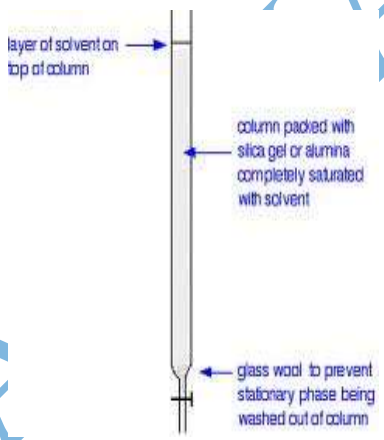
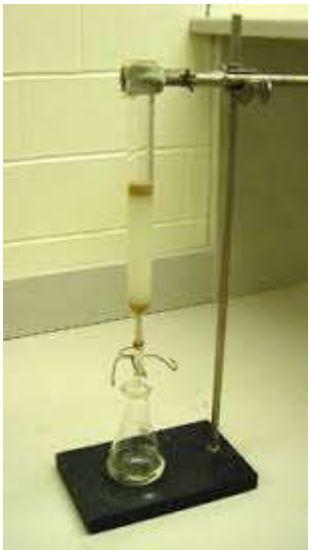
# *Column chromatography*

Column chromatography is adsorption type of chromatography .Column is a glass cylider with a tap at one end above which a disk of a cintered glass is located the benefit of it is to give a smooth , even & horizental surface & to prevent the adsorbent to pass out of the column. Such column is used for many types of chromatograpyh like ordinary adsorption chromatography , gel filteration & ion exchange kind of separation . The stationary phase is a powdered adsorbent which is placed in a vertical glass column . The mixture to be analysed is loaded on top of the column. The mobile phase is a solvent poured on top of the loaded column. The solvent flows down the column , causing the components of the mixture to distribute between the powdered adsorbent & the solvent , thus separating the components of the mixture so that as the solvent flows out of the bottom of the column, some components elute with early collections & other components elute with late fractions.

The columns differ in size (i.e length & diameter ) according to the purpose for which it is used.



## *Methods of packing the column*

There are two main methods for packing the column :

***1.Dry packing method*** : In this method the adsorbent is introduced as dry powder with the aid of vibration or packed with a special piston , after packing the powder a circular filter paper cut at a size equal to the circumference of the column is placed at the top of the powder surface , then the sample to be separated is introduced into the column on the round filter paper in a dry form , then another round filter paper is introduced on the powdered sample then the solvent is added gradually on the surface of the filter paper , the tap is opened & elution is continued .

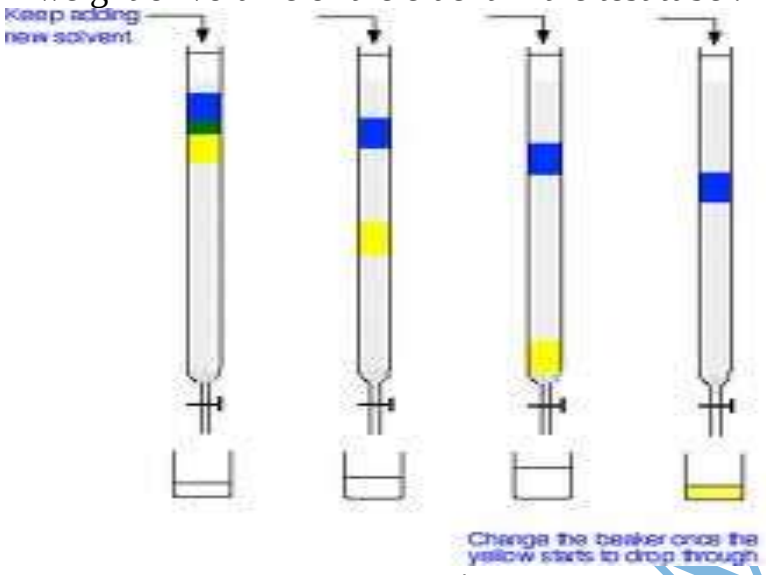
The disadvantage of this method is that any deffect will be formed in the column can not be corrected .

***2.Wet packing method*** : In this method the adsorbent is introduced into the column in the form of a slurry .

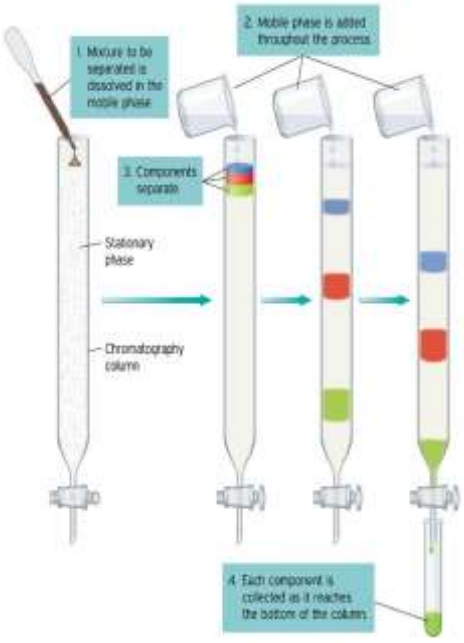
A well packed column is the one which should have no air bubbles with in the adsorbent & the adsorbent should be packed homogenously down the length of the column . Before packing a column it should be placed verticaly & should half filled with the solvent & if there are any air bubbles in the column they should be removed before adding the adsorbent . The eluent (solvent) is removed from the column through outlet & while doing this the outlet is adjusted to the flow needed . A slurry of adsorbent with the eluent is prepared the ratio of the adsorbent to the eluent is 1-3 or 1-4 depending on the adsorbent used . The slurry is packed in the column & during packing the tap is left opened to the flow required . It is very important that once the slurry is packed it should be done all at one time . The adsorbent should fill 2/3 of the hight of the column , & when the adsorbent column is to the required hight the solvent flow should continue & the top of adsorbent should be protected by a piece of filter paper which is cut to fit the inside of the column & the filter paper should rest on the top of the adsorbent . On the top of the filter paper a layer of acid washed sand is placed to insure an even layer of the sample . Before use 2-3 times the hight of adsorbenr should be passed through the column by passing eluent through the column to insure the cleaning of the adsorbent & the resting of particles. Then once the column is ready the eluent should be let to flow until it reaches the layer of the sand & then the sample is introduced to the column on the sand layer . The sample before introduction to the column should be dissolved in the minimum amount of the eluent . Once the sample is introduced onto the column then the flow is continued & the column should never be left to dry therefore a separatory funnel containing the eluent is placed on the top of the column to insure continuous flow of the eluent with out dryness of the column .

The fractions collected from the column are either large or small in volume . The small fractions are collected using fraction collector which is composed of a tray which contains hall in which the collecting test tubes are placed & the filled test

tube is replaced by a new one by rotation of the tray thruogh a timer or the difference in weight or volume of the eluent in the test tube .




رسم توضيحي **1**




رسم توضيحي **2**

## *Factors affect separation in column chromatography :*

1- Size of the sample-to the amount of adsorbent used . A ratio of 1:50 yield best separation . A ratio of 1:20 is used for a sample composed of 3 components .
2- Size of the column i.e length to the diameter .

3- Flow rate (1ml/min yield good separation) the flow rate depends on the flow of the tap & packing of the column (the finer the particle size of the adsorbent the more condensed the column is & the slower the flow of the eluent from the tap) .
4- Choice of the eluent .
5- Nature of the sample & adsorbent .Choice of the adsorbent & this means the activity & the particle size of the adsorbent (the finer the particles gives larger surface area) .

## *Types of operation ( elution ) :*

There are several ways to carry out a chromatographic process depending on how the sample the sample is introduced & moved through the stationary phase.

1- ***Elution*** : The sample is introduced as a thin layer at the top of the column. An innert mobile phase (gas or liquid) flows through the column carrying the components with it . The sample molecules distribute themselves between the two phases with each molecule spending a certain fraction of time in each phase depending on its distribution coefficient . The average rate of travel of all molecules of the same kind is a function of the distribution coefficient & the velocity of the mobile phase ( the elueting solvent).

***2- Dislpacement*** : The sample is introduced as a thin layer at the top of the column. In contrast to the innert mobile phase used in elution , an active mobile phase ( or one containing an active ingredient ) is used to displace the sample completely from the stationary phase . " The more tightly held sample components tend to displace the less tightly held components " . Zones of the components develop in the order of decreasing distribution coefficient , each zone displacing the one immediately preceding it until they are all forced out the end of the column .
The important advantages of displacement are that the sample is not diluted in large quantities & the column is used to its full capacity . On the other hand the zones tend to overlap to some extent & at the end of the run the column is loaded with the displacing agent which may be difficult to remove . For these reasons , it is not a popular technique .

***3- Frontal analysis*** : The sample is continuously introduced as a mixture with the mobile phase . By this method only the first component will be obtained pure therefore this method is not widely used .

***4- Gradient elution*** : This is the most widely used method of elution by which the sample is introduced as a thin film on the top of the column then the column is eluted starting from a non-polar solvent ex: hexane or benzen until all the soluble components emerge out of the column then a polar solvent is added gradually in increasing percentage ex : ethanol or methanol as 1% ethanol in hexane then increasing to 2% ,5% ,10% & so on . The percentage of the polar solvent should be increased only after all the soluble components in the preceding percentage emerge out of the column .

**Eluotropic series** is a listing of various compounds in order of eluting power for a given adsorbent. Such series are useful for determining necessary solvents needed for chromatography of chemical compounds. Normally such a series progress from non-polar solvents , such as n-hexane to a polar solvents in an eluotropic series depends both on the stationary phase as well as on the compound used , to determine the order.

## *Methods of detection of separation in column chromatography :*

There are several methods for detection of the separated zones in column chromatography depending on the nature of the sample :

1- Most common stationary phases are white or nearly colourless this make it possible to observe visually the band or zone of coulered components (depending on the colour ) .
2- Depending on the fluorescence : A number of organic compounds fluoresce in ultraviolet (UV) light therefore this detection is done by the use of UV lamp .
3- Depending on the use of colour-developing reagents : In a special technique called development chromatography , the column is eluted only until the first band appears at the end , then the column is extruded carefully &streaked with colour-developing reagents to indicate the position of the bands. The separated components can be extracted from the packing material if necessary .

4- Blind method : A number of small fraction are collected with small volumes then each fraction is analysed for its components using TLC or other techniques then similar fractions are combined together .

# *Thin layer chromatography (TLC)*

**Planar liquid chromatography (PLC)** involves the separation of mixtures on thin layers of adsorbents that are usually coated on glass , plastic, or aluminium sheets. The most common form of planar liquid chromatography is thin-layer chromatography (TLC); & this particular technique is the easiest , cheapest , & most widely used method for the isolation of natural products.

**TLC** is a kind of adsorption chromatography . The operations performed in TLC are essentially the same as in paper chromatography but instead of a piece of paper , a thin layer of finely devided adsorbent supported on a glass plate is used. Separation by TLC is effected by the application of the mixture or extracts as a spot or thin line onto a sorbent that has been applied on a backing plate . The plate is then placed into a tank with sufficient suitable solvent .The solvent then migrates up the plate through the sorbent by capillary action, a process known as development. As a consequence of development , compounds of a mixture will separate according to their relative polarities. Polarity is related to the type & number of functional groups present on a molecule capable of hydrogen-bonding.

## Analytical TLC

**1- Preparation of the layer** : The glass plate is usually flat & smooth , &different sizes & shapes of glass plates are used . Microscopic slides are cheep , readily availabe & adequate for small scale work . In any event the glass surface must be cleaned with a detergent & / or on organic solvent to remove any grease .

The thickness of the layer will determine the capacity of the system . Layers of 0.1-0.25 mm is satisfactory for analytical purpose . The primary concern is to obtain a layer of uniform thickness . Most thin layers are produced by spreading a film of an aquous slurry of the adsorbent over the entire surface . The slurry must be neither too thick nor too thin , or it will not spread properly . Silica gel when mixed with about twice its weight of water makes a very satisfactory slurry. The slurry after preparation should be spreaded on the plates & there are several types of spreaders used commercialy , but the well known manufacturers who have developed simple method for TLC are the Desaga & quickfit . In the Desaga method the plates are placed on a templet or tray which have flat surface & it should be placed on flat table , after fitting the plates on the templet , a spreader is used which is usually placed at the end of the templet & it has a rotating chamber through which the adsorbent is placed & there is a gauge to set the thickness of the adsorbent layer to be spreaded . The slurry is poured into the spreader & slowly & steadily moved along the row of plates to give a thin film of adsorbent on the glass plate . The plates are left on the templet until they are air dried & then they are activated . Activation is the removal of water molecules from the adsorbent by

placing the plates , after holding them in a rack , in the oven & heating it at 110 C for 30-60 minute . The activation process will change the type of chromatography from partition to adsorption type , because the water molecules which are found in the slurry will act as a stationary phase if not removed . The plates are left to cool & the edges are cleaned then it is kept in the desicator till use . Desicator is a well clossed chamber which contains a substance which has the ability to absorb moisture ex : silica gel blue .

**2- Preparation of the tank (jar)** : This include the following steps :

1- introduction of the solvent
2- lining of the tank .
3- saturation of the tank .

Solvents used in chromatagraphy should always be pure & dry . The amount of solvent used is calculated to give a solvent hight in the tank to about 1.5Cm.
The tank after introduction of the solvent should be lined with filter paper , this lining will ensure saturation of the tank , the tank is clossed tightly to ensure saturation .
Saturation of the tank is of great importance in separation because the migration of the component is greatly affected by the migration of the solvent system which in turn will evaporate from the plate if the tank is not saturated with the vapours of the solvent . The tank should be placed after preparation on a flat surface away from air current or heat or sun light because they affect saturation & hence the migration of the solute .

**3- Application of the sample** : Before application of the sample several parameters which should be adjusted ex:
-The base line (the line on which the sample will be spotted ) should be located & it is about 2-3 Cm above the lower edge of the plate & a mark is made with a fine needle to indicate its position . The base line level should be higher than the level of the solvent in the tank otherwise the sample will dissolve in the solvent before migration.
-The solvent front ( the level or hight to which the solvent will move or travel ) should also be adjusted .
-About 0.5 Cm is removed from both sides of the plate , this is necessary to prevent any adsorbent from the sides of the plate from absorbing solvent from lining of the tank & also for easiness of handling the plates .

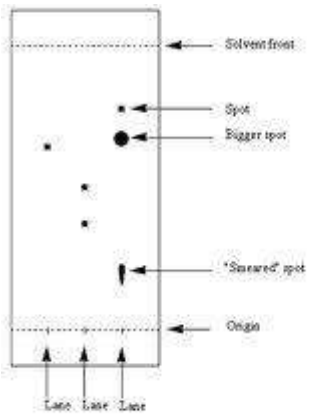
The sample is applied on the plate by the aid of a capillary or micro pipet , the first spot is applied about 1.5-2 Cm from the edge of the plate & about 1.5 Cm between two succesive spots . After each application the spot should be air dried so that to prevent diffusion of the spot . The concentration of the sample is very important to have a good separation because overloading the TLC plate will lead to streaking & if insufficient amount of the sample is applied then the trace

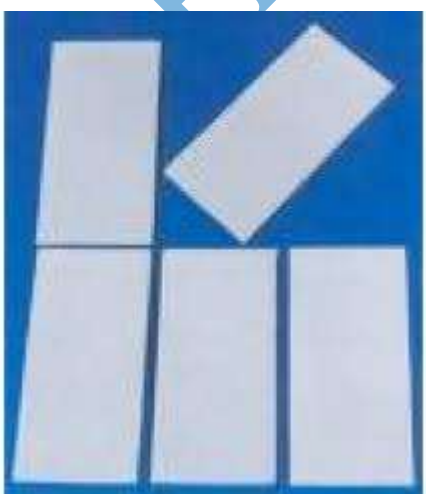
impurities may not be observed . After application & drying the spots , the plate is introduced in the tank and the tank is covered & the solvent is allowed to move or develop until it reaches the solvent front , then the plate is removed from the tank or jar & allowed to dry .

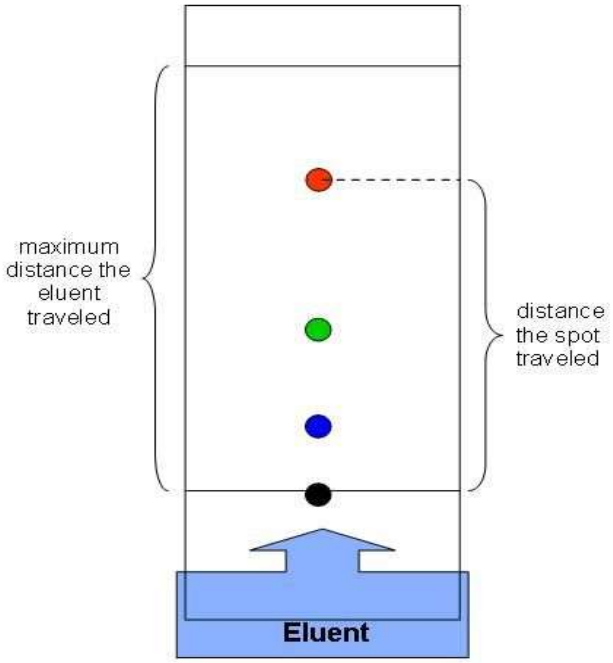
**4- Detection of spots** : There are several methods can be used to detect the separated spots ex :

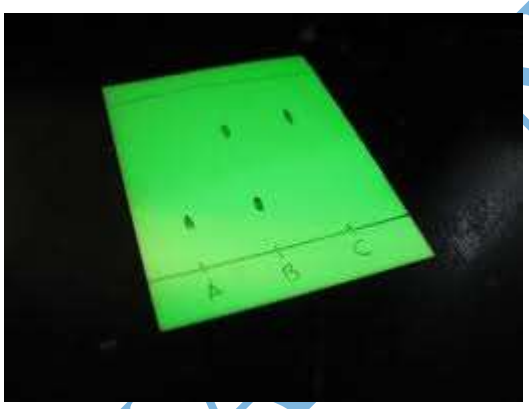
1- UV flouresence : the spots shows flouresence under UV light .
2- Iodine vapours : it is used extensively as a universal reagent for organic compounds which contain unsaturation .
3- Spraying the plate with concentrated $H_2SO_4$ which upon warming will chars the sample components leaving black spots .
4- Different spraying reagents are used to detect different compounds ex:
   - Dragendorff spray reagent used for detection of alkaloids.
   - Vanillin in $H_2SO_4$ spray reagent used for detection of terpenes .
   - Antimony trichloride spray reagent used for detection of steroids .
5- Coloured compounds are easily detected by their colour .

**The separation mode is shown in the following figures:**






**5- Measurments of chromatographic data :**

Several parameters should be measured to identify the separated spots .

One of the important methods used to identify the separated components is the use of standard material that could be spotted between the spots of the unknown components .

The important parameters measured are :

$R_f$ value = distance moved by the solute/distance moved by the solvent

$R_f$ value could be one or less than one but it can not be more than one because the sample components can not move above the solvent front .

If a standard (reference) material is used then another parameter is measured which is Rx value .

Rx value = distance moved by the sample/ distance moved by the reference
If Rx value =1 then the sample & the reference could be the same specially if these sample & reference give Rx value =1 in different solvent systems .
$HR_f$ value = $R_f$ value x 100
Finaly the developed plate is traced on tracing paper to be kept as a document. This tracing paper is called chromatogram on which all the materials & parameters used in the development of the plate is written ex:

- adsorbent used
- solvent system
- sample used
- reference material used
- spray reagent
- etc..

## Factors affecting migration of solute in TLC

These factors include the followings :
1- Nature of adsorbent – strong or active adsorbent
- medium activity
- weak activity

2- Nature of the solvent – following the eluting power of the solvent .
3- Nature of the solute – depending on the molecular weight , functional groups, configuration .

## Nature of adsorbent :

The adsorbing power of a material depends on the chemical nature of the surface, the particle size , & by its moisture content .
The particle size determines the surface area & this determines the active sites , the more fine a powder is the more is the surface area , so the adsorbent become stronger i.e by decreasing the particle size .In general chromatographic adsorbents are used at a standard particle size . The adsorbents used for TLC are of fine particle size than the adsorbents for column chromatography .

Examples of common adsorbents in a decreasing order of adsortion power:

- Alumina
- Silica gel
- Magnesia
- Calcium carbonate
- Sucrose
- Starch

- Powdered cellulose

It is usually prefrable to use an adsorbent with a binder like gypsom which is added to help in setting the adsorbent at a shorter time than the material with out a binder for this reason adsorbent having a binder should be immediately spread once the slurry is prepared other wise it hard to spread .

The adsorbents are classified into polar , medium polar , non polar . On a non polar ( weak ) adsorbent the interaction between the solute & the adsorbent depend on the physical forces (Vander Waals forces ) . In the case of polar adsorbent the dipole-dipole interaction & hydrogen bonding between the active site on the solid surface & the solute will act & it helps in retention . The polar adsorbent are very strong adsorbent & much stronger than the medium polar or the nonpolar adsorbent .

The strength of particular adsorbent may be changed by the particle size & its moisture content . The particle size determines the surface area of the powder & the number of active sites because solid surfaces increase with increasing the surface area , for this reason the more fine a powder, the more is the surface area. Polar adsorbent like silica gel contains free moisture which is held together by hydrogen bonding . By heating part or sometimes all the moisture may be removed , for this reason the nature of adsorbent may be changed after removal of water from & hence the type of chromatography from partition to adsorption type . In partition the adsorbent contains from 10-20% of free moisture while in the adsorption the adsorbent contains 4-6% of free moisture for this reason it is necessary to activate TLC plates after spreading the slurry to ensure the removal of water & to change the adsorbent to an active adsorbent & hence the system will be changed from partition to adsorption .



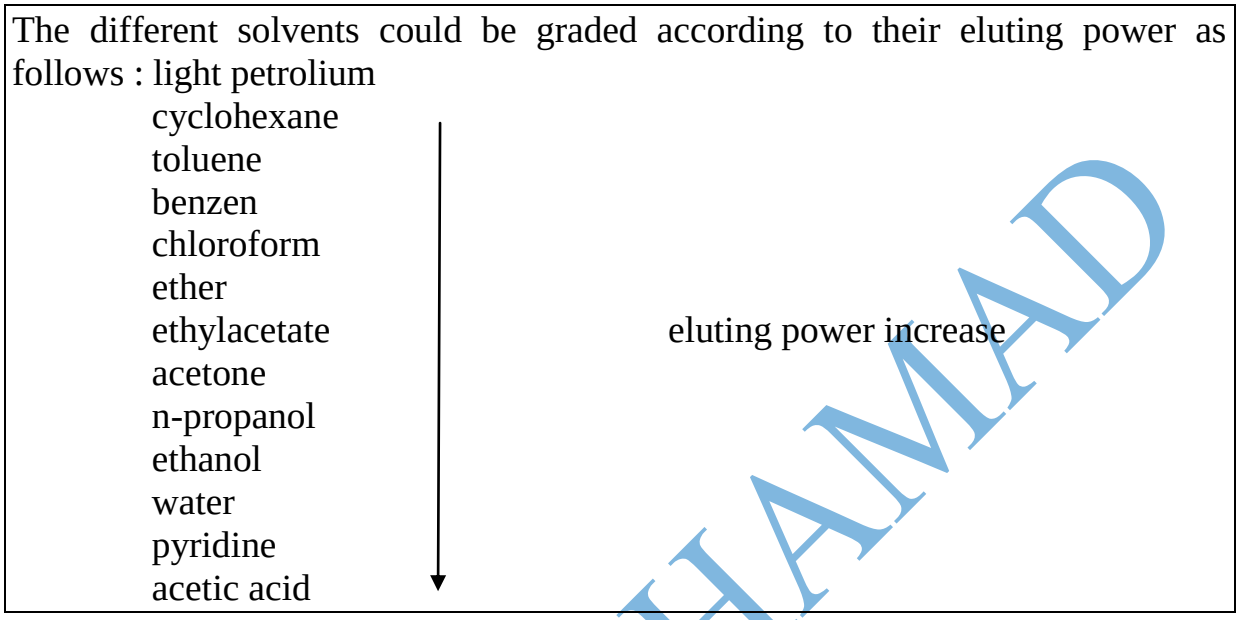
**Nature of the solvent** :

Solvents may be graded into a series according to their power of elution , the more polar a solvent is the greater is the eluting power of that solvent for this reason adsorption of a solute on an adsorbent will be very high starting from a very non polar solvent up the list of polarity this is because the solute is only slightly soluble in the solvent , we call this as the solute- solvent interaction is weak & therefore the solute- adsorbent interaction is higher & for this reason the solute will remain on the base line .

The weak migration if a solute is due to the weak solute-solvent interaction . In an open column chromatography ex : TLC when polar solvent is added to the

system it will push the solute up the plate depending upon the nature of the solute & therefore Rf value of the solute will be increased .

The different solvents could be graded according to their eluting power as follows : light petrolium




For open column chromatography like TLC a major solvent is always choosed in which the solute is slightly soluble , this solvent may be of moderate or non polar type , then another solvent is added to increase or change the $R_f$ value of the solute according to the nature of the solute to be separated ex : if strychnine alkaloid is separated on TLC, silica gel is used as adsorbent , abase solvent is of ethyl acetate is choosen . The plate is developed in this solvent & the Rf value is recorded . Ethyl acetate being a moderatly polar solvent , will not migrate strychnine alkaloid because the interaction between strychnine & silica gel (adsorbent) is higher than the interaction between ethylacetate & strychnine for this reason a more polar solvent like methanol is added to increase the polarity of the solvent & hence increase the $R_f$ value of strychnine . This what we mean by mixing of solvents i.e use solvent system ex : for the separation of strychnine the solvent system has been choosen which consist of ethyl acetate – methanol 98:2 .



## Nature of solute :

The **functional groups** play a big role in the migration of a solute . The presence of polar groups will lead to low migration . Also the **configuration & conformation** ex : cis & trans have effect on the migration because they will determine the way of attachment of the functional groups to the adsorbent .

Nonpolar proups: $CH_3$_ , $CH_3O$_ , Ph_ , $CH_3CH_2$_ ,----
Polar groups : -$CO_2H$ , -OH, -$NH_2$, -$SO_3H$, $PO_3H_2$ ----

The relationship between the **molecular weight** & adsorption has a rule which states that within a similar series the adsorption increase with an increase in the molecular weight & this what is called Traubes rule . As the adsorption increase the $R_f$ value will decrease .

Natural products may be "tracked" by running analytical TLC of fractions from other separation processes , such as column chromatography , HPLC. More than one solvent system should always be used for TLC separation , as even apparently "pure" spots may consist of several compounds with identical $R_f$ values . The similarity of different extracts from the same species can also be assessed in this way & the dicision to combine nonpolar & polar extracts can be made on the basis of identical or similar TLC chromatogram.

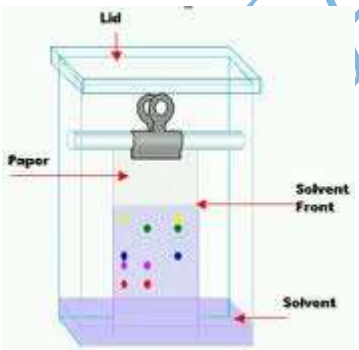
## Preperative TLC

This technique is used for qulitative as well as quantitative analysis . The size of the plates used here large ex : 20x20 or 20x40 Cm . In this teqnique usually a thick plates (preperative plates) are prepared with a thickness varying from 0.5-2mm . For preparation of such plates the same techniques disscused before in the analytical TLC are applied , the only difference is that the amount of the adsorbent in preperative TLC usually double the quantity of that used in the preparation of the slurry in analytical TLC .
The preperative plates are much thicker than analytical plates & so it should be dried at room temperature over night before activation in the oven to prevent cracking of the adsorbent layer . The sample is dissolved in the minimum amount of the solvent & then applied on to the plates at a distance of 1.5 Cm from the bottom of the plate in the form of a continuous line with the aid of a cappillary tube or automatic syringe & left to dry , then developed in the approperiat solvent . After development , the plates are left to dry then it is observed under UV light to locate the separated bands . If the components are not flourescent then one side of the plate is cut with a needle (the adsorbent is cut) & then the rest of the plate is covered with another glass plate & the exposed part is sprayed with a spray reagent to indicate the location of the separated bands . Then the glass plate is removed & the different components which are separated in the form of bands are removed or scrapped & the scrapped adsorbent is placed in a glass container (flask) & a solvent is added to each flask then it is shaken with the solvent to ensure the complete removal of the component from the adsorbent (elution) & then filtered & taken to dryness & estimated qualitatively & quntitatively .

# *Paper chromatography*

It is a kind of partition chromatography . The paper used for chromatography are usually Whattman No.1 filter paper . The paper commonly used consists of highly purified cellulose .

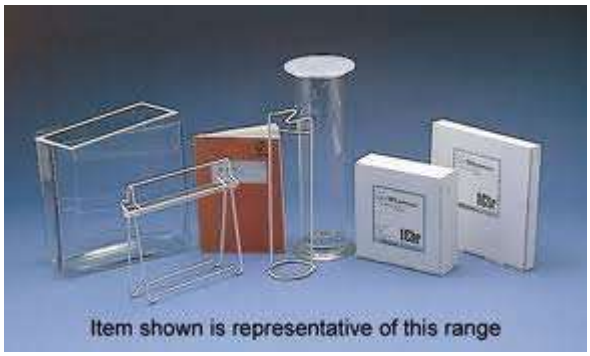
**Apparatus** : The apparatus required for paper chromatography consists of a support for paper , a solvent trough & an air – tight chamber in which the chromatogram is developed . The clossed chamber is necessary to prevent the evaporation of the volatile solvents from the large exposed area of the paper. The size of the chamber vary according to the size of the paper used . The paper is cut to the size required for each separation , paper are used as such or they may be washed or treated according to the required technique . The tank or chamber or jar used are usually round tanks which has ground glass lid & it should be saturated with the solvent before introduction of the paper . The solvent should always have a depth of 1.5 Cm in the tank .



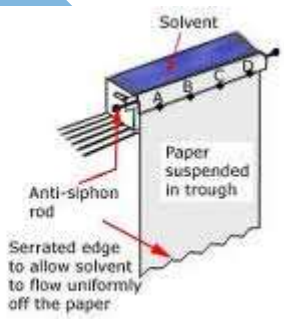

**Application of the sample spot** : Usually a line is drown at a distance which is 2Cm from the bottom of the paper , the line should be drawn with a pencil & any other coloured pencil or ink should not be used otherwise it will interfer with the separation .
The sample is then applied to this line with the aid of a hair drier & this is done for immediate evaporation of the sample giving a small circular spot on the base instead of letting the sample to evaporate by itself & so it will diffuse giving a big circular spots . The solvent front is also marked with a pencil , the first spot is applied at a distance of at least 1Cm from the edge of the paper . The sample should be compeletly dried before developing , the application is done with a capillary tube or micro pipet .

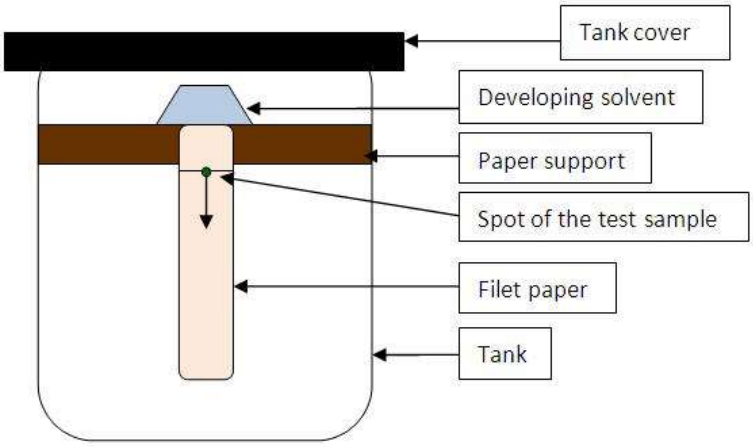
**Development** : Several developing techniques can be applied in paper chromatography .
**1-*Ascending development* :** This is the simplest & most popular type . The paper is suspended vertically with about 1-2 Cm above the surface of the solvent to

prevent diffusion of the sample downward into the solvent reservoir. The solvent ascends through the paper by capillary action . The rate of ascent is slow & decrease with time because of gravity , however , the slow rate enhances the possibility of achieving partition equilibrium & often results in compact sharply defined spots .

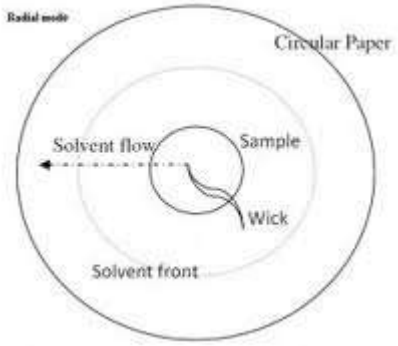



**2-*Descending development*** : This is done with a solvent trough at the top of the tank or chamber , the direction of flow is downward . To prevent rapid syphoning , the paper is folded into a U-shape with a short initial rise from the solvent trough . The method is much faster than the ascending development , longer pieces of paper may be used & large amount of solvents can be used if necessary for slow moving spots .

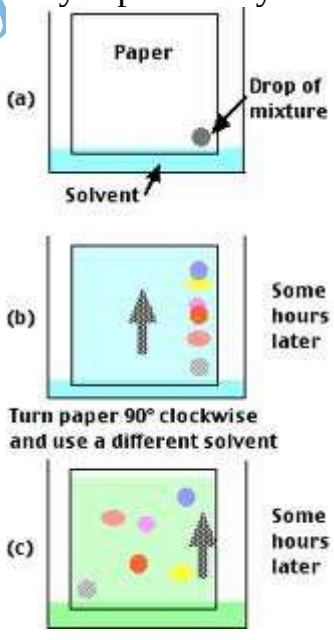



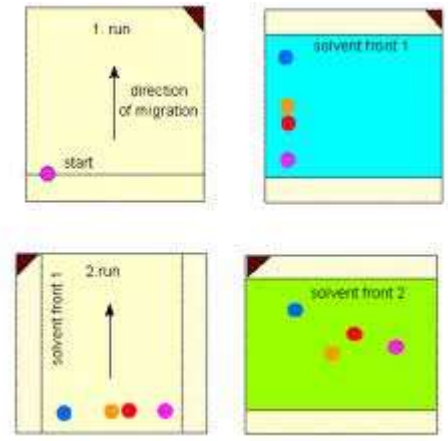



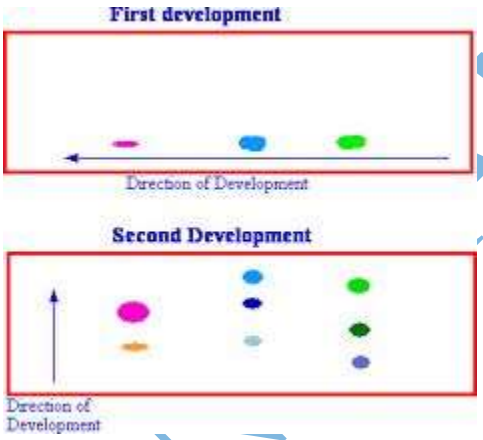
**3-*Radial or horizental development*** : In this method the sample is spotted around the center of the horizentally placed filter paper with a wick in the center to supply the solvent to the center of the paper from a supply trough below . The solvent move outward separating the components of the sample along radial paths forming circles of increasing diameter . A covered petri dish is an adequate container or the paper can be sandwiched between two glass plates , with a hole in the center of the upper one for the intoduction of the sample .




4-***Two dimentional development*** : In all previously described methods of chromatography the eluting solvent could flow in one direction only across the paper . On a plane surface such as a piece of paper , it is possible to carry out a sequential development in two directions . The sample is applied as a spot close to a corner . This is developed in the normal fashion by ascending or descending procedure until the fastest moving spot approaches the end of the paper . Then the paper is removed & after evaporating the solvent it is turned 90 degree & developed a second time with another solvent having different eluting properties In this manner , samples which could be only partially separated with either solvent alone may be compeletly separated by the combination of solvents.






**5-*Multiple development*** : It is a simple ascending development that is repeated several times between each two development the paper should be removed & dried then introduced again into the jar . This method enhance the separation by increasing the distance between two successive spots .

**Detection of the separated spots** : If the spots are not coloured the spots are detected either under UV lamp , or sprayed with a spray reagents .
Spots of separated antibiotics can be detected by applying the chromatogram on a seeded plate ( seeded with living microorganism ) & noticing the inhibition zones after 24 hours .

**Preperative paper chromatography** : In paper chromatography the same technique is followed as in TLC with one advantage that in paper type the separated components are easily removed by cutting the paper to the separated bands then each separated band is cut to small pieces to ensure the complete removal of sample when shaken with the solvent.

## Advantages of TLC over paper chromatography

1.small quantities are easily detected by TLC.

2.The spots are more compact & clear in TLC than paper chromatography.

3. We can use drastic reagent in TLC ex: concentrated $H_2SO_4$ in TLC but we can not use it on paper chromatography.

# *Gas – liquid chromatography (GLC)*

Gas-liquid chromatography is a partition type of chromatography where by the solute is partitioned between two phases a liquid stationary phase & a gas mobile phase . The innert carrier gas passes through a pressure regulator into a sample injector & mixing chamber . From here the carrier gas (mobile phase) carries the sample into the column .

The column may be packed with a porous solid coated with a thin film of a non-volatile liquid (stationary phase) , or it may be a long capillary tube with a thin coating of liquid on its wall . The sample components are separated as they pass through the column & one after the other pass through a detector which sends a signal to a recorder . Finally , the gas passes through a flowmeter & is exhausted to the atmosphere . A thermostated oven is provided for the column , injector , & detector , although the last two may be heated separately .

The sample injection point in the column is important part & usually there is a rubber stopper at the top of the column through which the syringe needle containing the sample passes to introduce the sample at the top of the column . The whole of the injection point unit should be at a higher degree than the rest of the column & it is usually kept 10 C above the temperature needed for the rest of the column , this is important to ensure the immediat evaporation of the liquid sample once it enters the column . The column oven may be set at any temperature required between 0-400 C . The injection unit has a separated heating control than the rest of the column .

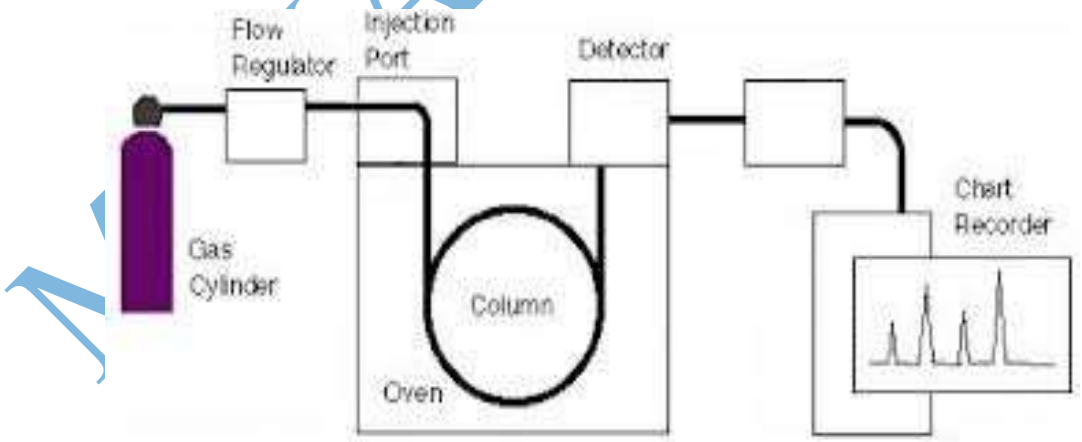



**Diagram of GLC 1**

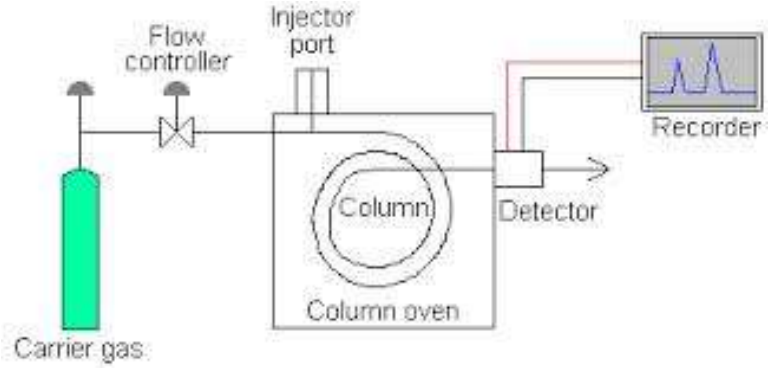



**Diagram of GLC 2**

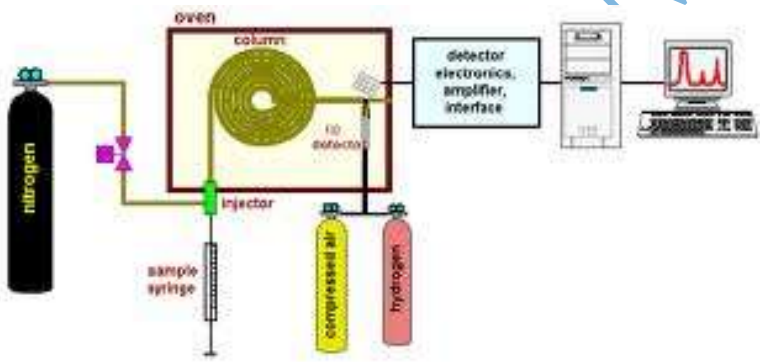



**Diagram of GLC 3**



**The instrument of GLC is composed of the following parts :**

1- **Carrier gas supply** : In GLC the carrier gas (which is supplied by a cylinder) provides trasportation for the sample components through the apparatus . It must be chemically innert & available in pure form & reasonable cost .The most commonly used gases are helium , nitrogen, argon, hydrogen & carbon dioxide . A high density is prefered for best fficiency & a low density for maximum speed . The flow control is obtained from a standard reducing valve & a soap film flow-meter is used almost exclusively to measure the flow rate .

2- **Columns** : there are two distinct types of columns in common use i.e packed & open tubular (capillary) . They are either stainless steal or glass column . The column is packed with the innert support which has been already coated with

the liquid phase . One end of the column is connected to the sample injector that means the place through which the sample or solute is introduced , the other end of the column is connected to the detector which will analyse the signal received.

3- **Solid support** : The ideal solid support should have a high specific surface ($1m^2/gm$) , & the surface must be chemicaly inert also wet able by the liquid phase so that it will spread in a thin layer of uniform thickness . In addition it must have thermal stability , mechnical strength & available in a uniform size , nearly spherical shaped particles ex : derivativesof diatomaceous earth .

4- **Detectors :** Detectors detects the emerging sample in the gas form usually the solute or sample emerge from the column in the form of a gas or steam .

**Types of detectors** :

1- Differential detectors : which measures instantaneous rate of emergence or concentration of the components each one alone .
2- Integral detectors : which accumulate the sample components & the signal gives the total amount which has emerged up to a given instant .

Signals from differential detectors are usually integrated for quantitative analysis , & the signals from integral detectors are differentiated to make them easier to interpret for qualitative analysis .
Detectors can also be classified as destructive or non destructive , depending on whether or not the sample components can be collected unchanged for further study.
**Hydrogen flame detector** : In this type hydrogen must be used as a carrier gas . it is burned as it emerge from the column yielding a nearly colorless flame . When an organic component emerges , the flame becomes yellow . The amount of the component is roughly proportional to the hight &/or luminosity of the flame . since most organic compounds are ionized in the flame an ion current can be collected between two oppositely charged electrodes . This is the principle of the flame ionization detector which is relatively simple & sensitive but of course , destroys the sample.
**Thermal conductivity detector** : The measurment of the thermal conductivity of a gas is based on the transfer of heat from a hot filament to a cooler surface . Thus the gas conduct heat from the filament to the wall . If a constant amount of electrical energy is supplied to the filament , its temperature will be a function of the thermal conductivity of the gas .

The thermal conductivity detector is simple , inexpensive , moderately sensitive , non-selective , non-destructive , very accurate if properly calibrated & more widely than any other .

**B-Ray detector** : The principle of operation is the same as that of the hydrogen flame ionization detector , except that ionization of the sample molecules is produced by another mechanism by the use of a radioactive source which emits B-rays . This detector is extremely sensitive but it is less accurate than other detectors & expensive .

## Qualitative analysis in GLC

One of the important parameters used in qualitative analysis in GLC is the retention time $t_R$ which means the time measured from the injection of the sample to the maximum of the elution peak , in another word , the residence time of a solute in the column . The $t_R$ is measured on the chromatogram paper from the time or point of injection to the peak of the solute . The point of injection is easily seen on the chromatogram paper from the emergence of the solvent peak . The solvent vaporizes immediately & emerge in the form of a peak which we consider it as the point of injection . The speed of the chart or paper is measured in Cm & then converted to time units . The retention time is the sum of the solute time of residence in the gas phase & the time of residence in the stationary phase .

The value of the retention time vary greatly with the length of the column , the percentage & nature of the stationary , the gas flow rate & column temperature ex : when the column is short , then the retention time is reduced because it will take shorter time for solute to emerge from 3ft column than 5ft column . Also temperature has a lot to do with the retention time , if the temperature is the right one needed for the evaporation of solute then the solute will emerge from the column otherwise the solute will not vaporize if the temperature is lower than its evaporation temperature & hence it will remain in the column .

The gas flow has also a big role in controling the retention time , if the flow of the gas increase the retention time decrease because the gas will flow faster carrying the vaporized solute & so the solute will emerge faster & hence the $t_R$ is decreased.

The type of liquid phase used affect the retention time , if the liquid phase is polar liquid then as we have seen in chromatography , polar solute will remain more in the polar liquid according to the fact that states (like dissolve like) & the same is for non-polar solutes & non-polar liquid phase .

Because of all these factors that affect the retention time it is preferable to choose another parameter which is the relative t which is measured relative to the standard material which is used at the same time . The standard material is known as internal standard .

$$\text{Relative tR} = \frac{\text{Retention time of the solute}}{\text{Retention time of the standard}}$$

Sometimes if there is a little difference between the solute & the standard tR then it is preferable to inject both the solute & the standard together in one injection , if the solute & the standard are the same then we should get one peak of double size .

## Quantitative analysis :

Quantitative estimation in GLC is done by measuring the area under the peak . This area is measured by several techniques :

1- Cut out the peak with a scissor & weigh the paper , then determine the weight of paper per unit area .
2- Use of planimeter .
3- Estimate the area by triangulation i.e draw tangents to the points of inflection on the peak sides & compute the area of the triangle formed with the base line.
4- Use a mechnical or electronic accessory to the recorder which will automatically integrate the area ( some devices operate directly from the detector ).
5- If the peak is too sharp (narrow) then the area can not be measured accurately , in this case we use the peak height as a measure of the amount provided a proper calibration factor is determined .

## Theoretical principles of GLC

Two general approaches have been very useful in rapid advance of GLC

1- **Plate theory** : This theory assumes that the column can be devided by hypothetical cross sections into identical segments , of such a length that with in each segment an “average” equilibrium distribution is achieved during the passage of the carrier gas . Each of these segments is called a “ theoretical plate” . Then a knowledge of the distribution coefficient & the carrier gas flow rate , it should be possible to predict the distributionof a solute along the column at any time .The column efficiency is defined as the number of theoretical plates in the column. The faster the equilibrium process , the smaller the plates & thus the greater the number of plates in the column. It is therefore important to know how to determine the number of plates a column possesses & the relationship of the number of theoretical plates in the column to the properties of the chromatogram.

Number of theoretical plates (n) = 16 ( $t_R$ / $\Delta t$)

$$\text{HETP} = \frac{\text{Column length}}{\text{Number of plates}}$$

HETP is the height equivalent of theoretical plates .

2- **Rate theory** : This theory conclude that as the velocity is increased elution will be faster but there will be less time to achieve equilibrium ( fewer transfer between the phases ) . Conversely as the velocity decreased the elution will be slower . This theory applies Van Deemter equation :

HETP = A + B/u + Cu
A= Eddy diffusion
B/u = longtudinal diffusion
Cu = mass transfer
U = the linear velocity of the carrier gas

A : deals with the passage that each molecule go through the column during separation & this depends on the packing of the column & size of the molecules because these factors will decrease the Eddy contribution .

B/u: depends on the gradient concentration & this also depends on the packing of the column , & there must be established equilibrium .

Cu: the shorter the time required for the solute to stay in one of the phases , the smallest the HETP .
Optimum velocity is the velocity which gives the smallest HETP & for each sample there is a specific optimum velocity .