EXPERIMENT 34

Lipids

Performance Goals

34–1 Determine whether a lipid is saturated or unsaturated.

34–2 Identify an unknown as stearic acid, olive oil, lecithin, or cholesterol.

CHEMICAL OVERVIEW

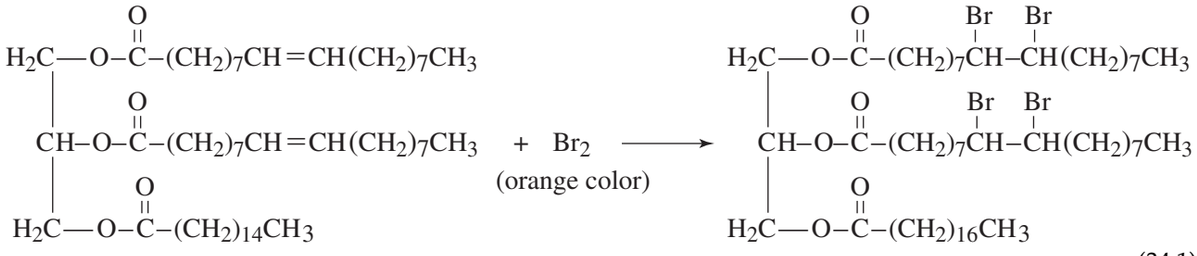
The word *lipid* comes from the Greek *lipos*, which means ''fat.'' Fats or lipids are defined as molecules that come from cells or tissues that are soluble in nonpolar solvents. Due to the definition being based mainly on solubility, lipids have a large range of structural diversity. This structural range can be further divided into four major categories: fatty acids (saturated and unsaturated), glycerides (glycerol-containing), nonglycerides (sphingolipids, steroids, waxes), and complex lipids (lipoproteins). In this experiment, three glycerol-containing lipids will be explored: a fat or oil, which contains glycerol and three fatty acids (saturated and unsaturated); lecithin, which contains glycerol; and two fatty acids and a phosphate attached to an amino alcohol. Cholesterol represents the steroid, nonglyceride lipids.

Fatty acids are carboxylic acids attached to long hydrocarbon chains of 10 to 20 carbons. Each fatty acid is further characterized by the length of the carbon chain and by the number of double bonds. Fatty acids with double bonds are unsaturated fatty acids, and those without double bonds are saturated fatty acids. The term *polyunsaturated fat* refers to a fatty acid that has multiple double bonds.

A qualitative test to determine saturation or unsaturation of fatty acids is to react the acid with a bromine solution in methlyene chloride. Bromine readily reacts with any alkene to dihalogenate. The bromine solution is reddish-brown in color and will become clear as the bromine reacts with any alkene present.

Saturated Fatty Acids	*Unsaturated Fatty Acids*
Animal fat, stearic acid	Oleic acid (contained in olive oil)
$CH_3(CH_2)_{16}COOH$	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
	Linoleic acid (contained in safflower oil)
	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$

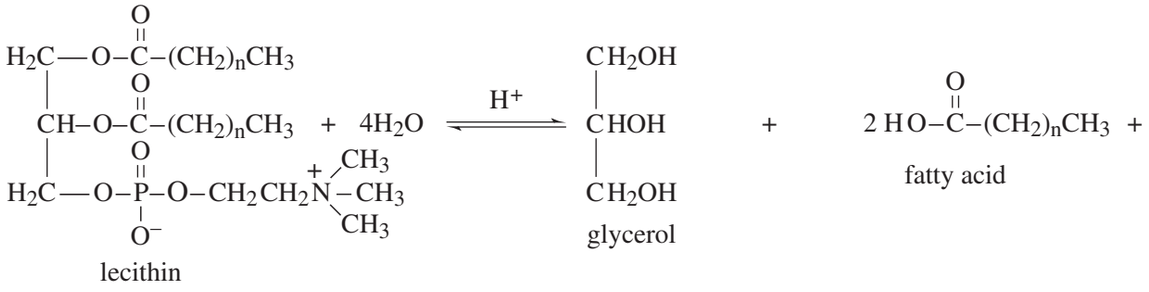
Glycerides are lipids that contain the molecule glycerol and fatty acids. Triglycerides, which contain one glycerol molecule and three fatty acids, are the main constituents of oils and animal fats. Fats tend to contain saturated fatty acids and come from animals, while oils contain unsaturated fatty acids and usually come from plants. A typical triglyceride found in olive oil consists of two oleic fatty acids and one palmitic fatty acid attached by an ester bond to glycerol. In this experiment, triglycerides containing unsaturated fatty acids will be identified by the bromine test.



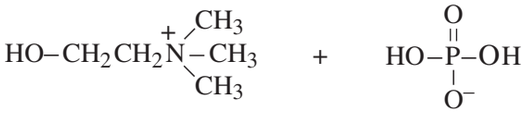
Olive oil *(colorless)* (34.1)

Another variation of triglycerides is phosphoglycerides, which are compounds that contain a phosphoryl group. When it is attached to a choline molecule, this lipid is designated as a lecithin.

A test for the presence of a phosphate can be used indirectly to differentiate between a phospholipid, cholesterol, and fat. Lecithin can be hydrolyzed in an acidic medium to give its four major constituents: glycerol, fatty acids, choline, and phosphate. The phosphate can then be detected using the molybdate test in which the appearance of a light-yellow precipitate indicates a positive test.



lecithin

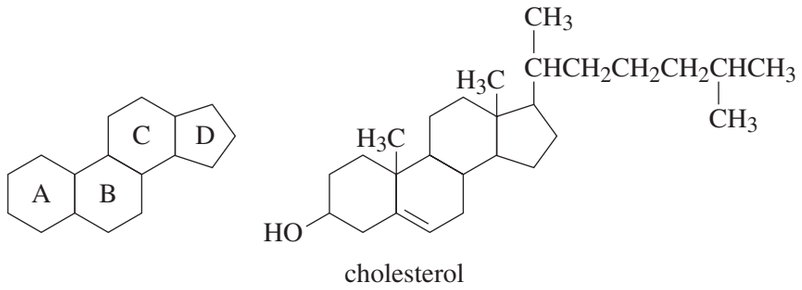


choline *phosphate* (34.2)

Steroids are classified as nonglycerol lipids that have a fused, four-ring skeleton system. Each ring is designated with a letter as shown below. An example of a common steroid is cholesterol.

The Lieberman–Buchard test is used to determine the presence of cholesterol. The reagent contains acetic anhydride and sulfuric acid, which reacts with the alcohol (OH group) and the alkene (double bond) in the

adjacent ring. A gradual color change from pink to lilac and then to deep green will be observed in a positive test.



cholesterol

SAFETY PRECAUTIONS AND DISPOSAL METHODS

Acids and bases are corrosive, and contact with your skin should be avoided. Any spilled acid or base should be washed off promptly. Sulfuric acid will burn when in contact with skin; wipe off the acid, then rinse the exposed area with plenty of cold water. Be sure to wear safety goggles or safety glasses while performing this experiment. Bromine, Br_2, causes severe burns on contact with the skin. Handle this reagent very carefully. Organic solvents are volatile and inhaling the vapors should be avoided.

Dispose of excess solids and liquids as directed by your instructor. Solutions containing precipitates should be collected in a waste container.

PROCEDURE

1. Bromine Test for Unsaturation

A. Prepare four test tubes by placing 5 drops, or a small amount of solid, of the following into separate test tubes: stearic acid, olive oil, lecithin, and cholesterol.

B. Add 1 mL of methlyene chloride to each of the test tubes. Then, add 2% Br_2 in methylene chloride to each sample drop by drop until a permanent orange-red color is obtained, or until 20 drops have been added. Record the final color.

2. Lieberman–Buchard Test for Cholesterol

A. Prepare four test tubes by placing 5 drops, or a small amount of solid, of the following into separate test tubes: stearic acid, olive oil, lecithin, and cholesterol. Add 3 mL of chloroform and 1 mL of acetic anhydride to each test tube. Next, add 1 drop of concentrated sulfuric acid to each mixture and record the color. Set aside for 5 minutes and record the color of each test tube.

3. Phosphate Test

A. Prepare four test tubes by placing 5 drops, or a small amount of solid, of the following into separate test tubes: stearic acid, olive oil, lecithin, and cholesterol.

B. Hydrolyze the compounds by adding 3 mL of 6 M nitric acid to each test tube.

C. Place each tube in a boiling hot water bath for 5 minutes. Remove the test tubes and let them cool to room temperature. Neutralize the samples by adding 3 mL of 6 M NaOH to each test tube. If a precipitate forms, the sample needs to be filtered.

D. Add 1 mL of molybdate solution to each test tube. Mix thoroughly and heat in a hot-water bath for 5 minutes. A light-yellow precipitate indicates that phosphate is present.

CAUTION

You need to have a *precipitate*; a yellow color is not a positive test.

4. Identification of an Unknown

Obtain an unknown from your instructor and perform tests 1 through 3 to determine the identity of your lipid as stearic acid, olive oil, lecithin, or cholesterol. Be sure to record the unknown number.

Name *Date* *Section*

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Advance Study Assignment

1. Write the equation for the reaction of linoleic acid with bromine.

2. a. What is the purpose of adding acid to the lecithin sample?

b. Write the four products of this reaction (from part 3b).

c. Which of the four products in part 3c is responsible for a positive test?

3. Identify the two unknowns below as stearic acid, olive oil, lecithin, or cholesterol, based on the following results.

a. The unknown reacts to give a green color in the presence of acetic anhydride and sulfuric acid.

b. The unknown gives negative results for tests 1 through 3.

Name _____ Date _____ Section _____

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Work Page

A. Bromine Test for Unsaturation

	Number of drops added	*Ending color*	*Saturated*	*Unsaturated*
Stearic acid				
Olive oil				
Lecithin				
Cholesterol				
Unknown				

B. Lieberman–Buchard Test for Cholesterol

	Initial color	*Ending color*	*Cholesterol present*
Stearic acid			
Olive oil			
Lecithin			
Cholesterol			
Unknown			

C. Phosphate Test for Lecithin

	Precipitate	*Phosphate present*
Stearic acid		
Olive oil		
Lecithin		
Cholesterol		
Unknown		

Unknown number _____ Identity of unknown _____

Name _____ Date _____ Section _____

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Report Sheet

A. Bromine Test for Unsaturation

	Number of drops added	*Ending color*	*Saturated*	*Unsaturated*
Stearic acid				
Olive oil				
Lecithin				
Cholesterol				
Unknown				

B. Lieberman–Buchard Test for Cholesterol

	Initial color	*Ending color*	*Cholesterol present*
Stearic acid			
Olive oil			
Lecithin			
Cholesterol			
Unknown			

C. Phosphate Test for Lecithin

	Precipitate	*Phosphate present*
Stearic acid		
Olive oil		
Lecithin		
Cholesterol		
Unknown		

Unknown number _____ Identity of unknown _____