

Diazepam Metabolism in Vitro: Michaelis-Menten Kinetics

Case Study

- How to change the name of the independent variable
- How to create a Michaelis-Menten enzyme kinetic model
- How to fit the model to the data
- How to produce the Lineweaver-Burke, Hanes and Eadie-Hofstee plots

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Diazepam Metabolism in Vitro: Michaelis-Menten Kinetics

Prerequisites

The prerequisite for this case study is having worked through the SAAM II introductory tutorial, "Getting Started with SAAM II Numerical."

What you will learn in this case study

- How to change the name of the independent variable "t" to "s".
- How to create a Michaelis-Menten enzyme kinetic model.
- How to fit the model to the data.
- How to produce the Lineweaver-Burke, Hanes and Eadie-Hofstee plots.

Data Required

The data file for this case study is

diazep_1.dat

The data file is discussed in the introduction.

Introduction

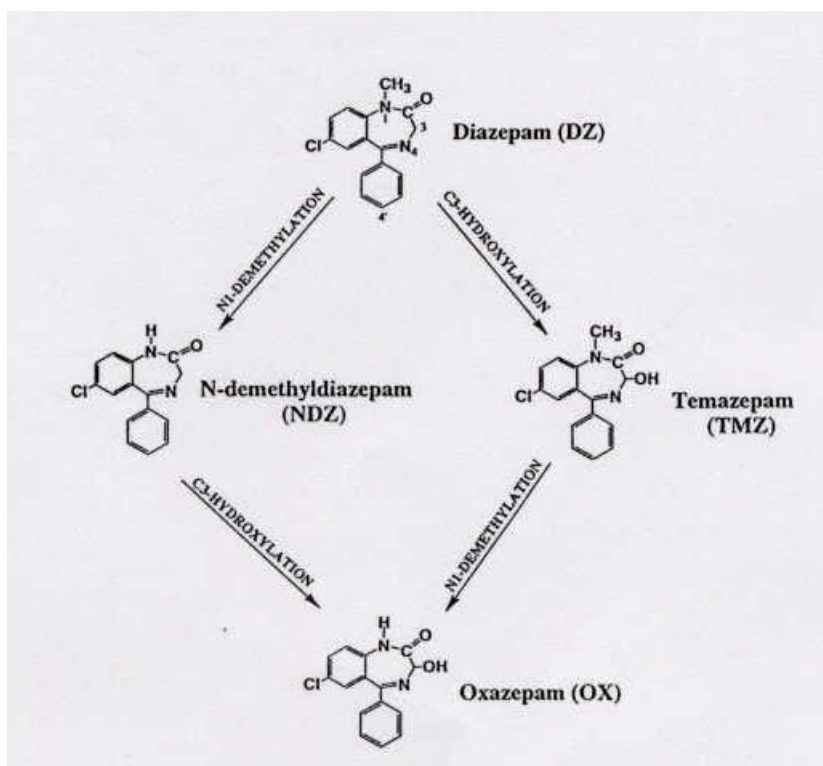
Analysis of processes involved in the transport and metabolism of a number of drugs and other compounds may require the use of models that incorporate the Michaelis-Menten equation which has the form:

$$v = \frac{V_{\max} * C}{K_m + C}$$

where v is the velocity of the reaction, C is the concentration of substrate, V_{\max} is the maximal velocity of the reaction, and K_m is the substrate concentration when $v = V_{\max} / 2$. The goal of an investigator is to estimate V_{\max} and K_m .

The use of human liver samples and microsomes has played an increasingly important role in identifying the different cytochrome P450 drug metabolizing enzymes, referred to as CYPs, and in elucidating drug metabolic pathways and rates during preclinical drug development and subsequent metabolism and pharmacogenetic investigations. This case study is based on an *in vitro* analysis of diazepam (Valium) metabolism using human liver microsomes [1]. As shown below, diazepam metabolism is mediated by a number

of P450 enzymes and proceeds by both N-dealkylation and C3-hydroxylation. This results in more kinetic complexity than if a single metabolic pathway and P450 enzyme were involved.



Investigators traditionally have transformed their data to estimate V_{max} and K_m values and to evaluate the appropriateness of Michaelis-Menten models. The Lineweaver-Burke is probably the most common transformation. In this so-called double reciprocal plot, one writes

$$\frac{1}{v} = \frac{K_m}{V_{max}} * \frac{1}{C} + \frac{1}{V_{max}}$$

In other words, one transforms the data so that the reciprocal of the velocity can be plotted as a function of the reciprocal of the substrate concentration. If a single Michaelis-Menten expression is appropriate for the data, the double reciprocal plot is a straight line from which it is easy to estimate V_{max} and K_m .

Two other linearizing plots are the Hanes and Eadie-Hofstee. The Hanes equation is:

$$\frac{C}{v} = \frac{K_m}{V_{max}} + \frac{1}{V_{max}} * C$$

and the Eadie-Hofstee equation is:

$$v = -K_m \left(\frac{v}{C} \right) + V_{\max}$$

The Hanes equation is also a straight line whose slope is $1/V_{\max}$ and intercept K_m/V_{\max} . The Eadie-Hofstee plot is a plot of v against v/s whose slope is K_m and intercept V_{\max} on the v -axis and V_{\max}/K_m on the v/s axis. The Eadie-Hofstee plot is useful in that it magnifies any departure of linearity (meaning there is more complexity than a single Michaelis-Menten term in the data) more than the Lineweaver-Burke plot

These and other transformations have a tendency to produce estimates of V_{\max} and K_m that are in error because the error structure in the data has not been handled properly in the transformation. More specifically, if d_1 is a datum with standard deviation sd_1 , then the standard deviation of $1/d_1$ is not $1/sd_1$. The result of these manipulations of the data is that smaller data end up with larger weights resulting in a skewing of the curve, and erroneous estimates of the desired parameters.

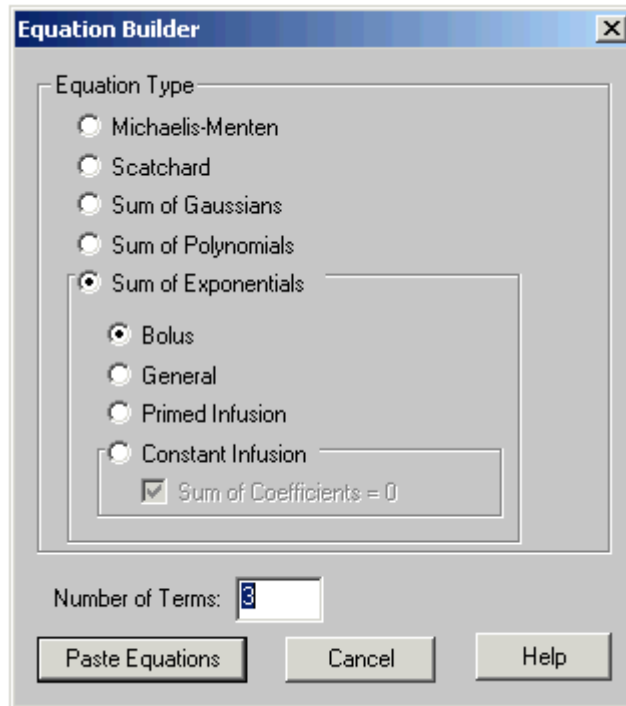
In this case study, you will learn how to fit the Michaelis-Menten model directly to your data thus eliminating the error problems caused by the transformations, and produce the desired transformation plots which are often more useful in presenting results.

1. Kato, R., Yamazoe, Y. "The importance of substrate concentration in determining cytochromes P450 therapeutically relevant in vivo." *Pharmacogenetics* 1994,4:359-62.

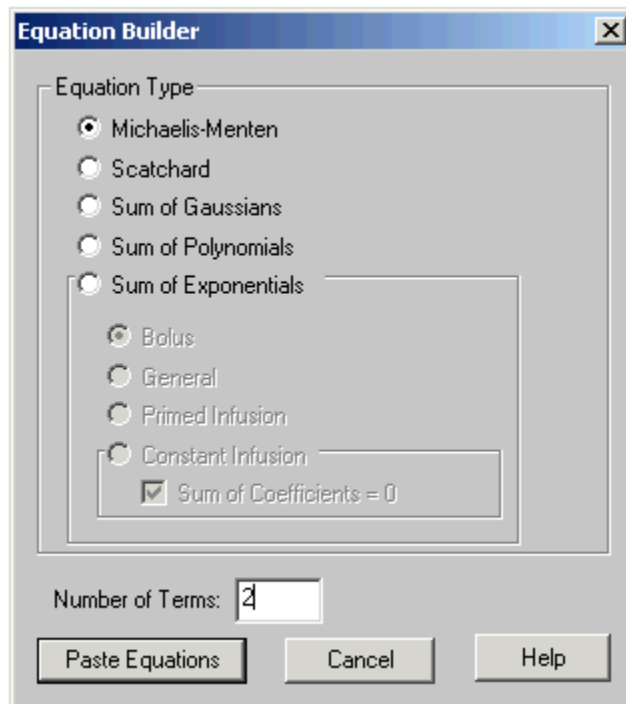
Computational Options

To create your Michaelis-Menten model, you have two choices. One is to use the **Equation Builder** in the **SAAM II Numerical Toolbox**. The other is to enter the equations directly, which is what we will do in this case study.

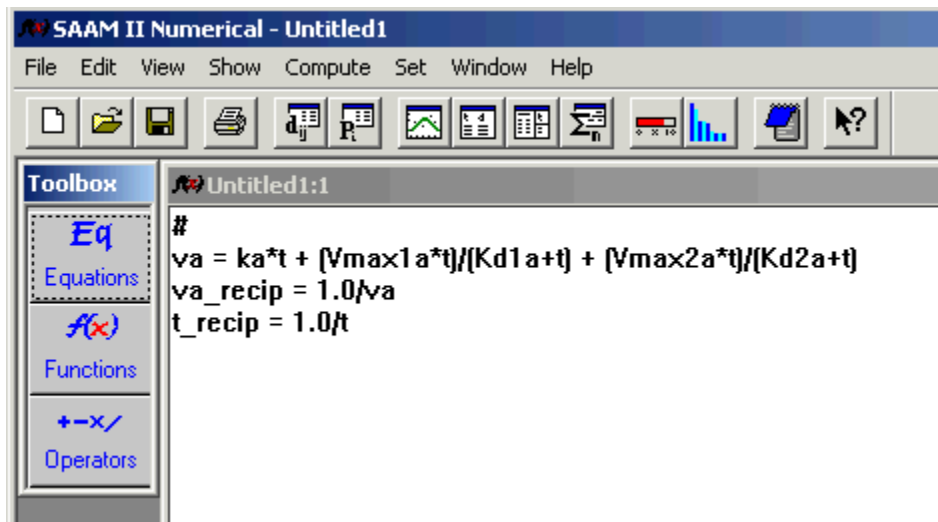
To use the **Equation Builder** in the **SAAM II Numerical Toolbox**, you click on **Equation Builder** tool. The **Equation Builder** dialog box will open as follows:



There are many pre-defined options available. If you select the **Michaelis-Menten** option and, for example, change the **Number of Terms** from “3” to “2”, the **Equation Builder** dialog box will appear as follows:



If you now click **Paste Equations**, the following equations will appear on the **SAAM II Numerical Working Canvas**:



The first equation is the velocity reaction containing the linear term $ka*t$ where ka is a parameter to be estimated, and two Michaelis-Menten terms where the two maximal velocities V_{max1a} and V_{max2a} and two half-saturation concentrations $Kd1a$ and $Kd2a$ (the equivalents of K_m in the Michaelis-Menten equation, and used interchangeably depending upon the situation) can be estimated from the data. In this expression, the independent variable is written t ; this is concentration rather than the time. The additional equations va_recip and t_recip let you set up the Lineweaver-Burke plot.

In this case study, we will not use the predefined equations because we will use “s” instead of “t” as the independent variable. In addition, besides the Lineweaver-Burke plot, we will also show how to create the Hanes and Eadie-Hofstee plots.

The data generated from the K_m and V_{max} estimates of Kato and Yamazoe (see [1] above) are shown below:

s	sample1
0	n
5	7.77E-02
10	0.135
20	0.238
40	0.352
60	0.446
80	0.549
100	0.655
120	0.694
140	0.743
160	0.852
180	0.871
220	0.99
240	1.039
260	1.047
280	1.043
300	1.129
320	1.101
340	1.153
360	1.185
380	1.274
400	1.291

Diazepam concentration is given in the “s” column and a measure of the velocity of the reaction is in the “sample1” column (the units are $\text{nmol min}^{-1}/\text{mg P450 protein}$). These are the data that will be described by the Michaelis-Menten model. However, to produce the Lineweaver-Burke, Hanes and Eadie-Hofstee plots, the data need to be transformed. The easiest way to do this is using a spreadsheet.

To produce the Lineweaver-Burke plot, you need the reciprocal of the sample. We will call this `smp1recip`. This can be calculated by taking the reciprocal of each sample. In the data shown below, these appear in the column “`smp1recip`”. For the Hanes plot, you need the quotient of the concentration and velocity, `s/sample1`. These are given in the column “`smp1sv`” column below. Finally, for the Eadie-Hofstee plot, you need the quotient of the sample divided by the velocity, `sample1/s`. These appear in the column “`smp1vs`”:

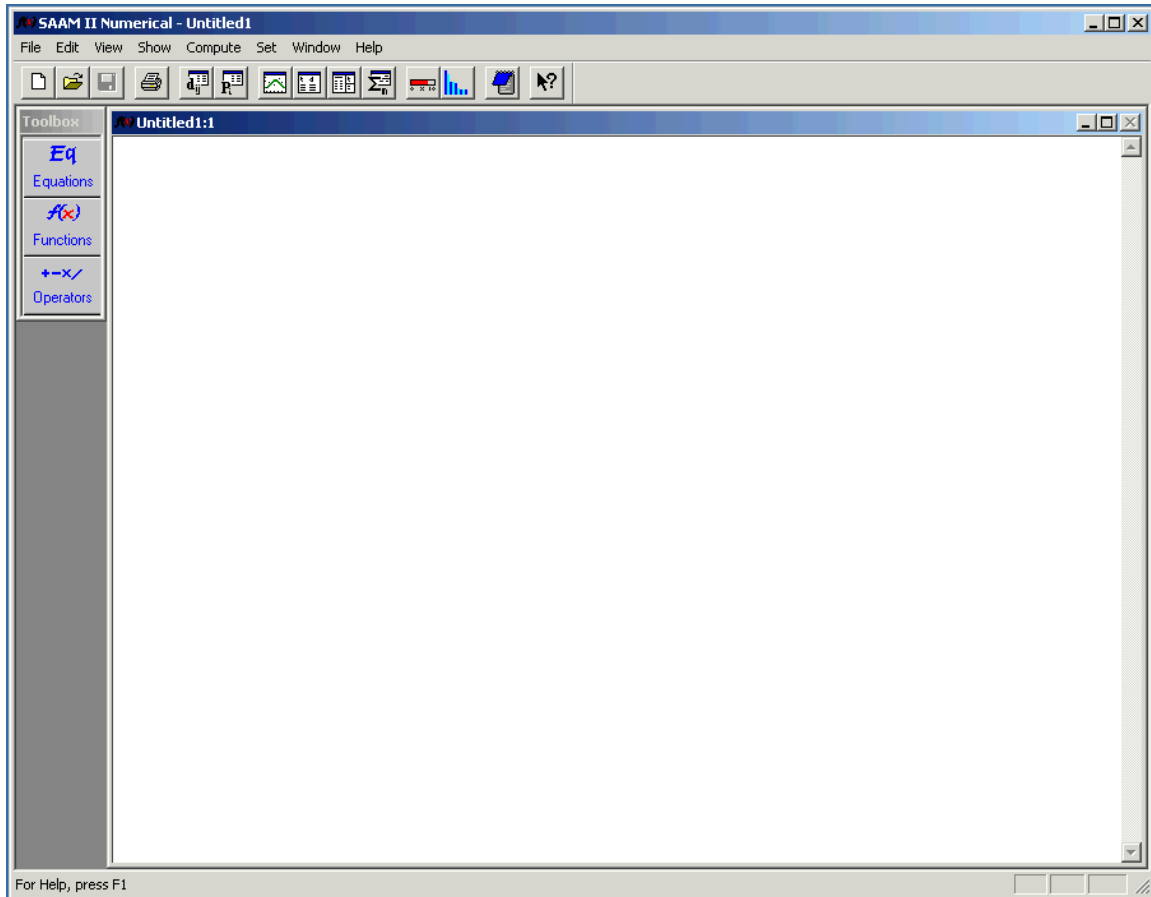
s	sample1	smp1recip	smp1sv	smp1vs
0	n	n	n	n
5	7.77E-02	12.870	64.350	0.01554
10	0.135	7.407	74.074	0.01350
20	0.238	4.202	84.034	0.01190
40	0.352	2.841	113.636	0.00880
60	0.446	2.242	134.529	0.00743
80	0.549	1.821	145.719	0.00686
100	0.655	1.527	152.672	0.00655
120	0.694	1.441	172.911	0.00578
140	0.743	1.346	188.425	0.00531
160	0.852	1.174	187.793	0.00533
180	0.871	1.148	206.659	0.00484
220	0.99	1.010	222.222	0.00450
240	1.039	0.962	230.991	0.00433
260	1.047	0.955	248.329	0.00403
280	1.043	0.959	268.456	0.00373
300	1.129	0.886	265.722	0.00376
320	1.101	0.908	290.645	0.00344
340	1.153	0.867	294.883	0.00339
360	1.185	0.844	303.797	0.00329
380	1.274	0.785	298.273	0.00335
400	1.291	0.775	309.837	0.00323

These are the data in the file **diazep_1.dat**.

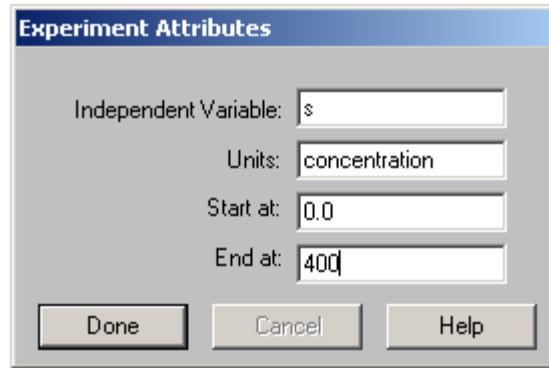
Part 1. Analyze data from a process believed to exhibit Michaelis-Menten kinetics.

In this part of the case study, you will plot the data to determine what kind of a Michaelis-Menten model you will use first. For the model, there are essentially three choices: a single Michaelis-Menten term, a single linear and a single Michaelis-Menten term, and two Michaelis-Menten terms. You would choose the first if the Lineweaver-Burke and Eadie-Hofstee plots of the data are straight lines. You would choose the second if the last part of the data plot is a straight line. You would choose the last if the last part of the curve of the data still exhibited curvature, and neither the Lineweaver-Burke nor Eadie-Hofstee plots are linear. Thus in examining the data, you will look at a plot of the data, and the Lineweaver-Burke and Eadie-Hofstee plots.

1. **Start the SAAM II Numerical** application. The **SAAM II Numerical** main window will open as follows:



2. Specify the experimental attributes.
 - a. In the **Set** menu, click **Experimental Attributes**. The **Experimental Attributes** dialog box will open.
 - b. In the **Independent Variable** box, rename “t” to “s”.
 - c. In the **Units** box, type “concentration”.
 - d. In the **End At** box, enter “400”. The **Experimental Attributes** dialog box will appear:



Experiment Attributes


Independent Variable: s

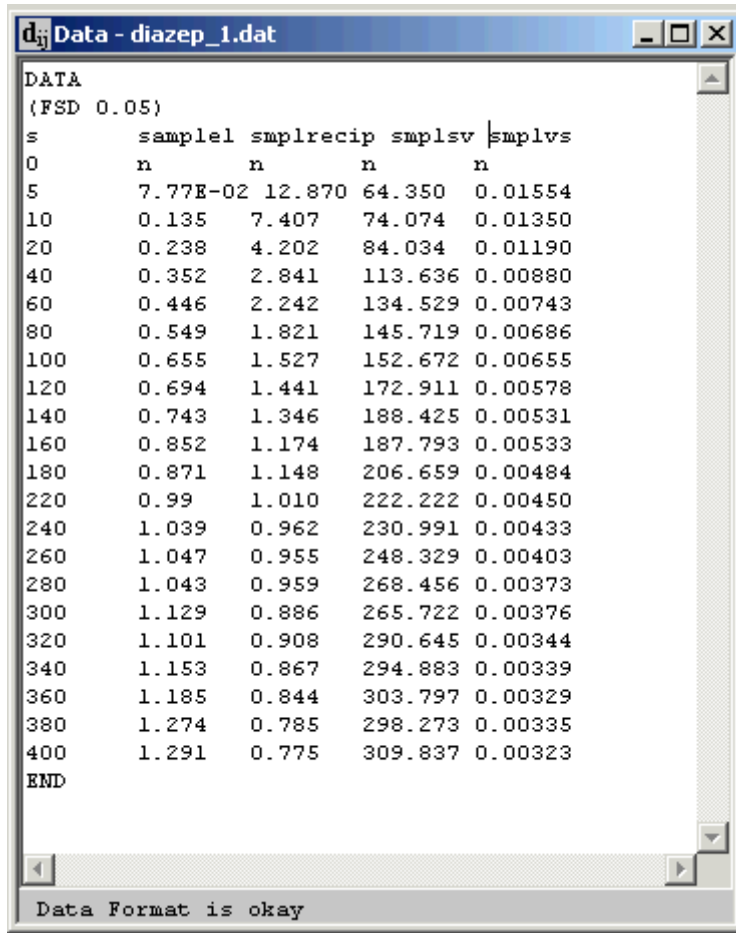
Units: concentration

Start at: 0.0

End at: 400

Done Cancel Help

- e. Click **Done**.
3. Add the data to your model.
 - a. In the **Show** menu, click **Data**, or alternatively, on the **SAAM II Toolbar**, click **Data** . The **Data** window will open.
 - b. In the **File** menu, click **Open**. The file **diazep_1.dat** should appear in the list (if it does not, find the folder where you put this data file).
 - c. Double-click **diazep_1.dat**. The data in this file will appear in the **Data** window as follows:



```


DATA
(FSD 0.05)
s      sample1 smp1recip smp1sv |smp1vs
      n        n         n      |n
0          7.77E-02 12.870 64.350 |0.01554
5          0.135   7.407  74.074 |0.01350
10         0.238   4.202  84.034 |0.01190
20         0.352   2.841 113.636 |0.00880
40         0.446   2.242 134.529 |0.00743
60         0.549   1.821 145.719 |0.00686
80         0.655   1.527 152.672 |0.00655
100        0.694   1.441 172.911 |0.00578
120        0.743   1.346 188.425 |0.00531
140        0.852   1.174 187.793 |0.00533
160        0.871   1.148 206.659 |0.00484
180        0.99    1.010 222.222 |0.00450
220        1.039   0.962 230.991 |0.00433
240        1.047   0.955 248.329 |0.00403
260        1.043   0.959 268.456 |0.00373
280        1.129   0.886 265.722 |0.00376
300        1.101   0.908 290.645 |0.00344
320        1.153   0.867 294.883 |0.00339
340        1.185   0.844 303.797 |0.00329
360        1.274   0.785 298.273 |0.00335
380        1.291   0.775 309.837 |0.00323
END
Data Format is okay

```

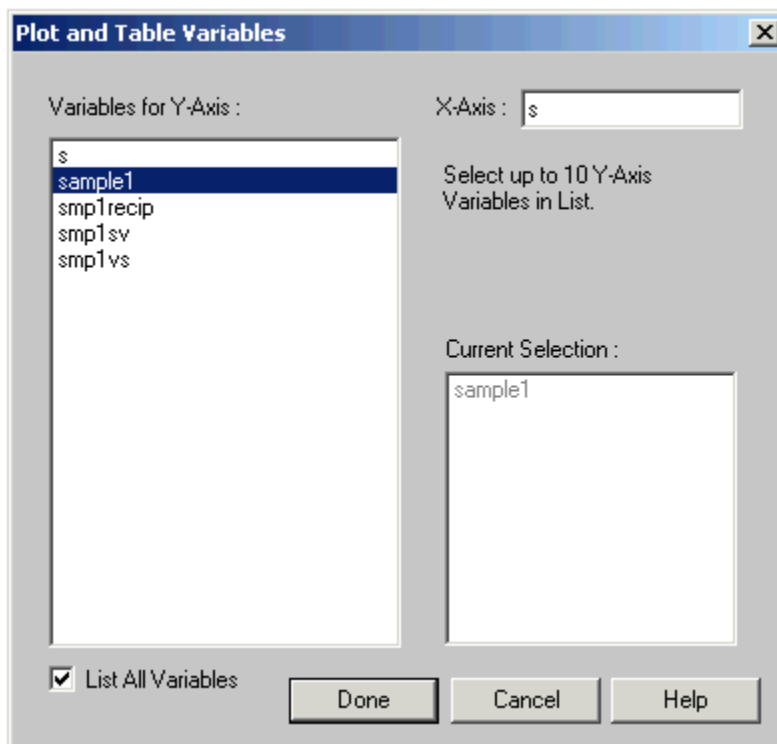


The data file. The contents of the data file have been explained in the introduction. The column “sample1” are the actual data while the remaining columns are transformed data that will permit the Lineweaver-Burke, Hanes and Eadie-Hofstee plots.



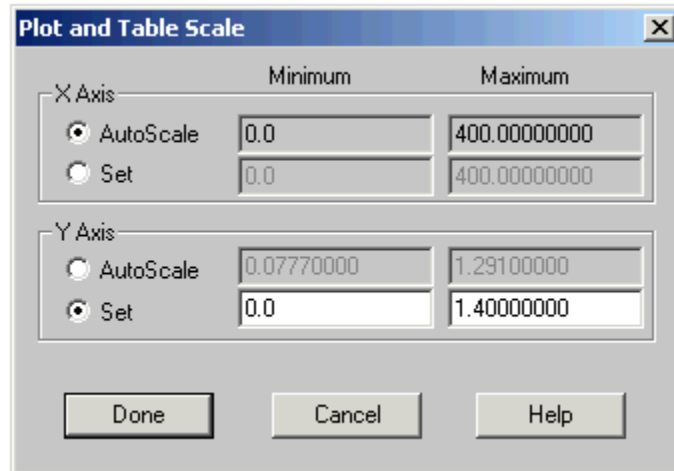
- d. Close the **Data** window.
4. View your data using a line plot.
 - a. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . The **Plot and Table Variables** dialog box will open.
 - b. Be sure the **List All Variables** check box is selected. (Click this box if it is not selected).

- c. Click **sample1** to move it to the **Current Selection** pane. The **Plot and Table Variables** dialog box will appear as follows:

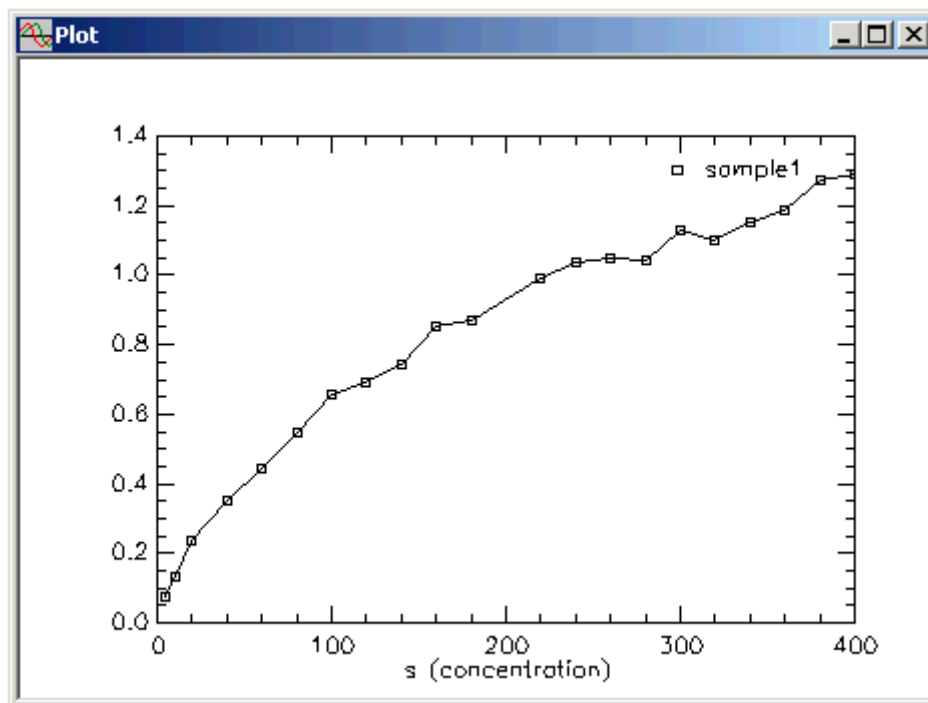


Note the name of the variable on the X-Axis is "s", the name of the independent variable you assigned in the **Experimental Attributes** dialog box.

- d. Click **Done**. A plot of the data will appear in the **Plot** window. It is useful to change the scale.
- e. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open. Change the Y-Axis scale as shown in the follow **Plot and Table Scale** dialog box:



- f. Click **Done**.
- g. In the **View** menu, click **Line Plot**. If the plot is not in linear mode, in the **View** menu, click **Semilog** to make the plot linear. Your plot will appear as follows:



You can see there is still some curvature towards the end of the data, so a linear component to your model (discussed below) will not be tested.

- h. Close the **Plot** window.



Line Plots. The data can be visualized as a linear or semilog plot after they have been loaded into the SAAM II **Data** window. This can be very useful in helping you decide with which model you would like to start your data analysis



5. View your data using the Lineweaver-Burke and Eadie-Hofstee plots.

Remember the Lineweaver-Burke equation is:

$$\frac{1}{v} = \frac{K_m}{V_{\max}} * \frac{1}{C} + \frac{1}{V_{\max}}$$

In our model, “s” will play the role of “C” in the equation. So the Lineweaver-Burke plot is a plot of “1/s” versus 1/v. The data have already been entered in the **Data** window transformed for this plot; they are called “smp1recip”. Thus to produce the Lineweaver-Burke plot, we need to specify “1/s”. This is done by entering an equation on the **Working Canvas**.

- a. Type the following on the **Working Canvas**:

```
#
# The following equation defines a variable for the Lineweaver-Burke plot.
# The addition of the term "0.00001" in the denominator for s_recip will
# prevent division by zero when s = 0.
#
s_recip = 1/(s + 0.00001)
#
```



The **Working Canvas** will appear, in part, as follows:

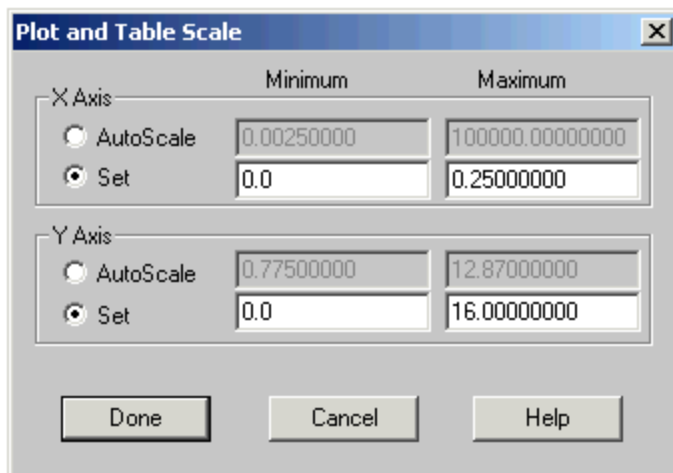
```
Untitled1:1
#
# The following equation defines a variable for the Lineweaver-Burke plot.
# The addition of the term "0.00001" in the denominator for s_recip will
# prevent division by zero when s = 0.
#
s_recip = 1/[s + 0.00001]
#
```



Comments in SAAM II Numerical. Comment lines can be added to your equation set by starting the comment line with the pound key “#”.



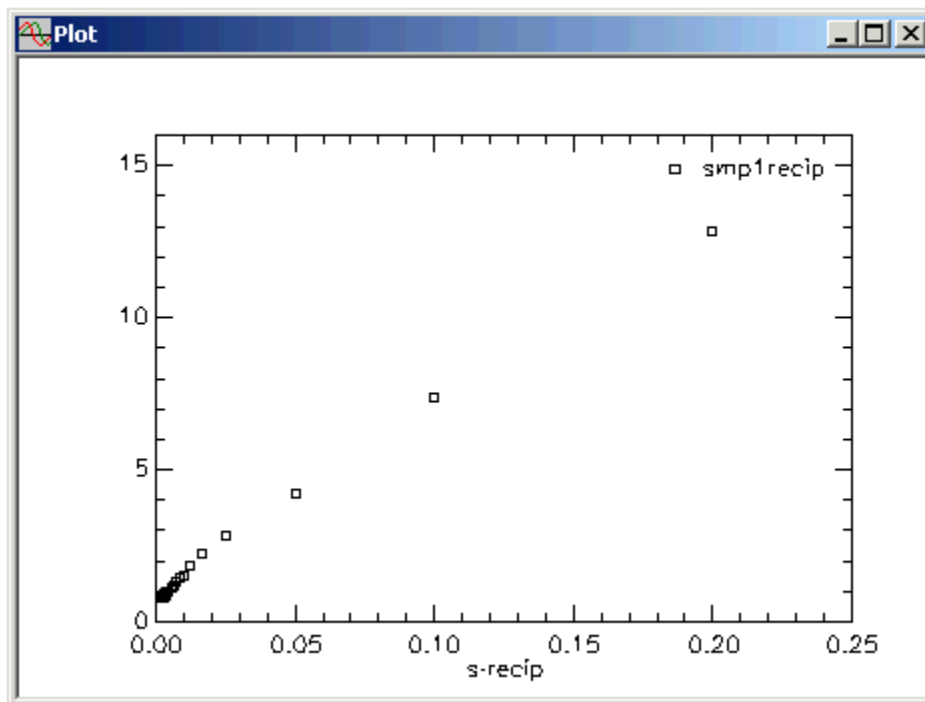
- b. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . Your previous plot of the data (no line plot) should appear.
- c. In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open. Be sure the **List All Variables** check box is selected. (Click this box if it is not selected).
- d. Type “s_recip” in the **X-Axis** box (this will change the variable on the X-Axis from “s” to “s_recip.”)
- e. Click **smp1recip** in the **Variable for Y-Axis** pane to move this to the **Current Selection** pane.
- f. Click **Done**. The **Plot** window will appear with nothing in it. This is because SAAM II remembers the plot scale you set previously when you examined the data. You need to reset the plot scale. In addition, you need to Solve in order to activate the equation “s_recip = 1/(s + 0.00001).”
- g. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II Toolbar**, click **Solve** .
- h. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open. Change the X-Axis and Y-Axis scales as shown in the following **Plot and Table Scale** dialog box:



Setting the X-Axis scale. The reason why the X-Axis scale had to be changed was because you were plotting (1/s) as the variable. The values for “s” range from “0” to “400”. Of course there is no “1/0” possible, so SAAM II substituted a very large number. The first datum for “s” was “5” the reciprocal of which is 0.2. Choosing “0.25” as the Maximum just gives a little more room on the plot.



- i. Click **Done**. Your plot will appear as follows:



You can see this plot is not linear, so more than one Michaelis-Menten term will be required in your model. If you create a plot as a line plot, the deviation, especially at the beginning, will be evident.

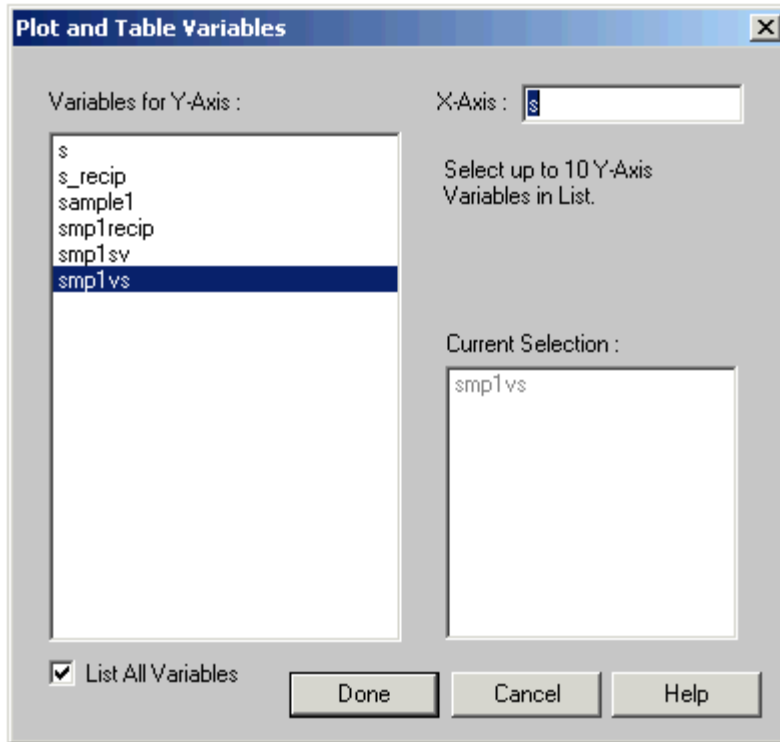
- j. Leave the **Plot** window open.

You will now examine the Eadie-Hofstee plot. Remember the Eadie-Hofstee equation is:

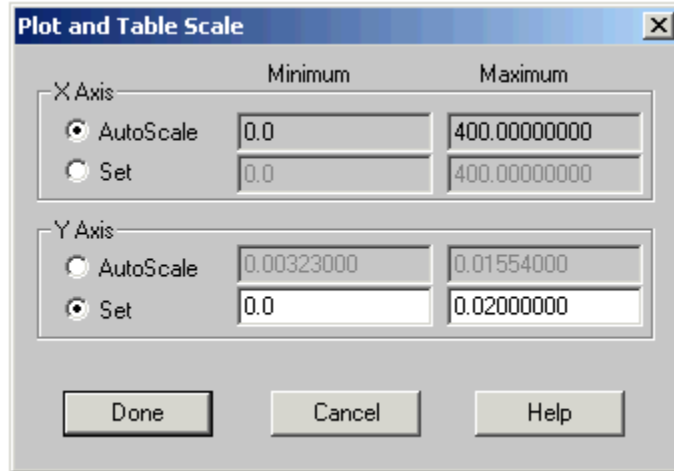
$$v = -K_m \left(\frac{v}{C} \right) + V_{\max}$$

Remembering “C” is replaced by “s” in our model, this is a plot of the data divided by “s” versus “s”. The corresponding “v/C” has been calculated in the **Data** window; it is in the column named “smp11vs”.

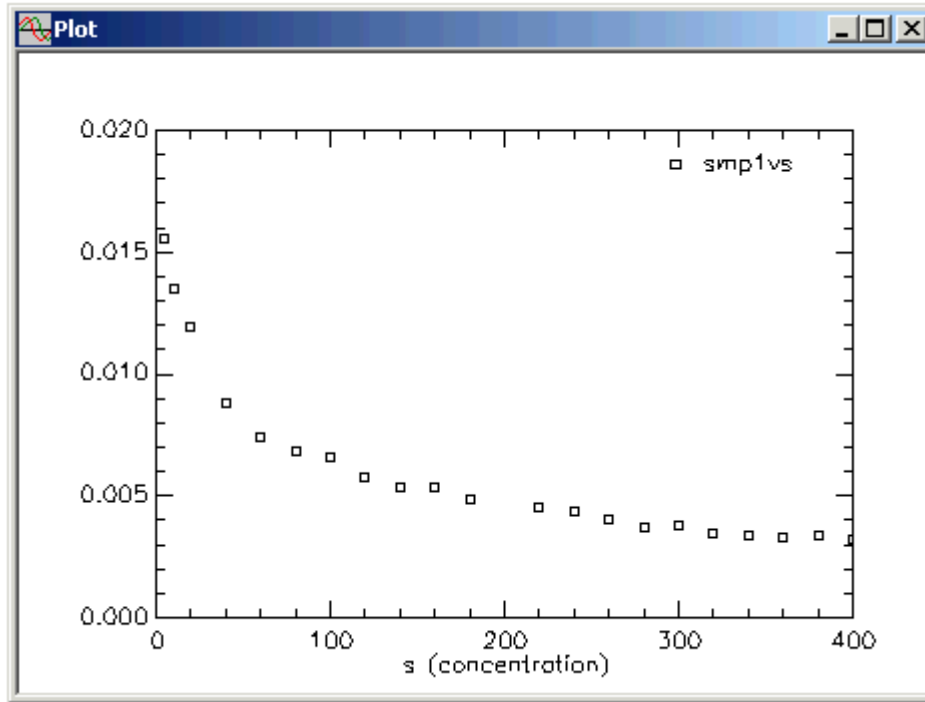
- k. In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open.
- l. Type “s” in the **X-Axis** box (this will return the variable on the X-Axis from to “s” from “s_recip.”)
- m. Click **smp11vs** in the **Variable for Y-Axis** pane to move this to the **Current Selection** pane. The **Plot and Table Variables** dialog box will appear as follows:



n. Click **Done**. You will need to reset your plot scale as follows:



o. Click **Done**. Your Eadie-Hofstee plot will appear as follows:



It is clear this plot is not linear; it is even more obvious than with the Lineweaver-Burke plot. Thus the data contain more complexity than a single Michaelis-Menten term.

- p. Close the **Plot** window.

Part 2. Specify the model of the data

Your pre-analysis of your data has led you to the conclusion that your initial model should contain two Michaelis-Menten terms. If you were unsure about whether a linear term was needed, you could first start with a linear plus a single Michaelis-Menten term. You will use the two Michaelis-Menten term model.

1. Specify the model for the data.

Type the following on the **Working Canvas** below your initial equation for “s_recip”:

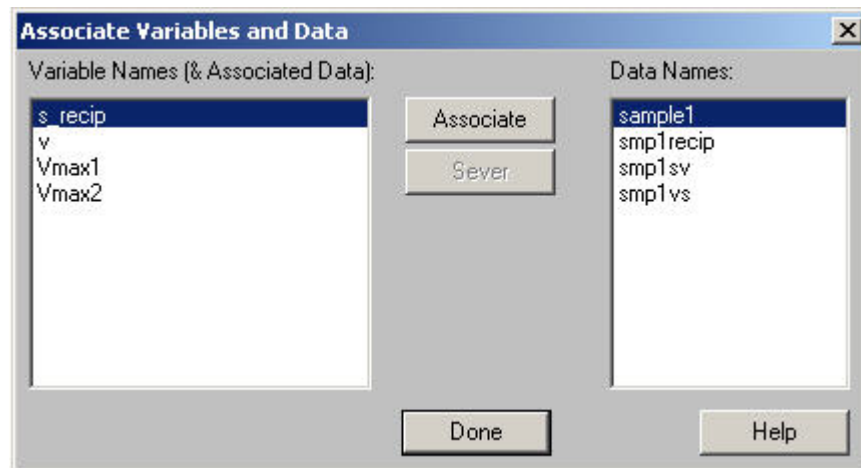
```
#
# Michaelis-Menten model with two terms:
#
Vmax1 = Vmax_CYP2C19
Vmax2 = Vmax_CYP3A4
#
# Vmax1 and Vmax2 are respectively Vmax for the fast and slow acting
```

```
# cytochrome P450 drug metabolizing enzymes. Vmax_CYP2C19 and
# Vmax_CYP3A4 will be two parameters to be estimated from the data.
#
v = (Vmax1*s)/(Km1 + s) + (Vmax2*s)/(Km2 + s)
#
```

Your **Working Canvas** will appear in part as follows:

```
#
#
# Michaelis-Menten model with two terms:
#
Vmax1 = Vmax_CYP2C19
Vmax2 = Vmax_CYP3A4
#
# Vmax1 and Vmax2 are respectively Vmax for the fast and slow acting
# cytochrome P450 drug metabolizing enzymes. Vmax_CYP2C19 and
# Vmax_CYP3A4 will be two parameters to be estimated from the data.
#
v = [Vmax1*s]/[Km1 + s] + [Vmax2*s]/[Km2 + s]
#
```

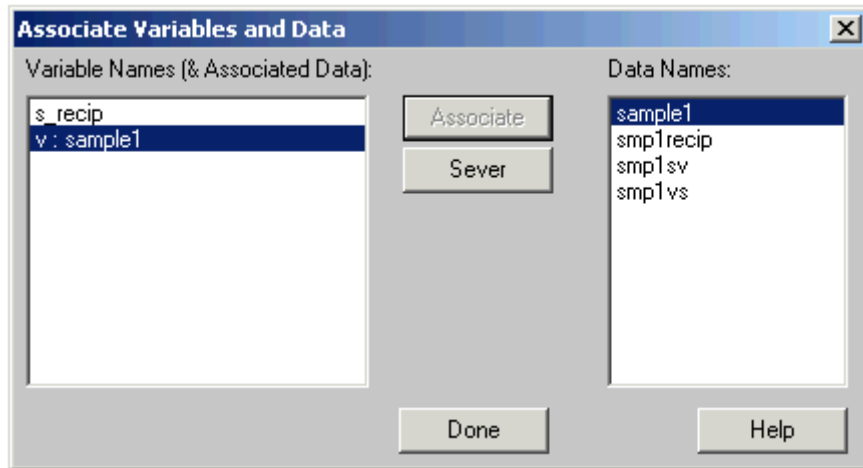
2. Associate the data and variable.
 - a. In the **Show** menu, click **Associations**. The **Associate Variables and Data** dialog box will open as shown in the following:




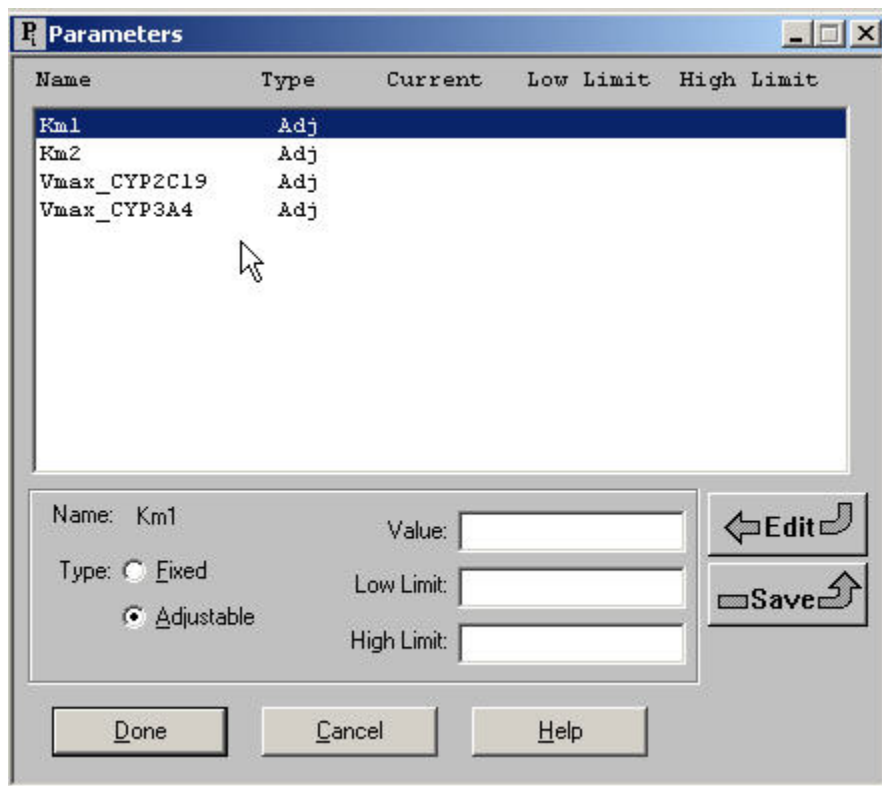
Note the entries in the **Data Names** box are the names of the columns in your data file.

- b. In the **Variable Names (&Associated Data)** box, click **v**.
 - c. In the **Data Names** box, click **sample1**.

- d. Click **Associate**. The **Associate Variables and Data** dialog box will appear as shown below:



- e. Click **Done**.
3. Enter the parameter values.
 - a. In the **Show** menu, click **Parameters**, or alternatively click the **Parameter**  tool on the **SAAM II Toolbar**. The **Parameters** dialog box will open as shown below:



There are four parameters to be estimated from the data, $V_{max_CYP2C19}$, V_{max_CYP3A4} , K_{m1} and K_{m2} . These are the four parameters describing the two Michaelis-Menten terms.

- b. Enter the parameters as shown in the following **Parameters** dialog box:



Name	Type	Current	Low Limit	High Limit
Km1	Adj	300.0000	30.0000	3000.0000
Km2	Adj	15.0000	1.5000	150.0000
Vmax_CYP2C19	Adj	1.0000	0.1000	10.0000
Vmax_CYP3A4	Adj	0.2000	0.0200	2.0000

Name: Km1 Value: 300.00000000

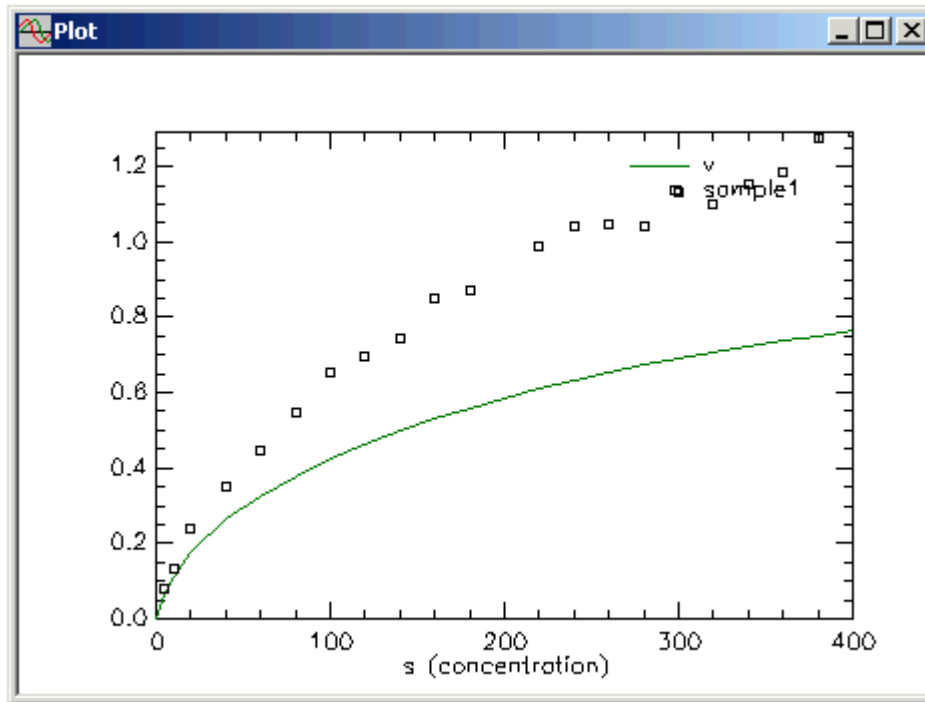
Type: Fixed Low Limit: 30.00000000

Adjustable High Limit: 3000.00000000

Buttons: Done, Cancel, Help, Edit, Save


- c. Click **Done**.
4. Solve the model, and view the solution.
- a. In the **Compute** menu, click **Solve**, or alternatively click the **Solve**  tool on the **SAAM II Toolbar**.
- b. Plot the data. In the **Show** menu, click **Plot**, or alternatively click the **Plot** tool  on the **SAAM II Toolbar**. The **Plot and Table Variables** dialog box will open. (Remember your last plot was the Eadie-Hofstee plot. However, you added the model equation after that plot. Thus when you plot after adding the equation, the previous plot will not open.) Be sure the **List All Variables** box is not checked.
- c. Click **v:sample1** to move these to the **Current Selection** window.

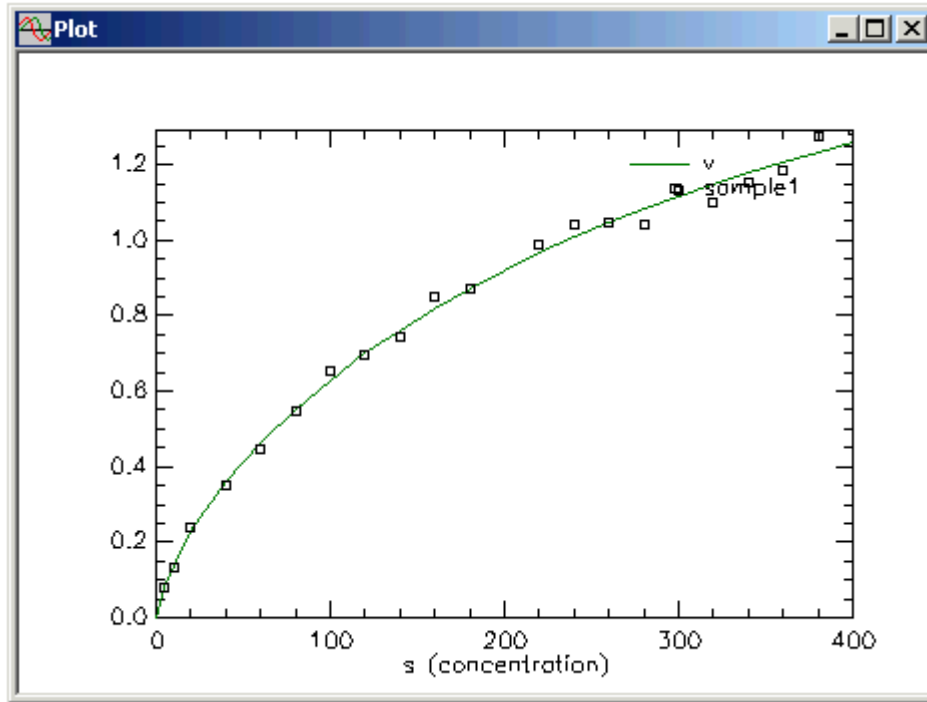
- d. Click **Done**. The following plot will appear:



Notice the autoscale option is active for the plot scale; you can change it if you wish to better visualize the last few data.

Leave the **Plot** window open.

5. Fit the model to the data, view the solution and statistics.
- a. In the **Compute** menu, click **Fit**, or alternatively click the **Fit**  tool on the **SAAM II Toolbar**. Your plot will be updated as follows:



- b. In the **Show** menu, click **Statistics**, or alternatively, click the **Statistics** tool on the **SAAM II Toolbar**. With the plot open, the **Working Canvas** will appear (after moving the open windows) as follows:

```

#
# The following equation defines a variable for the Lineweaver-Burke plot.
# The addition of the term "0.00001" in the denominator for s_recip will
# prevent division by zero when s = 0.
#
s_recip=1/[s + 0.00001]
#
# Michaelis-Menten model with two terms:
#
Vmax1 = Vmax_CYP2C19
Vmax2 = Vmax_CYP3A4
#
# Vmax1 and Vmax2 are respectively Vmax for the fast and slow acting
# cytochrome P450 drug metabolizing enzymes. Vmax_CYP2C19 and
# Vmax_CYP3A4 will be two parameters to be estimated from the data.
#
v = [Vmax1*s]/[Km1 + s] + [Vmax2*s]/[Km2 + s]
#
    
```

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
Km1	340.23555	8.06309e+001	2.36985e+001	170.11893	510.35217
Km2	17.37798	5.58266e+000	3.21249e+001	5.59957	29.15639
Vmax_CYP2C19	1.93212	1.32985e-001	6.88285e+000	1.65154	2.21269
Vmax_CYP3A4	0.22346	7.18224e-002	3.21417e+001	0.07192	0.37499
----- Derived Variables -----					
Vmax1	1.93212	1.32985e-001	6.88285e+000	1.65154	2.21269
Vmax2	0.22346	7.18224e-002	3.21417e+001	0.07192	0.37499

	Objective	Scaled Data Variance
v : sample1	-7.047256e+000	3.615331e-001

Total objective	-7.047256e+000	
AIC	-2.366594e+000	
BIC	-2.242246e+000	

You can see that the model provides a very good description of the data, and that the parameters are well estimated.

Using antibodies against specific CYP enzymes, Kato and Yamazoe were able to identify the enzyme with the lower K_m value as CYP 2C19, which demethylates diazepam, and the enzyme with the higher K_m value as CYP 3A4, which forms the 3C-OH metabolite and also participates in demethylation. These investigators also used their K_m and V_{max} results to resolve a discrepancy between previous *in vitro* studies, in which 3-OH was reported to be the major metabolite of diazepam, and the *in vivo* observation that diazepam was primarily demethylated. The following table which shows the relative contribution of the two CYP enzymes to diazepam metabolism indicated that this discrepancy resulted from the fact that much higher diazepam concentrations had been used for *in vivo* studies than are achieved in patients treated with this drug.

	[Diazepam]	CYP 2C19	CYP 3A4
<i>In Vitro</i>	100 μM	36%	64%
<i>In Vivo</i>	2 μM^*	70%	30%

* usual concentration in patients: 1 – 2 μM .

This demonstrates the utility of this type of detailed kinetic investigation and underscores the importance of conducting *in vitro* studies of drug metabolism at clinically relevant substrate concentrations. A final point of pharmacogenetic importance is that phenotypic poor metabolizers of diazepam have defective CYP 2C19 activity and only one Michaelis-Menten term is needed to characterize diazepam metabolism by liver slices from these individuals.

- c. Close the **Statistics** window. Leave the **Plot** window open.

Part 3. Create the Lineweaver-Burke, Hanes and Eadie-Hofstee plots.

While you examined the Lineweaver-Burke and Eadie-Hofstee plots of the data, you will now create these with the model predicted values as well as the Hanes plot.

1. Create the Lineweaver-Burke plot.

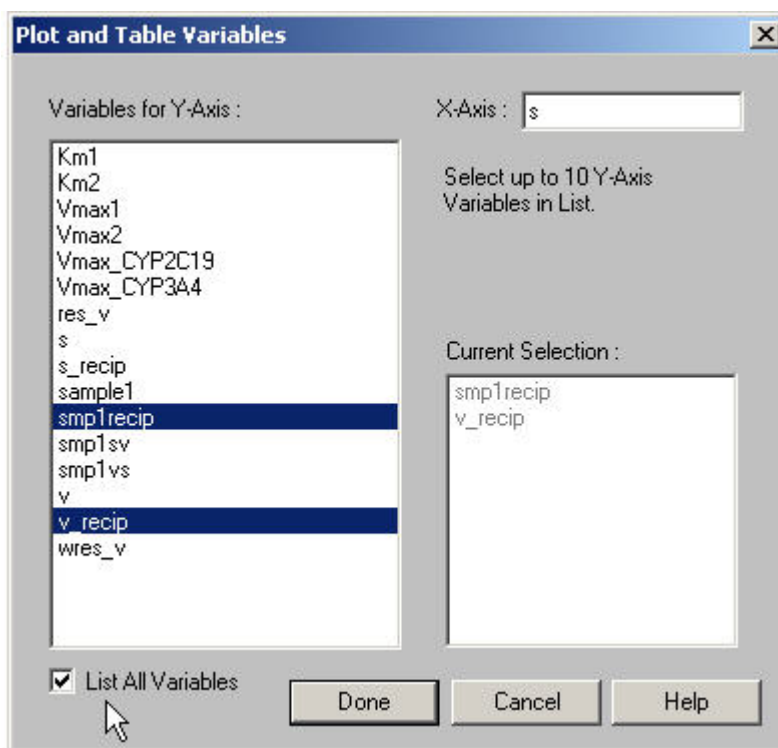
To produce the Lineweaver-Burke plot, you need to add another equation to produce the model calculated “1/v”.

- a. Type the equation “v_recip = 1/v” under your equation for “s1_recip”; your **Working Canvas** will appear as follows:

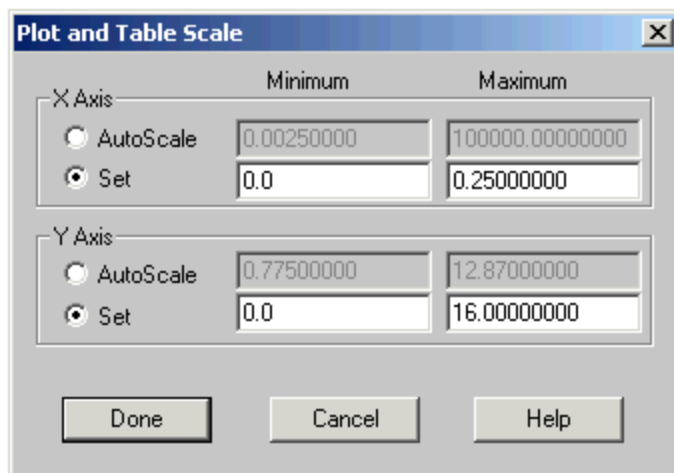
```
#  
# The following equation defines a variable for the Lineweaver-Burke plot.  
# The addition of the term "0.00001" in the denominator for s_recip will  
# prevent division by zero when s = 0.  
#  
s_recip=1/[s + 0.00001]  
v_recip = 1/v
```

Notice the plot will disappear. It has not been closed, and, as you will see, can be accessed from the **Window** menu.

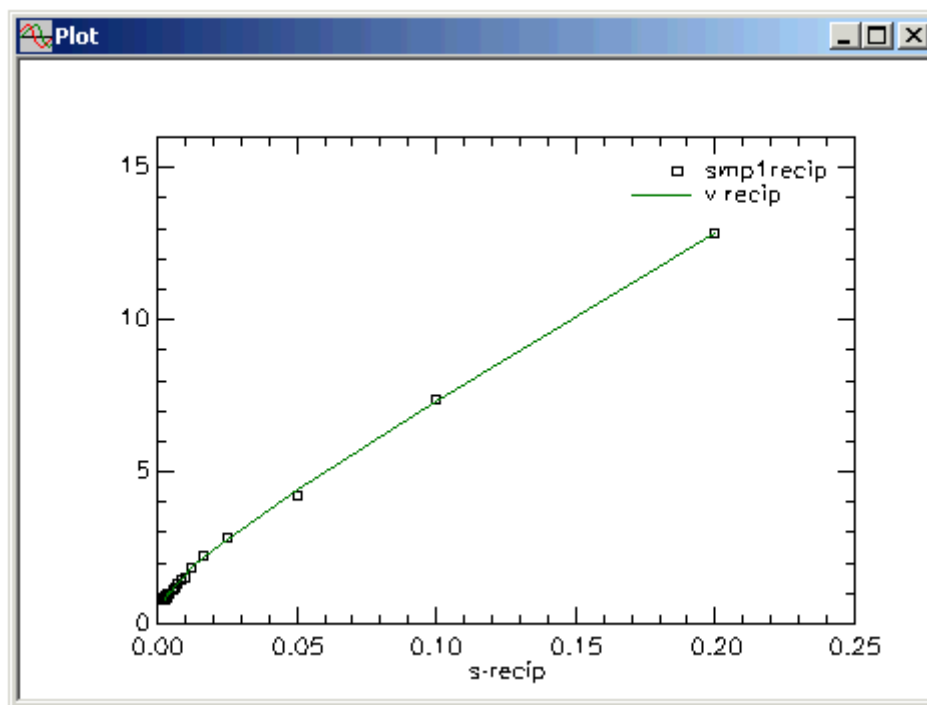
- Re-Solve the model. (This is necessary to activate the equation for v_recip. Remember the parameter values have not changed from those which produced the best fit to the data.)
- In the **Window** menu, click **Plot**. Your plot will reappear on the **Working Canvas**, and be active.
- In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open. Be sure the **List All Variables** check box is selected.
- Type "s_recip" in the **X-Axis** box.
- Click **v_recip**, then press **Ctrl** and **smp1recip**. The **Plot and Table Variables** dialog box will appear as follows:



- g. Click **Done**. Your plot will not be useful as, when you plotted the data, you need to change the scale.
- h. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.
- i. Set the X-Axis minimum and maximum values to 0.0 and 0.25 respectively. Set the Y-Axis minimum and maximum values to 0.0 and 16.0 respectively. The **Plot and Table Scale** dialog box will appear as follows:



- j. Click **Done**. Your Lineweaver-Burke plot will appear as follows:



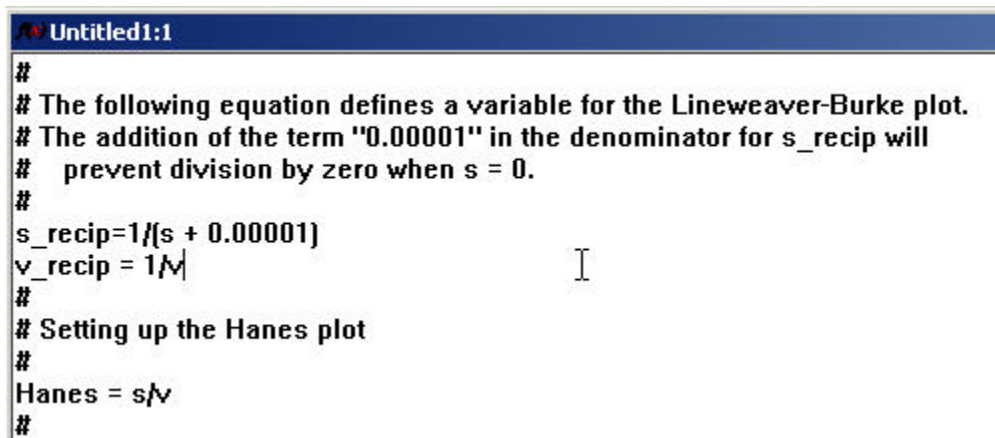
2. Create the Hanes plot

To create the Hanes plot, you need to add another equation for the model calculated value for “s/v”.

a. Type the following on your **Working Canvas**:

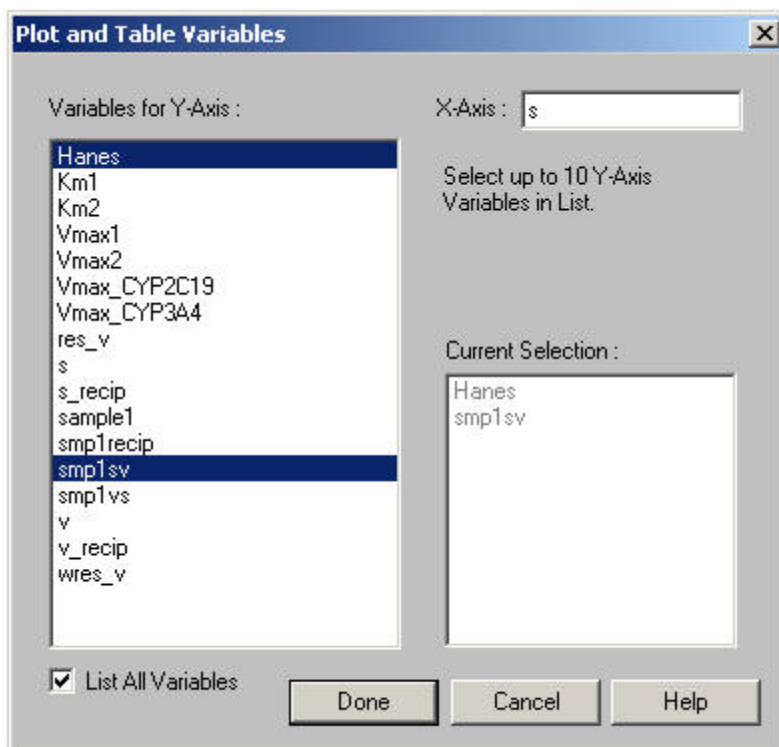
```
#
# Setting up the Hanes plot
#
Hanes = s/v
#
```

Your **Working Canvas** will appear as follows:

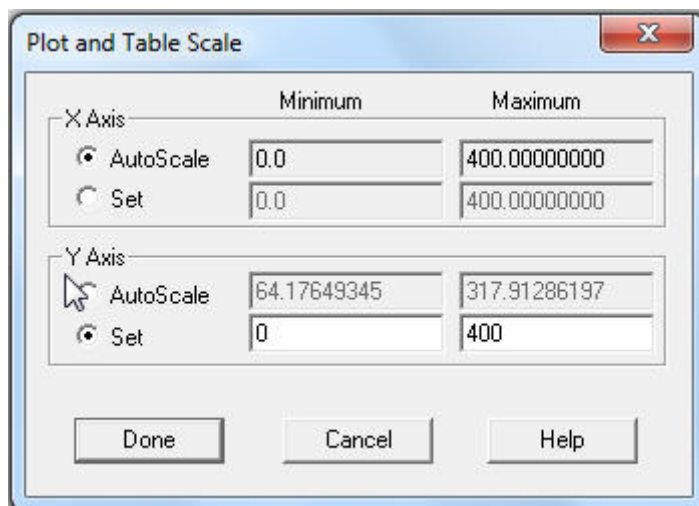


```
Untitled1:1
#
# The following equation defines a variable for the Lineweaver-Burke plot.
# The addition of the term "0.00001" in the denominator for s_recip will
# prevent division by zero when s = 0.
#
s_recip=1/[s + 0.00001]
v_recip = 1/v
#
# Setting up the Hanes plot
#
Hanes = s/v
#
```

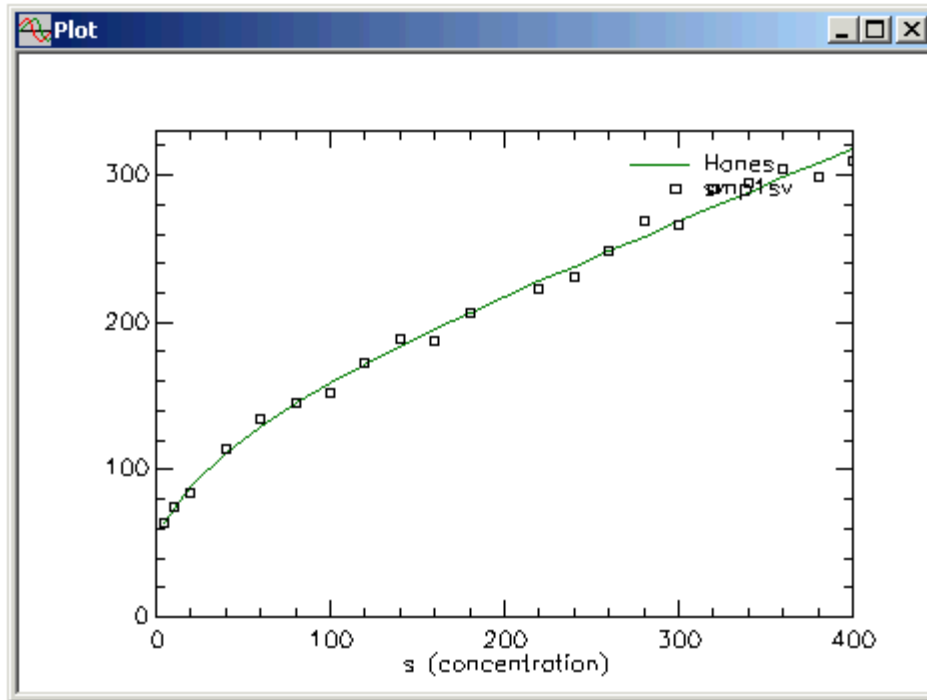
- b. Re-Solve the model.
- c. In the **Window** menu, click **Plot**. Your plot will reappear on the **Working Canvas**, and be active.
- d. In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open. Be sure the **List All Variables** check box is selected.
- e. Type “s” in the **X-Axis** box.
- f. Click **Hanes**, and then press **Ctrl** and **smp1sv**. The **Plot and Table Variables** dialog box will appear as follows:



- g. Click **Done**. Your plot will not be useful because the scale is set for the Lineweaver-Burke plot.
- h. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.
- i. Change the settings as follows:



- j. Click **Done**. Your Hanes plot will appear as follows:



3. Create the Eadie-Hofstee plot

To create the Eadie-Hofstee plot, you need to add another equation for the model calculated value for “v/s”.

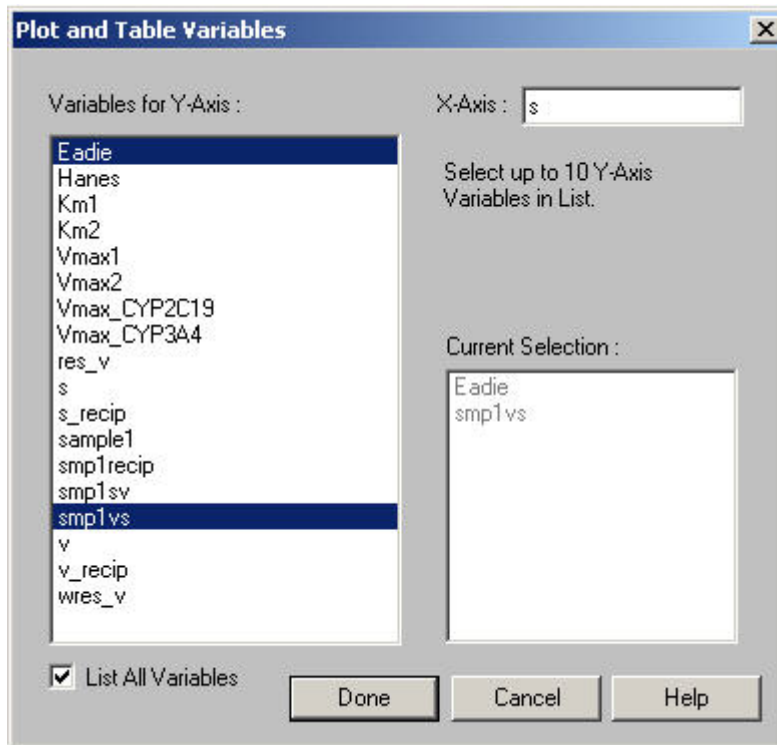
a. Type the following on your **Working Canvas**:

```
#
# Setting up the Eadie-Hofstee plot
#
Eadie = v/s
#
```

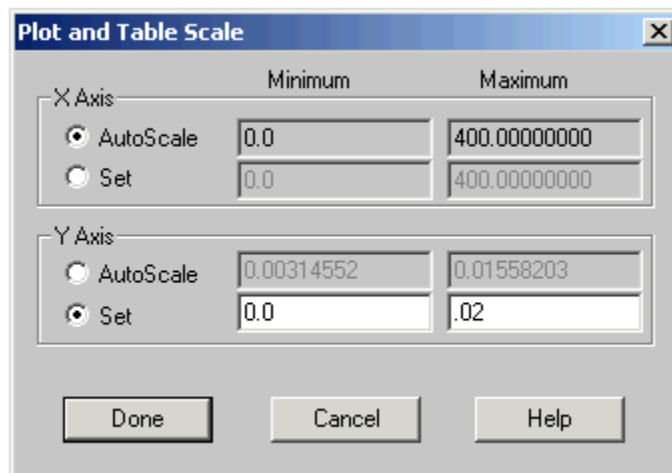
Your **Working Canvas** will appear as follows:

```
Untitled1:1
#
# The following equation defines a variable for the Lineweaver-Burke plot.
# The addition of the term "0.00001" in the denominator for s_recip will
# prevent division by zero when s = 0.
#
s_recip=1/(s + 0.00001)
v_recip = 1/v
#
# Setting up the Hanes plot
#
Hanes = s/v
#
# Setting up the Eadie-Hofstee plot
#
Eadie = v/s
#
# Michaelis-Menten model with two terms:
```

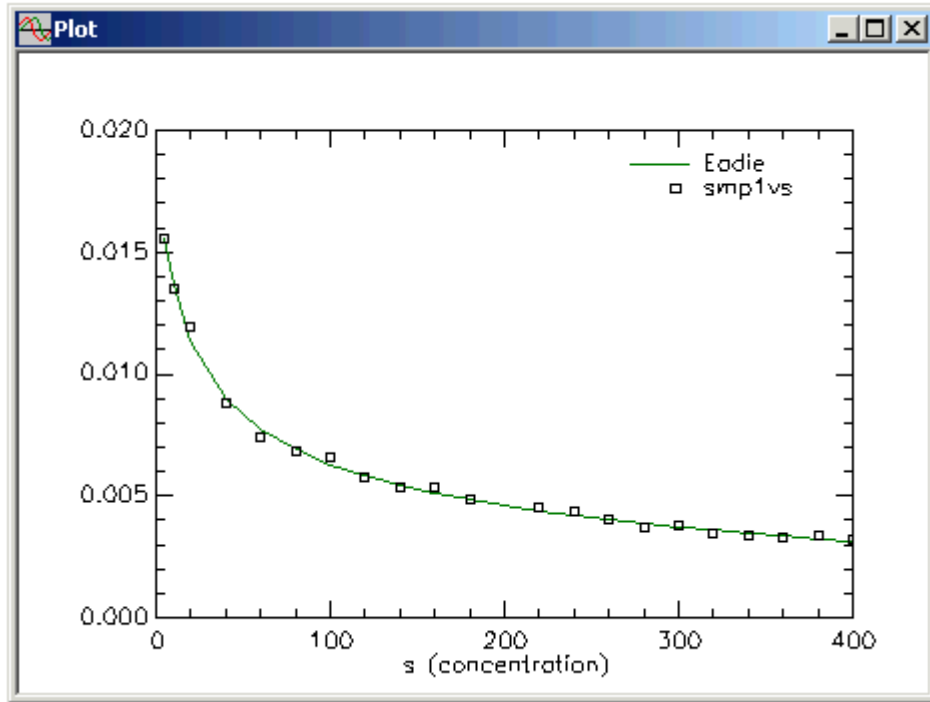
- b. Re-Solve the model.
- c. In the **Window** menu, click **Plot**. Your plot will reappear on the **Working Canvas**, and be active.
- d. In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open. Be sure the **List All Variables** check box is selected. Remember "s" is still the variable in the **X-Axis** box.
- e. Click **Eadie**, and then press **Ctrl** and **smp1vs**. The **Plot and Table Variables** dialog box will appear as follows:



- f. Click **Done**. Your plot will not be useful because the scale is set for the Hanes plot.
- g. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.
- h. Change the settings as follows:



- i. Click **Done**. Your Eadie-Hofstee plot will appear as follows:



j. Close all open windows.

Quit the **SAAM II Numerical** application. You can save the numerical study file if you wish.

Essential Points to Remember

- It is best to fit your “enzyme” kinetic data directly to a Michaelis-Menten model. That is, it is not necessary to transform your data to obtain the model parameter estimates (which is difficult if your model contains more than one Michaelis-Menten term.)
- You can easily plot your results in a transformed mode. This will require transforming your data, and writing the appropriate model equation.

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