ENZYMES

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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,
  - Neural pH ,
- Most enzymes are **highly substrate specific;**
- Activity of an enzyme can 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 E**nergy barrier** must be overcome (**Fig. 1**),

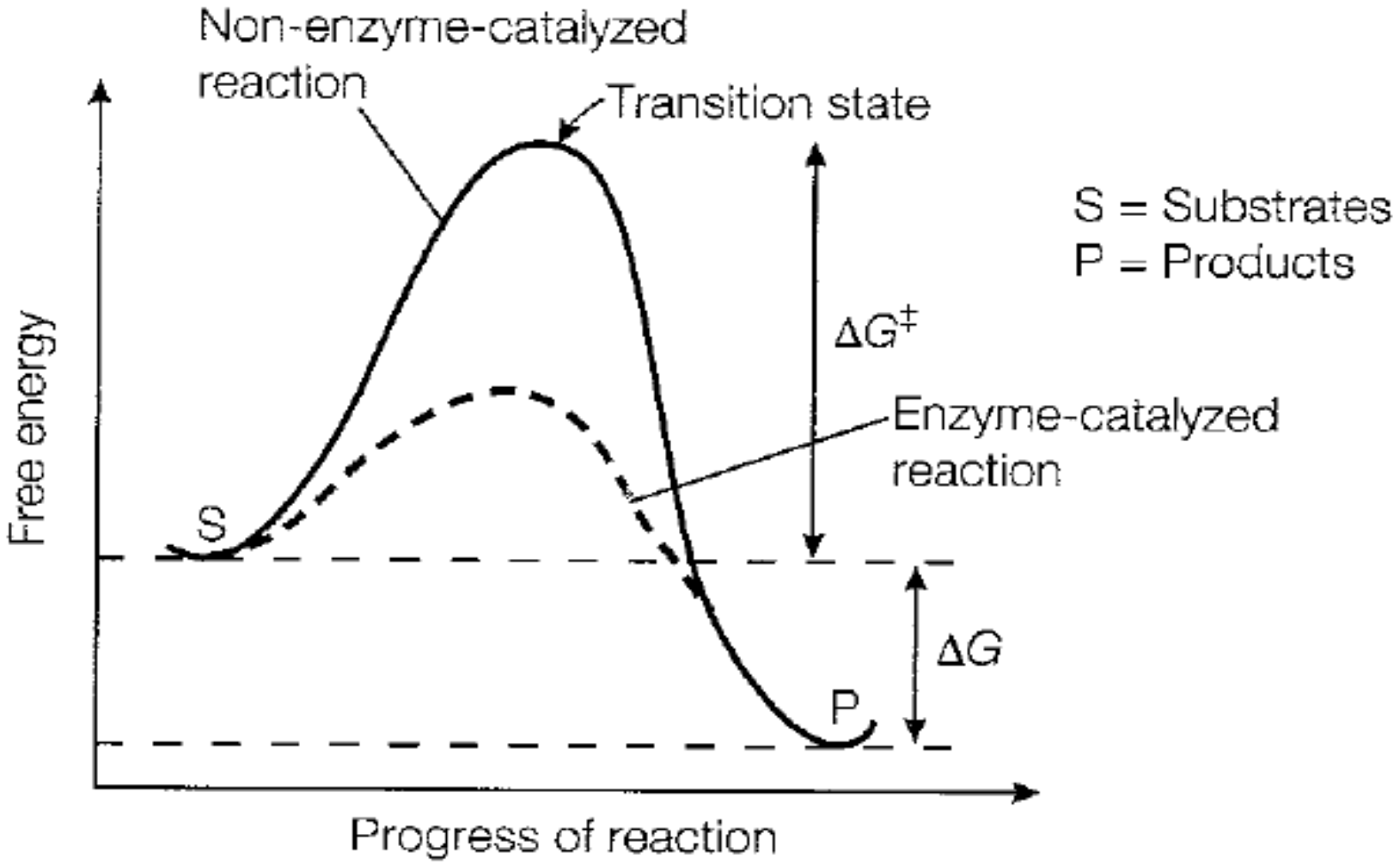
$$A + B = AB \longleftrightarrow C + D$$

- 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;

ΔG = 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 (ΔG)?

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

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

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

- Negative **ΔG** indicates that the reaction is thermodynamically favorable in the direction indicated
  - That it is likely to occur spontaneously, {**Fig. 1**};
- Positive **ΔG** 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 (**ΔG°**), defined [S] and [P] under specified biochemical conditions;

What is the general equation for enzyme catalyzed reaction?

- General expression for an enzyme catalyzed reaction:

E + S ⬅=====➔ ES ======➔ E + P

(***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;

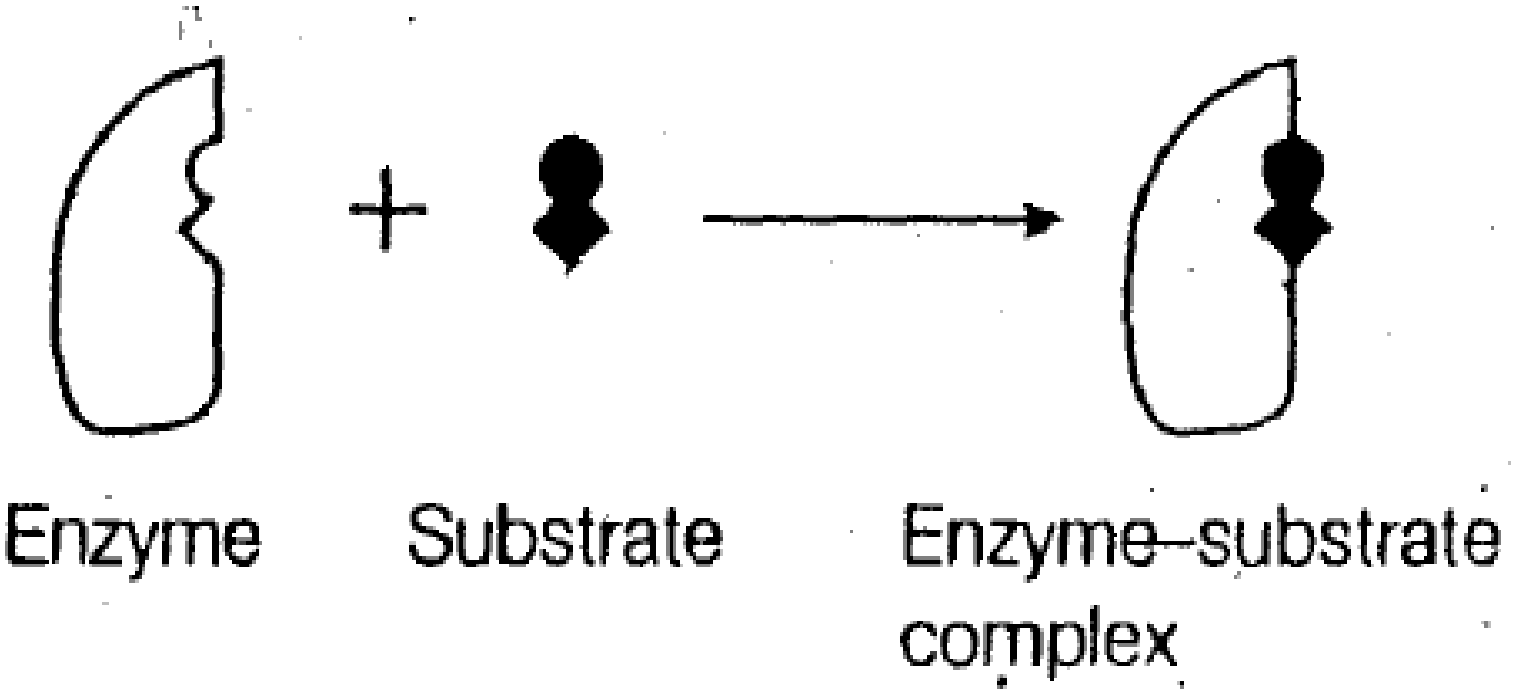
- Substrate binds in active site by multiple weak forces:
  - Electrostatic interactions,
  - Hydrogen bonds,
  - Van der Waals bonds,
  - Hydrophobic interactions,
  - Reversible covalent bonds,
- Binding of Substrate to Active site gives the Enzyme-Substrate complex (ES);
- Catalytically active residues 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

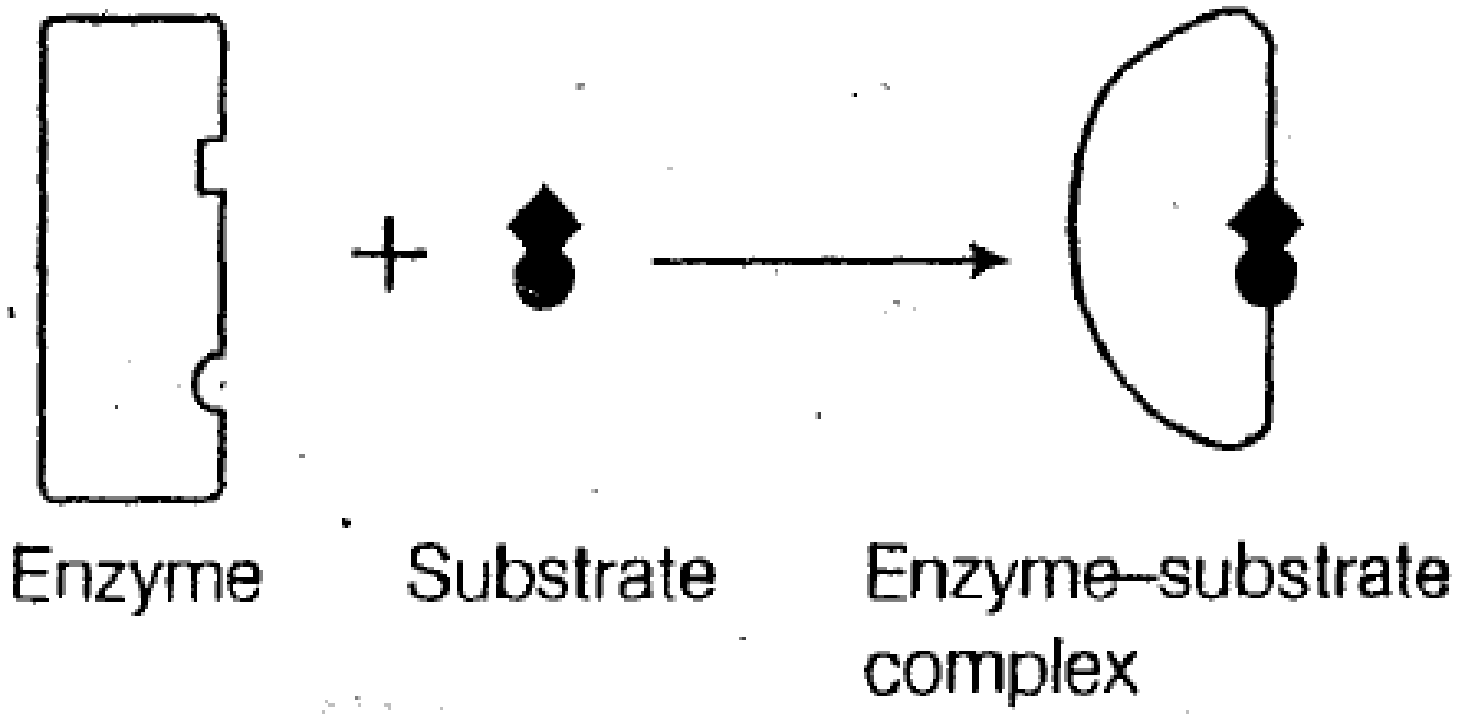


Briefly explain the Induced-Fit Model for Enzyme-Substrate binding

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

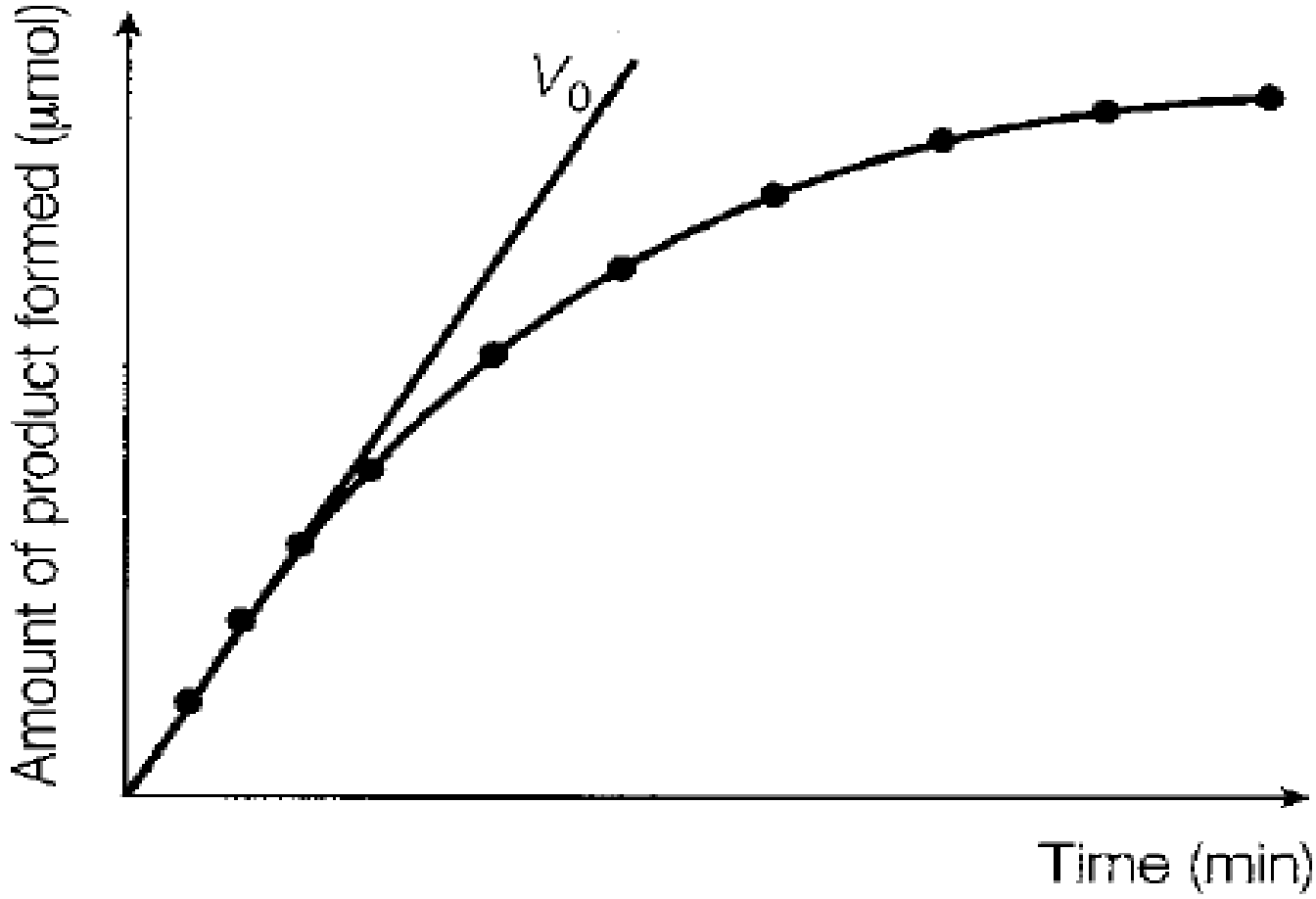
International System of Nomenclature is to Standardized and Rationalize Names of Enzymes:

- Enzymes are place 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 (**Vo**);
  - **Initial Velocity (Vo)** and is expressed as **μmol/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 {Vo = 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 (Vo)** 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 **Vo**

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

- An enzyme unit (U) is the amount of enzyme that catalyzes the transformation of 1.0μmol of Substrate per minute at 25°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 Vo is directly proportional to the concentration of the Enzyme,
- Increasing the amount of Enzyme increases Vo;
- Straight-line graph is obtained when Vo is plotted against Enzyme concentration;

How does [S] affect velocity of enzyme reaction?

- When Vo is plotted against [S] an 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 Vo,
  - It is a “First-order” reaction;
- **At higher [S]**:
  - Enzyme becomes saturated,
  - Increase in [S] lead to very small changes in Vo,
  - It is a “Zero-order” reaction;

Fig 3a: Graph of [S] vs Vo

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

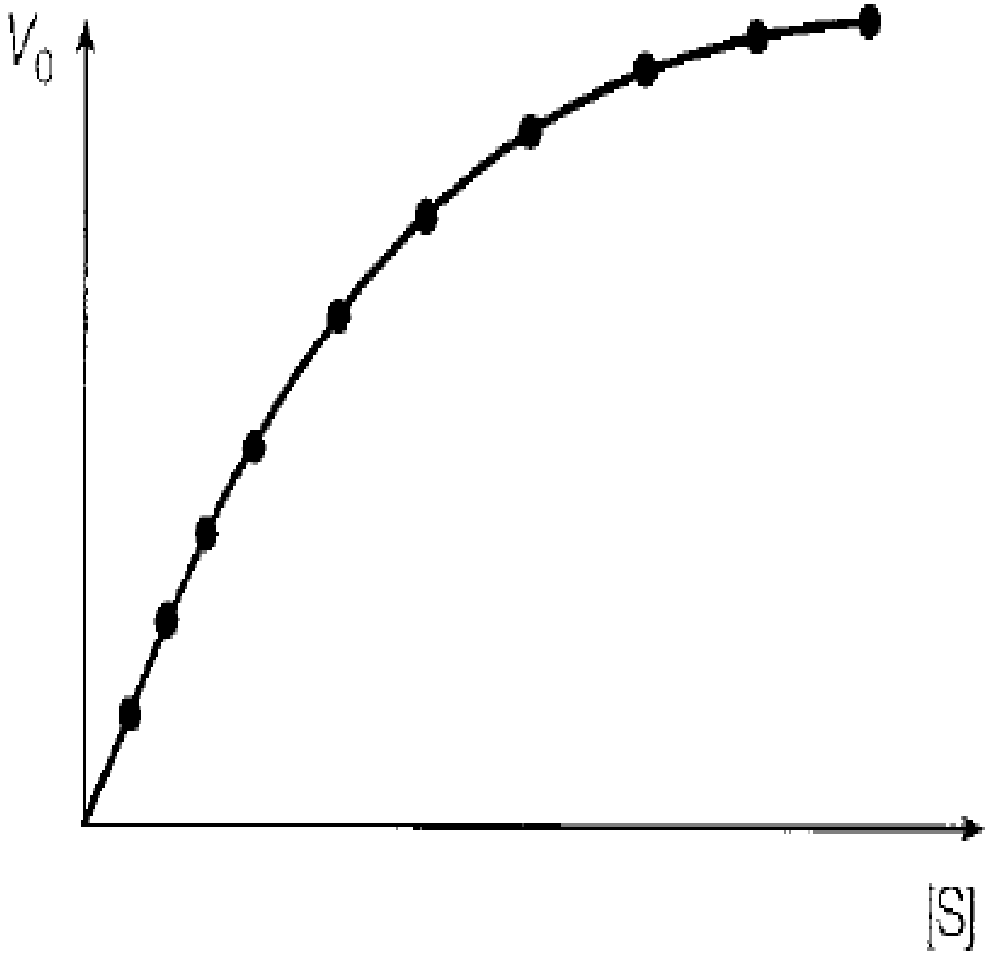
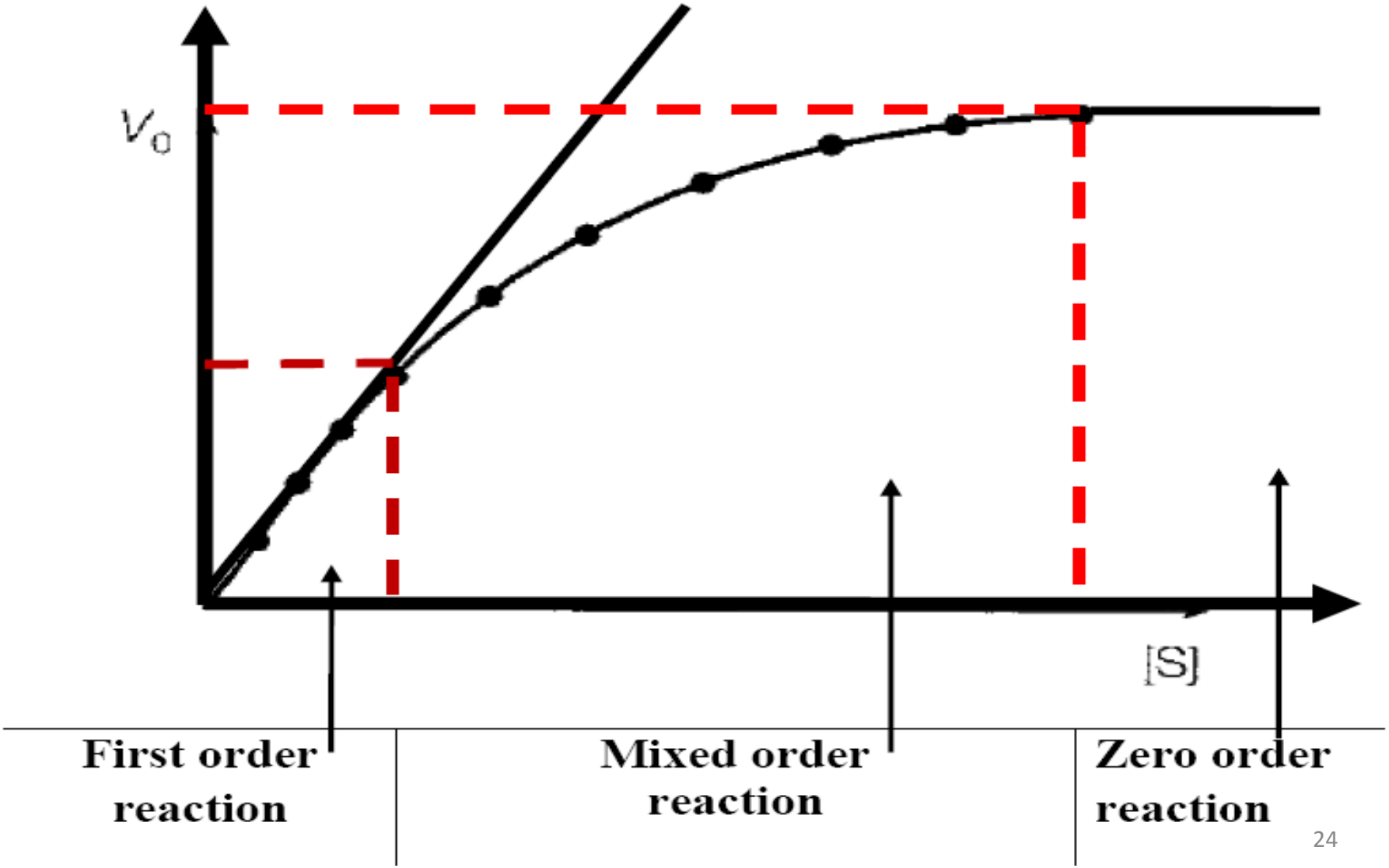


Fig. 3c: Graph of **[S]** against **Vo**; Diagram shows order of reactions: **First order, Mixed order, Zero order** with respect to [S]



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;

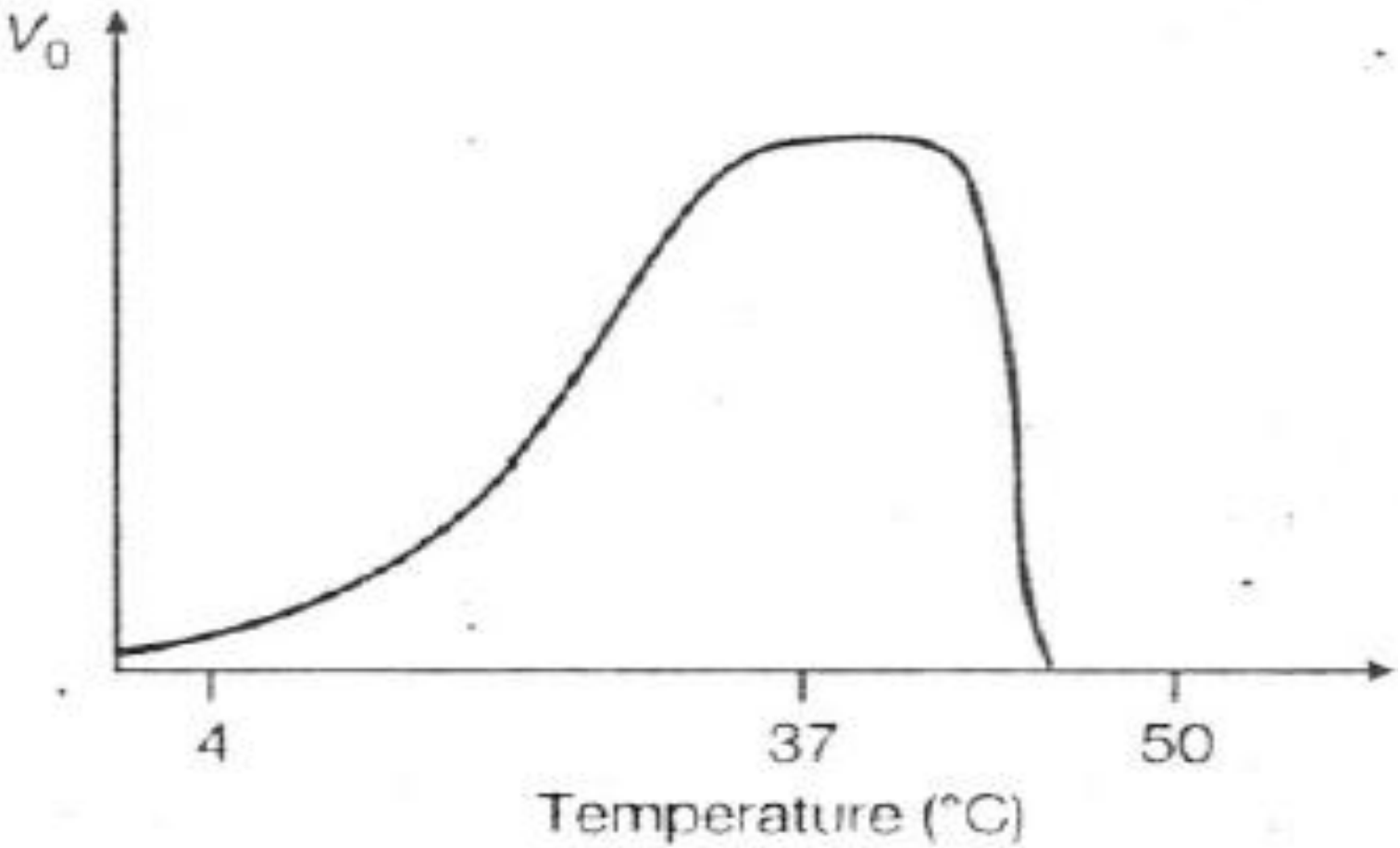
Fig 3b: Graph showing relationship between [S] and Vo: Graph can be separated into 3 sections:

- **At low [S]:** reaction is First-order with respect to **[S]**;
  - **Vo** is directly proportional to **[S]**
- **At mid [S]**: reaction is Mixed-order;
  - Proportionality of **Vo** to **[S]** is changing;
- **At high [S]**: reaction is Zero-order,
  - **Vo** is independent of **[S]**
  - Enzyme has its maximum velocity **(Vmax)**;
- Increasing **[S]** has no effect on **Vo**; 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 (Vo) 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 **Vo** gives a curve with well-defined optimum **T°C (Fig. 4a)**
- Temperature optimum: about 37°C,

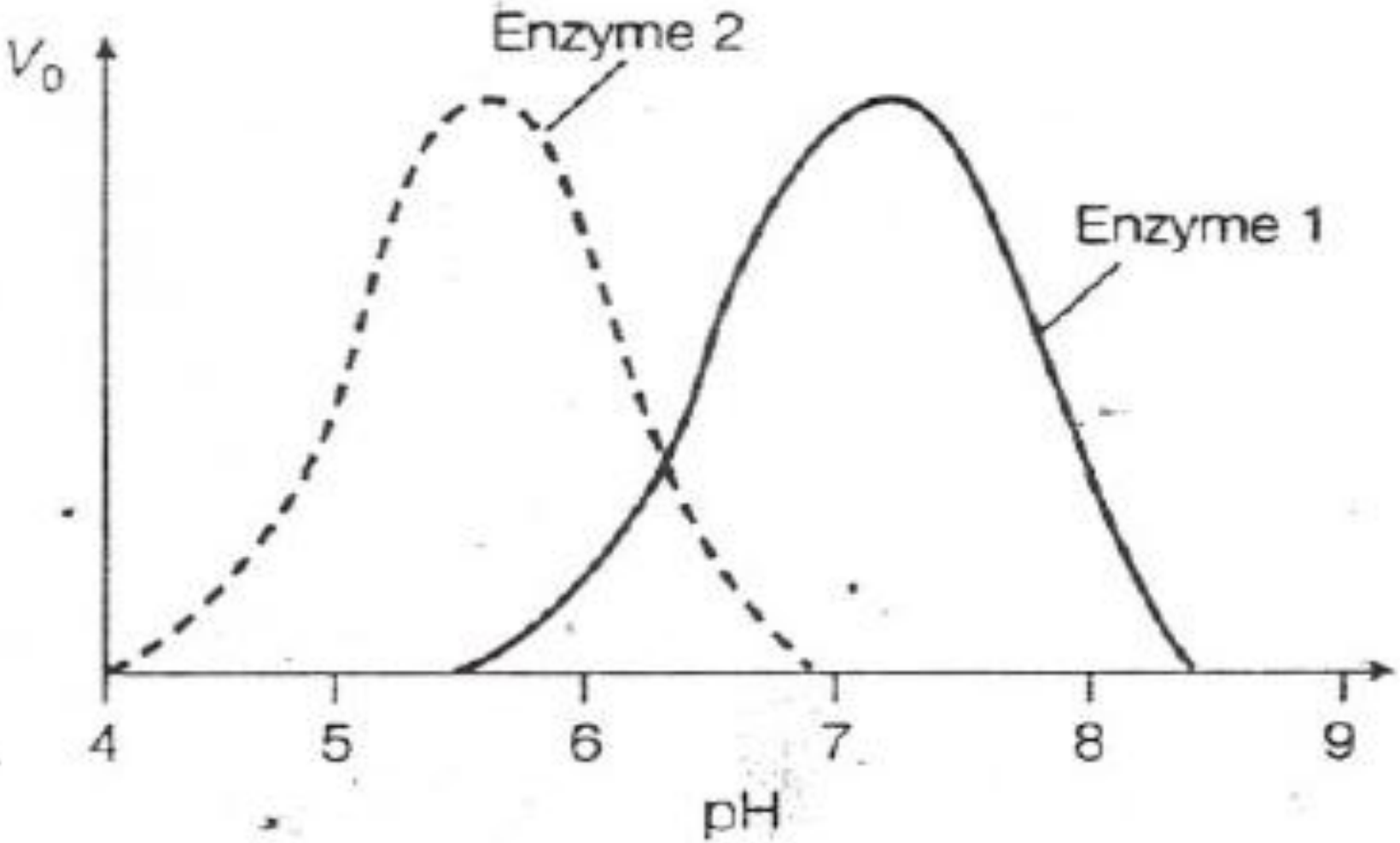
Fig. 4: Graph of **T** °C against **Vo** gives a curve with well-defined optimum **T°C**



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 Vo against pH for two different enzymes; Each graph is bell-shaped curve;



ENZYME KINETICS

- **General expression for an enzyme catalyzed reaction**:

E + S ⟵=====⟶ ES ======⟶ E + P

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

What does Michaelis-Menten equation represent?

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

What is the Michaelis-Menten equation?

Michaelis-Menten equation:

- Vo = Initial velocity,
- Vmax = Maximum velocity,
- [S] = Substrate conc.
- Km = 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 (Vo) of reaction must be used;

What is Km?

- **Km** is equal to the **[S]** at which the Initial velocity (**Vo**) is equal to **half** the Maximal velocity,

- That is: **Km = [S], when Vo = 0.5 Vmax**

- **Km** is a measure of the stability of **ES**-complex

How significant is Km?

- **Small value of Km**, means **high affinity** of the enzyme for the Substrate;
- **Large value of Km**, means **low affinity** of enzyme for the Substrate;
- **Km** is characteristic for a particular enzyme with a given substrate;
- **Km** is an important parameter in metabolic control in cells;
- **Km** 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 Km and Vmax 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) $$V_o = \frac{V_{max} \cdot [S]}{K_m + [S]}$$

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

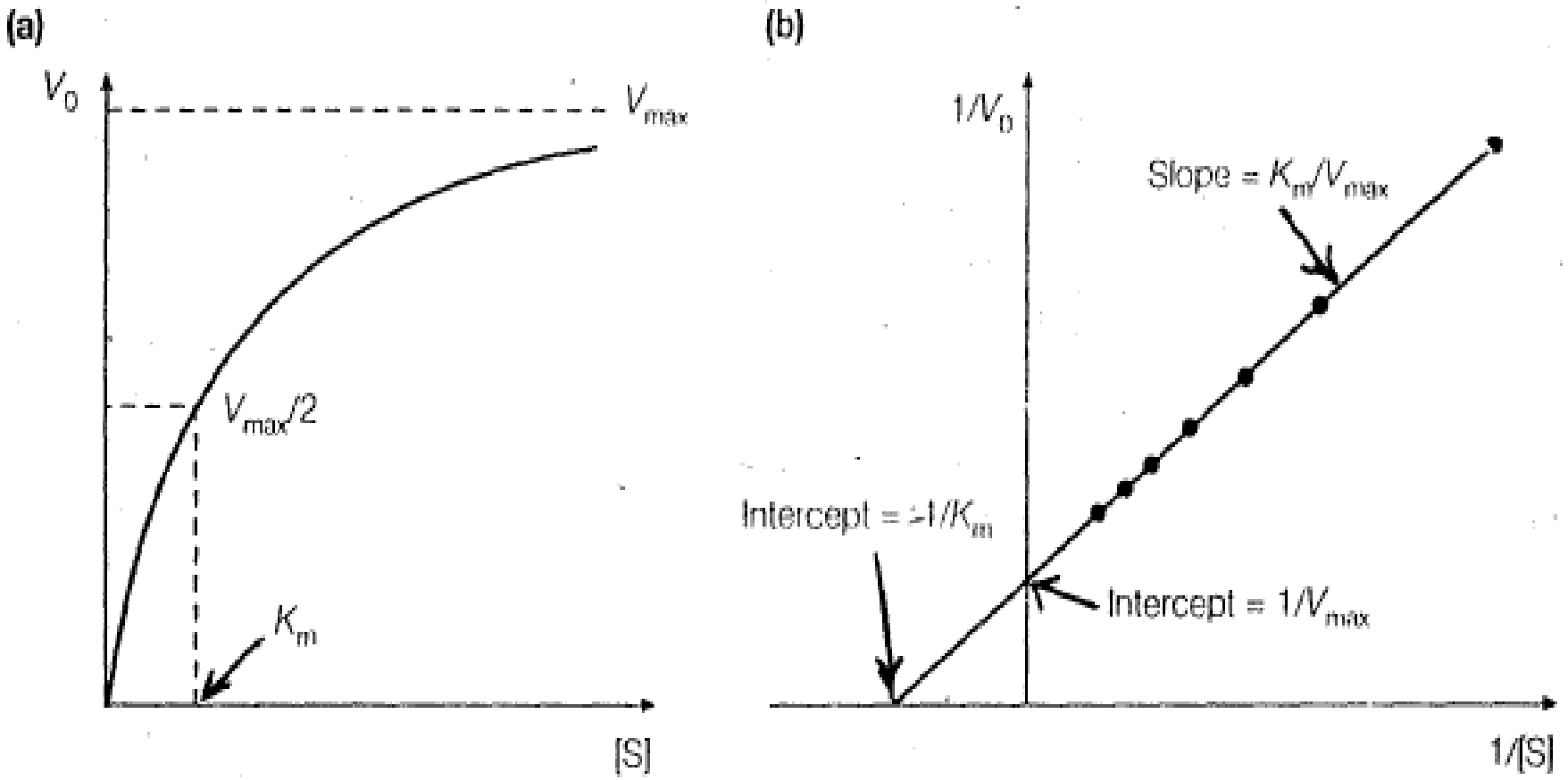
$$Y = mX + C$$

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

$$\mathbf{Y = MX + C}$$

- **Slope of graph: M = Km/Vmax,**
- **Intercept on Y-axis: C = 1/Vmax,**
- **Intercept on X-axis: when 1/Vo = 0, gives: – 1/Km**

Fig. 5a: Graph of [S] against Vo, show how to obtain Km;
Fig. 5b: Lineweaver-Burk plot (double-reciprocal plot) showing how to obtain Km



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]

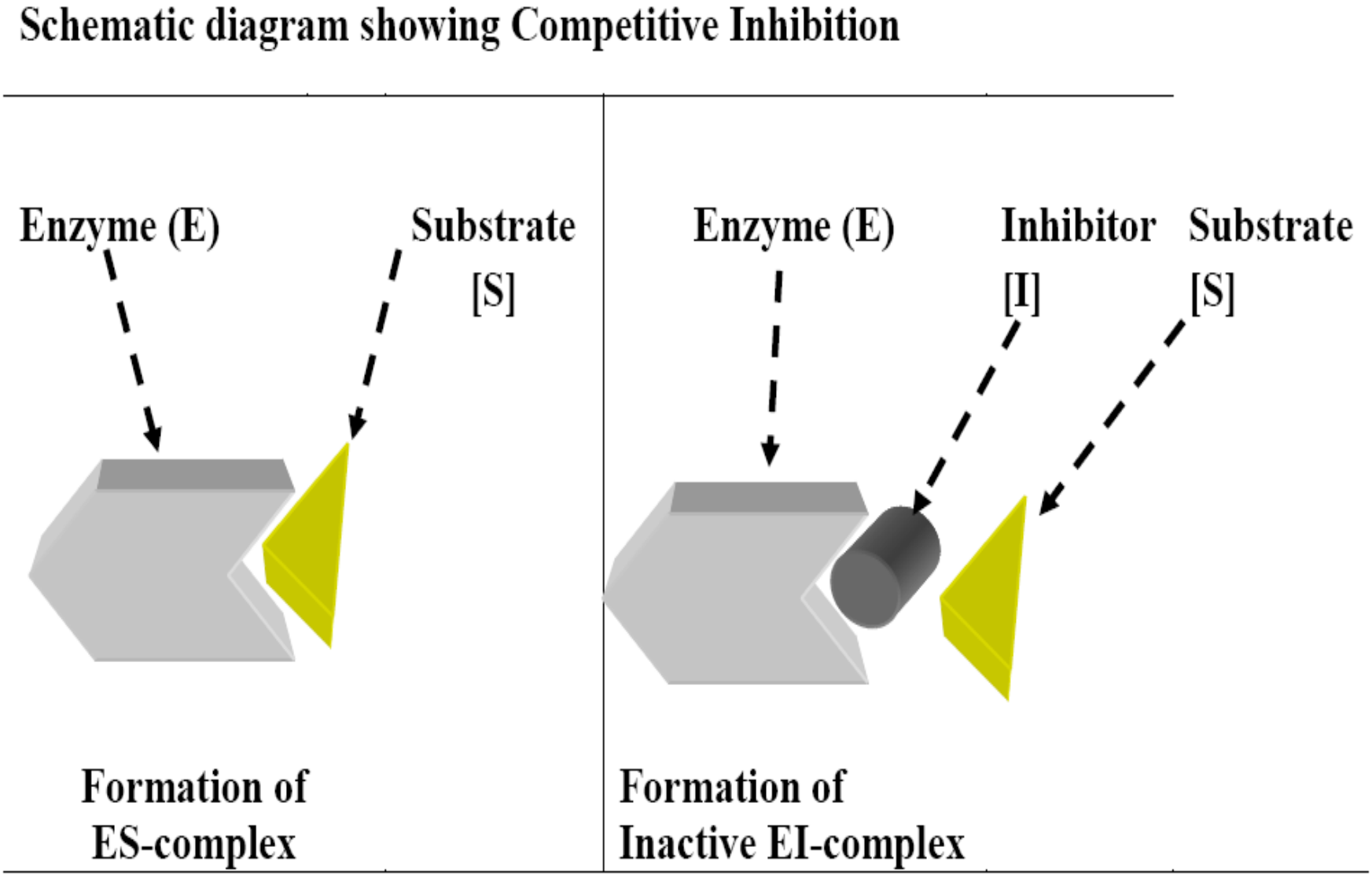
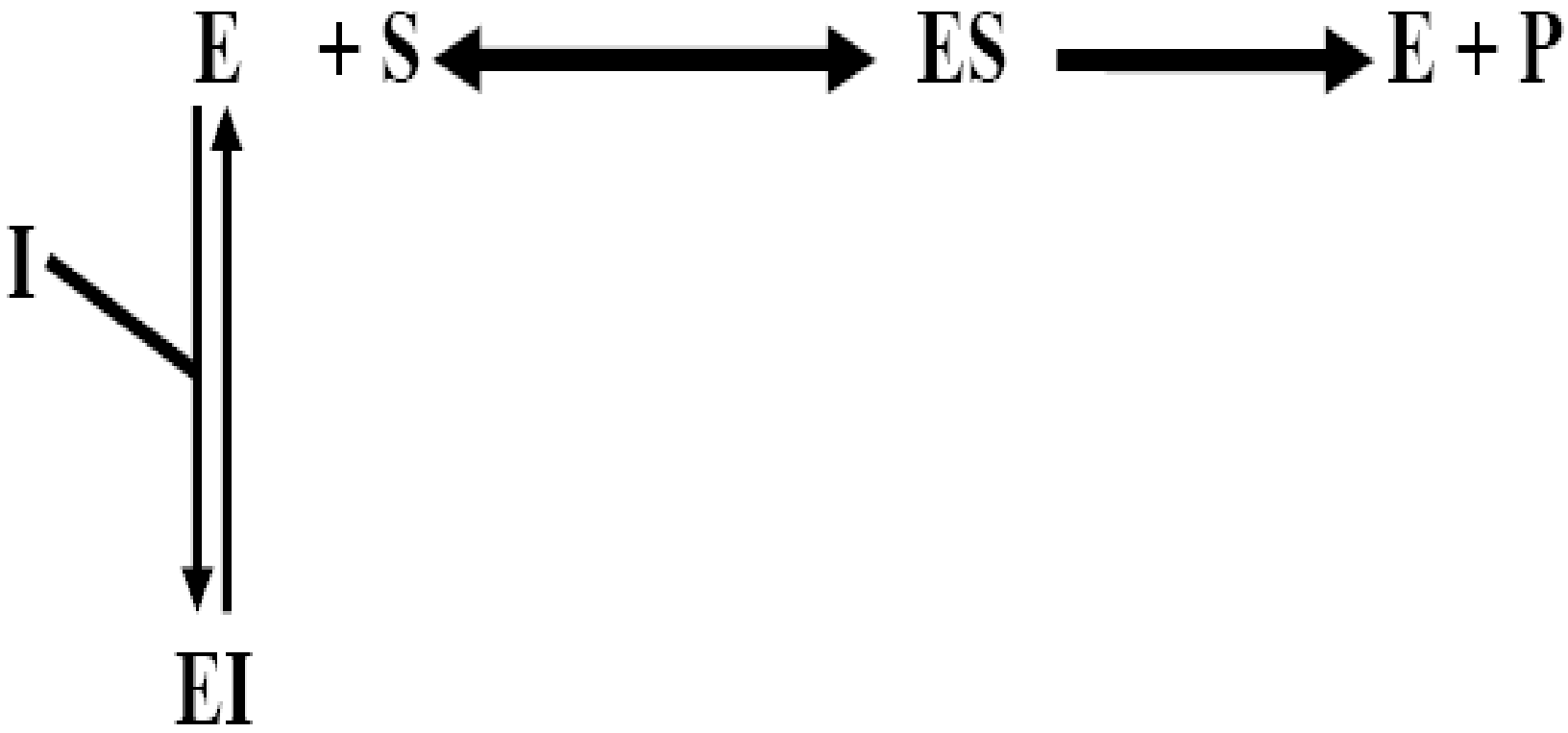


Fig. 6b: In competitive inhibition Enzyme (E) can bind to either Substrate [S] or Inhibitor [I]; it cannot bind to both at the same time.



Give examples of Competitive Inhibition

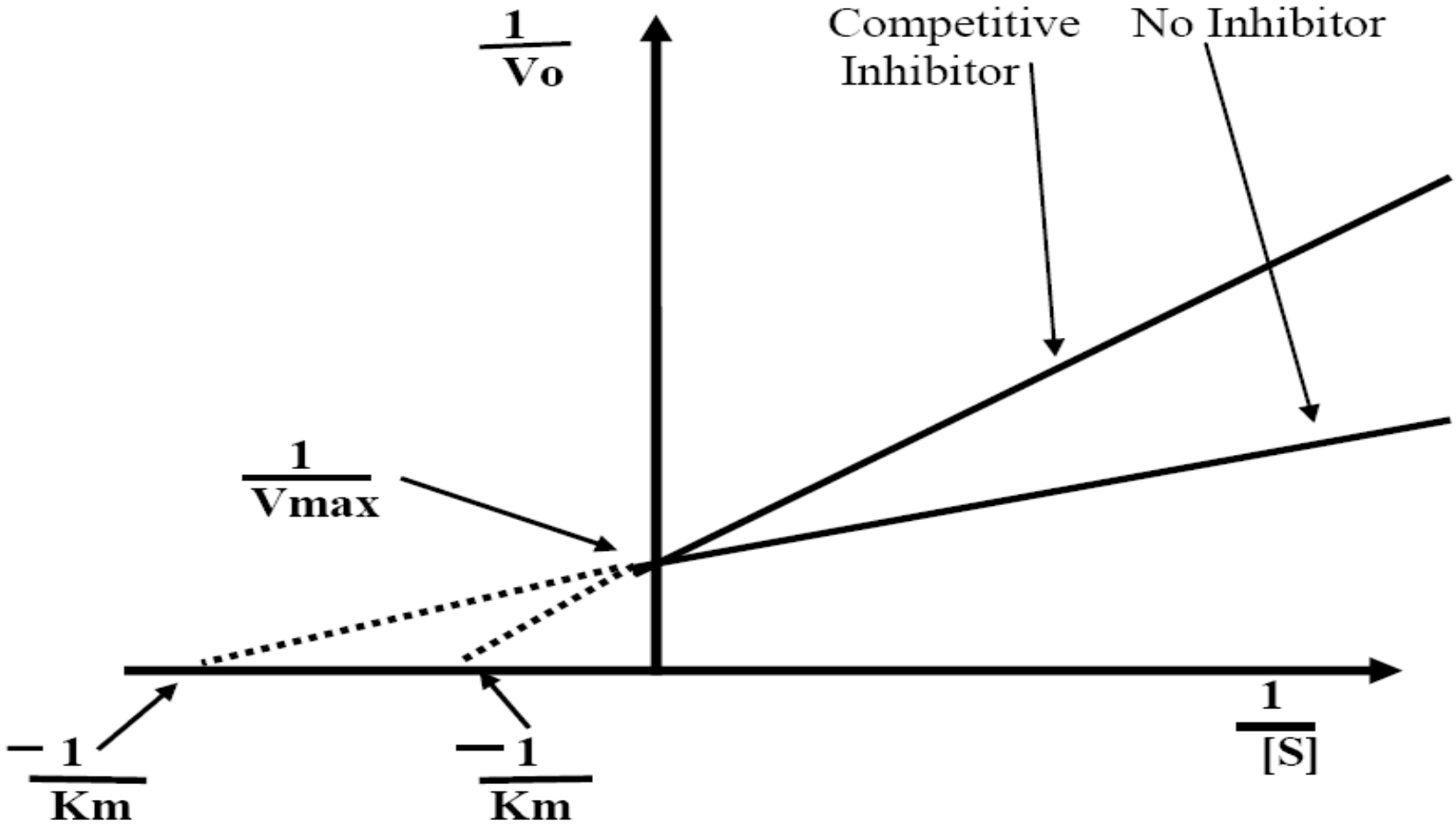
- Sulfanilamide competes with Para-Aminobenzoic Acid (PABA) in reaction catalyzed by **Dihydropteroate Synthetase** in the biosynthesis of Folate (Folic Acid);

- Methotrexate competes with Dihydrofolate in reaction catalyzed by **Dihydrofolate Reductase** (Methotrexate is structural analogue of Folate)

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

- Measure **Vo** 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 Vmax** of the Enzyme,
  - The **Km of the inhibited Enzyme increases**,
- Competitive inhibitor **increases** the **Km of an enzyme for its substrate**;

Fig. 7: Diagram of Lineweaver – Burk (Double-Reciprocal) plot showing effect of Competitive Inhibitor on Km and Vmax of an Enzyme.



Km of Enzyme with no Inhibitor	Km of Competitively Inhibited Enzyme (Km 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

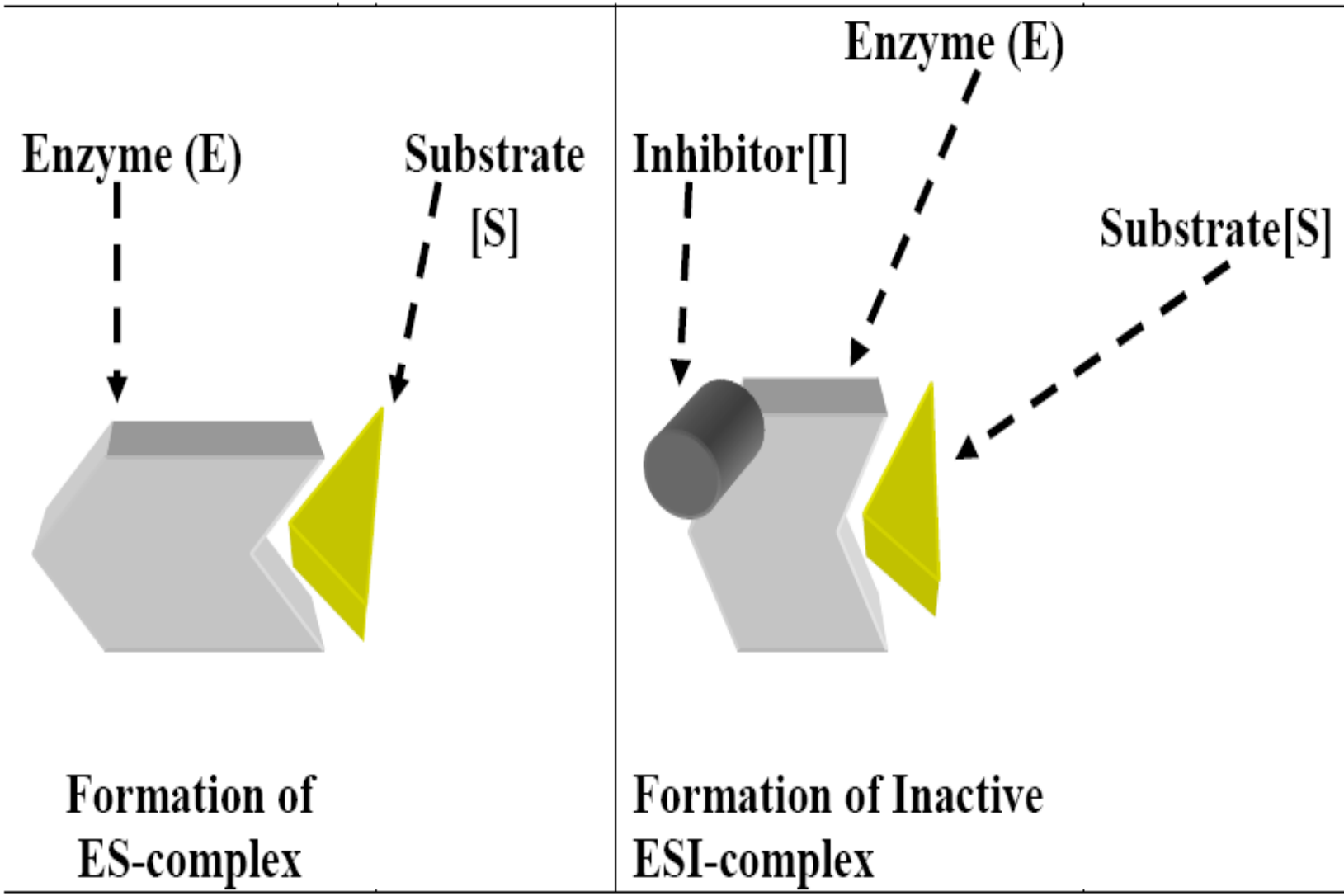
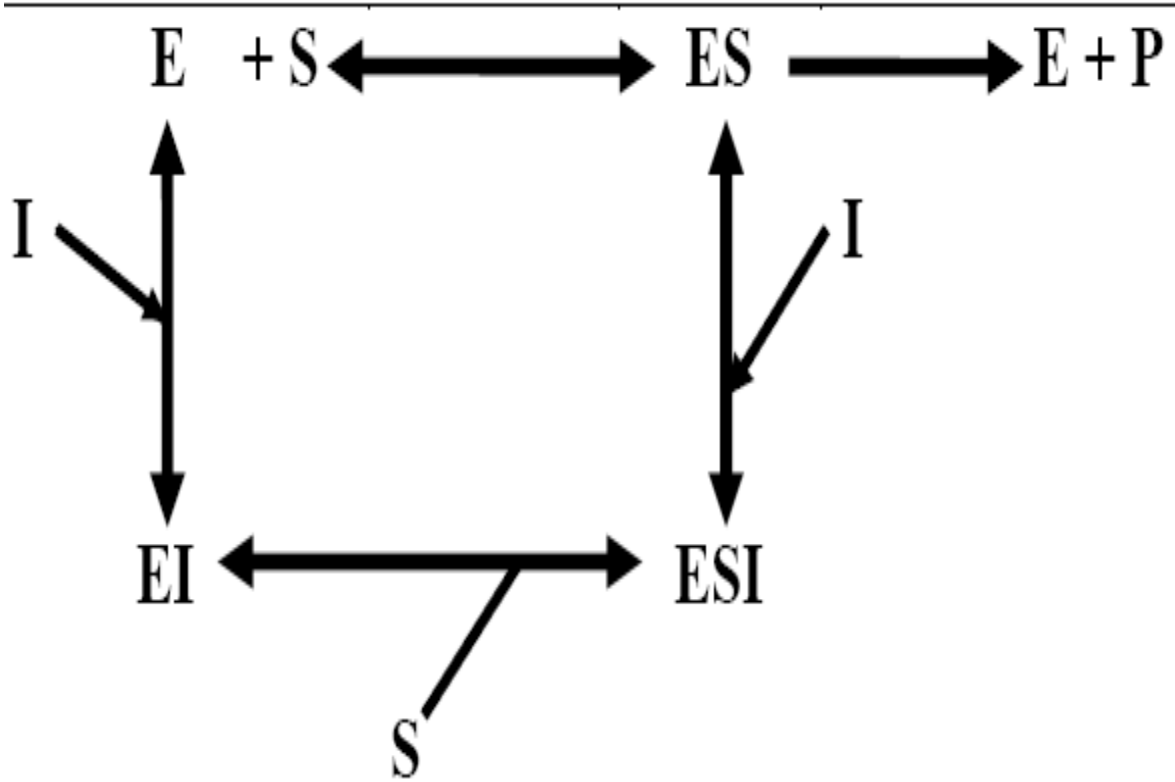


Fig. 8b:

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.

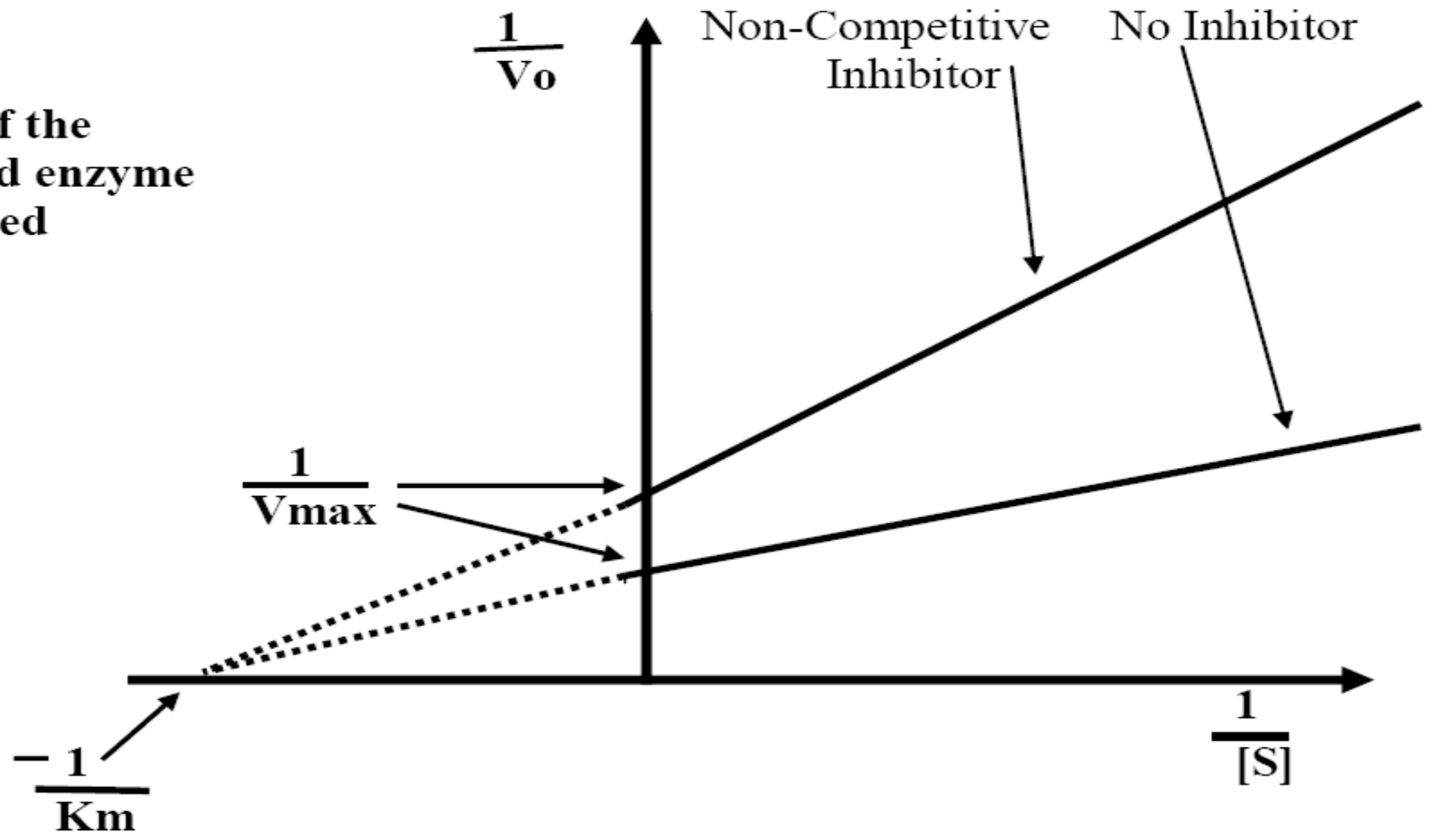


How can Michaelis-Menten constant be determined for Non-Competitive Inhibition of an enzyme?

- Measure **Vo** 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:
  - **Km** is not affected, thus affinity of the enzyme for Substrate in unchanged;
  - **Vmax** of inhibited enzyme is decreased;

Fig. 9:

Diagram of Lineweaver – Burk (Double-Reciprocal) plot showing effect of Non-Competitive Inhibitor on Km and Vmax of an Enzyme.



Km of Enzyme with no inhibitor and with non-competitive inhibition; Km 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 (Km).;
- How is Lineweaver-Burk Plot used to determine Km and Vmax 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 Km and Vmax of enzymes?
- List three irreversible inhibitors and the enzyme or enzymes affected by each;
- List five enzymes that are used for diagnosis;

REFERENCES

- Textbook of Biochemistry, with clinical correlations, Ed. By T. M. Devlin, 4th Edition.
- Harper's Illustrated Biochemistry 26th Edition; 2003; Ed. By R. K. Murray et. al.
- Biochemistry, By V. L. Davidson & D. B. Sittman. 3rd Edition.
- Hames BD, Hooper NM, JD Houghton; Instant Notes in Biochemistry, Bios Scientific Pub, Springer; UK.
- VJ Temple Biochemistry 1001: Review and Viva Voce Questions and Answers Approach:; Sterling Publishers Private Limited, 2012, New Delhi-110 – 020.