Analyzing Enzyme Kinetic Data with a Graphing Calculator

PART A: LINEWEAVER-BURK PLOTS

In discussing the properties of an enzyme, certain values, or parameters are determined experimentally under *steady state conditions*. These values are determined through kinetics studies and include:

V_{max}: The so-called maximal rate of the catalyzed reaction. The enzyme's active site is saturated.

K_m: The Michaelis constant. The substrate concentration at which the reaction rate is one-half its maximum value. Also known as the turnover number.

These values are determined experimentally by recording the progress of an enzyme-catalyzed reaction using fixed amounts of enzyme and a series of different substrate concentrations.

A typical data set looks table one where V_o is the initial reaction velocity and $[S]_o$ is the substrate concentration.

Table one
Steady-state enzyme kinetic data

$[S]_o$ (μmol L^{-1})	V_o (μmol L^{-1} min^{-1})
0.1	0.3
2.0	5.0
10.0	20.0
20.0	40.0
40.0	64.0
60.0	80.0
100.0	100.0
200.0	120.0
1000.0	150.0
2000.0	155.0

V_{max} and K_m can be determined from linear regression analysis of a plot of $1/V_o$ vs. $1/[S]_o$, a so-called Lineweaver-Burk plot.

$$\frac{1}{V}$$

$$\frac{1}{V_{max}}$$

$$\frac{-1}{K_m}$$
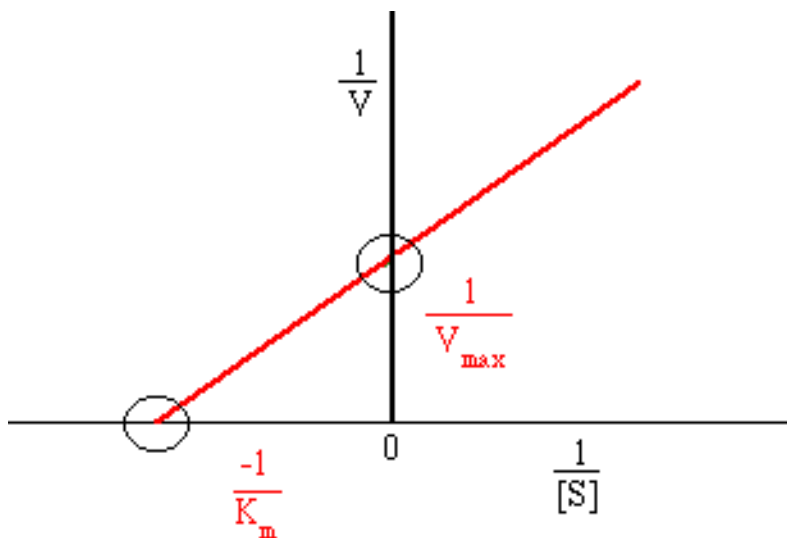
$$\frac{1}{[S]}$$

0

Figure one

A Lineweaver-Burk plot of enzyme kinetic data.

In a Lineweaver-Burk plot the inverse of the x and y-intercepts represent the kinetics constants K_m and V_{max} respectively.

Use the procedure below and a graphing calculator to determine the kinetics constants for the data in table one.

Procedure

- Enter the data from table one into your graphing calculator.

- Use the calculator to compute the inverse of the data sets entered in the above step and store this data in separate lists. This data represents the inverse values $1/V_o$ and $1/[S]_o$.

- Construct a scatterplot of $1/V_o$ vs. $1/[S]_o$.

```
2nd  Y=
Plots Off
ENTER
2nd  Y=
Plot1
ON
⊡ Xlist=L₃  Ylist=L₄ Mark: +
ZOOM
ZoomStat
```

- Determine the linear regression equation for the graph of $1/V_o$ vs. $1/[S]_o$ and record the equation coefficients.

```
STAT
CALC
LinReg(ax+b)
ENTER
L₃,L₄
ENTER
```

- Add the equation to the graph of the data.

```
Y=
Y1
VARS
Statistics
EQ
RegEQ
 GRAPH
```

- Adjust the graph window to include the x-intercept value.

```
WINDOW
Xmin = -Xmax
Xscl = Xmax-Xmin/10
Ymin = -Ymax
Yscal = Ymax-Ymin/10
```

- Determine the y-intercept value, which represents $1/V_{max}$.
- Calculate V_{max}. (91.80 μmol L^{-1} min^{-1} for the data in table one)

```
GRAPH
2nd  TRACE
Value
X=0
```

- Determine the x-intercept value which represents $-1/K_m$.
- Calculate K_m. (30.51 μmol L^{-1} for the data in table one)

```
GRAPH
2nd  TRACE
Zero
Use ◄  and ENTER to set the left
bound below the x-intercept.

Use ► and ENTER to set the right
bound above the x-intercept.

Use the arrow keys to place the
cursor close to the x-intercept.
and ENTER to guess.
```

Questions

Determine the kinetics constants for the following sets of data.

Set one

Steady-state enzyme kinetic data

$[S]_o$	V_o
(μmol L^{-1})	(μmol L^{-1} min^{-1})
0.5	50.0
1.0	75.0
2.0	100.0
3.0	112.5
10.0	136.4

Set two

Steady-state enzyme kinetic data

$[S]_o$	V_o
(μmol L^{-1})	(μmol L^{-1} min^{-1})
0.020	0.19
0.025	0.22
0.04	0.32
0.10	0.59
0.20	0.83
0.50	1.17
1.00	1.41
2.00	1.63

Enzyme catalyzed reaction rates are often affected by substances that inhibitor or interfere with the enzymes interaction with the substrate(s). There are three basic types of inhibition: noncompetitive, uncompetitive, and competitive inhibition.

The type of inhibition can be determined from graphical analysis of experimental data with Lineweaver-Burk plots. Inhibition experiments involve a series of experiments with fixed amount of inhibitor added to varying amounts of substrate. Lineweaver-Burk plots are constructed showing multiple lines for the various inhibitor concentrations.

The inhibition types and their graphical characteristics are summarized below and graphically in figure two.

| Inhibition type	Definition	Graphical characteristics
noncompetitive	The inhibitor binds the enzyme at a different site than the substrate causing a conformational change. The conformational change affects the rate of catalysis, but the overall turnoff remains constant.	The slopes of Lineweaver-Burk plots are different, as are the y-intercepts. Yet, the x-intercepts remain constant.
competitive	The inhibitor competes for the enzymes binding site with the substrate. The proportion of substrate molecules bound by the inhibitor reduces the rate of catalysis.	The slopes of Lineweaver-Burk plots are different, yet the y-intercepts are the same.
uncompetitive	The inhibitor binds the active site after the substrate. Binding of the inhibitor can stimulate the binding, but produces a non-productive complex.	The slopes of Lineweaver-Burk plots are the same and both intercepts are different.
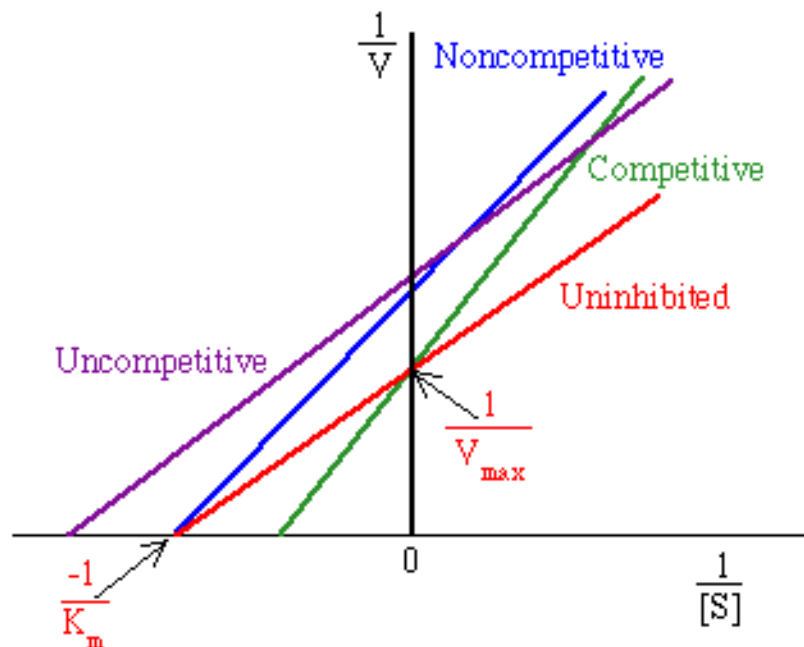
Figure two
Lineweaver-Burk plot showing inhibition experiments with fixed amounts of inhibitor added to varying amounts of substrate.

Table two
Enzyme inhibition data

	without inhibitor	with inhibitor
$[S]_o$ $\mu mol\ L^{-1}$	V_0 $\mu mol\ L^{-1}\ min^{-1}$	V_0 $\mu mol\ L^{-1}\ min^{-1}$
0.0000027	11.96	7.80
0.0000045	16.68	10.11
0.0000091	25.88	12.99
0.000027	38.87	16.04
0.000081	46.58	17.41

Use the procedure below and a graphing calculator to determine the type of inhibition for the data in table two.

<div align="center">

Procedure

</div>

- Enter the data from table two into your graphing calculator.

DIRECTIONS FOR TI-82/83
$\boxed{Y=}$ Clear all equations
$\boxed{STAT}$ EDIT
$L_1 = S_o$
$L_2 = V_o$ without inhibitor
$L_3 = V_o$ with inhibitor

- Use the calculator to compute the inverse of data sets entered in the above step and store this data in separate lists. This data represents the inverse values $1/V_o$ and $1/[S]_o$.

$L_4 = 1/L_1$
$L_5 = 1/L_2$
$L_6 = 1/L_3$

- Construct two scatterplots of $1/V_o$ vs. $1/[S]_o$.

$\boxed{2nd}$ $\boxed{Y=}$
Plots Off
$\boxed{ENTER}$
$\boxed{2nd}$ $\boxed{Y=}$
Plot1
ON
Xlist=L_4 Ylist=L_5 Mark: +
Plot2
ON
Xlist=L_4 Ylist=L_6 Mark: ·
$\boxed{ZOOM}$
ZoomStat

- Determine the linear regression equation for the graph of $1/V_o$ vs. $1/[S]_o$ and record the equation coefficients.
- Evaluate the equation coefficients and determine the inhibition type (*uncompetitive* in the case of the data from table two).

$\boxed{STAT}$
CALC
LinReg(ax+b)
ENTER
L_4, L_5
ENTER
$\boxed{STAT}$
CALC
LinReg(ax+b)
ENTER
L_4, L_6
ENTER

Questions

Determine the types of inhibition for the following sets of data:

Set one
Enzyme inhibition data

	without inhibitor	with inhibitor
$[S]_o$ $\mu mol\ L^{-1}$	V_o $\mu mol\ L^{-1}\ min^{-1}$	V_o $\mu mol\ L^{-1}\ min^{-1}$
0.000003	10.4	4.1
0.000005	14.5	6.4
0.00001	22.5	11.3
0.00003	33.8	22.6
0.00009	40.5	33.8

Set two
Enzyme inhibition data

	without inhibitor	with inhibitor
$[S]_o$ $\mu mol\ L^{-1}$	V_o $\mu mol\ L^{-1}\ min^{-1}$	V_o $\mu mol\ L^{-1}\ min^{-1}$
0.000003	10.4	2.1
0.000005	14.5	2.9
0.00001	22.5	4.5
0.00003	33.8	6.8
0.00009	40.5	8.1