



# Thiamine-Catalyzed Benzoin Condensation

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**PURPOSE OF THE EXPERIMENT** Use thiamine to catalyze the benzoin condensation of benzaldehyde.

**EXPERIMENTAL OPTIONS** Semi-Microscale Condensation ..... 4  
Microscale Condensation ..... 7

**BACKGROUND REQUIRED** You should be familiar with vacuum filtration, recrystallization, melting point measurement, and infrared spectroscopy.

**BACKGROUND INFORMATION** Wöhler and Liebig accidentally discovered the benzoin condensation in 1832. Their research was focused on **cyanohydrins**, the products obtained from addition of cyanide ion to aldehydes. Much to their surprise, Wöhler and Liebig observed that a new product formed when the reaction mixture contained an *aromatic* aldehyde. The research team determined the product to be an  $\alpha$ -hydroxy ketone called an **acyloin**. Further studies of this reaction indicated that cyanide was a catalyst in the reaction. The mechanism for the cyanide-catalyzed condensation of benzaldehyde is shown in Figure 1.

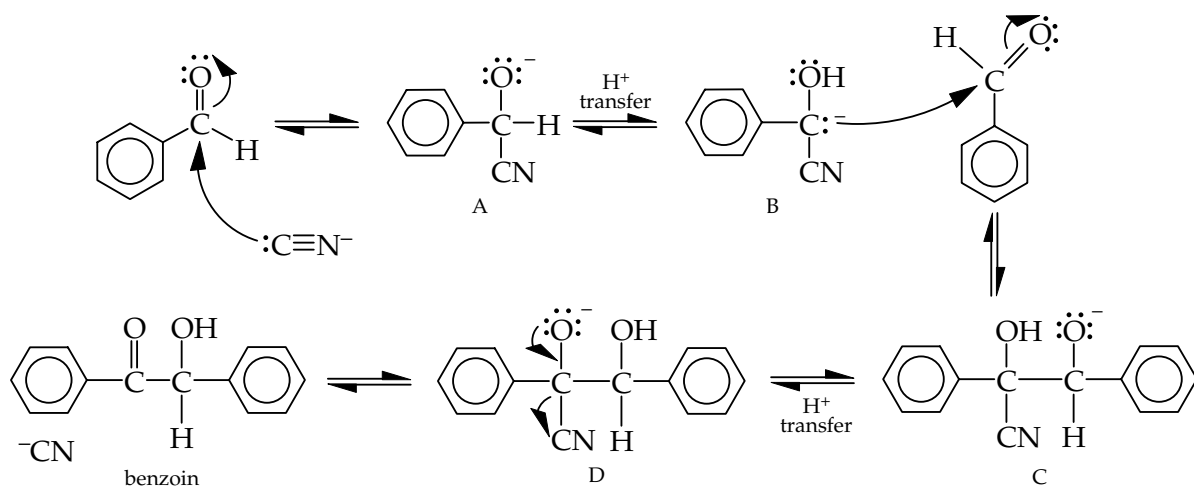


Figure 1 Cyanide-catalyzed benzoin condensation

In the first step of the reaction, the cyanide ion undergoes a reversible addition to the carbonyl of benzaldehyde, forming the oxyanion of the cyanohydrin, A. The electron-withdrawing effect of the cyano group increases the acidity of the  $\alpha$ -hydrogen atom on the carbon atom. Therefore, proton transfer from the  $\alpha$ -carbon to the oxygen forms carbanion B. This nucleophilic carbanion then adds to another molecule of benzaldehyde to yield a substituted cyanohydrin, C. Oxyanion C is in equilibrium with oxyanion D, formed through proton transfer from one oxygen to the other. Loss of cyanide stabilizes oxyanion D to form the acyloin, in this case benzoin.

The cyanide ion serves three functions in this reaction: (1) it acts as a nucleophile; (2) it increases the acidity of the  $\alpha$ -hydrogen on the carbon atom; (3) it acts as a leaving group after the condensation is completed.

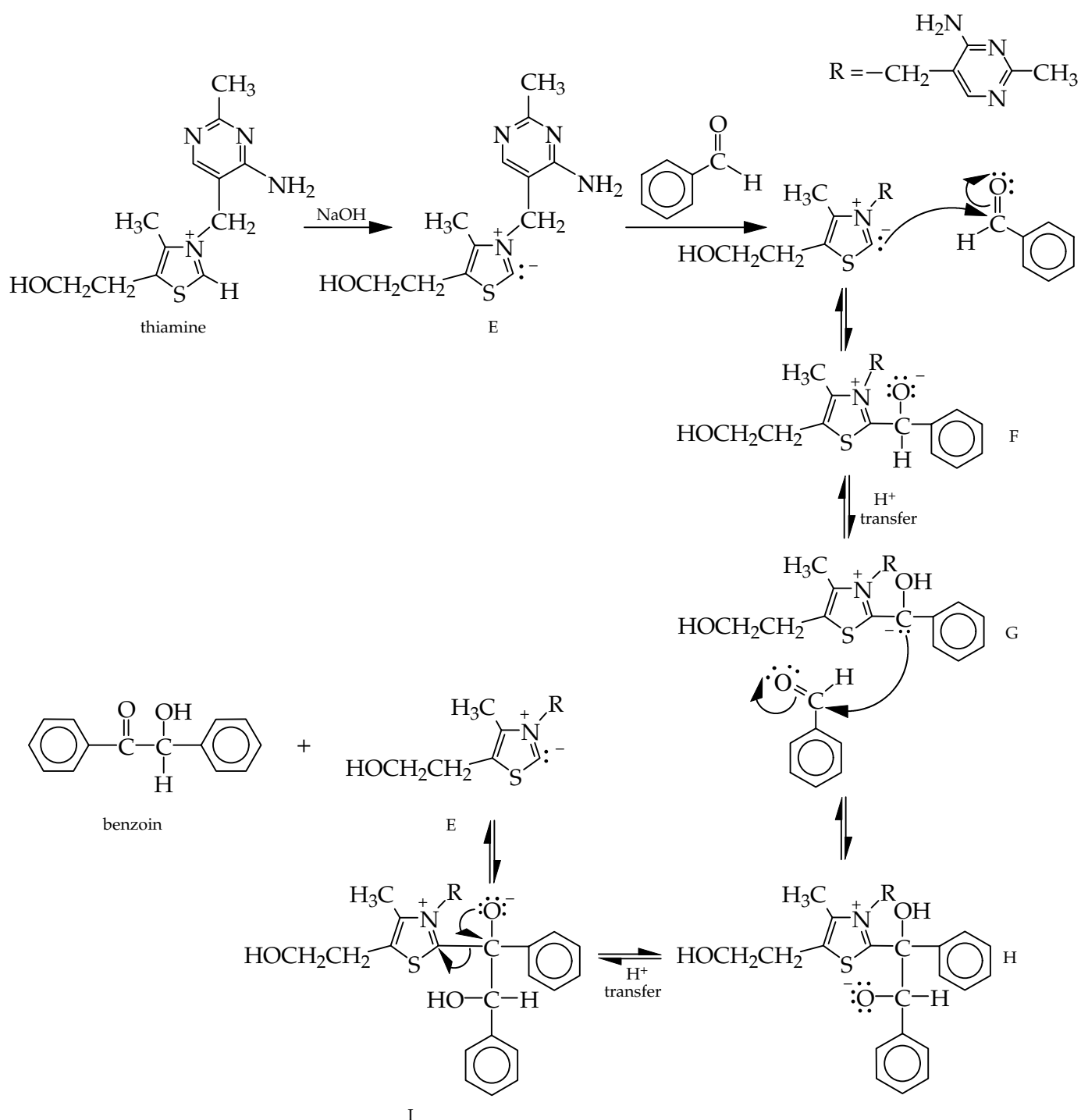
For over 100 years, the cyanide ion was the only observed chemical species that fulfilled all of the above functions. In 1958, Breslow discovered that in basic solutions, thiazolium salts are also effective catalysts for the benzoin condensation. What was of broader significance to Breslow's work was the recognition that several biological cofactors contain a thiazole ring. As a result, many biochemical reaction mechanisms could now be understood as analogs of the benzoin condensation.

High molar mass biopolymers called **enzymes** catalyze chemical reactions in living systems. An enzyme performs its catalytic function by bringing reactants together in the spatial relationship necessary for a reaction to occur. In other words, an enzyme provides the structural active site for a biochemical reaction. Most enzymes are proteins, which are polymers of amino acids. Amino acids at the active site provide catalytic function, serving as acids or bases in one or more steps of the reaction pathway. Enzymes often require additional small molecules called **coenzymes** as cocatalysts. Much of the bond breaking and bond making in biochemical reactions involve coenzymes. Many coenzymes are synthesized from vitamins.

Thiamine pyrophosphate (TPP) is a coenzyme found in humans and other animals. Many metabolic reactions, including the synthesis of  $\alpha$ -hydroxy ketones (acyloin condensations), require TPP. TPP is derived from vitamin B<sub>1</sub>, also known as thiamine. Thiamine will catalyze the benzoin condensation *in vitro* in the absence of an enzyme. The mode of action is similar to that of TPP under physiological conditions.

The mechanism of the thiamine-catalyzed benzoin condensation of benzaldehyde is shown in Figure 2. The steps are analogous to those in the cyanide-catalyzed mechanism.

Carbanion E forms when a hydroxide ion deprotonates the thiamine thiazole ring. Carbanion E, being a good nucleophile, undergoes a reversible addition to the benzaldehyde carbonyl, forming an alkylated thiazole derivative, F. The resonance effects of the thiazole ring increase the acidity of the  $\alpha$ -hydrogen atom on the carbon atom that is adjacent to the benzene ring. The increased acidity facilitates proton transfer from the  $\alpha$ -carbon to the oxygen, forming carbanion G. Carbanion G then adds to another molecule of benzaldehyde, forming oxyanion H. Oxyanion H is in equilibrium with oxyanion I, which can eliminate the catalyst and form benzoin.



**Figure 2** Thiamine-catalyzed benzoin condensation

In this experiment, you will synthesize benzoin from benzaldehyde using thiamine as a catalyst. You will characterize the product by melting point and by infrared spectroscopy.

## Semi-Microscale Condensation

### Equipment

250-mL beaker*	melting point capillary tubes
boiling chip <sup>†</sup>	microspatula
50-mL Erlenmeyer flask	Pasteur pipet, with latex bulb
25-mL filter flask, with vacuum tubing	reflux assembly <sup>†</sup> condenser, with tubing
filter paper, to fit Hirsch funnel	thermometer, -10 to 260 °C
glass stirring rod	25-mL round-bottom flask <sup>‡</sup>
10-mL graduated cylinder	sand bath <sup>§†</sup>
Hirsch funnel, with adapter	16 × 150-mm test tube
hot plate	2 utility clamps <sup>†</sup>
magnetic stir bar	watch glass
magnetic stirrer	

\*for ice-water bath

<sup>†</sup>for reflux method (option B, Part 1)

<sup>‡</sup>with stopper if using room-temperature method (option A, Part 1)

<sup>§</sup>stirring hot plate with crystallizing dish filled with sand or magnetic stirrer and electric flask heater filled with sand.

### Reagents and Properties

<i>substance</i>	<i>quantity</i>	<i>molar mass</i> (g/mol)	<i>d</i> (g/mL)	<i>mp</i> (°C)
benzaldehyde	4.0 mL	106.12	1.044	
benzoin*		212.25		135–137
95% ethanol	40 mL			
potassium bromide	0.100 g			
2.0M sodium hydroxide	3.0 mL			
thiamine hydrochloride	1.0 g	337.27		260
*product				

### Preview

- Place thiamine hydrochloride, distilled water, and 2.0M NaOH into a flask
- Add benzaldehyde to the flask
- Store for 2 days or fit the flask with a condenser and reflux the mixture for 75 min
- Cool the reaction mixture in an ice-water bath
- Use vacuum filtration to collect the crude product
- Weigh the crude product
- Recrystallize the crude product from 95% ethanol
- Use vacuum filtration to collect the purified product
- Dry and weigh the purified product
- Characterize the purified product

**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. Conducting the Benzoin Condensation

**NOTE 1:** Benzaldehyde, a suspected carcinogen, is also a certified food flavoring (almond extract). Food substances undergo a much higher scrutiny than do most other chemicals. Use normal precautions when working with benzaldehyde.

**NOTE 2:** Use either option *A* or *B*, as directed by your laboratory instructor. Higher yields are obtained using option *A*.

**NOTE 3:** If a stirrer is not available for this step, add a boiling chip to the flask.

**Caution:** 95% Ethanol is flammable and irritating. Keep away from flames. 2.0M NaOH is corrosive. Benzaldehyde is a suspected carcinogen and mutagen. [NOTE 1] Wear gloves when handling these compounds.

Place 1.00 g of thiamine hydrochloride into a 25-mL round-bottom flask. Add 2.0 mL of distilled or deionized water. Mix to dissolve.

Add 8.0 mL of 95% ethanol. Add a magnetic stir bar. Using a magnetic stirrer, stir the solution until it is homogeneous.

Use a Pasteur pipet to add 3.0 mL of 2.0M NaOH dropwise to the stirring solution over a 2-min period. Note that the solution initially turns a bright yellow color that then fades to pale yellow.

When the solution is pale yellow, add 4.0 mL of benzaldehyde. Stir until the mixture is homogeneous. [NOTE 2]

#### A. Conducting the Reaction at Room Temperature

Stopper the flask. Allow the reaction mixture to stand for 2–7 days. After this period of time, proceed to Step 2. **Isolating and Purifying the Product.**

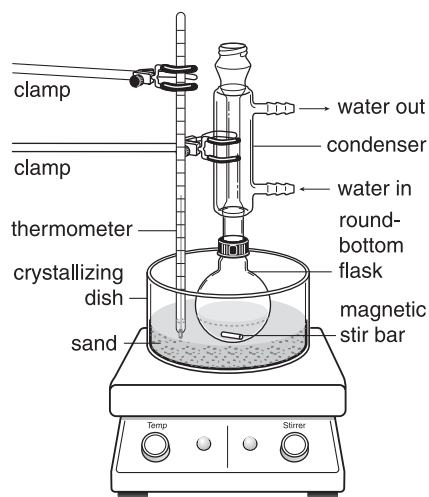
#### B. Conducting the Reaction by Reflux

Fit the flask with a water-jacketed condenser. Place the entire assembly in a sand bath, as shown in Figure 3.

Turn on the water to the condenser. Start the magnetic stirrer. [NOTE 3] Heat the reaction to a *gentle* boil and reflux for 75 min.

After the reflux period, remove the flask from the heat source. Allow the reaction mixture to cool to room temperature.

**Figure 3** Reflux apparatus with round-bottom flask



2. **Isolating and Purifying the Product** 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.

Place the reaction flask in the ice-water bath to crystallize the product. If necessary, scratch the bottom of the flask with a glass rod to induce crystallization. Once crystallization has started, allow the flask to remain in the ice-water bath for 5 min.

While the reaction mixture is cooling in the ice bath, assemble a vacuum filtration apparatus using a Hirsch funnel. Turn on the water to the aspirator. Moisten the filter paper with a few drops of distilled water.

Vacuum filter the reaction mixture. Rinse the crystals with 2 mL of the chilled water. Allow the product to air dry in the filter funnel for 5 min.

Transfer the crude product from the filter paper to a watch glass to finish drying. Weigh the product and record its mass.

For recrystallization, place the crude product in a 50-mL Erlenmeyer flask. Add 8.0 mL of 95% ethanol *for every gram* of crude product placed in the flask. Use a hot plate to heat the mixture gently until the ethanol boils. If the product does not completely dissolve, add more ethanol dropwise until all of the solid has dissolved in the boiling solvent.

Allow the solution to cool to room temperature. If necessary, scratch the bottom of the flask to induce crystallization. Then cool the solution in the ice-water bath for 5 min.

Filter the crystals using vacuum filtration. Allow the product to air dry in the filter funnel for 10 min.

Transfer the product from the filter paper to a watch glass to finish drying. Weigh the product and record the mass.

3. **Characterizing the Product** Measure the melting point of your dry product.

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

Prepare a KBr pellet of your product. Record the IR spectrum as directed by your laboratory instructor. Compare your spectrum to the reference spectrum of benzoin.

4. **Cleaning Up** Use the labeled collection containers provided by your laboratory instructor. Clean your glassware with soap or detergent.

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

## Microscale Condensation

### Equipment

250-mL beaker*	Pasteur pipet, with latex bulb
boiling chip	<i>reflux glassware, conical vial</i>
2 conical vials, 5.0-mL <sup>§ †</sup>	<i>assembly<sup>† ‡</sup></i>
25-mL filter flask, with vacuum tubing	condenser, with tubing
filter paper, to fit Hirsch funnel	thermometer, -10 to 260 °C
glass stirring rod	<i>reflux glassware, elastomeric</i>
10-mL graduated cylinder	<i>connector assembly<sup>† ‡</sup></i>
Hirsch funnel, with adapter	condenser, with tubing
hot plate	elastomeric connector
magnetic stir bar or spin vane	thermometer, -10 to 260 °C
magnetic stirrer	5.0-mL round-bottom flask <sup>† §</sup>
melting point capillary tubes	sand bath <sup>** †</sup>
micropipet, 100 to 1000- $\mu$ L	13 $\times$ 100-mm test tube
microspatula	watch glass

\*for ice-water bath

<sup>†</sup>use the glassware indicated by your laboratory instructor

<sup>‡</sup>for reflux method (option B, Part 1)

<sup>§</sup>with cap or stopper if using option A, Part 1

\*\*stirring hot plate with crystallizing dish filled with sand or magnetic stirrer and electric flask heater filled with sand

### Reagents and Properties

<i>substance</i>	<i>quantity</i>	<i>molar mass</i> (g/mol)	<i>d</i> (g/mL)	<i>mp</i> (°C)
benzaldehyde	1.00 mL	106.12	1.044	
benzoin*		212.25		135–137
95% ethanol	11 mL			
potassium bromide	0.100 g			
2.0M sodium hydroxide	0.5 mL			
thiamine hydrochloride	0.170 g	337.27		260
*product				

### Preview

- Place thiamine hydrochloride, distilled water, and 2.0M NaOH into a vial or flask
- Add benzaldehyde to the vial or flask
- Store for 2 days or fit the vial or flask with a condenser and reflux the mixture for 75 min
- Cool the reaction mixture in an ice-water bath
- Use vacuum filtration to collect the crude product
- Weigh the crude product
- Recrystallize the crude product from 95% ethanol
- Use vacuum filtration to collect the purified product
- Dry and weigh the purified product
- Characterize the purified product

**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. Conducting the Benzoin Condensation

**Caution:** 95% Ethanol is flammable and irritating. Keep away from flames. 2.0M NaOH is corrosive. Benzaldehyde is a suspected carcinogen and mutagen. [NOTE 1] Wear gloves when handling these compounds.

**NOTE 1:** Benzaldehyde, a suspected carcinogen, is also a certified food flavoring (almond extract). Food substances undergo a much higher scrutiny than do most other chemicals. Use normal precautions when working with benzaldehyde.

Place 0.170 g (170 mg) of thiamine hydrochloride into a 5-mL conical vial or a 5-mL round-bottom flask. Add 350  $\mu$ L of distilled or deionized water. Mix to dissolve.

Add 2.00 mL of 95% ethanol. Add a magnetic spin vane or stir bar. Using a magnetic stirrer, stir the solution until it is homogeneous.

While stirring, use a Pasteur pipet to add 0.5 mL of 2.0M NaOH dropwise to the solution over a 2-min period. Note that the solution initially turns a bright yellow color that then fades to pale yellow.

When the solution is pale yellow, add 1.00 mL of benzaldehyde. Stir until the mixture is homogeneous. [NOTE 2]

**NOTE 2:** Use either option A or B, as directed by your laboratory instructor. Higher yields are obtained using option A.

#### A. Conducting the Reaction at Room Temperature

Cap the vial or flask. Allow it to stand for 2–7 days. After this period of time, proceed to Step 2. **Isolating and Purifying the Product.**

#### B. Conducting the Reaction by Reflux

Depending upon your glassware, assemble the reflux apparatus shown in Figure 4(a) or 4(b). Place the entire assembly in a sand bath.

Turn on the water to the condenser. Start the magnetic stirrer. Heat the reaction to a *gentle* boil and reflux for 75 min.

After the reflux period, remove the vial or flask from the heat source. Allow the reaction mixture to cool to room temperature.

### 2. Isolating and Purifying the Product

**NOTE 3:** If you used a round-bottom flask for the reflux, transfer the reaction mixture to a vial before crystallizing the product.

Prepare an ice-water bath using a 250-mL beaker. Place 2 mL of distilled water into a test tube. Chill the water in the ice-water bath for later use.

Place the reaction vial in the ice-water bath to crystallize the product. [NOTE 3] If necessary, scratch the bottom and sides of the vial with a glass rod to induce crystallization. Once crystallization has started, allow the flask to remain in the ice-water bath for 5 min.

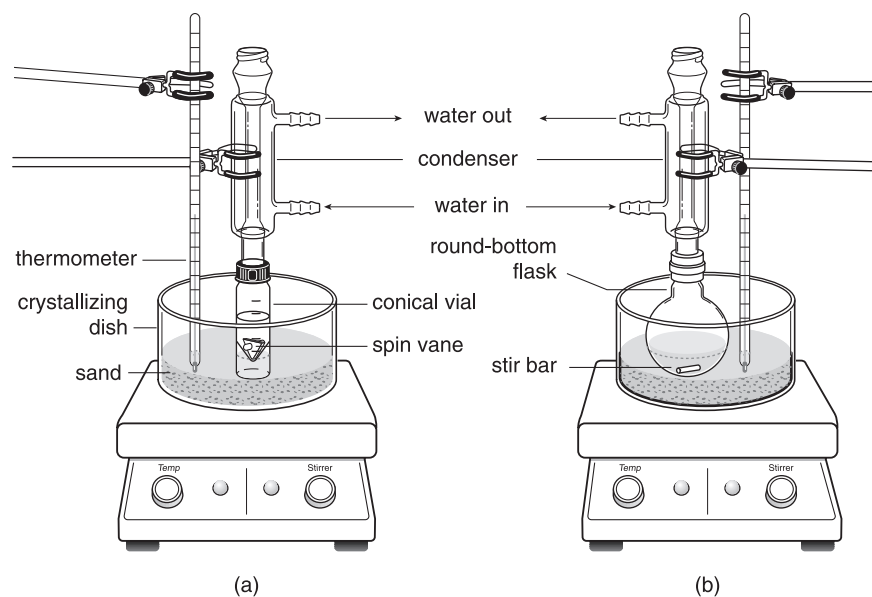
While the reaction mixture is cooling in the ice bath, assemble a vacuum filtration apparatus using a Hirsch funnel. Turn on the water to the aspirator. Moisten the filter paper with a few drops of distilled water.

Vacuum filter the reaction mixture. Rinse the crystals with 2 mL of the chilled water. Allow the product to air dry in the filter funnel for 5 min.

Transfer the crude product from the filter paper to a watch glass to finish drying. Weigh the product and record its mass.

For recrystallization, place the crude product in a 5.0-mL vial. Add 8.0 mL of 95% ethanol for *every gram* of crude product placed in the flask.

**Figure 4** Microscale reflux apparatus with (a) conical vial or (b) round-bottom flask and elastomeric connectors



Use a hot plate to heat the mixture gently until the ethanol boils. If the product does not completely dissolve, add more ethanol dropwise until all of the solid has dissolved in the boiling solvent.

Allow the solution to cool to room temperature. If necessary, scratch the bottom of the vial to induce crystallization. Then cool the solution in the ice-water bath for 5 min.

Filter the crystals using vacuum filtration. Allow the product to air dry in the filter funnel for 10 min.

Transfer the product from the filter paper to a watch glass to finish drying. Weigh the product and record its mass.

### 3. Characterizing the Product

Measure the melting point of your dry product.

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

Prepare a KBr pellet of your product. Record the IR spectrum as directed by your laboratory instructor. Compare your spectrum to the reference spectrum of benzoic acid.

### 4. Cleaning Up

Use the labeled collection containers provided by your laboratory instructor. Clean your glassware with soap or detergent.

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

- Post-Laboratory Questions**
1. Calculate the percent yield for your product, before and after recrystallization, using Equation 1.

$$\text{percent yield, \%} = \left( \frac{\text{actual yield, g}}{\text{theoretical yield, g}} \right) (100\%) \quad (\text{Eq. 1})$$

2. Calculate the percent recovery from the recrystallization, using Equation 2.

$$\text{percent recovery, \%} = \left( \frac{\text{mass after recrystallization, g}}{\text{mass before recrystallization, g}} \right) (100\%) \quad (\text{Eq. 2})$$

3. The benzoin molecule contains one chiral center, yet the product produced in this reaction is not optically active. Briefly explain.
4. Clearly label the IR spectrum of benzoin. Assign the appropriate vibrational modes to the following peaks:
  - (a)  $3400 \text{ cm}^{-1}$
  - (b)  $3052 \text{ cm}^{-1}$
  - (c)  $1676 \text{ cm}^{-1}$

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NAME

SECTION

DATE

*SYNT 737/Thiamine-Catalyzed Benzoin Condensation*

**Pre-Laboratory Assignment**

1. What safety precautions must be taken when using sodium hydroxide?
2. Briefly define the following terms:
  - (a) acyloin
  - (b) enzyme
  - (c) coenzyme
3. Calculate the number of moles of benzaldehyde and thiamine hydrochloride used in the Procedure. Explain why benzaldehyde is used for the theoretical yield calculation even though fewer moles of thiamine hydrochloride are present in the reaction.

## 12 SYNT 737/Thiamine-Catalyzed Benzoin Condensation

4. Calculate the theoretical yield for the benzoin condensation. Show your calculations here and in your laboratory notebook.