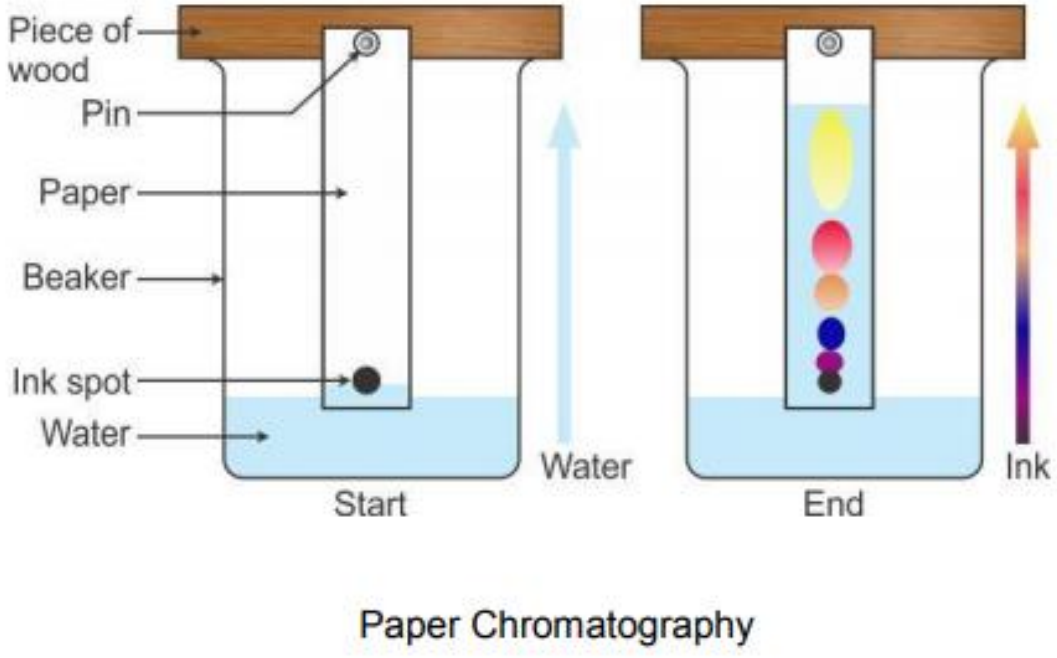
Paper Chromatography

By

Dr Gohar Taqi Kazimi

Department of Chemistry

University of Sargodha



Paper Chromatography

Paper Chromatography

It is one type of partition chromatography in which substances are distributed between two liquids, i.e., the stationary liquid (usually water) which is held in the fibers of the paper and called stationary phase; the other is the moving liquid or developing solvent and called the mobile phase.

PRINCIPLE OF SEPARATION

The principle involved is partition, where the substances are distributed or partitioned between two liquid phases. One phase is the water which is held in pores of filter paper used and other phase is that of mobile phase which moves over the paper. Separation of components depends on both their solubility in the mobile phase and their differential affinity to the mobile phase and stationary phase.

The principle can also be adsorption, between solid and liquid phases, where the stationary phase is the solid surface of paper and the liquid phase is of mobile phase. Paper impregnated with silica or alumina acts as adsorbent (stationary phase) and solvent as mobile phase.

Most of the applications of paper chromatography work on the principle of partition.

THEORY

Two types of forces operate when a drop of solution is applied on the filter paper and treated with a solvent.

Propelling force:

It tries to drag the substances in the direction of the flow of solvent. This depends upon;

- The rate of the solvent flow
- The solubility of the substance in the solvent
- The component with higher solubility will move rapidly along the filter paper than the less soluble component

Retarding force:

In paper chromatography the results are represented by Rf value which represents the movement or migration of solute relative to the solvent front.

FACTORS AFFECTING R_f VALUES:

- The temperature
- The solvent used
- Quality of the paper
- Techniques employed
- The distance travelled by the solvent and the solute
- Chemical reactions between the substance to be separated and the solution
- The concentration of the separated substance

Types of paper chromatography:

Ascending Paper Chromatography:

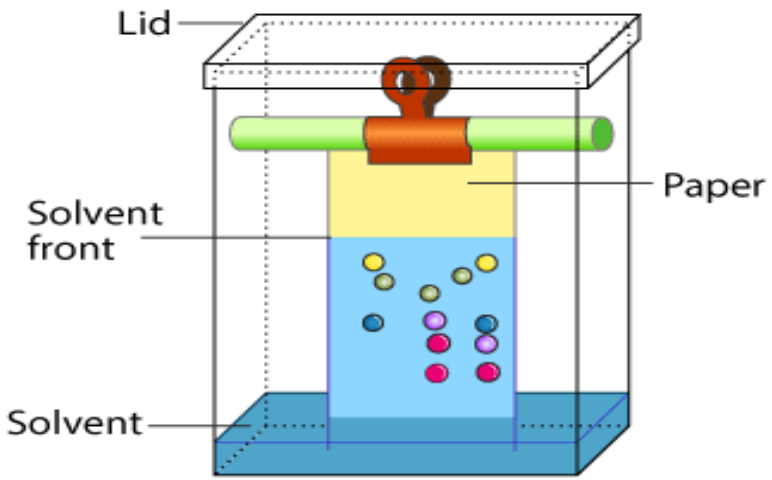
The techniques goes with its name as the solvent moves in an upward direction.

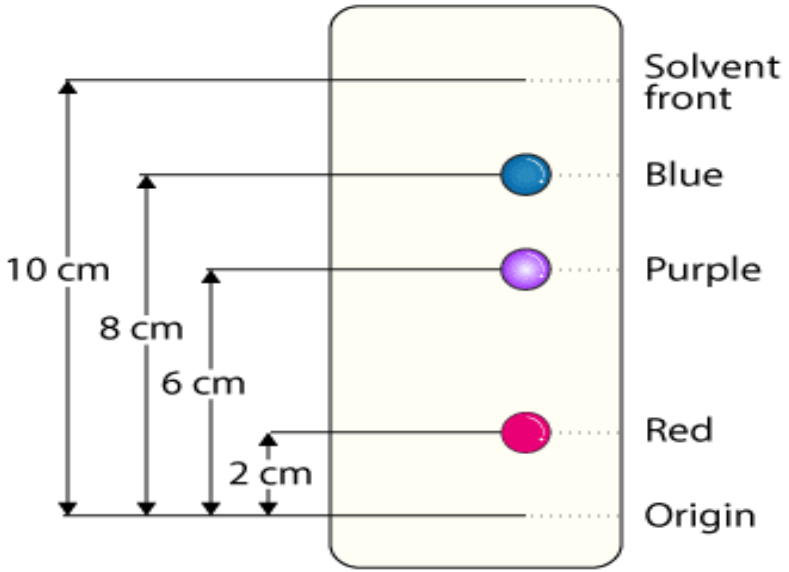
Apparatus: Well-sealed glass tank of suitable size and shape, Trough for mobile phase in upper portion Paper with upper end in the trough, Glass jar equilibrated with mobile phase prior to elution

Advantage: Apparatus is simple and easy to construct

Disadvantage: Slow development compared to descending technique.

Paper Chromatography





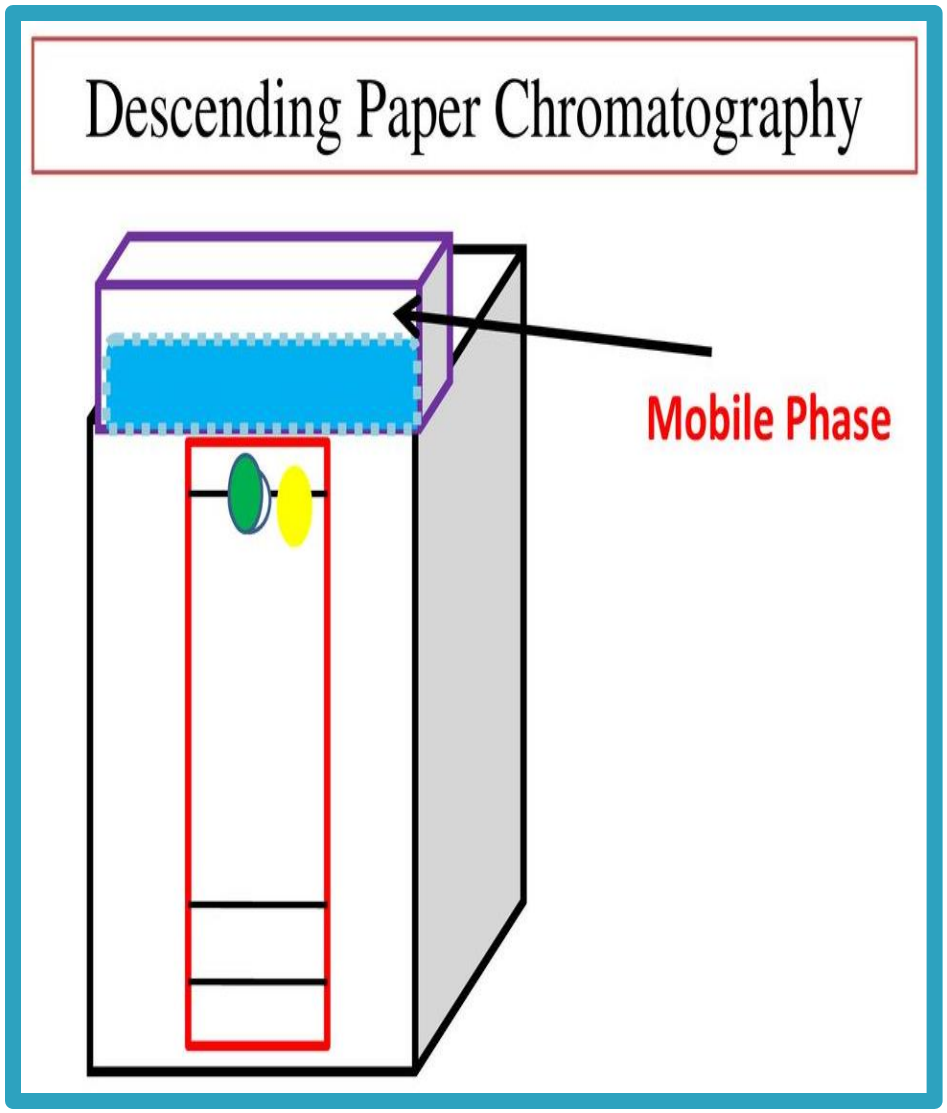
Descending Paper Chromatography:

The flow of the solvent is due to gravitational pull and capillary action is downwards hence the name descending paper chromatography.

Apparatus: Well-sealed glass jar containing mobile phase at the bottom of chamber. Hook to suspend paper in jar. Paper may also be rolled in jar, held together by staples, stings or plastic clips. Paper

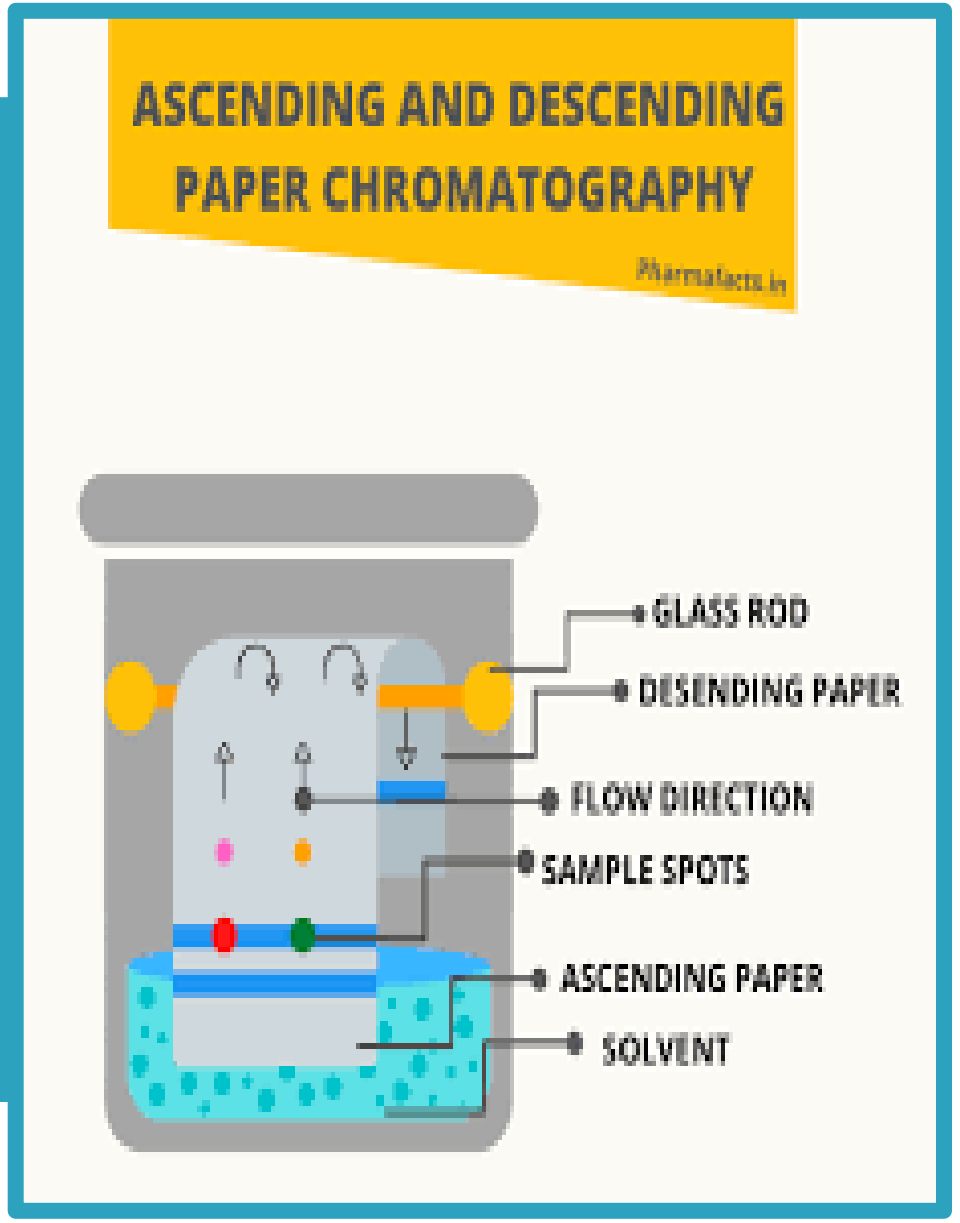
Advantages: Separation is quick and easy due to gravity and capillary action. This technique is preferred if Rf values of various constituents are almost same. Development can be continued indefinitely even through the solvent runs off at the other end of paper.

Disadvantage: The descending technique is a complex set up


Descending Paper Chromatography
Mobile Phase

Ascending–Descending Paper Chromatography:

In this chromatography movement of solvent occurs in two directions after a particular point. Initially, the solvent travels upwards on the paper which is folded over a rod and
after crossing the rod it continues with its travel in the downward direction



Two Dimensional Paper Chromatography:

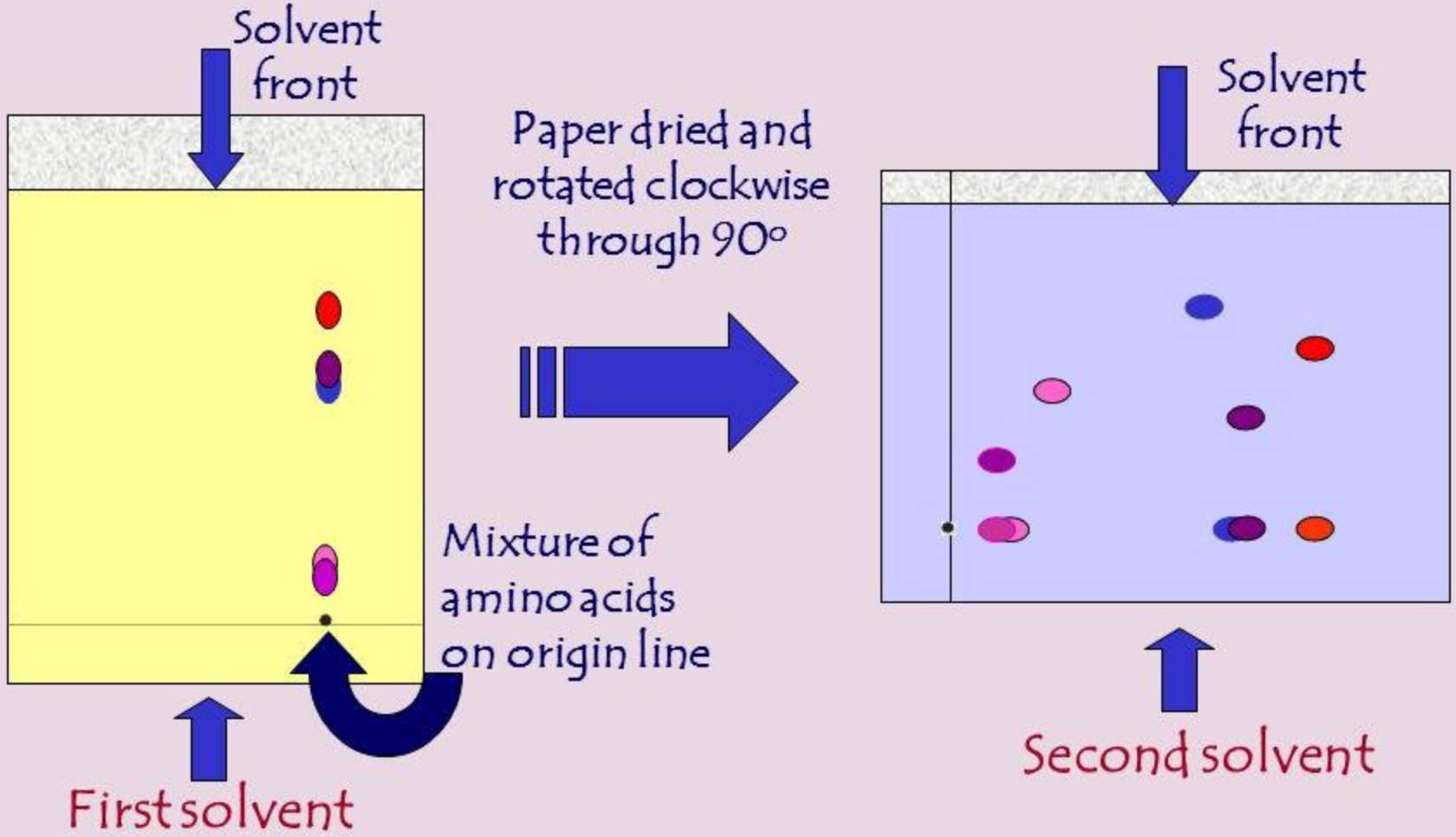
Substances which have the same r_f values can be resolved with the help of two-dimensional paper chromatography

Apparatus:

Rectangular/square filter paper

Well-sealed glass Jar

In this mode the samples are spotted to one corner of rectangular paper and allowed for first development. Then the paper is again immersed in mobile phase at right angle to previous development for second chromatogram.

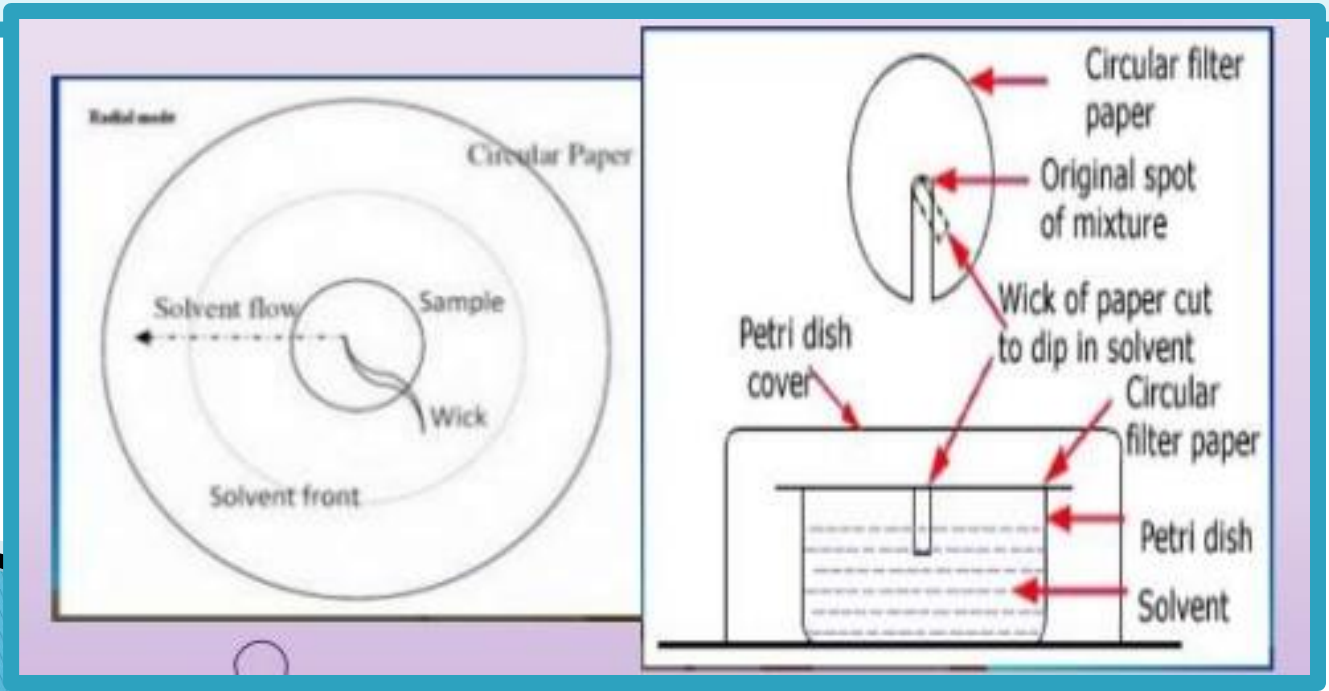


Two-way chromatography provides better separation of substances that behave in a similar fashion in the first solvent.
A second run in a different solvent resolves two very close spots more clearly

Radial or Circular Paper Chromatography

Procedure:

Spot is applied on filter paper using capillary. After the spot is dried, paper is fixed horizontally on the petri dish containing mobile phase. The wick at the center of paper dips into mobile phase in a petri dish and solvent rises through this wick. The solvent travels from center towards periphery of circular chromatography paper. The entire system is kept in a covered petri dish for development of chromatogram. Components get separated in the form of concentric circular zones.



Paper for Chromatography:

A wide variety of papers are available commercially in different sizes, shapes, porosities, thickness and chemical treatments.

Composition: Paper is composed of randomly directed cellulose fibers. Cellulose itself is a network of polymeric carbohydrate chains possessing hydrophilic character and cross linked by stable hydrogen bonded system.

Types

Glass Fiber Type Papers:

If very corrosive eluting conditions are required, a glass fiber type paper can be used. By special treatments, adsorption effects due to glass can be minimized.

Modified Papers: acid or base washed filter 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

Modified Cellulose Papers

Cellulose paper can be modified in several ways to alter its behavior. Several types of modified cellulose paper and their characteristics are as followed;

Types	Characteristics	Uses
Carboxyl papers	Exchange capacity of paper is increased by increasing carboxyl content by partial oxidation	For efficient separation of polar substances. Cationic separation of potential amines and amino acids
Acetylene papers	Such paper has hydrophobic property	Reverse phase chromatography of lipophilic substances like steroids, insecticides, pigments and metal cations

Whatman Papers

These are commonly used for chromatography purpose have a content of 99% α-cellulose. There are various types of Whatman papers available. The choice of this paper depends on the type of separation

Types

- Kieselguhr papers,
- Alumina papers,
- Zirconia papers,
- Silica papers

STATIONARY PHASES:

- **Aqueous stationary phase Water**
- Separation of moderately polar to very polar (ionic) mixture

- **Hydrophilic stationary phase**

Methanol, Formamide, Glycols, Cellosolves and Glycerol

- **Hydrophobic stationary phase**

Dimethylformamide, Kerosene, Aromatic and aliphatic hydrocarbons, oxygenated solvents

Mobile Phase:

Mixtures of two, three or more solvents, solutions of salts and solutions of buffers can be used as mobile phase.

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

Solvents used are listed in order of increasing polarity.

n-hexane < cyclohexane < carbon tetrachloride < Benzene < Toluene < Trichloroethylene < Diethyl ether < Chloroform < Ethyl acetate < n-butanol < n-propanol < Acetone < Ethanol < Methanol < Water

General criteria for good solvent system:

Rf values of sample should lie between 0.05 and 0.85 in the system .

The difference between the Rf values of any two components must be 0.05; the minimum value necessary in order to separate any two components.

The distribution ratios of the components in the solvent system should be independent of concentration so as to get circular spots.

The solvents should not undergo chemical reaction with any of the components of the sample mixture.

The solvent should not interfere with detection of spots on the developed chromatogram.

The composition of solvent system should not alter with time.

Suitable solvents for paper chromatography

If a pure solvent is not satisfactory, solubility of suitable polarity may be obtained by trying out mixtures in various proportions of solvents . Stationary phase Mobile phase
Water, Isopropanol-Ammonia-Water (9:2:1)
Water, n-butanol-Acetic acid-Water (4:1:5)
Water, Phenol saturated with water
Formamide, Chloroform Formamide Benzene
Formamide, Benzene-Cyclohexane Dimethyl Formamide
Liquid paraffin, Dimethyl Formamide-Methanol-Water (10:10:1)

Solvent Miscibility Table

SOLVENT NAME	WATER	METHANOL	ETHANOL	BUTANOL	CARBON TETRA CHLORIDE	CHLORO- FORM	METHANOIC ACID	PROPANONE
WATER	X	Y	Y	N.1	N.2	N.2	Y	Y
METHANOL	Y	X	Y	Y	Y	Y	R	Y
ETHANOL	Y	Y	X	Y	Y	Y	R	Y
BUTANOL	N.1	Y	Y	X	Y	Y	R	Y
CARBON TETRA CHLORIDE	N.2	Y	Y	Y	X	Y	N	Y
CHLORO-FORM	N.2	Y	Y	Y	Y	X	N	Y
METHANOIC ACID	Y	R	R	R	Y	Y	X	Y
PROPANONE	Y	Y	Y	Y	Y	Y	Y	X

Final Notes:

Chloroform, Tetrachloromethane and Butanol, cannot be diluted with water to form variable concentrations.

If water, an alcohol and chloroform (or tetrachloromethane) are mixed together, an upper layer of water and a bottom layer of alcohol + chloroform is formed. This shows that alcohol prefers to dissolve in chloroform rather than in water.

The following are solvents which can dissolve plastics, hence the chromatography of these must be performed in glass containers/test tubes.

Tetrachloromethane (Carbon tetrachloride) CCl_4
Trichloromethane (Chloroform) $CHCl_3$
Propanone (Acetone) CH_3COCH_3

Applications of Paper Chromatography

Some of the uses of Paper Chromatography in different fields are as below:

- To study the process of fermentation and ripening.
- To check the purity of pharmaceuticals.
- To inspect cosmetics.
- To detect the adulterants.
- To detect the contaminants in drinks and foods.
- To examine the reaction mixtures in biochemical laboratories.
- To determine dopes and drugs in humans and animals.
- Separation of mixtures of drugs
- Separation of carbohydrates, vitamins, antibiotics, proteins, etc.
- Identification of drugs Identification of impurities
- Analysis of metabolites of drugs in blood, urine etc.

PRACTICAL REQUIREMENTS

- Preparation of paper
- Preparation of sample solution
- Application of sample
- preparation of solvent
- Preparation of environment of glass jar
- Development
- Drying
- Detection
- Physical methods
- Chemical methods

Preparation of paper:

Cut the paper into desired shape and size depending upon work to be carried out . The starting line is marked on the paper with an ordinary pencil 5cm from the bottom edge. On the starting line marks are made 2cm apart from each other.

Preparation of sample solution:

Choice of suitable solvent for making solution is very important. Pure solutions can be applied directly on the paper but solids are always dissolved in small quantity of a suitable solvent. Biological tissues are treated with suitable solvents and their extracts obtained. Proteins can be precipitated with alcohol and salts can be removed by treatment with ion exchange resin.

Application of sample:

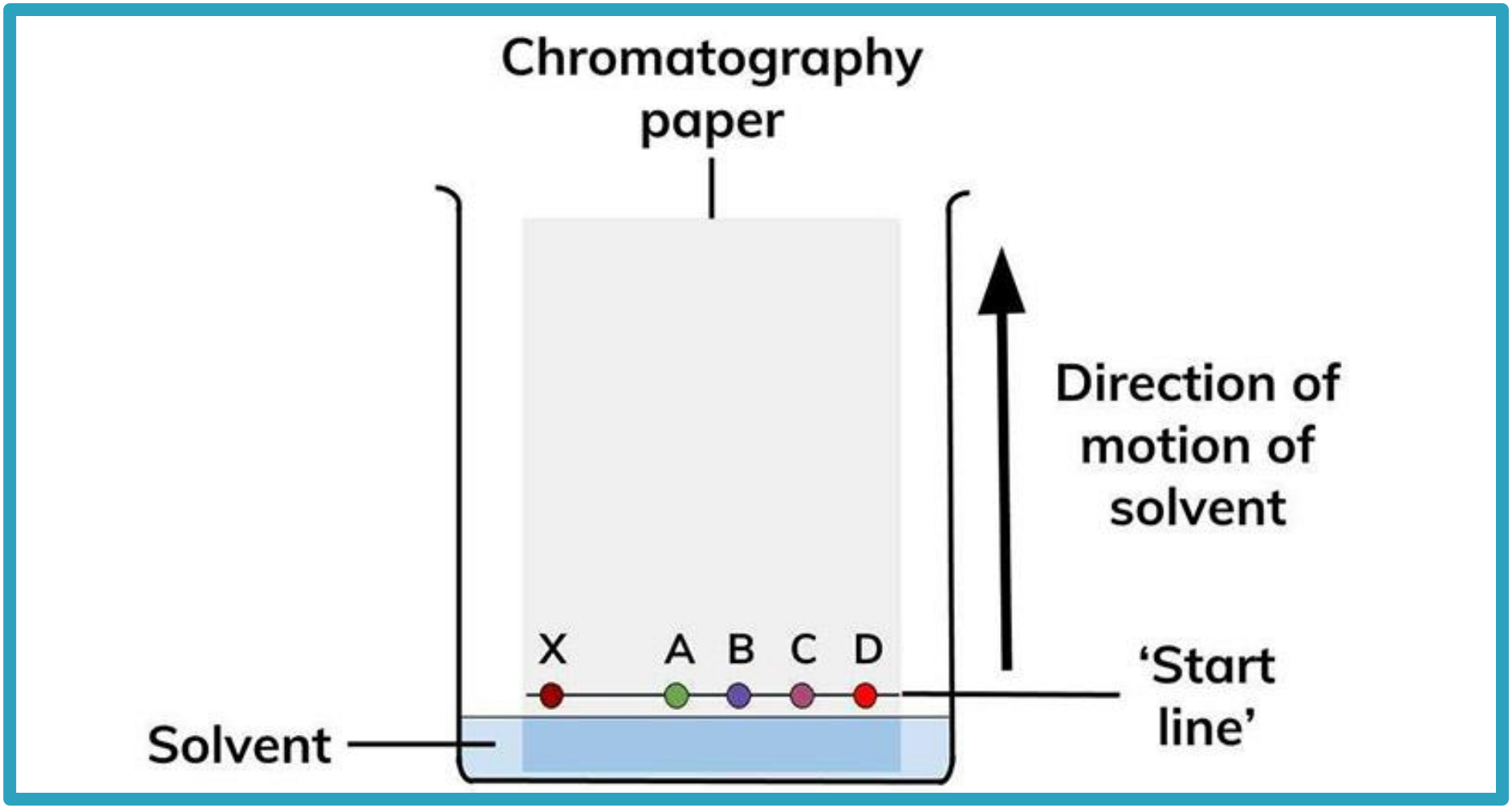
The sample to be applied is dissolved in the mobile phase and applied as a small spot on the origin line, using capillary tube or micropipette. Very low concentration is used to avoid larger zone. The spot is dried on the filter paper and is placed in developing chamber.

Preparation of solvent:

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.

Preparation of environment of glass jar:

The chromatographic jars may be made up of many materials like glass, plastic or stainless steel. Glass tanks are preferred most. They are available in various dimensional sizes depending upon paper length and development type. Chamber is filled with solvent and lid is closed. The chamber atmosphere is allowed to saturate with solvent vapor.


Chromatography paper
Direction of motion of solvent
X
A
B
C
D
'Start line'
Solvent

Development :

Several types of development are possible which increases the ease of operation. The paper is dipped in solvent in such a manner that the spots will not dip completely into the solvent. The solvent will rise up and it is allowed to run 2/3 rd. of paper height for better and efficient result.

Drying of chromatogram:

After the solvent has moved a certain distance for certain time, the chromatogram is taken out from the glass jar. Position of the solvent front is marked with a pencil. Paper is dried by cold or hot air depending on volatility of solvents. A simple hair dryer is a convenient device to dry chromatograms.

Detection :

Detection If the substances are colored they are visually detected easily. But for colorless substance, physical and chemical methods are used to detect the spot.

PHYSICAL METHODS

Iodine chamber method

UV chamber for fluorescent compounds – at 254 or at 365nm

CHEMICAL METHODS

SPRAYING METHODS

Spraying agents used are;

Ferric chloride

Ninhydrin in acetone

Dragendroff's reagents

3,5 dinitro benzoic acid

These methods are used for identification of; Phenolic compounds and tannins, Amino acids, Alkaloids, Cardiac glycosides