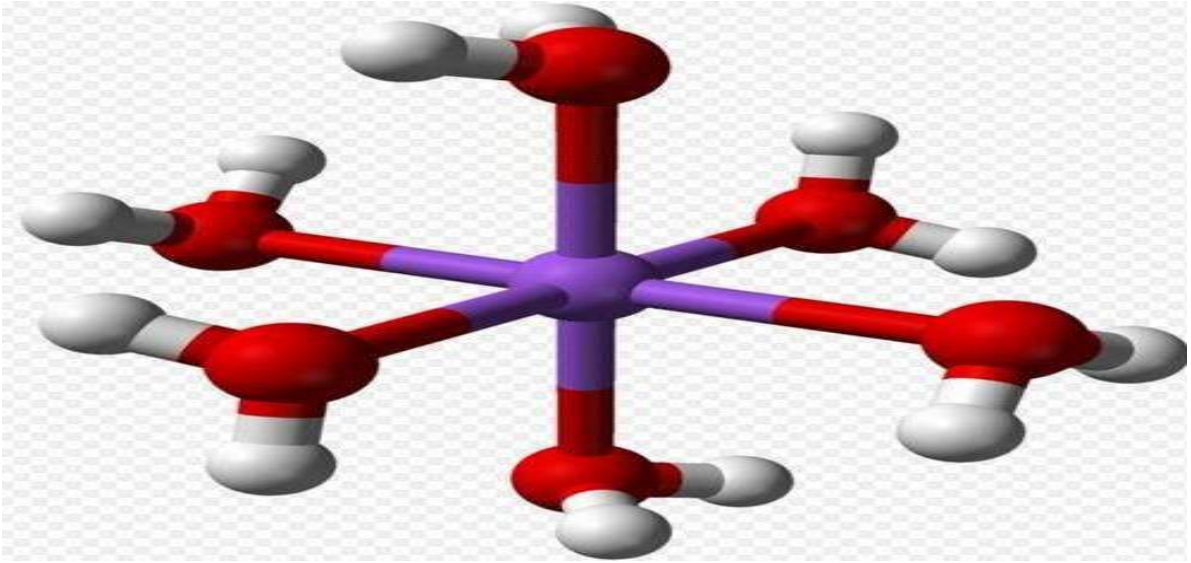
COMPLEXATION AND PROTEIN BINDING

B.JYOTHI
ASSISTANT PROFESSOR
DEPT. OF PHARMACEUTICS

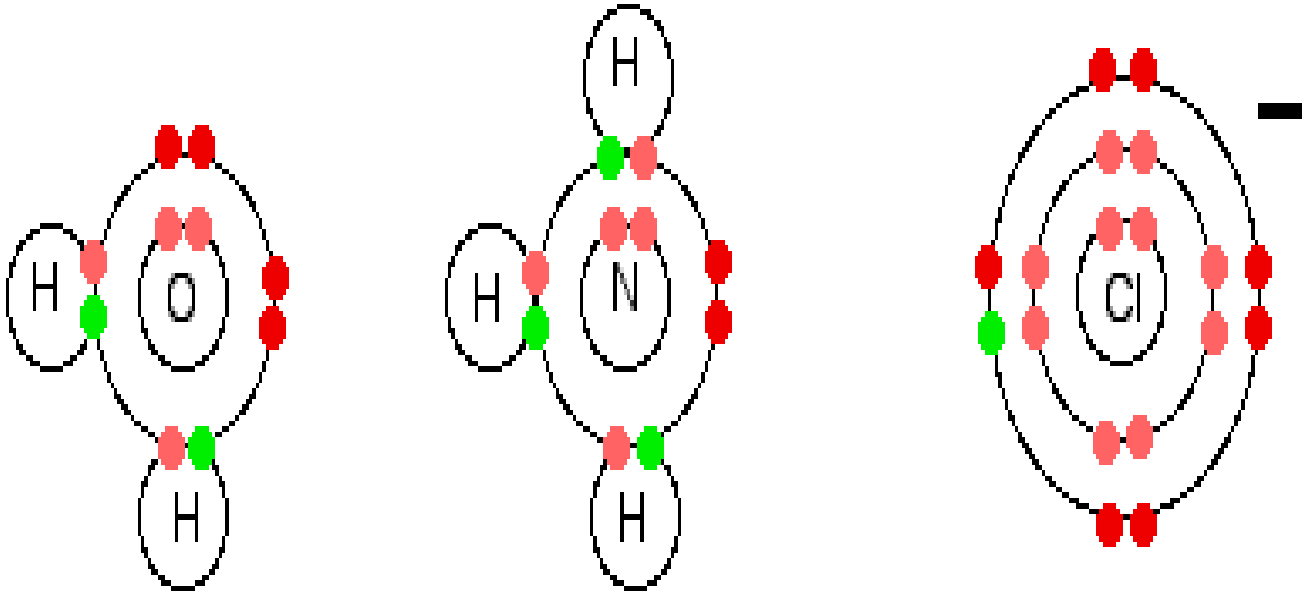
COMPLEXATION

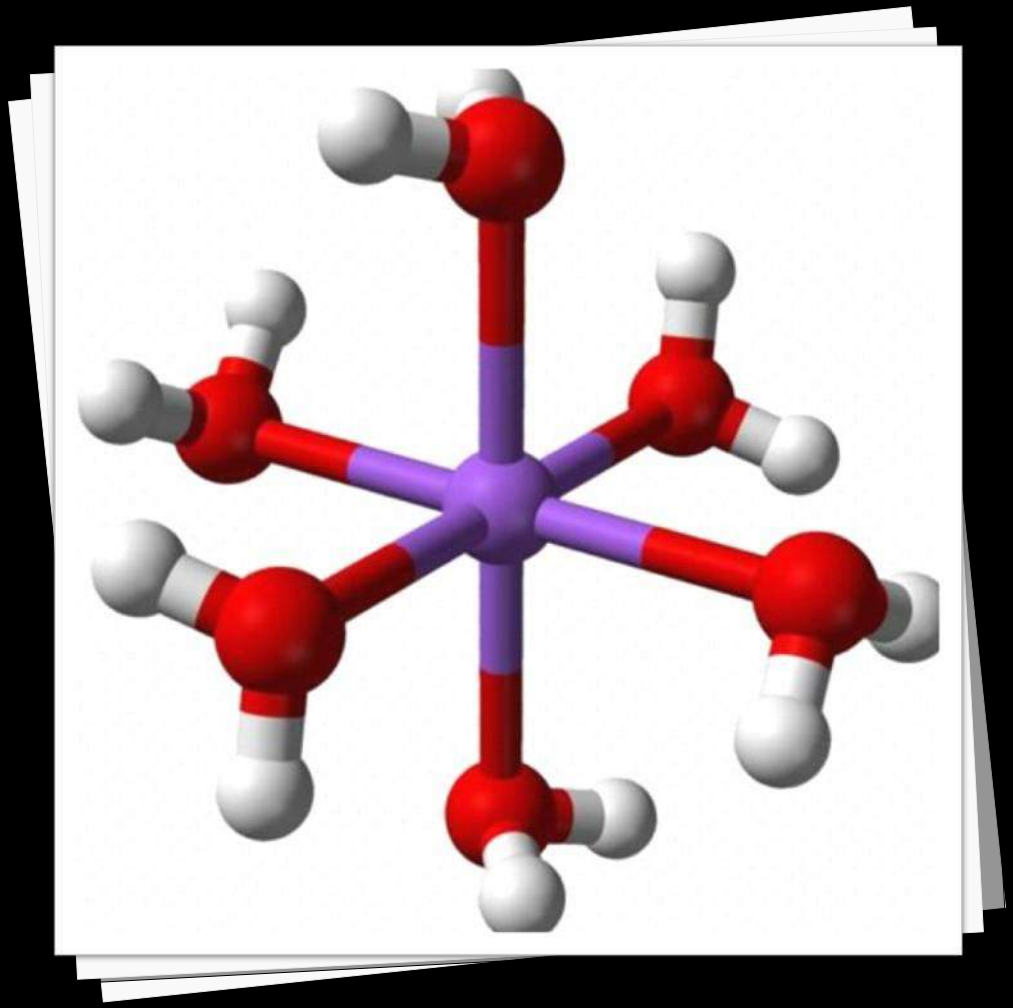
A ***coordination complex*** is the product of a Lewis acid-base reaction in which neutral molecules or anions (called ***ligands***) bond to a central metal atom (or ion) by ***coordinate covalent bonds***.



The nature of ligands

- Simple ligands include water, ammonia and chloride ions.
- What all these have got in common is active lone pairs of electrons in the outer energy level. These are used to form co-ordinate bonds with the metal ion.
- All ligands are lone pair donors. In other words, all ligands function as *Lewis bases*.

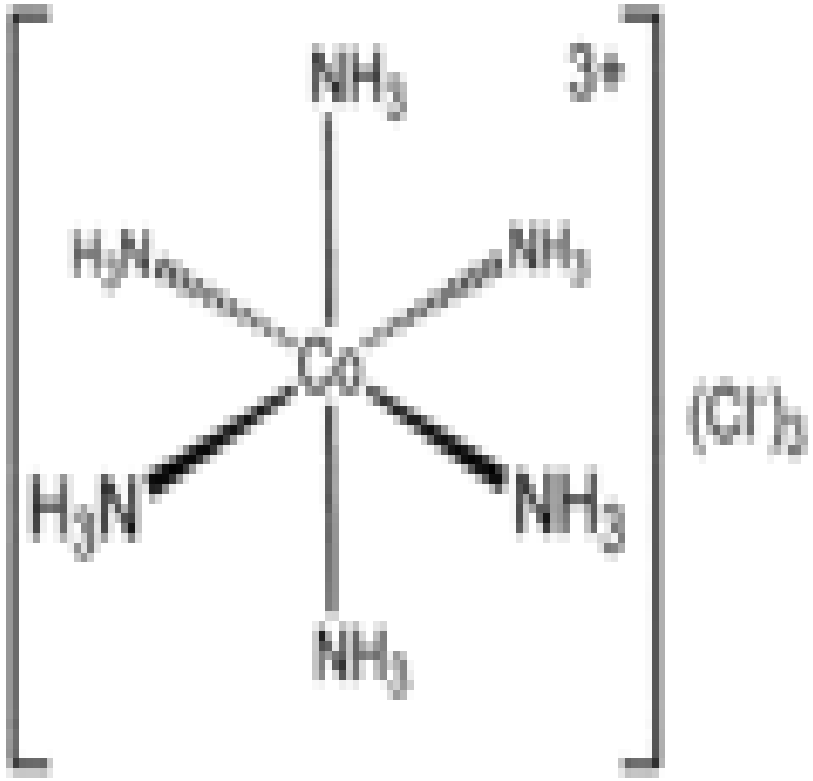




A **LIGAND** IS AN ION OR NEUTRAL MOLECULE THAT BONDS TO A CENTRAL METAL ATOM OR ION .

LIGANDS ACT AS LEWIS BASES (ELECTRON DONORS), AND THE CENTRAL METALS THEY BOND TO ACT AS LEWIS ACIDS (ELECTRON ACCEPTORS). .

COBALT(III) COMPLEX CONTAINING SIX AMMONIA LIGANDS, WHICH ARE MONODENTATE.



Hexamminecobalt (III) chloride

- Within a ligand, the atom that is directly bonded to the metal atom/ion is called the *donor atom*.
- A coordinate covalent bond is a covalent bond in which one atom (i.e., the donor atom) supplies both electrons
- If the coordination complex carries a net charge, the complex is called a *complex ion*.
- Compounds that contain a coordination complex are called *coordination compounds*.
- The *coordination number* is the number of donor atoms bonded to the central metal atom/ion.

COORDINATION COMPOUNDS

Identify the

b. **Lewi's base/Ligand**

c. **Lewi's acid/Metal ion**

d. **Donor atom**

e. **Coordination number**

In the following coordination complexes

$[Ag(NH_3)_2]^+$, $[Zn(CN)_4]^{2-}$, $[Ni(CN)_4]^{2-}$, $[PtCl_6]^{2-}$, $[Ni(NH_3)_6]^{2+}$

IMPORTANCE OF COMPLEXATION

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

- These properties include solubility, stability, partitioning, energy absorption and emission, and conductance of the drug
- Drug complexation can lead to beneficial propertiessuch as enhanced aqueous solubility (e.g. theophylline complexation with ethylenediamine to from aminophylline) and stability (e.g. inclusion complexes of labile drugs with cyclodextrins).

IMPORTANCE OF COMPLEXATION

- **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.**
- **Aqueous solubility of tetracycline decreases substantially when it complexes with calcium ions and coadministration of some drugs with antacids decreases absorption from the gastrointestinal tract.**
- **Drug complexation with hydrophilic compounds also can enhance excretion.**

IMPORTANCE OF COMPLEXATION

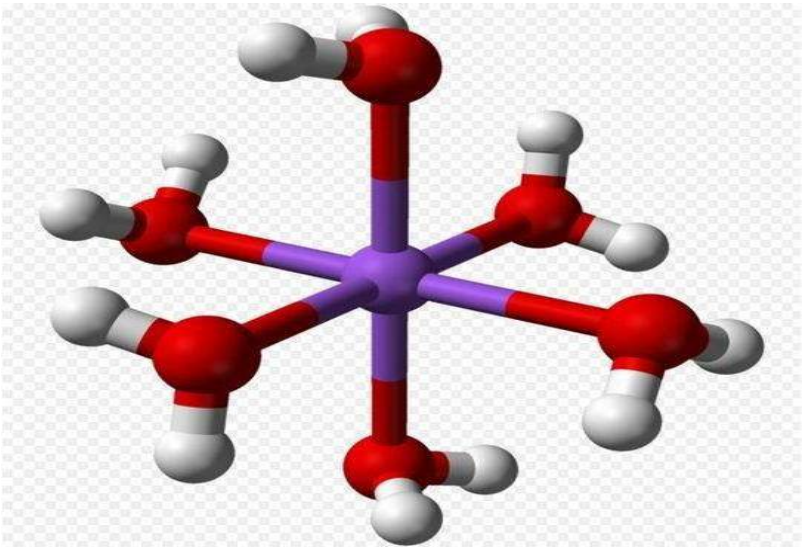
- **Complexes can alter the pharmacologic activity of the agent by inhibiting interactions with receptors.**

- **Complexation of a ligand with a substrate molecule can occur as a result of coordinate covalent bonding or one or more of the following noncovalent interactions:**

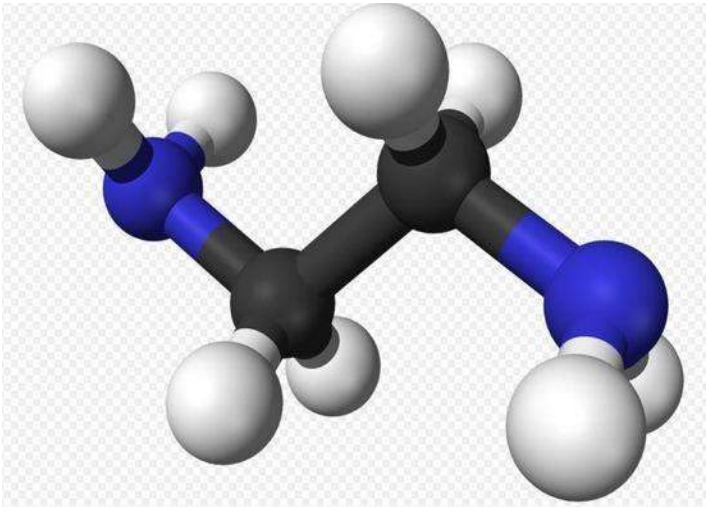
5. **Van der Waals forces**
6. **Dipolar forces**
7. **Electrostatic forces**
8. **Hydrogen bonding**
9. **Charge transfer**
10. **Hydrophobic interactions.**

LIGANDS

- **Ammonia, which has single pair of electrons (basic group) for bonding with metal ion, is called <u>unidentate ligand</u>.**
- **<u>Bidentate</u> - two basic groups. (ethylenediamine)**

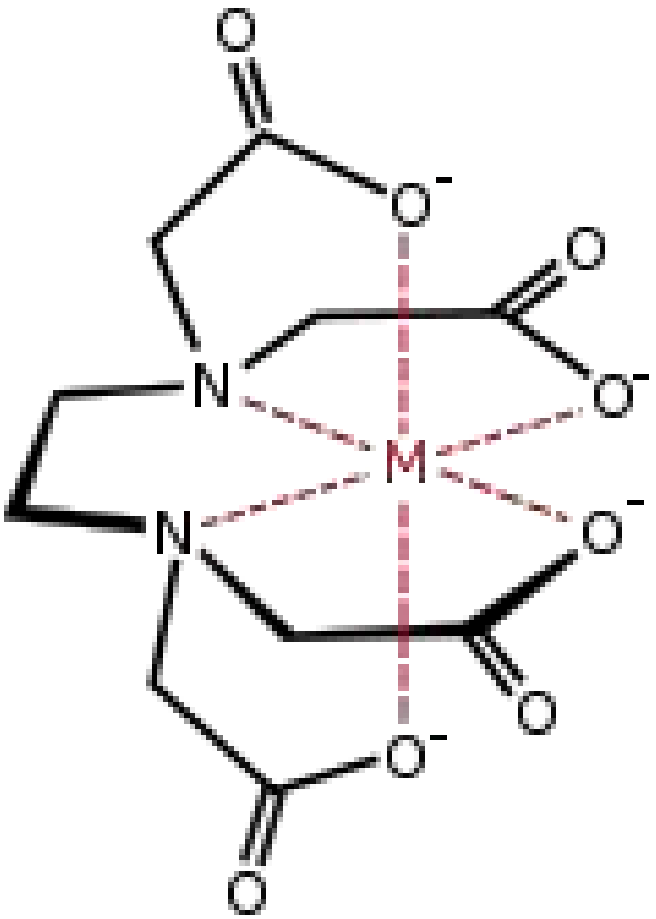


Six monodentate ligands attached



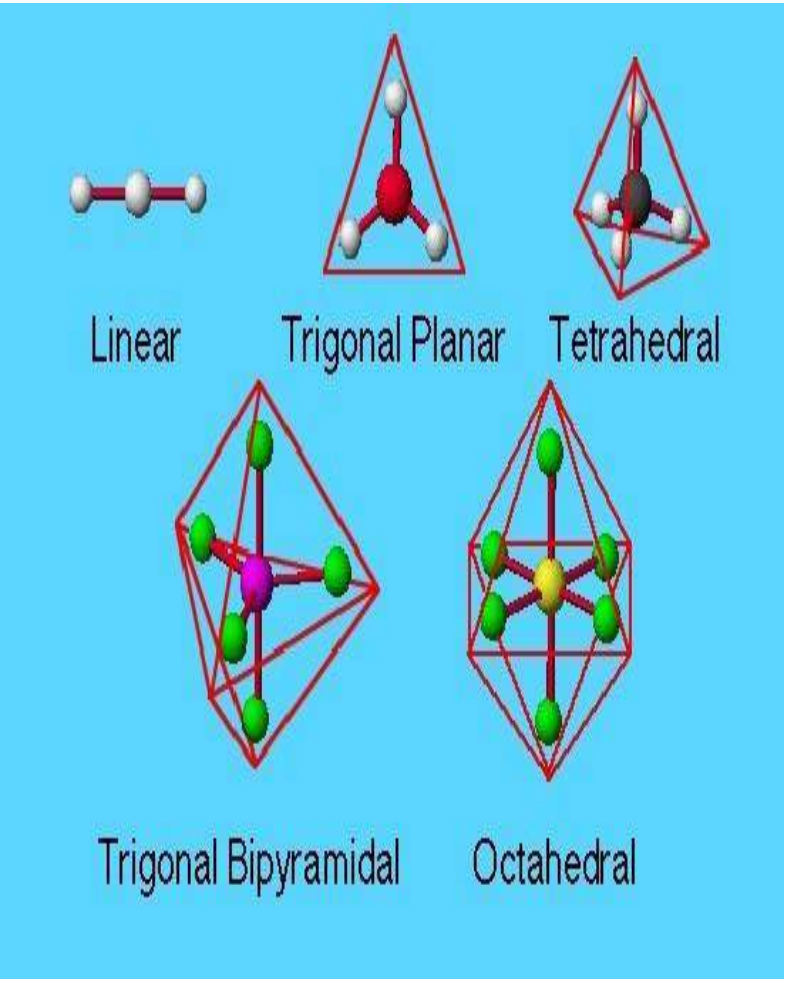
Bidentate ligand

LIGANDS



- **Multidentate or Polydentate – ligands with multiple binding sites (polymers); if the same metal ions binds with two or more sites.**
- **The complex form is called CHELATE**
- **Hexadentate - ethylenediaminetetra acetic acid (EDTA)- Has a total of six points (4:0 and 2: N) for attachment of metal ions.**

MOLECULAR STRUCTURES



Linear- O_2

Trigonal- BCl_3

Tetrahedral - CH_4

Square planar

$-Cu(NH_3)_4^{+2}$

Bipyramidal -

PF_5

Octahedral

$-Co(NH_3)_6^{+3}$

CHELATES

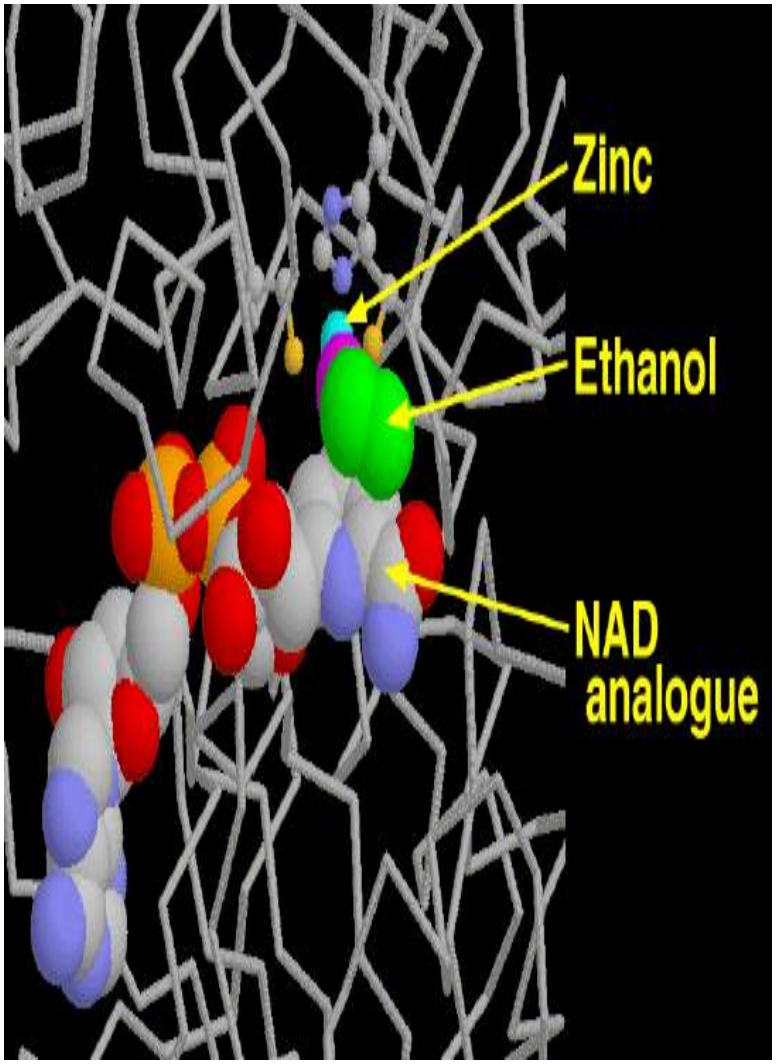
CHELATION

- **is the formation or presence of two or more separate coordinate bonds between a polydentate (multiple bonded) ligand and a single central atom.**

Two geometric forms

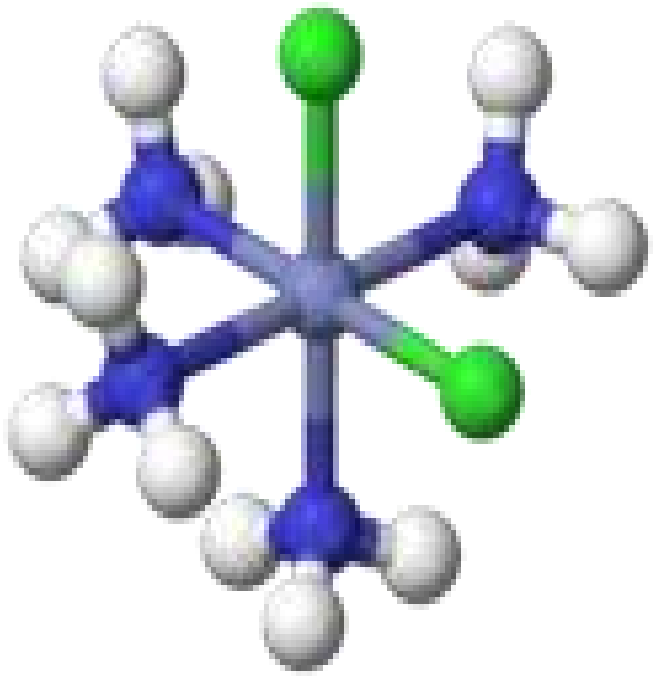
- a. *cis* isomer- 2 like ligands are adjacent
- Examples: alcohol dehydrogenase enzymes
  (contains Zinc)
- b. *trans* isomer- 2 like ligands are opposite each other
  Examples: vitamin B_{12} and hemeproteins

CHELATION

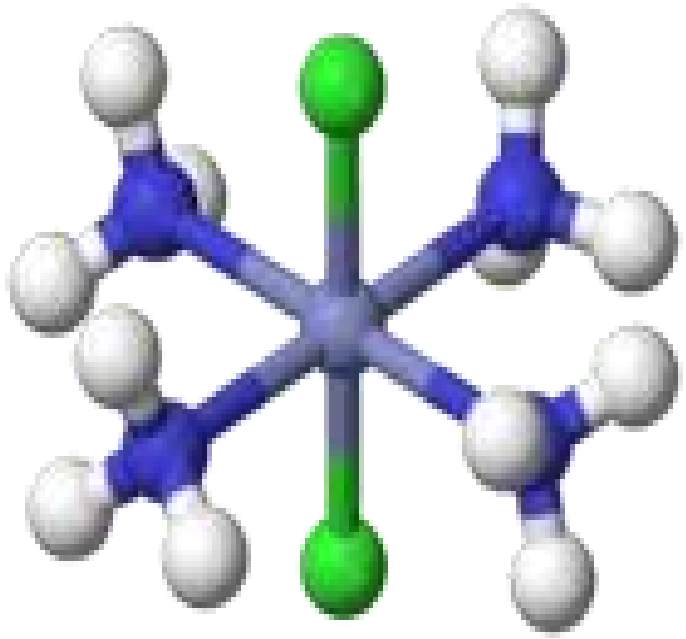


- **Alcohol dehydrogenase uses two molecular "tools" to perform its reaction on ethanol. The first is a zinc atom, which is used to hold and position the alcoholic group on ethanol. The second is a large NAD cofactor (constructed using the vitamin niacin), which actually performs the reaction.**

CHELATION

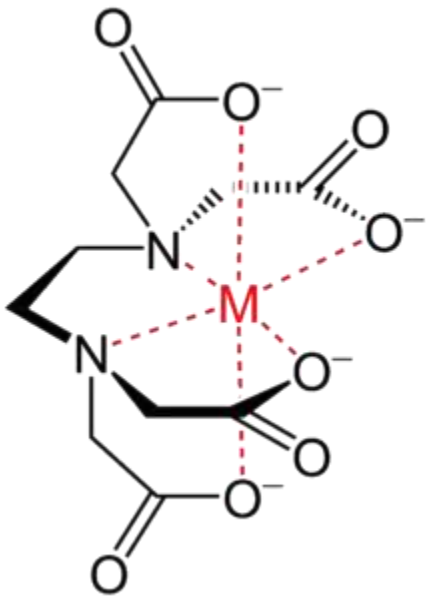


cis-
$[CoCl_2(NH_3)_4]^+$

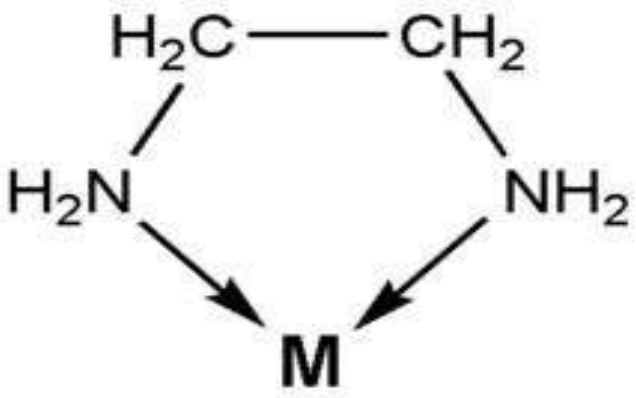


trans-
$[CoCl_2(NH_3)_4]^+$

CHELATES



EDTA



Ethylenediamine ligand, binding to a central metal ion with two bonds

Natural occuring chelates: chlorophyll, hemoglobin, albumin

ORGANIC MOLECULAR COMPLEXES

- Consists of constituents held together by weak forces of the donor-acceptor type or by hydrogen bonds

Examples:

- disulfiram, clomethiazole, tolnaftate - charge transfer complexes
- Quinhydrone of salicylic acid - quinhydrone complex
- Butesin picrate - a 2:1 complex of butesin and picric acid *(Picric acid complex)*
- Caffeine-gentisic acid complex - used in chewable tablet formulation
- Polymer complexes

ORGANIC MOLECULAR COMPLEXES

- Reaction of dimethylaniline and 2,4,6-trinitroanisole

COLD TEMPERATURE	HOT TEMPERATURE
Molar complex or organic coordination compound	Formation of salt or complexation
Secondary valence bond(not clear bond but rather attraction b/n 2 molecules	Primary valence bond
Weak donor-acceptor	Charge transfer

ORGANIC MOLECULAR COMPLEXES

- **Reaction between trinitrobenzene and benzene molecule.**

  -an example of *a charge transfer complexes (*one molecule polarizes the other resulting in a type of ionic interaction or charge transfer.

  -polar nitro groups of nitrobenzene induce a dipole in the readily polarizable benzene molecule(electrostatic attraction).

Organic complexes or molecular complexes are so weak that they cannot be seprarated from their solutions as definite compounds, with low energy of attraction and a a bond distance greater than 3 angstrom.

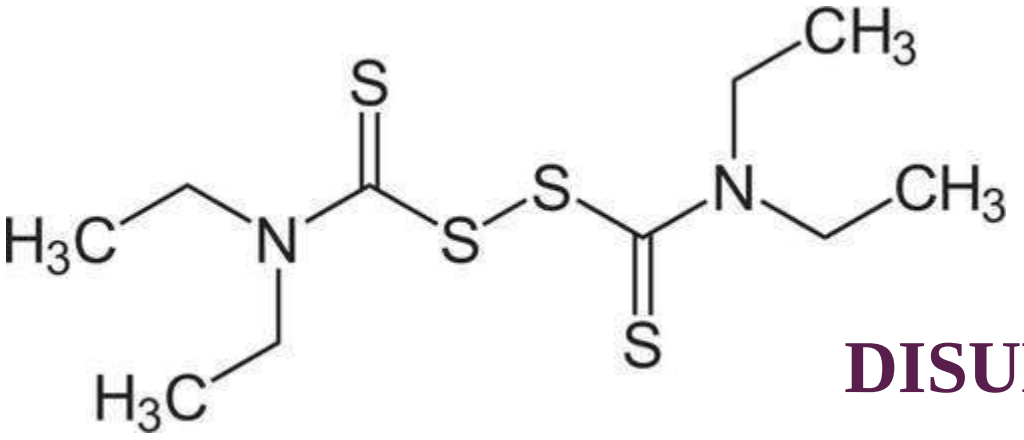
ORGANIC MOLECULAR COMPLEXES

- ***Reaction between a donor-acceptor complex of trinitrobenzene(D) and hexamethylbenzene(A).***

Molecular complexes- complexes bound together by van der Waals forces, dipole-dipole interactions and hydrogen bonding but lacking charge transfer.

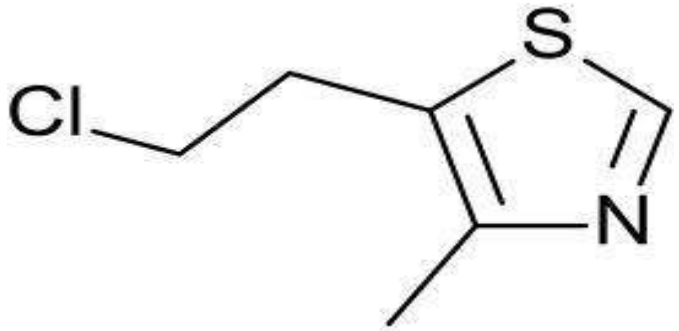
D···A	D+···A-
Weak Donor-acceptor complex or molecular complex	Charge transfer complex
London dispersion forces and dipole-dipole intrn	Resonance

ORGANIC MOLECULAR COMPLEXES

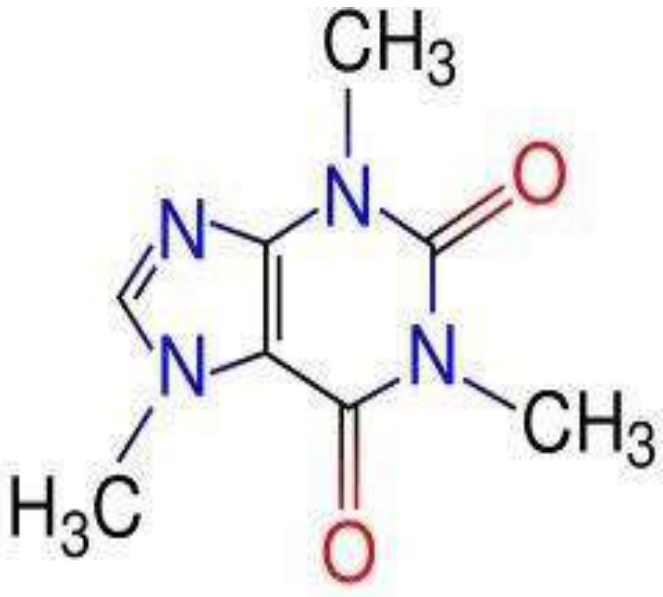


DISULFIRAM

CLOMETHIAZOLE



DRUG COMPLEXES



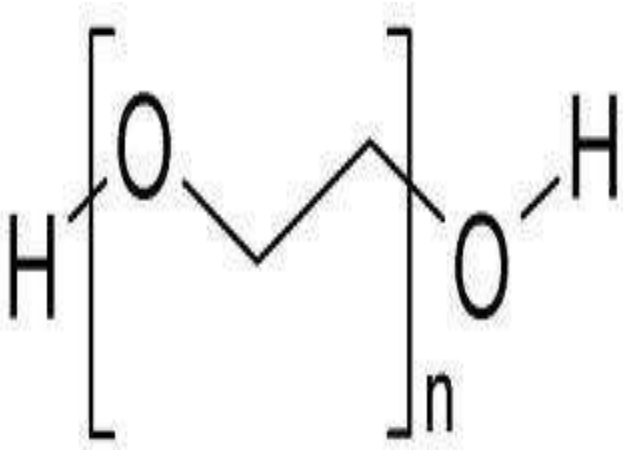
CAFFEINE

- Complexation of caffeine
- Polarized carbonyl group(rxn with acids)
- Nonpolar part(insolubility in water)
- Relative positive center
- Electrophilic or acidic nitrogen results in dipole-dipole interaction

DRUG COMPLEXES

- **Caffeine + organic acid anions** – more water soluble; example complex with xanthine.
- **Caffeine + organic acids** – less soluble but masks the bitter taste of caffeine.Ex – complex w/ gentisic acid formulated in extended release chewable tablet.
- Complexation of esters results from hydrogen bonding between nucleophilic carbonyl oxygen and an active hydrogen.
- Complexation of benzocaine with salicylates will decrease the boavailability of benzocaine while its comlexation with PEG will increase its absorption.

POLYMER COMPLEXES



POLYETHYLENE GLYCOL

- **PEG, polystyrene,carboxymethylcellulose and incompatibilities b/n carbowaxes, pluronics and tween with tannic acids, salicylic acids and phenols are due to their nucleophilic oxygens.**

POLYMER COMPLEXES

- **PVP binding with benzoic acid and nicotine derivatives – increases phosphate buffer solutions and decreases as the temperature is raised.**
- **Crosspovidone binds with acetaminophen, benzocaine, benzoic acid, caffeine, tannic acid, and papaverine HCl due to its dipolar character and porous structure.**
- **Polyolefin container interact with drugs depends linearly on the octanol water partition coefficient of the drug; which can result in loss of the active component in liq dosage forms**

POLYMER COMPLEXES

- **Dissolution rate of ajmaline is enhanced by complexation with PVP due to the aromatic ring of ajmaline and the amide groups of PVP to yield a dipole dipole induced complex.**
- **Incompatibilities in suspensions, emulions, suppositories and ointments may leaf to precipitation, flocculation, delayed biologic absirption, loss of preservative action and other undesirable physical, chemical and pharmacoloic effects.**

OTHER ORGANIC MOLECULAR COMPLEXES

Quinhydrone complexes – quindrone is formed by mixing alcoholic solutions of benzoquinone and hydroquinone forming green crystals.

Aqueous solution with quinhydrone, the complex disassociates into equivalent amounts of quinone and hydroquinone.

Picric acid complexes – such as Butesin picrate which combines the antiseptic property of picric acid and anesthetic property of Butesin used as a 1% ointment for burns and painful skin abrasions.

INCLUSION COMPOUNDS

- **A class of addition compounds where the constituent of the complex is trapped in the open *lattice or cagelike* crystal structure of the other of the other to yield a stable arrangement.**

Channel Lattice type – examples are deoxycholic acid with other complexes; urea and thiourea complexes and the starch-iodine solution.

Layer type- intercalate compounds b/n its layers. Example is the clay montmorillonite complexes

INCLUSION COMPOUNDS

Clathrates – crystallize in a *cage-like* lattice in which the coordinating compound is entrapped. Molecular size of the encaged component is important. Examples are

<u>Hydroquinone crystals</u> that traps methanol, CO2 and HCl but not smaller and larger molecules; and

<u>Warfarin sodium USP</u> is a clathrate of water, isopropyl alc and sodium warfarin.

INCLUSION COMPOUNDS

- **Monomolecular inclusion compounds** – involve entrapment of a single quest molecule in the cavity of one host molecule.

Gamma-Cyclodextrin accomodating mytomycin C and **beta-CD** accomodating indomethacin(inc reactivity) and retinoic acid(inc aq solubility), famotidine and tolbutamide(inc dissolution rate).

Cyclodextrin are used to **trap, stabilize and solubilize** sulfonamides, tetracyclines, morphine, aspirin, benzocaine, ephedrine, reserpine and testosterone.

INCLUSION COMPOUNDS

- Amorphous derivatives of beta-CD and gamma-CD –in complex with testosterone allow an efficient transport of hormone into the circulation via sublingual route.
- Water-soluble CCB diltiazem and ISDN complex with ethylated beta-CD- produce a sustained release effect .
- Femoxetine complex with beta-CD – oral liquid suspension bitter taste is suppressed.

PROTEIN BINDING

- Binding of drugs into proteins may
  - Facilitate the distribution of drugs into the body.
  - Inactivating the drug
  - Retarding the excretion of drug
- Interaction of a drug with proteins
  - Displacement of body hormones or coadministered agent.
  - Configurational change in the protein
  - Formation of drug-protein complex that is biologically active.

Important proteins: albumin and alpha1-acid glycoprotein

PROTEIN BINDING

- Measures free fraction or protein binding of drugs
- Ultrafiltration
- Ultracentrifugation
- Equilibrium dialysis
- Chromatography
- Spectrophotometry
- electrophoresis

METAL ION COORDINATE COMPLEXES

- **IRON COMPLEXES**

The ability of metal ions to coordinate with and then release ligands in some processes and to oxidize and reduce in other processes in biological system.

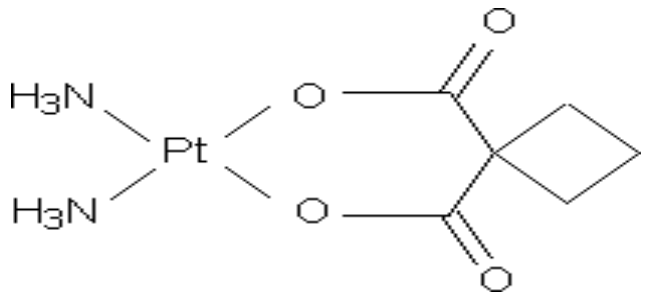
Example: iron (myoglobin and hemoglobin) – transport O_2

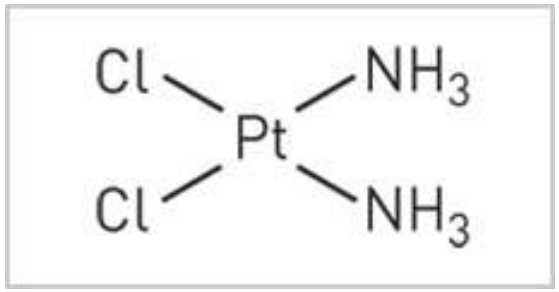
cytochrome – photosynthesis, respiratory system

- **PLATINUM COMPLEXES**

  Cisplatin and carboplatin – platinum II complexes that have prove to be the most useful agents in treatment of cancer.

- **CARBOPLATIN – less toxic to the peripheral nervous system and the kidneys.**
- **The decreased toxicity of carboplatin and the activity against cisplatin – resistant tumors have led to greater use of carboplatin.**

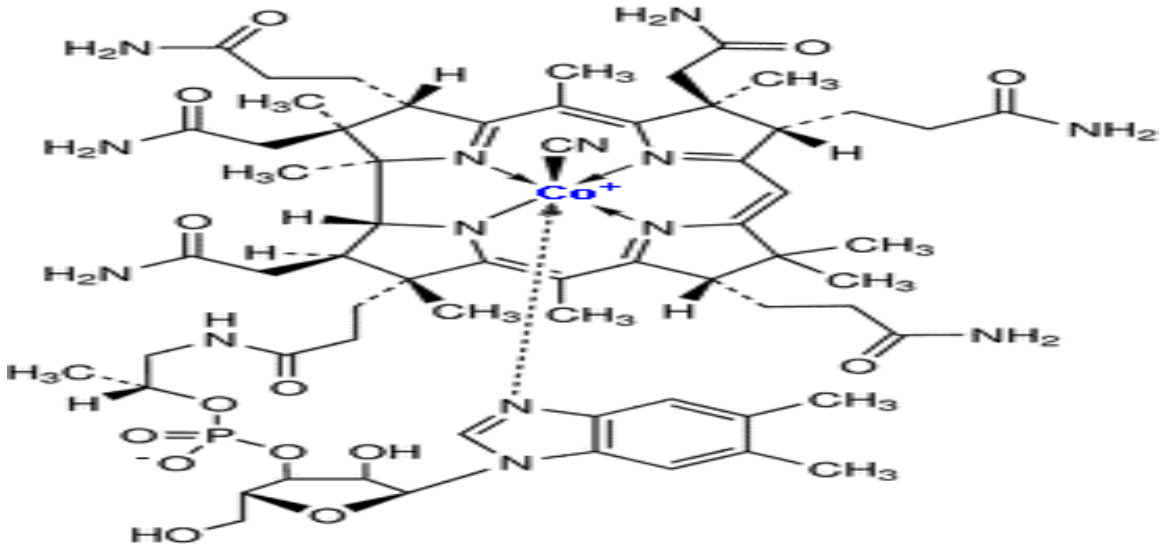




COPPER AND COBALT COMPLEXES

Copper ion – important proteins and enzymes (hemocyanin, superoxide dismutase and cytochrome oxidase).– forming colorless tetrahedral complexes.

Cobalt – the bilogical role of cobalt is largely confined to Vit B12 (cyanocobalamin)



ZINC COMPLEXES

- **Important metal ion that is present in many proteins confers structure and stability.**
- **Only metal ion found in crystalline insulin.**
- **Present in the enzymes carboxypeptidase and carbonic anhydrase.**

TOXIC HEAVY METAL COMPLEXES

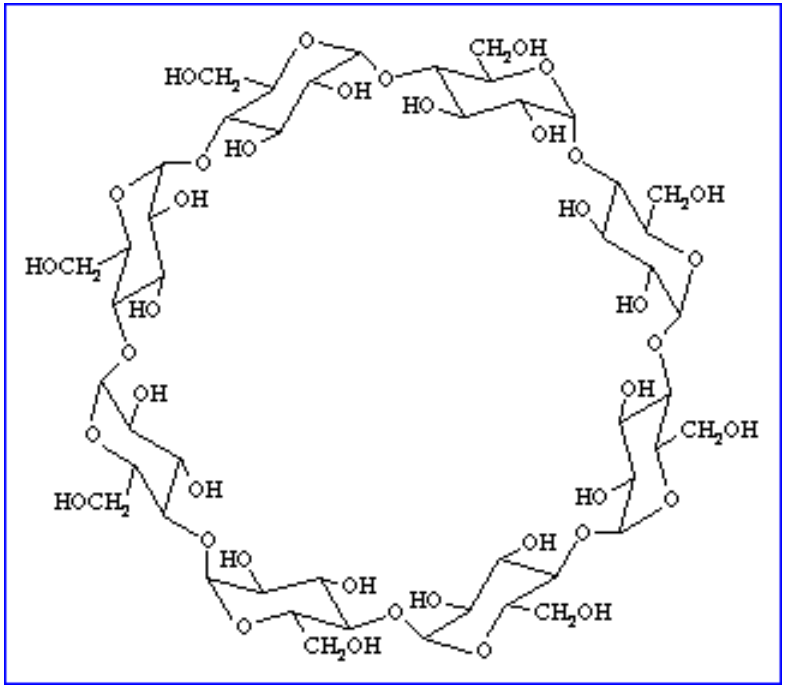
- **Because of the presence of lead in older paints and water and that of mercury in thermometers, poisoning incidences this metal ions are very common in pedriatic population.**

- **Lead and mercury toxicity – chelating agent such as dicalcium salt of EDTA and 2,3-dimercaptopropanol, also known as BAL, are the British Anti-Lewisite.**

CYCLODEXTRIN COMPLEXES

- **One of the most important molecular complexations is the interaction between molecules and cyclodextrin to form reversible inclusion complexes.**

- **Types:**
  - **Alpha**
  - **Beta**
  - **Gamma**



SUMMARY OF IMPROVEMENTS IN PROPERTIES OF SELECTED DRUG COMPOUNDS BY COMPLEXES WITH CYCLODEXTRINS.

Property	Drug Examples
Enhanced aqueous solubility	prostaglandins; ketoprofen and other (NSAIDs).
Improved stability	aspirin, atropine, procaine, digoxin, prostaglandin
Enhanced absorption and bioavailability	phenytoin, digoxin, barbiturates, sulfonamides, diuretics
Change from liq. To solid	nitroglycerin, methyl salicylate, oil
Decreased volatility	soluble vit. (A, D, K)
	iodine, camphor, menthol, chlorobutanol, salicylic acid
Improved taste and odor	prostaglandins, NSAIDs, thymol, chloramphenicol
Decreased stomach irritation	ASA, indomethacin, other NSAIDs
Inhibit RBC lysis	antibiotics, menandione,
Prevention of incompatibilities	vitamins

ION EXCHANGE RESINS

- **A method of complexation that is based on electrostatic interactions between the surface-bound ions on a solid particle (resin) and the oppositely charged ions in aqueous solution.**

- **Ion- exchange resins- are organic polymer-based or organic mineral particles with a positive or negative surface charge.**

- **Ion exchange resins are classified as cation exchangers or anion exchangers depending on the types of ions they can replace.**
- **Cation exchangers – replace surface-bound positively charged ions with similarly charged ions in solution.**
- **Anion exchangers – replace negatively charged ions on the surface with similarly changed ions in solution.**
- **Ion-exchange resins are used mainly for purification and drug delivery purposes.**

PROTEIN LIGAND INTERACTION

- **The interaction between small molecules such as drugs and proteins.**
- **Important in drug binding to receptor for pharmacologic activity, enzyme-substrate interaction in catalysis, antibody- antigen recognition and interaction between drugs and proteins in plasma that affects the distribution profile in the body.**
- **Many of the interactions between protein and low-molecular weight compounds occur in a reversible manner according to the following equilibrium.**

1. $[P] = [L] \longleftrightarrow [PL]$

2. $K_a = \frac{[PL]}{[P][L]}$

Where:

[P] – molar concentration of the protein

[L] – molar concentration of the ligand (or drug)

[PL] – molar concentration of protein-ligand complex

K_a – measure of affinity between the protein and the ligand (M^{-1} or liters per mole)

MECHACHANISMS OF INTERACTIONS

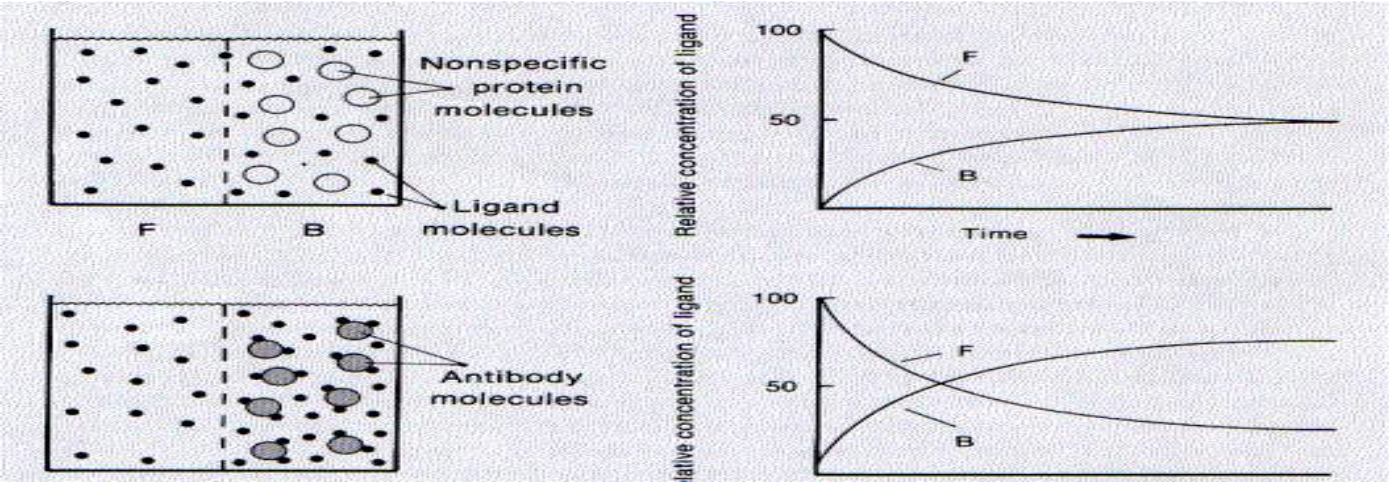
- **Proteins can interact with small molecules as a result of H-bonding between donor and acceptor functional group in amino acid sequence.**
- **Compose of different types of amino acids.**

Example:

- **H-bonding – proteins interact with small molecules.**
- **Electrostatic interactions – occur between charge amino acids with oppositely charge ligand molecules.**
- **Van der Waals interactions – dipole-dipole, dipole induced dipole, dispersion forces**
- **Hydrophobic interaction – interfacial phenomenon that results of attraction between nonpolar (hydrophobic) groups with water molecules.**

EXPERIMENTAL METHODS

- **Spectroscopic method**
- **Ultracentrifugation – "ultafiltration" separated with the aid of high centrifugal force.**
- **Gel filtration – first adapted for measurement of protein ligand interactions.**
- **Equilibrium dialysis – involves separation of compounds accdg. To size difference or MW using membranes with specific MW.**



PLASMA PROTEIN BINDING

- **Human plasma is composed of 200 known proteins.**

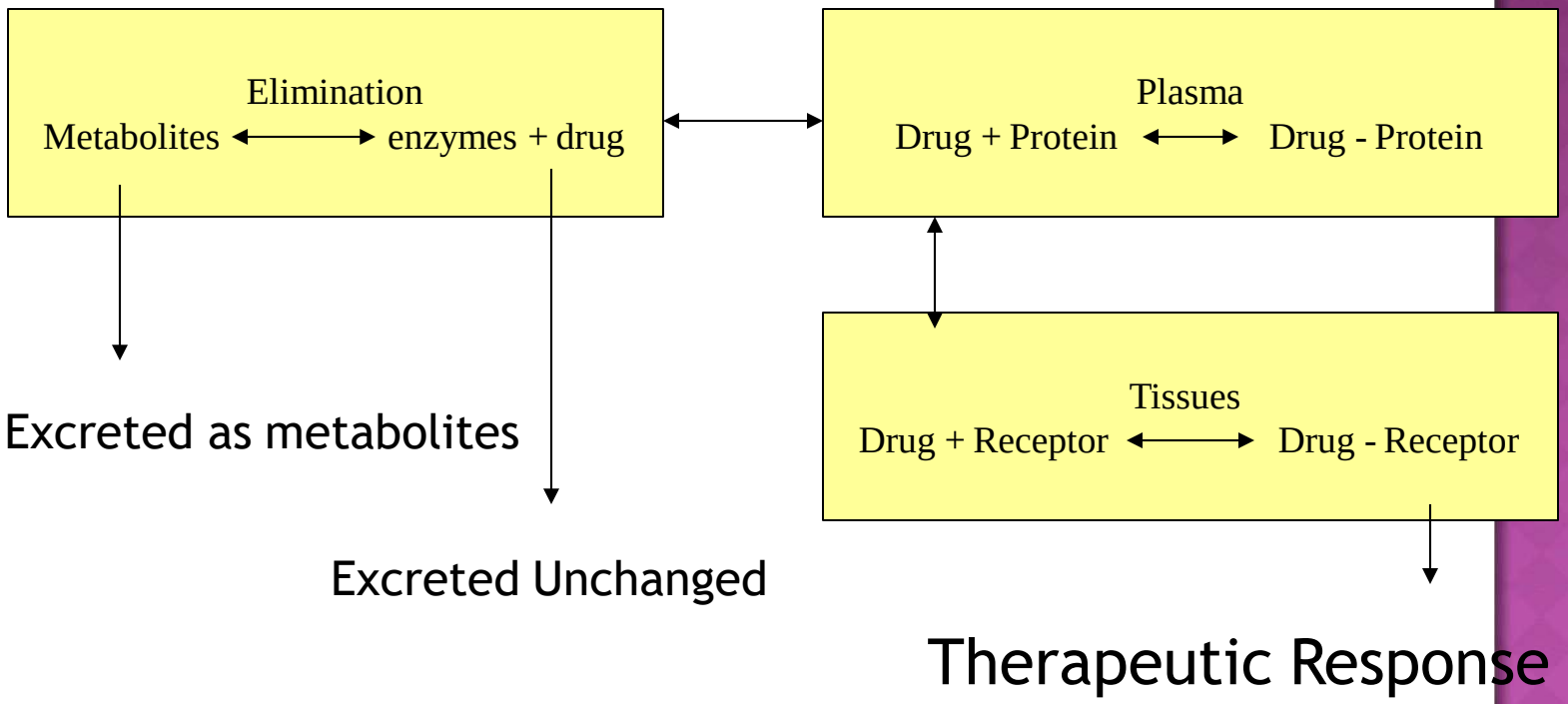
Functions:

- **Maintenance of osmotic pressure between ICF and ECF.**
- **Coagulation of Blood**
- **Immune reactions (antibodies)**
- **Transport of endogenous and exogenous compounds**
- **Function as enzymes or hormones.**

- **Since albumin is basic, acidic and neutral drugs will primarily bind to albumin. If albumin becomes saturated, then these drugs will bind to lipoprotein. Basic drugs will bind to the acidic alpha-1 acid glycoprotein. This is significant because various medical conditions may affect the levels of albumin, alpha-1 acid glycoprotein, and lipoprotein.**

SIGNIFICANCE OF PROTEIN BINDING

- **Interactions between plasma protein and drugs after systemic administration can have profound implications for the therapeutic outcomes.**



ANALYSIS OF PLASMA PROTEIN BINDING

- Mathematical analysis of protein drug interactions is performed to evaluate the binding activity or association constant (K_a) and measured by the number of binding sites (v)

Where:

- Aqueous buffer – pH 7.4
- Ionic strength – 0.16
- Temperature – 37 ^{o}C [98.6 ^{o}F]

Questions

IDENTIFY THE CENTRAL METAL ION, THE LIGANDS, AND THE COORDINATION NUMBER OF THE FOLLOWING COORDINATE COMPLEXES.

- A. $[Co(NH_3)_6]^{3+}$
- B. $[Cr(H_2O)_6]^{+3}$
- C. $[Ag(NH_3)_2]^{+}$
- D. $[Fe(CN)_6]^{4-}$

ANSWERS

A. The central metal ion is cobalt

The ligands are ammonia

The coordination number is 6

B. The central metal ion is chromium

The ligands are water molecules

The coordination number is 2

C. The central metal ion is silver

The ligands are ammonia

The coordination number is 2

D. The central metal ion is iron

The ligands are cyano

The coordination number is 6