

Experimental Determination of K_i Using the Dixon Plot

In the derivation below, V is used to represent V_{\max} and K is used to represent K_m .

The expression that describes the relationship of v to $[S]$, $[I]$ and various constants is given below:

$v = V[S]/(K(1 + [I]/K_i) + [S](1 + [I]/K_i'))$; the reciprocal of this relationship can be written as

$1/v = (K + [S])/V[S] + [I](K/K_i + [S]/K_i')/V[S]$, which means that at any fixed value of $[S]$, $1/v$ is linearly related to the value of $[I]$. (A plot of $1/v$ vs. $[I]$ is called a Dixon plot.)

If such a relationship is empirically studied at two different values of the fixed substrate concentration ($[S]_1$ and $[S]_2$), the two straight lines intersect when $1/v_1 = 1/v_2$. Using the above relationship for $1/v$, it can be shown that under these conditions

$$K + [S]_1/(V + [S]_1) + [I](K/K_i + [S]_1/K_i')/(V + [S]_1) = \\ K + [S]_2/(V + [S]_2) + [I](K/K_i + [S]_2/K_i')/(V + [S]_2)$$

Canceling the constant terms that appear on both sides of the equation leaves

$$K/V[S]_1 + [I](K/K_i)/V[S]_1 = K/V[S]_2 + [I](K/K_i)/V[S]_2, \text{ or}$$

$$K/V[S]_1 + [I](K/K_i)/V[S]_1 - K/V[S]_2 - [I](K/K_i)/V[S]_2 = 0, \text{ which can be factored according to}$$

$$K/V(1/[S]_1 + 1/[S]_2) + (K[I]/VK_i)(1/[S]_1 + 1/[S]_2) = 0 \text{ or } K/V(1/[S]_1 + 1/[S]_2)(1 + [I]/K_i) = 0.$$

Since by definition neither of the first two terms in the above relationship can have a value of zero, it must be true that at the point of intersection of the lines in a Dixon plot representing two different fixed values of $[S]$, $(1 + [I]/K_i) = 0$. This means that at the point of intersection, $[I] = -K_i$; thus a vertical line from the point of intersection to the $[I]$ axis provides an empirically determined value for K_i .