Eadie–Hofstee diagram

In Enzymology, an **Eadie–Hofstee diagram** (also **Woolf–Eadie–Augustinsson–Hofstee** or **Eadie–Augustinsson plot**) is a graphical representation of enzyme kinetics in which reaction velocity is plotted as a function of the velocity vs. substrate concentration ratio:

$$V = -K_m \frac{V}{[S]} + V_{\max}$$

Where, *v* represents reaction velocity, K_m is the Michaelis–Menten constant, [*S*] is the substrate concentration, and $V_{\max}$ is the maximum reaction velocity.

It can be derived from the Michaelis–Menten equation as follows:

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

invert and multiply with $V_{\max}$:

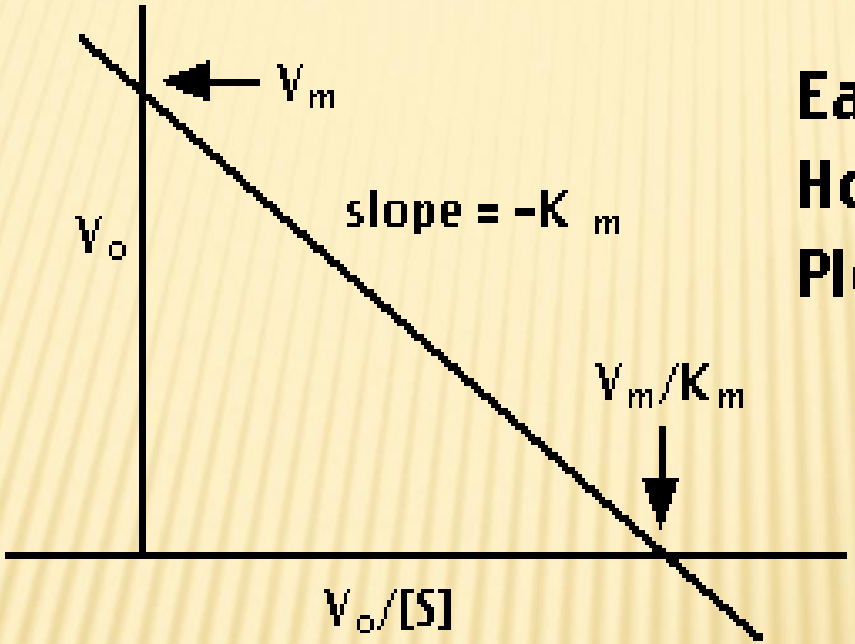
$$\frac{V_{\max}}{v} = \frac{V_{\max}(K_m + [S])}{V_{\max}[S]} = \frac{K_m + [S]}{[S]}$$

Rearrange:

$$V_{\max} = \frac{vK_m}{[S]} + \frac{v[S]}{[S]} = \frac{vK_m}{[S]} + v$$

Isolate v:

$$v = -K_m \frac{v}{[S]} + V_{\max}$$



Eadie-Hofstee Plot

A plot of v vs. v/[S] will yield V_{max} at the intercept with the *y*-axis and the slope is $-K_m$. Like other techniques that linearize the Michaelis–Menten equation, the Eadie-Hofstee plot was used historically for rapid identification of important kinetic terms like K_m and V_{max}, but has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible. It is also more robust against error-prone data than the Lineweaver–Burk plot, particularly because it gives equal weight to data points in any range of substrate concentration or reaction velocity. (The Lineweaver–Burk plot unevenly weights such points.) Both plots remain useful as a means to present data graphically.

One drawback from the Eadie–Hofstee approach is that neither ordinate nor abscissa represent independent variables: both are dependent on reaction velocity. Thus any experimental error will be present in both axes. Furthermore, the typical measure of goodness of fit, the correlation coefficient *R*, is not applicable.

Hanes–Woolf plot

In Enzymology, a Hanes–Woolf plot or Hanes-Wolff Plot is a graphical representation of enzyme kinetics in which the ratio of the initial substrate concentration [S] to the reaction velocity v is plotted against [S]. It is based on the rearrangement of the Michaelis–Menten equation shown below:

$$\frac{[S]}{v} = \frac{[S]}{V_{\max}} + \frac{K_m}{V_{\max}}$$

Where, K_m is the Michaelis–Menten constant and V_{max} is the maximum reaction velocity. It was first described by Barnet Woolf. Charles Samuel Hanes subsequently pointed out that the use of linear regression to determine kinetic parameters from this type of linear transformation is flawed, because it generates the best fit between observed and calculated values of 1/v, rather than v.

The equation can be derived from the Michaelis–Menten equation as follows:

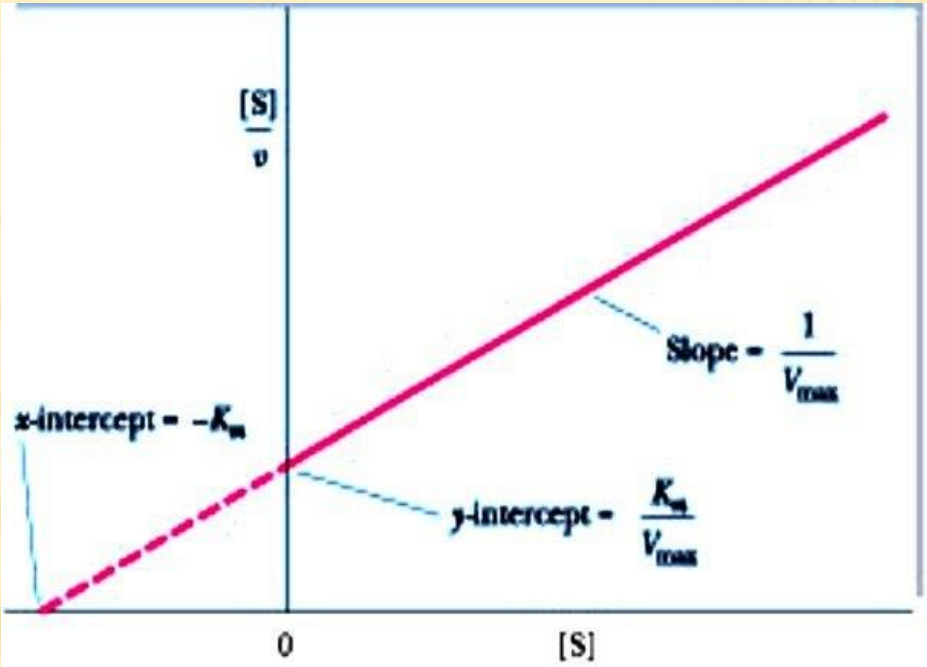
$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

invert and multiply by [S]:

$$\frac{[S]}{v} = \frac{[S](K_m + [S])}{V_{\max}[S]} = \frac{K_m + [S]}{V_{\max}}$$

Rearrange:

$$\frac{[S]}{v} = [S]\frac{1}{V_{\max}} + \frac{K_m}{V_{\max}}$$



Hanes–Woolf plot

As is clear from the equation, perfect data will yield a straight line of slope $\mathbf{1/V_{max}}$, a ***y*-intercept** of $\boldsymbol{K_m/V_{max}}$ and an ***x*-intercept** of $\boldsymbol{-K_m}$.

Like other techniques that linearize the Michaelis–Menten equation, the Hanes–Woolf plot was used historically for rapid determination of the important kinetic parameters K_m, V_{max} and V_{max}/K_m, but it has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible. It remains useful, however, as a means to present data graphically.

One drawback of the **Hanes–Woolf** approach is that neither ordinate nor abscissa represent independent variables: both are dependent on substrate concentration. As a result, the typical measure of goodness of fit, the correlation coefficient R, is not applicable.

Quick Summary of linear kinetic plots

	Lineweaver-Burk	Woolf- Hanes	Eadie-Hofstee
equation	reciprocal of M & M	L-B x [S]	L-B x $v_o V_{max}$
y-axis	$1 / v_o$	$[S] / v_o$	v_o
x-axis	1 / [S]	[S]	v_o / [S]
y-intercept	$1 / V_{max}$	K_M / V_{max}	V_{max}
x-intercept	$-1 / K_M$	$-K_M$	V_{max} / K_M
slope	K_M / V_{max}	$1 / V_{max}$	$- K_M$

Non-linear Regression Kinetics

- Nonlinear regression is a general technique to fit a curve through the experimental data.
- It fits data to any equation that defines Y as a function of X and one or more parameters.
- It finds the values of those parameters that generate the curve that comes closest to the data (minimizes the sum of the squares of the vertical distances between data points and curve).
- Except for a few special cases, it is not possible to directly derive an equation to compute the best-fit values from the data. Instead nonlinear regression requires a computationally intensive, iterative approach.

Nonlinear regression fits a mathematical model to the worker's data

Choices to make when fitting data with nonlinear regression

Step 1. Choose a model

Step 2. Choose (or review) initial values

Step 3. Decide whether to constrain any parameters

Step 4. Decide on a weighting scheme

Step 5. Decide how to handle replicate values (if any)

Step 6. Choose other options

How to determine Vmax and KM?

To determine Vmax and KM with Prism:

1. **Enter substrate concentrations into the X column and velocity into the Y column (entering replicates if one has them).**
2. **Click Analyze and choose built-in analyses. Pick nonlinear regression from the list of curves and regressions.**
3. **Choose more equations. Enter this equation as a new equation, or choose from the enzyme kinetics equation library.**

$$Y = (Vmax * X)/ (Km + X)$$

Learning of Prism 4 /Prism 5 (software) [GraphPad.com] is essential for enzyme kinetics analysis. GraphPad Prism is a commercial scientific 2D graphing and statistics software published by GraphPad Software, Inc., a privately-held California corporation.