Practical pharmacognosy
Second stage

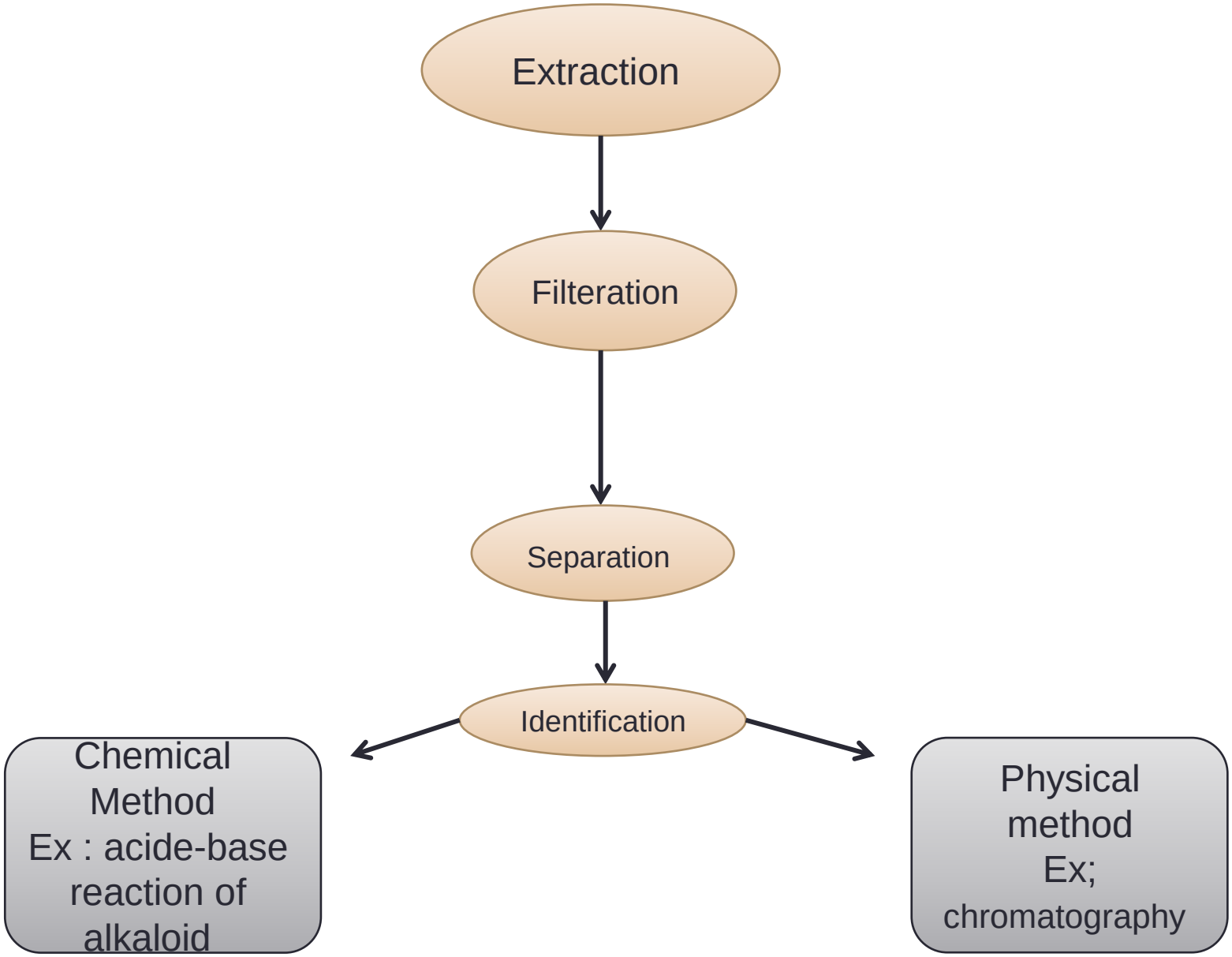
AL-Nahrain university
College of pharmacy

CHROMATOGRAPHY





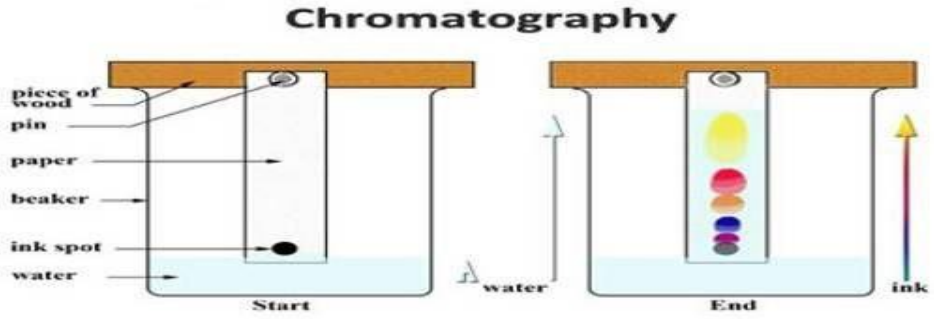
LAB 4


Extraction
Filteration
Separation
Identification
Chemical Method
Ex : acide-base reaction of alkaloid
Physical method
Ex;
chromatography

WHAT IS CHROMATOGRAPHY

- Chromatography is a **physical method** of separation in which the components to be separated are **distributed between two phases** One of which is **stationary (stationary phase)** while the other **(the mobile phase)** moves through it in a definite direction .
- The chromatographic process occurs due to differences in the distribution constant of the individual sample components.
- It is used for large and small quantities so it is used **quantitatively** and **qualitatively** and proved to be more effective from the other means of separation and identification.





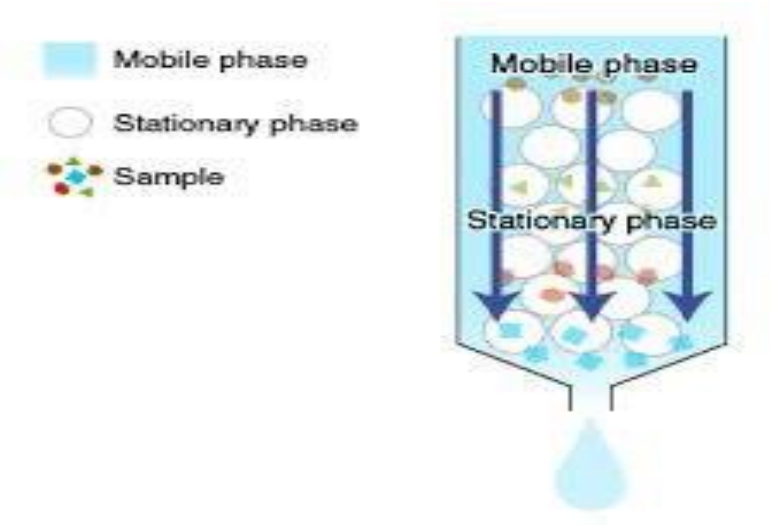
- **The separation of a mixture of compounds in chromatography to its components depends on the action of two forces ;**

1. **Mobile force (driving force) that will try to move the components of mixture.**
2. **Opposing force (stationary or retardation force) that will try to keep components in their places depending on many factors:**

a) Solubility in mobile phase.

b) Adsorption ability of component to be separated.

c) Ionic forces.



CHROMATOGRAPHY CLASSIFICATION

There are different types of chromatography classification :

a) Classification of chromatography **according to mobile phase**

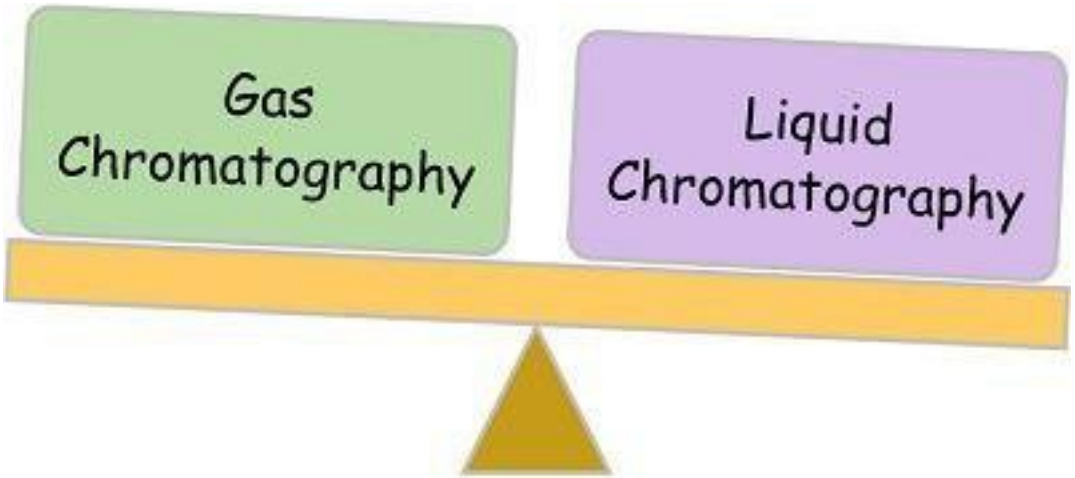
b) Classification **according to the packing of the stationary phase**

c) Classification **according to the force of separation**

Classification of chromatography according to mobile phase

1- Liquid chromatography: mobile phase is a liquid.

2- Gas chromatography: mobile phase is a gas.

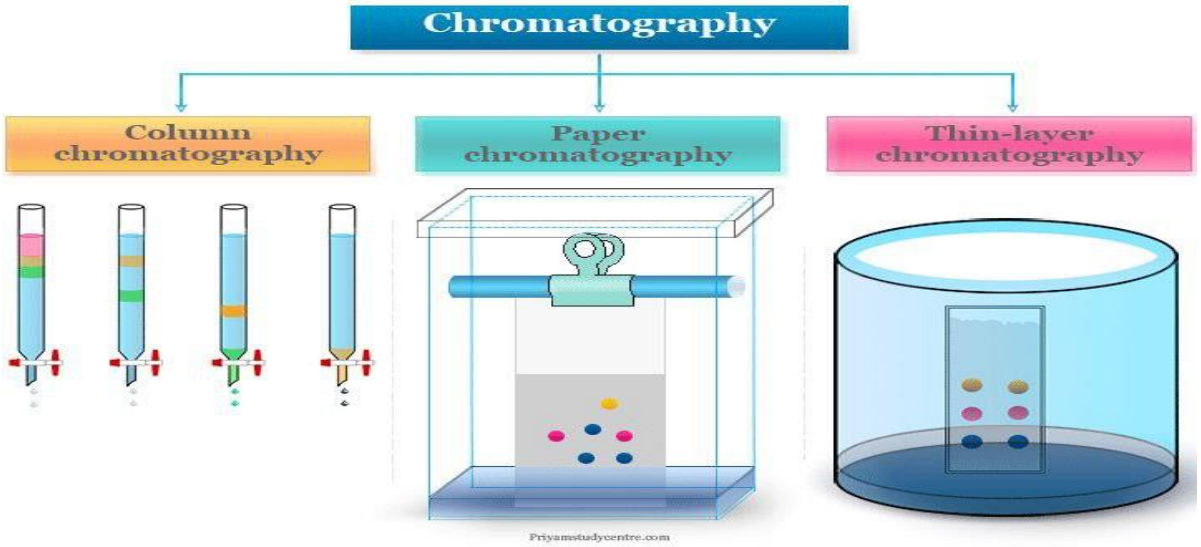


CLASSIFICATION ACCORDING TO THE PACKING OF THE STATIONARY PHASE

1-Thin layer chromatography (TLC): the stationary phase is a thin layer supported on glass, plastic or aluminum plates.

2- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support.

3- Column chromatography (CC): stationary phase is packed in a glass column



CLASSIFICATION ACCORDING TO THE FORCE OF SEPARATION

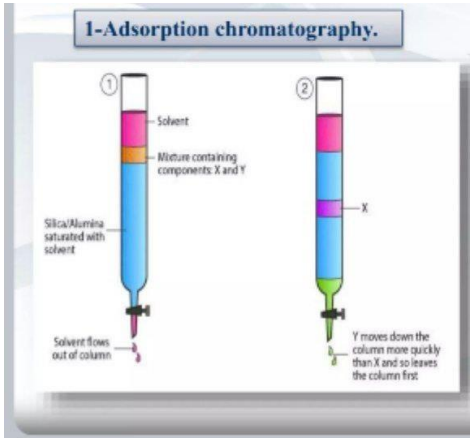
1- Adsorption chromatography.

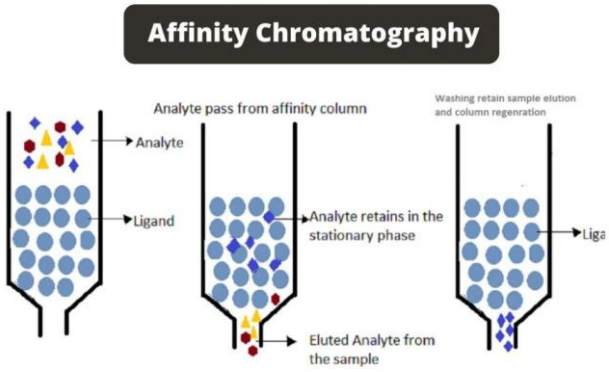
2- Partition chromatography

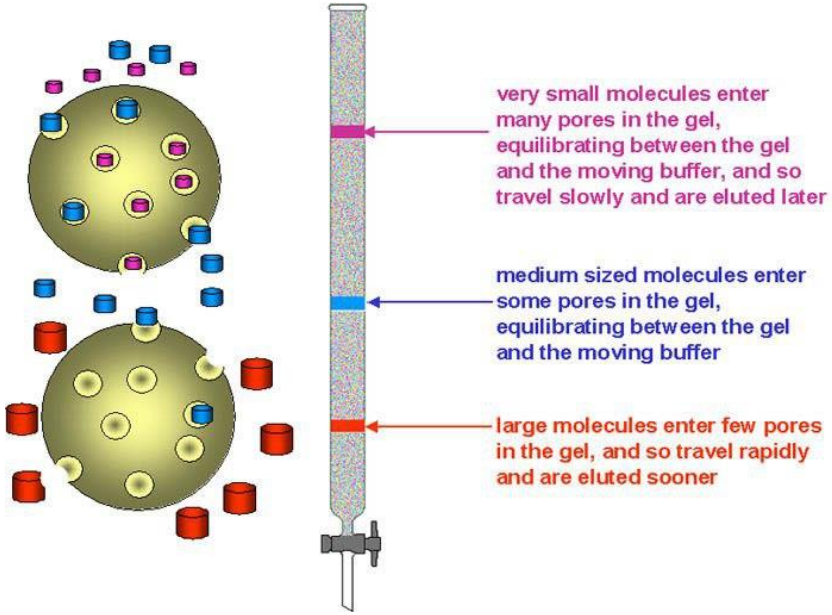
3- Ion exchange chromatography.

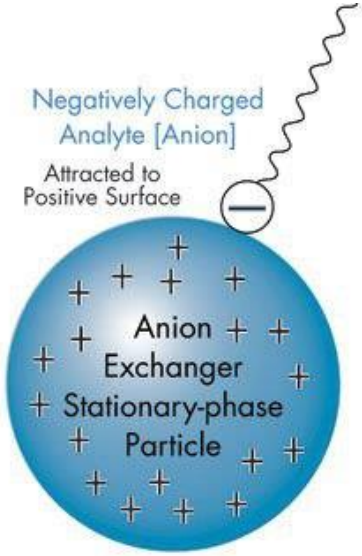
4- Gel filtration chromatography.

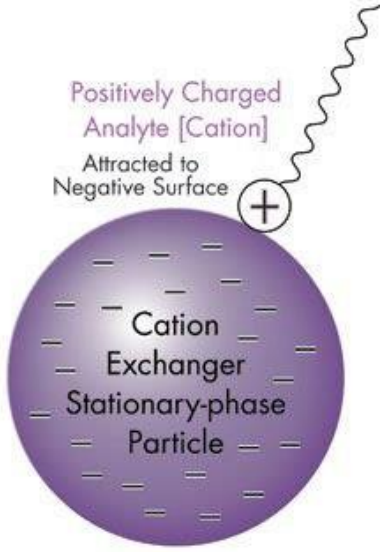
5- Affinity chromatography





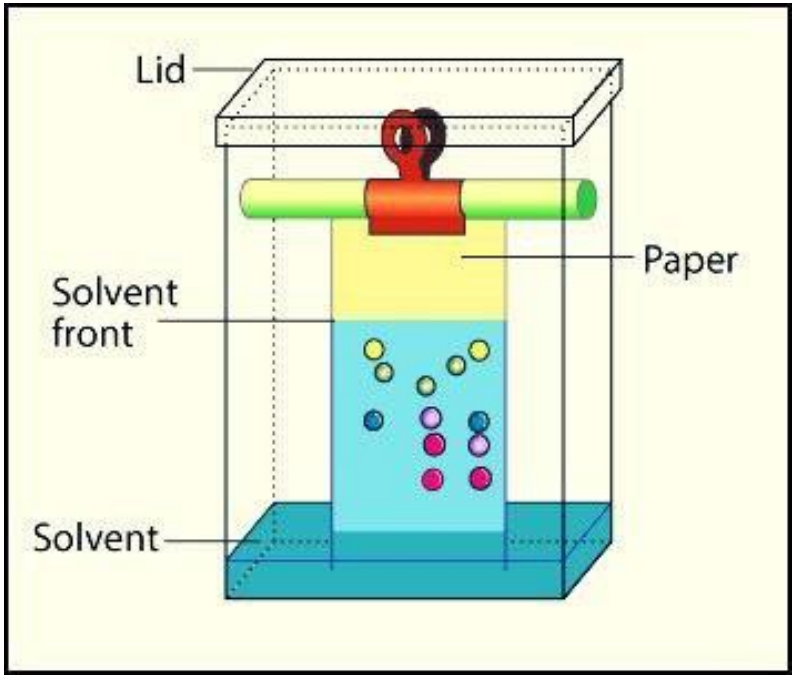






PAPER CHROMATOGRAPHY

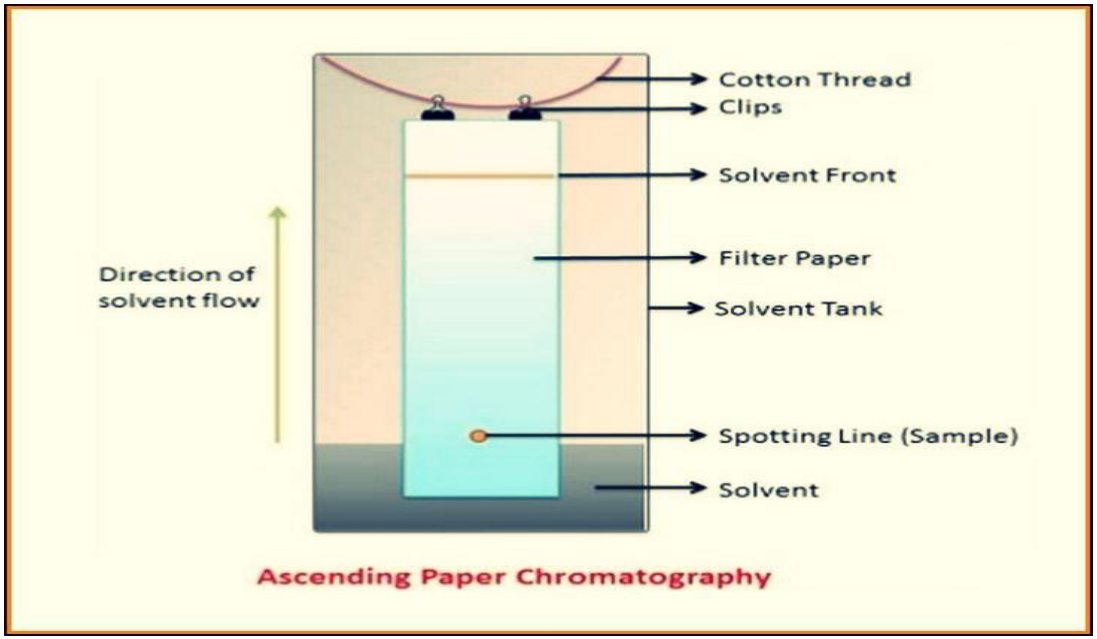
is a method of partition chromatography using filter paper strips as carrier or inert support. The factor governing separation of mixtures of solutes on filter paper is the partition between two immiscible phases. One is usually water adsorbed on cellulose fibers in the paper (stationary phase).The second is the organic solvent flows past the sample on the paper (stationary phase).



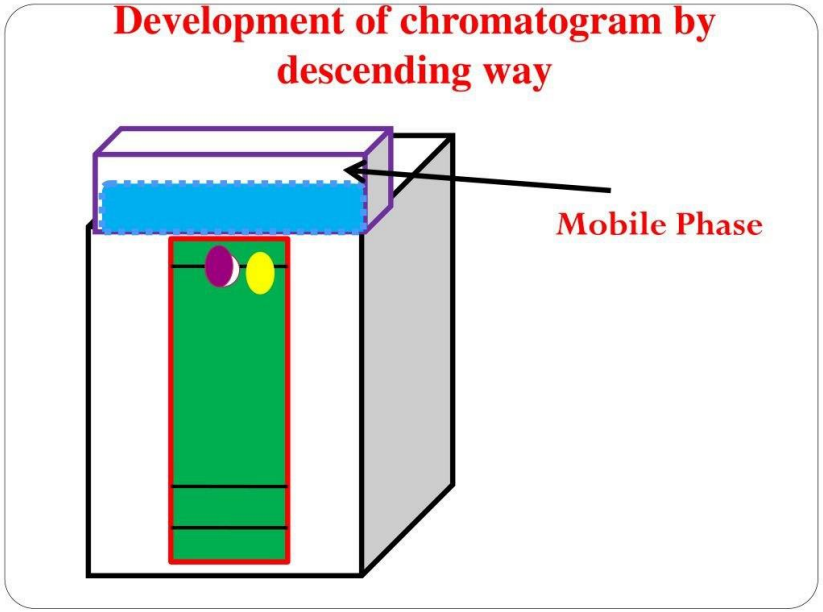
DEVELOPING TECHNIQUE IN THE PAPER CHROMATOGRAPHY

Developing: means separating mechanism (addition of mobile phase that cause separation)

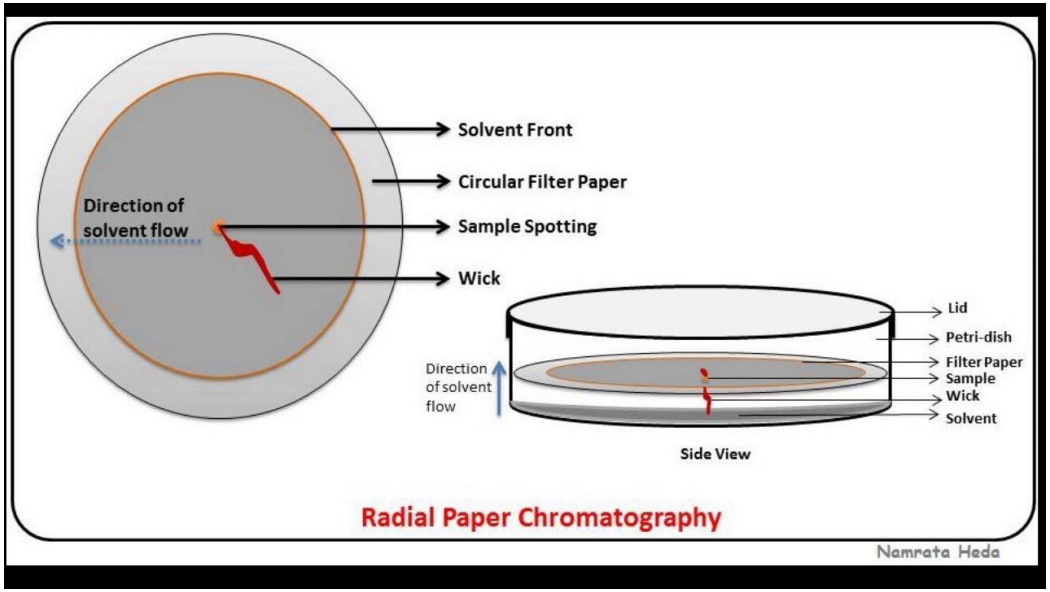
1. Ascending development : the paper will be dipped in the solvent mixture so that the solvent travels up the paper .



2. Descending development : the solvent will be supported at the top of the chamber . In this case the sample travels down the paper .



3. Radial development : Circular or horizontal paper chromatography is another technique used, in which circular filter paper bearing a wick at the center of the paper is placed in a petri dish and the solvent system supplementation is through the central wick.



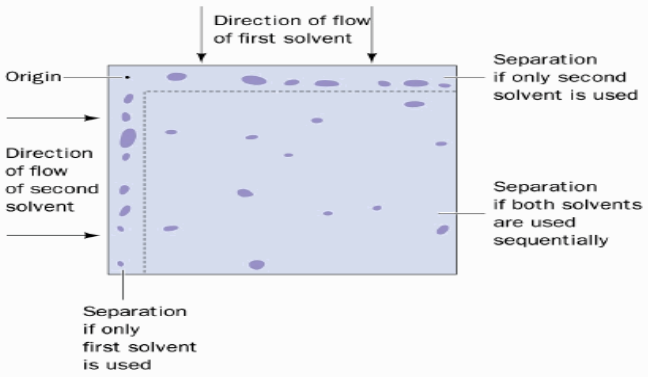
MULIPLE CHROMATOGRAPHY

Multiple chromatography includes all procedures in which the development is repeated after one development is completed.

A- muliple development : the chromatogram is repeatedly developed in the same direction and thus complete resolution of two or more substances which have Rf values close together can be obtained . As the mobile phase one can use either the same solvent system or different solvent system .

B- TWO-DIMENSIONAL CHROMATOGRAPHY

When large numbers of substances are to be separated on a single chromatogram. Development in a direction perpendicular to the first, and with a solvent system different from that used initially is often necessary. The sample is applied on one corner of a square piece of paper and after development with the first solvent; the paper is dried, rotated 90o and developed in the second direction. Usually, different types of solvents systems are used in each direction. It is essential that the first solvent be completely volatile.



Two-dimensional paper chromatography

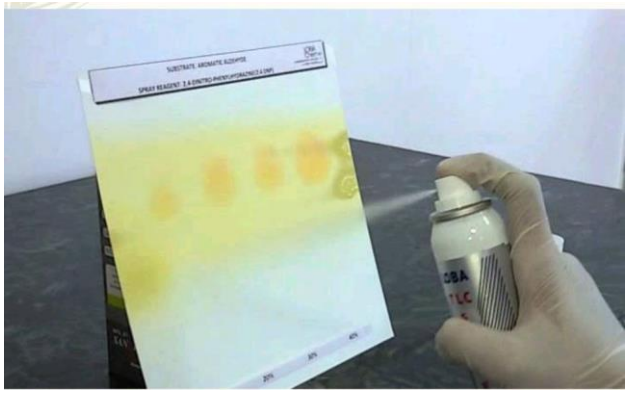
DETECTION METHODS IN PAPER CHROMATOGRAPHY

1) Chemical detection by using chemical reagents

2) Physical detection by using UV light.

3) Radioactive method: specific detection procedures when use to detect separated compound having some radioactivity or labeled compounds.

4) Biological methods by using certain microorganisms and are especially used for the detection of antibiotics.

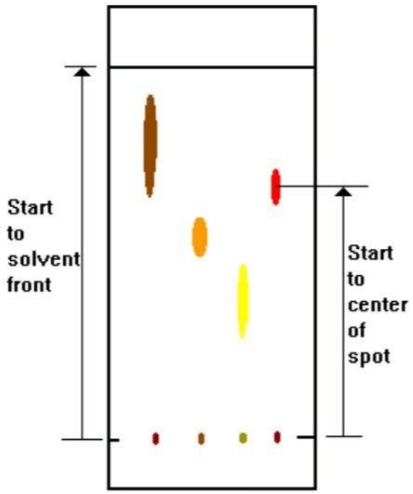




RETARDATION FACTOR (RF)

Can be defined as the distance moved or traveled by the compound to the distance moved by the solvent and it is constant for each compound when chromatography is carried out using the same technique. Mobile phase and the same conditions. Usually the RF value is used for the identification of the separated compound by comparison with the RF value of a standard .

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$



RF VALUE IS GOING TO CHANGE BY

1) Change the solvent.
2) Aging.
3) Impurities.
4) Temperature.
5) Saturation.
6) Solvent front must be uniform

IDENTIFICATION OF ISOLATED COMPOUNDS

- **Identifying the Spots by visualization, if the spots can be seen, outline them with a pencil.**
- **If no spots are obvious, the most common visualization technique is to hold the plate under a UV lamp.**
- **If no spots under UV light , spraying reagent is used .**
- **Then the RF (retention factor) value for each spot should be calculated .**
- **It is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates. Hence, known RF values can be compared to those of unknown substances to aid in their identifications.**

EXPERIMENT NO.1
CIRCULAR FILTER PAPER CHRONATOGRAPHY

1) Prepare a circular filter paper and insert a wick in the center of the paper. Mark four pencil dots (starting points) approximately 1cm from the wick.
2) Apply the sample on pencil dot (3different magic colors and ink).
3) Place a chromatographic paper over the dish that contains the mobile phase in such a way that develops to about 4-5 cm
4) 4) Remove the chromatogram, make the solvent front and dry at room temperature
5) 5) Examine the chromatogram by the daylight and calculate the Rf value for each separated spot.



IMPORTANT NOTES

- **The component which is more soluble in stationary phase moves slowly while the component which is more soluble in mobile phase which moves fast**

- **The advantages of paper chromatography : simple , inexpensive , the separation can be carried out under ordinary lab condition .**
- **The disadvantages of paper chromatography : time required is large , heating or corrosive reagents can not be used for detection .**

APPLICATION OF THE CHROMATOGRAPHY ?

