Enzymes may be defined as ***biocatalysts synthesized by living cells. They are protein in nature (exception – RNA acting***

as ribozyme), colloidal and thermolabile in character, and specific in their action.

In the laboratory, hydrolysis of proteins by a strong acid at

100°C takes at least a couple of days. The same protein is fully digested by the enzymes in gastrointestinal tract at body temperature (37°C) within a

couple of hours. This remarkable difference in the chemical reactions tak-ing place in the living system is exclusively due to enzymes. The very existence of

life is unimag-
inable without
the presence of
enzymes.

In the early days, the enzymes were given names by their discoverers in an arbitrary manner. For

example, the names pepsin, trypsin and chymotrypsin convey no information about the function of the enzyme or the nature of the substrate on

which they act. Sometimes, the suffix-***ase*** was added to the substrate for naming the enzymes e.g. lipase acts on lipids; nuclease on nucleic acids; lactase on lac-

tose. These are known as ***trivial names*** of the enzymes which, however, fail to give complete information of enzyme reaction (type of reaction,

cofactor requirement etc.)

Enzymes are sometimes considered under two broad categories : (a) **Intracellular enzymes** – They are functional

within cells where they are synthesized. (b) **Extracellular enzymes** – These enzymes are active outside the cell; all the digestive enzymes

belong to this group.

The International Union of Biochemistry (IUB) appointed an Enzyme Commission in 1961. This committee made a

thorough study of the existing enzymes and devised some basic principles for the classi-fication and nomenclature of enzymes. Since 1964, the ***IUB***

system of enzyme classification has been in force. Enzymes are divided into ***six major classes*** (in

1. **Oxidoreductases :** Enzymes involved in oxidation-reduction reactions.
2. **Transferases :** Enzymes that catalyse the transfer of functional groups.

3. **Hydrolases :** Enzymes that bring about hydrolysis of various compounds.

4. **Lyases :** Enzymes specialised in the addition or removal of water,

ammonia, CO_2 etc.

5. **Isomerases :** Enzymes involved in all the isomerization reactions.

6. **Ligases :** Enzymes catalysing the synthetic

reactions (*Greek* : ligate—to bind) where two molecules are joined together and ATP is used.

The important factors that influence the velocity of the enzyme reaction are discussed hereunder

1. Concentration of enzyme

As the concentration of the enzyme is increased, the velocity of the reaction proportion-

ately increases (*Fig.6.1*). In fact, this property of enzyme is made use in determining the serum enzymes for the diagnosis of diseases. By using a known volume

of serum, and keeping all the other factors (substrate, pH, temperature etc.) at the optimum level, the enzyme could be assayed in the laboratory.

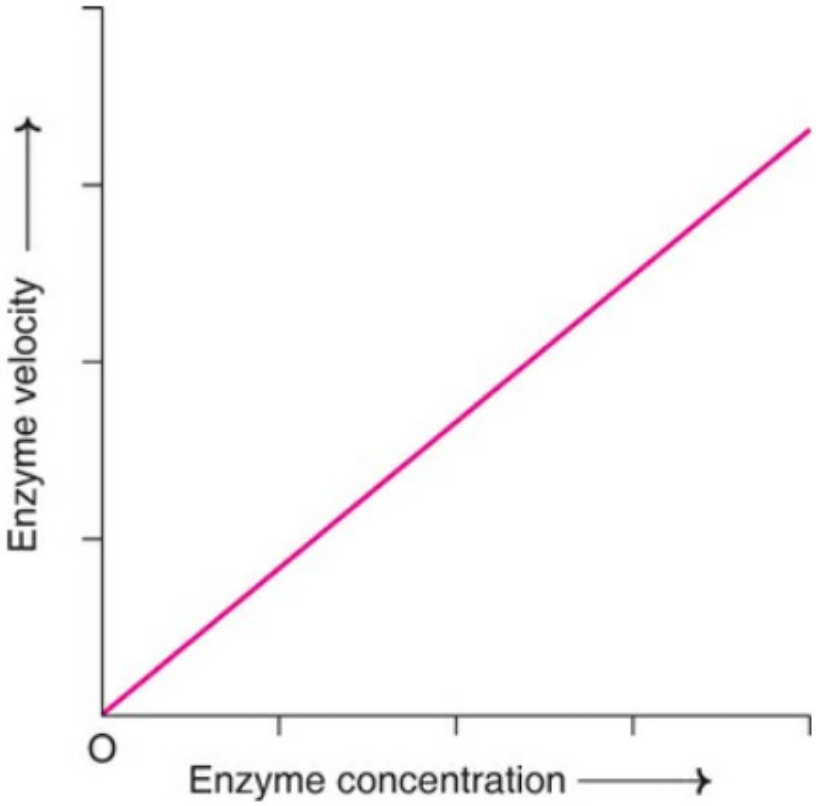

Fig. 6.1 Effect of enzyme concentration on enzyme velociy.

2. Concentration of substrate

Increase in the substrate concentration gradually ***increases the velocity of enzyme reaction*** within

the limited range of substrate levels. A rectangular hyperbola is obtained when velocity is plotted against the substrate concentration (*Fig.6.2*). Three

distinct phases of the reaction are observed in the graph (A-linear; B-curve; C-almost unchanged).

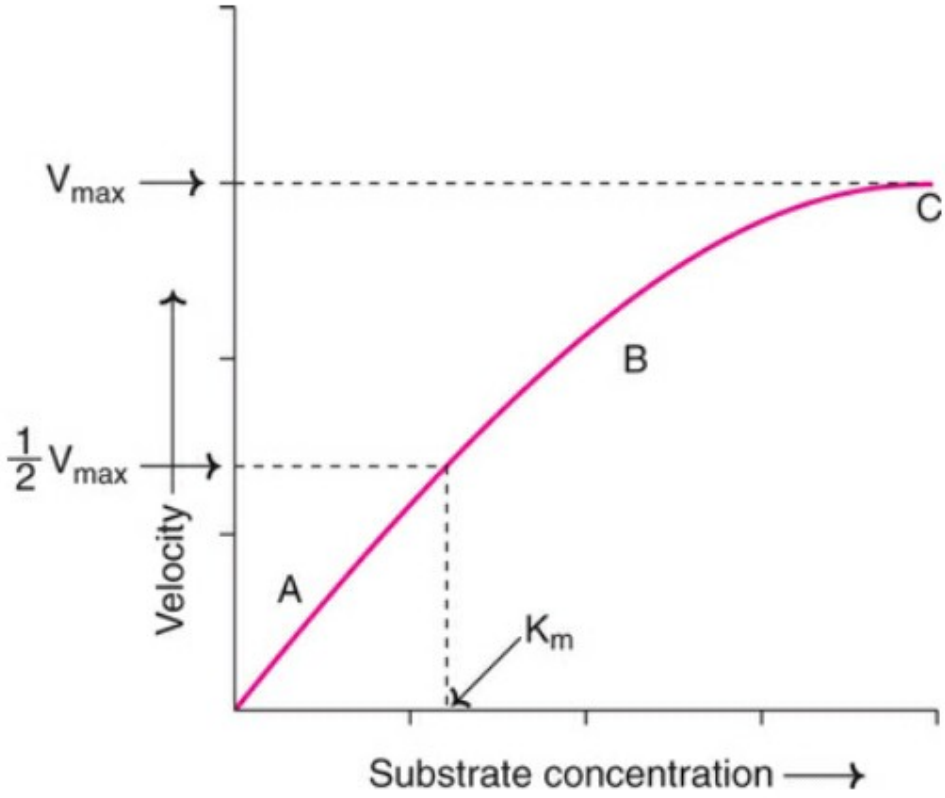

Fig. 6.2 Effect of substrate con-centration on enzyme velocity (A-linear; B-

curve; C-almost unchanged).

Order of reaction

When the velocity of the re-action is almost proportional to the substrate concentration

(i.e. [S] is less than K_m), the rate of the reaction is said to be ***first order*** with respect to substrate. When the [S] is much greater than K_m, the rate of reac-

tion is independent of substrate concentration, and the reaction is said to be ***zero order***.

Enzyme kinetics and K_m value

The enzyme (E)

and substrate (S) combine with each other to form an unstable enzymesubstrate complex (ES) for the formation of product (P).

$$\mathrm{E} + \mathrm{S} \underset{\mathrm{k_2}}{\overset{\mathrm{k_1}}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{\mathrm{k_3}} \mathrm{E} + \mathrm{P}$$

Here k_1, k_2 and k_3 represent the velocity constants for the respective reactions, as indicated by arrows.

K_m, the Michaelis-Menten constant (or ***Brig's and Haldane's constant***), is given by the formula

$$K_m = \frac{k_2 + k_3}{k_1}$$

The following equation is obtained after suitable algebraic manipulation.

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

equation (1)

where v = Measured velocity,

sured velocity (v) is equal to $\frac{1}{2}V_{max}$. Then the equation (1) may be substituted as follows

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{K_m + [S]}$$

$$K_m + [S] = \frac{2V_{max}[S]}{V_{max}}$$

$$\mathrm{K_m} + [\mathrm{S}] = 2\,[\mathrm{S}]$$

$$\mathrm{K_m} = [\mathrm{S}]$$

K stands for a constant and ***m*** stands for ***M***ichaelis (in $\mathrm{K_m}$).

$\boldsymbol{K_m}$ or the ***Michaelis-Menten constant***

is defined as the ***substrate concentration*** (expressed in moles/l) ***to produce half-maximum velocity*** in an enzyme catalysed reaction. It indicates

that half of the enzyme molecules (i.e. 50%) are bound with the substrate molecules when the substrate concentration equals the Km value.

K_m value is a constant and a ***characteristic feature of a given enzyme*** (comparable to a thumb impression or signature). It is a representative for measuring

the strength of ES complex. A ***low K_m value indicates a strong affinity between enzyme and substrate***, whereas a high Km value reflects a weak affinity

between them. For majority of enzymes, the K_m values are in the range of 10^{-5} to 10^{-2} moles. It may however, be noted that K_m is ***not dependent on***

the concentration of enzyme.

Lineweaver-Burk double reciprocal plot

For the determination of K_m value, the substrate saturation curve (***Fig.6.2***)

is not very accurate since V_{max} is approached asymptotically. By taking the reciprocals of the equation (1), a straight line graphic

representation is obtained.

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

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

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

The above equation is sim-

ilar to y = ax + b. Therefore, a plot of the reciprocal of the velocity $\left(\frac{1}{v}\right)$ vs. the reciprocal of the substrate concentration $\left(\frac{1}{[S]}\right)$ gives a straight line.

Here the slope is K_m/V_{max} and whose y intercept is $1/V_{max}$.

The Lineweaver-Burk plot is shown in ***Fig.6.3***. It is much easier to calculate the K_m from the in-

tercept on x-axis which is $-(1/K_m)$. Further, the double reciprocal plot is ***useful in understanding the effect of various inhibitions***

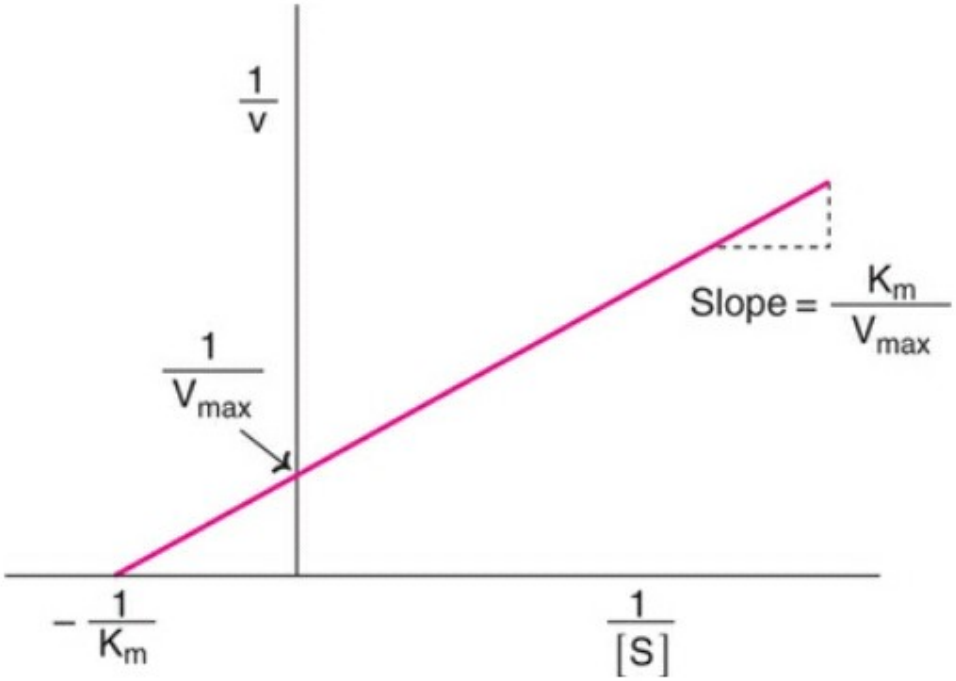

Fig. 6.3 Lineweaver-Burk double reciprocal plot.

3. Effect of temperature

Velocity of an

enzyme reaction increases with increase in temperature up to a maximum and then declines. A ***bell-shaped curve*** is usually observed (***Fig.6.4***).

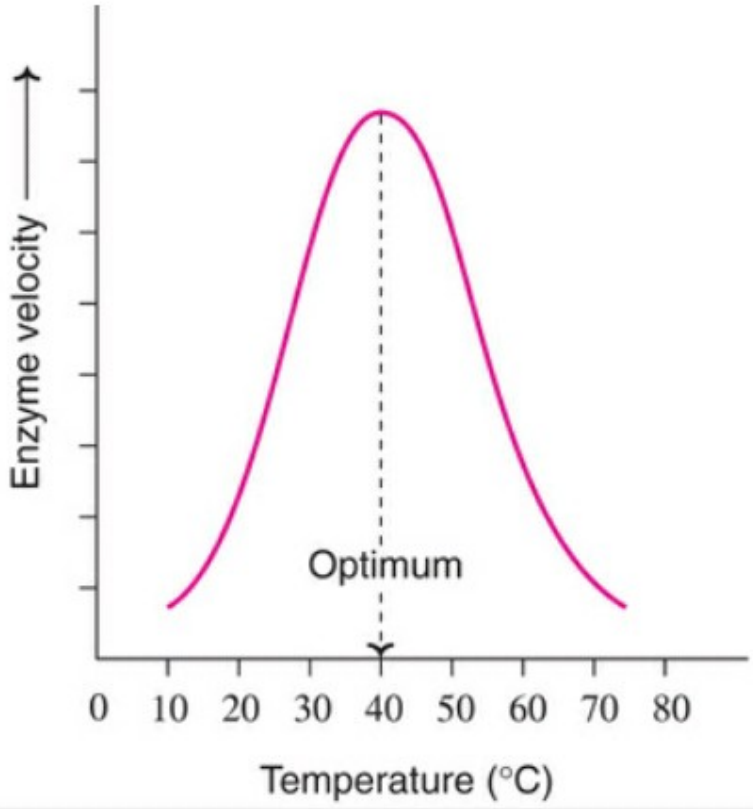

Fig. 6.4 Effect of temperature on enzyme velocity.

4. Effect of pH

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme ac-

tivity and a ***bell-shaped curve*** is normally obtained (***Fig.6.5***). Each enzyme has an optimum pH at which the velocity is maximum. Below and above this

pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive.

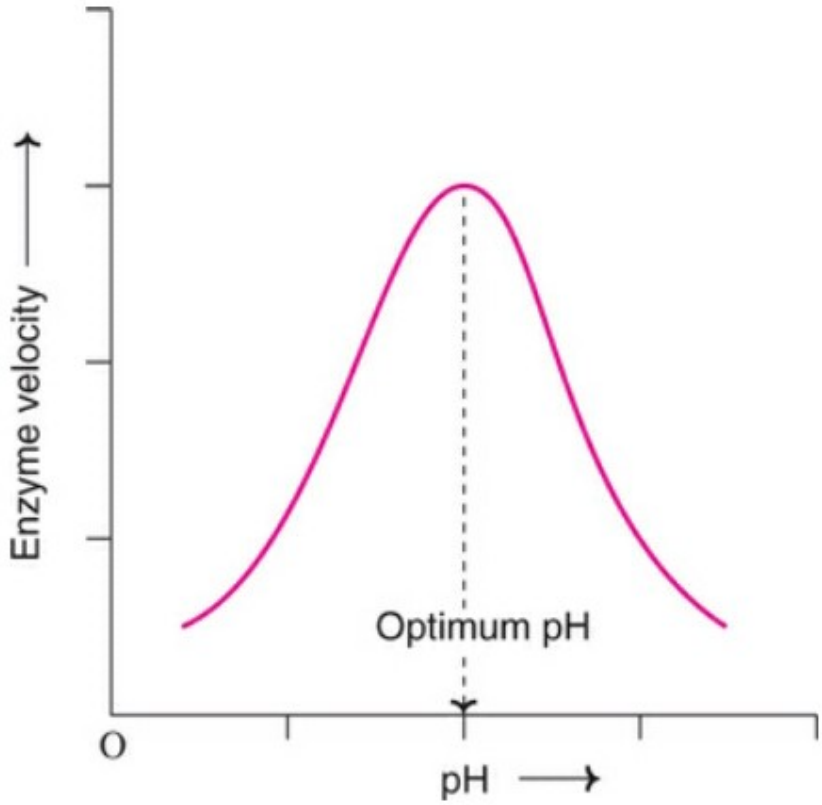

Fig. 6.5 Effect of pH on enzyme velocity.

Most of the enzymes of higher organisms show optimum activity around neutral pH (6–8). There are,

however, many exceptions like pepsin (1–2), acid phosphatase (4–5) and alkaline phosphatase (10–11). Enzymes from fungi and plants are most active

in acidic pH (4–6).

Hydrogen ions influence the enzyme activity by altering the ionic charges on the amino acids (particularly at the active site),

substrate, ES complex etc.

5. Effect of product concentration

The accumulation of reaction products generally decreases

the enzyme velocity. For certain enzymes, the products combine with the active site of enzyme and form a loose complex and, thus, inhibit the

enzyme activity. In the living system, this type of inhibition is generally prevented by a quick removal of products formed. The end product inhibi-

tion by feedback mechanism is discussed later.

6. Effect of activators

Some of the enzymes require certain inorganic ***metallic***

cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion

(Cl^-) for amylase. Metals function as activators of enzyme velocity through various mechanisms—combining with the substrate, formation of ES-metal com-

plex, direct participation in the reaction and bringing a conformational change in the enzyme.

Two categories of enzymes requiring met-

als for their
activity are
distinguished