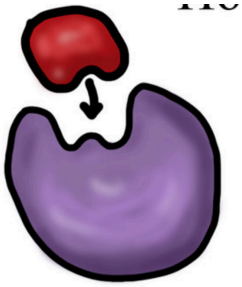
LAB: Investigating Enzyme Catalase : Determining the Rate of Reaction Using a Titration Method

BACKGROUND:

Enzymes are proteins produced by living cells, they act as catalysts in biochemical reactions.

 The Substrate is Hydrogen Peroxide. H_2O_2



The substrate (reactant) moves toward the enzyme's active site.

An example of an enzyme is a protein called **catalase**. It is shown as the purple structure above. Its shape is very important and unique. It's active site, location where the substrate fits is specific.

One function of catalase within cells is to prevent the accumulation of toxic levels of hydrogen peroxide (H_2O_2) formed as a by-product of metabolic processes.

The chemical reaction is triggered by the enzyme.

The enzyme releases the products.

The primary reaction catalyzed by catalase is the decomposition of H2O2 to form water and oxygen. Shown in the diagram above as blue and green.

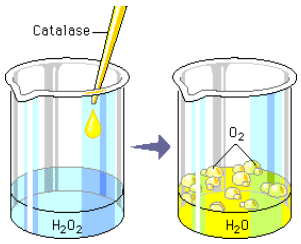
The reaction is shown below

2 H2O2 ----> 2 H2O + O2 (gas)

This reaction does occur spontaneously, but not at a very rapid rate. The enzyme catalase speeds up the reaction considerably.

INTRODUCTION:

In this investigation we will consider this enzyme and determine the rate of its activity. When you add the enzyme to hydrogen peroxide as shown in the diagram below you can see that the reaction rate is increased because the bubbles forming show the production of oxygen.



PREPARATION OF CATALASE:

We will need to extract the enzyme catalase from liver tissue for this investigation.

Catalase enzymes obtained from beef or chicken liver will also require the additional materials of a 250 mL beaker, cheesecloth, rubber band, and a **mortar** and **pestle**. The catalase solution is prepared as follows:

• Place a piece of fresh liver approximately $1\ cm^3$ in **size** in a mortar with **10 mL** of distilled water and grind (using a pestle) for **one minute**.

• Pour the solution through two layers of cheesecloth fixed (by rubber band) on the top of a 250 mL beaker.

• **Add 20 mL of distilled water to the macerated liver again in the mortar.** Pour this solution through the cheesecloth into the beaker used previously.

OVERVIEW:

In this experiment, a rate for this reaction will be determined.

The rate of a chemical reaction may be studied by **measuring the rate of disappearance of substrate; in this example, H2O2**

The rate is the amount of substrate used up over time.

Details:

In this experiment the disappearance of the substrate, H2O2, is measured as follows:

1. A catalase extract is mixed with substrate (H2O2) in a beaker. The enzyme catalyzes the conversion of H2O2 to H2O and O2(gas).

2. Before all of the H2O2 is converted to H2O and O2, the reaction is **stopped** by adding sulfuric acid (H2SO4).

The H2SO4 lowers the pH, denatures the enzyme, and thereby stops the enzyme's catalytic activity. The sulfuric acid is the ”Stop Solution”.

3. After the reaction is stopped, **the amount of substrate (H2O2) remaining in the beaker is measured.**

Potassium permanganate solution

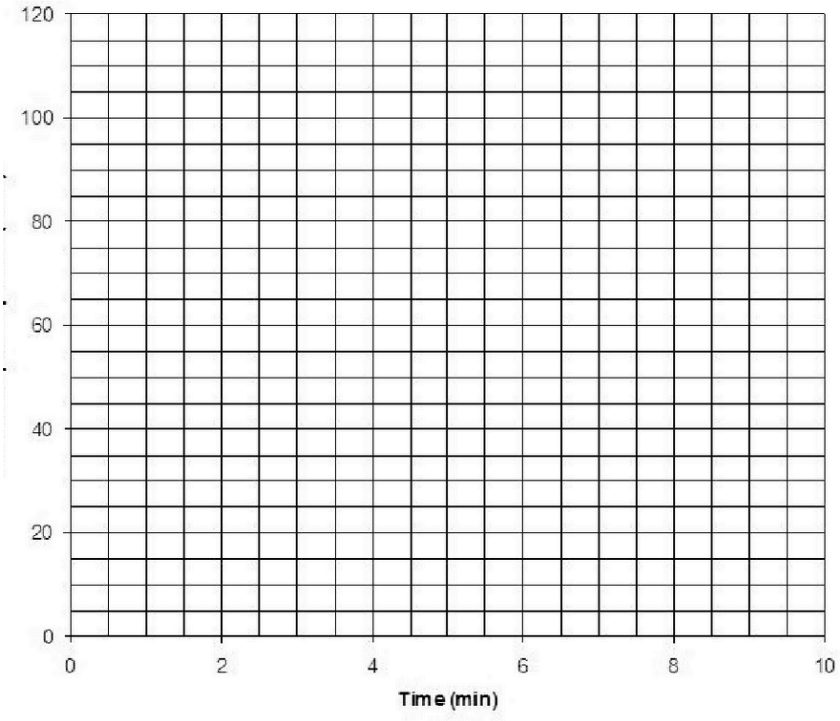
To assay this quantity, potassium permanganate is used.

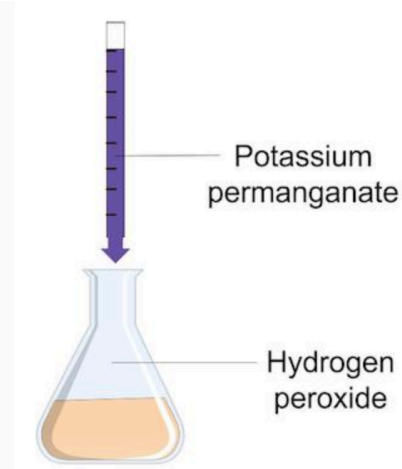
Potassium permanganate (KMnO4), in the presence of H2O2 and H2SO4, reacts as follows:

5 H2O2 + 2 KMnO4 + 3 H2SO4 **---->** K2SO4 + 2 MnSO4 + 8 H2O + 5 O2

Once the baseline amount is calculated you can subtract the remaining amount to determine the amount of H2O2 **used** up over time as a measure of rate.

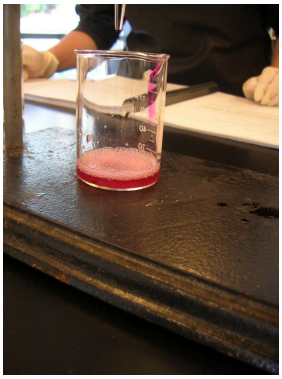
amount of H2O2 **used** up



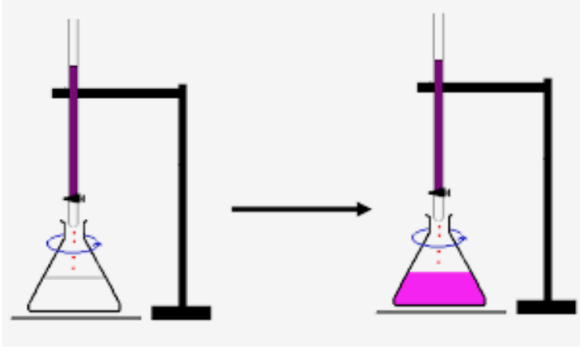


H2O2 is essential for this reaction. Once all the H2O2 has reacted, any more KMnO4 added will be in excess and will not be decomposed. The addition of excess KMnO4 causes the solution to have a **permanent pink** or **brown color**.

Therefore, the amount of H2O2 remaining is determined by adding KMnO4 until the KMnO4 color no longer disappears and the whole mixture stays a faint pink or brown, permanently. Add no more KMnO4 after this point.



The amount of KMnO4 added is a proportional measure of the amount of H2O2 remaining.



ESTABLISHING THE BASELINE

To determine the amount of H2O2 initially present in a 1.5% solution, one needs to perform all the steps of the procedure **without** adding catalase (enzyme) to the reaction mixture.

This amount is known as the **baseline** and is an index of the initial concentration of H2O2 in solution. In any series of experiments, a baseline should be established first.

PROCEDURE (Methods) FOR ESTABLISHING BASELINE

First thing is to pour 50 ml of 3% H2O2 in a 250 mL beaker and then add 50 ml of distilled water to dilute to a 1.5%.

1. Pour 10 mL of 1.5% H2O2 in a 250 mL beaker.

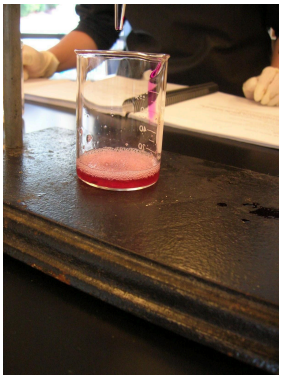
2. Pipet 1 mL using a p1,000 of H2O (instead of enzyme solution).

3. Pour 10 mL of H2SO4 Acid (5M). Using a graduated cylinder. *USE EXTREME CARE IN HANDLING ACIDS.*

4. Mix well.

5. **Remove 5 mL** and put into a small beaker and determine the amount of H2O2 present.

Use a burette to add KMnO4 a drop at a time to the solution until a persistent pink or brown color is obtained. Remember to gently swirl the solution after adding each drop. Check to be sure that you understand the calibrations on the burette.



THE ENZYME CATALYZED RATE OF H2O2 DECOMPOSITION

In this experiment, you will determine the rate at which a 1.5% H2O2 solution decomposes when catalyzed by the purified catalase extract.

To do this, you should determine how much H2O2 has been consumed after **10, 30, 60, 120, and 180 seconds**.

It is recommended that you run all of your experiments at the different time intervals and then at the completion, titrate them all at the same time.

<u>10 seconds</u>

1. Put 10 mL of 1.5% H2O2 in a 250-mL glass beaker.
2. Pipet 1 mL of catalase extract.
3. Swirl gently for 10 seconds.

4. At 10 seconds, Add 10 mL of H_2SO_4 (5M). Using a graduated cylinder. *USE EXTREME CARE IN HANDLING ACIDS.*

30 seconds

1. Put 10 mL of 1.5% H_2O_2 in a 250-mL glass beaker.

2. Pipet 1 mL of catalase extract.

3. Swirl gently for 30 seconds.

4. At 30 seconds, Add 10 mL of H_2SO_4 (5M). Using a graduated cylinder. *USE EXTREME CARE IN HANDLING ACIDS.*

60, 120, and 180 seconds

The methods are the same for these times. Each time, repeat steps 1 through 4, as above, except allow the reactions to proceed for 60, 120, and 180 seconds, respectively.

Each time, remove 5 mL from the beaker and transfer it to a new beaker so that you can titrate for the amount of H_2O_2 remaining.

Using a burette, to add $KMnO_4$, a drop at a time, to the solution until a persistent pink or brown color is obtained. Should the end point be overshot, there is sufficient sample left to repeat the titration.

THE ENZYME-CATALYZED RATE OF H_2O_2 DECOMPOSITION

Determine the **rate of the reaction (0 to 10)** and the rates between each of the time points.

$$\frac{\Delta Y}{\Delta X}$$

10-30 sec
30-60 sec
60-120 sec
120-180 sec
180-360 sec

Record the rates in your data table.
Units for reaction rate (mL of H_2O_2/sec)