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Isolating Clove Oil from Cloves Using Steam Distillation

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PURPOSE OF THE EXPERIMENT

Isolate clove oil from cloves by steam distillation and extraction. Use reactions with bromine, potassium permanganate, and iron(III) chloride to characterize the product. Analyze the product purity by thin-layer chromatography.

EXPERIMENTAL OPTIONS

BACKGROUND REQUIRED

You should be familiar with distillation, extraction, drying organic solvents, speeding evaporation of organic solvents, thin-layer chromatography, and general microscale techniques.

BACKGROUND INFORMATION

Simple and fractional distillations are carried out on miscible mixtures. Ideal mixtures follow **Raoult's law**: The total vapor pressure of the system is determined by adding together the products of the vapor pressure and the respective mole fraction of each compound. For a two-compound system, this relationship is shown in Equation 1, where P_T is the total vapor pressure, P_1^0 and P_2^0 are the vapor pressures of pure compounds 1 and 2, and X_1 and X_2 are the respective mole fractions.

$$P_T = P_1^0 X_1 + P_2^0 X_2 \qquad \text{(Eq. 1)}$$

Distillation can also be performed on mixtures in which the two compounds are *not* miscible. This process is called **codistillation**. When one of the compounds is water, the process is called **steam distillation**.

When two immiscible liquids are distilled, the total vapor pressure P_T above the liquid is equal to the sum of the vapor pressures of each compound. This relationship, known as **Dalton's law**, is shown in Equation 2.

$$P_T = P_1^0 + P_2^0 \qquad \text{(Eq. 2)}$$

02 01 00 99 98 — 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1

The respective mole fractions are *not* included in this equation because, in an ideal situation, each liquid vaporizes independently of the other. When P_T is equal to atmospheric pressure of 760 torr, compounds 1 and 2 begin to codistill, with each compound contributing to P_T.

Consider water as compound 1. The vapor pressure of pure water at its boiling point of 100 °C is 760 torr. Because compound 2 also contributes to P_T, the mixture will distill at a temperature less than 100 °C. The actual distillation temperature will depend on the vapor pressure of compound 2. Steam distillation offers an advantage in that volatile compounds that are unstable or have high boiling points can codistill with water at relatively low temperatures. This process avoids decomposition that might occur at the normal boiling point of the compound of interest. For example, eugenol, the major compound of clove oil, boils at a relatively high temperature of 254 °C. Steam distillation avoids this high temperature and results in the distillation of eugenol at a temperature slightly less than 100 °C.

In practice, steam distillation is usually carried out by one of two methods. In the first method, an excess of water is added to the compound of interest in a distilling flask. The mixture is then heated to the boiling point. The resulting vapor is condensed and collected in a receiving flask. The compound of interest is then separated from the water, often by extraction. In the second method, steam is bubbled into the compound of interest to effect the distillation. In this experiment, you will use the first method because it is easier to set up.

Clove oil belongs to a large class of natural products called the **essential oils**. Many of these compounds are used as flavorings and perfumes and, in the past, were considered to be the "essence" of the plant from which they were derived.

Cloves are the dried flower buds of the clove tree, *Eugenia caryophyllata*, found in India and other locations in the Far East. Steam distillation of freshly ground cloves results in clove oil, which consists of several compounds. Eugenol is the major compound, comprising 85–90 percent. Eugenol acetate comprises 9–10 percent. These structures are shown in Figure 1.

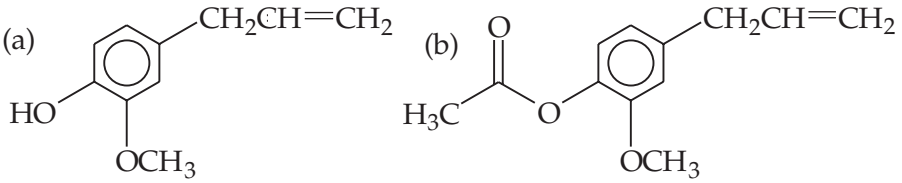
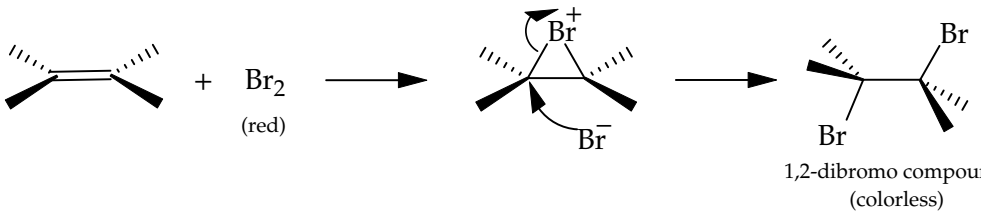


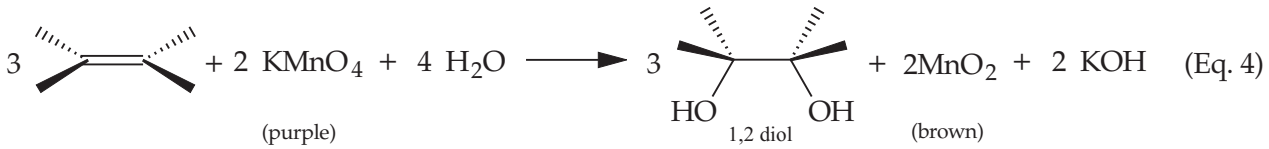
Figure 1 Structures for (a) eugenol and (b) eugenol acetate

Qualitative Tests Eugenol contains a carbon–carbon double bond and an aromatic hydroxyl group called a phenol. These functional groups provide the basis for simple chemical tests used to characterize the clove oil. A solution of bromine (Br_2) in dichloromethane decolorizes as Br_2 reacts with the double bond to form a colorless compound, as shown in Equation 3. A positive test is the disappearance of the red Br_2 color.

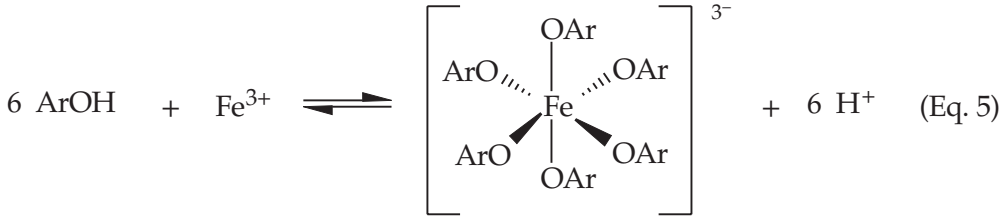


(Eq. 3)

A potassium permanganate ($KMnO_4$) solution can oxidize a double bond at room temperature to form a 1,2-diol with the simultaneous reduction of Mn^{7+} in $KMnO_4$ to Mn^{4+} in manganese dioxide (MnO_2), as shown in Equation 4. A positive test is the disappearance of the purple $KMnO_4$ and the appearance of MnO_2 as a muddy brown precipitate.



Phenols (Ar–OH) react with the Fe^{3+} ion in iron(III) chloride ($FeCl_3$) to give complexes that are blue, green, red, or purple, as shown in Equation 5. The color may last for only a few seconds or for many hours, depending on the stability of the complex.



In this experiment, you will steam distill clove oil from freshly ground cloves. Following the distillation, clove oil and water will be present in the receiving flask. Because clove oil will be a minor fraction of the distillate, the clove oil must be extracted from the water into an organic solvent such as dichloromethane. Removing the dichloromethane layer leaves clove oil as the product.

Semi-Microscale Steam Distillation

Equipment

boiling chips
Bunsen burner
cotton*
electric flask heater
50-mL Erlenmeyer flask, with stopper
glass stirring rod
10-mL graduated cylinder
50-mL graduated cylinder
marking pen
microspatula
mortar and pestle
3 Pasteur pipets, with latex bulb
powder funnel
sand bath†‡
125-mL separatory funnel§
standard-taper glassware
  Claisen connecting tube
  condenser, with adapter and tubing
  distilling head
  100-mL round-bottom flask
  2 round-bottom flasks, 50-mL
  thermometer, –10 to 260 °C, with adapter
support ring
2 support stands
3 utility clamps

*for Pasteur filter pipet
†or hot-water bath
‡sand in crystallizing dish on electric hot plate or sand in electric heating well with heat controller
§also use as addition funnel

Reagents and Properties

substance	*quantity*	*molar mass (g/mol)*	*bp (°C)*
cloves	5 g		
dichloromethane	21 mL	84.93	40
eugenol*		164.20	254
methanol	10 mL	32.04	64.7
sodium chloride, sat. solution	10 mL	58.44	
sodium sulfate, anhydrous	0.5 g	142.04	

*product

Preview

- Grind the cloves with a mortar and pestle
- Place the ground cloves and water in the distilling flask
- Assemble the steam distillation apparatus
- Distill the mixture
- Extract the clove oil into dichloromethane
- Dry the dichloromethane layer with anhydrous Na_2SO_4
- Remove the dichloromethane from the clove oil by distillation
- Weigh the clove oil

PROCEDURE

Chemical Alert

dichloromethane—*toxic and irritant*
eugenol—*irritant*
methanol—*flammable and toxic*
anhydrous sodium sulfate—*irritant and hygroscopic*

Caution: Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

1. Conducting Steam Distillation

Weigh 5 g of whole cloves. Grind them to a coarse powder, using a mortar and pestle. Reweigh the powder and record the mass.

Use a powder funnel to transfer the ground cloves to a 100-mL round-bottom flask. Add 40 mL of deionized or distilled water and a boiling chip to the flask. Mix well with a glass stirring rod. Mark the level of the mixture on the side of the flask.

Add 30 mL of water to a 50-mL round-bottom flask. Mark the level of the water on the side of the flask. Then discard the water from the flask.

Assemble the steam distillation apparatus shown in Figure 2 on the next page. Use the 100-mL round-bottom flask as the pot. Use the 50-mL round-bottom flask as the receiver. If a vacuum adapter is not used, *make certain there is an opening to the atmosphere.* Pour 100 mL of water into the addition funnel. Start the flow of water through the condenser.

Adjust a Bunsen burner flame to lessen the hot central cone. Heat the pot by waving the flame back and forth under the pot. [NOTE 1] Maintain a distillation rate of approximately one drop every 3–5 s.

Add water to the pot at 10-min intervals to keep the water level at the mark. Stop the distillation when approximately 30 mL of distillate has been collected.

NOTE 1: Do not heat the mixture too rapidly. The clove mixture tends to foam when rapidly heated. The burner flame can easily be added and withdrawn to control the heating rate.

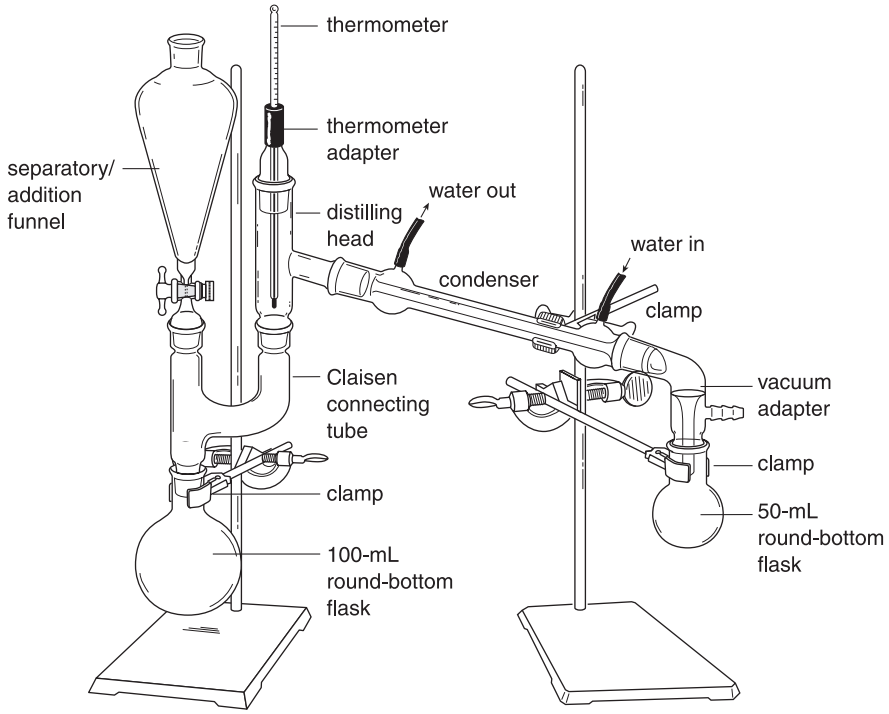


Figure 2 Semi-microscale steam distillation apparatus

2. Extracting the Clove Oil

Caution: Dichloromethane is toxic and irritating. Use a ***fume hood.*** Clove oil (eugenol) is irritating. Prevent eye, skin, and clothing contact.

Allow the receiver to cool to room temperature. Carefully pour the distillate from the receiver into a 125-mL separatory funnel. Add 10 mL of saturated NaCl solution.

Using a Pasteur pipet, carefully rinse the condenser and the inside neck of the receiving flask with 5 mL of dichloromethane. [NOTE 2] Swirl the flask gently to dissolve the remaining clove oil. Add this dichloromethane to the distillate in the separatory funnel.

NOTE 2: Significant amounts of clove oil will adhere to the condenser and the sides and neck of the receiving flask.

Cap the separatory funnel and gently swirl the contents for several seconds. *Vent the separatory funnel frequently.* After the pressure has been vented, shake the contents vigorously to thoroughly mix the two layers.

Swirl the separatory funnel. At the same time, gently tap the outside of the separatory funnel with your index finger to force into the bottom layer any droplets of dichloromethane that are adhering to the sides of the funnel.

Allow the layers to separate. Drain the lower dichloromethane layer into a 50-mL Erlenmeyer flask, making certain that none of the aqueous layer is transferred to the flask.

Rinse the condenser and the receiver with a second 5-mL portion of dichloromethane. Transfer the rinsing to the separatory funnel. Repeat the extraction of the aqueous layer.

Drain the second dichloromethane extract from the separatory funnel and combine it with the first one in the 50-mL Erlenmeyer flask. Repeat the rinsing and extraction process with a third 5- mL portion of dichloromethane. Combine the third extract in the same 50-mL Erlenmeyer flask.

Caution: Anhydrous sodium sulfate (Na_2SO_4) is irritating and hygroscopic. Do not inhale and ingest this compound.

Add approximately 0.5 g of anhydrous Na_2SO_4 to the flask containing the dichloromethane extracts. Stopper the flask. Allow the extracts to dry for 5 min.

Weigh a clean, dry 50-mL round-bottom flask to the nearest 0.001 g and record the mass. Using a Pasteur filter pipet, transfer the dried dichloromethane into the flask, making certain that no Na_2SO_4 is transferred with the solution. Use three additional 2-mL portions of dichloromethane to rinse the Na_2SO_4 and ensure complete transfer of the clove oil to the beaker.

Assemble a simple distillation apparatus using the 50-mL round-bottom flask as the pot. Add a boiling chip. Use a 40 °C sand bath or a hot-water bath to distill the dichloromethane away from the product.

When all of the dichloromethane has been distilled, cool the flask. Weigh it to the nearest 0.001 g and record the mass. Subtract the mass of the empty flask to obtain the mass of the clove oil.

Caution: Methanol is flammable and toxic. Keep away from flames or other heat sources. Prevent eye, skin, and clothing contact. Use a ***fume hood***.

Dissolve the clove oil in 10 mL of methanol. Proceed to the Characterizing the Product Section later in this module.

3. Cleaning Up Place your recovered materials in the appropriate labeled collection containers as directed by your laboratory instructor. Clean your glassware with soap or detergent.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Microscale Steam Distillation

Using Glassware with Elastomeric Connectors

Equipment

- 25-mL beaker
- boiling chip
- 15-mL centrifuge tube, with cap
- copper metal sponge
- cotton*
- 10-mL Erlenmeyer flask, with stopper
- 125-mL Erlenmeyer flask
- glass stirring rod
- glassware, with elastomeric connectors
  - distilling head/air condenser
  - distilling tube, with syringe port
  - 10-mL round-bottom flask
  - 1-mL syringe
- 10-mL graduated cylinder
- marking pen
- microburner
- microspatula
- mortar and pestle
- 3 Pasteur pipets, with latex bulb
- 1.0-mL pipet
- sand bath†
- support ring
- support stand
- thermometer, –10 to 260 °C, with adapter
- 2 utility clamps
- wire gauze

*for Pasteur filter pipet

†sand in crystallizing dish on electric hot plate or sand in electric heating well with heat controller

Reagents and Properties

substance	*quantity*	*molar mass (g/mol)*	*bp (°C)*
cloves	0.5 g		
dichloromethane	4 mL	84.93	40
eugenol*		164.20	254
ice			
methanol	1 mL	32.04	64.7
sodium chloride, sat. solution	1 mL	58.44	
sodium sulfate, anhydrous	50 mg	142.04	

*product

Preview

- Grind the cloves with a mortar and pestle
- Place the ground cloves and water in the distilling flask
- Assemble the steam distillation apparatus
- Distill the mixture
- Extract the clove oil into dichloromethane
- Dry the dichloromethane layer with anhydrous Na_2SO_4
- Remove the dichloromethane from the clove oil
- Weigh the clove oil

PROCEDURE

Chemical Alert

dichloromethane—*toxic and irritant*
eugenol—*irritant*
methanol—*flammable and toxic*
anhydrous sodium sulfate—*irritant and hygroscopic*

Caution: Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

1. Conducting Steam Distillation

Grind 10 whole cloves to a coarse powder, using a small mortar and pestle. Weigh 0.400–0.500 g of the powder and record the mass to the nearest 0.001 g.

Use a microspatula to carefully transfer the ground cloves to a 10-mL round-bottom flask. Add a boiling chip and 4 mL of deionized or distilled water. Mix well with a glass stirring rod. Mark the level of the mixture on the side of the flask.

Add 3 mL of water to a 15-mL centrifuge tube. Mark the level of the water on the side of the tube. Then discard the water from the tube.

Place a small plug of copper metal sponge in the distillation head to help prevent the mixture from foaming over into the centrifuge tube when the distilling flask is heated.

Fill a 125-mL Erlenmeyer flask three-quarters full with crushed ice. Place the centrifuge tube in the flask. Assemble the remainder of the distilling apparatus, as shown in Figure 3 on the next page. Start the flow of water through the condenser.

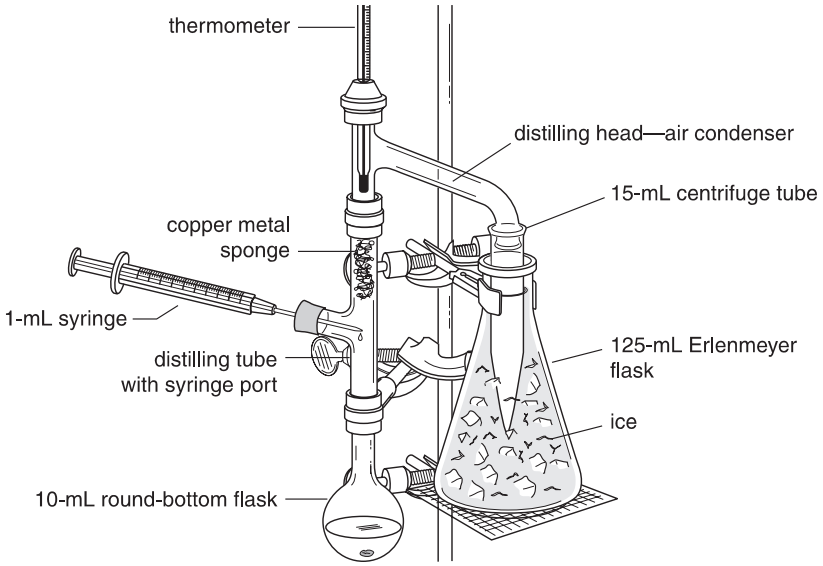


Figure 3 Microscale steam distillation apparatus using elastomeric connectors

NOTE 1: Do not heat the mixture too quickly. Rapid heating may cause the mixture to foam violently. A microburner flame can easily be added and withdrawn to control the heating rate.

Adjust a microburner flame to lessen the hot central cone. Heat the pot by waving the flame back and forth under the pot. [NOTE 1] Heat the mixture to maintain a distillation rate of approximately one drop every 5 s.

Draw 1.0 mL of water into the syringe. Add water dropwise to the pot every 5 min to keep the water level to the mark. Refill the syringe with water as needed. Add more ice, as needed, to the Erlenmeyer flask containing the centrifuge tube. Stop the distillation when 3 mL of distillate has been collected in the centrifuge tube.

2. Extracting the Clove Oil

Caution: Dichloromethane is toxic and irritating. Use a ***fume hood***. Clove oil (eugenol) is irritating. Prevent eye, skin, and clothing contact.

Remove the centrifuge tube from the flask. Add 1 mL of saturated NaCl solution.

Add 1 mL of dichloromethane to the centrifuge tube. Cap the tube and gently mix the layers, being careful to *vent the tube frequently.* After the initial pressure build-up has subsided, shake the centrifuge tube vigorously to mix the layers efficiently.

Swirl the tube. At the same time, gently tap the outside of the centrifuge tube with your index finger to force into the bottom layer any droplets of dichloromethane that are adhering to the sides of the tube.

Using a Pasteur pipet, remove the lower dichloromethane layer containing the clove oil into a 10-mL Erlenmeyer flask. Make certain that no water is transferred to the flask.

Repeat the extraction process two more times using 1-mL portions of dichloromethane. Combine all three dichloromethane extracts in the same 10-mL Erlenmeyer flask.

Caution: Anhydrous sodium sulfate (Na_2SO_4) is irritating and hygroscopic. Do not inhale and ingest this compound.

Add approximately 50 mg of anhydrous Na_2SO_4 to the flask containing the dichloromethane extracts. Stopper the flask. Allow the extracts to dry for 5 min.

Weigh a clean, dry 25-mL beaker to the nearest 0.001 g and record the mass. Using a Pasteur filter pipet, transfer the dried dichloromethane into the beaker, making certain that no Na_2SO_4 is transferred with the

solution. Use two additional 0.5-mL portions of dichloromethane to rinse the Na_2SO_4 and ensure complete transfer of the clove oil to the beaker.

In a ***fume hood***, place the beaker on the *surface* of a 40 °C sand bath to evaporate the dichloromethane. Use a gentle stream of air or nitrogen to speed the evaporation. [NOTE 2]

NOTE 2: When evaporating the dichloromethane, use a *gentle* stream of air or nitrogen, one you *barely* feel against your hand. A strong stream of air or nitrogen may blow the solution out of the beaker and product will be lost.

When all of the dichloromethane has been evaporated, weigh the beaker to the nearest 0.001 g and record the mass. Subtract the mass of the empty beaker to obtain the mass of the clove oil.

Caution: Methanol is flammable and toxic. Keep away from flames or other heat sources. Prevent eye, skin, and clothing contact. Use a ***fume hood***.

Dissolve the clove oil in 1 mL of methanol. Proceed to the Characterizing the Product Section later in this module.

3. Cleaning Up Place your recovered materials in the appropriate labeled collection containers as directed by your laboratory instructor. Clean your glassware with soap or detergent.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Microscale Steam Distillation

Using the Hickman Still

Equipment

25-mL beaker	marking pen
boiling chip	microburner
5 mL conical vial, with screw cap	microspatula
	mortar and pestle
condenser, with tubing	1-mL pipet†
copper metal sponge	4 Pasteur pipets, with latex bulb
cotton*	10-mL round-bottom flask
10-mL Erlenmeyer flask, with stopper	sand bath‡
	support ring
glass stirring rod	support stand
10-mL graduated cylinder	2 utility clamps
Hickman still	wire gauze

*for Pasteur filter pipet

†or adjustable micropipet

‡sand in crystallizing dish on electric hot plate or sand in electric heating well with heat controller

Reagents and Properties

substance	*quantity*	*molar mass (g/mol)*	*bp (°C)*
cloves	0.5 g		
dichloromethane	4 mL	84.93	40
eugenol*		164.20	254
methanol	1 mL	32.04	64.7
sodium chloride, sat. solution	0.5 mL	58.44	
sodium sulfate, anhydrous	50 mg	142.04	

*product

Preview

- Grind the cloves with a mortar and pestle
- Place the ground cloves and water in the distilling flask
- Assemble the steam distillation apparatus
- Distill the mixture
- Extract the clove oil into dichloromethane
- Dry the dichloromethane layer with anhydrous Na_2SO_4
- Remove the dichloromethane from the clove oil
- Weigh the clove oil

NOTE 1: Do not heat the mixture too rapidly. The clove mixture tends to foam when rapidly heated. The microburner flame can easily be added and withdrawn to control the heating rate.

PROCEDURE

Chemical Alert

dichloromethane—*toxic and irritant*

eugenol—*irritant*

methanol—*flammable and toxic*

anhydrous sodium sulfate—*irritant and hygroscopic*

NOTE 2: A standard Pasteur pipet can be used in a Hickman still model that has a built-in side port.

Caution: Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

1. Conducting Steam Distillation

Grind 10 whole cloves to a coarse powder, using a small mortar and pestle. Weigh 0.400–0.500 g of the powder and record the mass to the nearest 0.001 g.

Use a microspatula to carefully transfer the ground cloves to a 10-mL round-bottom flask. Add a boiling chip and 4 mL of deionized or distilled water. Mix well with a glass stirring rod. Mark the level of the mixture on the side of the flask.

Place a small plug of copper metal sponge in the neck of the Hickman still to help prevent the mixture from foaming over as it is heated.

Attach the Hickman still to the round-bottom flask. Assemble the remainder of the apparatus, as shown in Figure 4. Start the flow of water through the condenser.

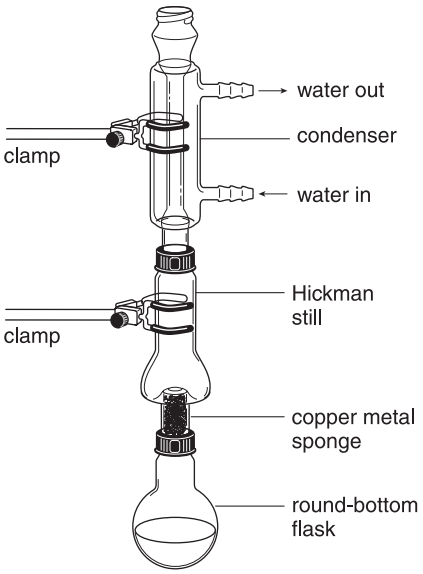


Figure 4 Microscale steam distillation apparatus using a Hickman still

Adjust a microburner flame to lessen the hot central cone. Heat the pot by waving the flame back and forth under the pot. [NOTE 1] Heat the mixture to maintain a distillation rate of approximately one drop every 5 s.

When the bottom portion of the Hickman still is full of distillate, remove the flame. Using a bent-tip Pasteur pipet, carefully remove the distillate from the Hickman still. [NOTE 2] Place the distillate in a 5-mL conical vial. Cap the vial to avoid spillage.

Using a clean Pasteur pipet, add water through the top of the condenser to keep the water level at the mark on the round-bottom flask. Again use the flame to distill the mixture. Continue the distillation until 3 mL of distillate has been collected in the 5-mL conical vial.

2. Extracting the Clove Oil

Caution: Dichloromethane is toxic and irritating. Use a ***fume hood***. Clove oil (eugenol) is irritating. Prevent eye, skin, and clothing contact.

Add 0.5 mL of saturated NaCl solution to the vial. Using a bent-tip Pasteur pipet and a 0.5-mL portion of dichloromethane, carefully wash down the inside walls of the Hickman still to remove residual clove oil that adhered to the glass. Transfer the dichloromethane to the 5-mL conical vial containing the distillate.

Repeat the rinsing with a second 0.5-mL portion of dichloromethane. Transfer the second portion of dichloromethane to the 5-mL conical vial.

Cap the vial tightly and gently mix the layers, being careful to *vent the vial frequently.* After the initial pressure build-up has subsided, shake the vial vigorously to thoroughly mix the layers.

Swirl the vial. At the same time, gently tap the outside of the vial with your index finger to force into the bottom layer any droplets of dichloromethane that are adhering to the sides of the vial.

Using a Pasteur pipet, transfer the lower dichloromethane layer containing the clove oil into a 10-mL Erlenmeyer flask. Make certain that no water is transferred to the flask.

Repeat the extraction process two more times, using 1-mL portions of dichloromethane. Combine all three dichloromethane extracts in the same 10-mL Erlenmeyer flask.

Caution: Anhydrous sodium sulfate (Na_2SO_4) is irritating and hygroscopic. Do not inhale and ingest this compound.

Add approximately 50 mg of anhydrous Na_2SO_4 to the flask containing the dichloromethane extracts. Allow it to dry for 5 min.

Weigh a clean, dry 25-mL beaker to the nearest 0.001 g and record the mass. Using a Pasteur filter pipet, transfer the dried dichloromethane into the beaker, making certain that no Na_2SO_4 is transferred with the solution. Use two additional 0.5-mL portions of dichloromethane to rinse the Na_2SO_4 and ensure complete transfer of the clove oil to the beaker.

In a ***fume hood***, place the beaker on the *surface* of a 40 °C sand bath to evaporate the dichloromethane. Use a gentle stream of air or nitrogen to speed the evaporation. [NOTE 3]

NOTE 3: When evaporating the dichloromethane, use a *gentle* stream of air or nitrogen, one you *barely* feel against your hand. A strong stream of air or nitrogen may blow the solution out of the beaker and product will be lost.

When all of the dichloromethane has been evaporated, weigh the beaker to the nearest 0.001 g and record the mass. Subtract the mass of the empty beaker to obtain the mass of the clove oil.

Caution: Methanol is flammable and toxic. Keep away from flames or other heat sources. Prevent eye, skin, and clothing contact. Use a ***fume hood***.

Dissolve the clove oil in 1 mL of methanol. Proceed to the Characterizing the Product Section on the next page.

3. Cleaning Up Place your recovered materials in the appropriate labeled collection containers as directed by your laboratory instructor. Clean your glassware with soap or detergent.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Characterizing the Product

Equipment

Bunsen burner	4 Pasteur pipets, with latex bulb
developing chamber*	pencil
10-mL graduated cylinder	6 test tubes, 13 × 100-mm
marking pen	3 × 7-cm TLC plate, silica gel, with fluorescent indicator
2 melting point capillary tubes†	
metric ruler	

*4-oz jar with lid or 250-mL beaker covered with aluminum foil

†for TLC micropipets

Reagents and Properties

compound	*quantity*	*molar mass (g/mol)*	*bp (°C)*
acetone	0.5 mL	58.08	56
bromine, 1% in dichloromethane	< 1 mL		
n-hexane	4.5 mL	86.18	69
iron(III) chloride, 1% aq.	< 1 mL	162.21	
methanol	6 mL	32.04	64.7
potassium permanganate, 0.05*M*	< 1 mL	158.04	

PROCEDURE

Chemical Alert

acetone—*flammable and irritant*
bromine—*toxic and oxidizer*
dichloromethane—*toxic and irritant*
eugenol—*irritant*
n-hexane—*flammable and irritant*
iodine—*toxic and corrosive*
iron(III) chloride—*toxic and corrosive*
methanol—*flammable and toxic*
potassium permanganate—*corrosive and oxidizer*

1. Analyzing Clove Oil by Chemical Tests

Caution: Clove oil (eugenol) is irritating. Methanol is flammable and toxic. Keep away from flames or other heat sources. Prevent eye, skin, and clothing contact. Do not inhale and ingest these compounds. Use a ***fume hood***.

Obtain six test tubes and label them 1–6. Label tubes 2, 4, and 6 "control". Add 1 mL of methanol to each of the six tubes.

Using a Pasteur pipet, add 5 drops of the methanol-clove oil solution to test tubes 1 and 3. Add 10 drops of the methanol-clove oil solution to test tube 5. Gently swirl each tube.

Testing with Bromine in Dichloromethane

Caution: Bromine (Br_2) is toxic and oxidizing. Dichloromethane is toxic and irritating. Prevent eye, skin, and clothing contact. Do not inhale and ingest these compounds. Use a ***fume hood***.

Using a Pasteur pipet, add a 1% Br_2 in dichloromethane solution dropwise to test tube 1. Record your observations after each drop is added. Note how many drops of the dichloromethane-Br_2 solution are needed until pale yellow coloration remains. [NOTE 1] Repeat this procedure using test tube 2.

NOTE 1: When all of the clove oil has reacted with Br_2, a pale yellow color will remain.

Testing with Potassium Permanganate

Caution: Potassium permanganate($KMnO_4$) is corrosive and oxidizing. Prevent eye, skin, and clothing contact. Do not inhale or ingest $KMnO_4$.

Using a Pasteur pipet, add three drops of 0.05*M* $KMnO_4$ to test tube 3 and record your observations. Repeat this procedure using test tube 4.

Testing with Iron(III) Chloride

Caution: Iron(III) chloride ($FeCl_3$) is toxic and corrosive. Prevent eye, skin, and clothing contact. Do not inhale or ingest $FeCl_3$.

Using a Pasteur pipet, add one drop of 1% $FeCl_3$ solution to test tube 5 and one drop to test tube 6. Record your observations.

2. Analyzing Clove Oil by Thin-Layer Chromatography

Caution: Acetone and *n*-hexane are flammable and irritating. Keep away from flames or other heat sources. Prevent eye, skin, and clothing contact. Do not inhale and ingest these compounds. Use a ***fume hood***.

Caution: Clove oil (eugenol) is irritating. Methanol is flammable and toxic. Keep away from flames or other heat sources. Prevent eye, skin, and clothing contact. Do not inhale and ingest these compounds. Use a ***fume hood***.

Obtain a 3 × 7-cm silica gel TLC plate from your laboratory instructor. Draw a *very faint* pencil line 1 cm from the bottom to mark the origin. Make two vertical marks that intersect the pencil line 0.5 cm from each edge of the plate and a third mark 1.5 cm from one edge.

Prepare micropipets for spotting the TLC plates by drawing out melting point capillary tubing. Using a micropipet, spot a standard sample of eugenol once on the middle mark, keeping the spot as small as possible. Using a new micropipet, spot your methanol-clove oil sample once on the left-hand mark. Using the same micropipet, spot your sample twice on the right hand mark, allowing the solvent to evaporate between spottings.

Prepare a developing chamber by pouring 4.5 mL of *n*-hexane and 0.5 mL of acetone into a 4-oz jar. [NOTE 2] Place the TLC plate into the chamber and attach the lid. Allow the eluent to develop the plate.

NOTE 2: Do not put filter paper or paper towel in the developing chamber. In this case, a better separation occurs without chamber saturation.

Caution: Ultraviolet radiation can cause severe eye damage. Wear goggles. Do not look directly into the UV lamp.

Iodine (I_2) is toxic and corrosive. Prevent eye, skin, and clothing contact. Do not inhale and ingest I_2. Use a ***fume hood***.

After developing the plate, *immediately* mark the eluent front. Dry the plate in a ***fume hood***. Visualize the chromatogram under short-wave UV light or in an I_2 chamber, as directed by your laboratory instructor. Use a pencil to circle the spots on your plate.

Measure the distance from the origin to the eluent front. Measure the distance from the origin to the center of each spot. [NOTE 3] Record your observations.

NOTE 3: Eugenol acetate should appear as a minor spot at a higher R_f than that of eugenol.

3. Cleaning Up

Place your recovered materials in the appropriate labeled collection containers as directed by your laboratory instructor. Clean your glassware with soap or detergent.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Post-Laboratory Questions

1. Calculate the percent yield of clove oil based upon the initial mass of the ground cloves.
2. Give your test results for the reaction of your eugenol product with each of the test reagents.
3. Complete the following reactions, giving the correct structure for each organic product.
   (a) eugenol + Br_2 ⟶
   (b) eugenol acetate + $KMnO_4$ ⟶
   (c) eugenol + $FeCl_3$ ⟶
   (d) eugenol acetate + $FeCl_3$ ⟶
4. Complete the following table after performing the TLC analysis on your clove oil sample. Indicate by a yes or no answer whether the spots are visible under UV light or I_2 vapors.

compound	R_f	*UV*	I_2
eugenol			
eugenol acetate			
other			

5. Using your R_fs, list the compounds in your clove oil in order of increasing polarity. Briefly explain your answer.

NAME SECTION DATE

TECH 722/Isolating Clove Oil from Cloves Using Steam Distillation

Pre-Laboratory Assignment

1. What precautions must be taken when mixing reagents in a separatory funnel or centrifuge tube?

2. Briefly define the following terms:
   (a) codistillation

   (b) steam distillation

   (c) Raoult's law

   (d) Dalton's law

   (e) essential oil

3. Why is steam distillation preferable to simple distillation for isolating high-boiling natural products?

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