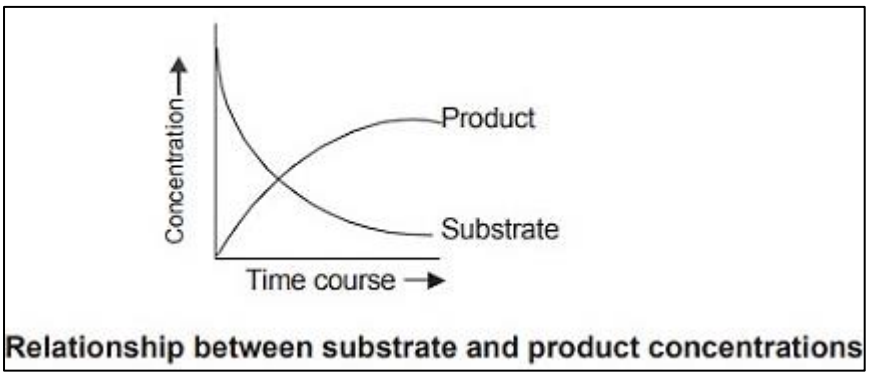
Enzyme Kinetics

Enzyme kinetics is the study of the chemical reactions that are catalysed by enzymes. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated. Studying an enzyme's kinetics in this way can reveal the catalytic mechanism of this enzyme, its role in metabolism, how its activity is controlled, and how a drug or an agonist might inhibit the enzyme.

Enzymes are usually protein molecules that manipulate other molecules—the enzymes' substrates. These target molecules bind to an enzyme's active site and are transformed into products through a series of steps known as the enzymatic mechanism.

$$E + S \rightleftarrows ES \rightleftarrows ES^* \rightleftarrows EP \rightleftarrows E + P$$

Relationship Between Substrate and Product Concentrations



Relationship between substrate and product concentrations

Although the velocity increases linearly with enzyme concentration, at constant enzyme Concentration, it increases hyperbolically as the substrate concentration increases. This indicates that the enzyme has a definite number of sites to combine with substrate. When all sites are occupied, no further rate enhancement occurs and the enzyme is saturated with the substrate.

DERIVATION OF M - M EQUATION

Leonor Michaelis and Mand L. Menten in 1913 proposed a successful explanation for the effect of substrate concentration on the enzyme activity. According to them the enzyme E, and the substrate S combines rapidly to form a complex, the enzyme substrate complex ES. This complex then breaks down relatively and slowly to form the product P of the reaction. These sequences of functions can be represented in the following equations

$$E + S \underset{K_2}{\overset{K_1}{\leftrightarrows}} ES$$

$$ES \underset{K_4}{\overset{K_3}{\leftrightarrows}} E + P$$

Step 1 K_1 & K_2 are the rate constants of the Forward and backward reactions (step 1)

Step 2 K_3 & K_4 are the rate constants of the forward and backward reactions respectively (step2)

This is true only for the enzyme reactions which fulfil the following conditions:

- Only a single substrate and a single product are involved.
- The reaction proceeds essentially to completion.
- The concentration the substrate is much greater than that of the enzyme in the system.
- An intermediate enzyme substrate complex is formed.
- The rate of decomposition of the substrate is proportional to the concentration of the
- enzyme substrate complex.

It is assumed that the concentration of S is much greater than that of E and that only initial velocities are measured, where only a small fraction of S has been converted. Under these conditions, concentration of P $\longrightarrow$ ES can be ignored. Applying law of mass action to the first step of the reaction in which K_1 and K_2 are the rate constants for the forward and backward reaction respectively,

The rate of forward reaction = $K_1[E]\,[S]$ ……(1)
The rate of backward reaction = $K_2[ES]$ ……(2)

Applying law of mass action to the second step of the reaction in which K_3 and K_4 are the rate constants for the forward and backward reaction respectively,

The rate of forward reaction $K_3[ES|$ .....(3)

The rate of backward reaction can be neglected. The total enzyme in the system can be represented as,

$$[Et] = [E] + [ES] \quad \text{..... (4)}$$

Where [E] is the un-combined free enzyme concentration. [ES] the enzyme substrate concentration and [Et] the total enzyme concentration. The velocity of the overall reaction is:

$$V = K_3\,[ES] \quad \text{……(5)}$$

This is the actual rate equation for the Overall reaction but it is not useful since neither K_3 nor [ES] can be measured directly. It is assumed that the reaction proceeds at steady state where the rate of formation of [ES] equals to the rate of degradation of [ES]. The rate of formation of ES, V_f is proportional to E and S as in any second order reaction.

$$V_f = K_1[E]\,[S]$$
$$= K_1([Et]-[ES])\,[S] \quad \text{……(6)}$$

The rate of disappearance of (ES), V_d is

$$V_d = K_2\,[ES] + K_3\,[ES]$$
$$= K_2 + K_3\,[ES] \quad \text{.....(7)}$$

Since in the steady state $V_d = V_f$, therefore:

$$K_1 ([Et]-[ES]) [S] = K_2 + K_3 [ES] \quad \text{.....(8)}$$

Rearranging this equation gives:

$$\frac{[S] ([Et]-[ES])}{[ES]} = \frac{K_2 + K_3}{K_1} = K_m \quad \text{.....(9)}$$

where Km is the Michaelis - Menten constant, a useful parameter characteristic of each enzyme and a substrate.

Rearranging this equation by solving for [ES]:

$$\frac{[S] [Et]-[S] [ES]}{[ES]} = K_m \quad \text{.....(10)}$$

$$K_m [ES] = [S] [Et] - [S] [ES]$$
$$K_m [ES] + [S] [ES] = [S] [Et]$$
$$[ES] (K_m + [S]) = [S] [Et]$$

$$[ES] = \frac{[Et] [S]}{K_m + [S]} \quad \text{.....(11)}$$

According to the previous equation (5),

$$V = K_3 [ES].$$

Substituting the value of [ES] in (11) in this equation we get:

$$V = \frac{K_3 [E_t] [S]}{K_m + [S]} \quad \text{.....(12)}$$

The maximal velocity V_{max} is equal to

$$V_{max} = K_3 [E_t] \quad \text{.....(13)}$$

Substituting the value of $K_3 [E_t]$ in the equation (12), the final Michaelis - Menten rate equation becomes,

$$V = \frac{V_{max} [S]}{K_m + [S]} \quad \text{.....(14)}$$

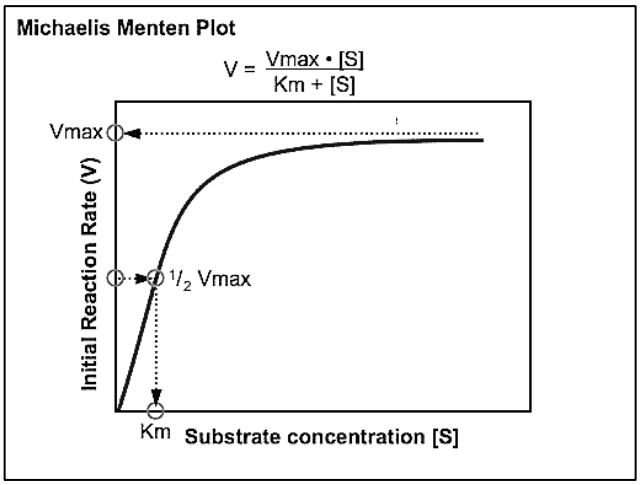
Now, when V is equal to half of the maximum velocity. i.e., $V = V_{max}/2$
Thus,

$$\frac{V_{max}}{2} = \frac{V_{max} [S]}{K_m + [S]} \quad \text{.....(15)}$$

Rearranging, $K_m + [S] = 2[S]$

Therefore, $K_m = [S]$ .....(16)

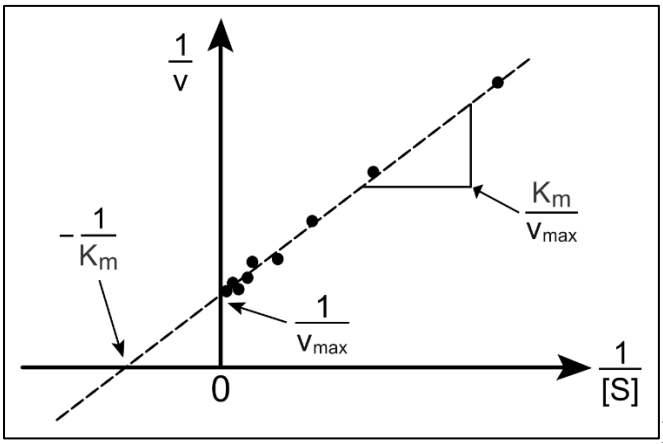
K_m Value Definition:



K_m is defined as the concentration of the substrate at which the velocity of the reaction is half-maximal. It is independent of enzyme concentration. The unit of K_m is moles per litre. Thus, Michaelis — Menten constant may be determined by a plot commonly known as M-M plot obtained by plotting substrate concentration [S] versus the rate of the reaction [V]

LINEWEAVER — BURK EQUATION

Transformation of the Michaelis - Menten equation: The value of K_m may be obtained more accurately from the Lineweaver – Burk equation which is obtained by taking the reciprocal of both sides of the Michaelis — Menten equation.



Lineweaver-Burk Plot

$$1/V = K_m + [S] / V_{max}\,[S] \quad \text{.....(1)}$$

Rearranging this equation, we have

$$1/V = K_m / V_{max}\,[S] + [S] / V_{max}\,[S] \quad \text{.....(2)}$$

which is further simplified to

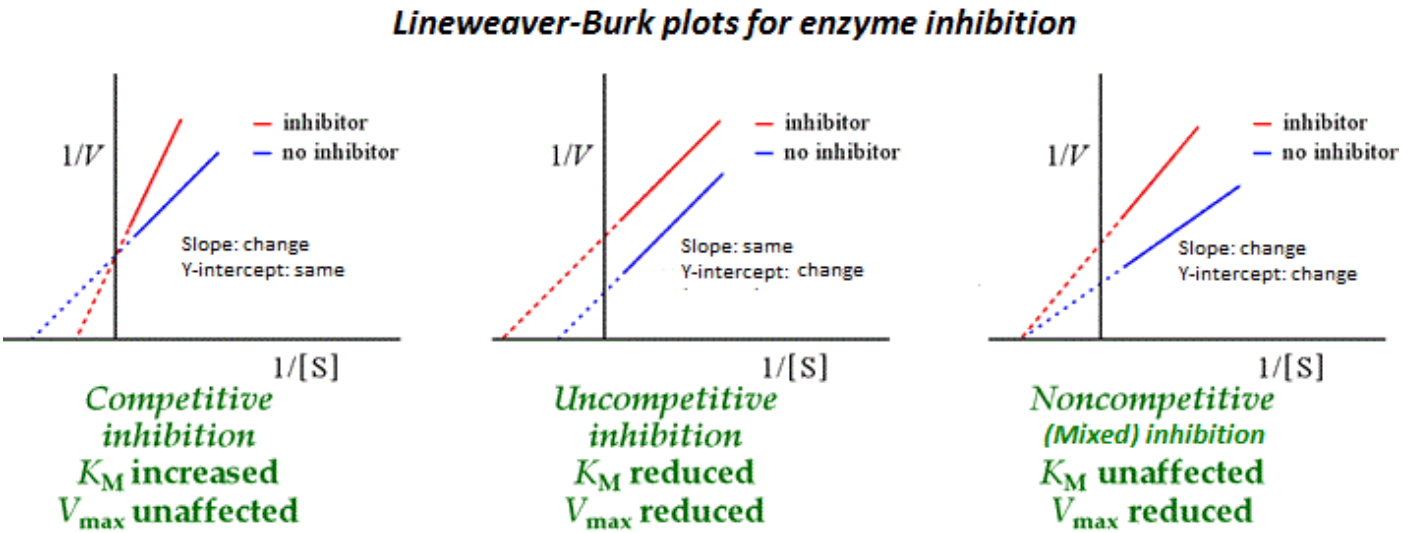
$$1/V = K_m / V_{max} .\ 1/[S] + 1/V_{max} \quad \text{.....(3)}$$

A plot of 1/V versus 1/S (a double reciprocal plot) yields a straight line with the slope of the K_m / V_{max} and ordinate intercept of $1 / V_{max}$. Since the slope and intercept are readily measured from the graph, the V_{max}, and K_m can be accurately determined.

The Lineweaver–Burk plot was widely used to determine important terms in enzyme kinetics, such as K_m and V_{max}, before the wide availability of powerful computers and non-linear regression software. The y-intercept of such a graph is equivalent to the inverse of V_{max}; the x-intercept of the graph represents $-1/K_m$. It also gives a quick, visual impression of the different forms of enzyme inhibition.

The double reciprocal plot distorts the error structure of the data, and it is therefore unreliable for the determination of enzyme kinetic parameters.

When used for determining the type of enzyme inhibition, the Lineweaver–Burk plot can distinguish **competitive**, **non-competitive** and **uncompetitive inhibitors**. Competitive inhibitors have the same y-intercept as uninhibited enzyme (since V_{max} is unaffected by competitive inhibitors the inverse of V_{max} also doesn't change) but there are different slopes and x-intercepts between the two data sets. Non-competitive inhibition produces plots with the same x-intercept as uninhibited enzyme (K_m is unaffected) but different slopes and y-intercepts. Uncompetitive inhibition causes different intercepts on both the y- and x-axes.



ES Complex Formation

According to Michaelis — Menten theory, the enzyme E combines with the substrate S to form an intermediate enzyme-substrate complex ES. This complex then breaks down into product P and enzyme E is regenerated. The enzyme can again combine with the fresh molecule of the substrate in a similar manner. The formation of the enzyme-substrate complex as an intermediate during the reaction has been proved by spectroscopic studies. So, a simple enzymatic reaction might be written as:

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

Where E, S and P represent enzyme, substrate and product respectively. ES and EP are complexes of the enzyme with substrate and product respectively. At the end of the reaction along with the required products the enzyme is regenerated in its original form and can involve in another round of catalysis. ES complex is a highly energised, transiently existing complex which can be easily degraded to form the product. In the formation of enzyme-substrate complexes, the substrate molecules attach at certain specific sites on the enzyme molecules. This specific point on enzyme molecules where the substrate molecules attach is known as active site or catalytic site. Active sites on the enzymes are usually provided by a certain functional group of amino acids present in the enzyme protein. For example, the free hydroxyl group of serine, phenolic group of tyrosine, sulfhydryl group of cysteine and imidazolyl group of histidine are some of the important catalytic groups present in enzyme active sites.