Isolation of Limonene from Orange Rind

CO₂ phase transitions
Extraction
Infrared spectroscopy

In this experiment, you will use liquid CO₂ to extract the essential oil limonene from orange rind. Following the isolation of limonene, you will determine its infrared spectrum and assign the major peaks observed in the spectrum to structural features present in the molecule. Mass spectrometry might also be used to determine structure and purity.

Required Reading
Review: Technique 4 (Handbooks and Catalogs)
Technique 8B (Filter Paper)
New: Phase transformations and critical points
Infrared spectroscopy
Mass spectrometry (optional)

Safety Precautions
Special care is required when the tube is in the water bath because the tube could explode or the lid could fly off due to the high pressure created in the centrifuge tube during the extraction. Use only the equipment and methods mentioned here, and do not, under any circumstance, use glass products as a substitution. It is highly recommended to perform this experiment in the hood.

Procedure

Preparing the Orange rind. Grate only the colored part of the peel of 1/2 of a medium-sized orange with the smallest grating surface of a standard cheese grater or zester into a pre-weighed weighing boat. Make sure you have at least 2.5 g of grated orange rind.

Extraction Vessel: Record the mass of a 15 mL centrifuge tube. Using a 20 cm piece of copper wire and a piece of 1.5 cm filter paper, make a solid trap. Wrap three coils of wire such that the apparatus fits inside the tube with the coils stopping near the beginning of the taper. Use the rest of the wire as a handle projecting up from the coils. All wire should be inside the tube. Cut off any extra wire. Put one piece of filter paper
between the coils, making a base to support the solid, and fold up the edges around the wire. Slide solid trap into tube. Add about 2.5 g of grated orange rind. Do not pack tightly.

**Extraction.** Fill the graduated cylinder two-thirds full with hot tap water (40-45°C) and place it under the hood. Fill the centrifuge tube with the finely crushed dry ice, tapping the bottom of the tube on the counter and adding more ice until the tube is full, then cap the centrifuge tube quickly and tighten it until the cap stops twisting. Lower the full centrifuge tube tapered end first into the graduated cylinder water bath waiting in the hood. The dry ice should become liquid CO$_2$ after a minute and remain in the liquid phase for about 3 minutes until all the gas has seeped out the top. If the CO$_2$ has not entered the liquid phase after 2 minutes, the centrifuge tube did not have a tight enough seal, and either the cap and tube should be replaced, or the dry ice was left in the tube too long without sealing the cap on.

After the all the liquid CO$_2$ is gone, uncap the tube. If necessary, rearrange the solid orange peel before the second extraction. A piece of wire can be used to break up the solid mass and create a channel to the bottom for liquid CO$_2$. Repeat the extraction by refilling the tube with dry ice, resealing the cap and putting the tube back in the water.

**Yield Determination.** Carefully remove the solid and the trap by pulling the wire handle with tweezers. Pre-weigh a small sample vial before collecting the extracted oil in it, and reweigh the vial after the oil has been collected. Calculate the weight of and the weight percentage recovery of the oil from the original amount of orange rind used.

**Spectroscopy.**

Infrared. Obtain the infrared spectrum of the oil as a pure liquid sample. (Technique 25, Section 25.2). It will be necessary to use a Pasteur pipet with a narrow tip to transfer a sufficient amount to the salt plates. Include the infrared spectrum in your laboratory report, along with an interpretation of the principal peaks.$^1$

Mass spectrometry. At the instructor’s option, determine the mass spectrum of the oil (Technique 28).

**Report**

Attach your infrared spectra to your report and label the major peaks with the type of bond or group of atoms that is responsible for the absorption. If you determined mass spectra, identify the important fragment ion peaks. Be sure to also include your weight percentage recovery calculation.

---

References