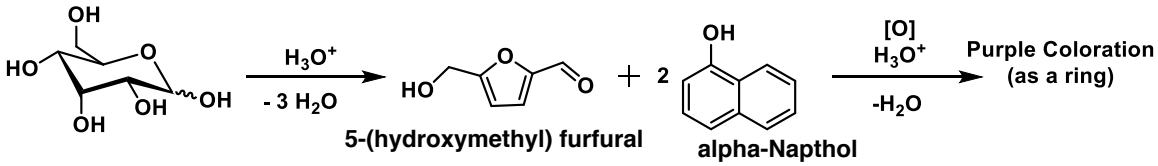
# Unknown carbohydrate Sample No.: Xxx, Yyy

# Principle for the Qualitative Analysis of Carbohydrates

1. **Molisch Test: -**
   All carbohydrates – monosaccharides, disaccharides, and polysaccharides – should give purple colour in presence of Molisch's reagent. In presence of, pentose sugars get dehydrated to furfural while hexose sugar get dehydrated to 5-hydroxymethylfurfural. Either of these aldehydes, if present, will condense with two molecules of naphthol (in Molisch's Reagent) to form a **purple-colored product** as a ring as illustrated below by the example of glucose.




**Scheme 1. Reaction of -naphthol with furfural derivatives.**

2. **Iodine Test: -**
   The 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**. The appearance of the deep blue color is due to the formation of the starch-iodine complex. Basically, iodide ion ($I^{3-}$) interacts with partially positively charged proton of hydroxyl group of sugar moiety. This test helps to distinguish starch, a polysaccharide from a batch of unknown carbohydrate samples.

3. **Fehling's Test: -**
   Fehling's Solution (deep-blue colored) is used to determine the presence of reducing sugars having aldehyde functional group. This test should be performed with **fructose**, **glucose**, **maltose**, **xylose**, and **sucrose**. Fehling's test differentiates between aldehydes and ketones. Aldehydes can be oxidized by the $Cu^{2+}$ ions in the presence of a strong base (alkaline) to form carbonic acids. Ketones cannot be oxidized by the same reagents. When the $Cu^{2+}$ oxidizes the aldehydes, it is reduced to $Cu^{+1}$ and forms the compound **$Cu_2O$ (Cuprous oxide), which is a reddish precipitate.** This helps to know if we have a reducing sugar (a sugar molecule with an aldehyde as the functional group).

   Fehling solution is prepared by mixing Fehling A [ blue-colored aqueous solution of Cu(II)$SO_4$] and Fehling B [ Colorless aqueous solution of Sodium Potassium Tartrate in an alkali like sodium hydroxide]. The tartrate ions prevent the formation of Cupric hydroxide by forming a complex with the cupric ions to keep them dissolved in the alkaline solution.


**Scheme 2. Chemical reaction of Fehling's solution with aldehyde**

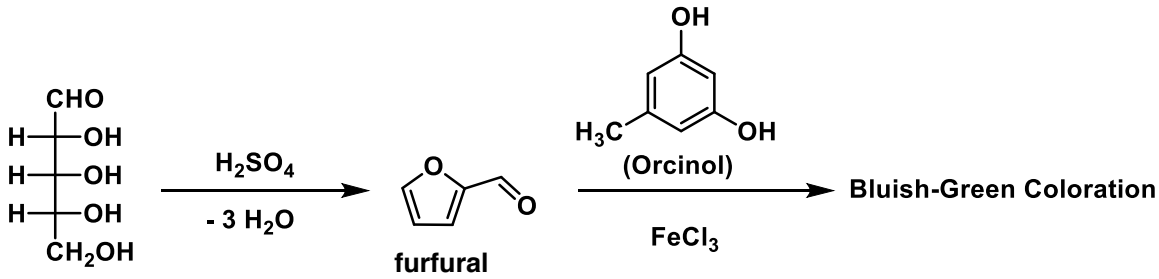
4. **Barfoed's Test: -**

Barfoed's reagent [Cupric acetate $Cu(OAc)_2$ in acetic acid (**acidic media**) ], is slightly acidic and is balanced so that it can **only be reduced by monosaccharides**. Disaccharides may also react with this reagent, but the reaction is much slower when compared to monosaccharides since for disaccharides first the glycosidic linkages need to be broken.. This test can be performed with **glucose**, **maltose**, and **sucrose**. **Reducing monosaccharides form red cuprous oxide ($Cu_2O$) within 5-6 min** and the precipitate formation **for disaccharides takes place after prolonged heating for 15-20 minutes.**


**Scheme 3. Chemical reaction of Barfoed's Test**

5. **Bial's Test: -**

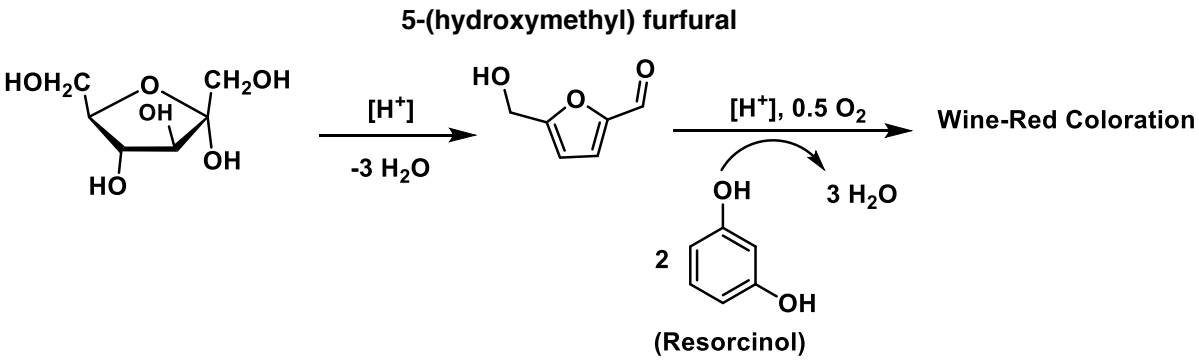
Bial's Test helps to determine the presence of pentoses (5C containing sugars). The components of this reagent are orcinol, HCl, and ferric chloride. In this test, the pentose is dehydrated to form furfural, which upon reacting with orcinol results in **bluish-green color** development. This test has to be performed with **ribose**, **xylose**, and **glucose**.




**Scheme 4. Chemical Reaction for Bial's Test**

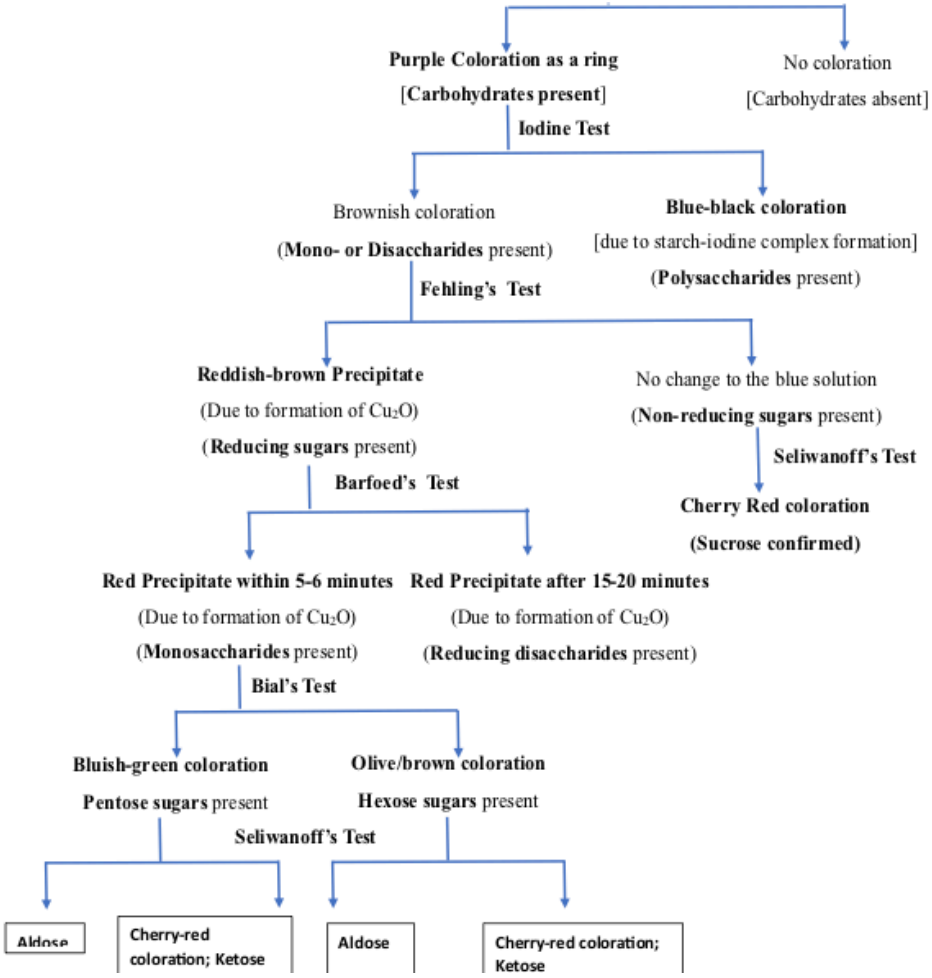
6. **Seliwanoff's Test: -**

Seliwanoff's Test distinguishes between aldose and ketose sugars. If the sugar contains a ketone group, it is a ketose and if it contains an aldehyde group, it is an aldose. Heating the ketoses give an **instantaneous cherry-red coloration**. This test has to be performed with **glucose, fructose, maltose,** and **sucrose.**




**Scheme 5. Chemical Reaction for Seliwaanoff's Test**

## Reactions in qualititative tests for carbohydrates



## Experimental Observation

For **unknown sample No.: 1**, the following tests were performed

| Name of the test | Observation | Comments |
|---|---|---|
| Molisch's test | Purple coloration | Carbohydrate present |
| Iodine test | No blue-black coloration | Starch absent |
| Fehling's test | Red cuprous oxide ppt | Reducing carbohydrate having aldehyde functional group present |
| Barfoed's test | Red cuprous oxide ppt appeared with 2-3 minutes | Monosaccharide present |
| Bial's test | No bluish green coloration formed | Pentose sugar absent |
| Seliwanoff's test | Cherry-red coloration did not form | Ketose sugar absent |

**Conclusion:** The above tests infer that **sample no. 1** is a reducing monosaccharide and hexose sugar, for example, glucose

For **unknown Sample No.: 2**, the following tests were performed.

| Name of the test | Observation | Comments |
|---|---|---|
| Molisch's test | Purple coloration | Carbohydrate present |
| Iodine test | No blue-black coloration | Starch absent |
| Fehling's test | Red cuprous oxide ppt | Reducing carbohydrate having aldehyde functional group present |
| Barfoed's test | Red cuprous oxide ppt appeared after 15 minutes | Disaccharide present |

**Conclusion:** The above tests infer that **sample no. 2** is reducing disaccharide, for example maltose, lactose etc.

For **unknown Sample No.: 3**, the following tests were performed.

| Name of the test | Observation | Comments |
|---|---|---|
| Molisch's test | Purple coloration | Carbohydrate present |
| Iodine test | No blue-black coloration | Starch absent |
| Fehling's test | Red cuprous oxide ppt | Reducing carbohydrate having aldehyde functional group present |
| Barfoed's test | Red cuprous oxide ppt appeared with 2-3 minutes | Monosaccharide present |
| Bial's test | bluish green coloration formed | Pentose sugar present |
| Seliwanoff's test | Cherry-red coloration did not form | Ketose sugar absent |

**Conclusion:** The above tests infer that unknown sample no. 3 is reducing pentose sugar, for example, xylose/ ribose etc

For **unknown Sample No.: 4**, the following tests were performed.

| Name of the test | Observation | Comments |
|---|---|---|
| Molisch's test | Purple coloration | Carbohydrate present |
| Iodine test | No blue-black coloration | Starch absent |
| Fehling's test | Red cuprous oxide ppt | Reducing carbohydrate having aldehyde functional group present |
| Barfoed's test | Red cuprous oxide ppt appeared with 2-3 minutes | Monosaccharide present |
| Bial's test | No bluish green coloration formed | Pentose sugar absent |
| Seliwanoff's test | Cherry-red coloration formed | Ketose sugar present |

**Conclusions:** The above tests infer that **sample no. 4** is reducing hexose sugar monosaccharide and also having keto functional group, for example could be fructose.

For **unknown sample No.: 5**, the following tests were performed.

| Name of the test | Observation | Comments |
|---|---|---|
| Molisch's test | Purple coloration | Carbohydrate present |
| Iodine test | No blue-black coloration | Starch absent |
| Fehling's test | No Red cuprous oxide ppt formed | Reducing carbohydrate having aldehyde functional group absent |
| Seliwanoff's test | Cherry-red coloration formed | Ketose sugar present |

**Conclusion:** The above tests infer that **sample no. 5** is non-reducing carbohydrate having ketose functional group, for example could be sucrose.