

## EXPERIMENT NO.12

# SEPARATION OF SUGARS BY PAPER CHROMATOGRAPHY

Aim: To perform separation of sugars by Paper chromatography.

Requirements (Apparatus):

- 1) Filter paper of appropriate dimensions which allow the application at specified points of the necessary quantities of the solution being examined and appropriate reference solutions
- 2) Graduated micro-pipettes capable of delivering micro-liter quantities say 10  $\mu$ l and less.
- 3) A reagent sprayer that will emit a fine spray and will not itself be attacked by the reagent.
- 4) An ultra-violet light, suitable for observation at short (254 nm) and long (365 nm) ultra-violet wavelengths.

Theory:

Paper chromatography is an analytical method used to separate colored chemicals or substances. It is primarily used as a teaching tool, having been chromatography methods, such as thin-layer chromatography (method of alkaloid analysis has a long history. It was first proposed in Russia by M.S. Tswett 1903 after the successful separation of a mixture of plant pigments. The colour of the tissue-paper is very important and can be compared against a standard. This is a c and also a quantitative method of analysis if the standard is scaled. The paper used is very similar to that for thin layer chromatography, but without the need of special coatings. The principle of separation is mainly partition rather than adsorption. A paper chromatography variant, two-dimensional chromatography involves using two solvents and rotating the paper  $90^\circ$  in between. This is useful for separating complex mixtures of compounds having similar polarity, for example, amino acids. The setup has three components. The mobile phase is a solution that travels up the stationary phase, due to capillary action. The mobile

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## Calculation

$$\text{Glucose Rf value} = \frac{4}{5.9} = 0.67$$

$$\text{Sucrose Rf value} = \frac{4.3}{6.7} = 0.70$$

$$\text{Fructose Rf value} = \frac{5.9}{5.7} = 0.68$$

(54)



phase is generally mixture of polar organic solvent with water, while the stationary phase is water, Paper is used to support stationary phase (water).

Paper chromatography has evolved over the years and has found widespread applications in paration of molecules of different polarities. Innumerable applications have been reported in analysis of different classes of compounds

such as Amino acids and organic acids Alkaloids, Polysaccharides, Proteins and peptides, Natural and artificial pigments, Inorganic cations and Plant extracts

#### PROCEDURE:

Paper chromatography is in practice as an exceedingly simple procedure. Cut a strip of Whatmann No. 1 chromatographic paper (30-40cm x 5 cm). A small drop of the material to be examined is placed about 2 in. (5 cm) from the edge of a rectangular sheet of filter paper and allowed to dry there. The edge of the paper was dipped in an appropriate liquid (the solvent) that moves up the paper by capillary action. As the liquid passes over the point of application. Each chemical compound is pulled along by the liquid at a speed, which is characteristic of that substance. By using this technique, a mixture of substances applied to the same spot can be separated from each other on the filter paper in a few hours because they move with the liquid at different speeds. Take out the paper gently, carefully mark the solvent front with pencil and allow the chromatogram to dry in well ventilated area. Measure and record the distance of each spot from the point of its application and calculate the R<sub>f</sub> value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

#### RESULT-

The R<sub>f</sub> value of diff. Sugars was found to be 0.67, 0.70, 0.65

