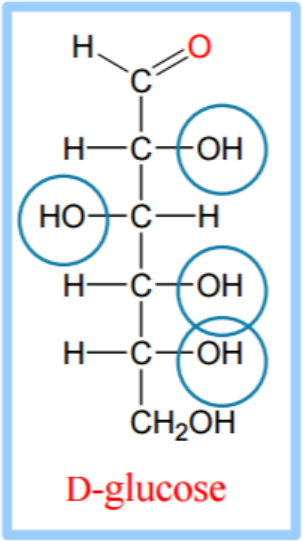
Qualitative tests of Carbohydrates-I-

BCH302 [Practical]

Carbohydrates:

- 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).
- Sugars ends with –ose**.**
- In human body, the D-glucose is used.



D-glucose

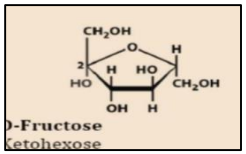
- **Biological role:**

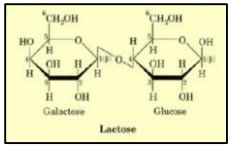
1. Are the **key source of energy** used by living things.
2. Also serve as extracellular structural elements as in cell wall of bacteria and plant.

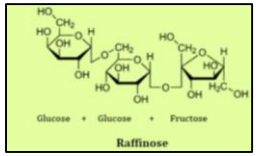
Classification of carbohydrates:

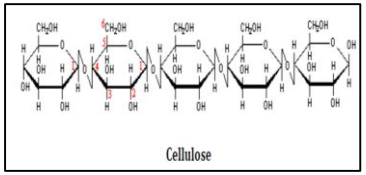
Carbohydrates

- **Simple**
  - One unite → Monosaccharides
  - Tow unite → Disaccharides
- **Complex**
  - 3-9 unites → Oligosaccharides
  - More than 9 unites → Polysaccharides







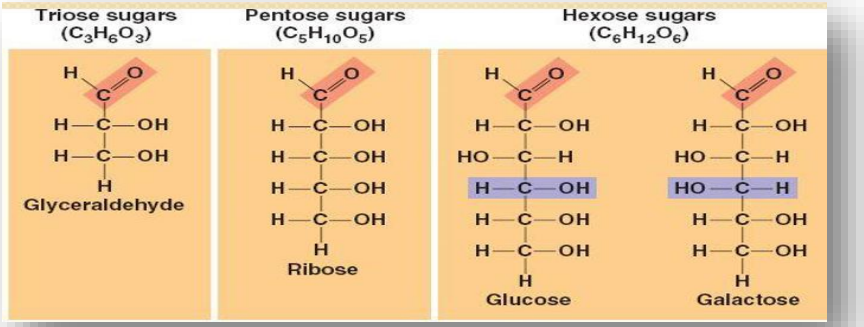


-Complex carbohydrates can be broken down into smaller sugar units through a process known as **hydrolysis.**

Classification of monosaccharide:

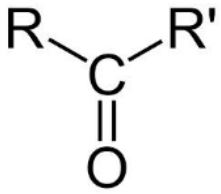
1. According to the number of carbon atoms:

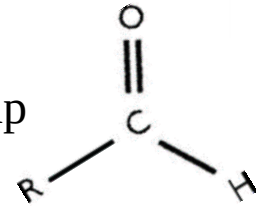
- Trioses (C-3).
- Tetroses (C-4).
- Pentoses (C-5).
- Hexoses (C-6).
- Heptoses (C-7).



2. According to the C=O function:

- **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.

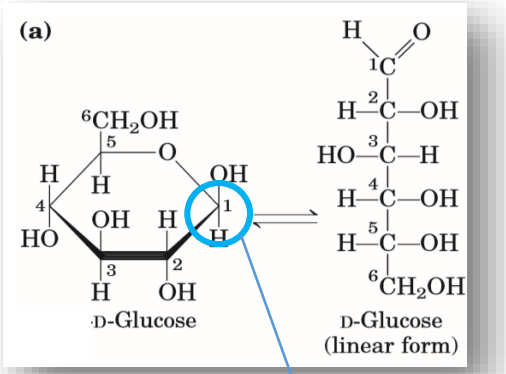




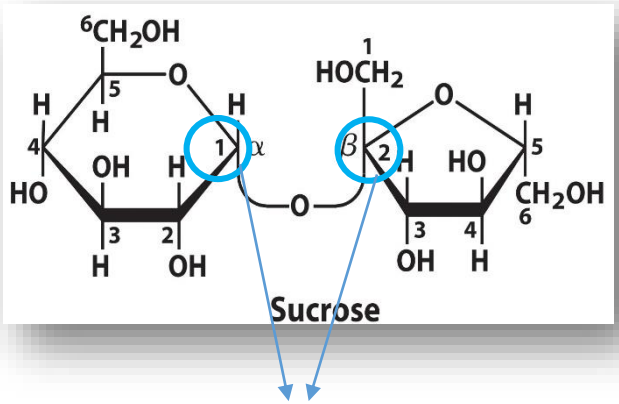
Reducing and non-reducing sugars:

- **Reducing and non Reducing sugar :**

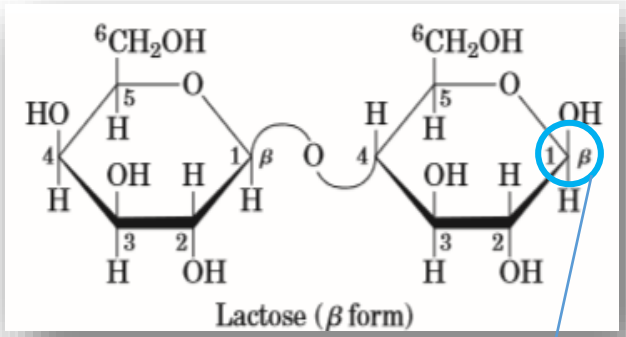
➔ If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can act as a reducing agent and is termed a reducing sugar.



FREE anomeric carbon
Reducing sugar



attached anomeric carbon
Non-Reducing sugar



FREE anomeric carbon
Reducing sugar

Reducing and non-reducing sugars cont’:

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

- **Some disaccharides** have exposed carbonyl groups and are also reducing sugars like **lactose**. While 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.

Solubility of carbohydrate:

- **Monosaccharide** and **disaccharid**e can be dissolved freely in water because water is a polar substance.

- **Polysaccharide** cannot be dissolved easily in water, because, it has high molecular weight , which give colloidal solutions in water.

Practical part

Qualitative tests of carbohydrates

1. Molicsh test: To identify the carbohydrate from other macromolecules.
2. Benedict test: for the presence of reducing sugars.
3. Barfoed's Test: for to distinguish between reducing monosaccharides, reducing disaccharides and non reducing di-polysaccharides.
4. Bial's Test: To distinguish between pentose monosaccharide and hexose monosaccharide (to detect pentoses).
5. Seliwanoff's Test: To distinguish between aldoses and ketoses (to detect ketoses).

Experiment 1 : Molisch test

Objective:

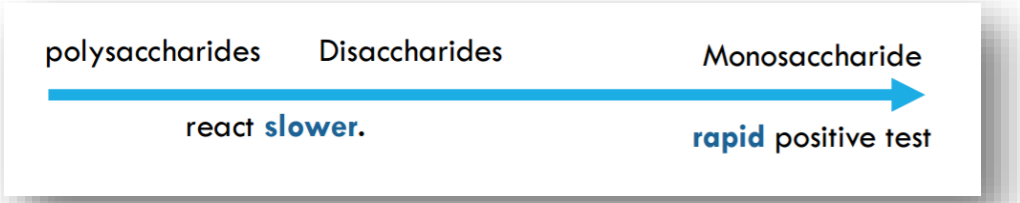
- To identify the carbohydrate from other macromolecules lipids and proteins (this test is specific for all carbohydrates).

Principle:

- **Two solutions are added :** H_2SO_4, α-naphthol

1- The test reagent (H_2SO_4) dehydrates pentose to form furfural and dehydrates hexoses to form 5-hydroxymethyl furfural.

2- The furfural and 5- hydroxymethyl furfural further react with α-naphthol present in the test reagent to produce a purple product.



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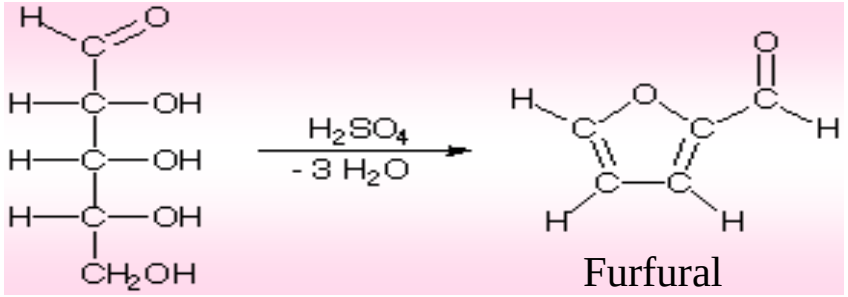
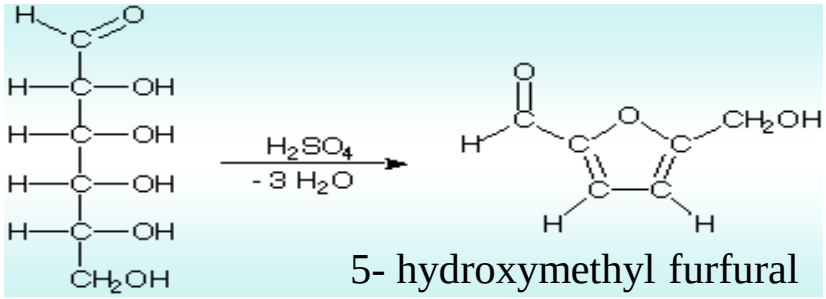


Furfural

Pentose sugar

α-naphthol

[Present in the reagent]



5- hydroxymethyl furfural

Hexose sugar

α-naphthol

Experiment 1 : Molisch test

Method:

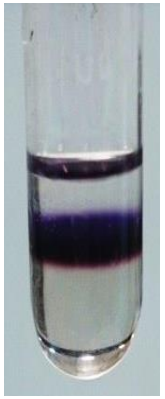
CAUTION

Concentrated sulfuric acid is extremely corrosive and can cause serious burns when not handled properly.

1. Two ml of a sample solution is placed in a test tube.
2. 0.5 ml 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.

Results:

Tube	Observation
Glucose	
Lactose	
Starch	



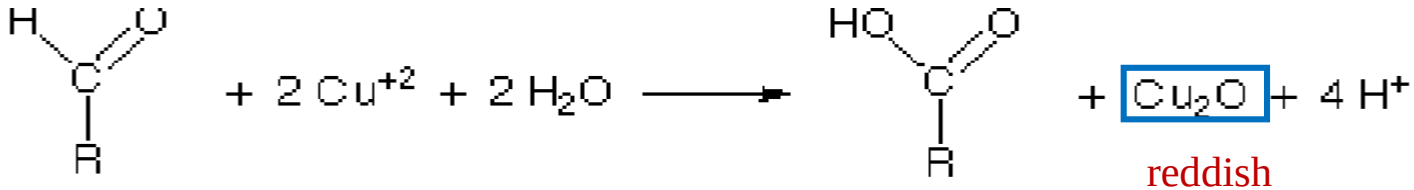
Experiment 2 : Benedict's Test

Objective:

- To distinguish between the reducing and non-reducing sugars (to detect the presence of reducing sugar).

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 oxide.



reducing sugar carboxylic acid reddish precipitate

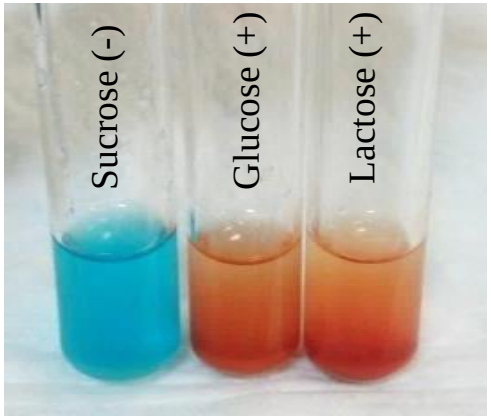
Experiment 2 : Benedict's Test

Method:

1. One ml of a sample solution is placed in a test tube.
2. Two ml of Benedict's reagent is added.
3. The solution is then heated in a boiling water bath for five minutes.

Results:

Tube	Observation
Glucose	
Lactose	
Sucrose	



Experiment 3 : Barfoed's test

Objective:

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

Principle:

- Barfoed's test used copper (II) ions in a slightly acidic medium.
- Reducing saccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.
- Different types of reducing sugars react at different rates. Reducing monosaccharides react quickly with Barfoed's reagent (acidic condition), but reducing **disaccharides react very slowly or not at all.**
- The non-reducing sugars give negative result.

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

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.)

Experiment 3 : Barfoed’s test

Method:

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

Results:

Tube	Observation
Glucose	
Lactose	
Sucrose	

Glucose (+)

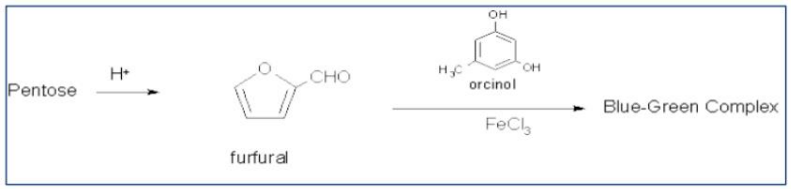
Experiment 4: Bial's test

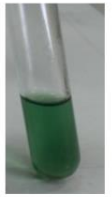
Objective:

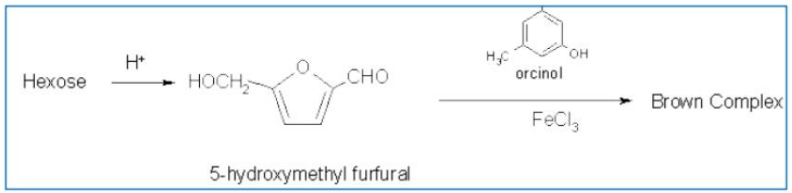
- To distinguish between pentose monosaccharide and hexose monosaccharide (to detect pentoses).

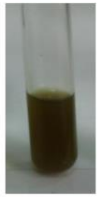
Principle:

- Bial's reagent (a solution of orcinol, HCl and ferric chloride).
- Bial's test uses concentrated HCl as a dehydrating acid and orcinol + traces of ferric chloride [FeCl3] 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.









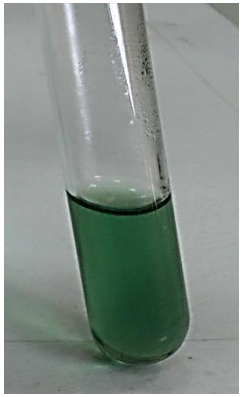
Experiment 4: Bial’s test

Method:

1. Put 2 ml of a sample solution in a test tube.
2. Add 2 ml of Bial's reagent to each tube.
3. Heat the tubes gently in hot water bath.
4. If the color is not obvious, more water can be added to the tube.

Results:

Tube	Observation
Glucose	
Ribose	



Ribose

(+)

Glucose

(-)

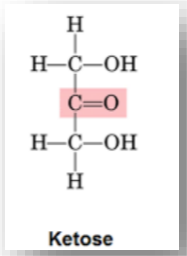
Experiment 5: Seliwanoff's test

Objective:

- To distinguish between aldoses and ketoses (to detect ketoses).

Principle:

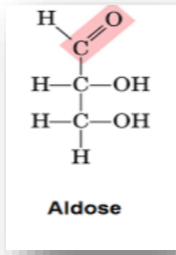
- Seliwanoff's Test uses 6M HCl as dehydrating agent and resoncinol as condensation reagent.

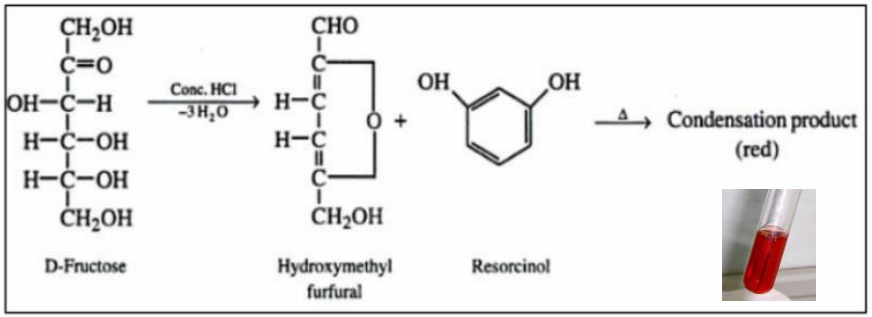


1. The test reagent dehydrates ketohexoses to form 5-hydroxymethylfurfural.

2. 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a cherry red product within two minutes.

3. Aldohexoses react to form the same product, but do so **<u>more slowly</u>** giving yellow to faint pink color.





Experiment 5: Seliwanoff's test

Method:

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

Results:

Tube	Observation
Glucose	
Fructose