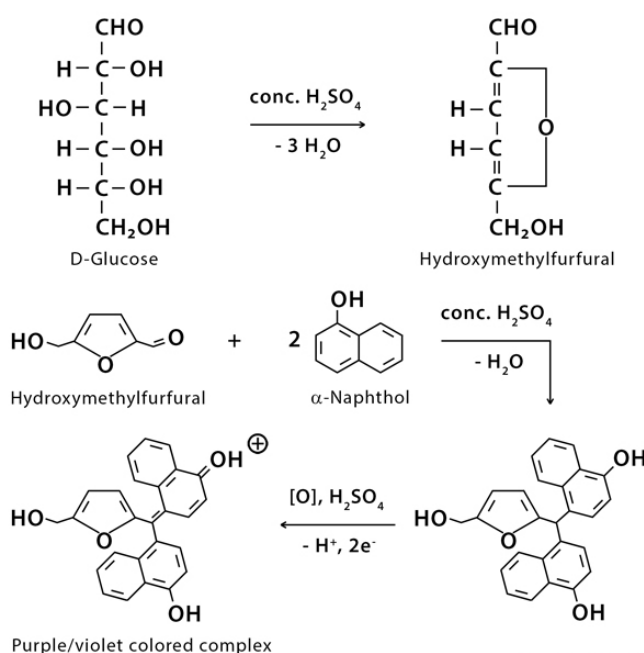


QUALITATIVE TESTS FOR CARBOHYDRATES

- In chemistry, qualitative analysis is **the determination of the chemical composition of a sample.**
- **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 coloured products. Pentoses yield furfural while Hexoses yield 5-Hydroxy methyl furfurals.

**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.



The formation of a purple ring is a positive indicator for Molisch's Test

Interpretation:

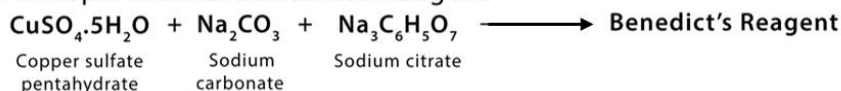
- This is a sensitive but a non-specific test and is given positive by all types of carbohydrates. If the oligosaccharides or polysaccharides are present they are first hydrolysed to mono saccharides which are then dehydrated to give the test positive.
- An appearance of reddish violet or purple coloured ring at the junction of two liquids is observed in a positive Molisch test.

2. BENEDICT'S TEST

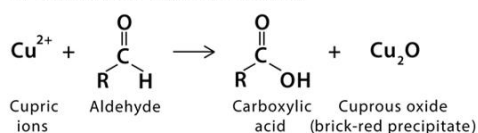
Principle:

- 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.

A. Preparation of Benedict's Reagent

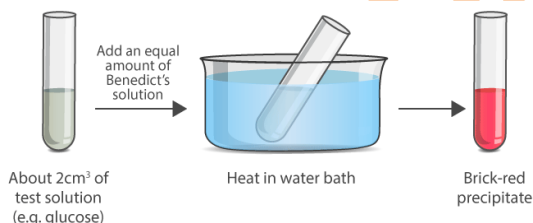


B. Benedict's Test Reaction



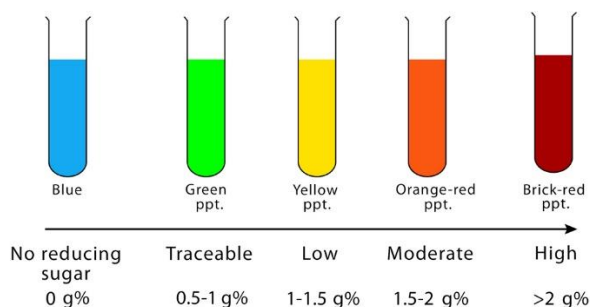
Procedure

- 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.



Interpretation:

- Benedict's test is a semi quantitative test. The colour of the precipitate gives a rough estimate of a reducing sugar present in the sample.
 - Green colour - 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% (+++++)

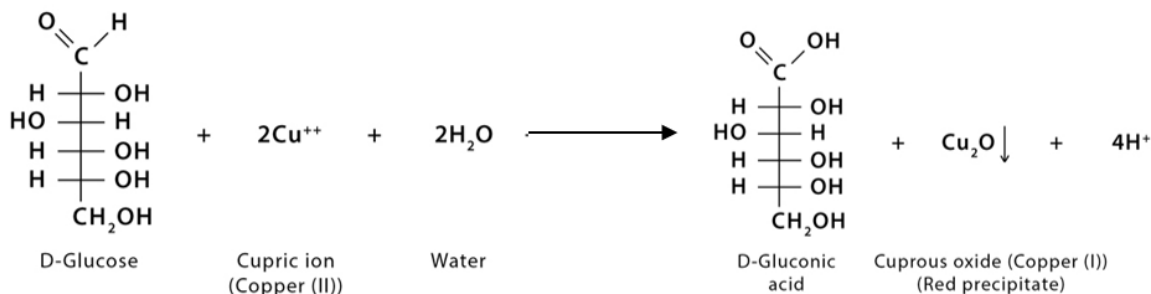


- Benedict's test is a semi quantitative test. The colour formed depends upon the amount of reducing sugar present in the mixture.

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.



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.



Solution remains blue
Carbohydrate is absent



Red precipitate is formed
Faster => Monosaccharide is present
Slower => Disaccharide is present

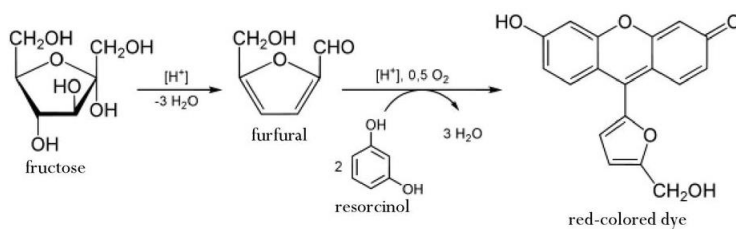
Interpretation:

- 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

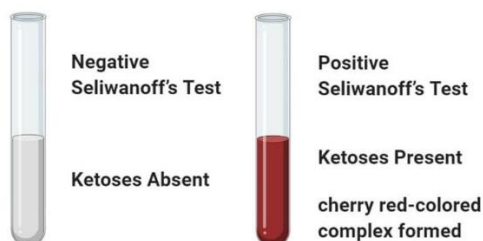
Principle:

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



Procedure:

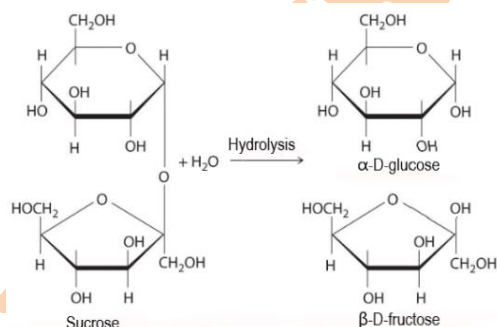
- To 3 ml of Seliwanoff reagent add 1ml of fructose.
- Boil for 30 seconds only.
- Cool the solution.
- A cherry red colour is observed in a positive reaction.

**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

**Procedure:**

- Add 2 drops of HCl and 1 drop of thymol blue to 5 ml of sucrose solution.
- The development of pink colour 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
- Formation of blue colour 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.

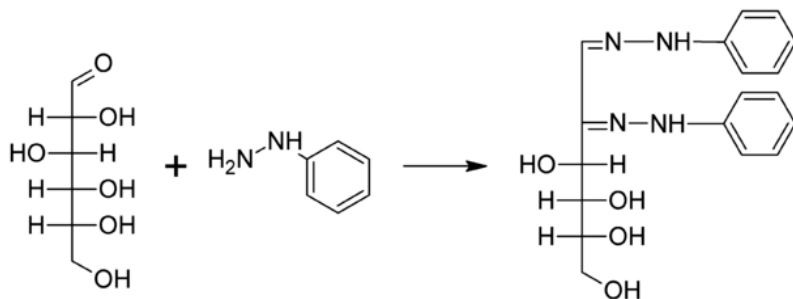
Interpretation:

- 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

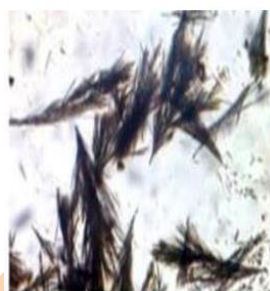
Principle:

- 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.



Procedure:

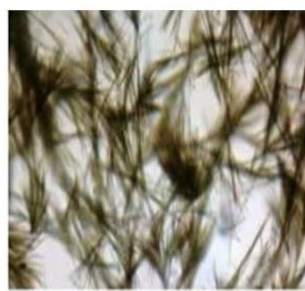
- Add 10 drops of glacial acetic acid to 5 ml of sugar solution in test tube.
- Then add a knife point of phenyl hydrazine hydrochloride and double the amount of sodium acetate crystals.
- Mix and warm a little to see that the solids are dissolved.
- Filter the solution in another test tube and keep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



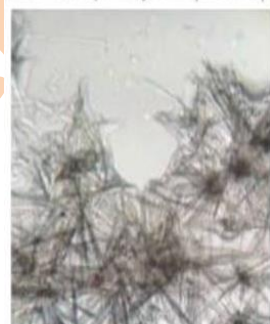
Needle shaped crystals (Glucose)



Needle shaped crystals (Fructose)



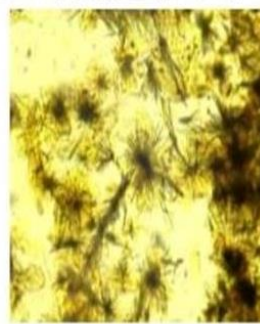
Needle shaped crystals (Mannose)



Is with thorny edge shaped crystals (Galactose)



Fine-long needle shaped crystals (Xylose)

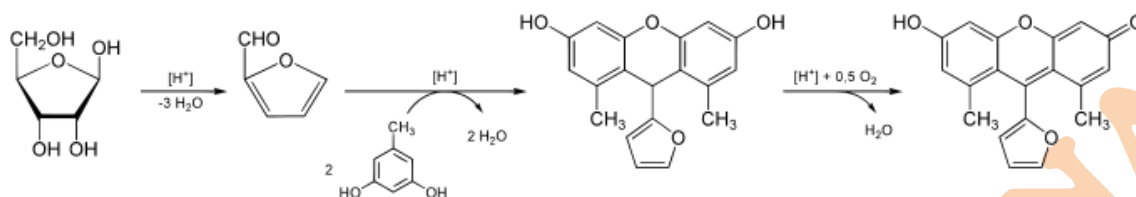


Sun flower shaped crystals (Maltos)

7. BIAL'S TEST

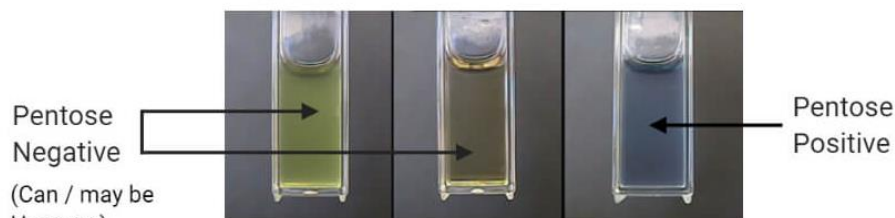
Principle:

- 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.



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.



Two negative tests (left, middle) and a positive test (right)

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 colours 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 colour obtained depends upon the length of the unbranched or linear chain available for complex formation



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

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

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, Erythro and Achrodextrins, formed as intermediates during hydrolysis of starch give violet, red and no colour with iodine respectively.

❖ Reaction 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

❖ Reaction 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

❖ Reaction 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

❖ Reaction for 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

❖ Reaction 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

❖ Reaction 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