Reducing sugar test

Third stage

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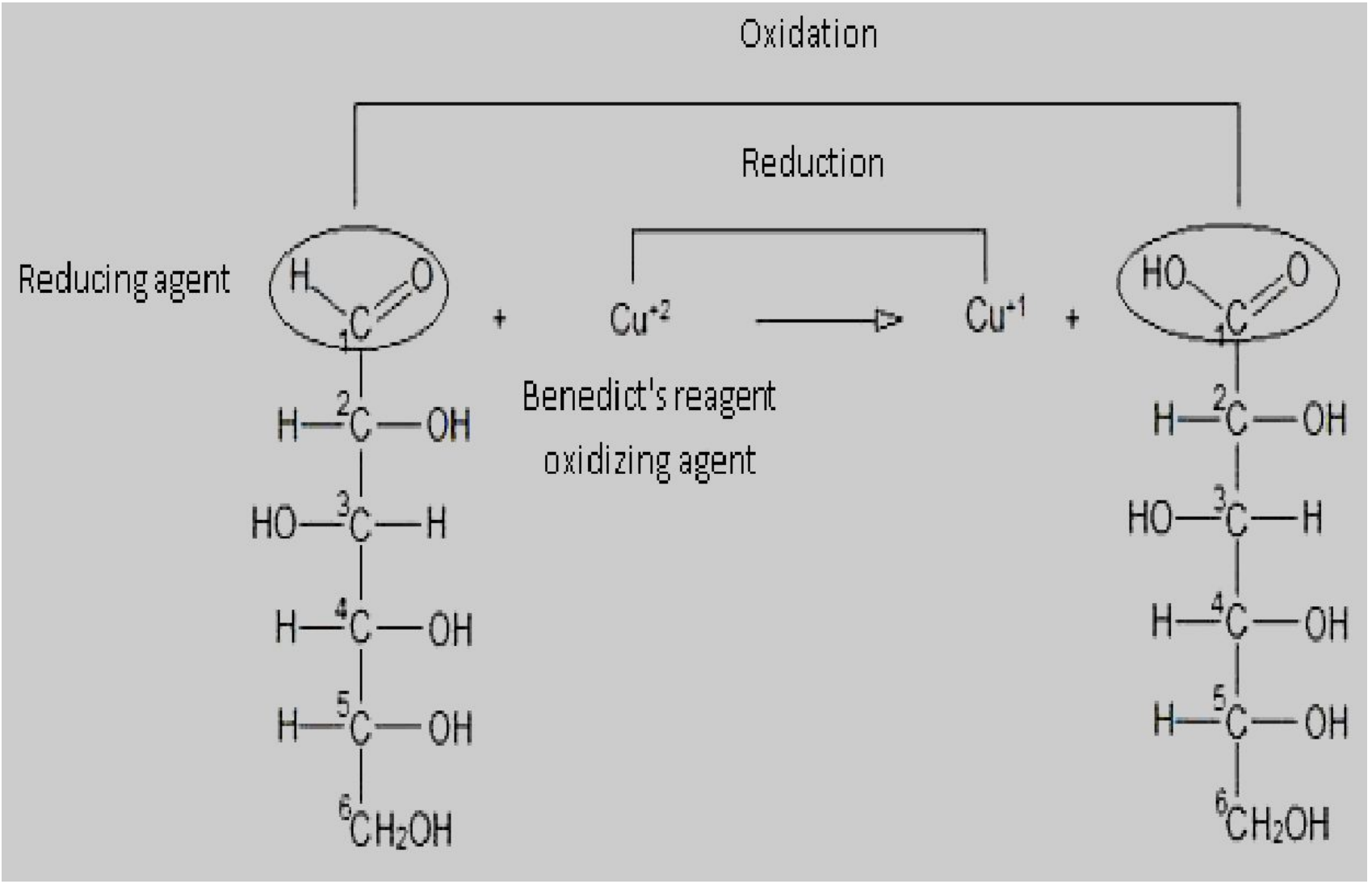
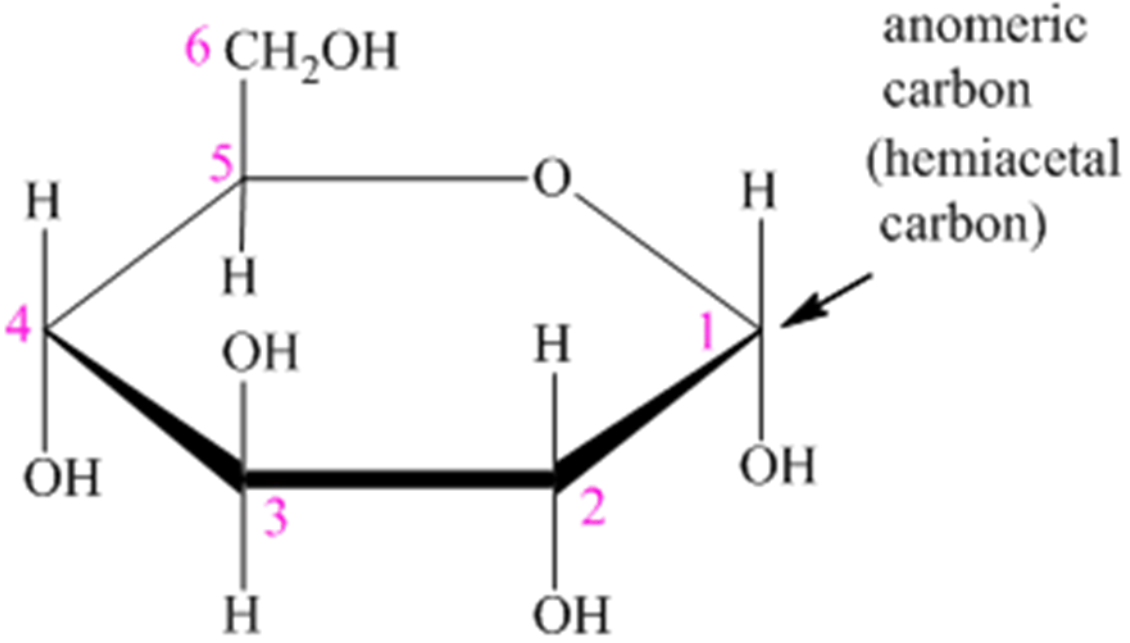
M.S.c Clinical Biochemistry

Reducing sugar

- A **reducing sugar** is any sugar that either has **free** an aldehyde group or ketone group that allows the sugar to act as a reducing agent.
- Reducing sugars can be oxidized by relatively mild oxidizing agents such as cupric ion (Cu2+). The carbonyl carbon (aldehyde group) is oxidized to a carboxylic acid, at the same time; cupric ion (Cu2+) is reduced to cuprous ion (Cu1+).

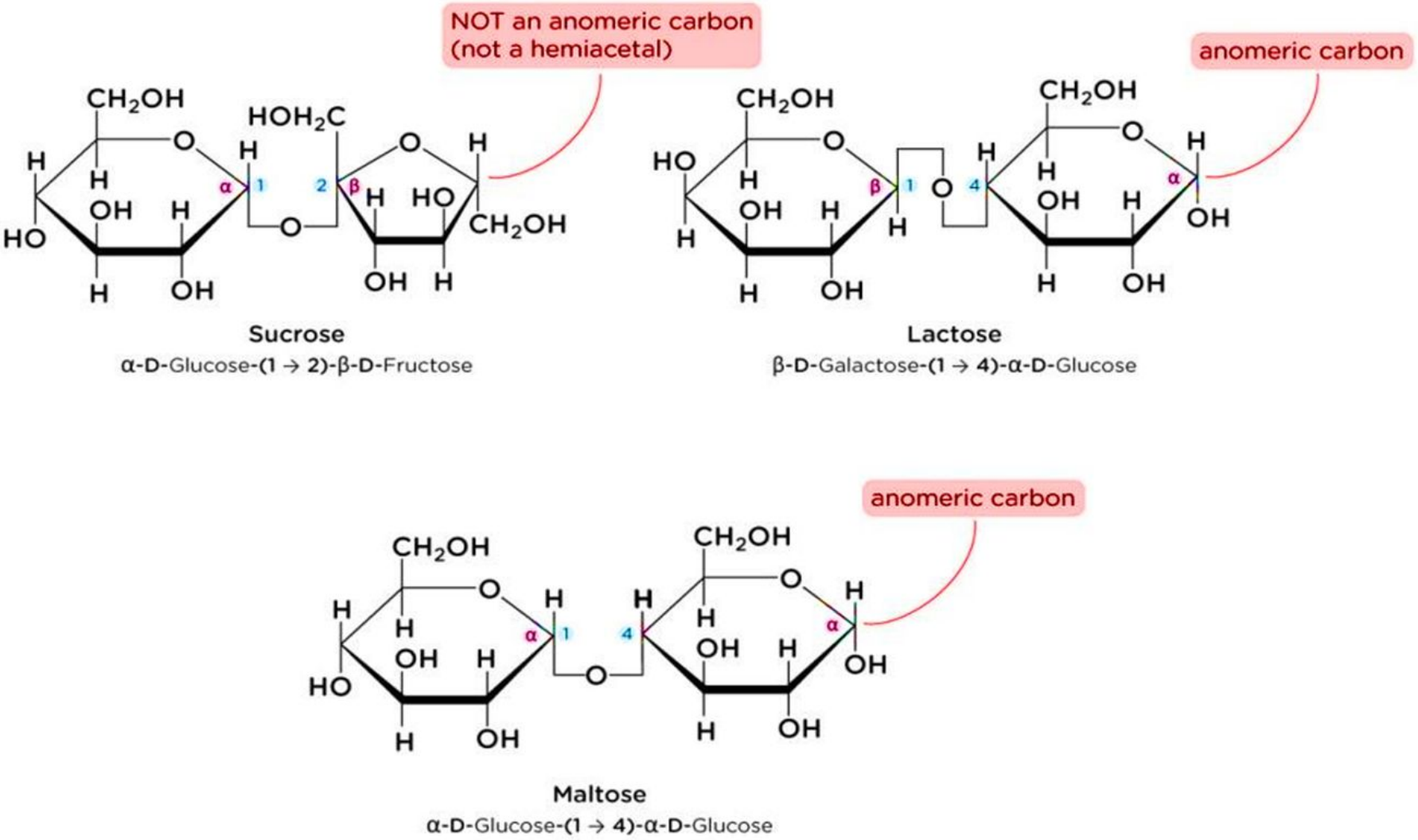

Oxidation
Reduction
Reducing agent
Cu+2
Cu+1
Benedict's reagent
oxidizing agent

- All monosaccharide's and disaccharides except **sucrose** are reducing sugar.



β-D-glucose

(reducing sugar)



Sucrose

α-D-Glucose-(1 → 2)-β-D-Fructose

Lactose

β-D-Galactose-(1 → 4)-α-D-Glucose

Maltose

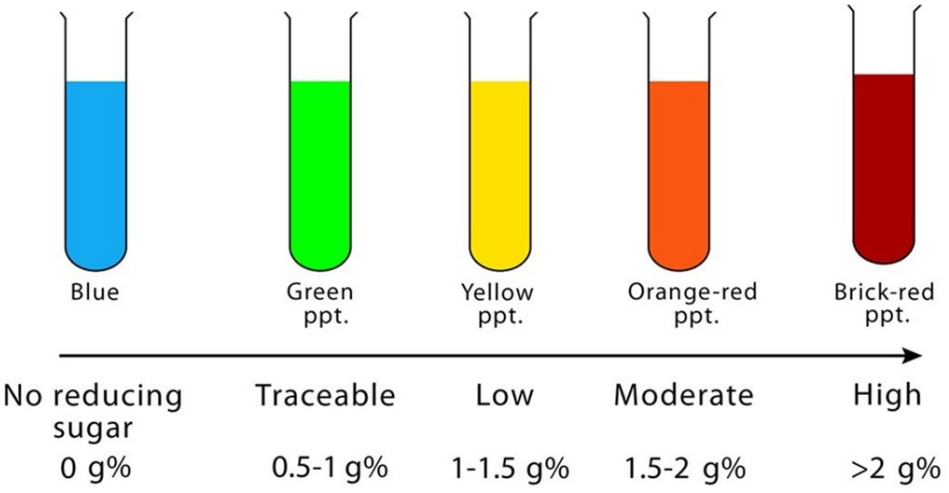
α-D-Glucose-(1 → 4)-α-D-Glucose

Benedict test

- Benedict's test is used to differentiate between reducing and non-reducing sugars.

- **Uses and Application of benedict's test**
- To detect the presence of glucose in blood and urine samples, where detection of excess of glucose indicate uncontrolled diabetes

- The test is semi-quantitative, since the color of the precipitate indicates approximate quantity of the sugar present in the sample.
- blue (with no reducing sugar present),
- green, yellow, orange, red, and then brick red or brown (with high concentration of reducing sugars).

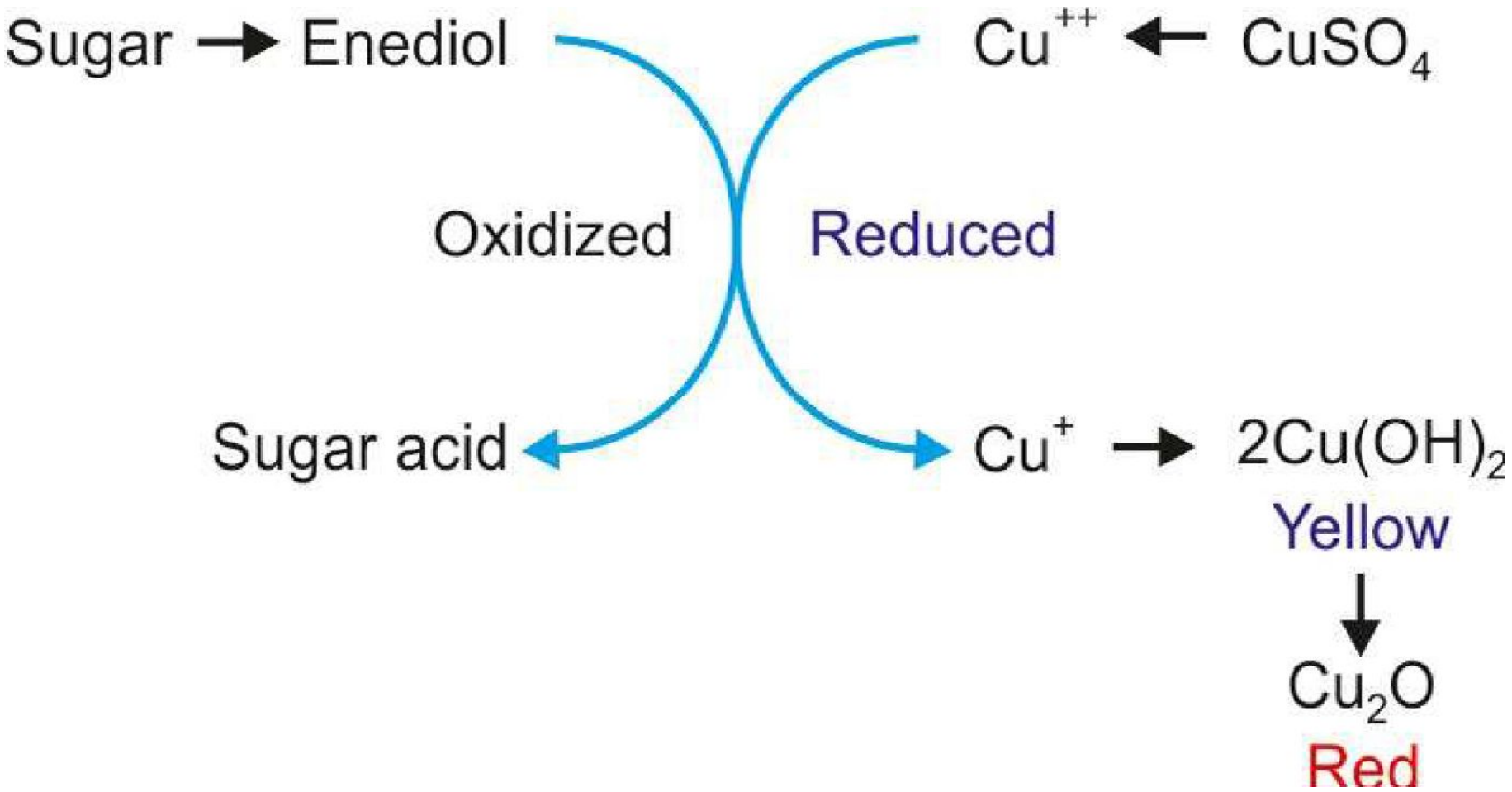


Benedict Reagent

- Benedict reagent solution compose of **Copper(II) sulfate** to provide cupric Cu^{+2} ion act as oxidizing ion. **Sodium citrate** in the reagent solution acts as a complexing agent which keeps Cu^{+2} in solution, otherwise Cu^{+2} combine with carbonate and precipitate as cupric carbonate before oxidation reduction reaction has occurred. **Sodium carbonate** serves to keep the solution alkaline (basic medium).

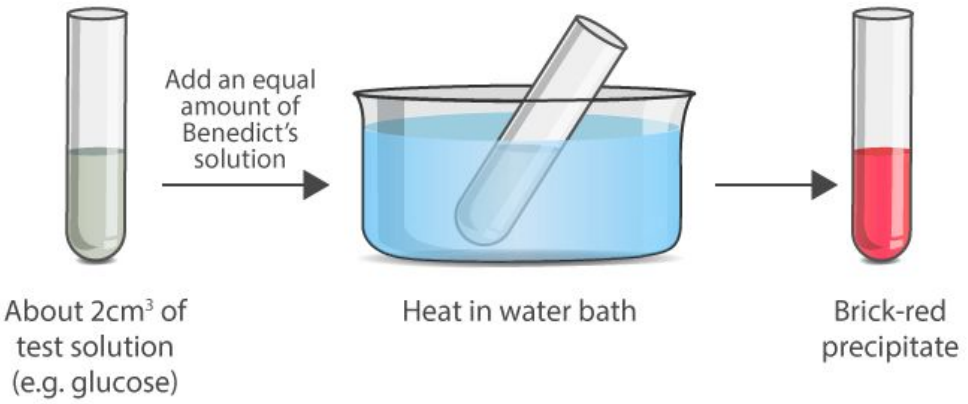
Principle:

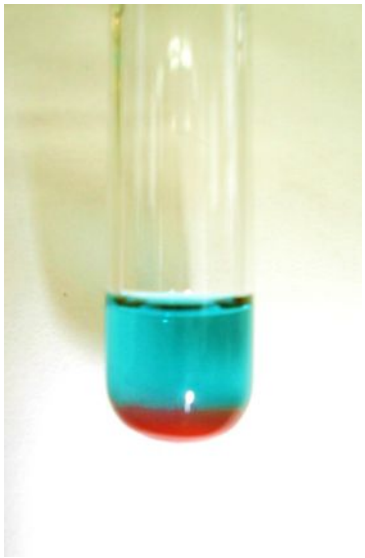
- The carbohydrates having a free or potentially free, aldehyde or ketone group can act as a reducing agent. Benedict's reagent appears **deep blue** in color and consists of copper sulphate mixed with sodium citrate and a weak alkaline, sodium carbonate. When reducing sugars are heated in the presence of alkaline they get converted to **enediols**, which are powerful reducing agents. Enediols reduce the cupric ions (**$Cu+_2$**) present in the Benedicts reagent to cuprous ions (**Cu+),** which get precipitated as insoluble red colored **cuprous oxide (Cu_2O).**


Sugar
Enediol
Cu^{++}
$CuSO_4$
Oxidized
Reduced
Sugar acid
Cu^{+}
$2Cu(OH)_2$
Yellow
Cu_2O
Red

procedure

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 4-10 min.
4. A positive test is indicated by: The formation of a reddish precipitate.



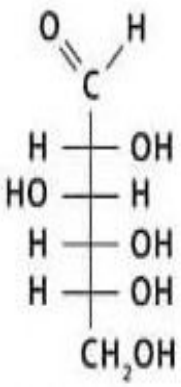


Barfoed test

- Barfoed’s test differentiates between reducing monosaccharide's and disaccharides. This is a reduction test in acidic medium

- **Principle:**
- Barfoed's reagent consists of copper acetate in dilute acetic acid. Since **acidic pH** is unfavorable for reduction, **monosaccharaides**, which are stronger reducing agents, react in about **1-2 min**, whereas reducing **disaccharides** take **7-12 min** to first get hydrolyzed in the acidic solution and then react. A thin red precipitate is formed at the bottom or sides of the tube. Thus, the difference in reducing strength can be detected.

Barfoed's Test Reaction

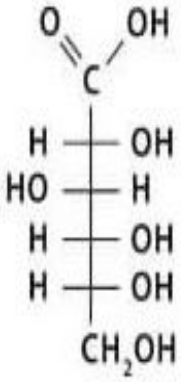


D-Glucose

+ $2Cu^{++}$ + $2H_2O$

Cupric ion (Copper (II))

Water



D-Gluconic acid

+ $Cu_2O\downarrow$ + $4H^+$

Cuprous oxide (Copper (I)) (Red precipitate)

Blue Solution

Carbohydrates absent

Red Precipitation

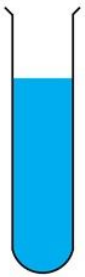
Within few minutes - monosaccharides

After 3 minutes- disaccharides

Procedure

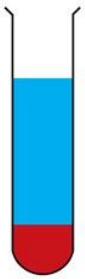
1. Add 2 ml of Barfoed's reagent to 1 ml of sample.
2. Keep the test tubes in boiling water bath for 1-2 min only.

- The boiling should not exceed 1-2 min, otherwise reducing disaccharides may be hydrolyzed and give a positive test result



Solution remains blue

Carbohydrate is absent



Red precipitate is formed

Faster => Monosaccharide is present

Slower => Disaccharide is present


THANK YOU
FOR YOUR
ATTENTION