3. Enzymes and Enzyme Kinetics

Read: Chapter 3
pp. 58 - 97

A. Introduction

- Enzymes are protein catalysts.
- Enzymes reduce the activation energy of a reaction involving substrate(s) which yields product(s).
- Enzymes do not affect the thermodynamic equilibrium of substrate(s) and product(s), only the kinetics.
- Enzymes generally are very specific.
- Their specificity and composition have led to the theory of the existence of an “active site,” a region on enzyme on which substrate(s) binds to undergo reaction.

- Subsequent crystallization evidence has supported the presence of active sites.
- In general, the reduction in activation energy is caused by either a proximity effect or an orientation effect.
  - proximity effect describes the tendency of enzymes to place reactants in close contact.
  - orientation effect describes the tendency of enzymes to place reactants in favorable orientation for reaction to proceed.

B. Enzyme Names

- Trivial names exist for many enzymes (e.g., chymosin, trypsin, papain)
- Common names involve adding the suffix “-ase” to the substrate of an enzymatic reaction (e.g., urease, pyruvate decarboxylase, cellulase).
- Formal names have been assigned by Enzyme Commission and are divided into seven classes. Enzyme numbers are comprised of 4 numbers separated by periods:

A.B.C.D

https://www.qmul.ac.uk/sbcs/iubmb/enzyme/

1: oxidoreductases

- involve transfer of hydrogen, oxygen or electrons between substrates
- require a hydrogen (or electron) donor and a hydrogen (or electron) acceptor

Example:

Common name: alcohol dehydrogenase

Formal name: alcohol:NAD^+ oxidoreductase
EC 1.1.1.1

2: transferases

- involve transfer of group other than oxygen or hydrogen

Example:

Common name: phosphoacetyl transferase

Formal name: acetyl CoA:phosphate acetyltransferase
EC 2.3.1.8

acetyl CoA + phosphate ⟶ CoA + acetylphosphate

3: hydrolases

- involve hydrolytic reactions, using water to break bonds

Example:

Common name: oxaloacetase

Formal name: oxaloacetate acetylhydrolase
EC 3.7.1.1

4: lyases

- involve the non-hydrolytic removal of groups from substrates

Example:

Common name: pyruvate decarboxylase

Formal name: pyruvate carboxylyase
EC 4.1.1.1

5: isomerases

- involve the isomerization of a molecule

Example:

Common name: lactate racemase

Formal name: lactate racemase
EC 5.1.2.1

(S)-lactate ⟶ (R)-lactate

6: ligases

- involve the synthesis of various bonds with the concomitant breakdown of energy-containing compounds like ATP.

Example:

Common name: aspartate ammonia ligase

Formal name: aspartate ammonia ligase
EC 6.3.1.1

$$ATP + \text{L-aspartate} + NH_3 \longrightarrow AMP + 2P_i + \text{L-asparagine}$$

7: translocases

- involve the movement of ions or other molecules across a membrane. Usually linked to another reaction.

Example:

Common name: Ferric-transporting ATPase

Formal name: ABC-type Fe^{3+} transporter
EC 7.2.2.7

$$ATP + H_2O + Fe^{3+}\text{-[iron-binding protein]}_{(side\ 1)} \rightarrow ADP + P_i + Fe^{3+}_{(side\ 2)} + \text{[iron-binding protein]}_{(side\ 1)}$$

C. Quantification of Enzymes

- Activity is usually used to quantify enzyme concentration. Activity is directly related to mass of material and the intrinsic catalytic "power" of the enzyme.
- A unit of activity is the amount of enzyme which results in the formation of a certain molar amount of product in a certain amount of time (at specified conditions of temperature, pH, buffer type, and substrate concentration).
- One International Unit of activity is the amount of enzyme required to produce 1.0 μmole of product in one minute.

- Specific activity normalizes enzyme activity by the mass of crude protein, and is a measure of the catalytic power of the enzyme on a per mass basis.

$$\text{specific activity} = \frac{\dfrac{\text{activity}}{\text{mass protein}}}{\dfrac{\text{mole product}}{(\text{mass protein})(\text{time})}}$$

- It is advantageous to measure product appearance rather than substrate disappearance since product concentration begins at zero.

When someone conducts an enzyme assay and reports “Enzyme Activity”, the activity is measured using excess substrates.

Therefore, enzyme activity is usually an indication of “V_{MAX}” of an enzyme (to be discussed later).

Example

A solution is measured for alcohol dehydrogenase activity by adding excess ethanol and NAD^+, and monitoring the formation of $NADH_2^+$ (which absorbs UV light at 340 nm with an molar extinction coefficient ε of 6.22 Abs Units·L/mmol). A 100 μL solution containing 33.5 mg/L protein is mixed with 900 μL buffer, NAD^+ and ethanol. After 1 minute, the absorbance of the mixture has increased by 0.46 AU.

a) What is the activity of alcohol dehydrogenase in the original protein solution?
b) What is the specific activity of alcohol dehydrogenase in the original protein solution?

Solution

One IU is equal to the amount of enzyme which generates one micromole of $NADH_2^+$ per minute.

a) What is the activity of alcohol dehydrogenase in the original protein solution?

Note that the “original protein solution” has been diluted by a factor of 10 as a result of the assay, and there are 1000 μmol in 1 mmol!

Solution (cont'd)

$$\frac{0.46\ \text{AU}}{\text{minute}} \times \frac{1000\ \mu\text{L assay soln}}{100\ \mu\text{L protein soln}} \times \frac{\text{mmol}}{6.22\ \text{AU}\cdot\text{L}} \times \frac{1000\ \mu\text{mol}}{\text{mmol}}$$

$$= 740.\ \mu\text{mol/L}\cdot\text{min} = \underline{740.\ \text{IU/L}}$$

b) What is the specific activity of alcohol dehydrogenase in the original protein solution?

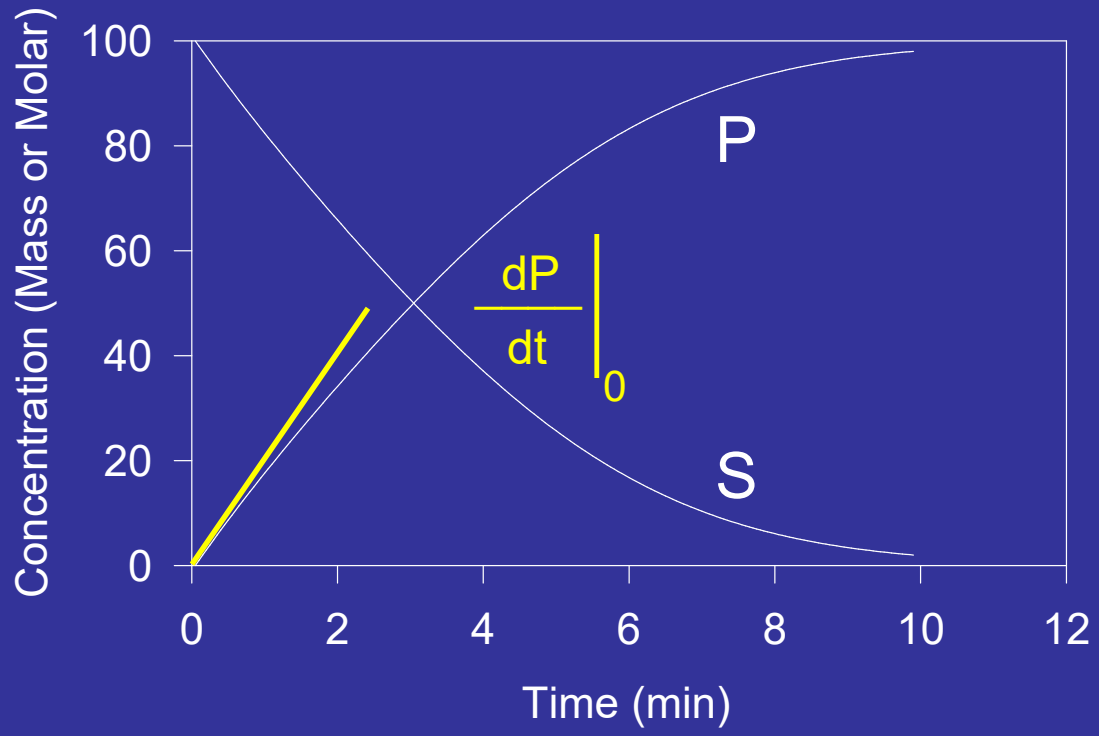
$$\frac{740\ \text{IU/L}}{33.5\ \text{mg protein/L}} = \underline{22.1\ \text{IU/mg}}$$

D. Models for Simple Enzyme Kinetics

1. An enzyme kinetics experiment

- Add a little enzyme into a beaker
- At time = 0, add some substrate into beaker
- Stir very well, and keep pH and temperature constant
- Measure disappearance of substrate or appearance of product with time

Result:

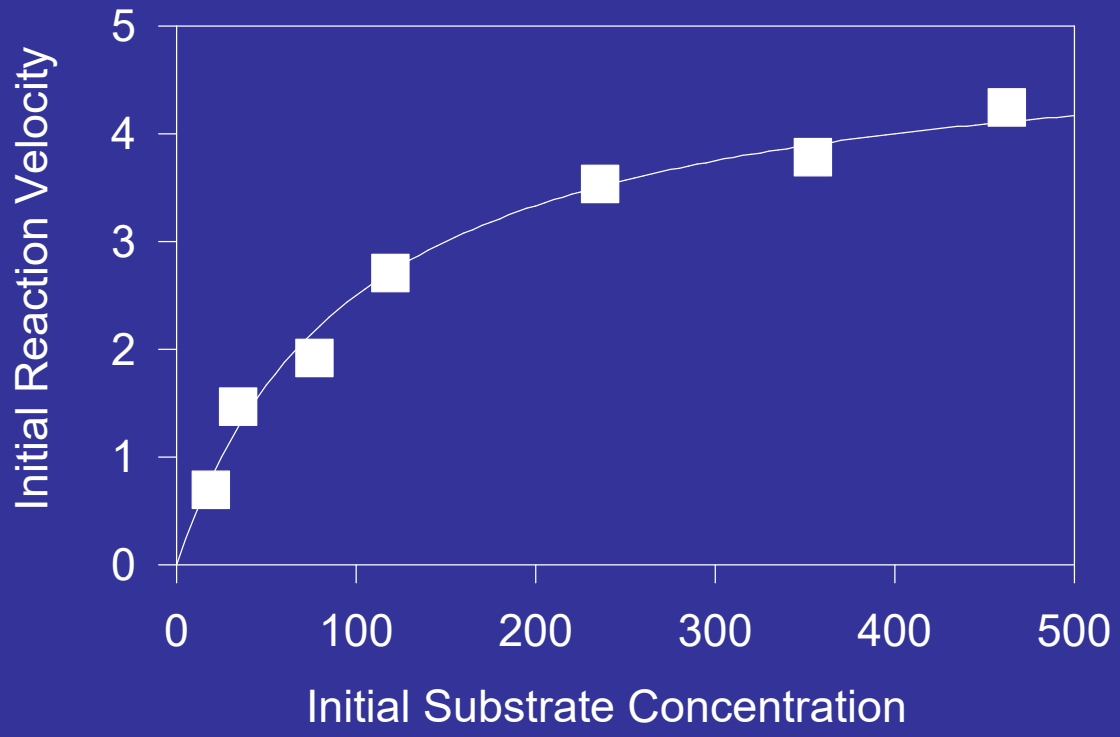


- Calculate v_0: "Initial Reaction Velocity"

$$\left.\frac{dP}{dt}\right|_0 \quad \text{or} \quad -\left.\frac{dS}{dt}\right|_0$$

- Repeat experiment for several different initial concentrations of substrate, keeping the enzyme concentration the same. (For simplicity, we will also keep initial product concentration zero)
- Plot $\left.\frac{dP}{dt}\right|_0$ versus S_0

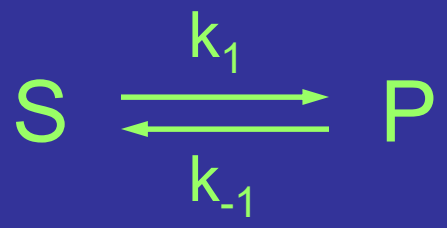
Result:



- This relationship was observed by Brown (1902) for invertase, converting sucrose into glucose and fructose

2. Mathematical representation of enzyme kinetics

a. First order kinetics ("one-step" reaction)



Mathematical Model:

$$-\frac{dS}{dt} = \frac{dP}{dt} = k_1 S - k_{-1} P$$

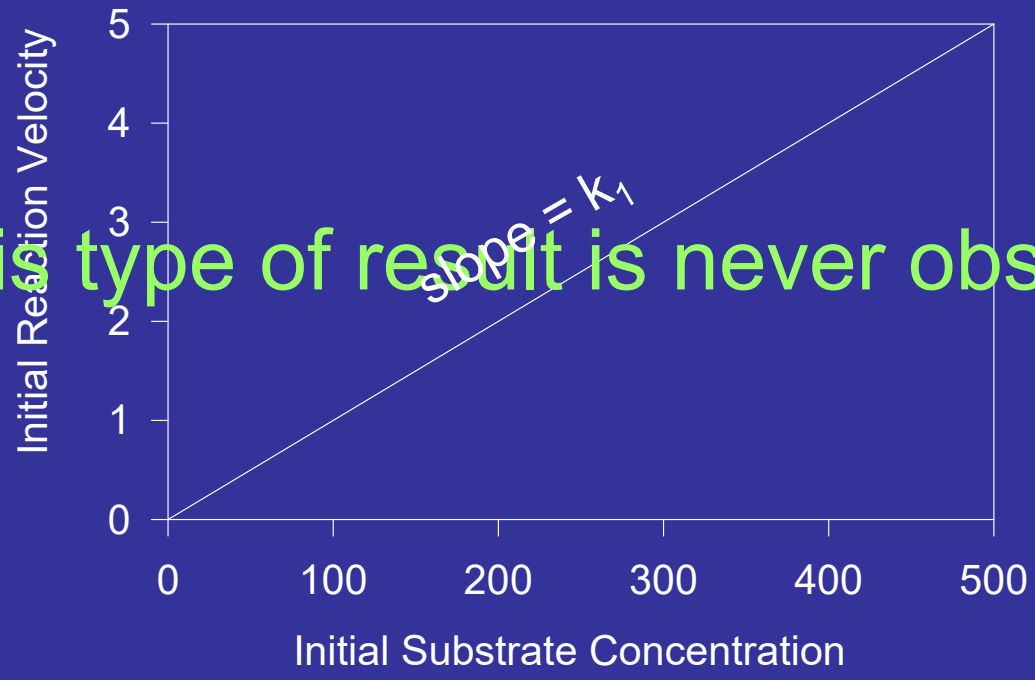
Notes

1) If we are concerned with the initial reaction rate, and the initial concentration of P is zero, then P < S throughout the experiment.
2) Most enzymatic reactions are thermodynamically favorable so $k_1 > k_{-1}$.
3) Considering 1) and 2) together means that $k_1S >> k_{-1}P$ for the time of interest.

$$-\frac{dS}{dt} = \frac{dP}{dt} \approx k_1S$$

- This equation predicts that the initial reaction rate should be proportional to the initial substrate concentration.

$$v_0 = \left.\frac{dP}{dt}\right|_0 = k_1 S_0$$



This type of result is never observed!

Therefore, enzyme catalysis must occur as a multi-step process.

b. Kinetics with enzyme-substrate complex

- Assumption that reaction #2 is irreversible is justified by observation that reaction of product (often) does not yield substrate.

Complex Dissociation Constant: $K_D = \frac{k_{-1}}{k_1}$

- The greater the value of K_D, the greater the tendency for the enzyme-substrate to dissociate (into enzyme and substrate).

Mathematical Model:

(4 components; thus 4 equations)

$$-\frac{dS}{dt} = k_1[E][S] - k_{-1}[ES]$$

$$-\frac{dE}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

$$\frac{dES}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

$$\frac{dP}{dt} = k_2[ES]$$

Notes:

1) The enzyme is not consumed in the reaction. Thus, throughout the experiment the total enzyme concentration ($[E_0]$) is a constant.
2) Moreover, enzyme exists either as free enzyme ([E]) or as the enzyme-substrate complex ([ES])
3) Considering 1) and 2) together means that:

$$[E_0] = [E] + [ES]$$

or

$$[E] = [E_0] - [ES]$$

Goals:

1) Find $\frac{dP}{dt}$ as f(S)

2) Find P, S, E, ES as f(t)

Methods of Solution:

1) Equilibrium between enzyme, substrate and enzyme-substrate complex is rapid.

   (Henri, Michaelis, Menten)

$$K_D = \frac{k_{-1}}{k_1} = K_M' = \frac{[E][S]}{[ES]}$$

$$K_M' = \frac{([E_0] - [ES])[S]}{[ES]}$$

$$[ES] = \frac{[E_0][S]}{K_M' + [S]}$$

Recall that $v = \frac{dP}{dt} = k_2[ES]$

So $v = \frac{dP}{dt} = \frac{k_2[E_0][S]}{K_M' + [S]}$

Define $V_{MAX} \equiv k_2[E_0]$

Then $\boxed{v = \frac{dP}{dt} = \frac{V_{MAX}[S]}{K_M' + [S]}}$

Michaelis-Menten Equation

2) The enzyme-substrate complex is in a “quasi-steady state”

(Briggs, Haldane)

$$\frac{dES}{dt} \approx 0$$

Since $\frac{dES}{dt} = -\frac{dE}{dt}$ (see rate equations)

$$\frac{dE}{dt} \approx 0$$

3 equations will be used

i) $\frac{dP}{dt} = k_2[ES]$

ii) $-\frac{dE}{dt} = 0 = k_1[E][S] - k_{-1}[ES] - k_2[ES]$

iii) $[E] = [E_0] - [ES]$

Combining ii) and iii):

$0 = k_1[E_0][S] - k_1[ES][S] - k_{-1}[ES] - k_2[ES]$

$0 = k_1[E_0][S] - (k_1[S] + k_{-1} + k_2)[ES]$

Rearranging for [ES]

$$[ES] = \frac{k_1[E_0][S]}{k_1[S] + k_{-1} + k_2}$$

Inserting into i):

$$v = \frac{dP}{dt} = \frac{k_2 k_1[E_0][S]}{k_1[S] + k_{-1} + k_2}$$

or

$$v = \frac{dP}{dt} = \frac{k_2[E_0][S]}{(k_{-1} + k_2)/k_1 + [S]}$$

Let

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

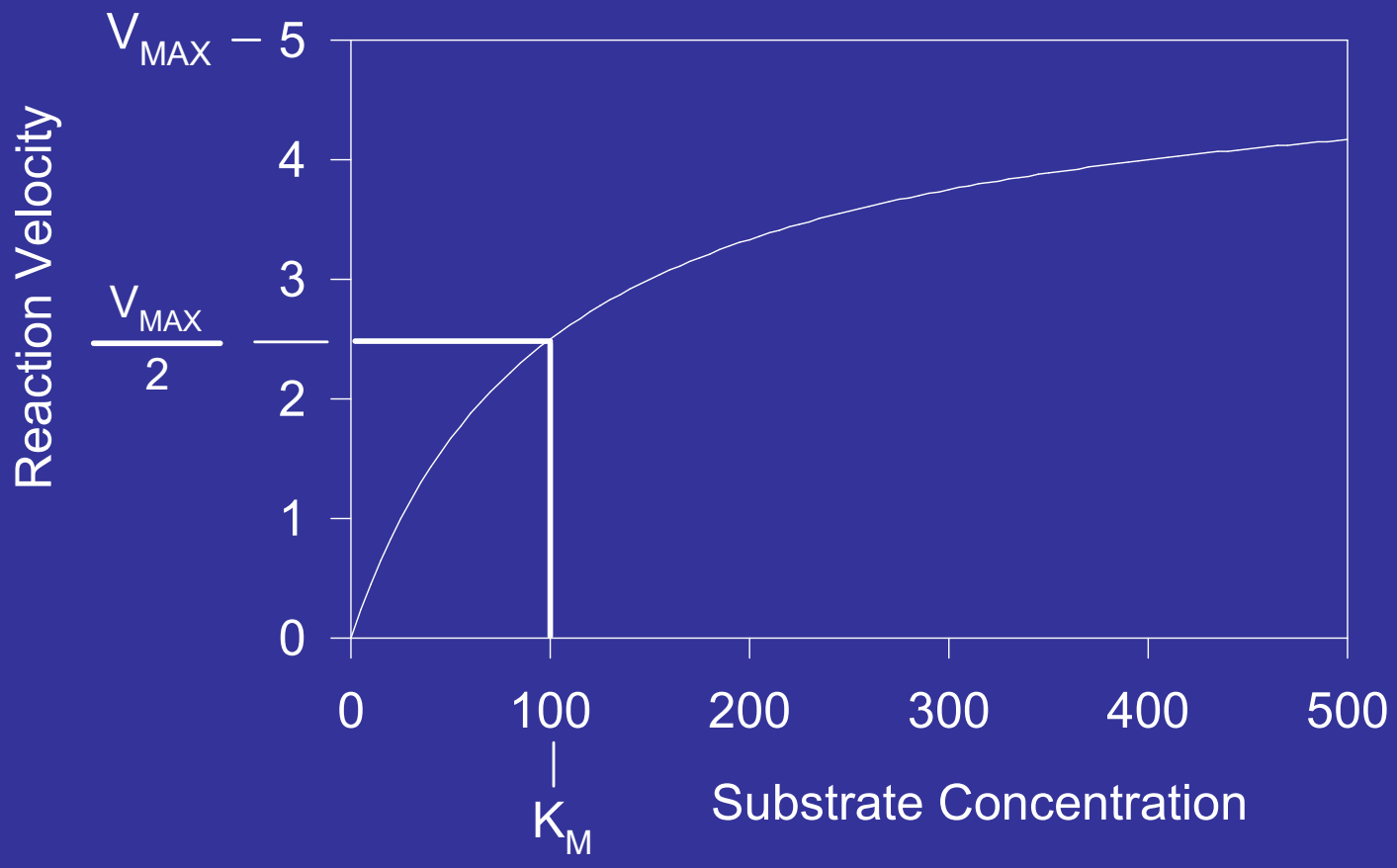
A “total” enzyme-substrate complex dissociation constant

And recalling definition of V_{MAX}

Then

$$v = \frac{dP}{dt} = \frac{V_{MAX}[S]}{K_M + [S]}$$

Results of two methods of solution:



Interpretation of parameters:

1) If S is large, $S >> K_M$, then

$$v = \frac{V_{MAX}[S]}{[S]} = V_{MAX}$$

2) If S is small, $S << K_M$, then

$$v = \frac{V_{MAX}[S]}{K_M}$$

3) If $S = K_M$, then

$$v = \frac{V_{MAX}K_M}{K_M + K_M} = \frac{V_{MAX}}{2}$$

K_M is equal to the substrate concentration which results in half of the maximum reaction rate.

Notes:

1) Usually only initial rate data are useful (t ~ 0) for finding values for V_{MAX} and K_M. However, once those values are determined, the Michaelis-Menten Equation can be used to find substrate concentration as a function of time.
2) The Briggs-Haldane assumption of quasi-steady-state cannot occur initially, since initial concentration of ES is zero.
3) Linearizations of Michaelis-Menten Equation can be used to find values of V_{MAX} and K_M. They are inherently flawed (statistically).

Integrate Michaelis-Menten to obtain S = f(t)

$$\frac{dP}{dt} = -\frac{dS}{dt} = \frac{V_{MAX}[S]}{K_M + [S]}$$

$$-\int_{S_0}^{S} dS \frac{K_M + [S]}{[S]} = \int_{0}^{t} V_{MAX} dt$$

$$\int_{S}^{S_0} \frac{dS\, K_M}{[S]} + \int_{S}^{S_0} dS = \int_{0}^{t} V_{MAX} dt$$

$$S_0 - S + K_M \ln(S_0/S) = V_{MAX}t$$

S as a function of time

3) Computer solution

Many computer software packages are available which solve sets of differential equations to yield concentrations as functions of time. Also, one may write his own simple program.

Recall the four differential equations:

$$-\frac{dS}{dt} = k_1[E][S] - k_{-1}[ES]$$

$$-\frac{dE}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

$$\frac{dES}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

$$\frac{dP}{dt} = k_2[ES]$$

Need initial conditions to solve: S_0, E_0, ES_0, P_0

A simple (!) method of solving is to approximate each differential equation with a difference equation:

$$\frac{dS}{dt} \approx \frac{\Delta S}{\Delta t} = \frac{S_{i+1} - S_i}{\Delta t}$$

$$\frac{dE}{dt} \approx \frac{\Delta E}{\Delta t} = \frac{E_{i+1} - E_i}{\Delta t}$$

$$\frac{dES}{dt} \approx \frac{\Delta ES}{\Delta t} = \frac{ES_{i+1} - ES_i}{\Delta t}$$

$$\frac{dP}{dt} \approx \frac{\Delta P}{\Delta t} = \frac{P_{i+1} - P_i}{\Delta t}$$

where i+1 refers to the value at a future time, and i refers to the value at the present time.

Thus, a new concentration (i+1) may be calculated from an old concentration (i):

$$\frac{S_{i+1} - S_i}{\Delta t} = -(k_1 E_i S_i - k_{-1} E S_i)$$

$$\frac{E_{i+1} - E_i}{\Delta t} = -(k_1 E_i S_i - k_{-1} E S_i - k_2 E S_i)$$

$$\frac{ES_{i+1} - ES_i}{\Delta t} = k_1 E_i S_i - k_{-1} E S_i - k_2 E S_i$$

$$\frac{P_{i+1} - P_i}{\Delta t} = k_2 E S_i$$

Or, in terms of i+1:

$$S_{i+1} = (-k_1 E_i S_i + k_{-1} ES_i)\Delta t + S_i$$

$$E_{i+1} = (-k_1 E_i S_i + k_{-1} ES_i + k_2 ES_i)\Delta t + E_i$$

$$*ES_{i+1} = (k_1 E_i S_i - k_{-1} ES_i - k_2 ES_i)\Delta t + ES_i$$

$$P_{i+1} = k_2 ES_i \Delta t + P_i$$

* or: $ES_{i+1} = E_0 - E_{i+1}$

For example, to calculate S, must step off time:

$$S_1 = (-k_1 E_0 S_0 + k_{-1} E S_0)\Delta t + S_0$$

$$S_2 = (-k_1 E_1 S_1 + k_{-1} E S_1)\Delta t + S_1$$

$$S_3 = (-k_1 E_2 S_2 + k_{-1} E S_2)\Delta t + S_2$$

$$S_4 = (-k_1 E_3 S_3 + k_{-1} E S_3)\Delta t + S_3$$

Solve the computer problem
(see Example 3.2, pp. 73-74)

$k_1 = 30$ L/g•min

$k_{-1} = 160$/min

$k_2 = 110$/min

$S_0 = 10.0$ g/L

$P_0 = 0$ g/L

$E_0 = 0.00875$ g enzyme/L

$ES_0 = 0$ g/L

```
/*

Program 1

Simple Enzyme Kinetics

*/

#include<stdio.h>
#include<conio.h>
main()
{
   FILE *fout;
   double kp1;                          //  units = L/g min
   double km1,k2;                       //  units = /minutes
   double Snew,Pnew,Enew,ESnew;         //  units = g/L
   double Sold,Pold,Eold,ESold;         //  units = g/L
   double deltatime;                    //  units = minutes
   double currenttime;                  //  units = minutes
   double endtime;                      //  units = minutes
   int count=0;

// Define Some Constants

   kp1 = 30;
   km1 = 160;
   k2 = 110.;
   endtime = 30;
   deltatime=0.00001;

// Set Initial Conditions

   Sold = 10.;
   Pold = 0.;
   Eold = 0.00875;
   ESold = 0.;
```

Must terminate program at some point.
We will end at a time of 30 minutes.

Must use a small value for Δt.
The smaller, the more accurate (careful of computational precision!). However, the smaller this value, the longer the program will take to run. Note: with this Δt and end time, the program will run through 3 million iterations (30/0.00001).

```
// Calculations

   fout=fopen("a:ex3a1.dat","w");
   currenttime = 0.;
   do
     {
     Snew = (-kp1*Eold*Sold+km1*ESold)*deltatime+Sold;
     Enew = (-kp1*Eold*Sold+km1*ESold+k2*ESold)*deltatime+Eold;
     ESnew = (kp1*Eold*Sold-km1*ESold-k2*ESold)*deltatime+ESold;
     Pnew = k2*ESold*deltatime+Pold;
     count++;
     if (count==25000)
       {
                fprintf(fout,"%7.2f %7.4f %7.4f %6.4f\n",currenttime,Snew,Pnew,(Sold-Snew)/deltatime);
                count=0;
       }
     Sold=Snew;
     Eold=Enew;
     ESold=ESnew;
     Pold=Pnew;
     currenttime+=deltatime;
     }
   while(currenttime<endtime);
   fclose(fout);
}
```

This is the meat of the program. Calculate the "new" values for each of the four components (e.g., S_1), given the "old" values for the components (e.g., S_0).

Send every 25000th time point (0.25 min) to a file.

Must update the values for each of the four components, so that next time through the loop, they will be the "old" values.

Results……..

Figure 1a

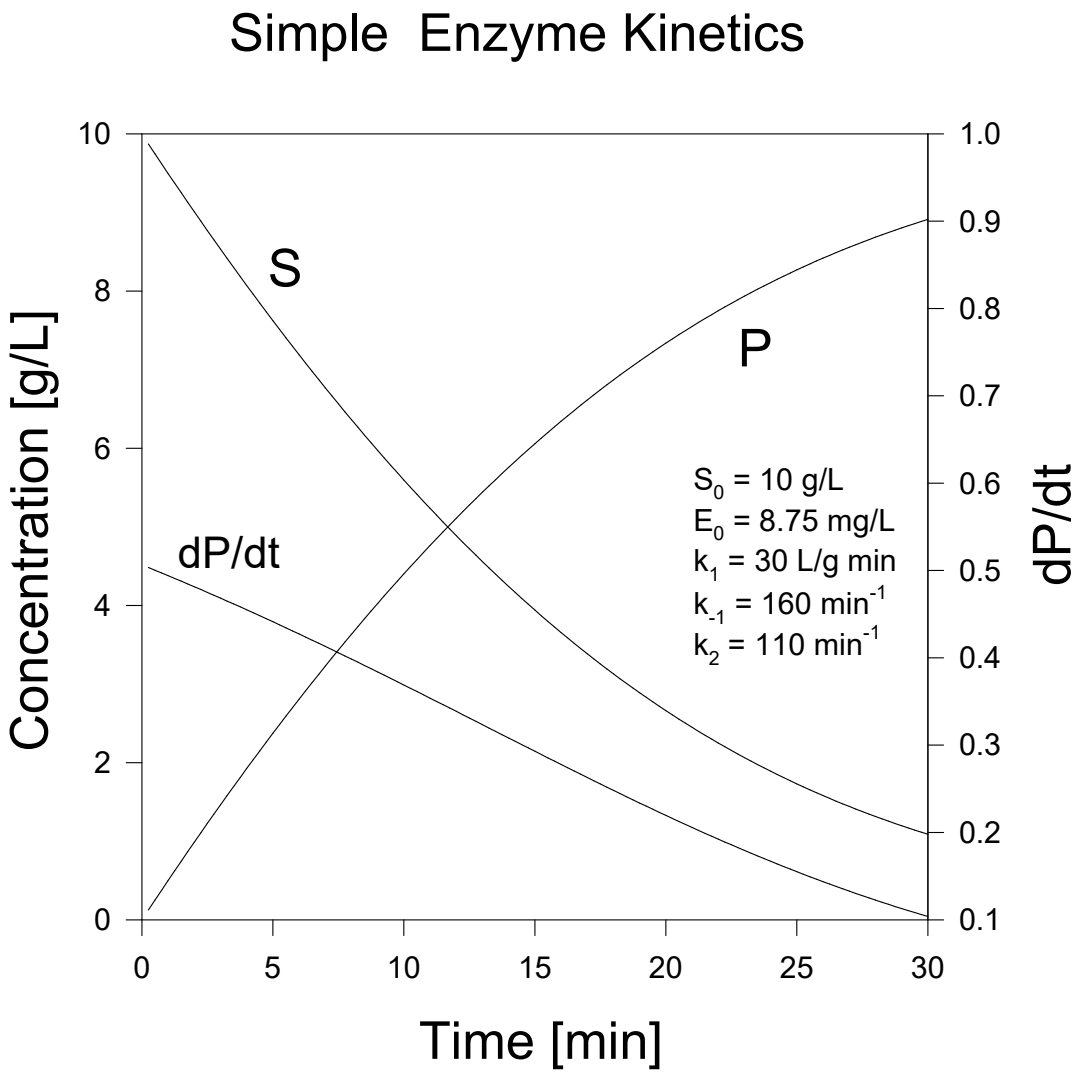

Simple Enzyme Kinetics
Concentration [g/L]
dP/dt
Time [min]
S
P
dP/dt
S_0 = 10 g/L
E_0 = 8.75 mg/L
k_1 = 30 L/g min
k_{-1} = 160 min^{-1}
k_2 = 110 min^{-1}
0
2
4
6
8
10
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0
5
10
15
20
25
30

Figure 1b

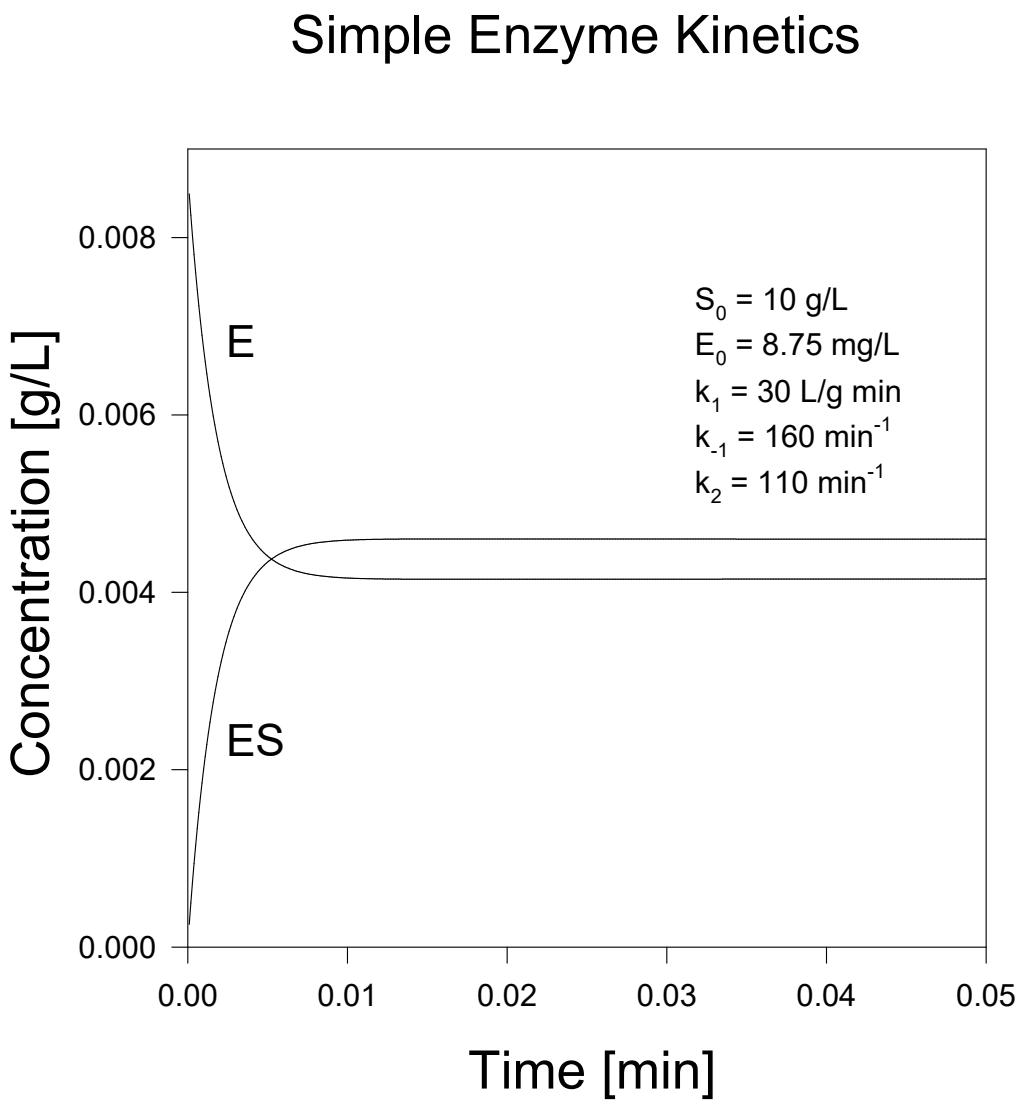
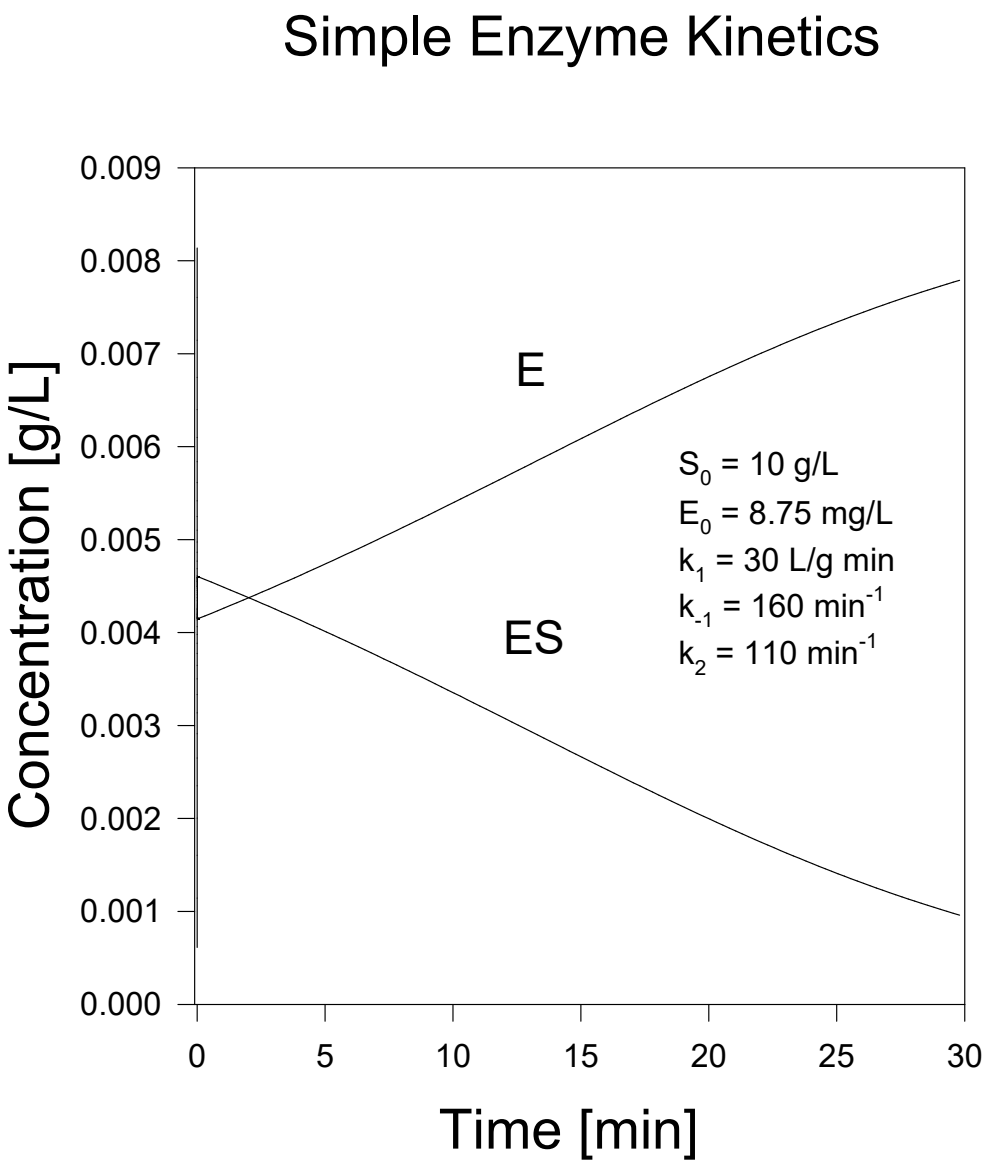

Simple Enzyme Kinetics
Concentration [g/L]
0.008
0.006
0.004
0.002
0.000
E
ES
S_0 = 10 g/L
E_0 = 8.75 mg/L
k_1 = 30 L/g min
k_{-1} = 160 min^{-1}
k_2 = 110 min^{-1}
0.00
0.01
0.02
0.03
0.04
0.05
Time [min]

Figure 1c


Simple Enzyme Kinetics
Concentration [g/L]
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0.000
E
ES
S0 = 10 g/L
E0 = 8.75 mg/L
k1 = 30 L/g min
k-1 = 160 min-1
k2 = 110 min-1
0
5
10
15
20
25
30
Time [min]

Notes:

1) dP/dt is not a constant (see Figure 1a). The reaction rate (i.e., the "reaction velocity") decreases with time. The rate of this decrease depends on the substrate concentration and the value of K_M. If the substrate concentration is very high initially (compared with K_M), then the rate of decline in dP/dt will be low.

   When we conduct our experiment (i.e., in a beaker), we must try our best to calculate the initial value of dP/dt, because in general the value of dP/dt will decrease the moment the experiment begins!

Notes:

2) Equilibrium cannot occur immediately (since ES=0 initially). The speed to equilibrium depends on the enzyme but is often quicker than can be measured.

Notes:

3) dES/dt is not equal to zero (in very short time scale – Figure 1b – and in very large time scale – Figure 1c). Thus, strictly speaking, the quasi-steady state assumption of Briggs and Haldane is not valid.

   It is invalid in a short time scale (up to 0.01 min in this particular case) because the reaction started with enzyme alone (ES=0).

Notes:

It is invalid at a long time scale because as the reaction proceeds, the concentration of S decreases. To maintain equilibrium, then, ES must decrease and/or E must increase. Since E+ES is a constant (E_0), both must occur.

$$K_D = \frac{k_{-1}}{k_1} = K_M' = \frac{[E][S]}{[ES]}$$

$$\frac{K_D}{[S]}\uparrow = \frac{[E]\uparrow}{[ES]\downarrow}$$

Notes:

4) Fortunately, we obtain the same parameters either from Michaelis-Menten or Briggs-Haldane (K_M and V_{MAX}), and they still very closely match the results from the differential equations. The time to reach equilibrium for most enzymatic reactions will be much shorter than we are capable of experimentally measuring.

Although the Michaelis-Menten and Briggs-Haldane equations may be developed from questionable premises, they work. Specifically, the two parameters (K_M and V_{MAX}) are readily calculable from simple experiments.

3. Determination of Michaelis-Menten Parameters

As described in Section 3.D.1, a series of batch experiments is conducted with different initial substrate concentrations, but with identical enzyme concentrations. Measure v_0 for each S_0.

The goal is to find the values for K_M and V_{MAX} which best fit the v_0 and S_0 data.

$$v_0 = \frac{V_{MAX} S_0}{K_M + S_0}$$

a. Hanes-Woolf Plot (also, Langmuir Plot)

Linearize the data in the following form:

$$\frac{S_0}{v_0} = \frac{K_M}{V_{MAX}} + \frac{S_0}{V_{MAX}}$$

Plot $\frac{S_0}{v_0}$ versus S_0 (y vs. x)

$$\text{Slope} = \frac{1}{V_{MAX}} \qquad \text{Intercept} = \frac{K_M}{V_{MAX}}$$

b. Lineweaver-Burke Plot

Linearize the data in the following form:

$$\frac{1}{v_0} = \frac{1}{V_{MAX}} + \frac{K_M}{V_{MAX}} \frac{1}{S_0}$$

Plot $\frac{1}{v_0}$ versus $\frac{1}{S_0}$

$$\text{Slope} = \frac{K_M}{V_{MAX}} \qquad \text{Intercept} = \frac{1}{V_{MAX}}$$

c. Eadie-Hofstee Plot

Linearize the data in the following form:

$$v_0 = V_{MAX} - K_M \frac{v_0}{S_0}$$

Plot v_0 versus $\frac{v_0}{S_0}$

Slope = $-K_M$ Intercept = V_{MAX}

Each of these three linearizations would provide precisely “correct” results if there were no random error associated with the measurement of v_0 and S_0. However, since such errors always occur, each of the linearizations will inappropriately weight one region of the data while essentially excluding another region. The best method of determining K_M and V_{MAX} is by a non-linear curve fit.

d. Non-linear curve fit

$$v_0^{CALC} = \frac{V_{MAX} S_0^{OBS}}{K_M + S_0^{OBS}}$$

$$\text{error} = \sum_{\substack{\text{all} \\ \text{data}}} \left(v_0^{CALC} - v_0^{OBS} \right)^2$$

$$\text{error} = \sum_{\substack{\text{all} \\ \text{data}}} \left(\frac{V_{MAX} S_0^{OBS}}{K_M + S_0^{OBS}} - v_0^{OBS} \right)^2$$

Goal: Find K_M and V_{MAX} which minimize error

Example of determining Michaelis-Menten Parameters

S (mmol/L)	V (mmol/Ls)	S/V	1/V	1/S	V/S
1.48	0.83	1.7831	1.2048	0.6757	0.5608
3.25	1.92	1.6927	0.5208	0.3077	0.5908
6.16	2.22	2.7748	0.4505	0.1623	0.3604
8.02	2.94	2.7279	0.3401	0.1247	0.3666
12.16	3.43	3.5452	0.2915	0.0822	0.2821
18.21	3.68	4.9484	0.2717	0.0549	0.2021

Example of determining Michaelis-Menten Parameters

	Slope	Intercept	Km	Vmax
Hanes-Woolf	0.1941	1.3181	6.79	5.15
Lineweaver-Burke	1.4903	0.1637	9.10	6.11
Eadie-Hofstee	-6.177	4.9342	6.18	4.93
Non-Linear Regression			6.34	5.05

Figure 2a

Enzyme Kinetics - Experimental Data

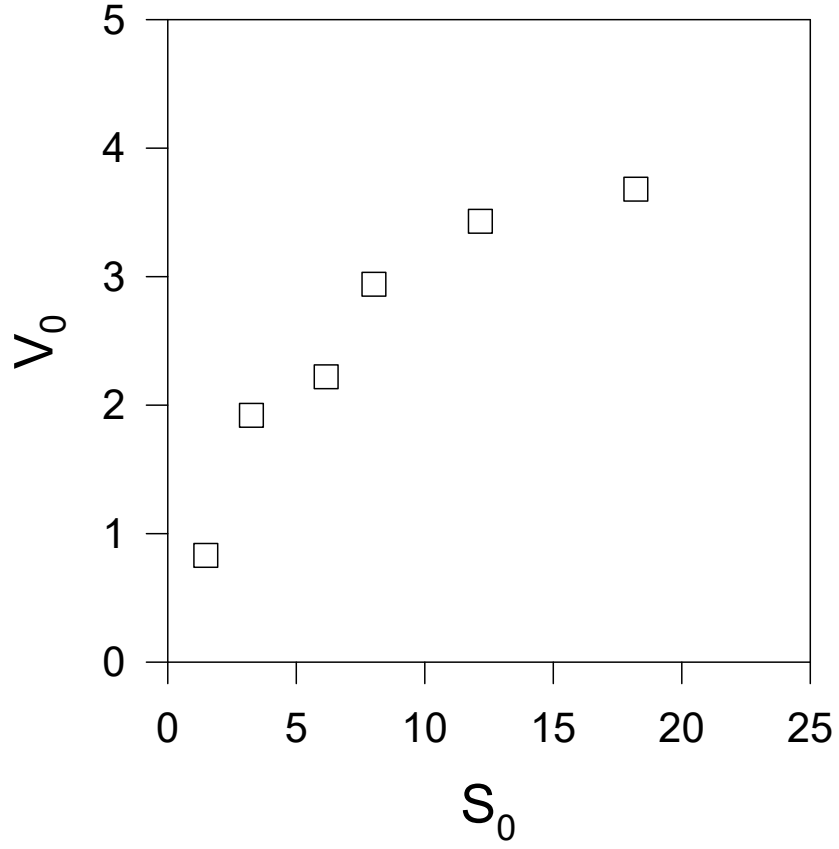


Figure 2b

Linearizations of Michaelis-Menten Equation

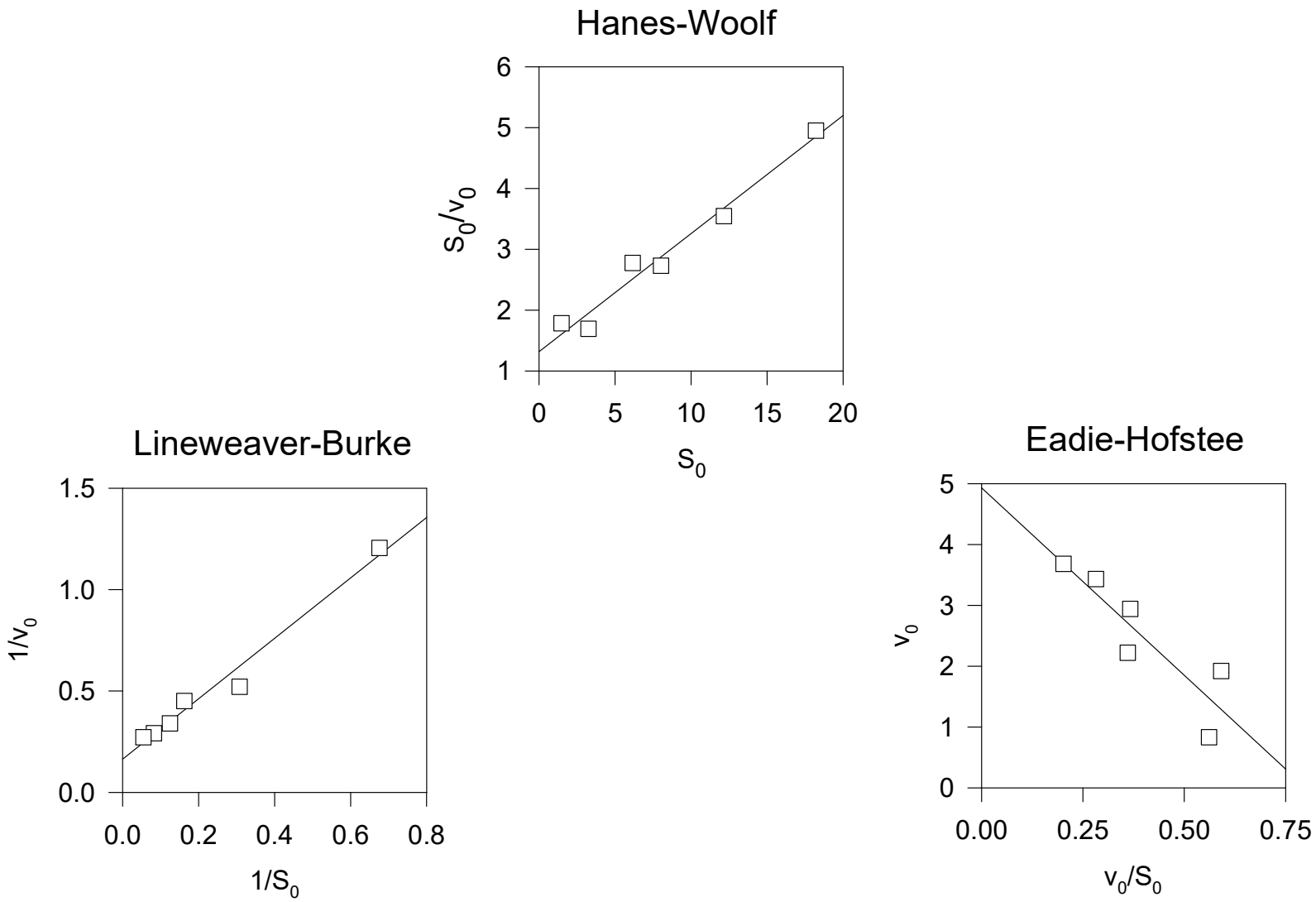
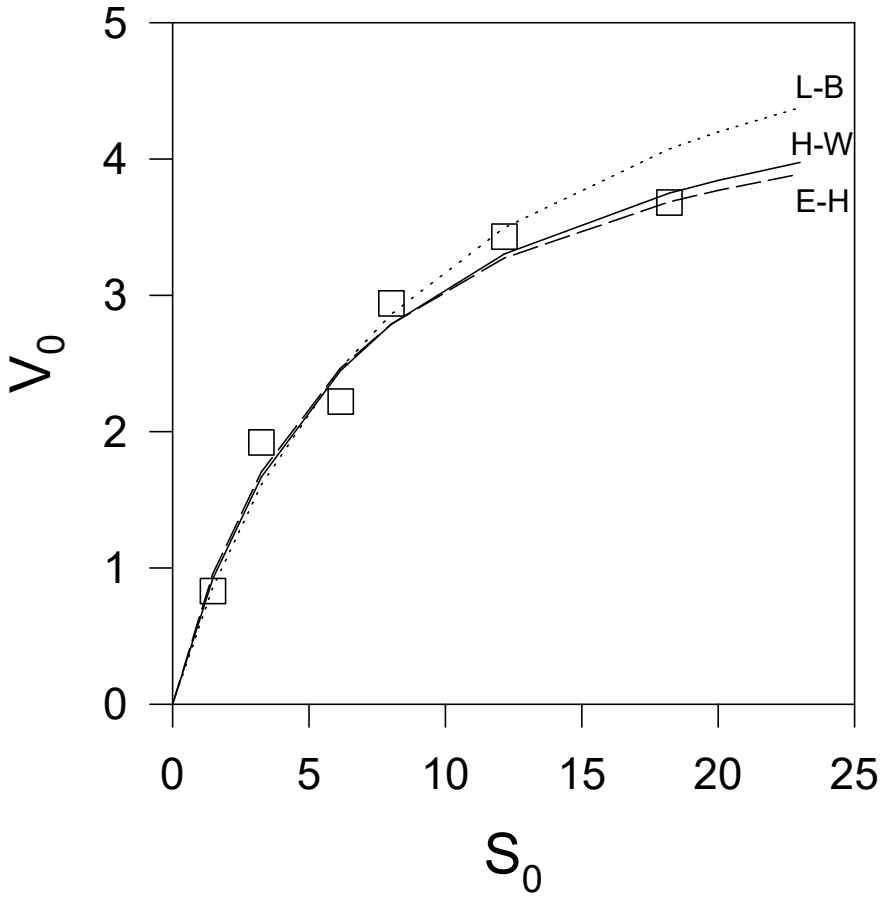


Figure 2c

Enzyme Kinetics - Model Fit



4. Some Related Kinetic Parameters

Note that $V_{MAX} \equiv k_2[E_0]$

V_{MAX} is not a very useful parameter to compare enzymes because it depends on the enzyme concentration!

What we would really like is a parameter which indicates the number of moles of substrate transformed per time per mole of active site - turnover number

The turnover number is merely k_2…just make sure that the units are correct….

Turnover Number $\equiv k_{CAT}$

$$= \frac{V_{MAX}\ (\text{mols/volume}\cdot\text{time})}{E_{ActSite}\ (\text{mols active site/volume})}$$

The time required for one catalytic cycle (per active site)

$$= \frac{1}{k_{CAT}}$$

Also, consider $\frac{dP}{dt} = \frac{V_{MAX}[S]}{K_M + [S]}$

or $\frac{dP}{dt} = \frac{k_{CAT}[S][E_{ActSite}]}{K_M + [S]}$

Recall if S is small, $S << K_M$, then

$$v = \frac{k_{CAT}[S][E_{ActSite}]}{K_M}$$

$\frac{k_{CAT}}{K_M}$ = pseudo first-order rate constant

Example

A purified enzyme is used in the conversion of A → B. The solution contains 5 mg/L of a dimeric enzyme having a total molecular weight of 82,340 g/mol (and two active sites). The molecular weight of A is 119 g/mol. If the V_{MAX} is found to be 0.13 g/Ls and K_M = 0.043 g/L, find the turnover number and the pseudo first-order rate constant.

$$k_{CAT} = \frac{V_{MAX}\ (\text{mols/volume}\cdot\text{time})}{E_{ActSite}\ (\text{mols active site/volume})}$$

$$\frac{(0.13\ \text{g/Ls})(\text{mol}/119\ \text{g})}{(5\ \text{mg/L})(\text{mol enz}/82{,}340\ \text{g})(\text{g}/1000\ \text{mg})(2\ \text{mol A site/mol enz})}$$

$= 9000\ s^{-1}$ ← turnover number
(the number of reactions per second at an active site)

$$\frac{1}{k_{CAT}} = 1.1 \times 10^{-4}\ \text{s} = 110\ \mu\text{s}$$

$$\frac{k_{CAT}}{K_M} = \frac{(9000\ s^{-1})}{(0.043\ g/L)(mol/119\ g)}$$

$$= 2.49 \times 10^7\ L/s \cdot mol$$

$$= 2.49 \times 10^4\ /smM$$ …typical units