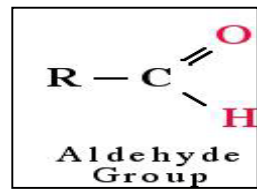
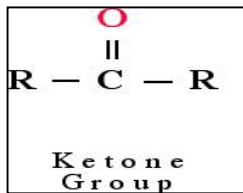


Qualitative tests of Carbohydrate



Carbohydrate :

- Carbohydrates are the **key source of energy** used by living things.
- Also serve as extracellular structural elements as in cell wall of bacteria and plant.
- Carbohydrates are defined as the polyhydroxy aldehydes or polyhydroxy ketones.
- Most , but not all carbohydrate have a formula $(CH_2O)_n$ (hence the name hydrate of carbon)
- In human body, the D-glucose is used.
- Simple sugars ends with –ose.



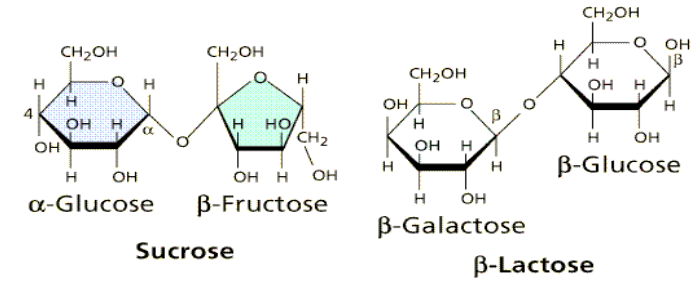
Several **classifications of carbohydrates** have proven useful, and are outlined in the following table.

Complexity	Simple Carbohydrates monosaccharides		Complex Carbohydrates disaccharides, oligosaccharides & polysaccharides		
Size	Tetrose C ₄ sugars	Pentose C ₅ sugars	Hexose C ₆ sugars	Heptose C ₇ sugars	etc.
C=O Function	Aldose sugars having an aldehyde function or an acetal equivalent. Ketose sugars having a ketone function or an acetal equivalent.				
Reactivity	Reducing sugars oxidized by Tollens' reagent (or Benedict's or Fehling's reagents). Non-reducing sugars not oxidized by Tollens' or other reagents.				

Classification :

1-Simple sugar (one unit) :

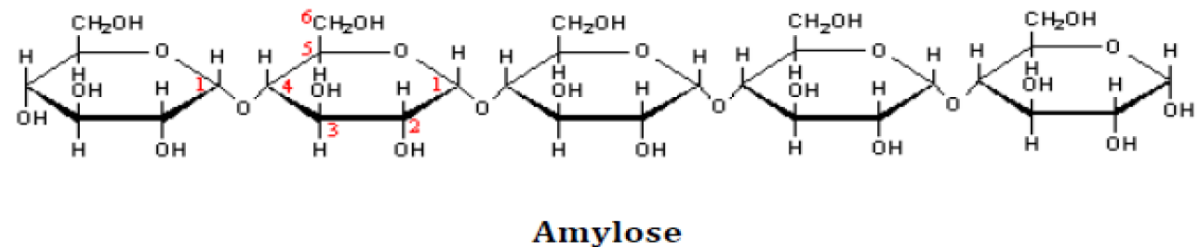
Monosaccharides contain **one** monosaccharide unit.



2-Complex sugar (more than one) :

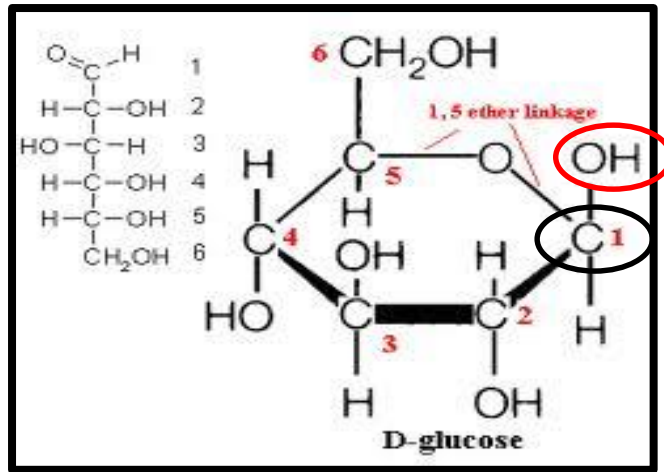
- **Disaccharides** contain **two** monosaccharide units.
- **Oligosaccharides** contain **3-9** monosaccharide units.
- **Polysaccharides** can contain more than 9 monosaccharide units.

-Complex carbohydrates can be broken down into smaller sugar units through a process known as **hydrolysis**.

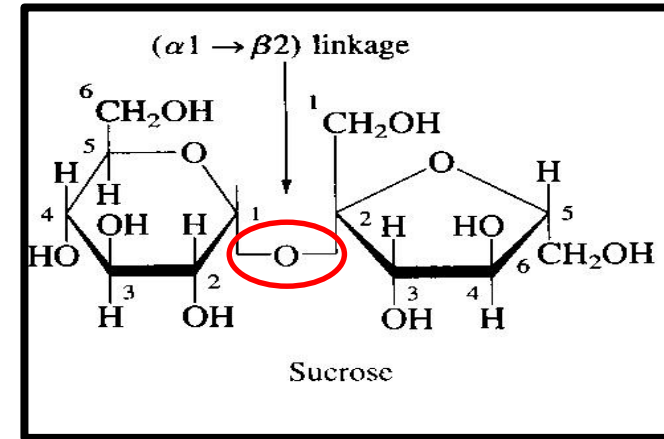


Reducing and non reducing sugars

Reducing and non reducing sugar :If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can act as a reducing agent and is termed a reducing sugar.



reducing



Non-reducing

Solubility of sugars [physical property]:

Monosaccharide and disaccharide **can** be dissolved freely in water because water is a polar substance, while polysaccharide **cannot** be dissolved easily in water, because, it has **high molecular weight**, which give colloidal solutions in water.



Chemical Properties of Carbohydrates:

- 1- **Molisch Test**: specific for carbohydrates.
- 2- **Benedict's Test**: presence of reducing sugars.
- 3- **Barfoed's Test**: test used for detecting the presence of monosaccharides.
- 4- **Bial's Test**: used to detect pentose [5C] monosaccharides.
- 5- **Seliwanoff's Test**: distinguish between aldoses and ketoses.

1.Molisch test:

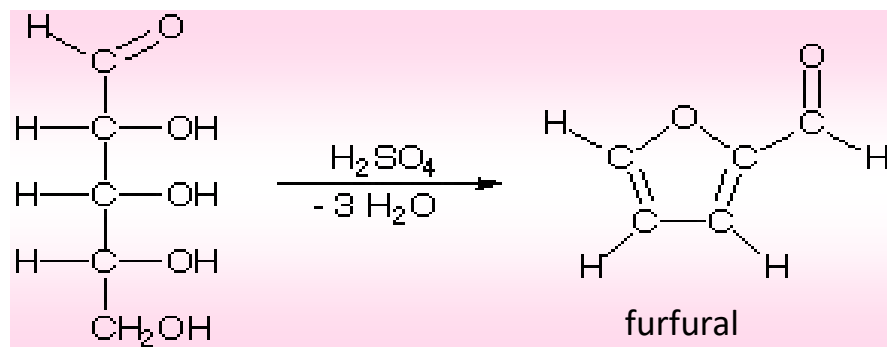
This test is specific for all carbohydrates Monosaccharide gives a **rapid** positive test, Disaccharides and polysaccharides react **slower**.

Objective:

To identify the carbohydrate from other macromolecules, lipids and proteins.

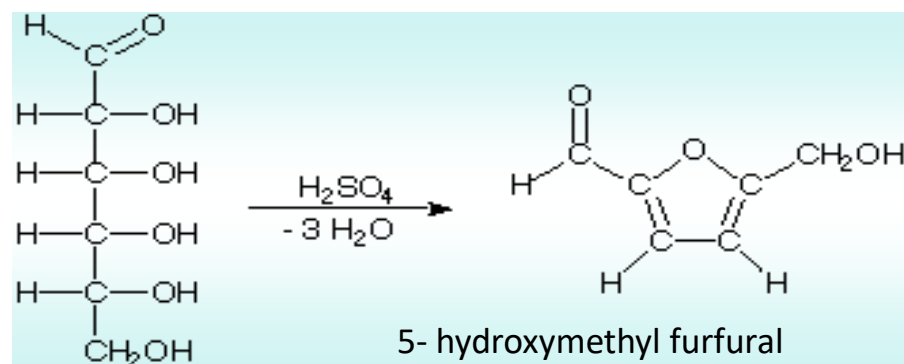
Principle:

- The test reagent(H₂SO₄) dehydrates pentose to form **furfural** and dehydrates hexoses to form **5-hydroxymethyl furfural**.
- The furfural and 5- hydroxymethyl furfural further react with α-naphthol present in the test reagent to produce a **purple ring**.



α -naphthol
[Present in the reagent]

Purple ring



α -naphthol

Purple ring

Method :

- 1-Two ml of a sample solution is placed in a test tube.
- 2-Two drops of the Molisch reagent (which α -naphthol in 95% ethanol) is added.
- 3-The solution is then poured slowly into a tube containing two ml of concentrated sulfuric acid so that two layers form, producing violet ring appear as liaison between the surface separations.

Tube	Observation
Glucose	
Lactose	
Starch	



2. Benedict's test:

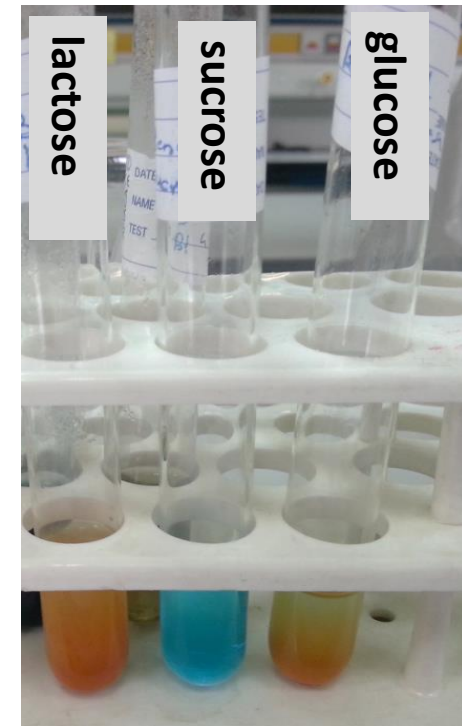
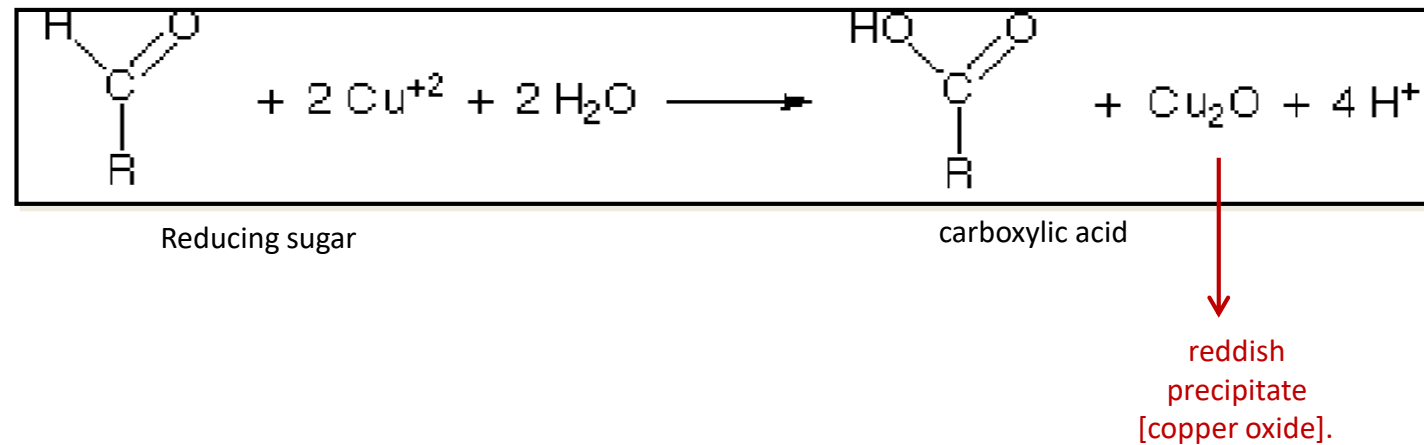
Objective:

To detect the presence of reducing sugars.

- **All monosaccharides** are reducing sugars; they all have a free reactive carbonyl group.
 - **Some disaccharides** have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution
- Large polymers** of glucose, such as starch, are **not** reducing sugars, since the concentration of hemiacetal groups is very low.

Principle :

- The copper sulfate (CuSO_4) present in Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar in **alkaline medium**.
- Reducing sugars are **oxidized** by the copper ion in solution to form a carboxylic acid and a **reddish precipitate** of copper (I) oxide.



Method :

- One ml of a sample solution is placed in a test tube.
- Two ml of Benedict's reagent is added.
- The solution is then heated in a boiling water bath for five minutes.
- A positive test is indicated by: The formation of a reddish precipitate.

Tube	observation
1-glucose	
2-lactose	
3-starch	

3.Barfoed's Test:

Objective :

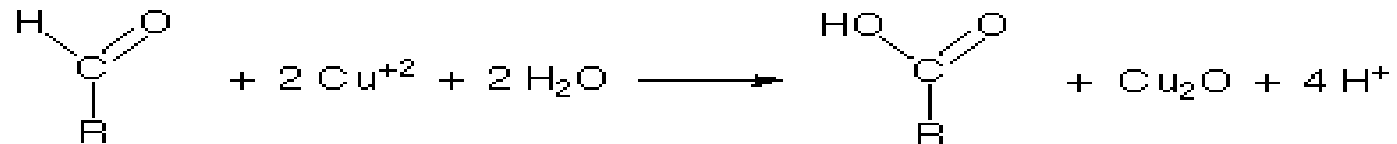
To distinguish between mono- , di- and poly saccharides.

Principle :

Barfoed's test used copper (**II**) ions in a **slightly acidic medium**.

Reducing **monosaccharides** are oxidized by the copper ion in solution to form a carboxylic acid and a **reddish** precipitate of copper (**I**) oxide within three minutes. Reducing **disaccharides** undergo the same reaction, but do so at a **slower** rate.

-The **nonreducing** sugars give negative result.



Method :

- Place one ml of a sample solution in a test tube.
- Add 3 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid).
- Heat the solution in a boiling water bath for 6 minutes(after the 3 min check the tubes).

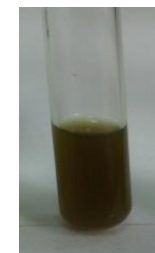
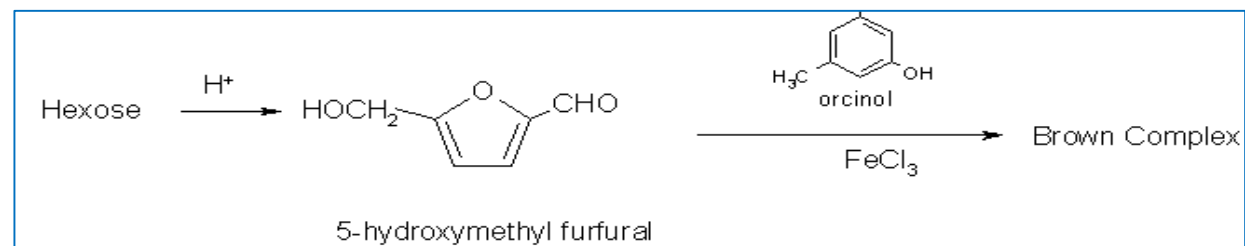
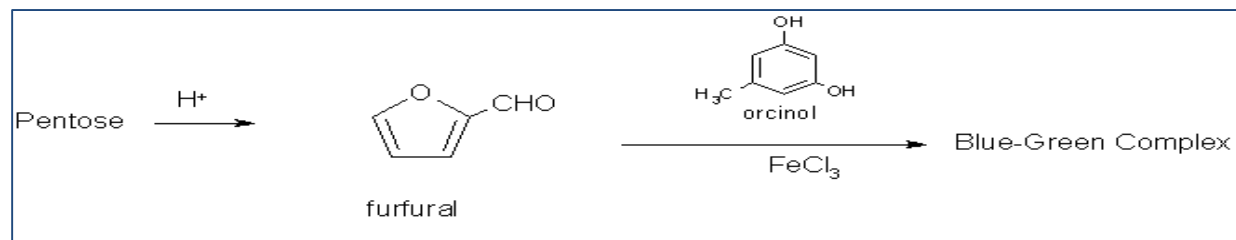
Tube	observation
glucose	
lactose	
starch	



4. Bial's Test:

Objective: To distinguish between pentose monosaccharide and hexose monosaccharide

Principle: Bial's test uses concentrated HCl as a dehydrating acid and orcinol + traces of ferric chloride [FeCl_3] as condensation reagent. 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 or green** product, while **hexoses** yield muddy-brown to grey condensation product.



Method :

- Put 2 ml of a sample solution in a test tube.
- Add 3 ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) to each tube.
- Heat the tubes gently in hot water bath for two minutes.
- If the color is not obvious, more water can be added to the tube.

Tube	observation
1-glucose	
2-ribose	

5.Seliwanoff's Test:

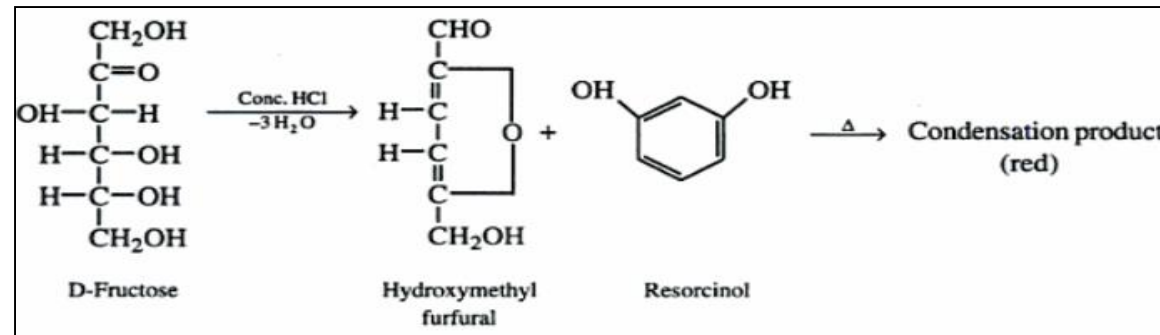
Objective:

used to distinguish between aldoses (like glucose) and ketoses (like fructose).

Principle:

Seliwanoff's Test uses 6M HCl as dehydrating agent and resorcinol as condensation reagent. The test reagent dehydrates **ketohexoses** to form 5-hydroxymethylfurfural. 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a **cherry red product** within two minutes.

-**Aldohexoses** react to form the same product, but do so more slowly giving yellow color.



Method :

- One half ml of a sample solution is placed in a test tube.
- Two ml of Seliwanoff's reagent (a solution of resorcinol and HCl) is added.
- The solution is then heated in a boiling water bath for two minutes.

Tube	observation
1-glucose	
2-fructose	

Questions:

- 1- Explain, although starch has free hemiacetal bond it gives negative Benedict test?
- 2- Why glucose (monosaccharide) and lactose (disaccharide) give positive Benedict test?
- 3- What is the difference between Benedict and Barfoed's reaction?
- 4- What are the carbohydrates' that give positive result with Seliwanoff ? why?