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Qualitative tests for Carbohydrates

OBJECTIVE

- To study the properties of carbohydrates
- To determine the identity of an unknown carbohydrate by carrying out a series of chemical reactions

GENERAL INTRODUCTION

- Carbohydrates are widely distributed in plants and animals; they have important structural and metabolic roles.
- Chemically carbohydrates are aldehyde or ketone derivatives of polyhydric alcohols
- **Glucose** is the most important carbohydrate; the major metabolic fuel of mammals (except ruminants) and a universal fuel of the fetus.
- It is the precursor for synthesis of all the other carbohydrates in the body.

CLASSIFICATION OF CARBOHYDRATES

(1) Monosaccharides are those carbohydrates that cannot be hydrolyzed into simpler carbohydrates. They may be classified as **trioses, tetroses, pentoses, hexoses,** or **heptoses,** depending upon the number of carbon atoms; and as **aldoses** or **ketoses** depending upon whether they have an aldehyde or ketone group.

CLASSIFICATION OF CARBOHYDRATES

(2)Disaccharides are condensation products of two monosaccharide units; examples are maltose and sucrose.

(3)Oligosaccharides are condensation products of three to ten monosaccharides.

(4)Polysaccharides are condensation products of more than ten monosaccharide units; examples are the starches and dextrins, which may be linear or branched polymers.

MONOSACCHARIDES OF BILOGICAL SIGNIFICANCE

MONOSACCHARIDE	ALDOSES	KETOSES
Trioses ($C_3H_6O_3$)	Glycerose (glyceraldehyde)	Dihydroxyacetone
Tetroses ($C_4H_8O_4$)	Erythrose	Erythrulose
Pentoses ($C_5H_{10}O_5$)	Ribose, Arabinose, Xylose	Ribulose, Xylulose
Hexoses ($C_6H_{12}O_6$)	Glucose, Galactose. Mannose	Fructose
Heptoses ($C_7H_{14}O_7$)	---------------	Sedoheptulose

DISACCHARIDES OF BILOGICAL SIGNIFICANCE

Sugar	Composition	Source
Isomaltose	*O*-α-D-glucopyranosyl-(1->6)-α-D-glucopyranose	Enzymic hydrolysis of starch (the branch points in amylopectin)
Maltose	*O*-α-D-glucopyranosyl-(1->4)-α-D-glucopyranose	Enzymic hydrolysis of starch (amylase); germinating cereals and malt
Lactose	*O*-α-D-galactopyranosyl-(1->4)-β-D-glucopyranose	Milk (and many pharmaceutical preparations as a filler)
Sucrose	*O*-α-D-glucopyranosyl-(1->2)-β-D-fructofuranoside	Cane and beet sugar, sorghum and some fruits and vegetables

POLYSACCHARIDES OF BIOLOGICAL SIGNIFICANCE

A) Homopolysacchrides	**Glucosan**	**Fructosan**	**Galactosan**
	Starch	Inulin	Agar
	Glycogen	-	-
	Dextrins	-	-
	Cellulose	-	-
B) Hetero polysaccharides	Non sulfated	Sulfated	Neutral polysaccharides
	Hyaluronic acid	Keratan sulfate	Blood group substances
	Chondroitin	Chondroitin sulfate	
		Dermatan sulfate	
		Heparin	

QUALITATIVE TESTS FOR CARBOHYDRATES

Preliminary Procedure

Obtain an unknown carbohydrate and prepare a 1% solution by dissolving 0.25 g of carbohydrate in 25 mL of deionized water.

1. MOLISCH TEST

Principle: Carbohydrates when treated with concentrated sulphuric acid undergo dehydration to give furfural derivatives. These compounds condense with Alpha naphthol to form colored products. Pentoses yield furfural while Hexoses yield 5-Hydroxy methyl furfurals.

1. MOLISCH TEST

Procedure:

Take 2 ml of carbohydrate solution in a clean and dry test tube. Add 2 drops of ethanolic Alpha Naphthol (Molisch reagent) and mix. Incline the test tube and add carefully 2 ml of concentrated sulphuric acid along the side of the test tube so as to form 2 layers.

1. MOLISCH TEST

Interpretation: This is a sensitive but a non-specific test and is given positive by all types of carbohydrates. If the oligosaccharides or polysaccharides are present they are first hydrolysed to mono saccharides which are then dehydrated to give the test positive.

1. MOLISCH TEST

An appearance of reddish violet or purple colored ring at the junction of two liquids is observed in a positive Molisch test.

2) BENEDICT'S TEST

Principle:

- Carbohydrates with free aldehyde or ketone groups have the ability to reduce solutions of various metallic ions.
- Reducing sugars under alkaline conditions tautomerise and form enediols.
- Enediols are powerful reducing agents.
- They reduce cupric ions to cuprous form and are themselves converted to sugar acids.
- The cuprous ions combine with OH- ions to form yellow cuprous hydroxide which upon heating is converted to red cuprous oxide.

2) BENEDICT'S TEST

Procedure

- Take 5 ml of Benedict's reagent.
- Add 8 drops of carbohydrate solution.
- Boil over a flame or in a boiling water bath for 2 minutes.
- Let the solution cool down.

(Negative Reaction)

(Positive Reaction)

2) BENEDICT'S TEST

Interpretation:

- Benedict's test is a semi quantitative test. The color of the precipitate gives a rough estimate of a reducing sugar present in the sample.
- **Green color - Up to 0.5 G% (+)**
- **Green precipitate - 0.5-1.0 G% (++)**
- **Yellow precipitate -1.0-1.5 G% (+++)**
- **Orange precipitate- 1.5-2.0 G% (++++)**
- **Brick red precipitate- > 2.0 G %(+++++)**

2) BENEDICT'S TEST

Benedict's test is a semi quantitative test. The color formed depends upon the amount of reducing sugar present in the mixture.

3)**BARFOED'S TEST**

Principle: Aldoses and ketoses can reduce cupric ions even in acidic conditions. This test is used to distinguish reducing mono saccharides from disaccharides by controlling pH and time of heating. Mono saccharides react very fast whereas disaccharides react very slowly.

3)BARFOED'S TEST

Procedure:

- To 2 ml of Barfoed's reagent, add 2 ml of carbohydrate solution.
- Keep the test tubes in the boiling water bath for 3 minutes.
- Cool under running water.
- Over-heating should be avoided.

A scanty brick red precipitate is observed in a positive reaction.

3)**BARFOED'S TEST**

Interpretation:

- The positive reaction indicates the presence of a reducing mono saccharide.
- On prolonged heating disaccharides can also give this test positive.
- Hence, the solution should be boiled for 3 minutes only.

4. SELIWANOFF'S TEST

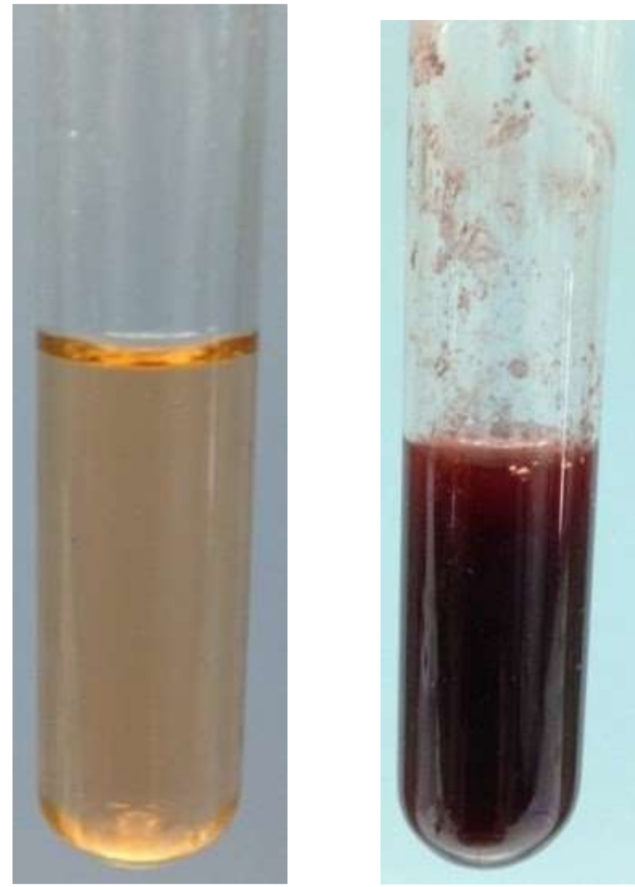
Principle:

Keto hexoses on treatment with hydrochloric acid form 5-hydroxy methyl furfural which on condensation with resorcinol gives a cherry red colored complex.

4. SELIWANOFF'S TEST

Procedure:

- To 3 ml of Seliwanoff reagent add 1ml of fructose.
- Boil for 30 seconds only.
- Cool the solution.



A cherry red color is observed in a positive reaction.

4. SELIWANOFF'S TEST

Interpretation:

- This test is given positive by ketohexoses so it is answered by fructose, sucrose and other fructose containing carbohydrates.
- This test distinguishes between glucose and fructose.
- Overheating of the solution should be avoided.
- Upon continuous boiling, aldoses get converted to ketoses and give a positive reaction with Seliwanoff reagent.

5.HYDROLYSIS TEST FOR SUCROSE

Principle:

- Sucrose on hydrolysis with HCl is converted to glucose and fructose.
- The presence of these two monosaccharides can be confirmed by Benedict’s and Seliwanoff test

5.HYDROLYSIS TEST FOR SUCROSE

Procedure:

- Add 2 drops of HCl and I drop of thymol blue to 5 ml of sucrose solution.
- The development of pink color indicates that the solution is acidic.
- Divide it in to two equal parts.
- Boil one portion for about one minute and then cool it under tap water.
- Neutralize both portions by adding 2% sodium carbonate drop by drop.

5.HYDROLYSIS TEST FOR SUCROSE

- Formation of blue color indicates neutralization.
- Perform Benedict's and seliwanoff's tests with the boiled portion.

Boiled portion gives positive test with Benedict's reagent, but the unboiled portion does not reduce Benedict's solution.

5.HYDROLYSIS TEST FOR SUCROSE

Interpretation:

- Sucrose is a non-reducing sugar, since it does not have free aldehyde or ketone group to cause reduction, hence it gives a negative reaction with Benedict's reagent.
- But upon boiling with HCl , sucrose is hydrolyzed to yield glucose and fructose, which give positive reactions with benedict and Seliwanoff reagents.

6. OSAZONE TEST

Principle:

- A solution of reducing sugar when heated with phenyl hydrazine, characteristic yellow crystalline compounds called Osazone are formed.
- These crystals have definite crystalline structure, precipitation time and melting point for different reducing sugars.

6. OSAZONE TEST

Procedure:

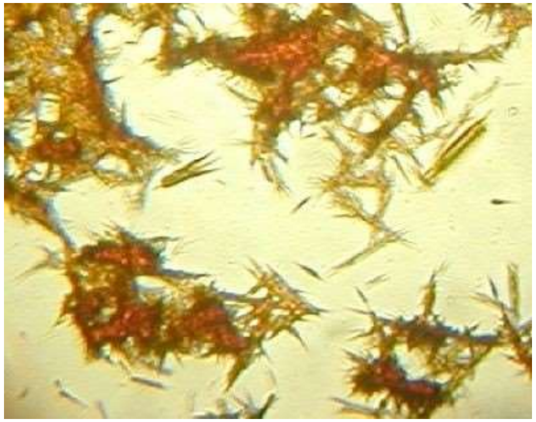
- Add 10 drops of glacial acetic acid to 5 ml of sugar solution in test tube.
- Then add a knife point of phenyl hydrazine hydrochloride and double the amount of sodium acetate crystals.
- Mix and warm a little to see that the solids are dissolved.

6. OSAZONE TEST

- Filter the solution in another test tube and keep the filtrate in a boiling water bath for 20 minutes.
- Allow the tube to cool slowly in the water bath without cooling it hurriedly under the tap to have better crystals.
- Examine the crystals under the microscope

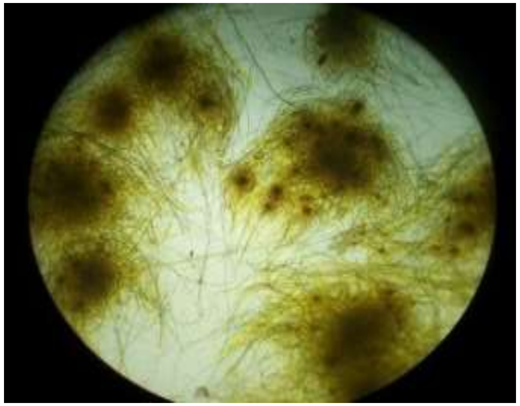
6. OSAZONE TEST

Needle shaped glucosazone crystals as viewed under the microscope



Galactosazone crystals as viewed under the microscope(Rhombic plates)

Sun flower shaped Maltosazone crystals as viewed under the microscope



Powder puff/hedge hog shaped crystals of lactose as viewed under the microscope

6. OSAZONE TEST

- Glucose, fructose and mannose produce the same Osazone because of the similarities in their molecular structure.
- Galactosazone crystals are formed in 7 minutes.
- Maltosazone crystals are formed in 10-15 minutes.

7. BIAL'S TEST

Principle:

- The test reagent dehydrates pentoses to form furfural.
- Furfural further reacts with orcinol and the iron ion present in the test reagent to produce a bluish product.

7. BIAL'S TEST

Procedure:

- 2 ml of a sample solution is placed in a test tube.
- 2 ml of Bial's reagent (a solution of resorcinol, HCl and ferric chloride) is added.
- The solution is then heated gently in a Bunsen Burner or hot water bath.

7. BIAL'S TEST

Interpretation:

- This test is specific for pentoses.
- Hexoses generally react to form green, red, or brown products

The formation of a bluish product. All other colors indicate a negative result for pentoses.

8. IODINE REACTION

- This is a test for polysaccharides

Principle :

Iodine forms a coordinate complex between the helically coiled polysaccharide chain and iodine centrally located within the helix due to adsorption. The color obtained depends upon the length of the unbranched or linear chain available for complex formation

8.IODINE REACTION

Left to right: Lugol's iodine, starch solution, starch solution with iodine.

Yellow-orange - negative. Purple-black -positive.

8.IODINE REACTION

Interpretation

Amylose- A linear chain component of starch, gives a deep blue color

Amylopectin- A branched chain component of starch, gives a purple color

Glycogen- Gives a reddish brown color

Dextrins- Amylo, Eryhthro and Achrodextrins, formed as intermediates during hydrolysis of starch give violet, red and no color with iodine respectively.

REACTIONS OF GLUCOSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Glucose is a carbohydrate
2)	Benedict's Test	Brick red ppt (Color depends on amount of sugar)	Glucose is a reducing carbohydrate
3)	Barfoed's test	Scanty red ppt at the bottom of test tube	Glucose is a reducing mono saccharide
4)	Seliwanoff's test	Cherry red color is not observed	Glucose is not a keto hexose
5)	Osazone test	Needle shaped crystals	Confirmatory test for glucose

REACTIONS OF FRUCTOSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Fructose is a carbohydrate
2)	Benedict's Test	Brick red ppt (Color depends on amount of sugar)	Fructose is a reducing carbohydrate
3)	Barfoed's test	Scanty red ppt at the bottom of test tube	Fructose is a reducing mono saccharide
4)	Seliwanoff's test	Cherry red color is observed	Fructose is a keto hexose
5)	Osazone test	Needle shaped crystals	Fructose , Glucose and Mannose form similar crystals

REACTIONS OF GALACTOSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Galactose is a carbohydrate
2)	Benedict's Test	Brick red ppt (Color depends on amount of sugar)	It is a reducing carbohydrate
3)	Barfoed's test	Scanty red ppt at the bottom of test tube	It is a reducing mono saccharide
4)	Seliwanoff's test	Cherry red color is not observed	It is not a keto hexose
5)	Osazone test	Rhombic plate shaped crystals	Confirmatory test for galactose

REACTIONS OF MALTOSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Maltose is a carbohydrate
2)	Benedict's Test	Brick red ppt (Color depends on amount of sugar)	It is a reducing carbohydrate
3)	Barfoed's test	No color change	It is not a mono saccharide
4)	Osazone test	Sun flower shaped crystals	Confirmatory test for Maltose

REACTIONS OF LACTOSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Lactose is a carbohydrate
2)	Benedict's Test	Brick red ppt (Color depends on amount of sugar)	It is a reducing carbohydrate
3)	Barfoed's test	No change in color	It is not a mono saccharide
4)	Osazone test	Powder puff/Hedge hog shaped crystals	Confirmatory test for Lactose

REACTIONS OF SUCROSE

S.NO.	TEST	OBSERVATION	INFERENCE
1)	Molisch Test	Purple ring at the junction of two liquids	Sucrose is a carbohydrate
2)	Benedict's Test	No color change	It is a non reducing carbohydrate
3)	Barfoed's test	No change in color	It is not a mono saccharide
4)	Seliwanoff test	Cherry red color	Keto hexose containing disaccharide
5)	Hydrolysis (Inversion) test	The hydrolytic products give positive reaction with Benedict's and Barfoed's reagents.	Confirmatory test for Sucrose
6)	Osazone test	No reaction	Sucrose does not form osazone crystals

IDENTIFICATION OF AN UNKNOWN CARBOHYDRATE

Step1- Perform Molisch test

Observation-Reddish violet ring at the junction of two liquids.

Inference- Carbohydrate is confirmed.

Step-2- Perform Iodine test

Observation- No change in color

Inference- Mono or disaccharide

Note- Blue, brown or red color is given by starch, glycogen and dextrins respectively

IDENTIFICATION OF AN UNKNOWN CARBOHYDRATE

Step-3- Perform Benedict’s test

Observation- Positive reaction (green, yellow, orange or red colored ppt)

Inference- The given carbohydrate is reducing in nature, it could be glucose, fructose, galactose, maltose or lactose.

Note- Sucrose gives a negative reaction with Benedict's reagent.

IDENTIFICATION OF AN UNKNOWN CARBOHYDRATE

Step-4- Perform Barfoed's test

Observation- A scanty red ppt at the bottom of the test tube

Inference- A reducing mono saccharide is present, it may be glucose, fructose, mannose or galactose

Note- The test is negative for disaccharides like, lactose, maltose and sucrose as well.

IDENTIFICATION OF AN UNKNOWN CARBOHYDRATE

Step-5- Perform Seliwanoff test

Observation- A cherry red color is observed

Inference- Fructose is confirmed**.**

Note- Glucose, galactose and mannose give a negative result with seliwanoff test.

Sucrose gives a positive reaction with seliwanoff test.

IDENTIFICATION OF AN UNKNOWN CARBOHYDRATE

Step-6- Perform osazone test (If fructose is confirmed, osazone test is not required)

Observations-

Needle shaped crystals- Glucose, fructose or mannose

Sun flower shaped crystals- Maltose is confirmed

Powder puff/ hedge hog crystals- Lactose is confirmed

IDENTIFICATION OF AN UNKNOWN CARBOHYDRATE

Step-7- Perform hydrolysis test

If the Benedict's, Barfoed's tests are negative and seliwanoff test is positive, the carbohydrate is essentially sucrose, for confirmation proceed with inversion/hydrolysis test

Observation- Benedict's and Barfoed's test react positively with hydrolytic products.

Inference- Sucrose is confirmed