Make up Lab: Synthesis and Quantitation of Acetylsalicylic Acid (Aspirin).
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Introduction:
Aspirin is a substance commonly found in medicine cabinets around the world. It is one of the least expensive and most useful drugs. The role that chemists have played in its development is a fascinating study of how the drug industry works.

The precursor to modern day aspirin was found in the bark of willow trees and used by people for thousands of years to alleviate pain. It was commonly brewed into a tea to soothe the sufferer. In the mid 1800s, the active ingredient (the chemical responsible for the analgesic affect), salicylic acid, was isolated and by 1870 methods had been developed to produce it on a wide scale basis. The structure of salicylic acid is shown in the figure below.

A white powder, it could be taken orally, but it had an unfortunate side effect. Salicylic acid is a relatively strong acid (pH of a saturated aqueous solution = 2.4) and causes irritation to the mouth, esophagus and stomach. In the late 1890s, a young chemist at Bayer named Felix Hoffman sought to improve this drug. His father suffered from rheumatoid arthritis, and the side affects of salicylic acid were preventing him from obtaining relief from the swelling and inflammation of the disease. Felix attempted to modify salicylic acid to make it more soluble in stomach acids by replacing the alcohol functional group with an ester. This involved a simple condensation reaction between an acid and an alcohol: in this case acetic anhydride reacts with the OH group on salicylic acid to produce acetyl salicylic acid.

This new compound proved to be immensely successful. It retained the pain relieving and fever reducing properties of salicylic acid, but was not as irritating to the stomach. When Hoffman revealed his work to his supervisors at Bayer, the company began to produce and market this new drug giving it the trade name Aspirin. Bayer aspirin is still a well-known name, even though the patent has long since run out, and aspirin is available under many trade names and in generic form.

Aspirin can be hydrolyzed to form salicylic acid. Hydrolysis is a reaction that causes the dissociation of water. The hydrolysis of an ester results in the formation of an alcohol and a carboxylic acid. When an O—C bond is broken the –H and –OH of the dissociated water adds to the two molecules where the bond was broken.

Aspirin is a mild analgesic (relief of pain) that works by blocking the synthesis of prostaglandins, a group of compounds produced in the body. These compounds can act directly on the heat-regulating centers of the central nervous system (producing fever), and constricting blood vessels which increases the body temperature because less heat can escape from the tissues into the blood. Prostaglandins also increase the permeability of capillaries allowing water to pass out of the capillaries and into the nearby tissues causing swelling and pain in parts of the body. Thus, by lowering the concentration of these compounds, aspirin reduces pain, fever, and inflammation. Other analgesics include acetaminophen (Tylenol) and ibuprofen (Advil and Naprin).
In this experiment, the hydrolysis will be done using a basic aqueous solution (thus simulating the alkaline conditions of the digestive tract where this reaction occurs in the body). Under these conditions, the salicylate dianion will be produced. Hydrolysis of an ester done in basic solution is called saponification. (It is the reaction by which soaps are made.)

\[
\text{Aspirin} + 3 \text{OH}^- \rightarrow \text{salicylate dianion} + \text{H}_2\text{O}
\]

In the second part of the experiment, the amount of hydrolyzed aspirin in a solution will be determined quantitatively using a spectrophotometric technique. The spectrophotometers you will be using produce and detect visible light (light in the 400-750 nm range). For this method to work then, it is necessary to produce a colored compound (a compound that will absorb light in the visible region). This can be done by adding a solution of Fe$^{3+}$(aq) to the hydrolyzed aspirin. At an appropriate pH, a 1:1 complex of Fe$^{3+}$ to hydrolyzed acid forms. This complex absorbs light of 520 nm, and therefore appears to be pink/purple in color.

\[
\text{Salicylate dianion} + \text{Fe(H}_2\text{O)}_6\text{Fe}^{3+} \rightarrow \text{complex} + 2 \text{H}_2\text{O}
\]
The Bausch and Lomb Spectronic 20 is a direct-reading, single-beam spectrophotometer that can be used to study absorbance in the range of 350-750 nm. The scale is scribed to read transmittance and absorbance.

In this instrument the white light of the tungsten lamp is reflected and dispersed (separated into various wavelengths) by the diffraction grating. The different wavelengths, or colors, leave the grating at different angles and thus are spread out in space as they arrive at the exit slit. The grating can be pivoted about an axis so that a particular wavelength is selected and focused on the exit slit. This light then passes through the sample to the phototube and is detected.

A full size version of this 24-well template will be provided.

Pre-Lab Quiz
Synthesis of Aspirin
Make-up Lab
Section #__ Day/Time_________
TA______________________________

1. What is Beer’s Law?

2. What organic functional groups are on aspirin?

3. What is a catalyst?

4. Draw a color wheel and use it to illustrate complementary colors. (See Exp. #3 Chemtrek)

5. A student weighs out 0.3966 g of salicylic acid and uses it to synthesize aspirin. 0.3224 g of aspirin are recovered. What is the % yield of aspirin?
Experimental:

Note: There is a 24-well template for this experiment. It will help you to organize your solutions in this experiment. See page 5 in the introduction.

Section A: Synthesis of Acetylsalicylic Acid (Aspirin)

\[
\text{Salicylic Acid } \quad \text{Acetic Anhydride } \quad \text{Acetylsalicylic Acid (Aspirin)}
\]

\[
\text{C}_7\text{H}_6\text{O}_3 (s) + \text{C}_4\text{H}_6\text{O}_3 (l) \rightarrow \text{C}_9\text{H}_8\text{O}_4 (s)
\]

In this experiment you will be synthesizing Aspirin from salicylic acid and acetic anhydride. (Acetic anhydride is the result of two molecules of acetic acid combining to form the anhydride and water.)

Question 1: What is a lacrimator?

1) Weigh 0.40 g salicylic acid in a clean, dry screw-top vial (record exact weight). Note: you do not need the top for this experiment.
2) Fill a Styrofoam cup from your drawer with boiling water (obtain from coffee urn in the front of the room) to make a hot water bath for the reaction. Set it near the hood by your workspace. Have a clamp ready to hold your vial in the hot water bath once all of the reagents have been added.

The next two steps should be done in the hood in the front of the room where the acetic anhydride and sulfuric acid are kept. Then the reaction container may be moved to the hot water bath near your workspace.

1. Slowly add 0.8 mL (d=1.082 g/mL) acetic anhydride to the salicylic acid in your vial using an air displacement pipette (do this in hood: acetic anhydride is a lacrimator) Note: If you do not feel confident regarding the use of an air displacement pipette ask your TA for help or read the description on page 11.
2. Add 1 drop of concentrated sulfuric acid (H$_2$SO$_4$) to the reaction mixture using a glass pipette. Gently swirl screw-top vial until the contents are well mixed.
5) Take the vial to your workspace, clamp it to a ring stand, and lower into your hot water bath.
6) Let sit in hot water bath 15-20 minutes.

Question 2: Is salicylic acid or acetic anhydride the limiting reactant in this reaction?

Question 3: What do you think sulfuric acid does in the reaction above? Hint: Why do you need only 1 drop?

While you are waiting for the reaction to progress make an ice bath and place a clean 8 mL pop-top vial full of distilled water into the ice bath to cool. (You can also go on to Section C and beyond.)

7) After 20 minutes slowly add approximately 2 mL of cold distilled water to the reaction mixture to decompose the remaining acetic anhydride (Caution: if water is added to the reaction mixture too fast the reaction will foam over.)
8) Cool the reaction mixture in an ice bath for 10-15 minutes. (Aspirin should crystallize).
9) Set-up the vacuum filtration apparatus. Your instructor will demonstrate how to use it. Make sure you place one of the disposable filter pieces (kept in the front of the room) on top of the plastic filter which is built into the Hirsch funnel.
10) Add 5 mL of cold water to the crystals and stir to break up any lumps.
11) Gently pour the liquid and the crystals into the Hirsch funnel. Use a straw spatula to scrape as many of the crystals as possible out of the screw top vial.
12) Transfer any remaining crystals from the screw top vial by adding small amounts of chilled water and swirling to suspend crystals.
13) After all crystals have been transferred rinse the crystals by adding a small amount of chilled water and stirring crystals in the funnel.
14) Let the crystals air dry in the funnel for 5 minutes by constantly pulling air through the funnel.
15) Transfer the crystals to a clean, pre-weighed, weighing boat. The crystals may still be a little damp at this point. Break them up with a spatula, so that they will air dry more quickly. 16) Weigh crystals.

**Question 4:** Calculate the percent yield of aspirin from the above reaction. Show your work.

**Purity Check:**
To check the purity of your aspirin sample place a spatula tip full of your synthesized aspirin (~0.01 g) in a clean, dry screw top vial. Add ~1 mL of 95% ethanol to completely dissolve the aspirin. 2 drops of 0.02 M FeCl₃ can then be added to the mixture. If the solution turns purple there is salicylic acid impurity still left in your sample.

**Question 5:** Why do you see a purple color if you have salicylic acid left in your product? Why don’t you see a purple color if you have pure aspirin? (Hint: What reaction from the introduction causes the color change?)

**Section B: Compare a Tablet of Commercial Aspirin With Your Sample**

1) Qualitatively test the water solubility of pure aspirin, and compare it with the water solubility of your synthesized aspirin.
2) Also compare the behavior of pure aspirin heated in a melting-point capillary with the behavior of your sample heated in a similar manner.

**Question 6:** Did the two samples differ? If so, why do you think this is the case?

**Question 7:** What was the melting point of your aspirin sample? What are the literature values for the melting point of aspirin and salicylic acid? What does this tell you about your sample?

**Section C: Formation of a Colored Complex from Aspirin**

Another way to analyze for the presence of aspirin is to convert it into a colored compound, and then quantitatively measure the intensity of the colored compound using a spectrophotometer. To obtain a colored compound, aspirin is first reacted with sodium hydroxide as shown in the reaction below. This produces a dianion with a structure similar to that of salicylic acid which is called the salicylate dianion.

```
\[
\text{Acetylsalicylic Acid (s)} + 2 \text{NaOH} \rightarrow \text{Salicylate Dianion (aq)} + 2 \text{Na}^+ + \text{H}_2\text{O} + \text{Acetic Acid (l)}
\]
```

The salicylate dianion is colorless, but when reacted (“complexed”) with iron (III) it forms a colored compound. We do not have time for you to produce the salicylate dianion from your aspirin, so a solution of this compound has been prepared for you by the stockroom. You will conduct the reaction of the salicylate dianion with iron (III).

**Question 8:** What does the term hydrolyzed mean?

**Question 9:** What do you think the structure of the salicylate di-anion is? (Hint: Which atoms will most easily leave the salicylic acid molecule in the presence of a base?)

**Section D: Preparation of Calibration Standards (Can be done in pairs)**

In this section you will be using the 0.1 mg/mL salicylate dianion also referred to as “hydrolyzed aspirin”) to make an Iron (III) salicylate dianion complex. This compound can then be analyzed to determine the amount of hydrolyzed aspirin in an unknown mixture.

The following dilutions are best prepared in a 24 well tray (Refer to the 24-well tray template on page 5). You will be using several different solutions in this experiment, and if you label the pipette tips used, then you can minimize cross contamination. The fine point marker in your kit can be used to mark the upper outside section of the pipette tip. For the section below you only need four pipette tips – one for the distilled water, one for 0.1 mg/mL salicylate dianion solution, one for the unknown diation solution, and one for the 0.02 M FeCl₃.
Before making the solutions, take a few moments to become familiar with the features of the air pipette and practice using distilled water. You can check your technique by weighing the amount of distilled water pipetted.

**Features of Air Displacement Pipette**

- **Dial**: used to set volume
  a) Put black lever in unlock position.
  b) Turn wheel on top of pipette to set the volume: volume is given in microliters (µl).

\[
500 \text{ µl} = 0.5 \text{ mL}.
\]
  c) Flip black lever back to lock.

**To make solutions:**
- Place tip on bottom of pipette, (to eject tip, push lever on side of pipette).
- Place pipette tip into solution and press down on wheel to FIRST stop. Slowly release, allowing fluid to be drawn in.
- To eject fluid, press down on wheel to first stop, then press to second stop to eject the last drop.

Using an air displacement pipette, prepare the following solutions:

<table>
<thead>
<tr>
<th>Table 1: Making Standard Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td><strong>Amount 0.1 mg/mL hydrolyzed aspirin (mL)</strong></td>
</tr>
<tr>
<td>Blank</td>
</tr>
<tr>
<td>Standard 1</td>
</tr>
<tr>
<td>Standard 2</td>
</tr>
<tr>
<td>Standard 3</td>
</tr>
<tr>
<td>Standard 4</td>
</tr>
<tr>
<td>Standard 5</td>
</tr>
</tbody>
</table>

| **Amount of 0.1 mg/mL Excedrin (mL)** |
| Unknown | 0.5 | 0 | 0.5 |

**Question 10**: What is the importance of preparing a “blank”?

**Question 11**: Calculate the concentrations of hydrolyzed aspirin in the solutions above in mg/L. Be sure to show at least one sample calculation.

(Note: For dilute aqueous solutions, mg/L is equivalent to “ppm” (parts per million))

**Section E: Spectrophotometric Determination of Aspirin (Can be done in Pairs)**

Set the wavelength on the Spec 20 to 520 nm.

**Question 12**: What is the color of the salicylate dianion Iron (III) complex? What is the color of 520 nm light? Does this make sense in light of the color of the salicylate dianion Iron (III) complex? Explain. (Hint: Refer to the background reading of Exp. 3.)

Make sure there is nothing in the sample compartment of the spectrophotometer. With the lid shut adjust the “zero control” knob (left knob) to read 0% T. (When there is no cuvette in the instrument, a shutter closes, and therefore no light should be reaching the detector). Wipe and clean the cuvette with the “blank” on the outside with a microwipe and place it in the black plastic cuvette holder. Then place the entire assembly into the sample compartment. The fully exposed side of the cuvette should face to the right. Shut the lid. Adjust the “light control” knob
(the knob on the right) until the indicator reading is 100% T (Absorbance = 0). What you have just done is calibrate the instrument to show infinite absorbance when the beam is blocked, and zero absorbance for the blank.

**Question 13:** What is the role of each of the three knobs on the Spectronic 20?

Next, remove the blank from the sample compartment and replace it with the cuvette containing standard #1. Measure the Absorbance to 3 significant figures using the best estimate for the final place. Record this in your notebook. Repeat with standard #2 through standard #5.

Your spectrophotometer scale can also be read in “% transmittance”, and sometimes this scale is easier to read. If your measurements are recorded in % Transmittance (%T), then this must be converted to Absorbance (A) for your graph. \[ A = -\log \left( \frac{\%T}{100} \right) \].

**Question 14:** What trend do you observe in the absorption results?

**Question 15:** What is the absorption law that governs this phenomenon? Explain.

**Question 16:** Plot a graph of Absorbance versus concentration (mg/L) for the various aspirin standards on the sheet of graph paper provided. Do this plot in pencil. This plot is your “standard calibration curve”. (Note: Each person should make his or her own graph.) Show your finished calibration curve to your TA before continuing.

Now measure the Absorbance value for your unknown aspirin sample. From the absorbance values of the unknown solution, determine its concentration by interpolation from the standard calibration curve.

**Question 17:** Indicate on the calibration graph the interpolation used to determine the concentration of hydrolyzed aspirin in your hydrolyzed Excedrin solution.

**Question 18:** What is the percentage of aspirin in Excedrin? (Hint: Notice that standard 5 is prepared in a similar manner to your unknown.)

**Question 19:** Does this agree with the amount of aspirin in Excedrin according to the label on the bottle? Remember to use the amount of aspirin listed on label and divide by the total weight of the tablet.