University of Mosul
College of Science
Chemistry Department

SEPARATION METHODS

IN CHEMICAL ANALYSIS

For Third Class

Second Course

Lecturer: Dr. Khalida mohammed Omer

Separating methods

In Chemical Analysis

Syllabus:-

1-Introduction to Analytical Separation :- Include

Separation, Classification of Separation techniques, Equilibrium Constant, General Theory of Separation , Separation Factor ….

2- Chromatography :- Include

History of chromatography ,Classification of Chrom. Methods, Types of Column ,Development of chromatogram ,Theory of Column efficiency , Application of Chrom.

3- Gas Chromatography :- Include

Introduction , Gas-Liquid Chrom. And Gas- Solid Chrom., Apparatus for Gas Chrom. , Carrier gas, Sample injection system , Types of Column , Detectors , Application of GLC (Qualitative and Quantitative Analysis) .

4- Liquid Chromatography and HPLC: Include

Adsorption Chrom , HPLC ,Principle ,Instrumentation ,….Ion exchange Chromatography .

5-Electrophoresis : Include

Types of Electrophoresis , Principle , Application , Capillary electrophoresis , Detectors in CE.

Refereces:-

1-Fundamental of Analytical Chemistry:-

by: Skoog & West . 9th ed (2013) ,p. 889-999.

2-Fundamental of Analytical Chemistry:-

by: Skoog & West . 8th ed (2004) ,p.908-1118.

3-Analytical Chemistry :-An Introduction :

by: Skoog ,West &Holler . 6th ed ((1994), p.486-520.

4-Principles of Instrumental Analysis :-

by: Skoog ,Holler & Grouch . 6th ed (2007) ,P.788-843.

5- An Introduction to chromatography :

By :David & Andrews , p.31 -59.

6-Analytical Chemistry:

by: Gray & Christian 6th ed (2004), p.541-642.

Separation Methods

In Chemical Analysis

Introduction to Analytical Separation

Separations are extremely important in Synthesis, in industrial chemistry , in the biomedical sciences, and in the chemical analysis

Analytical separation occur on a much smaller laboratory scale than in the industrial- scale. The separation methods include

Precipitation , distillation , extraction, masking agent , ….and various chromatographic techniques.

There are some important Definitions related to the topic of chemical separation. (must be understood) .

Separation: Is a processes of any scale that separates of mixture from each other without substantial chemical modification .

In some cases, a separation , may fully divided the mixture into pure constituents.

Or ,it is isolate or separate the analyte from potential interfering constituents.

Separation can carried out based on differences in chemical properties such as Size , Shape , Mass , or Chemical affinity between the constituent of mixture.

and often classified according the particular differences they use to a chive separation.

The Analyte : Is the substance to be separated from other using suitable separation methods.

Sample matrix: Is the medium containing an analyte to interest.

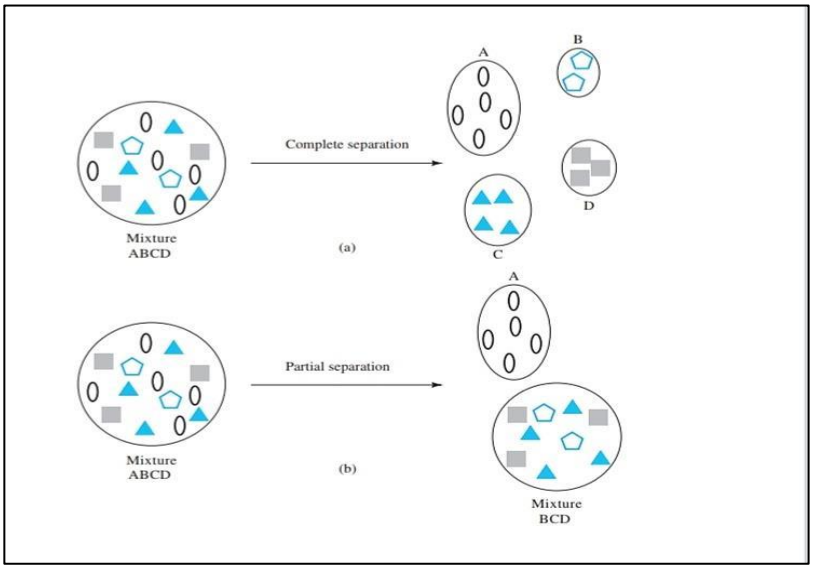
An Interference : Is a chemical species that causes a systematic error in analysis by enhancing Or attenuating the analytical signal.

The following is a simple diagram that illustrates the principle of separation:

In (a) a mixture of four components is completely separated.

In (b)a partial separation is shown.

Species A is isolated from the remaining mixture of B,C, and D.



As a consequence; most analytical methods require One or More preliminary steps to eliminate the effect of Interferents.

Few, if any analytical signal are so specific as to be totally free of Interferences

Tow General Methods are available for dealing with Interferences.

The First .

Makes use of a Masking Agent to immobilize or chemically bind the interferences in a form in which it no longer contributed to Or attenuates the signal from from the analyte

Example **/:-** Cyanide (CN^{-1}) is used to mask heavy metal such as (Cd, Co ,Cu ,Zn, Pd) and prevent their interference in the titration of Mg^{+2} and Ca^{+2} with EDTA.

A masking agent :-is reagent that chemically binds an interference and prevent it from causing errors in an analysis .

The Second

The second approach for avoiding the effect of an interferences Involves the Separating the analyte and the interferent as Separated Phases.

1- The classical way of performing this type of separation is based on Precipitation the analyte selectivity with an appropriate chemical reagent as hydrogen sulfide(H_2S).

2- Another method of removing the analyte as separated phase involve Electrolysis at **controlled electrode** potential .

3- Another method is based on converting the analyte a gaseous phase that can then be isolated by Distillation.

Chemical Species are generally **Separated** by converting them to **different phases** that can then to mechanically isolated

The Phases:

The phase definition are differ according to the separation method (technique) are used .

To simplify purpose , the phases gives number 1& 2 as the following table To emphases on their similarity.

For Example : The Stationary phase in **Chromatography** is resemblance to **Raffinate** in **Extraction** and **Retenate** in **Dialysis** .

Table 1:Explain the name of **Phases 1 and 2** in some methods .

Technique	Phase 1	Phase 2
Chromatography	Stationary phase	Mobile phase
Liquid –liquid Extraction	Raffinate	Extraction
Dialysis	Retenate	Diffusate

Classification of Separation techniques

As shown in the following table ,there are **Several Methods** of separation technique are used for dealing with **interferences** in an analysis , Including ; **Masking agent , Distillation , Solvent Extraction , Chromatography** . As shown below.

Table 2: Classification of separation techniques in general

Basis of Separation	Separation Technique
1-Size →	Filtration
→	Dialysis
→	Size _exclusion chromatography
2- Mass and Density →	Centrifugation
3- Complex formation →	Masking
4- Change in physical state →	Distillation
→	Sublimation
→	Recrystallization
5- Partitioning between phases →	Extraction
→	Chromatography

There are another table explain classification the separation methods according to The type of Method and Basis of Method

Note : Just for perusal

Separation Methods

Method	Basis of Method
1. Mechanical phase separation	
a. Precipitation and filtration	Difference in solubility of compounds formed
b. Distillation	Difference in volatility of compounds
c. Extraction	Difference in solubility in two immiscible liquids
d. Ion exchange	Difference in interaction of reactants with ion-exchange resin
2. Chromatography	Difference in rate of movement of a solute through a stationary phase
3. Electrophoresis	Difference in migration rate of charged species in an electric field

Classification of Separation Methods

There are several Manner of classification of separation methods (6 style):

1- Classification according the Phase .

There are **Two Phases**, the following table explain this ..

The First phase (Fundamental phase) , it is the Sample or contain of the sample.

The Second phase .

Classification according the phase

Fundamental phase (First)	Second Phase		
	Gas	Liquid	Solid
Gas	Thermal diffusion	Gas-Liq. Chrom. GLC	Gas-Solid. Chrom. GSC
Liquid	Distillation	1- Liq.-Liq. Chrom. LLC 2-Liq-Liq.extraction 3-Dialysis 4-Membrance filtration	1-Liq-Solid. chrom. LSC 2-Precipitation 3-Electro deposition 4-Recrystalization
Solid	Sublimation	Solubility extraction	_____

2- Classification the separation methods according the Type of Process. (Mechanical , Physical , Chemical) .

Other classification:

3- Sample introduction ⟹ Zonal or Continuous .

4- Contact phase ⟹ as a Whole or Intervene.

5- Nature of Sample ⟹ Aqueous or Non aqueous.

6- Nature of Sample ⟹ Ionic or Non ionic .

The equilibrium Constsnt (K_p)

When the separation system is reached to equilibrium state , the concentration ratio (Activity) for each component (Solute) in Two phases is stable and express in

$$(K_D)_A = \frac{[CA]_1}{[CA]_2} = \frac{\text{Conc. A in phase 1}}{\text{Conc. A in phase 2}} \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

The symbol C represented (Analytical Conc.) that is include all formulas (Ionic , Molecular , ……..).

$$Kp = \frac{[A]1}{[A]2} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

* Kp is used instead of K_D ,because K_D value can changed at conc. Changed.

* In order to obtain on constant amount (value) of Kp (partition coefficient), Activity is used instead concentration .

$$(Kp)_A = \frac{(\alpha A)1}{(\alpha A)2} = \frac{[A]1 * (\gamma A)1}{[A]2 * (\gamma A)2} \quad \ldots\ldots\ldots\ldots\ldots..(3)$$

γ is activity coefficient , When $\gamma = 1$ then Kp

$$(Kp) = \frac{[A]1}{[A]2} = \frac{(W_A)1 / M.wt_A}{V_1} \div \frac{(W_A)2 / M.wt_A}{V_2} \quad \ldots\ldots\ldots\ldots\ldots(4)$$

$$= \frac{(W_A)1 * V_2}{(W_A)2 * V_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..(5)$$

* $\frac{(W_A)1}{(W_A)2}$ is ratio of total amount called Distribution ratio Or Capacity factor and refer to it in $\acute{K}$

** The volume ratio for two phases is called Phase ratio and symbol in β, Which is $\beta = \frac{V2}{V1}$. then

$$Kp = \acute{K}\beta$$

Factors that control in choice of separation method

1- Amount of available sample and its nature .
2- Simplicity and high Selectivity .
3- Result accuracy , (The relative error must be less than 5%).

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*The Reason or resort to Separation and Analysis process.

1- Selectivity increase
2- Sensitivity increase .

** General Theory of Separation Efficiency

1- The goal of an analytical separation is to remove Either the Analyte OR the Interferent from the sample matrix .

2- To achieve a Separation there must be at least **One** important difference between the Chemical or Physical properties of the Analyte and Interferent.

3- A Separation also requires Selectivity .

⁂ Separation Efficiency (Separation Factor) .

A separation efficiency is influenced by :

1- The failure to remove all the Analyte.
2- The failure to remove all the Interferent .

- We define the Analyte Recovery , R_A , as :

$$R_A = \frac{CA}{(CA)^\circ} = \frac{Conc.of\ analyte\ after\ sep.}{Conc.of\ analyte\ befor\ sep.}$$

A recovery of 1.0 means that none of the analyte is Lost during the separation .

- The Interferent Recovery , R_I , as :

$$R_I = \frac{CI}{(CI)^\circ} = \frac{Conc.of\ interferent\ after\ sep.}{Conc.of\ interferent\ befor\ sep.}$$

The degree of separation is given by a

- **Separation Factor ($S_{I,A}$) :**

Which is the change in ratio of interferent to analyte caused by the separation .

$$S_{I,A} = \frac{RI}{RA} = \frac{CI/(CI)^\circ}{CA/(CA)^\circ} = \frac{CI/CA}{(CI)^\circ/(CA)^\circ}$$

In an ideal separation $R_A=1$, $R_I = 0$ and $S_{I,A} = 0$.

- Recovery (R) and Separation factor ($S_{I,A}$) are useful ways to evaluate the effectiveness of a Separation .

- In general , the separation factor should be approximately 10^{-7} ,for a quantitative analysis of a trace analyt in the present of a Macro interferent ,
- And 10^{-3} , when the analyte and interferent are present in approximately equal amount .
- Recoveries (R) and Separation factor ($S_{I,A}$) are useful ways to evaluated the effectiveness of a separation .

Example:// An analysis to determine the concentration of Cu in an industrial Alloy uses a procedure for which Zn is an interferent . When a sample containing 128.6 ppm Cu is carried through a separation to remove Zn, the conc. of Cu remaining is 127.2 ppm .when a 134.9 ppm solution of Zn is carried through the sep ., a conc. of 4.3 ppm remains . Calculate the recoveries for Cu and Zn and the separation factor .

Solution : / The recoveries for the analyte and interferent are :

$$R_{Cu} = \frac{127.2 \text{ ppm}}{128.6 \text{ ppm}} = 0.9891$$

And

$$R_{Zn} = \frac{4.3 \text{ ppm}}{134.9 \text{ ppm}} = 0.032$$

The Separation Factor is $S_{Zn,Cu} = \frac{R_{Zn}}{R_{Cu}} = \frac{0.032}{0.9891} = 0.032$

⁂ ((Requirement for a good Separation method))

1- A good method of separation should be Rapid , Simple and En expensive .
2- Should give quantitative recovery of analyte without loos or degradation .
3- Should yield a solution of analyte that is sufficiently concentrated to permit the final measurement to be made without the need for concentration .
4- Should generate little Or No laboratory **wests** .

⁂ "The relationship between Separation and Analysis "

1-Selection and preparation the sample .
2-Measurement the Sample .
3-Dissolve the Sample .
4-Preliminary treatments such as pH control .
5-Separation the desirable component .
6- Measurement the desirable component .
7- Results **Analysis** and **Report presentation** .

Chromatography

History of Chromatography :-

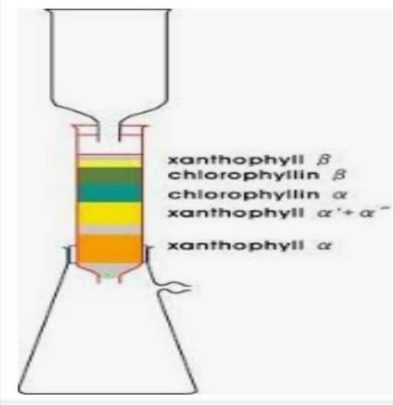
- **1512 –Branschwing .** Employed the principle of **GLC**(gas –liquid **chrom**) .
  in the purification of **Ethyl alcohol** from Olive o oil .
- **1903- Mikhail Tsweet : (Russian Botanist).**

Separated various plant pigments.

Chromatography was invented by the Russian scientist **Mikhail Tsweet in 1903. He** passes solution containing plant pigment , such as Chloraphylls and Xanthophylls a ,through glass columns packed with finely divided $CaCO_3$.

The separated species appeared as Colored bands on the column ,which Account for the name he chose for the method .

(Greek : Chroma meaning " Color " and Graphein meaning " To write"



But eventually methods were developed and there are several different types of chrom.

- 1931 - Lederer and Kuhn ------LC of carotenoids .
- 1938 - TLC and ion exchange .
- 1941 - Martin and synge –Developed Paper chromatography .
- 1050 - Reverse phase LC .
- 1953 - Martin and A.T. james develop GC .
- 1963 -Instrumental LC .

Chromatography Basics

Chromatography : Is a physical method of separation in which the components to be separated are distributed between Two phases ,
One of these phases is **Stationary phase** while other **Mobile phase** moves in definite direction .

- Chromatography is an analytical method that is widely used for **Separation** ,**identification** and **determination** of the chemical components in the complex mixture . .

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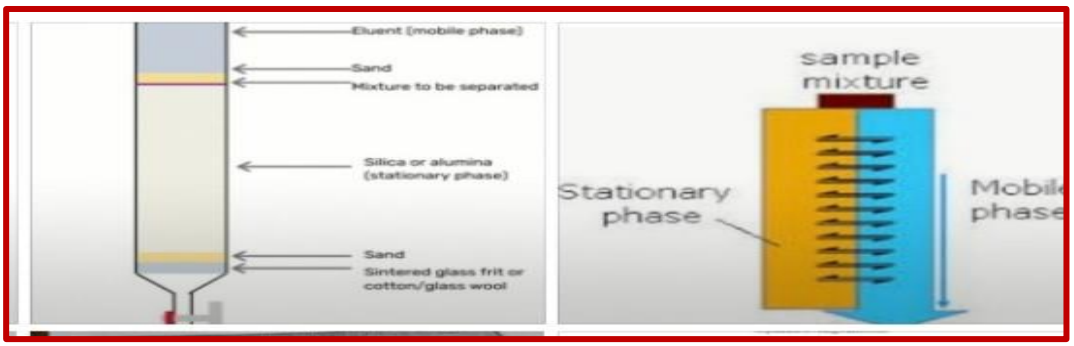
Mobile phase : (MP) :-

- Can be , Gas , **Liquid** , OR **Supercritical fluid** .
- Forced through immobile stationary phase .

Stationary phase : (SP):-

- Can be , **Liquid** **OR** **Solid** .
- Viscos liquid bonded on to surface of solid particles packed in column .
- Solid particles (e.g Silica gel) .

⁂ **The Two Phases** are chosen so that the components of the sample distribute b between the Mobile phase and Stationary phase to variety degree .as explain in the following figure .



Question : How you can describe the distribution the components of sample between two phases ?

Answer//

1. Components **more strongly retained** by SP move slowly with flow of MP.
2. Components **weakly retained** (held) by SP **move rapidly** .
3. Components Separated into discrete bands .
   - **U.V** detector used (or any suitable detector)
   - Separation based on **Affinity** of **SP** with sample .

" Purpose of Chromatography "

❖ **Analytical** ⟹ Determine chemical composition of sample (Qualitative and Quantitative)

❖ **Preparative** ⟹ Purify and collect one or more components of sample .

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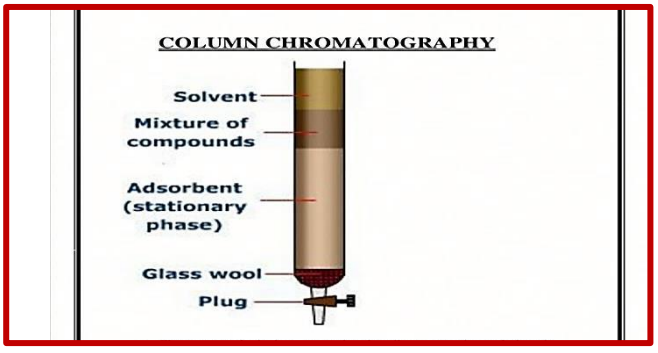
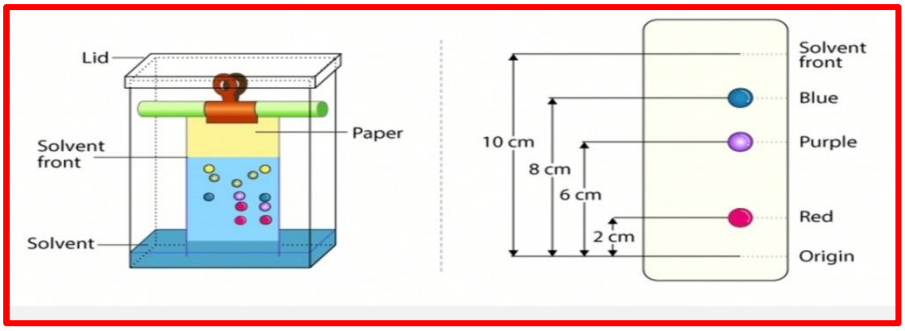
Classification of Chromatographic methods

Chromatographic methods are of two types :

1 : -Column Chromatography : The stationary phase is held in a narrow tube and the mobile phase is forced through the tube under pressure or gravity.



2 : - Planer Chromatography : The stationary phase is supported on a plate or in the Pores of a paper ;Here the mobile phase moves through the stationary phase by Capillary action or under the influence of gravity .as the following figure .



We will deal with Column chromatography only .

- ❖ Amore fundamental classification of chromatographic methods is based upon the **Type of phase** .

There are Three general categories of chromatography

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TABLE 31-4
Classification of Column Chromatographic Methods

General Classification	Specific Method	Stationary Phase	Type of Equilibrium
1. Gas chromatography (GC)	a. Gas-liquid (GLC)	Liquid adsorbed or bonded to a solid surface	Partition between gas and liquid
	b. Gas-solid	Solid	Adsorption
2. Liquid Chromatography (LC)	a. Liquid-liquid, or partition	Liquid adsorbed or bonded to a solid surface	Partition between immiscible liquids
	b. Liquid-solid, or adsorption	Solid	Adsorption
	c. Ion exchange	Ion-exchange resin	Ion exchange
	d. Size exclusion	Liquid in interstices of a polymeric solid	Partition/sieving
	e. Affinity	Group specific liquid bonded to a solid surface	Partition between surface liquid and mobile liquid
3. Supercritical fluid chromatography (SFC) (mobile phase: supercritical fluid)		Organic species bonded to a solid surface	Partition between supercritical fluid and bonded surface

Branches of chromatography according the mobile phase

1. Gas Chrom .(GC)
2. Liquid chrom.(LC)
3. Supercritical fluid Chrom. (SFC)

Note :- Liquid chrom.(LC) can be performed in **Column** & **Planer** .

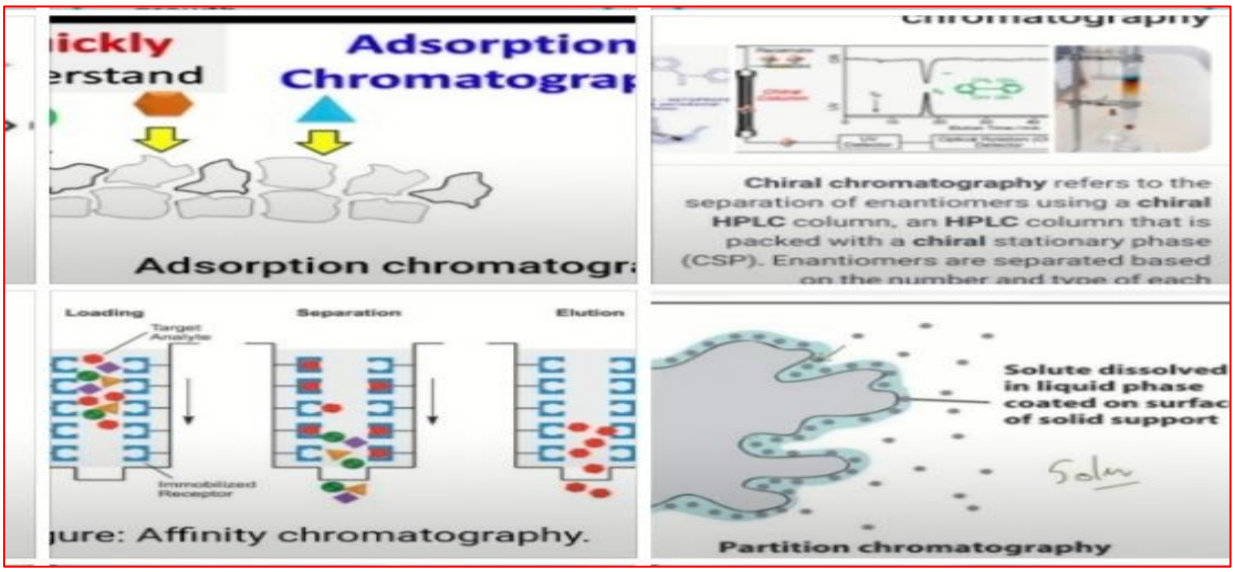
- But : **GC** and **SFC** are restricted to **column procedure** .

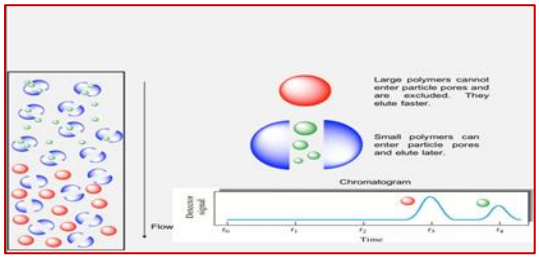
"Types of Liquid Chromatography "

The following classification of chromatographic method based on **mechanism of separation** .

Liquid Chromatography

Adsorption Chrom.	**Partition Chrom.**	**Size Exclusion Chrom.**	**Ion –Exchange Chrom.**	**Affinity Chrom.**	**Chiral Chrom.**
Competition between liquid mobile phase and solid adsorbent	Competition between liquid mobile phase & liquid stationary phase	Molecular Sieving	Competition between liquid and ionic stationary phase	Lock and Key mechanism	To separat Optical Isomers





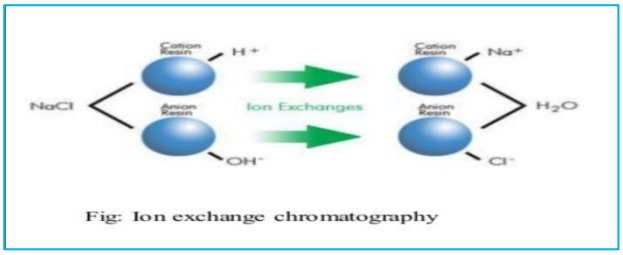


Fig: Ion exchange chromatography

"Chromatographic behavior Solutes"

The chromatographic behavior of solutes can be described by :

1- Retention Factor $\acute{K}_{(x)}$ $\acute{K} = \frac{tR-tm}{tm}$

2- Retention time tR $tR = \frac{VR}{U}$

3- Retention Volume VR VR =tR *U

- U = Rate of mobile phase

All of this represented a part of time that solute molecular spends in mobile phase.

Chromatogram

- If a **detector** that responds to solute concentration is **placed** at the **end** of the column and its ***signal is platted*** as a function of **time** (or **Volume** of added mobile phase) a series of symmetric peaks is obtained as shown in the figure:
- Such a plotter (Scheme) called a *Chromatogram* .
- It is used for Qualitative and Quantitative analysis.

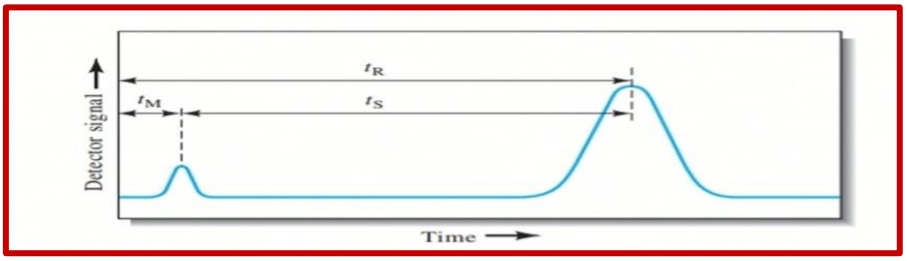
Chromatogram

* Qualitative Analysis

The **position** of peaks on the **time** axis can be used to **Identify** the component of the sample.

* Quantitative Analysis

The **areas** under the peaks provide a **quantitative measure** of a mount of each **species .**



A ***chromatogram***: is a plot of (or drown) of some function of solute concentration versus elution time or elution volume .

Development of chromatogram

There are Three main types of development of chromatogram

1-Frontal analysis
2-Elution analysis → **Can be** Isocartic technique or Gradient technique .
3-Displacement analysis

1 - Frontal analysis:- (Frontal → means the leading part of a zone)

* A procedure in which the sample(**Liquid or Gas**) is fed continuously into the chromatographic bed .
* In Frontal No **additional mobile phase** is used.
* This method in most cases give **information** only about the number of component that have been separated in the column.
* Frontal analysis may be used for Technical purpose , such as in the removal of slight amount of impurities in gases .

⁂ The leading part of **Zone** is called the **Front** ; hence the name of the method.

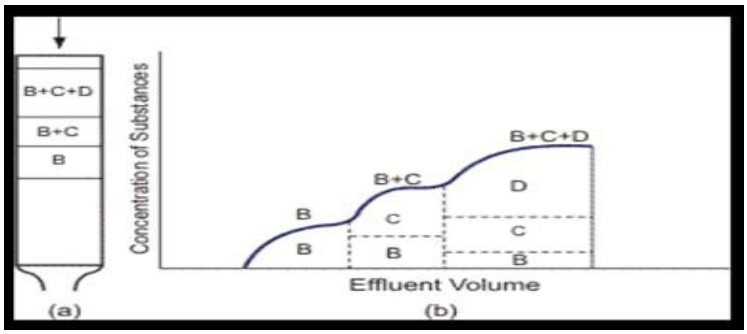


Figure showing the **Frontal analysis**

2- Elution analysis :_

* **This** type of analysis **Regard the best and more used** than other types .

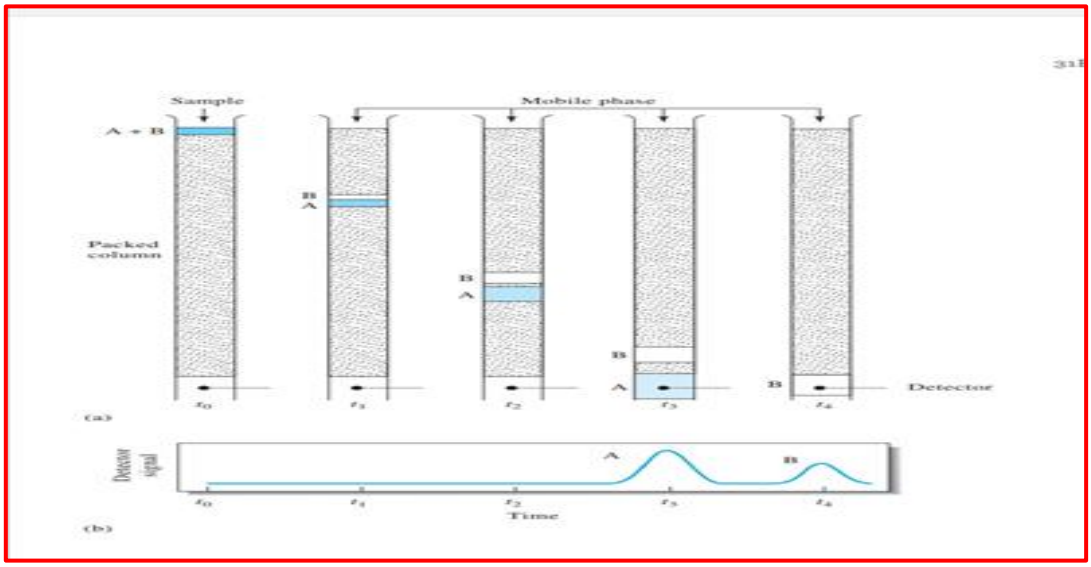
* **In this** type of analysis , the volume of sample should be rather **Small ,** so that Only a very small portion of the adsorbent is **Occupied** by the **Starting Zone**.

* The sample is dissolved in the mobile phase ,then is introduced at the **Top** of column, As the sample move down the column ,If the strength of each solutes interaction with the stationary phase is sufficiently different, the solute distribute themselves between the **Two phases ,** then separate into individual bands developing a Gaussion profile .

- Elusion: Is a process involves washing a species (solute) through a column by continuous addition of fresh solvent .

* Introduction of eluent (additional mobile phase) forces the solvent containing a part of the sample down the column , where further partition between the mobile and fresh portion of the stationary phase occurs .

A diagram showing the separation of a mixture of components A and B by column elution chromatography .



An eluent :- is a solvent used to carry the components of a mixture through A stationary phase .

3- Displacement analysis :-

*In this method it is also preferable to dissolve the sample in the same solvent which is was used for rinsing the column .

*After the mixture has been introduced into the column , where its component should be adsorbed in a less narrow zone at the top .

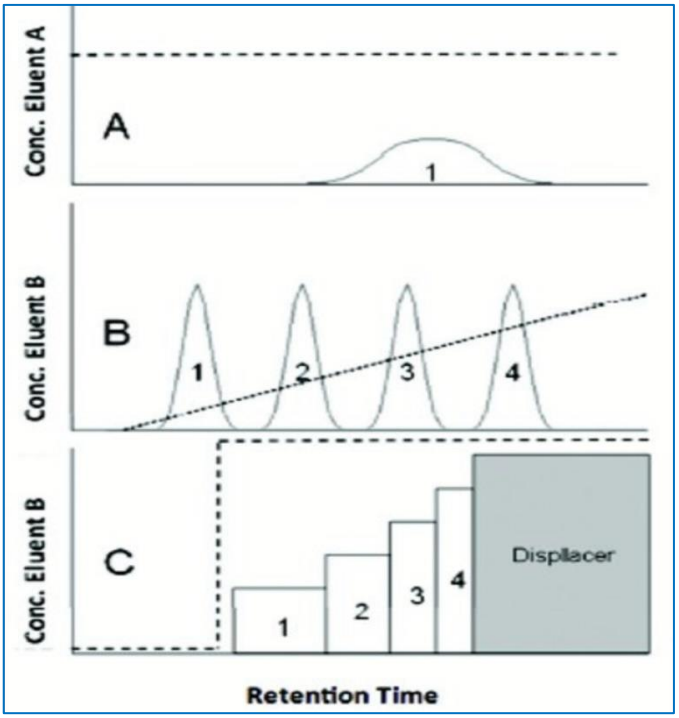
- After that adds a Solution containing a **Substance** which adsorbed more strongly

Than any component of the sample , which is called **Displacer (D)** ,pushes the Substances of the active sites on the adsorbent.

* The net result will be a distribution of these substances in the following order.

1- The **less strongly adsorbent** component will move immediately a head of the more strongly adsorbent components ,and **the most strongly adsorbed** will be followed by front of the **Displacer (D)**

The individual Zones will be contact with their neighbors at all times .
Since substance D is adsorbed mote than A & B , substance D can be used as dispacer to separate A & B .

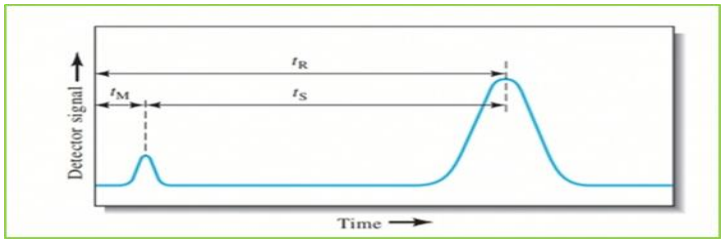


" The Chromatographic peak may be Characterized by many Ways "

1- Retention time - t_R

- **It is** the time spent *from* the Introduction of the sample (solute) to the **peak maximum.**
- **The retention time (t_R)** also can be measured **indirectly** as the volume of mobile phase eluting between the solutes introduction and the appearance of the solutes peak maximum . **This is Known as the Retention Volume : V_R .**

  Dividing the retention volume **V_R** by the mobile phase rate u ,gives the retention time **t_R** .



$$t_R = \frac{VR}{u} \qquad \text{or} \qquad VR = tR * u$$

2- Chromatographic Peaks Width :W

- Is determined by the intersection with the baseline of tangent lines drown through the inflection points on either side of the chromatographic peak .
- **Baseline Width** : is measured in **Units** of time or volume , depending on whether the retention time or retention volume is interest .

3- Dead or Void Time , t_M .

- Besides the solute peak also show a small peak eluted soon after the sample is injection into the mobile phase .
- **This peak** results from solutes **that** move through the column at the **Same rate** as the mobile phase
- **Since these** solutes do not **interact with the stationary phase (SP),** they are considered Non retained.

⁂ **The time or Volume** of mobile required to elute non retained component is called the **Void time, t_M or Void volume, V_M .**

$$V = \frac{L}{tM}$$ V =The average linear rate of solute .

$$u = \frac{L}{tM}$$ u =The average linear of mobile phase .

4- Distribution constant : K

- The distribution equilibrium involved in chromatography involve the transfer of an analyte between the mobile phase and stationary phase .

A mobile $\rightleftharpoons$ **A stationary phase**

The equilibrium constant **K** for this reaction is called Distribution constant .

$$K = \frac{Cs}{CM}$$

Where Cs is the m olar conc. of the solute in the stationary phase and C_M is the molar conc. of the solute in the mobile phase.

K is constant over a wide range of the solute concentration .

5- The capacity factor : $\acute{k}$ (The rate of solute migration)

- The **capacity factor** or ((**Retention factor**)), is an important parameter that is widely used to describe the migration rate of solutes on column .

For a solute **A** ,the retention factor $\acute{k}_A$ is defined as :

$$\acute{k}_A = \frac{tR - tM}{tM}$$

t_R and t_M are ready obtained from chromatogram .

1-When the **retention factor** for a solute is Much less than Unity, elution occurs so rapidly that accurate determination of the etention time tR is difficult .

2-When the **retention factor** is large than perhaps 20---30 , elution time become very long .

3- Idealy , the **retention factor** for the solutes in a mixture lie in the range 1 and 5 .

Example :// In a chromatographic analysis of Low- molecular weight acids ,butyric acid elutes with a retention time of 6.79 min., calculate the capacity factor $\acute{k}$ for butyric acid .

Solution :

$$\acute{k} = \frac{tR - tM}{tM}$$

$$\therefore \quad \acute{k} = \frac{6.79 - 0.3}{0.3} = 21.6$$

6- The Selectivity Factor (α) (Relative migration rates)

The selectivity factor α of a column for the two species A and B is defined as:

$$\alpha = \frac{KB}{KA}$$

Where K_B is the distribution constant for species B, and
KA is the distribution constant for species A.

- α is always greater than Unity
- A relationship between the selectivity factor and retention factor ḱ (capacity factor) is :

$$\alpha = \frac{\acute{k}\,B}{\acute{k}\,A}$$

An expression for the determination of α from an experimental chromatogram.

$$\alpha = \frac{(tR)B - tM}{(tR)A - tM}$$

The Selectivity factor for two analytes in a column provides a measure of how well the column will Separate the two .

Example : In the same chromatographic analysis for Low- molecular weight acids considered in the previous example, the retention time of 5.98 min. ,for iso butyric acid ,while butyric acid elutes with a retention time of 6.79 min., what is the selectivity factor for iso butyric acid and butyric acid ?

Solution : //

First , we must calculate the capacity factor (retention factor) for iso butyric acid ,using the void time from previous example .

$$\acute{k} = \frac{tR - tM}{tM}$$

$$= \frac{5.98 - 0.3}{0.3} = 18.9$$

From the previous example $\acute{k}$ for butyric acid = 21.6

The selectivity factor is :

$$\alpha = \frac{\acute{k} \quad butyric\ acid}{\acute{k}\ iso\ butyric\ acid} = \frac{21.6}{18.9} = 1.14$$

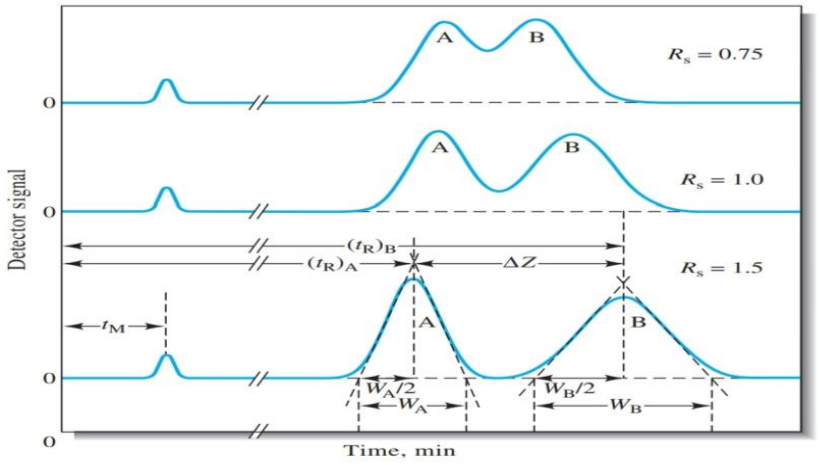
Note //we note that the value of α is more than 1.0 ,this indicates that
the separation process is good .

Chromatographic Resolution (R)

- The goal of chromatography is to separate a sample in to a series of chromatographic peaks ,each representing a single component of the sample .
- The resolution R of a column provides a quantitative measure of its ability to separate two analytes A and B .

Column resolution is define :

$$\mathbf{R} = \frac{(tR)B-(tR)A}{0.5\,(WA+WB)} = \frac{2\,\Delta\,tR}{WA+WB} \quad \text{.....(very important)}$$



1- It is evident from the figure that a resolution of **1.5** give an essentially complete separation of the two components (A& B), Whereas a resolution of **0.75** dose not .

2- At a resolution of **1.0** ,Zone A contain about **4%** B and Zone B Contains a similar amount of A.

3- At a resolution for **1.5** , the overlap is about **0.3%** .

Example : In a chromatographic analysis of a mixture consist of A&B solutes .A peak for A has a retention time of 8.36 min., with a baseline width of 0.96 min. ,whereas B elute at 9.54 min., with a baseline width of 0.64 min ., Calculate R (Resolution for A&B)

$$R = \frac{2\,\Delta\, tR}{WA+WB} = \frac{2[\,(tR)B-(tR)A\,]}{(WA+WB)}$$

❖ $$R = \frac{2\,(9.6-8.36)}{0.96-0.64} = 1.48$$

Methods for Describing Column Efficiency

1- A chromatographic column is made of **numerous** separated narrow layers called **theoretical plates**

2-At each plate , equilibration of solute between the mobile and stationary phase was assumed to take place

3-Movement of the solute down the column was as a stepwise transfer of equilibrated mobile phase one plate to the next .

Two related terms are widely used as **quantitative measure** of chromatography column efficiency .

1- Plate height **H**

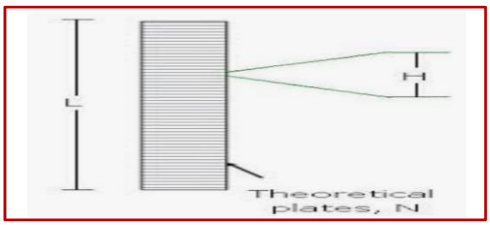
2- Number of theoretical plate **N**

The two are related by the equation .

$$N = \frac{L}{H} \quad \text{or} \quad H = \frac{L}{N}$$

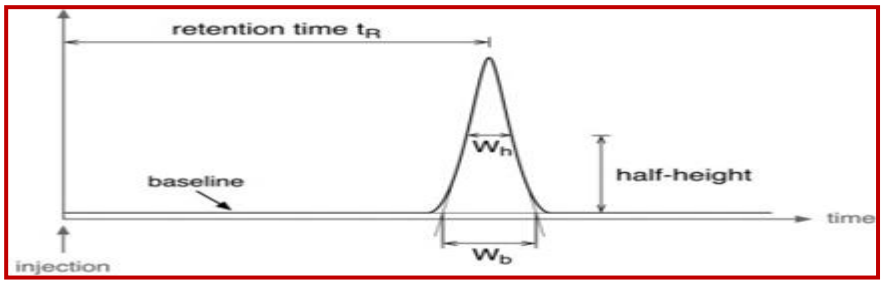
Where **L** is the length (usually in cm.) of the column

⁂ **The efficiency of column is great when H is small and N is large**



⁂ The number of theoretical plate N in a column can be obtained from the following relation .

$$N=16\left(\frac{tR}{W}\right)^2$$



Example : A chromatographic analysis of the pesticide , Dieldrin gives a retention time tR of 8.68 min. and a baseline width of 0.29 min. How many theoretical plate (N) involved in this separation ? The column used is 2.0 meters long ,What is the height plate ?

Solution :

$$N = 16\left(\frac{tR}{W}\right)^2 = 16\left(\frac{8.68}{0.29}\right) = 14300 \text{ plates}$$

$$H = \frac{L}{N} = \frac{(2.0 * 1000\, mm/m)}{14300} = 0.14 \text{ mm/plate}$$

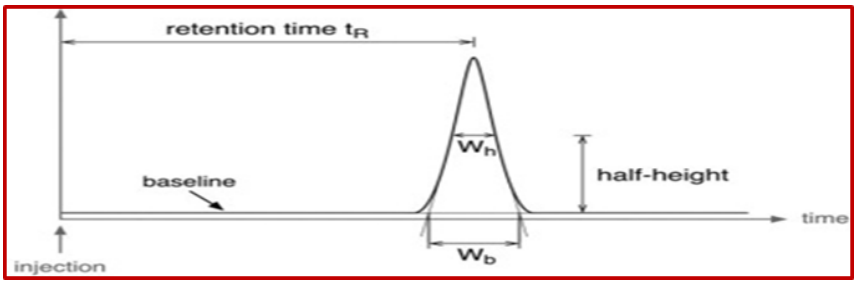
Theory of column Efficiency in Chromatography

The band broadening that occurs in column chromatography is the result of several factors ,which influence of **separation** .

1- Theoretical plates .

- **A** theoretical plate is derived from **distillation theory** .
- **Each** theoretical plate in chromatography can be representing a signal equilibrium step .
- **In reality ,** they are **a measure of efficiency** of a column ,
- **For high efficiency** , a large number of plates is necessary
- **The plate height , H** is the length of a column divided by the number of a **theoretical plates (N) .**

$$H = \frac{L}{N}$$



- **The number of plates or efficiency** can be obtained from a chromatogram ,from the expression .

$$N = 16 \left(\frac{tR}{W} \right)2$$

N= number of plate

tR = retention time

Wb = is the peak width

⁂ Retention volume VR may be used instead of tR .

2- Rate theory :-

This theory can be explain in Gas chrom. GC as the following .

Gas chromatography efficiency .

- **The Van Deemter Equation .**

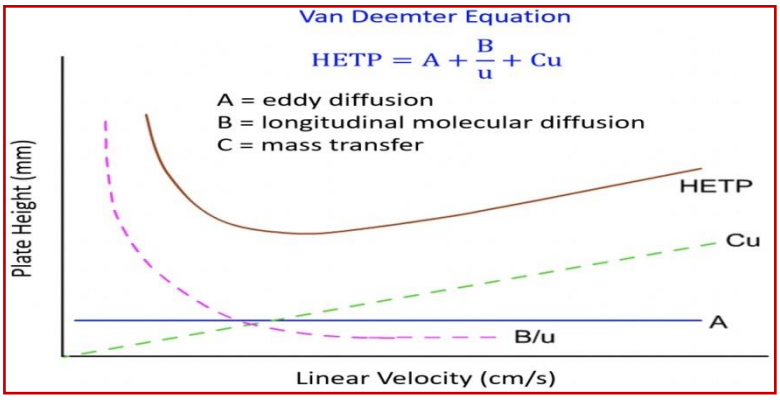
* -- **Van Deemter** showed for a packed gas chrom. column that the broadening of a packed is the **Summation effects** from several sources.

*--The **Van Deemter equation** expresses these in terms of the plate height , **H**

$$\text{HETP (H)} = A + \frac{B}{ú} + Cú$$

1- Where ú is the average velocity of the mobile phase .

2- A , B and C are factors which contribute to band broadening .



A: Random movement through stationary phase .
B: Diffusion in mobile phase .
C: Interaction with stationary phase .
H: Plate height .
ú : Average linear velocity.

Term A :
1-Molecules may travel <u>unequal distances</u> .
2-Independent of **ú.**
3- Depends on **size of stationary particles** or coating **TLC**

Term B :

1- The conc. of analyte is less at the **edges** of the band than at the **center** .
2- Analyte **diffuses** out from the **center** to the **edges** .
3- This causes **band broadening** .
4- Dependent of **ú** ,if the velocity of the mobile phase is **high** then the analyte spends less time in the column ,which decreases the effects of molecular diffusion .

Term C : (Resistance to mass transfer)

1- The analyte takes a certain amount of time to equilibrate between the stationary phase and mobile phase .
2- Dependent of **ú** , if the velocity of mobile phase is **high** ,then the analyte in the mobile phase move ahead of the analyte in the stationary phase .
The band of analyte is broadening .

Conclusion :

1- **Peaks** are broadening by → **Eddy diffusion**, **Molecular diffusion**, **Sloe mass transfer rates**

2- **Small** , **uniform particles** **Minimize Eddy diffusion** .
3- **Faster flow** decreases Molecular diffusion .

But increase Mass transfer effect . There will be an optimum flow .

Apllication of chromatography

- Chromatography is a powerful tool for **separation closely related chemical** species .
- In addition ,it can be employed for the **Qualitative and Quantitative** determination of separated species .

Qualitative analysis

1- A chromatogram provides **us** information a bout each species in A sample , namely ,its retention time (tR) ,or its position on the stationary phase after a certain elution period .

2- **It** is a widely used tool for **recognizing** the presence or absent of components of mixture containing a limited number of possible species whose identities are known .

3-Positive Spectroscopic identification would be impossible without a preliminary chromatographic separation on a complex .

1- Chromatography can provide useful quantitative information a bout the separated species .
2- Quantitative column chrom. is based upon a comparison of either the height or the area of the analyte peak with that standard .

(1) –Analysis based on Peak height .

(2) - Analysis based on Peak area .

1- Peak areas are a more satisfactory analysis variable than peak areas
2- Most modern chromatographic instruments are equipped with digital electronic that permit precise estimation of peak areas .

(3) - Calibration with standards .

1- The most method for quantitative chromatographic analysis involves the preparation of a series of standard solutions that approximate the composition of the unknown .

2- A plot of the data should yield a straight line passing through origin .

(4) - The internal – Standard method .

1- In this procedure ,a measured quantity of an Internal – Standard is introduced into each **Standard and Sample**.
2- The ratio of analyte peak areas (or Height) to internal – Standard areas (or Height) is the analytical parameter .

GAS CHROMATOGRAPHY
GC

Introduction :--

1- GC has been developed at around **1950** by Martin **and James** .

2- It is one of the most important of **newer development** in analytical chemistry .

3- It makes possible to separation of substances with boiling point up to 450 C^0. whereas the LC is suitable to separation of substances with b.p. 150 C^0 .

4- Gas chrom.as its name suggested ,is particulary suited for separation of Gases and Liquids or Solids in the gaseous state .

5- It is widely used for the determination of **Organic Compounds .**

Example : - The separation of **benzene** and **Cyclohexan** .(b.p 80.1 and 80.08) is extremely simple by **GC**., but it is impossible by conventional.

6- Within a few short year ,GC was used for the analysis of almost every type of organic compound .

7- Very complex mixture can be separated by this technique .when coupled with Mass spectrometry as a detection system .

There are two types of Gas Chom.

Gas- liquid chrom. GLC

Gas –solid chrom .

The more important of the two is Gas –liquid chrom.(GLC) used in the form of a capillary column.

1- Gas- Liquid Chromatography : GC

1- The mobile phase is a **gas** ,whereas the stationary phase is a **liquid** that is retaind on the surface of an inert solid by chemical bonding .

2- Gas –liquid chrom . finds widespread use in all fields of science ;its name is

usually shortened to gas chrom. (GC).

2- Gas –Solid Chromatography : GSC

1- The mobile phase is a gas ,whereas the stationary phase is a solid that retains The analytes by physical adsorption .

2- GSC has limited application owing to

A - Semi permanent retention of active or polar molecules .

B - Sever tailing of elution peak .

3- Thus ,this technique has not found widespread application except in the separation and determination of low - molecular –mass gases , such as air components , hydrogen sulfide H_2S ,carbon monoxide CO and nitrogen oxides

Apparatus for Gas Chrom.

The Gas chromatography apparatus consist of the following parts :-

1- Carrier gas in high pressure cylinder regulators and flow meters .such as

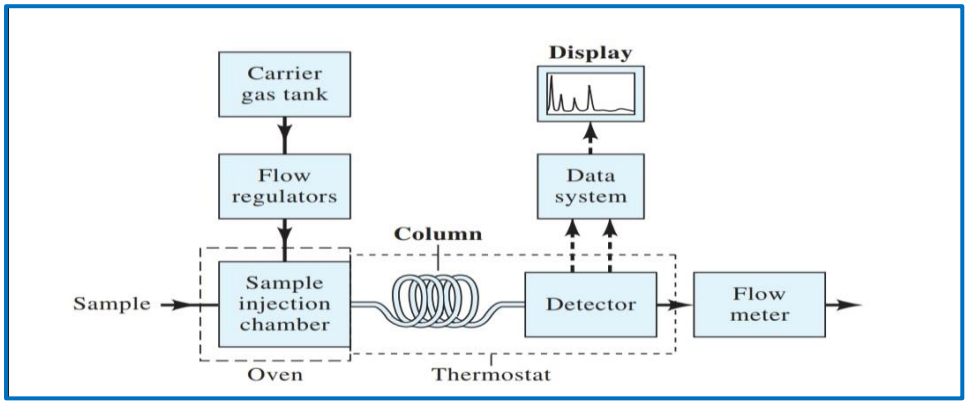
He ,H_2 , N_2 , Argon (Ar) .

2- Injector Sample injection system

3 - Column

4- Computer for recorder

The following figure explain the block diagram of a typical Gas Chrom.



1- *Carrier Gas supply (mobile phase).*

1)- The mobile phase in GC is **called carrier gas** and must be inert .

2)- **Helium** is the most common mobile phase **although** Ar , N_2 and H_2 are also used .

3)- **The choice** of which carrier gas to use is often determined by .

a- Instruments detector used .

b- The nature of the sample .

4)- These gases are available in pressurized tank.

5)- Inlet pressure usually range from 10 -50 **psi (1b/in^2)** above room Pressure .

6)- The mobile-phase velocity is usually within the

rang of

- **20 –150** ml /min. **For packed column** .
- **1--25** ml/ min. **For capillary column.**

2- *Sample Injection System* .

1-) **Column efficiency** required that the sample must be a suitable size and be introduced through a **" Plug "** as a vapor .

2-) **Slow injection** or **oversized sample** cause band spreading and poor resolution .

3-) **Calibrated microsyringes** , are used to inject liquid sample through a **rubber** or **silicon diaphragm** or **Septum** into a heated sample port located at the head of the column .

4-) **The sample port** is ordinary about **50 C^0** above the boiling point of the least volatile component of the sample .

5-) **For ordinary packed** analytical columns , **Sample size** range from a few tenths of microliter to 20 µL.

6-) **Capillary column** require samples that are smaller by a factor of **100** or more .

Table : Typical injection volumes for various column diameters .

Column Diameters	Maximum Injection Volumes (µL)
1/4 in. (packed column) 1/8 in. (packed column) Capillary Columns	100 20 0.1

3- *The Columns. (GC columns).*

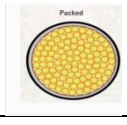
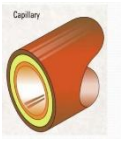
There are Two Types of column used in GC.

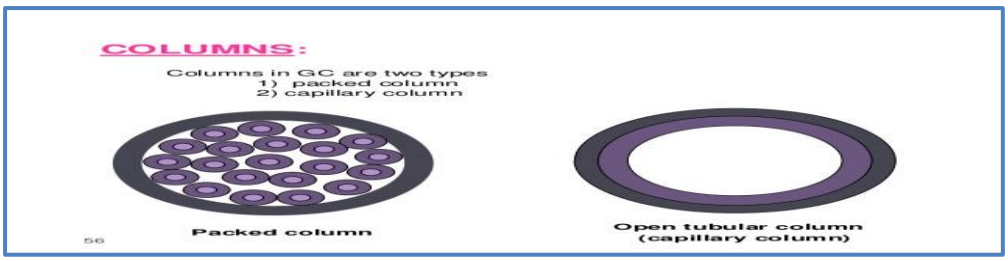
1- Packed column

2- Capillary or (Open tubular column)

* **packed columns** were the first type and were used for many years .

* **Capillry columns** are more commonly used today but paked column **still** used for application that not require high resolution or when increased capacity is needed .

Packed Column	**Capillary Column**
1- Used larg sampls size 2-Low resolution 3- Convenent to use 4- Cross section of this 1/8 in.diameter 	**1-Used small sample size** **2- High resolution** **3- Take care in use** **4- Cross section of this 0.1 –0.5 mm.** 



1- GC Column ,are typically very long . Length varying from **2 up to 300 ft or more .**

2- It is important for the column to be kept at **elavated temperature** during the run in order to prevent **condensation** of the sample component .

3- Such a long column is **wound** into a coil and fits nicety into a small oven probably constitutes about half of the total size of the instrument .

4- **Column temperature** is an important variable that must be controlled to a few tenths of a degree for preciese work.. Thus ,the column is housed in a thermostated oven .

→ **The optimu column temp. depend on the.**

1- Boiling point of the sample .
2- Degree of the separation required .

→ A chromatographic column provides a location for physical retaining the stationary phase .

⁂ The columns constraction also influences on the.

1- **The amount of sample that can be handel .**

2- **The efficiency of separation.**

3- **The number of analyte that can be easily separated .**

4- **The amount of time required for the separation.**

⁂ Packed columns

1- Packed columns are fabrecated from **glass** or **metal** tubing .

1----8 mm ID (Internal diameter)

1---10 m length

2- These tubes are densety packed with a uniform ,Solid support that is coated with a thin layer (1 µm) of the stationary phase .

3- **These** columns are ordinarily formed into **Coils** with a diameters of roughly **15 cm**. to permit convenient thermostating in an **oven .**

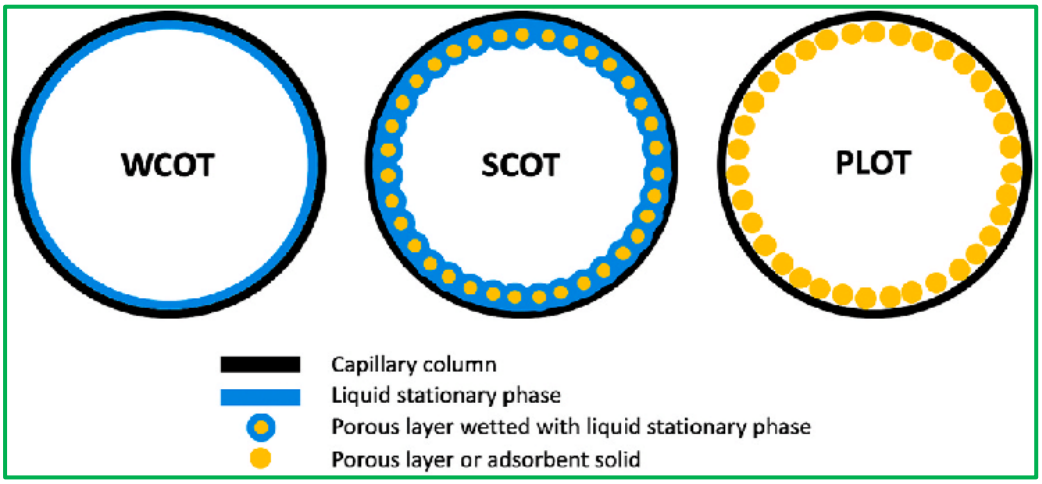
⁂ Capillary (or Open tubular) columns

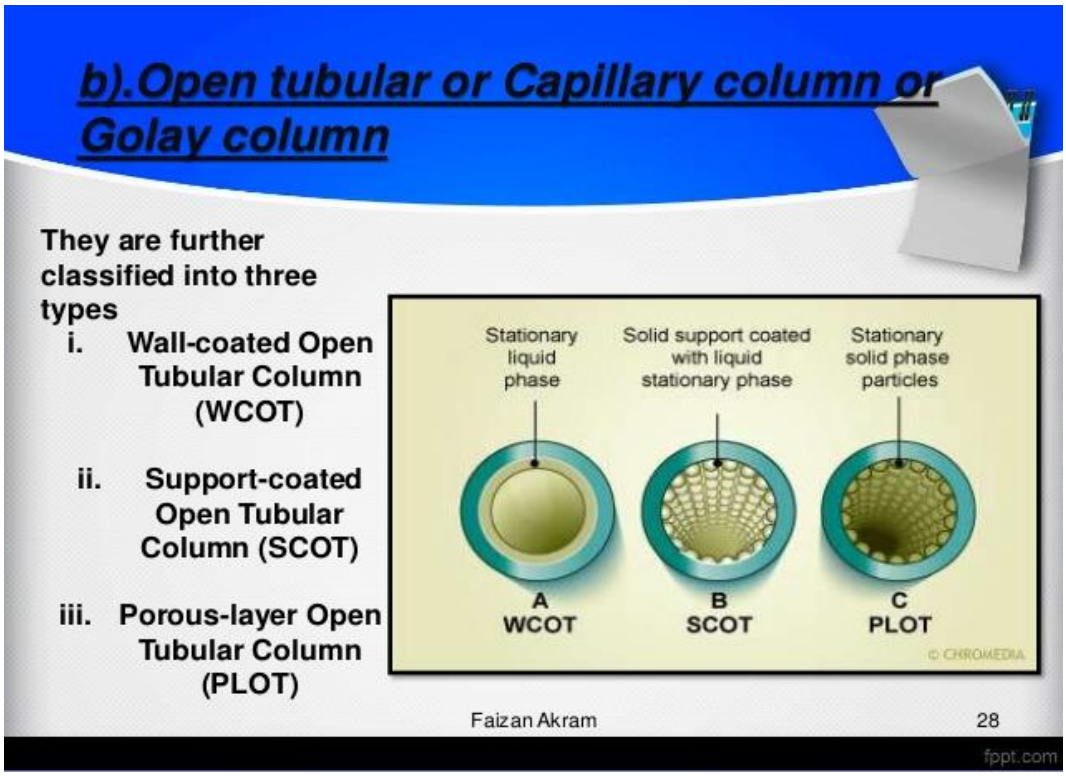
Note . Inner wall modified with thin film of liquid

0.3 - 0.5 mm ID

10 - 50 meter (may be up to 100 m)

Capillary Columns are of three principal types as in the following figure.





We will focus on only two types .

WCOT	SCOT
Wall –Coated Open tubular 1- Columns are simply capillary tubes 2- Coated with a thin layer of stationary phase . 3-Typically **0.25 µm** thick . 4- More efficiency ,but less capacity .	Support –Coated Open Tubular 1-The inner surface of the capillary tube Is lined with a thin film of support material . 2- Coated with a liquid stationary is attached to the capillary inner wall 3- Typically **30 µm** thick. 4-Less efficiency ,but more capacity .

Generally.The efficiency of a **SCOT** column is less than that of a **WCOT**

But : **SCOT** greater capacity than **WCOT.**

Stationary Phases (Liquid phase)

Selectivity in gas chrom. is influenced by the choice of stationary phase .

The main Criteria for selecting a stationary phase are :

1 - It should be chemically inert .
2- Thermally stable .
3- Low volatility (High b.p) .
4- Must have a appropriate polarity .

In general , nonpolar solutes are more easily separated with a nonpolar stationary, and polar solutes are easier to separate using a polar stationary phase .

Solid Support Materials

The packing ,or solid support in a packed column, serves to hold the liquid stationary in place must have the following characteristics .

1 - Must have high **specific area** of at least **$1m^2$ /g .**
2 - Should be **inert** at elevated temperatures .

3- Should be **uniformly and wetted** by the liquid .

4- Should be a good **mechanical strength** .

5- Should be consist of **Small**, **Uniform** , **Spherical particle** .

⁂ **In fact** .No substance that have these criteria is yet available .

Coating Support

To prepare the packing material .

1- The required amount of the stationary phase is dissolved in a suitable solvent such as **acetone** or **pentane** ,and a known **weight** of supporting medium added, **stirring** to get even distribution of the solution .

2- The solvent is then **evaporate by heating** and the medium completely dried

3- The next stage is **packing the column** ,it is important that the packing should be **uniform** .

4- The material is introduced into the column **a little by a little** or **Connected** with a **vibrator instrument** until it is filled .

5- **The two ends** are closed by a **small cotton** or **class wool** plug .

6- Then the column **coiled** in a **convenient shape** of oven.

Column forms include **Coiled tubes , U- Shape and W- shaped tube , but Coils** are the most commonly used .

⁂ Typical packed columns are **1 __10 m** long and **0.2__0.6 cm** ID

7- **A newly prepared** column should be conditioned at elevated temperature by passing **carrier gas** through it for **several hours** before used .

4- Detectors :-

1- **Detectors in GC** are designed to generate an electric signal when other than the carrier elutes from the column .

2- **The detector** indicate the presence and measure the amount of component in the column eluent .

Characteristics of the ideal detector .

The ideal detector for gas chrom. has the following characteristics.

1- Appropriate (adequate) **sensitivity** .
In general , the sensitivities of present –day detectors lie in the **10^{-8} _ 10^{-15}g** solute / s .
2- Good **stability** and **reproducibility**
3- **A linear response** to solutes that extents over several order .
4- A temperature range from **room temp. to at least 400 C^0** .
5- A **short response time** that is independent of flow rate .
6- **High reliability and ease of use** .
7- **Similarity in response** toward one or more classes of solutes .
8- **Nondestructive of sample .**

⁂ **In fact . No current detector exhibits** all these characteristics .

⁂ **Detectors** may be classified according to the type of chromatogram they produce ,which may be of the .

1- Integral type
2- Differential type

Some of the more common detectors :

1- Thermal conductivity detector TCD
2- Flame ionization detector FID
3- Electron capture detector ECD

Table 5: Features of GC Detectors. This table serve as a rough indication, it may be different depending on the compound chemic structure and analytical condition

Detector			Example of Detectable Compound	Example of Minimum Detectable Amount*
Universal Detector	Thermal Conductivity Detector	TCD	All compounds except for carrier gas	10 ppm (10 ng)
	Flame Ionization Detector	FID	Organic compounds	0.1 ppm (0.1 ng)
	Barrier Discharge Ionization Detector	BID	All compounds except for He and Ne	0.07 ppm (0.07 ng)
	Mass Spectrometer	MS	Ionized molecule	10 ppm (10 ng) in Scan mode 0.5 ppm (0.5 ng) in SIM mode 10 ppb (10 pg) in MRM mode
Selective High-sensitivity Detector	Electron Capture Detector	ECD	Organic Halogen compounds Organic mercury compounds	0.01 ppb (0.01 pg)
	Flame Photometric Detector	FPD	Sulfur compounds Organic phosphorus compounds Organic tin compounds	10 ppb (10 pg)
	Flame Thermionic Detector	FTD (NPD)	Organic phosphorus compounds Organic nitrogen compounds	0.1 ppb (0.1 pg) 1 ppb (1 pg)
	Sulfur Chemiluminescence Detector	SCD	Sulfur compounds	1 ppb (1 pg)

1- Thermal conductivity detector (TCD)

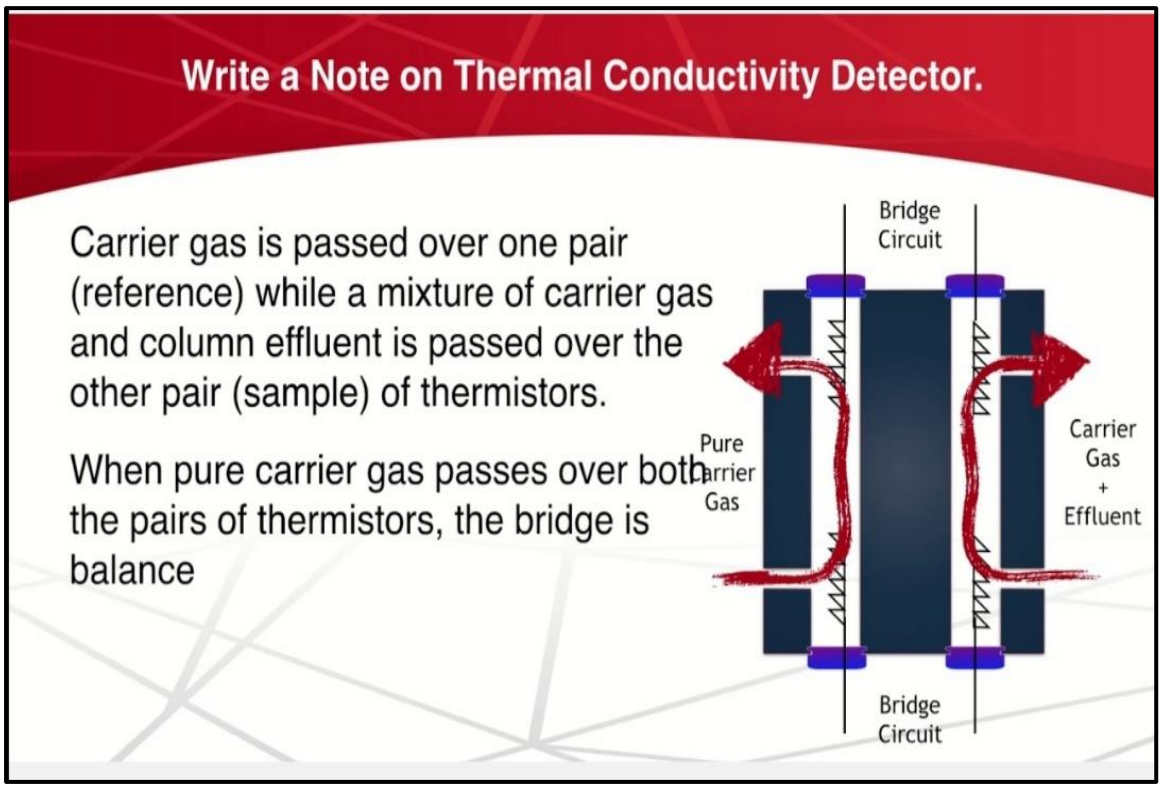
1-) One of the most important differential detector ,is the Khathrometer (Thermal cond. det.).

2-) In this instrument TWO identical cells ,made of Brass are used , each ONE containing a fine platinium , gold or tungsten wire.

3-) Helium (HE) is the mobile phase of choice when using a TCD ,due to its high thermal conductivity .

4-) The TCD operates on the principle of change in resistance of a heated wire .

TCD : A universal GC detector ,in which the signal is a change in the thermal conductivity of the mobile phase



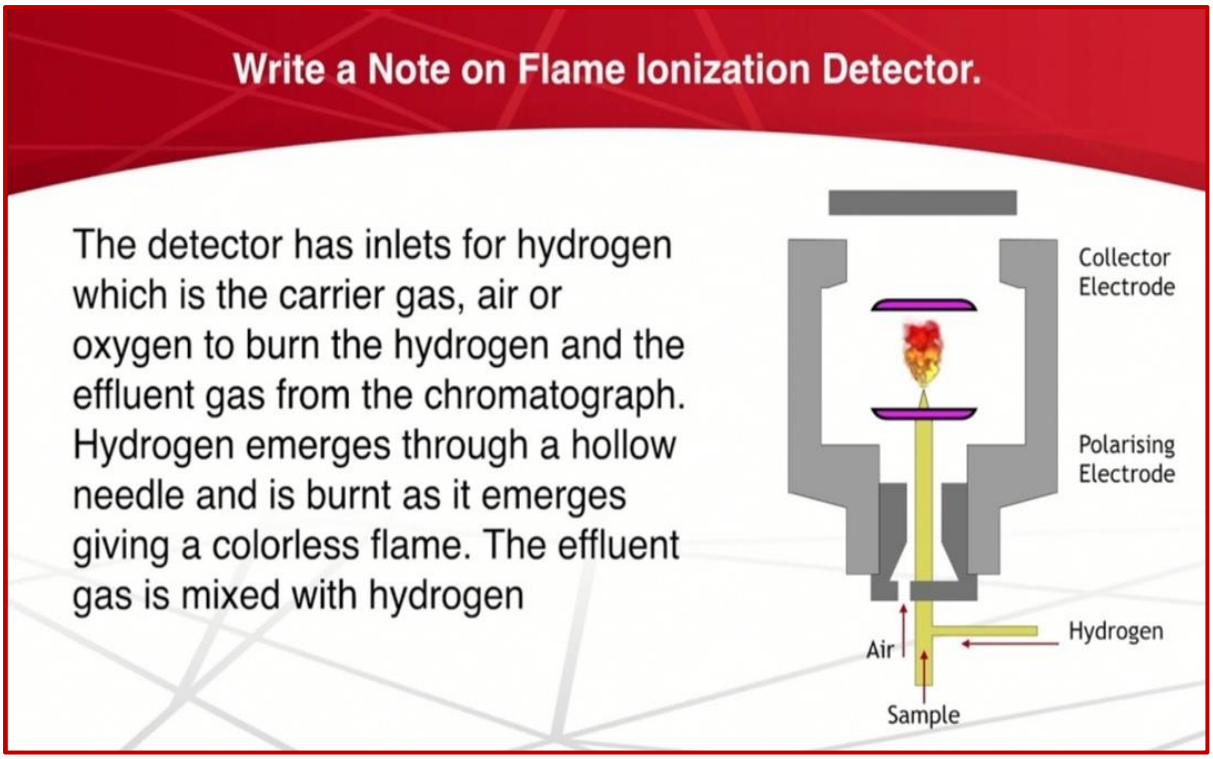
2- Flame –Ionization Detector : (FID)

1- FID perhaps the most widely used of all detectors for Gc.

2-Hydrogen (H_2), is used as the carrier gas with this detector .and the eluent is mixed with Oxygen (or Air) and combusted in a burner equipped with pair of electrode .

3- Detection involves monitoring the conducting of the combustion products .

FID : A nearly universal GC detector in which the solutes are combusted in H_2 / air flame ,producing a measurable current .



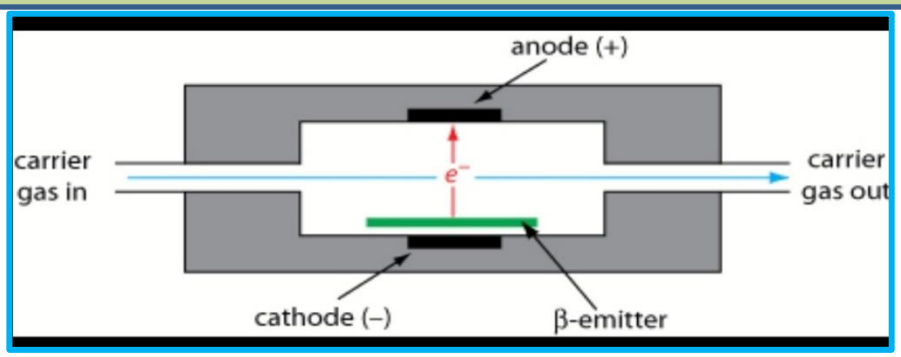
3- Electron capture detector (ECD)

1-The electron capture detector (ECD) has become **One** of the most widely used for Environmental sample, because this detector Selectivity responds to halogen – containing Organic compounds .

Such as Pesticides & Polychlorinated biphenyls .

2- The carrier gas for this detector is either pure nitrogen **(N_2)** or a Mixture of Argon and Methane **($A_r + CH_4$).**

ECD : A detector for GC that provides selectivity for solutes with **Halogen** and **Nitro functional gruops**



Hyphenated Methods

1- GC is often coupled with the selective techniques of spectrophotometric and electrochemistry .

Example : the **GC / MS** or **GC/IR** or **GC/ NMR**

These combined techniques are sometime called **Hyphenated methods** .

Hyphenated Methods

Hyphenated methods couple the separation capabilities of chromatography with quantitative and qualitative detection of spectral methods .

Application of GLC

GC has been widely applied to the separation and determination of the component in a variety of sample types .

1-Qualitative Analysis :

1-) In theory , retention time tR or VR (retention volume) useful for Identifying component in mixtures .

2-) In fact ,such data is limited by the number of variable that must Be controlled to obtain reproducible results .

3-) This limitation has been largely overcome by linking chromatography column directly with **IR ,MS ,NMR** spectrometers ,the resulting hyphenated instruments are powerful tools for identifying the components of complex mixture .

2- Quantitative Analysis : previously mention

Quantitative Analysis

GC is widely used for the analysis of a divers of sample in , **Environmental ,Clinical . Pharmaceutical ,Biochemical ,Forensic ,Food science and Petrochemical laboratories .**

1- Environmental analysis
2-Clinical analysis
3- Consumer goods
4- Petroleum industry

Liquid Chromatography

In liquid chromatography , the mobile phase is a Liquid solvent Containing the sample as a mixture of solutes .

There are several types of LC according to the type of stationary phase .

1- Adsorption or liquid –solid chromatography .

2- Partition or Liquid –Liquid chrom.

3-Ion –exchange chrom.

4-Size –exclusion chrom. which include .

a- Gel filtration ; is atype of size –exclusion chrom. ,in which the packing is hydrophilic .It is used for separate **Polar** species .

b- Gel permeation , a type of size –exclusion chrom., in which the packing is hydrophobic .It Is used to separate **non polar** species.

5- Affinity chromatography .

6- Chiral chromatography .

Adsorption chromatography

1- The separation of component from a mixture by adsorption from a moving liquid into the surface of powdered solid confined in the column
Is called column adsorption chrom.

2- When a mixture is applied to the top of column ,the liquid (or eluent)

Is passed through the column by gravity or by the applied or air pressure.

Choice the stationary phase (the adsorbent)

Silica gel , is regarded the most general material used ,the **aluminum oxide** (**Alumina)** .there are another adsorbent material have been used in special porpouse such as **wood charcoal .**

Adsorption have been classification according to selective strength of adsorption polarity .

A- Strong adsorbents : Such as Alumina , Silica gel ,Magnesium silicate and carbon.

B- Intermediate adsorbents: Include Ca-carbonate , Ca –phosphate and Mg –oxide .

C- Weak adsorbents: Such as Sucrose and Starch .

Mostly adsorbent are employed in this technique can be classified as

1- Acidic adsorbent , such as Silica gel and Magnesium silicate .These are used for Separating Acidic component in a mixture .

2- Basic adsorbent , such as Alumina ,it is used for separating of basic component .

Whereas Chemosorption for acidic samples occur on basic adsorbents.

Alumina (Aluminum Oxide)

There are Three types of alumina .

1- Neutral alumina (pH = 6.9 -7.1)
2- Basic alumina (pH = 10- 10.5)
3- Acidic alumina (pH = 3.5 – 4.5)

Mobile phase

There are several requirement for the choice of the solvent which is used as mobile phase

In liquid Chrom. LC

1- **Suitable polarity .**

2- Lower viscosity .

3- Stability to words the adsorbents

4-Its volatility for facility of solute recovery .

Polarity is the important object which can be changed to obtain the better separation.

A- **If a solvent is Too Polar** , movement becomes Too rapid and little or No separation of the components of mixture will result.

B- **If a solvent is not polar enough ,**non compound will elute from the **column**

Early : Lc (liquid Chrom.) . was performed in Glass column having inside diameter of perhaps 10 – 50 mm. (1-5 cm).

Length:	**50-500 cm.**
Particle size	**150 – 200 µm.**
Flow rates	**1- 10 ml / min. at best**

Attempts to speed up this classic procedure by application **vacuum** or **pressure**

Were not effective because Increase in flow rates were accompanied by increase in plate heights (H) and accompanying decrease in column efficiency .

⁂ **Notes about the the development in simple LC for develop HPLC.**

1- In the development of liquid chrom. ,it was realized that Large decrease in plate heights could be expected to accompany Decrease in the Partial size of packing .

2- It was not until the late 1960 , however ,that the **technology for Producing** and using **packing** with particle diameters as small as 3 to 10 µm was developed .

3-This technology requires the use instruments capable of high pumping pressures than the simple devices that preceded them.

4- Simultaneously , Detector were developed for continuous monitoring of column effluents.

5- ((The name high- performance liquid chrom. (HPLC) is often employed

To distinguish these newer procedures from the simple column chrom.))

Simple column chrom. , however still finds considerable use for preparative purposes. }

High – Performance Liquid Chromatography

(HPLC)

1- In 1964 *.J .Clavin Gidding* predicted improved liquid chrom. performance.

If one could use small particles under *increased flow pressure and that theoretically very high plate number could be achieved.*

Therefor classical liquid chrom. has largely been supplanted by the much more powerful and analytical useful form of **HPLC.**

It is the most widely used of all the analytical separation techniques

The reasons for the popularity of HPLC are.

1- Its sensitivity
2- Its ready adaptability to accurate quantitative determination .
3-Its ease of automation .
4- Its suitability for separating nonvolatile species or thermally fragile ones.
5- Its widespread applicability to substances that are important to industry ,to many Fields of science and to the public .

Example of such materials include .

((Amino acids ,proteins , nucleic acids , Hydrocarbons , Carbohydrates , Drugs Pesticides ,, Antibiotics , Steroids , , Metal –organic species and a variety of Organic substances .))

HPLC , is a type of chrom. that employs a liquid mobile phase and very finely stationary phase ,to obtain satisfactory flow rates , the liquid must be pressurized to several handred or more

Principles : Liquid chromatography is a well-established technique for the separation of substances. High performance liquid chromatography (HPLC) is a suitable method for the analysis of a wide range of application areas.

HPLC is **based on the distribution of the analyte (sample) between a mobile phase (eluent) and a stationary phase (packing material of the column)**. Depending on the chemical structure of the analyte, the molecules are retarded while passing the stationary phase.

Two criteria should be met .

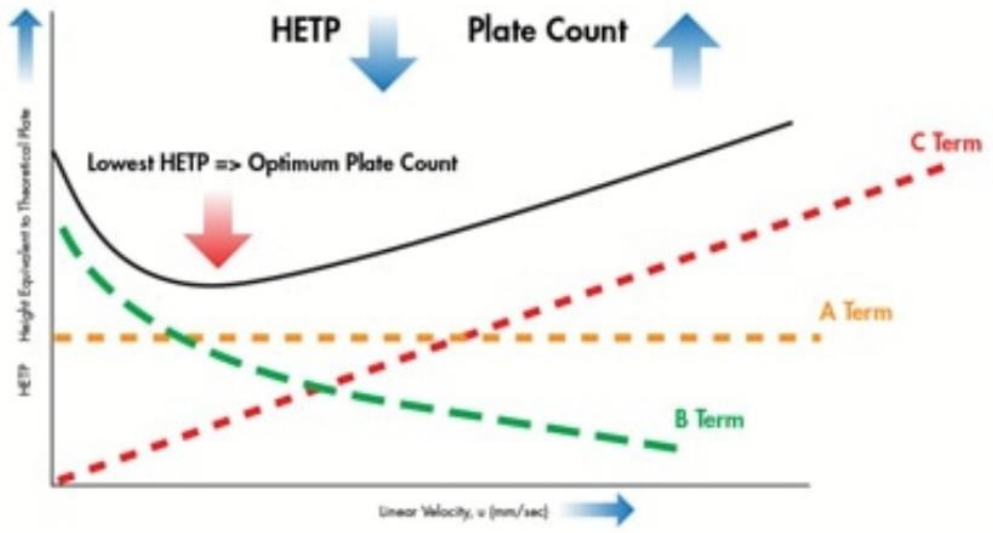
First : The packing should be finely divided and have high spherical requlirity to allow for optimum homogeneity and packing density ;

Second : The stationary phase should be in the form of a thin uniform film with no stagnant pools .

The first results in a small **A** value in the Van Deemter equation (Smaller eddy diffusion)

The second results in a small C value (more rapid mass transport between the phases -necessary for high flow rates).

Because the molecular diffusion in liquid ,the term B is small . Hence ,the detrimental (harmful) increase in H at low flow rates does not occurs . this is illustrated in the following figure .



Van Deemter plot HPLC

Stationary phase :

1- The particles have been developed that can be packed More homogeneously and provide improved efficiency .

2- The particles are High –purity silica ,and are typically 5-10 µm in diameter ,and may be 3.0 µm for high –speed chrom.

3- Pore size are in the 60 -100 A^0 range , although pore sizes of 300 A^0 or larger are Used for larger biomolecules to allow them to penetrate the pores .

Most HPLC is performed in the Liquid –Liquid (partition chrom.) mode

But ;adsorption chrom .is useful for many application

Types of particles used in HPLC

The most commonly used are :

1- Micro porous (diffusive particles)

2-Perfusion packing (macro porous)

3- Non porous packing

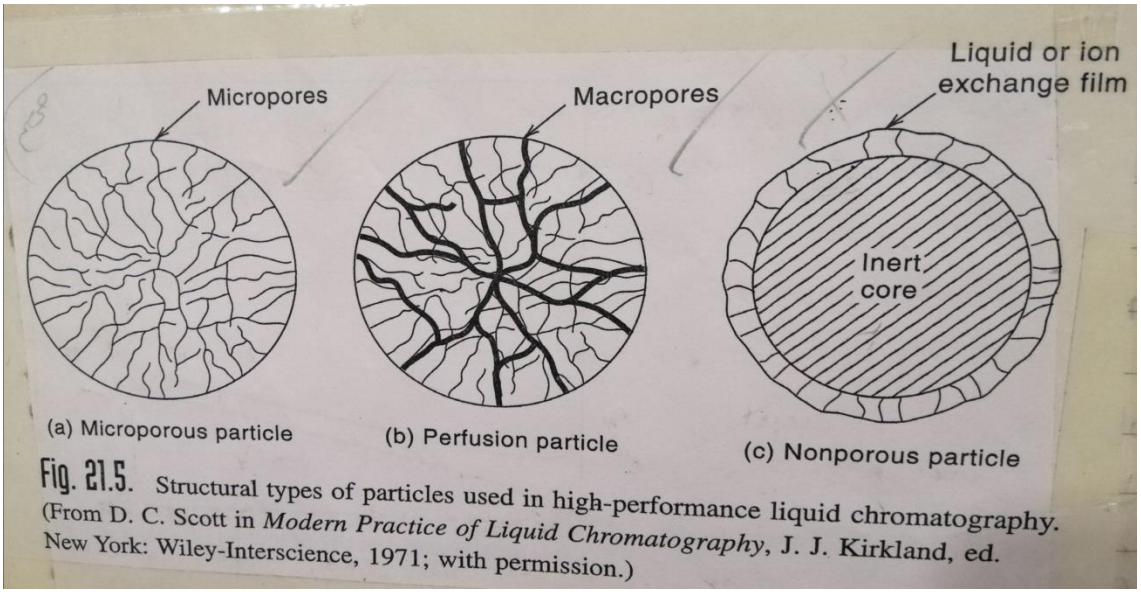
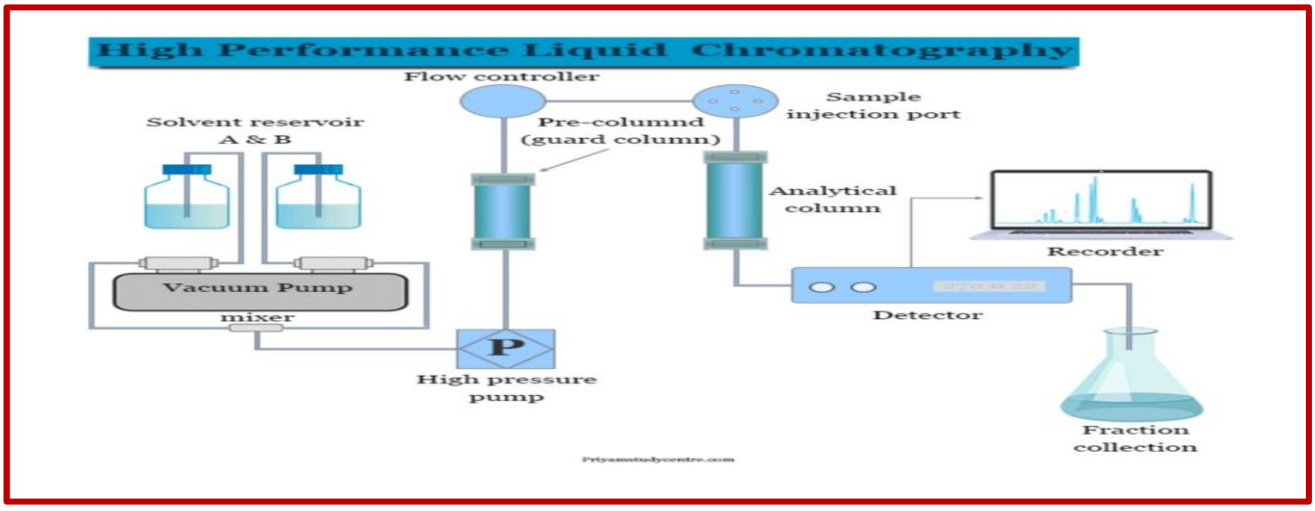


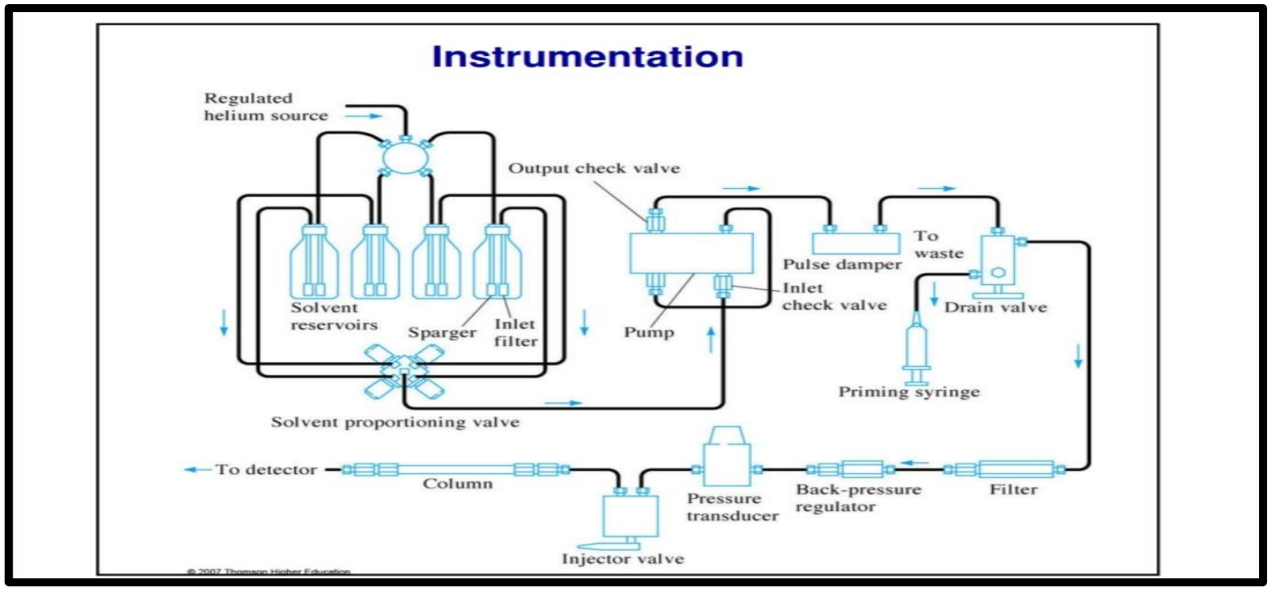
Fig. 21.5. Structural types of particles used in high-performance liquid chromatography. (From D. C. Scott in *Modern Practice of Liquid Chromatography*, J. J. Kirkland, ed. New York: Wiley-Interscience, 1971; with permission.)

Instrumentation :-

The following figure is a diagram showing the important components of a typicall HPLC instrument which are consist of five principal parts .

1- Mobile –phase reservoir and solvent treatment system .
2-Pumping system .
3-Sample Injection system .
4- Columns for HPLC .
5 – Detectors .





(1-)- Mobile- phase Reservoirs and Solvent treatment system.

a- A modern HPLC is equipped with one or more glass reservoirs, each one of which contains 500 ml or more of solvent .

b- Prevision are often included to remove dissolved gases and dust from the liquid .

An elution with a single solvent or a solvent mixture of constant composition is called Isocratic .

In gradient elution , two (and sometime more) solvent system that differ significantly in polarity are used .

C- Modern HPLC instruments are often equipped with proportioning valves that introduce liquids from two or more reservoirs at rates that vary continuously as Shown in figure.

An isocratic elution in HPLC is one in which the solvent composition remains constant

A gradient elution in HPLC is one in which the composition of the solvent is changed Continuously or in a series steps .