

Glucose



Galactose

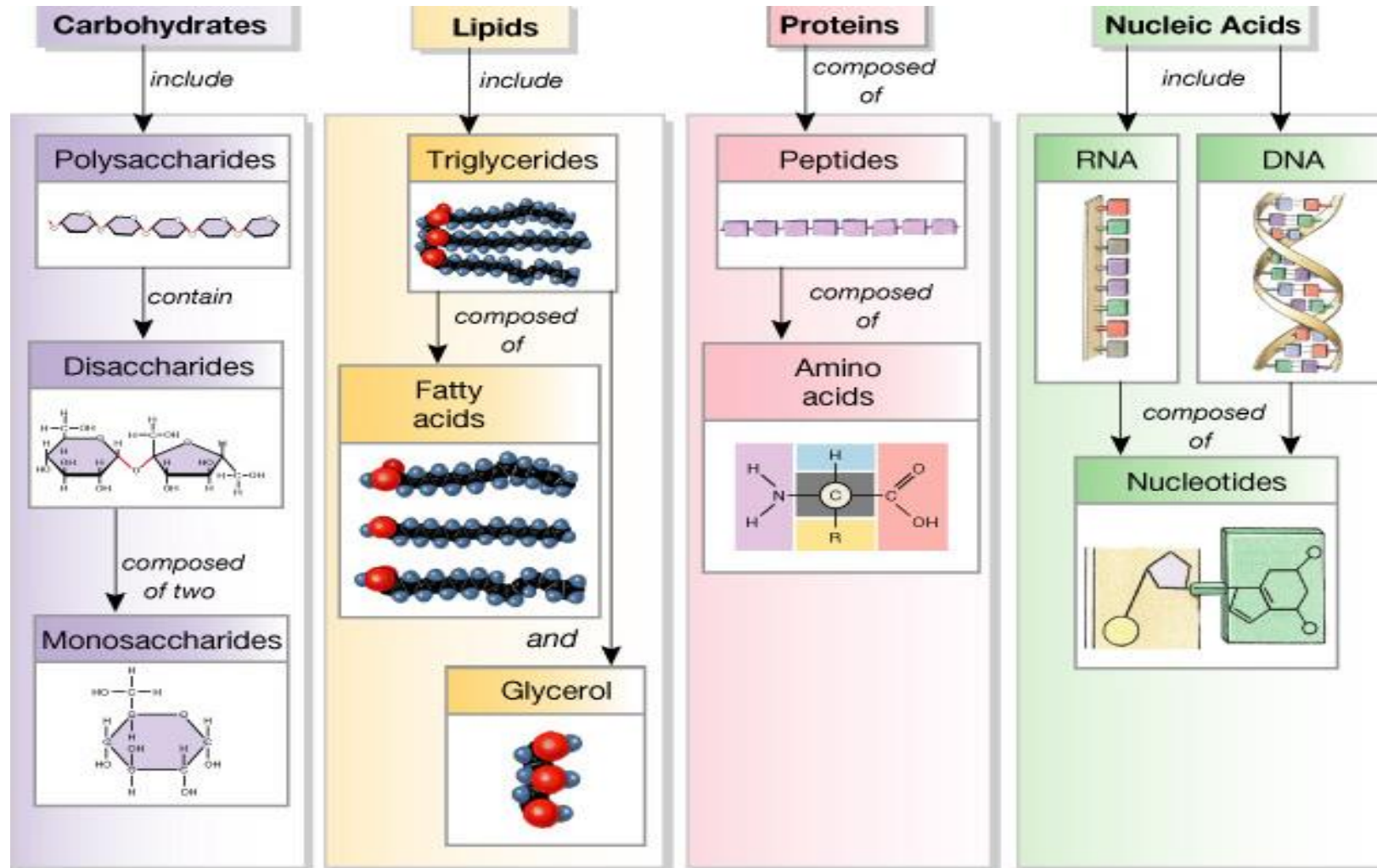


Fructose



# QUALITATIVE TESTS OF CARBOHYDRATE

# MACROMOLECULE



# 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.

## Structure:

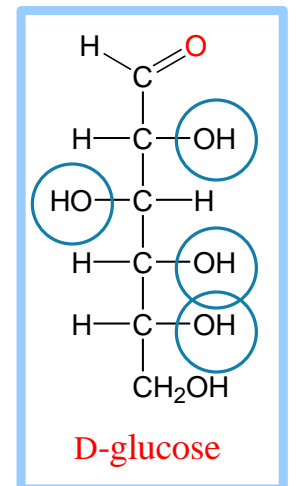
Carbohydrates are defined as the polyhydroxy aldehydes or polyhydroxy ketones.

Most , but not all carbohydrate have a formula

$(\text{CH}_2\text{O})_n$  (hence the name hydrate of carbon)

In human body, the D-glucose is used.

Simple sugars ends with -ose



# CLASSIFICATION

## Carbohydrates

### Simple sugar

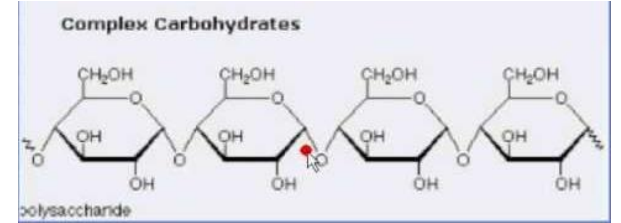
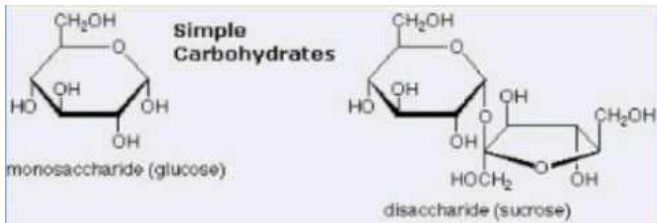
### Complex sugar

### Monosaccharides

### Disaccharides

### Oligosaccharides

### Polysaccharides



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

# CLASSIFICATION OF MONOSACCHARIDE

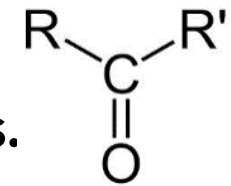
The number of carbon atoms

trioses (C-3)  
tetroses (C-4)  
**pentoses (C-5)**  
hexoses (C-6)  
heptoses (C-7)

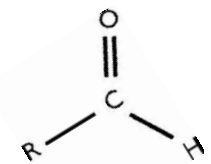
Triose	$C_3H_6O_3$
Tetroses	$C_4H_8O_4$
Pentoses	$C_5H_{10}O_5$
Hexoses	$C_6H_{12}O_6$

also be classified as ketoses or aldoses.

**A ketose** contains a carbonyl group attached to two R groups having one or more hydroxyl groups.

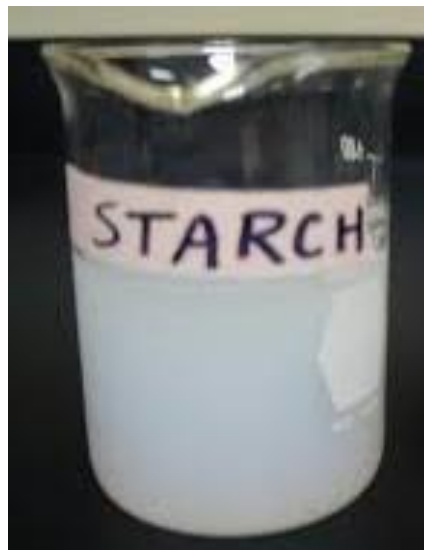


**An aldose** contains terminal aldehyde group in addition to R group containing -OH.



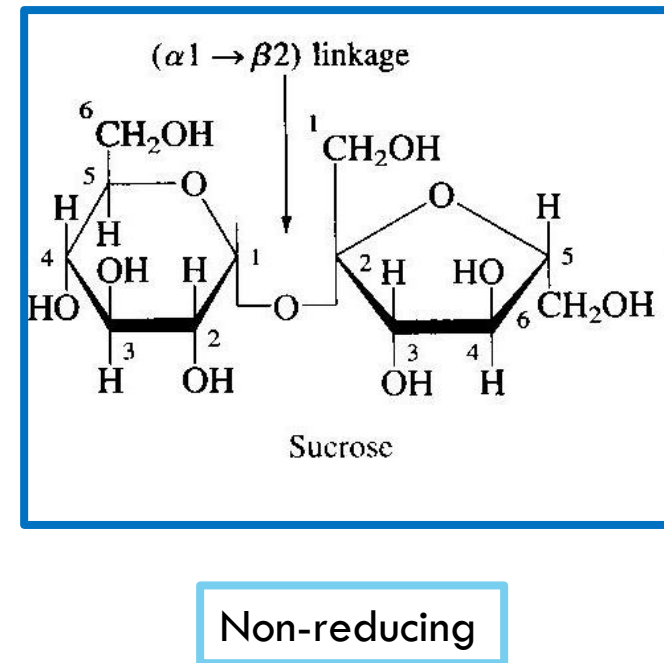
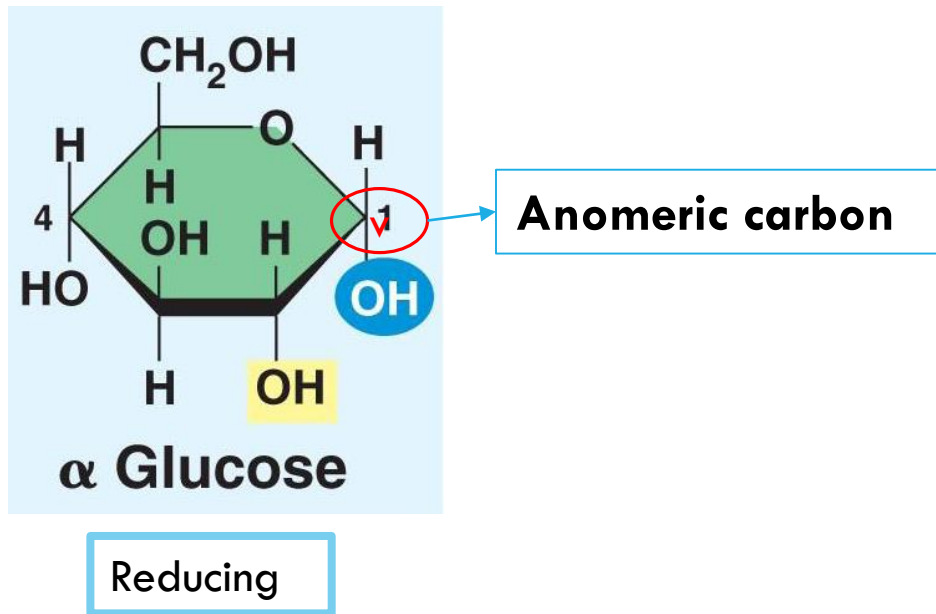
# SOLUBILITY OF CARBOHYDRATE

- **Monosaccharide and disaccharide can be dissolved freely in water, Why?**
- **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.



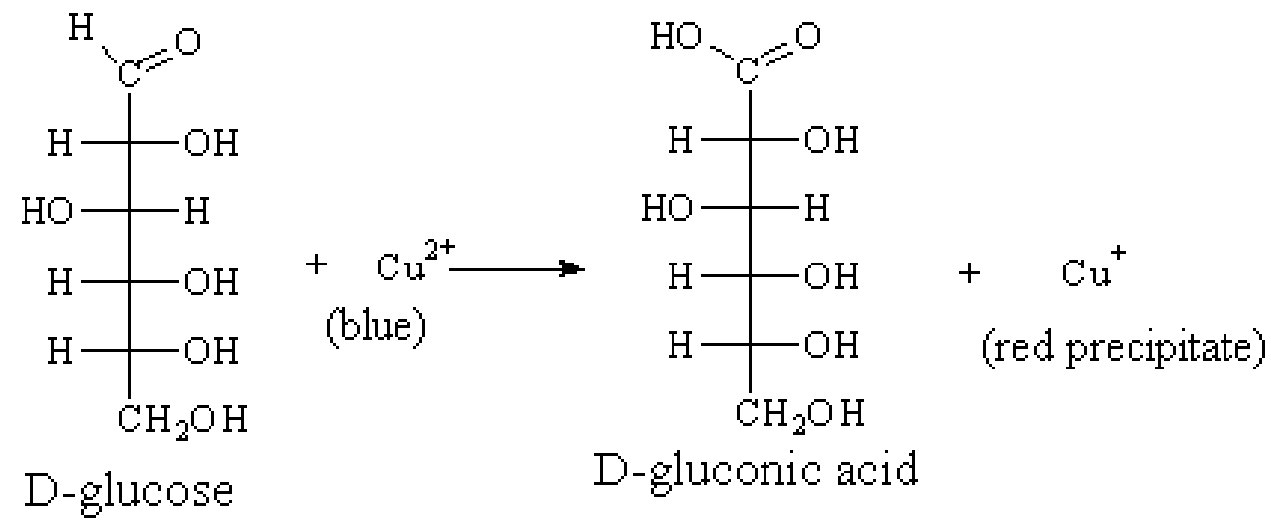
# REDUCING AND NON-REDUCING SUGARS

- **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.



# REDUCING SUGARS



# METHOD-QUALITATIVE TESTS

- The Objective of this lab is to know different methods for qualitative estimation and identification of carbohydrates

## Qualitative tests

### Molicsh test

To identify the carbohydrate from other macromolecules lipids and proteins

### Benedict

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

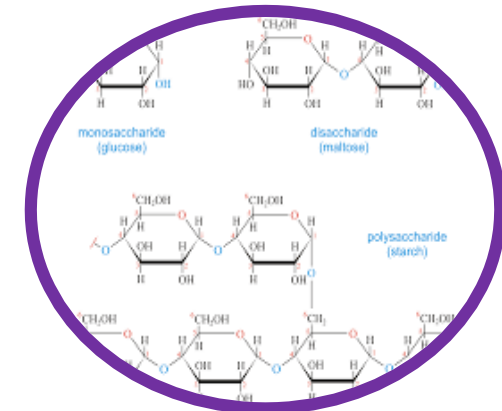
### Seliwanoff's Test

To distinguish between aldose and ketone sucrose

# MOLISCH TEST

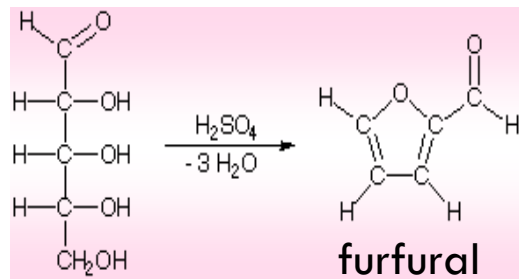
- **Objective:** To identify the carbohydrate from other macromolecules lipids and proteins.

- This test is specific for all carbohydrates.



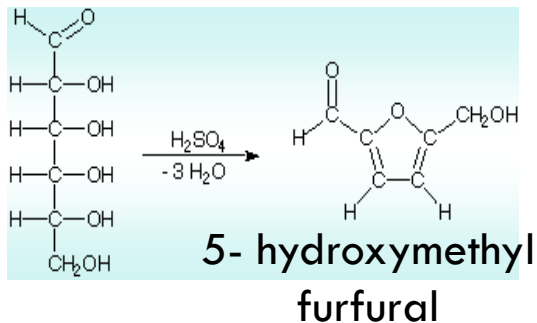
# MOLISCH TEST

- Two solutions are added :  $H_2SO_4$ ,  $\alpha$ -naphthol present
- Principle: 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  $\alpha$ -naphthol present in the test reagent to produce a purple product.



$\alpha$ -naphthol

Purple Ring



$\alpha$ -naphthol

Purple Ring



# MOLISCH TEST-METHOD

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

**2**-0.5 ml drops of the Molisch reagent (which  $\alpha$ -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.

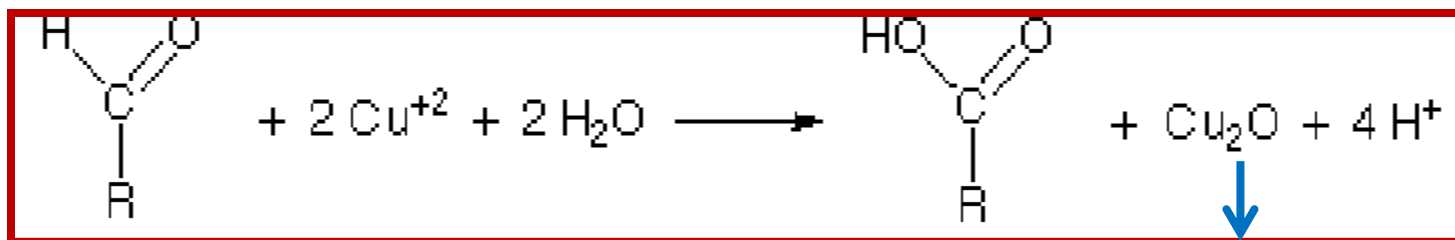
Tube	Observation
Glucose	
Lactose	
Starch	

# BENEDICT'S TEST

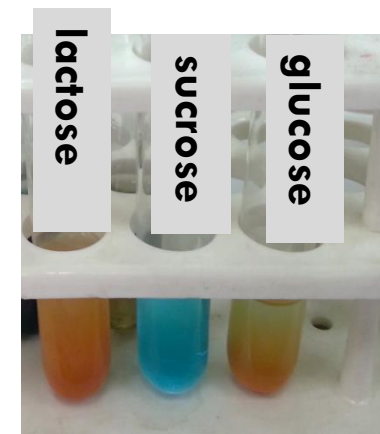
**Objective:** To distinguish between the reducing and non-reducing sugars.

**Principle:** The copper sulfate ( $\text{CuSO}_4$ ) 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.



reddish precipitate of copper



# 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.

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

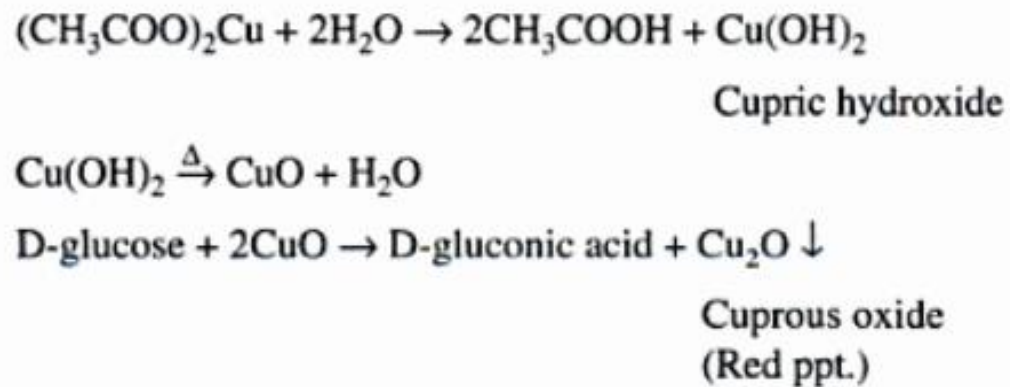
Tube	observation
1-glucose	
2-sucrose	
3-lactose	

# BARFOED'S TEST

**Objective:** This test is performed to distinguish between reducing monosaccharides, reducing disaccharides and non reducing disaccharides.

**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)





# BARFOED'S TEST

- Different types of reducing sugars react at different rates.
- Reducing monosaccharides react quickly with Barfoed's reagent, but reducing disaccharides react very slowly or not at all.
- Therefore, it is possible to distinguish between a reducing monosaccharide and a reducing disaccharide using Barfoed's reagent.

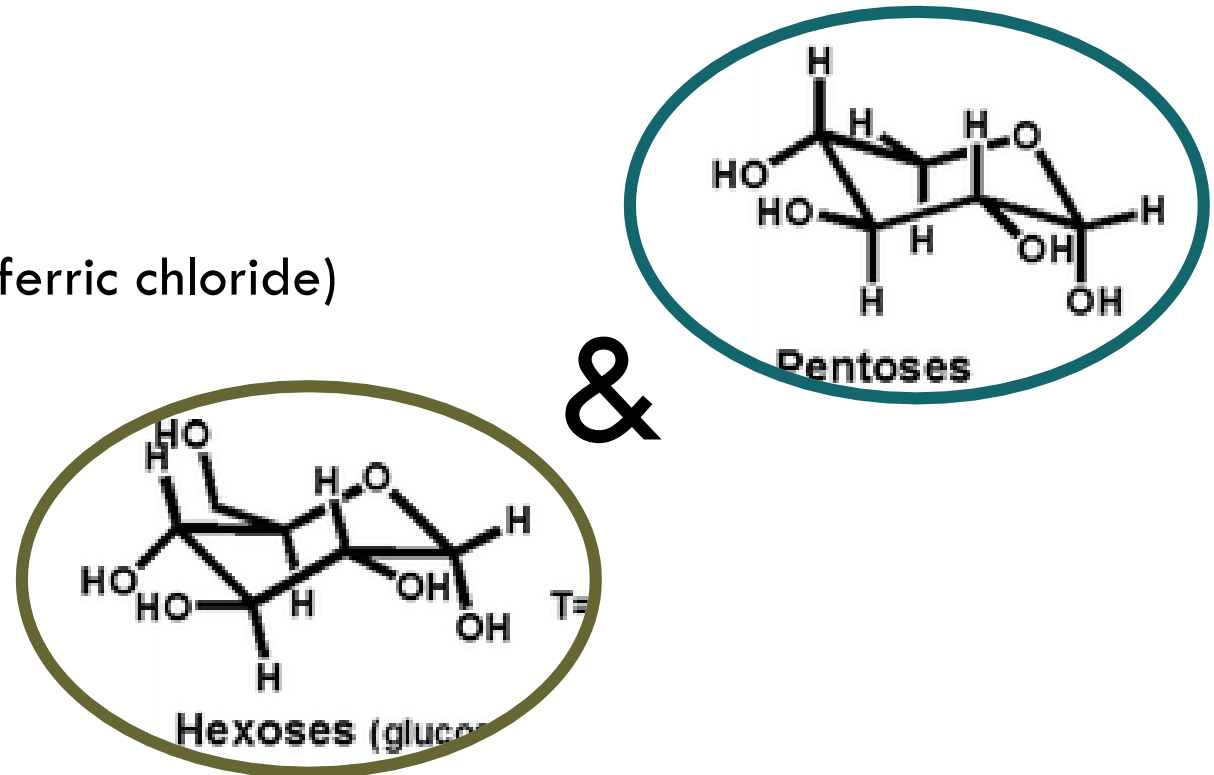
# BARFOED'S TEST-METHOD

- 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
1 -glucose	
2-sucrose	
3-lactose	

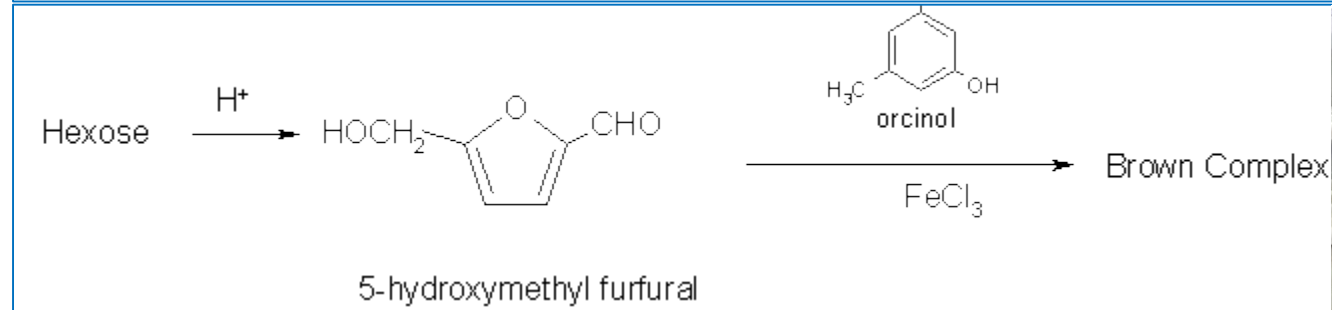
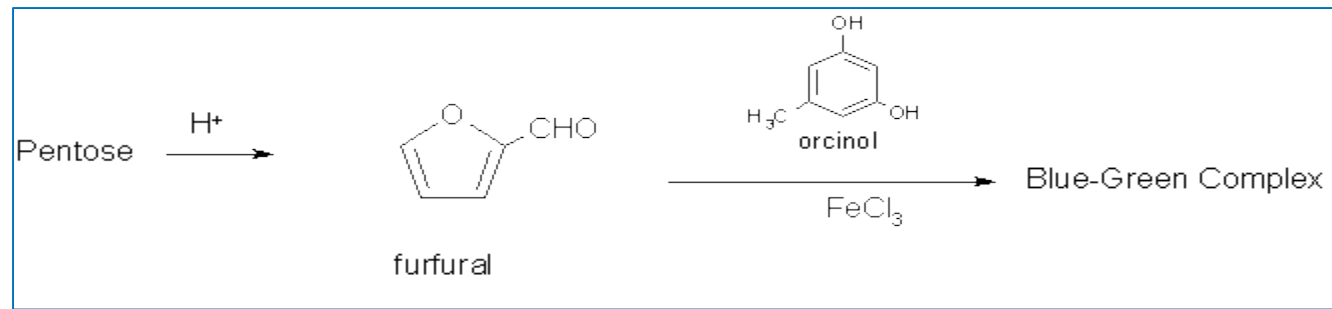
# BIAL'S TEST

- **Objective:** To distinguish between pentose monosaccharide and hexose monosaccharide
- **Reagents:**
- Bial's reagent (a solution of orcinol, HCl and ferric chloride)



# BIAL'S TEST-PRINCIPE

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.



# BIAL'S TEST-METHOD

1. Put 2 ml of a sample solution in a test tube.
2. Add 2 ml of Bial's reagent (a solution of orcinol, HCl and ferric chloride) 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.

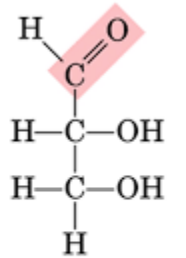
Tube	observation
1 -glucose	
2-ribose	

# SELIWANOFF'S TEST

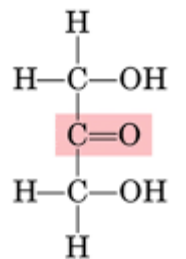
**Objective:** To distinguish between aldose and ketone.

**Principle:** Seliwanoff's Test uses 6M HCl as dehydrating agent and resorcinol 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 more slowly giving yellow to faint pink color.

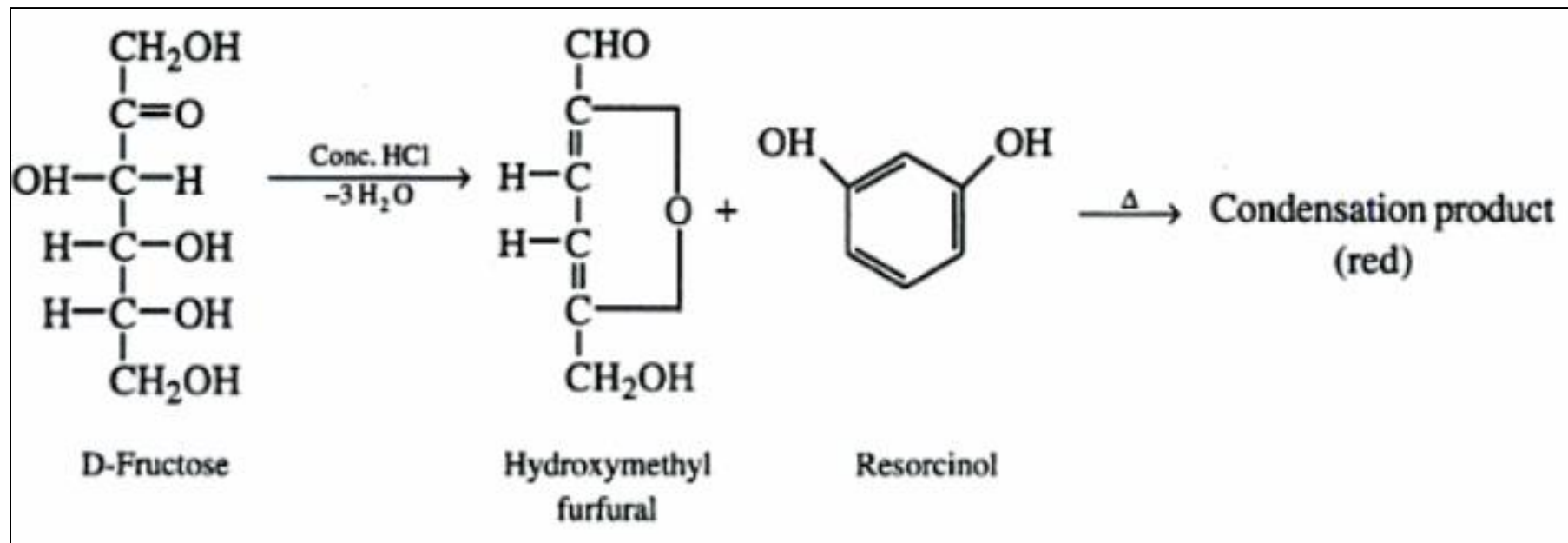


**Aldose**



**Ketose**

# PRINCIPLE



# SELIWANOFF'S TEST-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	