BP302T. PHYSICAL PHARMACEUTICS-I (Theory)

UNIT-IV - 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:

- 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
- **Organic molecular complexes**
  - Quinhydrone type
  - Picric acid type
  - Caffeine and other drug complexes
  - Polymer type
- **Non-Bonded or Inclusion/ occlusion compounds**
  - Channel lattice type
  - Layer type
  - Clathrates
  - Monomolecular type
  - Macromoleular type

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 –

- In inorganic metal complexes, the ligand provides only one site for binding with metal.
- The ammonia molecules in hexamminecobalt (III) chloride, as the compound [Co(NH3)6]3+ Cl3- , are known as the *ligands* and are said to be *coordinated* to the cobalt ion. The *coordination number* of the cobalt ion, or number of ammonia groups coordinated 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.

$$Co^{3+} + 6\ddot{N}H_3 = [Co(NH_3)_6]^{3+}$$

- Hybridization plays an important part in coordination compounds in which sufficient bonding orbitals are not ordinarily available in the metal ion.

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 pseudotetrahedral geometry.

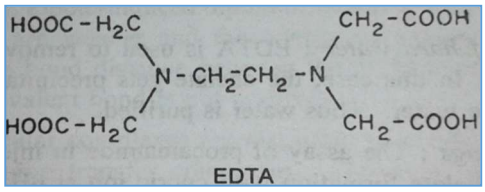


Fig 1. Structure of EDTA.

Olefin type -

- 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 are uses as catalyst in the manufacture of bulk drugs and analysis of drugs.

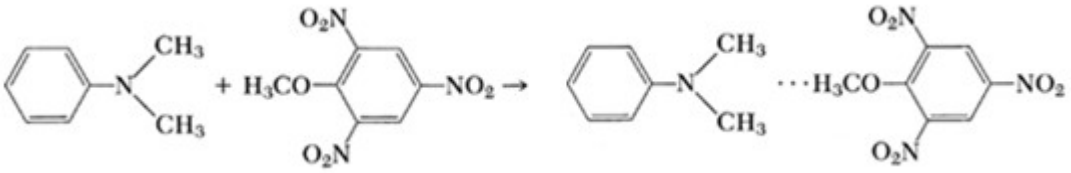
Aromatic type -

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

- Sigma (σ) complexes – sigma bond complexes involve in the formation of a sigma-bond between ion and a carbon of aromatic ring.
- "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.

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 but 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. The intermolecular bonding is quite higher compared to donor-acceptor type complexes. For example, the polar nitro groups of trinitrobenzene induce a dipole in the readily.

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.

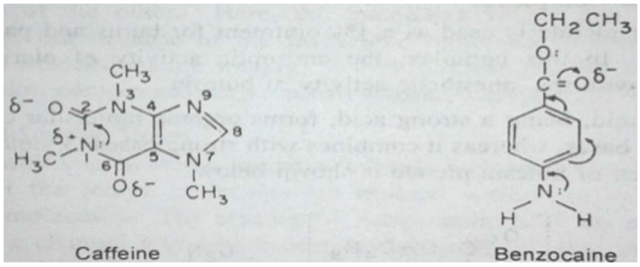


Fig 2. Structure of caffeine and Benzocaine.

Quinhydrone type –

- The molecular complex of this type is obtained by mixing alcoholic solutions of equimolar quantities of hydroquinone and benzoquinone.

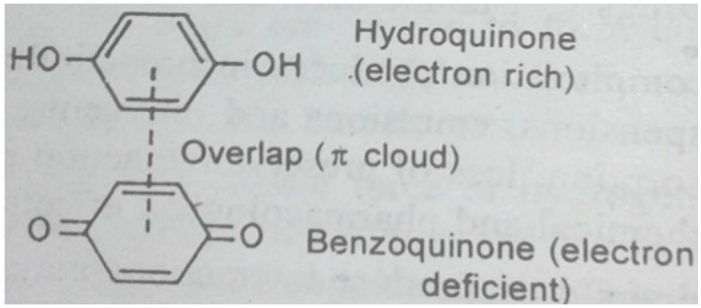


Fig 3. The complexes of hydroquinone and benzoquinone.

Polymers Type –

- Many pharmaceutical additives such as polyethylene glycols (PEGs), carboxymethyl cellulose (CMC) contain nucleophilic oxygen. These can form complexes with various drugs.
- E.g. Polymers: carbowaxes, pluronics etc. Drugs: tannic acid, salicylic acid, phenols etc.
- Carboxy methyl cellulose + Amphetamine – Poorly absorbed drugs.

Picric acid types –

- Picric acid, being a strong acid, forms organic molecular complexes with weak bases, whereas it combines with strong bases (anesthetic activity of butesin) to yield salts.

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.

Channel types –

- Channels are formed by crystallization of the host molecules, the guest component is usually limited to long, unbranched straight chain compounds.

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.

Clathrates -

- It is available as white crystalline powder, during crystallization, certain substances form a cage-like lattice in which the coordinating compound is entrapped.

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.

Applications of Complexation:

- ***Physical state:***
  - Complexation process improves processing characteristics by converting liquid to soild complex. β-cyclodextrine complexes with nitroglyerine.
- ***Volatility:***
  - Complexation process reduces Drug volatility for following benefits;
    - Stabilise system.
    - Overcome unpleasant odour (I_2 complexes with Poly Vinyl Pyrollidone, PVP).
- ***Solid state stability:***
  - Complexation process enhances solid state stability of drugs.
  - β-cyclodextrine complexes with Vitamin A and D.
- ***Chemical stability:***
  - Complex formation inhibit chemical reactivity (Mostly inhibit).
  - The hydrolysis of Benzocaine is decreased by complexing with Caffeine.
- ***Solubility:***
  - Complexation process enhances solubility of drug.
  - Caffeine enhances solubility of PABA (Para Amino Benzoic Acid) by complex formation.
- ***Dissolution:***
  - Complexation process enhances dissolution of drug.
  - β-cyclodextrine increases the dissolution of Phenobarbitone by inclusion complex.

- ***Partition co-efficient:***
  - Complexation process enhances the partition coefficient of certain drugs.
  - 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 aborption of Indomethacine and Barbiturates by complexing with β-cyclodextrine.
- ***Reduced toxicity:***
  - β-cyclodextrine reduces ulcerogenic effects of Indomethacine.
  - β-cyclodextrine reduces local tissue toxicity of Chlorpromazine.
- ***Antidote for metal poisoning:***
  - BAL (British Anti Lewisite) reduces toxicity of heavy metals by complexing with As, Hg and Sb.
- ***Drug actin through Metal Poisoning:***
  - 8-Hydroxy quinoline complexes with Fe exhibit greataer antimalaria activity.
- ***Antitubercular activity:***
  - PAS (Para Amino Salysylic acid) complexes with Cupric ion exhibit greater Antitubercular activity.
- ***Development of Novel Drug delivery system:***
  - The Comlexation 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.
- ***As Diagnostic agent:***
  - Ta^{90} complexes with citrate are used for diagnosis of Kidney and measurement of Glomerular Filtration Rate.

Methods of analysis Complexation:

Job's Method of Continuous Variation:

- As per the Job, the species possess several characteristics that are;
  - Dielectric constant.

- Refractive Index.
- Spectrophotometric extinction coefficient.

- Principle - When there is no complexation between the species, the value of property is additive. On complexation these properties changes but additive rule do not hold good. The change in the characteristics proves that the complexation has been taken place.
- Let's take two species A and B whose individual dielectric constant in solid form and Absorbance in solution form were measured. Then two species in both forms were mixed. The dielectric constant and absorbance were determined.
- The individual values are subtracted with mixed additive values and result was found out.
- If result is zero then no complexation and if result is not zero then there is complextaion.

pH Titration Method:

- Principle - 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.
- 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.

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 behavior of a solute between two immiscible liquids is expressed by distribution or partition co-efficient.
- <u>Principle</u> - When a solute complexes with an added substance, the solute distribution pattern changes depending on the nature of the complex.
- The complexation of iodine by potassium iodide.

  $I_2 + K^+I^-$ ---------- $K^+ I_3^-$
- The Equilibrium stability constant,

  $K = [K^+ I_3^-]/ [I_2] [K^+I^-]$

- The distribution coefficient of iodine between disulfide and water is 625.
- The K value of Iodine-Potassium iodide complex is 954.
- This change in distribution coefficient proves that the complexation has taken place.

Solubility Method:

- Principle - When the component in a mixture produce 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.

Spectroscopy Method:

- The study of donor acceptor (D-A) or charge transfer complexation is generally undertaken with absorption spectroscopy in the visible and UV regions of the spectrum.

$$D + A \underset{k_2}{\overset{k_1}{=\!=\!=\!=\!=\!=\!=\!=}} DA$$

- Where, D and A represents electron donor and acceptor, k_1 and k_2 are interaction rate constants.
- $K = k_1 / k_2$ = Equilibrium or stability constant for complexation.
- The absorbance A of the charge transfer band is measured at a definite wavelength and the constant K is obtained from the Benesi-Hildebrand equation.
- $A_0/A = (1/\varepsilon) + (1/K\varepsilon)\ (1/D_0)$
- Where, A_0 and D_0 are the initial concentration of acceptor and donor species in mole/litre. E is the molar absorptivity of the charge-transfer complex at its particular wavelength and K is the stability constant in litre/mole.
- A plot of A_0/A versus $1/D_0$ results in straight line with a slope of $1/K\varepsilon$ and an intercept of $1/\varepsilon$.
- The spectrometric method used to investigate the interaction of nucleic acid bases with catechol, epinephrine and isoproterenol.

Protein binding:

***Introduction*:**

- The interacting molecules are generally the macromolecules such as protein, DNA or adipose. The proteins are particularly responsible for such an interaction.
- The phenomenon of complex formation of drug with protein is called as protein binding of drug.

- As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to its pharmacokinetic and Pharmacodynamics inertness.
- Protein + drug ⇌ Protein-drug complex.
- Protein binding may be divided into - Intracellular binding. 2. Extracellular binding.

Mechanisms of protein drug binding:

- Binding of drugs to proteins is generally of reversible and irreversible.
- Reversible generally involves weak chemical bond such as: 1. Hydrogen bonds 2. Hydrophobic bonds 3. Ionic bonds 4. Van der Waal's forces.
- Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug.
- *Absorption* - As we know the conventional dosage form follow first order kinetics. So when there is more protein binding then it disturbs the absorption equilibrium.
- *Distribution* - A protein bound drug in particular does not cross the BBB, the placental barrier, the glomerulus. Thus protein binding decreases the distribution of drugs.
- *Metabolism* - Protein binding decreases the metabolism of drugs and enhances the biological half life. Only unbound fractions get metabolized. • e.g. Phenylbutazone and Sulfonamide.
- *Elimination* – Only the unbound drug is capable of being eliminated. Protein binding prevent the entry of drug to the metabolizing organ (liver) and to glomerulus filtration. • e.g. Tetracycline is eliminated mainly by glomerular filtration.
- *Systemic solubility of drug* – Lipoprotein act as vehicle for hydrophobic drugs like steroids, heparin, oil soluble vitamin.
- *Drug action* - Protein binding inactivates the drugs because sufficient concentration of drug cannot be build up in the receptor site for action. • e.g. Naphthoquinone.
- *Sustain release* – The complex of drug protein in the blood act as a reservoir and continuously supply the free drug. e.g. Suramin sodium-protein binding for antitrypanosomal action.
- *Diagnosis* – The chlorine atom of chloroquine replaced with radiolabeled I- 131 can be used to visualize-melanomas of eye and disorders of thyroid gland.

Factors affecting protein binding:

- Drug related Factor.
    - Physicochemical properties of drug Increase in lipophilicity increases the drug binding with the protein.

- Total concentration of drug – Alternation in drug and protein concentration alter the drug protein binding.

- Protein related Factors.
  - Physicochemical properties of protein – Lipoprotein bind with lipophilic drugs.
  - Quantity of protein – Disease state affect the concentration of protein in blood.
  - Number of binding sites – Albumin has more no of binding sites.
- Affinity and Magnitude of association constant.
- Drug Interaction.
  - Displacement reaction
  - Composition of drugs and normal body constituents.
  - Allosteric changes in protein molecules.
- Patient related factors.
  - Age – Noenata have low albumin content, thus less drug binding.
  - Disease state – Disease sate alter the drug binding.

Binding of drug to blood plasma proteins –

- The binding of drugs to plasma proteins is reversible.
- The extent or order of binding of drug to plasma proteins is: Albumin › ὰ1-Acid glycoprotein › Lipoproteins › Globulins.
- ***Binding of drug to human serum Albumin –***
  - It is the most abundant plasma protein (59 %).
  - Having M.W. of 65,000 with large drug binding capacity.
  - Both endogenous compounds such as fatty acid, bilirubin as well as drug bind to HSA.
  - Four different sites on HSA for drug binding.
  - Site I: warfarin and azapropazone binding site.
  - Site II: diazepam binding site.
  - Site III: digitoxin binding site.
  - Site IV: tamoxifen binding site.
- **Binding of drug to α1-Acid glycoprotein –**
  - It is called as orosomucoid. It has a M.W. 44,000.
  - Its plasma conc. range of 0.04 to 0.1 g %.
  - It binds to no. of basic drugs like imipramine, lidocaine, propranolol, and quinidine.

- **Binding of drug to Lipoproteins –**
  - Binding by Hydrophobic Bonds, Non-competative.
  - Mol wt: 2-34 Lacks dalton.
  - Lipid core composed of: Inside: triglyceride & cholesteryl esters. Outside: Apoprotein. e.g. Acidic: Diclofenac. Neutral: Cyclosporin A. Basic: Chlorpromazine.
  - Its types are LDL, HDL, VLDLand Chylomicrons.
- **Binding of drug to Globulins** –
  - α1 Globulin (Transcortine /Corticosteroid Binding globulin) - Steroidal drugs, Thyroxin & Cyanocobalamine (Vit B12).
  - α2 Globulin (Ceruloplasmine) - Vitamin A, D, E, K.
  - β1 Globulin (Transferin) - Ferrous ions.
  - β2 Globulin – Carotinoids.
  - γ Globulin – Antigens.

Kinetics of Protein Binding:

- An equation relating reaction velocity to Drug concentration (Mol/L) for a system where a Drug D binds reversibly to an Protein P of to form an Protein-Drug complex .
- This system can be represented schematically as follows:

  $P + D_F$ ======= PD
- Applying the law of mass action, the equilibrium or association constant (K) is;

  $K = [PD]/ [P] [D_F]$
- The [PD], [P] and [D] are the concentration of protein-drug complex, protein and drug in Mol/L.

  $K[P][D_F] = [PD]$
- Free protein concentration can obtain as;

  $[P_T] = + [PD]$

  $[P] = [P_T] \_ [PD]$
- $[P_T]$ is the total protein.
- Substituting the [P] in last equation, $K[P][D_F] = [PD]$

  $K ([P_T] – [PD]) [D_F] = [PD]$
- Where, D_F is the free drug.

  $K [P_T] [D_F] – K [PD] [D_F] = [PD]$

  $K [P_T] [D_F] = [PD] + K [PD] [D_F]$

  $K [P_T] [D_F] = [PD] (1+ K [D_F])$

$[PD] = (K [P_T] [D_F])/ (1+ K [D_F])$

$[PD]/ [P_T] = K [D_F]/ 1+ K [D_F]$

- Let R be expressed as moles of drug bound [PD] per mole of total protein $[P_T]$

  $R = [PD]/ [P_T] = K [D_F]/ 1+ K [D_F]$

- If V is the number of independent binding sites available then R,

  $R = V (K [D_F]/ 1+ K [D_F])$

  $1/R = 1/VK[D_F] + 1/V$

- The graph is plotted between 1/R versus $1/[D_F]$, called Klotz reciprocal plot, gives a straight line whose slope is 1/VK and intercept is V.

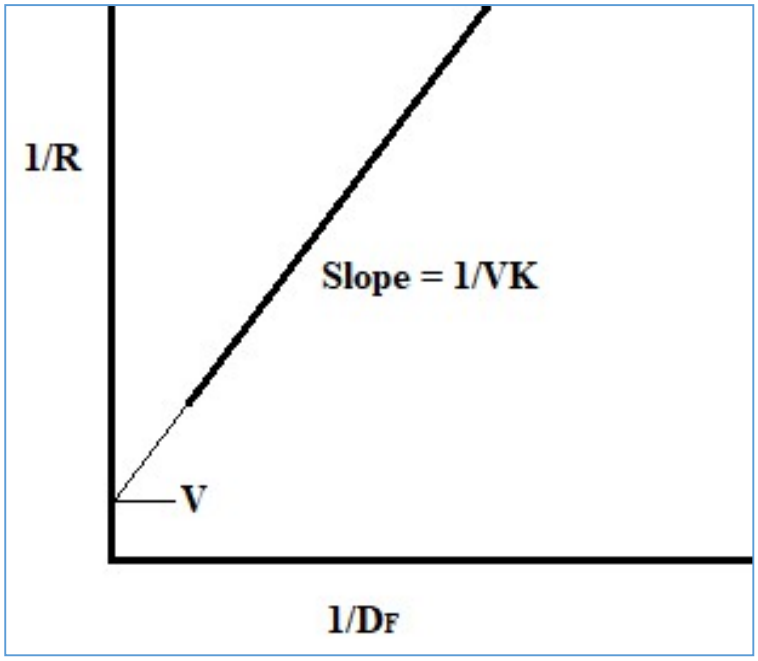


Fig 1. Klotz reciprocal plot.

$R + R K [D_F] = V K [D_F]$

$R/[D_F] = VK - RK$

Complexation and drug action:

- Protein binding inactivates the drugs because sufficient concentration of drug cannot be build up in the receptor site for action. • e.g. Naphthoquinone.
- Only free drug participate in drug action.
- Complexation can alter the pharmacological action of drug by interfering interaction with receptor.
- The action of drug to remove the toxic effect of metal ion from the human bodies is through the complexation reaction.

- It has been seen that in some instance complexation can also lead to poor solubility or decreased absorption of drug in the body, which decreases the bioavailability of drug in the blood. Thus the drug action gets altered.
- Drug complex with hydrophilic drug also enhance the drug elimination, thus helps in drug action termination and reduction in drug toxic action.
- Examples :
  - Tetracycline and Calcium – Poor absorbed complex.
  - Polar drug and complexing agent – Well absorbed lipid soluble complex.
  - Carboxy methyl cellulose and amphetamine – Poor absorbed complex.
  - PVP and I_2 – Better absorption.

Thermodynamic treatment of stability constants Complexes:

- The relationship between the standard free energy change of complexation and the over all stability constant K is related as;

  $\Delta G = -2.303RT \text{ Log } K$
- The Standard Enthalpy Change ΔH may be obtained from the slope of a plot of Log K Versus 1/T, thus the equation will be;

  $\text{Log } K = -(\Delta H/2.303R) \times (1/T) + \text{Constant}$
- When the value of K at two temperatures are known, the following equation can be written as;

  $\text{Log } (K_2/K_1) = -(\Delta H/2.303R) \times (T_2-T_1/T_1T_2)$
- The Standard entropy change may be obtained from the expression;

  $\Delta G = \Delta H - T\,\Delta S$
- As the stability constant for molecular complexation increases, ΔH and ΔS becomes more negative.
- As binding between the donor and receptor becomes stronger, ΔH becomes more negative.
- Since the specificity of interacting sites becomes negative, ΔS also become more negative.
- But the extent of change in ΔH is large enough to overcome the unfavourable entropy change resulting in negative ΔG value and hence complexation.

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