LAB 21: ASPIRIN AND ANALGESICS:
SYNTHESIS & ANALYSIS

PURPOSE: To synthesize aspirin from wintergreen or from salicylic acid.
To test the purity of aspirin by testing for the presence of phenols.
To use thin-layer chromatography to identify substances in analgesics.

SAFETY CONCERNS:
Always wear safety goggles. Acetic Anhydride and Acetic Acid are irritating to skin, eyes, and mucus membranes. If contacted, wash with soap and copious amounts of water.

ASPIRIN:
History:
The bark of the willow tree was used by ancient Native Americans to counter fever and pain. Europeans learned of willow bark’s medicinal properties in 1763 when clergyman Edward Stone presented his findings to the Royal Society of London. Willow bark extract was found to be a powerful drug with analgesic (pain reliever), antipyretic (fever reducer), and anti-inflammatory (reduces swelling) properties. In 1838 organic chemists isolated the active ingredient and identified it as salicylic acid, named from salix, Latin for the willow tree.

Although an effective remedy, the use of salicylic acid was limited because it damaged mucous membranes of the mouth and esophagus, and caused hemorrhaging of the stomach lining. It was determined that the acidic phenol group could be responsible for the damaging effects so in 1893 German chemist Felix Hoffman, working for the firm of Bayer, synthesized an ester of salicylic acid, acetylsalicylic acid. Acetylsalicylic acid was marketed by Bayer under the trade name of aspirin. During World War I the U.S. Government seized Bayer’s assets and sold them and the Bayer name to Sterling Products which became Sterling Winthrop Inc.

Biological Effect:
Aspirin works to relieve fever and pain by inhibiting the formation of prostaglandins, a class of 20-carbon acids that form at the site of an injury and are responsible for causing the inflammation and pain.

Aspirin works as a blood thinner by also inhibiting the formation of thromboxins which aid in the production of blood clots.

Aspirin should not be given to children who have influenza or chicken pox because of the risk of the rare but often fatal Reye’s Syndrome. Children suffering from these diseases should be given a nonaspirin pain reliever such as acetaminophen. Unless directed by a physician, aspirin should not be taken during the last 3 months of pregnancy.

Preparation and Reactivity:
Aspirin is easily prepared in the laboratory by esterification of salicylic acid with acetic acid.

\[
\text{Salicylic acid} + \text{Acetic acid} \rightarrow \text{Acetylsalicylic acid (Aspirin)} + \text{Water}
\]
Preparation of aspirin by treatment of salicylic acid with acetic anhydride, however, is much faster than traditional esterification so is more practical for us to do in a limited laboratory time.

\[
\text{Salicylic acid (138 g/mol)} + \text{Acetic Anhydride} \rightarrow \text{Acetylsalicylic acid (Aspirin) (180 g/mol)} + \text{Acetic Acid}
\]

Like salicylic acid, aspirin is an analgesic, antipyretic, and anti-inflammatory; but it is much less irritating to the stomach. Aspirin does cause slight gastrointestinal bleeding that can, over time, cause iron deficiency or gastric ulcers. These complications can be avoided with enteric-coated aspirin, which does not dissolve until reaching the small intestine. The basic conditions in the small intestine hydrolyze the acetylsalicylic acid to salicylic acid, which is absorbed into the bloodstream.

In commercial aspirin tablets about 325 mg (5 grains) of aspirin is bound together with inert materials called binders, whose function is to hold the tablet together. Some manufactures apply a micro-coating of hydroxypropyl methylcellulose to prevent the tablet from dissolving in the mouth but permit it to dissolve in the stomach. Sometimes buffers are added to combat stomach irritation. Color and flavor may also be added for market appeal.

Aspirin may be reconverted into salicylic acid in either of two ways; hydrolysis in an acidic solution which is the reversing of the equilibrium esterification to make it, or by hydrolysis with base (called saponification).

If aspirin is stored over a long period of time, especially under moist conditions, the hydrolysis reaction takes place, and the characteristic odor of vinegar (acetic acid) is easily detected. Generally, "old" aspirin tablets contain traces of acetic acid and salicylic acid.

Hydrolysis of aspirin with sodium hydroxide (saponification), followed by acidification of the sodium salt is a much faster method than acidic hydrolysis for converting aspirin into salicylic acid.

**Basic Hydrolysis; Saponification**
Salicylic acid can be readily detected by the presence of a violet color upon addition of iron (III) chloride, and the presence of salicylic acid in an aspirin tablet is an indication of its decomposition.

\[
\text{Salicylic acid} + \text{Fe}^{3+} \rightarrow \text{Phenol - Fe}^{3+} \text{ complex} (\text{purple})
\]

**OTHER ANALGESICS:**
Combination pain relievers often contain aspirin combined with other compounds with analgesic and antipyretic properties such as acetaminophen. Some also are mixed with caffeine.

**THIN-LAYER CHROMATOGRAPHY:**
Thin-layer chromatography (TLC) is a technique used to separate substances in a mixture. A TLC plate is typically a sheet of plastic, coated with a thin layer of a solid adsorbent such as silica gel. Small amounts of known and unknown substances are placed as small spots at one end of the TLC plate. Then the end of the plate with the spots is placed in a solvent contained in a developing chamber.

The solid silica layer on the TLC plate is called the **stationary phase**. The solvent or the **mobile phase** slowly moves up the silica layer on the TLC plate carrying the substances in the spot with it. The more soluble a substance is in the solvent, the higher the solvent will carry it up the plate. A substance that adheres strongly to the stationary silica gel moves only a short distance with the solvent. Thus, differences in the substances determine the distances they travel up the plate.

As the solvent front nears the top of the TLC plate, the plate is removed, marked, and dried. Then the substances are visualized. If they have colors, they can be seen directly. In this experiment, they are colorless. Because the silica material on the plate contains a fluorescent compound, ultraviolet light (254 nm) from a UV lamp can be used to visualize the substances. Compounds with aromatic rings will absorb the UV light and appear as dark spots on the plate.

A value called the **R_f** value can be calculated for each substance on a plate. The R_f is the distance that a substance moves on the plate divided by the distance the solvent moves. An unknown substance is identified if its R_f value matches the R_f value of one of the known substances used on the plate.

In this experiment of will use TLC to determine the R_f values for several known analgesics and to determine the purity of Aspirin product that you have made.
PROCEDURES:

ACTIONS:

I. SYNTHESIS OF ASPIRIN:

A. PREPARATION:

1. In the fume hood prepare a boiling water bath by heating about 150 mLs of water in a 400 mL beaker over a hot plate.

2. Carefully mass about 2.00g of salicylic acid and put it into a 125 mL Erlenmeyer flask.

3. Under the fume hood carefully add 5 mL of acetic anhydride to the flask of salicylic acid and swirl or stir to mix.

4. Slowly add 10 drops of 85% phosphoric acid, \((H_3PO_4)\) to the salicylic acid and acetic anhydride and mix well.

5. Place the flask and its contents in the boiling water bath under the hood and stir until all the solid dissolves.

6. Let react over boiling water bath for about 15 minutes. Then remove the flask from the hot water and let it cool to room temperature.

7. Cautiously add 20 drops of deionized water to the cool mixture.

8. When the reaction mixture has cooled to room temperature, add 50 mL of ice water directly to the reaction flask and then cool the mixture by placing the flask in an ice bath. Crystals of aspirin should form within 10 minutes or so.

B. COLLECTION OF ASPIRIN CRYSTALS

9. Set up a Buchner or Hirsh vacuum filtration apparatus. Insert filter paper and moisten with water to help it adhere to the funnel.

10. Turn on the vacuum source and pour the aspirin product onto the filter paper in the funnel.

11. Use a spatula to transfer any aspirin crystals left in the flask to the funnel and rinse the inside of the flask into the funnel with a 10 mL portion of ice cold water.

12. Spread the aspirin crystals out on the filter paper in the Buchner funnel and continue to draw air through the funnel to help dry the crystals.

13. Turn off the vacuum source and use a spatula to lift and transfer the filter paper with the aspirin residue on it to a watch glass. Allow the crystals to air dry.

14. Weigh your dry sample and determine the mass of your aspirin product to the accuracy of your balance. Report this as the actual yield on your report sheet.

NOTES:

1. The measurement does not need to be exactly 2.00g, however, you do need to know exactly how much you do have. Report whatever mass you obtain to the accuracy of your balance so that you can most correctly determine the percent yield of your product when you are done.

2. Acetic anhydride is irritating to the nose and sinus. Handle carefully.

3. Acetic anhydride is used in excess so the salicylic acid is the limiting reagent.

4. Keep your face away from the top of the flask: The acetic acid vapors that are produced are irritating.

5. The water is stopping the reaction by hydrolyzing any unused acetic anhydride into acetic acid. We are adding only a small amount of water at first as a safety precaution because the hydrolysis is exothermic and we don’t want it to get too hot.

6. As a general rule the water touching your reagents should be deionized water, however you may use iced tap water here to save time.

7. If no crystals appear, gently scratch the insides of the flask with a stirring rod.

8. Vacuum Filtration Apparatus:

9. The aspirin product will be the residue that remains on the filter paper.

10. The 10 mLs of ice water can be used a little at a time to make several small rinses. Several small rinses will be more effective at transferring product that one large rinse.

11. Air flowing through helps to partially dry the crystals.

12. You may need to let your crystals dry in your drawer until the next lab period to get an accurate weight for yield calculations and an accurate melting point in Part IIC.
C. CALCULATIONS:
15. Calculate the maximum possible grams of aspirin (theoretical yield) that could be made from the actual grams of salicylic acid with which you started.

16. Calculate the percent yield of aspirin you obtained. Turn in your product to your instructor.

II. ANALYSIS:
A. CHROMATOGRAPHY:
1. Obtain a silica TLC plate with fluorescent indicator cut to a size of 6 x 10 cm.

2. Draw a light line with pencil about 1 cm above the bottom of the short end of the plate. Mark 6 spots on the line equally spaced but away from the edges. Label the spots from A to F.

3. Using clean capillary pipettes, one for each substance, make tiny spots of each of the following substances on the appropriately labeled marks until there is sufficient sample spotted on the plate:
   - A: salicylic acid dissolved in ethanol
   - B: acetylsalicylic acid dissolved in ethanol (freshly made)
   - C: your prepared aspirin dissolved in ethanol
   - D: caffeine dissolved in ethanol
   - E: acetaminophen dissolved in ethanol
   - F: Extra Strength Excedrin dissolved in ethanol

4. Obtain a chromatography developing chamber, or make one from a 400 mL beaker, a piece of Saran wrap that covers, and a rubber band.

5. Carefully pour about a layer of chromatography solvent (50% ethyl acetate and hexane) into the chamber to a level of 0.5-0.6 cm and cover the chamber with a lid or saran wrap.

6. Carefully set the TLC plate in the solvent in the chromatography developing chamber. Cover and allow the beaker to remain undisturbed as the solvent moves up the plate.

7. When the solvent has risen almost to the top of the plate, remove the plate and draw a pencil line along the solvent front quickly before it dries.

8. When the solvent has evaporated from the TLC plate, observe it under UV light. Circle each spot with pencil and draw a picture of the spots on your report sheet.

9. Measure the distance from the origin to the solvent front and measure the distance from the origin to the center of each spot. Calculate the R value for each spot and record them next to each spot on your report sheet.

10. Determine and label the identity of each spot on the report sheet and formulate conclusions as to the purity of your prepared aspirin and as to the makeup of extra strength Excedrin.

11. Place used chromatography solvent in the organic waste solvent container.

Percent yield:
\[
\frac{\text{actual yield}}{\text{theoretical yield}} \times 100 = \% \text{ yield}
\]

Be sure you handle the plates at the edge only to avoid transferring substances from your fingers and to avoid rubbing the silica from the plate.

Any markings or labels must be drawn with pencil rather than pen to avoid separation of any ink pigments. Pencil markings and labels must also be made very lightly to avoid scraping the silica from the plate.

It is important that the solvent level is below the spots you place on the TLC plate so that the solvent does not simply wash the samples away.

Take a small crystal of your sample and dissolve it in a few drops of ethanol.

The developing chamber must be covered to avoid evaporation of the solvent from the plate and from the chamber itself.

The sides of the TLC plates should not touch the sides of the chamber. The solvent must rise up the plate from the bottom only and not from the sides of the plate.

Compounds containing an aromatic ring will appear dark under the UV light. Those without an aromatic ring will not appear at all. Salicylic Acid shows fluorescent blue under UV light.
B. TEST FOR PHENOLS:

12. Obtain 5 test tubes (any size) and label them A, B, C, D, & E.

13. Into tube A place 3 mL of deionized to use as a control. \(^{22}\)

14. Into tube B place 3 mL of 0.15% Salicylic acid solution.

15. Into tubes C, D, and E, place about 3 mL of deionized water and add a few crystals (the amount on the tip of a spatula) of the indicated solids:
   Tube C; add Commercial Aspirin (crushed).
   Tube D; add Aspirin product you prepared in Part I. \(^{23}\)
   Tube E; add known Acetylsalicylic acid.
   Stir each to mix well. \(^{24}\)

16. To each of the sample tubes A through E add 5 drops of 0.1 M ferric chloride (FeCl\(_3\)) solution. Mix well. Record your observations. \(^{25}\)

17. From your data make some conclusions as to the composition of your prepared aspirin as well as the other samples.

18. Conclude whether the Aspirin you made in Part I would be considered pure by USP standards. \(^{26}^{27}\)

19. From your results make some conclusions as to potential throat, esophagus or stomach irritation of your prepared aspirin.

\(^{22}\) The measurement does not need to be exact. You can determine 3 mls for the first tube with a graduated cylinder and then eyeball the height of water in each of the other tubes to match.

\(^{23}\) Although your sample is not yet dry, take a few crystals for this section. You are only taking a small amount so your final mass of product will not be significantly affected.

\(^{24}\) Mixing can be done by tapping the sides of the test tubes, by stirring with a glass rod, or by stoppering and shaking.

\(^{25}\) Any free salicylic acid either unreacted during synthesis or resulting from hydrolysis of aspirin, reacts with the Fe\(^{3+}\) ion to give a purple color. More salicylic acid in the sample results in a deeper color. A purple color resulting from addition of Fe\(^{3+}\) to aspirin or acetylsalicylic acid indicates that the product is either impure or that decomposition has taken place.

\(^{26}\) 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 United States Pharmacopeia (USP) standards. If the sample is darker, it is impure and not considered safe for ingestion. However, no matter what the results of the test, your laboratory-prepared aspirin must not be ingested!

\(^{27}\) The purity of aspirin can also be determined by measuring it’s melting point. Pure acetylsalicylic acid melts at 135°C. Salicylic acid melts at 157-159°C.
LAB 21: ASPIRIN & ANALGESICS:  
PRE LAB EXERCISES:  

1. The chemical name for Aspirin is ________________________________

2. Why are buffers added to some aspirin products?
   A. to make the pH of aspirin 7.4 to match the pH of blood
   B. to make the aspirin less acidic so therefore less irritating to the stomach.
   C. to make the pH of aspirin more acidic so it matches more the acidity of the stomach?
   D. to make the pH of aspirin more basic so that it matches more the acidity of the intestines.

3. A purple color when a compound is treated with FeCl₃ indicates what functional group is present?
   A. carboxylic acid  B. aromatic ring  C. amine  D. ester  E. phenol

4. When a silica TLC plate containing fluorescent indicator shows a dark spot when viewed under an ultraviolet light it means
   A. there is an analgesic or antipyretic present.
   B. there is a compound present that contains double bonds.
   C. there is a compound present that contains a ring.
   D. there is a compound present that contains an aromatic ring.

5. Aspirin that has been stored for a long time may have the odor of vinegar because
   A. Acetylsalicylic acid smells like vinegar.
   B. Aspirin hydrolyses in moist air to form salicylic acid and acetic acid and the salicylic acid smells like vinegar.
   C. Aspirin hydrolyses in moist air to form salicylic acid and acetic acid and the acetic acid smells like vinegar.

6. What role do the inactive ingredients play in an analgesic tablet?
   A. They hold the tablet together.  B. They add color.  C. They add flavor.
   C. They prevent tablets from dissolving too soon.  E. All of these.

7. Use structures (not formulas) to write the equation for the reaction of salicylic acid with acetic anhydride to form acetylsalicylic acid.

8. If 2.00g of salicylic acid reacts with an abundance of acetic anhydride, what is the maximum theoretical yield of acetylsalicylic acid that can be produced? Show calculations and circle your answer.
Lab 21: Aspirin & Analgesics:

Report:

I. Synthesis of Aspirin:

<table>
<thead>
<tr>
<th></th>
<th>Starting Reagent</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salicylic Acid</td>
<td>Acetylsalicylic Acid</td>
</tr>
<tr>
<td>Molar Mass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grams</td>
<td>(Actual grams used)</td>
<td>(Theoretical yield: grams expected: Show calculations)</td>
</tr>
<tr>
<td>Moles</td>
<td>(Actual Moles used: Show calculations)</td>
<td>(Theoretical moles expected:)</td>
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<tr>
<td>Yield, grams</td>
<td></td>
<td>(Actual experimental yield)</td>
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<tr>
<td>Percent Yield</td>
<td></td>
<td>(% yield: Show calculations)</td>
</tr>
</tbody>
</table>

Explanation/Analysis: Explain your results. Explain any anomalies.

II. Analysis: Tests for Quality (Verification of Purity)

A. Chromatography: Draw and label each spot.

<table>
<thead>
<tr>
<th>RF value</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salicylic Acid</td>
<td>Acetylsalicylic Acid</td>
<td>Student Prep</td>
<td>Caffeine</td>
<td>Acetaminophen</td>
<td>Excedrin</td>
</tr>
</tbody>
</table>

Summary & Conclusions:

Explanation/Analysis of Purity of prepared Aspirin from TLC: Make a conclusion about the purity of your prepared aspirin from your TLC data. Explain your results? Explain any anomalies.

Identity of Excedrin Components from TLC:

Identity of Excedrin Components from Product Label:
### B. Test for Phenols:

<table>
<thead>
<tr>
<th>A. Water (control)</th>
<th>B. 0.15 % Salicylic acid</th>
<th>C. Commercial Aspirin</th>
<th>D. Prepared Aspirin</th>
<th>E. Acetylsalicylic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test for Phenols</strong> (Color w/ FeCl₃)</td>
<td></td>
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<tr>
<td><strong>Conclusion</strong> (is a phenol present?)</td>
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<tr>
<td>(+ or -)</td>
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<tr>
<td><strong>Would it be USP approved?</strong> (Yes or No)</td>
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</table>

**Explanation/Analysis:**  Analyze the purity of your prepared aspirin from the FeCl₃ test. Is there any salicylic acid left or is it pure aspirin? Analyze the data and make judgments. Explain any anomalies.

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**Overall Conclusions/Explanation/Analysis:** Summarize and make a judgment regarding the purity of your prepared aspirin based on both the Chromatography (Part IIA) and the Test for Phenols (Part IIB).
LAB 21: ASPIRIN & ANALGESICS:  

NAME_________________  

RELATED EXERCISES:  

DATE_________________  

1. ___ An antipyretic serves to  
A. relieve pain.  B. reduce fever.  C. reduce swelling  D. all of these  

2. ___ An analgesic serves to  
A. relieve pain.  B. reduce fever.  C. reduce swelling  D. all of these

3. ___ The bark of the willow tree contains?  
A. Aspirin  B. Prostaglandins  C. Salicylic Acid  D. More than one of these  

4. ___ Which functional group of salicylic acid is thought to most irritate the stomach?  
A. phenol  B. carboxylic acid  C. ester  D. aromatic ring

5. ___ Aspirin works as a pain reliever by inhibiting the formation of  
A. thromboxins  B. anhydrides  C. prostaglandins  D. endorphins

6. ___ What quantity of aspirin is contained in most over-the-counter adult aspirin products?  
A. 1 g  B. 325 mg  C. 225 mg  D. varies widely

7. ___ The presence of salicylic acid in an aspirin tablet indicates  
A. the aspirin is fresh.  C. the aspirin contains large quantities of binder.  
B. the aspirin is old  D. the aspirin contains small quantities of binder.

8. In most fatal cases of aspirin poisoning, the dosage has been greater than 20 grams.  How many aspirin tablets are necessary to supply 20 grams of aspirin?  Show your work and circle your answer.  

9. You are stranded on an island but find a source of wintergreen oil.  You have a headache.  Use structures to show the reactions you could use to turn wintergreen (methyl salicylate) into aspirin.  

\[
\begin{align*}
\text{Methyl Salicylate} \\
\text{(In Wintergreen)}
\end{align*}
\]

REFERENCE SEARCH:  
10. Look up Aspirin in the Physician’s Desk Reference (PDR) or reliable medical web site and describe any side effects of concern.