

## Chem\*2580 Lecture 15

### Experimental Enzyme Kinetics: Linear Plots

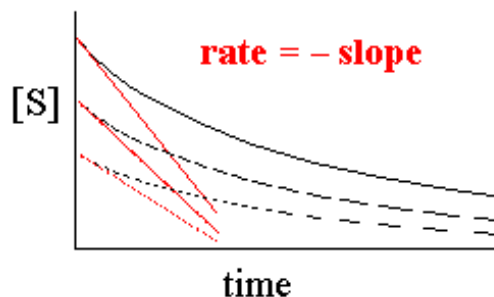
**Synopsis:** The kinetic behaviour of enzymes is described by the **Michaelis- Menten equation**, and the two characteristic constants associated with this equation,  $V_{\max}$  and  $K_M$ . Every enzyme has specific values for these constants, which must be measured experimentally. **Linear plots** provide the simplest means of fitting potentially error prone experimental values to the Michaelis-Menten equation. The **Wolf-Hanes** plot is preferable on statistically grounds, although the **Lineweaver-Burk** plot is also very widely used.

Reading: Lehninger (4th Ed) p.205-207; (3rd Ed) p.261-264; Horton p 141-142.

#### Experimental measurement of enzyme kinetic properties

The kinetic properties of enzymes may be characterized by **measuring reaction rates for a series of different substrate concentrations**. Each rate measured should be an **initial velocity**, either by taking the slope of a progress curve ([S] or [P] plotted versus time) at zero time, or by allowing the reaction to proceed for a very brief time and measuring extent of reaction. The reaction should be demonstrated to be linear over the short time interval.

A different progress curve is obtained for each initial substrate concentration, which is indicated by where the curve starts on the [S] axis. A slope is measured for each curve at  $t = 0$ . These slopes are the initial rates,  $v_0$  used in the **Michaelis-Menten plot**. **The Michaelis Menten Hyperbolic plot.**

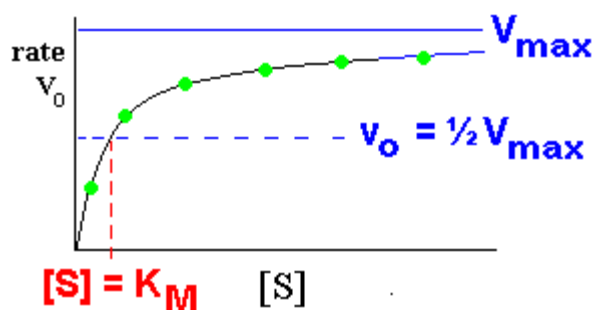


Initial rates taken from the progress curves are then plotted as a function of [S]. The graph follows a characteristic **hyperbolic** shape that matches the **Michaelis-Menten** equation.

$$v_0 = \frac{V_{\max} [S]}{K_M + [S]}$$

$V_{\max}$  is the limiting maximum rate as [S] tends to infinity.

Once  $V_{\max}$  has been determined we find the point on the curve where  $v_0 = \frac{1}{2} V_{\max}$ ; the concentration [S] at this point gives the value of  $K_M$ .



**$K_M$  and  $V_{max}$  tell you about the enzyme's properties as a catalyst.**

$V_{max}$  indicates catalytic rate when 100% of enzyme is occupied by substrate  
higher  $V_{max}$  means faster reaction, better catalysis.

$V_{max}$  is not a true constant - it is only constant if the **same amount of enzyme** is used for each rate measurement.  $V_{max}$  is proportional to the concentration of enzyme present:

$$V_{max} = k_2 [E]_{total}$$

From  $V_{max}$ , we can calculate the true constant  $k_2$ , the rate constant for the catalytic step of the two step enzyme reaction.  $k_2$  (sometimes written as  $k_{cat}$ ) is the **turnover number** of the enzyme.



$K_M$  indicates the **affinity of the substrate** for the enzyme.

**Low  $K_M$  means high affinity**, the enzyme binds this substrate **strongly**;

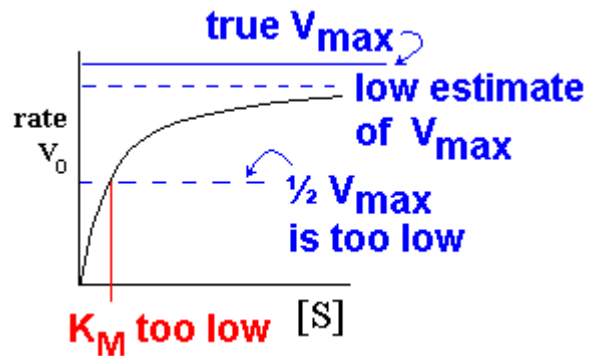
**High  $K_M$  means low affinity**, the enzyme binds this substrate **more weakly**.

**An enzyme that recognizes different substrates will have a different  $K_M$  for each substrate.**

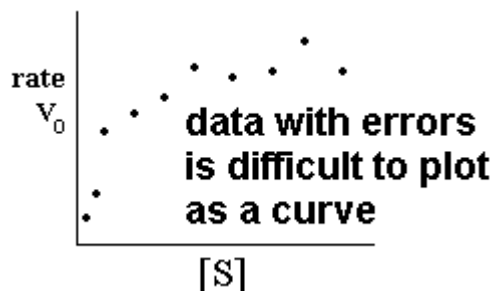
To measure  $K_M$  of substrate **B**, keep **[A] high and constant**, measure rate as a function of varying **[B]**.

**Measuring  $V_{max}$  and  $K_M$  can be tricky**

Unfortunately it's not as easy as it seems to estimate  $V_{max}$  by inspection of the hyperbolic plot, since the curve keeps creeping up even at very high  $[S]$ . Most people underestimate  $V_{max}$  by 10-20% when using this method. If the estimate of  $V_{max}$  is bad, the estimate of  $\frac{1}{2} V_{max}$  and  $K_M$  is also affected.



Real experimental data also tends to scatter off the theoretical curve due to measurement errors, making graphing the correct curve even more difficult.



## Linear Plots

Instead, the Michaelis-Menten equation may be rewritten to plot as a **straight line**, which is much easier for graphing experimental data. This is known as a **linear transform**.

### Lineweaver-Burk or Double Reciprocal Plot

Take **reciprocals** of both sides of the Michaelis-Menten equation and then cancel.

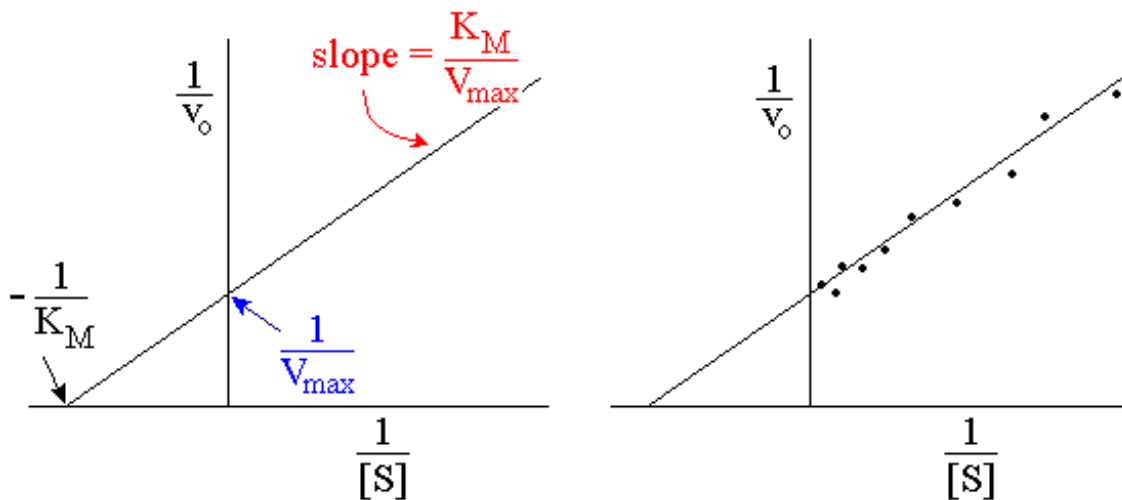
If we substitute **y** for  $1/v_o$  and **x** for  $1/[S]$ , this is now the standard equation for a straight line, with **slope** =  $K_M / V_{max}$  and **y intercept** =  $1 / V_{max}$ .

$$\frac{1}{v_o} = \frac{K_M + [S]}{V_{max} [S]}$$

$$\frac{1}{v_o} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = \underset{\text{slope}}{m} \cdot X + \underset{\text{y-intercept}}{b}$$

A straight line plot is much preferred over a curve, particularly when the data is slightly scattered due to experimental error. Slopes and intercepts are relatively easily obtained from a straight line graph. **The result is known as the Lineweaver-Burk plot, or Double Reciprocal plot**



Y-axis	X-axis	slope	y-intercept	x-intercept
$1 / v_o$	$1 / [S]$	$K_M / V_{max}$	$1 / V_{max}$	$- 1 / K_M$

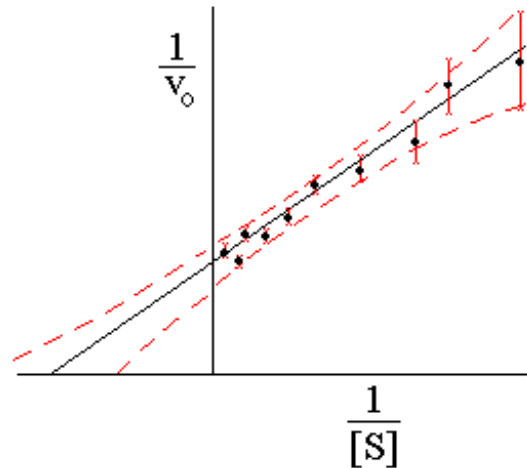
The Lineweaver-Burk plot is usually extrapolated to the negative **x**-axis. Although there's no data with negative **x** values, the negative **x**-intercept can be used to obtain  $K_M$ .  
 $X\text{-intercept} = - Y\text{-intercept} / \text{slope}$ .

**A hint for correlating axis labels and intercepts:** The y axis is  $1 / v_o$ , so its intercept is  $1 / V_{max}$ . The x axis is  $1 / [S]$  so its intercept is  $- 1 / K_M$ . (the negative sign corrects for the intercept having a negative value). Remember that  $K_M$  is a **concentration**, the concentration  $[S]$  that happens to give 50% of maximum rate or  $0.5 \times V_{max}$ .

## Other plots

There are several other ways of plotting enzyme data, but the Lineweaver-Burk method is the most widely used and should be known for that reason. However, it does suffer from one major problem due to skewing of the data when **reciprocals** are taken.

The smaller and more error prone experimental values become the largest values as reciprocals. The figure shows the error range for each measurement as red vertical bars. The rightmost values have an exaggerated error due to taking reciprocals of the data. The dotted red lines indicate the range of uncertainty of placement of the final straight line graph due to experimental error.



The **Wolf Hanes Plot** avoids taking reciprocal concentrations, and is the preferred method for **statistical correctness** of data analysis

Starting from the Lineweaver Burk equation, multiply both sides by  $[S]$ , then cancel:

The linear equation that results uses  $[S]$  values without taking reciprocals.

The X-axis is simply  $[S]$ , so a uniform series of concentrations in the experiment gives a uniformly spaced set of points in the data. The rightmost points relate to the larger and more accurate values of  $[S]$  and  $v_0$ , so there is no exaggeration of error on the right.

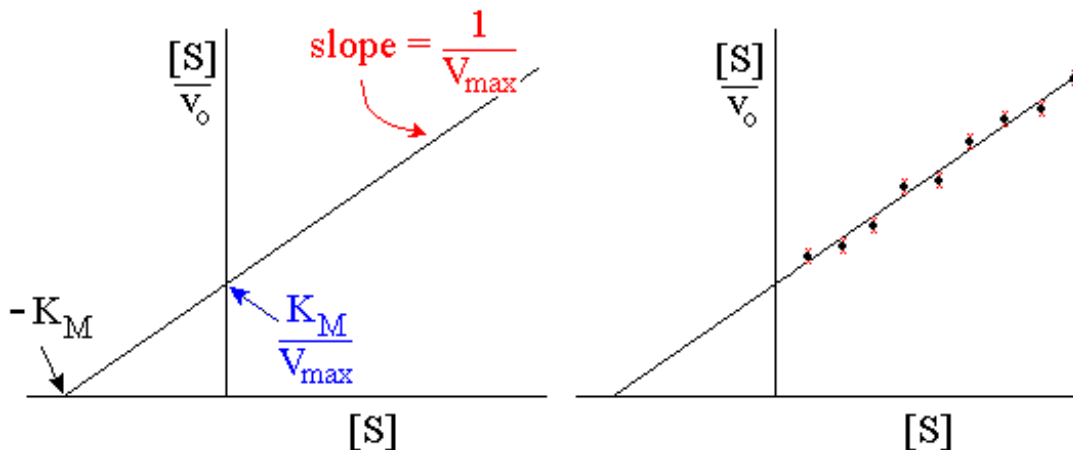
$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

multiply both sides by  $[S]$ :

$$\frac{[S]}{v_0} = \frac{K_M}{V_{max}} \frac{[S]}{[S]} + \frac{[S]}{V_{max}}$$

$$\frac{[S]}{v_0} = \frac{K_M}{V_{max}} + \frac{1}{V_{max}} [S]$$

$$y = \underset{\text{y-intercept}}{b} + \underset{\text{slope}}{m} \cdot X$$



Axes, slopes and intercepts for Woolf-Hanes plots:

Y-axis	X-axis	slope	y-intercept	x-intercept
$[S] / v_o$	$[S]$	$1 / V_{max}$	$K_M / V_{max}$	$- K_M$

### Eadie-Hofstee Plot

Start from the Lineweaver Burk equation and multiply both sides by  $v_o V_{max}$ , then cancel.

The only special merit of this method is that  $K_M$  is derived directly from the slope, and when data is scattered due to error, intercepts may be a bit inaccurate.

Data is skewed like the Lineweaver-Burk plot, because the smaller and least accurate points are plotted furthest from the y-axis, where they have an exaggerated effect on the slope.

$$\frac{1}{v_o} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

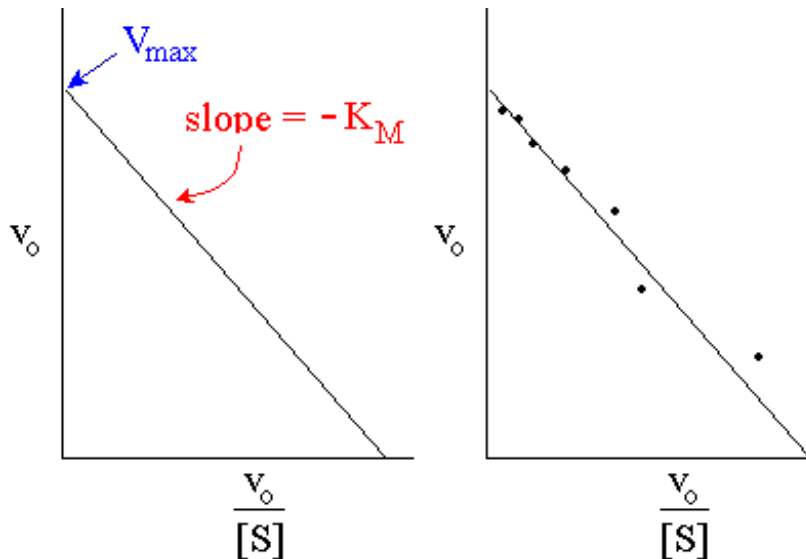
multiply both sides by  $v_o V_{max}$

$$\cancel{v_o} V_{max} \frac{1}{\cancel{v_o}} = \frac{K_M \cancel{v_o} V_{max}}{V_{max} [S]} + \frac{\cancel{v_o} V_{max}}{V_{max}}$$

$$V_{max} = K_M \frac{v_o}{[S]} + v_o$$

$$v_o = V_{max} - K_M \frac{v_o}{[S]}$$

$$y = \underset{\text{y-intercept}}{b} + \underset{\text{slope}}{m} \cdot X$$



Y-axis	X-axis	slope	y-intercept	x-intercept
$v_o$	$v_o / [S]$	$- K_M$	$V_{max}$	$V_{max} / K_M$