# VIVEKANANDA COLLEGE
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# KOLKATA-700063

NAAC ACCREDITED ‘A’ GRADE

Topic : Enzyme Kinetics
Course Title : Enzymes
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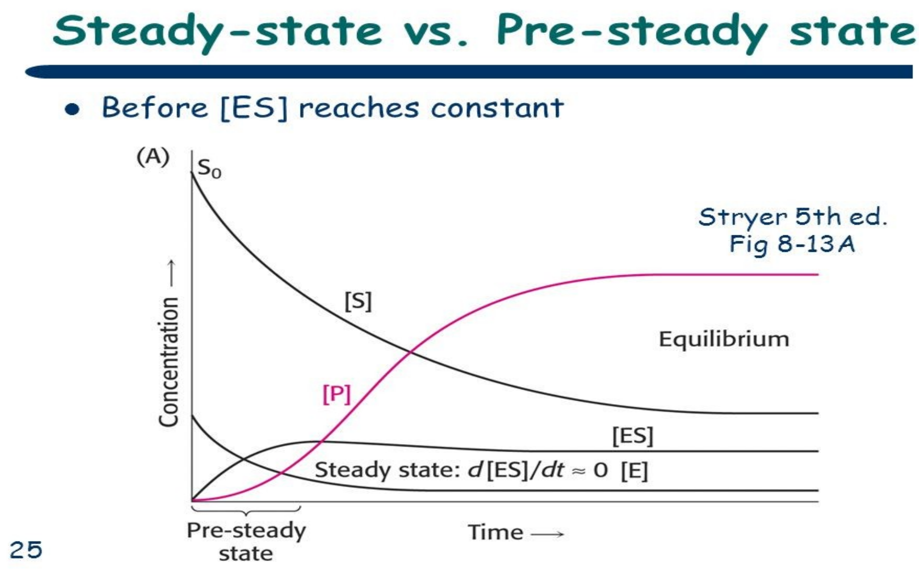
Name of the Department : Biochemistry

## Concept of pre steady state Kinetics

- Pre steady state represents the initial state or the starting period of the reaction.
- The concentration of the substrate is large excess and the ES complex assembles very rapidly.
- This state is too short and unstable ----passes over to the next state.
- Pre steady state kinetics is difficult to analyse.

## Steady state kinetics

- Steady state represents the second state or the post initial period of the reaction.
- It occurs immediately after the pre steady state.
- The concentration of the **enzyme-substrate complex** as well as the other reaction intermediate remain approximately **constant (steady)** over time.
- This state is relatively long and stable.
- Steady state kinetics is easy to analyse.
- The velocity of the enzyme catalysed reaction generally reflect the steady state.



Steady-state vs. Pre-steady state

- Before [ES] reaches constant

Stryer 5th ed.
Fig 8-13A

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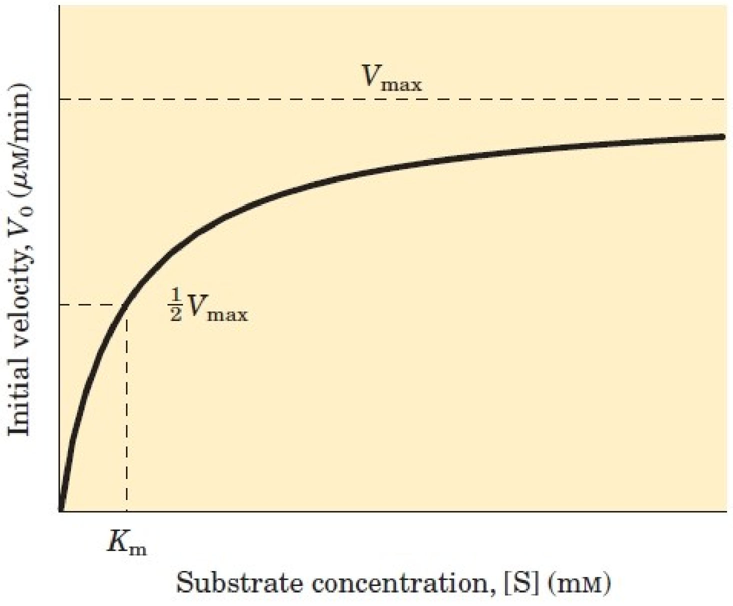
## Assumption of Steady State

- Under physiological condition, substrate is in great excess over enzyme.
- Rate of synthesis of ES must equal its rate of consumption over most of the course of the reaction -------[ES] maintains a steady state ------$d[\mathrm{ES}]/dt = 0$
- Steady state assumption was first proposed by Briggs and Haldane.

## Relationship between Initial Velocity ($V_0$) and Substrate concentration ([S])

- The rate of a reaction catalysed by an enzyme depends on the concentration of substrate, [S].
- In kinetics experiments, the initial velocity, $V_0$ is measured at [S] >> [E].
- The effect on $V_0$ of varying [S] ------ when [E] remains constant.



- At low [S], $V_0$ increases linearly with increase in [S].
- At higher [S], $V_0$ increases by smaller and smaller amounts in response to increases in [S].
- $V_0$ reaches to a plateau like region, $V_{max}$, maximum velocity

## Association and Dissociation constant

An enzymatic reaction can be represented as

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

E = Enzyme S= Substrate P = Product

- Here, $k_1$ is association rate constant
- **$k_1$/ $k_{-1}$** is the **association constant ($K_a$)**
- $k_{-1}$ is dissociation rate constant
- **$k_{-1}$/ $k_1$** is **dissociation constant ($K_d$)**

## Equilibrium constant - (mono substrate reaction)

$$S \leftrightarrow P$$

- Equilibrium constant ($K_{eq}$) = [P] / [S]

From thermodynamics in biochemical processes, the relationship between $K'_{eq}$ and $\Delta G'^{0}$ (under standard condition) can be described by the expression :

- $\Delta G'^{0} = - RT \ln K'_{eq}$

Where, R gas constant, 8.315J/mol. K

T absolute temperature, 298 K

**Large negative $\Delta G'^{0}$ value ------favourable reaction equilibrium ----spontaneous reaction**

## Michaelis Menten Equation Study

In 1913 a general theory of enzyme action and kinetics is developed by L. Michaelis and M.L. Menten.

## Michaelis Menten Hypothesis

- Only a single substrate and a single product are involved.
- The process proceeds essentially to completion.
- The concentration of the substrate is much greater than that of the enzyme.
- An intermediate enzyme substrate complex is formed.
- The rate of decomposition of the substrate is proportional to the concentration of the Enzyme Substrate complex.

## Derivation of Michaelis Menten Equation

To explain the behaviour of the enzyme, Michaelis-Menten proposed that one molecule of enzyme combined with one molecule of substrate to form ES complex which gives free enzyme and product P.

$$E+S \underset{k-1}{\overset{k1}{\rightleftharpoons}} ES \underset{k-2}{\overset{k2}{\rightleftharpoons}} E+P$$

- E = Enzyme, S = Substrate, ES = Enzyme-Substrate complex, P = Product
- $k_1$, $k_{-1}$, $k_2$, $k_{-2}$ are different rate constants.

- During the reaction, enzyme can remain as free enzyme as well as enzyme substrate complex -------
- $[E_{Total}] = [E_{free}] + [E_{as\ ES}]$
- Free or unbound enzyme i.e. $[E_{free}] = [E_T] - [ES]$

The derivation starts with the two basic steps of the formation and breakdown of ES.

- Rate of formation of ES complex = $k_1([E_T] - [ES])[S] + k_{-2}[E][P]$
- Rate of breakdown of ES complex = $k_{-1}[ES] + k_2[ES]$

From steady state assumption, $d[ES]/dt = 0$

- Rate of formation of ES = Rate of breakdown of ES

i.e. $k_1([E_T] - [ES])[S] + k_{-2}[E][P] = k_{-1}[ES] + k_2[ES]$

- During the initial stage of the reaction, [P] is very low in comparison to the [S]-----the term containing P can be neglected.

Therefore,

- $k_1 ([E_T]- [ES]) [S] = k_{-1}[ES] + k_2[ES]$

or, $k_1[E_T] [S] – k_1[ES][S] = (k_{-1} + k_2) [ES]$

- Adding the term $k_1[ES][S]$ to both sides of the equation ……

Then, $k_1[E_T] [S] = k_1[ES][S] + (k_{-1} + k_2) [ES]$

or, $k_1[E_T] [S] = [ES] (k_1[S] + k_{-1} + k_2)$

or, $[ES] = k_1[E_T] [S] / k_1[S] + k_{-1} + k_2$

- Now simplified further,

$[ES] = [E_T] [S] / [S] + (k_{-1} + k_2) / k_1$

The term $(k_{-1} + k_2) / k_1$ is defined as the **Michaelis constant, $K_m$**

□ $[ES] = [E_T] [S] / K_m + [S]$ ….Eqn (1)

- $V_0$ is determined by the breakdown of ES to form product
- which is determined by [ES] : $V_0 = k_2[ES]$

As [ES] is not easily measured experimentally, now $[ES] = V_0 / k_2$

- Substituting [ES] in Eqn (1),

$V_0 = k_2 [E_T] [S] / K_m + [S]$ ….Eqn (2)

- This equation can be further simplified. As maximum velocity occurs when total enzyme is saturated with substrate, i.e. $[ES] = [E_T]$
- $V_{max} = k_2[E_T]$.

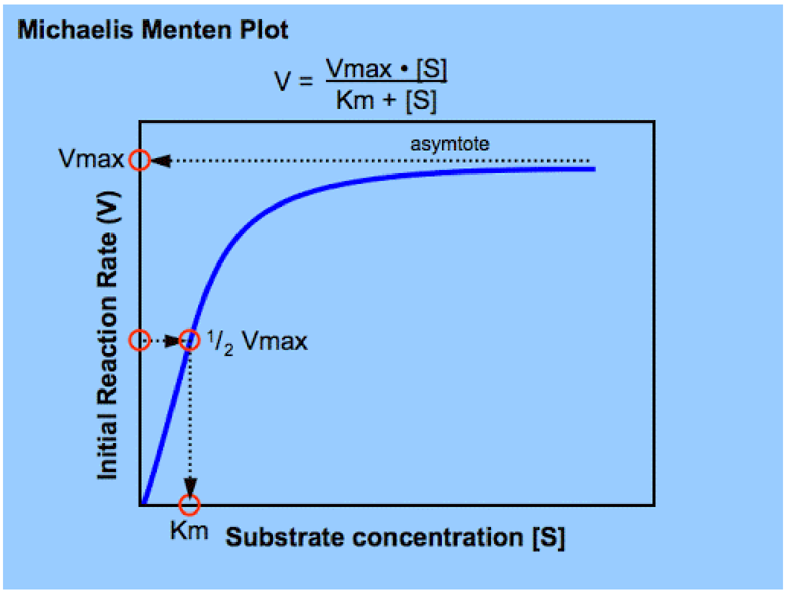
Substituting this in Eqn (2),

**$V_0 = V_{max} [S] / K_m + [S]$**

This is the **Michaelis-Menten rate equation** for one substrate-enzyme catalysed reaction.

$V_0$ is the initial rate or initial velocity when [S] is much greater than [E].

## Graphical representation of Michaelis-Menten Equation



- This is Rectangular hyperbolic in nature.
- Slope = $V_{max}/K_m$

Reference :

1. Lehninger : Principles of Biochemistry, 4th Edition, Nelson and Cox, W.H.Freeman and Company (New York).
2. Biochemistry, 3rd Edition, Voet and Voet, John Wiley & Sons
3. www.google.com