Al-Mustaqbal University
College of Health and medical techniques
Medical laboratories Techniques Department

First year chemistry practical Lectures

Presented by

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Seventh Lecture: Carbohydrates Tests

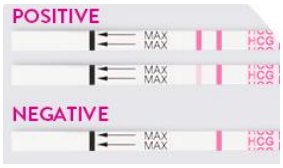
Tests for identification of Carbohydrates:

1-Molisch Test specific for carbohydrates.
2- Benedict's Test: presence of reducing sugars.
3. Barfoed's Test: test used for detecting the presence of monosaccharides.
4- Bial's Test: used to detect pentose [5C] monosacharides.
5 Seliwanoff's Test : used to distinguish between aldoses and ketoses.

1. **Qualitative analysis (What is in the sample?) :**

It is used to identify the presence or absence of certain chemical compounds or elements in a sample، such as presence of gold in a rock.

- **Positive and negative test as pregnancy strip test.**



2. Quantitative analysis (How much is in the sample?) means to finding the exact quantity of the chemical compounds or elements in different solutions or mixtures such as

3. Semi quantitative analysis؟

Benedict's Test is semi-quantitative.

Benedict's Test can give us an idea of how much reducing sugar is present in the sample. The greater the concentration of the reducing sugar, the greater the colour change in the flow diagram below. If we perform Benedict's Test for multiple samples under standardised conditions, (i.e. ensuring all solutions were given the same amount of time from when Benedict's solution was added to inspecting for the colour change) then we can estimate which solutions contain the greater concentration of reducing sugar in comparison to the other.

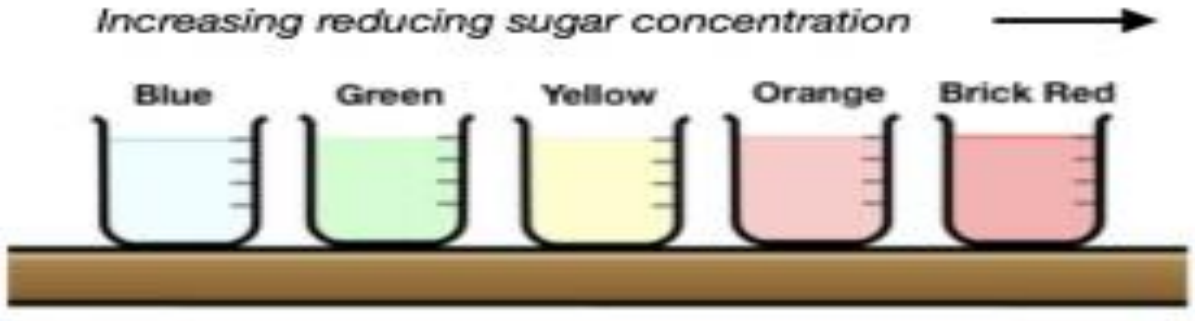


Fig 1. Colour Changes in Benedict's Test. The concentration of the reducing sugars in your sample dictates the extent of the precipitate formation and the colour change that you will observe.

1.Molisch test:

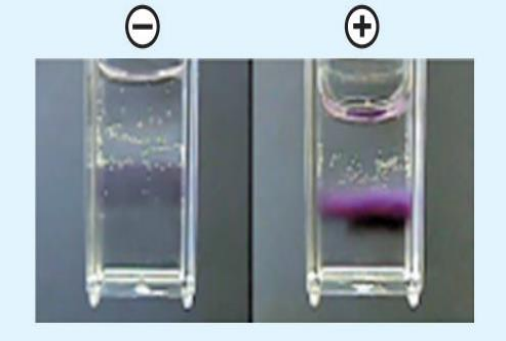
This test is specific for all carbohydrates .Monosaccharide gives a rapid positive test, Disaccharides and polysaccharides react slower.

Objective:

To identify the carbohydrate from other macromolecules, lipids and proteins.

Method:

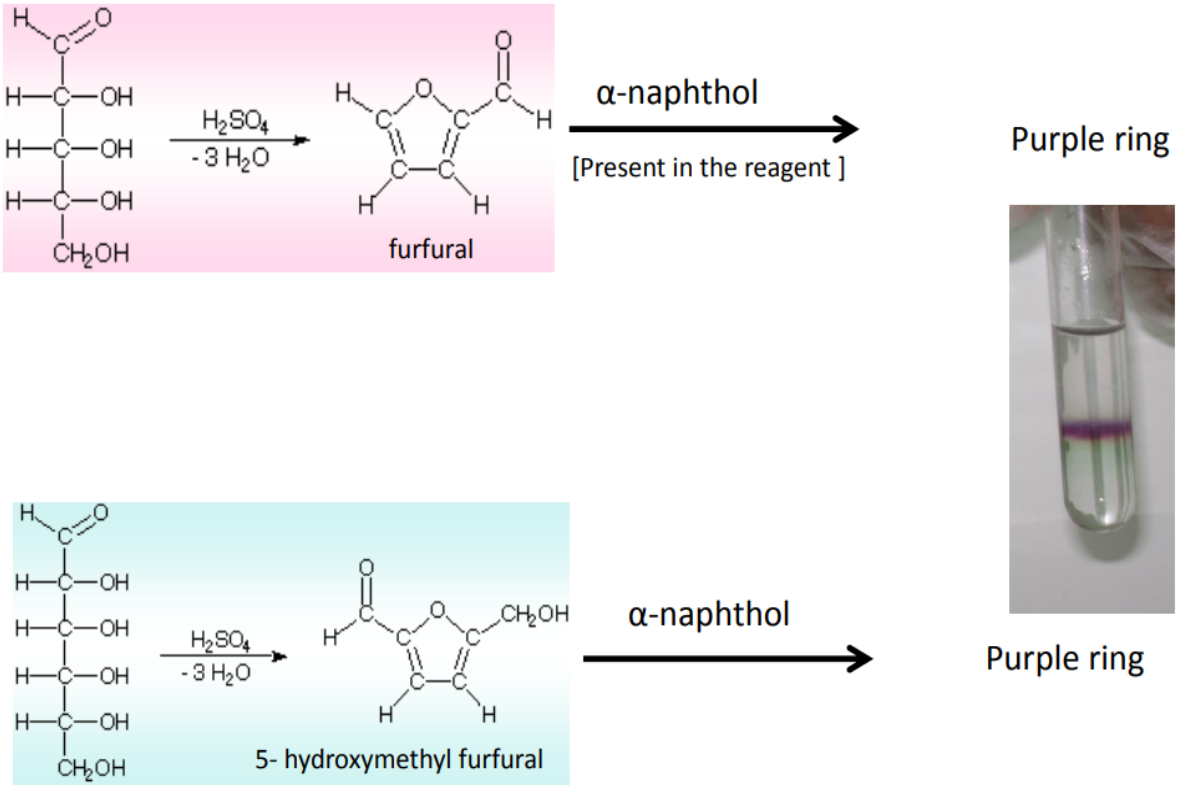
1. **Take 2ml of sample in dry test tube.**
2. **Take 2ml of distilled water in another tube as control.**
3. **Add 2-3 drops of Molisch's reagent to the solution & mix well.**
4. **Add 3 drops of H_2SO_4 get dehydrated to form furfural or derivative .**
5. **Observe colour change at the junction of two layers of solution , the sugar at the Upper and the acid at the bottom, and The separating surface purple colour indicates the presence of Carbohydrates (CHOs).**



Principle:

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

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



2.Benedict's test:

Objective:

Benedict's Test is a chemical analytical method used for the detection of reducing sugar in a solution. Benedict's Test is basicaly a qualitative test often used for **the differentiation of carbohydrates (saccharides/sugars) into reducing and non-reducing types**

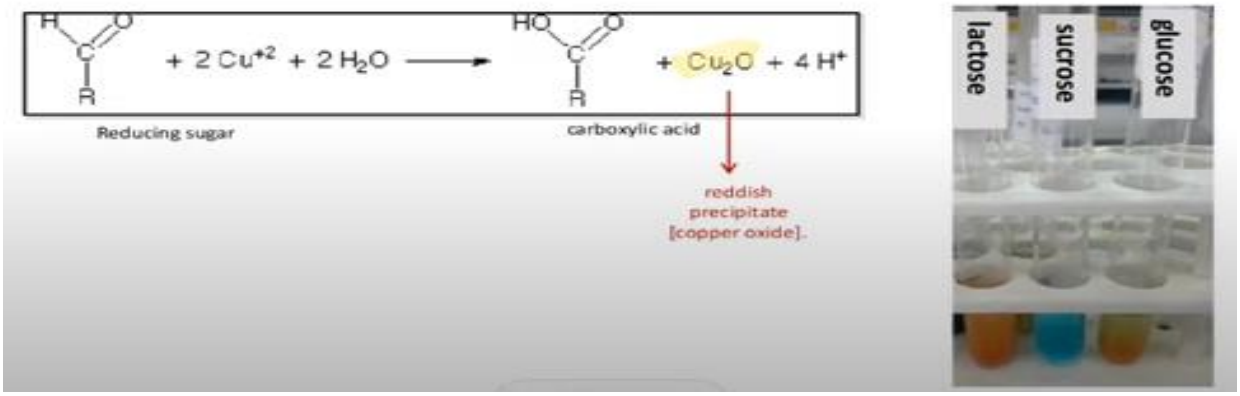
All monosaccharides are reducing sugars; they all have a free reactive carbonyl group.

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.

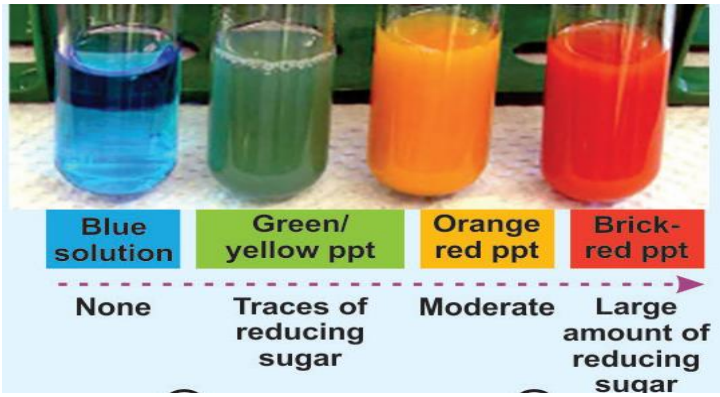
Principle:

Reducing sugars are oxidized in alkaline medium by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.



Method :

when 0.5 ml solution containing reducing sugar is boiled with 5.0 ml Benedict's reagent (blue color) for 5 minutes, brick red or green or yellow colored precipitate appears. This indicates the presence of reducing sugar in the sample. This test is applied for the detection of reducing sugars in urine in case of diabetes and galactosemia .

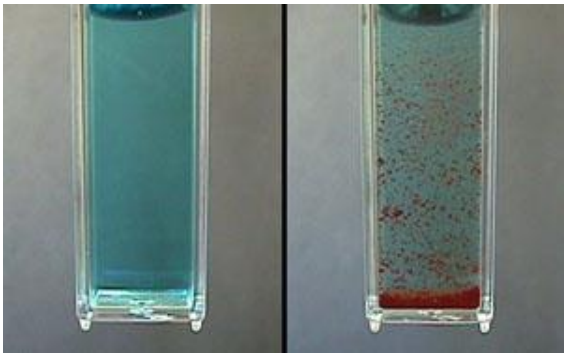


3.Barfoed's Test:

Objective:
To distinguish between mono-, di- and poly saccharides.

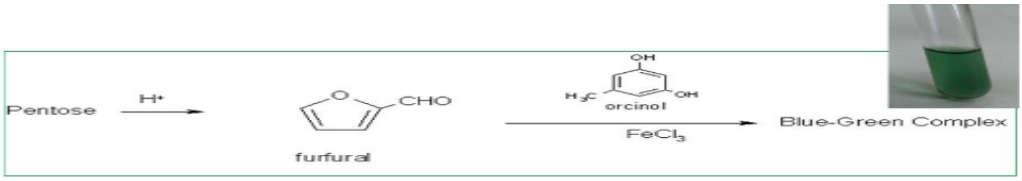
Principle:
Barfoed's test used copper (II) ions in a slightly acidic medium.
Reducing monosaccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide within three minutes.
Reducing disaccharides undergo the same reaction, but do so at a slower rate.

-The nonreducing sugars give negative result.



4. Bial's Test:

Objective:
To distinguish between pentose monosaccharide and hexose monosaccharide
Principle: Bial's test uses concentrated HCl as a dehydrating acid and orcinol - traces of ferric chloride [$FeCl_3$] 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.



5. Seliwanoff's Test:

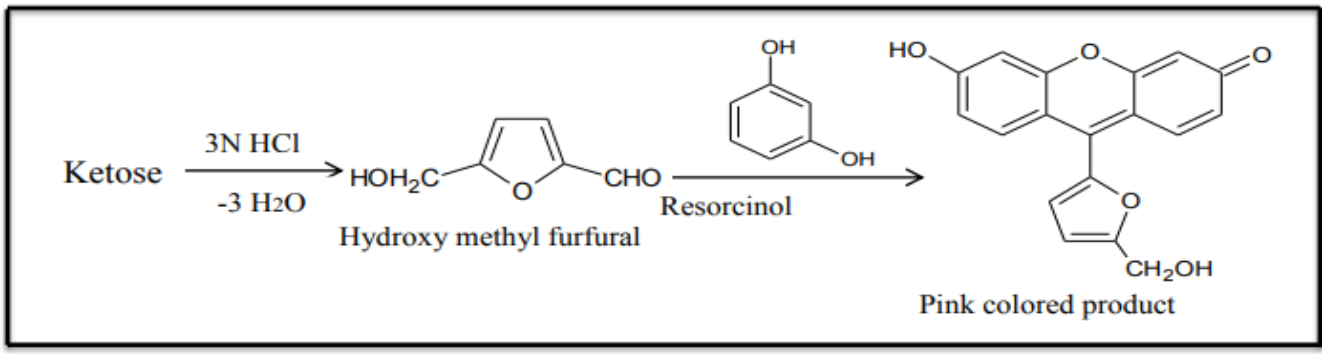
Objective:

used to distinguish between aldoses (like glucose) and ketoses (like fructose).

Principle:

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

-Aldohexoses react to form the same product, but do so more slowly giving yellow to faint pink color.



Method

1- Seliwanoff`s reagent is prepared by adding 0.05g of resorcinol in 500 ml concentrated hydrochloric acid 3N HCl.

2- Seliwanoff`s reagent(1 mL) is added to 1ml of sugar solution.

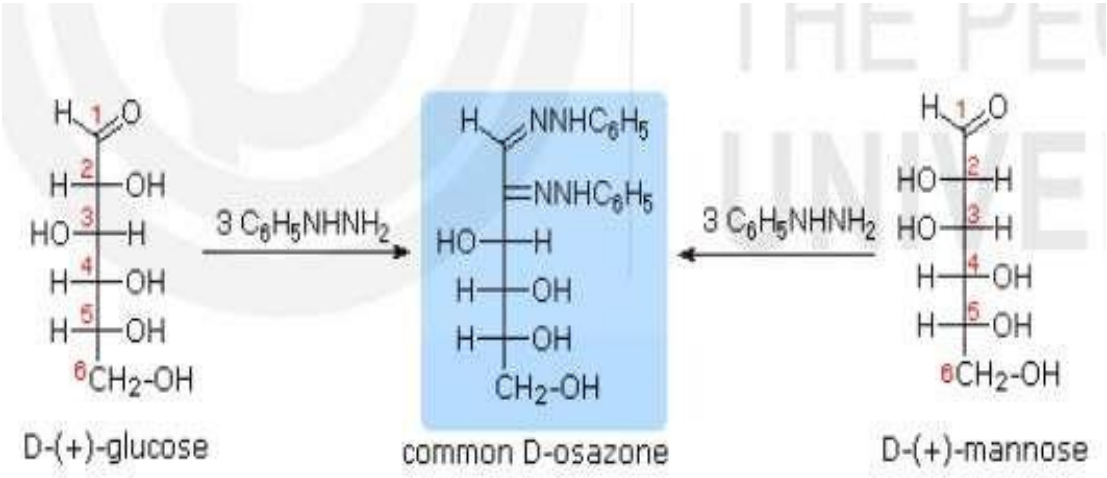
3- The solution is heated in a boiling water bath for 3-5 min. The change in the colour of the solution is noticed (pink coloured solution is present).

6. **Osazone test**:

This is the final and confirmatory test for qualitative analysis of carbohydrates

Principle

Reducing sugars upon reaction with Phenylhydrazine produces osazones, which are the characteristic derivatives of carbohydrates. These osazone derivatives have definite crystalline shape. These crystals make it easy to confirm the type of carbohydrate.



Osazone crystals