

Using SAAM II

Working with Samples

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Working with Samples

Prerequisites

The prerequisite for this tutorial is having worked through the **SAAM II** introductory tutorial, “Getting Started with **SAAM II Compartmental**.”

What you will learn in this tutorial

The purpose of this tutorial is to show you how to use the **Sample** tool beyond just linking your model to your data. Some examples of such linkage will be given in the Introduction. Other examples will be discussed in this tutorial. You will learn

- How to create PKPD models (Part 1)
- How to estimate the AUC of a sample using the SAAM II AUC option (Part 2)
- How to estimate the AUC using a sum of exponentials (Part 3)

Files Required

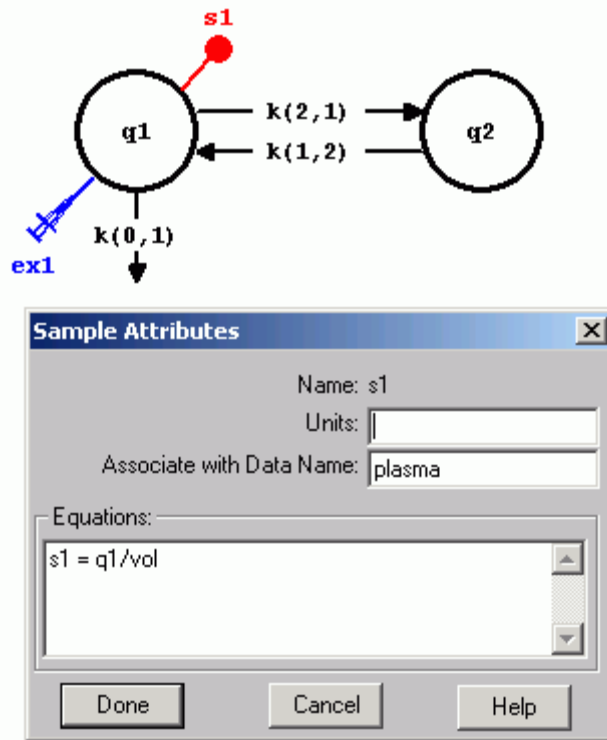
Study Files: The study file for this tutorial is

pkpd.stu
study_0.stu

These files are included as part of this tutorial. **study_0.stu** is the same study file that is installed in the **SAAM II** program folder and referred to in the **SAAM II** User Guide.

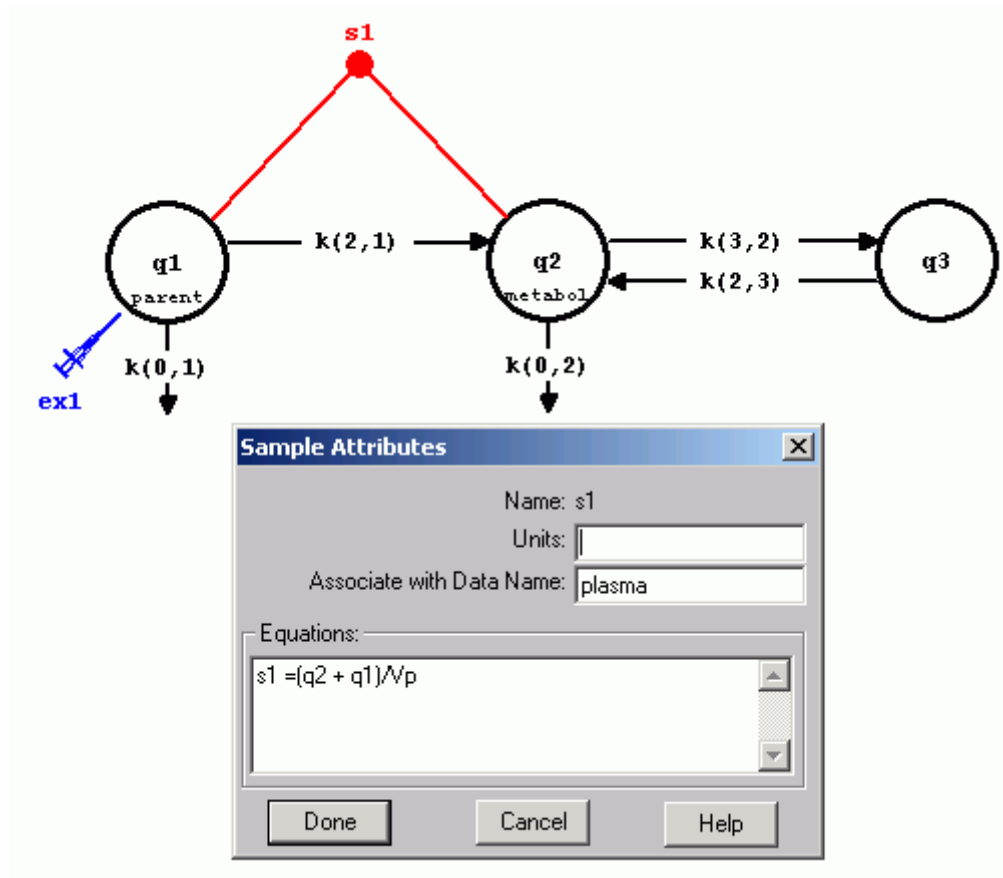
Introduction

This tutorial focuses on the different uses of the sample tool in the **SAAM II Toolbox**. Normally samples are used to link a compartment in your model to a set of data, and to specify the sample equation which makes the units of model (determined by the units of the input) consistent with the units of the data (determined by the experiment). The typical example is taken from **study_0** shown below:

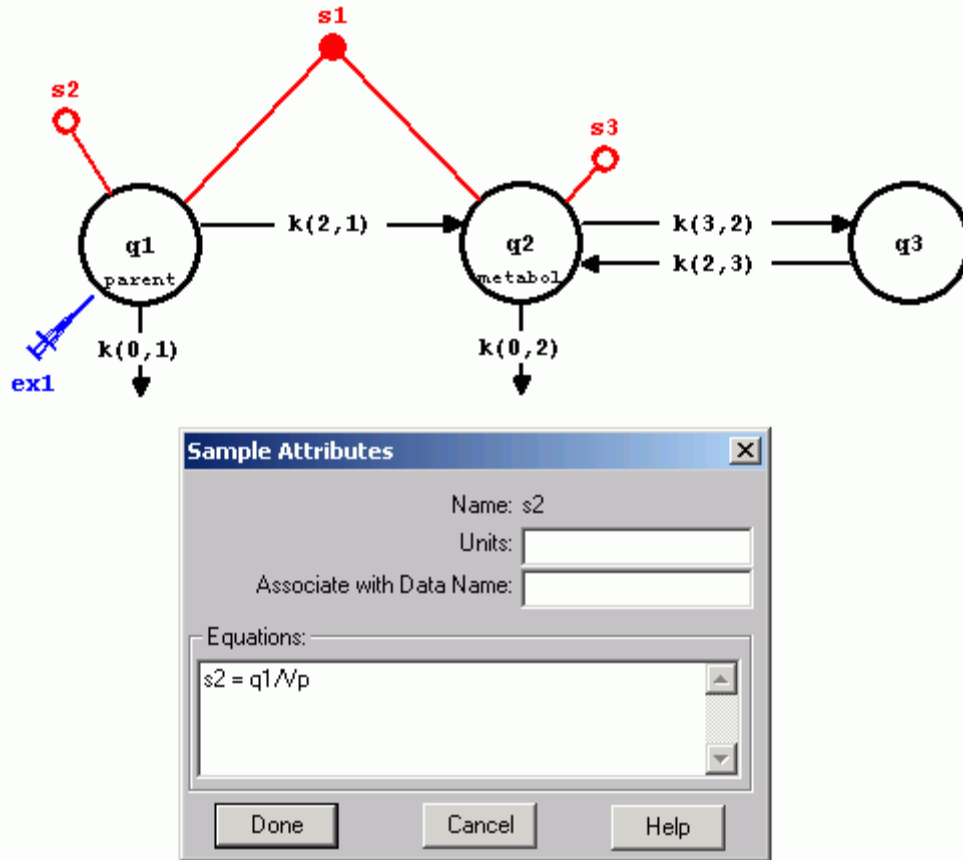


In terms of reproducing experiments, however, samples can be more complex, and serve purposes other than linking the model to data.

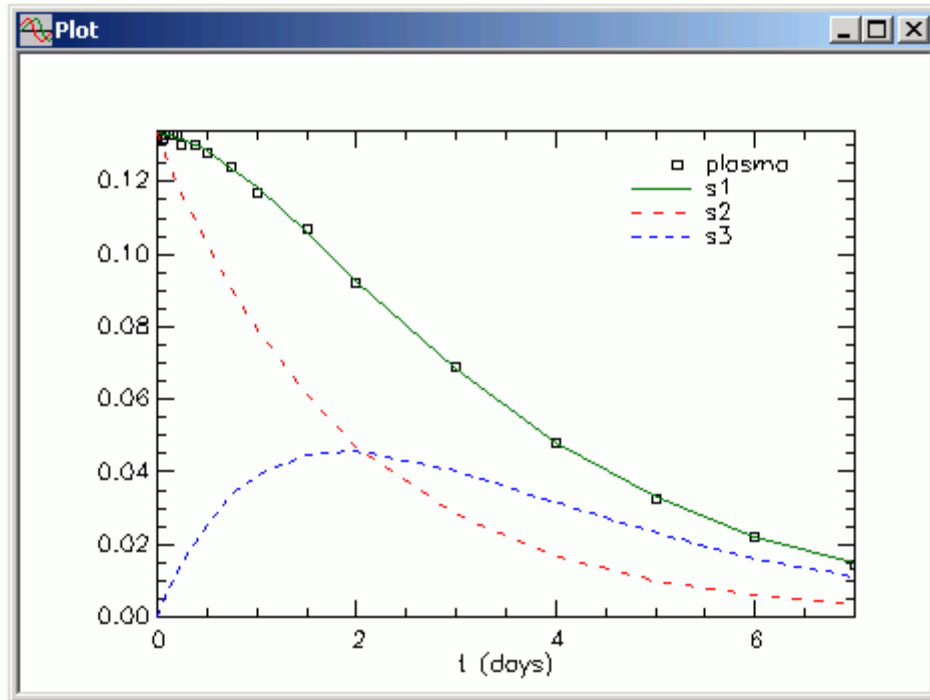
Consider the following example where a drug is given as a bolus into plasma. The drug is known to convert to a metabolite, but when taking plasma samples, only the sum of the drug and metabolite can be measured. The model and sample is shown as follows:



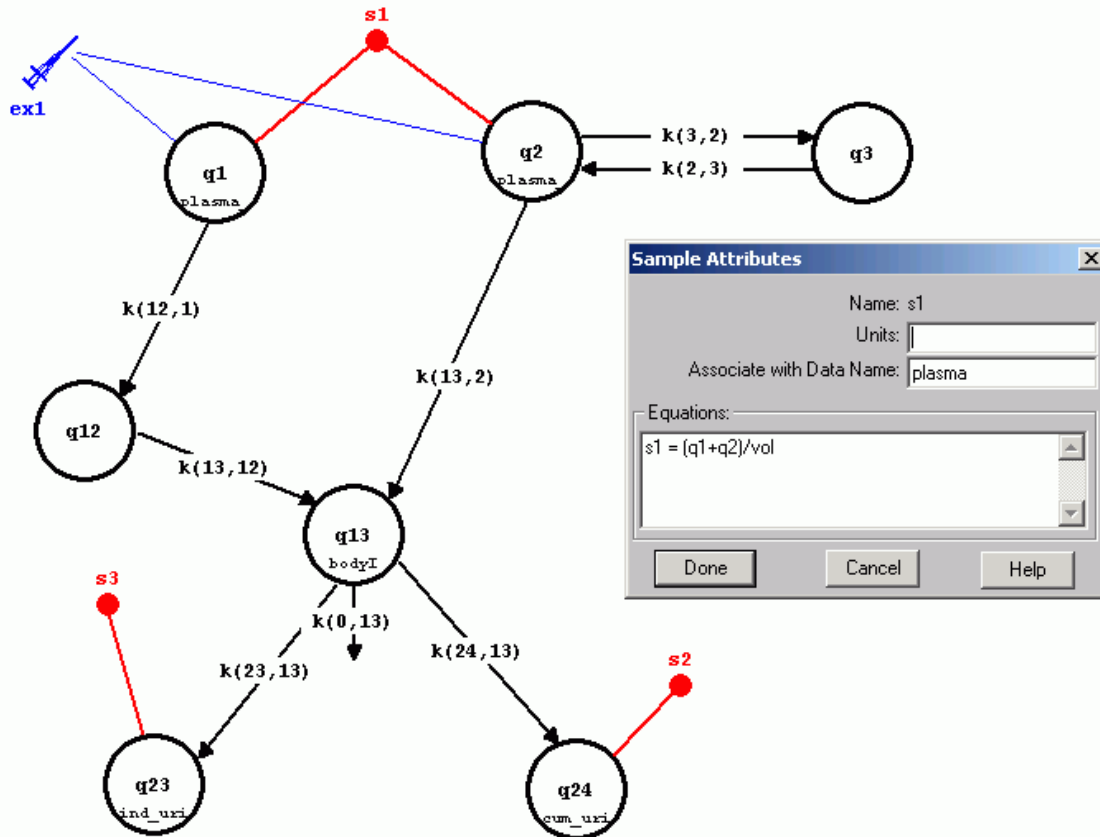
However, even though the data are the sum of the drug and its metabolite, the model is able to predict the behavior of the drug and metabolite separately. This can be done as shown in the following figure:



Here separate samples are associated with Compartments **q1** and **q2**. The sample equation, as shown above, is in terms of concentration. Thus **s2** and **s3**, which are not linked to any data because separate data on the drug and its metabolite do not exist, predict the behavior of the drug and its metabolite. If you plot, as shown below, **plasma**, **s1**, **s2** and **s3**, you can see the model predicted fit, and the contribution of **s2** and **s3** to the solution:

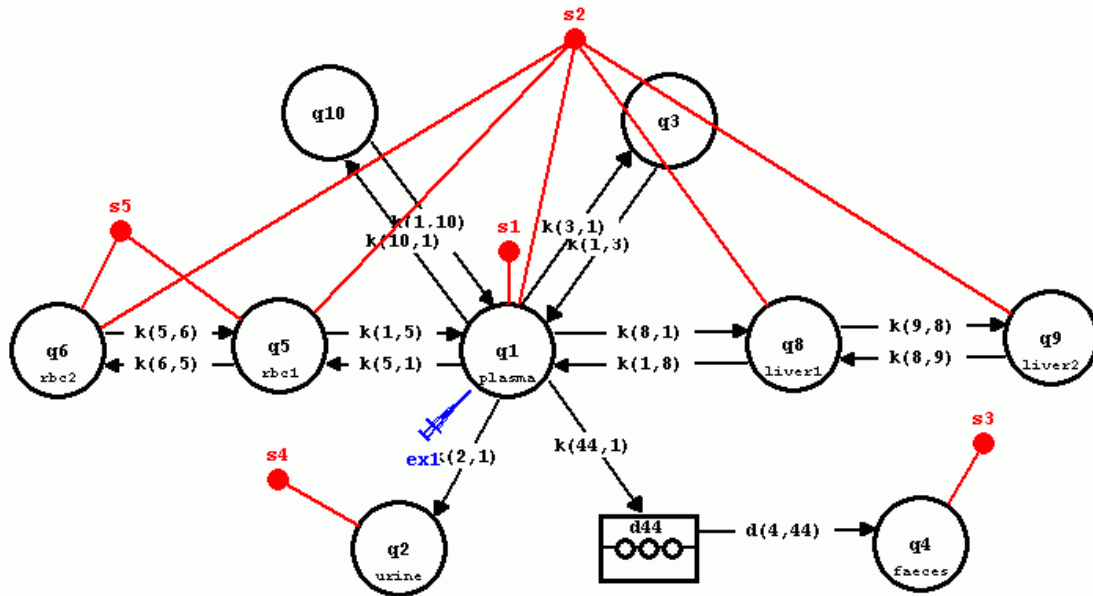


Another similar example comes when radioiodinated low-density lipoprotein (LDL) is injected into plasma. The plasma model contains two compartments; this reflects the kinetic heterogeneity of LDL. In addition, urine data are also collected, and analyzed in terms of individual and cumulative urine samples. This is an example of a single-input three-output experiment. A typical model looks like the following:



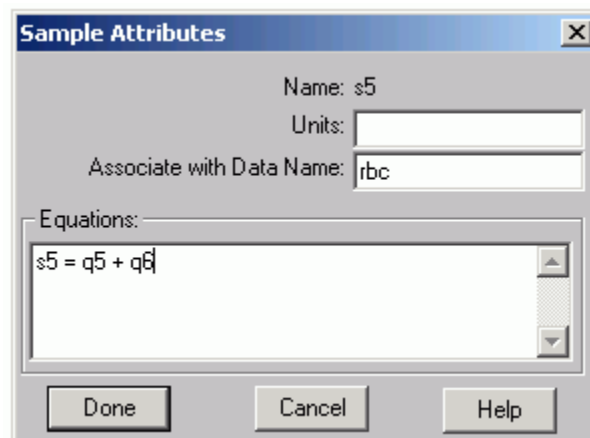
The input, **ex1**, is split between Compartments **q1** and **q2**; the sample equation, as shown in the figure above, is a sum of the radioactivity in Compartments **q1** and **q2** divided by the plasma volume. Samples **s2** and **s3** are linked to the cumulative and individual data respectively.

The final example comes from an experiment in which radiolabeled zinc was injected into plasma as a bolus. Serial plasma samples were taken. Radioactivity in red blood cells was also collected. Radioactivity in urine and faeces was measured. Using an external counter, radioactivity in the liver was calculated. This is very similar to MRI or PET studies where images over regions of interest are collected and test substances quantitated. Thus developing models of regions of interest is almost identical to the model for liver radioactivity. The model of the experiment appears as follows:

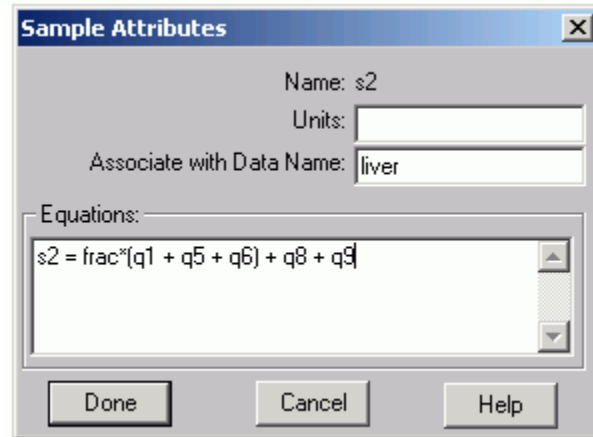


This model is obviously complex because of the nature of the data collected. Radioactive zinc was injected into Compartment **q1** as indicated by **ex1**. The plasma, urine and faeces data are linked through samples **s1**, **s4** and **s3**.

The red cell system consists of two compartments, Compartment **q5** and **q6**. Since total radioactivity in red cell is counted, the **Sample Attributes** dialog box appears as follows:



The liver system consists of two compartments, Compartment **q8** and **q9**. However, these are hypothesized to represent labeled zinc in liver cells while the counter sees both the cells and plasma. The **Sample Attributes** dialog box for the measurement as follows:



s2 is the measurement equation for the liver data. It consists of the zinc in the liver cells, “q8 + q9”. Total blood is given by “q1+ q5 + q6”, i.e. blood in plasma and the red cells. However, this is total blood. Only a fraction of total blood is in the liver. Hence *frac* is a parameter that estimates the fraction of total blood that is in the liver. It is known that around 20% of total blood is in the liver, so when fitting the model to the data, *frac* should be around 0.2, or 20%.

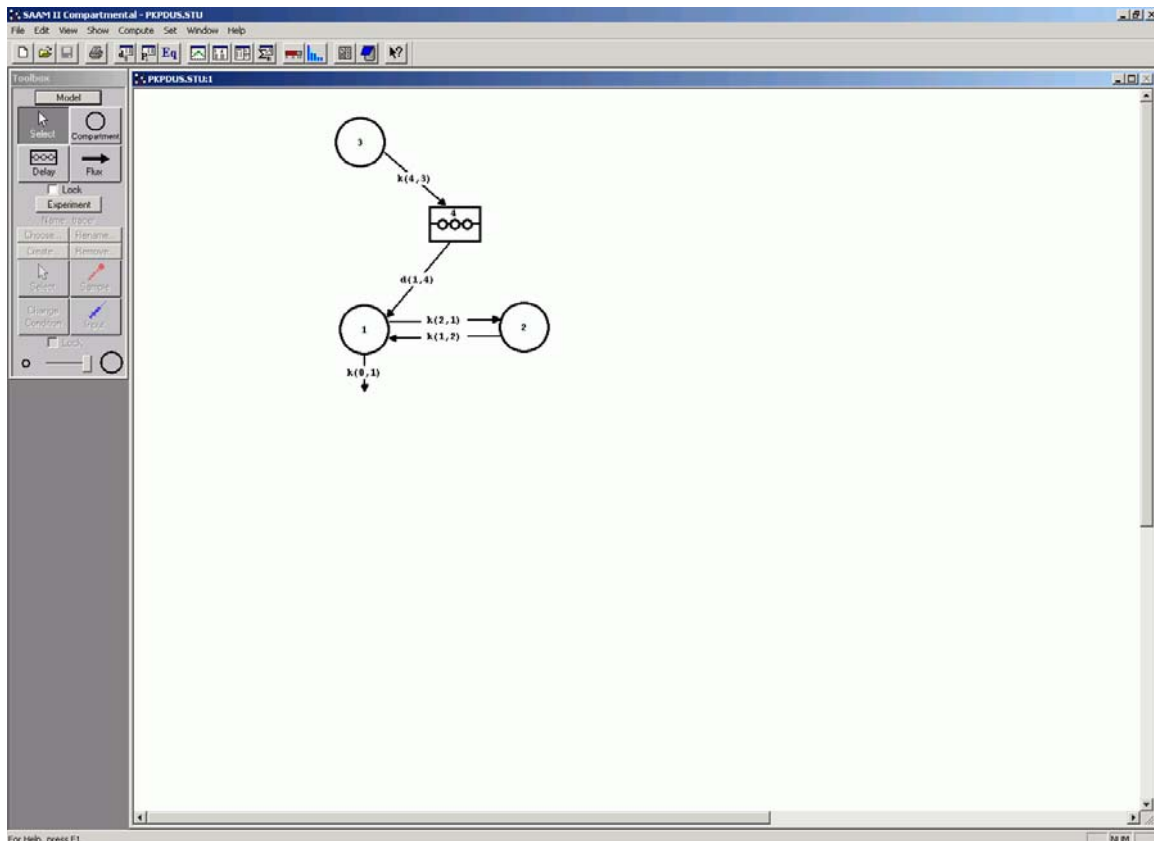
The above give some examples of how samples can be used to describe experimental data. The use of samples depends on the particular experiments.

In the following tutorials, we will describe how to create pharmacokinetic-pharmacodynamic (PKPD) models using the **Sample** tool. We will also give two examples of how to estimate area under the curve. This is discussed here because, in SAAM II, the area under the curve is calculated only for samples.

Part 1. Creating PKPD Models

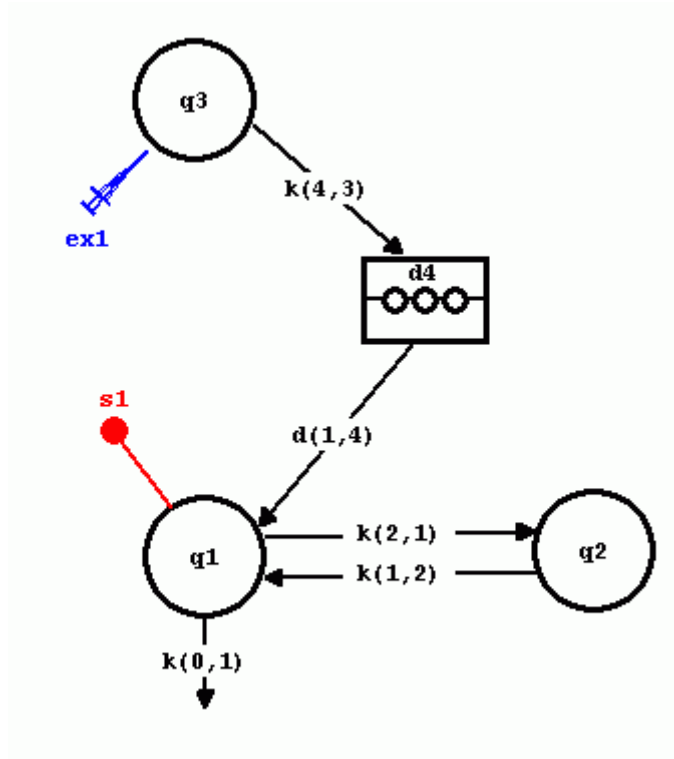
In this tutorial, you will create a PKPD model. The system model for the drug is two compartments. The drug is administered orally, and complete absorption is assumed. The PD model is a direct Emax model. The weighting scheme for the data is $1/y^2$ as specified by (GEN 0 1 2); the variance model is data-relative.

1. Start the SAAM II Compartmental application. The SAAM II Compartmental main window will open.
2. Open the SAAM II Compartmental study file **pkpd**.
 - a. The file **pkpd.stu** should appear in the file list; if it does not, find the folder where you put this file.
 - b. In the **File** menu, click **Open**. The SAAM II Compartmental main window will appear as shown below:

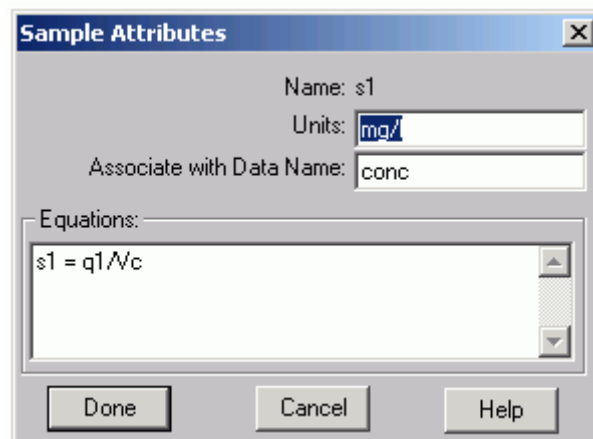


The system model, i.e. the behavior of the drug once in the systemic circulation, is described by Compartments 1 and 2. Compartment 3 is the compartment into which the drug is introduced; the upper part of the GI tract. Delay 4 simulates the transit time through the GI tract as the drug is absorbed.

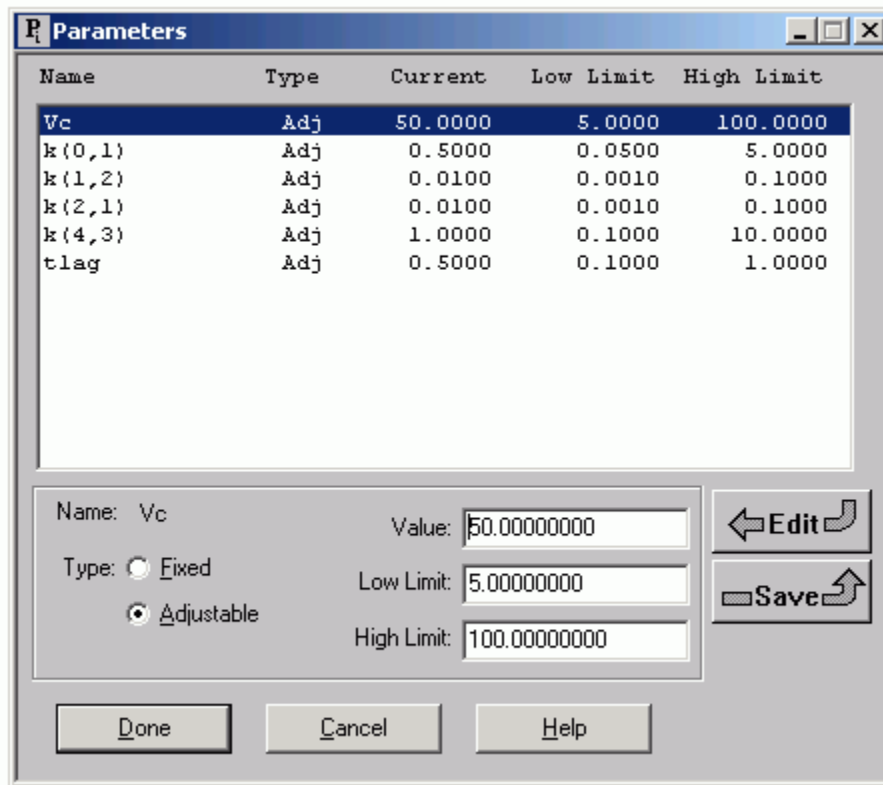
3. View the model and the experiment on the model. In the SAAM II Toolbox, click **Experiment**. The model of the experiment will appear on the **Drawing Canvas** as follows:





The experiment as been created to analyze the plasma data collected following a single dose of a tablet containing 400mg of the drug. The dose can be confirmed by opening the **Exogenous Input** dialog box. The **Sample Attributes** dialog box associated with s1 are shown below:

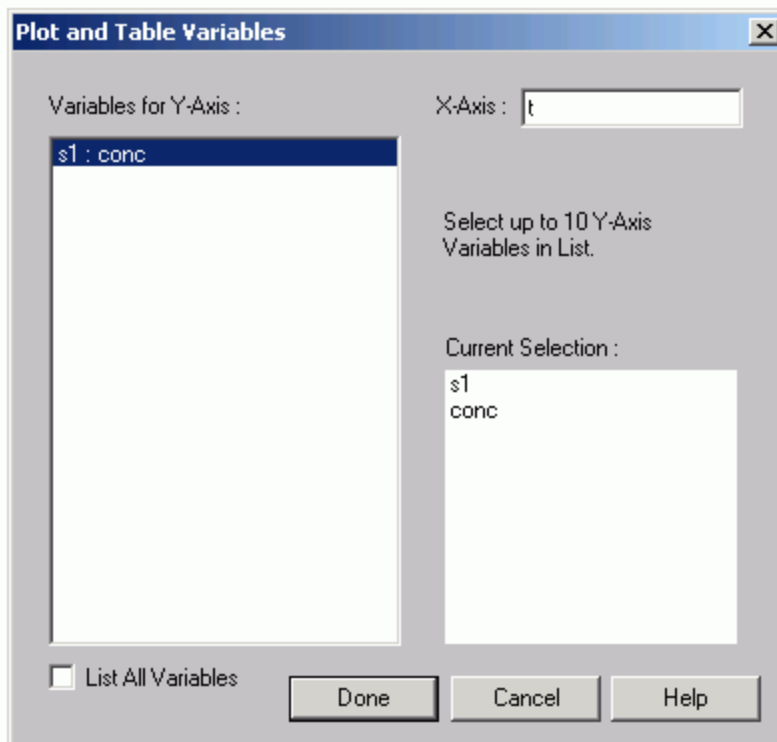


The sample is simply concentration in terms of mg/l, and the plasma data are named “conc”. The **Parameters** dialog box appears as follows:

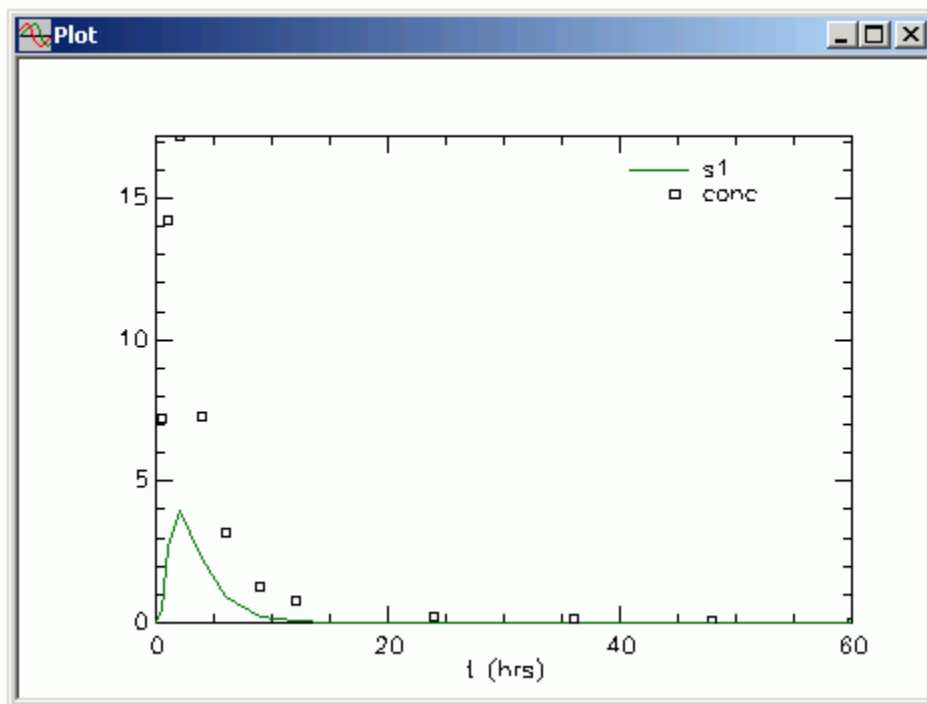


Click **Cancel** or **Done** to close the **Parameters** dialog box.


4. Solve the model, and view the solution.
 - a. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II Toolbar**, click **Solve** .
 - b. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . The **Plot and Table Variables** dialog box will open. Be sure the **List All Variables** check box is not selected.
 - c. Click **s1:conc** to move this to the **Current Selection** pane. The **Plot and Tables Variables** dialog box will appear as follows:

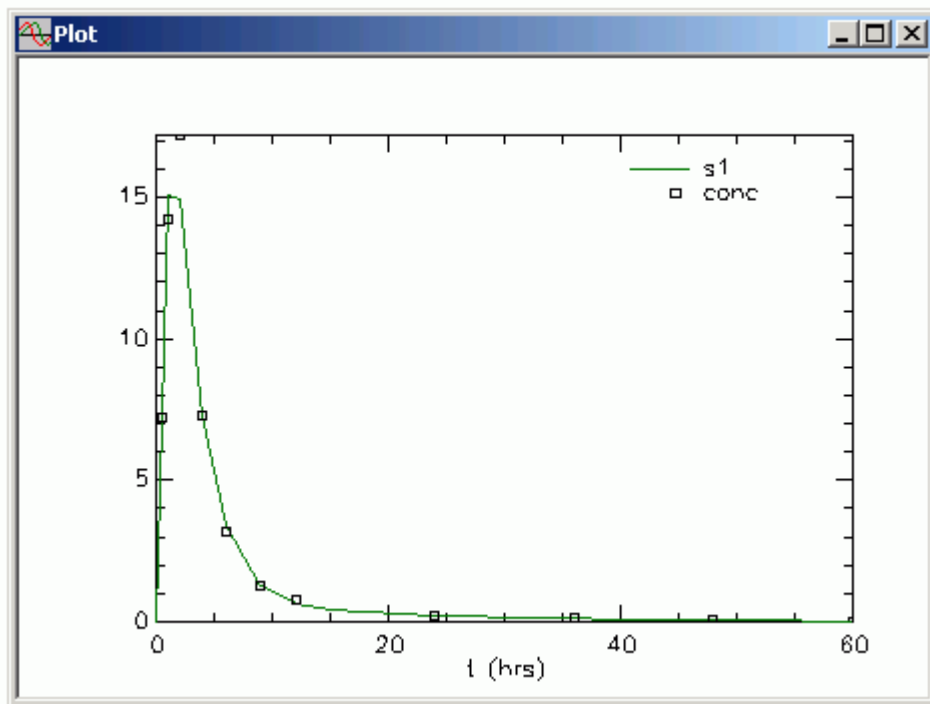


d. Click **Done**. Your plot will appear as shown below (in linear mode):




Leave the **Plot** window open.

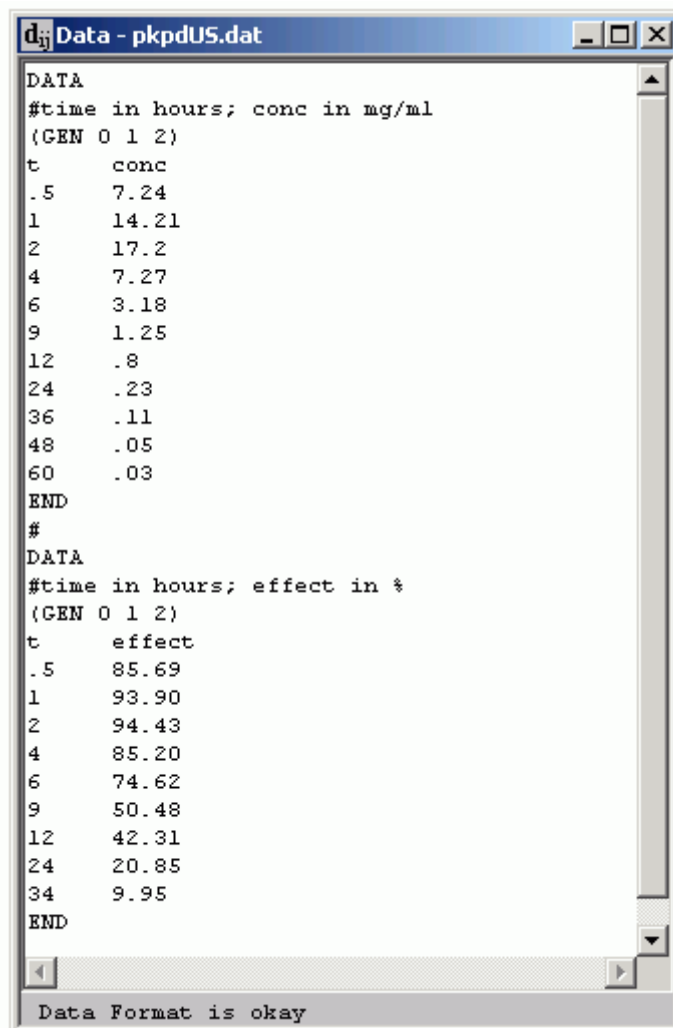
5. Fit the model to the data.
 - a. Fit the model to your data. In the **Compute** menu, click **Fit**, or alternatively, on the **SAAM II Toolbar** click **Fit** . Since the **Plot** window was open during the “Fit”, the **Plot** window will be updated as shown below:



- b. Close the **Plot** window.
6. Create the PD model.

Besides collecting plasma data, an effect was also measured. The key in PKPD models is linking the PK model to the effect, or PD, data.

- a. View the data. In the **Show** menu, click **Data**, or alternatively, on the **SAAM II Toolbar** click **Data** . The **Data** window will open as follows:



```
DATA
#time in hours; conc in mg/ml
(GEN 0 1 2)
t      conc
.5     7.24
1      14.21
2      17.2
4      7.27
6      3.18
9      1.25
12     .8
24     .23
36     .11
48     .05
60     .03
END
#
DATA
#time in hours; effect in %
(GEN 0 1 2)
t      effect
.5     85.69
1      93.90
2      94.43
4      85.20
6      74.62
9      50.48
12     42.31
24     20.85
34     9.95
END
```

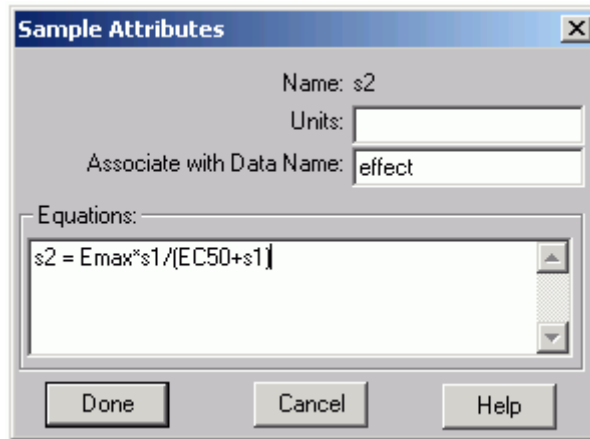
Data Format is okay

You can see there are two sets of data. One, “conc” is the plasma concentration of the drug. The other, “effect” is a measurement of an effect of the drug in terms of %. In the above, the weights assigned to both sets of data are the same. It is often the case that the “effect” data are known with less precision than the plasma data, and hence are assigned less weight.

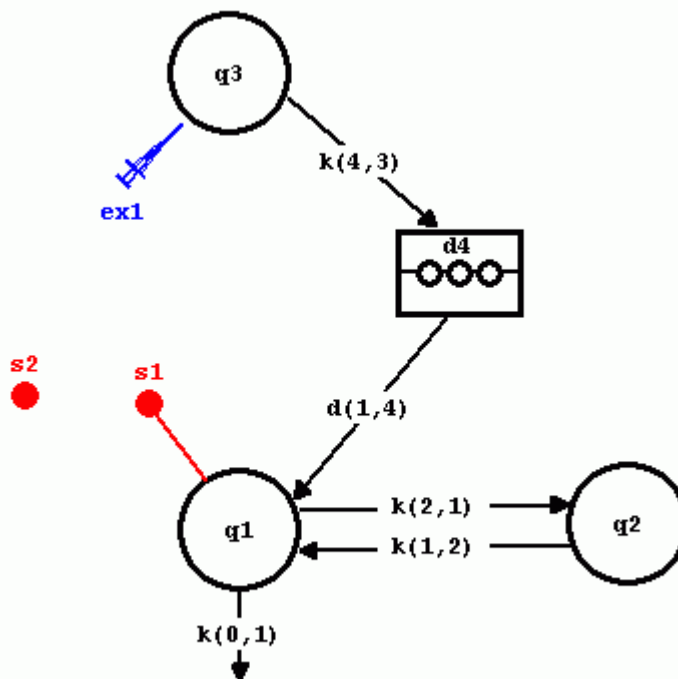
Close the **Data** window.

- b. In the **SAAM II Toolbox**, click **Sample**.
- c. Click Compartment **q1** and then click the **Drawing Canvas**. The sample **s2** will appear associated with Compartment **q1**.
- d. Double-click **s2** to open the **Sample Attributes** dialog box.
 - (1) Type “effect” in the **Associate with Data Name** box.

- (2) Type “ $s2 = E_{max} * s1 / (EC50 + s1)$ ” in the **Equations** box. The **Samples Attributes** dialog box will appear as follows:



- (3) Click **Done**. The model will appear as follows:



Notice **s2** is no longer associated with any compartment in the model. This is because the equation for **s2** does not contain any of the **qi**.



Samples and PKPD models. In SAAM II, the model is linked to the data via the **Sample** tool. When you create such samples, it does not matter which compartment you click. The reason is that the “link” equation involves a

plasma measurement, and hence will contain, as illustrated above, “s1” and not “q1”. If, on the other hand, you had written the sample equation

$$s2 = Emax * (q1/Vc) / (EC50 + (q1/Vc)),$$

s2 would be linked to Compartment q1 since “q1” appears in the equation; the equivalence is because “s1=q1/Vc”.

Obviously there are several different effect models that can be used; they can be specified in this fashion in SAAM II.



7. Enter the new parameters, solve the model, and view the solution.
 - a. Open the **Parameters** dialog box. Two new parameters, *Emax* and *EC50* will appear in the list. Enter the values as shown below (remember since you have previously Fitted the model to the plasma data, the system parameters are those from the best fit). The **Parameters** dialog box should appear as follows:

Name	Type	Current	Low Limit	High Limit
EC50	Adj	10.0000	1.0000	20.0000
Emax	Adj	80.0000	50.0000	200.0000
Vc	Adj	15.3199	5.0000	100.0000
k(0,1)	Adj	0.3605	0.0500	5.0000
k(1,2)	Adj	0.0745	0.0010	0.1000
k(2,1)	Adj	0.0691	0.0010	0.1000
k(4,3)	Adj	1.6776	0.1000	10.0000
tlag	Adj	0.2902	0.1000	1.0000

Name: Emax Value: 80

Type: Fixed Adjustable

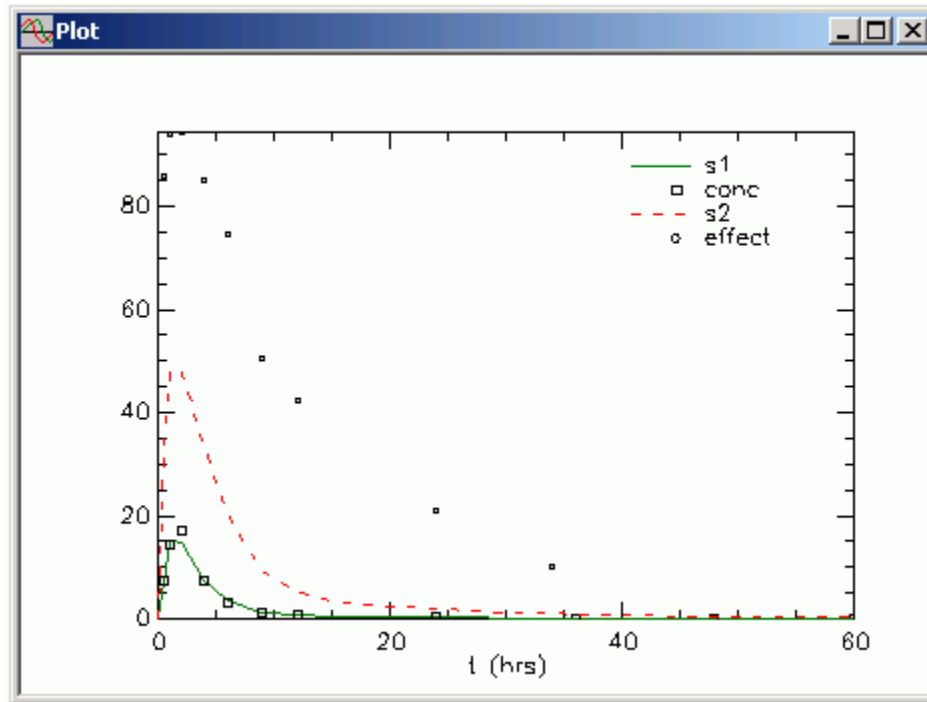
Low Limit: 50.00000000 High Limit: 200.00000000

Buttons: Done, Cancel, Help, Edit, Save

Notice for both *Emax* and *EC50* the **Low** and **High Limits** were separately set. Click **Done** to close the **Parameters** dialog box.

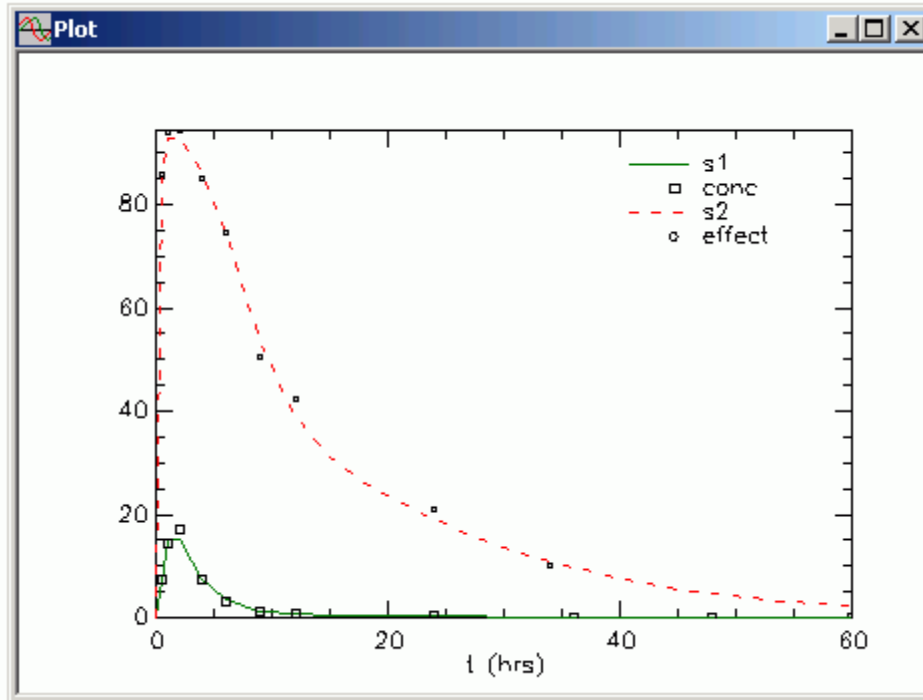
- b. Solve the model, and view the solution. In the **Plot and Tables Variables** dialog box, select both **s1:conc** and **s2:effect**. The plot of **s1:conc** (which will

not change from the previous best-fit solution) and **s2:effect** will appear as follows:



Leave the **Plot** window open.

8. Fit the model to the data. The **Plot** window will be updated as follows:



The **Statistics** window will appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
EC50	1.08787	1.12842e-001	1.03727e+001	0.84201	1.33373
E _{max}	99.47768	4.00656e+000	4.02759e+000	90.74815	108.20720
V _c	14.71656	1.92542e+000	1.30834e+001	10.52143	18.91169
k(0,1)	0.37365	3.71837e-002	9.95149e+000	0.29263	0.45467
k(1,2)	0.08218	6.77301e-003	8.24187e+000	0.06742	0.09694
k(2,1)	0.08067	1.42906e-002	1.77139e+001	0.04954	0.11181
k(4,3)	1.58733	5.38182e-001	3.39048e+001	0.41474	2.75993
t _{lag}	0.28901	6.64625e-002	2.29963e+001	0.14420	0.43382

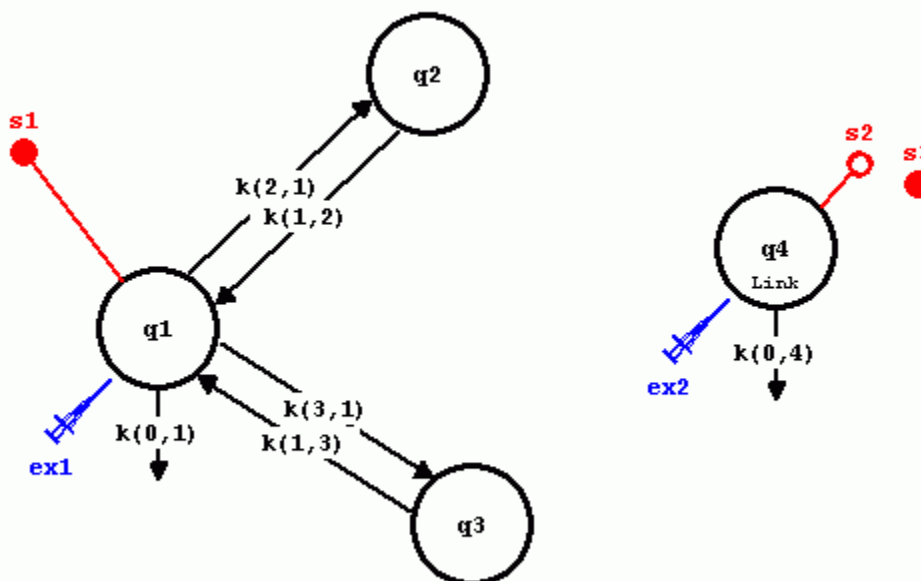
	Objective	Scaled Data Variance
s2 : effect	1.379369e+000	5.152349e-003
s1 : conc	-2.033751e+000	1.402546e-002

Total objective	-6.543828e-001	
AIC	1.091747e+000	
BIC	1.340680e+000	

Close the **Plot** and **Statistics** windows.

Quit the SAAM II Compartmental application. Do not save the changes to **pkpd.stu**.

This is an example of how a PKPD model can be created in SAAM II. The link between the PK model and the effect data comes via the sample equation s_2 containing the E_{max} model. There are many other options available to specify the link. Consider the following model:



This is a model in which a drug was introduced into plasma Compartment q_1 , and serial plasma samples were taken. The system model for the metabolism of the drug consists of Compartments q_1 , q_2 and q_3 . The sample s_1 is typical; it links the model with the plasma data. Compartment q_4 is the biophase or link compartment. It will be used to link the pharmacokinetics of the drug with the measured effect of the drug.

The input, ex_2 , into Compartment q_4 is defined " $ex_2=ki*s_1$ ". This links the pharmacokinetics of the drug with Compartment q_4 , and introduces a new parameter, ki which will be the compartment turnover. The sample s_2 is also typical; the sample equation is " $s_2 = q_4/V_4$ "; V_4 is a new parameter in the model. The sample is not linked to any data.

The pharmacodynamic equations are written in the **Equations Defined Here** pane in the **Equations** dialog box as shown below:

```

Eq Equations
-----
Equations Defined Elsewhere (read-only):
flux(2,1) = k(2,1) * q1
flux(1,2) = k(1,2) * q2
flux(3,1) = k(3,1) * q1
flux(1,3) = k(1,3) * q3
flux(0,4) = k(0,4) * q4
flux(0,1) = k(0,1) * q1
ex2.bolus = 0.0

Equations Defined Here:
E = Eo - Emax*Ce/(IC50 + Ce)
Ce = s2
Emax = Eo
ki = k(0,4)

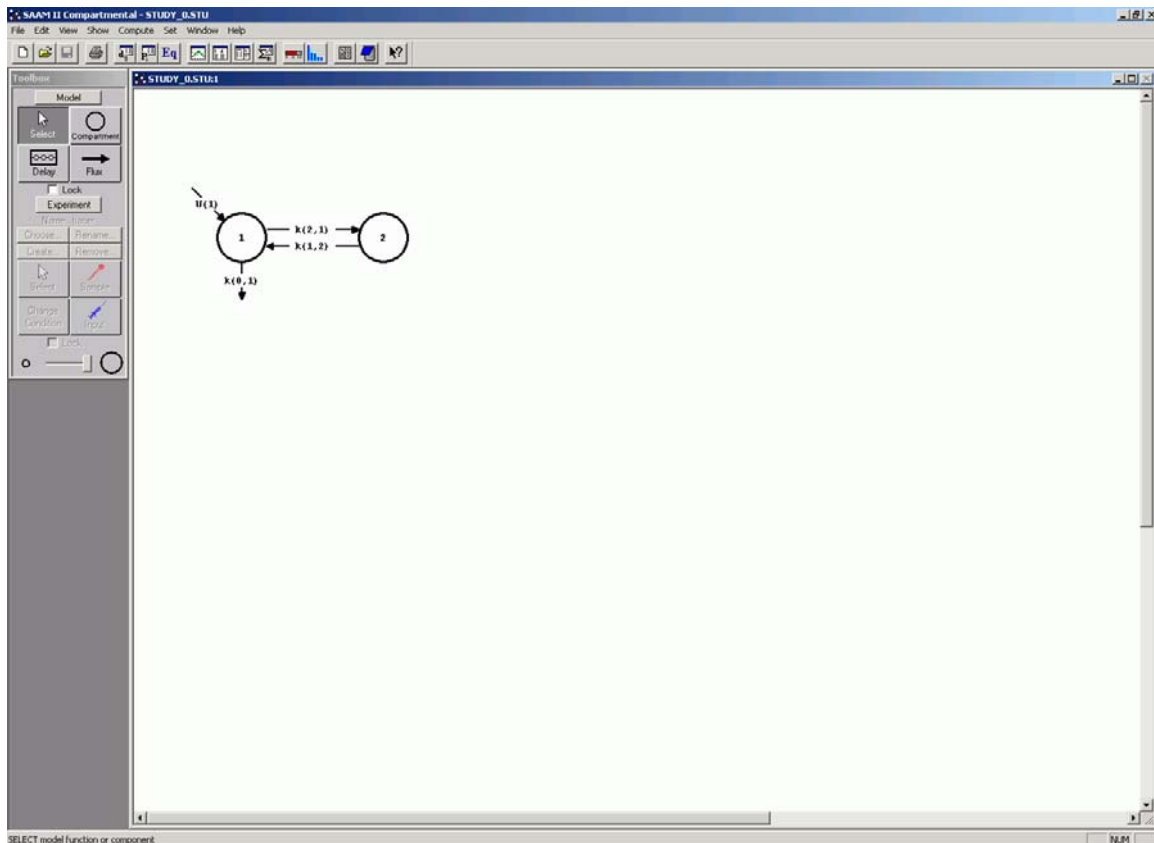
```

The first equation describes the relationship between the observed effect, E , the initial measurement of the drug effect, E_o , the concentration of drug in the link compartment, C_e , and the constants of the E_{max} model, E_{max} and IC_{50} . The second equation specifies C_e as equal to s_2 . The third equation specifies that the maximum drug effect, E_{max} , would simply consist of a complete reversal of the effect that was initially observed, E_o . In the last equation $k(0,4)$ is defined as a fractional clearance that is equal to k_i . In terms of Compartment **q4**, this is equivalent to an intercompartmental fractional clearance $k(4,1)$. However, the system model is structured without actual mass transfer between the pharmacokinetic model and the effect compartment. This allows the effect kinetic data to be analyzed without perturbing the pharmacokinetic “Fit.”

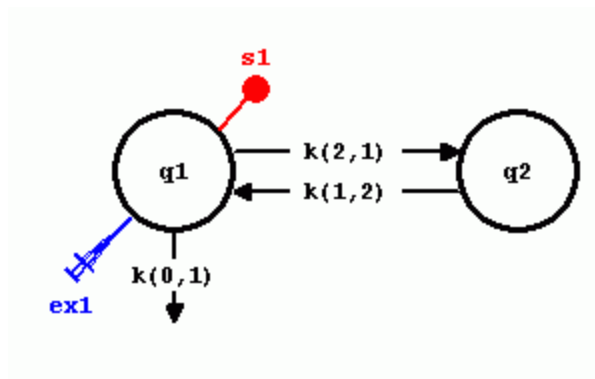
Part 2. Estimating the AUC for a Sample using the AUC Option in the Computational Settings Dialog Box.

There are two ways one can estimate area under the curve in the SAAM II **Compartmental** application. One way is to use the **AUC** command in the **Computational Settings** dialog box. This will be described in this tutorial.

1. Start the SAAM II **Compartmental** application. The SAAM II **Compartmental** main window will open.
2. Open the SAAM II **Compartmental** study file **study_0**.
 - a. The file **study_0.stu** should appear in the file list; if it does not, find the folder where you put this file.
 - b. In the **File** menu, click **Open**. The SAAM II **Compartmental** main window will appear as shown below:

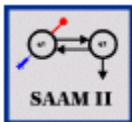
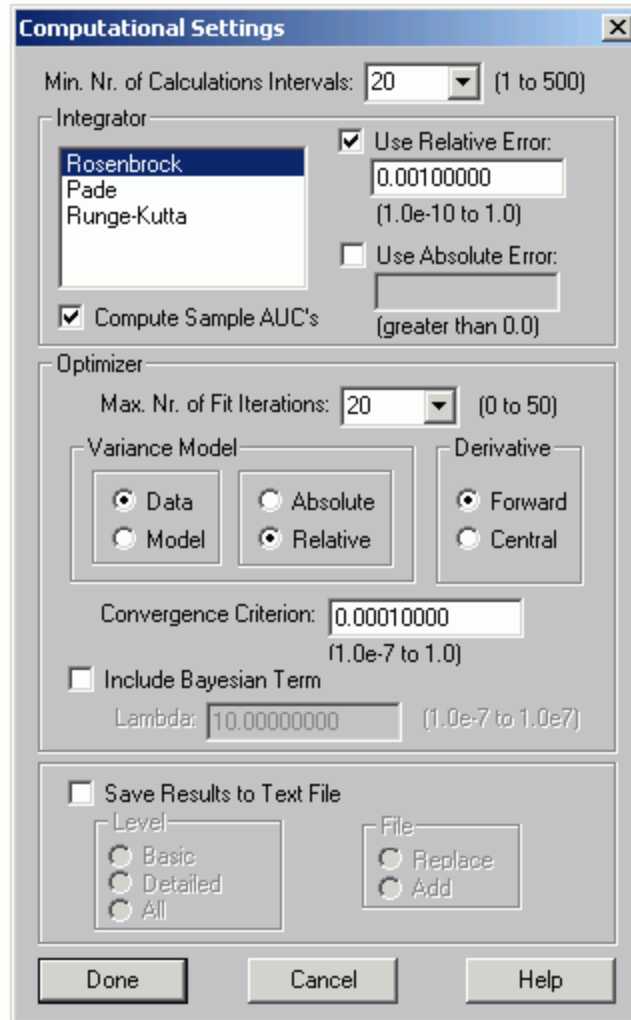


3. View the model and the experiment on the model. In the SAAM II **Toolbox**, click **Experiment**. The model of the experiment will appear on the **Drawing Canvas** as follows:



This is the experimental model developed and used in the **Getting Started with Compartmental** tutorial.

4. Estimate the area under **s1** and the first-moment (mean) area under **s1** for the duration of the experiment.
 - a. In the **Compute** menu, click **Computational Settings**. The **Computational Settings** dialog box will open.
 - b. In the **Integrator** pane, select **Compute Sample AUC's**. The **Computational Settings** dialog box will appear as follows:

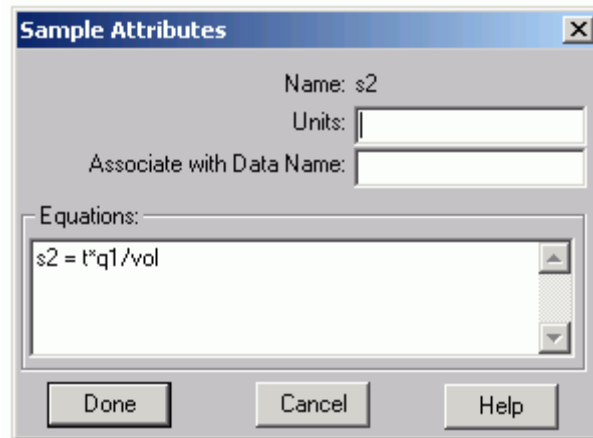


Sample AUC's. SAAM II will not estimate the sample area under the curve (AUC) unless this option is selected. SAAM II estimates AUC's only for samples. Thus, for example, if you wanted the AUC for Compartment **q2** in the model, you would need to put a sample on this compartment.

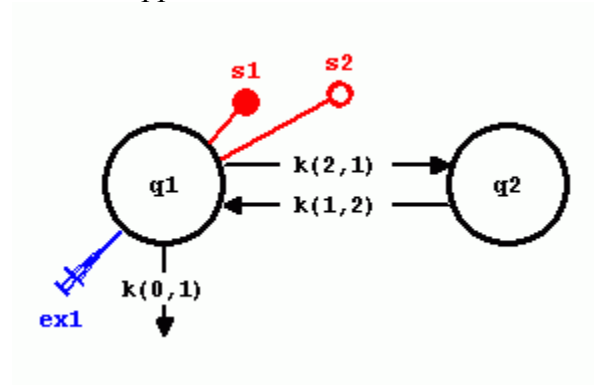


- c. Create a sample to estimate the first-moment (mean) area under **s1**.
 - (1) In the **SAAM II Toolbox**, click **Sample**.
 - (2) Click Compartment **q1** and then the **Drawing Canvas**. A sample **s2** will appear associated with Compartment **q1**.
 - (3) Double-click **s2** to open the **Sample Attributes** dialog box.

- (4) Type “ $s_2=t*q_1/vol$ ” in the **Equation** box. The **Sample Attributes** dialog box will appear as follows:



The model will appear as follows:



- (5) Click **Done**.



Samples and AUC's. The sample for the first-moment (mean) s_2 is written in terms of q_1 , and hence is associated with Compartment q_1 . The equation could have been written

$$s_2 = t * s_1$$

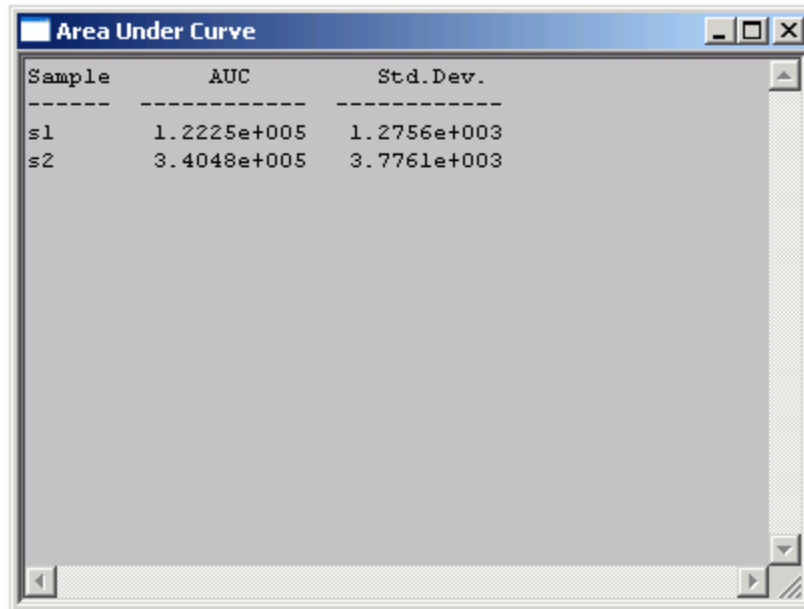
In that case, since q_1 does not appear directly in the equation, s_2 would no longer be associated with Compartment q_1 .



- d. Fit the model to the data. You can view the solution if you wish.

e. View the AUC's.

(1) In the **Show** menu, click **AUC's**. The **Area Under Curve** window will open as follows:



Sample	AUC	Std.Dev.
s1	1.2225e+005	1.2756e+003
s2	3.4048e+005	3.7761e+003

Because you have Fitted the model to the data, you will obtain standard deviations for the AUC's. If you had Solved the model, the standard deviations would not appear. Remember the AUC for **s1** is the area under the plasma curve, and the AUC for **s2** is the area under the first moment.



AUC's. When using the AUC option in the Computational Settings dialog box, the AUC will be calculated up to the end of the experiment. In this case, the numbers reported above are the AUC up to day 9. You can thus calculate the AUC over any time interval you wish (starting from time zero). To obtain an estimate of the AUC to time infinity, you need to set the time of the experiment appropriately long. This will be explained below.



(2) Close the **Area Under Curve** window.

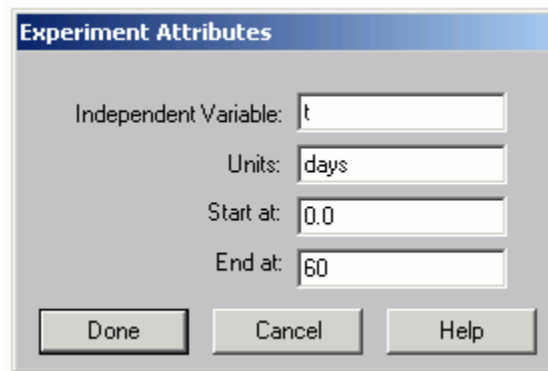
5. Estimate the AUC to “infinity”.

To obtain an estimate of the AUC to infinity, you need to set the time of the experiment to an appropriately long value. While there is no set rule on how to do this, a reasonable approach is the following.

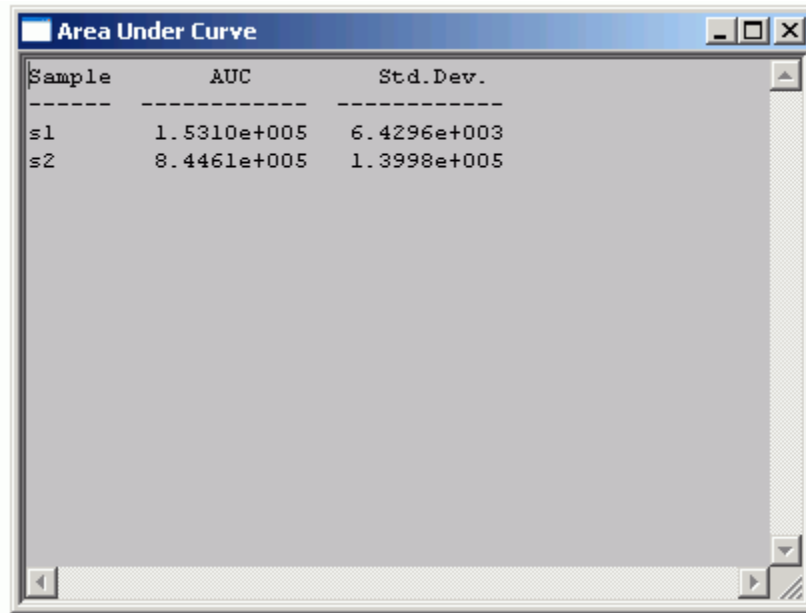
Calculate the inverse of the smallest rate constant in your model. By looking either in the **Parameters** dialog box or **Statistics** window, this value is approximately 0.175; the inverse is about 6.

Set the time of the experiment to 10 times this value, and Re-Fit the model to the data. In this case, the time will be set equal to 60. Then double this time, and Re-Fit the model to the data. The time will be 120. Keep doubling the time until there is no discernable difference between successive AUC calculations.

- a. In the **Set** menu, click **Experimental Attributes**.
- b. Change the length of the experiment from “9” to “60”. The **Experiment Attributes** dialog box will appear as follows:



- c. Click **Done**.
- d. Re-Fit the model to the data, and view the AUC's. The **Area Under Curve** window will appear as follows:

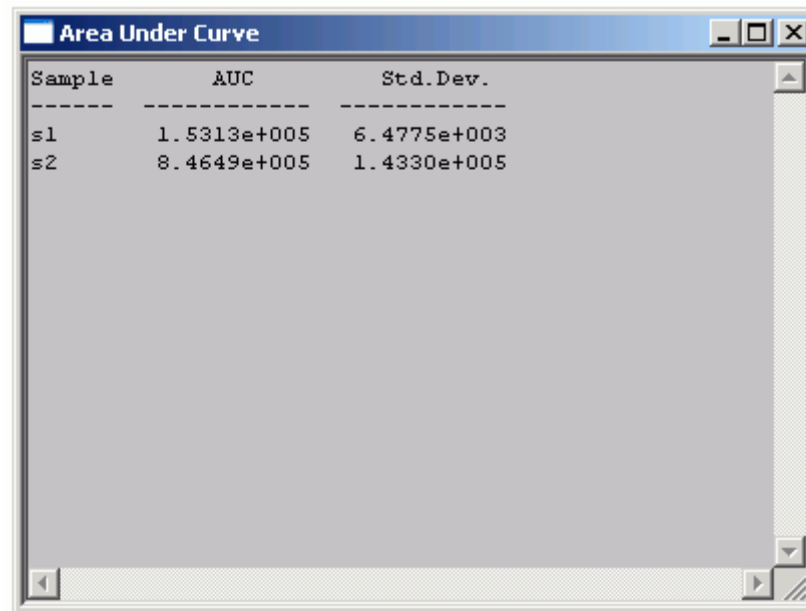


The screenshot shows a window titled "Area Under Curve" with a table containing the following data:

Sample	AUC	Std.Dev.
s1	1.5310e+005	6.4296e+003
s2	8.4461e+005	1.3998e+005

These numbers are obviously different from the previous estimates because the “integration” has gone from 9 to 60.

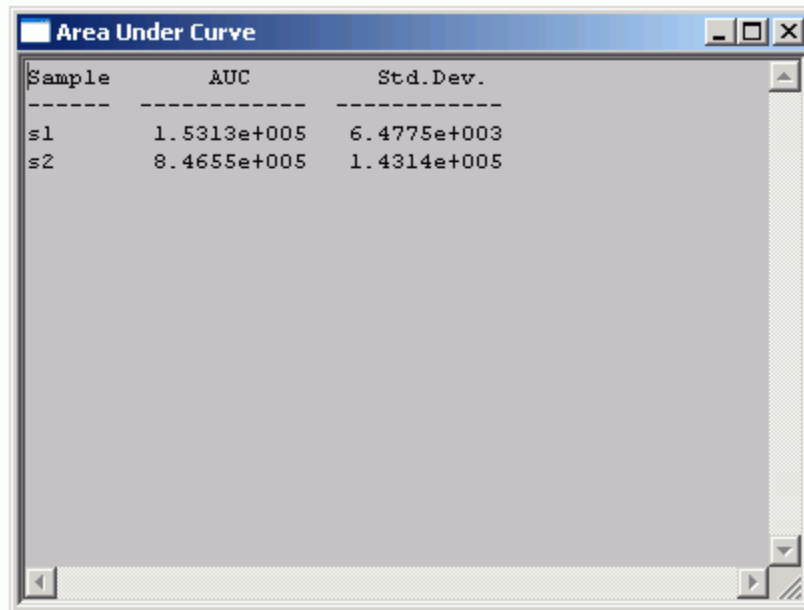
- e. Set the time of the experiment equal to 120. Re-Fit the model to the data and view the AUC's. The **Area Under Curve** window will appear as follows:



The screenshot shows a window titled "Area Under Curve" with a table containing the following data:

Sample	AUC	Std.Dev.
s1	1.5313e+005	6.4775e+003
s2	8.4649e+005	1.4330e+005

You can see now there is little difference; it is in the third significant figure for **s2**. If you set the time of the experiment equal to 240 and Re-Fit the model to the data, the **Area Under Curve** window will appear as follows:



Sample	AUC	Std.Dev.
s1	1.5313e+005	6.4775e+003
s2	8.4655e+005	1.4314e+005

Now there is no difference in **s1**, and the difference in **s2** is in the fourth significant figure. It is probably safe to stop at this stage.

- f. Close the **Area Under Curve** window.

Quit the **SAAM II Compartmental** application. Do not save the changes to **study_0.stu**.

Using this option may seem a little cumbersome if the area from zero to infinity is all that is desired. For a simple bolus injection into plasma, this can be the case. However, the option does allow you to estimate the area over different time intervals, something that can be important in observing the behavior of a substance in the body.

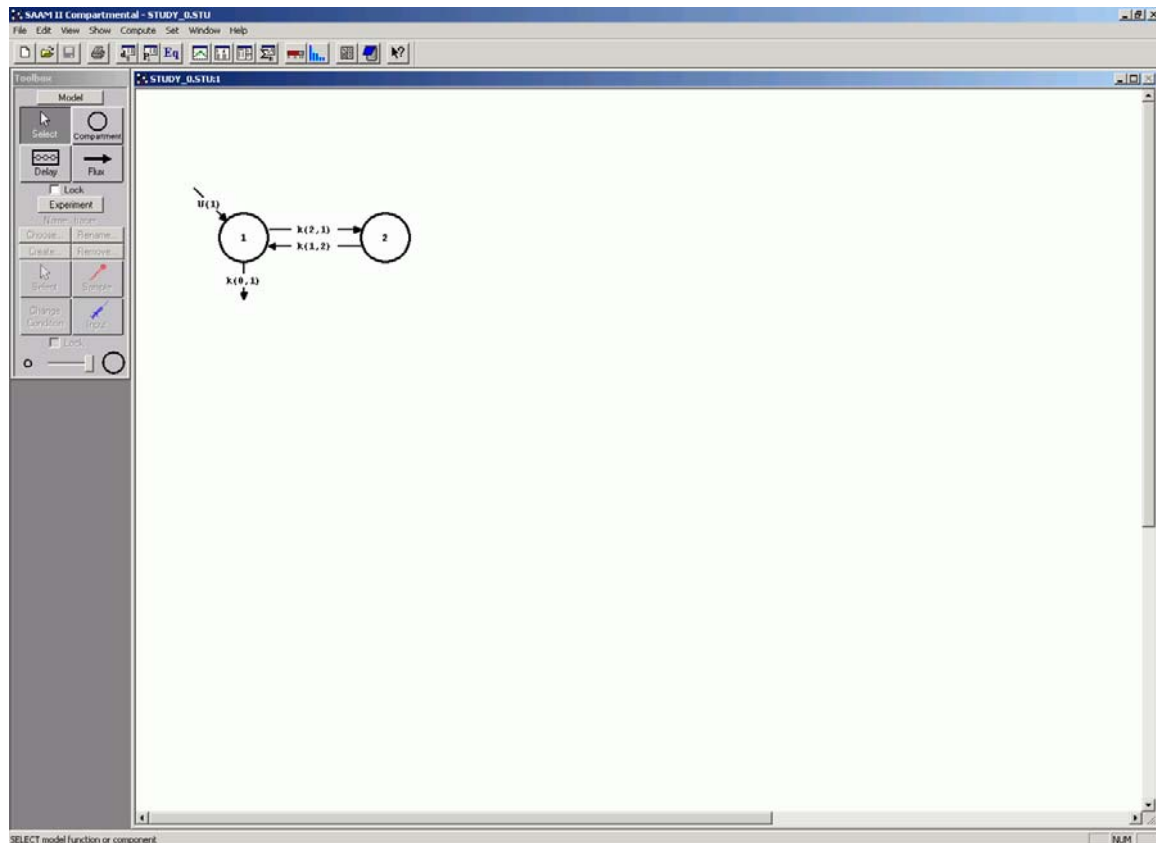
This option is really useful when you have inputs other than the bolus, especially multiple inputs. Writing equations to estimate areas in this case can be very tricky. Using other methods such as trapezoidal or log-trapezoidal rules with extrapolations can get even trickier.

For the simple bolus injection, sums of exponentials can be used in the **SAAM II Compartmental** application. This is explained in Part 3.

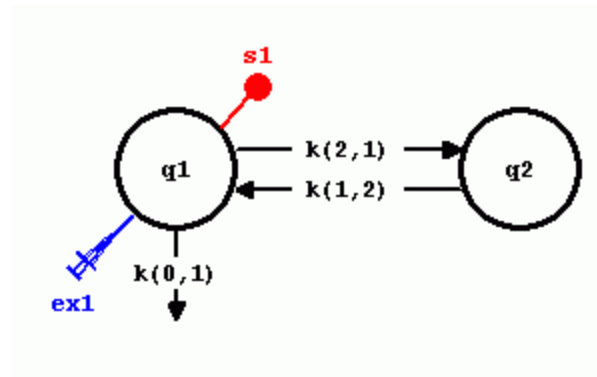
Part 3. Estimating the AUC for a Sample a Sum of Exponentials.

There are two ways one can estimate area under the curve in the SAAM II **Compartmental** application. One way is to use the **AUC** command in the **Computational Settings** dialog box as illustrated in Part 2. The other is to use sums of exponentials. This will be described in this tutorial.

1. Start the SAAM II **Compartmental** application. The SAAM II **Compartmental** main window will open.
2. Open the SAAM II **Compartmental** study file **study_0**.
 - a. The file **study_0.stu** should appear in the file list; if it does not, find the folder where you put this file.
 - b. In the **File** menu, click **Open**. The SAAM II **Compartmental** main window will appear as shown below:

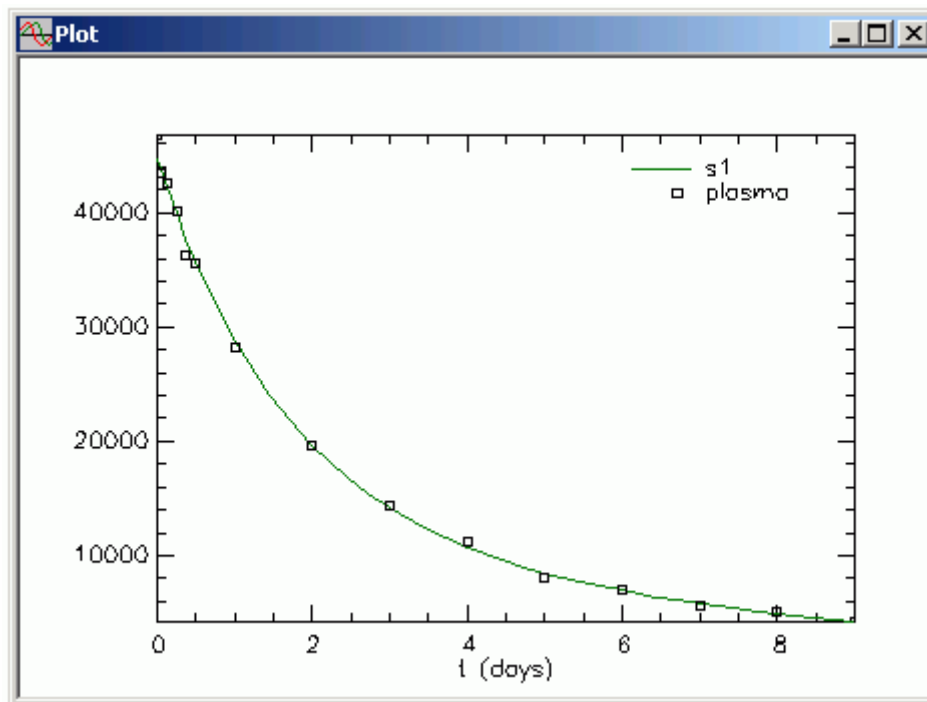


3. View the model and the experiment on the model. In the SAAM II **Toolbox**, click **Experiment**. The model of the experiment will appear on the **Drawing Canvas** as follows:



This is the experimental model developed and used in the **Getting Started with Compartmental** tutorial.

4. Since you have previously worked with **study_0**, you can proceed to Fit the model to the data, and view the solution. The plot of **s1:plasma** will appear as follows (in linear mode):



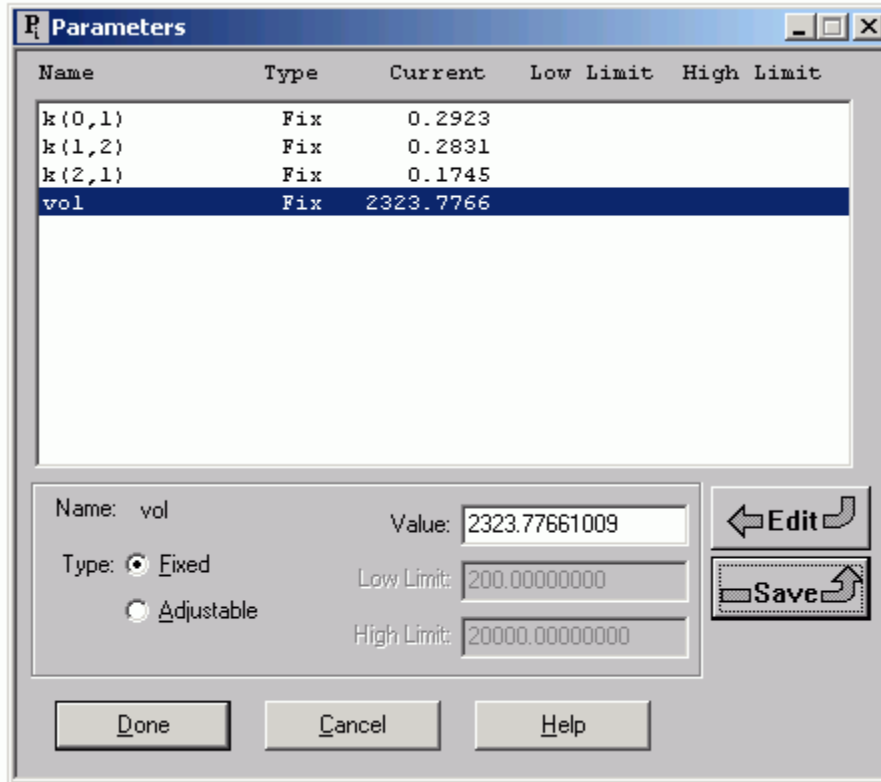
The statistics will appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
k(0,1)	0.29227	1.46155e-002	5.00071e+000	0.26010	0.32444
k(1,2)	0.28311	7.51183e-002	2.65335e+001	0.11777	0.44844
k(2,1)	0.17452	1.82636e-002	1.04652e+001	0.13432	0.21472
vol	2323.77661	3.85619e+001	1.65945e+000	2238.90219	2408.65103

	Objective	Scaled Data Variance
sl : plasma	1.336743e+001	1.113515e-001
Total objective	1.336743e+001	
AIC	7.935987e+000	
BIC	8.053995e+000	

Close the **Plot** and **Statistics** windows.

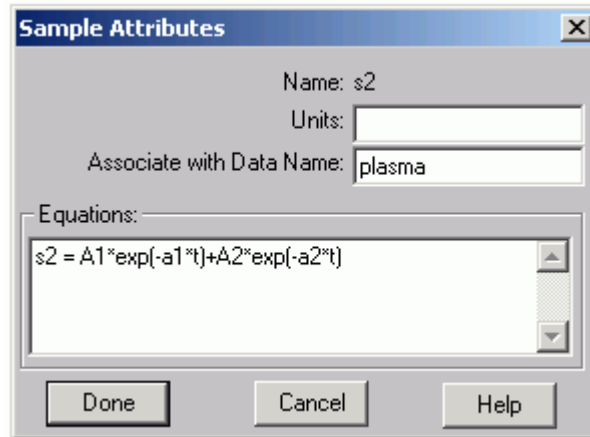
5. Estimate the area under the plasma curve and first-moment (mean) using a sum of exponentials.
 - a. Open the **Parameters** dialog box, and fix all model parameters. Select the first variable, click **Edit**, select **Fix**, and then click the **Save** button. Repeat for all variables. The **Parameters** dialog box will appear as follows:



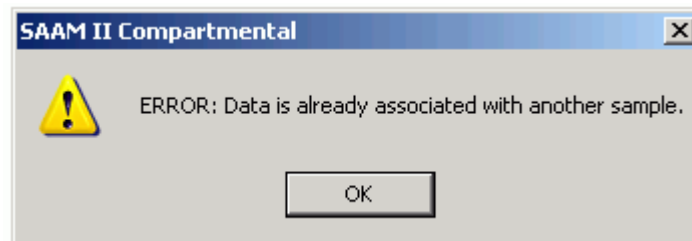
The reason you fix the model parameters is that, when you write your sum of exponentials, you introduce a new “numerical” model. The parameters of this model, as you will see, are adjustable so the compartmental model parameters must be fixed.

Click **Done** to close the **Parameters** dialog box.

- b. Create a sample to describe the plasma data by a sum of exponentials.
 - (1) In the **SAAM II Toolbox**, click **Sample**.
 - (2) Click Compartment **q1** and then the **Drawing Canvas**. A sample **s2** will be associated with Compartment **q1**.
 - (3) Double-click **s2** to open the **Sample Attributes** dialog box.
 - (3) Type “plasma” in the **Associate with Data Name** box.
 - (4) Type the equation “ $s2 = A1 \cdot \exp(-a1 \cdot t) + A2 \cdot \exp(-a2 \cdot t)$ ” in the **Equations** box. The **Sample Attributes** dialog box will appear as follows:



(5) Click **Done**. The following error message will appear:



Associating samples and data. In SAAM II, you cannot associate the same data name with more than one sample. To solve this problem, since the compartmental model and its sample **s1** has already been fitted, you can remove “plasma” from **s1** so that you can use it with **s2**.



(6) Click **OK**.

(7) In the **Sample Attributes** dialog box, click **Cancel**.

(8) Double-click **s1** to open the **Sample Attributes** dialog box.

(9) Remove “plasma” in the **Associate with Data Name** box.

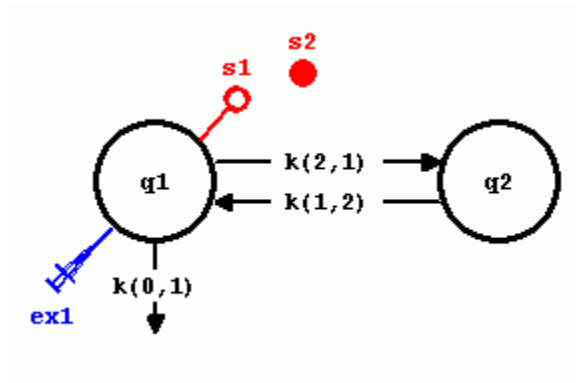
(10) Click **Done**. Because the sample equation remains, and the best fit parameters remain in the **Parameters** dialog box, the solution for **s1** will remain the best fit solution.

(11) Double-click **s2** to open the **Sample Attributes** dialog box.

(12) Type “plasma” in the **Associate with Data Name** box.

(13) Type the equation “ $s2 = A1*\exp(-a1*t)+A2*\exp(-a2*t)$ ” in the **Equations** box.

(14) Click **Done**. The model will appear as follows:



s1 is an open circle now because it is no longer associated with data. **s2** is a closed circle because it is associated with data. It is not connected to Compartment **q1** because **q1** does not appear in the equation for **s2**.

c. Estimate the area under the curve AUC and the mean area under the curve MAUC.

(1) In the **Show** menu, click **Equations**, or alternatively, on the **SAAM II Toolbar**, click **Equations** **Eq**. The **Equations** dialog box will open.

(2) Enter the following equations in the **Equations Defined Here** pane:

$$\begin{aligned} \text{AUC} &= A1/a1+A2/a2 \\ \text{MAUC} &= A1/a1^2+A2/a2^2 \end{aligned}$$

The **Equations** dialog box will appear as follows:

```

Eq Equations
-----
Equations Defined Elsewhere (read-only):
flux(1,2) = k(1,2) * q2
flux(2,1) = k(2,1) * q1
flux(0,1) = k(0,1) * q1
ex1.bolus = 0.0
ex1.infusion = 0.0
s2 = A1*exp(-a1*t)+A2*exp(-a2*t)
s1 = q1/vol

Equations Defined Here:
AUC = A1/a1+A2/a2
MAUC = A1/a1^2+A2/a2^2

```

(3) Close the **Equations** dialog box.



Sums of exponentials. Remember the number of exponential terms in your sum of exponentials equals the number of compartments in your model (in most cases). In this case, there are two compartments and hence two exponential terms. If there had been three compartments, there would have been three exponential terms. The formulas for AUC and AUMC are easy in this case because the mode of administration is a bolus. If the mode of administration were different, the number of exponential terms would still be two, but the expressions for AUC or AUMC could change depending upon the circumstances. In the case of multiple boluses or multiple infusions followed by a washout, the formulas can be very complex. For this reason, the scheme presented in Part 2 is more efficient.



- d. Enter the parameters for the sum of exponentials. Open the **Parameters** dialog box. Notice all the model parameters remain and are fixed. The new parameters are those for the sum of exponentials – $A1$, $A2$, $a1$ and $a2$. Enter the values as shown in the **Parameters** dialog box below:

Parameters

Name	Type	Current	Low Limit	High Limit
A1	Adj	40000.0000	4000.0000	4.000e+005
A2	Adj	10000.0000	1000.0000	1.000e+005
a1	Adj	0.5000	0.0500	5.0000
a2	Adj	0.0500	0.0050	0.5000
k(0,1)	Fix	0.2923		
k(1,2)	Fix	0.2831		
k(2,1)	Fix	0.1745		
vol	Fix	2323.7766		

Name: a2 Value: .05

Type: Fixed
 Adjustable

Low Limit: 0.00500000

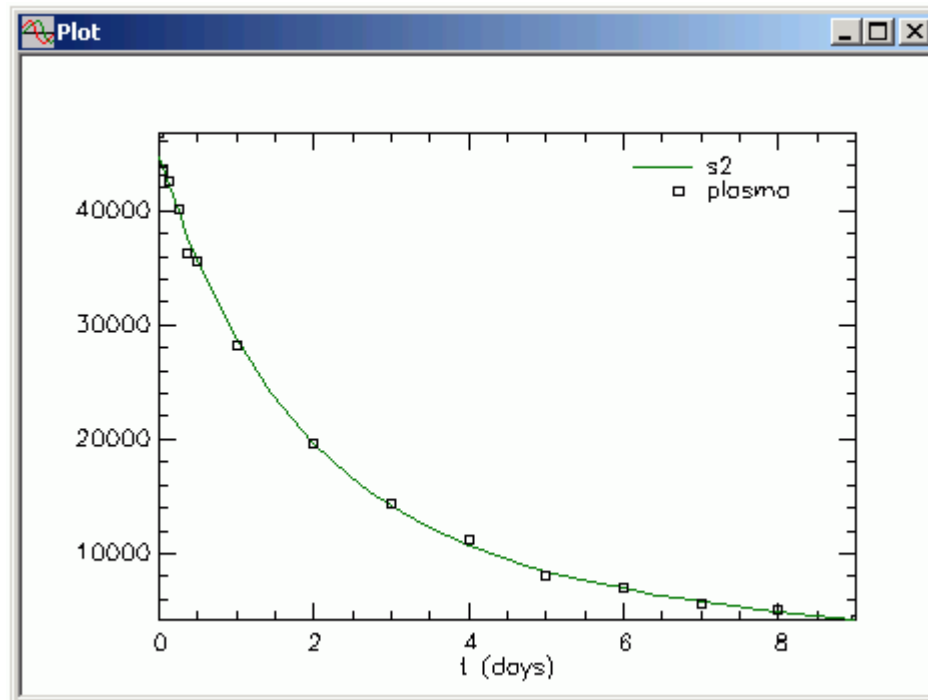
High Limit: 0.50000000

← Edit

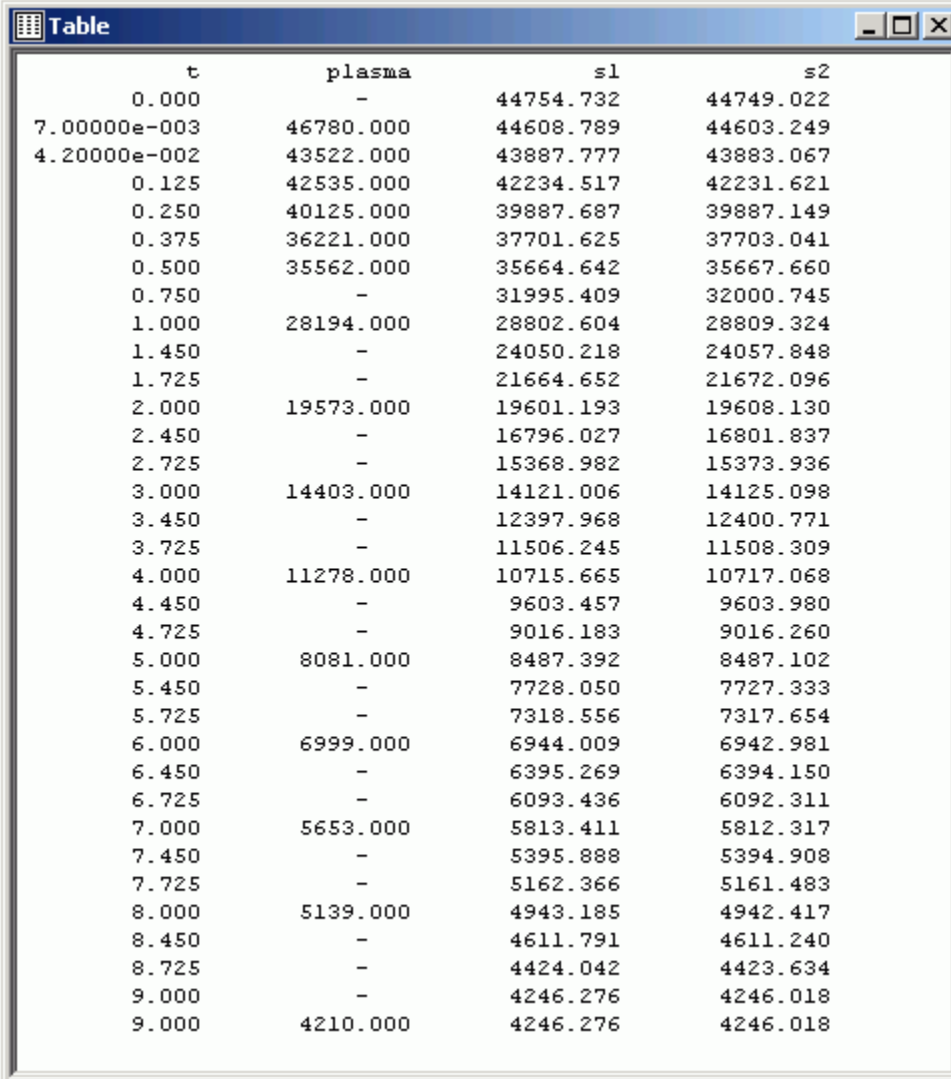
Save ↗

Done Cancel Help

- e. Fit the model to the data (remember the model now is the sum of exponentials). In the **Plot and Tables Variables Dialog Box**, select **s2:plasma** and click **Done**. The plot of **s2:plasma** will appear as follows:



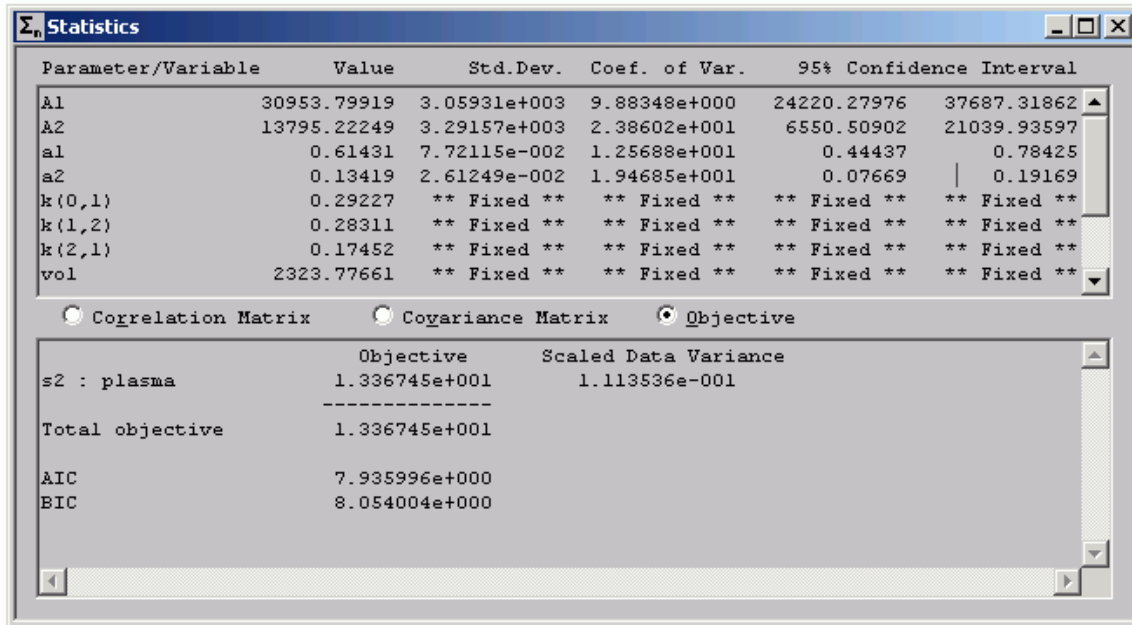
The plot, as expected, is essentially identical to the best fit of the two-compartment model to the data. You can actually verify this by plotting **s1**, **s2** and **plasma**. If you look at the table of values as shown below:



t	plasma	s1	s2
0.000	-	44754.732	44749.022
7.00000e-003	46780.000	44608.789	44603.249
4.20000e-002	43522.000	43887.777	43883.067
0.125	42535.000	42234.517	42231.621
0.250	40125.000	39887.687	39887.149
0.375	36221.000	37701.625	37703.041
0.500	35562.000	35664.642	35667.660
0.750	-	31995.409	32000.745
1.000	28194.000	28802.604	28809.324
1.450	-	24050.218	24057.848
1.725	-	21664.652	21672.096
2.000	19573.000	19601.193	19608.130
2.450	-	16796.027	16801.837
2.725	-	15368.982	15373.936
3.000	14403.000	14121.006	14125.098
3.450	-	12397.968	12400.771
3.725	-	11506.245	11508.309
4.000	11278.000	10715.665	10717.068
4.450	-	9603.457	9603.980
4.725	-	9016.183	9016.260
5.000	8081.000	8487.392	8487.102
5.450	-	7728.050	7727.333
5.725	-	7318.556	7317.654
6.000	6999.000	6944.009	6942.981
6.450	-	6395.269	6394.150
6.725	-	6093.436	6092.311
7.000	5653.000	5813.411	5812.317
7.450	-	5395.888	5394.908
7.725	-	5162.366	5161.483
8.000	5139.000	4943.185	4942.417
8.450	-	4611.791	4611.240
8.725	-	4424.042	4423.634
9.000	-	4246.276	4246.018
9.000	4210.000	4246.276	4246.018

you will see only minor differences due simply to the numerics of the software.

- f. View the statistics. The **Statistics** window will appear as follows:



Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
A1	30953.79919	3.05931e+003	9.88348e+000	24220.27976	37687.31862
A2	13795.22249	3.29157e+003	2.38602e+001	6550.50902	21039.93597
a1	0.61431	7.72115e-002	1.25688e+001	0.44437	0.78425
a2	0.13419	2.61249e-002	1.94685e+001	0.07669	0.19169
k(0,1)	0.29227	** Fixed **	** Fixed **	** Fixed **	** Fixed **
k(1,2)	0.28311	** Fixed **	** Fixed **	** Fixed **	** Fixed **
k(2,1)	0.17452	** Fixed **	** Fixed **	** Fixed **	** Fixed **
vol	2323.77661	** Fixed **	** Fixed **	** Fixed **	** Fixed **

	Objective	Scaled Data Variance
s2 : plasma	1.336745e+001	1.113536e-001
Total objective	1.336745e+001	
AIC	7.935996e+000	
BIC	8.054004e+000	

If you scroll in the **Parameter/Variable** pane, you will see the estimates for AUC and AUMC are:

	Value	SD	CoeffVar
AUC	153191.2	6.51E+03	4.25E+00
MAUC	848122	1.44E+05	1.70E+01

g. Close the **Statistics** window.

Quit the **SAAM II Compartmental** application. Do not save the changes to **study_0.stu**.

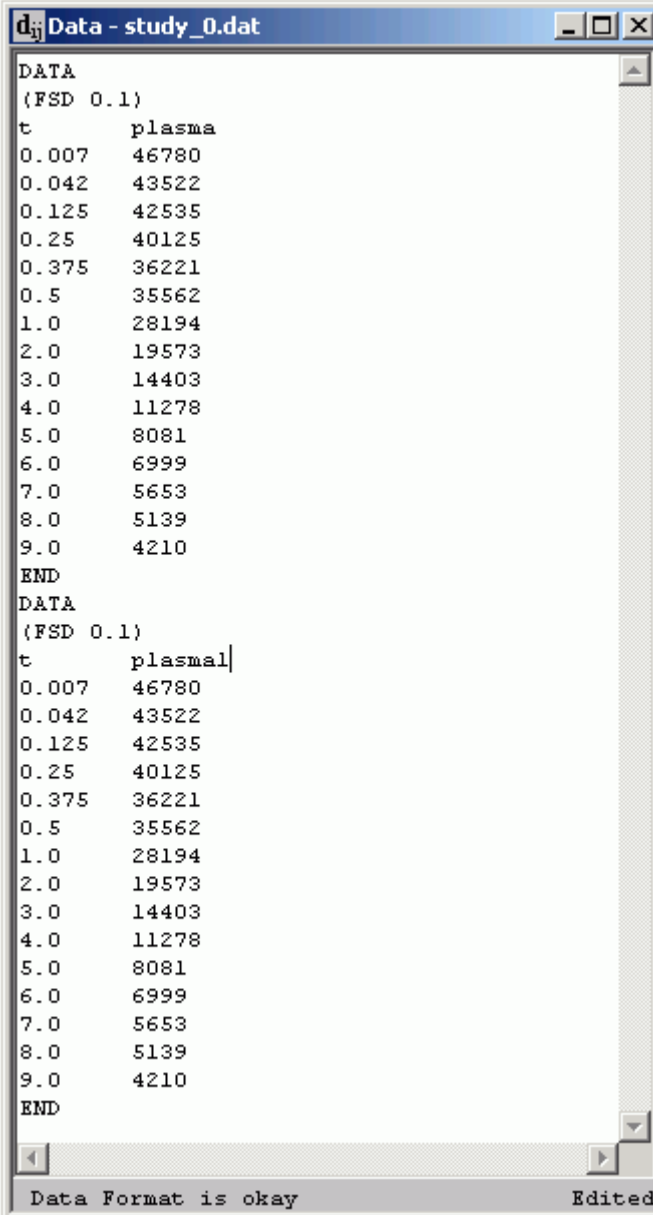


AUC calculations. Two points can be made in terms of the AUC calculations. If you worked through Part 2 as well as Part 3, how do the AUC calculations compare? The results are shown below:

Parameter	AUC Option		Sum of Exponential	
	Value	SD	Value	SD
AUC	153130	6478	153191	6514
MAUC	846550	143140	848122	144358

You can see there is very good correspondence, especially for AUC. For AUMC, in Part 2, you may want to extend the time of the experiment a little longer; the difference is in the third significant figure.

The other point dealt in this tutorial with the fact that “plasma” cannot be associated with two samples. Suppose you wanted to Fit the compartmental model and the sum of exponentials simultaneously. You can do this if you create another table for the plasma data, but give it a different name. Below is an example:



```
DATA
(FSD 0.1)
t      plasma
0.007  46780
0.042  43522
0.125  42535
0.25   40125
0.375  36221
0.5    35562
1.0    28194
2.0    19573
3.0    14403
4.0    11278
5.0    8081
6.0    6999
7.0    5653
8.0    5139
9.0    4210
END
DATA
(FSD 0.1)
t      plasma1
0.007  46780
0.042  43522
0.125  42535
0.25   40125
0.375  36221
0.5    35562
1.0    28194
2.0    19573
3.0    14403
4.0    11278
5.0    8081
6.0    6999
7.0    5653
8.0    5139
9.0    4210
END
Data Format is okay      Edited
```

Here the plasma data have been duplicated, but in the second table, they are named “plasma1”. In this situation, you can leave **s1** associated with “plasma” and associate **s2** with “plasma1”. Now there will be no error message. When you Fit the two models, there are in fact two models – the compartmental and sum of exponential -, to the data, you will get approximately the same final parameter values, but the statistics will be a little different. The reason is that

SAAM II creates only one objective function. Here with two models, there should be two. This is why we separated the models as we did.

