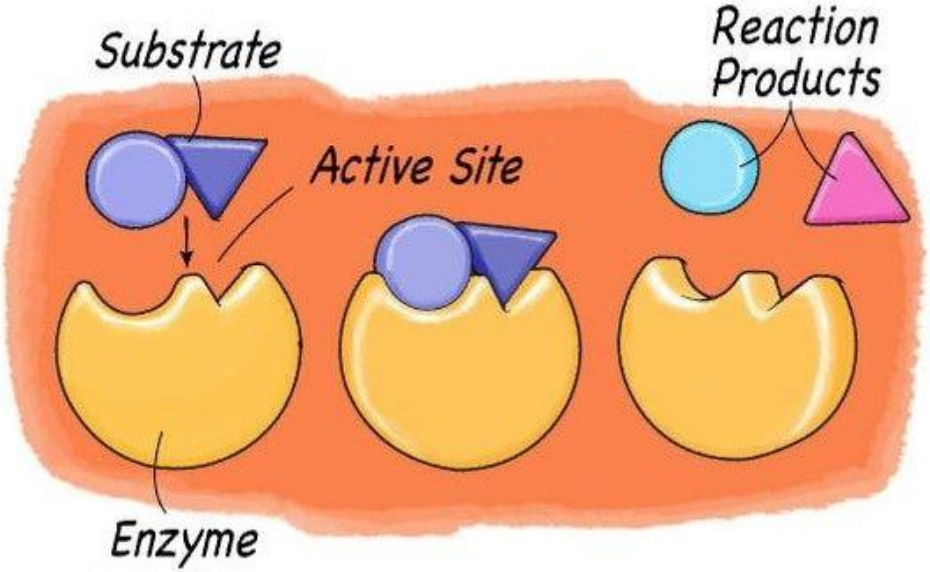
ENZYME KINETICS



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Enzyme Kinetics

- Enzyme Kinetics – Quantitative measurement of the rates of enzyme catalyzed reactions

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- The systematic study of factors that affect these rates

- Enzyme kinetics began in 1902 when Adrina Brown reported an investigation of the rate of hydrolysis of sucrose as catalyzed by the yeast enzyme inveratase.

- Brown demonstrated – when sucrose concentration is much higher than that of the enzyme, reaction rate becomes independent of sucrose concentration

Enzyme Kinetics

- Brown proposal – overall reaction is composed of two elementary reactions in which the substrate forms a complex with the enzyme that subsequently decomposes to products and enzymes.

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

- Here E, S, ES and P symbolize the enzyme, substrate, enzyme-substrate complex and products

Enzyme Function

- The **transition state** is a molecular intermediate between the substrate and its product
- X is the transition state **(Substrate → X → Product)**
- transition state has a higher free energy than both the substrate and its product
- stabilized upon the addition of an enzyme
- weak substrate binding, the enzyme's active site changes conformation such that it fits the transition state better than the initial substrate, hence has a **higher affinity** for this transition state.
- allows **more substrate molecules to be converted into products**.
- transition state has high energy and is unstable
- converts into a more stable product, which has a lower energy. The enzyme's active site has a **low affinity** for this product, so it dissociates and is released.

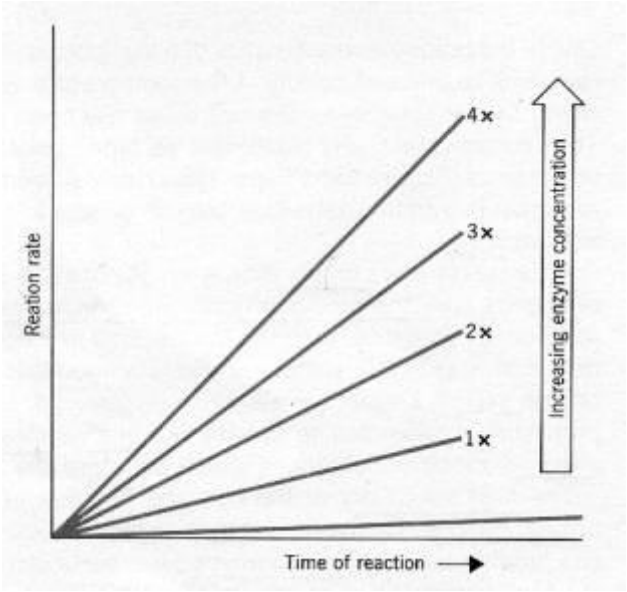
Factor affecting enzyme kinetics

1. Enzyme concentration
2. Temperature
3. Hydrogen ion concentration or pH
4. Substrate concentration
5. Inhibitors
6. Product concentration
7. Activators
8. Physical agents

Enzyme concentration

- The rate of enzyme catalyzed reaction is directly proportional to the concentration of enzyme.

- The plot of rate of catalysis versus enzyme concentrations a straight line



Temperature

- Increase with temperature
- Bell shape curve
- Q10 (temperature coefficient)- factor by which the rate of biological reaction increases for a 10ºC increase in temperature
- Optimum temperature
- Mostly at body temperature
- Some enzyme may be active above body temperature e.g. snake venom phosphokinase, muscle adenylate kinase, urease, enzymes in thermophillic bacteria

- Rise or fall in enzyme activity with temperature is prominent survival feature in “Cold blooded” animals
- In mammals- assumes physiological importance e.g. fever, hypothermia
- Mammals are warm blooded. Human body temp 37°C
- Reptiles are cold blooded. There temperature go down when the weather is cold and up when it is hot.

PH

- Optimum pH
- Most show at neutral pH (6-8)

Since enzymes are proteins pH changes affect.
1. Charged state of catalytic site
2. Conformation of enzyme molecules

- Trypsin- 7.6
- Pepsin- 2-2.5
- Acid phosphatase- 5
- Alkaline phosphatase- 9-10
- Enzymes from fungi- 4-6

Product concentration

- Accumulation - decreases the velocity
- In biological system this is prevented by quick removal of product

Activators

- Inorganic metallic cation/anions acts as activators by combining with substrate, ES complex, change in conformation of active site
- Metal activated enzymes- e.g. ATPase, Enolase
- Metalloenzyme- e.g.Pyruvate oxidase, cytochrome oxidase

Inhibitors

- Make active site unavailable to substrate or
- Change enzyme structure

CLINICAL USE	ENZYME INHIBITED	INHIBITOR
Epilepsy	GABA transaminase	Gama- vinyl GABA
Antidepressant	MAO	Tranylcypromine , phenelzine
Antihypertensive	ACE	Captopril, enalaprilat
Cardiac disorders	-ATPase	Cardiac glycosides
Gout	Xanthine oxidase	Allopurinol
Ulcer	, - ATPase	omeprazole

Physical agents

- Light, radiation (u.v., X- rays, gamma rays etc)

e.g. salivary amylase- activity increased by red/ blue light whereas decreased by u.v. light

Substrate concentration

- Rectangular hyperbola (*Michaelis plot*)
- Initial velocity- velocity when little substrate is reacted

Three phases of kinetics

1. In the **first phase**, substrate concentration is low and most of the enzyme molecules are free so they combine with the substrate molecules.

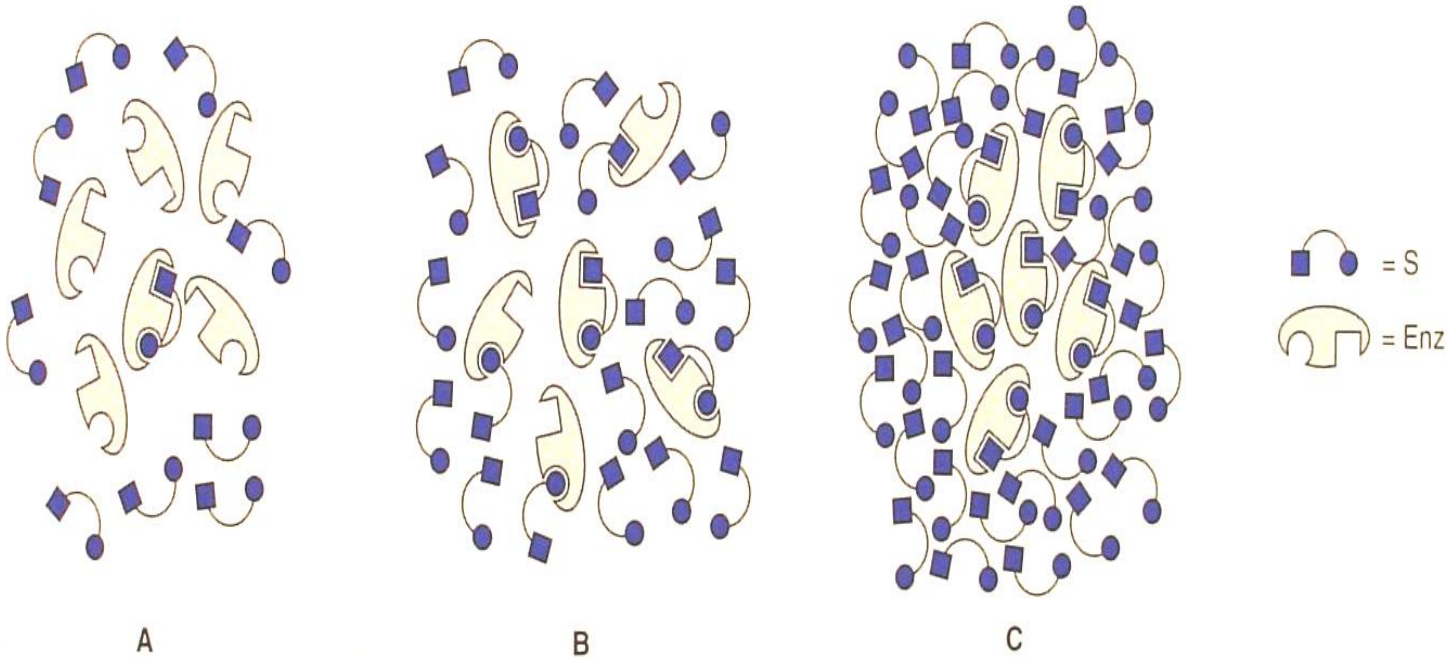
- velocity is proportional to substrate concentration.
- **It shows first-order kinetics**

2. In the **second phase,** half of the enzyme molecules are bound to substrate, so the velocity is not proportional to substrate concentration.

- **It shows mixed-order kinetics**

3. In the third phase, all the enzyme molecules are bound to substrate, so velocity remain unchanged because free enzyme is not available though the substrate is in excess.

- It shows **zero-order kinetics**



A. Low [S] B. 50% [S] or K_m C. High, saturating [S]

Rate-limiting Steps

- The **rate-limiting step** of any reaction is its slowest step, and this is what sets the pace of the entire reaction.
- **enzyme-substrate complex to the product** is normally **rate-limiting.**
- The rate of this step is **directly proportional** to the concentration of the enzyme-substrate complex.
- The concentration of the ES complex changes as the reaction progresses, and therefore the rate of product formation also changes.
- When the reaction reaches **equilibrium** (steady state phase), the ES concentration (and therefore the rate of reaction) remains relatively constant.

Reaction Kinetics

- When an enzyme is added to a substrate, the reaction that follows occurs in **three stages** with distinct kinetics:

Phase	Concentration of ES	Rate of product formation
Pre-steady state	**Rapid burst** of ES complexes form	Initially **slow**, waiting for ES to form, then speeds up
Steady-state (equilibrium)	ES concentration remains **constant** as it is being formed as quickly as it breaks down	**Constant** rate of formation, faster than the pre-steady state
Post-steady state	Substrate **depletes** so fewer ES complexes form	**Slow** as there are fewer ES complexes; slows down as substrate runs out

- The pre-steady state phase is very short, as equilibrium is reached within microseconds. Therefore, if you measure the rate in the first few seconds of a reaction, you will be measuring the reaction rate in ***the steady state***. This is the rate used in Michaelis-Menten Kinetics.

Steady State Assumption

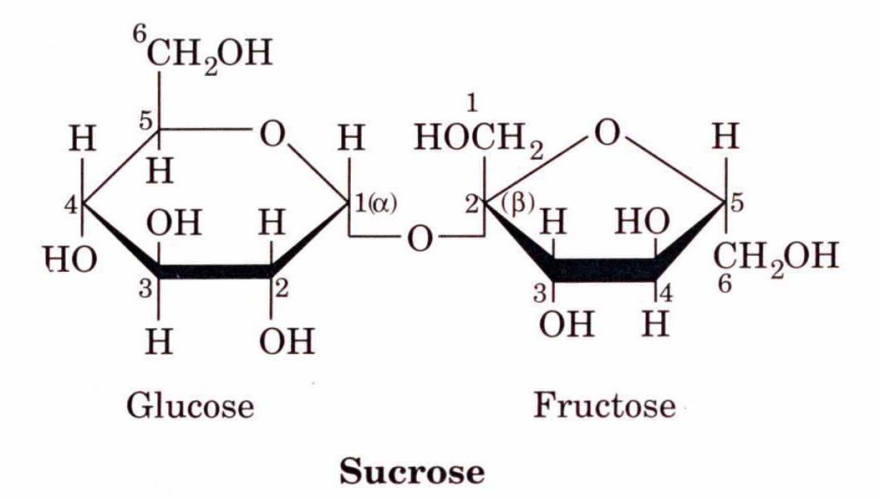
- The M-M equation was derived in part by making several assumptions. An important one was: **the concentration of substrate must be much greater than the enzyme concentration.**
- **I**n the situation where [S] >> [E] and at initial velocity rates, it is assumed that the changes in the concentration of the intermediate ES complex are very small over time.
- This condition is termed a **steady-state rate,** and is referred to as **steady-state kinetics. Therefore, it follows that the rate of ES formation will be equal to the rate ES breakdown.**

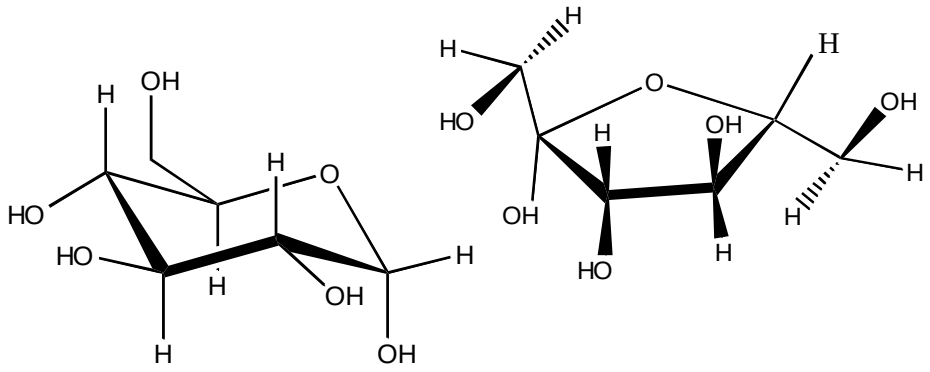
Kinetics of Enzymes

Enzymes follow zero order kinetics when substrate concentrations are high. Zero order means there is no increase in the rate of the reaction when more substrate is added.

Given the following breakdown of sucrose to glucose and fructose

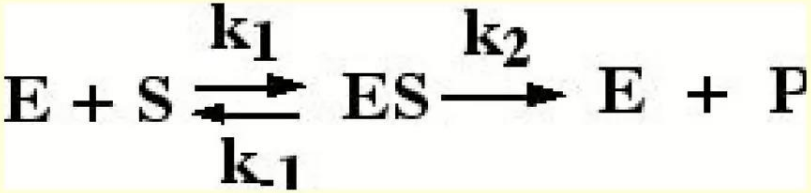
Sucrose + H_20 ⟶ Glucose + Fructose

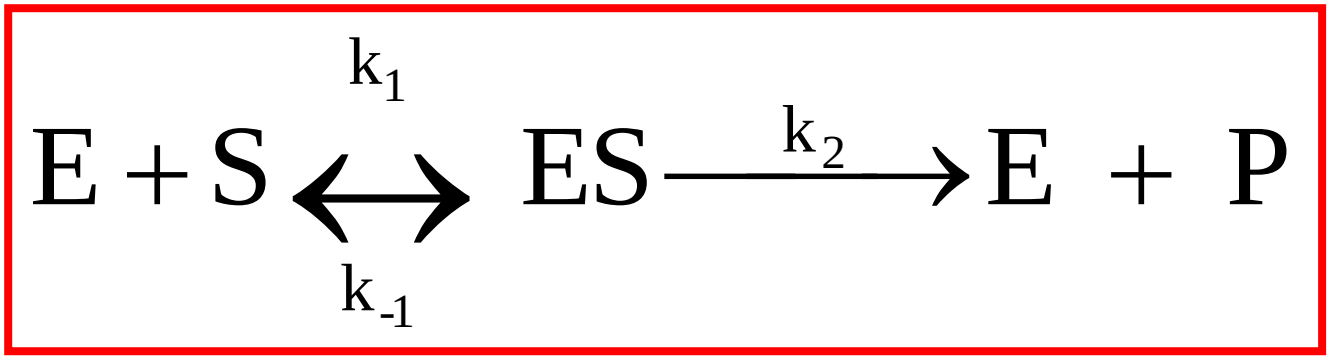




Michaelis and Menton Equation

- A model for enzyme kinetics was propsed by Michaelis and Menton in 1913.
- MM equation relates the initial rate of an enzyme catalysed reaction to the substrate concentration and a ratio of rate constants.
- It co-relates velocity with enzyme and substrate concentration.
- It has been derived for a single substrate-enzyme-catalysed reaction.





E = Enzyme S = Substrate P = Product

ES = Enzyme-Substrate complex

k_1 rate constant for the forward reaction

k_{-1} = rate constant for the breakdown of the ES to substrate

k_2 = rate constant for the formation of the products

Important notes about concentration of substrate:

a) Explanation of effect of substrate concentration:

1) At low substrate concentration, not all enzymes are saturated. So the rate of reaction will Increase.

2) At higher substrate concentration, all enzymes get saturated with substrates and any more Increase of substrate concentration will result in no increase in the rate of the reaction.

b) Michaelis-Menten Equation:

1) This equation describes the dependence of reaction velocity on substrate concentration.

2) Michaelis and Menten proposed that in any enzymatic reaction, the enzyme (E) combines with substrate (S) to form an enzyme-substrate (ES) complex.

3) ES then breaks down either to enzyme and substrate again or to enzyme and product (P).

Michaelis-Menten Equation

4) Michaelis and Menten equation describes how reaction velocity varies with substrate concentration as follows:

$$v = \frac{v_{max}[S]}{K_M + [S]}$$

Where: Vi = Initial reaction velocity

Vmax = maximal velocity

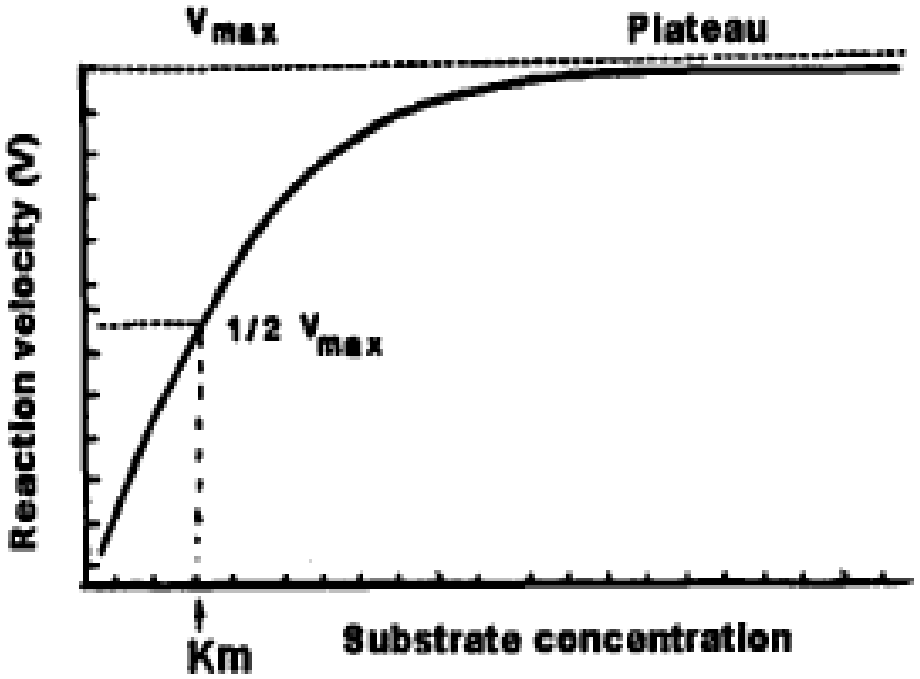
Km = Michaelis constants = $(k_1+k_2)/k_{-1}$

[S] = Substrate concentration

c) Michaelis constant (Km):

1) From the above equation, when substrate concentration (S) is equal to Km.

thus ***Km*** ***can be defined as:*** **substrate concentration that produces half maximum velocity.**



Michaelis Constant (K_m)

The substrate concentration that produces half the maximal velocity (Vmax/2) is known as **Michaelis constant (K_m)**

1. enzyme kinetic constant.
2. Indicates the substrate concentration required for the enzyme to work efficiently
3. Low K_m indicates high affinity of enzyme towards substrate. And vice-versa.

e.g. Hexokinase and glucokinase
K_m of hexokinase is low (1×10^{-5} M) whereas K_m of glucokinase is high (2.0×10^{-2} M)

4. Km is required when enzymes are used as drugs
5. Use of enzymes in immunodiagnostics (ELISA) require Km of the enzyme

Michaelis Constant (K_m)

- Km is a **unit of measurement for Michaelis constant**
- It is used to describe the affinity of an enzyme for its substrate.
- Km values are more or less constants for particular enzyme-substrate systems, but these may vary slightly with pH, temperature, ionic strength and also with types and amount of coenzymes when required for the reaction.
- The values of Km are measured in terms of molarity, typically ranging between 10 -3 to 10 -6 molar

Michaelis constants have been determined for many of the commonly used enzymes. The size of K_m tells us several things about a particular enzyme:

1. A small K_m indicates that the enzyme requires only a small amount of substrate to become saturated. Hence, the maximum velocity is reached at relatively low substrate concentrations.

2. A large K_m indicates the need for high substrate concentrations to achieve maximum reaction velocity.

- The substrate with the lowest K_m upon which the enzyme acts as a catalyst is frequently assumed to be enzyme's natural substrate, though this is not true for all enzymes.

Michaelis and Menton Equation

- In MM expression

  Total enzyme concentration= $[E_T] = [E] + [ES]$

  Free enzyme conc $[E] = [E_T] - [ES]$

  Substrate concentration = [S]

  Initial velocity = V_o, Velocity measured immediately after mixing E + S, at beginning of reaction (initial velocity), is called V_o.

  Maximum velocity = V_{max}

  Half V_{max} = K_m (substrate concentration)

  Km = substrate concentration that gives $V_o = 1/2\ V_{max}$.

Enzyme-catalyzed reactions show a hyperbolic dependence of V_o on [S]

At very low [S]:

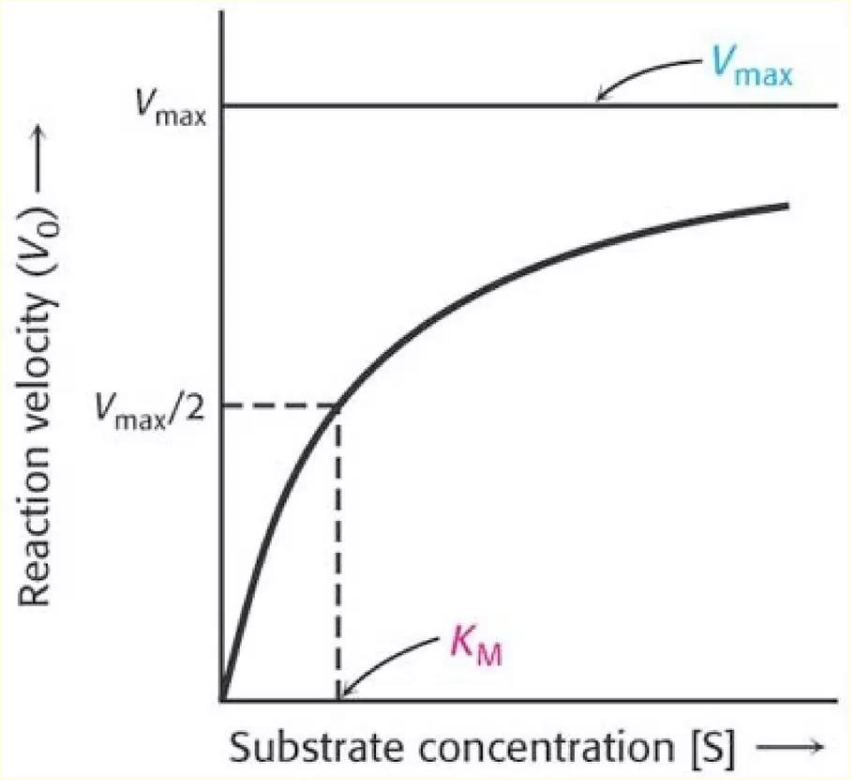
- V_0 is proportional to [S]; doubling [S] → double V_0.

2. In mid-range of [S], V_0 is increasing less as [S] increases (where V_0 is around 1/2 V_{max}).

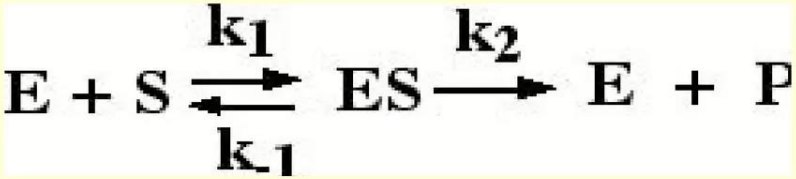
   Km = [S] that gives V_0 = 1/2 V_{max}.

3. At very high [S], V_0 is independent of [S]:

   $V_0 = V_{max}$.



Derivation



- Initial velocity $V_0 = k_2[ES]$
- Rate of formation of [ES] = k_1 [E][S]

  $= k_1([E_T]-[ES]).[S]$

  $= k_1[E_T][S] - [ES][S]$
- Rate of breakdown of [ES] $= k_{-1}[ES] + k_2[ES]$

  $= (k_{-1}+k_2)[ES]$
- Steady state:

  Rate of formation = Rate of breakdown

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

- separation of rate constants

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

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

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

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

$$[ES] = \frac{[E_T][S]}{[S] + \frac{k_{-1}+k_2}{k_1}}$$

$$\frac{k_{-1}+k_2}{k_1} = K_m \qquad [ES] = \frac{[E_T][S]}{[S] + K_m} \quad \text{..... (i)}$$

- Expressing Vo in term of [ES]:

  multiply k2 on both side of eq (i)

$$k_2.\,[ES] = \frac{k_2\,[E_T][S]}{[S] + K_m} \quad \ldots\ldots \text{(ii)}$$

As we know $V_o = k_2[ES]$

So, eq (ii) becomes

$$V_o = \frac{k_2\,[E_T][S]}{[S] + K_m} \quad \ldots\ldots \text{(iii)}$$

When [S] is greater, then V_o becomes V_{max} and

$$V_{max} = k_2\,[E_T]$$

So, eq (iii) becomes

$$Vo = \frac{Vmax\ [S]}{[S] + K_m}$$

Significance of MM equation

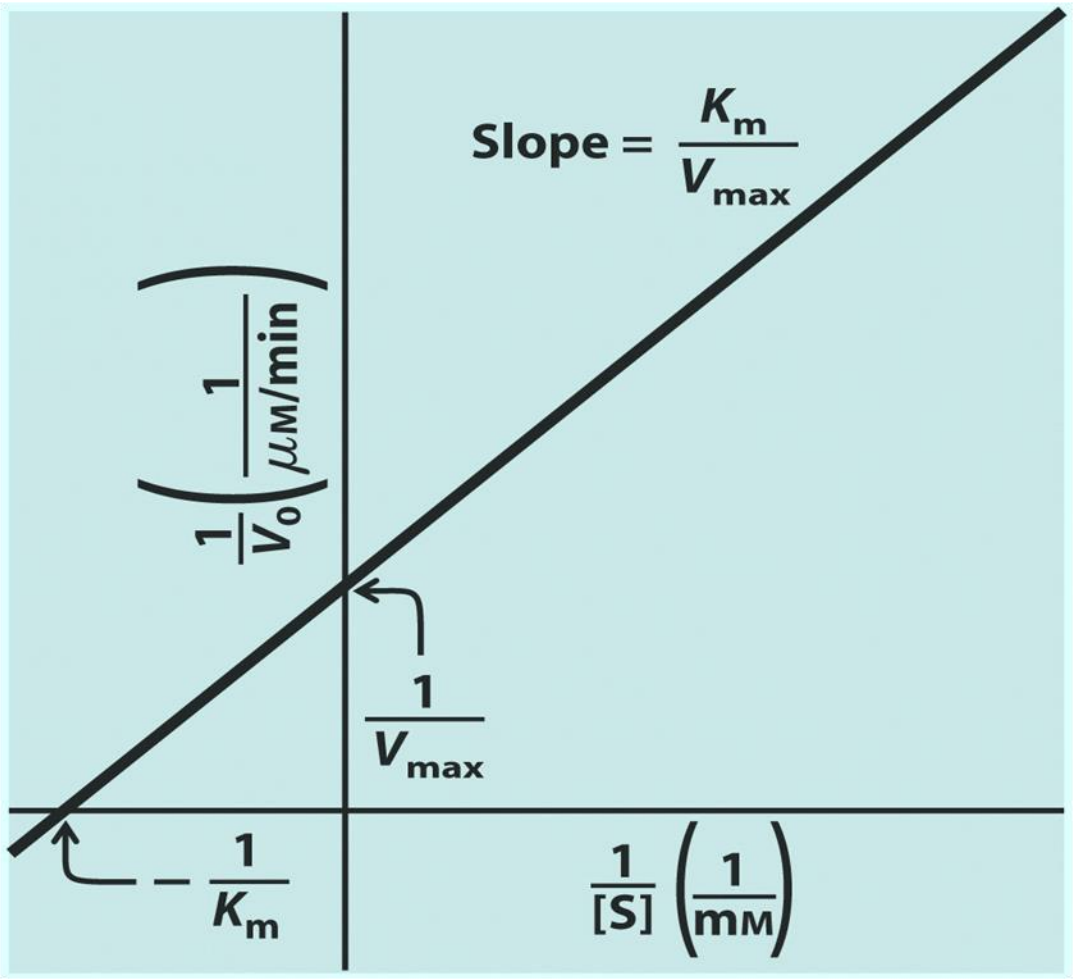
It describes

- kinetic behaviour of enzymes
- hyperbolic dependence of V_o on [S]
- independance of number of steps involved
- different enzymes have different K_m and V_{max}.
- K_m and V_{max} may be influenced by pH, temperature.
- K_m can be used as a relative measure of the affinity of the enzyme for each substrate (smaller K_m means higher affinity)
- in a metabolic pathways, K_m values may indicate the rate-limiting step (highest K_m means slowest step).
- V_{max} is independent of [S] at saturation.

Lineweaver-Burk (double reciprocal) **plot**

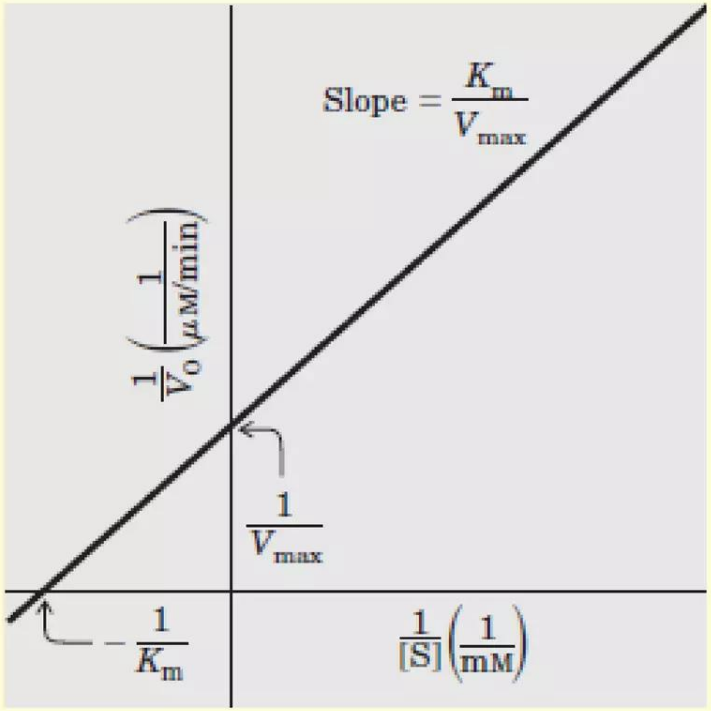
- A linear representation is more accurate and convinient for determining V_{max} and K_m.
- This equation is obtained by taking reciprocal of both the side of Michelis-Menton equation.
- 1/[S] vs. $1/V_o$

Lineweaver-Burk (Double Reciprocal) Plot



$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}$$

- A plot of 1/V versus 1/[S] is a straight line having a slope of K_{max}/V_{max} and an intercept of $1/V_{max}$ on the y-axis



When the substrate concentration becomes large enough to force the equilibrium to form completely all ES the second step in the reaction becomes rate limiting because no more ES can be made, and the enzyme-substrate complex is at its maximum value.

d) Important conclusions about Michaelis-Menten kinetics:

1) Substrates are usually present in physiological fluids in amounts nearly equal to Km values.

2) Km is a constant characteristic of an enzyme and its particular substrate. Km reflects the affinity of the enzyme for the substrate.

3) The smaller the Km value, the more active the enzyme:

i- Small (low) Km reflects a high affinity of the enzyme for substrate i.e. low concentration of substrate is needed to half saturate the enzyme.

ii- Large (high) Km reflects a low affinity to the enzyme for substrate i.e. high concentration of substrate is needed to half saturate the enzyme.


Thank You!