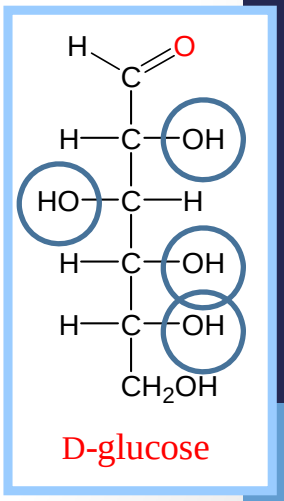
Qualitative tests of Carbohydrate

Introduction

- 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**



D-glucose

Classification

1-Simple sugar: (one unit)

Monosaccharides contain **one** monosaccharide unit.

2-Complex sugar (more than one):

Disaccharides contain **two** monosaccharide units.

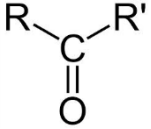
- **Oligosaccharides** contain **3-9** monosaccharide units.
- **Polysaccharides** can contain more than 9 monosaccharide units.

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

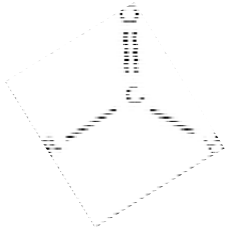
Monosaccharide

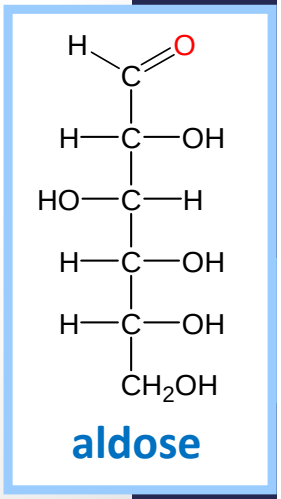
- They can be classified by the number of carbon atoms
- trioses (C-3)
- tetroses (C-4)
- **pentoses (C-5)**
- **hexoses** (C-6)
- heptoses (C-7)

- also be classified as ketoses or aldoses.
- **A ketose** contains a carbonyl group attached to two R groups having one or more hydroxyl groups.



- **An aldose** contains terminal aldehyde group in addition to R group containing -OH.





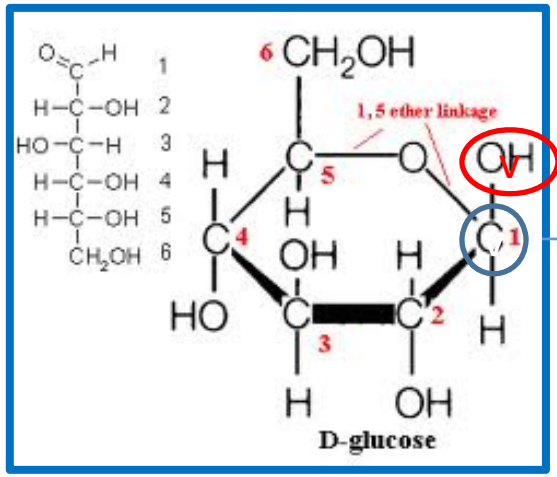
aldose

Solubility

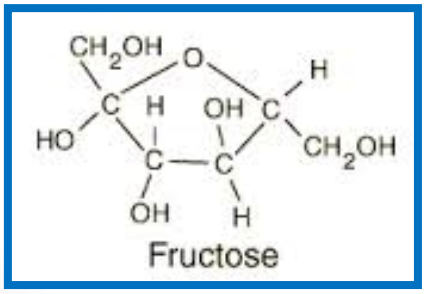
- Monosaccharide and disaccharide can be dissolved freely in water because water is a polar substance, while polysaccharide cannot be dissolved easily in water, because, it has high molecular weight , which give colloidal solutions in water soluble.

Reducing and non reducing sugars

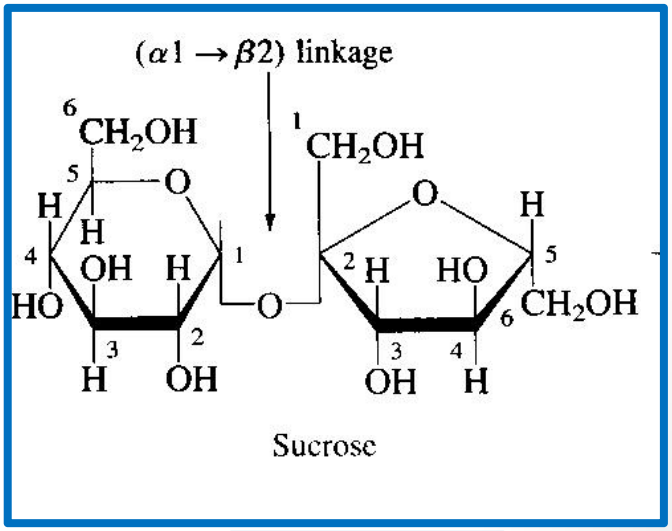
- Reducing and non reducing sugar :If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can act as a reducing agent and is termed a reducing sugar.



Anomeric carbon



reducing



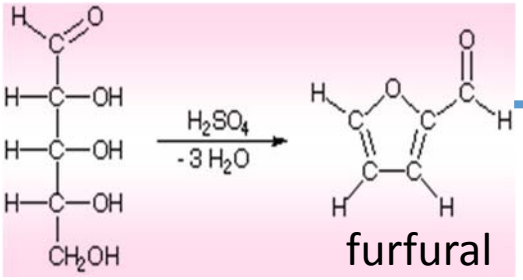
Non-reducing

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.

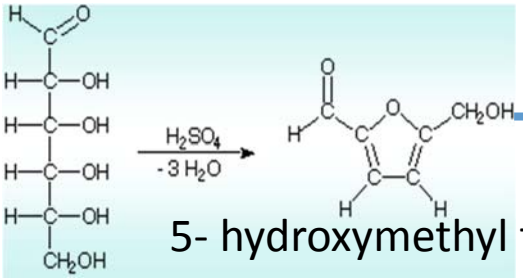
- **Principle**: The test reagent(**H2SO4**) dehydrates pentose to form furfural and dehydrates hexoses to form 5-hydroxymethyl furfural.
- The furfural and 5- hydroxymethyl furfural further react with α-naphthol present in the test reagent to produce a purple product.



furfural

α-naphthol

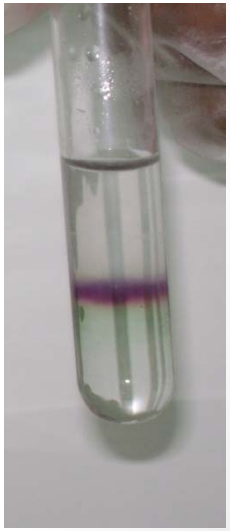
Purpel color



5- hydroxymethyl furfural

α-naphthol

Purpel color



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
1-glucose	
2-ribose	
3-sucrose	
4-starch	

Benedict's test

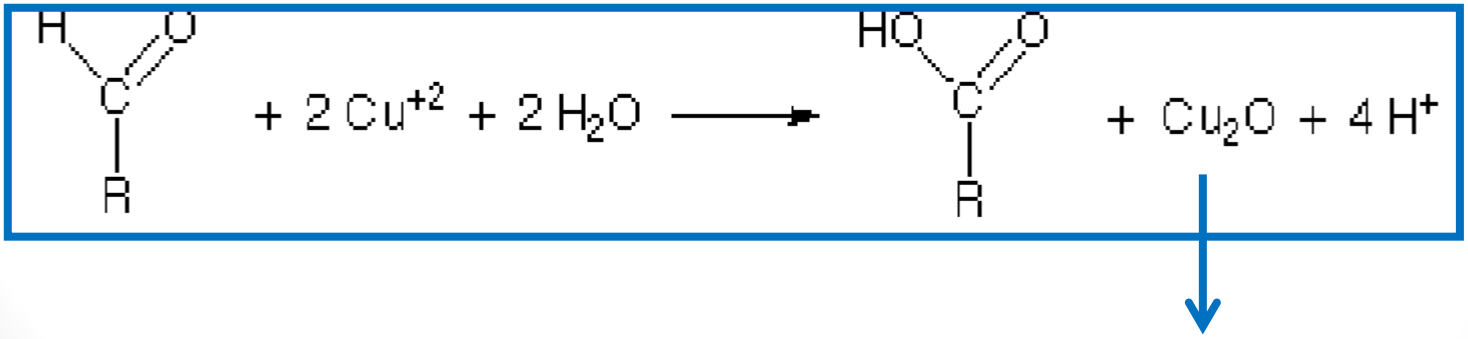
- Benedict's reagent is used as a test for the presence of reducing sugars.
- **All** monosaccharides are reducing sugars; they all have a free reactive carbonyl group.

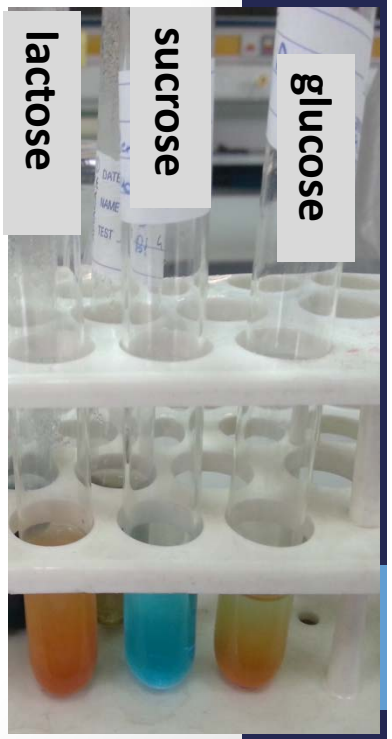
- **Some** disaccharides have exposed carbonyl groups and are also reducing sugars. Other disaccharides such as sucrose are non-reducing sugars and will not react with Benedict's solution.

- Large polymers of glucose, such as starch, are not reducing sugars
- **Objective**: To distinguish between the reducing and non-reducing sugars.

Benedict's test

- **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 oxide.





- 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-sucrose	
3-lactose	

Barfoed's Test

- This test is performed to distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.
- 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.

- Barfoed's reagent, cupric acetate in acetic acid , so in acidic medium , disacchride is a weaker reducing agent than monosacchride, so mono sacchride will reduce the copper in less time.

$$(CH_3COO)_2Cu + 2H_2O \rightarrow 2CH_3COOH + Cu(OH)_2$$

Cupric hydroxide

$$Cu(OH)_2 \xrightarrow{\Delta} CuO + H_2O$$

$$\text{D-glucose} + 2CuO \rightarrow \text{D-gluconic acid} + Cu_2O \downarrow$$

Cuprous oxide
(Red ppt.)

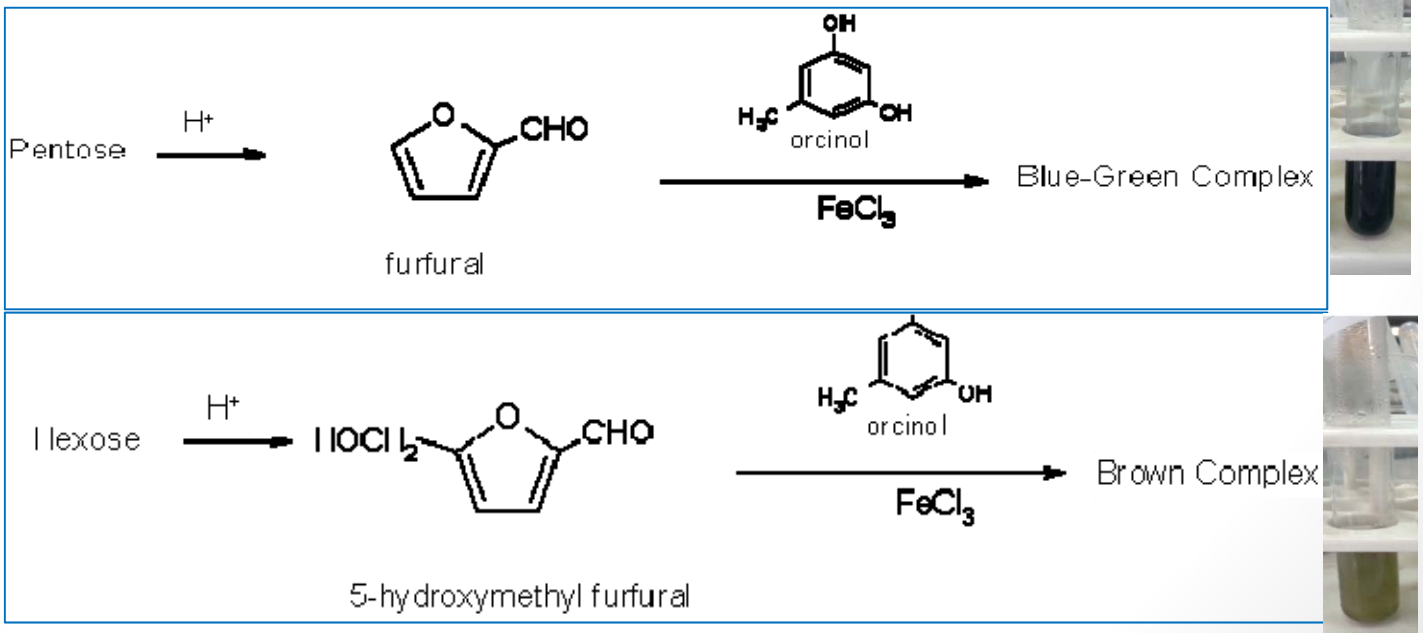
- Place one ml of a sample solution in a test tube.
- Add 3 ml of Barfoed's reagent (a solution of cupric acetate and acetic acid.
- Heat the solution in a boiling water bath for 6 minutes(after the 3 min check the tubes).

Tube	observation
1-glucose	
2-sucrose	
3-lactose	

Bial's Test

- This test is used to distinguish between pentose and hexose monosacharides.
- 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 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.



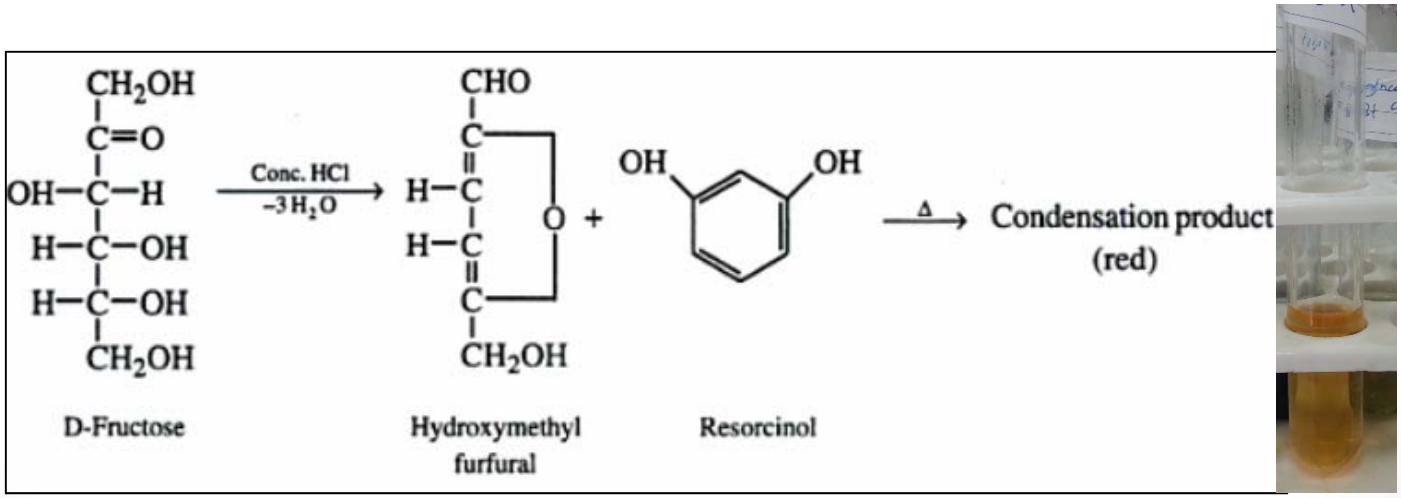
- Put 2 ml of a sample solution in a test tube.
- Add 2 ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) to each tube.
- Heat the tubes gently in hot water bath.
- If the color is not obvious, more water can be added to the tube.

Tube	observation
1-glucose	
2-ribose	
3-fructose	

Seliwanoff's Test

- This test is used to distinguish between aldoses (like glucose) and ketoses (like fructose).
- Objective: To distinguish between aldose and ketone sucrose.

- 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.



- 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	

Test	objective
Molisch test	To identify the carbohydrate from other macromolecules lipids and proteins
Benedict's test	Benedict's reagent is used as a test for the presence of reducing sugars.
Barfoed’s Test	to distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.
Bial’s Test	To distinguish between pentose monosaccharide and hexose monosaccharide
Seliwanoff's Test	To distinguish between aldose and ketone sugars