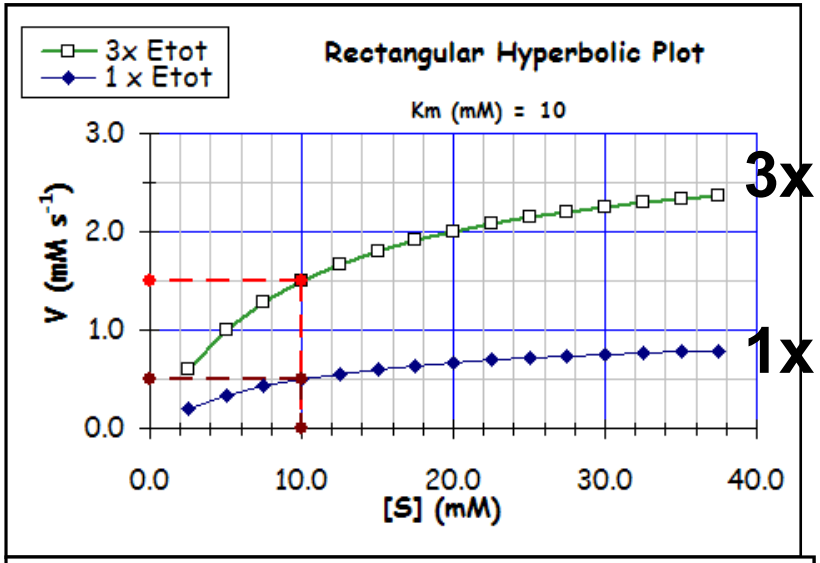
Enzyme Catalytic Kinetic Plots

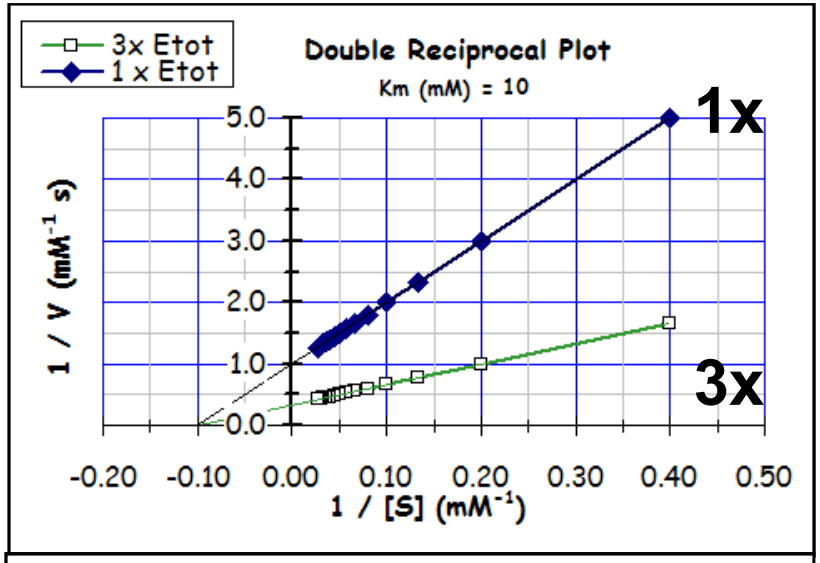
Rectangular Hyperbolic Plot

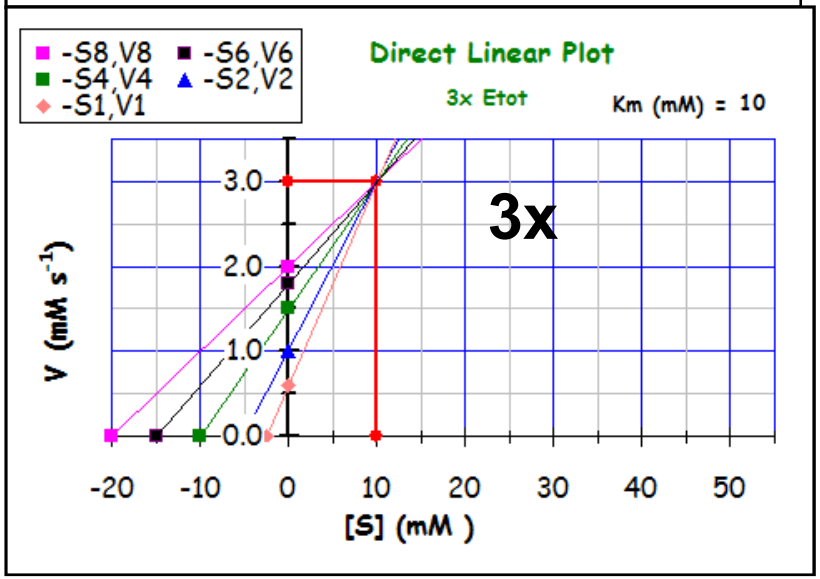
Double-Reciprocal (Lineweaver-Burk) Plot

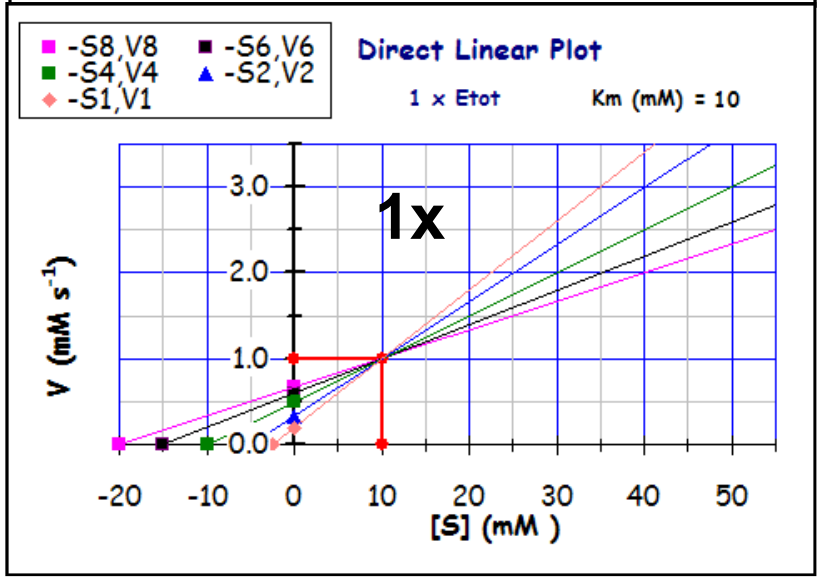
Direct Linear (Eisenthal-Cornish-Bowden) Plot

Enzyme Concentration Dependence `of Different Kinetic Data Plots

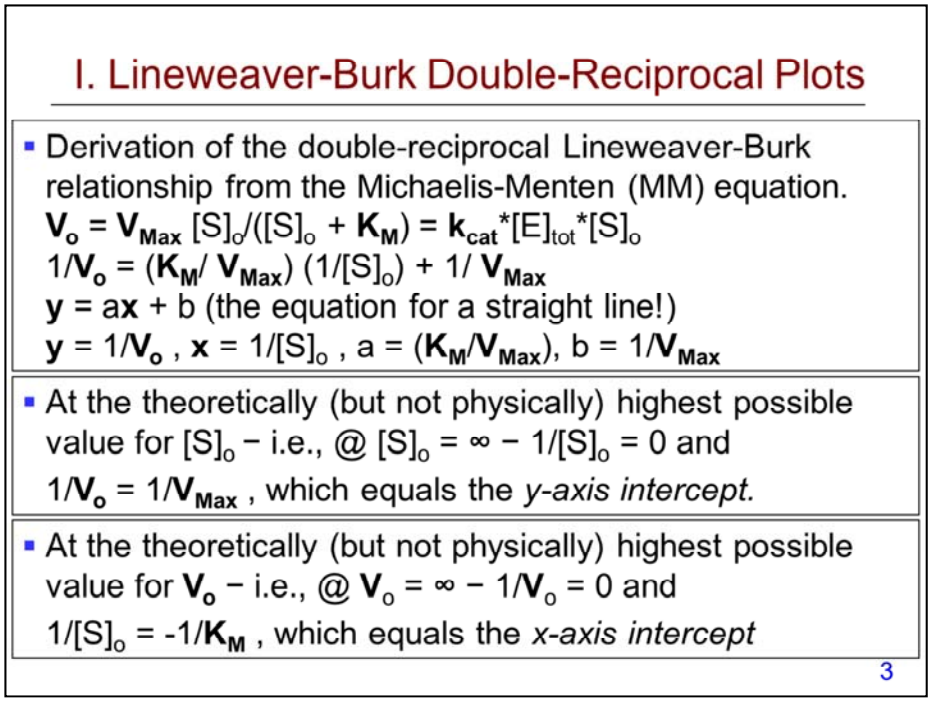








https://biosci.mcdb.ucsb.edu/biochemistry/sprdshts/Michaelis-Menten-kinetics.xls



- The most conventional way for analyzing enzyme kinetic rate data is to use a double-reciprocal (Lineweaver-Burk) plot. However, this plot has certain limitations, especially the kinetic data is very “noisy” or has a high level of experimental variation, which typically occurs when measured substrate concentrations and/or the product concentrations are very low.
- The double-reciprocal plot is generated by “inverting” the Michaelis-Menten (MM) equation and plotting the reciprocal values for !/Vo and 1/[S]o.
- If the enzyme kinetic data conforms to the MM equation, a linear relationship will be observed between 1/Vo and 1/[S]o.
- Even when enzyme kinetic data does not conform to MM kinetics, one can still emperically determine MM-like constants for kcat (the turnover number) and KM, the substrate concentration for half-maximal velocity.

Lineweaver-Burk "Double- Reciprocal" Plot

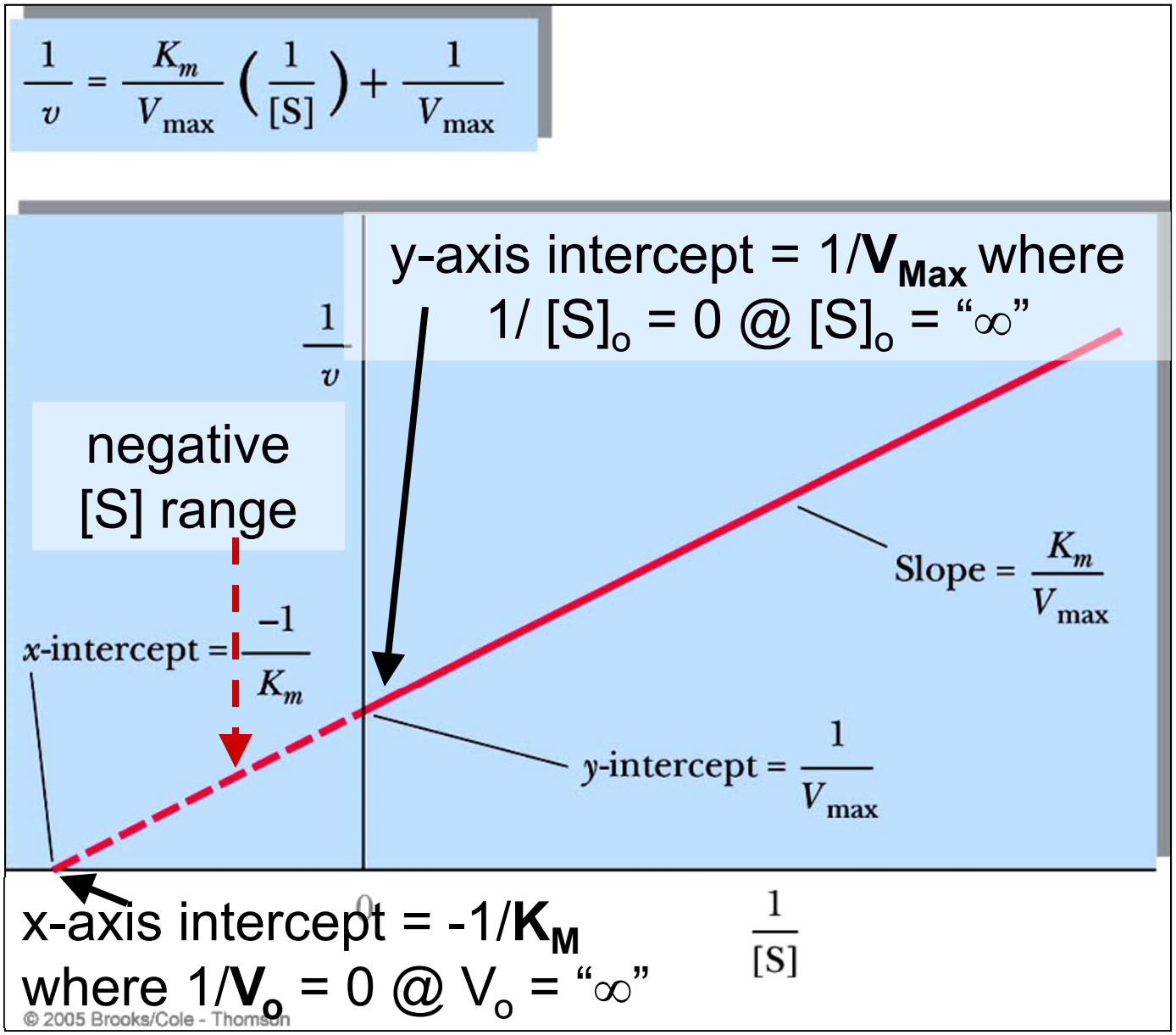


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Fig. 13-09,
p.419

II. Eisenthal-Cornish-Bowden Direct Linear Plot

- Derivation of direct-linear Eisenthal-Cornish-Bowden relationship:

$V_o = V_{Max}\,[S]_o/([S]_o + K_M)$

$V_{Max} = (V_o/[S]_o)\,K_M + V_o$

$y = ax + b$ (the equation for a straight line!)

$y = V_{Max}$, $x = K_M$, $a = (V_o/[S]_o)$, $b = V_o$

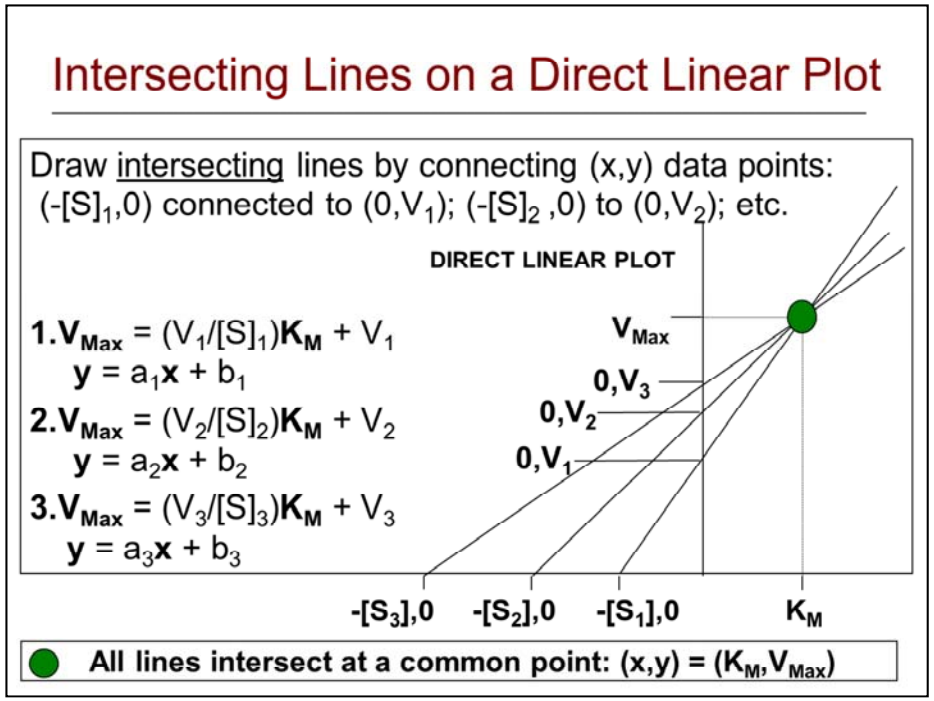
- By assuming that V_{Max} and K_M are variables (not constants), one finds a straight line equation for every pair of V_o and $[S]_o$ values:

1. $V_{Max} = (V_1/[S]_1)K_M + V_1$ $\quad y = a_1x + b_1$
2. $V_{Max} = (V_2/[S]_2)K_M + V_2$ $\quad y = a_2x + b_2$
3. $V_{Max} = (V_3/[S]_3)K_M + V_3$ $\quad y = a_3x + b_3$

- The direct linear (Eisenthal-Cornish-Bowden) plot is a rarely discussed analysis of enzyme kinetic data (see Chapter 8 of WWBH) because the enzyme data must conform to the MM equation and the empirical kinetic data must have very little noise for the plot to be interpretable. However, whenever these two conditions hold, the plot generates easily interpretable data.

After rearranging the MM equation the following mathematical relationships are found:

- Rearranged MM Equation: Vo = Vmax [S]o / ([S]o + KM), as found by multiply both sides by ([S]o + KM) / [S]o.
- Eisenthal-Cornish-Bowden Equation: Vmax = (Vo /[S]o) KM + Vo
- For a moment, assume that Vmax and KM are "variables" (not constants) and Vo / [S]o]and Vo are “constants” (not variables) in this equation.
- IF this were the case, this equation would describe a straight line for every pair of Vo and [S]o variables.
- “y" = a “x" + b, with “y" = Vmax “x" = KM a = (Vo / [S]o) and b = Vo
- For y = 0, x = -b/a = - Vo /(Vo/ [S]o) = -[S]o , For x = 0, y = b = Vo



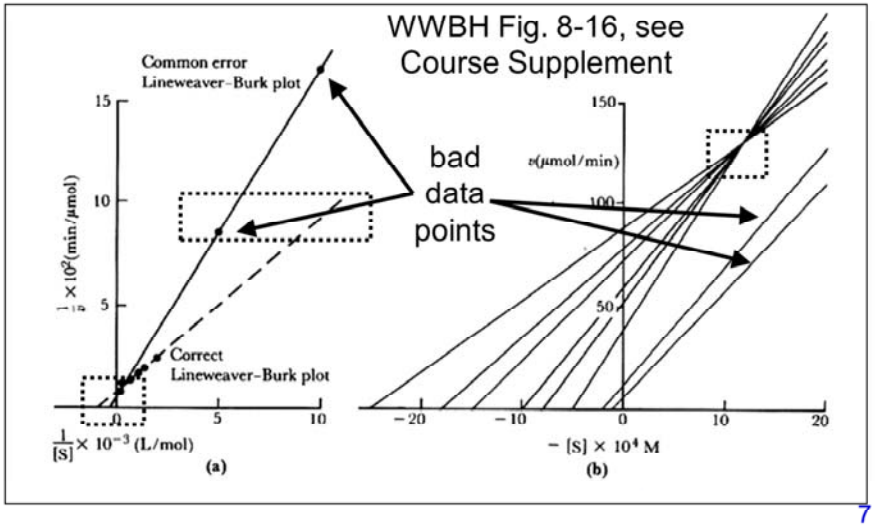
- Consider what happens with this equation when there are several values for initial substrate concentrations, [S]o, i.e., [S1], [S2], [S3], etc. -- and several corresponding values for the *initial velocity* of product formation, Vo, i.e., V1, V2, V3, etc.
- Each data pair point, defined by the 2 sets of (X,Y) coordinates falls on a unique line:

  For (-[**S1**],0) and (0, **V1**), line 1 = **Vmax** = (**V1**/[**S1**])**KM** + **V1**

  For (-[**S2**],0) and (0, **V2**), line 2 = **Vmax** = (**V2**/[**S2**])**KM** + **V2**

  For (-[**S3**],0) and (0, **V3**), line 3 = **Vmax** = (**V3**/[**S3**])**KM** + **V3**
- Each of these lines is different, BUT they all have one *common point of intersection* as defined by these (x,y) coordinates, (KM,Vmax); this point corresponds to the only real value for KM and Vmax.
- Thus, one predicts that all lines on a direct linear plot for a Michaelis-Menten enzyme will theoretically intersect at a single point.

Sensitivity of Double-Reciprocal and Direct Linear Plots to Data Errors



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- One difference between the double-reciprocal and the direct linear plots is the way in which bad data points affect the plots. A comparison between the two graphic representations direct is illustrated here with two "bad" data points (see Fig. 8.16, WWBH).
- The same data points are plotted on adjacent Lineweaver-Burk in the left graph of this figure. Two features of the direct linear plot are immediately evident by comparison.
- It is fairly difficult to know exactly where to draw the double-reciprocal line when the two bad data points are included (and given equal weight) and this affects greatly the intercept on the X-axis (**-1**/**Km**) but not the intercept on the Y-axis (**Vmax**).
- On a direct linear plot, the two bad data points generate two anomalous lines and it is easier to justify rejecting these two lines as bad data points because all of the other lines intersect at a common point corresponding to (**Vmax**,**KM**).

Pros and Cons of Different Kinetic Plots

A. Rectangular Hyperbolic Plot: $V_o = V_{Max}*[S]_o/([S]_o+K_M)$

- Pros: Easiest to interpret for in vivo enzyme kinetics since values for $[S]_{in\ vivo}$ tend to fluctuate near K_M.
- Cons: V_{Max} & K_M are impossible to determine exactly

B. Double-Reciprocal Plot:

$1/V_o = (K_M/V_{Max})*(1/[S]_o)+ 1/V_{Max}$

- Pros: V_{Max} determination based on "strong" data.
- Cons: K_M determination ultra sensitive to "bad" data.

C. Direct-Linear Plot: $V_{Max} = (V_o/[S]_o)*K_M + V_o$

- Pros: V_{Max} & K_M are easy to determine and "bad" data points are easy to spot & eliminate.
- Cons: Plot make no sense for a non-MM enzyme.