# Enzymes

**Enzymes:**

are specific protein catalysts that increase the rate of reaction without being changed in the overall process. (Enzymes are not consumed during the reaction).

- Enzymes (E) act on certain substances which are known as substrate (S) ⟶ they form an enzyme – substrate complex (ES) which is broken to give the product (P).

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

# Nomenclature of enzymes:

- Each enzyme is assigned two names:

## A) Recommended name:

i) Many enzymes have been named by adding the suffix ‘ase’

a) to the name of the substrate

(e.g., Maltose $\xrightarrow{\text{Maltase}}$ Glucose + Glucose

b) or to a word describing their action or activity.

ii) Some enzymes given trivial names.

(e.g., pepsin, trypsin)

**B) Systematic name:**

The international union of Biochemistry developed a system of nomenclature.

i) This system divide enzymes into six major classes:

1. Oxidoreductases
2. Transferases
3. Hydrolases
4. Lyases
5. Isomerases
6. Ligases

ii) The enzyme name has 2 parts:

The first name the substrate or substrates.

The second, ending in –ase, indicates the type of reaction catalyzed.

iii) This system give each enzyme a code number (EC) and it contains 4 digits separated by points.

first digit: Class

second digit: sub-class

third digit: sub-sub-class

fourth digit: serial number of the enzyme.

## TABLE 6–3 International Classification of Enzymes

| *No.* | *Class* | *Type of reaction catalyzed* |
|---|---|---|
| 1 | Oxidoreductases | Transfer of electrons (hydride ions or H atoms) |
| 2 | Transferases | Group transfer reactions |
| 3 | Hydrolases | Hydrolysis reactions (transfer of functional groups to water) |
| 4 | Lyases | Addition of groups to double bonds, or formation of double bonds by removal of groups |
| 5 | Isomerases | Transfer of groups within molecules to yield isomeric forms |
| 6 | Ligases | Formation of C—C, C—S, C—O, and C—N bonds by condensation reactions coupled to ATP cleavage |

Note: Most enzymes catalyze the transfer of electrons, atoms, or functional groups. They are therefore classified, given code numbers, and assigned names according to the type of transfer reaction, the group donor, and the group acceptor.

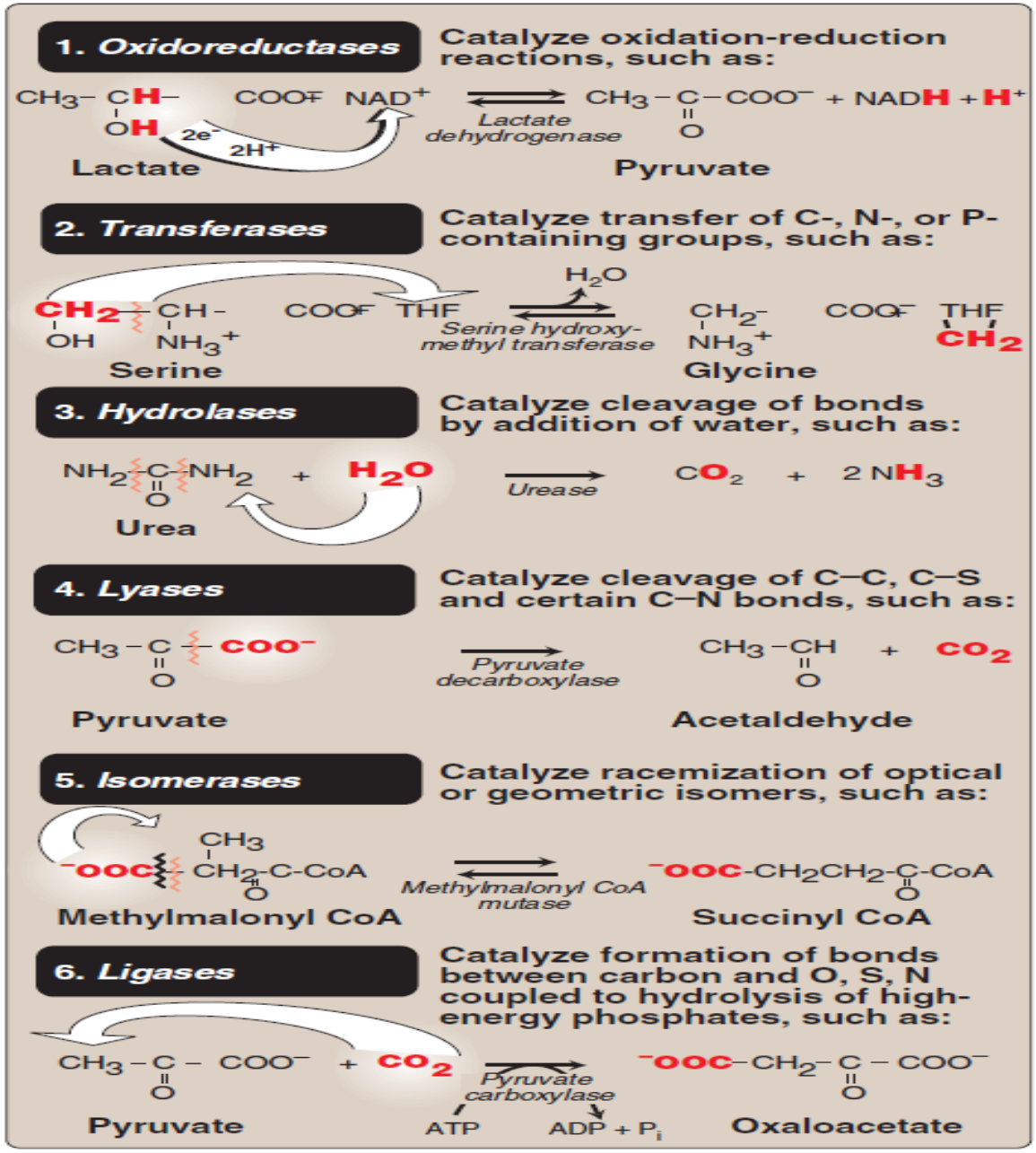



**Figure 5.1**
The six major classes of enzymes with examples. THF = tetrahydrofolate.

**General characterestices of enzymes:**

1)They are proteins.

2) High molecular weight proteins.

3) They act at specific PH and temperature.

4) Active site:

Enzyme molecule contain a special pocket or cleft called the active site.

(active site is relatively small portion of the total volume of an enzyme that bind the substrate).

-The active site contains amino acid side chains that participate in substrate binding and catalysis.

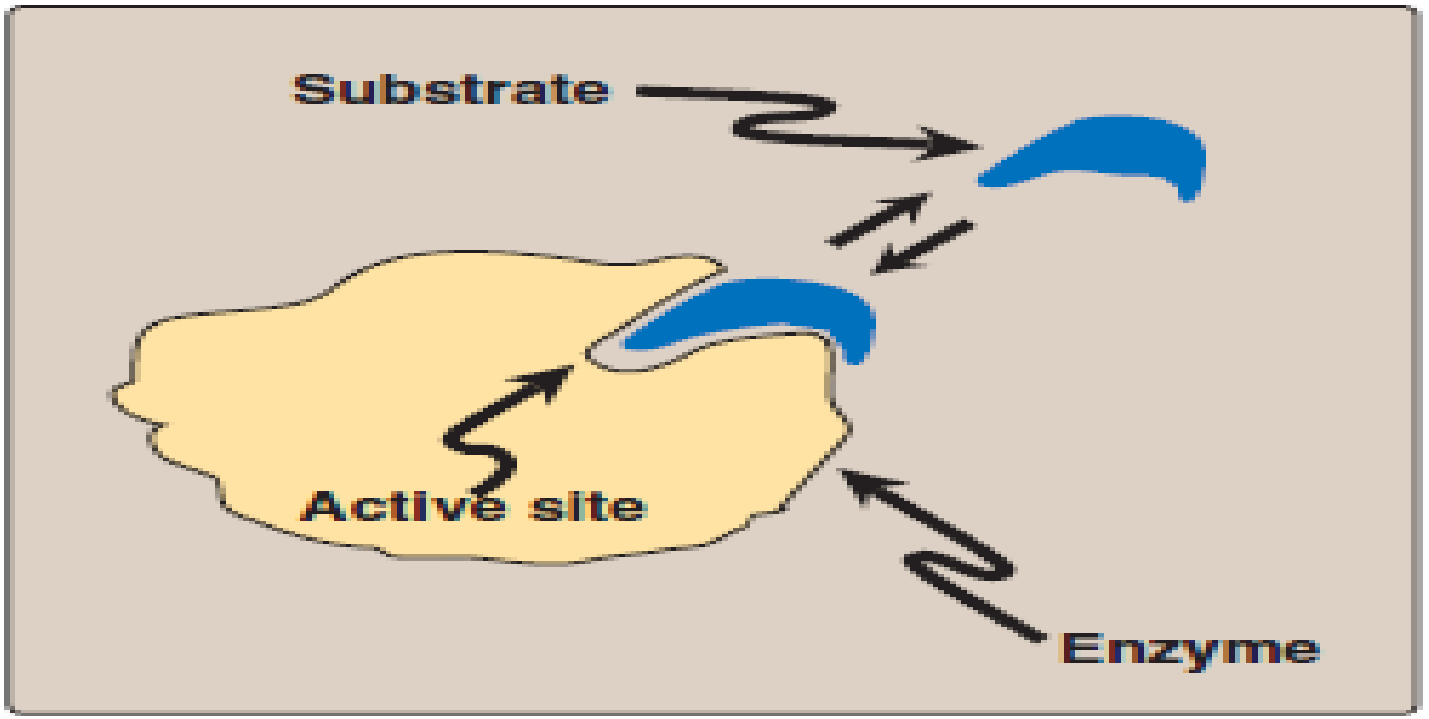



**Figure 5.2**
Schematic representation of an enzyme with one active site binding a substrate molecule.

## 5) Cofactors:

Enzymes

- Some enzymes depend for activity only on their structure as proteins (simple proteins)
- While other enzymes require one or more non-protein component known as **the cofactor** (these are conjugated proteins).

Cofactor

Inorganic ( metal ions e.g., $Zn^{+2}$, $Fe^{+2}$)

OR

Organic molecule called coenzymes

( Coenzymes are derivatives of vitamins)

example of coenzymes: $NAD^{+}$, FAD

## TABLE 6–1 Some Inorganic Elements That Serve as Cofactors for Enzymes

| | |
|---|---|
| $Cu^{2+}$ | Cytochrome oxidase |
| $Fe^{2+}$ or $Fe^{3+}$ | Cytochrome oxidase, catalase, peroxidase |
| $K^{+}$ | Pyruvate kinase |
| $Mg^{2+}$ | Hexokinase, glucose 6-phosphatase, pyruvate kinase |
| $Mn^{2+}$ | Arginase, ribonucleotide reductase |
| Mo | Dinitrogenase |
| $Ni^{2+}$ | Urease |
| Se | Glutathione peroxidase |
| $Zn^{2+}$ | Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B |

**Holoenzyme:** A complete, catalytically active enzyme together with its non- protein component (coenzyme or metal ion).

**Apoenzyme:** The protein part of an enzyme.( enzyme without its non-protein moeity).

**Apoenzyme + cofactor** → **Holoenzyme**

(catalytically inactive) → (active enzyme)

## 6) Specificity:

- Enzymes are highly specific, interacting with one or a few specific substrates and catalyzing only one type of chemical reaction.

Specificity →

- Absolute specificity:

(the enzyme can act only on one substrate)

- Group specificity:

(the enzyme can act on a group of related substrates).

## 7) Enzyme inhibitors:

Some substances combine with the enzyme and reduce its activity ⟹ These are known as inhibitors.

## 8) Enzyme activator:

Some substances combine with the enzyme and increase its activity ⟹ These are known as activators.

- Activators include:

a) Metal ions

b) HCl

$$\text{Pepsinogen} \xrightarrow{\text{HCl}} \text{Pepsin}$$

(inactive enzyme) (active enzyme)

c) Enzymes

$$\text{Trypsinogen} \xrightarrow{\text{enterokinase}} \text{Trypsin}$$

(inactive enzyme) (active enzyme)

## How do enzymes increase the rate of a reaction?

Enzymes increase rate of reaction by decreasing energy of activation ($\Delta G^{\neq}$) .

- Enzymes do not change $\Delta G^{\circ}$ (standard free energy change).

**Transition state:**

An unstable state in which new bonds are formed and old bonds are broken.

**Activation energy** ($\Delta G^{\neq}$) **:**

Difference in the energy level of S (or P) and the transition state.

**$\Delta G^{\circ}$ (standard free energy change):**

It is energy difference between substrate and product.

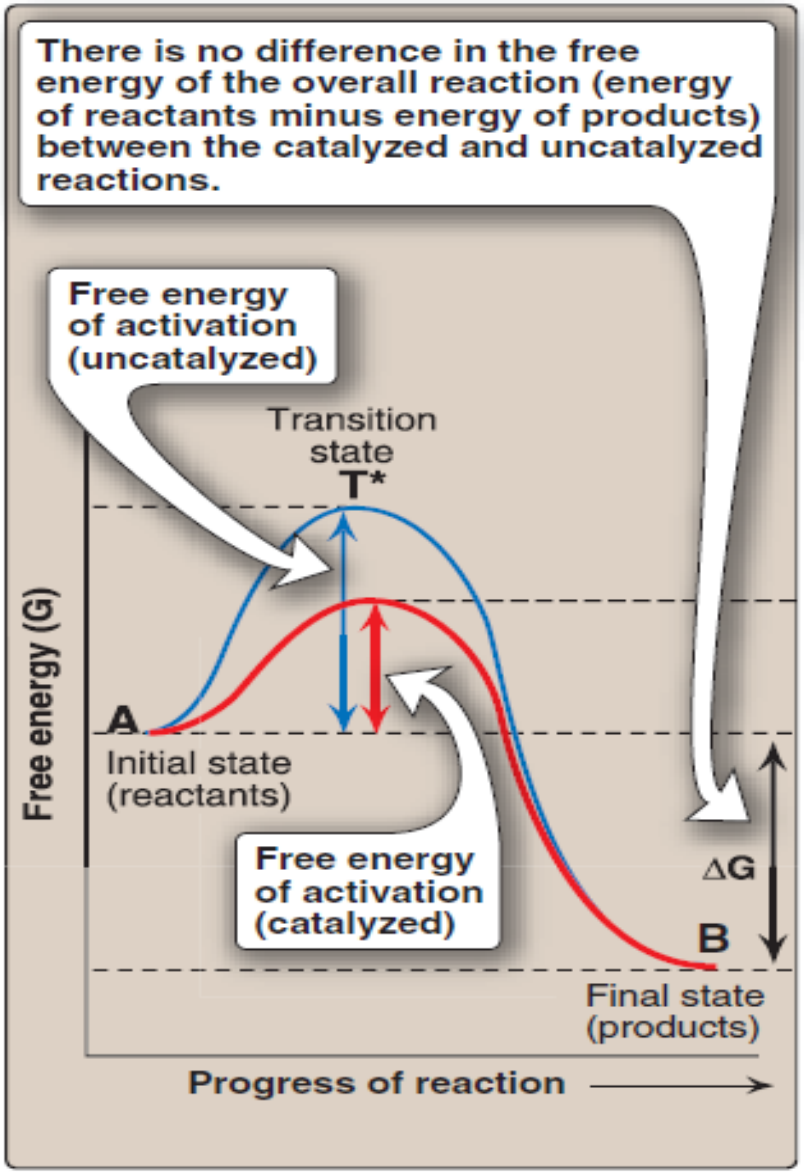



**Figure 5.4**

Effect of an enzyme on the activation energy of a reaction.

## Enzyme mechanism:

- During enzyme action, there is a temporary combination between enzyme and its substrate forming enzyme-substrate complex (ES complex).

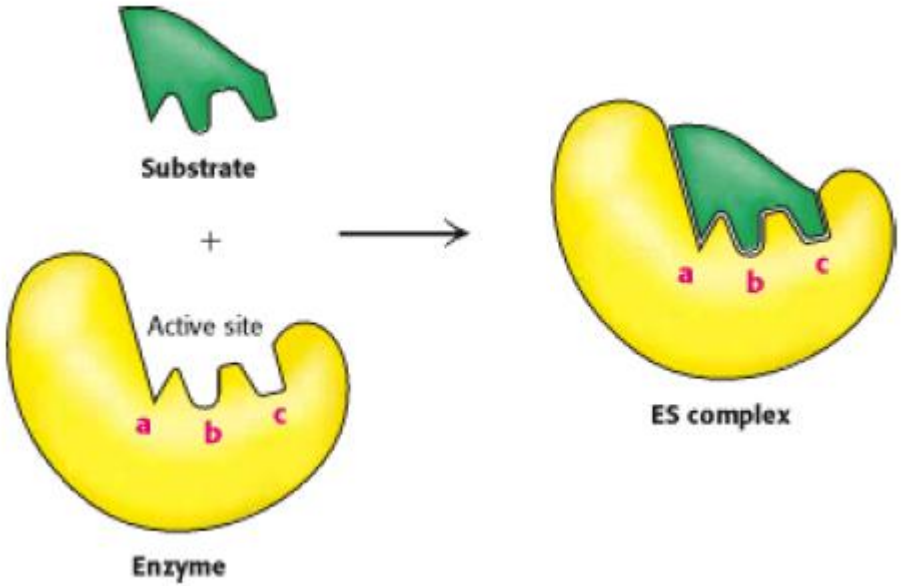
- This is followed by dissociation of this complex into enzyme and product.

$$E + S \rightleftharpoons ES \rightleftharpoons E + P$$

**Models to explain enzyme specificity towards substrate:**

**a) Lock and key model of enzyme-substrate binding:**

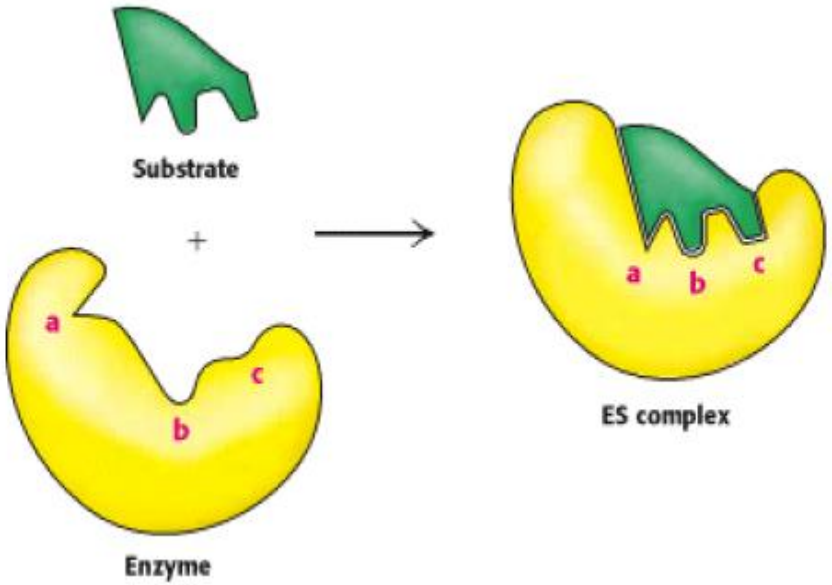
The active site of the unbound enzyme is complementary in shape to the substrate.



**b) Induced-fit model of enzyme-substrate binding:**

The enzyme change shape on the substrate binding.

- The active site forms a shape complementary to the substrate only after the substrate has been bound.



**Factors affecting reaction velocity of enzymes**:

1) substrate concentration
2) Enzyme concentration
3) pH
4) temperature
5) Inhibitors
6) Activators

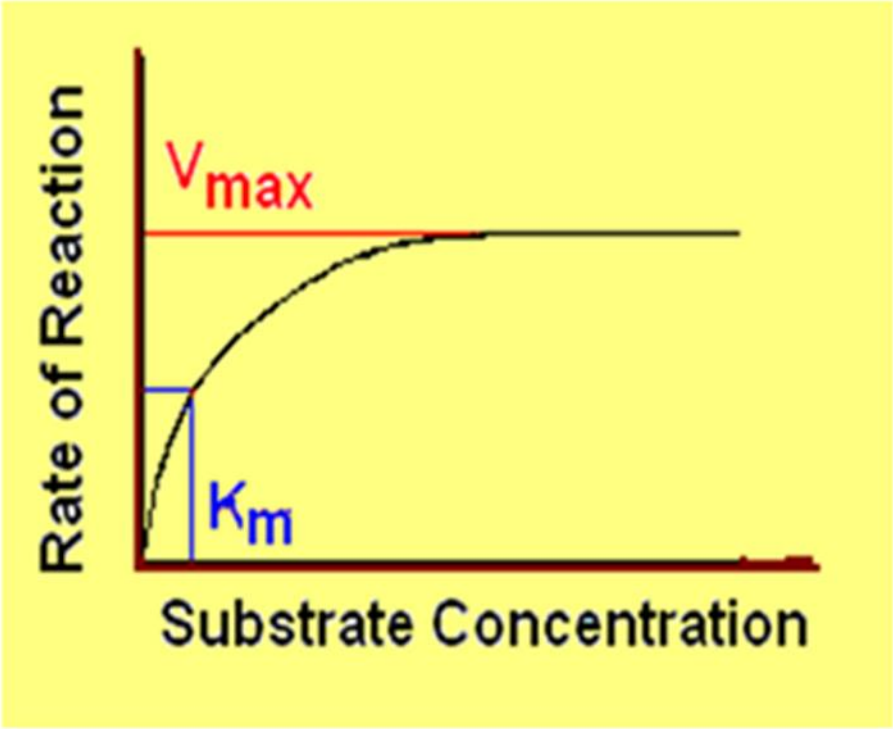
**1) Effect of substrate concentration:**

- velocity is usually expressed as μmol of product formed per minute.
- The rate of an enzyme-catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached.
- Increased substrate concentration after this point (Vmax) will not increase the rate.

[The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules present].

i.e., At **low substrate concentrations**, an increase in substrate concentration increases the rate of reaction because there are many active sites available to be occupied.

- At **high substrate concentrations,** the reaction rate levels off because most of the active sites are occupied.




Michaelis-Menten plot

- **Vmax** is the maximum reaction rate ( maximum velocity).
- **The Michaelis-Menton constant , Km** is the substrate concentration when the rate is ½ Vmax .

- Most enzymes show Michaelis-Menten kinetics.
- In Michaelis-Menten model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme.


where S is the substrate

E is the enzyme

ES is the enzyme–substrate complex

P is the product

- In Michaelis-Menten kinetics, the plot of initial reaction velocity ($v_o$) against substrate concentration ([S]), is **hyperbolic**.

**Michaelis-Menten equation:**

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

where vo = initial reaction velocity

Vmax = maximal velocity

Km = Michaelis constant

[S] = substrate concentration

**The Double-Reciprocal Plot (Lineweaver-Burk plot):**

- Transformations of the Michaelis-Menten equation give Lineweaver-Burk equation.

- Michaelis-Menten equation can be algebraically transformed into equations that are more useful in plotting experimental data.
- One common transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation

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

- Separating the components of the numerator on the right side of the equation gives

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

which simplifies to:

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

-This equation is called the **Lineweaver-Burk equation.**

- A plot of $1/V_0$ versus $1/[S]$ yields a straight line.
- This line has a slope of Km/Vmax, an intercept of $1/V_{max}$ on the $1/V_0$ axis, and an intercept of -1/Km on the $1/[S]$ axis.

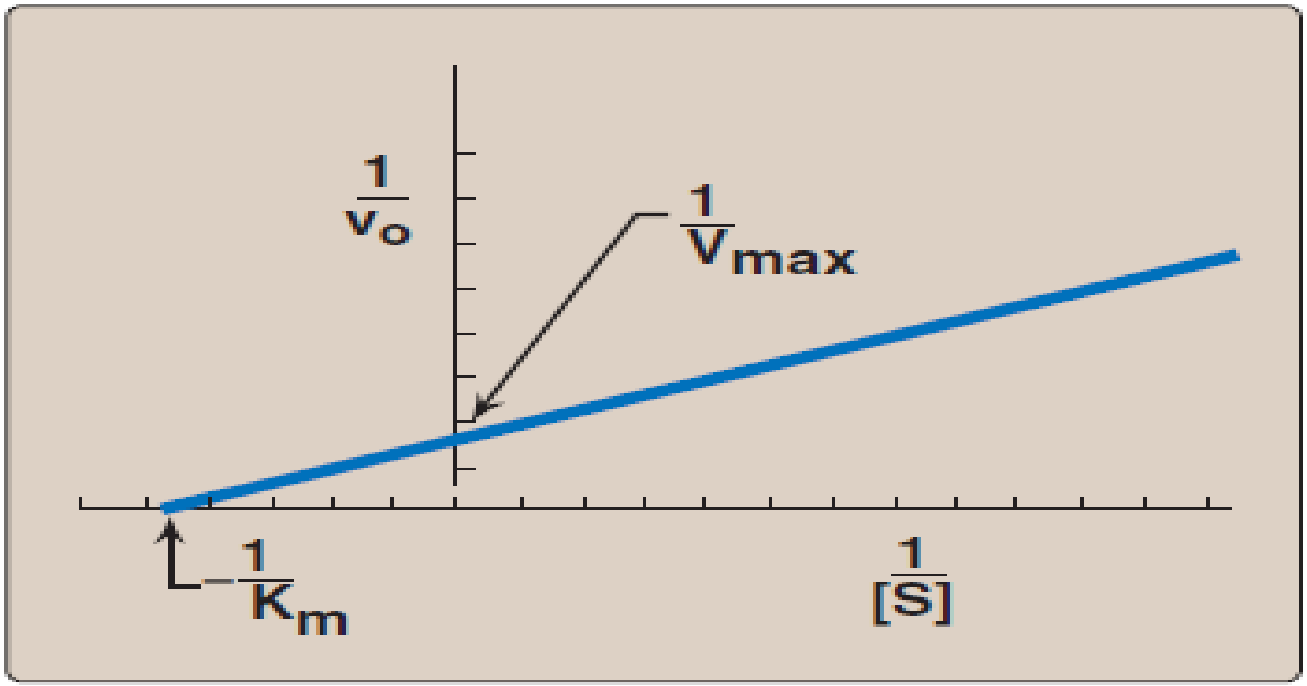
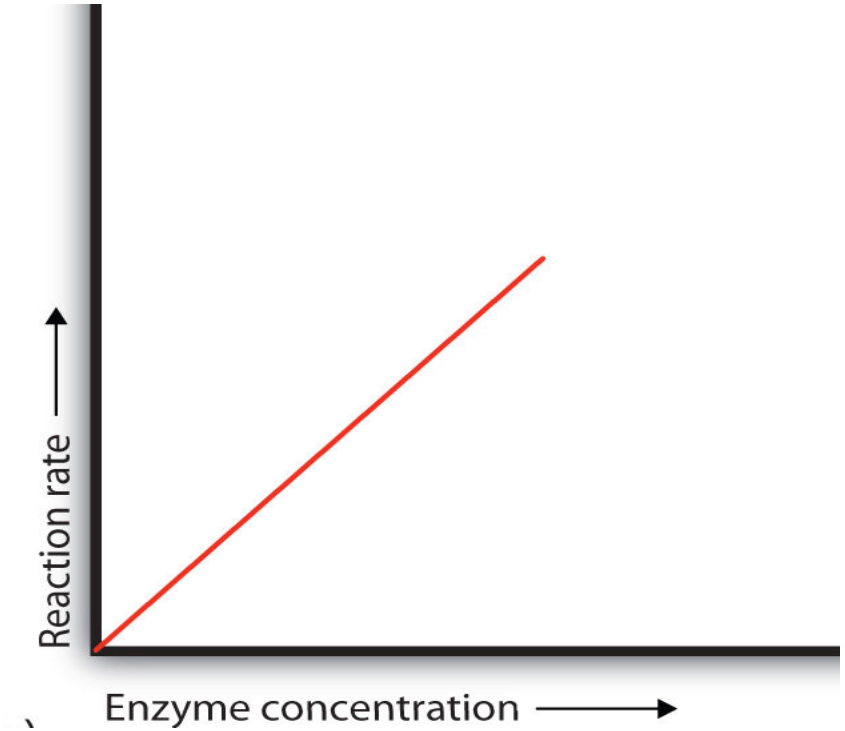
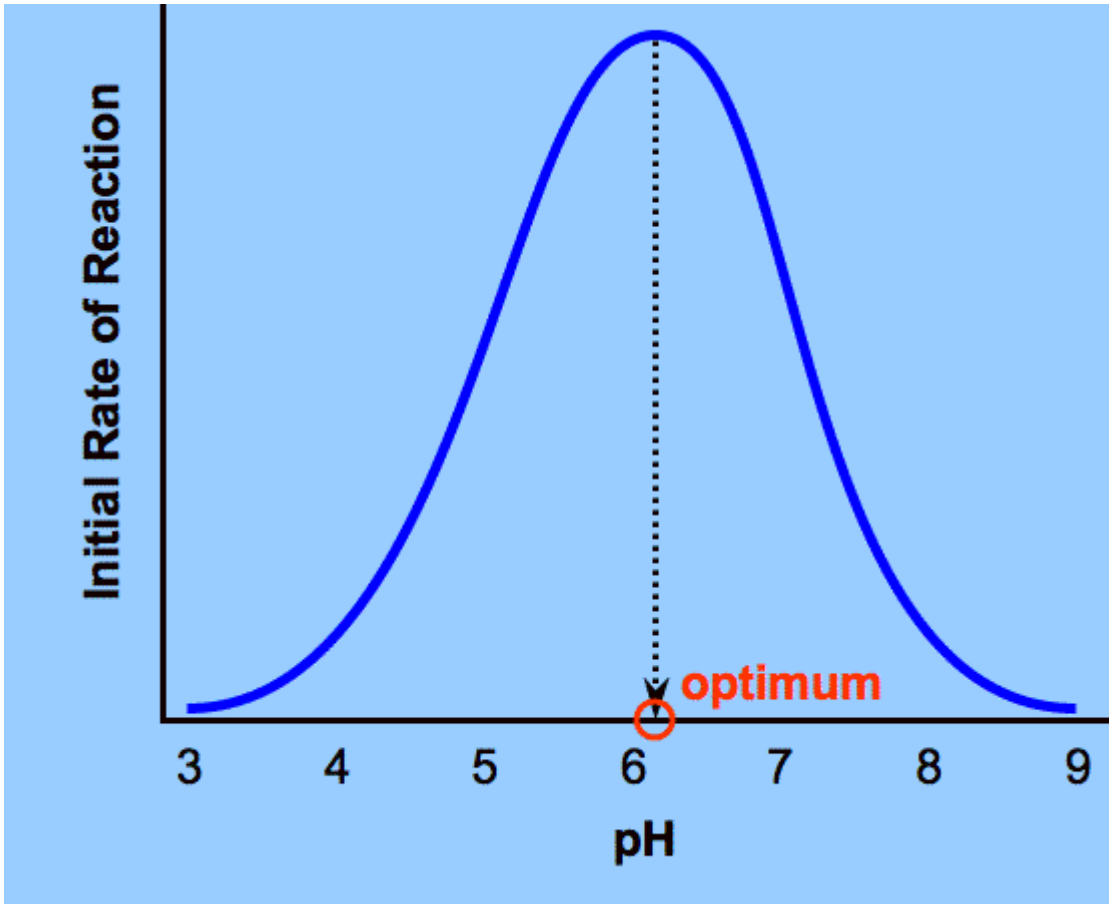



**Figure 5.11**
Lineweaver-Burk plot.

# 2) Effect of enzyme concentration:



# 3) Effect of PH:



## Optimum PH:

It is the PH at which the enzyme has its maximum activity.

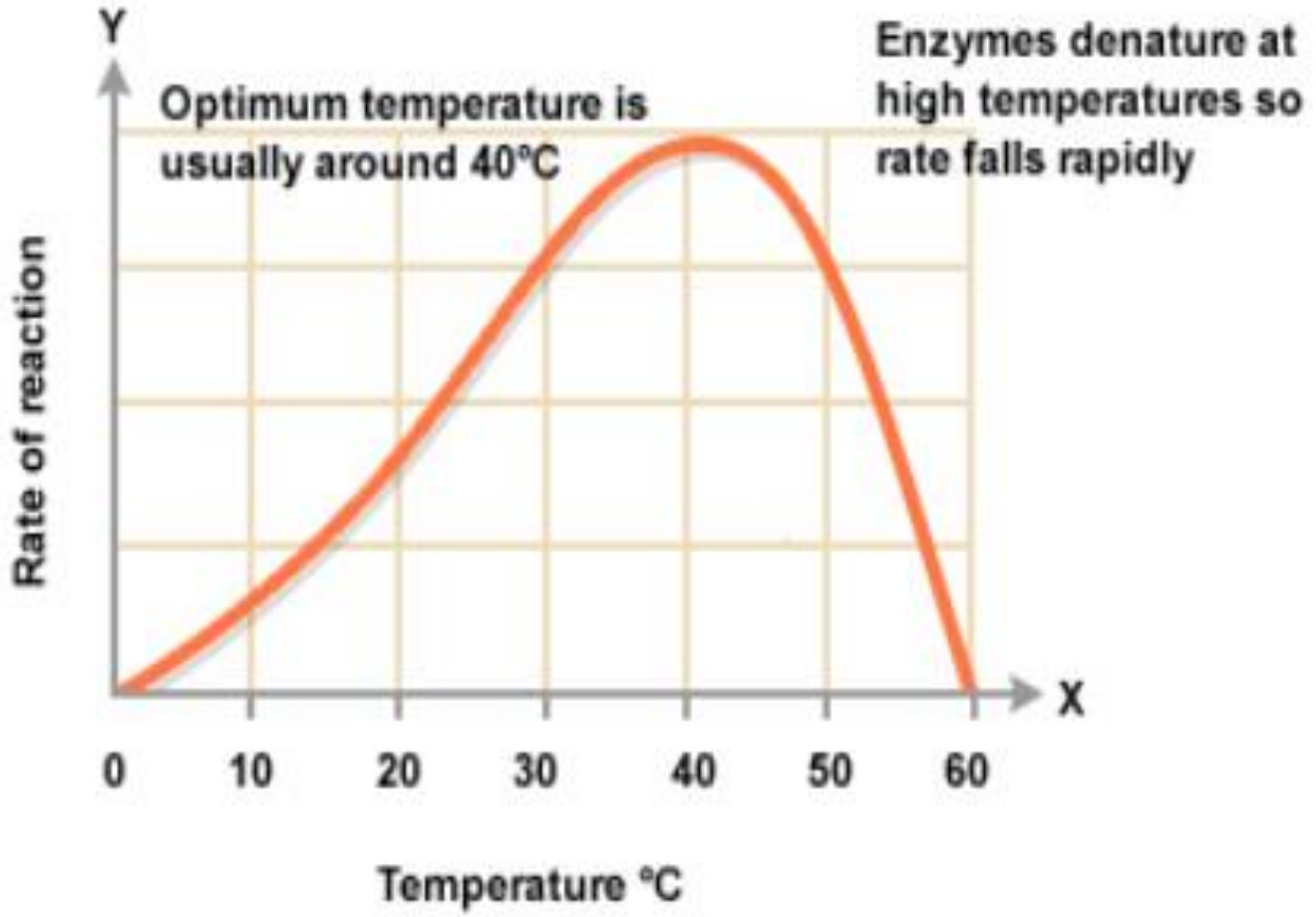
-Each enzyme has an optimal pH at which it is most efficient.

- The optimum pH varies for different enzymes.

For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment.

- A change in pH can alter the ionization of the groups of the amino acids.
- Extremes of pH can lead to denaturation of the enzyme,

# 4) Effect of temperature:



- The reaction velocity increases with temperature until a peak velocity is reached.

Optimum temperature:

It is the temperature at which the enzyme has its maximum activity.

- Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature-induced denaturation of the enzyme.

- Most enzymes are fully denatured at 70°C.

## 5) Effect of inhibitors:

Inhibitors: are chemicals that reduce the rate of enzymic reactions.

-Enzyme inhibitors are among the most important pharmaceutical agents.

There are two broad classes of enzyme inhibitors:

A) Irreversible inhibitors.

B) Reversible inhibitors

## A) Irreversible inhibitors:

- Combine with the functional groups of the enzyme in the active site, irreversibly (combine covalently to enzyme so as to permanently inactivate it).

- Most bind to a functional group in active site of enzyme to block that site.

**Example:**

Diisopropyl fluorophosphate (DFP) binds covalently to serine in serine proteases & acetylcholinesterase .

**B) Reversible inhibitors:**

Types of reversible inhibitors:

a) Competitive inhibitors

b) Noncompetitive inhibitors

c) Uncompetitive inhibitor

- The two most commonly encountered types of reversible inhibition are:

a) Competitive inhibition

b) Noncompetitive inhibition

## a) Competitive inhibition:

The inhibitor compete with the substrate to bind the active site.

-Competitive inhibitor structure usually resemble substrate.

The competitive inhibitor's action is proportional to its concentration. (The effect of a competitive inhibitor is reversed by increasing [S]).

Effect of competitive inhibitor on Km and $V_{max}$:

- Same Vmax
- increase Km

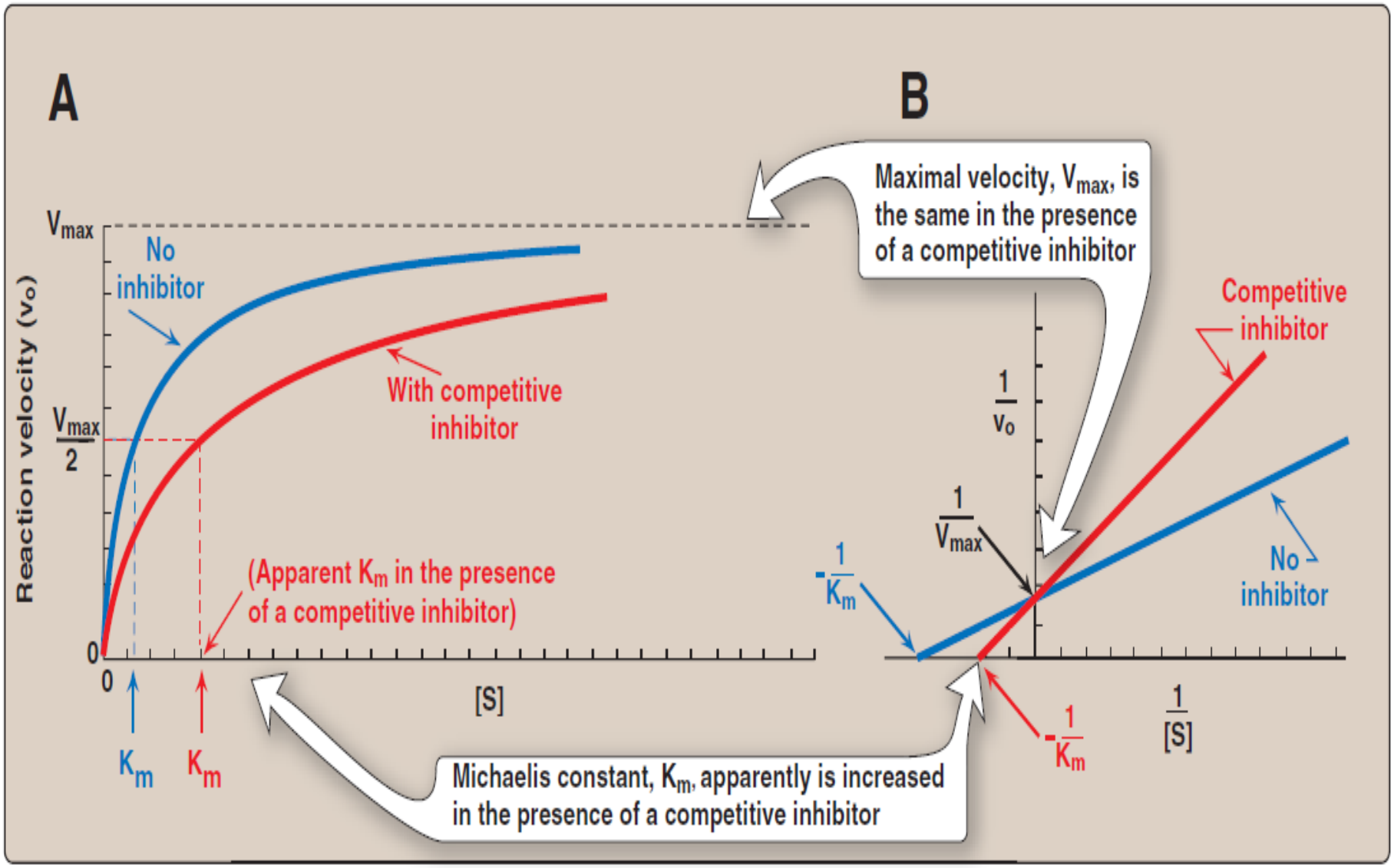



**Figure 5.12**

A. Effect of a competitive inhibitor on the reaction velocity ($v_o$) versus substrate ([S]) plot. B. Lineweaver-Burk plot of competitive inhibition of an enzyme.

## b) Noncompetitive inhibition:

-The inhibitor bind the enzyme at a site other than the active site.

- The noncompetitive inhibitor can bind either free enzyme or the ES complex.

-This type of inhibition not affected by the concentration of the substrate.

Effect of non-competitive inhibitor on Km and Vmax:

- decrease Vmax
- Same Km

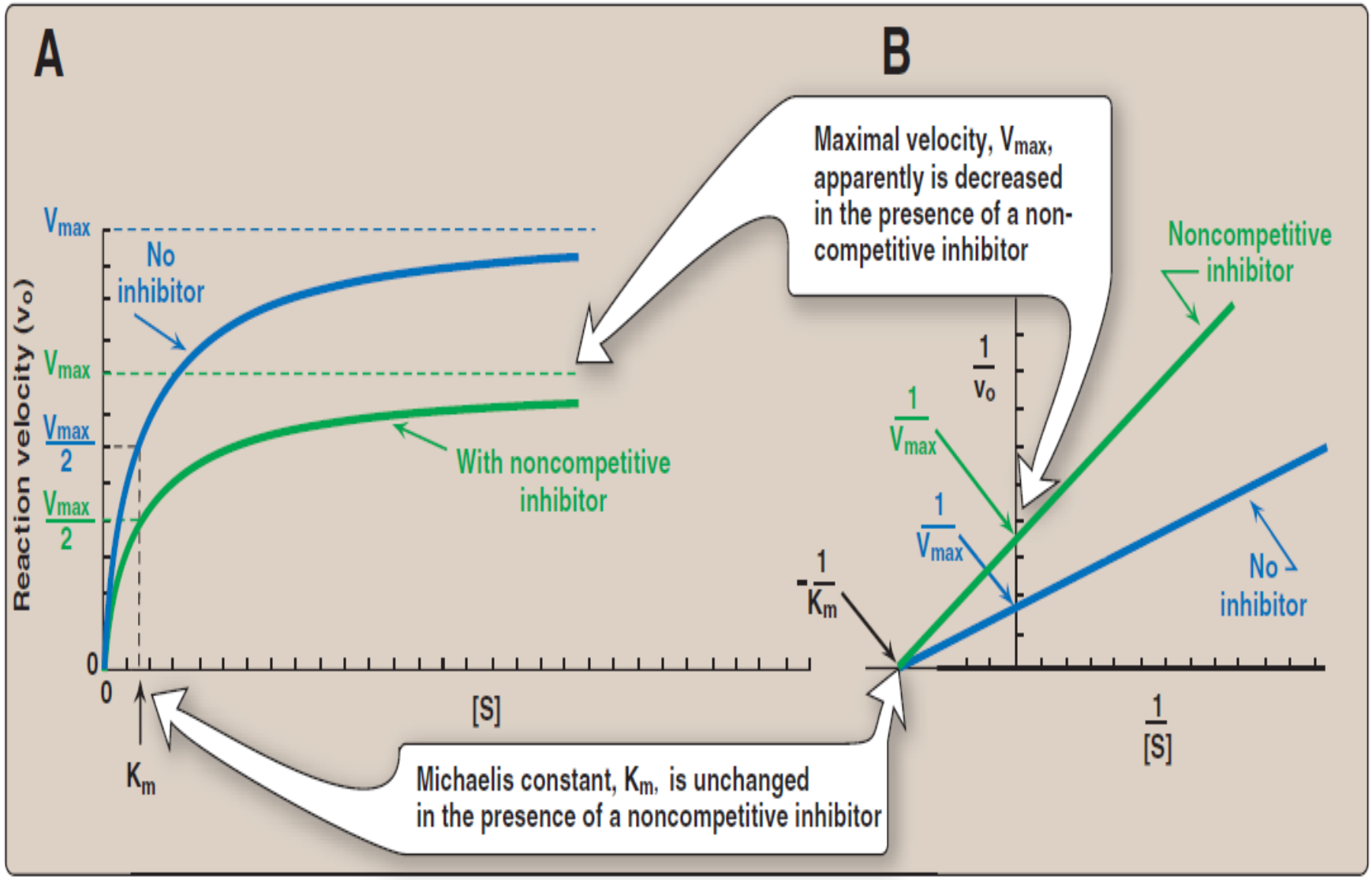



**Figure 5.14**

A. Effect of a noncompetitive inhibitor on the reaction velocity ($v_o$) versus substrate ([S]) plot. B. Lineweaver-Burk plot of noncompetitive inhibition of an enzyme.