# Qualitative tests used for carbohydrates biology essay



Carbohydrates are an essential component of our diet and an important source of energy for us. Most of the things generally included in our diet have a large ratio of carbohydrates present. Also the patients suffering from Diabetes excrete huge amounts of sugar in their urine which needs to be estimated. The purity of carbohydrates can also be checked by methods which can qualitatively estimate particular sugars. Hence the need for carbohydrate estimation arose.

We have been estimating carbohydrates qualitatively since school time but it was just confined to observing a colour change or a coloured precipitate to detect the presence of a carbohydrate. None of us ever thought that these qualitative tests are highly sensitive and provide much more information than just detecting the presence of a sugar.

In this study conducted, two qualitative tests used for carbohydrates were studied: Benedict's test and Seliwanoff's test. Benedict's test is a test used for detecting the presence of Reducing Sugars. The test was performed using Glucose which is the most common reducing sugar. Sucrose was used as a negative control. Different concentrations of glucose were tried ranging from 1. 6 M to 1. 6 mM and from 4% to 0. 25%. Also along with concentration the amount of sugar was varied to find the sensitivity limits and the limitations of the test. The result of Benedict's test is usually a brick red precipitate but with some modifications different colours of solutions and different amounts of precipitate could be observed with slight variation in the concentration and amount of sugar. This suggests that this test being Qualitative is not only an indicator of the presence or absence of a reducing sugar but can also be used to roughly estimate the concentration of sugar present. It can be https://assignbuster.com/qualitative-tests-used-for-carbohydrates-biology-essav/

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very useful in estimation of the concentration of sugar present in the urine of diabetic patients.

Seliwanoff's test is a qualitative test used for distinguishing between Aldoses and ketoses. Ketoses form a cherry red condensation product whereas Aldoses react to form a blue-green condensation product, which may further change to a peach product. The test was performed using fructose as the sample sugar. Various concentrations of Fructose were used ranging from 4% to 0. 01% to find the sensitivity limits and the limitations of the test. The test appeared to be sensitive even at 0. 01% showing a very faint red colour. There was a huge variation in the intensity of colour obtained at different concentrations of sugar. But the drawback of the test was that the red colour of the solution was not stable. It intensified with increase in the duration of time.

#### **2. INTRODUCTION**

Carbohydrates are the most abundant bio molecules on Earth. Each year, photosynthesis converts more than 100 billion metric tons of CO2 and H2O into cellulose and other plant products. Certain carbohydrates (sugar and starch) are a dietary staple in most parts of the world, and the oxidation of carbohydrates is the central energy-yielding pathway in most nonphotosynthetic cells. Insoluble carbohydrate polymers serve as structural and protective elements in the cell walls of bacteria and plants and in the connective tissues of animals.

Other carbohydrate polymers lubricate skeletal joints and participate in recognition and adhesion between cells. More complex carbohydrate

polymers covalently attached to proteins or lipids act as signals that determine the intracellular location or metabolic fate of these hybrid molecules, called glycoconjugates.

. Carbohydrates are polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis. Many, but not all, carbohydrates have the empirical

formula (CH2O)n; some also contain nitrogen, phosphorus, or sulphur. There are three major size classes of carbohydrates: monosaccharides, oligosaccharides, and polysaccharides

## Classification

Carbohydrates can be classified on the basis of the various characteristics they possess. They can be classified on the basis of the number of carbon atoms in the sugar chain, the terminal functional group in the chain, the number of sugar subunits and the reducing activity of the sugar units. Depending on various basis they are of several types:

## A. According to the number of carbon atoms in the sugar chain:

1. Trioses: contain 3 carbon atoms (e. g. glyceraldehyde).

2. Pentoses: contain 5 carbon atoms (e.g. ribose).

3. Hexoses: contain 6 carbon atoms (e. g. glucose).

## **B.** According to the terminal functional group in the sugar chain:

1. Aldoses: contain terminal aldehyde group (-CHO) (e. g. glucose).

2. Ketoses: contain terminal ketone group (C = O) (e. g. fructose).

#### **C.** According to the number of sugar subunits:

1. Monosaccharides: Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose.

2. Oligosaccharides: Consist of short chains of monosaccharide units(2-10) joined by characteristic linkages called glycosidic bonds. The most abundant are disaccharides, with two monosaccharide units. e. g. – sucrose (cane sugar), which consists of the six-carbon sugars- D-glucose and D fructose.

3. Polysaccharides: The polysaccharides are sugar polymers containing more than 20 or so monosaccharide units, and some have hundreds or thousands of units. Some polysaccharides, such as cellulose, are linear chains; others such as glycogen, are branched. Both glycogen and cellulose consist of recurring units of D-glucose, but they differ in the type of glycosidic linkage and have different properties and biological roles.

### **D.** According to the reducing activity of the sugar unit:

Carbohydrates that can undergo oxidation are called reducing sugars. This depends on the presence of an exposed carbonyl group.

 Reducing sugars: Certain sugars with a free carbonyl carbon can be oxidized by oxidizing agents such as ferric (Fe3) or cupric (Cu2) ion. The carbonyl carbon (anomeric carbon) is oxidized to a carboxyl group. Such sugars capable of reducing ferric or cupric ion are called Reducing sugars. e.
lactose, maltose.

2. Non-reducing sugars: Sugars like sucrose contain no free anomeric carbon atom since the anomeric carbons of both monosaccharide units are involved in the formation of glycosidic bond. Therefore it is a Non-Reducing sugar.

#### 2. 1 Biochemical Tests for Carbohydrates

The biochemical tests for carbohydrates can be divided into two categories:

Qualitative Tests- These tests are performed to detect the presence of carbohydrates in a test sample. They are also used to detect the type of carbohydrate present. There are different types of qualitative tests for different types of carbohydrates.

e. g. Fehling's and Benedict's test for reducing sugars, Seliwanoff's test for ketoses, lodine's test for starch, Bial's test for detecting pentoses etc. They are not very sensitive as compared to quantitative tests and cannot estimate the exact amount of carbohydrates present but show some difference in the observation with variation in the amount of carbohydrate hence, can be used to make a rough estimate of the amount of sugar present.

Quantitative Tests- These tests are the advanced form of qualitative tests and can be used to estimate the amount of carbohydrate present in a given sample. These tests use particular chemicals which form coloured complexes with sugars which can then be read at certain wavelengths using a spectrophotometer. Their absorbance can then be used to estimate the exact amount of carbohydrate present in the sample.

e. g. Anthrone test, DNS Method, Phenol- Sulphuric Acid Method etc.

### 2.11 Qualitative Test

Flow Chart for classifying an unknown carbohydrate

A. Molisch Test

Molisch test is used to distinguish between carbohydrates and noncarbohydrates. It is the preliminary test used to detect the presence of carbohydrates in a sample.

## Principle

It uses concentrated sulphuric acid as a Dehydrating acid which dehydrates all carbohydrates to form Furfural or 5-hydroxymethylurfural from reaction of sulphuric acid with pentoses and/or hexoses. These products condense with  $\alpha$ -naphthol to yield a purple condensation product.

## **B.** Iodine and Potassium Iodide Test

This is a test used particularly to detect starch and glycogen. Starch gives a blue-black colur with potassium iodide whereas glycogen gives reddishbrown colour.

## Principle

Starch contains  $\alpha$ -amylose, a helical saccharide polymer, and amylopectin.

Iodine forms a large complex polysaccharide with the  $\alpha$ -amylose helix,

producing a blue-black colour. Simple Oligosaccharides and Monosaccharides do not form this complex with lodine. Thus, the I2/KI test can be used to distinguish starches from other carbohydrates.

### C. Bial's Test

Bial's test is used to distinguish between pentoses and hexoses.

## Principle

This test uses concentrated hydrochloric acid as the dehydrating acid and orcinol with a trace of iron(III) chloride as the condensation reagent..

Pentoses subjected to the test yield a blue or green condensation product,

while hexoses yield a muddy brown to grey condensation product.

Pentose Dehydration Product Blue or Green condensation product

(Furfural)

Hexose Dehydration Product Muddy brown-Grey condensation product

(5-hydroxymethylfurfural)

D. Seliwanoff's Test

Seliwanoff's test is used to distinguish between aldoses and ketoses.

## Principle

This test uses 3N hydrochloric acid as the dehydrating agent as resorcinol as

the condensation reagent. When mixed with Seliwanoff's Reagent,

Ketopentoses react within 2 minutes to form a cherry red condensation

product. Aldopentoses react after 2 minutes to form a blue-green condensation product, which may further change to a peach product.

Fructose Hydroxy-methyl Cherry-Red Complex

Furfural

#### **Identifying Reducing Sugars**

All mono and disaccharides with a free aldehyde or keto group act as reducing agents in alkaline solutions. The reducing properties of sugars are dependent upon the presence of actual or potential aldehyde or ketone groups.

The enolization of sugars under alkaline conditions is an important consideration in reduction tests. The ability of a sugar to reduce alkaline test reagents depends on the availability of an aldehyde or keto group for reduction reactions. A number of sugars, especially disaccharides or polysaccharides have glycosidic linkages which involve bonding between each group, and hence there is no reducing group on the sugar; such as the case for sucrose, trehalose, inulin, glycogen, starch, and dextrin. In the case of reducing sugars, the presence of alkali causes extensive enolization especially at high pH and temperature. This leads to a higher susceptibility to oxidation reactions than at neutral or acidic pH. These sugars, therefore, become potential agents capable of reducing Cu+2 to Cu+, Ag+ to Ag and so forth. Reducing sugars can react with many different oxidizing agents. Fehling's test, Benedict's test and Barfoed's test have been used to distinguish between monosaccharides and disaccharides Monosaccharides fluctuate between a ring open form and a ring closed form. The ketone (-C= O) group, for Fructose and the aldehyde group (-CHO), for Glucose in the ring open forms can be reduced using these tests. Some sugar units in disaccharides also fluctuate between a ring open form and a ring closed form. These disaccharides are also reducing sugars because the ring open form has a ketone or aldehyde to react. Sucrose is one of the few disaccharides that do not have a ring open form so it is a non-reducing sugar.

Ring Open Fructose and Ring Closed Fructose Ring Open Glucose and Ring Closed Glucose

Reducing Sugars are oxidized by copper (II) ions. Benedict's reagent and Fehling's Reagent are mildly basic solutions whereas Barfoed's Reagent, a mildly acidic solution. The presence of red copper (I) oxide precipitate indicates that the saccharide has reduced the copper (I) ions.

#### E. Fehling's test

Principle

Fehling's test uses a mixture of fehling's solution A and B. Fehling's solution A consists of copper(II) sulphate dissolved in dilute sulphuric acid. Fehling's solution B is sodium potassium tartarate dissolved in dilute NaOH. Both the solutions are mixed in equal proportions and used as fehling's reagent. This reagent is used as a general test for detecting reducing sugars. A reducing sugar reduce copper(II) ions to copper(I) oxide, forming a red precipitate. 2 Cu++ + Cu+

Glucose

(Reducing Sugar)

Cu+Cu2O (red precipitate)

Cuprous Oxide

## F. Benedict's test

## Principle

Benedict's test uses a mixture of copper(II) sulphate, sodium citrate, and sodium carbonate in a mildly basic solution. This reagent is used as a general test for detecting reducing sugars. A reducing sugar reduce copper(II) ions to copper(I) oxide, forming a red precipitate.

CuSO4Cu++ + SO4-

(Copper Sulphate) (Cupric Ion) (Sulphate Ion)

2 Cu++ + Cu+

Glucose

(Reducing Sugar)

Cu+Cu2O (red precipitate)

#### Cuprous Oxide

### G. Barfoed's Test

Benedict's test gives positive test results for all reducing sugars. However, not all reducing sugars react at the same rate. With different oxidizing agents, disaccharides are considerably less reactive compared to monosaccharides. A positive Barfoed's test result is similar to that observed with Benedict's solutions. Monosaccharides give positive Barfoed's test results within 2-3 minutes, while disaccharides do not react under the same conditions.

## Principle

Barfoed's test uses copper(II) ions in a slightly acidic medium. If the reaction time is carefully monitored, this test can be used to distinguish reducing monosaccharides from reducing disaccharides. Reducing disaccharides cause the formation of copper(I) oxide after approximately 10 minutes.

(CH3COO) 2Cu + 2H2O 2 CH3COOH + Cu(OH)2

Cu(OH)2 CuO+ H2O

2CuO +Cu2O (red precipitate)

(Glucose)

Reducing Sugar

## 2. 12 Quantitative Tests

## A. Determination of Total Carbohydrate by Anthrone Method

## Principle

Carbohydrates are first hydrolysed into simple sugars using dilute

hydrochloric acid. In hot

acidic medium glucose is dehydrated to hydroxymethyl furfural. This

compound forms with

anthrone a green coloured product with an absorption maximum at 630 nm.

HCI anthrone

Carbohydrate furfural/hydroxymethyfurfural Green product

(630 nm)

## **B.** Phenol Sulphuric Acid Method for Total Carbohydrate

## Principle

In hot acidic medium carbohydrates are dehydrated to furfural/hydroxymethyl furfural. This forms a green coloured product with phenol and has absorption maximum at 490 nm.

H2SO4 phenol

Carbohydrate furfural/hydroxymethylfurfural Green product

(490 nm)

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## C. Determination of Reducing Sugars by Nelson-Somogyi Method

The Nelson-Somogyi method is one of the classical and widely used methods

for the quantitative determination of reducing sugars.

## Principle

The reducing sugars when heated with alkaline copper tartarate reduce the copper from the

cupric to cuprous state and thus cuprous oxide is formed. When cuprous oxide is treated with

Arsenomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place. The blue colour developed is compared with a set of standards in a colorimeter at 620 nm.

Cu2O+ Arsenomolybdate Molybdenum Blue

(Cuprous Oxide) (500 nm)

## **D. Estimation of Reducing Sugar by Dinitrosalicylic Acid Method**

This method is an alternative to Nelson-Somogyi method. It is a simple, sensitive and adoptable method during handling of a large number of samples at a time. However, enzymatic methods are usually preferred to DNS due to their specificity.

## Principle

3, 5-Dinitrosalicylic acid is an aromatic compound that reacts with reducing sugars to form 3-amino-5-nitrosalicylic acid, which absorbs light strongly at 540 nm.

3, 5-Dinitrosalicylic acid 3-amino-5-nitrosalicylic acid

## E. Determination of Glucose by Glucose Oxidase Method

Glucose is a widely distributed simple sugar with an active aldehyde group. Estimation of

glucose by glucose oxidase gives the true glucose concentration eliminating the interference

by other reducing sugars.

## Principle

Glucose oxidase catalyses the oxidation of alpha-D-glucose to D-glucono-1, 5 lactone (gluconic acid) with the formation of hydrogen peroxide. The oxygen liberated from hydrogen peroxide by peroxidase reacts with the Odianisidine and oxidises it to a red chromophore product.

Glucose + O2 H2O2 + Gluconic Acid

(glucose oxidase)

H 2O2 + O-dianisidine Red-coloured product

(peroxidase) (540 nm)

## **3. MATERIALS REQUIRED**

#### **I) Stock Solutions**

Glucose 1. 6 M

Glucose 4% (w/v)

Sucrose 0.1 M

Fructose 4% (w/v)

## II) Reagents

#### **Benedict's Reagent**

Step 1-Dissolve 173 g sodium citrate and 100 g sodium carbonate

in about 500 mL water.

Step 2-Heat to dissolve the salt

Step 3-Dissolve 17. 3 g copper sulphate in about 100 mL water and add it to

the above solution with stirring and make up the volume to 1 L with water.

#### Seliwanoff's Reagent

Dissolve 0. 05 gm resorcinol in 3N hydrochloric Acid.

#### **III) Miscellaneous**

Water Bath

Test Tubes

Clamps

Conical Flasks

Beakers

Containers

Test tube holders

## 4. METHODOLOGY

### 4.1 Benedict's Test

Step 1- Pipette out 1 ml of glucose solution in some test tubes so that there is a triplicate for each set.

Step 2- Pipette out 1ml of Distilled Water in one test tube which serves as blank and 1 ml of Sucrose solution (0. 1M) in another which serves as a negative control.

Step 3- Add 2 ml of Benedict's reagent in all the test tubes.

Step 4-Transfer all five tubes to the boiling water bath provided and record the time for the appearance of precipitate.

Step5- Make a subjective analysis of the colours observed. A scale of '+' to '+++++' can be used to record the depth of the colour, from lightest to darkest.

## 4. 11 Effect of variation in Carbohydrate Concentration (in M)

The concentration of glucose was varied from 1. 6 M to 1. 6 mM keeping the

reaction volume constant to 3 ml. The upper and the lower sensitivity limits

and the optimum molar concentration of glucose was found for the test through subjective analysis.

#### 4. 12 Effect of Reaction Volume

The reaction volume was reduced from 3 ml to 1. 5 ml for all the concentrations of glucose ranging from 1. 6 M to 1. 6 mM to find any difference in the sensitivity range and the optimum molar concentration of glucose for the test.

## 4. 13 Effect of variation in Carbohydrate Concentration (in %)

The concentration of glucose was varied from 4% to 0. 25%. Also the reaction volume was changed and the analysis was performed in two sets. The volume of reagent used was 5 ml whereas the volume of samples all concentrations in Set A and B were 50µl and 25µl respectively.

### 4. 14 Effect of variation in Sample Volume

The volume of glucose was varied for the same concentrations 4% to 0. 25% to see the changes in the colour of solution obtained and at the same time find the upper and lower limits at which the test still remains sensitive. The analysis was performed in 6 sets with the following volumes of glucose: 200µl, 100µl, 50µl, 25µl, 12. 5µl and 6. 25µl.

#### 4. 15 Effect of Reaction Volume on the results obtained.

Of the various sets tried 5 ml of Benedict's reagent and 200µl of sample gave the best results. To verify the consistency of results obtained the reaction volume was reduced. The analysis was performed in two sets. Set A with 2.5 ml of reagent and 100 $\mu$ l of sample and Set B with 1.25 ml of reagent and 50 $\mu$ l of sugar sample.

#### 4. 2 Seliwanoff's Test

Step 1- Pipette out 1 ml of fructose solution in test tubes so that there is a triplicate for each set.

Step 2-Pipette out 1ml of Distilled Water in one test tube which serves as blank.

Step 3- Add 2 ml of Seliwanoff's reagent in all the test tubes.

Step 4-Transfer all tubes to the boiling water bath for 2. 5 mins.

Step 5- Make a subjective analysis of the colours observed. A scale of '+' to

'+++++' can be used to record the depth of the colour, from lightest to darkest.

## 4. 21 Effect of variation in carbohydrate concentration (in %)

The concentration of fructose was varied from 4% to 0. 015% keeping the reaction volume constant to 3 ml. The upper and the lower sensitivity limits and the optimum concentration of fructose were found for the test through subjective analysis.

## **5. OBSERVATIONS**

### 5.1 Benedict's Test

## **5. 11 Effect of variation in Carbohydrate Concentration (in M)**

A brick red precipitate was observed for all the concentrations of sample ranging from 1. 6 M to 3. 125 mM. But the last concentration of 1. 6 mM showed a negligible amount of precipitate. Also once centrifuged the supernatant was found to be colourless for concentrations ranging from 1. 6 M to 0. 1 M. The supernatant was observed to be blue in concentrations ranging from 50 mm to 1. 6 mM.

Glucose

(M)

Colour Intensity

- 1.6
- 0.8
- 0.4
- 0.2
- 0.1
- 0.05
- 0. 02

<i>e</i>	5	
0. 012		
0. 006		
0. 003		
0.001		
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## 5. 12 Effect of variation in Reaction Volume

A brick red prcipitate was observed for all the concentrations of sample ranging from 1. 6 M to 6. 25 mM. Concentrations 3. 125 mM and 1. 5625 mM showed a negligible amount of precipitate. For concentrations 6. 25 mM to 0. 1 M there was an increase in the amount of precipitate observed with the highest amount formed in 0. 1 M of sample. For the next two concentrations i. e. 0. 2 M and 0. 4 M the amount of precipitate formed was almost equal but again reduced for 0. 8 M and 1. 6 M glucose sample.

Also after centrifugation the supernatant was found to be colourless for concentrations ranging from 1. 6 M to 0. 1 M. The supernatant was observed to be increasingly blue in concentrations ranging from 50 mm to 1. 5625 mM.

Glucose

(M)

Colour Intensity

- 1.6 0.8
- 0.4
- 0.2
- 0.1
- 0. 05
- 0.02
- 0. 012
- 0.006

0. 003

0.001

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## 5. 13 Effect of variation in carbohydrate concentrations (in %)

There was a brick red precipitate observed in for all the concentrations of sample ranging from 0. 25% to 4% in both the sets. The highest amount of precipitate was observed for 1% sample but there was no consistency in results seen.

Glucose (%)

Set

#### Set B

- 4.0
- 2.0
- 1.5
- 1.0
- 0.5
- 0.25

## 5. 14 Effect of variation in Sample Volume

There was a variation in the colours obtained at different concentrations and volumes of sugar sample used. But sets with 12. 5 $\mu$ l and 6. 25 $\mu$ l of sugar did not show any noticeable changes in colour.

Glucose

(%) Set A Set B Set C Set D Set E Set F

Set B

Set C

Set D		
Set E		
Set F		
4. 00		
2.00		
1.00		
0. 50		
0. 25		
reddish brown		
brown		
green		
greenish blue		
dull blue		
brown		
green		
greenish blue		
dull blue		

#### green

greenish blue

dull blue

blue

blue

greenish blue

dull blue

blue

blue

blue

dull blue

## 5. 15 Effect of reduction in the reaction volume

There was no change in the observations due to reduction in the reaction volume.

Glucose (%)

Set A Set B

- 4.0
- 2.0
- 1.0
- 0.5
- 0.25

reddish brown

brown

green

greenish blue

#### dull blue

reddish brown

brown

green

greenish blue

dull blue

#### 5. 2 Seliwanoff's Test

## 5. 21 Effect of variation in carbohydrate concentration (in %)

A cherry red colour is observed for all the concentrations of fructose used but the intensity of colour obtained decreases with decrease in concentration. The lowest concentration used (0. 015%) has a faint red colour. Also the colour of the solution intensifies with time if kept after boiling.

Fructose

(%)

**Colour Intensity** 

4.00

- 2.00
- 1.00
- 0.50

0.25 0.12 0.06 0.03 0.01 ++++++ +++++ ++++++++++

## 6. RESULTS AND DISCUSSION

## 6. 1 Benedict's Test

## 6. 11 Effect of variation in Carbohydrate Concentration (in M)

It can be observed that 0. 1 M is the optimum concentration of sugar for

Benedict's reaction with the reaction volume of 3 ml since the highest

amount of precipitate is formed at 0. 1 M.

Also the supernatant obtained after centrifugation is colourless which suggests that the reaction is completed and there is no unused reagent left.

Concentrations less than 0. 1 M show decreasing amounts of precipitate and the colour of the supernatant is also increasingly blue. This suggests that as the concentration of sugar is lowered the amount of unreacted Benedict's reagent increases which leaves the solution blue even after the reaction completes. 1. 6 mM sugar sample shows a negligible amount of precipitate formation which suggests that the reaction is not sensitive for concentrations lower than 1. 6 mM.

For concentrations higher than 0. 1 M the amount of precipitate formed again decreases with increase in concentration which suggests that the concentration is too high as compared to the amount of reagent used and hence no more precipitate is formed after the reaction completes.

#### 6. 12 Effect of variation in Reaction Volume

The reaction volume was reduced to half but has no effect on the results of the experiment. 0. 1 M is the optimum concentration of sugar for the reaction with the reaction volume of 3 ml since the highest amount of precipitate is formed at 0. 1 M.

Also the supernatant obtained after centrifugation is colourless which suggests that the reaction is complete and there is no unused reagent left.

Concentrations less than 0. 1 M show decreasing amounts of precipitate and the colour of the supernatant is also increasingly blue. This suggests that as the concentration of sugar is lowered, the amount of unreacted reagent increases which leaves the solution blue even after the reaction completes. 3mM and 1. 6 mM sugar samples show a negligible amount of precipitate formation which suggests that the reaction is not sensitive for concentrations lower than 3 mM with a reaction volume of 1. 5 ml.

For concentrations higher than 0. 1 M, the amount of precipitate formed again decreases with increase in concentration which suggests that the concentration is too high as compared to the amount of reagent used and hence no more precipitate is formed after the reaction completes.

## 6. 13 Effect of variation in carbohydrate concentrations (in %)

The highest amount of precipitate was formed in 1% sugar sample but there was no consistency in the observations due to incorrect preparation of reagent. During the preparation of reagent instead of mixing Sodium citrate and Copper Sulphate together in hot water they were dissolved separately and then mixed. Also the solution was not made up to 500 ml with distilled water rather measured amount of water was added to the solution to make it 500 ml which made the solution dilute and hence gave incorrect results.

### 6. 14 Effect of variation in Sample Volume

Different volumes of sugar were tried for all the different concentrations. There was a variation in the colours obtained. 200µl of sugar reacted with 5 ml of reagent showed maximum variation in colours at different concentrations: Reddish Brown, Brown, Green, Greenish blue and dull blue obtained at 4%, 2%, 1%, 0. 5% and 0. 25% respectively. Sets with 12. 5µl and 6. 25µl of sugar volume did not show any noticeable changes in colour which suggest that the volume of sugar solution was not enough for the reaction to take place.

### 6. 15 Effect of reduction in the reaction volume

Reduction in the reaction volume made no difference to the variation in colours obtained at different concentrations of sugar which suggests that Benedict's reagent and glucose can be reacted in this ratio in any volume to estimate the concentration of glucose present in an unknown sample.

### 6. 2 Seliwanoff's Test

## 6. 21 Effect of variation in carbohydrate concentration (in %)

A cherry red colour is observed for all the concentrations of Fructose used but the intensity of colour obtained decreases with decrease in concentration. The highest intensity is observed at 4% sugar concentration. 0. 015% which is the lowest concentration of sugar used gives a very faint red colour which cannot be taken as pos