25B.Sc (HONOURS) MICROBIOLOGY (CBCS STRUCTURE) CC-3: BIOCHEMISTRY (THEORY) SEMESTER –2 MCB-A-CC-2-3-TH TOTAL HOURS: 50 CREDITS: 4 Unit 1 Bioenergetics No. of Hours: 6 First and second laws of Thermodynamics. Definitions of Gibb’s Free Energy, enthalpy,and Entropy and mathematical relationship among them, Standard free energy change and equilibrium constant Coupled reactions and additive nature of standard free energy change, Energy rich compounds: Phosphoenolpyruvate, 1,3- Bisphosphoglycerate, Thioesters, ATP

Unit 2 Carbohydrates No. of Hours: 10 Families of monosaccharides: aldoses and ketoses, trioses, tetroses, pentoses, and hexoses. Stereo isomerism of monosaccharides, epimers, Mutarotation and anomers of glucose.Furanose and pyranose forms of glucose and fructose, Haworth projection formulae for glucose; chair and boat forms of glucose, Sugar derivatives, glucosamine, galactosamine, muramic acid, N- acetyl neuraminic acid, Disaccharides; concept of reducing and non-reducing sugars, occurrence and Haworth projections of maltose, lactose, and sucrose, Polysaccharides, storage polysaccharides, starch and glycogen. Structural Polysaccharides, cellulose, peptidoglycan and chitin

Unit 3 Lipids No. of Hours: 10 Definition and major classes of storage and structural lipids.Storage lipids.Fatty acids structure and functions.Essential fatty acids.Triacylglycerols structure, functions and properties. Saponification Structural lipids.Phosphoglycerides: Building blocks, General structure, functions and properties. Structure of phosphatidylethanolamine and phosphatidylcholine, Sphingolipids: building blocks, structure of sphingosine, ceramide. Special mention of sphingomyelins, cerebrosides and gangliosides Lipid functions: cell signals, cofactors, prostaglandins, Introduction of lipid micelles, monolayers, bilayers

Unit 4 Proteins No. of Hours: 10 Functions of proteins, Primary structures of proteins: Amino acids, the building blocks of proteins. General formula of amino acid and concept of zwitterion. Titration curve of amino acid and its Significance, Classification, biochemical structure and notation of standard protein amino acids Ninhydrinreaction.Natural modifications of amino acids in proteins hydrolysine, cystine and hydroxyproline, Non protein amino acids: Gramicidin, beta-alanine, D-alanine and D- glutamic acid Oligopeptides: Structure and functions of naturally occurring glutathione and insulin and synthetic aspartame, Secondary structure of proteins: Peptide unit and its salient features. The alpha helix, the beta pleated sheet and their occurrence in proteins, Tertiary and quaternary structures of proteins. Forces holding the polypeptide together. Human haemoglobin structure, Quaternary structures of proteins

Unit 5. Enzymes Structure of enzyme: Apoenzyme and cofactors, prosthetic group-TPP, coenzyme No. of Hours: 10 NAD,metal cofactors, Classification of enzymes, Mechanism of action of enzymes: active site, transition state complex and activation energy. Lock and key hypothesis, and Induced Fit hypothesis. Significance of hyperbolic, double reciprocal plots of enzyme activity, Km, and allosteric mechanism Definitions of terms – enzyme unit, specific activity and turnover number, Multienzymecomplex : pyruvate dehydrogenase; isozyme: lactate dehydrogenase, Effect of pH and temperature on enzyme activity. Enzyme inhibition: competitive- sulfa drugs; non-competitive-heavy metal salts

Unit 6. Vitamins Classification and characteristics with suitable examples, sources and importance

Name “ Enzyme “ Introduced by Frederick W. Kuhne.

Classification of Enzymes
The 7 major classes of enzymes with some important examples from some subclasses are
described b...

1. Oxidoreductases EC1
• This class comprises the enzymes which were earlier called dehydrogenases, oxidases,
peroxidases,...

2. Transferases EC2
• Catalyze the transfer or exchange of certain groups among some substrates
• In these are included th...

3. Hydrolases EC3
• Accelerate the hydrolysis of substrates
• These catalyze the hydrolysis of their substrates by adding ...

5. Isomerases EC5
• Facilitate the conversion of isomers, geometric isomers or optical isomers
• Alanine racemase, Cis-tra...

4. Lyases EC4
• Promote the removal of a group from the substrate to leave a double bond reaction or
catalyze its reverse ...

6. Ligases EC6
• Catalyze the synthesis of two molecular substrates into one molecular compound with the
release energy
• ...

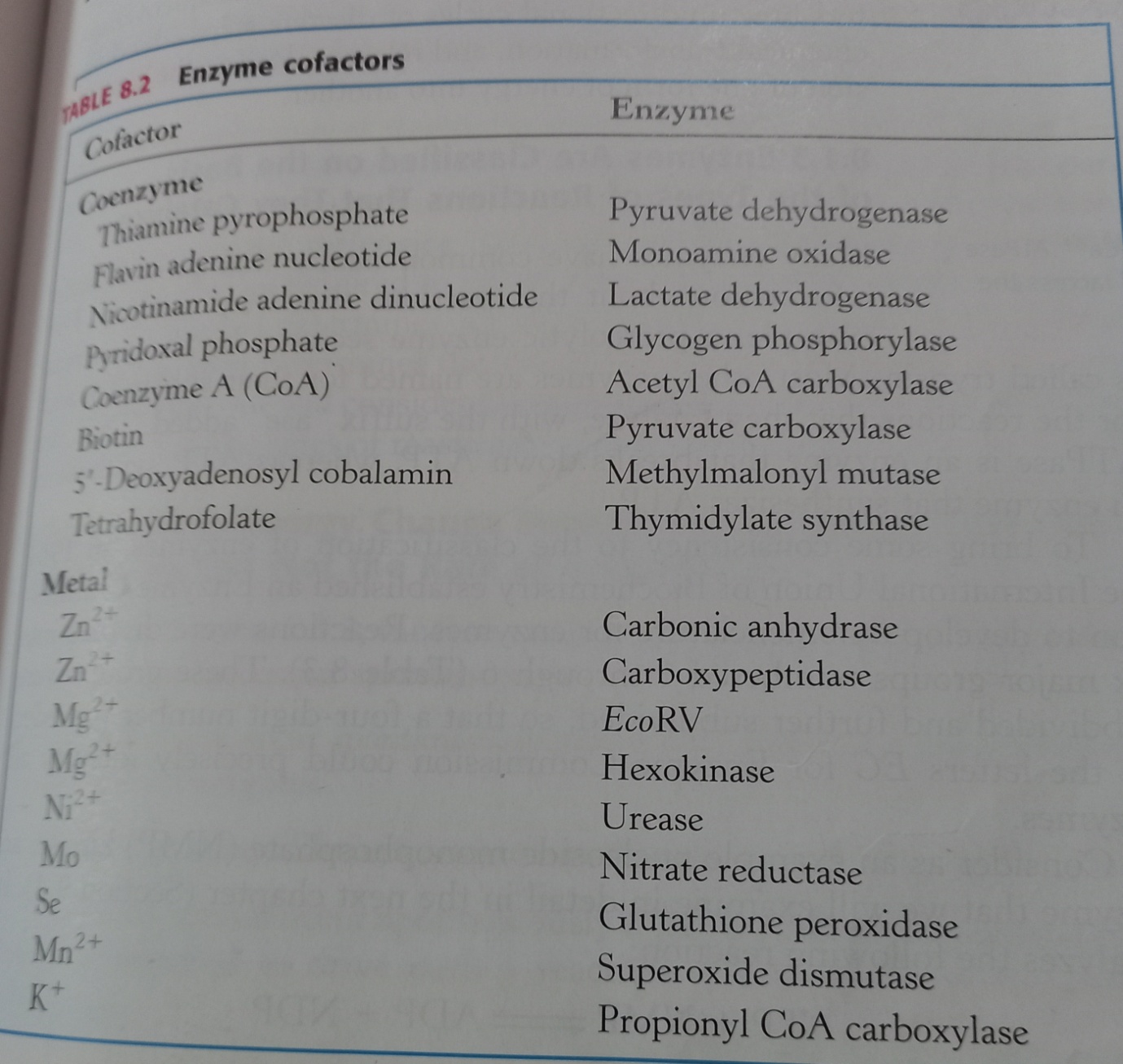
7. Translocase EC7
• Catalyze the movement of ions or molecules across membranes or their separation within
membranes
• he...

Active Site
As the substrate molecules are comparatively much smaller than the enzyme
molecules, there should be some spec... Properties of Active Site
1. The active site occupies a relatively small portion of the enzyme molecule
2. The active site...

Properties of Active Site
4. The active site binds the substrate molecule by relatively weak forces
5. The active sites in...

Nomenclature
• The first three numbers represent the class, subclass and sub-subclass to which an
enzyme belongs, and the ...

**Holo enzyme = Apo enzyme / Apo protein + Cofactor**



**Enzyme affect reaction rates, not equilibria**

**E + S = ES= EP= E + P**

**Activation Energy
 **

**Standard free energy change for the reaction is negetive means the free energy of the ground state of Product is lower than that of substrate. So Δ G is negetive for the equilibrium which favors the formation of product.**

**Transition State: A molecular moment in which events such as bond breakage , bond formation, charge development have proceeded to the precise point at which decomposition to either substrate or product is equally likely.**

**Activation Energy: The difference between the energy level of the ground state and the transition state is called activation energy.**

**Catalysis enhances the reaction rate by lowering the activation energy.**

Properties of Enzymes
1. Colloidal Nature
• On account of their large size, the enzyme molecules possess extremely low rat...

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Fischer’s Lock and Key Model
• also known as template model - proposed by Emil Fischer in 1898
• the union between the sub...

Fischer’s Lock and Key Model
• In fact, the enzyme-substrate union depends on a reciprocal fit between the molecular
struc...

Fischer’s Lock and Key Model
 

Koshland’s Induced Fit Model
• unfortunate feature of Fischer’s model is the rigidity of the active site
• Koshland presum...

Koshland’s Induced Fit Model
• As to the sequence of events during the conformational changes, 3 possibilities exist
1. Th...

**Enzyme is complementary to transition state.**

Koshland’s Induced Fit Model
 

Activation Energy
• All the chemical reactions in a biological system have an energy barrier which prevents
reactions from...

Activation Energy
 

Activation Energy
• It is important to remember that enzymes do not change the reaction’s ∆G
• In other words, they do not...

Michaelis Menten Hypothesis
Leonor Michaelis and Maud L. Menten (1913), while studying the hydrolysis of sucrose catalyzed...

E+S=ES K1. K-1, ES= EP K2, K-2, EP = E+ P K3

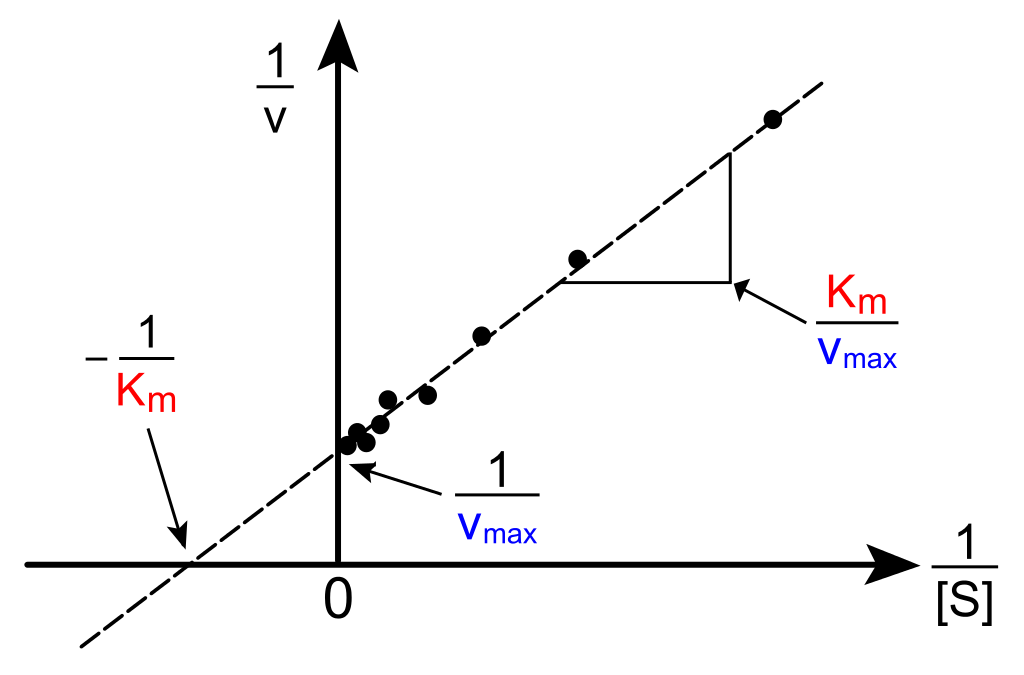
• The theory postulates that the enzyme (E) forms a weakly-bonded complex (ES) with the
substrate (S)
• This enyzme-substr...

1/v = Km + S / V max xS

1/V = Km/ Vmax x S + 1/Vmax

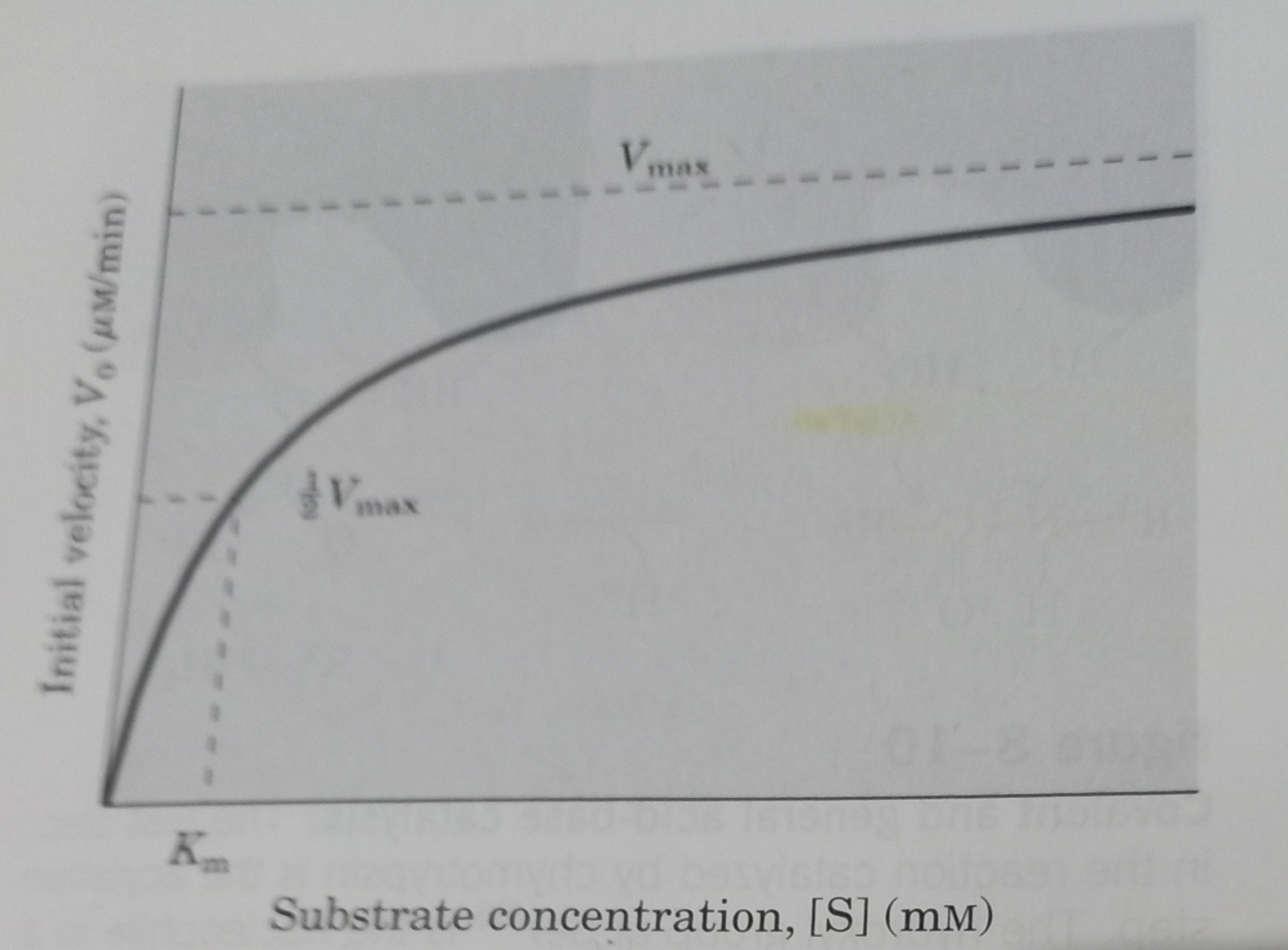
The Lineweave r–Burk plot puts 1/[S] on the [x-axis](https://en.wikipedia.org/wiki/Cartesian_coordinate_system) and 1/V on the [y-axis](https://en.wikipedia.org/wiki/Cartesian_coordinate_system).

.



{\displaystyle {1 \over V}={{K\_{m}+[S]} \over V\_{\max }[S]}={K\_{m} \over V\_{\max }}{1 \over [S]}+{1 \over V\_{\max }}}

• Km value is used as a measure of an enzyme’s affinity for its substrate. The lower the Km
value the higher the enzyme’s ...



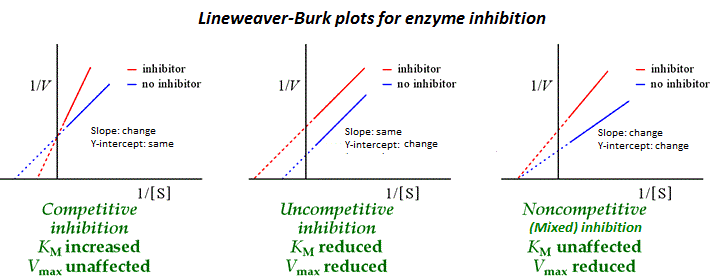
Unit Of Km = M ( molar) / mM ( milli molar) / µM ( micromolar)

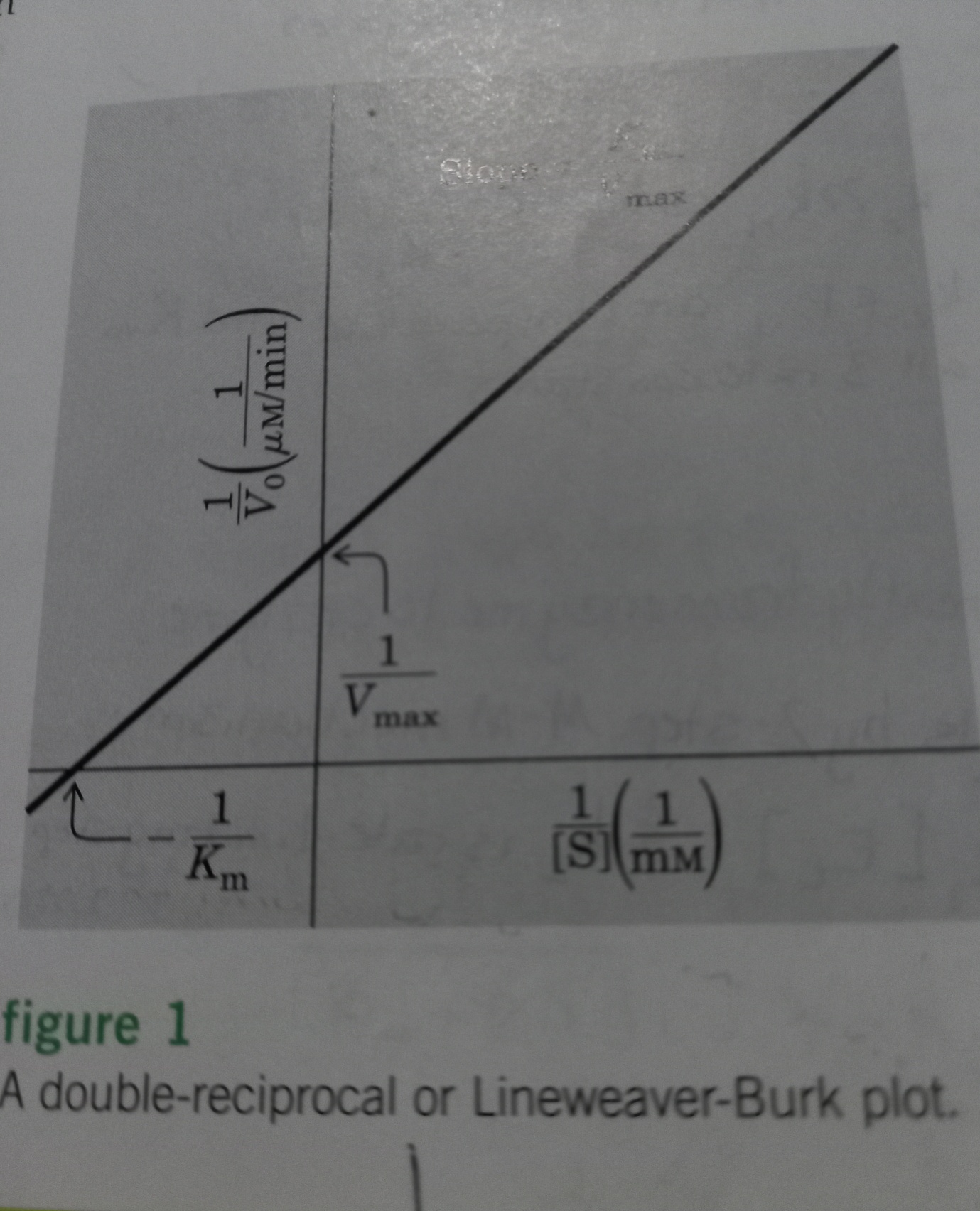
Unit of Vmax = Amount of product formed per unit time : micromole / minute

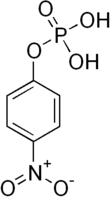
Biochemical reactions involving a single substrate are assumed to follow Michaelis-menten equation.

Kcat / turn over number: number of substrate molecules converted to product in a given unit time on a single enzyme molecule when the enzyme is fully saturated with substrate.

Kcat = Vmax/[ET]., unit = Second-1







Substrate concentration = 1mM

371.15 g / 1000 substrate is present in 1000 ml solvent for preparation of 1Mm solution

1000ml 1mM substrate solution contains 371.15/1000 g

1ml 1mM substrate solution contains 0.37115/1000 g

50 ml 1mM substrate solution contains 0.37115/1000g x 50

0.1M Glycine-NaOH buffer, pH 9.8

Para nitro phenyl P + Buffer = Paranitro phenol + inorganic P ( Pi)

Substrate concentration : 1 mM ( milli molar) , Total volume = 50ml, how much substrate will be required to prepare 50 ml substrate solution?

Substrate concentration in tube no 1 =

V1 xS1 = V2 XS2

V1= 0.1ml

V2 = 1ml

S1 = 1mM

S2 = 0.1 x 1 ml x mM/ 1ml

1---S2 = 0.1mM 1/S2 = 1/0.1mM = 10mM-1

2---- S2 = 0.2 mM

3---- S2 = 0.4 mM

4----S2 = 0.6 mM

5----S2 =0 .8mM

Velocity = Product formed per minute = product formed / 30min

Micro mole

|  |  |  |  |
| --- | --- | --- | --- |
| Tube no | Buffer ( ml) | Enzyme ( ml) | Substrate ( ml) |
| Blank | 0.8 | --- | 0.2 |
| 1 | 0.7 | 0.2 | 0.1 |
| 2 | 0.6 | 0.2 | 0.2 |
| 3 | 0.4 | 0.2 | 0.4 |
| 4 | 0.2 | 0.2 | 0.6 |
| 5 | 0.1 | 0.2 | 0.7 |
|  |  |  |  |
|  |  |  |  |
|  |  |  |  |

Temp= 37degree C, Time of incubation = 30min

After 30 min of incubation 2ml 1M NaOH solution will be added to each tube

At 440nm optical density is observed.

Preparation of standard curve of para nitro phenol

Stock concentration of para nitro phenol = 0.1mM ( milli molar)

Mol wt of para nitro phenol = 138.1 gm

Calculate the amount of para nitro phenol to prepare 0.1mM 25 ml solution.

0.1milli mole of paranitro phenol is present in 1000ml solution

138.1g/10000 paranitro phenol is present in 1000ml solution

1ml solution requires 138.1g/ 10000000 paranitro phenol

25 ml solution requires 138.1 x 25 g/ 107  =3452.5 g/ 107

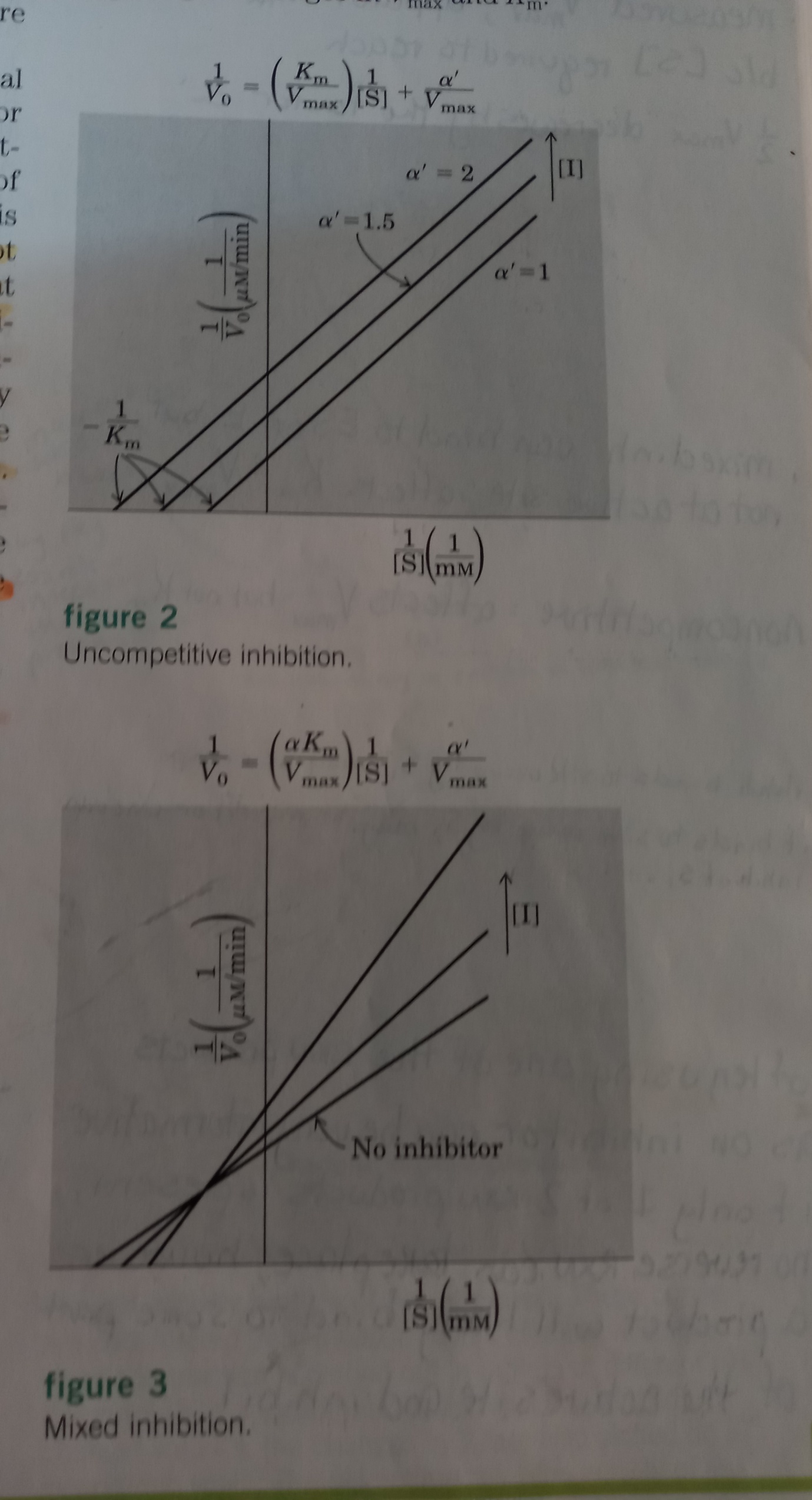
|  |  |  |
| --- | --- | --- |
| Test tube no | Volume of paranitro phenol  ( ml) | Volume of NaOH ( 1M)  (ml) |
| Blank | ---- | 3 |
| 1 | 0.1 | 2.9 |
| 2 | 0.2 | 2.8 |
| 3 | 0.4 | 2.6 |
| 4 | 0.6 | 2.4 |
| 5 | 0.8 | 2.2 |
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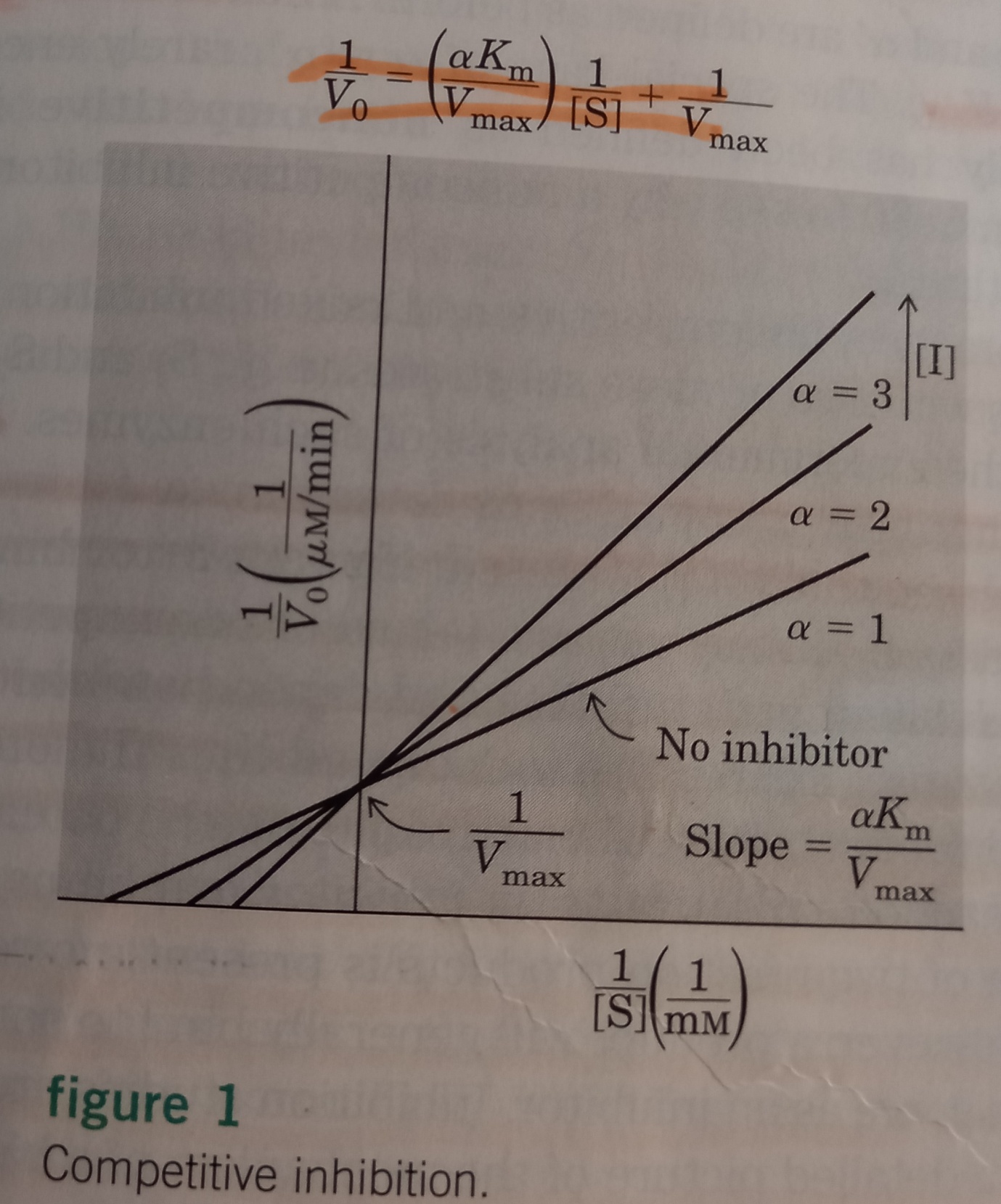
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| --- | --- | --- | --- | --- |
| Tube No | [S] micro molar | 1/ [S] micro molar-1 | V (micromole/min) | 1/V (micromole/min)-1 |
| 1 | 100 |  |  |  |
| 2 | 200 |  |  |  |
| 3 | 400 |  |  |  |
| 4 | 600 |  |  |  |
| 5 | 800 |  |  |  |
|  |  |  |  |  |

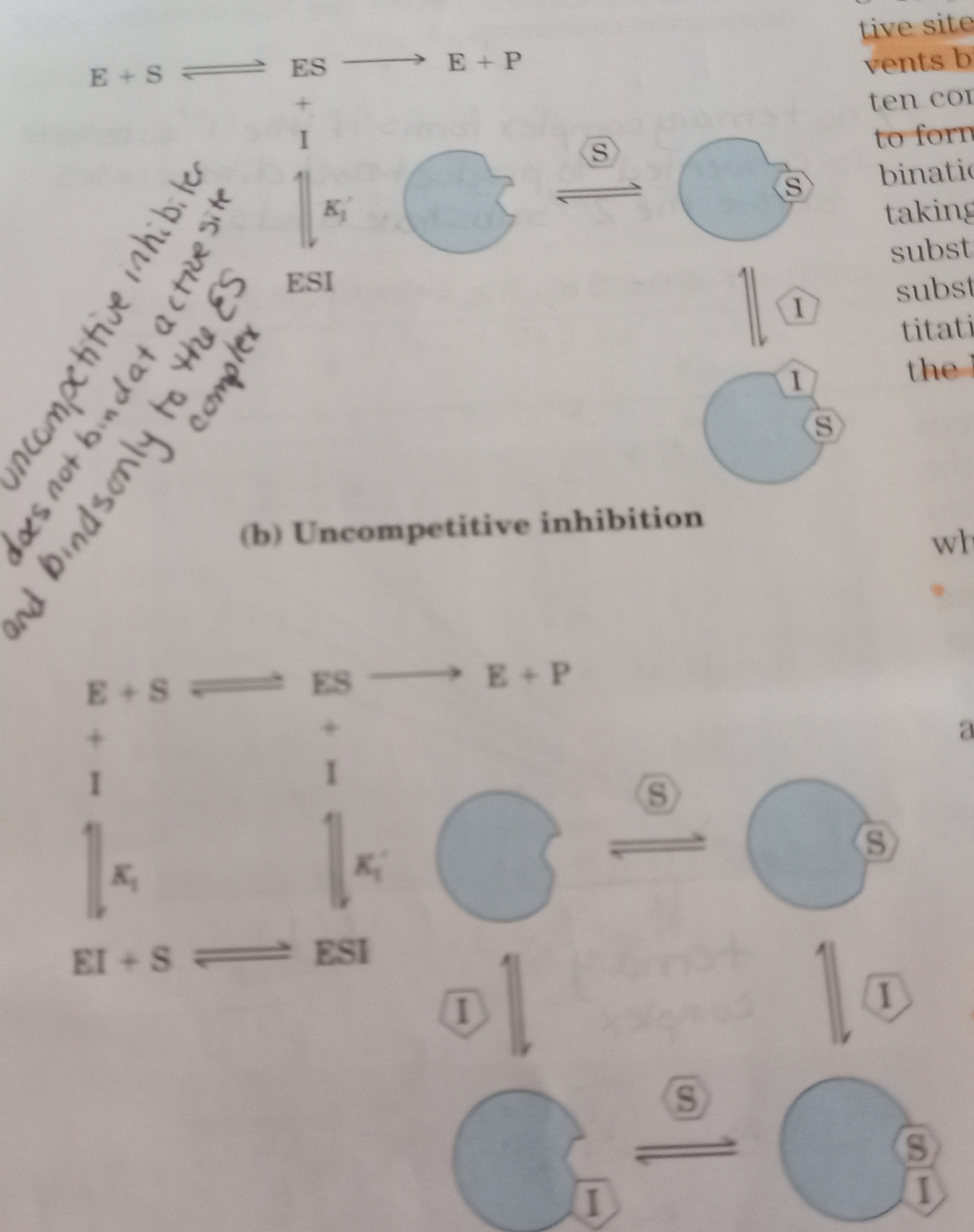
Para nitro phenol standard curve

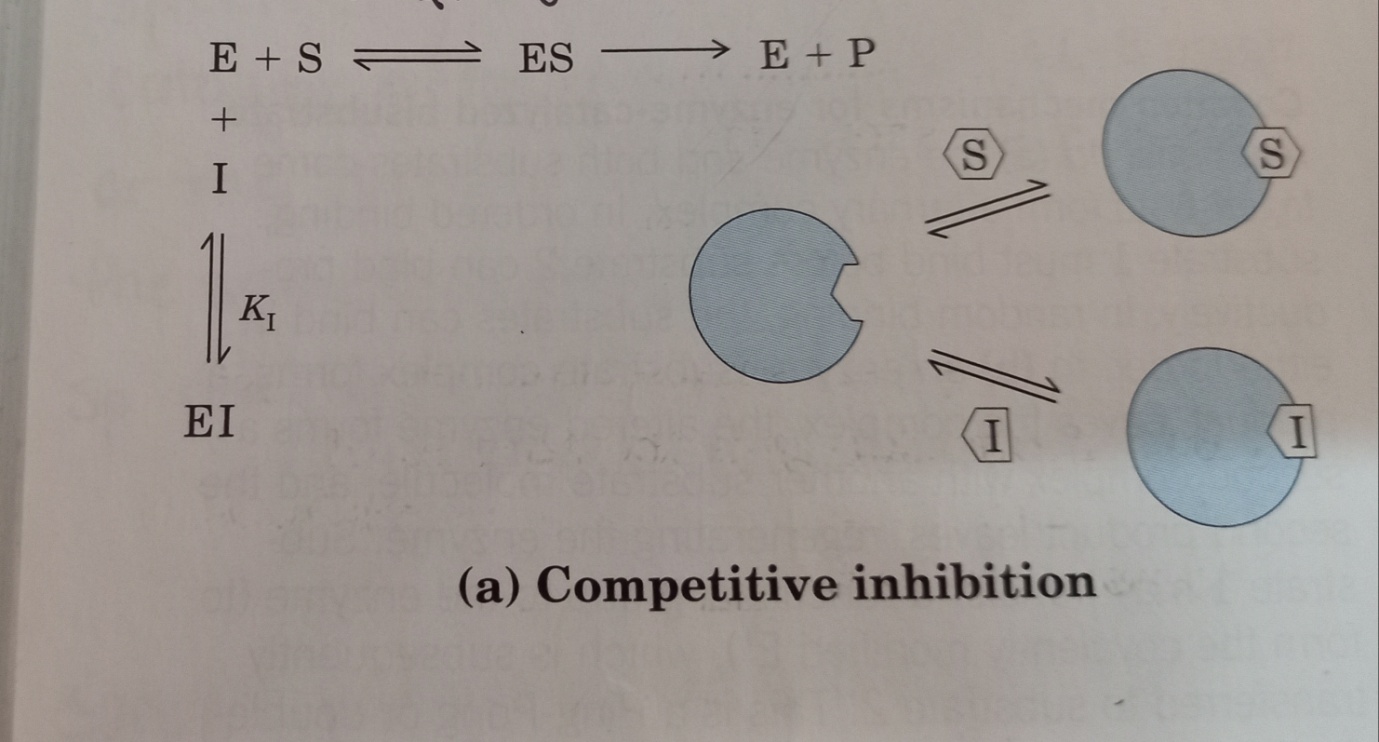
|  |  |  |
| --- | --- | --- |
| TubeNo | Amount of Para nitro phenol ( µmole/ml) micromole per millilitre | Optical density at 440 nm |
| Blank | 0.0 | 0.0 |
| 1 |  | 0.102 |
| 2 |  | 0.202 |
| 3 |  | 0.350 |
| 4 |  | 0.452 |
| 5 |  | 0.660 |
|  | --- |  |
|  |  |  |

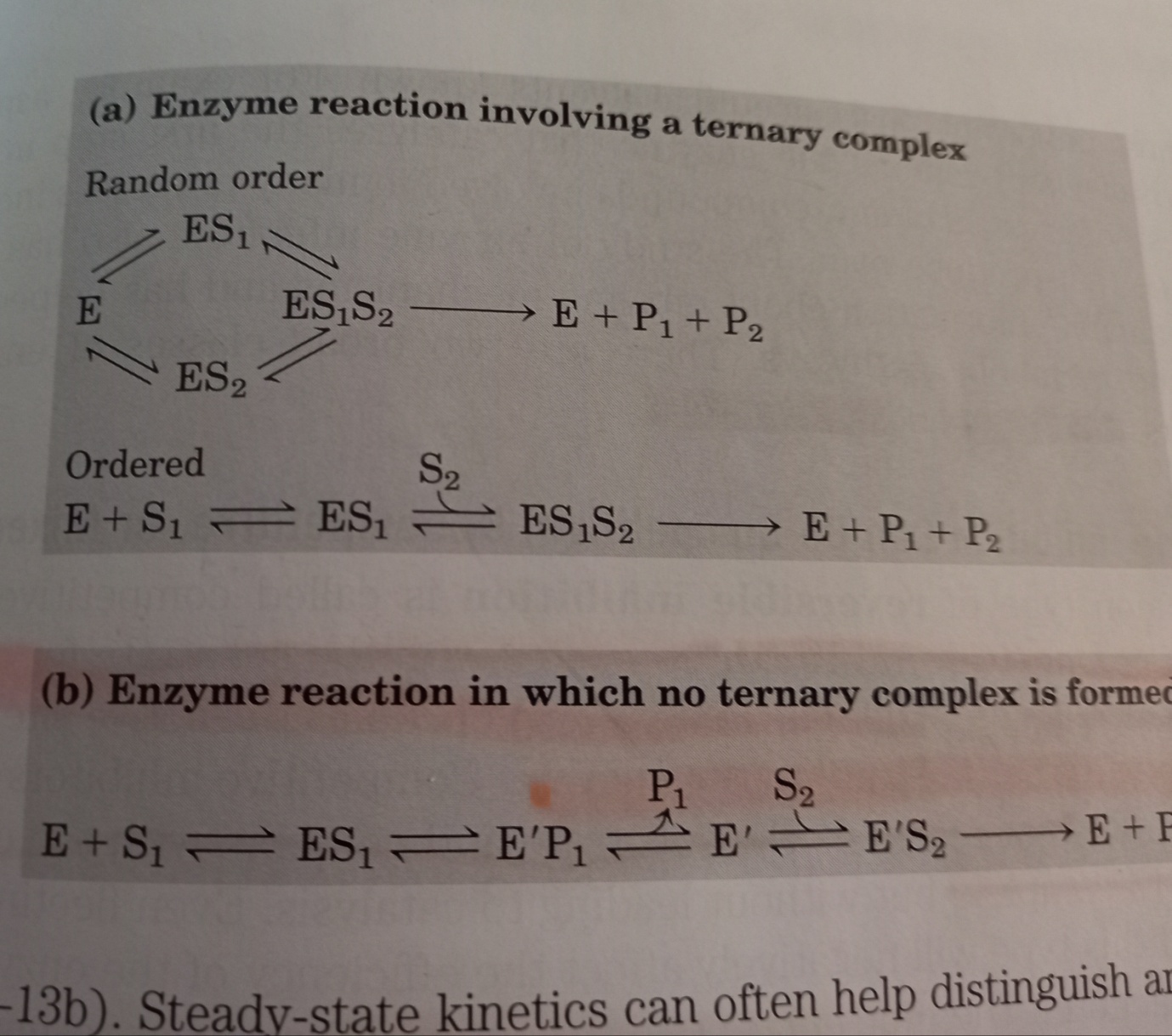
|  |  |  |
| --- | --- | --- |
| TubeNo | Concentration of paranitro phenol ( µg/ml) |  |
| Blank | 0.0 |  |
| 1 | 50 |  |
| 2 | 100 |  |
| 3 | 200 |  |
| 4 | 400 |  |
| 5 | 800 |  |
| unknown | --- |  |
|  |  |  |











|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Competitive | unncompetitive | Noncompetitive |  |
| Inhibitor binding | Occupies the active site | Binds at a site distinct from the substrate active site, binds only ES complex | Binds at a site distinct from the substrate active site, it will bind either E or ES |  |
| Michaelis Menten Equation | V0 = Vmax[S]  αKm +S  α = 1+ [I]  KI  KI = [E][I]  [EI] |  |  |  |
| Apparent Km (αKm) | Increses by the factor α | Deceases Km value | When binds E, effect is like competitive inhibition and binds ES , the effect is like uncompetitive inhibition |  |
| Apparent Vmax | Vmax remains same | Vmax value decreses |  |  |
| Excess substrate concentration |  |  |  |  |
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# Irreversible inhibitors

An irreversible inhibitor will bind to an enzyme so that no other [enzyme-substrate complexes](https://teaching.ncl.ac.uk/bms/wiki/index.php/Enzyme-substrate_complex) can form. It will bind to the [enzyme using](https://teaching.ncl.ac.uk/bms/wiki/index.php/Enzyme) a covalent bond at the active site which therefore makes the [enzyme denatured](https://teaching.ncl.ac.uk/bms/wiki/index.php/Enzyme). An example of an irreversible inhibitor is *diisopropyl fluorophosphate*which is present in nerve gas. It binds to the [enzyme](https://teaching.ncl.ac.uk/bms/wiki/index.php/Enzyme) and stops nerve impulses being transmitted. An example of where we use irreversible inhibitors in medicine is [penicillin](https://teaching.ncl.ac.uk/bms/wiki/index.php/Penicillin). Penicillin works by inhibiting the activity of the enzyme responsible for the creation of the bacterial [cell wall](https://teaching.ncl.ac.uk/bms/wiki/index.php/Cell_wall). This means that water can enter the bacterial cell, causing it to swell, burst and die termed lysis

Irreversible Inhibitor:

Group-specific reagents: Reacts with specific R group of amino acid.

Diisopropyl phosphofluoridate (DIPF)

Affinity labels: They are the molecules which are structurally similar to the substrate for the enzyme that covalently modify active site residue.

3-bromoacetol is an affinity label molecule for the enzyme Triose phosphate isomerise ( TIM). It mimics the normal substrate, dihyroxy acetone phosphate., by binding at the active site, then it covalently modifies.

Suicide inhibitor: Generates a chemically reactive intermediate that inactivates the enzyme through covalent modification. N, N-dimethylproperagylamine is inhibitor of Mono amine Oxidase ( MAO), the flavin prosthetic grp is being alkylated by this inhibitor at N5 position. MAO deaminates dopamine .

Penicillin irreversibly inactivates a key enzyme for bacterial cell wall synthesis.

|  |  |  |  |
| --- | --- | --- | --- |
| Name of the inhibitors | Name of substrate | Enzyme | Type of inhibition |
| Methotrexate | Tetrahydrofolate | Dihydrofolate reductase | Competitive |
| Diisopropylphosphofluoridate | Inhibitors of enzymatic hydrolysis | Acetylcholinesterase | Irreversible |
| Bromoacetol phosphate | Conversion of Dihydroxyacetone phosphate to glyceraldehydes 3 phosphate | Triosephosphate isomerase | Irreversible |
| N,N-dimethylpropargyl amine | Deamination of Dopamine or serotonine | Monoamineoxidase | Irreversible |
| Penicillin | Cross-linking during bacterial cell wall synthesis | Transpeptidase | Irreversible |
| Malonate | Inhibits the conversion of succinate to fumarate in TCA cycle | Succinate dehydrogenase (Complex II) | Competitive inhibitor |
| Arsenate | Function of alkaline phosphatase | Alkaline phoshatase | Competitive inhibitor |
|  |  |  |  |
|  |  |  |  |

Isozyme or Isoenzymes are enzymes that differ amino acid sequence yet catalyze the same reaction.Usually , these enzymes display different kinetic parameters, such as KM, or different regulatory properties. They are encoded by different genetic loci, which usually arise through gene duplication and divergence.

LDH-1: Present primarily in cardiac myocytes and erythrocytes.

▪

LDH-2: Present mostly in white blood cells.

▪

LDH-3: Present in highest quantity in lung tissue.

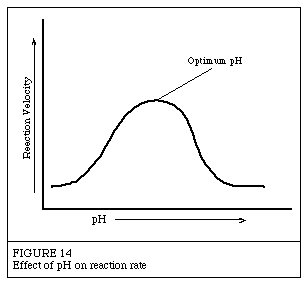
▪

LDH-4: Highest amounts found in pancreas, kidney, and placenta.

▪

LDH-5: Highest amounts found in liver and skeletal muscle.

Effect of pH



Effect of temperature

