

Cephalosporine Kinetics in Chinchilla

Case Study

- Using forcing functions in model development
- Developing an integrated system model
- Modeling single-input multiple-output experiments

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Cephalosporine Kinetics in Chinchilla

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 use forcing functions in model development
- How to develop an integrated system model
- How to model single-input multiple-output experiments

Data Required

The data file for this case study is

cephalosporine

This data file is Excel file. The contents of this file are included at the end of this case study. The data are also in a .dat file:

Cephalosporine.dat

Introduction

In this case study, you will develop a model that examines the distribution of an antibiotic (a cephalosporine) between plasma and middle ear fluid (MEF). You will first analyze the data for the drug in plasma using a 2-compartment model. The results will be used to create a forcing function which will represent the plasma concentration that drives the drug into both the right and left middle ear spaces. This will allow us to estimate the clearances into and out of the middle ear in this animal (chinchilla) model.

The data were generated by Ping Ji, a former graduate student in the Department of Pharmaceutics of the University of Minnesota, using animal # 1305. Sampling occurred at 3 sites, from plasma and both middle ears. A constant FSD of 0.05 may be associated with the error, as indicated in the data set

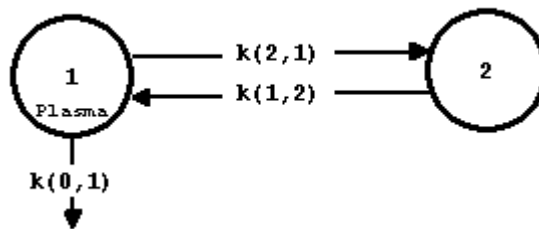
Time is in minutes, mass is in ng/kg, volume is in ml/kg and therefore concentration is ng/ml. The dosing regimen is an infusion in ng/min/kg given over 60 minutes for a total dose of 5mg/kg.

These unpublished data are from Ping Ji's thesis, University of Minnesota, 2004.

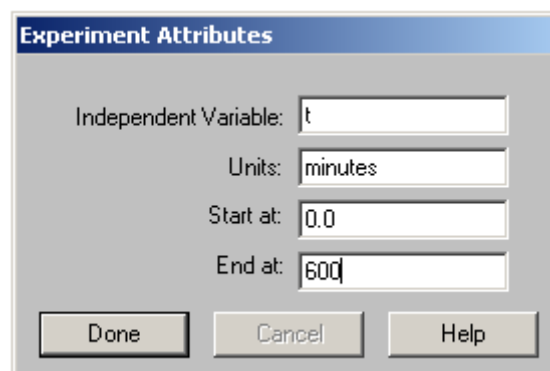
Part 1. Create the model for plasma cephalosporine kinetics.

The first step will be to create the model for plasma cephalosporin kinetics. Prior knowledge of cephalosporin metabolism indicates you should start your analysis using a two-compartment model.

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. Create the following system model on the **Drawing** canvas:

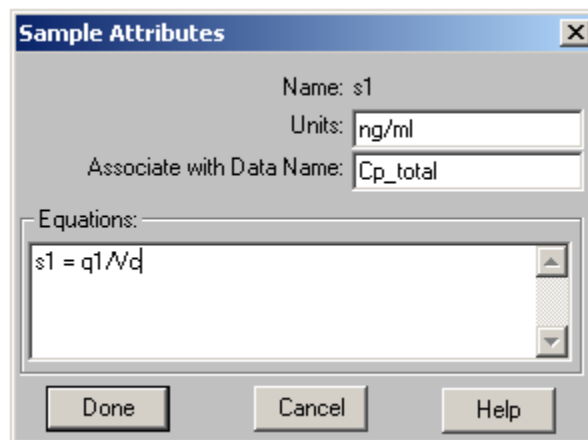


3. In the **SAAM II Toolbox**, click **Experiment**. Notice that the **Model** tools are unavailable and the **Experiment** tools are available. The **Experiment Attributes** dialog box will open.
 - a. Leave the entry in the **Units** box as “minutes”.
 - b. Enter “600” in the **End at** box. The **Experiment Attributes** dialog box will appear as follows:

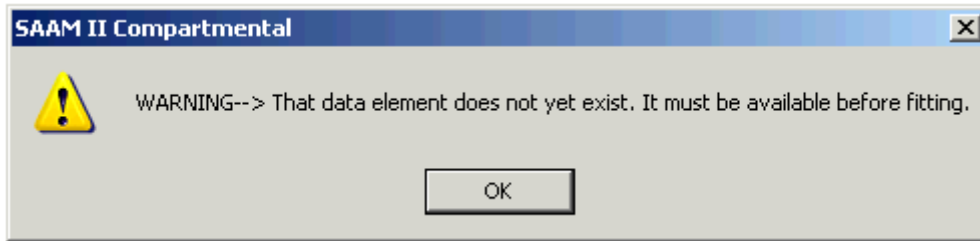


When you examine your data file, you will see the sampling schedule for plasma and the middle ears is different. Setting the **End at** time equal to 600

- will allow you to visualize the model predictions beyond the time frame of the experiment.
- c. Click **Done**. The **Create Experiment** dialog box will appear on the **Drawing Canvas**.
 - d. Click **Create**.
4. 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 “ng/ml” in the **Units** box.
 - e. Type “Cp_total” in the **Associate with Data Name** box.
 - f. Edit the sample equation “s1 = q1” to read “s1 = q1/Vc”. The **Sample Attributes** dialog box will appear as follows:



- g. Click **Done**. The following Warning message will appear:



Remember the Warning message appears because you have not entered your data yet.

- h. Click **OK**.
5. Create an input.
- 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. Type “input” in the **Name** box.
 - e. Type “ng/min/kg” in the **Units** box.
 - f. Click **Infusion** as the **Input Type**.
 - g. Enter “85333” in the **Constant Rate** box.
 - h. Enter “0” in the **Event Start** box.
 - i. Enter “60” in the **Event Stop** box.
 - j. Click **Add**. The **Exogenous Input** dialog box will appear as follows:

Exogenous Input

Name: Reference Name: Units:

Type	Initial	Constant	Start	Stop	Repeat Every	Nr. Repeats
Infusion	-	8.53e+4	0.000	60.000	-	-

Input Type:

Bolus
 Infusion
 Primed Infusion
 Equation

Initial Amount:

Constant Rate:

Event Start:

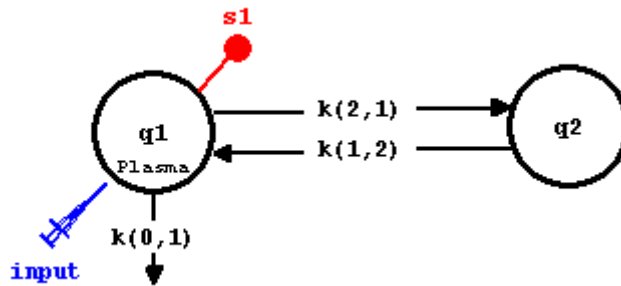
Event Stop:

Repeat Every:


Nr. of Repeats:

Equation:

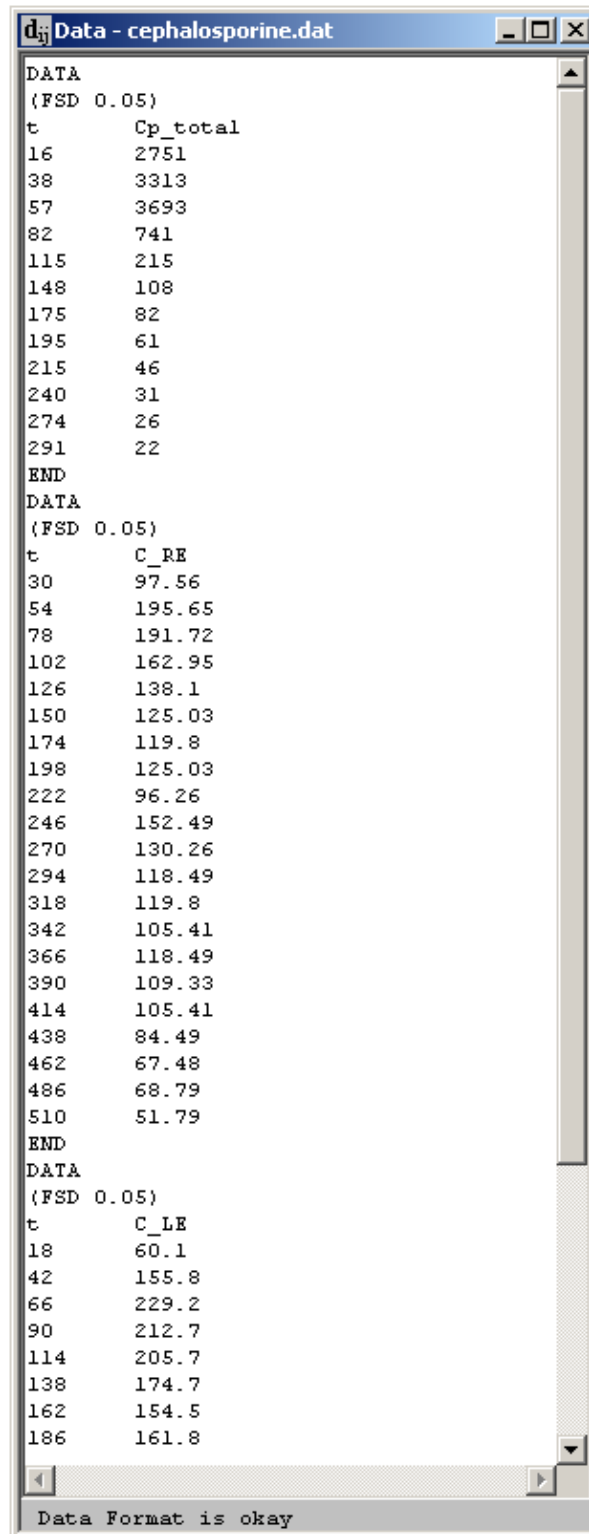
k. Click **Done**. The model will appear as follows:



Notice the name of your input syringe has changed from **ex1** to **input**; this is because you changed the **Name** in the **Exogenous Input** dialog box.

6. Add the data to your model.
 - a. In the **Show** menu, click **Data**, or alternatively, on the **SAAM II Toolbar**, click **Data** . The **Data** window will open.
 - b. In the **File** menu, click **Open**. The file **Cephalosporine** should appear in the list (if it does not, find the folder where you put this data file).

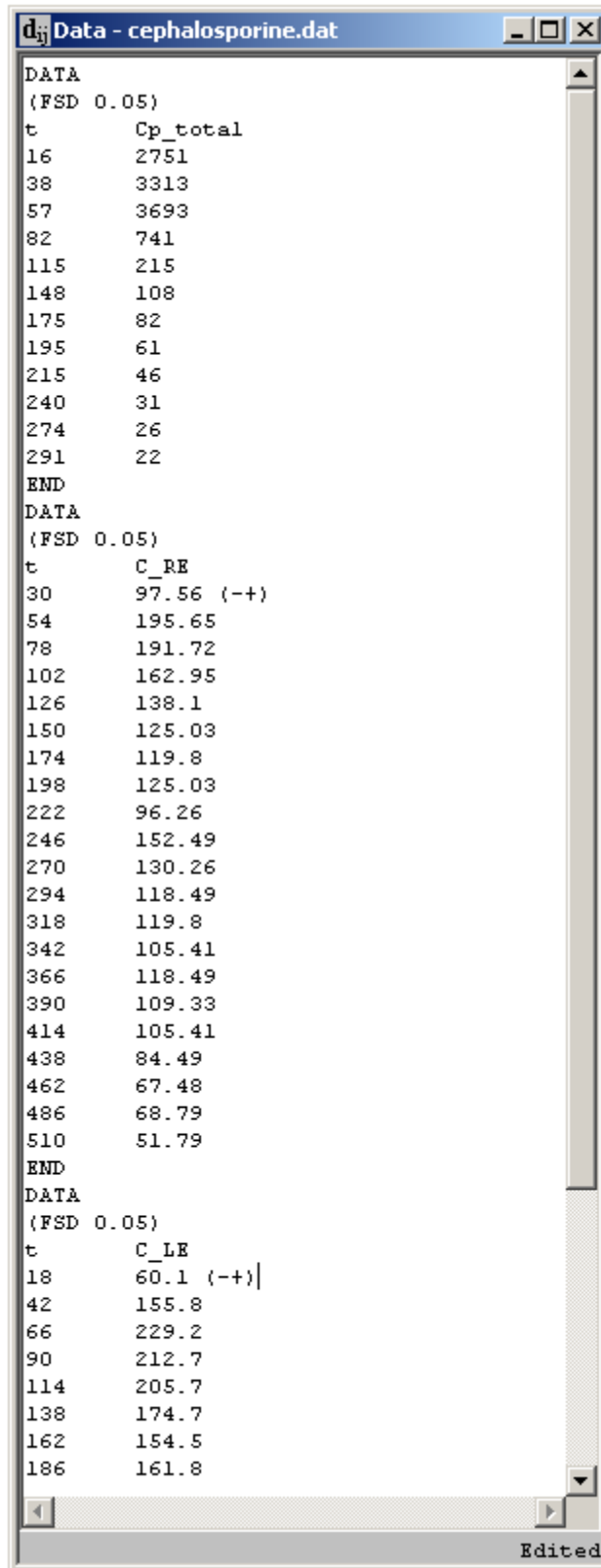
- c. Double-click **Cephalosporine**. The **Data** window should appear in part as follows:



```
d:\Data - cephalosporine.dat
DATA
(FSD 0.05)
t      Cp_total
16     2751
38     3313
57     3693
82     741
115    215
148    108
175    82
195    61
215    46
240    31
274    26
291    22
END
DATA
(FSD 0.05)
t      C_RE
30     97.56
54     195.65
78     191.72
102    162.95
126    138.1
150    125.03
174    119.8
198    125.03
222    96.26
246    152.49
270    130.26
294    118.49
318    119.8
342    105.41
366    118.49
390    109.33
414    105.41
438    84.49
462    67.48
486    68.79
510    51.79
END
DATA
(FSD 0.05)
t      C_LE
18     60.1
42     155.8
66     229.2
90     212.7
114    205.7
138    174.7
162    154.5
186    161.8
Data Format is okay
```

If you wish, you could have opened the Excel spreadsheet, and copied the data directly from the spread sheet into the **Data** window.

- d. Since the first part of your modeling exercise is to examine the plasma data only, you need to remove the right, C_RE, and left, C_LE, ear data. For the right ear, after the datum at 30 minutes, type “(-+)”. For the left ear, after the datum at 18 minutes, type “(-+)”. The **Data** window should appear in part as follows:



The screenshot shows a window titled "Data - cephalosporine.dat" with three data tables. Each table is preceded by "DATA" and "(FSD 0.05)".

t	Cp_total
16	2751
38	3313
57	3693
82	741
115	215
148	108
175	82
195	61
215	46
240	31
274	26
291	22

END

t	C_RE
30	97.56 (-+)
54	195.65
78	191.72
102	162.95
126	138.1
150	125.03
174	119.8
198	125.03
222	96.26
246	152.49
270	130.26
294	118.49
318	119.8
342	105.41
366	118.49
390	109.33
414	105.41
438	84.49
462	67.48
486	68.79
510	51.79

END

t	C_LE
18	60.1 (-+)
42	155.8
66	229.2
90	212.7
114	205.7
138	174.7
162	154.5
186	161.8

Edited


e. Close the **Data** window.

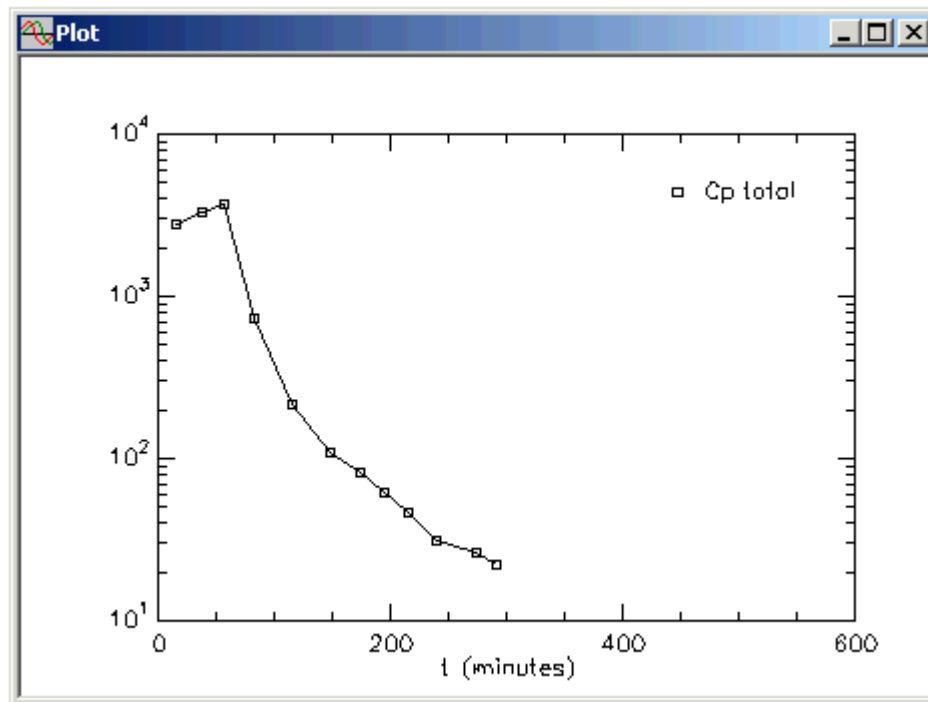
7. Obtain initial parameter estimates and enter these estimates.

For the plasma model, there are four parameters to estimate, the volume V_c and the three rate constants, $k(2,1)$, $k(1,2)$ and $k(0,1)$.

- a. Obtain initial parameter estimates.

To obtain initial parameter estimates, you will look first at the plasma data.

- (1) In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . The **Plot and Table Variables** dialog box will open. Be sure the **List All Variables** check box is not selected.
- (2) Click **s1:Cp_total** to move these to the **Current Selection** pane.
- (3) Click **Done**.
- (4) In the View menu, click both **Semilog** and **Line Plot**. Your plot should appear as follows:



You can see that the biphasic decay following the 60 minute infusion supports the choice of the two-compartment model.

You can estimate parameter values roughly based on the data, or use trial and error. In order to obtain a rough estimate of the initial (starting) values for V_c and $k(0,1)$, consider the fact that their product will be the clearance. If the AUC from 0 to infinity can be approximated from the linear-linear plot as the area of a corresponding rectangle, we can estimate the clearance as the dose /AUC. The AUC *roughly* appears to be about 3000 ng/ml (height) x 100 min (width) = 300,000 ng-min/ml. Then the estimated clearance would be:

$$\text{Estimated clearance} = 5,000,000 \text{ ng per kg} / 300,000 \text{ ng-min/ml} = 17 \text{ ml/min/kg} = k(0,1) * V_c$$

Using a value somewhere between 100 and 500 ml/kg for the V_c (typical values for cephalosporine), you would use a corresponding initial estimate for $k(0,1)$ of


$$k(0,1) = \text{clearance} / V_c = 0.17 \text{ to } 0.034 \text{ per min.}$$

Estimates for $k(2,1)$ and $k(1,2)$ can be obtained as the inverse of the time it takes to go from the maximal plasma concentration, approximately 3600, to half this value, 1800. The time is approximately 50 minutes (from the above plot), so estimates of .02 can be used.



The half-time method for parameter estimation. The half-time method for parameter estimation is discussed in the case study on Hydromorphone.



- b. In the **Show** menu, click **Parameters**, or alternatively, on the **SAAM II Toolbar**, click **Parameters** . The **Parameters** dialog box will open as shown below:

Name	Type	Current	Low Limit	High Limit
Vc	Adj			
k(0,1)	Adj			
k(1,2)	Adj			
k(2,1)	Adj			

Name: Vc Value:

Type: Fixed Low Limit:

Adjustable High Limit:

- c. Enter the following initial values for each of the model parameters as shown in the following:



Name	Type	Current	Low Limit	High Limit
Vc	Adj	300.0000	30.0000	3000.0000
k(0,1)	Adj	0.1000	0.0100	1.0000
k(1,2)	Adj	0.0200	0.0020	0.2000
k(2,1)	Adj	0.0200	0.0020	0.2000

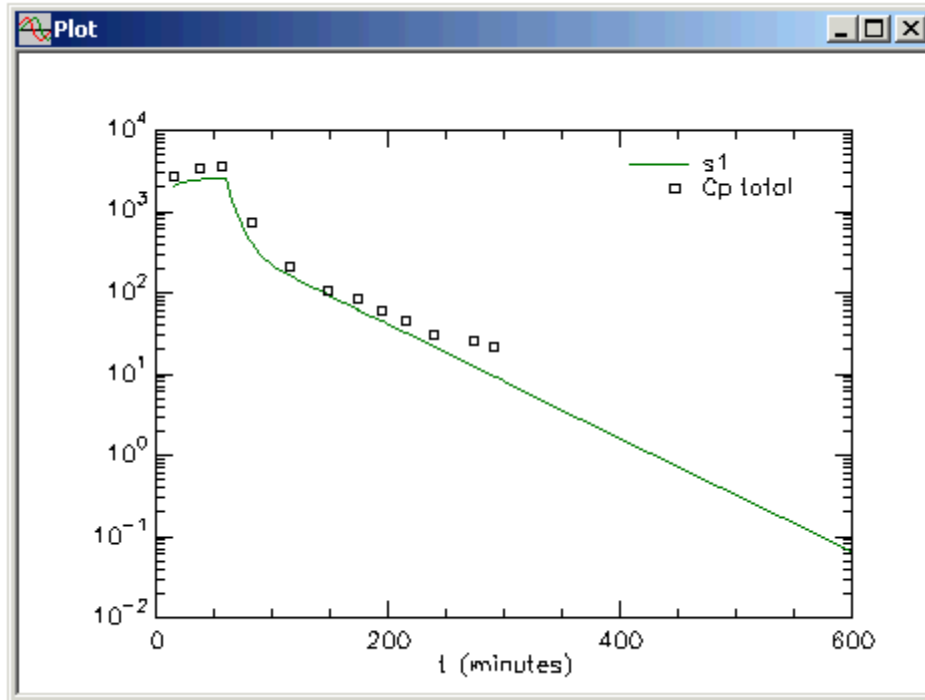
Name: k(1,2) Value: .02

Type: Fixed Adjustable

Low Limit: 0.00200000 High Limit: 0.20000000


Buttons: Done, Cancel, Help, Edit, Save

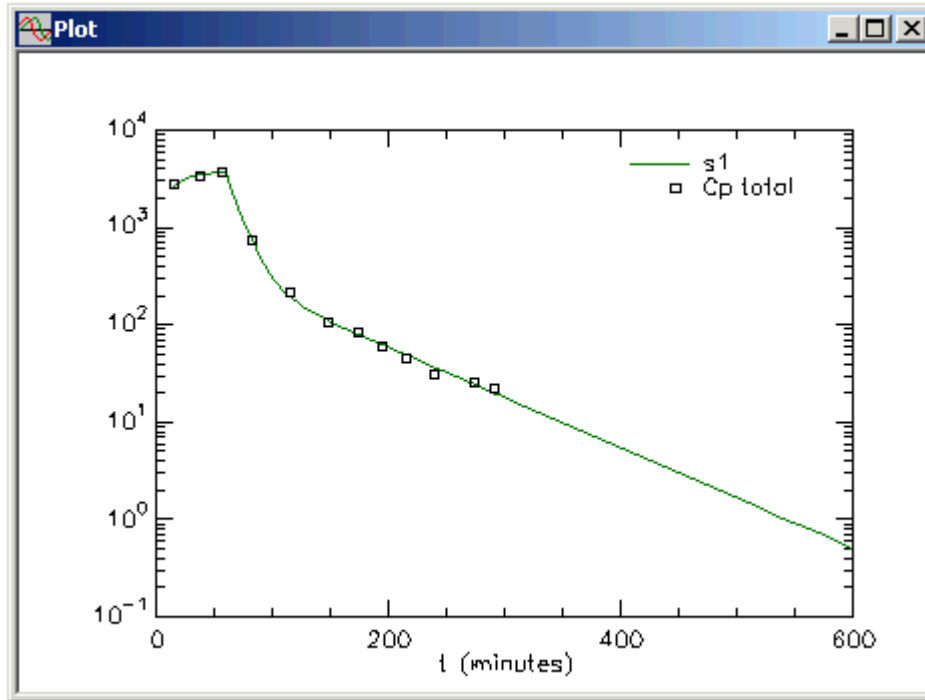
- d. Click **Done**.
8. Solve your model and view the solution.
 - a. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II Toolbar**, click **Solve** .
 - b. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . Because you have plotted **s1:Cp_total** previously (in semilog mode), your plot will appear as follows:



You can see that the initial parameter estimates are quite reasonable.

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** . When you have “Fitted” your model to your data, your plot will be updated as follows:



The Fit actually appears quite reasonable.

- b. View the statistics. The statistics associated with the fit are shown below:

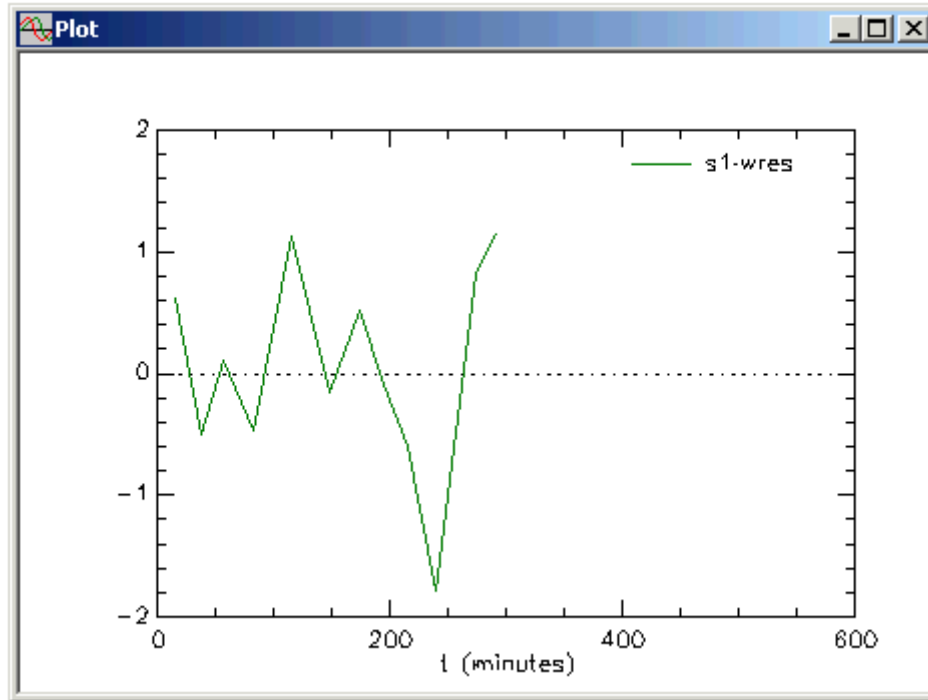
Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
Vc	295.17640	2.55806e+001	8.66620e+000	236.18759	354.16520
k(0,1)	0.07247	4.57120e-003	6.30804e+000	0.06193	0.08301
k(1,2)	0.01361	9.56495e-004	7.02887e+000	0.01140	0.01581
k(2,1)	0.00895	1.17341e-003	1.31151e+001	0.00624	0.01165

Parameter/Variable	Objective	Scaled Data Variance
s1 : Cp_total	6.301888e+000	3.299843e+000
Total objective	6.301888e+000	
AIC	4.486549e+000	
BIC	4.587571e+000	

Close the **Statistics** window; leave the **Plot** window open.

- c. View the weighted residuals.

The best way to visualize any potential problems with this model fit is to examine the weighted residuals (**s1_wres**). If you plot these (Y – Axis minimum and maximum of -2 and 2 respectively), you will obtain the following plot (you need to change the plot to linear):



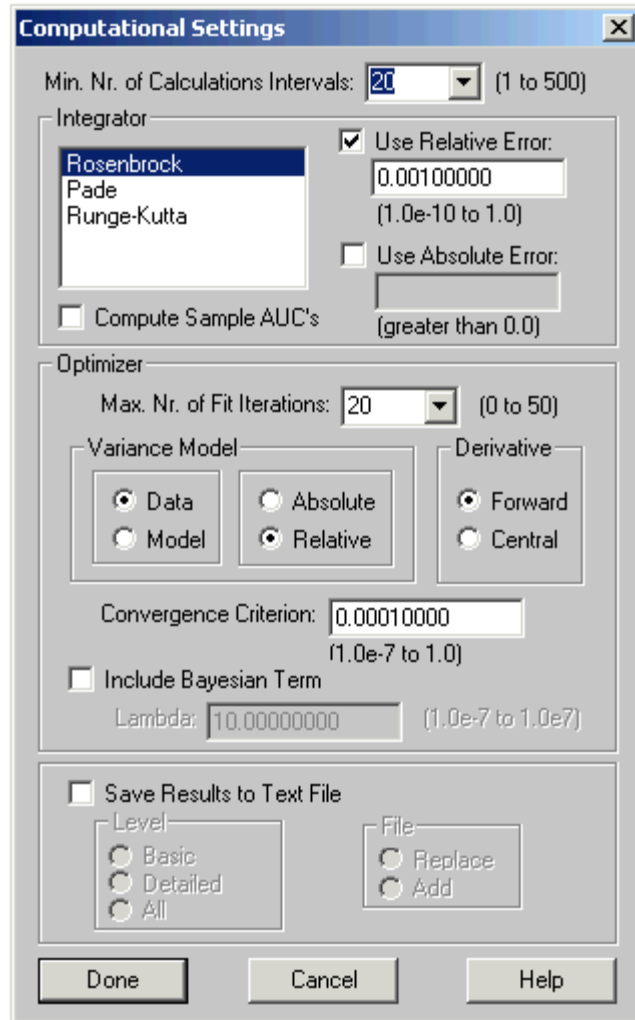
Return to the plot of **s1** and **Cp_total**. Close all open windows.



Stability of the parameter precision. To be sure of your statistical precision, you can refit the model the data, and see if you converge to the same results. Alternatively you can adopt the following strategy.

As part of the Fitting procedure, SAAM II calculated what are called numerical derivatives. These are approximations, and for the sake of speed and efficiency, the default in SAAM II is the “forward difference” method. The slower but often more accurate method is the “central difference” method.

An effective strategy, especially if your model is complicated, is first to fit using the forward difference method. Then, to estimate the final values, change to the central difference method. This is done in the **Computational Settings** dialog box in the **Compute** menu. This box appears as follows:



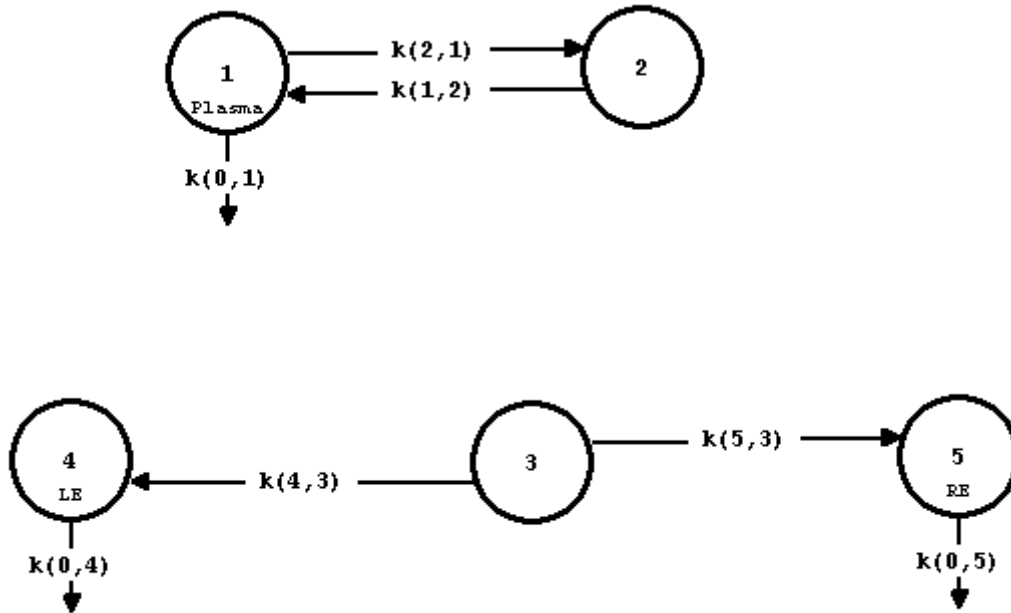
In the **Optimizer** box, you see the **Derivative** options. The **Forward** radio button is clicked. If you click “**Central**”, the method will change to the central. You can then fit your model to your data, and compare results. If you use the central method, then you should, in theory, always converge to the same answer if your model is well-posed.



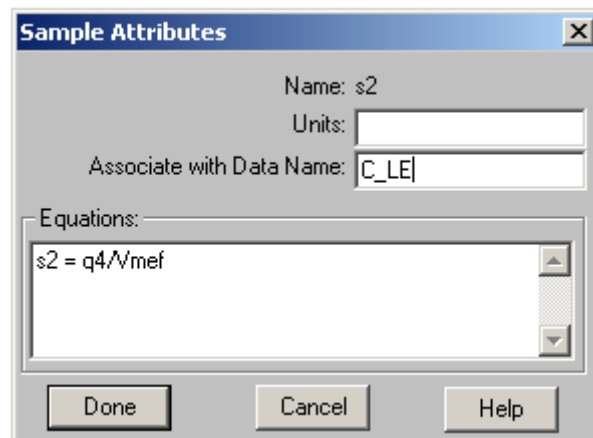
Part 2. Create a Forcing Function to analyze the middle ear data

In this part of the Case Study, you will use the model calculated plasma data as a forcing function to model the middle ear data.

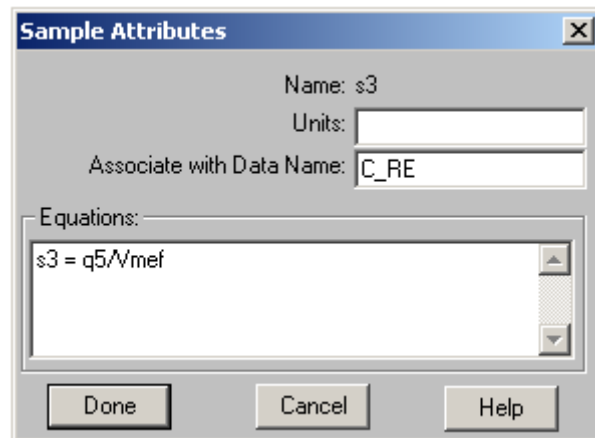
1. Create the middle ear data model.
 - a. In the **SAAM II Toolbox**, click **Model**. Add the middle ear data model as follows:



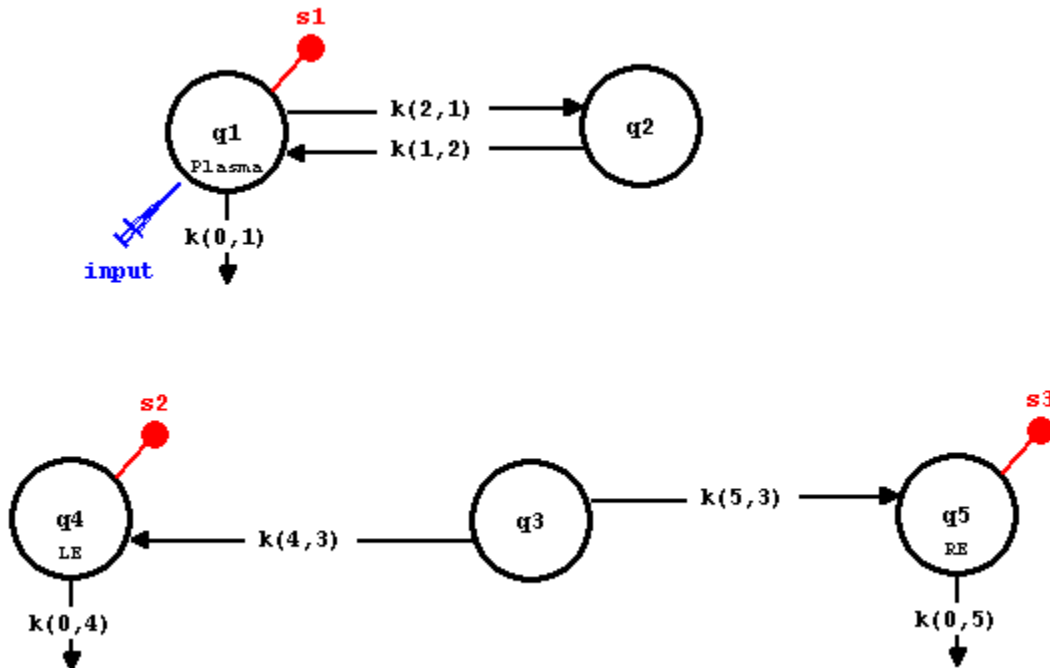
- b. In the **SAAM II Toolbox**, click **Experiment**.
- c. In the **SAAM II Toolbox**, click **Sample**. Click compartment **q4** and the **Drawing Canvas**. This will create sample **s2**.
- d. Open the **Sample Attributes** dialog box associated with **s2**. Associate the sample with **C_LE**, and edit the sample equation to read $s2 = q4/V_{mef}$. The **Sample Attributes** dialog box will appear as follows:



- e. Click **Done**.
- f. Create a sample on compartment **q5**, and associate this sample with **C_RE**. The **Sample Attributes** dialog box should appear as follows:

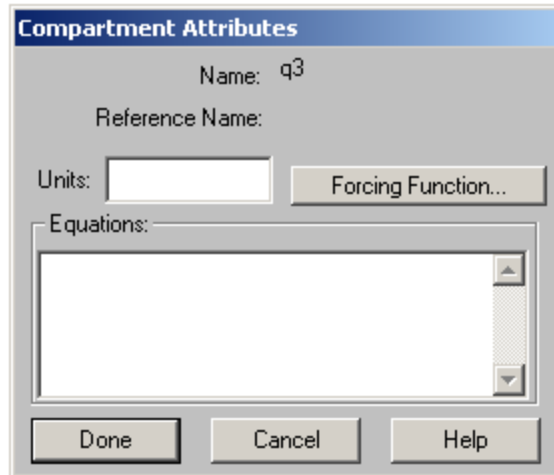


g. Click **Done**. Your model will appear as follows:

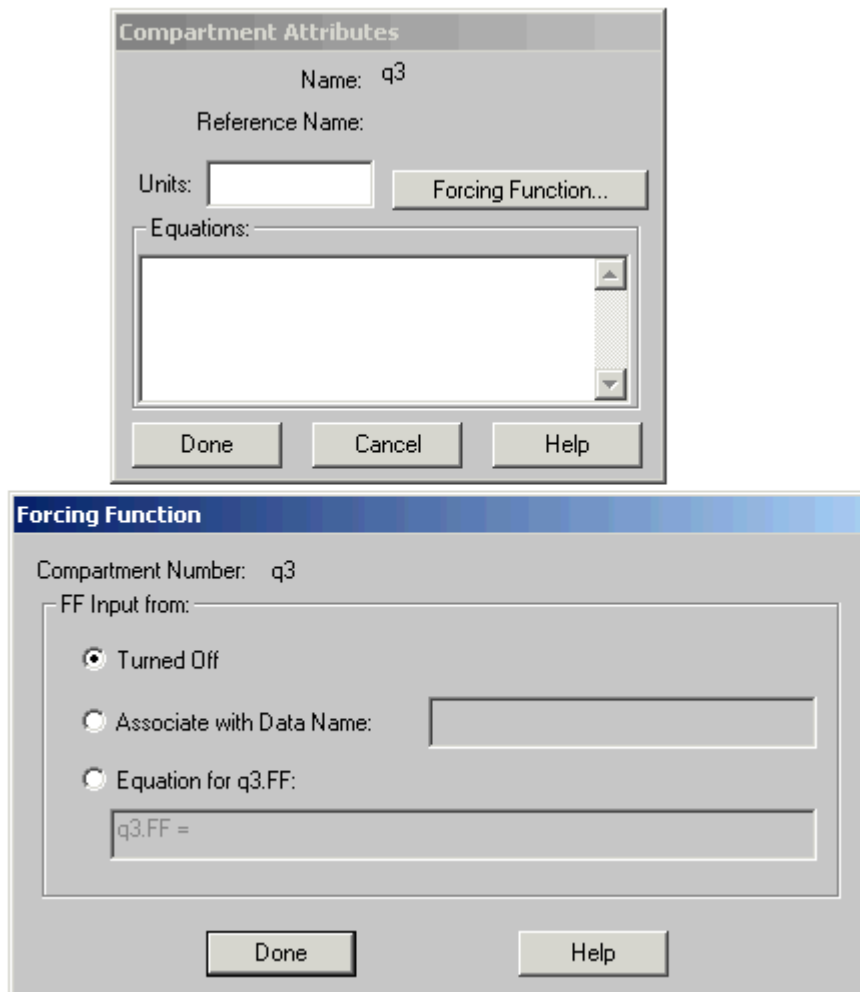


2. Create the forcing function.

- a. Double-click compartment **q3** to open the **Compartment Attributes** dialog box. The dialog box will appear as follows:

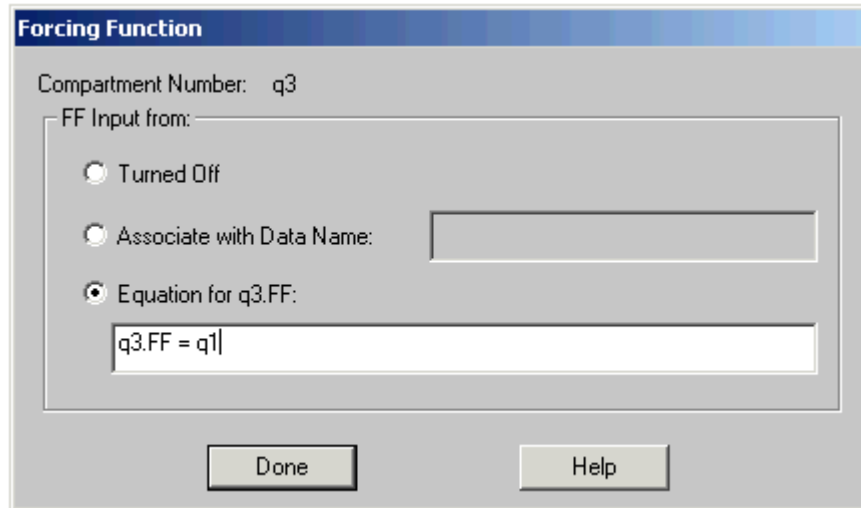


- b. Click **Forcing Function**. The **Forcing Function** dialog box will open as follows (with the **Compartment Attributes** dialog box):

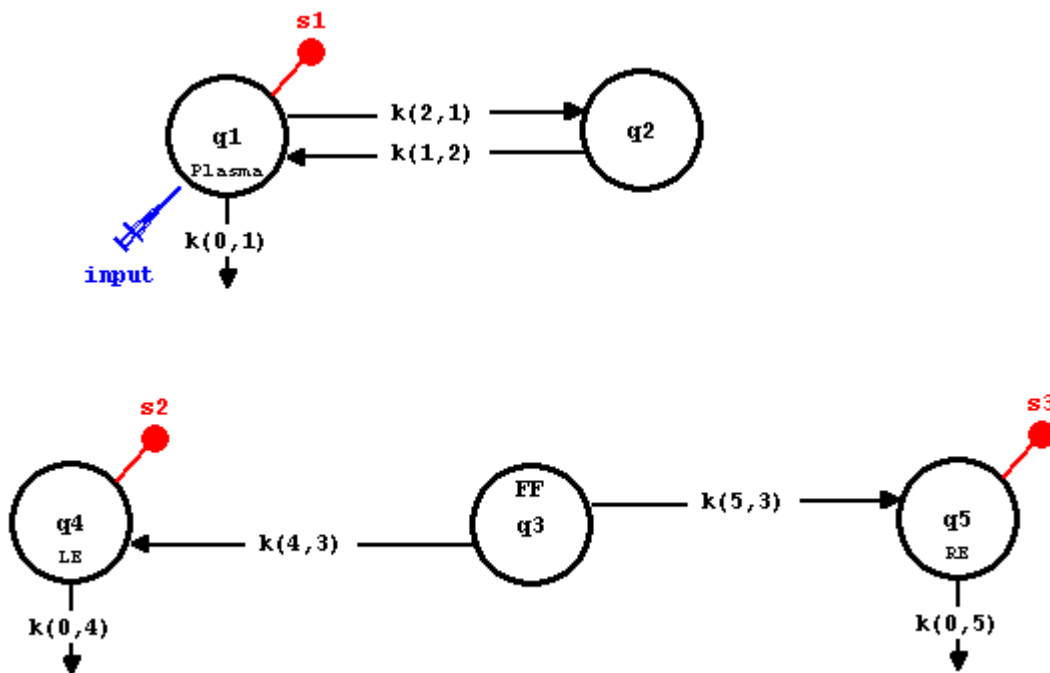


- c. Click the “**Equation for q3.FF**” radio button.

- d. Type “q3.FF = q1” in the **Equation** pane. The **Forcing Function** dialog box will appear as follows:



- e. Click **Done** first on the **Forcing Function** dialog box, and then the **Compartment Attributes** dialog box. Your model will appear as follows:



Notice the “FF” associated with compartment **q3**; this is to remind you that compartment **q3** now assumes the role of a forcing function.



Defining forcing functions. There are two ways in this case study that compartment **q3** can be defined as a forcing function. The method used here uses the model calculated value for plasma, **q1**. The advantage to this is that the forcing function is a smooth function.

An alternate way is to create a new data stream, and to use the data option (linear interpolation) to create the forcing function. To do this, you would open the table associated with **s1**. Depending upon the **Min Nr of Computational Intervals** you specify in the **Computational Settings** dialog box, this table can be extremely data rich. This table can be pasted into your **Data** window, and the “s1” column renamed to something like “ff”. The **Forcing Function** dialog box would appear:

You will have to be sure that the units are all correct. Remember the units of **q1** are ng/kg while the units for **s1** are ng/ml.

Finally, remember the role of the forcing function is to make sure compartments connected to the forcing function compartment “see” the forcing function and not the compartment. Thus the differential equation for **q4** is

$$\frac{dq_4}{dt} = k(4,3) \cdot q3.FF - k(0,4) \cdot q_4$$

and not

$$\frac{dq_4}{dt} = k(4,3) \cdot q_3 - k(0,4) \cdot q_4$$

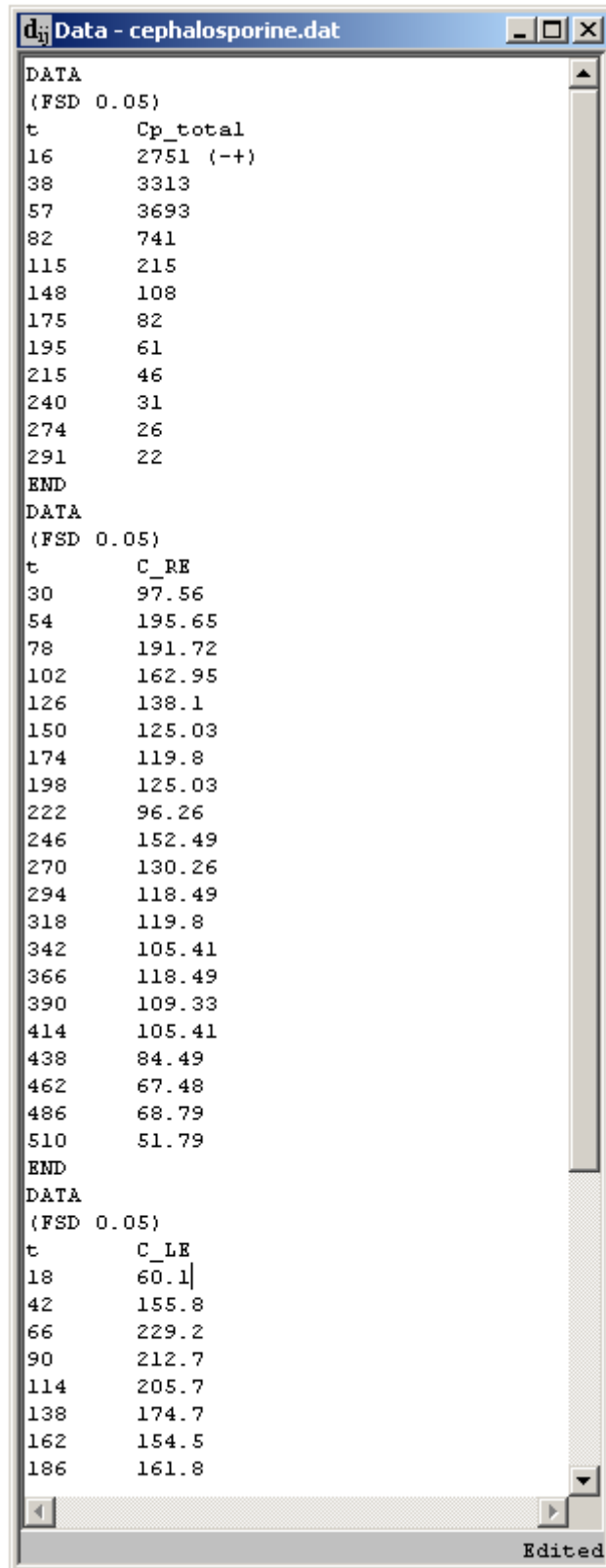
Since “ $q_3 = q_1$ ”, the actual differential equation is:

$$\frac{dq_4}{dt} = k_{43} \cdot q_1 - k_{40} \cdot q_4$$



3. Modify the data.

In the first part of the case study, you examined only the plasma data, and unweighted the middle ear data. Now you want to unweight the plasma data, and weight the middle ear data. Your modified data file should appear in part as follows:



DATA
(FSD 0.05)

t	Cp_total
16	2751 (-+)
38	3313
57	3693
82	741
115	215
148	108
175	82
195	61
215	46
240	31
274	26
291	22

END

DATA
(FSD 0.05)

t	C_RE
30	97.56
54	195.65
78	191.72
102	162.95
126	138.1
150	125.03
174	119.8
198	125.03
222	96.26
246	152.49
270	130.26
294	118.49
318	119.8
342	105.41
366	118.49
390	109.33
414	105.41
438	84.49
462	67.48
486	68.79
510	51.79

END


DATA
(FSD 0.05)

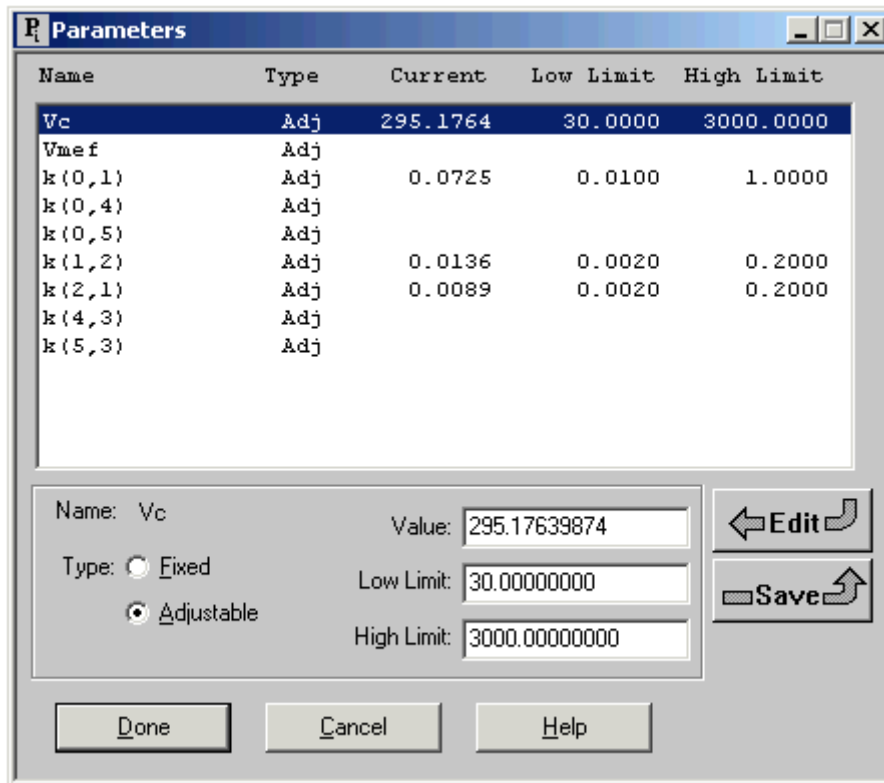
t	C_LE
18	60.1
42	155.8
66	229.2
90	212.7
114	205.7
138	174.7
162	154.5
186	161.8

Edited

4. Enter the parameter values.

Since **q1** is now a forcing function, you will want to fix the four parameters in this model. You will assume V_{mef} is known. The unknown parameters are the rate constants $k(4,3)$, $k(0,4)$, $k(5,3)$ and $k(0,5)$.

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



Name	Type	Current	Low Limit	High Limit
Vc	Adj	295.1764	30.0000	3000.0000
Vmef	Adj			
k(0,1)	Adj	0.0725	0.0100	1.0000
k(0,4)	Adj			
k(0,5)	Adj			
k(1,2)	Adj	0.0136	0.0020	0.2000
k(2,1)	Adj	0.0089	0.0020	0.2000
k(4,3)	Adj			
k(5,3)	Adj			

Name: Vc Value: 295.17639874

Type: Fixed Adjustable

Low Limit: 30.00000000

High Limit: 3000.00000000

Buttons: Edit, Save, Done, Cancel, Help

- b. Fix the parameters for the plasma model.
- c. Enter a value for V_{mef} equal to 1.64 ml/kg. This is approximately 1 ml in a 500g chinchilla, and was fixed based on the exact weight of the animal and the experimental conditions. Your **Parameters** dialog box should appear as follows:

Name	Type	Current	Low Limit	High Limit
Vc	Fix	295.1764		
Vmef	Fix	1.6400		
k(0,1)	Fix	0.0725		
k(0,4)	Adj			
k(0,5)	Adj			
k(1,2)	Fix	0.0136		
k(2,1)	Fix	0.0089		
k(4,3)	Adj			
k(5,3)	Adj			

Name: Vmef Value: 1.64

Type: Fixed Adjustable

Low Limit: 0.16400000 High Limit: 16.40000000

Buttons: Edit, Save, Done, Cancel, Help

- d. Enter the parameter estimates for the middle ear kinetics.

Although one might suspect that the kinetics for each ear are the same in which case the losses and transfers could be set equal to each other, in this model you will assume this is not the case, and let them be separate.

In addition, although the measurements of cephalosporine concentration in both ears is very good, the total amount of mass in each ear is very small compared to the rest of the body. Thus the transfer rate constants will be very small. The loss, however, will be somewhat larger in order to describe the kinetics.

You may have to hand-fit to obtain reasonable initial estimates. You can try 0.00001 as an initial estimate for both uptake rate constants $k(4,3)$ and $k(5,3)$, and 0.01 for the two losses $k(0,3)$ and $k(0,4)$. Your **Parameters** dialog box will appear as follows:



Name	Type	Current	Low Limit	High Limit
Vc	Fix	295.1764		
Vmef	Fix	1.6400		
k(0,1)	Fix	0.0725		
k(0,4)	Adj	0.0100	0.0010	0.1000
k(0,5)	Adj	0.0100	0.0010	0.1000
k(1,2)	Fix	0.0136		
k(2,1)	Fix	0.0089		
k(4,3)	Adj	1.000e-005	1.000e-006	1.000e-004
k(5,3)	Adj	1.000e-005	1.000e-006	1.000e-004

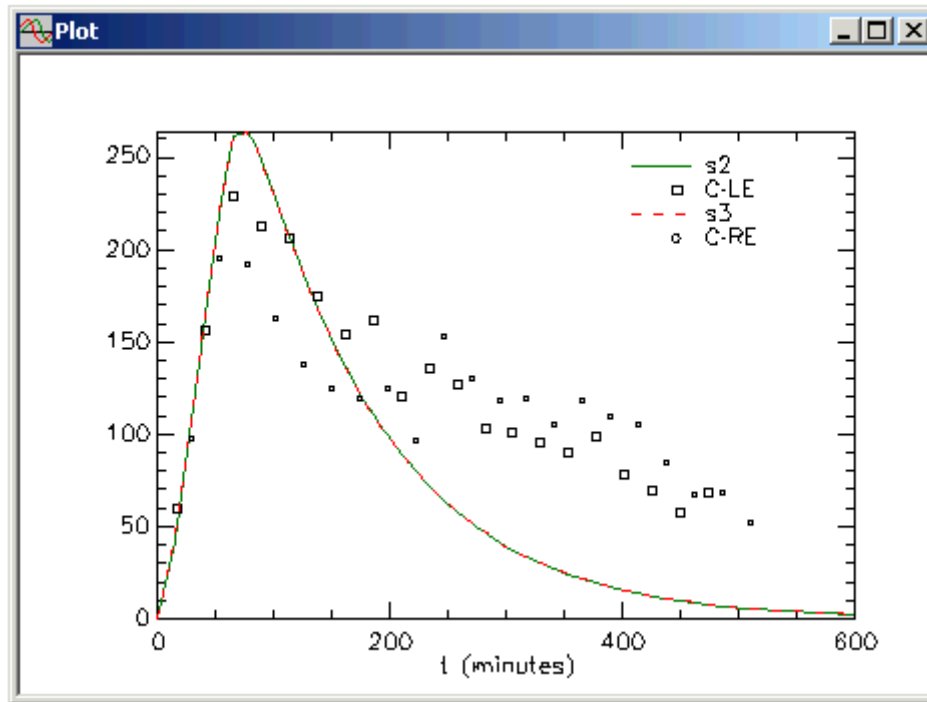
Name: k(4,3) Value: .00001

Type: Fixed Adjustable

Low Limit: 1.0000000e-006 High Limit: 0.00010000


Buttons: Done, Cancel, Help, Edit, Save

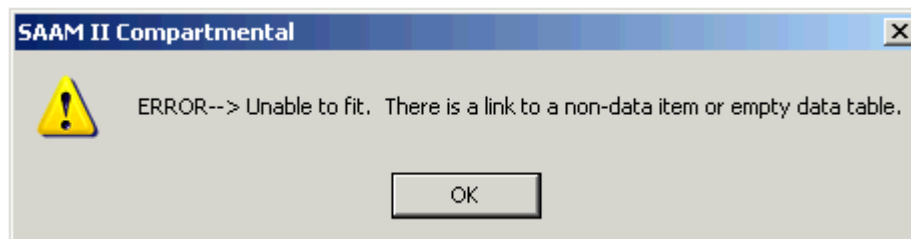
- e. Click **Done**.
5. Solve your model and view the solution.
 - a. In the **Compute** menu, click **Solve**, or alternatively, on the **SAAM II Toolbar**, click **Solve** .
 - b. In the **Show** menu, click **Plot**, or alternatively, on the **SAAM II Toolbar**, click **Plot** . Depending upon your previous plot, you will probably need to change the variables in the **Current Selection** pane in the **Plot and Table Variables** dialog box to **s2**, **s3**, **C_LE**, and **C_RE**. Your plot should appear as follows (in linear mode):



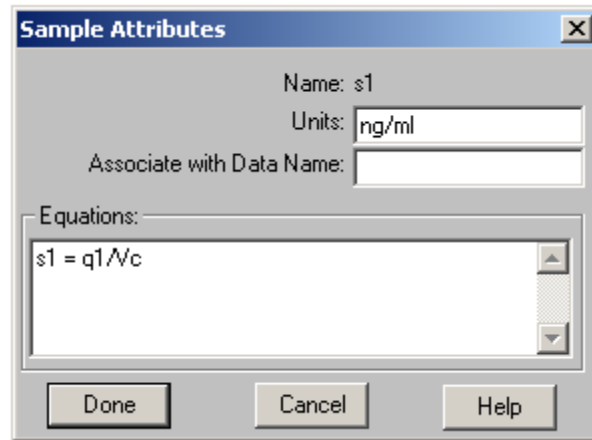
You can see that the initial parameter estimates are quite reasonable. You can try hand-fitting if you wish to see if you can improve the estimates.

Leave the **Plot** window open.

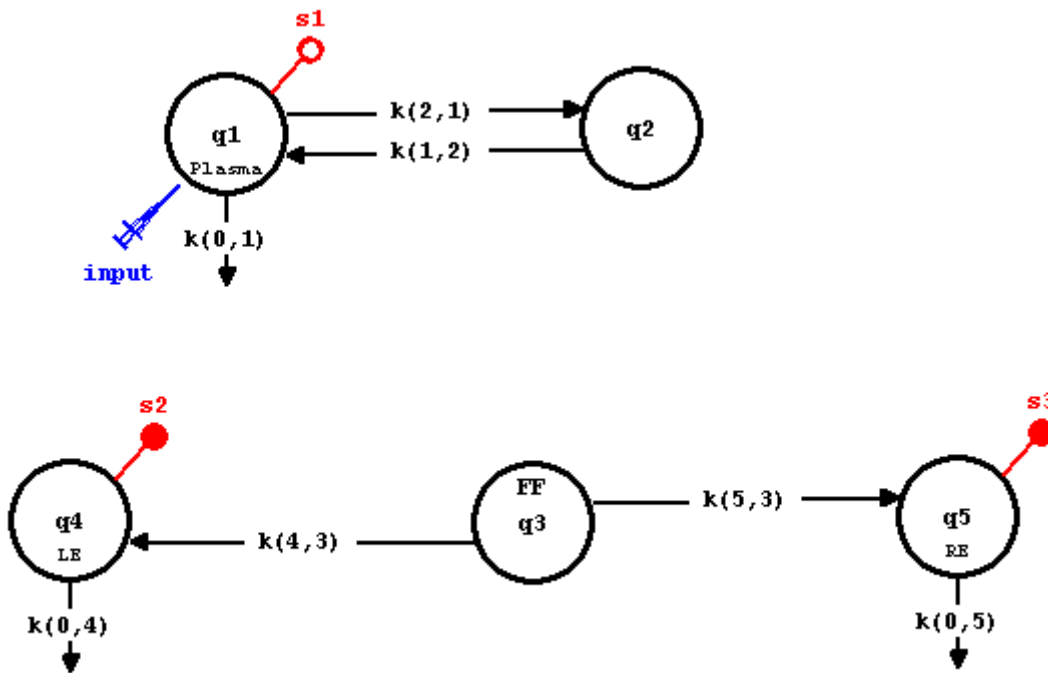
6. Fit the model to the data and view the solution.
 - a. In the **Compute** menu, click **Fit**, or alternatively, on the **SAAM II Toolbar**, click **Fit** . The following Error message will appear:



What is happening? Even though the parameters for the plasma model are fixed, there is still the association between **s1** and **Cp_total**. SAAM II thinks there should be weighted data in order to fit. But you have unweighted the **Cp_total** data since it should not be involved in the fit. To correct this error, you must open the **Sample Attributes** dialog box associated with **s1**, and delete **Cp_total**. The **Sample Attributes** dialog box will appear as follows:



Your model will appear as follows:



Notice the bullet associated with **s1** is open; this is because there are no longer data associated with this sample.

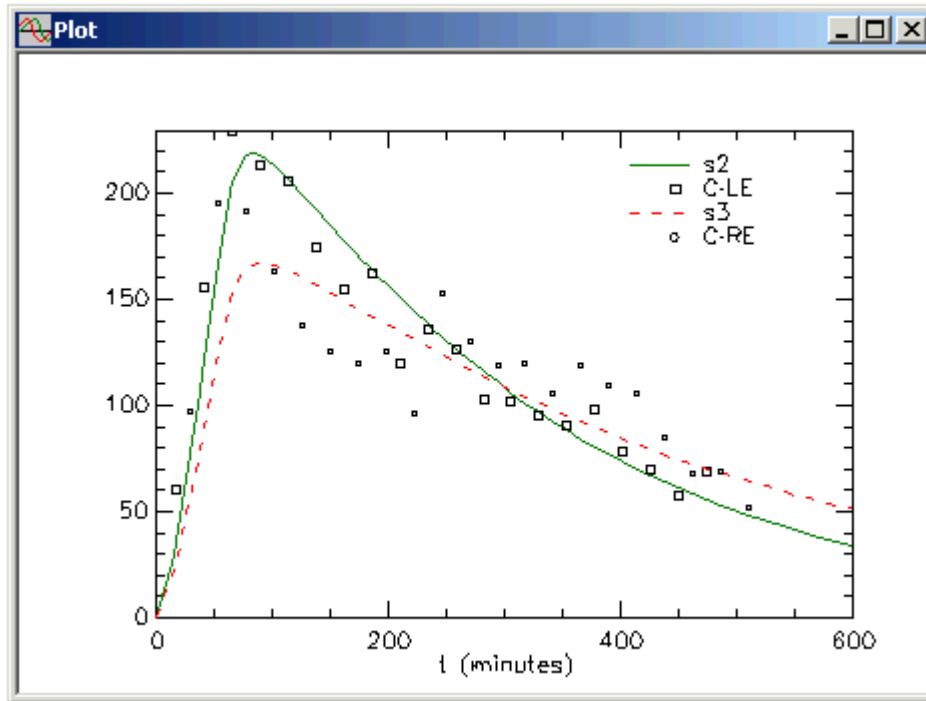



The forcing function and decoupling of the system. This case study illustrates how the forcing function can be used to decouple the system. In this case, drug enters the middle ear only from plasma, so once the plasma is correctly described by a model, one can concentrate on the middle ear models.

To be correct, then, you need only the shape of the plasma curve; the statistics associated with this shape should not be included in the statistics of the middle ear models.



- b. Re-Fit your model to the data. When you have “Fitted” your model to your data, your plot should be updated as follow:



- c. In the **Show** menu, click **Statistics**, or alternatively, on the **SAAM II Toolbar**, click **Statistics** . The **Statistics** window will appear as follows (you may need to scroll a little to see $k(5,3)$):

The screenshot shows the 'Statistics' window in SAAM II. It displays a table of parameter estimates and objective function values. The parameters listed are V_{mef} , $k(0,1)$, $k(0,4)$, $k(0,5)$, $k(1,2)$, $k(2,1)$, $k(4,3)$, and $k(5,3)$. The values for $k(0,4)$, $k(0,5)$, $k(4,3)$, and $k(5,3)$ are 0.00394, 0.00260, 6.67869e-006, and 4.81833e-006, respectively. The objective function values are also shown, including the total objective, AIC, and BIC.

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
V_{mef}	1.64000	** Fixed **	** Fixed **	** Fixed **	** Fixed **
$k(0,1)$	0.07247	** Fixed **	** Fixed **	** Fixed **	** Fixed **
$k(0,4)$	0.00394	2.93766e-004	7.44924e+000	0.00335	0.00454
$k(0,5)$	0.00260	3.55821e-004	1.37054e+001	0.00187	0.00332
$k(1,2)$	0.01361	** Fixed **	** Fixed **	** Fixed **	** Fixed **
$k(2,1)$	0.00895	** Fixed **	** Fixed **	** Fixed **	** Fixed **
$k(4,3)$	6.67869e-006	4.48978e-007	6.72254e+000	5.76842e-006	7.58896e-006
$k(5,3)$	4.81833e-006	4.45504e-007	9.24604e+000	3.91509e-006	5.72156e-006

	Objective	Scaled Data Variance
s3 : C_RE	3.725014e+000	1.850228e+001
s2 : C_LE	3.229628e+000	9.262909e+000

Total objective	6.954642e+000	
AIC	4.542601e+000	
BIC	4.667984e+000	

Record the values for $k(0,4)$, $k(0,5)$, $k(4,3)$ and $k(5,3)$; you will use these in Part 3.

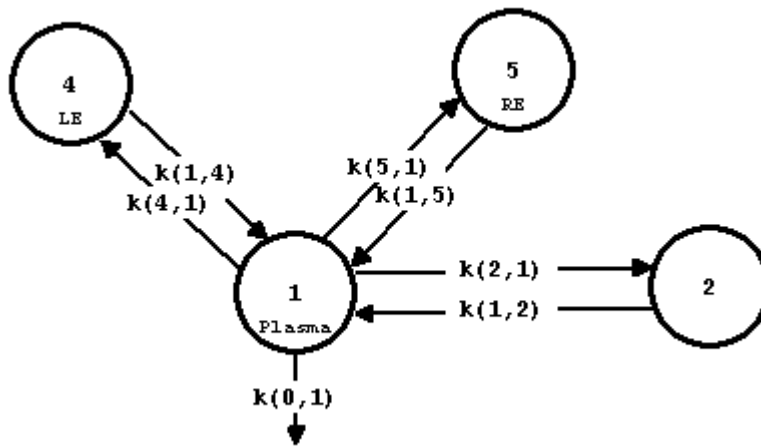
- c. Close all open windows.

At this point in your modeling exercise, you have a model which describes plasma and one which describes drug kinetics in both middle ears. It remains to put all this information together in an integrated system model.

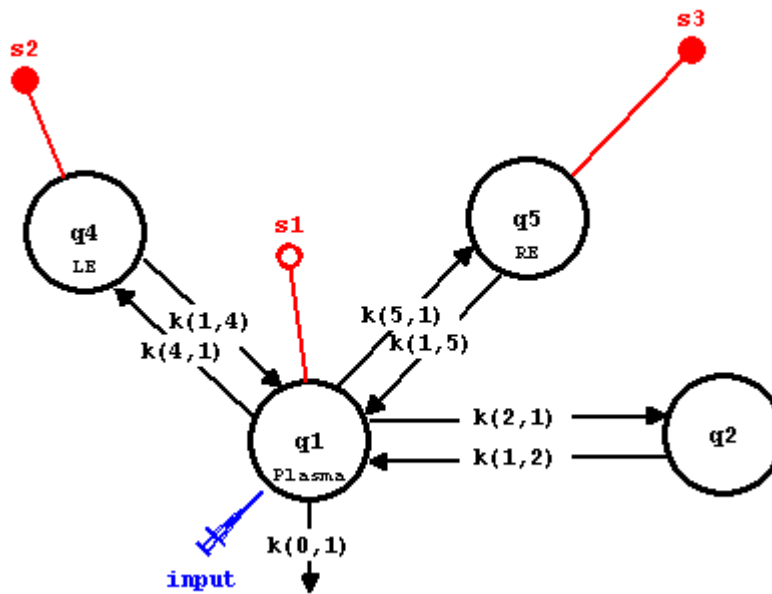
Part 3. Develop an integrated system model

In this part of the case study, you will combine the system and middle ear models into an integrated system model that will simultaneously describe all data. In this case, it will be quite easy because the uptake rate constants for the middle ear are extremely small compared with $k(0,1)$. Thus relative to plasma mass, the middle ear masses are negligible.

1. Create the integrated system model.
 - a. In the **SAAM II Toolbox**, click **Model**. Modify your model as follows:



- b. Click **Experiment** in the SAAM II Toolbox. You may have to move some items on the **Drawing Canvas**, but your model should look approximately as follows:



- 2. Re-Associate **Cp_total** with **s1** (the bullet should close). Reweight the **Cp_total** data.
- 3. Enter the parameter values.
 - a. When you open the **Parameters** dialog box, it will appear as follows:

The screenshot shows a 'Parameters' dialog box with a table of parameters and a configuration section for the selected parameter 'Vc'.

Name	Type	Current	Low Limit	High Limit
Vc	Fix	295.1764		
Vmef	Fix	1.6400		
k(0,1)	Fix	0.0725		
k(1,2)	Fix	0.0136		
k(1,4)	Adj			
k(1,5)	Adj			
k(2,1)	Fix	0.0089		
k(4,1)	Adj			
k(5,1)	Adj			

Configuration for Vc:

Name: Vc
Value: 295.17639900
Type: Fixed Adjustable
Low Limit: 30.00000000
High Limit: 3000.00000000

Buttons: Done, Cancel, Help, Edit, Save

Set all parameters which are fixed equal to adjustable EXCEPT *Vmef*. The **Parameters** dialog box should appear as follows:

Name	Type	Current	Low Limit	High Limit
Vc	Adj	295.1764	30.0000	3000.0000
Vmef	Fix	1.6400		
k(0,1)	Adj	0.0725	0.0100	1.0000
k(1,2)	Adj	0.0136	0.0020	0.2000
k(1,4)	Adj			
k(1,5)	Adj			
k(2,1)	Adj	0.0089	0.0020	0.2000
k(4,1)	Adj			
k(5,1)	Adj			

Name: k(2,1) Value: 0.00894705

Type: Fixed Adjustable

Low Limit: 0.00200000 High Limit: 0.20000000

Buttons: Done, Cancel, Help, Edit, Save

- b. Enter the middle ear parameters. In the integrated system model, $k(4,3)$ from Part 2 will become $k(4,1)$; $k(5,3)$ will become $k(5,1)$; $k(0,4)$ will become $k(1,4)$ and $k(0,5)$ will become $k(1,5)$. Enter these values. The **Parameters** dialog box should appear essentially as follows:

Name	Type	Current	Low Limit	High Limit
Vc	Adj	295.1764	30.0000	3000.0000
Vmef	Fix	1.6400		
k(0,1)	Adj	0.0725	0.0100	1.0000
k(1,2)	Adj	0.0136	0.0020	0.2000
k(1,4)	Adj	0.0040	4.000e-004	0.0400
k(1,5)	Adj	0.0030	3.000e-004	0.0300
k(2,1)	Adj	0.0089	0.0020	0.2000
k(4,1)	Adj	6.700e-006	6.700e-007	6.700e-005
k(5,1)	Adj	4.800e-006	4.800e-007	4.800e-005

Name: k(5,1) Value: 4.8e-06

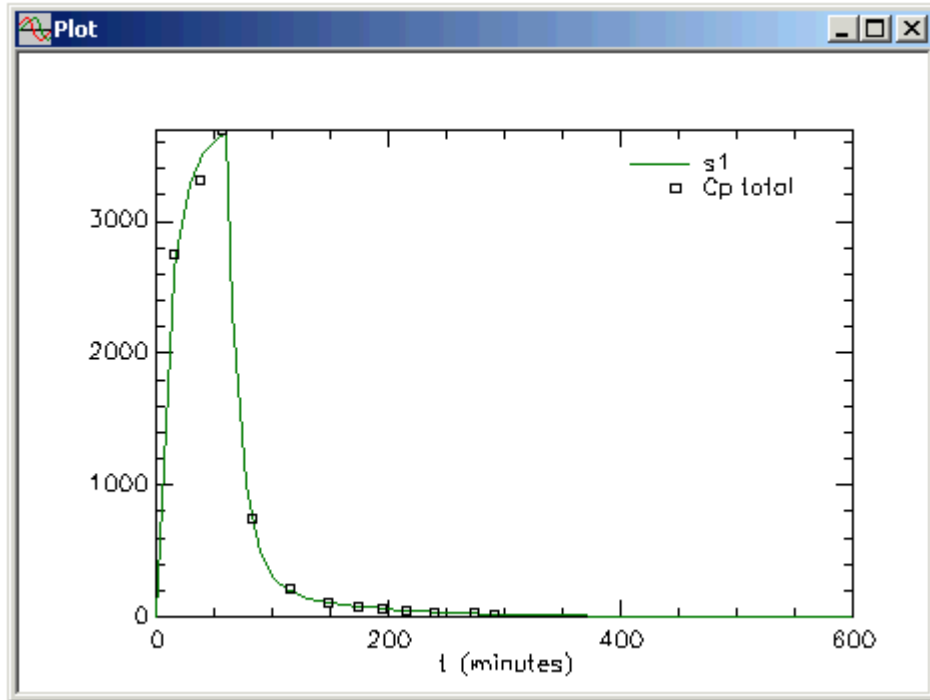
Type: Fixed Adjustable

Low Limit: 4.8000000e-007

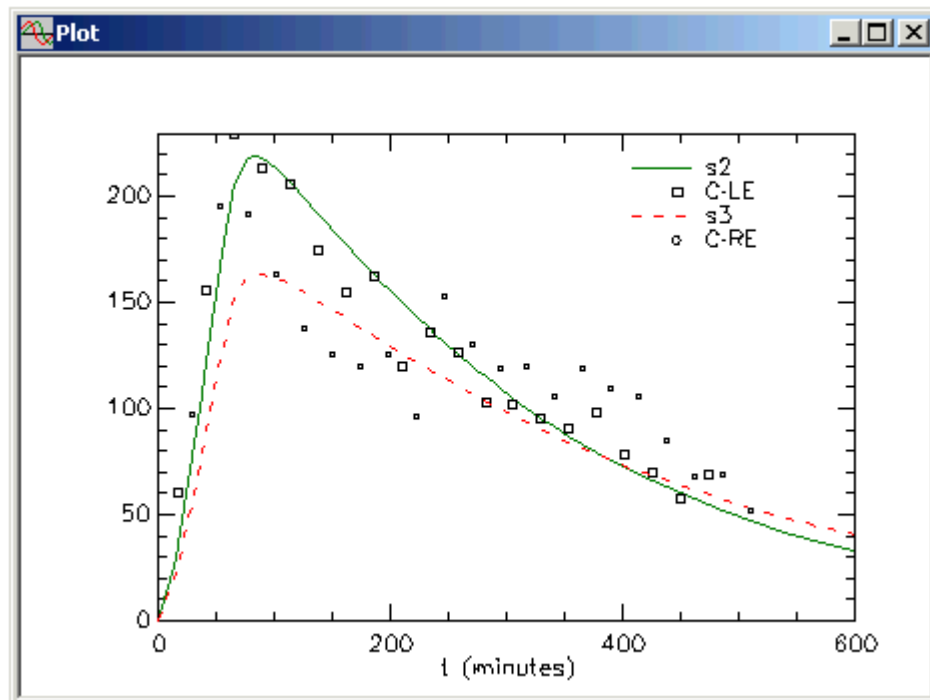
High Limit: 4.8000000e-005

Buttons: Done, Cancel, Help, Edit, Save

- c. Click **Done**.
4. Solve the model, and view the solution. Your plot of s1 and Cp_total should appear as follows (in linear mode):

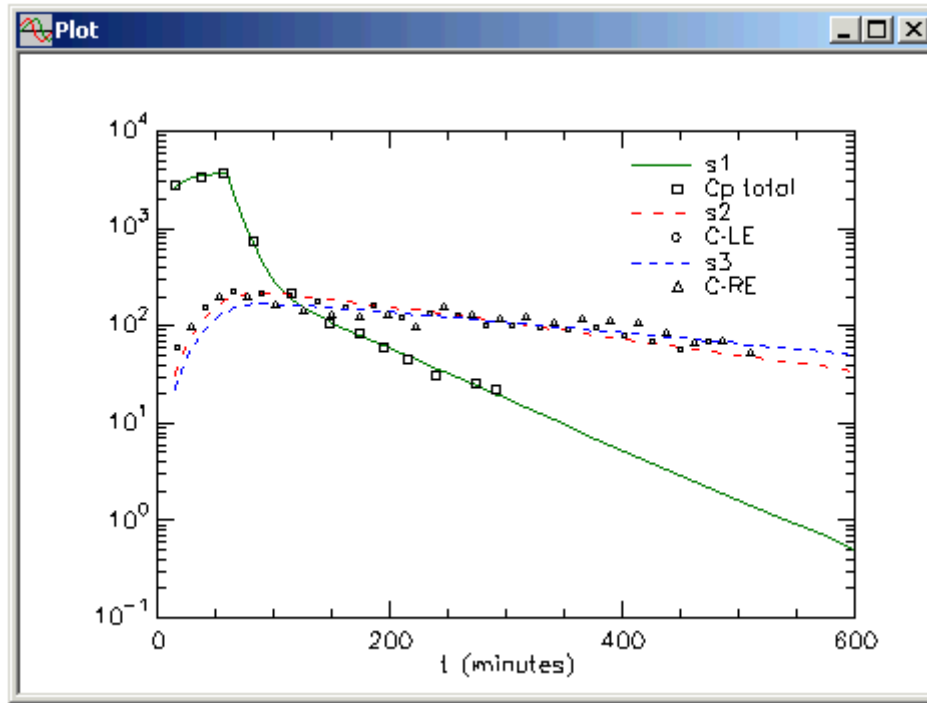


Your plot of the middle ear data should appear as follows:



As expected, these plots are excellent because you already have best-fit values for the parameters obtained from Parts 1 and 2. What remains is to Fit the model to the data to obtain the correct statistical information for the integrated system model.

5. Fit the model to the data. You should visualize your plots, but they will not have changed very much. If you wish you can also view the weighted residuals. A plot of all data in semi-log mode will appear as follows:



View the statistics. The **Statistics** window should appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
Vc	278.86810	2.18761e+001	7.84459e+000	234.76846	322.96775
Vmef	1.64000	** Fixed **	** Fixed **	** Fixed **	** Fixed **
k(0,1)	0.07598	4.46305e-003	5.87378e+000	0.06699	0.08498
k(1,2)	0.01377	8.58216e-004	6.23029e+000	0.01204	0.01550
k(1,4)	0.00393	2.95587e-004	7.52606e+000	0.00333	0.00452
k(1,5)	0.00259	3.62780e-004	1.40126e+001	0.00186	0.00332
k(2,1)	0.00957	1.15129e-003	1.20300e+001	0.00725	0.01189
k(4,1)	6.98525e-006	6.01411e-007	8.60972e+000	5.77288e-006	8.19763e-006

Objective	Scaled Data Variance
s3 : C_RE	2.873817e+000
s2 : C_LE	2.478657e+000
s1 : Cp_total	1.439496e+000

Total objective	6.791970e+000
AIC	4.522471e+000
BIC	4.726935e+000

The statistics associated with the integrated system model are excellent.

Close all open windows.

You may now **Quit** the **SAAM II Compartmental** application. You may save the study file for future reference if you wish.

Essential Points to Remember

- The forcing function is a powerful tool in model development.
- Forcing functions are used to decouple a system.
- Developing an integrated system model can be done in logical steps thus eliminating the need for ad hoc attempts that are often fruitless.

Data for this case study

DATA
(FSD 0.05)
t Cp_total
16 2751
38 3313
57 3693
82 741
115 215
148 108
175 82
195 61
215 46
240 31
274 26
291 22

END
DATA
(FSD 0.05)
t C_RE
30 97.56
54 195.65
78 191.72
102 162.95
126 138.1
150 125.03
174 119.8
198 125.03
222 96.26
246 152.49
270 130.26
294 118.49
318 119.8
342 105.41
366 118.49
390 109.33
414 105.41
438 84.49
462 67.48
486 68.79
510 51.79

END
DATA
(FSD 0.05)
t C_LE

18 60.1
42 155.8
66 229.2
90 212.7
114 205.7
138 174.7
162 154.5
186 161.8
210 120
234 135.7
258 126.7
282 103
306 101.4
330 95.7
354 90.1
378 98.5
402 78.1
426 69.5
450 57.8
474 68.8
END

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