

## BL483 Biochemical Techniques

### How to Make Double Reciprocal Plots for kinetic data in Excel

Making a graph (or chart as it is called in Excel) which displays the 4<sup>th</sup> quadrant requires that negative values for the x-axis (or 1/[S] axis in a double reciprocal plot) be displayed. It also requires that the linear regression line for the data set be extended into the 4<sup>th</sup> quadrant using negative x axis values.

1. Enter your data in Excel in columns with the substrate concentrations in the first column and initial velocities in the 2<sup>nd</sup> to 4<sup>th</sup> columns. Take the reciprocals of these data in the same columns directly below the data or in columns to the right of the data. You can do this by moving the cursor to a cell and entering the formula “=1/cell”, where cell is the position of the cell with the first substrate concentration such as A1, which can be entered by clicking on that cell when making the formula. Next copy the cell with the formula to the clipboard, then highlight the corresponding set of cells where the rest of the cells where the reciprocal data will go and pasting, which automatically increment the cell being used in the formula.
2. After adjusting the number of significant figures if you wish, highlight the cells with reciprocal data and click on the icon for the chart wizard and tell it that you want to create a chart with scatter data and no lines, on a new sheet (sometimes this is easier) by following through the steps in the wizard. You can also enter a title for the chart and the labels for x and y axis at this time. A chart will be created showing only the first quadrant and just the data points you highlighted as 3 series sets of data with different symbols.
3. Now highlight one of the points of a series and open the pull down menu for chart and click on “add trendline”. In the trendline dialog box, after you make sure that you have the linear regression type highlighted, click on the “options” tab, here check the boxes for the display of the equation of the line and its regression coefficient on the chart and in the “forecast” section increase the value of the “backward” parameter to a value of at least “5” or use your estimate of the x intercept, which will make the trendline go into the 4<sup>th</sup> quadrant with negative x values. You can also add a title to the trendline in this dialog box. When you click OK on this dialog box to close it, the graph will automatically adjust to display the 4<sup>th</sup> quadrant with the trendline in it.
4. Now by double clicking on the trendline you just made, you can change its pattern (so that all the trendlines will have a different pattern on the printed graph) and also adjust the length of the backward forecast so that the trendline reaches x-axis. You can keep doing this until the trendline just meets the x-axis. By dividing the y-intercept given in the equation of the line by the slope, you can get the exact value of the x-intercept and this value (or one that is a bit greater) can be entered as the “backward forecast” value to make the trendline just meet the x-axis in the chart. By double clicking on one of the data points, you will get a dialog box which allows you to increase the pt size of the symbol and adjust its color etc.
5. Repeat steps 3 and 4 for each data series.
6. If you want to adjust the x or y axis range to make the graph look nicer, you can double click on the axis and the select the “scale” tab to get a dialog box for making the range displayed what you want. If you have trouble getting the right place to double click, move the mouse over the axis and wait until a small message appears which says “value (X) axis”, then double click. If you want to move one of the text boxes with the equation of the line so that it is displayed more clearly and does not cover up the line or its data points, click on the text and then put mouse pointer on the outline of the box at one of the small “handles” and drag the box where you would like it.
7. Save the Excel file and then print the chart.