## Mathematical Modeling and Enzyme Kinetics

In attempting to better understand an experimental system, it is often useful to attempt to generate a mathematical equation that describes some aspect of the system. In order to be maximally useful, the equation should have parameters that are related to the physical properties of the system under investigation. An equation may fit experimental data well, but is generally useless as a mathematical model unless the parameters of the equation lend insight into the experimental system being examined.[1]

For kinetic studies of simple enzymatic reactions such as the one below (left), the usual mathematical model is the Michaelis-Menten equation (below, right):

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad\qquad v = \frac{V_{max}[S]}{K_m + [S]}$$
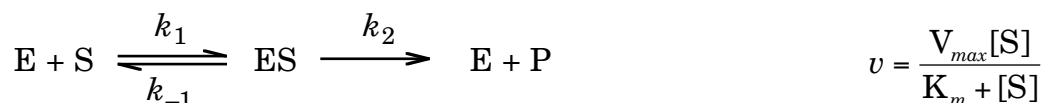
This equation has the form of a rectangular hyperbola, and has two asymptotic parameters: the V_{max} and the K_m. The V_{max} is usually interpreted to describe the velocity observed when the enzyme is fully saturated with substrate, and is a function of both the enzyme concentration and the catalytic rate constant of the enzyme. The K_m is a measure of the affinity of the enzyme-substrate interaction. Each parameter therefore has a well-defined physical meaning related to the properties of the enzyme involved.

When studying an enzyme, it is highly useful to measure the V_{max} and K_m values. In principle, the V_{max} and K_m values could be determined by measuring the velocity at two substrate concentrations, generating two equations with two unknowns that would allow solving for each unknown parameter.

Because many enzymes do not exhibit simple Michaelis-Menten kinetics, analyzing more data is necessary to determine which mathematical model is more likely to be correct. In addition, in the real world, experimental data rarely fit an equation exactly, which means that any experimental parameter determined from only two data points is likely to exhibit significant deviations from the true value. Most experimenters therefore measure the reaction velocity for a range of substrate concentrations, and then use all of the data to estimate the V_{max} and K_m values.

One well-accepted method for fitting data to a two-dimensional equation is to calculate the parameters that minimize the deviations of the dependent variable from the theoretical values. The least-squares linear regression algorithm allows the slope and *y*-intercept to be calculated exactly for any set of data. Unfortunately, as with many mathematical models, the Michaelis-Menten equation is not a linear

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[1] This is not to say that totally empirical equations, such as calibration curves, are useless. Empirical equations with predictive value are widely used. In general, however, a totally empirical equation has very limited use in determining the mechanism by which a process occurs, and is frequently useless for making predictions outside of a narrow range of values of the independent variables.

equation, and as with most non-linear equations, the parameters cannot be calculated exactly for any set of data. Several approaches have evolved to analyze enzymatic reaction data; two of these approaches will be discussed below.

In 1934, Hans Lineweaver and Dean Burk published a rearranged form of the Michaelis-Menten equation:[2]

$$\frac{1}{v} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$
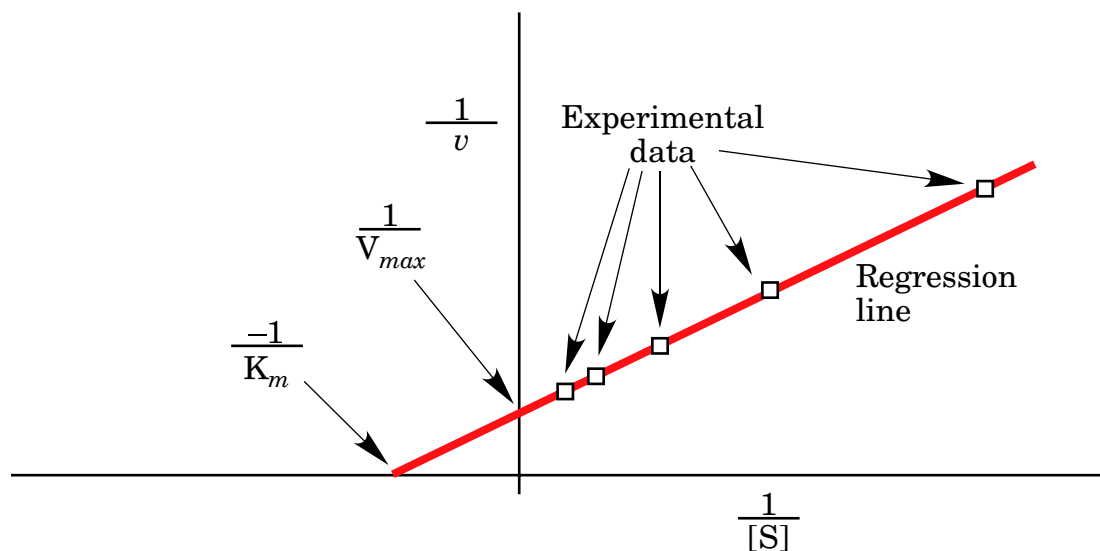
A plot of 1/*v versus* 1/[S] is linear, and allows the use of linear regression to solve for the slope (which is K_m/V_{max}) and the *y*-intercept (which is $1/V_{max}$). Unfortunately, the double-reciprocal plot tends to be heavily distorted by experimental errors, and in consequence frequently results in inaccurate and sometimes nonsensical V_{max} and K_m values.

An alternative method for estimating the V_{max} and K_m values is to use least-squares non-linear regression to fit the data to the Michaelis-Menten equation directly. The technique of least-squares non-linear regression differs somewhat from methods used for analysis of linear equations. Because least-squares non-linear regression equations cannot be solved analytically, it is necessary to make an initial estimate of the equation parameters, and then perform iterative calculations intended to

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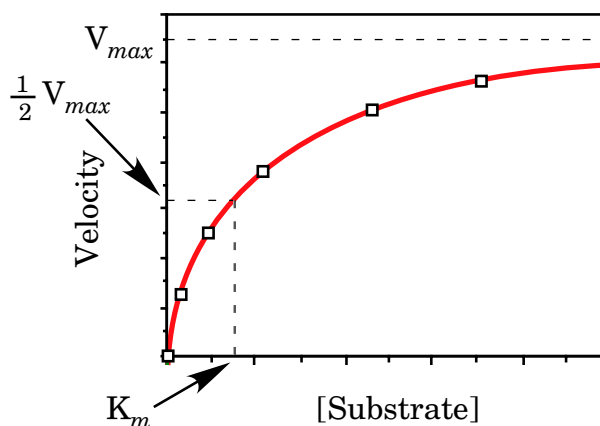
[2]Lineweaver, H. & Burk, D. (1934) "The Determination of Enzyme Dissociation Constants." *J. Am. Chem. Soc.* **56**, 658-666.

improve the fit between the theoretical and observed functions of the independent variables. Most computer programs (including Excel, see below) that allow this procedure have an algorithm for finding a minimum sum-of-the-squared errors.

Least-squares non-linear regression algorithms have some potential drawbacks. One potential problem is that, if the initial estimate of the parameters is too far from the correct value, the algorithm may fail to converge on an answer. A second potential problem is that the mathematical function may exhibit local minima of sum-of-the-squares, and therefore converge on an incorrect value (this is rarely observed for the rectangular hyperbola). Finally, for real data, it may be difficult or impossible to distinguish unambiguously between deviations due to experimental error and deviations due to an incomplete mathematical model.

When data fit a rectangular hyperbola fairly closely, they can be readily analyzed using either the Lineweaver-Burk plot or non-linear regression. However, real experiments may not yield data that are well behaved. You ***therefore need to look at the data _and_ at the resulting graphs***, and not merely accept (and report) the values calculated by Excel. ***There is no substitute for having an intelligent human look at the results*** of a kinetic (or any other kind of) experiment.

Using Non-linear Regression in Excel
1. **Office 2007**: Make sure that the **Solver** option is present in the **Data** tab. (If it is not present, click on the **Office** button, and then on **Excel Options**. Select **Add-ins** in the dialog box, and then click **Go** next to Manage Add-ins. Place a check box next to **Solver**, and click **Add**. Solver should appear in the **Analysis** section of the **Data** tab.)
2. **Office 2013**: Make sure that the **Solver** option is present in the **Data** tab (it should be an option on the right side of the ribbon. (If it is not present, click on the **File** tab, and then on **Options**. Select **Add-ins** in the dialog box, and then click the **Go** button next to Manage Add-ins. Place a check box next to **Solver**, and click **Add**. Solver should appear in the **Data** tab.)
3. Enter the data to be analyzed.
4. Create a column of squared errors (for the Michaelis-Menten equation, the formula would be: (C9-(D\$4*B9)/(D\$3+B9)))^2, where C9 is the velocity data point, B9 is the substrate concentration data point, and D\$4 and D\$3 are the V_{max} and K_m values, respectively. (Note that the \$ in from of the row number allows you to use "fill down" without changing the row reference.)
5. Create a cell that has the sum of the squared errors column (*e.g.*, D5).
6. Enter initial values for V_{max} and K_m cells. The values should be reasonably close to the actual values, whenever possible.
7. Select **Solver** from the **Data** tab. In the dialog box, Set Target Cell D5, Equal To: **Min** By Changing Cells \$D\$3 and \$D\$4. (Note that the cell references are specific for the example spreadsheet; you may need to use different ones.)
8. Click **Solve**.

An example spreadsheet for performing non-linear regression using the Michaelis-Menten equation is posted on the course website.