EXERCISE 6 Name ____________________________

What factors affect enzyme activity?

Objectives

After completing this exercise, you should be able to:

- Define and correctly use the following terms: enzyme, substrate, product, active site, enzyme activity, cofactor, enzyme progress curve, enzyme saturation, V_{max}
- Describe how the following factors affect enzyme activity: substrate concentration, temperature, pH, substrate specificity, and cofactors.

Prelab

Before you come to lab, read this entire exercise. You must also answer all questions and complete all assignments on the first 6 pages of this exercise. Your instructor will give you directions on when and where to turn in your work.

An enzyme is an organic molecule (usually a protein) that acts to speed up a specific chemical reaction. Without the necessary enzyme, most reactions within the cell take place so slowly as to be insignificant. Therefore, by controlling which enzymes are made, the DNA of a cell essentially controls which chemical reactions take place.

Enzymes speed up reactions by binding to reacting molecules, called **substrates**, to form an **enzyme-substrate complex**. The enzyme-substrate complex lines up the substrates in an orientation that stresses or distorts existing chemical bonds, leading to formation of the transition state. In the transition state, the substrates become more reactive and the metabolic reaction accelerates. The site of attachment and the surrounding parts of the enzyme that stress the substrate's bonds constitute the enzyme's **active site**. The reaction is complete when the **product** forms and the enzyme is released in its original condition. The enzyme is then ready to repeat the process with new substrate molecules.

Although enzymes speed up chemical reactions, each enzyme works most efficiently (causes the reaction to occur fastest) under a specific set of conditions. Because almost all enzymes are proteins, any factor that affects the shape of a protein may affect enzyme activity. For example, at high temperatures, proteins **denature**, or lose their normal shape. Also, the pH of a solution will affect the charge of acidic and basic amino acid side chains on a protein, affecting the interactions that lead to tertiary and quaternary protein structures. In addition to factors that affect protein shape, factors that affect substrate availability can also affect reaction rate. For example, as substrate concentration increases, the reaction rate also tends to increase. This is because the enzyme's active site is more likely to be occupied as substrate concentration increases. However, increasing substrate concentration cannot cause the reaction rate to increase indefinitely. Eventually, a point is reached where all available enzymes are saturated and working at maximum efficiency. Therefore, any additional increase in substrate concentration will not produce a corresponding increase in reaction rate. It is at this state of enzyme saturation that the maximum enzyme activity is observed, often referred to as V_{max}, for the enzyme's maximum velocity.

In this lab you will analyze the effects of substrate concentration, temperature, pH, substrate identity, and the presence or absence of cofactors on enzyme activity. One enzyme you will study is **glucose oxidase**. This enzyme can be commercially prepared and is routinely used to determine blood glucose levels. Abnormal levels of glucose in the bloodstream may be an indication of a variety of disorders, such as diabetes, hypoglycemia, insulin overdose, liver disease, or diseases of the thyroid or adrenal glands. The other enzyme that you will study is **catecholase**. **Catecholase** is found in many fruits and vegetables, and is responsible for the "browning" that occurs when these fruits and vegetables are cut or bruised.

Perform calculations for preparing solutions with different concentrations of glucose

The first enzyme you will study in lab is glucose oxidase. One of the substrates for glucose oxidase is glucose. In order to test how substrate concentration affects glucose oxidase activity, you will need to prepare 6 solutions containing different concentrations of glucose.

Your turn

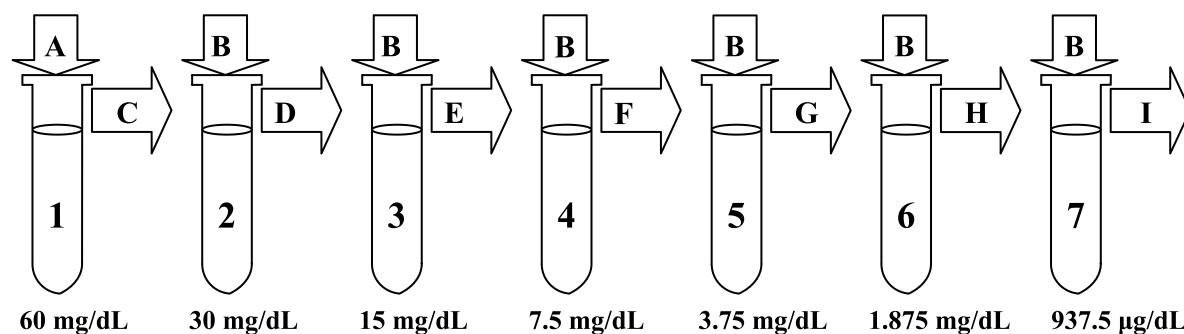
Starting with a stock solution containing 60 mg/dL of glucose, and using dH_2O as your diluting solution, you need to make 3 mL of each of the following glucose solutions: 30 mg/dL, 15 mg/dL, 7.5 mg/dL, 3.75 mg/dL, 1.875 mg/dL, and 937.5 µg/dL. Fill in the information below to show how you would prepare these solutions using a serial dilution. (Note: If you forgot how to do the calculations for a serial dilution, please consult the Prelab for Exercise # 2.)

What is df for this dilution series? _______________

What is V_2 for this dilution series? _______________

What is V_1 for this dilution series? _______________

Label the arrows in the diagram below **and in the diagram on page 7 of the Lab Procedures** to show how you would make the required solutions. Above each vertical arrow, write down the volume and type of liquid you will place in the tube (e.g. 13 mL of stock solution or 9 mL of dH_2O.) Below each horizontal arrow, write down how much solution you will transfer from one tube to the next (e.g. 5 mL):



Review how to determine and plot a best-fit line using linear regression analysis

In lab, you will examine several factors that affect the rate of the chemical reaction catalyzed by glucose oxidase. In this reaction, glucose, water and oxygen are the substrates; while gluconic acid and hydrogen peroxide are the products:

$$\text{Glucose} + \tfrac{1}{2} O_2 + H_2O \rightarrow \text{Gluconic Acid} + H_2O_2$$

If we want to know how fast this chemical reaction is occurring, we could measure how fast the substrates of the enzyme are used up, or we could measure how fast the products are formed. In your experiments, you will measure how quickly one of the products - hydrogen peroxide (H_2O_2) - is formed. To enable you do this, 2 compounds have been added to the glucose oxidase enzyme: 4-aminoantipyrine and phenol. When these 2 compounds react with hydrogen peroxide, a pink dye is formed:

H_2O_2 (***colorless***) + 4-aminoantipyrine + phenol → pink dye

Therefore, the faster hydrogen peroxide is formed, the faster the solution will turn pink.

Explain why the production of hydrogen peroxide (which is colorless) turns the reaction mixture pink:

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To estimate the rate of the reaction, you could simply eyeball how fast the reaction mixture turns pink. However, this would not be very accurate. A much more accurate way to measure the rate of the reaction involves using a spectrophotometer. A spectrophotometer (the Spec-20 in our lab) measures how much light of a specific wavelength is absorbed by a solution (**optical absorbance**). It turns out that the wavelength of light most strongly absorbed by the pink dye is 510 nm. As hydrogen peroxide is formed, the concentration of pink dye in the reaction mixture will increase, the pink color will get darker, and the optical absorbance at 510 nm (A_{510} values) will increase. Therefore, we can measure the rate of the reaction catalyzed by glucose oxidase by measuring how fast the A_{510} values of the reaction mixture increase. Once you have collected your data, you will analyze the relationship between time and A_{510} values using linear regression.

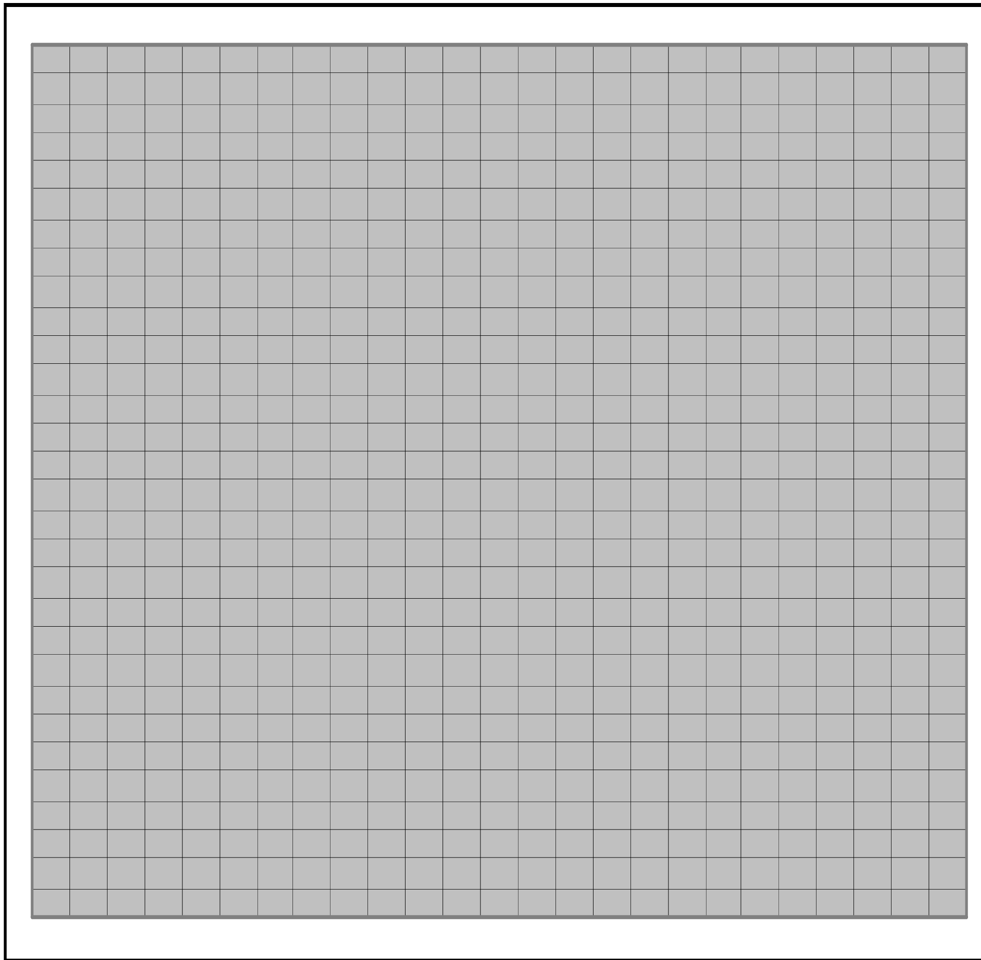
Your Turn

Using the data in the following table, plot a scatter diagram on the grid provided on the next page. Consult the "Graphing Check List" on p. 18 of the Prelab for Exercise #2 to make sure you have included all necessary information on your graph.

A_{510} values for a mixture of glucose oxidase, 4-aminoantipyrine, phenol, and glucose at selected time intervals

Time elapsed	Absorbance at 510 nm
0 sec	0
15 sec.	.085
30 sec.	.168
45 sec.	.256
60 sec.	.311
90 sec.	.505
2 min.	.565
3 min.	.602

⇒ **Note: Absorbance has no units. Also note that the time units in this table are not consistent. When you plot your graph, all time measurements must be listed using the same units (i.e. all in seconds or all in minutes.)**



Your Turn

Examine your scatter diagram and try to visualize the smooth line that would most closely match the 8 data points. This line is called an "**enzyme progress curve**". The **slope** of the line at any given point is the rate of the reaction, and is a measure of "**enzyme activity**". Notice that this line would have a steeper slope during the early time intervals (up to about 90 seconds), but would gradually "flatten out" as you move further towards the right side of the graph. Can you explain why this curve eventually flattens out over time?

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Actually, this is a fairly typical result when comparing two variables in a biological experiment. Often there is a linear (i.e. straight line) relationship between the variables when the independent variable has low and/or moderate values. But this relationship may "break down" as we approach extremely high (or in some cases extremely low) values of the independent variable, causing the "best fit" curve to "flatten out" (or in some cases to steepen). Therefore, although we could try to fit a straight line to all of the data points on our scatter diagram, we should look for signs that the linear relationship is "breaking down" **at the extreme ends of the curve**. In our example, because the best-fit line "flattens out" with the last two data points, you should fit a straight line to the **first six data points only**. It is only in this region where a true linear relationship exists.

Your Turn

Circle the six data points on your graph that lie in the region where time and A_{510} values appear to have a linear relationship (i.e. the points in the region before the line begins to "flatten out"). Using a calculator or computer, carry out linear regression to determine the best-fit straight line for these six data points. If you forgot how to carry out linear regression, consult the Prelab for Exercise #2.

Your regression analysis will give you the equation for the straight line that best fits your data. The general equation for a straight line can be written as follows:

$$y = mx + b$$

(Where m = the **slope** of the line, and b = the **y-intercept**)

In order to determine the equation of the straight line that best fits your data, you must determine its **slope** and **y-intercept** and then substitute these values into the equation above.

Linear correlation coefficient for your data ____________________

Based on the linear correlation coefficient, should you conclude that time and A510 values were linearly related during the first 90 seconds of the reaction? ______________________

Fill in the following values. **Be sure to use the correct units of measurement**.

Slope _______________ y-intercept ____________

Equation for this line ______________________________

Do not erase memory!

The linear regression equation tells you the exact mathematical relationship between the 2 variables, x and y. Therefore, if you know the value of one variable, you can substitute it into the equation and calculate the corresponding value of the other variable.

In addition, once you know the equation for the "best fit" straight line, it is fairly easy to plot the line on your scatter diagram. A spreadsheet program, such as Excel, can automatically plot the "trendline" for you. However, for this prelab you should plot the "best-fit straight line" on your scatter diagram using the following procedure:

1. Pick any 2 "x" values that lie within the region where your data indicate a linear relationship exists between the 2 variables (for your graph, this would be any time values between 0 and 90 seconds). It is usually best to pick values near the 2 extremes of the linear region.

2. For each x value, use the linear regression equation to calculate the corresponding y value. This will give you the coordinates for 2 points that lie on the linear regression line. (Note: If the data points for the regression line are stored in the calculator's memory, some calculators can automatically calculate the corresponding y value for any given x value and vice versa. This can save you some work. Detailed instructions on how to do this with the TI-36X calculator are on p. 4 of Appendix B.)

3. Now, plot these two points on your scatter diagram on the previous page. Use open circles or a different color when plotting these points so that you can visually distinguish these 2 points from the data points that you plotted earlier.

4. Finally, plot the best-fit line by drawing a straight line through the two points that you just added to your scatter diagram. Your line should extend within the region where your data suggest a linear relationship exists (i.e. between 0 and 90 seconds.)

Lab Procedures

I. Determine the effect of substrate concentration on enzyme activity.

⇒ **Be sure you do not confuse the enzyme (glucose oxidase) with the substrate (glucose)!**

1. Turn on the Spec-20 and set the wavelength to 510 nm. The Spec-20 must warm up for at least 15 minutes before it will give accurate measurements.

2. Examine the solutions you have been given. One should be labeled “ENZYME: Glucose Oxidase.” This is the ENZYME that will catalyze the reaction you will observe. Another solution should be labeled “GLUCOSE: Stock Solution (60 mg/dL).” This is one of the substrates for the reaction you will observe. Be very careful to use the correct solution for each part of the lab exercise.

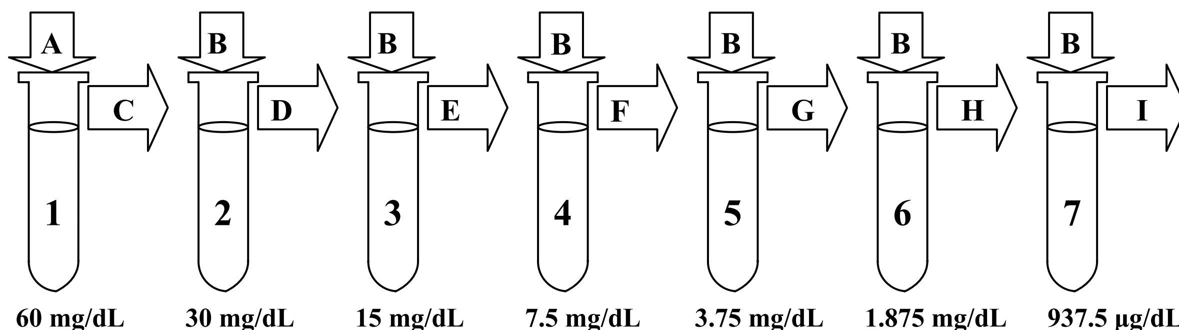
3. Using labeling tape and a Sharpie, label one 5 mL pipette “dH_2O” and another 5 mL pipette “Glucose.” Be very careful to use the “dH_2O” pipette only with distilled water and the “Glucose” pipette only with the glucose stock solution. Do not cross-contaminate any of your solutions.

⇒ **It is critical that the enzyme solution not be contaminated with glucose and that the glucose solution not be contaminated with enzyme! Contamination usually occurs because the same pipette has been used to measure both solutions or the tip of the pipettor has touched the other solution while dispensing. If you have accidentally cross-contaminated your solutions, or if either solution appears pink while in its original container, give the contaminated solution to your instructor and obtain uncontaminated solutions before you continue the activity. To avoid contaminating your enzyme solution with glucose, use only a micropipettor and clean tips to dispense the enzyme solution.**

4. Using labeling tape and a Sharpie, label 8 clean Spec-20 cuvettes as follows: label one cuvette “B” (for “blank”) and label the remaining 7 cuvettes with the numbers 1-7. When labeling Spec-20 cuvettes, never write on the cuvette! Use a small piece of label tape affixed to the top of the cuvette (so that it will not interfere with the spectrophotometer reading).

5. Add 3.0 mL of dH_20 (using the “dH_2O” pipet) and 2.0 mL of the enzyme (using a 1000 µL micropipettor with a clean tip) to the Spec-20 cuvette labeled “B" and set it aside. Later, this cuvette will be used to calibrate the Spec-20. (If this cuvette turns pink, it probably means that your blank cuvette has been contaminated with glucose and must be prepared again. You must locate the source of your contamination immediately to avoid making the same mistake again before continuing with the experiment.)

6. Starting with the stock solution containing 60 mg/dL of glucose, and using dH_2O as your diluting solution, you need to make 3 mL of each of the following glucose solutions: 30 mg/dL, 15 mg/dL, 7.5 mg/dL, 3.75 mg/dL, 1.875 mg/dL, and 937.5 µg/dL. As part of the Prelab, you were supposed to label the diagram below to show how you would prepare these solutions using the serial dilution technique. Ask your instructor to check your diagram before you proceed



7. Prepare your serial dilution of glucose in the Spec-20 cuvettes numbered 1 – 7 as follows:

   A. Use the pipette labeled "Glucose" to place the correct amount (v1 + v2) of stock solution in tube 1.
   B. Use the pipette labeled "dH_2O" to place the correct amount (v2) of dH_2O in tubes 2 - 7.
   C. Use the pipette labeled "Glucose" to transfer the correct amount (v1) of solution from tube 1 to tube 2.
   D. Mix the contents of tube 2 and then use a clean pipette to transfer the correct amount (v1) of solution from tube 2 to tube 3.
   E. Mix the contents of tube 3 and then use a clean pipette to transfer the correct amount (v1) of solution from tube 3 to tube 4.
   F. Mix the contents of tube 4 and then use a clean pipette to transfer the correct amount (v1) of solution from tube 4 to tube 5.
   G. Mix the contents of tube 5 and then use a clean pipette to transfer the correct amount (v1) of solution from tube 5 to tube 6.
   H. Mix the contents of tube 6 and then use a clean pipette to transfer the correct amount (v1) of solution from tube 6 to tube 7.
   I. Mix the contents of tube 7 and then use a clean pipette to discard the correct amount (v1) of solution from tube 7.

8. Decide which student in your group will be the timekeeper, which student will write down the absorbance levels measured (the note-taker), which student will add glucose oxidase enzyme to the Spec-20 cuvettes, and which student will operate the Spec-20. As you watch the readout while each tube is in the Spec-20, it will be constantly changing, so careful coordination in your group is necessary. The timekeeper will call out the times, the operator of the Spec-20 will call out the readings, and the note-taker will record the data. The note-taker must prepare an appropriately labeled table in his lab notebook to record the data, and this data must be transferred to every lab partner's notebook before starting Part II of the procedures.

9. Zero the Spec-20 following the instructions for the Spec-20 or the Spec-20D. Use the cuvette labeled "B", which you prepared in step 5, as your blank.

   Note: In the next step, the timekeeper should note the exact time that the glucose oxidase enzyme is added to the glucose. This is time zero. The first reading is at 15 seconds, so you have 15 seconds to mix the solutions, wipe off the cuvette with a Kimwipe, and place the cuvette in the Spec-20. You will be adding enzyme only to the diluted glucose solutions in cuvettes 2 – 7, not to the stock solution in cuvette # 1.

10. Add 2.0 mL of glucose oxidase enzyme to cuvette # 2 ONLY, and note the time. DO NOT add enzyme to more than one cuvette at a time! Quickly mix the glucose and enzyme solutions by sealing the end of the cuvette with Parafilm and gently inverting it 2-3 times.

11. After cleaning the cuvette with a Kimwipe to remove any fingerprints or spills, immediately place the cuvette into the Spec-20. Record the A_{510} values in your lab notebook every 15 seconds for 2 minutes, using a table such as the one below:

A_{510} Readings after Adding 2 mL of Glucose Oxidase to Varying Concentrations of Glucose

Cuvette	Glucose Concentration (mg/dL)	15 s	30s	45s	60 s	75 s	90 s	105 s	120 s
2									
3									
4									
5									
6									
7									

12. Repeat the procedure with the remaining 5 cuvettes (# 3 - 7).

13. Before disposing of your solutions, ask your instructor to check your absorbance measurements for plausibility.

14. Thoroughly clean your cuvettes by rinsing them several times with tap water and then with dH_2O. Place the cuvettes upside down in a test tube rack for later use.
    ⇒ **Remember: Never use abrasive cleansers or brushes when cleaning Spec-20 cuvettes. Rinse them with tap water and dH_20 only!!!!**

II. Determine the effect of temperature on enzyme activity.

1. Label four clean cuvettes 1 - 4 with labeling tape and a Sharpie. Add 2.0 mL dH20 and 1.0 mL of stock glucose solution to each cuvette. Place cuvette #1 in the ice bucket, #2 in the rack on your bench, #3 in the 37°C water bath, and #4 in the 65°C water bath to equilibrate the temperatures.

2. Check the thermometer in the lab to determine the lab room temperature for the cuvette left on your lab bench. Enter the temperature of each of the cuvettes in a clearly labeled table.

3. Label four test tubes (**not** Spec-20 cuvettes!) as follows: "ice", "room temperature", "37°C", "65°C". Add 2.0 mL of glucose oxidase enzyme solution to each of the four test tubes. Place the appropriate tube in the ice bucket, in a test tube rack on your lab bench, in a 37°C water bath, and in a 65°C water bath. Note the time and be ready to mix the enzyme tubes and the substrate tubes together after about 5 minutes.

4. Re-zero the Spec-20 using your “B” cuvette. Before proceeding, make sure all of the solutions have been in the ice bath/test tube rack/water baths for at least 5 minutes to allow them to reach the temperature of their surroundings. While waiting, you can be labeling beakers for Part III and/or planning and preparing for your experiment for Part IV. The note-taker can also prepare appropriately labeled tables for recording the data from this procedure. Also, don’t forget that this data must be transferred to every lab partner’s notebook before starting Part III of the procedures!

5. Pour the 2.0 mL of the cold glucose oxidase enzyme from the tube in the ice bucket into the cuvette # 1, which is also in the ice bucket. Cover with Parafilm, gently invert the cuvette several times, and return it to the ice. Note the time when the glucose oxidase enzyme was added and prepare to take absorbance readings every 15 seconds.

6. Keep cuvette #1 in the ice until about 10 sec. have elapsed since the enzyme was added. Then remove the cuvette and wipe it thoroughly with a Kimwipe (this will require more care than usual because condensation will tend to form on the outside of the cuvette). Quickly insert it into the Spec-20 and measure the absorbance at 15 sec. after the enzyme was added, as you did in the enzyme assay in Part I, above. (If you miss the 15 sec. mark, record the exact time with your absorbance and continue.)

7. Return the cuvette to the ice bucket. Remove the cuvette, dry it thoroughly, and measure the absorbance at 30, 45, 60, 75, 90, 105, and 120 seconds. Try to keep the cuvette on ice as much as is practical between measurements. Hold the cuvette by the top to avoid warming it with your hands.

8. Repeat this process with the other 3 cuvettes. The room temperature cuvette may remain in the Spec-20 until all of its measurements are made. Return the other two cuvettes to their respective water baths between readings. (If this is awkward, fill a beaker with the incubation water and bring the beaker to the Spec-20 during your measurements---the temperature will not deviate significantly over the time period that you will be taking your measurements.) Make sure you dry the cuvettes thoroughly before placing them in the Spec-20!

9. Thoroughly clean your cuvettes by rinsing them several times with tap water and then with dH_2O. Place the cuvettes upside down in a test tube rack for later use.
    ⇒ **Remember! Never use abrasive cleansers or brushes when cleaning Spec-20 cuvettes. Rinse them with tap water and dH_20 only!!!!**

III. Determine the effect of pH on enzyme activity

1. You have been provided with **4 unknown buffers labeled #1 - 4**. Label 4 clean cuvettes #1 - 4 and put 2.0 mL of the appropriate buffer in each cuvette using clean pipets to avoid cross-contamination.

2. Add 1.0 mL of glucose stock solution to each of the 4 cuvettes, seal with Parafilm, and mix by inverting the cuvette several times.

3. Use your reagent blank in cuvette "B" to re-zero the Spec-20.

4. After zeroing the Spec-20 with the "B" cuvette, add 2.0 mL of glucose oxidase enzyme to one of the 4 cuvettes containing buffered glucose solution, and note the time. Quickly seal the cuvette with Parafilm and mix the buffered glucose and enzyme solutions by covering the cuvette with Parafilm and inverting it 2-3 times. Wipe off the cuvette with a Kimwipe and place it into the Spec-20. Record the absorbance at 15, 30, 45, 60, 75, 90, 105, and 120 seconds in an appropriately labeled table in your lab notebook.

5. Repeat step 4 with the 3 remaining cuvettes and record your data.

6. Calibrate a pH meter and measure the pH of the reaction mixtures in cuvettes #1-4. If your pH probe will not fit into the cuvettes, pour some of each reaction mixture into a small beaker and take your readings. Record the pH values in your notebook.

7. Thoroughly clean your cuvettes by rinsing them several times with tap water and then with dH_2O. Place the cuvettes upside down in a test tube rack for later use.
   ⇒ **Remember! Never use abrasive cleansers or brushes when cleaning Spec-20 cuvettes. Rinse them with tap water and dH_20 only!!!!**

IV. Determine the specificity of the enzyme-substrate interaction

Galactose and mannose are isomers of glucose. All three of these monosaccharides have the formula $C_6H_{12}0_6$, but the arrangement of the atoms in each sugar differs.

So far, you have used glucose as the substrate for the reaction catalyzed by glucose oxidase. For this part of the lab, you have been provided with mannose and galactose solutions in the same concentration as the glucose stock solution. Design and carry out an experiment to determine if mannose and/or galactose can also act as substrates for the glucose oxidase enzyme.

V. Identify the cofactors required by the enzyme catacholase

In the first 4 parts of this lab, you examined how glucose oxidase activity is affected by 4 different variables: substrate concentration, temperature, pH, and substrate identity. Another variable that affects the activity of some enzymes is the presence of cofactors. A cofactor is a nonprotein substance that must be present in order for an enzyme to function normally. For example, some metal ions function as cofactors by helping to draw electrons away from substrate molecules, thereby making it easier for the reaction to proceed. When a cofactor is a nonprotein organic molecule, it is called a coenzyme. Many vitamins are parts of coenzymes.

Because plants are unable to physically escape from attacking pathogens, many rely on "chemical warfare" for protection. **Catecholase** is an enzyme that acts to protect many plants from pathogens. Catecholase catalyzes a reaction between catechol and O_2 (the substrates of this reaction.) As a result of the reaction, benzoquinone and water (the products of the reaction) are formed. Both catechol and catecholase are found in many plant cells. However, the reaction occurs only if the plant cells are damaged, which releases the catechol and catecholase and brings them into contact with the oxygen in the air. The benzoquinone that is formed during this reaction works as an antiseptic, protecting the plant against pathogens.

In this part of the lab, you will study the reaction catalyzed by catecholase in a test tube. As benzoquinone molecules are produced, they will react with each other to form long-chain brown pigments. The faster the reaction occurs, the faster benzoquinone will be produced, and the faster the reaction mixture will turn brown.

The source of catecholase in this experiment is a crude potato extract. Included in this extract are all the solutes of the potato cell, including cations such as Ca^{2+} , Mg^{2+} , and Cu^{2+} . These cations frequently serve as cofactors for a variety of enzymes. Chelators such as **ethylene diamine tetraacetic acid (EDTA)** and **phenylthiourea (PTU)** will be added to the reaction mixture in some test tubes to remove specific cations from solution. EDTA preferentially removes Ca^{2+} and Mg^{2+}, while PTU preferentially removes Cu^{2+}.

This part of the lab activity will be prepared as a demo by the instructor.

Instructor:

Label five test tubes and add: **Add Last:**

tube #	dH_2O (mL)	potato juice (mL)	EDTA (mL)	PTU (mL)	1% catechol (mL)
1	2.0	1.0	--	--	--
2	1.0	1.0	--	--	1.0
3	2.0	--	--	--	1.0
4	--	1.0	1.0	--	1.0
5	--	1.0	--	1.0	1.0.

Cover the tubes with Parafilm and mix by holding the Parafilm firmly in place with your thumb and inverting the tube a few times. This will ensure that the potato juice comes in contact with both oxygen and catechol.

Students:

Observe the 5 demonstration tubes on display in lab. Make an appropriately labeled table in your lab notebook to record your observations. Your table should show the contents of each tube, as well as the relative intensity of the brown color in each tube.

Clean up

Cleaning the Spec-20 cuvettes: Spec-20 cuvettes are not ordinary test tubes. They are expensive, and great care must be taken to avoid scratching them. Scratches interfere with the passage of light through the tube and that can lead to inaccurate results. Rinse the cuvettes thoroughly with tap water and then with dH_2O.

Clean your glassware.

All disposable glassware goes into the special glass disposal receptacle.

All instruments should be turned off and unplugged.

Wipe off your workspace with a damp paper towel.

Make sure everything that you have used is clean, put away, or discarded. Leave your work area in the same order that you found it in.

Ask your instructor to check your work area before you leave.

Postlab

Part I

1. On a sheet of graph paper, plot a scatter diagram showing how the A_{510} values changed over time when glucose oxidase was added to the solution containing 30 mg/dL of glucose. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis.

2. Now, on the same graph, plot scatter diagrams showing how the A_{510} values changed over time when the enzyme was mixed with the 5 other diluted glucose solutions. Use points of different shapes (e.g. triangles, squares) or colors to distinguish between the different substrate concentrations. Make sure that your axes are clearly labeled and include units of measurement.

3. Examine the graph you just prepared (containing the 6 scatter diagrams). For each substrate concentration, determine the time interval where the relationship between A_{510} values and time appears to be linear. Circle those points. Use linear regression to determine the equation for the best-fit straight line. This line is called the "**enzyme progress curve**". The **slope** of the line tells you the rate of the reaction, and is a measure of "**enzyme activity**". Finally, prepare a table like the one below and fill in the required information.

Substrate Concentration (mg Glucose/dL)	Equation for "best fit" straight line	Correlation Coefficient (r)	Slope of line, or enzyme activity (ΔA_{510} /sec.)
___________	_______________________	_______	_______________
___________	_______________________	_______	_______________
___________	_______________________	_______	_______________
___________	_______________________	_______	_______________
___________	_______________________	_______	_______________
___________	_______________________	_______	_______________

4. Plot the six best-fit lines on your graph. Label each line or include a figure legend to indicate the actual glucose (substrate) concentrations in mg/dL. Title your graph "Progress Curves for Glucose Oxidase at Different Substrate Concentrations".

5. On a second sheet of graph paper, prepare a scatter diagram showing how enzyme activity (the slopes of your progress curves) varies with substrate concentration. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis. Label the axes appropriately, including the units of measurement, and title your figure.

6. Note that the slope of each enzyme **progress curve** is a measure of the rate at which the product accumulates over time (i.e. the slope equals change in concentration of product divided by change in time). This rate is referred to **enzyme activity.** How does the slope of the enzyme progress curve change as the substrate concentration changes? What does this indicate about how substrate concentration affects enzyme activity?

7. V_{max} refers to the maximum enzyme activity that is approached as substrate concentration is increased. Estimate V_{max} for your solution of glucose oxidase.

Part II

8. Using an entire sheet of graph paper, plot a scatter diagram of A_{510} values versus time for each temperature tested. Plot the data for all 4 temperatures on the same graph. For each scatter diagram, determine the time interval where the relationship between A_{510} values and time appears to be linear. Circle those points and use linear regression to determine the equation of the "best fit" straight line for these points. Plot the 4 "best fit" straight lines on your graph. Label each line or include a key to indicate the temperature at which the enzyme activity was measured.

9. In an appropriately labeled table, record the equation for the "best fit" straight line, the correlation coefficient for this line, and slope of the line for each temperature.

10. On another sheet of graph paper, prepare a scatter diagram showing how enzyme activity (the slopes of your progress curves) varies with temperature. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis. Label the axes appropriately, including the units of measurement, and title your figure.

11. At what temperature does glucose oxidase have the greatest activity? Compare enzyme activity at room temperature (indicate actual room temperature) with enzyme activity at 37 ^{o}C. At which of these temperatures did you observe the most enzyme activity? Explain why you think this was so.

12. Describe the effect that each of the temperature extremes—0 ^{o}C and 65 ^{o}C-- had on enzyme activity. Explain why you think these temperatures would have these effects.

Part III

13. Using an entire sheet of graph paper, plot a scatter diagram of A_{510} values versus time for each pH tested. Plot the data for all 4 pH values on the same graph. For each scatter diagram, determine the time interval where the relationship between A_{510} values and time appears to be linear. Circle those points and use linear regression to determine the equation of the "best fit" straight line for these points. Plot the 4 "best fit" straight lines on your graph. Label each line or include a key to indicate the pH at which the enzyme activity was measured.

14. In an appropriately labeled table, record the equation for the "best fit" straight line, the correlation coefficient for this line, and slope of the line for each pH.

15. On another sheet of graph paper, prepare a scatter diagram showing how enzyme activity (the slopes of your progress curves) varies with pH. Use the entire sheet of paper for your scatter diagram and show the independent variable along the x axis and the dependent variable along the y axis. Label the axes appropriately, including the units of measurement, and title your figure.

16. At what pH does glucose oxidase have the greatest activity? At what pH did the enzyme have the least activity? Explain your results.

Part IV

17. Prepare a table in which you record the observations that you made of the effects of different substrates on the activity of glucose oxidase.

18. Draw the structures of β-D-glucose, β-D-mannose, and β-D-galactose. In what ways are their structures the same? In what ways are their structures different?

19. Based on your observations of the activity of glucose oxidase with these 3 substrates, would you classify this enzyme as highly specific? What do you suppose can cause this level of enzyme specificity?

Part V

20. The potato juice used in part IV contains catecholase (enzyme) and a small amount of catechol (substrate.) Adding additional catechol allows you to see a stronger reaction. Explain the design of this experiment by briefly describing the purpose of each tube (i.e. why it is included in the experiment):

    Tube 1 ____

    Tube 2 ____

    Tube 3 ____

    Tube 4 ____

    Tube 5 ____

21. Based on your results, does catecholase need any cofactors? If so, what cofactor(s) does it require?

22. Based on all the observations you made during this lab, do you think refrigeration could slow the browning of bruised or cut produce? Explain why or why not.

23. Cooks sometimes sprinkle sliced fruits with lemon juice to slow browning of the surface. Based on all the observations you made during this lab, propose a mechanism by which the browning of fresh fruits is delayed by lemon juice.