

Carbohydrate Tests :

Background :

carbohydrate are poly hydroxy organic compounds possessing aldehyde or ketone group in their geometry. They can be classify into three types

1- monosaccharides : such as pentoses (xylose) , hexoses (glucose , fructose and galactose)

2- disaccharides : sucrose , lactose and maltose .

3- polysaccharides : starch , glycogen and dextrin . all of these types serves as a source of energy .

For more information check the any text book of biochemistry .

The general test for carbohydrate is

- 1- Molisch's Test : is a sensitive chemical test for the presence of carbohydrates, based on the dehydration of the carbohydrate by sulfuric acid to produce an aldehyde, which condenses with two molecules of phenol (usually α -naphthol, resulting in a red- or purple-colored compound.

Procedure:

- Apply this test two different carbohydrate solutions of your own choice, preferably to one monosaccharide and one polysaccharide.

- Place 2 mL of a known carbohydrate solution in a test tube, add 1 drop of Molisch's reagent (10% α -naphthol in ethanol).

- Pour 1-2 mL of conc. H₂SO₄ down the side of the test tube, so that it forms a layer at the bottom of the tube.

- Observe the color at the interface between two layers and compare your result with a control test.

* A brown color due to charring must be ignored and the test should be repeated with a more dilute sugar solution.

2-Solubility Tests:

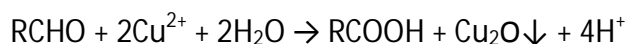
- Apply this test to all carbohydrates provided.

- Observe the solubility of the carbohydrates both in water and ethanol.

DO NOT depend on your solubility observations during identification of your unknown compound.

3-Barfoed's test: It was invented by Danish chemist Christen Thomsen Barfoed

Is a chemical test used for detecting the presence of monosaccharides. It is based on the reduction of copper(II) acetate to copper(I) oxide (Cu_2O), which forms a brick-red precipitate



(Disaccharides may also react, but the reaction is much slower.) The aldehyde group of the monosaccharide which normally forms a cyclic hemiacetal is oxidized to the carboxylate. A number of other substances, including sodium chloride, may interfere.

Preparing Reagent :

Barfoed's reagent consists of a 0.33 molar solution of neutral copper acetate in 1% acetic acid solution. The reagent does not keep well and it is therefore advisable to make it up when it is actually required.

Procedure :

To 1-2 mL of Barfoed's reagent, add an equal volume of sugar solution.

- Boil for 5 min. in a water bath and allow to stand.

You will observe a brick-red cuprous oxide precipitate if reduction has taken place.

This test is similar to the reaction of Fehling's solution to aldehydes.

3'- Benedict's reagent :(also called Benedict's solution): is a chemical reagent named after an American chemist, Stanley Rossiter Benedict .

Benedict's reagent is used as a test for the presence of reducing sugars. This includes all monosaccharides and many disaccharides, including lactose and maltose. Even more generally, Benedict's test will detect the presence of aldehydes , and alpha-hydroxy-ketones, including those that occur in certain ketoses. Thus, although the ketose fructose is not strictly a reducing sugar, it is an alpha-hydroxy-ketone, and gives a positive test because it is converted to the aldoses glucose and mannose by the base in the reagent.

The copper sulphate in Benedict's solution reacts with reducing sugars. Benedict's solution can be used to tell if there is a sugar in a substance such as glucose in starch .

Preparation of the Reagent :[One litre of Benedict's reagent can be prepared from 100 g of anhydrous sodium carbonate, 173 g of sodium citrate and 17.3 g of copper(II) sulfate pentahydrate.] . It is often used in place of Fehling's solution. Benedict's reagent contains blue

copper(II) ions (Cu^{2+}) which are reduced to copper(I) ions (Cu^+). These are precipitated as red copper(I) oxide which is insoluble in water.

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Benedict's Reagent provides a quantitative test for reducing sugars along with qualitative test. The color of the obtained precipitate gives an idea about the quantity of sugar present in the solution. A greenish precipitate indicates about 0.5% concentration; yellow precipitate indicates 1% concentration; orange indicates 1.5% and red indicates 2% or higher concentration.

To test for the presence of monosaccharides and reducing disaccharide sugars in food, the food sample is dissolved in water, and a small amount of Benedict's reagent is added. During a water bath, which is usually 4–10 minutes, the solution should progress in the colors of blue (with no glucose present), green, yellow, orange, red, and then brick red or brown (with high glucose present). A colour change would signify the presence of glucose. The common disaccharides lactose and maltose are directly detected by Benedict's reagent, because each contains a glucose with a free reducing aldehyde moiety.

Sucrose (table sugar) contains two sugars (fructose and glucose) joined by their glycosidic bond in such a way as to prevent the glucose isomerizing to aldehyde, or the fructose to alpha-hydroxy-ketone form. Sucrose is thus a non-reducing sugar which does not react with Benedict's reagent. Sucrose indirectly produces a positive result with Benedict's reagent if heated with dilute hydrochloric acid prior to the test, although after this treatment it is no longer sucrose. The acidic conditions and heat break the glycosidic bond in sucrose through hydrolysis. The products of sucrose decomposition are glucose and fructose, both of which can be detected by Benedict's reagent, as described above.

Starches do not react or react very poorly with Benedict's reagent, due to the relatively small number of reducing sugar moieties, which occur only at the ends of carbohydrate chains.

Benedict's reagent can be used to test for the presence of glucose in urine. Glucose found to be present in urine is an indication of diabetes mellitus. Once a reducing sugar is detected in urine, further tests have to be undergone in order to ascertain which sugar is present. Only glucose is indicative of diabetes.

4- Fehling's solution is a chemical test used to differentiate between water-soluble carbohydrate and ketone functional groups, and as a test for monosaccharides. The test was developed by German chemist Hermann von Fehling in 1849.

Preparing the Reagent : Fehling's is always prepared fresh in the laboratory. It is made initially as two separate solutions, known as Fehling's A and Fehling's B. Fehling's A is a blue aqueous solution of copper(II) sulfate, while Fehling's B is a clear solution of aqueous potassium sodium tartrate (also known as Rochelle salt) and a strong alkali (commonly sodium hydroxide).

Procedure:

- To 1 mL of Fehling's solution A (aqueous solution of CuSO_4) add 1 mL of Fehling solution B (solution of potassium tartrate).

- Add 2 mL of the sugar solution, mix well and boil.

Try to see the red precipitate of cuprous oxide that forms at the end of the reaction.

Equal volumes of the two mixtures are mixed to get the final Fehling's solution, which is a deep blue colour. In this final mixture, aqueous tartrate ions from the dissolved Rochelle salt chelate to Cu^{2+} (aq) ions from the dissolved copper(II) sulfate, as bidentate ligands giving the bistartratocuprate (II) complex as shown below. The tartarate ions, by complexing copper prevent the formation of $\text{Cu}(\text{OH})_2$ from the reaction of $\text{CuSO}_4 \cdot 2\text{H}_2\text{O}$ and NaOH present in the solution

Fehling's can be used to determine whether a carbonyl-containing compound is an aldehyde or a ketone. Fehling's solution is an oxidizing agent and the active reagent in the test. The compound to be tested is added to the Fehling's solution and the mixture is heated. Aldehydes are oxidized, giving a positive result, but ketones do not react, unless they are alpha-hydroxy-ketones.

The bistartratocuprate(II) complex oxidizes the aldehyde to a carboxylate anion, and in the process the copper(II) ions of the complex are reduced to copper(I) ions. Red copper(I) oxide then precipitates out of the reaction mixture, which indicates a positive result . (this is the same positive result as with Benedict's solution). A negative result is the absence of the red precipitate; it is important to note that Fehling's will not work with aromatic aldehydes; in this case Tollens' reagent should be used.

Fehling's can be used to screen for glucose in urine, thus detecting diabetes. Another use is in the breakdown of starch to convert it to glucose syrup and maltodextrins in order to measure the amount of reducing sugar, thus revealing the dextrose equivalent (DE) of the starch sugar.

Formic acid (HCOOH - methanoic acid) also gives a positive Fehling's test result, as it does with Tollens' test and Benedict's test also. This is because it is readily oxidizable to carbon dioxide and water.

For more information see Wikipedia, the free encyclopedia.

5- Seliwanoff's Test : A biochemical test to identify the presence of ketonic sugars, such as fructose in solution. It was devised by the Russian chemist F. F. Seliwanoff. A few drops of the reagent, consisting of resorcinol crystals dissolved in equal amounts of water and hydrochloric acid, are heated with the test solution and the formation of a red precipitate indicates a positive result.

Procedure :

-Heat 1 mL of sugar solution with 3 mL Seliwanoff's reagent (0.5 g resorcinol per liter 10% HCl) in boiling water.

- In less than 30 seconds, a red color must appear for ketoses.

- Upon prolonged heating, glucose will also give an appreciable color.

6- Bial's Test:

Bial's Test is to determine the presence of pentoses (5C sugars). The components of this reagent are resorcinol, HCl, and ferric chloride. In this test, the pentose is dehydrated to form furfural and the solution turns bluish and a precipitate may form. Perform this test with ribose and glucose.

Procedure:

- To 5 mL of Bial's reagent, add 2-3 drops of sugar solution and boil.

- Upon boiling, note the green-blue color formed.

Action of Alkali on Sugars:

Procedure:

- Heat 1 mL glucose solution with 1 mL 40% NaOH for 1 min.

- Cool and apply test for reducing sugars (e.g.; Fehling's Test).

- Apply a control test with glucose solution to observe the difference.

7- Iodine Test:

Iodine test is an indicator for the presence of starch. Iodine solution (iodine dissolved in an aqueous solution of potassium iodide) reacts with starch producing a blue-black color. Apply this test to all the polysaccharides provided.

Procedure:

- To 2-3 mL of polysaccharide solution, add 1-2 drops of iodine solution.

- Observe the different colors obtained for each of the polysaccharide solutions.