



Version 4.0

Step-by-Step Examples

Fitting Data to User-Defined Equations.

This article includes the following techniques:

- Entering a user-defined equation into Prism
- Specifying initial curve-fit values
- Trying out initial values (i.e., plotting the curve defined by the initial values)
- Constraining curve-fit parameters

Within the *Classic equations* and the *Prism equation library* are most of the curve-fit models that a biologist needs. Yet occasionally you may want to customize. For example, you may (a) need an equation of a form not built into Prism, (b) need to modify an existing equation, e.g., changing the two-site binding model to accommodate three sites, or (c) simply wish to rename the parameters in a built-in equation so that the curve-fit results will be labeled more appropriately.

In this example, we'll enter and fit data to a "user-defined" equation of a form not preprogrammed into Prism. The time course for total systemic absorption of a drug, expressed as the fraction (F) of the amount administered at a subcutaneous depot at time 0, is given by²

$$F = \frac{1}{(k_e/k_a) - 1} \left[e^{-k_e t / (k_e/k_a)} - e^{-k_e t} \right]$$

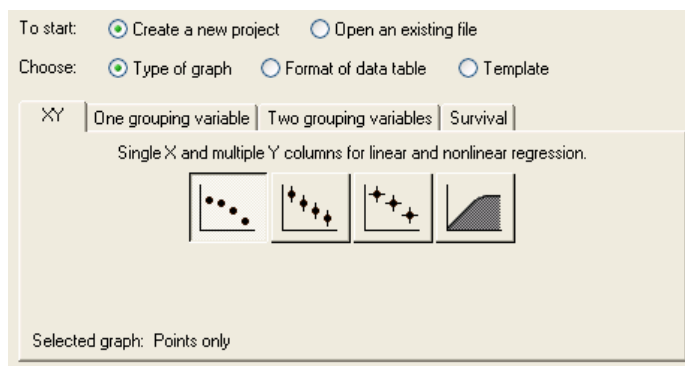
where t = time (min), and k_e and k_a are the first-order elimination and absorption rate constants (min^{-1}), respectively. We'll plot an example time course and fit the curve to the data to obtain an estimate of the rate constants.

¹ Adapted from: Miller, J.R., *GraphPad Prism Version 4.0 Step-by-Step Examples*, GraphPad Software Inc., San Diego CA, 2003. *Step-by-Step Examples* is one of four manuals included with Prism 4. All are available for download as PDF files at www.graphpad.com. While the directions and figures match the Windows version of Prism 4, all examples can be applied to Apple Macintosh systems with little adaptation. We encourage you to print this article and read it at your computer, trying each step as you go. Before you start, use Prism's **View** menu to make sure that the Navigator and all optional toolbars are displayed on your computer.

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² Neubig, R. R., The Time Course of Drug Action, in *Principles of Drug Action* (W.B. Pratt and P. Taylor, eds.), 3rd Edition, Churchill Livingstone Inc., 1990.

In the Welcome dialog, make the settings shown below.

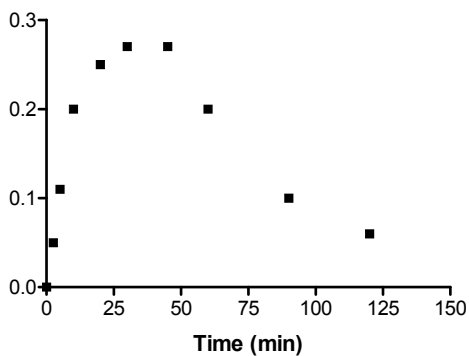


Enter the data as shown below.

	X Values	A
	Time (min)	Frac Abs
	X	Y
1	0.0	0.00
2	2.5	0.05
3	5.0	0.11
4	10.0	0.20
5	20.0	0.25
6	30.0	0.27
7	45.0	0.27
8	60.0	0.20
9	90.0	0.10
10	120.0	0.06

For this example, we're assuming that the Y values do not require correction by subtraction of a baseline—the minimum Y value is already 0. If you wish to subtract a baseline value from each Y value in a data set, read about automatic baseline correction in the Step-by-Step Example “Substrate-Velocity Curves and Lineweaver-Burk Plots”.

Click the yellow **Graphs** tab on the toolbar to view the graph:



Entering the User-Defined Equation

With the graph displayed, click on the **Analyze** button. From the **Curves & regression** category, select **Nonlinear regression (curve fit)**.

In the **Parameters: Nonlinear Regression (Curve Fit)** dialog box (**Equation** tab), choose **More equations** and, from the list below, click [**Enter your own equation**].

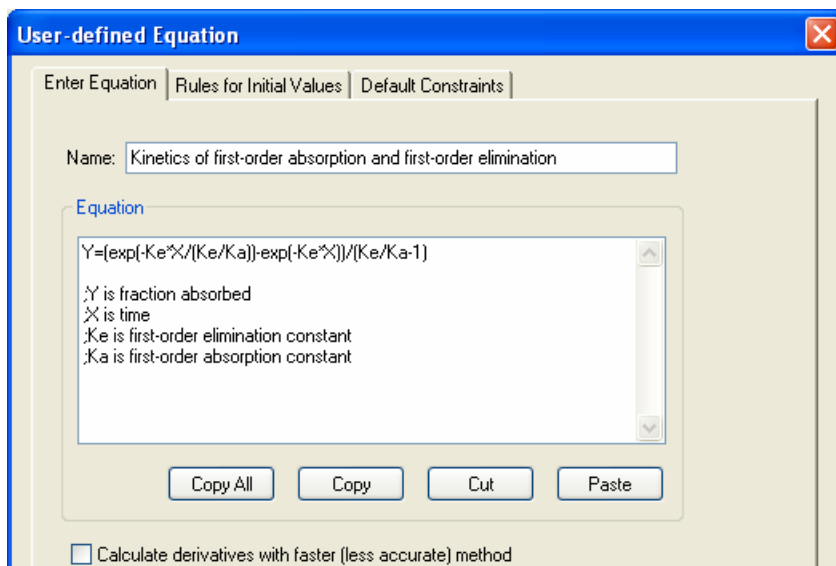
The **User-defined Equation** dialog is displayed. With the **Equation** tab selected, enter a **Name** for this curve-fit analysis (which will be added to the **More equations** list the next time you view it). Now enter your equation, in single-line form, into the **Equation** box. You are limited to certain types of equations and must adhere to a few simple syntax rules. Your equation must be of the form

$$Y = f(x)$$

Thus, you must designate the dependent and independent variables as Y and X, respectively, and you must write the equation so that Y is isolated on the left-hand side. The Prism *User's Guide* lists all the rules for entering user-defined equations in Prism, including the functions, or operators, that you may use. Our equation can be written on a single line as follows (the equation is not reduced to simplest terms, but that will help illustrate the use of an “intermediate” variable below):

$$Y=(\exp(-K_e*X/(K_e/K_a))-\exp(-K_e*X))/(K_e/K_a-1)$$

You can enter comments to prompt your memory or to help other users understand your equation by writing lines beginning with a semicolon.



If you prefer, you can break an equation into two or more expressions to make the resulting system simpler or more readable. Below, we define an intermediate variable

$$ratio = k_e / k_a$$

Prism will read that definition in the first line and then substitute k_e/k_a for “ratio” in the final equation:

Equation

```
ratio=Ke/Ka
Y=(exp[-Ke*X/ratio]-exp[-Ke*X])/[ratio-1]

;Y is fraction absorbed
;X is time
;Ke is first-order elimination constant
;Ka is first-order absorption constant
```

Your user-defined equation may be a variation of one of Prism's pre-programmed, or "classic", equations. In that case, you can keep manual entry of your equation to a minimum by copying the classic equation into the equation box. In the **Parameters: Nonlinear Regression (Curve Fit)** dialog box, choose an equation from the **Classic equations** list, then click the **View Equation...** button. In the **Classic Equation** dialog box, click **Copy All** to place the contents of the **Equation** window on the clipboard. Now you can move to the **User-defined Equation** dialog (from **Parameters: Nonlinear Regression (Curve Fit)**, choose **More equations...[Enter your own equation]**). Make sure the **Equation** tab is selected, then click **Paste** to enter the classic equation into the **Equation** window. Now type your modifications.

Entering Initial Values for Curve-Fit Parameters

When you're done entering your equation, click the **Rules for Initial Values** tab. Prism reads your equation and, after eliminating X, Y, and operators, arrives at the "parameters" to be fit—in this case, k_e and k_a . Curve fitting is an iterative, trial-and-error process of substituting values for the parameters in your equation in order to maximize the goodness of fit between your experimental data and the resulting substituted equation. Prism therefore must have an initial "guess" for the value of each parameter, that is, it must have a starting point for the process. When you use one of the *Classic equations*, Prism derives its own initial values, choosing numbers that are related to your experimental data by programmed "rules". For *User-defined equations*, Prism has no such rules, so you must provide either those rules or explicit initial values.

For each parameter, enter a constant value into the first column and a relational expression chosen from the drop-down list into the second column. Your choices fall into two categories:

Initial value, to be fit

This choice indicates that the value in the first column is to be used explicitly. Select this option when there is no rule in the second column that reasonably allows derivation of the initial value from your experimental data. The drawback of this choice is that you *may* have to provide a new initial value each time you run this analysis. For this step-by-step example, we will select this option, since no reasonable rule is available.

The initial value estimate generally does not have to be very close to the actual fitted value, particularly when the equation has few parameters to be fit and your data doesn't have too much scatter.

The initial values that you enter might come from (a) your past experience or the experience of other investigators, including published values, (b) alternative methods of computation, or (c) a trial process, whereby you allow Prism to generate a curve using the values you supply and check to see if the result is reasonable. We'll illustrate the latter process below.

Don't forget to avoid entering "illegal" initial values. In our example, the equation is undefined for $k_e = 0$, $k_a = 0$, and $k_e = k_a$.

A rule

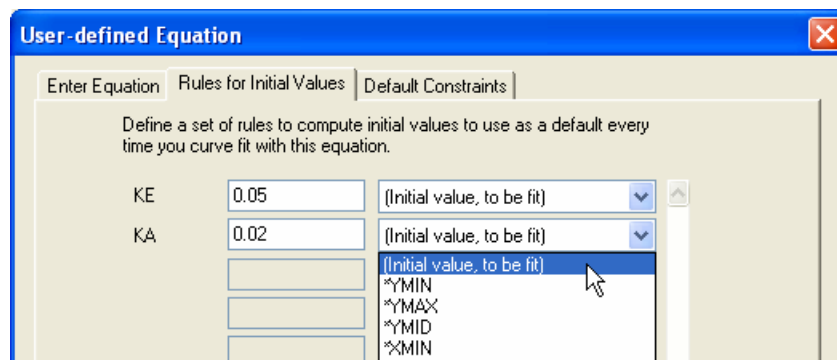
If you can find a rule on this list that allows Prism *consistently* to derive an initial value from your data (i.e., if the rule holds as your data varies within reason from experiment to experiment), this is the best choice. You won't have to enter a new initial value each time you run the curve-fit analysis.

Example

When collecting data for a sigmoidal dose-response curve, the input data are the logarithms of drug concentrations tested, and investigators typically choose those concentrations such that their logarithms will be distributed evenly over the curve. And the lower and upper plateaus of the idealized curve can be approximated by the lowest and highest responses measured, respectively. So good initial value rules for the for a three-parameter sigmoidal fit would be

LOGEC50:	1*Value of X at YMID
BOTTOM:	1*YMIN
TOP:	1*YMAX

Here are the entries for our example:



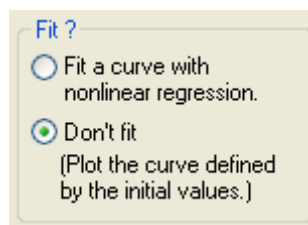
Click **OK** to return to the Parameters dialog.

Trying out Initial Values

Before doing the curve fit at all, we might like to know how close a curve generated using specific parameter values will come to our data.

Generating a trial curve is *not* mandatory. If the initial values produce a trial curve *very* far away from the data, Prism may not be able to figure out how to adjust the parameters for the next iteration, and it may abort the process. But ordinarily, the initial values need only be rough approximations of the fitted values, and it will not help to “fine-tune” them. If you are confident of your initial value settings, just go on to the next section.

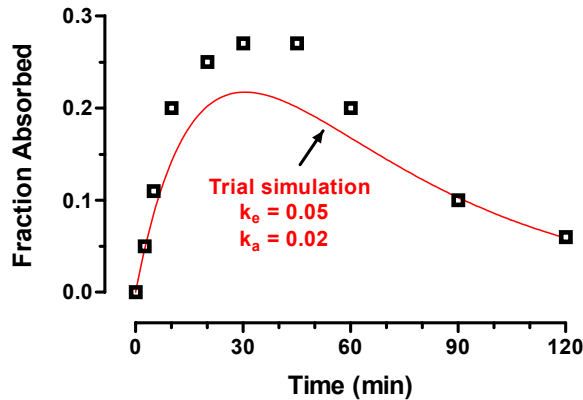
In the **Parameters: Nonlinear Regression (Curve Fit)** dialog box, choose to **...Plot the curve defined by the initial values.**



Click **OK** to generate the curve with k_e and k_a fixed to the specified initial values. Here is a partial view of the Results sheet.

Best-fit values	
KE	0.0500
KA	0.0200

Below is the graph (notations shown below were added using the text and drawing tools).



Since the curve is reasonably close to the points, you're ready to do the actual curve fit. Click the "Analysis parameters" button.



Reset the radio button to fit the curve.

Fit ?

Fit a curve with nonlinear regression.

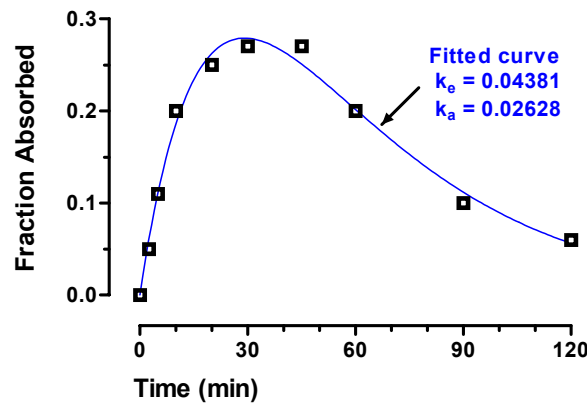
Don't fit
(Plot the curve defined by the initial values.)

Completing the Curve Fit

From the **Parameters: Nonlinear Regression (Curve Fit)** dialog, click **OK** to fit the curve. Click the yellow **Results** tab to display the fitted values for k_e and k_a .

Kinetics of first-order absorption	
Best-fit values	
KE	0.04381
KA	0.02628
Std. Error	
KE	0.001352
KA	0.0009958
95% Confidence Intervals	
KE	0.04069 to 0.04693
KA	0.02399 to 0.02858

Switch to the graph (notations in the figure below were added manually using the text and drawing tools).



Constraining Parameters

You may place limits on the value that a parameter can take on in the course of curve fitting. From the **User-defined Equation** dialog, select the **Default Constraints** tab. Prism displays the parameters for your equation. In the **Constraint** and **Value** boxed next to the parameter to be constrained, set a “rule” for the constraint. You may:

- Fix (hold constant) a curve-fit parameter. Prism will substitute the specified value as if you had written it directly into your equation, and it will fit only the remaining parameters. You may fix the value in the **Value** box...

Parameter	Constraint	Value
KE	Constant equal to	0.05

...or direct Prism to use, for each data set it fits, a value taken from the Y column heading on the table holding that data set (for an illustration, see the Step-by-Step Example “Computing K_i for a Competitive Enzyme Inhibitor”).

Parameter	Constraint	Value
KE	Data set constant (=column title)	

- Constrain a parameter to fall within a range.

- Must be less than
- Must be greater than
- Absolute value must be less than
- Must be between zero and

For example, make these settings to preclude the possibility of fitting either k_e or k_a to a negative value (physically unfeasible):

Parameter	Constraint	Value
KE	Must be greater than	0.0
KA	Must be greater than	0.0

- Stipulate that a parameter or parameters be shared, i.e., fitted to all data sets taken together.

Parameter	Constraint
KE	Shared value for all data sets

For an example of shared parameters, see the Step-by-Step Example “Computing K_i for a Competitive Enzyme Inhibitor”.

- Require that a parameter value both be shared over multiple data sets *and* fall within a range.

- Shared, and must be less than
- Shared, and must be greater than
- Shared, and abs. value less than
- Shared, and must be between zero and

- Limit the value of one parameter relative to another fitted parameter. Specify, for example, that the value of k_e must be at least twice that of k_a :

Constrain one parameter relative to another

<input checked="" type="checkbox"/>	KE	must be greater than	2.0	times	KA
<input type="checkbox"/>		must be greater than	1.0	times	

Setting constraints in the **User-defined Equation** dialog is only one of two ways to constrain parameters. It is appropriate when you want the constraint to apply each time you use that particular model. You can also set and change constraints for a particular nanalysis using the **Constraints** tab in the **Parameters: Nonlinear Regression (Curve Fit)**.