Graphs are a very useful way of expressing experimental data in biochemistry. Understanding the information contained in graphs will be important in understanding data in biochemical experiments. In this review, we will outline the preparation of a graph including the how numbers are represented and how axes are labelled. In many of your labs, you will be preparing graphs for presentation of your data. Several of your labs will be written in journal format. For these reports, we want you to prepare your graphs an acceptable "journal format”. These graphs, if done by hand on graph paper, will not be publication quality but at least the format will be publication quality. If you do the graphs through spreadsheet programs, it may be possible to have not only a journal format graph but also a publication quality graph. Do not do your graphs on the computer unless you get approval from your instructor.

**Construction of a graph:**

Each graph has a vertical axis (y-axis) that expresses the range of values for the dependent variable and a horizontal axis (x-axis) that expresses the range of values for the independent variable. The quality of your graph depends upon your following a set of simple rules. **ALL HAND-DRAWN GRAPHS ARE TO BE DONE IN INK.**

**Quality of graph paper:**

Good quality graph paper is required for all graphs in reports in biochemistry. This paper must be one of two types of engineering paper: 1 cm ruled in 1 mm divisions or 1 inch paper ruled in 20 (not 10) divisions. No other graph paper will be acceptable. Make the plot as large as possible using as much of graph paper as possible.

If you are using a spreadsheet program such as EXCEL to create graphs, you should consult with the instructor and review the information provided on EXCEL (short version and long version).

**Title or caption for graph:**

You will be required to give a **title or a caption** for your graphs. In scientific publications graphs have captions but no titles are given. Check with your instructor as to what will be required for your reports.

**Titles** are placed at the top of the graphs. They should be descriptive with enough information so the reader knows exactly what the graph is about. For example, for the buffer lab where histidine is titrated, an appropriate title could be, "Titration of the Side Chain of Histidine to Determine its pKa Value". A title that would not be acceptable would be "pH vs mole OH/mole histidine". This latter title just repeats the axis which are obvious anyway.
Captions: are to be at the bottom of the graphs which includes a figure number. The caption contains all the relevant information to describe the graph. The first line is usually an incomplete sentence. If followed by any further information, complete sentences must be used. For example, in the buffer lab for the histidine titration, the following caption would be appropriate:

Figure 1. Titration of histidine hydrochloride. The $pK_a$ value of the side chain determined from these data is 6.05.

Scale factors for the axes:

You must use a constant scale factor along the distance on each axis. For example, if you decide $1.0 \text{ cm} = 0.010 \text{ min}^{-1}$, then $5.0 \text{ cm} = 0.050 \text{ min}^{-1}$. A common error that some students make is not paying attention to this constant factor. Linear graphs will be nonlinear or have breaks in them at inappropriate values if the axes are constructed incorrectly.

Placement of zero on one axis or both axes:

It may not be necessary to have one (or both) axis start at 0.0. This is determined by the kind of experiment that you are doing. For instance, in the titration of histidine in the buffer experiment, you will have pH values as the dependent values on the y-axis that range from about 5 to about 8. In this case, the y axis should reflect the observed ranges of the measured pHs and not start at 0 (or end at 14). In this titration, the x-axis values will start at 0.0 moles of added NaOH so that a 0.0 value on the x-axis is appropriate. Ask your instructor if you have any trouble deciding about any graph you are about to prepare.

Labels for both axes:

Both axes must be labelled properly with appropriate significant figures and dimensions (units). Numbers must have the number of significant figures appropriate to the experiment. The units required varies from one journal to another. However, we require you to use a format which is simple and accepted by all scientific journals.

Significant figures: Appropriate for measurements. This is usually 2 or 3. For example, for the buffer lab, pH measurements between 1 and 9 should be expressed in 3 significant figures.

Number on axes: 0 to 1.0 in 0.1 or 0.2 increments
(in correct sig. figs.) 0 to 10.0 in 1.0 or 2.0 increments

For the maximum numbers on your axes, use ones that are divisible by 2 or 5 or 10. This makes points easier to locate. It is recommended to use the 0.2 (2.0) increments since the graph axes are less crowded.
Dimensions: EXPONENTIAL NOTATION IS NOT ALLOWED IN INDICATING NUMBERS ALONG EITHER AXIS.

The order of magnitude for a number is indicated in the axis title:

Example: $\beta$-CAROTENE (10^{-4} M)

This means that each number along the axis is to be multiplied by 10^{-4} M. This format alleviates confusion. The following example is one which is correctly written but is often interpreted by students as 10^4 M which is impossible(!):

$\beta$-CAROTENE x 10^4 M

For example: 0.235 x 10^{-4} x 10^4 M = 0.235

(actual concentration) (factor for graph) (value on graph)

Data points:

Data points are indicated using a circled dot. The size of the circle is an indication of the magnitude of the error in the data point. In journal articles, the data points are often indicated with a symbol only whose size is indicative of the magnitude of the error.

Lines or smooth curves?

Depending upon the experiment, you will have data points that define straight lines as well as data points that define a smooth curve with a well-defined mathematical function. You should know whether a line or curve is required for your graph.

For a straight line, use a clear plastic ruler and draw the best straight line that goes through the points. If the points do not all fit on the straight line, adjust the line so that you have as many points above the line as below it. Your eye will give you nearly the same line as the computer would do with a linear regression analysis.

For a curved line, you should use a flexible ruler and draw the best curve. If you are using a spreadsheet to create your graph, discuss with your instructor what mathematical function best describes the data points. Using this function, you will be able to have the computer draw the best curved line through your data. This is done very easily in EXCEL or QUATTRO PRO.
Determining the slope, y-intercept, and x-intercepts of a straight line:

To determine the slope of a straight line, pick two points \([x_1, y_1]\) and \([x_2, y_2]\) as far apart as possible on the line. The slope is defined as follows:

\[
\text{Slope} = \frac{Y_1 - Y_2}{X_1 - X_2}
\]

y-intercept = y value on line when line crosses y axis
(y value when x = 0)

x-intercept = x value on line when line crosses x axis
(x value when y = 0)

LINEWEAVER-BURK PLOT FOR LACTATE DEHYDROGENASE
(This is not a publication quality graph - why?)

Figure 1. Lineweaver-Burk plot for rabbit muscle lactate dehydrogenase. The \(K_m\) was found to be \(3.0 \times 10^{-5}\) M and the \(V_{max}\) 2.9 \(\times 10^{-6}\) M min\(^{-1}\).
Figure 1. Titration of histidine monohydrochloride with NaOH at room temperature. The $pK_a$ determined from these data is 6.20.