COMPLEXATION and PROTEIN BINDING

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COMPLEXATION

- Complexation is a term used to characterize the covalent or non covalent interactions between two or more compounds that are capable of independent existence.
- Complexation is the association between two or more molecules to form non bonded entity with a well-defined stoichiometry.
- Complexes are formed because of the donor acceptor mechanism.
- Coordination complexes consists of central atom or ion (coordination centre, usually metallic) and surrounded by array of bound neutral molecules or anions (called ligands).

COMPLEXATION

Once complexation occurs, the physical and chemical properties of the complexing species are altered. These properties include solubility, stability, partitioning, energy absorption and emission, and conductance of the drug.

1. Solubility
2. Stability
3. Absorption
4. Pharmacokinetics
5. Pharmacodynamics

LIGAND

- A ligand is an ion or molecule that binds to a central metal atom to form a complex.
- Ligands can be anions, cations, and neutral molecules.
- Ligands may be neutral or negatively charged species with electron pairs available.
- Occasionally, ligands can be cations (e.g. NO^+, $N_2H_5^+$) and electron-pair acceptors.

CLASSIFICATION of LIGAND

- Classification of ligands is on the basis of the number of binding sites with the central metal atom.

  - Mono dentate or Unidentate Ligands
  - Bidentate Ligands
  - Polydentate Ligands

CLASSIFICATION OF COMPLEXES

Metal ion complexes

- A. Inorganic type
- B. Chelates
- C. Olefin type
- D. Aromatic type
  - a. Pi (π) complexes
  - b. Sigma (σ) complexes

Organic molecular complexes

- A. Quinhydrone type complexes
- B. Picric acid type complexes
- C. Drug complexes
- D. Polymer type complexes

Inclusion/occlusion compounds

- A. Channel lattice type
- B. Layer type
- C. Clathrates
- D. Monomolecular type
- E. Macromolecular type

METAL ION COMPLEX

A metal ion complex consists of a central metal atom or ion bonded ligands

Inorganic type:

Each ligand donates a pair of electrons to form a coordinate covalent link between itself and the central ion having an incomplete electron shell.

In inorganic metal complex the ligand provides only one site for binding with metal.

Chelates:

Coordination compounds containing polydentate ligands are called as chelates (from Greek *chele*, "claw"), and their formation is termed *chelation*. Chelates are particularly stable and useful.

Some of the bonds in a chelate may be ionic or of the primary covalent type, whereas others are coordinate covalent links.

METAL ION COMPLEX

Olefin type:

Aqueous solution of metal ion with olefin forms water soluble olefin complexes.

Aromatic type:

Interaction of aromatic compound with the metal ion serves the basis for formation of aromatic complexes.

The bond formation i.e. π bond, σ bond between the metal ion and organic molecule like benzene, toluene governs the class of formation of complex.

Pi bonds are formed by the lateral overlap of two atomic orbitals whereas sigma bonds are a result of the head-to-head overlapping of atomic orbitals.

ORGANIC MOLECULAR COMPLEX

- An organic molecular complex consists of constituents held together 'by weak forces of the donor- acceptor type or by hydrogen bonds.
- Many organic complexes are so weak that they cannot be separated from their solutions as definite compounds, and they are often difficult to detect by chemical and physical means.

Quinhydrone Type:

The molecular complex that was, referred to as quinhydrone is formed by mixing alcoholic solutions of equimolar quantities of benzoquinone and hydroquinone.

Picric acid type complexes:

Picric acid being a strong acid forms organic molecular complex with weak bases.

Drug complexes:

Drug complex formation by the means of hydrogen bonding or induced dipole dipole forces are responsible for interaction between drug resulting into alteration or modification of physicochemical properties.

Polymer complexes:

Polymers containing nucleophilic oxygen can form complexes with various drugs. Owing to the repeating nature of a polymer molecule, many interacting sites may be present, which together will provide stronger bonding than a single covalent bond

INCLUSION/OCCLUSION COMPLEX

The class of addition compounds known as inclusion or occlusion compounds results more from the architecture of molecules than from their chemical affinity.

One of the constituents of the complex is trapped in the open lattice or cage-like crystal structure of the other to yield a stable arrangement.

Channel lattice type:

This is the type of host guest compound formed by crystallization of host compound. The guest molecule is usually limited to long, unbranched, linear chain compounds.

Layer type:

The crystals arrange in layers that can trap small molecules such as alcohols and glycols.

Their uses are currently limited but they can be useful in catalysis on account of a larger surface area.

Clathrate:

The clathrate crystallize in the form of 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.

Monomolecular type:

Monomolecular inclusion compounds involve the entrapment of a single guest molecule in the cavity of one host molecule.

Macromolecular type:

The atoms are arranged in three dimensions to produce cages and channels. Synthetic zeolites may be made to a definite pore size so as to separate molecules of different dimensions, and they are also capable of ion exchange.

APPLICATIONS OF COMPLEXATION

- To enhance or alter the physicochemical and biopharmaceutical properties of drug molecule.
- Solubility enhancement of poorly water soluble drug.
- To reduce the volatility of substance.
- Change in Absorption and bioavailability.
- Reduction of Adverse effect.
- Enhance stability of Vitamins.
- To reduce reactivity of compound.

- To reduce reactivity of compound
- For the colouration of materials.
- As an antidote in heavy metal poisoning.
- In electroplating.
- In the extraction of metals from their ores.
- In diagnosis of kidney function and glomerular filtration rate.
- As an anticoagulant.
- Development of sustain release drug delivery system.

STUDY OF COMPLEX

- **Two important parameters**
  - A determination of the stoichiometric ratio of ligand to metal or donor to acceptor
  - A quantitative expression of the stability constant for complex formation
- **Several method used to analyse the complex are**
  - Method of continuous variation
  - pH Titration Method
  - Distribution Method
  - Solubility Method
  - Spectroscopy Method

METHOD OF CONTINUOUS VARIATION

The stoichiometry of a metal ligand complexation reaction can be determined by three methods

Job's Method

Mole Ratio Method

Slope Ratio Method

Job's Method:

- A series of a solution are prepared with variable ratios of metal and ligand but with fixed total concentrations.
- An additive property that is proportional to concentration of the formed complex is measured and plotted against the mole fraction from 0 to 1 for one of the compounds of a mixture.
- A liner relationship is observed where no complexation occurs and if a complex forms between the two species, the value of the additive property will pass through a maximum (or minimum). Job's Method is restricted to formation of single complex.

Mole Ratio Method:

- A series of solutions are prepared with a fixed amount of the metal and variable amount of the ligand (or vice versa).
- The additive property that is proportional to the concentration of the formed complex is measured and plotted against the mole ratio of the component with the variable amounts.
- The complex formation is indicated by the change in the slope at the molar ratio that forms the complex. The calibration curve fattens out when there is no longer enough ligand to react with all the metal ions.
- Unlike Job's Method, the mole ratio method can be used to investigate the formation of higher complexes in solution.

Slope Ratio Method:

- Two set of solutions are prepared. The first set of solution contains a large excess of metal and a variable concentrations of ligand and the absorbance of formed complex is plotted against the ligand concentration and the slope of line is determined.
- A second set of solution is prepared with large excess of ligand and a variable concentration of metal. The absorbance of the formed complex is plotted against the metal concentration and the slope of the line is determined.
- The stoichiometric ratio of metal to ligand is inversely proportional to the ratio of slopes. The slope ratio method is limited to systems in which only a single complex is formed.

pH TITRATION METHOD

- This is one of the most reliable methods and can be used whenever the complexation is attended by a change in pH.
- Titration curves can be obtained by plotting the pH against the volume of base added to a solution of ligand alone and to another solution containing metal-ligand.
- The difference in pH for a given quantity of base indicates the occurrence of a complex.

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 constant so obtained by finding concentration of the component in two different phases is analyzed.
- The species common to both phases is the free or uncomplexed; the distribution law expresses only the concentration of free component whereas chemical analysis yields the total concentration of component in the aqueous phase.
- To obtain the concentration of component is the complex and hence the concentration of complex, one subtracts the free species from the total component of the aqueous phase.

SOLUBILITY METHOD

- According to the solubility method, excess quantities of the drug are placed in well-stoppered containers, together with a solution of the complexing agent in various concentrations, and the bottles are agitated in a constant temperature bath until equilibrium is attained.
- Aliquot portions of the supernatant liquid are removed and analysed to obtain the total drug concentration.
- The concentration of drug is plotted against the concentration of complexing agent.
- The addition of complexing agent raises the solubility of drug linearly owing to complexation. Once the solution becomes saturated with respect to complex and to the drug itself, complex continues to form and to precipitate from the saturated system as more complexing agent is added.
- Furthermore the entire excess drug has passed into the solution and has been converted to the complex. Although the solid drug has exhausted and the solution is no longer saturated, some of the drug remains uncomplexed in solution and it combines further with agent to form higher complexes.

SPECTROSCOPY METHOD

- Absorption spectroscopy in the visible and ultraviolet regions of the spectrum is commonly used to investigate electron donor–acceptor or charge transfer complexation.
- A solution exhibits a shift to a particular wavelength and peak is observed with intensity. When drug is analysed with non complexing solvent a curve is obtained with a single peak.
- No complex forms in electron acceptor component and donor organic solvent.
- The shift towards the UV region becomes greater as the electron donor solvent becomes a stronger electron releasing agent.

PROTEIN BINDING

- The phenomenon of complex formation between drug and protein is called as protein binding of drugs.
- **The binding of drugs to proteins contained in the body can influence their action in a number of ways. Proteins may**

  a) Facilitate the distribution of drugs throughout the body,

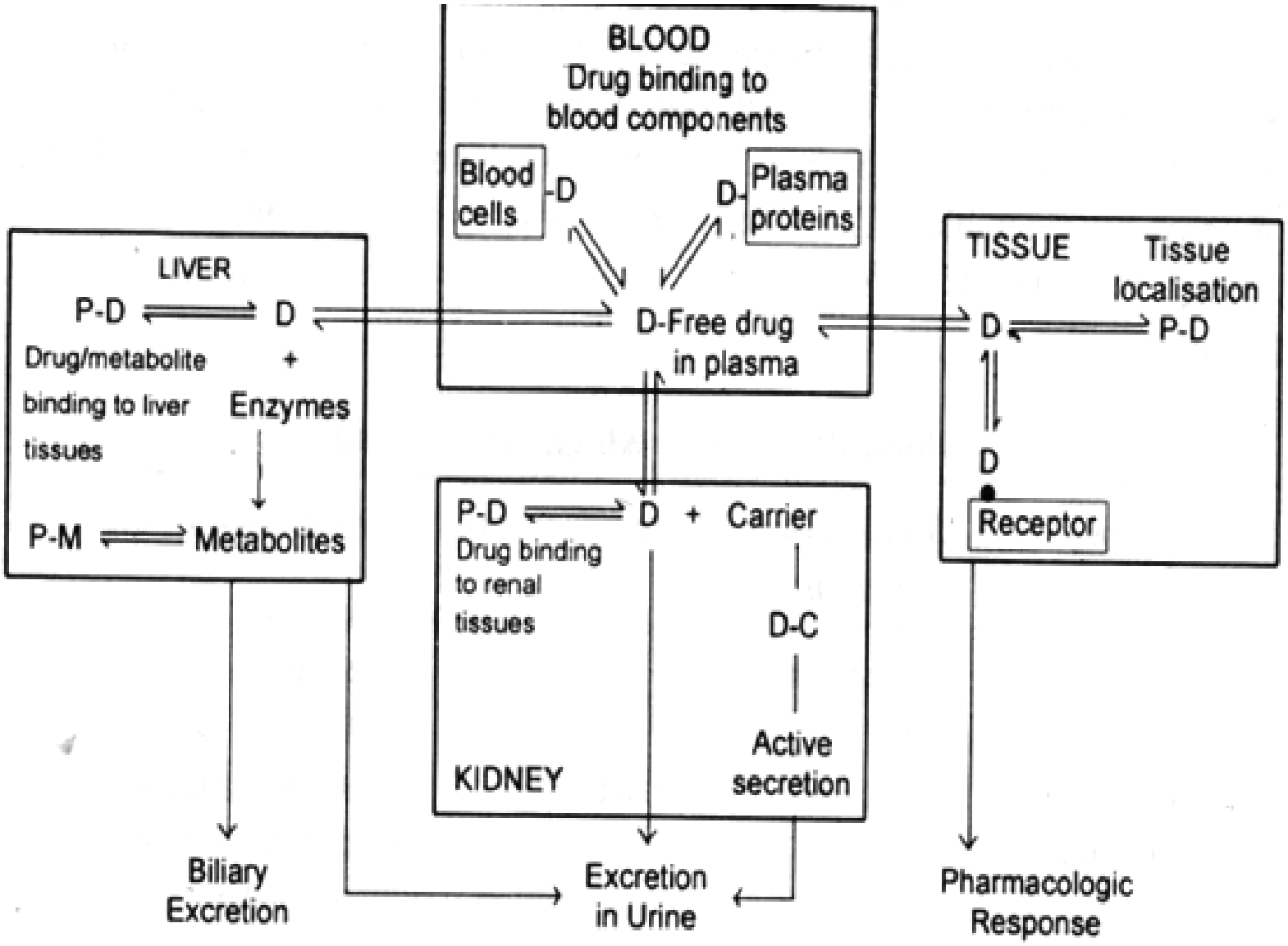
  b) Inactivate the drug by not enabling a sufficient concentration of free drug to develop at the receptor site, or

  c) Retard the excretion of a drug.
- **The interaction of a drug with proteins may cause**

  a) The displacement of body hormones,

  b) A configurational change in the protein,

  c) The formation of a drug-protein complex


BLOOD
Drug binding to blood components
Blood cells
D
D
Plasma proteins
D-Free drug in plasma
LIVER
P-D
D
Drug/metabolite binding to liver tissues
+
Enzymes
P-M
Metabolites
TISSUE
Tissue localisation
D
P-D
D
Receptor
P-D
D
+
Carrier
Drug binding to renal tissues
D-C
Active secretion
KIDNEY
Biliary Excretion
Excretion in Urine
Pharmacologic Response

- Depending on a specific drug's affinity for plasma protein, a proportion of the drug may become bound to plasma proteins, with the remainder being unbound.
- If the protein binding is reversible, then a chemical equilibrium will exist between the bound and unbound states, such that:

$$\text{Protein} + \text{drug} \rightleftharpoons \text{Protein-drug complex}$$

- The equilibrium constant, disregarding the difference between activities and concentrations, is

$$K = \frac{[\text{Protein Drug Complex}]}{[\text{Protein}][\text{ Drug}]}$$

- Irreversible drug binding though rare arises as a result of covalent binding and is often a reason for the carcinogenicity and tissue toxicity of the drug.

Protein Drug Binding

- Protein Drug Binding falls into

  Binding of drug to blood component like plasma protein

  Binding of drug to extra vascular tissue protein

- The binding of drug to plasma protein is reversible. The extent of bonding of drugs to various plasma proteins is: albumin > α_1 Acid Glycoprotein > Lipoprotein > Globulins.
- HSA has four different binding sites for drug binding

  Site I: Wafarin and azapropazone binding site

  Site II: Diazepam binding site

  Site III: Digitoxin binding site

  Site IV: Tamoxifen binding site

- Plasma protein binding is related to lipophilicity.
- In general, as compounds become more lipophilic, plasma protein binding becomes more significant, but there are many examples in which hydrophilic compounds are tightly bound and lipophilic chemicals (but unpredictably so) unless the compound is highly lipophilic, that is, log *P* of 4 or greater.
- For highly lipophilic compounds, significant plasma protein binding is common.

- Binding of drug to **α_1 Acid Glycoprotein** (AAG) involves hydrophobic bonds and this binding is also called as orosomucoid. It binds to number of basic drugs like imipramine, amitryptiline, propranolol, lidocaine etc.
- Because of high lipid content of **lipoprotein**, lipophilic drugs undergo hydrophobic bonding with lipoporoteins. A drug that binds to lipoprotein does so by dissolving in the lipid core of the protein and thus its capacity to bind depends on the lipid content. The binding of drugs to lipoprotein is non-competitive. A number of acidic, neutral and basic drugs bind to lipoprotein.

- Several **plasma globulins** have been identified and are labelled as α_1 – globulin that binds to number of steroidal drugs, α_2 – globulin that binds to fat soluble vitamins, β_1- globulin that binds to ferrous ion, β_2- Globulin that binds to carotinoids and γ globulin binds specifically to antigens.

FACTORS AFFECTING PROTEIN BINDING

- **Drug Related Factors**

  Physicochemical characteristic of drug

  Concentration of drug in the body

- **Protein Related Factors**

  Physicochemical characteristics of the protein for binding agent

  Concentration of protein for binding component

  Number of binding site on the binding agent

- **Drug Interaction**

  Competition between drug for the binding site

  Allosteric changes in protein molecule

- **Patient Related Factor**

  Age and Inter subject variation

  Disease state

DRUG RELATED FACTOR

- **Physicochemical characteristics of the drug:**

  Protein binding is directly related to the lipophilicity of drugs.

  The extent of binding increases with increase in lipophilicity.

- **Concentration of drug in the body:**

  The extent of protein drug binding can change with both changes in concentration of drugs as well as protein

PROTEIN RELATED FACTOR

- **Physicochemical characteristics of the protein for binding agent:**

  Lipoprotein and adipose tissue tend to bind lipophilic drugs by dissolving them in their liquid core. The physiologic pH remind the presence of active anionic and cationic groups on the albumin molecule to bind a variety of drugs

- **Concentration of protein for binding component:**

  Among the plasma proteins, binding predominantly occur with albumin as it is present in higher concentration in comparison to other plasma protein

- **Number of binding site on the binding agent:**

  Albumin has large number of binding sites as compared to other protein and is a high capacity binding component.

DRUG INTERACTION

- **Competition between drug for the binding site:**

  When two or more drug can bind to the same site, competition results between them for interaction with the binding site. A drug drug interaction for the common binding site is called as displacement interaction. The drug that gets displaced by another is called as the displaced drug and second drug as a displacer.

- **Allosteric changes in protein molecule:**

  The process involves alteration of protein structure by the drug or its metabolites thereby modifying its binding capacity.

PATIENT RELATED FACTOR

- **Age:**

  Modification of protein binding as influenced by age of the patient is mainly due to differences in the protein content in various age groups.

- **Inter subject variation:**

  These differences have been attributed to genetics and environmental factors.

- **Disease States:**

  Several pathologic conditions are associated with alteration in protein content.

STABILITY CONSTANT

- A **stability constant** is a measure of the strength of the interaction between the reagents that come together to form the complex.

- The **standard free energy change** of complexation is related to the overall stability constant K (or any of the formation constants) by the relationship

$$\Delta G = - 2.303RT \log K$$

- The **standard enthalpy change** ΔH may be obtained from the slope of a plot of log K versus $\frac{1}{T}$ following the expression

$$\log K = -\frac{\Delta H}{2.303RT} + \text{constant}$$

- When the values of K at two temperatures are known, the following equation may be used

$$\log \frac{K_2}{K_1} = \frac{\Delta H}{2.303R}\left(\frac{T_2 - T_1}{T_1 T_2}\right)$$

- The **standard entropy change** ΔS is obtained from the expression

$$\Delta G = \Delta H - T\Delta S$$

- Andrews and Keefer demonstrated that ΔH and generally ΔS become more negative as the stability constant for molecular complexation increases.
- Positive and negative thermodynamic interactions resulting from several kinds of interactions are tabulated below,

Type of Interaction	**Sign on**		**- ΔG is favoured by**
	ΔH	**ΔS**	
Electrostatic	~ 0	+	+ ΔS
Hydrophobic	+	+	large + ΔS
Chelation (polydentate ligand)	-	+	+ ΔS and/or - ΔH
Donor-acceptor (hydrogen bonding and chelation (monodentate ligand))	-	-	- ΔH
Unfolding of proteins	+	+	+ ΔS

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