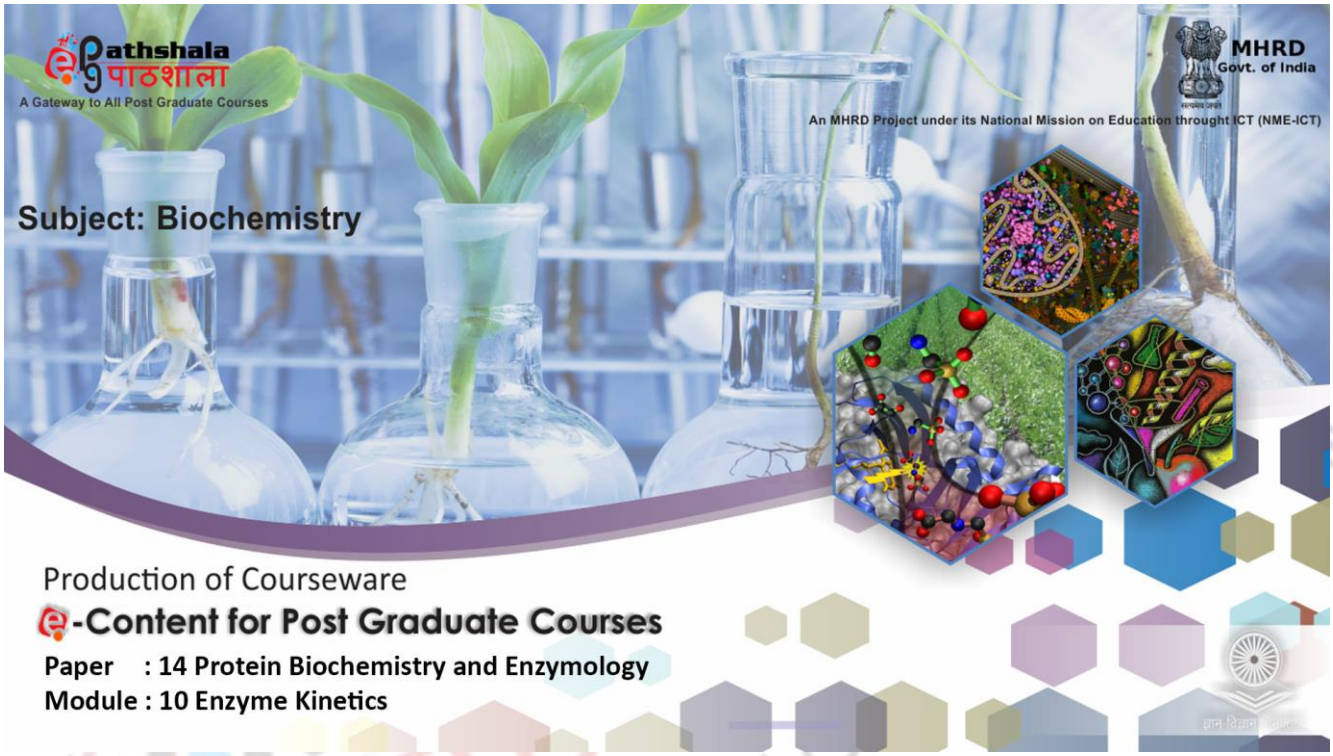

Production of Courseware

e-Content for Post Graduate Courses

Paper : 14 Protein Biochemistry and Enzymology

Module : 10 Enzyme Kinetics

Development Team

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Description of Module	
Subject Name	Biochemistry
Paper Name	
Module Name/Title	Enzyme Kinetics

1. Objectives

2. Enzymes as biological catalyst
3. Enzyme Catalysis
4. Understanding enzyme Kinetics
5. Equations for enzyme kinetics
6. Multisubstrate system
7. Enzyme Inhibition

2. Enzymes

Enzymes (proteins) catalyze a chemical reaction that takes place within a cell (but not always). All proteins, including enzymes are synthesized by ribosomes.

Enzymes are of primary importancein carrying out metabolic pathways, which otherwise would require high amount of energy (heat) in processing chains of chemical reaction, but enzymes (highly specific for substrate and reaction type)help to pull off these reactions at higher rate and at mild temperature and pressure.

An enzyme by joining with its specific substrate lowers the energy required for activation of that particular reaction (Activation energy). The reaction occurs, and the enzyme is released again unchanged to be used again.

3. Enzyme catalysis

CATALYST: it can be defined as any substance which performs its function by enhancing the reaction rate without itself being consumed as after the reaction completes, catalyst is now free for another reaction. A reaction which is driven by a catalyst is termed as catalyzed reaction, and the process is known as catalysis. Catalyst has certaincharacteristics described below:

1. It provides a different mechanism for the reaction and lowers the Gibbs energy of activation.

2. Initially an intermediate is formed with the reactant(s) by a catalyst which is released later on during the product formation step.

3. The catalyst cannot alter the thermodynamic equilibrium constant, which means that it is not able affect the enthalpies or Gibbs energies of both the reactants and products.

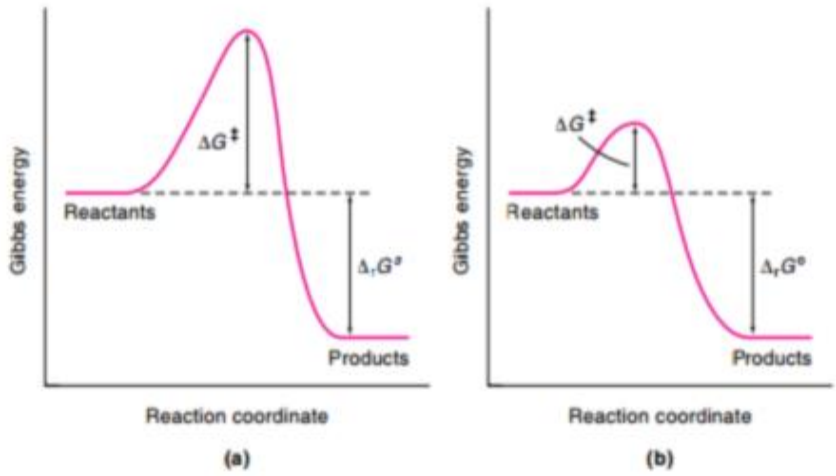


Fig.1 Gibb's energy in **(a)**Uncatlyzed reaction.**(b)**Catalyzed reaction.$\Delta_r G^o$ remains same in both cases.

Enzymes escalate chemical reaction without itself being chemically transformed and they also do not alter the equilibrium of the reaction. Alternative reaction pathways with lower energy barriers are introduced by enzymes for catalysis.***Catalytic Action***: Enzyme accelerates the rate of the reaction without shifting or changing its equilibrium, and in order to understand this "Transition state theory" (Eyring, 1935) was given which suggests, Reactants must overcome an energy barrier and pass through an activated complex before moving on to the reaction products.

A ⇌ B, can be designated by A⇌ X ⇌ B

here, X = transition state/activated complex

Enzyme introduces alternative pathways by following mechanism:

a.) Covalent catalysis: Catalytic functional group (nucleophile) attacks the substrate and forms covalent bond. Then electrons are withdrawn by an electrophilic catalyst resulting in rupture of the covalent bond, permitting further reaction and regeneration of enzyme based nucleophile. Eg. Decarboxylation of acetoacetate catalysed by primary amines.

b.) Acid base catalysis: A process involving partial proton transfer from an acid lowering the free energy of the transition state. A reaction is base catalysed, when the reaction rate is increased by partial proton abstraction by a base.Eg. Hydrolysis of peptide using chymotrypsin

c.) Proximity: The catalytic effectiveness is increased by bringing the substrate and catalytic groups closer (thereby increasing no. of collisions and reducing entropy. Eg. Imidazole-catalysed hydrolysis of esters, when the substrate and catalyst are brought in proximity to each other by hydrophobic interactions.

d.) Molecular distortion: The process in which the configuration of both enzyme and substrate, gets modified, upon binding of substrate is known as**induced fit.** Conformation of the substrate is changed in a way that it resembles the transition state; the conversion into the transition state is further facilitated by the stress created due to distortion of the substrate. The transition state is further stabilized by tight binding with enzyme and lowering activation energy requirement.

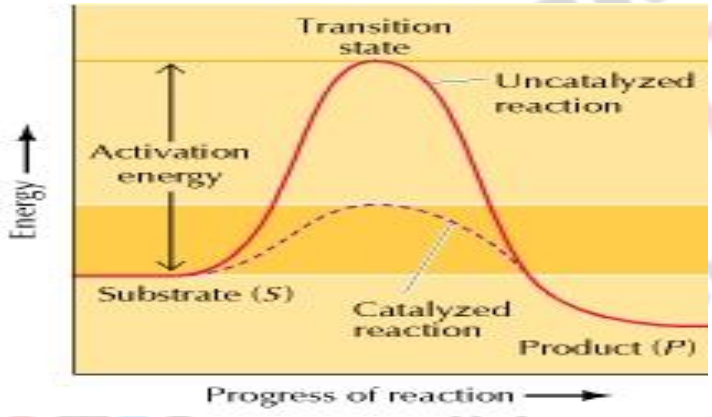


Fig.2 Energy diagram representing catalyzed and uncatalyzed reaction. In presence of catalyst (enzyme) the activation energy is lowered (compare the peaks of catalyzed and uncatalyzed reaction).

4. Enzyme kinetics

Enzyme kinetics, deals with enzyme reactions which are time-dependent and explains the mechanisms of enzyme catalysis and its regulation.

Let's understand enzyme kinetics as a function for the **concentration of the substrate** available for the enzyme.

- Start the experiment with a series of tubes which contains substrate, **[S]**.
- At time (t) zero, addsome amount of the enzyme.
- Wait for few minutes
- Then, measure the newly formed product concentration. We can also use spectrophotometer,If product absorbs light.

- At a time when the amount of substrate is greater than the amount of enzyme, then, the rate is the initial velocity of $\mathbf{V_i}$.

If we plot $\mathbf{V_i}$ as a function of **[S]**, following observations will be made:

- At low **[S]**, the initial velocity,$\mathbf{V_i}$, rises linearly with increasing **[S]**.
- When **[S]** increases, $\mathbf{V_i}$ settle down (rectangular hyperbolais formed).
- The asymptote shows $\mathbf{V_{max}}$ as the maximum velocity of the reaction.
- The substrate concentration which produces a $\mathbf{V_i}$ equal to one-half of $\mathbf{V_{max}}$ is called the Michaelis-Menten constant ($\mathbf{K_m}$**)**.

K_m is (approximately) inversely related to the maximum reaction velocity, or strength of binding between the enzyme and its substrate. Lower the K_m value, higher is the affinity for its substrate.

4.1 EQUATION OF ENZYME KINETICS

4.1.1 MICHAELIS MENTEN EQUATION

In 1913, Michaelis (1875–1949) and Menten (1879–1960, proceeded the work which was previously done by Frenchchemist V Henri (1872–1940), developed a mechanism to explain how the initial rate of enzyme-catalyzed reactions depends on the concentration.

Derivation of Michaelis-Menten equation: Few considerable assumptions can be made for the Michaelis-Menten equation derivation:

• Assuming that reverse reaction (P→ S) is negligible

• Assuming, there exists only a single central complex (ES). i.e. ES breaks down to E + P.
• Considering a situation when, [S] >> [E].Then the immediate interaction of S and E to form ES does not significantly affect free [S].
• Usually, ([S]-[ES])/[S] ≥ 99.9%

Considering the above assumptions, the reaction scheme is as follows:

Following are two parts for this reaction:
1) Formation of ES complex (a second order process)
2) The breakdown of ES complex to product P and free enzyme E (a first order process).

And the final Michaelis-Menten equation is as given below :

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

V_{max} = The maximum velocity achieved by the system, at maximum (saturating) substrate concentrations

K_M (the Michaelis constant)= substrate concentration at which the reaction velocity is 50% of the Vmax. Its unit is mM. It is also the concentration at which concentration of substrate is half the maximal velocity is observed.

[*S*] = concentration of the substrate *S*

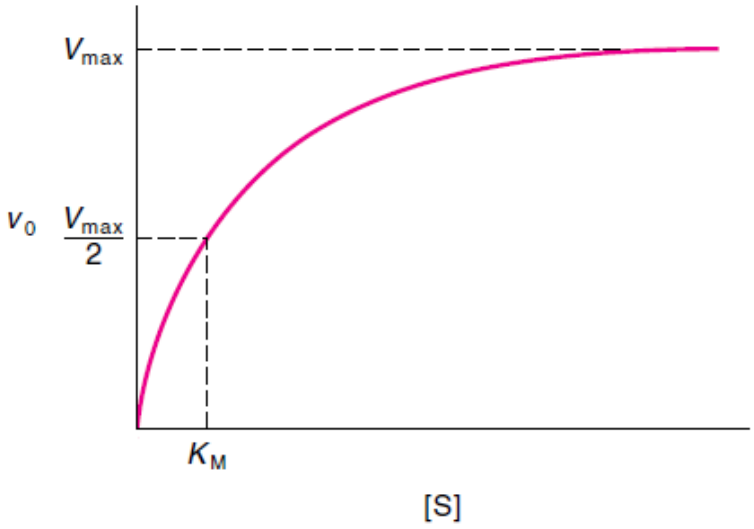


Fig 4.Graphical determination forV_{max} and K_M.

Line weaver burk plot

On the other hand, It is observed that in order to determine the value of V_{max} , the plot of V_0 vs. [S] is not useful since tracing the value of V_{max} under very high substrate concentrations is difficult. So, American chemists H. Line weaver and Burk employed the double-reciprocal plot. These are known as the **Lineweaver–Burk** plot. These are also called double-reciprocal plots.

$$\frac{1}{V_\circ} = \frac{K}{V_\circ[S]} + \frac{1}{V_{max}}$$

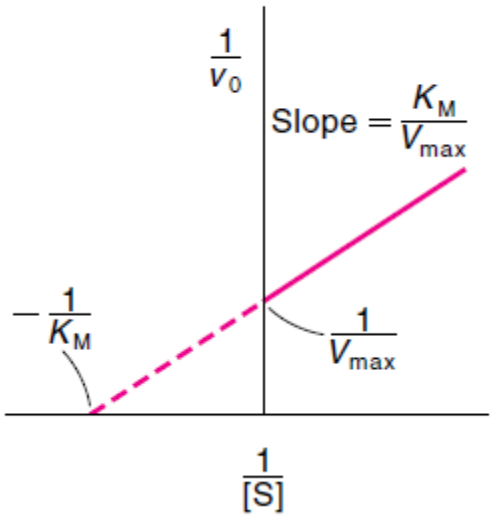


Fig 5 Lineweaver Burk plot

K_M and V_{max} is achieved from intercepts and slope of the straight line from graph.

One of the drawbacks of Lineweaver–Burk plot is that it is more susceptible to error. Another disadvantage is that at high substrate concentrations, the plot tends to compresses the data points into a small region and puts more emphasis on the points at lower substrate concentrations, which are often the least accurate.

Eadie–Hofstee plot

To overcome the drawbacks of Lineweaver Burke Plot, the **Eadie–Hofstee plot** was given.

Multiplying both sides of Michaelis-Menten equation by Vmax, we get,

$$v_\circ = -K_m \frac{V}{[S]} + V_{max}$$

The above equation represents a plot of $v_\circ$ versus $v_\circ$/ [S], called Eadie–Hofstee plot.

The plot is a straight line with slope equal to $-K_M$ and intercepts Vmax on the $v_\circ$ axis and V_{max}/K_M on $v_\circ$/[S] axis.

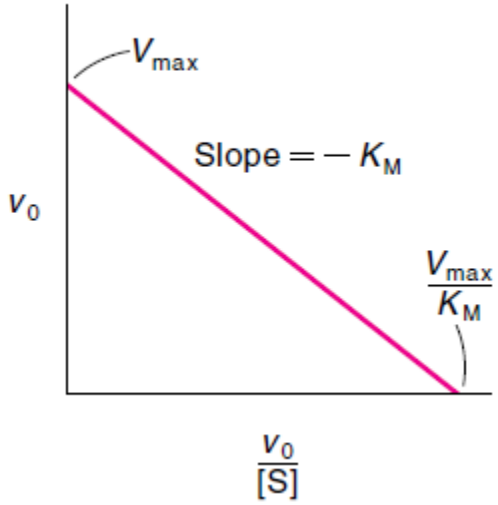


Fig.6 Eadie-Hofstee plot

SIGNIFICANCE OF K_M and V_{max}

In most cases, K_M value for enzymes lies between 10^{-1} and 10^{-7} . Value of K_M depends on particular substrate, and environmental conditions like ionic strength, pH and temperature.

The Michaelis constant, K_M has following meanings:

- It is the concentration of substrate at whichhalf ofthe active site gets occupied. Therefore, K_M provides a measure of the substrate concentration which is required for the significant catalytic process.
- f_{ES} refers to the fraction of sites filled. At any substrate concentration, f_{ES} can be measured when the K_M is known, from the following equation:

$$f_{ES}=\frac{v}{V_{max}}=\frac{[S]}{[S]+K_M}$$

K_M is described as $(k_{-1} + k_2)/k_1$. Considering a limiting case when, **$(k_{-1} >> k_2)$**, the ES complex breaks down to E and S much more rapidly than product formation. Under these circumstances:

$$K_M = \frac{k_{-1}}{k_1}$$

Dissociation constant of ES complex :

$$K_{ES} = \frac{[E][S]}{ES} = \frac{k_{-1}}{k_1}$$

K_M represents the affinity of the ES complex, only when k_{-1} is much greater than k_2.
High K_M implies weak binding, whereas a low K_M implies strong binding.
V_{max} gives the **turnover number** of an enzyme. By turnover number, it is meant the amount and number of substrate molecules that are converted into product by an enzyme molecule in a unit time, when the enzyme is fully saturated with substrate. It is equal to the kinetic constant k_2, which is also called k_{cat}. V_{max}, explains the turnover number of an enzyme if the concentration of active sites $[E]_T$ is known :

$$V_{max} = k_2 [E]_T$$

$$\text{hence, } k_2 = V_{max} / [E]_T$$

5. Multisubstrate System:

Until now, wehave studied single substrate enzyme catalysis, but in few instances, the process involves two or multi-substrates. Eg.

$$C_2H_5OH + NAD^+ \leftrightarrow CH_3CHO + NADH + H^+$$

The above reaction is alcohol dehydrogenase catalyzed. Alcohol dehydrogenase enzyme binds to both NAD+and the substrate which will be oxidized.

6. Enzyme Inhibition:

Inhibitors are a class of compounds which decrease or reduce the rate of an enzyme-catalyzed reaction. By studying enzyme inhibition, researchers have understood the nature of functional groups at the active site and the mechanism of specificity. Enzyme activity is usually regulated by the phenomenon called feedback mechanism where the end product is responsible for inhibiting the enzyme's activity. Infact, the amount of products formed is controlled by enzyme inhibition.
In irreversible inhibitions, inhibition gradually elevates with respect to time. When the irreversible inhibitor concentration exceeds the enzyme concentration, it results in complete inhibition.

Reversible Inhibition
Reversible inhibition can be of three types

- Competitive inhibition
- Non-competitive inhibition, and
- Uncompetitive inhibition

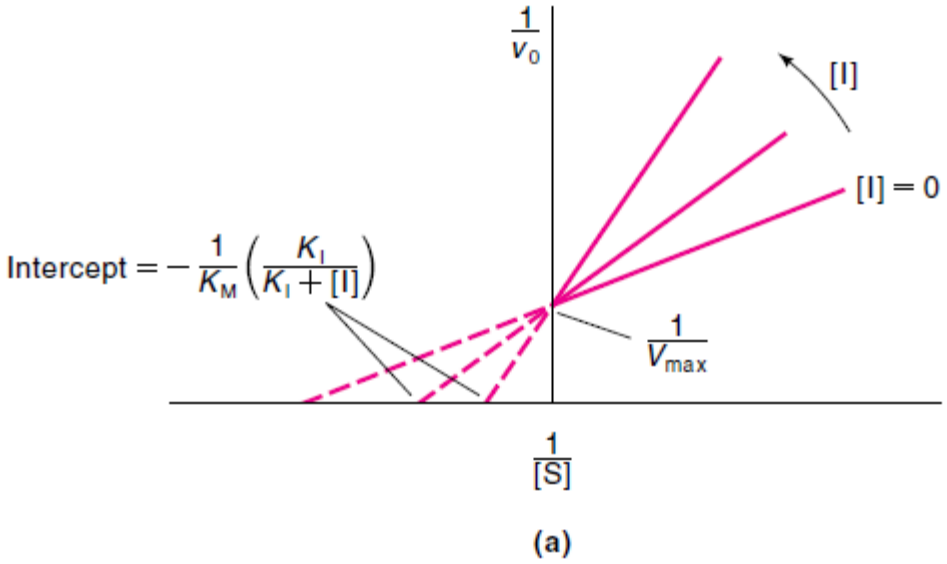
Competitive Inhibition: In this case, both the substrate S and the inhibitor I compete. Competitive inhibition can be reversed by increasing the concentration of the substrate.

$$E + S \underset{k_{-1}}{\overset{k_1}{<\!\!-\!\!>}} ES \xrightarrow{k_3} E + P$$

$$I + E \rightleftharpoons EI$$

The Line weaver Burk equation would be:

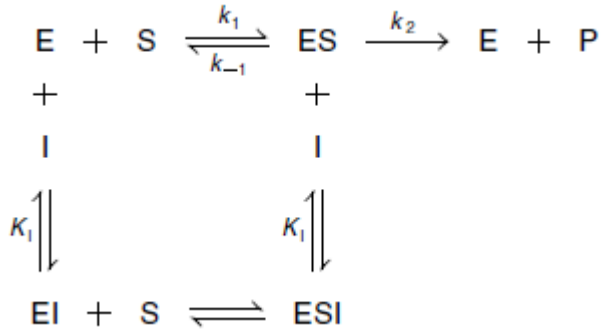
$$\frac{1}{v_\circ} = \frac{K_M}{V_{max}}\left(1 + \frac{I}{K_I}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$



(a)

Noncompetitive inhibition:

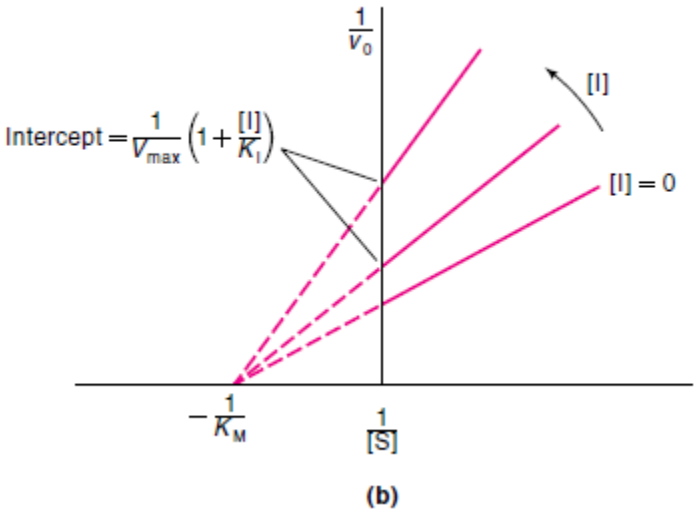
In non-competitive inhibition, the inhibitor may bind with both the free enzyme as well as the enzyme-substrate complex. But the inhibitor binds with enzyme at a site which is distinct from the substrate binding site. The binding of the inhibitor however does not affect the substrate binding, and vice versa. The reactions are:



The line weaver burk equation would be:

$$\frac{1}{v_\circ} = \frac{K_M}{V_{max}}\left(1 + \frac{I}{K_I}\right)\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{I}{K_I}\right)$$

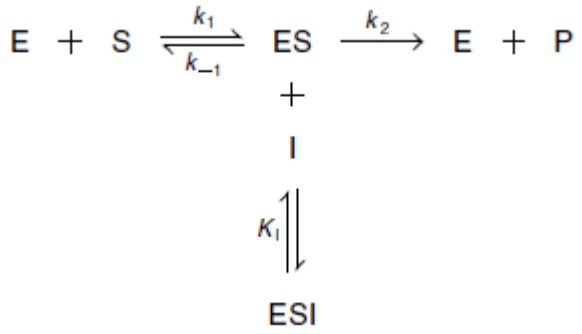
V_{max} reduced by 1+ [I] / K_I factor but K_M is unchanged



(b)

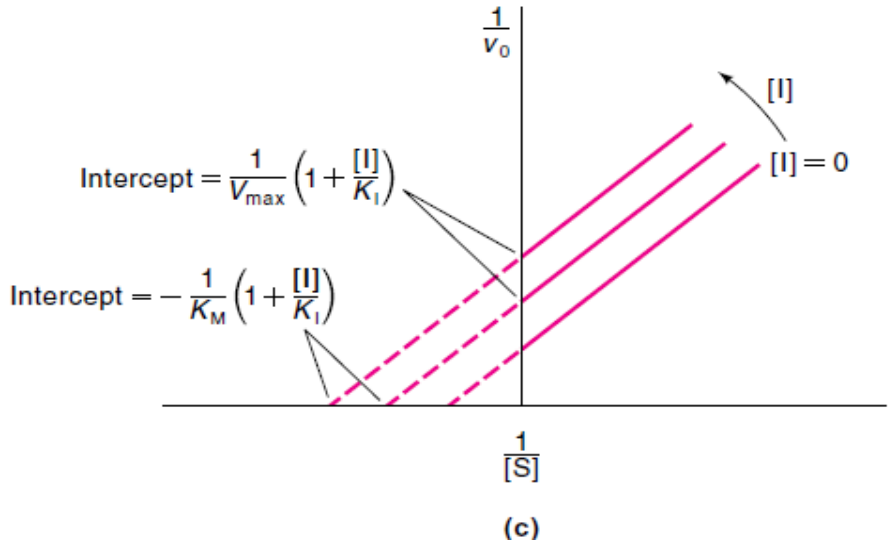
Uncompetitive Inhibition :

In Uncompetitive Inhibition, an inactive ESI complex is formed when Inhibitor reversibly binds to the enzyme–substrate [ES] complex. Here in this case inhibitor does not bind to the free enzyme.



Here in the above reaction, ESI complex does not form a product because 'I' does not interfere with the formation of ES. Again, unlike competitive inhibition, uncompetitive inhibition cannot be reversed by increasing the substrate concentration.

$$\frac{1}{v_\circ} = \frac{K_M}{V_{max}}\frac{1}{[S]} + \frac{1}{V_{max}}\left(1 + \frac{I}{K_I}\right)$$



(c)

FIG. 7 Lineweaver–Burk plots: (a) competitive inhibition, (b) noncompetitive inhibition, and(c) uncompetitive inhibition