**Experiment:5**

**General Color Tests for Carbohydrates**

**1/Molisch Test:**

\*It is a general test for carbohydrate.

\*It is effective for any compound which can be dehydrated to furfural or a substituted furfural (such as hydroxymethyl furfural) by concentrated sulfuric acid.

\*If the carbohydrate is an oligosaccharide (e.g. disaccharide, trisaccharide …etc) or a polysaccharide, the hydrolysis of the carbohydrate acetal linkage occurs simultaneously with the dehydration reaction (in polysaccharide the color develops slower).

\*The purple ring color is due to condensation products of furfural or it's derivatives with α-Naphthol.

Carbohydrate Furfural or it's derivatives.

Furfural or it's derivatives + α-Naphthol Purple ring.

\*A negative result by this reaction is a very good evidence of the absence of carbohydrates, but a positive test is indication to the probable presence of carbohydrate.

\*Thymol may be used as a reagent instead of α-Naphthol.

\*Thymol is more stable than α-Naphthol, and can be applied to insoluble carbohydrates like cellulose or wood.

**Procedure:**

1-To three separate test tubes, add 10 drops of 0.5% solutions of glucose, sucrose and starch respectively.

2-Dilute each sugar solution with 2 ml of water.

3-Add 2 drops of α-Naphthol solution to each tube and mix.

4-Incline the test tube slowly and carefully add 3ml of concentrated sulfuric acid down the side of the tube to form a layer below the sugar solution.

|  |  |  |  |
| --- | --- | --- | --- |
| **0.5% starch** | **0.5% sucrose** | **0.5% glucose** |  |
| 10 drops | 10 drops | 10 drops |
| 2 ml | 2 ml | 2 ml | **Water** |
| 2 drops | 2 drops | 2 drops | **α-Naphthol** |
| **Mix** |
| 3 ml | 3 ml | 3 ml | **Con. H2SO4** |

A purple ring at the interface is indicative of acarbohydrate.

**2/ Anthrone Test:**

\*It is another general test for carbohydrates.

\*Based on similar reactions in which anthrone is used instead of α-Naphthol in Molisch test to form the color product (green to blue green color).

Furfural or furfural derivatives + Anthrone colored compound green to blue green color

\*It is very sensitive, it will give a positive reaction with filter paper (cellulose).

\*It can be used for quantitative determination of glycogen, inulin and sugar of blood.

\*It can be used as qualitative test, since different sugars dehydrate at different rates and produce a variety of colors.

\*Furfural give green color but can be differentiated by the fact that the test is rapidly obscured by brown precipitate when the sample is diluted with 50% H2SO4 or glacial acetic acid.

**Procedure:**

1-To five separate test tubes, add 1 drop of : water, 0.5% glucose, 0.5% sucrose and 0.5% starch, respectively, and to the fifth test tube, add some filter paper.

2-Dilute each one with 1ml of water.

3-Add 3 ml of Anthrone reagent to each test tube.

4-Mix thoroughly by swirling, heat in a boiling water bath for 3 mins. Observe the color formed.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Filter paper** | **0.5%starch** | **0.5%sucrose** | **0.5%glucose** | **Blank** |
| Small piece | 1 drop | 1 drop | 1 drop | 1 drop |
| **Water** |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| **Anthrone reagent** |
| 3 ml | 3 ml | 3 ml | 3 ml | 3 ml |

Heat for 3 mins in boiling water bath.

Green to blue green = positive.

**Reducing Properties:**

\*A reducing sugar is sugar that has free or potentially free aldehydic or ketonic group.

\*When blue cupric hydroxide in alkaline liquid is heated, it's converted into insoluble black cupric oxide, but, if a reducing agent like certain sugars is present, the cupric hydroxide is reduced to insoluble yellow or red cuprous oxide.

Cu (OH)2 (cupric hydroxide) CuO (insoluble black sugar) + H2O

2 Cu (OH)2 + Reducing sugar Cu2O (insoluble yellow to red sugar) + 2H2O + O2

**1/Benedict's Test:**

\*Carbohydrate with a free or potentially free aldehyde or ketone group have reducing properties in alkaline solution.

\*In addition monosaccharides act as a reducing agent in weakly acid solution.

\*Benedict modified the original Fehling's test to produce a single solution which is more convenient for tests, as well as being more stable than Fehling's reagent.

\*Benedict's test is a rapid and general test for reducing sugar.

\*Benedict's reagent composed of copper sulphate and sodium citrate, made alkaline with sodium carbonate.

\*Citrate in Benedict's reagent act as a complexing agent to form deep, blue, stable, soluble complex ions with Cu++, this is done to prevent the precipitation of CuCO3.

\*The alkalinity of Benedict's solution (PH = 10.5) is due to the hydrolysis of sodium carbonate.

Reducing sugar + Benedict's reagent yellow to red ppt. + oxidation product

\*Sucrose does not reduce Benedict's solution, because it has no free aldehyde or ketone group.

**Procedure:**

a) 1-To seven separate test tubes, add 1 ml of: water, 1% glucose, 1% xylose, 1% fructose, 1% sucrose, 1% lactose and 1% starch respectively.

2-Add 5 ml of Benedict's reagent to each tube and mix.

3-Incubate all the tubes in boiling water bath for 5 mins. Allow to cool and observe changes in the color of solutions and color of any precipitates.

b)For sucrose and starch, do acid hydrolysis, then run again with Benedict's reagent.

a)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1%Starch | 1%Lactose | 1%Sucrose | 1%Fructose | 1%Xylose | 1%Glucose | Blank |
| 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml | 1 ml |
| Benedict's reagent |
| 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml |

Incubate in boiling water bath for 5 mins.

Observe changes in the color of solutions and formation of any precipitate.

**b)Hydrolysis step**

|  |  |  |
| --- | --- | --- |
| **1% Starch** | **1% Sucrose** |  |
| 5 ml | 5 ml |
| 10 drops | 10 drops | **HCL (3M )** |
| Incubate for 5 mins. In boiling water bath |
| Take 1 ml |
| 5 ml | 5 ml | **Benedict's Reagent** |

Incubate in boiling water bath for 5 mins. Compare the results with those obtained without acid treatment.

**2/Barfoed's Test:**

\*Barfoed's reagent composed of copper acetate in acetic acid.

\*By use of the Barfoed's reagent we can distinguish monosaccharides from disaccharides by controlling such conditions as PH and time of heating.

\*Monosaccharides are stronger reducing agents towards Cu++ than the disaccharide.

\*Under the acidic conditions of the Barfoed's test, the cuprous ion precipitates to the red cuprous oxide which settles to the bottom of the tube. (The solution still remains dark blue).

\*Aldose and ketose sugar reduce this reagent, but hexoses act more rapidly and more vigorously than reducing disaccharide.

\*Sucrose very easily hydrolyzed by the dilute acid reagent and the librated fructose is exceedingly reactive in this test.

\*This test is positive with solutions of all monosaccharides of con. 0.1% and above.

\*Disaccharide do not produce any reduction unless they are present in very high con.

**Procedure:**

a)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| 1%Starch | 1%Maltose | 1%Sucrose | 1%Fructose | 1%Xylose | 1%Glucose | Water |
| 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml |
| Barfoed's Reagent |
| 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml | 5 ml |
| Mix, incubate all the tubes in boiling water bath for 3.5 minsNote any change in color or clarity of the solution. |

Red Precipitate = positive

**b)Hydrolysis step**

|  |  |  |
| --- | --- | --- |
| **1% Starch** | **1% Sucrose** |  |
| 2 ml | 2 ml |
| 5 ml | 5 ml | **Barfoed's Reagent** |
| Incubate in boiling water bath for 3.5 minsNote any change in color formed or clarity of solution |

Red Precipitate = positive