Technique of Thin-Layer Chromatography

Experiment Title: Applying TLC As A Method to Monitor the Multistep Synthesis of Aspirin

You will be using the Pre and Post lab sheets posted on the web.

Introduction
Aspirin will be synthesized from methyl salicylate in two steps. Thin layer chromatography will be used to monitor the reaction for both steps of the synthesis. The first step requires the saponification of methyl salicylate to salicylic acid. The second step is the acetylation of salicylic acid with acetic anhydride to produce acetylsalicylic acid (aspirin).

Activities:
- Read the Introduction, Theory, Spotting the TLC Plate, Development, Visualization, and R_f Values sections of Experiment 7 in the Lab Guide, page 170-179.
- In the first part of the experiment you will determine an appropriate solvent system to separate methyl salicylate, salicylic acid and acetyl salicylic acid using standard solutions.
- Then you will saponify methyl salicylate with NaOH to produce salicylic acid. You will follow the progress of this experiment by TLC.
- You will isolate the crude salicylic acid and purify it by recrystallization before proceeding to the next step.
- You will then acylate the salicylic acid with acetic anhydride to produce acetylsalicylic acid. This reaction will be monitored by TLC.
- You will purify the acetylsalicylic acid and report a final melting point and % yield.
- You will obtain a 400 MHz 1H NMR spectrum of the salicylic acid isolated in the first step and acetylsalicylic acid isolated in the second step.

PreLab Exercise 1

Remember to check your syllabus to see which three questions to answer from the Prelab Exercise list.
PreLab Exercise 2

The prelab questions for PreLab Exercise 2 are listed below.

1. Why is it important to keep the cap on the acetic anhydride whenever it is not being used?

2. What is the purpose of adding the concentrated phosphoric acid to the reaction mixture in the synthesis of aspirin?

3. Why does the acylation take place at the phenolic position rather than the carboxylic acid position?

It is extremely important that you read the introductory material from Chapter 7 of the Lab Guide on thin-layer chromatography, because you will be using TLC to monitor the two-step synthesis of aspirin.

Analgesics are substances that relieve pain. The most common of these is aspirin, a component in more than 100 nonprescription drugs. Aspirin has wide spread use in medicine and over 30 million pounds of it are consumed each year in the United States. Willow leaves and bark have been used for centuries for their pain relieving and fever-reducing properties, however the active ingredient in these home remedies is salicylic acid, which over time irritates the stomach lining. In 1893 a German chemist Felix Hofmann synthesized acetyl salicylic acid that offered similar medicinal properties without the stomach irritation. Since the contents of the stomach are acidic, aspirin passes through unchanged and does not get absorbed until it reaches the basic environment of the intestines. Salicylic acid is a white crystalline compound that is commonly used in ointments and plasters for the removal of warts.

Aspirin is synthesized by acetylating salicylic acid to produce the corresponding acylated carboxylic acid. You will begin with methyl salicylate a component of oil of wintergreen that is used in flavoring candies. In the first of this two-step synthesis of aspirin, a methyl ester (methyl salicylate) is saponified to produce the corresponding carboxylic acid (salicylic acid). In this second step the phenolic functional group of salicylic acid is acetylated with acetic anhydride to produce a new ester (acetyl salicylic acid). Keep in mind that anhydrides decompose readily with the moisture in the air, so open and close the acetic anhydride bottle quickly and cover the containers used to transfer this reagent. Use all anhydrides in the hood and use gloves when handling them. You will be monitoring the progress of both reactions by TLC and identifying an appropriate solvent system for the separation of the starting material, intermediate product and final product of this multi-step synthesis.

To identify an unknown by TLC, the usual strategy is to find a stationary phase/mobile phase combination that will separate all the compounds you are analyzing. In this experiment, you will not vary the stationary phase or the silica gel, but will vary the polarity of the mobile phase by using differing ratios of the solvents hexane...
(nonpolar) and ethyl acetate (polar) to develop TLC plates spotted with standard solutions of the starting material and the two products in the two-step synthesis of aspirin. Once you and your lab group determine the optimal mobile phase that will give the best separation of these, you will use this composition to run a chromatogram of the reaction mixture to monitor the progress of both reactions. From the chromatogram, you should be able to determine whether or not the products have been produced by matching $R_f$'s of the standards to the compounds in the reaction mixture. You will need at least a 50% reaction yield after isolation and purification of the salicylic acid to carry out the second step in the synthesis.

**Procedure for TLC.**

You will be working in your research groups of 4 or 5 so choose the solvent composition(s) found below for the appropriate number of members in your group. Each group member will prepare two solvent compositions to be used as mobile phases for the separation of the methyl salicylate, salicylic acid, and acetylsalicylic acid.

Table 1. Mobile phase compositions for 5 group members

<table>
<thead>
<tr>
<th>Mobile Phase Composition Number</th>
<th>%Ethyl Acetate</th>
<th>%Hexane</th>
<th>$R_f$ Methyl salicylate</th>
<th>$R_f$ Salicylic Acid</th>
<th>$R_f$ Acetyl Salicylic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>60</td>
<td>40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Mobile phase compositions for 4 group members

<table>
<thead>
<tr>
<th>Mobile Phase Composition Number</th>
<th>%Ethyl Acetate</th>
<th>%Hexane</th>
<th>$R_f$ Methyl salicylate</th>
<th>$R_f$ Salicylic Acid</th>
<th>$R_f$ Acetyl Salicylic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>90</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>70</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Label the green lids of your two 4 oz. TLC jars with mixture composition numbers assigned in the diagram and table above. Use a 10 mL graduated cylinder to prepare 10 mL of each of the two compositions assigned. Don’t try to pour out the correct amount from the supply bottles, but rather use the plastic pipet attached to the supply bottle to transfer the solvent to better control the exact volume to be measured out. Working in the hood, pour each 10 mL mixture into the corresponding wide-mouth jar. Swirl to mix and then pour about half out so that the solvent level won’t be so high that it will wash compound spots off the TLC plate. (Dispose of the excess in the non-halogenated waste bottle.) Insert a piece of 4 cm filter paper into each jar so that it wraps around the inside wall of the jar and dips into the liquid. This creates a saturated vapor atmosphere that improves spot shape and reproducibility. Cap both jars. Using a lead pencil (not a pen) and a ruler, mark two plates as shown in Figure 7.13 in the lab guide. First, draw a light pencil line across the plate about 1 cm from the bottom of two TLC plates. Make four equally spaced vertical dashes on this line. The dashes should be about 5 mm from the edge and 5 mm apart. Then label the lanes at the top of the plate.

In a shorty vial, obtain a small amount of dichloromethane from the common shelf. Using a TLC spotting capillary tube from your desk, practice spotting on a paper towel using pure dichloromethane. After filling the capillary by dipping it in the liquid, touch it quickly to the towel so that the spot is no larger than 1 to 2 mm diameter. The smaller the spot, the better the final TLC analysis. After the solvent evaporates, you can apply more material in the same spot by again quickly touching the capillary to the surface at the same place.

Three people in your group of four or five should obtain just a few drops of one of the 1% or 2% solutions of the three standards: methyl salicylate, salicylic acid, and acetyl salicylic acid in shorty vials, properly labeled. Spot both of your plates with each of these three standards, placing the spot at the origin mark corresponding to each. Examine the plate under the UV light to see that enough of the compound has been applied by observing a visible dark purple dot, if it is not visible spot more. Using forceps, gently place one in each TLC bottle, being careful not to splash solvent up the plate (see Figure 7.9 in lab guide). Be sure that the spots are not below the solvent level or they will wash away into the solvent. Allow each plate to develop until the solvent front is approximately 1 cm from the top of the plate. Using forceps, remove the TLC plate and quickly mark the solvent front with a pencil. Allow the plates to dry in the hood.

Examine the plate under the UV lamp to see the components as dark spots against a bright orange or green-blue background. Outline the spots with a pencil. [The spots can also be visualized by putting the plate in an iodine chamber that can be found on the side shelf. After a few minutes sitting inside the closed bottle, compound spots turn brown.] Calculate the R_f value for the center of each spot as shown in Figure 7.11 in the lab guide and enter the value in Table 1 or Table 2 above. Tape the properly labeled TLC plates in your notebook using the wide sticky tape available on the side shelf. Cover the whole plate with tape.
Obtain $R_f$ values for the other mobile phase compositions form the other students in your group and enter them in Table 1 or 2 also. As a group, decide which composition gives the best separation, in other words: (1) it does not allow any of the compounds to remain on the baseline, (2) it does not allow any of the compounds to travel with the solvent front, and (3) it provides the greatest differences in the $R_f$ values for the compounds to be separated.

Cleaning Up. Do not dispose of the spotting capillaries; they are reusable!! They may be cleaned by dipping the ends into acetone and blotting the ends with a paper towel. Store them safely in the test tube labeled for TLC spotting capillary storage in your drawer for use in other experiments.

Used mixed solvents should be placed in the appropriate organics waste container in your hood. Return iodine chambers to the side-shelf.


**Saponification of Methylsalicylate**

You will saponify this ester to produce salicylic acid a precursor to acetylsalicylic acid. Methyl salicylate has two functional groups an ester and a phenol. Esters are easily hydrolyzed with base to the corresponding carboxylic acid. When a strong base such as NaOH is added to methyl salicylate three reactions occur: 1) ester hydrolysis, 2) phenoxide ion is formed, and 3) the carboxylic acid product is converted to its conjugate base. Addition of a strong acid in the reaction workup protonates both the phenolate and the carboxylate anions. Salicylic acid is insoluble in cold water so it can easily be isolated via filtration.

The hydrolysis reaction is very slow at room temperature but the reaction time can be shortened when the mixture is refluxed for a period of time. Refluxing occurs when the solvent in the reaction mixture is heated to boiling and resulting vapor is condensed and drops back into the reaction flask as a liquid after coming in contact with a condenser. You have already observed this behavior when you carried out the distillation experiment, however in that case you were interested in transferring the vapor to a second round bottom flask. In this case, you will be returning the vapor to the reaction flask as liquid solvent.

After allowing the reaction mixture to reflux you will obtain a sample for TLC to monitor the progress of the reaction. You can stop the reaction when the spot corresponding to the starting material is only slightly visible. You will begin the workup of the reaction by cooling and acidifying the reaction mixture and isolating the resulting solid product. Salicylic acid is only slightly soluble in cold water but is soluble in hot water so you will do your filtration while the mixture is cold. After isolating the product, you can take advantage of salicylic acid’s solubility in hot water and use this solvent for recrystallization.
**Experimental Procedure:** Have one member in the group make 20 mL of a 4 M sodium hydroxide solution in a 50 mL Erlenmeyer flask. When weighing out the NaOH pellets work quickly and do not touch them with your hands. The group will share this sodium hydroxide solution for their saponifications. Weigh out 230 mg of methyl salicylate into a large test tube 20 X 150 mm. Lay the test tube on the pan of the balance and have it rest on the two little dents designed to keep the tube from rolling. Add 3.5 mL of the 4 M sodium hydroxide solution to the tube containing the methyl salicylate and swirl to ensure that the two compounds mix. A white solid will quickly form. Add a 1/2 inch stir bar and clamp the tube and heat the water solution to reflux using your heating mantle. The tube is large compared to the volume of the solution so the walls of the tube will serve as the condenser. Reflux for 15 minutes, cool and check the reaction mixture by TLC. If the reaction has nearly gone to completion you can proceed to the work up section, if not reflux for an additional 5 – 10 minutes depending on amount of methyl salicylate remaining. Cool and check reaction mixture again by TLC.

**Workup:**
Place the reaction tube in a 250 mL beaker containing ice to keep the tube cool. Add 3 M sulfuric acid solution to the tube in 0.5 mL increments, (about 10 drops from a disposable pipet) until a heavy white precipitate forms and remains when the tube is stirred. Add an additional 10 drops of acid to ensure complete precipitation of the salicylic acid. Using a clean glass stirring rod test the pH of the salicylic acid solution to be sure it is acid using pH paper. Filter the cold solution using a Hirsh funnel and isolate the white solid.

The crude solid will be purified by recrystallization from water. Transfer the solid to a 10 mL Erlenmeyer flask and add 2 mL of water. Heat to boiling using a boiling stick to control bumping. Add water in small increments until all the solid is dissolved and then remove from the heat. Allow the solid to recrystallize by placing it in an ice bath. Once a significant amount of crystals have formed, cool the flask in an ice bath and then isolate the solid using vacuum filtration with a Hirsh funnel. Wash the crystals once with ice-cold water and remove the solvent using a vacuum. Transfer the crystals to a watch glass to dry until the next lab period.

**Yield and Characterization:**
Weigh the solid and obtain a melting point and obtain a $^1$H NMR spectrum of the solid.

**Acylation of Salicylic Acid**

Now you will carry out the second step of the synthesis and produce acetyl salicylic acid.

**Experimental Procedure**
Carry out this procedure in the Hood. Add 100 mg of salicylic acid produced in the TLC experiment to a clean 13 X 100 mm test tube. Add 250 µL of acetic anhydride
using a disposable syringe without the needle to the test tube containing the salicylic acid. (Have everyone in the your team use the same syringe for the addition of the acetic anhydride). Add one drop of 85% phosphoric acid from a Pasteur pipet and then loosely cork the top of the test tube. Shake the tube gently to dissolve the salicylic acid and then place the tube in a beaker with hot tap water. Leave the tube in the hot water for 15 minutes. Check temperature of water and if significant cooling has occurred replace with more hot water. Monitor the reaction by TLC. When the reaction has gone almost to completion remove the test tube from the water bath and add 700 µL of water from an adjustable pipet. Allow the solution to cool to room temperature. Crystallization of the crude product should begin. Now place the sample in an ice bath and force out the remaining crystals. Vacuum filter the crude product with a Hirsh funnel and allow it to dry on a piece of filter paper until the next lab period. Obtain the melting point of the solid and determine whether or not it further purification is needed. If the solid contains any starting material recrystallize the acetylsalicylic acid from ethyl acetate. After the recrystallized solid is dry obtain a second melting point and a $^1$H NMR spectrum.
PostLab Questions:

Remember to check your syllabus to see which three questions to answer from the Postlab Question list.

References:


Final Report

Tape Tables 1 and 2 into your In-Lab Data and Observations section. In your RESULTS AND DISCUSSION section, give the composition of the mobile phase your group chose and tell why, show explicitly how you calculated the Rf values. Describe the reproducibility of TLC Rf values. Discuss how your observed relative Rf’s corresponded to relative Rf’s predicted by you in the PreLab. Also include the % yield and weight of each purified product. You must also have an NMR spectrum for both products. Label the peaks and integrate all peaks. Include a discussion the interpretation of these spectra.