatically controllable from ambient temperature to 375°C. Since the detector contains only 5 millicuries of Nickel-63,the ECD is covered by a “General License”



Theory of Operation

- The radioactive Nickel 63 sealed inside the ECD detector emits electrons (beta particles) which collide with and ionize the make-up gas molecules (either nitrogen or P5).

This reaction forms a stable cloud of free electrons in the ECD detector cell.

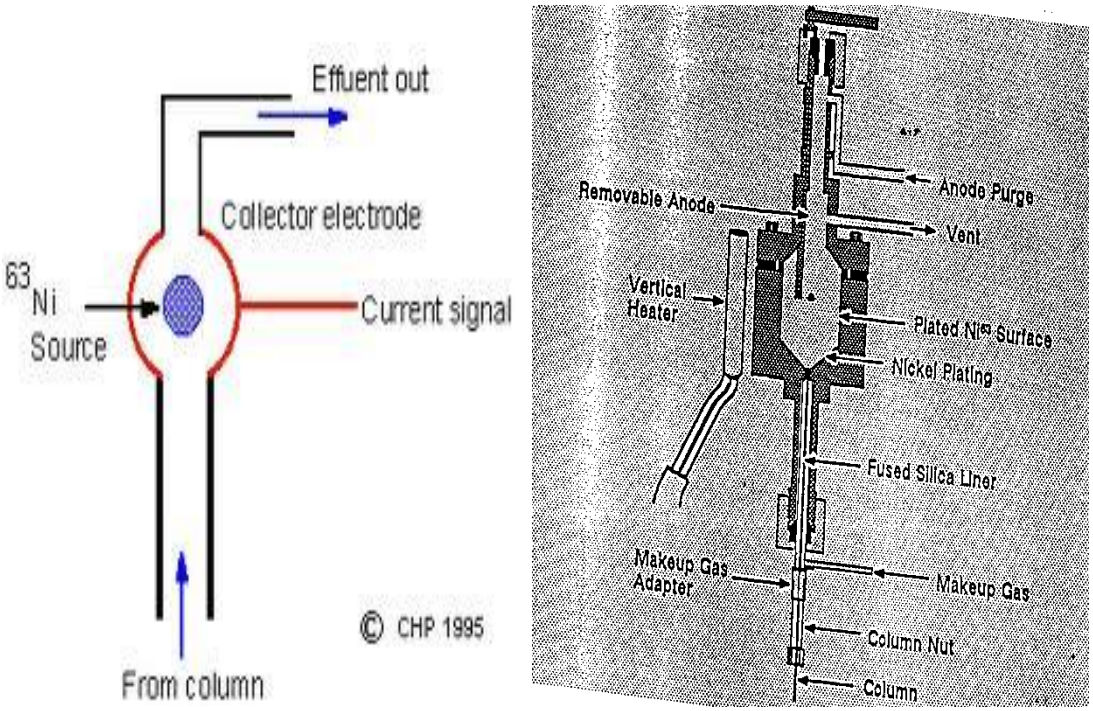
- The ECD electronics work to maintain a constant current equal to the standing current through the electron cloud by applying a periodic pulse to the anode and cathode.
- The standing current value is selected by the operator; the standing current value sets the pulse rate through the ECD cell. A standing current value of 300 means that the detector electronics will maintain a constant current of 0.3 nanoamperes through the ECD cell by periodically pulsing.
- If the current drops below the set standing current value, the number of pulses per second increases to maintain the standing current.
- When electronegative compounds enter the ECD cell from the column, they immediately combine with some of the free electrons, temporarily reducing the number remaining in the electron cloud.

- When the electron population is decreased, the pulse rate is increased to maintain a constant current equal to the standing current. The pulse rate is converted to an analog output, which is acquired by the data system.
- Unlike other detectors which measure an increase in signal response, the ECD detector electronics measure the pulse rate needed to maintain the standing current.

Example Pulse Trains

The pulse rate is increased in the presence of electronegative compounds Detector electronics pulse to maintain the standing current

ECD Detector Operational Diagram



- When electronegative compounds enter the ECD cell from the column, they immediately combine with some of the free electrons, temporarily reducing the number remaining in the electron cloud.
- When the electron population is decreased, the pulse rate is
- Increased to maintain a constant current equal to the standing current.
- The pulse rate is converted to an analog output, which is acquired by the data system.
- Unlike other detectors which measure an increase in signal response, the ECD detector electronics measure the pulse rate needed to maintain the standing current
- **The reaction occur inside ECD**

$$N_2 \xrightarrow{\beta} N_2^+ + e^-$$

$$AM + e^- \longrightarrow AM^-$$

$$AM^- + N_2^+ \longrightarrow AM + N_2$$

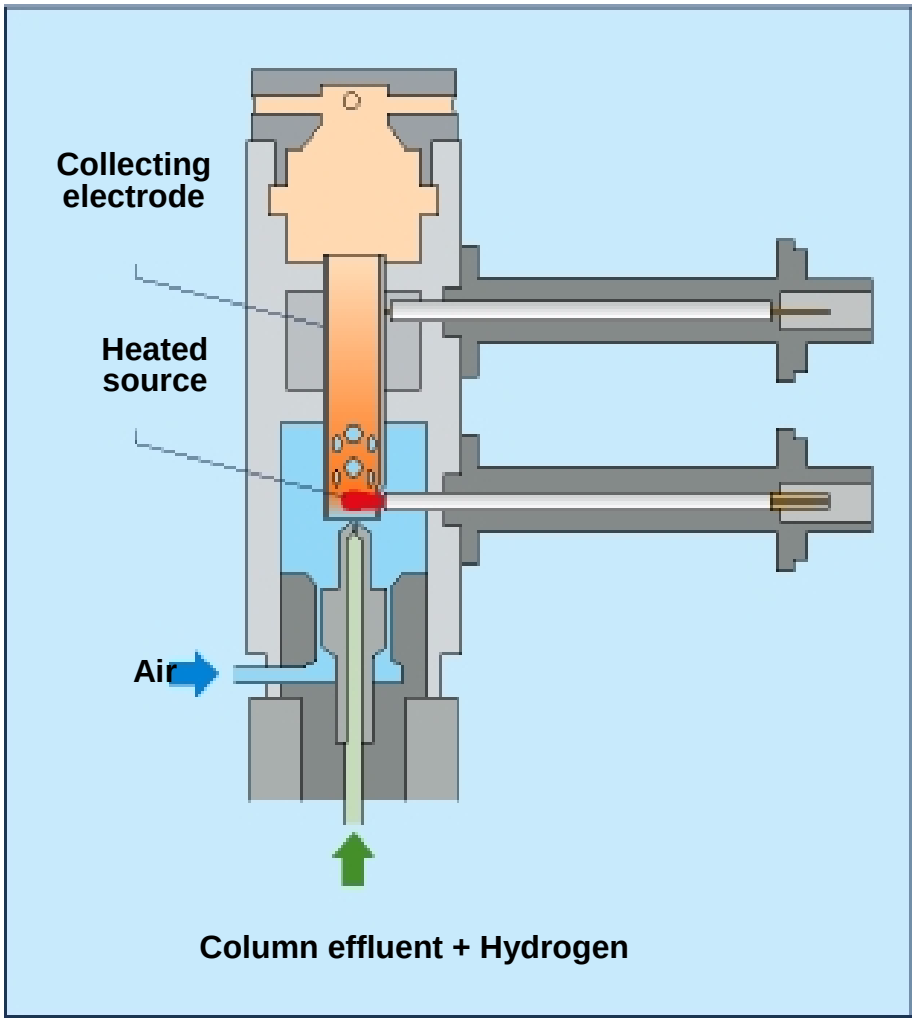
Example Pulse Trains

Detector electronics pulse to maintain the standing current

The pulse rate is increased in the presence of Electronegative compounds

3. Nitrogen/Phosphorus Detector - NPD

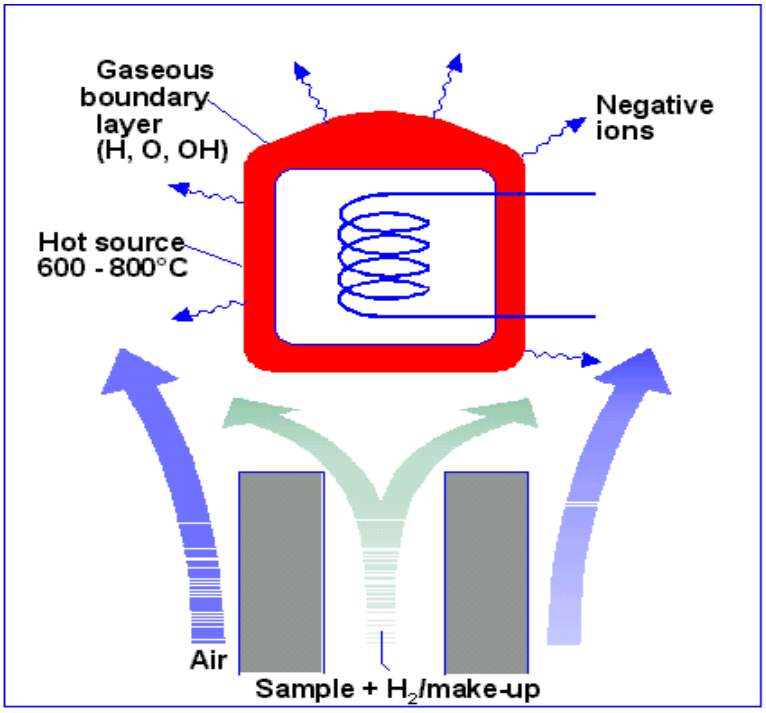
- The Nitrogen-Phosphorus Detector (NPD) has a linear response selective to organic compounds containing nitrogen and/or phosphorus.
- The NPD also responds tonormal hydrocarbons, but approximately 100,000 times less than nitrogen or phosphorus containing compounds.
- Due to its selectivity and sensitivity, the NPD is often used to detect pesticides, herbicides, drugs of abuse, and other trace compounds.
- Helium is the carrier gas of choice for the NPD detector, especially when other detectors are installed on the same GC
- The NPD is similar in design to the FID, except it uses a thermionic NPD bead to generate ions in hydrogen and air plasma.
- Like the FID, the NPD uses a stainless steel jet to deliver sample-laden carrier gas and hydrogen gas to the detector, and a positively charged collector electrode that also serves as the detector exhaust.
- The NPD bead is positioned between the jet and the collector electrode. The tip of the NPD jet is slightly different from that of the FID jet.



Theory of Operation

- Inside the NPD detector body, an electrically heated thermionic bead (NPD bead) is positioned between the jet orifice and the collector electrode.
- The bead is coated with an alkali metal which promotes the ionization of compounds that contain nitrogen or phosphorus.
- Hydrogen and air flows create hydrogen plasma around the hot NPD bead.
- When molecules containing nitrogen or phosphorus enter the plasma from the column and jet orifice, they undergo a catalytic surface chemistry reaction, producing thermionic electrons.
- The resulting ions are attracted to a positively charged collector electrode, then amplified and output to the data system.
- The hydrogen to air ratio is too lean to sustain a flame, therefore minimizing hydrocarbon ionization and contributing to the NPD detector's selectivity.

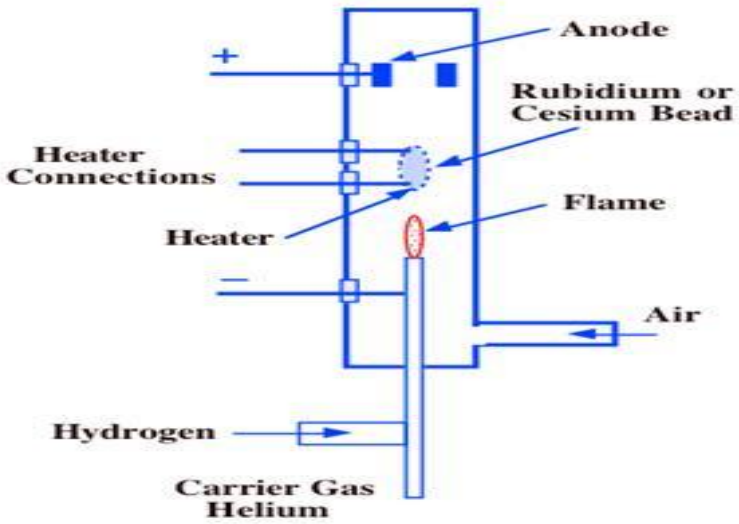
NPD: detection mechanism



Sample Decomposition ⟶ Electronegative products

(e.g. NO_2, CN, PO_2)

Electronegative species + Hot Source ⟶ Negative Ions



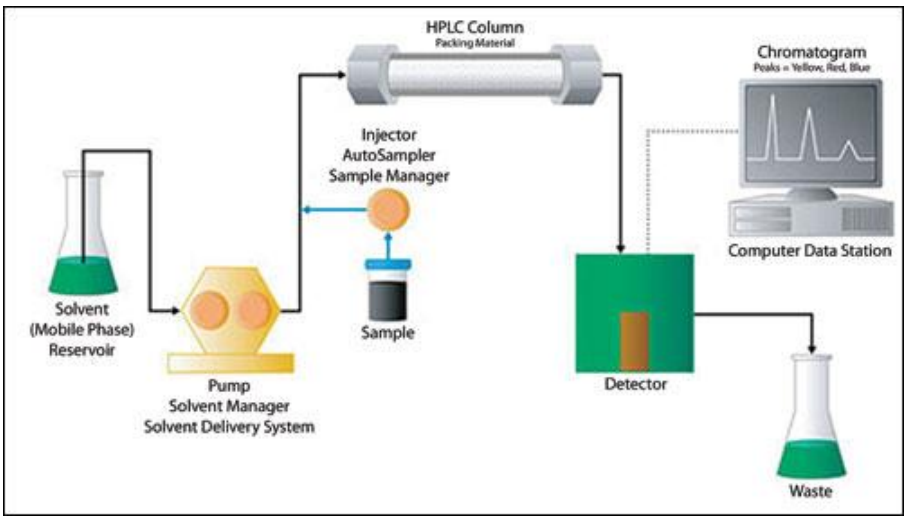
2-Liquid Chromatography

What the HPLC consist of?

1- Solvent Reservoir

2- Pump
3- Sample Manager
4- Column (separation)
5- Detector (Identification & Quantitation)
6- Data Handling

Schematic HPLC System



What is Requirements of Solvent Delivery Systems?

1- Stable flow with minimal pulsation
2- Wide flow rate range
3- High pressure capability
4- Chemically inert
5- High precision
6- Low internal volume

HPLC PUMB

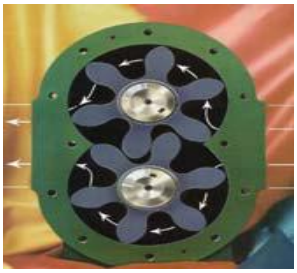
- High pressure pumps are needed to force solvents through packed stationary phase beds because the small particles in column need high pressure
- Must has stable flow

1- Displacement pump

(Screw-driven syringe pump): pulse free, small capacity (250 ml), no gradient elution

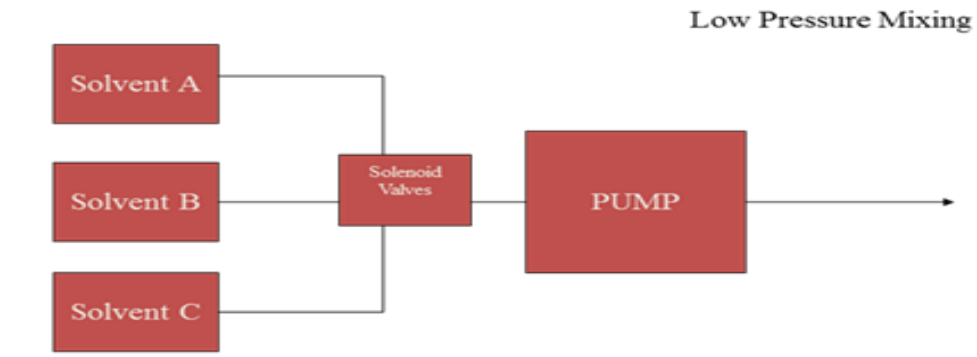
2- Reciprocating pump

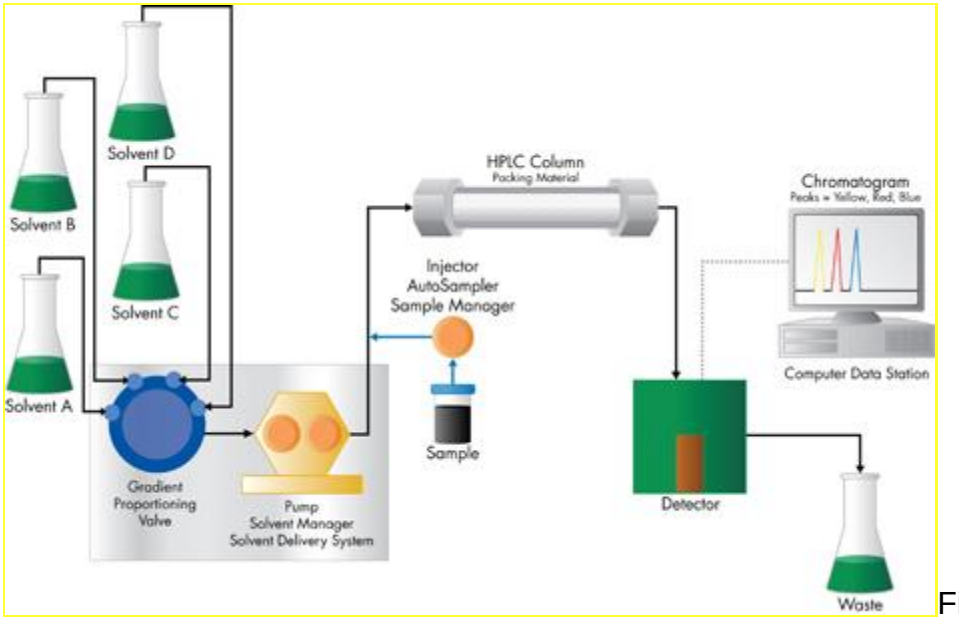
Most widely used. Small internal volume (35 ~ 400 µl), high pressure (105 psi), gradient elution, constant flow.



- **Types of mixing in solvent delivery system (Pump)**

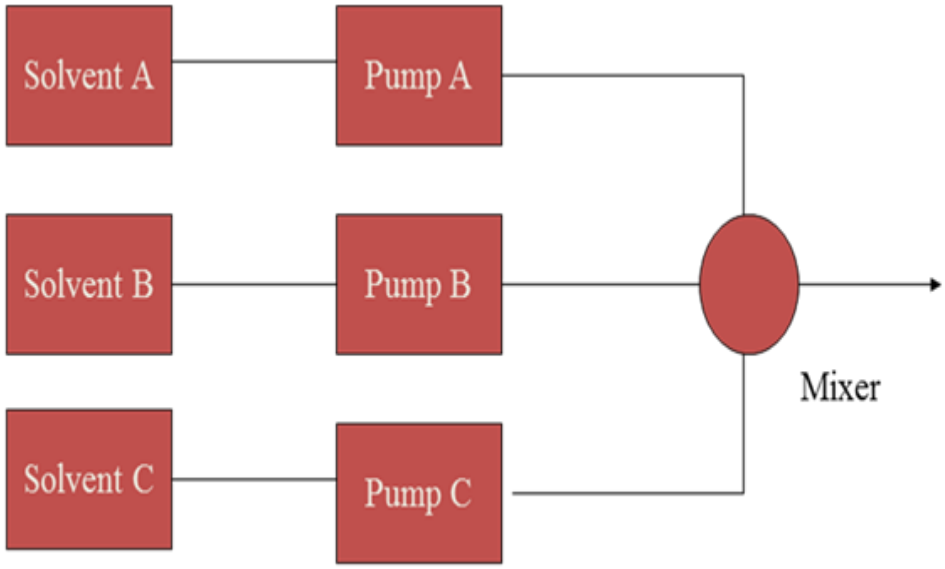
1- Low pressure Mixing

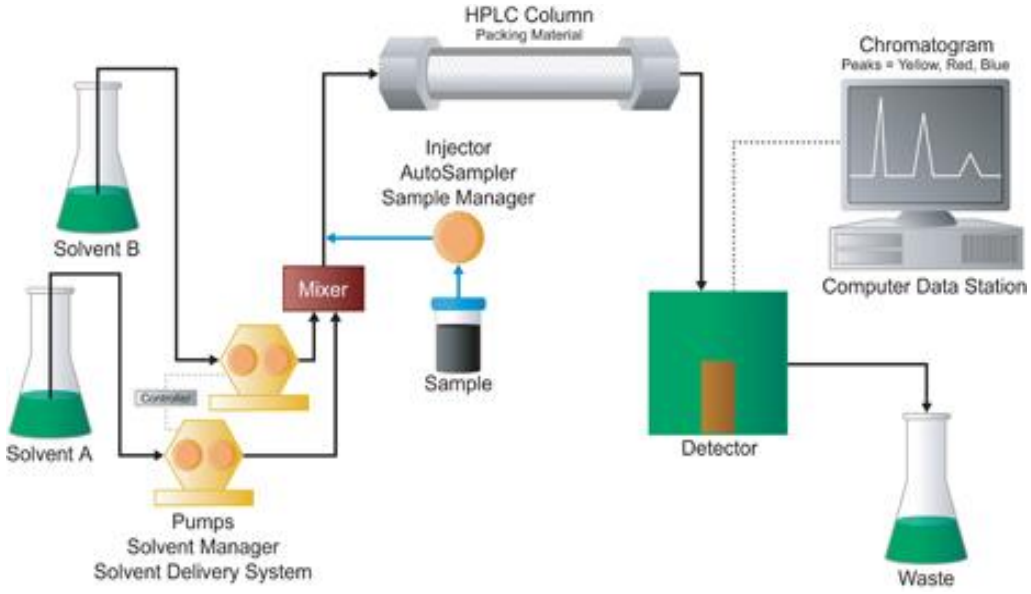




Fig(5)

2- Higher Pressure Mixing





Requirements of Sample Injection Devices

1- Introduce sample as narrow band
2- High precision - operator independent
3- High pressure capability
4- Chemically inert

What is the type of Elution using for separation of compounds in LC?

There are two type of elution in LC.

First one called Isocratic: where the mobile phase (one solvent or more) still in the same mixing (composition) form begging of injection until the run end.

Second Type called Gradient: where the mobile phase (more than on solvent) changes the mixing (composition) during the run, why we need this type? It is important when we need to separate mixture of compounds have different polarity.

Isocratic versus Gradient Elution

- **Isocratic elution has a constant mobile phase composition**
  - Can often use one pump
  - Mix solvents together ahead of time!
  - Simpler, no mixing chamber required
  - Limited flexibility, not used much in research
  - Mostly process chemistry or routine analysis.
- **Gradient elution has a varying mobile phase composition**
  - Uses multiple pumps whose output is mixed together
  - Often 2-4 pumps (binary to quaternary systems)
  - Changing mobile phase components changes the polarity index

- Can be used to subsequently elute compounds that were previously (intentionally) “stuck” on the column
- Some additional wear on the stationary phase
- Column has to re-equilibrate to original conditions after each run (takes additional time).

HPLC Column theory and types

What is the column?

Its tube must contain Packing matrixes (Stationary phase).

The major characters of any column:

1. Leak free
2. Minimum volume
3. Minimum Dead volume
4. Chemically inert according to separation system
5. We have many kind of column like(stainless steel good for high pressure resistance , Plastic and glass)

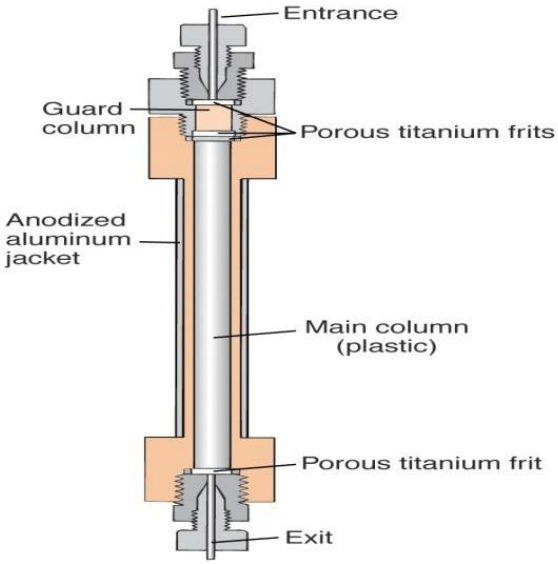
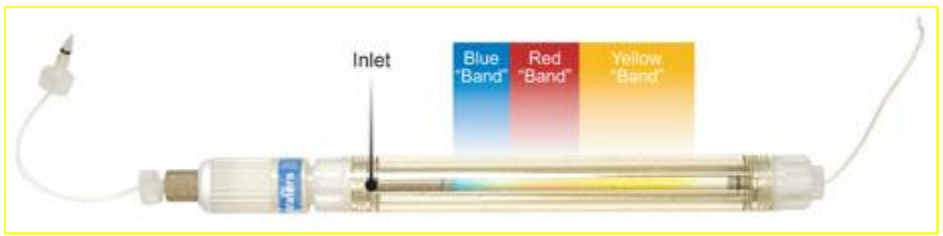


Diagram of HPLC column



Glass column

Stainless steel

To get will know about column we need to define the two terms which any column must be consist of:

1. **Mobile phase**: The fluid which carry out the components along the stationary phase to the detector and t may be Gas or Liquid
2. **The Stationary**: The bed in which happened adsorption for the components on it and it May be solid or gel or liquid but if liquid must be distributed on solid.
   - The Separation is based on the analyst's relative solubility between two liquid phases **(Stationary Phase)**
   - HPLC is largely the domain of packed columns

- Some research into micro bore /capillary columns is going on.
- Molecules move too slowly to be able to reach and therefore "spend time in" the Stationary phase of an open tubular column in HPLC.
  - The particle size of Stationary phases are usually about 1 to 20 μm in average diameter (often irregularly shaped)
- **In Adsorption chromatography**, there is no additional phase on the stationary phase particles (silica, alumina, Fluorosil).
- **In Partition chromatography**, the stationary phase is coated on to (often bonded) a solid support (C18, C8, divinyl -benzene resin)

So we have in HPLC two modes

1- Normal Phase

Polar Stationary phase and non polar solvent

2- Reverser phase

Non polar Stationary phase and polar solvent and we always use Reverser Phase

- C18 to about C8 terminations on the bonded phase.
- Phenyl and cyanoterminations on the bonded phase.

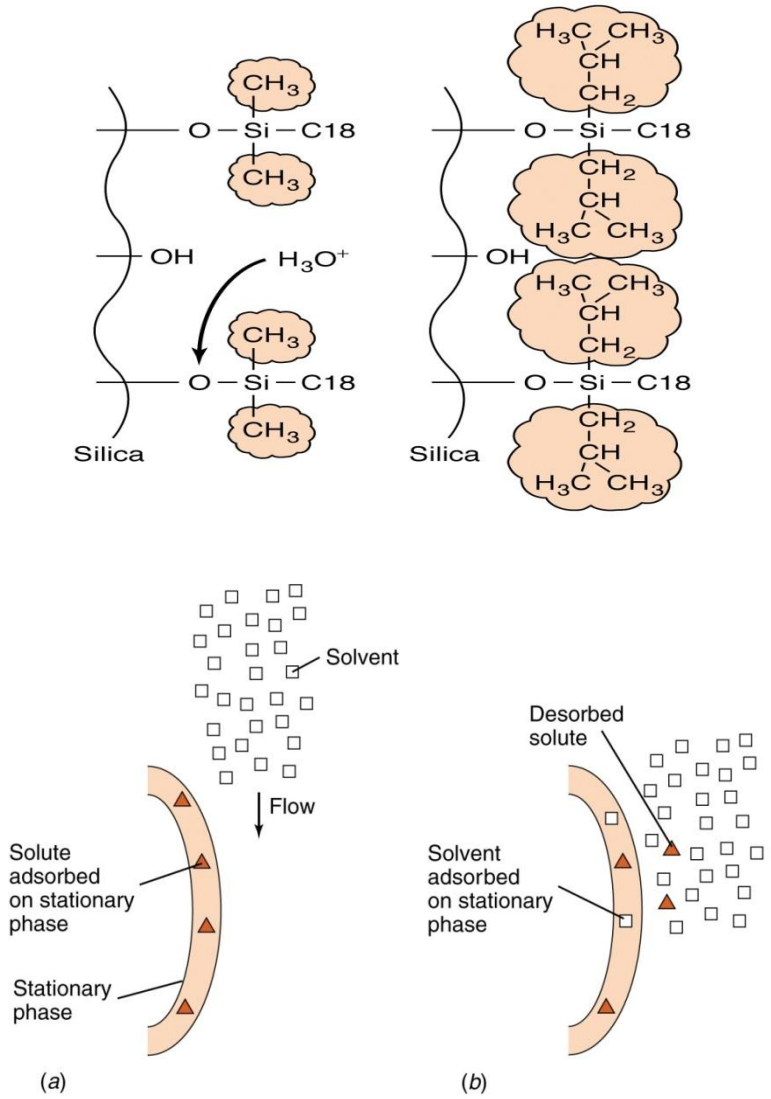


Diagram show how Adsorption occur on Stationary phase of column

Types of Bonded Phases

1- C-2 Ethyl Silyl - $Si\text{-}CH_2\text{-}CH_3$
2- C-8 Octyl Silyl $-Si\text{-}(CH_2)_7\text{-}CH_3$
3- C-18 Octadecyl Silyl - $Si\text{-}(CH_2)_{17}\text{-}CH_3$
4- CN Cyanopropyl Silyl $-Si\text{-}(CH_2)_3\text{-}CN$

2- The mobile phase

The mobile phase must do the following:

1- Solvate the analyte molecules and the solvent they are in
2- Be suitable for the analyte to transfer “back and forth” between during the separation process.

The mobile phase must be:-

1- Compatible with the instrument (pumps, seals, fittings, detector… etc).

2- Compatible with the stationary phase.
3- Readily available.
4- Adequate purity.
5- Not too compressible (causes pump/flow problems) & Free of gases (which cause compressibility problems).

The polarity Index and the mobile phase

- The polarity index is a measure of the relative polarity of a solvent. It is used for identifying suitable mobile phase solvents.
- The more polar your solvent is the higher the index.
- We should try to choose a polarity index for our solvent (or solvent mixture) that optimizes the separation of compounds usually the index is a starting point
- The polarity of any mixture of solvents to make a mobile phase can be modeled to give a theoretical chromatogram usually; optimization of solvent composition is experimental.

Table 25-2 Eluotropic series and ultraviolet cutoff wavelengths of solvents for adsorption chromatography on silica

Solvent	Eluent strength (ϵ°)	Ultraviolet cutoff (nm)
Pentane	0.00	190
Hexane	0.01	195
Heptane	0.01	200
Trichlorotrifluoroethane	0.02	231
Toluene	0.22	284
Chloroform	0.26	245
Dichloromethane	0.30	233
Diethyl ether	0.43	215
Ethyl acetate	0.48	256
Methyl *t*-butyl ether	0.48	210
Dioxane	0.51	215
Acetonitrile	0.52	190
Acetone	0.53	330
Tetrahydrofuran	0.53	212
2-Propanol	0.60	205
Methanol	0.70	205

The ultraviolet cutoff for water is 190 nm.

SOURCES: L. R. Snyder, in *High-Performance Liquid Chromatography* (C. Horváth, ed.), Vol. 3 (New York: Academic Press, 1983); *Burdick & Jackson Solvent Guide*, 3rd ed. (Muskegon, MI: Burdick & Jackson Laboratories, 1990).

TABLE 28-2 Properties of Common Chromatographic Mobile Phases

Solvent	Refractive Index[a]	Viscosity, cP[b]	Boiling Point, °C	Polarity Index, P'	Eluent Strength,[c] ε^0
Fluoroalkanes[d]	1.27–1.29	0.4–2.6	50–174	<–2	–0.25
Cyclohexane	1.423	0.90	81	0.04	–0.2
n-Hexane	1.372	0.30	69	0.1	0.01
l-Chlorobutane	1.400	0.42	78	1.0	0.26
Carbon tetrachloride	1.457	0.90	77	1.6	0.18
i-Propyl ether	1.365	0.38	68	2.4	0.28
Toluene	1.494	0.55	110	2.4	0.29
Diethyl ether	1.350	0.24	35	2.8	0.38
Tetrahydrofuran	1.405	0.46	66	4.0	0.57
Chloroform	1.443	0.53	61	4.1	0.40
Ethanol	1.359	1.08	78	4.3	0.88
Ethyl acetate	1.370	0.43	77	4.4	0.58
Dioxane	1.420	1.2	101	4.8	0.56
Methanol	1.326	0.54	65	5.1	0.95
Acetonitrile	1.341	0.34	82	5.8	0.65
Nitromethane	1.380	0.61	101	6.0	0.64
Ethylene glycol	1.431	16.5	182	6.9	1.11
Water	1.333	0.89	100	10.2	Large

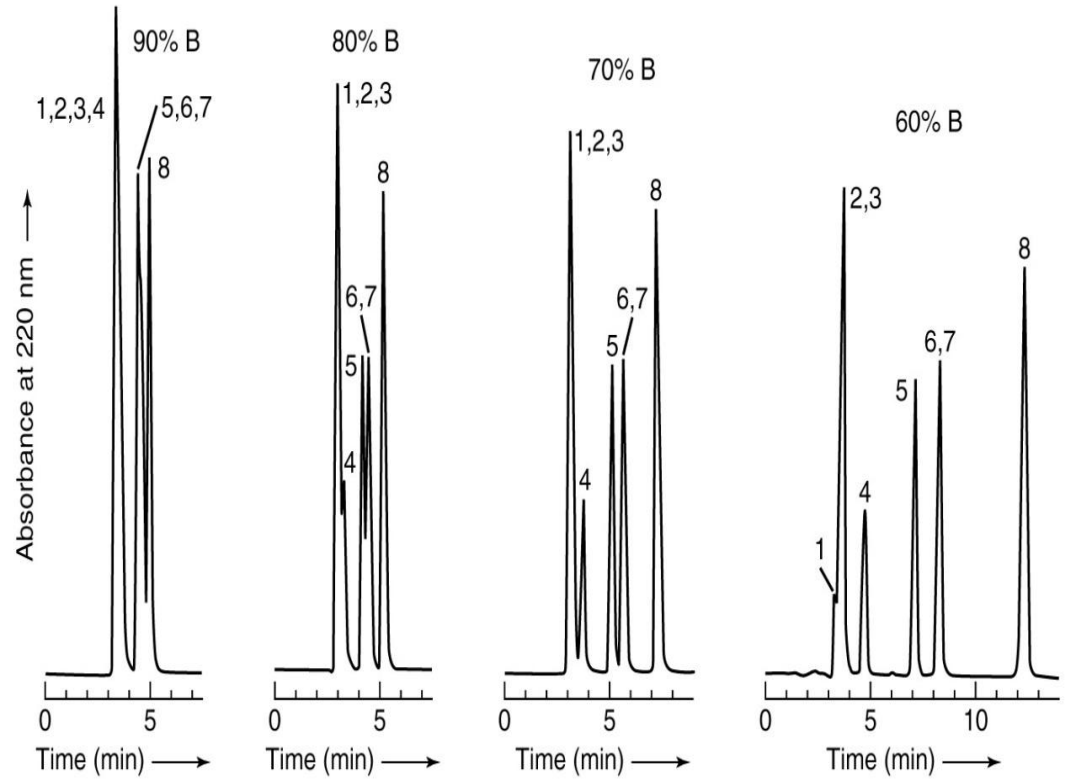
[a] At 25°C.

[b] The centipoise is a common unit of viscosity; in SI units, 1 cP = 1 $mN \cdot s \cdot m^{-2}$.

[c] On Al_2O_3. Multiplication by 0.8 gives ε^0 on SiO_2.

[d] Properties depend on molecular mass range of data given.

The effecting of mobile phase polarity in separation



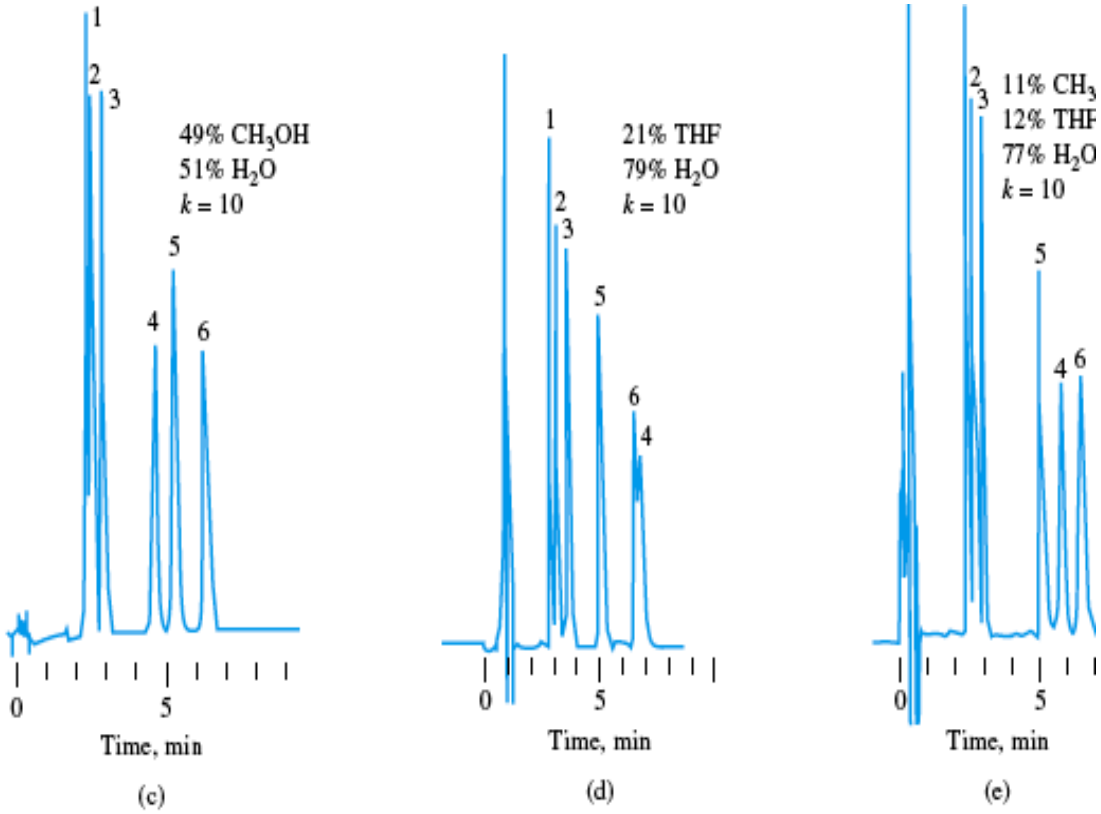
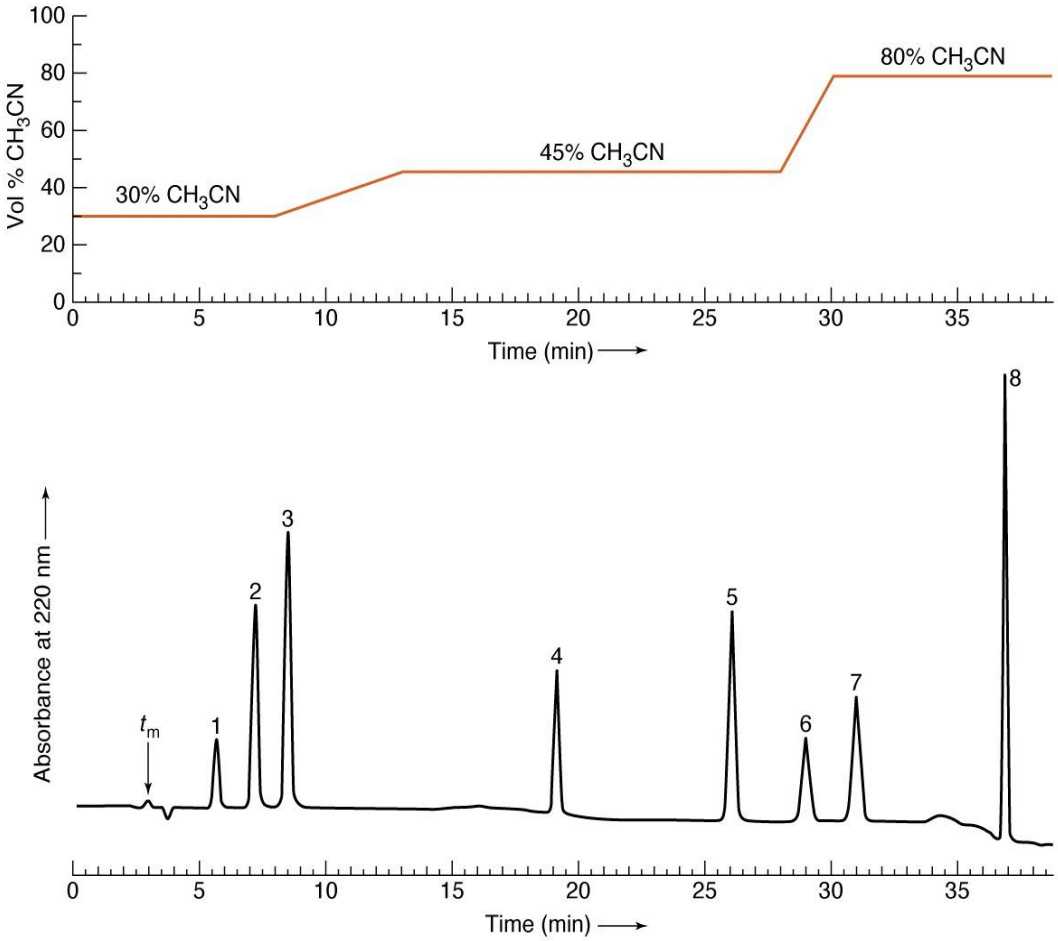


FIGURE 28-16 Systematic approach to the separation of six steroids. The use of water to adjust k is shown in (a) and (b). The effects of varying α at constant k are shown in (b), (c), (d), and (e). Column: 0.4 × 150 mm packed with 5 µm C_8 bonded, reversed-phase particles. Temperature: 50°C. Flow rate: 3.0 cm^3/min. Detector: UV 254 nm. THF = tetrahydrofuran. CH_3CN = acetonitrile. Compounds: (1) prednisone, (2) cortisone, (3) hydrocortisone, (4) dexamethasone, (5) corticosterone, (6) cortoexolone.

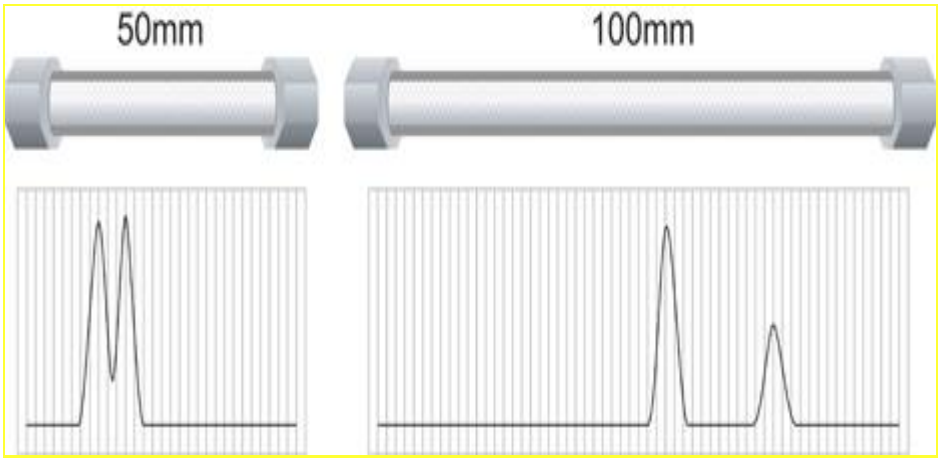
Diagram Showing how Gradient elution effecting separation



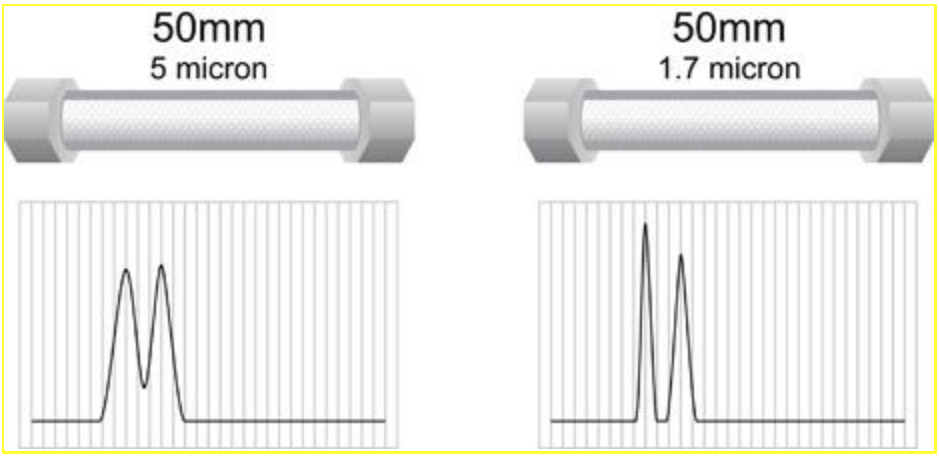
So we have in Chromatography very important factor called Resolution (RS). The Resolution factor is the power of separation of column, when the peak are will define in good separate so it good

When factor effect of separation of column

1- Column length when increase the resolution increase(RS)



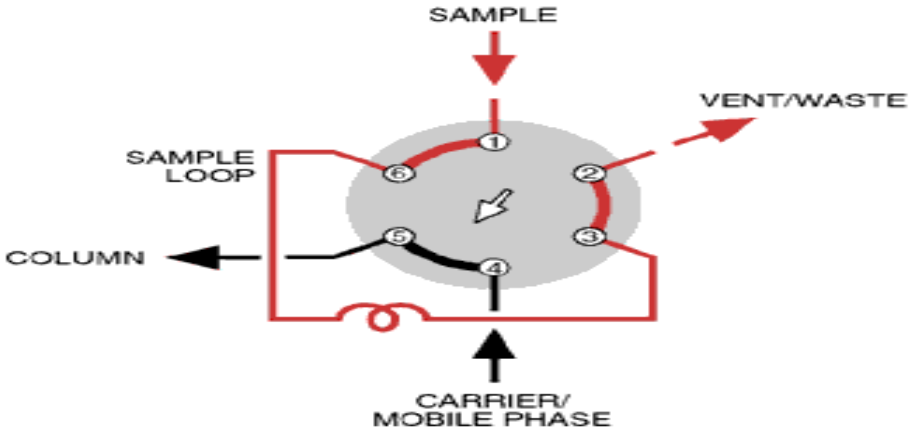
2- Particle size of column when increase the resolution increase (RS)

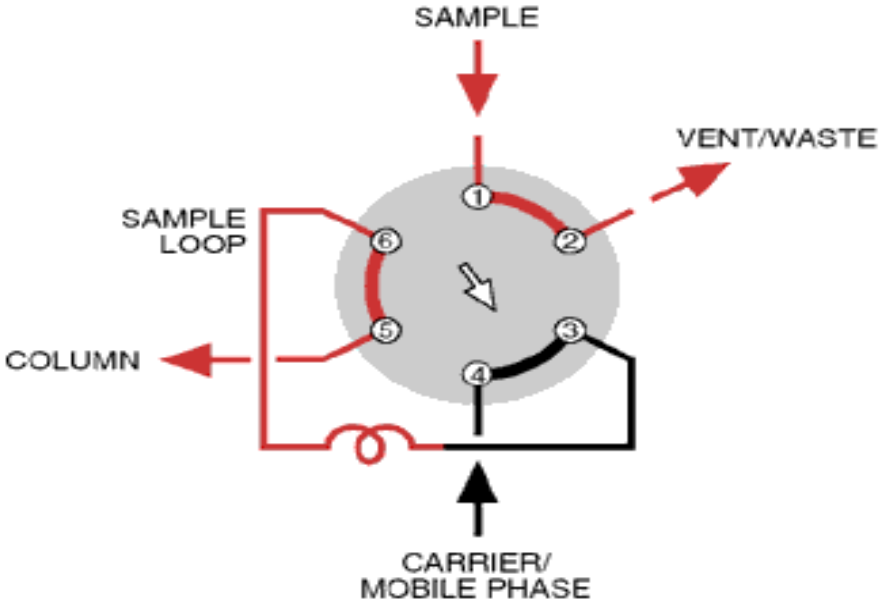


IN USP the good Resolution above 2 in the method application we will talk freely about good chromatography Plot

Injection in HPLC

- Usually 5 to 1000 µL volumes, all directly onto the column
- Not much worry about capacity since the columns have a large volume (packed).
- Injector is the last component before the column(s)
- A source of poor precision in HPLC
  - Errors of 2-3 %RSD are due just to injection
    - Due to capillary action and the small dimensions/cavities inside the injector
- 6-PORT Rotary Valve is the standard manual injector
- Automatic injectors are available
- Two positions, load and inject in the typical injector
- Injection loop internal volume determines injection volume it may be different volume due to the injection volume of the sample





Inject (move the sample loop into the mobile phase flow)

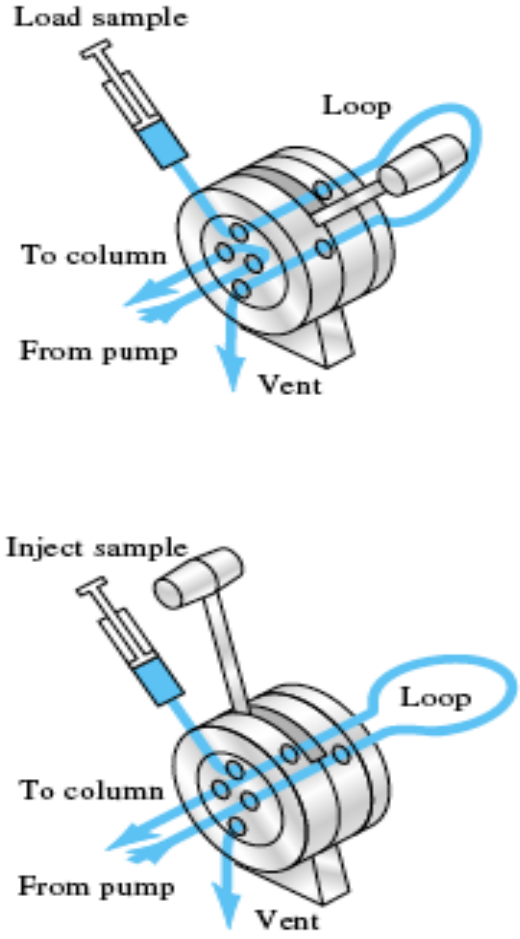


FIGURE 28-6 A sampling loop for LC. With the valve handle as shown on the left, the loop is filled from the syringe, and the mobile phase flows from pump to column. When the valve is placed in the position on the right, the loop is inserted between the pump and the column so that the mobile phase sweeps the sample onto the column. (Courtesy of Beckman-Coulter, Inc.)

Detection in HPLC

Mean specs in HPLC detector

- Must be solvent compatible, stable with mobile phase, column, etc.
- Universal
  - Respond to all analytes
- Analyte Specific
  - Respond to specific properties of analytes
- Non-destructive (most) expect (ELCD&MASS)
- High Sensitivity
- Negligible base line
- Large liner dynamic range
- Low dead volume
- Stable over long period of operation

TABLE 28-1 Performance of HPLC Detectors

HPLC Detector	Commercially Available	Mass LOD* (typical)	Linear Range† (decades)
Absorbance	Yes	10 pg	3–4
Fluorescence	Yes	10 fg	5
Electrochemical	Yes	100 pg	4–5
Refractive index	Yes	1 ng	3
Conductivity	Yes	100 pg–1 ng	5
Mass spectrometry	Yes	<1 pg	5
FTIR	Yes	1 µg	3
Light scattering	Yes	1 µg	5
Optical activity	No	1 ng	4
Element selective	No	1 ng	4–5
Photoionization	No	<1 pg	4

Sources: From manufacturer's literature; *Handbook of Instrumental Techniques for Analytical Chemistry*, F. Settle, ed., Upper Saddle River, NJ: Prentice-Hall, 1997; E. S. Yeung and R. E. Synovec, *Anal. Chem.*, 1986, *58*, 1237A.

*Mass LODs (limits of detection) depend on compound, instrument, and HPLC conditions, but those given are typical values with commercial systems when available.

†Typical values from the preceding sources.

- **Standard Absorbance Detector**

1. Single Beam UV-VIS instrument with a flow-through cell (cuvette)

2. Can use any UV-VIS with a special flow cell
   a. Extra connections lead to band-broadening if UV-VIS is far from HPLC column exit.
3. Usually utilize typical UV-VIS lamps and 254 nm default wavelength
   a. Can be set to other wavelengths (most)
   b. Simple filter detectors no longer widely used
   c. Adjustable wavelength units are cost-effective
4. Non-destructive, not-universal
   a. Not all compounds absorb light
   b. Can pass sample through several cells at several different wavelengths
5. Usually zeroed at the start of each run using an electronic software command. You can have real-time zeroing with a reference cell.

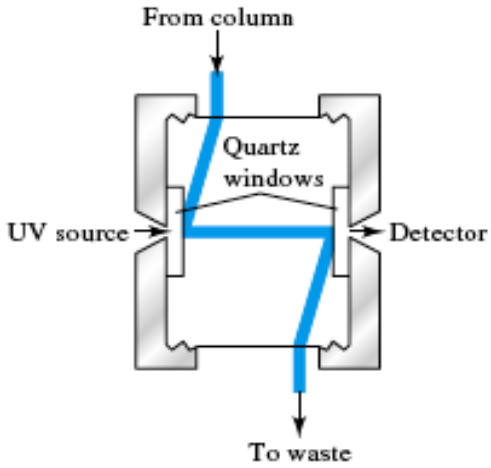


FIGURE 28-8 A UV-visible absorption cell for HPLC.

- **Diode Array Detector (DAD)**

1. The more common tool for research-grade HPLC instruments
2. Quite versatile
3. Advances in computer technology since ~1985 or so have lead to the development of Diode Array instruments
4. Non-destructive, non-universal
5. DAD scans a range of wavelengths every second or few seconds. At each point in the chromatogram one gets a complete UV-VIS spectrum!
6. Huge volumes of data
7. Detailed spectra for each peak and each region of each peak

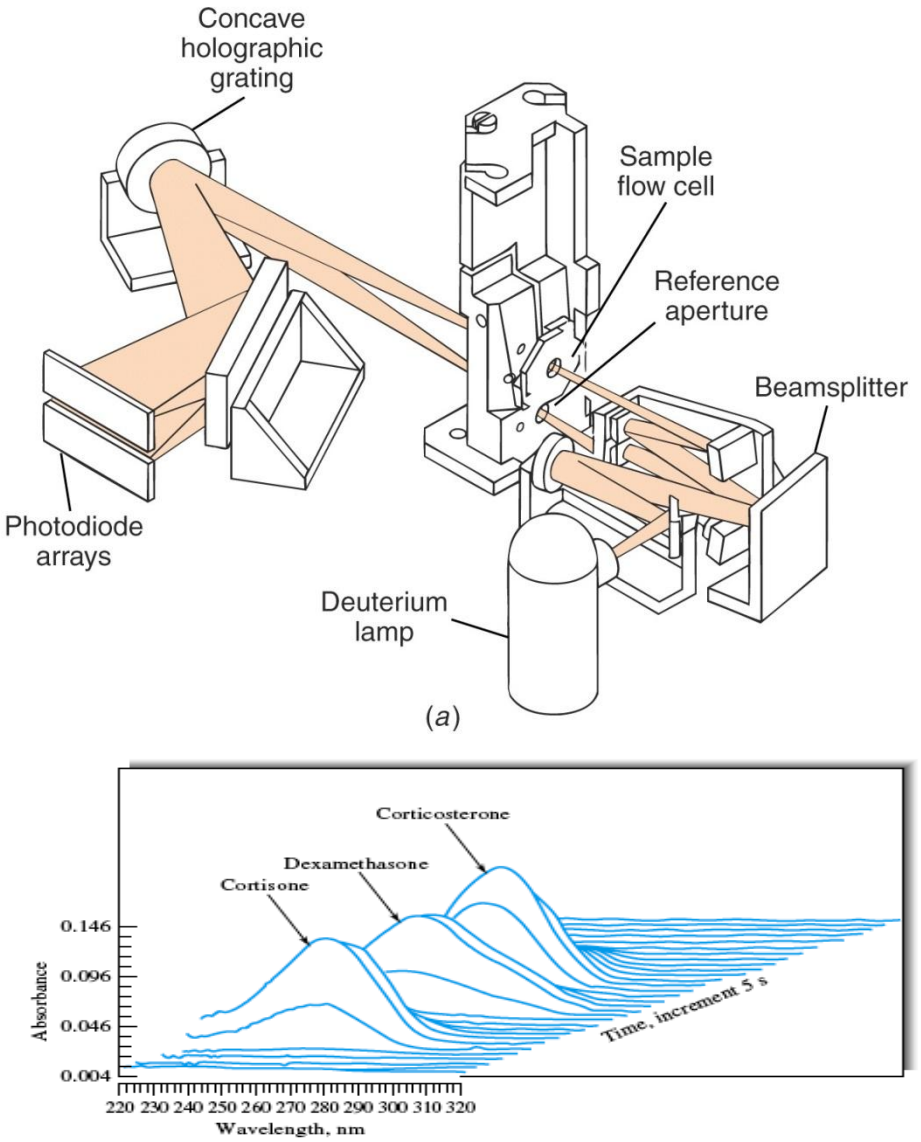


FIGURE 28-9 Absorption spectra of the eluent from a mixture of three steroids taken at 5-second intervals. (Courtesy of Hewlett-Packard Co., Palo Alto, CA.)

- **Refractive Index Detector**

1. One of a very few Universal HPLC detectors. Non-destructive
2. Responds to analytes changing the RI of the mobile phase requires a separate reference flow of mobile phase
3. Extremely temperature sensitive, usually heated sensitive to temp changes of +/- 0.001 °C
4. No longer really widely used absorbance detectors are relatively cheap.
5. Useful for process work, on-line monitoring, etc.

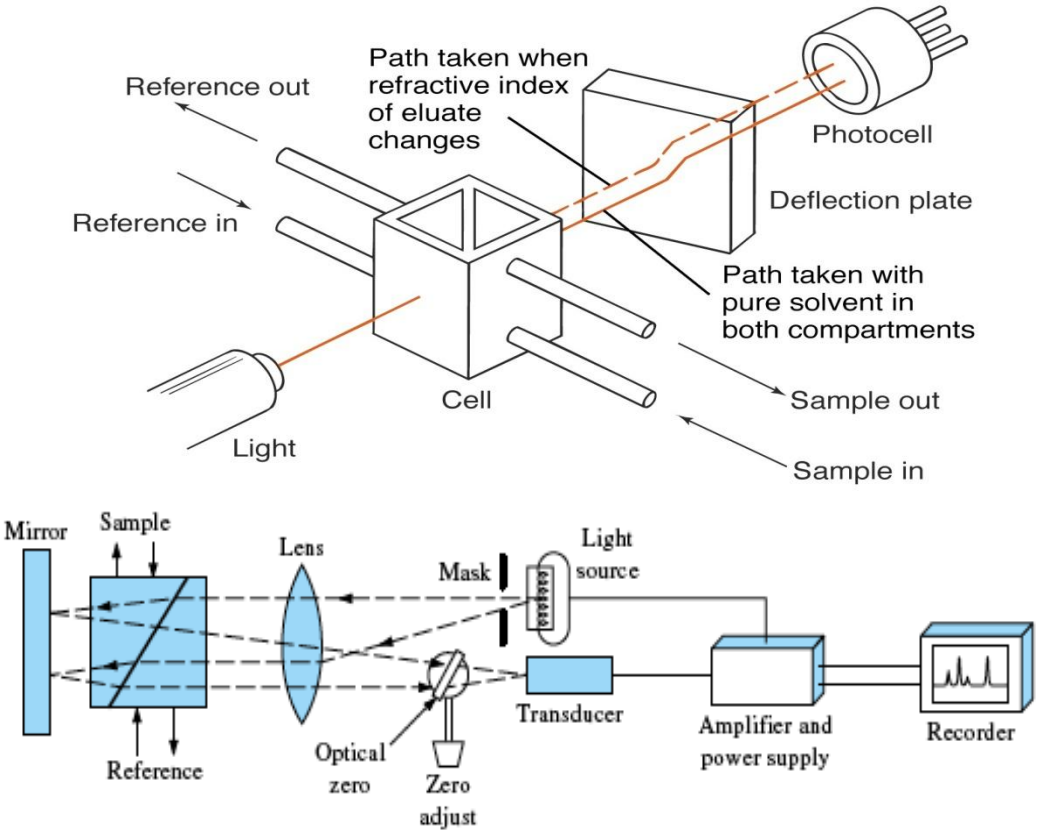
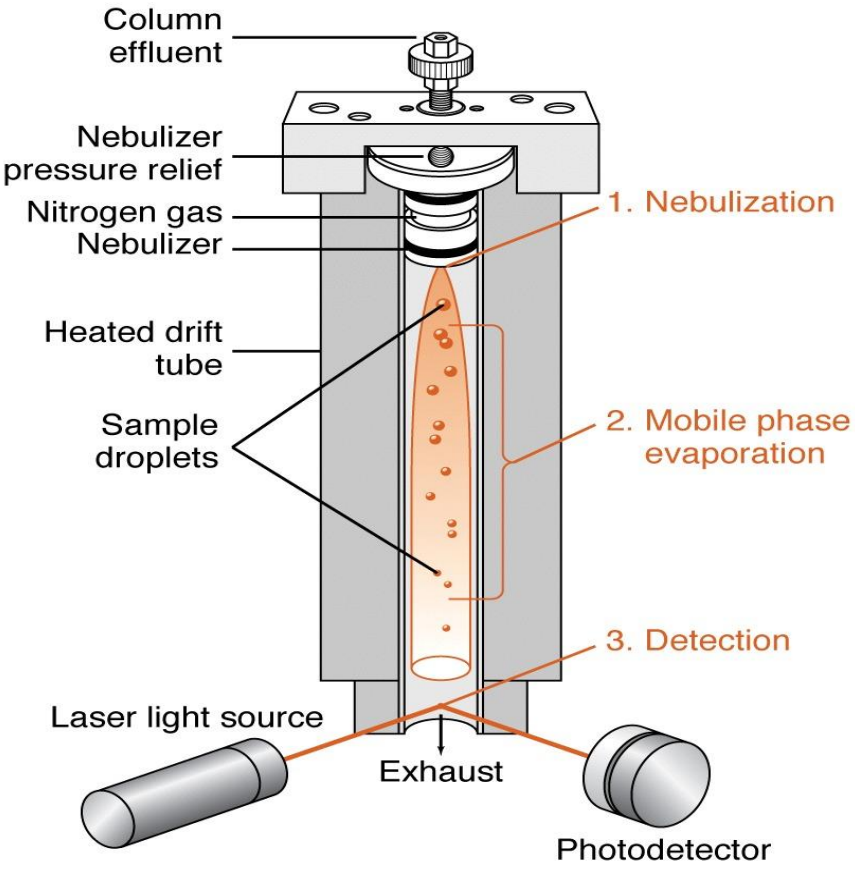


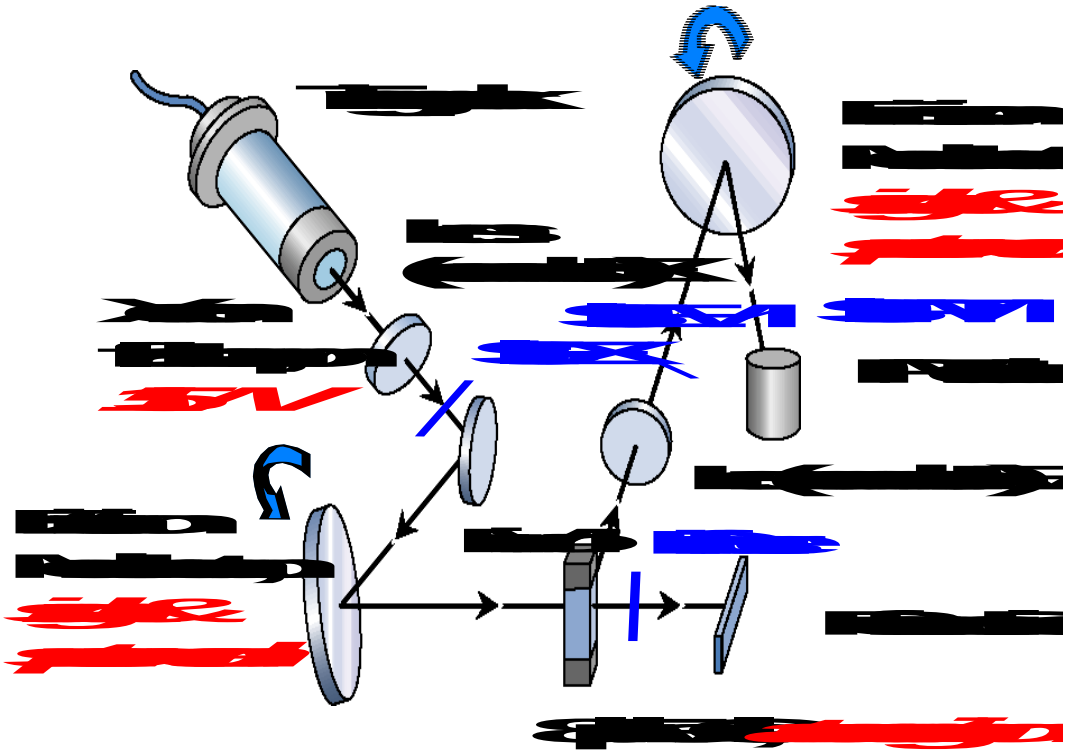
FIGURE 28-10 Schematic diagram of a differential refractive-index detector. (Courtesy of Waters Associates, Inc., Milford, MA.)

- **ELSD (Evaporative Light Scattering Detector)**

1. Universal, destructive
2. Useful for very large molecules, and a wide linear range
3. Analytes are de-solvated in the detector
4. Molecules pass through what is essentially a large cuvette for a UV-VIS instrument
5. The reduction in light intensity detected (due to scattering by the analytes) is measured
6. The larger and more concentrated a particular molecule is, the greater the scattering.



Florence Detector



3- FT-IR

Introduction

The infrared region of the electromagnetic spectrum lies between the visible and microwave regions. By convention, the infrared region is frequently divided into three sections :

Region	Range (cm^{-1})	Range (m)
Near-infrared	١٣٣٠٠ - ٤٠٠٠	0.75 -٢,٥
Mid-infrared	٤٠٠٠ - ٤٠٠	٢,٥ ــ ٢٥
Far-infrared	٤٠٠ - ١٠	٢٥ - ١٠٠٠

The mid-infrared, or fundamental vibrational region, is the most useful area of the spectrum for analytical spectroscopy

Analytical Principle:

A molecule may absorb infrared radiation of the appropriate frequency to excite it from one vibrational or rotational level to another. When a beam of infrared energy, covering a broad frequency range, passes through a sample, the energy at certain frequencies is absorbed by the sample. A graph of energy absorbed versus frequency is the absorption spectrum of the sample. The spectrum is characteristic of the particular molecule and its molecular motions .

For small molecules, bond lengths and angles can be determined from infrared spectra. For large molecules, the pattern of absorbance in the spectrum is characteristic of the functional groups in the molecule. The spectrum can be thought of as "fingerprint" of that chemical species, and therefore, infrared spectroscopy can be used to identify molecules (i.e., qualitative analysis). Spectroscopy can also be used for quantitative analysis because the intensity of absorption is proportional, among other things, to the amount of the species present.

The main component of an FT-IR spectrometer is the Michelson interferometer.

The interferometer contains a fixed mirror, a movable mirror, and a beam splitter. The beam splitter transmits half of the incident radiation to the moving

mirror and reflects the other half to the fixed mirror. The two beams are reflected by these mirrors back to the beam splitter, where they recombine.

When the fixed mirror and moving mirror are equidistant from the beams plitter, the amplitudes of all frequencies are in phase and recombine constructively. This position of zero path difference (ZPD), or zero retardation, is where the interferogram center burst occurs.

As the moving mirror is moved away from the beam splitter (retarded), an optical path difference is generated. As the position of the moving mirror changes, the two beams travel different distances within the interferometer before recombining. A pattern of constructive and destructive interference is generated based on the position of the moving mirror and the frequency of the retardation.

The intensity of the radiation varies in a complicated pattern as a function of mirror movement, and the output beam is the result of modulation by the interferometer. This modulated output beam is then directed through the sample compartment to the detector. At the detector it generates a continuous electrical signal called an interferogram.

Equipments

Spectrometer platform for the following dedicated applications :

On-board hardware diagnostics that continuously operates, ensuring optimal operation of the spectrometer.

Patented Dura glow™ high intensity air-cooled source provides high power and long life for continuous operation Internal polystyrene calibration standard for easy laboratory calibration Sealed and desiccated spectrometer enclosure with KBr windows; 750 g desiccant cartridge (largest in class) ensures spectra with minimal water vapor "artifacts" in the baseline

Spectral range: Mid/Near-IR

Detector: Cooled DTGS.

Chemical and reagents

- Fluorocarbon-113, (1,1,2-trichloro-1,2,2-trifluoroethane).
- Hydrochloric acid 1:1
- Sodium sulfate, anhydrous crystal.
- Silica gel (for TRPH)
- Reference material.

Practical

- Reference material preparation.
- Sample Preparation
- Software application.
- Data Handling.

4-TOC

Introduction

TOC Analyzer is a specialized version of TOC Analyzers optimized for use in municipal water applications, including raw and finished drinking waters. The Analyzer complies with (Standard Method 5310 C and EPA Method 415.3) and is accepted for compliance and regulatory monitoring under the USEPA's Disinfectants and Disinfection Byproducts Rule. It may be used as a standalone instrument, or paired with a high-capacity GE Auto sampler.

It is high-sensitive analyzer used to measure the concentration of total organic carbon (TOC), total inorganic carbon (TIC), and total carbon (TC = TOC + TIC) in water samples.

Analytical principles

The Analyzer is based on the oxidation of organic compounds to form carbon dioxide (CO_2) using UV radiation and a chemical oxidizing agent (ammonium per sulfate). Carbon dioxide is measured using a sensitive, selective membrane-based conduct metric detection technique. For each TOC measurement, the concentration of inorganic carbon species (CO_2, HCO_3 and CO_3 is determined and, after oxidation of the organic compounds, the total carbon (TC) content of the sample is measured. The concentration of the organic compounds is then calculated from the difference between the concentrations of TC and total inorganic carbon (TIC), generally referred to simply as inorganic carbon.

$$TOC = TC – IC$$

The Analyzer can be used to monitor water samples ranging from 4 parts per billion (ppb) TOC to 50 parts per million (ppm) TOC. The Analyzer is easy to operate, with extremely low maintenance, and no special training or chemical knowledge is required. The Analyzer is calibrated at the factory, and calibration remains stable for approximately one year. Recalibration and verification can be easily performed at the customer’s site.

Equipments

TOC Analyzer consists of six major subsystems:

1. Sample inlet system and sample pump, including the vial port for the Laboratory TOC Analyzer.
2. Chemical reagent subsystem, including reagent reservoirs and syringe pumps.
3. Oxidation reactor.
4. Measurement module, comprising:

- CO_2 transfer modules.
- Conductivity cells.

5. DI water loop, comprising
   - DI water reservoir.
   - Ion exchange resin (resin bed).
   - DI water pump.
6. Electronics subsystems, comprising:
   - Microprocessors and circuit boards.
   - Data outputs.

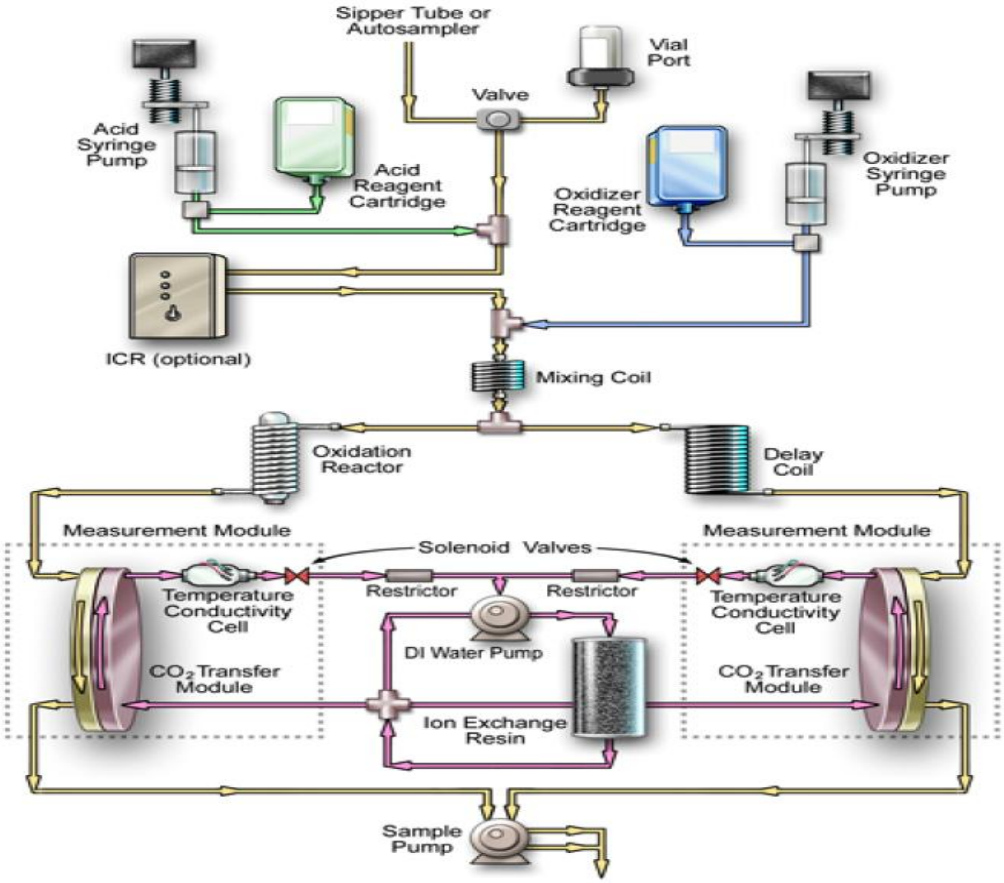


Figure 2: Analyzer Schematic — Laboratory Model with GE Autosampler

Sample Flow Path

The TOC Analyzers can measure discrete samples. This feature is specific to the

TOC Analyzer measures discrete samples by inserting a filled 40 ml sample vial into the vial port on the front of the Analyzer, or via a sipper tube.

Auto sampler can be used to introduce samples up to 120 vials in vial racks through the sample inlet port on the left side of the Analyzer.

After sample is introduced into the Analyzer, 6M phosphoric acid (H_3PO_4) (referred to as Acid in the user interface) is injected into the sample at the

programmed flow rate to reduce sample ph to between 2 and 3; this allows for accurate measurement of TOC and IC.

If the optional Inorganic Carbon Remover (ICR) unit is utilized, after acid is added to the sample, excess IC is removed by the ICR by vacuum degasification.

The acidified sample is then combined with 15% ammonium per sulfate ($(NH_4)_2S_2O_8$) (referred to as Oxidizer in the user interface) to promote oxidation of the organics. The sample travels through a mixing coil and on to a stream splitter.

The stream splitter divides the sample stream into two equal but separate flows. One stream is processed for the measurement of IC; the other is processed for measurement of TC.

The TC stream passes to an oxidation reactor where the sample is exposed to UV light. The combination of UV light and, depending on the application, per sulfate oxidizes the organic compounds in the sample, converting carbon to CO_2. The reactor is a spiral quartz tube wrapped around the UV lamp. The UV lamp emits light at 185 and 254 nm resulting in the formation of powerful chemical oxidizing agents in the form of hydroxyl radicals produced by the photolysis of water (eq. 1) and per sulfate (eq. 2, 3).

$$H_2O + h\nu\ (185\ nm) \rightarrow OH^{\bullet} + H^{\bullet} \qquad (1)$$

$$S_2O_8^{2-} + h\nu\ (254\ nm) \rightarrow 2\ SO_4^{\bullet-} \qquad (2)$$

$$SO_4^{\bullet-} + H_2O \rightarrow HSO_4^{-} + OH^{\bullet} \qquad (3)$$

The hydroxyl radicals ($OH^{\bullet}$) will completely oxidize organic compounds, converting the carbon atoms of the organic compound into CO_2.

$$\text{Organic Compounds} + OH^{\bullet} \rightarrow CO_2 + H_2O \qquad (4)$$

When the TOC concentration in the sample is low (<1 ppm), complete oxidation can usually be achieved using only the hydroxyl radicals from the photolysis of water (eq. 1) without the addition of per sulfate.

The IC stream passes through a delay coil, which is designed to make the total transit time of the IC stream through the Analyzer the same as the transit time of the TC stream through the Analyzer.

When the TC stream exits the oxidation reactor and the IC stream exits the delay coil, each stream moves to its respective CO_2 Transfer Module. The CO_2 Transfer Module is a patented design, utilizing a gas-permeable membrane that allows the transfer of CO_2 across the membrane. The membrane separates the sample side of the Analyzer from the DI side. The DI side of the Analyzer is a closed loop and consists of two conductivity cells—one for the TC stream and one for the IC stream—a DI water pump, DI water reservoir, and ion exchange resin (resin bed).

CO_2 from the sample passes through the membrane into the DI water supplied by the integrated DI Loop, while interfering compounds and other oxidation by-products are blocked by the membrane and remain on the sample side.

The CO_2 forms carbonic acid upon reaction with water, and the carbonic acid disassociates into hydrogen ions and bicarbonate ions:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \qquad (5)$$

DI water is continuously pumped through the DI side of the Analyzer, collecting the H^+ and HCO_3^- ions and H_2CO_3 and CO_2 molecules from the CO_2 transfer modules, delivering it to the conductivity cell for measurement.Then the ion exchange resin removes the HCO_3^- and other ions. The water is then pumped back to the CO_2 transfer module to repeat the sequence.

The TC and IC conductivity cells each contain a thermistor, and all conductivity readings are temperature corrected. The CO_2 from the TC and IC sample streams are measured by the respective conductivity cells, and the conductivity readings are used to calculate the concentration of TC and IC. Once the values are measured، TOC is calculated as:

$$TOC = TC - IC \qquad (6)$$

Major Accessories and Configurations

Inorganic Carbon Remover (ICR) Unit

For water samples containing high levels of inorganic carbon (IC) compared to the TOC levels (such as some municipal water systems, groundwater supplies, and RO permeates), improved accuracy in TOC measurements can be achieved by removing most of the IC prior to TOC measurement. The ICR consists of a vacuum degassing module, a vacuum pump, and a carbon and soda lime trap. In operation, the water sample is introduced into the Analyzer

and acid is added to the sample as usual. The stream is then directed through the vacuum-degassing module. Carbon dioxide produced from the reaction of bicarbonate and carbonate with acid is removed from the sample stream by the vacuum. The sample is returned to the Analyzer and is directed by the stream splitter for measurement of IC and TC. Approximately 98% of the IC is removed at concentrations up to 25 ppm. The activated carbon/soda lime trap prevents contamination of the sample stream from organic compounds and CO_2 in the atmosphere.

Typically, if the TOC is 10% or less of the IC concentration, the ICR will be required. For example, if the IC is (50 ppm), then use the ICR if the TOC is (5 ppm) or less for accurate TOC measurements. The Analyzer will calculate the TOC/IC ratio for each measurement; if this ratio is less than 0.1, a warning message will be issued and written to the Warnings/Errors list.

System Description

The TOC is designed to enable measurement of low-level organic contamination in water in the presence of large amounts of dissolved inorganic carbon. This situation is typically found in the effluent of reverse osmosis (RO) membrane units, as well as in some municipal waters.

Sources of IC include dissolved limestone and absorption of CO_2 from air. When the ratio of TOC to TC is less than 0.1, the accuracy and precision of the TOC result may become unacceptable. The Analyzer automatically detects this condition and will report a warning message. In order to accurately measure the TOC in this case, it is necessary to remove the IC before performing the measurement. Since the ratio of the non-ionic dissolved form (CO_2) to the ionic forms (HCO_3 and CO_3^{2-}) depends on pH, addition of acid causes all the IC to be present in the non-ionic dissolved CO_2 form:

$$2H^+ + CO_3^{2-} \rightarrow H^+ + HCO_3^- \rightarrow H_2O + CO_2 \qquad pH < 4$$

Once the IC is in the CO_2 form, it can be removed from the sample through a gas-permeable membrane using vacuum on the other side. The ICR uses this principle to remove nearly all the IC from the flowing sample stream.

The unit consists of a membrane module; a vacuum pump; a chemical trap; a switching valve; and associated electrical, hardware, and plumbing components. The chemical trap contains activated carbon and soda lime، and is used to purify the air used in the ICR.

Figure (1) shows a schematic of the ICR. The sample flows into the analyzer and is acidified using the acid reagent in the analyzer, then exits the Analyzer and flows through the Teflon® degasser. The vacuum pump draws air at reduced pressure through the activated carbon/soda lime trap and through the

degasser. Carbon dioxide produced from the acidified sample passes through the degasser into the air stream and leaves the ICR through the pump exhaust. The activated chemical trap prevents contamination of the sample stream from organic compounds and CO_2 in the atmosphere.

The switching valve is used to bypass the Teflon degasser, allowing normal TOC measurements even when the ICR is connected to the instrument. The vacuum sensor alerts the operator if a vacuum problem occurs.

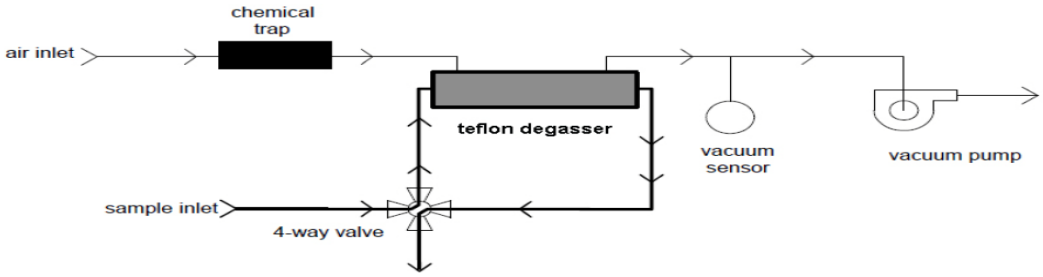


Figure 1: Flow Schematic of the ICR unit

Auto sampler System

The optional Auto sampler System consists of:

- Auto sampler.
- Software, running on a computer with a Windows® Operating System.
- Laboratory or Portable TOC Analyzer.
- Printer software controls both the Analyzer and the Auto sampler. Vials are screw-capped and have Teflon-lined septa. A stainless steel needle is used to transfer samples from the sample vials into the Analyzer. Results are displayed and stored by the computer via the software.

Practical

- Software application.
- Reference material preparation.
- Data Handling.
- Calibration steps.

5- AOX

Introduction

For **TOX determination**, the organic substances contained in a water sample are adsorbed by activated carbon. By adding sodium nitrate to the water sample and, after adsorption, washing the loaded carbon with halide-free sodium nitrate solution, inorganic halogen compounds are displaced from the carbon the loaded carbon is then combusted at 950°C in an oxygen flow. This

converts the organic halogen compounds into hydrogen halides, the concentration of which is determined, usually by coulometry.

$$TOX = AOX + POX + EOX,$$

Where:

TOX: Total organic halogen.

POX: Purgable organic halogen.

EOX: Extractable organic halogen.

AOX: Adsorbable organic halogen.

Analytical principles

Argentometric-coulometric titration with biamperometric end-point detection.

Reaction equation

(Argentometry): $Ag^+ + X^- \text{----> } AgX$

Where: X = Cl, Br, I

Coulometric (electrolytic) silver ion.

Generation (Anode): $Ag^- \rightarrow Ag^+ + e^-$

Silver ion „counting“: Coulometry ($q = I \times t$)

(Measuring the quantity of electric charge)

Faraday's law: $F = 96493\ As = m_{ag} / q$

Alternative to coulometry : Volumetry

Advantages of coulometry:

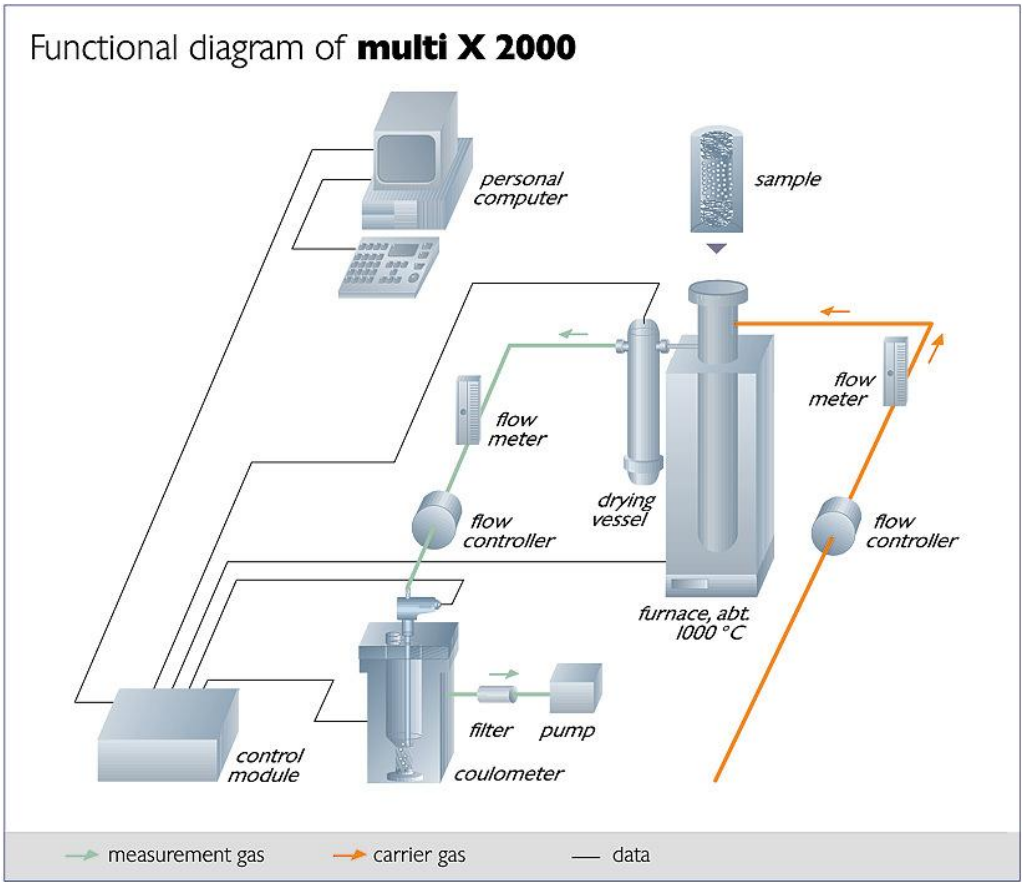
- Absolute method (no calibration needed).
- No standard solutions (titers, factors) needed.
- Small reagent quantities required (low cost, no disposal problems).
- Easy signal processing.
- High resolution of results.

Indication

Under defined conditions, the current flowing between two polarized silver electrodes is proportional to the concentration of Ag^+. A voltage is applied to two silver electrodes.

The "indicator" current flowing between the electrodes is converted into a quantity that controls titration.

Equipment



Functional Diagram of multi X 2000

Combustion System

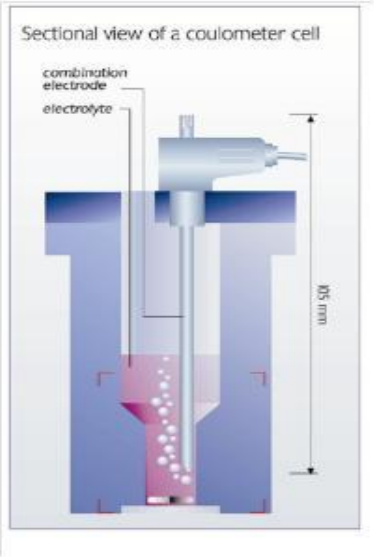
Vertical furnace with gas lock permits simple sample feeding. No complicated mechanical lock systems, and therefore no maintenance needed. Partial and full automation by easy retrofitting. Unequalled by any competitive system!

The quartz containers or fritted glass filters are introduced into the combustion system completely with the activated carbon or polycarbonate filters, respectively. The carbon therefore need not be ejected from the containers, which save a lot of work, prevents contamination and losses, and protects the combustion system from diversification Conversion for the determination of TOX (by the batch, sewage sludge or column method).

Coulometer cell

Patented Coulometer cell with self-cleaning silver anode the combination electrode needs neither a diaphragm nor a salt bridge. This ensures fast readiness for operation and requires no maintenance. A wide dynamic measuring range (0.1 – 250 µg absolute chloride) permits measurement of

samples with high TOX concentrations without dilution and without electrolyte exhaustion.



Electrolyte: Acetic acid of about 20 %, with stabilizer, ensures low evaporation; AgX is retained in solution finely dispersed; an activated carbon filter behind the cell prevents obnoxious odor.

Cooled coulometer cell option provides long-time stability during over-night.

Measurements and difficult climatic conditions; favorable signal-to-noise ratio; reduced drift.

Software

Windows

Controlling and monitoring of all measurement cycles and system parameters.

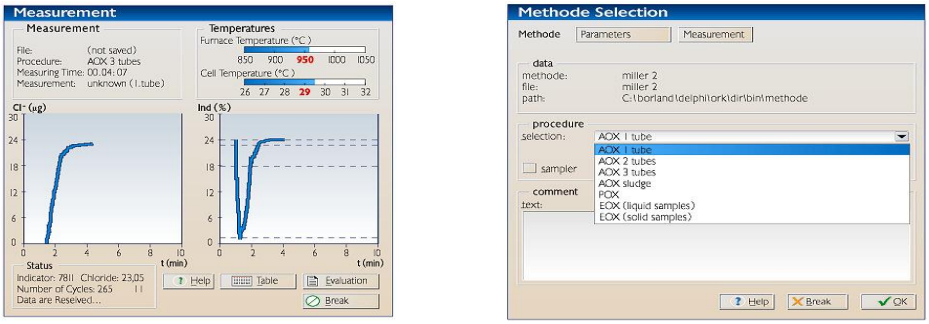
Graphic presentation of the measurement cycle, with continuous display of all important data and parameters on the screen.

Management and saving of measured data and parameters conforming to GLP; supplementary quality assurance software; printout of control charts.

Results output in terms of absolute data and concentrations; conversion of dilution steps.

Highly convenient and fail-safe operation.

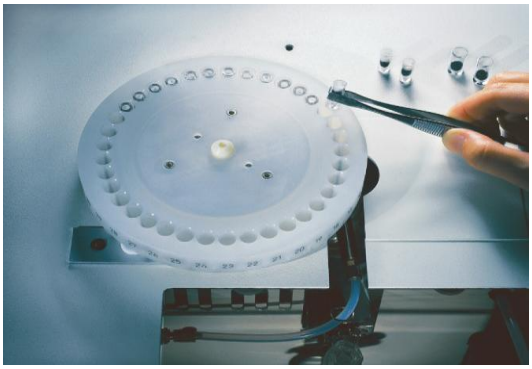
A diagnostic program permits the user to check the analyzer for faultless function at any time, and to locate any irregularities. Error messages are shown in plain text.



Graphic representation of measurement processes in TOX determination

Auto sampler

- Simple design, practically trouble-proof; no mechanical locks.
- The same auto sampler is suitable for the batch and column methods. Method conversion by simple exchange of the sample tray (25 or 36 places, respectively).
- Can be used with pneumatically controlled pre-combustion adapter for batch shaking or sewer sludge analysis; pre-combustion time can be selected via software.
- Automatic gas stop at the end of an analysis series minimizes operating costs.



General safety

European standards require that AOX measurements be made by professional institutions and duly qualified persons. This also includes the responsible, expert dealing with the potential hazards involved in all sampling, sample preparation and analysis procedures. Strictly observe all warnings and instructions given in this manual, in addition to any relevant general regulations and measures concerning labor safety and fire protection.

Potential Hazards

In working with the multi X® 2000, the following potential hazards must be considered:

- Handling of compressed gases
- Handling of oxygen
- Handling of toxic and caustic substances
- Handling of electrical equipment
- Handling of glass apparatus
- Handling of explosive substances
- High temperatures

Further Instructions

- The minimum precaution necessary is to wear safety goggles.
- The female standard ground joint of the combustion chamber, the sample airlock and the upper safety cover of the furnace as well as the furnace output end may reach temperatures of about 100 °C. Never touch these parts before they have cooled down.
- The various heads attached on top of the furnace have parts reaching far down into the furnace, which may heat up to more than 500 °C. To prevent inflammation, do not remove a head from the furnace before it has cooled down.
- When emptying the furnace, mind the temperature of the sample tubes falling out (risk of inflammation and burns).
- The AOX furnace is primarily designed for the combustion of activated carbon, but may also be used for paralyzing other substances. Avoid loading the furnace with solids or liquids that cannot be fixed in the fused-quartz sample tubes. Obtain information on whether the furnace is suitable for a particular solid or liquid before loading it.
- Take precautions against the formation of explosive mixtures before combusting substances liable to form such mixtures.

Chemical and reagents

Activated carbon: The adsorptive capacity of activated carbon is indicated by its iodine value. According to (AWWA Standard B 604), it should not be below 1050. Grain size distribution should satisfy the requirements of DIN 19 603. The blank value of the washed activated carbon should be less than 15 µg of chloride equivalent per gram of carbon.

Nitric acid: Conc. (HNO_3) = 10 mol/l

Nitric acid: about. 15%

Hydrochloric acid: Conc. (HCl) = 0.010 mol/l

Sulfuric acid: Density 1.84 g/ml

Oxygen: O_2

Nitrate stock solution: Dissolve 17 g of $NaNO_3$ in water, add 14 ml of concentrated nitric acid, and make up with water to a volume of 1000 ml.

Nitrate washing solution: Make up 50 ml of nitrate stock solution with water to a volume of 1000 ml.

Sodium sulphite (Na_2SO_3)

P- Chlorophenol stock solution: Dissolve 725 mg of p-chlorophenol (C_6H_5ClO) in 1000 ml of water. Pipette 5 ml of this solution into a 1000 ml volumetric flask and make up to volume with water. This solution contains 1 mg of organic chlorine per liter. P-Chlorophenol standard solutions: 1, Pipette 5, 10, 20, 25 ml of stock solution into a 100 ml volumetric flask, and make up to volume with water. This solution is of limited stability. For quality checks, only freshly prepared solution should be used.

Electrolyte solution: Pipette 8 ml of component solution b into a 100 ml volumetric flask, and make up to volume with component solution a.

Solution a: Pipette 200 ml of acetic acid, conc. ($C_2H_4O_2$) = 99%, into a 1000 ml volumetric flask, add 500 ml of water, add 4 ml of concentrated nitric acid, and make up to volume with water.

Solution b1: Stir 4 g of gelatin with 400 ml of water; allow swelling for 3 hours, and then dissolving while heating up to between 35 and 45 °C.

Solution b2: Dissolve 1.0 g of thymol and 0.3 g of thymol blue in 500 ml of methanol.

Solution b: Slowly stir the cooled-down (18 -22 °C) solution b1 into solution b2, filter into a 1000 ml volumetric flask, and make up to volume with water.

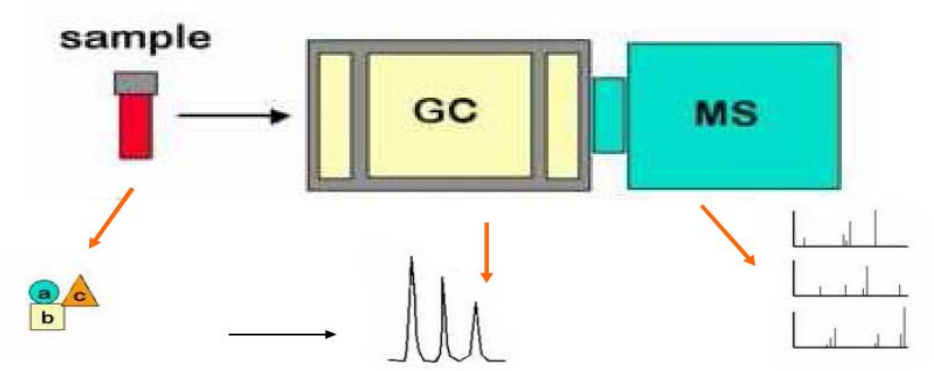
Stability of the electrolyte solution:

Solutions **a** and **b** can be kept in tightly closed bottles in the refrigerator for about 6 months. The readily mixed electrolyte solution should be kept in closed glass bottles for no more than 30 days at about (20 - 25 °C).

Practical

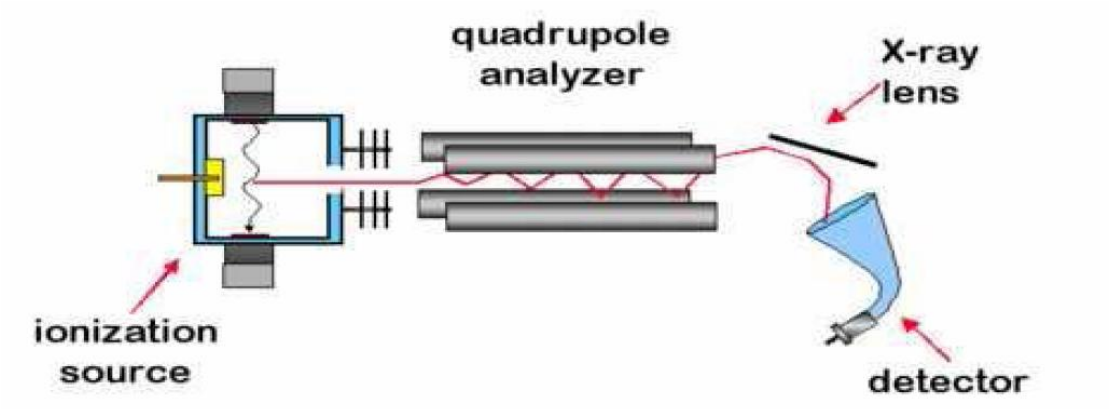
1. Sample preparation.
2. Software application.
3. Reference material preparation.
4. Data Handling.

6- Principles of GC/MS

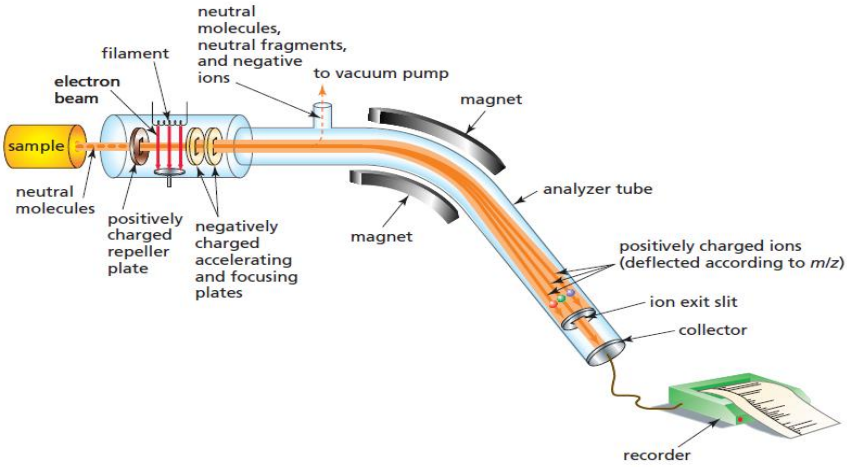


The GC / MS consist of:

1. GC which is responsible for the separation of compound mix
2. MS unit which consist of ion source for (ionization and fragmentation), and analyzer (mass filter) and detector for analyte detection.



1-There are many types of ion sources can be used for ionization and fragmentation. As electron ionization (EI), chemical ionization (CI), fast atom bombardment (FAB), electron spray ionization (ESI) and atmospheric pressure chemical ionization (APCI). Selecting the ion source depend on the technique be used and type of compound be detected. EI and CI used in GC and in GC EI for coarse fragmentation (library search) and CI for fine fragmentation. FAB, ESI, APCI used in LC. ESI used in polar compounds and APCI for non polar compounds.



Ion sources for GC

1- Electron Ionization

In electron ionization (EI), the analyte of interest, in the vapour phase, is bombarded with high-energy electrons (usually 70 eV)

(1 eV = 1.602 177 33 ×10^{-19} J). Analyte molecules absorb some of this energy (typically around 20 eV) and this causes a number of processes to occur. The simplest of these is where the analyte is ionized by the removal of a single electron. This yields a radical cation, termed the molecular ion ($M^{+\bullet}$), the m/z of which corresponds to the molecular weight of the analyte. This process typically requires some (10 eV) of energy and the ion so formed is therefore likely to possess around (10 eV) of excess energy which may bring about its fragmentation bond energies in organic molecules are typically around 4–5 eV. Two types of process may occur, i.e. Simple scission of bonds and, when certain spatial arrangements of atoms occur within the molecule, fragmentation after rearrangement of the molecular structure. The latter process produces ions which would not immediately be expected from a simple examination of the structure of the analyte molecule involved. The presence of rearrangement ions within a mass spectrum is usually highly significant in terms of deriving the structure of the analyte concerned. The processes occurring in electron ionization are summarized in Figure (1).

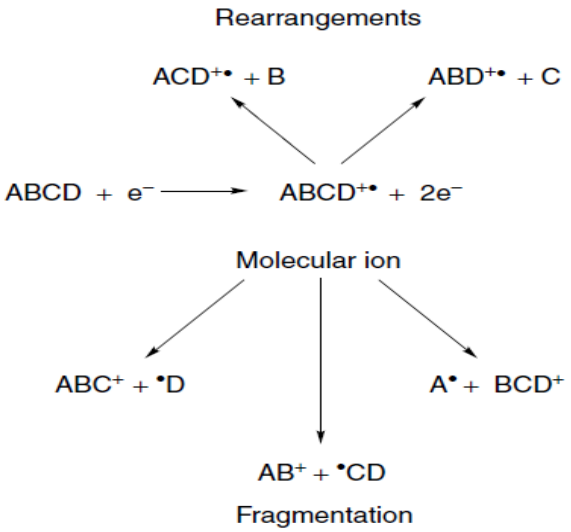


Figure (1) Processes occurring in the production of a mass spectrum by electron ionization.

2- Chemical Ionization

One of the major limitations of (EI) is that the excess energy imparted to the analyte molecule during electron bombardment may bring about such rapid fragmentation that the molecular ion is not observed in the mass spectrum. Under these circumstances, one of the most important pieces of analytical information is lost and the value of mass spectrometry is much reduced. Chemical ionization (CI) is a technique that has been developed specifically to enhance the production of molecular species, i.e. to reduce the fragmentation associated with ionization. A number of such techniques exist and these are known collectively as 'soft ionization techniques'. In this approach, analyte molecules, in the vapour phase (as with EI), are introduced into a mass spectrometer source containing a reagent gas. This mixture is then bombarded with electrons, as described above for (EI), and ionization occurs. Since the reagent gas is present in vast excess when compared to the analyte molecules (typically > 1000:1), it is the reagent gas, almost exclusively, which is ionized. Ion–molecule reactions then take place between the reagent gas ions and the neutral analyte molecules in the high-pressure regime of the mass spectrometer source. The specific reactions which take place depend upon the thermodynamics of the processes that are possible but typically lead to the formation of adducts of reagent ions with analyte molecules in relatively low energy processes which lead to little fragmentation.

The most commonly used reagent gases are methane, isobutane and ammonia, with the processes involved when methane is used being summarized in Figure (2). When interpreting spectra generated in this way it must be remembered that the m/z of the ion observed in the molecular ion region does not give the molecular weight directly as it arises from the combination of the analyte with an adduct. The mass of that adduct 1 in the

case of methane and isobutane, and 18 in the case of ammonia, must be subtracted from the m/z value observed.

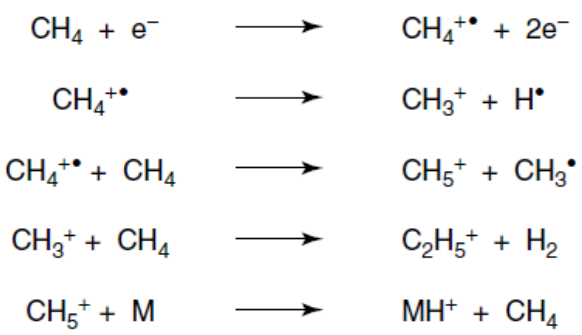


Figure (2) Processes occurring in chemical ionization mass spectrometry using methane as the reagent gas.

Ion separation (mass analyzer)

The mass analyzer is used to separate ions within a selected range of mass-to-charge (m/z) ratios. The analyzer is an important part of the instrument because of the role it plays in the instrument's accuracy and mass range.

1- Quadrupole analyzers

A classic quadruple is formed of four parallel metallic rods (L = 5–20cm) of hyperbolic cross-section in the interior of the assembly (Figure 16.8). These four rods are electrodes that are coupled in pairs. The separation between two diametrically opposed rods is designated as ($2r_0$). If a potential difference U is applied across the pairs, the potential $Ø_E$ for all points within the xy-plane, irrespective of z, will be given by:

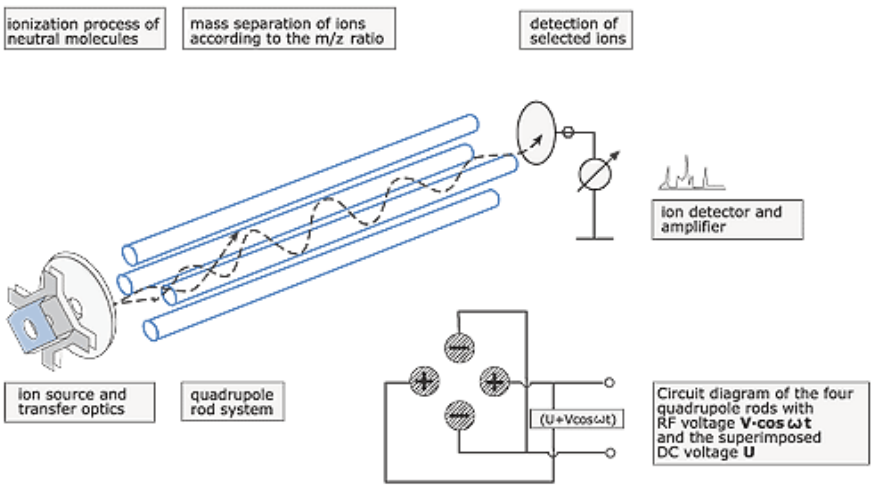
$$\Phi_E = U \cdot \frac{x^2 - y^2}{r_0^2}$$

$Ø_E$ remains between (+U) and (−U). For a homogeneous medium, the electric potential (E) is therefore zero all along the z-axis.

Expression 16.13 implies that in all xy-planes the points having the same potential are situated on the branches of an equilateral hyperbola of which the asymptotes are the straight lines (y=±x). The field lines are orthogonal to the equipotential curves for each point M_{xyz} of the space inside the quadruple. This field has the value:

$$\vec{E} = -\text{grad}\Phi_E$$

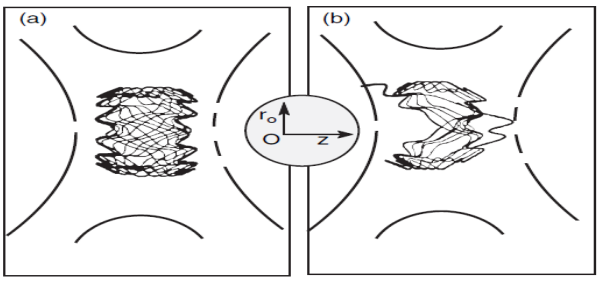
The voltage at the surface of each electrode inside the quadruple is given by: When a positive ion penetrates via the origin O into the filter maintained under vacuum, the components of its velocity vector in xyz-space, represented by _x_ y_ z_, will determine its trajectory. The central zone behaves like a tunnel along the z-axis, whose walls can attract or repel an ion depending upon the ion's position. The two positively charged electrodes focus it along the z-axis, which corresponds to the bottom of a potential valley (stability zone), while the two negatively charged bars, in contrast, have a defocusing effect (appearance of an mound of potential in the yz-plane).



2- Quadruple ion trap analyzers

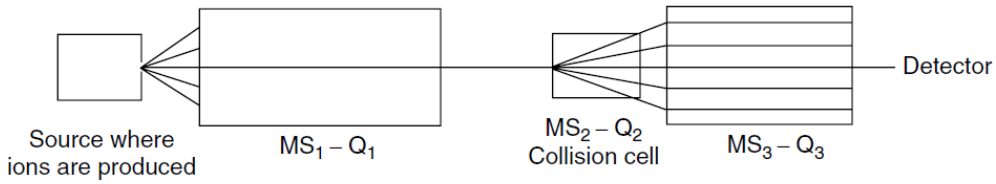
Other mass spectrometers are equipped with three-dimensional ion traps of which the geometry is much different to the quadruples previously described. In an ion-trap, the ions are confined between three electrodes (one toroidal and two end-caps), who's particular shape appears to result from a sort of an amorphosis of the four-bar set-up of a classic quadruple. As in the previous category they operate under the effect of a variable electric field (with or without a superimposed fixed field). Although they are, in appearance, physically simple devices, the fundamental principle of ion trap is complex. These ion trap detectors are sensitive, less costly than quadruples and compatible with different ionization techniques. The volume defined by the electrodes, named superior, inferior and annular, is simultaneously the ion source and the mass filter (Figure 16.11). These analyzers are almost exclusively linked with a separative technique (GC/MS). The functioning principles can be discussed as follows: the compound is submitted to a very brief electron bombardment yielding ions which subsequently penetrate the central part of the filter. A radiofrequency voltage is then applied to the annular electrode that confines these ions to the central space, by forcing them to follow complex trajectories in the presence of a low pressure of helium, of about (0.01 Pa), introduced to the trap as a form of shock absorber. To analyze the ions present, the radiofrequency amplitude is increased gradually to destabilize the ions. One by one, in the rising order of m/z ratio, the amplitude of oscillation of the ions grows in the axial direction until they are eventually ejected through a series of small holes pierced in one of the end-cap electrodes behind which stands an electron multiplier as detector

(Figure 5). If a reagent gas is introduced concurrently into the trap to react with the sample then a chemical ionization can also be obtained This small volume and high sensitivity instruments have, however, the inconvenience of operating over a narrow dynamic range and with an average reproducibility of spectra from the same sample, which represents a serious disadvantage in quantitative analysis.



MS - MS technique

In MS/MS technique two analyzer and collision cell are used. The first analyzer is used in choice interest ion (parent ion) and collision cell for the fragmentation of parent ion to daughter ions and the second analyzer as mass filter for daughter ions as in figure



Techniques

MS Scans

Full Scan

Scan from the (Q1 First Mass) to the (Q1 Last Mass). These scans are rich in spectral information, excellent for screening assays, ideal for identifying unknowns and for library searches.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	130.00	280.00					1.063

Selected Ion Monitoring

Scan selected ions only. Depending on the number of ions monitored and matrix interference, SIM sensitivity may be 5 to 50 times better than a full scan. SIM always give the maximum signal to noise ratio.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Neg.	321.00						0.500
2	Pos.	414.00						0.100

MS/MS Scans

Product Ion Scan

In Q1 select a precursor ion of interest; fragment it in Q2, and in Q3 scan for product ions in a selected mass range. This provides structural information about the precursor ion.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	219.00		50.00	250.00		5.000	0.500

Selected Reaction Monitoring

In Q1 scan for a precursor ion, in Q2 fragment the ions, and in Q3 scan for one specific product ion.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	414.00		219.00			10.000	0.500

Multiple Reaction Monitoring

In Q1 scan several precursor ions, in Q2 fragment the ions, and in Q3 scan for a specific product ion from each.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	452.00		225.00			5.000	0.500
2	Pos.	525.00		315.00			10.000	0.100
3	Pos.	633.00		380.00			15.000	0.100

Precursor Scan

In Q1 scan a mass range, in Q2 fragment the ions, and in Q3 check for a product ion of a particular mass. Use this to identify common functional groups or moieties for several analytes.

	Polarity	Q1 First Mass	Q1 Last Mass	Q3 First Mass	Q3 Last Mass	Capillary	Collision Energy	Req. Dwell Time
1	Pos.	360.00	420.00	191.00			5.000	0.120
2	Pos.	365.00	425.00	217.00			5.000	0.049

Detector

There are two types of detectors used in MS unit:

1- Electron multiplier

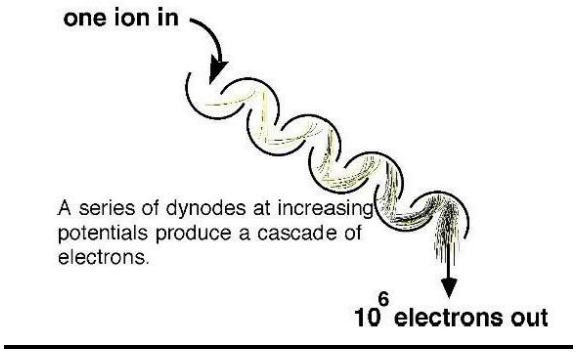
or

2- Photomultiplier tube.

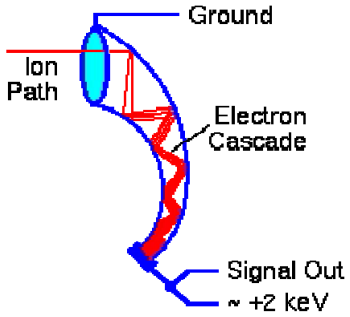
Electron multiplier more sensitive than Photomultiplier but its life shorter than Photomultiplier.

Electron Multiplier

An electron multiplier is made up of a series of dynodes maintained at ever increasing potentials. Ions strike the dynode surface, resulting in the emission of electrons. These secondary electrons are then attracted to the next dynode where more secondary electrons are generated, ultimately resulting in a cascade of electrons.



Electron multipliers can also be made from continuous dynode materials rather than discrete dynodes. This glassy material contains lead that provides conductivity comparable to the resistor chain in the discrete dynode electron multipliers.

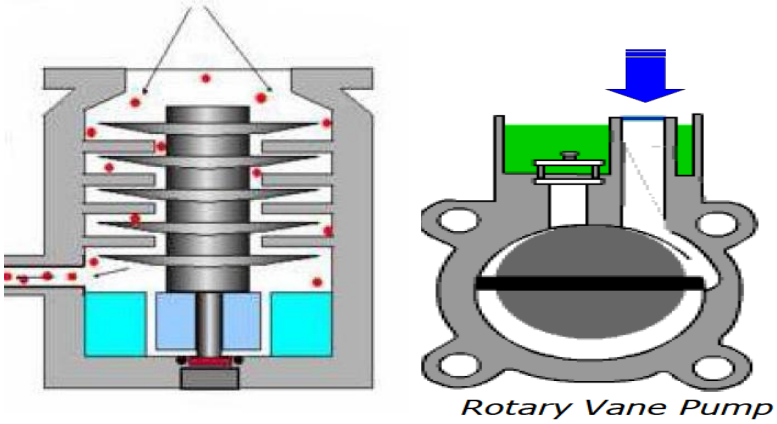


The Vacuum System

At the MS unit must be under vacuum at least from 10^{-4} – 10^{-6} this achieved free path from O_2, CO_2, H_2O, electric arch and this will achieved high sensitivity.

There two types of pumps into MS unit:

1. The rotary pump serves as our rough or fore pump. It can produce a vacuum of 10^{-2} to 10^{-4}Torr.
2. Turbo molecular or diffusion pump isused to achieve vacuums in the 10^{-5} Torr range.



7- Introduction to MS Ion Trap Theory

Mass Spectrometry

- Analytical technique used to identify compounds on the basis of their fragmentation pattern upon ionization.
- Samples are ionized and subsequently separated by their mass-to-charge ratios (m/z).
- A mass spectrum provides structural information about compounds which is used for their identification.

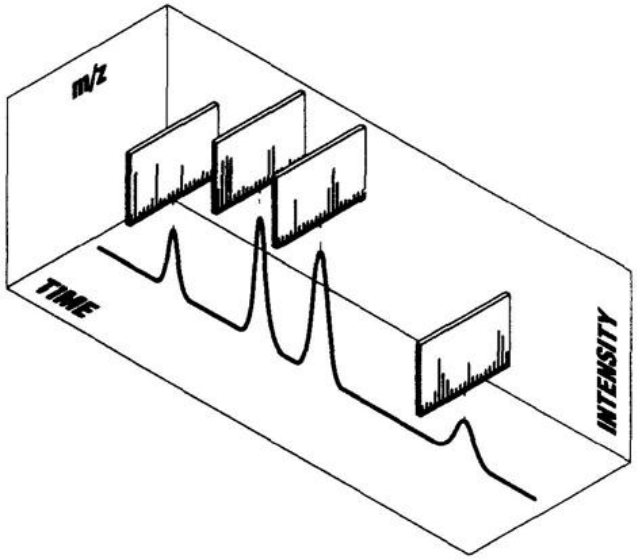
Gas Chromatography - Mass Spectrometry

Gas chromatography/mass spectrometry (GC/MS) is the combined technique of GC separation and MS detection.

The GC separates mixtures into discrete bands of compounds and then introduces those bands into the MS.

The analyte bands are ionized and MS analyses the ions generated in the selected mass range.

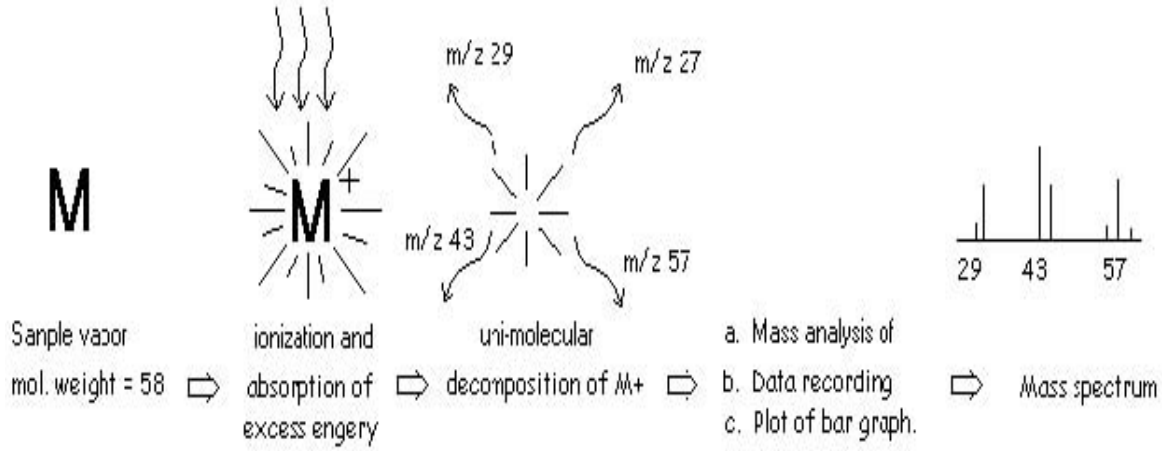
The GC/MS can provide data in three domains Time, Intensity and Mass spectrum



Mass Spectrometry Review

MS is an analytical technique for determining chemical composition and molecular structure.

The sample is converted into ions and neutral particles by an ionization process; the resulting ions are separated according to their mass-to-charge ratio (m/z).



The instrument consists of three major components:

1. Ion source: The purpose of the ion source is to provide the energy necessary to ionize the analyte molecules, while being maintained at a temperature high enough to prevent analyte condensation.

2. Mass analyzer for resolving the ions into their characteristics mass components according to their mass-to-charge ratio, (Magnetic sector, Quadruple and ion trap).
3. Detector system for detecting the ions and recording the relative abundance of each of the resolved ionic species. The detector used in GC/MS instrumentation is an electron multiplier. The detector collects the ions, which have been produced, and amplifies them into a measurable signal.

A data system controls instrument parameters and allows acquisition, manipulation. In addition, a sample introduction system is necessary to admit the samples to be studied to the ion source while maintaining the high vacuum; the advantage to using a GC as the inlet is that it separates complex mixtures into their individual components before the sample is introduced into the mass spectrometer. One of the drawbacks to using a GC as the inlet to an MS is the pressure differential. A GC operates at elevated pressures, but an MS operates under vacuum conditions (10^{-5} to 10^{-6} Torre) to allow for a long mean free path of movement of ions and to prevent any ion-molecule reactions.

Ion source:

The purpose of the ion source is to provide the energy necessary to ionize the analyte molecules, while being maintained at a temperature high enough to prevent analyte condensation.

The two types of ionization normally used in GC/MS are electron ionization (EI) and chemical ionization (CI).

Electron ionization (EI):

Molecules of the sample under analysis enter the ionization chamber in the vapor state. Positive ions are produced by passing a beam of electrons, obtained from tungsten or rhenium filaments, through the vapor, which is maintained at a pressure of (10^{-4} to 10^{-6}) mm of mercury. Provided the energy of the electron beam is greater than the ionization potential of the sample, the sample is ionized and/or fragmented, as represented by the following equation:

$$M + e^-_{70\ eV} \longrightarrow M^{+\bullet} + e^-_{50\ eV} + e^-_{thermal}$$

The molecular ion M+• can decompose to lower mass fragment, which may itself fragment or rearrange.

$$M^{+\bullet} \rightarrow A^{+\bullet} + B \rightarrow C^{+} + D^{\bullet}$$

Chemical ionization:

If a large excess of a reagent gas is employed together with the sample. As there is an excess of the reagent gas, the reagent molecules are preferentially ionized and the reagent ions then collide with the sample molecules and produce sample + reagent ions or in some cases protonated ions. This type of ionization is called chemical ionization and is a very gentle form of ionization. Very little fragmentation takes place and parent ions + a proton or a molecule of the reagent gas is produced. The molecular weight of the parent ion are thus easily obtained.

The most commonly used reagent gases are methane, isobutane, and ammonia.

Typical reactions for methane are shown in the following equations:

(EI) of methane, generates $CH_4^{+}\cdot$ which then reacts to give the Bronsted acid CH_5^{+};

$$CH_4^{+}\cdot + CH_4 \rightarrow CH_5^{+} + CH_3\cdot$$

If M in the source has a higher proton affinity than CH_4, the protonated species MH^{+} will be formed.

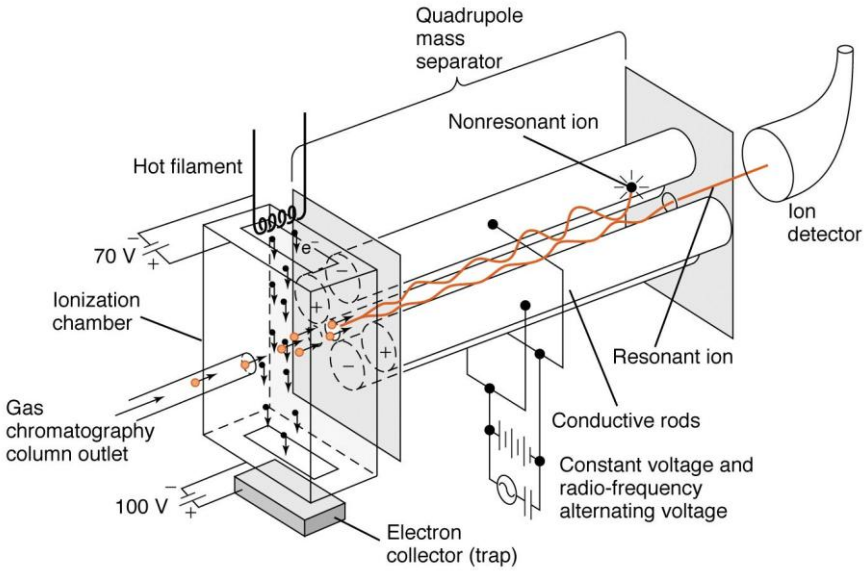
$$M + CH_5^{+} \rightarrow MH^{+} + CH_4$$

Mass analyzer

A mass spectrometer is classified according to the technique used to separate the ions.

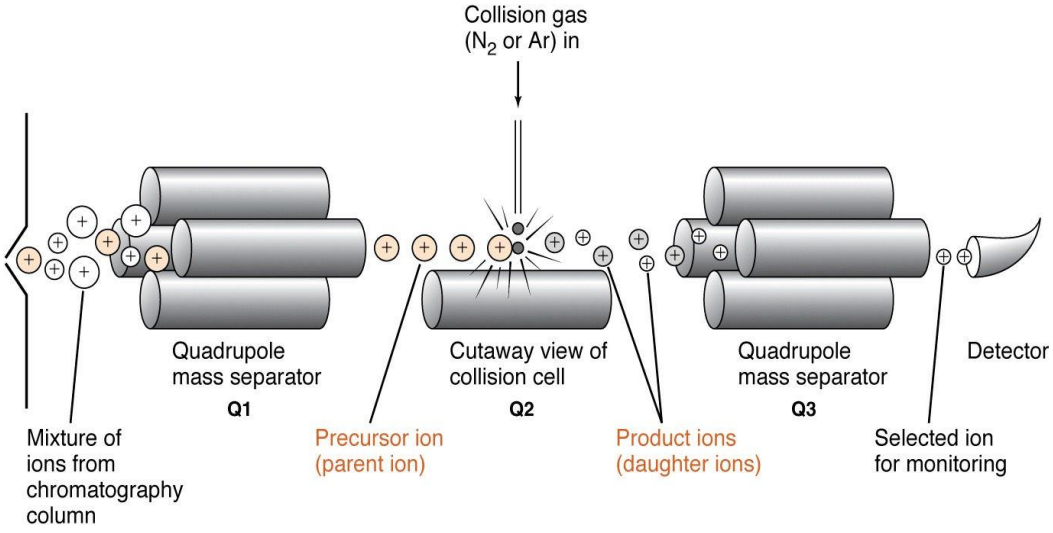
Quadruple Analyzers:

The instrument is based on four parallel rods in a square array. The ion beam is focused down the axis of the array and an electrical potential of fixed (DC) and radiofrequency (RF) components is applied to diagonally opposed rods. For a given combination of DC and RF components, ions of one specific m/z ratio have a stable path down the axis. All others are deflected to the sides and lost. Mass scanning is achieved by changing the DC and RF components of the voltage, while maintaining a fixed ratio. The quadruple analyzer is a mass filter because it separates ions on the basis of their m/z ratio.



Mass Spectrometry / Mass Spectrometry (MS/MS).

A typical analyzer array for doing MS/MS uses a linear arrangement of three quadruples between the ion source and the detector. The first and the third quadruples act as independent m/z analyzers, while the second (middle) quadruple acts as a collisional activation chamber through which ions from the first quadruple must pass before they enter the final quadruple.



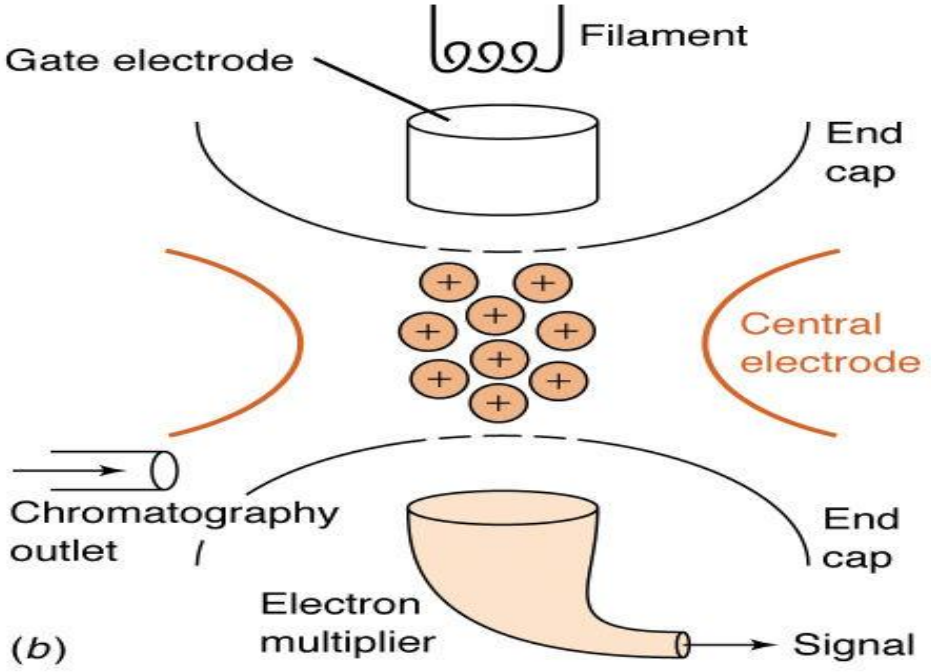
Ion - trap Analyzer

This quadruple-type device is composed of ring electrode placed between two end cap electrodes. The end caps are either held at ground potential or have an RF voltage applied to them, while an RF voltage is placed on the ring

electrode. As a result of these potentials, the hyperbolic surfaces of the three elements form a three dimensional quadruple analyzer.

Both ionization and mass analysis take place within the three-dimensional quadruple field.

In the ionization step, the RF voltage on the ring electrode is set low enough so that the ions within the mass range of interest are trapped within the device. Following ionization, mass analysis is accomplished through use of the “mass selective instability” mode of operation. That is, by raising the RF voltage on the ring electrode, ions of successively higher mass are ejected from the ion trap into an electron multiplier detector. In its most common application.



Options like Selected Ion Storage (SIS) and (MS/MS) can be performed on the ions stored in the ion trap before mass analysis takes place.

In MS/MS a parent ion is isolated and then dissociated by energetic collisions with helium buffer gas to form product ions. In (SIS), resonant waveforms are applied to eject unwanted ions within the stored mass range and fill the trap only with ions in the mass range of interest.

Detector:

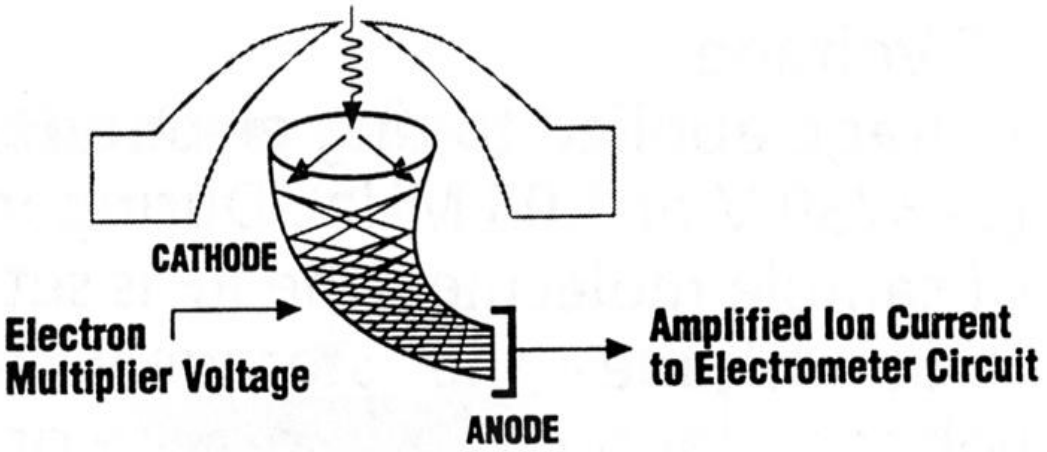
Electron Multiplier:

Electron Multiplier has the ability of surfaces that contain glass doped with about (10 –20%) lead oxide to expel more than one electron when a charged particle collides with it.

The interior surface of the electron multiplier that is located near the entrance is held at a highly negative potential (usually 1.2 to 3 kV); the exit end is referenced to ground (0 V). As each incoming ion collides with the multiplier surface, approximately two electrons are ejected from the surface. To the ejected electrons the remaining interior of the multiplier appears more positive than the entrance does, so that they are attracted further into the multiplier where they collide with the interior surface. Each electron ejected by the second collision also results in the ejection of two electrons, and this process continues down to the exit or last dynode of the multiplier.

Electrons generated in the last collision with the multiplier surface constitute the signal current output of the multiplier. This current is sent to an external electronic signal amplification circuit and finally to the data system.

The multiplier has a specific curve to prevent the cascade of electrons form traveling upward and creating a false signal.



Photomultiplier Detector:

Photomultipliers have been in use for a long time as detectors in radiation-based spectrometry such as IR and UV. Magnification of the signal in a photomultiplier is based on the same principle as that governing the electron multiplier, except that the inner surface of the photomultiplier is sensitive to photons rather than to charged particles. In order to use a photomultiplier as a detector in EIMS, the positive ions must cause the generation of photons that can be detected by the photomultiplier. This is accomplished by means of a fluorescent screen placed across the entrance to photomultiplier. As positive ions collide with the fluorescent screen, photons are produced in proportion to the number of ions present.

Vacuum System

The components of the mass spectrometer that cause ion formation, separation, and detection are contained in an ultraclean housing usually kept at moderately high vacuum.

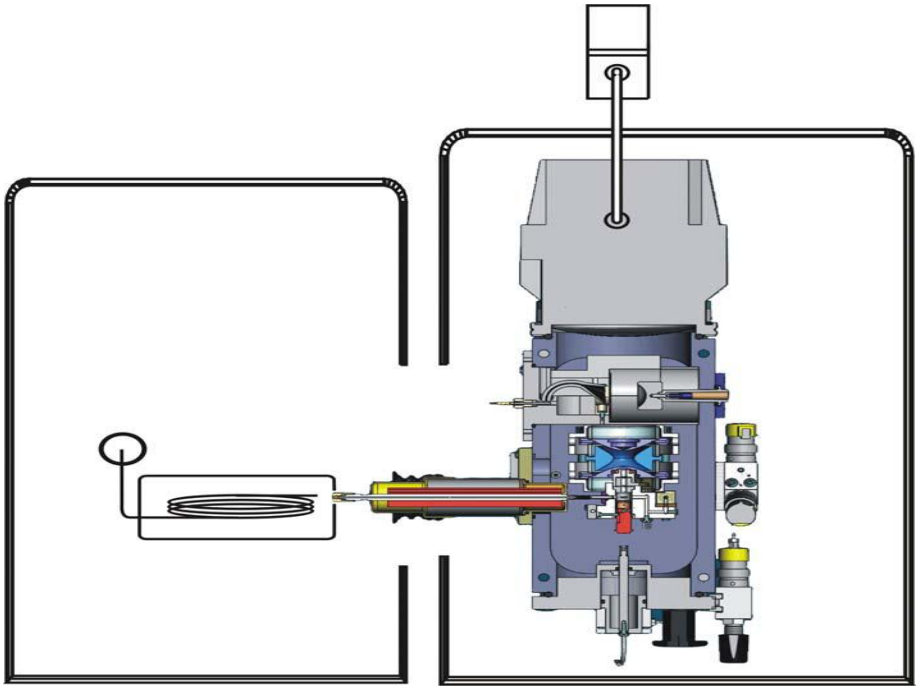
High vacuum ensures that, once the ions formed in the ion source begin to move toward the detector, they will not collide with other molecules because this could result in further fragmentation or deflect them from their desired path.

Nearly all fragmentation reactions occurring under these conditions are intermolecular (involving only the decomposition of individual ions) rather than intermolecular (involving the reaction of ions with other species that may be present). High vacuum also protects the metal and oxide surfaces of the ion source, analyzer, and detector from corrosion by air and water vapor, which could compromise the spectrometer's ability to form, separate, and detect ions.

Instrument equipment:

Overview:

Each subsystem of the 4000 Mass Spectrometer is described. The mass spectrometer is like an analyzer contained in a vacuum manifold surrounded by electronics components that drive the analyzer operation and acquire the resulting data.



Samples are first injected into the GC either manually or by way of an auto sampler. The sample is vaporized and the gas goes though a column in the GC oven. After separation in the column, the sample enters the mass spectrometer through a heated transfer line.

The MS analyzer consists of three parts: the source, ion trap, and detector. The samples flow from the transfer line into either an external source, where the sample is ionized, or directly into the ion trap for ionization. Once ionized, the ions are stored in the ion trap where they are systematically ejected for analysis. After ion ejection, the detector (consisting of a conversion dynode and electron multiplier) senses the ions.

Transfer Line:

A stainless steel tube directly couples the GC to the mass spectrometer.

The purpose of the transfer line is to keep the GC column warm as the column enters the mass spectrometer to avoid condensation of the sample, which could result in tailing.

If the sample is to be ionized in the external source, the transfer line is inserted into the source volume. If the sample is to be ionized in the trap, the transfer line is inserted into a hole in the trap close to an electron generating filament.

The transfer line temperature is set in the Temperature Dialog in System Control; the transfer line temperature should be set so that there is no cold spot between the GC column oven and the MS. In general, the transfer line temperature can be set as (± 20 °C). The maximum column operating temperature and not cause adverse chromatographic effects (e.g., retention time shifts or peak broadening)

After the sample stream passes through the transfer line it is ionized either in the ion trap or in the external source.

Analyzer:

The Analyzer consists of an Internal Ionization Assembly, the Ion Trap and a Conversion Dynode/Electron Multiplier Detector.

Internal Ionization Assembly

Electrons for ionization are produced and gated by an internal ionization source that resides just outside the ion trap's entrance electrode.

The source consists of a filament assembly and electron gate electrode

The filament assembly:

The filament assembly consists of two filaments.

Each filament is a rhenium ribbon. When sufficiently heated by electric current, the filament produces electrons by thermionic emission.

The filament emission current refers to the flow of emitted electrons from the filament. Emission current settings range from 5 to 100 µA.

The electron gate:

The electron gate is a cylindrical electrode that controls the entry of electrons into the ion trap cavity. When electrons emitted from the heated filament are not needed for ionization, the electron gate is held at (-150 Vdc) potential. An anodization layer insulates the electron gate from the filament end cap.

When the ion trap requires electrons, the electron gate potential changes from (-120 to +120 Vdc). The gate potential remains positive for a variable length of time, e.g., from (10 µsec to 65 msec). During this interval, the electrons are focused into the ion trap cavity with sufficient energy to achieve electron ionization of the sample molecules (or of the reagent gas molecules in the case of chemical ionization).

Ion Trap

The ion trap assembly consists of three electrodes contained in a heated oven.

The three electrodes are the entrance, ring and exit electrodes. These electrodes have hyperbolic inner surfaces that together form a cavity in which ionization, fragmentation, storage, and mass analysis take place.

There are holes in the center of both the entrance and exit end cap electrodes. The hole in the entrance electrode allows the entry of ionizing electrons when the system is configured for internal ionization. The hole in the exit end cap allows the exit of ions to the detector.

One of these holes in the entrance end cap also acts as the sample inlet to the ion trap in internal ionization modes.

The trap oven and its clamping plate hold the electrodes and spacers in place.

The RF generator assembly provides high voltage 1 mHz RF voltage that is applied to the RF ring electrode. Under the proper RF voltage, the ion trap electrodes create a three dimensional, hyperbolic electric field. This field is capable of trapping the ions in stable, periodic orbits. In the presence of helium damping gas, the ions are cooled towards the center of the trap. As the RF voltage increases, the ion trajectories become unstable in increasing order of mass to charge ratio. The ion trap ejects the ions and sends them to the conversion dynode and then to the electron multiplier for detection.

Detector

After ions are ejected from the trap, they are detected by a combination conversion dynode/electron multiplier detector. The detector is enclosed in a cylindrical stainless steel shield that prevents metastable ions from entering the source.

The conversion dynode is made up of a rounded stainless steel cup. If positive ions are to be detected, the conversion dynode is set to a large negative voltage (typically (-10 kV)). In this case, the secondary electrons will be attracted to the relatively positive multiplier. For negative ions, the conversion dynode is set to a large positive voltage, in which case positive ions from the dynode are attracted to the relatively negative multiplier.

The continuous-dynode electron multiplier consists of a lead-oxide/glass

A negative voltage of between (-800 and -3000V) is applied to the front end of the electron multiplier, referred to as the cathode. The back end of the cathode is held near ground potential, and is referred to as the anode.

Electrons or ions emitted from the conversion dynode strike the cathode with sufficient velocity to liberated additional electrons from the inner curving surface of the cathode. The increasingly positive potential gradient draws the ejected electrons into the electron multiplier, further accelerating them in the process. Because the electron multiplier is curved, the ejected electrons do not travel far before they again strike the inner surface of the multiplier, resulting in the emission of more electrons. This configuration produces a current of electrons that is accelerated toward ground potential at the exit end of the cathode.

The anode collects the electrons and passes the resulting ion signal to the ion amplifier that is mounted on the side of the vacuum manifold directly next to the multiplier. The ion current is proportional to the total number of ions that the ion trap ejects.

Vacuum System:

The analyzer is contained in a vacuum manifold maintained at a pressure of 10 μ Torr.

A turbo molecular pump provides the vacuum required. The turbo pump is backed by a mechanical rotary foreline pump,

The foreline pump has two purposes. The first is reducing the vacuum system pressure to a level that will allow the operation of the high vacuum turbomolecular pump. The second is maintaining the vacuum system pressure by removing the turbo molecular pump’s exhaust gases

A thermocouple gauge is used to measure the foreline pressure and an ion gauge to measure the vacuum manifold pressure.

Ion Gauge:

An ion gauge is present on the bottom of the vacuum manifold.

The ion gauge response depends on gas composition. A given pressure of air and water will give a different reading than that of helium. The ion gauge is meant to be a good indicator of vacuum conditions.

Thermocouple Gauge:

A thermocouple gauge is attached to the fore- line pump hose to measure pressure to check for gross leaks and fore- line pump failure.

If the Pump Spin Speed does not steadily increase, there may be a leak in the system. Large leaks will be indicated by a turbo speed less than 100%. Small leaks will show up by an increase in the pump current once at 100% or in the ion gauge pressure (See Diagnostics). Small leaks are diagnosed by changes in the ion gauge reading.

المراجع

- **تم الإعداد بمشاركة المشروع الألماني GIZ**
- **و مشاركة السادة :-**

➢ د/ سناء أحمد الإله	شركة مياه الشرب والصرف الصحى بالفيوم
➢ د/ شعبان محمد على	شركة مياه الشرب والصرف الصحى بالفيوم
➢ د/ حمدى عطيه مشالى	شركة مياه الشرب والصرف الصحى بالغربية
➢ د/ سعيد أحمد عباس	شركة مياه الشرب والصرف الصحى بالغربية
➢ د/ عبدالحفيظ السحيمى	شركة مياه الشرب بالقاهرة الكبرى
➢ د/ مى صادق	شركة مياه الشرب بالقاهرة الكبرى