Introduction

Lipids are naturally occurring substances that are arbitrarily grouped together on the basis of their insolubility in water (a polar solvent) and solubility in nonpolar solvents. Lipids include a wide variety of different substances, but are commonly subdivided into several classes based on structural similarities.

Discussion

A. Classes of Lipids

Triacylglycerols (Fats and Oils)

The triacylglycerols, commonly called fats and oils, are esters of glycerol (an alcohol) and fatty acids (long chain carboxylic acids). They are generally formed by a dehydration reaction as shown below in Figure 1. Triacylglycerols differ in the types of fatty acids attached to the glycerol backbone. The fatty acid always contains an even number of carbon atoms, commonly ranging between 10-20 carbon atoms long. The hydrocarbon chain on the fatty acid can be either saturated (contains only C-C single bonds), or unsaturated (containing one or more C=C bonds. Fats are solids, obtained primarily from animals, and contain a larger proportion of saturated fats, while oils are liquids obtained primarily from plants and contain a greater proportion of unsaturated fatty acids.

![Figure 1: Formation Reaction for Triacylglycerols](image1)

Phospholipids

Phospholipids are similar in structure to triacylglycerols but replace one fatty acid group with a phosphate group and an amino alcohol as shown in Figure 2. They are generally formed by a dehydration reaction similar to triacylglycerols.

![Figure 2: Typical Phospholid (lecithin)](image2)

Steroids

Steroids are lipids containing the core structure of 17 carbons fused in a ring structure containing three, six-member rings, and one five-member ring. The different functionality of steroids comes from the substituent groups attached to the core structure. Figure 3 below shows the structure of cholesterol, a typical steroid.
Miscellaneous

Many other types of lipids exist, but will not be examined in the lab. For example, waxes are esters of long chain alcohols and long chain carboxylic acids.

B. Physical Properties

The physical property that all lipids have in common is their insolubility in water, and solubility in nonpolar solvents. This property can be used to separate lipids from other compounds.

C. Chemical Properties

Polar vs Nonpolar - Sudan III

A typical test for nonpolar substances is a reaction with the dye Sudan III, which has a special attraction to nonpolar substances and thus is readily absorbed by lipids. It is also used in biology as a stain for lipids in plants seeds and animal tissues.

Saturated vs Unsaturated Fats

An easy way to test for unsaturation in fatty acids is simply adding a Br₂ solution to each fatty acid. As seen in Figure 4 below, bromine reacts via addition across the carbon-carbon double bond. Disappearance of the characteristic bromine color (red/orange/yellow) within a short period of time.

Acrolein Test

The Acrolein test is a general test for the presence of glycerol in a molecule. Potassium bisulfate is both a strong acid, and strong dehydrating reagent. When potassium bisulfate is heated with a fat, hydrolysis occurs, and the glycerol produced is dehydrated to form acrolein (CH₂=CHCH₃). Acrolein has a characteristic sharp irritating odor.

Lieberman-Burchard Test

This is a sensitive test for the presence of steroids in compounds. A color change from pinkish to blue/green is a positive test for steroids, and the intensity is roughly proportional to the amount of steroid present.
Laboratory 28: Properties of Lipids

Procedure

A. Lipid Extraction

1. Perform the extraction part of the test on the first day of lab, and the solubility tests, reaction with bromine, and acrolein tests on day two of the lab.

2. Choose either peanuts, chocolate chips, potatoe chips or sunflower seeds to perform the extraction on.

3. Weight out 5-6 grams of the substance to be tested.

4. Grind the substance to be tested in a mortar and pestle for 3-4 minutes to obtain a finely divided powder.

5. Tare a 125 mL Erlenmyer flask and transfer about 3 grams of the substance to be tested. Record the exact mass of the substance to be tested.

6. Pour approximately 20 mL of hexane in the Erlenmeyer flask. Stopper the flask and swirl the flask periodically over the next 15 minutes to mix the contents.

7. While the solvent extraction is proceeding, set up a filter funnel for gravity filtration. Measure and record the mass of the filter paper.

   (a) Fold a circle of filter paper in half. Fold it in half again and open it out into a cone. Tear off one corner of the outside folded edge. The tope edge of the cone which is to touch the funnel should not be torn. See Figure 5 below.

   ![](filter_paper.png)

   Figure 5: Filter paper apparatus.

   (b) Fit the opened cone into a funnel, placing the torn edge next to the glass. Wet with distilled water and press the top edge of the funnel against the paper forming a seal.

   (c) Use one of the setups suggested in Figure 6 below.

8. After 15 minutes, pour the contents of the Erlenmeyer flask into the funnel. do not overfill the paper filter cone. Use a spatula (or rubber policeman) to transfer any remaining solid.

9. When the filtration is complete, carefully remove the filter paper and place it on a watch glass. Place the watch glass on a piece of paper with your name on it to dry overnight.

10. Save the liquid portion of the filtration in a stoppered flask. Label the flask with your name.

11. After the residue is dry, record its mass.

12. You must complete the tests in the following sections before performing the tests on your extracted lipid.

13. Perform the solubility tests, reaction with bromine, and acrolein tests on the residue. Record your results.
B. Solubility - Water

1. Place 3 drops of liquid or a small pinch of solid samples listed in the data table in a test tube.
2. Add 2 mL of water to each test tube.
3. Mix each of the test tubes vigorously for 15 seconds. Wait 2 minutes. Sketch a picture of your results. In the sketch, label each liquid in the test tube. Note which pairs are miscible and which are not.
4. Add 5 drops of Sudann III solution to each test tube. Record the color and appearance of each test solution.
5. Discard of the contents of the test tubes in the waste container labeled "E28 - Lipid - Waste".

C. Solubility - Hexane

1. Place 3 drops of liquid or a small pinch of solid samples listed in the data table in a test tube.
2. Add 2 mL of hexane to each test tube.
3. Mix each of the test tubes vigorously for 15 seconds. Wait 2 minutes. Sketch a picture of your results. In the sketch, label each liquid in the test tube. Note which pairs are miscible and which are not.
4. Save your test tubes for use in the next section.

D. Reaction with Bromine

1. Use your saved test tubes from the Solubility - Hexane section or place 2 mL of hexane into each test tube and place 3 drops or a small pinch of each sample in the test tube.
2. Prepare a control/blank test tube using 2 mL of hexane.
3. Mix each of the test tubes vigorously for 15 seconds.
4. Do the following in the hood.
5. Be careful when pipetting the following solution, due to its high vapor pressure it has a tendency to spurt or drip from the pipette. Before pipetting fill and empty the bulb several times to equilibrate the pressure.
6. Add 5 drops of the bromine in dichloromethane solution (Br₂ in CH₂Cl₂) into each test tube.
7. Swirl the tubes gently to mix them.
8. Note the color of each tube at 10 seconds, 30 seconds and 1 minute after adding the bromine solution.
9. Record your results using the following codes (0 = no fading of bromine color, 1 = partial fading of bromine color, 2 = complete disappearance of the bromine color).
10. Discard of the contents of the test tubes in the waste container labeled "E28 - Lipid - Waste".
E. Acrolein Test

1. Place between 0.40 and 0.45 grams of potassium bisulfate in a clean dry test tube.

2. Add 2 drops or a small pinch of the material to be tested. Make sure the material to be tested is in physical contact with the potassium bisulfate.

3. Using a test tube holder, heat the bottom of the test tube over a bunsen burner flame. Hold the test tube at a 45 degree angle and gently agitate the mixture by shaking the tube while moving it into and out of the flame to control the rate of heating.

4. Continue heating until the potassium bisulfate is melted and a very slight darkening of the substance is noted. Stop heating.

5. Cautiously note the odor of the vapor coming off of the test tube by fanning. Acrolein should produce a characteristic sharp irritating odor.

6. Record the qualitative differences in odor as well as any differences in intensity.

7. Discard of the contents of the test tubes in the waste container labeled "E28 - Lipid - Waste".

F. Lieberman-Burchard Test

1. Place 3 drops of liquid or a small pinch of solid samples listed in the data table in a test tube.

2. Do the following in the hood.

3. Add 2 mL of chloroform to each test tube.

4. Add 10 drps of acetic anhydride to each test tube.

5. Add 2 drops of concentrated sulfuric acid to each tube.

6. Mix thoroughly.

7. After 5 minutes, note the color present in each test tube along with its relative intensity. Record your results.

8. Discard of the contents of the test tubes in the waste container labeled "E28 - Lipid - Waste".
## Results

### A. Lipid Extration

<table>
<thead>
<tr>
<th>Substance</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity of Lipid:</td>
<td></td>
</tr>
<tr>
<td>Serving Size (Label):</td>
<td></td>
</tr>
<tr>
<td>Amount of Fat/Serving (Label):</td>
<td></td>
</tr>
<tr>
<td>Mass of Original Sample (grams):</td>
<td></td>
</tr>
<tr>
<td>Mass of Filter Paper (grams):</td>
<td></td>
</tr>
<tr>
<td>Mass of Filter Paper + Residue (Lipid) (grams):</td>
<td></td>
</tr>
<tr>
<td>Mass of Residue (Lipid) (grams):</td>
<td></td>
</tr>
<tr>
<td>Appearance of Residue (Lipid):</td>
<td></td>
</tr>
<tr>
<td>Percent Lipid (show calc.):</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Results - Lipid Extraction
## B/C. Solubility Tests

<table>
<thead>
<tr>
<th>Substance</th>
<th>Solubility in Water</th>
<th>Miscible or Immiscible</th>
<th>Sudan III Test</th>
<th>Solubility in Hexane</th>
<th>Miscible or Immiscible</th>
</tr>
</thead>
<tbody>
<tr>
<td>ID Vegetable Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steric Acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Shortening</td>
<td></td>
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</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipid Extraction</td>
<td></td>
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</tr>
</tbody>
</table>

Table 2: Results - Solubility
Laboratory 28: Properties of Lipids

1. Which Lipids tested are soluble in water?

2. From your observations what do you conclude about the density of the lipids with respect to water (less, more, the same). Explain.

3. From your observations of the different lipids, what can you conclude about their solubility in Hexane? Explain.

4. What rule was learned in Hein Chapter 14 with regards to what type of substances are soluble with each other. Does the experiment above verify the rule? Explain.

5. Does the solubility behavior of the samples tested fit the pattern predicted based on the definition of lipids? Explain.

6. Did any molecules tested not fit the properties of lipids? Explain.
D. Bromine Test for Saturation/Unsaturation

<table>
<thead>
<tr>
<th>Substance</th>
<th>10 seconds</th>
<th>30 seconds</th>
<th>1 minute</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Vegitable Oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Steric Acid</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Cholesterol</td>
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<tr>
<td>Vegitable Shortening</td>
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</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Lipid Extraction</td>
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<td></td>
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</tbody>
</table>

Table 3: Results - Bromine Test
1. Write a complete chemical reaction showing how oleic acid reacts with Br₂.

2. Which substances are saturated?

3. Which substances are unsaturated?

4. Do the results of the bromine test match the information on the nutritional label? Explain.
### E/F. Acrolein Test and Lieberman-Burchard Test

<table>
<thead>
<tr>
<th>Substance</th>
<th>Acrolein (+/- and description)</th>
<th>Lieberman-Burchard (+/- and description)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetable Oil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stearic Acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
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<tr>
<td>Lipid Extraction</td>
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<td></td>
</tr>
</tbody>
</table>

Table 4: Results - Acrolein Test and Lieberman-Burchard Test
1. Which compounds tested positive for the Acrolein test?

2. Which compounds tested negative for the Acrolein test?

3. Did any compounds tested not match expectations? Explain.

4. Complete the following reaction: $\text{CH}_2\text{OHCHOHCH}_2\text{OH} \xrightarrow{\text{KHSO}_4} \text{ }$

5. Which compounds tested positive for the Lieberman-Burchard test?

6. Which compounds tested negative for the Lieberman-Burchard test?

7. Did any compounds tested not match expectations? Explain.
Prelab Questions

1. What functional group does the Bromine test give a positive result for? What is the evidence of a positive result?

2. What functional group does the Acrolein test give a positive result for? What is the evidence of a positive result?

3. What functional group does the Lieberman-Burchard test give a positive result for? What is the evidence of a positive result?

4. Complete the table below listing all of the chemical compounds used in lab. For organic compounds draw the structure, for inorganic compounds give the chemical formula.

<table>
<thead>
<tr>
<th>Name</th>
<th>Structure or Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromine</td>
<td></td>
</tr>
<tr>
<td>Potassium Bisulfate</td>
<td></td>
</tr>
<tr>
<td>Stearic Acid</td>
<td></td>
</tr>
<tr>
<td>Chloroform (trichloro methane)</td>
<td></td>
</tr>
<tr>
<td>Glycerol</td>
<td></td>
</tr>
<tr>
<td>Hexane</td>
<td></td>
</tr>
<tr>
<td>Oleic Acid</td>
<td></td>
</tr>
<tr>
<td>Sulfuric Acid</td>
<td></td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td></td>
</tr>
<tr>
<td>Acetic Anhydride- ((\text{CH}_3\text{CO})_2\text{O})</td>
<td></td>
</tr>
</tbody>
</table>