Complexation and Protein binding

INTRODUCTION:

- Complexation is the process of complex formation that is the process of characterization the covalent or non-covalent interactions between two or more compounds.
- The ligand is a molecule that interacts with another molecule, the Drug, to form a complex. Drug molecules can form complexes with other small molecules or with macromolecules such as proteins.
- A coordination complex is the product of a Lewis acid-base reaction in which neutral molecules or anions (called ligands) bond to a central metal atom (or ion) by coordinate covalent bonds
- Simple ligands include water, ammonia and chloride ions.

Once complexation occurs, the physical and chemical properties of the complexing species altered are;

- ❖ Solubility,
- ❖ Stability,
- ❖ Partition co-efficient,
- ❖ Energy absorption,
- ❖ Energy emission.
- ❖ Conductance of the drug.

Forces involved in complex formation:

- ❖Covalent bond.
- ❖Co-ordinate covalent bond.
- ❖Van der Waals force of dispersion.
- ❖Dipole-Dipole interaction.
- ❖Hydrogen bond.

Beneficial effects of complexation:

- Drug complexation, therefore, can lead to beneficial properties such as enhanced aqueous solubility (e.g., theophylline complexation with ethylenediamine to form aminophylline) and stability (e.g., inclusion complexes of labile drugs with cyclodextrins).
- Complexation also can aid in the optimization of delivery systems (e.g., ion-exchange resins) and affect the distribution in the body after systemic administration as a result of protein binding.
- In some instances, complexation also can lead to poor solubility or decreased absorption of drugs in the body.
- For some drugs, complexation with certain hydrophilic compounds can enhance excretion

CLASSIFICATION OF COMPLEXATION

A. Metal ion complexes

Metal ion includes the central atom as Drug and it interacts with a base (Electron-pair donor, ligand), forming co-ordination bonds between the species.

- Inorganic type
- Chelates
- Olefin type
- Aromatic type
    - Pi (π) complexes
    - Sigma (σ) complexes
    - "Sandwich" compounds

B. Organic molecular complexes

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

C. Non-Bonded or Inclusion/ occlusion compounds

- Channel lattice type
- Layer type
- Clathrates
- Monomolecular type
- Macromoleular type

A . Metal ion complexes:

- Metal ion includes the central atom as Drug and central metal atom or ion (cation) surrounded by a number of negatively charged ions or neutral molecules possessing lone pairs.
- The ions surrounding the metal are known as ligands. The number of bonds formed between the metal ion and ligand is called as co-ordination number

a) Inorganic type –

- In inorganic metal complexes, the ligand provides only one site for binding with metal.
- Ligands are generally bound to a metal ion by a covalent bond and hence called to be co-ordinated to the ion.
- The interaction between metal ion and the ligand is known as a Lewis acid-base reaction wherein the ligand (base) donates a pair of electron (to the metal ion, an acid) to form the co-ordinate covalent bond.

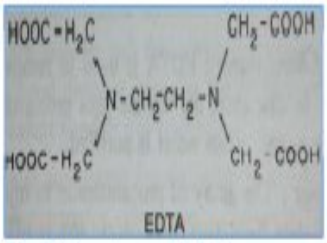
- For example, the ammonia molecules in hexamine cobalt (III) chloride, as the compound $[Co(NH_3)_6]^{3+} \cdot Cl3$ is called as the ligands and are said to be co-ordinated to the cobalt ion.
- The co-ordination number of the cobalt ion, or number of ammonia groups co-ordinated to the metal ions, is six.
- Other complex ions belonging to the inorganic group include $[Ag(NH3)_2]^+$, $[Fe(CN)_6]^{4-}$, and $[Cr(H2O)_6]^{3+}$.
- Each ligand donates a pair of electrons to form a coordinate covalent link between itself and the central ion having an incomplete electron shell.
- Hybridization plays an important part in coordination compounds in which sufficient bonding orbitals are not ordinarily available in the metal ion.
- Another example is interaction between silver and ammonia;

$$\underset{\text{Silver ion}}{Ag^+} + \underset{\text{Ammonia}}{2(:NH_3)} = \underset{\text{Silver-ammonia coordiniate complex}}{[Ag(NH_3)_2]^+}$$

- In this case silver metal ion interacts with ammonia to form silver-ammonia co-ordinate complex.
- Electron pair donating ligands such as H2O:, NC:, Cl: etc neutralizes co-ordinate complexes. The [Ag(NH3)2]+ complex is neutralized with Cl as [Ag(NH3)2]Cl.
- The co-ordination compounds through bonds with central metal atom and surrounding ligands plays important role in controlling the structure and functions of various enzymes in our body.

b)Chelates -

- The chelates are a group of metal ion complexes in which a substance (Ligands) provides two or more donor groups to combine with a metal ion.
- Some of the bonds in a chelate may be ionic or of the primary covalent type, whereas others are coordinate covalent links.
- When the ligand provides one group for attachment to the central ion, the chelate is called monodentate.
- Pilocarpine behaves as a monodentate ligand toward Co(II), Ni(II), and Zn(II) to form chelates of pseudo tetrahedral geometry.



- **Fig 1. Structure of EDTA.**

Applications of chelation:

- Chlorophyll and haemoglobin, two extremely important compounds, are naturally occurring chelates involved in the life processes of plants and animals.

• Albumin is the main carrier of various metal ions and small molecules in the blood serum. The amino-terminal portion of human serum albumin binds to Cu(II) and Ni(II) with higher affinity than that of dog serum albumin. This fact partly explains why humans are less susceptible to copper poisoning than are dogs. The binding of copper to serum albumin is important because this metal is possibly involved in several pathologic conditions.

c)Olefin type –

- Olefins belong to a family of organic compounds called hydrocarbons.
- They consist of different molecular combinations of the two elements, carbon and hydrogen.
- Another name for an olefin is an alkene. Alkenes contain one or more double bonds between the carbon atoms of the molecule. Olefins form different compounds based on their structure
- The aqueous solution of certain metal ions like Pt, Fe, Pd, Hg and Ag can absorb olefins such as ethylene to yield water soluble complexes.
- These complexes have limited use in pharmaceutical field, but have been used as a catalysts in the polymerisation of ethylene and propylene (unsaturated hydrocarbon) to form polyethylene and poly propylene respectively

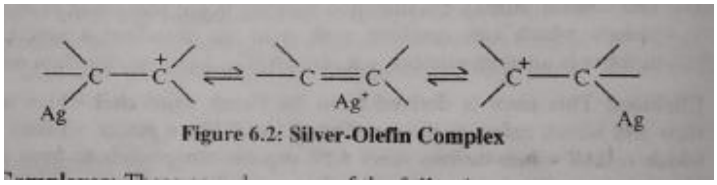


Figure 6.2: Silver-Olefin Complex

d)Aromatic type -

i. Pi (π) complexes – Aromatic bases (Benzene, toluene and Xylene) form pi-bond complexes with metal ions like Ag by Lewis acid-base reactions.

- Stability of complex depends on aromatic hydrocarbons basic strength

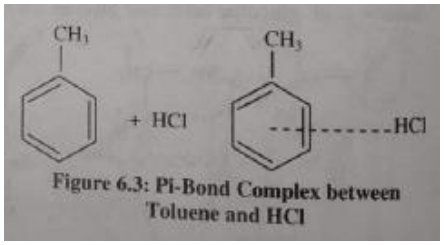


Figure 6.3: Pi-Bond Complex between Toluene and HCl

ii. Sigma (σ) complexes – sigma bond complexes involve in the formation of a sigma-bond between ion and a carbon of aromatic ring. The isolation of these highly reactive complex is difficult.

- For example. Toluene and HCl.$Alcl_3$ form a sigma complex

iii. Sandwich" compounds –

- The are relatively stable complexes involving in the delocalized covalent bond between the d-orbital of transition metal and a molecular orbit of the aromatic ring.
- For example, ferrocene or bisdicyclopentadienyl iron II is a sandwich complex.

- In this complex, one π electron each ring binds the metal atom and exhibit aromatic character

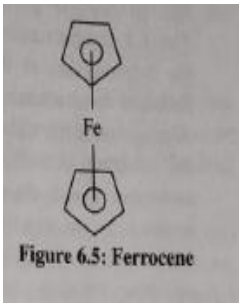


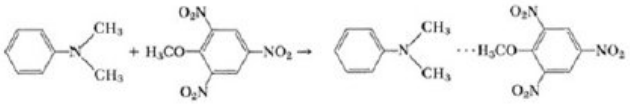
Figure 6.5: Ferrocene

B. Organic molecular complexes:

- Many organic complexes are so weak that they cannot be separated from their solutions as definite compounds.
- The energy of attraction between the constituents is probably less than 5 kcal/mole for most organic complexes.
- Because the bond distance between the components of the complex is usually greater than 3 Å, a covalent link is not involved.
- An organic coordination compound or molecular complex consists of constituents held together by weak forces of the donor–acceptor type or by hydrogen bonds.

❑ ***Donor Acceptor type –***

In this, the bond is between uncharged species and lacks charge transfer.
The dipole-dipole interaction and London dispersion forces (Dotted lines) make the complex stable. *Example - The compounds dimethylaniline and 2,4,6-trinitroanisole react in the cold to give a molecular complex.*



❑ ***The charge transfer Complexes –***

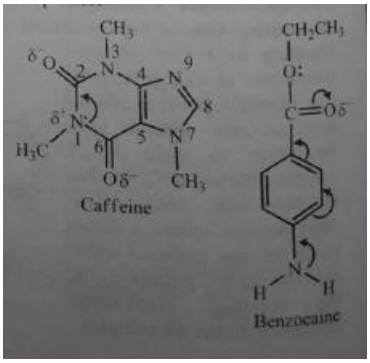
- In this one molecule polarizes the other, resulting in a type of ionic interaction or charge transfer, and these molecular complexes are often referred to as charge transfer complexes.
- The resonance makes the complex more stable.

a) Caffeine and other drug complexes -

- Drugs such as benzocaine, procaine and tetracaine form complexes with caffeine.
- A number of acidic drugs are known to form complexes with caffeine.

Mechanism

Dipole-dipole force or hydrogen bonding between the polarised carbonyl groups of caffeine and the hydrogen atom of the acid.



b) Quinhydrone type –

- The molecular complex of this type is obtained by mixing alcoholic solutions of equimolar quantities of hydroquinone and benzoquinone
- The 1:1 complex is formed when the π frame work of the electron deficient benzoquinone molecule overlaps with the π frame work of electron rich hydroquinone molecule
- Quinhydrone complex is used as an electrode in pH determination

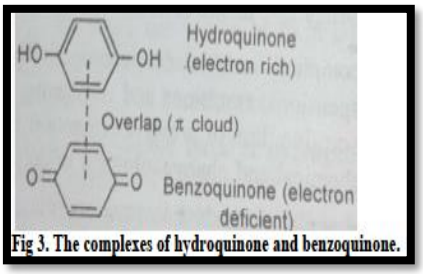


Fig 3. The complexes of hydroquinone and benzoquinone.

c)Polymers Type –

- Many pharmaceutical additives such as polyethylene glycols (PEGs), carboxymethyl cellulose (CMC) contain nucleophilic oxygen. These can form complexes with various drugs.

Agent	Drugs forming Complex
Polyethylene glycols	Salicylic acid, o-phthalic acid, acetyl salicylic acid, resorcinol, catechol, phenol, phenobarbital
Polyvinyl-pyrrolidone	Benzoic acid, salicylic acid, sodium salicylate, mandelic acid, sulfathiazole, chloramphenicol, phenobarbital
Sodium carboxy methyl cellulose	Quinine, benadryl, procaine, pyribenzamine

d)Picric acid types –

- Picric acid, being a strong acid, forms organic molecular complexes with weak bases,

Butesin has anaesthetic property and picric acid has antiseptic property. Butesin complexes with picric acid and form butesin- picrate ointment of the complex is used in treatment of burns.

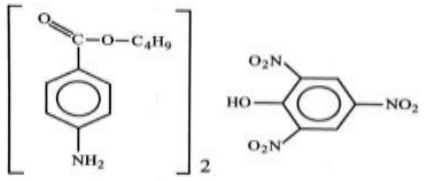


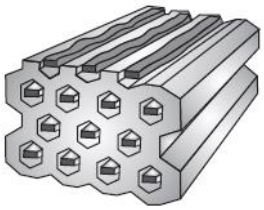
Figure 7.9: Butesin picrate complex

C. Inclusion Complexes:

- These complexes are also called occlusion compounds in which one of the components is trapped in the open lattice or cage like crystal structure of the other.
- The interaction in this case does not occur due to chemical reactivity, but because of the favourable molecular structure
- Weak interaction forces exist in these complexes

a) Channel types –

- Channels are formed by crystallization of host molecules.
- Host molecules are usually tubular channel (e.g., Deoxycholic acid, urea, thiourea, amylose).
- Guest component is usually long unbranched straight chain compounds (E.g. Paraffin, esters, acids, ethanol, etc)
- E.g., Urea- methyl Alpha lipoate Complex, starch-iodine complex
- These are useful for separation of isomers & analysis of dermatological creams.



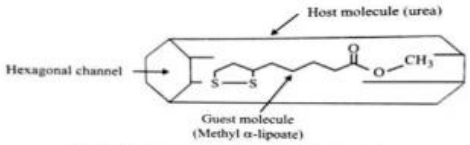


Figure 7.10 channel complex of methyl alpha lipoate with urea

b) Layer types –

- Compounds such as clays, montomorillorite (constituent of bentonite), can entrap hydrocarbons, alcohols and glycols.
- They form alternate monomolecular (monoatomic) layers of guest and host.
- Their uses are currently quite limited; however, these may be useful for catalysis on account of a larger surface area.

c)Clathrates -

- It is available as white crystalline powder, during crystallization, certain substances form a cage-like lattice in which the coordinating compound is entrapped.
- Chemical bonds are not involved in these complexes, and only the molecular size of the encaged component is of importance.
- The stability of a clathrate is due to the strength of the structure.
- Example- Hydroquinone form cage with hydrogen bonds and this can entrap methanol, CO2, HCl

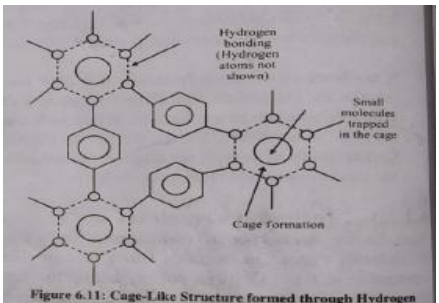


Figure 6.11: Cage-Like Structure formed through Hydrogen Bonding of Hydroquinone Molecules. Small Molecules are Entrapped in the Cages to form the Clathrate

d)Monomolecular types –

- Monomolecular inclusion compounds involve the entrapment of a single guest molecule in the cavity of one host molecule.
- Most of the host molecules are cyclodextrins.
  The interior of the cavity is relatively hydrophobic, whereas the entrance of the cavity is hydrophilic in nature

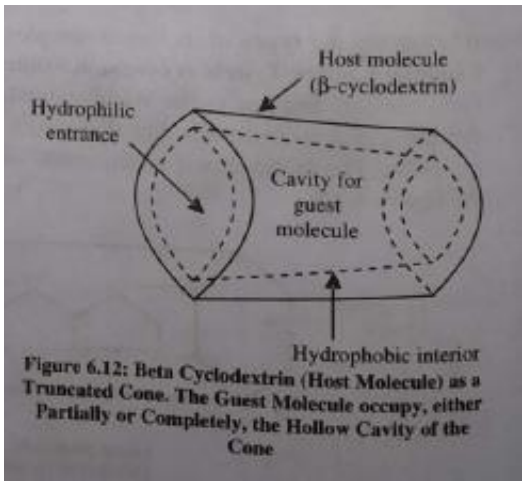


Figure 6.12: Beta Cyclodextrin (Host Molecule) as a Truncated Cone. The Guest Molecule occupy, either Partially or Completely, the Hollow Cavity of the Cone

<u>Applications of monomolecular complexes</u>

- Enhanced Solubility: Complexation with B-cyclodextrin enhances the solubility of retinoic acid (0.5mg/1t) up to 160mg/it.
- Enhanced Dissolution: Complexation with B-cyclodextrin enhances the dissolution rate of famotidine and tolbutamide.

- Enhanced Stability: Complexation with B-cyclodextrin enhances the stability of aspirin, benzocaine, ephedrine, and testosterone.
- Sustained Release: Complexation with ethylated B-cyclodextrin delays the release of diltiazem and isosorbide dinitrate for prolonged periods to provide a sustained effect

APPLICATIONS OF COMPLEXATION:

Physical state:

- Complexation process improves processing characteristics by converting liquid to solid complex.
  E.g-: β-cyclodextrin complexes with nitro-glycerine.

Volatility:

Complexation process reduces Drug volatility for following benefits;

- Stabilise system.
- Overcome unpleasant odour (*E.g-: I_2 complexes with Poly Vinyl Pyrrolidone, PVP*).

Solid state stability:

- Complexation process enhances solid state stability of drugs.
  E.g-: β-cyclodextrin complexes with Vitamin A and D.

Chemical stability:

- Complex formation inhibits chemical reactivity (Mostly inhibit).
  E.g-: The hydrolysis of Benzocaine is decreased by complexing with Caffeine.

Solubility:

- Complexation process enhances solubility of drug.
  E.g.-: Caffeine enhances solubility of PABA (Para Amino Benzoic Acid) by complex formation.
  The aqueous solubility of retinoic acid (0.5 mg/L), a drug used topically in the treatment of acne, is increased to 160 mg/L by complexation with β-CD.

Dissolution:

- Complexation process enhances dissolution of drug.
  E.g.-: β-cyclodextrine increases the dissolution of Phenobarbitone by inclusion Complex.

Partition co-efficient:

- Complexation process enhances the partition coefficient of certain drugs.
  E.g.-: Permanganate ion with benzene.

Absorption and Bioavailability:

- Complexation process *reduces the absorption of Tetracycline by complexing with cations like Ca^{+2}, Mg^{+2} and $Al^{+3.}$*
- Complexation process *enhances the absorption of Indomethacin and Barbiturates by complexing with β-cyclodextrin.*

Reduced toxicity:

- *β-cyclodextrin reduces ulcerogenic effects of Indomethacin.*

- *β-cyclodextrin reduces local tissue toxicity of Chlorpromazine.*

Antidote for metal poisoning:

- BAL (British Anti Lewisite) reduces toxicity of heavy metals by complexing with Arsenic, Mercury and Antimony.

Antitubercular activity:

- PAS (Para Amino Salicylic acid) complexes with Cupric ion exhibit greater Antitubercular activity.

Development of Novel Drug delivery system:

- The Complexation of drug with polymers used in the formulation of sustained drug delivery device.

Assay of Drugs:

- The complexometric titrations are used to assay of the drug containing the metal ion.

As therapeutic Tools:

- Both CITRATES and EDTA are used as preservation of blood as anti-coagulant.

Taste masking:

Cyclodextrins may improve the organoleptic characteristics of oral liquid formulations. The bitter taste of suspensions of femoxetine (antidepressant) is greatly suppressed by complexation of the drug with β-CD.

Modifying drug release:

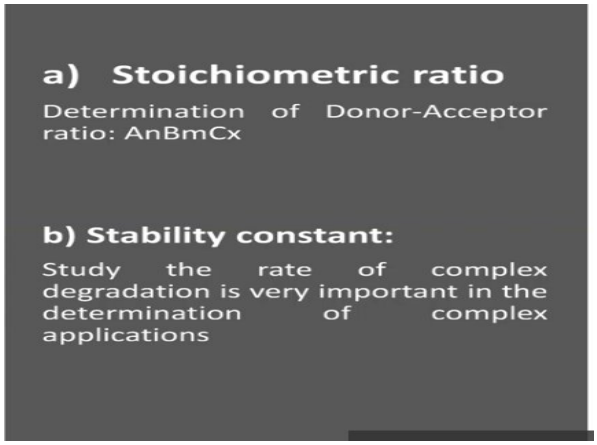
- The hydrophobic forms of β-CD have been found useful as sustained-release drug carriers. *The release rate of diltiazem (water-soluble calcium antagonist) was significantly decreased by complexation with ethylated β-CD.*
- The release rate was controlled by mixing hydrophobic and hydrophilic derivatives of β-CD at several ratios.

In diagnosis :

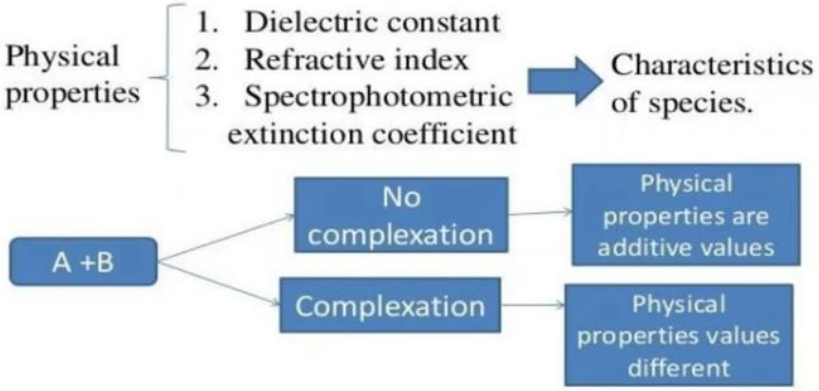
Technetium 90 (a radionuclide) is prepared in the form of citrate complex and this complex is used in diagnosis of kidney function and globular filtration rate.
Squibb (complex of a dye Azure A with carbacrylic cation exchange resin) is used for detection of achlorhydria due to carcinoma and pernicious anemia.

METHODS OF ANALYSIS OF COMPLEXATION:

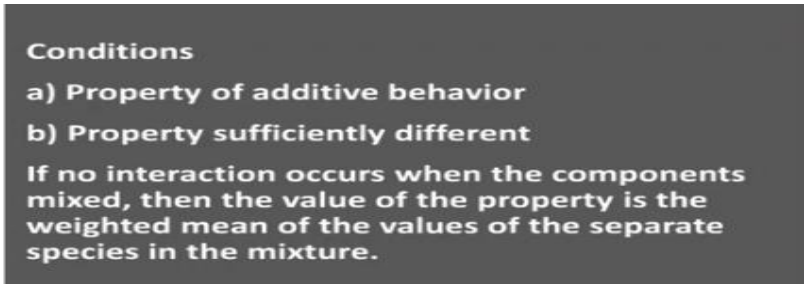
- A determination of the stoichiometric ratio of ligand to metal or donor to acceptor and a quantitative expression of the stability constant for complex formation are important in the study and application of co-ordination compounds. A limited number of the more important methods for obtaining these quantities are described below.
    - ❑ Method of Continuous Variation:
    - ❑ pH titration method
    - ❑ Distribution method
    - ❑ Solubility method



1. Method of Continuous Variation:

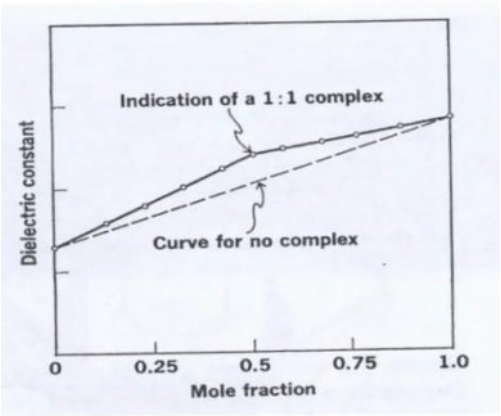


- In this we measured additive property.
- JOB suggested the use of additive property such as spectrophotometric extinction coefficient (dielectric constant & square of R.I) used for the measurement of complexation.



By using Dielectric constant

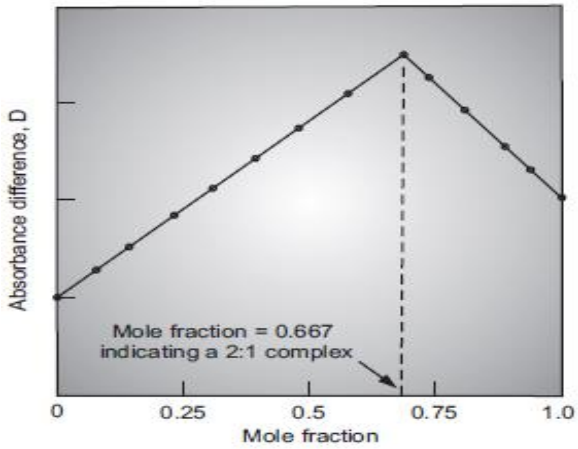
- This means that if the additive property, say dielectric constant, is plotted against the mole fraction from 0 to 1
- If there is no complexation then it will give linear relationship



- If solutions of two species A and B of equal molar concentration are mixed and if a complex form between the two species, the value of the additive property will pass through a maximum (or minimum) as shown by the upper curve in Figure
- For a constant total concentration of A and B, the complex is at its greatest concentration at a point where the species A and B are combined in the ratio in which they occur in the complex.
- The line show a change in slope occurs at the mole fraction corresponding to the complex.
- The change in slope occur at a mole fraction indicate a type of complex

By using spectrophotometric Absorbance

- In this method measure the absorbance of the solutions of various mole fraction in which the complex is form.
- Measure the absorbance of another same mole fraction of solution in which the complex is not form.
- Take the absorbance difference of this solutions (D) and plot a graph Vs mole fraction.
- The curves give the molar ratio of the complex
- Extrapolate the intersect point on x axis gives the concentration of mole fraction require to form stable complex.
- If we plot the graph of log [MAn] Vs log[A] then slope of line gives no. of ligand mole require to form complex & intercept gives the stability constant.



- ***The stability constant of the complex formation can be determined by a method described by Bent and French.***
- If the magnitude of the measured property, such as absorbance, is proportional only to the concentration of the complex MA_n, the molar ratio of ligand A to metal M and the stability constant can be readily determined. The equation for complexation can be written as

$$M + nA = MA_n$$

and the stability constant as

$$K = \frac{[MA_n]}{[M]\,[A]^n}$$

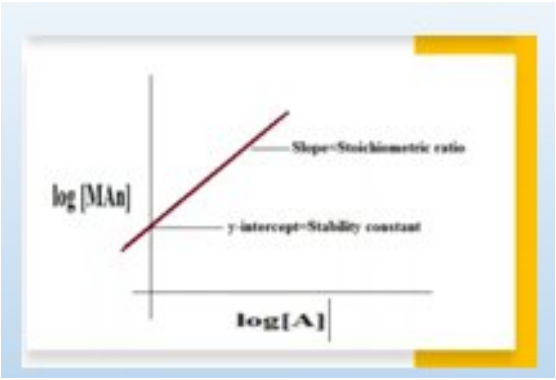
or, in logarithmic form,

$$\log [MA_n] = \log K + \log [M] + n \log [A]$$

where,

- ***[MAn] =concentration of the complex,***
- ***[M] =concentration of the uncomplexed metal,***
- ***[A] =concentration of the uncomplexed ligand,***
- ***n =number of moles of ligand combined with 1 mole of metal ion,***
- ***K =equilibrium or stability constant for the complex.***
- The concentration of a metal ion is held constant while the concentration of ligand is varied, and the corresponding concentration, [MAn], of complex formed is obtained from the spectrophotometric analysis.

If we plot the graph of log[MAn] Vs log[A] then slope of line gives no. of ligand mole require to form complex & intercept gives the stability constant



2. pH Titration Method

This method is applicable for that complex that produces the changes in pH on interaction. The significant change in pH will determine that complexation has been taken place.

- E,g, chelation of cupric ions by glycine molecules and chelation of calcium ions by EDTA

Principle –

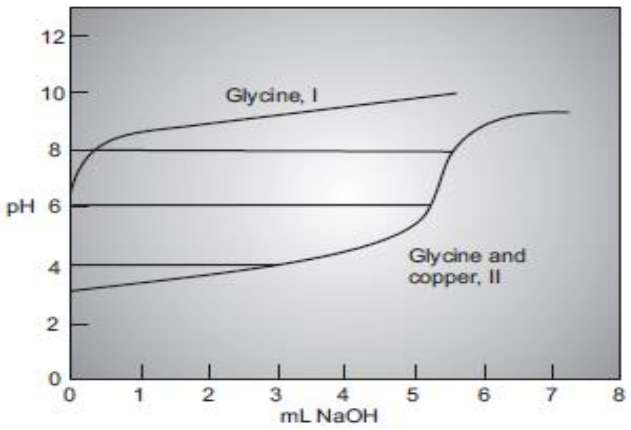
- The chelation of the cupric ion by glycine is represented as

$$Cu_2 + 2NH_3 + CH_2COO^- = Cu(NH_2CH_2COO)_2 + 2H^+$$

- In the reaction of equation since two protons are formed ,the addition of glycine to a solution containing cupric ions should result in a decrease in pH.

Method

- Let us take 75 ml of glycine solution and it is titrated with strong alkali NaOH solution. The pH was recorded. A graph was drawn between pH and volume of NaOH added.
- In another test, complex solution of glycine and copper salt is titrated. The change in pH with increments of NaOH solution also recorded. A graph was drawn between pH and volume of NaOH added.
- The two plots are compared and it is seen that the plot of glycine with copper is well below that of the pure glycine, which indicated that complexation is obtained throughout the titration range.



Calculation of Parameters

$\bar{n}$ the average number of ligand groups bound per metal ion is:

$$\bar{n} = \frac{(\text{total conc. of ligand bound})}{(\text{total conc. of metal ion})} \quad \ldots\ldots (5)$$

The horizontal distance calculated at any pH in terms of moles/litre of sodium hydroxide is equal to the total concentration of bound ligand. Since the initial total concentration of metal ion is known, $\bar{n}$ can be determined:

When $\bar{n} = 1$, the overall stability constant (β) can be determined by:

$$p[A] = 1/2 \log \beta \text{ at } \bar{n} = 1 \quad \ldots\ldots (6)$$

For estimating p[A]:

$$p[A] = pK_a - pH - \log([HA]_{initial} - [NaOH]) \quad \ldots\ldots (7)$$

Where,

p[A] = Concentration of bound ligand (glycine)

pK_a = Dissociation constant of ligand (glycine)

$[HA]_{initial}$ = Concentration of glycine at the initial stage at that pH value where $\bar{n} = 1$

[NaOH] = Horizontal distance in moles/litre of sodium hydroxide at the same pH

3.Distribution Method:

- The method of distributing a solute between two immiscible solvents can be used to determine the stability constant for certain complexes.
- The distribution behaviour of a solute between two immiscible liquids is expressed by distribution or partition co-efficient.
- ***Principle -*** When a solute complexes with an added substance, the solute distribution pattern changes depending on the nature of the complex.
- To explain this method, consider complexation between iodine and potassium iodide
- First iodine is added to immiscible system containing carbon disulphide and water
- The distribution coefficient in this system is calculated as

$$K = \frac{[I]_0}{[I]_W}$$

Where, K = Distribution coefficient

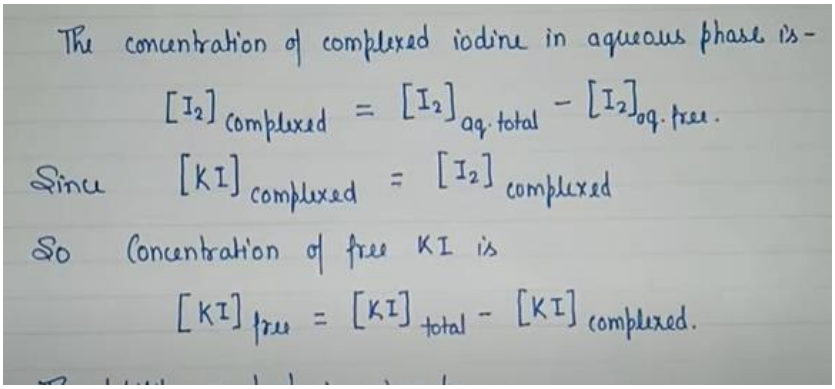
$[I]_o$ = Iodine concentration in organic phase, i.e., carbon disulphide

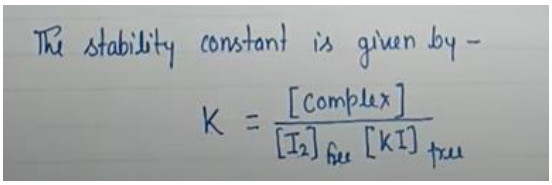
$[I]_w$ = Iodine concentration in aqueous phase

- In the next step, Iodine is added to an immiscible system containing carbon disulphide and aqueous solution of potassium iodide
- Iodine gets distributed between 2 phases
- Iodine and potassium iodide forms a complex where by following equilibrium reaction occurs

$$I_2 + I^- = I_3^-$$

- The concentration of free (un complexed) and total iodine along with concentration of potassium iodide in aqueous phase are determined
- The common species here is the free iodide, which could be obtained using distribution law

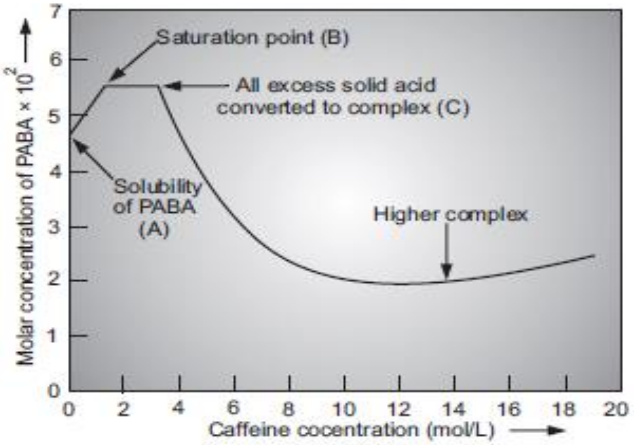


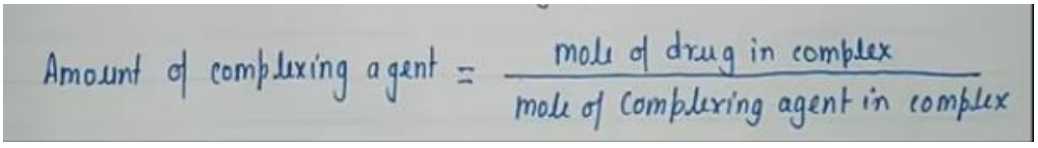


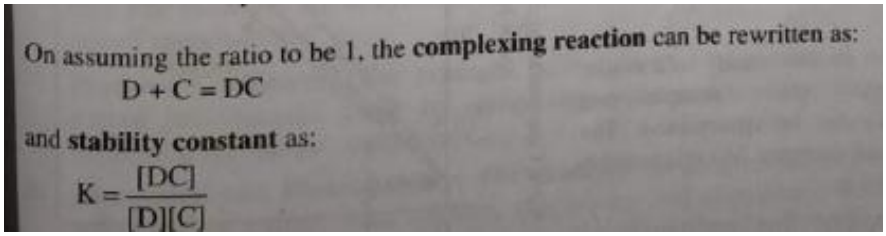
- The distribution method favours the complexation study of caffeine, polyvinyl pyrrolidone and polyethylene glycols with many acidic drugs like benzoic acid, salicylic acid and acetyl salicylic acid.

4. Solubility Method:

- ***Principle*** - When the component in a mixture produces a complex, the solubility of one of the components may be increased or decreased. The change in solubility is a sign of complexation.
- The experimental data can be used to analyse complexes in terms of donor-acceptor ratio and equilibrium stability constant.
- Example – PABA and Caffeine and Paracetamol – Caffeine.
- In this method, various concentrations of complexing agent in an aqueous vehicle are taken in well-stoppered containers.
- The drug in excess quantity is added in all the containers.
- Which are then placed at a constant temperature using a water bath and stirred until equilibrium
- On attaining equilibrium, from the supernatant liquid an aliquot portion is withdrawn to analyse the drug concentration in solution.

- *The solubility method was used to investigate the complexation of p-amino benzoic acid (PABA) by caffeine.*
- A plot between the drug solubility (PABA) at different molar concentrations of the complexing agent and the molar concentration of the complexing agent (Caffeine) is drawn
- The results of the study are shown here
- The drug solubility increases on increasing the concentration of complexing agent since a soluble complex is formed.
- 

- The point A at which the line crosses the vertical axis is the solubility of the drug in water.
- With the addition of caffeine, the solubility of PABA rises linearly owing to complexation. At point B, the solution is saturated with respect to the complex and to the drug itself.
- On further increasing the concentration of the complexing agent (caffeine), more complexes are formed which precipitate out from the solution (already saturated).
- The point C suggests that all the excess amount of drug (PABA) has been consumed for producing complexes.
- Adding more of the complexing agent forms one or more secondary complexes (higher complexes).
- (Since some of the PABA remains uncomplexed in solution, and it combines further with caffeine to form higher complexes)
- The curve gives the stoichiometry of the interaction.
- The complexing agent concentration can be known directly from the graph and is equal to:
- 

- 
On assuming the ratio to be 1, the **complexing reaction** can be rewritten as:

$$D + C = DC$$

and **stability constant** as:

$$K = \frac{[DC]}{[D][C]}$$

- Where,
- (DC) = Drug-complex concentration which is equal to the total drug in solution at point B minus the solubility of uncomplexed drug(A)
- [D] = Uncomplexed drug solubility
- [C] = Complexing agent concentration incorporated into to the system at point B minus the quantity of complexing agent in the drug complex (DC)
- For many caffeine complexes, the stability constants have been calculated using the Solubility method