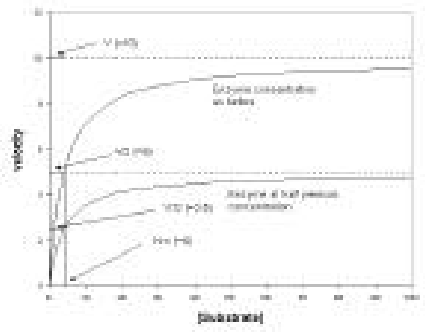
Graphical Analysis of Enzyme Data

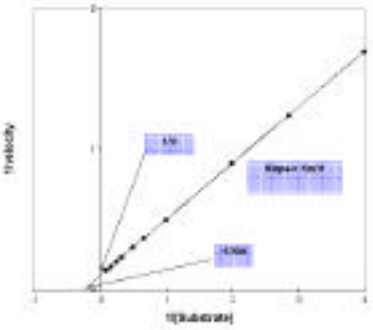
Science is about finding patterns in nature. Graphs help us to find patterns in quantitative data. In an enzyme assay, we look at product formation over time, so the simplest graph would be to plot product (P) or velocity (V) vs. substrate (S) concentration. This is called a Michaelis-Menten (M-M) plot, named after the researchers who established this method, based on an equation to describe the enzyme reaction: S becomes P in the presence of E.

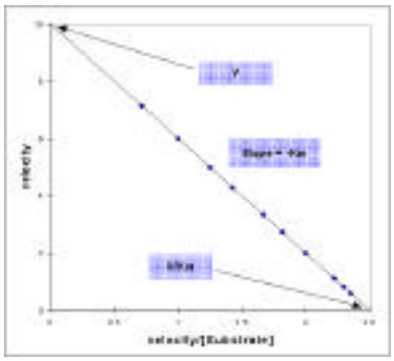


Note that the plot is actually a curve. Curves are hard to interpret, especially where they begin leveling off. Scientists tried to figure out how to get linear data, so they tried a variety of different approaches. Realizing that at early time reaction rates were more linear (near the intersection of the axes), they worked out ways to detect product formation with more accuracy, and began the field of enzyme kinetics. Scientists also used algebra to convert the formulas and see what patterns emerge when the data are plotted differently.

Reference for graph images: http://orion1.paisley.ac.uk/kinetics/contents.html

Lineweaver and Burk found that if you convert the Michaelis-Menten formula and then plot the n data, you CAN get a linear graph. What they had to do was plot the algebraic inverse of the product accumulation vs. substrate concentration (1/P vs. 1/S). When you reinspect the M-M plot, you find that the plateau where product formation levels off, is equivalent to the Vmax, maximum velocity. And, the value that is 1/2 that maximum is the Km, the affinity of the enzyme for its substrate.





Eadie and Hofstee found that if you plot product vs. the ratio of product/substrate, you also got a linear plot. This was especially helpful in the cases where endpoint assays were the only ones available (like our case with the catalase assay).

Scientists routinely test out a variety of formats for plotting data, not just looking at setting up axes with product and substrate various ways (direct, inverse, ratio), but also using semi-log or log-log papers (or calculating the logarithms for each data point). These graphs are also informative, helping us to see if the reaction is following simple or more complicated kinetics. For instance, DNA or protein migration during gel electrophoresis follows a log/linear pattern. Some scientists have begun using gradient gels to provide a sieve with logarithmic behavior to help in size analyses! The data we get helps us to clarify our ideas and form the new baseline for further experimentation.

Source: http://orion1.paisley.ac.uk/kinetics/Chapter_2/chapter2_7.html

Five: Graphing and arithmetic calculations are tools for data analysis …

Students will calculate the range, mean, variance and standard deviation of reaction times for each peroxide concentration.

Each student is given a copy of the spreadsheet containing the class data. The teacher will lead the students through the calculations and a discussion of the data for one of the concentrations of peroxide. The students can do the calculations for the remaining concentrations on their own, working in teams in the classroom and/or at home.

1. Look at the class data. Pick one concentration of peroxide (pick the concentration where the data seems to be spread the most). Ask students to examine and comment on the values entered by each group.

2. What is the **range** of the times recorded for the concentration? Identify the lowest and the highest times recorded, and calculate the difference. Students record by hand on their copies of the spreadsheet.

3. What factors do you think might account for the range of the data? Most often, students respond that everyone did not follow the same procedure or mistakes were made. Discuss these mistakes and then point out that these would be examples of human error.

4. Suggest to the students that even if everyone did follow the same procedure, exactly, there would still be a range of times. What sorts of factors could account for this? **Experimental error** (errors inherent in the experimental design) would account for this. Identify variables that were not, or could not be kept constant (constants that were not specified in the procedure). These variables have become sources of experimental error.

5. Calculate the average or **mean** time for that concentration. Students record by hand on their copies of the spreadsheet.

6. Why do we look at the range of the data as well as the mean? This gives us a better description of the sample. For example, let's say that the average height of students in a group is five feet and five inches tall. This could mean that every student in the class is five foot five inches tall, or it could mean that half of the students in the class are four foot five and the other half are six foot five and so one. We describe the class better by talking about both the mean and the range.

7. Even better, we can describe the **variance** of the data. How scattered is it? In the column to the right of the times on the spreadsheet, record the difference between the mean time and each time recorded. See the completed spreadsheet.

8. In the next column, square each of those values. Add the squares together and average. This value is the **variance**.

9. The square root of the variance is the **standard deviation** (SD) of the data. The standard deviation is a statistic that tells how tightly all the various examples are clustered around the mean in a set.

10. Students can express what the mean plus or minus one SD tells about the variance. A large SD tells you that the data are fairly diverse, while a small SD tells you the data are pretty tightly bunched together.