Method Sheet 40

Plotting a Lineweaver-Burk chart using Microsoft Excel

Overview

This method sheet explains how to plot a Lineweaver-Burk chart from enzyme activity data using Microsoft Excel. This type of chart is a useful tool in the drug discovery process as it helps to distinguish whether a new type of inhibitor works as a competitive, non-competitive or uncompetitive inhibitor. Competitive inhibitors bind to the active site of the enzyme, preventing access to the substrate. Non-competitive inhibitors bind to an allosteric site that is distinct from the active site. Uncompetitive inhibitors bind to the enzyme substrate complex, preventing release of the substrate. The line-fit equations from Lineweaver-Burk charts also enable easy calculation of K_m, which is a measure of the affinity of the enzyme for its substrate, and V_{max}, which is the maximum theoretical reaction rate of the enzyme. Unlike a standard Michaelis-Menten plot which charts reaction velocity [V] against substrate concentration [S] to yield a curve, the Lineweaver-Burk plot charts the reciprocals of these (i.e. 1/[V] vs 1/[S]) to yield a straight line. Determining where the lines for a reaction with and without a test inhibitor cross reveals which type of inhibitor is present in the reaction, as explained later.

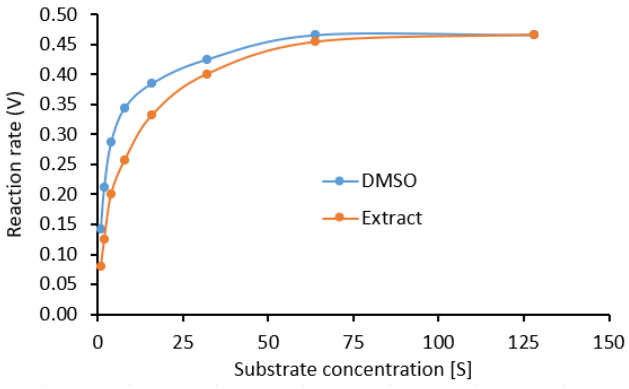
Method

1) You should have two plate readings, one at time = 0, just after adding the enzyme to the plate to start the reaction, and the other at a later timepoint when the wells containing the highest concentration of substrate are clearly yellow in colour.
2) Paste the values from both the first and second readings as tables of 8 x 12 cells in an empty Excel sheet.
3) Perform a background correction of the data by subtracting the values at time = 0 from the values taken at the second time point (see Method Sheet 20).
4) Skip the data normalisation step from Method Sheet 20 as we don't require it for the next part of the analysis.
5) You can analyse the data from one plate at a time if you like, but for your dissertation you will have to collate the data from several different experiments (on separate plates), so bring these data together into a separate sheet in Excel and calculate the average background corrected absorbance for each concentration tested (using the `=AVERAGE` function).
6) Align these values in a table with the concentrations tested in your dilution series in the first row, the second row containing the data for DMSO only and the third row for the Extract readings.
7) Make sure to NOT include the data for the zero concentration in your table, because this will cause an error in the inversion at a later step in the data analysis process.
8) The table should now look something like this:

1									
2		1	2	4	8	16	32	64	128
3	DMSO	0.142	0.211	0.286	0.344	0.385	0.425	0.466	0.466
4	Extract	0.080	0.125	0.200	0.257	0.332	0.400	0.454	0.466
5									

9) The values in the first row are the **substrate concentrations [S]**, and the values in the second and third rows are the **reaction velocities [V]**.

10) Creating a standard scatter plot of [V] vs [S] should yield a classic Michaelis-Menten curve for both the extract and DMSO dilution series, looking something like this:



11) Note that it is very difficult to guess from this chart where V_{max} and K_m are exactly.

12) Therefore, we will plot the reciprocal of both values on a different kind of chart, the **Lineweaver-Burk plot**, which plots **1/V vs 1/S**.

13) Create a new table below the first table of raw absorbance values, and use an Excel formula similar to the following to calculate the reciprocal of every value in the first table: `=1/B2`

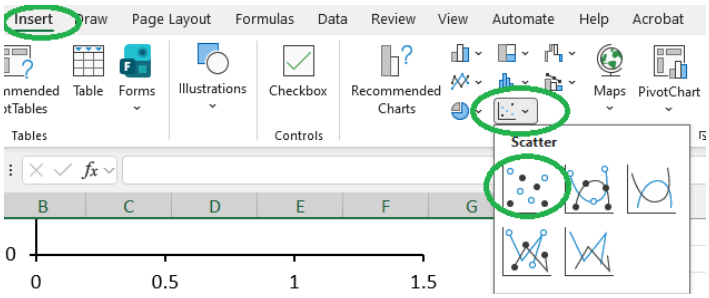
14) Remember to change the cell reference to match where the data are located in your own table.

15) The table will now change to something like this:

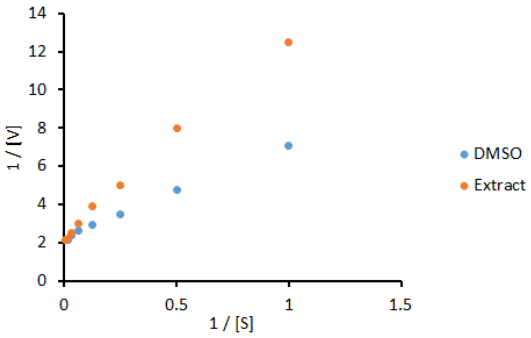
2		1	2	4	8	16	32	64	128
3	DMSO	0.142	0.211	0.286	0.344	0.385	0.425	0.466	0.466
4	Extract	0.080	0.125	0.200	0.257	0.332	0.400	0.454	0.466
5									
6									
7		1	0.5	0.25	0.125	0.0625	0.03125	0.01563	0.00781
8	DMSO	=1/B3	4.74	3.50	2.91	2.60	2.35	2.15	2.15
9	Extract	12.50	8.00	5.00	3.89	3.01	2.50	2.20	2.15

16) Highlight all three rows of the second table (the one containing the 1/V vs 1/S reciprocal data), and click on the 'Insert' tab of the main ribbon.

17) Click on the scatter plot chart icon and select the option for dots without lines.



18) Give the chart suitable labels and it should look something like this:

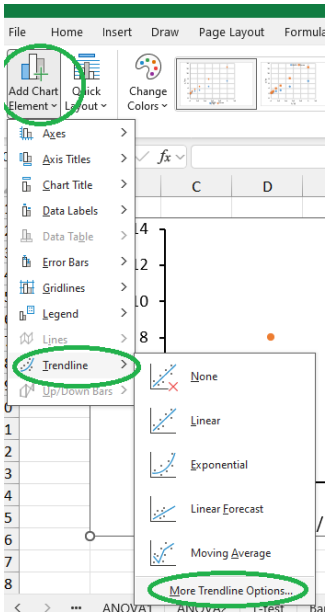


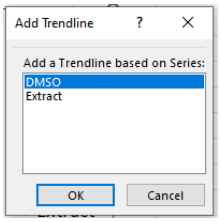
19) Now we have to insert a linear trendline for both the DMSO and the Extract data sets.

20) Click on the chart, then select the 'Chart Design' tab, and on the 'Add Chart Element' button at the left hand side of the ribbon, select 'Trendlines' and then 'More Trendline Options', as shown at right.

21) If 'More Trendline Options' does not become clickable straight away, click elsewhere, then try again.

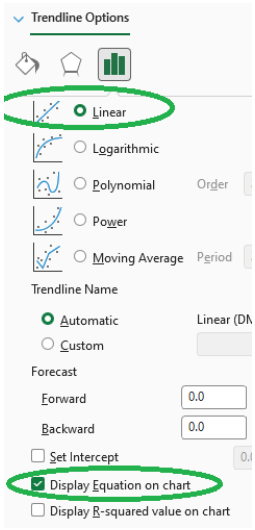
22) A small dialogue box will appear, asking you to choose which data series to create a trendline for.





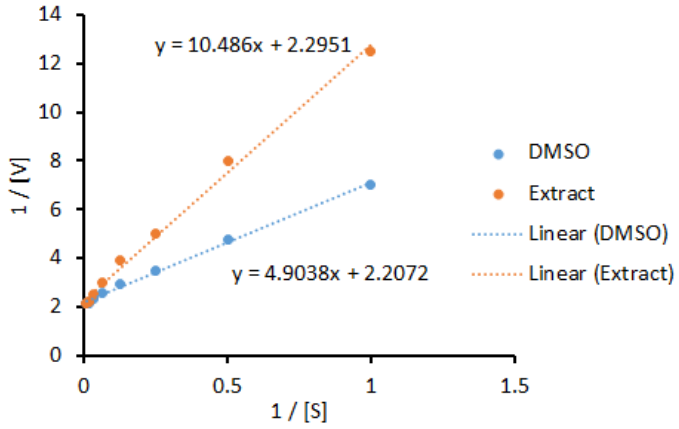
23) Click on the 'DMSO' option first, then click 'OK, and a new menu should appear at the right hand side of the screen.

24) Select the options for 'Linear' and 'Display equation on chart', but do not select any other options and do not use the 'Set intercept' function.



25) Click on the chart again and repeat the process for the Extract data set.

26) You should now have two trendlines on the chart between the dots, one for the DMSO dilution series, and one for the Extract dilution series, and an equation for both trend-lines, like this:



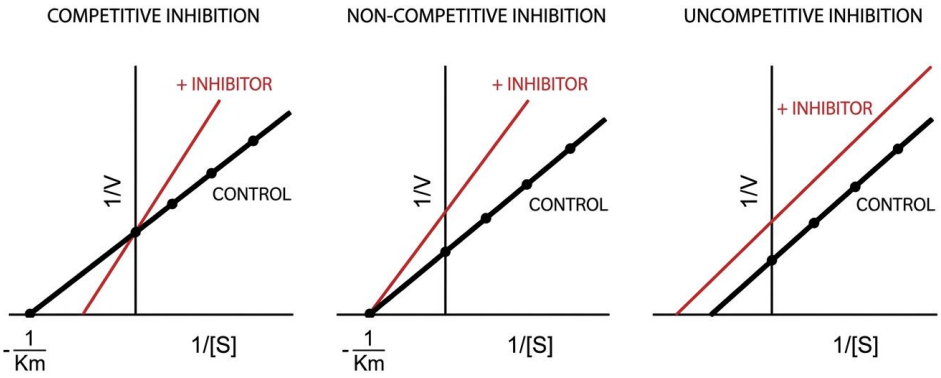
Interpretation of what the chart shows

1) To ascertain what type of inhibitor may be present in your hit extract, you should look primarily at where the two trend-lines on the Lineweaver-Burk plot intersect, as shown in the diagram below.

2) If the lines meet on the y-axis, it is a **competitive** inhibitor.

3) If the lines meet on the x-axis, it is a **non-competitive** inhibitor.

4) If the lines do not meet at all, it is an **uncompetitive** inhibitor.



5) We can also learn about the **K_m** of the inhibitor, by calculating where the line crosses the x-axis and taking the negative reciprocal of this number.

6) The K_m value tells us what concentration of substrate is necessary to achieve half of V_{max}, which in turn tells us something about how tightly the enzyme binds its substrate in the presence or absence of the inhibitor.

7) We can also use the equation of the trendline to calculate **V_{max}**, which is the maximum theoretical velocity of the reaction with unlimited substrate.

8) Vmax is simply the reciprocal of the 'c' value of the trendline equation, which is given by Excel on the chart in the `y = mx + c` format.

Notes

- Note that in this method, we are simplifying the process of measuring reaction rate by assuming that reaction rate will be proportional to the amount of absorbance measured at an early timepoint.
- This assumption will not be correct if you allow all the samples to become very yellow, so remember to read the plate before the reactions reach this point.
- If the lines on your chart are not very straight, check that you have performed the reciprocal function in Excel correctly.
- It may be helpful to extend the x-axis range of your chart to include negative numbers, since this may allow you to see more clearly where your trendlines may meet, to do so simply use the Format Chart Axis option then select the ‘Minimum’ x-axis value and choose a more appropriate number, such as -1.5.
- For best results do not allow the reaction to progress for too long, if the maximum absorbance values exceed 1.2, the readings are probably beyond the linear range of the spectrophotometer.