(B) Hanes – Woolf plot :

- It is also a modified version of MM equation.

Hanes – woolf equation :

$$\frac{[S]}{V} = \frac{1}{V_{max}}[S] + \frac{K_M}{V_{max}} \quad \text{... (i)}$$

From the equation :

Plot

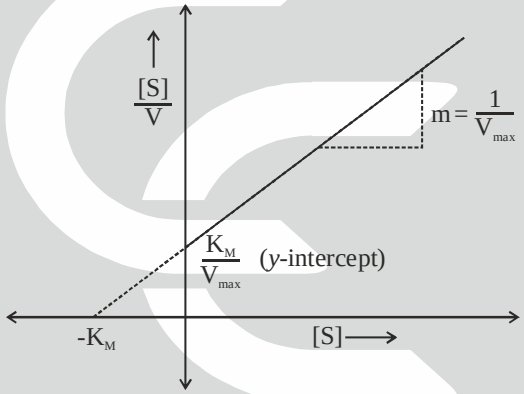
On y – axis $\rightarrow \frac{[S]}{V}$

On x – axis $\rightarrow$ [S]

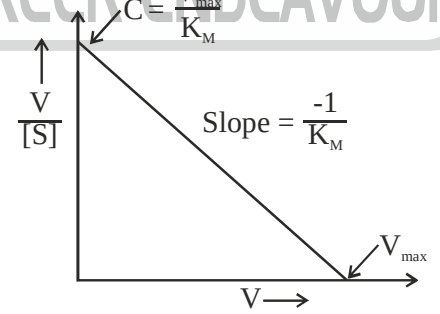
Comparing equation (i) with y = mx + C, we get

Slope, m $\rightarrow \frac{1}{V_{max}}$

y – intercept $\rightarrow \frac{K_M}{V_{max}}$



EADIE HOFSTEE PLOT :



$$\boxed{\frac{V}{[S]} = \frac{-V}{K_M} + \frac{V_{max}}{K_M}}$$

- Intercept on x-axis $\rightarrow V_{max}$.

SOLVED EXAMPLES

1. The best enzyme is that which has **[B.H.U.-2010]**

(a) Lowest Vmax / Km ratio (b) Vmax / Km ratio equal to 1

(c) Highest Vmax / Km ratio (d) None of the above

Soln. Km is the concentration of substrates when the reaction reaches half of Vmax. A small Km indicates high affinity since it means the reaction can reach half of Vmax in a small number of substrate concentration. This small Km will approach Vmax more quickly than high Km value. The ratio Vmax / Km is high means highest Vmax (increase the maximum velocity) and lowest Km (which increase the affinity of enzyme)

Correct option is (c)

2. Which of the following is true for enzyme catalyzed reaction? **[HCU-2015]**

(a) Enzymes force reactions to proceed in only one direction.

(b) Enzymes alter the equilibrium of the reaction.

(c) Enzymes alter the standard free energy of the reaction.

(d) Enzymes can couple energetically unfavorable reactions to favorable ones.

Soln. Catalysts lower the activation energy for reactions. The lower the activation energy for a reaction, the faster the rate. Thus enzymes speed up reactions by lowering activation energy. Many enzymes change shape when substrates bind.

Correct option is (d)

MEASURES OF ENZYME EFFICIENCY

- **Turnover Number (K_{cat}) :** It is equivalent to the no. of substrate molecules converted to product in a given unit of time on a single enzyme molecule when the enzyme is saturated with substrate.
- It is useful to define a more general rate constant, K_{cat}, to describe the limiting rate of any enzyme catalyzed reaction at saturation.
- If the reaction has several steps and one is clearly rate limiting, K_{cat} is equivalent to rate constant for that limiting step.
- For most of the enzymatic reaction, K_{cat} may be same as K_2 (rate constant for the formation of product).
- At saturation of substrate,

$V = V_{max}$

$V_{max} = [E_t]K_2$ or $V_{max} = K_{cat} \times [E_t]$

$$\boxed{K_2 = K_{cat} = \frac{V_{max}}{[E_t]}}$$

- It represents the kinetic efficiency of an enzyme.
- Its units is s^{-1}.
- For Eg. Carbonic anhydrase has a turnover number of 400,000 to 600,000 s^{-1} which means that each carbonic anhydrase molecule can produce up to maximum 600,000 molecules of product per second.

Specificity Constant $\left(\frac{K_{cat}}{K_M}\right)$:

- Specificity constant is a measure of how efficiently an enzyme converts substrates into products.
- K_{cat}, K_M allow us to evaluate the kinetic efficiency of enzymes, but either parameter alone is insufficient for this task.
- Two enzymes catalyzing different reactions may have the same K_{cat}, yet the rates of the uncatalyzed reactions

may be different and thus the rate enhancements brought about by the enzymes may differ greatly.

- Experimentally, the K_M for an enzyme tends to be similar to the cellular concenteration of its substrate.
- The best way to compare the catalytic efficiencies of different enzymes or the turnover of different substrates by the same enzyme is to compare the ratio K_{cat}/K_M for the two reactions which is called as specificity constant.

$$\text{Specificity constant} = \frac{K_{cat}}{K_M}$$

- Specificity constant as this is obtained by equating in MM equation as below.

$$V = \frac{K_{cat}}{K_M}[E_t][S] (\text{when } [S] <<< K_M[E] = [E_t])$$

- Its units are $M^{-1}S^{-1}$ (second order rate constant).
- This specificity constant is often used to measure enzyme efficiency.

Enzyme	$K_{cat}(s^{-1})$	$\frac{K_{cat}}{K_m}(M^{-1}s^{-1})$	Substrate
1. Acetyl cholinesterase	1.4×10^4	1.6×10^8	Acetyl choline
2. Catalase	4×10^7	4×10^7	H_2O_2
3. Carbonic Anhydase	4×10^5	1.5×10^7	HCO_3^-

1. At what [S] is the velocity (V) of an enzyme catalyzed reaction 25% of the V_{max} ?

Soln. The Michaelis – Menten equation,

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

$$V = \frac{1}{4}V_{max}$$

$$\frac{V_{max}}{4} = \frac{V_{max}[S]}{K_M + [S]}$$

$$K_M + [S] = 4[S]$$

$$K_M = 3[S]$$

$$[S] = \frac{K_M}{3}$$

2. An enzyme is discovered that catalyzes the chemical reaction,

$$\text{SAD} \rightleftharpoons \text{HAPPY}$$

A team of researchers sets out to study the enzyme, which they call happyase. They found that the turnover no. is 600 s^{-1} for happyase. They carried out several experiments.

When $[E_t]$ = 20 nM and [SAD] = 40 μ M, the reaction velocity, V is 9.6 μ Ms^{-1}. Calculate the K_M for the substrate SAD.

Soln. $K_{cat} = 600s^{-1}, E_t = 20nM = 0.020\,\mu M$

$$V_{max} = K_{cat}[E_t]$$

$$V_{max} = 600 \times 0.020\,\mu Ms^{-1}$$

$$V_{max} = 12\,\mu Ms^{-1}$$

Michaelis – Menten Equation,

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

$$9.6\,\mu M s^{-1} = \frac{12\mu M s^{-1} \times 40\,\mu M}{K_M + 40\,\mu M}$$

$$K_M + 40\,\mu M = \frac{480\mu M^2 s^{-1}}{9.6\,\mu M s^{-1}}$$

$$K_M + 40\,\mu M = 50\,\mu M$$

$$K_M = 10\,\mu M .$$

ORDER OF ENZYME KINETICS

(i) Zero order reaction :

Usually we observe the kinetics of enzymes in the post stabilization state when the ES complex has been stabilised, enzyme will follow zero order kinetics.

When the reaction rate is independent of the substrate concentration, the reaction is said to be of "zero order".

Rate = $k[S]^0$ (k is constant)

For zero order reaction, substrate must be present in an excess amount i.e. [S] >> K_m then Michaelis – Menten equation becomes

$V = \frac{V_{max} \times [S]}{[S] + K_m}$, since [S] >> K_m therefore we can ignore K_m in the denominator, hence

$V = \frac{V_{max} \times [S]}{[S]}$, we get $V = V_{max}$

Therefore, when $[S] >> K_M$, so $V = V_{max}$.

This means that the rate is equal to maximum velocity and is independent of the substrate concenteration. The reaction is zero-order kinetics.

(ii) First order reaction :

If the reaction rate is dependent only on the concentration of substrate, then it is first order reaction.

Rate = k[S], this occurs when [S] << K_M.

Michaelis – Menten equation

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

When [S] << K_M, MM equation reduces to $V = \frac{V_{max} \times [S]}{K_m}$

where $k = \frac{V_{max}}{K_m}$, hence

[Rate] V = k[S]

This means that the rate and the substrate concenteration are directly proportional to each other. The reaction is first – order kinetics. Usually the linear part of MM curve will depict the first order reaction.