BIOCHEMISTRY LAB # 5
SYNTHESIS of ASPIRIN

BACKGROUND

The production of new therapeutic drugs involves many steps. The first step in this process involves the identification of compounds that are active against a particular disease or have a particular function. These candidate compounds can be from natural sources, such as plants and fungi, or can be synthetic compounds made in the laboratory. The second step in the process, called optimization, involves modifying the structure of the candidate compound to improve its properties. Modifications are done to increase the potency, and selectivity of the compound while reducing its toxicity. Chemists use synthetic organic chemistry procedures to change functional groups in the candidate compound until they have obtained a compound with the desired properties. In the final, development, stage of the production process chemists check the suitability of the candidate compound for clinical trials and also optimize the synthetic route for large scale production of the compound.

Aspirin, known by the chemical name of acetylsalicylic acid, is a commonly used drug for relieving pain (analgesic), reducing fever (antipyretic), and reducing inflammation (anti-inflammatory). The precursor to aspirin, salicylic acid, was isolated from the bark of the willow tree in the early 19th century following the 18th century discovery that an extract from the bark of the willow tree could reduce fever. While salicylic acid was able to relieve pain and reduce fever, it also damaged the mucous membranes of the mouth and esophagus, and also caused bleeding in the stomach. In the late 19th century chemists found that they could reduce the undesirable properties of salicylic acid, but at the same time maintain the beneficial properties, by modifying the hydroxyl group on salicylic acid with an acetyl group to produce acetylsalicylic acid.

![Chemical structures](image)

Aspirin works by inhibiting a class of enzymes that are involved in the synthesis of prostaglandins. Prostaglandins are a type of lipid molecule that plays a role in the perception of pain and the onset of fever. In addition to aspirin other analgesics such as acetaminophen, ibuprofen, and naproxen can also be used to relieve pain and reduce fever. Some of these analgesics including aspirin are anti-inflammatory drugs and belong to a class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs).
PROCEDURE

The goal of this laboratory is to introduce you to the processes of chemical synthesis, isolation of product and the investigation of product purity. We will synthesize aspirin from salicylic acid, isolate the product by filtration, and check the purity of the synthesized product using thin layer chromatography (TLC).

TLC is a technique used to separate compounds in a mixture. In TLC a small amount of sample is placed as a spot on a plastic sheet that is coated with a thin layer of adsorbent material such as silica gel. This plastic sheet is called a TLC plate. The end of the plate with the sample is then placed in a developing chamber containing some solvent. The solvent slowly moves up the absorbent layer on the plate carrying the compounds in the sample with it. The distance that a compound travels depends on the solubility of the compound in the solvent. If a compound is highly soluble in the solvent then it will travel higher up on the plate than a compound that has a low solubility in the solvent. When the solvent has nearly reached the top of the plate it is removed from the developing chamber and allowed to dry. After drying, the positions of the compounds on the plate can be visualized, usually using an ultra-violet (UV) lamp. The compounds that were in the sample appear as dark spots on the plate.

A. PREPARATION OF ASPIRIN

Materials: 25 mL Erlenmeyer flask, 2 x 50 mL beaker, beral pipettes, hot plate, salicylic acid, acetic anhydride, 85% \( H_3PO_4 \) in a dropper bottle, ice, 10 mL graduated cylinder, filter, filter paper, spatula and drying oven. 
Caution: Acetic anhydride is irritating to the nose and sinus. Handle carefully.

A.1.

a) On a weigh paper, mass out 0.2 g of salicylic acid, record. Transfer salicylic acid into Erlenmeyer flask
b) Add 0.5 mL of acetic anhydride and cork the flask opening.
c) Add 2 drops of 85% phosphoric acid \((H_3PO_4)\) and re-cork the opening. Swirl the flask contents gently to mix. While holding the flask’s neck, carefully submerge the bottom surface of the flask in a hot water bath to heat the solution and dissolve the solids. The flask bottom should remain near the water surface and gently swirl until all the solid dissolves.
d) Remove the flask from the hot water bath, add 2 drops of \( H_2O \) dest. and let it cool to room temp.
e) Add 5 mL of chilled \( H_2O \) dest. to the cooled solution and re-cork the flask and swirl to mix. Place flask in an ice water bath for 10 minutes. Crystals of aspirin should form.

A.2. Collecting the Aspirin Crystals

a) Take a piece of filter paper, write group name with pencil on the rim and record its weight.
b) Place a funnel on another 50 ml beaker. Fold the filter paper into a cone, place into funnel and wet with a few drops of \( H_2O \) dest to make it stick to the sides.
c) Stir your crystallized reaction liquid with a fresh spatula and pour into the filter cone collecting the aspirin product on the filter paper in the funnel. Use the spatula to transfer any crystals left in the flask and then rinse the inside of the flask, and the spatula with 1 mL of chilled DI water to help transfer all the crystals to the funnel. Repeat this rinse two more times. DO NOT LOSE CRYSTALS!
d) Wait until all liquid has drained out of the filter, then lift the filter gently out of the cone and place it crystal side up onto a paper towel to remove liquid from the filter. DO NOT LOSE CRYSTALS!!! Move it to a new dry spot on the paper towel to help dry it out. Spread the crystal a little, then place the filter into the drying oven (50°C) rack, to continue drying the aspirin product for 10 minutes or so.
e) When paper is dry remove from the oven – DO NOT LOSE CRYSTALS- and weigh enter in data table.
f) Calculate mass of aspirin produced, by subtracting mass of filter paper from mass of e)
g) Calculate the yield of your reaction
B. QUALITY CONTROL

**Materials:** Plastic well plate, aspirin from part A, commercial aspirin tablets, acetylsalicylic acid, 0.15% (m/v) salicylic acid, pH indicator paper, glass stir rod and 1% FeCl₃.

B.1. Testing aspirin purity. Add a few crystals of
- your self-made aspirin
- the commercial aspirin
- laboratory grade acetylsalicylic acid
- to three wells of a well plate -TRY TO USE EQUAL AMOUNTS FROM ALL THREE PRODUCTS.
- Then add 10 drops of DI water to each well.
- Then place 10 drops of a prepared 0.15% salicylic acid solution to a fourth well. Now add 1-2 drops of 1% ferric chloride (FeCl₃) solution to each well.

Any free salicylic acid (unreacted during synthesis or resulting from hydrolysis in the breakdown of aspirin) reacts with the FeCl₃ to give a purple color -the more salicylic acid in the sample, the deeper the color! A purple solution 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 and compare the purity of the tested products to the reference sample of salicylic acid.

B2. Record your findings into your Data Report Sheet under B

C. ANALYSIS OF ANALGESICS USING CHROMATOGRAPHY

**Materials:** Developing chamber (beaker and glass lid), solvent (75% ethyl acetate and 25% hexane), TLC plates with silica gel, UV lamp (short wave), transfer pipettes, well plate, ethanol, aspirin product from part A, commercial aspirin, acetylsalicylic acid, acetaminophen and ibuprofen.

**Spotting the TLC plate**
Hold the TLC plate only between thumb and index finger on the side edges– oil from your fingers will interfere with the chromatography! Lightly draw a horizontal line approximately 1 cm from the bottom edge of the plate with a pencil. Mark 4 starting points, evenly spaced out, on that line. This is your starting line or origin.

Get a well plate and transfer 2-3 crystals of:
- Well 1 your synthesized aspirin
- Well 2 commercial aspirin
- Well 3 ibuprofen
- Well 4 acetaminophen

to wells 1-4 (draw a map so you won’t forget which is which!) in your well plate and add 5-6 drops of ethanol to each. Dip a clean 2 µL micro-capillary pipette into the first well. The solution will be drawn up the pipette by capillary action.

Touch the end of the pipette against the coated TLC plate at starting point 1. The solution will drain onto the coating. Tap lightly if necessary. When the spot is dry reapply 2 x more times on the same spot, however, the spot must be kept small rather than allowed to flow into a larger spot. Take a new micropipette for each new sample and repeat until all samples are applied.

Spots of analgesics on a TLC plate
Preparing the TLC Developing Chamber
There should be approximately 0.5 cm of ethyl acetate: hexane in the developing chamber.
Open the developing chamber and carefully lower the TLC plate into the jar. The solvent should not
reach up to the origin line on the plate. Cover the jar and allow the beaker to remain undisturbed as the
solvent moves up the TLC plate.

When the solvent has risen almost to the top of the plate, open the chamber and remove the TLC plate.
Quickly draw a pencil line along the solvent front. Allow the solvent to evaporate from the TLC plate.

C.1. To visualize the spots on the TLC plate, illuminate the dry plate with a UV light. Carefully circle
the outline of each spot with a pencil and place a small dot in the center of each spot. Record your
results.

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 using the equation given below and record these values.

\[
R_f = \frac{\text{distance traveled by analgesic}}{\text{distance traveled by solvent}}
\]

REPORT SHEET

A. PREPARATION OF ASPIRIN

A.1.a Mass of salicylic acid \( \quad \text{g} \)

Calculations
Calculate the maximum possible yield of the aspirin (in g) possible from the salicylic acid you used in
your reaction, using the following equation given below.

\[
\frac{\text{mass of salicylic acid (g)}}{138 \text{ g}} \times \frac{1 \text{ mole salicylic acid}}{1 \text{ mole aspirin}} \times \frac{180 \text{ g}}{1 \text{ mole aspirin}}
\]

A.2 a) mass of filter paper \( \quad \text{g} \)
A.2.e) mass of filter paper + aspirin \( \quad \text{g} \)
A.2.f) mass of produced aspirin \( \quad \text{g} \)

A.2.g) Calculate the percent yield of aspirin using the following equation.

\[
\% \text{ yield} = \frac{\text{mass of aspirin product (g)}}{\text{maximum possible yield of aspirin (g)}} \times 100 \quad \text{%}
\]

SHOW MATH:
B. TESTING THE PURITY OF YOUR ASPIRIN PRODUCT

<table>
<thead>
<tr>
<th>Sample Tested</th>
<th>B.1. Color with FeCl$_3$</th>
<th>Salicylic Acid Present? (Positive/Negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.15% salicylic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commercial aspirin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin from part A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

C. ANALYSIS OF ANALGESICS

C.1 a. Draw your TLC Plate

<table>
<thead>
<tr>
<th>Spot #</th>
<th>Analgesic substance</th>
<th>C.2. Distance Moved (cm)</th>
<th>C.3. $R_f$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Commercial Aspirin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Aspirin product</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Acetaminophen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Ibuprofen</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

QUESTIONS

(1). In part A.1. You chilled the reaction mixture in an ice water bath to form crystals of aspirin. Why do you think crystals formed when you chilled the reaction mixture?

(2). The percent yield you obtained is very likely to be below 100%. What factors do you think contributed to the reduction in percent yield?

(3). Based on your observations in part B provide a brief description of the purity of the synthesized aspirin product?

(4). Compare the $R_f$ value of your aspirin product to those of the other analgesics tested. Why are the values similar in some cases but not in other.
Prelab A - Aspirin Questionnaire

1. What is the full chemical name for Aspirin? ________________________________
2. Draw the Kekule structure of Aspirin.

3. What type of pharmacological substance class does Aspirin belong to? __________
4. Do you need a prescription to buy it? ____ Why or why not: ___________________________________________

5. Which enzyme does Aspirin inhibit? __________________
6. Aspirin lowers the level of which product? __________________
7. Where in nature can you find ‘natural Aspirin’? __________________
8. Make a Venn Diagram comparing/contrasting Acetyl salicylic Acid (ASA) and Salicylic Acid (SA)


10. Draw the structure of Arachidonic Acid in Kekule.

11. How long is its carbon skeleton of ARA? ____
12. What type of functional groups does ARA have? ________________________________
13. What type of molecule group (be as specific as possible) does ARA belong to?

14. What type of double bonds does it have?

15. ARA is the precursor for what type of hormone mediator class? _________________
16. Sketch the pathway of Prostaglandin synthesis with structure, insert enzymes and where NSAIDS intervene.
Prelab C - Chromatography

Image of a Thin Layer Chromatography of a chemical Rx

- Measure distance in mm from Start to Solvent Front: _____ mm
- Measure the distance the of all spots a, b, c and enter in the table below, calculate the Rf values

<table>
<thead>
<tr>
<th>Band</th>
<th>Distance migrated in mm</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>c</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

17. Aspirin, Caffeine, Ibuprofen, and Acetaminophen have the following Rf values:
Aspirin - 0.45, Caffeine - 0.08, Ibuprofen - 0.60, Acetaminophen - 0.24.
You run a Thin Layer Chromatogram (TLC) of an unknown over the counter pain reliever and observe two spots on the TLC plate. The first spot moved 0.35 cm from the origin while the second spot moved 2.2 cm from the origin. The solvent front moved 5 cm from the origin. What two substances are present in the pain reliever? Show your math:

Sketch the TLC from above: Lane 1 Aspirin, Lane 2 Ibuprofen, Lane 3 Acetaminophen and Lane 4 the unkown sample with relative distances....