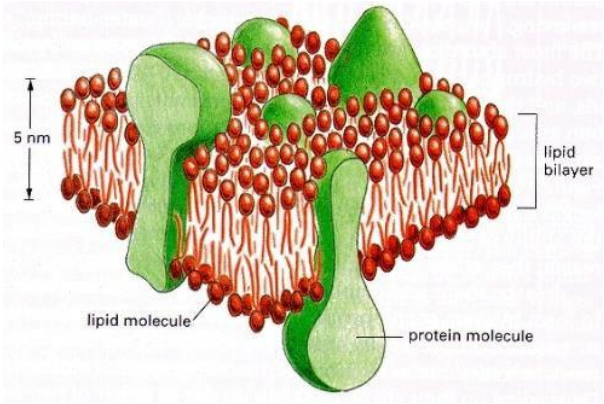
Qualitative tests of Lipids -I-

BCH302 [Practical]

Lipids

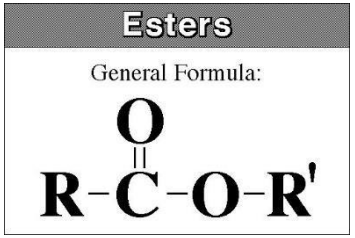
- It can be defined as **nonpolar** organic compound insoluble in polar solvent , but **soluble in organic solvents** such as benzene ,ether, chloroform.

- Lipids are esters of long chain **fatty acids** and alcohols.
- Fatty acids (F.A) are lipids' building blocks.



- **There are two types of fatty acids:**

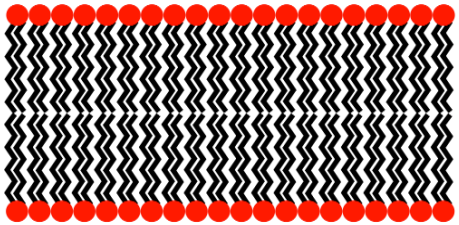
1. Saturated fatty acids.
2. unsaturated fatty acids.



- **Biological role of lipids:**

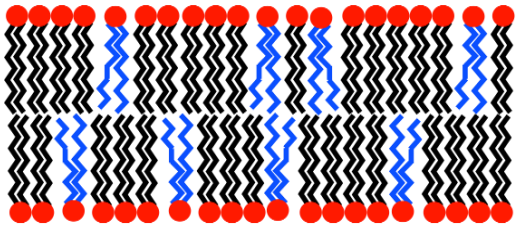
1. It presents in cell membranes.
2. An essential source of energy in the body. It give **more energy** than carbohydrate and proteins.

Types of fatty acids

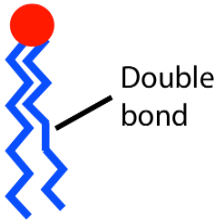


Saturated lipids only

Saturated

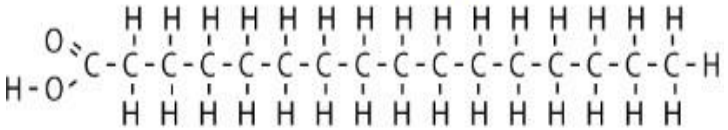


Mixed saturated and unsaturated

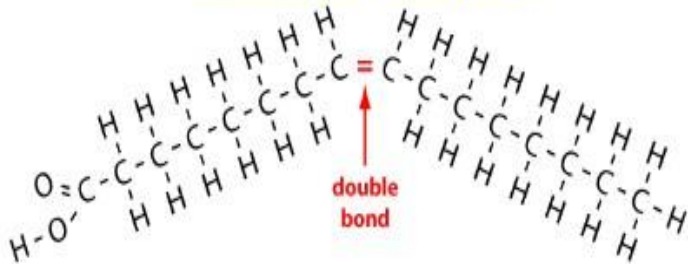


Monounsaturated

saturated fatty acid



unsaturated fatty acid



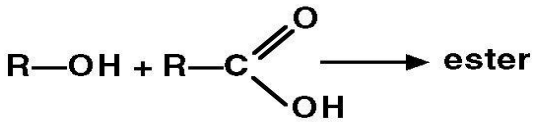
Classification of Lipids:

- **Lipids can be divided according to their chemical composition to:**

I) Simple lipids.
II) Compound (conjugated) lipids.
III) Derived lipids .

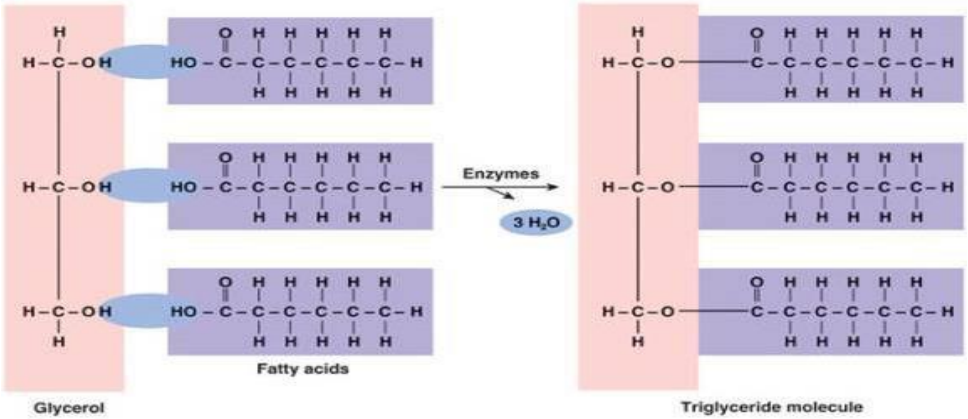
I) Simple lipids :

- **These compounds are:** esters of fatty acids with alcohol.



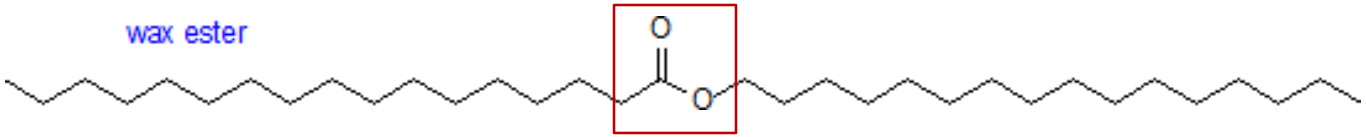
A. Neutral lipids:

Esters of fatty acids with alcohol (glycerol) , e.g.: Triacylglyceride.



B. Waxes:

Esters are formed from fatty acids and long chain alcohol which have only one hydroxyl group (mono hydroxyl alcohol) and are larger than Glycerol (high molecular weight) e.g.: Beeswax



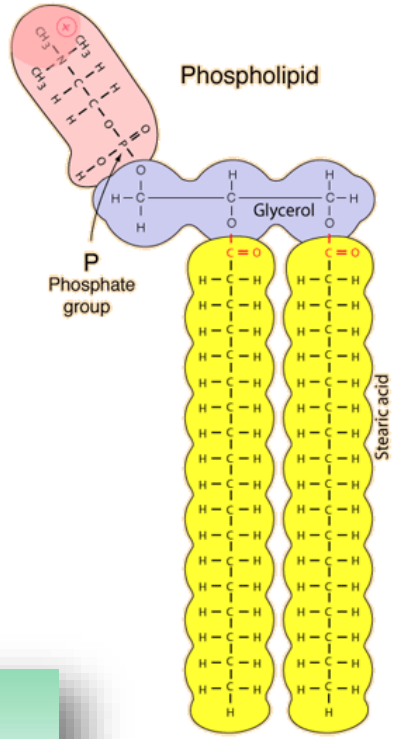
II) Compound (conjugated) lipids :

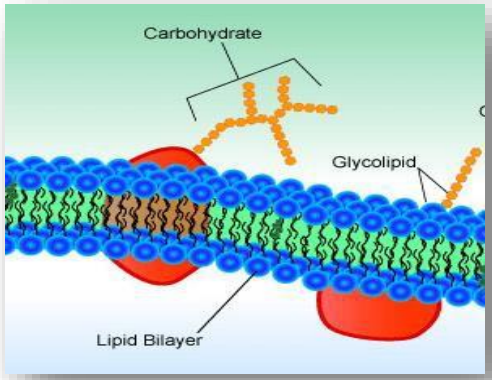
- **Lipids are linking with <u>other</u> compounds:**

a)Phospholipids:
Most phospholipids contain diglyceride, and phosphate group.

b)Glycolipids:
Lipids with a carbohydrate attached.

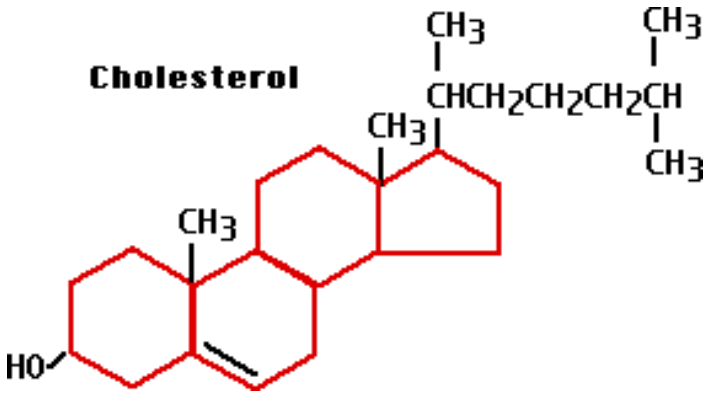
c)Proteolipids :
Any of a group of proteins to which a lipid molecule is attached.





III) Derived lipids :

- They are substances that are soluble in lipid or derived from the lipids by hydrolysis; for examples, cholesterol and fat soluble vitamins (A, K, E and D) .



Practical part

Qualitative tests of lipids

1. Solubility test.
2. Saponification test.
3. Separation of soap from the solution by salting out.
4. Formation of insoluble soap.

Experiment 1 : Solubility test

Objective:

- To test the solubility of oils in different solvent.

Principle:

- Fats are not dissolved in water due to their nature, which is non-polar (hydrophobic), but it is soluble in organic solvents such as chloroform, benzene, and boiling alcohol.

- Different lipids have ability to dissolve in different organic solvent.

- This property enable us to separate a mixture of fat from each other for example:
  - Phosphatidelipid can not dissolve in acetone.
  - Cerebroside and sphingomyline can not dissolve in the ether

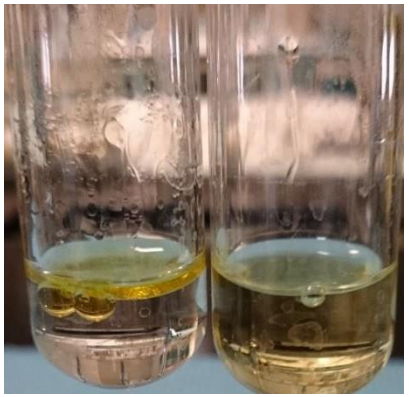
Experiment 1 : Solubility test

Method:

1. Place 0.5ml of oil in 6 test tubes clean, dry containing 4ml of different solvents (acetone, chloroform and ether and ethanol, cold ethanol and hot water).
2. Shake the tubes thoroughly, then leave the solution for about one minute.
3. Note if it separated into two layers , the oil are not dissolve; but if one layer, homogeneous transparent formed , oil be dissolved in the solvent.

Results:

Tube	Solvent	Degree of solubility
A	chloroform	
B	ether	
C	ethanol	
D	diluted acid	



dissolved not dissolved

Experiment 2 : Saponification test

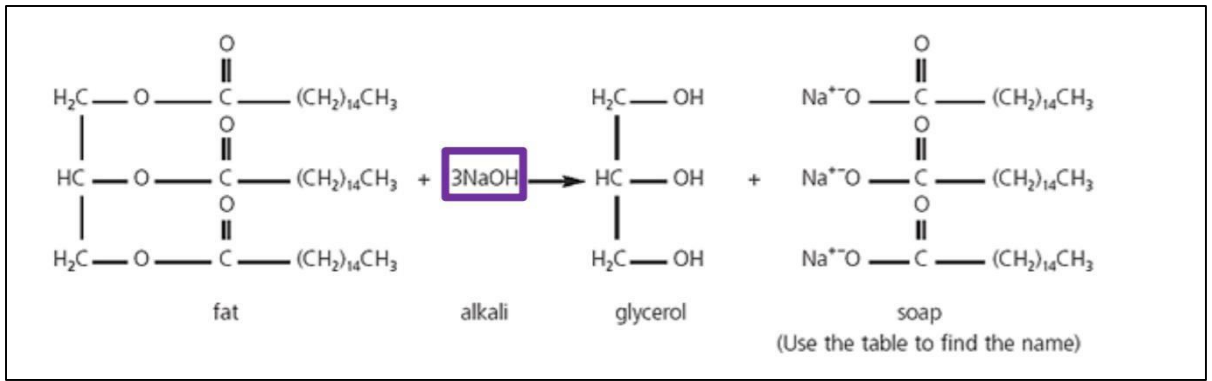
- TAG can be hydrolyzed into their component fatty acids and alcohols. This reaction can also be carried out in the laboratory by a process called saponification, where the hydrolysis is carried out in the presence of a strong base (such as NaOH or KOH).

Objective:

- To form the soap.

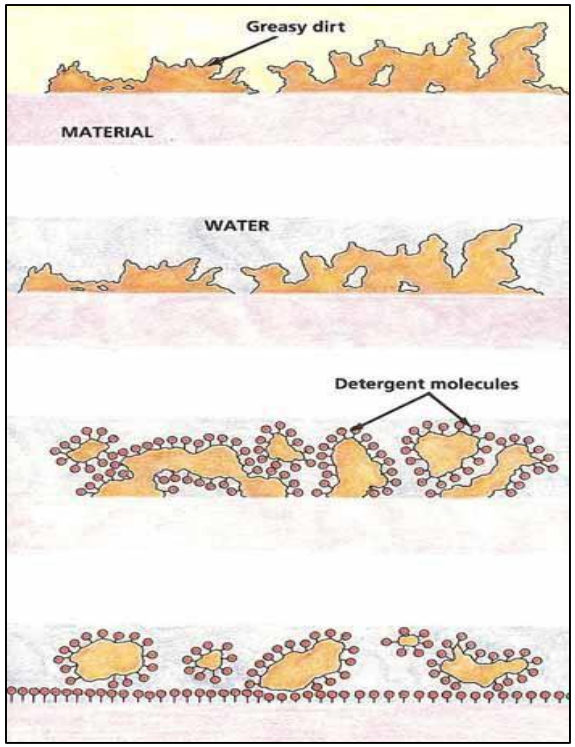
Principle:

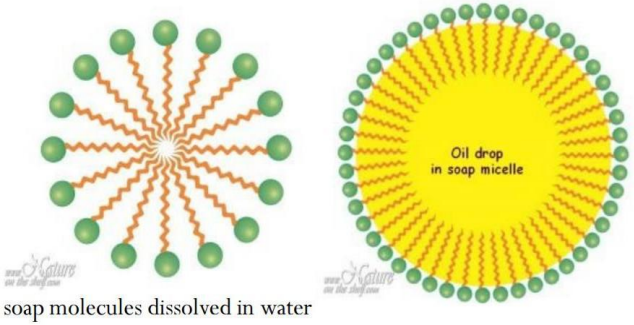
- Saponification is a process of hydrolysis of oils or fat with alkaline and result in glycerol and salts of fatty acids (soap).
- Soap can be defined as **mineral salts of fatty acids**



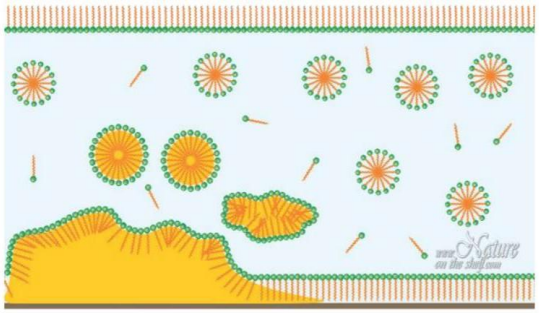
- Soap works on emulsification of oils and fats in the water as it works to reduce the attraction surface of the solution.

- When soap molecules are dissolved in water, the water-repelling hydrophobic tails cluster together while hydrophilic heads surround them arranging themselves in a spherical form toward water molecules.





soap molecules dissolved in water

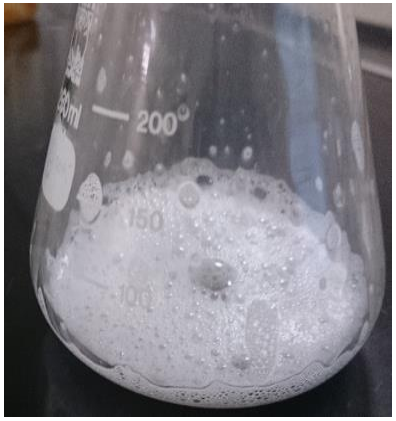


Experiment 2 : Saponification test

Method:

1. Place 2 ml of oil in a large flask.
2. Add 4 ml of alcoholic potassium hydroxide.
3. Boil the solution for 3 minutes. After this period, make sure it is perfectly saponification process, by taking a drop of the solution and mix with the water if oil separated indicates that the non-completion of the saponification. In this case, continued to boil until all the alcohol evaporates.
4. Take the remaining solid material (soap) and add about 30 ml of water and keep it for the following tests.
5. Shake the solution after it cools and noted to be thick foam.

Results:



Experiment 3 : Testing the separation of soap from the solution by salting out

Objective:

- To investigate the effect of NaCl on soap solubility.

Principle:

- To get the soap out of solution by salting out, when added solid sodium chloride to the solution until saturation; separated soap in the form of insoluble and floats above the surface.

- The NaCl solution provides Na+ and Cl- ions that bind to the polar water molecules, and help separate the water from the soap.
- This process is called salting out the soap.

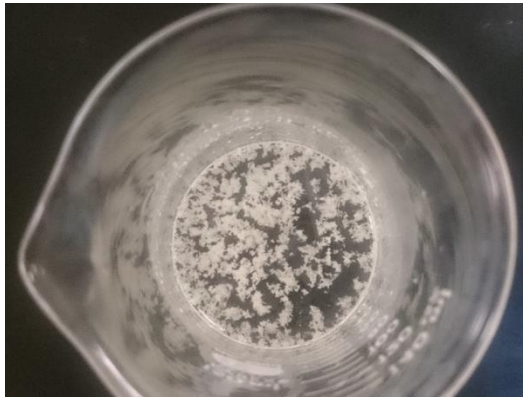
Experiment 3 : Testing the separation of soap from the solution by salting out

Method:

1. Place about 10 ml of soap in the beaker.
2. Then add small amounts of sodium chloride in batches, stirring until saturated solution.

Results:

Tube	Observation
Soap + NaCl	



Experiment 4: Test formation insoluble fatty acids salt (insoluble soaps)

Objective:

- To investigate the effect of different cations on soap solubility.

Principle:

- Working calcium, magnesium, lead or iron ions leads to the **deposition of soap** making it insoluble in water. These ions **replace the sodium or potassium present in soap**.
- Hard water contains significant quantities of Ca^{2+} , Mg^{2+} and some Fe^{3+} which react with the charged ends of the soaps to form insoluble salts of fatty acid.
- The insoluble salts of fatty acid that Ca^{2+} and Mg^{2+} form with soap anions cause **white precipitate to from.**

K^{+} soap + Ca^{+2}sulfate=> Ca^{+2} soap + K^{+} sulfate.

Potassium soap + calcium sulfate ➔ calcium soap + potassium sulfate.
(a white precipitate from calcium stearte or oleate is formed).

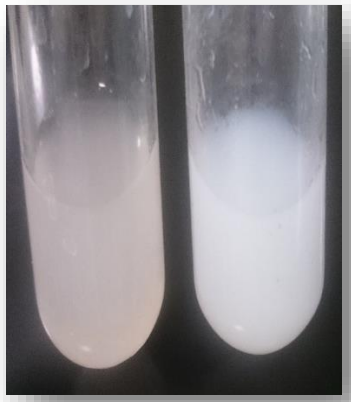
Experiment 4: Test formation insoluble fatty acids salt (insoluble soaps)

Method:

1. Add about 4 ml of distilled water to 2 ml of soap in two test tubes.
2. Add to the first tube a few drops of calcium chloride, to second tube MgCl.

Results:

Tube	Observation
$CaCl_2$	
$MgCl_2$	



Howe Work:

- **Why lipids are not soluble in acid ?**
- **What is the chemical composition of your soap?**
- **Why potassium hydroxide is used in saponification test?**