

Microbial and Enzyme Kinetics

Enzyme Kinetics Tutorial 3b

Rate measurements were made on a food enzyme with and without the presence of an inhibitor with the following results

| [S]/ μM | Initial Rate, $V_0/\mu\text{mol min}^{-1}$ | |
|--------------------|--------------------------------------------|----------------|
| | Without inhibitor | With Inhibitor |
| 25 | 8.20 | 5.65 |
| 50 | 12.86 | 8.16 |
| 75 | 15.51 | 9.68 |
| 100 | 17.95 | 11.04 |
| 150 | 20.02 | 13.56 |
| 200 | 22.64 | 15.71 |

Inhibitor concentration, $[I] = 20 \mu\text{M}$

1. Use either the Eadie-Hofstee or the Hanes plot to determine the type of inhibition
2. Determine K_m and V_0 for the enzyme without inhibition and the inhibitor parameter, K_I .
3. What further tests would be necessary to improve the accuracy of estimate of K_I ?
How would you calculate K_I in this case?

Rearrangement of Eadie-Hofstee and Hanes plots.

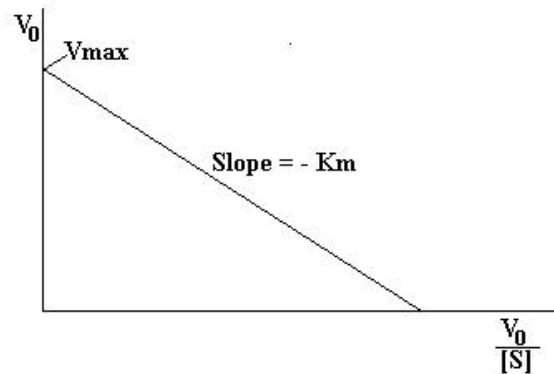
The Eadie-Hofstee and Hanes plots may be rewritten to simplify them

a. Eadie-Hofstee equation;

$$\frac{V_0}{[S]} = \frac{V_{\max}}{K_m} - \frac{V_0}{K_m}$$

can be rearranged to give

$$V_0 = V_{\max} - \frac{K_m V_0}{[S]}$$



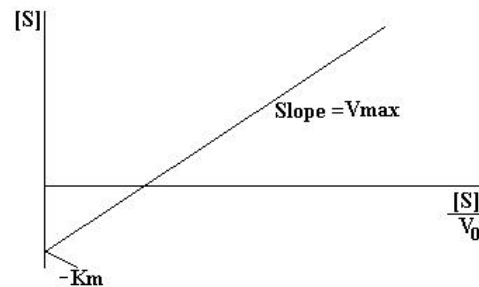
A graph of V_0 (y-axis) vs $V_0/[S]$ gives a straight line slope $-K_m$, intercept V_0 .

b. Hanes equation;

$$\frac{[S]}{V_0} = \frac{[S]}{V_{\max}} + \frac{K_m}{V_{\max}}$$

can be rearranged to give

$$[S] = \frac{V_{\max} [S]}{V_0} - K_m$$



A graph of $[S]$ (y-axis) vs. $[S]/V_0$ gives a straight line slope, V_{\max} , intercept $-K_m$.

Determining K_I

For systems where K_m is affected.

$$V_0 = \frac{V_{\max} [S]}{K_{MI} + [S]}$$

$$\text{where } K_{MI} = K_m \left(1 + \frac{[I]}{K_I} \right)$$

$$\text{rearranging gives } \frac{K_{MI}}{K_m} = 1 + \frac{[I]}{K_I}$$

A graph of K_{MI}/K_m will be a straight line, slope $1/K_I$ and intercept 1.

or K_I may be calculated directly from

$$K_I = \frac{[I]}{K_{MI}/K_m - 1}$$

For systems where V_{\max} is affected

$$V_0 = \frac{V_{MI} [S]}{K_m + [S]}$$

$$\text{where } V_{MI} = \frac{V_{\max}}{1 + [I]/K_I}$$

$$\text{rearranging gives } \frac{V_{\max}}{V_{MI}} = 1 + \frac{[I]}{K_I}$$

A graph of V_{\max}/V_{MI} will give a straight line of slope $1/K_I$ and intercept 1.

or K_I may be calculated directly from

$$K_I = \frac{V_{MI} \cdot [I]}{V_{\max} - V_{MI}}$$