ABSTRACT: An esterification reaction was performed in order to convert salicylic acid to acetylsalicylic acid, the prodrug and active ingredient in Aspirin. Salicylic acid is made less acidic by converting its alcohol functional group into an ester so that it is less damaging to the digestive system in the human body. The purpose of the experiment is to synthesize, isolate, and purify 2-acetoxybenzoic acid and analyze salicylic acid, crude product, and acetylsalicylic acid via Thin-Layer Chromatography to determine if pure aspirin was synthesized. The amount of crude aspirin synthesized was 3.029 grams and the amount of pure aspirin synthesized was 2.169. The theoretical yield was 2.520 grams. Thus, there was a percent error of 13.93 % and percent yield of 86.07%. TLC analysis showed that acetylsalicylic acid had a higher R\textsubscript{f} value than salicylic acid (.800 vs. .315 R\textsubscript{f} value, respectively). The salicylic acid was more polar because of its extra polar functional group and did not travel as far. Thus, pure aspirin was synthesized.

INTRODUCTION

2-Acetoxybenzoic acid, more commonly known as Aspirin, is a white, crystalline substance most commonly known for its pain-relieving qualities\textsuperscript{1,2}. Acetylsalicylic acid (active ingredient of Aspirin) is an acetyl derivative of salicylic acid and the prodrug of the active metabolite, salicylic acid.\textsuperscript{2} Aspirin is a salicylate drug because it is an ester of salicylic acid. It is commonly known for its pain relieving properties. However, it does not only serve as an analgesic but also as an antipyretic, anti-inflammatory, and antiplatelet medication\textsuperscript{2}. The main metabolite of acetylsalicylic acid, salicylic acid, is an essential part of the human metabolism\textsuperscript{3}. Salicylic acid is an integral part of pain management and was often used by ancient cultures, such as the Native Americans, who extracted the chemical from willow tree bark\textsuperscript{3}. This fundamental compound can cause stomach irritation and is bitter tasting, so a milder prodrug called acetylsalicylic acid was synthesized in 1893 by the German chemist Felix Hoffmann who worked for Bayer\textsuperscript{2,3,4}. Acetylsalicylic acid is a type of drug that is formulated deliberately so that it will deteriorate in the body into the active drug\textsuperscript{5}. This prodrug was developed because it is much less abrasive when delivered to the body and is much more easily absorbed\textsuperscript{6}. The active drug, salicylic acid, is the active metabolite because it is the form of the drug after the body has
processed it. Edward Stone of Oxford University discovered salicylic acid in 1763 from the bark of willow tree.\textsuperscript{4,5,6}

Aspirin works by suppressing the synthesis of prostaglandins and thromboxanes in the human body.\textsuperscript{3,4,5} Prostaglandins function as local hormones produced in the body that aid in the transmission of pain signals, regulate the hypothalamic thermostat, and inflammation.\textsuperscript{2} Thromboxanes are involved in the aggregation of platelets that form blood clots. It does this by the irreversible inactivation of prostaglandin-endoperoxide synthase (PTGS), also known as cyclooxygenase 2, an enzyme that is needed in the synthesis of prostaglandin and thromboxane.\textsuperscript{5} Aspirin serves as the acetylation agent where an acetyl group is covalently attached to a serine residue in the active site of the prostaglandin-endoperoxide synthase enzyme. The ability of aspirin to diminish inflammation is due to its inhibition of the synthesis of prostaglandins. Aspirin alters the oxygenase activity of prostaglandin synthetase by moving the acetyl group to a terminal amine group.\textsuperscript{4}

Though aspirin has numerous benefits, there are several adverse effects as well. It is particularly damaging to the stomach lining and there is an increased risk of gastrointestinal bleeding.\textsuperscript{3,5} The risk of stomach bleeding increases with use of drugs such as warfarin and alcohol.\textsuperscript{6} Large doses can cause a ringing in the ears, or tinnitus. Some people may have allergy-like symptoms including hives and swelling because of a possible salicylate intolerance.\textsuperscript{1} Aspirin can cause swelling of skin tissues (angioedema), increase risk of Reye’s syndrome and can cause hyperkalemia.\textsuperscript{1,2,3} Although most commonly known for its anti-inflammatory properties and pain-reducing qualities, acetylsalicylic acid is also an effective fever-reducer and has been shown to prevent the progression of existing cardiovascular issues such as heart attacks or strokes in low does on a long term basis. Aspirin’s antiplatelet effects come from its ability to inhibit the synthesis of thromboxane, which otherwise bind platelets together in areas where vessel damage has occurred.\textsuperscript{4} These platelets can clot together and become harmful otherwise. It also controls fevers through a similar mechanism (prostaglandin system) and the inhibition of PTGS that is not reversible.\textsuperscript{5}

Thin Layer Chromatography (TLC) is a chromatography technique that is used to separate mixtures that are non-volatile such as salicylic acid, acetylsalicylic acid, and the crude
acetylsalicylic acid product. A sheet is coated with an absorbent material such as silica gel and serves as the stationary phase. The samples are placed on the sheet and a solvent (mobile phase) moves up the stationary phase via capillary action. Various substances move up the stationary phase at different rates depending on their polarity and attraction to the stationary phase itself. Like substances dissolve like substances. Because silica gel is very polar, the affinity of polar substances to the silica gel will prevent them from moving very far up the TLC plate. Non-polar substances will move further up the TLC plate and be close to the solvent front. The hydroxyl groups present on the surface of silica gel can be modified so that they separate things in varying parameters depending on need. TLC is used to confirm the purity of acetyl salicylic acid and compare the polarity of other components of the reaction (salicylic acid and crude product). The solvent was a nonpolar 9:1 mixture of ethyl acetate and methylene chloride respectively.

The active ingredient of the drug Aspirin, acetylsalicylic acid can be synthesized through an esterification reaction between salicylic acid and acetic anhydride. This type of interaction involves a reaction of a carboxylic acid with an alcohol in order to form a carboxylate ester. Salicylic is a weak acid with an alcohol functional group attached to it. The products of the reaction between salicylic acid and acetic anhydride are acetylsalicylic acid and acetic acid.

MATERIALS AND METHODS

**Synthesis**

2.0 grams of salicylic acid, 5.0 mL of acetic anhydride and 5 drops of 85% phosphoric acid solution were placed into a 50 mL Erlenmeyer flask. A 70-80 °C hot water bath was prepared by placing a 250 mL beaker on a hot plate with a thermometer to monitor temperature. The 50 mL Erlenmeyer flask with the mixture of salicylic acid, acetic anhydride, and phosphoric acid was partially submerged in the water bath and heated for 15 minutes until vapors ceased to be released. After 10 minutes of heating the submerged flask passed, 2 mL of distilled water was added to the flask. Then, once the reaction reached completion the flask was removed and 20 mL of distilled water was added. The flask was left to cool to room temperature before being placed in an ice bath for 5 minutes to allow crystallization to occur. A vacuum filtration was set up and the mixture was filtered via vacuum filtration. Once the liquid had been drawn out of the mixture, the crystals were washed with 5 mL of cold, distilled water. This was repeated once
more. The vacuum filtration apparatus was left on for several minutes to aid in the drying of the solid product before it was weighed and recorded³.

**Purification**

About 5 mg of crude acetylsalicylic acid were set aside for TLC analysis. The remaining crude aspirin was added to a 125 mL Erlenmeyer flask. About 60 mL of hot ethanol/water solvent was added slowly to the crude aspirin in a warm water bath. Once the crystals dissolved, the flask was covered and left to cool to room temperature before it was placed in an ice bath for 10 minutes to fully crystallize. Then, the crystals were placed into a vacuum filter where they were subsequently rinsed with two 3 mL portions of cold deionized water and one 2 mL portion of cold ethanol³.

**TLC Analysis**

A developing chamber was made by using a 400 mL beaker and watch glass. 10 mL of 9:1 mixture of ethyl acetate and methylene chloride respectively was placed inside the beaker with a 110 mm filter paper in order to saturate the chamber with solvent vapors. The solvent was left to travel to the top of the filter paper before the silica gel coated TLC plate was placed inside of the beaker. 3 mg of each salicylic acid, crude product, and recrystallized product was place inside three separate small beakers and dissolved with 6 drops of TLC solvent. A different pipette was then used for each of the three samples to lightly spot the TLC plate at the light pencil hash mark about ½ inch from the bottom of the plate. The plate was left to develop until the solvent front was about ½ inch away from the top of the TLC plate. The plate was then removed from the developing chamber and the solvent front was promptly marked. The plate was left to dry before it was examined under UV light³.
RESULTS

Figure 1: Structure of Salicylic Acid, Acetic anhydride, and Acetylsalicylic acid

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Structure Image</th>
<th>Reference</th>
</tr>
</thead>
</table>

The structures of salicylic acid, acetic anhydride, and acetylsalicylic acid are pictured above with their functional groups clearly visible in red.

\[
\text{Mass} = \text{Density} \times \text{Volume} \quad (\text{Eq.1})
\]

\[
\text{Mass (g) acetic anhydride used} = (1.08 \text{ g/mL}) \times (5.00 \text{ mL}) \\
\text{Mass (g) acetic anhydride} = 5.40 \text{ g} 
\]

\[
\text{Mass of aspirin synthesized (g)} = (\text{Mass of aspirin and filter paper}) - (\text{Mass of filter paper}) \\
\text{Mass of aspirin synthesized (g)} = (3.159 \text{ g}) - (0.1300 \text{ g}) \\
\text{Mass of aspirin synthesized (g)} = 3.029 
\]

\[
\text{Mass of purified aspirin product (g)} = (\text{Mass of purified aspirin and filter paper}) - (\text{mass of filter paper}) \\
\text{Mass of purified aspirin product (g)} = (2.299 \text{ g}) - (0.1300 \text{ g}) \\
\text{Mass of purified aspirin product (g)} = 2.169 
\]

Table 1: Synthesis of Aspirin Data

<table>
<thead>
<tr>
<th>Property</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mass of salicylic acid used (g)</td>
<td>2.009</td>
</tr>
<tr>
<td>Volume of acetic anhydride used (mL)</td>
<td>5.000</td>
</tr>
<tr>
<td>Mass of acetic anhydride used (1.08 g/mL) used (g)</td>
<td>5.400</td>
</tr>
<tr>
<td>Mass of aspirin and filter paper (g)</td>
<td>3.159</td>
</tr>
</tbody>
</table>
The table above depicts the various masses and volumes of calculated and raw data in the synthesis of aspirin. 2.009 grams of salicylic acid was used with 5.000 mL of acetic anhydride. The calculated mass of acetic anhydride was calculated using it’s known density for a mass of 5.400 grams. The mass of aspirin and filter paper was 3.159 grams. The mass of the filter paper was .1300 grams. Thus, the calculated value of crude synthesized aspirin was 3.029 grams. Following purification, the calculated mass of the final aspirin product was 2.169 grams.

**Theoretical Yield**

\[
\text{2.0 g salicylic acid (1 mole/138.0 g) = 0.014 moles} \\
\text{5 mL acetic anhydride (1.08 g/mL) = 5.4 g} \\
\text{5.4 g (1 mole/102 g) = 0.05 moles} \\
\text{There is a smaller molar amount of salicylic acid so it is the limiting reagent.} \\
\text{Therefore, the theoretical yield of acetylsalicylic acid is 0.014 moles.} \\
\text{0.014 moles acetylsalicylic acid (180 g/mole) = 2.52 g}
\]

**Percent Error**

\[
\text{Percent Error} = \frac{\text{experimental mass - theoretical mass}}{\text{theoretical value}} \times 100% \\
\text{Percent Error} = \frac{2.169 - 2.520}{2.520} \times 100 \\
\text{Percent Error} = 13.92 \%
\]

**Percent Yield**

\[
\text{Percent Yield} = \frac{\text{experimental mass}}{\text{theoretical mass}} \times 100% \\
\text{Percent Yield} = \frac{2.169}{2.520} \times 100 \\
\text{Percent Yield} = 86.07 \%
\]

**Table 2: Theoretical Yield, Percent Error, and Percent Yield**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretical Yield (g)</td>
<td>2.520</td>
</tr>
<tr>
<td>Percent Error</td>
<td>13.93%</td>
</tr>
<tr>
<td>Percent Yield</td>
<td>86.07%</td>
</tr>
</tbody>
</table>

The calculated theoretical yield was 2.520 grams. Thus, the percent error was 13.93 % and the percent yield was 86.07%.

Figure 2: TLC Plate with Salicylic Acid, Crude Product, and Final Product under UV Light
Pictured above is the TLC plate with salicylic acid, crude product, and final purified produce under UV light, respectively. The final product (acetylsalicylic acid) traveled the furthest up the TLC plate. The salicylic acid travelled the smallest distance.

\[ \text{R}_f \text{Value} = \frac{\text{distance from start to center of substance}}{\text{distance from start to solvent front}} \]  \hspace{1cm} (Eq. 7)

\[ \text{R}_f \text{Value} = \frac{2.0 \text{ cm}}{6.35 \text{ cm}} \]

\[ \text{R}_f \text{Value} = 0.315 \]

Table 3: \( R_f \) Values of Salicylic Acid, Crude Product, and Final Product from TLC Analysis

<table>
<thead>
<tr>
<th>Substance</th>
<th>( R_f ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salicylic Acid</td>
<td>0.315</td>
</tr>
<tr>
<td>Crude Acetylsalicylic Acid</td>
<td>0.480</td>
</tr>
<tr>
<td>Pure Acetylsalicylic Acid</td>
<td>0.800</td>
</tr>
</tbody>
</table>

The salicylic acid travelled the smallest distance with an \( R_f \) value of 0.315. Crude acetylsalicylic acid had an \( R_f \) value of 0.480. The purified acetylsalicylic acid product traveled the furthest up the TLC plate with an \( R_f \) value of 0.800.

DISCUSSION

The esterification reaction is a term for a general reaction in which two reactants, an alcohol and an acid, form an ester in the final product\(^2\). This reaction can be used to synthesize aspirin from salicylic acid. These types of reactions are typically reversible, so most esterification reactions are equilibrium reactions. Le Chatelier’s principle is a pillar of modern chemistry that states that any change imposed on a system that is in equilibrium will cause the system to adjust to a new equilibrium in order to counteract the change\(^2\). The reaction is slow in pure acetic anhydride, therefore phosphoric acid was used as a catalyst for the reaction because it is a strong acid\(^2\). According to Le Chatelier’s principle, an excess amount of acetic anhydride
would force the equilibrium towards the desired product, acetylsalicylic acid. This mechanism would cause the reaction to favor the product side (aspirin and acetic acid). The solution was also heated in order to accelerate the approach to equilibrium.\(^2\)\(^3\)

Salicylic acid contains two acidic functional groups, a carboxylic acid and an a phenol group. The alcohol group (more specifically, the phenol group) in the salicylic acid participates in the reaction because it undergoes esterification and forms an acetylated ester. The human acid is acidic, but the acidity of salicylic acid is great and can thus be very damaging to the digestive system. It can cause gastric and intestinal bleeding as well as stomach ulcers to form. The acidi is to harsh on the lining of the stomach, so “covering up” or removing one of the acidic portions of salicylic acid and leaving the carboxylic acid part with an acetyl group makes it much less damaging to the body and makes absorption much easier.\(^2\) It is for this reason that acetylsalicylic acid is the active ingredient in Aspirin and serves as the prodrug. Aspirin works by irreversibly inhibiting cyclooxygenase 2 (COX-2) also known as PTGS and prevents the synthesis prostaglandins and thromboxane, which are involved in damage repair in tissues via inflammation, clotting, pain signaling, and temperature regulation.\(^5\)\(^6\)

The overall mechanism of reaction that is taking place in the synthesis of aspirin is much more complex than one would guess. Basically, an esterification reaction such as the synthesis of aspirin occurs when a carboxylic acid and an alcohol combine in a reaction to produce an ester. A molecule of water splits off and the remaining carboxylic acid and alcohol form the ester in its place. In the reaction, the phenoxide ion (OH on the ring) is stabilized by the electron withdrawing carbonyl group on the salicylic acid, making it a very stable nucleophile. The carbonyl carbon of the acetic anhydride is makes it an excellent electrophile because the leaving group or acetate ion is stabilized by the acidic conditions provided by the phosphoric acid catalyst. Firstly, protonation of acetic anhydride make it an even better electrophile. It takes a proton from phosphoric acid, leaving it with a negative charge. The nucleophile, salicylic acid attacks the carbonyl carbon on acetic anhydride and bonds. A bond forms between the carbonyl carbon of acetic anhydride and the oxygen (partial positive charge) from the –OH group of salicylic acid form a bond. Phosphoric acid deprotonates the intermediate and removes the hydrogen atoms that is bonded to the oxygen with the partial positive charge. This forms a tetrahedral intermediate and phosphoric acid is thus regenerated. An acetate anion is present and removes the hydrogen attached to oxygen on the intermediate. The removal of this hydrogen gives rise to an ester, and thus the product acetylsalicylic acid. Acetic acid is also formed. Phosphoric acid is essential in this reaction because it acts as a catalyst that (combined with heat) helps the reaction occur in a decent amount of time. It is a liquid acid and thus does not contain a large amount of water that would otherwise affect the yield of the reaction. It also has a strong conjugate base, which is important because this is a reversible reaction. The reaction was placed in a hot water bath and heated to 70-80 °C to help the reaction occur at a faster rate because adding heat to a system increases the energy present and particles move and collide at a faster rate. Otherwise, the reaction would take too far to long to react and the equilibrium would not favor the product side (aspirin and acetic acid). After heating the reaction, distilled water was added to help with recrystallization and to decompose any remaining acetic anhydride because it strongly reacts with water. There is remaining acetic anhydride because salicylic acid is the limiting reagent and acetic anhydride is present in excess. It is important to consider that
acetylsalicylic acid is not the only product that forms, acetic acid is another byproduct of the reaction. The objective is to isolate pure acetylsalicylic acid. A hot water/ ethanol mixture (about 20 mL hot solvent of water/ethanol per gram crude aspirin) is used to further purify aspirin by removing acetic acid. The acetic acid is very soluble in water and can be removed from aspirin, which is less polar and interacts with the ethanol portion of the mixture. A purified product is obtained after recrystallization of crude aspirin in the hot ethanol.

After Thin Layer Chromatography was performed, the determined $R_f$ values were .315, .480, and .800 for salicylic acid, crude aspirin, and purified aspirin respectively. An 86.07 % yield of purified acetylsalicylic acid was obtained. TLC analysis demonstrates that pure aspirin was synthesized. This is noted because of the high $R_f$ value of the pure aspirin. The solvent mixture allowed for the greatest separation between samples. Aspirin traveled very far up the solvent front because it is much less polar that salicylic acid because one of its acidic, or polar functional groups (-OH) was converted to an ester. Salicylic acid is much more polar because of its carboxylic acid group and the alcohol it contains. Therefore, salicylic acid was more attracted to the polar stationary phase (silica gel) and did not move as far up the TLC plate as acetylsalicylic acid. Aspirin was more attracted to the mobile phase (solvent that was relatively nonpolar) that the stationary phase. The crude product has a $R_f$ value that is between the salicylic acid and aspirin because of the presence of acetic acid that interacts with polar stationary phase.

There are many potential sources of error, including the constant threat of left over product on glassware. A large source of error could have been omitting to wash the newly synthesize crude aspirin crystals with cold distilled water three times. The crystals were only rinsed once with room temperature distilled water. This would could have added to the 13.93% error because a large amount of acetic anhydride may have not been removed, thus contaminating the product. Another source of error could have been that the entirety of the crude product (about 3 grams) was dissolved in the hot water/ethanol solvent and crystallized rather than one gram. Dealing with more crystals can maximize loss of product because you are dealing with a greater number of substance. A great deal of product could have been lost during vacuum filtration.
CONCLUSION

A total of 2.169 grams of pure aspirin was synthesize out of a possible yield of 2.52 grams. Thus, there was a 13.93% error and 86.07% product yield. TLC analysis further confirmed these results due to the observation that aspirin had a higher Rf value that salicylic acid (.800 vs. .315, respectively), thus demonstrating that the one of polar functional groups had been converted to an ester. This makes aspirin less acidic and therefore less damaging to the digestive system of the human body. In the future, special care should be given to the washing of the crystals with cold distilled water to maximize yield. Also, a stronger acid catalyst such sulfuric acid could be used to further increase the rate of reaction.

**Mechanism 1:** Reaction between salicylic acid, phosphoric acid, and acetic anhydride

**Mechanism 2:** Reaction of Water and byproducts
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Andra Postu
Organic Chemistry Lab II
Lab Partner: Michael Bible
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