UNIT 2A - PAPER 1

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UNIT 2A - PAPER 1
CHROMATOGRAPHY

1. Gas Chromatography
2. High Performance Liquid Chromatography
3. Thin Layer Chromatography
4. Column Chromatography

UNIT - 2A-1 | PAPER - 1

GAS CHROMATOGRAPHY

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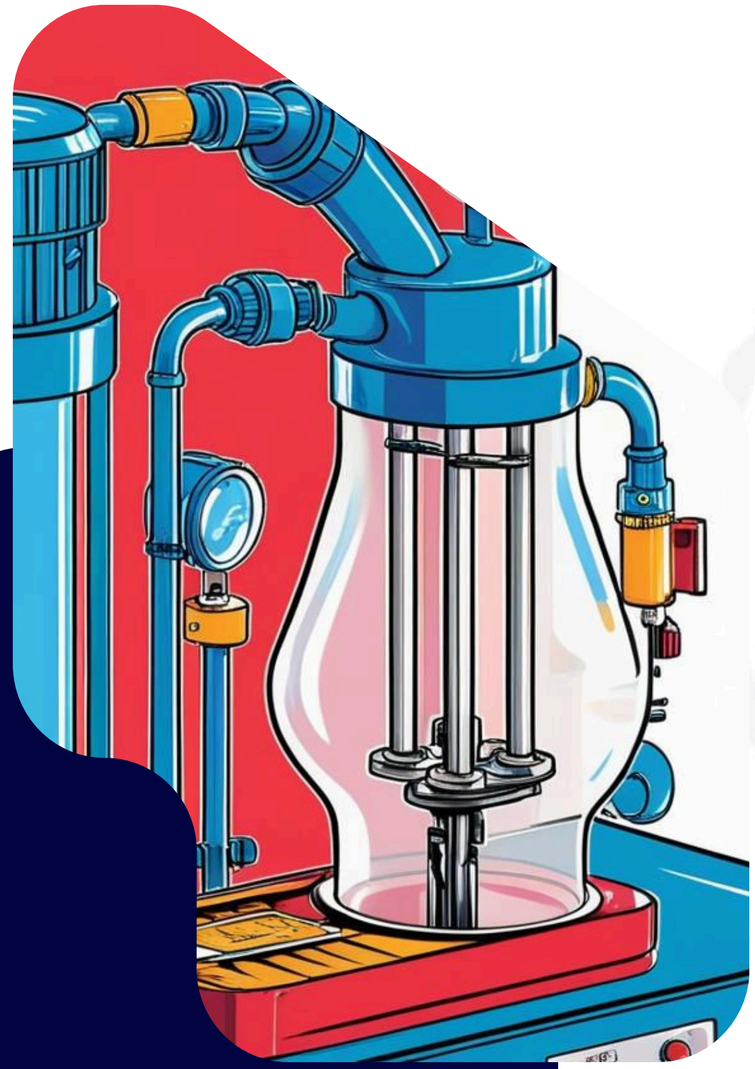
INTRODUCTION



- It is a process of separating component(s) from the given crude drug by using a gaseous mobile phase.

- It involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

TWO MAJOR TYPES

GAS-SOLID CHROMATOGRAPHY:

- Here, the mobile phase is a gas while the stationary phase is a solid.
- Used for separation of low molecular gases, e.g., air components, H_2S, CS_2 ,CO_2 ,rare gases, CO and oxides of nitrogen .

GAS-LIQUID CHROMATOGRAPHY:

- The mobile phase is a gas while the stationary phase is a liquid retained on the surface as an inert solid by adsorption or chemical bonding.

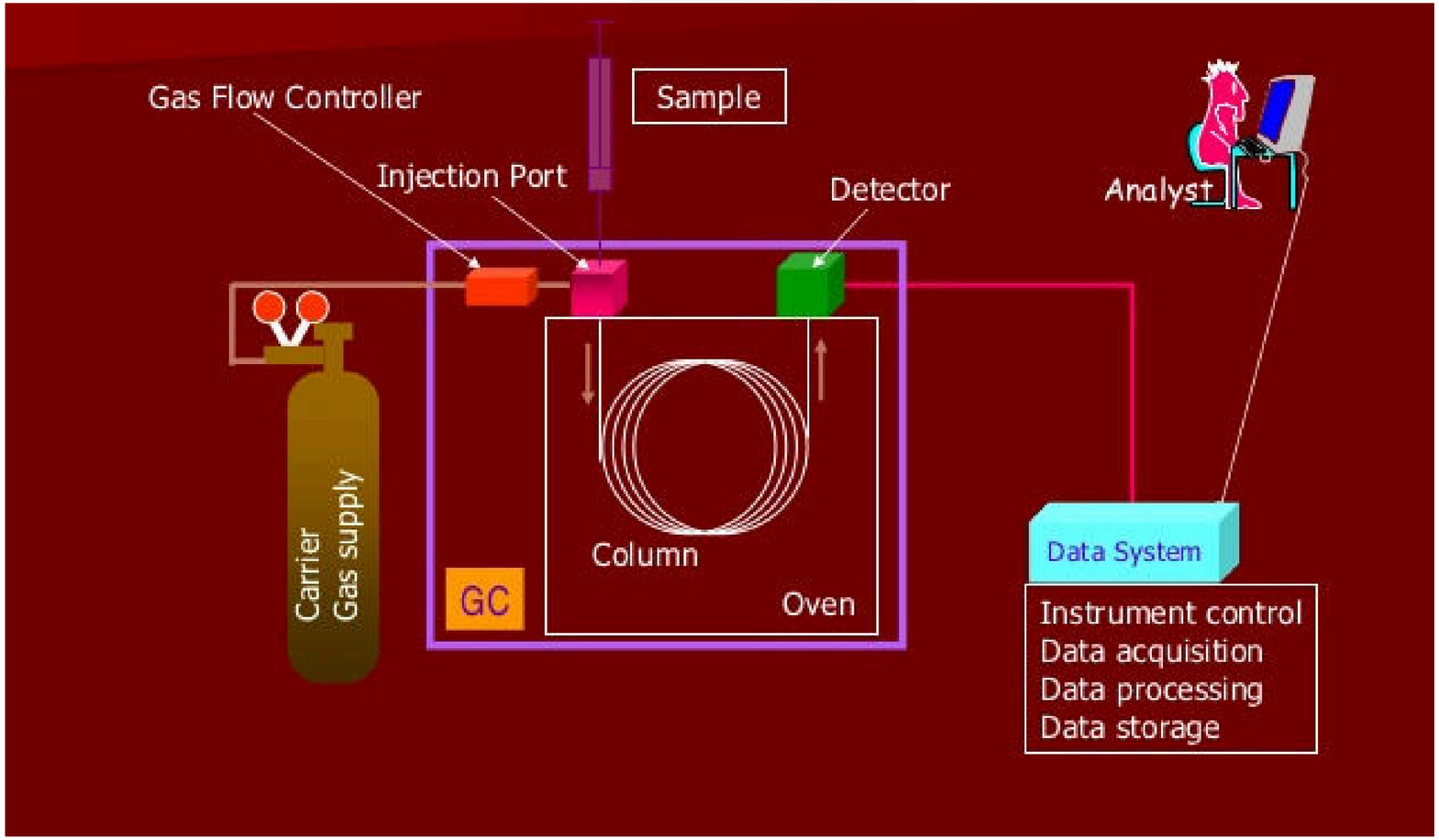
PRINCIPLES

- The principle of separation in GC is “partition.”
- The mixture of component to be separated is converted to vapour and mixed with gaseous mobile phase.
- The component which is more soluble in stationary phase travel slower and eluted later. The component which is less soluble in stationary phase travels faster and eluted out first.
- No two components has same partition coefficient conditions. So the components are separated according to their partition coefficient.
- Partition coefficient is “the ratio of solubility of a substance distributed between two immiscible liquids at a constant temperature.

INSTRUMENTATION

- Carrier gas
  - He (common), N2, H2, Argon
- Sample injection port
  - micro syringe
- Columns
- Detectors
- Thermal conductivity (TCD)
- Electron capture detector(ECD)
- Flame Ionization detector (FID)
- Flame photometric (FPD)

GAS CHROMATOGRAPH MAIN COMPONENTS



CARRIER GAS

- The cylinder/ gas tank is fitted with a pressure controller to control the pressure of gas, a pressure gauge that indicates the pressure, a molecular sieve to transfer filtered dry gas and a flow regulator to ensure a constant rate of flow of mobile phase to the column.
- It should meet the following criteria:
- Should be chemically inert
- Should be cheap and readily available
- Should be of high quality and not cause any fire accidents
- Should give best possible results

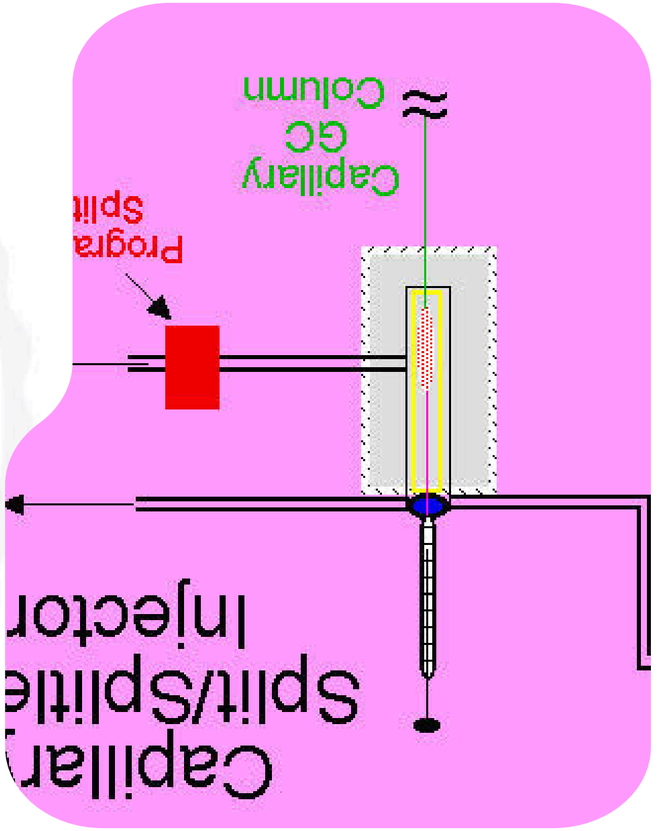
SAMPLING UNIT

- Sampling unit or injection port is attached to the column head.
- Since the sample should be in vapourized state, the injection port is provided with an oven that helps to maintain its temperature at about 20-50° C above the boiling point of the sample.
- Gaseous samples may be introduced by use of a gas tight hypodermic needle of 0.5-10 ml capacity.
- For Liquid samples , micro syringes of 0.1-100µL capacity may be used.

INJECTIONS OF SAMPLES INTO CAPILLARY COLUMNS

- **Split injections-** it splits the volume of sample stream into two unequal flows by means of a needle valve , and allow the smaller flow to pass on to the columns and the bigger part is allowed to be vented to the atmosphere. This technique is not suitable when highest sensitivity is required.

- **Splitless injectors-** They allow all of the sample to pass through the column for loading. Sample should be very dilute to avoid overloading of the column and a high capacity column such as SCOT or heavily coated WCOT columns should be used.



INJECTIONS OF SAMPLES INTO CAPILLARY COLUMNS

- **On column injectors:** A syringe with a very fine quartz needle is used. Air cooled to -20°c below the b.p. of the sample. After then the warmer air is circulated to vaporize the sample.

- **Automatic injectors:** For improving the reproducibility and if a large number of samples are to be analyzed or operation is required without an attendant, automatic injectors are used.

The solid samples are introduced as a solution or in a sealed glass ampoule, crushed in the gas stream with the help of a gas tight plunger, and the sample gets vapourized and flows into column under the influence of carrier gas.

COLUMN UNIT

- Columns are of different shapes and sizes that includes: “U” tube type or coiled helix type.
- They are mainly made of copper, stainless steel, aluminium, Glass, nylon and other synthetic plastics.

SUPPORT MATERIAL:-

- it’s main function is to provide mechanical support to the liquid phase. An ideal support should have a large surface area, chemically inert, should get uniformly wet with liquid phase, should be thermostable.
- Commonly used solid phases are: diatomaceous earth or kieselguhr, glass beads, porous polymers, sand,etc.

LIQUID PHASE

It should have the following requirements:

- It should be non-volatile
- Should have high decomposition temperature
- Should be chemically inert
- Should posses low vapour pressure at column temperature
- Should be chemically and structurally similar to that of the solute i.e., polar for polar solute.

EXAMPLES OF DIFFERENT LIQUID PHASE

CATEGORY	EXAMPLES
Non-polar hydrocarbon phases	Paraffin oil (nujol), silicon oil, silicon rubber gum (used for high temp of about 400°
Polar compounds (having polar groups like -CN, -CO and –OH)	Polyglycols (carbowaxes)
Liquids having hydrogen bonding	Glycol, glycerol, hydroxy acids

TYPES OF COLUMNS

There are two general types of columns:

Packed columns-In GLC, they are densely packed with finely divided, inert, solid support material (diatomaceous earth) coated with liquid stationary phase.

- In GSC, the columns are packed with adsorbents or porous polymers.
- Length- 1.5 - 10m
- internal diameter- 2 - 4mm.

Capillary columns-

- length ranges from 10-100m
- inner diameter is usually 0.1-0.5mm.

TYPES OF COLUMNS

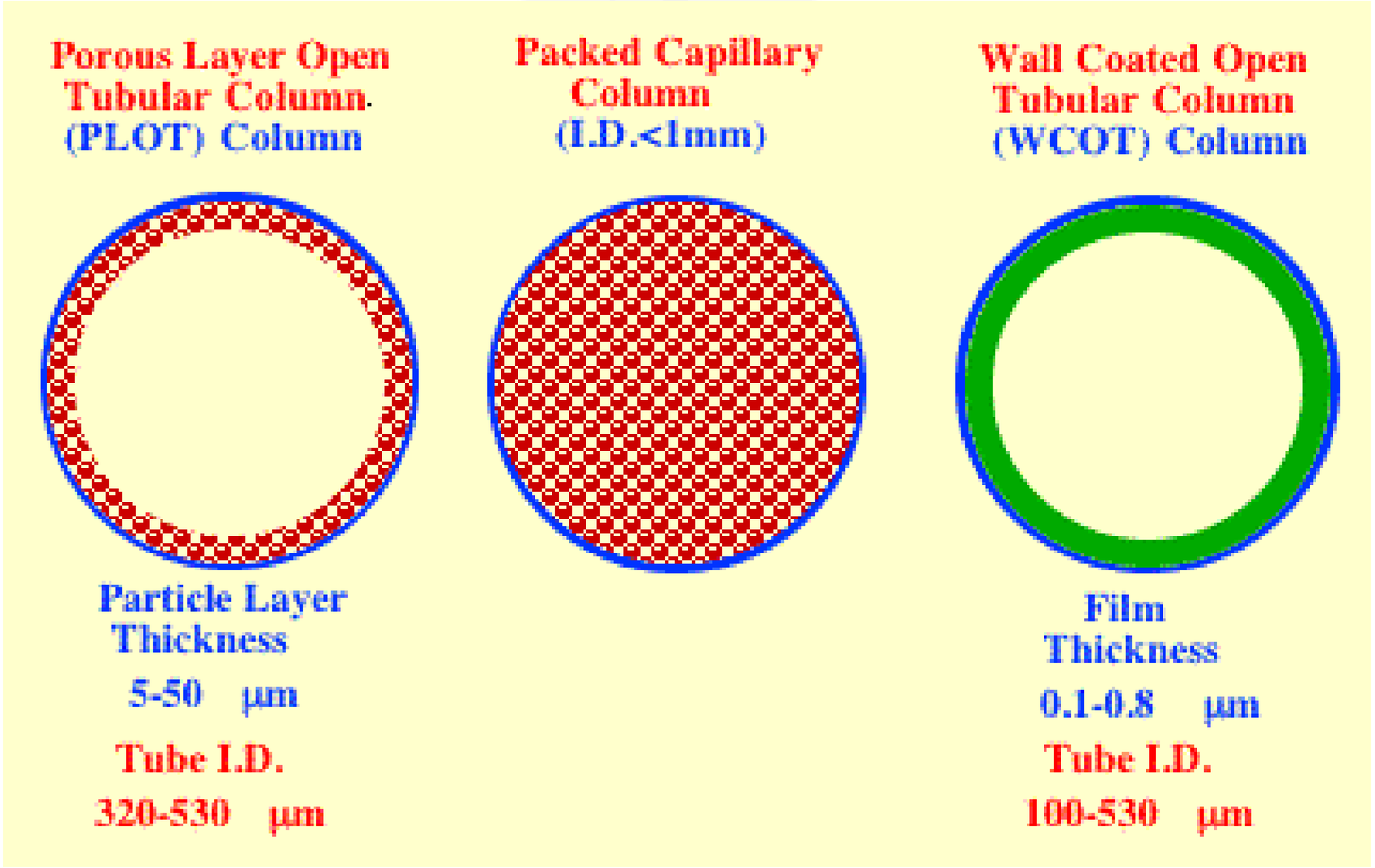
It is mainly of two types:

Wall-coated columns - consist of a capillary tube whose walls are coated with liquid stationary phase.

Support-coated columns- the inner wall of the capillary is lined with a thin layer of support material such as diatomaceous earth, onto which the stationary phase has been adsorbed. It is also known as PLOT (porous-layer open tubular columns).

SCOT columns are generally less efficient than WCOT columns. Both types of capillary column are more efficient than packed columns.

TYPES OF COLUMNS



TYPES OF COLUMNS

Equilibrium of the Column -

- The packed columns are equilibrated before introduction of the sample. This is done by allowing continuous flow of heated carrier gas through the columns for a specific duration of time (24hrs) at prescribed temperature.
- Ideally prepared and conditioned columns show a zero base line on the recorder upon passage of carrier gas alone.

Column Temperature -

- This can be controlled by jackets equipped with vapours of a boiling liquid, electrically heated metal blocks or circulating air baths.
- Compounds of low B.P- eluted at lower temperature
- Compounds of high B.P- boils at higher temperature resulting in broader and shallower peaks, require temperature programming.

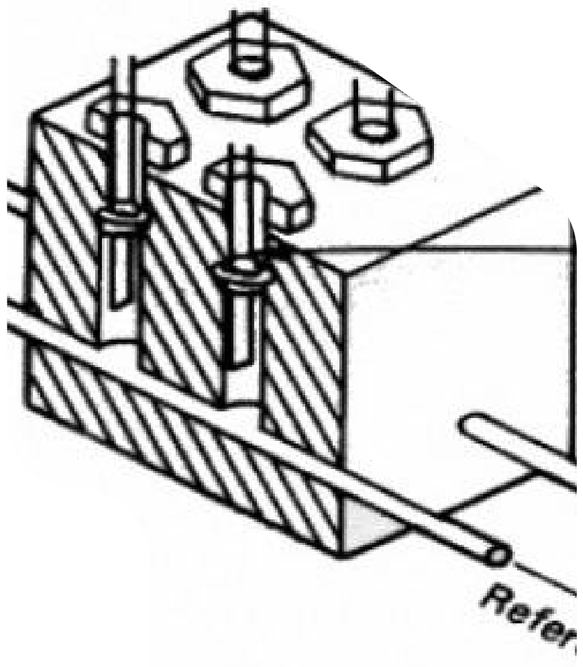
DETECTOR

- The eluted solute particles along with the carrier gas exit from the column and enter the detector.
- The detector then produces electrical signals proportional to the concentration of the components of solute.
- The signals are amplified and recorded as peaks at intervals on the chromatograph.

PROPERTIES OF AN IDEAL DETECTOR

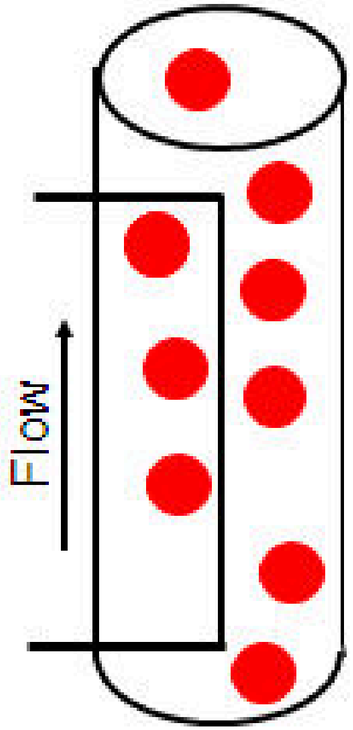
- Sensitive
- Operate at high T (0-400 °C)
- Stable and reproducible
- Linear response
- Wide dynamic range
- Fast response
- Simple (reliable)
- Nondestructive
- Uniform response to all analytes

THERMAL CONDUCTIVITY DETECTOR

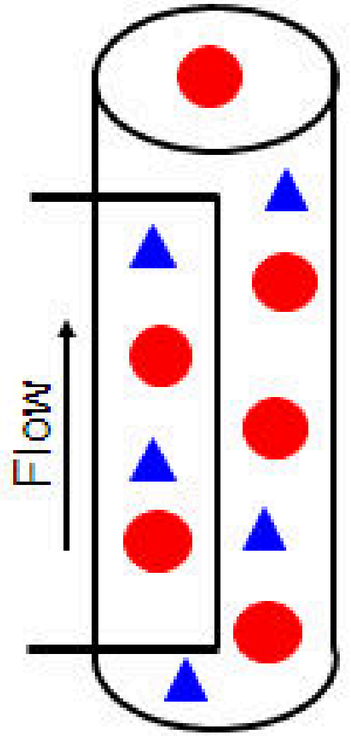


- “TCD is based upon the fact that the heat lost from a filament depends upon the thermal conductivity of the stream of surrounding gas as well as its specific heat.”
- When only carrier gas flows heat loss to metal block is constant, filament T remains constant.
- When an analyte species flows past the filament generally thermal conductivity changes, thus resistance changes which is sensed by Wheatstone bridge arrangement.
- The imbalance between control and sample filament temperature is measured and a signal is recorded.

The TCD is a nondestructive, concentration sensing detector. A heated filament is cooled by the flow of carrier gas.



When the carrier gas is contaminated by sample ▲, the cooling effect of the gas changes. The difference in cooling is used to generate the detector signal.



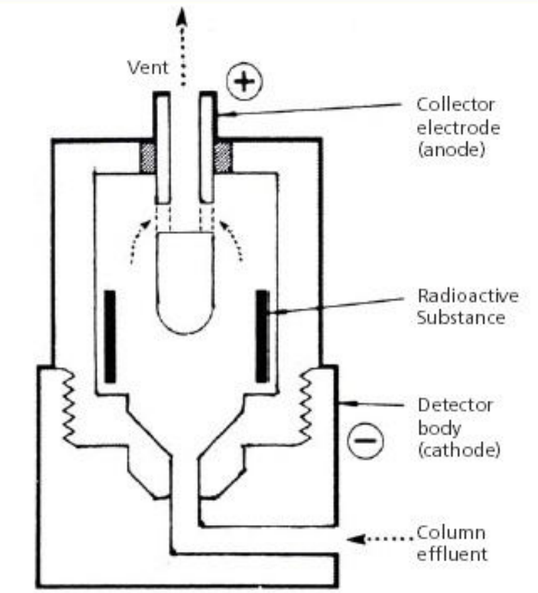
ADVANTAGES

- Simple and inexpensive
- Durable and posses long life
- Accurate results
- Non-selective, hence known as universal detectors

DISADVANTAGES

- Low sensitivity
- Affected by fluctuations in temperature and flow rate.

ELECTRON CAPTURE DETECTOR



Molecules of compounds, which posses affinity for electrons, differ in their electron absorbing capacities. This difference is utilized in this detector for identification of the compounds.

Working- A foil made up of a radioactive metal like Ni^{63} (β-emitter) is placed inside a Teflon coated cell which also contains a cathode and an anode.

- In the absence of organic species, the produced electrons migrate towards positive electrode and produce a certain constant standing current.
- When a sample/eluent is present it captures the electrons, elutes from column, there is a drop in this constant current.
- The potential across two electrodes is adjusted to collect all the ions and a steady saturation current, is therefore, recorded.

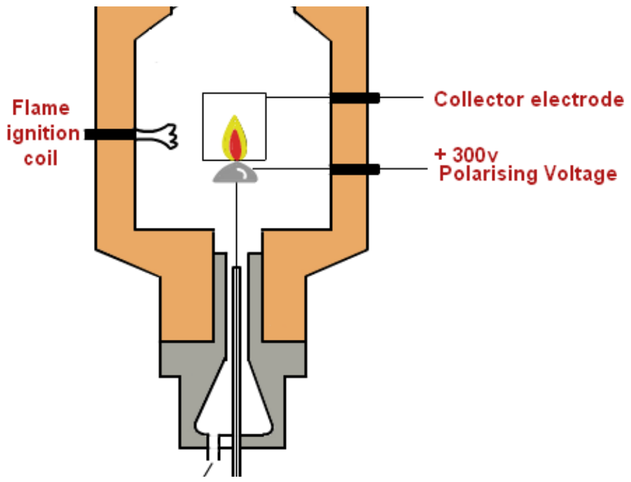
ADVANTAGES

- Highly selective
- Highly sensitive for the detection of compounds like halogens, quinones, peroxides, nitrites, etc.
- It is non-destructive
- More sensitive than TCD and FID.

DISADVANTAGES

- Least sensitive to compounds whose molecules have negligible affinity for electrons.
- Carrier gas used should be of pure form like pure nitrogen.

FLAME IONIZATION DETECTOR



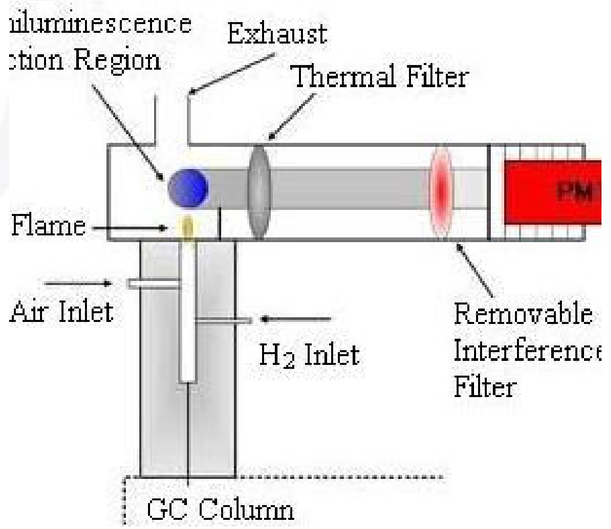
- This employs hydrogen flame that is maintained in a small cylindrical jet made up of platinum or quartz.
- Effluent from the column with helium or nitrogen as carrier gas are fed into the hydrogen flame, gets ignited and undergoes pyrolysis to produce ions.
- For detection of these ions, two electrodes are used that provide a potential difference.
- The ions produced are repelled by the positive electrode which hit the collector plate. The current produced in doing so is amplified and fed to an appropriate recorder.

FLAME PHOTOMETRIC DETECTOR

- It is a selective detector that is responsive to compounds containing sulphur or phosphorous
- The detection principle is the formation of excited sulphur (S_2^*) and excited hydrogen phosphorous oxide species (HPO*) in a reducing flame.
- A photomultiplier tube measures the characteristic chemiluminescent emission from these species. The optical filter can be changed to allow the photomultiplier to view light of 394 nm for sulphur measurement or 526 nm for phosphorus.

Applications:

- For detection of heavy metals like chromium, selenium, tin, etc, in organometallic compounds.
- Also for analysis of pesticides, coal, hydrogenated products as well as air and water pollutants.



WORKING

Fill the syringe with sample.

- Record the setting i.e., column temperature, detector temperature and injection port temperature.
- Introduce sample into the injection port by completely inserting the needle into the rubber septum. Note down the injection time.
- The sample gets vapourized due to higher temperature of injection port and is swept into column by carrier gas.
- This sample components now get distributed between the gas and stationary liquid phase depending upon their solubilizing tendencies.
- The components with minimal solubility move faster and those with maximum solubility travel slowly.
- The components leaving the column activate detector and recorder to give a plot.

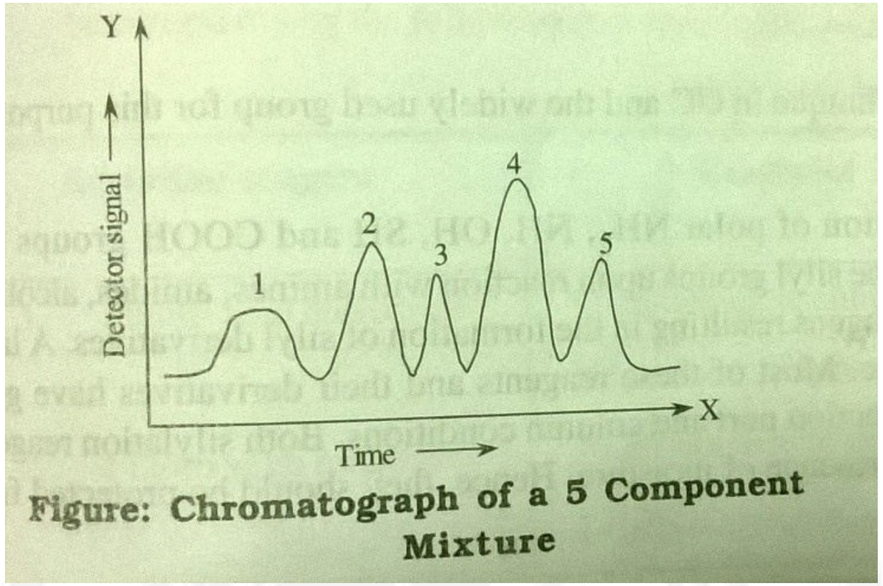


Figure: Chromatograph of a 5 Component Mixture

- The components that slowly leave the column give a bell shaped curve of shorter peak while the one which travels faster gives a bluntly pointed curve of larger peak.
- In above graph, the component that first emerges out of the column is component 4 followed by 2,5,3 and 1.
- The area under the curve is determined in order to obtain the percentage composition of the mixture.

EVALUATION

- HETP (height equivalent to theoretical plate)- It is the distance on the column in which equilibrium is attained between the solute in the gas phase and the solute in liquid phase. Larger the number of theoretical plates/ smaller the HETP, the more efficient the column is for separation.

  HETP = Length of column/n ; Where n = number of theoretical plates
- Retention Time: defined as the absolute time taken by a sample to show maximum peak after injecting.
- Retention Volume: defined as the volume of gas required to elute about half of the solute through the column.

  $VR = tR \times F$

  F =average volumetric flow rate (mL/min) ,estimated by using soap bubble meter (some gases dissolving in soap solution)

APPLICATIONS

- **Qualitative Analysis** – by comparing the retention time or volume of the sample to the standard / by collecting the individual components as they emerge from the chromatograph and identifying these compounds by other methods like UV, IR, NMR.

- **Quantitative Analysis**- area under a single component elution peak is proportional to the quantity of the detected component/response factor of the detectors. It is done by:

i. **Direct comparison method-**

$$A(\text{sample}) / A(\text{std}) = \alpha\, C(\text{sample})/C(\text{std})$$

Where, α is the response factor determined for every pure compound under given conditions.

APPLICATIONS

- **Calibration curve-** a graph is plotted by taking peak areas on Y axis and concentration of standard compound on X axis. Concentration of unknown sample is then determined by plotting its peak area on same graph.

II. **Internal standard method-** A known concentration of internal standard, which has similar retention characteristics as that of sample is added to both reference standard and test sample.

PHARMACEUTICAL APPLICATIONS

Quality control and analysis of drug products like antibiotics (penicillin), antivirals (amantidine), general anesthetics (chloroform, ether), sedatives/hypnotics (barbiturates), etc.

Assay of drugs – purity of a compound can be determined for drugs like :

- Atropine sulphate
- Clove oil
- Stearic acid
- In determining the levels of metabolites in body fluids like plasma, serum, urine, etc.

MISCELLANEOUS

- Analysis of foods like carbohydrates, proteins, lipids, vitamins, steroids, drug and pesticides residues, trace elements.
- Pollutants like formaldehyde, carbon monoxide, benzene, DDT etc.
- Dairy product analysis like milk, butter –for detection of aldehydes, milk sugars, ketones and fatty acids.
- Separation and identification of volatile materials, plastics, natural and synthetic polymers, paints, and microbiological samples.

UNIT - 2A-2 | PAPER - 1

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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INTRODUCTION

- HPTLC is a sophisticated & automated form of TLC
- Efficient separation in short time

PRINCIPLE

Adsorption

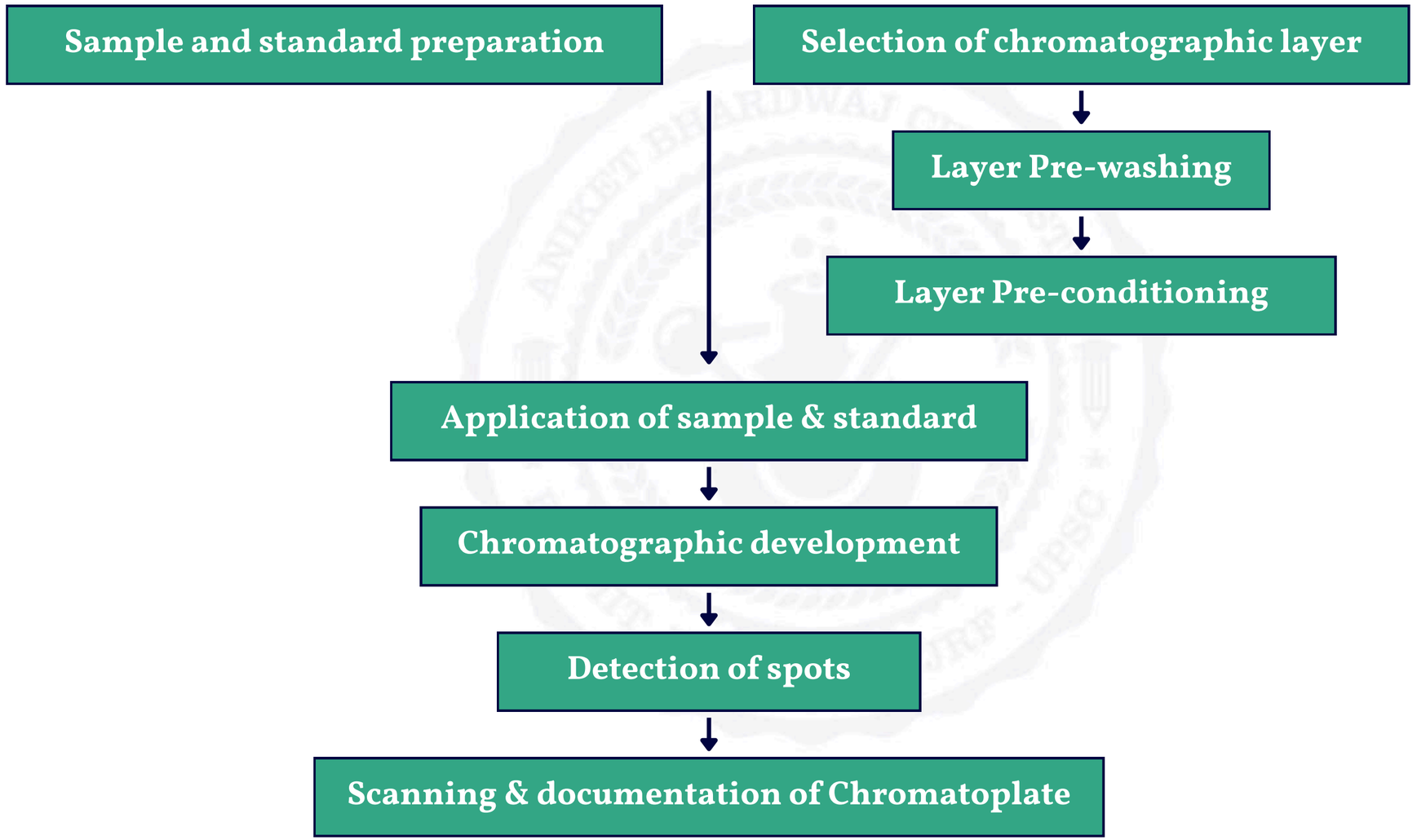
ADVANTAGES OF HPTLC OVER OTHER CHROMATOGRAPHIC METHODS

1. In HPTLC, simultaneous processing of sample and standard – better analytical accuracy & precision
2. Lower analysis time & less cost per analysis
3. HPTLC is very simple
4. In HPTLC, the sample preparation is simple
5. Solvent used in HPTLC needs no prior treatment like filtration & degassing
6. In HPTLC, the M.P consumption for sample is extremely low
7. HPTLC allows the use of corrosive & UV absorbing M.P

STEPS INVOLVED IN HPTLC

1. Sample preparation
2. Selection of chromatographic layer
3. Plates
4. Pre-washing
5. Conditioning
6. Sample application
7. Pre-conditioning
8. M.P
9. Chromatographic development
10. Detection of spots
11. Scanning & documentation

STEPS INVOLVED IN HPTLC



SAMPLE PREPARATION

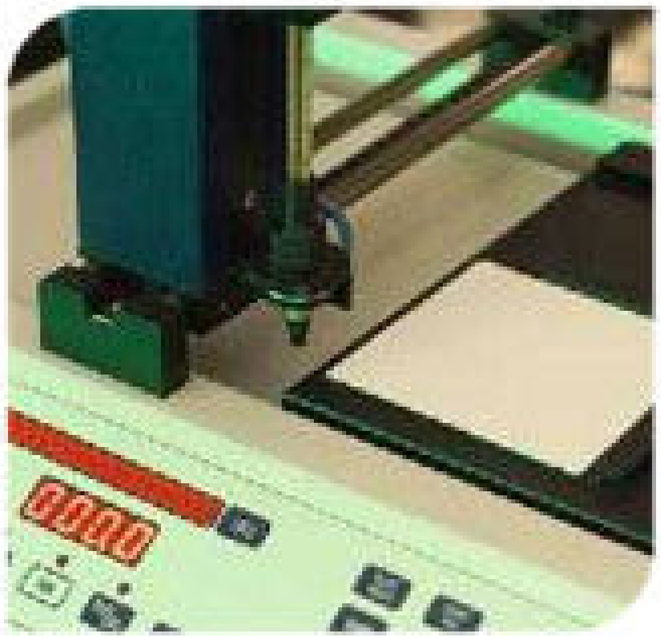
1. For normal phase chromatography using silica gel / alumina pre-coated plates, solvents – non polar
2. RP chromatography , usually polar solvents

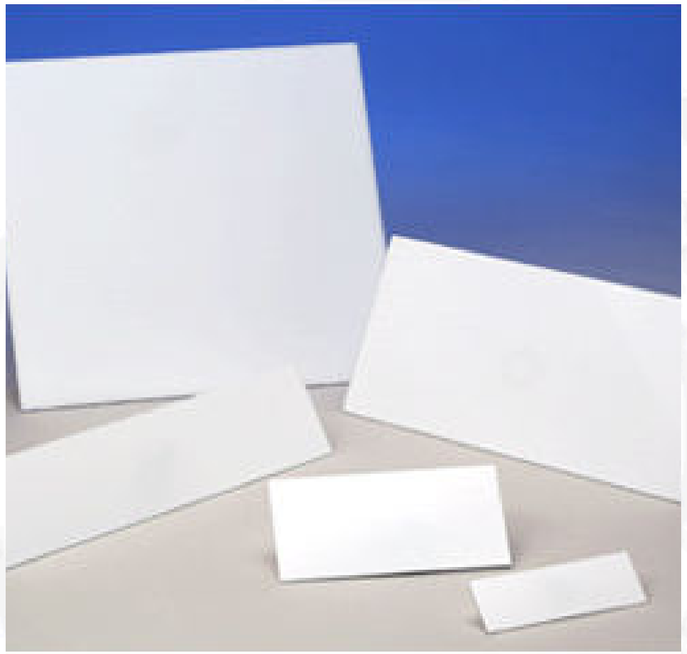
SELECTION OF CHROMATOGRAPHIC LAYER

1. Depends on the nature of material to be separated
2. Commonly used materials are Silica gel 60F, Alumina, Cellulose etc

PLATES

Generally, plates of 20 x 20cm or 5 x 7.5cm size having 100-250mm adsorbent thickness. Silica gel $60F^{254}$ pore size 6mm with fluorescent indicator is a coat material Basic difference in TLC & HPTLC plates is particle size of coated materials which is 5-20µm – TLC, 4-8 µm - HPTLC





PRE-WASHING

» to remove water vapors

» volatile impurities

Which might get trapped in the plates

To avoid this, plates are cleaned by using methanol as solvent by ascending or descending etc.

CONDITIONING

Plates activated by placing them in an oven at 120°C for 15 to 20 minutes.

Sample Application

- Application of 1.0 - 5µl for HPTLC
- Application carried out by Linomat applicator on the plates which give uniform, safe & std. results

PRE-CONDITIONING (CHAMBER SATURATION)

- For effective separation of sample
- For RP chromatography – saturate the chamber with methanol or polar solvent

MOBILE PHASE

Selection of appropriate M.P is based on the trial and error.

CHROMATOGRAPHIC DEVELOPMENT

Ascending, descending, horizontal, continuous, gradient, multidimensional…

HPTLC – migration distance of 5-6mm is sufficient, after development, plates removed & dried.

Common problems encountered during chro. Development are as follows…

1. **Tailing:** due to the presence of traces of impurities, this can be reduced by buffering the M.P

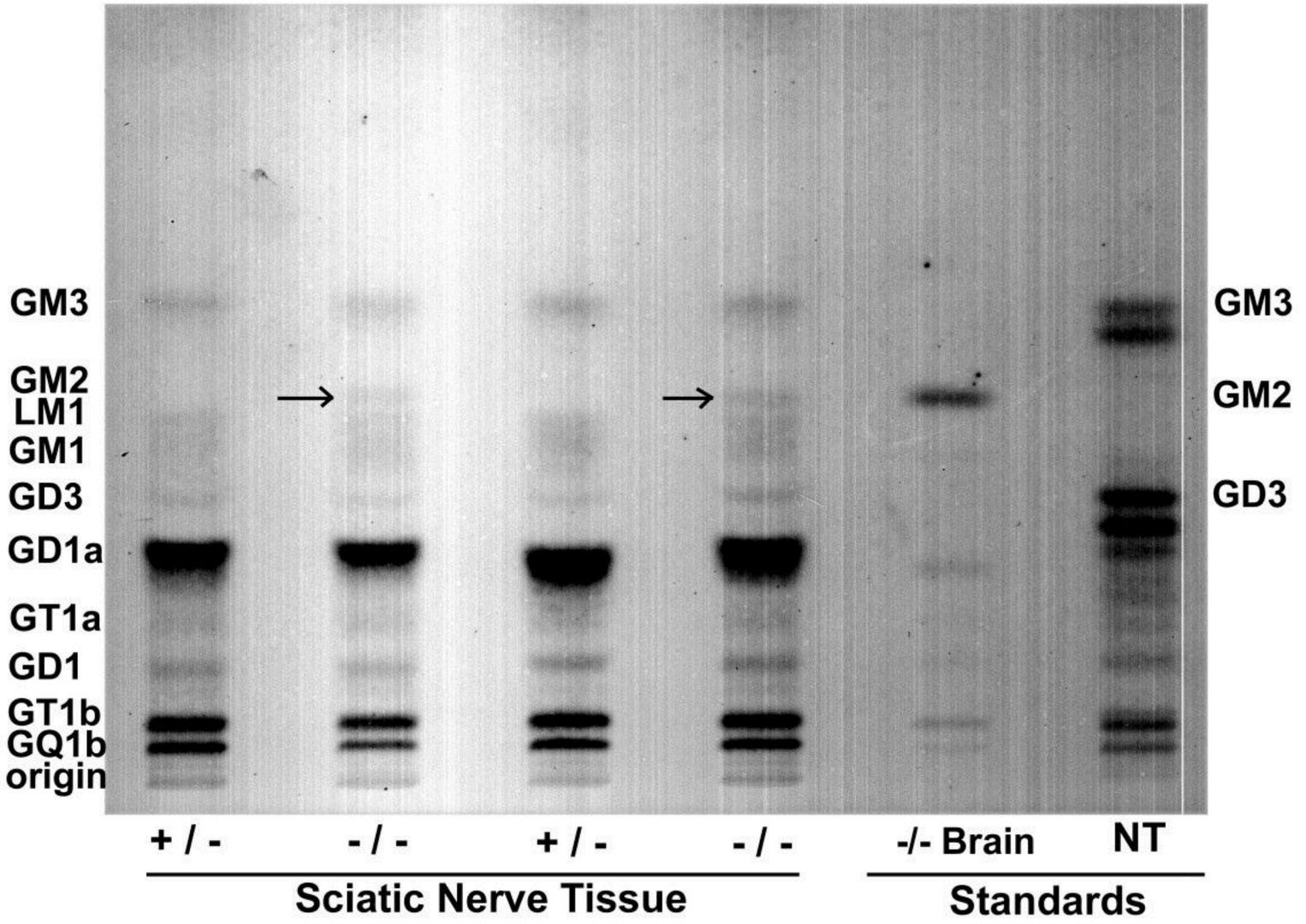
2. **Diffusion**: This is seen as zones on chromatographic plates. This may arise due to non-uniformity of M.P

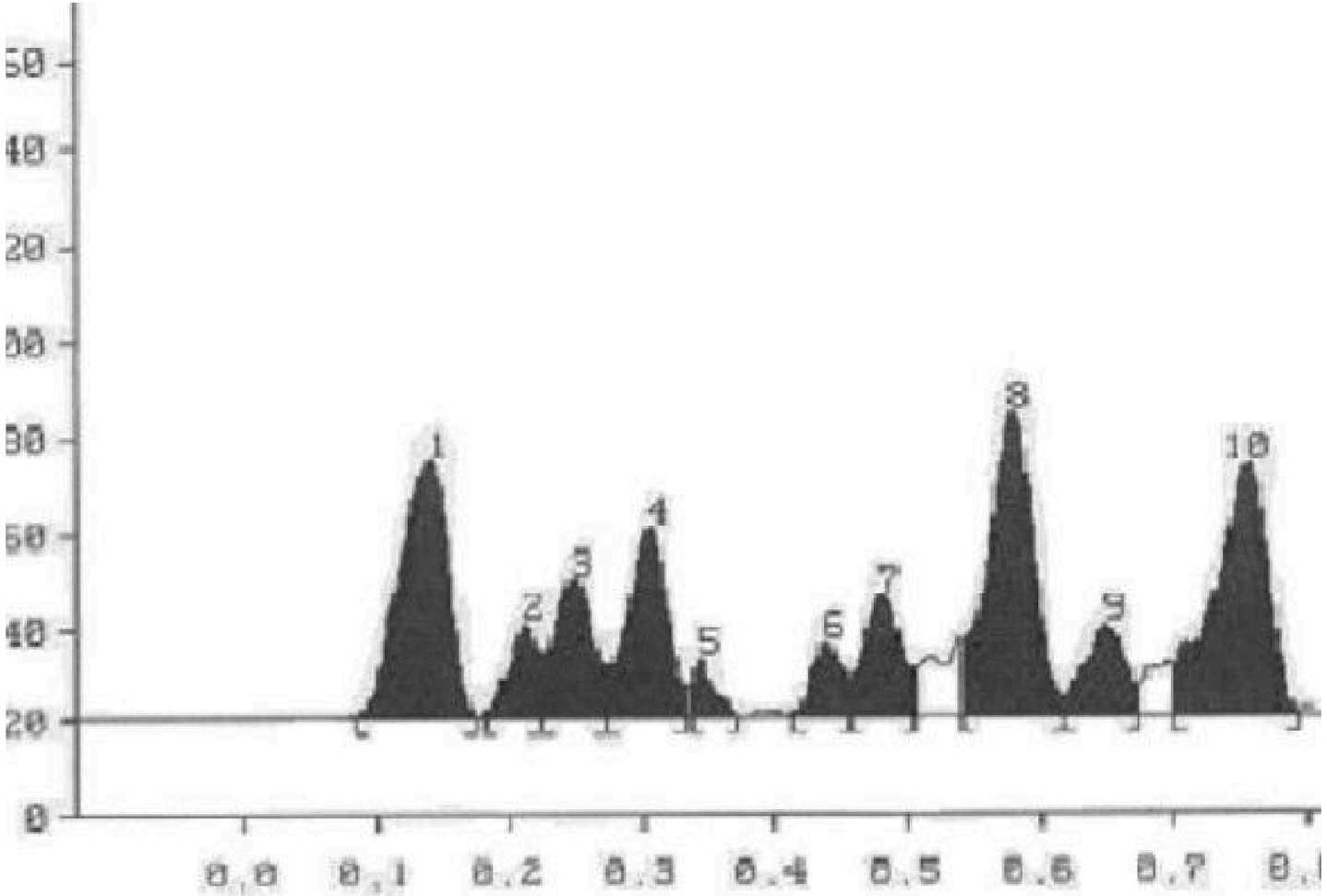
DETECTION OF SPOTS

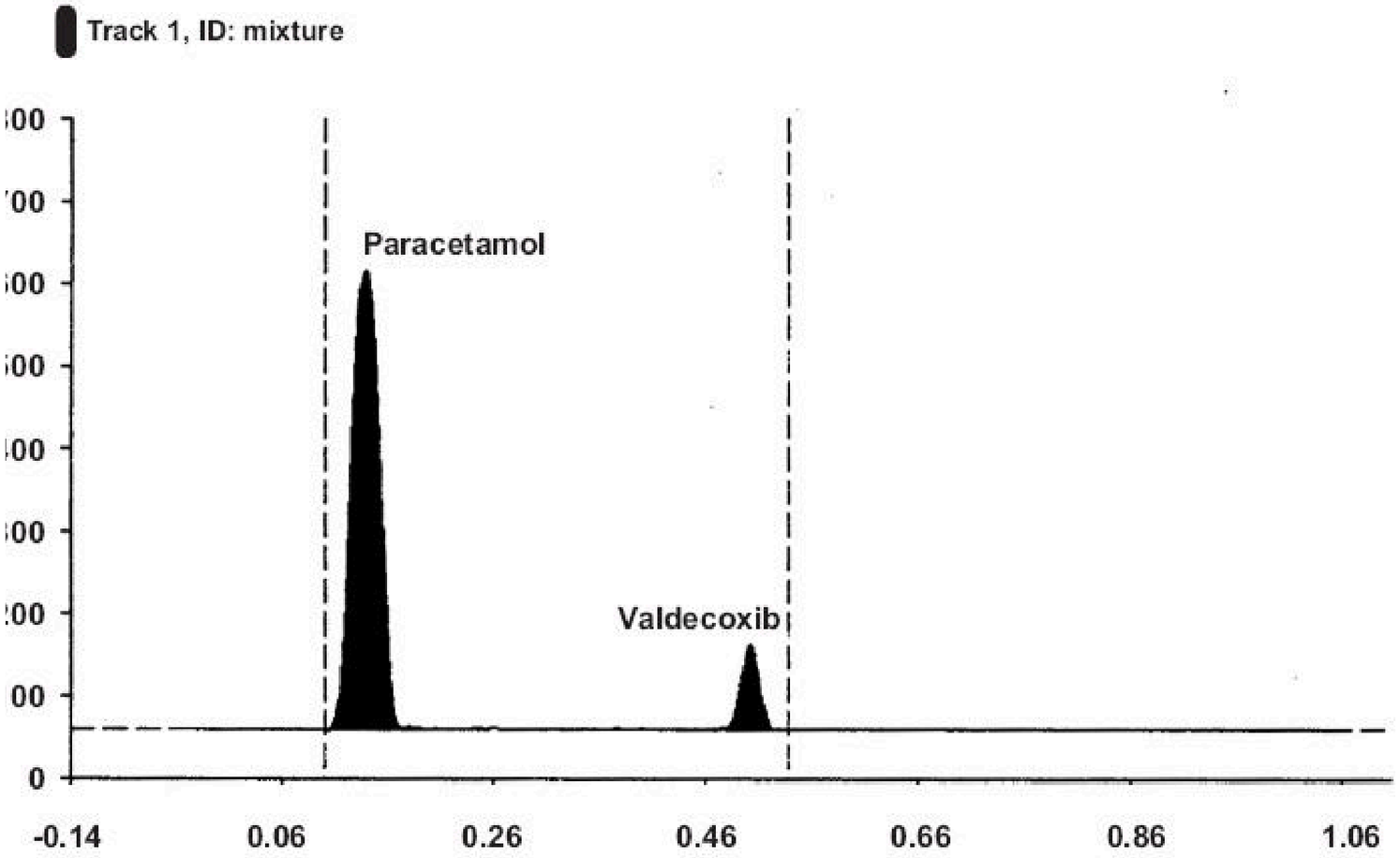
Detection can be done by iodine vapor in iodine chamber. Visual inspection at 254nm of UV region in UV cabinet

SCANNING & DOCUMENTATION

- HPTLC plates are scanned at selected UV regions WL by the instrument & the detected spots are seen on computer in the form of peaks.
- The scanner converts band into peaks & peak height or area is related to the concentration of the substance on the spot.







APPLICATIONS

- HPTLC rapidly gaining importance in several fields of science like
- Pharmaceuticals analysis
- Biochemistry
- Pharmacokinetics studies

UNIT - 2A-3 | PAPER - 1

THIN LAYER CHROMATOGRAPHY

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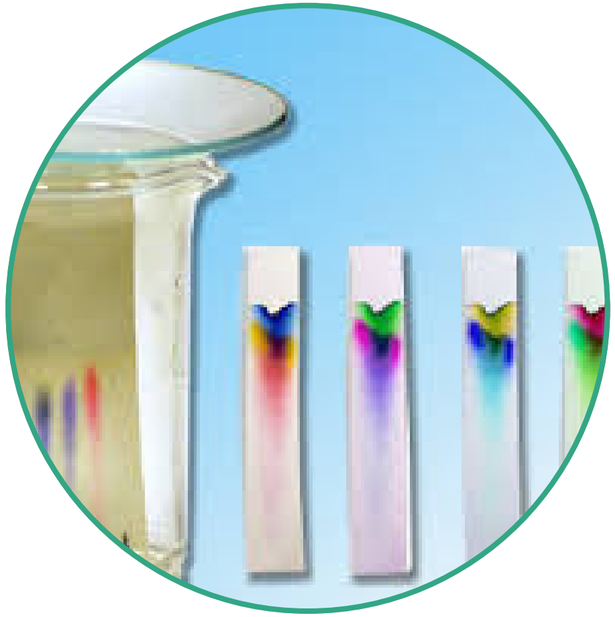
CHROMATOGRAPHY

Chromatography is essentially a group of technique for the separation of the compounds of mixtures by their continuous distribution between two phases, one of which is moving past the other.

- A solid stationary phase and a liquid mobile phase (adsorption chromatography)
- A liquid stationary phase and a liquid mobile phase (partition chromatography)



INTRODUCTION

- TLC is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorbent and a mobile phase in the form of liquid.
- Separation may also be achieved on the basis of partition, or a combination of partition and adsorption.

PRINCIPLE

- The principle of separation of TLC is adsorption.
- The compound having more affinity towards stationary phase travels slower, while the component having lesser affinity toward stationary phase travels faster.

ADSORBENT FOR TLC

In all chromatographic procedure, the separation takes place through neutral harmonisation of the stationary and mobile phase.

ADSORBENTS - INORGANIC ADSORBENT - ORGANIC ADSORBENT

The grain size of most TLC adsorbent lies between 5 and 50 micrometre.

A. Silica gel : It is amorphous and porous. Particle size for TLC is 10-40 micrometre.

B. Aluminas and other inorganic adsorbents :

   a. Alumina
   b. Kieselguhr
   c. Silicates (calcium and magnisium)
   d. Phosphates
   e. Bentonite

C. Organic : Cellulose derivative, starch, sucrose, mannitol etc.

MOBILE PHASE FOR TLC

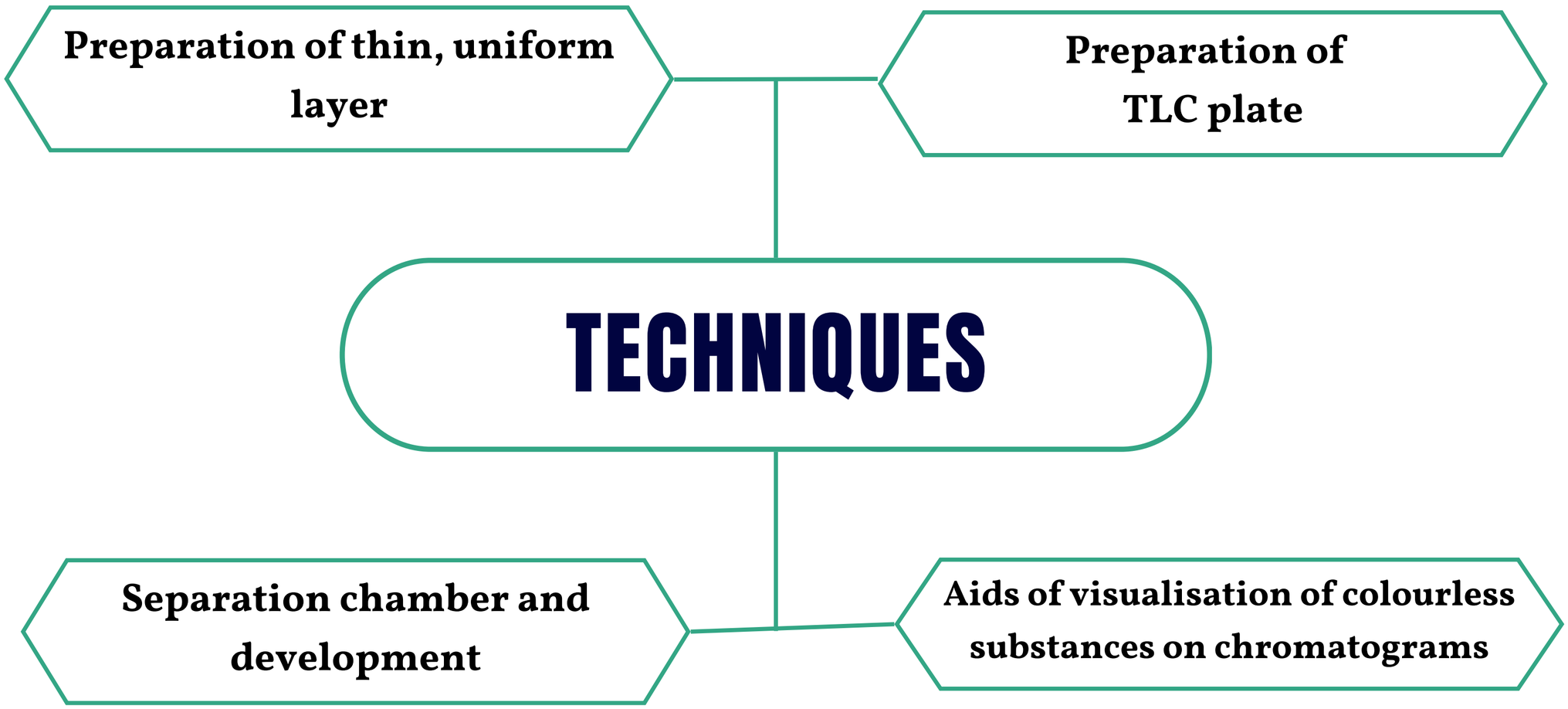
Depends upon various factors-

- Nature of the substance to be separated.
- Nature of stationary phase used.
- Mode of chromatography (Normal or Reverse phase)

FOLLOWING SOLVENTS USED IN INCREASING ORDER OR POLARITY

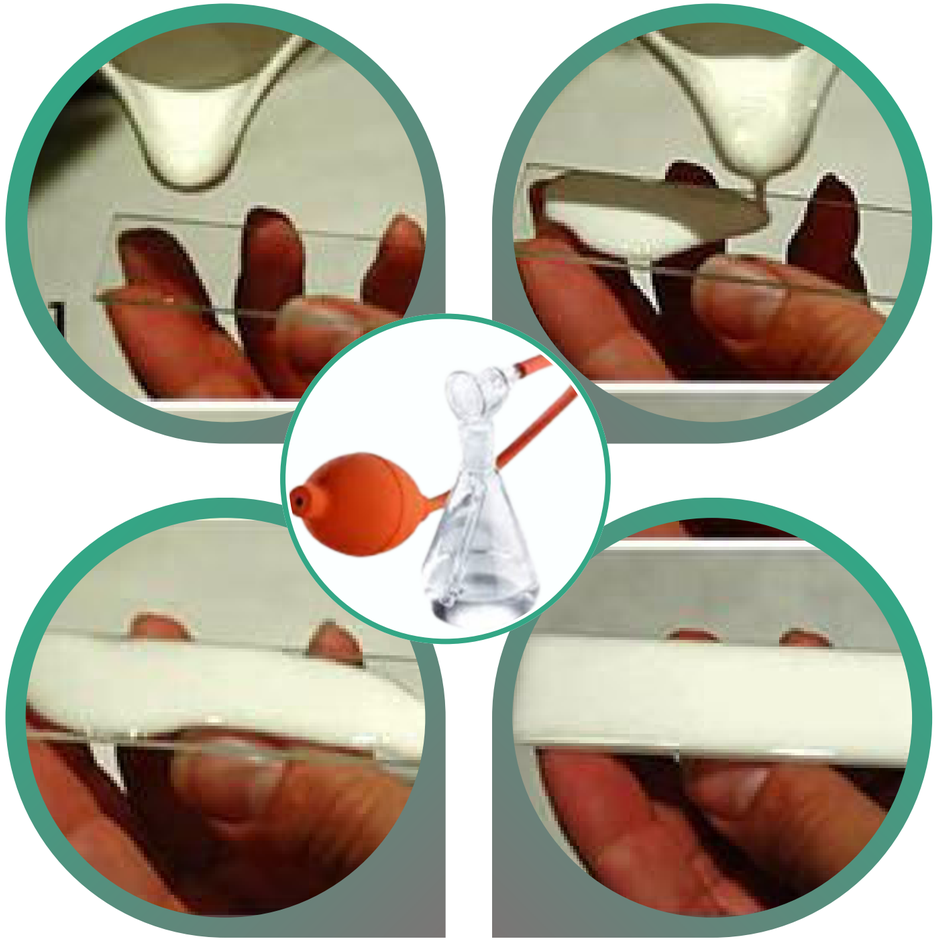
- n-hexane
- Cyclohexane
- Carbon tetrachloride
- Benzene
- Toluene
- Trichloroethylene
- Diethyl ether
- Chloroform
- Ethyl acetate

GENERAL TECHNIQUES IN TLC



PREPARATION OF THIN, UNIFORM LAYER

- Pouring Procedure
- Dipping Procedure
- Spraying Procedure
- Spreading Procedure

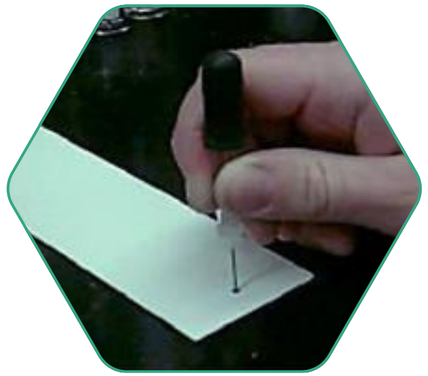


PREPARATION OF TLC PLATE

- Drying , storage and handling – First air dry then oven dry.
- Control and marking of the layer – Homogenous in transmitted light, no unevenness and no large grains.
- Application of the substance mixture for separation – no use of involatile solvents, concentration (0.1-1%)

  Spot application by – micro bulb pipette, graduated micro pipette, micro syringe.





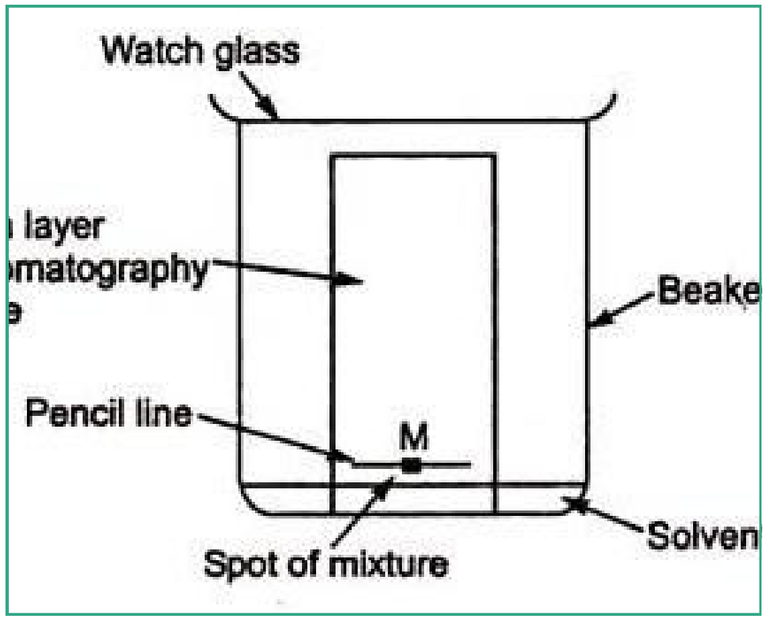

SEPARATION CHAMBER AND DEVELOPMENT

Types of chambers used are classified according to the nature of the development.

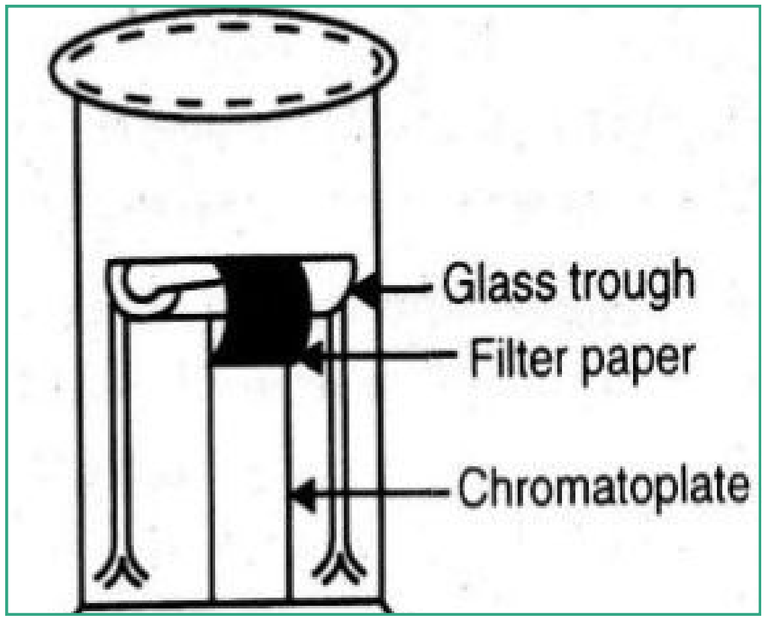
- Ascending development
- Descending development
- Horizontal development
- Thin-Layer electrophoresis

SEPARATION CHAMBERS

- Ascending procedure

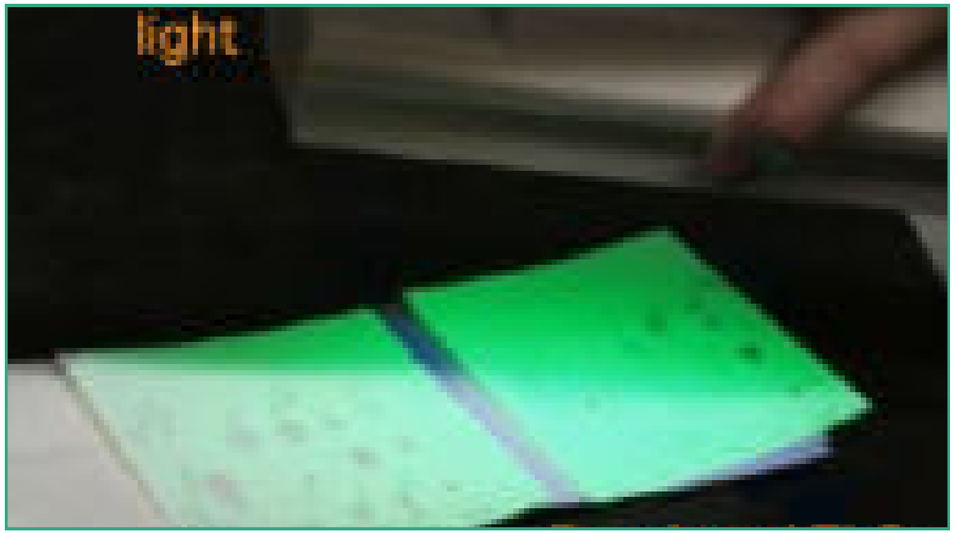


- Descending procedure



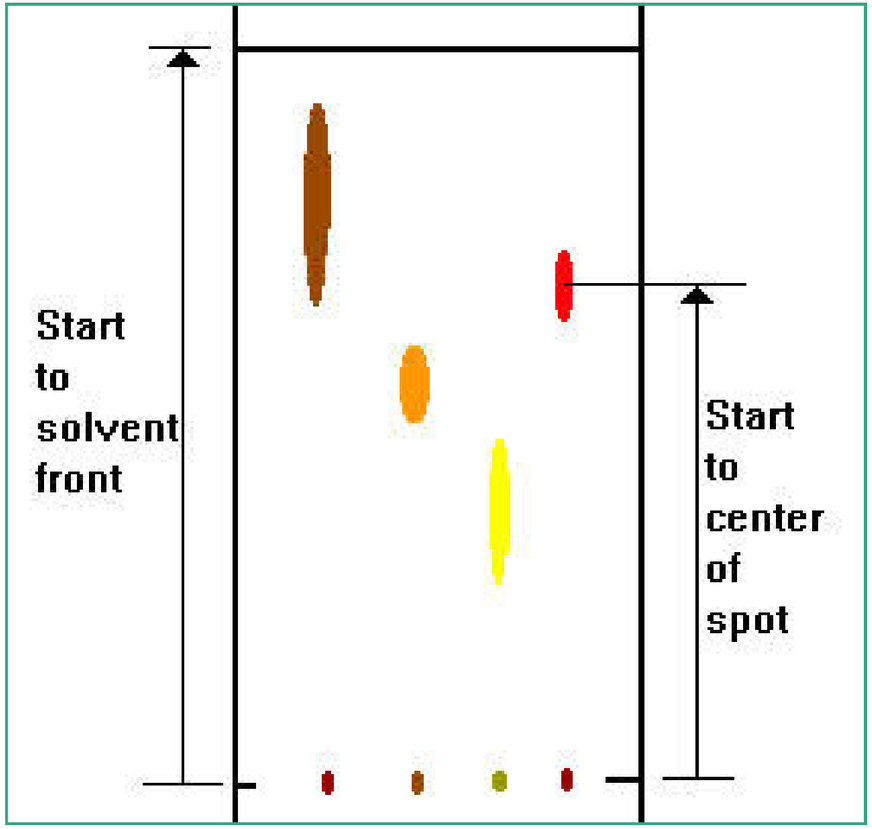
AIDS FOR VISUALISATION OF COLOURLESS SUBSTANCE ON CHROMATOGRAM

- UV lamp – for identifying fluorescent compound, the chromatograms are viewed in short and long wave uv light eg. Silica gel GF254.
- Spraying suitable reagents.



ANALYSIS OF SPOT

- Rf value will always be in the range 0 to 1.



STANDARD CONDITIONS IN TLC [AS PER I.P.]

- Dimension of the layer – 200×200 mm for analytical purpose, 100×200 mm for preliminary purpose, 400×200 mm for serial analysis.
- Layer thickness – 2 mm for preparative TLC, 150 µm for analytical separation.
- Drying of layer – Allow the coated plate to air dry, heat at 100-105 ̊C for at least 1 hour.
- Storage – plate must protect from laboratory fumes, store in dry condition.
- Starting point – 15 mm from lower edge, 15 mm between neighbouring points.
- Length of run – normally 100 mm i.e. from starting point to front.
- Chamber – with plane, ground glass cover with chamber saturation.
- Depth of immersion of layer – plate and the layer should dip only about 5 mm into the solvent.

ADVANTAGES OF TLC

- It is simple and fast procedure for all kinds of solid-liquid and liquid-liquid sample.
- TLC can be performed on analytical and preparative scale.
- It may be applied to almost the entire spectrum and chemical compounds.
- It can be employed for checking the chemical reaction in laboratory and synthesis in industrial scale.
- TLC can be used for identifying the adulteration of food as well as decomposition of food.
- Compound in trace amount can be detect by TLC.
- Requires less amount of substances.
- Less time consuming and take 15-45 minutes for developments.
- Even strong acids can be sprayed on plates.
- For many separation TLC plate is heated in oven for several hours.
- In TLC even corrosive reagent may be coated in plates.

DISADVANTAGES OF TLC

- The separation takes place in an open system or in open condition and hence there are chances that sample may be affected by the humidity and temperature.
- This method is used for the separation of non volatile compounds.
- More amount of mobile phase is required.
- Edge effect occur due to improper saturation of chamber.

APPLICATION

- Purity of any sample.
- Identification of compounds.
- Examination of reactions.
- Biochemical analysis.
- In chemistry.
- In pharmaceutical industry.
- In food and cosmetic industry.

UNIT - 2A-4 | PAPER - 1

COLUMN CHROMATOGRAPHY

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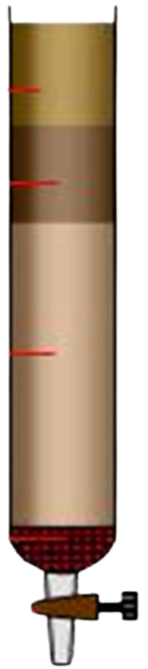
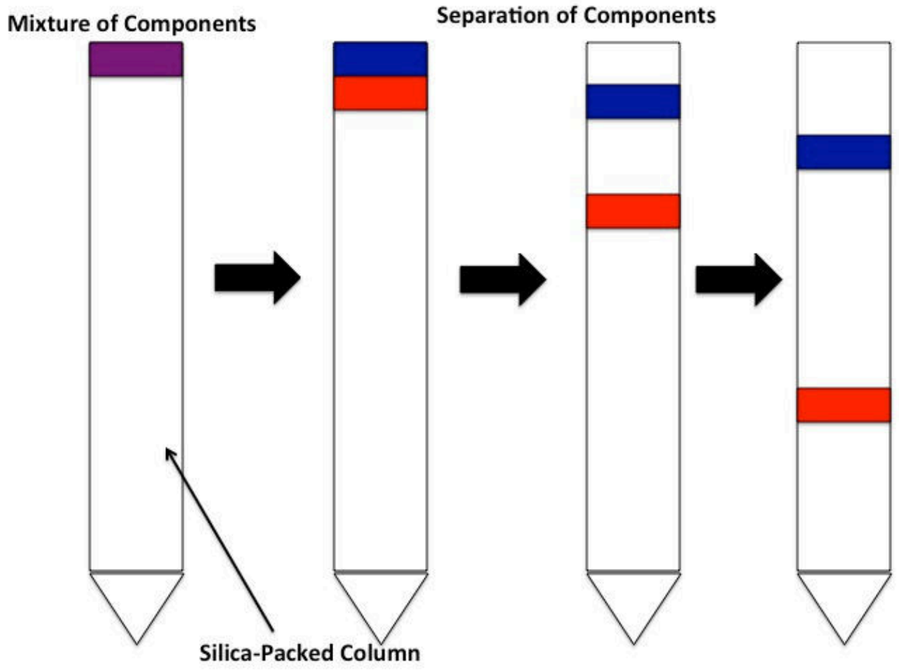
COLUMN CHROMATOGRAPHY

- Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase.
- There are a number of different kinds of chromatography, which differ in the mobile and the stationary phase used.



COLUMN CHROMATOGRAPHY

Column Chromatography was developed by the American chemist D.T Day in 1900, M.S. Tswett,the Polish botanist, in 1906 used adsorption columns in his investigations of plant pigments.



COLUMN CHROMATOGRAPHY

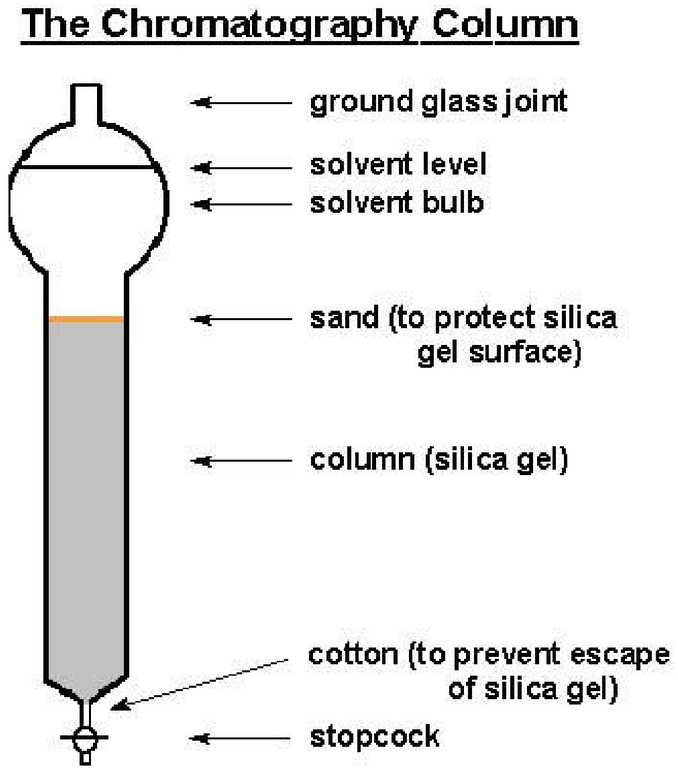
- Column chromatography is one of the most useful methods for the separation and purification of both solids and liquids.
- This is a solid - liquid technique in which the stationary phase is a solid & mobile phase is a liquid.

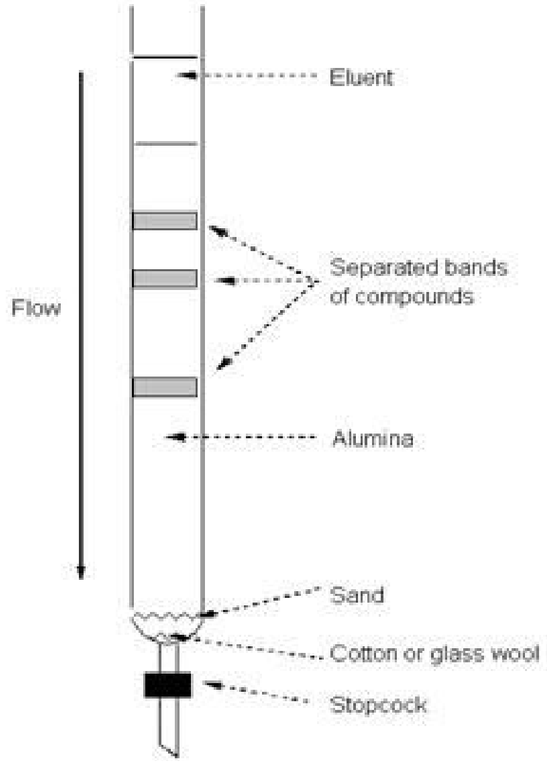
PRINCIPLE

- Adsorption
- Mixture of components dissolved in the M.P is introduced in to the column. Components moves depending upon their relative affinities.

COLUMN CHROMATOGRAPHY

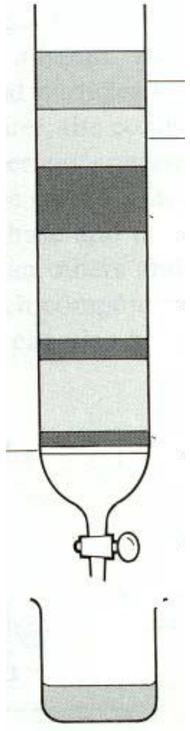
Adsorption column chromatography, the adsorbent, packed in a glass column, and a solvent, the mobile phase, that moves slowly through the packed column. A solvent used as a mobile phase is called an eluent.





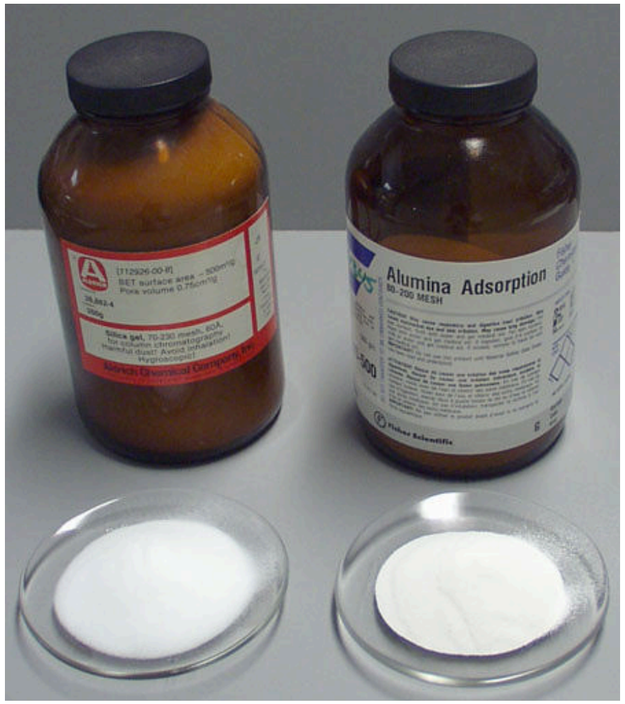
COLUMN CHROMATOGRAPHY

- A compound attracted more strongly by the mobile phase will move rapidly through the column, and elute from, or come off, the column dissolved in the eluent.
- In contrast, a compound more strongly attracted to the stationary phase will move slowly through the column.



EXPERIMENTAL ASPECTS OF COLUMN CHROMATOGRAPHY

- Adsorbents: The usual adsorbents employed in column chromatography are silica, alumina, calcium carbonate, calcium phosphate, magnesia, starch, etc.,
- Alumina is generally suitable for chromatography of less polar compounds. Silica gel gives good results with compounds containing polar functional groups.



ADSORBENT IN C.C SHOULD MEET FOLLOWING CRITERIA

- Particles should be spherical in shape & uniform in size
- Mechanical stability must be high
- They shouldn't react chemically
- It should be useful for separating for wide variety of compounds
- It should be freely available & inexpensive

(The particle size of the commercially available grade is in the range 50 – 200 µm.)

SELECTION OF STATIONARY PHASE

Success of chromatography depends upon proper selection of S.P, it depends on the following:

- Removal of impurities
- No. of components to be separated
- Length of the column used
- Affinity differences b/w components
- Quantity of adsorbent used

MOBILE PHASE

They act as solvent, developer & eluent. The function of a mobile phase are:

- As developing agent
- To introduce the mixture into the column – as solvent
- To developing agent
- To remove pure components out of the column – as eluent
- The choice of the solvent is depend on the solubility characteristics of the mixture. The solvents should also have sufficiently low boiling points to permit ready recovery of eluted material.
- However, polarity as seen the most important factor in adsorption chromatography.

Different mobile phases used: (in increasing order of polarity)

- Petroleum ether, carbon tetrachloride, cyclohexane, ether, acetone, benzene, toluene, esters, water, etc
- It can b e used in either pure form or as mixture of solvents

THE LENGTH OF THE COLUMN DEPENDS UPON:

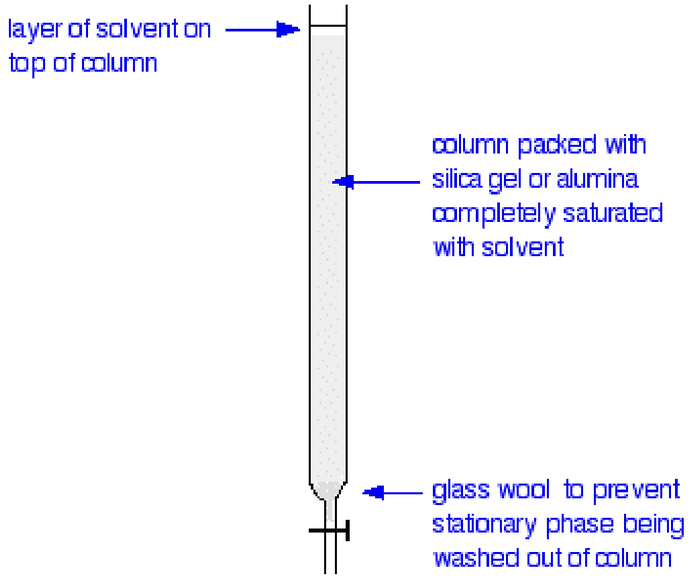
- Number of compounds to be separated
- Type of adsorbent used
- Quantity of the sample
- Affinity of compounds towards the adsorbent used
- Better separation will be obtained with a long narrow column than short thick column because number of plates will be more.

PREPARATION OF THE COLUMN

- It consists of a glass tube with bottom portion of the column – packed with glass wool/cotton wool or may contain asbestos pad,

» Above which adsorbent is packed

» After packing a paper disc kept on the top, so that the adsorbent layer is not disturbed during the introduction of sample or mobile phase.



PACKING TECHNIQUES IN C.C

- There are two types of preparing the column, they are:

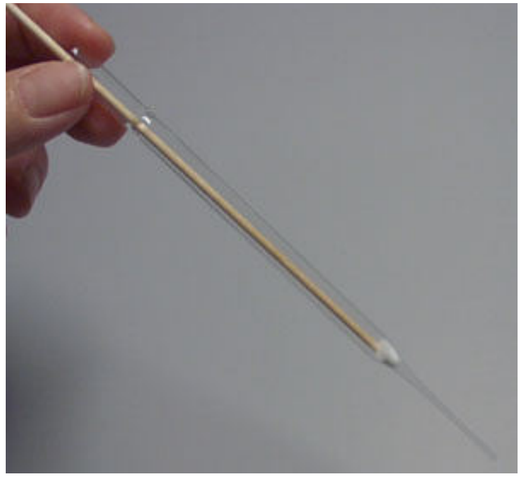
  i. Dry packing / dry filling

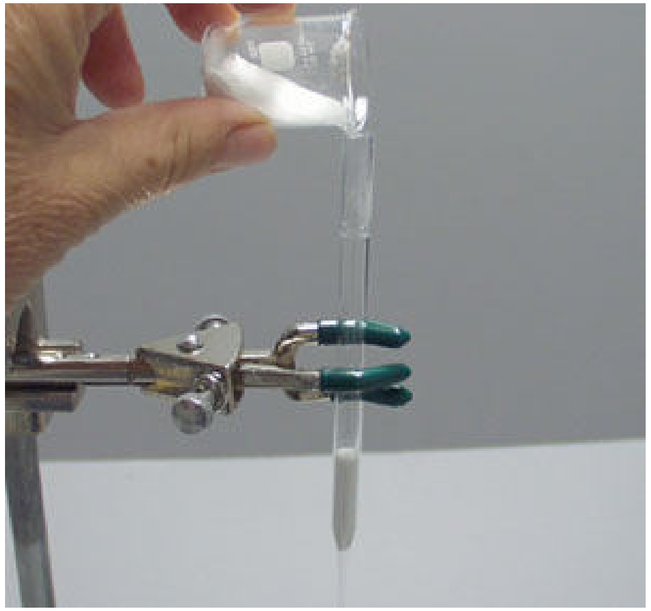
  ii. Wet packing / wet filling

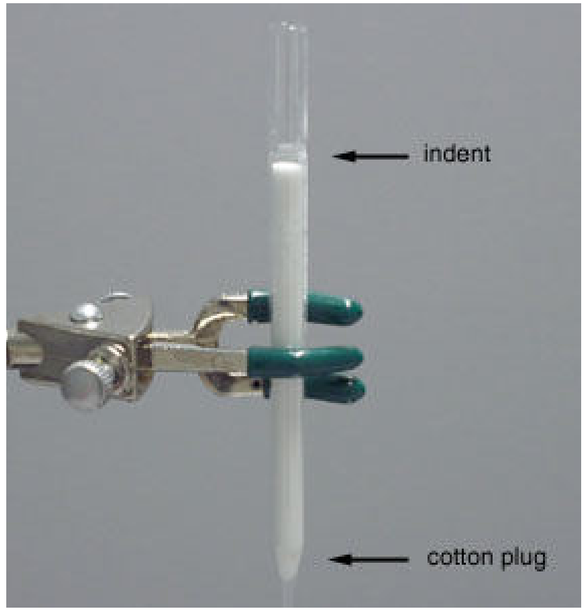
- The column should be free from impurity, before using column, it should be washed properly and dry it.
- Before filling column with stationary phase, cotton/glass wool is kept
- It should be uniformly filled

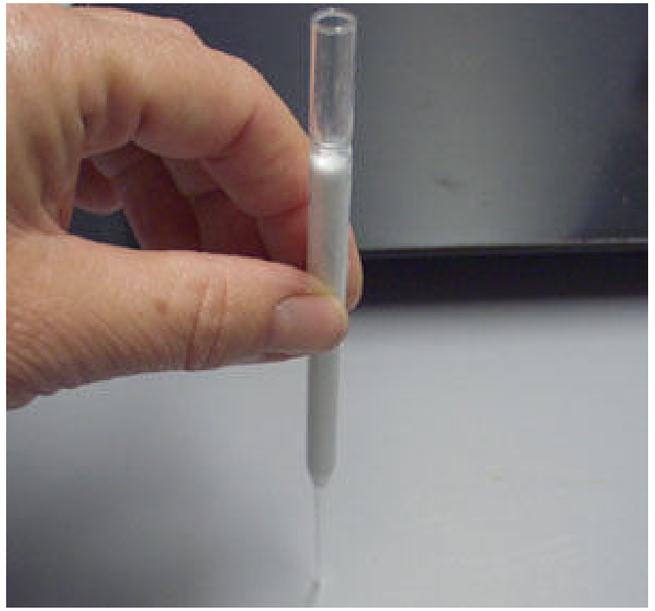
DRY PACKING TECHNIQUE

- Adsorbent is packed in the column in dry form
- Fill the solvent, till equilibrium is reached
- DEMERIT: Air bubbles are entrapped b/w M.P & S.P→ cracks appear in the adsorbent layer.
- After filling tapping can be done to remove void spaces.









WET PACKING TECHNIQUE

◊ Ideal & common technique

» The material is slurred with solvent and generally added to the column in portions.

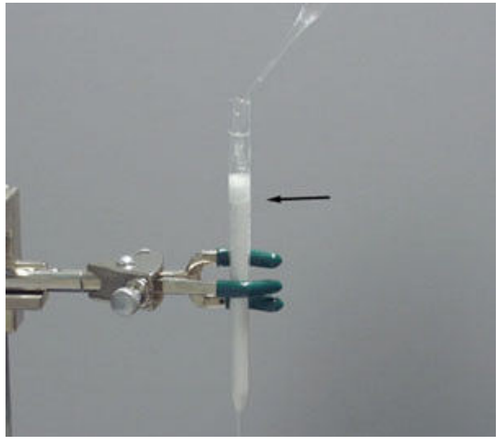
◊ S.P settles uniformly & no crack in the column of adsorbent.

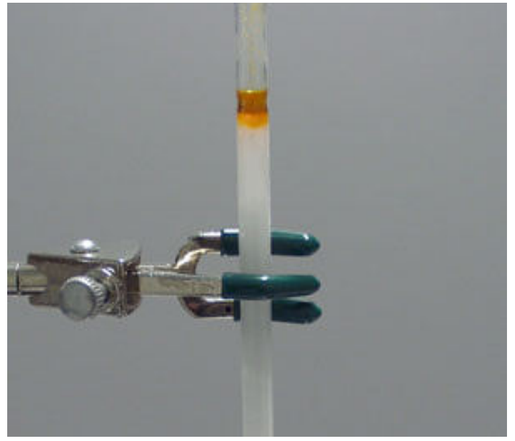
» solid settle down while the solvent remain upward.

» this solvent is removed then again cotton plug is placed.

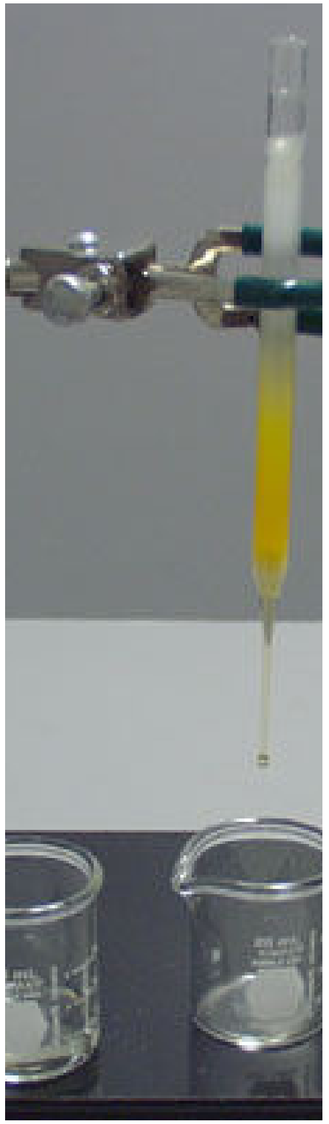
INTRODUCTION OF THE SAMPLE

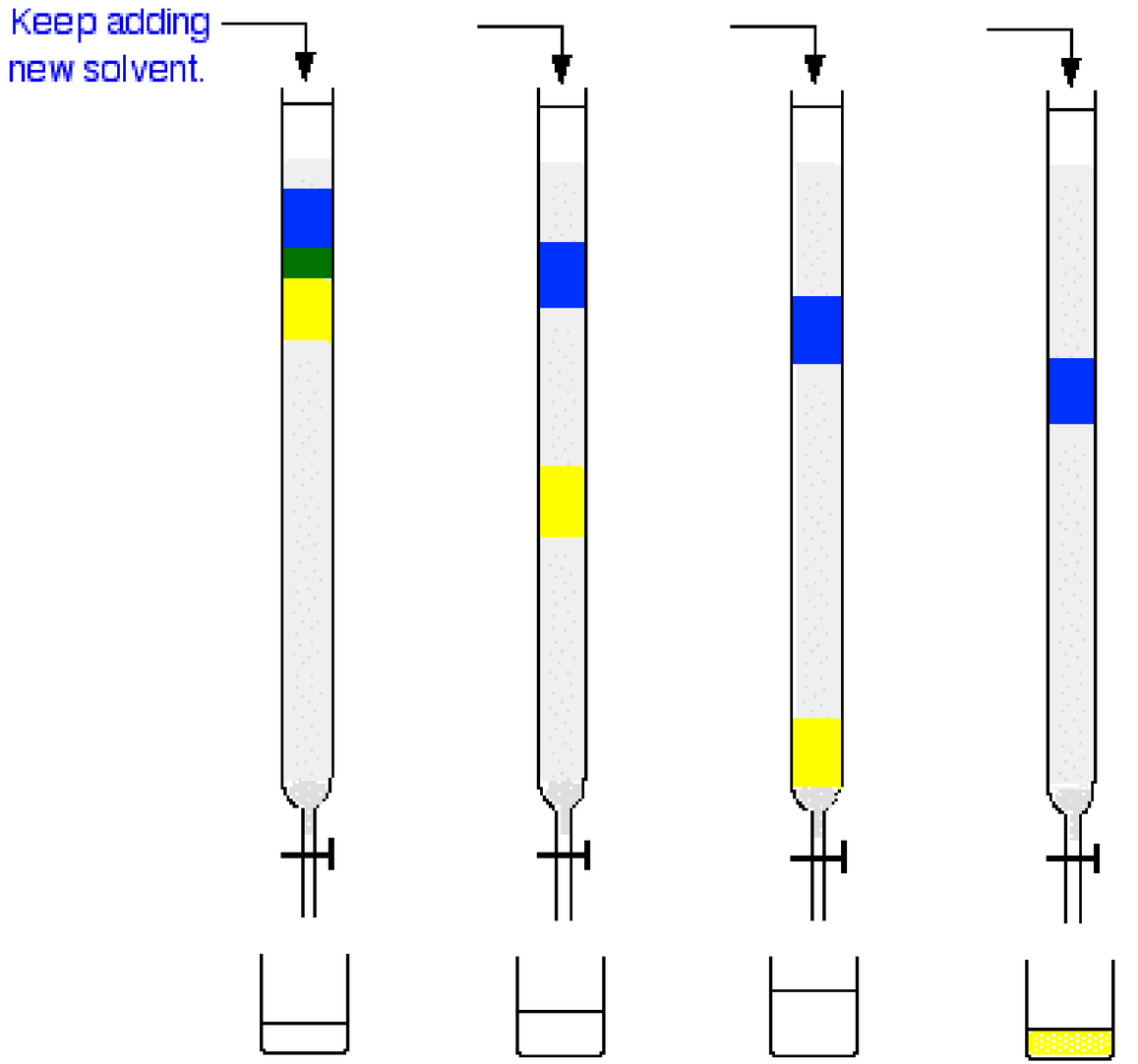
- The sample which is usually a mixture of components is dissolved in minimum quantity of the mobile phase.
- The entire sample is introduced into the column at once and get adsorbed on the top portion of the column.
- From this zone, individual sample can be separated by a process of elution.









DEVELOPMENT TECHNIQUE (ELUTION)

By elution technique, the individual components are separated out from the column. The two techniques are:

1. Isocratic elution technique : in this elution technique , same solvent composition or

   solvent of same polarity is used throughout the process of separation.

   Example: chloroform only

GRADIENT ELUTION TECHNIQUES

(gradient – gradually)

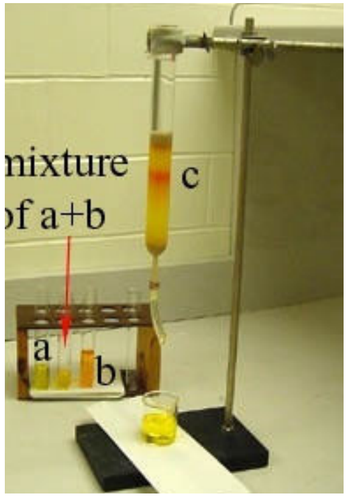
- Solvents of gradually ↑ polarity or ↑ elution strength are used during the process of separation.
- E.g. initially benzene, then chloroform, then ethyl acetate then chloroform

DETECTION OF COMPONENTS

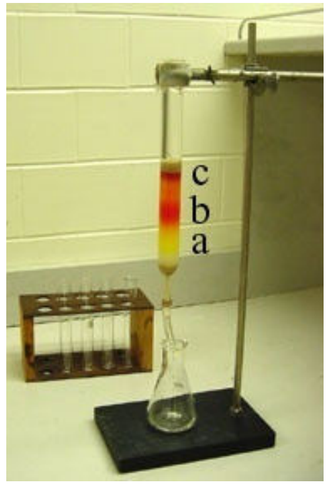
- If the compounds separated in a column chromatography procedure are colored, the progress of the separation can simply be monitored visually.

- If the compounds to be isolated from column chromatography are colorless. In this case, small fractions of the eluent are collected sequentially in labelled tubes and the composition of each fraction is analyzed by TLC.

- Eluting the sample: Components a, b, and c separate as column progresses.

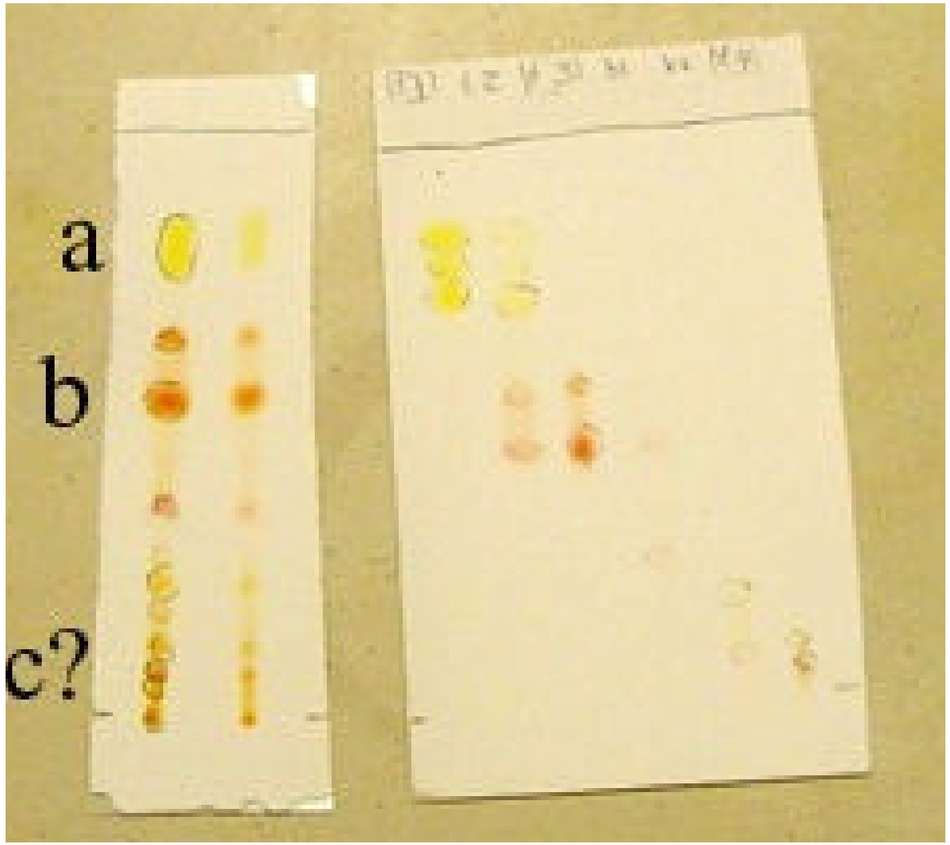


- Fractions can be collected in test tubes, vials, beakers, or Erlenmeyer flasks.



ANALYZING THE FRACTIONS

Analyze the fractions by thin-layer chromatography



FACTORS AFFECTING COLUMN EFFICIENCY

- Dimension of the column: column efficiency has been improved by increasing length/width ratio of the column.
- Particle size of column packing: separation to be improved by decreasing the particle size of the adsorbent.
- Activity of the adsorbent
- Temperature of the column: The speed of the elution increases at higher temperatures.
- Packing of the column
- Quality of solvents: solvents having low viscosities is giving better results.

APPLICATIONS

- Separation of mixture of compounds
- Purification process
- Isolation of active constituents
- Estimation of drugs in formulation
- solation of active constituents
- Determination of primary and secondary glycosides in digitalis leaf.
- Separation of diastereomers

ADVANTAGES OF C.C

- Any type of mixture can be separated
- Any quantity of mixture can be separated
- Wider choice of Mobile Phase
- Automation is possible

DISADVANTAGES OF C.C

- Time consuming
- more amount of Mobile Phase are required
- Automation makes the techniques more complicated & expensive

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