Qualitative Tests for Carbohydrates

Dr. Nesrin Mwafi

Biochemistry & Molecular Biology Department

Faculty of Medicine, Mutah University

Carbohydrates

- **Our Aim in this Lab:**

1. to characterize carbohydrates present in an unknown solution

2. To distinguish between different carbohydrates based on various chemical assays (aldose/ketose, reducing/non-reducing sugar, polysaccharides/simple sugars….etc.)

Classification of Carbohydrates

- Carbohydrates are “Sugars” or “Saccharides” consist of the empirical formula $(CH_2O)n$ where $n \geq 3$.

1. **Monosaccharides**: The building blocks of CHO which cannot be hydrolyzed into smaller units like glucose, galactose and fructose
2. **Disaccharides:** contain two monosaccharides covalently linked by glycosidic bond like sucrose which consists of glucose and fructose
3. **Oligosaccharides:** contain 3-10 units of monosaccharides covalently linked by glycosidic bond like Raffinose (a trisaccharide composed of galactose, glucose and fructose found in cabbage and broccoli
4. **Polysaccharides:** are polymeric molecules composed of long chains of monosaccharides linked together via glycosidic bonds like starch, cellulose and glycogen

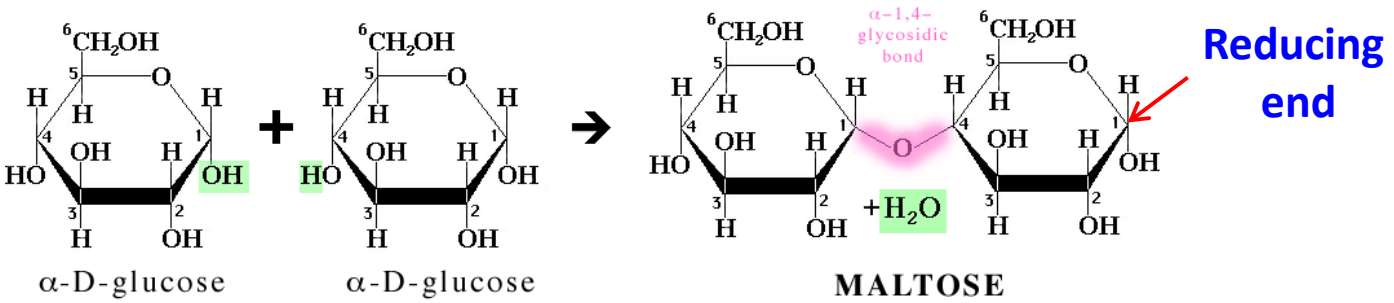
Monosaccharides

- They are classified according to the number of carbon atoms: trioses, tetroses, pentoses, **hexoses** …..etc.
- Also classified according to the chemical nature of the carbonyl group C=O either to Aldoses (the carbonyl group is an aldehyde) or Ketoses (the carbonyl group is a ketone)
- Sugar derivatives (modified monosaccharides): sugar acids, sugar alcohols, deoxy sugars and amino sugars

Monosaccharide	Sugar alcohol
Glyceraldehyde	Glycerol/glycerin
Ribose	Ribitol
Mannose	Mannitol
Glucose	Sorbitol

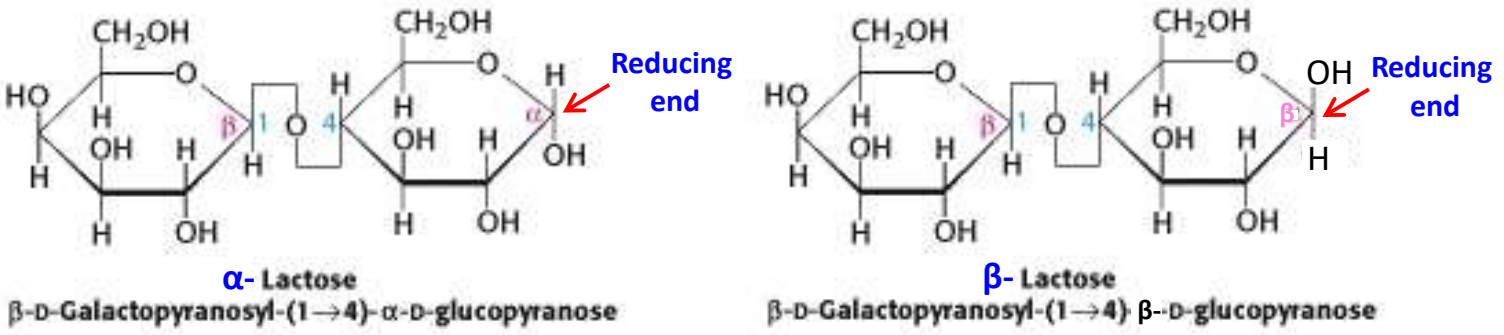
Disaccharides

1. **Maltose "malt sugar":** consists of two α-glucose units



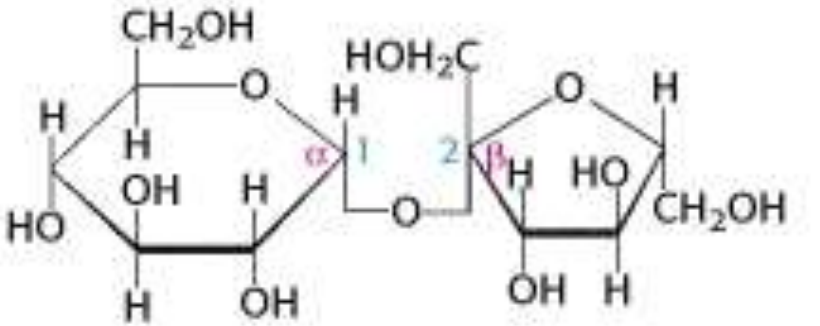
α-D-glucopyranosyl-(1⟶4)-α-D-glucopyranose

2. **Lactose "milk sugar":** consists of glucose & galactose



Disaccharides

3. Sucrose "table sugar": consists of glucose & fructose



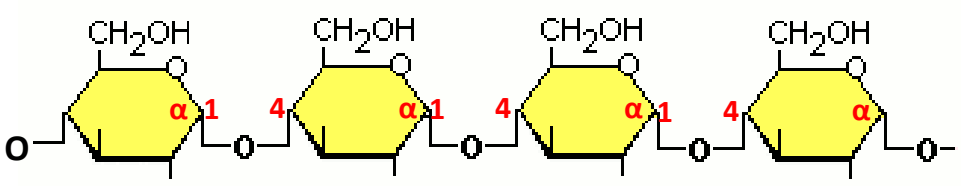
Sucrose
α-D-Glucopyranosyl-(1→2)-β-D-fructofuranose

- Sucrose is not a reducing sugar because the anomeric carbon of the second residue (the reducing end) is not free but involved in the glycosidic bond formation

Polysaccharides

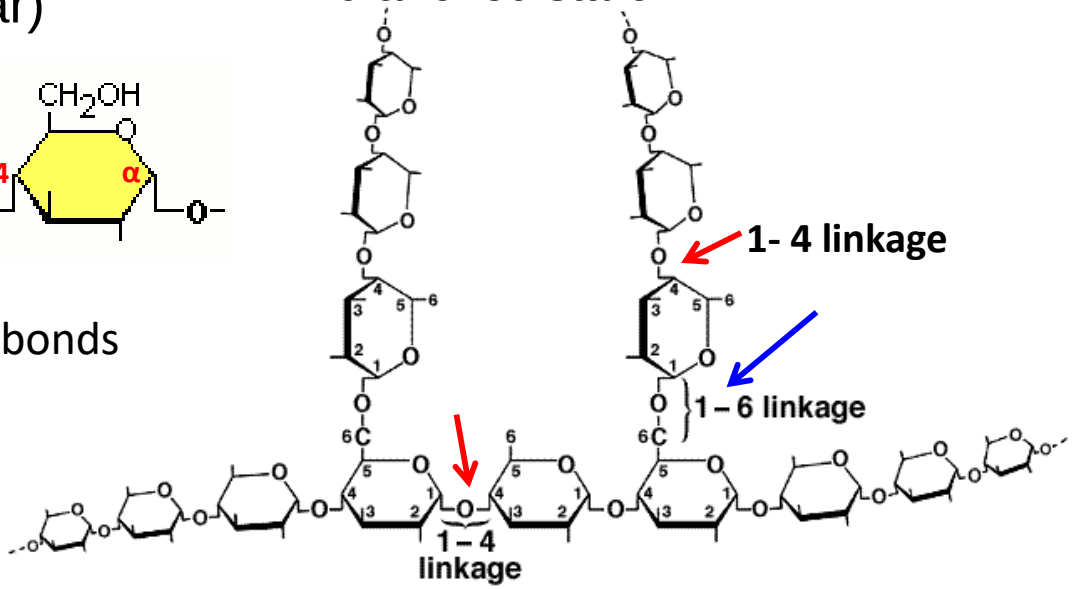
1. Starch: the storage polysaccharide in plants. It is a mixture of **amylose** (20%, unbranched) and **amylopectin** (80%, branched)

unbranched starch(linear)



Amylose : α (1 ⟶4) glycosidic bonds

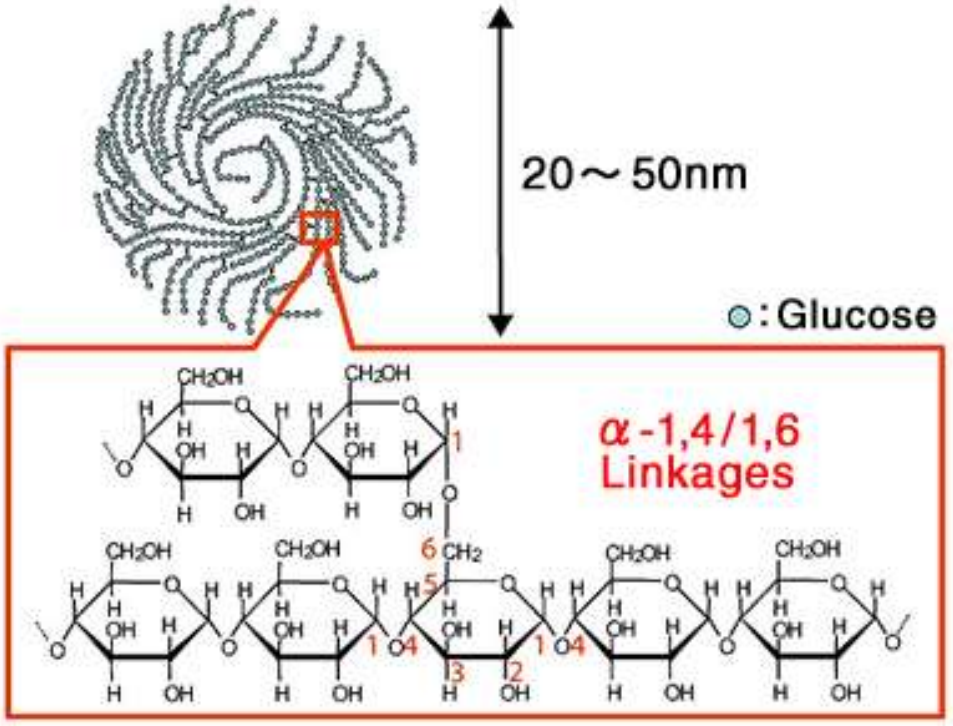
branched starch



Amylopectin: α (1⟶ 4) glycosidic bonds
with α (1 ⟶ 6) branch points

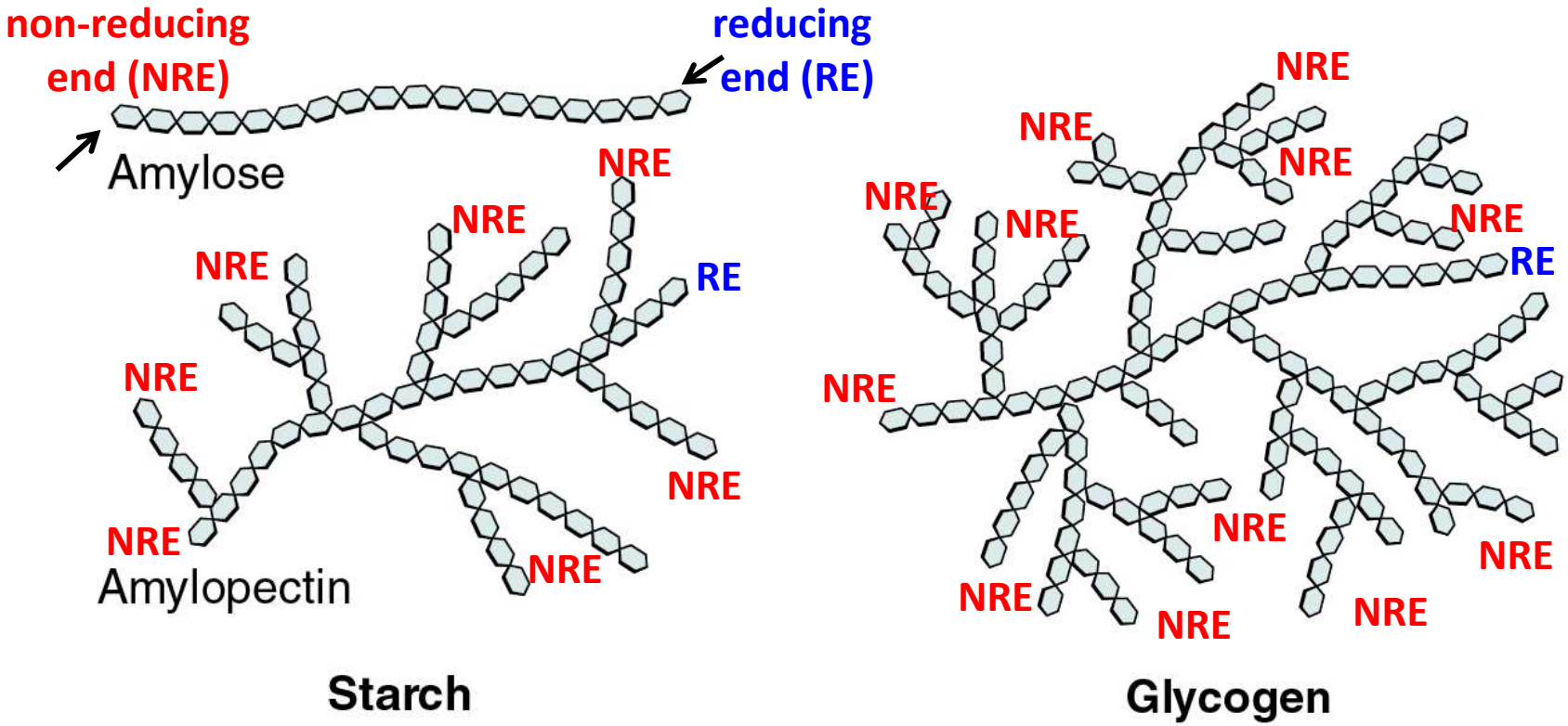
Polysaccharides

2. Glycogen: the storage polysaccharide in animals and human. It is highly branched polymer



Polysaccharides

- **Starch** and **glycogen** have one reducing end (the molecule end containing a free anomeric carbon C1). On the other hand, the branches ends are all called non-reducing ends



Reducing Sugar

- Sugars are classified as either reducing or non-reducing depending upon the presence of potentially **free anomeric carbon or carbonyl group**
- if the reducing groups are involved in the formation of glycosidic linkage, the sugar belongs to the non- reducing category like **sucrose**
- The reducing property is mainly due to the ability of these sugars to reduce metal ions such as copper to form insoluble cuprous oxide

  (cupric ion $Cu^{+2} \longrightarrow$ cuprous ion Cu+1)
- All monosaccharides are **reducing sugars**, along with some disaccharides, oligosaccharides, and polysaccharides

Tests for Carbohydrates

- There are a number of chemical tests for detection of carbohydrates, determining their properties and, possibly, their identity:

1. **Molisch Test**: the general test for carbohydrates

2. **Iodine Test**: used to differentiate simple sugars (mono/disaccharides) and polysaccharides

3. **Benedict's Test and Barfoed's Test**: used to differentiate reducing from non-reducing sugars.

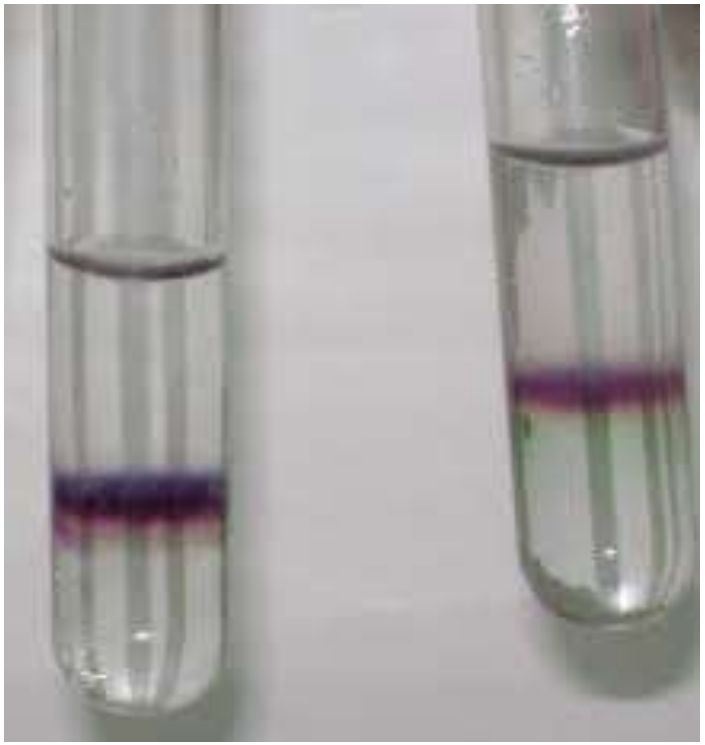
4. **Seliwanoff Test:** used to distinguish ketoses from aldoses

Molisch Test

- **Principle**: when carbohydrates are treated with **concentrated sulphuric acid** (H_2SO_4), they undergo dehydration to give furfural derivatives. These compounds condense with **α-naphthol** (Molisch reagent) to form colored products, generally a purple/violet ring at the interface of the two layers. Pentoses yield furfural while Hexoses yield 5-Hydroxy methyl furfurals

Molisch Test

- An appearance of reddish violet or purple colored ring at the junction of two liquids is observed in **a positive** Molisch test



Molisch Test

- **Procedure:** Take 2 ml of carbohydrate solution in a clean and dry test tube. Add 2 drops of Molisch reagent (α -naphthol) and mix. Tilt the test tube at a 45 degree angle and add carefully 2ml of concentrated sulphuric acid along the side of the test tube so as to form 2 layers.

Molisch Test

- **Interpretation:** This is a **sensitive** but a **non-specific** test and is given positive by all types of carbohydrates. If the oligosaccharides or polysaccharides are present they are first hydrolyzed to monosaccharides which are then dehydrated to give the test positive.

Iodine Test

- This is a test for polysaccharides such as starch
- **Principle**: Iodine forms a coordinate complex between the helically coiled polysaccharide chain and iodine centrally located within the helix due to adsorption. The color of the complex obtained depends upon the length of the unbranched or linear chain available for complex formation
- **Interpretation**: **Glycogen**: gives reddish brown color whereas **starch** gives deep blue color

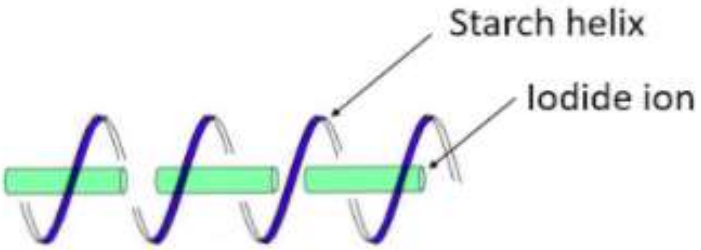
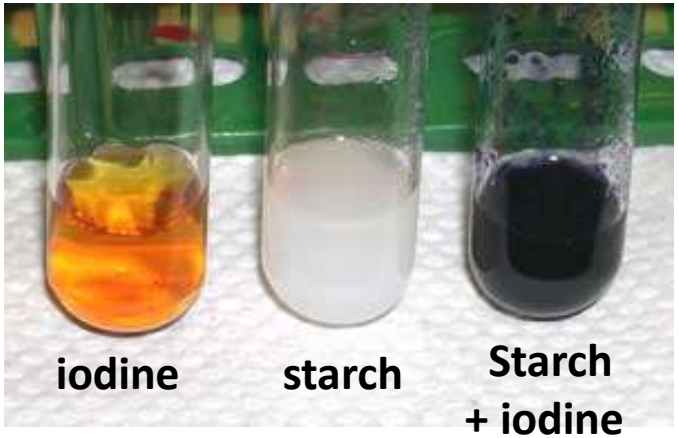
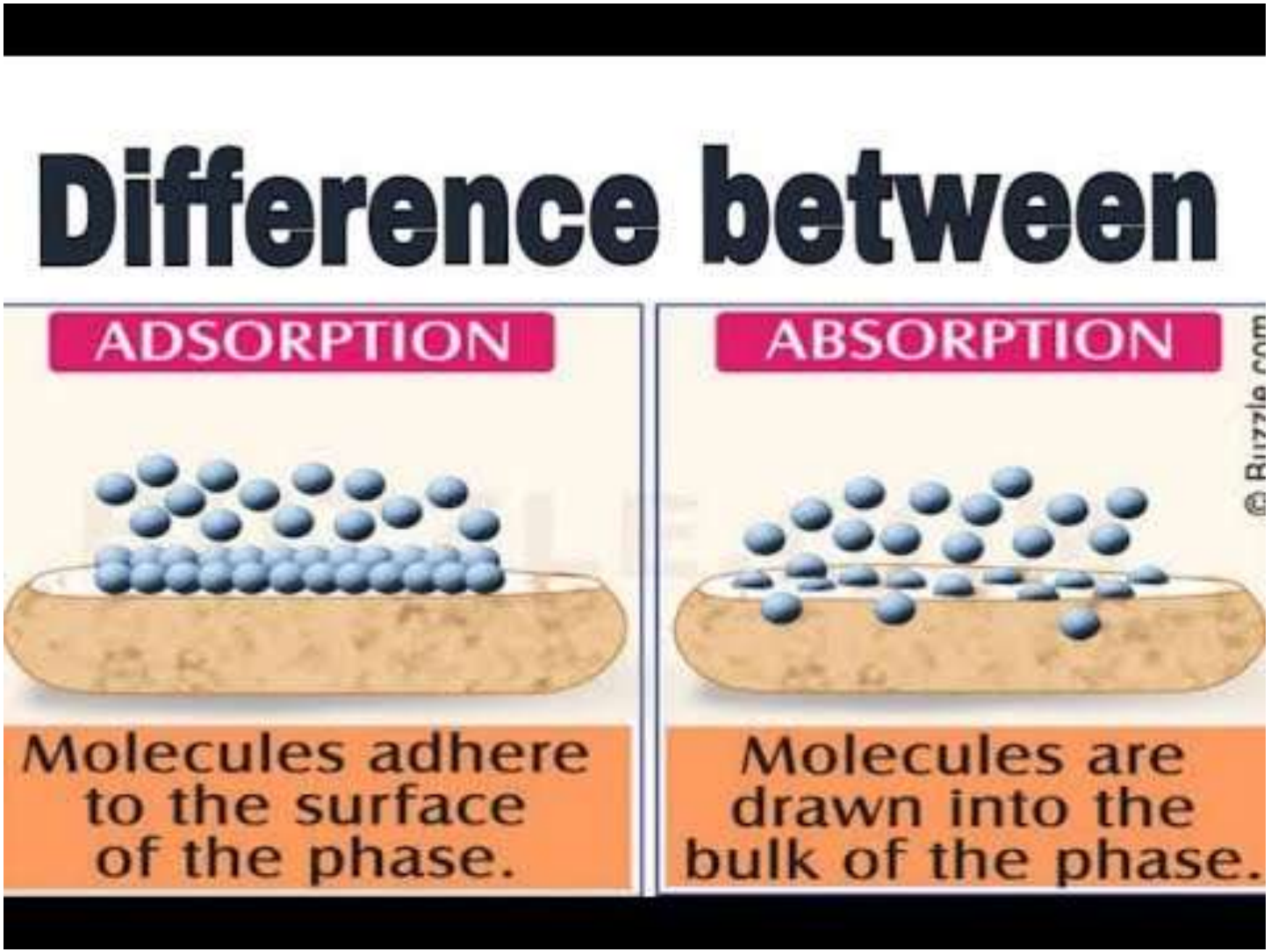


Fig.- Starch-iodine complex



Absorption / Adsorption



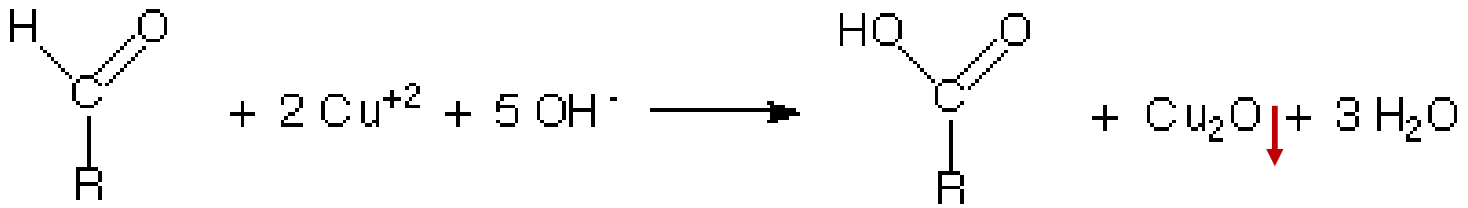
Iodine Test

- Monosaccharides and Disaccharides are two small to trap the iodine molecules and do not form dark colored complexes. Therefore, the iodine test can be used to distinguish between mono/disaccharides and polysaccharides

- **Procedure:** place 2 mL of each solution to be tested in a test tube. Add 2-3 drops of the Iodine reagent and mix. Record your results.

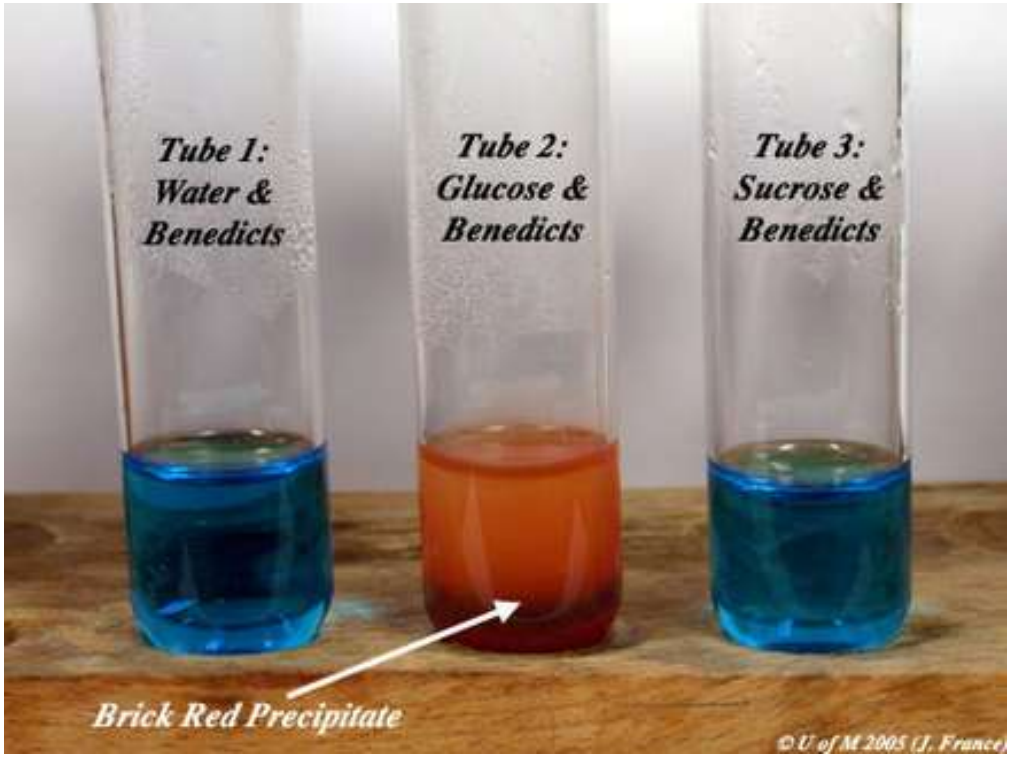
Benedict's Test

- This test is for reducing sugars which are capable of reducing metal ions in solution
- **Principle:** In this test, cupric ions (Cu^{+2})(aqua blue in solution) found as copper sulphate $CuSo_4$ are reduced to cuprous ions (Cu^{+1}) that form Cu_2O copper oxide under alkaline conditions which is a **brick-red precipitate**



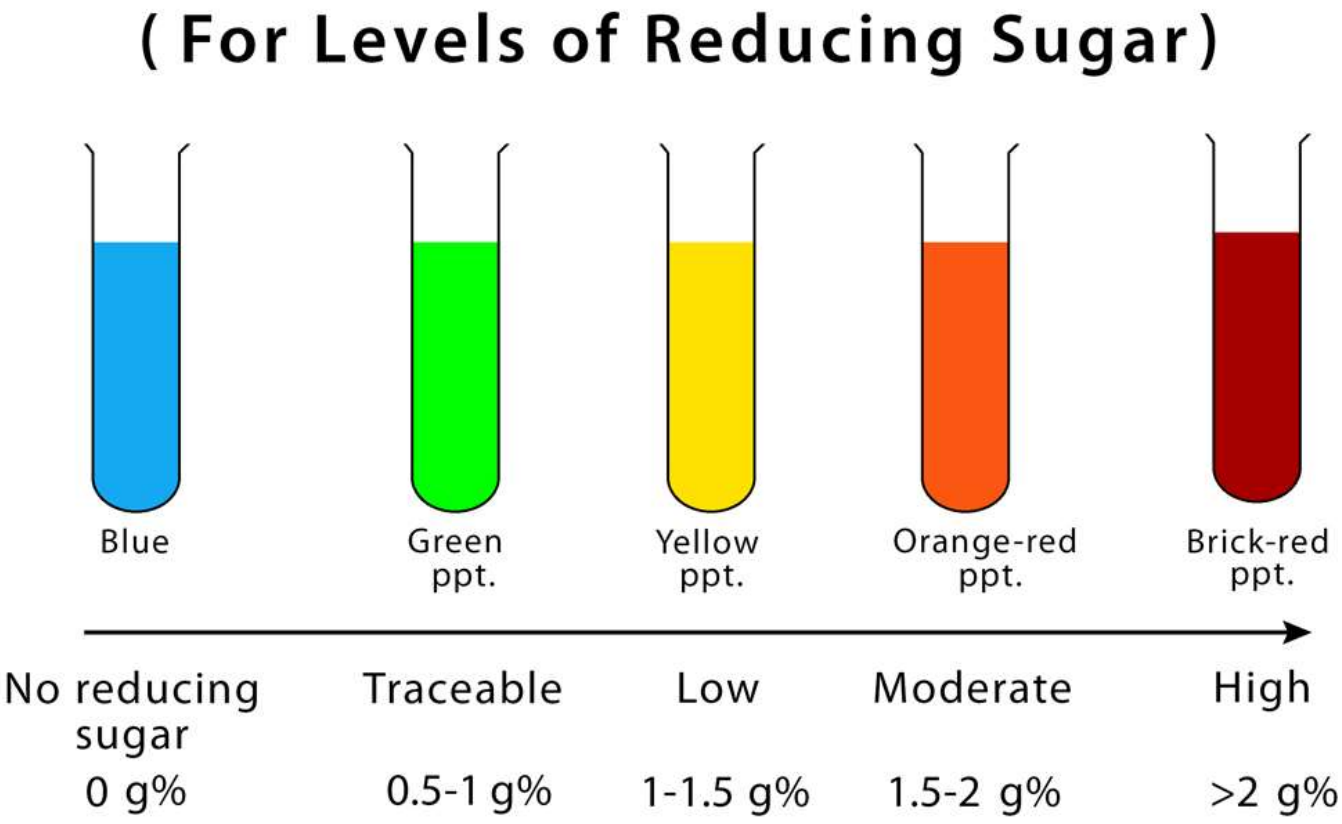
Benedict's Test

- **Procedure:** Add 8 drops of carbohydrate solution to 5 ml of Benedict's reagent and boil over a flame or in a boiling water bath for 2 minute. Let the solution cool down

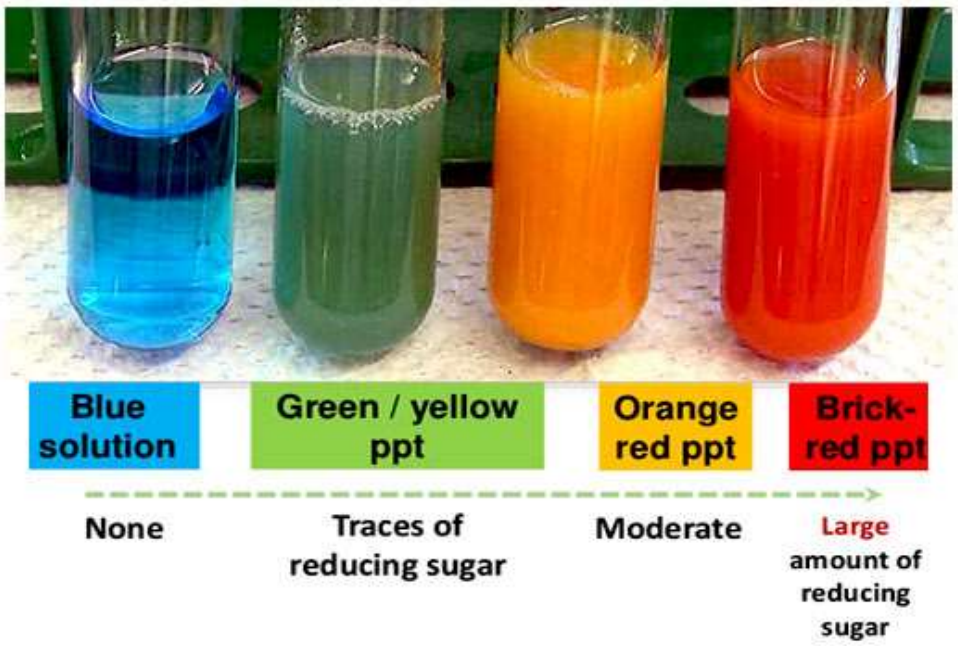


Benedict's Test

- Benedict's test is a semi quantitative test (Diabetes urine test). The color formed depends upon the amount of reducing sugar **(glucose)** present in the mixture



Benedict's Test



Urine strips for quick urine test for diabetes

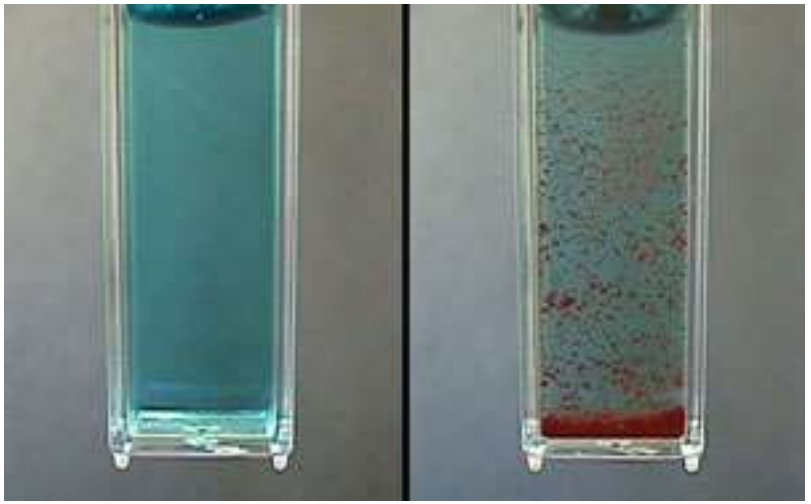
Barfoed's Test

- The Barfoed's test is similar to the Benedict's test but differs in the specific reagent used (copper (II) acetate in 1% acetic acid solution) which is less reactive than the Benedict's reagent
- **Principle:** reducing sugars can reduce cupric ions even in acidic conditions. This test is used to distinguish reducing monosaccharides from disaccharides by **controlling pH and time of heating**. Mono saccharides react very fast whereas disaccharides react very slowly
- As in Benedict's test, the primary reaction is the reduction of Cu^{+2} ions to Cu_2O which forms a brick red precipitate.

$$R\mathbf{CHO} + 2\mathbf{Cu^{2+}} + 2H_2O \rightarrow R\mathbf{COOH} + \mathbf{Cu_2O\downarrow} + 4H^+$$

Barfoed's Test

- **Procedure**: add 2 ml of carbohydrate solution to 2 ml of Barfoed's reagent and keep the test tube in the boiling water bath for 2-3 minutes **only**. Cool under running water



negative positive

A scanty brick red precipitate is observed in a positive reaction.

Barfoed's Test

- **Interpretation:**

1. The positive reaction indicates the presence of a reducing monosaccharide
2. On prolonged heating disaccharides can also give this test positive. Hence, the solution should be boiled for 3 minutes only.

Seliwanoff's Test

- **Principle:** Keto hexoses on treatment with hydrochloric acid form 5-hydroxymethyl furfural which on condensation with resorcinol (Seliwanoff reagent) gives a cherry red colored complex
- **Procedure**: to 3 ml of Seliwanoff reagent (resorcinol and HCL) add 1ml of sugar like fructose. Boil for 1- 2 min only then cool the solution

negative

positive

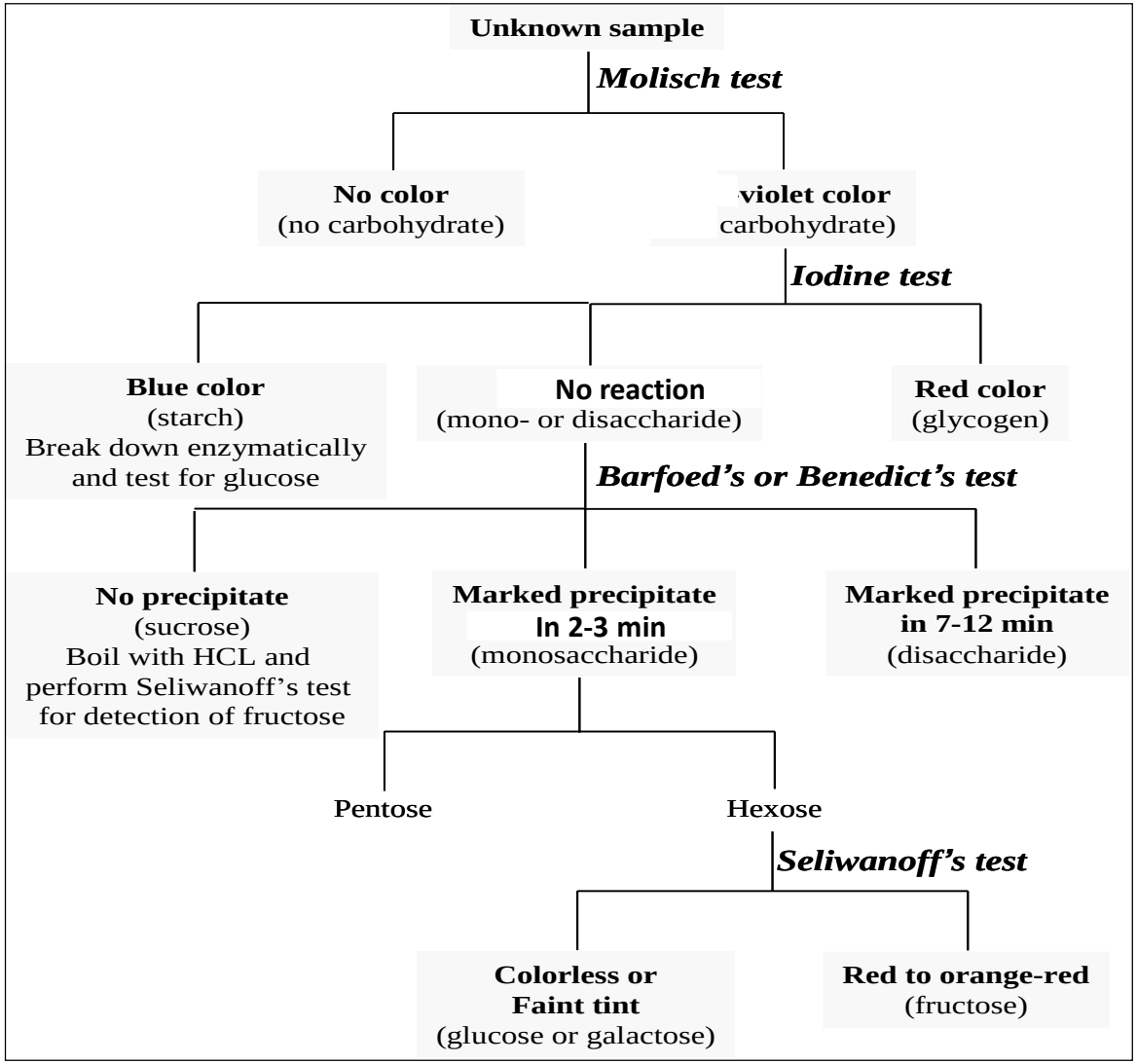
A cherry red color is observed in a positive reaction

Seliwanoff's Test

- **Interpretation:**

1. This test gives positive result with ketohexoses like fructose and fructose containing sugars (e.g. sucrose)
2. This test distinguishes between glucose and fructose
3. Overheating of the solution should be avoided as aldoses get converted to ketoses and give a false positive reaction with Seliwanoff reagent

Classification of Unknown Sample



Sugars Identification

Biochemical test	Glucose	Fructose	Maltose	Lactose	Sucrose
Molisch					
Benedict					
Barfoed					
Seliwanoff					

Sugars Identification

Biochemical test	Glucose	Fructose	Maltose	Lactose	Sucrose
Molisch	Positive	Positive	Positive	Positive	Positive
Benedict	Positive	Positive	Positive	Positive	Negative
Barfoed	Positive	Positive	Negative	Negative	Negative
Seliwanoff	Negative	Positive	Negative	Negative	Positive