- If the **Rf** value is **Zero**, it means that the distance travelled by solute is nil and the solute remains in the stationary phase and thus it is immobile.
- If the **Rf** value is **1**, it means that the solute has no affinity with the stationary phase and travels with the solvent front. For example, **Rf values depend on the temperature and solvent** used in the experiment, so same compound can have different **Rf** values in different solvents.

Exp. (1): Determination of retardation factor (Rf) of red ink paper chromatography:

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Procedure & Materials:

1) ***Prepare a strip of chromatographic paper (filter paper) and mark the origin line approximately 2cm from one end with a pencil.***
2) ***Place a drop of red ink onto the marked line of the filter paper strip.***
3) ***Pour about 10 ml of distilled water (D.W.) into a beaker.***
4) ***Dip one edge of the strip into the beaker of distilled water, ensuring that the top of the beaker is covered with a watch glass.***
5) ***Allow the strip to remain inside the beaker for a specific period of time.***
6) ***Measure the distance travelled by the red spot and the solvent line by using ruler, then calculate the Rf for the red ink spot by applying equation (1).***

Exp. (2): Separation of a colourless mixture from lead nitrate & silver nitrate using paper chromatography:

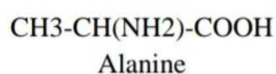
Procedure & Materials:

1) ***Prepare a strip of filter paper and mark the origin line (≈2cm) from one end with a pencil.***
2) ***Place a drop of a mixture containing two ions, lead and silver, onto the marked line of the filter paper strip.***
3) ***Pour about 10 ml of distilled water (D.W.) into a beaker.***
4) ***Dip one edge of the strip into the beaker of D.W., ensuring that the top of the beaker is covered with a watch glass.***

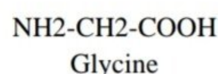
5) ***Allow the strip to remain inside the beaker for a specific period of time until the water rises along the paper and the developer (solvent front) reaches the upper end of the filter paper.***
6) ***Remove the filter paper from the beaker and leave it to dry, then mark the solvent front with a pencil.***
7) ***Take a 0.25M potassium chromate solution as a detector in a watch glass and dip the dried filter paper in the detector solution of K_2CrO_4; brown and yellow coloured zones will appear.***
8) ***Measure the distance travelled by the centre of the brown and yellow zones and also the solvent front using a ruler, then calculate the Rf value for the brown and yellow zones by applying equation (1).***

Exp. (3): Separation of a mixture of different amino acids using paper chromatography:

Amino acids serve as the fundamental building blocks of biological molecules. They possess a bifunctional nature, featuring a basic amino group at one end and a carboxylic acid group at the other. Reactions at these terminal groups can form peptide bonds, linking the amino terminal of one amino acid molecule to the carboxyl terminal of another. This process can be iterated to create long chains of amino acids. Short strings of amino acids are referred to as peptides, while longer chains are identified as proteins. Numerous amino acids play critical roles in biological contexts, including Methionine, Asparagine, Phenylalanine, Glutamine, Proline, Glycine, Threonine, Isoleucine, Tryptophan, Leucine, Valine, and Alanine.



Alanine



Glycine

Procedure & Materials:

1) ***Prepare a strip of filter paper and mark the origin line about 2cm from one end with a pencil.***
2) ***Prepare a solvent mixture of ethanol: water: NH_4OH with a ratio of 8:1:1, respectively.***
3) ***Prepare a developer reagent of 0.1% ninhydrin.***

4) ***Place one drop of a mixture containing amino acids onto the middle of the marked line of the strip.***
5) ***Pour about 10 ml of solvent into a beaker.***
6) ***Dip one edge of the strip into the beaker of solvent, ensuring that the spot of amino acid is not covered by the solvent, and the top of the beaker is covered with a watch glass.***
7) ***Allow the strip to remain inside the beaker for a specific period of time (approximately 30 min.) until the solvent rises along the paper and the developer (solvent front) reaches the upper end of the filter paper.***
8) ***Remove the filter paper from the beaker and leave it to dry; the separated amino acids will appear colourless. Then mark the solvent front with a pencil.***
9) ***Spray the dried filter paper with the developer reagent (ninhydrin) until the different amino acids become coloured.***