The Kinetics of Enzyme Reactions

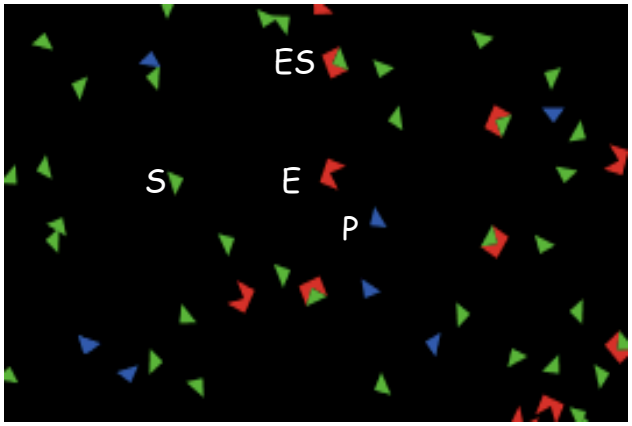
This activity will introduce you to the chemical kinetics of enzyme-mediated biochemical reactions using an interactive Excel spreadsheet or Excelet. A summarized chemical reaction with rate constants, k, is given below:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

reactants activated complex products

$$K_M = \frac{k_2 + k_{cat}}{k_1}$$

where S is the substrate that reacts with the enzyme, E, to make product, P. Here is a screenshot from a Netlogo model of the reaction above. The model is set-up to run as a Java applet. Go to the link listed below, click on the set-up button and then click the go/stop button. You can stop the model by clicking on the go/stop button a second time. Adjust the rate constants to see how they effect the reaction.



Link to Java applet:
http://academic.pgcc.edu/~ssinex/enzyme/Enzyme_modified.html

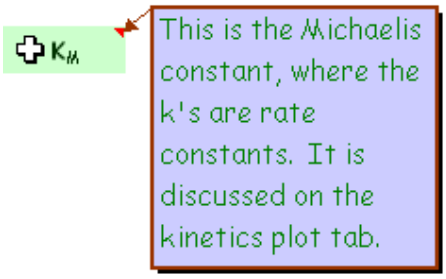
As the reaction proceeds, how do the concentrations change?

What happens to the enzyme at the end of the reaction?

Some Hints for First-time Excelet Users

You will run across a variety of interactive features as you navigate this spreadsheet that allows you to adjust variables, make selections and control items. The comment boxes supply valuable information, hints, and answers, so be sure to explore them!!!

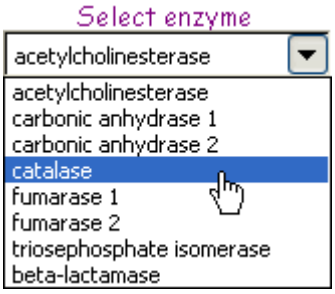
comment box (red triangle in upper right corner of cell) – place cursor on cell to view comments



option button (can only select one)

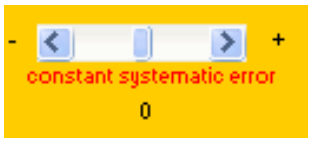
check box (can select any number of them)

drop down menu for selection

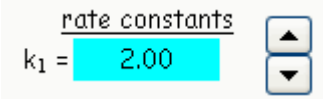


yellow cell – enter data by typing value in

scroll bar (slider) to select value

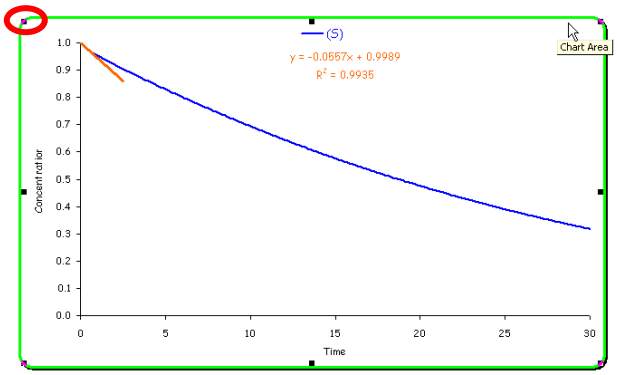


spinner to select value



Printing graphs only – click on the graph to highlight it, so that the little black squares appear on the edge of the chart.

Then press the print icon or select print from the file menu and a page-size graph will be printed.



To complete this task, you will need the interactive Excel spreadsheet at http://academic.pgcc.edu/~ssinex/excelets/enzyme_kinetics.xls.

The spreadsheet contains a number of worksheets that are accessed by clicking on the appropriate tab at the bottom of the screen as labeled below:

To use this spreadsheet, you will need the Analysis ToolPak and Solver Add-in installed in Excel. Go to the *needed Add-ins tab* and follow the instructions.

<u>A Short Review of Chemical Kinetics</u>

Consider some basic aspects of chemical kinetics. Go to the *review tab*.
Let's see how a catalyst works by examining a potential energy diagram. What does a catalyst do to the activation energy?

How about an enzyme?

How does the concentration of a reactant influence the rate of a chemical reaction? Describe and sketch the graphs including labeling the axes.

Zero order First order Second order

The rate as a function of concentration is an important graph to remember.

Enzyme Kinetics

Now, let's look at the behavior of an enzyme-mediated reaction. Go to the *kinetics plot tab*. Select an enzyme and note its substrate. Sketch the initial rate as a function of substrate concentration curve. Label the axes, the v_{max}, and K_M.

Enzyme: _____ Substrate: _____

How does the enzyme concentration compare to the substrate concentration?

Does the substrate for an enzyme matter? Explain.

How does the $(E)_o$ value influence the v_{max}?

How does the order of the reaction behave? Consider the behavior at the beginning of the graph and then later at large times. You may want to go review the rate as a function of concentration graphs you looked at earlier.

Now let's consider all the substances involved in the reaction. Go to the *S, P, & E over time tab*. Sketch and label the axes and substances for the concentration over time graph.

What do you notice about the enzyme concentration, (E), as the reaction goes to completion?

If the k_{cat} = 0, what does the reaction do?

If k_2 = 0, what would happen?

If k_1 = 0, what would happen?

Go to the *initial rate tab* and describe how the initial rate of reaction, v_o, is determined.

Data for enzyme kinetics follow the Michaelis-Menten equation, which is given below:

$$v_o = \frac{v_{max}(S)}{K_M + (S)}$$

where $v_{max} = k_{cat}(E)_o$ and $K_M = (k_2 + k_{cat})/k_1$. Describe how each of these are determined graphically. (Hint: see comments on the *kinetics plot tab*)

v_{max}

K_M

What does the v_{max} physically mean about the reaction?

To deal with the hyperbolic shape in an easier matter, a number of linear transformations have evolved. See the *transformed data tab* (all the errors are set at zero initially). What are the generalized linear equations <u>in terms of the variables in the Michaelis-Menten equation</u> for each transformation? (DO NOT record the specific regression equation found in the spreadsheet.) You may want to resize the window (View > Zoom… and set the size to see the entire screen) to be able to see the content of the entire screen. Explore this worksheet carefully!

Transformation	Generalized Linear Equation
Lineweaver-Burk	
Woolf Hanes	
Eadie-Hofstee	

These linear transformations are used to experimentally determine v_{max} and K_M.

What information (M-M equation) is obtained for each transformation equation?

Equation	Slope	y-intercept	x-intercept
Lineweaver-Burk			
Woolf Hanes			
Eadie-Hofstee			

Examining Experimental Error in the Data

Let's examine the influence of random and systematic error on the plots. You will want to watch the values of r^2, v_{max}, and K_M as the error is changed.

Increase the amount of random error and observe how it influences the three transformed plots. Be sure the systematic errors are both set at zero.

Type of Transformation	How does the slope respond?	How does the y-intercept respond?	Does the scatter in the data change?
Lineweaver-Burk			
Woolf Hanes			
Eadie-Hofstee			

Examine the constant systematic error and observe how it influences the three transformed plots. Set the random error back to zero.

Type of Transformation	How does the slope respond?	How does the y-intercept respond?	Does the scatter in the data change?
Lineweaver-Burk			
Woolf Hanes			
Eadie-Hofstee			

Examine the proportional systematic error and observe how it influences the three transformed plots. Set any other errors back to zero.

Type of Transformation	How does the slope respond?	How does the y-intercept respond?	Does the scatter in the data change?
Lineweaver-Burk			
Woolf Hanes			
Eadie-Hofstee			

The initial rate for lowest substrate concentration is the one influenced by the greatest amount of error. Play with the double the error spinner and decide which plot is influenced least by this error.

Least affected: _____

<u>The Kinetics of Enzyme Inhibitors</u>

Inhibitors, I, bind either to the enzyme, E, directly or to the enzyme intermediate, ES. They can render an enzyme functionless and hence impede an important biological reaction in a system. Go to the *inhibitors tab* and set a concentration of the inhibitor to (I) = 0.005 M. Beware how v_{max} and K_M respond to the inhibitor as you vary the strength of interaction between the enzyme and the inhibitor.

What happens to the rate of the enzyme reaction when an inhibitor is added?

How is each plot influenced if the inhibitors, I, binds to the enzyme, E, directly? This is known as a competitive inhibitor.

Type of Transformation	How does the slope respond?	How does the y-intercept respond?
Lineweaver-Burk		
Woolf Hanes		
Eadie-Hofstee		

How is each plot influenced if the inhibitors, I, binds to the enzyme intermediate, ES? This is known as an uncompetitive inhibitor and is rare by itself.

Type of Transformation	How does the slope respond?	How does the y-intercept respond?
Lineweaver-Burk		
Woolf Hanes		
Eadie-Hofstee		

Which transformed plot is the best to study the effect of an inhibitor on an enzyme? Why?

If the inhibitor binds to both E and ES (called noncompetitive), how do the plots respond?

Type of Transformation	How does the slope respond?	How does the y-intercept respond?
Lineweaver-Burk		
Woolf Hanes		
Eadie-Hofstee		

The Lineweaver-Burk plot of the transformed data is one of the most sensitive to experimental error compared to the other plots. However, it is one of the most influenced plots for studying the effects of inhibitors on enzymes. Thus you will see it used a lot in enzyme studies. It is the easiest transformation as well.

Now try your hand at analyzing some data by doing the assessment that follows.

Name_____

Assessment

1. Here is some data for the enzyme alcohol dehydrogenase (ADH) used to catalyze the conversion of ethanol (the substrate) to acetaldehyde (the product).

(S) M	v_o M/min
0.007	0.06
0.015	0.11
0.031	0.16
0.068	0.21
0.1	0.23
0.2	0.28
0.3	0.29
0.4	0.28

Use Excel to address the following and attach both graphs:

Plot v_o as a function of (S) and <u>estimate</u> the v_{max} and K_M. Explain how and label on the graph.

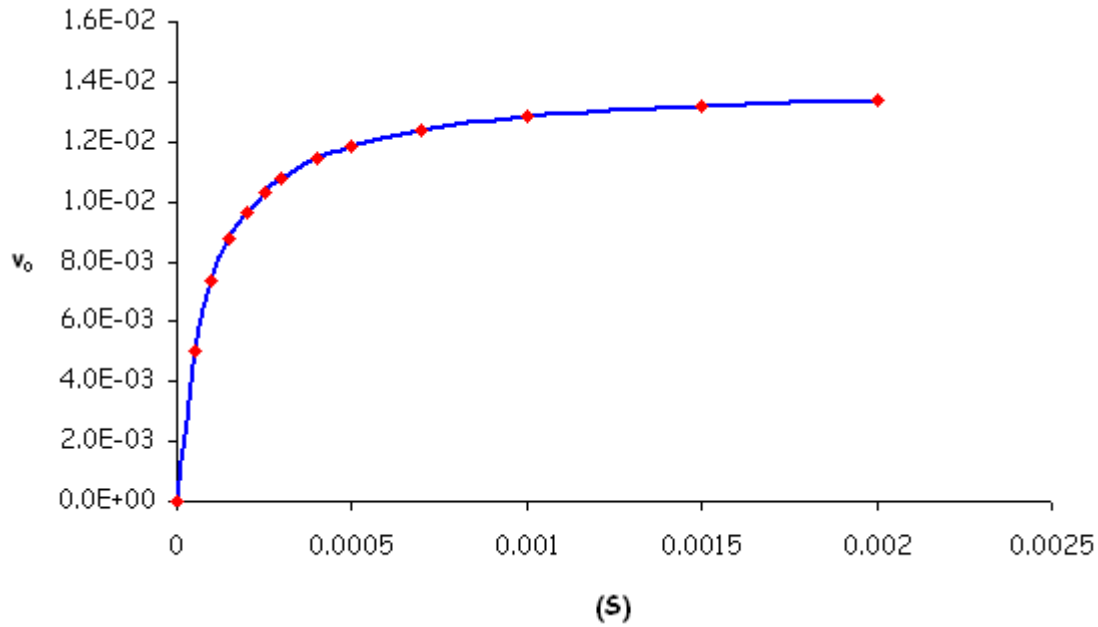
Plot a Lineweaver-Burk plot and determine the v_{max} and K_M. Show your calculations.

Data from K. Bendinskas, C. DiJiacomo, A. Krill, and E. Vitz, *Journal of Chemical Education*, **82** (7), 1068 (2005).

How close were your estimates to the values from the Lineweaver-Burk plot?

2. How does the concentration of an inhibitor, (I), for competitive inhibition influence: 1) the v_o as a function of (S); and 2) the Lineweaver-Burk plot? Sketch and label both graphs for no inhibitor and two different concentrations of inhibitor. So each graph will have three curves/lines on it and it will be LABELED with the conditions.

3. Consider the initial velocity, v_o, against substrate concentration plot given below to address the two questions. You may want to review the *kinetics plot tab*.



Where is the rate of the reaction the most sensitive to changes in (S)? Illustrate on graph.

Where is most or all of the active sites on the enzyme occupied? Illustrate on graph.

4. Using the *non-linear tab* analyze the data in the table below, address the questions below. (S) is in units of mmole/dm^3 and v_o in mmole/dm^3-min.

(S)	v_o
0.25	2.40
0.30	2.60
0.40	4.20
0.50	3.80
0.70	6.20
1.00	7.40
1.40	10.20
2.00	11.40

Estimate the v_{max} and enter the value in the yellow box for v_{max}.

v_{max} = _____

Estimate the K_M and enter the value in the appropriate yellow box. You may have to try several values to get the best fit.

K_M = _____

How well do the calculated v_o values fit the data points?

Now try a non-linear fit by using the Excel Solver following the directions in the upper right portion of the screen. Record the Solver values below:

v_{max} (Solver) = _____ K_M (Solver) = _____

Do these values provide a better fit? Explain.

Provide a possible explanation for the difference in the v_{max} values for the data set (manual vs. Solver fits).

Why does the non-linear analysis work better than the linear analysis? You may want to examine this data using the *linear fits tab*.