

ASPIRIN SYNTHESIS & ANALYSIS

INTRODUCTION

Since ancient times, the bark and leaves of willow trees have been used as a pain killer. The active component, salicylic acid (SA), can, however, cause stomach upset because its acidity ($pK_a = 2.97$) can be higher than the pH of the human stomach ($pH \sim 4$ after digestion is complete).

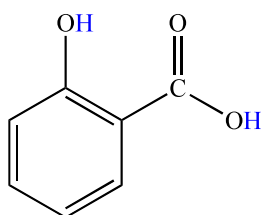


Figure 1. Salicylic Acid

Salicylic acid is a diprotic organic acid with two acidic functional groups: a *carboxylic acid* and a *phenol*. (Acidic hydrogen atoms are blue.) In comparison, the monoprotic acetylsalicylic acid (ASA, aspirin) is less acidic ($pK_a = 4.57$). The reason: an ester has replaced the acidic phenol in ASA.

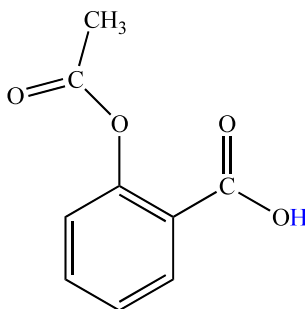


Figure 2. Acetylsalicylic Acid (ASA)

The synthesis of ASA from salicylic acid results in the formation of an ester functional group and, therefore, is called an **esterification**. The first step in this esterification is to create a **suspension** of salicylic acid (a solid at room temperature) in an excess of acetic anhydride (a liquid at room temperature). Acetic anhydride serves as both a reactant and a solvent. A **catalyst** is required for this reaction. Phosphoric acid, H_3PO_4 , donates a H^+ which binds to the

reaction complex. As a catalyst, H^+ is regenerated (not consumed) by the end of the reaction. As the reaction proceeds, the solid salicylic acid disappears and the acetylsalicylic acid product remains dissolved in the hot solution. Once all solid has disappeared (all the salicylic acid has been consumed) the reaction is complete.

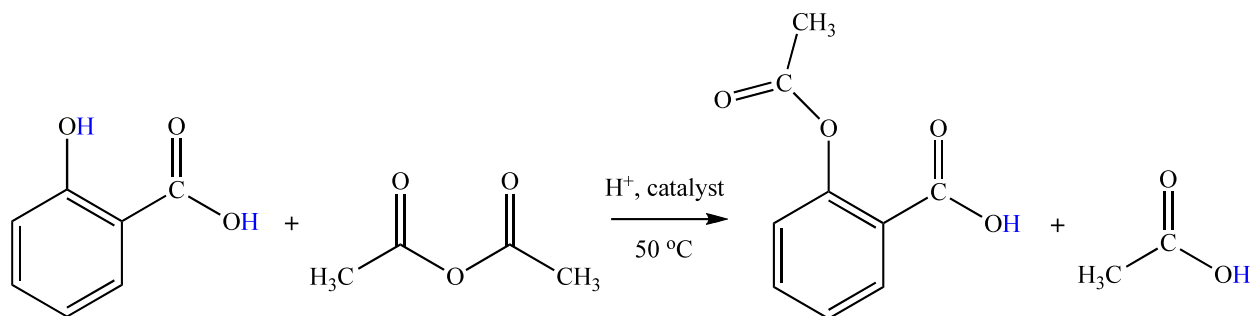


Figure 3. Formation of Acetylsalicylic Acid (ASA, a.k.a., Aspirin)

At this point the excess acetic anhydride must be **hydrolyzed** (split apart by the addition of water) to acetic acid. Acetic anhydride is *very* reactive toward water, so the hydrolysis must be done slowly – water should be added drop-wise.

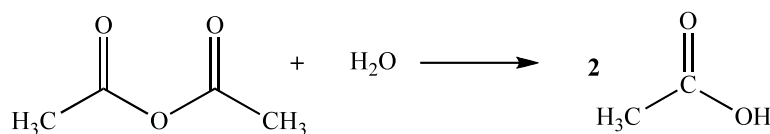


Figure 4. Hydrolysis of Acetic Anhydride

More water is then added and the flask is placed in an ice bath to lower the solubility and precipitate the ASA product. The product is then collected by filtration.

The product's purity is analyzed 3 ways:

- **Melting point** is a physical property inherent to a substance's identity. *Point* is actually a misnomer, substances actually melt over a range. The purer the substance, the smaller the melting range. The broader range of an impure sample (a mixture) results from the creation of a solution upon melting. A mixture's melting point is a **colligative property**, and is, therefore, dependent on the ratio of chemicals present. For this experiment, assume ASA is a solvent and any impurities play the role of solute. The more impurities, the lower the melting point. (Traditionally, this phenomenon is described as *freezing point depression*, but in this experiment the solvent, ASA, is a solid at room temperature and is heated to melt... not

cooled to freeze.) In this experiment, you will measure the melting point of your synthesized ASA product, salicylic acid, a crushed aspirin tablet, and pure ASA from a manufacturer.

- Samples with unreacted salicylic acid complex with Fe^{3+} to create a purple complex in aqueous solution. Pure ASA samples will remain colorless.
- Finally, the number of impurities in different samples will be determined by **thin layer chromatography**. Silica will serve as the *stationary phase* and an organic solvent will be the *mobile phase*. The different compounds in aspirin move up the TLC plate at different rates based the competing intermolecular interactions of the polar silica plate and the less polar solvent. More polar compounds *adsorb* more strongly to the silica and do not move as far up the TLC plate. Less polar components will favor the solvent and move higher. The TLC of the ASA you synthesize in lab will be compared with the TLCs of commercially prepared ASA, the salicylic acid starting material, caffeine, and an aspirin tablet.

A commercially prepared aspirin tablet contains mostly ASA, but other components such as caffeine, buffers, and starch binding agents are also added. *How much of an aspirin tablet is ASA?* How can we find out?

A solution of ASA is colorless and, therefore, does not absorb light in the visible region between 400 and 700 nm. After treatment with a strong base, a hydrolyzed and deprotonated ASA fragment (the conjugate base of salicylic acid, SA) is able to bind with Fe^{3+} ions in acidic solution. A solution of the resulting cationic Fe^{3+} -SA complex is deeply colored.

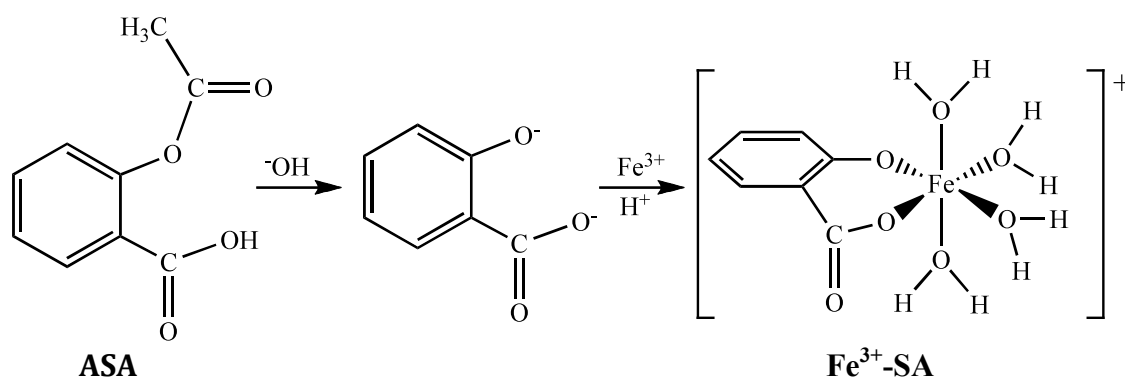


Figure 5. Synthesis of Fe^{3+} -SA

When white light (light containing all wavelengths from 400 to 700 nm) passes through a Fe^{3+} -SA solution, wavelengths corresponding to green light are strongly absorbed and the solution appears purple. This absorption is a result of the excitation of valence electrons to higher energy

electron orbitals. Therefore, the intensity of light at the absorbed wavelengths is reduced passing through solution; the amount of reduction is dependent on the concentration of the absorbing species and the distance the light travels through the solution (path length). This linear dependence is known as the Beer-Lambert Law (or "Beer's Law"):

$$(1) \quad A = \epsilon C \ell$$

A = absorbance (no units)

ϵ = molar absorptivity coefficient (units = L/mol-cm)

C = concentration of absorbing species (units = mol/L)

ℓ = path length (units = cm)

If the optical path length and molar absorptivity coefficient are held constant in an experiment, the absorbance varies with concentration alone. A plot of absorbance vs. concentration is known as a "Beer's Law Plot".

SAFETY PRECAUTIONS

Safety goggles, aprons, and gloves must be worn at all times in the laboratory. **Acetic anhydride** (a liquid at room temperature) is violently reactive toward water, flammable, corrosive, and can cause burns; add water slowly, prevent contact with eyes, skin, and clothing. Securely cap acetic anhydride when transferring it from the supply hood to the hood where you will work. Work with acetic anhydride *in the fume hood only*. The **Fe³⁺ solution** has been acidified to pH < 2 with hydrochloric acid and **sodium hydroxide** is a strong base. Both are corrosive and can cause burns; prevent contact with eyes, skin and clothing. Wash affected areas thoroughly with cold water. **Ethyl acetate** is flammable and harmful by inhalation, ingestion, and when in contact with skin. Any container holding ethyl acetate should be capped when not in use to prevent evaporation of the solvents, as they are harmful when inhaled. The **salicylic acid**, **acetylsalicylic acid**, and **aspirin tablets** used as unknowns may be contaminated through student activities and are NOT for internal use. Solutions containing ethyl acetate or Fe³⁺ must be placed in appropriate waste bottles and NEVER be poured down the drain. Report all spills, accidents, or injuries to your TA.

Before starting the experiment, the TA will ask you to do a quick demonstration or talk-through one of the following:

- 1) How to prepare a TLC plate? (no spotting necessary)
- 2) How to prepare a TLC developing chamber?
- 3) When do you need to remove the TLC plate from the developing chamber and how do you calculate R_f ?
- 4) How to set up a vacuum filtration?
- 5) How to prep a sample to take a melting point?
- 6) What color do you expect the Fe^{3+} test to turn the pure ASA and SA?
- 7) How to insert a cuvette into the spectrometer (what do you need to do to the cuvette before inserting it into the spectrometer)

Make sure you watch the videos on the course website and read the documents to prepare. These demonstrations will be done every week. Everyone will have presented at least one topic by the end of the quarter. The demonstrations should be short (>1 min) and will be graded.

PROCEDURE

This is a 2 week experiment. Part A must each be completed in a single laboratory session. Save a small amount of the aspirin tablet from Part A for Part B and C. Part B's work can be split between the two weeks. Plan your time accordingly.

Part A. Synthesis & TLC Analysis. *Work in pairs.*

Read "TLC" on the lab manual website before starting this section. Ask your TA to demonstrate the techniques.

TLC Preparation

1. Create two TLC plates (labeled #1 & #2). Each will be spotted with three samples.
 - Plate #1: Salicylic Acid (SA) reactant, Reaction Mixture before heating (Sample A) & after heating (Sample B)
 - Plate #2: Pure ASA, Aspirin tablet, & Caffeine

2. Round the bottom corners of the two TLC plates with scissors so the liquid eluent travels evenly up the plates. Use a pencil to lightly mark the origin line where the sample spots will be made.

3. Take a small amount of the salicylic acid (SA) starting material and dissolve as much as you can in 3 drops of ethyl acetate. Add another 10 drops of ethyl acetate. Spot the SA solution on the bottom left of Plate #1. Make the spot as small but as concentrated as possible (place a drop of solution, let it dry, and then repeat at least 3 times). Use a UV lamp to see if the spot is the right size (~1 mm diameter) before moving on. **Do not look directly into the UV lamp.** The other two spots on TLC plate #1 will be added later.
4. Prepare one TLC developing chamber: Add ethyl acetate into the TLC developing chamber until the liquid surface is below the origin line once the plate is placed in the container. A piece of filter paper should be stood against one wall inside of the flask. The purpose of the filter paper is to ensure that the atmosphere inside the chamber is saturated. Any vessel containing ethyl acetate should be capped when not in use to prevent its vapors from escaping into the laboratory.

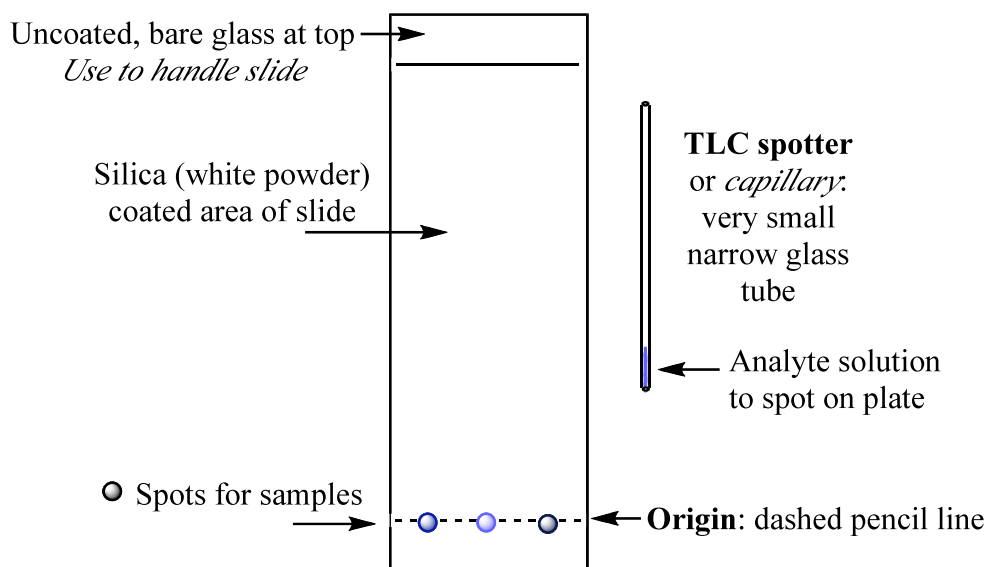


Figure 6. TLC Plate spotted with Samples.

5. Repeat the spotting process on Plate #2 with the aspirin tablet, pure ASA, & caffeine.

- Crush an aspirin tablet and place in a labeled, pre-weighed vial or test tube. Record the mass of the aspirin tablet. Do not lose this container or its contents, you will use them later.
- Take a small amount of the aspirin tablet and dissolve as much as you can in 3 drops of ethyl acetate. Add another 10 drops of ethyl acetate. Spot the solution on the left side of Plate #2 on the origin line.
- Repeat the process with a small portion of pure ASA & caffeine (separately). Spot these two solution in the middle and right side of the origin line of Plate #2.

Synthesis & TLC Development

6. In the fume hood and wearing gloves, combine ~ 1.5 g salicylic acid (record an accurate mass) and 3.5 mL acetic anhydride (from a graduated cylinder – cap the graduated cylinder when transferring between hoods) into a DRY vial. Add 5 drops H_3PO_4 to the suspension. Use a pipet to remove 2-3 drops of solution (Sample A).
7. Use a *new* TLC spotter and spot the removed solution on the middle of the origin line of Plate #1. (Spots should be evenly spaced and the right size so samples do not run together.)
8. Begin gently stirring and heating the mixture in a 50 °C water bath. Continue heating and stirring until all solid disappears (about 10 minutes).
9. Develop Plate #2 while the reaction goes to completion.
 - Place the plate in the prepared developing chamber. Make sure no edge of the TLC plate touches the filter paper. (If the filter paper touches any side of the TLC plate, the spots will be drawn sideways.) Solvent will begin to move up the plate. Once the ‘solvent front’ is within 1/2 cm of the top of the plate, remove the plate from the chamber and mark the **solvent front line** with a pencil. Do not let your solvent front travel to the top of the TLC plate.
 - Circle any visible spots on the TLC plate with a pencil, then use a UV lamp to see spots not otherwise visible. Measure the distance from the origin to the solvent front and from the origin to each spot. Take a digital picture of the plate to include with your report.

Record data neatly in a table and use the following equation to determine the R_f values of the various spots on the TLC plate:

$$R_f = \frac{\text{Distance from the origin to the center of the TLC spot (cm)}}{\text{Distance from the origin to the solvent front (cm)}}$$

10. After all solid disappears from the reaction mixture, use a pipet to remove 2-3 drops of solution. Use a *new* TLC spotter and spot the removed solution on the right of the origin line of Plate #1 (Sample B). Place the plate aside, you can develop it once you have the your ASA product drying in the oven (step #14).
11. Remove the vial from the water bath and carefully add 15 drops distilled H_2O . Wait one minute. If precipitation does not begin add 15 more drops of H_2O . If precipitation does not occur after 5 minutes, go on to the next step.
12. Once solid begins **precipitating** out of solution, add 15 mL distilled H_2O . The flask and its contents may now be removed from the fume hood. Break up any solids and cool in ice bath for 10 minutes.
13. To complete this step, read the document titled *Vacuum Filtration* on the lab manual website. Record the mass of a piece of filter paper. Collect the precipitate by **vacuum filtration** and wash with 2 mL chilled distilled H_2O . Discard the **filtrate** in the sink.
14. Dry the crystals for 15 minutes in the filter and then place them on a labeled preweighed watch glass in the oven for at least 10 minutes. You should check the temperature on the oven... if the temperature exceeds $65\text{ }^\circ\text{C}$, your product will decompose. (Note: While waiting for your product to dry in the oven, continue on with the next sections of the procedure.) Place Plate #1 into the developing chamber (repeat step #9).
15. Remove from the oven. Once cooled to room temperature, accurately record the mass. Collect this sample in a new vial labeled with the contents (ASA), your name, day and time of you lab section, and the date (with the year).

Note: Dispose of TLC spotters in the *Broken Glass Containers*. Do not throw them away in the trashcans. This is a health hazard for the janitorial staff. Liquids should be discarded in the containers in the hood labeled “Organic Waste”. Used TLC plates should be discarded in the containers in the hood labeled “Solid Waste”. Turn in the product for Part A to your TA.

Part B. Analysis I. *Work in pairs. Collect all data from this part into one table*

B.1 Melting Points

Take melting points: your ASA (product collected in Part A), aspirin tablet, pure ASA, and pure SA from the manufacturer.

1. Turn on the Mel-Temp. Check out a thermometer from the stockroom (the thermometers in lab do not go up to a high enough temperature).
2. For each sample, fill a small capillary tube with 2-3 mm of solid.
3. Keeping track of your samples, insert them into the Mel-Temp and monitor them, recording the temperatures at which melting begins and ends (i.e., the melting temperature range).







B.2 Fe³⁺ test

1. Obtain 4 test tubes and label them: your ASA, aspirin tablet, pure ASA, pure SA.
2. Place ~1 mL distilled H₂O into each test tube
3. Add a crystal or “pinch” of each sample to its test tube
4. Add 1 drop FeCl₃(aq) solution to each test tube.
5. Record your observations. Solutions should be discarded of in the hood labeled “Metal Containing Waste”.

Part C: Analysis II.

Creation of a Beer’s Law Plot. *Work in pairs.*

1. Obtain six sealed cuvettes containing the standard solutions and blank. The blank (labeled “B”) contains a clear yellow aqueous solution of acidic FeCl₃. The remaining cuvettes (labeled #1-5) contain standard solutions of Fe³⁺-SA prepared by the stockroom in the following manner:

- a) A **stock solution** was prepared by dissolving exactly 0.4000 g of pure ASA in approximately 10 mL of NaOH(aq) and heating the solution to a gentle boil. After cooling to room temperature, the solution was poured into a 250-mL volumetric flask and diluted to the mark with DI water.
 - b) The **standard solutions** (#1-5) were prepared by transferring exactly 1.00 mL, 2.00 mL, 3.00 mL, 4.00 mL, and 5.00 mL, respectively, of stock solution to clean 100-mL vol flasks, diluting to the mark with FeCl₃ solution, and then filling the appropriately labeled cuvette.
 - c) **Before coming to lab, calculate the concentration of ASA in cuvettes #1-5.**
2. Obtain a visible spectrometer from the stockroom. Use the USB cable to connect the visible spectrometer to the LabQuest2.
 3. Calibrate the spectrometer by clicking . The calibration dialog box will display the message: “Waiting....seconds for lamp to warm up.” (The minimum warm up time is one minute.) **Note: For best results, allow the spectrometer to warm up for at least three minutes.** Wipe the outside of the blank (containing FeCl₃(aq)) cuvette (labeled “B”) with a kimwipe and insert the cuvette in the sample compartment. Click **Finish Calibration** and then **OK**.
 4. Wipe the outside of the cuvette containing solution #5 with a kimwipe, place in the cuvette holder of the Spectrometer and click . Click  once the data collection is complete. Examine the graph and note the wavelength region of maximum absorbance. Remove the *rainbow* background spectrum by double clicking the rainbow background. Click  and store latest run.
 5. Go to the  screen, click on **Sensors > Data Collection**. Change Mode: *Events w/ Entry*, Name: *Concentration*, Units: *molarity*. Click **OK**.
 6. Wipe the outside of the cuvette containing solution #1 with a kimwipe, place in the cuvette holder of the Spectrometer, click  then click **Keep**. Type in your calculated value for the concentration of Fe-SA. Repeat with all remaining solutions.
 7. Click **Stop** once the absorbance values for all the standard solutions have been collected. Transfer the data to your ELN, title the plot and label the axes.

Absorbance of Fe-SA from an Aspirin Tablet. Work in pairs.

1. Transfer ~200 mg (record an accurate mass) of the crushed aspirin tablet from Part A to a clean 125-mL Erlenmeyer flask. Add 10-mL of 1M NaOH(aq) and heat the mixture to a gentle boil in a water bath. Rinse the walls of the flask with DI water to recover any hydrolyzed SA splattered on the walls. (Caution - hot glassware.)
2. Once the solution has cooled to room temperature, transfer it to a 100-mL vol. flask. Wash the walls of the Erlenmeyer flask with DI water, adding the washings to the volumetric flask. Fill the volumetric flask to the mark with DI water. Cap the flask and invert several times to mix the solution thoroughly. Allow any solid material to settle to the bottom of the flask.
3. Using the diluted solution from the 100-mL volumetric flask, rinse a 1-mL vol. pipet with a few drops and then pipet exactly 1.00-mL into a 50-mL volumetric flask. (Take care not to pipet any solids.) Fill the 50-mL volumetric flask to the mark with acidic FeCl₃ solution. Cap and invert to mix solution. The solution should be a purple color due to the Fe³⁺-SA ion formed.
4. Rinse a clean, empty cuvette with a few drops of the unknown solution and then fill the cuvette about 2/3 full with the solution. Record the absorbance for your unknown at λ_{max} .
5. When finished, pour the solutions in your two flasks and your cuvette into the designated waste containers in the hood labeled "Metal Containing Waste". (Return the original 6 cuvettes to the stockroom with your spectrometer.)

Make sure to clear your email address and password of the LabQuest2 so others can't access your email account. Shutdown the LabQuest2 and not simply put it to sleep. To shutdown the LabQuest2: press the home key, select System → Shut Down → OK.

CALCULATIONS

Part A. Synthesis & TLC

1. Calculate the percent yield of ASA synthesized in Part A.
2. How much excess reagent (in mL) is left over before the addition of water?
3. What are the similarities and differences in the TLC of the reaction mixture before heating and after completion?

4. Determine the R_f for all of the components on each slide. What is the R_f of ASA? SA?

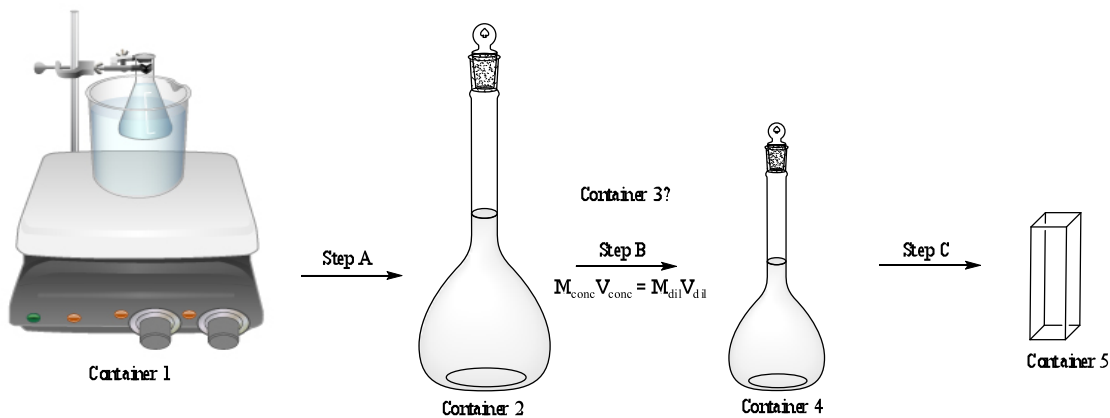
$$R_f = \frac{\text{Distance from the origin to the center of the spot (cm)}}{\text{Distance from the origin to the solvent front (cm)}}$$

Part B. Analysis I

5. Use the results from the TLCs, the melting points, and the Fe^{3+} tests to confirm if the product you synthesized is ASA.

Part C. Analysis II

- Using Excel, create a Beer's Law plot (absorbance vs. concentration).
- What is the value of the y-intercept on your Beer's Law plot? Provide possible explanations for why this number is not zero. (Take into account the sign of the value.)
- Using data from your Beer's Law Plot, what is the value of the molar extinction coefficient, ϵ , (also called the molar absorptivity coefficient) for the Fe-SA ion? (The length of the cuvette is 1.30 cm.) Find the mean value and standard deviation for ϵ .
- Using the Beer's Law plot, find the concentration of ASA in your unknown cuvette graphically and mathematically.
- The drawing below shows the steps for the preparation of the Fe-SA solution from the aspirin tablet. You need to think through these steps in order to find the mass % ASA in aspirin.



- What is container 3?
- What are the volumes of containers 1-4?
- What is step B called? What volumes are known in the equation underneath the arrow of step B? What are their values?

- d. In which container does a chemical reaction occur? What is that reaction?
 - e. What is done with the solution in container 4? What information is gained? How is it gained?
 - f. Label containers 2-5 with either M_{conc} or M_{dil} .
 - g. Which container(s) contain(s) all of the aspirin tablet?
6. Using the data from your Beer's Law Plot, calculate....
- a. The exact mass (in grams) of ASA in your aspirin unknown.
 - b. The mass percent of ASA in your aspirin unknown.

QUALITATIVE ERROR ANALYSIS

1. What modifications could be made to the procedure to better account for random (indeterminate) errors?
2. List three potential systematic (instrumental, methodological, or personal) errors that could be made in this experiment. (Note: Be specific, systematic errors are in the details. For example, losing your solution because you knocked over the cuvette is not a systematic error – it's a gross one.)
3. Did any gross errors occur? Did you mess up? Did the equipment or instrumentation fail?