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Carbohydrates

Introduction

As the name suggests, these are hydrates of carbon and their general formula is $C_nH_{2n}O_n$. Plants synthesize carbohydrates which serve chief nutrients for animals and are their main source of energy. These are classified as monosaccharides, disaccharide, oligosaccharides and polysaccharides.

Monosaccharides or simple sugars are either polyhydroxy aldehydes or ketones. These are further classified according to the number of carbon atoms in their structures as

- trioses ($C_3H_6O_3$), e.g. glycerol, dihydroxy acetone
- tetroses ($C_4H_8O_4$), e.g. therose, erythrose
- pentoses ($C_5H_{10}O_5$), e.g. xylose, lyxose, arabinose and ribose
- hexoses ($C_6H_{12}O_6$), e.g. fructose is ketone; glucose, glactose and mannose are aldoses
- heptoses ($C_7H_{14}O_7$), e.g. sedoheptulose is a ketose.

Disaccharides consist of two sugar molecules joined by glycoside bond and triglycerides and tetraglycerides consist of three or four simple sugars whereas oligosaccharides is up to eight simple sugar molecules and more than this number are polysaccharides. Sucrose (cane or beet root) is ketose and non-reducing, it consists of glucose and fructose. Reducing aldose disaccharides are lactose (milk sugar) contains glucose and glactose, maltose (malt sugar) contains 2 mols. of glucose.

Homoglycans consist of only one type of monosaccharide unit. Polysaccharides are inert i.e. do not ionize and serve as reserve material like starch in plants and glycogen in animals. Cellulose present in plants serves as structural support, Chitin is found in fungi and exoskeleton of arthropods.

Heteroglycans consist of two or more different monosaccharides or their derivatives e.g. mucous and hyaluronic acid.

Qualitative Tests

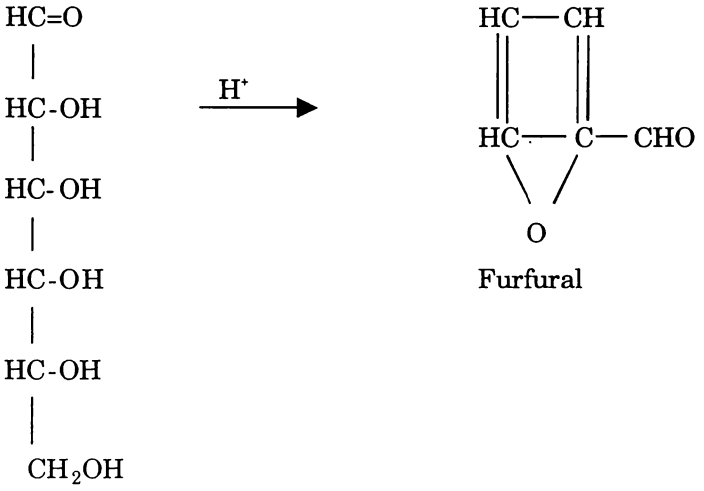
Requirements: 1% solution of glucose, fructose, glactose, sucrose, lactose, maltose, dextrin and sucrose for following tests.

Molish's Test

Reagent: α-nepthol 5% of α-nepthol in alcohol.

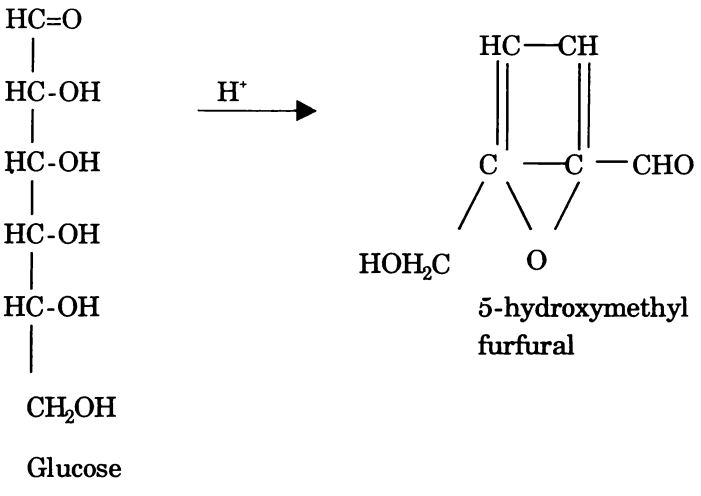
Procedure: To 2 ml of sugar solution add 2 drops of fresh 5% α-nepthol in alcohol and mix. Add 2 ml conc. H_2SO_4 by the side of test tube to form a layer below. A reddish-violet ring is formed at the junction of two layers. This is very sensitive and general test for all sugars. Proteins like casein and albumin also give this test positive.

Discussion: The red or violet colour develops due to condensation product of furfural or its derivatives like hydroxy methyl furfural.



Glucose

Furfural



Iodine Test

Reagents

- 0.01N Iodine solution in KI.
- 1% starch, glycogen or dextrin solution in hot water, cool to room temperature and add a few drops of 0.6 N HCl.

Procedure: A drop of solution of polysaccharide (1% starch, glycogen, or dextrin) is added to 3 ml Iodine solution.

Discussion: Starch gives blue colour while glycogen and dextrin gives brownish colour.

Iodine gives blue colour with starch due to adsorption of complex of iodine on starch. Other simple sugars do not give this test.

Benedict's Test

Dissolve 173 gm of sodium citrate and 100 gm sodium carbonate in 800 ml distilled water in graduated cylinder and raise the volume up to 850 ml with distilled water. Dissolve 17.3 gm of copper sulphate in 100 ml distilled water. To the previous solution of carbonate and citrate add this copper sulphate solution by constant stirring and make the volume to 1 litre with distilled water.

Procedure: To 5 ml of Benedict's reagent add 8 drops of sugar solution and heat in boiling water bath for a few minutes till the development of brick red precipitate. All reducing simple sugars (aldose or ketose) give the test positive while non-reducing sugars like sucrose and starch do not give the test.

Discussion: The cupric ions of the Benedict's reagent are reduced to brick red precipitate of Cuprous oxide by free aldehyde or ketone group of reducing sugar. Rest of the reducing sugars which have free aldehyde or ketose group give brick red precipitate of cuprous oxide. Benedict's reagent has copper citrate in sodium carbonate. It is very sensitive test and detects very small quantities of the reducing sugars. Non – reducing sugars like sucrose or starch do not give this test due to absence of free aldehyde or ketone group.

$$2Cu(OH)_2 + C_6H_{12}O_6 \longrightarrow 2CuOH + H_2O + \text{sugar}$$

$$2CuOH \longrightarrow Cu_2O + H_2O$$

Cuprous Hydroxide — Cuprous Oxide

Fehling's Test

Reagent

Soln. A: 138.6 gm powdered $CuSO_4.H_2O$ dissolved in 2 litres of distilled water.

Soln. B: 692 gm sodium potassium tartrate and 200 gm NaOH dissolved in 2 litres of distilled water at room temperature.

Procedure: Mix equal volume of Fehling's solution A and B. Take 5 ml of this solution and add 1 ml of the sugar sample. Heat in boiling water bath till, brick red precipitate appears.

Discussion: This test is also positive with reducing sugars with free aldehyde or ketone group. Non-reducing sugars like sucrose and starch do not give this test. Here too, sugar is oxidized at the expense of divalent copper which is reduced to monovalent form. Fehling's solution consists of copper sulphate and alkaline tartrate. Reduction occurs as follows:

$$2Cu(OH)_2 + C_6H_{12}O_6 \longrightarrow 2CuOH + H_2O + \text{Sugar}$$

cupric hydroxide + dextrose → cuprous hydroxide (yellow precipitate)

$$2CuOH \longrightarrow Cu_2O + H_2O$$

cuprous hydroxide (yellow precipitate) → cuprous oxide (brick red precipitate)

Cuprous hydroxide is yellow precipitate which changes to red precipitate of cuprous oxide.

Barfoed's Test

Barfoed's reagent: Add 24 gm of copper acetate in 450 ml boiling distilled water. If precipitate is formed, do not filter. Add to this 25 ml of 8.5% lactic acid to the hot solution. Shake, till the precipitate formed dissolves. Cool and dilute it to 500 ml.

Procedure: To 5 ml Barfoed's reagent add 0.5 ml sugar solution and heat in boiling water bath. The red-orange precipitate settles at room temperature after boiling for 5–7 minutes with reducing monosaccharide and 7–12 minutes with reducing disaccharides. Non–reducing disaccharide like sucrose may give slight precipitate due to hydrolysis and conversion to reducing monosaccharide.

Discussion: This test helps to distinguish between disaccharides and monosaccharides superficially. The reagent contains copper acetate in acetic acid and positive test is given by conversion of cupric oxide to cuprous oxide. A brown precipitate settles at the bottom on cooling at room temperature. This reagent contains copper acetate in acidic medium, so this test cannot be used for detection of sugars in urine or any fluid containing chlorides. In this test reduction is brought about in acidic solution. Monosaccharides give brown precipitate earlier and disaccharides after longer heating.

Seliwanoff's Test

Seliwanoff's reagent—add 0.05 g resorcinol in 100 ml dilute HCl (1:2).

Procedure: To freshly prepared Seliwanoff's reagent add a few drops of sugar solution and heat in boiling water bath for 10 minutes. Ketoses like fructose and sucrose give red colour while aldoses may give this test positive after prolonged heating due to conversion of aldoses to ketoses by catalytic action of hydrochloric acid.

Discussion: Ketoses give the test positive and develop bright colour, however aldoses may give this test on prolonged heating but colour developed is light red or pink. By heating fructose with HCl it gets converted to levulinic acid and hydroxyl methyl furfural and later on condensation of later with resorcinol gives positive reaction. Aldose sugar on heating with Seliwanoff's reagent for long time may develop colour because these are converted to ketoses in the presence of HCl. Sucrose gives positive test after it is hydrolyzed to glucose and fructose.

Anthrone Test

Anthrone reagent – 0.2% solution of anthrone in conc. H_2SO_4.

Procedure: To 2 ml anthrone reagent add a few drops of sugar solution.

Discussion: A blue green colour is given by glucose and polysaccharides containing glucose. Different colour develops with different sugars. This test is also used as basis of quantitative colorimetric estimation of sugars.

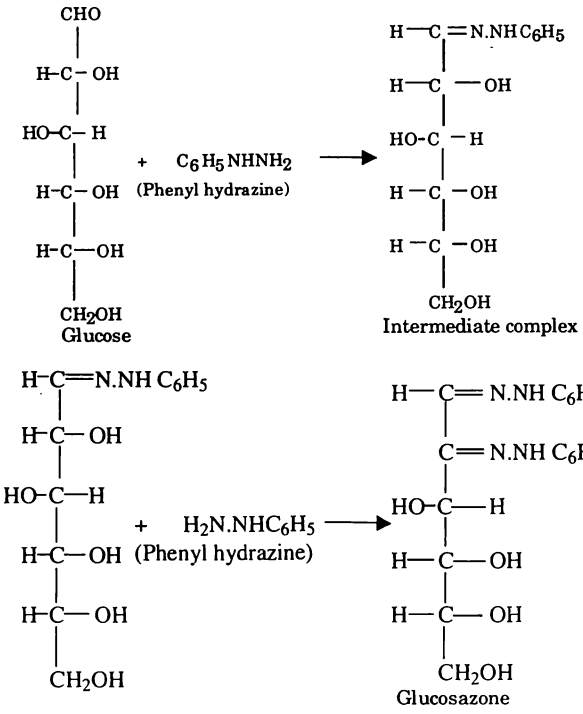
Osazone Test (Phenyl hydrazine test)

Phenyl hydrazine: Dissolve 80 gm of phenyl hydrazine hydrochloride and 200 gm of anhydrous sodium acetate in 100 ml distilled water. Keep in dark bottle and use freshly prepared solution.

Procedure: To 2 ml sugar solution add 5 ml freshly prepared phenyl hydrazine and keep in boiling water bath for 30 minute

or more till precipitate appears. Time taken for formation of crystals varies with different sugars. It may take 2 to 30 or 40 minutes. Cool to room temperature and observe the crystals under the microscope. Each sugar gives crystal of specific shape. Sometimes solution becomes concentrated on boiling and crystals appear only after the solution is diluted with water. Observe the shape of crystals under the microscope by taking a few crystals with brush on a slide containing a drop of water. Draw the shape of crystals for all the sugar solutions.

Discussion: Sugar molecule combines with two moles of phenyl hydrazine to form osazone crystals of bright yellow colour of microscopic size. Glucose and fructose give the same needle shaped crystals because of the same arrangement of H and OH group on 3, 5 and 6 carbons. Trisaccharides and polysaccharides do not respond to the reaction hence crystals are not formed (Fig. 3.1). To the phenyl hydrazine hydrochloride, sodium acetate is added to maintain the pH of the solution.



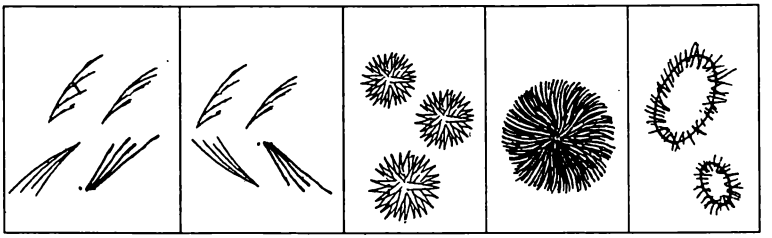


Fig. 3.1: Osazone crystals

Recording of the Observations

Test	Pentose	Glucose	Fructose	Galactose	Sucrose	Maltose	Lactose	Dextrin	Starch	Control (distilled water)
Molish's test										
Iodine test										
Benedict's test										
Fehling's test										
Barfoed's test										
Anthrone test										
Selwinoff's test										
Phenyl hydrazin test										
Time duration										
Shape of crystals										

Precautions

1. Use clean dry test tubes.
2. Use only given quantity of the reagents
3. Record your observations side by side.
4. While heating always keep the mouth of tube away from yourself.
5. Confirm your results with the teacher before throwing the contents of test tubes.
6. Add the solution gently by the side of the test tube for ring test.
7. Always do all the tests with control (distilled water) for comparison.

Objective Questions

1. What is a reducing sugar?
2. Which are non-reducing sugars?
3. Is Molish's test a confirmatory test for carbohydrates?
4. How to distinguish mono- and disaccharides?
5. What is grape sugar?
6. How to detect keto-group?
7. Is phenylhydrazine confirmatory test?
8. Which test distinguishes maltose and sucrose?
9. Which is sensitive test for reducing sugars?
10. Which is the easiest test to distinguish a sugar sample from any other substance?

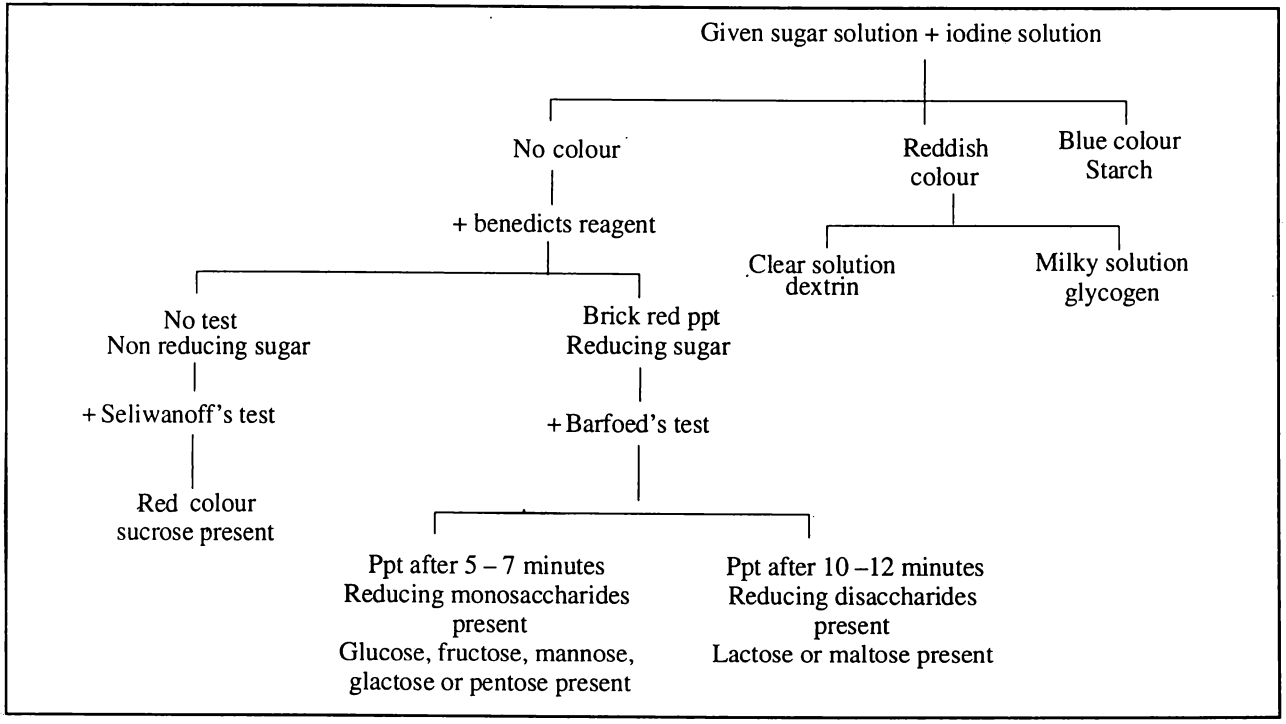
Scheme of Detection of Carbohydrates

Scheme for detection of carbohydrates is shown in Chart 3.1. Perform osazone test and confirm the sugar on the basis of shape of crystals.

Estimation of Glucose by Colorimeter

Requirements: Test tubes, water bath, 1.0% pure quality of glucose stock solution (10 mg/ml) in 0.2% Benzoic acid, colorimeter, Arsenomolybdate colour reagent, Alkaline copper reagent, pipettes, beaker.

Chart 3.1: *Scheme for detection of carbohydrates*



Reagents: Arsenomolybdate colour reagent: dissolve 100 gm of ammonium molybdate in 1800 ml distilled water and add 84 ml conc. H2SO4 and mix. Take 12 gm disodium orthoarsenate ($Na_2HAsO_4.7H_2O$) in 100 ml distilled water and mix it with previous solution and keep this at 37°C for 1 to 2 days. Store in a brown bottle.

Alkaline Copper Reagent

Solution A: dissolve 50 gm anhydrous sodium carbonate, 50 gm Rochelle salt, 40 gm sodium bicarbonate, 400 gm anhydrous sodium sulphate in 1600 ml distilled water and dilute it to 2 litres. Mix and filter if sediment is formed and store at room temperature.

Solution B: Dissolve 150 gm $CuSO_4,5H_2O$ in distilled water dilute to 1 litre and add 0.5 ml conc. H_2SO_4 and mix. Before using alkaline copper take 4 ml solution B and dilute to 100 ml with solution A, mix well and use.

Procedure: Preparation of standard curve: Dilute 1ml of glucose (stock solution) with 100 ml distilled water to get 0.1 mg/ml (dilution 1:100). Take 7 test tubes and add 0, 0.1, 0.2, 0.4, 0.8 and 1.6 of the diluted glucose solution. Make the volume in each tube 2.0 ml by adding distilled water. To each tube add 2.0 ml alkaline Copper solution, cover with marble and keep in boiling water bath for 5–6 minutes. Then cool the tubes under running tap water and to each add 1ml of Arsenomolybdate reagent. Mix well and let the blue colour develop. Read the tubes in spectrophotometer at 540 mμ. Plot a curve with salt concentration on the x-axis and OD on y-axis. It comes out as straight line.

Glucose Estimation in Blood

Take 1 ml of blood, add 7.0 ml distilled water and mix. Add 1 ml of 10% Sodium tungstate and 1 ml of 0.7 N H_2SO_4 drop-wise and mix well. The precipitate changes from red to brown let the precipitate settle and filter it. Collect the filtrate. From the filtrate take 0.5, 1.0 and 2.0 contents in three tubes and process as before and let the colour develop. Read all the contents in spectrophotometer at 540 mμ and find out the glucose content from the Standard curve and express the concentration in 100 ml of whole blood.

Discussion: The blood is deproteinized and filtrate contains only glucose as reducing substance. Phosphomolybdic acid is colour reagent. The extent of reduction is estimated by photometric method. In colorimeter/spectrophotometer the OD of coloured solution or its complex is index of depth of the colour, which obstructs the passage of light through the solution. From the concentration of such material in a given volume, calculate the amount of substance present in the sample.

Precautions

1. Prepare all the solutions accurately
2. While reading in the spectrophotometer, each time wash the tubes with distilled water.
3. Use clean and dry glassware.
4. Set the water bath at boiling temperature.
5. The colour of solution turns blue when arsenomolybdate reagent is added.
6. Measure all solutions accurately with pipette.
7. Record your results properly.

Record Observations

S.No.	Amount of glucose (ml)	Amount of benzoic acid (ml)	OD	Concentration of glucose (μg)

Objective Questions

1. Who first established this estimation?
2. How do you set the colorimeter?
3. What is the principle of colorimeter?
4. How do you make serial dilutions?
5. What is stock solution?

Estimation of Glycogen with Colorimeter

Requirements: Test tubes, water bath, spectrophotometer, anthrone reagent, glycogen standard solutions, protein free glycogen solution of unknown strength.

Procedure

Preparation of Standard Curve

Take seven tubes, keep first as blank control with 2 ml distilled water, from 2 to 7 add 10 μg, 20, 40, 80 and 160 μg standard glycogen. Add distilled water to make 2.0 ml and then add 4 ml anthrone to all tubes. Cover all the tubes with marble tops and keep in boiling water bath for 10 minutes. Cool to room temperature and read OD at 620 mμ against a reagent blank. Plot a curve taking concentration on x-axis and OD on y-axis. It comes out as straight line.

Preparation of Glycogen Extract

Take about 0.5 g liver and homogenize in 10 ml distilled water. Precipitate proteins by adding 10.0 ml of 20% Zn $(OH)_2$. Centrifuge to settle the proteins and take only the supernatant. This is liver glycogen extract.

To 0.5, 1.0 and 1.5 ml of extract add distilled water to make 2.0 ml and 4 ml Anthrone reagent and keep in boiling water bath for 10 minutes as before. Cool to room temperature and read at 620 mμ wavelength in spectrophotometer and interpret the glycogen content from the standard curve and represent in g.

Precautions

1. **Prep**are all the solutions accurately.

2. While reading the colorimeter, each time wash the tubes with distilled water.
3. Use clean and dry glassware.
4. Set the water bath at boiling temperature.
5. Measure all solutions accurately with pipette.
6. Record your results properly.
7. Handle homogenizer with care

Observations

S.No	Amount of glycogen content	Distilled water	OD	Concentration of glycogen

Objective questions

1. How proteins are precipitated?
2. Why liver tissue is taken?
3. Which sugars give anthrone test positive?
4. What is the function of conc. H_2SO_4 present in anthrone reagent

Separation Of Sugars by Paper Chromatography

Requirements: Cylindrical jar with lid, Whatman No 1 filter paper, given sugar solutions- glucose, xylose, lactose, mixture of all three sugars.

Solvent: Ethyl acetate, pyridine and water in the ratio 14:6:5 respectively.

Reagents used for locating: Phenyl diamine 0.5 g, stannous chloride 1.2 g, acetic acid 20 ml, and ethanol 50 ml.

Procedure: Take the solvent (25 ml) in the jar and cover it. On Whatman No 1 filter paper put a small drop of each sugar and the mixture near the base in a straight line. Put the paper in the jar, cover it and leave it aside for 3 hrs. By capillary action the solvent is drawn up and carries the substances with it for some distance. Substances are separated according to their relative solubilities in the solvent (Fig. 3.2). After that take out the paper and mark solvent front. Dry the paper and dip it in locating reagent mixture

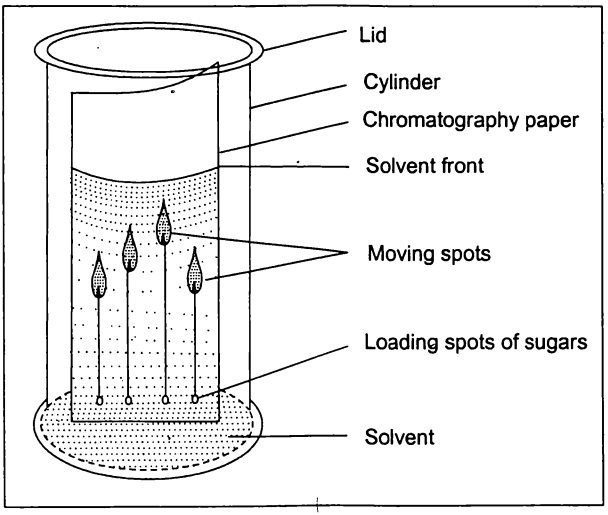


Fig. 3.2: *Paper chromatography chamber*

and then keep the paper in the oven at 100°C for 5–6 minutes to dry. All the spots will appear from dark yellow to other different shades. Find and compare R_F values.

$$\text{Rate of flow} = R_F = \frac{\text{Distance travelled by Sugar}}{\text{Distance travelled by Solvent}}$$

R_F Values of Sugars

Compounds	**Solvent**			
	1	**2**	**3**	**4**
D-Glucose	0.39	0.18	0.43	0.64
D-Mannose	0.45	0.20	0.46	0.69
D-Fructose	0.51	0.23	0.48	0.68
D-Arabinose	0.54	0.21	0.56	0.70
D-Ribose	0.59	0.31	—	0.76
Lactose	0.38	0.09	0.33	0.46
Sucrose	0.39	0.24	0.39	0.62
L-Ascorbic acid	0.24	0.38	0.59	0.84
Dehydroascorbic acid	0.28	0.48	—	0.88
D-Glacuronic acid	0.13	0.14	0.40	0.31
Raffinose	0.27	0.05	—	0.45

Solvents

1. Water saturated phenol + 1% NH_3 (HCN atmosphere).
2. n-butanol : glacial acetic acid : water (4:1:5 V/V) descending
3. n-butanol : glacial acetic acid : water (4:1:5 V/V) horizontal
4. i-propanol : pyridine : water : acetic acid (8:8:4:4 V/V) descending

Observations

Precautions

1. Take high quality filter paper Whatman No. 1.
2. Put the reagents properly using dropper.
3. Use clean and dry jar, pipettes, etc.
4. Run the column without any disturbance or shaking.
5. Always keep the lid of the jar covered.
6. Do not over dry the filter paper in the oven.

Objective Questions

1. What is R_F value and how do you calculate it?
2. What is the principle of chromatography?
3. How to choose the solvent for chromatography?
4. Why do you dry the filter paper?
5. What is the difference between ascending and descending paper chromatography?
6. What is capillary action?