Cheryl Coolidge

<div align="center">Guided Inquiry Activity
Enzyme Kinetics, Part One</div>

Why? Understanding the rate behavior of enzyme catalyzed reactions can help to explain how these biologically important reactions are controlled and regulated. The parameters V_{max} and Km give us important information about the speed and efficiency of enzyme catalyzed reactions.

Model 1 Review of kinetics and rate equations

Consider the reaction A → B. Starting with a 1M concentration of A, the time course of the reaction would appear like the graph below:
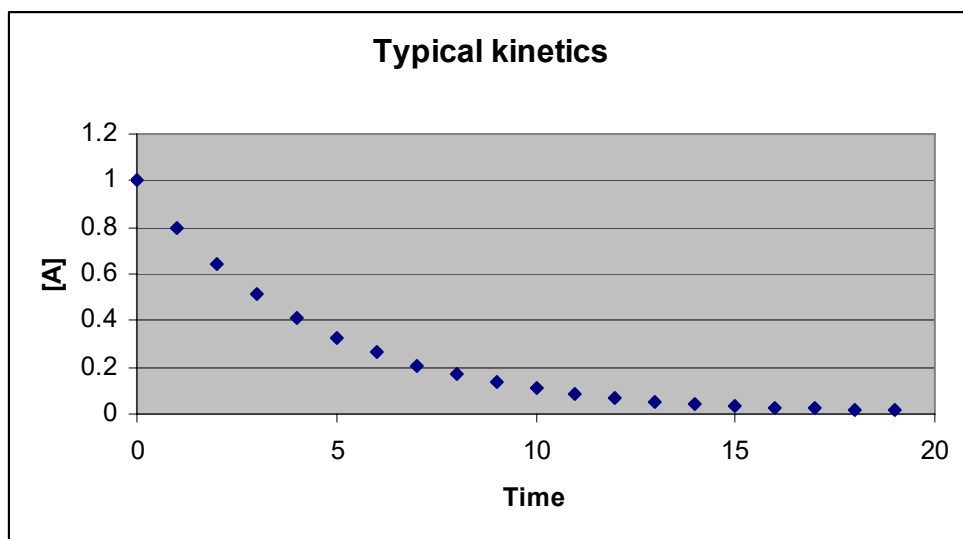
Critical thinking questions:

1. What happens to the concentration of A over time?
   a. Increases
   b. Decreases
   c. Stays constant

2. Chemically, what is happening to A as its concentration decreases?

3. What happens to the rate (change/time) of decrease of A over time?
   a. Increases
   b. Decreases
   c. Stays constant

4. Why do you think the rate of change behaves as you indicated in question 3?

5. How could you calculate the average rate of change in [A] over 20 minutes? What is this rate?

6. What is the rate of decrease for the time interval from 0 – 5 minutes?

7. From 5 – 10 minutes?

8. Do your answers in question 6 and 7 agree with your answer to question 3? Why or why not?

9. How could you measure the instantaneous rate?

A "rate equation" expresses the relationship between rate and concentration of reactants and takes the following form: Rate $= k[A]^x$, where k is the inherent rate constant. (The rate constant is an indicator of the potential speed of the reaction. If a reaction is very slow, the rate constant is much smaller than 1; if the rate constant is much larger than 1, then the reaction is very fast.) The brackets denote molar concentration, and x indicates the "reaction order" which must be determined experimentally. The overall reaction order is determined from the exponents:

Rate $= k[A]^0$ is zero order.

Rate $= k[A]^1$ is first order,

Rate $= k[A]^2$ is second order

Let's examine the relationship between rate and concentration for of the above order types by building a spreadsheet (see next page). Initially, we will assume that the rate constant has a value of 1. Columns B and C have numbers entered; columns D – F are created with formulas. Hint – in column C, you only have to enter the first 3 concentration points. You can the highlight them, place your cursor at the bottom right of cell C5 till you see a small cross, and then drag the pattern through cell C21. The value in cell B3 is "absolute"; you should enter it as B3 in your formulas so it does not adjust as you copy formulas. Create and copy your formulas using the above rate equations.

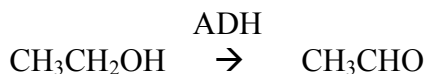| A	B	C	D	E	F
			rate		rate
2	k	conc A	zero	rate first	second
3	1	0.01	1	0.01	0.0001
4		0.02	1	0.02	0.0004
5		0.03	1	0.03	0.0009
6		0.04	1	0.04	0.0016
7		0.05	1	0.05	0.0025
8		0.06	1	0.06	0.0036
9		0.07	1	0.07	0.0049
10		0.08	1	0.08	0.0064
11		0.09	1	0.09	0.0081
12		0.1	1	0.1	0.01
13		0.11	1	0.11	0.0121
14		0.12	1	0.12	0.0144
15		0.13	1	0.13	0.0169
16		0.14	1	0.14	0.0196
17		0.15	1	0.15	0.0225
18		0.16	1	0.16	0.0256
19		0.17	1	0.17	0.0289
20		0.18	1	0.18	0.0324
21		0.19	1	0.19	0.0361

Critical thinking questions:

1. Create an XY graph of the zero order reaction. Highlight cells C3 through D21. Hit the graph icon and select XY graph with the dots connected. Add appropriate titles and axis labels. Save the graph for printing.

2. What is the relationship between rate and concentration?
   a. The rate is directly proportional to concentration.
   b. The rate is inversely proportional to concentration.
   c. The rate is independent of concentration.
   d. The rate is zero.

3. Now create the first order graph. Highlight only column C starting at cell C3. Place the cursor at the top of column E (E3). Hold down the Ctrl key, and highlight all of column E. Now create the first order graph as described in question 1.

4. What is the relationship between rate and concentration in a reaction that follows first order kinetics?
   a. The rate is independent of concentration.
   b. The rate increases linearly with concentration.
   c. The rate increases exponentially with concentration.
   d. The rate decreases linearly with concentration.

5. Now create the second order graph following the procedure described in question 3 (substituting column F for column E).

6. What is the relationship between rate and concentration in a reaction that follows second order kinetics?
   a. The rate is independent of concentration.
   b. The rate increases linearly with concentration.
   c. The rate increases exponentially with concentration.
   d. The rate decreases linearly with concentration.

7. Change the value of the rate constant in cell B3 to 2. What happens to the rates and the resulting graphs? Summarize the effect of the rate constant.

Model 2 The Michaelis Menton model of enzyme kinetics

Let's look at typical data for an enzyme catalyzed reaction. As you recall, an enzyme is defined as a biological catalyst, which speeds up a reaction and is not consumed in a reaction. In biological systems, the number of substrate molecules present is typically much larger than the number of enzyme molecules present.

The following table represents the rate of conversion of ethanol (the substrate) to acetaldehyde (the product) catalyzed by the enzyme alcohol dehydrogenase (ADH). This is the first reaction in the detoxification of ethanol in the liver (and acetaldehyde is implicated in hangovers). The data is adapted from the website below. (http://www.stetson.edu/~wgrubbs/datadriven/enzymekinetics/enzymewtg.html)

$$ CH_3CH_2OH \xrightarrow{\text{ADH}} CH_3CHO $$

Enter the data into a spreadsheet as shown below:

A	B	C	D
2	Trial	[Ethanol]	Rate, mM/min
3	1	0.007	0.06
4	2	0.015	0.11
5	3	0.031	0.21
6	4	0.068	0.26
7	5	0.100	0.28
8	6	0.200	0.29
9	7	0.300	0.29
10	8	0.400	0.29

To get a picture of the behavior, let's create a graph. Highlight cells C3 through D10, select the graph icon, choose an XY scatter graph with the dots connected, and add appropriate titles and axis labels. Save the graph for printing. In an enzyme catalyzed reaction, the reactant is typically referred to as the substrate.

Critical thinking questions:

1. What is the shape of the curve called?
   a. Linear
   b. Exponential
   c. Hyperbolic
   d. Parabolic

2. Indicate where the reaction follows first order kinetics.

3. Where are zero order kinetics observed?

4. What is the maximum rate the obtained in this reaction? (This is known as V_{max}, for maximal velocity.)

5. Refer back to question 2 on page 3. A zero order reaction is one where the rate is independent of reactant (substrate) concentration. From what you have previously learned about enzyme catalyzed reactions, why is a change from first order to zero order kinetics observed with an increase in substrate concentration?

The following derivation provides a model for the kinetics of an enzyme catalyzed reaction. You do not need to know all the steps of the derivation; just follow it through to get a feel for what each variable represents. We will only be working with the final equation.

In the following derivation, velocity (v), has the same meaning as rate. The substrate concentration, [S], is analogous to [A], the reactant concentration in the previous model. [E] represents the free enzyme, $[E_T]$ is total enzyme, [ES] is the enzyme substrate complex, and [P] is the concentration of product. k1, k2, and k3 are the rate constants for the forward reaction of E + S → ES, the forward reaction of ES → E + P, and the reverse reaction of ES → E + S, respectively. It is assumed that there is no appreciable reverse reaction of product back to the ES complex.

$$E + S \underset{k3}{\overset{k1}{\longleftrightarrow}} ES \overset{k2}{\longrightarrow} E + P$$
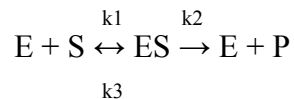
The rate of formation of the product is controlled by the conversion of ES to product. The rate can be expressed, then, as Rate = k_2[ES], or v (velocity) = k_2[ES].

The rate of formation of ES = k_1[E][S] and the rate of degradation of ES is the sum of k_2[ES] + k_3[ES] or $(k_2 + k_3)$[ES].

If the concentration of enzyme is significantly less than the concentration of substrate, then the concentration of ES is essentially constant and its rate of formation becomes equal to its rate of degradation:

$$k_1[E][S] = (k_2 + k_3)[ES]$$

Rearranging,

$$[ES] = \frac{k1 \times [E][S]}{(k2 + k3)}$$

The Michaelis constant $(K_m) = \frac{(k2 + k3)}{k1}$,

So $\quad [ES] = \frac{([E][S])}{Km}$

Free enzyme, $[E] = [E_T] - [ES]$

Substituting,

$$[ES] = \frac{([ET] - [ES]) \times [S]}{Km}$$

Solving for [ES]

$$\frac{([ES]xKm)}{[S]} = ([E_T] - [ES])$$

$$\frac{([ES]xKm)}{[S]} + \frac{([ES]x[S])}{[S]} = E_T$$

$$\frac{[ES] \times (Km + [S])}{[S]} = E_T$$

$$[ES] = \frac{ET \times [S]}{(Km + [S])}$$

Recall that velocity, v, $= k_2[ES]$

Substituting,

$$v = k_2 \; x \; \frac{ET \times [S]}{(Km + [S])}$$

The maximum velocity, V_{max}, is reached when all enzyme sites are filled with substrate, or $\quad V_{max} = k_2 \; x \; [E_T]$

Substituting gives the final form of the Michealis Menton equation:

$$v = \frac{V\max[S]}{Km + [S]}$$

Let us examine the rate curve predicted by the Michaelis Menton equation. We will arbitrarily use a value of 0.3 mmol/min for V_{max} and a value of 0.2 for Km (we'll figure out the units shortly). Create a spreadsheet as shown below. The substrate values in column B can be entered as a pattern as before. The velocities in column C should be calculated by entering and copying a formula using the Michaelis Menton equation; the value of Km and V_{max} should be entered as absolute cells in the formula.

A	B	C	D	E
	Substrate, M	velocity, mmol/min		
2			Km	Vmax
3	0	0.000	0.2	0.3
4	0.02	0.027		
5	0.04	0.050		
6	0.06	0.069		
7	0.08	0.086		
8	0.1	0.100		
9	0.2	0.150		
10	0.3	0.180		
11	0.4	0.200		
12	0.5	0.214		
13	0.6	0.225		
14	0.7	0.233		
15	0.8	0.240		
16	0.9	0.245		
17	1	0.250		
18	1.1	0.254		
19	1.2	0.257		
20	1.3	0.260		
21	2.3	0.276		
22	3.3	0.283		
23	4.3	0.287		
24	5.3	0.289		
25	6.3	0.291		
26	7.3	0.292		
27	8.3	0.293		
28	9.3	0.294		
29	10.3	0.294		
30	11.3	0.295		

Critical thinking questions:

1. Create a graph of substrate concentration vs. velocity. Select the graph icon and select the XY scatter plot with dots connected. Add appropriate titles and axis labels. Does the Michaelis Menton model produce a typical rate curve for an enzyme catalyzed reaction?

2. What is the asymptote of the hyperbola? What does this represent from the model?

3. Now change the value of Km to 4.0. What happens to the curve? What happens to the asymptote?

It is easier to compare the effect of changing one of the parameters of the equation if you can simultaneously see the graph that results from both the original value and the changed value. Since the graph is tied to the values in the cells, the changes you make to Km are automatically reflected in the graph. The following procedure shows you a way to see the graph that results from each value of Km simultaneously.

1. Place your cursor anywhere on column D. Insert a column by selecting the insert menu and choosing column.
2. Copy only the calculated values (not the underlying formulas) from column C into column D. Highlight all the cells in column C (C2 through C30), select edit, then copy. Put your cursor on cell D2 and then select paste. You will see a small clipboard at the lower right of the cells pasted in column D. Click on this clipboard and select values only. Fix the column width through the format menu.
3. Now change Km to 4. You will notice that only the values in column C change, but that column D reflects the values that resulted from a Km of 0.2.
4. Create a graph to show both. Highlight cells B3 through D30. Click the graph icon, select an XY graph with the dots connected. Add appropriate titles and axis labels. To indicate the Km used for each graph, right click on the completed graph. Select source data, then click on the series tab. Click on the word "series 1" and type Km = 4.0 where it says name, then OK. The select "series 2" and type Km = 0.2 in name, then OK.

Critical thinking questions

1. Use the final equation for the Michaelis Menton equation: $v = \dfrac{V\max[S]}{Km+[S]}$. Let's assume that you are at half maximal velocity, where $v = 0.5\ V_{max}$.. Replace v with $0.5\ V_{max}$, then gather like terms algebraically and solve for Km. See below to start.

$0.5\ V_{max} = \dfrac{V\max[S]}{Km+[S]}$

HINT – first multiply both sides by Km + [S], then divide by $0.5\ V_{max}$. What does V_{max} divided by $0.5\ V_{max}$ equal?

When you are done, you should have an equation that reads Km = ………
What, then, are appropriate units for Km?

2. Refer back to the graph that depicts the velocity curves for both values of Km. From the algebra above, you should have shown that Km = [S] when you made the substitution that you were at half maximal velocity. You know that V_{max} is 0.3, so what is $0.5\ V_{max}$?

3. Find that value of velocity on the y axis. Find the graph that represents a Km of 0.2. Since Km is equal to [S] at half maximal velocity, what is the value found for Km at this velocity? (HINT – drop a perpendicular to the x axis at one half V_{max}). What about when you set Km = 4? Obviously, in this example, you know Km because you set it.

4. In the graph below, what is V_{max}? What is Km? Approximate values are fine.
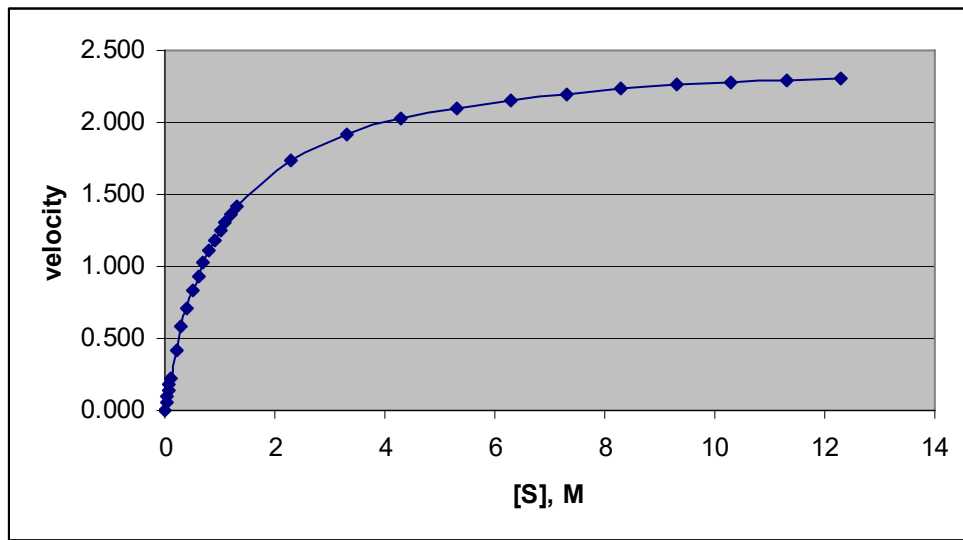
5. Km is the amount of substrate (reactant) required to get to half maximum speed. Does a Km of 0.2 or 4.0 represent a more efficient enzyme? Explain.