

# Analytical Chemistry

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## 1★ Separation Technique

### Chromatography

chroma → colour

Graphics → writing

overall term → writing with colours.

★ M. Tswit → first accidentally discovered chromatography

### (Chromatography)

It is a separation technique in which a multi-component mixture is separated into its individual parts or items.

★ Physically mix-up things or components in mixture (not chemically).

### Example:-

Separation of chlorophyll components on filter paper using acetone, that results into

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separation of

1. chlorophyll - a
2. chlorophyll - b
3. Xanthophylls
4. Carotenoids

In fact, all the chromatographic techniques are physical one involving various phenomena which is the basis of classification of chromatographic techniques.

**\* Types:** There are four types of chromatography depending upon the principle involved in separation of components.

### **\* Chromatography**

- 1 → Partition chromatography
- 2 → Adsorption " "
- 3 → Ion exchange "
- 4 → Gell Permeation/ Molecular sieve/ size exclusion chromatography

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## 1★ Partition Chromatography

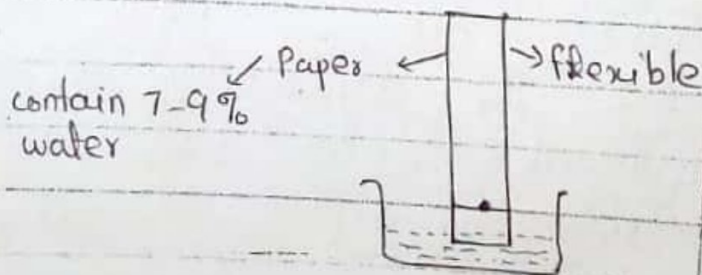
In partition chromatography the basic principle of separation is relative distribution of a solute between two liquids

**Example:**

Paper chromatography

### Paper chromatography

In paper chromatography, the separation of a mixture is performed using a mobile phase which is always liquid. A paper is a support.



The water contained in paper acts as a stationary phase while the mobile phase may be water

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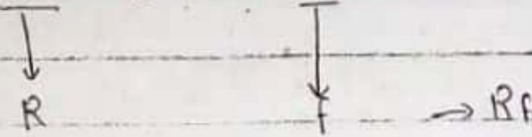
- \* water + alcohol
  - \* water + alcohol + acetic acid
  - \* water + acetic acid
  - \* water +  $\text{NH}_4\text{OH}$
  - \* water + alcohol +  $\text{NH}_4\text{OH}$
- and
- \* even polar organic (salt) solvent may act as mobile phase

As indicated earlier, that partition chromatography is relative distribution of a solute between two liquids, the stationary phase tries to stop the movement of solute while mobile phase acts opposite to the stationary phase.

As the solubility of different solutes vary considerably, the overall result of

- \* Mobile-Stationary phase is separation of the solutes into individual components.

## Retention Factor:



$R_f$  value =  $\frac{\text{Distance of component covered by it}}{\text{Distance covered by mobile phase}}$

$$R_f = \frac{D_{s-p}}{D_{M-P}} \quad \text{OR} \quad \frac{S_{SP}}{S_{MP}} = \frac{S_{org}}{S_{inorg}}$$

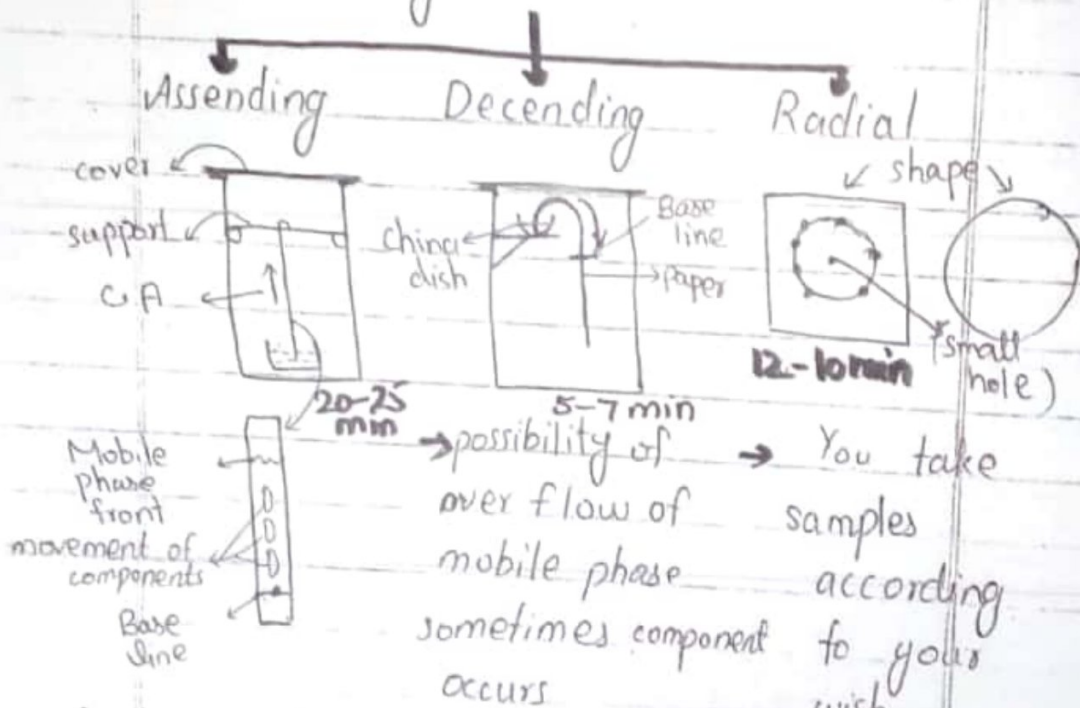
Annotations:

- $D_{s-p}$  is labeled as (Stationary phase)
- $D_{M-P}$  is labeled as (Mobile phase)
- $S_{SP}$  is labeled as (solubility)
- $S_{org}$  is labeled as (organic)
- $S_{inorg}$  is labeled as (inorganic)

- $R_f$  value is characteristic feature of a substance and a usually invariable.
- Its maximum value is 1 (one) and normal values are always less than one (1). [ $R_f = \frac{2\text{cm}}{10\text{cm}} = 0.2$ ]
- $R_f$  value is unit-less
- $R_f$  values can be utilized for qualitative identification.
- \* Chromium has maximum value of  $R_f = 0.999$

# Performance of paper chromatography:

Paper chromatography can be performed in three ways



(anti-gravity)  
 → Capillary action → Both C.A and Gravity action occurs (C.A)  
 → In this  $R_f$  value exist → In this  $R'$  value occur



→ commonly one sample contain in both of them



→ Many components separated simultaneously at a time

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Eluent  $\rightarrow$  Mobile phase

Elution  $\rightarrow$  Movement of mobile phase

\* speed of chromatography (paper) performed

Descending  $\rightarrow$  Radial  $\rightarrow$  Ascending

## 2\* Adsorption Chromatography

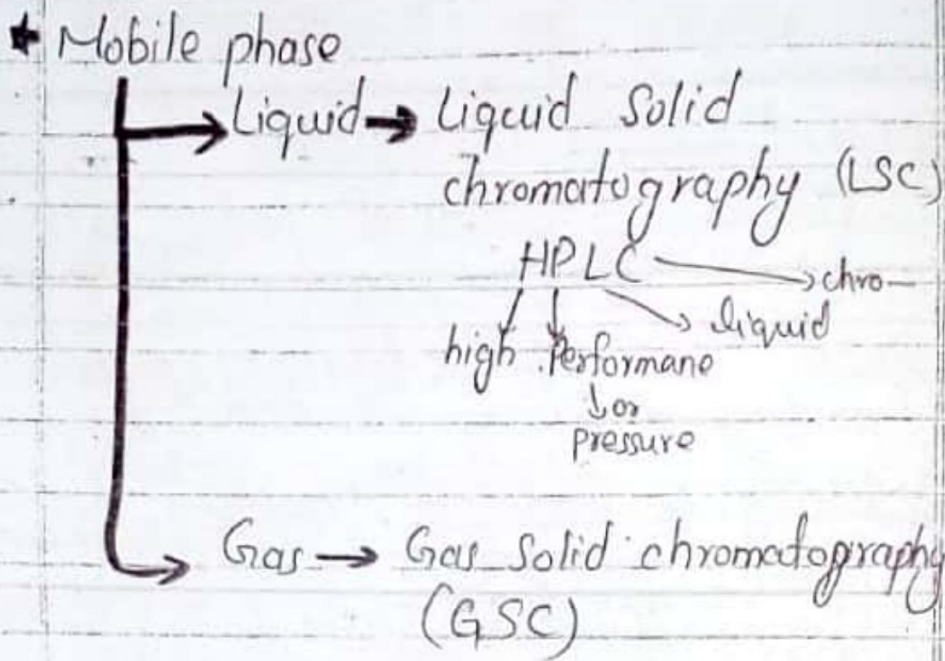
\* Stationary  $\rightarrow$  Adsorbent phase

\* Mobile phase  $\rightarrow$  Liquid / Gas

\* Adsorbents are always in solid form.

def:- case of

$\Rightarrow$  In  $\downarrow$  adsorption chromatography the stationary phase is always solid called adsorbent while the mobile phase can be a liquid or gas.



### Adsorbant:

There are several types of adsorbants like Talc (Talcum powder), Carbon black, Bone charcoal, Animal Charcoal, Diatomaceous Earth, Kieselghaur, Silica gell, Alumina ( $Al_2O_3$ )

↓  
hydrated  $SiO_2$

#### Adsorbant Colour

- Talc colourless
  - Carbon black black
  - Bone charcoal black
  - Animal charcoal black
- Diatomaceous and Kieselghaur are almost <sup>Earth</sup> same.



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→ In house-hold, dunk cake ashes are best adsorbant.

### Properties of adsorbant:

1. It must be very easily available.
2. It must be very cheap.
3. It must have an open structure to adsorb the components (It must be activated).
4. It must not react with any of the mobile phase or mobile phase components.
5. It must not react with any of sample components.
6. It should be easily used. It should be able to be easily used after activation.

★ As is clear from all above examples of adsorbant they will have no affinity for any of the substance

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there must be some cementing material incorporated in the adsorbant.

### Example:

In case of thin-layer chromatography (TLC)

Alumina or Silica gel

usually contains some cementing material, like

Gypsum (anhydrous  $\text{CaSO}_4$ )

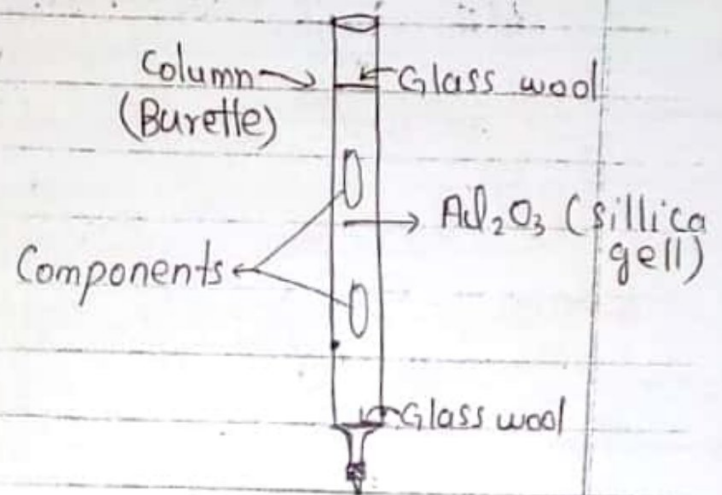
↳ Plaster of Paris → white colour

### Performance:

Adsorption chromatography is performed in several ways, the most common are

1. → Column Technique
2. → TLC
3. → Column Gas Chromatography

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(Column chromatography)



→ Glass wool help to retain the silica gell (because silica gell has fine particles)

TLC

## Thin layer chromatography

Mobile phase : Liquid  
stationary phase : Solid (Adsorbent)

As the name indicates, Thin layer chromatography, comprises following steps:

- a - Formation of a thin layer
- b - Elution (Resolution)
- c - Identification of Eluted components

### Formation of a thin layer

For the conduction of TLC a very fine layer of an adsorbent is made on a suitable material like glass, PVC plastic or some metals like aluminium or zinc. For this purpose, a TLC stand and TLC coaters are used.

TLC plates are fasten

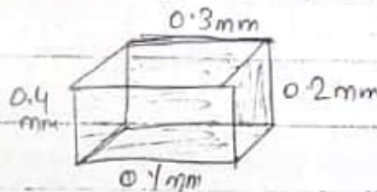
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in TLC stand, a slurry (paste) of adsorbent is made in water, is poured in TLC coater and dragged to form a thin layer on plates.

The homogenous plates are selected and kept in oven. The coating process is repeated twice or thrise to get sufficient amount of plates.

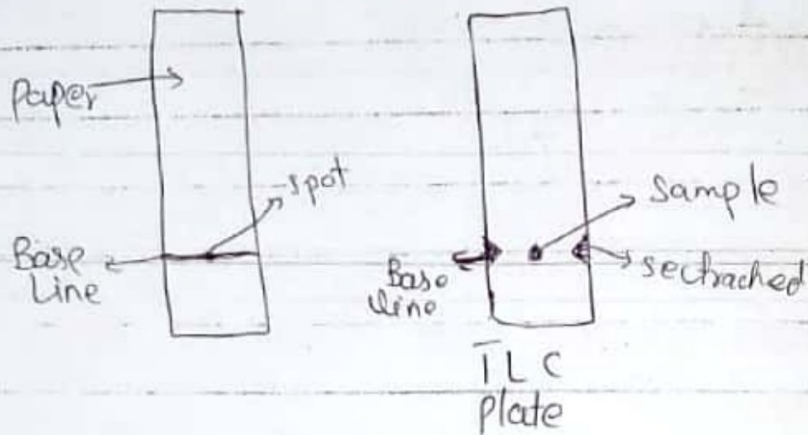
The plates are activated in an oven at  $110 \pm 5^\circ\text{C}$



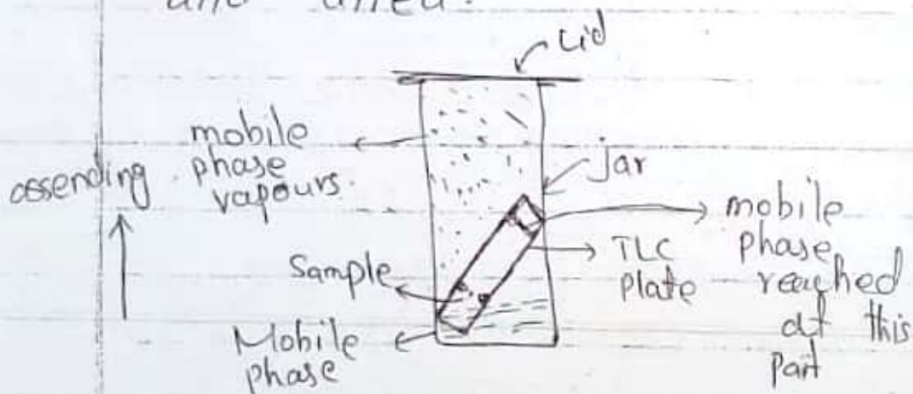
### Elution of Sample components:

For the elution of sample components, a solution of 2 to 3% sample is prepared in the requisite solvent and spotted on marked TLC plates. The sample is applied using Micro pipette or capillary Tube.

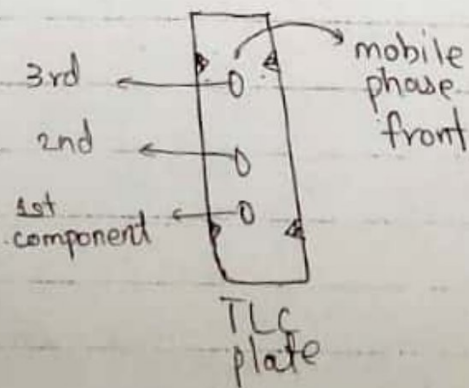
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Then sample is applied and dried.



~~is~~ Only ascending Technique is applied in TLC



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After drying, the applied sample, elution is conducted in a jar, having suitable mobile phase, covered with a lid. After an appropriate resolution, TLC plate is taken out and dried.

### Identification of eluted components: (in TLC and Paper chromatography)

When all the eluted components are coloured like mixture of dyes.

However when all eluted components are colourless, the identification become problematic.

In such a case, special techniques are used. The most commonly used are

1. Use of spray agents
2. Use of Iodine
3. Viewing under UV-rays (radiation)

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## 1\* Use of spray agents

Spray agents are those chemicals which can be sprayed on TLC/paper to develop colours. There is no typical universal spraying agent.

Several substances needs various types of spraying agents. In other words spraying agents are specific in nature.

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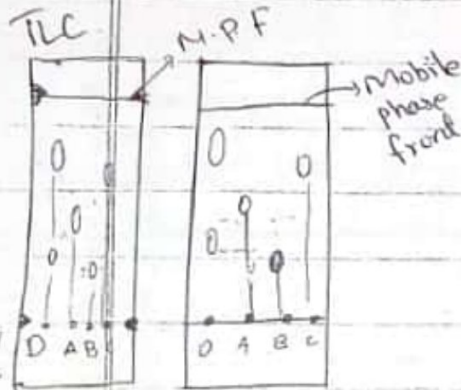
\* In case of colourless components various spray agents are used, they are also called locating agents

→ Table for locating agents

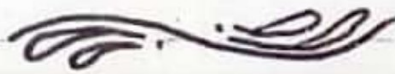


**\* Note \***

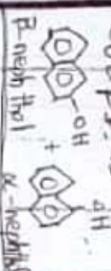
In most cases when sample components are colourless a comparative chromatographic, is used Technique

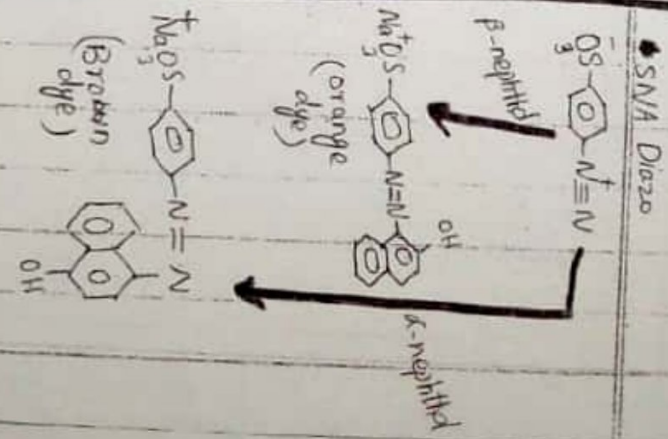


$B + A + C$   
 ↓      ↓      ↓  
 left    used    used  
 behind completely completely  
 (unreacted B was formed)



★ Table for locating agents and their suitability.

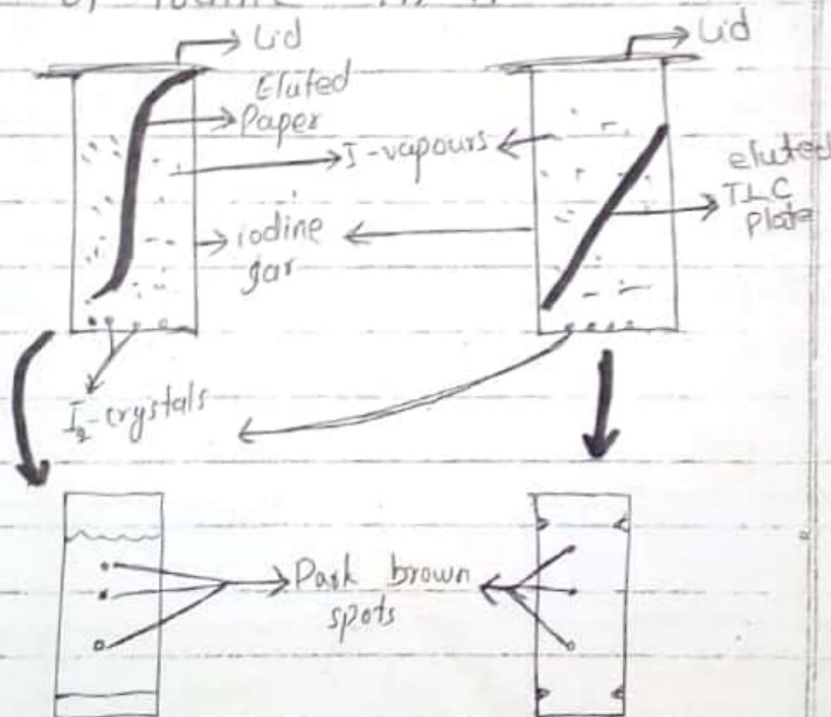
Sr#	Types of components	Locating agent	Suitability for	Colour
			Asymmetric	
1.	Amino acids	Ninhydrin	✓	Violet-pink-orange
2.	Sugars	conc. $H_2SO_4$	X	Brown-Black
3.	$Pb^{2+}$ & $Hg^{2+}$	K-I solution	✓	Yellow (PbI <sub>2</sub> ) Scarlet (HgI <sub>2</sub> )
4.	$Cu^{2+}$ & $Cd^{2+}$	$H_2S$ or $Na_2S$	✓	Black (CuS) Yellow (CdS)
5.	Couplers 	Diazonium salt e.g. SNA Diazo	✓	(p-naphthol) orange (o-naphthol) Brown
6.	$Fe^{3+}$ & $Co^{3+}$	$Na_3PO_4$ solution	✓	Yellow ( $FePO_4$ ) Violet ( $CoPO_4$ )



## 2\* Use of Iodine: (as a locating agent)

This is the most abundant method used for identification of colourless components.

The dried TLC plates or paper is kept in an iodine jar having a few crystals of iodine in it.



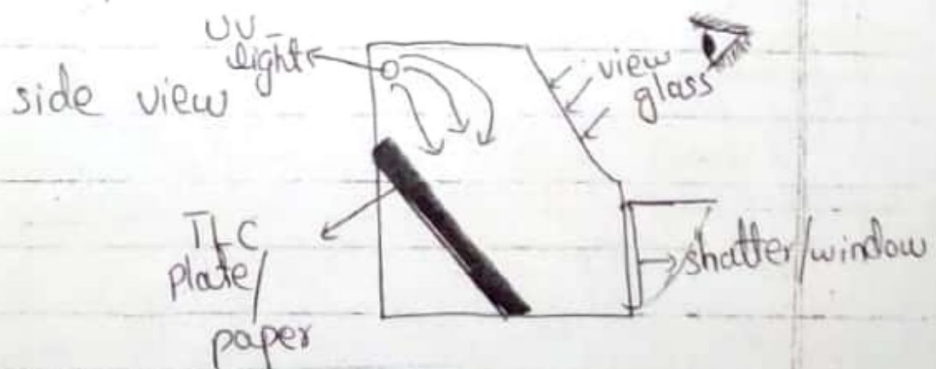
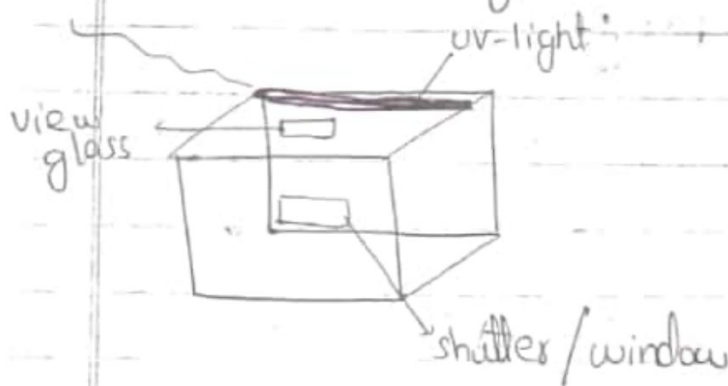
For the location of colourless components a few crystals of  $I_2$  are kept in a jar, then dried TLC plates or paper is kept in it, and the jar is covered with lid.

$I_2$ -vapours formed in jar, get associated with organic compounds by Van-wall forces and even can react with an isolated double bond. The net result is the formation of dark brown spots, that can be easily identified.

### 3. Viewing under UV-radiations.

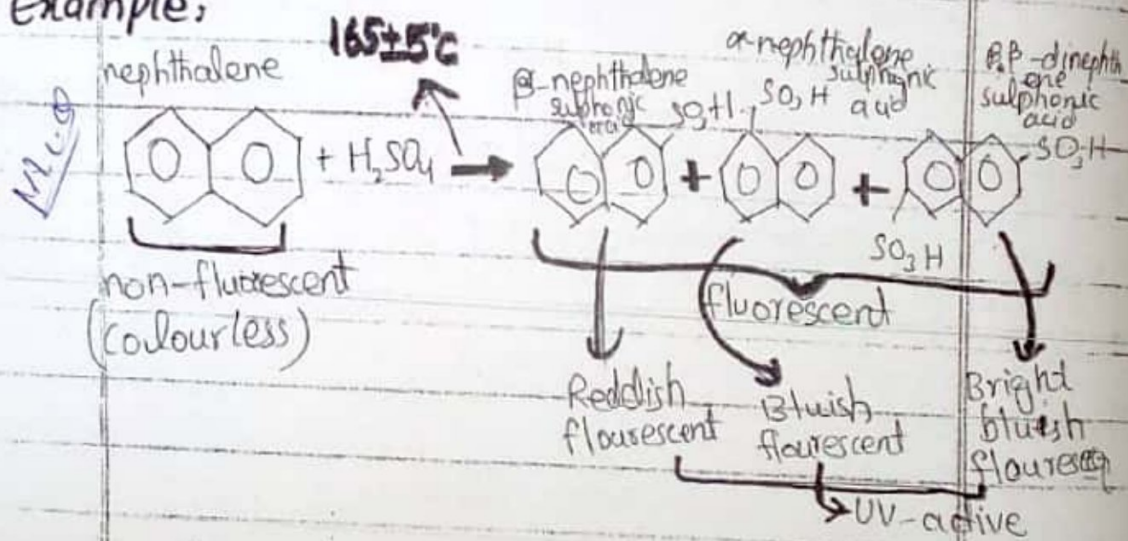
UV radiation as locating agent  
 UV-light " " "

→ The third method of identification of eluted samples is to view TLC plates or paper under UV-light.



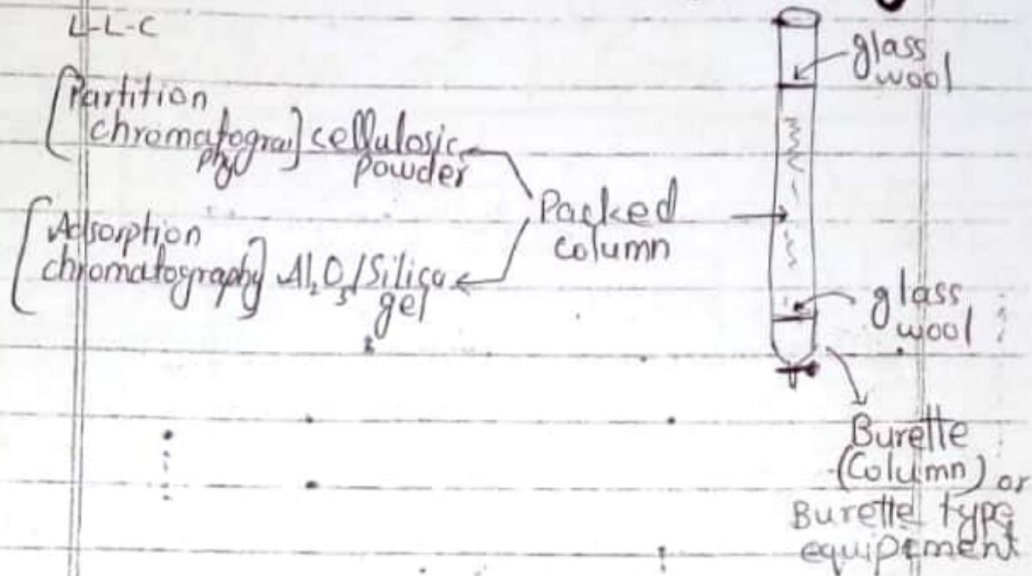
The plate or paper is kept in UV, the light is switched ON and the spots that are fluorescent are marked with a lead pencil and taken out and identified by comparison. This is non-destructive method.

Example:



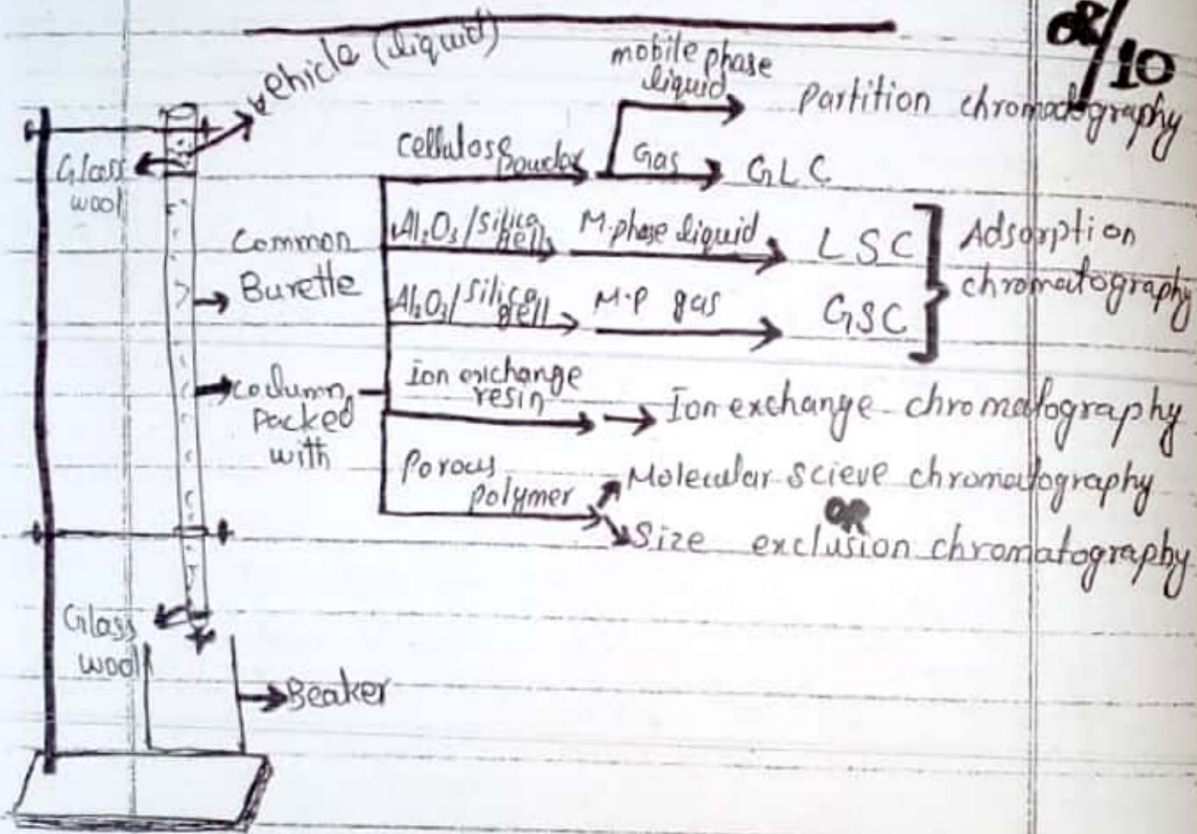
Now a days, fluorescent TLC plates are available, that can be used for identification of <sup>eluted</sup> non-fluorescent material as they will absorb fluorescence and look to be dark spots under UV-light.

# Column Chromatography



The chromatographic techniques used in a column depends upon its filling material. It may be Cellulosic powder, the chromatographic technique will be partition chromatography when mobile phase is liquid then it is called Liquid-Liquid Chromatography (LLC) when mobile phase is gas then technique is called Gas-Liquid Chromatography (GLC). However, when mobile phase is a liquid and high pressure is also used, then the technique is called (HPLC) High-Performance Liquid Chromatography.

OR High Pressure Liquid Chromatography.



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→ It is evident from above information that all types of chromatographic technique can be conducted in column.

→ vehicle → not adsorbent and not mobile phase



## Packing of Column (OR)

### Filling of column

For the conduction of column chromatography the most important feature is packing of column, for this purpose an inert material column like burette is selected.

A glass wool plug is inserted in bottom of the column, a slurry or past of requisite material (cellulose / Alumina / Silica gel / Ion exchange resin / porous polymer etc) is made in a vehicle (liquid) and poured into column upto a requisite column size and finally, another glass wool plug is inserted. The column is tapped well, to provide any air bubbles entrapped in it.

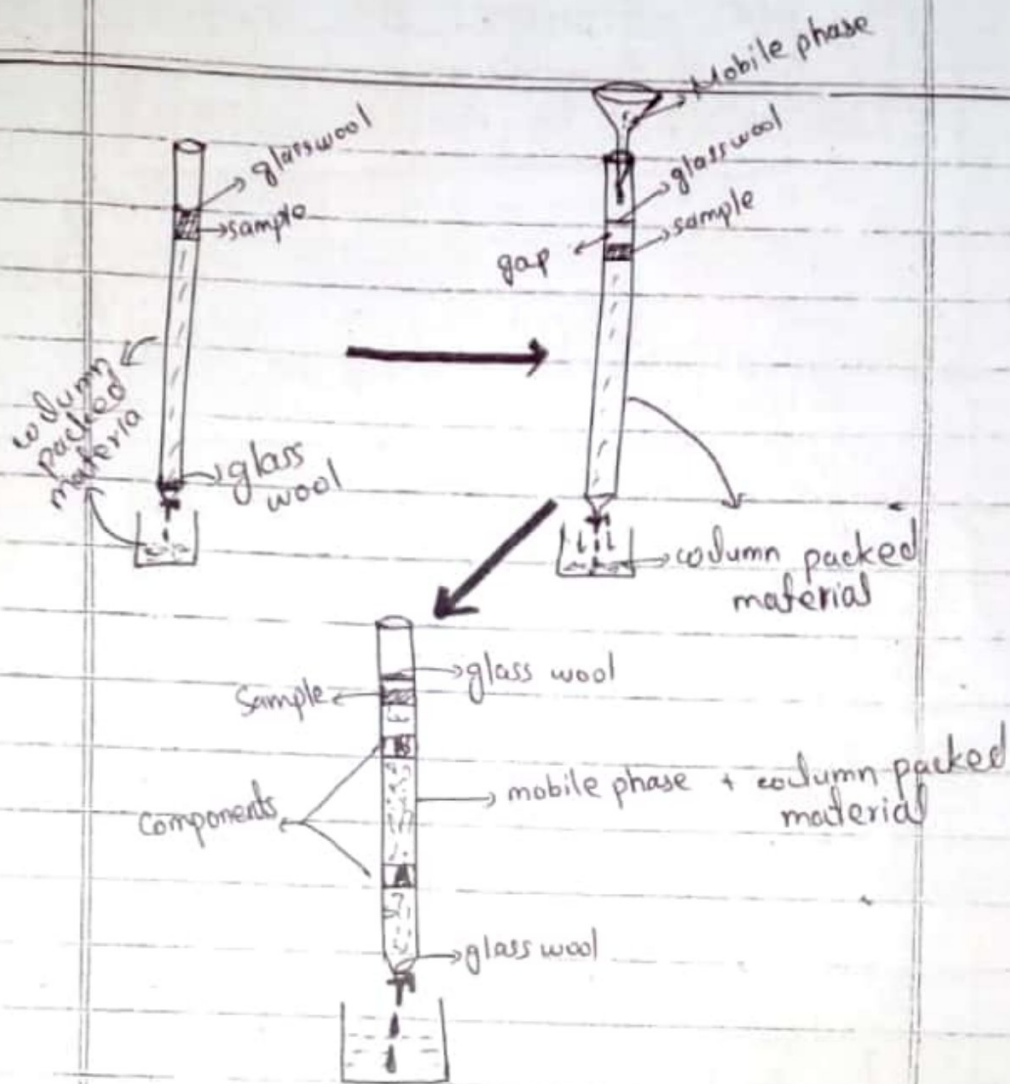
Now-a-days, several types of commercially prepared

Capillary columns and conventional columns are available for used in various types of instruments.

## Sample application:

After the preparation of column, the vehical liquid is drained upto glass wool plug. A sample of requisite material (material to be separated) is dissolved in minimum amount of solvent and poured on glass wool. The solution of the material should be **0.5mL - 2.0mL**.

The vehical is again drained shows that the sample is come in contact with packing material and then mobile phase is poured into column.



## Mobile phase & Elution

The choice of mobile phase depends upon, the nature of components to be separated as well as the material packed in column. In usual practice, the elution can be conducted

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by the change of molarity of mobile phase.

→ change of molarity

Acid mobile phase	Base mobile phase
↓	↓
0.5M to 4M	6M to 0.5M

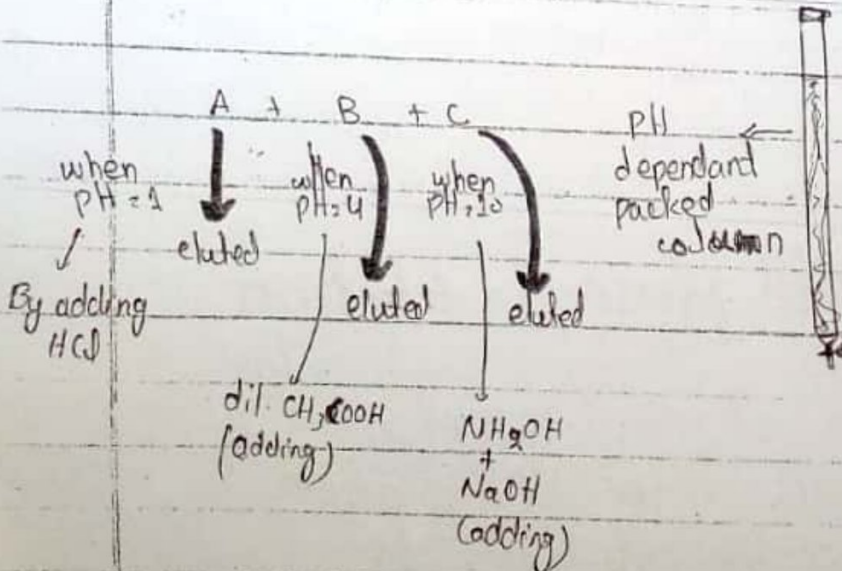
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→ change of polarity

H <sub>2</sub> O	Alcohol
↓	↓
high Polarity	Low Polarity

→ Change in pH

Acidic phase	OR	Basic
↓		↓
1 to 4 (pH)		9 to 12 (pH)



## Identification of colourless components:

In case of column chromatography, the identification of colourless components is an up-hill task. However, it is easy when column chromatography is conducted in an instrument like GLC, HPLC, GPC and GSC coupled with modern computers. These instruments have Pre-Fed Standards and they are able to identify colourless components qualitatively and quantitatively.

The main problem of identification of colourless components in a column in a lab work is over-come by.

1. Use of UV-Lamp
2. Use of Polarity of mobile phase
3. Use of pH gradient.