Aim 14

To separate Organic acids by Paper Chromatography

Requirements

Citric acid / Oxalic acid, Malic acid, succinic acid, tartaric acid, formic acid, n-butanol, bromophenol blue, distilled water, micropipette, beakers, Whatman's filter paper no. 1, separating funnel and tripod stand.

Preparation of reagents:

Load and prepare the solution of organic acid:

Citric acid = 10 mg/ml of distilled water

Succinic acid = 10 mg/ml of distilled water

Tartaric acid = 10 mg/ml of distilled water

Malic acid = 10 mg/ml of distilled water

Mixture = Add 10 mg of each acid in 10 ml of distilled water

Preparation of running solvent:

n-butanol, formic acid and distilled H_2O are taken in the ratio of 10:2:5 respectively and shaken well. Then, put it into a separating funnel and allow it to stand for some time so that solution gets separated into layers. Discard the lower layer and use the upper layer as running solvent.

Spraying Agent:

It is prepared by the dissolution of 0.04 % bromophenol blue in a clean and dry beaker in the 90 % ethanol.

Procedure

1. Cut the strip of Whatman's filter paper no. 1 (30 X 6 cm) or as per dimensions of the running chamber.
2. Draw a line at the end of the strip (2 cm above from end).
3. Mark two spots on the line at a equidistance from edges and distance of 3 cm from each other.
4. Load one spot of an organic acid such as Citric acid and another spot of a mixture of organic acid using capillary.
5. Now chromatogram is run in the solvent for approx. 15 hours and let it dry at room temperature.
6. The spot appears golden yellow colour while background appears navy blue or sky blue.
7. Draw the spot with the help of pencil and measure the distance travelled by solvents and respective acids.

Formula Used

R_f (retention factor) = $\frac{\text{Distance travelled by solute from the loading point}}{\text{Distance travelled by solvent from the loading point}}$

Precautions

1. Compound must be weighed accurately
2. The loading of the samples be done carefully at same point.