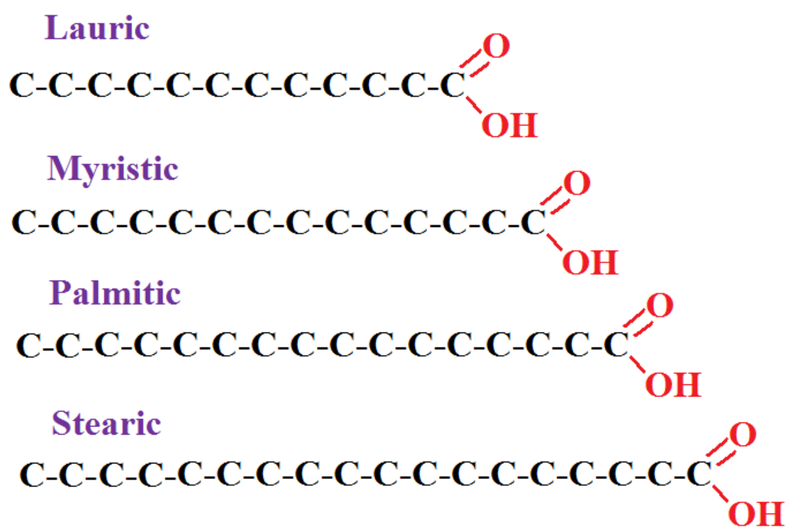
Lipids

The simplest lipids are the fatty acids. These are long chain hydrocarbons with carboxyl groups (COOH groups). We are interested in two groups of fatty acids: saturated and unsaturated fatty acids.

Saturated fatty acids are so called because each carbon atom in the chain holds all the possible hydrogen atoms it can. These lipids tend to be solids at room temperature. These are also the sorts of lipids found around organs in the human body, acting a cushions. The only bonds present between the carbon atoms are single bonds. The table, below, summarizes four of the saturated fatty acids; graphic representations are at the right of the table.

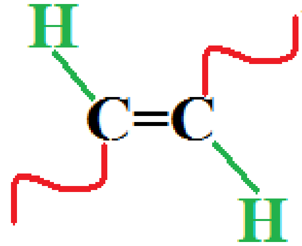
Lauric Acid
$C_{12}H_{24}O_2$
found in coconut oil
Myristic Acid
$C_{14}H_{28}O_2$
found in coconut oil
Palmitic Acid
$C_{16}H_{32}O_2$
found in lard
Stearic Acid
$C_{18}H_{36}O_2$
found in lard



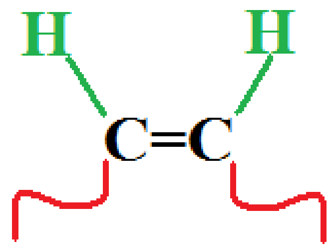
Unsaturated fatty acids do not have all the hydrogens they can hold, for there are occasional carbon-carbon double bonds in addition to the single bonds between carbon atoms. These fatty acids tend to be liquids at room temperature and are the primary type of lipid found in skin deposits.

Naturally occurring unsaturated fatty acids contain double bonds that are in the "cis" form and artificial unsaturated fatty acids contain double bonds that are in the "trans" form. The trans-fatty acids are found in oleo and margarine and have a high link with heart disease.

At right is a graphic of the two types of double bonds found in unsaturated fatty acids. The red squiggly lines represent the rest of the molecule. The top graphic shows the hydrogens across from each other in the double bond. This is the "trans" form. The bottom graphic shows the hydrogens on the same side of the double bond. This is the "cis" form.



When vegetable oils are partially hydrogenated to solidify them, invariably some cis (H's on same side of the double bond) fatty acids are altered (isomerized) to trans (H's on opposite side of double bond) fatty acids. “Hydrogenated” margarines contain 15-40% trans fatty acids. Elevated trans fatty acids in one's diet causes hypercholesterolemia which leads to increased coronary artery disease, atherosclerotic heart disease and coronary heart disease.



There are 4 unsaturated fatty acids that are important to remember, as well. These fatty acids (on the following page) are essential fatty acids.

There are three nomenclature items that need discussion, here, before examining the fatty acids: 1) the "n-" (enn minus) nomenclature, 2) the "ω" (omega) system of nomenclature and 3) the "Δ" (delta) system of nomenclature.

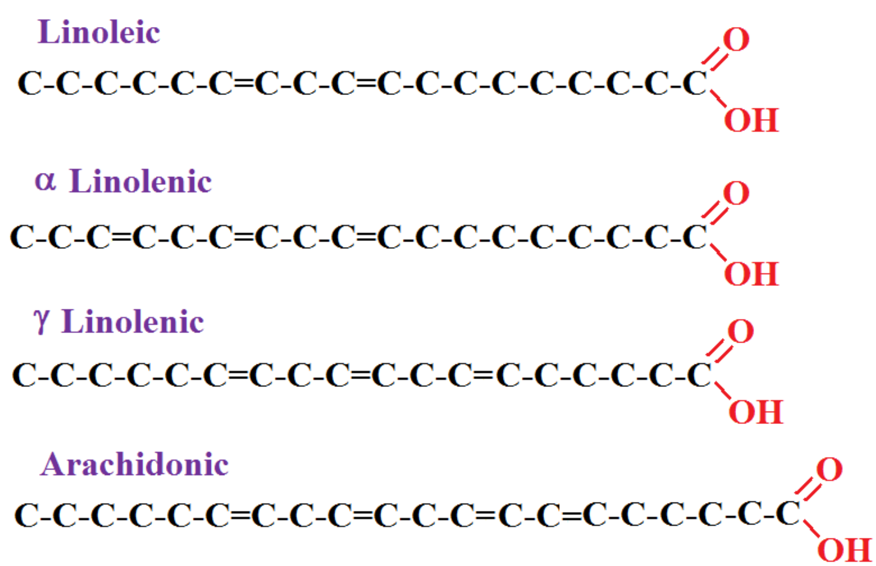
The "n-" system of nomenclature comes from going to the CH_3 end of the fatty acid molecule and counting in from the end (end - [e + d] = n) to the carbon with the first double bond.

The omega system is the same system, just another name. Both systems (n- and ω) were published at the same time by two different groups in two different journals. The former has become the “professional” format and the latter, the “lay” format.

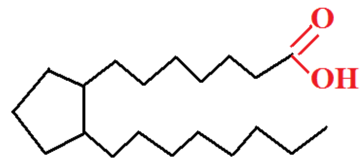
The delta (Δ) system of nomenclature of fatty acids, specifically unsaturated fatty acids, is equally as simple: the first number before the colon is the number of carbon atoms in the hydrocarbon chain. The number after the colon is the number of double bonds in the whole molecule between carbon atoms. One example using only these two descriptors is 20:4 to begin to identify arachidonic acid: 20 carbons and 4 double bonds.

The numbers superscripted above the delta (Δ) sign tell you the location of the double bonds, e.g., $^{\Delta 9, 12, 15}$: the double bonds are between carbons 9 and 10, 12 and 13, and 15 and 16, where we only identify the carbons by the lowest number in the double bonds per IUPAC nomenclature rules. Four essential unsaturated fatty acids are summarized and illustrated below.

Linoleic Acid
found in corn oil, soybean oil, cottonseed oil
n-6 or *w*6
$18:2^{\Delta 9, 12}$
α-Linolenic Acid
found in leafy vegetables and vegetable oils
n-3 or w3
$18:3^{\Delta 9, 12, 15}$
γ-Linolenic Acid
found in leafy vegetables and vegetable oils
n-6 or *w*6
$18:3^{\Delta 6, 9, 12}$
Arachidonic Acid
found in peanut oil, brain/nervous tissue
n-6 or w6
$20:4^{\Delta 5, 8, 11, 14}$



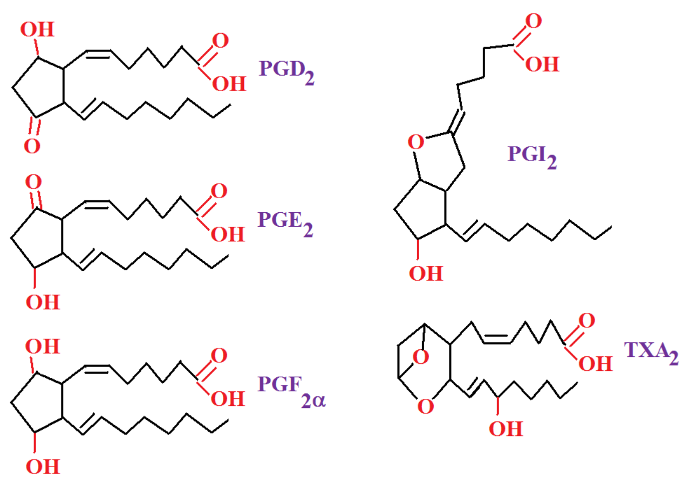
Arachidonic Acid Significance



Why is Arachidonic acid important? It is important because it is the precursor fatty acid for prostaglandin and leukotriene biosynthesis. These compounds are known as <u>eicosanoids</u>, i.e., compounds based off 20 carbons.

Prostaglandins mediate pain, smooth muscle contraction, fever, mucous production in the stomach, blood clotting, premenstrual syndrome and inflammation to name a few. One prostaglandin-mediated effect that is becoming more and more common due to hyperlipidemias is a skin rash. This rash is mediated by PGD_2 and follows niacin therapy for hyperlipidemia – it is easily treated by either taking an aspirin about 30 minutes beforehand or taking a slow release formula of niacin.

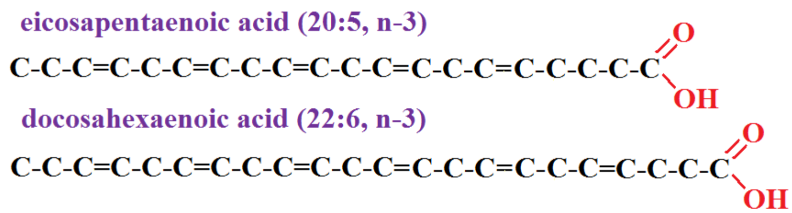
Prostaglandins are based off prostanoic acid (image top of page); representative PG's are shown, at right, prior to the table and leukotrienes (LT's) are illustrated on the middle right of the following page. There are nomenclature rules that follow prostaglandins (and LT's), too. PG is short for prostaglandin. The letter tells us about the ring constituents and the subscripted number tells us how many double bonds there are on the side chains. PG's may be inhibited at the level of synthesis with aspirin, ibuprofen or indomethacin; anti-leukotriene agents are now available for treating airway diseases.



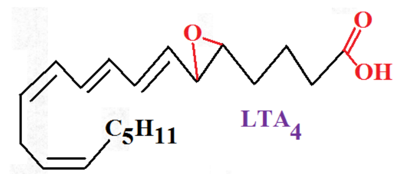
PGD_2	PGE_2	PGF_2	PGI_2	TXA_2
Mediates niacin skin flush	vasodilator	uterine muscle contractant	aka prostacyclin; vasodilator	vasoconstrictor; platelet aggregator

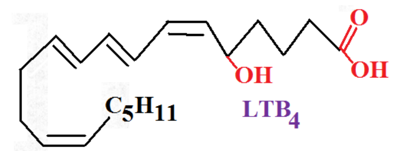
Note that arachidonic acid gives rise to the PG$_{\text{"2"}}$ series of prostaglandins – most of these are pro-inflammatory and can lead to serious consequences, e.g. myocardial infarction. Recently, two significant PUFA's (EPA and DHA) in fish oils have found more and more use for the treatment of various hyperlipidemias because they are n-3 fatty acids which produce the PG$_{\text{"3"}}$

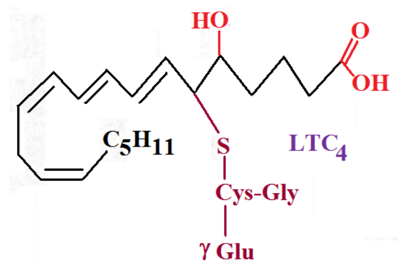
family which are primarily anti-inflammatory and, hence, heart healthy (EPA and DHA are illustrated at right).



LTB_4	LTC_4
chemotactic agent for PMN's (segmented neutrophils)	involved in allergy and anaphylaxis; more potent than HISTAMINE in shutting down airways and increasing swelling





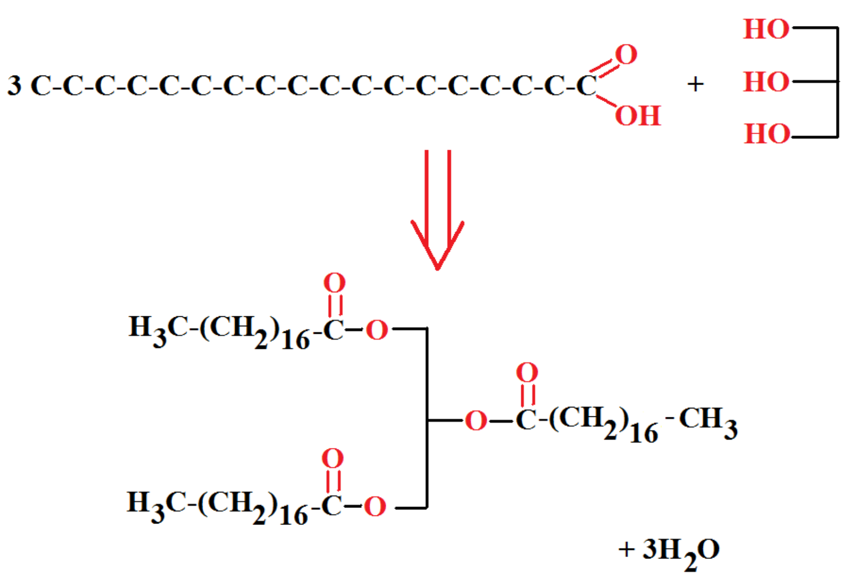


Triglycerides (TG's or TAG's)

TG is fairly self explanatory; TAG's are triacyl glycerols - the same thing, just a slightly different name. TAG's are made by condensing one molecule of glycerol with three molecules of fatty acids. The products are the triglyceride (in this case, tristearin) and 3 moles of water (bottom of page at right).

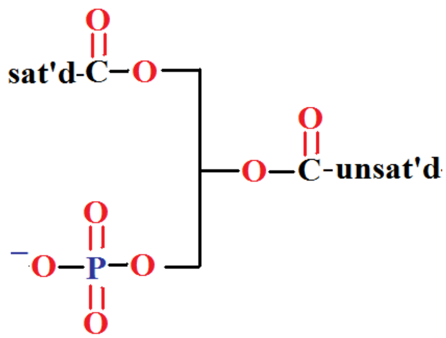
FA's in TG's

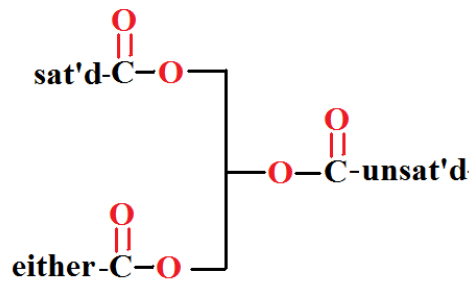
As a general rule, fatty acids align themselves on a glycerol molecule in such a manner that the #1 carbon has a saturated fatty acid bound to it, #2 carbon has an unsaturated fatty acid bound to it and #3 is fair game (top right following page).



Phospholipids

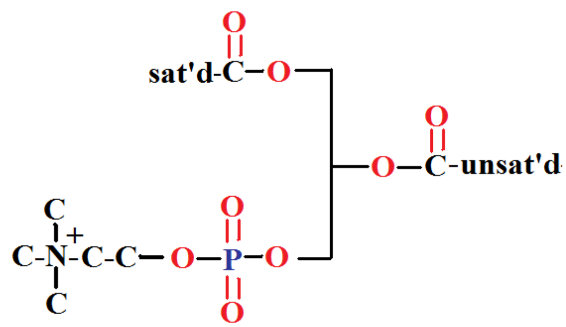
A lipid related to the TAG is the phospholipid (PL). Instead of a third fatty acid bound, proper, to the #3 carbon on glycerol, a phosphate is bound there. Other groups will bind with the phosphate. Figure lower left is representative of phosphatidic acid.





A representative illustration of phosphatidyl choline (PC) or lecithin is below center right. You may know the latter name as it is in non-dairy creamers as an emulsifying agent. PC is also important in cell membranes by assisting in membrane rigidity.

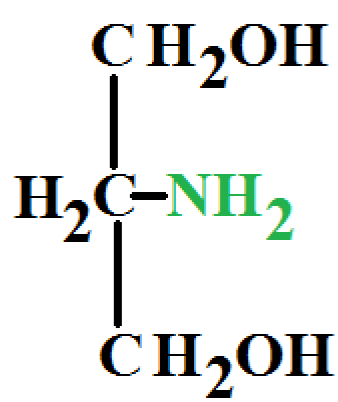
A related PL, cardiolipin, is a sort of "dimer" of PC. Cardiolipin is found in the inner mitochondrial membrane. This PL causes the inner mitochondrial membrane to be more fluid (more flexible) so that the protein complexes on electron transport will be brought closer together during active cellular respiration.



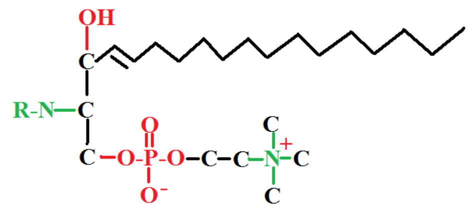
Sphingolipids

Differ from phospholipids (aka glycerophospholipids). Sphingosine is illustrated below right. Sphingolipids are found in high levels in brain tissue and in myelin sheath around peripheral nerves. Sphingomyelin is also found in amniotic fluid. Why are PC and sphingomyelin so important? Because clinically, a <u>L/S Ratio</u> can be used to determine maturity of a fetus for birth purposes. PC is required for surfactant in lung; surfactant not important in utero, BUT, it is for BREATHING. L/S ratio values and significance are summarized in the table, below.

An increase in PC comes with maturation of the lungs in utero as gestation comes to an end.	
L/S Ratio	**Week of Gestation**
< 1	Before 31st week
2	34th week
4	36th week
8	39th week (term ±)

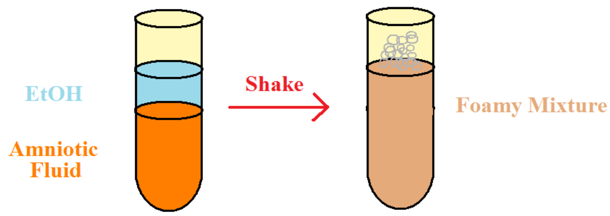


L/S of 2 or greater is ok for C-section. An L/S < 2 is NOT uniformly predictive of RDS. An L/S > 2 is associated with absence of RDS. A generic sphingomyelin structure is illustrated at right.



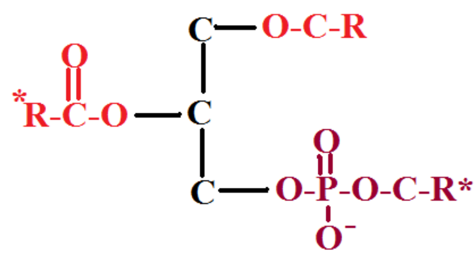
Foam Stability Test

Fetal surfactant in amniotic fluid "makes" foam in presence of EtOH, aka "shake test" (at immediate right). Foam is "USUALLY" indicative of an L/S of about 2.



Plasmalogens

Occur in membranes of nerve cells and muscle cells. A generic plasmalogen is illustrated just below right. R* in myelin is $-C-NH_3^+$. R* in cardiac muscle is the N,N,N-trimethyl-ammonium-methyl group (quaternary amine with 4 methyl groups). Both R*'s are found in mitochondrial membranes.

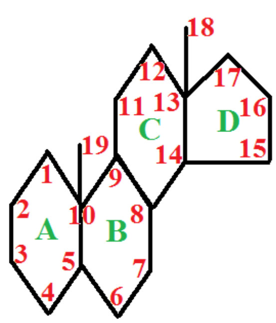


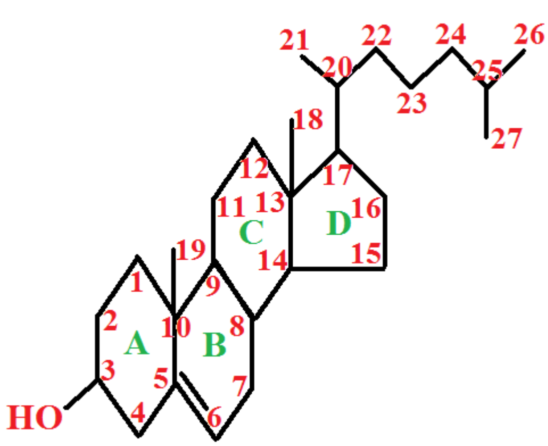
The Steroids

The steroids are ALL based off of a 4-fused ring system. The rings are labeled A thru D. Each carbon is numbered as shown in the graphic at bottom right.

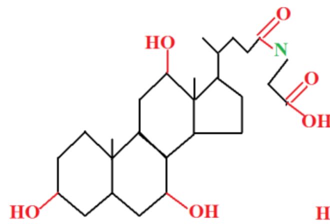
Cholesterol

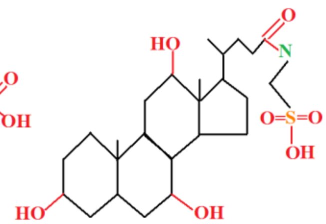
The primary steroid, i.e., that one compound from which all steroids are derived, is cholesterol (image bottom left). As you can see, the enumeration of cholesterol (once you are beyond the basic 4 ring system) is different than what one might expect. Carbons 18 and 19 are called bridging carbons (see bottom right image, too). On carbon #3 is an -OH group and between carbons 5 and 6 is a double bond. The hydrocarbon tail off carbon 17 is enumerated for you, above. In addition, the hydrocarbon chain will be modified by the body as necessary for the synthesis of specific steroids.

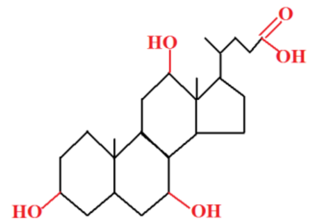




Digestive Detergents

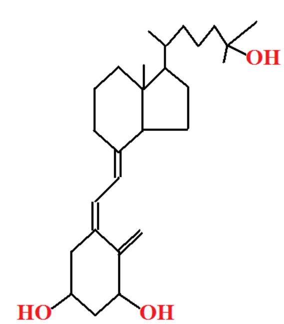

Glycocholic Acid


Taurocholic Acid


Cholic Acid

Cholesterol also plays an important role in digestion. The manner in which this occurs is that a derivative of cholesterol (cholic acid, at right) reacts with one of two amino acids or derivatives to form detergents. Detergents emulsify fat in the small bowel as small particles so that the enzymes in our small bowels may begin digesting the lipid.

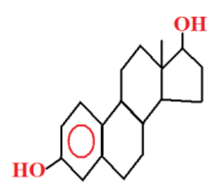
The two big bile salts, as these detergents are called, are glycocholic acid and taurocholic acid (image above right). Glycocholic acid is formed by the reaction of gly with cholic acid. Taurocholic acid is formed by reacting the oxidized product of cys, taurine, with cholic acid. Note that there is a polar, charged end and a nonpolar, uncharged, lipid-like end. It is due to these features that detergents work, i.e., the lipid-like end binds the lipid-like molecules and the polar end interacts with the water, in effect lifting the grease into the water.

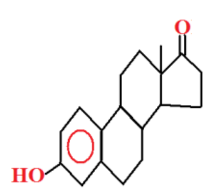


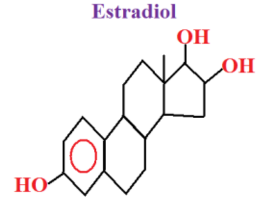
Important Steroid Hormones

The first is Vitamin D_3 (Image at right). This steroid is necessary for proper bone uptake of calcium and for adequate calcium metabolism in the body. The synthesis of this steroid begins in the skin with the photolysis of a cholesterol metabolite called 7-dehydrocholesterol. The synthesis of this "vitamin" is completed in the liver and kidney, respectively.

Sex Hormones

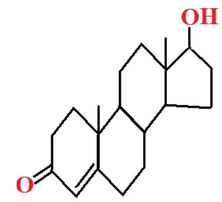

Estradiol


Estrone

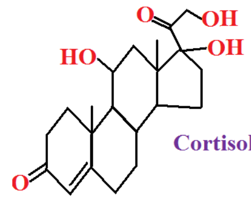

Estriol

Cholesterol is also the precursor for the steroid sex hormones. Note that each estrogen (estriol, estradiol and estrone) has an aromatic ring A (Image at right). This is due to an enzyme found in the biological fat layer in females (also found in men as they age and lose muscle mass; testosterone is shown in the image, top right of following page) called aromatase. Practically speaking, aromatase aromaticizes testosterone to the estrogens.

Other Steroid Hormones of Importance

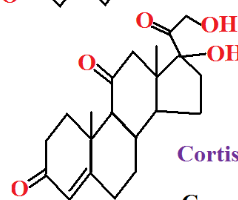


Cortisol is known as the muscle wasting hormone (2d image from top at right). This hormone kicks in as a stress hormone during starvation and causes muscle protein to be catabolized in such a manner that the carbon back bone of the individual amino acids is used to synthesize glucose.



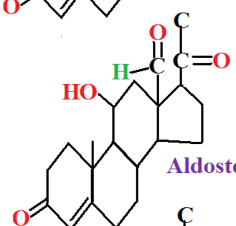
Cortisol

Cortisone (3d image from top, at right) we're familiar with as an anti-inflammatory agent.



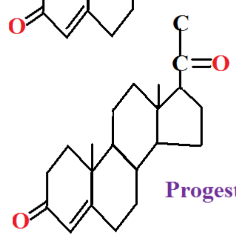
Cortisone

Aldosterone (4th image from top at right) is a mineralocorticoid that is responsible for directly regulating sodium/potassium ion regulation and indirectly with water balance and chloride ion regulation.



Aldosterone

Progesterone (bottom image at right) is the last female sex steroid hormone and is also the precursor for the synthesis of testosterone in the male.



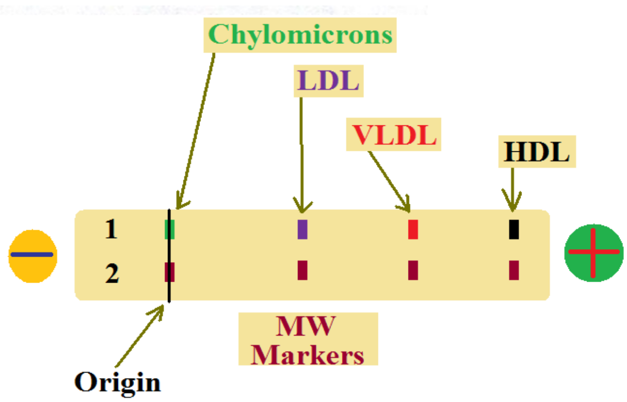
Progesterone

The Most Complex Lipids: The Lipoproteins of The Blood

Lipoproteins ≡ lipid complexed with water-soluble proteins. We are interested in 4 categories of lipoproteins: Chylomicrons, VLDL's, LDL's and HDL's (very low density lipoproteins, low density lipoproteins and high density lipoproteins). The table below describes the characteristics of the lipoproteins:

Classes of Lipoproteins				
	% Protein	% TAG	% PL	% Cholesterol esters
Chylomicrons	1-2	85-95	3-6	2-4
VLDL	6-10	50-65	15-20	16-22
LDL	18-22	4-8	18-24	45-50
HDL	45-55	2-7	26-32	15-20

Chylomicrons, by and large, are artifactual in the sense that they appear after we've eaten or if we have some sort of lipid metabolizing disease, e.g. diabetes mellitus, and are usually rapidly cleared from the blood stream under normal circumstances.



The LDL's are the cholesterol forms we hear about as the "bad" cholesterol. This portion is true as when we get too many LDL's, the cholesterol plates out in our arteries and forms atherosclerotic plaques.

While the HDL's are touted as the "good" cholesterol, it is important to remember that it is ONE form of the HDL that is the good cholesterol. ASIDE: Not all cholesterol is "bad" cholesterol. Remember, we need some cholesterol in our diets to synthesize steroid hormones. While our bodies WILL synthesize steroids from smaller molecules, they prefer cholesterol as the starting molecule.

LDL-Cholesterol

Note that LDL-cholesterol runs about half cholesterol. In many instances, it is difficult to obtain an actual lab analysis of the LDL-cholesterol. In that case, it may be calculated in one of two ways:

Method 1:

- LDL = $\text{Cholesterol}_{(\text{total})}$ - (VLDL + HDL)

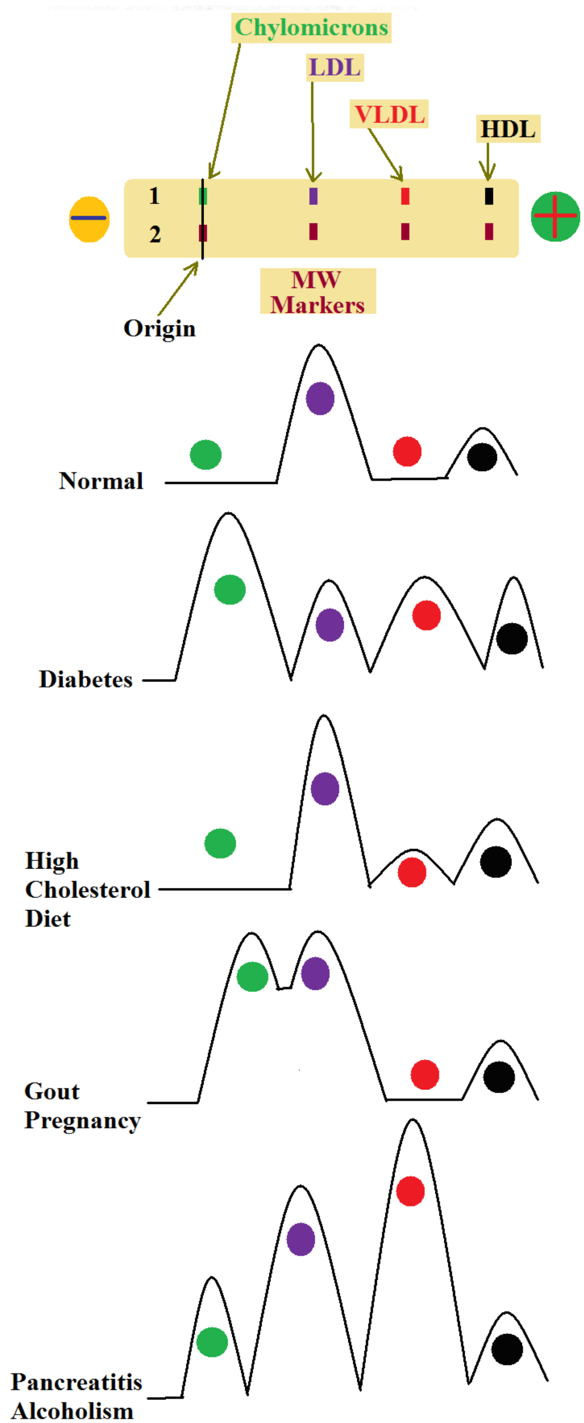
Method 2:

- LDL = $\text{Cholesterol}_{(\text{total})}$ - [(0.2*TAG) + HDL]

where TAG are the triglycerides in your blood.

These lipoproteins may be separated in a polyacrylamide gel or on paper that is in an appropriate buffer by applying an electrical current. This is called electrophoresis. As the molecules migrate into the gel under the influence of the current, they "are looking" for the region of the gel at that particular pH and charge where the medium is most "like themselves". Once these molecules reach that region, they stop migrating and may be analyzed. A representative graphic that demonstrates the separation of these lipoproteins at a pH of 8.6 is at the top of this page.

Note that with the exception of the Chylomicrons, the other lipoproteins are attracted to the positive side of the gel (or paper), indicating that their charges are more negative. The origin is the place where the samples are place in tiny wells in the gel. The Chylomicrons did not move from the origin.



Information like this is very useful, clinically. Patterns of lipoproteins may be detected by utilizing a gel scanner that uses light to detect how much of a particular lipoprotein is present. Some sample scans and their associated disease states are presented to the right.

Note that

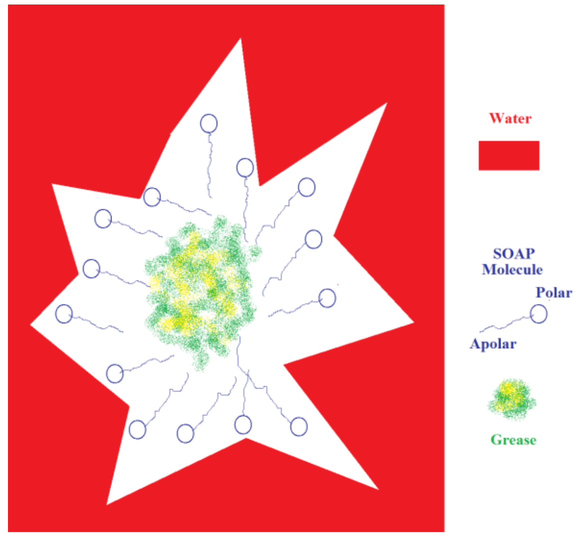
- **diabetics have elevated Chylomicrons and VLDL's with lowered LDL-cholesterol;**
- **those eating a high cholesterol diet have elevated LDL's and VLDL's;**
- **those with gout have elevated LDL's and VLDL's, as do pregnant women;**
- **those with pancreatitis or alcoholism have elevated Chylomicrons, LDL's and VLDL's.**

Saponification

Saponification is the process to make soap from fat – specifically from triglycerides. When lye (NaOH) is used, a hard soap is formed; when KOH is used, a soft soap is formed. In general, 3 moles of either base react with 1 mol of trigluceride to give 1 mol of glycerine and 3 mols of either “sodium soapate” or “potassium soapate”.

Micelles

Micelles form three-dimensional cages around substances. In this case, the detergent sets its polar portion towards the aqueous environment, while the apolar tail interacts with the grease (image at right), thereby “lifting” the grease out of whatever, e.g., fabric, skin, even in the bowel during fat digestion.



Rendering Lipids Esthetic

Freshly pressed olive oils, freshly pressed seed oils, freshly processed milk fat products Do NOT need further refinement.

Lipids containing carotenoids, lipoproteins, sterols, gums, lipopolysaccharides , pohosphoglycerides , lipid auto-oxidation products, polymerization products, anabolic steroid residues, lipid-soluble hydrocarbon pesticides, mycotoxins due to fungal invasions, foul smelling ketones and aldehydes , DO require further refinement.

The most common refining techniques are 1) Neutralization, 2) Degumming, 3) Bleaching, 4) Deodorizing and 5) Winterization.

NEUTRALIZATION

1. Addition of alkali produces oil-Insoluble soaps from FFA (Free Fatty Acids) called “foots” – when present in great quantities, they are called “soap stock”.
2. High Temperature steam distillation with greatly reduced pressures (vacuum) drives the removal of the volatile FA’s from the acylglycerols.
3. Liquid-liquid extraction of FFA with C_3H_8 has been reported – this is NOT financially feasible for human consumption.

DEGUMMING

Removes mucilaginous substances, proteins, phosphatides from crude fat. This is performed by:

1. Adding dilute H_3PO_4
2. Adding brine
3. Adding alkaline phosphate solutions
4. Steam

Many times, degumming is done to isolate crude lecithins to form edible emulsifiers for margarines, pastas and other fat-containing foods. After de-gumming, oils are centrifuged at high speeds to separate the PL's from the degummed oil. De-gummed oils last longer because carbohydrate-based gums are removed as a source of energy for micro-organisms

BLEACHING

Bleaching removes undesirable colors from oils. Conventional bleaching uses different "earths":

1. Bentonite
2. Activated clay
3. Natural clay
4. Activated charcoal

These compounds also adsorb:

1. Soap residues
2. Gum residues
3. Heavy metals (from hydrogenation – 3 BIG examples: Ni, Pt, Pd)
4. Phosphatides
5. Water

DEODORIZING

Removes naturally occurring and/or secondarily occurring (oxidative or hydrolytic) flavor/odoriferous compounds. Done by vacuum/steam distillation: 210-280°C and 1-6 mm Hg. Removes volatile FA's to point where 0.02-0.05% FA's are remaining.

Coconut and palm kernel oils need MAJOR deodorizing. Compounds in them include

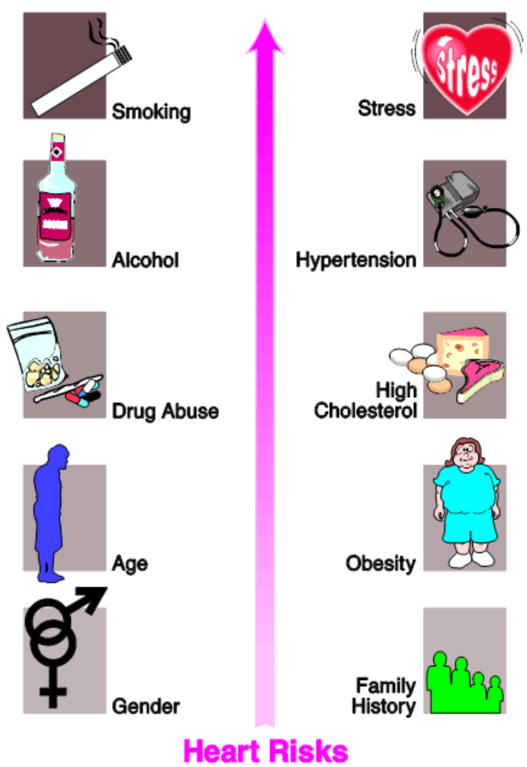
1. Aromatic methyl ketones
2. Unsaturated aldehydes
3. Ketoacids
4. Alcohols
5. Peroxide decomposition products
6. Hydroxy acids

Olive oil and some prime animal fats do not require deodorization as they have desirable odors and flavors.

WINTERIZATION

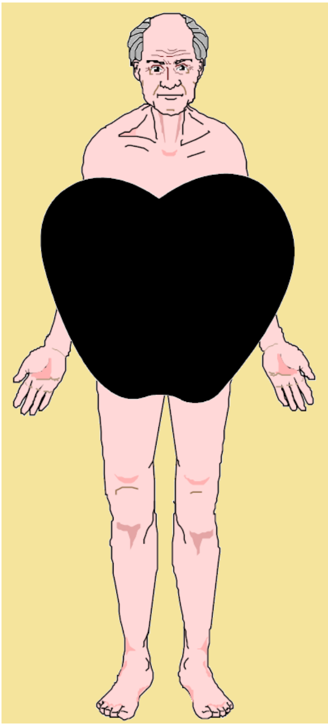
History: cottonseed oil legacy. When cottonseed oil was stored in tanks in winter, about 25% of it solidified. The oil left over ("winter oil") was used for bottling or for mayonnaise. Winterization has come to mean fats cooled to precipitate lipids that would cause clouding of the liquid lipid when stored at refrigerator temperature. Solids are centrifuged or filtered.

Liquid oil remaining evaluated by the "cold test". The cold test is defined as the oil rated by the amount of time required for the liquid fraction to become cloudy at 0°C. Olive oil, corn oil, sunflower seed oil usually are NOT winterized. Soybean oil is hydrogenated and winterized to remain clear at regular fridge temps since it's used as salad oil.

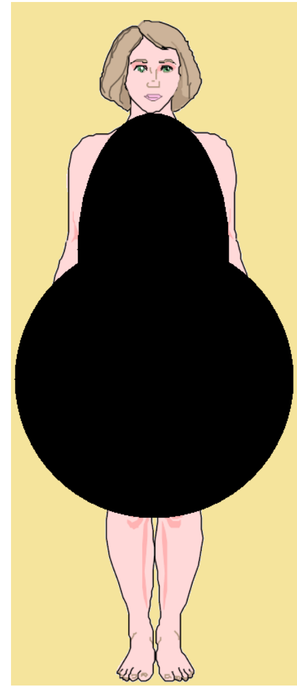


Clinical Applications of Lipids: Metabolic Syndrome -- Cardiac Disease Risk Factors

Metabolic syndrome is characterized by a group of metabolic risk factors in one person. They include: Abdominal obesity (excessive fat tissue in and around the abdomen); Atherogenic dyslipidemia (blood fat disorders — high triglycerides, low HDL cholesterol and high LDL cholesterol — that foster plaque buildups in artery walls); Elevated blood pressure; Smoking; Prothrombotic state (e.g., high fibrinogen or plasminogen activator inhibitor–1 in



the blood); Proinflammatory state (e.g., elevated C-reactive protein in the blood); age (the incidence of metabolic syndrome increases with age); ethnicity (African Americans and Mexican Americans are more prone to metabolic syndrome; African-American women are about 60 percent more likely than African-American men to have the syndrome); body mass index (BMI; kg/m^2; greater than 25 - the BMI is calculated as a measure of body fat compared to height and weight); insulin resistance or glucose intolerance (the body can't properly use insulin or blood sugar); personal or family history of diabetes (there is a greater risk for metabolic syndrome for those who have experienced diabetes during pregnancy (gestational diabetes) or who have a family member with type 2 diabetes); history of heavy drinking, stress, high-fat diet, post-menopausal status, and sedentary lifestyle.



Diagnostic: Apple (Image at top left) or Pear (Image at top right)?

There are no well-accepted criteria for diagnosing metabolic syndrome. Abdominal obesity (excessive fat tissue in and around the abdomen) seems to be a major player in metabolic syndrome. Your shape (apple or pear) is determined by measuring your true waist to widest hip ratio. A ratio greater than 0.8 indicates that you are an apple shape. A ratio less than 0.8 means you are a pear shape.

The apple shape is assigned to those who carry their excess adipose tissue around their abdomen; the pear shape is assigned to those who carry their excess adipose tissue around their hips. The pear shape is healthier. Slender people who are "sticks" with a ratio of 0.8 or greater are still classified as apples based on the ratio.

The National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) says: Women with waist-to-hip ratios of **more than 0.8** are at increased health risk because of their fat distribution. **Men** with waist-to-hip ratios of **more than 1.0** are at increased health risk because of their fat distribution .

Elevated waist circumference:

Men — Equal to or greater than 40 inches (102 cm); Women — Equal to or greater than 35 inches (88 cm). Waist measurements are actually more sensitive than BMI, although since BMI is so widely known, it is proving difficult to make the switch from BMI to waist measurements.

People with metabolic syndrome are at increased risk of coronary heart disease and other diseases related to plaque buildups in artery walls (e.g., stroke and peripheral vascular disease) and type 2 diabetes. Metabolic syndrome has become increasingly common in the United States. It's estimated that over 50 million Americans have it.

The dominant underlying risk factors for this syndrome appear to be abdominal obesity and insulin resistance. Insulin resistance is a generalized metabolic disorder, in which the body can't use insulin efficiently. This is why metabolic syndrome is also called the insulin resistance syndrome.

Other conditions associated with the syndrome include physical inactivity, aging, hormonal imbalance and genetic predisposition. Furthermore, the following are consistent with a diagnosis of metabolic syndrome:

Elevated triglycerides: Equal to or greater than 150 mg/dL

Reduced HDL ("good") cholesterol: Men — Less than 40 mg/dL;   Women — Less than 50 mg/dL

Elevated blood pressure: Equal to or greater than 130/85 mm Hg; Or use of antihypertensive medication (medication used to lower blood pressure).

Elevated fasting glucose: Equal to or greater than 100 mg/dL

Management

For managing both long- and short-term risk, lifestyle therapies are the first-line interventions to reduce the metabolic risk factors. These lifestyle interventions include:

Weight loss to achieve a desirable weight (BMI less than 25 kg/m^2); Increased physical activity, with a goal of at least 30 minutes of moderate-intensity activity on most days of the week; Healthy eating habits that include reduced intake of saturated fat, trans fat and cholesterol.

The primary goal of clinical management of metabolic syndrome is to reduce the risk for cardiovascular disease and type 2 diabetes. Then, the first-line therapy is to reduce the major risk factors for cardiovascular disease: stop smoking and reduce LDL cholesterol, blood pressure and glucose levels to the recommended levels.

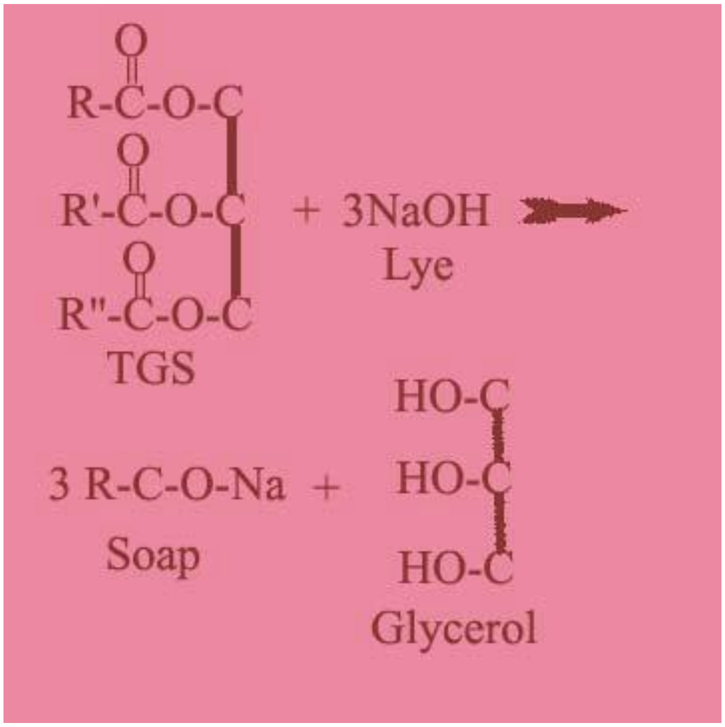
For more information in metabolic syndrome, refer to the following organizations and their websites: **National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III); The American Heart Association; The National Heart, Lung, and Blood Institute; World Health Organization (WHO); American Association of Clinical Endocrinologists (AACE).**

Experimental

<u>Saponification: The Production of Lye Soap</u>

Introduction

In this experiment, you will learn how chemical de-esterification works by hydrolyzing triglycerides (TGS) into soap and glycerol.



Saponification is the hydrolysis of a triglyceride (TGS or TAG) with NaOH or KOH resulting in two general products: glycerol and soap. The production of soap is one of the most ancient chemical syntheses. It, however, is not as old as fermenting grains for ethanol production.

Saponification follows the general reaction at right:

Note that 3 moles of NaOH are required for complete hydrolysis. This is because 3 fatty acids are used to form the TGS. Also note that R may equal R' which may equal R" OR R may not equal R' which may not equal R", e.g. tristearin is a triglyceride that con tains three molecules of stearic acid bound to the glycerine backbone; trilaurin is a triglyceride that contains three molecules of lauric acid bound to the glycerol backbone.

Soap is a mixture of sodium and potassium salts with different fatty acids. Soft soaps, potassium salts, are more water soluble than sodium salts and, therefore, easily lather. Liquid soaps and shaving creams are manufactured as potassium salts. Saturated fats, such as lard, produce hard soaps, while unsaturated fats, such as olive oil, yield soft soaps.

In this experiment, you will use alcoholic KOH to hydrolyze the TAG's in lard. Alcohol is used because it dissolves the KOH and the lipid. Additionally, sodium soap will also be made by adding a portion of your potassium salt to a saturated saline solution.

Materials and Methods

Materials

The table, below, summarizes the supplies you will need for this experiment:

Lard	Saturated saline	$FeCl_3$ solution	Litmus paper
20% alcoholic KOH	$CaCl_2$ solution	Reflux apparatus	Buchner funnel and filter paper
Distilled water	$MgCl_2$ solution	Heating mantle with Variac	Watch glass
Spatula	Disposable test tubes	Ring stand	Clamps

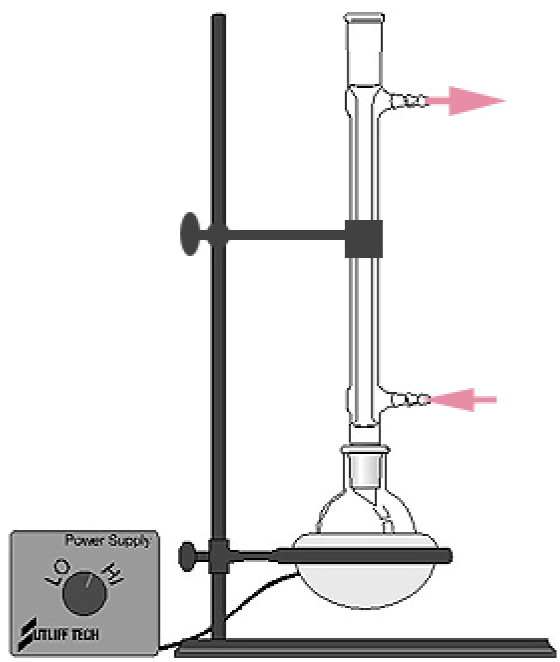
Method

Assemble a reflux apparatus as illustrated at bottom right (connect bottom pink arrow via tubing to the sink nozzle and run another hose from the top connector into the sink for a drain).

Place 10 grams (about a tablespoon) of lard into the round bottom flask. Add 40 mL of 20% alcoholic KOH solution to the lard.

Reflux for 30 minutes (what IS reflux?).   A lack of fat droplets indicates that saponification is complete.

After saponification, boil off the excess alcohol by pouring your saponified product into a beaker and boiling it CAREFULLY in the heating mantle – NO FLAMES!!!! ALCOHOL IS FLAMMABLE!!!!!!



The residue will become tacky and foamy and viscous on completion. Once the alcohol is cooked off, add 100 mL DISTILLED water and heat the mixture with stirring until you have a solution.

Pour half of your solution into an equal volume of saturated saline (that's 50 mL) with vigorous stirring. Large curds will develop. Filter the mixture in a Buchner funnel. Press the solid between paper towels to remove excess water.

Some of this soap will be used later – that which you do not use, you may keep if you so desire.

Test 2 portions of the 2 soaps for sudsing. Streak some lard lightly onto a watch glass in two lines and see if the 2 soaps will remove the grease. Does one do it better than the other? Which one?
Dissolve 3 small portions of your sodium soap in 1 mL distilled water; do the same with your potassium soap (you should have 6 tubes for this).

To these solutions, add 10 drops of the following into a tube, i.e., put 10 drops of the calcium chloride in one tube with sodium soap and in one tube with potassium soap; put 10 drops of the magnesium chloride in one tube with sodium soap and in one tube of potassium soap; put 10 drops of the iron(III) chloride in one tube of sodium soap and in one tube of potassium soap. Do any precipitates form?

Mix 1 mL each of your sodium soap and potassium soap with 1 mL of tap water. Does a precipitate form? What is the common name of these precipitates? Using litmus paper, determine the acidity or alkalinity of your two soap solutions.

Questions

1) In the space below, write the reaction for the hydrolyisis of tripalmitin with KOH.

2) What are the precipitates from the mineral solutions? Relate this to bath-tub ring.

3) Why is the saline solution important?

4) What do you conclude about the tap water in the lab? Is it hard or soft? Why or why not?

5) Based on your litmus paper reactions, what is the general pH of your 2 soap solutions?