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SECOND STAGE

TESTS FOR CARBOHYDRATES

Benedict's Test

It is a test for reducing sugars. Carbohydrates having free functional group, that is not involved in a glycosidic bond, give this test positive. All monosaccharides and disaccharides except sucrose give positive Benedict's test. This test is negative for polysaccharides.

Principle:

This test is based on the ability of reducing sugars to undergo oxidation in alkaline solutions. In the presence of an alkali, reducing sugars undergo tautomerization to form enediols. These enediols reduce the cupric ions (Cu^{+2}) to form cuprous ions (Cu^{+}). The cuprous ions from cuprous hydroxide. Upon heating, it is converted to cuprous oxide that forms precipitates.

The citrate ions present in the reagent release the cuprous ions slowly for reduction and prevent the formation of $Cu(OH)_2$ until the oxidation-reduction process is completed.

Reagents

Benedict's Qualitative Reagent is used that contains;

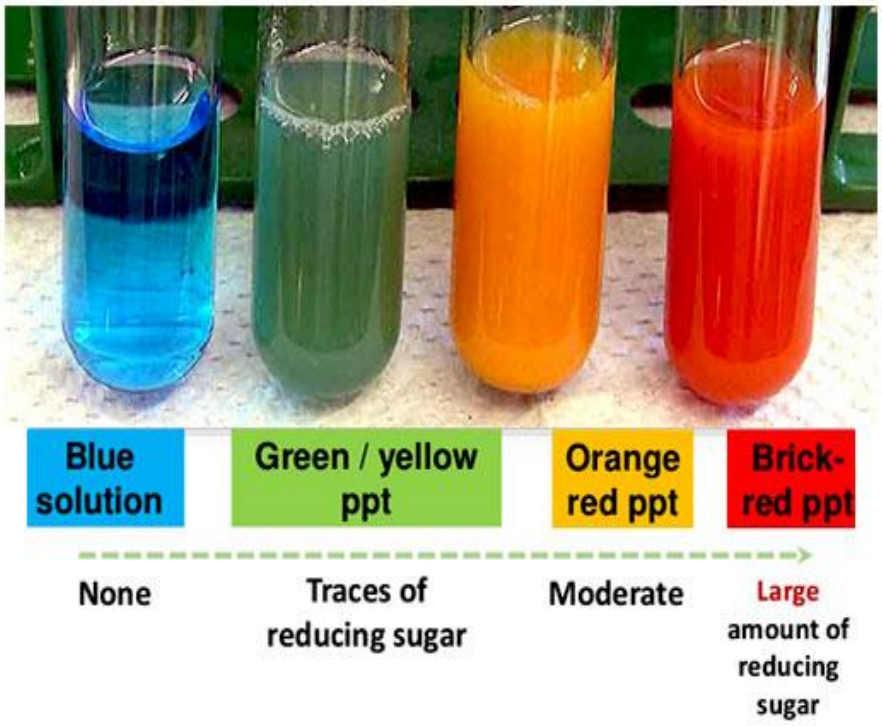
- Copper Sulfate (to provide cupric ions)
- Sodium Carbonate (to make solution alkaline)
- Sodium Citrate (to provide citrate ions)

Procedure of Benedict's Test

- Approximately 1 ml of sample is placed into a clean test tube.
- 2 ml (10 drops) of Benedict's reagent (CuSO4) is placed in the test tube.
- The solution is then heated in a boiling water bath for 3-5 minutes.
- Observe for color change in the solution of test tubes or precipitate formation

Results

- The precipitates of cuprous oxide indicate the presence of a reducing sugar in the test tube.



Points to Remember

- It is also a semi-quantitative test as the color of the precipitate is proportional to the concentration of reducing sugar in the test tube. Maximum concentration that can be tested in 2% at which brick-red precipitates are formed.
- This test is frequently used as a screening test for diabetes mellitus.
- The test is false positive for ascorbic acid, glutathione, uric acid, etc.

Barfoed's test:

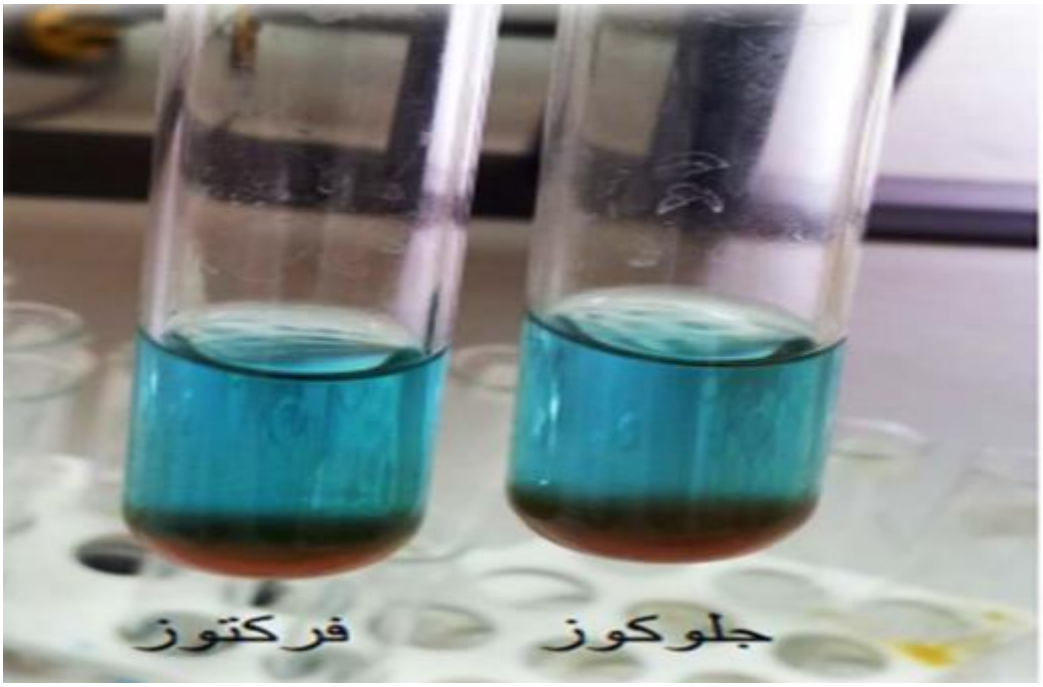
This test is used to distinguish reducing monosaccharides from reducing disaccharides. Since the monosaccharides reduce cupric ions (Cu^{+2}) faster than disaccharides even in slightly acidic solution. The rate of reduction depends upon the concentration of cupric ions and the time of heating.

Procedure:

1) Add 5 drops of the sugar solution to 15 drops of Barfoed's reagent in a test tube.

2) Boil for 3 minutes, and allow to stand. Report your observations.

Barfoed's reagent: Consists of 6.5% copper acetate in 1% acetic acid.



Points to Remember

1. Non-reducing disaccharides give a negative test with Barfoed's reagent, as the color of the solution remains blue without forming the red precipitate.
2. The Barfoed's test reaction is slow because it takes place in a weak acidic medium.

Seliwanoff's Test:

This test is used to distinguish an aldohexose from ketohexose. Heating with HCL dehydrates hexoses to hydroxymethyl furfural (HMF). Ketohexoses yield large amount of HMF and at faster rate than do aldohexoses. HMF form red condensation product with resorcinol.

Principle: Carbohydrates are dehydrated to form furfural derivatives by hydrochloric acid present in Seliwanoff's reagent. Furfural derivative of ketosugar condenses with resorcinol to form a chromogen (cherry red color).

Seliwanoff's reagent: 50 mg of resorcinol in 33 ml of concentrated hydrochloric acid and diluted to 100 ml with water.

Experiment	*Observation*	*Inference*
Take 3 ml of Seliwanoff's reagent in a test tube; add 1 ml of given solution. Boil for 30 seconds and allow it to cool at room temperature.	Cherry red color is formed. 	Given solution is a ketosugar

Points to Remember:

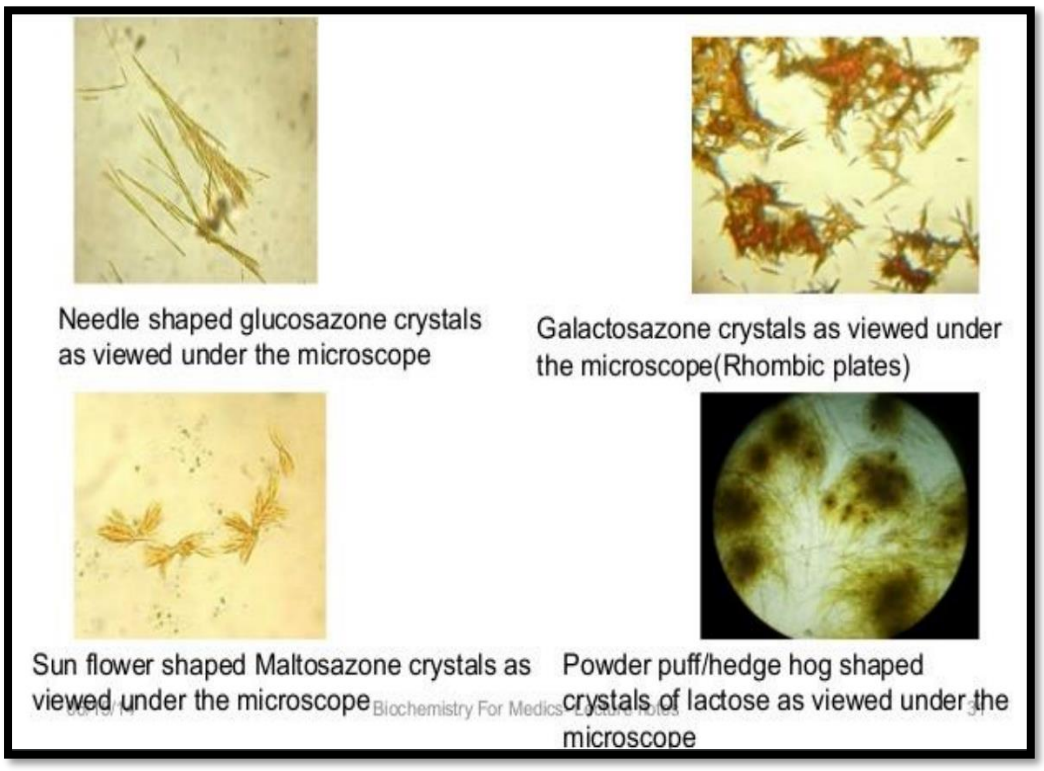
- This test is specific for ketohexoses only.
- Useful in differentiating aldohexoses and ketohexoses.
- The test will be answered by fructose, sucrose and other fructose containing carbohydrates.
- This test is very sensitive even for 0.1 % fructose. In the presence of glucose along with fructose sensitivity decreases.

Osazone test:

is a chemical test used to detect reducing sugars. This test even allows the differentiation of different reducing sugars on the basis of the time of appearance of the complex. This test is also termed Phenyl hydrazine test based on the reagent used for this test.

Procedure:

1- Add 3 mL of each sugar to a test tube and add a suitable amount of the solid mixture (phenylhydrazine hydrochloride and sodium acetate) using a small spatula, stirring well with a glass rod until the yellow-orange color appears.
2- Place the tubes in a boiling water bath for 30 minutes for monosaccharides and 45-60 minutes for reducing disaccharides.
3- Note that the precipitated osazone crystals for the monosaccharide will be at the bottom of the tube.
4- Cool the test tubes for reducing disaccharides under running water until osazone crystals are formed.
5- Place the crystals on glass slides and examine them under an electron microscope.



Points to Remember:

1- The increase in the amount of the mixture (phenylhydrazine hydrochloride and sodium acetate) more than the appropriate amount causes the formation of larger amounts of aniline with a brown color, which affects the crystal test and makes it unclear.
2- The decrease of less than the appropriate amount of the mixture (phenylhydrazine hydrochloride and sodium acetate) does not lead to the formation of osazone crystals.
3- The osazone test reaction occurs on the first and second carbon atoms to obtain the final product of the resulting crystal.
4- Osazone test is specific for reducing sugars only, and this means that non-reducing disaccharides such as sucrose do not give osazone test.

The test results for different carbohydrates are summarized below:

Test	*Molisch*	*Iodine*	*Benedict's*	*Barfoed's*	*Selivanoff's*
Glucose	+	-	+	+	-
Fructose	+	-	+	+	+
Lactose	+	-	+	-	-
Maltose	+	-	+	-	-
Sucrose	+	-	- (+ after acid hydrolysis)	-	+ after acid hydrolysis
Starch	+	+	- (+ after acid hydrolysis)	-	-

IDENTIFICATION OF UNKNOWN CARBOHYDRATE