LINE WEAVER- BURK DOUBLE RECIPROCAL PLOT:- For the determination of Km value, Substrate saturation curve is not very accurate since V_{max} is approached asymptotically

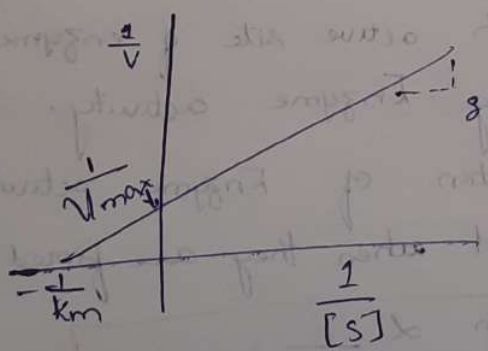
By taking reciprocal of equation ① in Km equation a straight line graph will be obtained.

$$\frac{1}{V} = \frac{Km + [S]}{V_{max}[S]}$$

$$\frac{1}{V} = \frac{Km}{V_{max}} \times \frac{1}{[S]} + \frac{[S]}{V_{max}[S]}$$

$$\frac{1}{V} = \frac{Km}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

$$y = ax + b$$



slope $= \frac{Km}{V_{max}}$

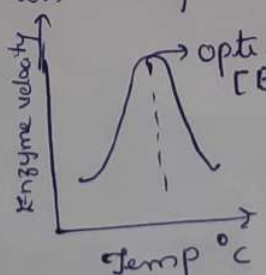
★ G-plot

plot of $\frac{1}{V}$ Vs $\frac{1}{[S]}$ gives Straight line

slope $= \frac{Km}{V_{max}}$

y Intercept $= \frac{1}{V_{max}}$

③ EFFECT OF TEMPERATURE:- Velocity of an enzyme reaction increase with increase in temperature up to a maximum and then declines (↓).



★ Taq DNA polymerase, Muscle adenylate kinase are active at 100°C (Exception).

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By taking reciprocal of equation (1) in K_m equation a straight line graph will be obtained.

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

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

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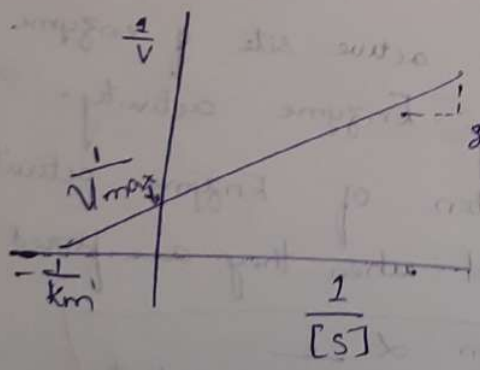
$$Y = ax + b$$



slope = $\frac{K_m}{V_{max}}$

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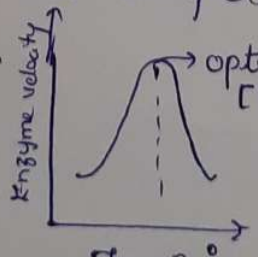


optimum temperature (35°c - 40°c) [Bell shaped curve]

★ Taq DNA polymerase, Muscle adenylate kinase are active at 100°c (Exception)

Let us assume that the measured velocity $V = \frac{1}{2} V_{max}$

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

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

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

$$K_m = 2[S] - [S]$$

* $\boxed{K_m = S}$

Michaelis-Menten constant is defined as the substrate concentration required to produce 1/2 maximum velocity in an enzyme catalysed reaction.

It indicates half of enzyme molecules are bound with the substrate molecules when substrate concentration equals K_m value.

K_m value is constant & characteristic feature of given enzyme.

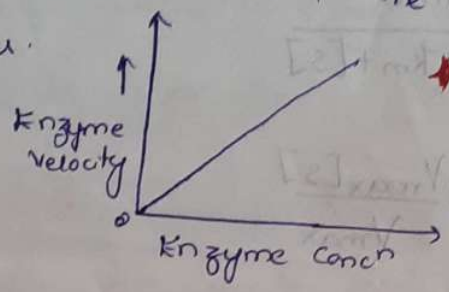
low K_m value → strong affinity between Enzyme & Substrate

high K_m value → low affinity

K_m is independent of concentration of enzyme

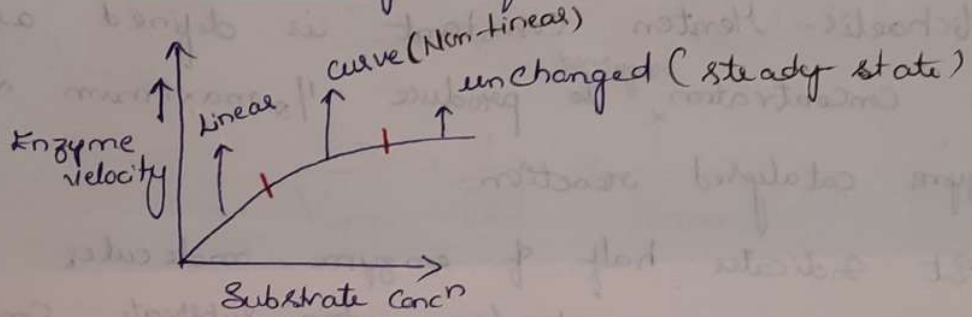
4 pat bits

CENTRATION OF ENZYME :- As the concentration of the nzyme is increased (↑), the velocity of reaction also increases.



Enzyme Conc^n ∝ Enzyme velocity

CONCENTRATION OF SUBSTRATE :- Increase in the substrate concentration gradually increases the velocity of enzyme reaction with in limited range of substrate levels.



Enzyme kinetics & Km value :- The enzyme [E] and substrate [S] combine with each other to form an unstable enzyme - substrate complex [ES] for formation of product.

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

$k_1, k_2, k_3 \longrightarrow$ velocity constants

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

K_m = Michaelis - Menten constant / Briggs & Haldanes Constant

The following equation after suitable algebraic manipulation.

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

v = measured velocity
V_{max} = Maximum Velocity
S = Substrate Concentration
K_m = Michaelis - Menten Constant.

(5) Isomerases :- These are the enzymes that catalyse Isomerisation reaction.

Ex:- Retind Isomerase, Keto Isomerase, phosphohexose Isom

$$A \longrightarrow A'$$

$$\text{Glucose-6-phosphate} \xleftarrow[\text{Isomerase}]{\text{phospho hexose}} \text{Fructose-6-phosphate.}$$

(6) Ligases :- These are the enzymes that catalyses Synthetic reactions where two molecules are joined together by using ATP.

$$A+B \xrightarrow[ATP \quad ADp+pi]{} A-B$$

Ex :- Succinate thiokinase, Glut amine Synthetase

FACTORS AFFECTING ENZYME ACTIVITY :- The following are the factors that affect enzyme activity.

1) Concentration of Enzyme
2) Concentration of Substrate
3) Concentration of product
4) Effect of temperature
5) Effect of pH
6) Effect of Activators
7) Effect of Time
8) Effect of Light and radiation

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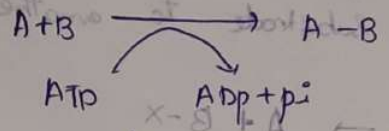

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