Green Isolation of Limonene

Essential oils are organic compounds that are extracted from natural sources and used in many products such as flavorings, fragrances, and cleaning products. The optically active enantiomer, D-limonene, is the major component of orange oil, which is found in the outer, colored portion of the rinds of oranges and other citrus fruits.

Traditionally essential oils have been extracted through the use of steam distillation or organic solvent extraction. During the past two decades, great strides have been made in technology that uses supercritical or liquid carbon dioxide in place of organic solvents. CO_2 is useful as a green alternative solvent because it provides environmental and safety advantages; it is nonflammable, relatively nontoxic, readily available, and environmentally benign. Although CO_2 is a greenhouse gas, when used as a solvent it is captured from the atmosphere, not generated, resulting in no net environmental harm. Large-scale CO_2 processing has had commercial success in many separation and extraction processes. The tunable solubility properties, low toxicity, and ease of removal of CO_2 have led to well established CO_2 technology for the extraction of various food products, including essential oils and hops, and for the decaffeination of coffee and tea.

Another major benefit of using carbon dioxide as a solvent is its accessible phase changes. Unlike other gases, relatively low temperatures and pressures can be used to form liquid and supercritical CO_2. As shown on the phase diagram in Figure 3, CO_2 sublimes at atmospheric pressure of 1.01 bar. The triple point of CO_2, where solid, liquid, and gas phases coexist in equilibrium, is achieved at 5.2 bar and -56.6 ºC. At or near this point, dry ice melts, forming liquid carbon dioxide. If the temperature and pressure are increased to the critical point (73.8 bar and 31.0 ºC), the CO_2 exists as a supercritical fluid and has no distinct liquid or vapor phase but properties that are similar to both.

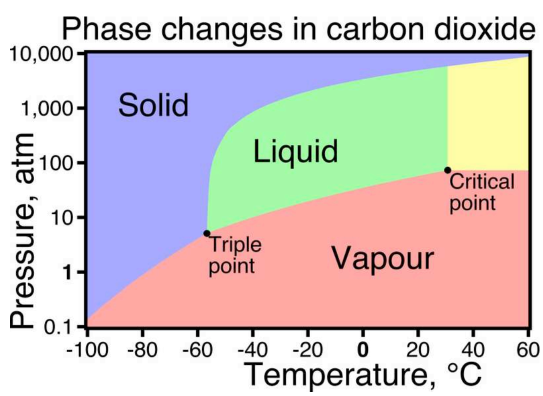

Figure 1: The temperature-pressure diagram for CO_2 shows phase transformations, triple point, and critical point.

Dry ice sublimes at atmospheric pressure and temperatures above -78 ºC. If the CO_2 is sealed in a vessel during sublimation, the internal pressure in the vessel increases. After the temperature and pressure have increased sufficiently, liquid carbon dioxide forms. Due to this accessible phase change, carbon dioxide can be used for bench top extraction processes. In many organic teaching laboratories, D-limonene is extracted through a solid/liquid extraction with pentane or methylene chloride or by a steam distillation. In this experiment you will be given the opportunity to extract D-limonene from orange rinds through the use of liquid CO_2. The greenness of the CO_2 extraction process can be compared with different extraction procedures through the evaluation of waste, purity, energy use, yield, and safety.

LEARNING OBJECTIVES

Students will:

-use experimental observations to differentiate among properties of solids, liquids, and gases. (3.1xxii)

-compare the observed phase changes of an experiment to a phase change diagram for carbon dioxide.

MATERIALS

Orange Peel	Dry Ice	Water
Zester	100 mL beaker	Centrifuge Tubes
Copper wire	Filter Paper	Plastic bottle
Scoop	Balance	

HAZARD INFORMATION

Chemical Name	Health Hazards	Physical Hazards	Environmental Hazards
Carbon Dioxide (gas)	Low	Gas under pressure	Low
Carbon Dioxide (solid)	low	Extremely cold solid	low

WASTE

Description of Material (include concentration)	Quantity per student	Hazard	Disposal Method
Orange peels		None	solid trash waste

PROCEDURE

SAFETY PRECAUTIONS: Due to the high pressure generated during the course of this experiment, there is some **RISK OF VESSEL RUPTURE AND/OR FLYING PROJECTILES**. Under no circumstances should any vessels other than the recommended ones be used. Safety procedures have been implemented to minimize the danger, but it is essential to follow these procedures to protect yourself and other students. Read all safety notes and entire procedure before beginning lab.

Additional Safety Notes:

Do not use any glass during this experiment. Substitution of glass centrifuge tubes or graduated cylinders could result in serious injuries if shattering should occur.

Always wear eye protection. Foreign material could become lodged in the eye if not covered.

Use gloves when working with dry ice. Contact with dry ice can damage skin tissues.

Do not liquefy CO_2 more than 5 times in the same centrifuge tube. After repeated liquefaction, the tube may become brittle and rupture.

NOTE: Due to the safety issues involved with the rapid increase of pressure during this procedure, it is important to read and understand the entire extraction procedure before beginning!

1. Prepare the orange rind – Grate only the colored part of the peel of ½ 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 0.75 g of grated orange rind.

2. Prepare the extraction vessel – Record the mass of a 15 mL centrifuge tube by weighing it in a small tared beaker. Make a solid trap (see Figure 2 A-C, YOUR INSTRUCTOR WILL GIVE YOU FURTHER INSTRUCTIONS). Add approximately 0.75 grams of grated orange rind (NOTE: Do not pack tightly) and record the total weight. Calculate the exact mass of orange peel added to the tube.

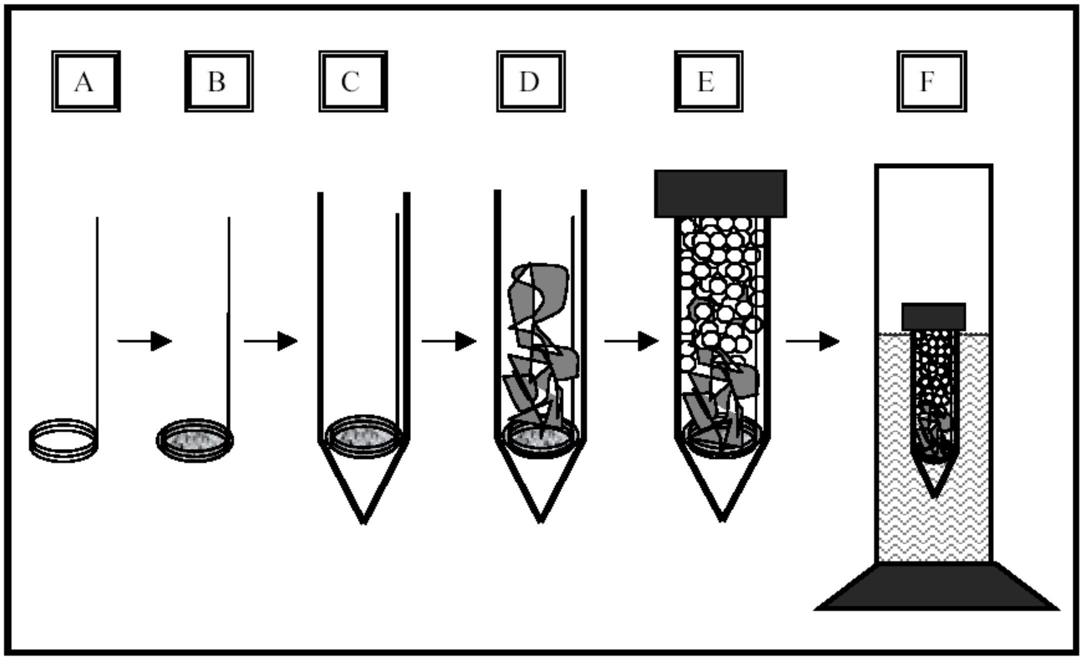

Figure 2: Illustration of the extraction procedure. A solid trap is constructed by (A) bending copper wire into coils and a handle, (B) placing filter paper or small-mesh metal screen between the wire coils, and (C) placing the solid trap in centrifuge tube. For extraction, (D) the grated orange peel is placed in the tube, and (E) the tube is filled with dry ice and sealed with a cap. (F) The prepared centrifuge tube is placed in the water in the plastic cylinder.

3. Prepare the extraction environment – Fill a plastic cylinder or large-mouth polycarbonate water bottle one half to two thirds full of warm (40-50º C) tap water. **Do not heat the water in the cylinder or add hot water later in the procedure.** Any sudden increase in temperature of surrounding water when the centrifuge tube is under pressure can cause the cap to blow off suddenly and violently. NOTE: Move items that should not get wet away from the cylinder because splashing may occur if the cap shoots off the tube.
4. Fill the rest of the centrifuge tube with crushed dry ice. **Remember to wear gloves!** Dry ice may be scooped up with the tube, added with a scoop, or poured into the tube from a beaker. Tap the tip of tube on the bench and add more dry ice until the tube is full. Twist cap on tightly until it stops turning. **If cap does not stop turning when tightened, remove the cap before proceeding**. Without a complete seal, the cap is likely to shoot off. Replace the cap with a new one before placing in water. If this does not provide a seal, replace the tube.

5. Immediately after capping, drop centrifuge tube, tapered end down, into the water in the cylinder (see Figure 2, F). Pressure will begin to build in the tube and gas will escape slowly from the region where the tube and the cap meet.

The plastic cylinder functions as a secondary container and protects you from possible injury. If the tube shatters or the cap shoots off, any projectiles will be directed straight up. **Do not place anything (including your face) above the cylinder.** Watch the extraction from the side, not the top, of the cylinder.

6. After 15 seconds, liquid CO_2 should appear. If no liquid has appeared after 1 minute, there is not a sufficient seal. Remove tube from cylinder, tighten cap, and put back in water. If repeated trials do not produce liquid, the cap or tube may need to be replaced. Liquid should boil and gas should escape for about 2 minutes and 45 seconds. NOTE: During this time, it is sometimes helpful to slowly rotate the cylinder on its base to prevent the centrifuge tube from freezing to the side of the cylinder. **Never remove the tube from the plastic cylinder when the CO_2 is liquid.** Tubes may rupture due to pressure and therefore must always remain in secondary containment.

7. As the liquid boils, it should pass through the peel and move to the bottom of the tube. If it cannot reach the bottom of the tube, the oil will deposit in the region of the tube containing the orange peel. This does not allow for isolation of the product.

8. After the liquid has evaporated and gas is no longer escaping, remove the tube from the cylinder with tweezers and open the cap. **Open centrifuge tubes slowly and only after the gas has escaped.** Opening tubes that are under pressure could result in the cap shooting a great distance.

9. 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. A third extraction can be performed in the same manner if desired. NOTE: The orange peel does not need to thaw between extractions.

10. Product (approximately 0.1 mL pale yellow oil) should be in the tip of the tube when the extraction is complete. Carefully remove the solid and the trap by pulling the wire handle with tweezers. If any solid remains in the tube, remove it with a spatula or wire. NOTE: Keep tube upright to avoid product loss.

11. Dry the outside of the tube with a paper towel, weigh the tube, and determine the mass of the product. Calculate percentage recovery based upon the yield of the product compared to the mass of rind used.

CLEANUP: The plastic tubes, filter paper, oranges and rinds can be thrown away and the water can go down the drain. The rest of the material is reusable and can be kept.

Student Name:____________________ Date: ____________________

Observations

Lab Questions

1. What is the percent yield? Show your calculation.

2. Carbon dioxide normally sublimes at room temperature. Why does it melt in the sealed container?

3. Based on question 2 can you explain what could happen that would cause this experiment to fail to make liquid CO_2 and therefore be unable to extract the limonene.

Green Question(s)

What are three advantages of using CO_2 as a solvent?

REFERENCES

Siena College Chemistry Department Lab Book. Limonene Extraction. Written in fall 2010.

Case, M., Sadlowski, C. Extraction of Limonene using CO_2. University of Vermont Laboratory Notebook.

TEACHER INFORMATION

REGENTS CORE

Teacher Tips:

-Having a trustworthy student grate/zest the oranges while you are talking to students about the experiment will save time

-You want to look for oranges that are bright in color and smell strongly when scratched into with a fingernail.

-Do not get deep into the rind of the orange when zesting. Once the color begins to fade deeper into the peel, a new area should be zested.

-Not all tubes will work. Finding good tubes is essential to the experiment working properly. Do not do anything to try and secure the tube tighter (tape, o-ring). This will increase the chances of the tube exploding.

Preparation:

- Time can be saved by preparing copper platforms before the experiment.

Answers to Questions

1. What is the percent yield? Show your calculation.
   Based on student data.

2. Carbon dioxide normally sublimes at room temperature. Why does it melt in the sealed container?

   Increased pressure in tube allows for liquid state to be formed.

3. Based on question 2 can you explain what could happen that would cause this experiment to fail to make liquid CO_2 and therefore be unable to extract the limonene.

   Lack of pressure buildup.

Green Question(s)

What are three advantages of using CO_2 as a solvent?
Readily available. Nontoxic.

WASTE

Chemical Name	CAS Number	Health Hazards	Physical Hazards	Environmental Hazards
Orange peel	None	None	None	none

1. How has this lab been modified to make it "greener"?

-This lab demonstrates an extraction experiment that uses a non-flammable, non-toxic solvent at standard temperature. Many extraction experiments involve the use of flammable or toxic solvents at elevated temperatures.

2. Which of the 12 principles of Green Chemistry were employed to make this lab greener?
Please check all that apply.

⊗ **Prevention** It's better to prevent waste than to treat or clean up waste afterwards.
❍ **Atom Economy** Design synthetic methods to maximize the incorporation of all materials used in the process into the final product.
❍ **Less Hazardous Chemical Syntheses** Design synthetic methods to use and generate substances that minimize toxicity to human health and the environment.
❍ **Designing Safer Chemicals** Design chemical products to affect their desired function while minimizing their toxicity.
⊗ **Safer Solvents and Auxiliaries** Minimize the use of auxiliary substances wherever possible make them innocuous when used.
⊗ **Design for Energy Efficiency** Minimize the energy requirements of chemical processes and conduct synthetic methods at ambient temperature and pressure if possible.
❍ **Use of Renewable Feedstocks** Use renewable raw material or feedstock rather whenever practicable.
❍ **Reduce Derivatives** Minimize or avoid unnecessary derivatization if possible, which requires additional reagents and generate waste.
❍ **Catalysis** Catalytic reagents are superior to stoichiometric reagents.
❍ **Design for Degradation** Design chemical products so they break down into innocuous products that do not persist in the environment.
❍ **Real-time Analysis for Pollution Prevention** Develop analytical methodologies needed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
⊗ **Inherently Safer Chemistry for Accident Prevention** Choose substances and the form of a substance used in a chemical process to minimize the potential for chemical accidents, including releases, explosions, and fires.[1]

[1] Retrieved from http://www.epa.gov/sciencematters/june2011/principles.htm