Enzyme Kinetics 2018

Learning Objectives

▶ **Enzyme Kinetics**

▶ **Enzyme Inhibition**

▶ **Drugs utilizing kinetics and inhibition and its clinical utility**

Enzyme Kinetics

the quantitative measurement of the rates of enzyme-catalyzed reactions and the systematic study of factors that affect these rates

Catalysts

▶ Increase rate of reaction by factor of 10^6

▶ Highly selective and specific

▶ Not changed as a result of catalysis

▶ Does not change the equilibrium constant

▶ **Enzymes Alter Only the Reaction Rate**

   and Not the Reaction Equilibrium

Factors affecting reaction velocity

Substrate concentration

▶ The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached

Temperature

▶ **Bell shaped curve**

▶ Increase of velocity with temperature

▶ Decrease of velocity with higher temperature

pH

- **Bell shaped curve**

- Effect of pH on the ionization of the active site
- Effect of pH on enzyme denaturation
- Variable pH optimum

Kinetic Order of Reaction

- Sum of the molar ratios of the reactants defines the **kinetic order** of the reaction
- First order
- Second order
- Pseudofirst order reaction

Michaelis Menten Equation

$$Vo = \frac{V_{max}\,[S]}{Km + [S]}$$
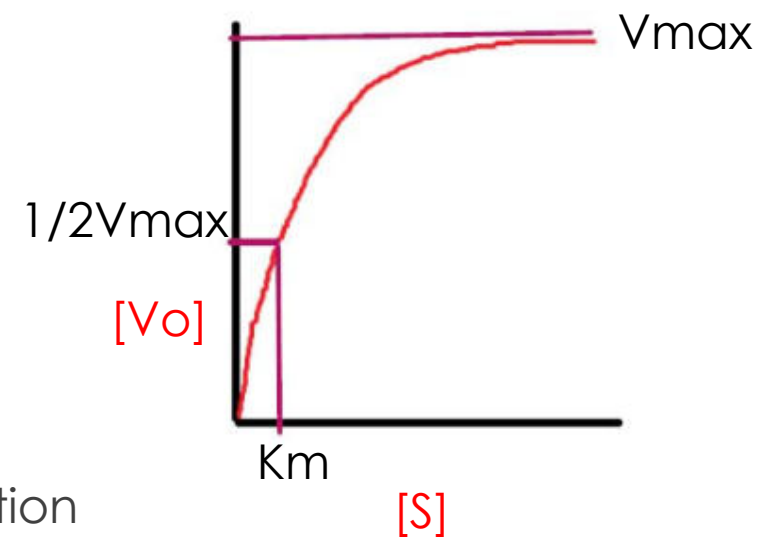
Relationship between initial velocity and substrate concentration

Km and its importance

▶ The Michaelis constant **Km** is the **substrate concentration**
at which **Vi** is half the maximal velocity **(Vmax/2)** attainable
at a particular concentration of the enzyme.

Unit?

▶ Reflects the **affinity of the enzyme** for that substrate:
   inverse relationship

▶ Specific for enzyme substrate combination

▶ **Order of reaction**

Clinical importance of Km

▶ **Hexokinase vs Glucokinase**

Difference between Hexokinase and Glucokinase

	Hexokinase	Glucokinase
Substrate specificity	All hexoses	Mainly Glucose
Km	Low (high affinity) *Works at normal glucose concentration*	High (low affinity) *works only when glucose levels are elevated*
Location	Universal	Mainly liver and Beta cells of pancreas
Vmax (rate of reaction)	Low	High
Glucose-6-PO4 (Allosteric inhibition)	Inhibits the enzyme	No inhibition
Insulin	No regulation	Positive regulation

Line-Weaver Burk plot

▶ **Double Reciprocal Graph**

▶ **Linear curve**

▶ $$\frac{1}{V_0} = \frac{K_m}{V_{max}\,[S]} + \frac{1}{V_{max}}$$

Linear form of Michaelis menten equation to Determine Km and Vmax.

Units of Enzyme activity

▶ Amount of substrate converted to product per unit time under standard conditions of pH and Temperature

▶ **IUB unit**: Katal (μmol/min)

▶ **SI Unit:** (mol/sec)

Relative activities of Enzymes

▶ **Specific activity (Vmax/protein concentration):** Impure Enzymes

▶ **Turnover number**(**Vmax/moles of enzyme**): Homogenous Enzymes

▶ **Catalytic constant, Kcat [Vmax/No. of active sites(St)]: unit time^{-1}**

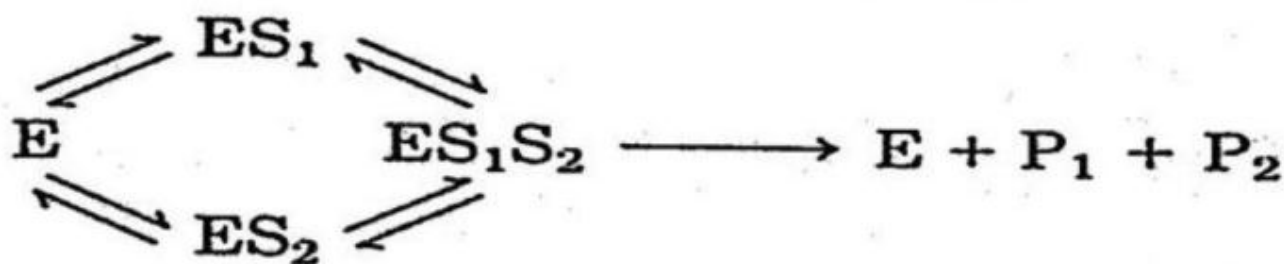▶ **Catalytic efficiency: Kcat/Km** (Carbonic anhydrase, ADA, acetylylcholinesterase)

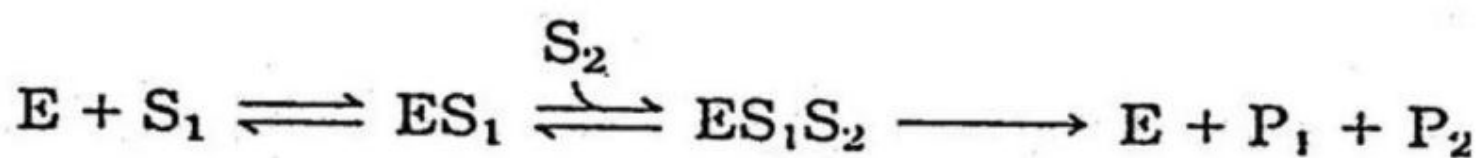# Two substrate Reactions(Bi Bi Reactions)

- **Sequential   :   Random**

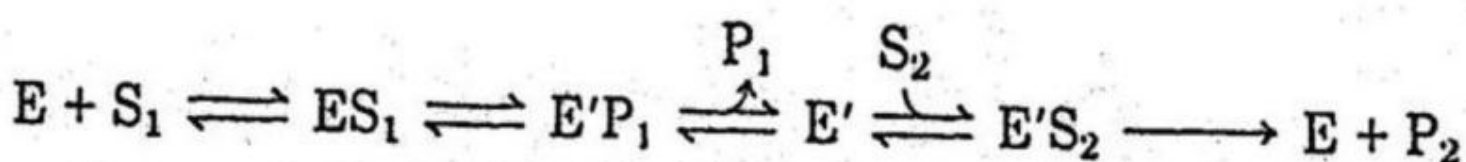   **Ordered**

- **Ping Pong: Double displacement reactions**

Two substrate Reactions

$$E \underset{\displaystyle ES_2}{\overset{\displaystyle ES_1}{\rightleftharpoons}} ES_1S_2 \longrightarrow E + P_1 + P_2 \qquad \text{Random}$$

$$E + S_1 \rightleftharpoons ES_1 \overset{S_2}{\rightleftharpoons} ES_1S_2 \longrightarrow E + P_1 + P_2 \qquad \text{Ordered/Sequential}$$

$$E + S_1 \rightleftharpoons ES_1 \rightleftharpoons E'P_1 \overset{P_1}{\rightleftharpoons} E' \overset{S_2}{\rightleftharpoons} E'S_2 \longrightarrow E + P_2 \qquad \text{Ping Pong/Double Displacement}$$

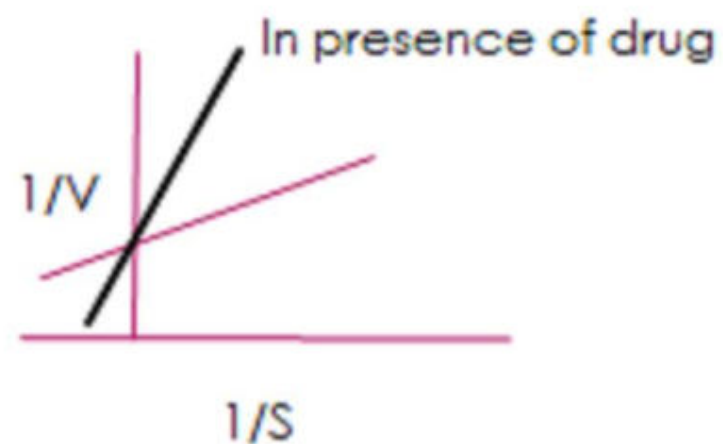# Enzyme Inhibition

Types of Inhibitions based on kinetics

- ▶ Competitive Inhibition

- ▶ Non-Competitive Inhibition

- ▶ Un-Competitive inhibition

Competitive Inhibition

- Binding at substrate binding site
- Inhibitor similar to substrate
- Km increased
- Vmax same

Clinical Application/Drugs

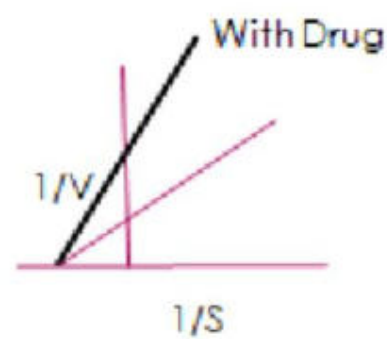- **Statin Drugs**

Competitive Inhibitors of HMG CoA reductase

- **Sulpha Drug (Str. Analogues of PABA)**

Inhibits Folic acid synthesis in Bacteria

- **Methanol Poisoning**

Non-Competitive Inhibition

► **Substrate and inhibitor binds at different sites**

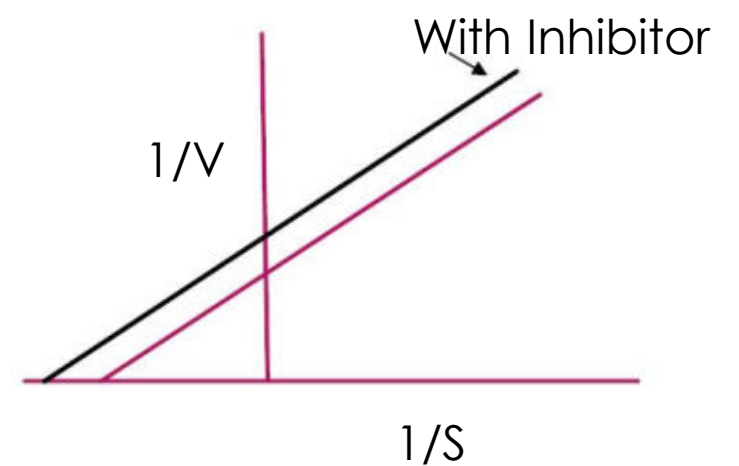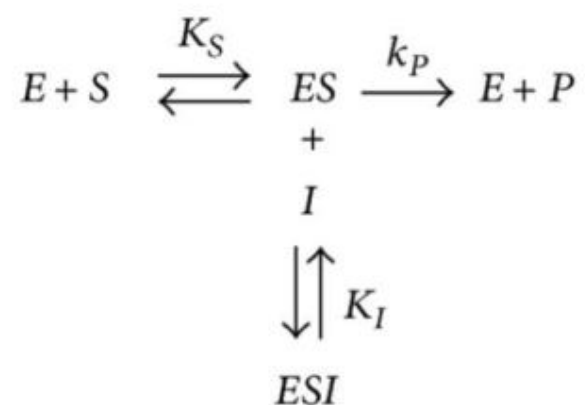► **Not structural analogues**

► **Decrease Vmax**

► **Km same**

Drugs/Toxins based on Non - Competitive Inhibition

► Ferrochelatase (Inhibition by **Lead**)

► Acetylcholinesterase (**Insecticides**)

► Cytochrome oxidase(**Cyanide**)

Uncompetitive Inhibition

▶ Inhibitor binds to ES Complex

▶ Both Km and Vmax decreases

$$E + S \underset{\longleftarrow}{\overset{K_S}{\longrightarrow}} ES \xrightarrow{k_P} E + P$$

$$\underset{ESI}{\overset{+}{\underset{I}{\big\updownarrow}}} K_I$$

1/V   With Inhibitor

1/S

Examples of Drugs showing Uncompetitive Inhibition

▶ Lithium (Inositol monophosphatase)

▶ Phenylalanine(Placental ALP)

Classification based on Reversibility

▶ **Reversible**

▶ **Irreversible**: Chemical modification or Covalent modification

Irreversible inhibitors Poison Enzymes

▶ **Diisopropylflurophosphate** (nerve gas): covalently binds acetylcholinestrase

▶ **Aspirin**(Cox)

▶ **Penicillin** (bacterial transpeptidase)

Mechanism Based Inhibition

▶ **Suicide Inhibition**

▶ Contains chemical group that is transformed by catalytic machinery

▶ Generates highly reactive group

▶ Binds covalently to catalytically essential residues

Drugs based on Suicide Inhibition

▶ **Allopurinol** (inhibits xanthine oxidase: Oxypurinol)

▶ **5 fluorouracil** (inhibits thymidylate synthase: FdUMP)

Transition state Analogs & Abzymes

▶ **Transition state analog: A molecule with shape similar to transition state**

▶ **Catalytic Antibodies**

▶ **Abzymes created using transition state analog as antigens**
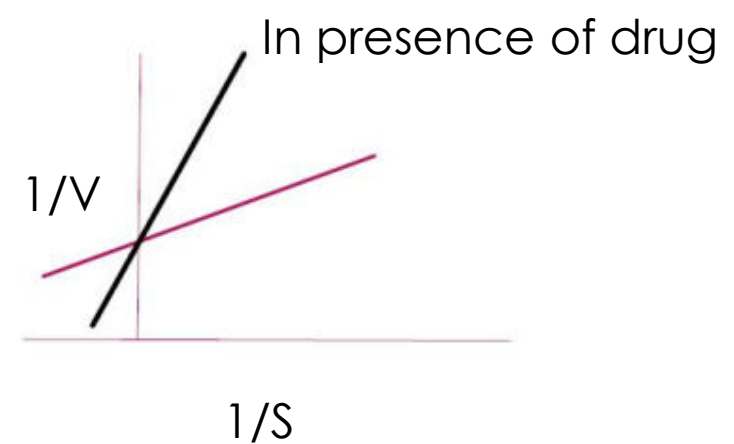
Clinical Scenario 1

A 45-year-old man presents to emergency with bradycardia, blurred vision, vomiting, increased and salivation. He is a farmer using OPC Spray for his field and pipe ruptured. Type of inhibition?

▶ **(A)** Competitive

▶ **(B)** Noncompetitive

▶ **(C)** Uncompetitive

▶ **(D)** Irreversible

Clinical scenario 2

▶ **A 35 year old lady comes to OPD with evening fatigue, eyelid drooping, dysphagia and slurred speech. A drug is administered with following effect. What is true**

a. Competitive: Vmax same, km increased

b. Competitive : Vmax same, km decreased

c. Non-competitive: Vmax decreased, Km same

d. Non-competitive: Vmax decreased, Km decreased

Clinical Scenario 3

A patient wants to go to Manali for trekking. He took a medicine for mountain sickness with following kinetics. What is the type of inhibition?

▶ **A.** Competitive

▶ B. Noncompetitive

▶ C. Uncompetitive

▶ D. Allosteric