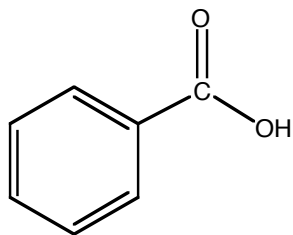


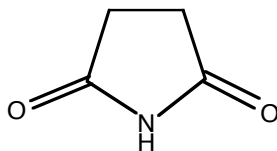
Experiment 2: Melting Points and the Identification of an Unknown and Cholesterol from Human Gallstones

Part 1. Melting Points and the Identification of an Unknown

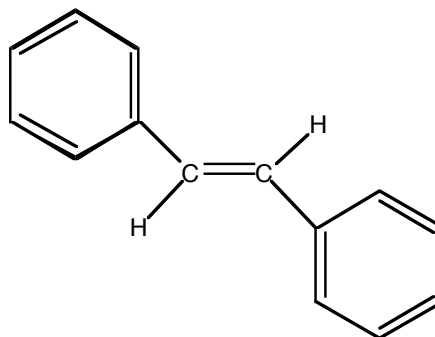
Read pp 116-127, Technique 10, (especially pages 123-124, 10.4) in *TOC* and **outline** the following procedure. You will determine the melting points of pure benzoic acid, succinimide and *trans*-stilbene.



Benzoic acid



Succinimide



***trans*-Stilbene**

Use the following procedure for each:

Obtain 0.5 g of the compound in a clean vial or test tube. Using a metal spatula, crush a portion of the solid to a fine powder on a clean, hard surface, such as a watchglass (do *not* crush the solid using a dirty mortar and pestle!). Push a melting point capillary into the powder, and force the powder down the capillary by tapping it or by dropping it through a long glass tube held vertically and resting on a hard surface. The column of solid should be no more than 2-3 mm in height, and it should be packed tightly. Mark the capillaries in the following manner (a marker will be provided):

- benzoic acid with **1** dot
- succinimide with **2** dots
- *trans*-stilbene with **3** dots

Your TA will demonstrate how to use the Mel-Temp. Use a setting of no higher than 45 on the Mel-Temp. Note at what temperature the first drops of liquid appear in the capillary and at what temperature the last trace of solid disappears (the *melting point range*). The melting points of up to three different samples can be obtained at the same time in the Mel-Temp. Determine the melting points of the three pure compounds simultaneously. Allow the Mel-Temp to cool to 80° C or lower before attempting a determination.

Your TA will provide you with a vial that contains either benzoic acid, succinimide or *trans*-stilbene. Write the code number in your notebook. In order to identify which compound is contained in your vial, use the following procedure:

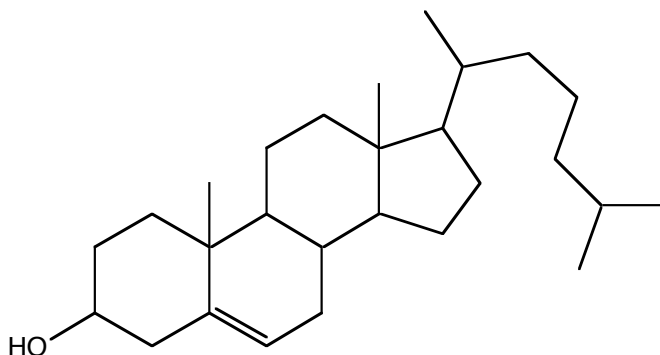
Weigh 0.25 g of the compound onto a clean watchglass, then return the vial to your TA. **Do not contaminate the contents of the vial!** Using a metal spatula, crush a portion of the solid to a fine powder. Make mixtures of your unknown with each pure substance in approximately 1:1 ratios on three different pieces of weighing paper. Label the pieces of weighing paper with dots as described above. To make the mixtures, place a small pile of the unknown substance next to an equal-sized pile of the pure substance on the

weighing paper, then mix them. Grind the mixture thoroughly for at least a minute using a metal spatula. Pack the mixtures into capillary tubes and label the capillaries with one, two and three dots. Determine the melting ranges of the three mixtures simultaneously.

Always place used capillary tubes into the GLASS waste boxes! The custodian who removes the paper trash might get cut by broken glass if capillaries and glassware are thrown into the waste basket. Place any leftover solid compounds into the appropriate **Laboratory Byproducts** jar.

Part 2. Cholesterol from Gallstones

The average human body contains 200-250 grams of cholesterol. This much maligned compound is actually a necessary ingredient for a variety of bodily functions. It



Cholesterol
m.p. 148-150°

is used in the synthesis of hormones and other steroids. It is a component of cell membranes where it contributes rigidity. The products of the breakdown of cholesterol are the bile salts, which aid in digestion.

As you will see in today's experiment, cholesterol is very insoluble in water. In order for it to be transported through the blood, it must combine with lipoproteins that increase its water solubility. The lipoproteins which transport the cholesterol have varying ratios of lipids (fats) and proteins and therefore have varying densities. The Low Density Lipoproteins, LDLs, transport the cholesterol from the liver to the different parts of the body where it can be put to good use. While our bodies require *some* cholesterol, *too much* cholesterol can cause problems if it is deposited from the LDLs onto the walls of the arteries, leading to atherosclerosis. For this reason, the LDLs are known as the "bad cholesterol". On the other hand, the High Density Lipoproteins, HDLs, scavenge the excess cholesterol in the bloodstream and transport it back to the liver where it can be broken down and excreted. For this reason, the HDLs are known as the "good cholesterol".

From the liver, excess cholesterol which is not broken down is transported to the gallbladder with *bile*, a substance that contains emulsifying agents and salts that are necessary for digestion. The gallbladder stores the bile and secretes it into the small intestine when it is needed. If too much cholesterol is present in the bile, it will precipitate in the gallbladder due to its low solubility in water. The solid cholesterol forms agglomerates, or "gallstones". In fact, the Greek words for bile, *chole*, and solid, *stereos*, combine to form the word *cholesterol*. In today's experiment, you will extract and purify cholesterol from gallstones.

When gallstones become large enough to irritate the gallbladder, causing pain, nausea, vomiting and obstruction of the bile ducts, they must be removed. Gallstone removal was traditionally accomplished by surgery; however, more recent developments have led to chemotherapies in which the gallstones are dissolved either by introduction of the solvent methyl *tert*-butyl ether directly into the gallbladder via catheter, or by oral administration of a bile acid. Fifteen years ago it was possible for us to obtain real gallstones from hospitals in the Boston area from which students could extract cholesterol. With the advent of these new dissolution techniques, it is no longer possible for us to obtain real gallstones, so you will extract cholesterol from "synthetic" gallstones. A jar of actual gallstones will be available for viewing purposes.

This extraction and crystallization of cholesterol from gallstones uses a *mixed solvent pair* for recrystallizing an impure compound. This technique is carried out by first dissolving the compound in a solvent in which it is quite soluble, then adding to the hot solution a solvent in which the compound is *insoluble* until the saturation point is reached. The saturation point is indicated by a cloudiness in the solution which will not dissipate with mixing. Once the saturation point is reached, the solution is removed from the heat source and allowed to stand at room temperature until crystals form.

Review pp 103-104, 9.3, in *TOC* and **outline** the following:

Place 100 mg of gallstones in a reaction tube (if necessary, crush large lumps with a mortar and pestle). Add 1.5 mL of 2-butanone and a boiling stick. Dissolve the gallstones as much as possible by gentle heating in an aluminum block. Keep the hot plate on a low setting. [**Note:** The aluminum block contains a hole for a thermometer; however, you should **never** place your thermometer in this hole as the Teflon coating on the thermometer could melt.] Set up the apparatus shown in Figure 1 on the [Figure Page, Expt. 2](#) (bring it to lab with you) but do **not** attach the connector to the vacuum. Do **not** use filter paper. After the solution has boiled for a minute or two, simply pour the hot mixture directly onto the filtering disk in the Hirsch funnel and allow the hot solution to filter by *gravity* into the reaction tube. Use about 0.3 mL more of hot 2-butanone to complete the transfer of material from the reaction tube and to wash the funnel.

Set a beaker of water on a hot plate, *clamp* a thermometer in the water and heat to 60-70°. Evaporate the solution in the reaction tube to 0.75 mL using the set-up shown in Figure 2 on the [Figure Page, Expt. 2](#). Partially open the vacuum outlet and place the reaction tube in the beaker of hot water. Shake the reaction tube gently back and forth during the evaporation. When the volume of the solution is approximately 0.75 mL, turn off the vacuum, remove the rubber septum and add 0.5 mL of methanol. Crystals should appear. Continue to add methanol until all of the crystals *just* re-dissolve. Do not add more than 1.5 mL total of methanol. Place a boiling stick in the reaction tube, and heat the solution to the boiling point. Add water dropwise until a very faint cloudiness appears which does not dissipate with shaking. At this point, the solution is saturated with cholesterol because cholesterol is insoluble in water and only slightly soluble in methanol.

Place the rubber septum loosely on the reaction tube and set it in a beaker. Allow it to cool undisturbed to room temperature, and then place the tube on ice. Collect the solid product by vacuum filtration using the set-up in Figure 1 *with* the connector attached to the vacuum. Use water to transfer the crystals from the reaction tube to the Hirsch funnel (you may also scrape them out with a spatula). Wash the crystals with very small amounts of water. Do not collect a second crop of crystals unless the yield is very low. A second crop is generally less pure than the first crop.

Scrape the crystals from the Hirsch funnel onto a clean watchglass or piece of filter paper and allow them to dry. Pour the filtrate, which contains 2-butanone and methanol, into the appropriate **Laboratory Byproducts** jar.

You will obtain the melting point and weight of your product next week after it has dried completely. You should write most of the Discussion for [Assignment #1](#) this week while Experiments 1 and 2 are fresh in your mind. Two days after your next lab period, you will be required to hand in this Discussion, as well as the Results for Experiment 2, to your TA.

Name _____ Date _____

T. A. _____ Lab period _____

Results and Calculations (to be handed in, along with the Discussion for Experiments 1&2, two days after the next lab period)

Melting ranges for:

benzoic acid _____

succinimide _____

trans-stilbene _____

unknown + benzoic acid _____

unknown + succinimide _____

unknown + *trans*-stilbene _____

Identification of unknown substance _____ Code # _____

Calculate the percent recovery of cholesterol from the gallstones.

Melting range for purified cholesterol: _____