Hydromorphone Kinetics

Case Study

- How to obtain initial parameter estimates for a two-compartment model
- How to write the equations for the noncompartmental parameters
- How to evaluate the area under the curve (AUC)
- How to investigate different two-compartment model structures (identifiability of the two compartment model)

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Hydromorphone Kinetics Following an Intravenous Bolus Injection: the Two-Compartment Model

Prerequisites

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

What you will learn in this case study

- How to obtain initial parameter estimates for a two-compartment model.
- How to write the equations for the noncompartmental parameters.
- How to evaluate the area under the curve.
- How to investigate different two-compartment model structures.

Data Required

The data file for this case study is

hydromorphone.dat

This data file is a text file. The contents of this file are included at the end of this case study.

Introduction

This case study will show you how to analyze data that are decaying biexponentially following a bolus injection of drug into plasma. It will show you how to obtain initial estimates for the model parameters. The equations for the noncompartmental model parameters will also be written. In addition, you will have the option to explore different structures for the two-compartment model and see which of the parameters change.

The data come from a study of hydromorphone kinetics[1]. Hydromorphone is a semisynthetic opiate agonist, similar in structure to morphine. It is used as an alternative to morphine for treating pain in cancer patients because it can be prepared for parenteral administration in more concentrated aqueous solutions and is about seven times more potent than morphine. Hydromorphone dosing is usually intravenous (iv), intramuscular or subcutaneous; in this study, the dosing is iv.

1 Hill, H.F., Coda, B. A., Tanaka, A. and Schaffer, R. "Multiple-dose evaluations of intravenous hydromorphone pharmacokinetics in normal human subjects." Anest. Analg. 1991, 72:330-336.

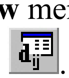
Part 1. Develop a two-compartment model for hydromorphone kinetics.

The first step will be to investigate the data and postulate a two-compartment model to describe the data following an iv bolus injection.

1. **Start** the **SAAM II Compartmental** application. The **SAAM II Compartmental** main window will open. In the **SAAM II Toolbox**, be sure the **Model** tools are available.

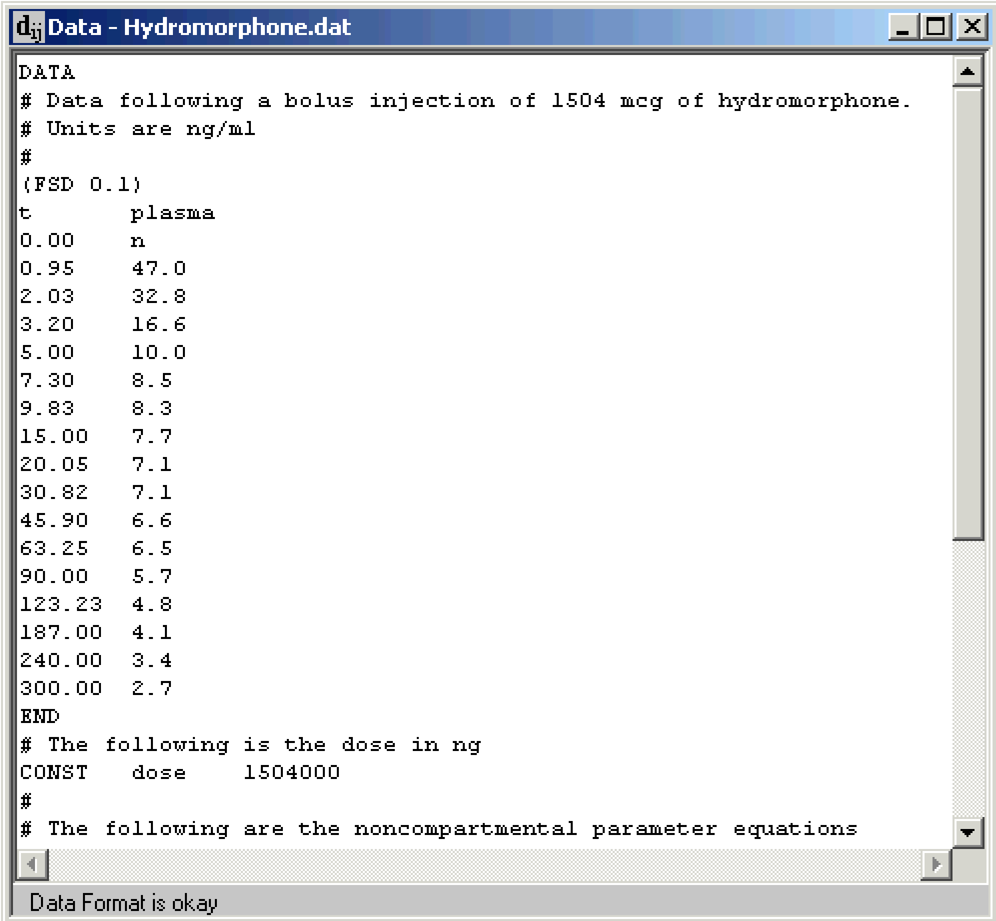
2. Investigate the data.

   The first step in any new model development process should be to investigate the data. This will give you an idea as to how many compartments will be needed. For example, if you plot your data following a bolus, and in semilog mode they appear as a straight line, then a single compartment will probably be adequate. If, on the other hand, they appear to decay at least biexponentially, then at least two compartments will be needed.

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

   c. Double-click **hydromorphone.dat**. The data file contains the plasma hydromorphone data following the bolus injection into plasma. The **Data** window will appear (in part) as follows:


Data - Hydromorphone.dat

```
DATA
# Data following a bolus injection of 1504 mcg of hydromorphone.
# Units are ng/ml
#
(FSD 0.1)
t        plasma
0.00     n
0.95     47.0
2.03     32.8
3.20     16.6
5.00     10.0
7.30     8.5
9.83     8.3
15.00    7.7
20.05    7.1
30.82    7.1
45.90    6.6
63.25    6.5
90.00    5.7
123.23   4.8
187.00   4.1
240.00   3.4
300.00   2.7
END
# The following is the dose in ng
CONST    dose     1504000
#
# The following are the noncompartmental parameter equations
```

Data Format is okay

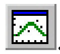
The weighting scheme is FSD so you can leave the variance model set as the default, data-relative.

If you scroll through the **Data** window, you will see that the dose for the iv bolus is entered as a constant using CONST. In addition, if you scroll to the end of the window, you will find the equations for the noncompartmental parameters entered as comments. You will use these equations later in the case study.

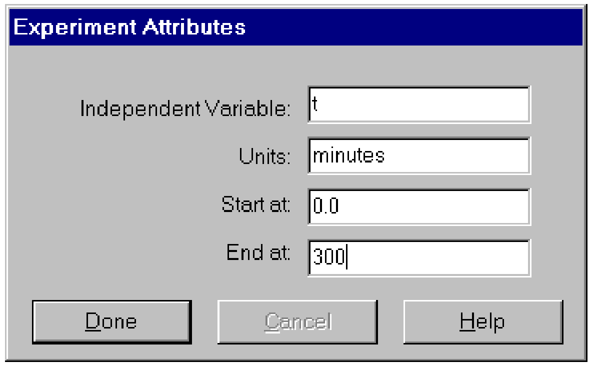
Entering comments in a Data file. You can enter comments in your data file if you precede each line with "#." In this data file, comments are provided which give information on the dose and units of the data. As noted, the noncompartmental parameter equations are also provided.

d. Close the **Data** window.

3. View your data using a line plot.

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

      (1) Enter "300" in the **End At** box. The **Experiment Attributes** dialog box will appear as follows:



      (2) Click **Done**. The **Plot and Table Variables** dialog box will open.

Experiment attributes. Normally you are used to seeing the **Experiment Attributes** dialog box when you switch from the **Model** to the **Experiment** tools. In this situation, no model has been created, so SAAM II does not know the duration of the experiment. When you try to plot your data, SAAM II must know when the experiment ends. For this reason, the **Experiment Attributes** dialog box opens at this point in the model building process.

   b. The **List All Variables** check box is selected. The reason the box is selected is because there are no samples specified yet.

   c. Click **plasma** to move it to the **Current Selection** pane.

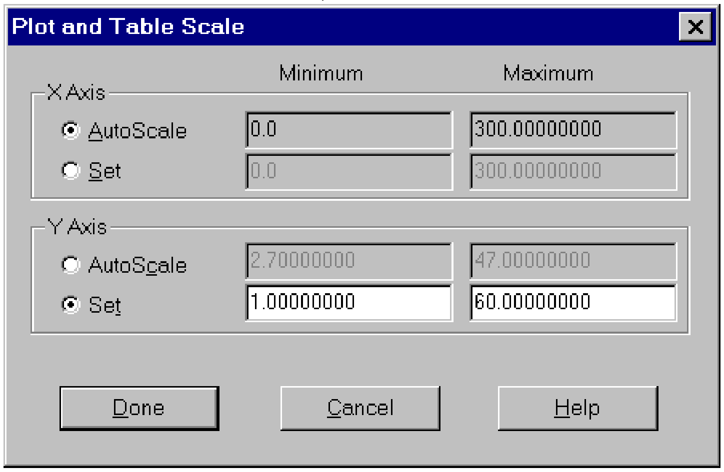
   d. Click **Done**. A plot of the plasma data will appear in the **Plot** window.

   e. In the **View** menu, click **Line Plot**.

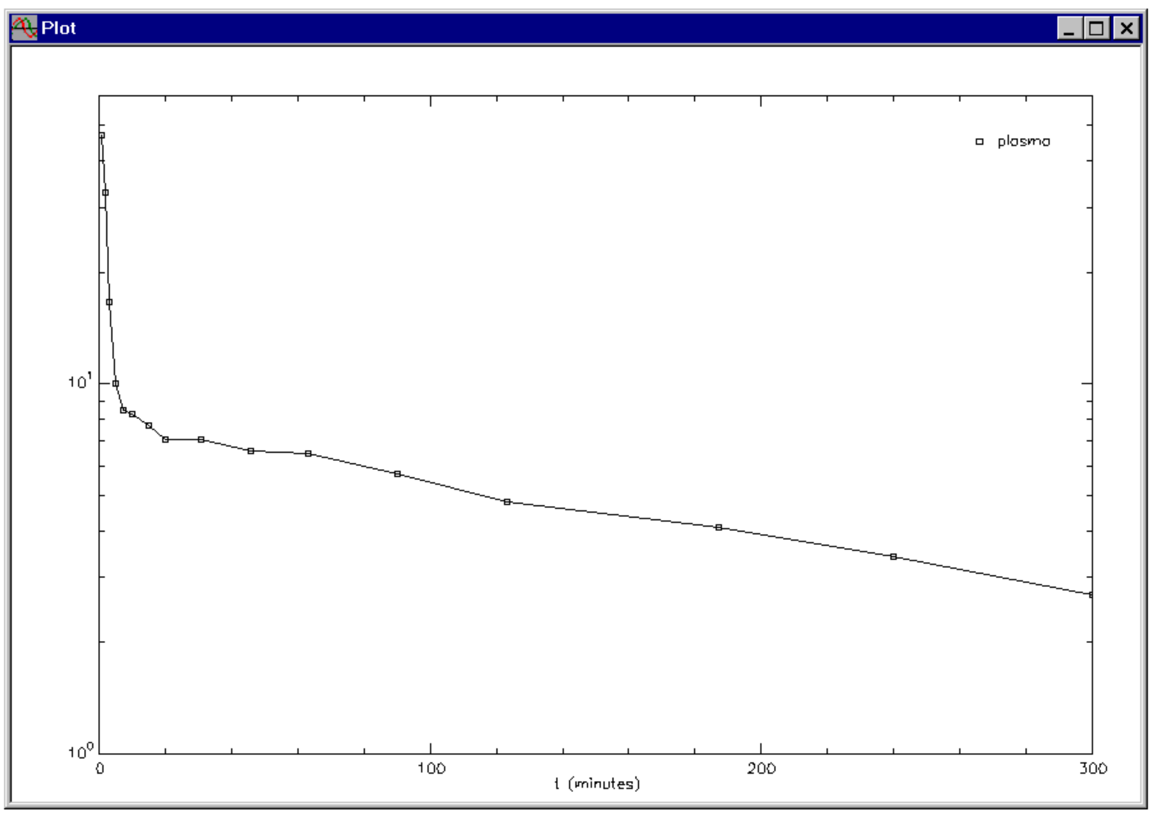
   f. In the **View** menu, click **Semilog**.

   g. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.

h. In the **Y Axis** pane, enter "1" in the **Minimum** box, and "60" in the **Maximum** box. The **Plot and Table Scale** dialog box will appear as follows:



i. Click **Done**. The following plot will appear:



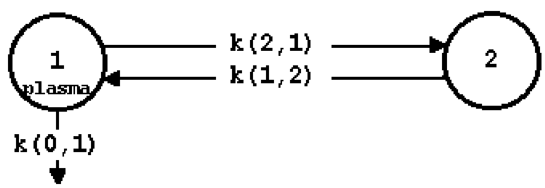
Line Plots. Using the line plot in semilog mode to connect your data can help you decide how many exponentials (compartments) will be needed for the model. In this case, it is clear that at least two exponentials or compartments will be needed.

It is also important to note that the first datum is at about 1 minute, and there are 4 samples by 5 minutes.

If these data had not been collected, i.e. if the first sample were drawn after 5 minutes, the data would appear to decay monoexponentially, and the researchers would have lost the valuable information concerning the rapid initial distribution of the drug. While initial mixing is always a problem, it is important to collect early samples if one wants to elucidate the details of the initial distribution of a drug, especially if this phase is very rapid which is the case here.

j. Close the **Plot** window.

Based on the above observations, the two-compartment model will be used to analyze these data. Create the following system model on the **Drawing Canvas**:



Part 2. Create the pharmacokinetic experiment on the model

1. In the **SAAM II Toolbox**, click **Experiment**. Notice that the **Experiment Attributes** dialog box does not open. This is because the attributes were specified when you did your line plot. However, the **Create Experiment** dialog box will appear on the **Drawing Canvas**. The choice of experiment **Types** is an **Experiment** or a **System**. **Experiment** is selected.

   a. Enter “hydromorphone” in the **New Name** box. The **Create Experiment** dialog box will appear as follows:

b. Click **Create**.

Experiment vs. System. The **Create Experiment** dialog box is where you create your experiment. There are two types of experiments - "Experiment" and "System".

The **Experiment** type will invoke SAAM II's differential equation machinery. When you create this type of experiment, your compartmental model will be interpreted by SAAM II as a system of differential equations. Once parameter values and inputs have been specified, SAAM II can Solve the system of differential equations, and can Fit your model to your data. This type of experiment is designed to deal with time dependent data following an experimental (exogenous) input into the system.

The **System** experiment creates a system of algebraic equations whose coefficients are the *k(i,j)* of your compartmental model. The System model is used primarily in tracer-tracee experiments to describe the tracee data in the steady-state.

The default **New Name** is "Exper". This can be changed to a name of your choice.

3. Create a sample on Compartment **1**.

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

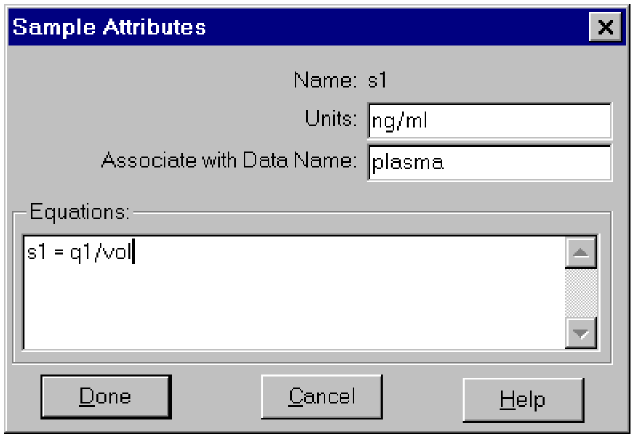
   b. Click Compartment **q1**, and then click on the **Drawing Canvas**. The sample **s1** will appear.

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

   d. Type "ng/ml" in the **Units** box.

   e Type "plasma" in the **Associate with Data Name** box. (Notice "plasma" is in lower case.)

   f Edit the sample equation "s1=q1" to read "s1=q1/vol" in the **Equations** box. The **Samples Attributes** dialog box will appear as shown below:



g Click **Done**.

4. Create an input into Compartment **1**.

   The dose of hydromorphone was 1504 mg. Since the units of the data are in ng/ml, the dose must be consistent with the data. Thus the dose is 1,504,000 ng. As described above, the dose is entered as a constant in the data file, and hence the input will be written as an equation.

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

   b. Click Compartment **q1**, and then click on the **Drawing Canvas**. The input **ex1** will appear.

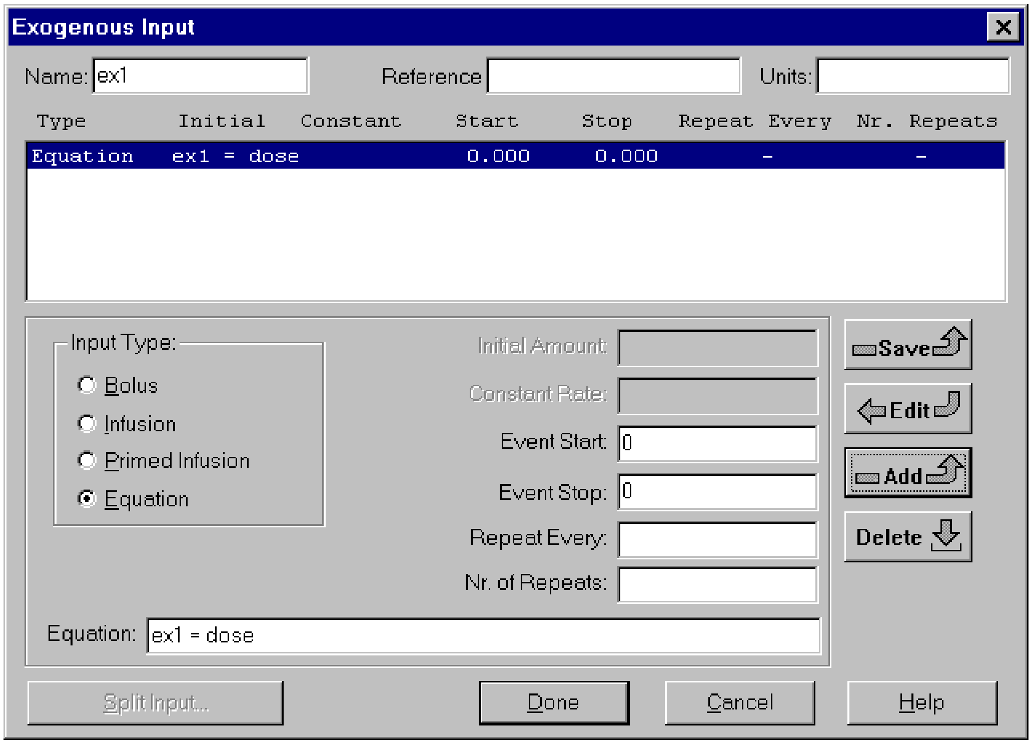
   c. Double-click **ex1** to open the **Exogenous Input** dialog box.

   d. Be sure equation is selected as the input type. In the **Equation** pane, type "ex1=dose".

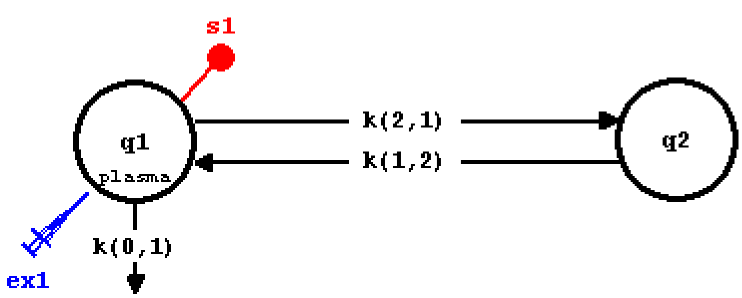
   e. In the **Event Start** box, enter "0".

   f. In the **Event Stop** box, enter "0".

   g. Click **Add**. The **Exogenous Input** dialog box will appear as follows:



h. Click **Done**. Your model will appear as follows:



Equation input. It is frequently the case that one would like have the dose specified in the data file. This can be done in SAAM II using the CONST feature. Constants such as doses can be entered in this way. To create the bolus input equal to the dose, you write the input as a equation equal to the constant dose. The bolus is created by making the start and stop time equal.

Part 3. Defining Derived Variables (Noncompartmental parameters).

The parameters for your model are the rate constants *k(2,1), k(1,2), k(0,1)* and the volume *vol*. These are called the primary parameters. The rate constant *k(0,1)* is a fractional measure of the elimination rate of the drug, and it has units of inverse time (in this case, 1/hour).

From these parameters, the equations for the noncompartmental parameters can be written. For example, the clearance is defined as

$$Cl = k(0,1) * vol$$

Clearance *Cl* is a derived variable, as it is a function of the primary parameters *k(0,1)* and *vol*. As you recall, parameters are variables that appear in the equations characterizing the model or experiment, but were not given numerical values. SAAM II automatically determines the model parameters. Following a successful "fit," the optimal values and statistical information about their precision appear in the **Statistics** window. In addition, the statistical information about the derived variables appears in the **Derived Variables** pane.

Besides clearance, there are a number of other noncompartmental pharmacokinetic parameters that can be estimated from the two-compartment model with a bolus injection into plasma. These are

```
Cmax=dose/vol
half_time=log(2)/k(0,1)
r=sqrt((k(2,1)+k(1,2)+k(0,1))^2-(4*k(1,2)*k(0,1)))
alpha=(k(2,1)+k(1,2)+k(0,1)+r)/2
beta=(k(2,1)+k(1,2)+k(0,1)-r)/2
A=(dose/vol)*((alpha-k(1,2))/(alpha-beta))
B=-(dose/vol)*((beta-k(1,2))/(alpha-beta))
AUC=dose/(vol*k(0,1))
AUMC=(A/alpha^2)+(B/beta^2)
Syst_MRT=AUMC/AUC
MRT_Cpt1=1/k(0,1)
Cl=dose/AUC
Vss=Cl*Syst_MRT
```

Included in this list are the parameters characterizing the solution of the system of two differential equations represented by the two-compartment model. More specifically, the solution *s1* can be written:

$$s1=A*exp(-alpha*t)+B*exp(-beta*t)$$

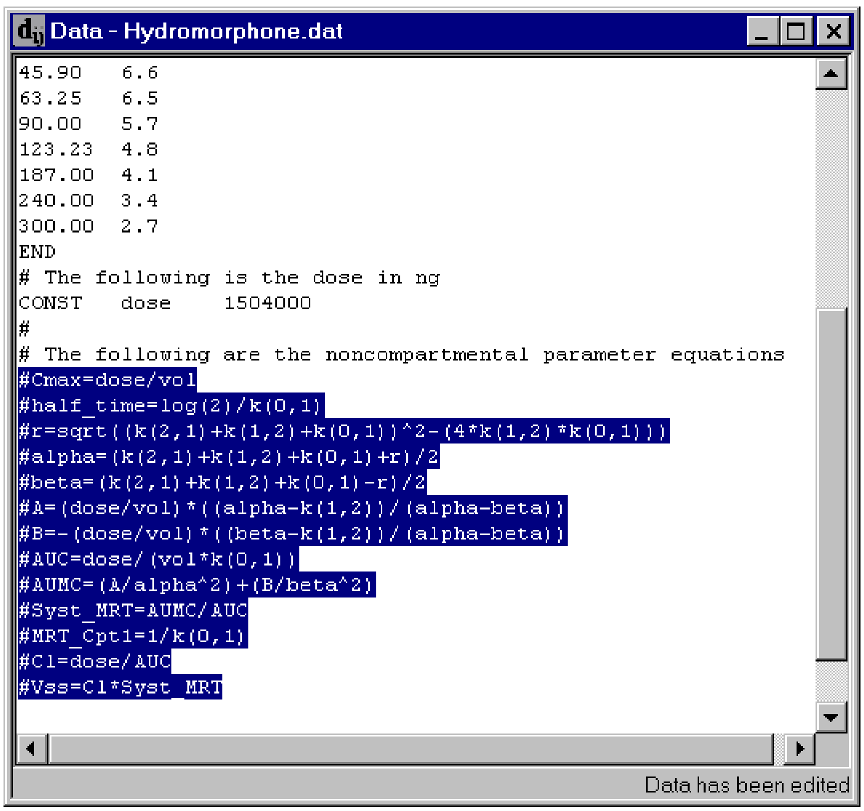
If you were to fit a sum of two exponentials to these data, you would obtain the above values for *A, B, alpha* and *beta*.

Notice also that there are two residence time calculations. Syst_MRT is the mean residence time in the system, i.e. the average time a hydromorphone particle spends in the system before being irreversibly lost. MRT_Cpt1 is the average time a hydromorphone particle spends in plasma.

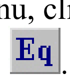
You can enter these parameters directly. For convenience, they can be cut and pasted from the **Data** file to the **Equations** dialog box as described below.

1. In the **Show** menu, click **Data**, or alternatively, on the **SAAM II Toolbar**, click **Data** . The **Data** window will open.

2. Scroll down through the **Data** window until all the noncompartmental parameters are available. Select and copy the noncompartmental parameters. The selected information will appear in the **Data** window as follows:

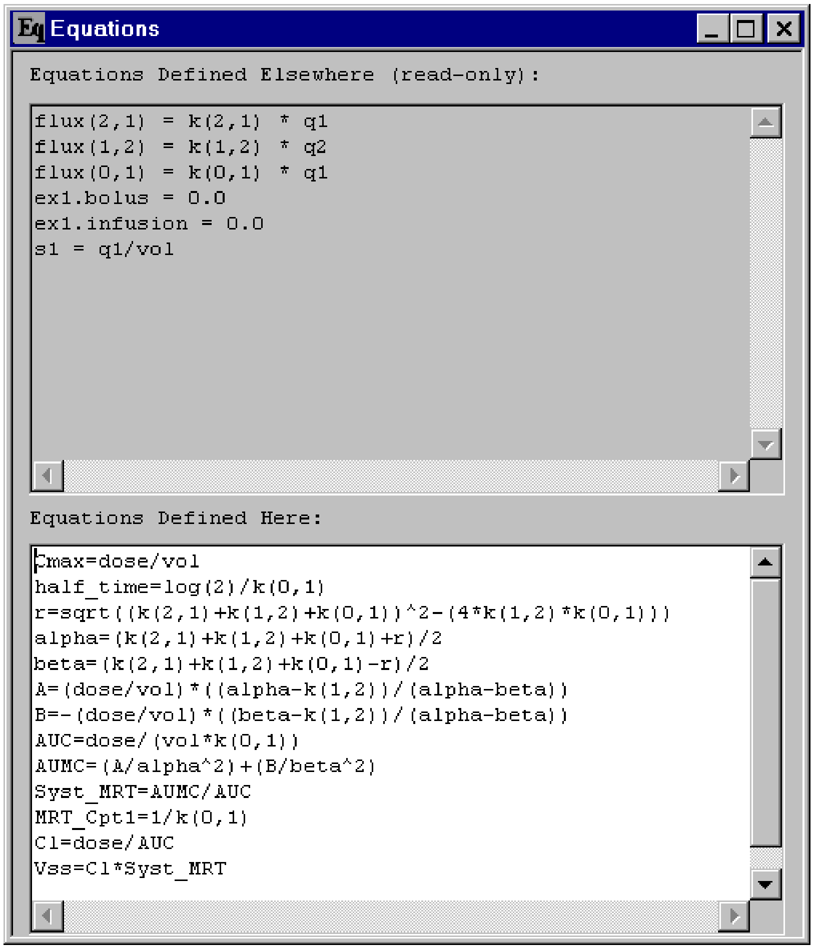


3. Close the **Data** window.

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

5. Paste the equations in the **Equations Defined Here** pane in the **Equations** dialog box.

6. Remove the "#" from in front of each equation. The **Equations** dialog box will appear as follows:



SAAM II

Equation syntax. There are two points to remember about equation syntax in SAAM II. First, while it may be appealing to define half time as t_1/2, this is improper syntax, and SAAM II will display an error message. The other point is that the natural log is "log," not "ln." If you define, for example, t_half=ln(2)/k(0,2), "ln(2)" will be interpreted as a parameter instead of an algebraic operation and will appear as a parameter in the **Parameters** dialog box.

7. Close the **Equation** dialog box.

Part 4. Enter the parameter values, solve the model, fit the model to data, view the solution and record the results.

Before you can Solve (simulate) your model or Fit your model to your data, you must provide numerical estimates for the primary parameters of your model. These are the parameters that appear in the **Parameters** dialog box.

1. Enter the parameter values.

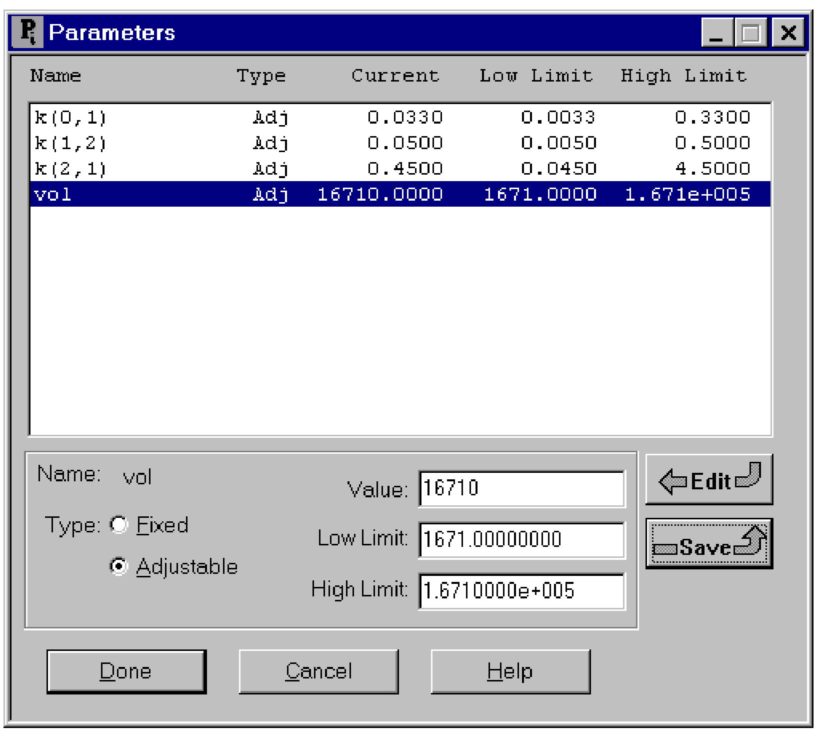
   a. In the **Show** menu, click **Parameters**, or alternatively, on the **SAAM II Toolbar,** click **Parameter** . The **Parameters** dialog box will open.

   b. If *k(0,1)* is not selected, double-click *k(0,1)* to select it. Enter “0.033” in the **Value** box and click **Save**.

   c. Double-click *k(1,2)*. Enter “0.05” in the **Value** box and click **Save**.

   d. Double-click *k(2,1)*. Enter “0.45” in the **Value** box and click **Save**.

   e. Double-click *vol*. Enter “16710” in the **Value** box and click **Save**. The **Parameters** dialog box will appear as follows:



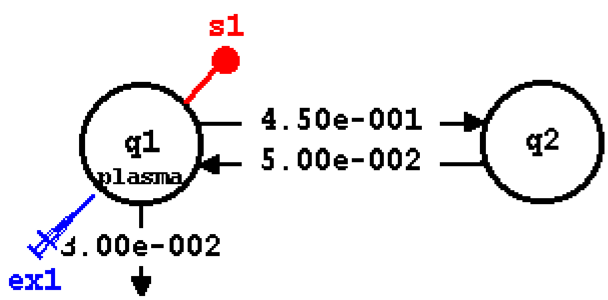
   f. Click **Done**.

Initial parameter estimates. How to obtain the initial estimates for the parameters is explained in the Appendix 1 to this case study. How these estimates are obtained applies to any experiment in which you are using a two-compartment model following a bolus injection of a drug.

2. 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 **View** menu, select **Model Labels** and click **Values**. Your model will appear as follows:



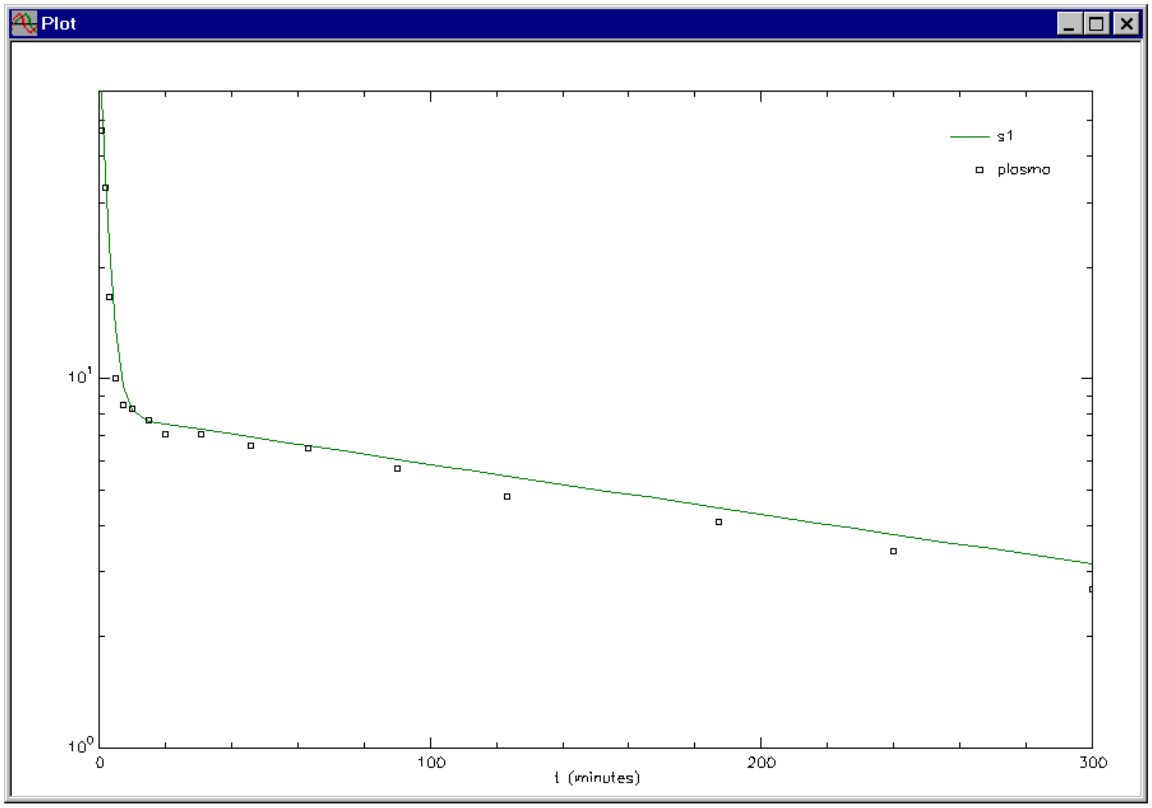
Model labels. Model Labels gives you three options to label the transfers in your model. One is the name, *k(i,j)* which is the default label. The other is **Values**; this labels the transfers with their current values. In the event that a transfer is non-linear, the current value is the zero time value. The final option is no label at all. Labeling with the parameter value is useful since it lets you know the current value of each parameter without having to open the **Parameters** dialog box.

   c. Plot the data. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar,** click **Plot** . Since you have previously plotted the data, the plot of the data will appear.

   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 cleared.

e. Click **s1:plasma** to move it to the **Current Selection** pane.

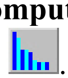
f. Click **Done**. If your plot does not appear in semi-log mode, in the **View** menu, click **Semilog**. Your plot will appear as follows:

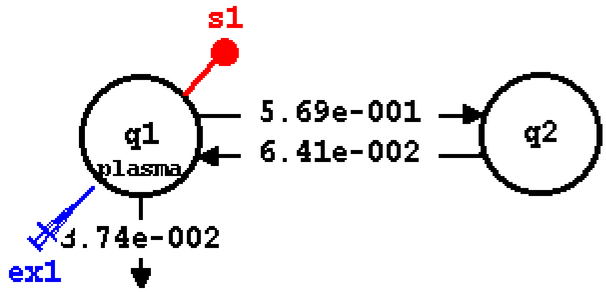


The initial estimate for the parameters are reasonable, so you can proceed to Fit your model to your data.

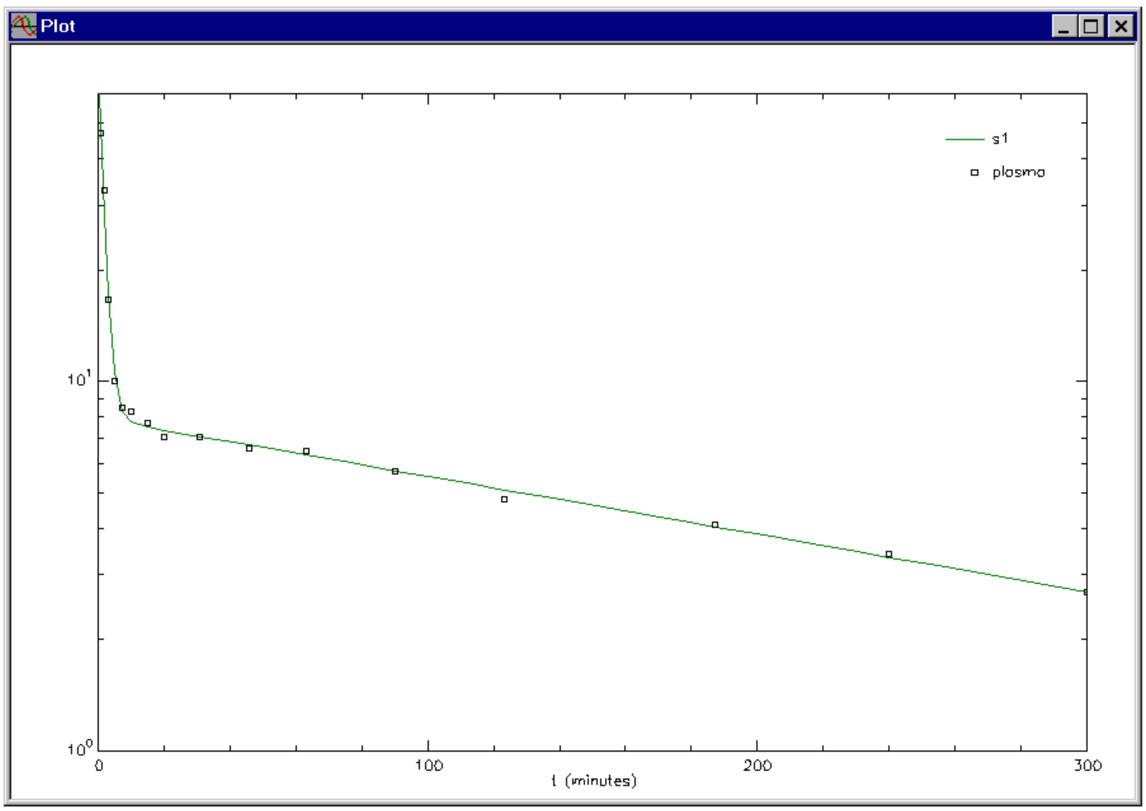
g. Leave the **Plot** window open.

3. Fit your model to your data and view the solution and the statistics.

a. In the **Compute** menu, click **Fit**, or alternatively, on the **SAAM II Toolbar** click, **Fit** . Your model will be updated as follows:

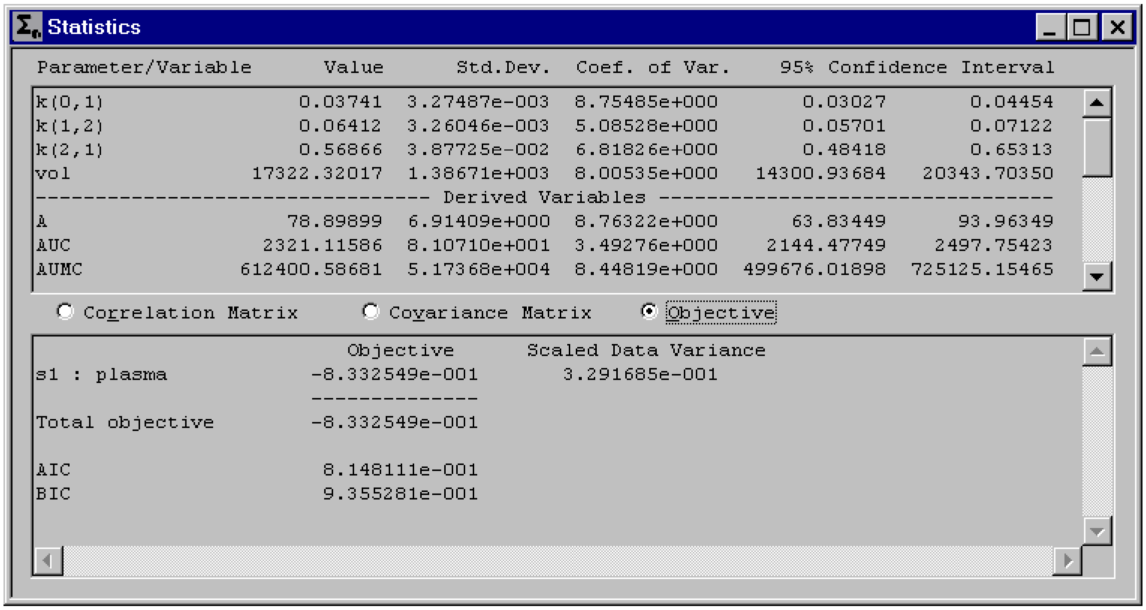


b. Since your **Plot** window is already open, your plot will be updated and appear as follows:



The "fit" is satisfactory in that there are no obvious systematic deviations between the model predictions and the data.

c. In the **Show** menu, click **Statistics**, or alternatively, on the **SAAM II Toolbar** click, **Statistics** . The **Statistics** window will open as follows:



You can see that the statistics are quite reasonable in that no parameter has a large error. You will have to scroll through the **Parameter/Variable** pane to see all of the pharmacokinetic noncompartmental parameters.

4. Record the results in the **Notes** window (optional).

   You may record the results of your modeling exercise using the **Notes** window if you wish. You may also omit this part of the case study since the contents of the **Notes** window are included as Appendix 2.

   If you do not do this part of the case study, close the **Statistics** and **Plot** windows before proceeding. If you do this part of the case study, leave these windows open.

   a. In the **Show** menu, click **Notes**, or alternatively, on the **SAAM II Toolbar** click **Notes** . The **Notes** window will open.

   b. Type the text "Fit of two-compartment model to data with loss from Compartment 1."

   c. In the **Statistics** window, select all the information for all the parameters.

   d. In the **Edit** menu, click **Copy**.

   e. Click in the **Notes** window to make it the current window. In the **Edit** menu, click **Paste**. Add the titles to the columns (e.g. value, SD, Coeff of Var, etc.)

   f. In the **Statistics** window, select the information for the objective function.

g. Click in the **Notes** window. Click below the statistical information. In the **Edit** menu, click **Copy**. Your **Notes** window should appear as follows:

Notes

```
Fit of two-compartment model to data with loss from compartment 1
                        value    SD            Coeff Var          95% confidence
k(0,1)                0.03741  3.27487e-003  8.75485e+000       0.03027        0.04454
k(1,2)                0.06412  3.26046e-003  5.08528e+000       0.05701        0.07122
k(2,1)                0.56866  3.87725e-002  6.81826e+000       0.48418        0.65313
vol               17322.32017  1.38671e+003  8.00535e+000   14300.93684    20343.70350
--------------------------------- Derived Variables ---------------------------------
A                    78.89899  6.91409e+000  8.76322e+000      63.83449       93.96349
AUC                2321.11586  8.10710e+001  3.49276e+000    2144.47749     2497.75423
AUMC             612400.58681  5.17368e+004  8.44819e+000  499676.01898   725125.15465
B                     7.92541  1.96436e-001  2.47856e+000       7.49741        8.35340
Cl                  647.96421  2.26318e+001  3.49276e+000     598.65376      697.27465
Cmax                 86.82440  6.95060e+000  8.00536e+000      71.68035      101.96844
MRT_Cpt1             26.73345  2.34047e+000  8.75485e+000      21.63400       31.83290
Syst_MRT            263.83887  1.36718e+001  5.18189e+000     234.05055      293.62719
Vss              170958.14482  4.43532e+003  2.59439e+000  161294.42408   180621.86557
alpha                 0.66658  4.15640e-002  6.23540e+000       0.57602        0.75714
beta                  0.00360  1.81290e-004  5.03870e+000       0.00320        0.00399
half_time            18.53022  1.62229e+000  8.75485e+000      14.99555       22.06489
r                     0.66298  4.15183e-002  6.26235e+000       0.57252        0.75344


                          Objective       Scaled Data Variance
s1 : plasma            -8.332549e-001        3.291685e-001
                       --------------
Total objective        -8.332549e-001

AIC                     8.148111e-001
BIC                     9.355281e-001
```

Two or three compartments? At this point, you have successfully fit the two-compartment model to your data, and estimated the primary parameters and the noncompartmental parameters. How do you know the two-compartment model is the appropriate model? In this case, if you try to add a third compartment, you will find that the three-compartment model is not supported by these data. This will be revealed when you see that the two new rate constants cannot be estimated. Thus you are safe in concluding that the two-compartment model is appropriate.

h. Close all windows.

Part 5. Estimate areas under the curves.

One is often interested in the “area under the curve.” This usually refers to the area under the plasma concentration-time curve. Sometimes, however, it is useful to obtain other areas such as areas under the different compartments qi in the model or functions of the area under the plasma concentration-time curve such as the first-moment, or "mean" area under the curve.

In this part of the case study, SAAM II's ability to compute the area under a curve will be discussed. This will show you how to evaluate any of the areas you wish.

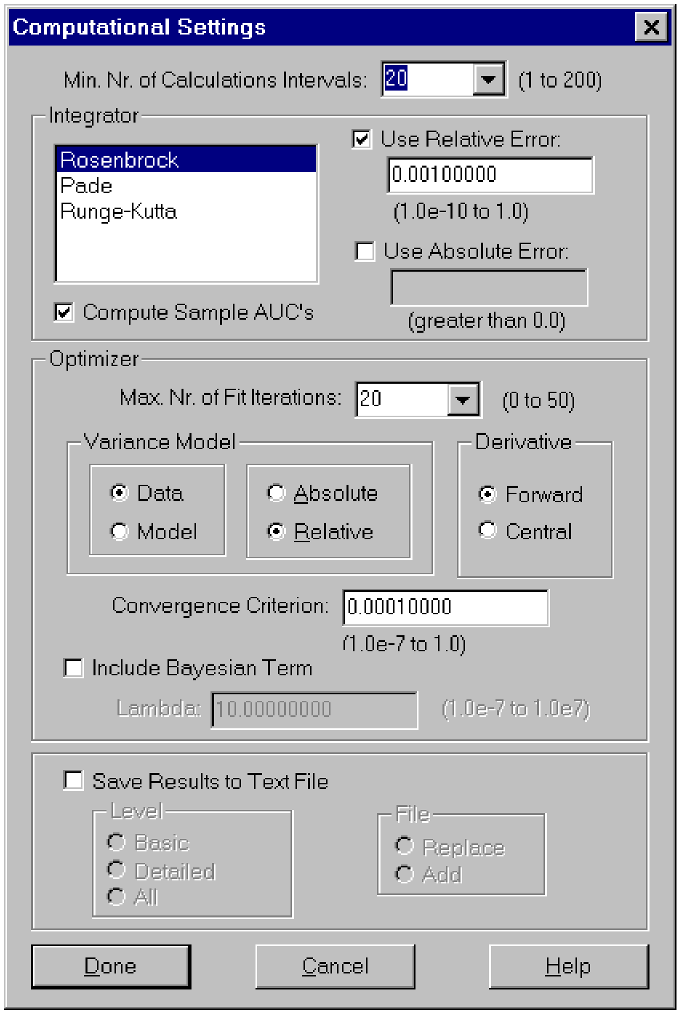
Under normal circumstances, you probably will not have the noncompartmental equations defined. In this case, you do, so as a reference point, remember that the AUC calculated above is 2321.12 with a standard deviation of 8.1. This will allow you to compare SAAM II's calculations with those obtained from the noncompartmental equations.

1. Estimate the area under the concentration-time curve (**s1**) using the **Compute Sample AUC's** feature in SAAM II.

    a. In the **Compute** menu, click **Settings**. The **Computational Settings** dialog box will open.

Computational Settings. In order to open the **Computational Settings** dialog box, all other windows and dialog boxes must be closed.

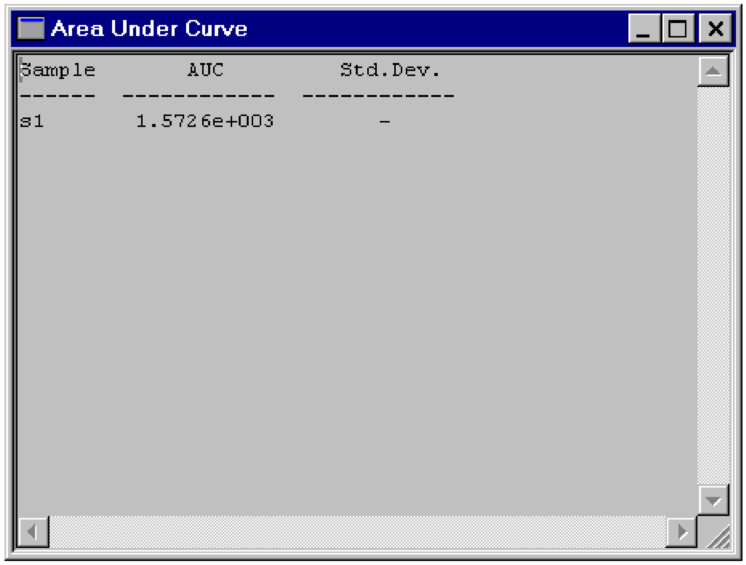
    b. Select the **Compute Sample AUC's** check box. The **Computational Settings** dialog box will appear as follows:



c. Click **Done**.

d. Solve your model.

e. In the **Show** menu, click **AUC's**. The **Area Under Curve** window will open as shown below:



Using the Compute Sample AUC's feature in SAAM II. To use the **Compute Sample AUC's** feature in SAAM II, you have to select this option in the **Computational Settings** window. The calculated AUC values can be viewed by clicking **AUC's** in the **Show** menu.

You will notice two things about the value returned above. First, it is equal to 1573 which is less than the value you calculated previously which was 2321. Second, there is no statistical information.

The reason why the value for the AUC is less than 2321 is that when you calculate sample AUC using the **Compute Sample AUC's** feature in SAAM II, the value calculated will be the value at the end of the experiment, in this case 300 minutes. Thus to estimate the true AUC, you need to extend the time of the experiment until the area under the tail portion of the curve is negligible. A rule of thumb is to set the end of the experiment equal to 100 times the inverse of the smallest rate constant. Here the smallest rate constant is *k(0,1)* which is 0.037. The inverse is 27, and 100 times that is 2700 minutes. To be sure you are close to the true AUC, you should double that number to see if there is a difference in AUC; if there is, you must continue to double the duration of the experiment until the difference in AUC values is small.

The reason why there are no statistics is that you have not fitted your model to the data. When you fit your model to your data, statistics on the AUC will be returned.

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

g. Change the time of the experiment.

   (1) In the **Set** menu, click **Experiment Attributes**. The **Experiment Attributes** dialog box will open.

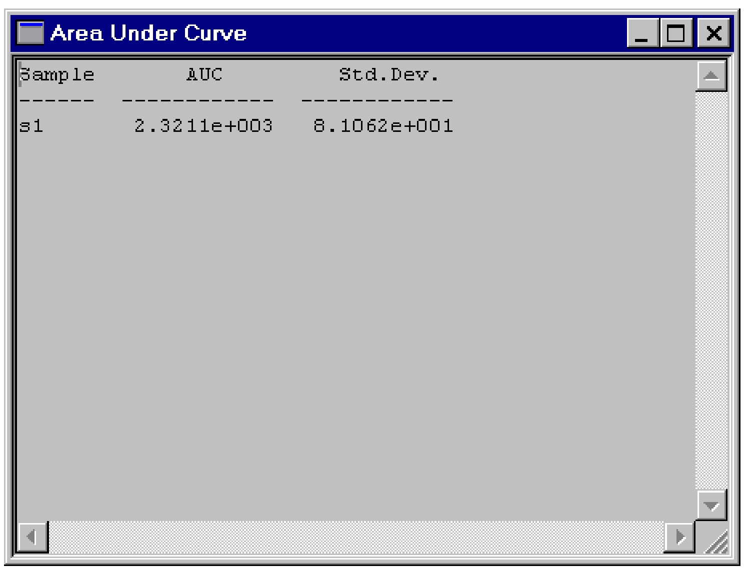
   (2) Change the **End At** time from “300” to “2700”.

   (3) Click **Done**.

h. Re-Solve the model, and view the AUC. The value now is 2321.0 which is essentially identical to the one obtained using the model parameters.

i. Change the **End At** time of the experiment from “2700” to “5400”. Re-Solve the model and view the AUC. The AUC is now 2321.1; the difference between integrating to 2700 and 5400 is 0.1 so it is safe to stop.

j. Fit the model to your data, and view the AUC. The **Area Under Curve** window will appear as follows:



When you Fit your model to your data, you will obtain an estimated standard deviation for the AUC.

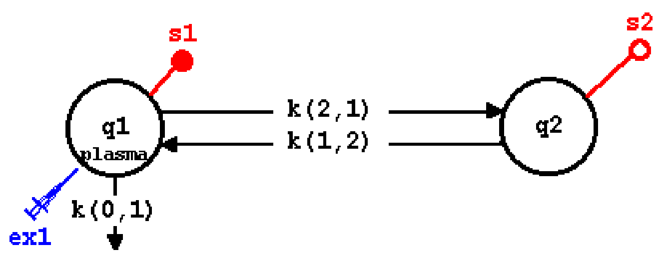
SAAM II *Area under the curve calculation.* It may appear somewhat cumbersome to evaluate the area under the curve using this method. It requires a few steps to be sure that you have integrated long enough to obtain the total area. The advantage of this method, however, is that you can obtain the area up to any time you wish simply by changing the time the experiment ends. Thus you can investigate, for example, the time at which the area has reached a certain percentage of its total area.

k. Close the **Area Under Curve** window.

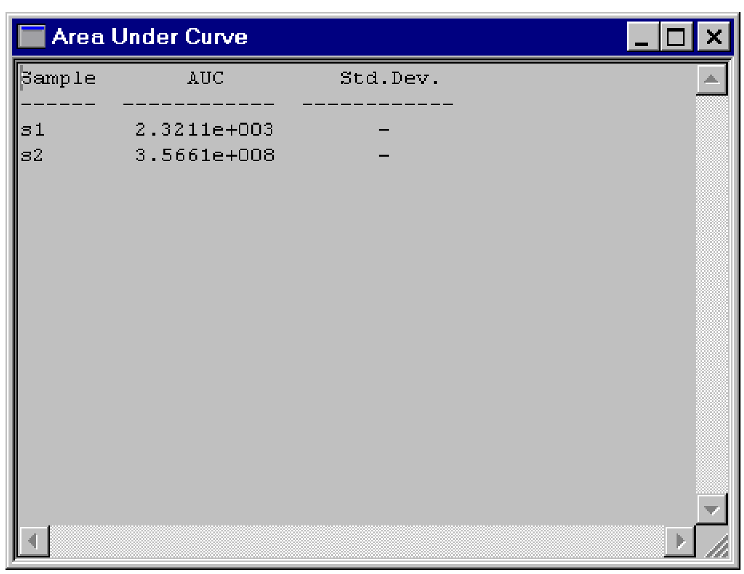
2. Estimate the area under **q2**.

In order to estimate the area under **q2**, the solution of the differential equation q2'(t), you must first put a sample on it. Then when you Solve or Fit, the area will be automatically calculated.

a. Put a sample on **q2**. Your model will appear as follows:



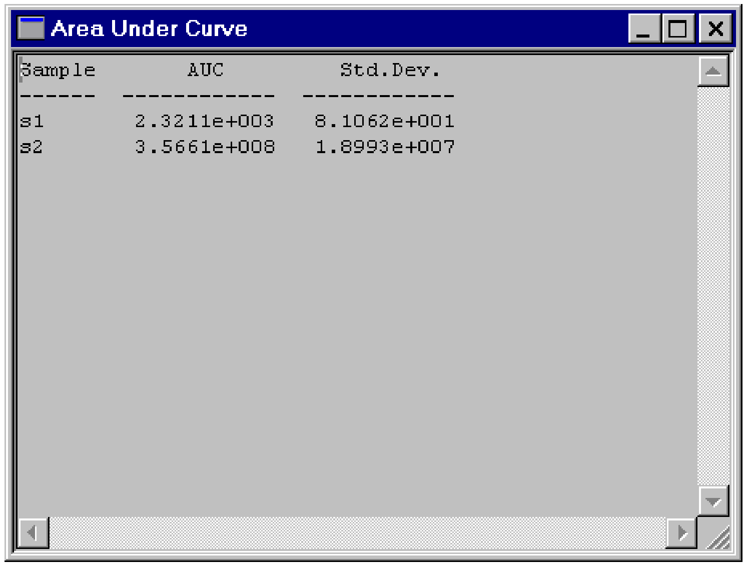
b. Solve your model, and view the AUC. The **Area Under Curve** window will appear as follows:



Sample	AUC	Std.Dev.
s1	2.3211e+003	-
s2	3.5661e+008	-

Notice that there are no statistics; this is because you "Solved," not "Fitted," your model to your data.

c. Fit the model to the data, and view the AUC. The **Area Under Curve** window will appear as follows:



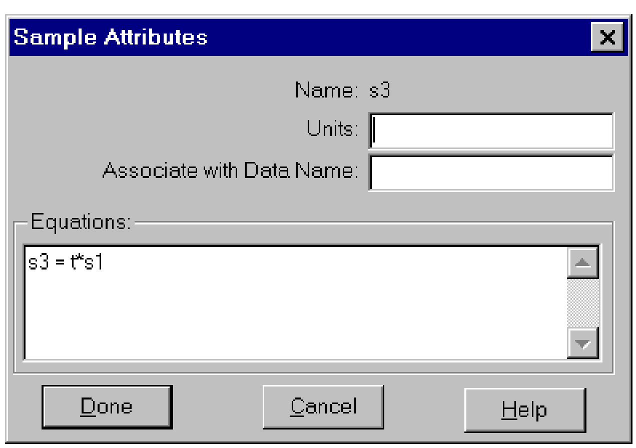
d. Close the **Area Under Curve** window.

3. Estimate area under the first moment curve, AUMC.

The area under the first moment curve, or the mean area under the curve, is needed to estimate some of the noncompartmental parameters such as the system mean residence time. To estimate AUMC, you need to create a new sample as follows.

a. Create a sample on Compartment **q1**. Sample **s3** will be created.

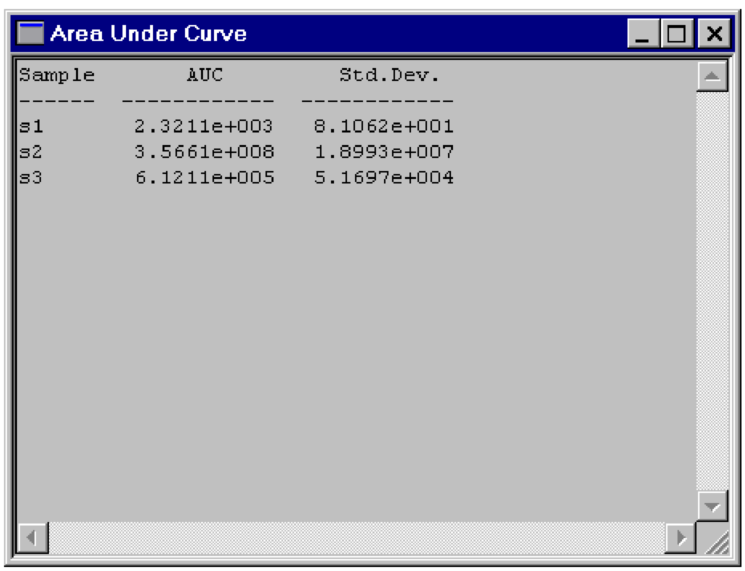
b. Open the **Sample Attributes** dialog box, and edit the sample equation to read “s3=t*s1”. The **Sample Attributes** dialog box will appear as follows:



c. Click **Done**. You will notice **s3** is no longer connected to **q1**; this is because **q1** does not appear in the sample equation.

Estimating AUMC. In SAAM II, the compute area under the curve option will calculate $\int_0^{t_{end}} si(t)dt$ where t_{end} is the time at which the experiment ends (and set by the user - this is what you changed so that $\int_0^{t_{end}} si(t)dt$ would approximate $\int_0^{\infty} si(t)dt$.) When you write the sample equation s3 = t*s1, you are approximating $\int_0^{t_{end}} t \cdot s1(t)dt$ which is exactly AUMC.

d. Fit the model to the data, and view the area under the curve calculations. The **Area Under Curve** window will appear as follows:


Area Under Curve

Sample	AUC	Std.Dev.
s1	2.3211e+003	8.1062e+001
s2	3.5661e+008	1.8993e+007
s3	6.1211e+005	5.1697e+004

Because of the way in which **s3** was defined, the AUC for **s3** is actually the AUMC for the concentration-time curve. The value for **s3**, the AUMC, is 612110 with a standard deviation of 51697. These values compare favorably with the values calculated originally of 612,400 with a standard deviation of 51,737. These values are very close; the differences have to do with what is called round-off error in the numerical calculations.

e. (Optional) Copy the contents of the **Area Under Curve** window and paste the information in the **Notes** window. If you do not do this part, reset the time of the experiment to 300, and delete samples **s2** and **s3**. Re-Solve the model.

Open the **Notes** window. Title the section "SAAM II AUC calculations." Copy and pasate the contents of the **Area Under Curve** window into the **Notes** window. This part of your **Notes** window will appear as follows:

```
SAAM II AUC calculations

Sample        AUC           Std.Dev.
------    ------------   ------------
s1         2.3211e+003    8.1062e+001
s2         3.5661e+008    1.8993e+007
s3         6.1211e+005    5.1697e+004
```

f. Close the **Notes** and **Area Under Curve** windows. Reset the time of the experiment to 300, and delete samples **s2** and **s3**. Re-Solve the model.

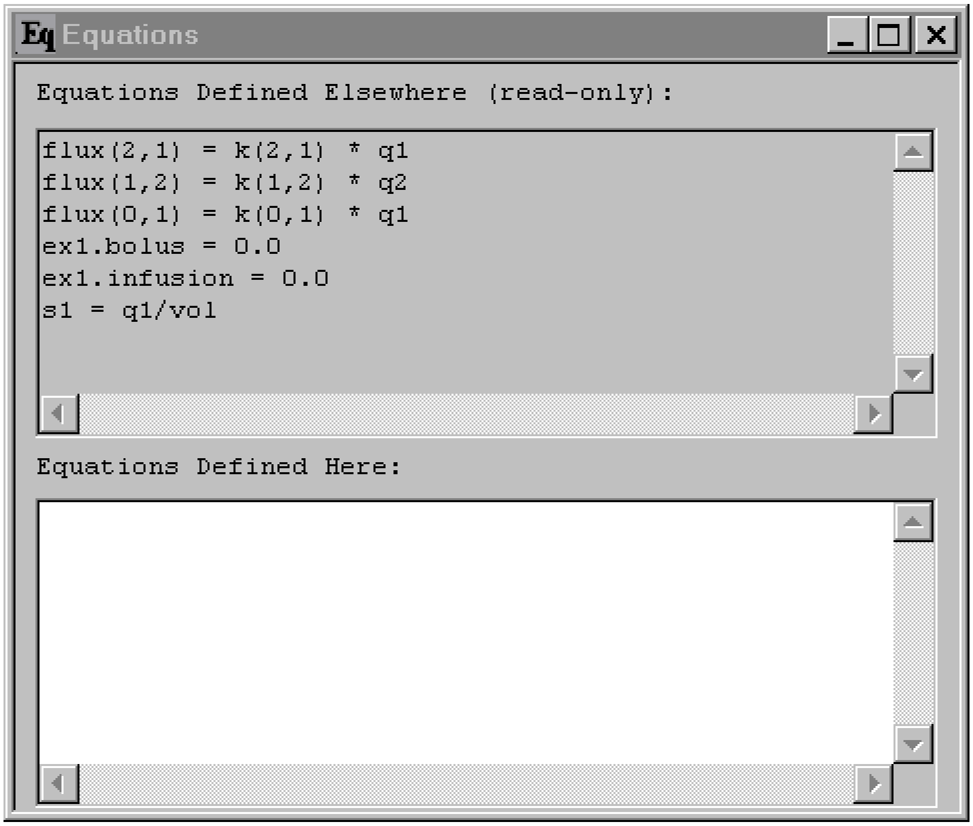
If you wish, you may save the study file for future use. It is recommended you **Save** the SAAM II study file as **Two_Compartment_Template**. You may proceed to Part 6 which is optional. Otherwise, **Quit** the **SAAM II Compartmental** application. The contents of the **Notes** window are contained in Appendix 2 (Appendix 2 also contains the notes from the next part of this case study.)

The two-compartment model template. You can use this as a template for the two compartment model where, following a bolus injection of drug iv, you want to estimate both the model parameters and noncompartmental parameters. You will need to provide the new data and initial parameter estimates; the model's equations do not change.

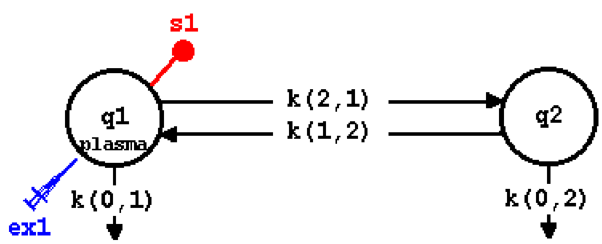
Part 6. Explore different structures for the two-compartment model - *a priori* identifiability (optional).

To explore different structures for the two-compartment model, you need first to remove the noncompartmental equations from the **Equations** dialog box.

1. Remove the noncompartmental equations from the **Equations Defined Here** pane in the **Equations** dialog box. The **Equations** dialog box will appear as follows:

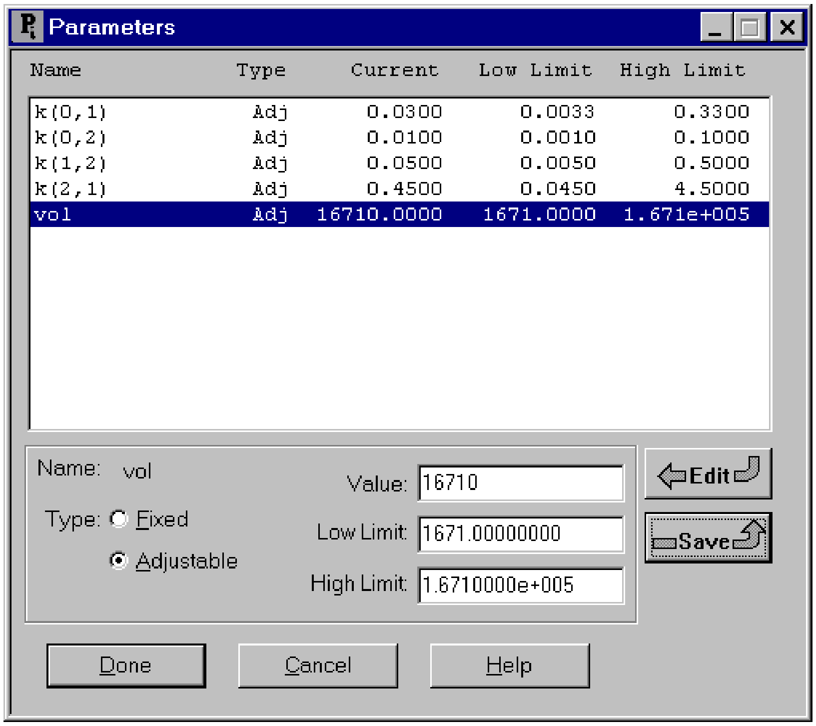


2. Modify your system model by adding a loss *k(0,2)*. Your model should appear as follows:



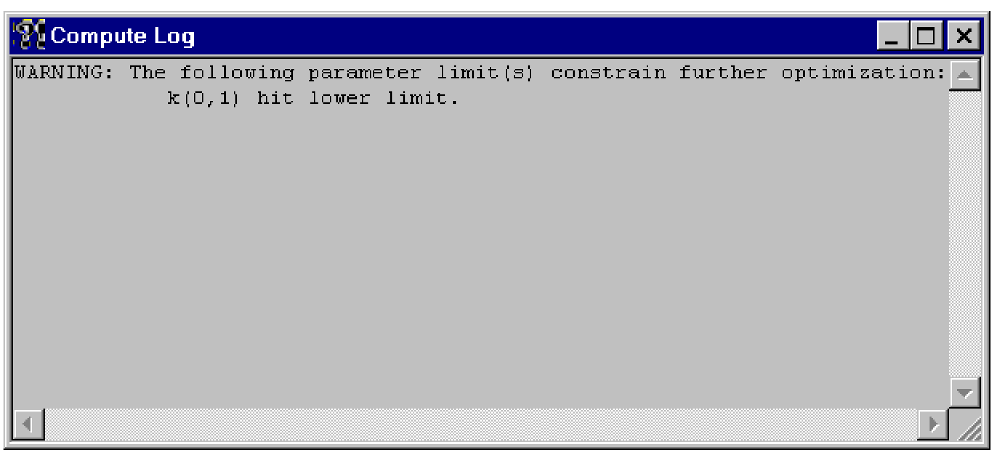
Your model now has five parameters instead of the original four.

3. Open the **Parameters** dialog box, and enter the initial values as shown below:



4. Solve the model, and view the solution. When you do, you will see that the model predicted values are decaying more rapidly than the data. Change *k(0,1)* to 0.01 and *k(0,2)* to 0.005. When you re-solve the model, it is still decaying a little too fast. You can continue hand-fitting the model to the data if you wish.

5. Fit the model to the data. When you do, you will get a warning message as shown below:



As the warning implies, *k(0,1)* has hit a lower limit. If you change the **Low Limit** for *k(0,1)* and fit again, you will get the same warning message. You can continue, but it is clear that *k(0,1)* is heading towards zero.

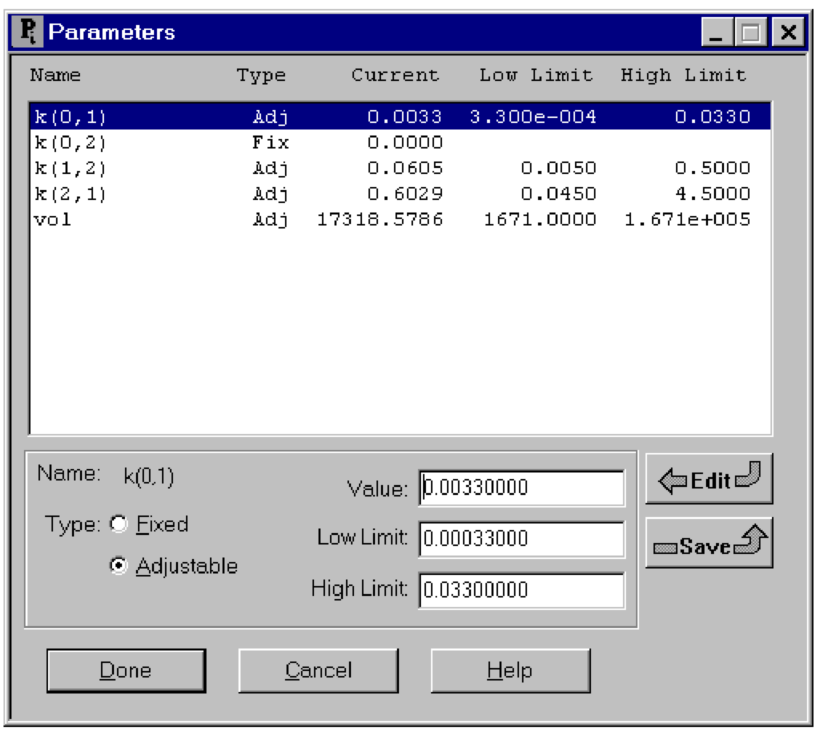
The reason is that you cannot estimate the five parameters in the model from the data; this model is *a priori* nonidentifiable as explained in Appendices 1 and 3.

SAAM II

Warning messages. Warning messages in SAAM II are designed to help you in your modeling exercise when the program detects a problem. In this case, when a parameter hits a limit, it can mean either that you are near a solution and have to adjust the limit, or your parameter cannot be estimated from the data. When the same warning message appears time after time when you change a limit, it is a good sign you will not be able to estimate all model parameters, and will have to resort to alternative strategies.

In this particular situation, it is possible to get a warning message that the covariance matrix is unreliable, and no statistical information is available. This warning results again from the fact that, as noted above, there are more parameters in the model than can be estimated from the data.

6. Open the **Parameters** dialog box, set *k(0,2)* equal to zero, and fix it. Reset the low and high limits for *k(0,1)*. The **Parameters** dialog box will appear as follows:



7. Fit the model to the data. You will get a warning that *k(0,1)* hit an upper limit. Adjust the upper limit, and fit the model again. As a rule of thumb, you can double the limit. In this case, you can double the upper limit, but the low limit

will remain unchanged. If your upper limit increases substantially from your original estimate, you should readjust your low limit also so that the high limit is about 10 times greater than the value and the low limit is about 0.1 times the value. You should obtain a successful fit. View the statistics - you will see they are identical to your first successful fit because, with *k(0,2)* equal to zero, the model is the same as the original model.

8. (Optional) Open the **Notes** window, and add the statistics from the last Fit. Enter the title "Best fit of model with k(0,2) = 0." This part of your **Notes** window will appear as follows:

```
Best fit of model with k(0,2) = 0

                        value    SD              Coeff Var           95% confidence
k(0,1)                0.03739  3.27285e-003  8.75375e+000       0.03026        0.04452
k(0,2)           0.00000e+000   ** Fixed **   ** Fixed **   ** Fixed **    ** Fixed **
k(1,2)                0.06411  3.26014e-003  5.08492e+000       0.05701        0.07122
k(2,1)                0.56844  3.87544e-002  6.81773e+000       0.48400        0.65287
vol               17327.35156  1.38688e+003  8.00399e+000   14305.60304    20349.10008

                          Objective      Scaled Data Variance
s1 : plasma            -8.332469e-001       3.291711e-001
                       --------------
Total objective        -8.332469e-001

AIC                     8.148151e-001
BIC                     9.355321e-001
```

Close the **Notes** window.

9. Fix *k(0,1)* and Re-Solve the model.

   a. Open the **Parameters** dialog box.

   b. Set *k(0,1)* equal to zero, and fix it.

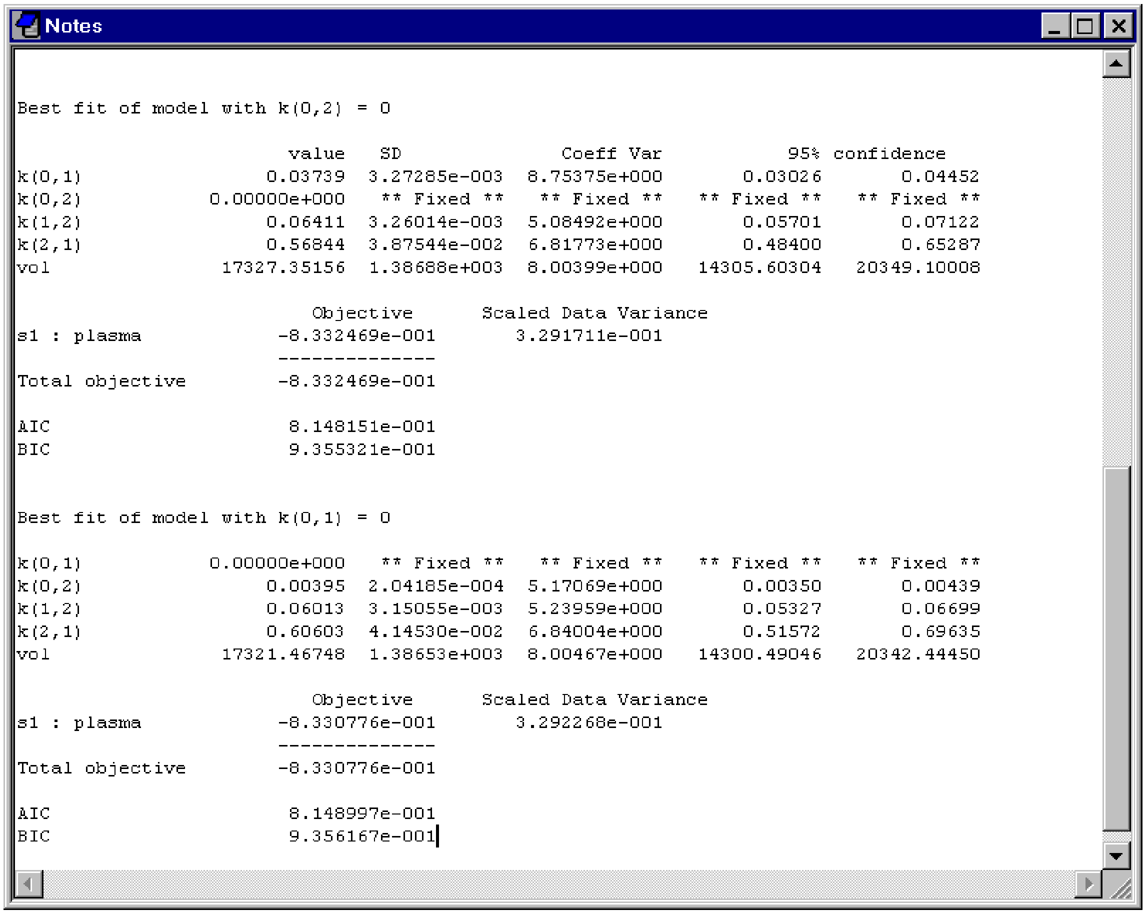
   c. Set *k(0,2)* adjustable, and enter 0.01, 0.001 and 0.1 as the **Value**, **Low Limit** and **High Limit** respectively.

   d. Close the **Parameters** dialog box.

10. Fit the model to the data.

If you plot the results, you will see that the "Fit" is identical to your previous "Fit" where *k(0,2)* was zero. If you look at your parameter values, you will see, in comparing the two cases with first k(0,2) = 0 and then k(0,1) = 0, that the exchange rate constants are different, but the value of the objective function is essentially the same. You have obtained an identical solution, one with the loss from compartment 1, and the other with the loss from compartment 2!

If you wish, you may record the results in the **Notes** window as you did above, otherwise you may proceed to step 11. If you record you results, title this part "Best fit of model with k(0,1) = 0." This part of the **Notes** window will appear as follows:



```
Notes

Best fit of model with k(0,2) = 0

                       value     SD            Coeff Var          95% confidence
k(0,1)               0.03739  3.27285e-003  8.75375e+000      0.03026       0.04452
k(0,2)          0.00000e+000    ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(1,2)               0.06411  3.26014e-003  5.08492e+000      0.05701       0.07122
k(2,1)               0.56844  3.87544e-002  6.81773e+000      0.48400       0.65287
vol              17327.35156  1.38688e+003  8.00399e+000  14305.60304   20349.10008

                         Objective      Scaled Data Variance
s1 : plasma            -8.332469e-001      3.291711e-001
                       --------------
Total objective        -8.332469e-001

AIC                     8.148151e-001
BIC                     9.355321e-001


Best fit of model with k(0,1) = 0

k(0,1)          0.00000e+000    ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(0,2)               0.00395  2.04185e-004  5.17069e+000      0.00350       0.00439
k(1,2)               0.06013  3.15055e-003  5.23959e+000      0.05327       0.06699
k(2,1)               0.60603  4.14530e-002  6.84004e+000      0.51572       0.69635
vol              17321.46748  1.38653e+003  8.00467e+000  14300.49046   20342.44450

                         Objective      Scaled Data Variance
s1 : plasma            -8.330776e-001      3.292268e-001
                       --------------
Total objective        -8.330776e-001

AIC                     8.148997e-001
BIC                     9.356167e-001
```

Close the **Notes** window.

11. Perform a series of "Fits" with *k(0,2)* <u>fixed</u> at different values. To do this, set *k(0,1)* as adjustable with an initial estimate of 0.02. You can pick any value for *k(0,2)* between 0 and 0.0039. Try three examples with *k(0,2)* fixed at the following values:

    k(0,2) = 0.001
    k(0,2)= 0.002
    k(0,2)= 0.003

    You will see that in each case you obtain a "Fit" that is identical to the others, and that the value for the objective function is essentially the same for all of the "Fits."

12. Set k(0,2) equal to 0.006, and fit the model to the data. As you lower the **Low Limit** on *k(0,1)*, you will get a series of warnings that *k(0,1)* has hit the low limit. It wants to go to zero. Set *k(0,1)* equal to zero (remember biology prevents *k(0,1)* from becoming negative). When you fit the model to the data, you will not obtain a good fit.

Interval identifiability. This two-compartment model, the model with the two exchange rate constants and two losses, is called interval identifiable. This means that while there may be an infinite number of solutions, i.e. you can set *k(0,2)* to an infinite number of values, these values must lie within certain bounds to obtain a good "fit" of the model to the data. Interval identifiability is described in Appendix 3 for the two-compartment model. It is sometimes the case that, even though the full two-compartment model is not identifiable, the intervals in which the parameters lie provide valuable information.

13. Introducing fixed parameter constraints.

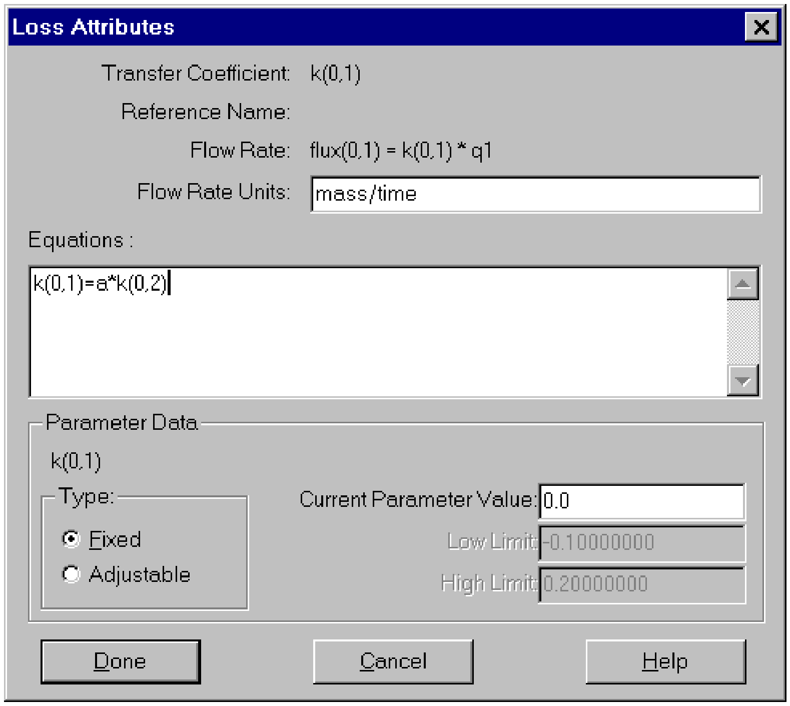
    Suppose that in this situation, you know that the full two-compartment model was appropriate to analyze these pharmacokinetic data. You have just learned that you cannot estimate all five parameters. As you have seen, if you have precise knowledge about one parameter, you can fix it, and obtain a good fit (assuming that your known parameter lies in the correct interval.)

    Suppose instead you know from the literature that there is a relationship between the losses *k(0,1)* and *k(0,2)*. For example, suppose k(0,1)=a*k(0,2) where *a* is known. You can enter this information in your model. The result will be that the number of parameters to estimate is 4 and your model is identifiable.

    How to do this when *a* is assumed to be equal to 3 is explained below.

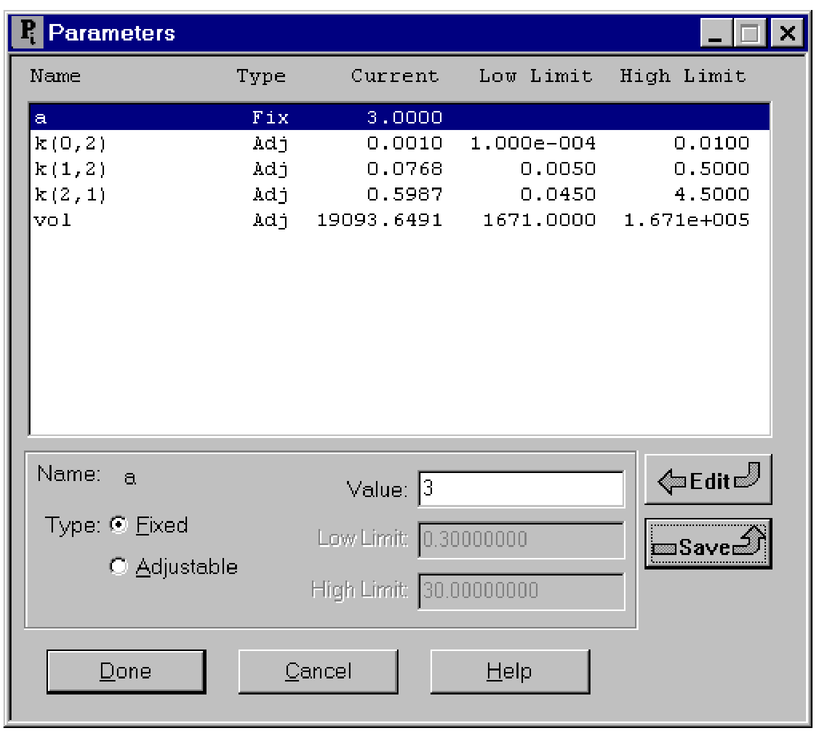
    a. Be sure the **Experiment** tools are available. Double-click *k(0,1)* to open the **Loss Attributes** dialog box.

    b. In the **Equations** pane, type "k(0,1)=a*k(0,2)". The **Loss Attributes** dialog box will appear as follows:



c. Click **Done**.

d. Open the **Parameters** dialog box. Enter "3" as the **Value** for *a*, and fix it. Enter "0.001" as an initial estimate for *k(0,2)*; set *k(0,2)* as an adjustable parameter. The **Parameters** dialog box will appear as follows:



Click **Done**.

e. Fit the model to the data. When you view the solution, you will see from the plot that the fit is the same as all others you have obtained in this part of the case study. When you view the statistical information, you will see that the error estimates for the parameters are fine also.

What you should also notice is that while the values of the rate constants are changing, the value of the objective function is remaining essentially the same. This illustrates the multi-solution nature of the general two-compartment model.

If you wish, you can record the results in the **Notes** window with the heading "Best fit of the model to the data with k(0,1) = 3*k(0,2)." If you do not record your results, you may proceed to step 14. Your **Notes** window will appear in part as follows:

```
Best fit of model with k(0,1) = 0

k(0,1)          0.00000e+000   ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(0,2)               0.00395  2.04185e-004  5.17069e+000       0.00350       0.00439
k(1,2)               0.06013  3.15055e-003  5.23959e+000       0.05327       0.06699
k(2,1)               0.60603  4.14530e-002  6.84004e+000       0.51572       0.69635
vol              17321.46748  1.38653e+003  8.00467e+000   14300.49046   20342.44450

                          Objective      Scaled Data Variance
s1 : plasma           -8.330776e-001       3.292268e-001
                      --------------
Total objective       -8.330776e-001

AIC                    8.148997e-001
BIC                    9.356167e-001


Best fit of the model to the data with k(0,1) = 3*k(0,2)

a                    3.00000   ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(0,2)               0.00304  1.54312e-004  5.07958e+000       0.00270       0.00337
k(1,2)               0.06107  3.21343e-003  5.26191e+000       0.05407       0.06807
k(2,1)               0.59699  4.12733e-002  6.91359e+000       0.50706       0.68691
vol              17320.81535  1.38655e+003  8.00514e+000   14299.77569   20341.85501
--------------------------------- Derived Variables ---------------------------------
k(0,1)               0.00911  4.62935e-004  5.07958e+000       0.00810       0.01012


                          Objective      Scaled Data Variance
s1 : plasma           -8.332526e-001       3.291692e-001
                      --------------
Total objective       -8.332526e-001

AIC                    8.148122e-001
BIC                    9.355292e-001
```

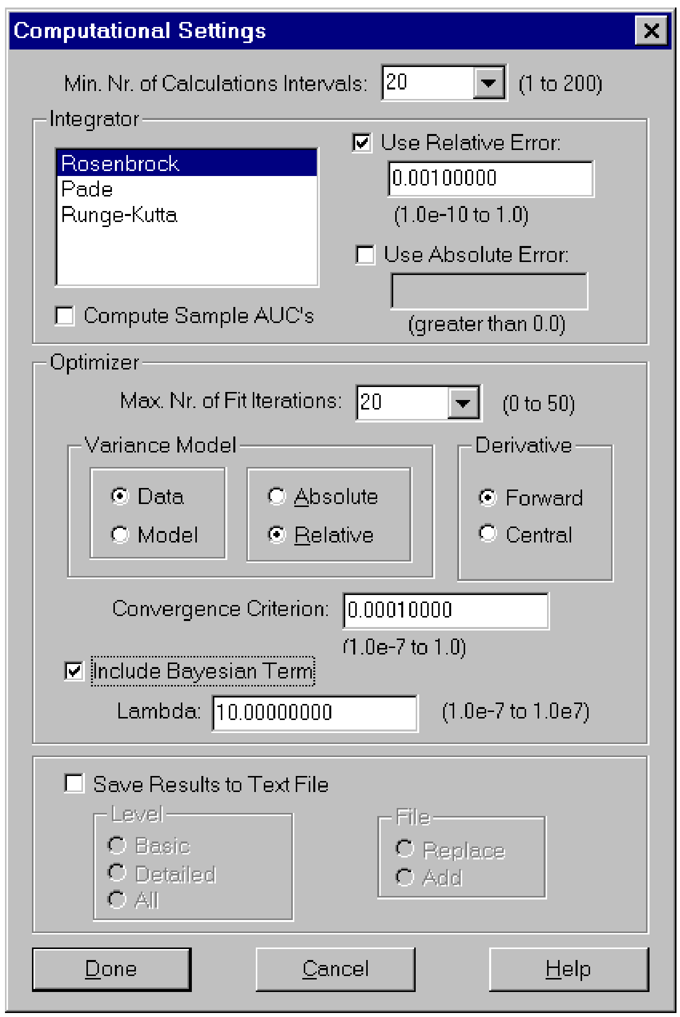
Close the **Notes** window.

14. Using Bayesian parameters.

    Suppose you know that there is a relationship between *k(0,1)* and *k(0,2)*, but you do not know it precisely. In the above, for example, suppose you know that k(0,1) = a*k(0,2), but instead of knowing that *a* is precisely 3, from the literature you know that *a* is approximately 3 with a standard deviation of 1. The question is how to incorporate the uncertainty about *a* into the model. This is done in SAAM II by using the Bayesian option.

    a. In the **Compute** menu, click **Settings**. The **Computational Settings** dialog box will open.

       (1) Select the **Include Bayesian Term** check box. The **Computational Settings** dialog box will appear as follows:



       (2) Click **Done**.

    b. Open the **Parameters** dialog box.

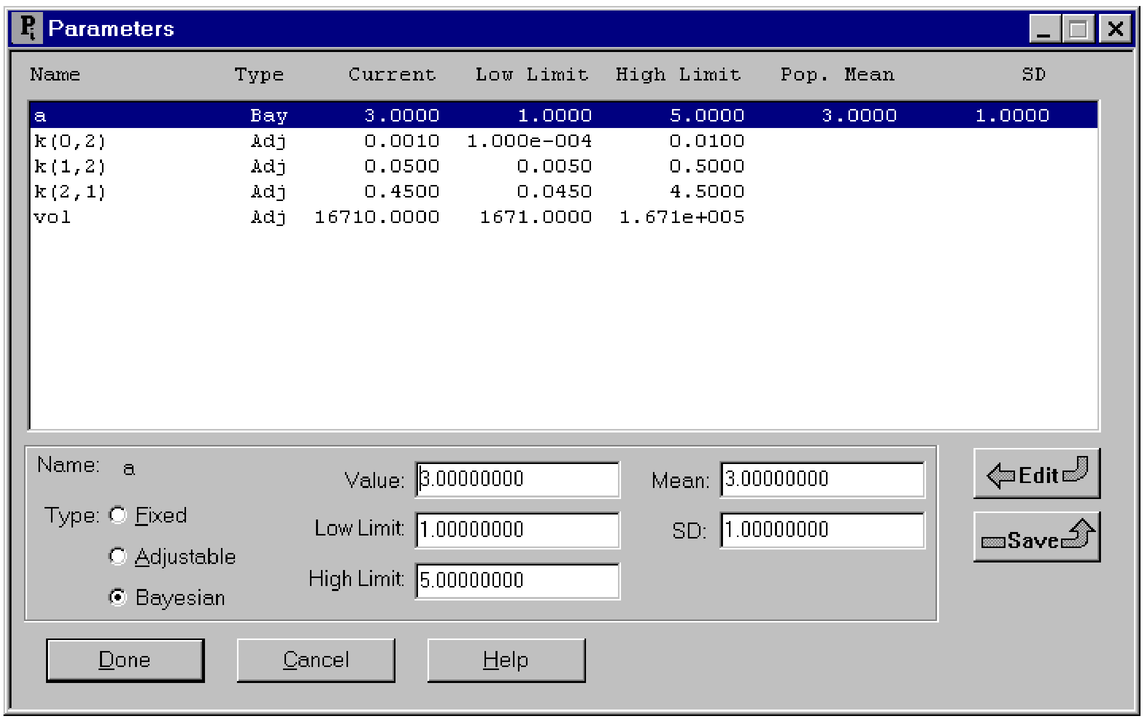
(1) Be sure *a* is selected (if it is not, double-click *a* to select it.)

(2) Select the **Bayesian** option.

(3) Enter: "3" in the **Value** box.
"1" in the **Low Limit** box.
"5" in the **High Limit** box.
"3" in the **Mean** box.
"1" in the **SD** box.

(4) Click **Save**.

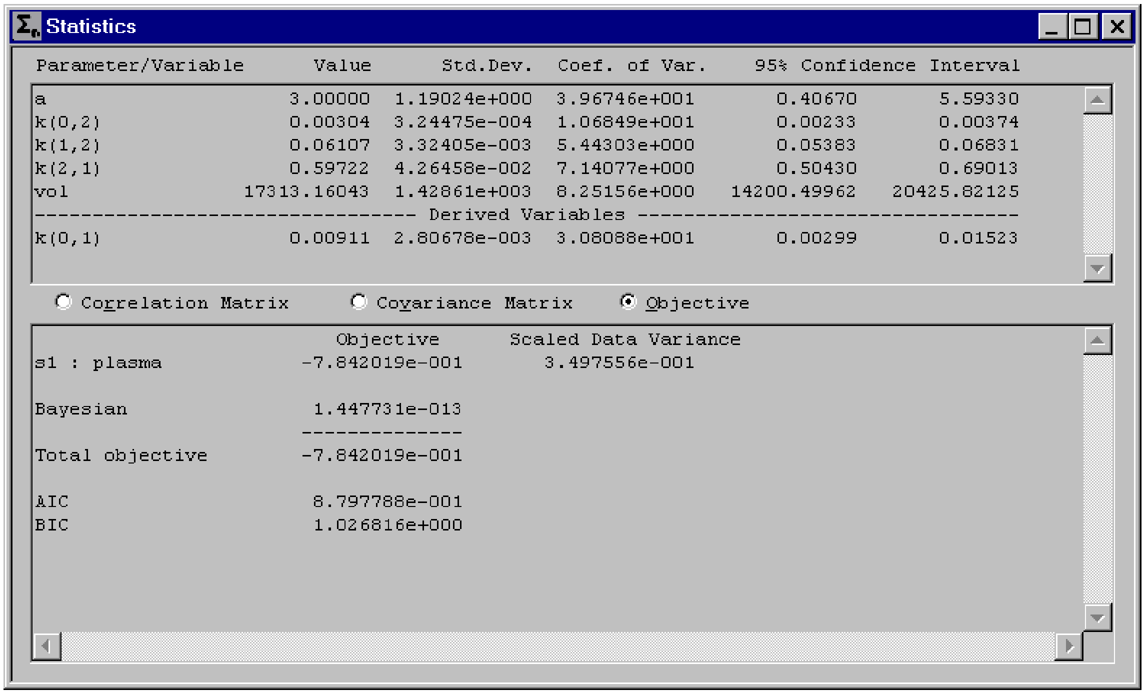
(5) Set the initial estimates for the other parameters as shown in the **Parameters** dialog box as follows:



(6) Click **Done**.

c. Solve the model, and view the solution. It is reasonable.

d. Fit the model to the data, view the solution and statistics. The solution in terms of the plot is the same as for all other successful "Fits." The **Statistics** window will appear as follows:



Now even though the "Fit" is identical to the rest, the statistical information is different. The reason is the addition of the Bayesian parameter *a*. This adds some uncertainty that was not present before. This is reflected in the uncertainties in the rate constants which are a little higher than in the previous fits.

e. If you wish, you can record the results in the **Notes** window with the title "Best fit of the model to the data with k(0,1) = a*k(0,2) where a=3 is Bayesian with SD = 1." If you do not record your results, **Quit** the **SAAM II Compartmental** application.Your **Notes** window will appear in part as shown below:

Notes

```
Best fit of the model to the data with k(0,1) = a*k(0,2)where a=3 is Bayesian with SD = 1.

a                      3.00000  1.19024e+000  3.96746e+001       0.40670       5.59330
k(0,2)                 0.00304  3.24475e-004  1.06849e+001       0.00233       0.00374
k(1,2)                 0.06107  3.32405e-003  5.44303e+000       0.05383       0.06831
k(2,1)                 0.59722  4.26458e-002  7.14077e+000       0.50430       0.69013
vol                17313.16043  1.42861e+003  8.25156e+000   14200.49962   20425.82125
-------------------------------- Derived Variables --------------------------------
k(0,1)                 0.00911  2.80678e-003  3.08088e+001       0.00299       0.01523

                          Objective      Scaled Data Variance
s1 : plasma            -7.842019e-001       3.497556e-001

Bayesian                1.447731e-013
                       --------------
Total objective        -7.842019e-001

AIC                     8.797788e-001
BIC                     1.026816e+000
```

If you wish, you may save this study file for future use. For convenience, the contents of the **Notes** window are included in Appendix 2; this should help you review the modeling analysis you have just completed.

Quit the **SAAM II Compartmental** application.

Essential Points to Remember

- Both the compartmental and non-compartmental pharmacokinetic parameters for the two-compartment model can be obtained from the compartmental analysis using standard equations.
- The AUC option in SAAM II enables the area under the curve to be calculated for any compartment, but the length of the experiment must be increased to ensure all of the area is estimated.
- Parameter identifiability limits the number of parameters that can be specified for a given compartmental model. As a general rule, the number of compartments in a model cannot exceed the number of exponential phases in the data, and the number of model parameters cannot exceed the number of exponents and coefficients in the multiexponential describing the data.

Appendix 1: Obtaining Initial Parameter Estimates for the Two-Compartment Model (Two-Exponential Model)

The key to understanding how initial parameter estimates can be obtained for the two-compartment (or two-exponential) model lies in understanding the notion of half-life. The half-life is defined as the time it takes for one-half of the material remaining in a system to irreversibly leave the system.

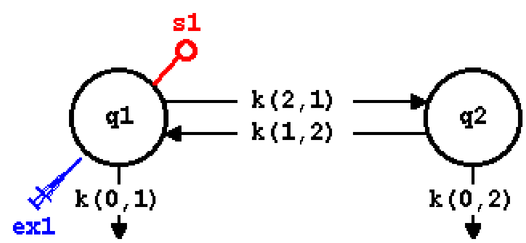
In this appendix, it is assumed that you have worked through the appendix explaining how to obtain initial parameter estimates for the one-compartment model; this appendix follows the case study on cadralazine kinetics. The ideas presented in this appendix extend those presented for the one-compartment (one-exponential) model. In this situation, unlike the one-exponential case, the data following the bolus injection into plasma do not appear as a straight line when plotted semi-logarithmically. However, as will be seen, the biexponential decay is a combination of two monoexponential decays.

In this appendix, you will learn how to obtain the initial estimates in the following two situations:

- bolus injection of drug into plasma, and serial plasma samples taken; and
- constant infusion of drug into plasma, and serial plasma samples taken.

The bolus injection will be discussed first. Three different techniques that can be used to obtain initial parameter estimates will be discussed.

To begin, some information concerning the structure of the general two-compartment model and sums of exponentials is necessary. The general two-compartment model is given below:



Model 1. The general two-compartment model with five parameters.

If the sample equation s1 is given by s1=q1/vol, then there are five parameters in this model: *vol, k(2,1), k(1,2), k(0,1)* and *k(0,2)*. If the input into Compartment **q1** is a bolus, then it is known that the solution to the system of differential equations represented by the model is an expression of the form:

$$s_1(t) = \frac{q1}{vol} = A_1 \cdot e^{-\alpha_1 t} + A_2 \cdot e^{-\alpha_2 t} \quad (1)$$

while if the input into compartment **q1** is a constant infusion, the solution is:

$$s_1(t) = \frac{q1}{vol} = A_0 + A_1 \cdot e^{-a_1 t} + A_2 \cdot e^{-a_2 t} \quad A_0 + A_1 + A_2 = 0 \qquad (2)$$

For both sums of exponentials, there are four parameters: A_1, A_2, a_1 and a_2. This is true in the case of (2) because of the equation constraining the sum of the parameters equal to zero.

It is known that for a data set that is biexponentially decaying (i.e. following a bolus injection) or biexponentially rising (i.e. following a constant infusion) that the four parameters of the exponential model are unique (called *a priori* or uniquely identifiable). However, the five parameters of the general two-compartment model are not uniquely identifiable. In this case, there are an infinite number of solutions. Why is this the case?

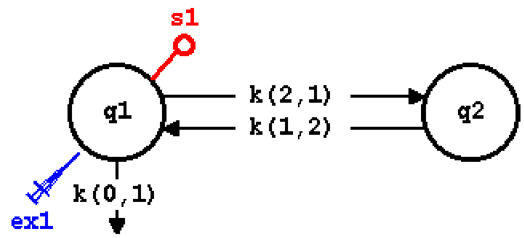
The reason why this is the case comes from the relationship between the A_1, A_2, a_1 and a_2 and the *vol, k(2,1), k(1,2), k(0,1)* and *k(0,2)*. This is important since it provides the equations by which the initial parameters for the two-compartment model can be obtained. The relationship comes from *a priori* identifiability analysis [2].

The relationship for the bolus injection is the following:

$$\begin{aligned} k(1,1) &= \frac{A_1 \cdot a_1 + A_2 \cdot a_2}{A_1 + A_2} \\ k(2,2) &= \frac{A_2 \cdot a_1 + A_1 \cdot a_2}{A_1 + A_2} \\ k(1,2) \cdot k(2,1) &= \frac{A_1 \cdot A_2 \cdot (a_1 + a_2)^2}{(A_1 + A_2)^2} \\ vol &= \frac{d}{A_1 + A_2} \end{aligned} \qquad (3)$$

where $k(1,1) = k(2,1) + k(0,1)$ and $k(2,2) = k(1,2) + k(0,2)$, and *d* is the amount in the bolus. Thus the parameters that can be uniquely identified as *vol, k(1,1), k(2,2)* and the product *k(1,2)k(2,1)*. The relationship for the *k(i,j)* is the same for the constant infusion experiment; the expression for *vol* is different as discussed below.

If the general two-compartment model is not uniquely identifiable, what about the commonly used two-compartment model shown as follows:



Model 2. The two-compartment model with no loss from compartment 2.

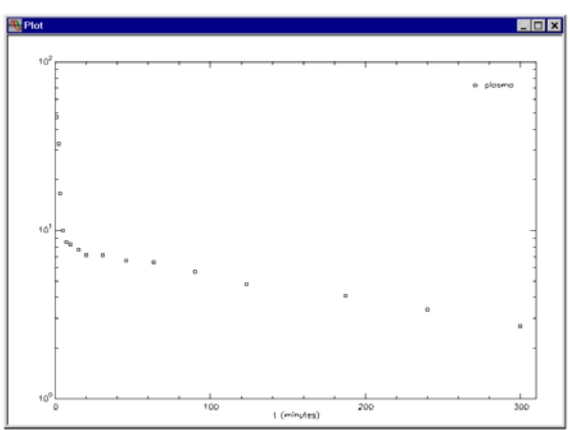
In this case, there is no loss *k(0,2)*. This means *k(2,2)=k(1,2)*. Since *k(2,2)* is uniquely identifiable and since it now equals *k(1,2)*, *k(1,2)* uniquely identifiable. Since the product *k(1,2)·k(2,1)* is identifiable and *k(1,2)* is known, one can solve for *k(2,1)* making it uniquely identifiable. Since *k(1,1)=k(0,1)+k(2,1)*, and *k(2,1)* is known, one can solve for *k(0,1)* making it uniquely identifiable. Thus the parameters for the two-compartment model, Model 2, are uniquely identifiable, and can be obtained directly from the coefficients A_1 and A_2 and exponentials a_1 and a_2 of the sum of two exponentials using (3).

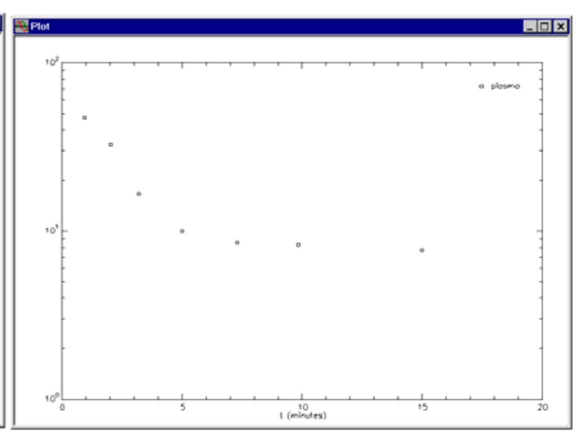
In what follows, initial parameter estimates for Model 2 will be discussed for the bolus and constant infusion inputs.

Part 1. Bolus injection into plasma

Three methods to estimate *k(2,1), k(1,2), k(0,1)* and *vol* will be given. The formal curve peeling method, as discussed in [1], will be given first. This is somewhat tedious, so two quicker methods will be discussed. If neither of these quicker methods work, you can always rely on the formal curve peeling method.

If you plot the hydromorphone data in semi-log form, you will obtain the following:

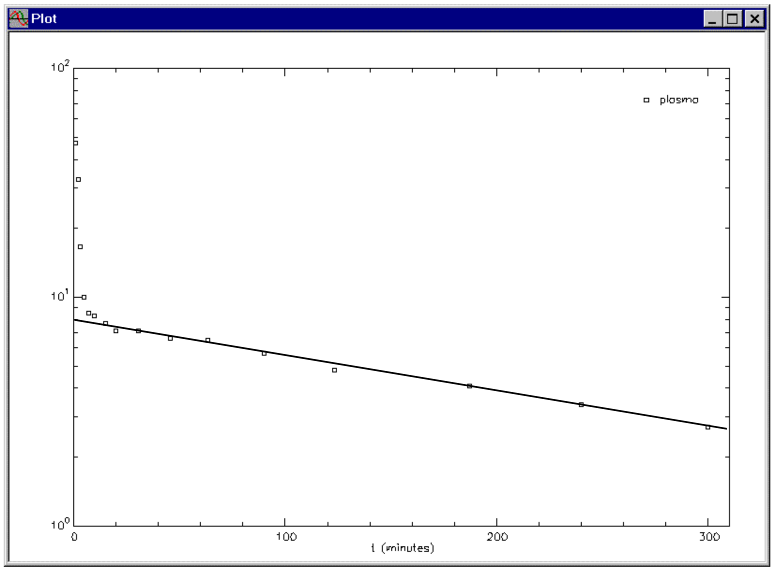




The left plot shows the all of the data. Since the initial decay is very rapid, it is useful to expand this portion of the plot. The right plot shows the initial decay to 20 minutes. It is interesting to note that the break in the curve occurs at about 5 minutes. Thus if one waited until 5 minutes or later to draw the first plasma sample, the data would decay monoexponentially.

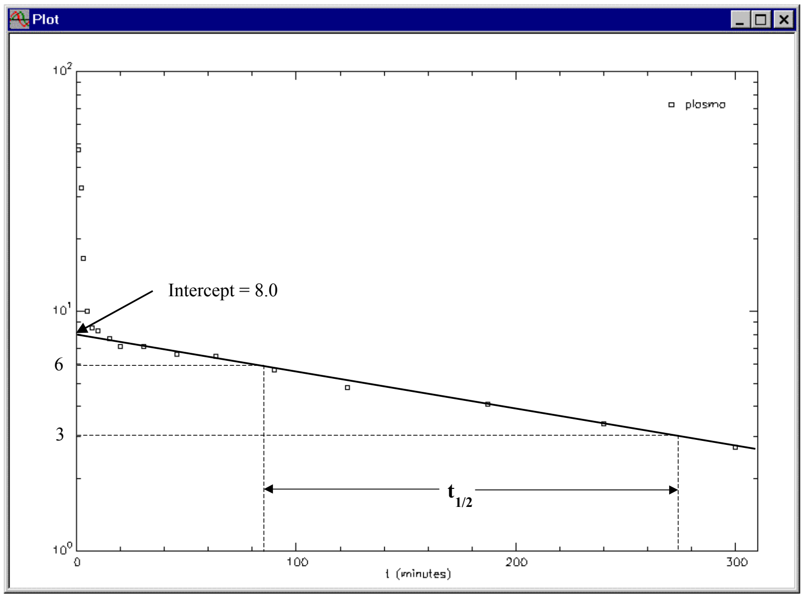
Curve Peeling

Following the description in Chapter 3 of [1], the first step in formal curve peeling is to draw a line through the final decaying portion of the data making sure this line is extended to intersect with the y-axis. This is shown in the following figure:



Note the intersection of this line with the y-axis, as shown in the following figure, is 8. This provides an estimate for A_2. You can now calculate the half-life of the terminal slope. This is shown in the following figure as the time it takes to go from 6 (at about 85 minutes) to 3 (at about 275 minutes) which is approximately 190 minutes. The half-life is thus ln(2)/190, or 0.0036. This provides an estimate for a_2. Thus we have the second term of (1):

$$A_2 \cdot e^{-a_2 t} = 8 \cdot e^{-0.0036 \cdot t} \quad (4)$$



The next step is to subtract this amount from each datum. The point is that *s1(t)* will describe each datum. Since *s1(t)* is a sum of two exponentials, both exponentials will contribute to the datum. Thus for the i^th datum, $s_1(t_i) = A_1 e^{-a_1 t_i} + A_2 e^{-a_2 t_i}$. In formal curve peeling, the contribution of the second exponential, $A_2 e^{-a_2 t}$, is subtracted from each datum. For this example, the results are shown below:

t	plasma	plasma - 8exp(-0.0036*t)
0.95	47.0	39.0
2.03	32.8	24.9
3.20	16.6	8.7
5.00	10.0	2.2
7.30	8.5	0.7
9.83	8.3	0.5
15.00	7.7	0.1
20.05	7.1	-0.3
30.82	7.1	-0.1
45.90	6.6	-0.1
63.25	6.5	0.1
90.00	5.7	-0.1
123.23	4.8	-0.3
187.00	4.1	0.1
240.00	3.4	0.1
300.00	2.7	0.0

Again, remembering the model is

$$s_1(t) = A_1 \cdot e^{-a_1 t} + A_2 \cdot e^{-a_2 t} \qquad (5)$$

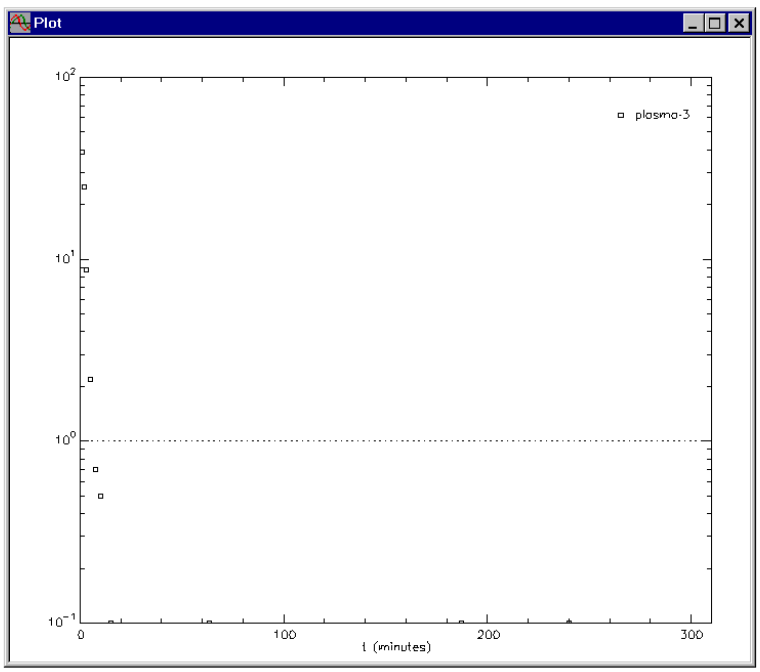
the result of this subtraction is a set of modified data that can be described by the first exponential:

$$s_1^{\mathrm{mod}}(t) = A_1 \cdot e^{-a_1 t} \qquad (6)$$

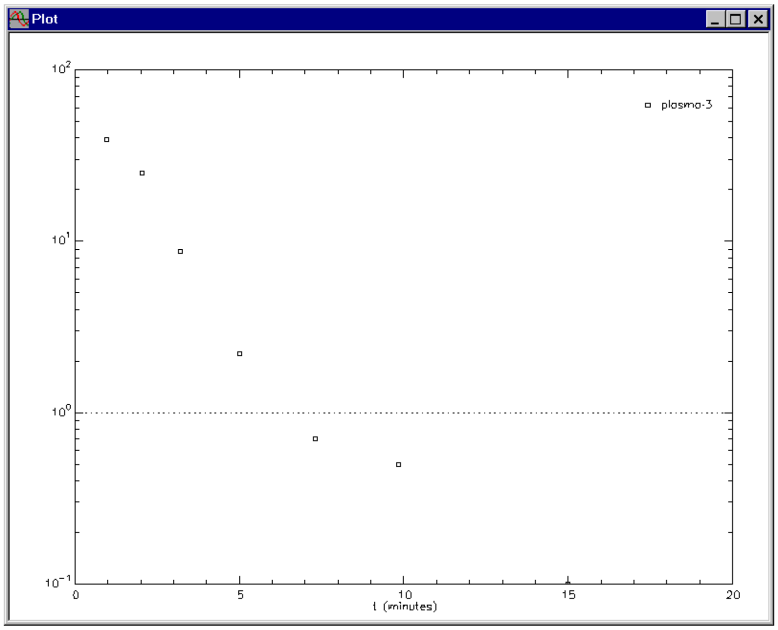
That is, if you plot the modified data given in the previous table, the initial portion should decay monoexponentially.

Why just the initial portion? The reason is the term $A_1 e^{-a_1 t}$ decays rapidly and hence its contribution to the data in the tail portion of the curve is much, much less than $A_2 e^{-a_2 t}$. That is, in the tail portion of the curve, numerically $A_1 e^{-a_1 t}$ is much smaller than $A_2 e^{-a_2 t}$. Since $A_2 e^{-a_2 t}$ predominates in the tail portion, after a certain point you would expect the modified data to be near zero, and be both positive and negative. This is exactly the case as you can see in the previous table.

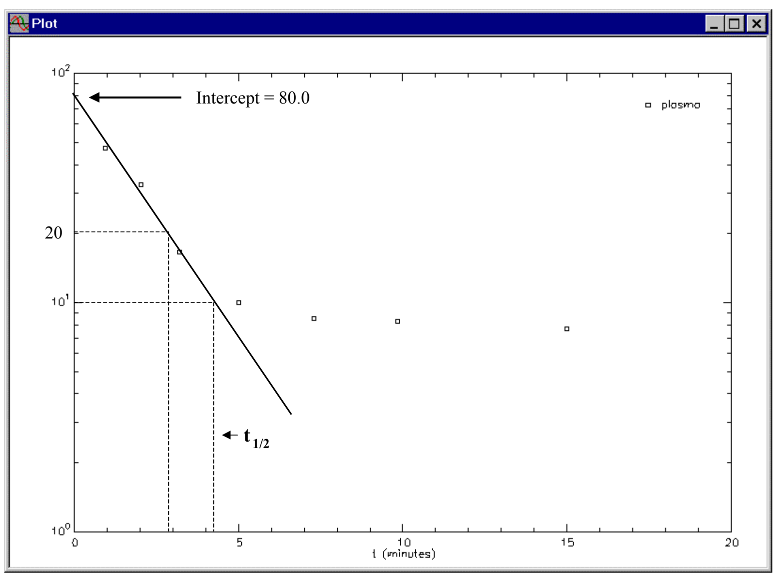
If you now plot the modified data, you will obtain the following:



This plot is not informative since the initial decay is rapid. If the modified data are plotted with a 20 minute time scale, you will obtain the following:



Now you see the initial data do appear to decay monoexponentially. You can now repeat the above process to obtain estimates for A_1 and a_1. This is shown in the following figure:



The intercept of the tangent line is at 80. This provides an estimate for A_1. The half-life, here measured as the time to go from 20 to 10, is about 1.2 minutes. Thus an estimate for a_1 can be obtained from ln(2)/2 which is 0.58.

If you are using (1) as your model, these will provide the initial estimates for the model parameters A_1, A_2, a_1 and a_2. If you are using Model 2, estimates for the rate constants and volumes can be found using (3):

$$k(2,2) = k(1,2) = \frac{A_2 \cdot a_1 + A_1 \cdot a_2}{A_1 + A_2} = 0.056$$

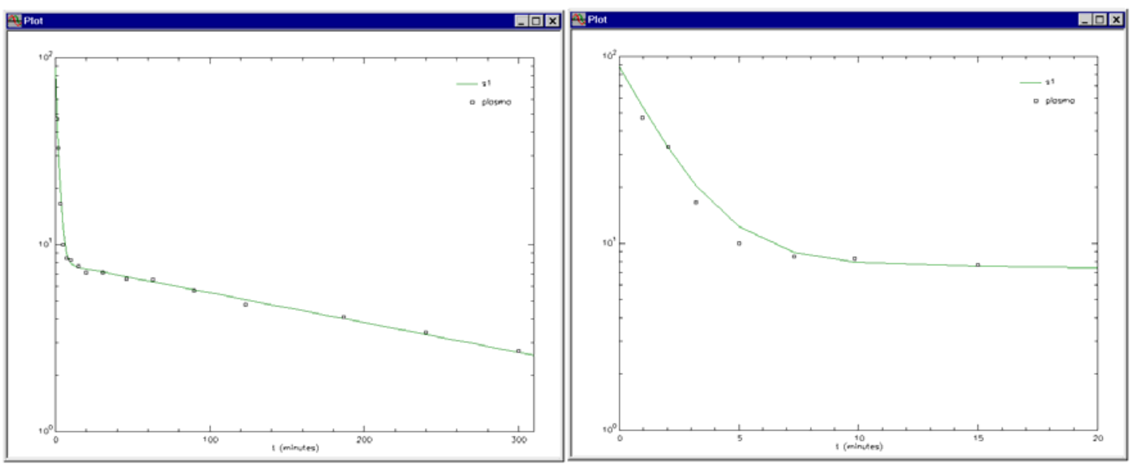
$$k(2,1) = \frac{A_1 \cdot A_2 \cdot (a_1 - a_2)^2}{k(1,2) \cdot (A_1 + A_2)^2} = 0.49 \tag{7}$$

$$k(0,1) = k(1,1) - k(2,1) = \frac{A_1 \cdot a_1 + A_2 \cdot a_2}{A_1 + A_2} - k(2,1) = 0.038$$

The dose is 1,504,000ng. Hence an estimate for *vol* can be obtained as follows:

$$vol = \frac{1504000}{88} = 17090 \tag{8}$$

If you put these initial estimates into your model and Solve, you will obtain the following solution:



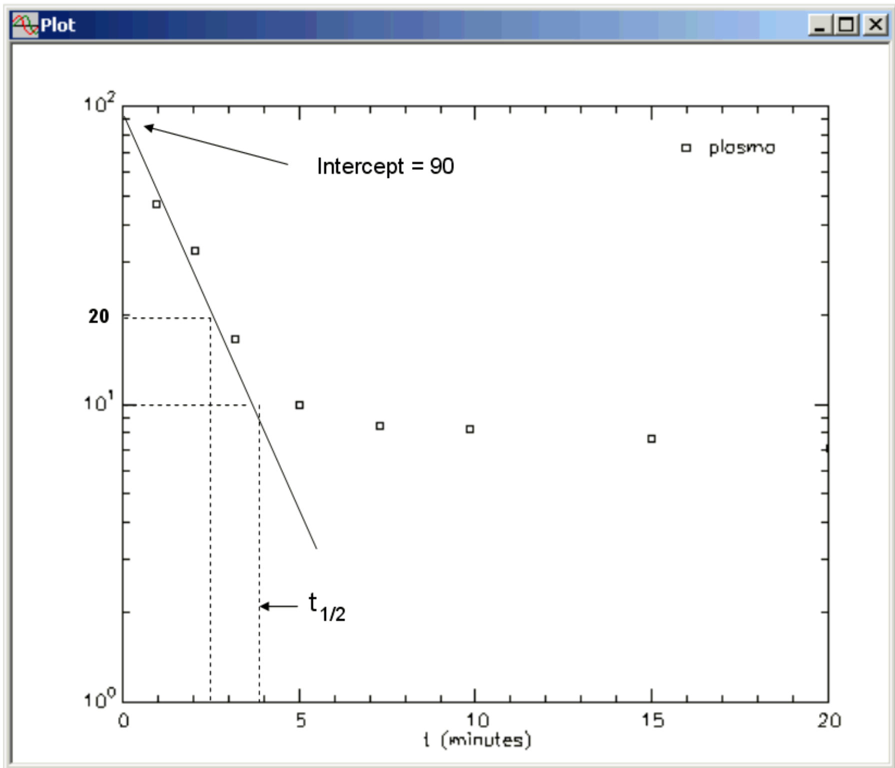
These estimates are clearly satisfactory.

<u>Quick Curve Peeling</u>

There is a quicker method to obtain the initial parameter estimates that avoids the formal subtraction of $A_2 e^{-a_2 t}$ from the data.

The first step is to draw the line through the final decay, and estimate A_2 and a_2 as above; it will be exactly the same. Then, rather that subtracting this component from each datum, you can simply draw a tangent line through the initial decay. In this case, you can estimate the half-life but the intersect with the y-axis will be $A_1 + A_2$ instead of A_1. Knowing A_2, you can obtain an estimate for A_1 simply by subtracting A_2 from this intercept.

Since the initial decay is so rapid it is difficult to estimate on the full scale. It is more convenient to use the expanded scale from 0 to 20 (for example) shown in the following figure. Estimating $A_1 + A_2$ and the half-life are shown in the following figure:



The intercept of 90 gives an estimate for $A_1 + A_2$. Knowing an estimate for A_2 is 8, an estimate for A_1 is 82. The estimate of 0.0036 for a_2 is obtained as described previously.

From the above, you can see that the half-life of the initial decay is about 1.3 minutes (slightly longer than the formal method because $A_2 e^{-a_2 t}$ has not been subtracted from the data.) An estimate for *a1* is thus ln(2)/1.3 or 0.53. The rate constants can be estimated:

$$
\begin{aligned}
k(2,2) &= k(1,2) = \frac{A_2 \cdot a_1 + A_1 \cdot a_2}{A_1 + A_2} = 0.050 \\
k(2,1) &= \frac{A_1 \cdot A_2 \cdot (a_1 - a_2)^2}{k(1,2) \cdot (A_1 + A_2)^2} = 0.45 \\
k(0,1) &= k(1,1) - k(2,1) = \frac{A_1 \cdot a_1 + A_2 \cdot a_2}{A_1 + A_2} - k(2,1) = 0.033
\end{aligned}
\tag{9}
$$

and

$$
vol = \frac{1504000}{90} = 16710 \tag{10}
$$

These estimates are a little different from the formal curve peeling method, but they are close enough to begin your modeling exercise.

Technical remark:

There are many different kinds of biexponentially decaying data. They range from data such as the hydromorphone whose initial decay is extremely rapid followed by a long, well-defined final decay, to data which display a very subtle biexponential nature. The subtle biexponential nature means it is difficult to be sure there is actually a second exponential in the data. At the extremes, one has to be aware that curve peeling, either formal or quick, may produce estimates for A_1, A_2, a_1 and a_2 that are very sensitive to how you draw your tangent lines. While you will produce estimates of these parameters, it could turn out that one of the *k(i,j)*, especially *k(0,1)*, may end up with a negative value. If this happens, you need to revisit your tangent lines, especially the one characterizing the initial decay.

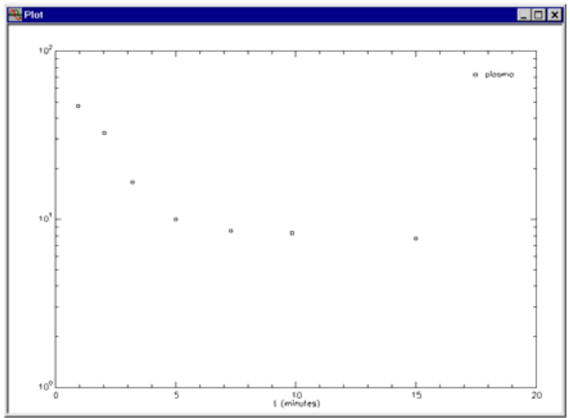
End of technical remark:

A Quick Method

There is a quick method that can be used that doesn't involve curve peeling. However, it does not always produce satisfactory estimates, and you may have to resort to one of the two curve peeling methods. It also requires some experience in using the method since in many cases, you will have to adjust some of the parameters before proceeding with your modeling exercise. But it is quick, and provides insight into which model parameters affect different parts of the model predicted curve.

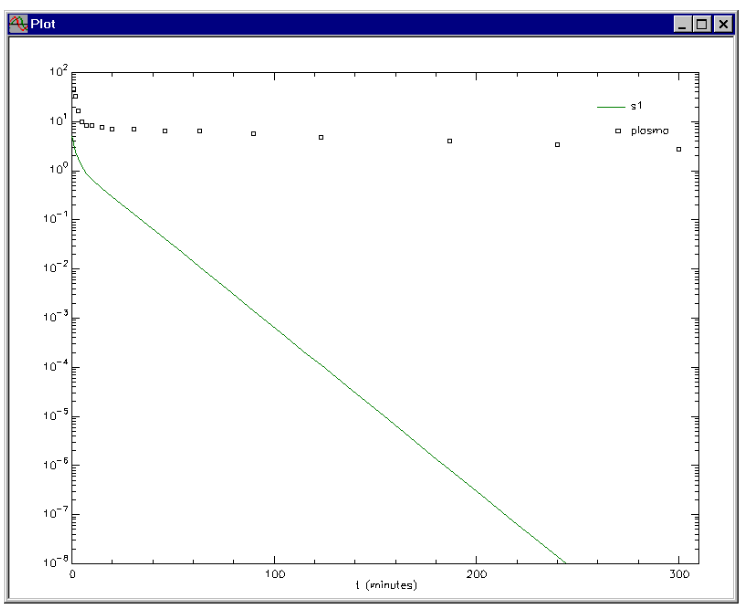
The idea is the following. You can look for the time at which the break in the curve (data) occurs, and use the inverse of that number as initial estimates for all the *k(i,j)* of the two-compartment Model 2.

If you look at the original data, reproduced in the following figure on an expanded scale, you will see the break in the curve occurs at about 5 minutes. The reciprocal is 0.2, so you can use this value as the estimate for the rate constants.

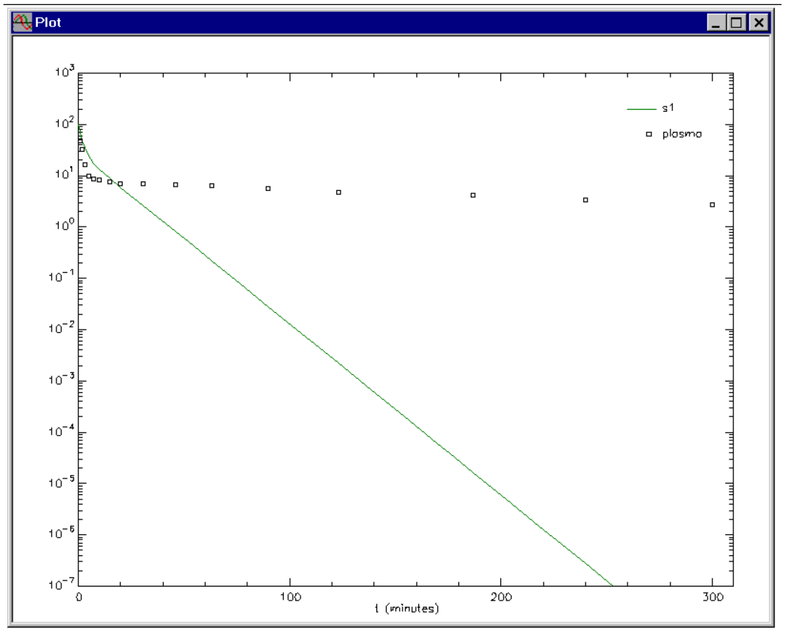


There are two different methods to estimate *vol*. One is the previous method where you can extrapolate the initial decay to time zero. The other is simply to use the first datum; this assumes that the first datum is representative of the zero-time extrapolation. For the latter, remembering the dose is 1504000ng and the first datum is 47ng/ml, an estimate for *vol* is 32,000 (you should note this is very different from the estimates above; this is because the initial decay is extremely rapid in this example meaning the estimate based on the first datum will be much less reliable than extrapolating to time zero on the y-axis.)

If you use these initial estimates and solve your model, you will obtain the solution shown in the following figure:



While this does not appear very good, the initial decay does have the characteristics of the initial decay of the data. The estimate for *vol*, which actually scales the model predictions, is off by a factor of about 2 (which you can tell by looking where the model predicted value intersects the y-axis, and where a line extrapolated through the data would intersect the y-axis.) Thus change the estimate of *vol* from 30000 to 15000, and re-solve. You will obtain the solution shown in the following figure:

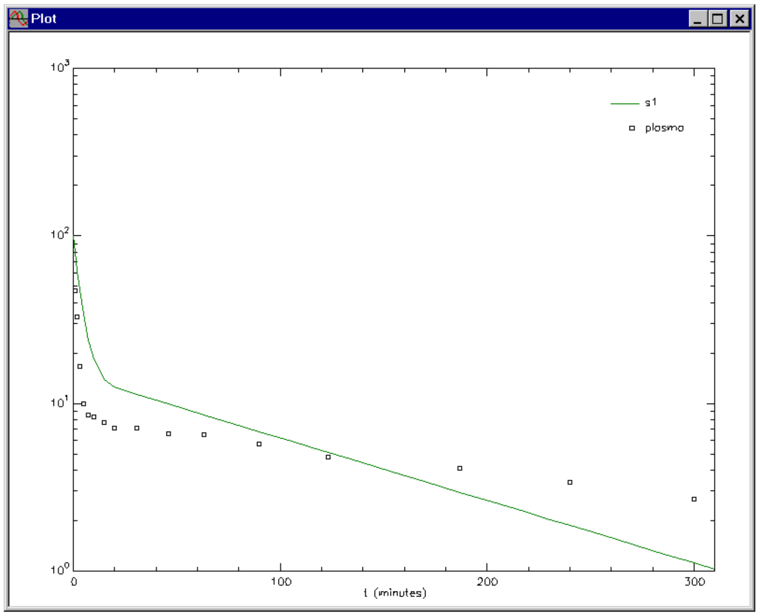


Obviously the shape of the curve has not been adjusted, but the scale is about right. What is happening now between the model predictions and the data is the following:

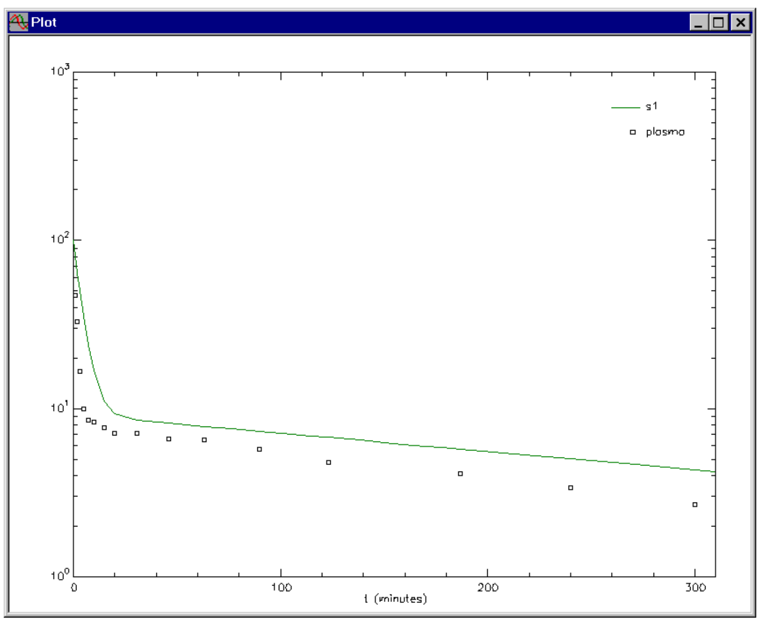
- The initial decay is not rapid enough. Since *k(0,1)* partially controls the final decay, this parameter cannot be increased. Thus *k(2,1)* needs to be increased.
- The final decay is far too rapid. Thus both *k(0,1)* and *k(2,1)* need to be decreased. Which decreases and by how much depends upon how much material remains in compartment 2. It is this compartment that provides the material for the tail portion of the curve.

As a rule of thumb, in adjusting the parameters by hand, it is a good idea to adjust one at a time rather than in groups. In situations such as the present where the estimates are not very accurate, it is best to change them by a factor of no more than 5. As a rule of thumb, as the estimates improve, it is best to change them by a factor of 2. This will allow you to watch the changes in the model solution, and make better estimates of the adjustments you need to make to the parameters.

The following figure shows is result of two simulations. In the first, *k(0,1)* was reduced to 0.05 (1/4 of the original estimate), and in the second, *k(1,2)* was changed to 0.05 (1/4 of the original estimate). You can see that there is a substantial improvement.

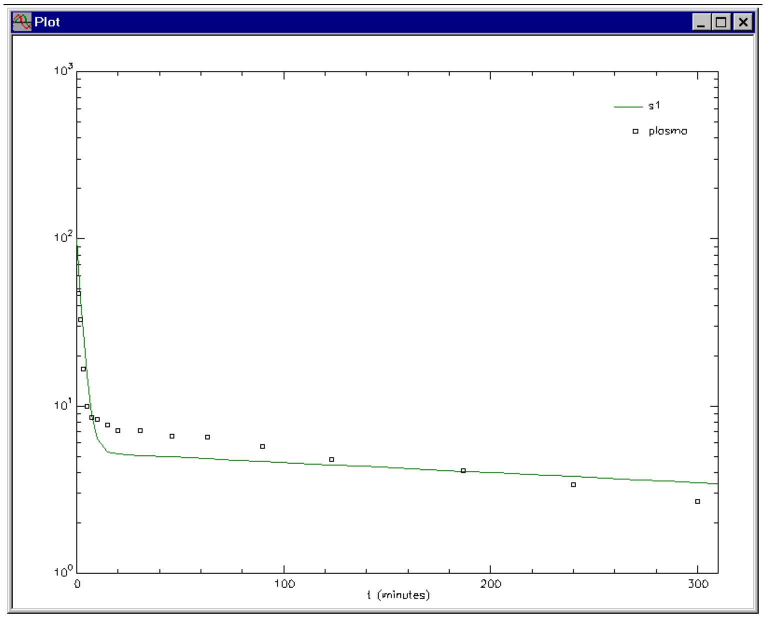


The following is the solution of the model obtained by reducing *k(0,1)* and *k(1,2)* to 0.025:



While not perfect, the shape of the bend and tail portion is quite reasonable. You might try nudging k(0,1) slightly larger, say to 0.03, to see what happens.

To deal with the initial decay, increase k(2,1) from 0.2 to 0.4. The solution you will obtain is shown in the following figure:



This is certainly good enough to proceed. So from the original quick method estimates for the parameters, with 6 simulations you have succeeded in obtaining not only reasonable estimates for the parameters, but a knowledge of which parameter affects which portion of the curve!

In summary, the steps to obtain the initial estimates for the two-compartment Model 2 following a bolus injection are

Formal curve peeling

- Plot the data on semi-log paper.
- Draw a straight line through the tail portion of the data; extend the line to intersect with the y-axis.
- Calculate the half-life $t_{1/2}$ as described above to obtain an estimate for a_2 in (1).
- Note where the line intersects the y-axis, this provides an estimate for A_2 in (1).
- Subtract $A_2 e^{-a_2 t}$ from each datum.
- Plot the modified data on semi-log paper.
- Draw a straight line through the initial decay extending is to the y-axis. Where it intersects the y-axis is an estimate for A_1.
- Calculate the half-life $t_{1/2}$ as described above to obtain an estimate for a_1 in (1).
- Estimate the model parameters from (3).

Quick curve peeling

- Plot the data on semi-log paper.
- Draw a straight line through the tail portion of the data; extend the line to intersect with the y-axis.
- Calculate the half-life $t_{1/2}$ as described above to obtain an estimate for a_2 in (1).

- Note where the line intersects the y-axis, this provides an estimate for A_2 in (1).
- Draw a straight line through the initial decay extending is to the y-axis. Where it intersects the y-axis is an estimate for A_1+A_2.
- Calculate the half-life $t_{1/2}$ as described above to obtain an estimate for a_1 in (1).
- Calculate A_1 by subtracting A_2 from A_1+A_2.
- Estimate the model parameters from (3).

<u>*Quick method*</u>

- Plot the data on semi-log paper.
- Estimate the time at which the break in the data appears (the move from the dominance of one exponential to the second.
- Calculate the reciprocal of this time.
- Use this number as estimates for the rate constants *k(2,1)*, *k(1,2)* and *k(0,1)*.
- Estimate the volume as the quotient of the bolus dose divided by the first datum or the initial tangent line decay value extrapolated to time zero.

To practice the method, you can try exercise 2 in Chapter 3 of [1].

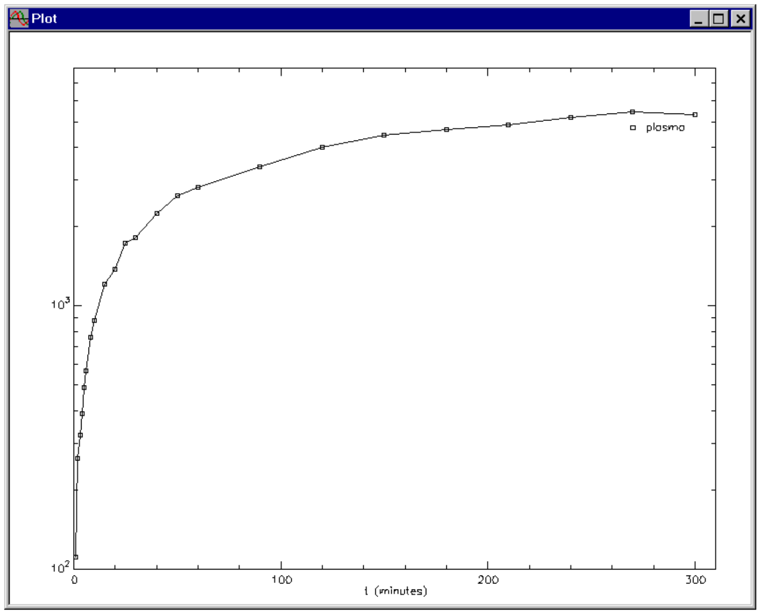
Part 2. Constant infusion into plasma

The case when the drug is administered as a constant infusion into a two-compartment model is not quite as straightforward as the bolus injection. First, the equation for a biexponential rise is

$$s_1(t) = A_0 + A_1 e^{-a_1 \cdot t} + A_2 e^{-a_2 \cdot t} \qquad A_0 + A_1 + A_2 = 0 \tag{11}$$

The counterpart of the curve peeling methods does not apply as described above without a preliminary step.

Consider the following data (plotted in semilog and connected using the **Line Plot** option in the **View** menu) which were obtained following a constant infusion of 400,000 units/minute for 310 minutes:



These data are rising biexponentially to a plateau value. What happens if you subtract the plateau value from the data? You will obtain:

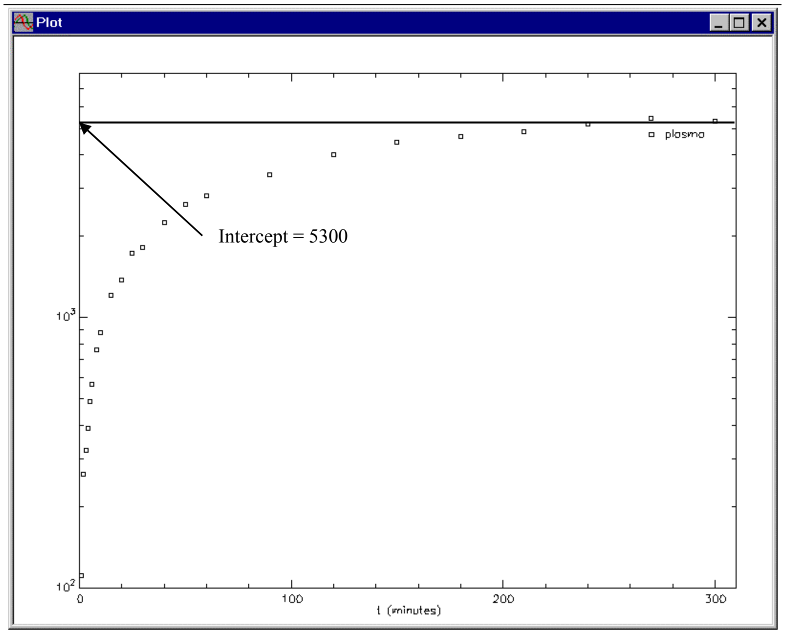
$$s_1^*(t) = A_0 + A_1 e^{-a_1 \cdot t} + A_2 e^{-a_2 \cdot t} - A_0 = A_1 e^{-a_1 \cdot t} + A_2 e^{-a_2 \cdot t} \tag{12}$$

since A_0 is the plateau value. However, since there is the constraint equation $A_0 + A_1 + A_2 = 0$, this will be negative. But

$$s_1^{\mathrm{mod}}(t) = -(A_1 e^{-a_1 \cdot t} + A_2 e^{-a_2 \cdot t}) \tag{13}$$

will decay biexponentially with a y-axis intercept of $-(A_1 + A_2)$. This situation will now exactly parallel that of the biexponential decay!

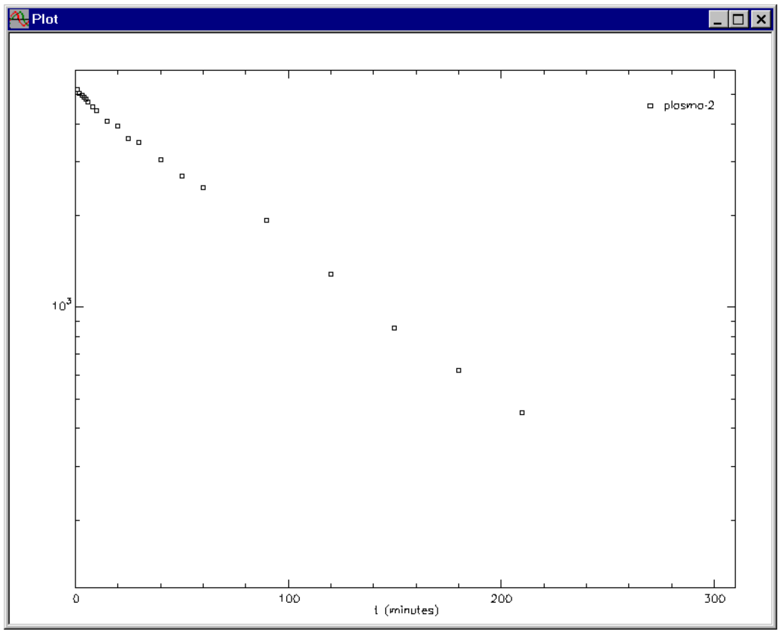
Using the data in the previous figure, the following figure illustrates how the plateau concentration is estimated:



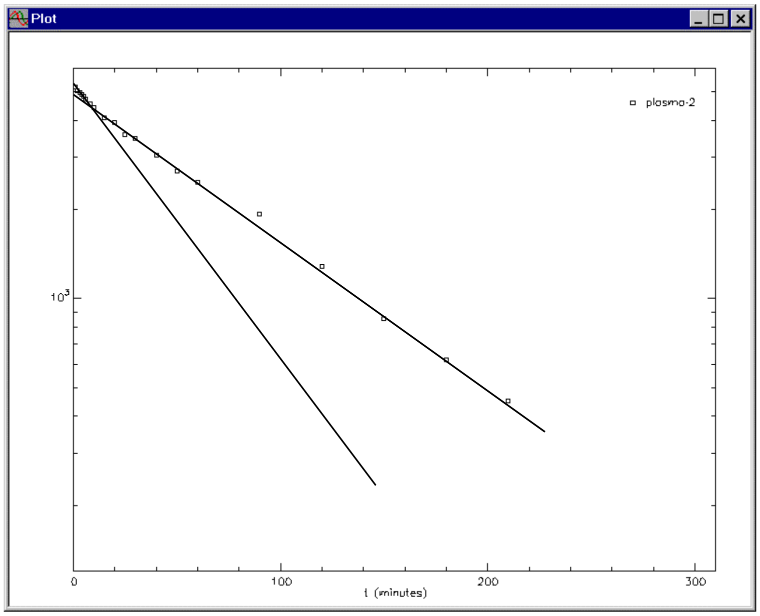
For this set of data, a plateau value of 5300 is estimated. Subtracting this value from each datum, and multiplying the result by -1 gives the following set of modified data:

t	plasma	-(plasma-5300)
1	111	5189
2	264	5036
3	324	4976
4	391	4909
5	490	4810
6	569	4731
8	763	4537
10	879	4421
15	1210	4090
20	1377	3923
25	1728	3572
30	1815	3485
40	2238	3062
50	2613	2687
60	2829	2471
90	3366	1934
120	4015	1285
150	4446	854
180	4678	622
210	4850	450
240	5214	86
270	5463	-163
300	5319	-19

If you plot the modified data, you will obtain the following figure:



It is interesting in that these data might appear to decay monoexponentially. This is an example of the more subtle biexponential decay discussed in the technical remark earlier in this appendix. In fact if you draw the tangent lines for the quick curve peeling method, you will see the biexponential as follows:



With these modified data, you can proceed to use either the formal or quick curve peeling methods to estimate the A_1, A_2, a_1 and a_2, and hence the rate constants of the model.

For the volume *vol*, an estimate is obtained using the equation for the clearance rate *CR* as follows:

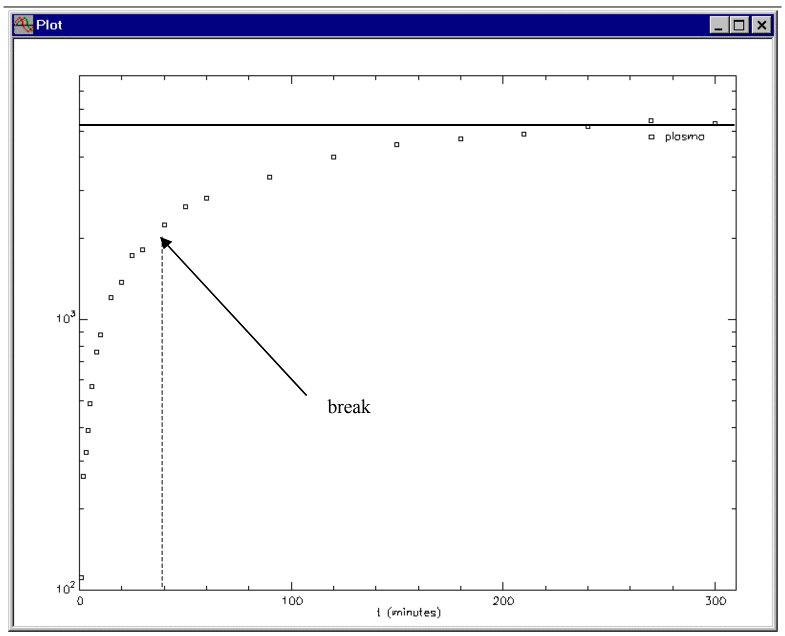
$$CR = \frac{\text{infusion rate}}{\text{plateau}} = \frac{400000}{5300} = 75.5 \qquad (14)$$

This is the same equation used in the monoexponential rise. With your estimate for *k(0,1)*,

$$vol = \frac{CR}{k(0,1)} \qquad (15)$$

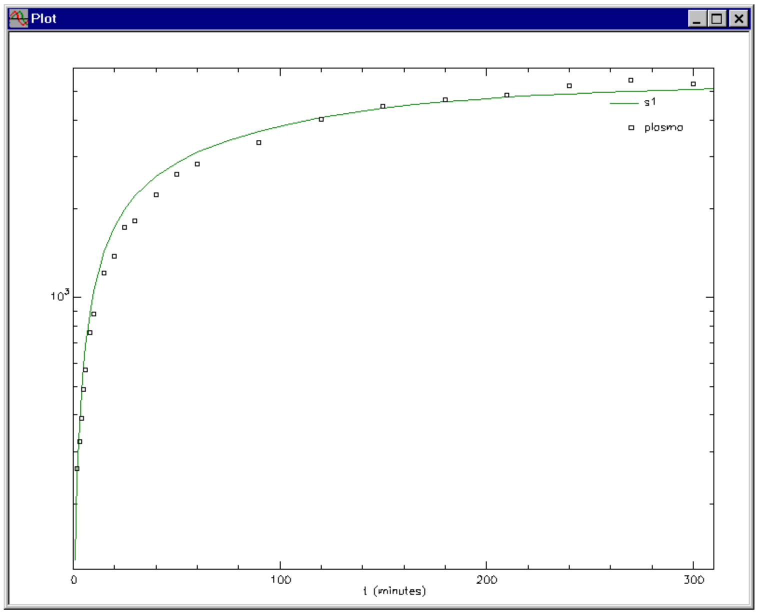
<u>A Quick Method</u>

Is there a counterpart to the quick method? The answer is yes, and it works as follows. You can estimate where the break in the rising data is (this is somewhat arbitrary); this is shown in the following figure:



As indicated in the figure, the break occurs at about 40 minutes. Estimates for the rate constants can be obtained from the inverse of 40 which is 0.025. An estimate for the volume can be obtained using the equation for clearance rate (14) as described above. Using 0.025 as an estimate for *k(0,1)* in (15), an estimate of 3020 for *vol* would be obtained.

The following figure shows the simulation with *k(0,1)=k(2,1)=k(1,2)=0.025* and *vol* equal 3020:



Clearly these estimates are quite reasonable, and you can proceed with your modeling exercise.

In summary, the steps to obtain the initial estimates for the two-compartment Model 2 following a constant infusion are:

Formal curve peeling or quick curve peeling

- Plot the data on semi-log paper.
- Draw a horizontal straight line to estimate the plateau value; extend the line to the y-axis to obtain an estimate for A_0.
- Subtract A_0 from each datum, and multiply the result by -1.
- Plot the modified data on semi-log paper, and proceed as above with either the formal or quick curve peeling method.
- Plot the modified data on semi-log paper.

Quick method

- Plot the data on semi-log paper.
- Draw a line to estimate the plateau (this will help in evaluating where the break occurs.)
- Estimate the time at which the break in the data appears.
- Calculate the reciprocal of this time.
- Use this number as estimates for the rate constants *k(2,1)*, *k(1,2)* and *k(0,1)*.
- Estimate the volume as the quotient of the clearance rate and *k(0,1)*.

Part 3. Short infusion into plasma:

In many cases, an intravenous drug dose is not given as a bolus but a short (5 to 60 minute) infusion. This is used to increase safety by lowering the peak plasma concentrations to which the subject is exposed. In this case, plasma concentrations after the infusion is stopped may exhibit a multi-exponential form. The exponents a_1 and a_2 can be estimated by either curve peeling method as described in Part 1 for the bolus injection data. A vertical line parallel to the y-axis then can be drawn at the end-infusion time and uncorrected intercept estimates made. If the end infusion time is (τ), equations developed by Loo and Riegelman [3] can then be used to "correct" these values so that they are the same that would have been obtained had the drug dose been administered as a bolus injection:

$$
\begin{aligned}
A_1 &= \frac{a_1 \tau}{1 - e^{-a_1 \tau}} A_1' \\
A_2 &= \frac{a_2 \tau}{1 - e^{-a_2 \tau}} A_2'
\end{aligned}
\qquad (16)
$$

where A'_1 and A'_2 are the uncorrected intercepts measured at the end of infusion (infusion time = τ).

References:

1. Atkinson, A. J. Jr., Daniels, C.E., Dedrick, R.L., Grundzinskas, C.V., and Markey, S.P. *Principles of Clinical Pharmacology.* Eds. Academic Press. New York, NY, 2001.

2. Cobelli, C., Foster, D., and Toffolo, G. *Tracer Kinetics in Biomedical Research.* Kluwer Academic/Plenum Press, New York, NY, 2000.

3. Loo, J.C.K., and Riegelman, S. *Assessment of Pharmacokinetic Constants from Postinfusion Blood Curves Obtained after I.V. Infusion. J. Pharm. Sci. 1970; 59: 53-55.*

Appendix 2: Contents of the Notes window for this Case Study

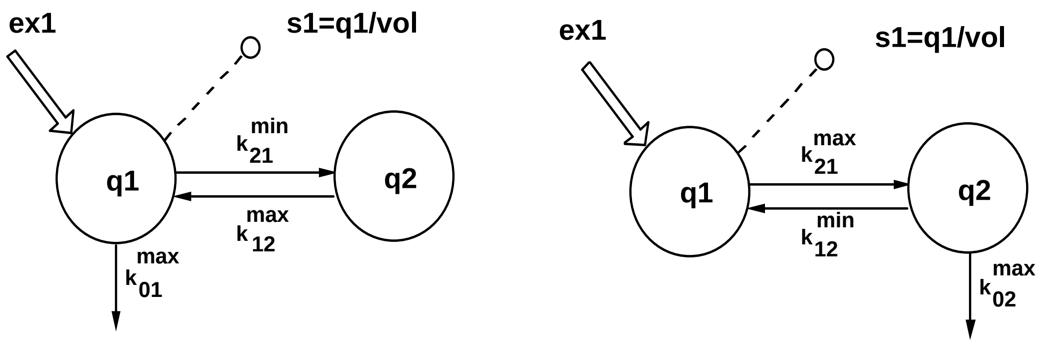
```
Fit of two-compartment model to data with loss from compartment 1
                       value   SD             Coeff Var         95% confidence
k(0,1)               0.03741  3.27487e-003  8.75485e+000      0.03027       0.04454
k(1,2)               0.06412  3.26046e-003  5.08528e+000      0.05701       0.07122
k(2,1)               0.56866  3.87725e-002  6.81826e+000      0.48418       0.65313
vol              17322.32017  1.38671e+003  8.00535e+000  14300.93684   20343.70350
--------------------------------- Derived Variables ---------------------------------
A                   78.89899  6.91409e+000  8.76322e+000     63.83449      93.96349
AUC               2321.11586  8.10710e+001  3.49276e+000   2144.47749    2497.75423
AUMC            612400.58681  5.17368e+004  8.44819e+000 499676.01898  725125.15465
B                    7.92541  1.96436e-001  2.47856e+000      7.49741       8.35340
Cl                 647.96421  2.26318e+001  3.49276e+000    598.65376     697.27465
Cmax                86.82440  6.95060e+000  8.00536e+000     71.68035     101.96844
MRT_Cpt1            26.73345  2.34047e+000  8.75485e+000     21.63400      31.83290
Syst_MRT           263.83887  1.36718e+001  5.18189e+000    234.05055     293.62719
Vss             170958.14482  4.43532e+003  2.59439e+000 161294.42408  180621.86557
alpha                0.66658  4.15640e-002  6.23540e+000      0.57602       0.75714
beta                 0.00360  1.81290e-004  5.03870e+000      0.00320       0.00399
half_time           18.53022  1.62229e+000  8.75485e+000     14.99555      22.06489
r                    0.66298  4.15183e-002  6.26235e+000      0.57252       0.75344

                          Objective      Scaled Data Variance
s1 : plasma            -8.332549e-001       3.291685e-001
                       --------------
Total objective        -8.332549e-001

AIC                     8.148111e-001
BIC                     9.355281e-001


SAAM II AUC calculations

Sample       AUC         Std.Dev.
------  ------------  ------------
s1       2.3211e+003   8.1062e+001
s2       3.5661e+008   1.8993e+007
s3       6.1211e+005   5.1697e+004


Best fit of model with k(0,2) = 0

                       value   SD             Coeff Var         95% confidence
k(0,1)               0.03739  3.27285e-003  8.75375e+000      0.03026       0.04452
k(0,2)          0.00000e+000   ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(1,2)               0.06411  3.26014e-003  5.08492e+000      0.05701       0.07122
k(2,1)               0.56844  3.87544e-002  6.81773e+000      0.48400       0.65287
vol              17327.35156  1.38688e+003  8.00399e+000  14305.60304   20349.10008

                          Objective      Scaled Data Variance
s1 : plasma            -8.332469e-001       3.291711e-001
                       --------------
Total objective        -8.332469e-001

AIC                     8.148151e-001
BIC                     9.355321e-001


Best fit of model with k(0,1) = 0

k(0,1)          0.00000e+000   ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(0,2)               0.00395  2.04185e-004  5.17069e+000      0.00350       0.00439
k(1,2)               0.06013  3.15055e-003  5.23959e+000      0.05327       0.06699
k(2,1)               0.60603  4.14530e-002  6.84004e+000      0.51572       0.69635
vol              17321.46748  1.38653e+003  8.00467e+000  14300.49046   20342.44450

                          Objective      Scaled Data Variance
s1 : plasma            -8.330776e-001       3.292268e-001
                       --------------
```

```
Total objective       -8.330776e-001

AIC                    8.148997e-001
BIC                    9.356167e-001


Best fit of the model to the data with k(0,1) = 3*k(0,2)

a                    3.00000   ** Fixed **   ** Fixed **   ** Fixed **   ** Fixed **
k(0,2)               0.00304  1.54312e-004  5.07958e+000       0.00270       0.00337
k(1,2)               0.06107  3.21343e-003  5.26191e+000       0.05407       0.06807
k(2,1)               0.59699  4.12733e-002  6.91359e+000       0.50706       0.68691
vol              17320.81535  1.38655e+003  8.00514e+000   14299.77569   20341.85501
--------------------------------- Derived Variables ---------------------------------
k(0,1)               0.00911  4.62935e-004  5.07958e+000       0.00810       0.01012


                        Objective      Scaled Data Variance
s1 : plasma           -8.332526e-001       3.291692e-001
                      --------------
Total objective       -8.332526e-001

AIC                    8.148122e-001
BIC                    9.355292e-001


Best fit of the model to the data with k(0,1) = a*k(0,2)where a=3 is Bayesian with SD =
1.

a                    3.00000  1.19024e+000  3.96746e+001       0.40670       5.59330
k(0,2)               0.00304  3.24475e-004  1.06849e+001       0.00233       0.00374
k(1,2)               0.06107  3.32405e-003  5.44303e+000       0.05383       0.06831
k(2,1)               0.59722  4.26458e-002  7.14077e+000       0.50430       0.69013
vol              17313.16043  1.42861e+003  8.25156e+000   14200.49962   20425.82125
--------------------------------- Derived Variables ---------------------------------
k(0,1)               0.00911  2.80678e-003  3.08088e+001       0.00299       0.01523

                        Objective      Scaled Data Variance
s1 : plasma           -7.842019e-001       3.497556e-001

Bayesian               1.447731e-013
                      --------------
Total objective       -7.842019e-001

AIC                    8.797788e-001
BIC                    1.026816e+000
```

Appendix 3: Interval Identifiability of the Two-Compartment Model

The full two-compartment model, i.e. the model with four rate constants *k(i,j)* and the volume parameter *vol*, used in the last part of this case study, while not uniquely identifiable, is an example of an interval identifiable model [1]. It is interval identifiable because you can determine the interval in which the parameters must lie in order to obtain a best fit of the model to the data. Sometimes this information is of value in kinetic studies.

The bounds for the parameters are summarized in the following figure. When *k(0,2)* is zero (its low limit), the maximum values for *k(0,1)* and *k(1,2)*, and the minimum value for *k(2,1)* are obtained. When *k(0,1)* is zero (its low limit), the maximum values for *k(0,2)* and *k(2,1)*, and the minimum value for *k1,2)* are obtained.



The bounds for the rate constants *k(i,j)* are therefore

$$0 \le k(0,1) \le k(0,1)^{max}$$
$$0 \le k(0,2) \le k(0,2)^{max}$$
$$k(2,1)^{min} \le k(2,1) \le k(2,1)^{max}$$
$$k(1,2)^{min} \le k(1,2) \le k(1,2)^{max}$$

With your knowledge of the permissible intervals, you can now revisit this study and this time fix one of the other rate constants. If your fixed value is within the interval, you will obtain a “fit;” if your fixed value is outside the interval, you will not be able to obtain a “fit.”

1. Cobelli, C., Foster, D., and Toffolo, G. *Tracer Kinetics in Biomedical Research*. Kluwer Academic/Plenum Publishers. New York, NY 2000

Data for this case study

The data file for this case study includes the formulas for the noncompartmental parameters.

```
DATA
# Data following a bolus injection of 1504 mcg of hydromorphone.
# Units are ng/ml
#
(FSD 0.1)
t       plasma
0.00    n
0.95    47.0
2.03    32.8
3.20    16.6
5.00    10.0
7.30    8.5
9.83    8.3
15.00   7.7
20.05   7.1
30.82   7.1
45.90   6.6
63.25   6.5
90.00   5.7
123.23 4.8
187.00 4.1
240.00 3.4
300.00 2.7
END
# The following is the dose in ng
CONST       dose    1504000
#
# The following are the noncompartmental parameter equations
#Cmax=dose/vol
#half_time=log(2)/k(0,1)
#r=sqrt((k(2,1)+k(1,2)+k(0,1))^2-(4*k(1,2)*k(0,1)))
#alpha=(k(2,1)+k(1,2)+k(0,1)+r)/2
#beta=(k(2,1)+k(1,2)+k(0,1)-r)/2
#A=(dose/vol)*((alpha-k(1,2))/(alpha-beta))
#B=-(dose/vol)*((beta-k(1,2))/(alpha-beta))
#AUC=dose/(vol*k(0,1))
#AUMC=(A/alpha^2)+(B/beta^2)
#Syst_MRT=AUMC/AUC
#MRT_Cpt1=1/k(0,1)
#Cl=dose/AUC
#Vss=Cl*Syst_M
```

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