Lipids

Lipids are heterogeneous organic substances that have solubility in non-polar organic solvents such as benzene, ether, chloroform, etc. but they do not dissolve in water. The functions of lipids are :

1- Storing energy (A form of energy stock) .
2- Acting as structural components of cell membranes .

There are different types of lipids contain non-polar structures similar to hydrocarbons, which are given an oily or waxy state insoluble in water.

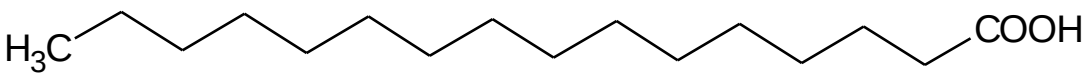
Lipids are divided by chemical properties into types :

1- **Simple lipids** : contains fats , oils , and waxes.
2- Compound lipids : contains phospholipids , glycolipids , sphingolipids .
3- Derivative lipids : contains cholesterol (Steroids) and fat-soluble vitamins.

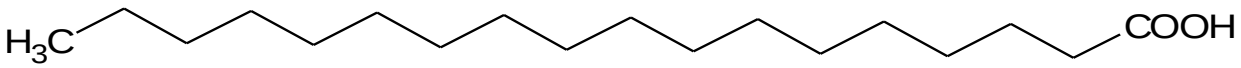
fatty acids :

A fatty acid is a carboxylic acid with a long aliphatic chain which is involved in the structure of simple and compound lipids, which is either saturated or unsaturated. Fatty acids differ by length, often categorized as short to very long. There are examples of fatty acids :

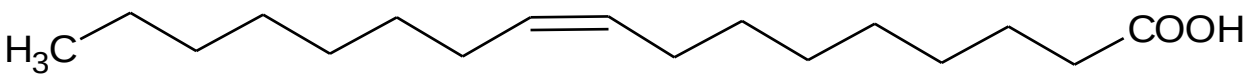
16:0 Palmitic acid



18:0 Stearic acid



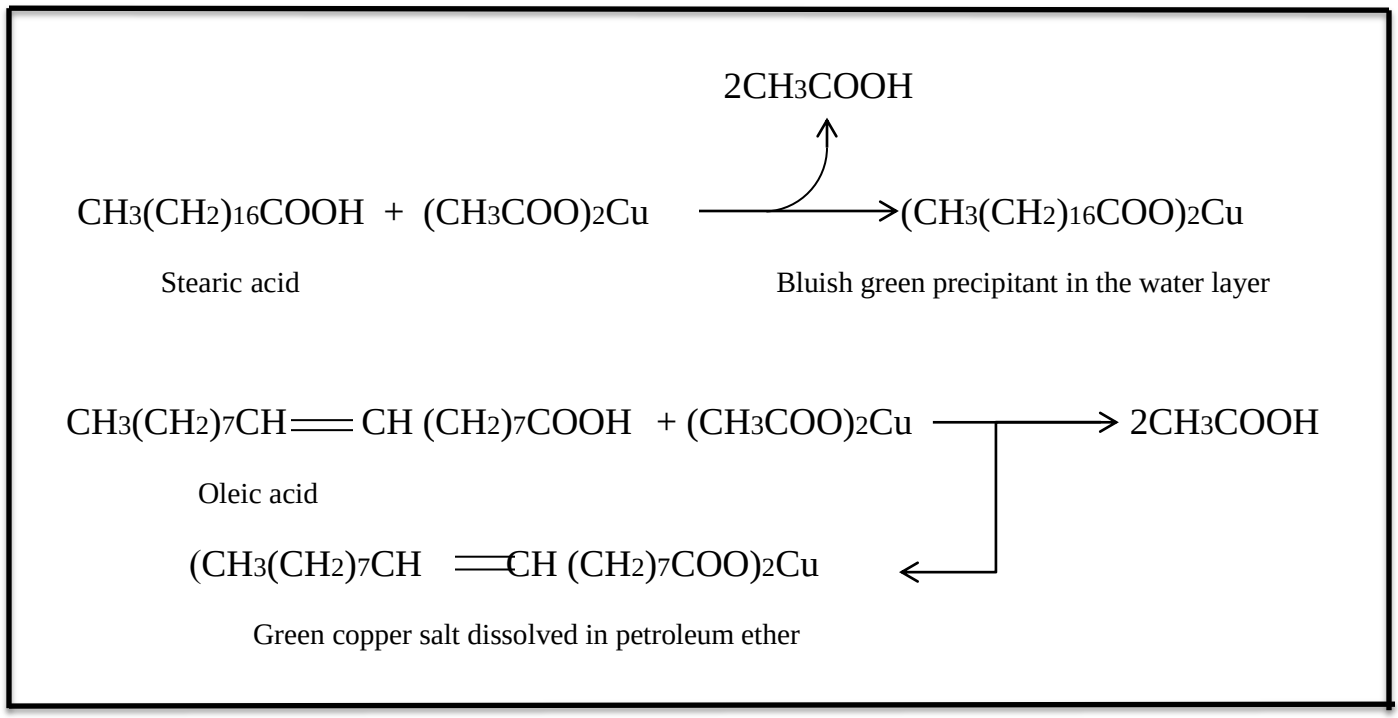
18:1 cis Δ^9 Oleic acid



Qualitative tests of lipid :

1-Detection of saturation by copper acetate:

Triglyceride does not interact with copper acetate solution but the saturated fatty acids interact with this solution and give bluish green precipitant in the water layer, while the unsaturated fatty acids give green copper salt dissolved in petroleum ether (organic layer).



Method :

1- In clean and dry test tube, 10 drops of 10% copper acetate are added without shaking the tube to 1ml of unsaturated fatty acid (oleic acid), then 1ml of saturated fatty acid (stearic acid) is added in the other test tube and the 10 drops of 10% copper acetate are added in the same tube (without shaking the tube).

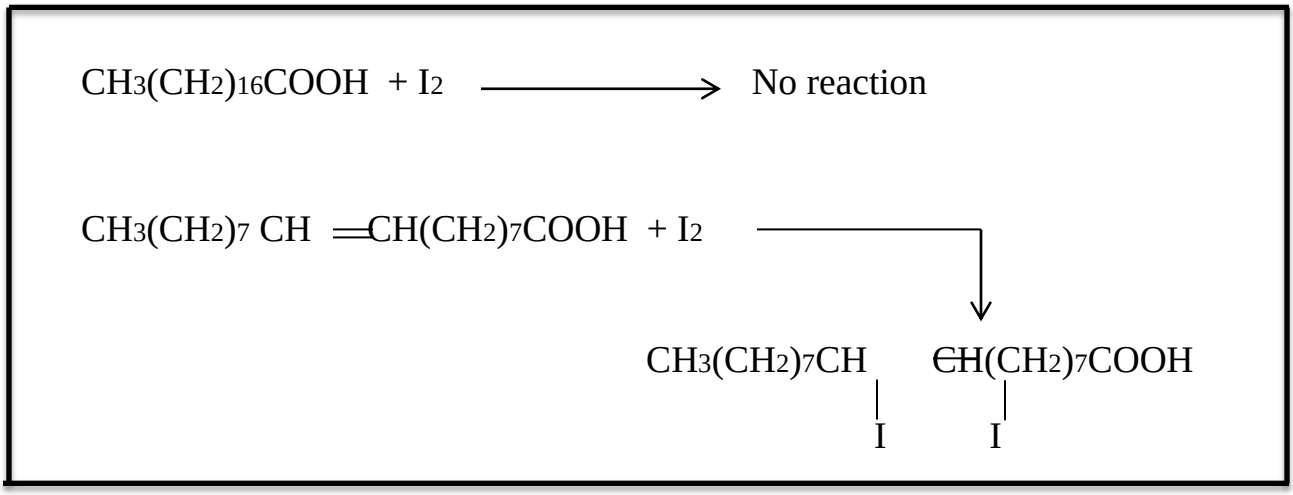
2- The different between two tubes is noted that the first reaction is given green copper salt dissolved in petroleum ether and the second reaction is given bluish green precipitant in the water layer.

Caution :

Do not shake the test tube because it will give a heavy emulsion.

2- Iodine test :

Fatty acids found in animal fats are fully saturated while those in plant`s oils (or vegetable oils) contain one or more of double bonds. When the iodine solution is added to the unsaturated fatty acid, it will lead to disappear the iodine color because of saturating the double bonds, but the iodine color does not disappear when it added to the saturated fatty acid.

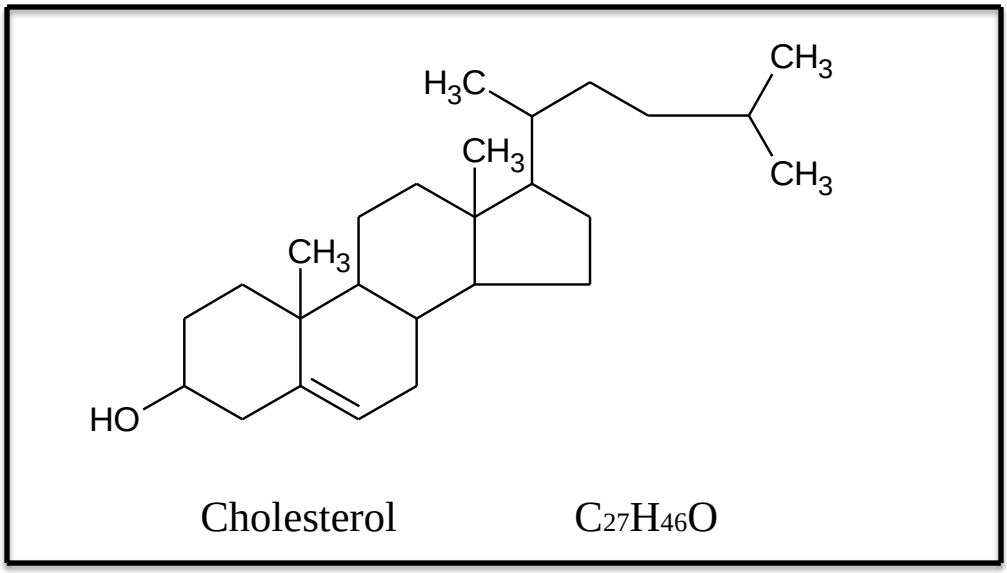


Method :

1- In clean and dry test tube, Iodine solution is added drop by drop to the two test tubes, the first one contain saturated fatty acid and the other one contain unsaturated fatty acid.
2- The tubes are shaken after added the drops of iodine solution until the color of iodine disappears.
3- The different between two tubes is noted that the color of iodine does not disappear in the first tube while in the second tube the color disappears.

3- Qualitative tests of cholesterol:

Cholesterol is a steroid lipid built from four linked hydrocarbon rings. It is an amphipathic molecule, with a polar head group (the hydroxyl group at C-3) and nonpolar hydrocarbons body (the steroid nucleus and the hydrocarbon side chain at C-17), about as long as a 16- carbon fatty acid in its extended form.



Cholesterol $C_{27}H_{46}O$

When steroids that contain unsaturated bonds are treated in non-aqueous conditions with strong acids, they interact yield distinct color outputs depending on the conditions of the experiment, the resulting colors show significant differences from one compound to another, as well as the mechanical reaction is complicated.

There are the tests used to detect cholesterol:

1- *Salkowaki test* :

It is an important test used to detect cholesterol depending on the colors (distinct and clear colors) that yield from reaction cholesterol with concentrated sulfuric acid. In order for this detection to be successful, the tubes must be dry and the solutions should be non-aqueous.

Method:

1- In clean and dry test tube, 1 ml of H_2SO_4 is added to 1ml of cholesterol and it is shaken well.
2- The separating of two layer is noted, the top layer is red and the bottom layer is green.

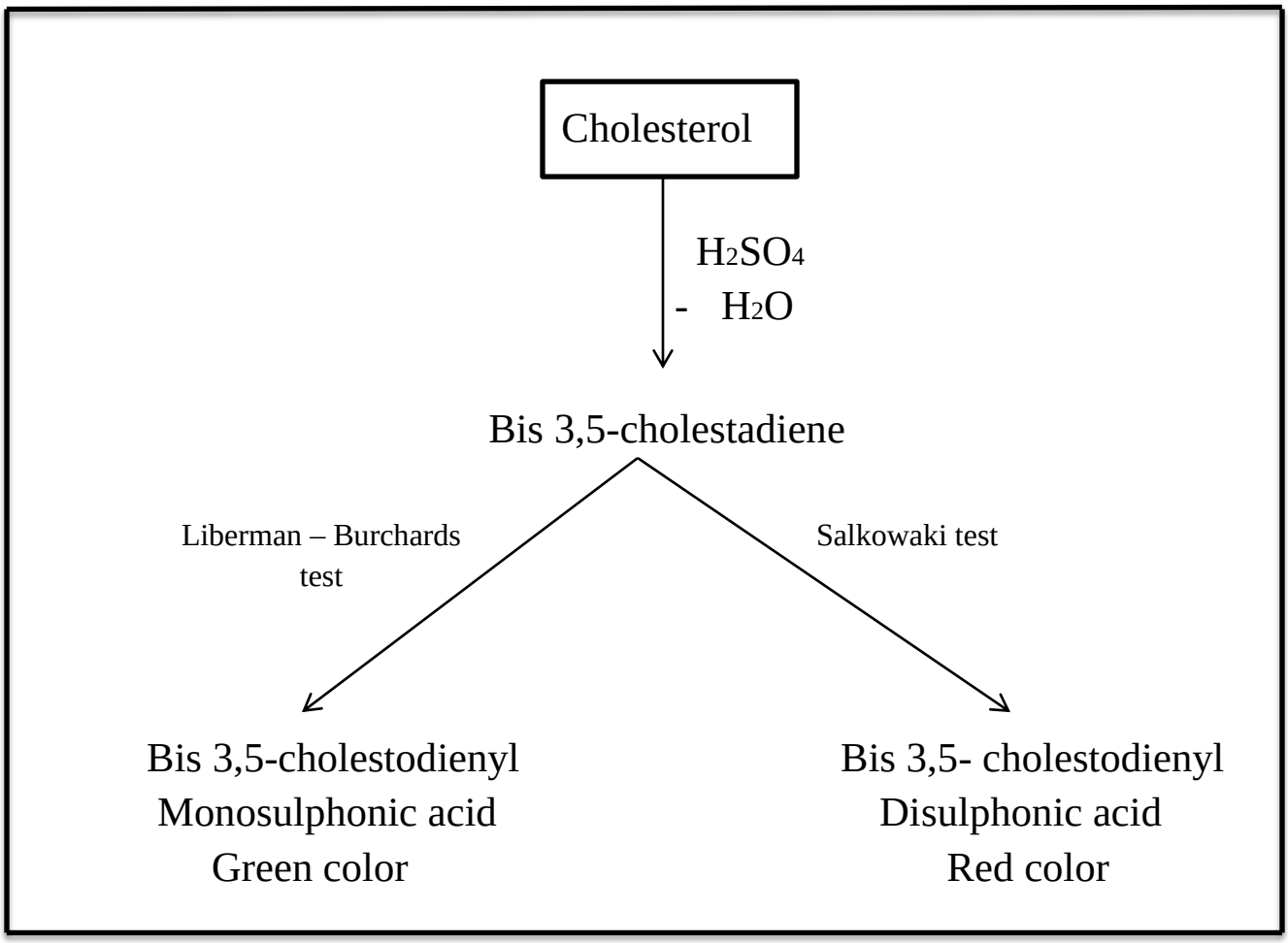
2 - *Liberman – Burchards test :*

It is another detection of cholesterol using acetic acid with the concentrated sulfuric acid, which are added to the cholesterol, where the pink color is appeared and changes to violet and then to the bluish green. It can used to

measure the amount of cholesterol by measuring the intensity of color (quantitative estimation).

Method :

1- In clean and dry test tube, 1 ml of acetic acid is added to 1ml of 5% cholesterol which is dissolved in the chloroform with 2 drops of H_2SO_4 with shaking.
2- The change of color is noted.

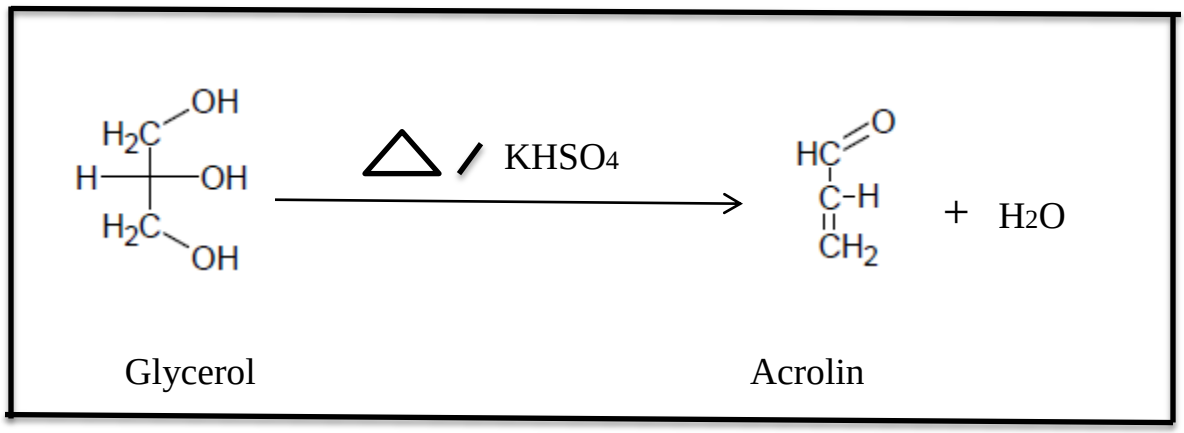


4-Acrolin test:

It is an important test for glycerol where two molecules of water are lost by heating with a dehydrated substance $KHSO_4$ and it turns into a volatile substance with a smell that is similar to the smell of burned fat (unsaturated aldehyde) is called Acrolin . This test is distinctive for glycerol whether it is free or combined with fatty acids.

Method :

1- In clean and dry test tube, 0.5ml of glycerol is put with 0.5ml of $KHSO_4$.
2- The tube is heated and the change of the solution in the tube is noted.



5-Rancidity of lipids:

It is a term called to lipids which left exposed to the air at a normal temperature and has a foul taste and foul smell because it contains volatile fatty acids (VFA), where there is a change in the physical and chemical properties of fats. There are two types of rancidity:

1- **Hydrolytic rancidity:**
It is caused by enzymes or microorganisms causing the release of volatile fatty acids (VFA) with a short chain and they have smell not desirable as it happens in butter. The factors that help to occur this type of rancidity are (heat and humidity).

2- **Oxidative rancidity:**
It occurs especially in oils containing unsaturated fatty acids where these acids are oxidized and converted into compounds with a short chain (ketones, aldehydes and volatile fatty acids (VFA)), causing their own smell. The factors that help to occur this type of rancidity are oxygen, light and heat .

Method :

1- A solution contain 2 drops 1% of phenol naphthalene that added to 0.5% NaOH is prepared.

2- The old and new lipid are put in two test tubes, then the previous solution is added to each tube drop by drop and are observed colors that are formed in both tubes.

6-determination of acid value for lipids:

Lipid is rancid as a result of storage. As a result of this, the amount of fatty acids found in fat gives a large indicator about the age and quality of lipid.

(The acid value): It is the number of milligrams of potassium hydroxide needed to equalize free fatty acids found in one gram of lipid.

Method:

1- In clean and dry conical flask, 2 ml 12% of olive oil is added, then 2 drops of phenol naphthalene are added to it .

2- The solution in the conical flask is titrated with 0.1N of KOH until the pink color is appeared (equivalence point).

Calculation :

1- Calculation of weight of oil :

12	100
X	2

X = 0.42 g (weight of oil in the sample).

2- Calculation the volume of base :

$$\mathbf{N} = \frac{\text{wt.}}{\text{M.wt}} \times \frac{1000}{V_{ml}}$$

$$0.1 = \frac{\text{wt.}_{KOH}}{56} \times \frac{1000}{V_{ml}}$$

Wt. = M gm

Multiply (M x 1000) to convert it into milligrams.

M is represents the number of milligrams KOH that needed to equalize free fatty acids found in (0.24) gram of lipid.

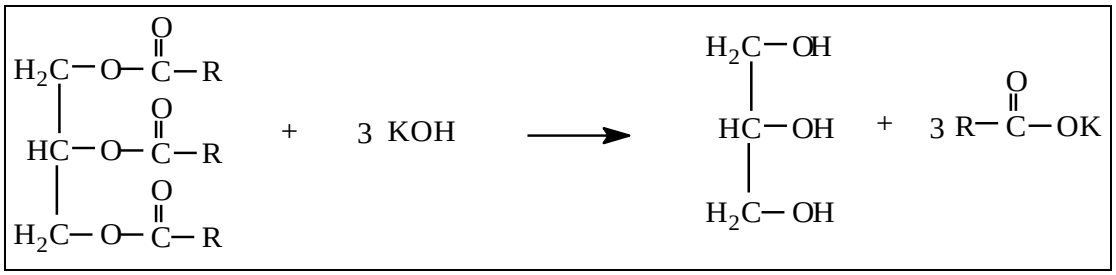
M	0.24
X	1

X = ? mg

X is represents the number of milligrams (KOH) that needed to equalize free fatty acids found in 1 gram of lipid.

7-The saponification value:

Saponification is a process that involves conversion of fat or oil into soap and alcohol in the presence of aqueous alkali. Oils can be saponified with KOH to form soap and glycerol as follows:



Saponification number:

It is the number of milligrams of potassium hydroxide KOH needed to equalize free fatty acids that produced by the complete decomposition of one gram of lipid. At the condensation process, the triglyceride is treated with a strong base, which cleaves the ester bond, releasing glycerol and potassium salts of fatty acids (soap).

The saponification value gives important information about the nature of fatty acids found in lipid.

Method:

1- In clean and dry beaker, 1 gm of lipid is weighted and put in it and dissolved in a suitable solvent.

2- The contents of the beaker are transfer to 250 ml conical flask by washing the beaker several times with small amounts of solvent that used, then 25ml of potassium hydroxide 0.5 mol/L KOH is added.

3- At the same time, another conical flask containing all the components of the first conical flask is prepared without adding lipid (called the blank flask).

4- The process of condensation for two conical flasks is done for 30 min ,then the conical flasks are cooled by leaving them at room temperature.

5- The contents of the two conical flasks are titrated against 0.5 mol/L hydrochloric acid with phenolphthalein as an indicator.

Calculation :

The difference in reading between two conical flasks gives the number of millimeters of KOH required for the saponification of 1 gm of lipid.

M.Wt of KOH = 56 gm/mol.

As the three molecules of fatty acid are removed from the triglyceride, so:

$$\text{Saponification value (S)} = \frac{3 \times 56 \times 1000}{\text{Average molecular weight of fat}}$$

$$\text{Average molecular weight of fat} = 3 \times 56 \times 1000 / S$$