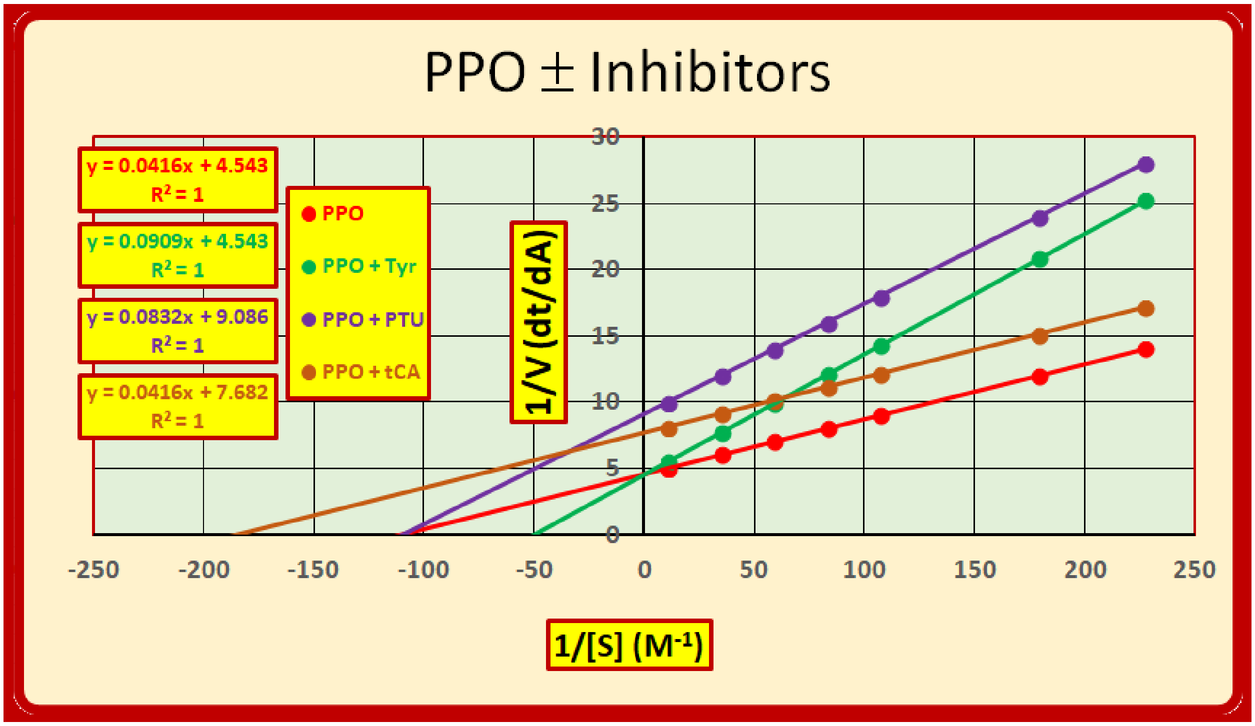
All,

I've received a number of inquiries regarding the potato polyphenol oxidase dry lab. I figure this is the best way to address the issues.

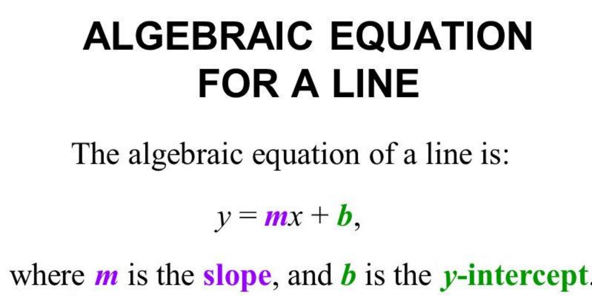
First off, your Lineweaver-Burke ought to look something like this when you're done with it (more or less):



Where the **red line is <u>without</u> inhibitor**, the **green line is with Tyr**, the **purple line is with PTU** and the **burnt orange line is with tCA**.

Note that the line equations are color-coded along with the legend and the lines; also note the labels for the X and Y axes; note, too, the range on the X axis so as to accommodate visualizing the X intercept.

Second, do you note that the line equations are all of the form **y = m x + b**?, i.e., the algebraic form of a straight line (image at right).



ALGEBRAIC EQUATION FOR A LINE

The algebraic equation of a line is:

$$y = mx + b,$$

where m is the **slope**, and b is the **y-intercept**.

For the un-inhibited line, the line equation is y = 0.0416 x + 4.543. This equation was generated by Excel in the trend line insertion pop-up; also, the backwards extension of the line to the x intercept was, likewise, manually generated in the trend line insertion pop-up. The y intercept (b) is the reciprocal of the V_{max} of the un-inhibited enzyme. Remember that the reciprocal of ANYTHING is $\frac{1}{\text{ANYTHING}}$, i.e., the reciprocal of 2 = $\frac{1}{2} = 0.5$. That means that to obtain the V_{max} of the un-inhibited enzyme, one must take the reciprocal of the y intercept:

$$\frac{1}{4.543 \text{ Minutes per Absorbance Units}} = 0.2201 \text{ Absorbance Units per Minute.}$$

To obtain the K_M of the enzyme, there are two options: Option #1 is to simple set y equal to 0 and solve for x: 0 = 0.0416 x + 4.543; x = -109.207. The result, however, is the negative reciprocal of the K_M. In order to obtain the K_M, then, simply take the negative reciprocal of the value:

$$K_M = -\left(\frac{1}{-109.207}\right) = 0.009157 \text{ M or } 9.157*10^{-3} \text{ M.}$$

Option #2 is to remember that m = slope = $\frac{K_M}{V_{max}}$, p. 17 of 25 in the experiment. Since you know V_{max} from above and the slope (from the line equation), simply re-arrange the slope equation and solve for K_M: $(\text{slope})(V_{max}) = K_M$.

$$\left(\frac{0.0416 \text{ moles*Minute}}{\text{L*Absorbance Units}}\right)\left(\frac{0.2201 \text{ Absorbance Units}}{\text{Minute}}\right) = 0.009156 \text{ M or } 9.156*10^{-3} \text{ M}$$

That takes care of K_M and V_{max}.

It doesn't, Third, however, take care of either $\frac{1}{[S]}$ or $\frac{1}{V}$ data. That data is fairly straight-forward: for $\frac{1}{[S]}$ data, simply take the reciprocal of all the [S] data, <u>**EXCEPT for the 0 M catechol data**</u>, and record it in a new Excel column.

For the $\frac{1}{V}$ data, you have two options to determine those values: the first option is to determine the reaction velocities as per the experimental write-up (p. 22 of 25 in experiment): $V = \frac{A_{480\text{ nm}}^{30\text{ seconds}} - A_{480\text{ nm}}^{0\text{ seconds}}}{0.5 \text{ minutes}}$ and, **then determine** the reciprocals for the $\frac{1}{V}$ data for all four (4) trials and set them up in Excel columns adjacent to the $\frac{1}{[S]}$ column:

	1/V			
1/[S]	PPO	PPO + Tyr	PPO + PTU	PPO + tCA
227.3317	14	25.19836	27.98863	17.139
179.2548	12	20.83009	23.99104	15.139
107.1394	9	14.27769	17.99464	12.139
83.10096	8	12.09355	15.99584	11.139
59.0625	7	9.909419	13.99705	10.139
35.02404	6	7.725284	11.99825	9.139
10.98558	5	5.54115	9.999451	8.139

Your second option (since you're using Excel and you might as well take advantage of it) is to rearrange the velocity equation as follows:

$$\frac{1}{V} = \frac{0.5 \text{ minutes}}{A_{480\text{ nm}}^{30\text{ seconds}} - A_{480\text{ nm}}^{0\text{ seconds}}}$$

And, then, put your $\frac{1}{V}$ data in columns adjacent to the $\frac{1}{[S]}$ data (per the above table) so that you can graph it.

Once your linear graph is constructed, determine the inhibition pattern, determine the K_M and V_{max} values, print it out and you're done!