

# **ENZYMES**

**UNIVERSITY OF PNG**

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**DISCIPLINE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY**

**BMLS II / B Pharm II / BDS II**

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# What are enzymes?

- Enzymes are organic catalysts that increase the rates of chemical reactions without changes in the enzymes during the process;
- Enzyme reactions occur under mild conditions, such as:
  - Body temperature,
  - Atmospheric pressure,
  - Neutral pH ,
- Most enzymes are **highly substrate specific**;
- Activity of an enzyme can be regulated;
- Most enzymes are proteins, except a few catalytically active **RNA molecules** (Ribozymes);

## How is Activation Energy related to Transition state?

- For chemical reaction to proceed **Energy barrier** must be overcome (Fig. 1),

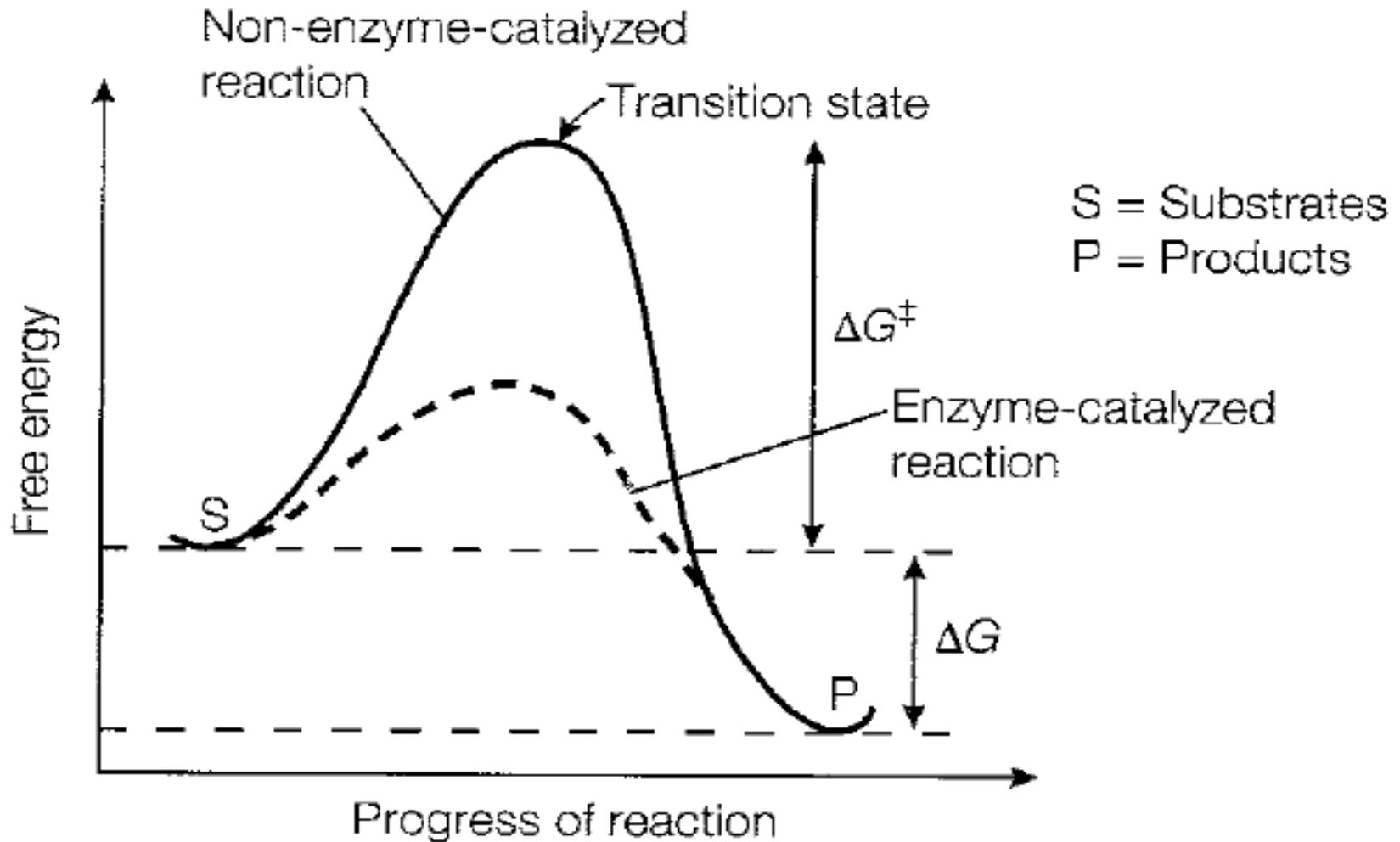


- Energy is needed to transform substrate into **“Transition state (AB)”**,
- Transition state has the **highest “Free energy”** of any component in the reaction pathway;
- **“Gibbs” Free Energy of Activation ( $\Delta G^\ddagger$ )** is the **difference in Free energy between Transition state and Substrate** (Fig. 1)

# Fig. 1: Diagram showing energy changes in Enzyme reaction

$\Delta G^\ddagger$  = Gibbs Free Energy of Activation;

$\Delta G$  = Free Energy or Gibbs Free Energy;



## Is Gibbs Free Energy of Activation affected by enzyme?

- Enzyme decreases Gibbs Free Energy of Activation;
- Enzyme stabilizes Transition state of a chemical reaction;
- Enzyme does not alter the energy levels of substrates and products;
- Enzyme increases the rate at which reaction occurs, but has no effect on overall change in energy of the reaction;
- Enzyme increases the rate of chemical reaction by decreasing the Gibbs Free Energy of Activation ( $\Delta G^\ddagger$ )  
{See Fig. 1};

## Is Gibbs free energy of activation ( $\Delta G^\ddagger$ ) the same as Gibbs free energy change ( $\Delta G$ )?

- Gibbs Free Energy change ( $\Delta G$ ) is different from Gibbs Free Energy of Activation ( $\Delta G^\ddagger$ ) {Fig. 1};
- Gibbs Free Energy change ( $\Delta G$ ) is the free energy change between SUBSTRATE [S], AND PRODUCT [P];

$$\Delta G = \text{Free energy of [S]} - \text{Free energy of [P]}$$

- $\Delta G$  indicates if a reaction is energetically favorable or not
- $\Delta G$  is independent of the path of the chemical reaction,
- $\Delta G$  provides no information about the rate of a chemical reaction since the rate of the chemical reaction is governed by  $\Delta G^\ddagger$

- Negative  $\Delta\mathbf{G}$  indicates that the reaction is thermodynamically favorable in the direction indicated
  - That it is likely to occur spontaneously, {**Fig. 1**};
- Positive  $\Delta\mathbf{G}$  indicates that the reaction is not thermodynamically favorable and requires an input of energy to proceed in the direction indicated;
- Energy can be achieved by coupled reactions;
- Standard Free Energy change ( $\Delta\mathbf{G}^\circ$ ), defined [S] and [P] under specified biochemical conditions;

## What is the general equation for enzyme catalyzed reaction?

- General expression for an enzyme catalyzed reaction:



*(Where E = Enzyme; S= Substrate; ES = Enzyme-Substrate Complex; P = Product);*

- Concept of “Active site” or “Catalytic site” or “Substrate-binding” site is needed to understand formation of **ES-complex**

# What is the Active site or Catalytic site of an enzyme?

- Active site or Catalytic site of an enzyme:
  - Region that binds Substrate(s) and converts it into Product(s);
  - Relatively small part of the whole enzyme molecule;
  - Three-dimensional entity formed by amino acid residues that can lie far apart in the linear polypeptide chain;

- Substrate binds in active site by multiple weak forces:
  - Electrostatic interactions,
  - Hydrogen bonds,
  - Van der Waals bonds,
  - Hydrophobic interactions,
  - Reversible covalent bonds,
- Binding of Substrate to Active site gives the Enzyme-Substrate complex (ES);
- Catalytically active residues within the active site acts on Substrate, forming “Transition state” and then Products, which are released;

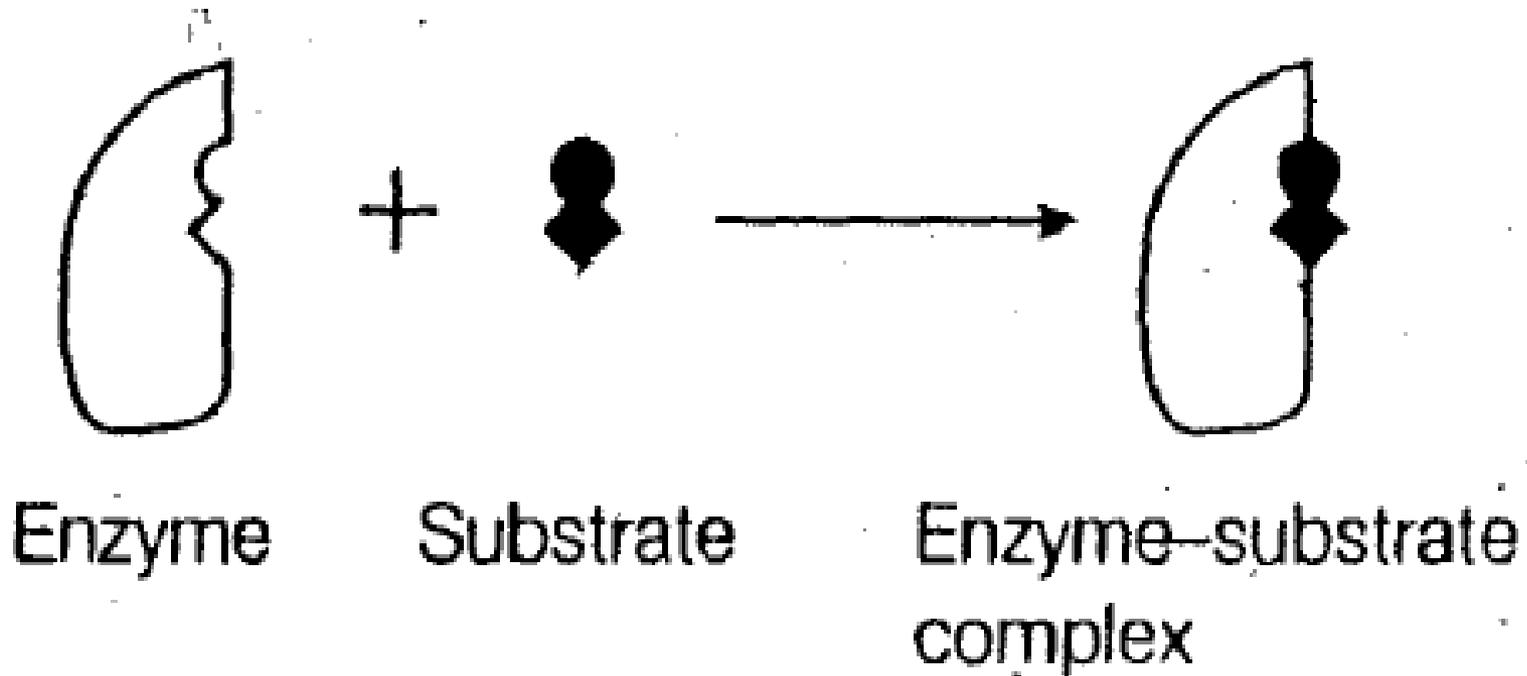
## Briefly explain the Lock-and-Key Model for Enzyme-Substrate binding

### Lock-and-Key Model: (Fig. 2a)

- Proposed by Emil Fischer in 1894,
- According to this model the shape of the Substrate and the Active site on the enzyme fit together like a Key into its Lock;
- Both shapes are considered Rigid and Fixed, and perfectly complement each other when brought together in the right alignment;

## Fig 2a: Diagram of Lock-and-Key model

Binding of Substrate (S) to Enzyme (E) to form ES complex



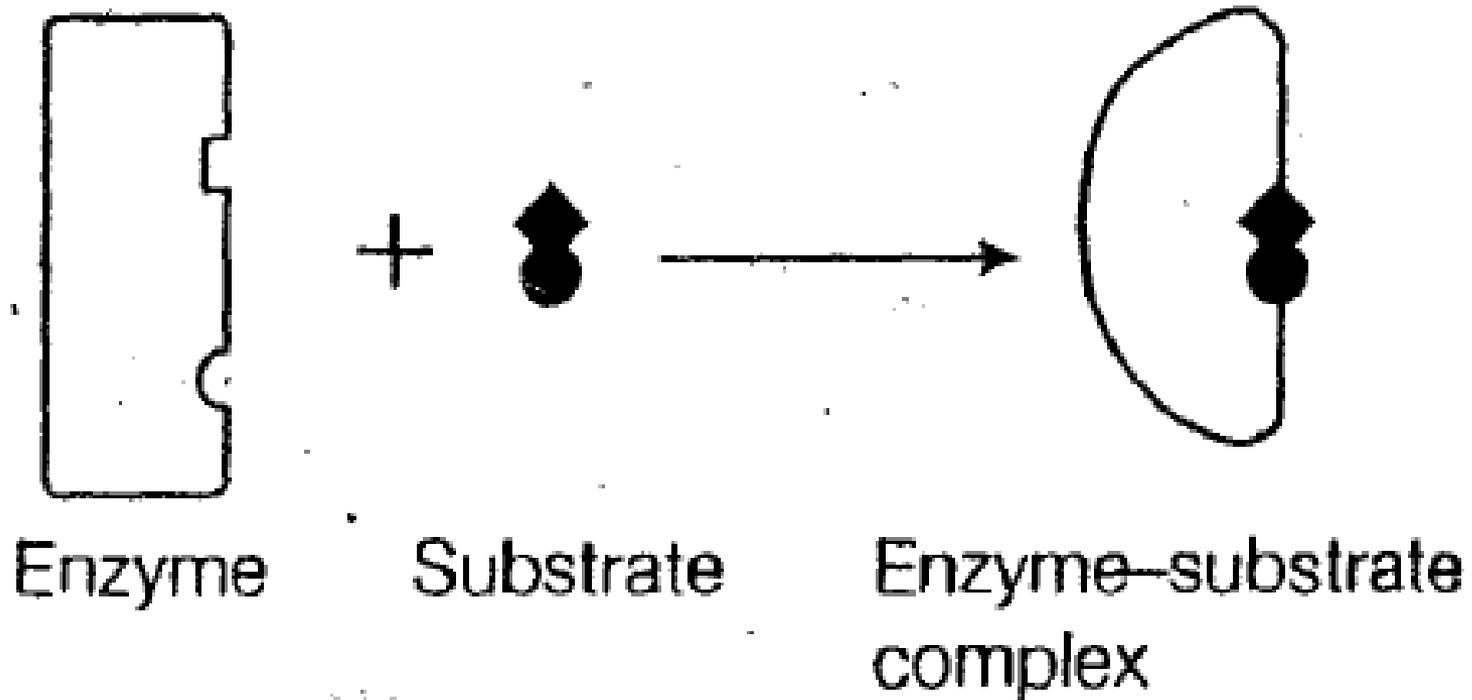
## Briefly explain the Induced-Fit Model for Enzyme-Substrate binding

### Induced-Fit Model: (Fig. 2b)

- Proposed in 1958 by Daniel Koshland, Jr
- Binding of Substrate Induces a conformational change in the Active site of the enzyme;
- Enzyme may distort the Substrate, forcing it into a conformation similar to that of the “Transition state”

## Fig 2a: Diagram of Lock-and-Key model

Binding of Substrate (S) to Enzyme (E) to form ES-complex



# Nomenclature of enzymes

## Some enzymes have common names:

- Many enzymes are named by adding suffix “-ase” to the name of their substrate; Example:
- **Urease:** enzyme that catalyzes hydrolysis of Urea,
- **Maltase:** enzyme that catalyzes hydrolysis of Maltose,
- Some enzymes, such as **Trypsin** and **Chymotrypsin**, have names that do not denote their substrate;
- Some other enzymes have several alternative names;

# International Classification of Enzymes

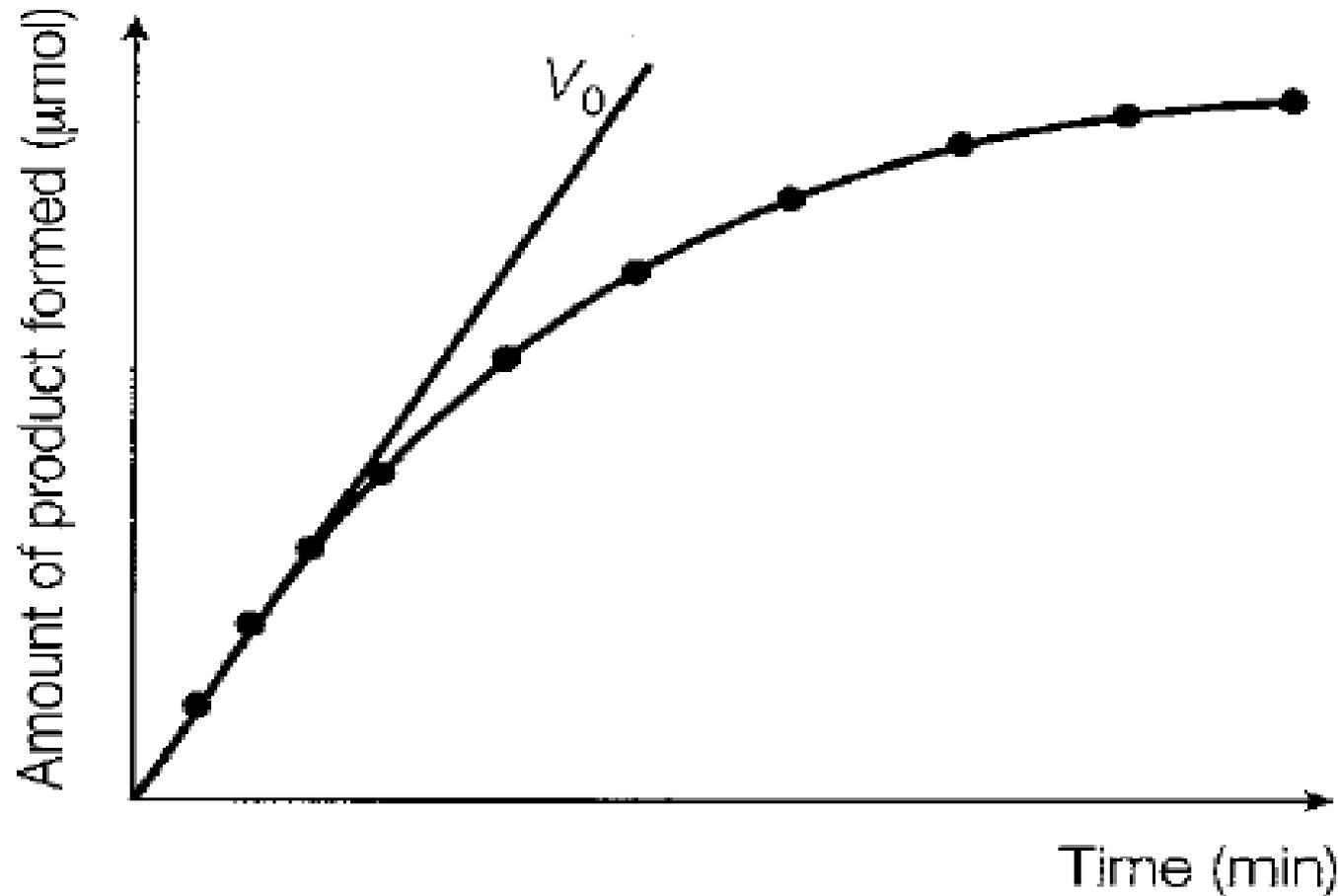
International System of Nomenclature is to Standardized and Rationalize Names of Enzymes:

- Enzymes are placed into one of Six Major classes based on the type of reaction catalyzed:
- Each enzyme is identified uniquely by using a Four-Digit Classification number
- Six Major classes are:
  - Oxido-Reductases,
  - Transferases
  - Hydrolases
  - Lyases,
  - Isomerases,
  - Ligases or Synthases

## What is the velocity or rate of an enzyme reaction?

- Velocity or Rate of Enzyme-catalyzed reaction is the change in the amount of Substrate or Product per unit time;
- Velocity of Enzyme is measured under “**Steady-State**” conditions, when the amount of Substrate is very large compared to amount of enzyme;
  - Velocity is reported as the value at time zero (**V<sub>o</sub>**);
  - **Initial Velocity (V<sub>o</sub>)** and is expressed as  $\mu\text{mol}/\text{min}$
- Velocity is fastest at time zero, which is the point when no product has been formed (**Fig. 3**)

**Fig. 3: Diagram showing Amount of Product formed and Time for an enzyme catalyzed reaction { $V_0$  = Initial Velocity}**



- **Fig. 3** shows Typical graph of Product formed against Time for an Enzyme-catalyzed reaction;
- Initial period of rapid product formation gives the linear portion of the graph;
- Slowing-down of the enzyme velocity as Substrate is used up and/or as the enzyme loses activity;
- **Initial Velocity ( $V_o$ )** is obtained by drawing a straight line through the linear part of the curve, starting at the zero time-point
- Slope of the straight line is equal to  **$V_o$**

## How is the activity of an enzyme expressed (Enzyme units)?

- An enzyme unit (U) is the amount of enzyme that catalyzes the transformation of  $1.0\mu\text{mol}$  of Substrate per minute at  $25^{\circ}\text{C}$  under optimal conditions for that enzyme;
- Activity of an Enzyme, is the Total Units of Enzyme in the sample,
- Specific Activity is the Number of Units per milligram of Protein (**units/mg**)
- Specific Activity is a measure of the purity of an enzyme;
- During the purification of an enzyme, the Specific Activity increases and becomes maximal and constant when the enzyme is pure

## How does Enzyme concentration affect reaction velocity?

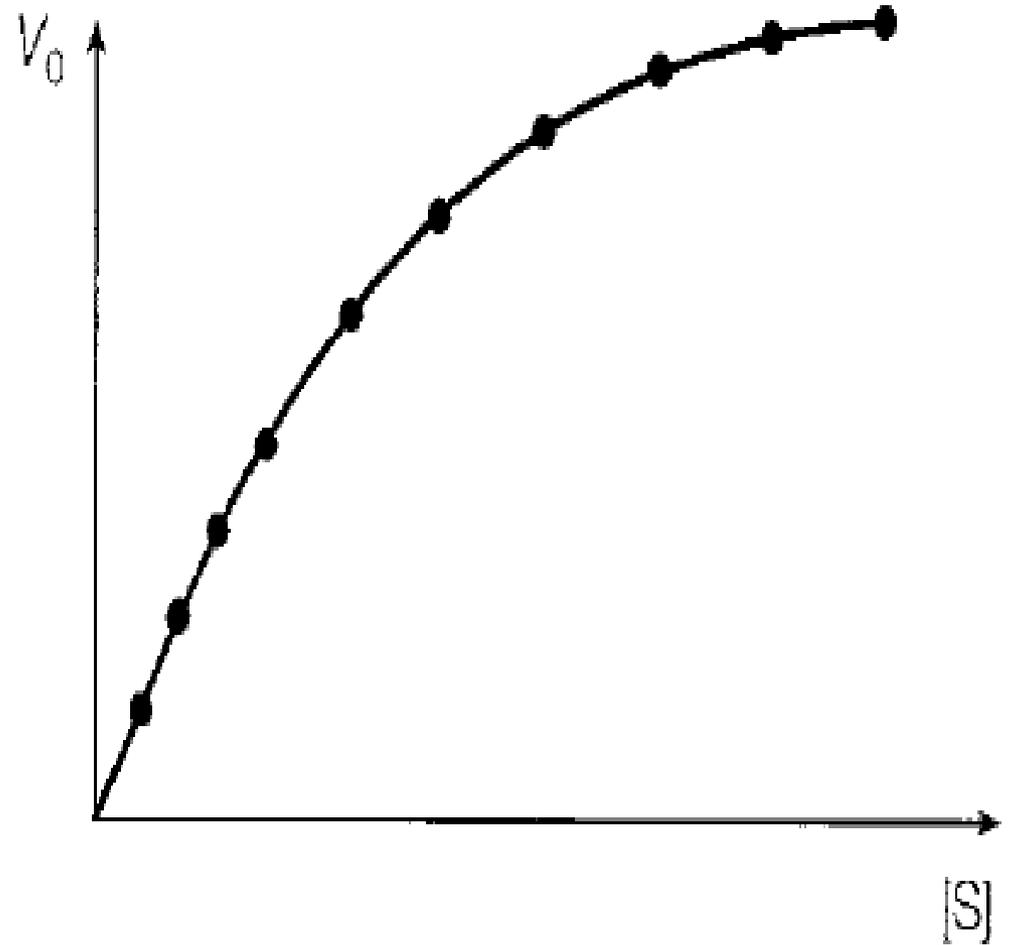
- When Substrate concentration is constant, the  $V_o$  is directly proportional to the concentration of the Enzyme,
- Increasing the amount of Enzyme increases  $V_o$ ;
- Straight-line graph is obtained when  $V_o$  is plotted against Enzyme concentration;

## How does [S] affect velocity of enzyme reaction?

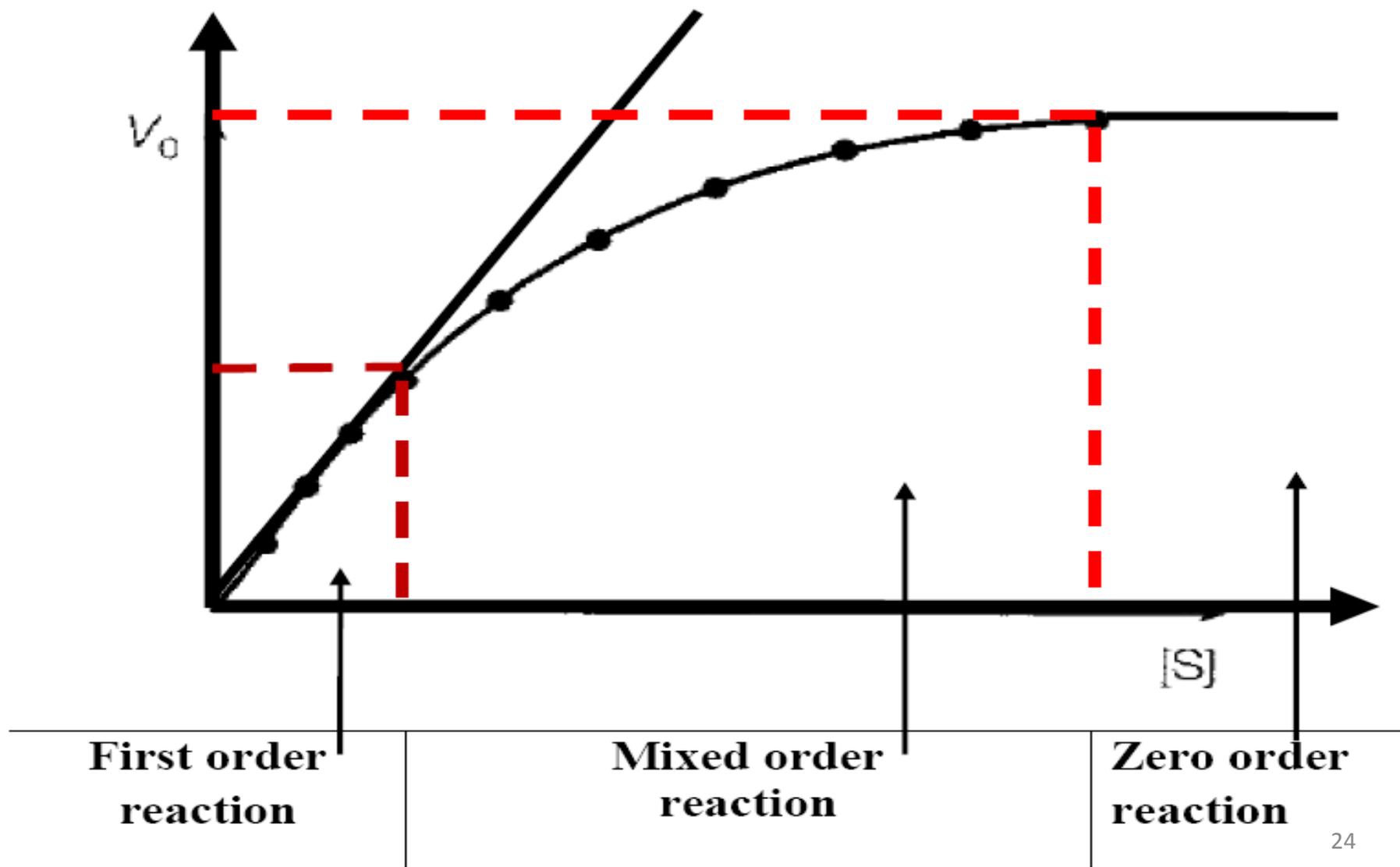
- When  $V_o$  is plotted against [S] a Hyperbolic curve is obtained (**Fig. 3a**)
  - The curve can be separated into sections based on concentration of the Substrate [S] (**Fig. 3b**)
- **At low [S]:**
  - Doubling of [S] will lead to doubling of the  $V_o$ ,
  - It is a “First-order” reaction;
- **At higher [S]:**
  - Enzyme becomes saturated,
  - Increase in [S] lead to very small changes in  $V_o$ ,
  - It is a “Zero-order” reaction;

## Fig 3a: Graph of [S] vs $V_o$

- For constant amount of Enzyme [E];
- Graph [S] vs  $V_o$  gives Hyperbolic curve;
- Curve has three sections, based on [S]; (see Fig. 3b)



**Fig. 3c:** Graph of  $[S]$  against  $V_o$ ; Diagram shows order of reactions: **First order, Mixed order, Zero order** with respect to  $[S]$



# What is a First-order reaction?

## First order reaction:

- For a given amount of enzyme, the Velocity of the reaction is directly proportional to the  $[S]$ ;
- Increasing the  $[S]$  also cause increase in Velocity of reaction;
- Relationship between Velocity and  $[S]$  is Linear

## What is a zero order reaction?

### Zero order reaction:

- For a given amount of Enzyme **[E]**, the Velocity of the reaction is Constant and Independent of Substrate **[S]** concentration;
- Increasing the Substrate concentration **[S]** has no effect on reaction velocity;
- Addition of more substrate will not speed up the reaction;

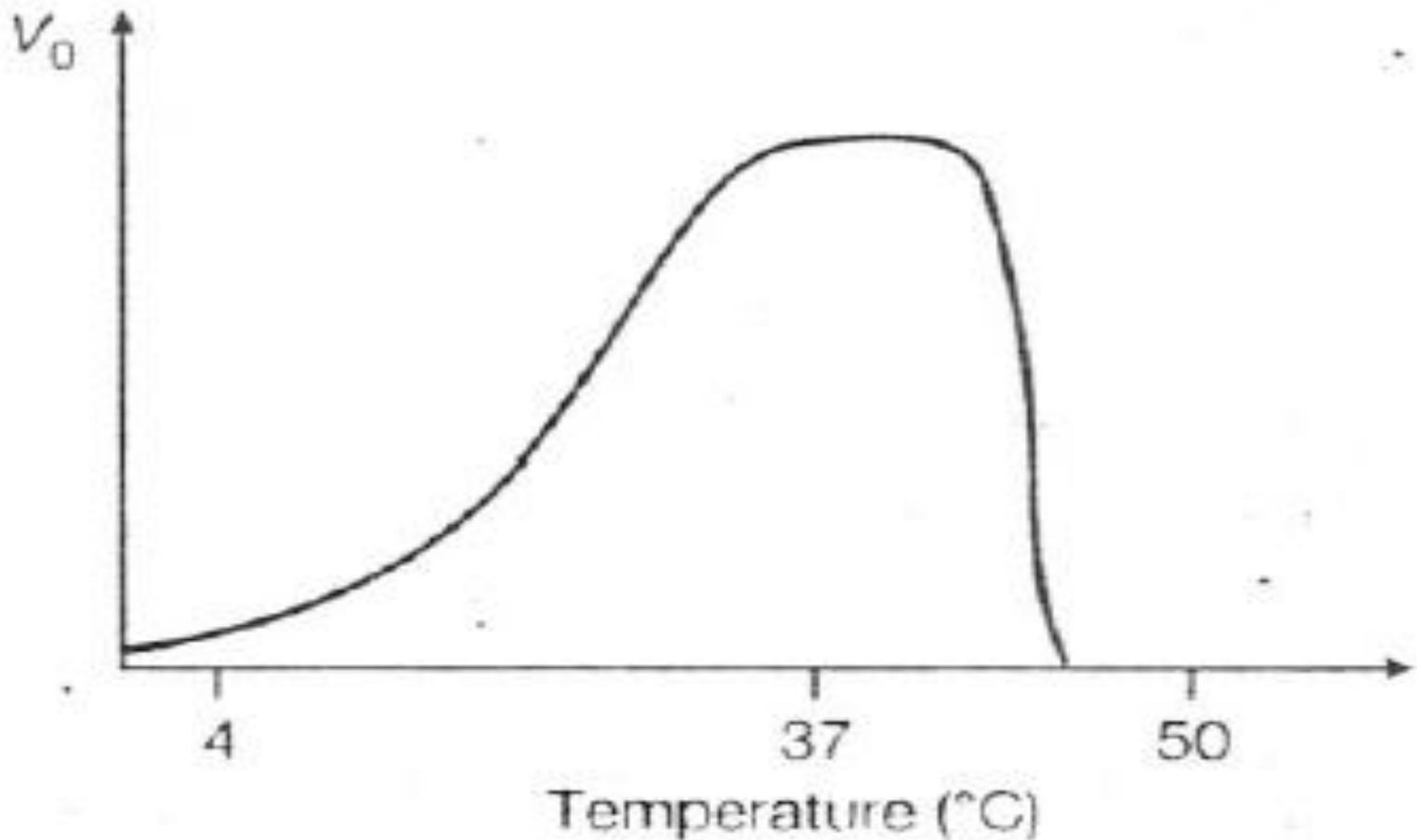
**Fig 3b: Graph showing relationship between [S] and  $V_o$ :  
Graph can be separated into 3 sections:**

- **At low [S]:** reaction is First-order with respect to [S];
  - **$V_o$**  is directly proportional to [S]
- **At mid [S]:** reaction is Mixed-order;
  - Proportionality of  **$V_o$**  to [S] is changing;
- **At high [S]:** reaction is Zero-order,
  - **$V_o$**  is independent of [S]
  - Enzyme has its maximum velocity ( **$V_{max}$** );
- Increasing [S] has no effect on  **$V_o$** ; enzyme is saturated;
- These enzymes are called **Michaelis-Menten** enzymes

# How does Temperature (T) affect rate of enzyme reaction?

- T °C affects rate of enzyme reactions in two ways:
- **First:** Rise in T °C increases rate ( $V_o$ ) of reaction;
- **Second:** Increasing T °C above a certain value causes inactivation of enzyme (denature of enzyme) and thus reduces the rate of reaction;
- Most enzymes are Thermo-labile,
  - In Hypothermia most enzyme reactions are depressed, causing reduction in metabolism;
- Graph of T °C against  $V_o$  gives a curve with well-defined optimum T °C (**Fig. 4a**)
- Temperature optimum: about 37°C,

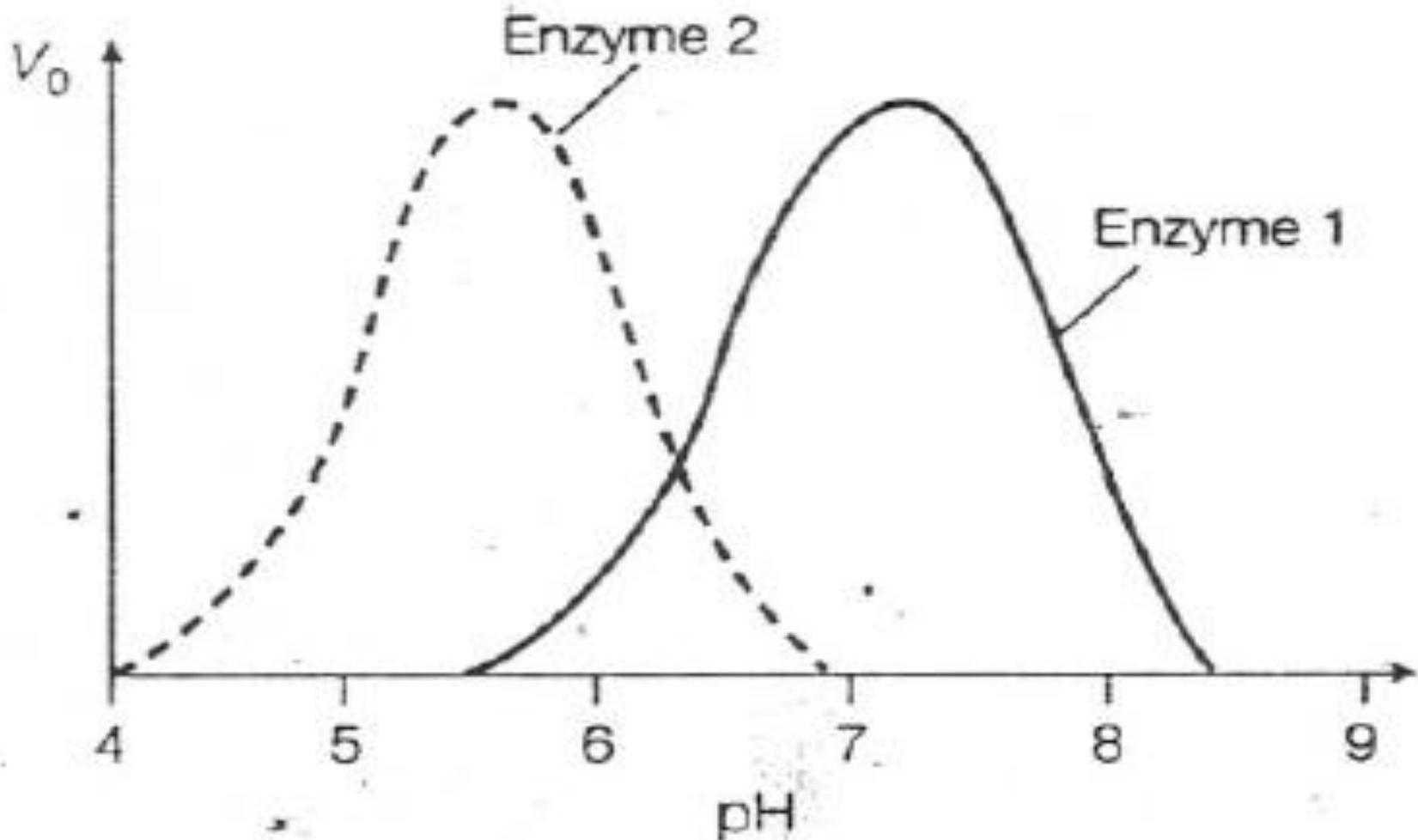
**Fig. 4:** Graph of  $T$  °C against  $V_o$  gives a curve with well-defined optimum  $T$ °C



# How does pH affect rate of enzyme reaction?

- Each enzyme has an Optimum pH at which rate of reaction is maximum;
- Small deviations in pH from optimum value lead to reduced rate caused by changes in ionization of groups at the active site of enzyme;
- Larger deviations in pH lead to denature of enzyme due to interference with weak non-covalent bonds that maintaining the three-dimensional structure;
- Optimum pH of most enzymes is around 6.8, but there is diversity in pH optima, due to different functions;
- Digestive enzyme Pepsin has acidic pH of stomach (pH 2)

**Fig. 4b: Graph of  $V_0$  against pH for two different enzymes; Each graph is bell-shaped curve;**



# ENZYME KINETICS

- General expression for an enzyme catalyzed reaction:



*(Where E = Enzyme; S= Substrate; ES = Enzyme-Substrate Complex;  
P = Product)*

## What does Michaelis-Menten equation represent?

- Relationship between Initial velocity ( $V_0$ ) and substrate concentration  $[S]$ ;

# What is the Michaelis-Menten equation?

## Michaelis-Menten equation:

- $V_0$  = Initial velocity,
- $V_{max}$  = Maximum velocity,
- $[S]$  = Substrate conc.
- $K_m$  = Michaelis constant,

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

## What are the 3 basic assumptions of Michaelis-Menten equation?

1. **[S]** is very large compared to **[E]**; Enzyme is saturated with substrate, no free enzyme is available;
2. **[ES]** complex is in a “Steady-State”, (i.e., Rate of formation of ES complex is equal to its rate of breakdown);
3. Initial velocity ( $V_0$ ) of reaction must be used;

## What is $K_m$ ?

- **$K_m$**  is equal to the **[S]** at which the Initial velocity ( **$V_o$** ) is equal to **half** the Maximal velocity,
- That is:  **$K_m = [S]$ , when  $V_o = 0.5 V_{max}$**
- **$K_m$**  is a measure of the stability of **ES**-complex

## How significant is $K_m$ ?

- **Small value of  $K_m$** , means **high affinity** of the enzyme for the Substrate;
- **Large value of  $K_m$** , means **low affinity** of enzyme for the Substrate;
- **$K_m$**  is characteristic for a particular enzyme with a given substrate;
- **$K_m$**  is an important parameter in metabolic control in cells;
- **$K_m$**  values are near to the concentration of substrates in cells;

# What is Lineweaver-Burk Plot (Double-Reciprocal Plot)?

- Lineweaver-Burk Plot (Double-Reciprocal plot) is used to determine  $K_m$  and  $V_{max}$  for an enzyme;
- Michaelis-Menten equation is rearranged to give Double-reciprocal equation;
- Equation is similar to that for a straight-line:

$$Y = MX + C,$$

$$(a) \quad V_o = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

$$(b) \quad \frac{1}{V_o} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$Y = mX + C$$

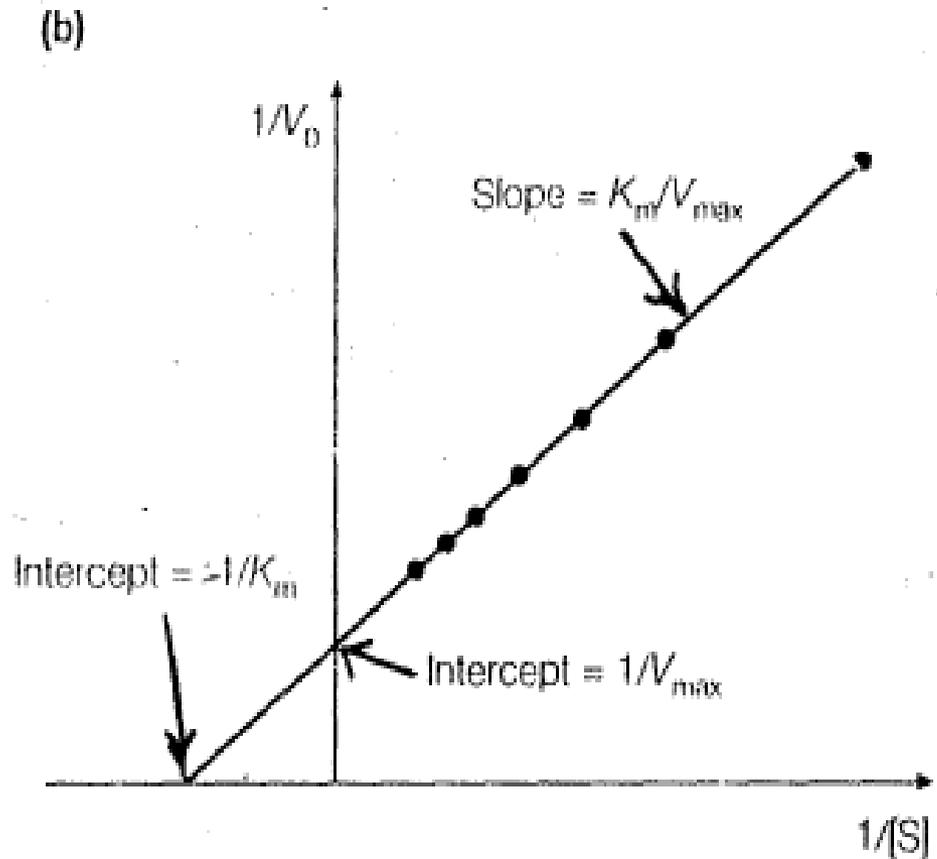
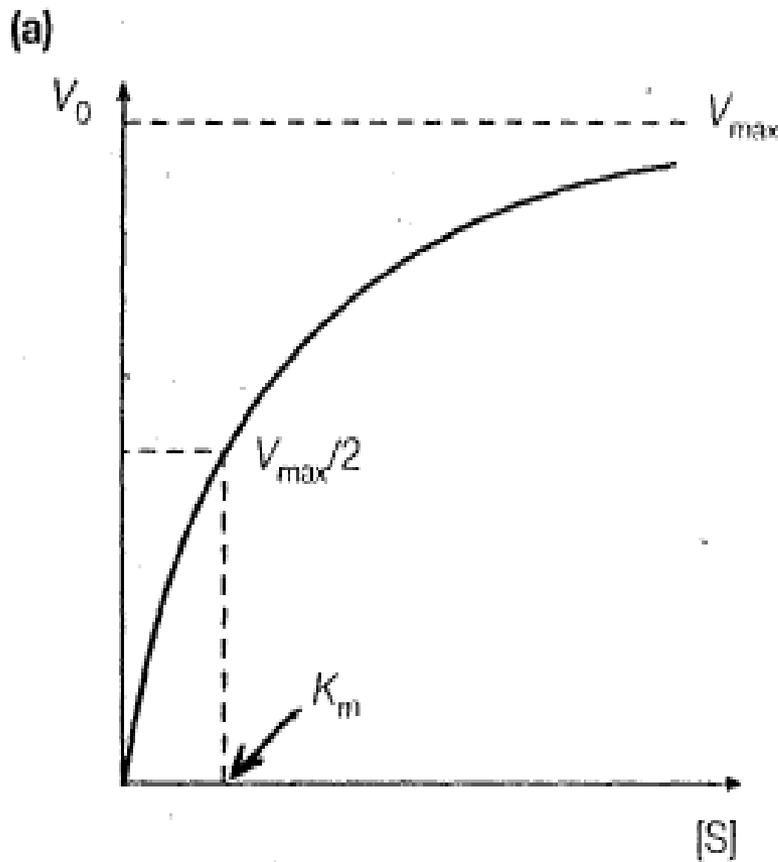
- Equation is used to plot the Double-reciprocal graph
- Information that can be obtained from graph (**Fig. 5**):

$$Y = MX + C$$

- **Slope of graph:  $M = K_m/V_{max}$ ,**
- **Intercept on Y-axis:  $C = 1/V_{max}$ ,**
- **Intercept on X-axis: when  $1/V_o = 0$ , gives:  $-1/K_m$**

**Fig. 5a:** Graph of  $[S]$  against  $V_0$ , show how to obtain  $K_m$ ;

**Fig. 5b:** Lineweaver-Burk plot (double-reciprocal plot) showing how to obtain  $K_m$



# ENZYME INHIBITION

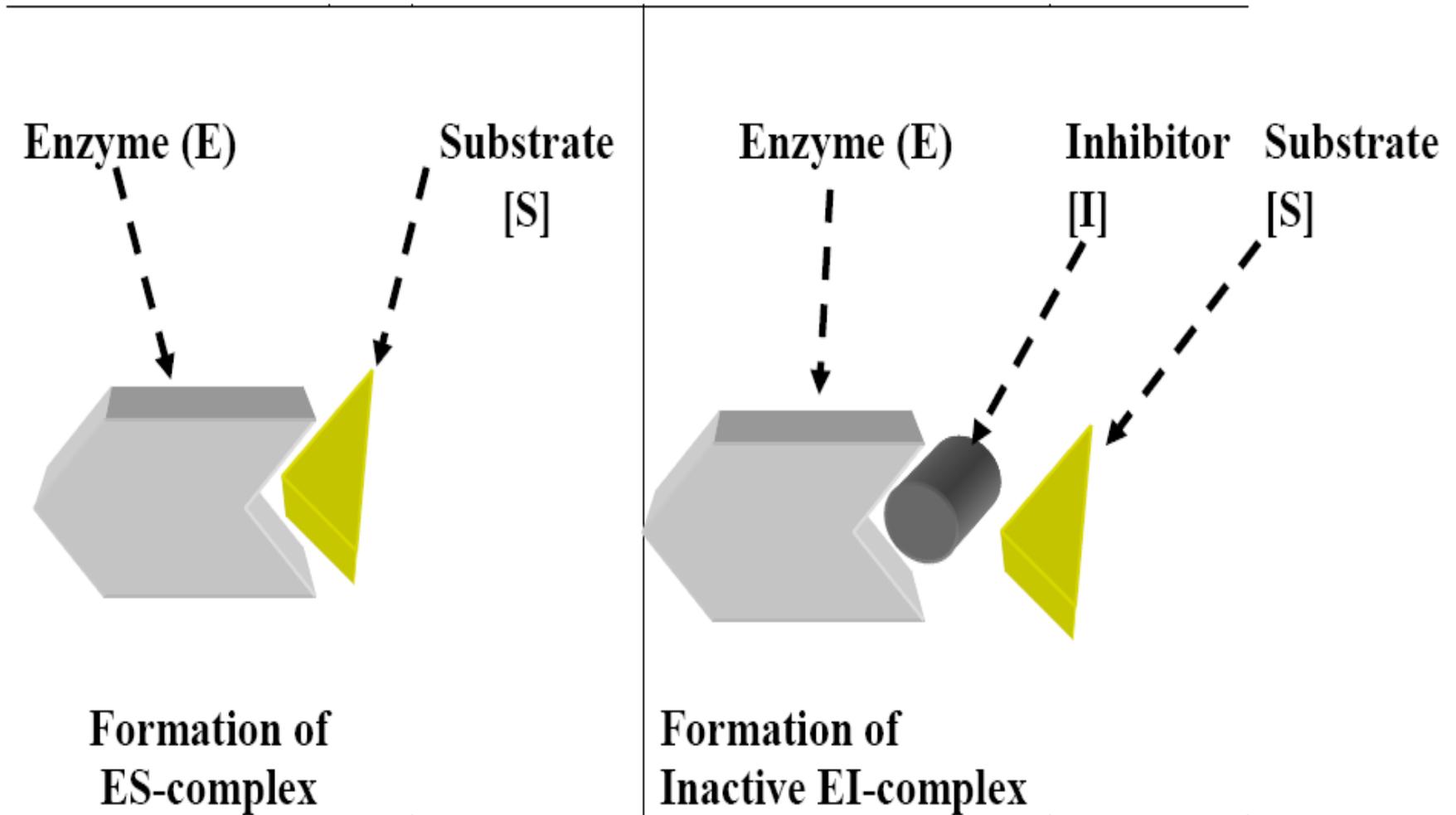
- Some compounds can inhibit enzyme reactions;
- Two main types of Enzyme inhibition are:
  - **Reversible Inhibition,**
  - **Irreversible Inhibition,**
- Reversible inhibition can be subdivided into:
  - **Competitive inhibition,**
  - **Non-competitive inhibition,**

## What is competitive inhibition?

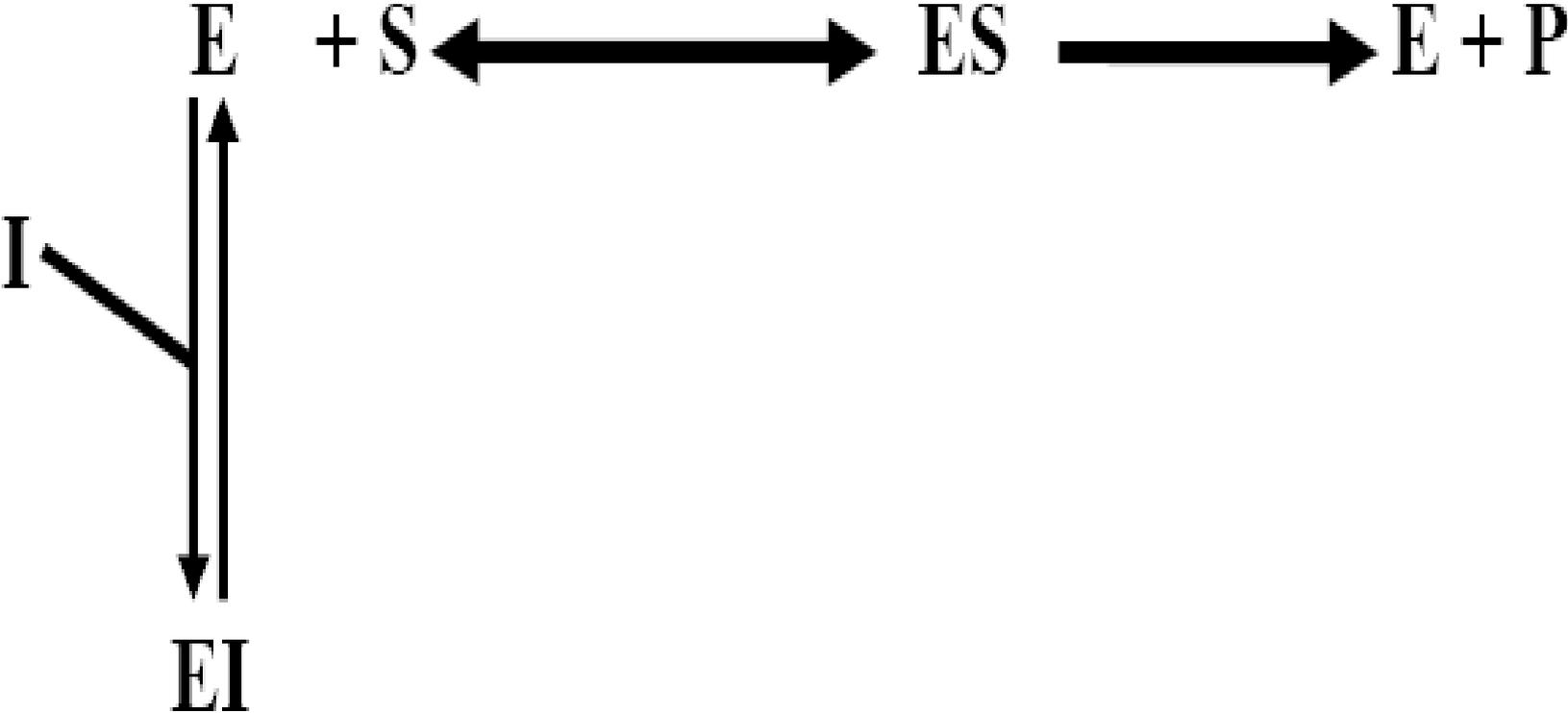
- **Competitive inhibition:** Substrate [S] and Inhibitor [I] competes for the binding site of the enzyme;
- Competitive Inhibitor:
  - Has structural similarities to the Substrate,
  - Competes with substrate for enzyme active site,
  - Binds reversibly to active site of Enzyme,
- Enzyme may bind either Substrate [S] or Inhibitor [I], but not both at the same time;
- High [S] displaces competitive inhibitor from active site;
- **Fig. 6** illustrates binding of [S] and Inhibitor [I] to binding site of Enzyme [E];

# Fig. 6a: Binding of [S] and [I] to binding site of Enzyme [E]

Schematic diagram showing Competitive Inhibition



**Fig. 6b: In competitive inhibition Enzyme (E) can bind to either Substrate [S] or Inhibitor [I]; it cannot bind to both at the same time.**



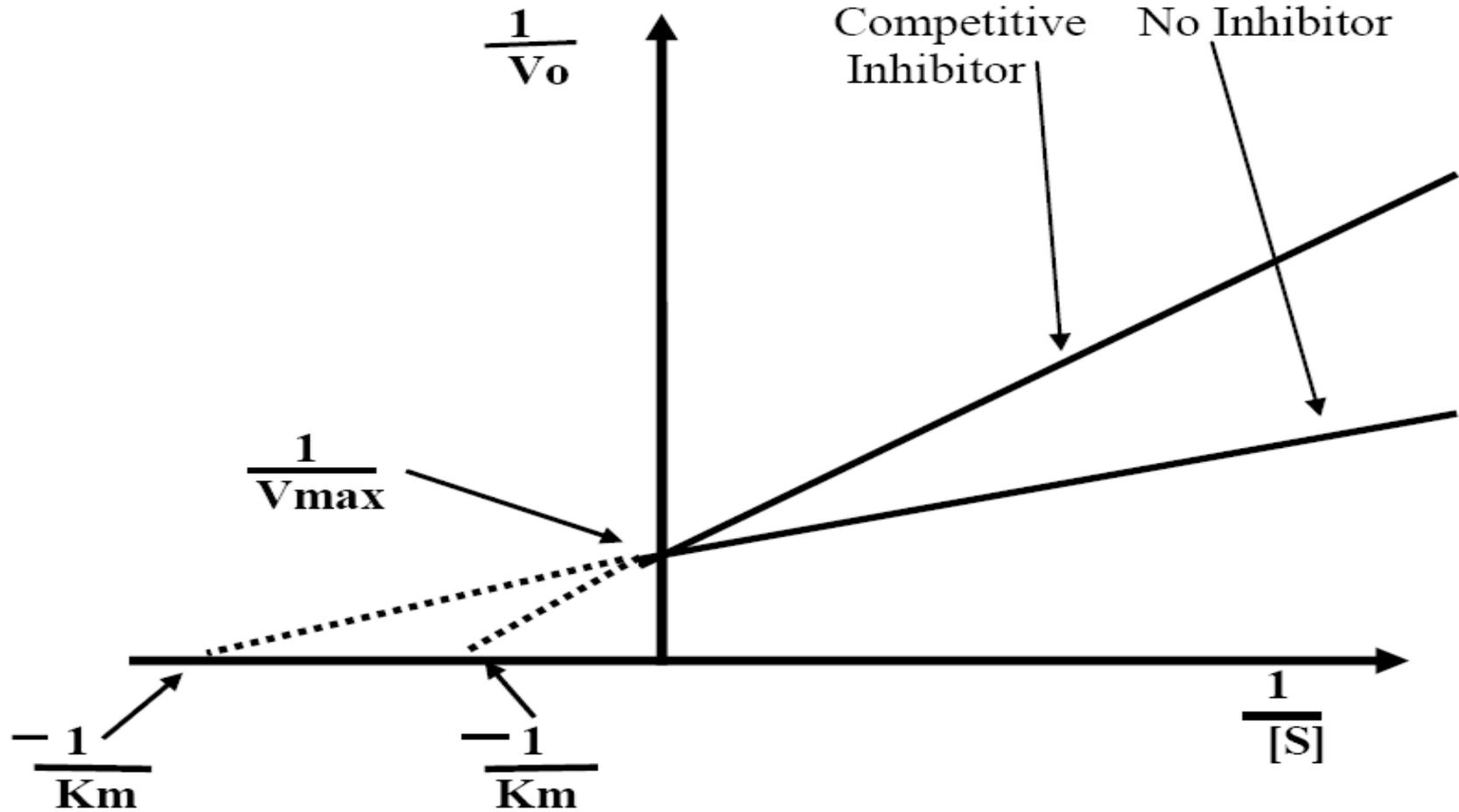
## Give examples of Competitive Inhibition

- Sulfanilamide competes with Para-Aminobenzoic Acid (PABA) in reaction catalyzed by **Dihydropteroate Synthetase** in the biosynthesis of Folate (Folic Acid);
- Methotrexate competes with Dihydrofolate in reaction catalyzed by **Dihydrofolate Reductase** (Methotrexate is structural analogue of Folate)

## How can Michaelis-Menten Constant ( $K_m$ ) be determined in Competitive Inhibition of an enzyme?

- Measure  $V_o$  at different  $[S]$  in the absence and presence of a fixed concentration of the Competitive Inhibitor  $[I]$ ;
- **Draw Lineweaver-Burk plot** for Inhibited and Non-inhibited enzyme (**Figs. 7**);
- **Interpretation of the results:**
  - There is **no change in  $V_{max}$**  of the Enzyme,
  - The  **$K_m$  of the inhibited Enzyme increases**,
- Competitive inhibitor **increases the  $K_m$  of an enzyme for its substrate**;

**Fig. 7:** Diagram of Lineweaver – Burk (Double-Reciprocal) plot showing effect of Competitive Inhibitor on  $K_m$  and  $V_{max}$  of an Enzyme.



**$K_m$  of Enzyme  
with no Inhibitor**

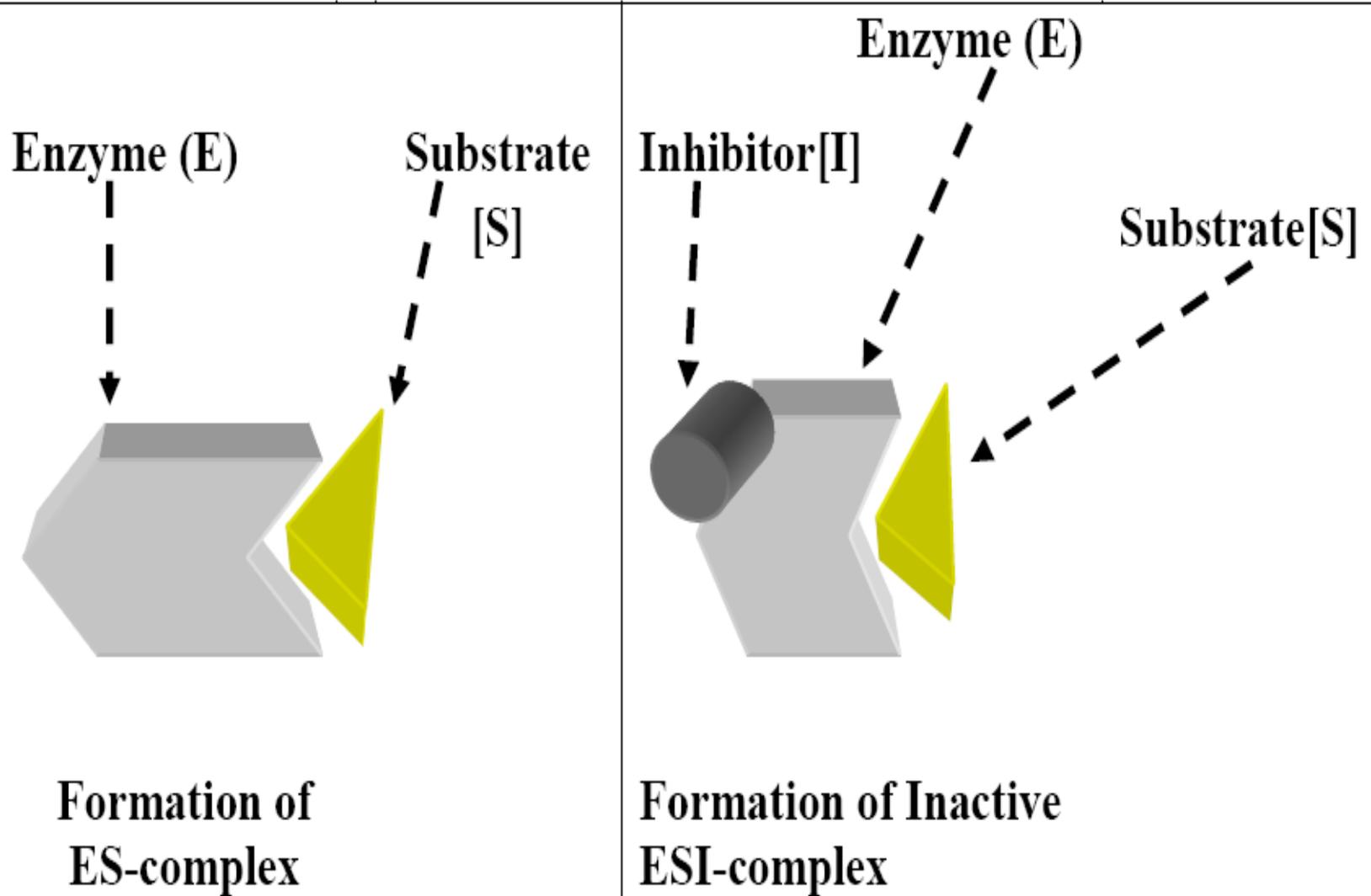
**$K_m$  of Competitively  
Inhibited Enzyme ( $K_m$  is  
Increased)**

## What is Non-Competitive inhibition (Give example)?

- Non-competitive inhibitor binds reversibly to a site other than Active Site of the enzyme, (**Fig. 8a**);
- Enzyme can bind Inhibitor, Substrate or both the Inhibitor and Substrate together (**Fig. 8b**);
- Effects of non-competitive inhibitor cannot be overcome by increasing  $[S]$ ;
- Example of Non-competitive inhibitor:
  - Inhibition of Renin by Pepstatin

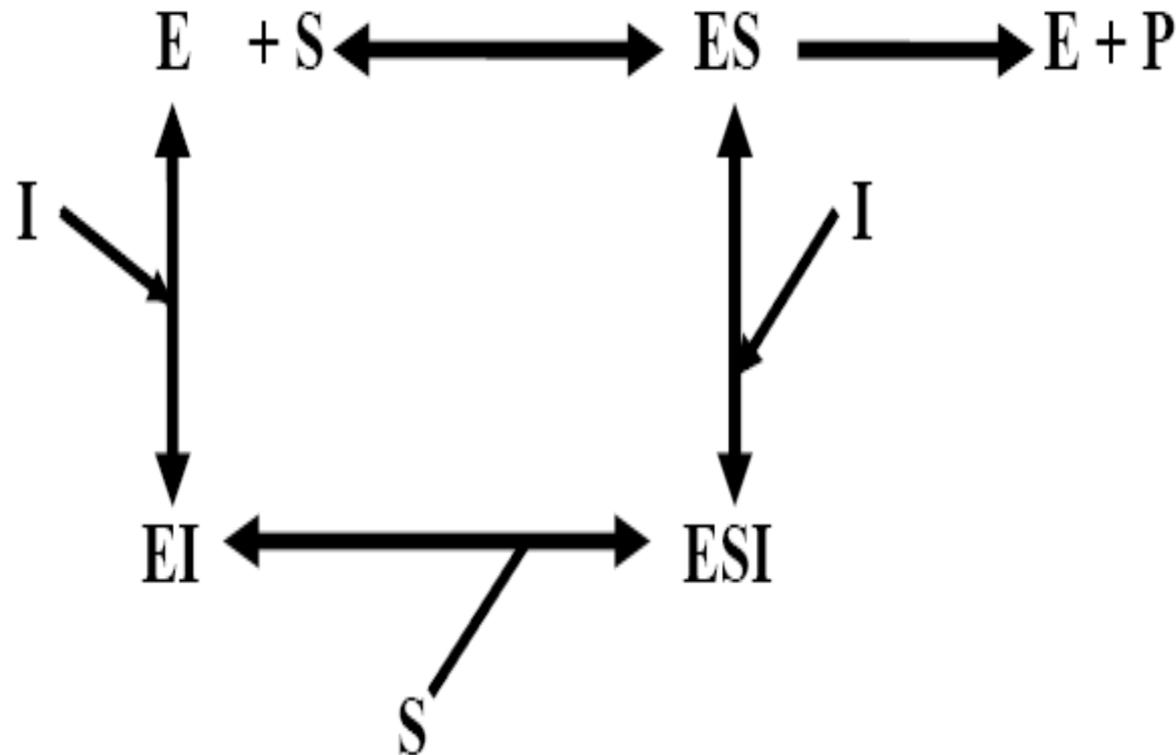
**Fig. 8a:**

**Schematic diagram showing Non-Competitive Inhibition**



**Fig. 8b:**

In non-competitive inhibition Enzyme (E) has two sites: Substrate binding site and Inhibitor binding site. E binds to either Substrate [S] or Inhibitor [I]; Enzyme can bind to both [S] and [I] at the same time.

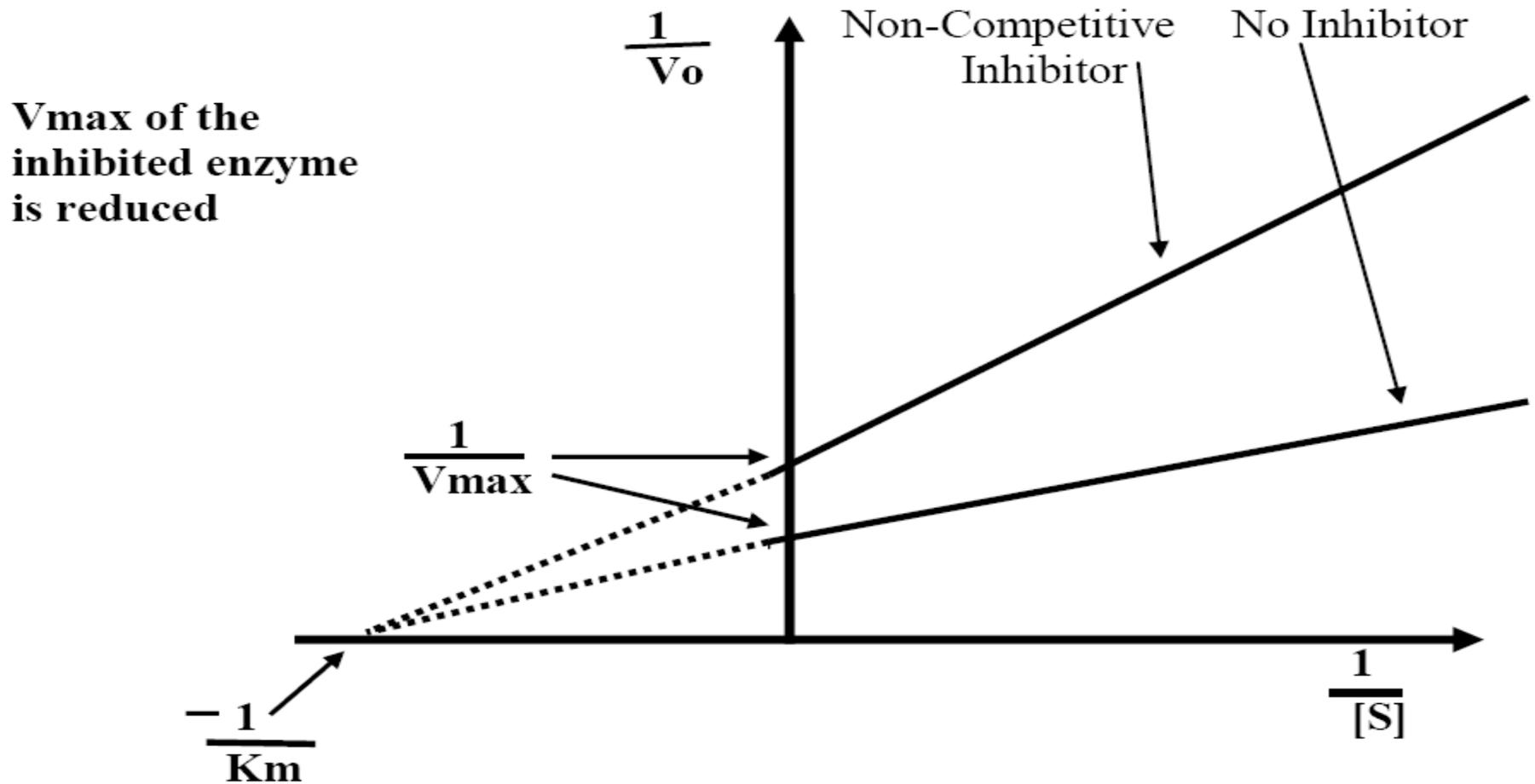


## How can Michaelis-Menten constant be determined for Non-Competitive Inhibition of an enzyme?

- Measure  **$V_o$**  of the enzyme at different **[S]** in the absence and presence of a fixed concentration of non-competitive inhibitor;
- **Draw Lineweaver-Burk plot** for Inhibited and Non-inhibited enzyme (**Fig. 9**)
- Interpretation of results:
  - **$K_m$**  is not affected, thus affinity of the enzyme for Substrate is unchanged;
  - **$V_{max}$**  of inhibited enzyme is decreased;

**Fig. 9:**

Diagram of Lineweaver – Burk (Double-Reciprocal) plot showing effect of Non-Competitive Inhibitor on  $K_m$  and  $V_{max}$  of an Enzyme.



$K_m$  of Enzyme with no inhibitor and with non-competitive inhibition;  $K_m$  does not change

## What is Irreversible inhibition (Give examples)?

- Irreversible Competitive inhibitors inhibit enzymes by binding very tightly to the Active Sites of enzymes;
- Some toxic, naturally occurring and manufactured compounds are irreversible enzyme inhibitors;
- Examples:
  - Penicillin inhibits Trans-peptidase needed in development of bacterial membrane;
    - It prevents normal growth of bacteria;
  - Aspirin inhibits Cyclooxygenase required for synthesis of Eicosanoids;
  - Allopurinol inhibits Xanthine Oxidase required for the degradation of Purines

## Give examples of enzymes that are used in diagnosis

- **Acid Phosphatase:** Tumor Marker in cancer of Prostate;
- **Alanine Aminotransferase (ALT):** used in LFT;
- **Aspartate Aminotransferase (AST):** use in LFT, Cardiac Function, Myocardial damage;
- **Alkaline Phosphatase (ALP):** use in LFT, Cholestatic liver disease; a marker of Osteoblast activity in bone disease;
- **Amylase:** Indicator of cell damage in acute Pancreatitis;
- **Creatine Kinase (CK-MB):** use in Myocardial damage;

- **Gamma-Glutamyl Transpeptidase (GGT):** used in LFT, Hepato-Biliary damage and Alcoholism;
- **Lactate Dehydrogenase (LDH):** Muscle damage
- Cholinesterase involved in impulse transmission at neuromuscular and synaptic junctions, and
- **Plasma Cholinesterase (Pseudo-Cholinesterase):**  
It is involved in hydrolysis of Succinylcholine, a muscle-relaxant used in Anesthesia;
  - Can be used in diagnosis of poisoning with Pesticides, Insecticides, Organo-Phosphorus compounds;

## STUDY QUESTIONS

- How does enzymes affect (a) Gibbs free energy of activation; (b) Gibbs free energy?
- Use 2 models to describe how an enzyme can bind substrate to active site;
- Define the following (a) Enzyme units; (b) Specific activity of an enzyme;
- Explain how substrate concentration affects rate of enzyme-catalyzed reaction;
- State the significance of Michaelis-Menten constant ( $K_m$ );
- How is Lineweaver-Burk Plot used to determine  $K_m$  and  $V_{max}$  of an enzyme?
- Use graph to explain competitive and noncompetitive inhibition of enzymes;
- How is competitive inhibition different from non-competitive inhibition, in the way they affect  $K_m$  and  $V_{max}$  of enzymes?
- List three irreversible inhibitors and the enzyme or enzymes affected by each;
- List five enzymes that are used for diagnosis;

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