GENERAL REACTIONS OF CARBOHYDRATES

MOLISCH'S REACTION:

PRINCIPLE:

Sugars on reaction with dehydrating agents like concentrated strong acids (concentrated H_2SO_4) yield furfural and furfural derivatives, such as hydroxymethyl furfural, which condense with α-naphthol and give a reddish violet ring.

REAGENTS:

1. Molisch's Reagent:
   - i- α-naphthol, 5-1.
   - ii- Ethyl alcohol 95/
2. Concentrated H_2SO_4
3. Original solution (O.S.) – containing a carbohydrate.

PROCEDURE:

To 2ml of sugar solution (original solution) add 2 to 3 drops of Molisch's reagent. Mix thoroughly. Carefully pore 5 ml concentrated H_2SO_4 along the side of the test tube. Acid being heavier will form a layer beneath the sugar solution. The formation of a reddish violet ring at the junction of the two liquids indicates the presence of carbohydrates. This test is very sensitive and is given by all the carbohydrates.

REACTIONS GIVEN BY MONOSACCHARIDES AND DISACCHARIDES

1. COPPER REDUCTION TESTS:

Carbohydrates which give reduction tests have free aldehyde or ketonic groups, and are called "reducing sugars".

PRINCIPLE:

Alkaline copper reagents (Benedict's and Fehling's reagents) are reduced by the reducing sugars with the formation of yellow, orange or red precipitate.

The reaction of acid copper reagents (Barfoed's reagent) with reducing sugars is slow and can be used to distinguish monosaccharides from disaccharides.

a. FEHLING'S TEST:

1. Fehling's reagent:
   - i. Solution A - Copper sulphate solution, and
   - ii. Solution B - Alkaline tartrate solution.

   These solutions are preserved in separate bottles. Fehling's reagent is freshly prepared by mixing equal volumes of solution-A with solution-B.

2. Original solution (O.S.) – containing a carbohydrate.

PROCEDURE:

To 1ml of sugar solution (original solution) in a test tube, add 1ml of Fehling's reagent. Mix and boil carefully. The production of yellow or brownish-red precipitate of cuprous oxide indicates the presence of reducing sugars in the sample.

b. BENEDICT'S TEST:

1. Benedict's reagent:
   i. Copper sulphate,
   ii. Sodium Citrate, and
   iii. Sodium Carbonate.
2. Original solution (O.S.) – containing a carbohydrate.

PROCEDURE:

To 5ml of Benedict's reagent in a test tube add 8 drops of sugar solution (original solution). Mix thoroughly and heat to boil for 2 minutes. Allow the tube to cool. The solution, in addition to formation of a precipitate, will change colour from blue to green, yellow, orange or red depending upon the amount of reducing sugar present. This test can be used as a rough quantitative test for the clinical evaluation as shown in the following table:

OBSERVATIONS	CONCENTRATION OF SUGAR IN THE ORIGINAL SOLUTION (%)	CLINICAL EVALUATION
No colour change (Blue)	0.0%	Nil
Green coloured solution with no precipitate.	0.1%	Traces
Green coloured solution with yellow precipitate.	0.1 - 0.5%	+
Olive green coloured solution with yellow precipitate.	0.5 - 1%	++
Yellow orange coloured precipitate.	1 - 2%	+++
Brick red coloured precipitate.	2% or more.	++++

c. BARFOED'S TEST:

1. Barfoed's Reagent:
   i. Copper acetate, and
   ii. Acetic acid.
2. Original solution (O.S.) – containing a carbohydrate.

PROCEDURE:

To 5ml of Barfoed's reagent in a test tube add 0.5ml of sugar solution (original solution). Mix thoroughly and place it in the boiling water bath. Note the time when signs of reduction i.e., formation of a red precipitate of cuprous oxide first appears in the test tube.

The monosaccharides start forming precipitates in less than 7 minutes where as the precipitates appearing after 7 minutes indicate the presence of disaccharides in the solution.

2. OSAZONE TEST (PREPARATION OF OSAZONES)

REAGENTS:

1. Phenylhydrazine mixture consisting of:
   i. Phenylhydrazine, and
   ii. Anhydrous sodium acetate.
2. Original solution (O.S.) – containing a carbohydrate.

PROCEDURE:

Take phenylhydrazine mixture and fill about half an inch of test tube with it. Add 3 to 5ml of sugar solution (original solution) to the test tube. Mix thoroughly and place it in a boiling water bath. Note the appearance of yellow crystalline precipitate in the test tube. Remove and allow the test tube to cool slowly. Do not cool the test tube under tap water. Examine the crystals under the microscope and make a drawing. Draw only what you see.

Osazones of glucose are formed in 10-15 minutes where as those of disaccharides take upto 45 minutes. Sucrose does not react with phenylhydrazine to form crystalline compounds called osazones.

SUGAR	OSAZONE	SHAPE
Glucose	Glucosazone	Needle shaped.
Fructose	Fructosazone	Needle shaped.
Galactose	Galactosazone	Fluffy ball shaped.
Lactose	Lactosazone	Fluffy ball shaped.
Maltose	Maltosazone	Sunflower shaped.

SPECIAL REACTIONS GIVEN BY THE INDIVIDUAL SUGARS

TEST FOR FRUCTOSE (FREE OR COMBINED):

1. SELIWANOFF'S TEST:

PRINCIPLE:

Fructose on heating with the HCl , rapidly forms furfural , which on reaction with resorcinol gives red coloured compounds.

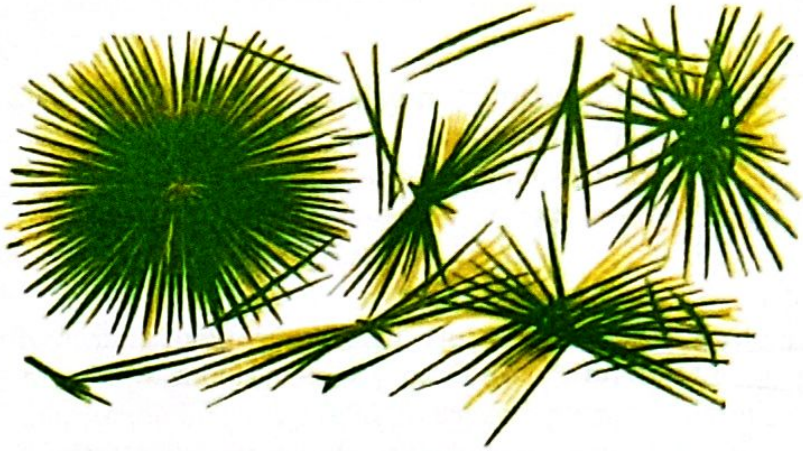
REAGENT:

1. Seliwanoff's Reagent:
   i. Resorcinol, and
   ii. Concentrated HCl.
2. Original solution (O.S.) – containing a carbohydrate.

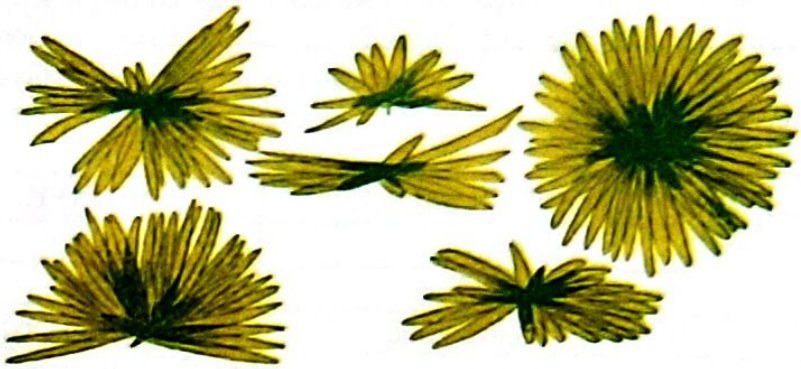
PROCEDURE:

To 3ml of Seliwanoff's reagent in a test tube add 3 drops of a original solution (fructose solution) and heat the mixture to just boiling. A positive reaction is indicated by the production of a red colour.

Ketoses free or combined (Fructose or Sucrose), give a red colour. Prolonged boiling with aldoses is bound to give a false positive result, as the aldoses also start giving a similar reaction.



Glucosazone or Fructosazone



Maltosazone

Lactosazone

OSAZONES

HYDROLYSIS OF SUCROSE

Sucrose does not reduce Benedict's, Barfoed's or Fehling's reagents, nor does it form any osazone. Sucrose, upon hydrolysis, takes one molecule of water and breaks down into two molecules of monosaccharides i.e., glucose and fructose.

PROCEDURE:

To 3ml of sugar solution (original solution) in a test tube add few drops of concentrated HCl. Mix carefully and gently heat to boil. Cool under tap water. Add 1ml 5% NaOH drop by drop to the test tube. Heat to boil again. On completion of hydrolysis the solution will turn yellowish, indicating the presence of a reducing sugar.

After acid hydrolysis it gives positive copper reduction tests.

REACTIONS GIVEN BY POLYSACCHARIDES

The polysaccharides are complex carbohydrates. They have high molecular weight. Few of them are appreciably digested in the alimentary canal by human beings.

They are not reducing sugars and so cannot reduce Benedict's reagent. Also they cannot form osazones. When starch and glycogen are boiled with dilute acids, they are hydrolyzed to glucose. The intermediate products formed during the course of hydrolysis, are dextrins and maltose.

1. IODINE TEST:

REAGENTS:

a. 0.01N Iodine solution.
b. Original solution (O.S.) – containing a carbohydrate.

PROCEDURE:

To 3ml of the starch solution (original solution) in a test tube, add 1 - 2 drops of the dilute iodine solution. Observe the production of a blue colour. The blue colour produced disappears on heating and it reappears on cooling the solution.

Starch, glycogen and higher dextrins on reaction with dilute iodine solution form coloured compounds as shown in the table below:

Starch	Blue colour.
Amylodextrin.	Purple colour.
Erythrodextrin.	Red colour.
Glycogen.	Red colour.
Achrodextrin (lower dextrins), Disaccharides (Maltose) and Monosaccharides.	No change in colour.

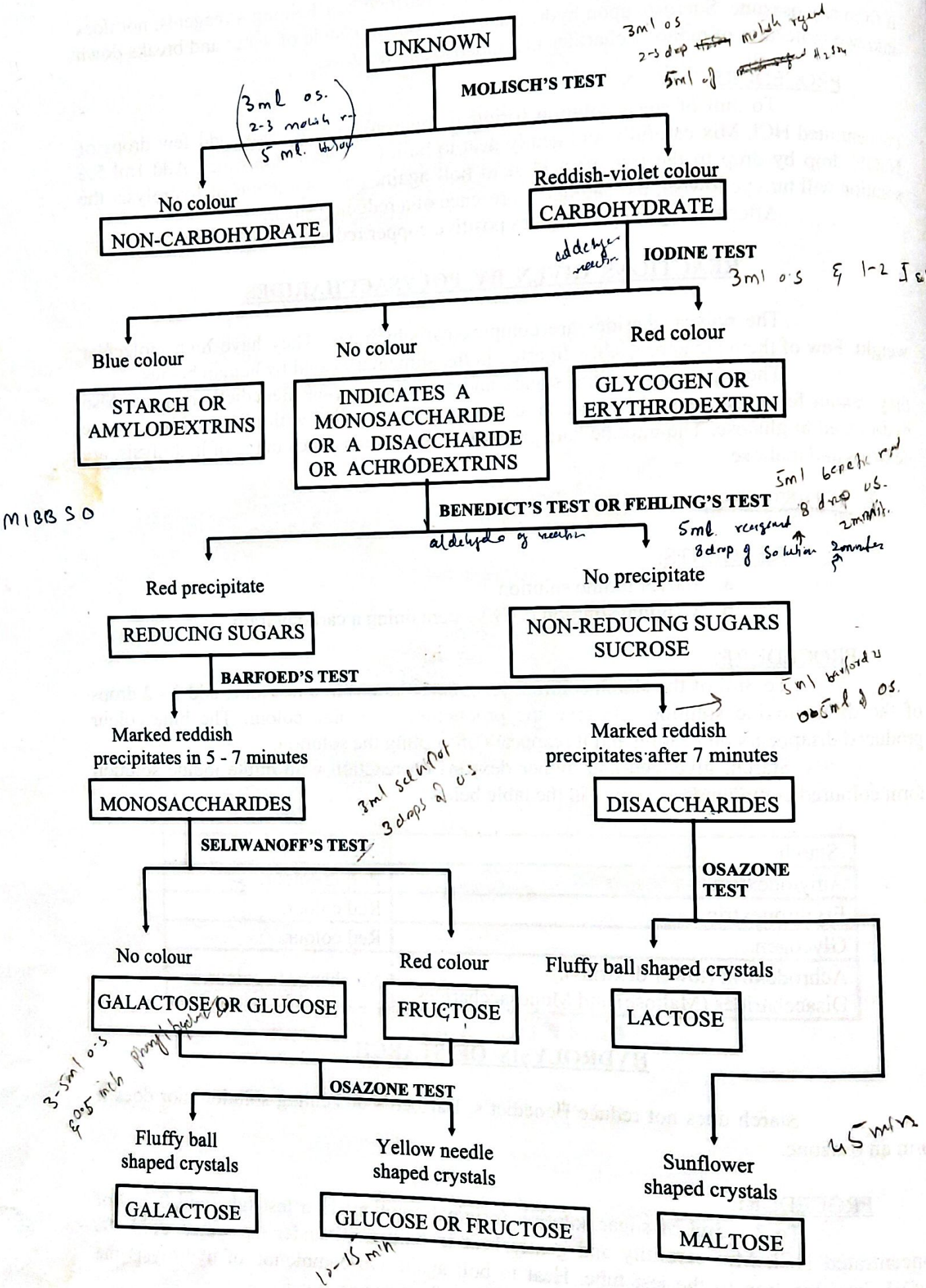
HYDROLYSIS OF STARCH

Starch does not reduce Benedict's, Barfoed's or Fehling solution, nor does it form an osazone.

PROCEDURE:

To 3 - 5ml of sugar solution (original solution) in a test tube add 0.5ml of concentrated HCl. Mix carefully and gently heat to boil. Cool under tap water. Add 5% NaOH drop by drop to the test tube. Heat to boil again. On completion of hydrolysis the solution will turn yellowish, indicating the presence of a reducing sugar.

QUALITATIVE TESTS FOR IDENTIFICATION OF CARBOHYDRATES.



QUESTION / ANSWERS

1. **Describe a test for the detection of sugar in urine?**

A. Benedict's Test – To 5 ml Benedict's Reagent add 8 drops of urine, mix and heat to boil for 2 minutes. The change in the colour of solution indicates the presence of sugar in the urine.

2. **What is the composition of Benedict's Reagent?**

A. Benedict's Reagent contains : Sodium citrate, Sodium carbonate, Cupric sulphate and distilled water.

3. **What is the use of Benedict's Test?**

A. Benedict's Test is used to detect the presence of reducing sugars in any solution.

4. **What is positive Benedict's Test?**

A. A positive Benedict's Test gives various shades of colour from green, yellow, orange, red and brick-red colour depending upon the increasing concentration of reducing sugars in the solution.

5. **What is the clinical significance of a positive Benedict's Test?**

A. A positive Benedict's Test indicates the presence of reducing sugars particularly glucose in the urine. Being a qualitative test it indicates a rough estimate of glucose in the urine sample. This can be helpful in diagnosing and monitoring the level of glucose in patients with diabetes mellitus.

6. **What is the pH required for Benedict's and Barfoed's Test?**

A. pH for Benedict's Test is alkaline and for Barfoed's Test it is acidic.

7. **What is reducing sugar?**

A. All the sugars which have free aldehyde or ketone group are known as reducing sugars. They reduce the cupric (Cu^{++}) to cuprous (Cu^{+}) Ions.

8. **Name the reducing sugars?**

A. All the monosaccharides and disaccharides (i.e., glucose, fructose, galactose, lactose and maltose) except sucrose are the reducing sugars.

9. **What is a non-reducing sugar?**

A. The sugars which do not have any free aldehyde or ketone group are called non-reducing sugars e.g., sucrose and polysaccharides.

10. **Why sucrose being disaccharide is a non-reducing sugar?**

A. Sucrose being disaccharide consisting of glucose and fructose, both are reducing sugars but the aldehyde group of glucose and ketone group of fructose is linked together in glucosidic linkage, thus there is no free reducing group available.

11. **What is the principle of Molisch's Test?**

A. Carbohydrates are converted into furfural by concentrated H_2SO_4, which acts as a dehydrating agent. Furfurals form a coloured complex with α- naphthol (Molisch's Reagent) producing a violet ring at the junction of two liquids.

12. **What is the use (or significance of Molisch's Test?**

A. It is used for detection of carbohydrates in any solution.

13. **How much is ++ sugar in the urine?**

A. 0.5 – 1%

14. **What is the significance of Barfoed's Test?**

A. Copper in acid medium in Barfoed's reagent is reduced slowly and can be used to distinguish between monosaccharides and disaccharides e.g., A positive Barfoed's Test within or less than 7 minutes indicates the presence of monosaccharides, whereas, a positive Barfoed's Test after or more than 7 minutes indicates the presence of disaccharides in any solution i.e., it is faster in monosaccharides than in disaccharides.

15. **What is the composition of Seliwanoff's Reagent?**

A. It contains : i- Resorcinol, and ii- HCl.

16. **What is the significance of Seliwanoff's Reagent?**

A. Positive Seliwanoff's Test indicates the presence of keto- sugars, thus it helps in differentiating between aldo- and keto- sugars.

17. **What is the principle of Seliwanoff's Test?**

A. The HCl in Seliwanoff's Reagent acts as a dehydrating agent, converting keto- sugars into furfurals, which form a cherry-red coloured complex with Resorcinol.

18. **What is the use of Iodine Test?**

A. It indicates the presence of polysaccharides in any solution.

19. **What are the products of hydrolysis of sucrose, lactose and maltose?**

A. i- Sucrose → Glucose + Fructose,
ii- Lactose → Glucose + Galactose,
iii- Maltose → Glucose + Glucose.

EXPERIMENT No. 2 Date: _____

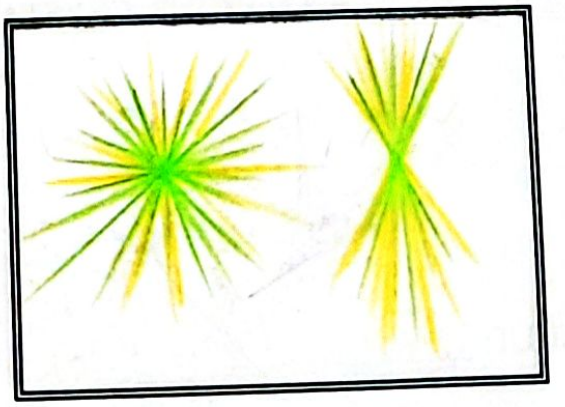
DETECTION OF GLUCOSE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Glucose solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Violet ring at junction of two liquids	Carbohydrates indicated
2	IODINE TEST	No change in colour	Monosaccharides & Disaccharides
3	BENEDICT'S TEST	Brick red colour ppt	Reducing sugar
4	BARFOED'S TEST	Red ppt of cuprous oxide in 5-7 min	Monosaccharide confirm
5	SELIWANOFF'S TEST	No 22/1/20	May be glucose or galactose.
6	OSAZONE TEST	Yellow needle like crystals	Glucose

SKETCH OF THE OSAZONE CRYSTALS



RESULT: This is glucose soln needle shaped crystals

EXPERIMENT No. 3 Date:

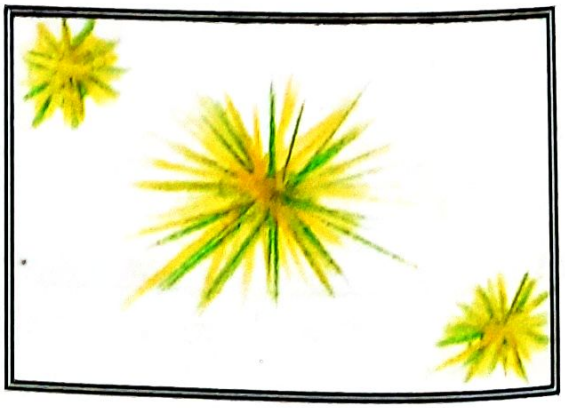
DETECTION OF FRUCTOSE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Fructose solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Reddish violet ring is formed	Carbohydrates present.
2	IODINE TEST	No change in colour	Monosaccharide and disaccharide
3	BENEDICT'S TEST	No change in colour	Reducing sugar absent.
4	BARFOED'S TEST	Red ppt in 7 minutes.	Monosaccharides
5	SELIWANOFF'S TEST	Red colour	fructose
6	OSAZONE TEST	yellow needle shaped crystals.	fructose confirm

SKETCH OF THE OSAZONE CRYSTALS



RESULT: Fructose (yellow needle) crystal.

EXPERIMENT No. 4 Date: _____

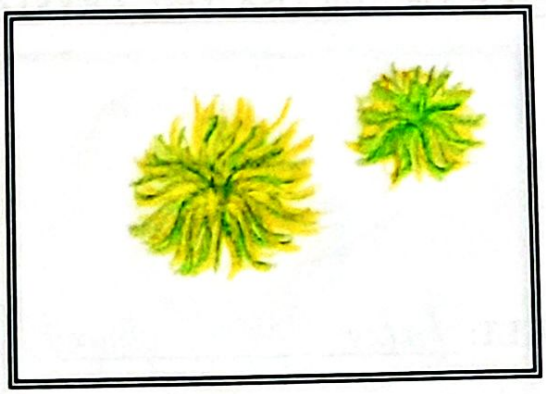
DETECTION OF GALACTOSE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Galactose solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	A reddish violet ring is formed	Carbohydrate is present
2	IODINE TEST	No change in colr	Mono and disaccharide prst
3	BENEDICT'S TEST	Yellow orange ppt	reducing sugar present
4	BARFOED'S TEST	Red ppt in 7 min	Monosaccharides present
5	SELIWANOFF'S TEST	No change in colr	Keto sugar is present
6	OSAZONE TEST	fluffy shaped crystals	galactose is present

SKETCH OF THE OSAZONE CRYSTALS



RESULT: galactose confirm. fluffy shaped crystals

Date: _____

EXPERIMENT No. 5

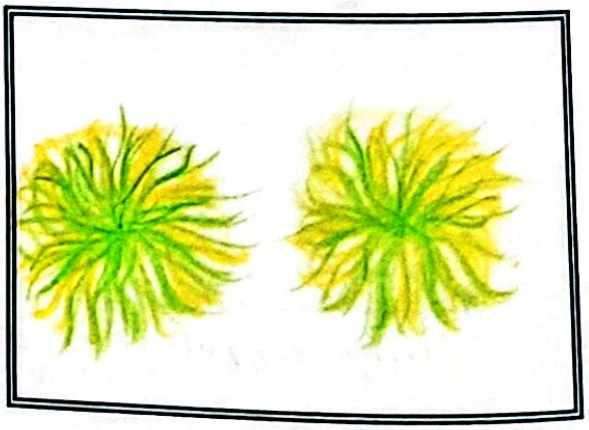
DETECTION OF LACTOSE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Lactose solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Violet ring at junction	Carbohydrate
2	IODINE TEST	No change in colour	mono and disaccharide
3	BENEDICT'S TEST	Red ppt formation	Reducing sugar present
4	BARFOED'S TEST	Red ppt after 7 min	Disaccharide present
5	SELIWANOFF'S TEST	No change in colour	keto sugar excluded. aldo sugar present
6	OSAZONE TEST	fluffy ball crystals	Lactose confirm

SKETCH OF THE OSAZONE CRYSTALS



RESULT: Latose (fluffy-shaped) crystals

EXPERIMENT No. 6 Date: _____

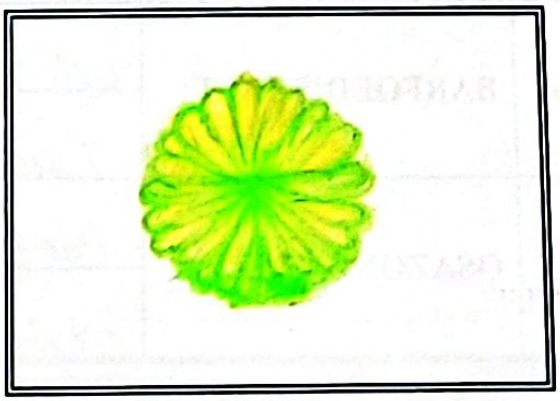
DETECTION OF MALTOSE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Maltose solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Violet reddish ring is formed at junction	Carbohydrate is present.
2	IODINE TEST	No change is colr	Mono and di-saccharide present
3	BENEDICT'S TEST	Red ppt is formed	Reducing sugar present
4	BARFOED'S TEST	Red ppt is formed after 7 mints	diasaccharide is present
5	SELIWANOFF'S TEST	No change in colr	Keto sugar is excluded
6	OSAZONE TEST	Sunflower shaped crystals formed	Maltose confirm

SKETCH OF THE OSAZONE CRYSTALS



RESULT: Maltose (sunflower shaped crystals)

Date:_____

EXPERIMENT No. 7

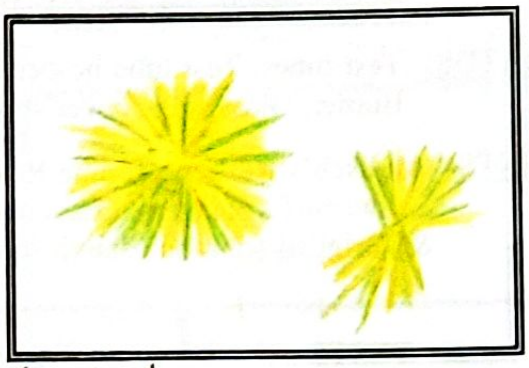
DETECTION OF SUCROSE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Sucrose solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Reddish violet ring appeared	Carbohydrate present
2	IODINE TEST	No change in colr	Disaccharide present
3	BENEDICT'S TEST	No change in colr	Reducing sugar exclude sucrose prst.
4	BARFOED'S TEST	No change in colr	Reducing sugar excluded sucrose prt
5	SELIWANOFF'S TEST	Red cherry colour appeared	combioned keto sugar present
6	PERFORM THE FOLLOWING TESTS AFTER HYDROLYSIS.		
a	BENEDICT'S TEST	Red ppt is formed	Reducing sugar present
b	BARFOED'S TEST	ppt is formed before 7 minutes	Monosaccharide is present
c	OSAZONE TEST	Needle shaped crystals formed	Glucose & fructose sucrose confirm

SKETCH OF THE OSAZONE CRYSTALS



RESULT: Sucrose ?confirm (glucose + fructose)
needle shaped crystals

22/1/20

EXPERIMENT No. 8 Date: _____

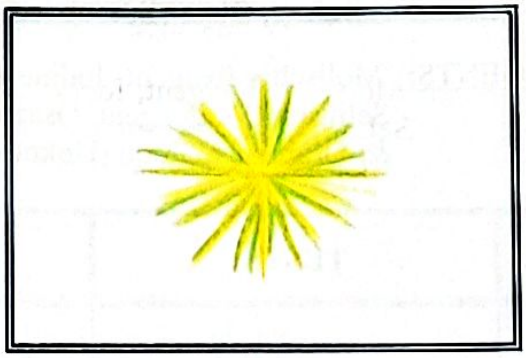
DETECTION OF STARCH IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Starch solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Red violet ring is formed at junction	Carbohydrates confirmed
2	IODINE TEST	Blue colour appear	Polysaccharide present
3	BENEDICT'S TEST	No ppt is formed	Reducing sugar is absent
4	BARFOED'S TEST	No ppt is formed	Reducing sugar is absent
5	SELIWANOFF'S TEST	No change in colour	Keto sugar is excluded
6	PERFORM THE FOLLOWING TESTS AFTER HYDROLYSIS.		
a	BENEDICT'S TEST	Red ppt is formed	Reducing sugar present.
b	BARFOED'S TEST	ppt formed before 7 minutes	monosaccharide is present.

SKETCH OF THE OSAZONE CRYSTALS



RESULT: starch confirmed (needle shaped crystals formed)

22/1/20

EXPERIMENT No. 1 Date: _____

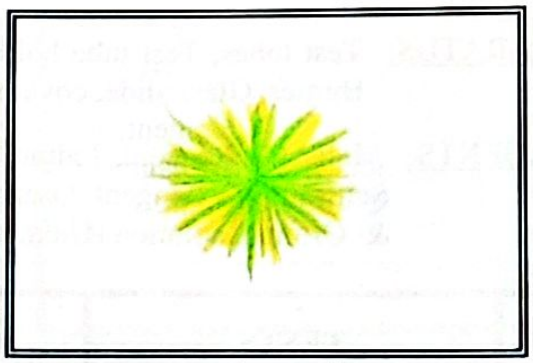
DETECTION OF UNKNOWN CARBOHYDRATE IN A GIVEN SOLUTION.

APPARATUS: Test tubes, Test tube holder, Test tube Rack, Pipettes, Beaker, Water bath, Burner, Glass slide, cover slip and Microscope.

REAGENTS: Molisch's Reagent, Iodine Reagent, Benedict's Reagent, Barfoed's Reagent, Seliwanoff's Reagent, Osazone Mixture, Conc. HCl, Conc. H_2SO_4, 5% NaOH & Original solution (Unknown Carbohydrate solution).

No.	TESTS	OBSERVATION	INFERENCE
1	MOLISCH'S TEST	Reddish violet ring	Carbohydrate pres
2	IODINE TEST	No change in colour	Monosaccharide & Disaccharide pres
3	BENEDICT'S TEST	Red ppt is formed	Reducing sugar is present.
4	BARFOED'S TEST	Red ppt is formed with in 7min	Monosaccharide is present.
5	SELIWANOFF'S TEST	Cherry red colour is present.	keto sugar is present.
6	PERFORM THE FOLLOWING TESTS AFTER HYDROLYSIS OF THE UNKNOWN SOLUTION (IF REQUIRED)		
A	BENEDICT'S TEST		
B	BARFOED'S TEST		
C	OSAZONE TEST		

SKETCH OF THE OSAZONE CRYSTALS



RESULT: fructose needle shaped crystals confirmed.

22/1/20