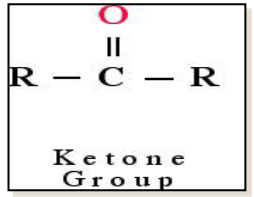
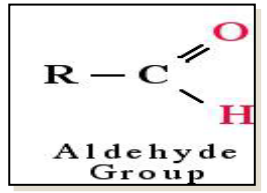
Qualitative Tests of Carbohydrate-I

Introduction:

- Carbohydrates are defined as the **polyhydroxy aldehydes or polyhydroxy ketones.**
- Most , but not all carbohydrate have a formula (CH2O)n (hence the name hydrate of carbon).
- In human body, the D-glucose is used.
- Simple sugars ends with –ose.





- **Biological role:**
- 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.

Classification of carbohydrates:

- 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 based on complexity:

1. **Simple sugar (one unit) :**

Monosaccharides contain one monosaccharide unit.

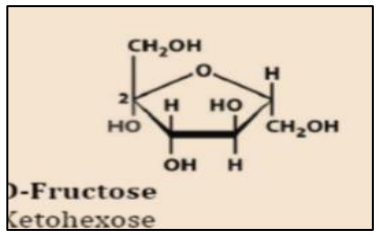
2. **Complex sugar (more than one) :**
    1. **Disaccharides** contain two monosaccharide units.
    2. **Oligosaccharides** contain 3-9 monosaccharide units.
    3. **Polysaccharides** can contain more than 9 monosaccharide units.

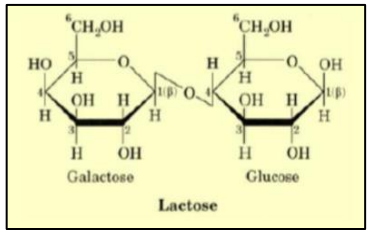
- Complex carbohydrates can be broken down into smaller sugar units through a process known as **hydrolysis.**

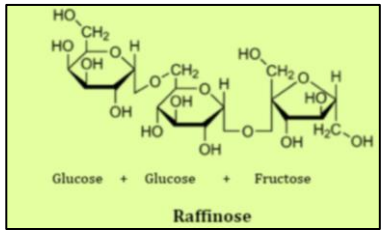
Classification based on complexity:

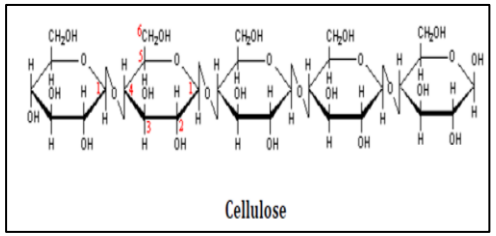
Carbohydrates

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





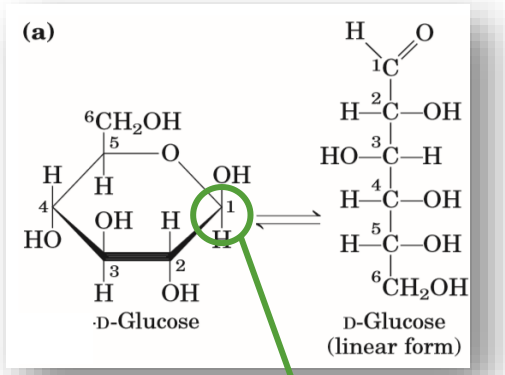




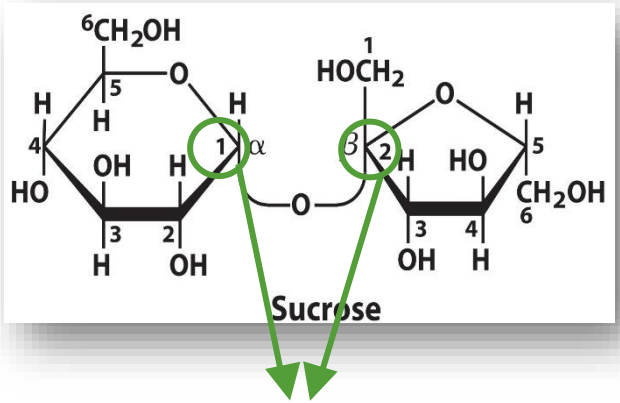
Classification based on reactivity:

- **Reducing and non Reducing sugar :**

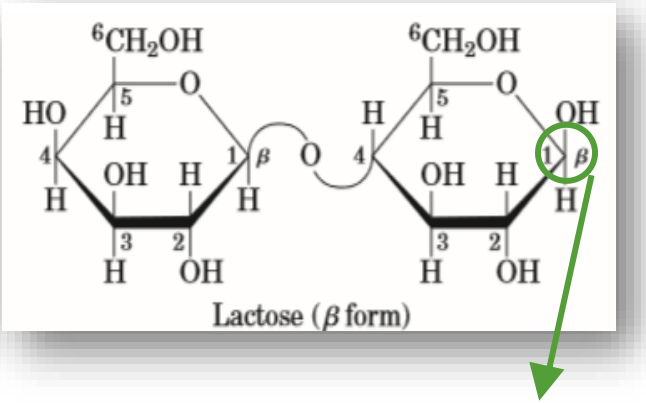
If the oxygen on the anomeric carbon of a sugar is not attached to any other structure, that sugar can act as a **reducing agent** and is termed a **reducing sugar.**



FREE anomeric carbon
Reducing sugar



attached anomeric carbon
Non-Reducing sugar



FREE anomeric carbon
Reducing sugar

Classification based on reactivity 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 sugars [physical property]:

- **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

Chemical properties 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 (detect reducing monosaccharides).
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 I : Molisch test

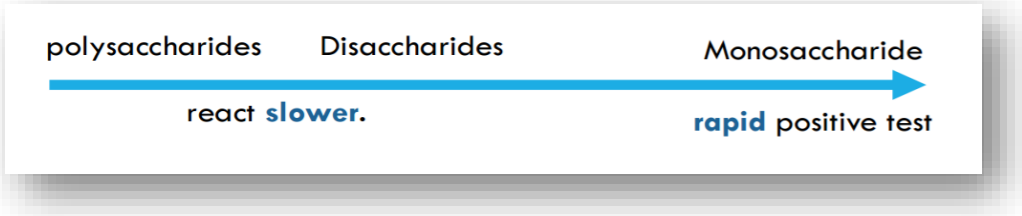
➢ **Objective:**

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 and 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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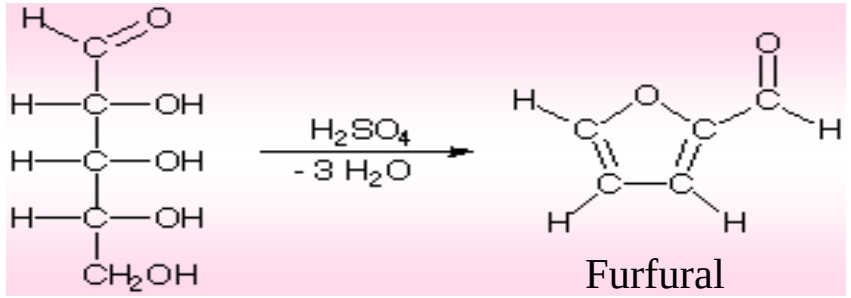
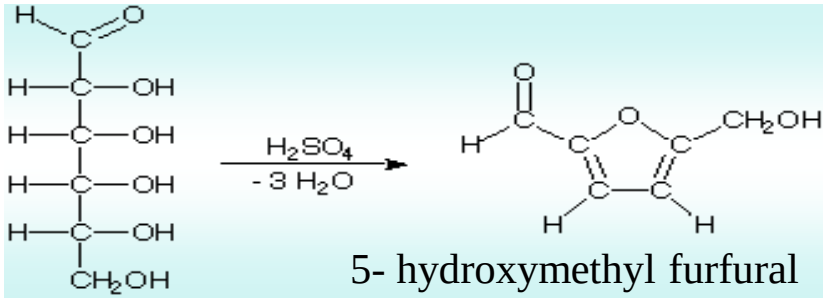


Furfural

Pentose sugar

α-naphthol

[Present in the reagent]



5- hydroxymethyl furfural

Hexose sugar

α-naphthol

Experiment I : Molisch test

CAUTION

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

- **Method:**

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

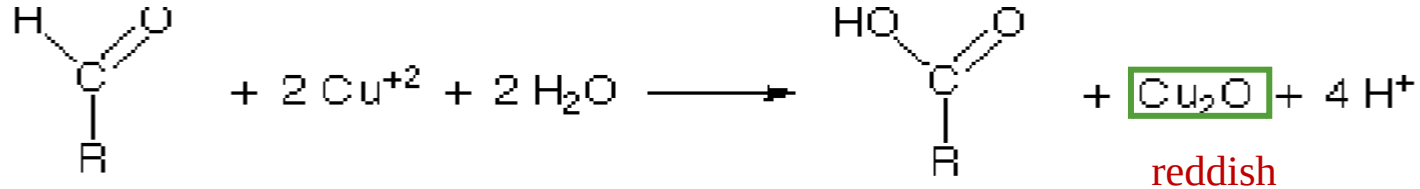
Experiment 2 : Benedict's test

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.
- The non-reducing sugars give negative result.



reducing sugar carboxylic acid reddish precipitate

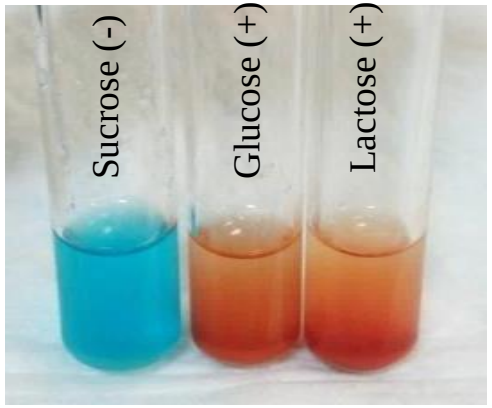
Experiment 2 : Benedict's test

- **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.
4. A positive test is indicated by: The formation of a reddish precipitate.

- **Results:**

Tube	Observation
Glucose	
Lactose	
Sucrose	



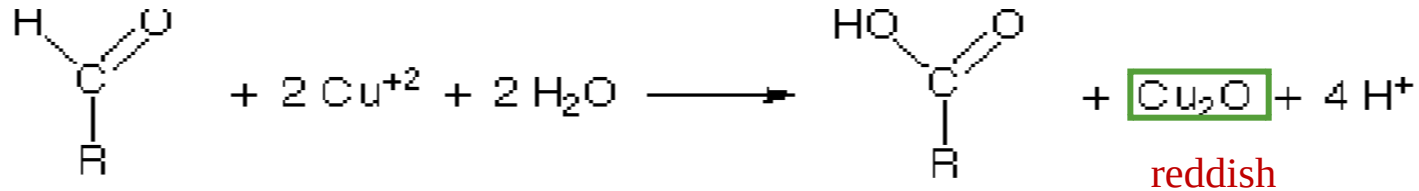
Experiment 3 : Barfoed's test

➢ **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.



reducing sugar carboxylic acid reddish precipitate

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	
Starch	

Glucose (+)

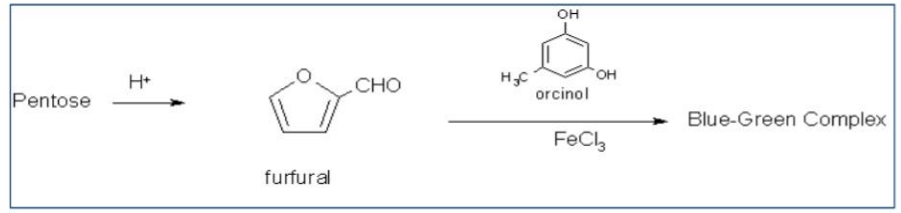
Experiment 4 : Bial's test

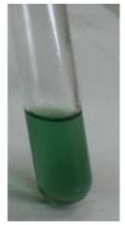
➢ **Objective:**

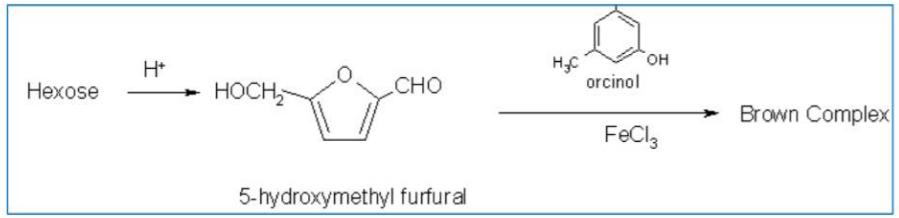
To distinguish between pentose monosaccharide and hexose monosaccharide (to detect pentoses).

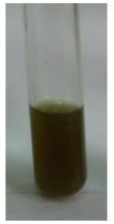
➢ **Principle:**

- Bial's reagent (orcinol, concentrated HCl as a dehydrating acid and 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.









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	

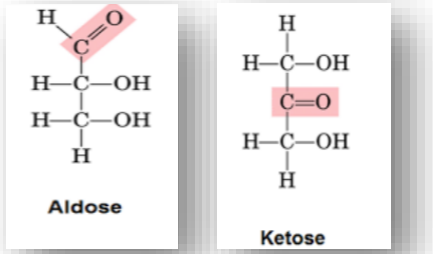
Ribose
(+)

Glucose
(-)

Experiment 5 : Seliwanoff's test

➢ **Objective:**

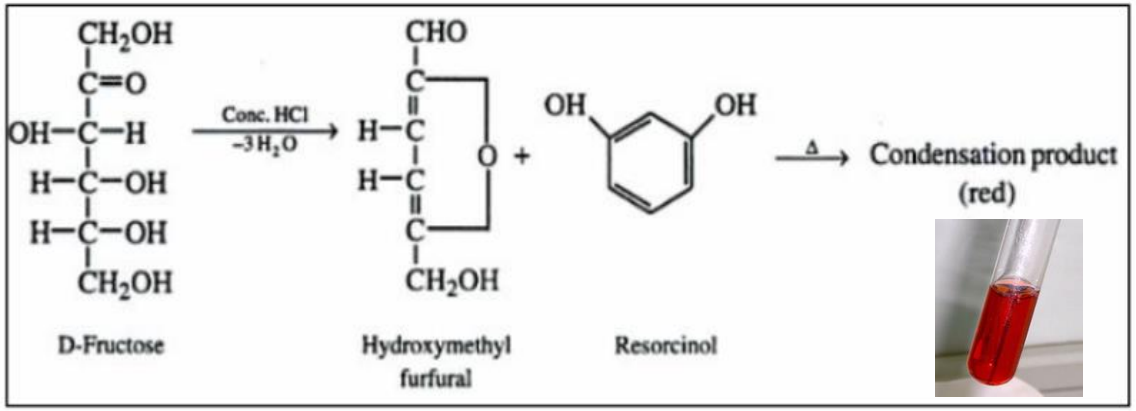
To distinguish between aldoses and ketoses (to detect ketoses).



➢ **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 ➔ 5-hydroxymethylfurfural further condenses with resorcinol present in the test reagent to produce a cherry red product within **two minutes.**
2. Aldohexoses react to form the same product, but do so **more slowly** giving yellow to faint pink color.



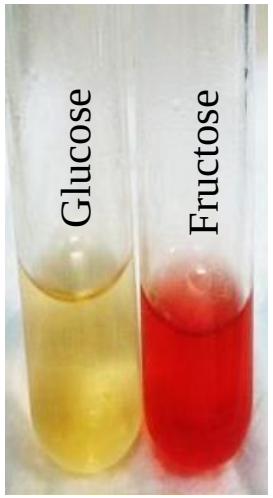
Experiment 5 : Seliwanoff's test

- **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	



Homework:

- **Explain, although starch has free hemiacetal bond it gives negative Benedict test?**
- **What is the difference between Benedict and Barfoed's reaction?**