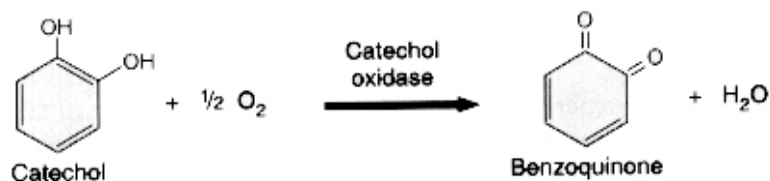


Enzyme Activity

Enzymes act as catalysts for the chemical reactions that occur in living organisms, allowing reactions to occur in the milliseconds necessary to maintain life. Chemically, enzymes are proteins. Each specific enzyme has a unique physical structure that is essential for its function. The shape of each specific enzyme "fits" the shape of the reacting molecule(s) for which the enzyme serves as a catalyst. Because of the enzyme "fit", the reacting molecules are brought together at the appropriate bonding sites. The enzyme, therefore, "lowers" the activation energy of the chemical reaction.

In a reaction catalyzed by an enzyme, the reacting molecules are called the **substrate**. The substrate molecules combine with the active site of the enzyme forming a temporary complex called the **enzyme-substrate complex**. As the chemical reaction takes place and the **products** are formed, the enzyme is released, unchanged from its original structure. Since the enzyme is not consumed or changed by the chemical reaction, it can be used over and over to catalyze additional substrate molecules.

In this laboratory you will observe the activity of the enzyme, catechol oxidase, an enzyme which catalyzes the conversion of the chemical catechol to a brown pigmented substance, benzoquinone. Catechol oxidase is found in the cells of many organisms. You have probably observed this reaction a number of times when you have cut potatoes or many fruits and left them out on the counter. They turn a rusty or brownish color. The potato is a good source for this enzyme, and we will obtain our enzyme from potatoes for this laboratory.



Since catechol is colorless and the product, benzoquinone, is a rusty-brown color, the chemical reaction is easy to detect. In addition, the intensity of the pigment produced is a reflection of the amount of catechol that is converted. This, in turn, can tell you how effectively the catalyst, catechol oxidase, has worked under a set of experimental conditions.

Materials Required For the Entire Class:

Hot plates

Hot water bath at 40°C

Hot water bath at 60°C

Stock buffer solutions, with pipette assembly, of:

pH 2

pH 4

pH 6

pH 7

pH 8

Ice

Stock bottle of pH7 Buffer – kept cold

Stock bottle of distilled water – kept cold

Test tubes (17 per group)

Test tube racks (3 per group)

Culture dishes with crushed ice for the potato juice-extract (the catechol oxidase source)

Medicine droppers

Test tube clamps

China markers (grease pencil)
600-ml Beakers (3 per group)
Thermometers
Dropper Bottles of Catechol (**Note: Poison!**)
50-ml beaker for 35 ml of potato juice extract (the catechol oxidase source)
250-ml beaker for cold distilled water

Catechol is a poison. Use caution handling this chemical. Avoid contact with the solution. If a spill occurs, immediately wipe up the spill with dry paper towels and then use disinfectant solution on the spill site. Be sure to notify your instructor of the spill and your clean-up procedures.

Materials Required to Prepare the Catechol Oxidase Extract (For the Class):

2 potatoes, peeled and cut into chunks
1 Blender
700 ml cold distilled water
1 1000-ml beaker
1 Large funnel
Several layers of cheesecloth

Preparation of the Catechol Oxidase Extract (This will be done in advance or by the lab staff, your instructor or by student volunteers)

- Peel and chunk the potatoes. Put the peeled and chunked potatoes in the blender. Add 700 ml of cold distilled water and blend at high speed for 2 minutes.
- Line the large funnel with several layers of cheesecloth and place the funnel in the 1000 ml beaker, which has been placed in a container of ice. Filter the potato juice through the beaker
- The filtrate is your potato juice-catechol oxidase extract. It will be referred to simply as "catechol oxidase" throughout the exercises.

Note: Potatoes also contain catechol, so you will need to keep the catechol oxidase extract on ice at all times during the laboratory to retard any natural chemical reaction which might occur.

Establishing a Reference Reaction.

Since you are testing conditions that affect the activity of the enzyme, catechol oxidase, you will need to determine, qualitatively, the reaction that occurs when you perform your experiments. To provide you with an idea of what to expect, you can establish a reference reaction for comparison purposes throughout the exercises. For this lab a reference standard can be established by performing a catechol oxidase-catechol reaction with no additional experimental conditions. You will also set up a control for a negative reaction standard.

Please note that the hue of the benzoquinone is not critical; you are interested in the intensity of the color. Some of the other chemicals used in the set of exercises may affect the color of the benzoquinone some of the time, but not the intensity. Do not be concerned when this occurs.

Procedure for the reference reaction

1. Label one test tube catechol oxidase and a second test tube water
2. Fill both tubes 1/4 full of distilled water
3. Add 60 drops of catechol to both tubes
4. Add 60 drops of catechol oxidase (potato juice) to just the catechol oxidase tube
5. Agitate both test tubes

6. After 5 minutes, shake both tubes again and observe the results.
 - The intensity of the pigment in the catechol oxidase tube will be your reference standard for the maximum reaction. You will call this intensity a “5”
 - The intensity of the pigmentation (colorless) in the water tube will be your control. This intensity will be a “0”.

For the following exercises, you will use a graded intensity scale of 0 (no reaction) → 5 (maximum reaction) to measure the activity of the enzyme, catechol oxidase.

Exercise I: The Effect of Temperature on Enzymatic Activity

Within limits, the rate of a chemical reaction mediated by an enzyme will increase as the temperature increases. However, enzymes are proteins and high temperatures denature proteins. The maximum enzyme activity occurs at the temperature just below the point where the enzyme is denatured. At temperatures above this point, enzyme activity declines rapidly since the shape of the denatured protein has been altered. It is probably not just a coincidence that the optimum temperature for the enzymes of many living organisms is about 38°C.

In this exercise you will determine the effect of temperature on the activity of the enzyme, catechol oxidase at five different temperatures (ice water, room temperature, 40°C, 60°C and Boiling water).

Setting up the experimental temperature conditions

1. There are two hot water bathes set up in the room:
 - One set at 40°C
 - One set at 60°C
2. Set up a boiling water bath using a 600-ml beaker and one of the hot plates located on the side counters. When the water boils, turn the hot plate down to a “simmer”.
3. Fill a 600-ml beaker with crushed ice. Add water.
4. Fill a 600-ml beaker with room temperature water (about 25°C)

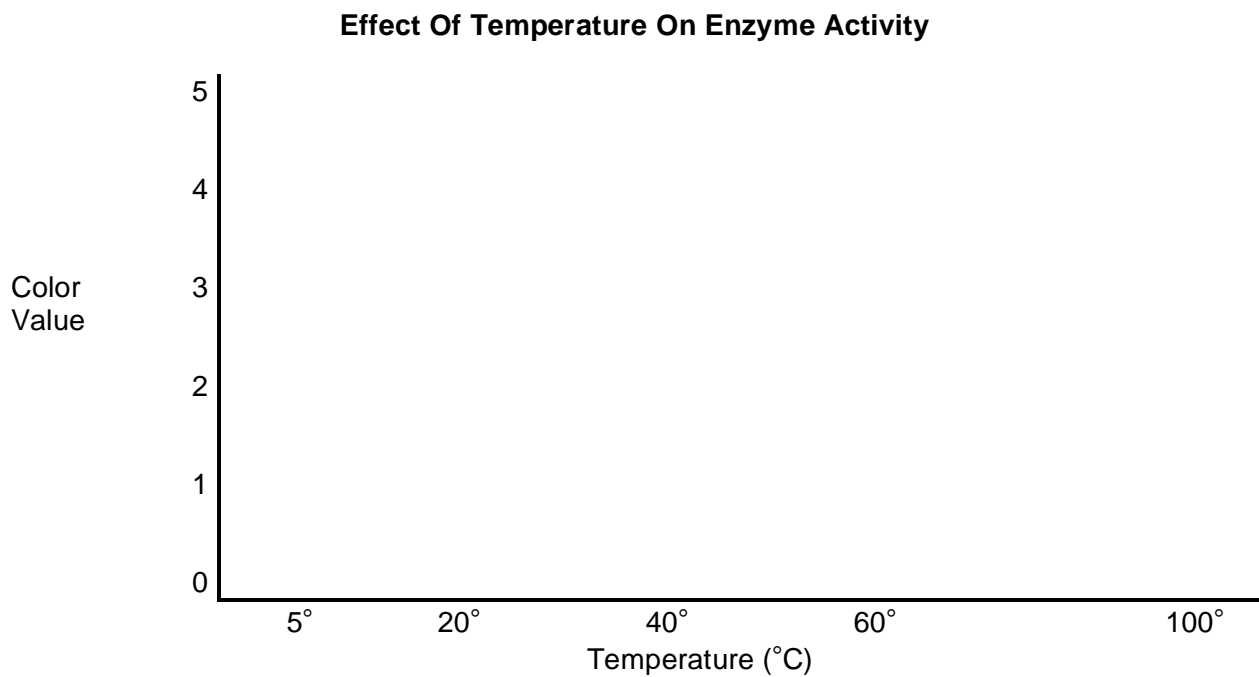
Procedure

1. Label 5 test tubes 1 through 5.
2. Fill each tube 1/4 full of distilled water
3. Add 10 drops of catechol oxidase (potato juice) to each test tube.
4. Put Tube 1 in the ice water.
Put Tube 2 in the beaker of room temperature water.
Put Tube 3 in the 40°C hot water bath.
Put Tube 4 in the 60°C hot water bath.
Put Tube 5 in the boiling water.
5. Incubate the tubes at the designated temperatures for 10 minutes. This is to bring the tubes to the appropriate temperature. **Do not collect any data yet.**
4. After incubating the tubes for 10 minutes, add 10 drops of catechol to each test tube and continue to incubate the tubes at the designated temperatures.
5. At 5-minute intervals shake the tubes and record the color intensity (on a scale of 0 → 5) for each of the 5 tubes in the table below. Do your readings for a total of 20 minutes.

Do not bring your test tubes back to your laboratory table for the observations. They should remain at the experimental temperature until you have completed your 20 minute reading.

Time (min)	Temperature in °C (intensity from 0 → 5)				
	Ice Water	Room Temp	40	60	100
0					
5					
10					
15					
20					

Plot the data for your 20-minute results of the effect of temperature on the activity of catechol oxidase on the following graph.



What, according to your data, is the optimum temperature for the enzyme, catechol oxidase?

How did the 100°C condition affect the enzyme, catechol oxidase?

Exercise II: The Effect of Enzyme Concentration on Reaction Rate

When the substrate (in this case, catechol) is present in sufficient quantity so that it does not limit the reaction, the rate of the chemical reaction is usually directly proportional to the concentration of the enzyme (in this case, catechol oxidase).

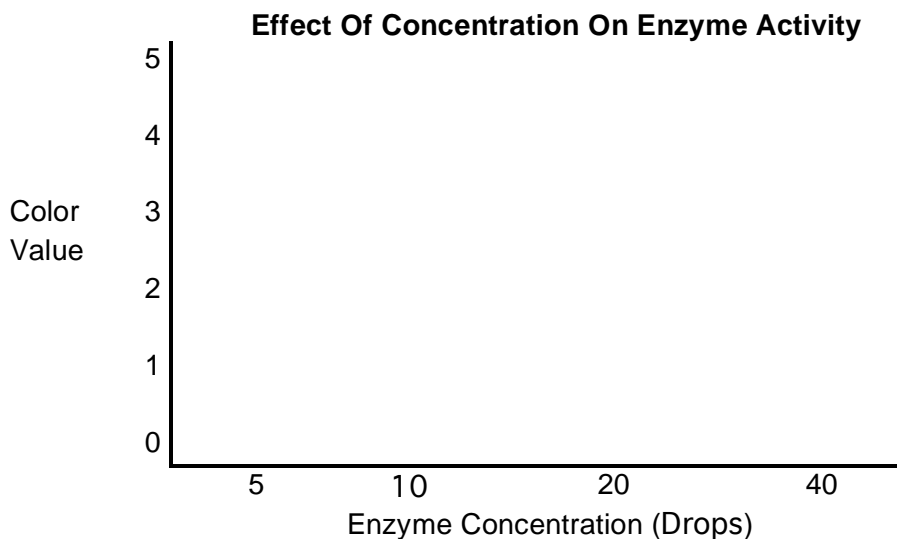
Procedure

1. Label 4 test tubes 1 through 4.
2. Fill each tube 1/4 full of distilled water.
3. Add the following amounts of catechol oxidase to the 4 test tubes.
 - Add 5 drops of catechol oxidase to tube 1
 - Add 10 drops of catechol oxidase to tube 2
 - Add 20 drops of catechol oxidase to tube 3
 - Add 40 drops of catechol oxidase to tube 4
4. Add 10 drops of catechol to each tube and agitate.
5. Record the intensity for each tube in the table below. This will be time "0".
6. At 5 minute intervals shake and record the color intensity (on a scale of 0 → 5) for each of your 4 test tubes. Do readings for 20 minutes.

Time (min)	Concentration (intensity from 0 → 5)			
	5 drops	10 drops	20 drops	40 drops
0				
5				
10				
15				
20				

Explain the results you have recorded above, with reference to the effect of concentration on enzyme activity.

Plot the data for your 20-minute results of the effect of concentration on the activity of catechol oxidase on the following graph.



Does the concentration of the enzyme affect the overall reaction or does the concentration affect just the rate at which the reaction proceeds? In other words, given enough time would all of your experimental tubes reach the same intensity (the overall reaction)?

Exercise III: The Effect of pH on Enzyme Activity

The hydrogen ion concentration (pH) of a solution can affect an enzyme's three dimensional structure in much the same way as high temperatures can, thereby affecting the ability of an enzyme to function. Most enzymes function best near neutral pH ranges. Some enzymes, like those of the human stomach, require an acid pH to function, while others function in basic pH ranges.

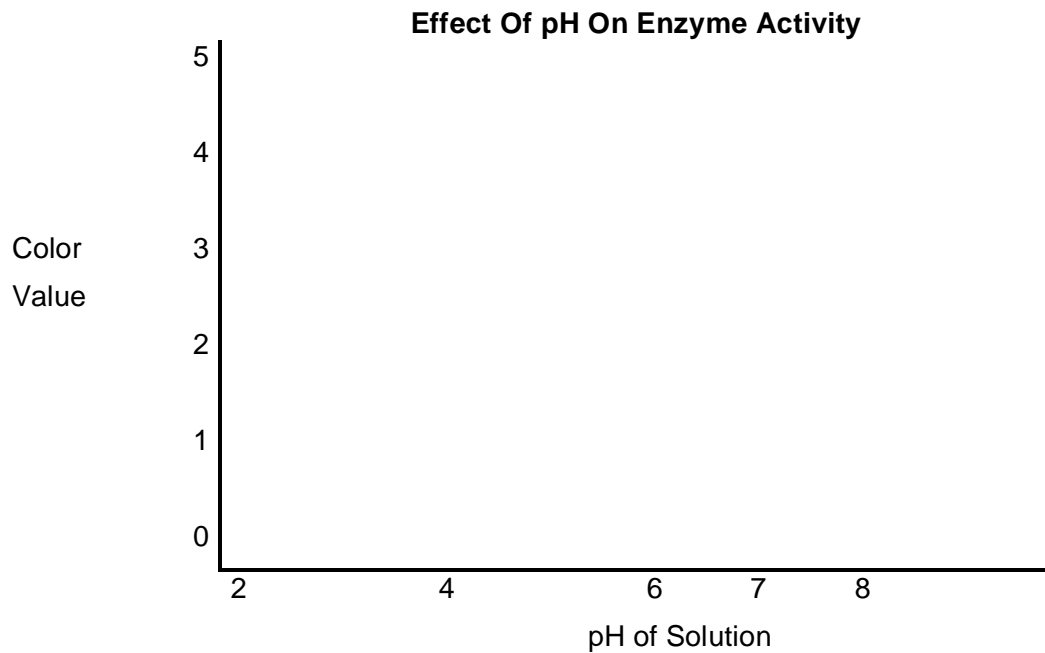
This exercise will help you to determine the optimum pH for the enzyme, catechol oxidase, as well as illustrating the effect that the pH of a solution has on the enzyme.

Procedure

1. Label 5 test tubes 1 through 5.
2. Take the numbered test tubes in a test tube rack to the stock table where the buffer solutions are located. Each buffer solution should have a 10-ml pipette with a pipette pump attached; do not mouth pipette. Use only the designated pipette for each of the buffer solutions to avoid contaminating the buffers. Add the buffer solutions to the proper tubes and return to your lab table.
3. Add the buffers in the following way:
 - Fill tube 1 1/4 full with the pH 2 buffer solution.
 - Fill tube 2 1/4 full with the pH 4 buffer solution.
 - Fill tube 3 1/4 full with the pH 6 buffer solution.
 - Fill tube 4 1/4 full with the pH 7 buffer solution.
 - Fill tube 5 1/4 full with the pH 8 buffer solution.
4. Return to your lab table with the buffer-filled test tubes.
5. Add 10 drops of catechol oxidase to each of the 5 test tubes.
6. Now add 10 drops of catechol to each of the 5 test tubes and shake each tube.
7. Record the color intensity (on a scale of 0 → 5) for each of the tubes. This is time "0".
8. Continue to shake the tubes and record the color intensity at 5 minute intervals for 20 minutes.

Time (min)	Intensity from 0 → 5				
	Tube Number (pH of tube in parentheses)				
	1 (2)	2 (4)	3 (6)	4 (7)	5 (8)
0					
5					
10					
15					
20					

Plot the data for your results for the 20 minute time interval on the following graph.



What is the optimum pH for the enzyme, catechol oxidase?

What do you think the optimum pH would be for pepsin, an enzyme found in your stomach?

When you have completed all of the exercises, rinse out your test tubes and put them in the "used test tube" container. Clean any other glassware and put it in its proper location. Return all of your remaining supplies to the designated locations and clean your table top thoroughly.