

Aspirin and Other Analgesics

Goals

- Use an esterification reaction to synthesize aspirin.
- Purify the crude aspirin sample.
- Test the purity of prepared aspirin and commercial aspirin products.
- Determine the physical and chemical properties of aspirin.
- Use thin-layer chromatography to separate and identify substances in analgesics.

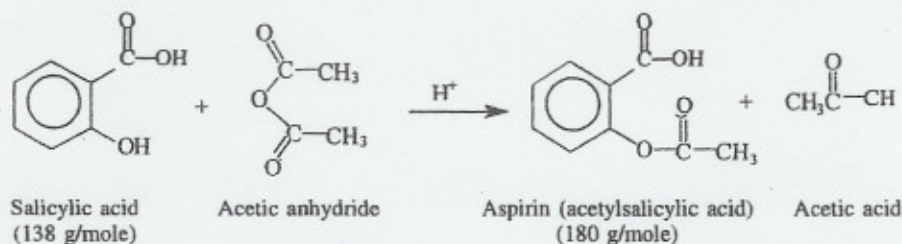
Discussion

In the 18th century, an extract of willow bark was found useful in reducing fevers (antipyretic) and relieving pain and inflammation. Although salicylic acid was effective at reducing fever and pain, it damaged the mucous membranes of the mouth and esophagus, and caused hemorrhaging of the stomach lining. At the turn of the century, scientists at the Bayer Company in Germany noted that salicylic acid contained a phenol group that might cause the damage. They decided to modify the salicylic acid by forming an ester with a two-carbon acetyl group. The resulting substance was acetylsalicylic acid, or ASA, which we call aspirin. Aspirin acts by inhibiting the formation of prostaglandins, 20-carbon acids that form at the site of an injury and cause inflammation and pain.

In commercial aspirin products, a small amount of acetylsalicylic acid (300 mg to 400 mg) is bound together with a starch binder and sometimes caffeine and buffers to make an aspirin tablet. The basic conditions in the small intestine break down the acetylsalicylic acid to yield salicylic acid, which is absorbed into the bloodstream. The addition of a buffer reduces the irritation caused by the carboxylic acid group of the aspirin molecule.

A. Preparation of Aspirin

Aspirin (acetylsalicylic acid) can be prepared from acetic acid and the hydroxyl group on salicylic acid. However, this is a slow reaction. The ester forms rapidly when acetic anhydride is used to provide the acetyl group. *The aspirin you will prepare in this experiment is impure and must not be taken internally!*



Using the following equation, the maximum amount (yield) of aspirin that is possible from 2.00 g of salicylic acid can be calculated.

$$\begin{aligned}
 2.00 \text{ g salicylic acid} &\times \frac{1 \text{ mole salicylic acid}}{138 \text{ g}} \times \frac{1 \text{ mole aspirin}}{1 \text{ mole salicylic acid}} \times \frac{180 \text{ g}}{1 \text{ mole aspirin}} \\
 &= 2.61 \text{ g aspirin (possible)}
 \end{aligned}$$

Laboratory 29

Suppose the total amount of aspirin you obtain has a mass of 2.25 g. A percentage yield can be calculated as follows:

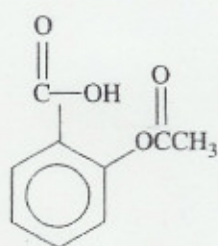
$$\% \text{ Yield} = \frac{\text{g aspirin obtained}}{\text{g aspirin calculated}} \times 100 = 86.2\% \text{ yield of aspirin product}$$

B. Testing Aspirin Products

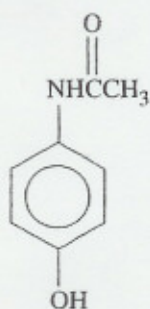
The purity of the crude sample and the recrystallized aspirin product can be tested with ferric chloride, FeCl_3 . The Fe^{3+} ion reacts with the phenol group on salicylic acid and gives a purple color. This test can also be used to determine the purity of commercially prepared aspirin. Sometimes old aspirin breaks down to give salicylic acid and acetic acid. Then the aspirin in the bottle smells like vinegar and should be discarded.

C. Analysis of Analgesics

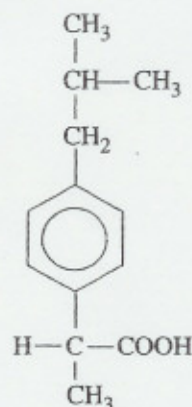
Aspirin is one of several analgesics that are used to relieve pain. Other analgesics include acetaminophen, ibuprofen, and naproxen. Many aspirin products include caffeine. These products including aspirin are used to reduce fever, which means they are also antipyretics. However, aspirin also has anti-inflammatory properties and may reduce the risk of a heart attack.



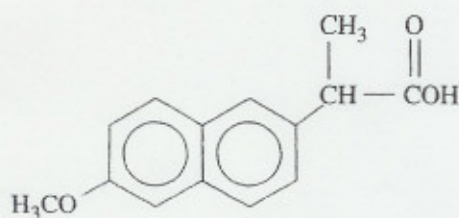
Aspirin



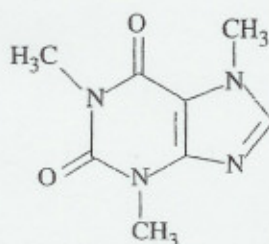
Acetaminophen



Ibuprofen



Naproxen



Caffeine

Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a technique used to separate substances in a mixture. A TLC plate is typically a sheet of plastic, coated with a thin layer of a solid adsorbent such as silica gel.

Small amounts of known and unknown substances are placed as small spots at one end of the TLC plate. Then the end of the plate with the spots is placed in a solvent contained in a developing chamber.

The solid silica layer on the TLC plate is called the *stationary phase*. The solvent or the *moving phase* slowly moves up the silica layer on the TLC plate carrying the substances in the spot with it. The more soluble a substance is in the solvent, the higher the solvent will carry it up the plate. A substance that adheres strongly to the stationary silica gel moves only a short distance with the solvent. Thus, differences in the substances determine the distances they travel up the plate.

As the solvent front nears the top of the TLC plate, the plate is removed, marked, and dried. Then the substances are visualized. If they have colors, they can be seen directly. In this experiment, they are colorless. Because the silica material on the plate contains a fluorescent compound, ultraviolet light (254-nm) from a UV lamp can be used to visualize the substances, which appear as dark spots on the plate.

Calculating R_f Values

A value called the R_f value can be calculated for each substance on a plate. The R_f is the distance that a substance moves on the plate divided by the distance the solvent moves. An unknown substance is identified if its R_f value matches the R_f value of one of the known substances used on the plate. (See Figure 29.1.)

$$R_f = \frac{\text{distance substance moves}}{\text{distance solvent moves}}$$

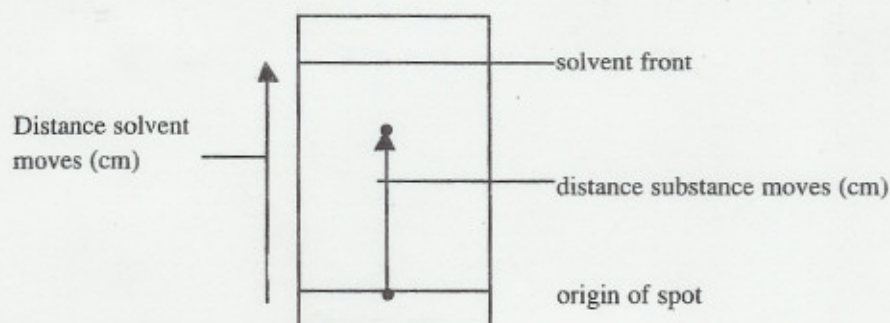


Figure 29.1 Distances moved by a substance and solvent on a TLC plate

In this experiment, you will use TLC to determine the R_f values for several known analgesics. You will also determine the R_f values and identify the types of analgesics in a variety of over the counter drugs used to relieve pain.

Experimental Procedures

Experimental Procedures

WEAR PROTECTIVE GOGGLES AT ALL TIMES!



A. Preparation of Aspirin

Materials: 125-mL Erlenmeyer flask, 400-mL beaker, hot plate or Bunsen burner, ice, salicylic acid, acetic anhydride, 5- or 10-mL graduated cylinder, stirring rod, pan or large beaker, dropper, 85% H_3PO_4 in a dropper bottle, Büchner filtration apparatus, filter paper, spatula, watch glass

- A.1 Weigh a 125-mL Erlenmeyer flask. Add 2.00 g of salicylic acid and reweigh. *Working in the hood, carefully* add 5 mL of acetic anhydride to the flask.

Caution: Acetic anhydride is irritating to the nose and sinus. Handle carefully.

Slowly add 10 drops of 85% phosphoric acid, H_3PO_4 . Stir the mixture with a stirring rod. Place the flask and its contents in a boiling water bath and stir until all the solid dissolves.

Remove the flask from the hot water and let it cool to room temperature. *Working in the hood, cautiously* add 20 drops of water to the cooled mixture.

**KEEP YOUR FACE AWAY FROM THE TOP OF THE FLASK:
ACETIC ACID VAPORS ARE IRRITATING.**

When the reaction is complete, add 50 mL of cold water. Cool the mixture by placing the flask in an ice bath for 10 minutes. Stir. Crystals of aspirin should form. If no crystals appear, gently scratch the sides of the flask with a stirring rod.

Collecting the Aspirin Crystals

Some Büchner filtration apparatuses should be set up in the lab. Add a piece of filter paper. Place the funnel in the filter flask making sure that the neck fits snugly in a rubber washer. Moisten the filter paper. Turn on the water aspirator and pour the aspirin product onto the filter paper in the Büchner funnel. Push down gently on the funnel to create the suction needed to pull the water off the aspirin product. The aspirin crystals will collect on the filter paper. See Figure 29.2.

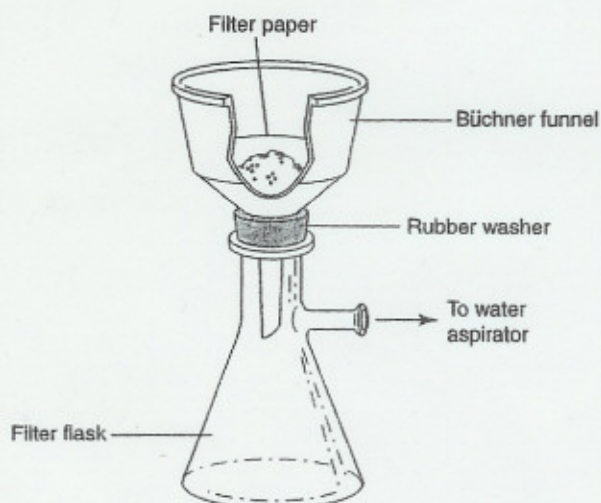


Figure 29.2 Apparatus for suction filtration with a Büchner funnel

Use a spatula to transfer any crystals left in the flask. Rinse the inside of the flask with a 10-mL portion of ice cold water to transfer all the crystals to the funnel. Wash the aspirin crystals on the filter paper with two 10-mL portions of cold water.

Spread the aspirin crystals out on the filter paper and draw air through the funnel. This helps dry the crystals. Turn off the water and use a spatula to lift and transfer the filter paper and aspirin to a paper towel. Don't touch it; it may still contain acid. Allow the crystals to air dry.

A.2 Weigh a clean, dry watch glass. Transfer the aspirin crystals to the watch glass and reweigh.

Calculations

A.3 Calculate the mass of salicylic acid.

A.4 Calculate the maximum yield of aspirin possible from the salicylic acid.

A.5 Calculate the mass of the aspirin you collected.

A.6 Calculate the percent yield of aspirin.

A.7 If a melting point apparatus is available, determine the melting point of your aspirin product. Pure aspirin has a melting point of 135°C. Salicylic acid melts at 157–159°C. Compare the melting point of your aspirin with the known melting points of aspirin and salicylic acid.

B. Testing Aspirin Products

Materials: Test tubes, spatula, aspirin from part A, commercial aspirin tablets, buffered aspirin, acetylsalicylic acid; 0.15% (m/v) salicylic acid, pH indicator paper, stirring rod, 1% FeCl_3 , 10% NaOH, 10% HCl, 400-mL beaker, hot plate or Bunsen burner, blue litmus paper

B.1 **pH of aspirin** Place 3 mL of 0.15% salicylic acid in the first test tube. In test tubes 2–5, place a few crystals (the amount on the tip of a spatula) of the following substances and add 3 mL of water to each:

- | | |
|---------------------------------|--------------------------------|
| 1. 0.15% Salicylic acid | 4. Aspirin product from part A |
| 2. Commercial aspirin (crushed) | 5. Acetylsalicylic acid |
| 3. Buffered aspirin (crushed) | |

Stir each mixture and touch the stirring rod to a piece of pH indicator paper. Compare the color of the paper to the chart on the container. Record the pH of each. *Save these test tubes and samples for part B.2.*

B.2 **Testing aspirin purity** To each of the samples from B.1, add 5 drops of 1% ferric chloride (FeCl_3) solution. Any free salicylic acid (unreacted during synthesis or resulting from hydrolysis in the breakdown of aspirin) reacts with the FeCl_3 to give a purple color. The more salicylic acid in the sample, the deeper the color. This indicates that the product is impure or that decomposition has taken place.

The maximum salicylic acid allowed in commercially prepared aspirin products is 0.15%. If the sample test has a lighter color than a 0.15% standard, the sample would be considered *pure* by USP standards. If the sample is darker, it is *impure* and not safe for ingestion. However, no matter what

the results of the test, your laboratory-prepared aspirin must not be ingested. Record the colors. Compare the purity of the tested products to the reference sample of salicylic acid.

C. Analysis of Analgesics

Materials: 400-mL beaker (developing chamber), Saran wrap, rubber band to fit beaker, solvent (75% ethyl acetate and 25% hexane), TLC plate with silica gel, UV lamp (short wave 254 nm), micropipettes, spot plates, dropper bottle containing 1% solutions in ethanol of aspirin, ibuprofen, acetaminophen, naproxen, caffeine, over the counter drugs, ruler

tylenol

Preparing the TLC Developing Chamber

Obtain a 400-mL beaker, a piece of Saran wrap that covers, and a rubber band. Carefully pour a small amount of solvent into the beaker to a level of 0.5 – 0.6 cm. *It is important that the solvent level is below the spots you place on the TLC plate.* Cover the beaker with Saran wrap and secure with a rubber band.

Spotting the TLC Plate

Obtain a TLC plate that is 6 cm × 10 cm. *Be sure you handle the plates at the edge only to avoid transferring substances from your fingers.* Draw a light line with pencil about 1 cm above the end. This is your starting line or *origin*. Mark 6 dots on the line equally spaced. Label the dots from 1 through 6.

Place a few drops of each of the 1% solutions in a spot plate. Number the wells as follows.

- | | | |
|-------------|--------------|--------------------------|
| 1. aspirin | 2. ibuprofen | 3. acetaminophen |
| 4. naproxen | 5. caffeine | 6. over the counter drug |

Using clean capillary pipettes, one for each substance, spot a tiny amount of each substance on a dot. Lightly tap the micropipette to deliver a small amount. When dry you can apply again. The spot must be kept small rather than allowed to flow into larger spots. (See Figure 29.3.)



Figure 29.3 Spots of analgesics on a TLC plate

Placing TLC Plate in Developing Chamber

Carefully set the plate in the solvent in the beaker you prepared as the developing chamber. *The solvent must be lower than the origin on the plate.* Cover the beaker with Saran. Allow the beaker to remain undisturbed as the solvent moves up the TLC plate. (See Figure 29.4.)

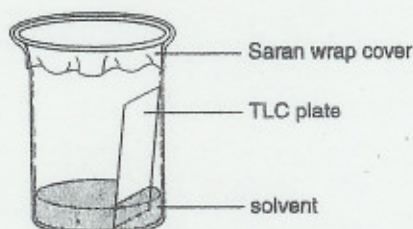


Figure 29.4 A developing chamber containing TLC plate.

When the solvent has risen almost to the top of the plate, open the chamber and draw a pencil line along the solvent front. Remove the plate and allow the solvent to evaporate in the hood. Place used solvent in the organic solvent container.

- C.1 Observe the TLC plate under UV light. Circle each spot. Draw a picture of the spots on your TLC plate.
- C.2 Measure the distance from the origin to the solvent front. Measure the distance from the origin to the center of each spot.
- C.3 Calculate the R_f value for each analgesic.
- C.4 Identify the analgesics in the over the counter pain reliever.

Report Sheet - Lab 29

A. Preparation of Aspirin

A.1 Mass of flask

Mass of flask and salicylic acid

A.2 Mass of watch glass

Mass of watch glass and crude aspirin product

Calculations

A.3 Mass of salicylic acid

A.4 Possible (maximum) yield of aspirin

(Show calculations.)

A.5 Mass of aspirin

A.6 Percent yield

(Show calculations.)

A.7 Melting point ($^{\circ}\text{C}$) of aspirin product (optional)

Questions and Problems

Q.1 Write the structural formula for aspirin. Label the ester group and the carboxylic acid group.

Q.2 If a typical aspirin tablet contains 325 mg aspirin (the rest is starch binder), how many tablets could you prepare from the aspirin you made in lab?

Report Sheet - Lab 29**B. Testing Aspirin Products**

Samples Tested	B.1 pH	B.2 Color with FeCl ₃
1. 0.15% Salicylic acid		
2. Commercial aspirin brand:		
3. Buffered aspirin brand:		
4. Aspirin from A		
5. Acetylsalicylic acid		

Questions and Problems

Q.3 Give an explanation for any differences in the pH values in the samples you tested in part B.1.

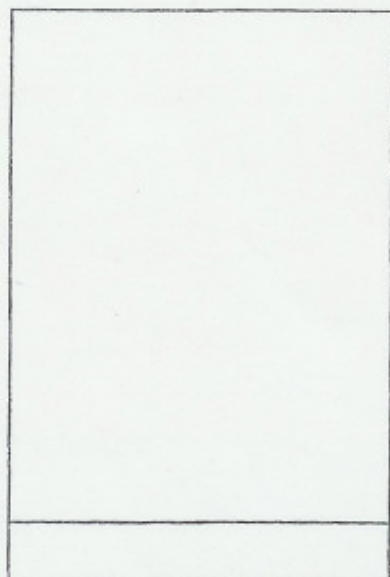
Q.4 How does the pH of buffered aspirin product compare to the pH of the nonbuffered aspirin product?

Q.5 What substance is present if the FeCl₃ test gives a purple color? Which sample is the most impure?

Q.6 Aspirin that has been stored for a long time may give a vinegar odor and give a purple color with FeCl₃. What reaction would cause this to happen?

Report Sheet - Lab 29**C. Analysis of Analgesics**

C.1



C.2 Distance moved by solvent _____ cm

Spot #	Analgesic substance	C.2 Distance moved	C.3 R_f value
1			
2			
3			
4			
5			
6			

C.4 Substance(s) present in over the counter pain reliever

According to your R_f values, what substance(s) can you identify as present in the over the counter pain reliever in your analysis?

Substance 1 _____

Substance 2 _____