The Chemistry of Lipids

Experiment #8

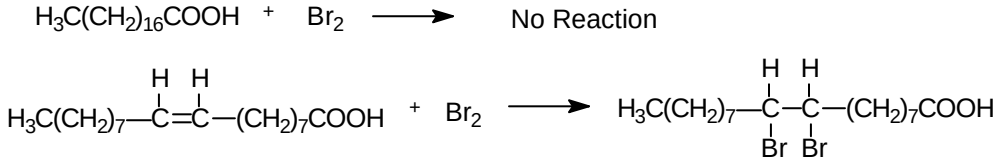
Objective

To observe the solubility of lipids in polar and nonpolar solvents and to compare saturated and unsaturated fats in their chemical reaction with bromine. The percent fat in a food will be determined by extraction of the fat and weighing it.

Introduction

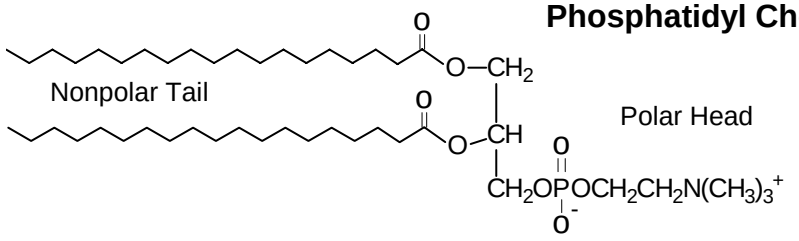
Lipids, by definition, are natural substances that do not mix with water but dissolve in organic solvents. There are several classes of lipids, including: fatty acids, waxes, triacylglycerols (fats and oils), phospholipids and steroids. The fatty acids are usually not free in nature, but are components of triacylglycerols, waxes and phospholipids. Animal fats or vegetable oils (especially palm oil) are used to make soap. You prepared soap in an earlier experiment.

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 and are susceptible to chemical attack. One substance that readily attacks these double bonds is the element bromine (Br_2). The product of this reaction is a brominated 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, where iodine is used in place of bromine for this reaction.



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.

Phosphatidyl Choline (Lecithin)



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

Cholesterol reacts with acetic anhydride when sulfuric acid is used as a catalyst for dehydration, to form a series of colors from red through violet and blue-green. The products of the reaction that produce these colors are not well understood.

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 bromine they will absorb (or react with). You will study a color test for cholesterol and determine the lipid content of 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.

Note: All heating should be done with hot plates.

Procedure

Part A. Solubility of Some Lipids.

You will need 6 large and 6 small test tubes containing the four test lipids as outlined in the table on the Report Sheet. To avoid confusion in the procedure, refer to the table at the beginning of the Report Sheet. There will be 1 large tube and 2 small tubes containing each test lipid. The large tube (left column of table) will contain lipid with petroleum ether; the small tubes (2 right columns) will contain lipid with either ethanol or water.

1. Add 3 drops of oleic acid (a liquid) to 2 small test tubes and 1 large test tube and label them OA (for oleic acid).

2. Using a spatula, add a small amount (equivalent in mass to 3 drops of liquid) of stearic acid (a solid) to each of 2 clean small test tubes and 1 clean large test tube and label them SA (for stearic acid).

3. Add 3 drops of vegetable oil to 2 clean small test tubes and 1 clean large test tube and label them VO.

4. Using a spatula, add a small amount (the size of a pencil eraser) of vegetable shortening to 1 clean large test tube and label it VS. Add a small amount of lard to another clean large test tube and label it Lard.

5. Add about 3 mL of lecithin solution to 1 large test tube and label it PL (for phospholipid).

6. Add about 3 mL of petroleum ether to each of the large test tubes containing the different lipids: OA, SA, VO, VS and PL. Shake each tube well and record the solubilities and observations in the table on the Report Sheet. What happens to the lecithin?

7. Add about 3 mL of ethanol to one small tube labeled SA, add 3 mL ethanol to one small tube labeled OA, and add 3 mL ethanol to one small tube labeled VO. Shake each tube well to see if the lipid dissolves in alcohol. Record solubility of each lipid in ethanol in the table on the Report Sheet.

8. Add about 3 mL of deionized water to each remaining small test tube (not the tube you added ethanol to) and shake well to see if each lipid dissolves in water. Record your observations in the table on the Report Sheet.

[**Note**: Save petroleum ether mixtures for part B]. Dispose of the water and ethanol solutions in the sink. You will need detergent to remove the lipid residues from the small test tubes.

Part B. Measuring Unsaturation of Lipids with Bromine/Water

Note: Bromine/Water Solution is very caustic; use it in the hood and avoid getting it on your skin.

1. Using the labeled solutions of lipids in petroleum ether that you prepared above, add 5 drops of bromine/water solution (under the hood) to the test tubes, one at a time, and shake the tube well using a cork to stopper the test tube. Does the yellow color of bromine disappear? If it doesn't there are no double bonds (unsaturation) in the test compound. If the yellow color disappears add another 5 drops of bromine/water solution and shake well.

2. Record your observations for each tube in the table on the Report Sheet. Notice there will be two layers of liquid, the organic solvent (petroleum ether) and the water from the bromine water solution. Be especially observant with the lecithin mixture.

3. **Dispose of these solutions in the "Halogenated" Organic Waste Container in the fume. Do not pour the contents of these tubes into the sinks.**

Part C. Lieberman-Burchard Test for Cholesterol

Caution: Acetic anhydride and concentrated sulfuric acid are very caustic. Handle them with care and use them only in the fume hood.

1. Place 10 drops of 1 % solution of cholesterol in chloroform in a small test tube. Add 5 drops of acetic anhydride and mix well. Then add 3 drops of concentrated sulfuric acid and mix well again. Record any color changes that take place. Allow the tube to stand for 5 minutes and note any further changes in color.
2. Add a small amount (size of pencil eraser) of lard to another small test tube and dissolve this in about 2 mL of chloroform. After the lipid completely dissolves, add 5 drops of

acetic anhydride and mix well. Then add 3 drops of concentrated sulfuric acid and mix again. Take note of any color changes that take place over the next 5 minutes.

3. Add 2 drops of vegetable oil to a clean small test tube and dissolve it in 10 drops of chloroform. After dissolving it, add 5 drops of acetic anhydride and mix well. Then add 3 drops of concentrated sulfuric acid, mix well again and take note of any color changes over the next 5 min.

4. Record all observations on the Report Sheet.

Part D. Extraction of Lipids from Foods.

{See diagram at the end of this section}

1. Accurately weigh a small amount (10 to 20 g) of solid food, such as potato chips, bran muffin, donut or croissant. You should note and record any nutrition facts on the bag or container of food, if it is given.

2. Record the weight of your sample on the Report Sheet.

3. Crush or crumble the food sample into small pieces and place it in a large Erlenmeyer flask. (See diagram on next page).

4. 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.

5. While you are waiting for the lipids to dissolve, weigh a clean 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.

6. Record the mass of the beaker to the nearest 0.01 g on the Report Sheet.

7. Carefully decant the petroleum ether from the flask containing your food sample to the weighed beaker. (Pour only the clear liquid, not the solid portion of the food).

***** Dispose of the remaining solid food in the Solid Organic Waste container in the fume hood *****

8. Place the beaker on a hot plate or water bath in the hood to evaporate all of the petroleum ether.

DO NOT ATTEMPT TO EVAPORATE THE PETROLEUM ETHER WITH A FLAME!!!

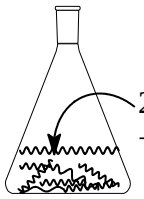
9. When you think it has all evaporated, check to see that there is no longer a strong smell

characteristic of the petroleum ether, and allow the beaker to cool. You may put it in a large beaker with some cold water. Do not get water inside the beaker of lipid.

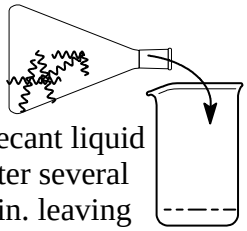
10. After the beaker has cooled to room temperature, make sure it is dry on the outside and weigh it again.

11. Record the weight on the Report Sheet.

12. Calculate the mass of lipid extracted from the food sample and determine the weight percent of fat in that food. How does your value compare with the value on the food wrapper (if it was available)?

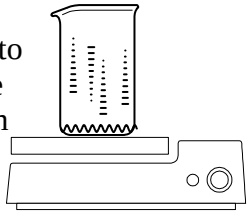


25 mL Petroleum ether
+ 10 g crushed food



Decant liquid after several min. leaving solids in flask

Heat on hot plate to evaporate petroleum ether



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Chemistry of Lipids

Experiment #8 Pre-Lab Exercise

1. Describe the differences between saturated fatty acids and unsaturated fatty acids in terms of their chemical structures. What is a polyunsaturated fatty acid?

2. What kinds of chemical reactions might be observed with unsaturated fatty acids that would not be observed with saturated fatty acids. Show the chemical reaction that would take place for the unsaturated fatty acid.

3. Cholesterol is the most abundant steroid in the human body. Describe at least 3 important functions for cholesterol in the body, either direct functions or as a precursor for other substances.

Name _____ Section _____

4. What are *trans*-fatty acids? In what kinds of foods are these *trans*-fatty acids found? Are *trans*-fatty acids saturated or unsaturated fatty acids?

5. Many processed foods are labeled "cholesterol free". From what food sources would these foods be made? Animal or vegetable?

Name _____ Section _____

The Chemistry of Lipids

Experiment #8 Data & Report Sheet

Parts A. Solubility of Lipids

	Large Tubes	Small Tubes	
	Petroleum Ether	Ethanol	Water
Stearic Acid			
Oleic Acid			
Vegetable Oil			
Veg. Shortening		xxx	xxx
Lard		xxx	xxx
Lecithin Observation?		xxx	xxx

1. From the results of these solubility tests, how would you classify ethanol as a solvent relative to water and petroleum ether? Polar, nonpolar, or both?

Part B. Test for Lipid Unsaturation with Bromine

	Stearic Acid	Oleic Acid	Vegetable Oil	Vegetable Shortening	Lard	Lecithin
Color after adding Br_2						
Saturated or Unsaturated						

2. Explain the difference in the color of bromine solution in the tube with oleic acid compared to the tube with stearic acid? What is happening if the color changes?

Name _____ Sec _____

3. Did you observe any change in color of the bromine solution in the tube with vegetable oil compared to vegetable shortening? Explain your observations. How is vegetable shortening made? Read the label to see if there is any indication.

Part C. Test for Cholesterol

	Cholesterol Solution	Lard	Vegetable Oil
Color Changes Observed			

4. From your results in the test for cholesterol, which of the food lipids that you tested contain cholesterol?

Part D. Extraction of Lipid from Food

From what food item are you extracting lipids? _____

Step		
1	Mass of Food Sample	_____ g
10	Mass of Beaker and Lipid	_____ g
6	Mass of Empty Beaker	_____ g
	Mass of Lipid Extracted (Subtract mass of empty beaker from mass of beaker + lipid)	_____ g

Weight percent of lipid in food: _____ percent
(100% x mass of lipid extracted/total mass of food sample used for extraction)

5. Determine the number of Calories (kilocalories) of energy derived from fat in 100 g of this food by multiplying 9 Cal/g fat times the answer for % lipid obtained above. How does your result compare with the amount indicated on the label?