PART 1

Introduction to Basic Laboratory Techniques

1 **EXPERIMENT 1**

Solubility

Solubility

Polarity

Acid-base chemistry

Critical-thinking application Nanotechnology

Having a good comprehension of solubility behavior is essential for understanding many procedures and techniques in the organic chemistry laboratory. For a thorough discussion of solubility, read the chapter on this concept (Technique 10) before proceeding, as an understanding of this material is assumed in this experiment.

In Parts A and B of this experiment, you will investigate the solubility of various substances in different solvents. As you are performing these tests, it is helpful to pay attention to the polarities of the solutes and solvents and to even make predictions based on them (see "Guidelines for Predicting Polarity and Solubility," Technique 10, Section 10.4). The goal of Part C is similar to that of Parts A and B, except that you will be looking at miscible and immiscible pairs of liquids. In Part D, you will investigate the solubility of organic acids and bases. Section 10.2B will help you understand and explain these results.

In Part E, you will perform several exercises that involve the application of the solubility principles learned in Parts A–D of this experiment. Part F is a unique nanotechnology experiment that also relates to solubility.

REQUIRED READING

New:	Technique 5	Measurement of Volume and Weight
	Technique 10	Solubility

SUGGESTED WASTE DISPOSAL

Dispose of all wastes containing methylene chloride into the container marked for halogenated waste. Place all other organic wastes into the non-halogenated organic waste container.

NOTES TO THE INSTRUCTORS

In Part A of the procedure, it is important that students follow the instructions carefully. Otherwise, the results may be difficult to interpret. It is particularly important that consistent stirring is done for each solubility test. This can be done most easily by using the larger-style microspatula found in your drawer.

We have found that some students have difficulty performing Critical-Thinking Application 2 on the same day that they complete the rest of this experiment. Many students need time to assimilate the material in the experiment before they can complete this exercise successfully. One approach is to assign Critical-Thinking

Applications from several technique experiments (for example, Experiments 1–3) to a laboratory period after students complete the individual technique experiments. This provides an effective way of reviewing some of the basic techniques.

PROCEDURE

NOTE: It is very important that you follow these instructions carefully and that consistent stirring be done for each solubility test.

Part A. Solubility of Solid Compounds

Place about 40 mg (0.040 g) of benzophenone into each of four *dry* test tubes.[1] (Don't try to be exact: You can be 1–2 mg off and the experiment will still work.) Label the test tubes and then add 1 mL of water to the first tube, 1 mL of methyl alcohol to the second tube, and 1 mL of hexane to the third tube. The fourth tube will serve as a control. Determine the solubility of each sample in the following way: Using the rounded end of a microspatula (the larger style Technique 2, Figure 2.10), stir each sample continuously for 60 seconds by twirling the spatula rapidly. If a solid dissolves completely, note how long it takes for the solid to dissolve. *After 60 seconds* (do not stir longer), note whether the compound is soluble (dissolves completely), insoluble (none of it dissolves), or partially soluble. You should compare each tube with the control in making these determinations. You should state that a sample is partially soluble only if a significant amount (at least 50%) of the solid has dissolved. For the purposes of this experiment, if it is not clear that a significant amount of solid has dissolved, then state that the sample is insoluble. If all but a couple of granules have dissolved, state that the sample is soluble. An additional hint for determining partial solubility is given in the next paragraph. Record your results in your notebook in the form of a table, as shown below. For those substances that dissolve completely, note how long it took for the solid to dissolve.

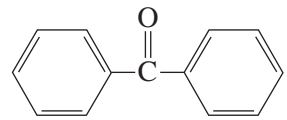
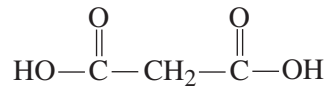
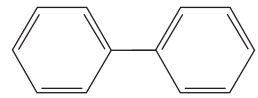
Although the instructions just given should enable you to determine if a substance is partially soluble, you may use the following procedure to confirm this. Using a Pasteur pipet, carefully remove most of the solvent from the test tube *while leaving the solid behind.* Transfer the liquid to another test tube and then evaporate the solvent by heating the tube in a hot water bath. Directing a stream of air or nitrogen gas into the tube will speed up the evaporation (see Technique 7, Section 7.10). When the solvent has completely evaporated, examine the test tube for any remaining solid. If there is solid in the test tube, the compound is partially soluble. If there is no, or very little, solid remaining, you can assume that the compound is insoluble.

Now repeat the directions just given, substituting malonic acid first and then biphenyl for benzophenone. Record these results in your notebook.

Part B. Solubility of Different Alcohols

For each solubility test (see table below), add 1 mL of solvent (water or hexane) to a test tube. Then add one of the alcohols, *dropwise*. Carefully observe what happens as you add each drop. If the liquid solute is soluble in the solvent, you may see tiny horizontal lines in the solvent. These mixing lines indicate that solution is taking place. *Shake the tube after adding each drop*. While you shake the tube, the liquid that was added may break up into small balls that disappear in a few seconds. This also indicates that solution is taking place. Continue adding the alcohol with shaking until you have added a total of 20 drops. If an alcohol is partially soluble, you will observe that at first the drops will dissolve, but eventually a second layer of liquid (undissolved alcohol) will form in the test tube. Record your results (soluble, insoluble, or partially soluble) in your notebook in table form.

[1] *Note to the instructor:* Grind up the benzophenone flakes into a powder.

	Solvents		
Solid Organic Compounds	**Water (highly polar)**	**Methyl Alcohol (intermediate polarity)**	**Hexane (nonpolar)**
Benzophenone 			
Malonic acid 			
Biphenyl 			

	Solvents	
Alcohols	**Water**	**Hexane**
1-Octanol $CH_3(CH_2)_6CH_2OH$		
1-Butanol $CH_3CH_2CH_2CH_2OH$		
Methyl alcohol CH_3OH		

Part C. Miscible or Immiscible Pairs

For each of the following pairs of compounds, add 1 mL of each liquid to the same test tube. Use a different test tube for each pair. Shake the test tube for 10–20 seconds to determine if the two liquids are miscible (form one layer) or immiscible (form two layers). Record your results in your notebook.

Water and ethyl alcohol

Water and diethyl ether

Water and methylene chloride

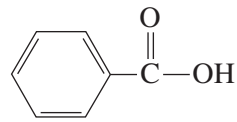
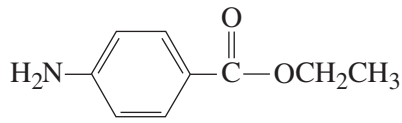
Water and hexane

Hexane and methylene chloride

Part D. Solubility of Organic Acids and Bases

Place about 30 mg (0.030 g) of benzoic acid into each of three *dry* test tubes. Label the test tubes and then add 1 mL of water to the first tube, 1 mL of 1.0 *M* NaOH to the second tube, and 1 mL of 1.0 *M* HCl to the third tube. Stir the mixture in each test tube with a microspatula for 10–20 seconds. Note whether the compound is soluble (dissolves completely) or is insoluble (none of it dissolves). Record these results in table form. Now take the second tube containing benzoic acid and 1.0 *M* NaOH. While stirring, add *M* HCl dropwise until the mixture is acidic. Test the mixture with litmus or pH paper to determine when it is acidic.[2] When it is acidic, stir the mixture for 10–20 seconds and note the result (soluble or insoluble) in the table.

Repeat this experiment using ethyl 4-aminobenzoate and the same three solvents. Record the results. Now take the tube containing ethyl 4-aminobenzoate and 1.0 *M* HCl. While stirring, add 6.0 *M* NaOH dropwise until the mixture is basic. Test the mixture with litmus or pH paper to determine when it is basic. Stir the mixture for 10–20 seconds and note the result.

		Solvents	
Compounds	**Water**	***1.0* M *NaOH***	***1.0* M *HCl***
Benzoic acid 		Add 6.0 *M* HCl	
Ethyl 4-aminobenzoate 			Add 6.0 *M* NaOH

Part E. Critical-Thinking Applications

1. Determine by experiment whether each of the following pairs of liquids are miscible or immiscible.

   Acetone and water

   Acetone and hexane

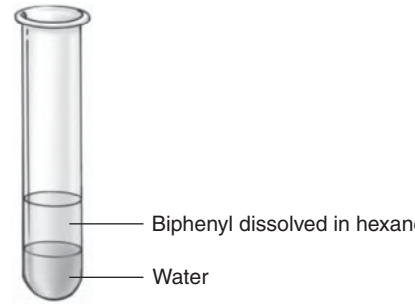
   How can you explain these results, given that water and hexane are immiscible?

2. You will be given a test tube containing two immiscible liquids and a solid organic compound that is dissolved in one of the liquids.[3] You will be told the identity of the two liquids and the solid compound, but you will not know the relative positions of the two

[2]Do not place the litmus or pH paper into the sample; the dye will dissolve. Instead, place a drop of solution from your spatula onto the test paper. With this method, several tests can be performed using a single strip of paper.

[3]The sample you are given may contain one of the following combinations of solid and liquids (the solid is listed first): fluorene, methylene chloride, water; triphenylmethanol, diethyl ether, water; salicylic acid, methylene chloride, 1 *M* NaOH; ethyl 4-aminobenzoate, diethyl ether, 1 *M* HCl; naphthalene, hexane, water; benzoic acid, diethyl ether, 1 *M* NaOH; *p*-aminoacetophenone, methylene chloride, 1 *M* HCl.

liquids or in which liquid the solid is dissolved. Consider the following example, in which the liquids are water and hexane and the solid compound is biphenyl.



a. Without doing any experimental work, predict where each liquid is (top or bottom) and in which liquid the solid is dissolved. Justify your prediction. You may want to consult a handbook such as *The Merck Index* or the *CRC Handbook of Chemistry and Physics* to determine the molecular structure of a compound or to find any other relevant information. Note that dilute solutions such as 1 *M* HCl are composed mainly of water, and the density will be close to 1.0 g/mL. Furthermore, you should assume that the density of a solvent is not altered significantly when a solid dissolves in the solvent.

b. Now try to prove your prediction experimentally. That is, demonstrate which liquid the solid compound is dissolved in and the relative positions of the two liquids. You may use any experimental technique discussed in this experiment or any other technique that your instructor will let you try. In order to perform this part of the experiment, it may be helpful to separate the two layers in the test tube. This can be done easily and effectively with a Pasteur pipet. Squeeze the bulb on the Pasteur pipet and then place the tip of the pipet on the bottom of the test tube. Now withdraw only the bottom layer and transfer it to another test tube. Note that evaporating the water from an aqueous sample takes a very long time; therefore, this may not be a good way to show that an aqueous solution contains a dissolved compound. However, other solvents may be evaporated more easily (see p.). Explain what you did and whether or not the results of your experimental work were consistent with your prediction.

3. Add 0.025 g of tetraphenylcyclopentadienone to a dry test tube. Add 1 mL of methyl alcohol to the tube and shake for 60 seconds. Is the solid soluble, partially soluble, or insoluble? Explain your answer.

Part F. Nanotechnology Demonstration[4]

In this exercise, you will react a thiol (R-SH) with a gold surface to form a **self-assembled monolayer (SAM)** of thiol molecules on the gold. The thickness of this layer is about 2 nm (nanometer). A molecular system like this with dimensions at the nanometer level is an example of **nanotechnology.** Molecular self-assembly is also the key mechanism used in nature for the creation of complex structures such as the DNA double helix, proteins, enzymes, and the lipid bilayer of cell walls.

[4]This experiment is based on the self-Assembled Monolayer Demonstration Kit, produced by Asemblon, Inc., 15340 NE 92nd St., Suite B, Redmond, WA 98052; phone: 425–558–5100. Dr. Daniel Graham, a principal scientist and founder of Asemnlon, suggested this demonstration for inclusion in this book and helped to write the experiment.

The thiol that is used in this experiment is 11-mercaptoundecan-l-ol, $HS(CH_2)_{11}OH$. The self-assembly of this thiol onto gold is caused by an interplay between the attraction of sulfur and gold and the drive to minimize the energy of the system by packing the alkane chain of the thiols into an optimal arrangement. The bond energy of the sulfur–gold bond is about 45 kcal/mol, the strength of a partial covalent bond. As more thiols come to the surface of the gold, the interaction between the alkane chains becomes increasingly important. This is caused by the van der Waals attraction between the methylene groups (CH_2), which packs the chains close together in a crystalline-like monolayer. The process of self-assembly occurs quickly (within seconds) and results in the formation of an ordered surface that is only one molecule thick. This surface is referred to as a self-assembled monolayer.

The thiol used in this experiment consists of a terminal mercapto group (-SH), a spacer group (chain of CH_2 units), and a head group (-OH). Different head groups can be used, which makes thiol SAMs powerful surface engineering tools. Because a hydroxyl group attracts water, it is said to be hydrophilic. Since the hydroxyl group is positioned on the outer surface of the SAM, the outer surface takes on the properties of the head group and is also hydrophilic.

The first step in this experiment is to use a butane torch to clean the gold slide (glass plate coated with gold). The purpose of this step is to remove hydrocarbons from the air that have deposited on the gold surface over time. If the slide is dipped into water immediately after being cleaned, the gold surface should be coated with water. This occurs because the pure gold surface is a high-energy surface, which attracts the water molecules. Within a few minutes, the gold surface will be covered with hydrocarbons. In this experiment, you will wait a few minutes after the slide has been cleaned with the butane torch. The slide will then be dipped into water and wiped dry with tissue paper. You will print a word on the gold slide using a specially prepared pen containing the thiol. After rinsing the slide in water again, you will observe what has occurred on the surface of the slide.

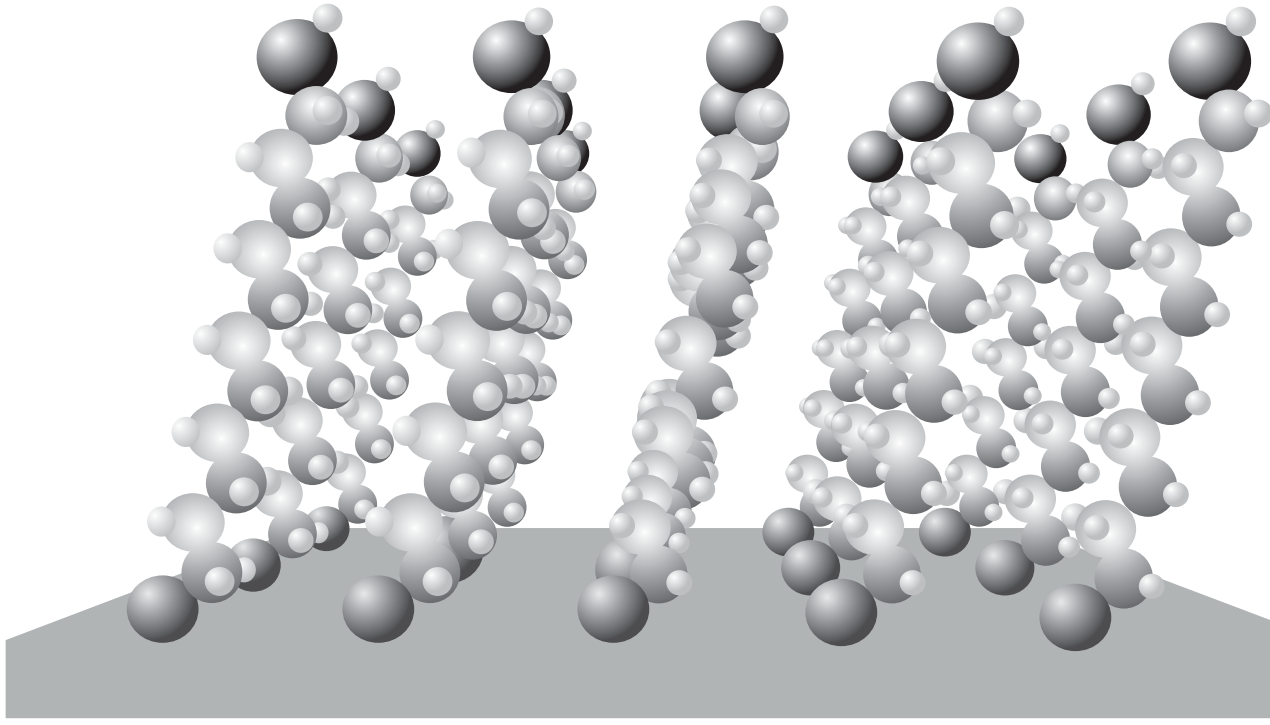
PROCEDURE

NOTE: Your instructor will first "erase" the gold using a butane torch.

CAUTION

When handling the gold slide, it is important to avoid touching the surface. Touching the surface can transfer contaminants from your fingers or gloves that can interface with the experiment. If you inadvertently touch the surface and leave fingerprints or other contaminants on it, you can clean the slide by rinsing it, with methanol and then acetone until the slide is clean.

Select a gold-coated slide that has been flamed by your instructor. Your should wait several minutes after the slide has been cleaned before proceeding with the next step. Holding the gold-coated slide in one hand **by the outer edges**, rinse the slide by completely dipping it in a beaker filled with deionized water. The water should roll off the slide when tilted. If the water droplets stick, gently wipe the slide off with a tissue paper and dip the slide in water again. Repeat this process until the slide comes out mostly dry. Gently wipe the slide completely dry with tissue paper. Breathe gently across the slide as if you were trying to fog up a window. Immediately after breathing on the slide, look at it before the moisture from your breath has evaporated. No writing should appear on the slide. If it does, your instructor should repeat the "erasing" step with the butane torch. Then repeat the rinsing procedure



Self-assembled monolayer of 11-mercaptoundecan-1-ol.

described above until the slide comes out mostly dry. Gently wipe it completely dry with tissue paper.

Place the slide with the gold side up on a flat surface. Take the Asemblon thiol pen and print a word of your choice. For best results, you should use gentle constant pressure and write in large block letter. The ink should wet the surface, and the lines in each letter should be continuous. The thiol assembly happens almost instantaneously, but to get good letter shapes the ink must completely wet all parts of each letter. If the ink does not adhere to a given part of a letter as you write it, go over it again with the pen. Let the ink sit on the gold surface for 30 seconds. Carefully pick up the slide by the edges at one end without touching the gold surface. Dip the slide into the beaker filled with deionized water and pull it out. Repeat this rinsing procedure four or five times.

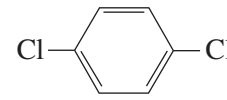
Look at the slide and record what you see. Water should adhere to the letters that were written, and the rest of the slide should remain dry. Letters that have a closed loop often trap water within the loop due to the high surface tension of water. If this occurs, try shaking off the excess water. If water still remains in the loops, take a piece of wet tissue paper and gently wipe across the surface. This should remove the water within the loops, but not the water that adheres to the letters.

REPORT

Part A

1. Summarize your results in table form.
2. Explain the results for all the tests done. In explaining these results, you should consider the polarities of the compound and the solvent and the potential for hydrogen bonding. For example, consider a similar solubility test for *p*-dichlorobenzene in hexane. The test indicates that *p*-dichlorobenzene is soluble in hexane. This result can be explained

by stating that hexane is nonpolar, whereas *p*-dichlorobenzene is slightly polar. Because the polarities of the solvent and solute are similar, the solid is soluble. (Remember that the presence of a halogen does not significantly increase the polarity of a compound.)



***p*-Dichlorobenzene**

3. There should be a difference in your results between the solubilities of biphenyl and benzophenone in methyl alcohol. Explain this difference.
4. There should be a difference in your results between the solubilities of benzophenone in methyl alcohol and benzophenone in hexane. Explain this difference.

Part B

1. Summarize your results in table form.
2. Explain the results for the tests done in water. In explaining these results, you should consider the polarities of the alcohols and water.
3. Explain, in terms of polarities, the results for the tests done in hexane.

Part C

1. Summarize your results in table form.
2. Explain the results in terms of polarities and/or hydrogen bonding.

Part D

1. Summarize your results in table form.
2. Explain the results for the tube in which 1.0 *M* NaOH was added to benzoic acid. Write an equation for this giving complete structures for all organic substances. Now describe what happened when 6.0 *M* HCl was added to this same tube, and explain this result.
3. Explain the results for the tube in which 1.0 *M* HCl was added to ethyl 4-aminobenzoate. Write an equation for this. Now describe what happened when 6.0 *M* NaOH was added to this same tube, and explain.

Part E

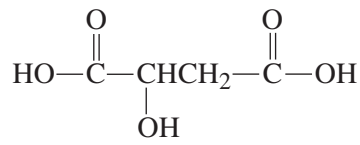
Give the results for any Critical-Thinking Applications completed, and answer all questions given in the Procedure for these exercises.

Part F

Record what you see after writing on the plate and dipping it into deionized water.

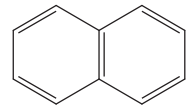
QUESTIONS

1. For each of the following pairs of solute and solvent, predict whether the solute would be soluble or insoluble. After making your predictions, you can check your answers by looking up the compounds in *The Merck Index* or the *CRC Handbook of Chemistry and Physics*. Generally, *The Merck Index* is the easier reference book to use. If the substance has a solubility greater than 40 mg/mL, you conclude that it is soluble.
   a. Malic acid in water



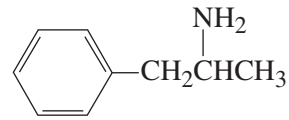
Malic acid

b. Naphthalene in water



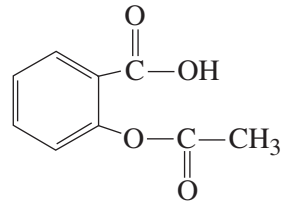
Naphthalene

c. Amphetamine in ethyl alcohol



Amphetamine

d. Aspirin in water

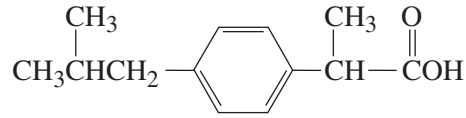


Aspirin

e. Succinic acid in hexane (*Note:* the polarity of hexane is similar to petroleum ether.)

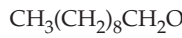
Succinic acid

f. Ibuprofen in diethyl ether



Ibuprofen

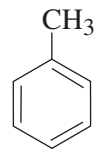
g. 1-Decanol (*n*-decyl alcohol) in water



1-Decanol

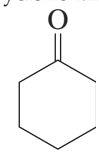
2. Predict whether the following pairs of liquids would be miscible or immiscible:

a. Water and methyl alcohol
b. Hexane and benzene
c. Methylene chloride and benzene
d. Water and toluene



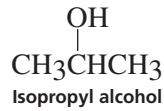
Toluene

e. Cyclohexanone and water



Cyclohexanone

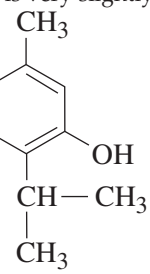
f. Ethyl alcohol and isopropyl alcohol



Isopropyl alcohol

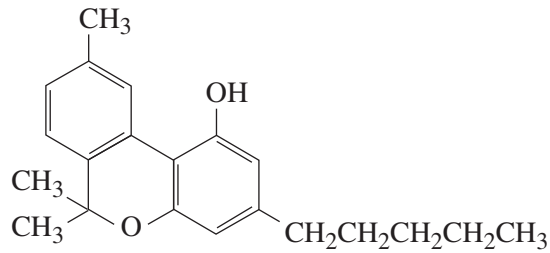
3. Would you expect ibuprofen (see 1f) to be soluble or insoluble in 1.0 *M* NaOH? Explain.

4. Thymol is very slightly soluble in water and very soluble in 1.0 *M* NaOH. Explain.



Thymol

5. Although cannabinol and methyl alcohol are both alcohols, cannabinol is very slightly soluble in methyl alcohol at room temperature. Explain.



Cannabinol

Questions 6–11 relate to Part F. Nanotechnology Demonstration

6. Why do the letters stay wet while the rest of the surface is dry?

7. Immediately after flame-cleaning the gold surface, water will adhere to the surface when the slide is dipped in water. If this water is cleaned off the slide and the slide is allowed to sit in the air for several minutes, water will no longer adhere to the surface when the slide is rinsed in water. Explain why.

8. A hydroxyl group on the end of the molecule makes the surface of the gold hydrophilic. How would a methyl group affect the surface? What is this effect called?

9. Why does heating the slide with a butane torch "erase" the writing?

10. How is this exercise different than writing on a glass surface with a crayon or wax pencil?

11. Why does water sometimes stick in the middle of some letters like P, O, or B, where there should not be any thiol?

2 **EXPERIMENT 2**

Crystallization

Crystallization

Vacuum filtration

Melting point

Finding a crystallization solvent

Mixture melting point

Critical-thinking application

The purpose of this experiment is to introduce the technique of crystallization, the most common procedure used to purify crude solids in the organic laboratory. For a thorough discussion of crystallization, read Technique 11 before proceeding, as an understanding of this method is assumed in this experiment.

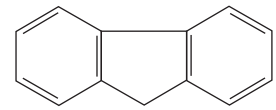
In Part A of this experiment, you will carry out a crystallization of impure sulfanilamide using 95% ethyl alcohol as the solvent. Sulfanilamide is one of the sulfa drugs, the first generation of antibiotics to be used in successfully treating many major diseases, such as malaria, tuberculosis, and leprosy (see the essay "Sulfa Drugs," that precedes Experiment 46).

In Part A of this experiment, and in most of the experiments in this textbook, you are told what solvent to use for the crystallization procedure. Some of the factors involved in selecting a crystallization solvent for sulfanilamide are discussed in Technique 11, Section 11.5. The most important consideration is the shape of the solubility curve for the solubility vs. temperature data. As can be seen in Technique 11, Figure 11.2, the solubility curve for sulfanilamide in 95% ethyl alcohol indicates that ethyl alcohol is an ideal solvent for crystallizing sulfanilamide.

The purity of the final material after crystallization will be determined by finding the melting point of your sample. You will also weigh your sample and calculate the percentage recovery. It is impossible to obtain a 100% recovery. This is true for several reasons: There will be some experimental loss, the original sample is not 100% sulfanilamide, and some sulfanilamide is soluble in the solvent even at 0°C. Because of this last fact, some sulfanilamide will remain dissolved in the **mother liquor** (the liquid remaining after crystallization has taken place). Sometimes it is worth isolating a second crop of crystals from the mother liquor, especially if you have performed a synthesis requiring many hours of work and the amount of product is relatively small. This can be accomplished by heating the mother liquor to evaporate some of the solvent and then cooling the resultant solution to induce a second crystallization. The purity of the second crop will not be as good as the first crop, however, because the concentration of the impurities will be greater in the mother liquor after some of the solvent has been evaporated.

In Part B, you will be given an impure sample of the organic compound fluorene (see structure that follows). You will use an experimental procedure for determining which one of three possible solvents is the most appropriate. The three solvents will illustrate three very different solubility behaviors: One of the solvents will be an appropriate solvent for crystallizing fluorene. In a second solvent,

fluorene will be highly soluble, even at room temperature. Fluorene will be relatively insoluble in the third solvent, even at the boiling point of the solvent. Your task will be to find the appropriate solvent for crystallization and then perform a crystallization on this sample.



Fluorene

You should be aware that not all crystallizations will look the same. Crystals have many different shapes and sizes, and the amount of mother liquor visible at the end of the crystallization may vary significantly. The crystallizations of sulfanilamide and fluorene will appear significantly different, even though the purity of the crystals in each case should be very good.

In Part C of this experiment, you will determine the identity of an unknown using the melting point technique. The **mixture melting point** technique is introduced in this section.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Technique 10	Solubility
New:	*Technique 8	Filtration, Sections 8.3 and 8.5
	Technique 9	Physical Constants of Solids: The Melting Point
	*Technique 11	Crystallization: Purification of Solids

SUGGESTED WASTE DISPOSAL

Dispose of all organic wastes into the nonhalogenated organic waste container.

Part A. Macroscale Crystallization

This experiment assumes a familiarity with the general macroscale crystallization procedure (see Technique 11, Section 11.3). In this experiment, step 2 in Figure 11.4 (removal of insoluble impurities) will not be required. Although the impure sample may have a slight color, it will also not be necessary to use a decolorizing agent (see Technique 11, Section 11.7). Leaving out these steps makes the crystallization easier to perform. Furthermore, very few experiments in this textbook require either of these techniques. If a filtration or decolorizing step is ever required, you may consult Technique 11, which describes these procedures in detail.

Pre-lab Calculations

1. Calculate how much 95% ethyl alcohol will be required to dissolve 0.75 g of sulfanilamide at 78°C. Use the graph in Technique 11, Figure 11.2, to make this calculation. The reason for making this calculation is so that you will know ahead of time the approximate amount of hot solvent you will be adding.
2. Using the volume of solvent calculated in step 1, calculate how much sulfanilamide will remain dissolved in the mother liquor after the mixture is cooled to 0°C.

To dissolve the sulfanilamide in the minimum amount of hot (boiling or almost boiling) solvent, you must keep the mixture at (or near) the boiling point of 95% ethyl alcohol during the entire procedure. You will likely add more solvent than the amount you calculated, as some solvent will evaporate. The amount of solvent is calculated only to indicate the approximate amount of solvent required. You should follow the procedure to determine the correct amount of solvent needed.

PROCEDURE

Preparations. Weigh 0.75 g of impure sulfanilamide and transfer this solid to a 25-mL Erlenmeyer flask.[1] Note the color of the impure sulfanilamide. To a second Erlenmeyer flask, add about 15 mL of 95% ethyl alcohol and a boiling stone. Heat the solvent on a *warm* hot plate until the solvent is boiling.[2] Because 95% ethyl alcohol boils at a relatively low temperature (78°C), it evaporates quite rapidly. Setting the temperature of the hot plate too high will result in too much loss of solvent through evaporation.

Dissolving the Sulfanilamide. *Before heating the flask containing the sulfanilamide*, add enough hot solvent with a Pasteur pipet to barely cover the crystals. Then heat the flask containing the sulfanilamide until the solvent is boiling. At first this may be difficult to see, because so little solvent is present. Add another small portion of solvent (about 0.5 mL), continue to heat the flask, and swirl the flask frequently. You may swirl the flask while it is on the hot plate or, for more vigorous swirling, remove it from the hot plate for a few seconds while you swirl it. When you have swirled the flask for 10–15 seconds, check to see if the solid has dissolved. If it has not, add another portion of solvent. Heat the flask again with occasional swirling until the solvent boils. Then swirl the flask for 10–15 seconds, frequently returning the flask to the hot plate so that the temperature of the mixture does not drop. Continue repeating the process of adding solvent, heating, and swirling until all the solid has dissolved completely. Note that it is essential to add just enough solvent to dissolve the solid—neither too much nor too little. Because 95% ethyl alcohol is very volatile, you need to perform this entire procedure fairly rapidly. Otherwise, you may lose solvent nearly as quickly as you are adding it, and the procedure will take a very long time. The time from the first addition of solvent until the solid dissolves completely should not be longer than 10–15 minutes.

Crystallization. Remove the flask from the heat and allow the solution to cool *slowly* (see Technique 11, Section 11.3, Part C, for suggestions). Cover the flask with a small watch glass, or stopper the flask. Crystallization should begin by the time the flask has cooled to room temperature. If it has not, scratch the inside surface of the flask with a glass rod (not fire-polished) to induce crystallization (see Technique 11, Section 11.8). When it appears that no further crystallization is occurring at room temperature, place the flask in a beaker containing ice water (see Technique 6, Section 6.9). Be sure that both water and ice are present and that the beaker is small enough to prevent the flask from tipping over.

Filtration. When crystallization is complete, vacuum filter the crystals using a small Büchner funnel (see Technique 8, Section 8.3, and Figure 8.5). (If you will be performing the Optional Exercise at the end of this procedure, you must save the mother liquor from this filtration procedure. Therefore, the filter flask should be clean and dry.) Moisten the filter paper with a few drops of 95% ethyl alcohol, and turn on the vacuum (or aspirator) to the fullest

[1]The impure sulfanilamide contains 5% fluorenone, a yellow compound, as the impurity.
[2]To prevent bumping in the boiling solvent, you may want to place a Pasteur pipet in the flask. Use a 50-mL flask so that the Pasteur pipet does not tip the flask over. This is a convenient method because a Pasteur pipet will also be used to transfer the solvent.

extent. Use a spatula to dislodge the crystals from the bottom of the flask before transferring the material to the Büchner funnel. Swirl the mixture in the flask and pour the mixture into the funnel, attempting to transfer both crystals and solvent. You will need to pour the mixture quickly, before the crystals have completely resettled on the bottom of the flask. (You may need to do this in portions, depending on the size of your Büchner funnel.) When the liquid has passed through the filter, repeat this procedure until you have transferred all the liquid to the Büchner funnel. At this point, there will usually be some crystals remaining in the flask. Using your spatula, scrape out as many of the crystals as possible from the flask. Add about 2 mL of *ice-cold* 95% ethyl alcohol (measured with a calibrated Pasteur pipet) to the flask. Swirl the liquid in the flask and then pour the remaining crystals and alcohol into the Büchner funnel. This additional solvent helps transfer the remaining crystals to the funnel, and the alcohol also rinses the crystals already on the funnel. This washing step should be done whether or not it is necessary to use the wash solvent for transferring crystals. If necessary, repeat with another 2-mL portion of ice-cold alcohol. Wash the crystals with a total of about 4 mL of ice-cold solvent.

Continue drawing air through the crystals on the Büchner funnel by suction for about 5 minutes. Transfer the crystals onto a preweighed watch glass for air drying. (Save the mother liquor in the filter flask if you will be doing the Optional Exercise.) Separate the crystals as much as possible with a spatula. The crystals should be completely dried within 10–15 minutes. You can usually determine if the crystals are still wet by observing whether or not they stick to a spatula or stay together in a clump. Weigh the dry crystals and calculate the percentage recovery. Compare the color of the pure sulfanilamide to the impure sulfanilamide at the beginning of the experiment. Determine the melting point of the pure sulfanilamide and the original impure material. The literature melting point for pure sulfanilamide is 163°C – 164°C. At the option of the instructor, turn in your crystallized material in a properly labeled container.

Comments on the Crystallization Procedure

1. Do not heat the crude sulfanilamide until you have added some solvent. Otherwise, the solid may melt and possibly form an oil, which may not crystallize easily.
2. When you are dissolving the solid in hot solvent, the solvent should be added in small portions swirling and heating. The procedure calls for a specific amount (about 0.5 mL), which is appropriate for this experiment. However, the actual amount you should add each time you perform a crystallization may vary, depending on the size of your sample and the nature of the solid and solvent. You will need to make this judgment when you perform this step.
3. One of the most common mistakes is to add too much solvent. This can happen most easily if the solvent is not hot enough or if the mixture is not stirred sufficiently. If too much solvent is added, the percentage recovery will be reduced; it is even possible that no crystals will form when the solution is cooled. If too much solvent is added, you must evaporate the excess by heating the mixture. Using a nitrogen or air stream directed into the container will accelerate the evaporation process (see Technique 7, Section 7.10).
4. Sulfanilamide should crystallize as large, beautiful needles. However, this will not always happen. If the crystals form too rapidly or if there is not enough solvent, they will tend to be smaller, perhaps even appearing as a powder. Compounds other than sulfanilamide may crystallize in other characteristic shapes, such as plates or prisms.
5. When the solvent is water or when the crystals form as a powder, it will be necessary to dry the crystals longer than 10–15 minutes. Overnight drying may be necessary, especially when water is the solvent.

Optional Exercise. Transfer the mother liquor to a tared (preweighed) 25-mL Erlenmeyer flask. Place the flask in a warm water bath and evaporate all the solvent from the mother liquor. Use a stream of nitrogen or air directed into the flask to speed up the rate of evaporation (see Technique 7, Section 7.10). Cool the flask to room temperature and dry the outside. Weigh the flask with solid. Compare this to the weight calculated in the Prelab Calculations. Determine the melting point of this solid and compare it to the melting point of the crystals obtained by crystallization.

Part B. Selecting a Solvent to Crystallize a Substance

In this experiment, you will be given an impure sample of fluorene.[3] Your goal will be to find a good solvent for crystallizing the sample. You should try water, methyl alcohol, and toluene. After you have determined which is the best solvent, crystallize the remaining material. Finally, determine the melting point of the purified compound and of the impure sample.

PROCEDURE

Selecting a Solvent. Perform the procedure given in Technique 11, Section 11.6 with three separate samples of impure fluorene. Use the following solvents: methyl alcohol, water, and toluene.

Crystallizing the Sample. After you have found a good solvent, crystallize 0.75 g of impure fluorene using the procedure given in Part A of this experiment. Weigh the impure sample carefully and be sure to keep a little of it for later determination of the melting point. After filtering the crystals on the Büchner funnel, transfer the crystals to a preweighed watch glass and allow them to air-dry. If water was used as the solvent, you may need to let the crystals sit out overnight to dry, because water is less volatile than most organic solvents. Weigh the dried sample and calculate the percentage recovery. Determine the melting point of both the pure sample and the original impure material. The literature melting point for pure fluorene is 116°C – 117°C. At the option of the instructor, turn in your crystallized material in a properly labeled container.

Part C. Mixture Melting Points

In Parts A and B of this experiment, the melting point was used to determine the purity of a known substance. In some situations, the melting point can also be used to determine the identity of an unknown substance.

In Part C, you will be given a pure sample of an unknown from the following list:

Compound	Melting Point (°C)
Acetylsalicylic acid	138–140
Benzoic acid	121–122
Benzoin	135–136
Dibenzoyl ethylene	108–111
Succinimide	122–124
o-Toluic acid	108–110

Your goal is to determine the identity of the unknown using the melting-point technique. If all of the compounds in the list had distinctly different melting points, it would be possible to determine the identity of the unknown simply by determining its melting point. However, each of the compounds in this list has a melting point that is close to the melting point of another compound in the list. Therefore, determining the melting point of the unknown will allow you to narrow down the choices to two compounds. To determine the identity of your compound, you must perform mixture melting points of your unknown and each of the two

[3] The impure fluorene contains 5% fluorenone, a yellow compound.

compounds with similar melting points. A mixture melting point that is depressed and has a wide range indicates that the two compounds in the mixture are different.

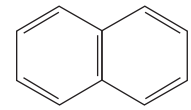
PROCEDURE

Obtain an unknown sample and determine its melting point. Determine mixture melting points (see Technique 9, Section 9.4) of your unknown and all compounds from the previous list that have similar melting points. To prepare a sample for a mixture melting point, use a spatula or a glass stirring rod to grind equal amounts of your unknown and the known compound in a watch glass. Record all melting points and state the identity of your unknown.

Part D. Critical-Thinking Application

The goal of the exercise is to find an appropriate solvent to crystallize a given compound. Rather than do this experimentally, you will try to predict which one of three given solvents is the best. For each compound, one of the solvents has the desired solubility characteristics to be a good solvent for crystallization. In a second solvent, the compound will be highly soluble, even at room temperature. The compound will be relatively insoluble in the third solvent, even at the boiling point of the solvent. After making your predictions, you will check them by looking up the appropriate information in *The Merck Index*.

For example, consider naphthalene, which has the following structure:



Naphthalene

Consider the three solvents ether, water, and toluene. (Look up their structures if you are unsure. Remember that ether is also called diethyl ether.) Based on your knowledge of polarity and solubility behavior, make your predictions. It should be clear that naphthalene is insoluble in water, because naphthalene is a hydrocarbon that is nonpolar and water is very polar. Both toluene and ether are relatively nonpolar, so naphthalene is most likely soluble in each of them. One would expect naphthalene to be more soluble in toluene, because both naphthalene and toluene are hydrocarbons. In addition, they both contain benzene rings, which means that their structures are very similar. Therefore, according to the solubility rule "Like dissolves like," one would predict that naphthalene is very soluble in toluene. Perhaps it is too soluble in toluene to be a good crystallizing solvent? If so, then ether would be the best solvent for crystallizing naphthalene.

These predictions can be checked with information from *The Merck Index.* Finding the appropriate information can be somewhat difficult, especially for beginning organic chemistry students. Look up *naphthalene* in *The Merck Index.* The entry for *naphthalene* states, "Monoclinic prismatic plates from ether." This statement means that naphthalene can be crystallized from ether. It also gives the type of crystal structure. Unfortunately, sometimes the crystal structure is given without reference to the solvent. Another way to determine the best solvent is by looking at solubility-vs.-temperature data. A good solvent is one in which the solubility of the compound increases significantly as the temperature increases. To determine whether the solid is too soluble in the solvent, check the solubility at room temperature. In Technique 11, Section 11.6, you were instructed to add 0.5 mL of solvent to 0.05 g of compound. If the solid completely dissolved, then the solubility at room temperature was too great. Follow this same guideline here. For naphthalene, the solubility in toluene is given as 1 g in 3.5 mL. When no temperature is given, room temperature is assumed. By comparing this to the 0.05 g in 0.5 mL ratio, it is clear that naphthalene is too soluble in toluene at

room temperature for toluene to be a good crystallizing solvent. Finally, *The Merck Index* states that naphthalene is insoluble in water. Sometimes no information is given about solvents in which the compound is insoluble. In that case, you would rely on your understanding of solubility behavior to confirm your predictions.

When using *The Merck Index,* you should be aware that alcohol is listed frequently as a solvent. This generally refers to 95% or 100% ethyl alcohol. Because 100% (absolute) ethyl alcohol is more expensive than 95% ethyl alcohol, the cheaper grade is usually used in the chemistry lab. Finally, benzene is frequently listed as a solvent. Because benzene is a known carcinogen, it is rarely used in student labs. Toluene is a suitable substitute; the solubility behavior of a substance in benzene and toluene is so similar that you may assume any statement made about benzene also applies to toluene.

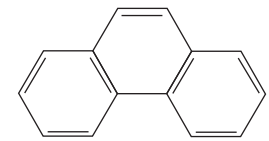
For each of the following sets of compounds (the solid is listed first, followed by the three solvents), use your understanding of polarity and solubility to predict

1. The best solvent for crystallization
2. The solvent in which the compound is too soluble
3. The solvent in which the compound is not sufficiently soluble

Then check your predictions by looking up each compound in *The Merck Index.*

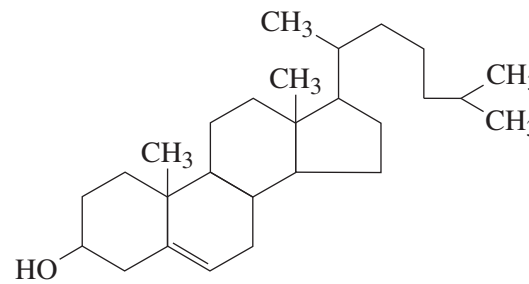
1. Phenanthrene; toluene, 95% ethyl alcohol, water

Phenanthrene



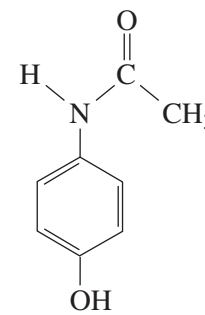
2. Cholesterol; ether, 95% ethyl alcohol, water

Cholesterol

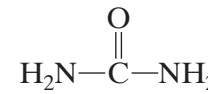


3. Acetaminophen; toluene, 95% ethyl alcohol, water

Acetaminophen



4. Urea; hexane, 95% ethyl alcohol, water



Urea

REPORT

Part A

1. Report the melting points for both the impure sulfanilamide and the crystallized sulfanilamide and comment on the differences. Also, compare these to the literature value. Based on the melting point of the crystallized sulfanilamide, is it pure? Also comment on the purity based on the color of the crystals. Report both the original weight of the impure sulfanilamide and the weight of the crystallized sulfanilamide. Calculate the percentage recovery and comment on several sources of loss.

2. If you completed the Optional Exercise (isolating the solid dissolved in the mother liquor), do the following:

- **a.** Make a table with the following information:
  - **i.** Weight of impure sulfanilamide used in the crystallization procedure
  - **ii.** Weight of pure sulfanilamide after crystallization
  - **iii.** Weight of sulfanilamide plus impurity recovered from the mother liquor (see Part A, Optional Exercise)
  - **iv.** Total of items ii and iii (total weight of sulfanilamide plus impurity isolated)
  - **v.** Calculated weight of sulfanilamide in the mother liquor (see Part A, Pre-Lab Calculations)
- **b.** Comment on any differences between the values in items i and iv. Should they be the same? Explain.
- **c.** Comment on any differences between the values in items iii and v. Should they be the same? Explain.
- **d.** Report the melting point of the solid recovered from the mother liquor. Compare this to the melting points of the crystallized sulfanilamide. Should they be the same? Explain.

Part B

1. For each of the three solvents (methyl alcohol, water, and toluene), describe the results from the tests for selecting a good crystallizing solvent for fluorene. Explain these results in terms of polarity and solubility predictions (see "Guidelines for Predicting Polarity and Solubility," Technique 10, Section 10.2A).

2. Report the melting points for both the impure fluorene and the crystallized fluorene and comment on the differences. What is the literature value for the melting point of fluorene? Report the original weight of both the impure fluorene and the weight of the crystallized fluorene. Calculate the percentage recovery, and comment on several sources of loss.

3. The solubility of fluorene in each solvent used in Part B corresponds to one of the three curves shown in Technique 11, Figure 11.1. For each solvent, indicate which curve best describes the solubility of fluorene in that solvent.

Part C

Record all melting points and state the identity of your unknown.

Part D

For each compound assigned, state your predictions along with an explanation. Then give the relevant information from *The Merck Index* that supports or contradicts your predictions. Try to explain any differences between your predictions and information found in *The Merck Index*.

QUESTIONS

1. Consider a crystallization of sulfanilamide in which 10 mL of hot 95% ethyl alcohol is added to 0.10 g of impure sulfanilamide. After the solid has dissolved, the solution is cooled to room temperature and then placed in an ice-water bath. No crystals form, even after scratching with a glass rod. Explain why this crystallization failed. What would you have to do at this point to make the crystallization work? You should assume that starting over again with a new sample is not an option. (You may need to refer to Technique 11, Figure 11.2.)
2. Benzyl alcohol (bp 205°C) was selected by a student to crystallize fluorenol (mp 153°C–154°C) because the solubility characteristics of this solvent are appropriate. However, this solvent is not a good choice. Explain.
3. A student performs a crystallization on an impure sample of biphenyl. The sample weighs 0.5 g and contains about 5% impurity. Based on his knowledge of solubility, the student decides to use benzene as the solvent. After crystallization, the crystals are dried and the final weight is found to be 0.02 g. Assume that all steps in the crystallization were performed correctly, there were no spills, and the student lost very little solid on any glassware or in any of the transfers. Why is the recovery so low?

3 EXPERIMENT 3

Extraction

Extraction

Critical-thinking application

Extraction is one of the most important techniques for isolating and purifying organic substances. In this method, a solution is mixed thoroughly with a second solvent that is **immiscible** with the first solvent. (Remember that immiscible liquids do not mix; they form two phases or layers.) The solute is extracted from one solvent into the other because it is more soluble in the second solvent than in the first.

The theory of extraction is described in detail in Technique 12, Sections 12.1–12.2. You should read these sections before continuing this experiment. Because solubility is the underlying principle of extraction, you may also wish to reread Technique 10.

Extraction is a technique used by organic chemists, but it is also used to produce common products with which you are familiar. For example, vanilla extract, the popular flavoring agent, was originally extracted from vanilla beans using alcohol as the organic solvent. Decaffeinated coffee is made from coffee beans that have been decaffeinated by an extraction technique (see essay "Caffeine," that precedes Experiment 11). This process is similar to the procedure in Part A of this experiment, in which you will extract caffeine from an aqueous solution.

The purpose of this experiment is to introduce the macroscale technique for performing extractions and allow you to practice this technique. This experiment also demonstrates how extraction is used in organic experiments.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Technique 10	Solubility
New:	*Technique 12	Extraction
	Essay	Caffeine

SPECIAL INSTRUCTIONS

Be careful when handling methylene chloride. It is a toxic solvent, and you should not breathe its fumes excessively or spill it on yourself.

In Part B, it is advisable to pool the data for the distribution coefficients and calculate class averages. This will compensate for differences in the values due to experimental error.

SUGGESTED WASTE DISPOSAL

You must dispose of all methylene chloride in a waste container marked for the disposal of halogenated organic wastes. Place all other organic wastes into the nonhalogenated organic waste container. The aqueous solutions obtained after the extraction steps must be disposed of in the container designated for aqueous waste.

Part A. Extraction of Caffeine

One of the most common extraction procedures involves using an organic solvent (nonpolar or slightly polar) to extract an organic compound from an aqueous solution. Because water is highly polar, the mixture will separate into two layers or phases: an aqueous layer and an organic (nonpolar) layer.

In this experiment, you will extract caffeine from an aqueous solution using methylene chloride. You will perform the extraction step three times using three separate portions of methylene chloride. Because methylene chloride is more dense than water, the organic layer (methylene chloride) will be on the bottom. After each extraction, you will remove the organic layer. The organic layers from all three extractions will be combined and dried over anhydrous sodium sulfate. After transferring the dried solution to a preweighed container, you will evaporate the methylene chloride and determine the weight of caffeine extracted from the aqueous solution. This extraction procedure succeeds because caffeine is much more soluble in methylene chloride than in water.

Prelab Calculation In this experiment, 0.170 g of caffeine is dissolved in 10.0 mL of water. The caffeine is extracted from the aqueous solution three times with 5.0-mL portions of methylene chloride. Calculate the total amount of caffeine that can be extracted into the three portions of methylene chloride (see Technique 12, Section 12.2). Caffeine has a distribution coefficient of 4.6 between methylene chloride and water.

PROCEDURE

NOTE: To obtain good results, you should make all weighings accurately, preferably on a balance that is accurate to within 0.001g.

Preparation. Add exactly 0.170 g of caffeine and 10.0 mL of water to a screwcap centrifuge tube. Cap the tube and shake it vigorously for several minutes until the caffeine dissolves completely. It may be helpful to heat the mixture slightly to dissolve all the caffeine.

Extraction. Using a Pasteur pipet, transfer the caffeine solution to a 125-mL separatory funnel. (Don't forget to close the stopcock!) Using a 10-mL graduated cylinder, obtain 5.0 mL of methylene chloride and add to the separatory funnel. Stopper the funnel and hold it as shown in Technique 12, Figure 12.6. Hold the stopper in place *firmly* and invert the separatory funnel. While the funnel is inverted, release the pressure by slowly opening the stopcock. Continue inverting and venting until the "whoosh" is no longer audible. The two layers must now be mixed thoroughly so that as much caffeine as possible is transferred from the aqueous layer to the methylene chloride layer. However, if the mixture is mixed too vigorously, it may form an emulsion. Emulsions look like a third frothy layer between the original two layers, and they can make it difficult for the layers to separate. Follow these instructions carefully to prevent the formation of an emulsion. Shake the mixture gently by inverting the funnel repeatedly in a rocking motion. Initially, a good rate of shaking is about one rock per two seconds. When it is clear that an emulsion is not forming, you may shake the mixture more vigorously, perhaps one time per second. (Note that it is usually not prudent to shake the heck out of it!) Shake the mixture for at least one minute. When you have finished mixing the liquids, place the separatory funnel in the iron ring and let it stand until the layers separate completely.[1] Place a 50-mL Erlenmeyer flask under the separatory funnel and remove the top stopper on the funnel. Allow the bottom (organic) layer to drain slowly by partially opening the stopcock. When the interface between the upper and lower phases just begins to enter the bore of the stopcock, close the stopcock immediately.

Repeat this extraction two more times using 5.0 mL of fresh methylene chloride each time. Combine the organic layer from each of these extractions with the methylene chloride solution from the first extraction.

Drying the Organic Layers. Dry the combined organic layers over granular anhydrous sodium sulfate, following the instructions given in Technique 12, Section 12.9, "Drying Procedure with Anhydrous Sodium Sulfate." Read these instructions carefully and complete steps 1–3 in "A. Macroscale Drying Procedure." Step 4 described in the next section of this experiment.

Evaporation of Solvent. Transfer the dried methylene chloride solution with a clean, dry Pasteur pipet to a dry, preweighed 50-mL Erlenmeyer flask, while leaving the drying agent behind. Evaporate the methylene chloride by heating the flask in a hot water bath at about 45°C.[2] This should be done in a hood and can be accomplished more rapidly if a stream of dry air or nitrogen gas is directed at the surface of the liquid (see Technique 7, Section 7.10). When the solvent has evaporated, remove the flask from the bath and dry the outside of the flask. Do not leave the flask in the water bath for a long time after the solvent has evaporated because the caffeine may sublime. When the flask has cooled to room temperature, weigh it to determine the amount of caffeine that was in the methylene chloride solution. Compare this weight with the amount of caffeine calculated in the Prelab Calculation.

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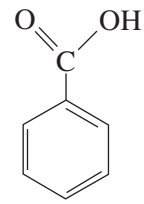
[1] If an emulsion has formed, the two layers may not separate on standing. If they do not separate after about 1–2 minutes, first try swirling the separatory funnel to break the emulsion. If this does not work, try method 5 in Technique 12, Section 12.10.

[2] A more environmentally friendly procedure is to use a rotary evaporator (see Technique 7, Section 7.11). With this method, the methylene chloride is recovered and can be reused.

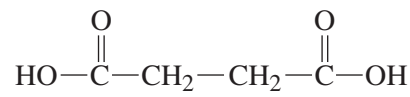
Part B. Distribution of a Solute Between Two Immiscible Solvents

In this experiment, you will investigate how several different organic solids distribute themselves between water and methylene chloride. A solid compound is mixed with the two solvents until equilibrium is reached. The organic layer is removed, dried over anhydrous sodium sulfate, and transferred to a tared container. After evaporating the methylene chloride, you will determine the weight of the organic solid that was in the organic layer. By finding the difference, you can also determine the amount of solute in the aqueous layer. The distribution coefficient of the solid between the two layers can then be calculated and related to the polarity of the solid and the polarities of the two liquids.

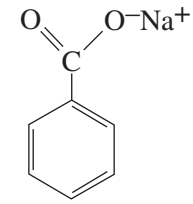
Three different compounds will be used: benzoic acid, succinic acid, and sodium benzoate. Their structures are given below. You should perform this experiment on one of the solids and share your data with two other students who worked with the other two solids. Alternatively, data from the entire class may be pooled and averaged.



Benzoic acid



Succinic acid



Sodium benzoate

PROCEDURE

NOTE: To obtain good results, you should make all weighings accurately, preferably on a balance that is accurate to within 0.001g.

Place 0.100 g of one of the solids (benzoic acid, succinic acid, or sodium benzoate) into a screw-cap centrifuge tube. Add 4.0 mL of methylene chloride and 4.0 mL of water to the tube. Cap the tube and shake it for about 1 minute. The correct way to shake is to invert the tube and right it in a rocking motion. A good rate of shaking is about one rock per second. When it is clear that an emulsion is not forming, you may shake it more vigorously, perhaps two to three times per second. Check for undissolved solid. Continue shaking the tube until all the solid is dissolved.

Allow the centrifuge tube to sit until the layers have separated. Using a Pasteur pipet, you should now transfer the organic (bottom) layer into a test tube. Ideally, the goal is to remove all of the organic layer without transferring any of the aqueous layer. However, this is difficult to do. Try to squeeze the bulb so that when it is released completely, you will draw up the amount of liquid that you desire. If you have to hold the bulb in a partially depressed position while making a transfer, it is likely that you will spill some liquid. It is also necessary to transfer the liquid in two or three steps. First, depress the bulb completely so that as much of the bottom layer as possible will be drawn into the pipet. Place the tip of the pipet squarely in the **V** at the bottom of the centrifuge tube and release the bulb slowly. When making the transfer, it is essential that the centrifuge tube and the test tube are held next to each other. A good technique for this is illustrated in Figure 12.8. After transferring the first portion, repeat this process until all of the bottom layer has been transferred to the test tube. Each time, depress the bulb only as much as is necessary and place the tip of the pipet in the bottom of the tube.

Dry the organic layer over granular anhydrous sodium sulfate, following the instructions given in Technique 12, Section 12.9, "Drying Procedure with Anhydrous Sodium Sulfate." Read these instructions carefully and complete steps 1–3 in the "Microscale Drying Procedure." Step 4 is described in the next paragraph.

Transfer the dried methylene chloride solution with a clean, dry Pasteur pipet to a dry, preweighed test tube, leaving the drying agent behind. Evaporate the methylene chloride by heating the test tube in a warm water bath while directing a stream of dry air or nitrogen gas at the surface of the liquid. When the solvent has evaporated, remove the test tube from the bath and dry the outside of the tube. When the test tube has cooled to room temperature, weigh the test tube to determine the amount of solid solute that was in the methylene chloride layer. Determine by difference the amount of the solid that was dissolved in the aqueous layer. Calculate the distribution coefficient for the solid between methylene chloride and water. Because the volume of methylene chloride and water was the same, the distribution coefficient can be calculated by dividing the weight of solute in methylene chloride by the weight of solute in water.

Optional Exercise. Repeat the previous procedure using 0.075 g of caffeine, 3.0 mL of methylene chloride, and 3.0 mL of water. Determine the distribution coefficient for caffeine between methylene chloride and water. Compare this to the literature value of 4.6.

Part C. How Do You Determine Which One is the Organic Layer?

A common problem that you might encounter during an extraction procedure is not knowing for sure which layer is organic and which is aqueous. Although the procedures in this textbook often indicate the expected relative positions of the two layers, not all procedures will give this information and you should be prepared for surprises. Sometimes, knowing the densities of the two solvents is not sufficient, because dissolved substances can significantly increase the density of a solution. It is very important to know the location of the two layers, because usually one layer contains the desired product and the other layer is discarded. A mistake at this point in an experiment would be disastrous!

The purpose of this experiment is to give you some practice in determining which layer is aqueous and which layer is organic (see Technique 12, Section 12.8). As described in Section 12.8, one effective technique is to add a few drops of water to each layer after the layers have been separated. If a layer is water, then the drops of added water will dissolve in the aqueous layer and increase its volume. If the added water forms droplets or a new layer, then it is the organic layer.

PROCEDURE

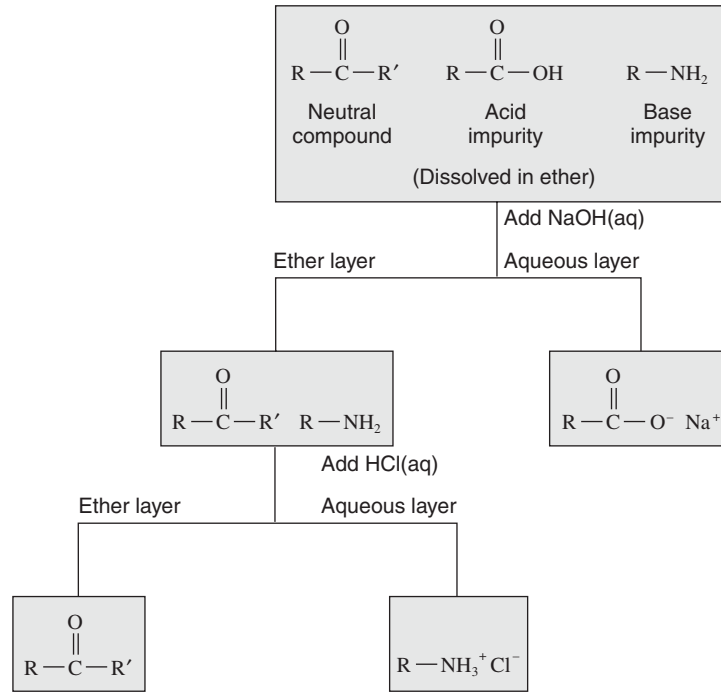
Obtain three test tubes, each containing two layers.[3] For each tube, you will be told the identity of the two layers, but you will not be told their relative positions. Determine experimentally which layer is organic and which layer is aqueous. Dispose of all these mixtures into the waste container designated for halogenated organic wastes. After determining the layers experimentally, look up the densities of the various liquids in a handbook to see if there is a correlation between the densities and your results.

Part D. Use of Extraction to Isolate a Neutral Compound From a Mixture Containing an Acid or Base Impurity

In this experiment, you will be given a solid sample containing an unknown neutral compound and an acid or base impurity. The goal is to remove the acid or base by extraction and isolate the neutral compound. By determining the melting point of the neutral compound, you will identify it from a list of possible compounds. There are many organic reactions in which the desired product, a neutral compound, is contaminated by an acid or base impurity. This experiment illustrates how extraction is used to isolate the product in such a situation.

[3]The three mixtures will likely be (1) water and *n*-butyl chloride, (2) water and *n*-butyl bromide, and (3) *n*-butyl bromide and saturated aqueous sodium bromide.

In Technique 10, "Solubility," you learned that organic acids and bases can become ions in acid–base reactions (see Section 10.2 B "Solutions in Which the Solute Ionizes and Dissociates"). Before reading on, review this material if necessary. Using this principle, you can separate an acid or base impurity from a neutral compound. The following scheme, which shows how both an acid and a base impurity are removed from the desired product, illustrates how this is accomplished:



Flowchart showing how acid and base impurities are removed from the desired product.

The neutral compound can now be isolated by removing the water dissolved in the ether and evaporating the ether. Because ether dissolves a relatively large quantity of water (1.5%), the water must be removed in two steps. In the first step, the ether solution is mixed with a saturated aqueous NaCl solution. Most of the water in the ether layer will be transferred to the aqueous layer in this step (see Technique 12, Section 12.9). Finally, the remainder of the water is removed by drying the ether layer over anhydrous sodium sulfate. The neutral compound can then be isolated by evaporating the ether. In most organic experiments that use a separation scheme such as this, it would be necessary to perform a crystallization step to purify the neutral compound. In this experiment, however, the neutral compound should be sufficiently pure at this point to identify it by melting point.

The organic solvent used in this experiment is ether. Recall that the full name for ether is diethyl ether. Because ether is less dense than water, this experiment will give you practice in performing extractions where the nonpolar solvent is less dense than water.

The following procedure provides instruction on removing an acid impurity from a neutral compound and isolating the neutral compound. It contains an additional step that is not normally part of this kind of separation scheme: The aqueous layers from each extraction are segregated and acidified with aqueous HCl. The purpose of this step is to verify that the acid impurity has been removed completely from the ether layer. In the Optional Exercise, the sample contains a neutral compound with a base impurity; however, a detailed

procedure is not given. If you are assigned this exercise, you must create a procedure by using the principles discussed in this introduction and by studying the following procedure for isolating the neutral compound from an acid impurity.

PROCEDURE

Isolating a Neutral Compound from a Mixture Containing an Acid Impurity. Add 0.36 g of an unknown mixture to a screw-cap centrifuge tube.[4] Add 10.0 mL of ether to the tube and cap it. Shake the tube until all the solid dissolves completely. Transfer this solution to a 125-mL separatory funnel.

Add 5.0 mL of 1.0 *M* NaOH to the separatory funnel and shake for 30 seconds, using the same procedure described in Part A. Let the layers separate. Remove the bottom (aqueous) layer and place this in an Erlenmeyer flask labeled "1st NaOH extract." Add another 5.0-mL portion of 1.0 *M* NaOH to the funnel and shake for 30 seconds. When the layers have separated, remove the aqueous layer and put it in an Erlenmeyer flask labeled "2nd NaOH extract."

While stirring, add 6 *M* HCl dropwise to each of the two test flasks containing the NaOH extracts until the mixtures are acidic. Test the mixtures with litmus or pH paper to determine when they are acidic. Observe the amount of precipitate that forms. What is the precipitate? Does the amount of precipitate in each flask indicate that all the acid impurity has been removed from the ether layer containing the unknown neutral compound?

The drying procedure for an ether layer requires the following additional step, which is not included in the procedure for drying a methylene chloride layer (see Technique 12, Section 12.9, "Saturated Salt Solution"). To the ether layer in the separatory funnel, add 5.0 mL of saturated aqueous sodium chloride. Shake for 30 seconds and let the layers separate. Remove and discard the aqueous layer. Pour the ether layer (without any water) from the *top* of the separatory funnel into a clean, dry Erlenmeyer flask. Now dry the ether layer over granular anhydrous sodium sulfate (see Technique 12, Section 12.9, "Drying Procedure with Anhydrous Sodium Sulfate"). Complete steps 1–3 in "A. Macroscale Drying Procedure." Step 4 is described in the next paragraph of this experiment.

Transfer the dried ether solution with a clean, dry Pasteur pipet to a dry, preweighed Erlenmeyer flask, leaving the drying agent behind. Evaporate the ether by heating the flask in a warm water bath. This should be done in a hood and can be accomplished more rapidly if a stream of dry air or nitrogen gas is directed at the surface of the liquid (see Technique 7, Section 7.10).[5] When the solvent has evaporated, remove the flask from the bath and dry the outside of the flask. Once the flask has cooled to room temperature, weigh it to determine the amount of solid solute that was in the ether layer. Obtain the melting point of the solid and identify it from the following table:

	Melting Point (°C)
Fluorenone	82–85
Fluorene	116–117
1, 2, 4, 5-Tetrachlorobenzene	139–142
Triphenylmethanol	162–164

[4]The mixture contains 0.24 g of one of the neutral compounds given in the following table and 0.12 g of benzoic acid, the acid impurity.
[5]See footnote 3.

Part D. Critical-Thinking Application

Optional Exercise: Isolating a Neutral Compound from a Mixture Containing a Base Impurity. Obtain 0.36 g of an unknown mixture containing a neutral compound and a base impurity.[6] Develop a procedure for isolating the neutral compound, using the previous procedure as a model. After isolating the neutral compound, obtain the melting point and identify it from the list of compounds given above.

PROCEDURE

1. Add 4 mL of water and 2 mL of methylene chloride to a screw-cap centrifuge tube.
2. Add 4 drops of solution A to the centrifuge tube. Solution A is a dilute aqueous solution of sodium hydroxide containing an organic compound.[7] Shake the mixture for about 30 seconds, using a rapid rocking motion. Describe the color of each layer (see the following table).
3. Add 2 drops of 1 *M* HCl. Let the solution sit for 1 minute and note the color change. Then shake for about 1 minute, using a rapid rocking motion. Describe the color of each layer.
4. Add 4 drops of 1 *M* NaOH and shake again for about 1 minute. Describe the color of each layer.

		Color
Step 2	**Aqueous**	
	Methylene chloride	
Step 3	**Aqueous**	
	Methylene chloride	
Step 4	**Aqueous**	
	Methylene chloride	

REPORT

Part A

1. Show your calculations for the amount of caffeine that should be extracted by the three 5.0-mL portions of methylene chloride (see Prelab Calculation).
2. Report the amount of caffeine isolated. Compare this weight with the amount of caffeine calculated in the Prelab Calculation. Comment on the similarity or difference.

PartB

1. Report in table form the distribution coefficients for the three solids: benzoic acid, succinic acid, and sodium benzoate.
2. Is there a correlation between the values of the distribution coefficients and the polarities of the three compounds? Explain.

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[6]The mixture contains 0.24 g of one of the neutral compounds given in the list on this page and 0.12 g of ethyl 4-aminobenzoate, the base impurity.

[7]Solution A: Mix 25 mg of 2, 6-dichloroindophenol (sodium salt) with 50 mL of water and 1 mL of 1 *M* NaOH. This solution should be prepared the same day it is used.

3. If you completed the Optional Exercise, compare the distribution coefficient you obtained for caffeine with the corresponding literature value. Comment on the similarity or difference.

Part C

1. For each of the three mixtures, report which layer was on the bottom and which one was on the top. Explain how you determined this for each mixture.
2. Record the densities for the liquids given in a handbook.
3. Is there a correlation between the densities and your results? Explain.

Part D

1. Answer the following questions about the first and second NaOH extracts.
   a. Comment on the amount of precipitate for both extracts when HCl is added.
   b. What is the precipitate formed when HCl is added?
   c. Does the amount of precipitate in each tube indicate that all the acid impurity has been removed from the ether layer containing the unknown neutral compound?
2. Report the melting point and weight of the neutral compound you isolated.
3. Based on the melting point, what is the identity of this compound?
4. Calculate the percent recovery for the neutral compound. List possible sources of loss.

If you completed the Optional Exercise, complete steps 1–4 for Part D.

Part E

Describe fully what occurred in steps 2, 3, and 4. For each step, include (1) the nature (cation, anion, or neutral species) of the organic compound, (2) an explanation for all the color changes, and (3) an explanation for why each layer is colored as it is. Your explanation for (3) should be based on solubility principles and the polarities of the two solvents. (*Hint:* It may be helpful to review the sections in your general chemistry textbook that deal with acids, bases, and acid–base indicators.)

REFERENCE

Kelly, T. R. "A Simple, Colorful Demonstration of Solubility and Acid/Base Extraction." *Journal of Chemical Education, 70* (1993): 848.

QUESTION

1. Caffeine has a distribution coefficient of 4.6 between methylene chloride and water. If 52 mg of caffeine are added to a conical vial containing 2 mL of water and 2 mL of methylene chloride, how much caffeine would be in each layer after the mixture had been mixed thoroughly?

4 **EXPERIMENT 4**

A Separation and Purification Scheme

Extraction

Crystallization

Devising a procedure

Critical-thinking application

There are many organic experiments in which the components of a mixture must be separated, isolated, and purified. Although detailed procedures are usually given for carrying this out, devising your own scheme can help you understand these techniques more thoroughly. In this experiment, you will devise a separation and purification scheme for a three-component mixture that will be assigned to you. The mixture will contain a neutral organic compound and either an organic acid or base in nearly equal amounts. The third component, also a neutral compound, will be present in a much smaller amount. Your goal will be to isolate in pure form *two* of the three compounds. The components of your mixture may be separated and purified by a combination of acid–base extractions and crystallizations. You will be told the composition of your mixture well in advance of the laboratory period so that you will have time to write a procedure for this experiment.

This experiment can be performed at two different scales. In Experiment 4A, the procedure calls for 1.0 g of the assigned mixture, and the extraction procedures are carried out with a separatory funnel. In Experiment 4B, the extraction procedures are performed with a centrifuge tube using 0.5 g of the assigned mixture. Your instructor will tell you which procedure to follow.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

New:	*Technique 11	Crystallization: Purification of Solids
	*Technique 12	Extractions, Separations, and Drying Agents

SUGGESTED WASTE DISPOSAL

Dispose of all filtrates that may contain 1, 4-dibromobenzene or methylene chloride into the container designated for halogenated organic wastes. All other filtrates may be disposed of into the container for nonhalogenated organic wastes.

Experiment 4 is based on a similar experiment developed by James Patterson, North Seattle Community College, Seattle.

NOTES TO THE INSTRUCTOR

Students must be told the composition of their mixture well in advance of the laboratory period so that they have enough time to devise a procedure. It is advisable to require that students turn in a copy of their procedure at the beginning of the lab period. You may wish to allow enough time for students to repeat the experiment if their procedure doesn't work the first time or if they want to improve on their percentage recovery and purity. If you allow enough time for students to perform this experiment just once, it will be helpful to put out pure samples of the compounds in the mixtures so students can try out different solvents to determine a good solvent for crystallizing each compound.

4A EXPERIMENT 4A

Extractions with a Separatory Funnel

PROCEDURE

Advanced Preparation. Each student will be assigned a mixture of three compounds.[1] Before coming to the laboratory, you must work out a detailed procedure that can be used to separate, isolate, and purify *two* of the compounds in your mixture. You may not be able to specify all the reagents or the volumes required ahead of time, but the procedure should be as complete as possible. It will be helpful to consult the following experiments and techniques:

Experiment 1, "Solubility," Part D

Experiment 3, "Extraction," Part D

Technique 10, Section 10.2B

Technique 12, Sections 12.9 and 12.11

The following reagents will be available: 1 *M* NaOH, 6 *M* NaOH, 1 *M* HCl, 6 *M* HCl, 1 *M* $NaHCO_3$, saturated sodium chloride, diethyl ether, 95% ethanol, methanol, isopropyl alcohol, acetone, hexane, toluene, methylene chloride, and anhydrous sodium sulfate. Other solvents that can be used for crystallization may also be available.

Separation. The first step in your procedure should be to dissolve about 1.0 g (record exact weight) of the mixture in the minimum amount of diethyl ether or methylene chloride. If more than about 10 mL of a solvent is required, you should use the other solvent. Most of the compounds in the mixtures are more soluble in methylene chloride than diethyl ether; however, you may need to determine the appropriate solvent by experimentation. Once you have selected a solvent, this same solvent should be used throughout the procedure when

[1]Your mixture may be one of the following: (1) 50% benzoic acid, 40% benzoin, 10% 1, 4-dibromobenzene; (2) 50% fluorene, 40% *o*-toluic acid, 10% 1, 4-dibromobenzene; (3) 50% phenanthrene, 40% methyl 4-aminobenzoate, 10% 1, 4-dibromobenzene; or (4) 50% 4-aminoacetophenone, 40% 1, 2, 4, 5-tetrachlorobenzene, 10% 1, 4-dibromobenzene. Other mixtures are given in the Instructor's Manual, along with some suggestions about these mixtures.

an organic solvent is required. If you use diethyl ether, you must use two steps to dry the organic layer. First, the organic layer must be mixed with saturated sodium chloride (see Technique 12, Section 12.9, Saturated Salt Solution), and then the liquid dried over anhydrous sodium sulfate (see Technique 12, Section 12.9, Drying Procedure with Anhydrous Sodium Sulfate). For all extraction procedures in this experiment, you should use a separatory funnel.

Purification. To improve the purity of your final samples, you should include a backwashing step at the appropriate place in your procedure. See Technique 12, Section 12.11 for a discussion of this method. Crystallization will most likely be required to purify both of the compounds you isolate. To find an appropriate solvent, you should consult a handbook. You can also use the procedure in Technique 11, Section 11.6 to determine a good solvent experimentally. Note that diethyl ether or other very low boiling solvents are not generally good solvents for performing a crystallization. If you use water as a solvent, you will need to let the crystals air-dry overnight. Your procedure should include at least one method for determining if you have obtained both compounds in a pure form. Hand in each compound in a labeled vial.

When performing the laboratory work, you should strive to obtain a high recovery of both compounds in a highly pure form. If your procedure fails, modify it and repeat the experiment.

REPORT

Write out a complete procedure by which you separated and isolated pure samples of two of the compounds in your mixture. Describe how you determined that your procedure was successful and give any data or results used for this purpose. Calculate the percentage recovery for both compounds.

4B EXPERIMENT 4B

Extractions with a Screw-Cap Centrifuge Tube

PROCEDURE

Follow the procedure given in Experiment 4A, except for the following changes in the "Separation" and "Purification" sections. Dissolve about 0.5 g of the assigned mixture in the minimum amount of diethyl ether or methylene chloride.[2] If more than about 4 mL of a solvent is required, you should use the other solvent. For all extraction procedures in this experiment, you should use a screw-cap centrifuge tube.

Remember that when you are removing one of the layers, you should always remove the bottom layer from the centrifuge tube.

[2]See footnote 1.

5 **EXPERIMENT 5**

Chromatography

Thin-layer chromatography

Column chromatography

Following a reaction with thin-layer chromatography

Chromatography is perhaps the most important technique used by organic chemists to separate the components of a mixture. This technique involves the distribution of the different compounds or ions in the mixture between two phases, one of which is stationary and the other moving. Chromatography works on much the same principle as solvent extraction. In extraction, the components of a mixture are distributed between two solvents according to their relative solubilities in the two solvents. The separation process in chromatography depends on differences in how strongly the components of the mixture are adsorbed to the stationary phase and how soluble they are in the moving phase. These differences depend primarily on the relative polarities of the components in the mixture.

There are many types of chromatographic techniques, ranging from thin-layer chromatography, which is relatively simple and inexpensive, to high-performance liquid chromatography, which is very sophisticated and expensive. In this experiment, you will use two of the most widely used chromatographic techniques: thin-layer and column chromatography. The purpose of this experiment is to give you practice in performing these two techniques, to illustrate the principles of chromatographic separations, and to demonstrate how thin-layer and column chromatography are used in organic chemistry.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

New:	*Technique 19	Column Chromatography
	Technique 20	Thin-Layer Chromatography

SPECIAL INSTRUCTIONS

Many flammable solvents are used in this experiment. Use Bunsen burners for making micropipets in a part of the lab that is separate from where the solvents are being used. The thin-layer chromatography should be performed in the hood.

SUGGESTED WASTE DISPOSAL

Dispose of methylene chloride in the container designated for halogenated organic wastes. Dispose of all other organic solvents in the container for nonhalogenated organic solvents. Place the alumina in the container designated for wet alumina.

NOTES TO THE INSTRUCTOR

The column chromatography should be performed with activated alumina from EM Science (No. AX0612-1). The particle sizes are 80–200 mesh, and the material is Type F-20. The alumina should be dried overnight in an oven at 110°C and stored in a tightly sealed bottle. Alumina more than several years old may need to be dried for a longer time at a higher temperature.

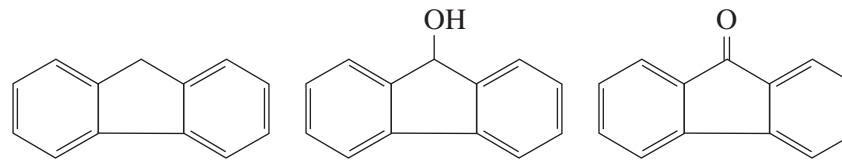
For thin-layer chromatography (TLC), use flexible silica-gel plates from Whatman with a fluorescent indicator (No. 4410 222). If the TLC plates have not been purchased recently, they should be placed in an oven at 100°C for 30 minutes and stored in a desiccator until used. If you use different alumina or different thin-layer plates, try out the experiment before using it with a class. Other materials than those specified here may give different results from those indicated in this experiment.

Grind up the fluorenone flakes into smaller pieces for easier dispensing. Commercially available fluorenol is often contaminated with fluorenone and fluorene, and fluorenone is often contaminated with fluorene. If iodine is used to visualize the spots in Part A, these contaminants will likely be invisible. However, if a UV lamp, which is more sensitive, is used, the contaminants will likely be visible. These compounds can be purified by crystallization (see Instructor's Manual), and the contaminants will then likely be invisible even when the spots are visualized under a UV lamp. It is best to use iodine to visualize the spots in Part C even if the fluorenone is pure. Since iodine is not as sensitive as a UV lamp, students will observe a more gradual change in the intensities of the spots for the two compounds when iodine is used.

5A EXPERIMENT 5A

Thin-Layer Chromatography

In this experiment, you will use thin-layer chromatography (TLC) to separate a mixture of three compounds: fluorene, fluorenol, and fluorenone:



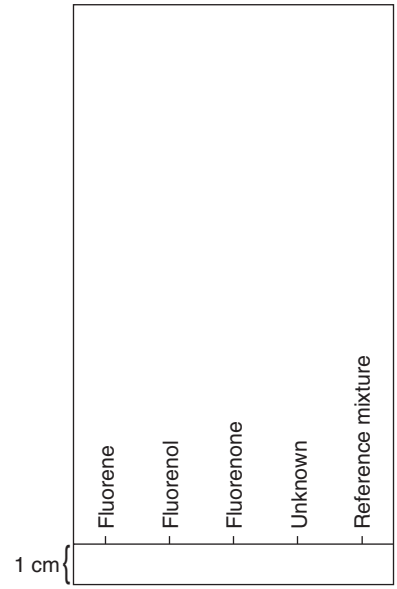
Fluorene Fluorenol Fluorenone

Based on the results with known samples of these compounds, you will determine which compounds are found in an unknown sample. Using TLC to identify the components in a sample is a common application of this technique.

PROCEDURE

Preparing the TLC Plate. Technique 20 describes the procedures used for thin-layer chromatography. Use a 10-cm 3 5.3-cm TLC plate (Whatman Silica-Gel Plates No. 4410 222). These plates have a flexible backing, but should not be bent excessively. They should be

handled carefully or the adsorbent may flake off them. Also, they should be handled only by the edges; the surface should not be touched. Using a lead pencil (not a pen), lightly draw a line across the plate (short dimension) about 1 cm from the bottom (see figure). Using a centimeter ruler, move its index about 0.6 cm in from the edge of the plate and lightly mark off five 1-cm intervals on the line. These are the points at which the samples will be spotted.



Prepare five micropipets to spot the plate. The preparation of these pipets is described and illustrated in Technique 20, Section 20.4. Prepare a TLC development chamber with methylene chloride (see Technique 20, Section 20.5). A beaker covered with aluminum foil or a wide-mouth, screw-cap bottle is a suitable container to use (see Technique 20, Figure 20.4). The backing on the TLC plates is thin, so if it touches the filter paper liner of the development chamber at any point, solvent will begin to diffuse onto the adsorbent surface at that point. To avoid this, be sure that the filter paper liner does not go completely around the inside of the container. A space about 2.5 inches wide must be provided. (*Note:* This development chamber will also be used for Parts C and D in this experiment.)

On the plate, starting from left to right, spot fluorene, fluorenol, fluorenone, the unknown mixture, and the standard reference mixture, which contains all three compounds.[1] For each of the five samples, use a different micropipet to spot the sample on the plate. The correct method of spotting a TLC plate is described in Technique 20, Section 20.4. Take up part of the sample in the pipet (don't use a bulb; capillary action will draw up the liquid). Apply the sample by touching the pipet *lightly* to the thin-layer plate. The spot should be no larger than 2 mm in diameter. It will usually be sufficient to spot each sample once or twice. If you need to spot the sample more than once, allow the solvent to evaporate completely between successive applications and spot the plate in exactly the same position each time. Save the samples in case you need to repeat the TLC.[2]

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[1]*Note to the instructor:* The individual compounds and the reference mixture containing all three compounds are prepared as 2% solutions in acetone. The unknown mixture may contain one, two, or all three of the compounds dissolved in acetone.

[2]After you have developed the plate and seen the spots, you will be able to tell if you need to rerun the TLC plate. If the spots are too faint to see clearly, you need to spot the sample more. If any of the spots show tailing (Technique 19, Section 19.12), then less sample is needed.

Developing the TLC Plate. Place the TLC plate in the development chamber, making sure that the plate does not come in contact with the filter paper liner. Remove the plate when the solvent front is 1–2 cm from the top of the plate. Using a lead pencil, mark the position of the solvent front. Set the plate on a piece of paper towel to dry. When the plate is dry, place the plate in a jar containing a few iodine crystals, cap the jar, and warm it *gently* on a hot plate until the spots begin to appear. Remove the plate from the jar and lightly outline all the spots that became visible with the iodine treatment. Using a ruler marked in millimeters, measure the distance that each spot has traveled relative to the solvent front. Calculate the R_f values for each spot (see Technique 20, Section 20.9). Explain the relative positions of the three compounds in terms of their polarities. Identify the compound or compounds that are found in the unknown mixture. If your instructor requests it, submit the TLC plate with your report.

5B EXPERIMENT 5B

Selecting the Correct Solvent for Thin-Layer Chromatography

In Experiment 5A, you were told what solvent to use for developing the TLC plate. In some experiments, however, it will be necessary to determine an appropriate development solvent by experimentation (see Technique 20, Section 20.6). In this experiment, you will be instructed to try three solvents for separating a pair of related compounds that differ slightly in polarity. Only one of these solvents will separate the two compounds enough so that they can be easily identified. For the other two solvents, you will be asked to explain, in terms of their polarities, why they failed.

PROCEDURE

Preparation. Your instructor will assign you a pair of compounds to run on TLC, or you will select your own pair.[3] You will need to obtain about 0.5 mL of three solutions: one solution of each of the two individual compounds and a solution containing both compounds. Prepare three thin-layer plates in the same way as you did in Experiment 5A, except that each plate should be 10-cm × 3.3-cm. When you mark them with a pencil for spotting, make three marks 1 cm apart. Prepare three micropipets to spot the plates. Prepare three TLC development chambers as you did in Experiment 5A, with each chamber containing one of the three solvents suggested for your pair of compounds.

Developing the TLC Plate. On each plate, spot the two individual compounds and the mixture of both compounds. For each of the three samples, use a different micropipet to spot the sample on the plates. Place each TLC plate in one of the three development cham-

[3]*Note to the instructor:* Possible pairs of compounds are given in the following list. The two compounds to be resolved are given first, followed by the three developing solvents to try: (1) benzoin and benzil; acetone, methylene chloride, hexane; (2) vanillin and vanillyl alcohol; acetone, 50% toluene–50% ethyl acetate, hexane; (3) diphenylmethanol and benzophenone; acetone, 70% hexane–30% acetone, hexane. Each compound in a pair should be prepared individually and as a mixture of the two compounds. Prepare all of them as 1% solutions in acetone.

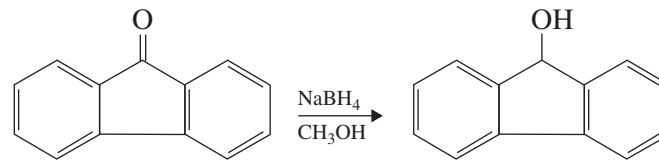
bers, making sure that the plate does not come in contact with the filter paper liner. Remove each plate when the solvent front is 1–2 cm from the top of the plate. Using a lead pencil, mark the position of the solvent front. Set the plate on a piece of paper towel to dry. When the plate is dry, observe it under a short-wavelength UV lamp, preferably in a darkened hood or a darkened room. With a pencil, lightly outline any spots that appear. Next, place the plate in a jar containing a few iodine crystals, cap the jar, and warm it *gently* on a hot plate until the spots begin to appear. Remove the plate from the jar and lightly outline all the spots that became visible with the iodine treatment. Using a ruler marked in millimeters, measure the distance that each spot has traveled relative to the solvent front. Calculate the R_f values for each spot. If your instructor requests it, submit the TLC plates with your report.

Which of the three solvents resolved the two compounds successfully? For the two solvents that did not work, explain, in terms of their polarities, why they failed.

5C **EXPERIMENT 5C**

Monitoring a Reaction with Thin-Layer Chromatography

Thin-layer chromatography is a convenient method for monitoring the progress of a reaction (see Technique 20, Section 20.10). This technique is especially useful when the appropriate reaction conditions have not yet been worked out. By using TLC to follow the disappearance of a reactant and the appearance of a product, it is relatively easy to decide when the reaction is complete. In this experiment, you will monitor the reduction of fluorenone to fluorenol:



Fluorenone **Fluorenol**

Although the appropriate reaction conditions for this reaction are already known, using TLC to monitor the reaction will demonstrate how to use this technique.

PROCEDURE

Preparation. Work with a partner on this part of the experiment. Prepare two thin-layer plates in the same way as you did in Part A, except that one plate should be 10-cm × 5.3-cm and the other one, 10-cm × 4.3-cm. When you mark them with a pencil for spotting, make five marks 1 cm apart on the first plate and four marks on the second plate. During the reaction, you will be taking five samples from the reaction mixture at 0, 15, 30, 60, and 120 seconds. Three of these samples should be spotted on the larger plate and two of them on the smaller one. In addition, each plate should be spotted with two reference solutions, one containing fluorenone and the other, fluorenol. Using a pencil to make very light marks, indicate at the top of each plate where each sample will be spotted so that you can keep track of them. Write the number of seconds and an abbreviation for the two reference compounds. Use the same TLC development chamber with methylene chloride that you used in Part A. Prepare seven micropipets to spot the plates.

Running the Reaction. Once sodium borohydride has been added to the reaction mixture (see next paragraph), take samples at the times just indicated. Because this must be done in such a short time, you must be well prepared before starting the reaction. One person should be the timekeeper, and the other person should take the samples and spot the plates. Spot each sample once, using a different pipet for each sample.

Place a magnetic stirring bar (Technique 7, Figure 7.8A or 7.8C) into a 25-mL Erlenmeyer flask. Add 0.40 g of fluorenone and 8 mL of methanol to the flask. Place the flask on a magnetic stirrer. Stir the mixture until all the solid has dissolved. Now take the first sample (the "0 second" sample) and spot the plate. Using smooth weighing paper, weigh 0.040 g of sodium borohydride and immediately add it to the reaction mixture.[4] If you wait too long to add it, the sodium borohydride will become sticky, as it absorbs moisture from the air. Begin timing the reaction as soon as the sodium borohydride is added. Use the micropipets to remove samples of the reaction mixture at the following times: 15, 30, 60, and 150 seconds. Use a different micropipet each time and spot a TLC plate with each sample. On each plate, also spot the two reference solutions of fluorenone and fluorenol in acetone. After developing the plates and allowing them to dry, visualize the spots with iodine, as described in Part A. Make a sketch of your plates and record the results in your notebook. Do these results indicate that the reaction went to completion? In addition to the TLC results, what other visible evidence indicated that the reaction went to completion? Explain.

Optional Exercise: Isolation of Fluorenol. Using a Pasteur pipet, transfer the reaction mixture to another 25-mL Erlenmeyer flask, leaving the magnetic stirring bar behind. Add 2 mL of water and heat the mixture almost to boiling for about 2 minutes. Allow the flask to cool slowly to room temperature in order to crystallize the product. Then place the flask in an ice-water bath for several minutes to complete crystallization. Collect the crystals by vacuum filtration, using a small Büchner funnel (see Technique 8, Section 8.3). Wash the crystals with three 2-mL portions of an ice-cold mixture of 80% methanol and 20% water. After the crystals are dry, weigh them and determine their melting point (literature, 153–154°C).

5D **EXPERIMENT 5D**

Column Chromatography

The principles of column chromatography are similar to those of thin-layer chromatography. The primary difference is that the moving phase in column chromatography travels downward, whereas in TLC the solvent ascends the plate. Column chromatography is used more often than TLC to separate relatively large amounts of compounds. With column chromatography, it is possible to collect pure samples of the separated compounds and perform additional tests on them.

In this experiment, fluorene and fluorenone will be separated by column chromatography using alumina as the adsorbent. Because fluorenone is more polar than fluorene, fluorenone will be adsorbed to the alumina more strongly. Fluorene will elute off the column with a nonpolar solvent hexane, whereas fluorenone will not come off until a more polar solvent (30% acetone–70% hexane) is put on the column. The purities of the two separated compounds will be tested by TLC and melting points.

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[4]Note to the instructor: The sodium borohydride should be checked to see whether it is active: Place a small amount of powdered material in some methanol and heat it gently. If the hydride is active, the solution should bubble vigorously. If using an old bottle, it is also good to check the material for stickiness due to absorption of water. If it is too sticky, it can be difficult for students to weigh it out.

PROCEDURE

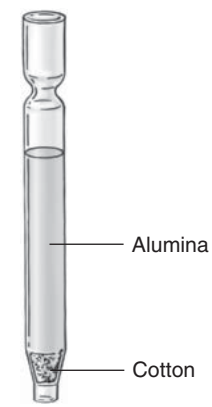
Advance Preparation. Before running the column, assemble the following glassware and liquids. Obtain four dry test tubes (16-mm × 100-mm) and number them 1 through 4. Prepare two dry Pasteur pipets with bulbs attached. Place 9.0 mL of hexane, 2.0 mL of acetone, and 2.0 mL of a solution of 70% hexane–30% acetone (by volume) into three Erlenmeyer flasks. Clearly label and stopper each flask. Place 0.3 mL of a solution containing fluorene and fluorenone into a small test tube.[5] Stopper the test tube. Prepare one 10-cm × 3.3-cm TLC plate with four marks for spotting. Use the same TLC development chamber with methylene chloride that you used in Part A. Prepare four micropipets to spot the plates.

Prepare a chromatography column packed with alumina. Place a loose plug of cotton in a Pasteur pipet (5¾-inch) and push it gently into position using a glass rod (see figure chromatography column, below for the correct position of the cotton). *Do not ram the cotton tightly, because this may result in the solvent flowing through the column too slowly.* Using a file, score the Pasteur pipet about 1 cm below the cotton plug. To break the tip off the pipet, put your thumbs together at the place on the pipet that you scored and push quickly with both thumbs.

CAUTION

Wear gloves or use a towel to protect your hands from being cut while breaking the pipet.

Add 1.25 g of alumina (EM Science, No. AX0612-1) to the pipet while tapping the column gently with your finger.[6] When all the alumina has been added, tap the column with your finger for several seconds to ensure that the alumina is tightly packed. Clamp the column in a vertical position so that the bottom of the column is just above the height of the test tubes you will be using to collect the fractions. Place test tube 1 under the column.



Chromatography column.

[5]*Note to the instructor:* This solution should be prepared for the entire class by dissolving 0.3 g of fluorene and 0.3 g of fluorenone in 9.0 mL of a mixture of 5% methylene chloride–95% hexane. Store this solution in a closed container to prevent evaporation of solvent. This will provide enough solution for 20 students, assuming little spillage or other types of waste.

[6]As an option, students may prepare a microfunnel from a 1-mL disposable plastic pipet. The microfunnel is prepared by (1) cutting the bulb in half with scissors, and (2) cutting the stem at an angle about ½ inch below the bulb. This funnel can be placed in the top of the column (Pasteur pipet) to aid in filling the column with alumina or with the solvents (see Technique 19, Section 19.6).

Running the Column. Using a Pasteur pipet, add 3 mL of hexane to the column. The column must be completely moistened by the solvent. Drain the excess hexane until the level of hexane reaches the top of the alumina. Once hexane has been added to the alumina, the top of the column must not be allowed to run dry. If necessary, add more hexane.

NOTE: It is essential that the liquid level not be allowed to drain below the surface of the alumina at any point in this procedure.

When the level of the hexane reaches the top of the alumina, add the solution of fluorene and fluorenone to the column using a Pasteur pipet. Begin collecting the eluent in test tube 2. Just as the solution penetrates the column, add 1 mL of hexane and drain until the surface of the liquid has reached the alumina. Add another 5 mL of hexane. As fluorene elutes off the column, some solvent will evaporate, leaving solid fluorene on the tip of the pipet. Using a Pasteur pipet, dissolve this solid off the column with a few drops of acetone. It may be necessary to do this several times, and the acetone solution is also collected in tube 2.

After you have added all the hexane, change to the more polar solvent (70% hexane–30% acetone).[7] When changing solvents, do not add the new solvent until the last solvent has nearly penetrated the alumina. The yellow band (fluorenone) should now move down the column. Just before the yellow band reaches the bottom of the column, place test tube 3 under the column. When the eluent becomes colorless again, place test tube 4 under the column and stop the procedure.

Tube 2 should contain fluorene and tube 3, fluorenone. Test the purities of these two samples using TLC. You must spot the solution from tube 2 several times in order to apply enough sample on the plate to be able to see the spots. On the plate, also spot the two reference solution containing fluorene and fluorenone. After developing the plate and allowing it to dry, visualize the spots with iodine. What do the TLC results indicate about the purities of the two samples?

Using a warm water bath (40–60°C) and a stream of nitrogen gas or air, evaporate the solvent from test tubes 2 and 3. As soon as all the solvent has evaporated from each of the tubes, remove them from the water bath. There may be a yellow oil in tube 3, but it should solidify when the tube cools to room temperature. If it does not, cool the tube in an ice-water bath and scratch the bottom of the test tube with a glass stirring rod or a spatula. Determine the melting points of the fluorene and fluorenone. The melting point of fluorene is 116–117°C and of fluorenone is 82–85°C.

REPORT

Experiment 5A

1. Calculate the R_f values for each spot. Include the actual plate or a sketch of the plate with your report.
2. Explain the relative R_f values for fluorene, fluorenol, and fluorenone in terms of their polarities and structures.
3. Give the composition of the unknown that you were assigned.

Experiment 5B

1. Record the names and structures of the two compounds that you ran on TLC.
2. Which solvent resolved the two compounds successfully?
3. For the other two solvents, explain, in terms of their polarities, why they failed.

[7]Sometimes the fluorenone also moves through the column with hexane. Therefore, be sure to change to test tube 3 if the yellow band starts to emerge from the column.

Experiment 5C

1. Make a sketch of the TLC plate or include the actual plate with your report. Interpret the results. When was the reaction complete?
2. What other visible evidence indicated that the reaction went to completion?
3. If you isolated the fluorenol, record the melting point and the weight of this product.

Experiment 5D

1. Describe the TLC results on the samples in test tubes 2 and 3. What does this indicate about the purities of the two samples?
2. Record the melting points for the dried solids found in tubes 2 and 3. What do they indicate about the purities of the two samples?

QUESTIONS

1. Each of the solvents given should effectively separate one of the following mixtures by TLC. Match the appropriate solvent with the mixture that you would expect to separate well with that solvent. Select your solvent from the following: hexane, methylene chloride, or acetone. You may need to look up the structures of solvents and compounds in a handbook.
   a. 2-Phenylethanol and acetophenone
   b. Bromobenzene and p-xylene
   c. Benzoic acid, 2, 4-dinitrobenzoic acid, and 2, 4, 6-trinitrobenzoic acid
2. The following questions relate to the column chromatography experiment performed in Experiment 5D.
   a. Why does the fluorene elute first from the column?
   b. Why was the solvent changed in the middle of the column procedure?
3. Consider the following errors that could be made when running TLC. Indicate what should be done to correct the error.
   a. A two-component mixture containing 1-octene and 1, 4-dimethylbenzene gave only one spot with an R_f value of 0.95. The solvent used was acetone.
   b. A two-component mixture containing a dicarboxylic acid and tricarboxylic acid gave only one spot with an R_f value of 0.05. The solvent used was hexane.
   c. When a TLC plate was developed, the solvent front ran off the top of the plate.

6 EXPERIMENT 6

Simple and Fractional Distillation

Simple distillation

Fractional distillation

Gas chromatography

Distillation is a technique frequently used to separate and purify a liquid component from a mixture. Simply stated, distillation involves heating a liquid mixture to its boiling point, where liquid is rapidly converted to vapor. The vapors, richer in the more volatile component, are then condensed into a separate container. When

Experiment 6 is based on a similar one developed by James Patterson, North Seattle Community College, Seattle.

the components in the mixture have sufficiently different vapor pressures (or boiling points), they can be separated by distillation.

The purpose of this experiment is to illustrate the use of distillation for separating a mixture of two volatile liquids with different boiling points. Each mixture, which will be issued as an unknown, will consist of two liquids from the following table.

Compound	Boiling Point (°C)
Hexane	69
Cyclohexane	80.7
Heptane	98.4
Toluene	110.6
Ethylbenzene	136

The liquids in the mixture will be separated by two different distillation techniques: simple and fractional distillation. The results of these two methods will be compared by analyzing the composition of the **distillate** (the distilled liquid) using gas chromatography. You will also construct a graph of the distillation temperature versus the total volume of distillate collected. This graph will allow you to determine the approximate boiling points of the two liquids and to make a graphic comparison of the two different distillation methods.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

New:	*Technique 14	Simple Distillation
	*Technique 15	Fractional Distillation, Azeotropes
	Technique 22	Gas Chromatography

SPECIAL INSTRUCTIONS

Many flammable solvents are used in this experiment; therefore, do not use any flames in the laboratory.

Work in pairs on this experiment. Each pair of students will be assigned an unknown containing two liquids found in the previous table. One student in the pair should perform a simple distillation and the other student, a fractional distillation. The results from these two methods will be compared.

SUGGESTED WASTE DISPOSAL

Dispose of all organic liquids in the container for nonhalogenated organic solvents.

NOTES TO THE INSTRUCTOR

The apparatus for the fractional distillation procedure should be insulated as described in the procedure; otherwise, heat loss may make it impossible to complete the distillation. The most convenient way to measure the temperature during the distillation is to use a Vernier LabPro interface with a laptop computer and stainless steel temperature probe (or thermocouple). See the Instructor's Manual for additional comments about suitable temperature probes for this experiment. If you use the Vernier LabPro interface, you will need to give students instructions on how to use this. If a thermometer is used, the temperature will be most accurate if a partial immersion mercury thermometer is used. See the Instructor's Manual for additional comments about the use of other kinds of thermometers in this experiment.

Prepare unknown mixtures consisting of the following pairs of liquids: hexane–heptane, hexane–toluene, cyclohexane–toluene, and heptane–ethylbenzene. For each mixture, use an equal volume of both liquids. Distillation of these mixtures should provide a good contrast between the two distillation methods. It is important that you read the Instructor's Manual for helpful hints about these mixtures. You should try out the experimental setup that the students will be using with the heptane-ethylbenzene mixture to make sure that the heating device will get hot enough to distill ethylbenzene in a reasonable amount of time.

Unless the samples are analyzed by gas chromatography immediately after the distillation, it is essential that the samples be stored in leak-proof vials. We have found GC-MS vials to be ideal for this purpose.

The gas chromatograph is prepared as follows: column temperature, 140°C; injection temperature, 150°C; detector temperature, 140°C; carrier gas flow rate, 100 mL/min. The recommended column is 8 feet long with a stationary phase such as Carbowax 20M.

You should determine retention times and response factors for the five compounds given in the table provided at the beginning of this experiment. Because the data in this experiment are expressed as volume, the response factors should also be based on volume. Inject a mixture containing equal volumes of all five compounds and determine the relative peak areas. Choose one compound as the standard and define its response factor to be equal to 1.00. Calculate the other response factors based on this reference. Typical response factors are given in footnote 2.

PROCEDURE

You should work in pairs on this experiment. Each pair of students will be assigned an unknown mixture containing equal volumes of two of the liquids from the table shown at the beginning of the experiment on p. One student should perform a simple distillation on the mixture, and the other should perform a fractional distillation.

Apparatus. If you are performing a simple distillation, assemble the apparatus shown in Figure 14.1. If performing a fractional distillation, assemble the apparatus shown in Technique 15, Figure 15.2. In each apparatus, use a 50-mL round-bottom flask as the distilling flask and replace the receiving flask with a 25-mL graduated cylinder. It will be easier to assemble the apparatus in a secure manner if you use plastic joint clips (see Technique 7, Section 7.1, Part A). Carefully note the position of the thermometer in Technique 14, Figure 14.1 and Technique 15,

Figure 15.2. The bulb of the thermometer or the bottom of the temperature probe must be placed below the sidearm, or it will not read the temperature correctly.

If performing the fractional distillation, pack the fractionating column (condenser with the larger inner diameter) with 3.6 g of stainless steel cleaning-pad material. The easiest way to pack the column is to cut several strands of the cleaning pad with the correct weight. Using a long wire with a bent end, pull the cleaning pad through the condenser. After releasing the long wire, use a metal spatula or glass stirring rod to adjust the position of the packing. Do not pack the material too tightly at any one place in the condenser.

CAUTION

You should wear heavy cotton gloves when handling the stainless steel cleaning pad. The edges are very sharp and can easily cut into the skin.

Insulate both the fractionating column and the distilling head by wrapping them with a single thickness of cotton pad. Hold the cotton pad in place by completely wrapping it with aluminum foil (shiny side in).

For either the simple or fractional distillation, place several boiling stones into the 50-mL round-bottom flask. Also add 28.0 mL of the unknown mixture (measured with a graduated cylinder) to the flask. Use a heating mantle for heating.

Distillation. These instructions apply to both the simple and fractional distillations. Start circulating the cooling water in the condenser and adjust the heat so that the liquid boils rapidly. During the initial stages of the distillation, continue to maintain a rapid boiling rate. As the hot vapors rise, they will gradually heat up the glassware and, in the case of the fractional distillation, the fractionating column as well. Because the mass of glass and other materials is fairly large, it will take 10–20 minutes of heating before the distillation temperature begins to rise rapidly and approaches the boiling point of the distillate. (Note that this may take longer for the fractional distillation.) When the temperature begins to level off, you should soon see drops of distillate falling into the graduated cylinder.

NOTE: For the remainder of the distillation, it is very important to regulate the temperature of the heating mantle so that the distillation occurs at a rate of about 1 drop per 2 seconds. If the distillation is performed more rapidly than this, you may not achieve good separation between the liquids.

Now you may need to turn down the heat control to achieve the desired rate of distillation. In addition, it may be helpful to lower the heating mantle slightly below the round-bottom flask for a minute or so to cool the mixture more quickly. You should also begin recording the distillation temperature as a function of the total volume of distillate collected. If you are using a temperature probe with the Vernier LabPro interface, you will need to hit the "Start Collecting" button on the screen and the temperature will be monitored by the computer. Beginning at a volume of 1.0 mL, record the temperature at every 1.0-mL interval, as determined by the volume of distillate in the 25-mL graduated cylinder. After you have collected 4 mL of distillate, remove the graduated cylinder and collect the next few drops of distillate in a small leak-proof vial.[1] Label the vial "4-mL sample." Cap the vial tightly; otherwise, the more volatile component will evaporate more rapidly, and the composition of the mixture will change. Resume collecting the distillate in the graduated cylinder. As the distillation temperature increases, you may need to turn up the heat control to maintain the same rate of

[1]We have found GC-MS vials ideal for this purpose.

distillation. After the first component has distilled, it is possible that the distilling temperature will drop significantly. Continue to record the temperature and volume data. When you have collected a total of 20 mL of distillate, take another small sample of distillate in a second small vial. (If the total volume of distillate that you can collect is less than 20 mL, take the last few drops as your second sample.) Cap the vial and label it "20-mL sample." Then continue the distillation until there is a small amount (about 1.0 mL) of liquid remaining in the distilling flask.

NOTE

Do not distill to dryness! A dry flask may crack if it is heated too hot.

The best way to stop the distillation is to turn off the heat and immediately lower the heating mantle.

ANALYSIS

Distillation Curve. Using the data you collected for the distillation temperature and the total volume of distillate, construct separate graphs for the simple and fractional distillations. Plot the volume in 1.0-mL increments on the *x*-axis and the temperature on the *y*-axis. Comparing the two graphs should make clear that the fractional distillation resulted in a better separation of the two liquids. Using the graph for the fractional distillation, estimate the boiling points of the two components in your mixture by noting the two regions on the graph where the temperatures leveled off. From these approximate boiling points, try to identify the two liquids in your mixture (see table shown at the beginning of this experiment). Note that the observed boiling point for the first component may be somewhat higher than the actual boiling point, and the observed boiling point for the second component may be somewhat lower than the actual boiling point. The reason is that the fractionating column may not be efficient enough to completely separate all of the pairs of liquids in this experiment. Therefore, it may be easier to identify the two liquids in your mixture from the gas chromatograph, as described in the next section.

Gas Chromatography. Gas chromatography is an instrumental method that separates the components of a mixture based on their boiling points. The lower-boiling component passes through the column first, followed by the higher-boiling components. The actual length of time required for a compound to pass through the column is called the **retention time** of that compound. As each component comes off the column, it is detected, and a peak is recorded that is proportional in size to the amount of the compound that was put on the column.

Gas chromatography can be used to determine the compositions of the two samples that you collected in the small vials. The instructor or a laboratory assistant may either make the sample injections or allow you to make them. In the latter case, your instructor will give you adequate instructions beforehand. A reasonable sample size is 2.5μL. Inject the sample into the gas chromatograph and record the gas chromatogram. Depending on how effectively the two compounds were separated by the distillation, you may see one or two peaks. The lower-boiling component has a shorter retention time than the higher-boiling one. Your instructor will provide you with the actual retention times for each compound so that you can identify the compound in each peak. This will enable you to identify the two liquids in your mixture.

Once the gas chromatogram has been obtained, determine the relative areas of the two peaks (see Technique 22, Section 22.11). You can calculate this by triangulation, or the instrument may do this electronically. In either case, you should divide each area by a response factor to account for differences in how the detector responds to the different compounds.[2] Calculate the percentages of the two compounds in both samples. Compare these results for the simple and fractional distillations.

REPORT

Distillation Curve

Record the data for the distillation temperature as a function of the volume of distillate. Construct a graph for these data (see "Analysis," above). Compare the graphs for simple and fractional distillations of the same mixture. Which distillation resulted in a better separation? Explain. Report the approximate boiling points for the two compounds in your mixture and identify the compounds, if possible.

Gas Chromatography

For both the 4-mL sample and the 20-mL sample, determine the relative areas of the two peaks, unless there is only one peak. Divide the areas by the appropriate response factors and calculate the percentage composition of the two compounds in each sample. Compare these results for the simple and fractional distillations of the same mixture. Which distillation resulted in a better separation? Explain. Identify the two compounds in your mixture. If your instructor requests it, turn in the gas chromatograms with your report.

7 EXPERIMENT 7

Infrared Spectroscopy and Boiling-Point Determination

Infrared spectroscopy

Boiling-point determination

Organic nomenclature

Critical-thinking application

The ability to identify organic compounds is an important skill that is frequently used in the laboratory. Although there are several spectroscopic methods and many chemical and physical tests that can be used for identification, the goal of this experiment is to identify an unknown liquid using infrared spectroscopy and a boiling-point determination. Both methods are introduced in this experiment.

[2]Because response factors are instrument specific, you will be given the response factors for your instrument. Typical response factors obtained on a GowMac 69-350 gas chromatograph are hexane (1.50), cyclohexane (1.80), heptane (1.63), toluene (1.41), and ethylbenzene (1.00). These response factors were determined by injecting a mixture of equal volumes of the five liquids and determining the relative peak areas.

REQUIRED READING

New:	Technique 4	How to Find Data for Compounds: Handbooks and Catalogs
	Technique 13	Physical Constants of Liquids: The Boiling Point and Density, Part A. "Boiling Points and Thermometer Correction"
	Technique 25	Infrared Spectroscopy

SPECIAL INSTRUCTIONS

Many of the unknown liquids used for this experiment are flammable; therefore, do not use any flames in the laboratory. Also be careful when handling all of the liquids because many of them are potentially toxic.

This experiment can be performed individually with each student working on one unknown. However, the opportunity to learn is greater if students work in groups of three. In this case, each group is assigned three different unknowns. Each student in the group obtains an infrared spectrum and performs a boiling-point determination on one of the unknowns. Subsequently, the student shares this information with the other two students in the group. Then each student analyzes the collective results for the three unknowns and writes a laboratory report based on all three unknowns. Your instructor will inform you whether you should work alone or in groups.

SUGGESTED WASTE DISPOSAL

If you have not identified the unknown by the end of the laboratory period, you should return the unknown liquid to your instructor in the original container in which it was issued to you. If you have identified the compound, dispose of it in either the container for halogenated waste or the one for nonhalogenated waste, whichever is appropriate.

NOTES TO THE INSTRUCTOR

If you choose to have students work in groups of three, be sure to assign unknowns that differ both in structure *and* functional group, with at least one aromatic compound in each set. If the experiment is performed early in the year, students may have some difficulty in finding the structures of the compounds that are in the list of possible unknowns and may need help. For each unknown, compounds with boiling points as much as 5°C higher than the experimental boiling point should be considered, because student-determined boiling points are frequently low. This will depend on the method used and the skill of the person performing the technique. *The Merck Index*, the *CRC Handbook of Chemistry and Physics*, and the lecture textbook can all be helpful in determining these structures. Technique 4, "How to Find Data for Compounds: Handbooks and Catalogs," provides helpful information for students just beginning to use handbooks. The nuclear magnetic resonance (NMR) portion of the experiment is optional.

We suggest that access to the NMR be granted only after a plausible solution has been tendered. If you do not have an NMR, there are several online databases where you can obtain a printed copy of the spectrum to hand to students.

The best way to perform the boiling-point determination is to use a Vernier LabPro interface with a laptop computer and stainless-steel temperature probe (or thermocouple). See the Instructor's Manual for additional comments about suitable temperature probes for this experiment. If you use the Vernier LabPro interface, you will need to give students instructions on how to use this. If a thermometer is used, the results will be more accurate with a partial immersion mercury thermometer than with nonmercury ones. If you use a partial immersion mercury thermometer, you do not need to perform a stem correction.

PROCEDURE

Part A. Infrared Spectrum

Obtain the infrared spectrum of your unknown liquid (see Technique 25, Section 25.2). If you are working in a group, provide copies of your spectrum for everyone in your group. Identify the significant absorption peaks by labeling them *right on the spectrum* and include the spectrum in your laboratory report. Absorption peaks corresponding to the following groups should be identified:

C—H (SP^3)

C—H (SP^2)

C—H (aldehyde)

O—H

C=O

C=C (aromatic)

aromatic substitution pattern

C—O

C—X (if applicable)

N—H

Part B. Boiling-Point Determination

Perform a boiling-point determination on your unknown liquid (see Technique 13, Section 13.2). Your instructor will indicate which method to use. Depending on the method used and the skill of the person performing the technique, boiling points can sometimes be slightly inaccurate. When experimental boiling points are inaccurate, it is more common for them to be lower than the literature value. The difference may be as much as 5°C, especially for higher-boiling liquids and if you use a non-mercury thermometer. If you use a Vernier LabPro interface with a stainless-steel temperature probe or a partial immersion mercury thermometer, the results should be within 1–2°C. Your instructor may be able to give you more guidance about what level of accuracy you can expect.

Part C. Analysis and Report

Using the structural information from the infrared spectrum and the boiling point of your unknown, identify this liquid from the list of compounds given in the table included with this experiment. If you are working in a group, you will need to do this for all three compounds. In order to make use of the structural information determined from the infrared spectrum, you will need to know the structures of the compounds that have boiling points close to the value you experimentally determined. You may need to consult *The Merck Index* or the

CRC Handbook of Chemistry and Physics. It may also be helpful to look up these compounds in the index of your lecture textbook. If there is more than one compound that fits the infrared spectrum and is within a few degrees of the experimental boiling point, you should list all of them in your laboratory report.

In your laboratory report, include (1) the infrared spectrum with the significant absorption peaks identified *right on the spectrum,* (2) the experimental boiling point for your unknown, and (3) your identification of the unknown. Explain your justifications for making this identification and write out the structure of this compound.

Optional Exercise: NMR Spectrum. Your instructor may ask you to determine the nuclear magnetic resonance spectrum of your unknown liquid (see Technique 26, Section 26.1). Alternatively, your instructor may issue you a previously-run spectrum of your compound. You should provide structural assignments for all of the groups of hydrogens that are present. Do this *right on the spectrum.* If you have correctly determined the identity of your unknown, all the groups of hydrogens (and their chemical shifts) should fit your structure. Include the properly labeled spectrum in your report and explain why it fits the suggested structure.

List of Possible Unknown Liquids

Compound	BP (°C)	Compound	BP (°C)
acetone	56	butyl acetate	127
2-methylpentane	62	2-hexanone	128
sec-butylamine	63	morpholine	129
isobutyraldehyde	64	3-methyl-1-butanol	130
methanol	65	hexanal	130
isobutylamine	69	chlorobenzene	132
hexane	69	2, 4-pentanedione	134
vinyl acetate	72	cyclohexylamine	135
1, 3, 5-trifluorobenzene	75	ethylbenzene	136
butanal	75	*p*-xylene	138
ethyl acetate	77	1-pentanol	138
butylamine	78	propionic acid	141
ethanol	78	pentyl acetate	142
2-butanone	80	4-heptanone	144
cyclohexane	81	2-ethyl-1-butanol	146
isopropyl alcohol	82	*N*-methylcyclohexylamine	148
cyclohexene	83	2, 2, 2-trichloroethanol	151
isopropyl acetate	85	2-heptanone	151
triethylamine	89	heptanal	153
3-methylbutanal	92	isobutyric acid	154
3-methyl-2-butanone	94	bromobenzene	156
1-propanol	97	cyclohexanone	156
heptane	98	dibutylamine	159
tert-butyl acetate	98	cyclohexanol	160
2, 2, 4-trimethylpentane	99	butyric acid	162
2-butanol	99	furfural	162
formic acid	101	diisobutyl ketone	168

Compound	BP (°C)	Compound	BP (°C)
2-pentanone	101	furfuryl alcohol	170
2-methyl-2-butanol	102	octanal	171
pentanal	102	decane	174
3-pentanone	102	isovaleric acid	176
propyl acetate	102	limonene	176
piperidine	106	1-heptanol	176
2-methyl-1-propanol	108	benzaldehyde	179
1-methylcyclohexene	110	cycloheptanone	181
toluene	111	1,4-diethylbenzene	184
sec-butyl acetate	111	iodobenzene	186
pyridine	115	1-octanol	195
4-methyl-2-pentanone	117	methyl benzoate	199
2-ethylbutanal	117	methyl phenyl ketone	202
methyl 3-methylbutanoate	117	benzyl alcohol	204
acetic acid	118	4-methylbenzaldehyde	204
1-butanol	118	ethyl benzoate	212
octane	126		

ESSAY

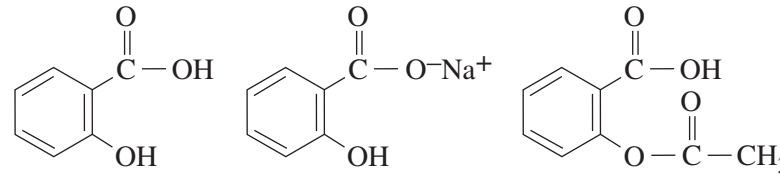
Aspirin

Aspirin is one of the most popular cure-alls available today. It is a powerful **analgesic** (relieves pain), **antipyretic** (reduces fever), **anti-inflammatory** (reduces swelling), and **antiplatelet** (slows blood-clotting) drug. Although its history as a modern medicine began only a little over a century ago, its medicinal origins actually lie in folk remedies, some of which were recognized as early as 3000 BC. Early Greek, Roman, Egyptian, Babylonian, and Chinese medical treatises recognized the ability of extracts of the willow and other salicylate-containing plants, such as meadowsweet and myrtle, to alleviate fever, pain, and inflammation. The use of meadowsweet extracts was common throughout the Middle Ages. Aspirin first appeared as a commercially available tablet in 1899. By the late 1950s, over 15 billion tablets were consumed each year. The commercial introduction of acetaminophen (Tylenol) in 1956 and of ibuprofen in 1962 caused a temporary decline in the use of aspirin. However, new uses have been found for the drug in treating heart disease ("baby aspirin"), and its popularity remains strong. Since it was first made available to the general public, it is estimated that over a trillion aspirin tablets have been consumed by patients seeking relief.

The modern history of aspirin began on June 2, 1763, when Edward Stone, a clergyman, read a paper to the Royal Society of London entitled, "An Account of the Success of the Bark of Willow in the Cure of Agues." By *ague,* Stone was referring to what we now call malaria, but his use of the word *cure* was optimistic; what his extract of willow bark actually did was to dramatically reduce the feverish symptoms of the disease. He was promoting his new malaria cure as a substitute for "Peruvian Bark," an imported and expensive remedy, which we now know

contains the drug quinine. Almost a century later, a Scottish physician found that Stone's extract could also relieve the symptoms of acute rheumatism.

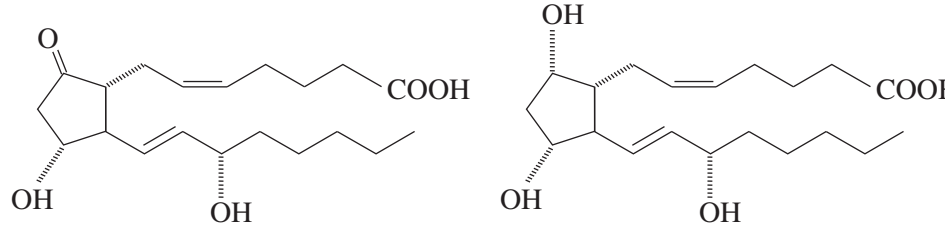
Soon thereafter, organic chemists working with willow bark extract and flowers of the meadowsweet plant (which gave a similar compound) isolated and identified the active ingredient as salicylic acid (from *salix,* the Latin name for the willow tree). The substance could then be chemically produced in large quantities for medical use. It soon became apparent that using salicylic acid as a remedy was severely limited by its acidic properties. The substance irritated the mucous membranes lining the mouth, esophagus, and stomach. The first attempts to circumvent this problem by using the less acidic sodium salt (sodium salicylate) were only partially successful. This substance was less irritating, but had such an objectionable sweetish taste that most people could not be induced to take it. The breakthrough came at the turn of the century (1893) when Felix Hofmann, a young chemist working for the German company Bayer, devised a practical route for synthesizing acetylsalicylic acid, which was found to have all the same medicinal properties without the highly objectionable taste or the high degree of mucosal-membrane irritation. Bayer called its new product "aspirin," a name derived from *a-* for acetyl and the root *-spir,* from the Latin name for the meadowsweet plant, *spirea.*



Salicylic acid **Sodium salicylate** **Acetylsalicylic acid (Aspirin)**

The history of aspirin is typical of many of the medicinal substances in current use. Many began as crude plant extracts or folk remedies, the active ingredients of which were isolated and their structure determined by chemists, who then improved on the original.

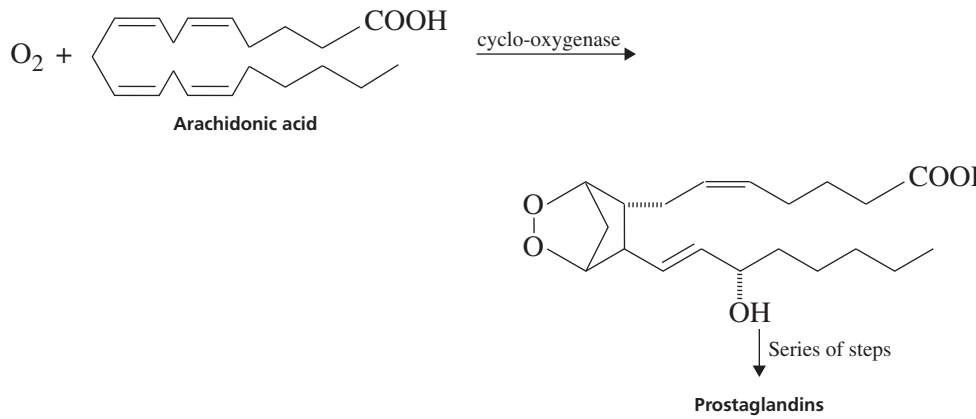
Through the research of J. R. Vane and others in the 1970s, aspirin's mode of action has largely been explained. A whole new class of compounds, called **prostaglandins**, has been found to be involved in the body's immune responses. Their synthesis is provoked by interference with the body's normal functioning by foreign substances or unaccustomed stimuli.



Prostaglandin E_2 **Prostaglandin $F_{2\alpha}$**

These substances are involved in a wide variety of physiological processes and are thought to be responsible for evoking pain, fever, and local inflammation. Aspirin has recently been shown to prevent bodily synthesis of prostaglandins and thus to alleviate the symptomatic portion (fever, pain, inflammation, menstrual cramps) of the body's immune responses (that is, the ones that let you know

something is wrong). Research suggests that aspirin may inactivate one of the enzymes responsible for the synthesis of prostaglandins. The natural precursor for prostaglandin synthesis is **arachidonic acid**. This substance is converted to a peroxide intermediate by an enzyme called **cyclo-oxygenase**, or prostaglandin synthase. This intermediate is converted further to prostaglandin. The apparent role of aspirin is to attach an acetyl group to the active site of cyclo-oxygenase, thus rendering it unable to convert arachidonic acid to the peroxide intermediate. In this way, prostaglandin synthesis is blocked.



Aspirin tablets (5-grain size) are usually compounded of about 0.32 g of acetylsalicylic acid pressed together with a small amount of starch, which binds the ingredients. Buffered aspirin usually contains a basic buffering agent to reduce the acidic irritation of mucous membranes in the stomach, because the acetylated product is not totally free of this irritating effect. Bufferin contains 0.325 g of aspirin together with calcium carbonate, magnesium oxide, and magnesium carbonate as buffering agents. Combination pain relievers usually contain aspirin, acetaminophen, and caffeine. Extra-Strength Excedrin, for instance, contains 0.250 g aspirin, 0.250 g acetaminophen, and 0.065 g caffeine.

In the late 1980s scientists discovered that small daily doses of aspirin were effective in reducing the risk of blood-clotting diseases. "Baby aspirin" tablets contain about 25% (0.082 g) of the amount of acetylsalicylic acid that is contained in a regular aspirin tablet. These small tablets are often prescribed to survivors of heart attacks and strokes to prevent a reoccurrence. As an antiplatelet drug, aspirin prevents tiny red blood cells (platelets) from clumping together or clotting. Clotting in arteries can initiate the events that lead to arteriosclerosis. If blood clots block arteries or break loose and travel to the heart or the brain, heart attacks and strokes can occur.

Some persons are allergic to aspirin and cannot tolerate it or other salicylate-based medicines. In other people, aspirin may cause gastric irritation or ulcers and bleeding in the stomach. For this reason, doctors often prefer to prescribe acetaminophen (Tylenol). When treating the children, aspirin should also be avoided in favor of Tylenol, due to known link between aspirin consumption and Reye's Syndrome, a disease which can be fatal, however, acetaminophen does not have any antiplatelet activity and cannot prevent or deter clotting diseases in susceptible adults. Finally, with some diseases, aspirin simply provides superior relief of pain and inflammation and is preferred over any of the newer analgesics. Following its decline in the mid-twentieth century, aspirin has undergone a resurgence and is once again a top seller in the analgesic marketplace.

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8 **EXPERIMENT 8**

Acetylsalicylic Acid

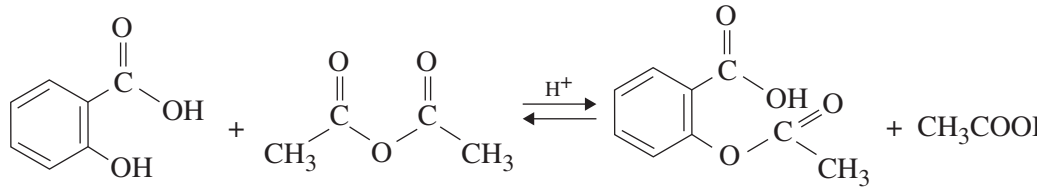
Crystallization

Vacuum filtration

Melting point

Esterification

Aspirin (acetylsalicylic acid) can be prepared by the reaction between salicylic acid and acetic anhydride:



Salicylic acid **Acetic anhydride** **Acetylsalicylic acid** **Acetic acid**

In this reaction, the **hydroxyl group** (—OH) on the benzene ring in salicylic acid reacts with acetic anhydride to form an **ester** functional group. Thus, the formation of acetylsalicylic acid is referred to as an **esterification** reaction. This reaction requires the presence of an acid catalyst, indicated by the H^+ above the equilibrium arrows.

When the reaction is complete, some unreacted salicylic acid and acetic anhydride will be present along with acetylsalicylic acid, acetic acid, and the catalyst. The technique used to purify the acetylsalicylic acid from the other substances is called **crystallization**. The basic principle is quite simple. At the end of this reaction, the reaction mixture will be hot, and all substances will be in solution. As the solution is allowed to cool, the solubility of acetylsalicylic acid will decrease, and it will gradually come out of solution, or crystallize. Because the other substances are either liquids at room temperature or are present in much smaller amounts, the crystals formed will be composed mainly of acetylsalicylic acid. Thus, a separation of acetylsalicylic acid from the other materials will have largely been accomplished. The purification process is facilitated by the addition of water after the crystals have formed. The water decreases the solubility of acetylsalicylic acid and dissolves some of the impurities.

To purify the product even more, a recrystallization procedure will also be performed. In order to prevent the decomposition of acetylsalicylic acid, ethyl acetate, rather than water, will be used as the solvent for recrystallization.

The most likely impurity in the product after purification is salicylic acid itself, which can arise from incomplete reaction of the starting materials or from **hydrolysis** (reaction with water) of the product during the isolation steps. The hydrolysis reaction of acetylsalicylic acid produces salicylic acid. Salicylic acid and other compounds that contain a hydroxyl group on the benzene ring are referred to as **phenols**. Phenols form a highly colored complex with ferric chloride (Fe^{3+} ion). Aspirin is not a phenol, because it does not possess a hydroxyl group directly attached to the benzene ring. Because aspirin will not give the color reaction with ferric chloride, the presence of salicylic acid in the final product is easily detected. The purity of your product will also be determined by obtaining the melting point.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	*Technique 8	Filtration, Sections 8.1–8.6
	*Technique 9	Physical Constants of Solids: The Melting Point
New:	Technique 5	Measurement of Volume and Weight
	Technique 6	Heating and Cooling Methods
	*Technique 7	Reaction Methods, Sections 7.1, 7.4–7.6
	*Technique 11	Crystallization: Purification of Solids
	Essay	Aspirin

SPECIAL INSTRUCTIONS

This experiment involves concentrated sulfuric acid, which is highly corrosive. It will cause burns if it is spilled on the skin. Exercise care in handling it.

SUGGESTED WASTE DISPOSAL

Dispose of the aqueous filtrate in the container for aqueous waste. The filtrate from the recrystallization in ethyl acetate should be disposed of in the container for nonhalogenated organic waste.

PROCEDURE

Preparation of Acetylsalicylic Acid (Aspirin). Weigh 2.0 g of salicyclic acid (*MW* = 138.1) and place this in a 125-mL Erlenmeyer flask. Add 5.0 mL of acetic anhydride (*MW* = 102.1, *d* = 1.08 g/ml), followed by 5 drops of concentrated sulfuric acid, and swirl the flask gently until

CAUTION

Concentrated sulfuric acid is highly corrosive. You must handle it with great care.

the salicylic acid dissolves. Heat the flask gently on the steam bath or in a hot-water bath at about 50°C (see Technique 6, Figure 6.4) for at least 10 minutes. Allow the flask to cool to room temperature, during which time the acetylsalicylic acid should begin to crystallize from the reaction mixture. If it does not, scratch the walls of the flask with a glass rod and cool the mixture slightly in an ice bath until crystallization has occurred. After crystal formation is complete (usually when the product appears as a solid mass), add 50 mL of water and cool the mixture in an ice bath.

Vacuum Filtration. Collect the product by vacuum filtration on a Büchner funnel (see Technique 8, Section 8.3, and Figure 8.5). A small amount of additional cold water can be used to aid in the transfer of crystals to the funnel. Rinse the crystals several times with small portions of cold water. Continue drawing air through the crystals on the Büchner funnel by suction until the crystals are free of solvent (5–10 minutes). Remove the crystals for air drying. Weigh the crude product, which may contain some unreacted salicylic acid, and calculate the percentage yield of crude acetylsalicylic acid (*MW* = 180.2).

Ferric Chloride Test for Purity. You can perform this test on a sample of your product that is not completely dry. To determine if there is any salicylic acid remaining in your product, carry out the following procedure. Obtain three small test tubes. Add 0.5 mL of water to each test tube. Dissolve a small amount of salicylic acid in the first tube. Add a similar amount of your product to the second tube. The third test tube, which contains only solvent, will serve as the control. Add 1 drop of 1% ferric chloride solution to each tube and note the color after shaking. Formation of an iron–phenol complex with Fe(III) gives a definite color ranging from red to violet, depending on the particular phenol present.

Optional Exercise: Recrystallization.[1] Water is not a suitable solvent for crystallization because aspirin will partially decompose when heated in water. Follow the general instructions described in Technique 11, Section 11.3, and Figure 11.4. Dissolve the product in a minimum

[1]Crystallization is not necessary. The crude product is quite pure and is sometimes degraded by the crystallization (as judged by $FeCl_3$).

amount of hot ethyl acetate (no more than 2–3 mL) in a 25-mL Erlenmeyer flask, while gently and continuously heating the mixture on a steam bath or a hot plate.[2]

When the mixture cools to room temperature, the aspirin should crystallize. If it does not, evaporate some of the ethyl acetate solvent to concentrate the solution and cool the solution in ice water while scratching the inside of the flask with a glass rod (not a fire-polished one). Collect the product by vacuum filtration, using a Büchner funnel. Any remaining material can be rinsed out of the flask with a few milliliters of cold petroleum ether. Dispose of the residual solvents in the waste container for non-halogenated organic waste. Test the aspirin for purity with ferric chloride as described above. Determine the melting point of your product (see Technique 9, Sections 9.5–9.8). The melting point must be obtained with a completely dried sample. Pure aspirin has a melting point of 135−136°C.

Place your product in a small vial, label it properly Technique 2, Section 2.4, and submit it to your instructor.

ASPIRIN TABLETS

Aspirin tablets consist of acetylsalicylic acid pressed together with a small amount of inert binding material. Common binding substances include starch, methylcellulose, and microcrystalline cellulose. You can test for the presence of starch by boiling approximately one-fourth of an aspirin tablet with 2 mL of water. Cool the liquid and add a drop of iodine solution. If starch is present, it will form a complex with the iodine. The starch–iodine complex is a deep blue-violet. Repeat this test with a commercial aspirin tablet and with the acetylsalicylic acid prepared in this experiment.

QUESTIONS

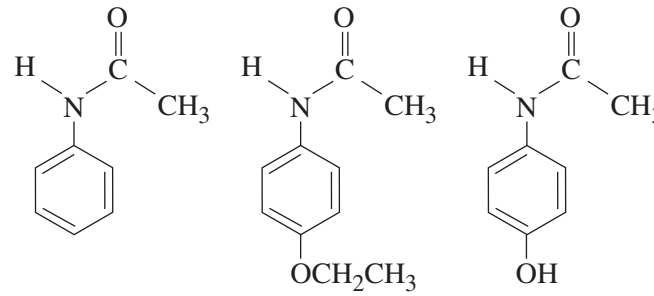
1. What is the purpose of the concentrated sulfuric acid used in the first step?
2. What would happen if the sulfuric acid were left out?
3. If you used 5.0 g of salicylic acid and excess acetic anhydride in the preceding synthesis of aspirin, what would be the theoretical yield of acetylsalicylic acid in moles? in grams?
4. What is the equation for the decomposition reaction that can occur with aspirin?
5. Most aspirin tablets contain five grains of acetylsalicylic acid. How many milligrams is this?
6. A student performed the reaction in this experiment using a water bath at 90°C instead of 50°C. The final product was tested for the presence of phenols with ferric chloride. This test was negative (no color observed); however, the melting point of the dry product was 122–125°C. Explain these results as completely as possible.
7. If the aspirin crystals were not completely dried before the melting point was determined, what effect would this have on the observed melting point?

[2]It will usually not be necessary to filter the hot mixture. If an appreciable amount of solid material remains, add 5 mL of additional ethyl acetate, heat the solution to boiling, and filter the hot solution by gravity into an Erlenmeyer flask through a fluted filter. Be sure to preheat the short-stemmed funnel by pouring hot ethyl acetate through it (see Technique 8, Section 8.1, and Technique 11, Section 11.3). Reduce the volume until crystals appear. Add a minimum additional amount of hot ethyl acetate until the crystals dissolve. Let the filtered solution stand.

ESSAY

Analgesics

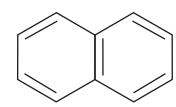
Acylated aromatic amines (those having an acyl group, $R-\overset{\overset{O}{\|}}{C}-$, substituted on nitrogen) are important in over-the-counter headache remedies. Over-the-counter drugs are those you may buy without a prescription. Acetanilide, phenacetin, and acetaminophen are mild analgesics (relieve pain) and antipyretics (reduce fever) and are important, along with aspirin, in many nonprescription drugs.



Acetanilide **Phenacetin** **Acetaminophen**

The discovery that acetanilide was an effective antipyretic came about by accident in 1886. Two doctors, Cahn and Hepp, had been testing naphthalene as a possible **vermifuge** (an agent that expels worms). Their early results on simple worm cases were very discouraging, so Dr. Hepp decided to test the compound on a patient with a larger variety of complaints, including worms—a sort of shotgun approach. A short time later, Dr. Hepp excitedly reported to his colleague, Dr. Cahn, that naphthalene had miraculous fever-reducing properties.

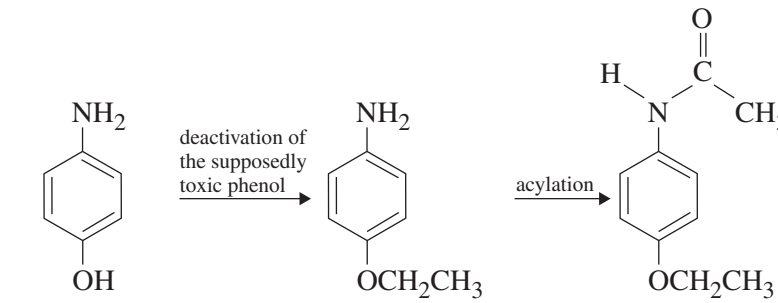
In trying to verify this observation, the doctors discovered that the bottle they thought contained naphthalene apparently did not. In fact, the bottle brought to them by their assistant had a label so faint as to be illegible. They were sure that the sample was not naphthalene, because it had no odor. Naphthalene has a strong odor reminiscent of mothballs. So close to an important discovery, the doctors were nevertheless stymied. They appealed to Hepp's cousin, who was a chemist in a nearby dye factory, to help them identify the unknown compound. This compound turned out to be acetanilide, a compound with a structure not at all like that of naphthalene. Certainly, Hepp's unscientific and risky approach would be frowned on by doctors today; and to be sure, the Food and Drug Administration (FDA) would never allow human testing before extensive animal testing (consumer protection has greatly progressed). Nevertheless, Cahn and Hepp made an important discovery.



Naphthalene

In another instance of serendipity, Cahn and Hepp's publication, describing their experiments with acetanilide, caught the attention of Carl Duisberg, director

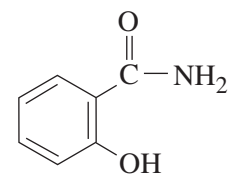
of research at the Bayer company in Germany. Duisberg was confronted with the problem of how to profitably get rid of nearly 50 tons of *p*-aminophenol, a by-product of the synthesis of one of Bayer's other commercial products. He immediately saw the possibility of converting *p*-aminophenol to a compound similar in structure to acetanilide by putting an acyl group on the nitrogen. It was then believed, however, that all compounds having a hydroxyl group on a benzene ring (that is, phenols) were toxic. Duisberg devised a scheme of structural modification of *p*-aminophenol to synthesize the compound phenacetin. The reaction scheme is shown here.



***p*-Aminophenol** **Phenacetin**

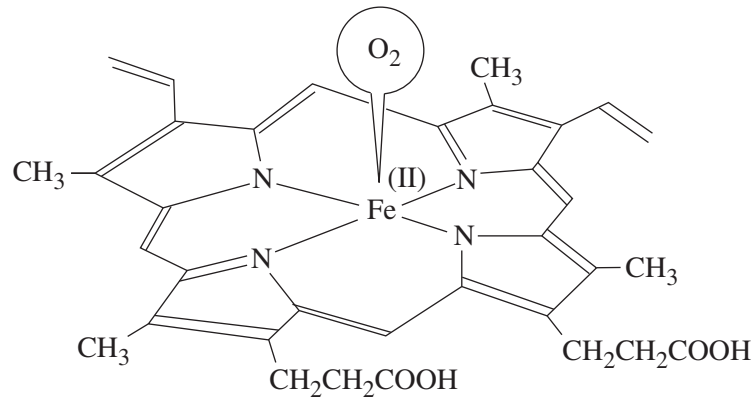
Phenacetin turned out to be a highly effective analgesic and antipyretic. A common form of combination pain reliever called an APC tablet was once available. An APC tablet contained **A**spirin, **P**henacetin, and **C**affeine (hence, **APC**). Phenacetin is no longer used in commercial pain-relief preparations, as it was discovered that not all aromatic hydroxyl groups lead to toxic compounds. Today the compound acetaminophen is very widely used as an analgesic in place of phenacetin.

Another analgesic, structurally similar to aspirin, that has found some application is **salicylamide**. Salicylamide is an ingredient in some pain-relief preparations, although its use is declining.



Salicylamide

Upon continued or excessive use, acetanilide can cause a serious blood disorder called **methemoglobinemia**. In this disorder, the central iron atom in hemoglobin is converted from Fe(II) to Fe(III) to give methemoglobin. Methemoglobin will not function as an oxygen carrier in the bloodstream. The result is a type of anemia (deficiency of hemoglobin or lack of red blood cells). Phenacetin and acetaminophen cause the same disorder, but to a much lesser degree. Because they are also more effective as antipyretic and analgesic drugs than acetanilide, they are preferred remedies. Acetaminophen is marketed under a variety of trade names, including Tylenol, Datril, and Panadol, and is often successfully used by people who are allergic to aspirin.



Heme portion of blood-oxygen carrier, hemoglobin

More recently, a new drug has appeared in over-the-counter preparations. This drug is **ibuprofen**, which was initially marketed as a prescription drug in the United States under the name Motrin. Ibuprofen was first developed in England in 1964. The United States obtained marketing rights in 1974. Ibuprofen is now sold without a prescription under several brand names, which include Advil, Motrin, and Nuprin. Ibuprofen is principally an anti-inflammatory drug, but it is also effective as an analgesic and an antipyretic. It is particularly effective in treating the symptoms of rheumatoid arthritis and menstrual cramps. Ibuprofen appears to control the production of prostaglandins, which parallels aspirin's mode of action. An important advantage of ibuprofen is that it is a very powerful pain reliever. One 200-mg tablet is as effective as two tablets (650 mg) of aspirin. Furthermore, ibuprofen has a more advantageous dose–response curve, which means that taking two tablets of this drug is approximately twice as effective as one tablet for certain types of pain. Aspirin and acetaminophen reach their maximum effective dose at two tablets. Little additional relief is gained at doses above that level. Ibuprofen,

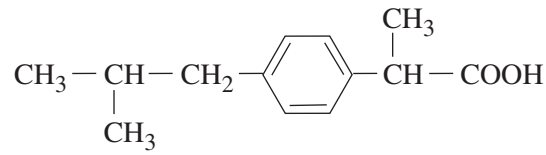
Analgesics and Caffeine in Some Common Preparations

	Aspirin	**Acetaminophen**	**Caffeine**
Aspirin*	0.325 g	—	—
Anacin	0.400 g	—	0.032 g
Bufferin	0.325 g	—	—
Cope	0.421 g	—	0.032 g
Excedrin (Extra-Strength)	0.250 g	0.250 g	0.065 g
Tylenol	—	0.325 g	—
B. C. Tablets	0.325 g	—	0.016 g
Advil	—	—	—
Aleve	—	—	—
Orudis	—	—	—

Note: Nonanalgesic ingredients (e.g., buffers) are not listed.

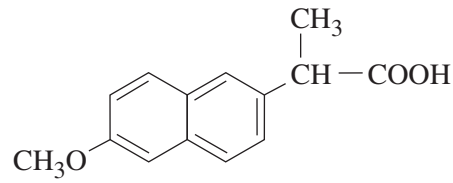
*5-grain tablet (1 grain = 0.0648 g).

however, continues to increase its effectiveness up to the 400-mg level (the equivalent of four tablets of aspirin or acetaminophen). Ibuprofen is a relatively safe drug, but its use should be avoided in cases of aspirin allergy, kidney problems, ulcers, asthma, hypertension, or heart disease.

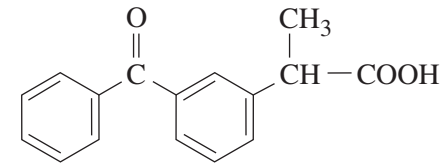


Ibuprofen

The Food and Drug Administration has also approved two other drugs with similar structures to ibuprofen for over-the-counter use as pain relievers. These new drugs are known by their generic names, **naproxen** and **ketoprofen**. Naproxen is often administered in the form of its sodium salt. Naproxen and ketoprofen can be used to alleviate the pain of headaches, toothaches, muscle aches, backaches, arthritis, and menstrual cramps, and they can also be used to reduce fever. They appear to have a longer duration of action than the older analgesics.



Naproxen



Ketoprofen

Salicylamide	Ibuprofen	Ketoprofen	Naproxen
—	—	—	—
—	—	—	—
—	—	—	—
—	—	—	—
—	—	—	—
—	—	—	—
0.095 g	—	—	—
—	0.200 g	—	—
—	—	—	0.220 g
—	—	0.0125 g	—

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9 EXPERIMENT 9

Acetaminophen

Vacuum filtration

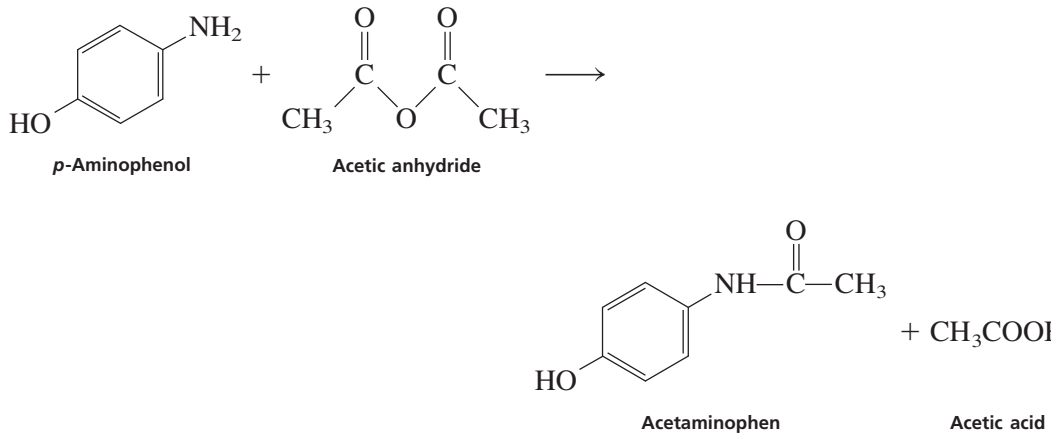
Decolorization

Crystallization

Preparation of an amide

Preparation of acetaminophen involves treating an amine with an acid anhydride to form an amide. In this case, *p*-aminophenol, the amine, is treated with acetic anhydride to form acetaminophen (*p*-acetamidophenol), the amide.

The crude solid acetaminophen contains dark impurities carried along with the *p*-aminophenol starting material. These impurities, which are dyes of unknown



***p*-Aminophenol** **Acetic anhydride** **Acetaminophen** **Acetic acid**

structure, are formed from oxidation of the starting phenol. Although the amount of the dye impurity is small, it is intense enough to impart color to the crude acetaminophen. Most of the colored impurity is destroyed by heating the crude product with sodium dithionite (sodium hydrosulfite $Na_2S_2O_4$). The dithionite reduces double bonds in the colored dye to produce colorless substances.

The decolorized acetaminophen is collected on a Büchner funnel. It is further purified by crystallization from a mixture of methanol and water.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Techniques 5 and 6	
	Technique 7	Reaction Methods, Section 7.4
	*Technique 8	Filtration, Sections 8.1–8.5
	*Technique 9	Physical Constants of Solids: The Melting Point
New:	*Technique 11	Crystallization: Purification of Solids
	Essay	Analgesics

SPECIAL INSTRUCTIONS

Acetic anhydride can cause irritation of tissue, especially in nasal passages. Avoid breathing the vapor and avoid contact with skin and eyes. *p*-Aminophenol is a skin irritant and is toxic.

SUGGESTED WASTE DISPOSAL

Aqueous solutions obtained from filtration operations should be poured into the container designated for aqueous wastes. This includes the filtrate from the methanol and water crystallization steps.

NOTES TO THE INSTRUCTOR

The *p*-aminophenol acquires a black color upon standing due to air oxidation. It is best to use a recently purchased sample, which usually has a gray color. If necessary, black material can be decolorized by heating it in a 10% aqueous solution of sodium dithionite (sodium hydrosulfite) prior to starting the experiment.

PROCEDURE

Reaction Mixture. Weigh about 1.5 g of *p*-aminophenol (*MW* = 109.1) and place this in a 50-mL Erlenmeyer flask. Using a graduated cylinder, add 4.5 mL of water and 1.7 mL of acetic anhydride (*MW* = 102.1, *d* = 1.08 g/ml). Place a magnetic stir bar in the flask.

Heating. Heat the reaction mixture, with stirring, directly on a hot plate, using a thermometer to monitor the internal temperature (about 100°C). After the solid has dissolved (it may dissolve, precipitate, and redissolve), heat the mixture for an additional 10 minutes at about 100°C to complete the reaction.

Isolation of Crude Acetaminophen. Remove the flask from the hot plate and allow the flask to cool to room temperature. If crystallization has not occurred, scratch the inside of the flask with a glass stirring rod to initiate crystallization (see Technique 11, Section 11.8). Cool the mixture thoroughly in an ice bath for 15–20 minutes and collect the crystals by vacuum filtration on a small Büchner funnel (see Technique 8, Section 8.3). Rinse the flask with about 5 mL of ice water and transfer this mixture to the Büchner funnel. Wash the crystals on the funnel with two additional 5-mL portions of ice water. Dry the crystals for 5–10 minutes by allowing air to be drawn through them while they remain on the Büchner funnel. During this drying period, break up any large clumps of crystals with a spatula. Transfer the product to a watch glass and allow the crystals to dry in air. It may take several hours for the crystals to dry completely, but you may go on to the next step before they are totally dry. Weigh the crude product and set aside a small sample for a melting-point determination and a color comparison after the next step. Calculate the percentage yield of crude acetaminophen (*MW* = 151.2). Record the appearance of the crystals in your notebook.

Decolorization of Crude Acetaminophen. Dissolve 2.0 g of sodium dithionite (sodium hydrosulfite) in 15 mL of water in a 50-mL Erlenmeyer flask. Add your crude acetaminophen to the flask. Heat the mixture to about 100°C for 15 minutes, with occasional stirring with a spatula. Some of the acetaminophen will dissolve during the decolorization process. Cool the mixture thoroughly in an ice bath for about 10 minutes to reprecipitate the decolorized acetaminophen (scratch the inside of the flask if necessary to induce crystallization). Collect the purified material by vacuum filtration on a small Büchner funnel, using small portions (about 5 mL total) of ice water to aid the transfer. Dry the crystals for 5–10 minutes by allowing air to be drawn through them while they remain on the Büchner funnel. You may go on to the next step before the material is totally dry. Weigh the purified acetaminophen and compare the color of the purified material to that obtained above.

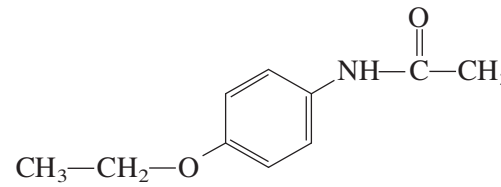
Crystallization of Acetaminophen. Place the purified acetaminophen in a 50-mL Erlenmeyer flask. Crystallize the material from a solvent mixture composed of 50% water and 50% methanol by volume. Follow the crystallization procedure described in Technique 11, Section 11.3. The solubility of acetaminophen in this hot (nearly boiling) solvent is about 1 g/5 mL. Although you can use this as a rough indication of how much solvent is required to dissolve the solid, you should still use the technique shown in Figure 11.4, to determine how much solvent to add. Add small portions of hot solvent until the solid dissolves. Step 2 in Figure 11.4 (removal of insoluble impurities) should not be required in this crystallization. When the solid has dissolved, allow the mixture to cool slowly to room temperature.

When the mixture has cooled to room temperature, place the flask in an ice bath for at least 10 minutes. If necessary, induce crystallization by scratching the inside of the flask with a glass stirring rod. Because acetaminophen may crystallize *slowly* from the solvent, it is necessary to cool the flask in an ice bath for the 10-minute period. Collect the crystals using a Büchner funnel as shown in Technique 8, Figure 8.5. Dry the crystals for 5–10 minutes by allowing air to be drawn through them while they remain on the Büchner funnel. Alternatively, you may allow the crystals to dry until the next laboratory period.

Yield Calculation and Melting-Point Determination. Weigh the crystallized acetaminophen (*MW* = 151.2) and calculate the percentage yield. This calculation should be based on the original amount of *p*-aminophenol used at the beginning of this procedure. Determine the melting point of the product. Compare the melting point of this final product with that of the crude acetaminophen. Also compare the colors of the crude, decolorized, and pure acetaminophen. Pure acetaminophen melts at 169.5–171°C. Place your product in a properly labeled vial and submit it to your instructor.

QUESTIONS

1. During the crystallization of acetaminophen, why was the mixture cooled in an ice bath?
2. In the reaction between *p*-aminophenol and acetic anhydride to form acetaminophen, 4.5 mL of water were added. What was the purpose of the water?
3. Why should you use a minimum amount of water to rinse the flask while transferring the purified acetaminophen to the Büchner funnel?
4. If 1.30 g of *p*-aminophenol is allowed to react with excess acetic anhydride, what is the theoretical yield of acetaminophen in moles? in grams?
5. Give two reasons why the crude product in most reactions is not pure.
6. Phenacetin has the structure shown. Write an equation for its preparation, starting from 4- ethoxyaniline.



ESSAY

Identification of Drugs

Frequently, a chemist is called on to identify a particular unknown substance. If there is no prior information to work from, this can be a formidable task. There are several million known compounds, both inorganic and organic. For a completely unknown substance, the chemist must often use every available method. If the unknown substance is a mixture, then the mixture must be separated into its components and each component identified separately. A pure compound can often be identified from its physical properties (melting point, boiling point, density, refractive index, and so on) and a knowledge of its functional groups. These groups can be identified by the reactions that the compound is observed to undergo or by spectroscopy (infrared, ultraviolet, nuclear magnetic resonance, and mass spectroscopy). The techniques necessary for this type of identification are introduced in a later section.

A somewhat simpler situation often arises in drug identification. The scope of drug identification is more limited, and the chemist working in a hospital trying to identify the drug in an overdose or the law enforcement officer trying to identify a suspected illicit drug or poison usually has some prior clues to work from. So does the medicinal chemist working for a pharmaceutical manufacturer who might be trying to discover why a competitor's product may be better.

Consider a drug overdose case as an example. The patient is brought into the emergency ward of a hospital. This person may be in a coma or hyperexcited state, have an allergic rash, or clearly be hallucinating. These physiological symptoms are

themselves a clue to the nature of the drug. Samples of the drug may be found in the patient's possession. Correct medical treatment may require a rapid and accurate identification of a drug powder or capsule. If the patient is conscious, the necessary information can be elicited orally; if not, the drug must be examined. If the drug is in the form of a tablet or capsule, the process is often simple because many drugs are coded by a manufacturer's trademark or logo, by shape (round, oval, or bullet shape), by formulation (tablet, gelatin capsules, or time-release microcapsule), and by color. Some drugs also bear an imprinted number or code.

It is more difficult to identify a powder, but such identification may be easy under some circumstances. Plant drugs are often easily identified because they contain microscopic bits and pieces of the plant from which they are obtained. This cellular debris is often characteristic for certain types of drugs, and they can be identified on this basis alone. A microscope is all that is needed. Sometimes chemical color tests can be used as confirmation. Certain drugs give rise to characteristic colors when treated with special reagents. Other drugs form crystalline precipitates of characteristic color and crystal structure when treated with appropriate reagents.

If the drug itself is not available and the patient is unconscious (or dead), identification may be more difficult. It may be necessary to pump the stomach or bladder contents of the patient (or corpse) or to obtain a blood sample. These samples of stomach fluid, urine, or blood would be extracted with an appropriate organic solvent, and the extract would be analyzed.

Often the final identification of a drug, as extracted from stomach fluid, urine, or blood hinges on some type of **chromatography**. Thin-layer chromatography (TLC) is often used. Under specified conditions, many drug substances can be identified by their R_f values and by the colors that their TLC spots turn when treated with various reagents or when observed under certain visualization methods. In the experiment that follows, TLC is applied to the analysis of an unknown analgesic drug.

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10 EXPERIMENT 10

TLC Analysis of Analgesic Drugs

Thin-layer chromatography

In this experiment, thin-layer chromatography (TLC) will be used to determine the composition of various over-the-counter analgesics. If the instructor chooses, you may also be required to identify the components and actual identity (trade name) of an unknown analgesic. You will be given two commercially prepared TLC plates with a flexible backing and a silica-gel coating with a fluorescent indicator. On the first TLC plate, a reference plate, you will spot five standard compounds often used in analgesic formulations. In addition, a standard reference mixture containing four of these same compounds will be spotted. Ibuprofen is omitted from this standard mixture because it would overlap with salicylamide after the plate is developed. On the second plate (the sample plate), you will spot Naproxen sodium as an additional standard and four commercial analgesic preparations in order to determine their composition. At your instructor's option, one or more of these may be an unknown.

The standard compounds will all be available as solutions of 1 g of each dissolved in 20 mL of a 50:50 mixture of methylene chloride and ethanol. The purpose of the first reference plate is to determine the order of elution (R_f values) of the known substances and to index the standard reference mixture. Several of the substances have similar R_f values, but you will note a different behavior for each spot with the visualization methods. On the sample plate, the standard reference mixture will be spotted, along with Naproxen sodium and several solutions that you will prepare from commercial analgesic tablets. These tablets will each be crushed and dissolved in a 50:50 methylene chloride–ethanol mixture for spotting.

Reference Plate		**Sample Plate**	
Acetaminophen	(Ac)	Naproxen sodium	(Nap)
Aspirin	(Asp)	Sample 1*	(1)
Caffeine	(Cf)	Sample 2*	(2)
Ibuprofen	(Ibu)	Sample 3*	(3)
Salicylamide	(Sal)	Sample 4*	(4)
Reference mixture	(Ref)	Reference mixture	(Ref)

*At the instructors' option, one or more of the samples may be an unknown.

Two methods of visualization will be used to observe the positions of the spots on the developed TLC plates. First, the plates will be observed while under illumination from a short-wavelength ultraviolet (UV) lamp. This is best done in a darkened room or in a fume hood that has been darkened by taping butcher paper or aluminum foil over the lowered glass cover. Under these conditions, some of the spots will appear as dark areas on the plate, while others will fluoresce brightly. This difference in appearance under UV illumination will help to distinguish the substances from one another. You will find it convenient to outline very lightly in *pencil* the spots observed and to place a small **x** inside those spots that fluoresce.

For a second means of visualization, iodine vapor will be used. Not all the spots will become visible when treated with iodine, but some will develop yellow, tan, or deep brown colors. The differences in the behaviors of the various spots with iodine can be used to further differentiate among them.

It is possible to use several developing solvents for this experiment, but ethyl acetate with 0.5% glacial acetic acid added is preferred. The small amount of glacial acetic acid supplies protons and suppresses ionization of aspirin, ibuprofen, and naproxen sodium, allowing them to travel upward on the plates in their protonated form. Without the acid, these compounds do not move.

In some analgesics, you may find ingredients besides the five mentioned previously. Some include an antihistamine and some contain a mild sedative. For instance, Midol contains N-cinnamylephedrine (cinnamedrine), an antihistamine, and Excedrin PM contains the sedative methapyrilene hydrochloride. Cope contains the related sedative methapyrilene fumarate. Some tablets may be colored with a chemical dye.

REQUIRED READING

Review:	Essay Analgesics	
New:	Technique 19	Column Chromatography, Sections 19.1–19.3
	Technique 20	Thin-Layer Chromatography
	Essay	Identification of Drugs

SPECIAL INSTRUCTIONS

You must first examine the developed plates under ultraviolet light. After comparisons of *all* plates have been made with UV light, iodine vapor can be used. The iodine permanently affects some of the spots, making it impossible to go back and repeat the UV visualization. Take special care to notice those substances that have similar R_f values; these spots each have a different appearance when viewed under UV illumination or a different staining color with iodine, allowing you to distinguish among them.

Aspirin presents some special problems because it is present in a large amount in many of the analgesics and because it hydrolyzes easily. For these reasons, the aspirin spots often show excessive tailing.

SUGGESTED WASTE DISPOSAL

Dispose of all development solvent in the container for nonhalogenated organic solvents. Dispose of the ethanol–methylene chloride mixture in the container for halogenated organic solvents. The micropipets used for spotting the solution should be placed in a special container labeled for that purpose. The TLC plates should be stapled in your lab notebook.

NOTES TO THE INSTRUCTOR

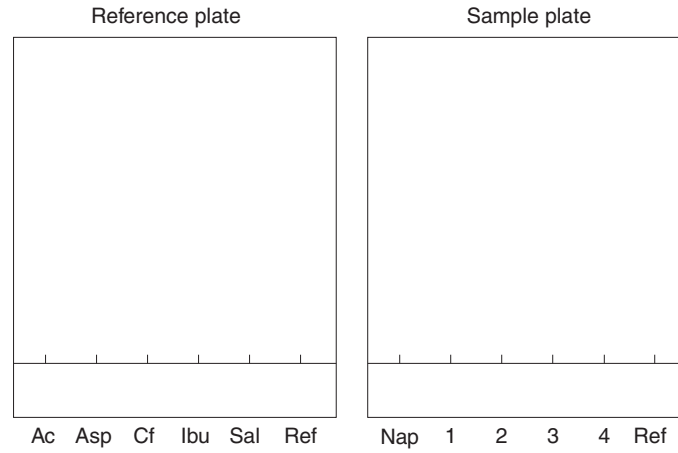
If you wish, students may work in pairs on this experiment, each one preparing one of the two plates.

Perform the thin-layer chromatography with flexible Silica Gel 60 F-254 plates (EM Science, No. 5554-7). If the TLC plates have not been purchased recently, you should place them in an oven at 100°C for 30 minutes and store them in a desiccator until used. If you use different thin-layer plates, try out the experiment before using them with a class. Other plates may not resolve all five substances.

Ibuprofen and salicylamide have approximately the same R_f value, but they show up differently under the detection methods. For reasons that are not yet clear, ibuprofen sometimes gives two or even three spots. Naproxen sodium has approximately the same R_f as aspirin. Once again, however, these analgesics show up differently under the detection methods. Fortunately, naproxen sodium is not combined with aspirin or ibuprofen in any current commercial product.

PROCEDURE

Initial Preparations. You will need at least 12 capillary micropipets to spot the plates. The preparation of these pipets is described and illustrated in Technique 20, Section 20.4. A common error is to pull the center section out too far when making these pipets, with the result that too little sample is applied to the plate. If this happens, you won't see *any* spots. Follow the directions carefully.



Preparing TLC Plates.

After preparing the micropipets, obtain two 100-cm × 6.6-cm TLC plates (EM Science Silica Gel 60 F-254, No. 5554-7) from your instructor. These plates have a flexible backing, but they should not be bent excessively. Handle them carefully or the adsorbent may flake off. Also, you should handle them only by the edges; the surface should not be touched. Using a lead pencil (not a pen), *lightly* draw a line across the plates (short dimension) about 1 cm from the bottom. Using a centimeter ruler, move its index about 0.6 cm in from the edge of the plate and lightly mark off six 1-cm intervals on the line (see figure above). These are the points at which the samples will be spotted. If you are preparing two reference plates, it would be a good idea to mark a small number **1** or **2** in the upper right-hand corner of each plate to allow easy identification.

Spotting the Reference Plate. On the first plate, starting from left to right, spot acetaminophen, then aspirin, caffeine, ibuprofen, and salicylamide. This order is alphabetic and will avoid any further memory problems or confusion. Solutions of these compounds will be found in small bottles on the supply shelf. The standard reference mixture (Ref) also found on the supply shelf, is spotted in the last position. The correct method of

spotting a TLC plate is described in Technique 20, Section 20.4. It is important that the spots be made as small as possible, (ca. 1–2 mm in diameter). With too much sample, the spots will tail and will overlap one another after development. With too little sample, no spots will be observed after development. The optimum applied spot should be about 1–2 mm (1/6 in.) in diameter. If scrap pieces of the TLC plates are available, it would be a good idea to practice spotting on these before preparing the actual sample plates.

Preparing the Development Chamber. When the reference plate has been spotted, obtain a 16-oz wide-mouth, screw-cap jar (or other suitable container) for use as a development chamber. The preparation of a development chamber is described in Technique 20, Section 20.5. Because the backing on the TLC plates is very thin, if they touch the filter paper liner of the development chamber *at any point,* solvent will begin to diffuse onto the absorbent surface at that point. To avoid this, you may either omit the liner or make the following modification.

If you wish to use a liner, use a very narrow strip of filter paper (approximately 5 cm wide). Fold it into an L shape that is long enough to traverse the bottom of the jar and extend up the side to the top of the jar. TLC plates placed in the jar for development should *straddle* this liner strip, but not touch it.

When the development chamber has been prepared, obtain a small amount of the development solvent (0.5% glacial acetic acid in ethyl acetate). Your instructor should prepare this mixture; it contains such a small amount of acetic acid that small individual portions are difficult to prepare. Fill the chamber with the development solvent to a depth of about 0.5–0.7 cm. If you are using a liner, be sure it is saturated with the solvent. Recall that the solvent level must not be above the spots on the plate or the samples will dissolve off the plate into the reservoir instead of developing.

Development of the Reference TLC Plate. Place the spotted plate (or plates) in the chamber (straddling the liner if one is present) and allow the spots to develop. If you are doing two reference plates, both plates may be placed in the same development jar. Be sure the plates are placed in the developing jar so that their bottom edge is parallel to the bottom of the jar (straight, not tilted); if not, the solvent front will not advance evenly, increasing the difficulty of making good comparisons. The plates should face each other and slant or lean back in opposite directions. When the solvent has risen to a level about 0.5 cm from the top of the plate, remove each plate from the chamber (in the hood) and, using a lead pencil, mark the position of the solvent front. Set the plate on a piece of paper towel to dry. It may be helpful to place a small object under one end to allow optimum air flow around the drying plate.

UV Visualization of the Reference Plate. When the plate is dry, observe it under a short-wavelength UV lamp, preferably in a darkened hood or a darkened room. Lightly outline all of the observed spots with a pencil. Carefully notice any differences in behavior between the spotted substances. Several compounds have similar R_f values, but the spots have a different appearance under UV illumination or iodine staining. Currently, there are no commercial analgesic preparations containing any compounds that have the same R_f values, but you will need to be able to distinguish them from one another to identify which one is present. Before proceeding, make a sketch of the plates in your notebook and note the differences in appearance that you observed. Using a ruler marked in millimeters, measure the distance that each spot has traveled relative to the solvent front. Calculate R_f values for each spot (see Technique 20, Section 20.9).

Analysis of Commercial Analgesics or Unknowns (Sample Plate). Next, obtain half a tablet of each of the analgesics to be analyzed on the final TLC plate. If you were issued an unknown, you may analyze four other analgesics of your choice; if not, you may analyze five. The experiment will be most interesting if you make your choices in a way that gives a wide spectrum of results. Try to pick at least one analgesic each containing aspirin, acetaminophen, ibuprofen, a newer analgesic, and, if available, salicylamide. If you have a favorite analgesic, you may wish to include it among your samples. Take each analgesic half-tablet,

place it on a smooth piece of notebook paper, and crush it well with a spatula. Transfer each crushed half-tablet to a labeled test tube or a small Erlenmeyer flask. Using a graduated cylinder, mix 15 mL of absolute ethanol and 15 mL of methylene chloride. Mix the solution well. Add 5 mL of this solvent to each of the crushed half-tablets and then heat each of them *gently* for a few minutes on a steam bath or sand bath at about 100°C. Not all of the tablet material will dissolve, because the analgesics usually contain an insoluble binder. In addition, many of them contain inorganic buffering agents or coatings that are insoluble in this solvent mixture. After heating the samples, allow them to settle and then spot the clear liquid extracts (1–4) on the sample plate. Spot the standard solution of naproxen on the left-hand edge, and spot the standard reference solution (Ref) on the right-hand edge of the plate (see figure above). Develop the plate in 0.5% glacial acetic acid–ethyl acetate as before. Observe the plate under UV illumination and mark the visible spots as you did for the first plate. Sketch the plate in your notebook and record your conclusions about the contents of each tablet. This can be done by directly comparing your plate to the reference plate(s)—they can all be placed under the UV light at the same time. If you were issued an unknown, try to determine its identity (trade name).

Iodine Analysis. Do not perform this step until UV comparisons of all the plates are complete. When ready, place the plates in a jar containing a few iodine crystals, cap the jar, and warm it gently on a steam bath or warm hot plate until the spots begin to appear. Notice which spots become visible and note their relative colors. You can directly compare colors of the reference spots to those on the unknown plate(s). Remove the plates from the jar and record your observations in your notebook.

QUESTIONS

1. What happens if the spots are made too large when preparing a TLC plate for development?
2. What happens if the spots are made too small when preparing a TLC plate for development?
3. Why must the spots be above the level of the development solvent in the developing chamber?
4. What would happen if the spotting line and positions were marked on the plate with a ballpoint pen?
5. Is it possible to distinguish two spots that have the same R_f value but represent different compounds? Give two different methods.
6. Name some advantages of using acetaminophen (Tylenol) instead of aspirin as an analgesic.

ESSAY

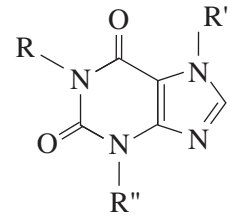
Caffeine

The origins of coffee and tea as beverages are so old that they are lost in legend. Coffee is said to have been discovered by an Abyssinian goatherd who noticed an unusual friskiness in his goats when they consumed a certain little plant with red berries. He decided to try the berries himself and discovered coffee. The Arabs soon

cultivated the coffee plant, and one of the earliest descriptions of its use is found in an Arabian medical book circa A.D. 900. The great systematic botanist Linnaeus named the plant *Coffea arabica.*

One legend of the discovery of tea—from the Orient, as you might expect—attributes the discovery to Daruma, the founder of Zen. Legend has it that he inadvertently fell asleep one day during his customary meditations. To be assured that this indiscretion would not recur, he cut off both eyelids. Where they fell to the ground, a new plant took root that had the power to keep a person awake. Although some experts assert that the medical use of tea was reported as early as 2737 BC in the pharmacopeia of Shen Nung, an emperor of China, the first indisputable reference is from the Chinese dictionary of Kuo P'o, which appeared in AD 350. The nonmedical, or popular, use of tea appears to have spread slowly. Not until about AD 700 was tea widely cultivated in China. Tea is native to upper Indochina and upper India, so it must have been cultivated in these places before its introduction to China. Linnaeus named the tea shrub *Thea sinensis;* however, tea is more properly a relative of the camellia, and botanists have renamed the shrub *Camellia thea.*

The active ingredient that makes tea and coffee valuable to humans is **caffeine**. Caffeine is an **alkaloid**, a class of naturally occurring compounds containing nitrogen and having the properties of an organic amine base (alkaline, hence, *alkaloid*). Tea and coffee are not the only plant sources of caffeine. Others include kola nuts, maté leaves, guarana seeds, and, in small amount, cocoa beans. The pure alkaloid was first isolated from coffee in 1821 by the French chemist Pierre Jean Robiquet.



XANTHINES
Xanthine R = R' = R" = H
Caffeine R = R' = R" = CH_3
Theophylline R = R" = CH_3, R' = H
Theobromine R = H, R' = R" = CH_3

Caffeine belongs to a family of naturally occurring compounds called **xanthines**. The xanthines, in the form of their plant progenitors, are possibly the oldest known stimulants. They all, to varying extents, stimulate the central nervous system and the skeletal muscles. This stimulation results in an increased alertness, the ability to put off sleep, and an increased capacity for thinking. Caffeine is the most powerful xanthine in this respect. It is the main ingredient of the popular No-Doz keep-alert tablets. Although caffeine has a powerful effect on the central nervous system, not all xanthines are as effective. Thus, theobromine, the xanthine found in cocoa, has fewer central nervous system effects. It is, however, a strong **diuretic** (induces urination) and is useful to doctors in treating patients with severe water-retention problems. Theophylline, a second xanthine found in tea, also has fewer central nervous system effects but is a strong **myocardial** (heart muscle) stimulant; it **dilates** (relaxes) the coronary artery that supplies blood to the heart. Its most important use is in the treatment of bronchial asthma, because it has the properties of a **bronchodilator** (relaxes the bronchioles of the lungs). Because it is also a **vasodilator** (relaxes blood vessels), it is often used in treating hypertensive headaches. It is also used to alleviate and to reduce the frequency of attacks of **angina pectoris** (severe chest pain). In addition, it is a more powerful diuretic than theobromine.

One can develop both a tolerance for the xanthines and a dependence on them, particularly caffeine. The dependence is real, and a heavy user (>5 cups of coffee per day) will experience lethargy, headache, and perhaps nausea after about 18 hours of

abstinence. An excessive intake of caffeine may lead to restlessness, irritability, insomnia, and muscular tremor. Caffeine can be toxic, but to achieve a lethal dose of caffeine, one would have to drink about 100 cups of coffee over a relatively short period.

Caffeine is a natural constituent of coffee, tea, and kola nuts (*Kola nitida*). Theophylline is found as a minor constituent of tea. The chief constituent of cocoa is theobromine. The amount of caffeine in tea varies from 2% to 5%. In one analysis of black tea, the following compounds were found: caffeine, 2.5%; theobromine, 0.17%; theophylline, 0.013%; adenine, 0.014%; and guanine and xanthine, traces. Coffee beans can contain up to 5% by weight of caffeine, and cocoa contains around 5% theobromine. Commercial cola is a beverage based on a kola nut extract. We cannot easily get kola nuts in this country, but we can get the ubiquitous commercial extract as a syrup. The syrup can be converted into "cola." The syrup contains caffeine, tannins, pigments, and sugar. Phosphoric acid is added, and caramel is added to give the syrup a deep color. The final drink is prepared by adding water and carbon dioxide under pressure, to give the bubbly mixture. Before decaffeination, the Food and Drug Administration required a "cola" to contain some caffeine (about 0.2 mg per ounce). In 1990, when new nutrition labels were adopted, this requirement was dropped. The Food and Drug Administration again currently requires that a "cola" contain *some* caffeine, but limits this amount, to a maximum of 5 milligrams per ounce. To achieve a regulated level of caffeine, most manufacturers remove all caffeine from the kola extract and then re-add the correct amount to the syrup. The caffeine content of various beverages is listed in the accompanying table.

Given the recent popularity of gourmet coffee beans and espresso stands, it is interesting to consider the caffeine content of these specialty beverages. Gourmet coffee certainly has more flavor than the typical ground coffee you may find on any grocery store shelf, and the concentration of brewed gourmet coffee tends to be higher than ordinary drip-grind coffee. Brewed gourmet coffee probably contains something on the order of 20–25 mg of caffeine per ounce of liquid. Espresso coffee is a very concentrated, dark-brewed coffee. Although the darker roasted beans used for espresso actually contain less caffeine per gram than regularly roasted beans, the method of preparing espresso (extraction using pressurized steam) is more efficient, and a higher percentage of the total caffeine in the beans is extracted. The caffeine content per ounce of liquid, therefore, is substantially higher than in most brewed coffees. The serving size for espresso coffee, however, is much smaller than for ordinary coffee (about 1.5–2 oz per serving), so the total caffeine available in a serving of espresso turns out to be about the same as in a serving of ordinary coffee.

Amount of Caffeine (mg/oz) Found in Beverages

Brewed coffee	12–30	Tea	4–20
Instant coffee	8–20	Cocoa (but 20 mg/oz of theobromine)	0.5–2
Espresso (1 serving = 1.5–2oz)	50–70	Coca-Cola	3.75
Decaffeinated coffee	0.4–1.0		

Note: The average cup of coffee or tea contains about 5–7 ounces of liquid. The average bottle of cola contains about 12 ounces of liquid.

Because of the central nervous system effects from caffeine, many people prefer **decaffeinated** coffee. The caffeine is removed from coffee by extracting the whole beans with an organic solvent. Then the solvent is drained off, and the beans are steamed to remove any residual solvent. The beans are dried and roasted to

bring out the flavor. Decaffeination reduces the caffeine content of coffee to the range of 0.03% to 1.2%. The extracted caffeine is used in various pharmaceutical products, such as APC tablets.

Among coffee lovers there is some controversy about the best method to remove the caffeine from coffee beans. **Direct contact** decaffeination uses an organic solvent (usually methylene chloride) to remove the caffeine from the beans. When the beans are subsequently roasted at 200°C, virtually all traces of the solvent are removed, because methylene chloride boils at 40°C. The advantage of direct contact decaffeination is that the method removes only the caffeine (and some waxes), but leaves the substances responsible for the flavor of the coffee intact in the bean. A disadvantage of this method is that all organic solvents are toxic to some extent.

Water process decaffeination is favored among many drinkers of decaffeinated coffee because it does not use organic solvents. In this method, hot water and steam are used to remove caffeine and other soluble substances from the coffee. The resulting solution is then passed through activated charcoal filters to remove the caffeine. Although this method does not use organic solvents, the disadvantage is that water is not a very selective decaffeinating agent. Many of the flavor oils in the coffee are removed at the same time, resulting in a coffee with a somewhat bland flavor.

A third method, the **carbon dioxide decaffeination process,** is being used with increasing frequency. The raw coffee beans are moistened with steam and water, and they are then placed into an extractor where they are treated with carbon dioxide gas under very high temperature and pressure. Under these conditions, the carbon dioxide gas is in a **supercritical** state, which means that it takes on the characteristics of both a liquid and a gas. The supercritical carbon dioxide acts as a selective solvent for caffeine, thus extracting it from the beans.

There are, however, benefits to ingesting caffeine. Small amounts of caffeine have been found to be helpful in controlling weight, alleviating pain, and reducing the symptoms of asthma and other breathing problems. Recently, studies on mice indicate that caffeine may help to reverse or slow the development of Alzheimer's disease in mice. Other studies on humans indicate that caffeine may reduce the likelihood of developing Parkinson's disease and reduce the risk of colon cancer.

Another problem, posed by the beverage tea, is that in some cases persons who consume high quantities of tea may show symptoms of Vitamin B_1 (thiamine) deficiency. It is suggested that the tannins in the tea may complex with the thiamine, rendering it unavailable for use. An alternative suggestion is that caffeine may reduce the levels of the enzyme transketolase, which depends on the presence of thiamine for its activity. Lowered levels of transketolase would produce the same symptoms as lowered levels of thiamine.

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11 EXPERIMENT 11

Isolation of Caffeine from Tea Leaves

Isolation of a Caffeine

Isolation of a natural product

Extraction

Sublimation

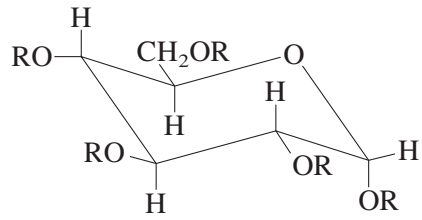
In this experiment, caffeine is isolated from tea leaves. The chief problem with the isolation is that caffeine does not exist alone in tea leaves, but is accompanied by other natural substances from which it must be separated. The main component of tea leaves is cellulose, which is the principal structural material of all plant cells. Cellulose is a polymer of glucose. Because cellulose is virtually insoluble in water, it presents no problems in the isolation procedure. Caffeine, on the other hand, is water-soluble and is one of the main substances extracted into the solution called tea. Caffeine constitutes as much as 5% by weight of the leaf material in tea plants.

Tannins also dissolve in the hot water used to extract caffeine from tea leaves. The term **tannin** does not refer to a single homogeneous compound or even to substances that have a similar chemical structure. It refers, rather, to a class of compounds that have certain properties in common. Tannins are phenolic compounds having molecular weights between 500 and 3000. They are widely used to tan leather. They precipitate alkaloids and proteins from aqueous solutions. Tannins are usually divided into two classes: those that can be **hydrolyzed** (react with water) and those that cannot. Tannins of the first type that are found in tea generally yield glucose and gallic acid when they are hydrolyzed. These tannins are esters of gallic acid and glucose. They represent structures in which some of the hydroxyl groups in glucose have been esterified by digalloyl groups. The nonhydrolyzable tannins found in tea are condensation polymers of catechin. These polymers are not uniform in structure; catechin molecules are usually linked at ring positions 4 and 8.

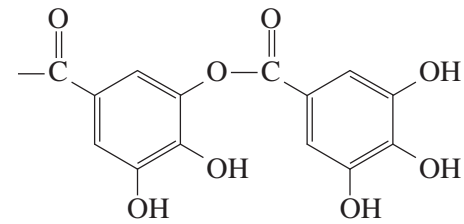
When tannins are extracted into hot water, some of these compounds are partially hydrolyzed to form free gallic acid. The tannins, because of their phenolic groups, and gallic acid, because of its carboxyl groups, are both acidic. If sodium

carbonate, a base, is added to tea water, these acids are converted to their sodium salts, which are highly soluble in water.

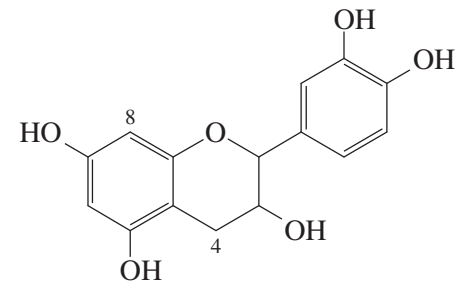
Although caffeine is soluble in water, it is much more soluble in the organic solvent methylene chloride. Caffeine can be extracted from the basic tea solution with methylene chloride, but the sodium salts of gallic acid and the tannins remain in the aqueous layer.



Glucose if R = H
A tannin if some R = digalloyl

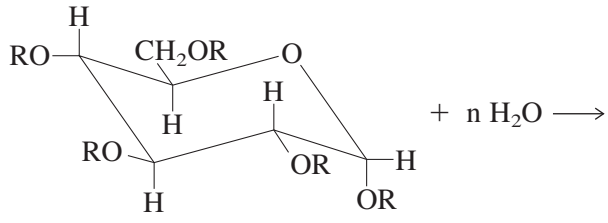


A digalloyl group

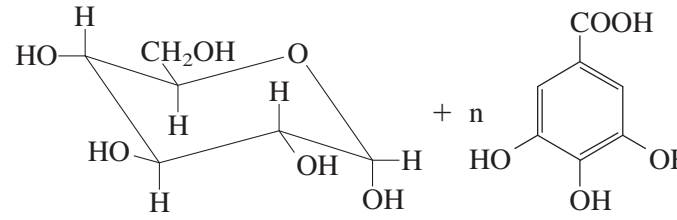


Catechin

The brown color of a tea solution is due to flavonoid pigments and chlorophylls and to their respective oxidation products. Although chlorophylls are soluble in methylene chloride, most other substances in tea are not. Thus, the methylene chloride extraction of the basic tea solution removes nearly pure caffeine. The methylene chloride is easily removed by evaporation (bp 40°C) to leave the crude caffeine. The caffeine is then purified by sublimation.

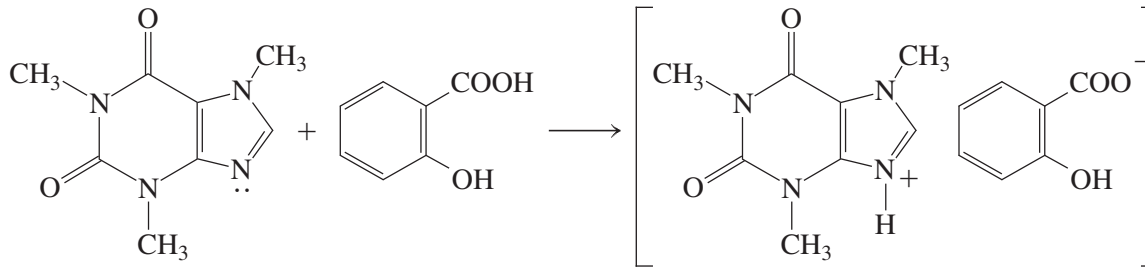


R = digalloyl



Glucose **Gallic acid**

Experiment 11A outlines the isolation of caffeine from tea using macroscale techniques. An optional procedure in Experiment 11A allows the student to convert caffeine to a **derivative**. A derivative of a compound is a second compound, of known melting point, formed from the original compound by a simple chemical reaction. In trying to make a positive identification of an organic compound, it is often necessary to convert it to a derivative. If the first compound, caffeine in this case, and its derivative have melting points that match those reported in the chemical literature (a handbook, for instance), it is assumed that this is no coincidence and that the identity of the first compound, caffeine, has been established conclusively.



Caffeine is a base and will react with an acid to give a salt. With salicylic acid, a derivative **salt** of caffeine, caffeine salicylate, can be made to establish the identity of the caffeine isolated from tea leaves.

In Experiment 11B, the isolation of caffeine is accomplished using microscale methods. In this experiment, you will be asked to isolate the caffeine from the tea contained in a single tea bag.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Techniques 5 and 6	
	*Technique 7	Reaction Methods, Sections 7.2 and 7.10
	*Technique 9	Physical Constants of Solids: The Melting Point
New:	*Technique 12	Extractions, Separations, and Drying Agents, Sections 12.1–12.5 and 12.7–12.9
	*Technique 17	Sublimation
	Essay	Caffeine

SPECIAL INSTRUCTIONS

Be careful when handling methylene chloride. It is a toxic solvent, and you should not breathe it excessively or spill it on yourself. In Experiment 11B, the extraction procedure with methylene chloride calls for two centrifuge tubes with screw caps. Corks can also be used to seal the tubes; however, the corks will absorb a small amount of the liquid. Rather than shake the centrifuge tube, you can conveniently accomplish agitation with a vortex mixer.

SUGGESTED WASTE DISPOSAL

You must dispose of methylene chloride in a waste container marked for the disposal of halogenated organic waste. When you are discarding tea leaves, do not put them in the sink; they will clog the drain. Dispose of them in a waste container. Dispose of the tea bags in a waste container, not in the sink. The aqueous solutions obtained after the extraction steps must be disposed of in a waste container labeled for aqueous waste.

11A EXPERIMENT 11A

Isolation of Caffeine from Tea Leaves

PROCEDURE

Preparing the Tea Solution. Place 5 g of tea leaves, 2 g of calcium carbonate powder, and 50 mL of water in a 100-mL round-bottom flask equipped with a condenser for reflux (see Technique 7, Figure 7.6). Heat the mixture under gentle reflux, being careful to prevent any bumping, for about 20 minutes. Use a heating mantle to heat the mixture. Shake the flask occasionally during this heating period. While the solution is still hot, vacuum filter it through a fast-filter paper such as E&D No. 617 or S&S No. 595 (see Technique 8, Section 8.3). A 125-mL filter flask is appropriate for this step.

Extraction and Drying. Cool the filtrate (filtered liquid) to room temperature, and using a 125-mL separatory funnel, extract it (see Technique 12, Section 12.4) with a 10-mL portion of methylene chloride (dichloromethane). Shake the mixture vigorously for 1 minute. The layers should separate after standing for several minutes, although some emulsion will be present in the lower organic layer (see Technique 12, Section 12.10). The emulsion can be broken and the organic layer dried at the same time by passing the lower layer *slowly* through anhydrous magnesium sulfate, according to the following method. Place a small piece of cotton (not glass wool) in the neck of a conical funnel and add a 1-cm layer of anhydrous magnesium sulfate on top of the cotton. Pass the organic layer directly from the separatory funnel into the drying agent and collect the filtrate in a dry Erlenmeyer flask. Rinse the magnesium sulfate with 1 or 2 mL of fresh methylene chloride solvent. Repeat the extraction with another 10-mL portion of methylene chloride on the aqueous layer remaining in the separatory funnel, and repeat the drying, as described above, with a *fresh* portion of anhydrous magnesium sulfate. Collect the organic layer in the flask containing the first methylene chloride extract. These extracts should now be clear, showing no visible signs of water contamination. If some water should pass through the filter, repeat the drying, as described above, with a fresh portion of magnesium sulfate. Collect the dried extracts in a dry Erlenmeyer flask.

Distillation. Pour the dry organic extracts into a 50-mL round-bottom flask. Assemble an apparatus for simple distillation (see Technique 14, Figure 14.1), add a boiling stone, and remove the methylene chloride by distillation on a steam bath or heating mantle. The residue in the distillation flask contains the caffeine and is purified by crystallization and sublimation. Save the methylene chloride that was distilled; you may use some of it in the next step. The remaining methylene chloride must be placed in a waste container marked for halogenated waste; it must *not* be discarded in the sink.

Crystallization (Purification). Dissolve the residue from the methylene chloride extraction of the tea solution in about mL of the methylene chloride that you saved from the distillation. You may have to heat the mixture on a steam bath or heating mantle to dissolve the solid. Transfer the solution to a 25-mL Erlenmeyer flask. Rinse the distillation flask with an additional mL of methylene chloride and combine this solution with the contents of the Erlenmeyer flask. Add a boiling stone and evaporate the now light-green solution to dryness by heating it on a steam bath or a hot plate *in the hood*.

The residue obtained on evaporation of the methylene chloride is next crystallized by the mixed-solvent method (see Technique 11, Section 11.10). Using a steam bath or hot plate, dissolve the residue in a small quantity (about 2 mL) of hot acetone and add, dropwise, just enough low-boiling (bp 30°–60°C) petroleum ether to turn the solution faintly cloudy.[1] Cool the solution and collect the crystalline product by vacuum filtration, using a small Büchner funnel. A small amount of petroleum ether can be used to help in transferring the crystals to the Büchner funnel. A second crop of crystals can be obtained by concentrating the filtrate. Weigh the product (an analytical balance may be necessary). Calculate the weight percentage yield (see Technique 2, end of Section 2.2) based on the 5 g of tea originally used and determine the melting point. The melting point of pure caffeine is 238°C. Note the color of the solid for comparison with the material obtained after sublimation.

Sublimation of Caffeine. Caffeine can be purified by sublimation (see Technique 17). Assemble a sublimation apparatus as shown in Figure 17.2C. If it is available, the apparatus shown in Figure 17.2A will give superior results. Insert a 15-mm × 125-mm test tube into a No. 2 neoprene adapter, using a *little* water as a lubricant, until the tube is fully inserted. Place the crude caffeine into a 20-mm × 150-mm sidearm test tube. Next place the 15-mm × 125-mm test tube into the sidearm test tube, making sure they fit together tightly. Turn on the aspirator or house vacuum and make sure a good seal is obtained. At the point at which a good seal has been achieved, you should hear or observe a change in the water velocity in the aspirator. At this time, also make sure that the central tube is centered in the sidearm test tube; this will allow for optimal collection of the purified caffeine. Once the vacuum has been established, place small chips of ice in the inner test tube to fill it.[2] When a good vacuum seal has been obtained and ice has been added to the inner test tube, heat the sample gently and carefully with a microburner to sublime the caffeine. Hold the burner in your hand (hold it at the *base,* not by the hot barrel) and apply heat by moving the flame back and forth under the outer tube and up the sides. If the sample begins to melt, remove the flame for a few seconds before you resume heating. When sublimation is complete, remove the burner and allow the apparatus to cool. As the apparatus is cooling and before you disconnect the vacuum, remove the water and ice from the inner tube using a Pasteur pipet.

When the apparatus has cooled and the water and ice has been removed from the inner tube, you may disconnect the vacuum. The vacuum should be removed carefully to avoid dislodging the crystals from the inner tube by the sudden rush of air into the apparatus. *Carefully* remove the inner tube of the sublimation apparatus. If this operation is done carelessly, the sublimed crystals may be dislodged from the inner tube and fall back into

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[1]If the residue does not dissolve in this quantity of acetone, magnesium sulfate may be present as an impurity (drying agent). Add additional acetone (up to about 5 mL), gravity-filter the mixture to remove the solid impurity, and reduce the volume of the filtrate to about 2 mL. Now add petroleum ether as indicated in the procedure.

[2]It is very important that ice not be added to the inner test tube until the vacuum has been established. If the ice is added before the vacuum is turned on, condensation on the outer walls of the inner tube will contaminate the sublimed caffeine.

the residue. Scrape the sublimed caffeine onto weighing paper, using a small spatula. Determine the melting point of this purified caffeine and compare its melting point and color with the caffeine obtained following crystallization. Submit the sample to the instructor in a labeled vial, or, if the instructor directs, prepare the caffeine salicylate derivative.

THE DERIVATIVE (OPTIONAL)

The amounts given in this part, including solvents, should be adjusted to fit the quantity of caffeine you obtained. Use an analytical balance. Dissolve 25 mg of caffeine and 18 mg of salicylic acid in 2 mL of toluene in a small Erlenmeyer flask by warming the mixture on a steam bath or hot plate. Add about 0.5 mL (10 drops) of high-boiling (bp 60°–90°C) petroleum ether or ligroin and allow the mixture to cool and crystallize. It may be necessary to cool the flask in an ice-water bath or to add a small amount of extra petroleum ether to induce crystallization. Collect the crystalline product by vacuum filtration, using a Hirsch funnel or a small Büchner funnel. Dry the product by allowing it to stand in the air, and determine its melting point. Pure caffeine salicylate melts at 137°C. Submit the sample to the instructor in a labeled vial.

11B EXPERIMENT 11B

Isolation of Caffeine from a Tea Bag

PROCEDURE

Preparing the Tea Solution. Place 20 mL of water in a 50-mL beaker. Cover the beaker with a watch glass and heat the water on a hot plate until the water is almost boiling. Place a tea bag into the hot water so that it lies flat on the bottom of the beaker and is covered as completely as possible with water.[3] Replace the watch glass and continue heating for about 15 minutes. During this heating period, it is important to push down *gently* on the tea bag with a test tube so that all the tea leaves are in constant contact with water. As the water evaporates during this heating step, replace it by adding water from a Pasteur pipet.

Using a Pasteur pipet, transfer the concentrated tea solution to two centrifuge tubes fitted with screw caps. Try to keep the liquid volume in each centrifuge tube approximately equal. To squeeze additional liquid out of the tea bag, hold the tea bag on the inside wall of the beaker and roll a test tube back and forth while exerting *gentle* pressure on the tea bag. Press out as much liquid as possible without breaking the bag. Combine this liquid with the solution in the centrifuge tubes. Place the tea bag on the bottom of the beaker again and pour 2 mL of hot water over the bag. Squeeze the liquid out, as just described, and transfer this liquid to the centrifuge tubes. Add 0.5 g of sodium carbonate to the hot liquid in each centrifuge tube. Cap the tubes and shake the mixture until the solid dissolves.

Extraction and Drying. Cool the tea solution to room temperature. Using a calibrated Pasteur pipet (Technique 5, Section 5.4), add 3 mL of methylene chloride to each centrifuge tube

[3]The weight of tea in the bag will be given to you by your instructor. This can be determined by pouring out the contents of several bags of tea and determining the average weight. If this is done carefully, the tea can be returned to the bags, which can be restapled.

to extract the caffeine (see Technique 12, Section 12.7). Cap the centrifuge tubes and gently shake the mixture for several seconds. Vent the tubes to release the pressure, being careful that the liquid does not squirt out toward you. Shake the mixture for an additional 30 seconds with occasional venting. To separate the layers and break the emulsion (see Technique 12, Section 12.10), centrifuge the mixture for several minutes (be sure to balance the centrifuge by placing the two centrifuge tubes on opposite sides). If an emulsion still remains (indicated by a green brown layer between the clear methylene chloride layer and the top aqueous layer), centrifuge the mixture again.

Remove the lower organic layer with a Pasteur pipet and transfer it to a test tube. Be sure to squeeze the bulb before placing the tip of the Pasteur pipet into the liquid, and try not to transfer any of the dark aqueous solution along with the methylene chloride layer. Add a fresh 3-mL portion of methylene chloride to the aqueous layer remaining in each centrifuge tube, cap the tubes, and shake the mixture in order to carry out a second extraction. Separate the layers by centrifugation, as described previously. Combine the organic layers from each extraction into one test tube. If there are visible drops of the dark aqueous solution in the test tube, transfer the methylene chloride solution to another test tube using a clean, dry Pasteur pipet.If necessary, leave a small amount of the methylene chloride solution behind in order to avoid transferring any of the aqueous mixture. Add a small amount of granular anhydrous sodium sulfate to dry the organic layer (see Technique 12, Section 12.9). If all the sodium sulfate clumps together when the mixture is stirred with a spatula, add some additional drying agent. Allow the mixture to stand for 10–15 minutes. Stir occasionally with a spatula.

Evaporation. Transfer the dry methylene chloride solution with a Pasteur pipet to a dry, preweighed 25-mL Erlenmeyer flask, while leaving the drying agent behind. Evaporate the methylene chloride by heating the flask in a hot-water bath (see Technique 7, Section 7.10). This should be done in a hood and can be accomplished more rapidly if a stream of dry air or nitrogen gas is directed at the surface of the liquid. When the solvent is evaporated, the crude caffeine will coat the bottom of the flask. Do not heat the flask after the solvent has evaporated, or you may sublime some of the caffeine. Weigh the flask and determine the weight of crude caffeine. Calculate the weight percentage recovery (see Technique 2, end of Section 2.2) of caffeine from tea leaves, using the weight of tea given to you by your instructor. You may store the caffeine by simply placing a stopper firmly into the flask.

Sublimation of Caffeine. Caffeine can be purified by sublimation (see Technique 17, Section 17.5). Follow the method described in Experiment 11A. Add approximately 1.0 mL of methylene chloride to the Erlenmeyer flask and transfer the solution to the sublimation apparatus using a clean, dry Pasteur pipet. Add a few more drops of methylene chloride to the flask in order to rinse the caffeine out completely. Transfer this liquid to the sublimation apparatus. Evaporate the methylene chloride from the outer tube of the sublimation apparatus by gently heating it in a warm-water bath under a stream of dry air or nitrogen.

Assemble the apparatus as described in Experiment 11A, or use the apparatus shown in Figure 17.2A if it is available. Be sure that the inside of the assembled apparatus is clean and dry. If you are using an aspirator, install a trap between the aspirator and the sublimation apparatus. Turn on the vacuum and check to make sure that all joints in the apparatus are sealed tightly. Place *ice-cold* water in the inner tube of the apparatus. Heat the sample gently and carefully with a microburner to sublime the caffeine. Hold the burner in your hand (hold it at its base, not by the hot barrel) and apply the heat by moving the flame back and forth under the outer test tube and up the sides. If the sample begins to melt, remove the flame for a few seconds before you resume heating. When sublimation is complete, discontinue heating. Remove the cold water and remaining ice from the inner tube and allow the apparatus to cool while continuing to apply the vacuum.

When the apparatus is at room temperature, remove the vacuum and *carefully* remove the inner tube. If this operation is done carelessly, the sublimed crystals may be dislodged from the inner tube and fall back into the residue at the bottom of the outer test tube. Scrape the sublimed caffeine onto a tared piece of smooth paper and determine the weight of caffeine recovered. Calculate the weight percentage recovery (see Technique 2, end of Section 2.2) of caffeine after the sublimation. Compare this value to the percentage recovery determined after the evaporation step. Determine the melting point of the purified caffeine. The melting point of pure caffeine is 236°C; however, the observed melting point will be lower. Submit the sample to the instructor in a labeled vial.

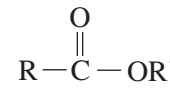
QUESTIONS

1. Outline a separation scheme for isolating caffeine from tea (Experiment 11A or Experiment 11B). Use a flowchart similar in format to that shown in Technique 2.
2. Why was the sodium carbonate added in Experiment 11B? Why was calcium carbonate added in Experiment 11A?
3. The crude caffeine isolated from tea has a green tinge. Why?
4. What are some possible explanations for why the melting point of your isolated caffeine may be lower than the literature value (236°C)?
5. What would happen to the caffeine if the sublimation step were performed at atmospheric pressure?

ESSAY

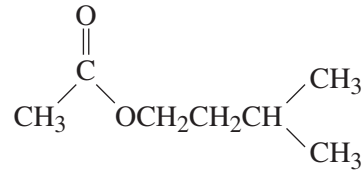
Esters—Flavors and Fragrances

Esters are a class of compounds widely distributed in nature. They have the general formula

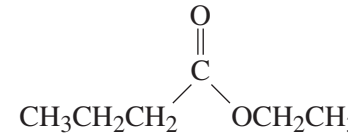


The simple esters tend to have pleasant odors. In many but not all cases, the characteristic flavors and fragrances of flowers and fruits are due to compounds with the ester functional group. An exception is the case of the essential oils. The **organoleptic** qualities (odors and flavors) of fruits and flowers may often be due to a single ester, but more often, the flavor or the aroma is due to a complex mixture in which a single ester predominates. Some common flavor principles are listed in Table 1. Food and beverage manufacturers are familiar with these esters and often

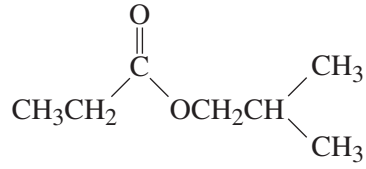
TABLE 1 Ester Flavors and Fragrances



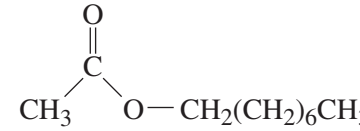
Isoamyl acetate
(banana)
(alarm pheromone of honeybee)



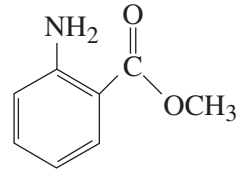
Ethyl butyrate
(pineapple)



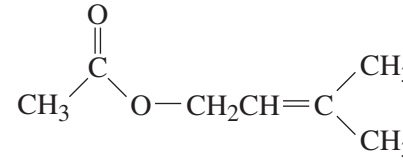
Isobutyl propionate
(rum)



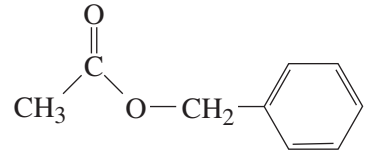
Octyl acetate
(oranges)



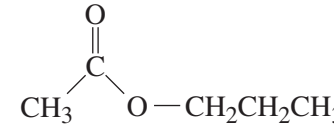
Methyl anthranilate
(grape)



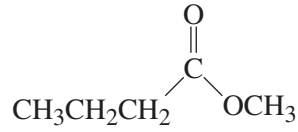
Isopentenyl acetate
("Juicy Fruit")



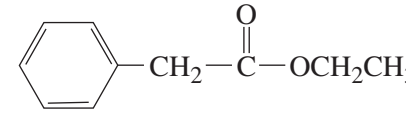
Benzyl acetate
(peach)



***n*-Propyl acetate**
(pear)



Methyl butyrate
(apple)



Ethyl phenylacetate
(honey)

TABLE 2 Artificial Pineapple Flavor

Pure Compounds	%	Essential Oils	%
Allyl caproate	5	Oil of sweet birch	1
Isoamyl acetate	3	Oil of spruce	2
Isoamyl isovalerate	3	Balsam Peru	4
Ethyl acetate	15	Volatile mustard oil	1
Ethyl butyrate	22	Oil of cognac	5
Terpinyl propionate	3	Concentrated orange oil	4
Ethyl crotonate	5	Distilled oil of lime	2
Caproic acid	8		19
Butyric acid	12		
Acetic acid	5		
	81		

use them as additives to spruce up the flavor or odor of a dessert or beverage. Many times, such flavors or odors do not even have a natural basis, as is the case with the "juicy fruit" principle, isopentenyl acetate. An instant pudding that has the flavor of rum may never have seen its alcoholic namesake; this flavor can be duplicated by the proper admixture, along with other minor components, of ethyl formate and isobutyl propionate. The natural flavor and odor are not exactly duplicated, but most people can be fooled. Often, only a professional taster, a trained person with a high degree of gustatory perception, can tell the difference.

A single compound is rarely used in good-quality imitation flavoring agents. A formula for an imitation pineapple flavor that might fool an expert is listed in Table 2. The formula includes 10 esters and carboxylic acids that can easily be synthesized in the laboratory. The remaining seven oils are isolated from natural sources.

Flavor is a combination of taste, sensation, and odor transmitted by receptors in the mouth (taste buds) and nose (olfactory receptors). The stereochemical theory of odor is discussed in the essay that precedes Experiment 15. The four basic tastes (sweet, sour, salty, and bitter) are perceived in specific areas of the tongue. The sides of the tongue perceive sour and salty tastes, the tip is most sensitive to sweet tastes, and the back of the tongue detects bitter tastes. The perception of flavor, however, is not so simple. If it were, it would require only the formulation of various combinations of four basic substances—a bitter substance (a base), a sour substance (an acid), a salty substance (sodium chloride), and a sweet substance (sugar)—to duplicate any flavor! In fact, we cannot duplicate flavors in this way. Humans possess about 9,000 taste buds. The combined response of these taste buds is what allows perception of a particular flavor.

Although the "fruity" tastes and odors of esters are pleasant, they are seldom used in perfumes or scents that are applied to the body. The reason for this is chemical. The ester group is not as stable under perspiration as the ingredients of the more expensive essential-oil perfumes. The latter are usually hydrocarbons (terpenes), ketones, and ethers extracted from natural sources. Esters, however, are used only for the cheapest toilet waters, because on contact with sweat they undergo hydrolysis, giving organic acids. These acids, unlike their precursor esters, generally do not have a pleasant odor.

Butyric acid, for instance, has a strong odor like that of rancid butter (of which it is an ingredient) and is a component of what we normally call body odor. It is this substance that makes foul-smelling humans so easy for an animal to detect when downwind of them. It is also of great help to the bloodhound, which is trained to follow small traces of this odor.

Ethyl butyrate and methyl butyrate, however, which are the *esters* of butyric acid, smell like pineapple and apple, respectively.

A sweet, fruity odor also has the disadvantage of possibly attracting fruit flies and other insects in search of food. Isoamyl acetate, the familiar solvent called banana oil, is particularly interesting. It is identical to a component of the alarm **pheromone** of the honeybee. Pheromone is the name applied to a chemical secreted by an organism that evokes a specific response in another member of the same species. This kind of communication is common among insects who otherwise lack means of exchanging information. When a honeybee worker stings an intruder, an alarm pheromone, composed partly of isoamyl acetate, is secreted along with the sting venom. This chemical causes aggressive attack on the intruder by other bees, who swarm around the intruder. Obviously, it wouldn't be wise to wear a perfume compounded of isoamyl acetate near a beehive. Pheromones are discussed in more detail in the essay preceding Experiment 45.

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Shreve, R. N.; Brink, J. *Chemical Process Industries,* 4th ed.; McGraw-Hill: New York, 1977.

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12 EXPERIMENT 12

Isopentyl Acetate (Banana Oil)

Esterification

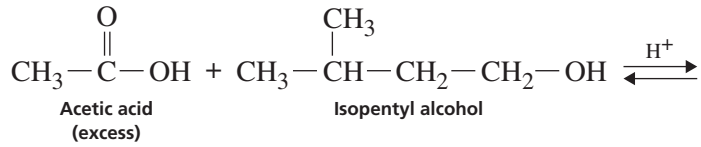
Heating under reflux

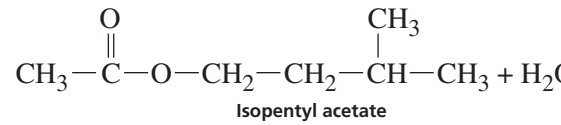
Separatory funnel

Extraction

Simple distillation

In this experiment, you will prepare an ester, isopentyl acetate. This ester is often referred to as banana oil, because it has the familiar odor of this fruit.





Isopentyl acetate is prepared by the direct esterification of acetic acid with isopentyl alcohol. Because the equilibrium does not favor the formation of the ester, it must be shifted to the right, in favor of the product, by using an excess of one of the starting materials. Acetic acid is used in excess because it is less expensive than isopentyl alcohol and more easily removed from the reaction mixture.

In the isolation procedure, much of the excess acetic acid and the remaining isopentyl alcohol are removed by extraction with sodium bicarbonate and water. After drying with anhydrous sodium sulfate, the ester is purified by distillation. The purity of the liquid product is analyzed by determining the infrared spectrum.

REQUIRED READING

Sign in at www.cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Techniques 5 and 6	
New:	*Technique 7	Reaction Methods
	*Technique 12	Extractions, Separations, and Drying Agents
	Technique 13	Physical Constants of Liquids, Part A. Boiling Points and Thermometer Correction
	*Technique 14	Simple Distillation
	Essay	Esters—Flavors and Fragrances

If performing the optional infrared spectroscopy, also read

Technique 25,	Part A

SPECIAL INSTRUCTIONS

Be careful when dispensing sulfuric and glacial acetic acids. They are very corrosive and will attack your skin if you make contact with them. If you get one of these acids on your skin, wash the affected area with copious quantities of running water for 10–15 minutes.

Because a 1-hour reflux is required, you should start the experiment at the very beginning of the laboratory period. During the reflux period, you may perform other experimental work.

SUGGESTED WASTE DISPOSAL

Any aqueous solutions should be placed in a container specially designated for dilute aqueous waste. Place any excess ester in the nonhalogenated organic waste container.

NOTES TO THE INSTRUCTOR

This experiment has been carried out successfully using Dowex 50X2-100 ion exchange resin instead of the sulfuric acid.

PROCEDURE

Apparatus. Assemble a reflux apparatus, using a 25-mL round-bottom flask and a water-cooled condenser (refer to Technique 7, Figure 7.6,). Use a heating mantle to heat. In order to control vapors, place a drying tube packed with calcium chloride on top of the condenser.

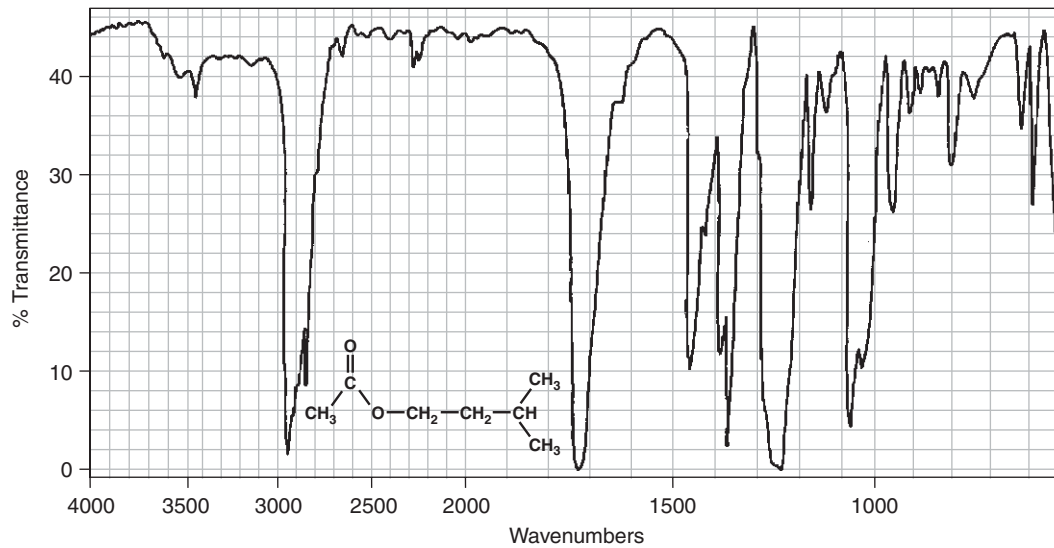
Reaction Mixture. Weigh (tare) an empty 10-mL graduated cylinder and record its weight. Place approximately 5.0 mL of isopentyl alcohol in the graduated cylinder and reweigh it to determine the weight of alcohol. Disconnect the roundbottom flask from the reflux apparatus and transfer the alcohol into it. Do not clean or wash the graduated cylinder. Using the same graduated cylinder, measure approximately 7.0 mL of glacial acetic acid ($MW = 60.1$, $d = 1.06$ g/mL) and add it to the alcohol already in the flask. Using a calibrated Pasteur pipet, add 1 mL of concentrated sulfuric acid, mixing *immediately* (with swirling), to the reaction mixture contained in the flask. Add a corundum boiling stone and reconnect the flask. Do not use a calcium carbonate (marble) boiling stone because it will dissolve in the acidic medium.

Reflux. Start water circulating in the condenser and bring the mixture to a boil. Continue heating under reflux for 60–75 minutes. Then disconnect or remove the heating source and allow the mixture to cool to room temperature.

Extractions. Disassemble the apparatus and transfer the reaction mixture to a separatory funnel (125-mL) placed in a ring that is attached to a ring stand. Be sure that the stopcock is closed and, using a funnel, pour the mixture into the top of the separatory funnel. Also be careful to avoid transferring the boiling stone, or you will need to remove it after the transfer. Add 10 mL of water, stopper the funnel, and mix the phases by careful shaking and venting (see Technique 12, Section 12.4, and Figure 12.6). Allow the phases to separate and then unstopper the funnel and drain the lower aqueous layer through the stopcock into a beaker or other suitable container. Next, extract the organic layer with 5 mL of 5% aqueous sodium bicarbonate just as you did previously with water. Extract the organic layer once again, this time with 5 mL of saturated aqueous sodium chloride.

Drying. Transfer the crude ester to a clean, dry 25-mL Erlenmeyer flask and add approximately 1.0 g of anhydrous granular sodium sulfate. Cork the mixture and allow it to stand for 10–15 minutes while you prepare the apparatus for distillation. If the mixture does not appear dry (the drying agent clumps and does not "flow," the solution is cloudy, or drops of water are obvious), transfer the ester to a new clean, dry 25-mL Erlenmeyer flask and add a new 0.5-g portion of anhydrous sodium sulfate to complete the drying.

Distillation. Assemble a distillation apparatus using your smallest roundbottom flask to distill from (see Technique 14, Figure 14.1). Use a heating mantle to heat. Preweigh (tare) and use another small round-bottom flask, or an Erlenmeyer flask, to collect the product. Immerse the collection flask in a beaker of ice to ensure condensation and to reduce odors. You should look up the boiling point of your expected product in a handbook so you will know what to anticipate. Continue distillation until only one or two drops of liquid remain in the distilling flask. Record the observed boiling point range in your notebook.



Yield Determination. Weigh the product and calculate the percentage yield of the ester. If your instructor requests it, determine the boiling point using one of the methods described in Technique 13, Sections 13.2 and 13.3.

Spectroscopy. If your instructor requests it, obtain an infrared spectrum using salt plates (see Technique 25, Section 25.2). Compare your spectrum with the one reproduced in the text. Interpret the spectrum and include it in your report to the instructor. You may also be required to determine and interpret the proton and carbon-13 NMR spectra (see Technique 26, Part A and Technique 27, Section 27.1). Submit your sample in a properly labeled vial with your report.

QUESTIONS

1. One method of favoring the formation of an ester is to add excess acetic acid. Suggest another method, involving the right-hand side of the equation, that will favor the formation of the ester.
2. Why is the mixture extracted with sodium bicarbonate? Give an equation and explain its relevance.
3. Why are gas bubbles observed when the sodium bicarbonate is added?

4. Which starting material is the limiting reagent in this procedure? Which reagent is used in excess? How great is the molar excess (how many times greater)?
5. Outline a separation scheme for isolating pure isopentyl acetate from the reaction mixture.
6. Interpret the principal absorption bands in the infrared spectrum of isopentyl acetate or, if you did not determine the infrared spectrum of your ester, do this for the spectrum of isopentyl acetate shown in the previous figure. (Technique 25 may be of some help.)
7. Write a mechanism for the acid-catalyzed esterification of acetic acid with isopentyl alcohol.
8. Why is glacial acetic acid designated as "glacial"? (*Hint:* Consult a handbook of physical properties.)

ESSAY

Terpenes and Phenylpropanoids

Anyone who has walked through a pine or cedar forest, or anyone who loves flowers and spices, knows that many plants and trees have distinctively pleasant odors. The essences or aromas of plants are due to volatile or **essential oils**, many of which have been valued since antiquity for their characteristic odors (frankincense and myrrh, for example). A list of the commercially important essential oils would run to over 200 entries. Allspice, almond, anise, basil, bayberry, caraway, cinnamon, clove, cumin, dill, eucalyptus, garlic, jasmine, juniper, orange, peppermint, rose, sandalwood, sassafras, spearmint, thyme, violet, and wintergreen are but a few familiar examples of such valuable essential oils. Essential oils are used for their pleasant odors in perfumes and incense. They are also used for their taste appeal as spices and flavoring agents in foods. A few are valued for antibacterial and antifungal action. Some are used medicinally (camphor and eucalyptus) and others as insect repellents (citronella). Chaulmoogra oil is one of the few known curative agents for leprosy. Turpentine is used as a solvent for many paint products.

Essential-oil components are often found in the glands or intercellular spaces in plant tissue. They may exist in all parts of the plant, but are often concentrated in the seeds or flowers. Many components of essential oils are steam-volatile and can be isolated by steam distillation. Other methods of isolating essential oils include solvent extraction and pressing (expression) methods. Esters (see essay "Esters—Flavors and Fragrances," that precedes Experiment 12) are frequently responsible for the characteristic odors and flavors of fruits and flowers, but other types of substances may also be important components of odor or flavor principles. Besides the esters, the ingredients of essential oils may be complex mixtures of hydrocarbons, alcohols, and carbonyl compounds. These other components usually belong to one of the two groups of natural products called **terpenes** or **phenylpropanoids**.

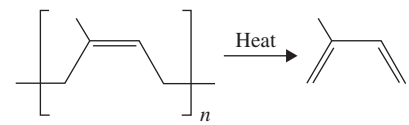
TERPENES

Chemical investigations of essential oils in the nineteenth century found that many of the compounds responsible for the pleasant odors contained exactly 10 carbon atoms. These 10-carbon compounds came to be known as terpenes if they were hydrocarbons and as **terpenoids** if they contained oxygen and were alcohols, ketones, or aldehydes.

Eventually, it was found that minor and less volatile plant constituents containing 15, 20, 30, and 40 carbon atoms also exist. Because compounds of 10 carbons were originally called terpenes, they came to be called **monoterpenes**. The other terpenes were classified in the following way.

Class	No. of Carbons	Class	No. of Carbons
Hemiterpenes	5	Diterpenes	20
Monoterpenes	10	Triterpenes	30
Sesquiterpenes	15	Tetraterpenes	40

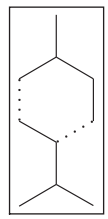
Further chemical investigations of the terpenes, all of which contain multiples of five carbons, showed them to have a repeating structural unit based on a five-carbon pattern. This structural pattern corresponds to the arrangement of atoms in the simple five-carbon compound isoprene. Isoprene was first obtained by the thermal "cracking" of natural rubber.

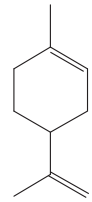


Natural rubber **Isoprene**

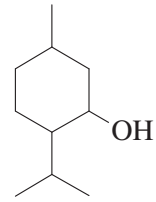
As a result of this structural similarity, a diagnostic rule for terpenes, called the **isoprene rule**, was formulated. This rule states that a terpene should be divisible, at least formally, into **isoprene units**. The structures of a number of terpenes, along with a diagrammatic division of their structures into isoprene units, is shown in the following full-page figure. Many of these compounds represent odors or flavors that should be very familiar to you.

Modern research has shown that terpenes do not arise from isoprene; it has never been detected as a natural product. Instead, the terpenes arise from an important biochemical precursor compound called **mevalonic acid** (see the biochemical scheme that follows). This compound arises from acetyl coenzyme A, a product of the biological degradation of glucose (glycolysis), and is converted to a compound called isopentenyl pyrophosphate. Isopentenyl pyrophosphate and its isomer 3, 3-dimethylallyl pyrophosphate (double bond moved to the second position) are the five-carbon building blocks used by nature to construct all the terpene compounds.

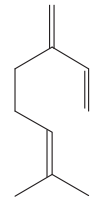




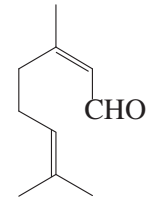
Limonene
(citrus)



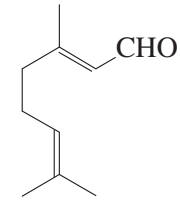
Menthol
(mint)



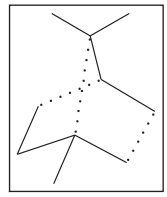
Myrcene
(bayberry)

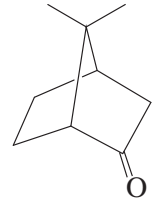


Citronellal
(citronella)

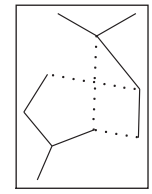


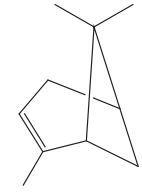
Citral
(lemongrass)



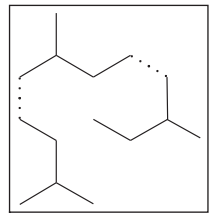


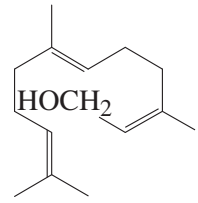
Camphor
(camphor)



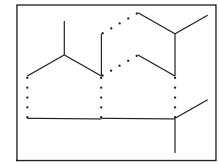


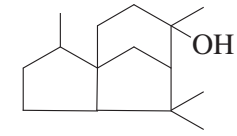
α-pinene
(pine turpentine)



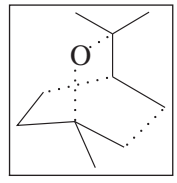


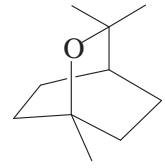
Farnesol
(lily of the valley)



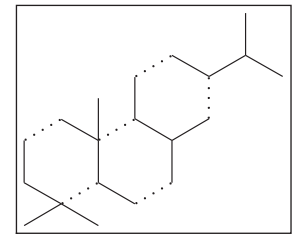


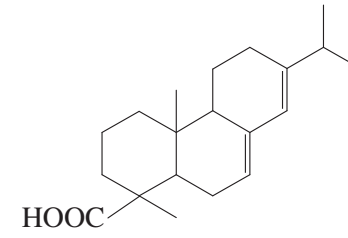
Cedrol
(cedar)



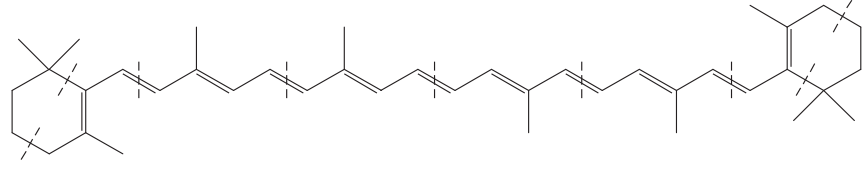


1,8-Cineole
(eucalyptus)



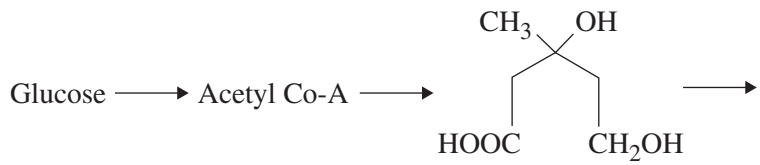


Abietic acid
(pine rosin)

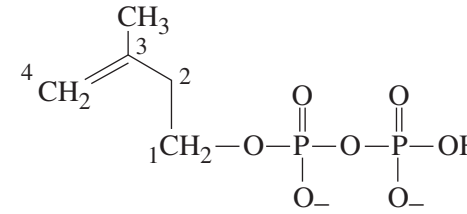


β-Carotene
(carrots)

Selected terpenes.



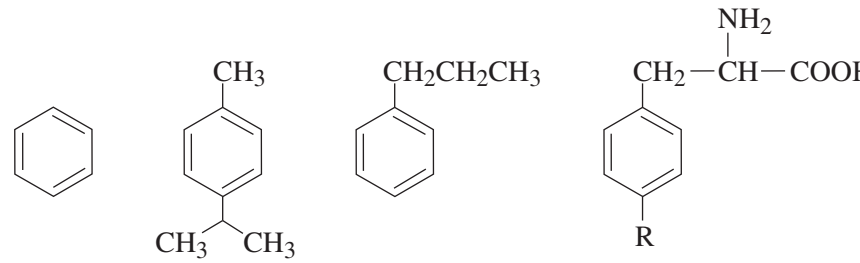
Mevalonic acid



Isopentenyl pyrophosphate

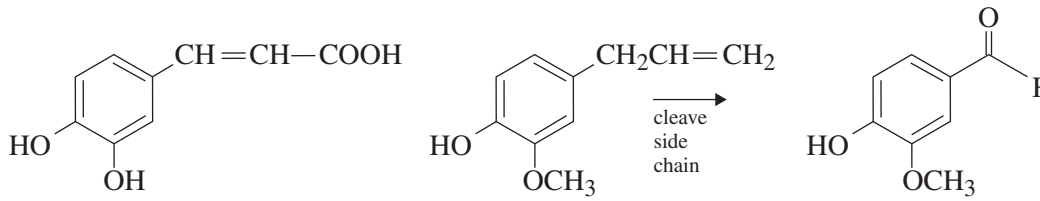
PHENYLPROPANOIDS

Aromatic compounds, those containing a benzene ring, are also a major type of compound found in essential oils. Some of these compounds, such as *p*-cymene, are actually cyclic terpenes that have been aromatized (had their ring converted to a benzene ring), but most are of a different origin.



Benzene | ***p*-Cymene** | **Phenylpropane** | **Phenylalanine & Tyrosine** (R = H) (R = OH)

Many of these aromatic compounds are **phenylpropanoids**, compounds based on a phenylpropane skeleton. Phenylpropanoids are related in structure to the common amino acids phenylalanine and tyrosine, and many are derived from a biochemical pathway called the **shikimic acid pathway**.



Caffeic acid (coffee) | **Eugenol (cloves)** | **Vanillin (vanilla)**

It is also common to find compounds of phenylpropanoid origin that have had the three-carbon side chain cleaved. As a result, phenylmethane derivatives, such as vanillin, are also quite common in plants.

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13 EXPERIMENT 13

Isolation of Eugenol from Cloves

Use of a handbook

Steam distillation

Extraction

Infrared spectroscopy

In this experiment, you will steam distill the essential oil eugenol from the spice cloves. Following the isolation of eugenol, you will determine its infrared spectrum and assign the major peaks observed in the spectrum to structural features present in the molecule. If NMR spectroscopy is available, your instructor may also have you determine the proton or carbon-13 NMR spectra and interpret them as well.

Prior to coming to class, you should look up the structure of eugenol and its physical properties in a handbook such as *The Merck Index* or the *CRC Handbook of Chemistry and Physics.* Technique 4 will provide you some guidance in finding this information.

REQUIRED READING

Sign in at www .cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Techniques 4 and *12	
New:	Essay	Terpenes and Phenylpropanoids
	Technique 18	Steam Distillation

SPECIAL INSTRUCTIONS

Foaming can be a serious problem if you use finely ground spices. It is recommended that you use clove buds in place of the ground spice. However, be sure to cut or break up the large pieces or crush them with a mortar and pestle.

SUGGESTED WASTE DISPOSAL

Any aqueous solutions should be placed in the container specially designated for aqueous wastes. Be sure to place any solid spice residues in the garbage can, as they will plug the sink if disposed of there.

NOTES TO THE INSTRUCTOR

If ground spices are used (not recommended), you may want to have the students insert a Claisen head between the round-bottom flask and the distillation head to allow extra volume in case the mixture foams.

PROCEDURE

Apparatus. Using a 100-mL round-bottom flask to distill and a 50-mL round-bottom flask to collect, assemble a distillation apparatus similar to that shown in Technique 14, Figure 14.1. Use a heating mantle to heat. The collection flask may be immersed in ice to ensure condensation of the distillate.

Preparing the Spice. Weigh approximately 3.0 g of your spice onto a weighing paper and record the exact weight. If your spice is already ground, you may proceed without grinding it; otherwise, break up the seeds using a mortar and pestle or cut larger pieces into smaller ones using scissors. Mix the spice with 35–40 mL of water in the 100-mL round-bottom flask, add a boiling stone, and reattach it to your distillation apparatus. Allow the spice to soak in the water for about 15 minutes before beginning the heating. Be sure that all the spice gets thoroughly wetted. Swirl the flask gently, if necessary.

Steam Distillation. Turn on the cooling water in the condenser and begin heating the mixture to provide a steady rate of distillation. If you approach the boiling point too quickly, you may have difficulty with frothing or bump-over. You will need to find the amount of heating that provides a steady rate of distillation but avoids frothing and/or bumping. A good rate of distillation would be to have one drop of liquid collected every 2–5 seconds. Continue distillation until at least 15 mL of distillate have been collected.

Normally, in a steam distillation the distillate will be somewhat cloudy due to separation of the essential oil as the vapors cool. However, you may not notice this and still obtain satisfactory results.

Extraction of the Essential Oil. Transfer the distillate to a separatory funnel and add 5.0 mL of methylene chloride (dichloromethane) to extract the distillate. Shake the funnel vigorously, venting frequently. Allow the layers to separate.

The mixture may be spun in a centrifuge if the layers do not separate well. Stirring gently with a spatula sometimes helps to resolve an emulsion. It may also help to add about 1 mL of a saturated sodium chloride solution. For the following directions, however, be aware that the saturated salt solution is quite dense, and the aqueous layer may change places with the methylene chloride layer, which is normally on the bottom.

Transfer the lower methylene chloride layer to a clean, dry Erlenmeyer flask. Repeat this extraction procedure with a fresh 5.0-mL portion of methylene chloride and place it in the same Erlenmeyer flask used to place the first extraction. If there are visible drops of water, you need to transfer the methylene chloride solution carefully to a clean, dry flask, leaving the drops of water behind.

Drying. Dry the methylene chloride solution by adding about 1 g of granular anhydrous sodium sulfate to the Erlenmeyer flask (see Technique 12, Section 12.9). Let the solution stand for 10–15 minutes and swirl it occasionally.

Evaporation. While the organic solution is being dried, obtain a clean, dry medium-sized test tube and weigh (tare) it accurately. Decant a portion (about one- third) of the dried organic layer to this tared test tube, leaving the drying agent behind. Add a boiling stone and, working in a hood, evaporate the methylene chloride from the solution by using a gentle stream of air or nitrogen and heating to about with a water bath (see Technique 7, Section 7.10). When the first portion is reduced to a small volume of liquid, add a second portion of the methylene chloride solution and evaporate as before. When you add the final portion, use small amounts of clean methylene chloride to rinse the drying agent, allowing you to transfer all of the remaining solution into the tared test tube. Be careful to prevent any of the sodium sulfate from being transferred.

CAUTION

The stream of air or nitrogen must be very gentle, or you will blast your solution out of the test tube. In addition, do not overheat the sample, or your sample may "bump" out of the tube. Do not continue the evaporation beyond the point where all the methylene chloride has evaporated. Your product is a volatile oil (that is, liquid). If you continue to heat and evaporate, you will lose it. It would be better to leave some methylene chloride than to lose your sample.

Yield Determination. When the solvent has been removed, reweigh the test tube. Calculate the weight percentage recovery of the oil from the original amount of spice used.

SPECTROSCOPY

Infrared. Obtain the infrared spectrum of the oil as a pure liquid sample (see Technique 25, Section 25.2). It may be necessary to use a Pasteur pipet with a narrow tip to transfer a sufficient amount to the salt plates. If even this fails, you may add one or two drops of carbon tetrachloride (tetrachloromethane) to aid in the transfer. This solvent will not interfere with the infrared spectrum. Include the infrared spectrum in your laboratory report, along with an interpretation of the principal peaks.

Nuclear Magnetic Resonance. If your instructor requests it, determine the nuclear magnetic resonance spectrum of the oil (see Technique 26, Part A).

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 NMR spectra, assign the peaks to either hydrogen or carbon atoms and explain any splitting patterns. Be sure to also include your weight percentage recovery calculation.

QUESTIONS

1. Using a handbook such as the *CRC Handbook of Chemistry and Physics* or *The Merck Index*, look up the following properties of eugenol:

melting point	density	solubility in water, chloroform, ethanol, and diethyl ether
boiling point	refractive index	

2. Using the *Physicians' Desk Reference* (PDR) or the *PDR for Nonprescription Drugs and Dietary Supplements*, find a medical use for eugenol (oil of cloves).
3. A terpene named caryophyllene is the major by-product in oil of cloves. Find the structure of caryophyllene in a handbook and show how it fits the "terpene rule" (see essay "Terpenes and Phenylpropanoids") that precedes this experiment.
4. Find a boiling point for caryophyllene. If the reported boiling point is not at atmospheric pressure, correct it to 760 mmHg (see Technique 13, Section 13.2).
5. Caryophyllene is a chiral molecule. Look up the specific rotation of caryophyllene; then draw its structure and identify any stereocenters by placing an asterisk next to them. Is eugenol chiral?
6. Why is steam distillation rather than a simple distillation used to isolate eugenol?
7. Why does the newly condensed steam distillate appear cloudy?
8. After the drying step, what observations will allow you to determine if the product is "dry" (that is, free of water)?
9. A natural product (*MW* = 150) distills with steam at a boiling temperature of 99°C at atmospheric pressure. The vapor pressure of water at 99°C is 733 mmHg.
   a. Calculate the weight of natural product that codistills with each gram of water at 99°C.
   b. How much water must be removed by steam distillation to recover this natural product from 3.0 g of a spice that contains 10% of the desired substance?

ESSAY

Stereochemical Theory of Odor

The human nose has an almost unbelievable ability to distinguish odors. Just consider for a few moments the different substances you can recognize by odor alone. Your list should be long. A person with a trained nose, a perfumer, for instance, can often recognize even individual components in a mixture. Who has not met at least one cook who could sniff almost any culinary dish and identify the seasonings and spices that were used? The olfactory centers in the nose can identify odorous substances even in small amounts. Studies have shown that with some substances, as little as one 10-millionth of a gram (10^{-7} g) can be perceived. Many animals, for example, dogs and insects, have an even lower threshold of smell than humans do (see essay Pheromones: Insect Attractants and Repellents, that precedes Experiment 45).

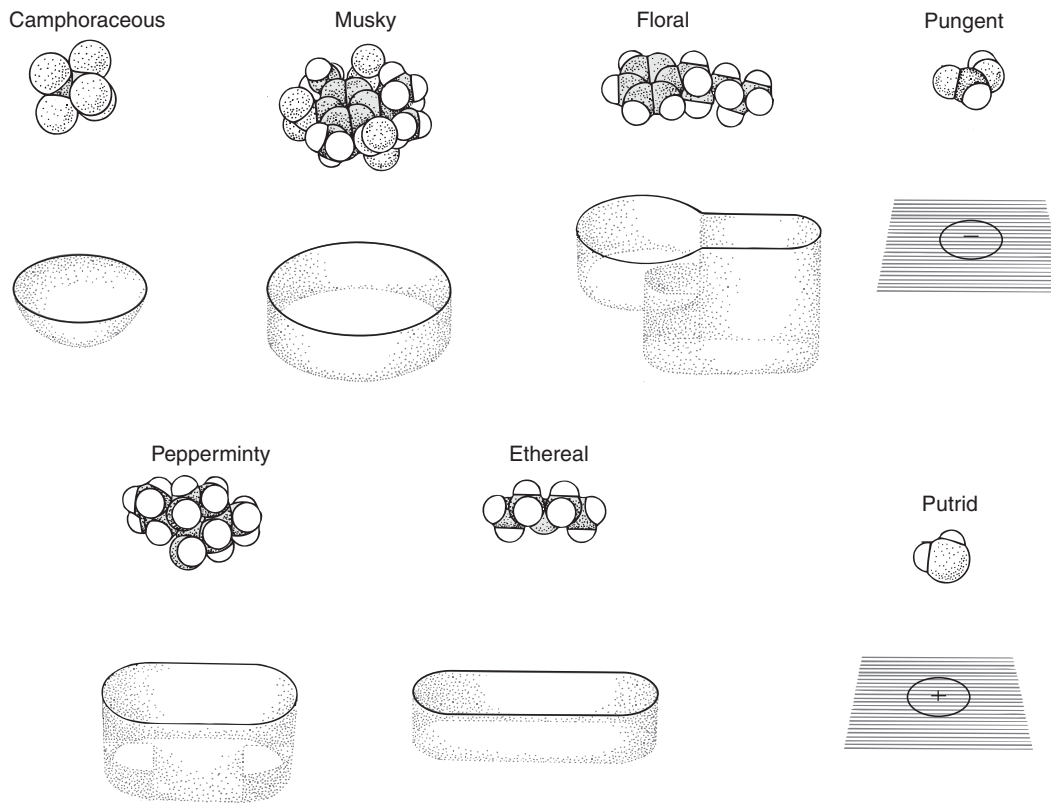
Many theories of odor have been proposed, but few have persisted very long. Strangely enough, one of the oldest theories, although in modern dress, is still the most current theory. Lucretius, one of the early Greek atomists, suggested that substances having odor gave off a vapor of tiny "atoms," all of the same shape and size, and that these atoms gave rise to the perception of odor when they entered pores in the nose. The pores would have to be of various shapes, and the odor perceived would depend on which pores the atoms were able to enter. We now have many similar theories about the action of drugs (receptor-site theory) and the interaction of enzymes with their substrates (the lock-and-key hypothesis).

A substance must have certain physical characteristics to have the property of odor. First, it must be volatile enough to give off a vapor that can reach the nostrils. Second, once it reaches the nostrils, it must be somewhat water soluble, even if only to a small degree, so that it can pass through the layer of moisture (mucus) that covers the nerve endings in the olfactory area. Third, it must have lipid solubility to allow it to penetrate the lipid (fat) layers that form the surface membranes of the nerve cell endings.

Once we pass these criteria, we come to the heart of the question. Why do substances have different odors? In 1949, R. W. Moncrieff, a Scot, resurrected Lucretius' hypothesis. He proposed that in the olfactory area of the nose is a system of receptor cells of several types and shapes. He further suggested that each receptor site corresponded to a different type of primary odor. Molecules that fit these receptor sites would display the characteristics of that primary odor. It would not be necessary for the entire molecule to fit into the receptor, so for larger molecules, any portion might fit into the receptor and activate it. Molecules having complex odors would presumably be able to activate several different types of receptors.

Moncrieff's hypothesis has been strengthened substantially by the work of J. E. Amoore, who began studying the subject as an undergraduate at Oxford in 1952. After an extensive search of the chemical literature, Amoore concluded that there were only seven basic primary odors. By sorting molecules with similar odor types, he even formulated possible shapes for the seven necessary receptors. For instance, from the literature he culled more than 100 compounds that were described as having a "camphoraceous" odor. Comparing the sizes and shapes of all these molecules, he postulated a three-dimensional shape for a camphoraceous receptor site. Similarly, he derived shapes for the other six receptor sites. The seven primary receptor sites he formulated are shown in the figure below, along with a typical prototype molecule having the appropriate shape to fit the receptor. The shapes of the sites are shown in perspective. Pungent and putrid odors were not thought to require a particular shape in the odorous molecules, but, rather, to need a particular type of charge distribution.

You can verify quickly that compounds with molecules of roughly similar shape have similar odors if you compare nitrobenzene and acetophenone with benzaldehyde or *d*-camphor and hexachloroethane with cyclooctane. Each group of substances has the same basic odor *type* (primary), but the individual molecules differ in the *quality* of the odor. Some of the odors are sharp, some pungent, others sweet, and so on. The second group of substances all have a camphoraceous odor, and the molecules of these substances all have approximately the same shape.

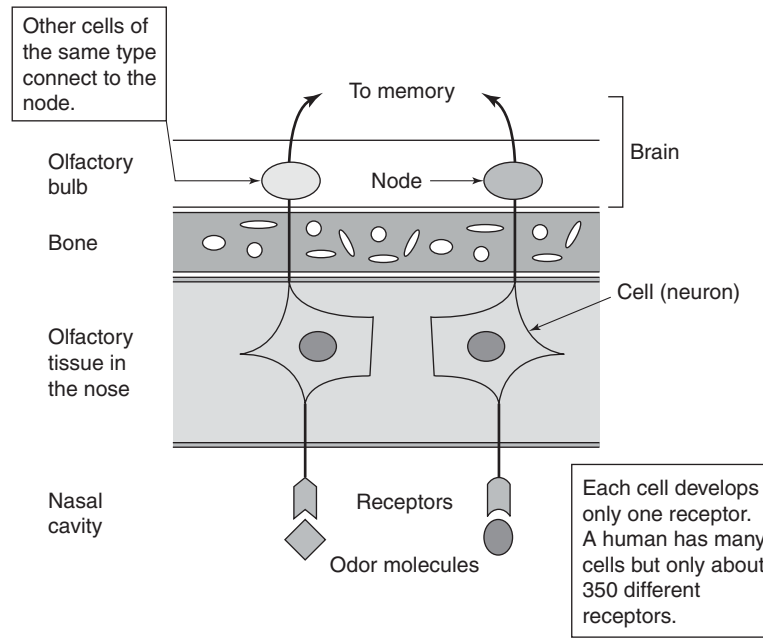


From "The Stereochemical Theory of Odor," by J. E. Amoore, J. W. Johnston Jr., and M. Rubin. Copyright © 1964 by Scientific American, Inc. All rights reserved.

An interesting corollary to Amoore's theory is the postulate that if the receptor sites are chiral, then optical isomers (enantiomers) of a given substance might have *different* odors. This circumstance proves true in several cases. It is true for (−)- and (+)-carvone; we investigate the idea in Experiment 14 in this textbook.

The theory of odor changed dramatically in 1991 as a result of the biochemical research of Richard Axel and Linda Buck, who was a postdoctoral student in Axel's research group. Subsequently, Buck founded her own group that continued research on the nature of the sense of smell. In 2004, Axel and Buck won the Nobel Prize in Physiology or Medicine for their combined work during the previous decade.

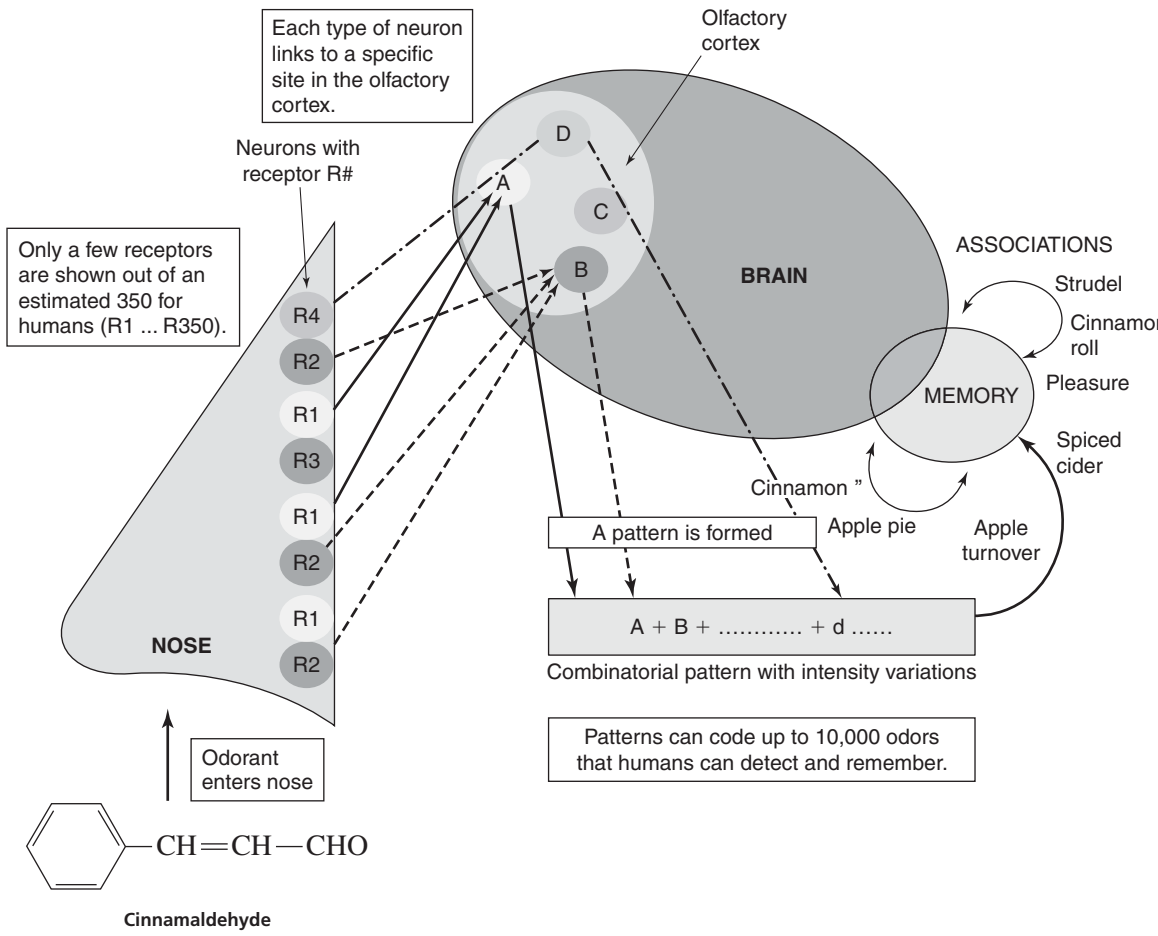
The 1991 paper, based on research conducted with mice, described a family of membrane-spanning receptor proteins found in a small area of the upper nose called the olfactory epithelium. Mice have genes that can encode as many as 1000 types of receptor proteins. Subsequent work has estimated that humans, who have a lesser-developed sense of smell than mice, encode only about 350 of these receptor proteins. Each of these protein receptors is located on the surface of the olfactory epithelium and is connected to a single nerve cell (neuron) located in the epithelium. The neuron "fires" or sends a signal when an odorant molecule binds to the active site of the protein. The signal is carried across the bones of the skull and into a node in an area of the brain called the olfactory bulb. The signals from all receptors are processed in the olfactory bulb and sent to the memory area of the brain where recognition of the odor takes place. The figure below (Odor Receptors in the Nose) shows a schematic of the olfactory region.



Odor receptors in the nose.

The signals from all of the types of protein receptors are collected, or integrated, in the olfactory bulb. The node (a postulated feature) is a common connection where the signals from each type of cell are collected and sent to memory, each with an intensity proportional to the numbers of cells that were stimulated by the odorant molecules. Because a given odorant molecule should be capable of binding to more than one type of receptor and because many odors are composed of more than one type of molecule, the signal sent to memory should be a complex combinatorial pattern consisting of contributions from several nodes, each with a different intensity value. This system should allow a human to recognize as many as 10,000 odors and for mice to recognize many more. The memory region in the brain can also make associations based on a given pattern. For instance, cinnamaldehyde can be recognized as the odor of the spice cinnamon, but it can also be associated with other items such as apple pie, cinnamon rolls, apple strudel, spiced cider, and, of course, pleasure. A figure showing these associations, but limited in that only a few receptors are represented, is shown in the figure Nobel Prize Theory.

Although our modern understanding of the detection of odor has evolved to become a more highly detailed theory than the one proposed by Lucretius, it would appear that his fundamental hypothesis was correct and has even withstood the scrutiny of modern science.



Nobel prize theory of the detection of odors (Axel and Buck, 2004).

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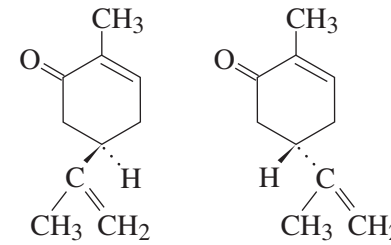
14 EXPERIMENT 14

Spearmint and Caraway Oil: (+)- and (–)-Carvones

Stereochemistry
Gas chromatography
Polarimetry
Spectroscopy
Refractometry

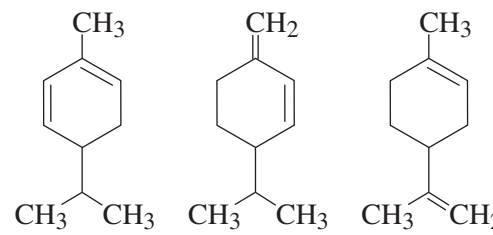


(R)-(–)-Carvone from spearmint oil **(S)-(+)-Carvone from caraway oil**

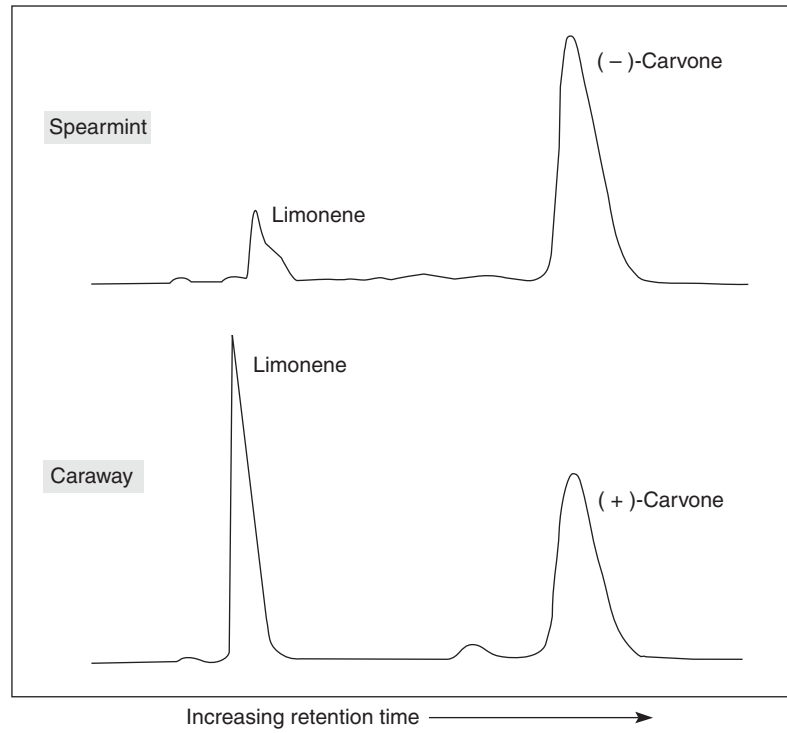
In this experiment, you will compare (+)-carvone from caraway oil to (–)-carvone from spearmint oil, using gas chromatography. If you have the proper preparative-scale gas-chromatographic equipment, it should be possible to prepare pure samples of each of the carvones from their respective oils. If this equipment is not available, your instructor will provide pure samples of the two carvones obtained from a commercial source, and any gas chromatographic work will be strictly analytical.

The odors of the two enantiomeric carvones are distinctly different from each other. The presence of one or the other isomer is responsible for the characteristic odors of each oil. The difference in the odors is to be expected, because the odor receptors in the nose are chiral (see essay "Stereochemical Theory of Odor," that precedes this experiment). This phenomenon, in which a chiral receptor interacts differently with each of the enantiomers of a chiral compound, is called **chiral recognition**.

Although we should expect the optical rotations of the isomers (enantiomers) to be of opposite sign, the other physical properties should be identical. Thus, for both (+)- and (–)-carvones, we predict that the infrared and nuclear magnetic resonance spectra, the gas-chromatographic retention times, the refractive indices, and the boiling points will be identical. Hence, the only differences in properties you should observe for the two carvones are the odors and the signs of rotation in a polarimeter.



α-Phellandrene **β-Phellandrene** **Limonene**



Caraway oil contains mainly limonene and (+)-carvone. The gas chromatogram for this oil is shown in the figure above. The (+)-carvone (bp 203°C) can easily be separated from the lower-boiling limonene (bp 177°C) by gas chromatography, as shown in the figure. If one has a preparative gas chromatograph, the (+)-carvone and limonene can be collected separately as they elute from the gas chromatography column. **Spearmint oil** contains mainly (–)-carvone with a smaller amount of limonene and very small amounts of the lower-boiling terpenes, α- and β-phellandrene. The gas chromatogram for this oil is also shown in the figure. With preparative equipment, you can easily collect the (–)-carvone as it exits the column. It is more difficult, however, to collect limonene in a pure form. It is likely to be contaminated with the other terpenes, because they all have similar boiling points.

REQUIRED READING

Review:	Technique 25	Infrared Spectroscopy
New:	Technique 22	Gas Chromatography Technique
	Technique 23	Polarimetry
	Essay	Stereochemical Theory of Odor

If performing any of the optional procedures, read, as appropriate:

Technique 13	Physical Constants of Liquids, Boiling Points
Technique 24	Refractometry

Technique 26	Nuclear Magnetic Resonance Spectroscopy
Technique 27	Carbon-13 Nuclear Magnetic Resonance Spectroscopy

SPECIAL INSTRUCTIONS

Your instructor will either assign you spearmint or caraway oil, or have you choose one. You will also be given instructions on which procedures from Part A you are to perform. You should compare your data with those of someone who has studied the other enantiomer.

NOTE: If a gas chromatograph is not available, this experiment can be performed with spearmint and caraway oils and pure commercial samples of the (+)- and (–)-carvones.

If the proper equipment is available, your instructor may require you to perform a gas-chromatographic analysis. If preparative gas chromatography is available, you will be asked to isolate the carvone from your oil (Part B). Otherwise, if you are using analytical equipment, you will be able to compare only the retention times and integrals from your oil to those of the other essential oil.

Although preparative gas chromatography will yield enough sample to do spectra, it will not yield enough material to do the polarimetry. Therefore, if you are required to determine the optical rotation of the pure samples whether or not you perform preparative gas chromatography, your instructor will provide a prefilled polarimeter tube for each sample.

NOTES TO THE INSTRUCTOR

This experiment may be scheduled along with another experiment. It is best if students work in pairs, each student using a different oil. An appointment schedule for using the gas chromatograph should be arranged so that students are able to make efficient use of their time. You should prepare chromatograms using both carvone isomers and limonene as reference standards. Appropriate reference standards include a mixture of (+)-carvone and limonene and a second mixture of (–)-carvone and limonene. The chromatograms should be posted with retention times, or each student should be provided with a copy of the appropriate chromatogram.

The gas chromatograph should be prepared as follows: column temperature, 200°C; injection and detector temperature, 210°C; carrier gas flow rate, 20mL/min. The recommended column is 8 feet long with a stationary phase such as Carbowax 20M. It is convenient to use a Gow-Mac 69-350 instrument with the preparative accessory system for this experiment.

You should fill polarimeter cells (0.5 dm) in advance with the undiluted (+)- and (–)-carvones. There should also be four bottles containing spearmint and caraway oils and (+)- and (–)-carvone. Both enantiomers of carvone are commercially available.

PROCEDURE

Part A. Analysis of the Carvones

The samples (either those obtained from gas chromatography, Part B, or commercial samples) should be analyzed by the following methods. Your instructor will indicate which methods to use. Compare your results with those obtained by someone who used a different oil. In addition, measure the observed rotation of the commercial samples of (+)-carvone and (–)-carvone. Your instructor will supply prefilled polarimeter tubes.

Analyses to Be Performed on Spearmint and Caraway Oils

Odor. Carefully smell the containers of spearmint and caraway oil and of the two carvones. About 8–10% of the population cannot detect the difference in the odors of the optical isomers. Most people, however, find the difference quite obvious. Record your impressions.

Analytical Gas Chromatography. If you separated your sample by preparative gas chromatography in Part B, you should already have your chromatogram. In this case, you should compare it to one done by someone using the other oil. Be sure to obtain retention times and integrals or obtain a copy of the other person's chromatogram.

If you did not perform Part B, obtain the analytical gas chromatograms of your assigned oil—spearmint or caraway—and obtain the result from the other oil from someone else. Your instructor may prefer to perform the sample injections or have a laboratory assistant perform them. The sample injection procedure requires careful technique, and the special microliter syringes that are required are very delicate and expensive. If you are to perform the injections yourself, your instructor will give you adequate instruction beforehand.

For both oils, determine the retention times of the components (see Technique 22, Section 22.7). Calculate the percentage composition of the two essential oils by one of the methods explained in the section.

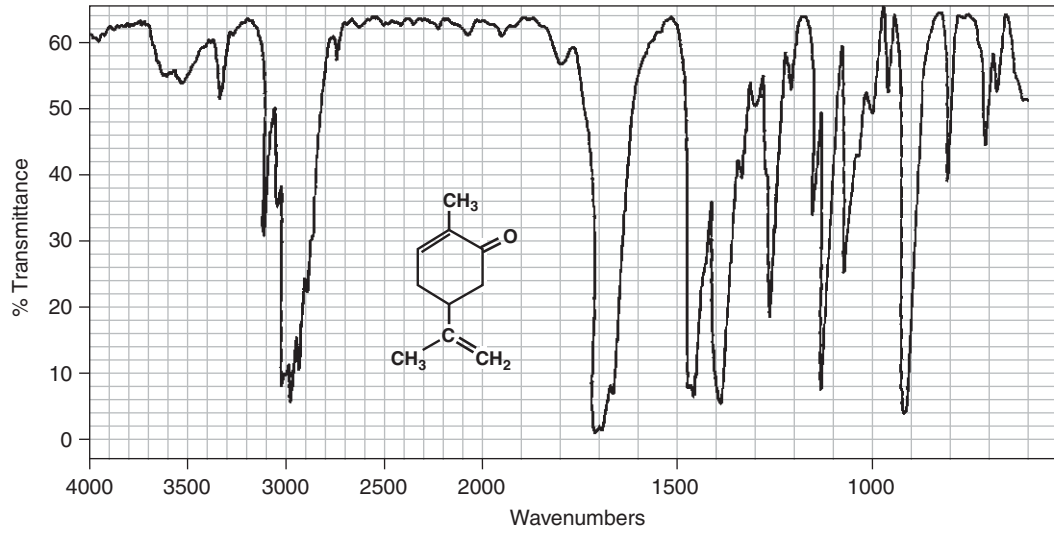
Analyses to Be Performed on the Purified Carvones

Polarimetry. With the help of the instructor or assistant, obtain the observed optical rotation α of the pure (+)-carvone and (–)-carvone samples. These are provided in prefilled polarimeter tubes. The specific rotation $[\alpha]_D$ is calculated from the first equation in the Technique 23, Section 23.2. The concentration c will equal the density of the substances analyzed at 20°C. The values, obtained from actual commercial samples, are 0.968 g/mL for (+)-carvone and 0.9593 g/mL for (–)-carvone. The literature values for the specific rotations are as follows: $[\alpha]_D^{20} = +61.7°$ for (+)-carvone and –62.5° for (–)-carvone. These values are not identical because trace amounts of impurities are present.

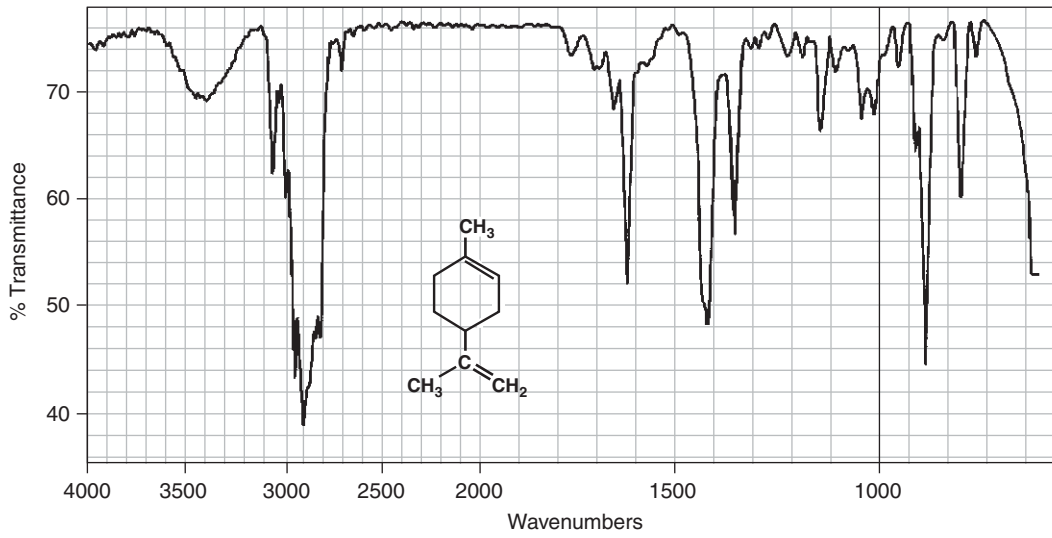
Polarimetry does not work well on the crude spearmint and caraway oils because of the presence of large amounts of limonene and other impurities.

Infrared Spectroscopy. Obtain the infrared spectrum of the (–)-carvone sample from spearmint or of the (+)-carvone sample from caraway (see Technique 25, Section 25.2). Compare your result with that of a person working with the other isomer. If your instructor requests it, obtain the Infrared spectrum of the (+)-limonene, which is found in both oils. If possible, determine all spectra using neat samples. If you isolated the sample by preparative gas chromatography, it may be necessary to add 1–2 drops of carbon tetrachloride to the sample. Thoroughly mix the liquids by drawing the mixture into a Pasteur pipet and expelling several times. It may be helpful to draw the end of the pipet to a narrow tip in order

to withdraw all the liquid in the conical vial. As an alternative, use a microsyringe. Obtain a spectrum on this solution, as described in Technique 25, Section 25.7.

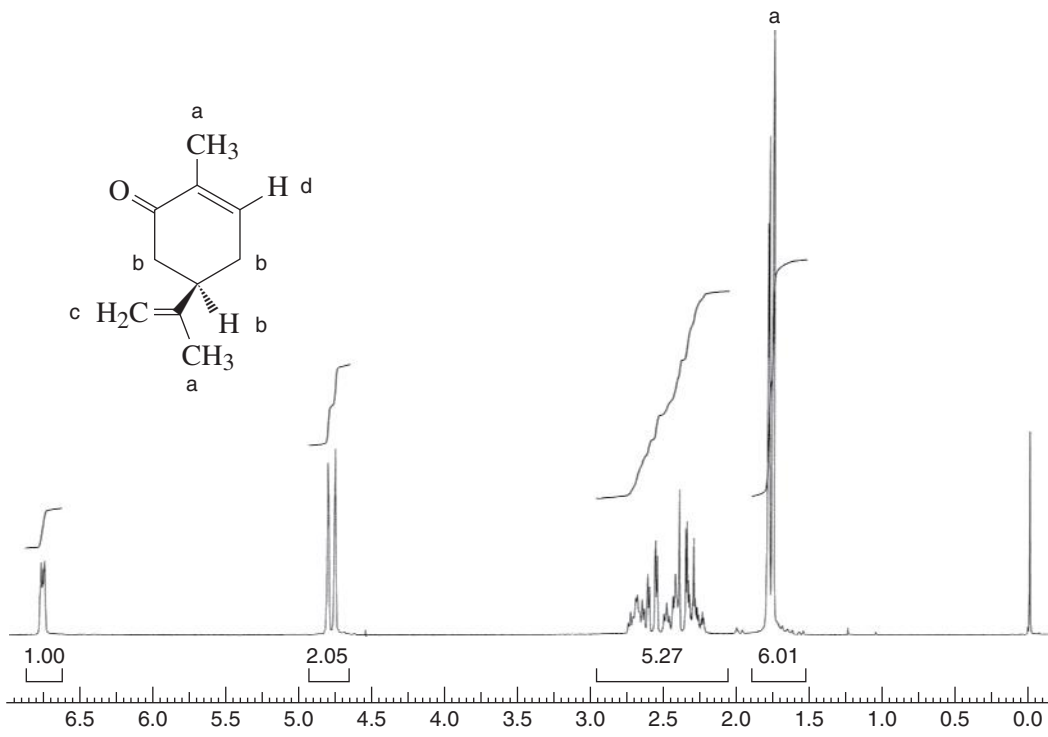


Infrared Spectrum of carvone (neat).

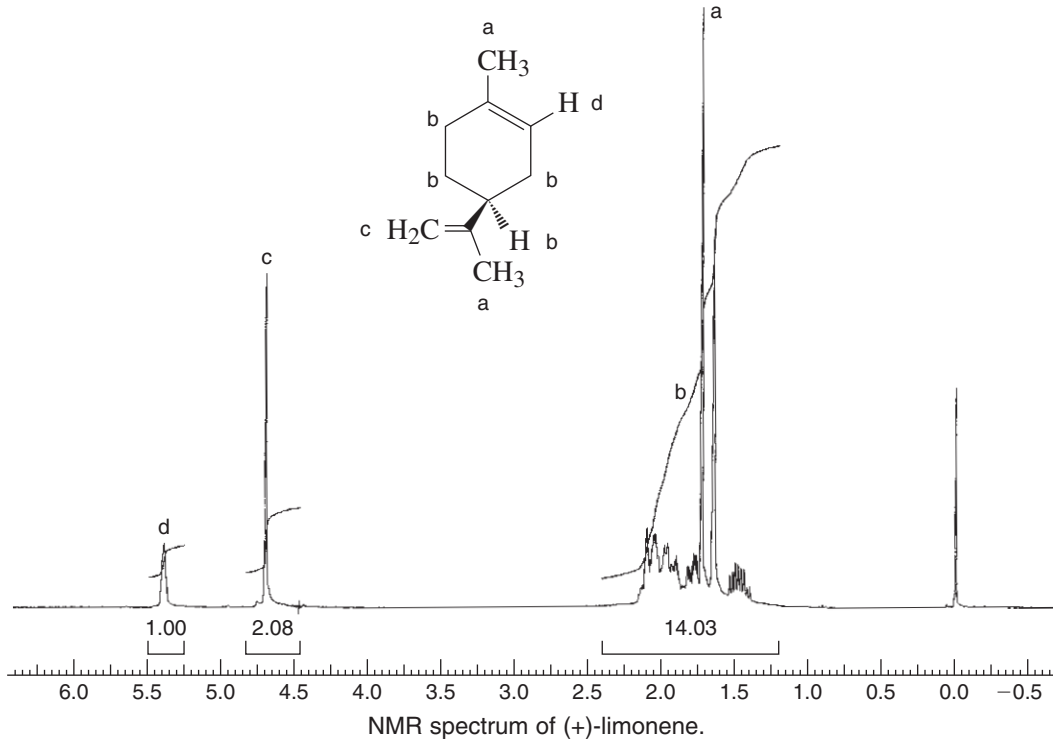


Infrared spectrum of limonene (neat).

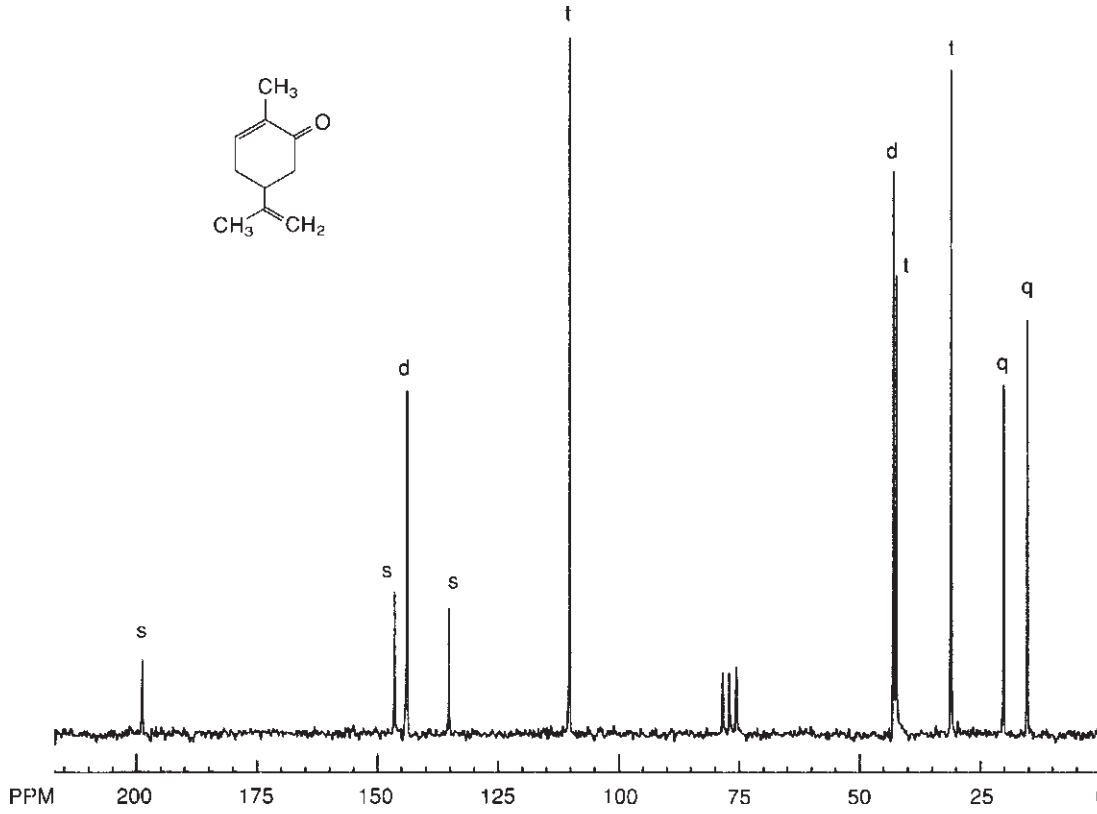
Nuclear Magnetic Resonance Spectroscopy. Using an NMR instrument, obtain a proton NMR spectrum of your carvone. Compare your spectrum with the NMR spectra for (–)-carvone and (+)-limonene shown in this experiment. Attempt to assign as many peaks as you can. If your NMR instrument is capable of obtaining a carbon-13 NMR spectrum, determine a carbon-13 spectrum. Compare your spectrum of carvone with the carbon-13 NMR spectrum shown in this experiment. Once again, attempt to assign the peaks.



NMR spectrum of (–)-carvone from spearmint oil.



NMR spectrum of (+)-limonene.



Decoupled carbone-13 spectrum of carvone, $CDCl_3$. Letters indicate appearance of spectrum when carbons are coupled to protons (s = singlet, d = doublet, t = triplet, q = quartet).

Boiling Point. Determine the boiling point of the carvone you were assigned. Use the micro boiling-point technique (see Technique 13, Section 13.2). The boiling points for both carvones are 230°C at atmospheric pressure. Compare your result to that of someone using the other carvone.

Refractive Index. Use the technique for obtaining the refractive index on a small volume of liquid, as described in Technique 24, Section 24.2. Obtain the refractive index for the carvone you separated (Part B) or for the one assigned. Compare your value to that obtained by someone using the other isomer. At 20°C, the (+)- and (–)-carvones have the same refractive index, equal to 1.4989.

Part B. Separation by Gas Chromatography (Optional)

The instructor may prefer to perform the sample injections or have a laboratory assistant perform them. The sample injection procedure requires careful technique, and the special microliter syringes that are required are delicate and expensive. If you are to perform the sample injections, your instructor will give you adequate instruction beforehand.

Inject 50μL of caraway or spearmint oil onto the gas-chromatography column. Just before a component of the oil (limonene or carvone) elutes from the column, install a gas-collection tube at the exit port, as described in Technique 22, Section 22.11. To determine when to connect the gas-collection tube, refer to the chromatograms prepared by your instructor. These chromatograms have been run on the same instrument you are using under the same conditions. Ideally, you should connect the gas-collection tube just before the limonene or carvone elutes from the column. You should remove the tube as soon as all the

component has been collected, but before any other compound begins to elute from the column. You can accomplish this most easily by watching the recorder as your sample passes through the column. The collection tube is connected (if possible) just before a peak is produced, or as soon as a deflection is observed. When the pen returns to the baseline, remove the gas-collection tube.

This procedure is relatively easy for collecting the carvone component of both oils and for collecting the limonene in caraway oil. Because of the presence of several terpenes in spearmint oil, it is somewhat more difficult to isolate a pure sample of limonene from spearmint oil (see chromatogram). In this case, you must try to collect only the limonene component and not any other compounds, such as the terpene, which produces a shoulder on the limonene peak in the chromatogram for spearmint oil.

After collecting the samples, insert the ground joint of the collection tube into a 0.1-mL conical vial, using an O-ring and screw cap to fasten the two pieces together securely. Place this assembly into a test tube, as shown in Figure 22.11. Put cotton on the bottom of the tube and use a rubber septum cap at the top to hold the assembly in place and to prevent breakage. Balance the centrifuge by placing a tube of equal weight on the opposite side (this could be your other sample or someone else's sample). During centrifugation, the sample is forced into the bottom of the conical vial. Disassemble the apparatus, cap the vial, and perform the analyses described in Part A. You should have enough sample to perform the infrared and NMR spectroscopy, but your instructor may need to provide additional sample to perform the other procedures.

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QUESTIONS

1. Interpret the infrared spectra for carvone and limonene and the proton and carbon-13 NMR spectra of carvone.
2. Identify the chiral centers in α-phellandrene, β-phellandrene, and limonene.
3. Explain how carvone fits the isoprene rule (see essay "Terpenes and Phenylpropanoids," that precedes Experiment 13).
4. Using the Cahn–Ingold–Prelog sequence rules, assign priorities to the groups around the chiral carbon in carvone. Draw the structural formulas for (+)- and (–)-carvone with the molecules oriented in the correct position to show the R and S configurations.
5. Explain why limonene elutes from the column before either (+)- or (–)-carvone.
6. Explain why the retention times for both carvone isomers are the same.
7. The toxicity of (+)-carvone in rats is about 400 times greater than that of (–)-carvone. How do you account for this?

ESSAY

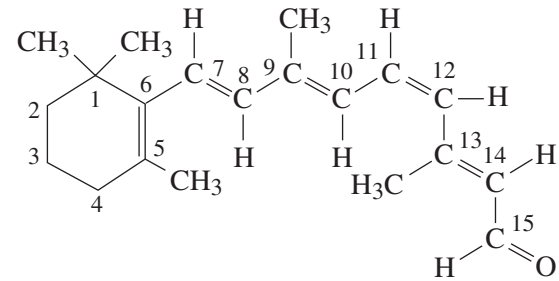
The Chemistry of Vision

An interesting and challenging topic for chemists to investigate is how the eye functions. What chemistry is involved in detection of light and transmission of that information to the brain? The first definitive studies on how the eye functions were begun in 1877 by Franz Boll. Boll demonstrated that the red color of the retina of a frog's eye could be bleached yellow by strong light. If the frog was then kept in the dark, the red color of the retina slowly returned. Boll recognized that a bleachable substance had to be connected somehow with the ability of the frog to perceive light.

Most of what is now known about the chemistry of vision is the result of the elegant work of George Wald, Harvard University; his studies, which began in 1933, ultimately resulted in his receiving the Nobel Prize in biology. Wald identified the sequence of chemical events during which light is converted into some form of electrical information that can be transmitted to the brain. Here is a brief outline of that process.

The retina of the eye is made up of two types of photoreceptor cells: **rods** and **cones**. The rods are responsible for vision in dim light, and the cones are responsible for color vision in bright light. The same principles apply to the chemical functioning of the rods and the cones; however, the details of that functioning are less well understood for the cones than for the rods.

Each rod contains several million molecules of **rhodopsin**. Rhodopsin is a complex of a protein, **opsin**, and a molecule derived from Vitamin A, 11-*cis*-retinal (sometimes called **retinene**). Little is known about the structure of opsin. The structure of 11-*cis*-retinal is shown here.

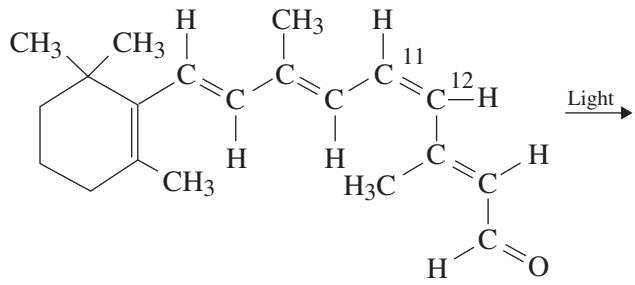


11-*cis*-Retinal

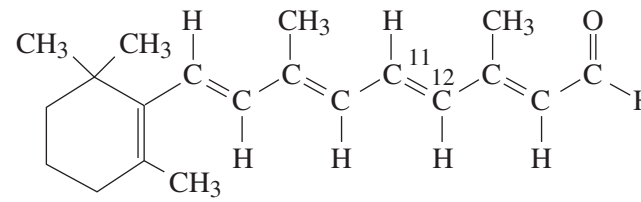
The detection of light involves the initial conversion of 11-*cis*-retinal to its all-*trans* isomer. This is the only obvious role of light in this process. The high energy of a quantum of visible light promotes the fission of the π bond between carbons 11 and 12. When the π bond breaks, free rotation about the σ bond in the resulting radical is possible. When the π bond re-forms after such rotation, all-*trans*-retinal results. All-*trans*-retinal is more stable than 11-*cis*-retinal, which is the reason the isomerization proceeds spontaneously in the direction shown.

The two molecules have different shapes because of their different structures. The 11-*cis*-retinal has a fairly curved shape, and the parts of the molecule on either side of the *cis* double bond tend to lie in different planes. Because proteins have complex and specific three-dimensional shapes (tertiary structures), 11-*cis*-retinal associates with the protein opsin in a particular manner. All-*trans*-retinal has an elongated shape, and the entire molecule tends to lie in a single plane. This different shape for the molecule, compared with that for the 11-*cis* isomer, means that all-*trans*-retinal will have a different association with the protein opsin.

In fact, all-*trans*-retinal associates very weakly with opsin because its shape does not fit the protein. Consequently, the next step after the isomerization of retinal is the dissociation of all-*trans*-retinal from opsin. The opsin protein undergoes a simultaneous change in conformation as the all-*trans*-retinal dissociates.

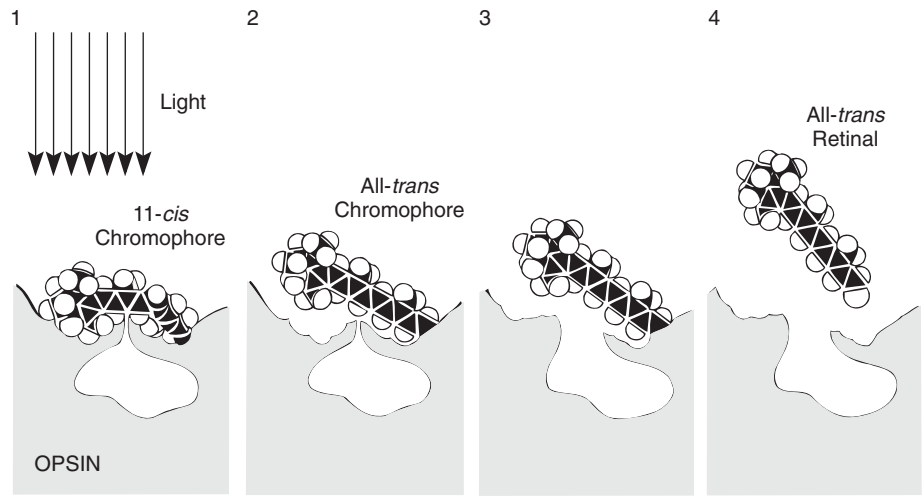


11-*cis*-Retinal

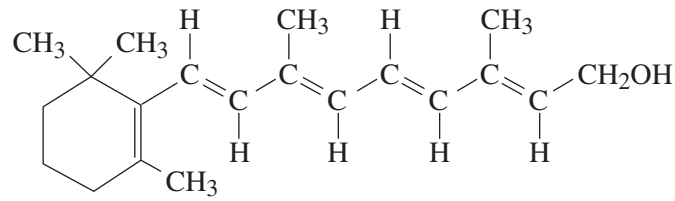


All-*trans*-Retinal

At some time after the 11-*cis*-retinal–opsin complex receives a photon, a message is received by the brain. It was originally thought that either the isomerization of 11-*cis*-retinal to all-*trans*-retinal or the conformational change of the opsin protein was an event that generated the electrical message sent to the brain. Current research, however, indicates that both these events occur too slowly relative to the speed with which the brain receives the message. Current hypotheses invoke involved quantum mechanical explanations, which regard it as significant that the chromophores (light-absorbing groups) are arranged in a very precise geometrical pattern in the rods and cones, allowing the signal to be transmitted rapidly through space. The main physical and chemical events Wald discovered are illustrated in the Figure below for easy visualization. The question of how the electrical signal is transmitted still remains unsolved.

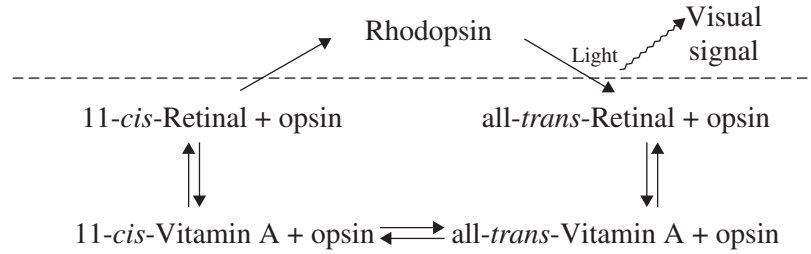


Wald was also able to explain the sequence of events by which the rhodopsin molecules are regenerated. After dissociation of all-*trans*-retinal from the protein, the following enzyme-mediated changes occur. All-*trans*-retinal is reduced to the alcohol all-*trans*-retinol, also called all-*trans*-Vitamin A.



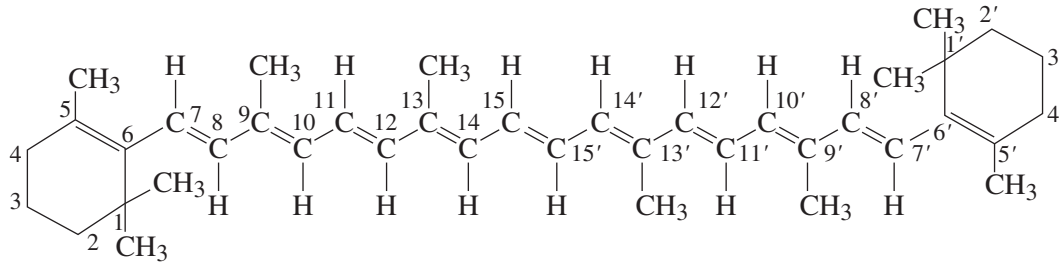
All-*trans*-Vitamin A

All-*trans*-Vitamin A is then isomerized to its 11-*cis*-Vitamin A isomer. After the isomerization, the 11-*cis*-Vitamin A is oxidized back to 11-*cis*-retinal, which forthwith recombines with the opsin protein to form rhodopsin. The regenerated rhodopsin is then ready to begin the cycle anew, as illustrated in the figure.



By this process, as little light as 10^{-14} of the number of protons emitted from a typical flashlight bulb can be detected. The conversion of light into isomerized retinal exhibits an extraordinarily high quantum efficiency. Virtually every quantum of light absorbed by a molecule of rhodopsin causes the isomerization of 11-*cis*-retinal to all-*trans*-retinal.

As you can see from the reaction scheme, the retinal derives from Vitamin A, which requires merely the oxidation of a—CH_2OH group to a—CHO group to be converted to retinal. The precursor in the diet that is transformed to Vitamin A is β-carotene. The β-carotene is the yellow pigment of carrots and is an example of a family of long-chain polyenes called **carotenoids**.



***β*-Carotene**

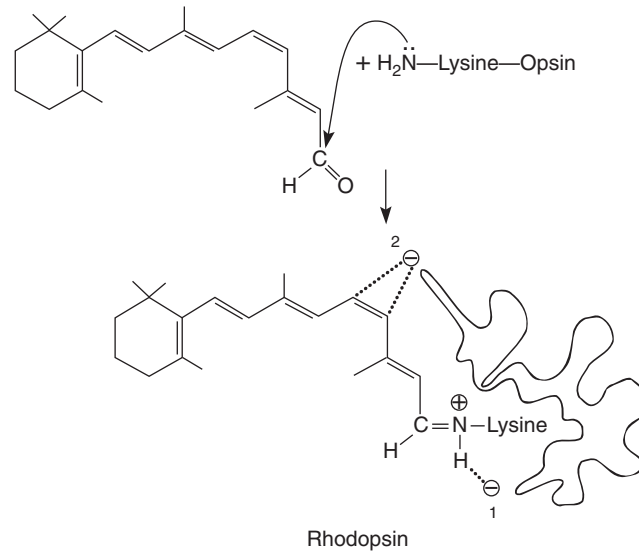
In 1907, Willstätter established the structure of carotene, but it was not known until 1931–1933 that there were actually three isomers of carotene. The α-carotene differs from β-carotene in that the α isomer has a double bond between C_4 and C_5 rather than between C_5 and C_6, as in the β isomer. The γ isomer has only one ring, identical to the ring in the β isomer, whereas the other ring is opened in the γ form between C_1' and C_6'. The β isomer is by far the most common of the three.

The substance β-carotene is converted to Vitamin A in the liver. Theoretically, one molecule of β-carotene should give rise to two molecules of this vitamin by cleavage of the C_{15}–C_{15}' double bond, but actually only one molecule of Vitamin A is produced from each molecule of carotene. The Vitamin A thus produced is converted to 11-*cis*-retinal within the eye.

Along with the problem of how the electrical signal is transmitted, color perception is also currently under study. In the human eye, there are three kinds of cone cells, which absorb light at 440, 535, and 575 nm, respectively. These cells discriminate among the primary colors. When combinations of them are stimulated, full-color vision is the message received in the brain.

Because all these cone cells use 11-*cis*-retinal as a substrate trigger, it has long been suspected that there must be three different opsin proteins. Recent work has begun to establish how the opsins vary the spectral sensitivity of the cone cells, even though all of them have the same kind of light-absorbing chromophore.

Retinal is an aldehyde, and it binds to the terminal amino group of a lysine residue in the opsin protein to form a Schiff base, or imine linkage (>C=N–). This imine linkage is believed to be protonated (with a plus charge) and to be stabilized by being located near a negatively charged amino acid residue of the protein chain. A second negatively charged group is thought to be located near the 11-*cis* double bond. Researchers have recently shown, from synthetic models that use a simpler protein than opsin itself, that forcing these negatively charged groups to be located at different distances from the imine linkage causes the absorption maximum of the 11-*cis*-retinal chromophore to be varied over a wide enough range to explain color vision.



Rhodopsin

Whether there are actually three different opsin proteins, or whether there are just three different conformations of the same protein in the three types of cone cells, will not be known until further work is completed on the structure of the opsin or opsins.

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15 EXPERIMENT 15

Isolation of Chlorophyll and Carotenoid Pigments from Spinach

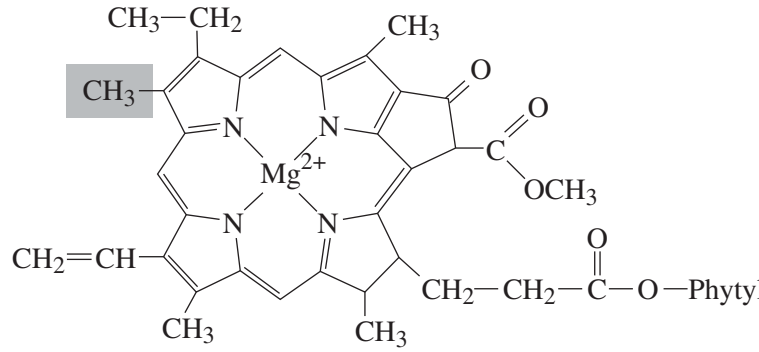
Isolation of a natural product

Extraction

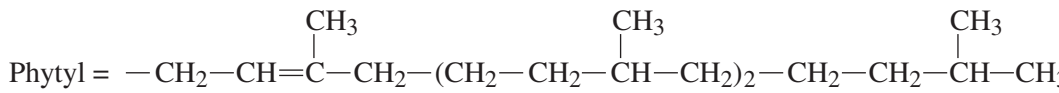
Column chromatography

Thin-layer chromatography

Photosynthesis in plants takes place in organelles called **chloroplasts**. Chloroplasts contain a number of colored compounds (pigments) that fall into two categories: **chlorophylls** and **carotenoids**.



Chlorophyll *a*



Carotenoids are yellow pigments that are also involved in the photosynthetic process. The structures of α and **β-carotene** are given in the essay preceding this experiment. In addition, chloroplasts also contain several oxygen-containing derivatives of carotenes, called **xanthophylls**.

In this experiment, you will extract the chlorophyll and carotenoid pigments from spinach leaves using acetone as the solvent. The pigments will be separated by column chromatography using alumina as the adsorbent. Increasingly polar solvents will be used to elute the various components from the column. The colored fractions collected will then be analyzed using thin-layer chromatography. It should be possible for you to identify most of the pigments already discussed on your thin-layer plate after development.

Chlorophylls are the green pigments that act as the principal photoreceptor molecules of plants. They are capable of absorbing certain wavelengths of visible light that are then converted by plants into chemical energy. Two forms of these pigments found in plants are **chlorophyll *a*** and **chlorophyll *b*.** The two forms are identical, except that the methyl group that is shaded in the structural formula

of chlorophyll *a* is replaced by a —CHO group in chlorophyll *b.* **Pheophytin *a*** and **pheophytin *b*** are identical to chlorophyll *a* and chlorophyll *b,* respectively, except that in each case the magnesium ion, Mg^{2+}, has been replaced by two hydrogen ions, $2H^{+}$.

REQUIRED READING

Sign in at www .cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	Techniques 5 and 6	
	Technique 7	Reaction Methods, Section 7.9
	*Technique 12	Extractions, Separations, and Drying Agents, Sections 12.5 and 12.9
	Technique 20	Thin-Layer Chromatography
New:	*Technique 19	Column Chromatography
	Essay	The Chemistry of Vision

SPECIAL INSTRUCTIONS

Hexane and acetone are both highly flammable. Avoid the use of flames while working with these solvents. Perform the thin-layer chromatography in the hood. The procedure calls for a centrifuge tube with a tight-fitting cap. If this is not available, you can use a vortex mixer for mixing the liquids. Another alternative is to use a cork to stopper the tube; however, the cork will absorb some liquid.

Fresh spinach is preferable to frozen spinach. Because of handling, frozen spinach contains additional pigments that are difficult to identify. Because the pigments are light-sensitive and can undergo air oxidation, you should work quickly. Samples should be stored in closed containers and kept in the dark when possible. The column chromatography procedure takes less than 15 minutes to perform and cannot be stopped until it is completed. It is important, therefore, that all of the materials needed for this part of the experiment are prepared in advance and that you are thoroughly familiar with the procedure before running the column. If you need to prepare the 70% hexane–30% acetone solvent mixture, be sure to mix it thoroughly before using.

SUGGESTED WASTE DISPOSAL

Dispose of all organic solvents in the container for nonhalogenated organic solvents. Place the alumina in the container designated for wet alumina.

NOTES TO THE INSTRUCTOR

The column chromatography should be performed with activated alumina from EM Science (No. AX0612-1). The particle sizes are 80–120 mesh, and the material is Type F-20. Dry the alumina overnight in an oven at 110°C and store it in a tightly sealed bottle. Alumina more than several years may need to be dried for a longer time at a higher temperature. Depending on how dry the alumina is, solvents of different polarity will be required to elute the components from the column.

For thin-layer chromatography, use flexible silica-gel plates from Whatman with a fluorescent indicator (No. 4410 222). If the TLC plates have not been purchased recently, place them in an oven at 110°C for 30 minutes and store them in a desiccator until use.

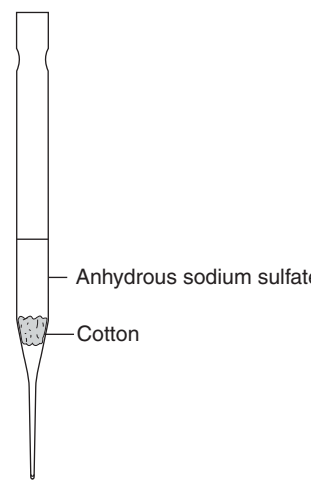
If you use different alumina or different thin-layer plates, try out the experiment before having students conduct it in class. Materials other than those specified here may give different results than indicated in this experiment.

PROCEDURE

Part A. Extraction of the Pigments

Weigh about 0.5 g of fresh (or 0.25 g of frozen) spinach leaves (avoid using stems or thick veins). Fresh spinach is preferable, if available. If you must use frozen spinach, dry the thawed leaves by pressing them between several layers of paper towels. Cut or tear the spinach leaves into small pieces and place them in a mortar along with 1.0 mL of cold acetone. Grind with a pestle until the spinach leaves have been broken into particles too small to be seen clearly. If too much acetone has evaporated, you may need to add an additional portion of acetone (0.5–1.0 mL) to perform the following step. Using a Pasteur pipet, transfer the mixture to a centrifuge tube. Rinse the mortar and pestle with 1.0 mL of cold acetone and transfer the remaining mixture to the centrifuge tube. Centrifuge the mixture (be sure to balance the centrifuge). Using a Pasteur pipet, transfer the liquid to a centrifuge tube with a tight-fitting cap (see "Special Instructions" if one is not available).

Add 2.0 mL of hexane to the tube, cap the tube, and shake the mixture thoroughly. Then add 2.0 mL of water and shake thoroughly with occasional venting. Centrifuge the mixture to break the emulsion, which usually appears as a cloudy, green layer in the middle of the mixture. Remove the bottom aqueous layer with a Pasteur pipet. Using a Pasteur pipet, prepare a column containing anhydrous sodium sulfate to dry the remaining hexane layer, which contains the dissolved pigments. Put a plug of cotton into a Pasteur pipet (5¾-inch) and tamp it into position using a glass rod. The correct position of the cotton is shown in the Figure on this page. Add about 0.5 g of powdered or granular anhydrous sodium sulfate and tap the column with your finger to pack the material.



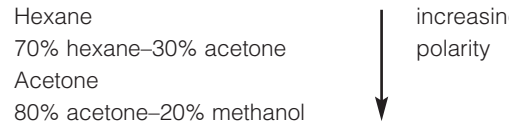
Column for drying extract.

Clamp the column in a vertical position and place a dry test tube (13-mm × 100-mm) under the bottom of the column. Label this test tube with an *E* for *extract* so that you don't confuse it with the test tubes you will be working with later in this experiment. With a Pasteur pipet, transfer the hexane layer to the column. When all the solution has drained, add 0.5 mL of hexane to the column to extract all the pigments from the drying agent. Evaporate the solvent by placing the test tube in a warm-water bath (40–60°C) and directing a stream of nitrogen gas (or dry air) into the tube. Dissolve the residue in 0.5 mL of hexane. Stopper the test tube and place it in your drawer until you are ready to run the alumina chromatography column.

Part B. Column Chromatography

Introduction. The pigments are separated on a column packed with alumina. Although there are many components in your sample, they usually separate into two main bands on the column. The first band to pass through the column is yellow and consists of the carotenes. This band may be less than 1 mm wide, and it may pass through the column rapidly. It is easy to miss seeing the band as it passes through the alumina. The second band consists of all the other pigments discussed in the introduction to this experiment. Although it consists of both green and yellow pigments, it appears as a green band on the column. The green band spreads out on the column more than the yellow band, and it moves more slowly. Occasionally, the yellow and green components in this band will separate as the band moves down the column. If this begins to occur, you should change to a solvent of higher polarity so that they came out come out as one band. As the samples elute from the column, collect the yellow band (carotenes) in one test tube and the green band in another test tube.

Because the moisture content of the alumina is difficult to control, different samples of alumina may have different activities. The activity of the alumina is an important factor in determining the polarity of the solvent required to elute each band of pigments. Several solvents with a range of polarities are used in this experiment. The solvents and their relative polarities follow:



A solvent of lower polarity elutes the yellow band; a solvent of higher polarity is required to elute the green band. In this procedure, you will first try to elute the yellow band with hexane. If the yellow band does not move with hexane, you then add the next more polar solvent. Continue this process until you find a solvent that moves the yellow band. When you find the appropriate solvent, continue using it until the yellow band is eluted from the column. When the yellow band is eluted, change to the next more polar solvent. When you find a solvent that moves the green band, continue using it until the green band is eluted. Remember that occasionally a second yellow band will begin to move down the column before the green band moves. This yellow band will be much wider than the first one. If this occurs, change to a more polar solvent. This should bring all the components in the green band down at the same time.

Advance Preparation. Before running the column, assemble the following glassware and liquids. Obtain five dry test tubes (16-mm × 100-mm) and number them 1 through 5. Prepare two dry Pasteur pipets with bulbs attached. Calibrate one of them to deliver a volume of about 0.25 mL (see Technique 5, Section 5.4). Place 10.0 mL of hexane, 6.0 mL of 70% hexane–30% acetone solution (by volume), 6.0 mL of acetone, and 6.0 mL of 80% acetone–20% methanol (by volume) into four separate containers. Clearly label each container.

Prepare a chromatography column packed with alumina. Place a *loose* plug of cotton in a Pasteur pipet (5¾-inch) and push it *gently* into position using a glass rod (see Figure above, Column for Drying Extract for the correct position of the cotton). Add 1.25 g of alumina (EM Science, No. AX0612-1) to the pipet[1] while tapping the column gently with your finger. When all the alumina has been added, tap the column with your finger for several seconds to ensure that the alumina is tightly packed. Clamp the column in a vertical position so that the bottom of the column is just above the height of the test tubes you will be using to collect the fractions. Place test tube 1 under the column.

NOTE: Read the following procedure on running the column. The chromatography procedure takes less than 15 minutes, and you cannot stop until all the material is eluted from the column. You must have a good understanding of the whole procedure before running the column.

Running the Column. Using a Pasteur pipet, slowly add about 3.0 mL of hexane to the column. The column must be completely moistened by the solvent. Drain the excess hexane until the level of hexane reaches the top of the alumina. Once you have added hexane to the alumina, the top of the column must not be allowed to run dry. If necessary, add more hexane.

NOTE: It is essential that the liquid level not be allowed to drain below the surface of the alumina at any point during the procedure.

When the level of the hexane reaches the top of the alumina, add about half (0.25 mL) of the dissolved pigments to the column. Leave the remainder in the test tube for the thin-layer chromatography procedure. (Put a stopper on the tube and place it back in your drawer). Continue collecting the eluent in test tube 1. Just as the pigment solution penetrates the column, add 1 mL of hexane and drain until the surface of the liquid has reached the alumina.

Add about 4 mL of hexane. If the yellow band begins to separate from the green band, continue to add hexane until the yellow band passes through the column. If the yellow band does not separate from the green band, change to the next more polar solvent (70% hexane–30% acetone). When changing solvents, do not add the new solvent until the last solvent has nearly penetrated the alumina. When the appropriate solvent is found, add this solvent until the yellow band passes through the column. Just before the yellow band reaches the bottom of the column, place test tube 2 under the column. When the eluent becomes colorless again (the total volume of the yellow material should be less than 2 mL), place test tube 3 under the column.

Add several mL of the next more polar solvent when the level of the last solvent is almost at the top of the alumina. If the green band moves down the column, continue to add this solvent until the green band is eluted from the column. If the green band does not move or if a diffuse yellow band begins to move, change to the next more polar solvent. Change solvents again if necessary. Collect the green band in test tube 4. When there is little or no green color in the eluent, place test tube 5 under the column and stop the procedure.

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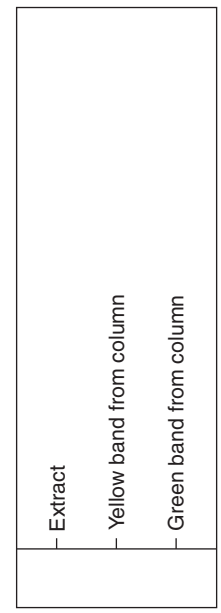
[1]As an option, students may prepare a microfunnel from a 1-mL disposable plastic pipet. The microfunnel is prepared by (1) cutting the bulb in half with scissors, and (2) cutting the stem at an angle about ½-inch below the bulb. This funnel can be placed in the top of the column (Pasteur pipet to aid in filling the column with alumina or with the solvents (see Technique 19, Section 19.6.

Using a warm-water bath (40°–60°C) and a stream of nitrogen gas, evaporate the solvent from the tube containing the yellow band (tube 2), the tube containing the green band (tube 4), and the tube containing the original pigment solution (tube E). As soon as all the solvent has evaporated from each of the tubes, remove them from the water bath. Do not allow any of the tubes to remain in the water bath after the solvent has evaporated. Stopper the tubes and place them in your drawer.

Part C. Thin-Layer Chromatography

Preparing the TLC Plate. Technique 20 describes the procedures for thin-layer chromatography. Use a 10-cm × 3.3-cm TLC plate (Whatman Silica-Gel Plates No. 4410 222). These plates have a flexible backing, but should not be bent excessively. Handle them carefully, or the adsorbent may flake off them. Also, you should handle them only by the edges; the surface should not be touched. Using a lead pencil (not a pen), *lightly* draw a line across the plate (short dimension) about 1 cm from the bottom (see Figure). Using a centimeter ruler, move its index about 0.6 cm in from the edge of the plate and lightly mark off three 1-cm intervals on the line. These are the points at which the samples will be spotted.

Prepare three micropipets to spot the plate. The preparation of these pipets is described and illustrated in Technique 20, Section 20.4. Prepare a TLC development chamber with 70% hexane–30% acetone (see Technique 20, Section 20.5). A beaker covered with aluminum foil or a wide-mouth, screw-cap bottle is a suitable container to use (see Technique 20, Figure 20.5). The backing on the TLC plates is thin, so if they touch the filter paper liner of the development chamber *at any point,* solvent will begin to diffuse onto the absorbent surface at that point. To avoid this, be sure that the filter paper liner does not go completely around the inside of the container. A space about 2 inches wide must be provided.



Preparing the TLC plate.

Using a Pasteur pipet, add 2 drops of 70% hexane–30% acetone to each of the three test tubes containing dried pigments. Swirl the tubes so that the drops of solvent dissolve as much of the pigments as possible. The TLC plate should be spotted with three samples: the extract, the yellow band from the column, and the green band. For each of the three samples, use a different micropipet to spot the sample on the plate. The correct method of spotting a TLC plate is described in Technique 20, Section 20.4. Take up part of the sample in the pipet (don't use a

bulb; capillary action will draw up the liquid). For the extract (tube labelled E) and the green band (tube 4), touch the plate once *lightly* and let the solvent evaporate. The spot should be no longer than 2 mm in diameter and should be a fairly dark green. For the yellow band (tube 2), repeat the spotting technique 5–10 times, until the spot is a definite yellow. Let the solvent to evaporate completely between successive applications and spot the plate in exactly the same position each time. Save the liquid samples in case you need to repeat the TLC.

Developing the TLC Plate. Place the TLC plate in the development chamber, making sure that the plate does not come in contact with the filter paper liner. Remove the plate when the solvent front is 1–2 cm from the top of the plate. Using a lead pencil, mark the position of the solvent front. As soon as the plates have dried, outline the spots with a pencil and indicate the colors. This is important to do soon after the plates have dried, because some of the pigments will change color when exposed to the air.

Analysis of the Results. In the crude extract, you should be able to see the following components (in order of decreasing values):

Carotenes (1 spot) (yellow orange)

Pheophytin *a* (gray, may be nearly as intense as chlorophyll *b*)

Pheophytin *b* (gray, may not be visible)

Chlorophyll *a* (blue green, more intense than chlorophyll *b*)

Chlorophyll *b* (green)

Xanthophylls (possibly three spots: yellow)

Depending on the spinach sample, the conditions of the experiment, and how much sample was spotted on the TLC plate, you may observe other pigments. These additional components can result from air oxidation, hydrolysis, or other chemical reactions involving the pigments discussed in this experiment. It is common to observe other pigments in samples of frozen spinach. It is also common to observe components in the green band that were not present in the extract.

Identify as many of the spots in your samples as possible. Determine which pigments were present in the yellow band and which were present in the green band. Draw a picture of the TLC plate in your notebook. Label each spot with its color and its identity, where possible. Calculate the R_f values for each spot produced by chromatography of the extract (see Technique 20, Section 20.9). If your instructor requests it, submit the TLC plate with your report.

QUESTIONS

1. Why are the chlorophylls less mobile on column chromatography, and why do they have lower R_f values than the carotenes?
2. Propose structural formulas for pheophytin *a* and pheophytin *b*.
3. What would happen to the values of the pigments if you were to increase the relative concentration of acetone in the developing solvent?
4. Using your results as a guide, comment on the purity of the material in the green and yellow bands; that is, did each band consist of a single component?

ESSAY

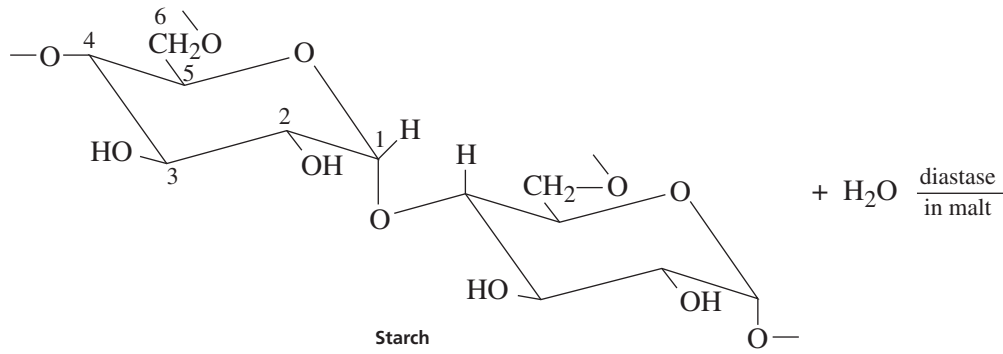
Ethanol and Fermentation Chemistry

The fermentation processes involved in making bread, making wine, and brewing are among the oldest chemical arts. Even though fermentation had been known as an art for centuries, it was not until the nineteenth century that chemists began to understand this process from the point of view of science. In 1810, Gay-Lussac discovered the general chemical equation for the breakdown of sugar into ethanol and carbon dioxide. The manner in which the process took place was the subject of much conjecture until Louis Pasteur began his thorough examination of fermentation. Pasteur demonstrated that yeast was required in the fermentation. He was also able to identify other factors that controlled the action of the yeast cells. His results were published in 1857 and 1866.

For many years, scientists believed that the transformation of sugar into ethanol and carbon dioxide by yeasts was inseparably connected with the life process of the yeast cell. This view was abandoned in 1897, when Büchner demonstrated that yeast extract would bring about alcoholic fermentation in the absence of any yeast cells. The fermenting activity of yeast is due to a remarkably active catalyst of biochemical origin, the enzyme zymase. It is now recognized that most of the chemical transformations that occur in living cells of plants and animals are brought about by enzymes. "These enzymes" are organic compounds, generally proteins, and establishment of the structures and reaction mechanisms of these compounds is an active field of present-day research. Zymase is now known to be a complex of at least 22 separate enzymes, each of which catalyzes a specific step in the fermentation reaction sequence.

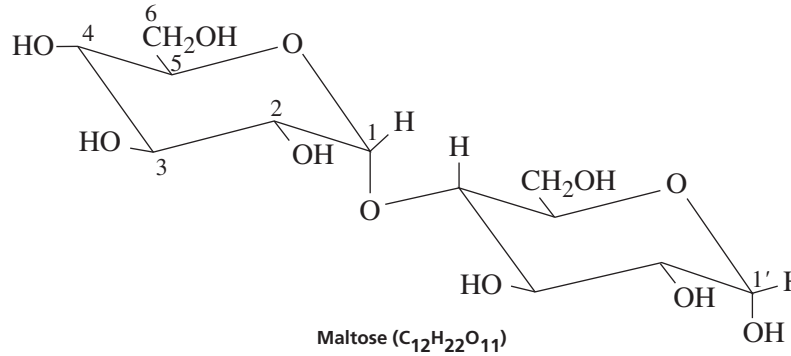
Enzymes display an extraordinary specificity—a given enzyme acts on a specific compound or a closely related group of compounds. Thus, zymase acts on only a few select sugars and not on all carbohydrates; the digestive enzymes of the alimentary tract are equally specific in their activity.

The chief sources of sugars for fermentation are the various starches and the molasses residue obtained from refining sugar. Corn (maize) is the chief source of starch in the United States, and ethyl alcohol made from corn is commonly known as **grain alcohol**. In preparing alcohol from corn, the grain, with or without the germ, is ground and cooked to give the **mash**. The enzyme diastase is added in the form of **malt** (sprouted barley that has been dried in air at and ground to a powder) or of a mold such as *Aspergillus oryzae.* The mixture is kept at until all the starch has been converted to the sugar **maltose** by hydrolysis of ether and acetal bonds. This solution is known as the **wort**.



Starch

This is a glucose polymer with 1,4- and 1,6- glycosidic linkages. The linkages at C-1 are α.



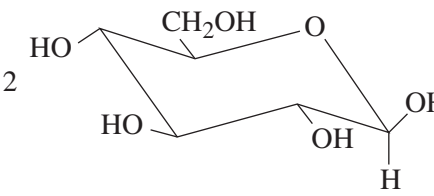
Maltose ($C_{12}H_{22}O_{11}$)

The α linkage still exists at C-1. The —OH is shown α at the 1′ postion (axial), but it can also be *ß* (equatorial).

The wort is cooled to 20°C and diluted with water to 10% maltose, and a pure yeast culture is added. The yeast culture is usually a strain of *Saccharomyces cerevisiae* (or *ellipsoidus*). The yeast cells secrete two enzyme systems: maltase, which converts the maltose into glucose, and zymase, which converts the glucose into carbon dioxide and alcohol. Heat is liberated, and the temperature must be kept below 35°C by cooling to prevent destruction of the enzymes. Oxygen in large amounts is initially necessary for the optimum reproduction of yeast cells, but the actual production of alcohol is anaerobic. During fermentation, the evolution of carbon dioxide soon establishes anaerobic conditions. If oxygen were freely available, only carbon dioxide and water would be produced.

After 40–60 hours, fermentation is complete, and the product is distilled to remove the alcohol from solid matter. The distillate is fractionated by means of an efficient column. A small amount of acetaldehyde (bp 21°C) distills first and is followed by 95% alcohol. Fusel oil is contained in the higher-boiling fractions. The fusel oil consists of a mixture of higher alcohols, chiefly 1-propanol, 2-methyl-1-propanol,3-methyl-1-butanol, and 2-methyl-1-butanol. The exact composition of fusel oil varies considerably; it particularly depends on the type of raw material that is fermented. These higher alcohols are not formed by fermentation of glucose. They arise from certain amino acids derived from the proteins present in the raw material and the yeast. These fusel oils cause the headaches associated with drinking alcoholic beverages.

$$\text{Maltose} + H_2O \xrightarrow{\text{maltase}} 2$$



***β*-D-(+)-Glucose**
(*α*-D-(+)-Glucose, with an axial —OH, is also produced.)

$$\underset{C_6H_{12}O_6}{\text{Glucose}} \xrightarrow{\text{zymase}} 2\,CO_2 + 2\,CH_3CH_2OH + 26\ \text{kcal}$$

Industrial alcohol is ethyl alcohol used for nonbeverage purposes. Most commercial alcohol is denatured to avoid payment of taxes, the biggest cost in the price of liquor. The denaturants render the alcohol unfit for drinking. Methanol, aviation fuel, and other substances are used for this purpose. The difference in price between taxed and nontaxed alcohol is more than $20 a gallon. Before efficient synthetic processes were developed, the chief source of industrial alcohol was fermented blackstrap molasses, the noncrystallizable residue from refining cane sugar (sucrose). Most industrial ethanol in the United States is now manufactured from ethylene, a product of the "cracking" of petroleum hydrocarbons. By reaction with concentrated sulfuric acid, ethylene becomes ethyl hydrogen sulfate, which is hydrolyzed to ethanol by dilution with water. The alcohols 2-propanol, 2-butanol, 2-methyl-2-propanol, and higher secondary and tertiary alcohols are also produced on a large scale from alkenes derived from cracking.

Yeasts, molds, and bacteria are used commercially for the large-scale production of various organic compounds. An important example, in addition to ethanol production, is the anaerobic fermentation of starch by certain bacteria to yield 1-butanol, acetone, ethanol, carbon dioxide, and hydrogen.

For additional information on the production of ethanol, see the essay Biofuels that precedes Experiment 25. In this essay, the production of ethanol from corn for use in automobiles is discussed, along with the production of ethanol from other sources such as plant cellulose.

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Seevers, M. H.; Davis, V. E.; Walsh, M. J. Morphine and Ethanol Physical Dependence: A Critique of a Hypothesis. *Science* **1970,** *170,* 1113.
Yamanaka, Y.; Walsh, M. J.; Davis, V. E. Salsolinol, an Alkaloid Derivative of Dopamine Formed in Vitro during Alcohol Metabolism. *Nature* **1970,** *227,* 1143.

16 **EXPERIMENT 16**

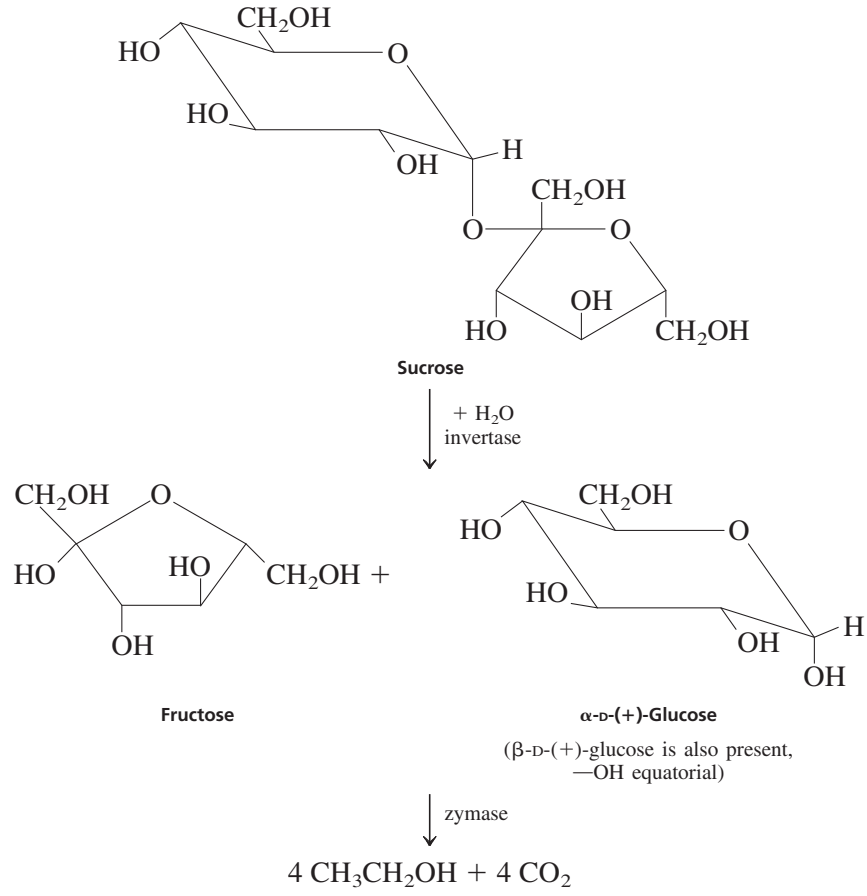
Ethanol from Sucrose

Fermentation

Fractional distillation

Azeotropes

Either sucrose or maltose can be used as the starting material for making ethanol. Sucrose is a disaccharide with the formula $C_{12}H_{22}O_{11}$. It has one glucose molecule combined with fructose. Maltose consists of two glucose molecules. The enzyme **invertase** is used to catalyze the hydrolysis of sucrose. **Maltase** is more effective in catalyzing the hydrolysis of maltose. The hydrolysis of maltose is discussed in the



essay, "Ethanol and Fermentation". **Zymase** is used to convert the hydrolyzed sugars to alcohol and carbon dioxide. Pasteur observed that growth and fermentation were promoted by adding small amounts of mineral salts to the nutrient medium. Later it was found that before fermentation actually begins, the hexose sugars combine with phosphoric acid, and the resulting hexose–phosphoric acid combination is then degraded into carbon dioxide and ethanol. The carbon dioxide is not wasted in the commercial process because it is converted to dry ice.

The fermentation is inhibited by its end-product ethanol; it is not possible to prepare solutions containing more than 10–15% ethanol by this method. More-concentrated ethanol can be isolated by fractional distillation. Ethanol and water form an azeotropic mixture consisting of 95% ethanol and 5% water by weight, which is the most concentrated ethanol that can be obtained by fractionation of dilute ethanol–water mixtures.

REQUIRED READING

Sign in at www .cengage.com to access Pre-Lab Video Exercises for techniques marked with an asterisk.

Review:	*Technique 8	Filtration, Sections 8.3 and 8.4
	Technique 13	Physical Constants of Liquids, Part A. Boiling Points and Thermometer Correction
New:	Technique 13	Physical Constants of Liquids, Part B. Density
	*Technique 15	Fractional Distillation, Azeotropes
	Essay	Ethanol and Fermentation Chemistry

SPECIAL INSTRUCTIONS

Start the fermentation at least 1 week before the period in which the ethanol will be isolated. When the aqueous ethanol solution is to be separated from the yeast cells, it is important to transfer carefully as much of the clear, supernatant liquid as possible, without agitating the mixture.

SUGGESTED WASTE DISPOSAL

Discard all aqueous solutions in the waste container marked for the disposal of aqueous waste. Filter Aid may be discarded in the trash containers.

NOTES TO THE INSTRUCTOR

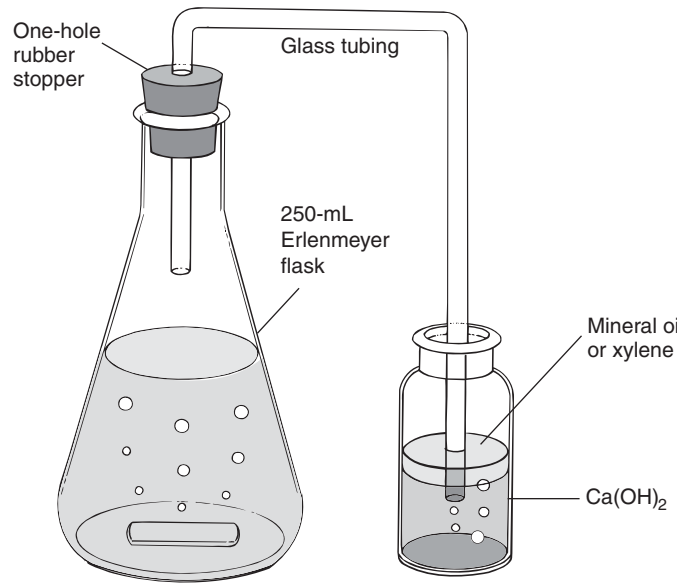
It may be necessary to use an external heat source to maintain a temperature of 30–35°C. Place a lamp in the hood to act as a heat source.

This experiment can also be performed without doing the fermentation. Provide each student with 20 mL of a 10% ethanol solution. This solution is used in place of the fermentation mixture in the fractional distillation section of the Procedure.

PROCEDURE

Fermentation. Place 20.0 g of sucrose in a 250-mL Erlenmeyer flask. Add 175 mL of water warmed to 25–30°C, 20 mL of Pasteur's salts,[1] and 2.0 g of *dried* baker's yeast. Shake the contents vigorously to mix them and then fit the flask with a one-hole rubber stopper with a glass tube leading to a beaker or test tube containing a saturated solution of calcium hydroxide.[2] Protect the calcium hydroxide from air by adding some mineral oil or xylene to form a layer above the calcium hydroxide (see figure below). A precipitate of calcium carbonate will form, indicating that CO_2 is being evolved. Alternatively, a balloon may be substituted for the calcium hydroxide trap. Oxygen from the atmosphere is excluded from the chemical reaction by these techniques. If oxygen were allowed to continue in contact with the fermenting solution, the ethanol could be further oxidized to acetic acid or even all the way to carbon dioxide and water. As long as carbon dioxide continues to be liberated, ethanol is being formed.

Allow the mixture to stand at about 30–35°C until fermentation is complete, as indicated by the cessation of gas evolution. Usually about week is required. After this time, *carefully* move the flask away from the heat source and remove the stopper. Without disturbing the sediment, transfer the clear, supernatant liquid solution to another container by decanting.



Apparatus for fermentation experiment.

If the liquid is not clear, clarify it by the following method. Place about 1 tablespoon of Filter Aid (Johns-Manville Celite) in a beaker with about 100 mL of water. Stir the mixture vigorously and then pour the contents into a Büchner funnel (with filter paper) while applying a vacuum, as in vacuum filtration (see Technique 8, Section 8.3). This procedure will cause a thin layer of Filter Aid to be deposited on the filter paper (see Technique 8, Section 8.4).

[1]A solution of Pasteur's salts consists of potassium phosphate, 2.0 g; calcium phosphate, 0.20 g; magnesium sulfate, 0.20 g; and ammonium tartrate, 10.0 g, dissolved in 860 mL water.
[2]Alternatively, you can cover the flask opening with saran wrap or other plastic wrap, using a rubber band to hold the plastic wrap firmly in place.

Discard the water that passes through this filter. The decanted liquid containing the ethanol is then passed through this filter under gentle suction. The extremely tiny yeast particles are trapped in the pores of the Filter Aid. The liquid contains ethanol in water, plus smaller amounts of dissolved metabolites (fusel oils) from the yeast.

Fractional Distillation. Assemble the apparatus shown in Technique 15, Figure 15.2; select a round-bottom flask that will be filled between half and two-thirds full by the liquid to be distilled. Insulate the distilling head by covering it with a layer of cotton held in place with aluminum foil. Use a heating mantle for the heat source. Pack the condenser (the one in your kit that has a larger diameter) uniformly with about 3 g of stainless-steel cleaning sponge (no soap!) (see Experiment 6).

CAUTION

You should wear heavy cotton gloves when handling the stainless-steel sponge. The edges are very sharp and can easily cut into the skin.

Add about 10 g of potassium carbonate to the filtered solution for each 20 mL of liquid. After the solution has become saturated with potassium carbonate, transfer it to the round-bottom flask of the distillation apparatus. It is important to distill the liquid **slowly** through the fractionating column to get the best possible separation. This can be done by carefully following these instructions: As ethanol moves up the distillation column, it will not wet the stainless-steel sponge and you will not be able to see the ethanol. After all of the ethanol has begun moving up the column, water will begin to enter the column. Since water will wet the stainless-steel sponge, you will be able to see the water gradually moving up the column. To get a good separation, you should control the temperature in the distilling flask so that it takes about 10–15 minutes for the water to move up the column. Once ethanol reaches the top of the column, the temperature in the distillation head will increase to about 78°C and then rise gradually until the ethanol fraction is distilled. Collect the fraction boiling between 78°C and 84°C and discard the residue in the distillation flask. You should collect about 4–5 mL of distillate. The distillation should then be interrupted by removing the apparatus from the heat source.

Analysis of Distillate. Determine the total weight of the distillate. Determine the approximate density of the distillate by transferring a known volume of the liquid with an automatic pipet or graduated pipet to a tared vial. Reweigh the vial and calculate the density. This method is good to two significant figures. Using the preceding table, determine the percentage composition by weight of ethanol in your distillate from the density of your sample. The extent of purification of the ethanol is limited because ethanol and water form a constant-boiling mixture, an azeotrope, with a composition of 95% ethanol and 5% water.

Percentage Ethanol by Weight	Density at 20°C (g/mL)	Density at 25°C (g/mL)
75	0.856	0.851
80	0.843	0.839
85	0.831	0.827
90	0.818	0.814
95	0.804	0.800
100	0.789	0.785

Calculate the percentage yield of the alcohol and submit the ethanol to the instructor in a labeled vial.[3]

QUESTIONS

1. Write a balanced equation for the conversion of sucrose into ethanol.
2. By doing some library research, see whether you can find the commercial method or methods used to produce **absolute ethanol**.
3. Why is the air trap necessary in the fermentation?
4. How does acetaldehyde impurity arise in the fermentation?
5. The diethylacetal of acetaldehyde can be detected by gas chromatography. How does this impurity arise in fermentation?
6. Calculate how many milliliters of carbon dioxide would be theoretically produced from 20 g of sucrose at 25°C and 1 atmosphere pressure.

[3]A careful analysis by flame-ionization gas chromatography on a typical student-prepared ethanol sample provided the following results:

Acetaldehyde	0.060%
Diethylacetal of acetaldehyde	0.005%
Ethanol	88.3% (by hydrometer)
1-Propanol	0.032%
2-Methyl-1-propanol	0.092%
5-Carbon and higher alcohols	0.140%
Methanol	0.040%
Water	11.3% (by difference)