

Paper Chromatography

By- Dr Ekta Khare

Paper Chromatography

- Paper chromatography definition explains that is an inexpensive and powerful analytical technique, which requires a piece of paper or strips serving as an adsorbent in the stationary phase across which a particular solution is allowed to pass.

Or

- **Paper chromatography (PC) is a type of planar chromatography whereby chromatography procedures are run on a specialized paper.**
- PC is considered to be the simplest and most widely used of the chromatographic techniques because of its applicability to isolation, identification, and quantitative determination of organic and inorganic compounds.
- It was first introduced by German scientist Christian Friedrich Schonbein (1865).
- This analytical tool employs very few quantities of material.

Principle of Paper Chromatography

- Paper chromatography is a form of liquid chromatography where the basic principle involved can be either:
 - partition chromatography
 - adsorption chromatography
- Paper Partition chromatography: In paper chromatography separation of component is distributed between phases of liquid.
- Here, one phase of liquid is water that is held amidst the pores of filter paper and the other liquid is the mobile phase that travels along with the filter paper.
- Separation of the mixture is the result that is obtained from the differences in the affinities towards the water and mobile phase when travelling under capillary action between the pores of the filter paper.
- Paper Adsorption chromatography: Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase.

Instrumentation of Paper chromatography

- Stationary phase & papers used
- Mobile phase
- Developing Chamber
- Detecting or Visualizing agents

1. STATIONARY PHASE AND PAPERS

- Whatman filter papers of different grades like No.1, No.2, No.3, No.4, No.20, No.40, No.42 etc
- In general the paper contains 98-99% of α -cellulose, 0.3 – 1% β -cellulose.

Other modified papers

- Acid or base washed filter paper
- Glass fiber type paper.
- Hydrophilic Papers – Papers modified with methanol, formamide, glycol, glycerol etc.
- Hydrophobic papers – acetylation of OH groups leads to hydrophobic nature, hence can be used for reverse phase chromatography.
- Impregnation of silica, alumina, or ion exchange resins can also be made.

... Instrumentation of Paper chromatography

2. PAPER CHROMATOGRAPHY MOBILE PHASE

- Pure solvents, buffer solutions or mixture of solvents can be used.

3. CHROMATOGRAPHIC CHAMBER

- The chromatographic chambers are made up of many materials like glass, plastic or stainless steel.
- Glass tanks are preferred most.
- They are available in various dimensional size depending upon paper length and development type.
- The chamber atmosphere should be saturated with solvent vapor.

Steps in Paper Chromatography

- In paper chromatography, the sample mixture is applied to a piece of filter paper, the edge of the paper is immersed in a solvent, and the solvent moves up the paper by capillary action. The basic steps include:

Selection of Solid Support

- Fine quality cellulose paper with defined porosity, high resolution, negligible diffusion of the sample, and favoring good rate of movement of solvent.

Selection of Mobile Phase

- Different combinations of organic and inorganic solvents may be used depending on the analyte.
- **Example.** Butanol: Acetic acid: Water (12:3:5) is a suitable solvent for separating amino acids.

Saturation of Tank

- The inner wall of the tank is wrapped with filter paper before the solvent is placed in the tank to achieve better resolution.

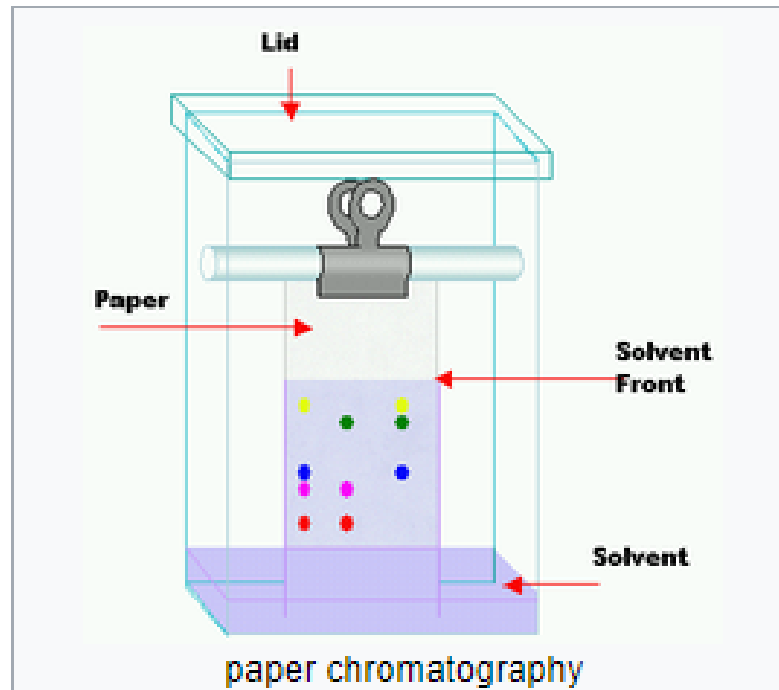
Sample Preparation and Loading

- If the solid sample is used, it is dissolved in a suitable solvent. Sample (2-20ul) is added on the baseline as a spot using a micropipette and air dried to prevent the diffusion.

Development of the Chromatogram

- Sample loaded filter paper is dipped carefully into the solvent not more than a height of 1 cm and waited until the solvent front reaches near the edge of the paper.

Paper chromatography



Acronym PC

Classification [Chromatography](#)

Analytes chromatography is a technique used for separation of the parts of a mixture of either gas or liquid solution

Other techniques

Related [Thin layer chromatography](#)

Different types of development techniques can be used:

ASCENDING DEVELOPMENT

- Like conventional type, the solvent flows against gravity.
- The spots are kept at the bottom portion of paper and kept in a chamber with mobile phase solvent at the bottom.

DESCENDING TYPE

- This is carried out in a special chamber where the solvent holder is at the top.
- The spot is kept at the top and the solvent flows down the paper.
- In this method solvent moves from top to bottom so it is called descending chromatography.

ASCENDING – DESCENDING DEVELOPMENT

- A hybrid of above two techniques is called ascending-descending chromatography.
- Only length of separation increased, first ascending takes place followed by descending.

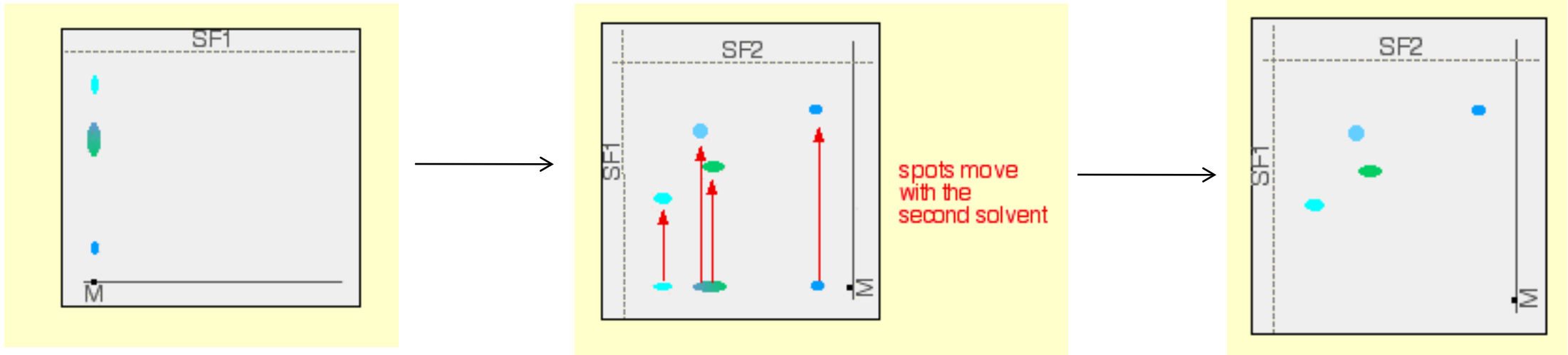
CIRCULAR / RADIAL DEVELOPMENT

- Spot is kept at the centre of a circular paper.
- The solvent flows through a wick at the centre & spreads in all directions uniformly.

TWO-DIMENTIONAL CHROMATOGRAPHY

- This helps in resolving substances that have similar Rf values.

TWO-DIMENSIONAL CHROMATOGRAPHY



Drying of Chromatogram

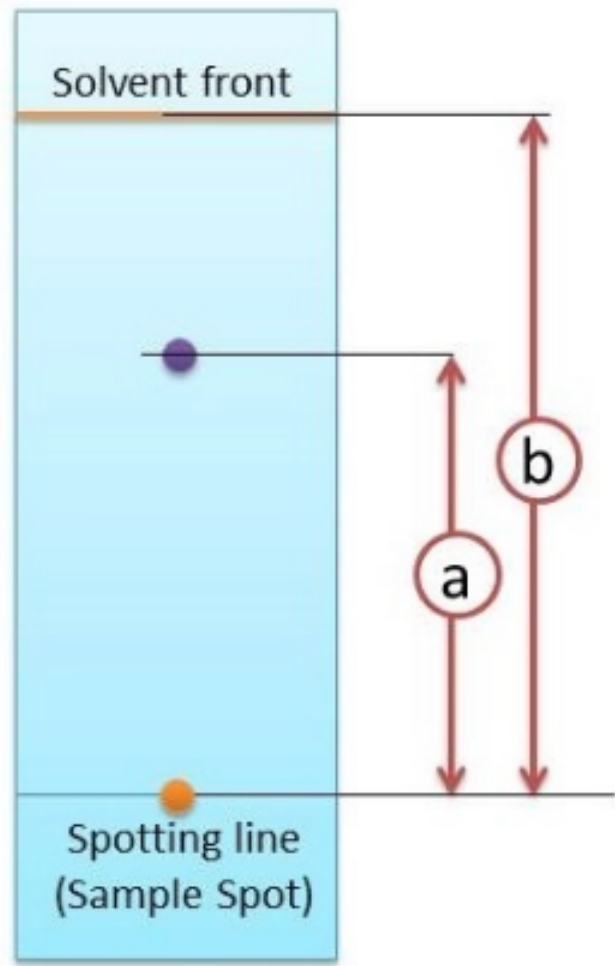
- After the development, the solvent front is marked and left to dry in a dry cabinet or oven.

Detection

- Colorless analytes were detected by staining with reagents such as iodine vapor, ninhydrin, etc.
- Radiolabeled and fluorescently labeled analytes were detected by measuring radioactivity and fluorescence respectively.

R_f values

- Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the baseline.
- The distance traveled relative to the solvent is a constant for a particular compound as long as other parameters such as the type of paper and the exact composition of the solvent are constant.
- The distance traveled relative to the solvent is called the R_f value.
- Thus, in order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, “R_f value” is calculated for each separated component in the developed chromatogram.



$$R_f = \frac{\text{distance travelled by the component}}{\text{distance travelled by the solvent}}$$
$$= \frac{a}{b}$$

Calculating the Retention Factor Value

Applications of Paper Chromatography

- To check the control of purity of pharmaceuticals,
- For detection of adulterants,
- Detect the contaminants in foods and drinks,
- In the study of ripening and fermentation,
- For the detection of drugs and dopes in animals & humans
- In analysis of cosmetics
- Analysis of the reaction mixtures in biochemical labs.

Advantages of Paper Chromatography

- Simple
- Rapid
- Paper Chromatography requires very less quantitative material.
- Paper Chromatography is cheaper compared to other chromatography methods.
- Both unknown inorganic as well as organic compounds can be identified by paper chromatography method.
- Paper chromatography does not occupy much space compared to other analytical methods or equipments.
- Excellent resolving power

Limitations of Paper Chromatography

- Large quantity of sample cannot be applied on paper chromatography.
- In quantitative analysis paper chromatography is not effective.
- Complex mixture cannot be separated by paper chromatography.
- Less Accurate compared to HPLC or HPTLC