Extract and Evaporation: Separating the Components of a Mixture

Background Reading

- Lab safety and keeping a notebook: Chaps 1&2, Pg 1-18.
- Extraction and Washing: Chapt 15, Pg 110-122.

Background Scenario

One of the many places organic chemists are employed is in private laboratories associated with consulting firms. The purpose of these labs is generally to provide chemical analysis or synthesis of material for customers. The first few labs in CHM 220 will teach you the basic techniques needed to analyze and purify mixtures to determine the individual components. This first lab will involve determining the composition of a possible pharmaceutical preparation.

Watchdog agencies exist which monitor the pharmaceuticals sold over the counter in the United States. These agencies will use private laboratory firms to analyze products suspected of violating enforced standards. For example, a new brand of analgesic may appear on drugstore shelves at a low price. The label indicates that the tablets were manufactured in the United States by a legitimate pharmaceutical company. However, the labels may reveal discrepancies and the appearance/quality of the tablets could lead an investigator to suspect that they might be counterfeit. Such knockoffs of a domestic product can be manufactured cheaply elsewhere and smuggled into the US, where they are sold for high profits. This laboratory exercise places you in the position of analyzing a suspected counterfeit analgesic preparation.

The label on the suspected bottle lists the ingredients per tablet as aspirin (200 mg), acetaminophen (250 mg), and sucrose (50 mg). The sucrose is an inactive ingredient used to make the tablets more palatable to children. The watchdog agency hiring your laboratory firm has reason to believe that this product does contain aspirin, sucrose and another active component, but they are 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 their presence in an analgesic drug would not be detected by the consumer. But acetanilide and phenacetin are banned in the US because of their toxicity.

Your goal as the laboratory technician is to analyze the suspected 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 will have two weeks to complete your investigation.

Scientific Methodology

The above scenario presents two problems for you to solve in this week's and next week's experiments: (1) Is the composition of the drug preparation as stated on the label accurate? (2) What is the identity of the unknown component in the mixture? You will concentrate on the first problem this week, following the course of action described in the procedure. Because of potential material losses during your manipulations, you must allow for some margin of error in deciding whether the percent composition derived from the label is accurate. For the purposes of this experiment, a range of ±2% for the stated amount of sucrose is close enough to indicate that the label is accurate. For the aspirin and the unknown, a range of ±5% will suffice. As always, you should start with a working hypothesis (Purpose), gather and interpret data, change your hypothesis if the evidence (data) does not support it, arrive at a conclusion and report your results.

Information on Painkilling Drugs from Antifebrin to Tylenol

Analgesic drugs reduce pain and 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 (Figure 1.) you can see that acetaminophen is structurally related to both phenacetin and acetanilide, whose painkilling effects were discovered in the late nineteenth century.

In 1886 a pair of clinical assistants, 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 stock room until they found one that worked. When one of them came across an ancient bottle labeled NAPHTHALENE, they tried it out on a patient who had every malady in the book, including worms. Treatment did not 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, they noticed that the white substance in the bottle was nearly odorless. Since naphthalene has a strong mothball odor, Hepp suspected that the bottle was mislabeled and sent it to his cousin, a chemist at a local dye factory, for analysis. The tests showed that the compound was not naphthalene but acetanilide.

![Figure 1. Structures of analgesic/antipyretic compounds](image)

Acetanilide proved to have painkilling as well as fever-reducing properties and was soon being marketed under the proprietary name antifebrin. Unfortunately, some patients using 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 a 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 someone 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 group as shown in Figure 2.

![Figure 2. Bayer's Synthesis of Phenacetin](image)

Incorporation of an acetyl group (CH₃CO-) 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 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 hydroxyl group is acetaminophen, which has proved to be a safer drug than either acetanilide or phenacetin. In the body, acetanilide and phenacetin are both converted to acetaminophen, which is now believed to be the active form of all three drugs.

Understanding the Experiment

Most organic reaction products and many commercial preparations are mixtures containing a number of different substances. To obtain a pure compound from such a mixture, you must separate the desired compound from the other components of the mixture by taking advantage of differences in their physical and chemical properties. For example, substances having 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 into water-soluble salts to be separated from water insoluble compounds in the mixture.

In this week's experiment, you will separate the components of a simulated pharmaceutical preparation, making use of the different acid-base and solubility properties of the individual mixture components. The mixture will contain aspirin, sucrose and an unknown organic compound that may be either acetanilide or phenacetin. These compounds have the following solubility characteristics:

1. Sucrose is soluble in water but insoluble in the non-polar organic solvent dichloromethane CH₂Cl₂ (also called methylene chloride).
2. Aspirin is soluble in dichloromethane but relatively insoluble in water. Sodium hydroxide can remove an acidic hydrogen from aspirin to make a salt, sodium acetylsalicylate, which is insoluble in dichloromethane but soluble in water (Figure 3.).
3. Acetanilide and phenacetin, like aspirin are soluble in dichloromethane and insoluble in water. They are not converted into salts by sodium hydroxide.

From the above information, it should be clear that combining your solid mixture with dichloromethane will result in all of the aspirin and unknown dissolving while the sucrose remains insoluble. At this point, the sucrose can be isolated from the rest of the mixture components by filtration. The aspirin can be selectively removed from the dichloromethane solution by extraction with an aqueous sodium hydroxide solution which will convert the aspirin into a water-soluble salt (Figure 3.). The salt will be much more soluble in water than in dichloromethane so when the aqueous solution comes in contact with the dichloromethane, the sodium move into the aqueous layer where it will be better solvated. The unknown will remain in the dichloromethane solution. To regenerate the aspirin, the basic aqueous solution can be acidified with HCl. The HCl will reprotonate the sodium acetylsalicylate to form aspirin which is insoluble in water. The aspirin will precipitate out of the aqueous solution and can be collected by filtration.

To recover the unknown solid, the dichloromethane will be evaporated under reduced pressure, using a rotovap (short for rotary evaporator). The rotovap works under reduced pressure (partial vacuum) which reduces the boiling point of the solvent. This allows the dichloromethane to "boil off" at room temperature. The importance of not heating the solution is that some organic compounds are heat sensitive. They begin to decompose when heated. Using the rotovap to remove the dichloromethane is a common technique which we will use throughout the organic chemistry sequence. The above information should allow you to draw a flow diagram which summarizes the separation process. This flow diagram should be in your lab notebook.The masses of the dried components of your original mixture can be used to determine the percent composition of that mixture.

Procedure:

Separation of Sucrose. Accurately weigh about 3 g of solid mixture and transfer it to a clean, dry 125 mL Erlenmeyer flask. Add 50 mL of dichloromethane to the flask. Stir the mixture thoroughly to dissolve as much solid as possible. Carefully use a stirring rod to break up any lumps or granules. Using a preweighed piece of
filter paper, vacuum filter the solution. Gently wash the solids with 5 mL of fresh dichloromethane. Weigh the sucrose when it is completely dry. It should not smell like dichloromethane. Be sure to record the mass of sucrose in your lab notebook.

Separation of Aspirin. Transfer the dichloromethane filtrate to a clean separatory funnel and extract it with two 25 mL portions of 0.5 M sodium hydroxide solution. Because the dichloromethane solution will be the bottom layer (why?), you will have to transfer each layer to a different container (be sure to label the containers) and return the dichloromethane to the separatory funnel for the second extraction. Combine the two aqueous extracts in the same container and save the dichloromethane layer for isolation of the unknown.

Add 10 mL of 6 M HCl slowly and with stirring to the combined aqueous extracts. Test the pH of the solution and add more acid if needed to bring the pH to 2 or lower. Cool the mixture in an ice/water bath and collect the aspirin by vacuum filtration. Wash the aspirin with a small amount of water to remove any salts from the acidic solution (where do the salts come from?). Let the aspirin dry on the filter paper for a couple of minutes with the aspirator running, then dry the aspirin to a constant mass. Weigh the aspirin and record the mass in your lab notebook.

Isolation of the Unknown Component. Pour the dichloromethane solution into a preweighed 100 mL round bottom (RB) flask. Turn on the aspirator leading to the rotovap and close the vent stopcock at the end of the condenser coils. Place your thumb over the end where the flask gets attached to verify that a vacuum is being pulled.

![Figure 4. Rotary Evaporator](image)

Place RB flask containing the dichloromethane solution on the rotovap and once the vacuum is holding the flask in place, turn on the rotovap (the flask will begin to rotate). As the flask get colder, lower it into the water bath to speed the evaporation. When all of the dichloromethane has been removed, raise the flask out of the water bath and turn off the rotovap. Slowly open the stopcock at the end of the condensor coils to release the vacuum. If the vacuum is released too fast, the rush of air will blow some of your unknown solid out of the flask. Once the vacuum has been broken, the flask can be removed from the rotovap and weighed. This will give you the mass of unknown compound. Carefully transfer the solid from the RB flask to a vial to be kept until next week when we will determine the identity of the unknown.

Discussion/Write-up

State the problem and your initial hypothesis. Report your percent recovery. Briefly discuss the technique of extraction. State how you can determine which of two solutions will be the bottom layer when two solutions are not miscible. State your final conclusion and report how the evidence supports this conclusion.

Questions:

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

2. Describe and explain the possible effects on your results of the following experimental errors or variations, in each case specifying the component(s) whose percentages would be too high or too low if: (a) After adding dichloromethane to the initial mixture, you did not stir the mixture long enough. (b) During NaOH extraction you failed to mix the aqueous and organic layers thoroughly. (c) You mistakenly extracted the dichloromethane solution with 0.5 M HCl rather than 0.5 M NaOH.

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?

4. Acetaminophen is a weaker acid than carbonic acid (H₂CO₃), but aspirin is a stronger acid than carbonic acid. Prepare a flow diagram showing a procedure for separating a mixture of sucrose, aspirin and acetaminophen.
5. Write balanced chemical equations for the reactions involved when aspirin dissolved in aqueous NaOH and when aspirin is precipitated from a sodium acetylsalicylate solution by addition of HCl. Assuming that both reactions are spontaneous under standard conditions, label the stronger acid, stronger base, weaker acid and weaker base in each equation.