EXPERIMENT 11

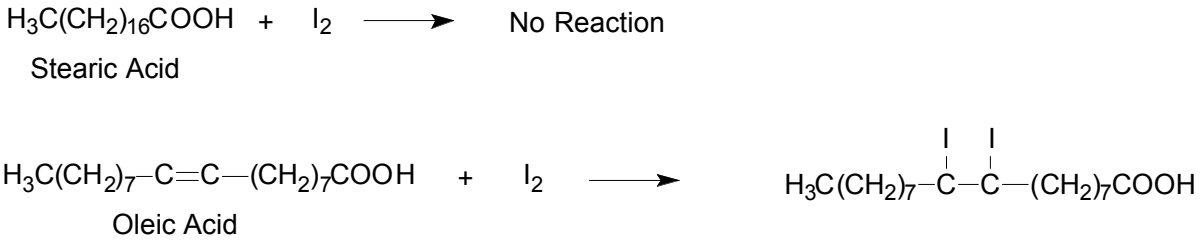
The Chemistry of Lipids

INTRODUCTION

Lipids, by definition, are natural substances that do not mix with water but dissolve in organic solvents. There are several classes of lipids, including: fatty acids, waxes, triacylglycerols (fats and oils), phospholipids and steroids. The fatty acids are usually not free in nature, but are components of triacylglycerols, waxes and phospholipids. Animal fats or vegetable oils (especially palm oil) are used to make soap. In the **saponification** process a solution of sodium hydroxide (lye) is heated with fats to hydrolyze the ester bonds that link the fatty acids to glycerol. There are 3 acids linked to each glycerol, hence the name tri-acyl-glycerol. Reaction with sodium hydroxide results in formation of the sodium salt of the fatty acids, which is known as soap. The chemical reaction for saponification is:

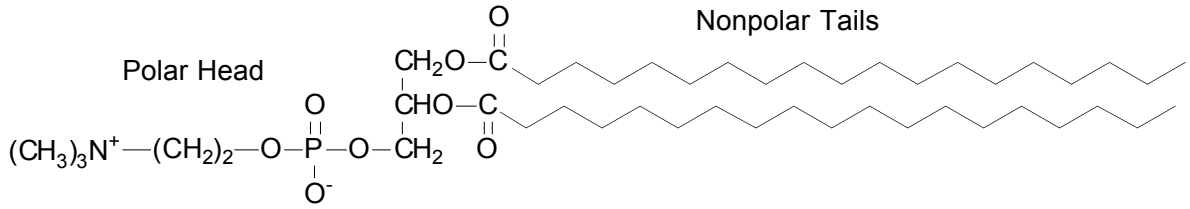
Fatty acids are classified as **saturated or unsaturated**, according to whether they have carbon-carbon double bonds or not. The carbon-carbon double bonds are sites where the molecules are not saturated with hydrogen. The double bond is very susceptible to chemical attack. One substance that readily attacks these double bonds is the element iodine (I_2). The product of this reaction is an iodinated fatty acid (see the reaction below). One measure of the degree of unsaturation of fats and oils used by food chemists is known as the **iodine number**.

Phospholipids, such as lecithin, have a polar or charged portion and a nonpolar portion



consisting of the long chain fatty acids within the same molecule. Consequently the polar or charged portion of these molecules will mix with water and the nonpolar portion repels the water but mixes with lipids.

These phospholipids will form small globules (more or less spherical) in water that make the water look opaque or milky. When lipids mix with water, this is known as **emulsification** and



Phosphatidyl Choline (Lecithin)

the mixture is known as an **emulsion**. This is what gives milk its opaque appearance. This is also the process by which cells form membranes, where the **phospholipid membrane** acts as a barrier between the watery areas inside and outside the cell.

In this lab you will be testing the solubility of some lipids, in particular the fatty acids, triacylglycerols and phospholipids. You will also determine the degree of unsaturation of some lipids by determining how much iodine they will absorb (or react with). You will also determine the lipid content of some foods by extracting the fats and oils into a nonpolar organic solvent, evaporating the solvent and measuring the amount of lipid extracted.

CAUTION!! MAKE SURE THERE ARE NO OPEN FLAMES NEAR YOU WHEN DOING THIS EXPERIMENT. PETROLEUM ETHER IS VERY FLAMMABLE.

MATERIALS NEEDED

Lecithin (solid), ethanol, petroleum ether, glycerol, stearic acid, oleic acid, vegetable oil, 1% starch solution in water, 0.5% iodine solution in petroleum ether, food samples for extraction of lipids, test tubes, 250 mL Erlenmeyer flask, and 50 mL or 100 mL beaker.

PROCEDURE

Part 1. Solubility of Lipids

Add about 0.1 g of lecithin to a small beaker and add about 25 mL of deionized water. Swirl or stir the mixture until the lecithin dissolves or becomes suspended in the water. While you are waiting for the lecithin to dissolve, set up 9 small test tubes and 6 large test tubes that are clean and dry. You will be testing solubility in 3 different solvents: water, ethanol (alcohol) and petroleum ether (a nonpolar organic solvent). You also will be observing the effect of lecithin, a phospholipid, on the ability of lipids to mix with water.

A) Add a few drops of glycerol to each of 3 separate small test tubes. The structure of glycerol is shown in the introduction. In which of the 3 solvents would you expect glycerol to dissolve? Add about 2 or 3 mL of water to one of the test tubes containing glycerol, shake it and see if the glycerol dissolves. Add 2 or 3 mL of ethanol to a second test tube containing glycerol, shake it and see if the glycerol dissolves. Add the same amount (2 or 3 mL) of petroleum ether to a third test tube containing glycerol, shake it and see if the glycerol dissolves.

Caution! Avoid getting petroleum ether on your skin or clothes.

Record your results in your notebook. You should set up a table for the solubilities as shown on the next page.

Solubility of Lipids

Tubes to Use6	7Large Tubes6		7Small Tubes6		Add to Water
	Water	Lecithin	Ethanol	Petroleum Ether	NaOH Soln

Glycerol					
Stearic Acid					
Oleic Acid					
Vegetable Oil					

B) Repeat the solubility test above for the following lipids, in place of the glycerol. Add a pinch (a small amount on the end of a spatula) of stearic acid (a solid) to each of two small test tubes and two large test tubes and label them SA (for stearic acid). Add a few drops of oleic acid to each of two small and two large test tubes and label them OA. Add a few drops of vegetable oil to each of two small and two large test tubes and label them VO.

Test the solubility of each of these as you did for the glycerol by adding 2 or 3 mL of either ethanol or petroleum ether (separately) to the small test tubes containing stearic acid, oleic acid and vegetable oil. Shake each to determine whether it dissolves in either of these two solvents. You should describe in your notebook what happens to each lipid when solvent is added.

Add 2 or 3 mL of either water or lecithin solution (separately) to the large tubes containing these lipids and vigorously shake each to determine whether it dissolves. Look closely at the tubes and describe what happens in your notebook. Does each dissolve in water? Do you see the same thing happen when lecithin solution is added to the lipids (compared to when you added water)?

CAUTION! Sodium Hydroxide (NaOH) is caustic, avoid getting the sodium hydroxide on your skin or clothes.

After you have recorded these observations, add about 2 mL of 1.0 M NaOH solution to the tubes containing lipid and water. Shake again. Record in your notebook what happens when you add sodium hydroxide to each lipid in water. Give an explanation for these results. What have you formed by adding the sodium hydroxide solution to the fatty acids? Do you get the same result with vegetable oil? Why or why not?

Part 2. Measuring Unsaturation of Lipids with Iodine.

Add about 1 g of stearic acid to one clean large test tube and about 1 mL of oleic acid to another clean large test tube. Be sure to label the tubes to avoid confusion. Add 2 or 3 mL of petroleum ether to each test tube and swirl to dissolve each fatty acid. Do you notice any difference in these solutions? Add several drops (5 to 10) of starch solution to each tube as an indicator that turns blue when all the sites of unsaturation (double bonds) have reacted with iodine. What happens to the starch solution when you add it to the petroleum ether solution? Swirl the tubes and allow the starch solution to settle to the bottom of each tube before proceeding.

Pour 5 to 10 mL of 0.5% iodine (I_2) solution (in petroleum ether) into a small beaker; note the color. Add a few (2 or 3) drops of the iodine solution to the test tube containing stearic acid and swirl to mix thoroughly. Does the color disappear? Does the starch solution in the bottom of the tube change color? If the starch solution changes to a deep blue color, that means there is excess iodine in the solution and there are no double bonds to react with the iodine. If there is no change in the color of the starch solution at the bottom, place a stopper on the tube and shake it to mix the two layers of liquid. If there is still no change in color of the iodine solution, add a few more drops of the iodine solution and shake again. Record the number of drops needed to just cause the iodine solution to change color. How many drops of iodine solution were needed to completely react with the stearic acid?

Now add a few drops of iodine solution to the tube containing oleic acid in petroleum ether. Do you see any change in the oleic acid/petroleum ether solution? Is there any change in the starch solution at the bottom of the tube. Swirl the solution well to get the two layers in the tube to mix. Continue adding the iodine solution to the oleic acid solution, a few drops at a time, mixing well after each addition to determine whether the starch solution changes color in the bottom of the tube. Keep track of the number of drops of iodine solution needed to get the starch solution in the bottom of the tube to change to a deep blue color.

Part 3. Extraction of Lipids from Foods.

Weigh a small amount of solid food, such as potato chips, bran muffin, donut or

croissant. You should use between 10 and 20 grams of food. Place this food sample in a large Erlenmeyer flask and break the sample into small pieces to facilitate the extraction of lipids (larger surface area). Add 25 mL of petroleum ether to the flask containing your food sample and swirl the flask for several minutes to get the lipids to dissolve in the petroleum ether.

While you are waiting for the lipids to dissolve, weigh a clean, dry 100 mL beaker as accurately as possible. Be sure the balance is set at zero before placing the beaker on the pan to weigh it. Record the mass of the empty beaker to the nearest 0.01 g in your notebook. Carefully decant the petroleum ether from the flask containing your food sample to the weighed beaker. **Avoid getting the solid portion of the food into the beaker, only the clear liquid**. Place the beaker on a hot plate in the hood to evaporate all of the petroleum ether. When you think it has all evaporated, check to see that there is no longer a strong odor characteristic of petroleum ether in the beaker. There may be a mild odor of the lipid. After the beaker has cooled to room temperature, weigh it again and record the weight of the beaker plus lipid. The difference between the weight of the beaker containing the extracted lipid and the empty beaker will give the weight of the lipid you extracted from the food sample.

Calculate the percent weight of fat in your food sample by dividing the weight of the lipid by the weight of the original food sample and multiply by 100%.

$$\text{Percent Lipid in Food Sample} = \frac{\text{Weight of lipid extract (g)}}{\text{Wt. of original food sample (g)}} \times 100\%$$

You can determine the number of Calories (kilocalories) of energy derived from fat in 100 g (about 3.5 ounces) of this food by multiplying 9 Cal/g fat times the answer for percent lipid obtained above. Record in your notebook the amount of Calories you would expect to get from a full serving of your food (a typical serving is usually considered to be 100 g, unless noted otherwise). How many ounces in 100 g? How much of this food would you normally eat for a single serving?

<u>Questions to answer in your notebook.</u>

1. What are some common commercial uses for glycerol? How can glycerol be obtained commercially (see introduction)? Would you expect glycerol to be toxic to humans?

Explain.

2. What was the substance formed when you added sodium hydroxide to the mixture of stearic or oleic acid in water?

3. Is there any difference in the amount of iodine solution needed to complete the reaction with oleic acid compared to the amount needed for the stearic acid solution? How do you explain this difference?

4.* Oleic acid and vegetable oil are both liquid lipids. Would you say they both have the same chemical properties? Explain your answer using observations from this lab regarding solubility and reaction with NaOH and the iodine test for unsaturation.