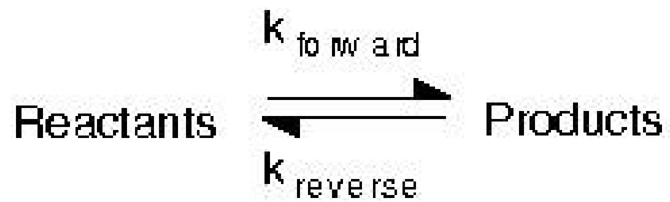
Equilibrium and Rate



$$\frac{d[P]}{dt} = k_{forward} * [R]$$

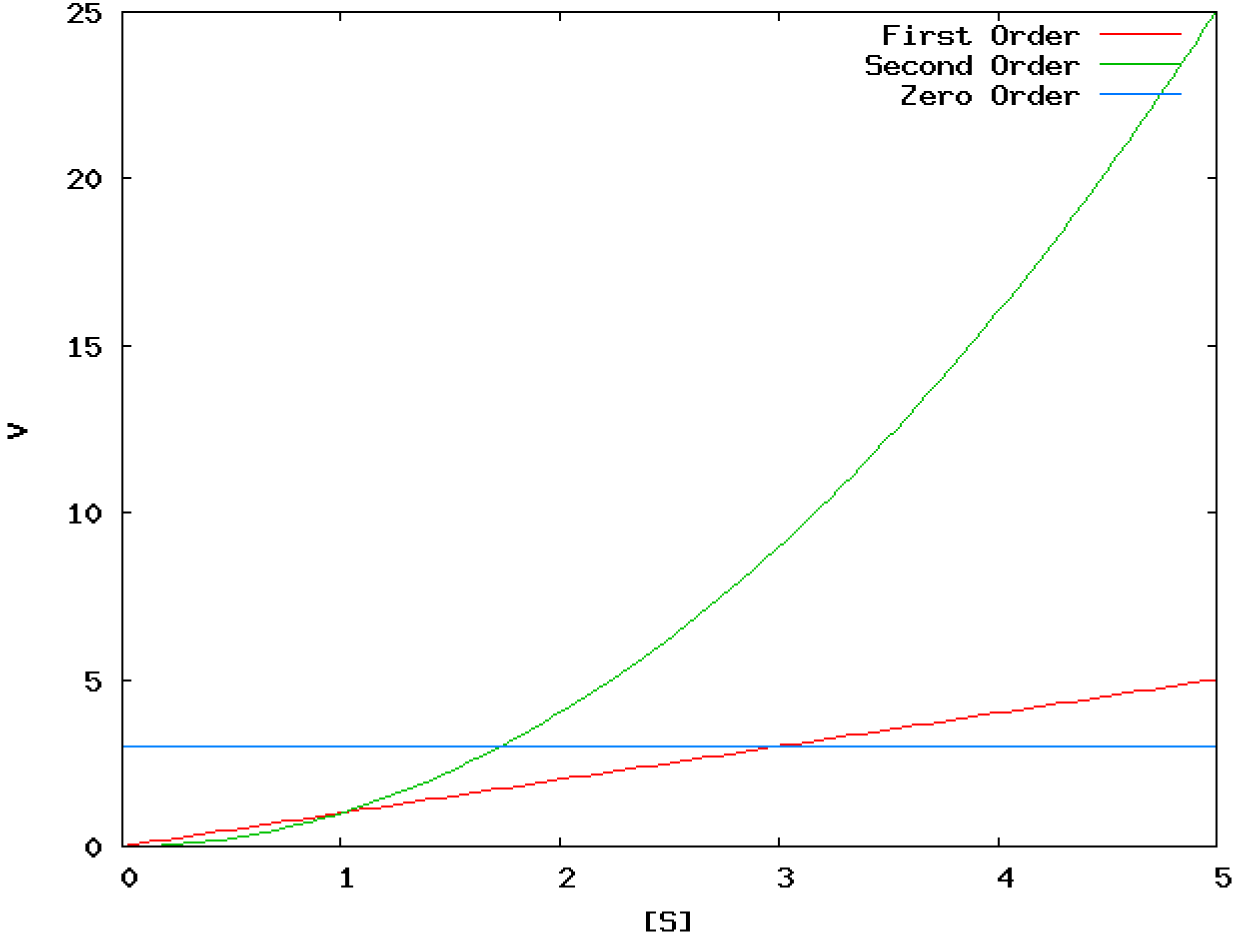
$$\frac{d[R]}{dt} = k_{reverse} * [P]$$

- At equilibrium $\frac{d[P]}{dt} = \frac{d[R]}{dt}$

$$k_{forward} * [R] = k_{reverse} * [P]$$

$$\frac{[P]}{[R]} = \frac{k_{forward}}{k_{reverse}} = K_{eq}$$

Initial Rate and Reaction Order



Derivation of Michaelis-Menton

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E^*S \xrightarrow{k_{cat}} E + P$$

- Steady-state approximation

$$\frac{d[E*S]}{dt} = k_1[E][S] - (k_{cat} + k_{-1})[E*S] = 0$$

$$k_1[E][S] = [E*S](k_{cat} + k_{-1})$$

$$[E*S] = [E][S]\left(\frac{k_1}{k_{cat} + k_{-1}}\right)$$

- By definition: $$K_M = \frac{k_{cat} + k_{-1}}{k_1}$$

$$K_M = \frac{[E][S]}{[E*S]}$$

Derivation of Michaelis-Menton

- Substitute

$$K_M = \frac{([E]_{total} - [E*S])[S]}{[E*S]}$$

- Solve for [E*S]

$$K_M * [E*S] = ([E]_{total} - [E*S]) * [S]$$

$$K_M * [E*S] + [S] * [E*S] = [E]_{total} * [S]$$

$$[E*S](K_M + [S]) = [E]_{total} * [S] \qquad [E*S] = \frac{[E]_{total} * [S]}{K_M + [S]}$$

$$V_0 = k_{cat}[E*S] = \frac{k_{cat}[E]_{total}[S]}{K_M + [S]}$$

$$V_0 = V_{max}\, when\, [E*S] = [E]_{total} \qquad V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Meaning of Equation, Constants

- K_M is the Michaelis constant
  - Same units as K_d, related to K_d
- V_{max} is maximum rate of enzyme reaction
  - Can be used to calculate k_{cat} if $[E]_{total}$ is known
- $[S] << K_M$, $V_0 = k_{cat}/K_M[E]_{total}[S]$
  - k_{cat}/K_M is second order rate constant
  - Limited by diffusion?
- $[S] << K_M$, $V_0 = V_{max}$

Lineweaver-Burk Plots

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

$$\frac{1}{V_0} = \frac{K_M + [S]}{V_{max}[S]}$$

$$\frac{1}{V_0} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

- Plot $1/V_0$ versus $1/[S]$
- Slope = K_M/V_{max}
- Y-Intercept = $1/V_{max}$
- X-Intercept = $-1/K_M$

Competitive Inhibition

$$V_0 = k_{cat}[E*S] \qquad [E*S] = \frac{[E][S]}{K_M}$$

$$[E]_{total} = [E] + [E*S] + [E*I]$$

$$K_I = \frac{[E][I]}{[E*I]} \qquad [E*I] = \frac{[E][I]}{K_I}$$

$$[E]_{total} = [E*S] + [E] + \frac{[E][I]}{K_I}$$

$$[E*S] = [E]_{total} - [E](1 + \frac{[I]}{K_I})$$

Competitive Inhibition

$$[E*S] = [E]_{total} - [E](1 + \frac{[I]}{K_I})$$

$$[E*S] = [E]_{total} - \frac{[E*S]K_M}{[S]}(1 + \frac{[I]}{K_I})$$

$$[E*S][S] + [E*S]K_M(1 + \frac{[I]}{K_I}) = [E]_{total}[S]$$

$$k_{cat}[E*S]([S] + K_M(1 + \frac{[I]}{K_I})) = k_{cat}[E]_{total}[S]$$

$$V_0 = \frac{V_{max}[S]}{K_M(1 + \frac{[I]}{K_I}) + [S]}$$

Competitive Inhibition

- Increase in apparent KM
- No change in Vmax
- At [I] = 0, simplifies to Michaelis-Menton

Uncompetitive Inhibition

$$V_0 = k_{cat}[E*S]$$

$$[E*S] = \frac{[E][S]}{K_M}$$

$$[E]_{total} = [E*S] + [E] + [E*S*I]$$

$$K_I = \frac{[E*S][I]}{[E*S*I]}$$

$$[E]_{total} = [E*S] + \frac{[E*S]K_M}{[S]} + \frac{[E*S][I]}{K_I}$$

$$\frac{[E]_{total}}{1 + \frac{K_M}{[S]} + \frac{[I]}{K_I}} = [E*S]$$

$$\frac{[E]_{total}[S]}{K_M + [S](1 + \frac{[I]}{K_I})} = [E*S]$$

Uncompetitive Inhibition

$$V_0 = \frac{V_{max}[S]}{K_M + [S](1 + \frac{[I]}{K_I})}$$

$$V_0 = \frac{\frac{V_{max}}{(1 + \frac{[I]}{K_I})}[S]}{\frac{K_M}{(1 + \frac{[I]}{K_I})} + [S]}$$

- Decreases apparent V_{max}
- Decreases apparent K_M