

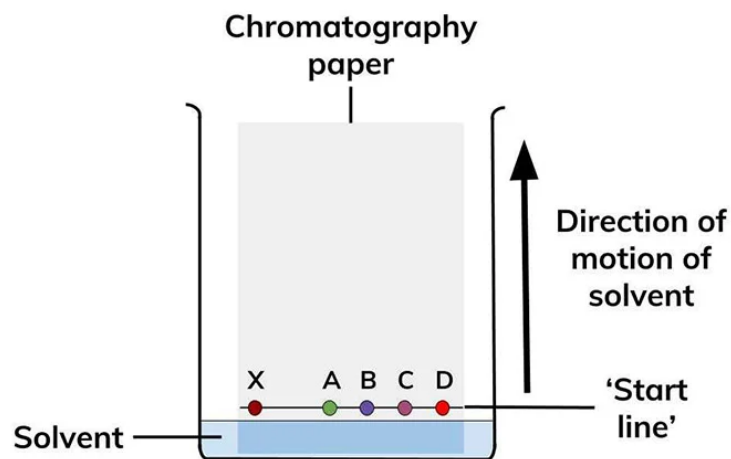
Paper Chromatography

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❖ What Is Paper Chromatography?

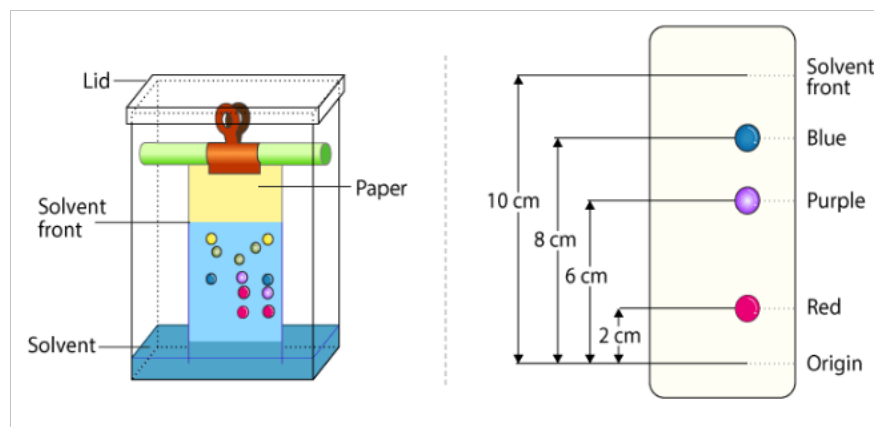
Chromatography technique that uses paper sheets or strips as the adsorbent being the stationary phase through which a solution is made to pass is called paper chromatography. It is an inexpensive method of separating dissolved chemical substances by their different migration rates across the sheets of paper. It is a powerful analytical tool that uses very small quantities of material. Paper chromatography was discovered by Synge and Martin in the year 1943.



❖ Paper Chromatography Principle:

The principle involved can be partition chromatography or adsorption chromatography. Partition chromatography because the substances are partitioned or distributed between liquid phases. The two phases are water held in pores of the filter paper and the other phase is a mobile phase which passes through the paper. When the mobile phase moves, the separation of the mixture takes place. The compounds in the mixture separate themselves based on the differences in their affinity towards stationary and mobile phase solvents under the capillary action of pores in the paper. Adsorption chromatography between solid and liquid phases, wherein the solid surface of the paper is the stationary phase and the liquid phase is the mobile phase.

Paper Chromatography Diagram:



❖ Instrumentation Of Paper Chromatography:

- 1) Stationary phase & papers used
- 2) Mobile phase
- 3) Developing Chamber
- 4) 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.

2. Paper Chromatography Mobile Phase:

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

Examples-

- Hydrophilic mobile phase
- Isopropanol: ammonia: water 9:1:2
- Methanol : water 4:1
- N-butanol : glacial acetic acid : water 4:1:5
- Hydrophobic mobile phases
- dimethyl ether: cyclohexane kerosene : 70% isopropanol

The commonly employed solvents are the polar solvents, but the choice depends on the nature of the substance to be separated.

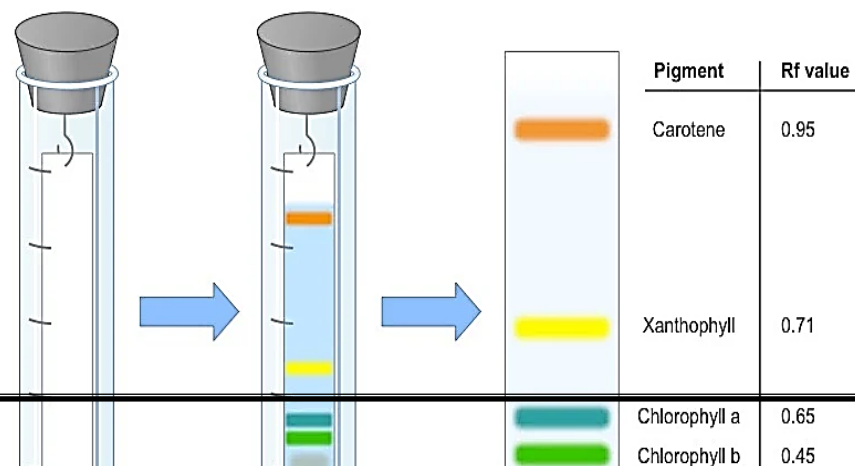
If pure solvents do not give satisfactory separation, a mixture of solvents of suitable polarity may be applied.

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.

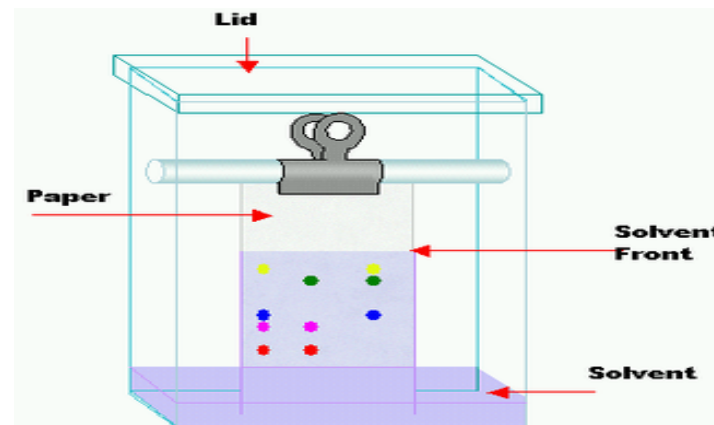


❖ Paper Chromatography Procedure:

Below we have explained the procedure to conduct Paper Chromatography Experiment for easy understanding of students.

- 1) **Selecting a suitable type of development:** It is decided based on the complexity of the solvent, paper, mixture, etc. Usually ascending type or radial paper chromatography is used as they are easy to perform. Also, it is easy to handle, the chromatogram obtained is faster and the process is less time-consuming.
- 2) **Selecting a suitable filter paper:** Selection of filter paper is done based on the size of the pores and the sample quality.
- 3) **Prepare the sample:** Sample preparation includes the dissolution of the sample in a suitable solvent (inert with the sample under analysis) used in making the mobile phase.
- 4) **Spot the sample on the paper:** Samples should be

spotted at a proper position on the paper by using a capillary tube.



5)

Chromatogram

development:

Chromatogram development is spotted by immersing the paper in the mobile phase. Due to the capillary action of paper, the mobile phase moves over the sample on the paper.

- 6) **Paper drying and compound detection:** Once the chromatogram is developed, the paper is dried using an air drier. Also, detecting solution can be sprayed on the chromatogram developed paper and dried to identify the sample chromatogram spots.

❖ Types Of Paper Chromatography

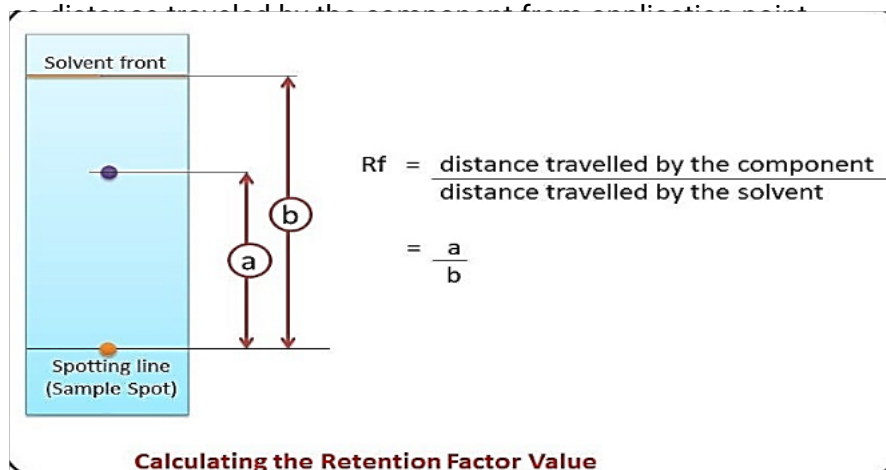
- i. Paper Adsorption Chromatography
- ii. Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase.

- iii. Paper Partition Chromatography
- iv. Moisture / Water present in the pores of cellulose fibers present in filter paper acts as stationary phase & another mobile phase is used as solvent In general paper chromatography mostly refers to paper partition chromatography.

❖ Rf values:

Some compounds in a mixture travel almost as far as the solvent does; some stay much closer to the base line. The distance travelled 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 travelled relative to the solvent is called the Rf value.

Thus, in order to obtain a measure of the extent of movement of a component in a paper chromatography experiment, "Rf value" is calculated for each separated component in the developed chromatogram. An Rf value is a number that is defined



❖ Advantages Of Paper Chromatography:

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

❖ Limitations Of Paper Chromatography:

- i. Large quantity of sample cannot be applied on paper chromatography.
- ii. In quantitative analysis paper chromatography is not effective.
- iii. Complex mixture cannot be separated by paper chromatography.

iv. Less Accurate compared to HPLC or HPTLC

and Sham K. Anand.

iv. <https://microbenotes.com/paper-chromatography/>

❖ Paper Chromatography Applications:

There are various applications of paper chromatography. Some of the uses of Paper Chromatography in different fields are discussed below:

- i. To study the process of fermentation and ripening.
- ii. To check the purity of pharmaceuticals.
- iii. To inspect cosmetics.
- iv. To detect the adulterants.
- v. To detect the contaminants in drinks and foods.
- vi. To examine the reaction mixtures in biochemical laboratories.
- vii. To determine dopes and drugs in humans and animals.

❖ References:

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- ii. Pharmaceutical analysis volume second by Ashutosh kar.
- iii. Instrumental method of chemical analysis by G.R.Chatwal

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