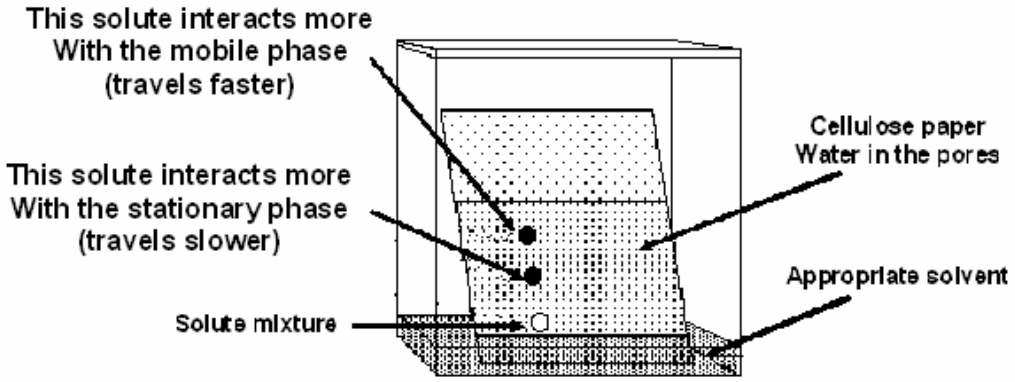
Chromatographic Separation of sugars.

Principle:

The term chromatography comes from the earlier times when the technique was used for the separation of colored plants pigments. Chromatography is a technique for separation of closely related groups of compounds. The separation is brought about by differential migration along a porous medium and the migration is caused by the flow of solvent.

Within limits chromatography can be divided into two types : partition and adsorption chromatography .Paper chromatography is an example of liquid-liquid chromatography .

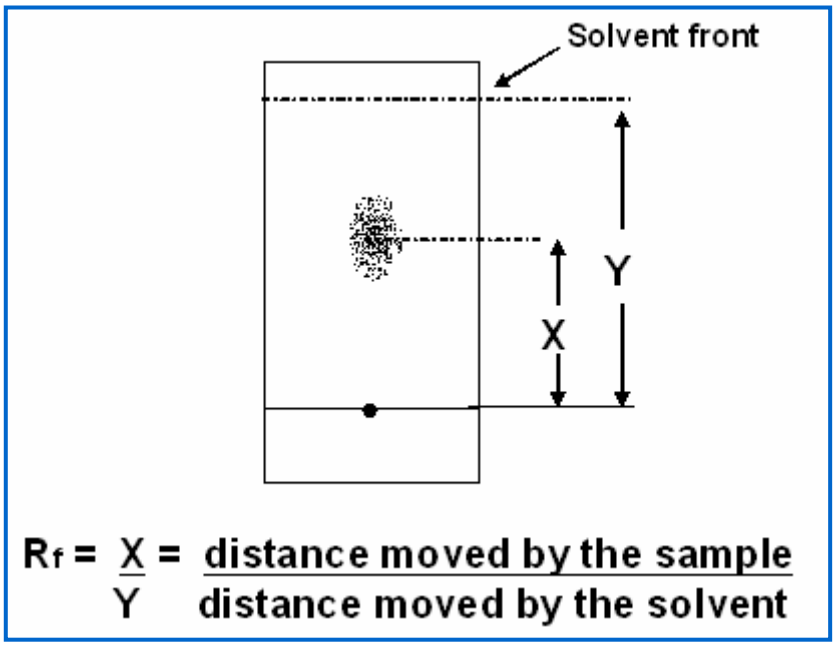


Illustrative scheme for Paper chromatography

In this type of chromatography separation is due to differential partition of solutes between two liquid phases .One liquid phase is bound to the porous medium for example, the water bound in the cellulose paper, this phase is referred to as, the stationary phase. The other liquid phase, the mobile phase flows along the porous medium .As the mobile phase flows over the solute mixture, the individual solutes partition themselves between the aqueous stationary phase and the organic mobile phase relative to their solubilities in the two phases. The more soluble a solute

in the mobile phase, the faster it will travel along the paper, and conversely, the mobile phase must be a mixture in which the compounds to be separated are soluble or partially soluble .In paper chromatography solute or solute mixture is spotted in solution along a base line on a sheet of filter paper(whatman No. 1).The mobile phase(solvent) is allowed to flow over the spots either ascending the paper by capillary action or descending the paper by gravity.

The separation is measured in terms of a unit called R_f (relative rates of flow) with respect to the solvent front.
The figure below explains how to calculate this value.



The Rf value of a compound in a particular solvent system is constant under identical conditions of the experiment, e.g. temperature, pH, etc.

Because most compound are colorless the spots are visualized after separation by specific reagent. The location reagent is applied by spraying the paper or rapidly dipping it in a solution of the reagent in a volatile solvent. Viewing under ultraviolet light is also useful since some compound which absorb it strongly show up as dark spots against the florescent background of the paper.

Materials:

Paper : Usually whatman No. 1 filter paper is used because of its known

Solvents: [a] Water-saturated phenol + 1% ammonia
[b] n-butanol-acetic acid-water (4:1:5 v/v)
[c] isopropanol-pyridine-water-acetic acid (8:8:4:1 v/v)

Spray reagent:

A. Ammoniacal silver nitrate:

add equal volumes of NH_4OH to a saturated solution of $AgNO_3$ and dilute the methanol to give a final concentration of 0.3M.After spraying the developed chromatograms,place them in an oven for 5-10 minutes, when the reducing sugars appear as brown spots.

B. Alkaline permanganate:

Prepare aqueous solution of $KMNO_4$ (1%) containing 2 % $NaCO_3$.After spraying with this mixture, the chromatograms are kept at 100C for a few minutes, when the sugar spots appear as yellow spots in purple background.

C. Aniline diphenylamine reagent:

Mix 5 volumes of 1% aniline and 5 volumes of 1% diphenylamine in acetone with 1 volume of 85% phosphoric acid .after spraying the dried chromatograms with this solution the spots are visualized by heating the paper at 100C for a few minutes.

D.Resorcinol reagent:

Mix 1% ethanolic solution of resorcinol and 0.2N HCl (1:1 v/v).Spray the dried chromatograms and visualize spots by heating at 90C.

Procedure:

1.Place sufficient solvent into the bottom of the tank. Cover the led and allow the tank to be saturated with the solvent.

2.Take a sheet of whattman 1 chromatography paper (about 9 x 10 cm) and place it on a piece of clean paper on a bench.

3. Draw a fine line with a pencil along the width of the paper and about 1.5cm from the lower edge.

4.Along this line place four equality spaced (about 2cm apart) small circles with a pencil.

5.Label the paper at the top with the name of each of the sugars and label the last unknown.

6.Use a fine capillary or tooth pick to place the drops of the solutions of the sugars, glucose, fructose, maltose, lactose and the mixture .

7.After spotting, dry the paper with hot air dryer for one minute, repeat this step again.

8.Place the spotted paper in the chromatographic tank and make the development by using the ascending technique.

9.Close the tank with lid, allow the solvent to flow for about 30-45 minutes.

10.Remove the paper and immediately mark the position of the solvent front with a pencil.

11. After the chromatogram has dried, spray the paper with the locating reagent.

12.you need to put the paper on the hot plate at low temperature or expose it to the hot air dryer, until the colored spots appear. the colors are stable for some weeks if kept in the dark and away from acid vapors.

13. Circle the position of each spot with pencil.

14. Calculate the Rf value for each spot and also for the spots the mixture contained.

- General summary of the behavior of the various sugars to these reagents are given below:

Sugars	a	b	c	d
Aldohexoses	+	+	+	pink
Ketohexoses	+	+	+	red
Aldopentoses	+	+	+	Blue,green
Ketopentoses	+	+	+	-
Deoxy sugars	-	+	+	-
Glycosides	+	-	-	-
Amino sugars	+	+	+	-

- The table below Rf values of some sugars in the solvents previously mentioned. They are only for comparative purposes, since Rf Varies with physical parameters.

Sugar	Solvent a	Solvent b	Solvent c
Glucose	0.39	0.18	0.64
Galactose	0.44	0.16	0.62
Fructose	0.51	0.25	0.68
Ribose	0.59	0.31	0.76
Deoxy ribose	0.73	-	-
Lactose	0.38	0.09	0.46
Maltose	0.36	0.11	0.50
Sucrose	0.39	0.14	0.62

Name: ***No.***

Experiment 7:

Results Sheet

1- Draw a sketch of your chromatogram.
2-Calculate R_f values for each spot of the mixture being separated.
3-By comparing the R_f values of the mixture along with those for the standards, state what sugars does this mixture contain?