Thin layered chromatographic separations of Glucose, Fructose and Sucrose.

Object : To separate Glucose and Fructose from the given mixture by using thin layer chromatography.

Principle :

It is an adsorption type of chromatography and is used for qualitative determination. Silica gel and alumina are most commonly used as an adsorbent/ stationary phase. The moving/mobile phase may be a pure solvent or a mixture of solvents. Components present in the solutions are separated on the basis of their polarity.

Chemicals Required :

1. Glass plate
2. Beaker
3. Silica gel G
4. Ethyl acetate
5. Dil. H_2SO_4
6. Capillary tube
7. Distilled water.

Procedure :

1. Prepare a slurry of silica gel and apply it on glass plate.
2. Let the plate dry for half an hour or until the silica dries.
3. Draw 2 lines from each end at the distance of 2cm.
4. Spot the given solution on the stationary phase with the help of a capillary tube.
5. In a beaker containing ethyl acetate, dip the end of glass plate where the spotting has been done such that the spot does come in contact with the mobile phase.
6. Leave it for some time so that the solvent runs till the line drawn in the opposite end.
7. After that take it out of the beaker and spray with dil. H2SO4.
8. Heat the plate on a hot plate at 70-80^{o}C for half an hour.
9. The black spots of Glucose and Fructose can be seen on the plate.
10. Measure their distance from the lower line and calculate their Rf value.

COLUMN CHROMATOGRAPHIC SEPARATION OF PIGMENTS FROM GREEN LEAVES

Object : Column chromatographic separation of pigments from green leaves.

Principle : The success of a separation by column chromatography depends on the choice of the stationary and mobile phases. The stationary phase material is filled in a column. Any of the three possible mechanisms: partition, adsorption or ion exchange can be employed by the use of a particular type of the stationary phase inside the column. For example, for the separation based on adsorption a n adsorbent is packed in the column.

The choice of the mobile phase depends on the nature of the substance and how strongly it is adsorbed. In a number of cases such as alumina and silica gel as the adsorbent, the mobile phase is generally a non-polar solvent such as petrol and benzene because polar groups such as hydroxyl-(OH) group in water and in ethanol would cause desorption. Eluents containing two or more solvents may be used for better results. In such cases the polarity is increased by adding a polar solvent with a non-polar one.

Requirements :

1. Chromatographic column
2. Beaker
3. Conical flask
4. Wash bottle
5. Calcium carbonate
6. Benzene
7. Petroleum ether
8. Ethyl alcohol

PROCEDURE :

1. **Preparation of the Extract:** Take 5-10 g of fresh grass (or leaves of a green plant) cut it up into fine pieces in a mortar, grind for about 30 seconds, add 10 ml of ethyl alcohol and 20 ml of petroleum ether, grind again. Decant the liquid into a separatory funnel after filtering through glass wool placed in an ordinary funnel. Add 10 ml alcohol and 20 ml petroleum ether again to the mortar containing grass, grind, and transfer the liquid after decantation to the separatory funnel containing the first fraction. Shake gently. A light green emulsion may form if shaken vigorously. Allow to settle the layers. The bottom layer is water-ethanol layer and the upper layer of petroleum-ether contains grass extract. Remove the bottom layer and wash the petroleum layer with water for 3 or 4 times until the layer is clear. Remove the aqueous layer.

   The extract is now free from alcohol but contains water in very small amounts. Transfer the upper layer containing the extract to a dry conical flask. To this, add anhydrous sodium sulphate (dried by heating in an oven hot plate before use), shake the flask and leave it over for about 15 minutes to remove any water present with the extract. Transfer the extract to a clean and dry test tube, cover it and take it for chromatography.
2. **Preparation of column:** Take a glass column or a burette of about 20cm in length and 7-8 mm diameter tube. Place a small wad of cotton wool as the column support. Pack the column with anhydrous calcium carbonate (dried by heating in a china dish over a burner), tap it regularly with a glass rod. Add the adsorbent in small portions and gently press down until a column of 8-

10 cm has been uniformly packed. Place a small wad of cotton wool at the top of the calcium carbonate column and use it for chromatography.

3. Take the uniformly packed column containing calcium carbonate and fix it in a stand vertically.
4. Take 1-2 ml of dried extract of leaves, drip into the column in the form of a thin layer of solution, and allow to run evenly into the adsorbent until: a green zone 3-4 mm deep is formed at the top of the column. This is known as the loading of the sample.
5. Add the developer (benzene) to the column and allow the developer through the column packing till separate bands are observed.
6. Note the colour of different bands and their order.

RESULT AND DISCUSSION :

The bands observed on the column are of different colours. The uppermost thin yellowish-green zone is chlorophyll-b, below this is the bluish-green zone of chlorophyll-a, the next orange-yellow zone contains xanthopylls and the lowest orange zone contains carotenes. The carotenes are least adsorbed by the adsorbent and can be easily washed out of the column.

Three main interactions are to be considered in column chromatography: the activity of the adsorbent, the polar behaviour of the substance and the polarity of the eluting solvent. This experiment is based on the results of the inventor of the technique of chromatography, M. Tswett, who applied the technique to separate various plant pigments using calcium carbonate as the stationary phase packed in a column.

References:

1. College Practical Chemistry, VK Ahluwalia, S Dhingra, A Gulati, Universities Press.
2. Advanced Practical Organic Chemistry, OP Agrawal, Krishna Publication.