BCH 371
Modern Concepts in Biochemistry

# Supplement 7
# Enzyme Kinetics

Enzymes are an important group of proteins found in all living organisms. An enzyme represents a special case of a protein-ligand interaction in which binding of the ligand to the protein leads to a chemical reaction. The ligand in this case is referred to as a substrate (S), and the resulting chemical reaction leads to the formation of a product (P). The reactions catalyzed by enzymes are usually quite simple and involve just one or two substrates and one or two products. An enzyme does not alter the overall energetics or thermodynamics of a reaction. However, in any chemical reaction, a certain amount of activation energy is required to get to a transition state where covalent bonds can be broken or formed and the substrate can be converted to the product. An enzyme changes the path of the reaction so that less activation energy is required. Like other catalysts, an enzyme is not permanently altered by its participation in a chemical reaction. As a result, an individual enzyme molecule can be used again and again.

## A. Basic Enzyme Kinetics

The term **kinetics** in the context of a chemical reaction refers to the **rate** at which it occurs. Consider a simple enzyme-catalyzed reaction in which a **substrate (S)** is converted to a **product (P**).

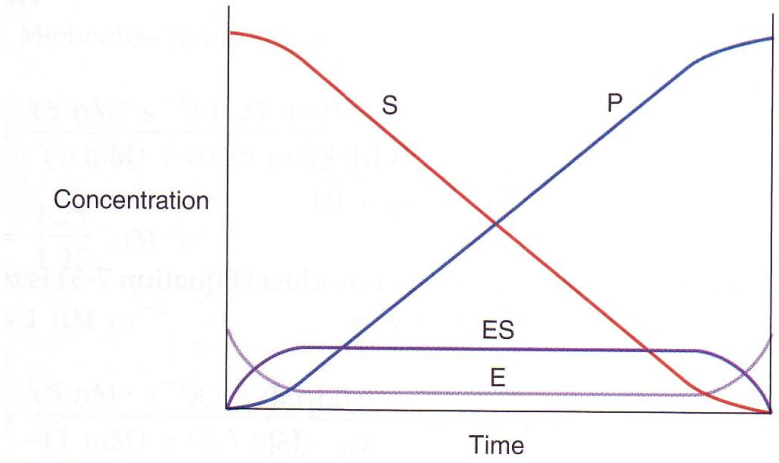
S ----------> P
Enzyme E

The rate of the reaction might be described in terms of the rate at which the substrate disappears or the rate at which the product is formed.

The reaction S -----> P actually involves several steps. The enzyme (E) and the substrate (S) first combine to form an enzyme-substrate complex (ES). A chemical reaction then occurs, leading the formation of an enzyme product complex (EP). The product and the enzyme then separate to release the free product (P) and to regenerate the free enzyme (E). Thus the simple reaction given above can be described more precisely as:

E + S -----> ES -----> EP -----> E + P

The changes that occur in the concentrations of E, ES, S, and P with time are summarized in the following figure.




Once E and S have combined to form ES, there is a period of **steady state kinetics** in which the substrate concentration gradually declines and the product concentration gradually increases.

The rate of a chemical reaction can be expressed mathematically as the change in substrate or product concentration with time. This is often called the **velocity** of the reaction (V) and can be written as:

$$V = \frac{d[S]}{dt} = \frac{d[P]}{dt}$$

In the simple case shown here, where one substrate molecule is converted to one molecule of product, these two numbers are exactly the same. In practice, the kinetics of some enzymes are determined by measuring changes in substrate concentration while the kinetics of other enzymes are determined by measuring changes in product concentration, depending on which molecule is easier to detect quantitatively.

## B. Reaction Order

The velocity of a chemical reaction depends on two factors: the concentration of the limiting reactant and a **proportionality or rate constant (k)** which is an intrinsic property of the molecules themselves. For the simple reaction S -----> P,

$$V = \frac{d[S]}{dt} = k[S]$$

This is referred to **first-order or unimolecular reaction**.

Some of the enzyme-catalyzed reactions that occur within cells are more complex. Consider a reaction in which two molecules of a substrate A combine to give one molecule of a product B.

$$A + A \text{ -----> } B$$

In this case, the reaction depends on two molecules of A reacting with one another.

$$V = k [A] [A] = k [A]^2$$

This is a **second order or bimolecular reaction**.

In a similar way, suppose molecule of C combines with a molecule of D to give molecule E.

$$C + D \text{ -----> } E$$

$$V = \frac{d [C] [D]}{d t} = k [C] [D]$$

This is also a second order or bimolecular reaction. Higher order reactions involving three substrates (**termolecular reactions**) are rare and reactions involving four substrates are unknown.

## C. Measuring the Initial Velocity of an Enzyme-Catalyzed Reaction

The rate of an enzyme-catalyzed reaction is usually determined by measuring the amount of product formed or the amount of substrate consumed as a function of time. Measurement of this rate does not require a completely purified enzyme but it does require a sensitive assay for either the product or substrate. The rate of the reaction depends on several factors such as the concentration of the enzyme, the structure of the substrate, the concentration of the substrate, the physical environment, including the temperature, pH, and ionic strength (salt concentration) of the solution, and the presence of other molecules that might act as activators or inhibitors of the reaction.

Suppose you can measure the rate of a particular enzyme as an increase in absorbance in a particular wavelength as the product is formed. The results on the next page show the data that were collected over two minutes in both a table and a graph.

**Table 4.2** Absorbance versus Time

| Time (sec) | Absorbance |
|---|---|
| 0 | 0 |
| 15 | 0.05 |
| 30 | 0.10 |
| 45 | 0.15 |
| 60 | 0.20 |
| 75 | 0.24 |
| 90 | 0.27 |
| 105 | 0.30 |
| 120 | 0.32 |

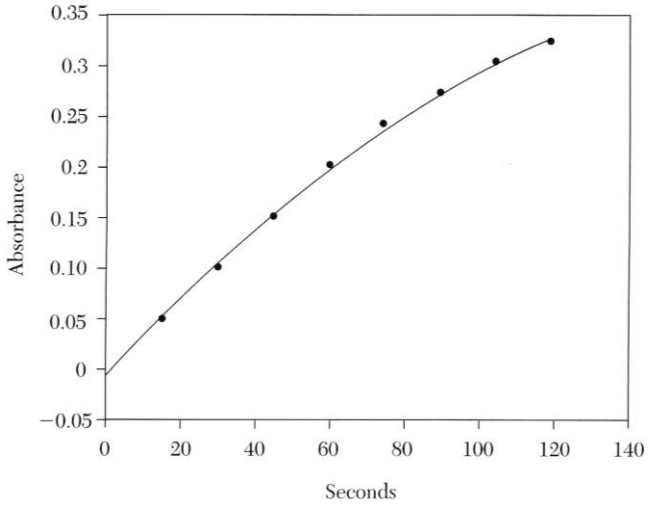
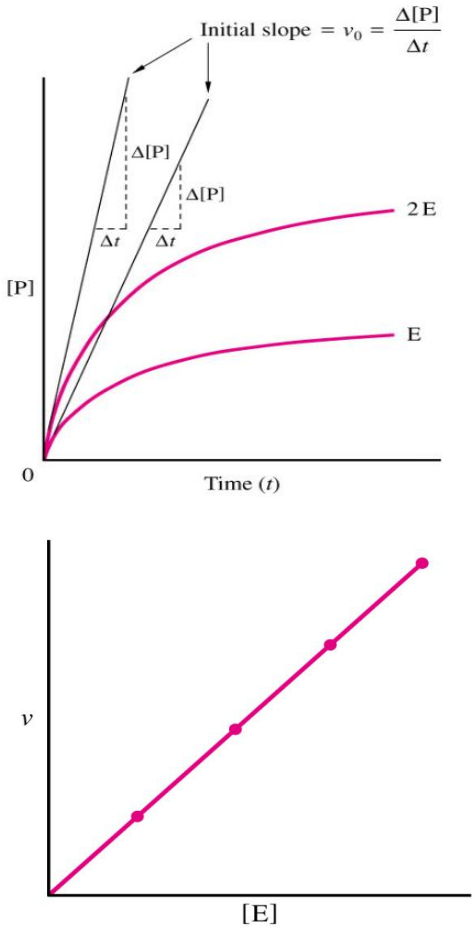


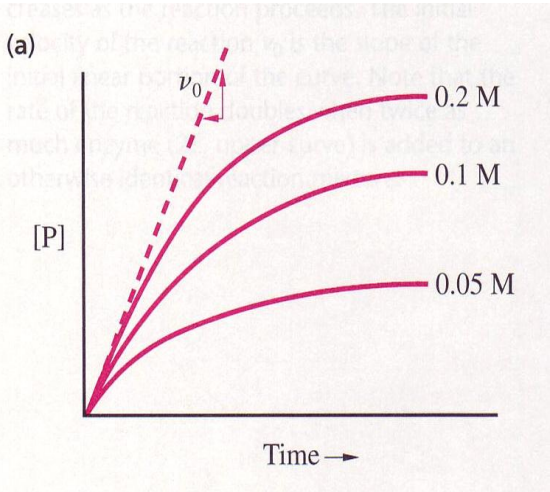

**Figure 4.3** Absorbance vs. time for enzyme-catalyzed reactions

Notice that the amount of absorbance due to the product is initially 0, but it then increases with time as the reaction proceeds. Also notice that although the rate at which the absorbance increases is constant for the first 60 seconds (0.05 A every 15 seconds), the rate then begins to slow down. This is typical of many enzyme-catalyzed reactions. As a result, it is common to focus only on the initial rate of the reaction or the **initial velocity or $V_o$**.

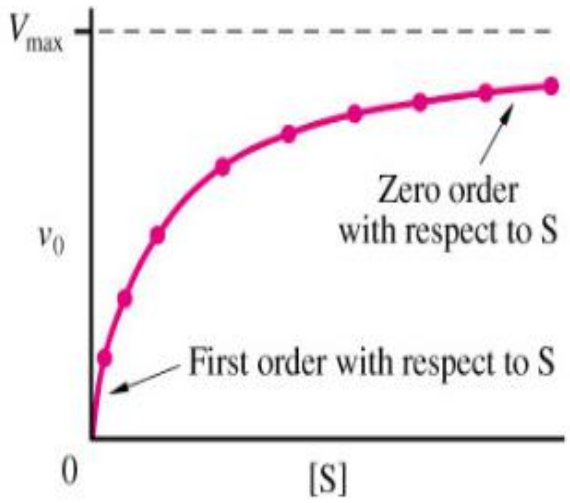
The initial velocity of a reaction is a function of the amount of enzyme. As shown in the next two figures, if you add more enzyme to the reaction mixture containing a certain concentration of substrate, the reaction goes two or three times as fast.




The initial velocity of an enzyme-catalyzed reaction also depends on the substrate concentration. Suppose you set up a reaction in which there is a certain fixed amount of enzyme and then add varying amounts of substrate (0.05 M, 0.1 M, 0.2 M, etc.). When the amount of product is then measured as a function of time, the following results might be obtained.



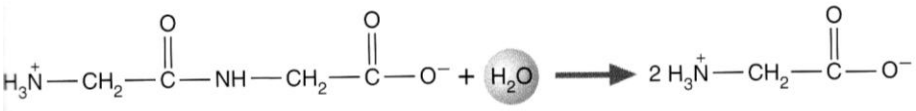
Note that initial rate of the reaction ($v_o$ here) increases as the substrate concentration increases and is fastest with the highest substrate concentration. This analysis can be extended by using additional substrate concentrations. When all of the initial velocity values are plotted on the Y axis as a function of substrate concentration [S] on the X axis, the following graph is obtained.




The reaction is said to be **first order** with respect to S during the first few points of the curve because the rate is directly dependent the substrate concentration. At the upper end of the curve, the reaction is said to be **zero order** with respect to S because adding more S has no effect on the rate of the reaction. This is referred to as **substrate saturation**. The maximum rate of reaction is referred to as the **maximal velocity or $V_{max}$.**

## D. Practice Problems

1. Consider the following reaction in which the dipeptide glycylglycine undergoes hydrolysis to form two individual glycine molecules.



Suppose the reaction was measured with different concentrations of glycylglcine and water, and the following results were obtained, where the rate is given in millimoles per second. .

| [Glycylglycine] | [$H_2O$] | Rate |
|---|---|---|
| 0.1 | 0.1 | $1 \times 10^2$ |
| 0.2 | 0.1 | $2 \times 10^2$ |
| 0.1 | 0.2 | $2 \times 10^2$ |
| 0.2 | 0.2 | $4 \times 10^2$ |

What is the order of the reaction?

2. Sucrase is an enzyme that catalyzes the hydrolysis of sucrose to form glucose and fructose. Suppose you set up a reaction and measure the amount of sucrose remaining in the solution after different periods of time. The following results were observed.

| time (min) | sucrose concentration (M) |
|---|---|
| 0 | 0.5011 |
| 30 | 0.4511 |
| 60 | 0.4038 |
| 90 | 0.3626 |
| 120 | 0.3148 |
| 180 | 0.2674 |

Plot the substrate concentration as a function of time and determine if the reaction is linear for the full 180 minutes. What is the initial velocity ($V_o$) of the reaction?

3. An enzyme-catalyzed reaction was measured with a fixed amount of enzyme and different amounts of substrate. The following data were obtained.

| substrate concentration (M) | velocity (μmole/min) |
|---|---|
| $6 \times 10^{-6}$ | 20.8 |
| $1 \times 10^{-5}$ | 29 |
| $2 \times 10^{-5}$ | 45 |
| $6 \times 10^{-5}$ | 67.6 |
| $1.8 \times 10^{-4}$ | 87 |

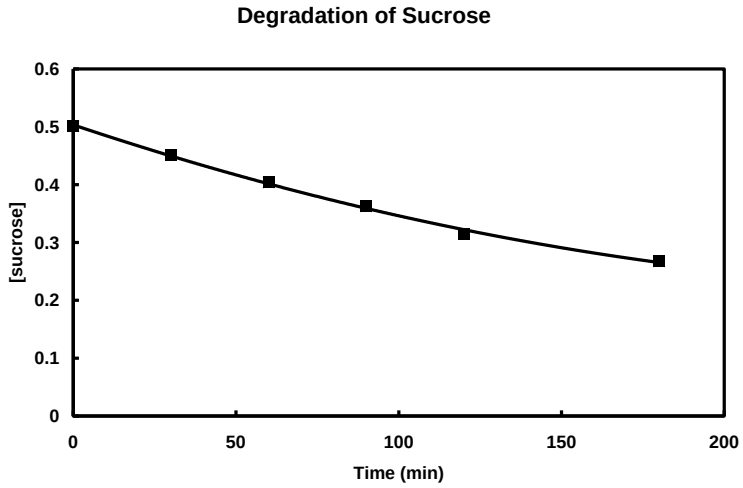
Make a graph in which you plot velocity as a function of [S]. Does the enzyme show substrate saturation? What is the apparent maximal velocity ($V_{max}$)?

## E. Answers to Practice Problems

1. To answer this question, you need to determine if the rate of the reaction varies with the concentration of a particular substrate. In this case, notice that when glycylglycine concentration is doubled, the rate increases by two-fold. Likewise, when the water concentration is doubled, the rate also increases by two-fold. This indicates that reaction depends both on the concentration of glycylglycine and the concentration of water. It is said to be first order with respect to glycylglycine and water individually and so is a second order reaction overall. That is,

$$V = [\text{glycylglycine}]\,[H_2O]$$

2. When you plot the concentration of the substrate on the Y axis and time on the X axis, the following graph is obtained.




Note that while there is a steady decrease in the sucrose concentration with time, it is not linear for the entire three hour period and the best-fit line is a smooth curve rather than a straight line. You can also see that substrate degradation is not linear by just by looking at the data. The change in the sucrose concentration from 0 to 30 minutes was 0.5011 – 0.4511 = 0.0500. The change from 30 minutes to 60 minutes was 0.4511 – 0.4038 = 0.0473, which is close to the first interval but a little slower. The change from 120 to 180 was 0.3148 - 0.2674 = 0.0474 but over a total of 60 minutes. The reaction is clearly slowing down.

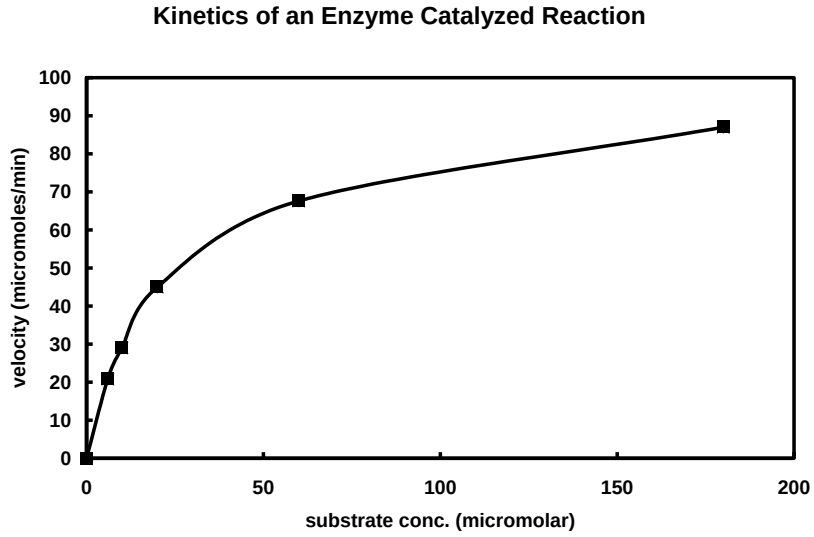
The initial velocity thus should be determined from the first 30 minutes of the reaction. There was a change of 0.0500 moles/liter in 30 minutes, so

$$V = \frac{0.0500 \text{ moles}}{\text{liter} \times 30 \text{ min}}$$

$$= \frac{0.00167 \text{ moles}}{\text{liter min}} = \frac{1.67 \text{ mmole}}{\text{liter min}}$$

Note: in some cases, velocity may be expressed as a change in the concentration of the substrate or product, in which case there will be a unit of volume (liter or ml) in the denominator. In other cases, velocity may be expressed only as a change in absorbance or in the amount of a substrate or product per unit of time.

3. When you plot velocity on the Y axis as a function of substrate concentration on the X axis, you get the following graph.




Note that the substrate concentrations were expressed in micromolar (μM) so they would spread out nicely along the axis. A point at 0,0 was added so that the line would start at the origin and a smooth curve was fitted to the data points. The enzyme does appear to show substrate saturation in the sense the velocity increases as the substrate concentration increases but then approaches a plateau. The maximal rate of the reaction is about 100 μmoles/min, but it is hard to determine this value precisely from this type of graph.

## F. The Michaelis-Menten Model

The basic features of an enzyme-catalyzed reaction can be explained using a kinetic model first developed by Michaelis and Menten. This model is based on the reversible formation of an enzyme-substrate complex:

$$E + S \underset{k_{-1}}{\overset{k_1}{<=====>}} ES \underset{k_{-2}}{\overset{k_2}{<=====>}} E + P$$

Each of the steps in the reaction has its own rate constant. Because binding of the enzyme and substrate to form the enzyme-substrate complex occurs through noncovalent interactions, the rate constants $k_1$ and $k_{-1}$ are usually high. Because the conversion of substrate to product requires the formation or breakage of covalent bonds, the rate constant $k_2$ is usually much smaller. The initial velocity ($V_o$) is thus determined primarily by the concentration of ES and $k_2$.

$$V_o = k_2 [ES]$$

Although the actual concentration of [ES] will depend on the concentration of [E] and [ES], one of basic assumptions of the **Michaelis Menten model** is that system rapidly goes to a **steady state condition** like that shown on page 2 where [ES] is effectively constant in a particular reaction. Another basic assumption of the model is that the reverse reaction can be ignored. That is, we assume that $k_{-2}$ or P are essentially 0. Using this model and some additional assumption described in the text, Michaelis and Menten derived an expression for the relationship between the substrate concentration [S] and the actual initial velocity $V_o$. This **Michaelis Menten equation** is:

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

This equation has the basic form of a hyperbolic function and gives the saturation curve shown on page 6. In this expression, $\mathbf{V_{max}}$ is again the maximal rate of reaction. The rate of the reaction will be maximal when there is sufficient substrate present to maintain <u>all</u> of the enzyme molecules in the reaction in the form of ES. Under this condition, [ES] = [E] and

$$V_{max} = k_2[E]$$

$V_{max}$ is a measure of the catalytic efficiency of the enzyme. To compare different enzymes, the term $\mathbf{k_{cat}}$ or **turnover number** is often used, where

$$k_{cat} = k_2 = \frac{V_{max}}{[E]}$$

The reason of doing this is that it allows different enzymes to be compared, even if their concentrations in the test reactions are not the same.

The term $\mathbf{K_m}$ in the Michaelis Menten equation is a constant called the **Michaelis constant**, which is a characteristic of each particular combination of enzyme and substrate. The Michaelis constant is numerically defined as:

$$K_m = \frac{k_2 + k_{-1}}{k_1}$$

$K_m$ thus effectively summarizes the rate constants for the <u>dissociation</u> of the ES complex in the numerator and the <u>formation</u> of the ES complex in the denominator. Another way to say this is that $K_m$ is a measure of the stability of the enzyme substrate complex. For most enzymes, where binding is much faster than catalysis, $K_m$ becomes equal to the binding constant for the enzyme and substrate. A high $K_m$ value indicates a relatively unstable enzyme-substrate complex. A low $K_m$ value indicates a relatively stable enzyme-substrate complex. $K_m$ thus also indicates the affinity of the enzyme for a particular substrate.

The constants $k_{cat}$ and $K_m$ are characteristic of a particular enzyme with a certain substrate and often combined to give a measure of overall catalytic efficiency of that enzyme.

$$\text{Efficiency} = \frac{k_{cat}}{K_m}$$

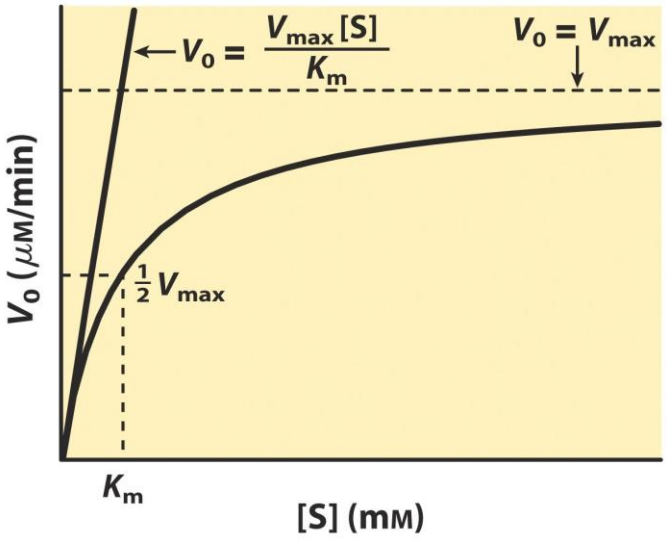
The following table summarizes the kinetic characteristics of several important enzymes.

**TABLE 11.1 Michaelis–Menten parameters for selected enzymes, arranged in order of increasing efficiency as measured by $k_{cat}/K_M$**

| Enzyme | Reaction Catalyzed | $K_M$ (mol/L) | $k_{cat}(s^{-1})$ | $k_{cat}/K_M$ $[(mol/L)^{-1}s^{-1}]$ |
|---|---|---|---|---|
| Chymotrypsin | Ac–Phe–Ala $\xrightarrow{H_2O}$ Ac–Phe + Ala | $1.5 \times 10^{-2}$ | 0.14 | 9.3 |
| Pepsin | Phe–Gly $\xrightarrow{H_2O}$ Phe + Gly | $3 \times 10^{-4}$ | 0.5 | $1.7 \times 10^{3}$ |
| Tyrosyl-tRNA synthetase | Tyrosine + tRNA $\longrightarrow$ tyrosyl-tRNA | $9 \times 10^{-4}$ | 7.6 | $8.4 \times 10^{3}$ |
| Ribonuclease | Cytidine 2′, 3′ cyclic phosphate $\xrightarrow{H_2O}$ cytidine 3′-phosphate | $7.9 \times 10^{-3}$ | $7.9 \times 10^{2}$ | $1 \times 10^{5}$ |
| Carbonic anhydrase | $HCO_3^- + H^+ \longrightarrow H_2O + CO_2$ | $2.6 \times 10^{-2}$ | $4 \times 10^{5}$ | $1.5 \times 10^{7}$ |
| Fumarase | Fumarate $\xrightarrow{H_2O}$ malate | $5 \times 10^{-6}$ | $8 \times 10^{2}$ | $1.6 \times 10^{8}$ |

## G. Determination of $V_{max}$ and $K_m$

The kinetic parameters $V_{max}$ and $K_m$ can be determined in several ways. The simplest is through an examination of a graph of initial velocity ($V_o$) as a function of substrate concentration. The graph shows a simple hyperbolic curve in which the velocity gradually increases and approaches a maximal value at high substrate concentrations. $V_{max}$ can be estimated directly from the graph. $K_m$ can also be estimated from the graph because it is equal to the substrate concentration at 0.5 $V_{max}$.




Note that when $V_o$ is equal to ½ $V_{max}$, the Michaelis Menten equation can be written as

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

If you divide both sides of this equation by $V_{max}$, you get

$$\frac{1}{2} = \frac{[S]}{K_m + [S]}$$

Solving for $K_m$,

$$K_m + [S] = 2[S]$$

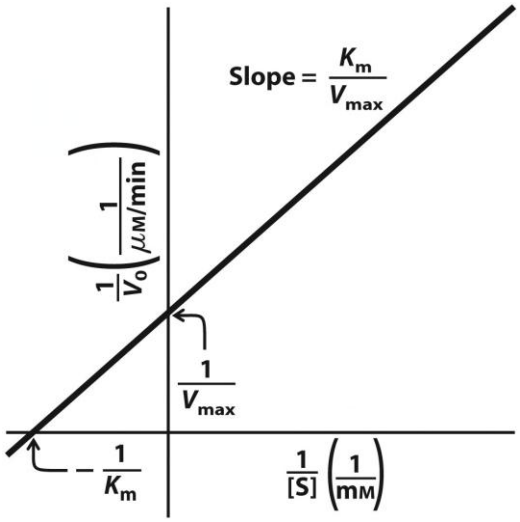
$$K_m = [S]$$

Enzymes with a low $K_m$ value reach 1/2 $V_{max}$ at a low substrate concentration, while enzymes with a high $K_m$ reach $V_{max}$ at a high substrate concentration.

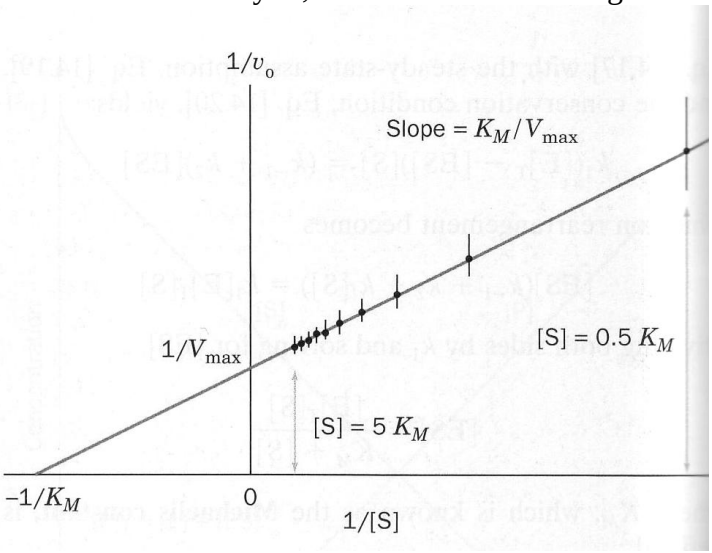
While $V_{max}$ and $K_m$ can be estimated from hyperbolic curves, the values obtained are often compromised by the need to use high substrate concentrations to define $V_{max}$. Consequently, several linear transformations of the Michaelis-Menten equation are often used. The most commonly used of these is the **double-reciprocal** or **Lineweaver-Burke plot**. This is based on a simple algebraic rearrangement of the terms in the Michaelis Menten equation to give the following expression.

$$\frac{1}{V_o} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

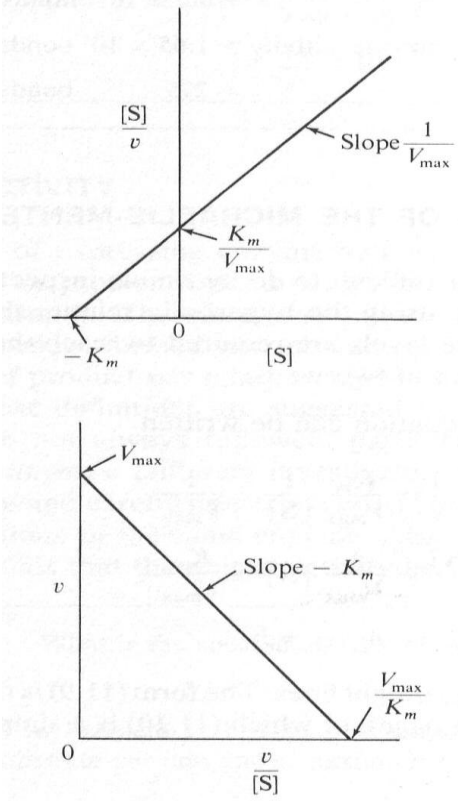
Note that this equation has the form of a straight line (y = mx + b), where m is the slope and b is the intercept. A plot of $1/V_o$ as a function of 1/[S] gives a straight line with a Y intercept of $1/V_{max}$. The slope is $K_m/V_{max}$. The X intercept is actually $-1/K_m$ which is also useful to know.



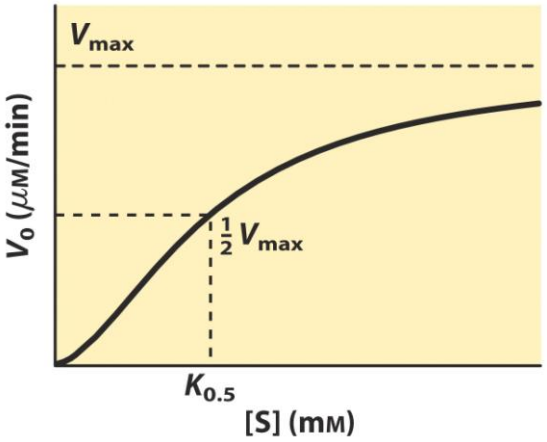

Although the Lineweaver-Burke plot is commonly used in biochemistry, it is flawed in the sense that it gives greatest weight to the most inaccurate points. That is, to the slowest reactions which occur at the lowest substrate concentrations. This leads to larger errors in the data analysis, as shown in the next figure.




Consequently, two other linear transformations are often used instead. In a **Hanes-Woolfe plot**, $[S]/V_o$ is plotted as a function of [S]. In an **Eadie-Hofstee plot**, $V_o$ is plotted as a function of $V_o/[S]$. Both of these graphs with the corresponding slopes and intercepts are shown below.




While many enzymes show simple Michaelis-Menten kinetics and can be analyzed in this way, others show more complex kinetics. Some enzymes give a sigmoidal curve rather than a hyperbolic curve. In this case, the rate of reaction does not increase linearly with the substrate concentration. Rather the rate gradually accelerates as the substrate concentration increases. The rate then gradually decelerates as the substrate concentration increases even further. The term $K_m$ is usually not applied to this situation because the Michaelis Menten model and its corresponding rate constants do not apply. This term is replaced by $K_{0.5}$ (the substrate concentration at half maximal velocity). For enzymes that show this behavior, $K_{0.5}$ and $V_{max}$ can still be estimated graphically. However, the linear transformations cannot be done. Enzymes that exhibit sigmoidal kinetics do so because of positive cooperativity. These enzymes normally consist of several identical subunits. Interactions between these subunits give rise to the complex kinetics as shown in the following figure.




## G. Practice Problems

1. An enzyme catalyzed reaction has a $K_m$ of 1 mM and a $V_{max}$ of 5 nM/sec. What is the reaction velocity when the substrate concentration is a) 0.25 mM; b) 1.5 mM; or c) 10 mM?

2. The following chart shows the $K_m$ values for chymotrypsin with two different substrates.

| Substrate | $K_M$ (M) |
|---|---|
| *N*-acetylvaline ethyl ester | $8.8 \times 10^{-2}$ |
| *N*-acetyltyrosine ethyl ester | $6.6 \times 10^{-4}$ |

   a) Which substrate has the higher affinity for the enzyme?
   b) Which substrate is likely to give the higher value of $V_{max}$?

3. The enzyme hexokinase acts on both glucose and fructose. The $K_m$ and $V_{max}$ values are given in the following table.

| Substrate | $K_M$ (M) | $V_{max}$ (relative) |
|---|---|---|
| Glucose | $1.0 \times 10^{-4}$ | 1.0 |
| Fructose | $7.0 \times 10^{-4}$ | 1.8 |

   Compare and contrast the interaction of the enzyme with these two substrates.

4. The following data were collected as part of a kinetic study of an enzyme. Without graphing the data, estimate the $K_m$ for the reaction.

| [S] (μM) | $v_0$ (μM/min) |
|---|---|
| 50 | 10 |
| 100 | 19 |
| 150 | 31 |
| 200 | 38 |
| 300 | 55 |
| 400 | 62 |
| 800 | 68 |
| 1000 | 70 |

5. The velocity of an enzyme-catalyzed reaction was measured at several substrate concentrations. Calculate $K_m$ and $V_{max}$ for the reaction.

| [S] (μM) | $v_0$ (mM · $s^{-1}$) |
|---|---|
| 0.25 | 0.75 |
| 0.5 | 1.20 |
| 1.0 | 1.71 |
| 2.0 | 2.18 |
| 4.0 | 2.53 |

6. Kinetic data were obtained for the enzyme β-galactosidase, which catalyzes the following reaction. 1 μg of purified enzyme was incubated with varying amounts of lactose and the amount of lactose left was measured after various time intervals.

lactose + $H_2O$ ---> glucose + galactose

| lactose concentration (mM) | initial velocity (μmol/min) |
|---|---|
| 1 | 10.0 |
| 2 | 16.7 |
| 4 | 25 |
| 8 | 33.3 |
| 16 | 40.0 |
| 32 | 44.4 |

a. Using graph paper, make a graph of the data in which you plot initial velocity as a function of substrate concentration. Use this graph to estimate $V_{max}$ and $K_m$ for the enzyme and substrate.

b. Calculate 1/S and 1/V for each entry in the data table on the last page. Make a second graph in which you plot 1/initial velocity as a function of 1/substrate concentration. Use this graph to estimate $V_{max}$ and $K_m$ for the enzyme and substrate.

c. Which set of values is likely to be the more accurate? What are the sources of error in each case?

## H. Answers to Practice Problems

1. To answer this problem, use the Michaelis-Menten equation to solve for the initial velocity. Note that some books use $V_o$ or $V_{max}$ to indicate the initial velocity but others use $v_o$ or $V_{max}$. Likewise, some use $K_m$ and others use $K_M$. It does not make any difference.

(a) $$v_0 = \frac{(5\ \text{nM} \cdot \text{s}^{-1})(0.25\ \text{mM})}{(1\ \text{mM}) + (0.25\ \text{mM})}$$

$$= \frac{1.25}{1.25}\ \text{nM} \cdot \text{s}^{-1}$$

$$= 1\ \text{nM} \cdot \text{s}^{-1}$$

(b) $$v_0 = \frac{(5\ \text{nM} \cdot \text{s}^{-1})(1.5\ \text{mM})}{(1\ \text{mM}) + (1.5\ \text{mM})}$$

$$= \frac{7.5}{2.5}\ \text{nM} \cdot \text{s}^{-1}$$

$$= 3\ \text{nM} \cdot \text{s}^{-1}$$

(c) $$v_0 = \frac{(5\ \text{nM} \cdot \text{s}^{-1})(10\ \text{mM})}{(1\ \text{mM}) + (10\ \text{mM})}$$

$$= \frac{50}{11}\ \text{nM} \cdot \text{s}^{-1}$$

$$= 4.5\ \text{nM} \cdot \text{s}^{-1}$$

2. a. N-acetyl-tyrosine ethyl ester has the lower $K_m$ value and so would have a higher affinity for the enzyme.

b. The value of $V_{max}$ is not related to the $K_m$ and so no conclusion can be drawn from these data.

3. The higher $K_m$ indicates that hexokinase has a lower affinity for fructose compared to glucose. However, once the substrate is bound to the enzyme, fructose is more rapidly converted to the products.

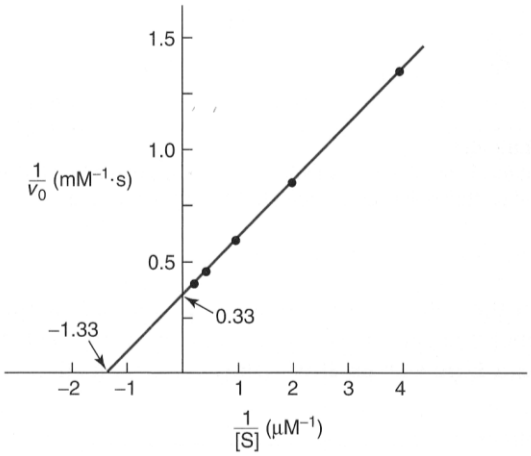
4. The $V_{max}$ for the reaction appears to be about70 μM/min. 0.5 $V_{max}$ would therefore be 35 μM/min. The substrate concentration that yields an initial velocity of 35 is 200 μM, so this corresponds to the $K_m$.

5. Here is the solution to this question.

**Solution**

Calculate the reciprocals of the substrate concentration and velocity, then make a plot of $1/v_0$ versus 1/[S] (a Lineweaver–Burk plot).

| $1/[S]$ ($\mu M^{-1}$) | $1/v_0$ ($mM^{-1} \cdot s$) |
|---|---|
| 4.0 | 1.33 |
| 2.0 | 0.83 |
| 1.0 | 0.58 |
| 0.5 | 0.46 |
| 0.25 | 0.40 |




The intercept on the 1/[S] axis (which is equal to $-1/K_M$) is $-1.33\ \mu M^{-1}$. Therefore,

$$K_M = -\left(\frac{1}{-1.33\ \mu M^{-1}}\right) = 0.75\ \mu M$$

The intercept on the $1/v_0$ axis (which is equal to $1/V_{max}$) is $0.33\ mM^{-1} \cdot s$. Therefore,

$$V_{max} = \frac{1}{0.33\ mM^{-1} \cdot s} = 3.0\ mM \cdot s^{-1}$$

6. Here is the solution to this problem.

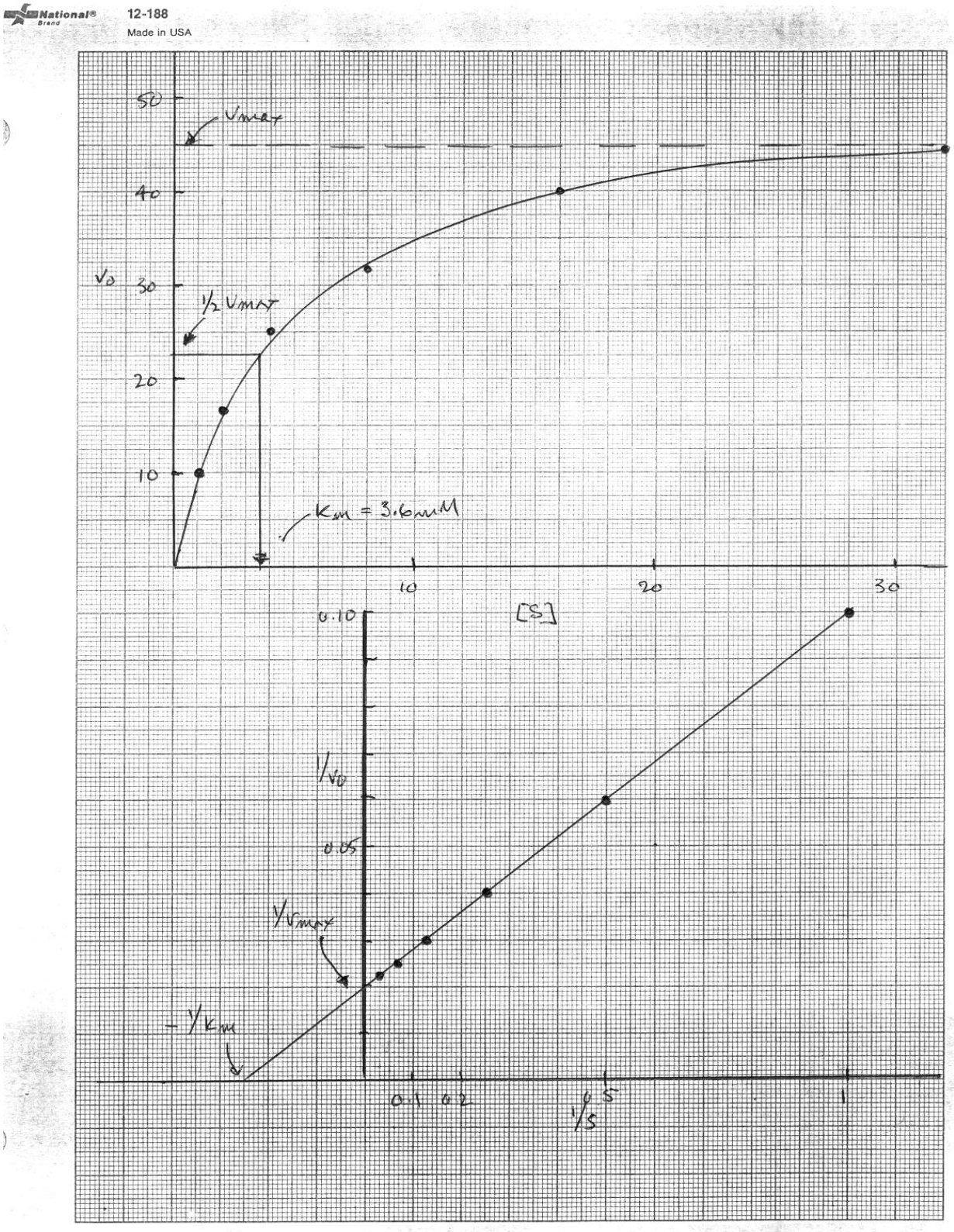
a. Plot $V_o$ as a function of substrate concentration, as shown by the next graph. $V_{max}$ appears to be about 45 μmole/min. 1/2 $V_{max}$ is 22.5 μmole/min, which corresponds to a substrate concentration of 3.6 mM. This is the apparent $K_m$.

b. To make a Lineweaver-Burke plot, calculation 1/S and $1/V_o$.

| lactose concentration (mM) | 1/S | initial velocity (μmol/min) | $1/V_o$ |
|---|---|---|---|
| 1 | 1 | 10.0 | 0.1 |
| 2 | 0.5 | 16.7 | 0.060 |
| 4 | 0.25 | 25 | 0.04 |
| 8 | 0.125 | 33.3 | 0.03 |
| 16 | 0.0625 | 40.0 | 0.025 |
| 32 | 0.0312 | 44.4 | 0.0225 |

Plot $1/V_o$ as a function of 1/S as shown on the next page. Be sure to allow room to extrapolate back across the Y axis. From the graph, $1/V_{max}$ is 0.02, so $V_{max}$ is 50 μmole/min. $-1/K_m$ is -0.25, so $K_m$ is 4 mM.

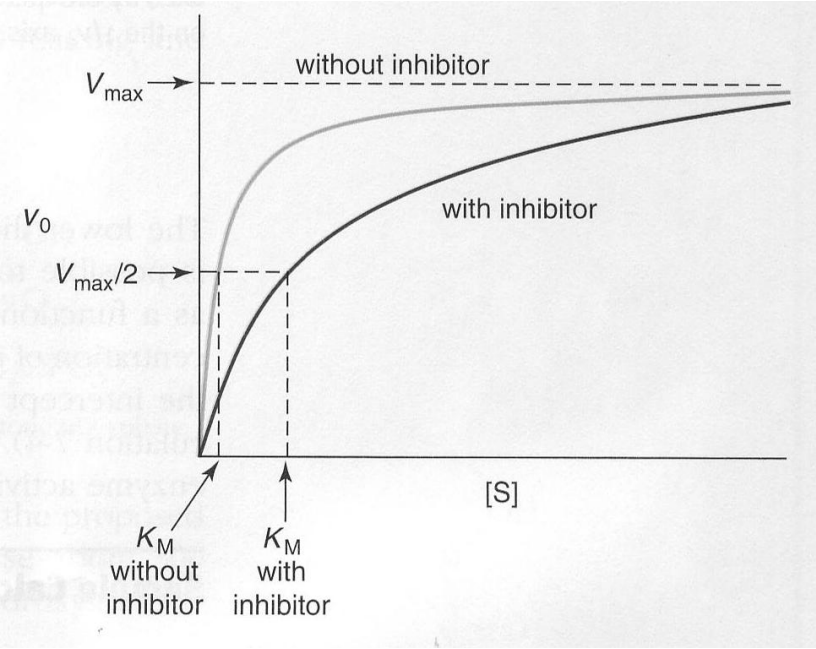
c. The first set of values is lower than the second, although the second is likely to be more accurate. In the first plot, it is necessary to estimate where $V_{max}$ will be, and an under or over estimation of $V_{max}$ will affect the apparent $K_m$ value. The major source of error in the Lineweaver Burke plot is the uneven spacing of the points, which tends to give the least accurate points (lowest substrate concentrations and slowest velocities) greater value.


National Brand
12-188
Made in USA
50
40
30
20
10
$V_0$
$V_{max}$
$\frac{1}{2} V_{max}$
$K_m = 3.6 mM$
10
20
30
[S]
0.10
$1/V_0$
0.05
$1/V_{max}$
$-1/K_m$
0.1
0.2
1/5
5
1
0 Millimeters to the Centimeter

## I. Inhibition Kinetics

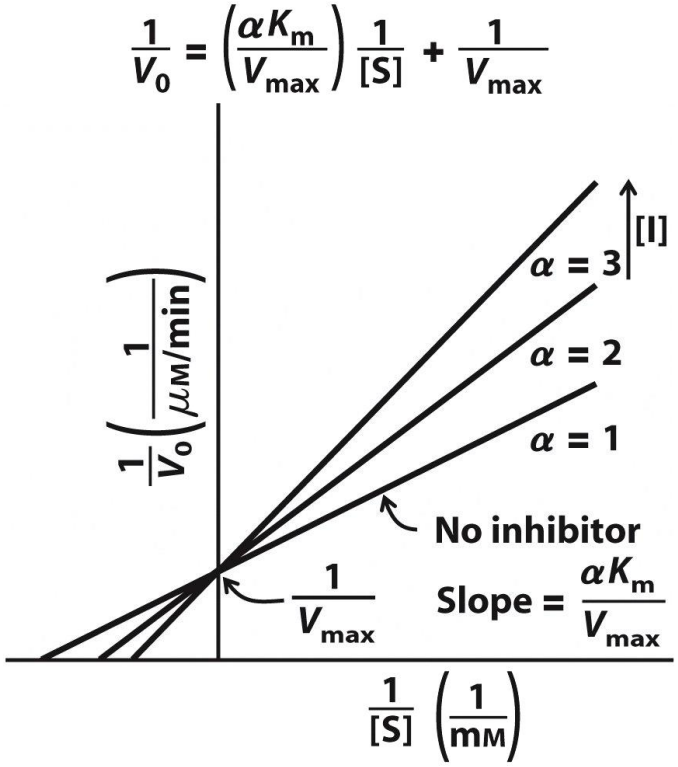
Many enzymes are subject to regulation. This often involves the binding of other ligands or **effectors** either to the active site or to secondary regulatory sites. Binding of these ligands often leads **inhibition** of enzyme activity and a reduction in the initial velocity at a particular substrate concentration. However, in some cases, ligand binding can lead to **activation** and a increase in the initial velocity at a particular substrate concentration.

Most effectors that bind at the active site are molecules that resemble the substrate. This usually leads to **competitive inhibition** because the inhibitor is structurally so similar to the substrate that it competes with it for binding to this site. Competitive inhibition can be demonstrated through a kinetic analysis of enzyme activity in the presence of the inhibitor. In the presence of an inhibitor, the apparent $K_m$ for the enzyme increases but the $V_{max}$ is not affected. This is shown in the following graph is which $V_o$ is plotted as a function of substrate concentration.



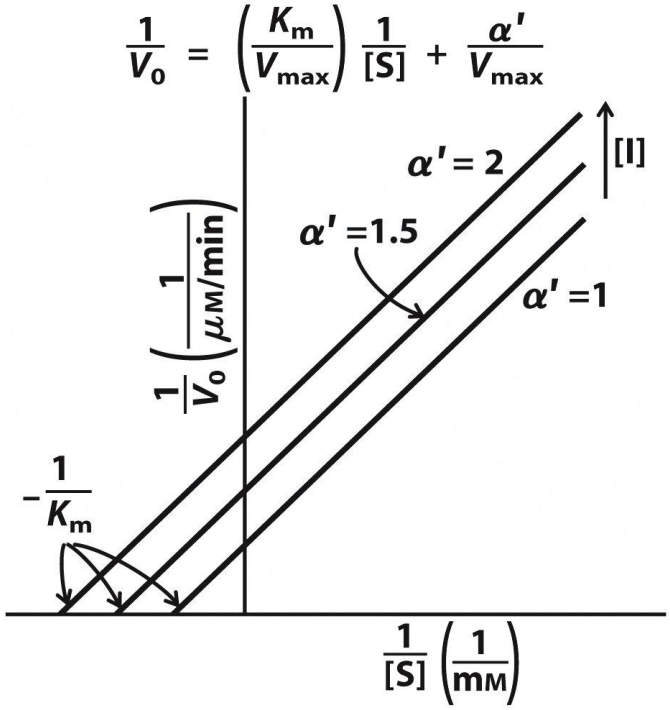

The effect of competitive inhibition can also be seen in a Lineweaver Burke plot, as shown on the next page.. In this case, when the velocity of the reaction is determined with a series of different inhibition concentration, a set of lines is produced that intersect on the Y axis at $1/V_{max}$. The slope increases by a constant factor ($\alpha$) that is characteristic of that inhibitor. The apparent $K_m$ also increases. This can be seen in terms of the points at which the lines intersect the X axis. $-1/K_m$ gets smaller and smaller, which means that the apparent $K_m$ gets larger and larger.

Competitive inhibition:

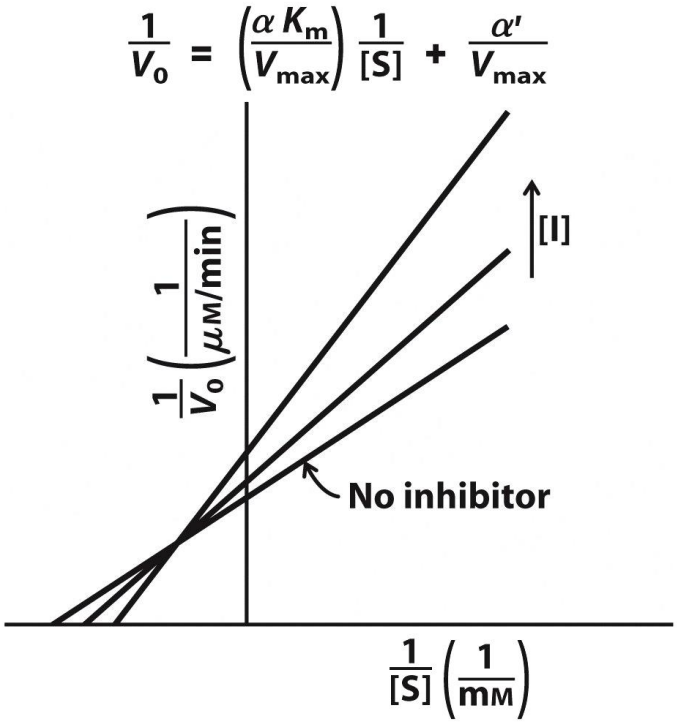



Regulation at a secondary site can occur in one of two ways. In some cases the effector binds only to the enzyme substrate complex (ES). This results in **uncompetitive inhibition.** There is a decrease in both $K_m$ and $V_{max}$, but their ratio stays the same. Uncompetitive inhibition gives a series of parallel lines in a Lineweaver-Burke plot as shown on the next page. In other cases, the effector binds both to the free enzyme (E) and the enzyme substrate complex (ES). This results in **mixed inhibition**. There is a decrease in $V_{max}$ and an increase in $K_m$, giving a series of intersecting lines but not at the Y intercept in a Lineweaver-Burke plot. **Noncompetitive inhibition** is a special form of mixed inhibition in which $V_{max}$ decreases but $K_m$ stays the same. In this case, the lines intersect at $-1/K_m$ on the X axis. Uncompetitive and mixed forms of inhibition most commonly occur with enzymes that have two substrates.

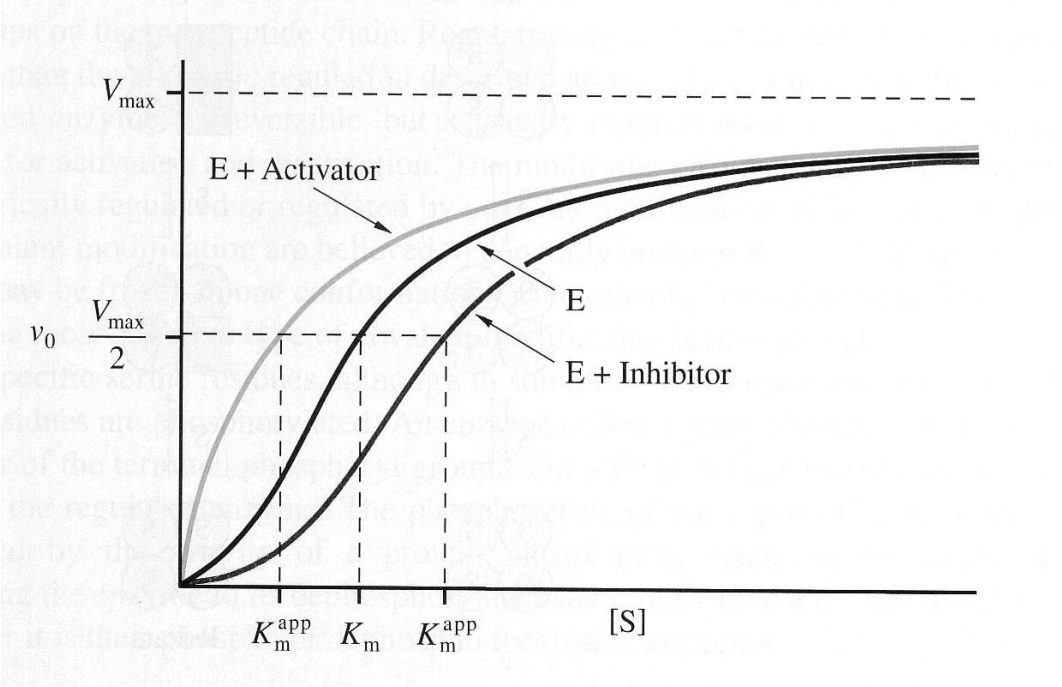
Uncompetitive Inhibition:




Mixed Inhibition:



Allosteric enzymes exhibit sigmoidal kinetics as a result of interactions between the subunits. Some effectors of allosteric enzymes decrease or increase the apparent $K_m$ or $K_{0.5}$ without changing $V_{max}$. Others decrease or increase $V_{max}$ without changing $K_{0.5}$. The following figure shows the first case where Vmax remains the same even as




## J. Practice Problems

1. The following data were obtained for a particular enzyme in the absence and in the presence of a particular inhibitor. Graph the data and determine the nature of the inhibition.

| Substrate concentration, m*M* | 2.0 | 3.0 | 4.0 | 10.0 | 15.0 |
|---|---|---|---|---|---|
| Micromoles of product formed per hour (no inhibitor) | 13.9 | 17.9 | 21.3 | 31.3 | 37.0 |
| Micromoles of product formed per hour (inhibitor present) | 8.8 | 12.1 | 14.9 | 25.7 | 31.3 |

2. The cytochrome P450 family of enzymes are membrane-bound monooxygenases that can detoxify organic compounds. The following data were obtained for the enzyme P450 A34 with the sedative midazolam as a substrate. The results for the standard reactions were compared to those with ketoconazaole, an commonly used anti-fungal agent as the inhibitor. Determine Km and Vmax for the reaction and determine the mode of inhibition.

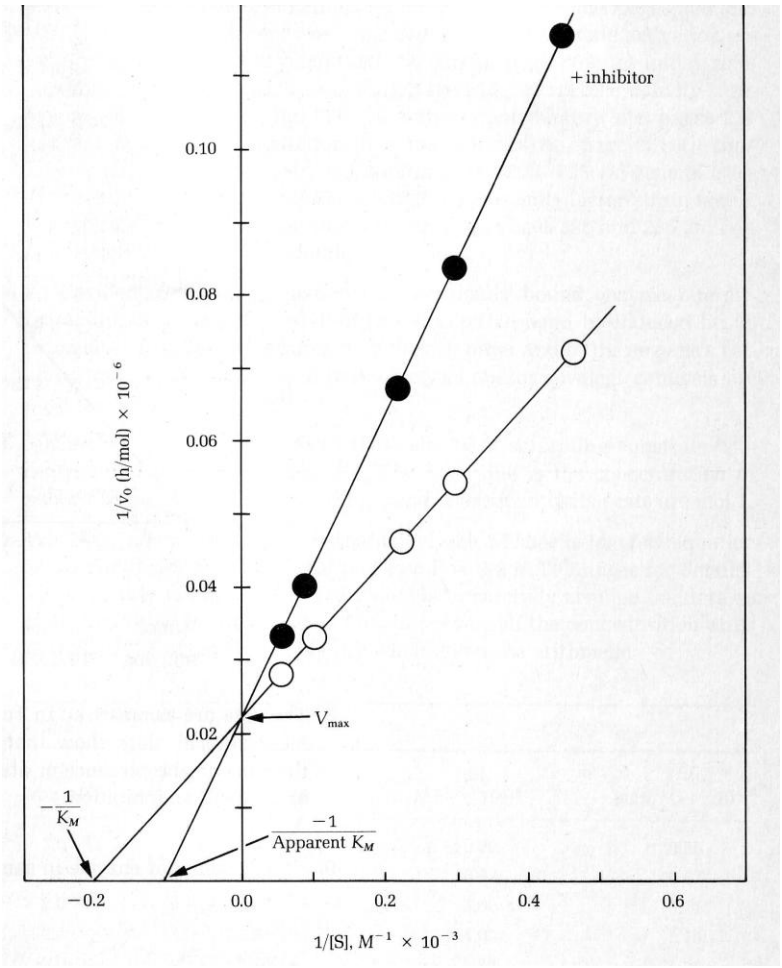
| Midazolam ($\mu$M) | Rate of product formation (pmol $l^{-1}$ $min^{-1}$) | Rate of product formation in the presence of 0.1 $\mu$M ketoconazole (pmol $l^{-1}$ $min^{-1}$) |
|---|---|---|
| 1 | 100 | 11 |
| 2 | 156 | 18 |
| 4 | 222 | 27 |
| 8 | 323 | 40 |

## K. Answers to Practice Problems

1. To answer this question, first calculate $1/V_o$ and $1/S$ for both reactions. Then make a Lineweaver-Burke plot.

*Table 9-7*

| | | No inhibitor | | Inhibitor present | |
|---|---|---|---|---|---|
| [S], $M \times 10^3$ | $1/[S]$, $M^{-1} \times 10^{-3}$ | $v_0$, (mol/h) $\times 10^6$ | $1/v_0$, (h/mol) $\times 10^{-6}$ | $v_0$, (mol/h) $\times 10^6$ | $1/v_0$, (h/mol) $\times 10^{-6}$ |
| 2.0 | 0.50 | 13.9 | 0.072 | 8.8 | 0.114 |
| 3.0 | 0.33 | 17.9 | 0.056 | 12.1 | 0.083 |
| 4.0 | 0.25 | 21.3 | 0.047 | 14.9 | 0.067 |
| 10.0 | 0.10 | 31.3 | 0.032 | 25.7 | 0.039 |
| 15.0 | 0.07 | 37.0 | 0.027 | 31.3 | 0.032 |

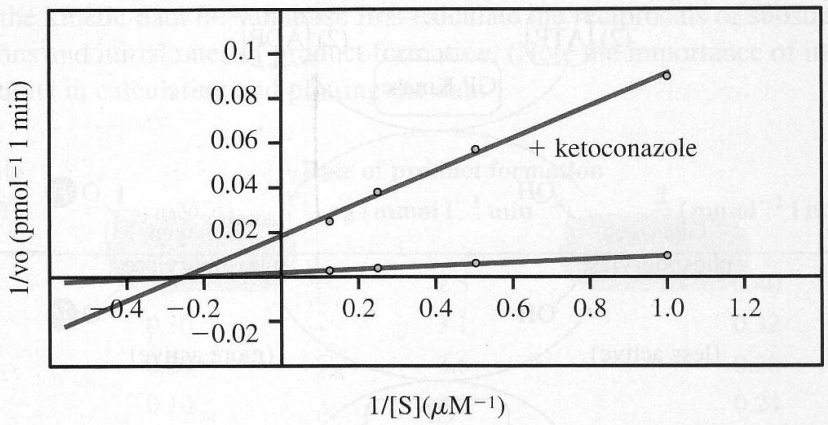



The results indicate that the inhibitor causes competitive inhibition.

2. Here is the solution to this problem.

(a) To plot the kinetic data for P450 3A4, first calculate the reciprocals of substrate concentrations and initial rates of product formation. The data are plotted in the double reciprocal plot and are shown with the dashed line.

| **Midazolam** | | **Rate of product formation** | |
|---|---|---|---|
| **[S] ($\mu$M)** | **1/[S] ($\mu M^{-1}$)** | **$v_0$ (pmol $l^{-1}$ $min^{-1}$)** | **$1/v_0$ ($pmol^{-1}$ l min)** |
| 1 | 1 | 100 | 0.01 |
| 2 | 0.5 | 156 | 0.0064 |
| 4 | 0.25 | 222 | 0.0045 |
| 8 | 0.125 | 323 | 0.0031 |




Vmax is obtained by taking the reciprocal of 1/Vmax from the y intercept (Figure 5.6).

$$1/\text{Vmax} = 0.0025 \text{ pmol}^{-1} \text{ l min, so Vmax} = 400 \text{ pmol l}^{-1} \text{ min}^{-1}$$

Km is obtained by taking the reciprocal of $-1/\text{Km}$ from the x intercept

$$-1/\text{Km} = -0.3 \ \mu\text{M}^{-1}, \text{ so Km} = 3.3 \ \mu\text{M}$$

(b) The reciprocals of the substrate concentration and activity in the presence of ketoconazole are given in the table.

| **Midazolam [S] ($\mu$M)** | **1/[S] ($\mu$M$^{-1}$)** | **Rate of product formation in the presence of 0.1 $\mu$M ketoconazole $v_0$ (pmol l$^{-1}$ min$^{-1}$)** | **1/$v_0$ (pmol$^{-1}$ l min)** |
|---|---|---|---|
| 1 | 1 | 11 | 0.091 |
| 2 | 0.5 | 18 | 0.056 |
| 4 | 0.25 | 27 | 0.037 |
| 8 | 0.125 | 40 | 0.025 |

The plot of the data (solid line) is given in the double reciprocal plot shown in (a). There is an increase in the y intercept and no apparent change in the x intercept. From the double reciprocal plot, it appears that ketoconazole is a noncompetitive inhibitor (see Figure 5.12). These inhibitors are characterized by an apparent decrease in Vmax (increase in 1/Vmax) with no change in Km.