Enzyme Kinetics: Pre-Activity Assignment

Read the sections of your textbook related to enzyme kinetics. **Find** a general chemistry book (or your general chemistry notes) and **review** basic reaction kinetics. Record answers to the Pre-Activity Assignment on your own paper or in an electronic document, using your text as a reference.

1. What is the difference between "K" and "k" in reaction kinetics? Why is it important that we are clear when we write "k" and "K"?

2. Rate laws for reaction mechanisms' elementary steps are based only on the number of substrates. (No reaction orders are necessary.) Write the rate law for a reaction with a single substrate and the rate law for a reaction with two substrates.

3. What is the simplified reaction used by Leonor Michaelis and Maud Menten to describe an enzymatically-catalyzed reaction? (This question is NOT: What is the Michaelis-Menten equation?)

4. What assumptions were Menten and Michaelis making when they used this reaction?

5. Why do enzymologists only look at the initial velocity when studying enzyme reaction rates?

6. G.E. Briggs and J.B.S. Haldane added the steady-state assumption to Michaelis-Menten kinetics in 1925. What is the steady-state assumption and why is it important to our understanding of reaction kinetics?

7. There are typically many, many equations in this section of most Biochemistry textbooks. Which one of them is the actual Michaelis-Menten equation? Write it down.

8. What is the definition of the V_{max} of an enzyme?

9. What is the technical definition of K_m? Give a "practical definition" for this constant.

10. What is the definition of K_d (dissociation constant)? When is the K_m the same as a K_d and when is it not?

11. What is the k_{cat} of an enzyme? What does this tell us?

12. What is the best way to compare catalytic efficiencies of different enzymes?

13. Give an example of an enzyme with a catalytic efficiency near 10^8–10^9 $M^{-1}s^{-1}$. What is the rate limiting step in this type of reaction?

Enzyme Kinetics Activity

Content Learning Objectives

Students will be able to:

- Draw uncatalyzed and catalyzed velocity vs concentration of reactant/substrate graphs
- Determine V_{max} and K_m from Michaelis-Menten graphs and explain what those values tell us and what they don't tell us
- Compare V_{max}, K_m, k_{cat} and k_{cat}/K_m values to determine relative properties of enzymes

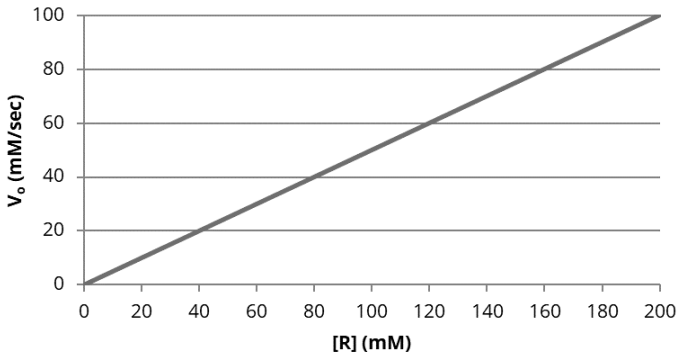
Process Learning Objectives

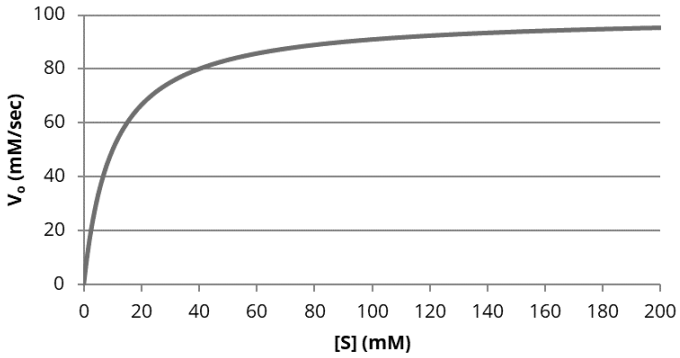
Students will make gains in:

- *Information Processing*: Students will interpret data from graphs.
- *Communication*: Teams should work to ensure that everyone on the team expresses ideas verbally while doing the activity.

Model 1: Two graphs of the velocity of a reaction (y-axis) vs the concentration of the reactant/substrate (x-axis).

The graph on the left is for a non-enzymatically catalyzed reaction (reactant) and the graph on the right is for an enzymatically catalyzed reaction (substrate).

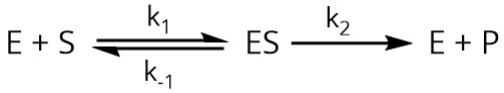




Questions

1. Compare the two graphs in **Model 1**. What is different about the reactions shown in the graphs and why does that make the shapes of the graphs different?

Model 2: The simplified reaction used by Michaelis and Menten to describe an enzymatically-catalyzed reaction.



2. Using your knowledge from General Chemistry and your answers to Pre-Activity Assignment #2 …
   - …write the rate law for the formation of the ES complex

   - …write the rate law for the dissociation of the ES complex

   - …write the rate law for the formation and release of the product

3. You may have written the same thing for the second and third bullet above. The "k" you write in your rate laws is the same as the "k" as shown in **Model 2**. Rewrite the rate laws in the spaces above using the appropriate "k's".

4. Based on what you wrote above, if k_2 is much faster than k_{-1}, will the ES complex result in product?

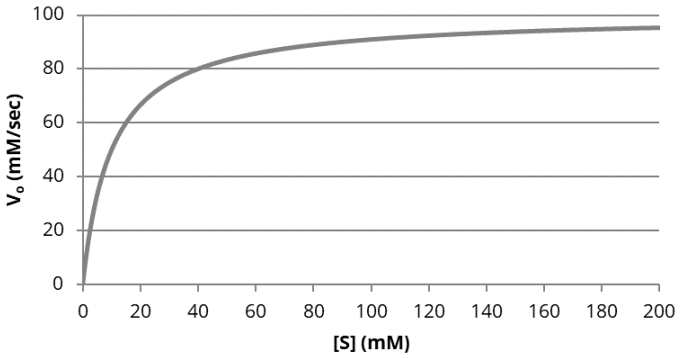
5. What will happen if k_{-1} is much faster than k_2?

We are not going to go through the entire derivation of the Michaelis-Menten equation from the rate laws above to the equation shown below. However, I would like you to be aware that this is how Leonor Michaelis and Maud Menten were able to derive this equation. They were able to start with basic general chemistry principles and use those (with some simplifications) to come up with their equation. This equation was THEN verified experimentally.

6. The Michaelis-Menten equation is shown below. Define each of the symbols in this equation.

$$V_o = \frac{V_{max}[S]}{K_m + [S]}$$

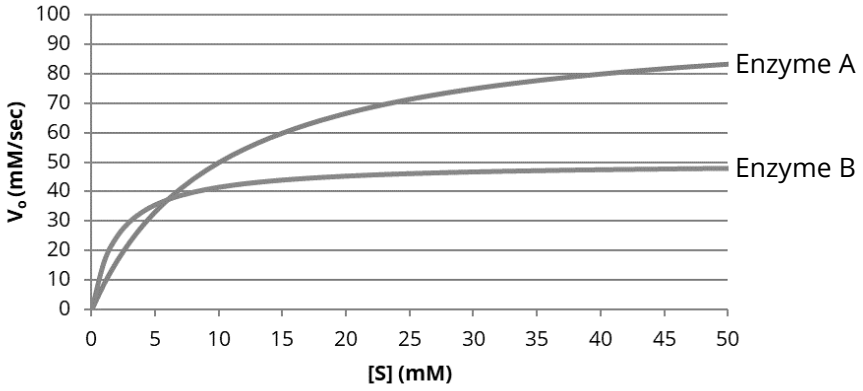
Model 3: Michaelis-Menten Graph



7. The Michaelis-Menten equation can be graphed. When the V_o is plotted vs the [S], the graph seen in **Model 3** is formed. This is called the Michaelis-Menten graph (or plot). **Determine the V_{max}** for the enzyme used to collect the data in **Model 3**. **Mark the V_{max}** on the graph.

8. Use the "practical definition of K_m" (from Pre-Activity Assignment #9) to **determine the K_m** for the enzyme used to collect the data in **Model 3**. **Mark the K_m** on the graph. ***What are the units on K_m?***

Model 4: Two different enzymes had their kinetic parameters measured and graphed.



9. Which enzyme in **Model 4** has the higher V_{max}? What does the relative V_{max} tell you about the enzymes?

10. Which enzyme in **Model 4** has the higher K_m? What does the relative K_m tell you about the enzymes?

Model 5: Equations for K_d and K_m.

The dissociation constant (K_d) for an ES complex is the rate of the dissociation of the complex divided by the rate of the formation of the complex. It DOES not include any conversion of the complex to product. The K_m for an enzyme is the sum of the rates of the disappearance of the ES complex (ES going to E + S or EP) divided by the rate of the formation of the ES complex. In the simplified reaction from Model 2, this is the sum of k_2 and k_{-1} divided by k_1.

$$K_d = \frac{k_{-1}}{k_1} \qquad K_m = \frac{(k_{-1} + k_2)}{k_1}$$

11. Is the K_m of an enzyme the same as the K_d? (*Yes or No?)*

12. Individually, using the equation in **Model 5**, circle your choice from below to fill in the blank in the following sentence:

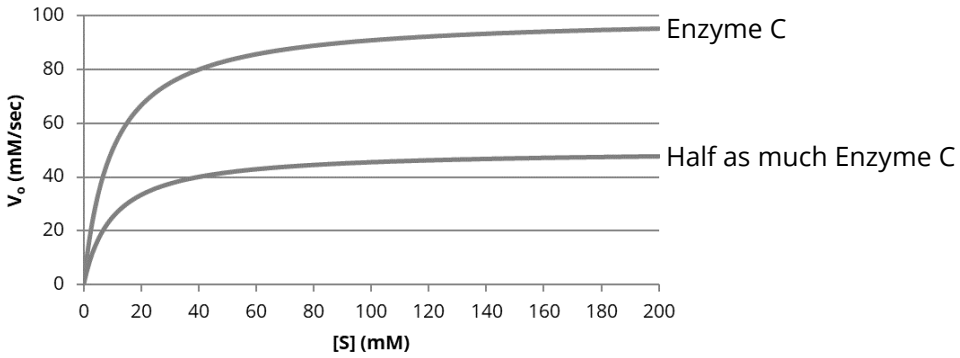
    If k_2 is _____ than k_{-1}, then the K_m becomes k_{-1}/k_1 and is the same as the dissociation constant for the enzyme.

    much greater than *greater than* *the same as* *less than* *much less than*

13. Explain your choice to your team and come to a team consensus of the correct answer.

Model 6: Two Michaelis-Menten plots.

The top line (Enzyme C) is the same as **Model 3**. The experiment that generated the bottom line was the same as the top line, only with HALF as much enzyme.



14. Using the data in **Model 6**, what are the V_{max} and K_m when only half as much enzyme is used? Compare your answers to your answers from **Model 3**. Are they the same?

15. Because of the dependence of V_{max} on the amount of enzyme used in the experiment, enzyme kinetists normally compare the k_{cat} of enzymes instead of the V_{max}. For simple reactions, k_{cat} will be the rate of the rate limiting step. (This is not true for more complicated reaction mechanisms.) The k_{cat} is a first-order rate constant with units of 1/time (or time^{-1}). It is a measure of the number of product molecules produced per unit time. It can be measured by dividing the V_{max} by the total concentration of enzyme. If the concentration of Enzyme C was 40 mM in the first experiment and 20 mM in the second experiment, what is the k_{cat} of the enzyme in **Model 6**?

16. Although k_{cat} is an improvement over V_{max}, to truly compare the efficiency of enzymes you also need to account for how often binding leads to a reaction, which is measured by the K_m of the enzyme. k_{cat}/K_m is called the specificity constant of an enzyme and is the true measure of catalytic efficiency. ***Use the data below to determine the specificity constants.***

Enzyme	k_{cat} (sec^{-1})	K_m (M)	Specificity Constant ($M^{-1}sec^{-1}$)
1	1.4×10^{3}	1.5×10^{-4}	
2	4.0×10^{5}	2.6×10^{-1}	
3	9.7×10^{4}	9.5×10^{-3}	
4	3.8×10^{3}	2.5×10^{-2}	

17. Which enzyme produces the most product per unit time (k_{cat})?

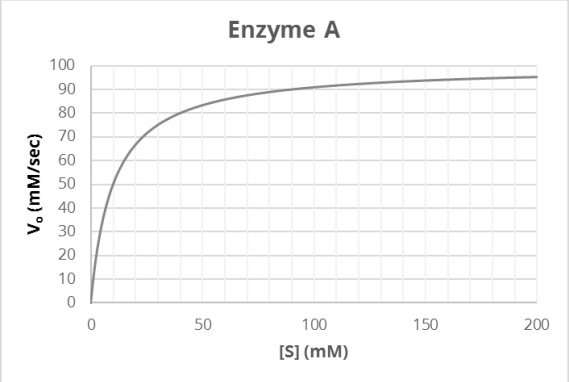
18. Which enzyme likely binds most tightly to its substrate (K_m)?

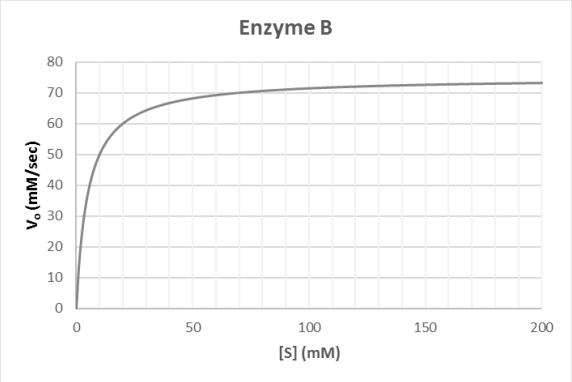
19. Which enzyme is the most catalytically efficient (specificity constant)?

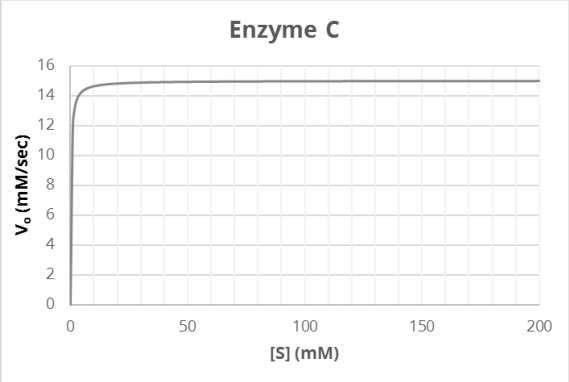
20. Based on your answers to #17-19, what makes an enzyme catalytically efficient?

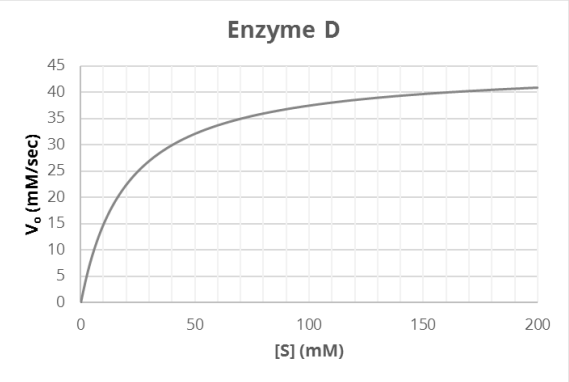
Skill Exercise

Below are Michaelis-Menten graphs for four different enzymes. The concentrations of the enzymes are as follows: Enzyme A = 50 mM; Enzyme B = 50 mM; Enzyme C = 100 mM; Enzyme D = 10 mM.









1. Fill in the following table. Think about how many significant figures you should have and don't forget to include units!

	Enzyme A	Enzyme B	Enzyme C	Enzyme D
V_{max}				
K_m				
k_{cat}				
Specificity Constant				

2. If all four of these enzymes catalyzed the same reaction and you wanted to use this reaction as part of a manufacturing process, which enzyme would you choose to use? **Explain your answer**.