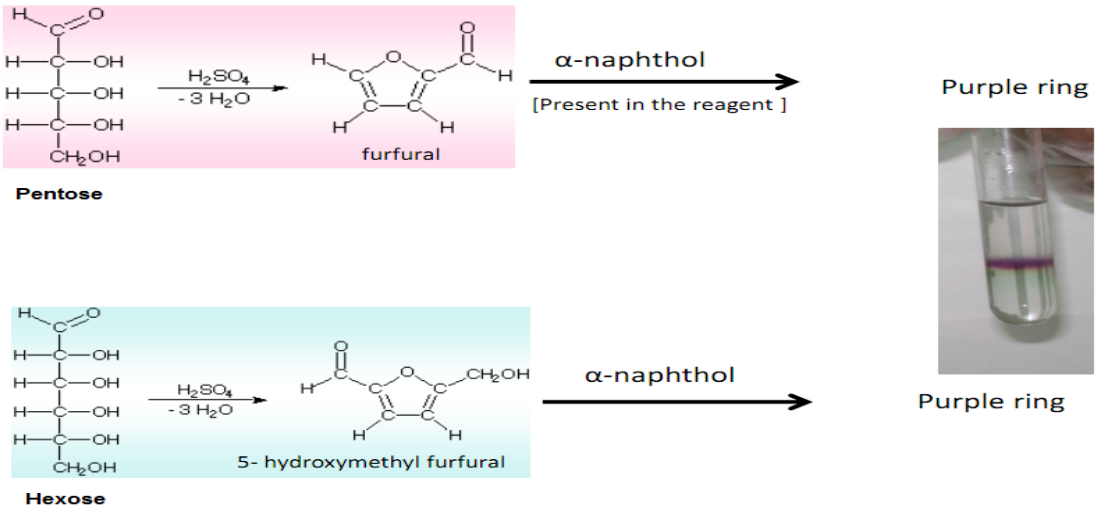
Exp.No.1 Molisch test

Principle:

Molisch's test is a general test for all carbohydrates. In this test, carbohydrates when reacted with conc. H2SO4 get dehydrated to form furfural and its derivatives.

Reactions:

This test is based on the fact that pentoses and hexoses are dehydrated by conc. **H2SO4** acid to form **Furfural** or Hydroxyl methyl furfural, respectively. Furfurals react with α-naphthol present in the test reagent to produce a purple product.



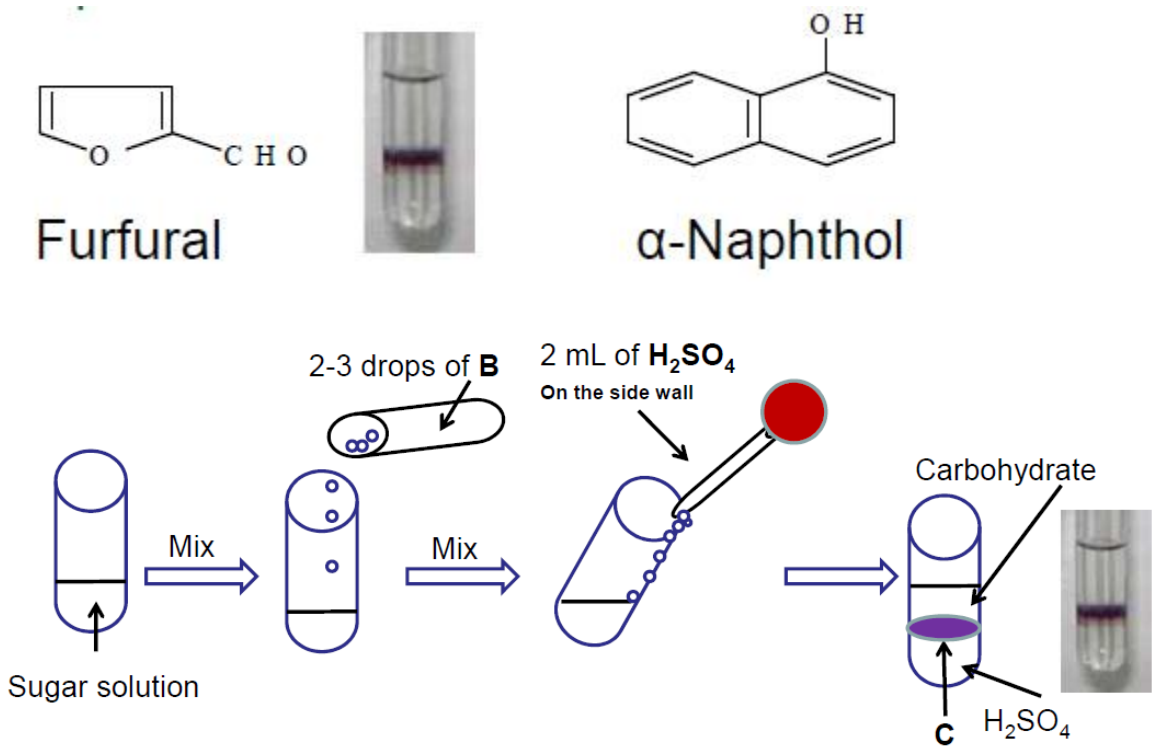
Reagents for Molisch's test:

- test solution: 5 % Glucose, 5 % Sucrose, 5 % Starch
- Molisch's reagent (5 % α naphthol in ethanol)
- H2SO4
- Dry test tubes
- pipettes

Procedure of Molisch's test:

1. Take 2ml of sample in dry test tube.
2. Take 2ml of distilled water in another tube as control.
3. Add 2-3 drops of Molisch's reagent to the solution.

4. Gently pipette 1ml conc. H2SO4 along the side of the tube so that two distinct layers are formed.
5. Observe color change at the junction of two layers.
6. Appearance of purple color indicates the presence of carbohydrates.



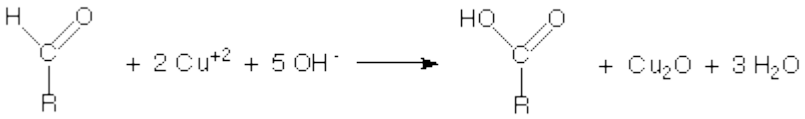
Exp.No. 2 Benedict's test

Is Specific to reducing sugars, sugars with a free aldehyde or ketone group) such as Fructose, Glucose, Galactose , Lactose & Maltose. Shows positive test. except sucrose.

Benedict reagent is formed from (sodium carbonate Na_2CO_3 + sodium citrate dihydrate$Na_3C_6H_5O_7$ + copper sulfate pent hydrate $CuSO_4.5H_2O$).

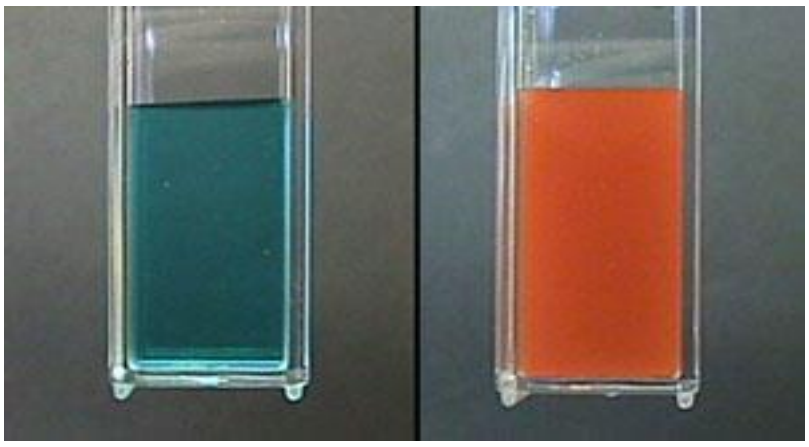
Reactions:

Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.



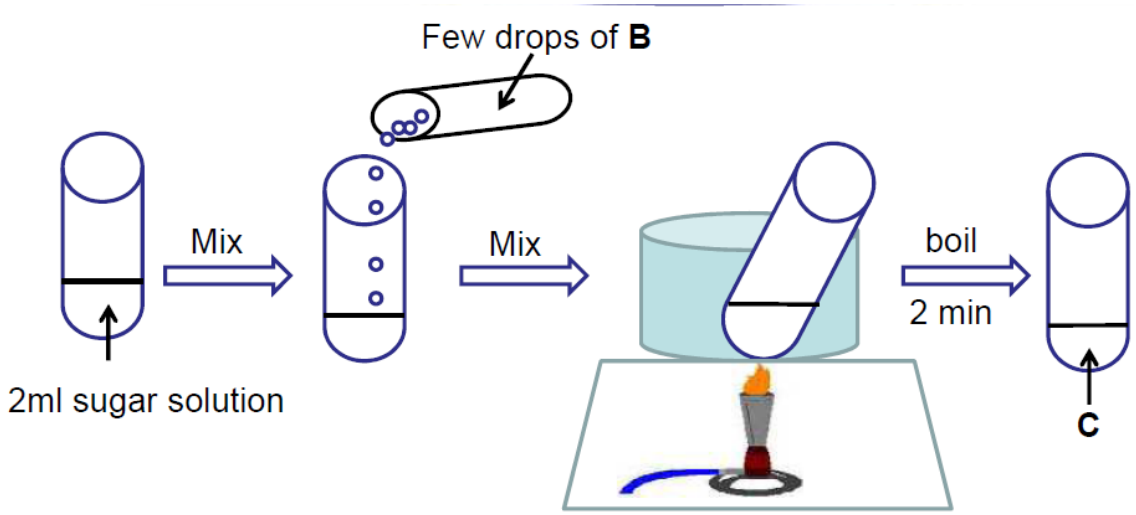
How to perform the test:

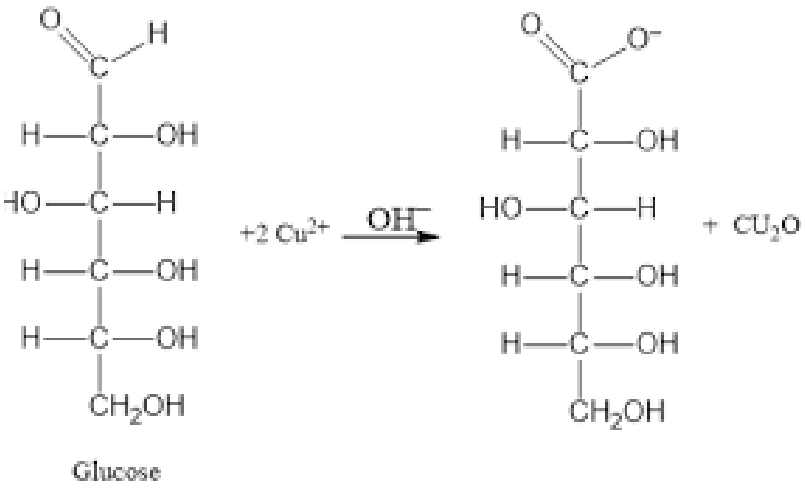
One ml of a sample solution is placed in a test tube. Two ml of Benedict's reagent (a solution of sodium citrate and sodium carbonate mixed with a solution of copper sulfate) is added. The solution is then heated in a boiling water bath for three minutes.



a negative test (left) and a positive test (right)

The copper sulfate (CuSO4) present in Benedict's solution reacts with electrons from the aldehyde or ketone group of the reducing sugar to form cuprous oxide (Cu_2O), of ***yellowish orange or red ppt.***

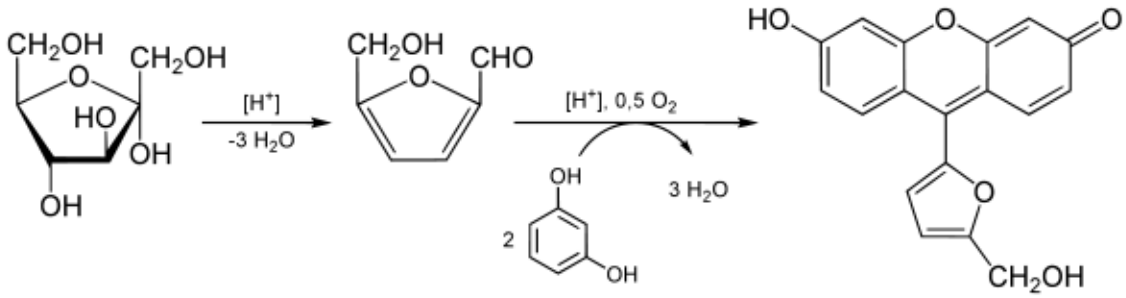




Glucose

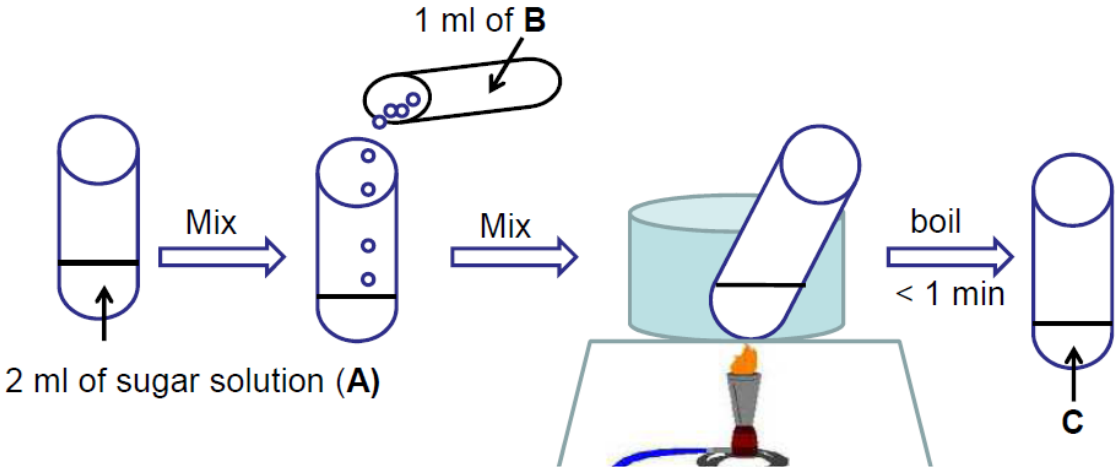
Exp.No. 3 Seliwanoff's test

Is Specific to Keto sugars (Fructose ,Sucrose). to differentiate (distinguish) between aldoses and ketoses saccharides. When a ketose is heated with a strong mineral acid, hydroxyl methyl furfural is formed. This compound (Sucrose or Fructose) forms a red complex with the organic compound, resorcinol. Seliwanoff's reagent [Contains resorcinol + conc. HCl]



Procedure &observation:

- To 5drops of carbohydrate solution in a test tube, add 10dropse to Selivanoff's reagent.
- Mix well, and boil it up for 3 min only.
- Observe ***red color***.

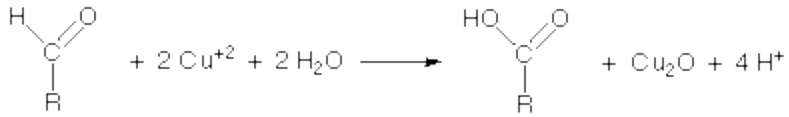


Exp.No. 4 Barfoed's Test

Is Specific to Mono-saccharide**s** , To differentiate between Monosaccharides (+ve) and Disaccharides (-ve).

Barfoed reagent is formed from:-

[Cu(CH3COO)2 + CH3COOH]. Reducing mono saccharides are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I).

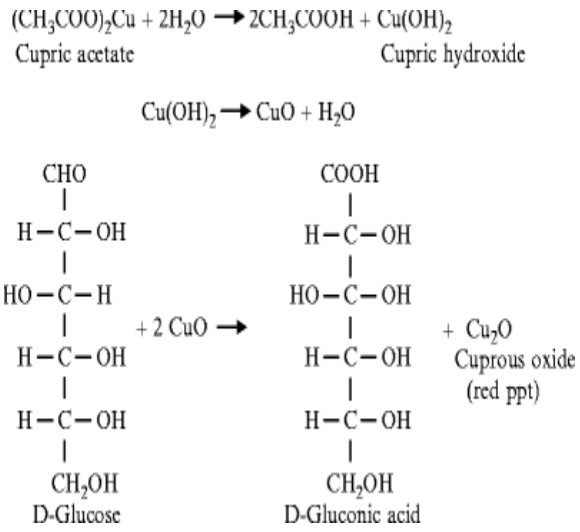


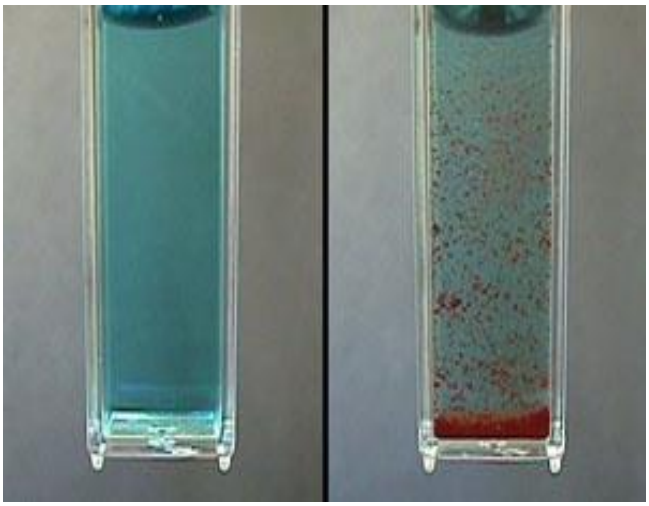
How to perform the test:

One ml of a sample solution is placed in a test tube. one ml of Barfoed's reagent (a solution of cupric acetate and acetic acid) is added. The solution is then heated in a boiling water bath for three minutes.

A positive test is indicated by:

The formation of a reddish precipitate within three minutes.



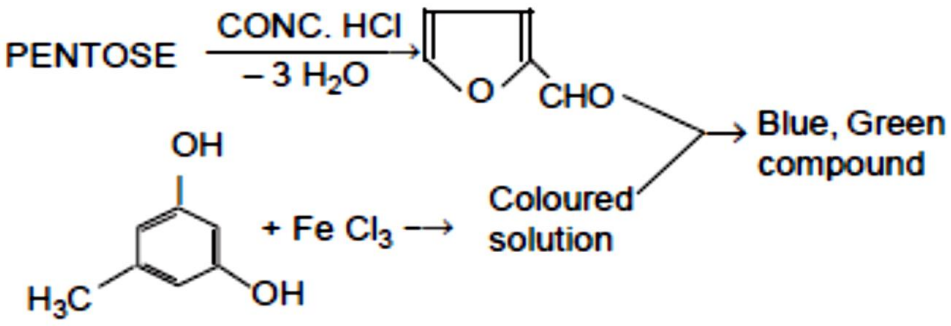


Exp.No. 5 Bial's Test

Is Specific to Pentose's sugar Shows positive test (+ ve) , To differentiate between Pentose's sugar and hexose sugar (-ve) .

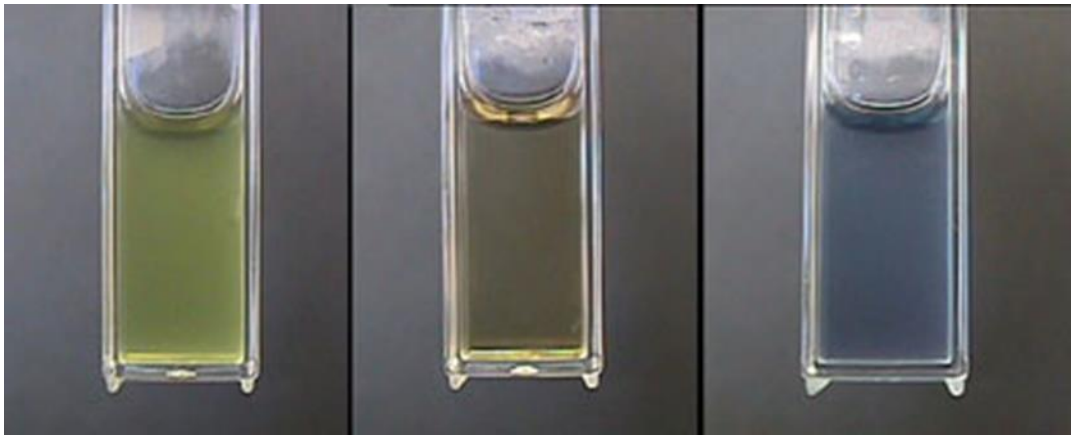
Reactions:

The reagent dehydrates pentoses to form furfural. Furfural further reacts with (Orcinol and ferric chloride) present in the reagent to produce a bluish product



How to perform the test:

2 ml of a sample solution is placed in a test tube. 2 ml of Bial's reagent (a solution of orcinol , HCl and ferric chloride) is added. The solution is then heated in hot water bath. If the color is not obvious, more water can be added to the tube. The formation of a bluish product. All other colors indicate a negative result for pentoses. Note that hexoses generally react to form green, red, or brown products.



Exp.No.6 Iodine's test

Principle:

Iodine test is **Specific to Starch**

To differentiate between Mono, Disaccharides (-ve), and Starch (+ve).

The structure of starch is a such that the molecule is coiled.

Iodine reagent (I_2 + KI), which interact specifically with these coiled molecules to form a **blue colored complex**. A positive iodine assay indicates the presence of starch, so if the color turns dark blue, this is a positive result.

Procedure & observation:

- To 2 mL of carbohydrate solution in a test tube, add 1-2 drops of Iodine solution

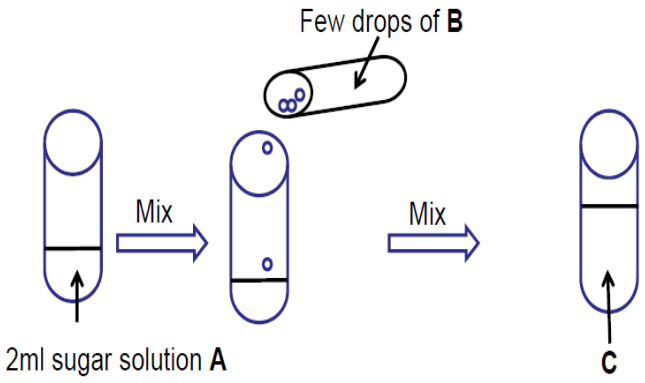
-Mix well, a deep blue color appears.

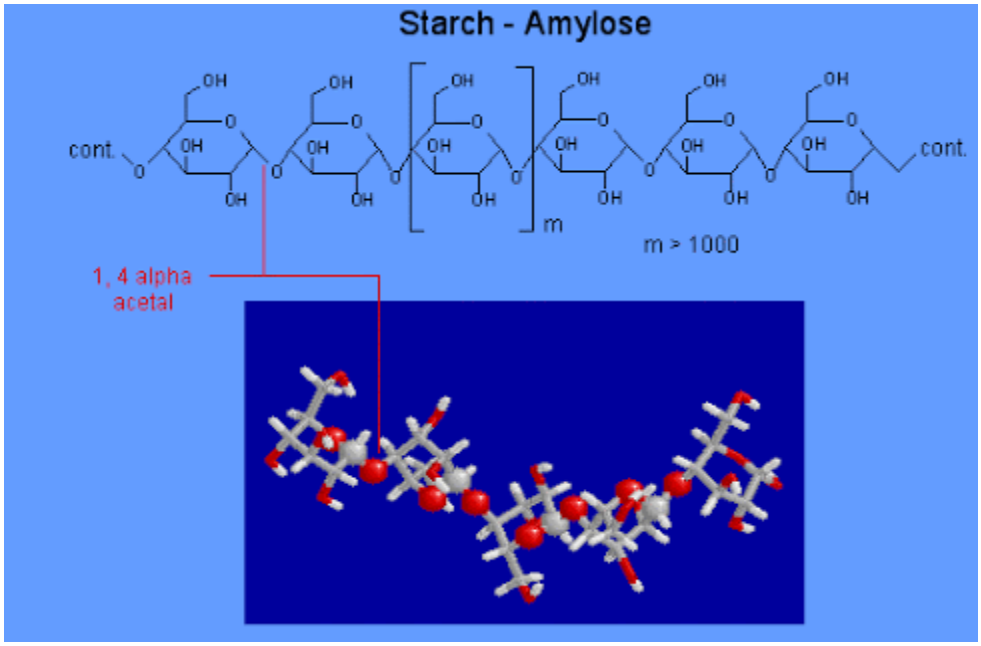
-Warm up, the color disappears. (why ?)

- Cool down, the color re-appears

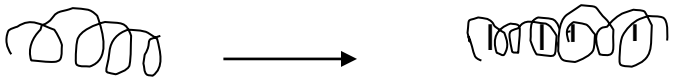
- Add NaOH (5%) the color disappear

- Add HCl the color re-appears





I_2 +



$3I_2 + 6\ NaOH \longrightarrow 5\ NaI + NaIO_3 + 3H_2O$

$NaIO3 + HCl \longrightarrow HIO_3 + NaCl$

$NaI + HCl \longrightarrow HI + NaCl$

$HIO_3 + HI \longrightarrow I_2 + H_2O$