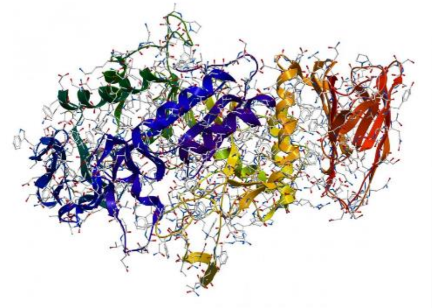


CEN-CHE 422
ENZYME ENGINEERING

ENZYME KINETICS

(Saturation Kinetics)

$$E + S \leftrightarrow ES \leftrightarrow E + P$$

1. The substrate binds to the enzyme
2. The substrate is converted to product

$$\mathrm{E} + \mathrm{S} \leftrightarrow \mathrm{ES} \leftrightarrow E + P$$

1) **The first step is fast, the reaction quickly reaches equilibrium**

2) **The second step is slower, thus rate-limiting step**

Michaelis-Menten Kinetics

(MM assumption: rapid equilibrium assumption)

$$\boxed{r = \frac{r_{\max} C_S}{Km + C_S}}$$

$$E + S \overset{k_1}{\underset{k_{-1}}{\leftrightarrow}} ES \overset{k_2}{\underset{k_{-2}}{\leftrightarrow}} E + P$$

Assumption 1. **The formation of ES complex is a rapid equilibrium reaction**

Dissociation constant:

$$Km = \frac{k_{-1}}{k_1} = \frac{C_E . C_S}{C_{ES}}$$

Assumption 2. **Decomposition of ES complex to product is an irreversible reaction**

$$E + S \overset{k_1}{\underset{k_{-1}}{\leftrightarrow}} ES \xrightarrow{k_2} E + P \qquad k_2 >> k\text{-}_2$$

substrate consumption rate ($-r_S$)= product formation rate (r_P)

$$r = \frac{dCp}{dt} = - \frac{dCs}{dt}$$

$r=k_2C_{ES}$

$$r = \frac{dCp}{dt} = k_2C_{ES}$$

C_{ES}= ?

Relate reaction rate to measurable quantities???

Enzyme mass balance:

C_{Eo}=total amount of enzyme present in both the free and combined form

$C_{Eo} = C_E + C_{ES}$ ***or*** $C_E = C_{Eo} - C_{ES}$

$$Km = \frac{C_E C_S}{C_{ES}} = \frac{(C_{Eo} - C_{ES})C_S}{C_{ES}} \Rightarrow C_{ES} = \frac{C_{Eo} C_S}{Km + C_S}$$

$r = k_2 C_{ES}$

$$r = \frac{k_2 C_{Eo} C_S}{K_m + C_S}$$

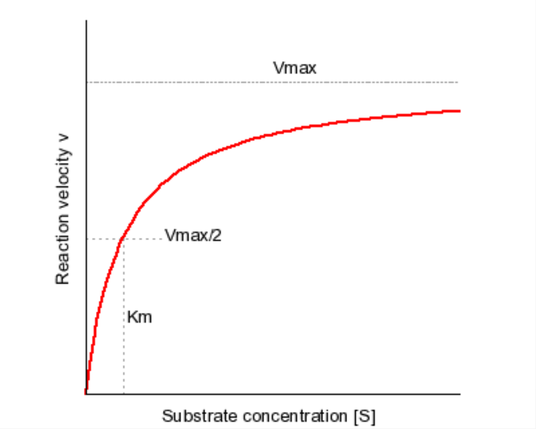
$k_2 C_{Eo} = r_{max}$

max reaction rate: the rate when all enzyme binds to substrate ($C_{Eo} = C_{ES}$)

Substituting r_{max} into the rate equation:

$$\boxed{r = \frac{r_{max} C_S}{Km + C_S}}$$

Henry-Michaelis-Menten Equation



where;

C_s = free substrate concentration

r = reaction rate

r_{max} = maximum reaction rate

K_m = Michaelis-Menten constant

Michaelis-Menten Kinetics

(Briggs-Haldane Approach)

$$E + S \leftrightarrow ES \leftrightarrow E + P$$

In most systems, initial concentration of substrate is quite higher than the concentration of enzyme (C_{So} >> C_{Eo})

k_1 k_2

k_{-1}

Thus, the ES concentration will rapidly approach a steady-state - that is, after an initial burst phase, its concentration will not change appreciably until a significant amount of substrate has been consumed.

This is called ***quasi-steady-state*** approximation

Shortly after the reaction started:

$$\frac{dC_{ES}}{dt} = 0$$

k_1 k_2

The net rate of formation ES complex is zero

or

formation rate of ES = decomposition rate of ES

$$\frac{dC_{ES}}{dt} = k_1 C_E C_S - (k_{-1} + k_2) C_{ES}$$

$$\frac{dC_{ES}}{dt} = k_1 C_E C_S - k_{-1} C_{ES} - k_2 C_{ES} = 0$$

$$C_{ES} = \frac{k_1 C_E C_S}{(k_{-1} + k_2)}$$

Enzyme mass balance : $C_{Eo} = C_E + C_{ES}$

$$C_{Eo} = C_E + \frac{k_1 C_E C_S}{(k_{-1} + k_2)}$$

$$C_E = \frac{C_{Eo}}{1 + \dfrac{k_1 C_S}{k_{-1} + k_2}}$$

$$C_{ES} = \frac{k_1 C_S}{(k_{-1} + k_2)} x \frac{C_{Eo}}{1 + \dfrac{k_1 C_S}{k_{-1} + k_2}} = \frac{k_1 C_S C_{Eo}}{(k_{-1} + k_2) + k_1 C_S} = \frac{C_{Eo} C_S}{\dfrac{k_{-1} + k_2}{k_1} + C_S}$$

$$r = \frac{dC_p}{dt} = k_2 C_{ES}$$

Here:
Cs = free substrate concentration
r = reaction rate
r_{max} = maximum reaction rate
K_m = Michaelis-Menten constant

$$C_{ES} = \frac{C_{Eo} C_S}{Km + C_S}$$

$$r = k_2 C_{ES} = \frac{k_2 C_{Eo} C_S}{Km + C_S}$$

$$\boxed{r = \frac{r_{\max} C_S}{Km + C_S}}$$

Henry-Michaelis-Menten Equation

Assumption: MM equation is valid for $C_{So} >> C_{Eo}$
This assumption looses its validity with the consumption of substrate i.e. later in reaction (as the time progresses)

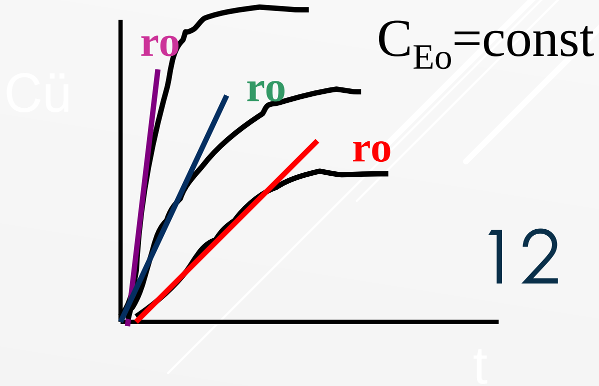
$$\boxed{r_o = \frac{r_{\max} C_{S\,o}}{Km + C_{S\,o}}}$$

Thus, the MM equation is expressed in terms of initial values.

Initial reaction rate

Reaction rate at t=0 and can be found from the slope of $(t;C_P)$ or $(t;C_S)$ plot at zero time

$$r = \frac{dC_P}{dt}_{t=0} = -\frac{dC_S}{dt}_{t=}$$



DEPENDENCE OF MM EQUATION ON ENZYME CONCENTRATION

$$r = \frac{k_2 C_{Eo} C_S}{Km + C_S}$$ ⇨

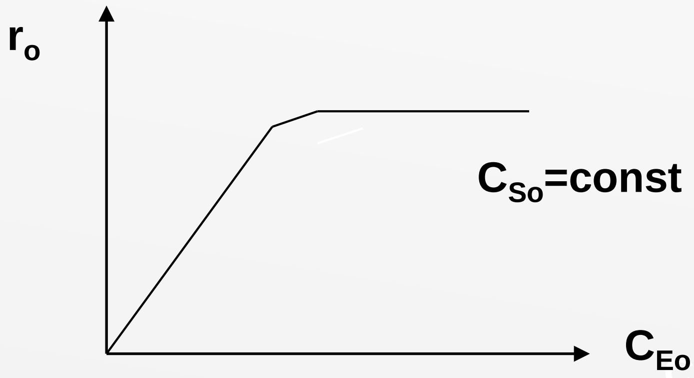
Enzyme concentration ↗

Reaction rate ↗

However $C_{Eo} \geq Cso$ ⇨

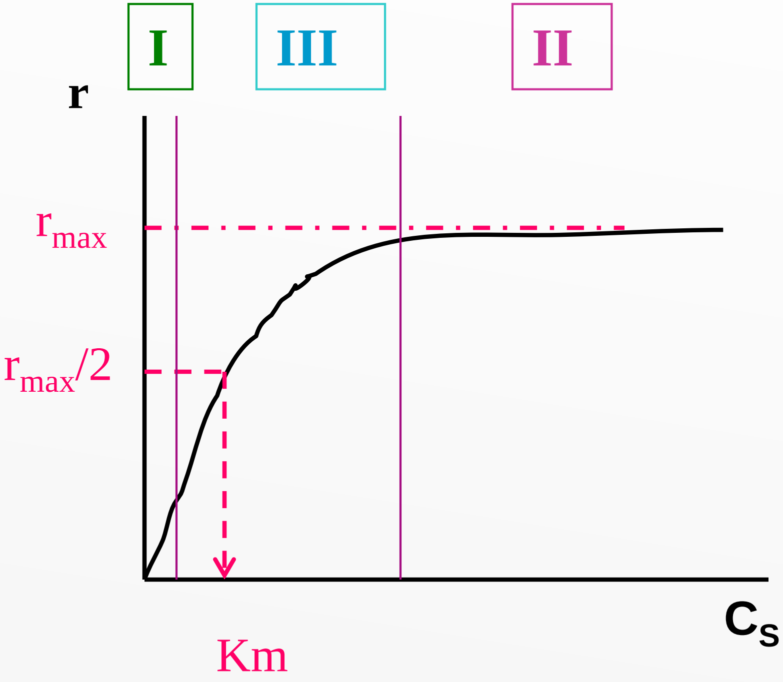
Enzyme concentration ↗

Reaction rate →



REACTION ORDER

$$r = \frac{r_{\max} C_S}{Km + C_S}$$



I.Region: Low substrate concentration

C_S << K_m ⇒ 1st order

$$r = \frac{r_{\max}}{K_m} C_S$$

III. Region: Transitation

Special point: ⇒ C_S=K_m

K_m ⇒ **Substrate concentration at** $r = r_{max}/2$

II. Region: High substrate concentration

C_S >> K_m ⇒ 0th order

$r = r_{max}$