

# Theophylline Kinetics Following a Short Intravenous Infusion

## Case Study

- How to create a two-compartment model
- How to obtain initial parameter estimates
- How to evaluate the “Fit”
- How to add a third compartment
- How to compare the results of two different models
- How to write some noncompartmental parameters

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## **Theophylline Kinetics Following a Short Intravenous Infusion**

### **Prerequisites**

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

### **What you will learn in this case study**

- How to create a two-compartment model
- How to obtain initial parameter estimates
- How to evaluate the "Fit"
- How to add a third compartment
- How to compare the results of two different models
- How to write some noncompartmental parameters.

### **Data Required**

The data file for this case study is

**theo.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 short infusion 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.

Since the 1930's theophylline has been used as a bronchodilator to treat patients with bronchial asthma. Although oral doses are used in ambulatory patients, this drug is often administered intravenously as aminophylline to patients with acute attacks of severe asthma. Potentially lethal central nervous system toxicity and cardiac arrhythmias have occurred when this drug is given by rapid intravenous injection, so current recommendations for intravenous are to infuse a 6 mg/kg dose of aminophylline (equivalent to 4.8 mg/kg theophylline) over 20 minutes.

In terms of pharmacokinetic analysis, the result of administering theophylline to humans by intravenous infusion is that the drug exhibits a two-compartment pattern of

distribution in which the central compartment approximates expected values for extracellular fluid space. However, when anesthetized dogs were given bolus injections of this drug, a three-compartmental model is needed for pharmacokinetic analysis<sup>1</sup>. The central compartment of this model is blood volume and the intercompartmental clearances for the peripheral compartments equal the blood flow to these compartments. It is likely that the exceptionally rapid distribution of theophylline to the brain and heart account for the toxicity that has been encountered when this drug has been injected intravenously at too rapid a rate.

Because bronchial asthma affects young women during their child-bearing years, a serial pharmacokinetic studies were conducted in a group of pregnant asthmatic women to establish what dose modifications might be appropriate for these patients<sup>2</sup>. The data for this exercise were taken from a study that was conducted in one of the women between the 36<sup>th</sup> and the 38<sup>th</sup> week of her pregnancy. Subtherapeutic doses were administered. Even though only a 5-minute infusion time was used, the data were only adequate to characterize a two-compartment pharmacokinetic model.


1. Belknap SM, Nelson JE, Ruo TI, Frederiksen MC, Worwag EM, Shin S-G, Atkinson AJ Jr: Theophylline distribution kinetics analyzed by reference to simultaneously injected urea and inulin. *J Pharmacol Exp Ther* 1987;243:963-9.
2. Frederiksen MC, Ruo TI, Chow MJ, Atkinson AJ Jr. Theophylline pharmacokinetics in pregnancy. *Clin Pharmacol Ther* 1986;40:321-8.

### **Part 1. Develop a Two Compartment Model for Theophylline Kinetics.**

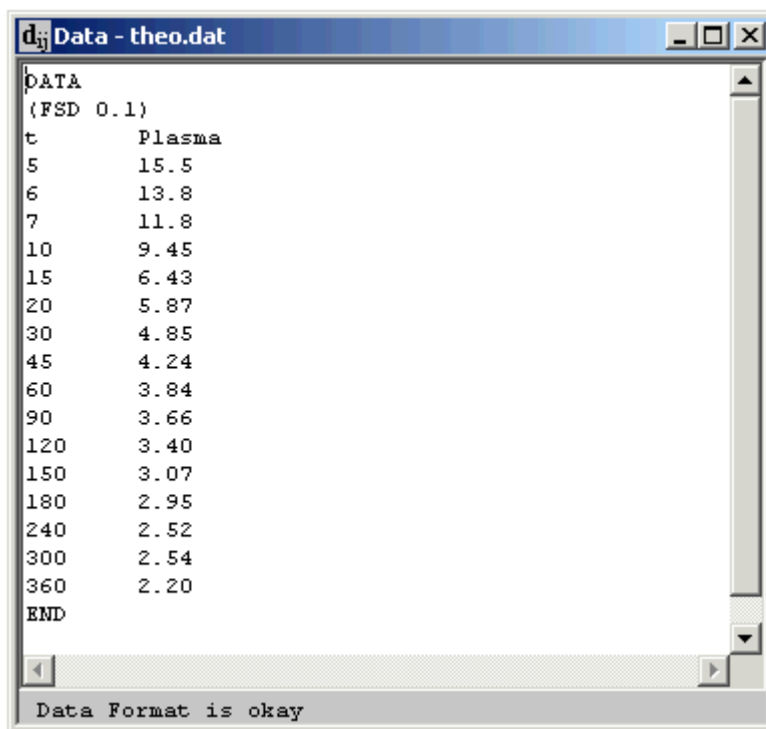
The first step will be to investigate the data and postulate a two compartment model to describe the data following iv as a short iv infusion.

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 the data following a bolus or short infusion, 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 biphasically, 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 “**theo.dat**” should appear in the list (if it does not, find the folder where you have put this data file).
- c. Double-click **theo.dat**. The data file contains the plasma theophylline data following the 5 minute constant infusion. The plasma data appear in the column called “Plasma”. The **Data** window will appear as follows:



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

- d. Close the **Data** window.
2. View the 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 “360” in the **End At** box. The **Experiment Attributes** dialog box will appear:

(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 **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 the data, SAAM II must know when the experiment ends. For this reason, the **Experiment Attributes** dialog box opens at this point in the case study.

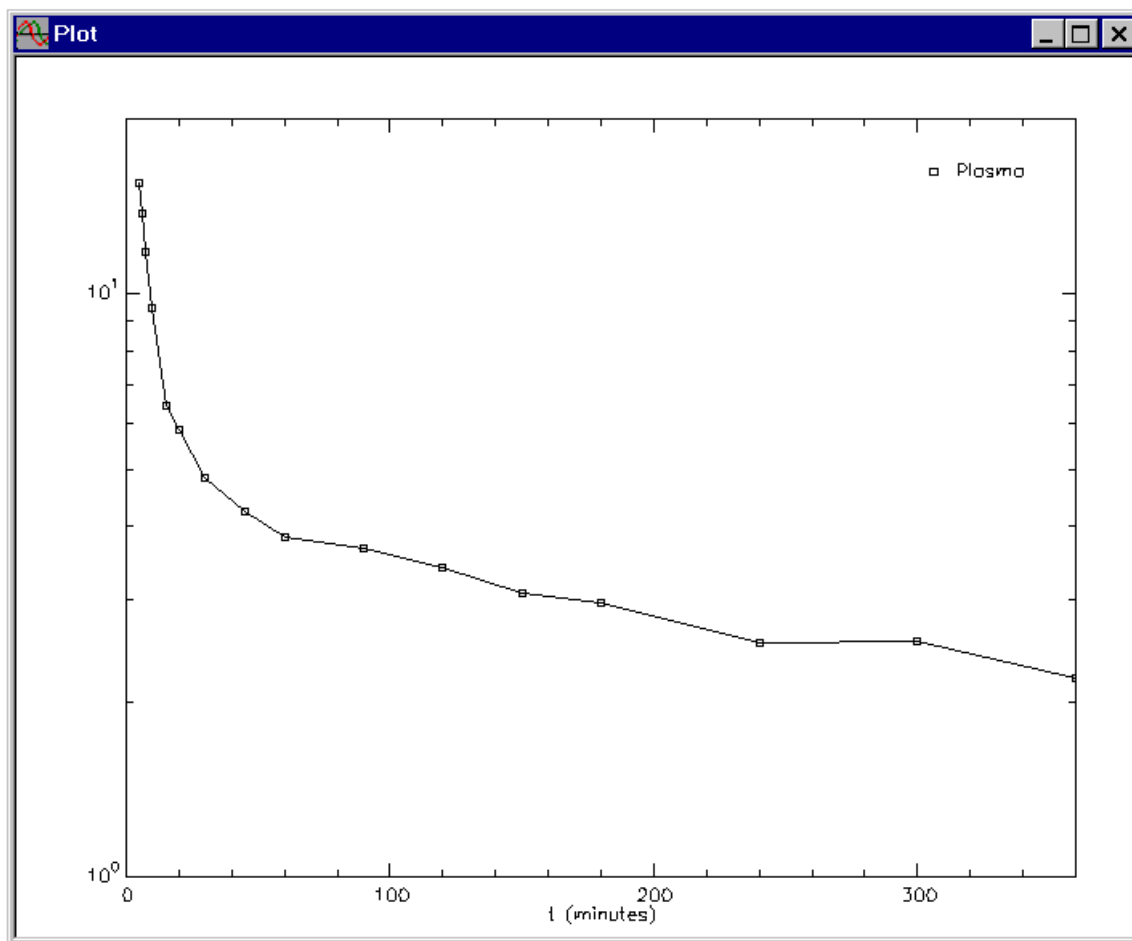


- b. The **List All Variables** check box is selected. The reason the box is selected is because there are no samples specified yet.
- c. Click **Plasma** to move it to the **Current Selection** pane.
- d. Click **Done**. A plot of the plasma data will appear in the **Plot** window.
- e. In the **View** 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, click **Set**. Enter “1” in the **Minimum** box, and “20” in the **Maximum** box. The **Plot and Table Scale** dialog box will appear as follows:

**Plot and Table Scale**

	Minimum	Maximum
X Axis		
<input type="radio"/> AutoScale	0.0	360.00000000
<input checked="" type="radio"/> Set	0.0	360.00000000
Y Axis		
<input type="radio"/> AutoScale	2.20000000	15.50000000
<input checked="" type="radio"/> Set	1.00000000	20.00000000

i. Click **Done**. The line plot of the data will appear as follows:





*Line Plots.* Using the line plot in semilog mode to connect the 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.

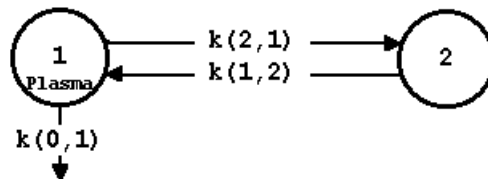
It is also important to note that the first datum, taken at 5 minute, occurs when the infusion has just ended. It is possible that mixing may not be complete.

The break in the curve appears around 40 minutes, so as described in Appendix 1, initial estimates for the rate constants of the two compartment model will be 0.025. An initial estimate for the volume of compartment 1 can be obtained by dividing the total dose by the 5 minute datum. This is  $200/15.5$  which is approximately 13.

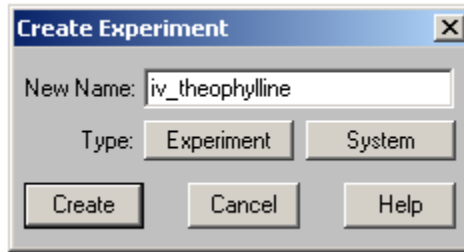
More information on obtaining initial parameter estimates for the two compartment model is provided in the Appendix 1.



- j. Close the **Plot** window.
3. Create the following system model on the **Drawing Canvas**:



4. In the **SAAM II Toolbox**, click **Experiment**. Notice that the **Model** tools are unavailable and the **Experiment** tools are available. Notice also the **Experiment Attributes** dialog box does not open. This is because the experimental attributes have already been specified. However, the **Create Experiment** dialog box will open.
  - a. Be sure the **Experiment** is selected. Type “iv\_theophylline” in the **New Name** box. The **Create Experiment** dialog box will appear as follows:



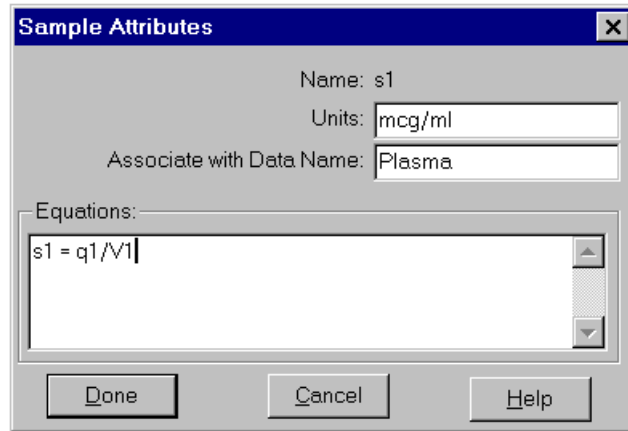
- b. Click **Create**.
5. Create a sample.
- a. In the **SAAM II Toolbox**, click **Sample**.
  - b. Click compartment **q1**, then click on the **Drawing Canvas**. The sample **s1** will appear.
  - c. Double-click **s1** to open the **Sample Attributes** dialog box.
  - d. Type “mcg/ml” in the **Units** box.



*Units.* The units of the data are micrograms/milliliter. Normally this would be written  $\mu\text{g/ml}$  or  $\mu\text{g/mL}$ . SAAM II does not accept  $\mu$  for micro. Writing the units  $\text{mg/ml}$  would be normally interpreted as micrograms/ml. Thus writing the units  $\text{mcg/ml}$  is a convenient way to denote the microgram.



- e. Type “Plasma” in the **Associate with Data Name** box.
- f. Edit the sample equation “ $s1 = q1$ ” to read “ $s1 = q1/V1$ ”. The **Sample Attributes** dialog box will appear:



Sample Attributes

Name: s1

Units: mcg/ml

Associate with Data Name: Plasma

Equations:

s1 = q1/V1

Done Cancel Help

- g. Click **Done**.
6. Create an input.
- An intravenous dose of 200 mg of theophylline was infused over 5 minutes.
- 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. Select **Infusion** as the **Input Type**.
  - e. Enter “40” in the **Constant Rate** box.
  - f. Enter “0” in the **Event Start** box.
  - g. Enter “5” in the **Event Stop** box.
  - h. Click **Add**. The **Exogenous Input** dialog box will appear:

**Exogenous Input**

Name:  Reference:  Units:

Type	Initial	Constant	Start	Stop	Repeat Every	Nr. Repeats
Infusion	-	40.000	0.000	5.000	-	-

Input Type:

Bolus  
 Infusion  
 Primed Infusion  
 Equation

Initial Amount:

Constant Rate:


Event Start:

Event Stop:

Repeat Every:

Nr. of Repeats:

Equation:

- i. Click **Done**.
7. Enter parameter values.
    - a. In the **Show** menu, click **Parameters**, or alternatively, on the **SAAM II Toolbar**, click **Parameters** . The **Parameters** dialog box will open.
 

The parameter *V<sub>1</sub>* should be selected. If it is not selected, double-click *V<sub>1</sub>*. Be sure the **Adjustable** option is selected.
    - b. Enter “13” in the **Value** box, “5” in the **Low Limit** box, “30” in the **High Limit** box, and click **Save**.
    - c. Double-click *k(0,1)* to select it.
    - d. Enter “0.025” in the **Value** box, and click **Save**.
    - e. Double-click *k(2,1)* to select it.
    - f. Enter “0.025” in the **Value** box, and click **Save**.
    - g. Double-click *k(1,2)* to select it.
    - h. Enter “0.025” in the **Value** box, and click **Save**.

When you have finished, the **Parameters** dialog box should appear as follows:

Name	Type	Current	Low Limit	High Limit
V1	Adj	13.0000	5.0000	30.0000
k(0,1)	Adj	0.0250	0.0025	0.2500
k(1,2)	Adj	0.0250	0.0025	0.2500
k(2,1)	Adj	0.0250	0.0025	0.2500

Name: k(0,1) Value: .025

Type:  Fixed  Adjustable

Low Limit: 0.00250000 High Limit: 0.25000000

Buttons: Done, Cancel, Help, Edit, Save

- i. Click **Done**.



*Initial parameter estimates.* The initial parameter estimates were obtained as described in the comments following the line plot shown previously.



8. Solve the model and view the solution.
  - a. In the **View** menu, select **Model Labels**, and click **Values**.

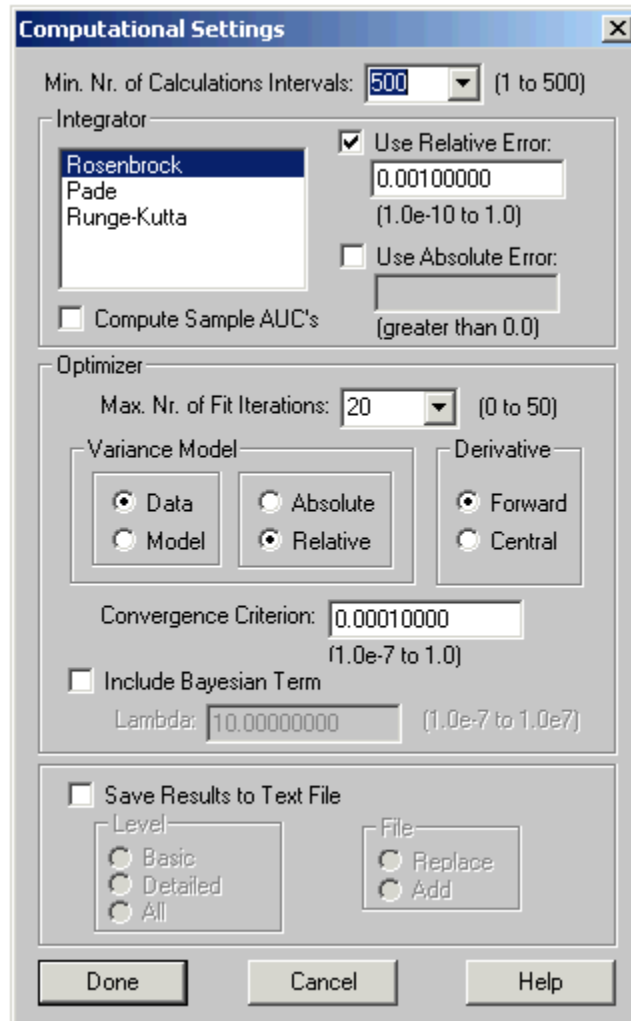


*Label "Values".* In the model, the default name for the transfers is  $k(i,j)$ . You have the option of displaying the numerical values of the parameters. This can help you keep track of the modeling exercise.

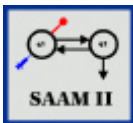


- b. Set the **Minimum Number of Calculation Intervals** equal to 500.

- (1) In the **Compute** menu, click **Settings**. The **Computational Settings** dialog box will open.
- (2) Enter “500” in the **Minimum Number of Calculation Intervals** box. The **Computational Setting** dialog box will appear as follows:




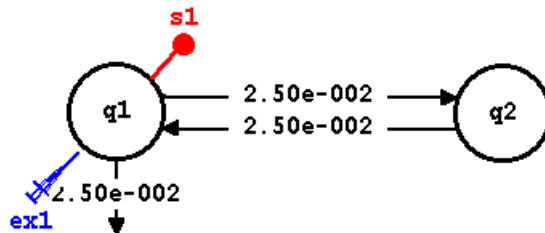
- (3) Click **Done**.




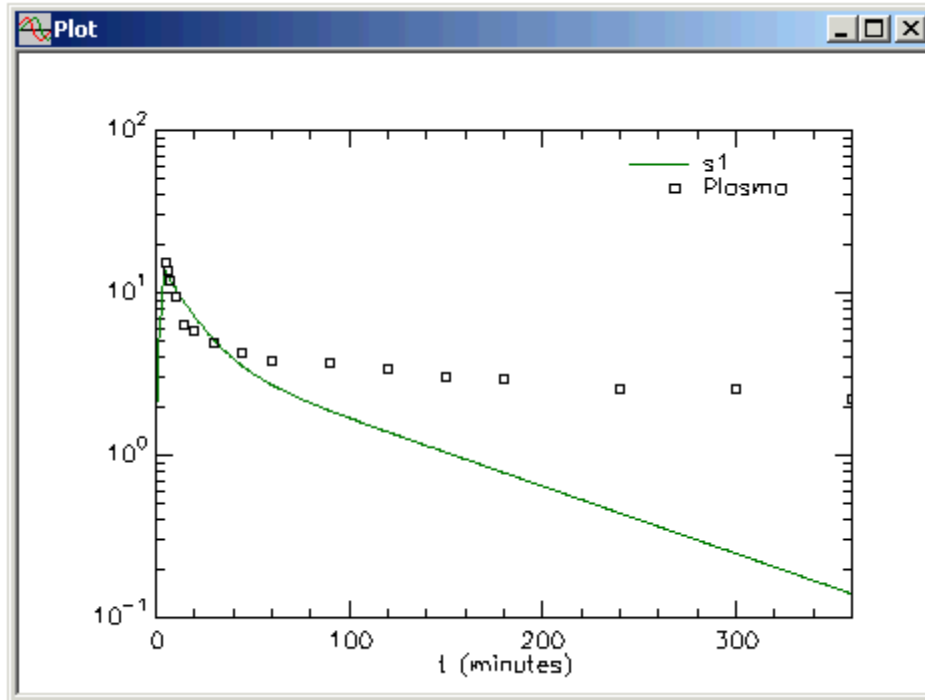
*Minimum Number of Calculation Intervals.* In order to see the details of the model solutions, especially the initial rise during the 5 minute infusion, you must set the **Maximum Number of Calculation Intervals** to a large number such as the maximal 500.



- c. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II Toolbar**, click **Solve** . The model will appear as follows with the parameter names replaced by their numerical values:



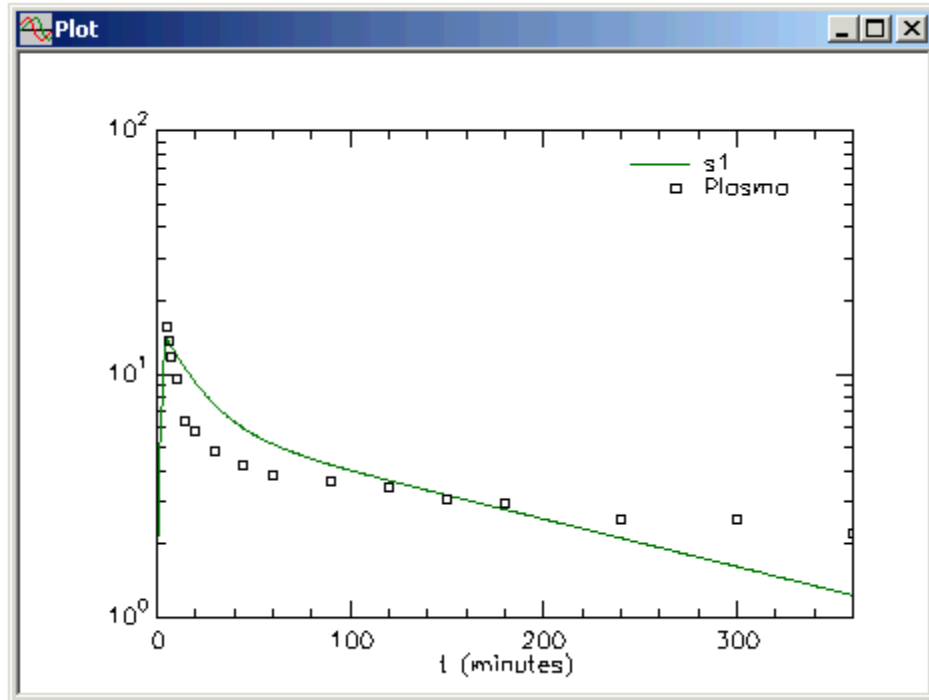
- d. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar** click **Plot** . The **Plot and Table Variables** dialog box will open.
- e. In the **Plot and Tables** dialog box, clear the **List All Variables** check box to list only those variables associated with data.
- f. Click **s1:Plasma**; these will move to the **Current Selection** pane.
- g. Click **Done**. The plot will appear as follows:



Notice in the plot that the autoscale option is active in the **Plot and Table Scale** dialog box. Remember you set the Y axis scale when you did the line plot. The reason why autoscale is now active is that the model calculated values fall below 2, the minimum value set previously for the line plot.


Notice also the details of the rising portion of the curve; if the **Minimum Number of Calculation Intervals** was set at the default value of 20, you would not see this rise.

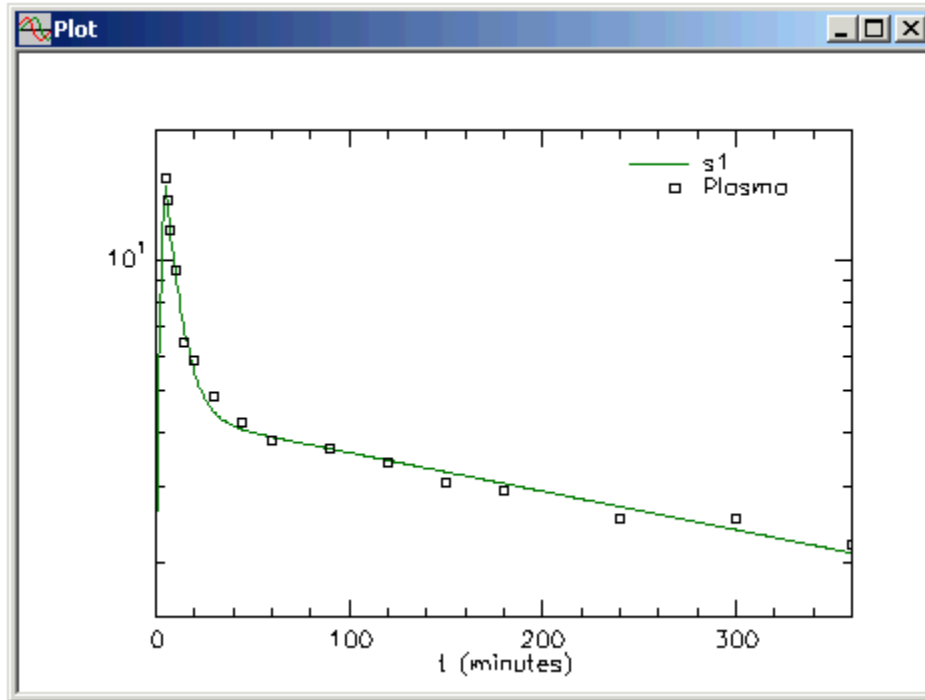
The initial estimates are reasonable, but the value for  $k(0,1)$  is obviously too large. If you wish, you can try hand-fitting to improve the estimates. For example, if you set  $k(0,1)$  equal to 0.01, the plot will change as shown below:




You may continue to hand-fit if you wish. However, these estimates are close enough so that you can fit the model to the data.

Leave the **Plot** window open.

9. Fit the model to the data and view the solution.
  - a. In the **Compute** menu, click **Fit**, or alternatively, on the **SAAM II Toolbar**, click **Fit** . (You may receive a warning about a parameter value reaching a limit - if you do, change the appropriate limit, and Re-Fit the model to the data). When you have "Fitted" the model to the data, the plot should appear as follow (in this plot, the **Plot and Table Scale** has been adjusted - the Y Axis minimum and maximum are respectively 1.5 and 20):



Leave the **Plot** window open.

- b. In the **Show** menu, click **Statistics**, or alternatively, on the **SAAM II** **Toolbar**, click **Statistics** . The **Statistics** window should appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
V1	10.52577	5.68639e-001	5.40235e+000	9.28681	11.76472
k(0,1)	0.00848	7.20158e-004	8.49540e+000	0.00691	0.01005
k(1,2)	0.03394	2.68420e-003	7.90867e+000	0.02809	0.03979
k(2,1)	0.09968	1.01351e-002	1.01679e+001	0.07759	0.12176

Correlation Matrix   
 Covariance Matrix   
 Objective

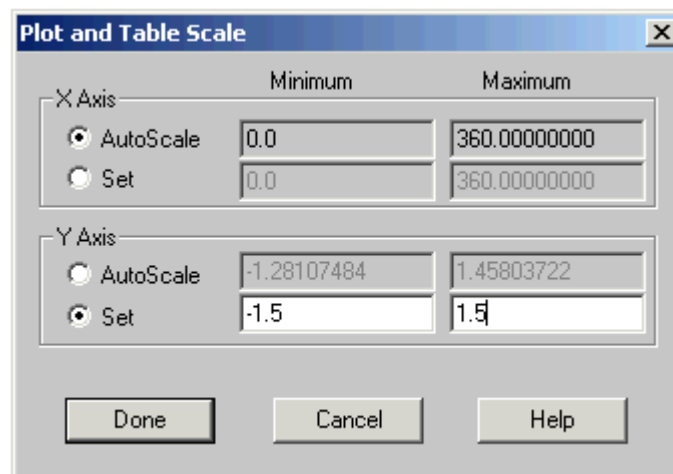
	Objective	Scaled Data Variance
s1 : Plasma	-1.929872e+000	2.957759e-001
Total objective	-1.929872e+000	
AIC	2.665026e-001	
BIC	3.872196e-001	

It is apparent from the Coefficients of Variation in the **Statistics** window that the model parameters are all well defined. The largest coefficient of variation is 10%.

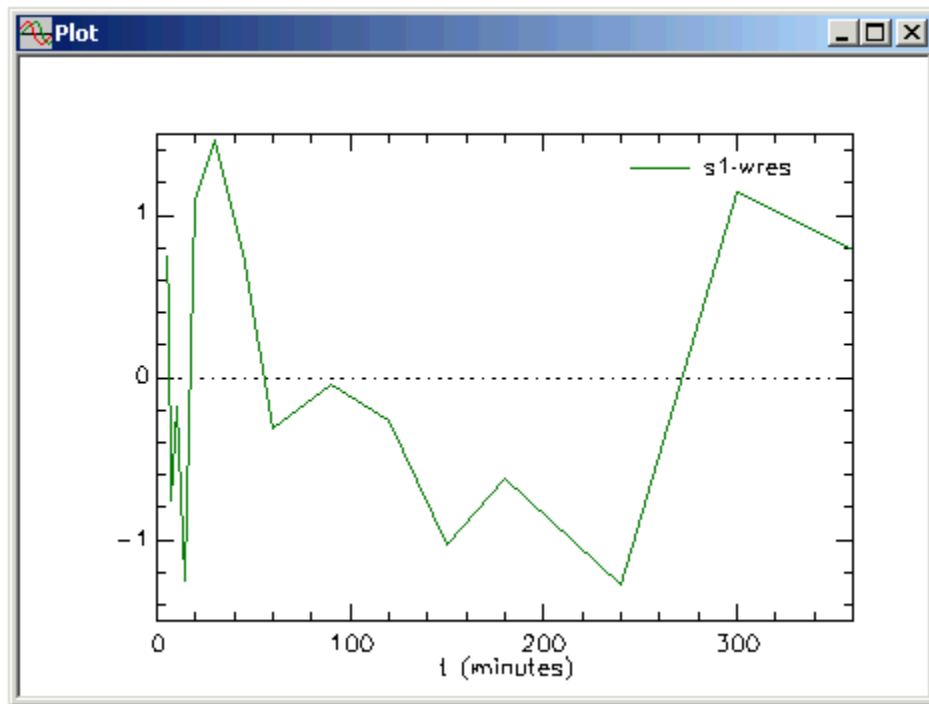
Record the values for AIC and BIC; these will be used later to help determine whether a two-compartment or three-compartment model is most appropriate for these data.

Close the **Statistics** window.

- c. View the weighted residuals (remember the **Plot** window is still open.)
  - (1) Click on the **Plot** window to make it the current window.
  - (2) In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open.
  - (3) Select the **List All Variables** check box.
  - (4) Click **s1\_wres**. This variable will move to the **Current Selection** pane.
  - (5) Click **Done**. The plot is not informative because the Y-Axis scale was set manually, and the plot is semi-log. These need to be changed.
  - (6) In the **View** menu, click **Semilog** to return the plot to a linear plot.
  - (7) In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.
  - (8) In the **Y Axis** pane, select **Set**. Enter “-1.5” and “1.5” respectively in the **Minimum** and **Maximum** boxes. The **Plot and Table Scale** dialog box will appear as follows:



(9) Click **Done**. The plot of the weighted residuals will appear as follows:



While the weighted residuals do line in a band close to -1 and 1, it is clear there are systematic deviations in the beginning and mid-part of the curve.


(10) Return your plot to **s1:Plasma**. You will have to reset the Y Axis scale to a minimum and maximum of 1.0 and 20 respectively.



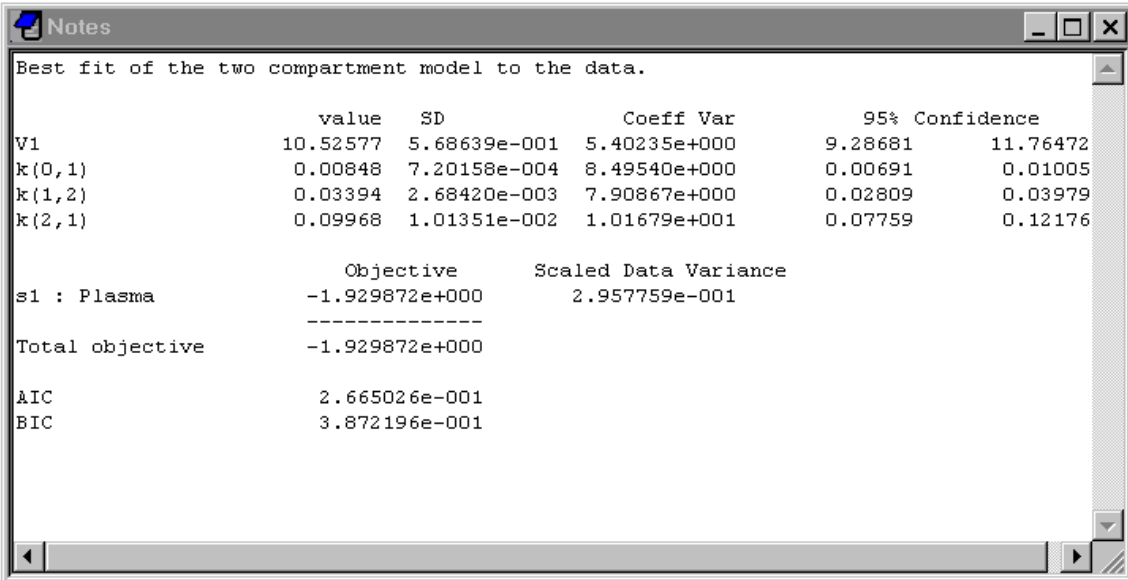
*Runs test.* You can if you wish perform the runs-test for goodness-of-fit. How to do this is explained in Appendix 2.



10. (Optional) Record the results in the **Notes** window. The contents of the **Notes** window is given in Appendix 3.

- 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 “Best fit of the two compartment model to the data.”
- c. In the **Statistics** window, select all the information in the **Parameter/Variables** pane.

- d. In the **Edit** menu, click **Copy**.
- e. Click in the **Notes** window. In the **Edit** menu, click **Paste**. Title the columns for parameters, values, etc. as shown in the figure below.
- f. In the **Statistics** window, select the information for the objective function. In the **Edit** menu, click **Copy**.
- g. Click in the **Notes** window. Click below the statistical information. Paste the information from the objective function. The **Notes** window will appear as follows:



Best fit of the two compartment model to the data.

	value	SD	Coeff Var	95% Confidence	
V1	10.52577	5.68639e-001	5.40235e+000	9.28681	11.76472
k(0,1)	0.00848	7.20158e-004	8.49540e+000	0.00691	0.01005
k(1,2)	0.03394	2.68420e-003	7.90867e+000	0.02809	0.03979
k(2,1)	0.09968	1.01351e-002	1.01679e+001	0.07759	0.12176


	Objective	Scaled Data Variance
s1 : Plasma	-1.929872e+000	2.957759e-001
-----		
Total objective	-1.929872e+000	

AIC	2.665026e-001
BIC	3.872196e-001

## Part 2. Estimate Clearance and Volume Parameters from the Model.

The clearance and volume parameters can be calculated directly from the compartmental model parameters.

1. Enter the equations for the pharmacokinetic parameters calculated from the model parameters.
  - a. In the **Show** menu, click **Equations**, or alternatively, on the **SAAM II Toolbar**, click **Equation** . The **Equations** dialog box will open.
  - b. Enter the following equations in the **Equations Defined Here** pane in the **Equations** dialog box.

$$CL_e = V1 * k(0,1)$$

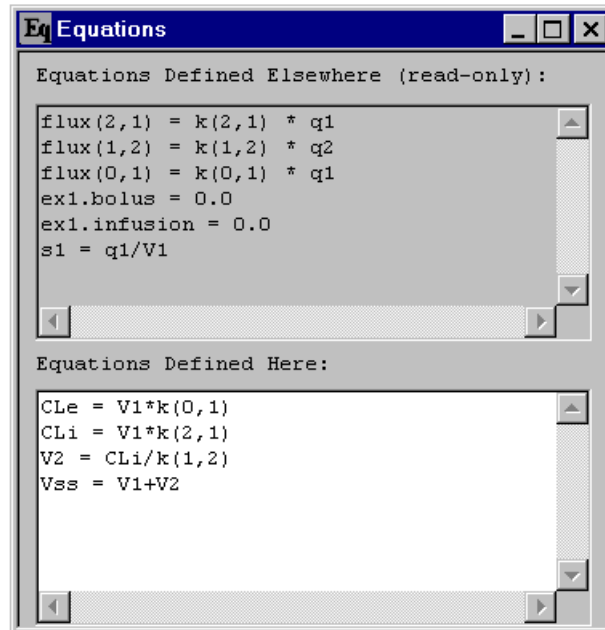
$$CL_i = V1 * k(2,1)$$

$$V2 = CLi/k(1,2)$$

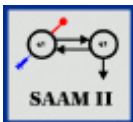
$$V_{ss} = V1+V2$$

The first two equations are clearance, one from “plasma” Compartment 1 and the other from the extravascular Compartment 2.  $V2$  is the “volume” of Compartment 2 while  $V_{ss}$  is the “steady state” volume, or equivalent volume of distribution.

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



- c. Close the **Equations** dialog box.
2. Re-Fit the model to the data. The Fit will not change, but you will obtain statistical information on the pharmacokinetic parameters.
    - a. Open the **Statistics** window. You can see there is very little difference in the parameter values and their error estimates.



*Objective function information.* You may notice that the objective function information changes a little between the first “Fit”, recorded in the notes, and this “Fit”. The reason why this happens is because in the first “Fit”, you started from another initial set of parameter estimates than this “Fit” above which actually started from a “best Fit”.

SAAM II, as do many other software tools, uses formulas to calculate derivatives required in the fitting process. These are approximation, and can be somewhat sensitive to the initial starting values.

Technically, to obtain the most accurate and reproducible parameter values and errors, you should fit the model to the data. Then, in the **Computational Settings** window in the **Compute** menu, select the **Central** option for the **Derivative** calculation. The central option is more accurate than the default forward option, but it takes longer to compute. This is why the forward option is set as the default.



If you are not copying your results in the **Notes** window, close the **Statistics** window and proceed to **Part 3**.

- b. (Optional) In the **Statistics** window, and select and copy the information in the **Parameter/Variable** pane. Close the **Statistics** window.
- c. Open the **Notes** window, and paste this information over the statistical information you had originally on the parameters. The **Notes** window should appear as follows:

Best fit of the two compartment model to the data.

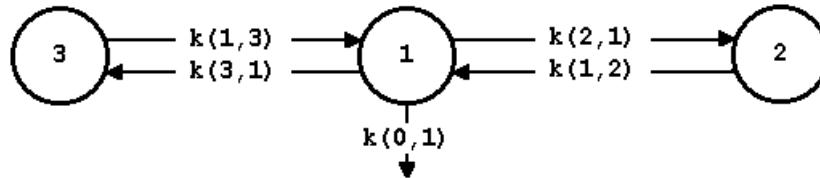
	value	SD	Coeff Var	95% Confidence	
V1	10.52577	5.68639e-001	5.40235e+000	9.28681	11.76472
k(0,1)	0.00848	7.20158e-004	8.49540e+000	0.00691	0.01005
k(1,2)	0.03394	2.68420e-003	7.90867e+000	0.02809	0.03979
k(2,1)	0.09968	1.01351e-002	1.01679e+001	0.07759	0.12176
----- Derived Variables -----					
CLe	0.08923	5.20824e-003	5.83705e+000	0.07788	0.10057
CLi	1.04918	6.47472e-002	6.17121e+000	0.90811	1.19025
V2	30.91290	1.41931e+000	4.59133e+000	27.82049	34.00532
Vss	41.43867	1.44656e+000	3.49085e+000	38.28689	44.59045
-----					
	Objective	Scaled Data Variance			
s1 : Plasma	-1.929872e+000	2.957759e-001			
-----					
Total objective	-1.929872e+000				
AIC	2.665026e-001				
BIC	3.872196e-001				

- d. Close the **Notes** window.

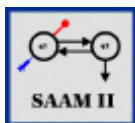
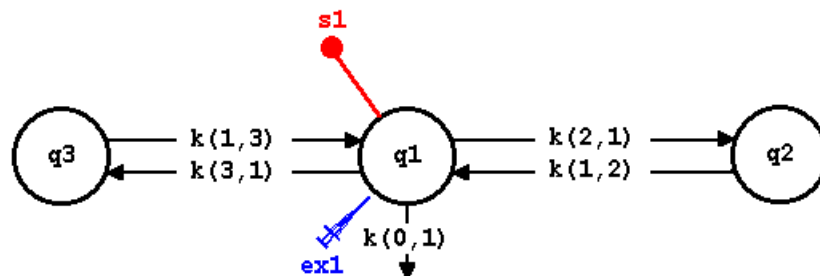
### Part 3. Add a Third Compartment to the Model

This part of the case study will show you how to add a third compartment to the model.

1. In the **SAAM II Toolbox**, click **Model** to make these tools available.
2. Modify the system model on the **Drawing** canvas as follows:



3. In the **SAAM II Toolbox**, click **Experiment**. Notice that the **Model** tools are unavailable and the **Experiment** tools are available. Notice also that the **Experiment Attributes** dialog box does not open. This is because the experiment attributes have already been specified. The model will appear as follows (you may have to move some of the objects):



*Modifying a system model.* Suppose you have created the system model, and the experiment on the model. That is, you have specified the experiment attributes. In addition, if you have edited the data file, the information will remain. When you modify the system model by adding more compartments or transfers, the experiment attributes do not change. What changes will change is the information required to solve the model, or fit the model to the data. When you add additional transfers, you must specify the initial values and bounds (if they are adjustable). In this above case, you have added a third compartment, compartment 3, and two transfers,  $k(3,1)$  and  $k(1,3)$ . Thus to solve the model, you need only provide initial estimates and the bounds for these parameters.



4. Enter the parameter values.




*Obtaining initial parameter estimates.* When you build the model structure as we are doing in the case study, you need to provide initial estimates for the new parameter, in this situation,  $k(3,1)$  and  $k(1,3)$ . You can use the best estimates from the two-compartment model as follows to obtain these estimates. In the case of the two compartment model, the best estimates are:  $V1 = 10.5$ ,  $k(0,1) = 0.009$ ,  $k(2,1) = 0.1$  and  $k(1,2) = 0.034$ . You now need initial estimates for  $V1$ ,  $k(2,1)$ ,  $k(1,2)$ ,  $k(3,1)$ ,  $k(1,3)$  and  $k(0,1)$ . Let us assume Compartment 2 is the rapidly turning over compartment.

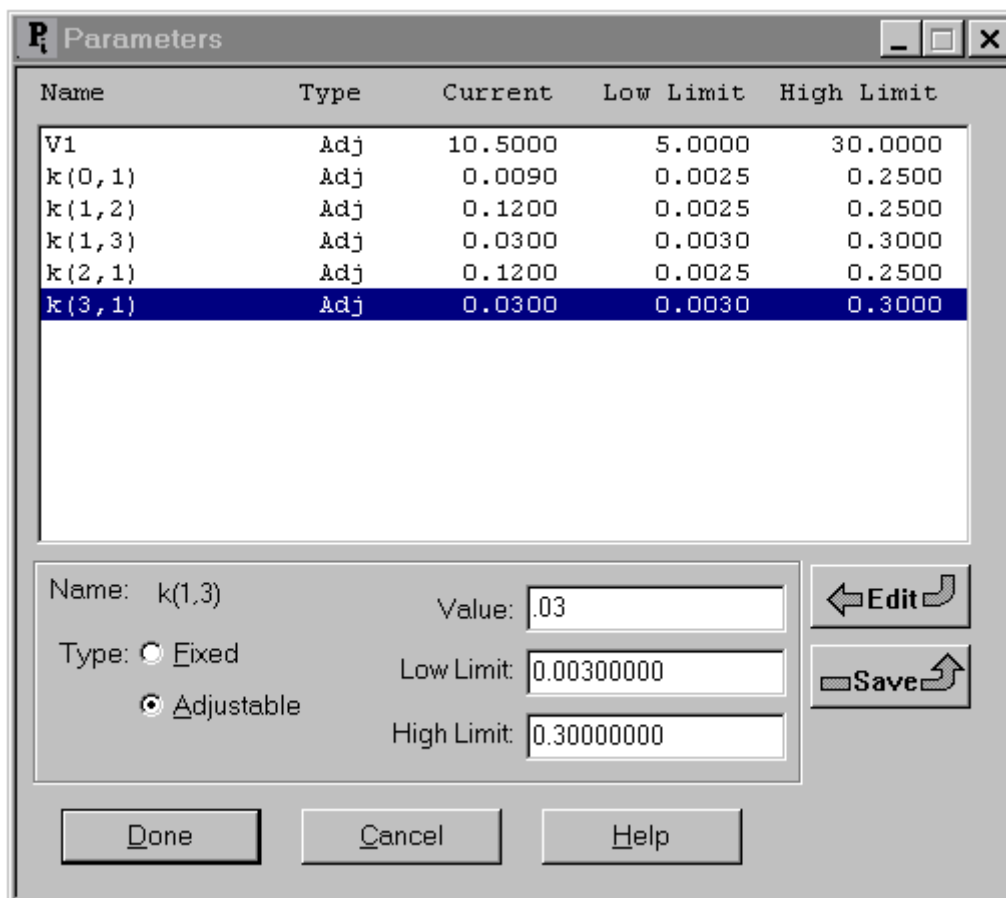
- i. Initial estimates for  $k(2,1)$  and  $k(1,2)$ . Initial estimates for  $k(2,1)$  and  $k(1,2)$  can be obtained by doubling the average between  $k(2,1)$  and  $k(1,2)$  from the best “Fit” of the two-compartment model. This number is about 0.12.
- ii. Initial estimates for  $k(3,1)$  and  $k(1,3)$ . Initial estimates for  $k(3,1)$  and  $k(1,3)$  can be obtained by halving the average between  $k(2,1)$  and  $k(1,2)$  from the best “Fit” of the two-compartment model. This number is about 0.03.
- iii. Initial estimates for  $V1$  and  $k(0,1)$ . Initial estimates for  $V1$  and  $k(0,1)$  can be obtained by using the best fit values for the two compartment model. These are 10.5 and 0.009 respectively.

This rule of thumb in terms of obtaining initial parameter estimates works most of the time. When it does not, you may need to hand-fit to adjust some of the initial estimates.



- a. In the **Show** menu, click **Parameters**, or alternatively, on the **SAAM II Toolbar**, click **Parameters** . The **Parameters** dialog box will open. The parameter  $V1$  should be selected. If it is not selected, double-click  $V1$ . Be sure the **Adjustable** option is selected.
- b. Enter “10.5” in the **Value** box, and click **Save**. (There is no need to adjust the low and high limit.)
- c. Double-click  $k(0,1)$  to select it.
- d. Enter “0.009” in the **Value** box, and click **Save**. (There is no need to adjust the low and high limit.)
- e. Double-click  $k(2,1)$  to select it.

- f. Enter “0.12” in the **Value** box, and click **Save**. (There is no need to adjust the low and high limit.)
- g. Double-click  $k(1,2)$  to select it.
- h. Enter “0.12” in the **Value** box, and click **Save**. (There is no need to adjust the low and high limit.)
- i. Double-click  $k(3,1)$  to select it.
- j. Enter “0.03” in the **Value** box, and click **Save**.
- k. Double-click  $k(1,3)$  to select it.
- l. Enter “0.03” in the **Value** box, and click **Save**. The **Parameters** dialog box will appear as follows:



The screenshot shows a dialog box titled "Parameters" with a table of parameters and a configuration section for the selected parameter  $k(1,3)$ .

Name	Type	Current	Low Limit	High Limit
V1	Adj	10.5000	5.0000	30.0000
$k(0,1)$	Adj	0.0090	0.0025	0.2500
$k(1,2)$	Adj	0.1200	0.0025	0.2500
$k(1,3)$	Adj	0.0300	0.0030	0.3000
$k(2,1)$	Adj	0.1200	0.0025	0.2500
$k(3,1)$	Adj	0.0300	0.0030	0.3000

Configuration for  $k(1,3)$ :

Name:  $k(1,3)$  Value: .03

Type:  Fixed  Adjustable

Low Limit: 0.00300000

High Limit: 0.30000000

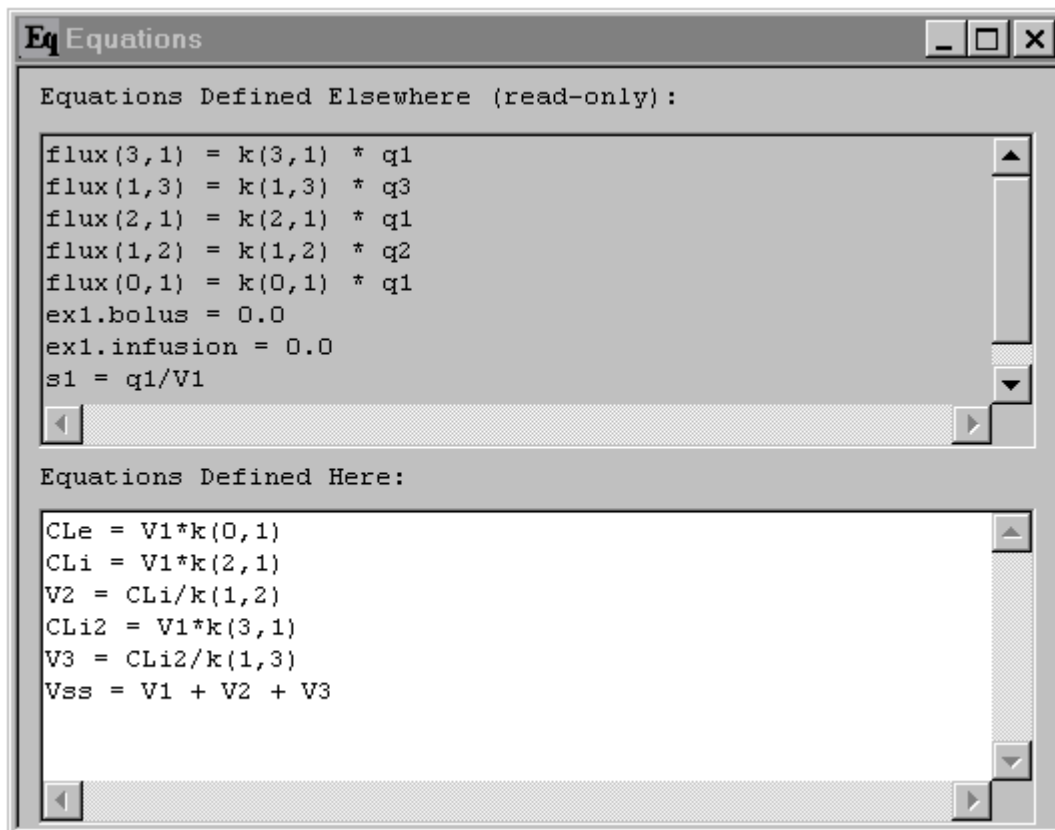
Buttons: Done, Cancel, Help, Edit, Save

- m. Click **Done**.
5. Modify the clearance and volume parameters.

Since you have added a new compartment to the model, you need to modify the pharmacokinetic parameter equations as follows:


$$\begin{aligned} CL_e &= V_1 * k(0,1) \\ CL_i &= V_1 * k(2,1) \\ V_2 &= CL_i / k(1,2) \\ CL_{i2} &= V_1 * k(3,1) \\ V_3 &= CL_{i2} / k(1,3) \\ V_{ss} &= V_1 + V_2 + V_3 \end{aligned}$$

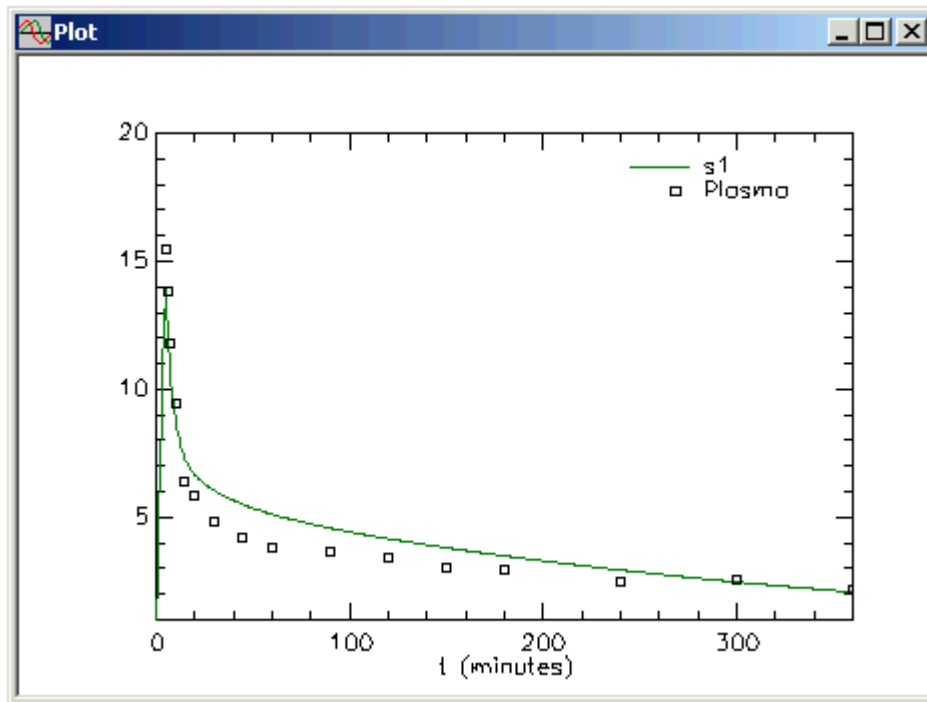
Notice the first three equations do not change. The next two are added to accommodate the third compartment, and the last to calculate the correct  $V_{ss}$ . When you have finished, the **Equations** dialog box will appear as follows:




6. Solve the model and view the solution.
  - a. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II**

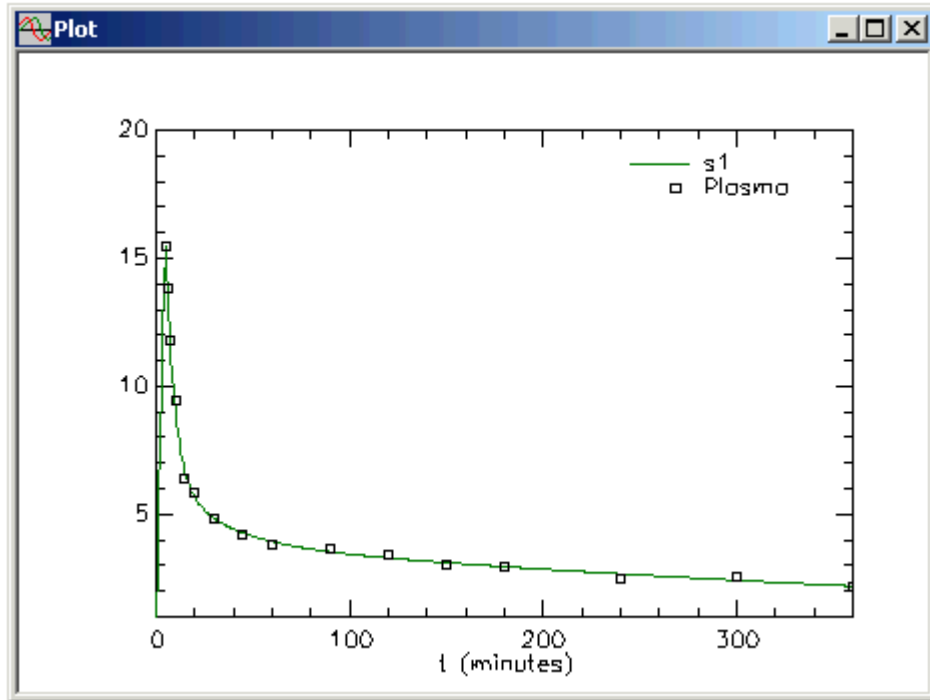
**Toolbar**, click **Solve** .

- b. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . The plot of **s1** and **Plasma** will appear as follows (you will probably have to reset the **Plot and Table Scale**; the following has a **Y Axis minimum** and **maximum** of 1.0 and 20 respectively):




The initial parameter estimates are sufficient that we can proceed to fit the model to the data. Leave the **Plot** window open.

7. Fit the model to the data and view the solution.
- a. In the **Compute** menu, click **Fit**, or alternatively, on the **SAAM II Toolbar**, click **Fit** . When you have “Fitted” the model to the data, the plot should appear as follows:



Leave the **Plot** window open.

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

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
V1	9.35622	6.14759e-001	6.57059e+000	7.98645	10.72599
k(0,1)	0.00837	9.47165e-004	1.13129e+001	0.00626	0.01048
k(1,2)	0.08196	2.84491e-002	3.47103e+001	0.01857	0.14535
k(1,3)	0.01869	4.97204e-003	2.66051e+001	0.00761	0.02977
k(2,1)	0.09310	1.89612e-002	2.03657e+001	0.05086	0.13535
k(3,1)	0.05024	2.12469e-002	4.22949e+001	0.00289	0.09758
----- Derived Variables -----					
CLe	0.07833	6.44809e-003	8.23150e+000	0.06397	0.09270
<input type="radio"/> Correlation Matrix <input type="radio"/> Covariance Matrix <input checked="" type="radio"/> Objective					
		Objective	Scaled Data Variance		
s1 : Plasma		-2.856577e+000	1.405015e-001		
		-----			
Total objective		-2.856577e+000			
AIC		-7.185020e-002			
BIC		9.715358e-002			

The statistics are not as good as the two-compartment model; the largest coefficient of variation is just over 40%. Yet the model predicted values do

better than the two-compartment model in describing the kinetics around the break in the curve.




*Interpreting the statistics.* In interpreting the statistical information in terms of the parameter precision, you expect less precision when you add complexity to the model. But the question is, besides the primary parameters changing, how to the pharmacokinetic parameters change?

You have seen the biggest change is about a 40% error in  $k(3,1)$ . In terms of the pharmacokinetic parameter, the largest error is in  $V2$ ; the error in  $V3$  is only 15%. Finally the addition of the third compartment had little effect on  $Vss$  either in terms of the numerical estimate or the parameter precision.

Notice in this case the values for AIC and BIC are both lower for the three-compartment model when compared with the two-compartment model indicating the three-compartment model is more appropriate.



If you are not recording your results in the **Notes** window, close the **Statistics** window, and proceed to **Step 8**.

- c. (Optional) In the **Statistics** window, in the **Parameter/Variable** pane, select the parameters and derived variables.
- d. In the **Edit** menu, click **Copy**.
- e. In the **Show** menu, click **Notes**, or alternatively on the **SAAM II Toolbar** click **Notes** . The **Notes** window will open.
- f. In the **Notes** window, type “Best fit of a three-compartment model to the data.”
- g. In the **Edit** menu, click **Paste**. The statistical information will be pasted into the **Notes** window.
- h. In the **Statistics** window, in the **Objective** pane, select the s1:Plasma objective function, and the AIC and BIC criteria.
- i. Return to the Notes window. In the **Edit** menu, click **Paste**. The last part of the **Notes** window will appear as follows:

```

Notes
-----
AIC          2.665026e-001
BIC          3.872196e-001

Best fit of a three compartment model to the data

V1           9.35622   6.14765e-001   6.57065e+000   7.98644   10.72600
k(0,1)       0.00837   9.47258e-004   1.13140e+001   0.00626   0.01048
k(1,2)       0.08196   2.84493e-002   3.47103e+001   0.01857   0.14535
k(1,3)       0.01869   4.97213e-003   2.66055e+001   0.00761   0.02977
k(2,1)       0.09310   1.89605e-002   2.03650e+001   0.05086   0.13535
k(3,1)       0.05024   2.12474e-002   4.22957e+001   0.00289   0.09758
-----
Derived Variables
-----
CLe          0.07833   6.44874e-003   8.23232e+000   0.06397   0.09270
CLi          0.87110   1.54672e-001   1.77560e+001   0.52646   1.21573
CLi2         0.47001   1.83550e-001   3.90520e+001   0.06104   0.87899
V2           10.62805   4.65225e+000   4.37733e+001   0.26219   20.99390
V3           25.15006   3.98683e+000   1.58522e+001   16.26685   34.03326
Vss          45.13432   2.19006e+000   4.85232e+000   40.25456   50.01409

Objective      Scaled Data Variance
s1 : Plasma    -2.856578e+000      1.405015e-001
-----
Total objective -2.856578e+000

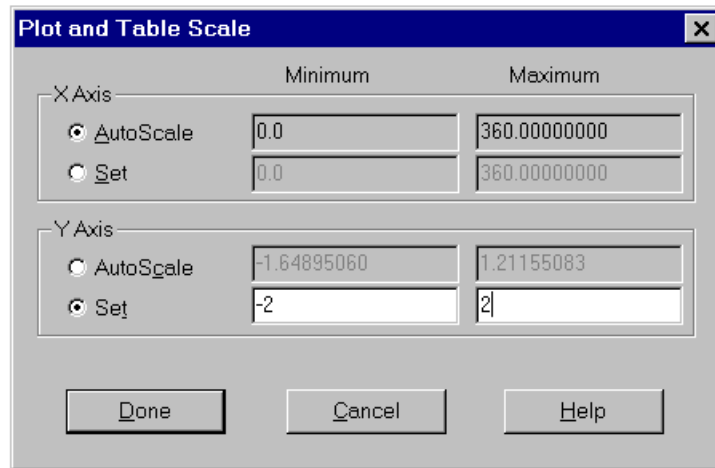
AIC           -7.185023e-002
BIC           9.715355e-002

```

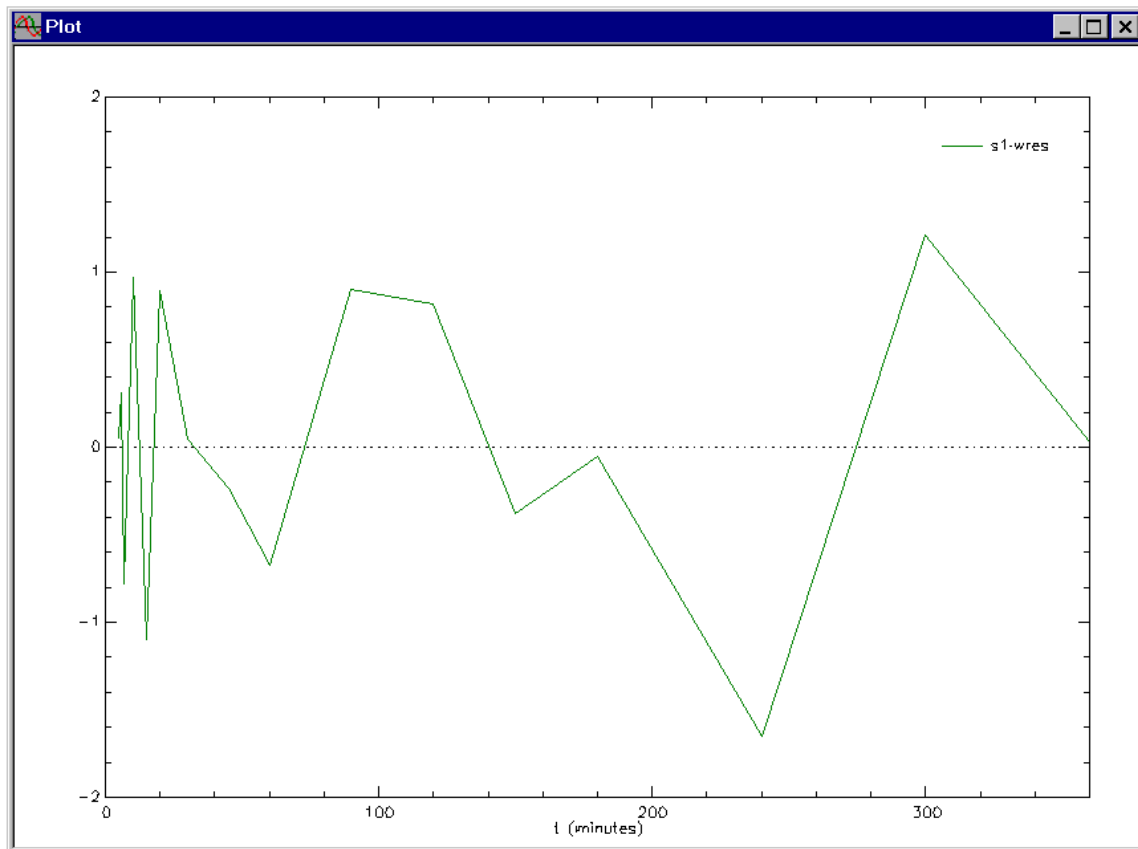
The contents of the **Notes** window are included as Appendix 3.

- j. Close the **Notes** and **Statistics** windows. Leave the **Plot** window open.
8. View the residuals.
    - a. Click on the **Plot** window to make it the current window.
    - b. In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open.
    - c. Select the **List All Variables** check box.
    - d. Click **s1\_wres**. This variable will move to the **Current Selection** pane.
    - e. Click **Done**. The plot is not informative since it is in semi-log mode and the Y axis scale has been set for **s1** and **Plasma**.
    - f. In the **View** menu, click **Semilog** to return to a linear plot. It is necessary to reset the scale.
    - g. In the **Set** menu, click **Plot/Table Scale**. The **Plot and Table Scale** dialog box will open.

- h. In the **Y Axis** pane, select **Set**. Enter “-2” in the **Minimum** box, and “2” in the **Maximum** box. The **Plot and Table Scale** dialog box will appear as follows:



- i. Click **Done**. The plot of the weighted residuals will appear as follows:



The residuals have improved considerably with no apparent systematic deviation. In addition, they lie in a band almost between -1 and 1 which, for a

good fit of the data with the proper weights assigned, is in line with statistical theory.



*Which model is best?* You have two pieces of information to determine which of the two or three-compartment model is most appropriate to describe the these data. First are the residuals. To complete the modeling exercise, you should perform a runs test on the residuals (or weighted residuals) of both the two and three-compartment models (this is done in Appendix 2). Second is an analysis of the parsimony parameters, AIC and BIC. In this case, parsimony criteria support the three-compartment model

Finally, to complete the exercise, you should try a four compartment model. If you do (following the above rules for obtaining initial parameter estimates), in most cases you will have problems fitting with different parameter values hitting lower limits. In the event that you choose a set of initial values where you actually fit, you will find that some of the parameters have error estimates greater than 100%, and the value for AIC and BIC is larger than for the three compartment model.



You may save the study file if you wish for future use.

**Quit** the **SAAM II Compartmental** application.

### Essential Points to Remember

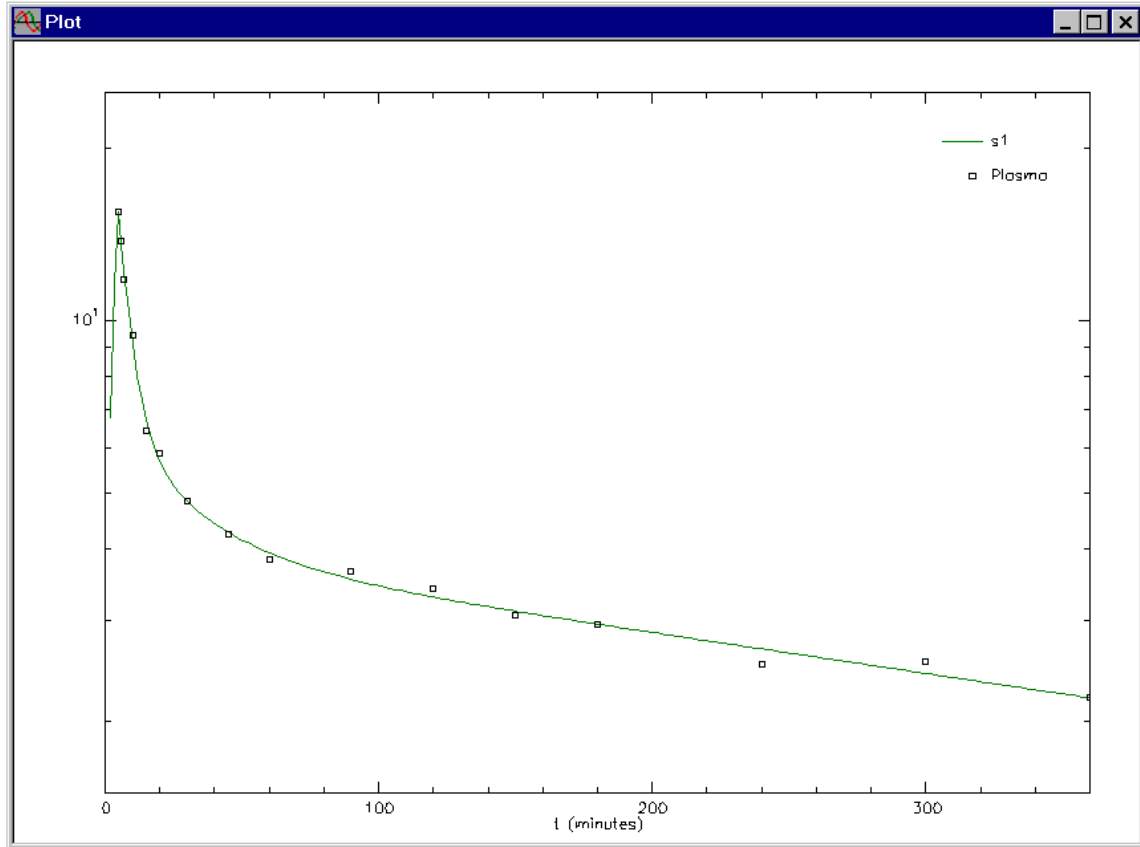
- Pharmacokinetic parameters can be defined as part of the model
- When necessary, it is easy to add a new compartment to the model.
- There are convenient ways to obtain initial parameter estimates for the model; these include adding model complexity.
- When adding a compartment to the model, you need to check all the new parameter values and their precision including the derived parameters.

### Modeling Notes

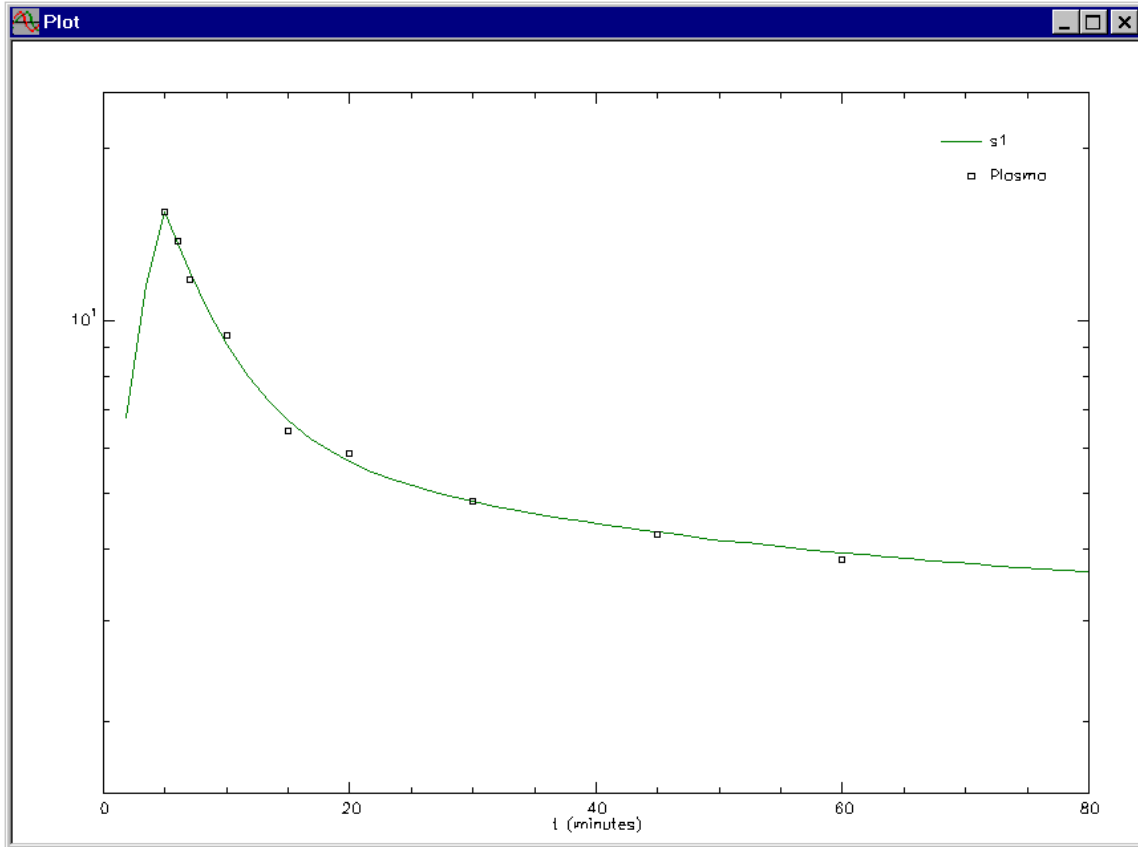
There is a very important but subtle point to be learned in this case study. That is the drug was input as a 5 minute constant infusion, not a bolus. You notice when you plot the data you do not see this. The reason is because of the resolution of SAAM II's plots. The resolution of the plots is determined by the **Minimum Number of Calculation Intervals**, a number used by SAAM II to determine when values of the model solution

should be saved for plotting or tabular purposes. You previously set this number to its maximum value of 500.

Below is the best fit of the three-compartment model to the data.



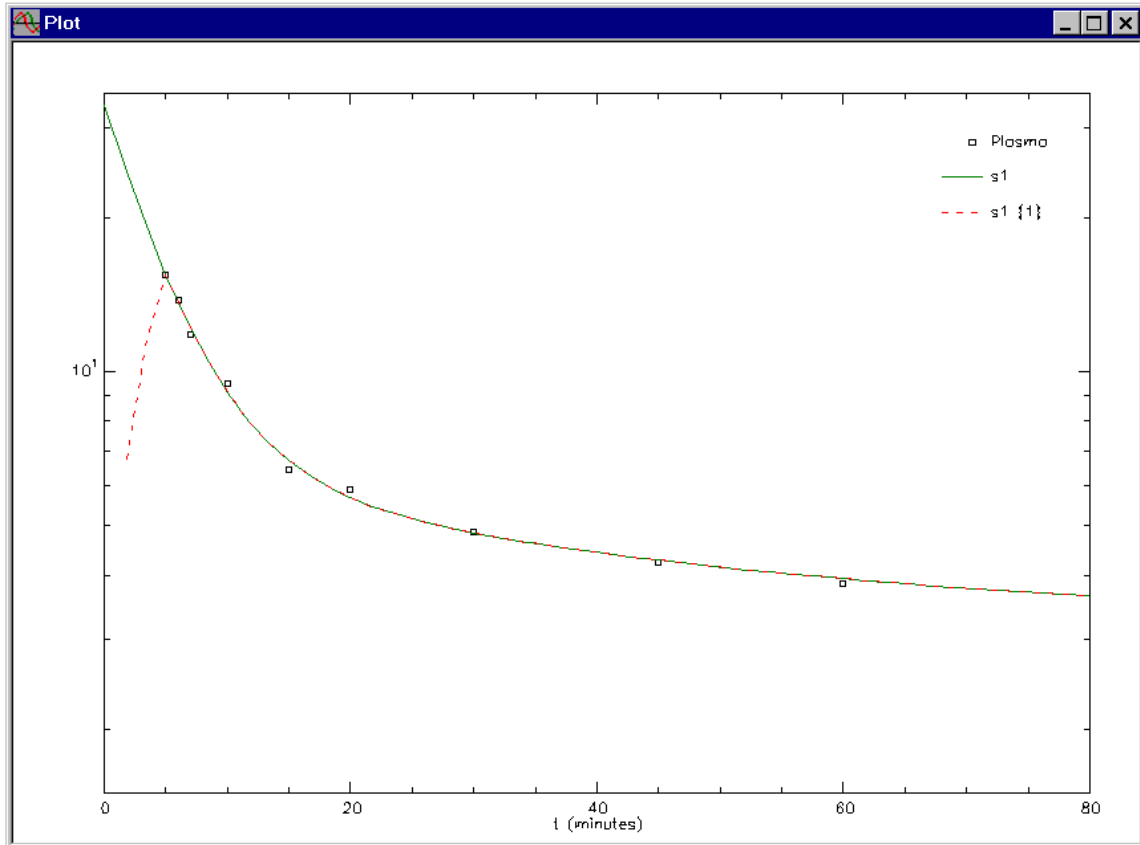
If you expand the initial part of the curve:



You can clearly see the rising portion of the curve starting from zero!

Yet when the initial parameter estimates were obtained using any of the curve peeling methods, a bolus injection was assumed. While incorrect, it did yield sufficiently close initial estimates to proceed.

Suppose you ignored the fact that this was an infusion, and treated the input as a bolus. To see what happens, this can be simulated by changing the input format from an infusion to a bolus. The first part of the curve will appear (comparing solutions):



You can see a big difference in the beginning. What is affected? The estimated volume  $V_I$ ! When the input is described by the 5 minutes infusion,  $V_I$  is estimated to be 9.4L while if the input is regarded as a bolus,  $V_I$  is estimated to be 6.0L. This is more than 30% lower, and ALL pharmacokinetic parameters estimated with a volume term will be in error by this percentage.

The point is: SAAM II can simulate exactly what was done in the laboratory; there is no need to try and do something different!

**Data for this case study**

DATA  
(FSD 0.1)  
t      Plasma  
5      15.5  
6      13.8  
7      11.8  
10     9.45  
15     6.43  
20     5.87  
30     4.85  
45     4.24  
60     3.84  
90     3.66  
120    3.40  
150    3.07  
180    2.95  
240    2.52  
300    2.54  
360    2.20  
END

### 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.

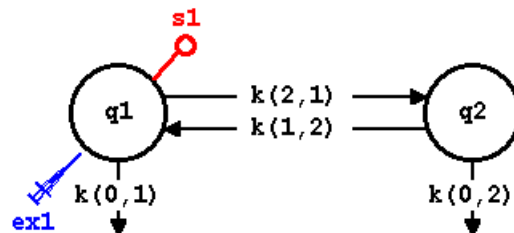
In this appendix, it is assumed that you have worked through the appendix explaining how to obtain initial parameter estimates for the one-compartment model; this appendix follows the case study on cadralazine kinetics. The ideas presented in this appendix extend upon those presented for the one-compartment (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 semilogarithmically. 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  $s_1$  is given by  $s_1 = q_1 / \text{vol}$ , then there are five parameters in this model:  $\text{vol}$ ,  $k(2,1)$ ,  $k(1,2)$ ,  $k(0,1)$  and  $k(0,2)$ . If the input into compartment  $q_1$  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:

$$s_1(t) = \frac{q_1}{\text{vol}} = A_1 \cdot e^{-a_1 t} + A_2 \cdot e^{-a_2 t} \quad (1)$$

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

$$s_1(t) = \frac{q_1}{vol} = A_0 + A_1 \cdot e^{-a_1 t} + A_2 \cdot e^{-a_2 t} \quad A_0 + A_1 + A_2 = 0 \quad (2)$$

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<sup>2</sup> that for a set of 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?

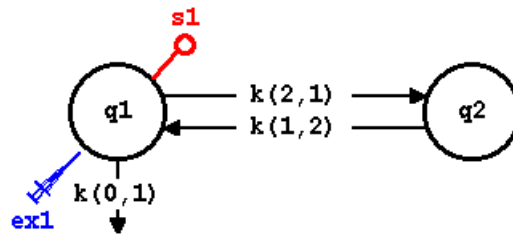
The reason why this is the case comes from the relationship between the  $A_1$ ,  $A_2$ ,  $a_1$  and  $a_2$  and the  $vol$ ,  $k(2,1)$ ,  $k(1,2)$ ,  $k(0,1)$  and  $k(0,2)$ . This is important since it provides the equations by which the initial parameters for the two-compartment model can be obtained.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

The relationship for the bolus injection is the following:

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

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.

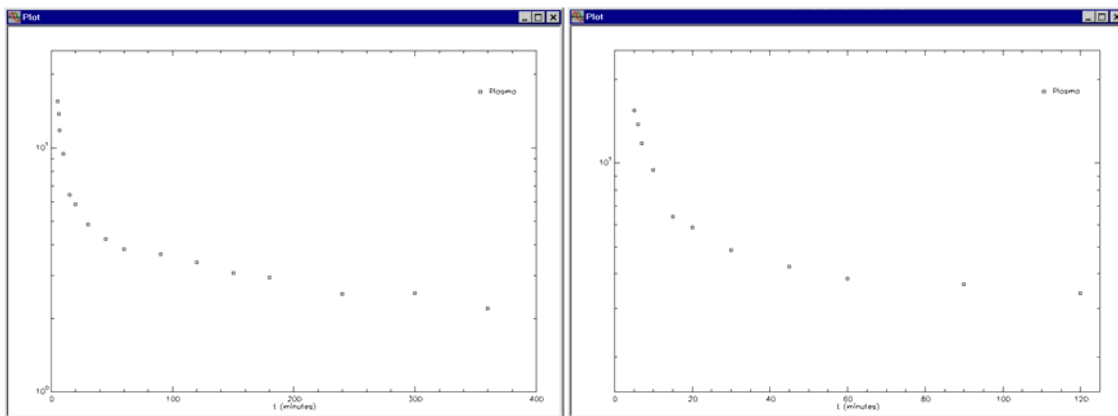
In this case, there is no  $k(0,2)$ . Thus  $k(2,2)=k(1,2)$  making  $k(1,2)$  uniquely identifiable. Since the product  $k(1,2)k(2,1)$  is identifiable and  $k(1,2)$  is known, one can solve for  $k(2,1)$  making it uniquely identifiable. Since  $k(1,1)=k(0,1)+k(2,1)$ , and  $k(2,1)$  is known, one 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 theophylline data in semi-log form, you will obtain the following:

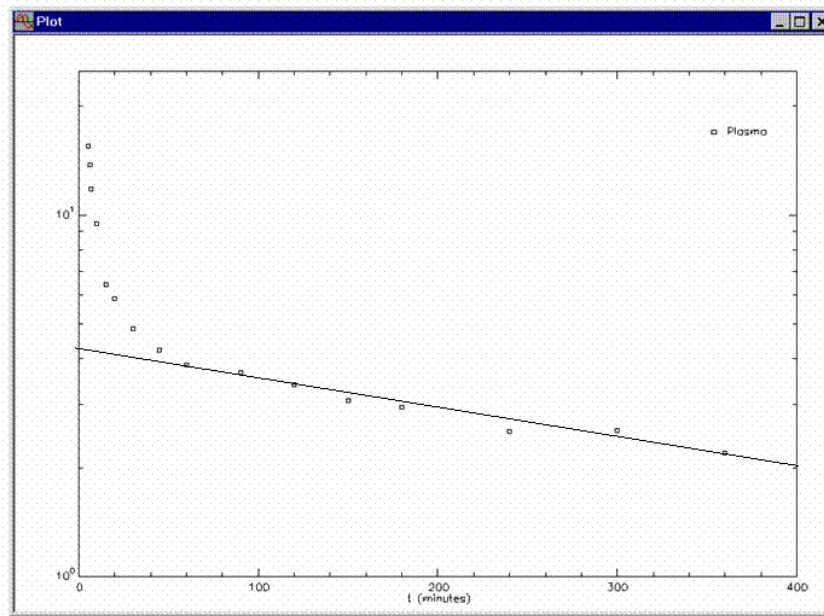


The left plot shows the totality of the data (the **Plot/Table Scale** has been set with a maximum of 370 for the X Axis, and the Y Axis going from 1.5 to 25). It is useful to

expand the initial portion of the plot to see some of the details of the start of the decay. You can clearly see the effect of the 5 minute infusion protocol rather than a bolus in this plot.

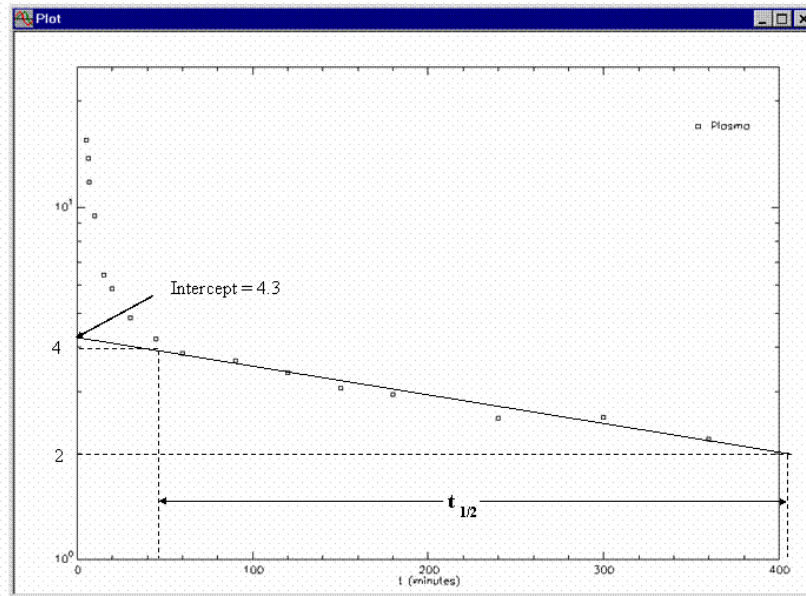
### Curve Peeling

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 intersect of this line with the y-axis, as shown in the following figure, is 4.3. 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 4 (at about 45 minutes) to 2 (at about 410 minutes) which is approximately 365 minutes. The half-life is thus  $\ln(2)/365$ , or 0.0019. This provides an estimate for  $a_2$ . Thus we have the second term of (1):

$$A_2 \cdot e^{-a_2 t} = 4 \cdot e^{-0.0019 t} \quad (4)$$



The next step is to subtract this amount from each datum. The point is that  $sI(t)$  will describe each datum. Since  $sI(t)$  is a sum of two exponentials, both exponentials will contribute to the datum. Thus for the  $i^{\text{th}}$  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-4.3*exp(-0.0019*t)
5	15.5	11.24065658
6	13.8	9.548741645
7	11.8	7.556811367
10	9.45	5.230928742
15	6.43	2.250820135
20	5.87	1.730334354
30	4.85	0.788245502
45	4.24	0.292371485
60	3.84	0.00329079
90	3.66	0.035867234
120	3.4	-0.023334317
150	3.07	-0.163661294
180	2.95	-0.10449728
240	2.52	-0.2053995
300	2.54	0.108240614
360	2.2	0.030243341

Again, remembering the model is:

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

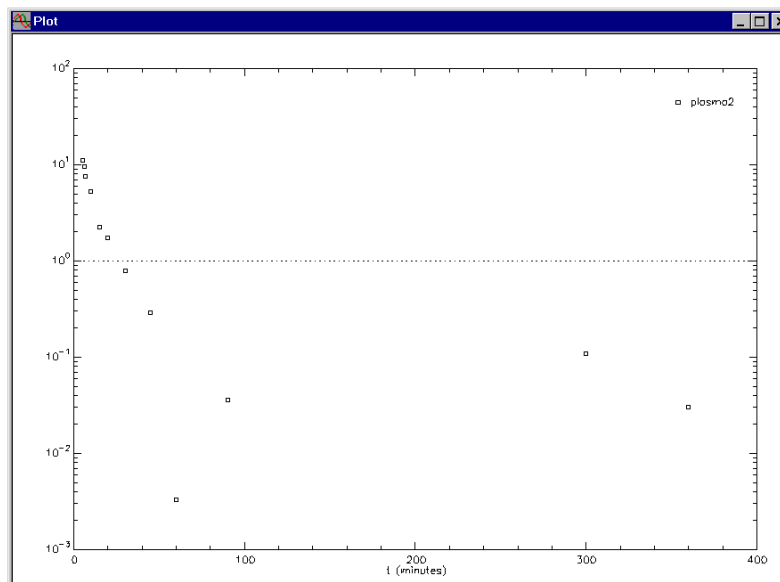
the result of this subtraction is a set of modified data that can be described by the first exponential:

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

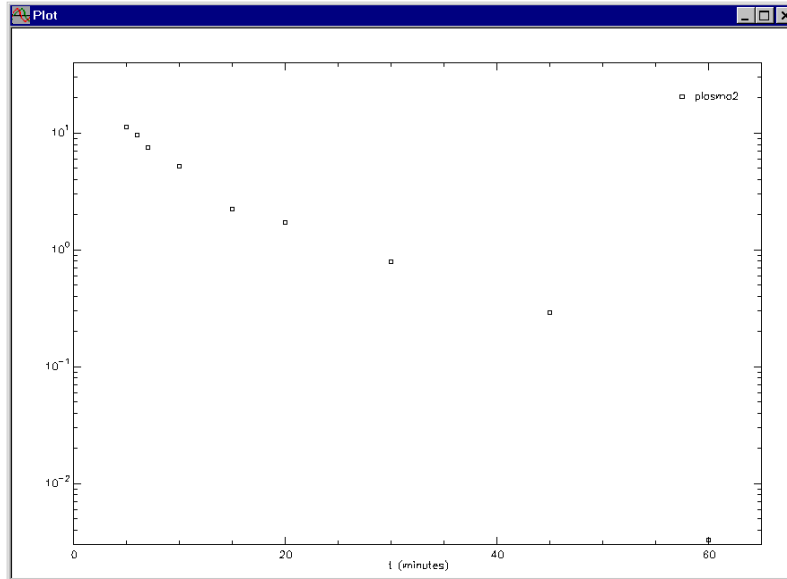
That is, if you plot the modified data given in the previous table, the initial portion should decay monoexponentially.

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.

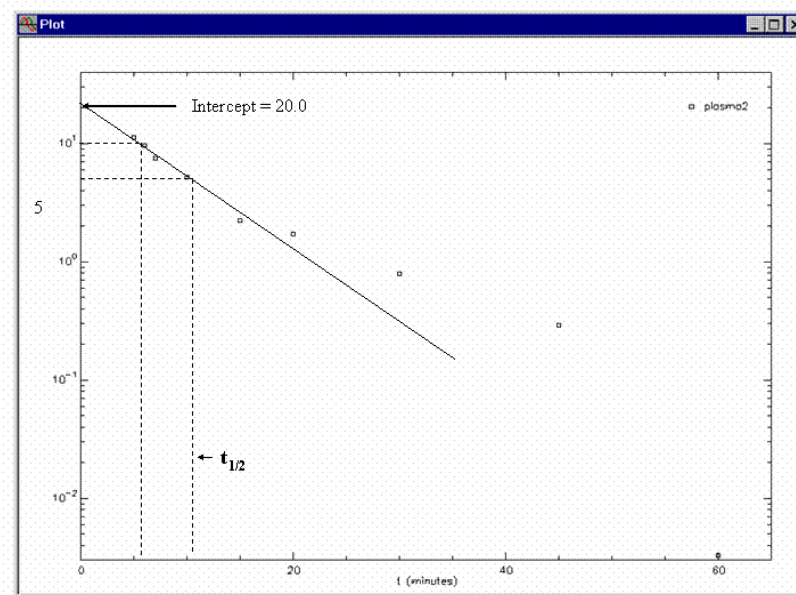
If you now plot the modified data, you will obtain the following:



This plot is not informative since the initial decay is rapid. If the modified data are plotted in the 60 minute time scale, you will obtain the following:



Now you see the initial data do appear to decay monoexponentially although there is an indication there may be a break around 10 - 20 minutes. You can now repeat the above process to obtain estimates for  $A1$  and  $a1$ . This is shown in the following figure:



The intercept of the tangent line is at 20. This provides an estimate for  $A1$ . The half-life, here measured as the time to go from 10 to 5, is about 5 minutes. Thus an estimate for  $a1$  can be obtained from  $\ln(2)/5$  which is 0.14.

If you are using (1) as your model, these will provide the initial estimates for the model parameters  $A1$ ,  $A2$ ,  $a1$  and  $a2$ . If you are using Model 2, estimates for the rate constants and volumes can be found using (3):

$$k(2,2) = k(1,2) = \frac{A2 \cdot a1 + A1 \cdot a2}{A1 + A2} = 0.0263$$

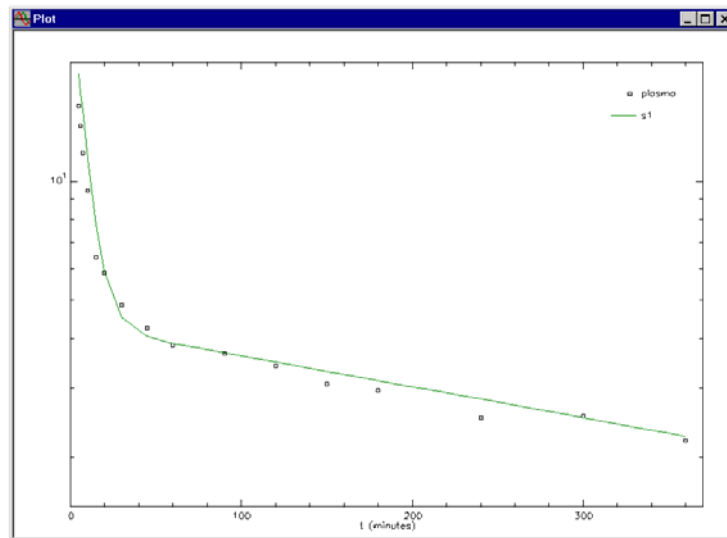
$$k(2,1) = \frac{A1 \cdot A2(a1 - a2)^2}{k(1,2) \cdot (A1 + A2)^2} = 0.106 \quad (7)$$

$$k(0,1) = k(1,1) - k(2,1) = \frac{A1 \cdot a1 + A2 \cdot a2}{A1 + A2} - k(2,1) = 0.0096$$

The dose is 1504000ng. Hence an estimate for *vol* can be obtained:

$$vol = \frac{200}{24.3} = 8.23 \quad (8)$$

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



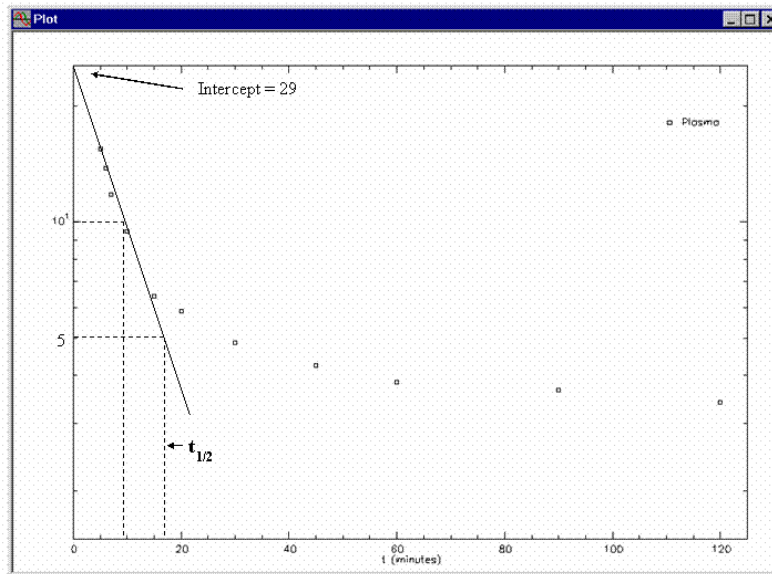
These estimates are clearly satisfactory.

### Quick Curve Peeling

There is a quicker method to obtain the initial parameter estimates that avoids the formal subtracting 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 than 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 intercept 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.

To obtain better resolution for the estimates, you can operate on an expanded scale from 0 to 120 (for example). Estimating  $A1 + A2$  and the half-life are shown below:



The intercept of 29 gives an estimate for  $A1 + A2$ . Knowing an estimate for  $A2$  is 4.3, an estimate for  $A1$  is 24.7 which is slightly higher than the estimate of 20 obtained above. The estimate of 0.0019 for  $a2$  is obtained as described previously.

From the above, you can see that the half-life of the initial decay is about 5 minutes. An estimate for  $a1$  is thus  $\ln(2)/5$  or 0.14. The rate constants can be estimated:

$$k(2,2) = k(1,2) = \frac{A2 \cdot a1 + A1 \cdot a2}{A1 + A2} = 0.0224$$

$$k(2,1) = \frac{A1 \cdot A2(a1 - a2)^2}{k(1,2) \cdot (A1 + A2)^2} = 0.108 \quad (9)$$

$$k(0,1) = k(1,1) - k(2,1) = \frac{A1 \cdot a1 + A2 \cdot a2}{A1 + A2} - k(2,1) = 0.0115$$

and

$$vol = \frac{200}{29} = 6.7 \quad (10)$$

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 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  $A1$ ,  $A2$ ,  $a1$  and  $a2$  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.

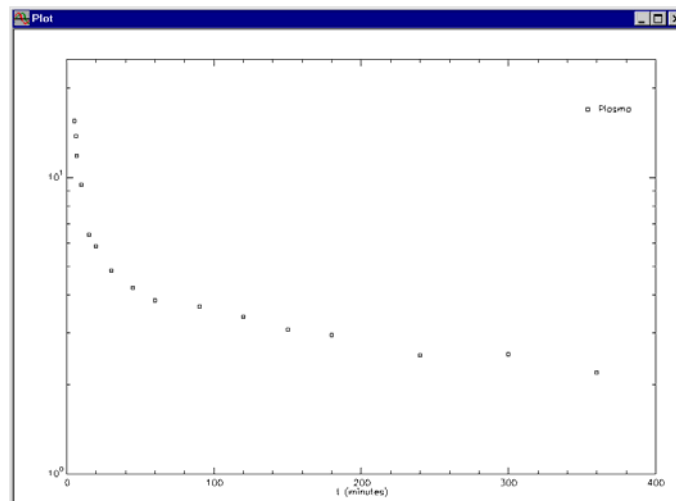
*End of technical remark:*

### 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.

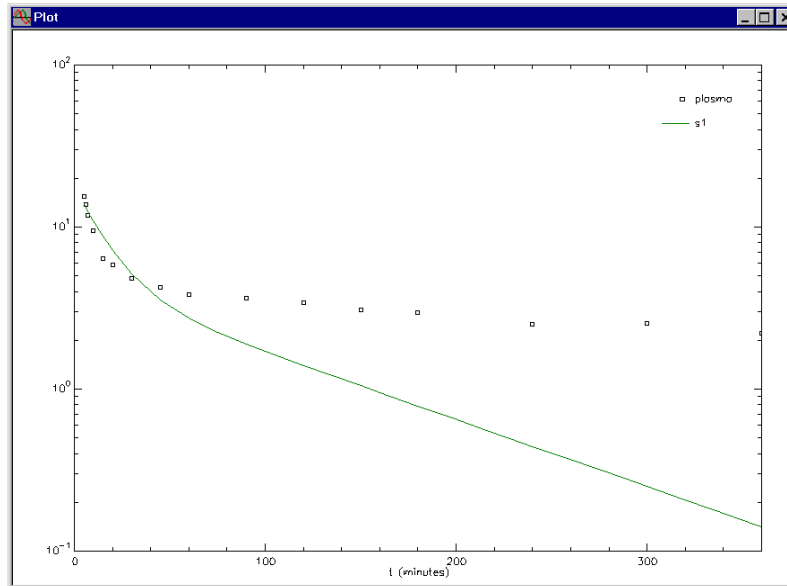
If you look at the original data, reproduced in the following figure, you will see the break in the curve occurs at about 40 minutes. The reciprocal is 0.025, so you can use this value as the estimate for the rate constants.



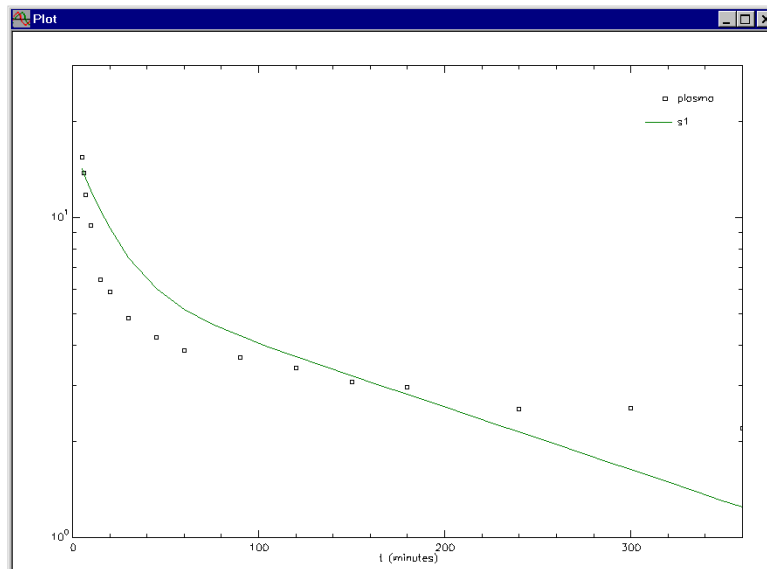
There are two different methods to estimate  $vol$ . One is the previous method where you can extrapolate the initial decay to time zero. The other is simply to use the first datum;

this assumes that the first datum is representative of the zero-time extrapolation. For the latter, remembering the dose is 200mg and the first datum is 15.5mg/L, an estimate for  $vol$  is 12.9.

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 initial decay is described pretty well. What is off is the final slope. You need to decrease  $k(0,1)$  from 0.025 to, for example, 0.01. You will obtain:



Now things are getting closer, but the initial decay is not rapid enough (meaning  $k(2,1)$  is too small and needs to be increased) and the final decay is still too fast (meaning  $k(1,2)$  needs to be decreased). If you wish, you can experiment changing these values one parameter at a time. But the initial estimates now are sufficient to proceed with fitting your model to your data.

When hand-fitting, as a rule of thumb it is a good idea to adjust one parameter at a time rather than in groups. In situations where the estimates are not very accurate, it is best to change them by a factor of no more than 5. As the estimates become better, 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.

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 it 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 it 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).

#### Quick method

- 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].

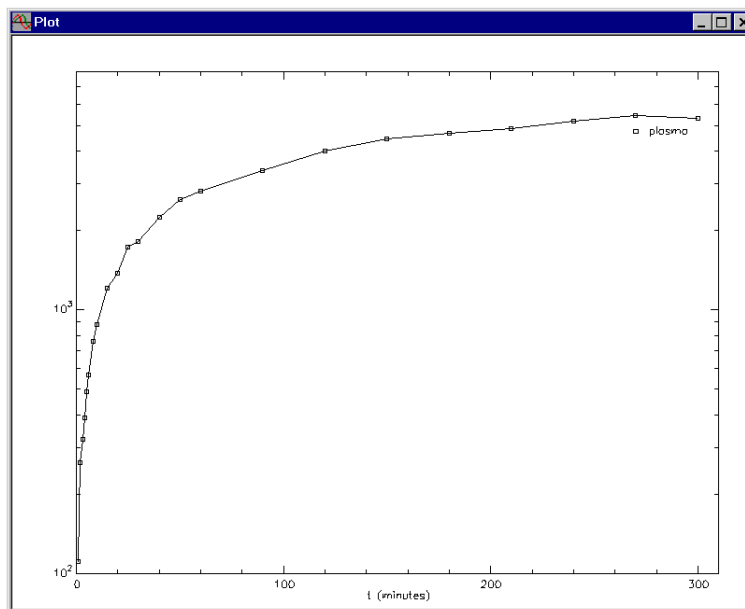
## Part 2. Constant infusion into plasma

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

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

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:

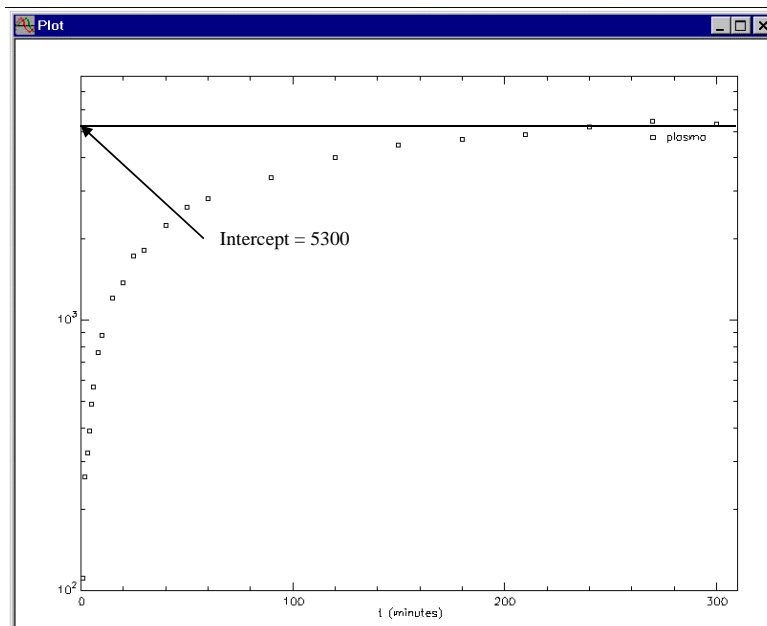
$$s_1^*(t) = A_0 + A_1 e^{-a_1 t} + A_2 e^{-a_2 t} - A_0 = A_1 e^{-a_1 t} + A_2 e^{-a_2 t} \quad (12)$$

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

$$s_1^{\text{mod}}(t) = -(A_1 e^{-a_1 t} + A_2 e^{-a_2 t}) \quad (13)$$

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

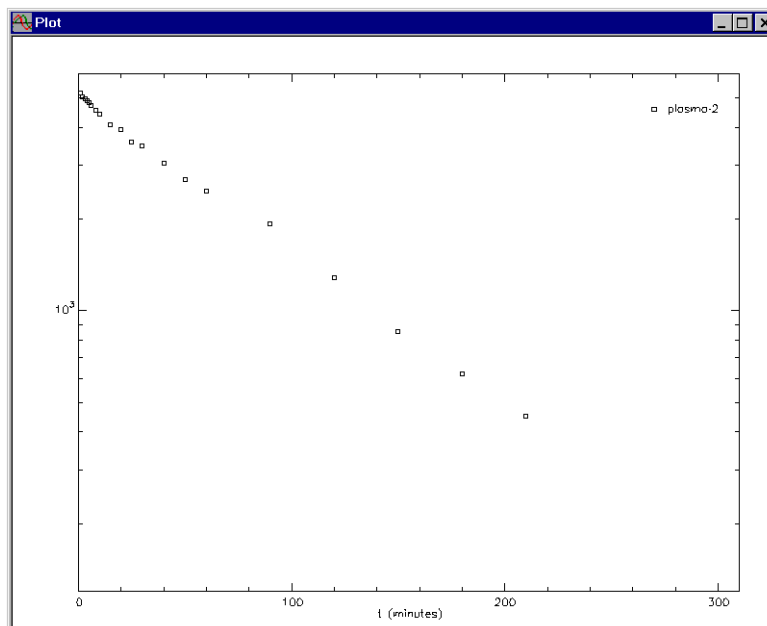
Using the data in the previous figure, the following figure illustrates what is:



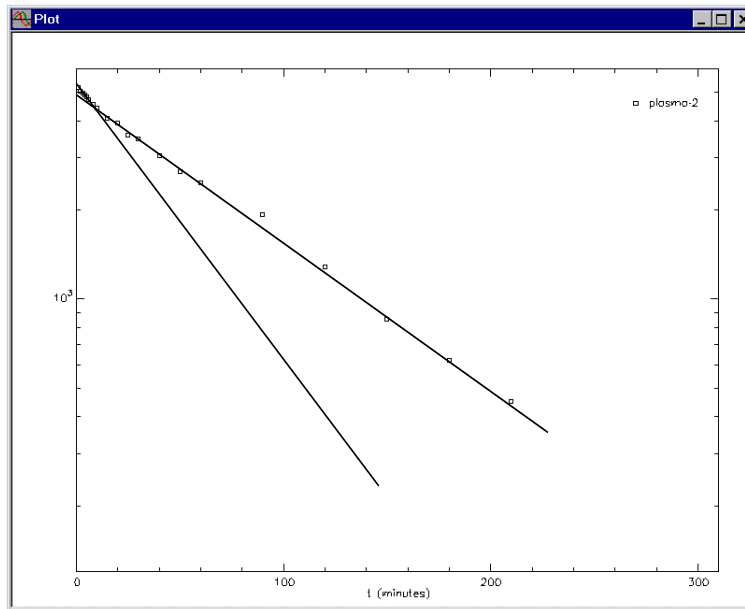
For this set of data, a plateau value of 5300 is estimated as shown in the figure above. Subtracting this value from each datum, and multiplying the result by -1 gives the following set of modified data:

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  $A1$ ,  $A2$ ,  $a1$  and  $a2$ , 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.

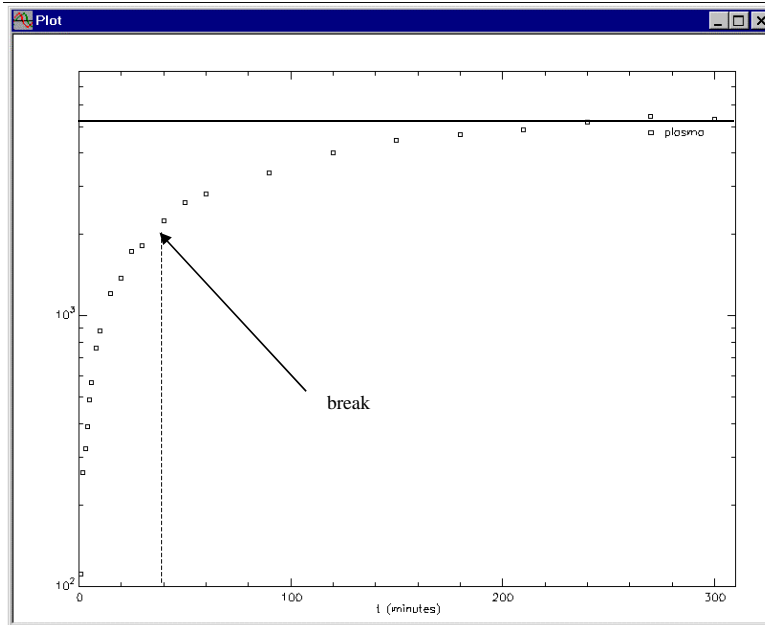
$$CR = \frac{\text{infusion rate}}{\text{plateau}} = \frac{400000}{5300} = 75.5 \quad (14)$$

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

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

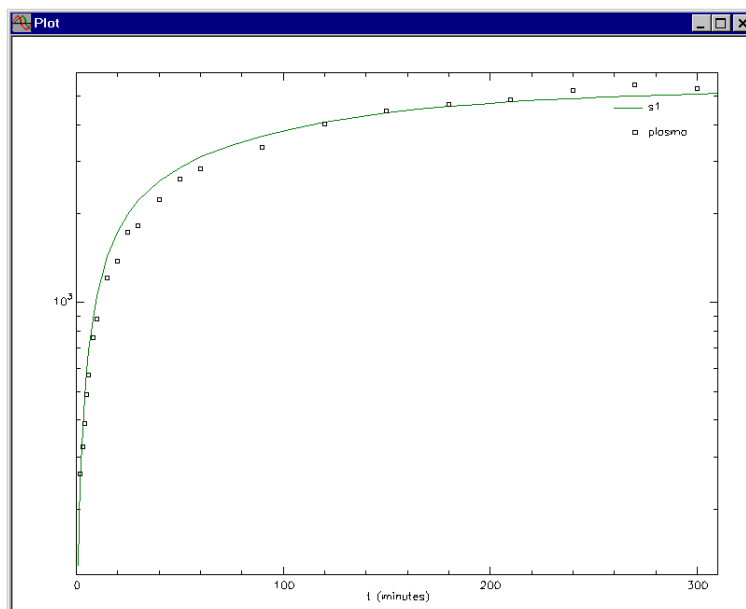
### A Quick Method

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 are (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 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  $k(0,1)$ .

1. Principles of Clinical Pharmacology. AJ Atkinson, Jr, CE Daniels, RL Dedrick, CV Grundzinskas and SP Markey, Eds. Academic Press. New York, NY, 2001.
2. Cobelli, C, D Foster and G Toffolo. Tracer Kinetics in Biomedical Research. Kluwer Academic/Plenum Press, New York, NY, 2000.

Appendix 2: The Runs Test for Goodness-of-Fit

The runs test for the two-compartment and three-compartment models following the best-fit of both models to the data is performed as described below. If you do not know how to perform or interpret the runs test, please consult any standard book on statistics or biostatistics.


The two tables of weighted residuals are shown below. The left hand table are the weighted residuals from the best fit of the two-compartment model to the data; the right hand table are from the best fit of the three-compartment model.

t	sl_wres
0.000	-
5.000	-
5.000	0.740
6.000	0.408
7.000	-7.64218e-001
10.000	-1.72853e-001
15.000	-1.25144e+000
20.000	1.100
30.000	1.460
45.000	0.776
60.000	-3.13151e-001
90.000	-4.06904e-002
120.000	-2.64162e-001
150.000	-1.03459e+000
180.000	-6.16727e-001
240.000	-1.27992e+000
300.000	1.137
360.000	-
360.000	0.781

t	sl_wres
0.000	-
5.000	-
5.000	5.48842e-002
6.000	0.312
7.000	-7.81359e-001
10.000	0.973
15.000	-1.09321e+000
20.000	0.902
30.000	5.32685e-002
45.000	-2.31965e-001
60.000	-6.70214e-001
90.000	0.907
120.000	0.824
150.000	-3.75380e-001
180.000	-5.37663e-002
240.000	-1.64735e+000
300.000	1.211
360.000	-
360.000	3.05203e-002

The tables were obtained as follows. Just before you Fit your model to your data:

1. Set the **Minimum Number of Calculation Intervals** equal to “1”.
2. Fit the model to the data.
3. In the **Show** menu, click **Table**, or alternatively, on the **SAAM II Toolbar**, click **Table** . Assuming the **Plot** window is already open with a plot of the weighted residuals, the table shown above will appear.

To perform the runs test, you must calculate the Z variable for each of the two fits. From the table, there are 16 weighted data points used in the “Fit”; thus for the runs test, N = 16.

Calculate the Z variable for the two-compartment model.

- N, the number of data, is 16.
- The number of positive and negative residuals,  $n^+$  and  $n^-$ , is respectively 7 and 9.
- The number of runs R equals 5. (Remember a run is a sequence of positive or negative residuals; a run can consist of only one, or several, positive or negative residuals).
- Estimate the mean, variance and standard deviation from the formulas shown below:

$$\mu = \frac{2n^+n^-}{N} + 1 = 8.88 \quad \sigma^2 = \frac{2n^+n^-(2n^+n^- - N)}{(N-1)N^2} = 3.61 \quad \sigma = 1.90$$

- Calculate the Z variable:

$$Z = \frac{R - \mu}{\sigma} = -2.04$$

For a 5% significance level to see whether the “Fit” should be accepted or not, Z should be in the interval [-1.96,1.96]. Since it is not, the test for goodness-of-fit indicates that the “Fit” cannot be accepted.

Calculate the Z variable for the three-compartment model.

- N, the number of data, is 16.
- The number of positive and negative residuals,  $n^+$  and  $n^-$ , is respectively 9 and 7.
- The number of runs R equals 9. (Remember a run is a sequence of positive or negative residuals; a run can consist of only one, or several, positive or negative residuals).
- Estimate the mean, variance and standard deviation from the formulas shown below:

$$\mu = \frac{2n^+n^-}{N} + 1 = 8.88 \quad \sigma^2 = \frac{2n^+n^-(2n^+n^- - N)}{(N-1)N^2} = 3.61 \quad \sigma = 1.90$$

- Calculate the Z variable:

$$Z = \frac{R - \mu}{\sigma} = -0.06$$

For a 5% significance level to see whether the “Fit” should be accepted or not,  $Z$  should be in the interval  $[-1.96, 1.96]$ . Since it is, the test for goodness-of-fit indicates that the “Fit” can be accepted.

As you saw in this case study, the AIC and BIC indicated that the three-compartment model was most appropriate. The test for goodness-of-fit confirms this conclusion. The important point to remember is that you should take advantage of all available information – parameter precision, results of the runs test and parsimony criteria - in making a final judgment.

## Appendix 3: Contents of the Notes window for this Case Study

Best fit of the two compartment model to the data

	value	SD	Coeff Var	95% Confidence	
V1	10.53187	5.68684e-001	5.39965e+000	9.29281	11.77092
k(0,1)	0.00847	7.19655e-004	8.49714e+000	0.00690	0.01004
k(1,2)	0.03391	2.68275e-003	7.91116e+000	0.02807	0.03976
k(2,1)	0.09954	1.01196e-002	1.01661e+001	0.07749	0.12159
----- Derived Variables -----					
CLe	0.08920	5.21059e-003	5.84157e+000	0.07785	0.10055
CLi	1.04836	6.47016e-002	6.17168e+000	0.90739	1.18934
V2	30.91513	1.42021e+000	4.59390e+000	27.82076	34.00950
Vss	41.44700	1.44750e+000	3.49241e+000	38.29317	44.60082

	Objective	Scaled Data Variance
s1 : Plasma	-1.929837e+000	2.957864e-001
-----		
Total objective	-1.929837e+000	
AIC	2.665203e-001	
BIC	3.872373e-001	

Best fit of a three compartment model to the data

	value	SD	Coeff Var	95% Confidence	
V1	9.35622	6.14758e-001	6.57058e+000	7.98645	10.72599
k(0,1)	0.00837	9.47258e-004	1.13140e+001	0.00626	0.01048
k(1,2)	0.08196	2.84493e-002	3.47103e+001	0.01857	0.14535
k(1,3)	0.01869	4.97213e-003	2.66055e+001	0.00761	0.02977
k(2,1)	0.09310	1.89605e-002	2.03650e+001	0.05086	0.13535
k(3,1)	0.05024	2.12474e-002	4.22957e+001	0.00289	0.09758
----- Derived Variables -----					
CLe	0.07833	6.44875e-003	8.23233e+000	0.06397	0.09270
CLi	0.87110	1.54672e-001	1.77561e+001	0.52646	1.21573
CLi2	0.47001	1.83550e-001	3.90521e+001	0.06104	0.87899
V2	10.62805	4.65224e+000	4.37733e+001	0.26219	20.99390
V3	25.15006	3.98684e+000	1.58522e+001	16.26683	34.03328
Vss	45.13432	2.19006e+000	4.85231e+000	40.25457	50.01407

	Objective	Scaled Data Variance
s1 : Plasma	-2.856578e+000	1.405015e-001
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Total objective	-2.856578e+000	
AIC	-7.185023e-002	
BIC	9.715355e-002	