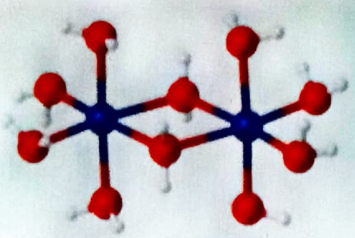

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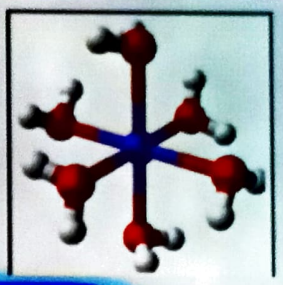
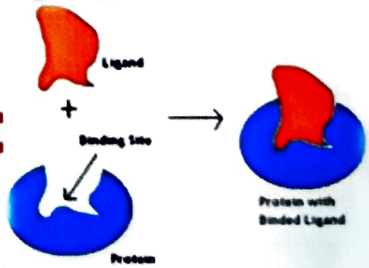


COMPLEXATION AND PROTEIN BINDING

Points to be covered in this topic

1. INTRODUCTION
2. CLASSIFICATION OF COMPLEXATION
3. APPLICATION
4. METHODS OF ANALYSIS
5. PROTEIN BINDING
6. CRYSTALLINE STRUCTURE
7. THERMODYNAMIC TREATMENT OF STABILITY CONSTANTS



❑ INTRODUCTION

- **Complexes or co-ordination** compounds result from a donor–acceptor mechanism or **Lewis acid–base reaction** between two or more different **chemical components.**
- The term complexation is used to **characterize the covalent** or **non-covalent interactions** between two or more **compounds capable** of **independent existence.**
- The **ligand, a molecule, interacts** with substrate, the molecule, to form a complex. Drug molecules can form **complexes with other small molecules** or **with macromolecules.**

CLASSIFICATION OF COMPLEXATION

Metal ion or co-ordination complexes

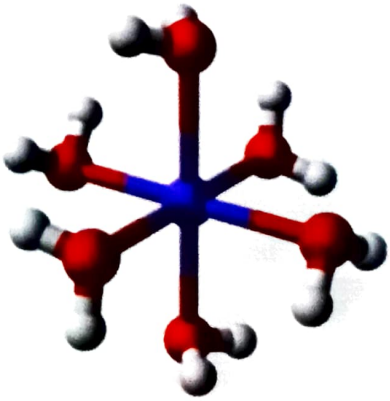
- Inorganic type
- Chelates
- Olefin type
- Aromatic type
  - (i) Pi (π) complexes
  - (ii) Sigma (σ) complexes
  - (iii) Sandwich compounds

Organic molecular complexes

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

Inclusion/Occlusion compounds

- Channel lattice
- Layer type
- Clathrates
- Monomolecular type
- Macromolecular type



❑ CLASSIFICATION OF COMPLEXATION

❖ Metal Ion or Co-ordination Complexes

A satisfactory understanding of **metal ion complexation** is based upon a **familiarity with atomic structure** and molecular forces, and electronic structure as well as **hybridization.** The co-ordination complex or metal complex is a structure **made-up of a 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.**

➢ Inorganic Complexes

Ligands are **generally bound to a metal ion** by a covalent bond and hence **called to be coordinated 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 **coordinate covalent bond.**

✓ **For example :-**

The ammonia molecules in **Hexamine cobalt (III) chloride,** as the compound $[Co(NH_3)_6]^{3+} \cdot Cl_3$ is called 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** $[Ag(NH_3)_2]^+$, $[Fe(CN)_6]^{4-}$ **and** $[Cr(H_2O)_6]^{3+}$.

➢ Chelates

- A substance containing **two or more donor groups** may combine with a metal to form a special type of **complex known as a chelate.**
- 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.**

✓ **For example :-**

Pilocarpine behaves as a monodentate ligand toward **Co(II), Ni(II),** and **Zn(II)** to form chelates of **pseudo tetrahedral geometry.**

✓ **Applications of chelation :-**

- **Chlorophyll and hemoglobin,** 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 synthetic chelating agent **ethylene diamine tetra acetic acid (EDTA)** has been used to tie-up or sequester iron and copper ions.
- In the process of sequestration, the **chelating agent and metal ion form a water-soluble compound.**
- **EDTA is widely used to sequester** and **remove Calcium ions from hard water.**

➢ 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.** Some have **short chains** with only two, **three or four carbons, such as ethylene.** Others form long chains or **closed ring structures.**

➤ Aromatic Type

✓ Pi (π) complexes :-

The **example of Pi complex** is interaction of **local anesthetic Bupivacaine** and its structural analogs such as **2,6-dimethylaniline,** and **N-methyl-2, 6-dimethylacetanilide,** and **Cocaine,** with several electron deficient aromatic moieties. In solution, the anesthetic, its analogs and **cocaine are electron donors** and form **π–π charge transfer complexes** with **strong aromatic acceptors.**

The **concentrations of free Bupivacaine**, its analogs and of cocaine are reduced from solution Via binding to aromatic-functionalized silica. The **rapid binding of bupivacaine** (1) and its analogs 2, 6-dimethylaniline (2) and 2, 6-dimethylacetanilde (3), respectively, and of cocaine (4), by the acceptor molecules.

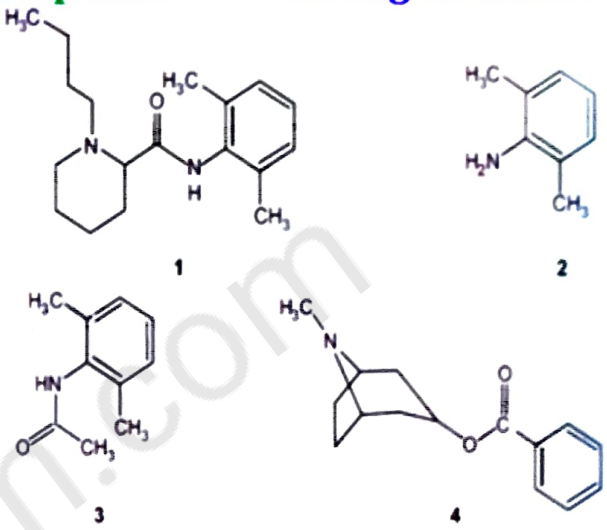


Fig.- Pi Complex Interaction in Bupivacaine and its Structural Analogs

✓ Sigma (σ) complexes:-

- **An Arenium ion** is a **cyclohexadienyl cation** that appears as a reactive intermediate in electrophilic **aromatic substitution.**
- This complex is also called a **Wheland intermediate** or a **sigma complex or σ-complex.**
- The **smallest Arenium ion** is the **Benzenium ion ($C_6H_7^+$),** which is **protonated benzene.**

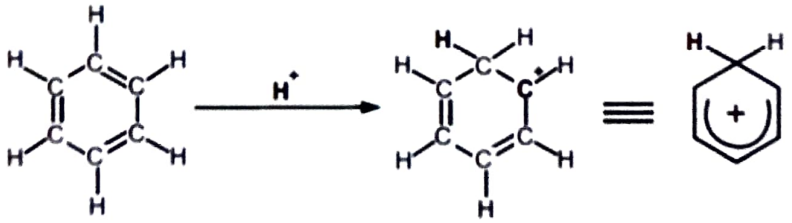


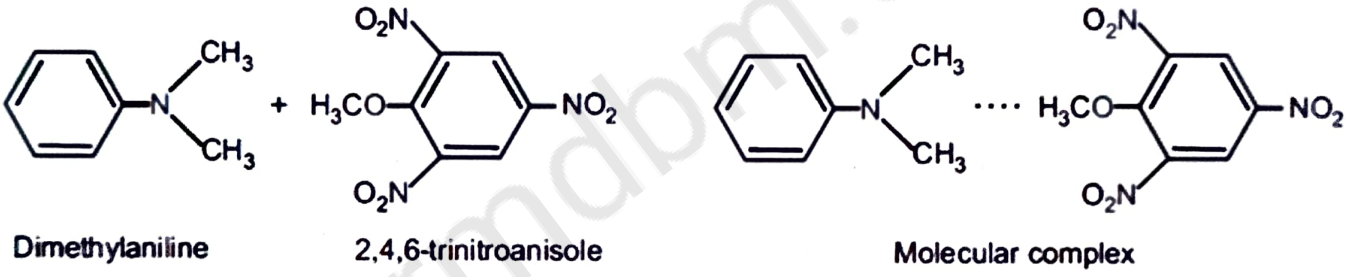
Fig.- Sigma Complex in Benzene

✓ Sandwich compounds:-

A sandwich compound is a **metal bound by haptic covalent bonds** to two arene ligands. The arenes have **formula C_nH_n,** substituted derivative (for example $C_n(CH_3)_n$) and heterocyclic derivatives **(for example BC_nH_{n+1}).** Because metal is **usually situated** b/w 2 rings, it is **said to be "sandwiched".**

Organic Molecular Complexes

- An **organic molecular complex** consists of constituents held together. The **forces involved are of donor** and acceptor type or **by hydrogen bonds.**
- There is a difference between **complexation and the formation of organic compounds.**
- **For example, Dimethyl aniline and 2,4,6-trinitroanisole** react at low temperature to **give a molecular complex.**
- **The dotted line in the complex**, indicates that the **two molecules are held together** by a **weak secondary valence force.**
- It is not to be **considered as a clearly defined** bond but rather as an overall attraction between the **two aromatic molecules.**



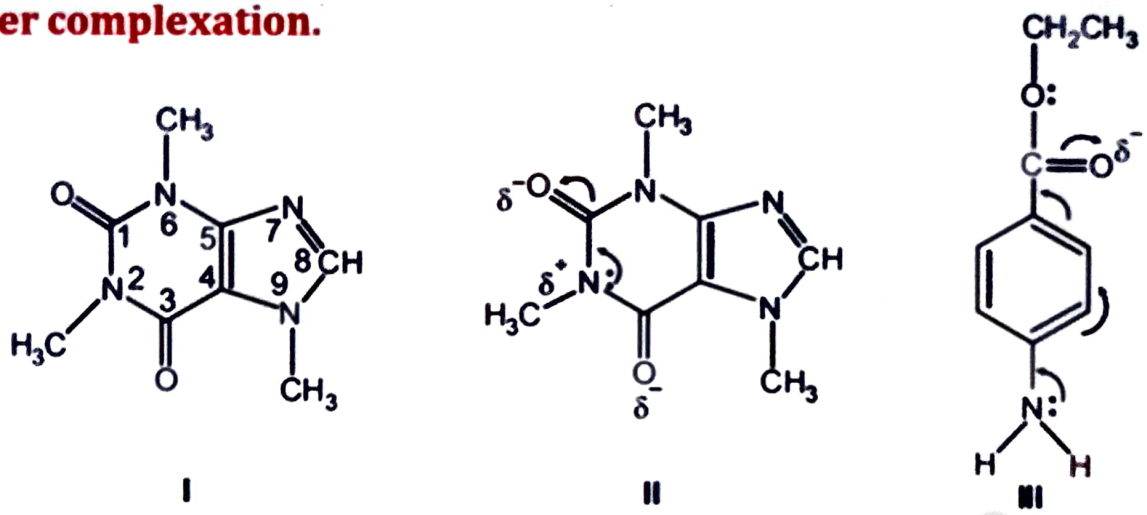
Dimethylaniline 2,4,6-trinitroanisole Molecular complex

Fig. - Molecular Complex Formation Through Weak Secondary Valence Force

Drug Complexes

- In the formation of drug complex **degree of interaction** depends upon **certain effects.**
- For example, the **complexing of caffeine** with **several acidic drugs.**
- The interaction between **Caffeine and Sulfonamide or Barbiturate** is a **dipole-dipole force** or **Hydrogen bonding** between the polarized carbonyl groups of caffeine and the **Hydrogen atom of the acid.**
- The **secondary interaction occurs** between the **non-polar parts** of the molecules and the resultant complex is **"squeezed out"** of the aqueous **phase due to the great internal pressure of water.**

- There are **no activated hydrogens** on caffeine; the **hydrogen at the number 8 position is very weak (Ka = 1×10^{-14})** and **is not likely to enter complexation.**



I II III

Fig.- Complexing Sites in Caffeine (I and II) and Benzocaine (III)

➢ Polymer Complexes

- **The polymers containing nucleophilic oxygens such as polyethylene** glycols, polystyrene, carboxymethylcellulose and similar can form **complexes with various drugs.**
- **The examples of this type** include incompatibilities of carbowaxes, pluronics, and tweens with **tannic acid, salicylic acid, and phenol.**
- The **interactions may occur** in **suspensions, emulsions, ointments, and suppositories** and are manifested as a **precipitate, flocculate,** delayed **biologic absorption, loss of preservative action,** or other undesirable physical, chemical, and **pharmacological effects.**
- The **interaction of Povidone (PVP)** with ionic and neutral aromatic **compounds is affected by several factors** that affect the **binding to PVP** of **substituted Benzoic acid** and **Nicotine derivatives.**
- **Ionic strength** has no influence but the **binding increases in** phosphate buffer solutions and **decreases as the temperature is raised.**
- **Crospovidone, a cross-linked insoluble PVP,** can bind drugs owing to its **dipolar character and porous structure.**

Pharmaceutical examples of molecular organic complexes

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
Oxytetracycline and Tetracycline	γ-butyrolactone, Sodium salicylate, Sodium saccharin, Caffeine

➢ Inclusion Compounds

- The **inclusion or occlusion compounds** results from the **architecture of molecules.**
- 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

The **bile acids especially cholic acids** form a complex of **deoxycholic acid** in combination with **paraffin, organic acids, esters, ketones, and aromatic compounds** and with solvents such as **ether, alcohol, and dioxane.** The crystals of **deoxycholic acid** are arranged to form a channel into which **the complexing molecule can fit.**

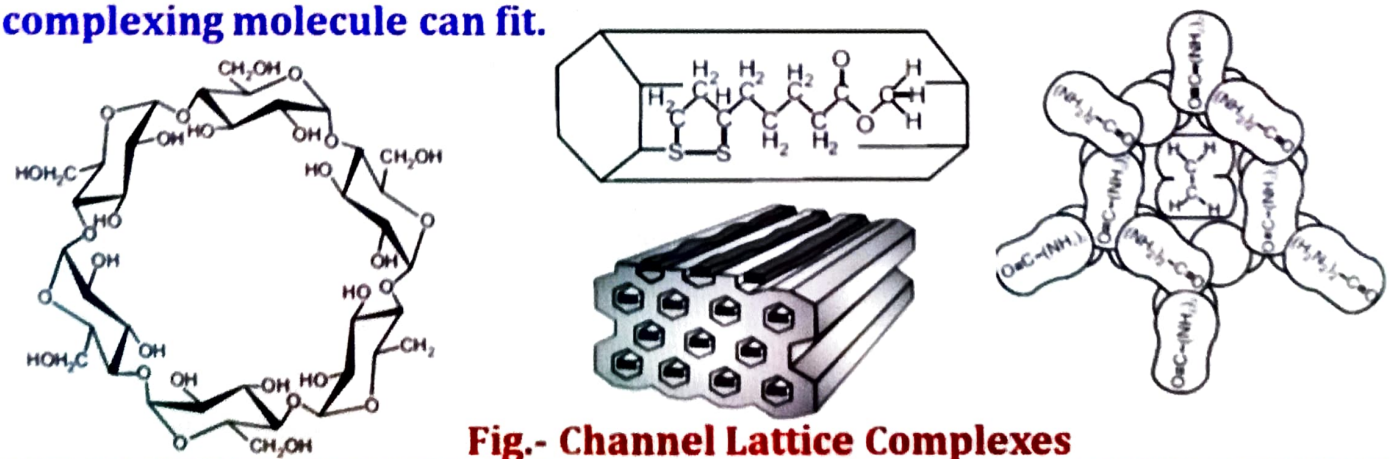


Fig.- Channel Lattice Complexes

❑ CLASSIFICATION OF COMPLEXATION

❖ Organic Molecular Complexes

➢ Layer Type

Some other examples includes **clay montmorillonite,** the **principal constituent of bentonite,** can trap hydrocarbons, **alcohols, and glycols** between the layers of their lattices. **Graphite can also intercalate** compounds **between its layers.**

➢ Clathrates

The **clathrates 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. The **stability of a clathrate** is due to the **strength of the structure**. The **highly toxic agent hydroquinone (quinol) crystallizes** in a **cage like hydrogen-bonded structure.**

❖ Monomolecular Inclusion Compounds

- Inclusion compounds are of channel - and cage-type (clathrate) and mono- and macro molecular type **Monomolecular inclusion compounds** involve the **entrapment of a single guest molecule** in the **cavity of one host molecule.**
- Monomolecular host structures are **represented by the cyclodextrins (CD).**
- **Cyclodextrins are cyclic oligomers** of glucose that can form water-soluble inclusion complexes with small molecules and **portions of large compounds.** These complexes are **biocompatible and do not elicit** any immune responses and have **low toxicities in animals and humans.**

❑ APPLICATIONS OF COMPLEXATION

❖ Solubility enhancement

- 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-Cyclodextrin**
- Derivatives of the natural **crystalline Cyclodextrin** have been developed to improve aqueous **solubility and to avoid toxicity.**

❖ Bioavailability enhancement

- **Dissolution rate** plays an important role in bioavailability of drugs, **fast dissolution usually favours absorption.**
- The dissolution rates of Famotidine (Used in the treatment of gastric and duodenal ulcers) and that of Tolbutamide **(Oral antidiabetic drug)** is increased by **complexation with β- Cyclodextrin.**

❖ Modifying drug release

The **hydrophobic forms of β-Cyclodextrin** 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-Cyclodextrin**. The release rate was controlled by mixing **hydrophobic and hydrophilic** derivatives of **Cyclodextrin at several ratios.**

❖ 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-Cyclodextrin.**

❑ APPLICATIONS OF COMPLEXATION

❖ Administration of therapeutic agents

- Some therapeutic agents **administered only** as complexes due to **physicochemical limitations.**
- For example, **iron complex with ferrous sulphate** and **carbonate and insulin complex** with **Zn and Vitamin-B_{12}**

❖ Use of ion exchange

- Cholestyramine resin **(quaternary ammonium anion exchange resin)** is used to **relief pruritus,** the resin exchange chloride ion **from bile** result in increased elimination of **bile through faeces.**

❖ 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 **glomerular filtration rate.**
- **Squibb (complex of a dye Azure A** with acrylic cation exchange resin) is used for **detection of achlorhydria** due to **carcinoma and pernicious anemia.**

❖ Complexation as a therapeutic tool

- Complexing agents are used **for variety of uses.**
- Many of them are related to **chelation of metal ion.**
- One of the important uses is **preservation of blood.**
- EDTA and citrates are used for **in-vitro to prevent clotting.**
- For example, anticoagulant acid citrate dextrose solution and anticoagulant **sodium citrate solution.**
- **Citrates act by chelating calcium** ion in blood as it depletes body calcium.

❑ APPLICATIONS OF COMPLEXATION

❖ Treatment of poisoning

Therapeutic procedure involves complexation to **minimize poisoning.** It is **possible by two pathways. First by absorption of toxicants** from GIT using **complexing and adsorbing agent** and second by inactivation of **toxic material systemically** and enhanced **elimination of toxic substance** through use of dialysis.

➢ Arsenic and mercury poisoning

The most **effective agent is BAL (Dimercaprol).** The arsenical dimercaprol is shown as: $CH_2SCHSAs\text{-}RCH_2OH$. Two **Sulphahydryl groups** chelate with metal and a free OH group promotes water solubility.

➢ Lead poisoning

Treatment of choice for **acute/chronic lead poisoning** is I.V. administration of calcium or **disodium complex of EDTA.** This **complex chelates** ions which exhibit a higher affinity of **EDTA than do the calcium.**

➢ Radioactive materials

Poisoning with **radioactive materials** particularly with long **biological half-life** encounters problems that metal has toxic effect and body suffer from radiation damage. **Uranium and Plutonium exposure** have been successfully **treated with CaNaEDTA.**

➢ Dialysis and complexation in poisoning

Removal of poisons from **systemic circulation** can be done by artificial kidney or by **peritoneal dialysis.** Dialyzing fluid is injected into **peritoneal cavity continually and circulated** into and out of the cavity. The toxic material diffuses through **wall of the blood vessel** into the fluid **present in the cavity.** Efficiency of this **procedure is improved** by using **principle of complexation.**

❑ METHODS OF ANALYSIS OF COMPLEXATION

❖ Method of Continuous Variation

The use of an additive property such as the spectrophotometric extinction coefficient such as dielectric constant or the square of the refractive index may also be used for the measurement of complexation. If the property for two species is sufficiently different and if no interaction occurs when the components are mixed, then the value of the property is the weighted mean of the values of the separate species in the mixture. This means that if the additive property, say dielectric constant, is plotted against the mole fraction from 0 to 1 for one of the components of a mixture where no complexation occurs, a linear relationship is observed.

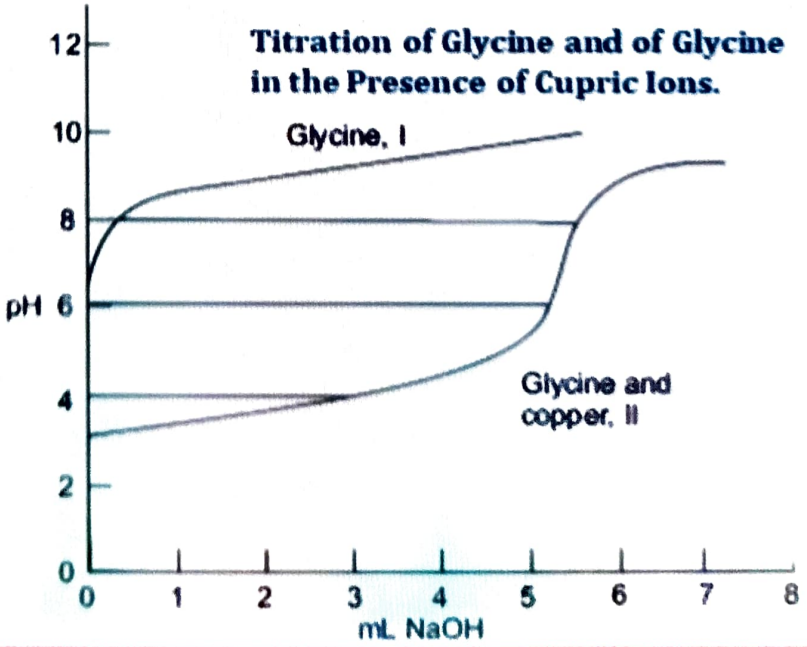
❖ pH Titration Method

This is most reliable method and used whenever the complexation is attended by a change in pH. 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.

The potentiometric titration curves are obtained from the results of data obtained by adding a strong base to a solution of glycine and to another solution containing glycine and a copper salt.



❑ METHODS OF ANALYSIS OF COMPLEXATION

❖ Distribution Method

The **method of distributing** a solute between **two immiscible solvents** can be used to determine the stability constant **for certain complexes.** The complexation of **iodine by potassium iodide** may be used as an example to illustrate the method. The equilibrium reaction in its **simplest form is**

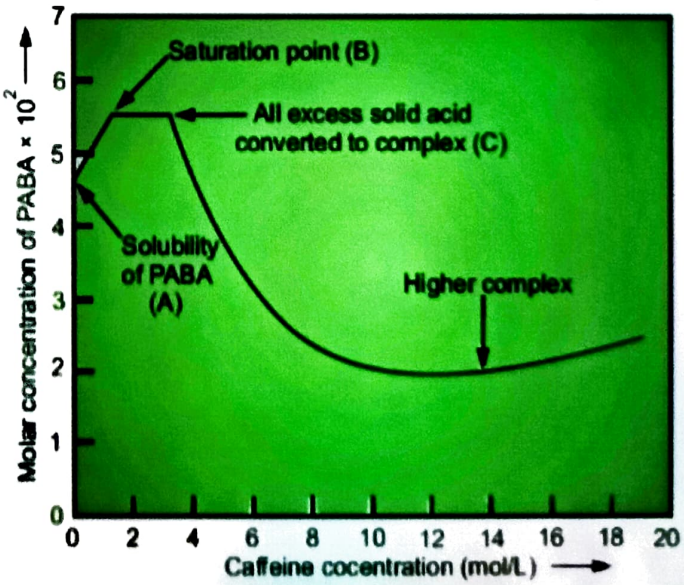
$$I_2 + I^- \rightleftarrows I^1_3$$

➢ **Additional steps also occur in polyiodide formation**

For example, $2I^- + 2I_2 \rightleftarrows I_6^{2-}$ may occur at **higher concentrations**, but it need not be considered here. **Higuchi investigated** the complexing action of caffeine, polyvinylpyrrolidone, and polyethylene glycols on **many acidic drugs,** using the **partition or distribution method.**

❖ 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 analyzed. The solubility method was used to **investigate the complexation** of **p-amino benzoic acid (PABA) by caffeine.**

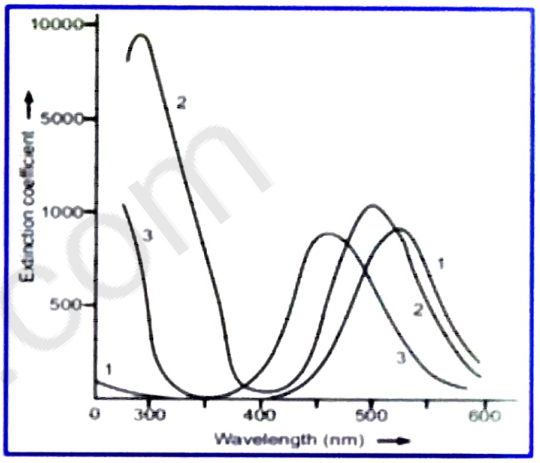


The Solubility of *Para*-Aminobenzoic Acid (PABA) in the Presence of Caffeine

❑ METHODS OF ANALYSIS OF COMPLEXATION

➢ Spectroscopy and Change Transfer Complexation Method

Absorption spectroscopy in the visible and ultraviolet regions of the spectrum is commonly used to investigate **electron donor–acceptor** or charge **transfer complexation.** When iodine is analyzed in a non-complexing **solvent such as CCl_4,** a curve is obtained with a single **peak at about 520 nm.** The solution is violet. A **solution of iodine in benzene** exhibits a **maximum shift to 475 nm,** & a new peak of considerably higher intensity for charge shifted **band appear at 300 nm.** A solution of iodine in diethyl ether shows **still greater shift to lower wavelength** and the appearance of a new maximum. **These solutions are red to brown.**



Absorption Curves of Iodine in the Non-complexing Solvent : (1) CCl4 and the complexing solvents (2) benzene and (3) diethyl ether

❖ Other Methods

➢ ^{1}H-NMR method :-

- Complexation of caffeine **with L-tryptophan in aqueous solution** was investigated by **using ^{1}H-NMR spectroscopy. Caffeine interacts with L-tryptophan at a molar ratio** of **1:1 by parallel stacking.** Complexation is a result of **polarization and π – π interactions** of the **aromatic rings. The tryptophan,** which is presumed to be the **binding site in serum albumin** for certain drugs, **can interact with caffeine even as free amino acid.**

➢ Circular dichroism :-

- The coil–helix transition of **polyadenylic acid** induced by the binding of the **Catecholamines, Norepinephrine** and **Isoproterenol,** using circular dichroism.
- Most mRNA molecules contain regions of **polyadenylic acid,** which are thought to increase the **stability of mRNA** and to favor **genetic code translation.**

➢ Infrared spectroscopy :-

The **infrared spectroscopy** was also used to **investigate the hydrogen-bonded complexes involving polyfunctional** bases **such as proton donors.** This is a very precise technique for determining the **thermodynamic parameters** involved in **hydrogen-bond formation** and for **characterizing the interaction sites** when the molecule has **several groups available** to form hydrogen-bonded. Caffeine forms **hydrogen-bonded complexes** with various **proton donors: phenol, phenol derivatives, aliphatic alcohols, and water.**

❑ PROTEIN BINDING

"The **phenomenon of complex formation** of drugs with proteins is called **protein binding".** A protein bound drug is **neither metabolized** nor excreted hence it is **pharmacologically inactive.**

❖ Types of protein binding :-

1. **Reversible binding :-** Involves **weak chemical bonds** such as H-bonds, hydrophobic bonds, ionic bonds, **Vander Waal's forces.**
2. **Irreversible binding :-** Arises as a result of **covalent bonding** & is often a reason for **carcinogenicity or toxicity of drug.**

❖ Significance of protein binding :-

- Absorption- protein binding with drugs **decreases free drug conc** & disturbs abs equilibrium. **Decrease in Distribution of drugs.**
- **Decrease metabolism** by preventing entry of drug to metabolizing organs & **enhances biological half life.**
- Only unbound drug is capable of **being eliminated.**
- Diagnosis of diseases or disorders by using radio active substance.
- Site specific drug delivery of **hydrophilic moieties.**

❖ Kinetics of protein binding :-

- If 'P' represent protein & 'D' represent the drug then applying law of mass action to reversible protein binding, the equation will be :-

$$P + D \rightleftharpoons PD$$

The association constant, Ka ⟶

$$Ka = [PD] / [P]/ [D] \quad \text{or} \quad [PD] = Ka [P] [D]$$

The 'Pt' is the total conc of protein present, unbound and bound

$$Pt = [PD]+[P]$$

To study the behaviour of drugs, a determinable ratio 'r' is as follows

$$r = \text{Moles of drug bound}/ \text{total moles of protein}$$

$$r = [PD] / Pt$$

$$r = Ka [D] / Ka [D] + 1$$

The above equation holds when there is only one binding site on protein & protein drug complex is 1:1 Complex.

❖ Measurement of protein binding :-

➢ Equilibrium dialysis

- Equilibrium dialysis is used to determine the extent of binding of a compound to plasma proteins.
- A semi-permeable membrane separates a protein-containing compartment from a protein-free compartment.
- The system is allowed to equilibrate at 37°C.
- The test compound present in each compartment is quantified by LC-MS/MS.

❖ Measurement of protein binding :-

➢ Dynamic dialysis

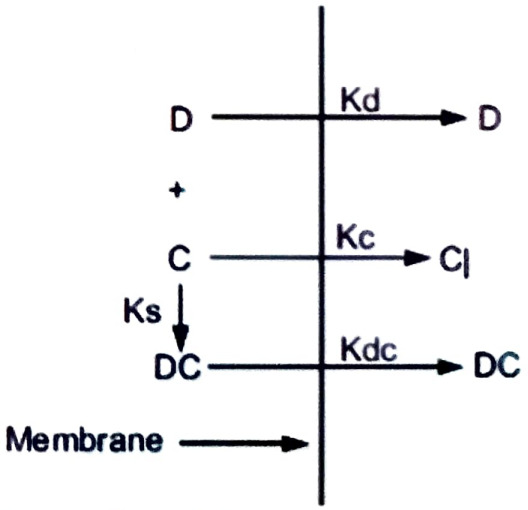
Dynamic dialysis is an exciting **new technology** that utilizes **fluid dynamics** to **increase purification efficiency** and improve large **buffer handling**, ideal for the production of **fragile proteins, viscous fluids and polymer gels**, such as **hyaluronic acid.**

➢ Ultrafiltration

- **Protein ultrafiltration is a pressure-driven** membrane process used for the concentration and/or **purification of protein solutions.**
- Ultrafiltration membranes typically have mean pore **size between 10** and **500 Å,** which is intermediate between reverse **osmosis and microfiltration.**

❑ COMPLEXATION AND DRUG ACTION

- The Fig. depicts **transfer of Drug(D), Complex(DC), and complexing agent (C)** across biological membrane. With subsequent dissociation of complex after transfer.
- The **rate of transfer** of **total drug on the right side of the membrane** is a function of **rate of transport of drug in its free and complex form.**
- If **transport rate of complex is more than drug,** the diffusion will be aided by complex formation.
- If **complexing agent is not diffusible rate of appearance of drug** will be a function of transfer of free (uncomplexed) drug.
- If the **complex is not transported, diffusion is retarded by complexation.**

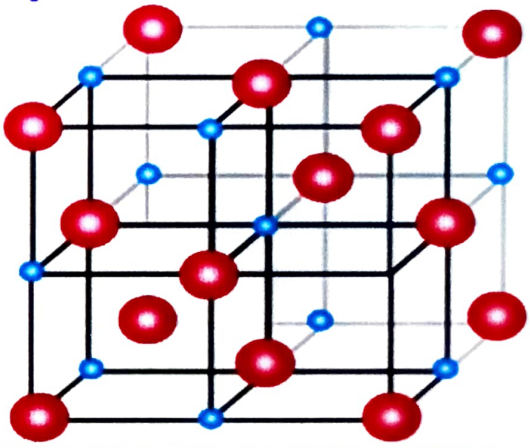


Transfer of Moieties Across Biological Membrane

- The mechanism by which complex formation can affect the passage compound include **alteration of o/w partition coefficient, apparent solubility, effective size of drug, change in the charge of the drug and alteration in diffusion of drug.**
- One of the best example of this class is the **interference of Calcium ions** with the **intestinal absorption of Tetracycline.**
- Other examples wherein **absorption was decreased due to formation of complex** include oral administration of **Neomycin and Kanamycin with bile salt.**

❑ CRYSTALLINE STRUCTURES OF COMPLEXES

- Complex or co-ordination **compounds cover the range** from quite simple inorganic salts to **elaborate metal-organic hybrid** materials and intricate **bioactive metalloproteins.**
- Their present uses and their **potential applications** are diverse due to their compositions, their molecular and **crystal structures** and their chemical and **physical properties.**
- Besides their use as **chemical reactants, complex compounds** are considered for extraction processes and as active agent in **remedies and for drug delivery.**



❑ THERMODYNAMIC TREATMENT OF STABILITY CONSTANTS

- The thermodynamics of **metal ion complex formation** provides much significant information. In particular, it is useful in **distinguishing between enthalpic and entropic effects.**

- **Enthalpic effects** depend on **bond strengths and entropic effects** have to do with **changes in the order/disorder of the solution as a whole**.
- The stability constant of metal complexes are related to thermodynamic properties such as free energy **charge (ΔG), enthalpy (ΔH), & entropy change (ΔS).**

✓ **These values can be computed by usual equation**

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

The standard enthalpy change (ΔH) obtained from slope of plot of log K vs 1/T

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

✓ **Standard entropy change is**

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

If stability constant **increase= ΔH & ΔS become negative**

- The standard enthalpy change can be determined by **Calorimetry** or by using the **Van't Hoff equation,**
- As binding between donor & acceptor is stronger then **ΔH also become negative.**