Using SAAM II

Multiple Input-Output Experiments

Multiple Input-Output Experiments

Prerequisites

The prerequisite for this tutorial is having worked through the **SAAM II** introductory tutorial, "Getting Started with **SAAM II Compartmental**." It will be helpful to have worked through the Using SAAM II Tutorial "Using Delays – Part 1."

What you will learn in this tutorial

The purpose of this tutorial is to show you have to deal with experiments with multiple inputs and multiple outputs. You will learn how to

- Create multiple input-output experiments using the **Duplicate** command (Part 1).
- Create multiple input-output experiments using **Create Experiment** in the **SAAM II Toolbox** (Part 2).

Files Required

Study Files: The study files for this tutorial are

io_duplicate.stu
io_expt.stu
io_expt_final.stu

The study file **io_duplicate.stu** is saved for convenience and can be used with Part 1. The study file **io_expt.stu** will be used for Part 2. The study file **io_expt_final.stu** is the study file created in Part 2.

Data File: The data file for this tutorial is

io.dat

This file is included as part of this tutorial.

Introduction

There are many instances when your experimental protocol may call for multiple inputs. Examples in metabolic kinetics come from multiple tracer studies. For example, carbon and hydrogen labeled glucose can be injected (two-inputs) and both tracers followed in plasma samples (two-outputs). In pharmacokinetic studies, an example would be the iv and oral administration of a drug in the same individual. Normally this is done at different times so the assumption has to be made that the subject is in the same “state” in both experiments. More recently, it has become possible to label a drug with a stable isotope. The drug and isotopically labeled drug can then be administered iv and orally (two-inputs), and plasma levels of drug and stable isotope followed (two-outputs). Finally, the increased use of tools such as MRI and PET is making multiple input-output studies more common. Multiple input-output studies will become more important especially in the pharmaceutical industry as the process of drug discovery and development becomes more sophisticated.

There are two ways multiple input-output studies can be created in SAAM II. One uses the model duplication command in the **Edit** menu. Here, as illustrated in Part 1, you can create a model, and then duplicate the model structure. You have the option of having corresponding rate constants set equal, or they can be different. In Part 1, you will see that you can create a model for the iv system. Then you can duplicate this model, and add to it components to describe absorption.

The other method uses the **Create Experiment** option in the **SAAM II Toolbox**. Here you first create the system model. In the system model, you can have inputs at different sites. SAAM II does the book keeping via the **Create Experiment** option. You create a different experiment for each input.

The most common experiment is the single input experiment. When you create your experiment, the compartment names change from, for example, **1** to **q1**. When you create multiple experiments, **q1** changes to, for example, **q1.1** for a second experiment, **q1.2** for a third experiment, etc. Using this option makes it much easier to keep track of the individual inputs and outputs.

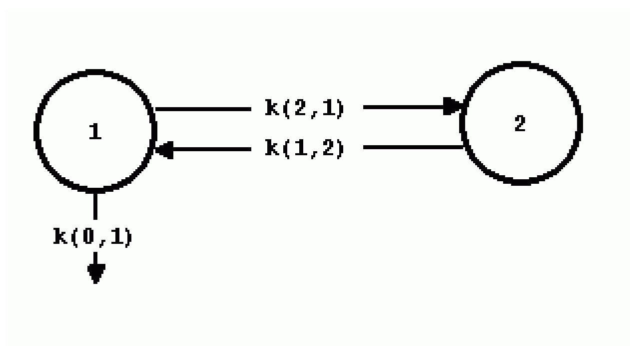
The data to be used in this tutorial come from a pharmacokinetic study in which a drug was first given iv, and then later via a suspension into the upper GI tract.

Part 1. Creating a Multiple Input-Output Experiment Using the Duplicate Command

In this tutorial, you will simultaneously analyze data following an iv administration and oral suspension administration of a drug using the Duplicate command. In the iv experiment, the drug was infused for 20 minutes while in the suspension, it was given as a bolus.

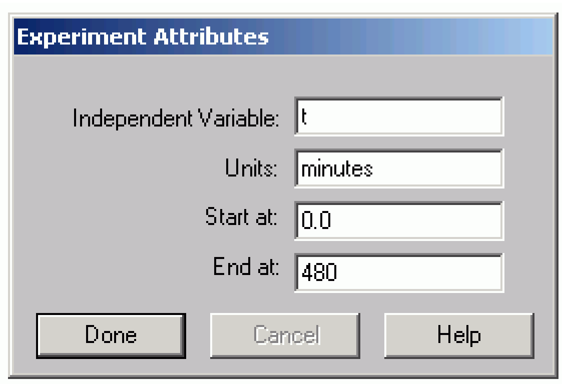
1. **Start** the **SAAM II Compartmental** application. The **SAAM II Compartmental** main window will open.

2. Create the following system model for the iv data on the **Drawing Canvas**:

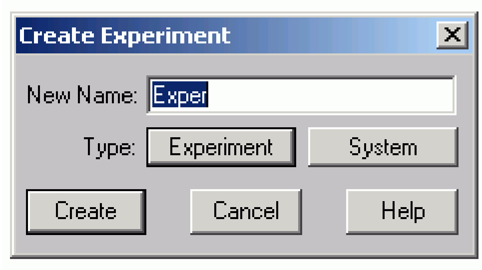


3. In the **SAAM II Toolbox**, click **Experiment**. The **Experiment Attributes** dialog box will open.

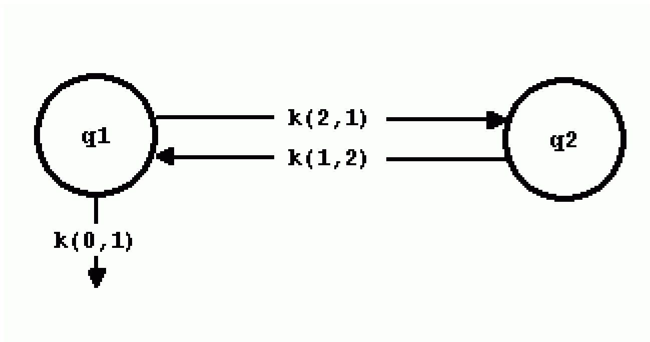
   a. Type "480" in the **End At** box. **The Experiment Attributes** dialog box should appear as follows:



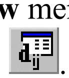
   b. Click **Done**. The **Create Experiment** dialog box will open as follows:



c. Click **Create**. The model will appear on the **Drawing Canvas** as follows:

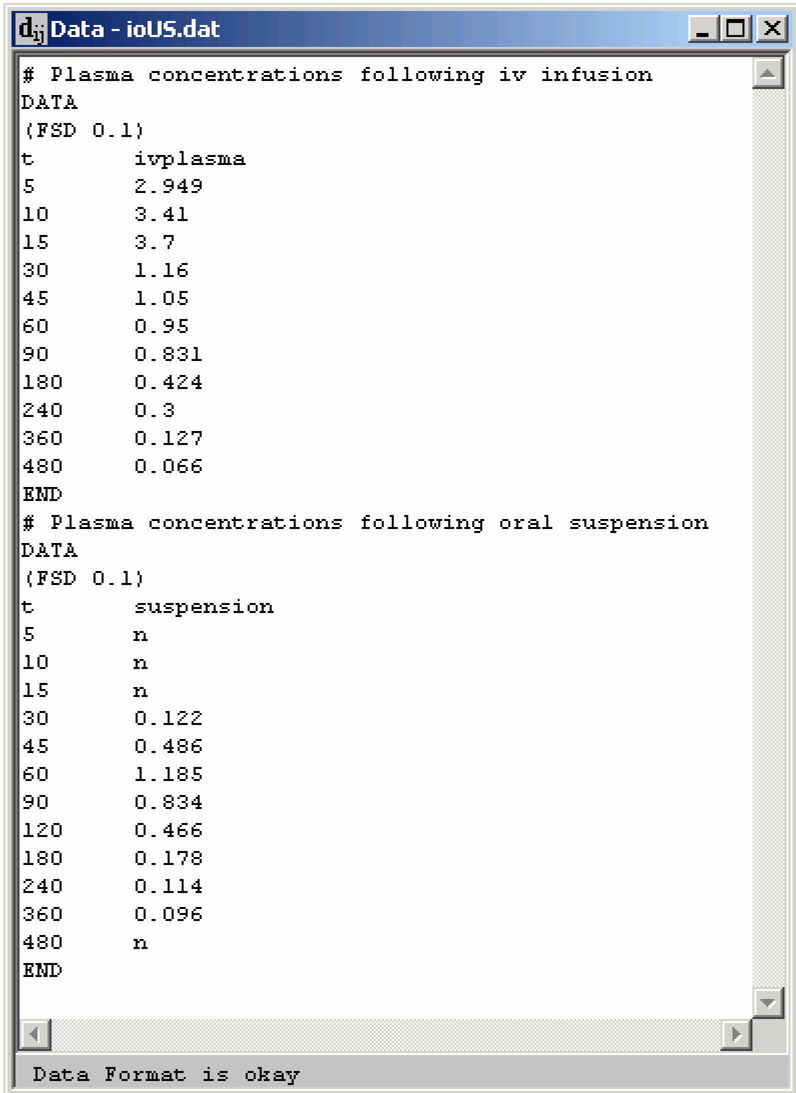


4. Add the data to your model.

   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 **io.dat** should appear in the list (if it does not, find the folder where you have put this data file).

   c. Double-click **io.dat**. The data file contains drug data following the 20 minute constant infusion, “ivplasma”, and following the oral administration, “suspension.” The oral data will be used later. The **Data** window appears as follows:



Data - ioUS.dat

```
# Plasma concentrations following iv infusion
DATA
(FSD 0.1)
t        ivplasma
5        2.949
10       3.41
15       3.7
30       1.16
45       1.05
60       0.95
90       0.831
180      0.424
240      0.3
360      0.127
480      0.066
END
# Plasma concentrations following oral suspension
DATA
(FSD 0.1)
t        suspension
5        n
10       n
15       n
30       0.122
45       0.486
60       1.185
90       0.834
120      0.466
180      0.178
240      0.114
360      0.096
480      n
END
```

Data Format is okay

The weighting scheme is FSD; you can leave the variance model set as the default data-relative.

d. Close the **Data** window.

5. Create a sample and an input

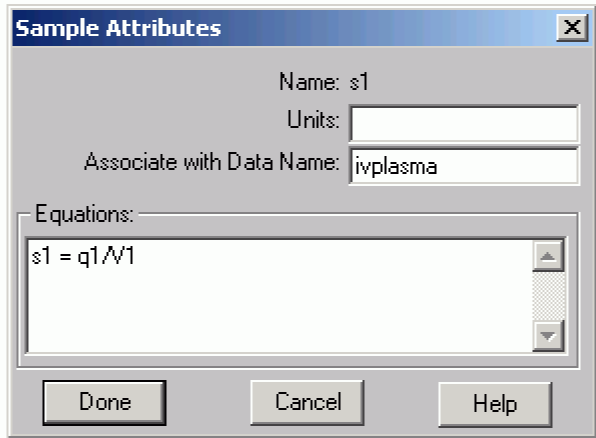
   a. In the **SAAM II Toolbox**, click **Sample**.

   b. Click Compartment **q1** and then the **Drawing Canvas**. The sample **s1** will appear associated with Compartment **q1**.

   c. Double-click **s1** to open the **Sample Attributes** dialog box.

   d. Type "ivplasma" in the **Associate with Data Name** box.

e. Edit the sample equation to read “s1=q1/V1”. The **Sample Attributes** dialog box will appear as follows:



f. Click **Done**.

g. In the **SAAM II Toolbox**, click **Input**.

h. Click Compartment **q1** and then the **Drawing Canvas**. The input **ex1** will appear associated with Compartment **q1**.

i. Specify the 20 minute infusion.

(1) Double-click **ex1** to open the **Exogenous Input** dialog box.

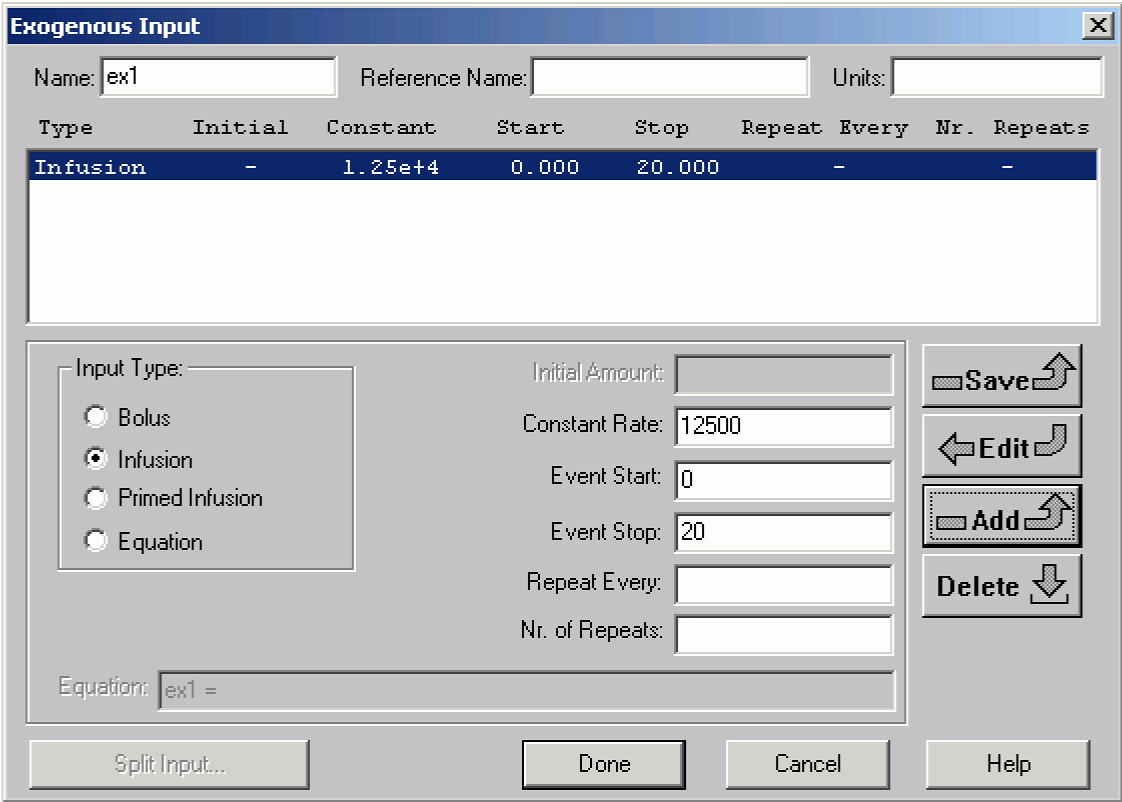
(2) Select **Infusion** in the **Input Type** pane.

(3) Type “12500” in the **Constant Rate** box.

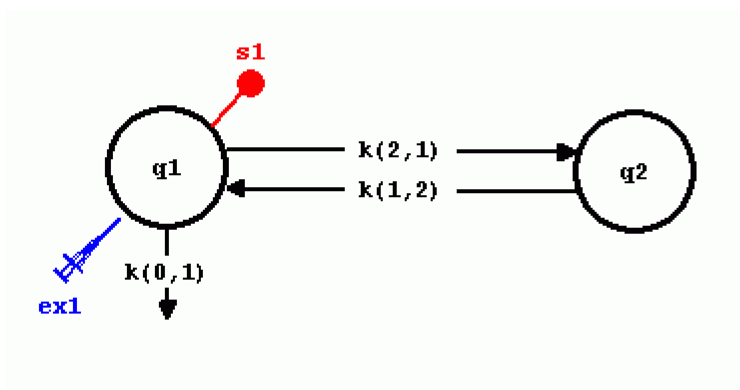
(4) Type “0” in the **Event Start** box.

(5) Type “20” in the **Event Stop** box.

(6) Click **Add**. The **Exogenous Input** dialog box should appear as follows:



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



6. Enter the parameter values.

   a. In the **Show** menu, click **Parameters**, or alternatively, on the **SAAM II Toolbar,** click **Parameters** . The **Parameters** dialog box will open.

   The parameter *V1* should be selected. If it is not selected, double-click *V1*. Be sure the **Adjustable** option is selected.

b. Type “13000” in the **Value** box, “2000” in the **Low Limit** box, “20000” in the **High Limit** box, and click **Save**.

c. Double-click *k(0,1)* to select it.

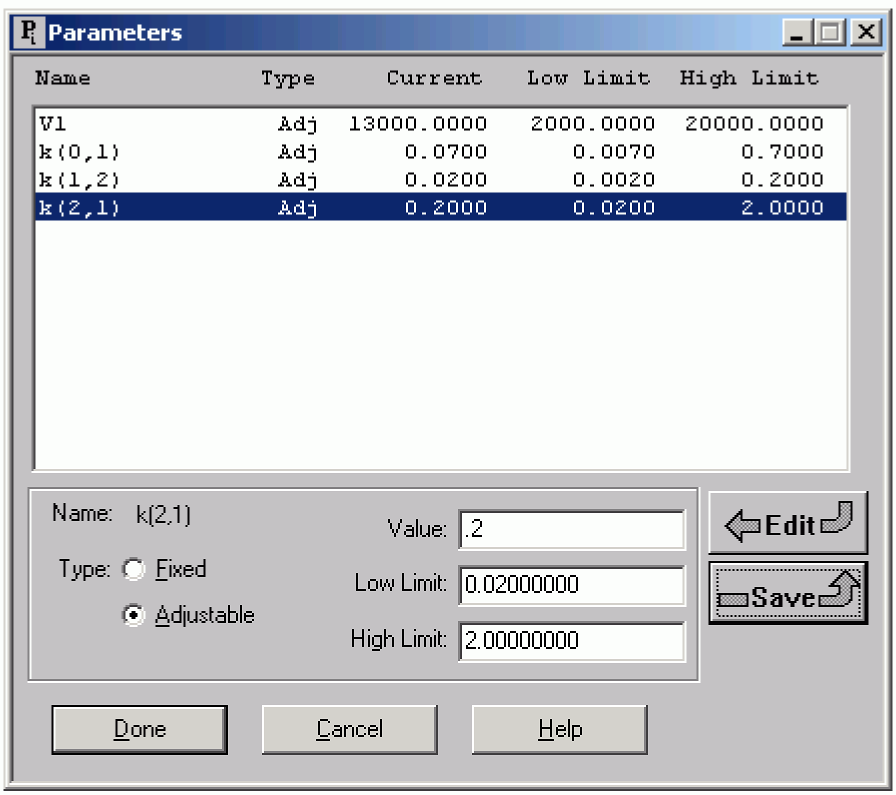
d. Type “0.07” in the **Value** box, and click **Save**.

e. Double-click *k(1,2)* to select it.

f. Type “0.02” in the **Value** box, and click **Save**.

g. Double-click *k(2,1)* to select it.

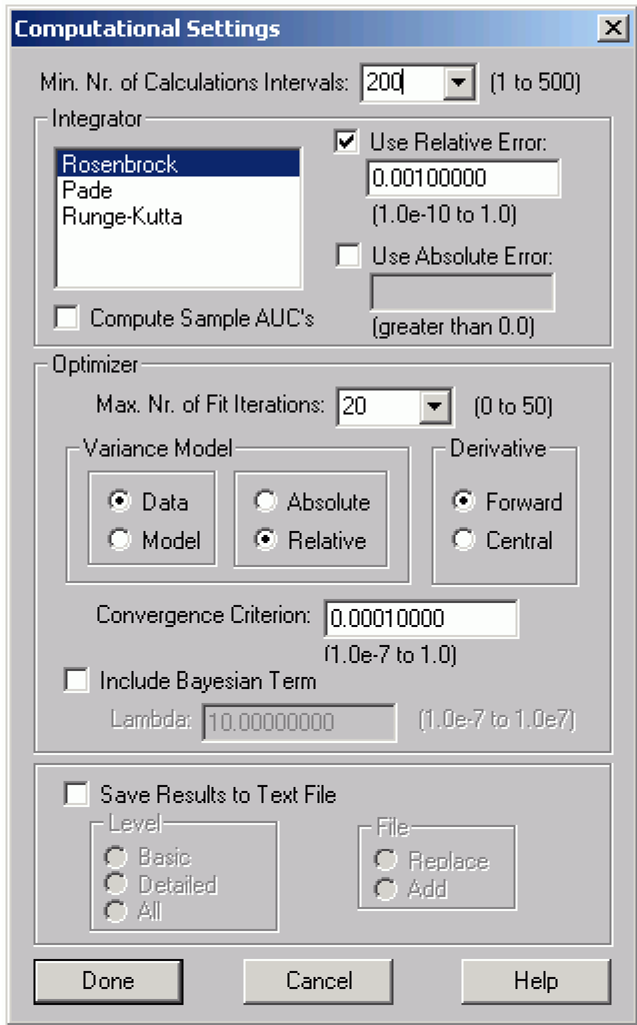
h. Type “0.2” in the **Value** box, and click **Save**. The **Parameters** dialog box should appear as follows:



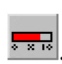
i. Click **Done**.

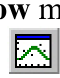
7. Solve the model and view the solution.

a. It will be helpful to have better resolution of your plots than the default. In the **Compute** menu, click **Settings**. The **Computational Settings** dialog box will open. Change the entry in the **Min. Nr. of Calculation Intervals** from “20” to “200”. The **Computational Settings** dialog box should appear as follows:



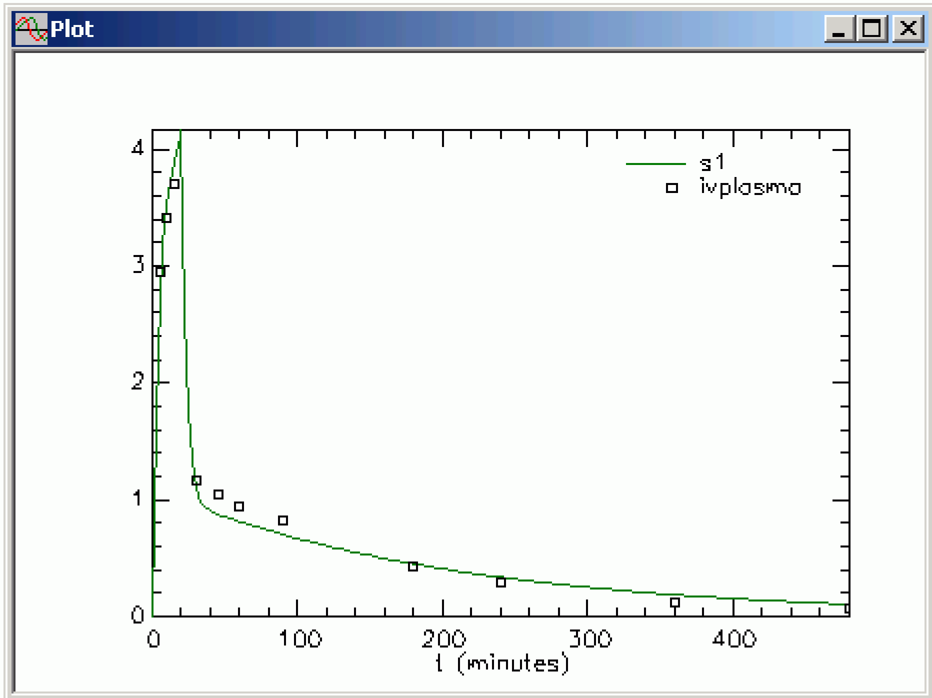
Click **Done**.

b. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II Toolbar,** click **Solve** .

c. 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.

d. Click **s1:ivplasma**; it will move to the **Current Selection** pane.

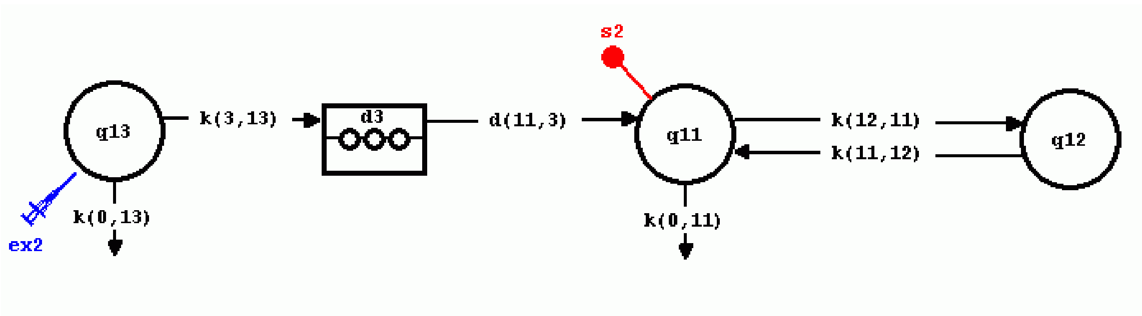
e. Click **Done**. The following plot will appear (in linear mode):



f. Close the **Plot** window.

You have now created the model which will be used to analyze the iv plasma data. The system model for the oral suspension data will now be created assuming once the drug enters plasma it behaves the same as the drug introduced using the iv infusion.

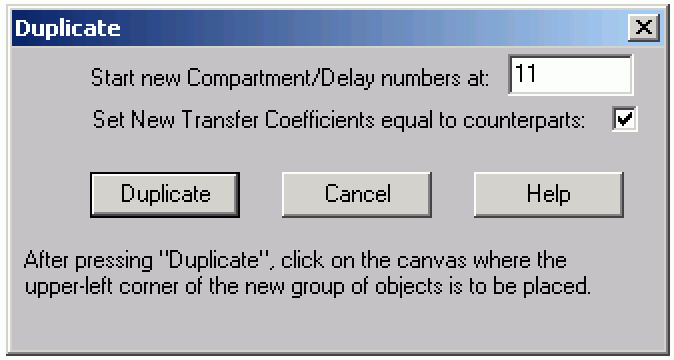
8. Create the following system model for the oral suspension data using the **Duplicate** command.



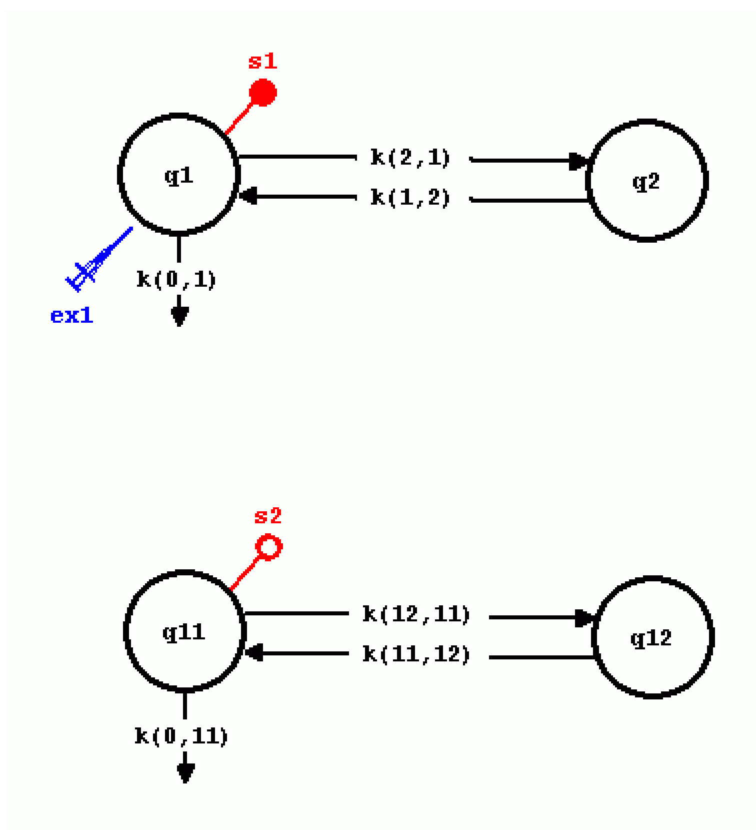
a. While pressing the **CTRL** key, click **q1**, **q2** and **s1**.

b. In the **Edit** menu, click **Duplicate**. The **Duplicate** dialog box will open.

c. Change the entry in the **Start new Compartment/Delay numbers at** box equal to "11". Select the **Set New Parameters Transfer Coefficients equal to counterparts** box. The **Duplicate** dialog box will appear as follows:



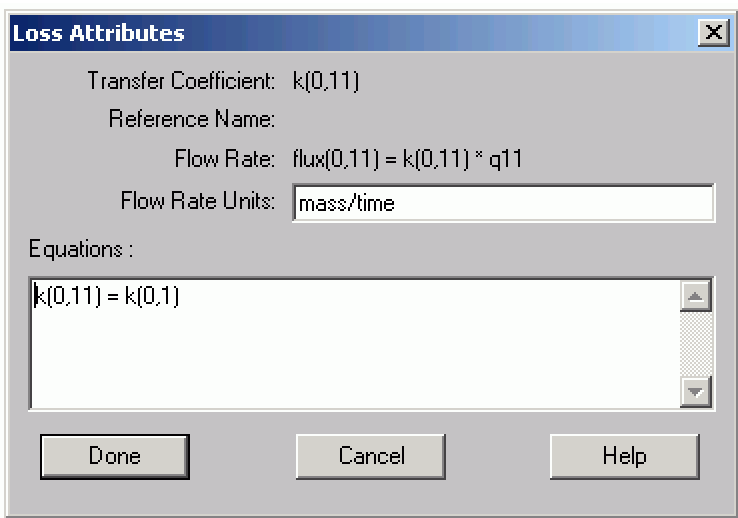
d. Click **Duplicate**. As instructed in the **Duplicate** dialog box, click on the Drawing Canvas approximately where you would like the duplicated model to appear. The following models will appear on the **Drawing Canvas** (after some rearrangement):



Duplicating. The **Duplicate** command allows you to duplicate a model, or a portion of a model. There are several features that are important.

- The duplicated sample **s2** will appear associated with Compartment **q11** (in this case). It is not, however, associated with data and hence the circle is open. In addition, the equation for the sample is "s2=q11", that is, the equation for sample **s1** does not carry over to **s2**.
- You have the option of setting the compartment number from where the duplicated model will start. For this reason, the order in which you click the compartments as you begin the duplication process can be important.
- You have the option of having the parameters of the duplicated model be different. In that case, the structure is preserved but the parameters, *k(0,11), k(12,11)* and *k(11,12)* would appear in the **Parameters** dialog box. The other option, the one chosen here, is to set the new parameters' transfer coefficients equal to their counterparts.

To see how this works, double-click, for example, k(0,11). The **Loss Attributes** dialog box will appear as follows:



SAAM II has defined "k(0,11)=k(0,1" thus saving you the trouble of having to do this for every parameter in the duplicated model. (Of course, you can remove this equation if you wish in which case *k(0,11)* would appear as a parameter in the **Parameters** dialog box.)

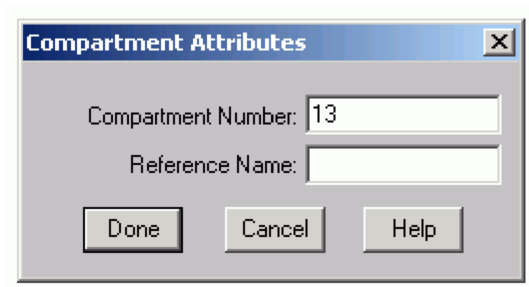
If the option of setting the parameters equal to their counterparts is chosen, it is necessary to Solve the original model first in order for the model to be created internally in SAAM II.

e. Create the model for oral absorption.

(1) In the **SAAM II Toolbox**, click **Model**. The **Model** tools are now available.

(2) In the **SAAM II Toolbox**, click **Compartment**.

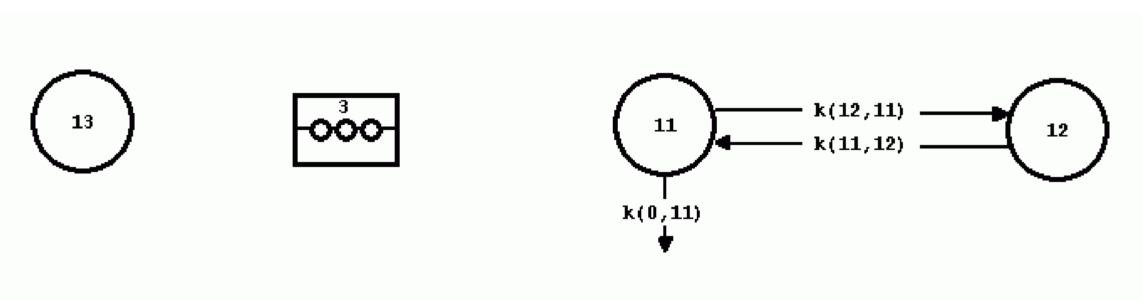
(3) Click on the **Drawing** canvas where you would like the new compartment to appear. Compartment **3** will appear. Double-click Compartment **3** to open the **Compartment Attributes** dialog box. Change the number of the compartment from "3" to "13". The **Compartment Attributes** dialog box will appear as follows:



Click **Done**.

(4) In the **SAAM II Toolbox**, click **Delay**.

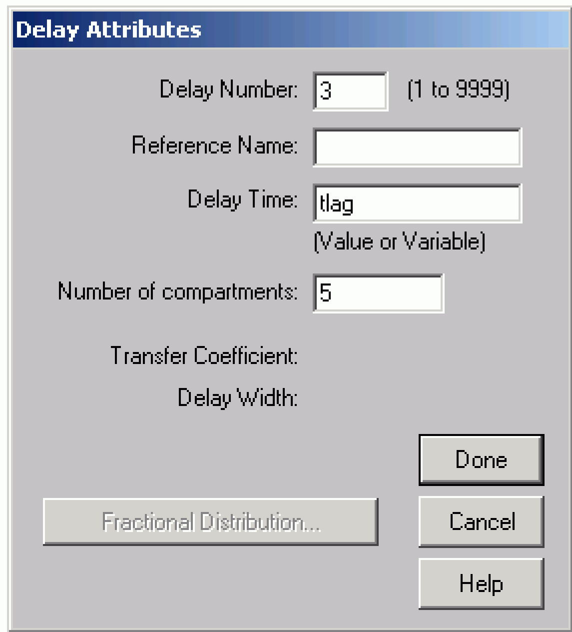
(5) Click on the **Drawing Canvas** where you would like the delay to be located. Place it between Compartments **13** and **11**. Your model for the oral dose should appear as follows:



(6) Double-click Delay **3**. The **Delay Attributes** dialog box will open.

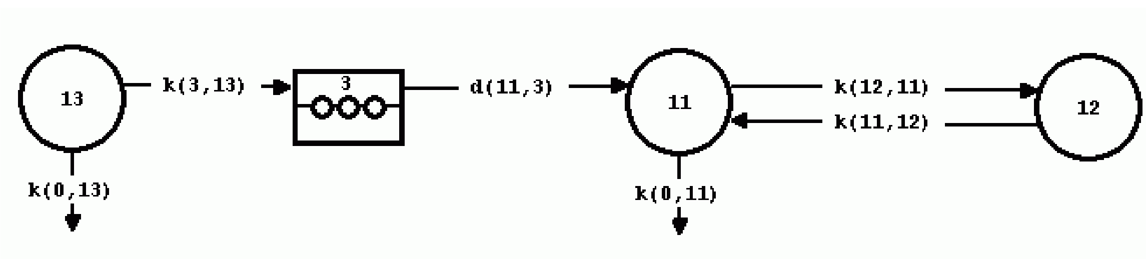
(7) Change the entry in the **Delay Time** box to "tlag". *tlag* will become an adjustable parameter equal to the delay time in absorption.

(8) Change the entry in the **Number of compartments** box to "5". The **Delay Attributes** dialog box should appear as follows:



(9) Click **Done**.

(10) Add the fluxes *k(0,13)*, *k(3,13)* and *d(11,3)*. The model will appear as follows:



Remember the output from the delay is denoted by "d" to signify the fraction of the material in the delay moving along this transfer pathway. In this case, *d(11,3)* is equal to 1.

9. Create an input into Compartment **q13**.

   a. In the **SAAM II Toolbox**, click **Experiment** to make these tools available.

   b. Click **Input**.

   c. Click Compartment **q13** and then the **Drawing Canvas**. The input **ex2** will appear associated with Compartment **q13**.

d. Double-click **ex2** to open the **Exogenous Input** dialog box. Be sure "bolus" is selected as the **Input Type**.

e. In the **Initial Amount** box, type "2.5e+05".

f. Click **Add**.

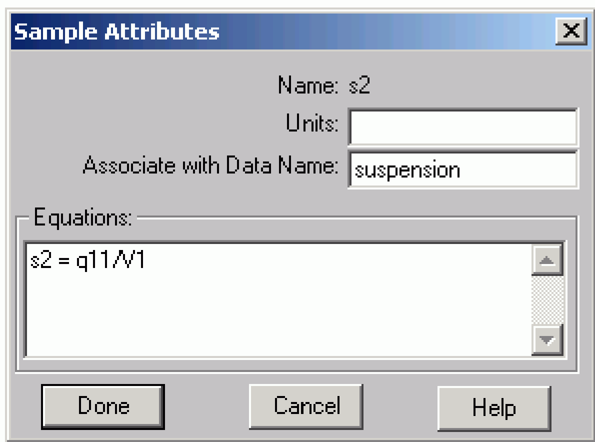
g. Click **Done**.

10. Associate **s2** with the oral data, and change the sample equation.

a. Double-click **s2** to open the **Sample Attributes** dialog box.

b. Type "suspension" in the **Associate with Data Name** box.

c. Edit the sample equation to read "s2=q11/V1" The **Sample Attributes** dialog box will appear as follows:



d. Click **Done**. Notice it is assumed that the volume of Compartments **q1** and **q11** are the same.

11. Specify the new parameter values.

There are three new parameters to specify, *tlag*, *k(0,13)* and *k(3,13)*. Compartment **q13** is the compartment into which the suspension is introduced, and hence is really a part of the delay which is the GT tract. Thus Compartment **q13** should turn over at approximately the same rate as the compartments in the delay. These turn over at the rate "tlag/5", i.e. the delay time divided by the number of compartments in the delay.

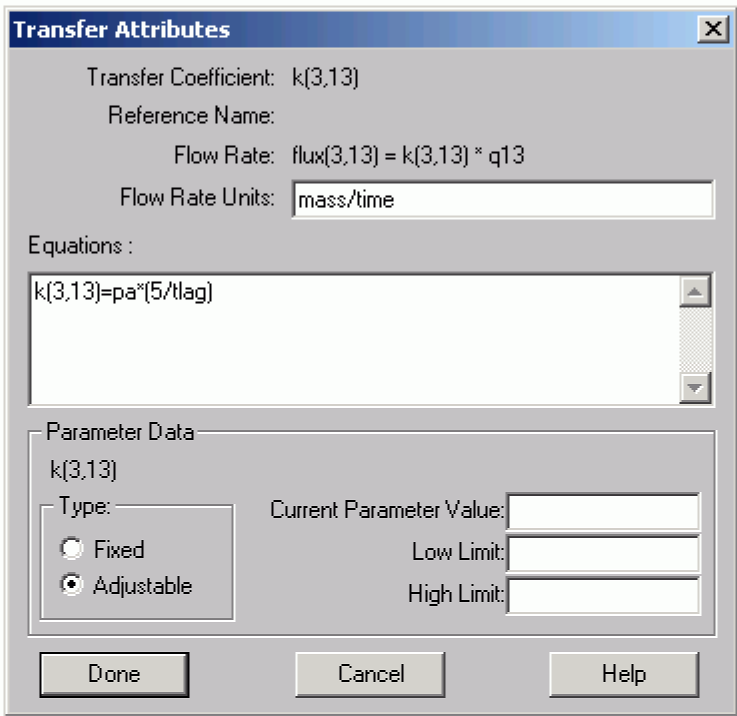
However not all of the suspension may be absorbed. Define a new parameter "pa" equal to the fraction absorbed. Then you can write:

k(3,13) = pa*(5/tlag)
k(0,13)=(1-pa)*(5/tlag)

This will allow you to estimate the fraction "pa", or percent "pa*100", of the drug in suspension absorbed.

a. Double-click *k(3,13)* to open the **Transfer Attributes** dialog box.

b. In the **Equation** pane, type the equation "k(3,13) = pa*(5/tlag)". The **Transfer Attributes** dialog box will appear as follows:



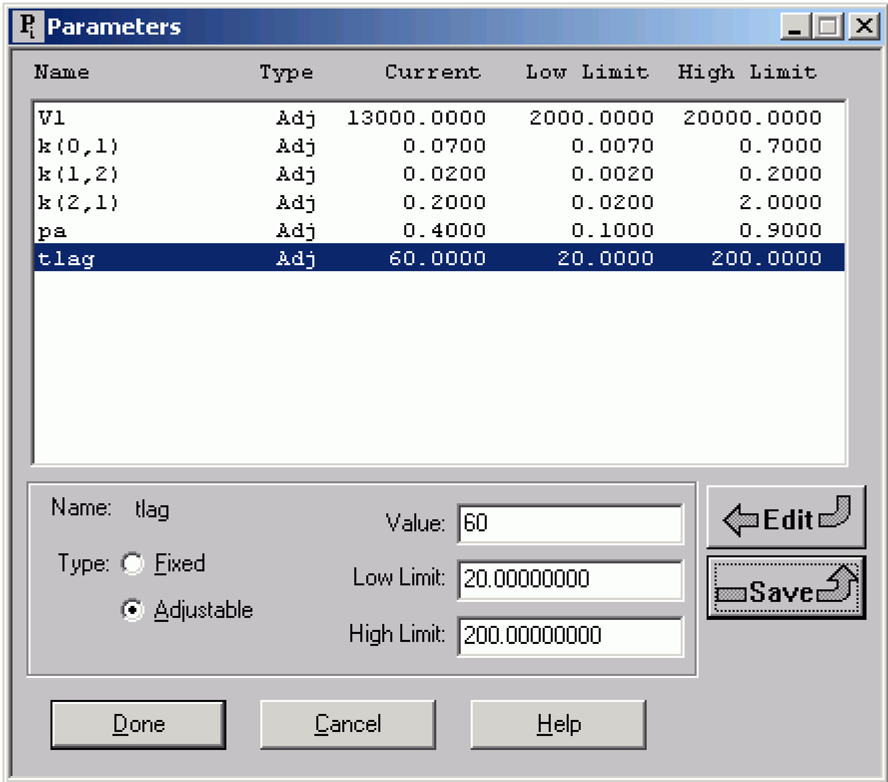
c. Click **Done**.

d. Double-click *k(0,13)* to open the **Loss Attributes** dialog box.

e. In the **Equation** pane, type the equation "k(0,13) = (1-pa)*(5/tlag)".

f. Click **Done**.

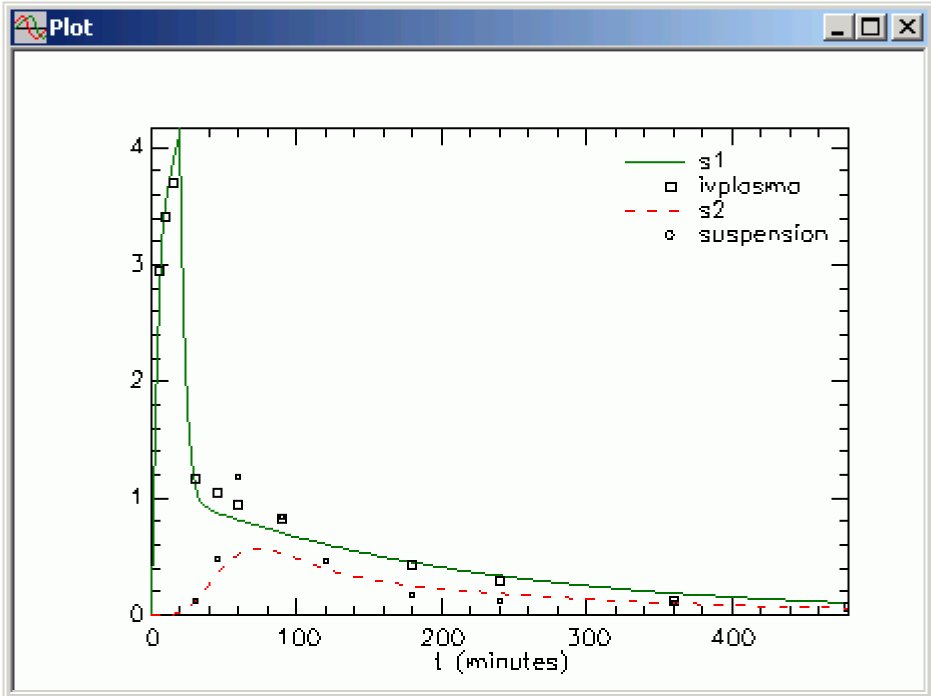
g. Open the **Parameters** dialog box, and enter values as shown below for *pa* and *tlag*.



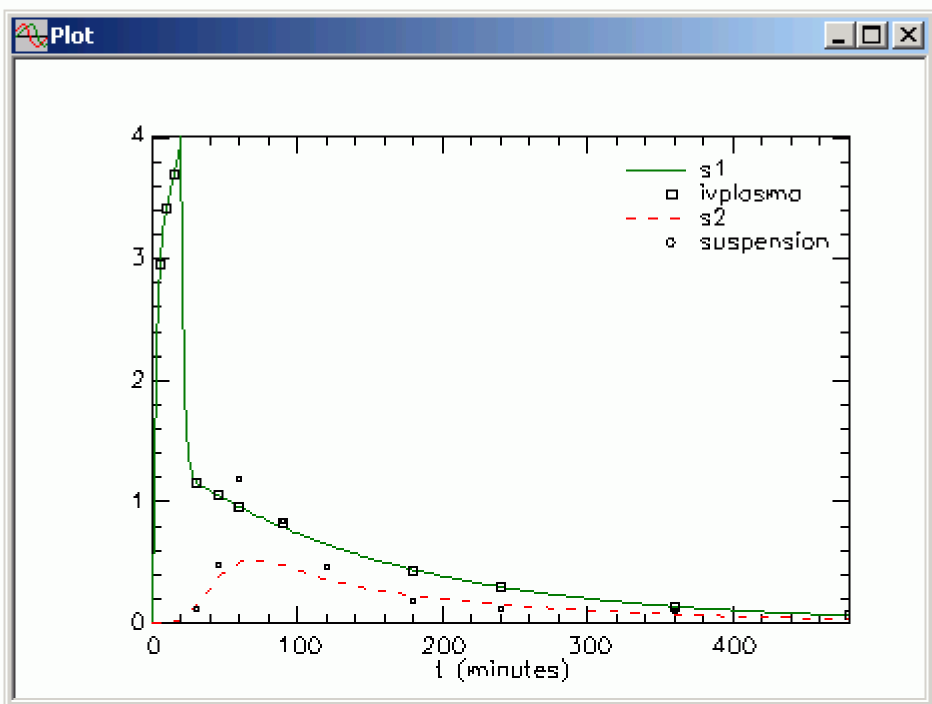
Notice the default **Low** and **High Limits** have not been used for *pa* or *tlag*. The reason for *pa* is that it is a fraction, and cannot exceed "1". The reason for *tlag* is that the lag time cannot be longer than the experiment itself.

h. Click **Done**.

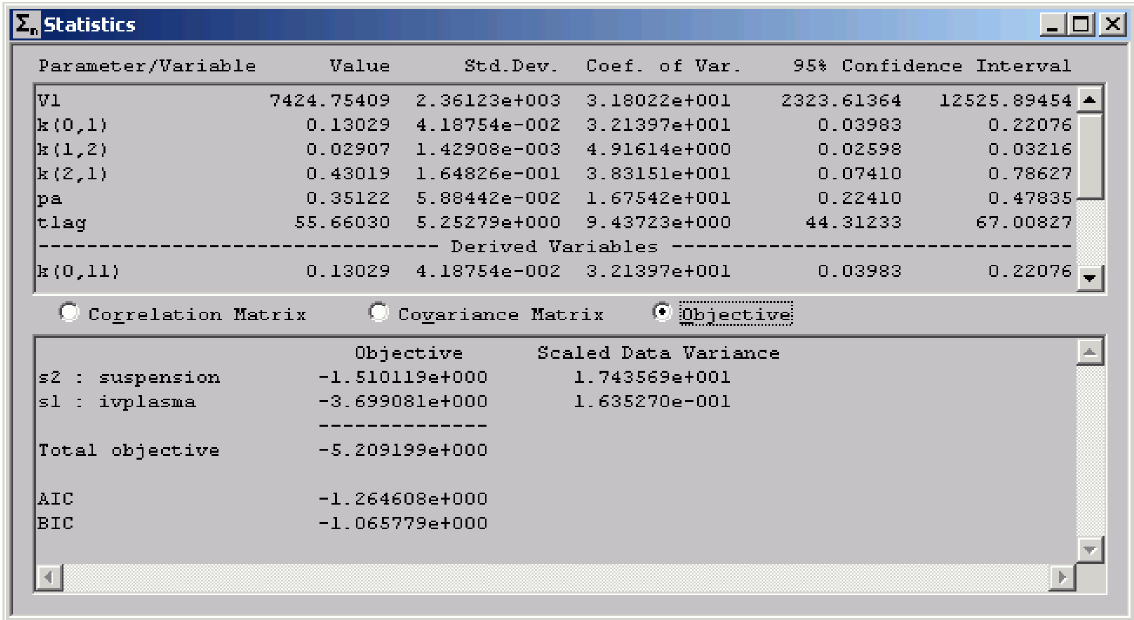
12. Solve the model and view the solution. The plot of **s1:ivplasma** and **s2:suspension** should appear as follows (in linear mode):



13. Fit the model to the data. The plot will be updated as follows:



The statistics will appear as follows:


Statistics

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
V1	7424.75409	2.36123e+003	3.18022e+001	2323.61364	12525.89454
k(0,1)	0.13029	4.18754e-002	3.21397e+001	0.03983	0.22076
k(1,2)	0.02907	1.42908e-003	4.91614e+000	0.02598	0.03216
k(2,1)	0.43019	1.64826e-001	3.83151e+001	0.07410	0.78627
pa	0.35122	5.88442e-002	1.67542e+001	0.22410	0.47835
tlag	55.66030	5.25279e+000	9.43723e+000	44.31233	67.00827
-------- Derived Variables --------					
k(0,11)	0.13029	4.18754e-002	3.21397e+001	0.03983	0.22076

Correlation Matrix   Covariance Matrix   Objective

	Objective	Scaled Data Variance
s2 : suspension	-1.510119e+000	1.743569e+001
s1 : ivplasma	-3.699081e+000	1.635270e-001

Total objective	-5.209199e+000	
AIC	-1.264608e+000	
BIC	-1.065779e+000	

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

Quit the **SAAM II Compartmental** application. You may save this study file if you wish. The study file **io_duplicate.stu** is this study before the Fit.

Multiple input-output study. In studies such as this one where, on different occasions, a drug is administered iv and later orally (or in this case, as a suspension), there are two essential assumptions made when analyzing the data simultaneously:

Assumption 1: The subject is in the same "condition" during the two separate studies. That is, the determinants of how the drug is metabolized are the same on both days of the study.

Assumption 2: The metabolism of the drug that is absorbed into the systemic circulation is identical to that when the drug is introduced directly by iv.

For each such study, these assumptions must be considered if the "system model" is to be assumed identical for both studies.

Part 2. Creating a Multiple Input-Output Experiment Using Create Experiment

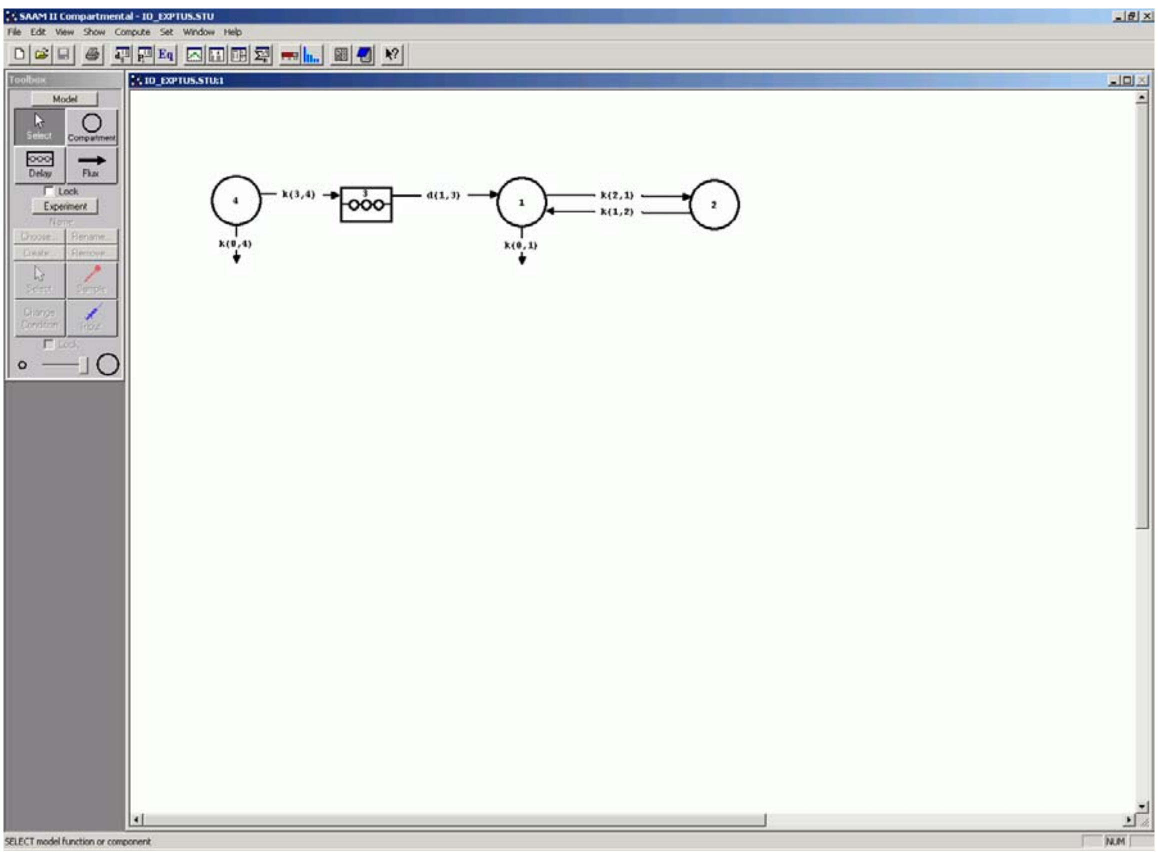
In this tutorial, you will simultaneously analyze data following an iv administration and oral suspension administration of a drug by creating two separate experiments. In the iv experiment, the drug was infused for 20 minutes while in the suspension, it was given as a bolus.

1. **Start** the **SAAM II Compartmental** application. The **SAAM II Compartmental** main window will open.

2. Open the **SAAM II Compartmental** study file **io_expt.stu**.

   a. The file **io_expt.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:

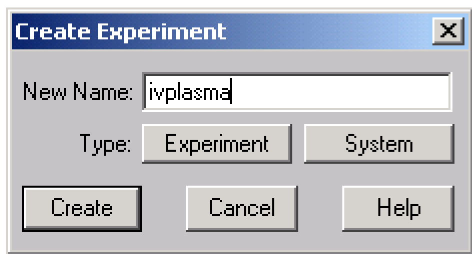


   This is the system model which has been proposed to describe the drug data following an iv administration into plasma, Compartment **1**, and into the GI tract, Compartment **4**. Compartments **1** and **2** are proposed as the system model, i.e. to describe the drug kinetics in the body. Compartment **4** is proposed to be the upper end of the GI tract, the compartment into which the drug suspension is administered. The Delay **3** represents transit through the GI tract. The characteristics of the delay have been set in that the delay time is a parameter *tlag*, and the number of the compartments in the delay has been set equal to 5. You can confirm this by double-clicking on Delay **3**, and examining the **Delay Attributes** dialog box. Also the time of the experiment has been set to 480 minutes. Thus when you click on **Experiment** in the **SAAM II Toolbox**, the **Experiment Attributes** dialog box will not open as is normally the case; the **Create Experiment** dialog box will open.

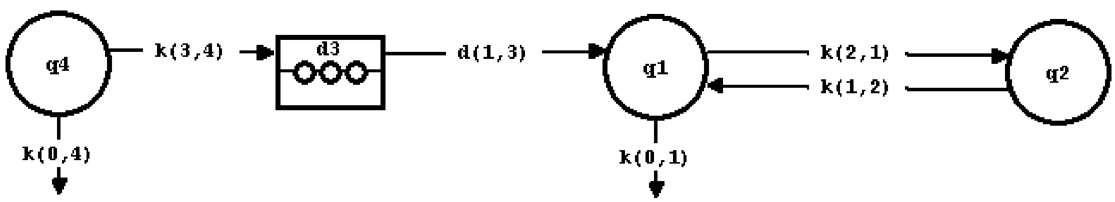
3. Create the iv experiment.

   a. In the **SAAM II Toolbox**, click **Experiment**. The **Create Experiment** dialog box will open.

   b. Change the name of the experiment from "Exper" to "ivplasma". The **Create Experiment** dialog box will appear as follows:



   c. Click **Create**. The model will appear as follows:



4. Create the experimental input and sample for the iv experiment.

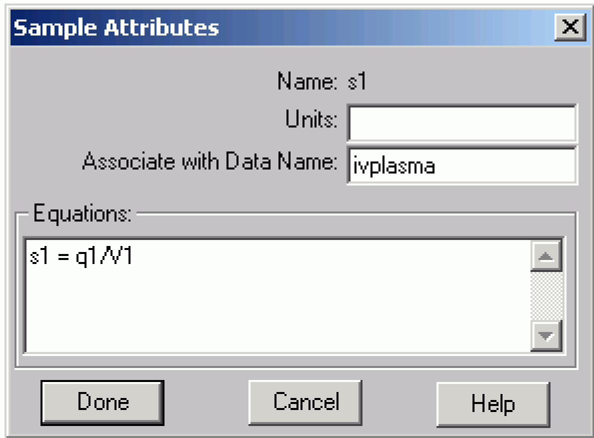
   a. In the **SAAM II Toolbox**, click **Sample**.

   b. Click Compartment **q1** and then the **Drawing Canvas**. The sample **s1** will appear associated with Compartment **q1**.

   c. Double-click **s1** to open the **Sample Attributes** dialog box.

   d. Type "ivplasma" in the **Associate with Data Name** box.

e. Edit the sample equation to read “s1=q1/V1”. The **Sample Attributes** dialog box will appear as follows:



f. Click **Done**.

g. In the **SAAM II Toolbox**, click **Input**.

h. Click Compartment **q1** and then the **Drawing Canvas**. The input **ex1** will appear associated with Compartment **q1**.

i. Specify the 20 minute infusion.

(1) Double-click **ex1** to open the **Exogenous Input** dialog box.

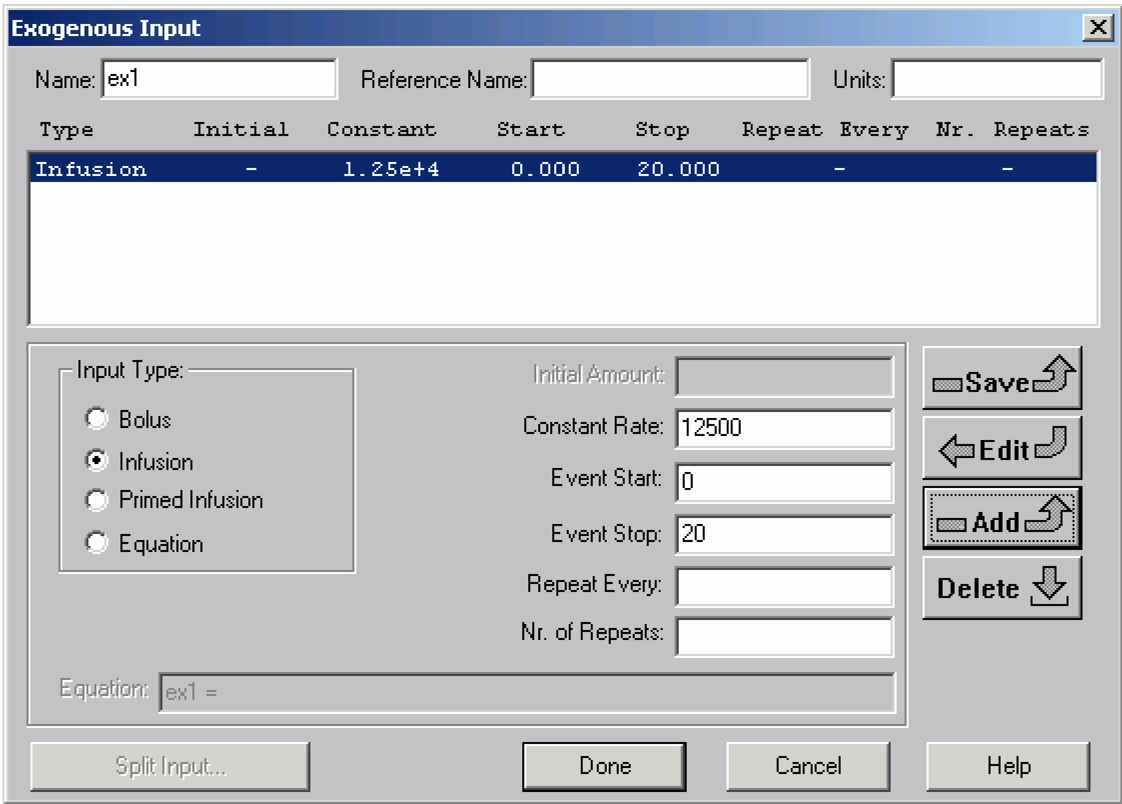
(2) Select **Infusion** in the **Input Type** pane.

(3) Type “12500” in the **Constant Rate** box.

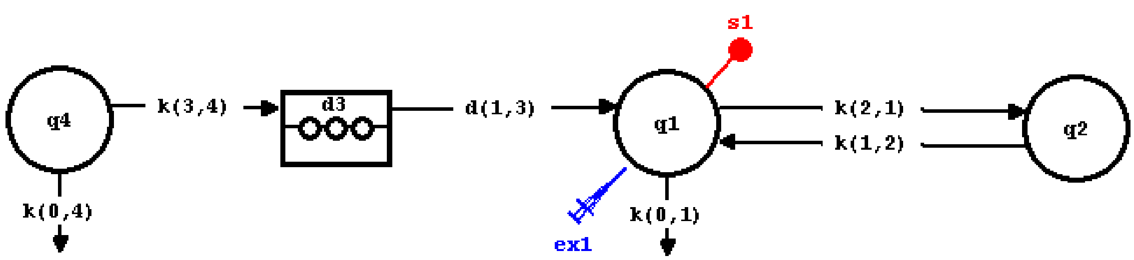
(4) Type “0” in the **Event Start** box.

(5) Type “20” in the **Event Stop** box.

(6) Click **Add**. The **Exogenous Input** dialog box should appear as follows:



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

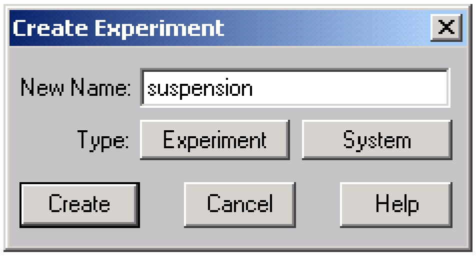


This is the model to describe the iv plasma data following the 20 minute constant infusion of drug. Notice that neither Compartment **q4** nor Delay **d3** will play any role whatsoever in describing the iv plasma data.

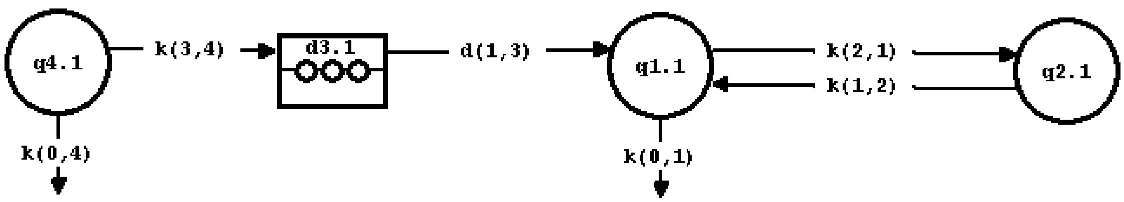
5. Create the experiment for introducing the drug in suspension.

   a. In the **SAAM II Toolbox**, click **Create**. The **Create Experiment** dialog box will open.

   b. Change the name of the experiment from “Exper” to “suspension”. The **Create Experiment** dialog box will appear as follows:



c. Click **Create**. The model will appear as follows:



Notice the compartment numbers have changed from, for example, **q4** to **q4.1**. The ".1" means this is an experiment separate from the case where these is no such label. This is how SAAM II does its book keeping for multiple experiments.

6. Create the experimental input and sample for the suspension experiment.

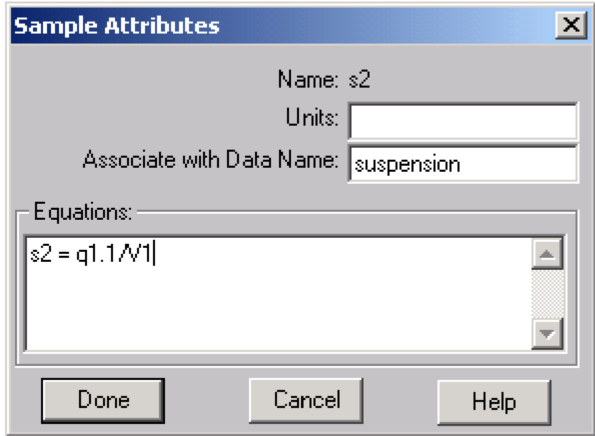
   a. In the **SAAM II Toolbox**, click **Sample**.

   b. Click Compartment **q1.1** and then the **Drawing Canvas**. The sample **s2** will appear associated with Compartment **q1.1**.

   c. Double-click **s2** to open the **Sample Attributes** dialog box.

   d. Type "suspension" in the **Associate with Data Name** box.

   e. Edit the sample equation to read "s2=q1.1/V1. The **Sample Attributes** dialog box will appear as follows



f. Click **Done**.

g. In the **SAAM II Toolbox**, click **Input**.

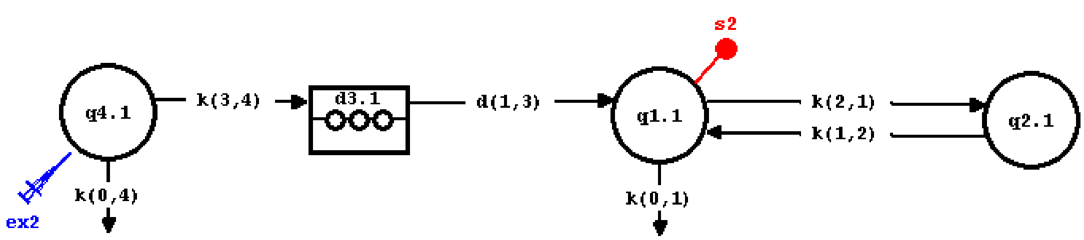
h. Click Compartment **q4.1** and then the **Drawing Canvas**. The input **ex2** will appear associated with Compartment **q4.1**.

i. Double-click **ex2** to open the **Exogenous Input** dialog box. Be sure “bolus” is selected as the **Input Type**.

j. In the **Initial Amount** box, type “2.5e+05”.

j. Click **Add**.

l. Click **Done**. The model will appear as follows:



This is the model to describe the suspension data following the bolus introduction of the drug as a suspension in the upper GI. Now both Compartment **q4** and Delay **d3** will play any role in describing the suspension data. The data can be analyzed together because the “iv” experiment will analyze these data while the above will analyze the suspension data. The parameters in common are *k(2,1), k(1,2), k(0,1)* and *V1*.

7. Enter the parameter values.

Besides the system model parameters *k(2,1), k(1,2), k(0,1)* and *V1*, there are the parameters describing absorption. These are *tlag*, the time of the delay, and *k(3,4)* and **k(0,4)**.

Compartment **4** is the compartment into which the suspension is introduced, and hence is really a part of the delay which is the GT tract. Thus Compartment **4** should turn over at approximately the same rate as the compartments in the delay. These turn over at the rate "tlag/5", i.e. the delay time divided by the number of compartments in the delay.

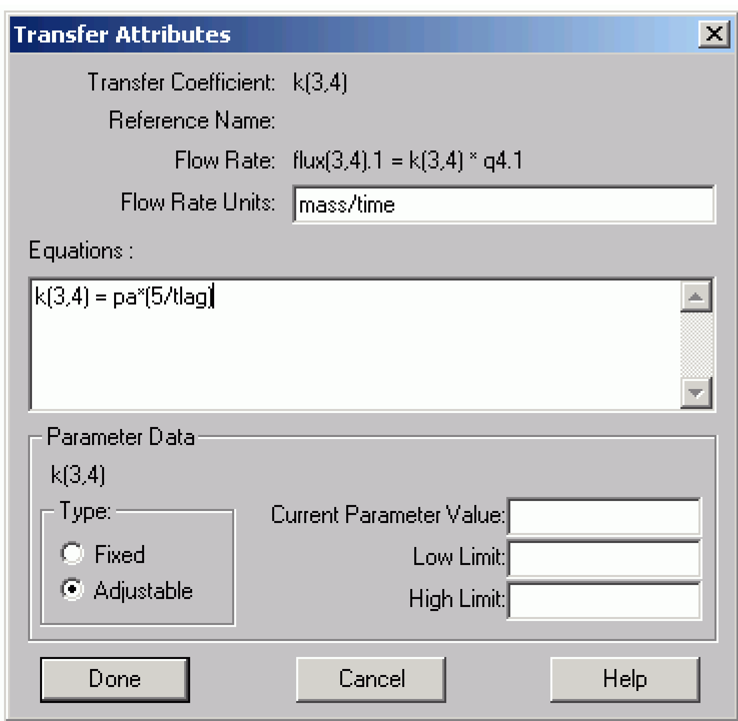
However not all of the suspension may be absorbed. Define a new parameter "pa" equal to the fraction absorbed. Then you can write:

k(3,4) = pa*(5/tlag)
k(0,4)=(1-pa)*(5/tlag)

This will allow you to estimate the fraction, or percent, of the drug in suspension absorbed.

a. Double-click *k(3,4)* to open the **Transfer Attributes** dialog box.

b. In the **Equation** pane, type the equation "k(3,4) = pa*(5/tlag)". The **Transfer Attributes** dialog box will appear as follows:



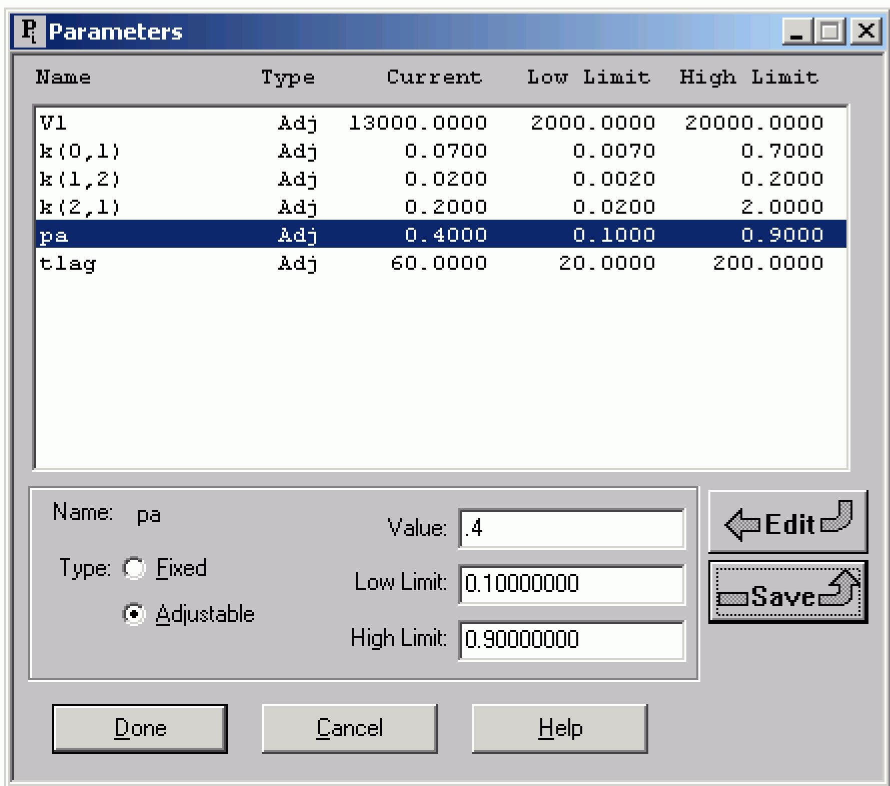
c. Click **Done**.

d. Double-click *k(0,4)* to open the **Loss Attributes** dialog box.

e. In the **Equation** pane, type the equation “k(0,4) = (1-pa)*(5/tlag)”.

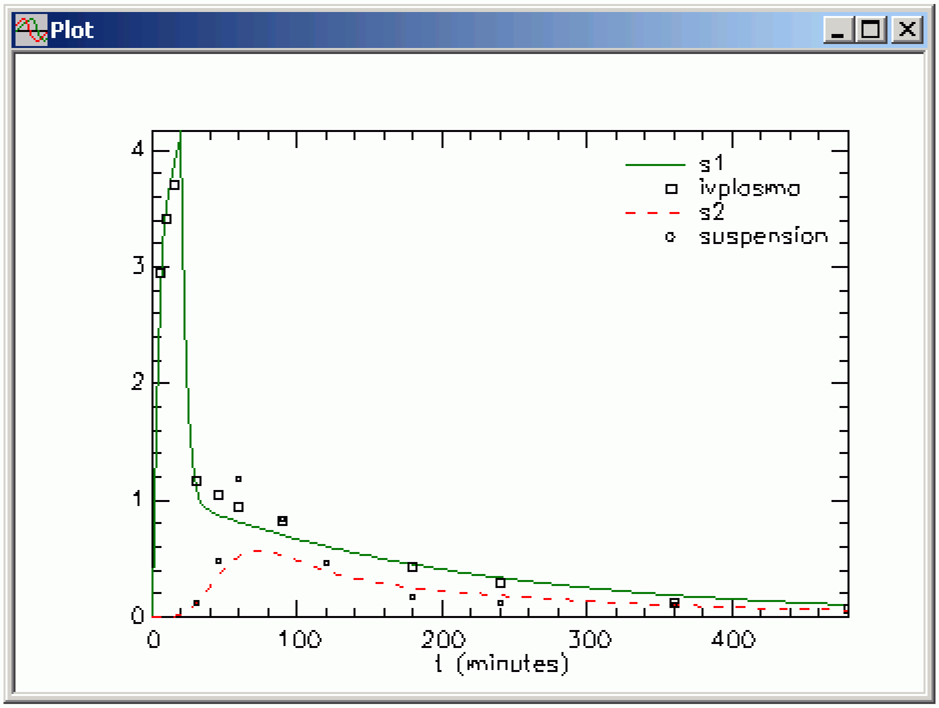
f. Click **Done**.

g. Open the **Parameters** dialog box. The value for *tlag* is already in the study file. Enter the remaining parameters as shown in the **Parameters** dialog box below:

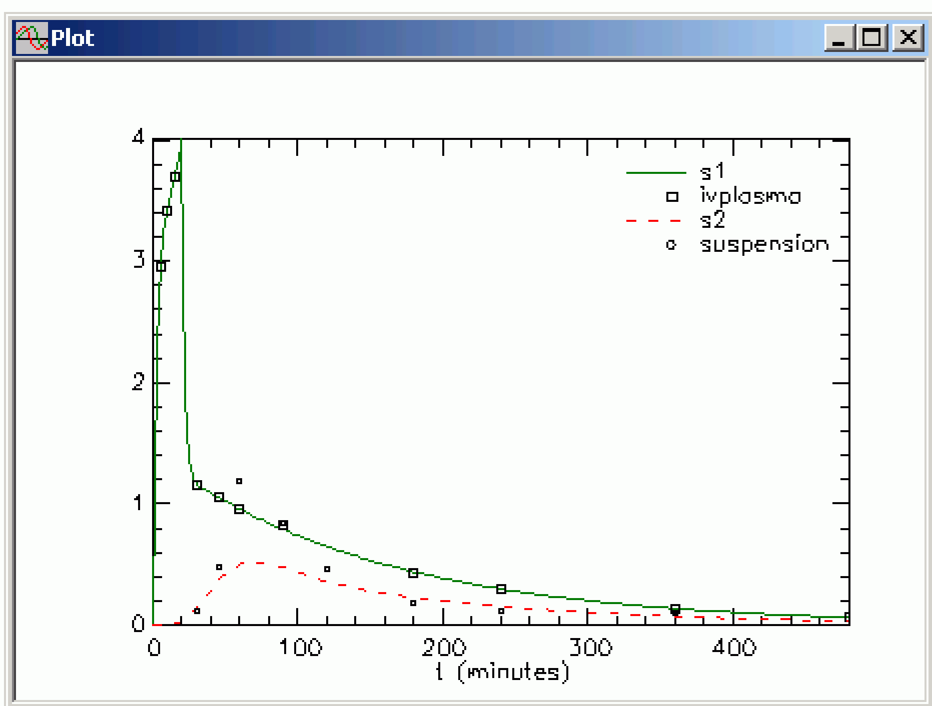


h. Click **Done**. Notice the default **Low** and **High Limits** have not been used for *V1*, *pa* or *tlag*. The reason for V1 is that it is known with more accuracy than the default **Low** and **High Limits** would indicate. The reason for *pa* is that it is a fraction, and cannot exceed “1”. The reason for *tlag* is that the lag time cannot be longer than the experiment itself.

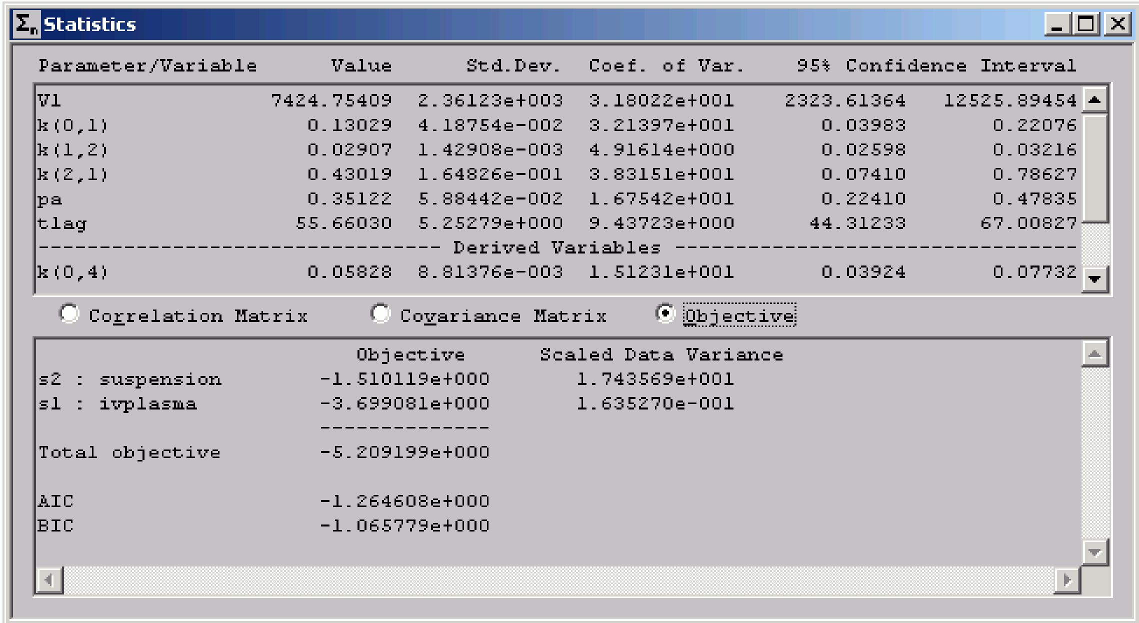
8. Solve the model and view the solution. The plot of **s1:ivplasma** and **s2:suspension** will appear as follows:



9. Fit the model to the data. The plot will be updated as follows:



The statistics will appear as follows:



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

Quit the **SAAM II Compartmental** application. Do not save the changes to **io_expt.stu**. The study file with the parameter values before the fit has been saved as **io_exp_final.stu**.

Multiple input-output study. In studies such as this one where, on different occasions, a drug is administered iv and later orally (or in this case, as a suspension), there are two essential assumptions made when analyzing the data simultaneously:

Assumption 1: The subject is in the same "condition" during the two separate studies. That is, the determinants of how the drug is metabolized are the same on both days of the study.

Assumption 2: The metabolism of the drug that is absorbed into the systemic circulation is identical to that when the drug is introduced directly by iv.

For each such study, these assumptions must be considered if the "system model" is to be assumed identical for both studies.