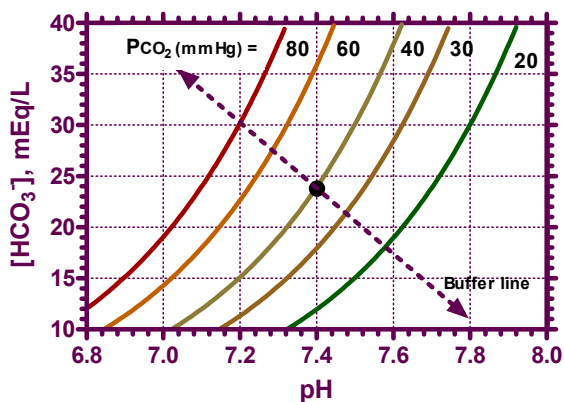


## Creating a Family of Theoretical Curves

It is common to show relationships between three or more variables as a set, or “family” of curves on a single graph. For each curve, one independent variable is plotted continuously, while the other is held constant. The curve set is produced by incrementing the second variable arbitrarily. An example is the acid-base diagram<sup>2</sup>, a family of curves relating  $[\text{HCO}_3^-]$ ,  $\text{PCO}_2$ , and pH in blood.



### Acid-Base Diagram

Physicians plot blood gas and chemistry measurements on the diagram and analyze acid-base disturbance/compensation based on displacement from the normal point (black circle). Respiratory and metabolic mechanisms are deduced by resolving displacements into components parallel to the “buffer line” and parallel to the  $\text{PCO}_2$  isobars, respectively.

Prism’s curve generation analysis allows you to produce a family of theoretical curves quickly and easily. In this article, we’ll create the graph shown above.

<sup>1</sup> 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](http://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.

©2003 GraphPad Software, Inc. All rights reserved. GraphPad Prism is a registered trademark of GraphPad Software, Inc. Use of the software is subject to the restrictions contained in the software license agreement.

<sup>2</sup> Davenport, H.W., *The ABC of Acid-Base Chemistry*, Chicago, University of Chicago Press, 1975.

---

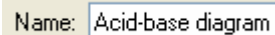
## Generating and Graphing the Curves

It isn't necessary to have a data table containing numbers in order to generate theoretical curves in Prism; you need only an open project file. If you're beginning a new file, accept the default settings in the Welcome dialog, click **OK**, then click the **Analyze** button.

In the **Analyze Data** dialog, choose the **Simulate and generate** radio button, then select **Create a family of theoretical curves** from the list to the right.

Prism can generate curves based upon either its built-in equations (curve-fit models) or user-defined equations. We'll do the latter. In the **Parameters: Create a Family of Theoretical Curves** dialog, select the **More equations** radio button and then choose **Enter your own equation** from the list below.

Prism opens the **User-defined Equation** dialog. Give your user-defined equation a name.



Name: Acid-base diagram

Each curve we'll generate is based upon a modified Henderson-Hasselbalch equation.

$$pH = pK + \log \frac{[HCO_3^-]}{0.03 \cdot PCO_2}$$

Before entering an equation into Prism, we must isolate the intended ordinate (Y value) on the left,

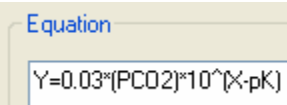
$$[HCO_3^-] = 0.03 \cdot PCO_2 \cdot 10^{(pH-pK)}$$

then indicate the dependent and independent variables by substitution with Y and X.

$$y = 0.03 \cdot PCO_2 \cdot 10^{(x-pK)}$$

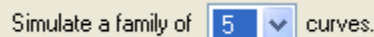
$PCO_2$  will assume a different value for each curve in the family. We could substitute the constant value 6.1 for pK, but for the sake of illustration later, we'll treat it as a variable parameter.

In the **User-defined Equation** dialog (**Enter Equation** tab), type the equation and then click **OK** to return to the Parameters dialog.



Equation  
Y=0.03\*(PCO2)\*10^(X-pK)

Prism identifies  $PCO_2$  and pK as the parameters whose values you must fix for each curve. Choose to **Simulate a family of 5 curves**.



Simulate a family of 5 curves.

Enter the parameter values for the first curve (**Curve A**).

Parameters for curve: Curve A

Copy Previous Parameters

	Value
PCO2	20.0
PK	6.1

Switch to **Curve B**, then click **Copy Previous Parameters**, which restores the last-entered parameter values in order to save typing. Change the value of PCO<sub>2</sub>.

Parameters for curve: Curve B

Copy Previous Parameters

	Value
PCO2	30.0
PK	6.1

Continue in this manner until PCO<sub>2</sub> values have been entered for all five curves, A-E. The values for this example are 20, 30, 40, 60, and 80.

We left pK as a variable parameter in the user-defined equation just to illustrate the **Copy Previous Parameters** feature. We could have fixed that value in the **User-defined Equation** dialog.

Equation

$$Y=0.03*(PCO2)^*10^{(X-6.1)}$$

At the top of the dialog, choose to plot each curve from **X = 6.8** to **X = 8.0**. Here are all the dialog settings after entry of all curve parameters:

**Parameters: Create a Family of Theoretical Curves**

X values  
 Create curve with 150 line segments. Starting at X= 6.8 Ending at X= 8.0

Equation list  
 Classic equations  More equations

[Enter your own equation.]  
 [Select an equation from the Prism equation library.]  
 [Import an equation from a Prism file or template.]  
 Kinetics of first-order absorption and first-order elimination  
 Acid-base diagram  
 Michaelis-Menten

Simulate a family of 5 curves.

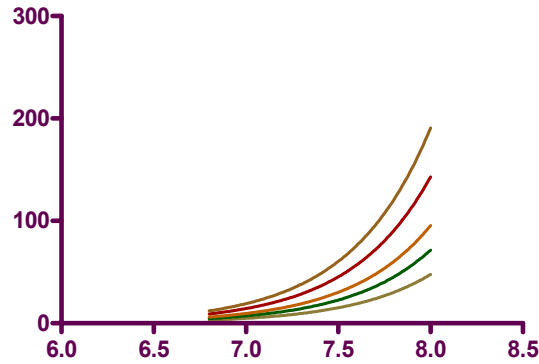
Parameters for curve: Curve E

Copy Previous Parameters

	Value
PCO2	80.0
PK	6.1

Click **OK** to generate the curves and display the graph.

### Theoretical curve:Curve

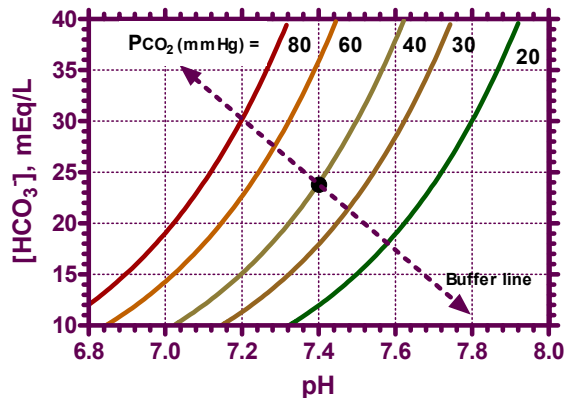


If the curves don't look right, and you think you made a mistake, click the "Analysis parameters" button to return to the Parameters dialog.



## Completing the Graph

Here is another look at our intended graph:



Click **Change... Size & Frame** to open the **Format Axes** dialog. Verify that the **General** tab is selected, then choose **Frame with grid** from the drop-down list under **Frame & axes**.

In the same dialog, choose the **X axis** tab. Remove the check from the **Auto** box under **Range** and change the values for **Minimum** and **Maximum**. Under **Tick options**, set the values for major and minor ticks as shown below:

Range		Tick options		
<input type="checkbox"/> Auto		Major ticks:	Interval: <input type="text" value="0.2"/>	Starting at: <input type="text" value="6.8"/>
Minimum: <input type="text" value="6.8"/>		Minor ticks:	# intervals: <input type="text" value="5"/>	Spacing: <input type="text" value="Equal"/>
Maximum: <input type="text" value="8.0"/>		All ticks:	Direction: <input type="text" value="Down"/>	Length: <input type="text" value="Short"/>

Choose the **Left Y axis** tab, then adjust the range and tick settings as follows:

Range		Tick options			
<input type="checkbox"/> Auto		Major ticks:	Interval: <input type="text" value="5.0"/>	Starting at: <input type="text" value="10.0"/>	
Minimum: <input type="text" value="10.0"/>		Minor ticks:	# intervals: <input type="text" value="5"/>	Spacing: <input type="text" value="Equal"/>	
Maximum: <input type="text" value="40.0"/>		All ticks:	Direction: <input type="text" value="Left"/>	Length: <input type="text" value="Short"/>	

Delete or modify the graph title as desired, and click once on the X and Y titles to edit them, using the superscript and subscript text buttons when necessary.



The text labels in the plot area are made using the text tool.



Click on the text tool button, then click once on the graph where you wish to insert the label. Then type the text. When done typing, click somewhere else to leave the text-insertion mode. Remember that you can then click the text label once to select it and make fine position changes using the arrow keys on your keyboard.

You may wish to place a white background behind the text labels in the plot area to avoid clashing with the gridlines. Click to select the text item and then choose **Change... Selected text...** In the Format Text dialog, click the **Borders and Fill...** button. In the **Format Object** dialog, Set **Interior...Fill** to match the plot area color.

The “buffer line” may be added using the arrow drawing tool.



After you draw, position, and adjust the length of the arrow, double-click it to open the **Format Object** dialog and adjust **Thickness, Style,** and **Direction.**

You can add a “normal” point to the graph by switching to the data table and entering coordinates for the point (e.g., X = 7.4, Y = 24). Return to the graph and choose **Change... Add Data Sets....** In the **Add Data Sets to Graph** dialog, verify that the correct data table (Data 1) is listed under **Data sets to add,** and click **OK.** Finally, double-click on the point symbol to open the **Format Symbols and Lines** dialog and change the size and shape of the symbol, if desired.