

# EXPERIMENT 11

**Study of simple reactions of carbohydrates, fats and proteins and detection of their presence in given foodstuffs.**

---

## 11.1 OBJECTIVES

After performing this experiment, you should be able to :

- *test the presence of carbohydrates, proteins and fats in the given food samples;*
- *establish whether the given carbohydrate is reducing or non reducing.*
- *distinguish between saturated and unsaturated fats.*

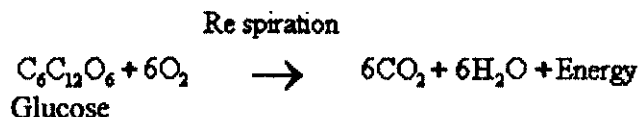
## 11.2 WHAT YOU SHOULD KNOW

### 11.2.1 Carbohydrates

Carbohydrates are polyhydroxy aldehydes or ketones and the substances which yield these on hydrolysis. Carbohydrates are classified as :

- i) Monosaccharides - glucose, fructose - ( $C_6H_{12}O_6$ )
- ii) Disaccharides - sucrose, maltose - ( $C_{12}H_{22}O_{11}$ )
- iii) Polysaccharides - starch, cellulose, glycogen.

All the carbohydrates, except cellulose, are hydrolysed in our body to glucose which on slow oxidation, in presence of oxygen, liberates large amount of energy. The oxidation of glucose can be represented as :



Thus carbohydrates (sugars and starch) are the main source of energy in our body. The cellulose acts as a roughage. The carbohydrates which reduce Fehling's solution and Tollen's reagent are called

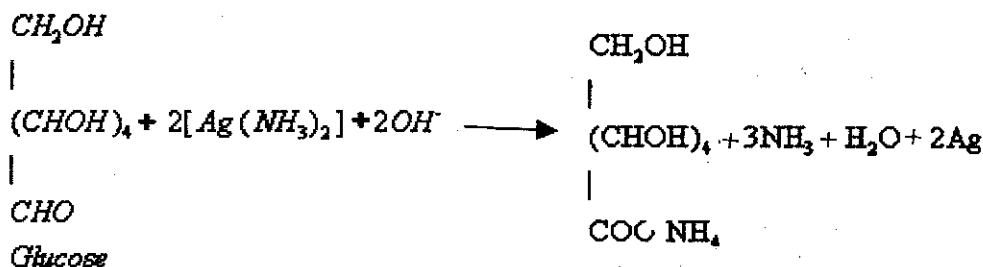
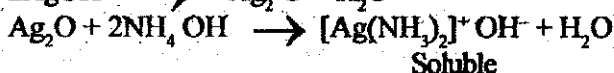
reducing sugars. All the Monosaccharides and most of the disaccharides are reducing sugars. Sucrose (cane-sugar) is a non-reducing sugar and hence does not reduce Fehling's solution.

### Test for Carbohydrates

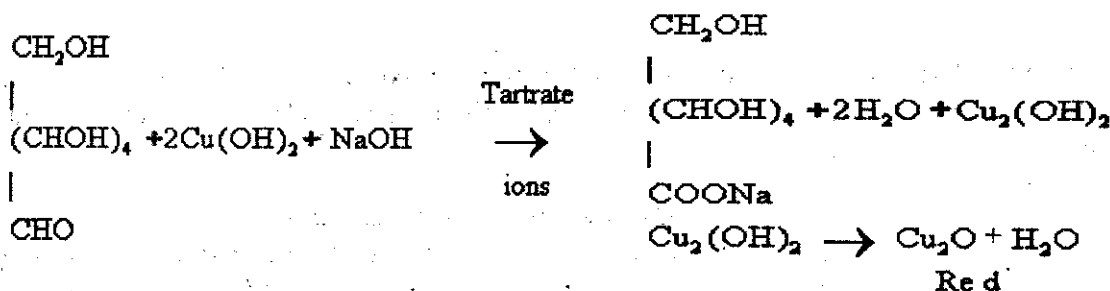
1. **Molisch's Test** (for all carbohydrates) : When an aqueous solution or suspension of a sample containing a carbohydrate is treated with - naphthol (Molisch's reagent), followed by the addition of few drops of conc. sulphuric acid along the sides of the test tube a red-violet ring appears at the junction of two layers. This happens because concentrated sulphuric acid converts carbohydrates to furfural or its derivatives which reacts with -naphthol to give a coloured (red-violet) product.

2. **Tollen's Test** (for reducing sugars) : Any aldose or ketose having a free carbonyl groups reduces Tollen's reagent to metallic silver. This test is commonly known as silver mirror test as a kind of mirror is obtained on the walls of the test tube.

The reactions involved in this test are as follows



3. **Fehling's Test** (for reducing sugars): When equal quantities of Fehling's solution A and B are mixed with an aqueous solution of a reducing sugar and the mixture is heated on a boiling water bath, a reddish precipitate of cuprous oxide is formed. The following reactions are involved in the test.



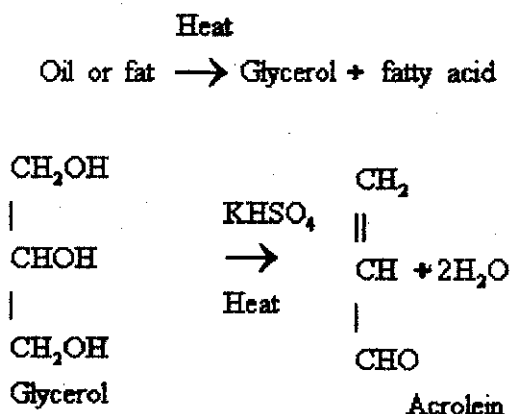
4. **Iodine Test for starch** : Starch gives a purple-blue colour when treated with iodine. It is due to the adsorption of iodine on the surface of starch. The colour gets discharged on heating and reappears on cooling.

### 11.2.2 Fats :

Fats are triesters of long chain fatty acids and glycerol. One of the function of fats in the body is to provide energy. Fats provide twice as much energy as that provided by the same amount of carbohydrates. Fats are insoluble in water. During the process of digest, fats are hydrolysed into simple substances, like fatty acids and glycerol which further break down into still simpler substances. Sources of fats in our food are butter, ghee, cheese, milk, egg-yolk, meat, nuts, groundnut and soyabean etc.

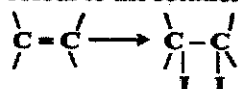
#### Tests for Fats

1. **Spot Test** : When a sample containing fats is pressed or rubbed on a clean white sheet of paper, a greasy spot (translucent spot) appears.
2. **Solubility Test** : This test is based on the fact that fats are soluble in organic solvents like chloroform, alcohol etc., but are insoluble in water. A small amount of the sample is mixed with a few drops of water, chloroform and alcohol in different test tubes and inferences are drawn on the basis of the miscibility and immiscibility of the sample with the water or organic solvents.
3. **Acrolein Test** : When a sample containing fat is heated with solid potassium bisulphate ( $\text{KHSO}_4$ ) in a test tube, a pungent irritating odour of acrolein is produced according to the following chemical reaction;



4. **Huble's Test (for determination of unsaturation in fats)** : This test is based on the fact that greater the amount of unsaturation in a fat or an oil, greater is the amount of Huble's solution required to produce a permanent violet colour.

For this equal amount of saturated fat (cotton seed oil) and unsaturated fat or oil (linseed oil) are taken in two different test tubes and Huble's solution is added to each test tube dropwise. The number of drops of Huble's solution required for getting a permanent colour are counted in each case. More the number of drops of Huble's solution, greater is the unsaturation in an organic compound. Iodine from Huble's solution adds up across the unsaturation sites of oil or fat giving a colourless product and a little more of iodine gives a permanent violet colour to the solution.



### 11.2.3 Proteins

Proteins are complex nitrogenous organic compounds of high molecular mass. These help in the growth and maintenance of living body and in transmission of genetic information. Proteins are made up of amino acids as the basic building blocks. In all, there are 20 different amino acids required by the body. Some of these are called essential amino acids as they cannot be synthesized by the organisms. These must form a part of our food.

#### Tests for Proteins

1. **Biuret Test** : When a sample of protein is treated with a solution of copper sulphate in alkaline medium, a violet colour appears. The intensity of the colour also indicates the relative amount of protein present in different samples.
2. **Ninhydrin Test** : When a few drops of a 0.1% alcoholic solution of ninhydrin, are added to a very dilute solution / suspension of protein and the contents are boiled for 1-2 minute, a red-purple colour appears.
3. **Xanthoprotein Test** : When an aqueous solution of protein is treated with a few drops of nitric acid and contents are allowed to stand; a deep yellow colour appears.
4. **Million's Test** : A few drops of milk are warmed with mercuric nitrate in presence of dil. sulphuric acid and cooled. One drop of sodium nitrate solution is added and the mixture is again warmed. A red colour appears showing the presence of proteins in milk.

### 11.3 MATERIAL REQUIRED

(1) Apparatus	(2) Chemicals
Test tubes with stand, Burner	Fehling's solutions - A and B, Molisch reagent ( - Naphthol, Silver nitrate, Ammonium hydroxide, Iodine solution, Ethyl alcohol, Hubles's solution, Potassium bisulphate, dilute sulphuric acid, Mercuric nitrate, Sodium nitrate, Nitric acid, Ninhydrin solution (0.1% in alcohol), Sodium hydroxide, Copper sulphate, and some food samples like : milk, sucrose, glucose, wheat flour, vegetable oil, ghee, butter, cheese potato pieces, lemon, grapes, banana, eggs etc. 5% solution of egg white protein.

## 11.4 HOW TO PERFORM THE EXPERIMENT

### 11.4.1 Worksheet No. 1 : Test for Carbohydrates

Experiment	Observation	Inferences
<b>For Carbohydrates</b> <b>(i) Molisch's test :</b> Take 2-3 mL of aqueous solution or suspension of the sample. Add a few drops of Molisch's reagent and shake it. Then add 5-6 drops of conc. $H_2SO_4$ slowly along the side of the test tube.		
<b>ii) Tollen's test:</b> Take a test tube and clean it thoroughly with NaOH solution. Then wash the tube with excess of water to remove NaOH. Take 2-3 mL of aqueous solution of carbohydrate in the test tube and add 2-3 mL of freshly prepared Tollen's reagent to it. Keep the test tube in a boiling water bath for about 10 minutes.		
<b>iii) Fehling's Test:</b> Take a small amount of crushed food items (1g) or 2-3 mL of aqueous solution of the sample (nearly 5%) . Add 2 mL each of Fehling's solution A and Fehling's solution B. Keep the test tube in a boiling water bath.		
<b>iv) Iodine Test for Starch :</b> Take a small amount (1-2g) of the food sample in a test tube and add a few drops of dilute iodine solution.		

## 11.4.2 Worksheet = 2: Test for Fats

Experiment	Observation	Inference
<b><u>For Fats</u></b> <b>i) Spot Test :</b> Take a food item to be tested and press or rub it on a clean white sheet of paper and hold it against light.		
<b>ii) Solubility test:</b> Take a small amount (50mg) of the given sample in a test tube. Add 2-3 mL water in it and shake. Observe if it has dissolved in it. Similarly check the solubility of the compound in alcohol, $\text{CHCl}_3$ and $\text{CCl}_4$ separately.		
<b>iii) Acrolein Test :</b> Heat a small amount (2-3g) of sample with same amount (2-3g) of solid $\text{KHSO}_4$ in a test tube. Bring the mouth of the test tube near the nose and smell the gas evolved, if any, only superficially.		
<b>iv) Huble's test for unsaturation:</b> Take two test tubes labelled as 'A' and 'B'. Add about 3-4 mL of chloroform to each test tube. Put some samples (2-3g) of unsaturated fat (linseed oil) in test tube 'A' and saturated fat (cottonseed oil) in test tube 'B'. Add Huble's solution to each test tube dropwise and keep on adding while counting the drops, until a permanent violet colour appears in each test tube.		

**11.4.3 Worksheet = 3 : Test for Proteins**

Experiment	Observations	Inference
<b><u>For Proteins</u></b> <b>i) Biuret Test :</b> Take one mL of milk or any other protein containing sample in a test tube. Make it alkaline by adding a few drops (4-5) of NaOH solution. Then add 4-5 drops of dil. solution of $\text{CuSO}_4$ .		
<b>ii) Ninhydrin Test:</b> Take about 1 mL of 5% solution of egg white in a test tube. Add to it 4-5 drops of 0.1% ninhydrin solution. Boil the contents for about one minute and cool it.		
<b>iii) Xanthoprotein Test :</b> Take 2-3 grams of glutenin of wheat in a test tube. Add 2-3 mL of conc. $\text{HNO}_3$ to it. Shake the contents and keep aside.		
<b>iv) Millon's Test :</b> Take about 1 mL of milk in a test tube. Add 1-2 drops of mercuric nitrate solution and one drop of dil. $\text{H}_2\text{SO}_4$ . Boil and cool the contents and then add 1 drop of $\text{NaNO}_3$ solution. Warm the mixture again and cool it.		

## 11.5 PRECAUTIONS

1. Smell the gases coming out of reactions carefully.
2. Some compounds are highly corrosive. For example, phenol can cause blisters and burn your skin when it falls on it. Immediately resort to first aid.
3. Use a water bath for warming the contents. Do not heat organic the compounds on a direct flame, these can catch fire easily.
4. Neutral ferric chloride must be prepared fresh (procedure is given in appendix.)
5. Tollen's reagent must be freshly prepared (Procedure is given in the appendix)
6. Fehling's solution should be prepared by mixing equal amounts for Fehling's solution A and Fehling's solution B required for the test.

## 11.6 Observation

Record your observations in the blank worksheet in the following manner :

1. .... Are found in the given food stuffs.

Test	Glucose	Table 11.1	Sucrose	Starch
1. Solubility in water	Soluble		Soluble	Insoluble
2. Fehling's test				
3. Tollen's test				
4. Molisch's test				
5. Benedict's test				
6. Iodine test				



Table 11.2

Sl. No.	Test	Fat (Saturated)		Fat (Unsaturated)
		Animal	Vegetable	
1.	Spot test			
2.	Solubility test (in org. solvent)			
3.	Acrolein test			
4.	Huble's test			

### 11.7 CONCLUSIONS

Following conclusions are drawn based on test

#### For Carbohydrates :

- The given sample / does not / contain carbohydrates.
- The given sample contains reducing / non-reducing sugar.

#### For Fats :

- The sample does not contain fat.
- The sample contains saturated / unsaturated fat.

#### For Proteins :

- The given sample contains proteins.
- The given sample does not contain proteins.

#### Common :

- The given sample contains fat and protein body.
- The given sample contains carbohydrates and protein.
- The given sample contains carbohydrates, fat and protein.

### 11.8 CHECK YOUR UNDERSTANDING

Q. 1 Why are carbohydrates known as 'hydrates of carbon' ?

-----  
-----

Q. 2. Define carbohydrates.

-----

Q. 3 What is the general formula of polysaccharides ?

-----

Q. 4 What is meant by the term reducing sugar ?

-----

Q. 5 Name the compound formed as red precipitate when a carbohydrate is treated with Fehling's solution.

-----

Q. 6 Name the carbohydrate which is not digested in our body.

-----

Q. 7 Which carbohydrate forms blue colour product when treated with iodine ?

-----

Q. 8 What are fats ?

-----

Q. 9 What products are obtained on hydrolysis of fats ?

-----

Q. 10 Write down the structural formula of Acrolein.

-----

Q. 11 Name four sources of fats ?

-----

Q. 12 What is the difference in the physical state of saturated and unsaturated fats at room temperature ?

-----

- Q. 13 A fat required more of Huble's solution of giving a permanent violet colour in the mixture ?  
What inference will you draw from this observation ?
- 

- Q. 14 Define proteins.
- 

- Q. 15 What is the main role of proteins in our body ?
- 

- Q. 16 Name the bonding which unite amino acids to form a protein.
- 

### 11.9 NOTE FOR THE TEACHER

1. Ensure that students work themselves with at least one sample of each kind of carbohydrates, (Glucose, Sucrose and starch), saturated and unsaturated fats.
2. After the students have performed the tests of all the samples, they may be asked to identify the unknown substance and write full details in the experiments sheet.
3. The reactions in all the experiments occur very easily. The students may be asked to perform slow addition and pay keen observation.

### 11.10 CHECK YOUR ANSWERS

Ans. 1 Carbohydrates are called as hydrates of carbon because proportion of hydrogen and oxygen in carbohydrates is similar to that in water.

Ans. 2 Carbohydrates are polyhydroxy aldehydes or ketones and the substances which yield polyhydroxy aldehydes or ketones on hydrolysis.

Ans. 3  $C_6H_{14}O_5$

Ans. 4 Sugars having free carbonyl group can reduce Tollen's reagent Fehling's solution to give metallic silver and  $Cu_2O$  respectively. Such sugars are called reducing sugars.

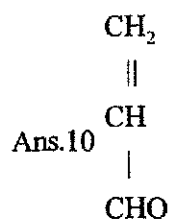
Ans. 5  $Cu_2O$  (Cuprous oxide)

Ans. 6 Cellulose

Ans. 7 Starch.

Ans. 8 Fats are triesters of long chain fatty acids and glycerol.

Ans. 9 Fatty acid and glycerol



Ans. 11 Cotton seed oil, coconut, milk, ghee

Ans. 12 Saturated fats are solids and unsaturated fats are liquids at room temperature.

Ans. 13 The given fat has more unsaturation.

Ans. 14 Proteins are complex nitrogenous organic compounds.

Ans.15 Proteins are helpful in growth and maintenance of our body tissues and also regulate various physiological activities.

Ans. 16 Amino acids join through peptide bonds making a polypeptide chain (protein).