

CHM 112 Lab Procedure 9:

LIPIDS: SAPONIFICATION (THE PROPERTIES AND SYNTHESIS OF SOAP)

Objectives:

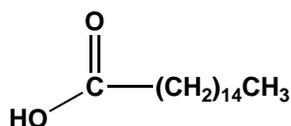
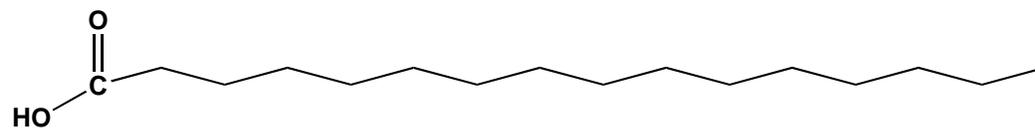
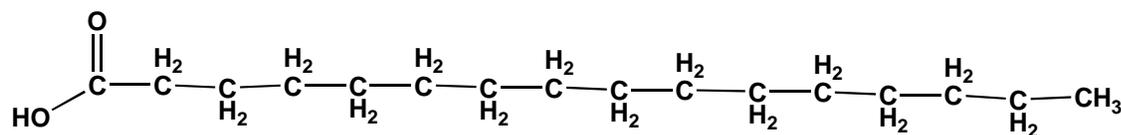
- to examine the interaction of water and oil in the presence and absence of soap and detergent
- to examine the interaction of soap and detergent solutions with different metal ion solutions
- to prepare a soap from common lipid sources
- to determine the alkalinity of a commercially-prepared soap

Lipids are one of the four major classes of biomolecules (along with carbohydrates, proteins and nucleic acids). They store energy in the body for use when food is not readily available. They are integral for cell membranes to separate compartments within the cell, and can act as chemical messengers in the endocrine system and throughout the body.

Lipids are naturally occurring organic molecules that can dissolve in non-polar solvents, like ether. The other biomolecules (proteins, carbohydrates and nucleic acids) are polar and therefore much more soluble in a polar solvent like water.

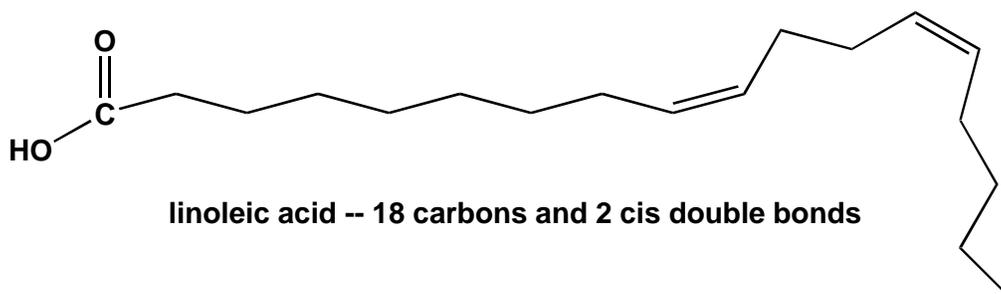
Many lipids are esters or amides of **fatty acids**. Fatty acids are defined as a long-chain carboxylic acid; the ones found in animals and plants usually have 12-22 carbons in the chains. Almost all fatty acids have an **even** number of carbons in the chain.

Here is **palmitic acid**, a fairly common fatty acid. Notice that, along with the structural method to writing the molecule, there are also a couple shorthand methods as well.



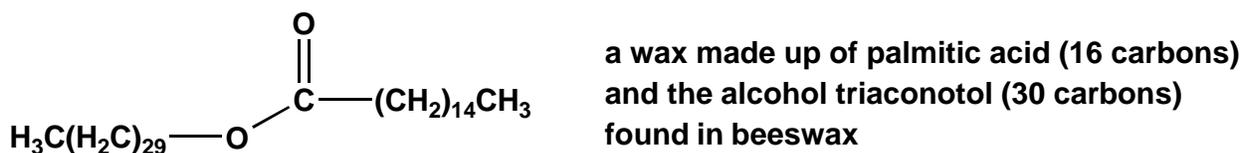
palmitic acid - a fatty acid with 16 carbons in the chain

In palmitic acids, all the carbons are connected with single bonds – the molecule is a **saturated fatty acid**. Other fatty acids are **unsaturated**, that is, they contain double bonds in the chain. Common unsaturated fatty acids include oleic acid with 18 carbons and 1 double bond, linoleic with 18 carbons and 2 double bonds and linolenic, with 18 carbons and 3 double bonds. All natural double bonds in fatty acids are **cis-** double bonds.



Notice with the saturated fatty acids (like palmitic acid), the carbons fall in somewhat of a straight line, but in the unsaturated fatty acids, the cis double bond creates “kinks” in the structure of the fatty acid. This is the reason that lipids containing a high percentage of saturated acids tend to be solid, but as the percentage of unsaturated fatty acids increases, the more fluid the lipid. The “kinks” in the unsaturated fatty acids keep them from packing close together, and this keeps unsaturated lipids fluid.

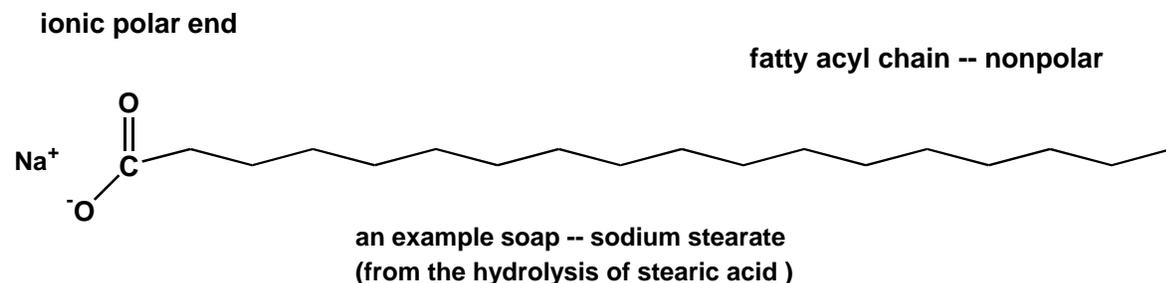
These fatty acids can combine with other molecules in several different ways. A **wax** is the ester of a fatty acid with a long chain alcohol. Usually the fatty acid has an even number (16-36) carbons and the alcohol also has an even number of carbons (24-36). This creates a very long and very non-polar ester molecule. In fact, these waxes are found in materials like bee honeycombs and the feathers of aquatic birds so that the birds stay dry.



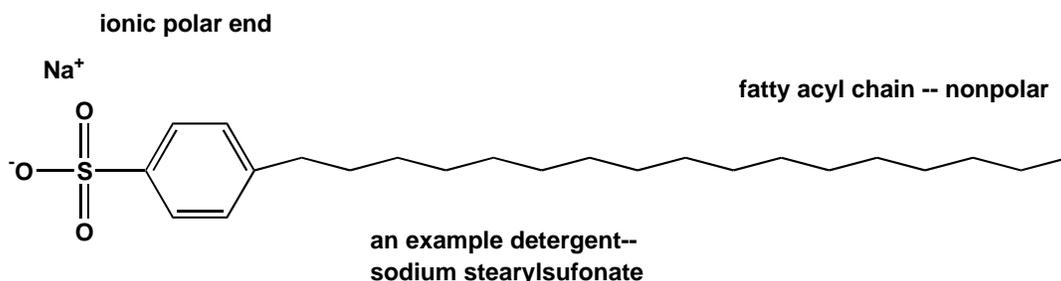
Animal fats and vegetable oils contain abundant **triacylglycerol** (TAG) – the form in which most dietary lipids and storage lipids occur. TAGs are also fatty acid esters (like waxes) but the fatty acids are esterified to the tri-alcohol **glycerol**. Each glycerol molecule has three fatty acid ester links.

Notice that the three fatty acids are in the carboxylate ion form with a negative charge. These negatively charged ions are countered by the positive sodium ions. Notice that these are NOT attached to each other, but are in ionic form in aqueous solution, much the same way NaCl dissociates into Na^+ and Cl^- ions when placed into aqueous solution.

These fatty acid salts are called **soaps**, and have two very different ends. The ionic end is very polar and is hydrophilic. The fatty acyl chain is very nonpolar and hydrophobic. Soaps are called **amphipathic** molecules due to this dual nature.



While **soaps** are produced from natural fats and oils, related molecules, **detergents**, are produced from petroleum chemicals. They have the same hydrophobic and hydrophilic ends and are able to remove dirt and grease in the same manner. Instead of carboxylic acids, however, many detergents are the salts of inorganic acids such as **sulfonic acid**.



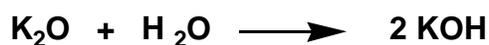
This is an **anionic detergent**, because there is a negative charge on the sulfonate ion. Soaps produced from carboxylic acids are also anionic soaps, due to their negative charge. Other detergents that are cationic (made from quaternary ammonium salts) and nonionic (such as polyethers) are also involved in cleaning.

This dual nature of a soap or detergent molecule is responsible for the ability of soaps to clean. The fatty acyl chain attracts the nonpolar dirt and grease. The hydrophilic end is able to dissolve in water. The hydrophobic end of a soap or detergent avoids water, so the hydrophobic ends of many soap molecules will collect together with their hydrophilic heads pointing out in to the aqueous solution, and their hydrophobic tails clustered together in a little hydrophobic microenvironment. This clustering is called a **micelle**, and these little balls often surround a particle of grease or dirt, thus

maintaining the hydrophobic core of the micelle. This is the way soaps work. Your textbook contains some good illustrations of soap micelles.

In “hard” water, divalent and trivalent cations (such as Mg^{+2} , Ca^{+2} and Fe^{+3}) will precipitate out the fatty acid salts as insoluble precipitates. That is why it takes more soap to clean in hard water than in “soft” water. Enough soap must be added to precipitate out all the cations, and then the additional soap is able to perform its cleaning function. Detergents are sometimes able to avoid this problem, as they are not as easily precipitated by metal ions.

In earlier times, soaps were prepared by hand, not in large industrial processes. Commercial lye (NaOH – it’s found in commercial drain cleaners!) could be used, but if this was not available, wood ash could be used. After wood is burned, the ash contains potassium oxide, K_2O . When this K_2O is mixed with water, the strong base potassium hydroxide (KOH) is formed.



This base was used to saponify tallow or any other available fat or oil to make soap.

Soap may vary in composition – soap made from olive oil is called **Castile soap**, and if potassium salt is produced instead of the sodium salt it is called a **soft soap** (this terminology doesn’t mean “liquid” soap). Many different perfumes, germicides and colors may be added, and air may be whipped into the soap to make it float.

The soap you will be making in lab is different than what is purchased commercially in stores. For one thing, commercial bars of soap are often a mixture of soaps and detergents; this soap is a completely vegetable (or plant) based soap. Also, in the commercial saponification reaction, the glycerol (or glycerin) that is produced is removed (it is often worth more to the company than the soap itself), and other (less expensive) materials are added to the soap to provide the qualities of glycerol.

The amphipathy of soap molecules has contributed to the understanding of how membranes work within the cell. Lipids (although not triacylglycerols) are the major component of membranes, and these membrane lipids also have a dual nature. Instead of forming micelles, these lipids form a **lipid bilayer**, sheet-like bilayers in which the hydrophobic tails of individual lipid molecules project into the core of the sheet and the polar head groups faces the aqueous environment on either side of the membrane. **Glycerophospholipids**, where one fatty acid ester is replaced by a phosphate ester to a polar head group, and **sphingolipids**, where the glycerol of TAGs is replaced by the polar amino acid **sphingosine**, are the major lipid components of most biological membranes.

In this procedure, you will compare the properties of soap and detergents in their ability to solubilize motor oil, that is, to wrap up the motor oil in micelles. You will also compare the ability of soap and detergent to be effective (that is, to foam up) in the presence of different cations. You will also prepare soap, using sodium hydroxide (NaOH) and you will look at properties of a commercial soap (Ivory soap).

Procedure:

Part A: Role of soaps and detergents in solubilizing oil

1. In three clean (although not necessarily dry) test tubes: add 5 mL deionized water. Add just 5 drops oil (motor oil) to form a visible layer on top of the water.
 - a. To the first tube, add ~1 mL (20 drops) of soap solution.
 - b. To the second tube, add 1 mL detergent solution.
 - c. The third tube is a negative control; add 20 drops of deionized water.
2. Stopper all three tubes and shake vigorously for 10 seconds.
3. Observe the results for all three tubes, and record your observations. How much foam formed? Did any of the tubes form more foam than the others? What type of lipid aggregate probably formed in the tubes containing soap and detergent?
4. Record your observations and conclusions in your lab notebook.

Part B: Interaction of soap and detergent with solutions of cations

1. Set up and label 5 clean (although not necessarily dry) test tubes, and add 40 drops (~2 mL) soap solution to each tube.
2. Add:
 - a. To tube #1, add 10 drops Na⁺ cation (shown on stock bottle as NaCl)
 - b. To tube #2, add 10 drops K⁺ cation (shown on stock bottle as KCl)
 - c. To tube #3, add 10 drops NH₄⁺ cation (shown on stock bottle as NH₄Cl)
 - d. To tube #4, add 10 drops Ca²⁺ cation (shown on stock bottle as CaCl₂)
 - e. To tube #5, add 10 drops Fe³⁺ cation (shown on stock bottle as FeCl₃)
3. Stopper the tubes and shake vigorously for 10 seconds. Observe the results for each tube and record all observations. Did the soap foam up with all the cations? Which cation/soap solution foamed the least? Those soap solutions with cations that did not foam were not forming micelles in the presence of those cations. Those cations precipitated the soap solution.
4. Repeat Steps 1-3 using detergent solution instead of soap solution. Were there any detergent/cation solutions that behaved differently than the soap/cation solutions?

(The presence of divalent (2+) and trivalent (3+) cation species in a water supply contribute to "hard water," which eliminates lather and decreases cleaning effectiveness. Many commercial cleaning agents contain additives that will eliminate the divalent and trivalent cation species by precipitating them out of the water.)

5. Clean out the test tubes (or use different, clean test tubes) and repeat step 1 of this section, but alter the procedure by adding 10 drops of sodium phosphate (Na₃PO₄) to the soap or detergent solutions and mixing. Add 10 drops of the cation solutions

listed above to the appropriate tubes (step 2), stopper the tubes and shake vigorously for 10 seconds. Observe the results and record the observations, particularly if there is a difference from the previous results without the sodium phosphate.

Part C: Preparation of Soap

The lye used in this procedure (a 25% (w/v) solution of NaOH) is corrosive. Do not splash this solution or get it on you!

Do not use any metal utensils (like a scoopula) in this procedure. The sodium hydroxide solution will react with the metal. Use only glass or wooden utensils in this procedure.

1. Set up a hot plate and thermometer as shown/described by your instructor.
2. In a clean and dry 250-mL beaker prepare a mixture:

18.1 g coconut butter (sometimes called coconut oil; coconut oil/coconut butter is NOT the same as cocoa butter)

21.1 g olive oil

21.1 g vegetable shortening

Tare the beaker and then add each fat in succession; taring again after each fat is measured. Measure these by **WEIGHT**, not by volume. If any or all of the masses are over by a little, that's OK. Do not weight out any less than these masses.

3. In a clean and dry 50-mL beaker, measure out **30 g of 25% sodium hydroxide** (NaOH). This can be done by taring the empty beaker and adding 30 g of 25% NaOH. This is NOT the same as 30 mL of sodium hydroxide. Make this measurement by **WEIGHT**, not by volume. If the mass is under by a little, that's OK. Do not weight out any more than 30 g of NaOH.
4. Melt the fat mixture in the beaker; the solution temperature should not exceed 75 °C. Also heat the beaker of sodium hydroxide until it reaches about the same temperature as the fat mixture. It does not have to be the exact same temperature – as long as the fat is melted and the NaOH solution is warm.
5. When the two beakers are at the same temperature (at least 60 °C, but less than 75 °C), GENTLY (CAREFULLY! VERY CAREFULLY!) pour the sodium hydroxide solution into the fat mixture. The mixture will become cloudy.
6. Mix (use a glass stirring rod or wooden applicator sticks) this emulsion for at least **thirty-five minutes** (but less than 45 minutes), keeping the temperature between 60 °C and 75 °C. This mixture will require attention to make sure that the temperature does not exceed 75 °C or drop below 60 °C. If you stir too vigorously, the soap will not have the right consistency.
7. After thirty-five minutes, the mixture should resemble thick frosting or pudding.
8. If desired, you may add coloring or scent at this point. This soap is a light cream color without added coloring.
 - a. Adding blue or green coloring would turn the soap a pinkish-gray color (it has to do with the reaction of the sodium hydroxide with the organic molecules

- that are the blue and green dyes). The best choice is a red or yellow tint. To add coloring, add about two or three drops and mix thoroughly. More may be added if desired.
- b. Adding 2-3 drops produces a fairly recognizable scent. Add 2-3 drops, and mix thoroughly. More may be added if desired.
9. Pour the mixture into two Styrofoam cups, half of the mixture in each cup (one cup for each student in the pair).
 10. Cover each cup with plastic wrap, and set in your equipment locker **for at least one week**. This "curing" time is absolutely essential. Corrosive NaOH is still present in the mixture even after 45 minutes of "cooking;" the saponification reaction continues in the cup after the soap is poured. As soap cures, this hydroxide reacts to yield a mild soap.
 11. Clean up your materials. Since there is still unreacted 25% sodium hydroxide in the beaker and on other equipment, you may wish to wear gloves to clean up.
 - a. Scrape any incomplete ("raw") soap from the beaker onto a paper towel and throw into the trash. Do not put down the sink, as any sodium hydroxide present may damage sink pipes.
 - b. The beaker will still have unreacted fat and hydroxide and will seem greasy. Rinse the beaker with a small amount (less than 10 mL) of 5% acetic acid (vinegar is also acceptable for this purpose) before washing the beaker in hot, soapy water. The 5% acetic acid solution may go down the sink.
 12. Several tests on the characteristics of soap are to be performed using a high-grade commercial soap (Ivory flakes will be available). Do NOT perform these test on the soap you have prepared; the saponification reaction is not yet complete and results on your prepared soap would be in error.

The pH indicator used (phenolphthalein) for these tests is one you should be familiar with from General Chemistry. Phenolphthalein is colorless in a solution that is pH 8 or below, and bright pink in a solution with pH above 8.
 13. **Many people make soap in their kitchen, for themselves, as a hobby or to sell. There are thousands of different recipes for soap, and each type of has slightly different characteristics. If you are interested in pursuing soapmaking on your own, check your local public library for books on the subject, or the many different Internet sites on the subject.**

References:

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Lab partner(s) _____

Notebook points/initials _____

Date _____

Name _____

**REPORT FOR LAB PROCEDURE 9:
LIPIDS: SAPONIFICATION
(SYNTHESIS AND PROPERTIES OF SOAP)**

Part I: Role of Soaps and Detergents in Solubilizing Oil

Tube	Observations
#1: water, oil, and soap	
#2: water, oil and detergent	
#3: water and oil (negative control)	

Part II: Interaction of soap and detergents with cation solutions

1. Soap or detergent + cations

Cation solution	Observation with soap	Observation with detergent
Tube #1: Na ⁺		
Tube #2: K ⁺		
Tube #3: NH ₄ ⁺		
Tube #4: Ca ²⁺		
Tube #5: Fe ³⁺		

2. Soap or detergent + sodium phosphate + cations

Cation solution	Observation with soap	Observation with detergent
Tube #1: Na ⁺		
Tube #2: K ⁺		
Tube #3: NH ₄ ⁺		
Tube #4: Ca ²⁺		
Tube #5: Fe ³⁺		

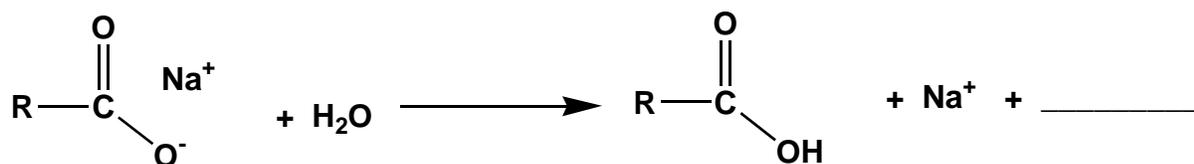
Part III. Preparation of Soap

After your soap is poured and your bench is cleaned up, perform these tests with a high-quality commercial soap (Ivory flakes will be available in the lab). Do **not** perform these tests on your own soap.

A. Alkalinity of soap

- Add three drops phenolphthalein solution (pH indicator solution) to 5 mL of water. What color (if any) does the solution turn? _____.
- Now dissolve a small piece of the Ivory soap (about the size of a pea) in the solution. Does the color change or intensify? _____.
- What type of solution does the soap create (acidic or basic)? _____.

In saponification, the fatty acid carboxylate ions are formed (in the presence of the base). These carboxylate ions are the conjugate bases of the fatty acids, and therefore, able to accept a proton. When placed into water (a proton donor), these conjugate bases are able to accept protons from any source (including water). Complete this equation to answer the remaining questions.



What is this other species (an anionic species) that is formed? _____

Would this reaction account for the color of the solution with phenolphthalein that you saw?

Keeping this reaction and its results in mind, do you expect that there is a reasonable possibility that any soap solution would have a pH of 7 or lower? Why did you answer that way?
