UNIT => 5

Enzyme

- Enzyme are biological catalysts that speed up the rate of the biochemical reaction.
- Most of the enzyme are protein in nature.

→ Properties of Enzymes

- Biological catalyst
- Speeds up rate of reaction
- Enzymes are protein
- Enzymes are specific to a substrate of reaction
- Enzymes are reversible & can catalyzed a reaction going both ways.
- Enzymes are denatured by -change in temperature or pH.

→ Nomenclature of Enzyme

i> Enzyme acted on the substrate.

- The molecules upon which enzyme acts is known as substrate.
- then naming the enzyme by adding the suffix -ase in the name of substrate.

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suffix

eg ⇒ Substrate — -ase

Maltose → Maltase
lactose → lactase
Lipid → Lipase

i) we add -ase in suffix at the name of process

suffix

eg ⇒ Process — -ase

DNA polymerization → DNA polymerase

→ International union of Biochemistry (IUB)

- IUB established in 1955.
- They formed 'Enzyme commission'

↓

⇒ During future enzymes, how we will gave names & classify it.

→ classification of Enzymes (Six types/divided)

1) → Oxidoreductases
- those enzymes involved in oxidat^n-Reduct^n

eg ⇒ Glucose-6-Phosphate dehydrogenase

2) → Transferases
- Group Enzymes involved in group transfer

eg ⇒ Hexokinase, Transketolase

3) → Hydrolyses → involved in hydrolysis

eg ⇒ Maltase, lactase.

4) → Lyases → involve in the process of add^n or removal of group from the substrate

eg ⇒ Aldolase, fumerase

5) → Isomerases → enzymes involved to interconversion of isomer.

eg ⇒ Phosphotriose Psomerase.

6) → Ligases → enzyme involved in joining of two molecules using ATP.

eg ⇒ DNA Ligase, Succinate thiokinase.

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Enzyme kinetics :

- Enzyme kinetics is the study of the chemical reactions that are catalysed by enzyme.
- The target molecules means substrate binds to enzyme active site & transformed into product through a series of step known as enzymatic mechanism.

→ Factors affecting rate of reaction :

i) The collision or kinetic theory :

- collision theory states that the two molecules approaches within a bond forming distance of one another for reaction. So, the molecules need to sufficient kinetic energy to remove the barrier towards each other & form the product by reaching transition state.

↓

[old bond break new bond form]

eg ⇒ $AB + C \rightleftharpoons [A \cdots B \cdots C] \rightarrow A\text{-}C + B$

ii) Activation Energy :

- The energy require to activate or remove the energy barrier b/w between the substrate to reach at transition state is known as Activation energy.

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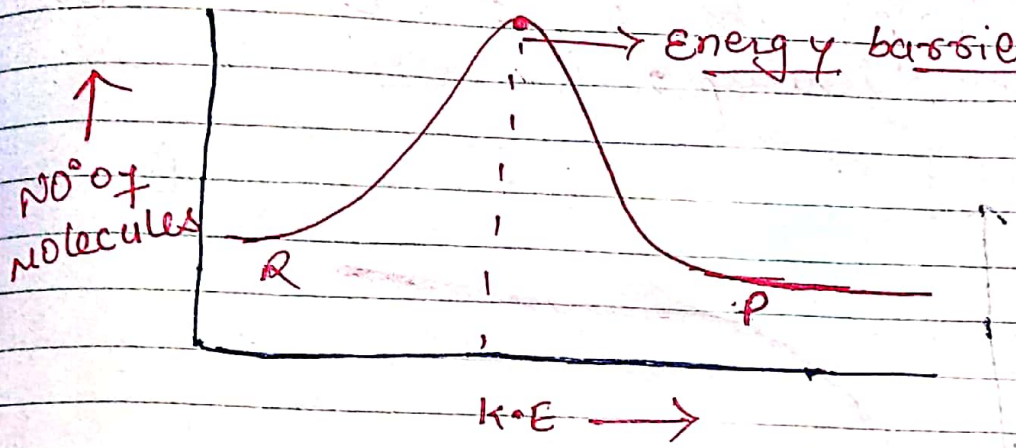
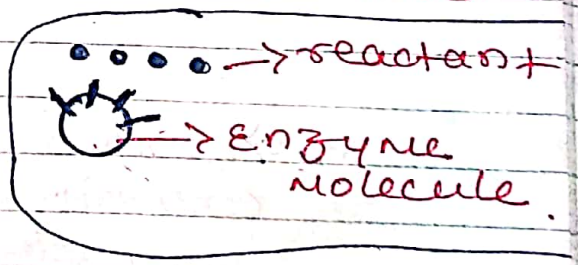
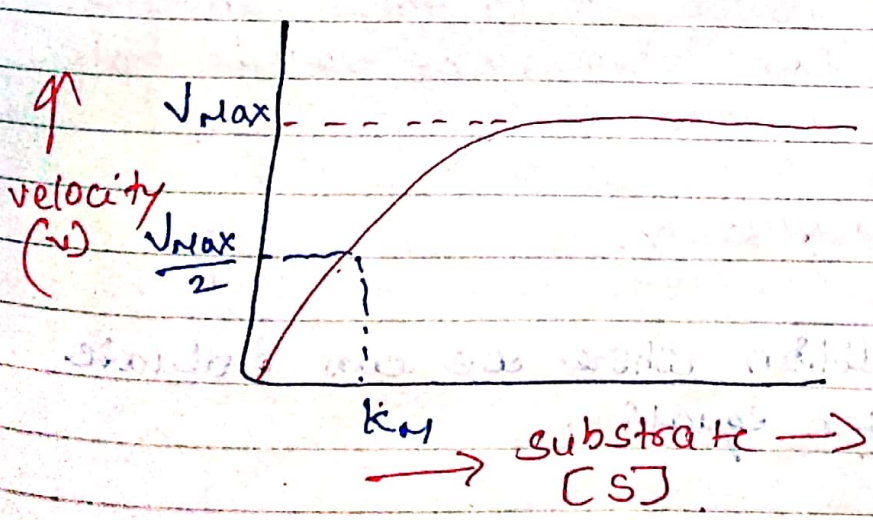
In a reaction there is an optimum number of atoms that collide colloids to form the product & the energy of this optimum atom is known as activation energy.



→ conc. of the substrate affect enzyme activity :

- The conc. of substrate is increase, the substrate molecule combined with active sites of enzyme molecule & no more active sites available, On this stage it reaches to no further inc. in the rate of reaction





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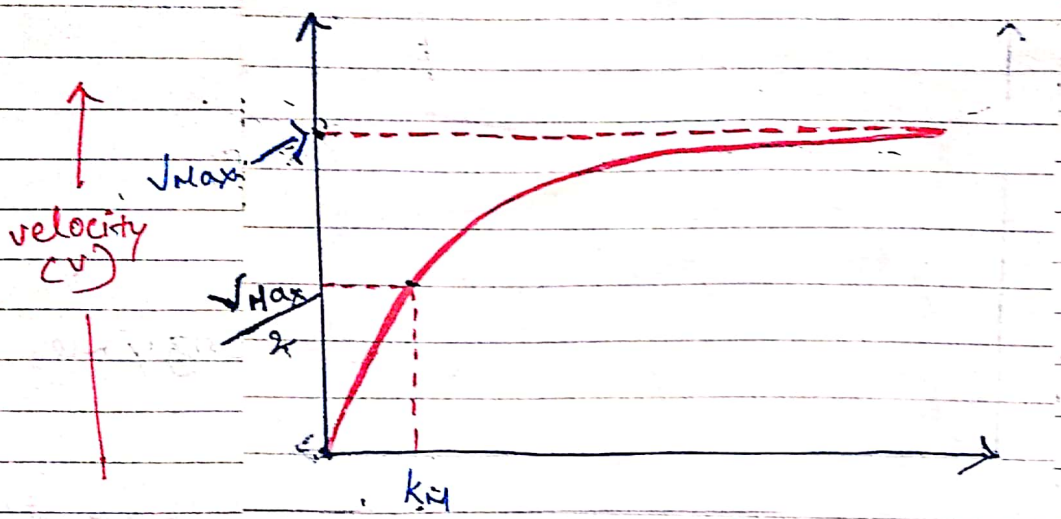
ENZYME KINETICS

Michaelis – Menten Equation :

- Michaelis – Menten eqn gives the Mathematical relationship between initial velocity reaction (V_i) & substrate conc. [S] shown graphically,



The equation $V_i = \frac{V_{MAX}[S]}{K_M + [S]}$

K_M ⇒ Michaeli's const. it is the substrate constant when velocity of rxn is half of maximum velocity.

V_{MAX} ⇒ Maximum velocity.

- There are 3 condition where we can evaluate Michaeli's – Menten equation.

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i) when [S] is much less than K_M :

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

$$V_i = \frac{V_{max} \cdot [S]}{K_M} \quad [\because \text{neglect } + [S]]$$

$$V_i \propto \left(\frac{V_{max}}{K_M}\right) \cdot [S]$$

Hence, initial velocity (V_i) $\propto$ [S].
→ substrate.

ii) when substrate is much greater than K_M :

$$V_i = \frac{V_{max} \cdot [S]}{[S]} \quad [\because \text{neglect} \rightarrow K_M]$$

$$\boxed{V_i = V_{max}}$$

iii) when [S] is equal to K_M.

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

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

$$\boxed{V_i = \frac{V_{max}}{2}}$$

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Lineweaver – Burke Plot

- When V_{max} is ploted against S in Meachaeli's Menten eqn it is difficult to fix the point of V_{max} (Max. velocity)

- This difficulty is overcome by reciprocal of ~~V & [S]~~ V & [S] then plot. The resulting curve is linear & is called Lineweaver-burke plot. Then K_M & V_{max} can be easily obtained from the plot.

$$V_i = \frac{V_{max} \times [S]}{K_M + [S]}$$ [From Mechaeli's Menten eqn]

$$\frac{1}{V_i} = \frac{K_M + [S]}{V_{max} \times [S]}$$ [reciprocal it]

$$\frac{1}{V_i} = \frac{K_M}{V_{max} \times [S]} + \frac{[S]}{V_{max} \times [S]}$$

$$\frac{1}{V_i} = \frac{K_M}{V_{max} \times [S]} + \frac{1}{V_{max}}$$

- This is an eqn for straight line

$$y = ax + b$$

$\boxed{y = \frac{1}{V_i}}$, $\boxed{x = \frac{1}{[S]}}$, $\boxed{a = \frac{K_M}{V_{max}}}$, $\boxed{b = \frac{1}{V_{max}}}$

y-axis, x-axis.

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- So, $\frac{1}{v_i}$ is plotted as a funct^n of $\frac{1}{[S]}$ gives a straight line whose intercept is $\frac{1}{V_{max}}$ & slope is $\frac{K_M}{V_{max}}$.

- Now in eqn $y = ax + b$

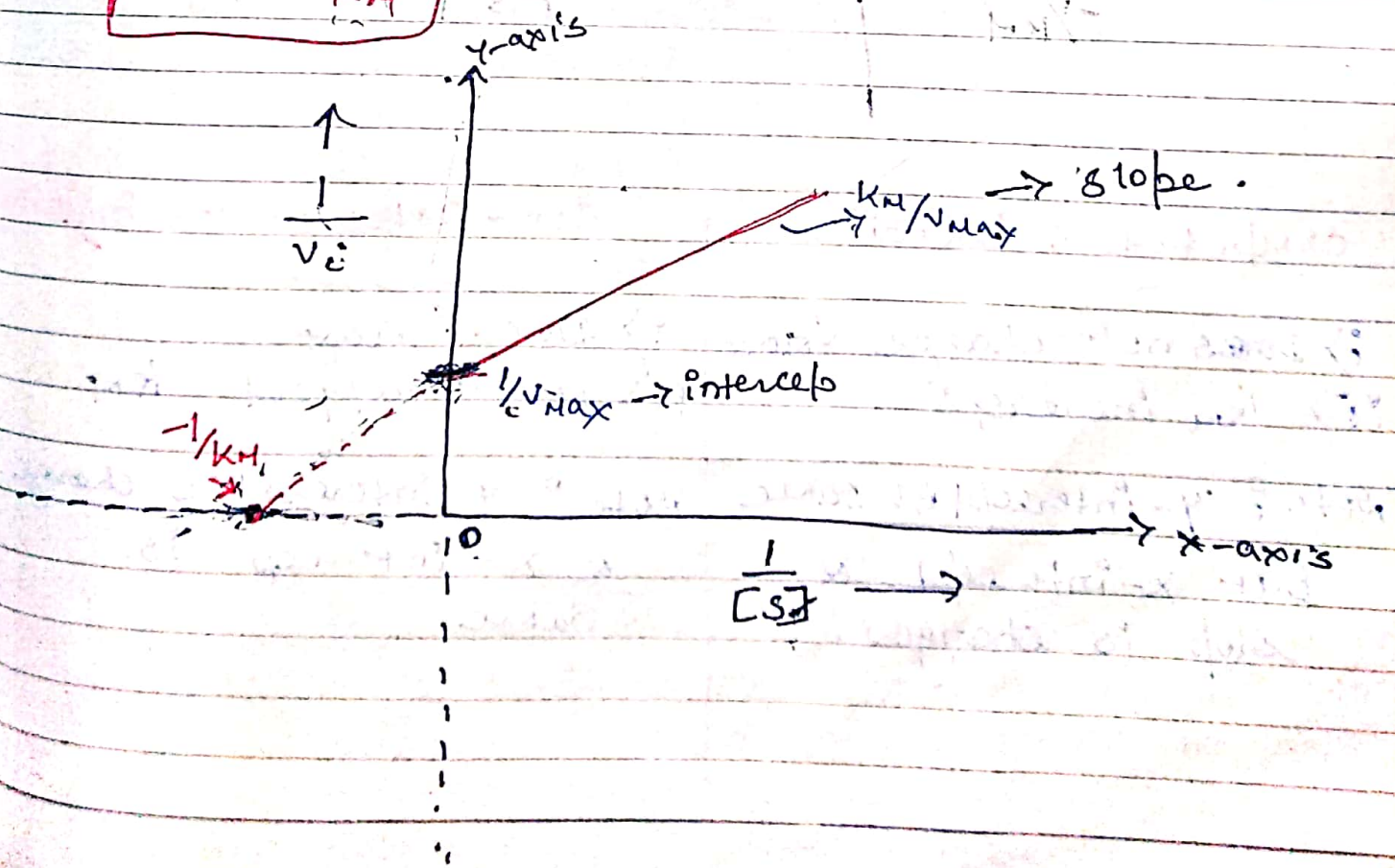
put, $y = 0$

$0 = ax + b$

$-ax = b$.

$x = \frac{-b}{a}$ $\Rightarrow$ $x = \frac{-1}{V_{max}} \times \frac{V_{max}}{K_M}$.

$$x = \frac{-1}{K_M}$$



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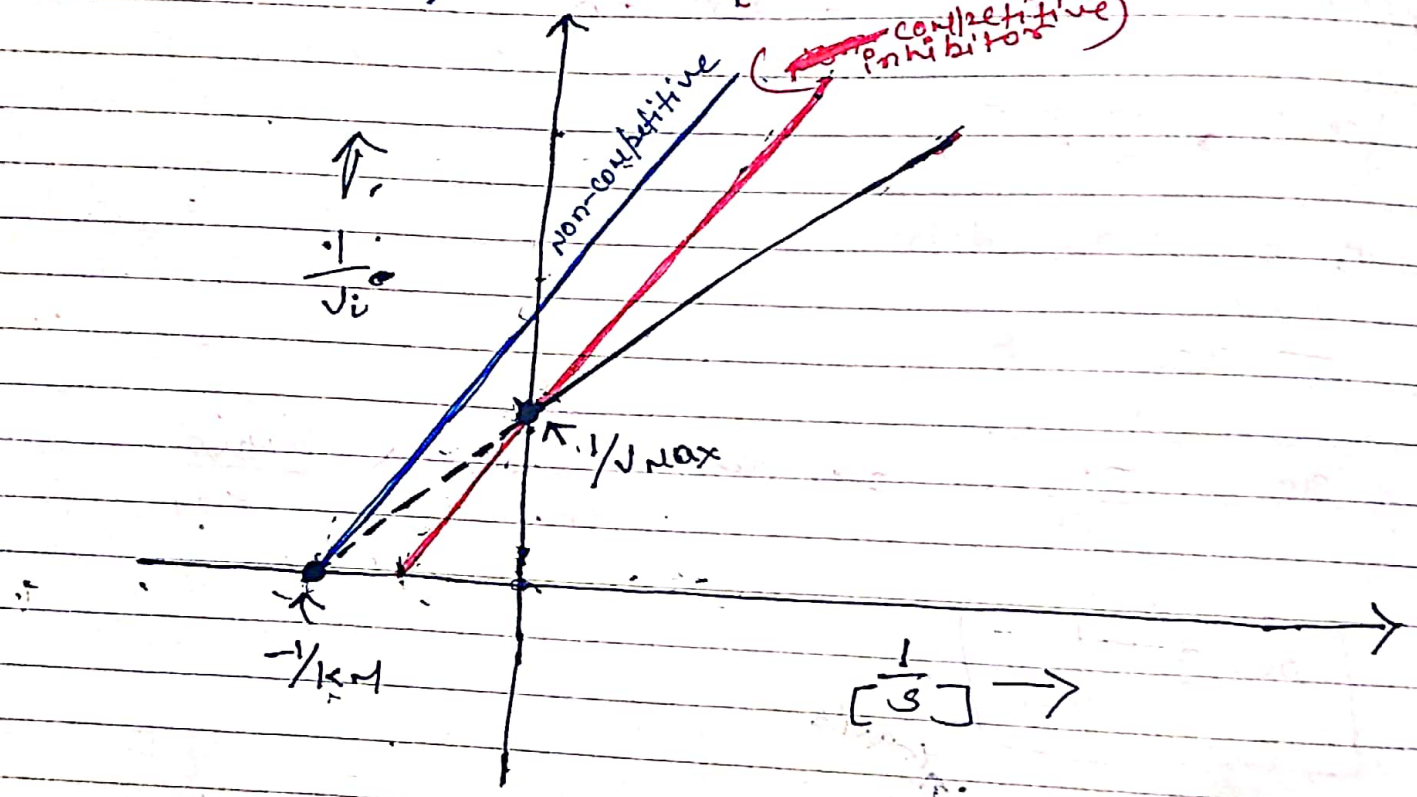
• The line weaver-burk plot particularly useful for calculating inhibitor.

→ Enzyme inhibition

① → competitive inhibitor
② → Non-competitive inhibitor.



competitive inhibitor	Non-competitive inhibitor
i) Does not change V_{max}	i) Dec. V_{max}.
ii) K_M increases	ii) Doesn't affect K_M
Note: y-intercept is same but x-intercept & slop is changes.	Note: y-intercept is change & x-intercept is same

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→ Competitive inhibitor : (irreversible inhibitor)

- In lineweaver burk plot the V_{max} (V_{max}. velocity) doesn't change but the K_M (Michaelis menten const.) is increase.
- The V_{max} at y-intercept is same but the K_M at x-intercept is change.

eg :→ Di-iso/propyl fluoro/phos/phate inhibits Acetylcholinesterase.

→ Non-competitive inhibitor : (reversible inhibitor)

- In line weaver burk plot the V_{max}. is decreases but doesn't change in K_M.
- The V_{max} at y-intercept is change but the K_M at x-intercept is same.

eg :→ Monoamine oxidase.

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