1. Catalase and the Decomposition of Hydrogen Peroxide

Introduction

When hydrogen peroxide decomposes, the products are oxygen and water. Although this reaction is spontaneous in the sense of thermodynamics, it is very slow under ordinary conditions. The rate is accelerated, however, by such catalysts as aqueous Fe^{3+} and I^{-} ions. An enzyme called *catalase* is a biological catalyst for this reaction. This protein (Ebbing/Gammon, Chapter 25) is such an effective catalyst that 1 mg will cause the same rate of decomposition of hydrogen peroxide as 2 kg of Fe^{3+} ions.

Catalase, a protein with a molecular mass of about 5.4×10^5 g/mol, contains a heme group like the one found in hemoglobin. It occurs in such diverse things as mammalian liver, potatoes, and baker's yeast. Baker's yeast will be used in this experiment.

Purpose

During this experiment, you will measure the amount of oxygen that is released during the catalyzed decomposition of hydrogen peroxide. This measurement will allow you to calculate the original concentration of the hydrogen peroxide.

Concept of the experiment

The quantity of oxygen that is evolved will allow you to determine the molarity of the solution of hydrogen peroxide (H_2O_2). To do so, you will need to calculate the number of moles of gas by using the volume of the evolved gas, the atmospheric pressure, Dalton's law of partial pressures (you will be collecting the gas over water), the temperature of the gas, and the ideal gas law.

When you use Dalton's law of partial pressures, you will need the vapor pressure of water. The vapor pressure of water at various temperatures can be found in the table in the experiment "The Decomposition of Potassium Chlorate."

Procedure

Getting started

1. Work with a partner.
2. Obtain a 10-mL transfer pipet, a thermometer, a vial (fitted with a short length of copper wire), a 250-mL Erlenmeyer flask, a 500-mL Erlenmeyer flask, a 400-mL beaker, two stoppers with attached plastic tubing, and a screw pinch clamp.
3. Obtain about 25 mL of the H_2O_2 solution whose concentration is to be determined. You also will need a small quantity of baker's yeast.
4. Obtain and record the atmospheric pressure.

Assembling the apparatus

1. Use the diagram in Figure 1.1 as a guide in assembling the apparatus. Use the 250-mL Erlenmeyer flask as flask A, the 500-mL Erlenmeyer flask as flask B, and the 400-mL beaker as beaker D. Put a pinch clamp near the beaker end of tube C.
2. Clamp flask A to a ring stand.
3. Add 400 mL of water to flask B and 300 mL of water to beaker D. Mark the water level in flask B with a marking pencil.
4. Place the entire apparatus at least 1 ft from the edge of the laboratory bench to minimize the chance of knocking the apparatus off the bench.
5. Fill tube C with water. To do so, remove the stopper from flask A and loosen the pinch clamp on tube C. Draw water into the tube from the beaker by using a rubber suction bulb on the glass tube that was in flask A. If air bubbles remain in the tube, they may be removed by siphoning water back and forth from flask B to beaker D. Siphoning will occur when the flask and the beaker are alternately raised and lowered. Do *not* try to siphon by sucking on the tube with your mouth.

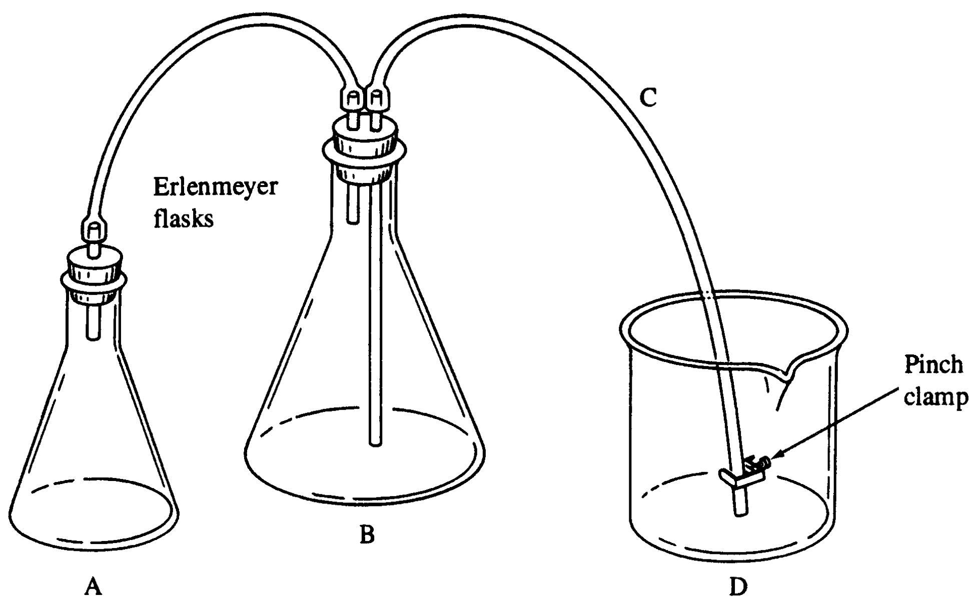


FIGURE 1.1
The arrangement of glassware and tubing.

6. Use siphoning to bring the water level in flask B back to the 400-mL mark that you placed on the flask. Tighten the pinch clamp while the water is at that level.

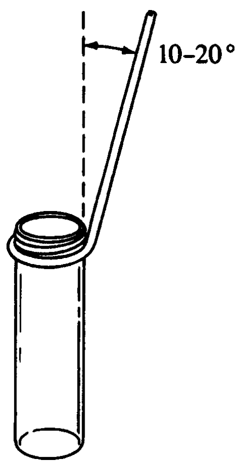
Testing for leaks

1. Return the stopper to flask A, putting it *firmly* in place, and open the pinch clamp.
2. Raise beaker D. If water flows continuously from the beaker to flask B, there is a leak in your apparatus. Make sure the stoppers in the flasks are pushed in as much as possible. If necessary, ask your laboratory instructor for help.
3. When your apparatus is free of leaks, bring the water level in flask B back to the 400-mL mark that you made.
4. Tighten the pinch clamp.

Setting up the vial

1. Angle the copper wire attached to the vial so that it is about 10°–20° from a vertical position, as shown in Figure 1.2. The wire will prevent the vial from tipping over when the vial is in flask A. However, the bent wire will allow the vial to tip slightly so that a solution in flask A can enter the vial when the flask is tilted.
2. Remove the stopper from flask A and add 135 mL of water.
3. To test the position of the wire, use crucible tongs to lower the vial gently into flask A until the *upright* vial rests on the bottom of the flask. Use the tongs to tip the vial until the wire touches the wall of the flask. The mouth of the vial should be above the water level in the upright flask. When the flask is tilted, water should flow into the vial if the wire has been bent properly.

FIGURE 1.2
The vial and the attached piece of copper wire.



4. Once the wire is positioned properly, remove the vial from the flask, discard the water in the vial and the flask, and dry the interior and exterior of the vial.

Doing the reaction

1. Pipet 10.0 mL of the solution of H_2O_2 into flask A.
2. Add 125 mL of distilled water to flask A from a graduated cylinder. Swirl gently to obtain a homogeneous solution.
3. Using a metal spatula, add baker's yeast to the vial to a depth of about 1/4 inch. Wipe the exterior of the vial carefully to remove any of the yeast that may be there.
4. Make sure that flask A is clamped to the ring stand.
5. Using crucible tongs, lower the vial gently into flask A until the *upright* vial rests on the bottom of the flask.
6. Return the stopper to flask A and recheck the system for leaks with the method that you used earlier.
7. Adjust the pressure inside the apparatus to atmospheric pressure with the following procedure. Open the pinch clamp. Raise beaker D until the water level in that beaker is exactly equal to that in flask B. Water will siphon into or out of the beaker until the internal and external pressures are equal. Close the clamp tightly.

8. Pour all of the water out of beaker D, but do not dry the beaker. Place the end of tube C (still filled with water) back in the beaker.
9. Keeping the pinch clamp closed for the moment, remove flask A from its clamp, and tilt the flask carefully so that the solution in the flask flows into the vial. Do not allow the solution to touch the rubber stopper.
10. Gas evolution will begin immediately. After about 1 min, loosen the pinch clamp. Water will be forced from flask B to beaker D. Agitate flask A gently and continuously until no more effervescence occurs.
11. Clamp the flask to the ring stand and allow another 5 min for the heat generated by the reaction, if any, to dissipate.
12. Adjust the pressure inside the apparatus to atmospheric pressure, using the method in Step 7.
13. Loosen the stopper in flask B, insert the thermometer into the flask, and measure the temperature of the gas (not the water). The temperature of the gas should agree with the temperature of the water in beaker D to within 2°C. If it does not, more time should have been allowed for the heat of the reaction to dissipate. Record the temperature of the gas to the nearest degree.
14. Carefully measure the volume of the water in the beaker using a 1-L graduated cylinder. Record the result.
15. Discard the solution in flask A, rinse the flask and the vial, dry the interior and exterior of the vial, and replace the water in flask B and beaker D.
16. Repeat Steps 1 through 14. If the volumes of the evolved gas in the two trials differ by more than 10 mL, another trial is necessary.

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Date:	....	Student name:	....
Course:	....	Team members:	....
Section:	....		....
Instructor:	....		....

Prelaboratory assignment

1. a. What chemical reaction will occur during this experiment?

   b. What quantities must be measured in this experiment?

2. a. Why is it necessary to equalize the water levels in flask B and beaker D before you measure the volume of displaced water?

   b. Just before you allow the reaction to begin, you are asked to discard the water in beaker D. Why are you forbidden to dry the beaker?

3. A quantity of O_2 is collected over water at 23°C and an atmospheric pressure of 744 mmHg. What is the pressure of the gas?

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Results

Atmospheric pressure (mmHg): ....

Trial	**1**	**2**	**3**
Volume of H_2O_2 solution (mL)	....	....	....
Temperature of the gas (°C)	....	....	....
Volume of water displaced (mL)	....	....	....
Vapor pressure (mmHg)	....	....	....
Pressure of the gas (mmHg)	....	....	....

Questions

1. a. Calculate the number of moles of gas that evolved in each trial.

b. Using the mean number of moles of the gas, calculate the molarity of the H_2O_2 solution.

2. The solutions of hydrogen peroxide that we can purchase in a pharmacy or a supermarket are 3% H_2O_2 by mass. Using any reasonable assumption that you wish, calculate the percentage of H_2O_2 by mass in the solution that you used in this experiment. Make sure that you state your assumption.