Experiment 12 –Lipids

Lipids are a class of biological molecules that are insoluble in water and soluble in nonpolar solvents. There are many different categories of lipids and each category has different components present in its structure.

Fatty acids are components of many types of lipids. Fatty acids are carboxylic acids with very long hydrocarbon chains, usually 12-18 carbon atoms long. Even though these carboxylic acids can hydrogen bond with water, they are insoluble because of the length of their hydrocarbon chains. Fatty acids can be saturated or unsaturated. A saturated fatty acid contains no carbon-carbon double bonds, so it is "saturated" with hydrogen. Unsaturated fatty acids contain one or more *cis* double bonds. (Very few naturally occurring fatty acids contain *trans* double bonds.) The presence of *cis* double bonds has an important effect on the melting point of the fatty acid. *Cis* double bonds form rigid kinks in the fatty acid chains (remember that there is no rotation around a double bond), and the result is that unsaturated fatty acids can not line up very well to give a regularly arranged crystal structure. Saturated fatty acids, on the other hand, line up in a very regular manner. The result of this is that saturated fatty acids have high melting points and are usually solids at room temperature. Unsaturated fatty acids, however, have low melting points and are usually liquids at room temperature. The names, structures, and melting points of some common fatty acids are shown in the table below.

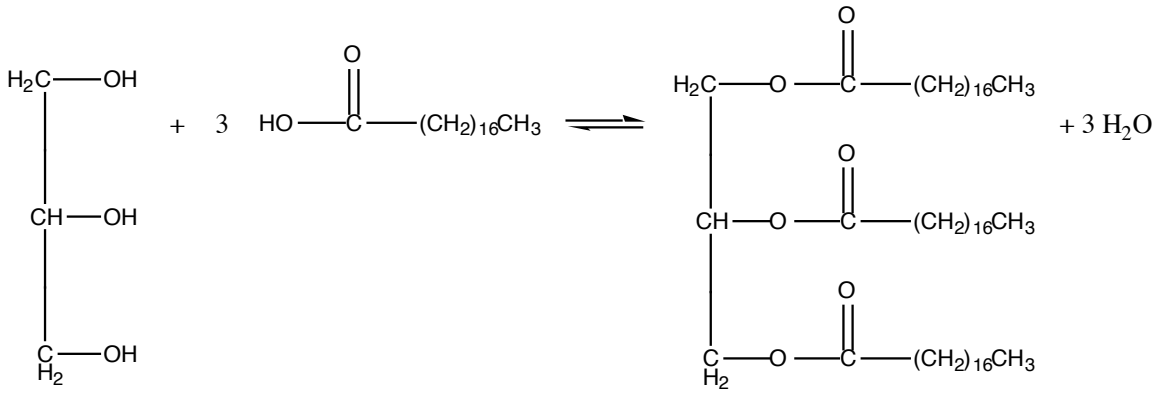
Structures of Common Fatty Acids			
Name	Number of carbons	Structure	Melting Point (°C)
Saturated Fatty Acids			
Lauric acid	12	$CH_3(CH_2)_{10}COOH$	44
Myristic acid	14	$CH_3(CH_2)_{12}COOH$	58
Palmitic acid	16	$CH_3(CH_2)_{14}COOH$	63
Stearic acid	18	$CH_3(CH_2)_{16}COOH$	70
Unsaturated Fatty Acids (all double bonds are *cis*)			
Palmitoleic acid	16	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$	-1
Oleic acid	18	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$	4
Linoleic acid	18	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	-5
Linolenic acid	18	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$	-11

Waxes are lipids that are used in nature as protective coatings. Structurally, a wax molecule is an ester of a long-chain alcohol and a long-chain fatty acid. Naturally occurring waxes are mixtures of different molecules. There are natural waxes present on the surfaces of many fruits and leaves, in beeswax, and on the feathers of aquatic birds.

Fats and oils both belong to a class of molecules called **triacylglycerols** or **triglycerides**. Fats usually come from animal sources and are solids at room temperature, and oils are generally from plant sources and are liquids at room temperature. Triglycerides are triesters of glycerol and three fatty acid molecules. The fatty acids in the triglyceride can be the same or different. Naturally occurring fats and oils are typically mixtures of different triglycerides. The melting point of a particular fat or oil depends on the proportions of saturated and unsaturated fatty acid components present. For example, butter (which is a fat) contains about 30% unsaturated fatty acids and about 70% saturated fatty acids and cholesterol. Corn oil contains about 88% unsaturated fatty

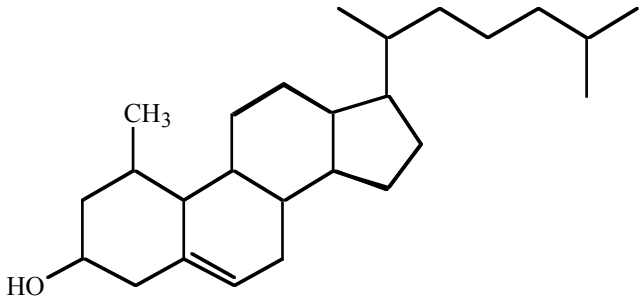
acids and about 12% saturated fatty acids. In general, the higher the degree of unsaturation, the lower the melting point of the fat or oil.

Shown below is the reaction of a molecule of glycerol with three molecules of stearic acid to form a triglyceride molecule and three molecules of water.



Glycerol Stearic Acid Glyceryl tristearin

Cholesterol is a steroid and has a very different structure from other types of lipids. The structure of cholesterol is shown on the next page. It is classified as a lipid because it is nonpolar and therefore insoluble in water.



Cholesterol

Phospholipids contain a charged phosphate and a charged amino alcohol in addition to having long nonpolar chains. Therefore, they have a dual nature – one end of the molecule is charged and therefore compatible with water, and the other end is nonpolar and therefore compatible with nonpolar substances. Phospholipids are the main components of cell membranes, where they are arranged in a lipid bilayer. The charged ends face the solvent (water), and the nonpolar ends face each other in the interior of the membrane. Phospholipids can be further classified as **glycerophospholipids** or **sphingolipids**. Glycerophospholipids contain glycerol, 2 fatty acids, a phosphate group, and an amino alcohol. Phosphate-containing sphingolipids contain sphingosine, one fatty acid, a phosphate group, and an amino alcohol.

Glycolipids contain either glycerol or sphingosine, fatty acids, and one or more monosaccharides. **Fat-soluble vitamins** are also classified as lipids, simply because they are fat-soluble. Your textbook shows structures of all of these types of lipid molecules.

Properties of Lipids

In this experiment, you will study the solubility of several lipids in water and in a less polar solvent, methylene chloride (CH_2Cl_2). You will also test several lipids with bromine to determine the degree of unsaturation of the lipid.

You may remember that alkenes react with bromine to give dibrominated compounds. The bromine adds to the double bond, and one bromine atom is placed on each side of the double bond. The resulting compound contains only single bonds. Many substances can add across a C=C double bond, such as H_2, Cl_2, Br_2, HCl, HBr, HI, and H_2O. However, bromine is especially useful since it has a color (the other molecules are colorless). The addition product of bromine, however, is colorless. You can therefore tell if bromine is reacting with something based on the color of the solution: if the orange color of bromine fades as it is added to the other reactant, then the bromine is reacting with one or more double bonds. The more double bonds present, the more bromine will be needed to completely react with the substrate. If the orange color of bromine remains when just one drop of it is added to a reactant, it means that the bromine did not react with anything and therefore there are no C=C double bonds present in the substrate. In this experiment, you will add a bromine solution one drop at a time to various lipids until the orange color persists (which means that it no longer fades away). The number of drops of bromine needed for the solution to remain orange will tell you the relative degree of unsaturation of the compounds you are comparing.

Safety Precautions:

- Wear your safety goggles.
- Use the bromine in the hood and do not breathe its vapors.
- Avoid contact with the bromine solution – it can cause burns.

Waste Disposal:

- All waste must be placed in the **organic** waste containers (which have a pink label) in one of the fume hoods.

Procedure

Part 1 – Models of Triacylglycerols (Triglycerides)

1. Construct molecular models of glycerol and 3 molecules of acetic acid. Draw the condensed structural formula of each.
2. Use the models from step 1 to make a model of a triglyceride that contains all of the above molecules. You will need to form 3 ester bonds. Three molecules of water are removed in this process. Write the equation for this reaction on your report sheet – this is the formation of glyceryl acetate. The structure of this triglyceride is similar to the structure of fats and oils, except fats and oils have much longer carbon chains.

3. Using the model of glyceryl triacetate, hydrolyze the ester bonds by adding one molecule of water to each. Write the equation for this reaction. This hydrolysis reaction is usually catalyzed by acid or an enzyme.

Part 2 - Physical Properties of Lipids and Fatty Acids

4. Label 7 clean, dry test tubes. Put a small sample of each of the following lipids in separate test tubes: olive oil, safflower oil, stearic acid, oleic acid, lecithin, cholesterol and vitamin A. If the lipid is a solid, use a very small amount on the tip of a spatula. If the lipid is a liquid, use 5 drops. If the vitamin A is given as a capsule, you will need to puncture it and squeeze out some of the liquid inside to test it.
5. Classify each of the lipids as a triglyceride, fatty acid, steroid, or phospholipid.
6. Describe the appearance and odor, if any, of each of the lipids.
7. Add 20 drops of methylene chloride to each tube and shake each of the tubes to mix the solutions well. Determine whether each of the lipids is soluble or insoluble in methylene chloride. Record your observations on the report sheet. **Save these solutions for part 3!**
8. Label 7 clean test tubes. (The tubes do not have to be dry this time.) Put a small sample of each of the lipids in separate test tubes, as you did in step 4.
9. Add about 2 mL of deionized water to each test tube and shake each tube to mix thoroughly. Determine whether each of the lipids is soluble or insoluble in water. Record your observations on the report sheet.

Part 3 - Bromine Test

10. For this part, you will need the samples from step 7. To each solution, add 1% bromine solution drop by drop, shaking the tube after each drop, until the solution remains orange. If you add 1 drop of bromine and the orange color of the bromine does not disappear, do not add any more bromine. If your sample contains no double bonds, it will not react with bromine and the orange color of bromine will not disappear, even if only one drop of bromine is added. If your sample contains double bonds, it will react with the added bromine, and the orange color will disappear. The more double bonds present in the sample, the more bromine will be needed to react with the sample. The orange color of bromine remains in the solution when all of the double bonds have reacted.
11. Record your observations – you will need to notice whether the orange color fades rapidly or persists in each case.

Questions

1. What functional group is present in a triglyceride?
2. What functional group is present in a fatty acid?
3. Draw the structure of oleic acid.
4. Draw the structure of glyceryl triolein.
5. What do lipids have in common?
6. What type of solvent would be needed to remove an oil spot? Why?
7. The melting point of stearic acid is 70°C, and the melting point of oleic acid is 4°C. Explain in detail why their melting points are so different.
8. Based on part 3 of the experiment, which oil is more unsaturated, safflower oil or olive oil? Explain.
9. Which should have a higher melting point, safflower oil or olive oil? Explain your reasoning.
10. What components are present in a phosphoglyceride?

11. Why is it that phosphoglycerides are found in cell membranes?