

# **GENERAL TESTS FOR CARBOHYDRATE**

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# Introduction

- \* Carbohydrates are of great importance to human beings.
- \* They are major part of our diet, providing 60-70% of total energy required by the body.
- \* Chemically, Carbohydrates are aldehydes and ketones of polyhydroxy alcohols.
- \* Commonly found carbohydrates are glucose, fructose, sucrose, starch etc.

# Introduction

- \* They are classified into monosaccharaides (Glucose, Fructose, Ribose), disaccharides (Lactose, Maltose, Sucrose) and Polysaccharides (Starch, Cellulose, Glycogen etc.)

# 1. Molisch's Test



- It is a general test for carbohydrates.
- A positive Molisch Test indicates the presence of carbohydrate in a given test solution.

# 1. Molisch's Test



- All Carbohydrates **except amino sugars** give Molisch test positive.
- Disaccharides and Polysaccharides are easily hydrolysed by Concentrated  $\text{H}_2\text{SO}_4$  into monosaccharide and therefore give a positive test.

# Procedure of Molisch Test

Test	Observation	Inference
<p>Take 1 ml of carbohydrate solution in a test tube. Add 1-2 drops of 1% alcoholic <math>\alpha</math>-Naphthol solution to it. Mix it well and then add 2 ml concentrated <math>H_2SO_4</math> from side of the test tube and observe.</p> <p><b>(DO NOT SHAKE)</b></p>	<p>Violet coloured ring is obtained at the junction of the two liquids.</p>	<p>Carbohydrates present in the given solution.</p>

**Ring of any other colour is due to impurities.**

# PRINCIPLE OF MOLISCH'S TEST

- When Carbohydrate reacts with concentrated  $\text{H}_2\text{SO}_4$  undergoes dehydration to give hydroxymethyl furfural derivative, which condenses with 2 molecules of alcoholic  $\alpha$ -Naphthol to form **Purple** coloured complex.

## 2. Iodine Test

- ▣ This is a specific test for polysaccharides (Ex. Starch) which adsorb iodine and form coloured complex.
- ▣ Starch gives **blue** colour while glycogen gives **Reddish brown** colour.
- ▣ The most commonly available polysaccharides is Starch which is mixture of amylose and Amylopectin.



## 2. IODINE TEST

- The individual glucose units in Amylose are linked by  $\alpha$ -1,4 glycosidic linkages.
- Amylopectine has branching points contributed by  $\alpha$ -1,6 glycosidic bonds.
- Starch is insoluble in cold water but forms a colloidal solution in hot water.
- Starch has no detectable reducing activity.

## 2. Iodine Test

Test	Observation	Inference
Take 2ml carbohydrate solution in a test tube, then add 1-2 drops of dilute iodine solution to it. Mix it well and observe.	Blue or Violet Colour obtained	Polysaccharides present.

## 2. Iodine Test

Test	Observation	Inference
<p><b><u>Effect of Heating and cooling :</u></b></p> <p><b>(i) Take 2ml carbohydrate solution in a test tube. Then add 1-2 drops of dilute iodine solution to it. Mix it well, heat it and observe.</b></p>	<p><b>Blue or Violet Colour disappear</b></p>	<p><b>Due to heat, helical coiled structure of Amylose is disrupted, Iodine dissociated out, so blue or violet colour disappear.</b></p>
<p><b>(ii) Cool it at room temp. or under running tap water and observe</b></p>	<p><b>Blue Colour reappear</b></p>	<p><b>On cooling, helical coiled structure of Amylose again reformed, so blue colour reappears.</b></p>

## 2. Iodine Test

Test	Observation	Inference
<p><b><u>Effect of Alkali and Acid :</u></b></p> <p><b>(i) Take 2ml carbohydrate solution in a test tube. Then add 1-2 drops of dilute iodine solution to it. Mix it well and then add 1ml of 10% NaOH solution to it. Mix it well and observe.</b></p>	<p><b>Blue or Violet Colour disappear</b></p>	<p><b>Due to alkali (NaOH), iodine molecule are taken up by alkali to form sodium hypo iodide complex (NaOI), so blue or violet colour disappear.</b></p>
<p><b>(ii) After colour disappear then add same amount of glacial acetic acid mix well and observe.</b></p>	<p><b>Blue Colour reappear</b></p>	<p><b>When acid is added, iodine liberated from sodium hypoiodide (NaOI) complex reacts with polysaccharide solution to form blue or violet colour.</b></p>

# Principle of Iodine Test

- o Iodine forms a coordinate complex between the helically coiled polysaccharides chain and iodine centrally located within the helix due to adsorption.
- o The colour obtained depends upon the length of the unbranched or linear chain available for complex formation.

### 3. Benedict's Test Before hydrolysis



Test	Observation	Inference
Take 5 ml Benedic's reagent and then add 8 drops of carbohydrate solution. Mix it well, boil it for 2 min, mix well and observe.	No coloured precipitate obtained.	Non-reducing Sugars are present

## 4. Starch Hydrolysis Test

Test	Observation	Inference
<p>Take 2 ml starch solution then add 2 ml conc. HCl. Boil it for 5 min then cool under tap water and then add 2 ml of 40% NaOH solution. Check with red litmus paper. Then perform Benedict's Test with this hydrolysate.</p> <p><b>(i) Benedict's Test After hydrolysis</b></p>	<p>G/Y/O/R coloured precipitate obtained.</p>	<p>After hydrolysis reducing Sugars are present</p>

# Results



- **The Given solution is of Carbohydrates, Polysaccharides, Non-Reducing Sugar, Starch present.**