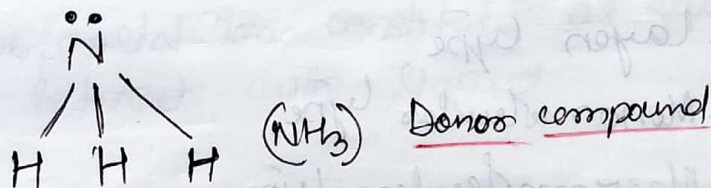# UNIT-4
Complexation & Protein Binding

⟶ Complexation is defined as an association of two or more chemical species, resulting a formation of complexes.

⟶ Complexes generally results from a donor-acceptor mechanism.

eg- NH_3 (Donor ⟶ Ligands (which has lone pair)

$$\overset{\displaystyle \ddot{N}}{\underset{\displaystyle H \quad H \quad H}{\Big|\Big|\diagdown}}$$ (NH₃) Donor compound

⟹ The Donor compound is a non-metallic atom or ion, which can donate an electron pair.

⟹ The acceptor is usually a metallic ion (atom) or a neutral atom is capable of accepting a pair of electrons. eg- Co (Cobalt).

So, Substrate (acceptor) molecules reffered as central atom and Donor reffered as ligands, which attached to the central atom.

Classification

1) Metal ion complexes / Coordinate complexes.

├─ Inorganic type

├─ Chelates

├─ Olefin types

├─ Aromatic types.

2) Organic Molecular complexes

+ Quinhydrone type
+ Picric acid type
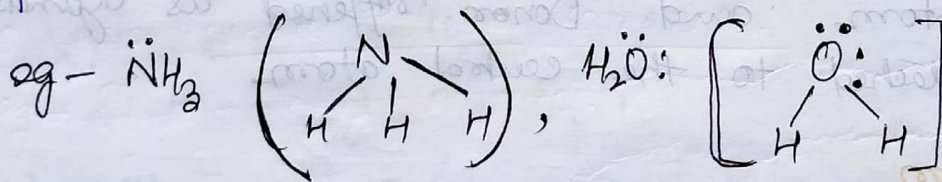+ Caffeine and other drug complexes
+ Polymer type

3) Inclusion / Oclusion compounds

+ Clathrate
+ Channel lattic type
+ Layer type
+ Monomolecular type.
+ Macromolecular type.

Donor (Ligands) : which has lone pair and attached with central atom.
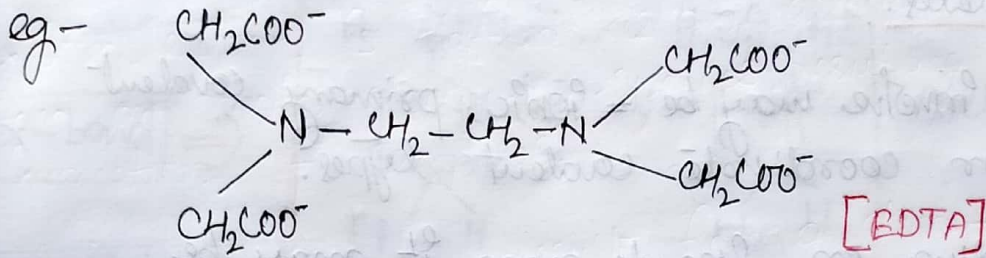
Types

① Monodentate : which provide only one centre for attachement to the central atom.

eg – $\ddot{N}H_3$ $\left(\begin{array}{c} N \\ H \quad H \quad H \end{array} \right)$, $H_2\ddot{O}:$ $\left[\begin{array}{c} \ddot{O}: \\ H \quad H \end{array} \right]$

② Bidentate ligand : Those ligands which provide two centre for attachement to the central metal ion (atom)

eg – $_2H\ddot{N}$ ~~~ CH_2-CH_2 ~~~ $\ddot{N}H_2$ (ethylenediamine)
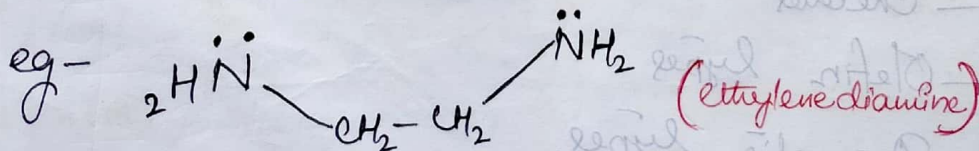
(iii) <u>Polydentate ligand</u> : Those ligand which provide two or more than two centre for attachment to the central atom. (Hexadentate)

eg-

$$CH_2COO^-$$
$$\searrow$$
$$N-CH_2-CH_2-N$$
$$\nearrow \quad\quad\quad\quad \searrow CH_2COO^-$$
$$CH_2COO^- \quad\quad\quad CH_2COO^-$$

[EDTA]
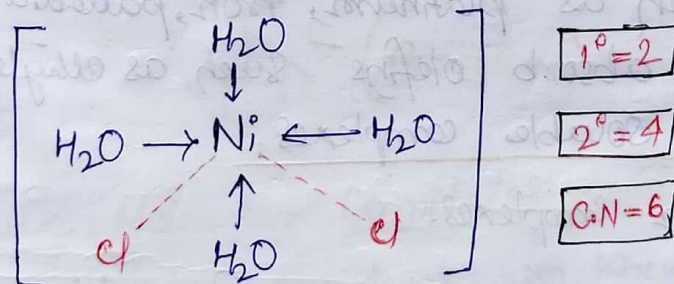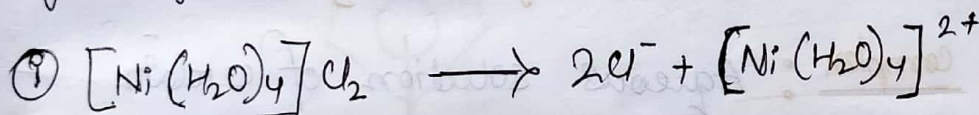
<u>CLASSIFICATION</u>

① <u>Metal ion Complexes / Coordinate Complex</u>

In this type, metal ion <u>constitutes</u> as central <u>atom</u> and <u>interact</u> with ligand.
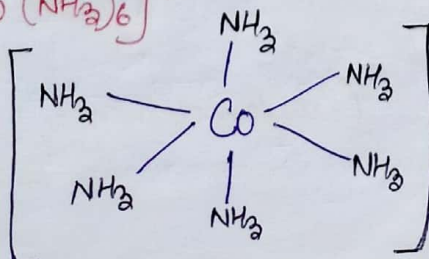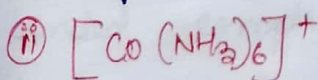
a) <u>Inorganic Complexes</u> : In this complex, ligands are <u>Inorganic</u> in nature, which attached with metal ion.

eg- Ligands use → $\ddot{N}H_3$, $H_2\ddot{O}:$, $C\equiv \ddot{N}$

① $[Ni(H_2O)_4]Cl_2 \longrightarrow 2Cl^- + [Ni(H_2O)_4]^{2+}$

$$
\begin{bmatrix}
& H_2O & \\
& \downarrow & \\
H_2O \rightarrow & Ni & \leftarrow H_2O \\
& \uparrow & \\
Cl & H_2O & Cl
\end{bmatrix}
$$

$1° = 2$
$2° = 4$
$C.N = 6$

In which, ligands attached with metal atom with coordinate bond. & Cl attached with simple ionic bond.

• <u>Valencies</u>

② $[Co(NH_3)_6]^+$

$$
\begin{bmatrix}
& NH_3 & \\
NH_3 & | & NH_3 \\
\searrow & Co & \swarrow \\
NH_3 & | & NH_3 \\
& NH_3 &
\end{bmatrix}
$$

• Primary valencies (1°) → Ionic bond
• Secondary valencies (2°) → Coordinate bond
   (Total no. of ligands)
• Coordination no. → Max. no. of atom binding to the central atom.

(ii) **Chelates :** A substances containing two or more donor group may combine with a metal ion to form a complex known as chelates.

→ Bonds involve may be = ionic, primary covalent type or coordinate covalent types.

→ Depending on ligands group, it may be bidentate, tridentate, hexadentate or polydentate.

→ when metal atom (central) attached with these ligands, it forms cyclic structure (closed).

eg — EDTA, ethylenediameme. etc.

$$H_2\ddot{N} \longrightarrow M \longleftarrow \ddot{N}H_2$$
$$CH_2 - CH_2$$

→ central metal atom

(ethylenediamine)
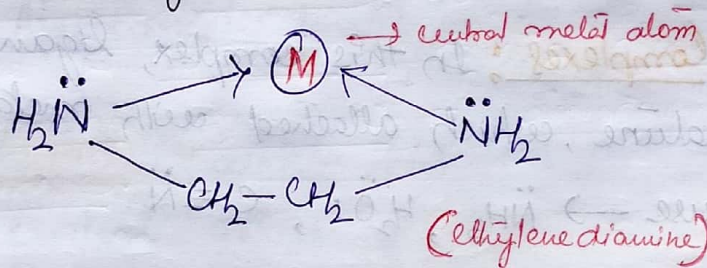
(iii) **Olefin complex :** Aqueous solution of certain metal ions such as platinum, iron, palladium, silver etc can absorb olefins such as ethylene to yield water soluble complexes.

eg — Silver – Olefin complexes.

(iv) **Aromatic Complexes :** They are formed by interaction of metal ions as acceptors with aromatic molecules such as benzene, toluene, xylene.
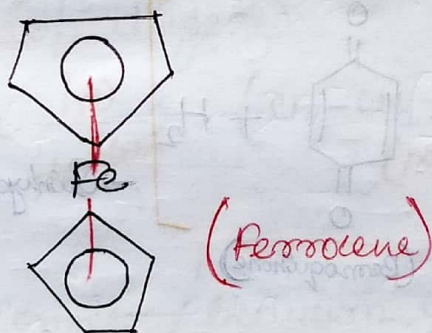
- π bond
- α bond
- Sandwich compound

$\boxed{\pi \text{ bond}} \Rightarrow$ + HCl = -- HCl

$\boxed{\sigma-\text{bond}} \Rightarrow$

$AlCl_4^-$
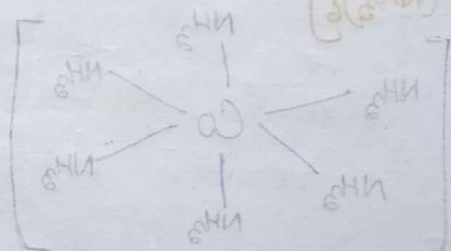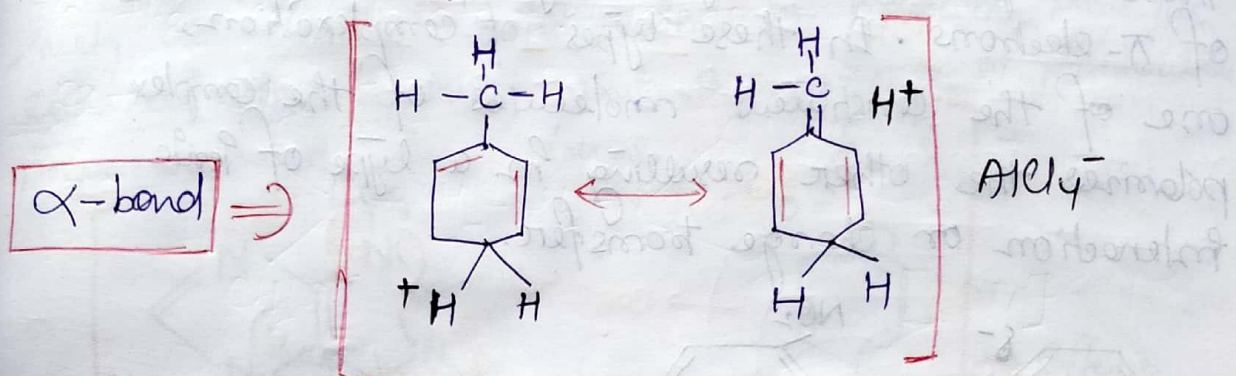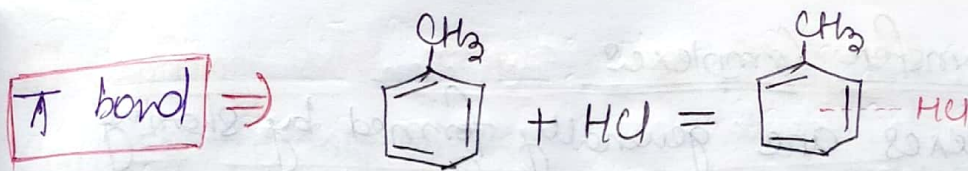
⊙ Sigma-bond complex of toluene with $HCl-AlCl_3$

$\boxed{\text{Sandwich compound}} \Rightarrow$ This are relatively stable complexes involving a delocalized covalent bond between the d-orbital of a transition metal and a molecular orbital of aromatic ring.


(Ferrocene)

② <u>Organic Molecular Complexes</u>

Organic molecular complexes, also known as addition complexes are formed by the union of two organic molecules held together by electrostatic forces, ionic, covalent and also by hydrogen bonded complexes.

Charge transfer Complexes

These complexes are generally formed by sharing of π-electrons. In these types of complexation, one of the constituent molecules of the complex polarizes the other resulting in a type of ionic interaction or charge transfer.
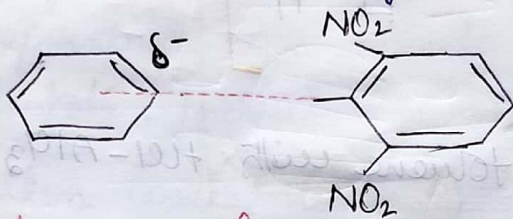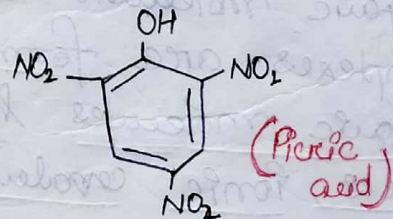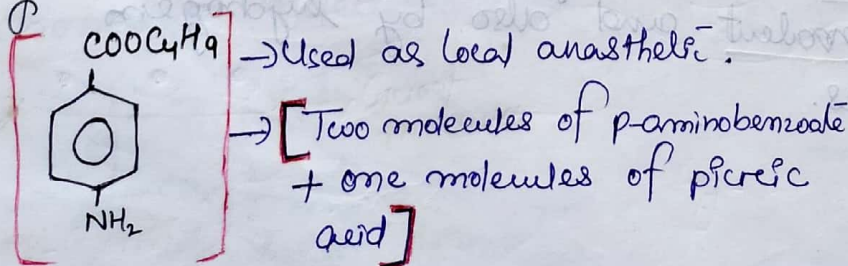

(Charge-transfer complex between benzene and tri-nitro benzene)

① Quinhydrone Complex : The molecular complex of this type is obtained by mixing alcoholic solutions of equimolar quantities of benzoquinone and hydroquinone.

(Hydroquinone)   (Benzoquinone)   → Quinhydrone
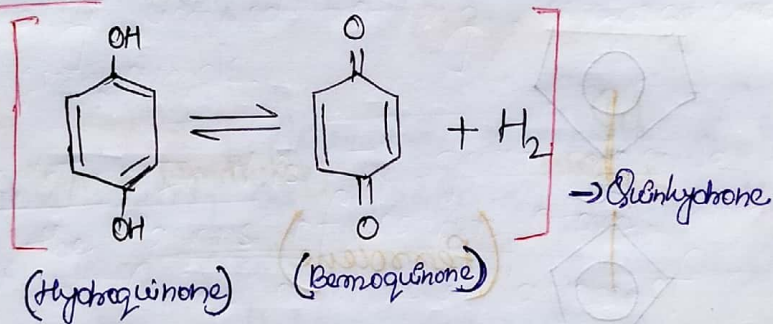
ⓘ Picric acid Complexes : Picric acid (2,4,6-trinitrophenol), being a strong acids, forms organic molecular complexes with weak bases.

eg - Butesin picrate

→ Used as local anasthetic.

→ [Two molecules of p-aminobenzoate + one molecules of picric acid]

(Picric acid)

(ii) Caffeine and other drug molecules:

Caffeine forms complexes with a number of drugs such as bemocaine, tetracaine or procaine and this enhances the stability and appearance of pharmaceuticals preparation of these drugs.

→ Caffeine Complexes.

(Caffeine)   (Bemocaine)
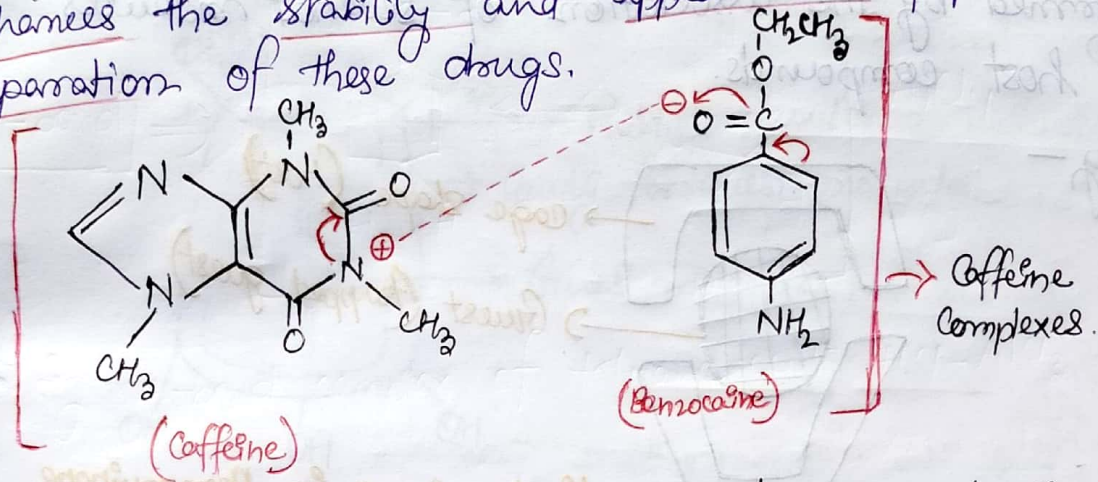
⇒ It involves dipole-dipole forces / hydrogen bonding.

(iv) Polymer type Complex: Many pharmaceutical additives such as polyethylene glycols (PEGs), Carboxymethyl cellulose (CMC) etc. can form complexes with a large number of drugs.

eg - $n\ CH_2 = CH_2 \xrightarrow[\text{polymerisation}]{h\nu} \{CH_2-CH_2-CH_2-CH_2-CH_2\}$

(ethene)   (polythene)
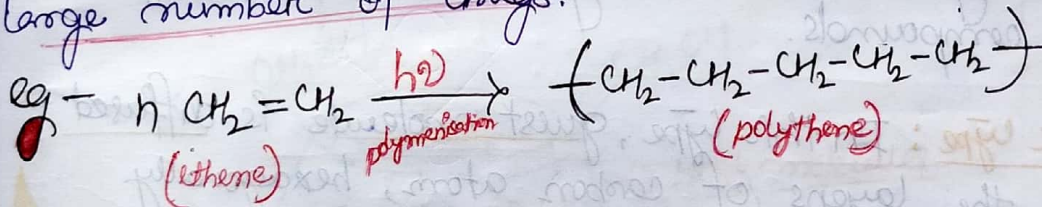
3) Inclusion Complex / Occlusion compounds

In these complexes, one of the components is entrapped (trapped) in the open lattice or cage like crystal structure of the other.

→ Here, component is known as Guest molecules and open lattice and cage like structure is known as host molecules in which guest molecule trapped.

→ No involvement of any type of bond, so also called no-bond complexes.

(i) **Clathrates** : Clathrates are inclusion compounds in which a molecule of guest compound get entrapped within the cage like structure formed by the association of several molecules of host compounds.

eg —

→ cage shape (host)

→ Guest (trapped guest)
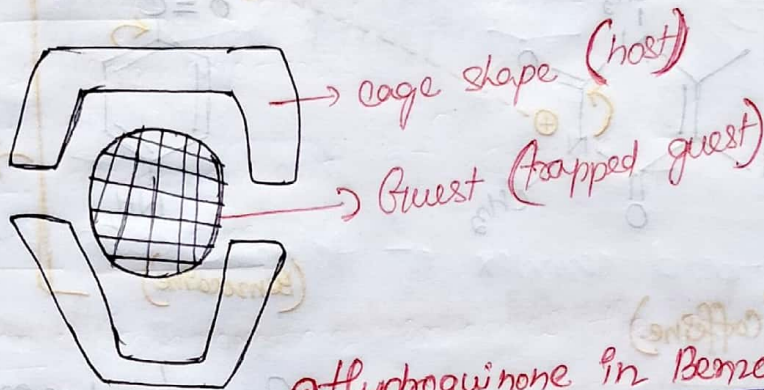
• Hydroquinone in Benzoquinone

(ii) **Channel lattice type** : Channels are formed by crystallization of the host molecules in which the guest molecules can fit, the guest component is usually limited to long, unbranched straight chain compounds.

(iii) **Layer type** : In this type, guest molecule is diffused between the layers of carbon atom, hexagonally oriented to form alternate layers of guest and host molecules. eg — clays, montmorillonete.
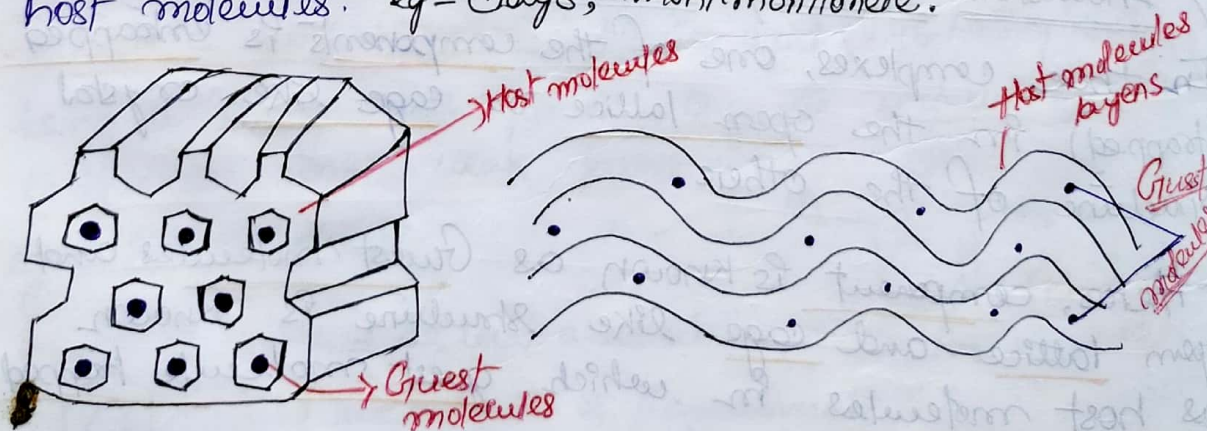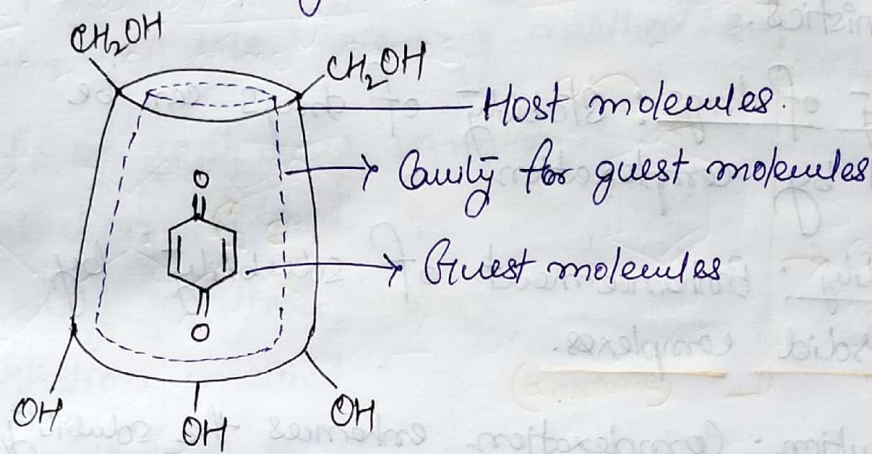

→ Host molecules

→ Guest molecules

Host molecules layers

Guest molecules

Fig — Channel lattice type

Fig — Layere type

(iv) **Monomolecular type :** In this complexes, a single guest molecules is entrapped in the cavity of host molecules. eg - Cyclodextrin complextion.

- Host molecules.
→ Cavity for guest molecules
→ Guest molecules

(v) **Macromolecular complexes :** In this complexes, more than one guest molecule is entrapped in the cavity of host molecules. eg - Cyclodextrin as a host molecules.

→ Host molecules
→ Guest molecules
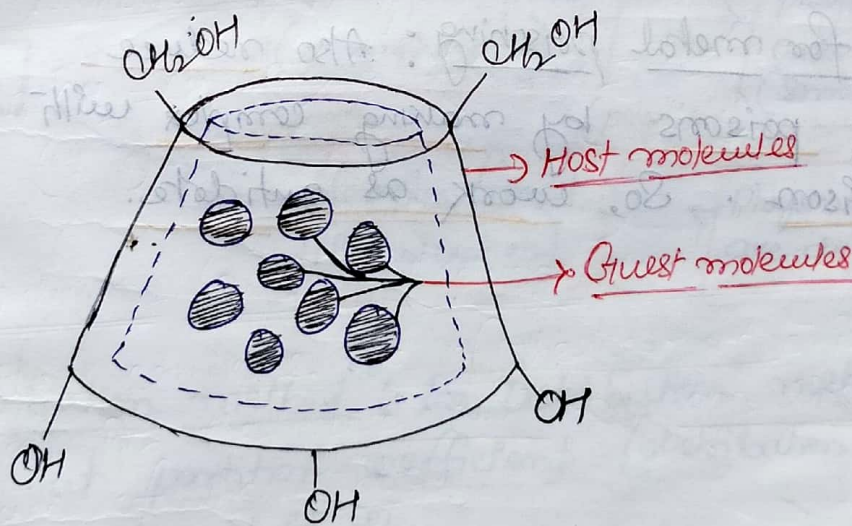
Applications of Complexation

(i) **Physical state**: To convert liquid substances to solid complexes and improve its processing characteristics.

(ii) **Stability of drugs**: Stability of drugs can be enhanced by complexation.

(iii) **Solubility**: Enhancement of solubility by using solid complexes.

(iv) **Dissolution**: Complexation enhances the solubility thus enhancing the dissolution of drugs.

(v) **Absorption & Bioavailability**: Complexation helps in increasing the absorption and bioavailability of drug in the body.

(vi) **Antidote for metal poisoning**: Also reduce toxicity of poisons by making complex with metal poison. So, work as antidote.
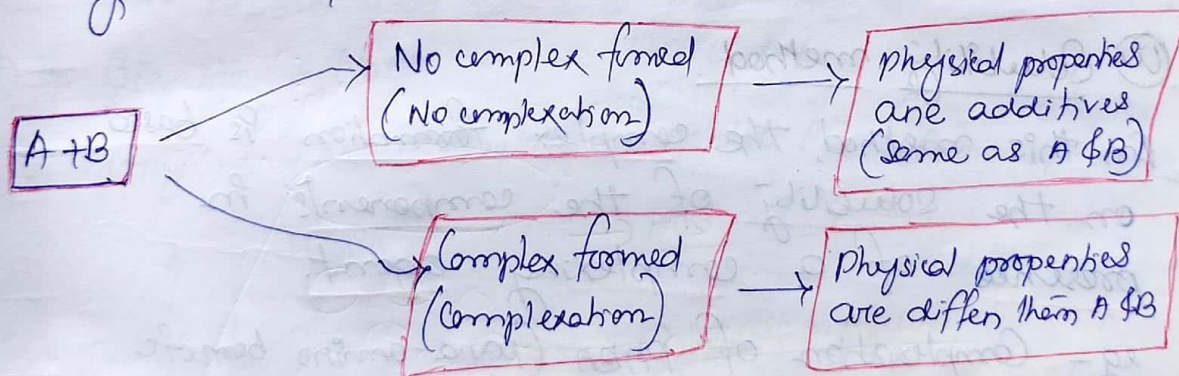
Methods of analysis of Complexation

After complexation process, we have to know whether the complex is made or not?
for this, we use following methods :—

① Method of continuous variation.
② Distribution method.
③ Solubility method
④ PH titration method.

① Method of continuous variation

we know that, when two or more species associated, they formed a complex and due to formation of complex their physical properties would have changed such as Dielectric constant, refractive index etc.

$A + B$

→ No complex formed (No complexation) → physical properties are additives (same as A & B)

→ Complex formed (Complexation) → Physical properties are differ them A & B

② Distribution method : In Distribution method, we find out partition coefficient (distribution coefficient).

eg. $K = \dfrac{X \ in \ oil}{X \ in \ water}$

Now, If in distribution method, complex formed them its solubility increase in oil/water on due to it, its partition coefficient's value change.

Let,

① → when complex is not formed

Iodine (I_2) in 30ml CCl_4 & 30 ml water

for this, let Partition coefficient $(k) = a$

② → when complex is formed/not formed (check)

Iodine (I_2) in 30ml CCl_4 & 30 ml KI

for this, let Partition Coefficient $(k) = b$

If complex is formed in 2nd condition,
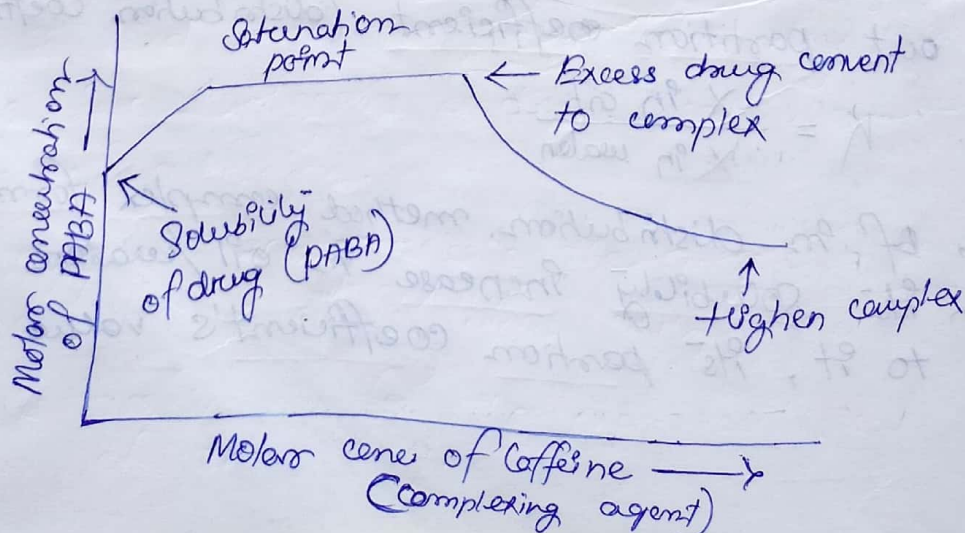   then $\boxed{a \neq b}$
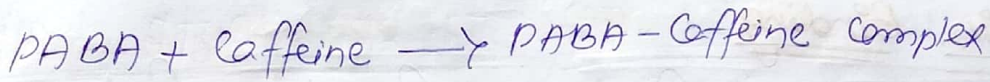
If complex is not formed in 2nd condition
   then $\boxed{a = b}$ (same in second as first)

⓲ Solubility method

In this method, the complex formation is based
on the solubility of the components in
presence of a complexing agent.

eg - Complexation of PABA (para-amino benzoic
acid) by caffeine.

Molar conc of Caffeine →
(complexing agent)

PABA + Caffeine ⟶ PABA-Caffeine complex

$$K = \frac{[PABA-Caffeine]}{[PABA][Caffeine]}$$

If, K (constant) value increase ⟶ complexation ↑
K (constant) value decrease ⟶ complexation ↓

④ PH titration Method : when complex formed
(complexation happened) then also is pH change.
So, firstly we check pH before complexation, then
after complexation if the value of the pH change
means complex formed and if not change means
complex not formed.

example: Chelation of cupric ion by glycine.

① ⟶ Glycine + NaOH ⟶ Basic pH

② ⟶ Glycine + NaOH + Cu^{2+} ⟶ Acidic pH (means complex formed, pH change)

pH ↑

10
9
8 — Basic
7
6
5
4 — Acidic
3
2
1
0

(Glycine)

(Glycine + Cuppen complex)

Volume of NaOH ⟶
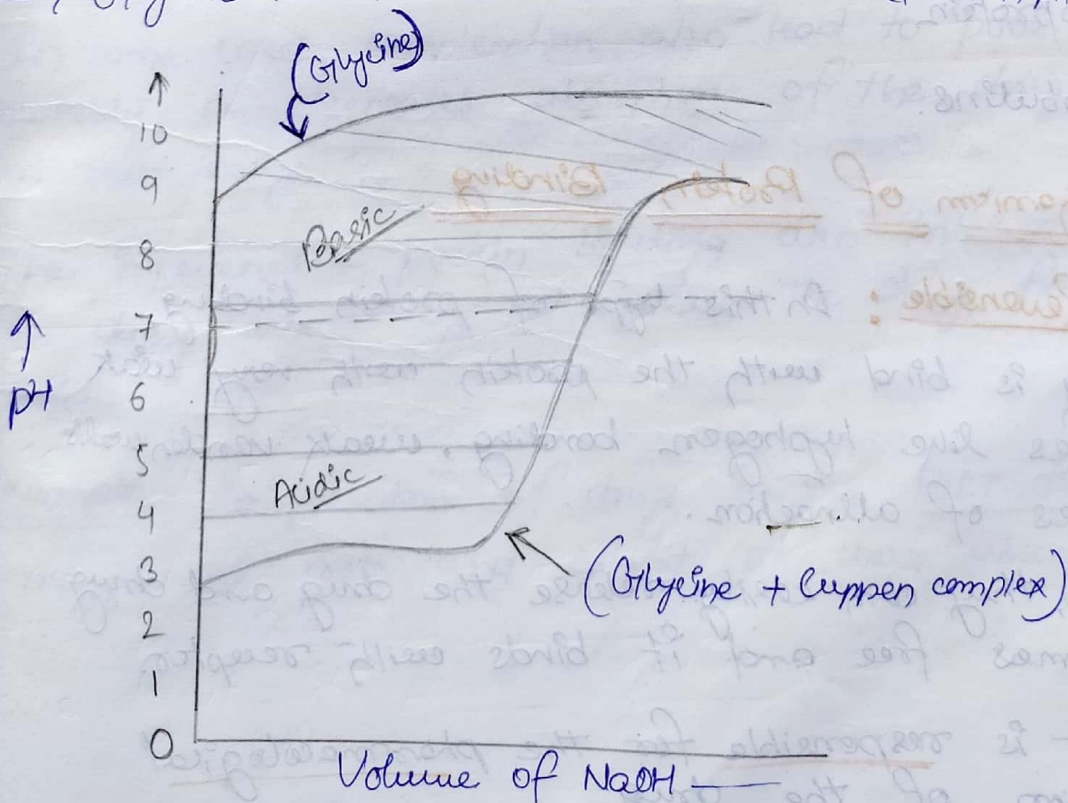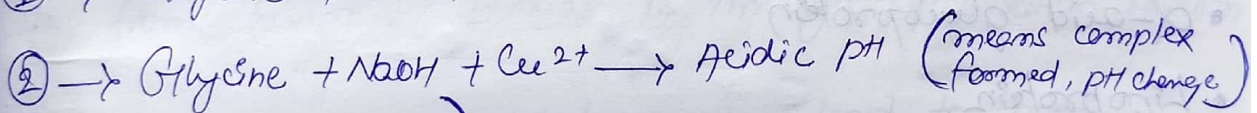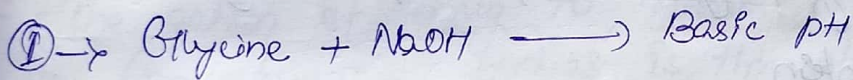
Protein Binding

The phenomena of complex formation of drug with protein is called as protein - drug binding (protein binding)

eg →

protein binding process (complex formation)

(Protein drug binding)

protein site   drugs
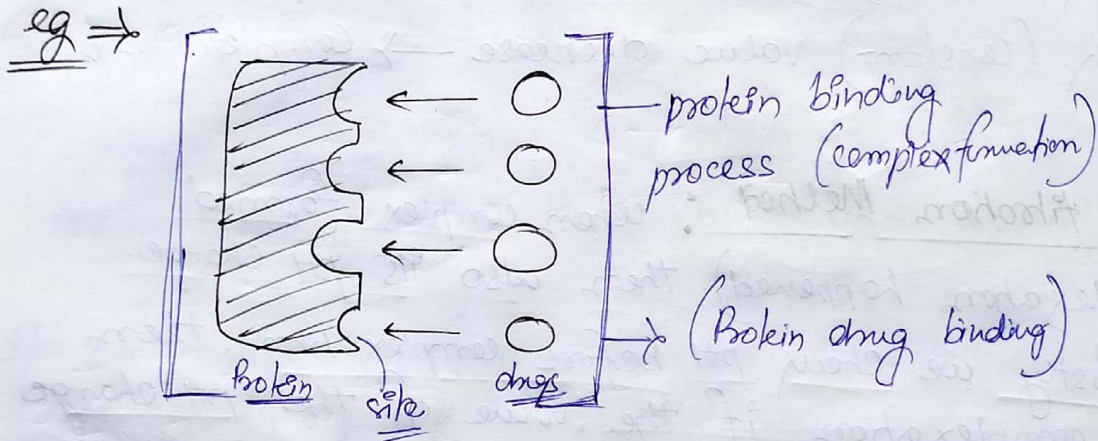
The proteins which involves in this complexation are :— (Blood protein)

- Albumin (Mostly drug attached with albumin)
- α-acid glycoprotein
- Lipoprotein
- Globulins.

Mechanism of Protein Binding

① Reversible : In this type of protein binding, drug is bind with the protein with very weak forces like hydrogen bonding, weak vanden walls forces of attraction.

→ So, they can easily release the drug and drug becomes free and it binds with receptor.

→ It is responsible for the pharmacological action of the drug.

(11) **Irreversible :** In this type of protein binding, the drug bind with the protein with strong bond like covalent bond.

→ Drug after binding with protein cannot release and drug donot become free so they donot give any pharmacological action.

Complexation and Drug action

→ Complexes can alter the pharmacological activity of the agents by inhibiting interaction with receptor.

→ Protein binding complex also affect absorption, metabolism and drug action by interfering with receptor site of action. (inactivate the drug)

→ The action of drug to remove toxic metals from human bodies is through complexation reaction

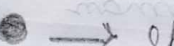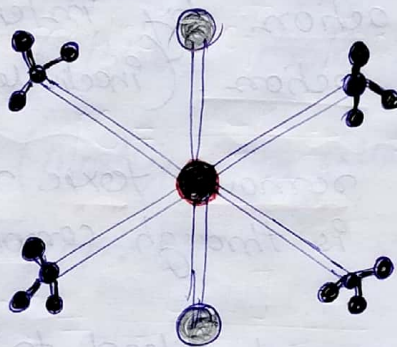⊛ In some cases, complexation also lead to poor solubility or decreases absorption of the drugs in the body.
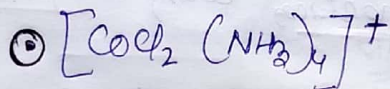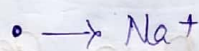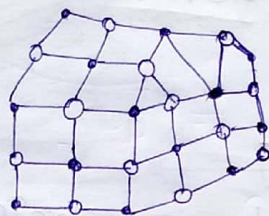
⊛ The irreversible protein binding also inhibit the drug action by not release the drug from protein.

example : Complexation of drug in the GIT fluids may alter rate and extent of drug absorption.

Crystalline Structures of Complexes

A crystalline structure is any structure of ions, molecules or atoms that are held together in a ordered, three dimensional arrangement.

eg - NaCl.

$0 \rightarrow Cl^-$

$\bullet \longrightarrow Na^+$

$\odot \left[CoCl_2 (NH_3)_4\right]^+$

$\bullet \rightarrow Co$

$\blacksquare \rightarrow$

$\bullet \rightarrow$ $\left[\begin{array}{c} \end{array}\right]$ (NH_3)

$\bullet \rightarrow Cl$

$\Rightarrow$ Complex compounds cover the range from quite simple inorganic salts to elaborate metal organic hybrid materials.

$\Rightarrow$ Their present uses and their potential applications are diverese due to their composition, molecular and crystalline structure and their physical and chemical properties.

Thermodynamic treatment of stability constants

The stability constants of the metal complexes are related to thermodynamic properties such as free energy (ΔG), Enthalpy (ΔH) and entropy change (ΔS)

$$\boxed{\Delta G = \Delta H - T\Delta S} \longrightarrow \text{standard entropy change obtained from this eq}^n.$$

where,

ΔG = Gibbs free energy.

ΔH = Enthalpy.

T = Temperature.

ΔS = Entropy.

◉ If $\Delta G = -ve$, then Rate of complexation increases.

⚮ if, Rate of complexation increases, then stability constant increases.

($\Delta G = -ve$) $\propto$ Rate of complexation ↑ $\propto$ Stability constant ↑

• If $\Delta G = +ve$, then Rate of complexation decreases, then stability constant decreases.

($\Delta G = +ve$) $\propto$ Rate of complexation ↓ $\propto$ Stability constant ↓
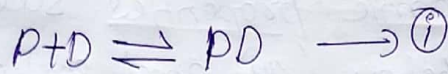
• If Temperature ↑ = $\Delta G \Rightarrow -ve$

Temperature ↓ = $\Delta G \Rightarrow +ve$

Stability constant : It is an equilibrium constant for the formation of a complex in solution.

⇒ It is the measure of the strength of the interaction between the reagent that come together to form the complex.

Kinetics of Protein-Drug Binding.

If p is the protein & b is the drug. Then applying law of mass action —

$$P + D \rightleftharpoons PD \longrightarrow \textcircled{1}$$

At equilibrium, $K_a = \dfrac{[PD]}{[P][D]} \longrightarrow \textcircled{ii}$

$$[PD] = K_a[P][D] \longrightarrow \textcircled{iii}$$

If 'P_T' total conc. of protein present

$$P_T = [PD] + [P] \longrightarrow \textcircled{iv}$$

If r is the no. of moles of drug bound to total moles of protein, then —

$$r = \dfrac{[PD]}{[P_T]} = \dfrac{[PD]}{[PD] + [P]} \longrightarrow \textcircled{v}$$

$$r = \dfrac{[PD]}{[PD] + [P]}$$

$$= \dfrac{K_a[P][D]}{K_a[P][D] + [P]}$$

$$= \dfrac{K_a[P][D]}{[P](K_a[D] + 1)}$$

$$= \dfrac{K_a[D]}{K_a[D] + 1} \longrightarrow \textcircled{vi}$$

If more than 1 or N No. of binding sites are available then,

$$r = \dfrac{N K_a[D]}{K_a[D] + 1}$$