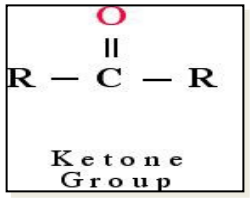
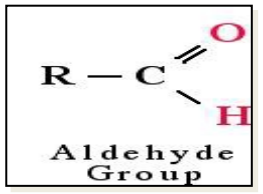
Qualitative tests of Carbohydrate

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.

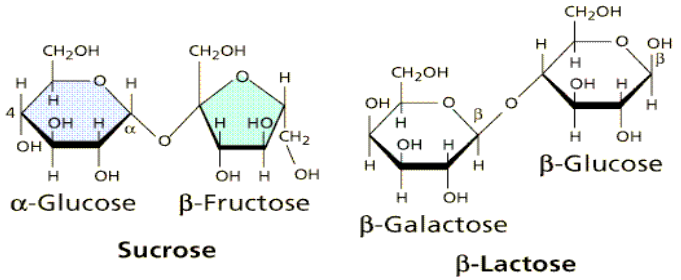




Several **classifications of carbohydrates** have proven useful, and are outlined in the following table.

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 :



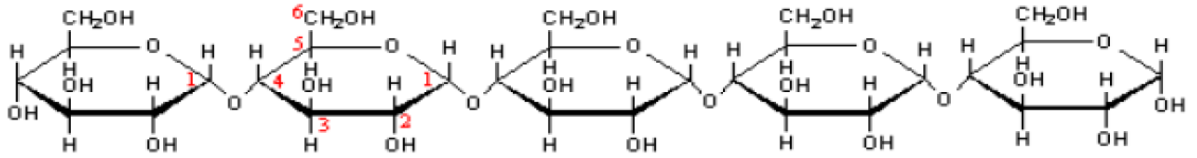
1-Simple sugar (one unit) :

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.

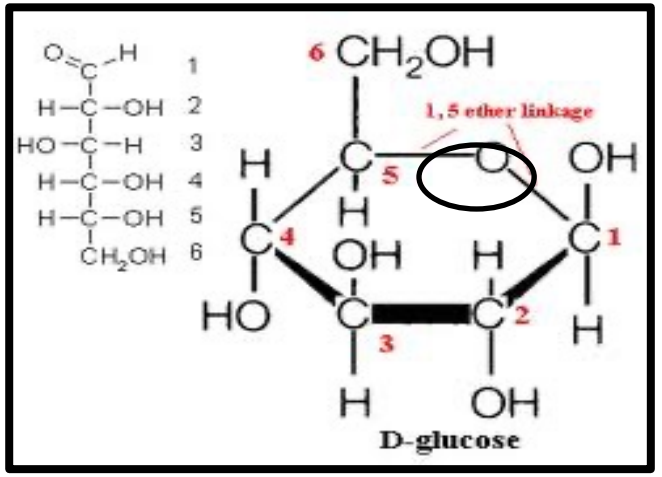
-Complex carbohydrates can be broken down into smaller sugar units through a process known as **hydrolysis**.



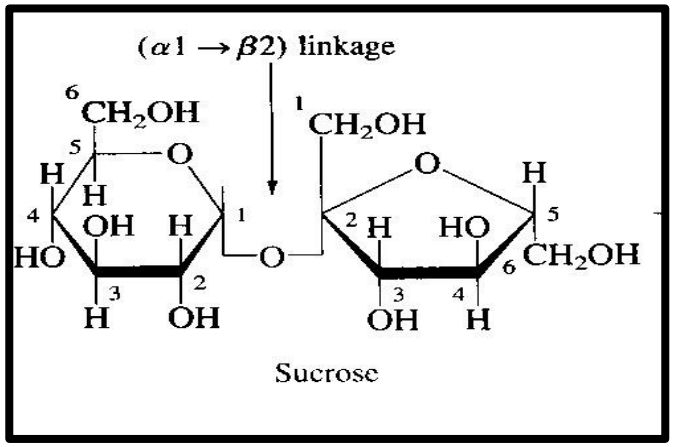
Amylose

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] monosacharides.

5- **Seliwanoff's Test**: distinguish between aldoses and ketoses.

1.Molisch test:

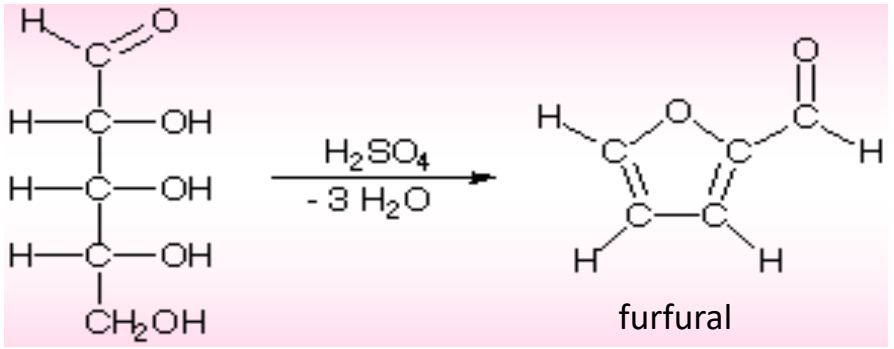
This test is specific for all carbohydrates Monosaccharide gives a **rapid** positive test, Disaccharides and polysaccharides react **slower**.

Objective:

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

Principle:

- The test reagent(H_2SO_4) 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**.

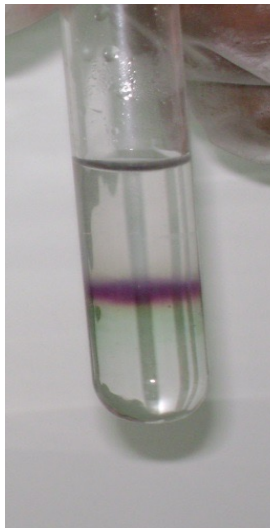


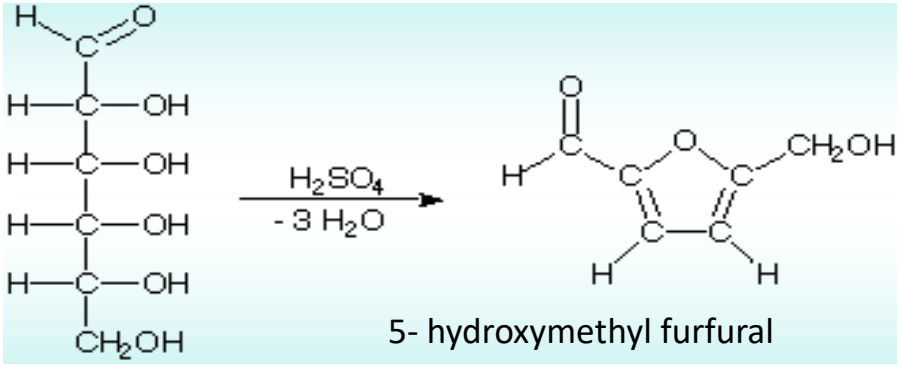
furfural

α-naphthol

[Present in the reagent]

Purple ring





5- hydroxymethyl furfural

α-naphthol

Purple ring

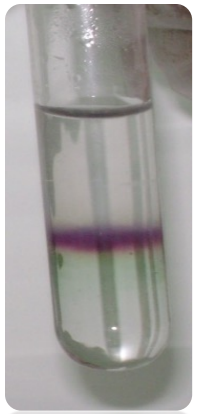
Method :

1-Two ml of a sample solution is placed in a test tube.

2-Two drops of the Molisch reagent (which α-napthol 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.

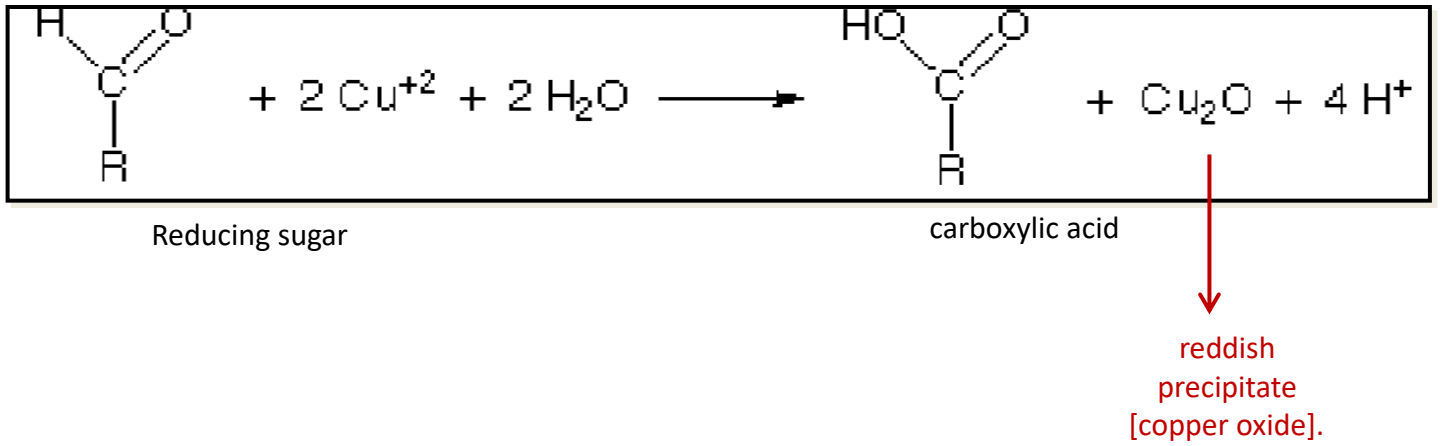
- **All monosaccharides** are reducing sugars; they all have a free reactive carbonyl group.

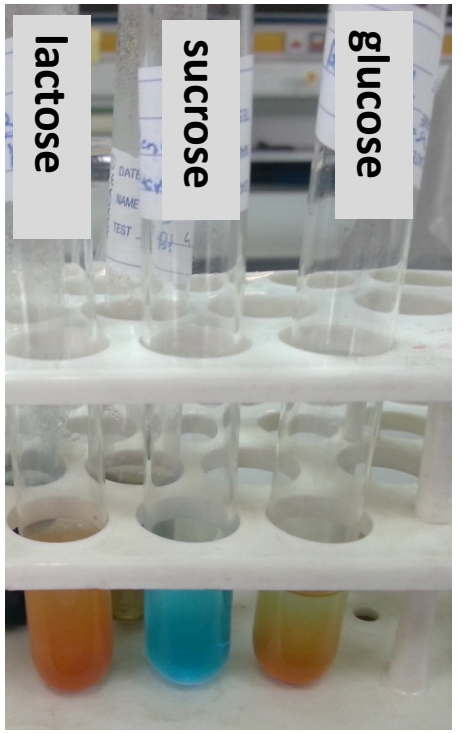
- **Some disaccharides** have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution

-**Large polymers** of glucose, such as starch, are **not** reducing sugars, since the concentration of hemiacetal groups is very low.

Principle :

- The copper sulfate (CuSO4) 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 :

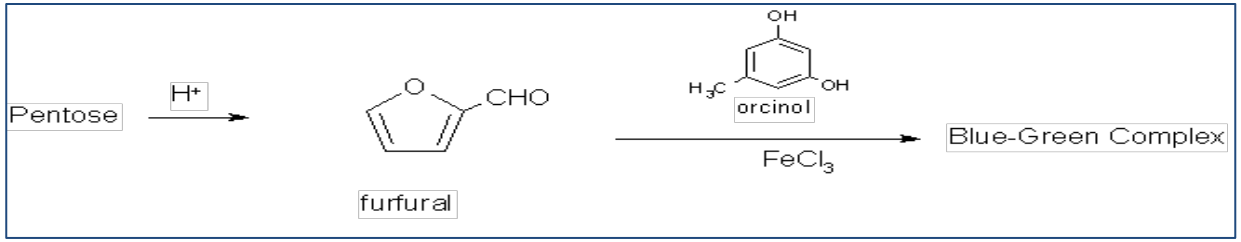
- Place one ml of a sample solution in a test tube.
- Add 3 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid).
- Heat the solution in a boiling water bath for 6 minutes(after the 3 min check the tubes).

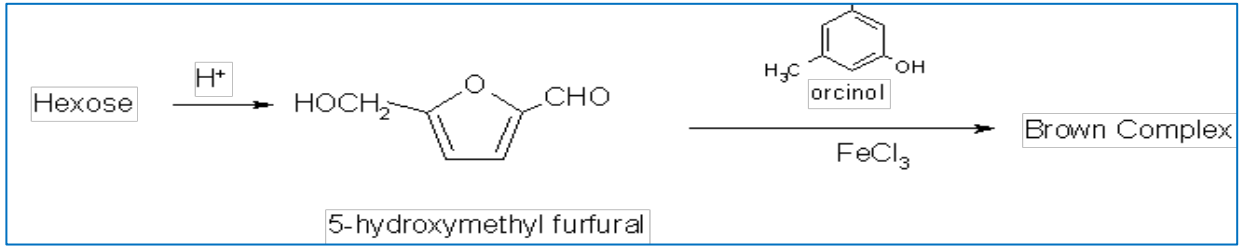
Tube	observation
glucose	
lactose	
starch	

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.
- If the color is not obvious, more water can be added to the tube.

Tube	observation
1-glucose	
2-ribose	

5.Seliwanoff's Test:

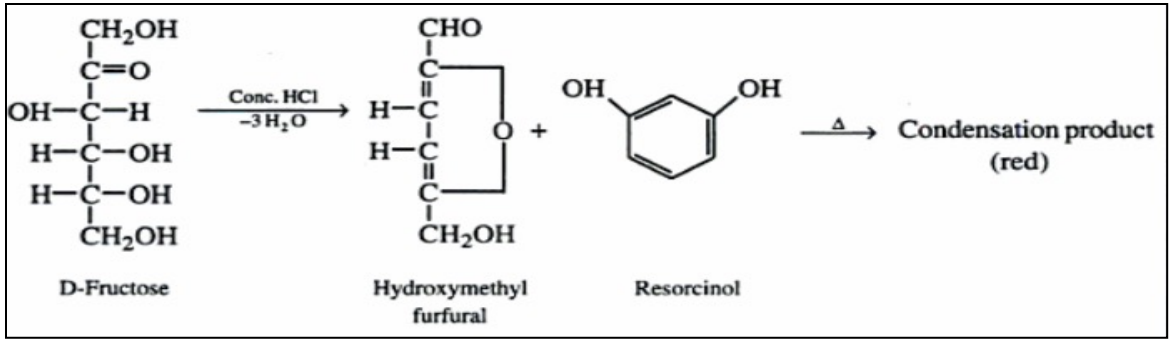
Objective:

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

Principle:

Seliwanoff's Test uses 6M HCl as dehydrating agent and resoncinol 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 to faint pink 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- Name the complex formed by the addition of concentrated sulfuric acid to sugar solution and explain the reaction?

2- Why sucrose gives negative Benedict test?

3- Explain, although starch has free hemiacetal bond it gives negative Benedict test?

4- Why glucose (monosaccharide) and lactose (disaccharide) give positive Benedict test?

5- What is the difference between Benedict and Barfoed's reaction?

6- What are the carbohydrates' that give positive result with Seliwanoff ? why?