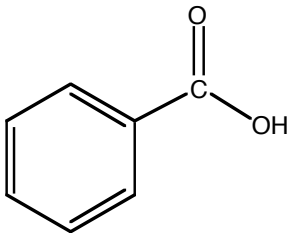
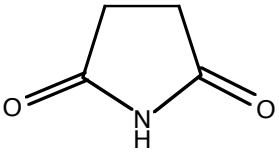
Experiment 2: Melting Points and the Identification of an Unknown *and* Cholesterol from Human Gallstones

Part 1. Melting Points and the Identification of an Unknown

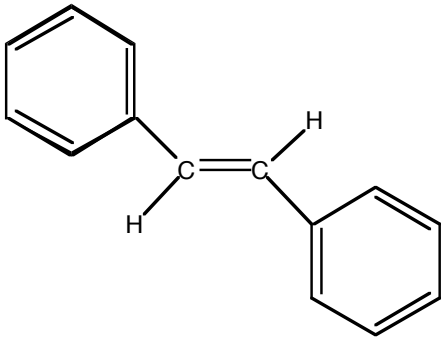
Read pp 211-220, Chapter 14, (especially page 218, 14.5) in *LTOC* and view a video about this technique at http://www.macmillanlearning.com/Catalog/studentresources/mohrig4e/. Select **Videos**, then choose **14.1 How to Pack a Melting Point Tube** (you may stop watching when she starts to talk about sealing the tube).



Benzoic acid



Succinimide



***trans*-Stilbene**

You will determine the melting points of pure benzoic acid, succinimide and *trans*-stilbene. Use the following procedure for each (**outline** these steps):

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 DigiMelt (see Figure 14.2 in *LTOC* on page 214). Refer to the attached instructions (these instructions are also posted in the lab). You do not need to outline these instructions. Set the Start Temp to 105°, the Stop Temp to 135° and the Ramp Rate to 2° per minute. 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 DigiMelt. Determine the melting points of the three pure compounds simultaneously. Allow the DigiMelt to cool to 80° before attempting a determination.

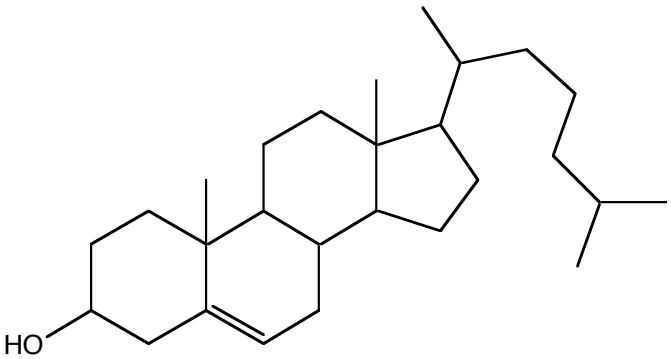
Your TA will provide you with a vial that contains either benzoic acid, succinimide or *trans*-stilbene. Write the code number in your notebook. In order to identify which compound is contained in your vial, use the following procedure:

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 one 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 housekeeping staff who remove 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.



Cholesterol
m.p. 148-150°

It 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 that 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 that 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. Twenty-five 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 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.

The 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 *in*soluble until the saturation point is reached. The saturation point is indicated by a cloudiness in the solution that 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 226-227 in *LTOC* and view video **15.1 Recrystallization** at http://www.macmillanlearning.com/Catalog/studentresources/mohrig4e/. **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 on a hot plate (no temperature probe) in an aluminum block. Set the temperature of the hot plate to 250°. Set up the apparatus shown in Figure 1 on 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 an additional 0.3 mL of hot 2-butanone to complete the transfer of material from the reaction tube and to wash the funnel.

Turn off the hot plate and, using the cloth towel in your drawer, carefully remove the hot aluminum block. Attach a temperature probe, set a beaker of water on the hot plate and place the probe in the water. You will evaporate the solution in the reaction tube to 0.75 mL using the set-up shown in Figure 2 on Figure Page, Expt. 2. Clamp the reaction tube *above* the water bath and only partially open the vacuum outlet. Lower the reaction tube into the water bath. Turn on the hot plate and set the temperature to 60°C. 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 in a water bath. 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 in 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.

If you were not able to obtain all the dipole moments of the molecules from Part 2 of Experiment 1 last week, continue to work with others using the Spartan program.

You will obtain the melting point and weight of your cholesterol 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.

Instructions for using the DigiMelt apparatus

(you may bring this sheet to lab with you)

Read about melting points on pages 211-220 in *LTOC* before using the DigiMelt.

1. Turn on the DigiMelt using the power button on the back. Wait until the temperature of the device is displayed.
2. Push the yellow Start Temp button and the yellow LED next to Start should flash. Use the blue button with the up arrow/number 2 or down arrow/number 3 to set the starting temperature (about 10 degrees below melting point). If you press and hold the button, the temperature will move by single degrees, then by five degrees.
3. Push the yellow Stop Temp button and the yellow LED next to Stop should flash. Use the arrow buttons to set the stop temperature (about 10 degrees above melting point).
4. Push the yellow Ramp Rate button and the yellow LED next to Ramp should flash. Use the arrow buttons to set the rate at which you want the temperature to increase. Usually 2° per minute is appropriate.
5. Push the Ramp Rate button again, and the device will display its current temperature.
6. Push the Start/Stop button and the red LED next to Preheat should light. The oven will heat up quickly to the Start temperature and stop.
7. When the red Ready LED becomes lit, insert your capillary/capillaries with sample into the hole(s) above the lens.
8. Push the Start/Stop button and the red LED next to Melt should light. The temperature should now increase at the rate that you set for the ramp. Observe your sample(s) at this time.
9. Record in your notebook the melting range(s) for the compound(s).
10. To finish before the Stop temperature is reached, push the Start/Stop button. The red LED next to Cooling should light, indicating the oven has turned off. If the device reaches the Stop temperature before you push the Start/Stop button, the oven will shut off and the Cooling LED will light.

The highest temperature that the instrument will reach is 260°C. If it reaches this temperature, the oven will shut off and it will cool down.

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: _____