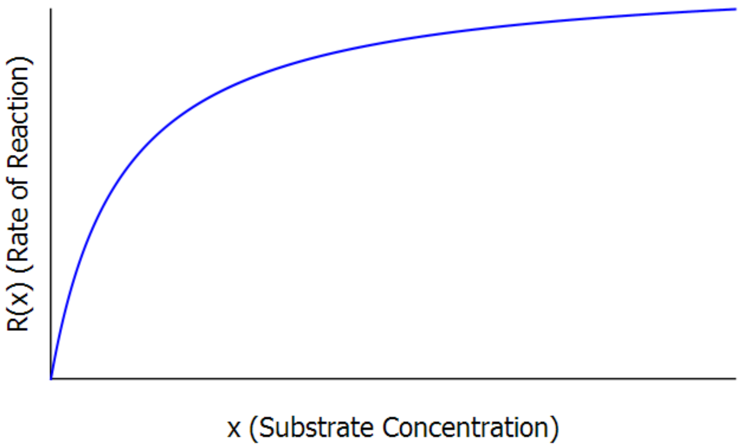
Name: ______________________________ Section: ____________

Collaborators: ____

Lab 6: Enzyme Kinetics

Enzyme kinetics is the study of chemical reactions that are catalyzed by enzymes. Such chemical reactions start with molecules known as **substrates** and convert them into new molecules known as **products**. Protein molecules known as **enzymes** facilitate the conversion of substrate molecules into product molecules. The rate of reaction, $R(x)$, for conversion of substrate to product depends on x, the amount of substrate in the reaction. For many enzyme-catalyzed reactions, the function modeling reaction rate, $R(x)$, has roughly the shape below.



1. For positive values of x, is the derivative $R'(x)$ positive, negative, or zero? Justify your answer.

2. From the graph above, it appears that as x gets large, the function $R(x)$ levels off to approach a horizontal line. What does that tell you about the derivative $R'(x)$ for large x?

3. From the graph on page 1, it appears that when x is small, the function $R(x)$ is closely approximated by a linear function. What does that tell you about the derivative $R'(x)$ for small values of x.

In biochemistry, one of the widely used models for enzyme kinetics is the **Michaelis-Menten kinetics model**. This model gives the rate of conversion $R(x)$ (in millimoles/sec) of substrate to product as a function of x, the amount of substrate in the reaction (in millimoles). The Michaelis-Menten model describes the reaction rate with an equation of the form:

$$R(x) = \frac{Ax}{x + B}$$

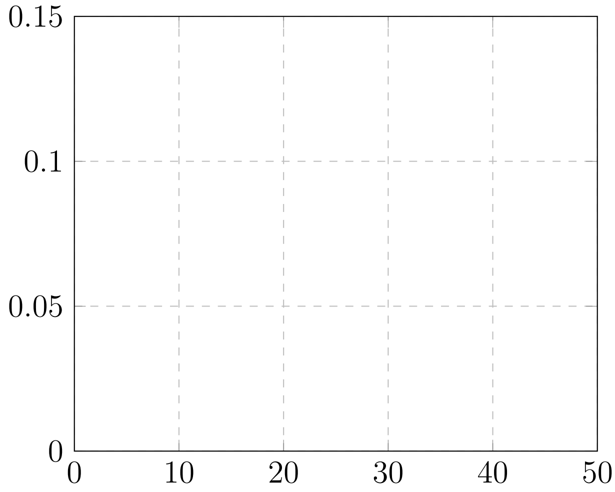
where A and B are fixed positive constants that depend on the particular substrate and enzyme used in the chemical reaction.

In this lab, we will explore the rate of the chemical reaction behind the browning of apples after exposure to air. Substrates in the apple known as *phenols* are converted to dark colored products responsible for browning after catalyzation with the enzyme *o-diphenol oxidase*. Experimentally phenols are converted to products at a rate of

$$R(x) = \frac{0.1x}{x + 1.25}$$

millimoles per second when there are x millimoles of phenols present in the apple.

4. Sketch a graph of $R(x)$ on the domain $0 \leq x \leq 50$, labeling your axes with units.



5. Use the quotient rule to find the derivative function $R'(x)$.

6. As the amount x of substrate in a reaction increases, the reaction rate $R(x)$ also increases. Therefore $R(x)$ is an increasing function, which means its derivative must be positive. Verify, using your answer from question 5, that $R'(x)$ is positive for all $x \geq 0$.

Experiments in enzyme kinetics suggest that when x is large, i.e. the amount of substrate is large relative to the amount of enzyme, then the rate of reaction $R(x)$ is nearly constant. Recall that the derivative of a constant function is 0.

7. Using your equation for $R'(x)$ from problem 5, show that as x gets big, $R'(x)$ approaches zero. This tells us that as x gets big, $R(x)$ is nearly constant.

Above you showed that for large x, the reaction rate $R(x)$ is nearly constant (i.e. the graph of $R(x)$ is close to a horizontal line). You will now find the constant value that $R(x)$ is approaching.

8. Using the function for $R(x)$, complete the following table of values. Round to 4 decimal places.

amount of phenols, x (in mM)	10	100	1000	10000	100000
reaction rate, $R(x) =$ (in mM/s)					

9. Based on the table above, estimate the value of

$$\lim_{x \to \infty} \frac{0.1x}{x + 1.25}.$$

10. Use the rules you have learned for taking limits to evaluate the exact value of this limit:

$$\lim_{x \to \infty} \frac{0.1x}{x + 1.25}$$

The limit you evaluated in question 10 tells us the reaction rate when the amount of substrate is unlimited. Since you showed in question 6 that the function $R(x)$ is always increasing, the reaction rate can never exceed this limiting value. For this reason, the limiting value is known as the **maximal reaction rate**.

11. Find the amount of phenol substrate for which the reaction rate is exactly half of the maximal reaction rate. That is, find x so that $R(x)$ is $1/2$ times your answer from question 10.

Your solutions to questions 10 and 11 are related to the constants A and B from the general Michaelis-Menten kinetics model

$$R(x) = \frac{Ax}{x + B}.$$

The constant A in the Michaelis-Menten model is equal to the maximal reaction rate and the constant B is the amount of substrate for which the reaction rate is half of the maximal reaction rate. In our model $R(x)$:

$$R(x) = \frac{0.1x}{x + 1.25},$$

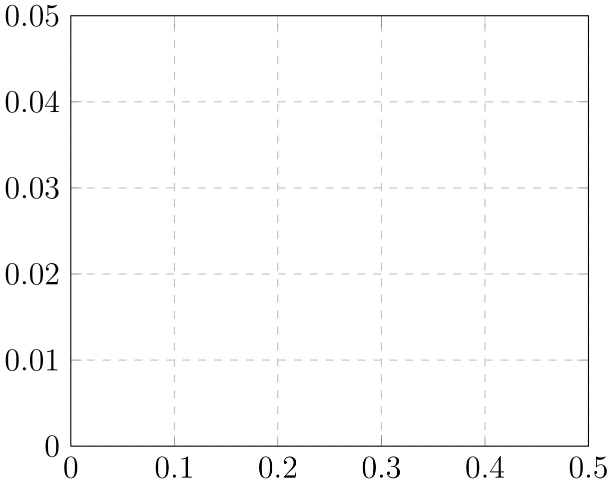
the value of A is 0.1 and B is 1.25. Knowing this, you can check your answers for questions 9 and 10. Did your solutions give the correct values for A and B?

12. Use your equation for $R'(x)$ to complete the following table. Round to three decimal places.

x	0	0.0001	0.001	0.01	.1	1	10
$R'(x)$							

For general reactions, experiments suggest that when x is small, i.e. the amount of substrate is small relative to the amount of enzyme, then the rate of reaction $R(x)$ is nearly linear. Recall that the derivative $R'(a)$ is the slope of the tangent line to the graph of $R(x)$ at $x = a$. From the table you completed in problem 12, these slopes are all approximately .080 when $x = 0, 0.0001$, or 0.001. In other words, when x is very small, the function $R(x)$ looks a lot like the line $f(x) = 0.08x$.

13. On the axes below, draw both $R(x)$ and the line $f(x) = 0.08x$ for $0 \leq x \leq 0.5$.



We have shown that our model of the browning reaction in apples satisfies some basic properties, which are related to the derivative $R'(x)$:

- As the amount of substrate increases, the reaction rate increases (i.e. $R(x)$ is an increasing function, so $R'(x)$ is positive).
- When the amount of substrate is large, the reaction rate is nearly constant (i.e. for x large, $R'(x)$ approaches 0).
- When the amount of substrate is small, the reaction rate is nearly linear (i.e. for small x, $R'(x)$ is nearly constant).

You will now show that these properties always hold for a Michaelis-Menten model.

14. For a general Michaelis-Menten model

$$R(x) = \frac{Ax}{x + B}$$

where A and B are fixed positive constants, find the derivative $R'(x)$.

15. Show that $R(x)$ is an increasing function by explaining why $R'(x)$ is positive for all $x > 0$.

16. Show that when the amount of substrate is large, the reaction rate is nearly constant by showing that for big values of x, $R'(x)$ approaches 0.

17. Show that when the amount of substrate is small, the reaction rate is nearly linear by showing that for small x, $R'(x)$ is nearly constant.

Participation	/5
Correctness	/5
Total	/10