3.

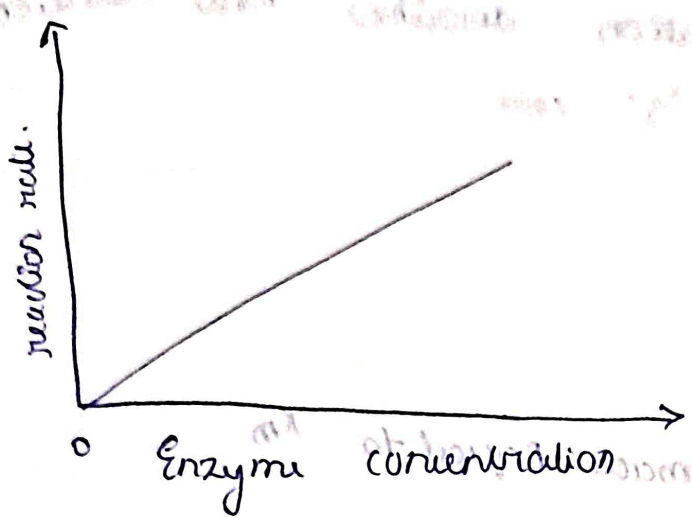
Factors affecting Enzyme activity

- Enzyme concentration
- Substrate concentration
- temperature
- pH (Hydrogen ion concentration)
- product accumulation
- Time.
- presence of activators.
- presence of inhibitors.

the rate of velocity of a reaction (V) is the no. of substrate molecules converted to product per unit time.

(1) Enzyme Concentration.

- rate of rxn or velocity (V) is directly proportional to the enzyme concentration, when sufficient substrate is present.
- This properly made use of in determining the level of particular enzyme in plasma, serum or tissue.

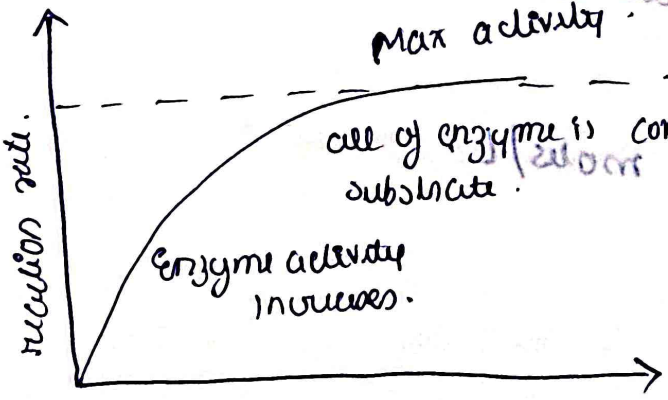


(Straight line graph.)

2. Effect of Substrate Concentration.

- As substrate conc is increased, velocity increases until it reaches a max value - Vmax.
- Vmax is the maximum velocity obtained in presence of excess substrate.
- The levelling off at high [S] conc. reflects the saturation of all binding sites of 'E' with 'S'.

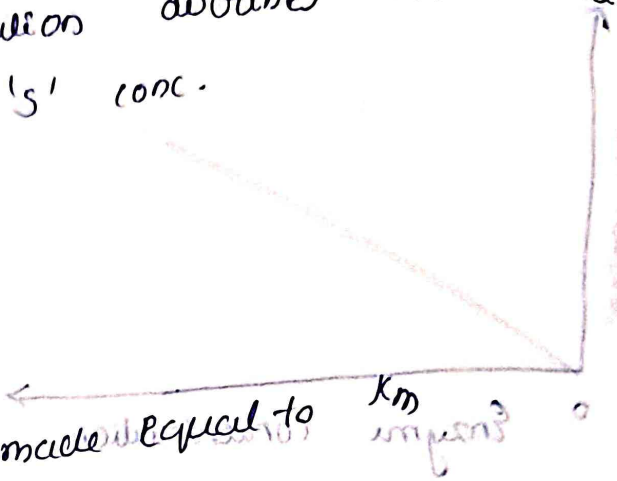
hyperbolic graph



the plot of Vo - initial vel. vs Substrate conc. is hyperbolic

- Michaelis menton equation describes how reaction velocity varies with 'S' conc.

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



- when conc. of [S] is made equal to K_m (Michaelis constant).

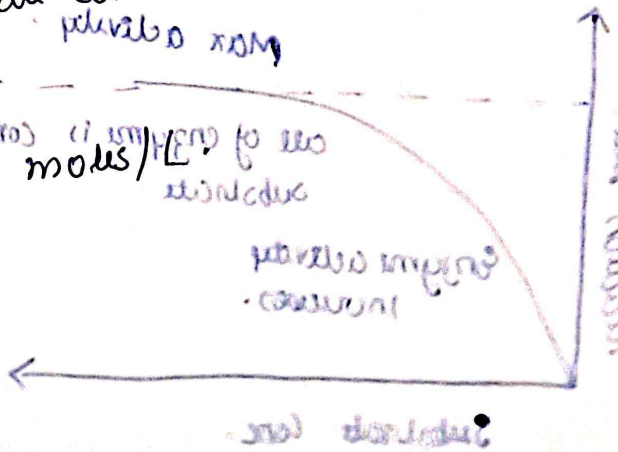
$$V = \frac{1}{2} V_{max}$$

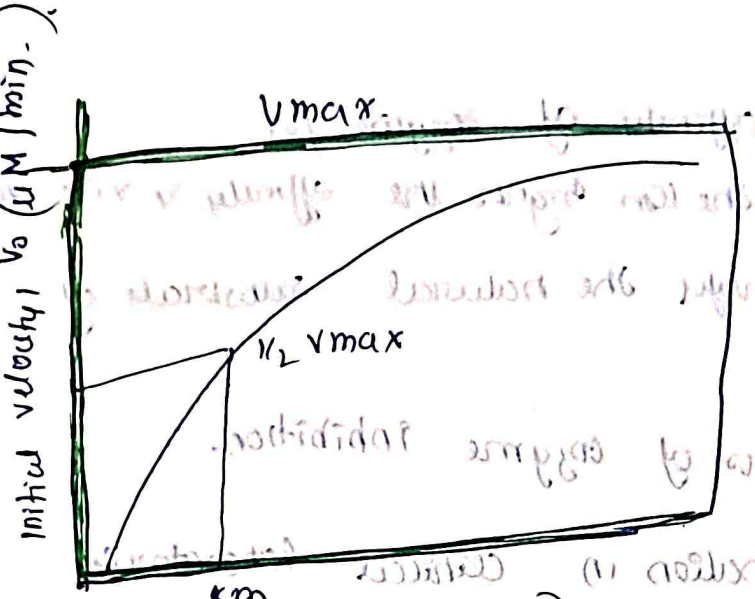
{ Velocity at which half the number of enzyme is saturated with the substrate - $\frac{1}{2} V_{max}$ }

VVImpt.

What is K_m?

- K_m is the substrate conc. at half maximum velocity.
- It denotes that half of enzyme molecules (50%) are bound with the substrate at that particular substrate conc.
- K_m is expressed in moles/L.





Km values of most of enzymes are within the range 10^{-5} to 10^{-2} moles/L.

- Km is the signature of the enzyme, or

∴ It is constant for an enzyme and is the characteristic feature of a particular enzyme for a specific substrate.

Significance of Km:

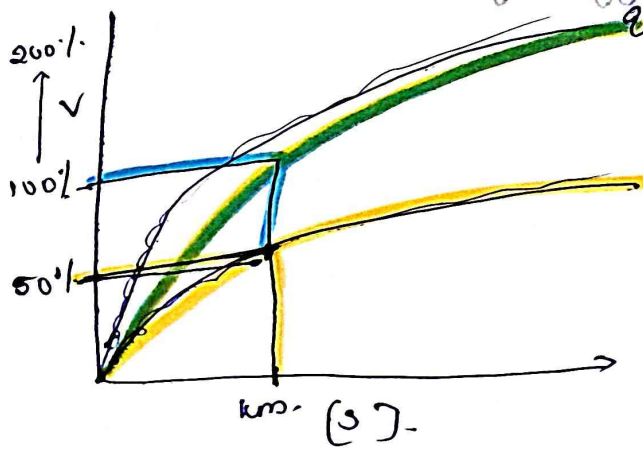
(1) Km denotes the affinity of enzyme for Substrate. - Lower the Km higher the affinity & vice versa.

(2) Km help to identify the natural substrate of an enzyme.

(3) It is the evaluator of enzyme inhibition.

(4) For enzyme estimation in clinical laboratories Km of the enzyme gives an idea of the amount of S to be added to the reaction medium.

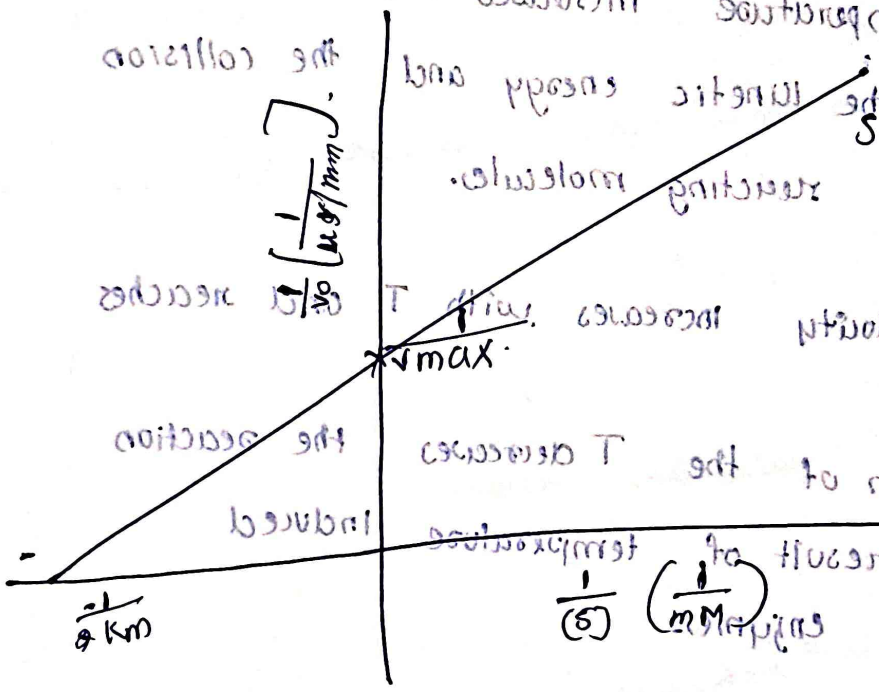
- Km is independent of enzyme conc. = If enzyme conc. is doubled, v_{max} will be doubled. But Km will remain exactly same.



Lineweaver – Burk plot or Double reciprocal plot.

- If the reciprocal $(1/x)$ of the Michaelis – Menten equation is done, after algebraic simplification the following equation results.

$$\frac{1}{Vs} = \frac{Km}{Vmax} \frac{1}{[S]} + \frac{1}{Vmax}$$

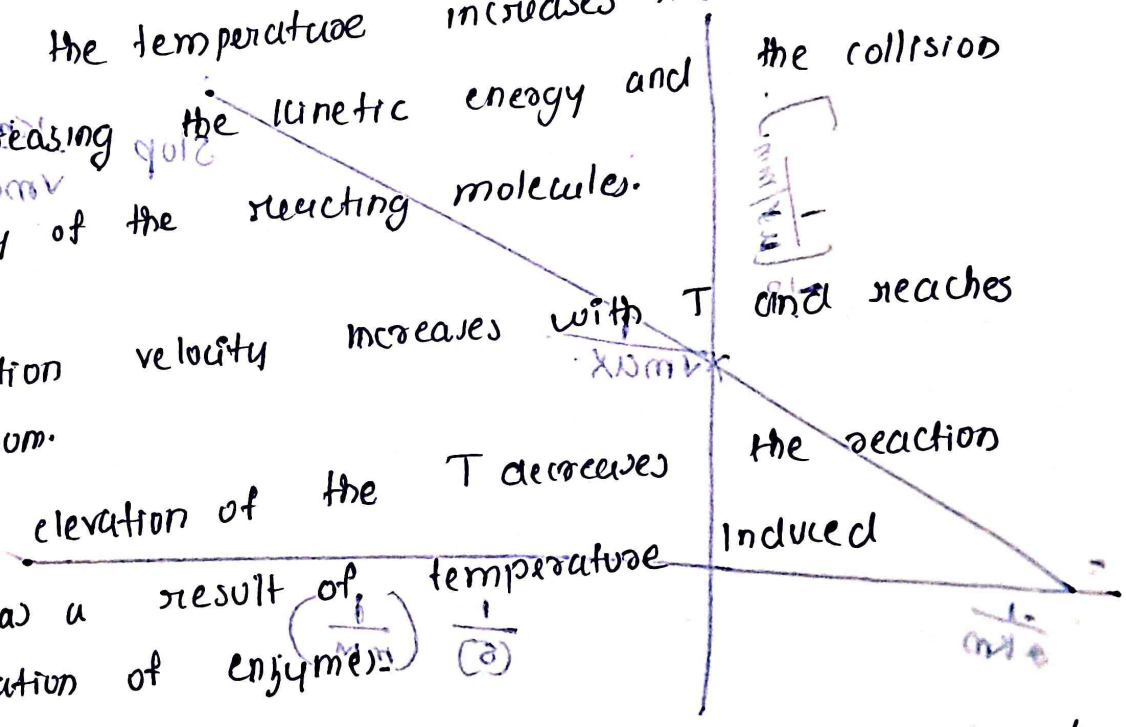


- Biggest advantage to using the double reciprocal plot is a more accurate determination of V_{max} and hence K_m.
- also useful in characterizing effects of Enzyme inhibition.

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

3. temperature.

- Raising the temperature increases the reaction rate by increasing the kinetic energy and the collision frequency of the reacting molecules.
- The reaction velocity increases with T and reaches a maximum.
- Further elevation of the T decreases the reaction velocity as a result of temperature induced denaturation of enzymes.

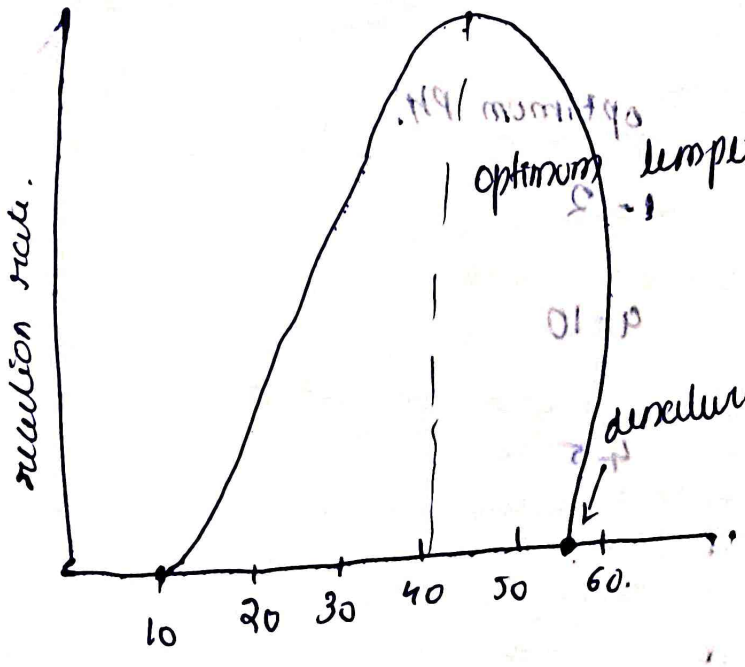


- optimum temperature: T at which maximum amount of substrate is converted to product per unit time.
- most human enzymes have optimum T around 37°C.

- when the temperature is more than 50°C, enzymes start to denature and consequent loss of tertiary structure of protein
- enzyme activity decreased.

- The temperature coefficient (Q_{10}) is the factor by which the rate of catalysis is increased by a rise in 10°C.

The graph of reaction velocity against T is bell shaped.

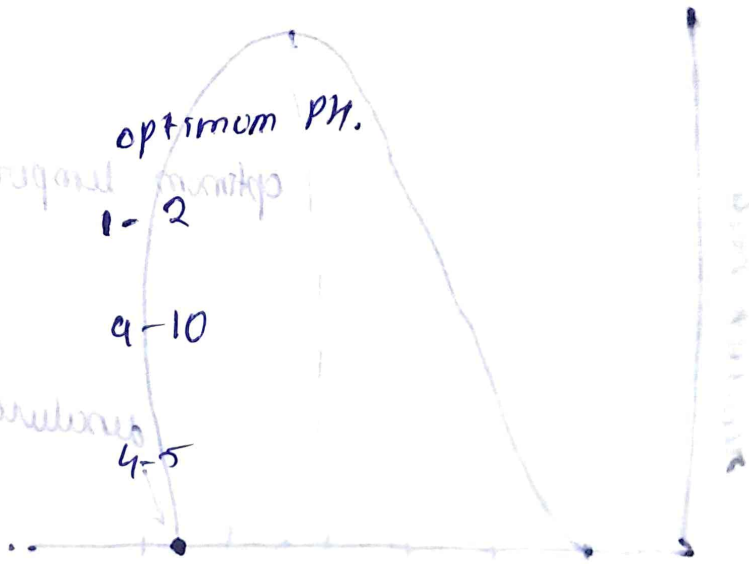


4. Effect of pH.

- Enzymes are most active at optimum pH.
- Amino acids with acidic or basic side-chains at the active site have the proper charges when the pH is optimum.
- Enzyme activity is lost at low / high pH as tertiary structure is disrupted.

Usually Enzymes have optimum pH between 6 & 8.

Important Exceptions.

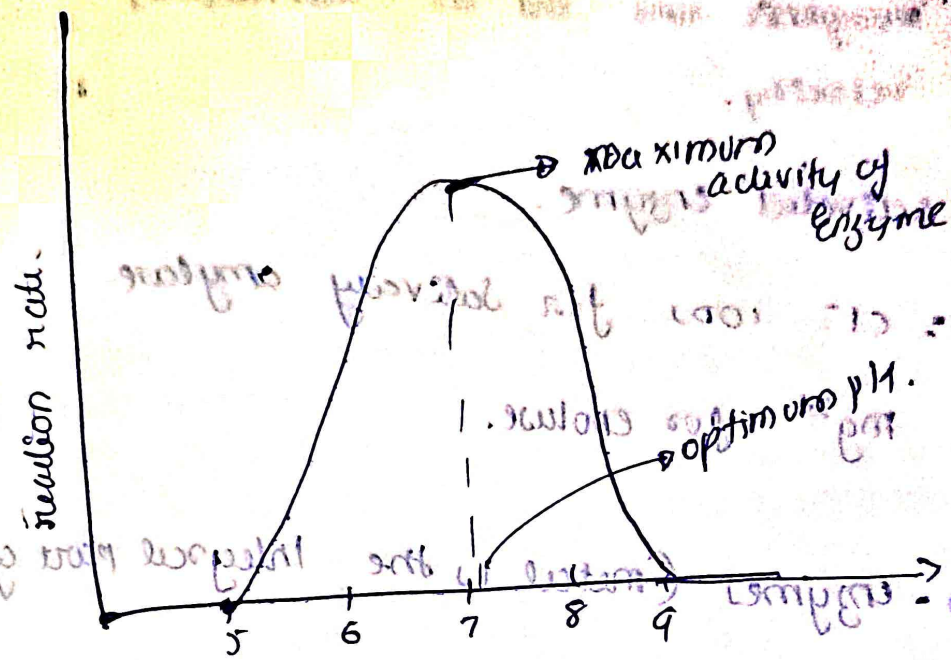


Enzyme.

pepsin.

alkaline phosphatase.

acid phosphatase.



• Bell shaped graph. ✓ (mark for this)

pH.

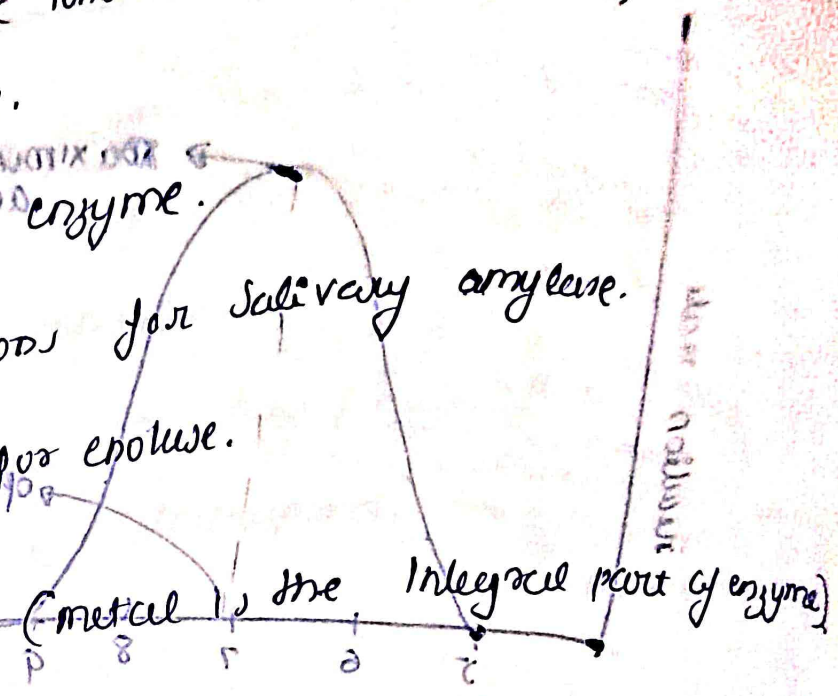
eg: copper in reaction

5. effect of activators.

- certain inorganic ions act as activators, increase velocity.
- metal activated enzyme.

eg: Cl^- ions for salivary amylase.

Mg^{2+} for epoluse.



- metallo-enzymes (metal is the integral part of enzyme)

eg: copper in tyrosine.

6. Effect of Inhibitors.

- presence of inhibitors decrease velocity.

7. effect of product accumulation.

- when product ↑ increased the reaction is slowed, stopped or reversed.

8. effect of time.

- velocity decreases with time.