Cholesterol from Human Gallstones


Prelab Exercise
Refer to your lecture text. Show structures of products predicted for the reaction of cholesterol with: 1) Br₂; 2) H₂ (Pd/C); 3. PCC (pyridinium chlorochromate) in CH₂Cl₂. Show stereochemistry at the new bonds formed.

Introduction
In this experiment, cholesterol will be isolated from human gallstones. Cholesterol is an unsaturated alcohol containing 27 carbon atoms and 46 hydrogen atoms:

![Cholesterol structure](image)

It is a solid (mp 148.5°C) and is insoluble in water but soluble in boiling ethanol and dioxane.

The gall bladder is attached to the undersurface of the liver just below the rib cage. It retains bile produced by the liver and feeds it into the upper part of the small intestine as needed for digestion. Bile consists primarily of bile acids, which are carboxylic acids closely resembling cholesterol and which aid in the digestion of fats by functioning as emulsifying agents. The gall bladder also harbors free cholesterol. If the concentration of cholesterol in the bile exceeds a certain critical level, it will come out of solution and agglomerate into particles that grow to form gallstones. An amateur geologist given a bottle of gallstones to identify once labeled them a "riverbed conglomerate"—and indeed they do resemble stones in color, texture, and hardness. They come in a variety of shapes and colors and can be up to an inch in diameter.

As gallstones collect, they irritate the lining of the gall bladder, causing severe pain, nausea, and vomiting. The stones can block the bile duct and lead to fatal complications. Formerly, the remedy was major surgery. Now, the gallstones are disintegrated in the gall bladder and the entire organ removed through a small incision in the navel. Consequently, it may soon be impossible to obtain whole human gallstones.

In the average human, approximately 200 g of cholesterol is concentrated primarily in the spinal cord, brain, and nerve tissue. Insoluble in water and plasma, it is transported in the bloodstream bound to lipoproteins, which are proteins attached to lipids (fats). Recent research has divided these lipoproteins, when centrifuged, into two broad classes—high-density (HDL) and low-density (LDL) lipoproteins. A relatively high concentration of HDL bound to cholesterol seems to cause no problems and in fact is beneficial, but a high ratio of LDL cholesterol leads to the deposition of cholesterol both in the gall bladder (resulting in gallstones) and on the walls of the arteries (causing a plaque that cuts off blood flow and hastens hardening of the arteries or atherosclerosis).
Mounting evidence points to unsaturated fats such as those found in vegetable oils as favoring the HDL-cholesterol bond, while LDL-cholesterol formation is speeded by saturated fats such as those found in animals. The HDL cholesterol level goes down with smoking or eating large amounts of sugar. It goes up with regular exercise and with the consumption of moderate amounts of alcohol (a glass of wine per day). The 1985 Nobel prize in physiology or medicine went to Michael Brown and Joseph Goldstein for their pioneering work on LDL- and HDL-cholesterol.

The average American woman at age 75 has a 50% chance of developing gallstones, while for a man of the same age the chance is only half as great. Gallstones and coronary heart disease are also much more common in overweight people. Almost 70% of the women in certain Native American tribes get gallstones before the age of 30, whereas only 10% of black women are afflicted. Swedes and Finns have gallstones more often than Americans; the problem is almost unknown among the Masai people of East Africa.

**Cautions**

Some peroxycarboxylic acids are explosive; the reagent used in the present experiment is a particularly stable and convenient peroxycarboxylic acid that is quite safe to handle.

**Cholesterol from Gallstones**

Cholesterol is a white crystalline compound that melts at 148-149°C; crushed gallstones are usually dirty brown in color. The gallstones are treated with hot 2-butanone, which dissolves the cholesterol but not the impurities (principally bilirubin, a metabolite of hemoglobin).

![Chemical Structure of Bilirubin](image)

While the butanone is hot, the mixture is filtered, using the Hirsch funnel, to remove the insoluble impurities. Not much cholesterol will crystallize when the butanone solution is cooled because cholesterol is very soluble in this solvent, but if some water is added to the hot butanone solution, the solubility of cholesterol in the mixed solvent will be decreased. On cooling, a higher yield of cholesterol will be obtained. The product is isolated by filtration on the Hirsch funnel.

**Isolation Procedure**

Crushed gallstones can be found on the hooded shelf. Do not breathe the dust as the gallstones may not be sterile. Weigh out 100 mg and return the remainder to the Gallstone supply bottle.

Place the 100 mg in a 10 x 100 mm reaction tube and add 1.5 mL of 2-butanone. Insert a boiling stick and dissolve the solid by gentle heating on a hot sandbath.
Also, place a test tube with 0.4 mL of 2-butanone in the sand bath. Start the Varistat around 50 and increase only if necessary. While this is heating, set up a Hirsch funnel in a separate ring stand with 1.3 cm filter paper on the plastic frit inside to keep it clean. Make sure to use the thick-walled vacuum tubing in your drawer. Attach this to the vacuum so there is gentle suction, and filter the hot solution through the Hirsch funnel.

When filtering is complete, use the 0.4 mL of hot 2-butanone to complete the transfer and wash the funnel. Transfer the solution from the filter flask to a clean reaction tube, and dispose of the bilirubin on the filter paper in the trash bin.

Clean the Hirsch funnel and insert a new piece of filter paper. Turn on the nitrogen in the hood, place a pipet at the end of the tubing to direct a stream of N\textsubscript{2} gas to evaporate the solution to approximately 0.75 mL.

Now add 0.5 mL of methanol to the reaction tube. Place a boiling stick in the reaction tube, heat the solution to boiling, and then add water drop-wise until a very faint cloudiness appears. This will take about 5 drops and appear whitish in appearance. It produces a solution saturated with cholesterol at the boiling point because cholesterol is not very soluble in methanol.

Cap the tube with a size 0 cork, wrap it in some insulating material (cotton batting or a paper towel), and place it in a beaker to cool undisturbed to room temperature. Then cool the tube in ice and collect the cholesterol on the Hirsch funnel by vacuum filtration. Save the filtrate.

If desired, a second crop of less pure material can be collected by concentrating the filtrate to the point of saturation just as in the original crystallization. After the crystals are dry, determine the weight and melting point of the cholesterol, and calculate the percent recovery of the cholesterol from the human gallstones. The percent recovery should around 50%. Analyze according to your assignment sheet and instructions for product analysis in the Lab Guide.

As you leave lab, go by the stockroom and examine the bottle of whole gallstones so that you can answer PostLab question 2 below.

**Analysis**
Analyze your final product by IR.

**Cleaning Up**
The solvents 2-butanone and methanol should be placed in the organic solvents container, and the bilirubin on the filter paper and the small cork should be placed in the trash bin.

**Postlab Questions**
1. Cholesterol is an alcohol. Why is it more soluble in organic solvents than in water?
2. Explain the appearance of natural gallstones now that you know the composition of the two main components of gallstones.