BCM 101
BIOCHEMISTRY
Week 7 Practical
"Chemistry of lipids"

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The term "**lipids**" applies to a class of compounds that are soluble in organic solvents (e.g. alcohol and chloroform) and insoluble in water. Lipids are essential components of every living cell.
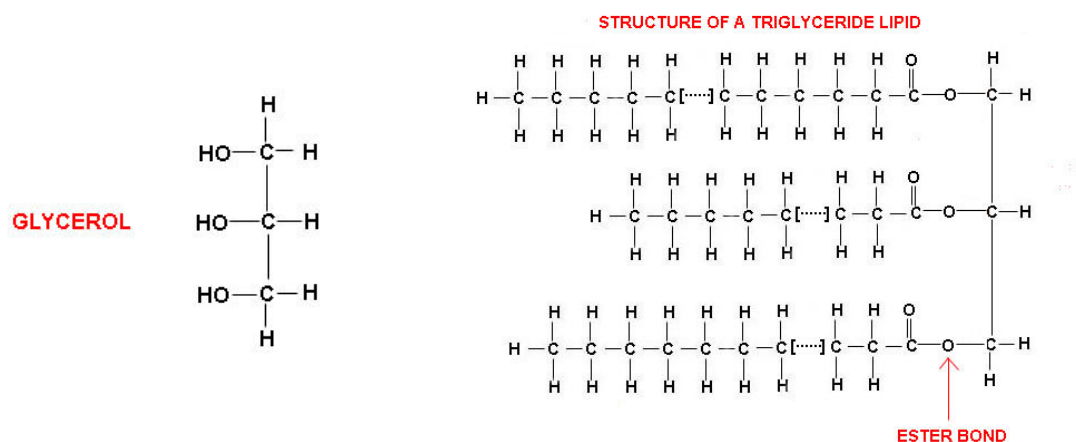
The **aim** of this practical session is to:
1. Obtain a simplified knowledge about lipids.
2. Perform different tests showing some lipid characteristics.

Classification of lipids

Lipids are classified into:
I. Simple lipids:
They are esters of fatty acids with various alcohols. They include **oils** and **fats** which are esters of fatty acids with **glycerol** (i.e. triglycerides).
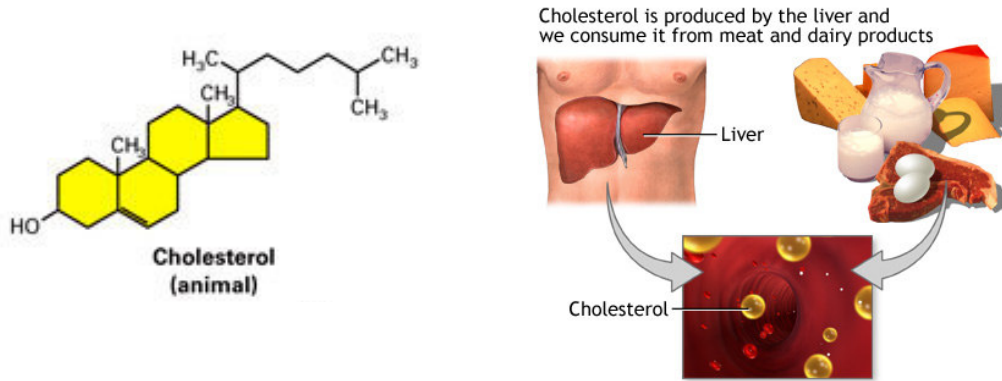

II. Complex lipids:
They contain (in addition to fatty acids and alcohols) additional groups as **phosphate** (e.g. phospholipids), **carbohydrates** (e.g. glycolipids) and **proteins** (e.g. lipoproteins).

III. Precursor and derived lipids:

They include fatty acids, cholesterol, steroid hormones, fat-soluble vitamins (vitamin A, D, E & K) and eicosanoids, which include prostaglandins, leukotrienes and thromboxanes.

Cholesterol (animal)

Cholesterol is produced by the liver and we consume it from meat and dairy products

Biological functions of lipids:

1. They provide energy storage and metabolic fuels.

2. Cholesterol is a precursor of steroid hormones, bile acids (which help in digestion of dietary fats) and vitamin D.

Steroid hormone (testosterone)

Bile acid (deoxycholic acid)

Vitamin D₃
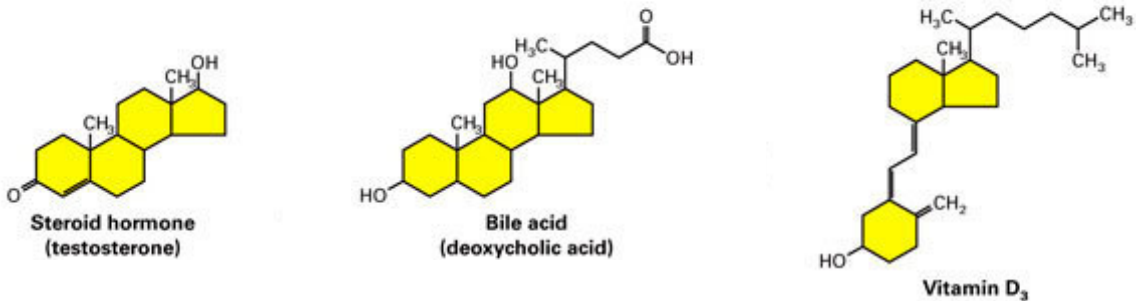
3. They act as functional and structural components of the cell membrane.

4. Lipoproteins (e.g. LDL & HDL) are a mean for transporting lipids in blood.

5. Imbalance in lipid metabolism can lead to major clinical problems such as obesity and atherosclerosis.

Normal artery

Artery narrowed by atherosclerosis

Blood flow

Plaque
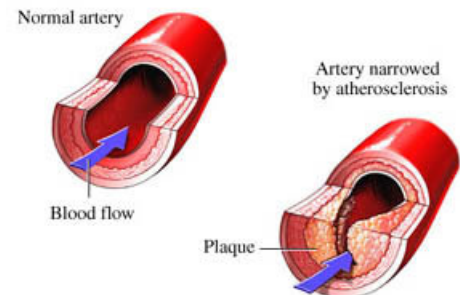
Practical

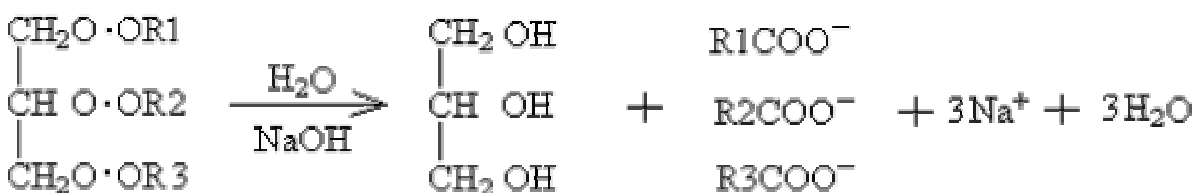## 1. Test for solubility of lipids in polar & non-polar solvents:

– Add about 2 ml of the provided **oil sample** to 5 ml of **water** in a test tube and try to mix oil with water. By shaking, oil and water mix initially; however, they gradually separate out to form 2 layers.
– Repeat the experiment using **chloroform** instead of water.
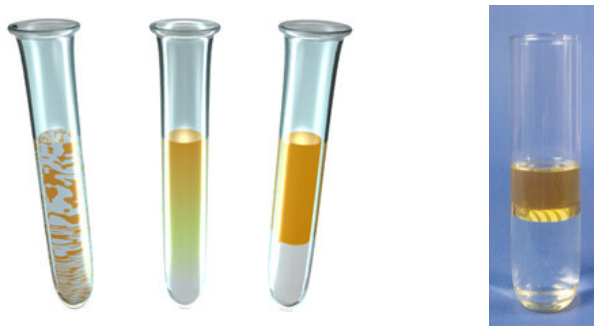
– Oil dissolves in the organic solvent "**chloroform**" but not in water because oils contain long hydrocarbon tails which are hydrophobic in nature.

2. Saponification of oils:

Saponification of oils means the hydrolysis of triglycerides in the oil in presence of an alkaline medium (e.g. NaOH) into glycerol and fatty acids with the production of sodium salt of fatty acids or "**soap**".

$$
\begin{array}{ccc}
\begin{matrix} CH_2O \cdot OR1 \\ | \\ CH\ O \cdot OR2 \\ | \\ CH_2O \cdot OR3 \end{matrix} & \xrightarrow[NaOH]{H_2O} & \begin{matrix} CH_2\ OH \\ | \\ CH\ OH \\ | \\ CH_2\ OH \end{matrix} \quad + \quad \begin{matrix} R1COO^- \\ R2COO^- \\ R3COO^- \end{matrix} + 3Na^+ + 3H_2O
\end{array}
$$

– Place 5 ml of the provided oil sample in a 250 ml beaker.
– Add 15 ml of alcoholic NaOH solution.

boiling water bath

Oil + alcoholic NaOH → soap

- Cover the beaker with a watch glass and heat on a boiling W.B.
- Stir the reaction mixture from time to time using a glass rod until a semi-solid mass is formed.
- Put a small amount of this mass in a test tube containing about 5 ml of water and shake well; a **froth** is obtained indicating the presence of **soap**.
- In another test tube, prepare a soap solution by dissolving a small amount of the formed soap in water. Add few drops of $CaCl_2$ or $MgSO_4$ to this solution and shake well; note the formation of insoluble Ca or Mg soaps without any froth. This explains why soap is not effective in hard water which contains calcium and magnesium; for soap to be effective, it must be soluble in water.

$$2\ RCOO^- + Ca^{2+} \longrightarrow (RCOO)_2Ca \downarrow ppt$$

3. Detection of cholesterol in food extracts:

- **Salkowski test:** to 2 ml of the provided chloroformic extract of canned food, add an equal volume of concentrated sulfuric acid; a yellow to brick-red color is formed indicating the presence of cholesterol.
- **Liebermann-Burchard test**: add 10 drops of acetic anhydride and 2 drops of concentrated sulfuric acid to 2 ml chloroformic extract of canned food; a bluish-green color is formed indicating the presence of cholesterol.
  N.B. Colors are due to sulfonation, acetylation and rearrangement in cholesterol molecule.

5. Detection of cholesterol in a lipid mixture by thin layer chromatography (TLC):

Thin layer chromatography (**TLC**) is based on the separation of a mixture of compounds as they migrate (with the help of a solvent system) through a thin layer of adsorbent material. When using a non-polar solvent system, polar compounds are held back by the adsorbent while non-polar compounds advance further.
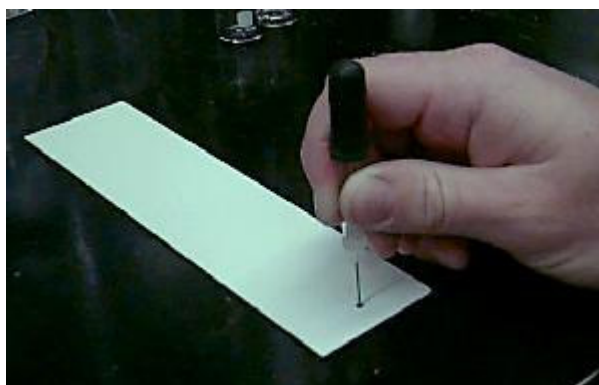
− With your pencil, draw a line about 1 cm from the bottom edge of a silica gel chromatography sheet.

− On this line, apply 2 spots: the first for the lipid mixture and the second for cholesterol as a reference. N.B. mark with your pencil "**M**" for the mixture and "**C**" for cholesterol.

− Put the TLC sheet vertically in a beaker containing 10 ml of a non-polar solvent system composed of petroleum ether : diethylether : glacial acetic acid (70:30:1).

− Cover the beaker immediately with aluminum foil.

− Allow the solvent to rise to the appropriate level (about 1 cm from the upper end of the sheet).

− Remove the TLC sheet from the beaker and immediately draw a line with your pencil at the **front line** (level reached by the solvent).

- Allow the solvent to evaporate and place the TLC sheet vertically in another beaker containing few crystals of iodine; cover the beaker with a watch glass, iodine will sublime and colorize the lipid spots with a yellow color.

- Remove the TLC sheet and encircle spots with a pencil.

- Record the Rf value for each spot.
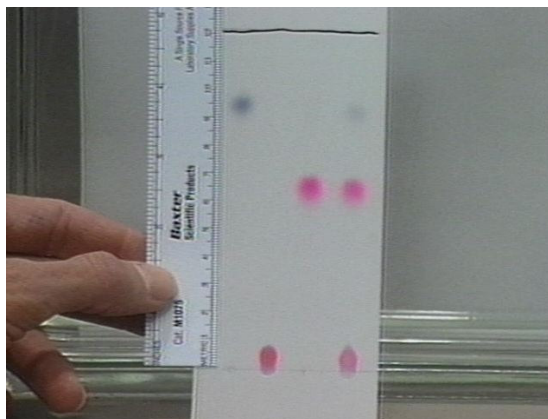
$$Rf = \frac{\text{distance traveled by the spot}}{\text{distance traveled by the solvent}}$$

- Note that if the lipid mixture contains cholesterol, a spot with the same Rf value of reference cholesterol is obtained.
- Comment on the polarity of compounds in the separated spots.

Student Name: .... **Student number:** ....
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1. Test for solubility of lipids in polar and non-polar solvents:

The provided lipid is soluble in ………………………….……………
because…………………………………………………………………………
……………………………………………………………………………...…..

2. Saponification of oils:

What is the chemical nature of soap ? …………….……………………

3. Detection of cholesterol in food extract:

Salkowski test: …………………………………………………………

Liebermann-Burchard test: …………………………………………….

Comment: ……………………………………………………………….

4. Detection of cholesterol in a lipid mixture by TLC:

Rf value of cholesterol spot = …………………………………………

Does the mixture contain cholesterol ? …………………………………