Acetaminophen: The Acetylation of *p*-Aminophenol

prepared by **Joe Jeffers,** Ouachita Baptist University

PURPOSE OF THE EXPERIMENT

Prepare acetaminophen by making an amide of *p*-aminophenol.

EXPERIMENTAL OPTIONS

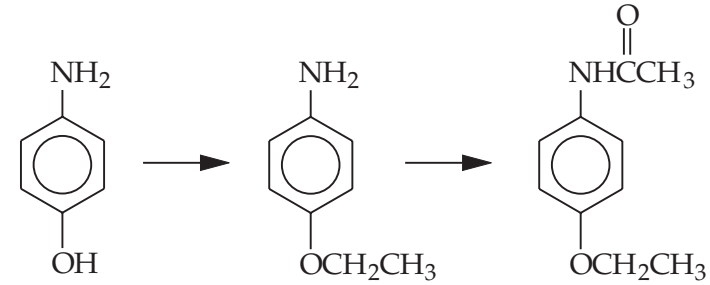
BACKGROUND REQUIRED

You should be familiar with reflux, vacuum filtration, recrystallization, thin-layer chromatography (TLC), melting point measurement, and infrared spectroscopy (IR).

BACKGROUND INFORMATION

In 1886, doctors accidentally discovered that acetanilide was effective as a fever reducer. However, while acetanilide was safe when used occasionally, continued use led to methemoglobinemia, a blood disorder resulting in reduced ability to carry oxygen to the tissues.

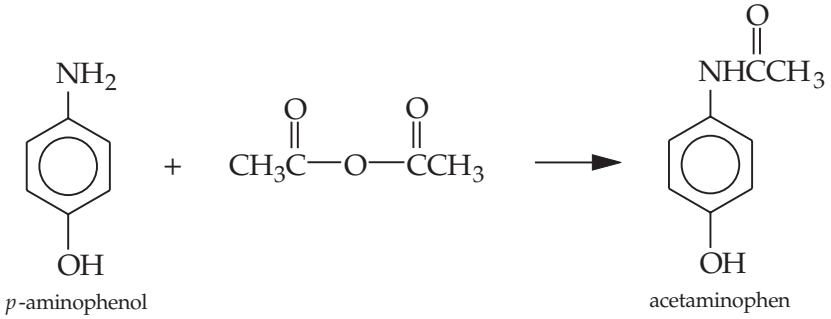
Carl Duisberg of the Bayer Company in Germany had a huge stockpile of *p*-aminophenol. Building on the reported results of acetanilide, Duisberg considered the possibility of acetylating *p*-aminophenol to produce a marketable product. Because phenols were widely considered to be toxic, Duisberg first converted the phenol group into an ether, then acetylated the amine, making phenacetin, as shown in Equation 1.



p-aminophenol phenacetin (Eq. 1)

Phenacetin had fever-reducing qualities similar to acetanilide without the high risk of methemoglobinemia.

In 1949, scientists discovered that the body converts phenacetin to acetaminophen. Furthermore, they found that acetaminophen was just as effective as phenacetin as a fever reducer and pain reliever, but less toxic. Acetaminophen can be made from *p*-aminophenol by reaction with acetic anhydride, as shown in Equation 2.



p-aminophenol acetaminophen (Eq. 2)

Acetaminophen is widely marketed as Tylenol® and Datril® in the United States and as Panadol® in Europe and Australia.

In this experiment, you will synthesize acetaminophen from *p*-aminophenol and acetic anhydride. You will use thin-layer chromatography (TLC) to determine the extent of the reaction. You will characterize your product by melting point and by infrared spectroscopy.

Semi-Microscale Synthesis

Equipment

25-mL beaker
2 beakers, 250-mL*
250-mL beaker, with aluminum foil or plastic wrap cover†
Bunsen burner
25-mL filter flask, with vacuum tubing
filter paper
glass rod
10-mL graduated cylinder
heater–stirrer
Hirsch funnel, with adapter
magnetic stir bar
melting point capillary tube
micropipet, 100 to 1000-µL
microspatula
2–3 open-ended capillary tubes
2 Pasteur pipets, with latex bulb
pencil
ruler
sand bath‡
standard taper glassware condenser, with tubing 10-mL round-bottom flask
stirring hot plate
support stand
2 test tubes, 13 × 100-mm
thermometer, –10 to 260 °C
TLC plate, silica gel, 2.5 × 7.5-cm, with fluorescent indicator
2 utility clamps
watch glass

*one each for the ice-water bath and the boiling-water bath

†for TLC developing chamber

‡sand in crystallizing dish on electric hot plate or sand in electric heating well with heat controller

Reagents and Properties

substance	*quantity*	*molar mass (g/mol)*	*mp (°C)*	*bp (°C)*
acetaminophen*		151.17	169–172	
acetic anhydride	0.550 mL	102.09		138
p-aminophenol	0.500 g	109.13	188–190	
ethyl acetate with 0.5% acetic acid	6 mL			
methanol	2.5 mL			
potassium bromide†	0.100 g			

*product
†for IR

Preview

- Add *p*-aminophenol, acetic anhydride, and distilled or deionized water to a reflux apparatus
- Use the sand bath to reflux the reaction for 20 min
- Monitor the completeness of the reaction using TLC
- Crystallize the product and collect the crystals by vacuum filtration
- Recrystallize the product from methanol–water
- Collect and dry the crystals
- Measure the melting point
- Collect an IR spectrum

PROCEDURE

Caution: Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

Always use caution in the laboratory. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Avoid ingesting any of the reagents.

1. Setting Up the Reaction

Assemble a reflux apparatus using a 10-mL round-bottom flask as a pot, as shown in Figure 1.

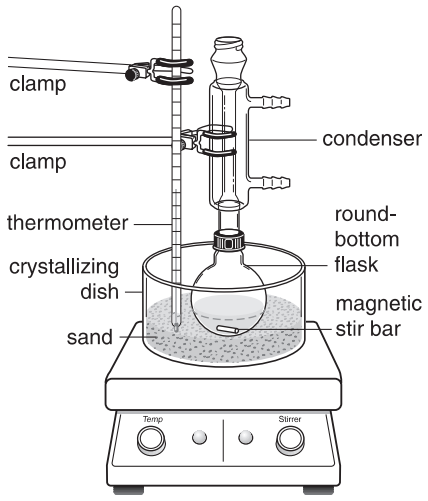


Figure 1 Semi-microscale reflux apparatus

Caution: Acetic anhydride is corrosive and a lachrymator. *p*-Aminophenol is toxic and irritating.

Put 0.500 g (500 mg) of *p*-aminophenol into the 10-mL round-bottom flask. Add 0.550 mL (550 μL) of acetic anhydride, 1.5 mL of distilled or deionized water, and a magnetic stir bar. Reassemble the reflux apparatus without delay.

2. Conducting the Reaction

Place the reaction flask in a sand bath and secure the flask with a clamp. Turn on the stirring hot plate.

Heat the reaction to 110–115 °C. [NOTE 1] Reflux at this temperature for 20 min. Monitor the sand temperature so the reaction does not overheat.

NOTE 1: The *p*-aminophenol will not dissolve until the temperature is close to 110 °C.

In the meantime, prepare an ice-water bath using a 250-mL beaker. Pour 10 mL of distilled water into a test tube. Chill the water in the ice-water bath for later use.

3. Preparing for Thin-Layer Chromatography

Caution: Do not use a Bunsen burner near flammable reagents such as ethyl acetate and methanol.

While the reaction is refluxing, use open-ended capillary tubing and a Bunsen burner to prepare 3–5 micropipets for TLC spotting.

Obtain a 2.5 × 7.5-cm silica gel TLC plate. Using a ruler as a straightedge, draw a *very faint* pencil line across the plate, 1 cm from the bottom. Make three small vertical lines that intersect the horizontal line at 6, 12, and 18 mm from the left side of the plate.

Using a pencil, label the plate from left to right below the vertical lines with "pAP", "Rxn", and "AAP". Do not cut through the silica gel with your marks.

Obtain standard samples of *p*-aminophenol and acetaminophen from your laboratory instructor. Using a micropipet, spot *p*-aminophenol at the position labeled pAP. Discard the micropipet.

Using a new micropipet, spot acetaminophen at the position marked AAP. Discard the micropipet.

Caution: Ethyl acetate is flammable and irritating.

Prepare a developing chamber by adding 6 mL of 0.5% acetic acid in ethyl acetate to a 250-mL beaker. Add filter paper to the chamber to act as a wick. Cover the chamber with aluminum foil or plastic wrap and set it aside in a ***fume hood.***

4. Analyzing the Reaction

After 20 min of reflux, remove the reaction flask from the heat. Transfer the reaction mixture, while it is still warm, to a 25-mL beaker. Allow the reaction mixture to cool to room temperature.

Use a micropipet to spot the reaction mixture on the TLC plate at the position marked Rxn.

Place the TLC plate in the developing chamber. Cover the chamber with the foil (or plastic wrap). Develop the plate until the eluent is within 1 cm of the top of the plate.

Remove the plate from the chamber. Immediately mark the eluent front with a pencil. Keep the plate in the ***fume hood*** for 1 min to allow the eluent to evaporate.

Caution: Ultraviolet radiation can cause severe damage to the eyes. Wear UV protective goggles. Do not look directly into the UV lamp.

View the plate under UV light to visualize the spots. Use a pencil to circle each spot. Note the relative amounts of *p*-aminophenol and acetaminophen from the Rxn spot.

5. Recovering the Product

If product crystals do not appear in the 25-mL beaker, scratch the walls of the beaker with a glass rod to induce crystallization. Put the beaker into the ice-water bath for 15 min to complete the crystallization.

Set up a vacuum filtration apparatus using a Hirsch funnel and a 25-mL filter flask. Wet the filter paper with water and turn on the vacuum.

Collect the product crystals by vacuum filtration. Rinse the beaker with 1 mL of cold water and add the rinse to the Hirsch funnel. Rinse the crystals with an additional 1 mL of cold water. Draw air through the funnel for 10 min to partially dry the crystals.

Transfer the crystals back into the 25-mL beaker.

6. Recrystallizing the Product

Set up a boiling-water bath using a 250-mL beaker.

Caution: Methanol is flammable and toxic.

Prepare a methanol–water recrystallizing solvent by combining 2.5 mL of methanol and 2.5 mL of distilled water in a test tube. Heat the solvent in the boiling-water bath.

Dissolve the crude acetaminophen in a *minimum* amount of hot solvent. Allow the solution to cool slowly to room temperature. Complete the recrystallization by cooling the beaker in the ice-water bath for 5–10 min.

Collect the product crystals by vacuum filtration using a Hirsch funnel. Transfer the crystals to a watch glass. Allow the crystals to dry. [NOTE 2]

NOTE 2: A drying oven set at 100–110 °C may be used to dry the crystals. If the crystals are air dried, it is best to dry them overnight.

7. Characterizing the Product

Measure the melting point of your product.

Caution: Potassium bromide (KBr) is irritating and hygroscopic.

Obtain an infrared spectrum of your product either by preparing a KBr pellet or as indicated by your laboratory instructor.

8. Cleaning Up

Use the labeled collection containers provided by your laboratory instructor.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Microscale Synthesis

Equipment

2 beakers, 250-mL*
250-mL beaker, with aluminum foil or plastic wrap cover†
Bunsen burner
25-mL filter flask, with vacuum tubing
filter paper
glass rod
10-mL graduated cylinder
Hirsch funnel, with adapter
melting point capillary tube
micropipet, 100 to 1000-µL
microspatula
2–3 open-ended capillary tubes
2 Pasteur pipets, with latex bulb
pencil
reflux glassware, elastomeric connectors assembly‡
- air condenser
- elastomeric connectors
- magnetic stir bar
- 5-mL long-necked round-bottom flask
- 5-mL vial or 10-mL beaker

reflux glassware, conical vial assembly‡
- condenser
- 5-mL conical vial
- magnetic spin vane

ruler
sand bath§
stirring hot plate
support stand
2 test tubes, 13 × 100-mm
–10 to 260 °C thermometer
TLC plate, silica gel, 2.5 × 7.5-cm, with fluorescent indicator
2 utility clamps
watch glass

*one each for the ice-water bath and the boiling-water bath

†for TLC developing chamber

‡use glassware provided by your laboratory instructor

§sand in crystallizing dish on electric hot plate or sand in electric heating well with heat controller

Reagents and Properties

substance	*quantity*	*molar mass (g/mol)*	*mp (°C)*	*bp (°C)*
acetaminophen*		151.17	169–172	
acetic anhydride	0.225 mL	102.09		138
p-aminophenol	0.200 g	109.13	188–190	
ethyl acetate with 0.5% acetic acid	6 mL			
methanol	2.0 mL			
potassium bromide†	0.100 g			

*product

†for IR

Preview

- Add *p*-aminophenol, acetic anhydride, and distilled or deionized water to a reflux apparatus
- Use the sand bath to reflux the reaction for 15 min

- Monitor the completeness of the reaction using TLC
- Crystallize the product and collect the crystals by vacuum filtration
- Recrystallize the product from methanol–water
- Collect and dry the crystals
- Measure the melting point
- Collect an IR spectrum

PROCEDURE

Caution: Wear departmentally approved safety goggles at all times while in the chemistry laboratory.

Always use caution in the laboratory. Many chemicals are potentially harmful. Prevent contact with your eyes, skin, and clothing. Avoid ingesting any of the reagents.

1. Setting Up the Reaction

Assemble the reflux apparatus shown in Figure 2 that corresponds to your glassware. Add a stir bar or a spin vane.

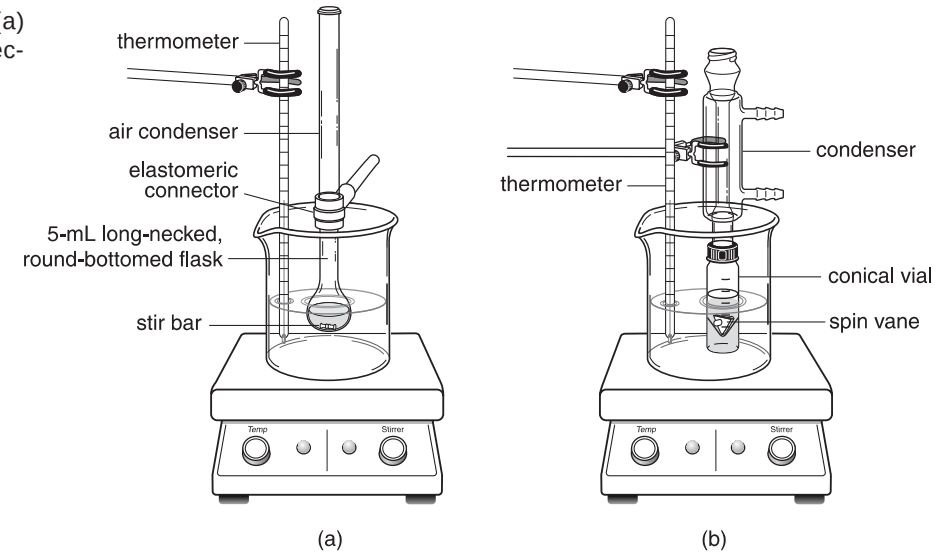


Figure 2 Reflux apparatus for (a) glassware with elastomeric connectors or (b) conical vial

Caution: Acetic anhydride is corrosive and a lachrymator. *p*-Aminophenol is toxic and irritating.

Put 0.200 g (200 mg) of *p*-aminophenol into the 5-mL round-bottom flask or 5-mL conical vial. Add 0.225 mL (225 µL) of acetic anhydride and 0.6 mL (600 µL) of distilled or deionized water. Reassemble the reflux apparatus without delay.

2. Conducting the Reaction

Place the reaction flask (vial) in a sand bath and secure the flask with a clamp. Turn on the stirring hot plate.

Heat the reaction to 110–115 °C. [NOTE 1] Reflux at this temperature for 15 min. Monitor the sand temperature so the reaction does not overheat.

NOTE 1: The *p*-aminophenol will not dissolve until the temperature is close to 110 °C.

In the meantime, prepare an ice-water bath using a 250-mL beaker. Pour 5 mL of distilled water into a test tube. Chill the water in the ice-water bath for later use.

3. Preparing for Thin-Layer Chromatography

Caution: Do not use a Bunsen burner near flammable reagents such as ethyl acetate and methanol.

While the reaction is refluxing, use open-ended capillary tubing and a Bunsen burner to prepare 3–5 micropipets for TLC spotting.

Obtain a 2.5 × 7.5-cm silica gel TLC plate. Using a ruler as a straightedge, draw a *very faint* pencil line across the plate, 1 cm from the bottom. Make three small vertical lines that intersect the horizontal line at 6, 12, and 18 mm from the left side of the plate.

Using a pencil, label the plate from left to right below the vertical lines with "pAP", "Rxn", and "AAP". Do not cut through the silica gel with your marks.

Obtain standard samples of *p*-aminophenol and acetaminophen from your laboratory instructor. Using a micropipet, spot *p*-aminophenol at the position labeled pAP. Discard the micropipet.

Using a new micropipet, spot acetaminophen at the position marked AAP. Discard the micropipet.

Caution: Ethyl acetate is flammable and irritating.

Prepare a developing chamber by adding 6 mL of 0.5% acetic acid in ethyl acetate to a 250-mL beaker. Add filter paper to the chamber to act as a wick. Cover the chamber with aluminum foil or plastic wrap and set it aside in a ***fume hood.***

4. Analyzing the Reaction

After 15 min of reflux, remove the heat from the flask (or vial). If you used a round-bottom flask, transfer the reaction mixture, while it is still warm, to a vial or small beaker. Allow the reaction mixture to cool to room temperature.

Use a micropipet to spot the reaction mixture on the TLC plate at the position marked Rxn.

Place the TLC plate in the developing chamber. Cover the chamber with the foil (or plastic wrap). Develop the plate until the eluent is within 1 cm of the top of the plate.

Remove the plate from the chamber. Immediately mark the eluent front with a pencil. Keep the plate in the ***fume hood*** for 1 min to allow the eluent to evaporate.

Caution: Ultraviolet (UV) radiation can cause severe damage to the eyes. Wear UV protective goggles. Do not look directly into the UV lamp.

View the plate under UV light to visualize the spots. Use a pencil to circle each spot. Note the relative amounts of *p*-aminophenol and acetaminophen developed from the Rxn spot.

5. Recovering the Product

If product crystals do not appear in the vial (or beaker), scratch the walls of the vessel with a glass rod to induce crystallization. Put the vessel into the ice-water bath for 15 min to complete the crystallization.

Set up a vacuum filtration apparatus using a Hirsch funnel and a 25-mL filter flask. Wet the filter paper with water and turn on the aspirator.

Collect the product crystals by vacuum filtration. Rinse the vial (beaker) with 0.5–1 mL of cold water and add the rinse to the Hirsch funnel. Rinse the crystals with an additional 0.5–1 mL of cold water. Draw air through the funnel for 10 min to partially dry the crystals.

Transfer the crystals back to the 5-mL vial.

6. Recrystallizing the Product

Set up a boiling-water bath using a 250-mL beaker.

Caution: Methanol is flammable and toxic.

Prepare a methanol–water recrystallizing solvent by combining 2.0 mL of methanol and 2.0 mL of distilled water in a test tube. Heat the solvent in the boiling-water bath.

Dissolve the crude acetaminophen in a *minimum* amount of hot solvent. Allow the solution to cool slowly to room temperature. Complete the recrystallization by cooling the beaker in the ice-water bath for 5–10 min.

Collect the product crystals by vacuum filtration using a Hirsch funnel. Transfer the crystals to a watch glass. Allow the crystals to dry. [NOTE 2]

NOTE 2: A drying oven set at 100–110 °C may be used to dry the crystals. If the crystals are air dried, it is best to dry them overnight.

7. Characterizing the Product

Measure the melting point of your product.

Caution: Potassium bromide (KBr) is irritating and hygroscopic.

Obtain an infrared spectrum of your product either by preparing a KBr pellet or as indicated by your laboratory instructor.

8. Cleaning Up

Use the labeled collection containers provided by your laboratory instructor.

Caution: Wash your hands thoroughly with soap or detergent before leaving the laboratory.

Post-Laboratory Questions

1. Calculate your percent yield of acetaminophen. Remember to adjust your theoretical yield to the actual amount of *p*-aminophenol you used in the experiment.
2. Do your TLC results indicate that the reaction went to completion? Briefly explain.
3. Does your measured melting point indicate that your product is pure? Briefly explain.
4. Does your IR spectrum indicate that the starting material was acetylated? Briefly explain.

NAME SECTION DATE

SYNT/746Acetaminophen: The Acetylation of p-Aminophenol

Pre-Laboratory Assignment

1. What precautions should you take when working with:
   (a) acetic anhydride

   (b) methanol

2. What is the limiting reagent in this reaction? (Acetic anhydride density is $d = 1.082$ g/mL.) Show your calculations here and in your laboratory notebook.

3. Calculate the theoretical yield of acetaminophen in this experiment. Show your calculations here and in your laboratory notebook.

4. How can TLC allow you to determine if your reaction has gone to completion? Briefly explain.

5. *p*-Aminophenol reacts with acetic anhydride at the amino group rather than at the phenol group. Briefly explain why the amino site is preferred over the phenol site.

ISBN 0-53497-774-X