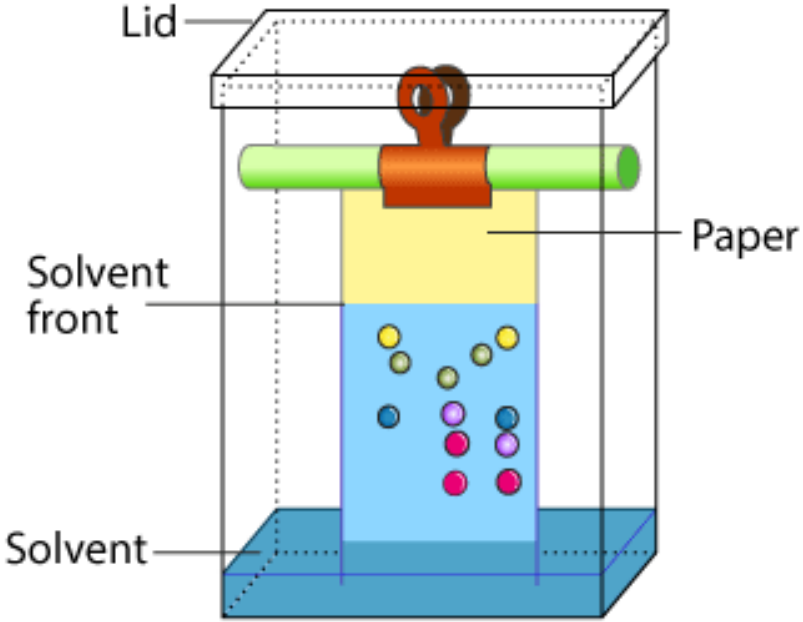
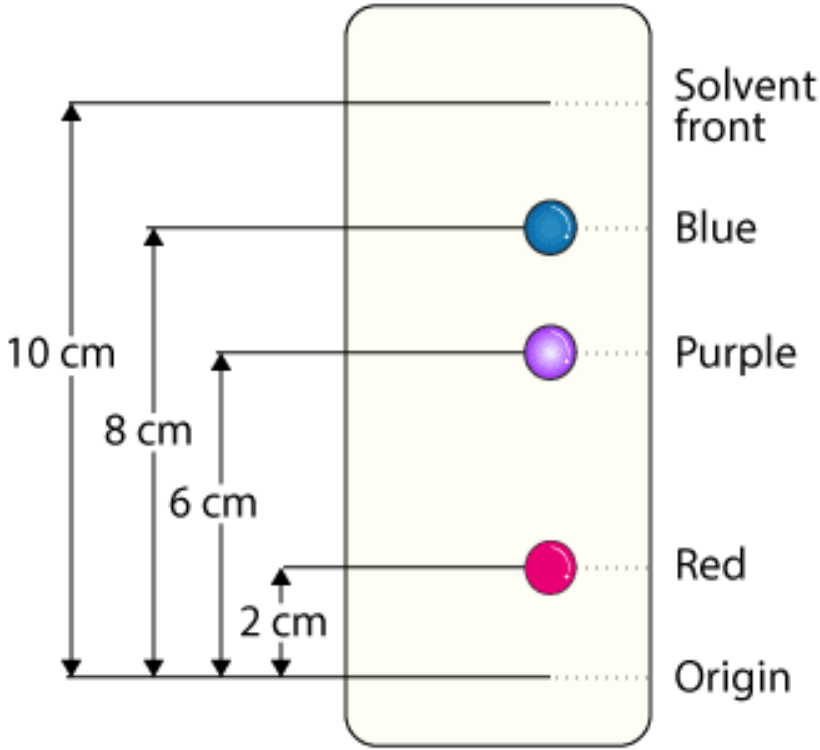
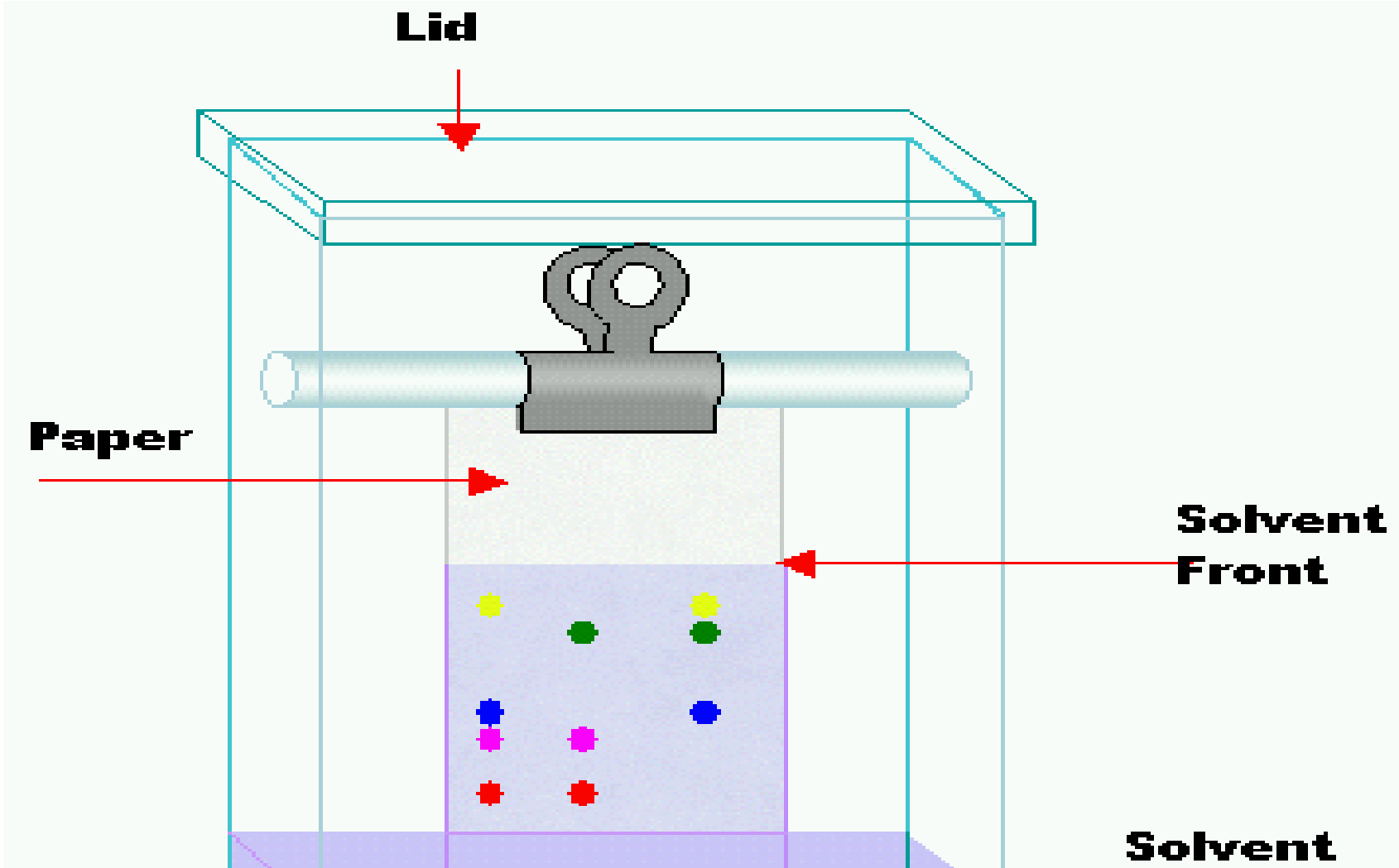
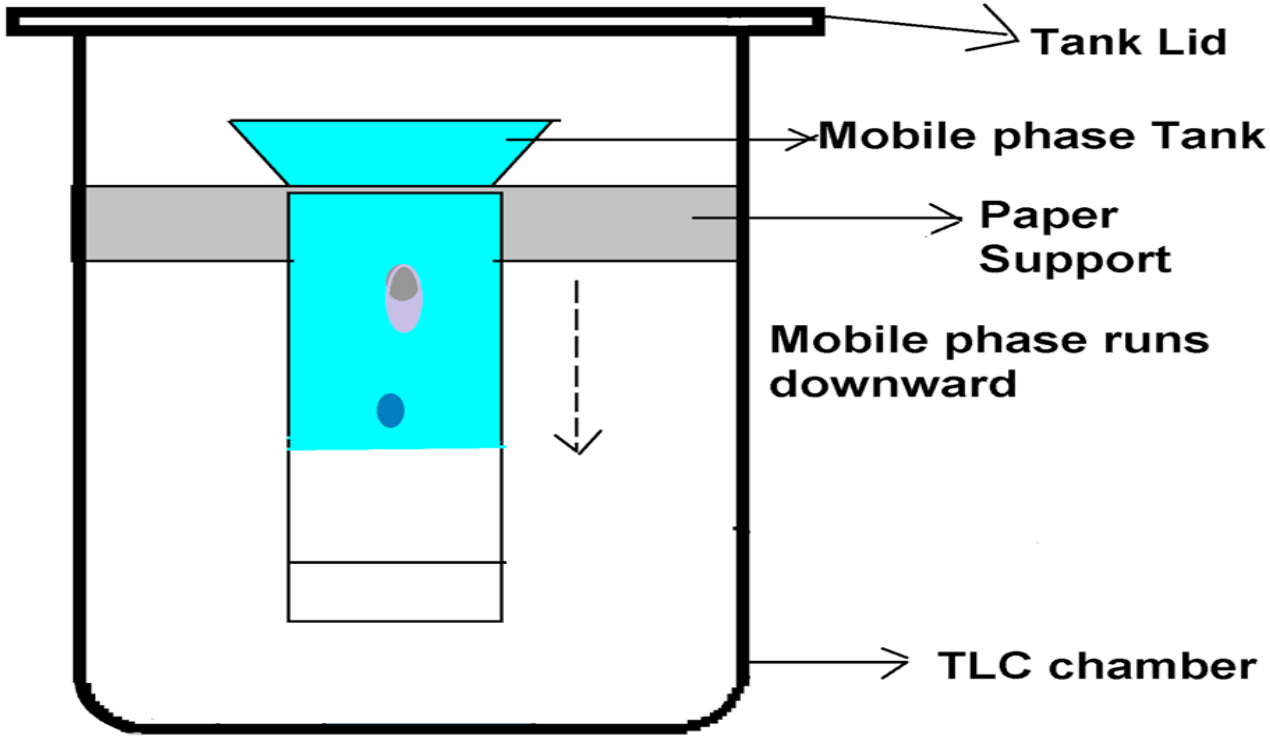
PAPER CHROMATOGRAPHY






Lid
Paper
Solvent
Front
Solvent

Descending Chromatograpphy

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Paper chromatography is generally used for the separation of water-soluble organic and inorganic compounds or highly polar compounds, such as amino acids and sugars. Mixtures of cations and mixtures of anions, in the solution form, are also separated by paper chromatography.

In paper chromatography, the stationary phase is water, which is supported by the paper. The paper used for paper chromatography contains 22% by weight of water which is the stationary phase. The mobile phase for paper chromatography is generally a polar organic solvent and water.

In paper chromatography, the stationary phase is a liquid (water) and the mobile phase is also a liquid (organic solvent + water). For this reason, paper chromatography is also called liquid–liquid chromatography. These two liquids constitute a two phase system (and can be considered as equivalent to two immiscible liquids taken in a separating funnel). The compounds in a mixture (e.g., A + B) undergo partition or distribution between the two liquid phases. Further, the compounds of the mixture A and B have different values of partition or distribution coefficients. The differences in the partition coefficients form the basis for separating mixtures of compounds using paper chromatography. For this reason, paper chromatography is one kind of **partition chromatography**.

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31 ET	225

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3.5.2 APPLICATION OF

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3.5.1 Nature of Paper—Support, Stationary Phase

Paper: Stationary phase The paper used for paper chromatography is made from cotton cellulose. It is commercially available in different thicknesses and densities and in different shapes, such as rectangular, circular or strip-form. The paper used for paper chromatography is a type of filter paper, and generally those manufactured by the Whatmann brand name are used (Table 3.4). For general paper chromatography work, the Whatmann No.1 grade of paper is used. This paper consists of a mass of small cellulose fibres randomly matted together to form a 3-dimensional network with relatively large open spaces. That is, the paper has a porous character. In these spaces lie the water molecules (22% by weight of the paper). These water molecules constitute the stationary phase of the paper chromatography set-up. The water molecules are held to the cellulose by adsorption forces. This water is incorporated into the paper at the time of manufacture.

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Table 3.4 Characteristics of Whatmann Chromatography Papers

Grade	Flow rate of Water	Density g/cm	Thickness mm

forces. This water is incorporated into the paper at the time of

Table 3.4 Characteristics of Whatmann Chromatography Papers

Grade	Flow rate of Water in mm/30 min	Density g/cm	Thickness mm
20	85	0.58	0.16
2	115	0.54	0.18
1	130	0.54	0.16
3 MM	130	0.56	0.33
3	130	0.49	0.38
4	180	0.46	0.20
17	190	0.50	0.88
31 ET	225	0.36	0.53

The role of the paper is that of an inert support for the water stationary phase. Thus, overall, the paper used for paper chromatography constitutes the stationary phase. It should be noted that in all chromatographic techniques with liquid as the

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Fig. 3.18 Descen

3.5.2 Application of sample, solvent systems: Mobile phase

Technique of paper chromatography Whatmann No.1 paper of a suitable size and shape is chosen. At one end of the paper, at a distance of about 3–4 cm, draw a line with a pencil. On this line the sample solution/s to be analyzed is spotted. Consider a sample solution containing two compounds A and B (which may be amino acids, sugars, cations, anions, etc.) The solution is spotted at the marked

Mobile phase The mobile phase, which is generally a polar organic solvent and water mixture, is taken in a closed glass chamber. This chamber is made of glass and is rectangular or cylindrical. The mobile phase liquid may be placed in a specially designed trough fixed at the top end of the chamber or it may be placed in the bottom of the chamber. When the liquid mobile phase is placed at the top end of the chamber, this technique is known as descending paper chromatography; whereas if the liquid mobile is placed in the bottom of the chamber, it is known as ascending paper chromatography (Fig. 3.18). The descending paper chromatography technique is more commonly used.

The mobile phase is generally mixture of solvents but it always contains water as a component. Some typical useful mobile phases are given in Table 3.5.

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Table 3.5 Common Mobile Phases in Paper Chromatography

Compounds	Mobile Phase solvent system
Separation of amino acids	Butanol : acetic acid: H_2O (4 : 1 : 5)
Separation of sugars	Ethyl acetate: Pyridine : H_2O (2 : 1 : 2)
Separation of mixture of Cu, Ni, Mn, Co chlorides	Acetone : Conc. HCl : H_2O (87 : 8 : 5)
Separation of halides F^-, Cl^-, Br^-, I^-	Pyridine : H_2O (90 : 10)

...ing paper chromatography technique is used (Fig. 3.18), the spotted paper, from its spotted end, is cautiously inserted into the trough containing the mobile phase and fixed in position with a glass rod. The paper hangs down from the top of the chamber. The mobile phase percolates through the fibres of the paper by capillary action and also by gravity. As the mobile phase moves down (descends), the compounds A and B also move downwards, but they move at different speeds and thus get separated. In general, the movement of the solvent of the mobile phase (**solvent front**) is faster than the compounds A and B. The movement of the compounds A and B by the mobile phase solvent is known as the development of the paper. The development generally requires about 24 hrs (for standard size sheets). When the solvent front has moved nearly 4/5 of the length of the paper, the paper is taken out of the chamber, the solvent front marked with a pencil and the paper is allowed to air dry. If the compounds A and B are coloured compounds, they are directly visible. These spots are circled with a pencil. If the

paper, from its spotted end, is carefully dipped into the mobile phase solvent contained in the bottom of the chamber and the paper is held in a vertical position by hanging it from a hook or clip fixed to the top cover of the chamber. The mobile phase solvent (moves up or ascends) percolates through the fibres of the paper by capillary action only.

As the mobile phase ascends on the paper, the compounds A and B also move upwards, but at different speeds. The paper is developed till the solvent front reaches 4/5 of the paper length, the paper is taken out of the chamber, the solvent front marked, and the spots from A and B are located and marked.

$$\text{Partition Coefficient of Compound B} = \frac{\text{Conc. of B in sta. phase}}{\text{Conc. of B in mob. phase}}$$

$$\text{Partition Coefficient of Compound A} = \frac{\text{Conc. of A in sta. phase}}{\text{Conc. of A in mob. phase}}$$

The partition coefficient of A is greater than that of B. For chromatographic separation it is necessary that the compounds in a mixture must have different partition coefficients. Now, compound B is more soluble in the mobile phase (which is the moving phase), its concentration is more in the mobile phase, and it moves faster relative to A. Compound A has more solubility (more affinity) in the stationary phase (a phase that does not move), its concentration is more in the stationary phase, and therefore it moves slowly relative to B. Thus, compounds A and B get separated on the paper (Fig. 3.20).

3.5.4 Detection of the spots

Locating the spots on the paper: Locating reagents or colour Reagents

The developed paper is taken out of the chamber, the solvent front is marked, and the paper is air-dried. If the compounds in the mixture analyzed are all coloured, they are directly visible. The spots due to the compounds are circled

mixture analyzed are colourless, two general methods are used for their detection or location: (1) Use of UV light, (2) Use of colour reagents. (1) The developed paper is seen under a UV light. The compounds are observed as fluorescing or coloured and their position is marked by circling them with a pencil. (2) The use of colour reagents is commonly adopted. Table 3.6 shows the colour reagents used to detect different classes of compounds or ions on the paper.

Table 3.6 Locating Reagents in Paper Chromatography

Nature of the mixture	Colour reagents	Colour
Amino acids	Ninhydrin	Purple
Sugars	Aniline phthalate	Brown
Cations such as Co, Mn, Ni, Zn	Diphenyl carbazide	Purple, Pale pink, Red, Pink
Anions F^-, Cl^-, Br^-, I^-	Silver nitrate/ fluorescein	Brown

The paper is sprayed with the chosen colour reagent and the compounds appear as coloured spots, which are marked with pencil.

The paper chromatography results are expressed in terms of R_f values, i.e., retardation factor values (Fig. 3.21).

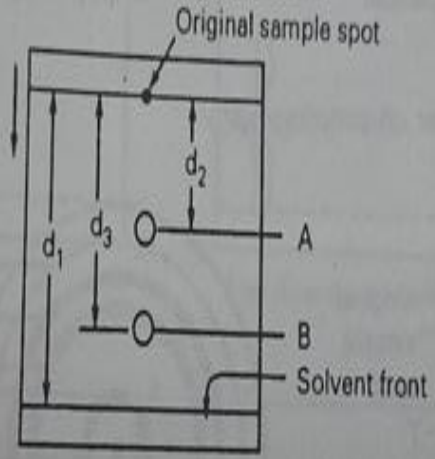


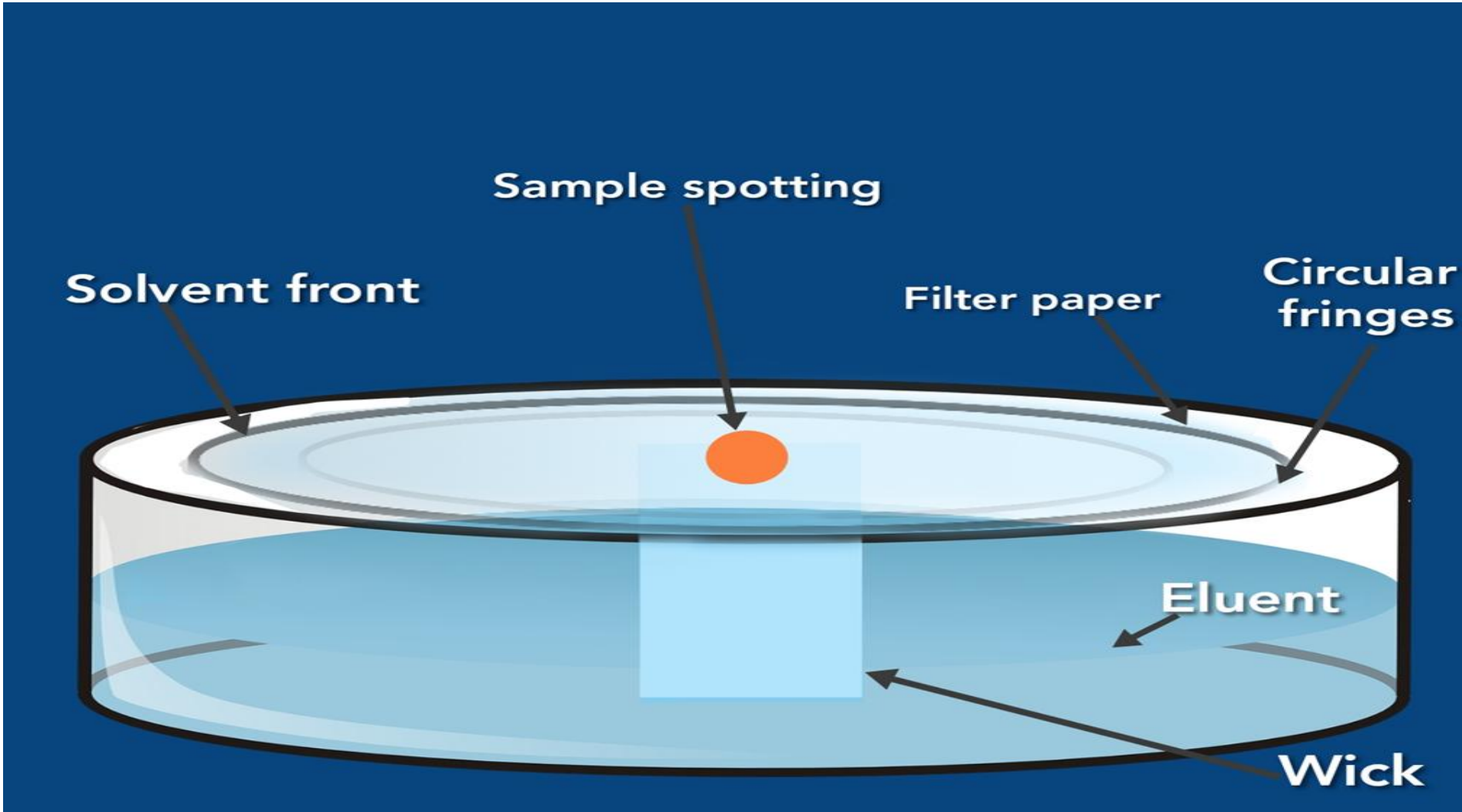
Fig. 3.21 *R_f values in paper chromatography*

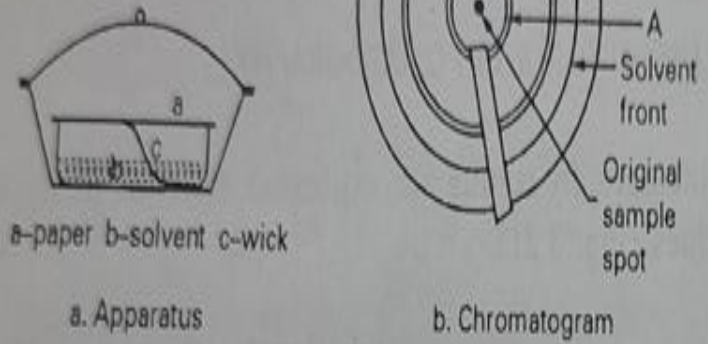
$$R_f(\mathrm{A}) = \frac{\text{Distance moved by Compound A}(d_2)}{\text{Distance moved by the Solvent front }(d_1)}$$

the development. After the development is complete, i.e., when the solvent front has moved to about 4/5 of the length of the paper, it is removed, the solvent front is marked, the paper is dried and sprayed with a colour reagent and the compound spots are visualized. The R_f values of the compounds are then calculated.

Horizontal paper chromatography (Fig. 3.23)

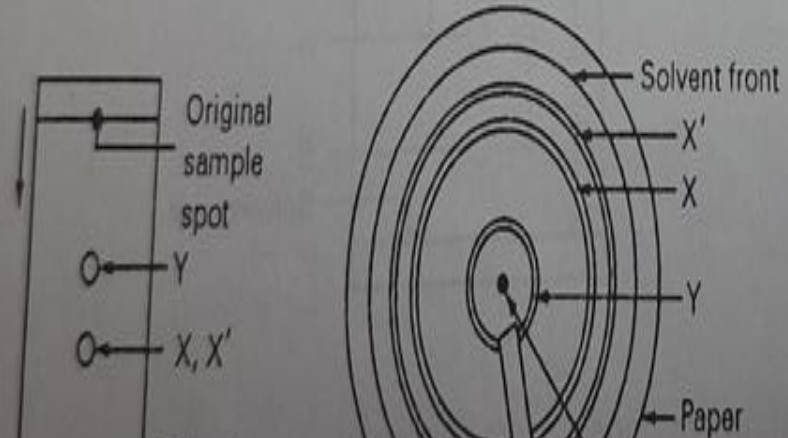
In this method, the sample spot is placed at the centre of a circular-shaped paper. The mobile phase solvent is placed at the bottom of a circular chamber. The spotted paper is held horizontally on the chamber. The solvent for development is applied at the spot by a capillary tube or wick, (which is a cut portion of the circular paper) from which it spreads out radially. The compounds in the mixture spread into a series of concentric bands. In the descending and ascending paper chromatography methods, the compounds in the mixture appear as circular or oval spots after separation.


Sample spotting
Solvent front
Filter paper
Circular fringes
Eluent
Wick

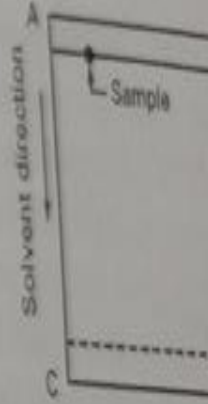


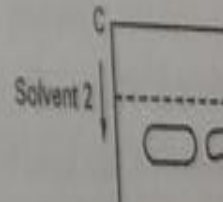
a. Apparatus b. Chromatogram

Fig. 3.23 *Horizontal paper chromatography*



with close R_f values (such as the mi
protein is hydrolyzed) the one-dimens
chromatographic methods do not giv
complex mixtures with close R_f value
used (Fig. 3.25).





The advantage of horizontal paper chromatography lies in its greater resolution. The sample is spread over a greater area now. It is equivalent to using a dilute solution of the sample. The resolution is always better for dilute solutions in all chromatographic techniques. In concentrated solutions, closely situated R_f spots may overlap due to "tailing effects". Thus, a sample solution containing X, X′ and Y may show only two spots in the descending or ascending paper chromatography methods, but three bands in horizontal paper chromatography (Fig. 3.24).

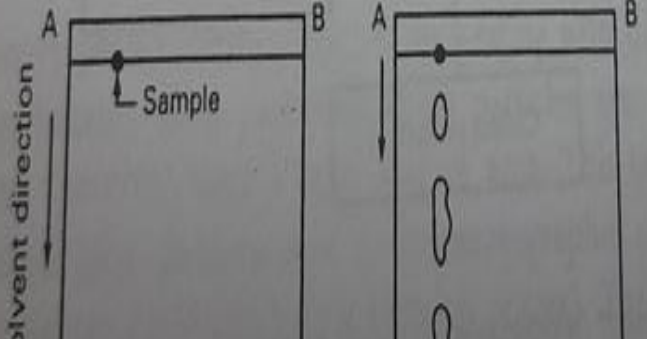
3.5.7 TWO-DIMENSIONAL PAPER CHROMATOGRAPHY AND APPLICATIONS

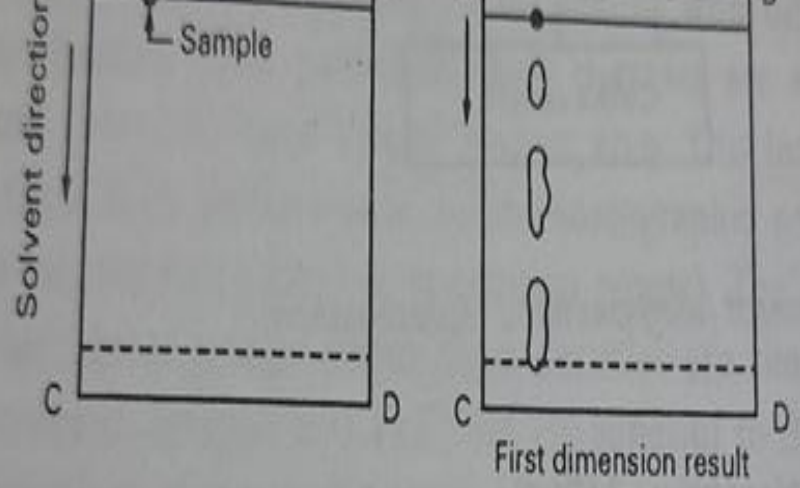
Two-dimensional paper chromatography

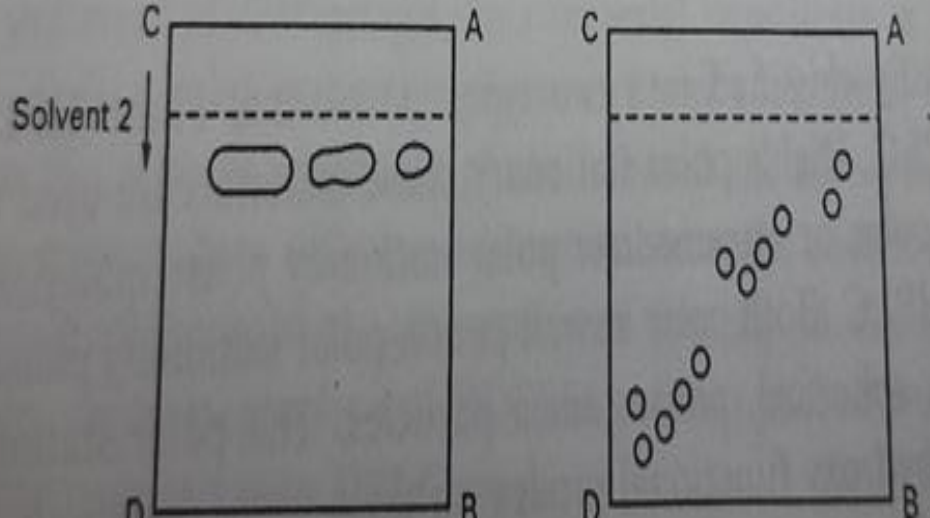
Two-dimensional paper chromatography

In the descending, ascending and horizontal paper chromatography, the solvent moves only once on the paper and in one direction only. These techniques may therefore be called one-dimensional paper chromatography.

In the case of mixtures which are complex, i.e., a larger number of compounds with close R_f values (such as the mixture of amino acids that results when a protein is hydrolyzed) the one-dimensional descending, ascending and horizontal chromatographic methods do not give good separations. For the separation of complex mixtures with close R_f values, two-dimensional paper chromatography is used (Fig. 3.25).







phase : phenol :
each. Detection
of the spots: red

Experiment 2

paper chromat

Conc. HCl : H

diphenyl carba

Procedure A square sheet of Whatmann No.1 paper, marked ABCD at its four corners, is used and the sample containing amino acids is spotted at one corner (A corner). The development of the paper with solvent 1 (n-butanol:acetic acid:water 12:3:5 v/v), with the edge of the paper AB immersed in the solvent trough and carrying out descending paper chromatography, gives partial separation as shown in Fig. 3.25. Three broad spots are seen. The sheet is removed and dried (chemical reagent spraying should not be done at this stage to visualize the spots). This constitutes first dimension chromatography. The paper is turned by 90° now, placed in another paper chromatography chamber with the CA end of the paper fixed into the trough containing solvent 2 (phenol:water 500:125 g) and descending paper chromatography is done. This constitutes second dimension chromatography. After the development is complete, the paper is dried and sprayed with ninhydrin. The separation is complete and clear, and all the component amino acids show up individually (11 different amino acids). The spot pattern is compared with the similar 2-D paper chromatography of known standards and mixtures, by the use of R_f values (Fig. 3.25).

The separation of amino acids, cations and anions by paper chromatography is illustrated by the following three experiments. The relevant experimental parameters

APPLICATIONS

- 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 ....

ADVANTAGES OF P.C

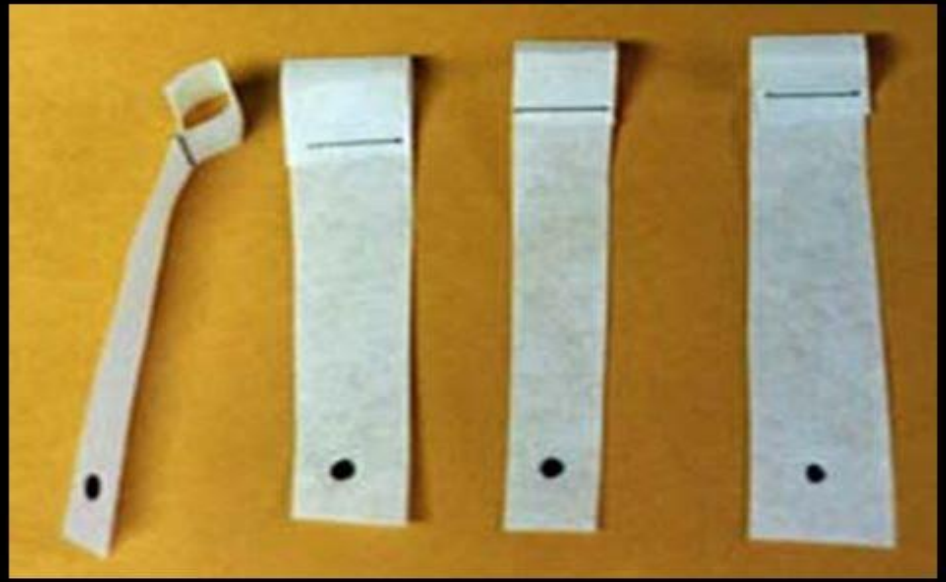
Simple ,rapid ,inexpensive ,excellent resolving power

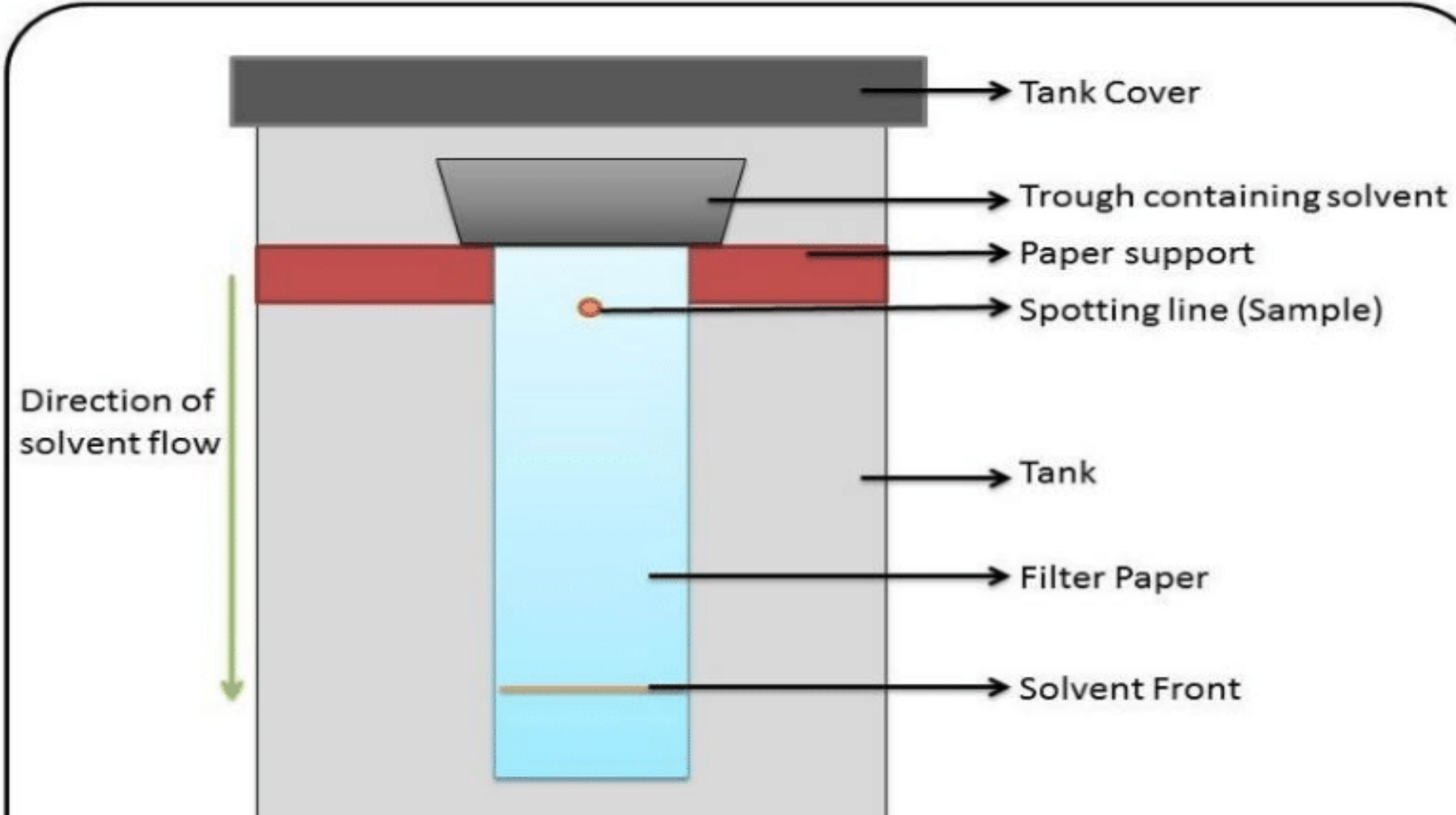
PRECAUTIONS IN P.C

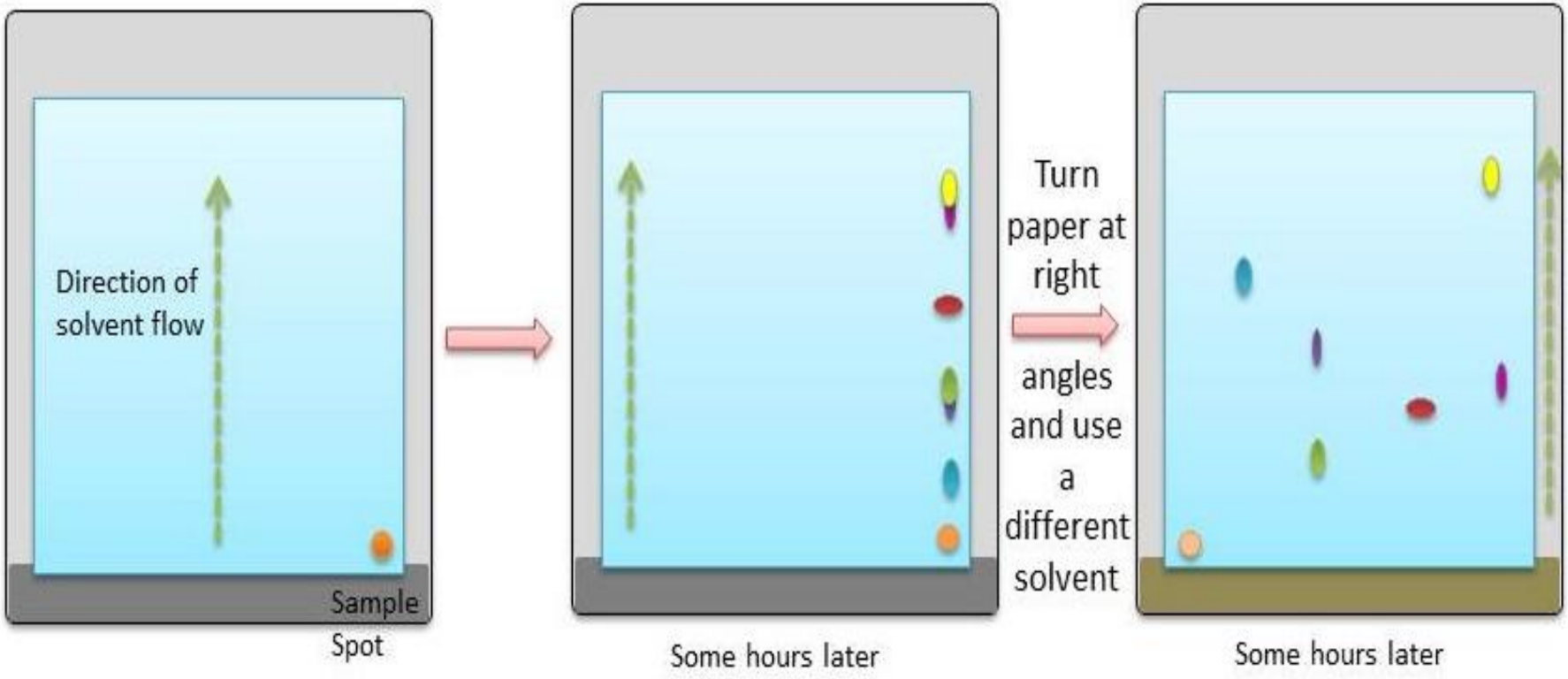
Establishing the vapor solvent equilibrium

Stability of solvent mixture is first ensured

SEPARATION OF AMINO ACIDS BY PAPER CHROMATOGRAPHY




Tank Cover
Trough containing solvent
Paper support
Spotting line (Sample)
Direction of solvent flow
Tank
Filter Paper
Solvent Front



2-Dimensional Paper Chromatography