AL-RASHEED PHARMACY Dpt.

3rd year 1st semester

Biochemistry

Lab 3

Benedict's Test
Barfoed's Test
Iodine Test

Tests based on reducing properties of carbohydrates

Benedict's Test

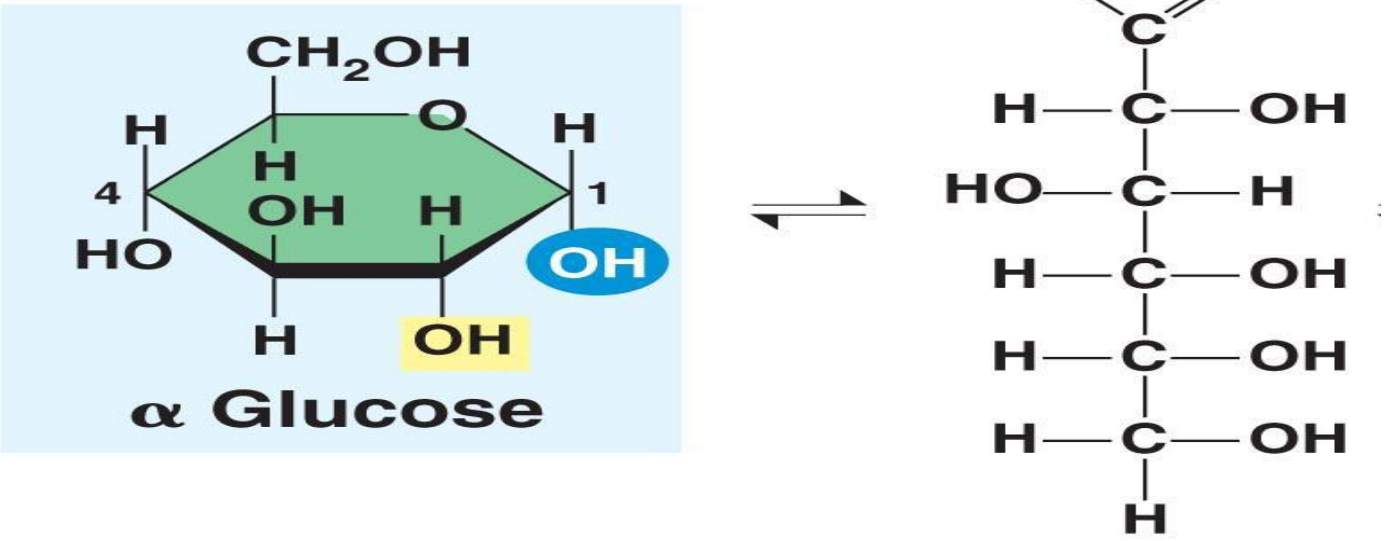
Barfoed's Test

Benedict's Test

Introduction

Reducing sugar is any carbohydrate which is capable of being oxidized to form carboxyl group(-COOH) and causes the reduction of other substances. Reducing sugars are carbohydrates that can act as reducing agents due to the presence of free aldehyde groups or free ketone groups.

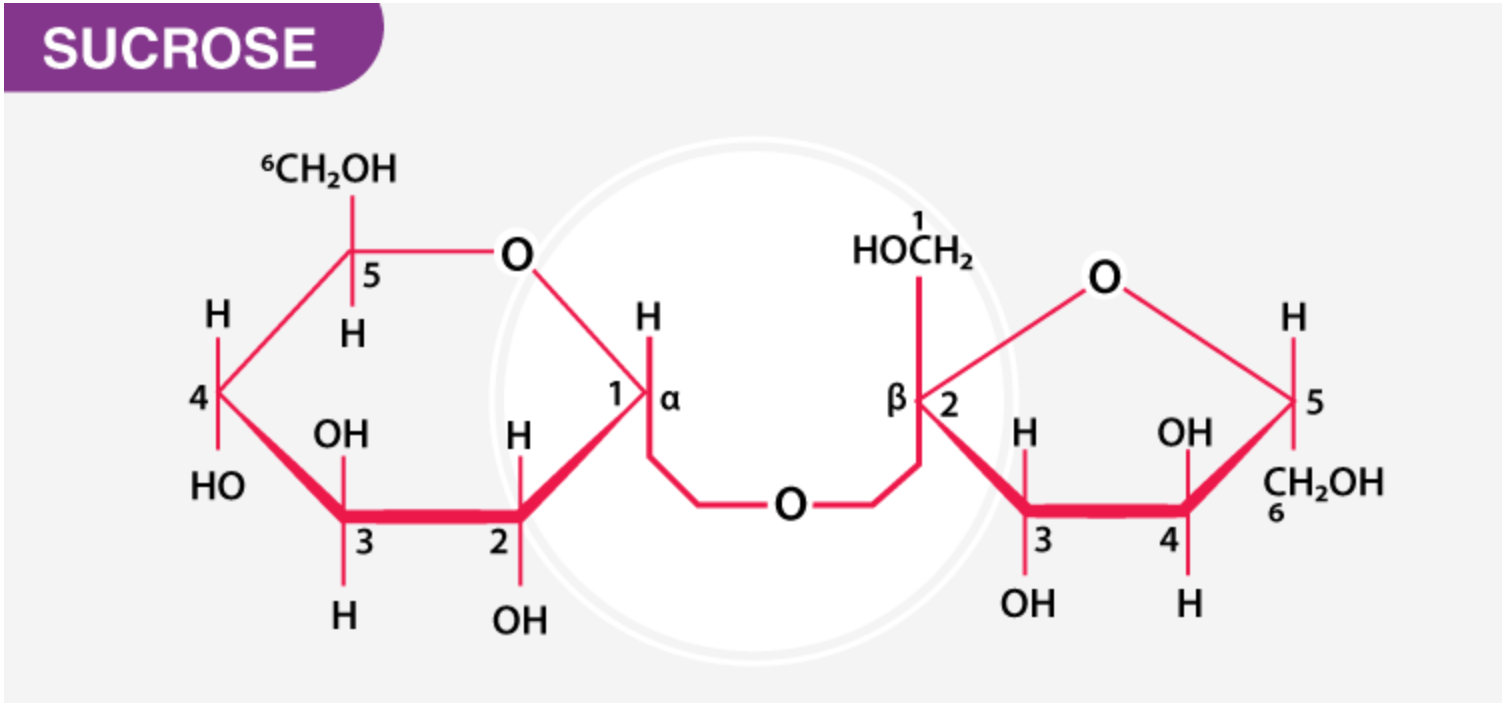
Structure of reducing sugar



Non reducing sugar

Non-reducing sugars are any type of carbohydrate which are unable to be oxidized and do not reduce other substances. Non-reducing sugars are carbohydrates that cannot act as reducing agents due to the absence of free aldehyde groups or free ketone groups.

Structure of non reducing sugar



Benedict's Test Definition

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.

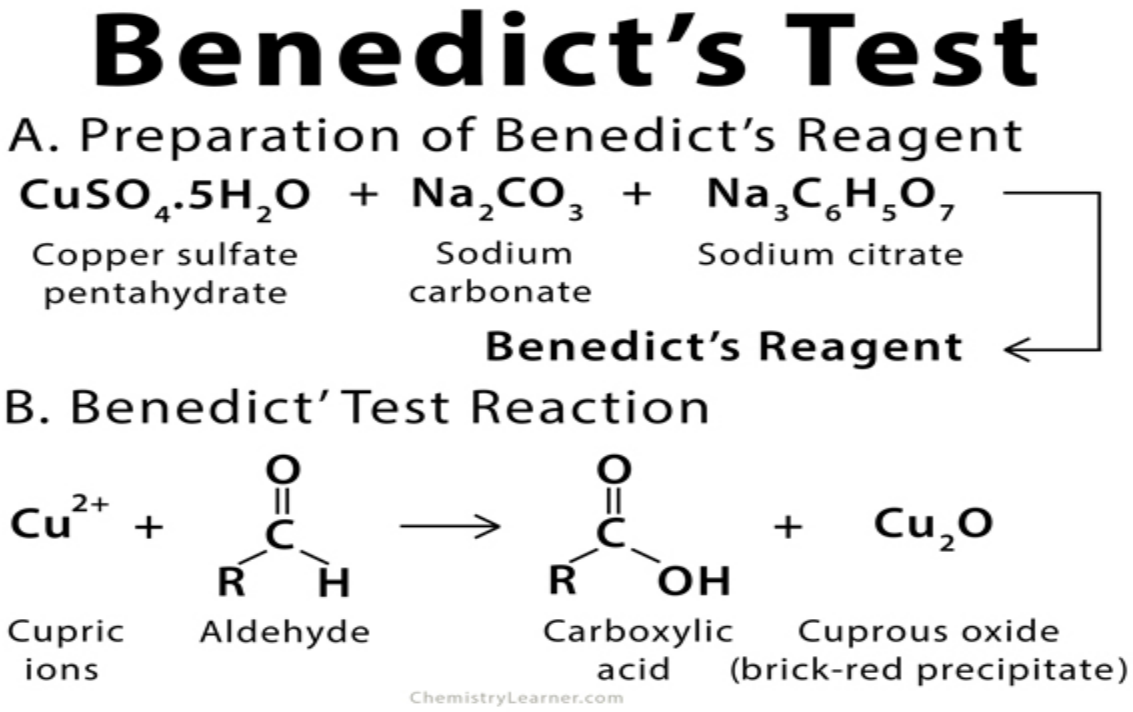
Objectives of Benedict's Test

- To determine the presence or absence of reducing sugar in the solution.

Principle of Benedict's Test

This test is based on the ability of reducing sugars to undergo oxidation in alkaline solutions. In the presence of an alkali, reducing sugars 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.

Benedict's Test
Reaction



Benedict's Reagent

Benedict's solution is a deep-blue alkaline chemical which consists of copper sulfate pentahydrate ($CuSO_4$. $5H_2O$), sodium carbonate (Na_2CO_3), sodium citrate ($Na_3C_6H_5O_7$) and distilled water. Sodium carbonate renders alkaline conditions which are required for the reaction, while sodium citrate is a complexing agent which complexes with the copper (II) ions to avoid degradation into copper (I) ions during storage.

Procedure

- Pipette out 2 ml (10 drops) of Benedict's reagent and placed it in the clean test tube
- Approximately 1 ml of sample is added to Benedict's reagent.
- The test tube is placed over the boiling water bath for 3-5 minutes .
- Observe for color change in the solution of test tubes or precipitate formation.

Observations

Brick-red precipitate is formed at the bottom of the test tube.

Results

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

Barfoed's Test

Barfoed's Test Definition

It is a differentiating test to distinguish between monosaccharides and disaccharides. Barfoed's test is also based on the reducing ability of sugar. However, sucrose also gives this test positive as it undergoes hydrolysis in the presence of an acid. Monosaccharides give early positive test while the disaccharides give late positive.

Objectives of Barfoed's Test

- To detect reducing carbohydrates.
- To distinguish reducing monosaccharides from disaccharides.

Principle of Barfoed's Test

The Barfoed reagent is made up of copper acetate in a dilute solution of acetic acid.Monosaccharides, which are strong reducing agents, react in about 1-2 min. However, the reducing disaccharides take a longer time of about 7-8 minutes, having first to get hydrolyzed in the acidic solution and then react with the reagent. Once the reaction takes place, thin red precipitate forms at the bottom the tube. The difference in the time of appearance of precipitate thus helps distinguish reducing monosaccharides from reducing disaccharides.

Procedure of Barfoed's Test

- Take 2 ml of Barfoed's reagent in a test tube
- Add 2 ml of the test solution to the above test tube
- Mix the solutions
- Hold the test tube on boiling water bath for minutes
- Allow to cool at room temperature
- Look for the precipitates
- If no precipitates are formed, boil for an additional 10 minutes
- Allow to cool and look for the precipitates

Observations

1-Red precipitates are formed after the first 5 minutes

2-Red precipitates are formed after additional boiling

Results

1-Formation of red precipitates after the initial first 5 minutes indicates the presence of a monosaccharide

2-If precipitates are formed after 15 minutes, a disaccharide is present in the test solution

Test for detection polysaccharides

Iodine Test

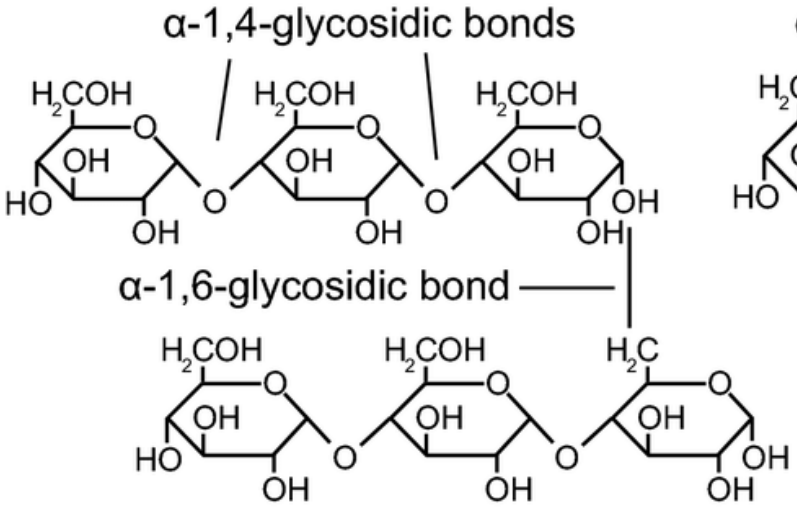
Iodine Test Definition

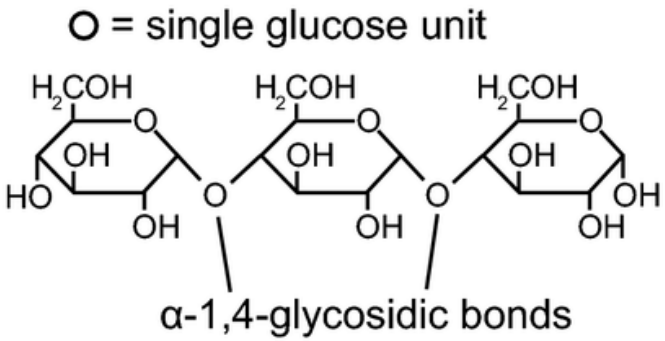
This test is specific for polysaccharides. This test is used to differentiate polysaccharides from the rest of carbohydrates. It is given positive by starch and glycogen.

Structures of polysaccharides

amylopectin

amylose





Objectives of Iodine Test

To detect the presence of polysaccharides, primarily starch.

Principle of Iodine Test

- The iodine test is based on the absorptive properties possessed by large polysaccharide molecules. The glucose chains in most of polysaccharides are organized to form helices. The space between the turns of the helix can hold small iodine molecules. This is seen with amylase chains found in starch. Glycogen and amylopectin can also absorb these iodine molecules on their surface. The absorptive property of polysaccharides decreases upon heating.
- Blue or red-colored iodine complexes are formed in this test.

Procedure of Iodine Test

- Take 2 ml of the given solution in a test tube
- Add 2-3 drops of iodine reagent in the above test tube
- Wait for some time

Observations

- When the iodine is added to the solution, the color of the solution changes. It may give the following colors;
- Blue
- Reddish-purple
- Reddish-brown

Result

If the color of the solution changes on adding iodine, it represents that polysaccharide is present in the solution. The nature of polysaccharide is detected based on the color formed.

- If blue color appears, amylase or starch is present in the solution
- If reddish-purple color appears, dextrin is present
- If reddish-brown color appears, glycogen is present

THANK YOU FOR LISTINING

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