

# Aim 16

## To separate different Sugars by Paper Chromatography

### Requirements

Sucrose, glucose, fructose, n-butanol, acetic acid, water, aniline, diphenylamine, orthophosphoric acid, separating funnel, tripod stand, Whatman's filter paper no.1, beaker, capillary tube, funnel, filter paper, and distilled water.

### Preparation of standard sugar solutions:

1. Four beakers of 50 ml capacity are taken and mark them as 1 to 4.
2. 10 mg of each standard sugar sample is mixed well in 10 ml of distilled water in a separate beaker.

### Preparation of running solvent:

1. Take n- butanol: acetic acid: water in a ratio of 4: 1: 5.

n-butanol = 300 ml

Acetic acid = 75 ml

Water = 375 ml

Mix it and make 750 ml of value.

2. Shake well and transfer the above solution in a separating funnel. Discard the lower layer and use the upper layer as running solvent.

### Preparation of spraying agent:

1. Mix 2 ml of aniline in 48 ml of n-butanol and 2 ml of diphenylamine in 48 ml of n-butanol and add 10 ml of orthophosphoric acid to it.

2. After mixing precipitates are formed and filtered out. Use filtrate as spraying agent.

### **Formula Used**

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

### **Procedure**

1. Take the strip of Whatman's filter no. 1.
2. Mark a line using a pencil at 2-2.5 cm from the bottom.
3. Mark two points on the line at equidistance from the edges a distance of 3 cm away from each other.
4. Load given sugar mixture sample on one spot and on other spot standard sugar solution was loaded. Dry it using drier. Repeat the same process for 15-20 times.
5. After loading, hang the strip in chromatography chamber using glass rod.
6. Hanging should be done in such a way so that the lower portion of the strip was dipped in the solvent while loaded sample spots remain outside the running solvent.
7. Leave the chamber undisturbed for overnight.
8. Spray the spraying agent on chromatographic paper, dry under oven at 50°C spot appear on the chromatogram.
9. Mark the spots using pencil now distance travelled by solvent and respective sugar sample are measured and recorded.