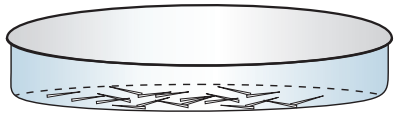
UNIT-11

# TESTS FOR CARBOHYDRATES FATS AND PROTEINS



EXPERIMENT 11.1

## Aim

To study the characteristics of carbohydrates, fats and proteins in pure form and detection of their presence in the given foodstuffs.

## I. TEST FOR CARBOHYDRATES, FATS AND PROTEINS IN PURE FORM

### A. Tests for Carbohydrates

#### Theory

Carbohydrates are optically active polyhydroxy aldehydes, polyhydroxy ketones or compounds, which give these units as hydrolysis product. Starch, cellulose and sugars are the familiar examples of carbohydrates. Carbohydrates are classified on the basis of number of polyhydroxy aldehyde or ketone units obtained from them on hydrolysis. Three broad classes are as follows :

(i) *Monosaccharides :* These cannot be hydrolysed further to polyhydroxy aldehydes or ketones.

(ii) *Oligosaccharides :* These yield 2-10 monosaccharide units on hydrolysis. Common amongst these are disaccharides, which produce two monosaccharide units.

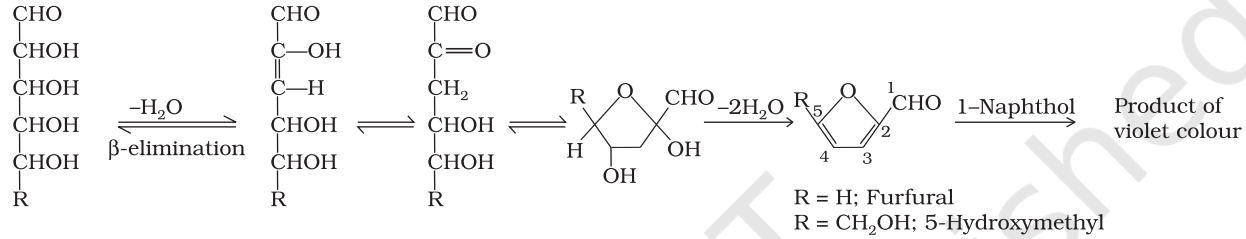
(iii) *Polysaccharides :* These yield large number of monosaccharide units on hydrolysis.

Monosaccharides are further classified on the basis of number of carbon atoms and functional group present in them. If a monosaccharide contains aldehydic group it is called **aldose**. If it contains keto group it is called **ketose**. **Carbohydrates of all classes give Molisch's test**. Carbohydrates, which are sweet in taste, are called sugars. Glucose, fructose (fruit sugar) and sucrose (table sugar) are examples of sugars. Sugars are classified into two major categories: reducing sugars and non-reducing sugars. Reducing property of sugars is detected by the three tests namely Fehling's test, Benedict's test and Tollen's test.

Prepare 1% stock solution of glucose, fructose and sucrose in separate beakers and divide each of the solutions into test tubes marked A, B, C and D etc. and perform the following tests.

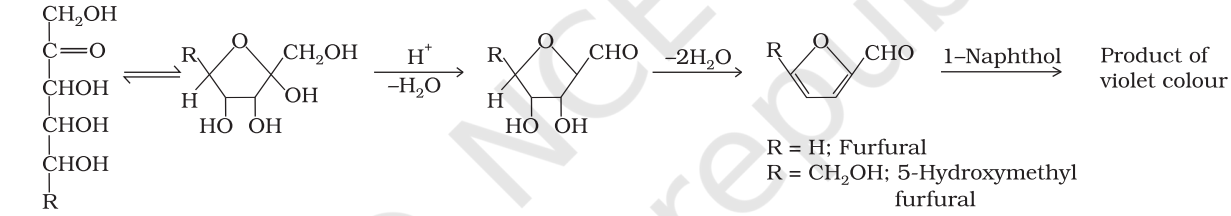
## I. Theory of Molisch's test

On adding concentrated sulphuric acid to the aqueous solution of carbohydrate containing alcoholic solution of 1-naphthol, a deep violet colour appears at the junction of the two liquids. Concentrated sulphuric acid hydrolyses glycosidic bonds of carbohydrate to give monosaccharides which are dehydrated to an aldehyde known as furfural which undergoes reaction with 1–naphthol to give a unstable condensation product of deep violet colour. This test may be given by some other organic compounds also. The following reaction takes place :




R = H; Aldopentose
R = $CH_2OH$; Aldohexose

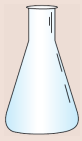
R = H; Furfural
R = $CH_2OH$; 5-Hydroxymethyl furfural




R = H; Ketopentose
R = $CH_2OH$; Ketohexose

R = H; Furfural
R = $CH_2OH$; 5-Hydroxymethyl furfural

## Material Required



- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One

- Glucose, fructose, sugar (sucrose) : As per need
- Alcoholic solution of 1-Naphthol : As per need
- Concentrated $H_2SO_4$ : As per need

## Procedure

Add 2-3 drops of alcoholic solution of 1% 1-naphthol in test tube 'A' and then pour 2 mL conc. $H_2SO_4$ down the sides of the test tube so that it forms a separate layer at the bottom of the test tube. The formation of a purple ring at the interface of the two layers confirms the presence of carbohydrates.

## II. Theory of test for reducing sugars

### A. Fehling's test and Benedict test

Black copper (II) oxide is formed on heating a suspension of copper hydroxide in alkaline solution.

*Alcohol* 

*$H_2SO_4$* 

**Hazard Warning**

- *In high concentration 1-Naphthol is extremely destructive to all body tissues.*

$$Cu(OH)_2 \longrightarrow \underset{\text{Copper (II) oxide (black)}}{CuO} + H_2O$$

If some reducing agent is present in the reaction medium, then orange-red copper (I) oxide is precipated.

$$2Cu(OH)_2 \longrightarrow \underset{\text{Copper (I) oxide (Orange-red)}}{Cu_2O} + 2H_2O + [O]$$

Reducing sugars contain aldehydic group or α-hydroxy ketonic group, therefore in alkaline medium reduce $Cu^{2+}$ ions. But if the reaction is carried out directly in the presence of an alkali then, copper (II) hydroxide gets precipitated. To overcome this problem, copper (II) ions are complexed with tartarate ions (Fehling's reagent) or citrate ions (Benedict's solution). Both the complex ions are soluble in alkaline medium and yield $Cu^{2+}$ ions in such a low concentration that solubility product of cupric hydroxide is not reached.

Reducing sugars react with Fehling's reagent according to the following reaction:

$$RCHO + 2\,Cu^{2+} + 5OH^- \longrightarrow RCOO^- + \underset{\text{(orange-red precipitate)}}{Cu_2O} + 3H_2O$$

The discharge of blue colour due to $Cu^{2+}$ ions and appearance of orange-red precipitate of $Cu_2O$, indicates the reducing property of sugars.

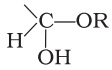
$$\text{D-Glucose} + Cu^{2+}\text{ (citrate)} \xrightarrow{Na_2CO_3(aq)} \text{D-Gluconic acid} + \underset{\text{(orange-red precipitate)}}{Cu_2O}$$

$$\text{D-Maltose} + Cu^{2+}\text{(tartarate)} \xrightarrow{NaOH\ (aq)} \text{D-Maltobionic acid} + \underset{\text{(orange-red precipitate)}}{Cu_2O}$$

$$\text{Sucrose (disaccharide)} \xrightarrow[\text{solution}]{\text{Fehling's}} \text{No reaction}$$

Sometimes, the cuprous precipitate comes down as yellow cuprous hydroxide, but on warming this is converted to orange-red copper (I) oxide.

In some cases, this reaction may be used as a quantitative analytical process for the determination of reducing sugars in blood and urine etc.

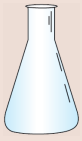
All monosaccharides are reducing sugars. Disaccharides which contain a free hemi-acetal group (  ) are also mild reducing sugars. Most naturally occurring disaccharides are reducing sugars (sucrose is an exception).

### B. Tollen's test

Tollen's reagent is ammoniacal solution of silver nitrate. A reducing sugar, reduces silver ion to metallic silver which gets deposited on the inner surface of the test tube in the form of silver mirror. The reaction occurs as follows:

$$RCHO + 2\,[Ag(NH_3)_2]^+ + 2OH^- \longrightarrow 2Ag + RCOONH_4 + H_2O + 3NH_3$$

## Material Required



- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One
- Water bath : One
- Bunsen burner : One

- Fehling's solutions A and B : As per need
- Benedict's reagent : As per need
- Tollen's reagent : As per need

## Procedure

### A. Fehling's test

Mix 1 mL each of Fehling's solutions A and B in a test tube and add the mixture to test tube B. Heat the content of the test tube on a water bath. The formation of a orange-red precipitate indicates the presence of reducing sugar.

### B. Benedict's test

Add 1 mL of Benedict's reagent to test tube C and heat the mixture to boiling in a water bath for 2 minutes. The formation of a orange-red precipitate due to the formation of copper (I) oxide indicates the presence of reducing sugar.

*Resorcinol* 

*Furfural* 

### C. Tollen's test

Prepare Tollen's reagent by adding sodium hydroxide solution dropwise to 1 mL aqueous silver nitrate solution to get the precipitate of silver oxide. Now add ammonium hydroxide solution while shaking the mixture so that initially formed silver oxide precipitate dissolves. Add the reagent to the sugar solution contained in a test tube and warm the reaction mixture on a water bath. Formation of silver mirror on the walls of the test tube shows the presence of reducing sugar.

### Caution!

*Never heat the test tube on direct flame as it may* ***cause explosion****.*

## III. Theory of test to distinguish Monosaccharide from Disaccharide

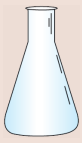
### Barfoed's test

The reagent is cupric acetate in acetic acid solution. It is weakly acidic and is reduced by only monosaccharides. Prolonged boiling may hydrolyse disaccharides and false positive test may be obtained. Monosaccharides react with this reagent within 5 minutes to give a brick red precipitate of copper (I) oxide. Disaccharides take a longer time to react because aldehyde function is masked in the acetal linkage.

$$\underset{\text{Monosaccharide}}{RCHO} + 2Cu^{2+} \longrightarrow RCOOH + \underset{\text{Brick-red}}{Cu_2O}$$

The precipitate of cuprous oxide obtained is less dense and its colour is brick-red instead of orange-red.

## Material Required



- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One
- Water bath : One
- Bunsen burner : One

- Glucose, fructose, sugar (sucrose) : As per need
- Barfoed's reagent : As per need

## Procedure

Take 10 drops of 1% sugar solution in a test tube and add 1 mL Barfoed's reagent. Heat the the content of the test tube in a water bath to boiling for five minutes. The formation of orange-

red precipitate indicates positive test for monosaccharides. Disaccharides do not give this test.

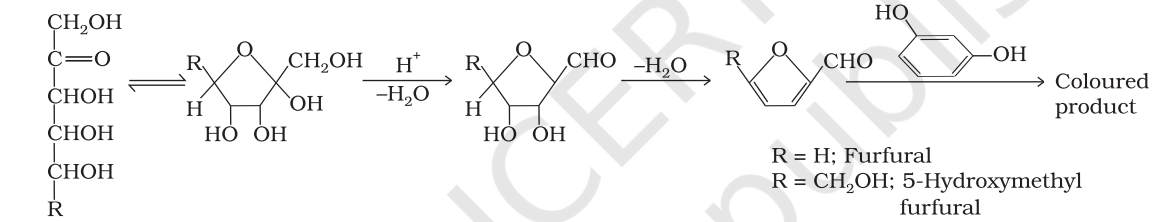
### Test for Sucrose

Hydrolyse the sucrose for performing the test by adding 5 drops of concentrated HCl to 5 mL of 1% sucrose solution and heating the mixture in a boiling water bath. Cool the mixture and add NaOH solution to obtain neutral or slightly alkaline solution. Perform the tests for reducing sugar and Seliwanoff's test given below with the hydrolysed product and record your results.

## IV. Test to distinguish Ketose from Aldose

### Seliwanoff's test

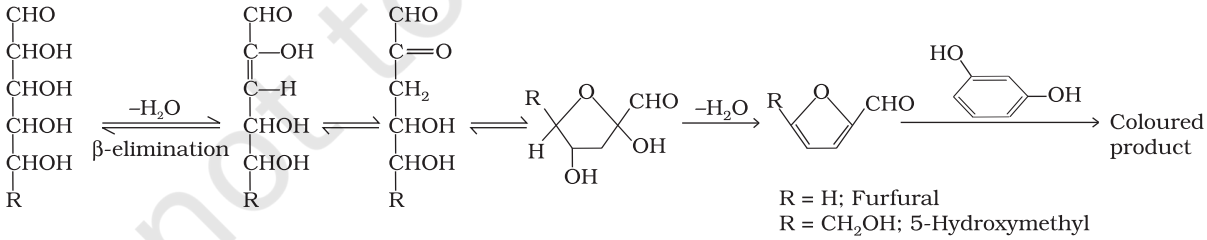
Ketoses dehydrate very rapidly under acidic conditions to give furfural, which reacts with resorcinol (1,3-dihydroxy benzene) to give a coloured product.




R = H; Ketopentose
R = $CH_2OH$; Ketohexose

R = H; Furfural
R = $CH_2OH$; 5-Hydroxymethyl furfural

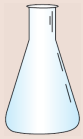
Ketohexoses give red colour and ketopentoses give blue-green colour. Aldoses take longer time to produce colour because under the same conditions, aldoses form furfural slowly, probably because β-elimination is required before dehydration to furfural. Therefore prolonged heating should be avoided.




R = H; Aldopentose
R = $CH_2OH$; Aldohexose

R = H; Furfural
R = $CH_2OH$; 5-Hydroxymethyl furfural

## Material Required



- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One
- Water bath : One
- Bunsen burner : One

- Glucose, fructose, sugar (sucrose) : As per need
- Seliwanoff's reagent : As per need

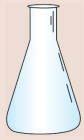
### Procedure

Add 2 mL of Seliwanoff's reagent to 10 drops of 1% sugar solution taken in a test tube. Heat the test test tube in boiling water for two minutes. Ketohexoses give red colour. Ketopentose gives blue-green colour. Aldoses do not give colour within two minutes.

### V. Theory of test for Polysaccharides (Starch)

Starch gives blue colour with iodine solution due to the formation of a complex known as starch iodide complex. Starch is present in wheat, rice, maize, potatoes etc.

## Material Required



- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One
- Water bath : One
- Bunsen burner : One

- Starch solution : As per need
- Iodine solution : As per need

### Procedure

**Iodine test**

*Iodine* 

Make a suspension of (0.5 g) starch in 5 mL water and pour it in 50 mL boiling water to get an aqueous colloidal solution. To this add a few drops of aqueous iodine solution. The appearance of blue colour indicates the presence of starch.

## B. Test for Oils and Fats

### Theory

These are the esters of glycerol and long chain fatty acids and are known as triglycerides. Triglycerides which are liquids at room

temperature are oils and those that are solids are called fats. Oils are of plant origin and fats are of animal origin. Triglycerides in which three acyl groups are same are called simple triglycerides and a triglyceride in which three acyl groups are different is called mixed triglyceride. Many naturally occurring fatty acids contain two or three double bonds. The fats from which these come are called polyunsaturated fats or oils. while oils are glycerides of unsaturated fatty acids. Fats and oils are insoluble in water.

On heating with potassium hydrogen sulphate, oils and fats give characteristic odour of acrolein. This is the test for glycerol present either free or combined as an ester. On heating with potassium hydrogen sulphate, glycerol is dehydrated and acrolein is formed which has a pungent odour. The reaction is as follows :

$$\begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \xrightarrow[KHSO_4]{\text{Heat}} \begin{array}{l} CH_2 \\ \| \\ CH \\ | \\ CHO \end{array} + H_2O$$

## Material Required

- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One
- Water bath : One
- Bunsen burner : One

- Mustard oil/ghee : As per need
- Potassium hydrogen-sulphate : As per need

## Procedure

Add a few crystals (0.5g) of dry potassium hydrogen sulphate to 3 mL of mustard oil/ghee taken in a test tube and heat the content of the test tube gently. A pungent smell confirms the presence of an oil or a fat.

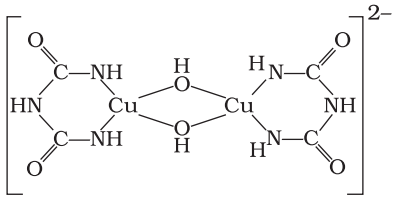
## C. Tests for Proteins

### Theory

Proteins are complex organic compounds containing nitrogen and are made up of amino acids. Proteins are present in egg albumin, soya beans, pulses, fish, milk etc. Their presence can be confirmed by several tests. Due to the presence of characteristic side chains in them, certain amino acids exhibit typical colour reactions that form the basis for their identification. Proteins also respond to the colour reactions of amino acids, but can be distinguished from amino acids by biuret reaction, and coagulation reaction.

## I. Biuret test for peptide bonds

Alkaline copper sulphate reacts with compounds containing two or more peptide bonds to form complexes of violet colour.

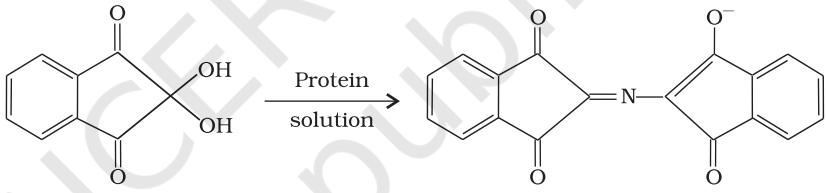


and $[Cu(NHCONHCONH)_2]^{2-}$

The name of the test comes from the name of the compound, biuret, which gives this test. The reaction is not absolutely specific for peptide bond because many compounds containing two carbonyl groups linked through nitrogen or carbon atoms give a positive result.

## II. Ninhydrin reaction

Ninhydrin is a powerful oxidizing agent and reacts with proteins to give a blue-violet compound called Rhumann's purple.




Ninhydrin — Rhumann's purple (blue violet)

***Note :*** *Ammonia, primary amines, amoniacids and peptides also react with ninhydrin.*

## III. Xanthoproteic reaction

Aromatic groups of either the free aminoacid or protein, undergo nitration on heating with concentrated nitric acid. The salts of these derivatives are orange in colour.

## Material Required

- Test tubes : As per need
- Test tube stand : One
- Test tube holder : One
- Beaker (100 mL) : One
- Water bath : One
- Bunsen burner : One

- Egg albumin/casein
- Ninhydrin
- Copper reagent
- Concentrated $HNO_3$
- Alcohol

: As per need

## Procedure

### A. Biuret test

Prepare 0.5% (w/*V*) solution of casein or egg albumin in 0.1 M NaOH solution. Take 2-3 mL of the solution and add about 2 mL of 10% sodium hydroxide solution to it. Add a few drops of copper reagent and warm the mixture for about 5 minutes. Appearance of violet colour due to the formation of a complex species of $Cu^{2+}$ ions with - CONH – group confirms the presence of protein in the sample.


### B. Ninhydrin reaction

Take 2-3 mL of an aqueous solution of egg albumin in a test tube. Add 3-4 drops of ninhydrin solution to it and heat. Appearance of blue colour indicates the presence of protein.

### C. Xanthoproteic test

Take 1 mL of an aqueous solution of egg albumin in a test tube and add a few drops of concentrated nitric acid. Heat the reaction mixture for a few minutes on a Bunsen flame. A yellow colour appears. Cool the test tube under running tap and add a few drops of 10M sodium hydroxide solution, an orange colour appears.

## II. Test for Carbohydrates, Fats and Proteins in Foodstuffs

(i) Take the samples of milk, wheat flour, rice and gram flour powder of legumes to test for the presence of carbohydrates, fats and proteins.

(ii) Take 0.5 mL sample of milk to carry out each of the tests.

(iii) For wheat flour, rice flour, gram flour and legume powder, add 100 mg of the sample in 10 mL of distilled water and boil the suspensions, to get a colloidal solution. Perform the tests with this collidal solution and record the results in Table 11.1.

**Table 11.1 : Test for carbohydrates, fats and proteins in the different samples of food materials**

| Sample | Carbohydrates Present/Absent | Fats Present/Absent | Protein Present/Absent |
|---|---|---|---|
| Milk | | | |
| Wheat flour | | | |
| Rice flour | | | |
| Gram flour | | | |
| Legumes | | | |

From the experiments, it will be observed that food materials like wheat flour, gram flour and legumes contain carbohydrates and proteins. The rice flour contains carbohydrates, while milk contains fats and proteins. Similarly, other food materials may be tested for the presence of carbohydrates, fats and proteins.

## Precautions

(a) Shake the mixture thoroughly while preparing the extract of gram, wheat and rice flour.
(b) Always use fresh reagents to carry out the tests.
(c) Use only required quantities of reagents.

## Discussion Questions

(i) How will you distinguish between sucrose and glucose?

(ii) Explain why does fructose reduce Fehling's solution and Tollen's reagent inspite of the presence of ketonic group?

(iii) What is the role of tartarate and citrate ions in Fehling's reagent and Benedict's reagent respectively?