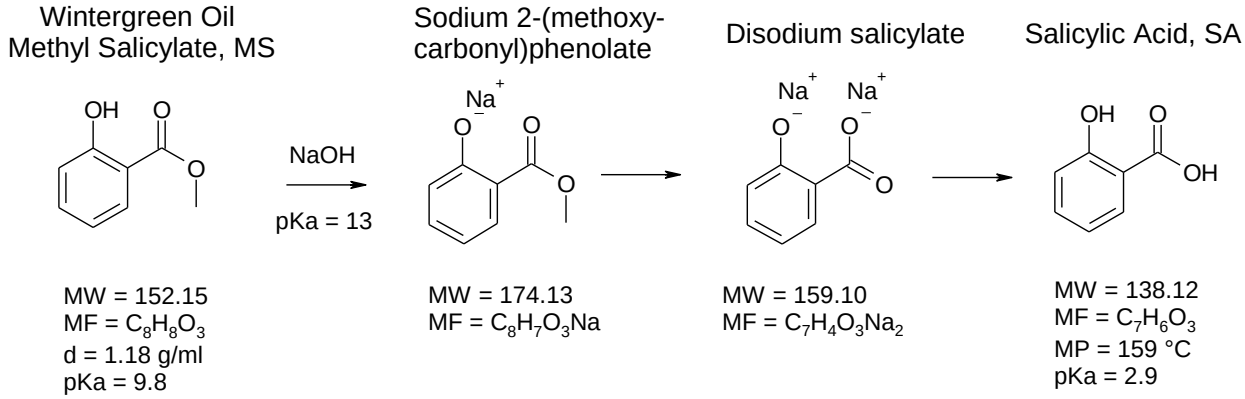
Acetylation of Salicylic Acid: Step 2 of Aspirin Synthesis

Background:

Our new company is preparing to manufacture aspirin by a new synthetic route. This is a new "green" process from wintergreen oil. There is a procedure developed intended for manufacturing on a large scale (100 kg) that requires testing for reproducibility as regards to yield and purity of the API. The two step synthesis encompasses aqueous base hydrolysis of wintergreen oil (methyl salicylate, MS) to salicylic acid (SA) followed by acetylation to acetylsalicylic acid (aspirin, ASA).

Scheme 1: Synthesis of Aspirin

STEP 1: Hydrolysis of methyl salicylate



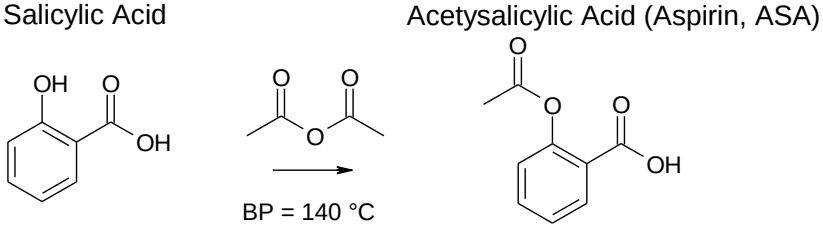
MW = 152.15
MF = $C_8H_8O_3$
d = 1.18 g/ml
pKa = 9.8

MW = 174.13
MF = $C_8H_7O_3Na$

MW = 159.10
MF = $C_7H_4O_3Na_2$

MW = 138.12
MF = $C_7H_6O_3$
MP = 159 °C
pKa = 2.9

STEP 2: Acetylation of Salicylic Acid



MW = 138.12
MF = $C_7H_6O_3$
MP = 159 °C

MW = 180.16
MF = $C_9H_8O_4$
MP = 135 °C
pKa = 3.5

Procedure for Step 2: Acetylation of Salicylic Acid to Acetylsalicylic Acid (ASA, Aspirin)

Important: Check off each step as you complete that step

- ☐ Place a filled DI water squeeze bottle into a bucket of ice to cool.
- ☐ Place a weighing boat on top of a top load balance and press tare.
- ☐ Weigh ~3.0 g of salicylic acid into the weighing funnel using a spatula.
    - Record the mass to the nearest 0.01 g: _____
- ☐ Remove the center rubber septum from the 3-neck round bottom flask, add a magnetic stir bar to the reaction vessel.
- ☐ Place a powder funnel into the port and add the weighed salicylic acid to the 100 mL reaction vessel.
- ☐ Using the lab jack, raise the stirrer and heating mantle until the heating mantle touches the bottom of the reaction flask.
- ☐ Using a 6 mL pipette, transfer 6.0 mL of acetic anhydride into the reaction flask through the open port and replace the rubber septum stopper.

    The instructor will demonstrate the correct handling of the pipette.
- ☐ Turn on the stirrer to make a slurry mixture.
- ☐ Using a re-pipette, add 100 µL (0.100 mL) of 85% phosphoric acid through the stoppered port and re-stopper. Eject the pipette tip into the waste container.
- ☐ Ensure that the tip of the thermocouple probe is in contact with the slurry. See Picture1.

Picture 1

Picture 2

☐ The power switch to the J-Kem Controller, which controls the heating mantle, has been turned on and pre-set to 85°C. **Do not flip the switch on the left side**.

☐ Turn the power dial from "OFF" to "50—500 mL" for your position number (see Picture 2). Record the start time: _____

- ☐ Describe any changes to the reaction mixture.

  _____

☐ Heat until the reaction temperature reaches 75-77 °C. Record the time: _____

☐ Reset the J-Kem Controller to "10—100 mL". Heat the reaction mixture for 25 min.

During the assigned heating time, perform the following calculations and record the re**sults in Table 1.**

- ☐ moles of salicylic acid = mass of salicylic acid (g) / Molar Mass (g/ mole) of salicylic acid

  = _____ moles Record in Table 1 on line (**1**).

- ☐ theoretical yield of ASA = moles of SA × Molar Mass (g/ mole) of ASA

  = _____ g Record in Table 1 on line (**2**).

☐ After heating the reaction for the assigned time, turn off the J-Kem Controller by turning the **knob—not the switch—** counter clockwise to OFF. Record the time: _____

☐ Use the lab jack to lower the stirrer, then remove the heating mantle and replace with the empty crystallizing dish**.**

Warning: the reaction flask and the heating mantle will still be hot!

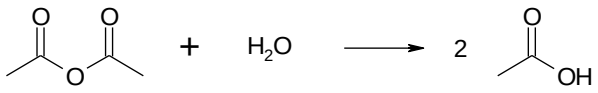
☐ Using the lab jack, raise the stirrer and continue stirring the reaction mixture.

- Describe any changes to the reaction mixture that have occurred.

  _____

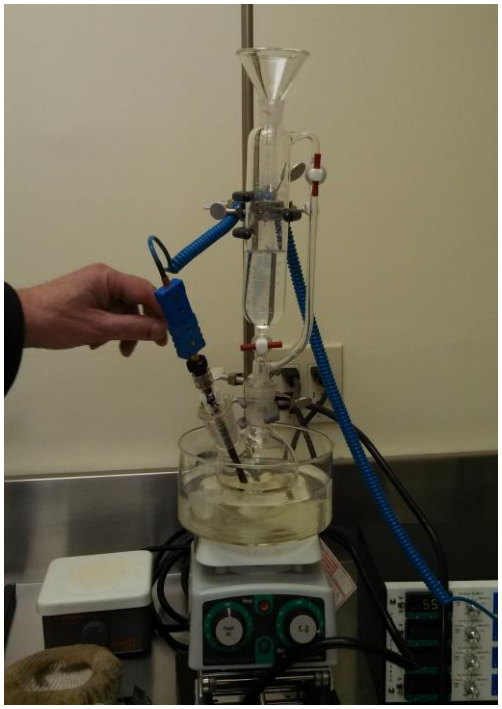
☐ When the reaction has cooled to 50 °C, using the re-pipette, add 1.0 mL DI water slowly in 100 µL (0.100 mL) portions over 3-5 minutes through the stoppered port. Observe the temperature.

WARNING: The hydrolysis of acetic anhydride is exothermic and no more than 0.1 mL of water should be added at any time to control the amount of heat produced.



☐ Ensure that the addition funnel stopper is in the closed horizontal direction. Add 15 mL of DI water to the addition funnel. After you have added the first 1.0 mL, add the 15 mL of DI water to the reaction vessel all at once through the stoppered port. What happens to the temperature?

☐ Fill the crystallizing dish no more than half-way with tap water to make a water bath. See Picture 3.



Picture 3

- If no crystals appear as the flask cools, see the instructor for a few aspirin seed crystals. Add the seed crystals through the stoppered port to help initiate crystallization.

- Add lots of ice to the water bath and continue stirring the reaction mixture until it cools to 5°C or after 15 minutes, whichever comes first. Notify the instructor that the crystallization is complete.
- The instructor will help you with the following steps:
  - Using the lab jack, lower the stirrer and ice bath from the reaction flask.
  - Remove the additional funnel and lay it aside carefully.
  - Remove the thermocouple and lay it aside.
  - Turn off the J-Chem Controller for your position.
  - Carefully remove the reaction flask from the apparatus.
  - One member of the team should hold the reaction flask upright so that it does not tip and spill your product.
- Place a 60 mL sintered glass filter funnel onto a cone rubber adapter set on a 250-mL filter flask that is securely clamped on a ring stand on the lab bench. Attach the side arm of the flask to a vacuum source and turn on the vacuum.
- **Gently** swirl the reaction flask and then pour the contents of the reaction flask into the filter funnel. See Picture 4.

Picture 4

- ☐ Using the chilled DI water squeeze bottle, rinse the reaction product off the sides of the reaction flask, swirl gently, and transfer any remaining solids to the filter. Repeat twice.
- ☐ Using a magnetic retriever, carefully remove the stir bar from the reaction product if it has dropped into the filter flask. Holding the magnetic retriever over the filter funnel, carefully rinse down the stir bar so that the rinse water deposits any residual product into the filter.
- ☐ Turn off the vacuum to the filter flask. Using the **ice cold** DI water bottle, wash the solid product with approximately 20 mL of **cold DI water**. Turn on the vacuum. Repeat once
  - ☐ What is being washed from the product? _____
- ☐ When no solution is observed dripping from the filter funnel, use a spatula to break up the product cake in the funnel and allow air to continue passing through the product for 5 min. Turn off the vacuum to the filter flask.
- ☐ Label a small crystallizing dish with your names. Weigh the labeled crystallizing dish and record its mass to the nearest 0.01 g. Tare mass: _____ g
- ☐ Using a spatula, carefully transfer the entire solid product to the weighed crystallizing dish. Reweigh the dish. Weight of dish and product:_____.
- ☐ Place a Kimwipe and rubber band on top of the crystallizing dish to prevent contamination.
- ☐ Place the covered crystallizing dish into the vacuum oven and dry at 90°C under vacuum for 30 minutes.
- ☐ Turn off the vacuum and carefully remove the crystallizing dish from the oven.

  Warning: the crystallizing dish will be very hot!
- ☐ Allow the crystallizing dish to cool to room temperature. Remove the Kimwipe and rubber band, weigh the crystallizing dish to the nearest 0.01 g. Gross mass: _____ g

- Calculate the amount of product in the crystallizing dish. Net mass: _____ g
  Record the net mass in Table 1 on line (**3**).
  - Why did the weight change?
  - Can a yield be calculated? Why or why not? _____
  - Where is the rest of the yield? (Hint: Solubility of ASA in water is 2 g/L).
- Replace the kimwipe and rubber band to prevent spillage and contamination.
- Proceed to the analytical section for acetylsalicylic acid, then return to complete Table 1.

Table 1

	Product	Acetylsalicylic Acid
0	Molar Mass (g/mole)	180.16
1	Amount of salicylic acid (moles)	
2	Theoretical Yield of acetylsalicylic acid (g)	
3	Dried/Net Mass (g)	
4	% Purity (HPLC)	
5	Actual Yield of acetylsalicylic acid (g)	
6	Amount of acetylsalicylic acid (moles)	
7	Acetylation % Yield	

Analytical Procedures

IR (identity)

The infrared spectrum of each organic compound is unique to the types and number of bonds in the molecule. Obtain an IR spectrum for acetylsalicylic acid and compare to known identified spectra.
Sample preparation:

Running a Background IR Spectrum

- Place one salt plate in the sample holder and lay the holder flat on the bench top.
- Place a second salt plate over the first.
- Place the cover over the plates and insert the holder into the IR spectrophotometer.
- Record the background spectrum.

Preparing a Sample in Acetone

- Using the spatula, transfer a very small amount of your sample to the agate mortar. Note each spatula is marked with line to provide guidance on the amount of sample required.
- Grind the sample to a very fine powder using the agate pestle.
- Add 4 drops of Acetone to dissolve the sample.
- Place one salt plate flat on the bench top.
- Place one drop of the Acetone / sample solution on the face of the salt plate.
- Allow to evaporate and the sample to crystallize.
- Carefully place the salt plate sandwich into the sample holder.
- Carefully place the cover over the plates and insert the holder into the IR spectrophotometer.
- Record the sample spectrum.
  - Select instrument from the menu
  - Select scan background or scan sample
  - Enter the sample ID: background or unknown ID
  - Click OK to begin scan
  - Select View and Label Peaks from the menu
  - Print a copy of each spectrum
- After obtaining the spectrum, disassemble the holder and wipe both plates clean with a Kimwipe.
- Place a few drops of isopropanol on each plate and wipe clean with a new Kimwipe.

MP (identity and purity)

Each crystalline organic compound has a unique melting point related to the stability of its crystal lattice energy. Measure the melting point of each dried crystalline solid that was obtained and compare to known values.

- Dip the open end of a melting point capillary into the pile of crystals.
- Invert and tap the tube on the desk to collect the crystals at the bottom.
- Place the capillary into the melting point apparatus.
- Turn the unit on and watch the sample through the sight glass.
- Record the temperature when the sample begins to melt and when fully melted
- Record melting point range on the table of Assay result

Aspirin Analysis by HPLC

The Experiment: Your team will prepare samples of aspirin for qualitative and quantitative analysis by HPLC.

Preparing the Sample

- In a 10 mL volumetric flask, add approximately 6 mg of the aspirin that you synthesized. Record the exact mass to the nearest 0.01 mg: _____ mg. Give this value to the instructor.
- Dissolve and dilute to the mark with HPLC solvent. Use a plastic transfer pipette to add the final amount.
- Label the flask.
- Seal the flask with a stopper. Place your thumb on the stopper, invert the flask and gently swirl. Repeat this 5 times.
- Filter through a 0.45µ syringe filter into a labelled HPLC vial (¾ full).
- Record the information for your sample in Table 2:

Table 2

Sample Name	Sample Amount (g)	Dilution Volume (flask size in mL)

Inputting Data into the HPLC Data System

- Put the HPLC vial into the autosampler, noting the vial location number: _____
- Input the sample name, sample amount, and dilution volume into the run sequence file.
- Once all the samples are in the autosampler and the sample information is added to the run sequence, start the HPLC and the samples will be analyzed.

Calculating the Amount of Aspirin in a Synthesized Sample

- As each sample is analyzed on the HPLC a report will be printed that provides the peak area, the mg/ mL of aspirin in your sample and its purity. Record purity in Table on line (**4**)
- Calculate the actual yield of acetylsalicylic acid produced (**5**), the amount of acetylsalicylic acid synthesized (**6**), and the % yield from your reaction (**7**) in Table 1. Remember:
  - moles of acetylsalicylic acid (**7**) = Actual Yield (**5**) / Molar Mass (**0**)
  - % Yield (**7**) = moles of product (**6**) / moles of starting material (**1**) × 100%

    or
  - % Yield (**7**) = Actual Yield (**5**) / Theoretical Yield (**2**) × 100%

S2S Team Report

Methyl salicylate identification (yes or no)

Tests	Team 1	Team 2	Team 3	Team 4
IR conformity				

Step 1 Salicylic Acid Analyses

Tests	Team 1	Team 2	Team 3	Team 4
MP (135 lit)				
Spectrophotometric Weight % Assay				
% Yield				

Step 2 Acetylation

Salicylic Acid identification

Tests	Team 1	Team 2	Team 3	Team 4
MP				

Step 2 Aspirin Assays

Tests	Team 1	Team 2	Team 3	Team 4
MP-ID				
HPLC Area % Purity				
Yield				

What was the reproducibility of the reaction yield?

How did the ID and quality of the methyl salicylate compare to the commercial grade?

What other assays might be of value in determining the quality of the products?

What recommendations does the synthesis team have to make?