VIVEKANANDA COLLEGE
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NAAC ACCREDITED ‘A’ GRADE

Topic	: Enzyme Kinetics
Course Title	: Enzymes
Paper	: CC4
Unit	: II
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Name of the Department	: Biochemistry

Lineweaver – Burk plot

From Michaelis-Menten equation

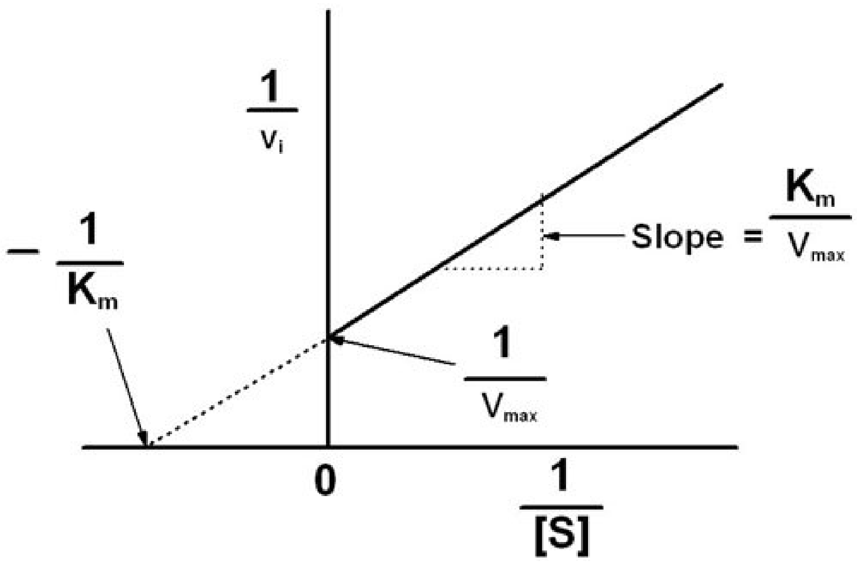
$\mathbf{V_0 = V_{max} [S] / K_m + [S]}$

or, $1/V_0 = K_m + [S] / V_{max} [S]$

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

This is **Lineweaver - Burk equation** -------A straight line equation

- Michaelis – Menten hyperbolic graph does not give the accurate value of V_{max}.
- Hence **inverse of velocity and inverse of substrate concentration gives a straight line**.
- This plot is known as **Double Reciprocal plot or Lineweaver – Burk plot**.



From this straight line plot, V_{max} and K_m value are accurately measured.

Eadie – Hofstee plot

- In LB plot, the extrapolation across the 1/V axis to determine the value of $-1/K_m$ reaches the edge of the graph before reaching the 1/[S] axis.
- This problem is circumvented by Eadie – Hofstee (EH) plot.
- The Eadie – Hofstee plot does not require a long extrapolation to calculate K_m.

EH Equation :

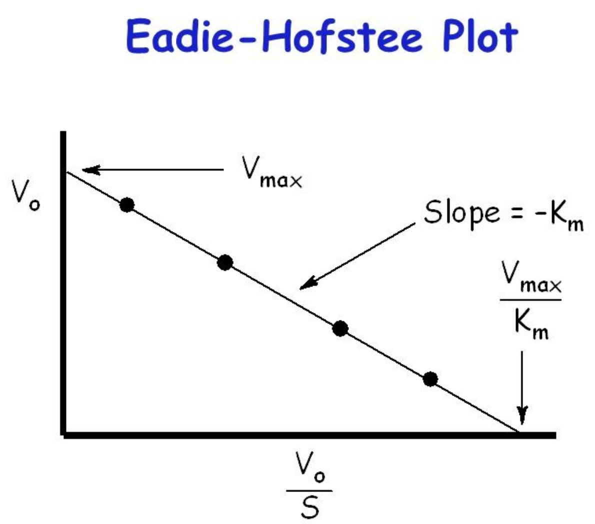
$1/V_0 = K_m / V_{max} [S] + 1/V_{max}$ …….LB equation

Multiplying $V_0 . V_{max}$ in both sides

$V_{max} = K_m (V_0 /[S]) + V_0$

or $\mathbf{V_0 = - K_m (V_0 / [S]) + V_{max}}$ …….EH equation

By plotting V_0 vs $V_0/[S]$, EH plot gives K_m and V_{max} value

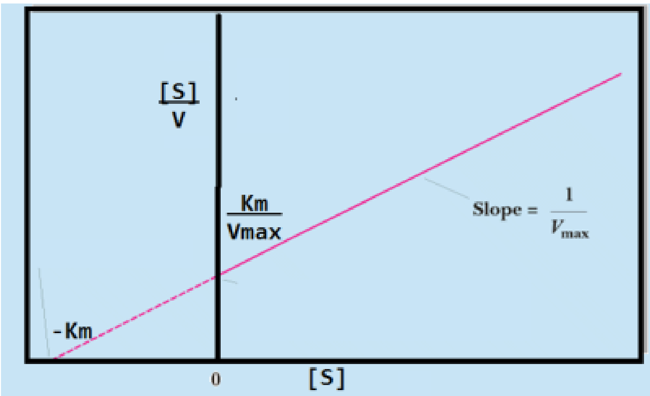


Hanes plot

- The LB plot is further modified to give a straight line that intercepts Y-axis only.
- Hanes equation is derived from the LB equation:

  $1/V = K_m / V_{max} [S] + 1 / V_{max}$
- Multiplying by [S] on both sides,

  $[S] / V = K_m / V_{max} + [S] / V_{max}$ …….Hanes equation
- **Hanes plot** is a graphical representation of enzyme kinetics in which the ratio of the initial substrate concentration [S] to the reaction velocity V is plotted against [S].



- The Hanes plot was used for rapid determination of the important kinetic parameters K_m and V_{max}.

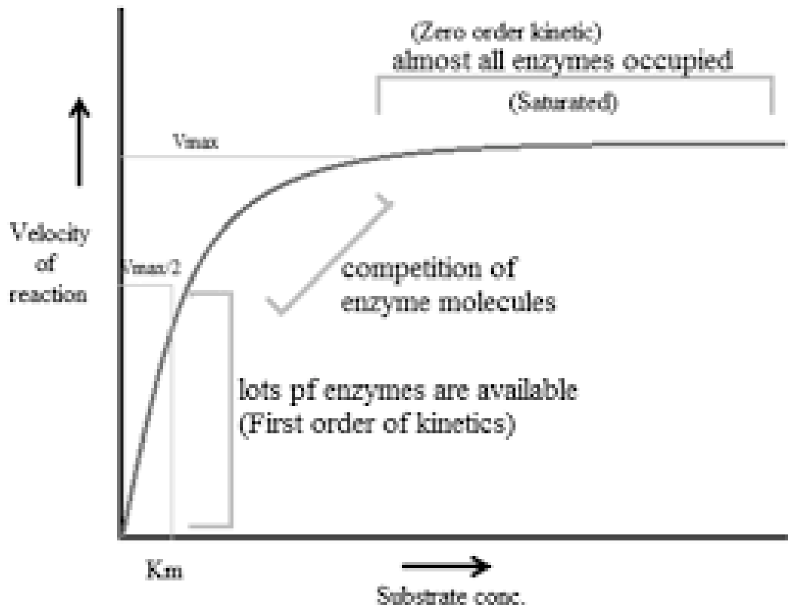
K_m (Michaelis constant)

- K_m is constant for particular enzyme and substrate.
- K_m does not change with [S]
- K_m changes with change in pH or temperature of the reaction
- When $V_0 = ½ V_{max}$, $K_m = [S]$ (from Michaelis-Menten equation)
- Unit of K_m will be [S], expressed as Molar term
- It indicates the affinity of the enzyme for its substrate

- When an Enzyme acts on more than one Substrate, the Substrate for which K_m is least, is the best substrate for that particular enzyme.

V_{max} (Maximum Velocity)

- The maximal velocity of a reaction, V_{max}, occurs at high [S].
- The enzyme is fully saturated with the substrate i.e. total enzymes entirely form ES complex.
- $V_{max} = k_2[E_T]$ (follows Michaelis-Menten mechanism)



K_{cat} and Turn over number

- Turn over no. is defined as number of S molecules converted to P in a given unit of time on a single enzyme molecule -----when E is saturated with S.

- The rate constant k_2 is also equal to the turn over number of the enzyme

- Catalytic constant (**k_{cat}**) of an enzyme also called **turn over number**

- It is the number of reaction processes (turnovers) that each active site catalyses per unit time.

- The larger k_{cat} ---- the more favourable the reaction towards product

- Unit of turn over number is min^{-1}

- $k_{cat} = V_{max} / [E_T]$ ------ ($k_2 = k_{cat}$)

k_{cat} / K_m

- Kinetic parameter (k_{cat} / K_m) provides a **measure of the catalytic efficiency**.

- The higher the k_{cat} / K_m, the more "efficient" the enzyme

- $k_{cat} / K_m = k_2 / K_m = k_2 k_1 / k_{-1} + k_2$

- This ratio is **maximal** when **$k_2 >> k_{-1}$** ----formation of P from ES is fast compared to its decomposition back to S and E.

- □ $k_{cat} / K_m = k_1$

- It is a second order rate constant for the formation of ES

- Unit $M^{-1}s^{-1}$

- It is sometimes called **specificity constant**

References :

1. Lehninger : Principles of Biochemistry, 4th Edition, Nelson and Cox, W.H.Freeman and Company (New York).

2. Biochemistry, 3rd Edition, Voet and Voet, John Wiley & Sons

3. www.google.com