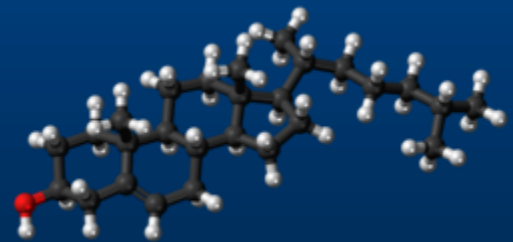
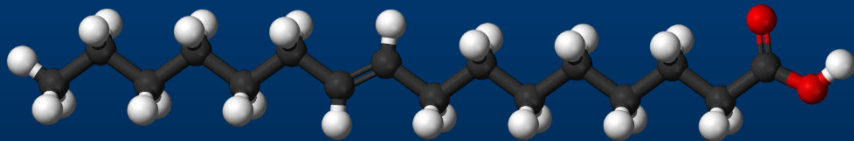




Lipids



The aims of the activities

- Learning about the structure and composition of lipids.
- Determination of the acid number as a measure of freshness of fat for consumption.

Lipids

Insoluble in water, usually the esters of higher fatty acids with alcohols mono-, di- or polyhydric.

- Energy magazine
- Thermal pillows and amortization
- Biological membrane components
- Biologically active compounds

Lipids

Lipids are divided into subgroups based on their chemical structure:

Simple lipids:

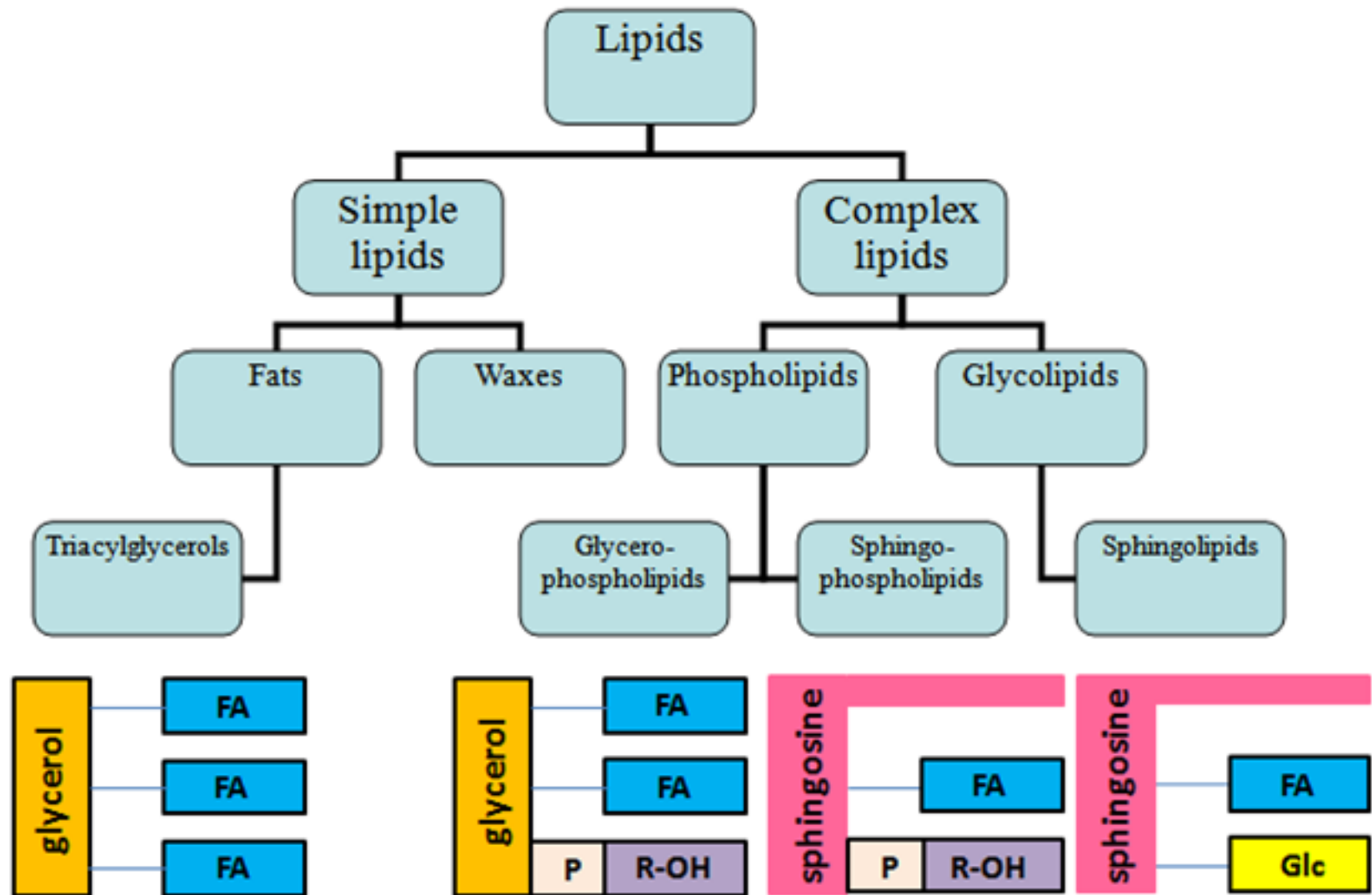
- **Fats** – esters of fatty acids with glycerol
- **Waxes** – esters of fatty acids with higher alcohols

Complex lipids: (including also other additional groups)

- **Phospholipids** – have phosphate residue, nitrogenous base or other compounds.
- **Glycolipids** – esters containing also saccharides, **do not have** phosphate residue.

Derivatives of lipids:

- Isoprene compounds
- Steroids



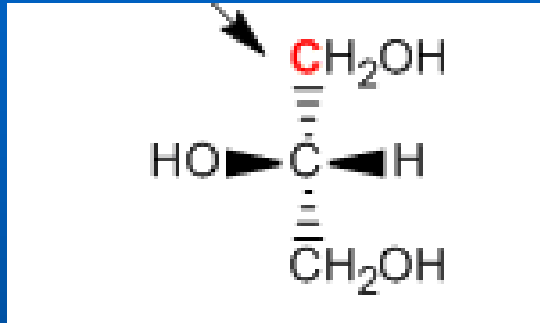
Fats (acylglycerols)

Esters of higher fatty acids and glycerol

Glycerol - C1 - α

C2 - β

C3 - α



1. Monoacylglycerols

2. Diacylglycerols

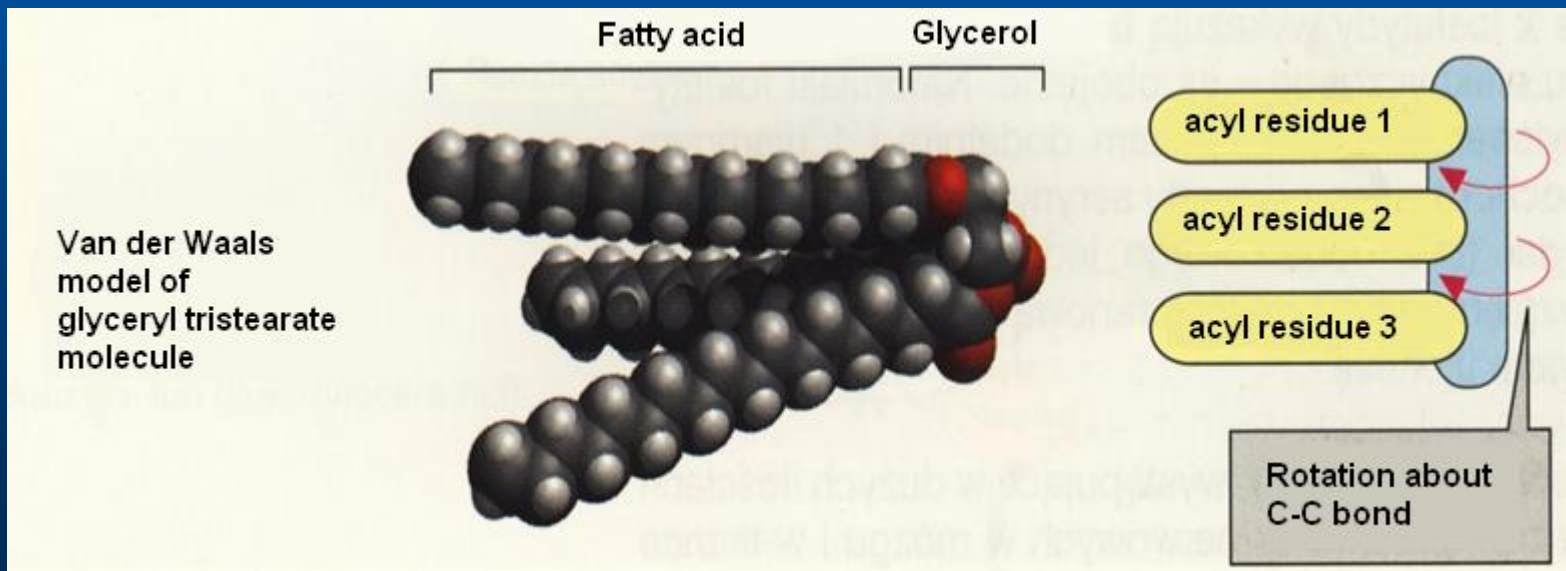
3. Triacylglycerols

- simple (contain one kind of fatty acid)
- mixed (two or three different fatty acids are present in the molecule)

Fats

Fats are hydrophobic, insoluble in water, do not create dispersed micelle.

They are soluble in: chloroform, benzene, ether and hot ethanol.



Properties of fats

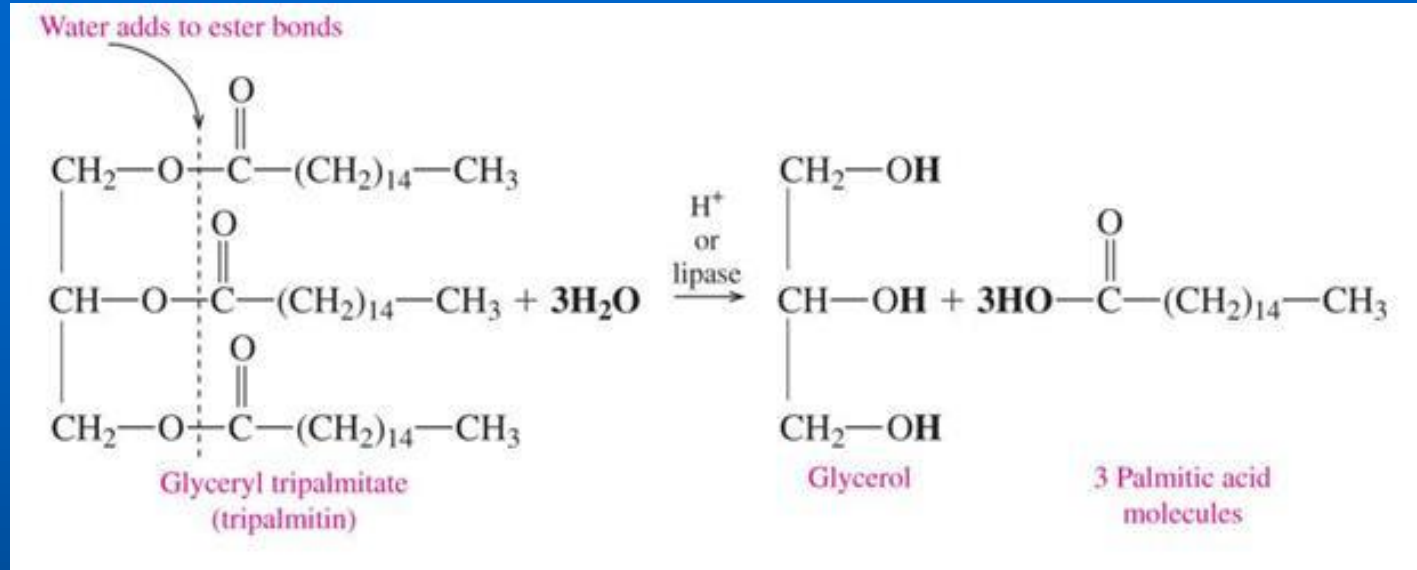
Hydrolysis:

I. **Enzymatic** (lipase) \rightarrow glycerol + FFA

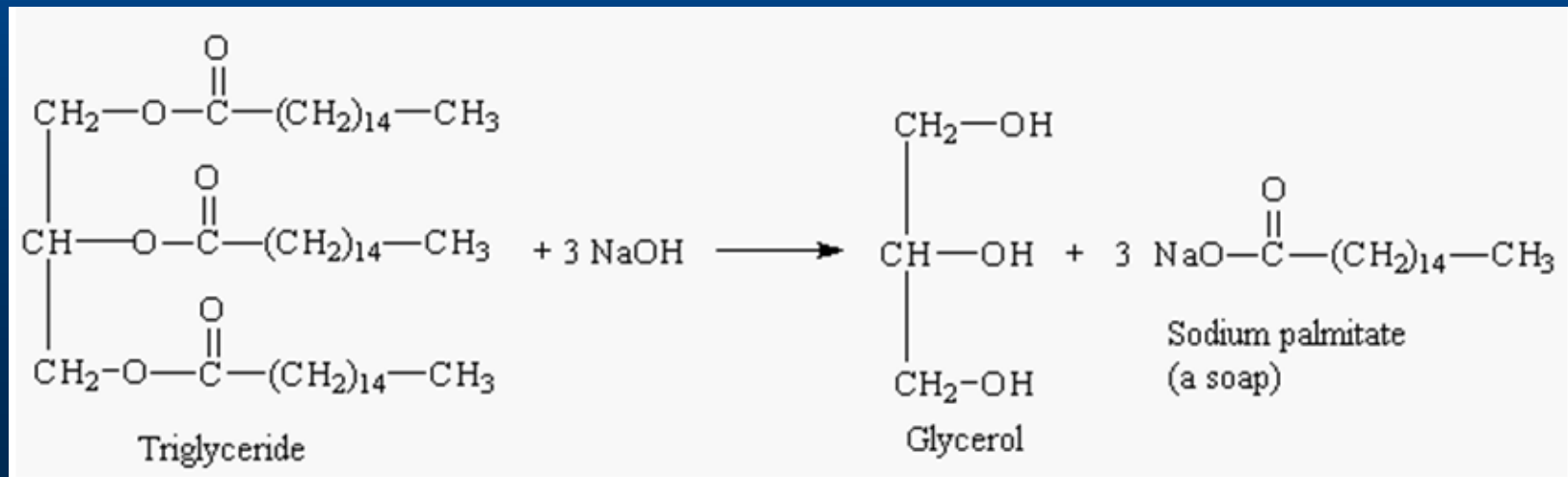
II. **Acid** \rightarrow glycerol + FFA

III. **Alkaline** \rightarrow glycerol + soap

Acidic/enzymatic hydrolysis of glycerol tripalmitate



Saponification – alkaline hydrolysis of glycerol tristearate



Fatty acids

FAs usually have even number of carbon and unbranched chains.

Fatty acid chain has a hydrophobic nature, carboxyl groups are polar.

Higher fatty acids do not pass through the mitochondrial membrane (transporter - carnitine).

- Saturated
- Unsaturated

Common fatty acids

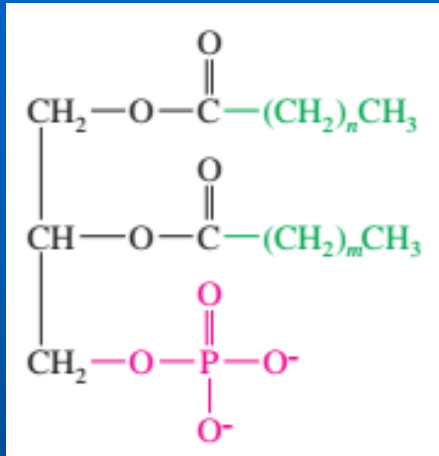
Structural Formula	Chemical Name
$C_{15}H_{31}COOH$	Palmitic acid
$C_{17}H_{35}COOH$	Stearic acid
$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	Palmitoleic acid
$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	Oleic acid
$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	Linoleic acid
$CH_3(CH_2-CH=CH)_3(CH_2)_7COOH$	Linolenic acid
$CH_3(CH_2)_3(CH_2-CH=CH)_4(CH_2)_3COOH$	Arachidonic acid
$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$	Erucic acid

Phospholipids

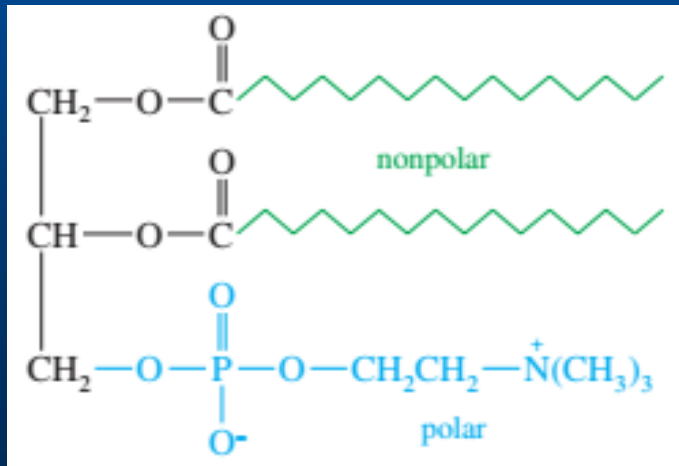
Major components of cell membranes

- **glicerophospholipids** (phosphoglycerides):
 - biologically active factors involved in blood clotting
 - important components for the formation of lipid bilayers of cells
 - key components of the membranes of muscles, nerves and brain
- **sphingophospholipids**
 - important constituent of the myelin sheath surrounding the axon of all nerve cells

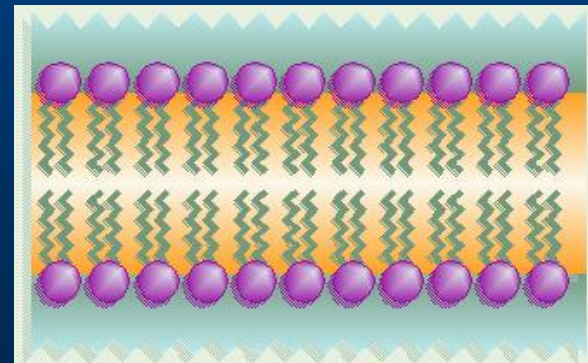
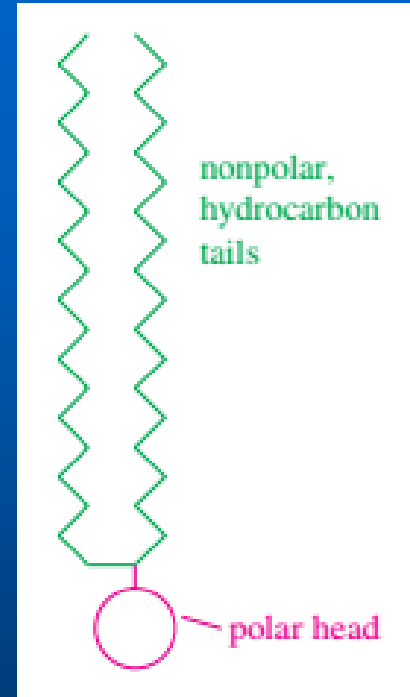
Glycerophospholipids



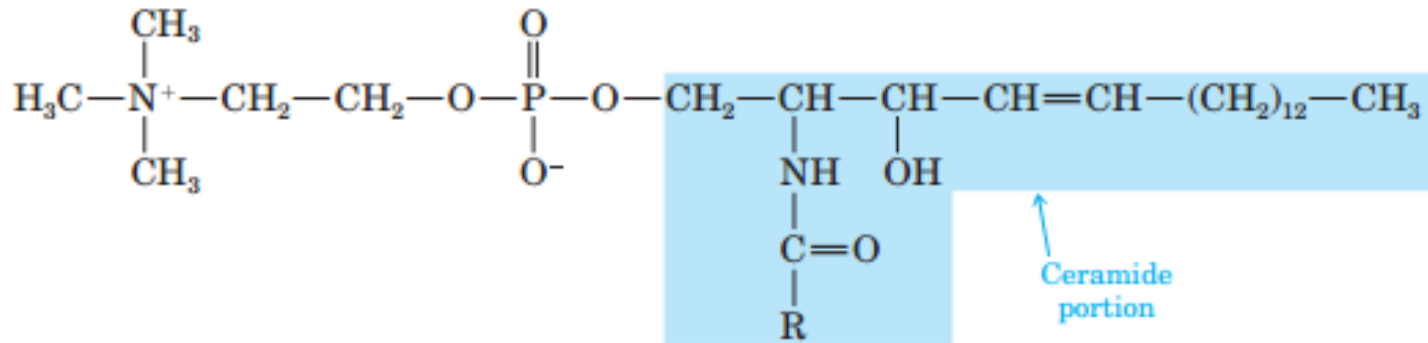
Phosphatidic acid



Lecithin (phosphatidyl choline)



Sphingophospholipids



A sphingomyelin
(a sphingolipid)



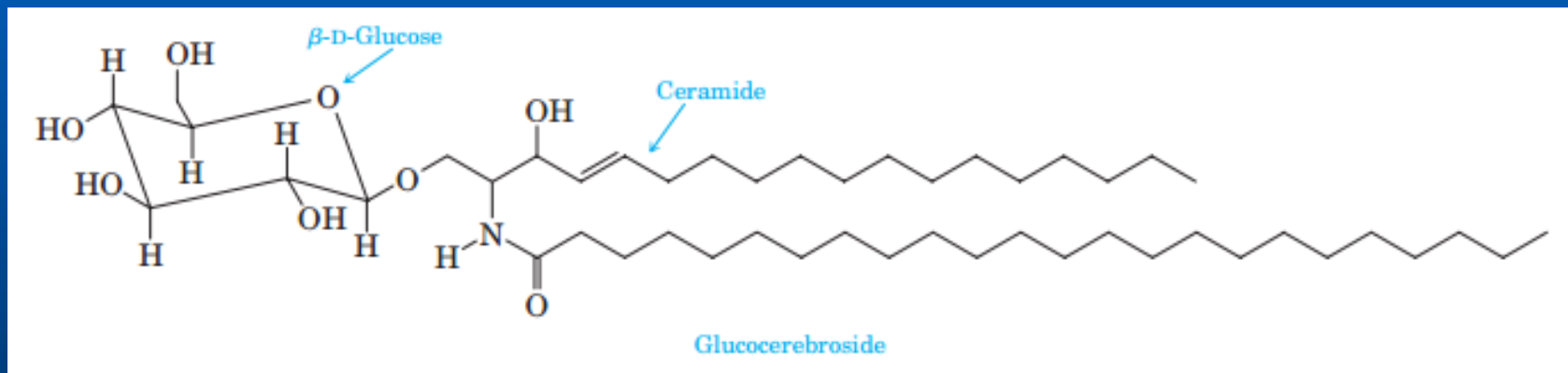
Sphingomyelin



Glycolipids

■ cerebrosides

- present in nerve and brain cells



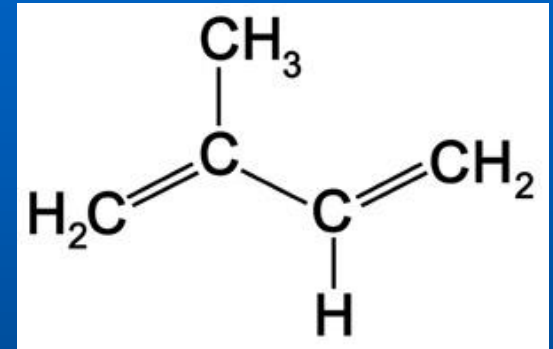
■ gangliosides

- very common as part of nerve cells' outer membranes (sugar sequence leads to cell recognition and communication)

Lipid derivatives

Isoprenoids

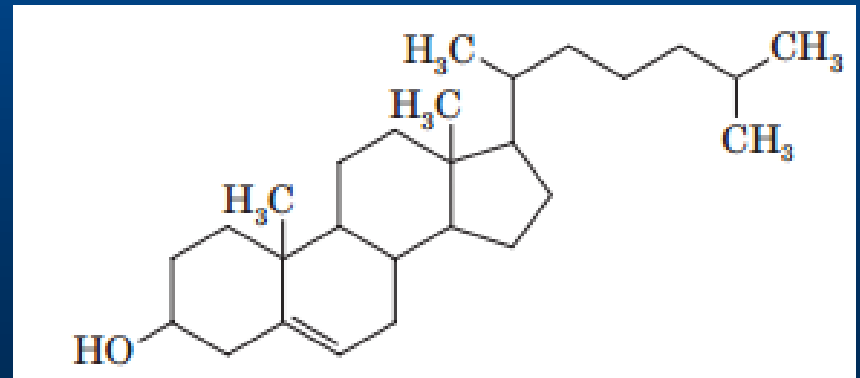
Vitamins: A, E, K



Steroids

Cholesterol is the most abundant steroid precursor of :

- bile acids
- Vitamin D
- steroid hormones



Task 1

Determination of acid number

- **Acid number (AN):** the mass of KOH [mg] that is required to neutralize **free fatty acids** contained in 1g of fat.

$$AN = a \times 5,611 / g$$

- AN is an indicator of fat freshness. It is determined to assess the rancidity of the fat.

WARNING: This task will be done at the end if all the burners are off !

Task 1

Determination of acid number

Procedure:

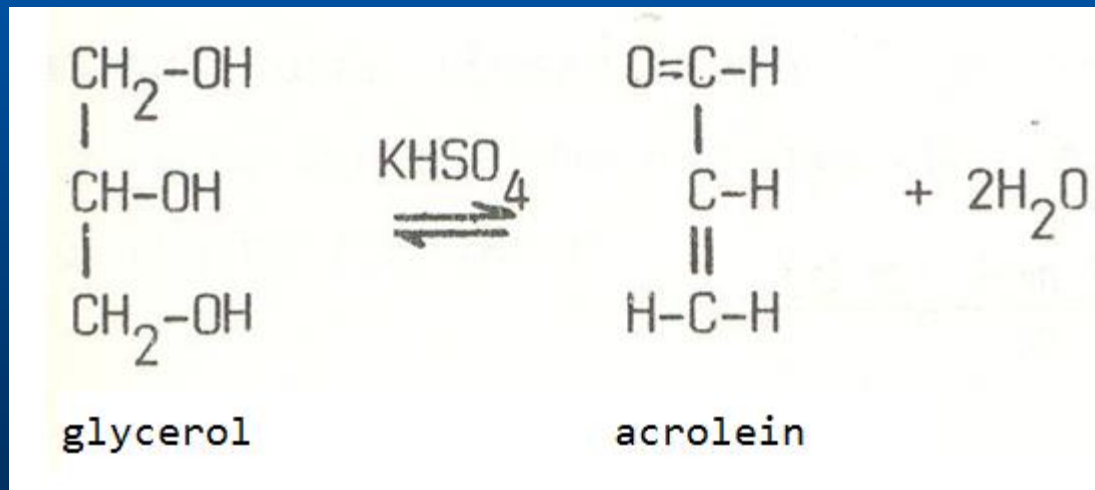
1. Add 5 g of fat (butter) to the flask and heat until it has melted.
2. **Carefully (!)** add 25 cm³ of the alcohol-ether mixture and mix it.
3. Add 5 drops of phenolphthalein and titrate with 0.1 M NaOH to achieve a persistent colour of the indicator (pink)
4. Calculate the acid number (AN) of the fat using the formula.

WARNING: This task will be done at the end if all the burners are off !

Task 2

Dehydration of glycerol to acrolein

- During heating of fat in the presence of potassium hydrogen sulfate (KHSO_4) glycerol loses two molecules of water and it is converted to an unsaturated aldehyde acrolein with an unpleasant smell.



- Acrolein is one of the products of fat rancidity.

Task 2

Dehydration of glycerol to acrolein

Procedure:

1. Add a pinch of potassium bisulfite and 2 drops of oil to the test tube.
2. Carefully heat the tube until the appearance of an irritating odor characteristic of acrolein.
3. Put a strip of filter paper moistened with a solution of diamminesilver (I) complex at the mouth of the test tube.

Filter paper becomes black because vapor emitted acrolein reduces Ag^+ ions to colloidal silver.

Task 3

Detection of the presence of unsaturated bonds

- Unsaturated fatty acids contain multiple bonds, which can bind oxygen (under the influence of KMnO_4) leading to degradation of fatty acids in place of the bond.
- As a result of the oxidation of unsaturated FAs the compounds with shorter chains are created.

Task 3

Detection of the presence of unsaturated bonds

Procedure:

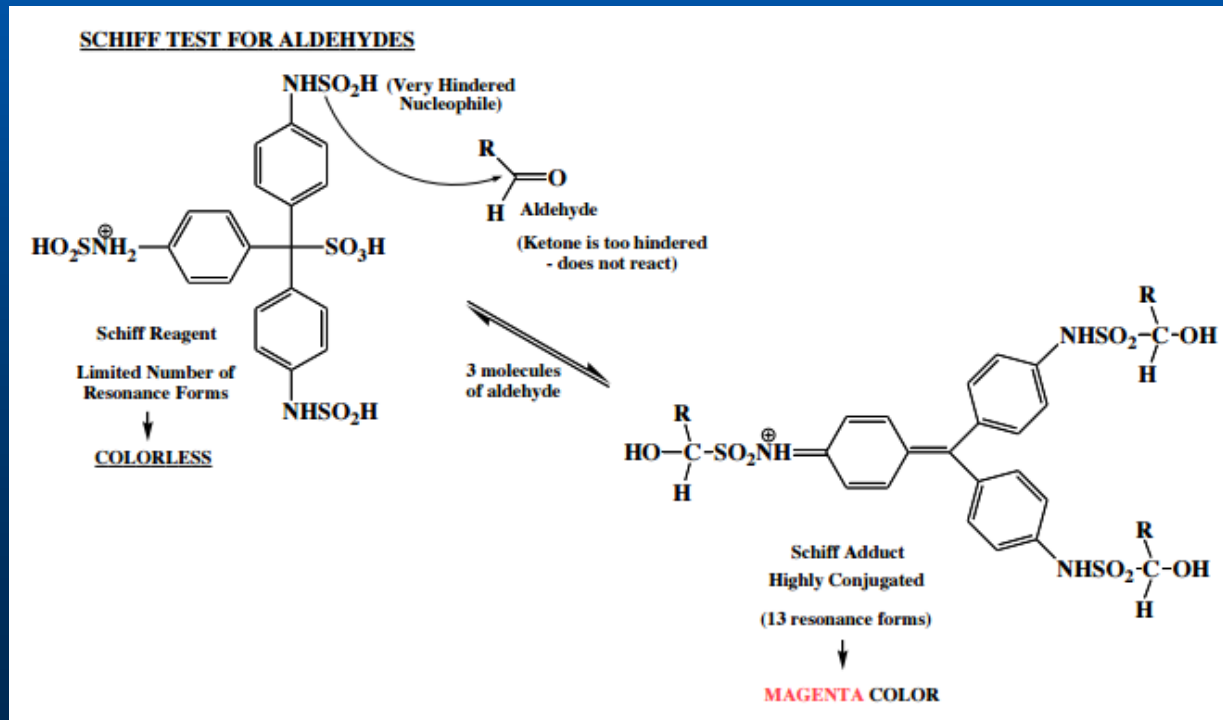
1. Add 5 cm³ of Na₂CO₃ (0,5 mol/dm³) and one drop of oil to the test tube.
2. Heat the sample in a water bath at approx. 50°C for 2 minutes.
3. Add KMnO₄ (0.1 mol/dm³) drop by drop shaking the tube after the addition of each drop until a persistant **slightly pink colour** of the solution appears.

Task 4

Detection of the presence of rancidity products

a) ALDEHYDES

Aldehydes are detected in the reaction with Schiff reagent.



Task 4

Detection of the presence of rancidity products

a) ALDEHYDES

Procedure:

1. Add 2 cm³ of saturated NaCl solution to both test tubes **A** and **B**.
2. Add 0,5 cm³ of **fresh oil** to the test tube **B** and 0,5 cm³ of **rancid oil** to the test tube **A**.
3. Heat test tubes A and B over the burner until they start boiling.
4. Add 5 drops of Schiff reagent to each tube.
5. Compare the developed colours of the samples.

Task 4

Detection of the presence of rancidity products

b) FFAs

- The presence of FFAs causes the decrease of the pH of the solution.
- Bromophenol blue is an acid–base indicator and its useful range lies between pH 3.0 and 4.6:
 - at $\text{pH} \leq 3.0$ is yellow
 - at $\text{pH} \geq 4.6$ is blue

Task 4

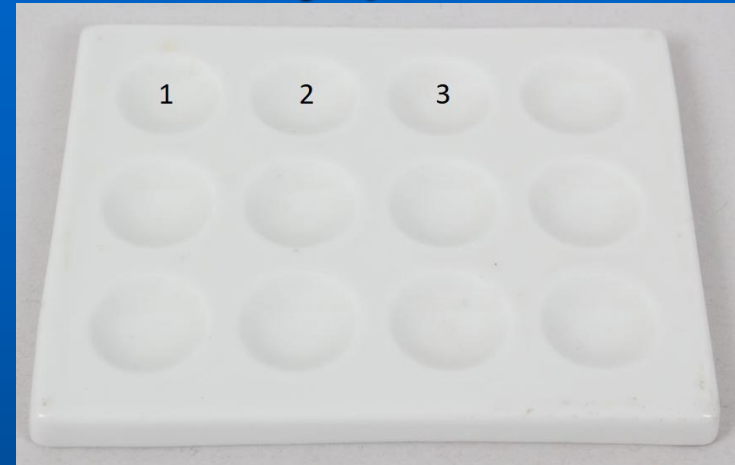
Detection of the presence of rancidity products

b) FFAs

Procedure:

1. Take a porcelain plate and add drops as follows:

- 1) to the 1st well: 3 drops of distilled water,
 - 2) to the 2nd well: 2 drops of distilled water and 1 drop of rancid oil,
 - 3) to the 3rd well: 2 drops of distilled water and 1 drop of fresh oil.
2. Add 1 drop of bromophenol blue to all three wells.



Tasks 5, 6 and 7

Examination of fats solubility

- Simple lipids are insoluble in water, while they are soluble in the apolar organic solutions.
- Similar properties have dyes, like Sudan III.
- Compounds, having in their structure hydrophilic and hydrophobic parts, act as detergents and facilitate the formation of an emulsion of the fat in aqueous phase.

REMINDER

1. We start with task # 2
2. We won't do task # 1 unless **everyone reports** that all the other tasks have been completed.