EXPERIMENT

Separating the Components of “Panacetin”

Extraction and Evaporation

Separation Methods.

Operations

OP-7a  Heat Sources
OP-17  Centrifugation
OP-18a  Liquid–Liquid Extraction
OP-19  Evaporation
OP-4  Weighing
OP-5  Measuring Volume
OP-6  Making Transfers
OP-16  Vacuum Filtration
OP-26  Washing and Drying Solids

Before You Begin

1. Read the experiment carefully.
2. Read operations OP-7a, OP-17, OP-18a, and OP-19. Read or review the other listed operations as necessary.

Scenario

Your supervisor has received the following message from a drug watchdog agency, the Association for Safe Pharmaceuticals (ASP).

Greetings:

Our roving agent in Southern California, Sam Surf, recently purchased some Panacetin—an analgesic drug preparation—at a drugstore in San Diego. According to the label on the bottle, the Panacetin tablets were manufactured in the United States by a legitimate pharmaceutical company, but Sam detected some discrepancies on the label and flaws in the tablets themselves that made him suspect they might be counterfeit. Such illegal knockoffs of a domestic drug can be manufactured cheaply elsewhere and smuggled into the United States, where they are sold at a big profit margin.

The label on the bottle lists the ingredients per tablet as aspirin (200 mg), acetaminophen (250 mg), and sucrose (50 mg). Aspirin and acetaminophen are presumably the active ingredients, while sucrose is an inactive ingredient used to make the tablets more palatable to children. But counterfeit drugs may contain less of an active ingredient than claimed, the wrong active ingredient, or no active ingredients at all. We have reason to believe that Panacetin does contain aspirin, sucrose, and another active component, but we’re

See the Scenario in the experiment “The Effect of pH on a Food Preservative” if you don’t understand the purpose of the Scenarios.
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not sure what that component is or whether the amounts listed on the label are accurate. The unknown component is probably a chemical relative of acetaminophen, either acetanilide or phenacetin. Both of these kill pain as effectively as acetaminophen, so the consumer wouldn’t notice their presence in an analgesic drug. But acetanilide and phenacetin are banned in the United States because of their toxicity, and we would like to keep them off the market.

We want your Consulting Chemists Institute to analyze this drug preparation to find out what percentages of aspirin, sucrose, and the unknown component it contains, and whether the unknown is acetanilide or phenacetin. You have two weeks to complete your investigation.

Les Payne, Director of Operations, ASP

Applying Scientific Methodology

The Scenario presents two problems for you to solve in this experiment and the next: (1) Is the composition of Panacetin as stated on the label accurate? (2) What is the identity of the unknown component in Panacetin? You will concentrate on the first problem in this experiment, following the course of action described in the Directions. Because of possible material losses, you must allow some margin for error in deciding whether the percentage composition derived from the label (10% sucrose, 40% aspirin, 50% unknown component) is accurate. For the purposes of the experiment, ranges of 6–14% sucrose, 30–50% aspirin, and 40–60% of the unknown component are close enough to indicate that the label is reasonably accurate. You should start with a working hypothesis, gather and interpret evidence, change your hypothesis if the evidence does not support it, arrive at a conclusion, and report your results.

Painkilling Drugs, from Antifebrin to Tylenol

Analgesic drugs reduce pain; antipyretic drugs reduce fever. Some drugs, including aspirin and acetaminophen, do both. Many of the common over-the-counter analgesic–antipyretic drug preparations contain aspirin, acetaminophen, or combinations of these substances with other ingredients. For example, acetaminophen is the active ingredient of Tylenol, and Extra Strength Excedrin contains aspirin, acetaminophen, and caffeine. From their molecular structures, you can see that acetaminophen is chemically related to both acetanilide and phenacetin, whose painkilling effects were discovered late in the nineteenth century.

In 1886, two clinical assistants named Arnold Cahn and Paul Hepp were looking for something that would rid their patients of a particularly unpleasant intestinal worm. The trick was to find a drug that would kill the worm but not the patient, and their method—not a very scientific one—was to test the chemicals in their stockroom until they found one that worked. When someone came across an ancient bottle labeled NAPHTHALENE, they tried it out on a patient who had every malady in the book, including worms. It didn’t faze the worms, but it reduced the patient’s fever dramatically. Before Cahn and Hepp went out on a limb and endorsed naphthalene...
as a cure-all for fevers, someone noticed that the white substance in the bottle was nearly odorless. Since naphthalene has a strong mothball-like odor, Hepp suspected that the bottle was mislabeled and sent it to his cousin, a chemist at a nearby dye factory, for analysis. The tests showed that the new drug was not naphthalene at all, but acetanilide.

Acetanilide proved to have painkilling as well as fever-reducing properties and was soon being marketed under the proprietary name Antifebrin. Unfortunately, some patients who used Antifebrin developed a serious form of anemia called methemoglobinemia, in which hemoglobin molecules are altered in a way that reduces their ability to transport oxygen through the bloodstream. Even though Antifebrin is now considered too toxic for medicinal use, its discovery did much to stimulate the development of safer and more effective analgesic–antipyretic drugs.

About six months after the discovery of Antifebrin, a similar drug was developed as the result of a storage problem. Carl Duisberg, director of research for the Friedrich Bayer Company, had to get rid of 50 tons of para-aminophenol—a seemingly useless yellow powder that was a by-product of dye manufacturing. Rather than pay a teamster to haul the stuff away, Duisberg decided to change it into something Bayer could sell. After reading about Antifebrin, he reasoned that a compound with a similar molecular structure might have similar therapeutic uses. Duisberg knew that a hydroxyl (OH) group attached to a benzene ring is characteristic of many toxic substances (for example, phenol), so he decided to “mask” the hydroxyl group in para-aminophenol with an ethyl (CH₃CH₂—) group, as shown in the margin. Incorporation of an acetyl (CH₃CO—) group then yielded phenacetin, which proved to be a remarkably effective and inexpensive analgesic–antipyretic drug. Until recently, phenacetin was used in APC tablets (which contained aspirin, phenacetin, and caffeine) and in other analgesic–antipyretic drug preparations. It is no longer approved for medicinal use in the United States because it may cause kidney damage, hemolytic anemia, or even cancer in some patients.

Ironically, the substance Duisberg would have obtained had he not masked the hydroxy group is acetaminophen, which has proven to be a safer drug than either acetanilide or phenacetin. In the body, acetanilide and phenacetin are both converted to acetaminophen, which is believed to be the active form of all three drugs.

**Understanding the Experiment**

Most natural products and many commercial preparations are mixtures that contain a number of different substances. To obtain a pure compound from such a mixture, the desired compound is separated from the other components of the mixture by taking advantage of differences in physical and chemical properties. For example, substances that have very different solubilities in a given solvent may be separated by extraction or filtration, and liquids with different boiling points can be separated by distillation. Acidic or basic substances are often converted to water-soluble salts, which can then be separated from the water-insoluble components of a mixture.

In this experiment, you will separate the components of a simulated pharmaceutical preparation, Panacetin, making use of their solubilities and acid–base properties. Panacetin contains aspirin, sucrose, and an unknown...
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device component that may be either acetanilide or phenacetin. These substances have the following characteristics:

- Sucrose is insoluble in the organic solvent dichloromethane (CH₂Cl₂, also called methylene chloride).
- Aspirin, acetanilide, and phenacetin are soluble in dichloromethane but relatively insoluble in water.
- Aspirin reacts with sodium bicarbonate to form a salt, sodium acetylsalicylate, which is insoluble in dichloromethane and soluble in water.
- Acetanilide and phenacetin are not converted to salts by sodium bicarbonate.

Mixing the Panacetin with dichloromethane should therefore dissolve the aspirin and the unknown component, leaving the sucrose behind as an insoluble solid that can be removed by filtration or (as in this experiment) by centrifugation. Aspirin can be removed from the dichloromethane solution by extraction with an aqueous solution of sodium bicarbonate. The base converts aspirin to its sodium salt, as shown in the margin. This salt will migrate from the dichloromethane layer, in which it is insoluble, to the aqueous layer, in which it is soluble. The unknown component will stay behind in the dichloromethane layer. After separation of the layers, aspirin can be recovered by precipitation from the aqueous layer with hydrochloric acid followed by vacuum filtration. The unknown component can then be isolated by evaporating the solvent from the dichloromethane solution.

This separation process is summarized by the following flow diagram:
You can estimate the percentage composition of Panacetin from the masses of the dried components. Careful work is required to obtain accurate results in this experiment; errors can arise from incomplete mixing with dichloromethane, incomplete extraction or precipitation of aspirin, incomplete drying of the recovered components, and losses in transferring substances from one container to another.

Dichloromethane may be harmful to the environment, especially when released into groundwater. The Environmental Protection Agency (EPA) classifies it as a priority pollutant and has established a Maximum Contaminant Level (MCL) of 5 parts per billion (ppb) for its concentration in drinking water. See the “Chemistry and the Environment” section for information about priority pollutants, MCLs, and other environmental topics.

**DIRECTIONS**

**Separation of Sucrose.** Accurately weigh [OP-4] about 0.400 g of Panacetin and transfer it to a clean, dry 15-mL screw-cap centrifuge tube (tube A) that has been tared (preweighed). Measure [OP-5] 8.0 mL of dichloromethane into the centrifuge tube. Dissolve as much solid as possible in this solvent by shaking vigorously for several minutes. If necessary, use a wooden applicator stick to break up any solid lodged in the tip. Balancing the centrifuge tube against one of equal mass, centrifuge [OP-17] the mixture for at least three minutes, and then transfer [OP-6] the liquid to a second screw-cap centrifuge tube (tube B), leaving all of the solid behind. Leave centrifuge tube A (uncapped) under a fume hood until you have finished the rest of the experiment, to allow any traces of dichloromethane to evaporate. Then weigh it and calculate the mass of the sucrose it contains. Record the mass of the sucrose in your laboratory notebook. If requested, submit the sucrose to your instructor in a tared and labeled vial.

**Separation of Aspirin.** Extract [OP-18a] the dichloromethane in centrifuge tube B with two separate 4-mL portions of 5% sodium bicarbonate. For each extraction, use a stirring rod to stir the liquid layers until any fizzing subsides before you stopper and shake the centrifuge tube. After the first extraction, transfer [OP-6] the bottom (dichloromethane) layer to a labeled 20-mL beaker, transfer the top (aqueous) layer to a second small beaker, and return the bottom layer to the centrifuge tube. After the second extraction, transfer the dichloromethane layer to a tared 4-dram screw-cap vial, and combine the second aqueous layer with the first one. Save the dichloromethane solution in the vial for the following step, “Isolation of the Unknown Component.”

A Greener Way: Diethyl ether is less harmful to health and the environment than is dichloromethane, so it can be used as the extraction solvent in place of dichloromethane. Just remember that the ether layer separates on top of the water layer rather than below it. If you use dichloromethane, it is best to evaporate the organic layer under vacuum and recover the solvent, which can then be recycled.

**Safety Notes**

- **Dichloromethane** may be harmful if ingested, inhaled, or absorbed through the skin. There is a possibility that prolonged inhalation of dichloromethane may cause cancer. Minimize contact with the liquid and do not breathe its vapors.

See “Chemical Hazards” in the Laboratory Safety section for an explanation of the hazard symbols.

- **Fire**
- **Health**

**Fire**

**Health**

**Reactivity**

dichloromethane

**Take Care!** Avoid contact with dichloromethane; do not breathe its vapors.

**Stop and Think:** Does all of the solid dissolve? If not, why not?

**Stop and Think:** What components of Panacetin are in the transferred liquid?

**Take Care:** A gas is evolved, so don’t shake too vigorously.

**Stop and Think:** Which component of Panacetin is in the transferred liquid? In the dichloromethane layer? If you don’t shake the centrifuge tube long enough, how might that affect your results?
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Slowly add 1.0 mL of 6 M hydrochloric acid to the combined aqueous extracts while stirring with a glass rod. Test the pH of the solution with pH paper by using your stirring rod or the closed end of a capillary melting-point tube to transfer a small drop of the supernatant liquid (liquid near the surface) to a strip of pH paper. Add more acid, if necessary, to bring the pH to 2 or lower. Cool the mixture in an ice/water bath for at least five minutes, collect the aspirin by vacuum filtration [OP-16], and wash it on the filter [OP-26a] with cold water. Let the aspirin dry on the filter for a few minutes with the aspirator running, then dry [OP-26b] it to constant mass. Weigh the aspirin and record its mass in your lab notebook. Submit the aspirin to your instructor in a vial labeled with the experiment number, the name of the product, its mass, your name, the current date, and any other information required by your instructor.

**Isolation of the Unknown Component.** Under the hood, evaporate [OP-19] the solvent from the dichloromethane solution while heating [OP-7a] it in a warm-water bath. Discontinue evaporation when only a solid residue remains or when no more solvent evaporates. Let the unknown component dry [OP-26b] to constant mass, and weigh it.

Calculate your percent recovery, dividing the sum of the masses of all components by the mass of Panacetin that you started with. Calculate the approximate percentage composition of Panacetin, based on the total mass of components recovered. (These percentages should add up to 100%.) In your report, be sure to include the information specified in the “Report” section of “The Effect of pH on a Food Preservative” and any other information requested by your instructor.

**Exercises**

1. (a) Describe any evidence that a chemical reaction occurred when you added 6 M HCl to the solution of sodium acetylsalicylate. (b) Explain why the changes that you observed took place.

2. Describe and explain the possible effect on your results of the following experimental errors or variations. In each case, specify the component(s) whose percentage(s) would be too high or too low. (a) After adding dichloromethane to Panacetin, you didn’t stir or shake the mixture long enough. (b) During the NaHCO₃ extraction, you failed to mix the aqueous and organic layers thoroughly. (c) You mistakenly extracted the dichloromethane solution with 5% HCl rather than 5% NaHCO₃. (d) Instead of using pH paper, you neutralized the NaHCO₃ solution to pH 7 using litmus paper.

3. Although acetanilide and phenacetin are not appreciably acidic, acetaminophen (like aspirin) is a stronger acid than water. What problem would you encounter if the unknown component were acetaminophen rather than acetanilide or phenacetin, and you extracted the aspirin with 5% NaOH? Explain, giving equations for any relevant reactions.

4. Acetaminophen is a weaker acid than carbonic acid (H₂CO₃), but aspirin is a stronger acid than carbonic acid. Prepare a flow diagram like the one in this experiment, showing a procedure that can be used to separate a mixture of sucrose, aspirin, and acetaminophen.
5. Write balanced reaction equations for the reactions involved (a) when aspirin dissolves in aqueous NaHCO₃ and (b) when aspirin is precipitated from a sodium acetylsalicylate solution by HCl. Assuming that both reactions are spontaneous under standard conditions, label the stronger acid, stronger base, weaker acid, and weaker base in each equation.

**Other Things You Can Do**

(Starred items require your instructor’s permission.)

*1. Carry out the minilab “Extraction of Iodine by Dichloromethane” to help you visualize what happens during an extraction.

2. Write a short research paper about the chemistry and physiological effects of analgesic–antipyretic drugs using such sources as the *Kirk–Othmer Encyclopedia of Chemical Technology* [Bibliography, A8] and titles from section L of the Bibliography.