Shree H. N. Shukla Institute of Pharmaceutical Education and Research, Rajkot

B. Pharm
Semester-III

Subject Name: Physical pharmaceutics-I
Subject Code: BP302TP

CHAPTER-4- COMPLEXATION AND PROTEIN BINDING

SYLLABUS:

Introduction, Classification of Complexation, Applications, methods of analysis, protein binding, Complexation and drug action, crystalline structures of complexes and thermodynamic treatment of stability constants.

- The course deals with the various physical and physicochemical properties, and principles involved in dosage forms/formulations.
- Theory and practical components of the subject help the student to get a better insight into various areas of formulation research and development, and stability studies of pharmaceutical dosage forms.

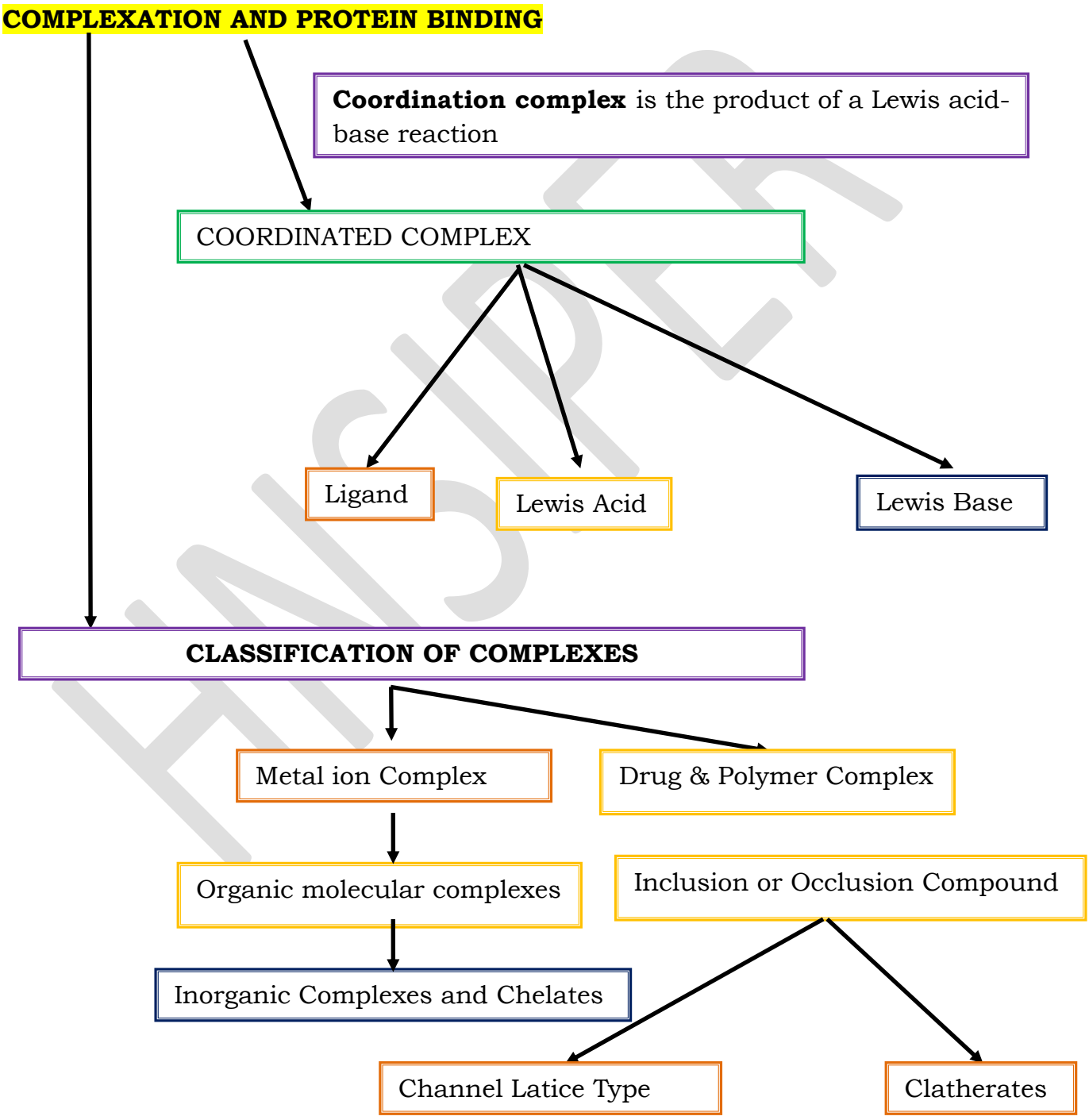
Learning objectives

- Understand the nature of the intra and intermolecular forces that are involved in stabilizing molecular and physical structure.
- Understand the differences in these forces and their relevance to different types of molecules.
- Appreciate the differences in the strengths of the intermolecular forces that are responsible for the stability structures in the different states of matter.
- Understands properties of gaseous states.

COMPLEXATION AND PROTEIN BINDING

TOPIC: WHAT IS COMPLEXATION AND PROTEIN BINDING?

Ans:



Detailing:

Classification of Complexes

I. Metal ion complexes

A. Inorganic type
B. Chelates
C. Olefin type
D. Aromatic type
1. Pi (π) complexes
2. Sigma (σ) complexes
3. "Sandwich" compounds

II. Organic molecular complexes

A. Quinhydrone type
B. Picric acid type
C. Caffeine and other drug complexes
D. Polymer type

III. Inclusion/occlusion compounds

A. Channel Lattice Type
B. Layer type
C. Clathrates
D. Monomolecular type
E. Macromolecular type

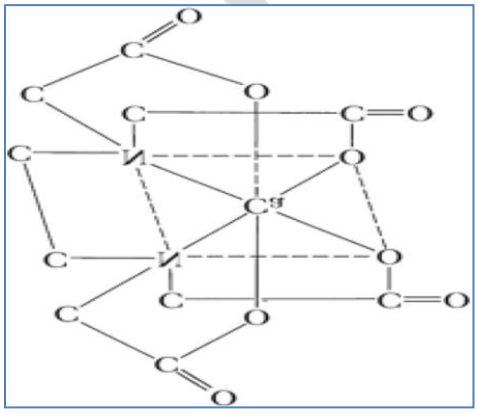
Inorganic Complexes

- The ammonia molecules in hexamminecobalt (III) chloride, as the compound [Co(NH3)6]3+ Cl3 is called, 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.
- For example, Ligands such as H2O H3 , C -, or l- donate a pair of electrons in forming a complex with a metal ion, and the electron pair enters one of the unfilled orbitals on the metal ion.
- Hybridization plays an important part in coordination compounds in which sufficient bonding orbitals are not ordinarily available in the metal ion.

- Table 1 shows some compounds in which the central atom or metal ion is hybridized differently and the geometry those results.

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.***
- Molecules with two and three donor groups are called *bidentate* and *tridentate*, respectively.
- Ethylenediaminetetraacetic acid has six points for attachment to the metal ion and is accordingly *hexadentate*; however, in some complexes, only four or five of the groups are coordinated.
- The synthetic chelating agent ethylenediaminetetraacetic acid (Fig. 10-1) has been used to tie up or *sequester* iron and copper ions so that they cannot catalyze the oxidative degradation of ascorbic acid in fruit juices and in drug preparation. Chelation places stringent steric requirements on both metal and ligands. Ions such as Cu(II) and Ni(II), which form square planar complexes, and Fe(III) and Co(III), which form octahedral complexes, can exist in either of two geometric forms. Because of this isomerism, only *cis-coordinated ligands*— ligands adjacent on a molecule—will be readily replaced by reaction with a chelating agent.
- Vitamin B12 and the hemoproteins are incapable of reacting with chelating agents because their metal is already coordinated in such a way that only the *trans*coordination positions of the metal are available for complexation. In contrast, the metal ion in certain enzymes, such as alcohol dehydrogenase, which contains zinc, can undergo chelation, suggesting that the metal is bound in such a way as to leave two *cis* positions available for chelation.



Coordination Number	Orbital Configuration	Bond Geometry	Example Formula	Example Structure
2	sp	Linear	O_2	O—O
3	sp^2	Trigonal	BCl_3	Cl, B—Cl, Cl
4	sp^3	Tetrahedral	CH_4	H, C, H, H, H
4	dsp^2	Square planar	$Cu(NH_3)_4^{2}$	NH_3, Cu, NH_3, NH_3, NH_3
5	dsp^3	Bipyramidal	PF_5	F, P, F, F, F, F
6	d^2sp^3	Octahedral	$Co(NH_3)_6^{3}$	NH_3, Co, NH_3, NH_3, NH_3, NH_3

One Word Question Answer

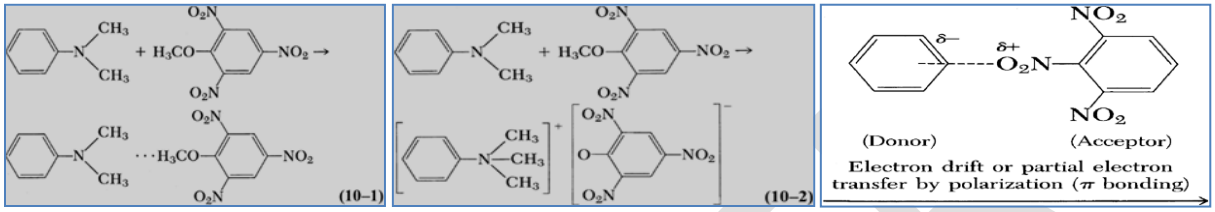
SR NO.	QUESTION	ANSWER
1	What is product of a Lewis acid-base reaction in which neutral molecules or anions (called ***ligands***) bond to a central metal atom (or ion) by ***coordinate covalent bonds.***	Coordination complex
2	ligands are lone pair donors. In other words, all ligands function as ?	Lewis Base
3	Any nonmetallic atom or ion, whether free or contained in a neutral molecule or in an ionic compound, that can donate an electron pair can serve as ?	Donor
4	The constituent that accepts a share in the pair of electrons, is frequently a metallic ion, although it can be a neutral atom.	Acceptor
5	Dipolar, and induced dipolar types are formation of?	Intermolecular force
6	Which of the cobalt ion, or number of ammonia groups coordinated to the metal ions, is six, is called?	Coordination Number
7	When the ligand provides one group for attachment to the central ion, the chelate is called?	Monodentate
8	Fe(III) and Co(III), which form?	Octahedral Complex

Detailing:

Organic Molecular Complexes

An organic coordination compound or molecular complex consists of constituents held together by weak forces

1- Hydrogen bonds. The compounds dimethyl aniline and 2,4,6-trinitroanisole react in the cold to give a molecular complex:



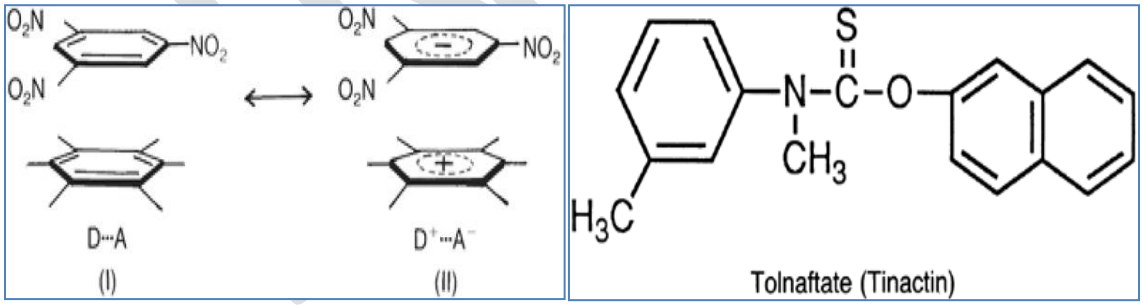
2- Electron donor–acceptor mechanism:-

- The type of bonding existing in molecular complexes in which hydrogen bonding plays no part is not fully understood, but it may be considered as *el**ectron donor–acceptor mechanism***

3 - Charge transfer complexes.

- One molecule polarizes the other, resulting in a type of ionic interaction or charge transfer,
- For example, the polar nitro groups of trinitrobenzene induce a dipole in the readily polarizable benzene molecule, and the electrostatic interaction that results leads to complex formation:

4- London dispersion forces and dipole–dipole interactions contribute more to the stability of the complex. A resonance interaction is shown in Figure 10-2 .



- Trinitrobenzene is the acceptor, A, molecule and hexamethylbenzene is the donor, D.
- On the left side of the figure, weak dispersion and dipolar forces contribute to the interaction of A and D; on the right side of the figure, the interaction of A and D results from a significant transfer of charge, making the electron acceptor trinitrobenzene negatively charged (A-) and leaving the donor, hexamethylbenzene, positively charged (D+).

- The overall donor–acceptor complex is shown by the double-headed arrow to resonate between the uncharged D … A and the charged D+ Amoieties.
- 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.
- 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 A, In both charge transfer and donor–acceptor complexes, Charge transfer complexes are of importance in pharmacy. Iodine forms 1:1 charge transfer complexes with the drugs disulfiram, chlomethiazole, and tolnaftate.
- These drugs have recognized pharmacologic actions of their own: Disulfiram is used against alcohol addiction, clomethiazole is a sedative–hypnotic and anticonvulsant, tolnaftate is an antifungal agent.
- Each of these drugs possesses a nitrogen–carbon–sulfur moiety. and a complex may result from the transfer of charge from the pair of free electrons on the nitrogen and/or sulfur atoms of these drugs to the antibonding orbital of the iodine atom.
- Thus, by tying up iodine, molecules containing the N—C==S moiety inhibit thyroid action in the body.

Drug Complexes

- Caffeine is complexing with a number of acidic drugs. such as a sulfonamide or a barbiturate to a dipole–dipole force or hydrogen bonding between the polarized carbonyl groups of caffeine and the hydrogen atom of the acid.
- A secondary interaction probably occurs between the nonpolar parts of the molecules, and the resultant complex is “squeezed out” of the aqueous phase owing to the great internal pressure of water.
- These two effects lead to a high degree of interaction.
- Caffeine forms complexes with organic acid *anions* that are more soluble than the pure xanthine, but the complexes formed with organic acids, such as gentisic acid, are less soluble than caffeine alone.
- Such insoluble complexes provide caffeine in a form that masks its normally bitter taste and should serve as a suitable state for chewable tablets.

One Word Question Answer

SR NO.	QUESTION	ANSWER
1	The energy of attraction between the constituents is probably for most organic complexes	less than 5 kcal/mole
2	One molecule polarizes the other, resulting in a type of ionic is called?	Ionic Charge Transfer
3	Caffeine forms complexes with ?	organic acid *anions*
4	Clomethiazole is included in which category?	Sedative & Convulsant
5	Which comples is masks its normally bitter taste?	Caffine Complex

Detailing:

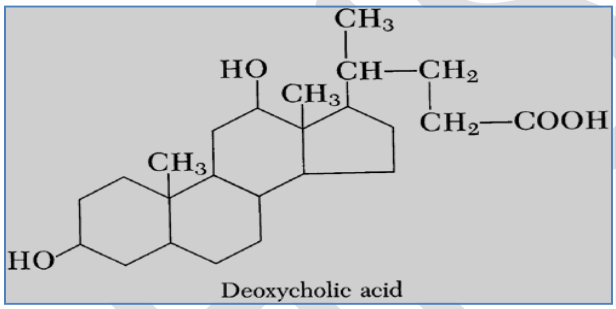
Polymer Complexes

- Polyethylene glycols, polystyrene, carboxymethylcellulose, and similar polymers containing nucleophilic oxygens can form complexes with various drugs, can be attributed to these interactions.
- The interactions that may occur in Suspensions, Emulsions, Ointments, Suppositories.
- The incompatibilities of certain polyethers, such as the Carbowaxes, Pluronics, and Tweens with tannic acid, salicylic acid, and phenol may be manifested as a precipitate, flocculate, delayed biologic absorption, loss of preservative action, undesirable physical, chemical, and pharmacologic effects.

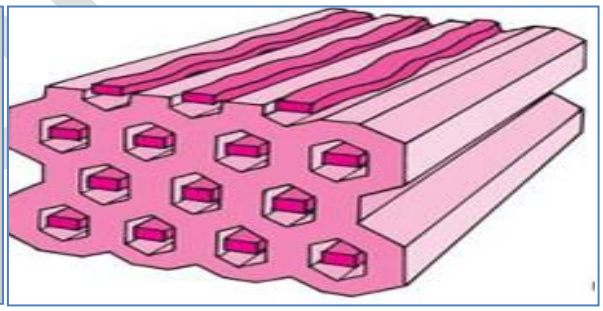
Inclusion or Occlusion Compounds

Channel Lattice Type

- The *cholic acids* (bile acids) can form a group of complexes principally involving deoxycholic acid in combination with paraffins, 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. 10-3).



Deoxycholic acid



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, that is, to the high energy that must be expended to decompose the compound.
- Powell and Palin made a detailed study of clathrate compounds and showed that the highly toxic agent hydroquinone (quinol) crystallizes in a cage like hydrogen-bonded structure, as seen in Figure 10-4.

- The holes have a diameter of 4.2 A and permit the entrapment of one small molecule to about every two quinol molecules.
- Small molecules such as methyl alcohol, CO2, and HCl may be trapped in these cages, but smaller molecules such as H2and larger molecules such as ethanol cannot be accommodated.

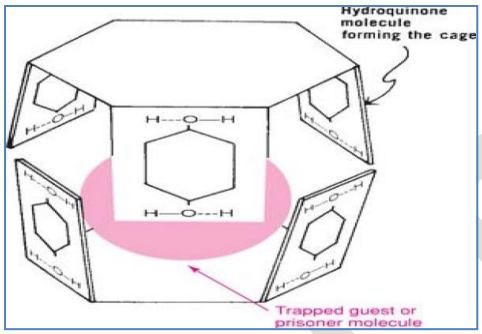


Fig. 10-4. Cage like structure formed through hydrogen bonding of hydroquinone molecules. Small molecules such as methanol are trapped in the cages to form the clathrate

Monomolecular Inclusion Compounds: Cyclodextrins

- 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). These compounds are cyclic oligosaccharides containing a minimum of six D-(+)-glucopyranose units attached by α-1,4 linkages.
- The natural α-, β-, and γ-cyclodextrins (α-CD, β-CD, and γ-CD, respectively) consist of six, seven, and eight units of glucose, respectively.
- The cyclodextrin structure exists as a truncated cone, which is seen in Figure 10-5***a***; it can accommodate molecules such as mitomycin C to form inclusion compounds (Fig. 10-5***b***).
- The interior of the cavity is relatively hydrophobic because of the CH2 groups, whereas the cavity entrances are hydrophilic owing to the presence of the primary and secondary hydroxyl groups.
- β-CD and γ-CD are the most useful for pharmaceutical technology owing to their larger cavity size (internal diameter almost 6 A and 8 A, respectively). Water inside the cavity tends to be squeezed out and to be replaced by more hydrophobic species.
- Thus, molecules of appropriate size and stereochemistry can be included in the cyclodextrin cavity by hydrophobic interactions.

One Word Question Answer

SR NO.	QUESTION	ANSWER
1	CMC, Polyester can form complexes with various drugs is called?	Polymer Complex
2	a group of complexes principally involving deoxycholic acid in combination with paraffins are called?	Channel Lattice type
3	Monomolecular host structures are represented by which molecule?	Beta Cyclodextrine
4	β-CD and γ-CD their larger cavity size from?	6A and 8A
5	Molecules crystallize in the form of a cage like lattice in which the coordinating compound is entrapped, is called?	Clatherates
6	How many unit of glucose required to form γ-CD?	8 Unit of Glucose

- Complexation does not ordinarily involve the formation of covalent bonds. Some drugs may be too large to be accommodated totally in the cavity. As shown in Figure 10-5***b***, mitomycin C interacts with γ-CD at one side of the torus.
- Thus, the aziridine ring of mitomycin C is protected from degradation in acidic solution.

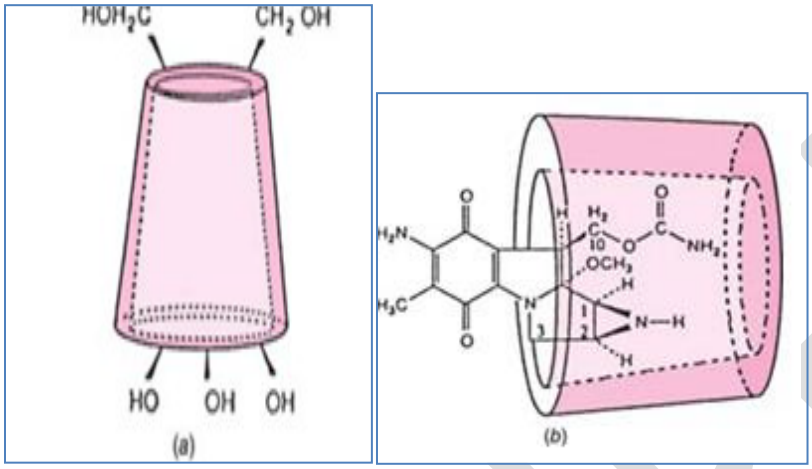
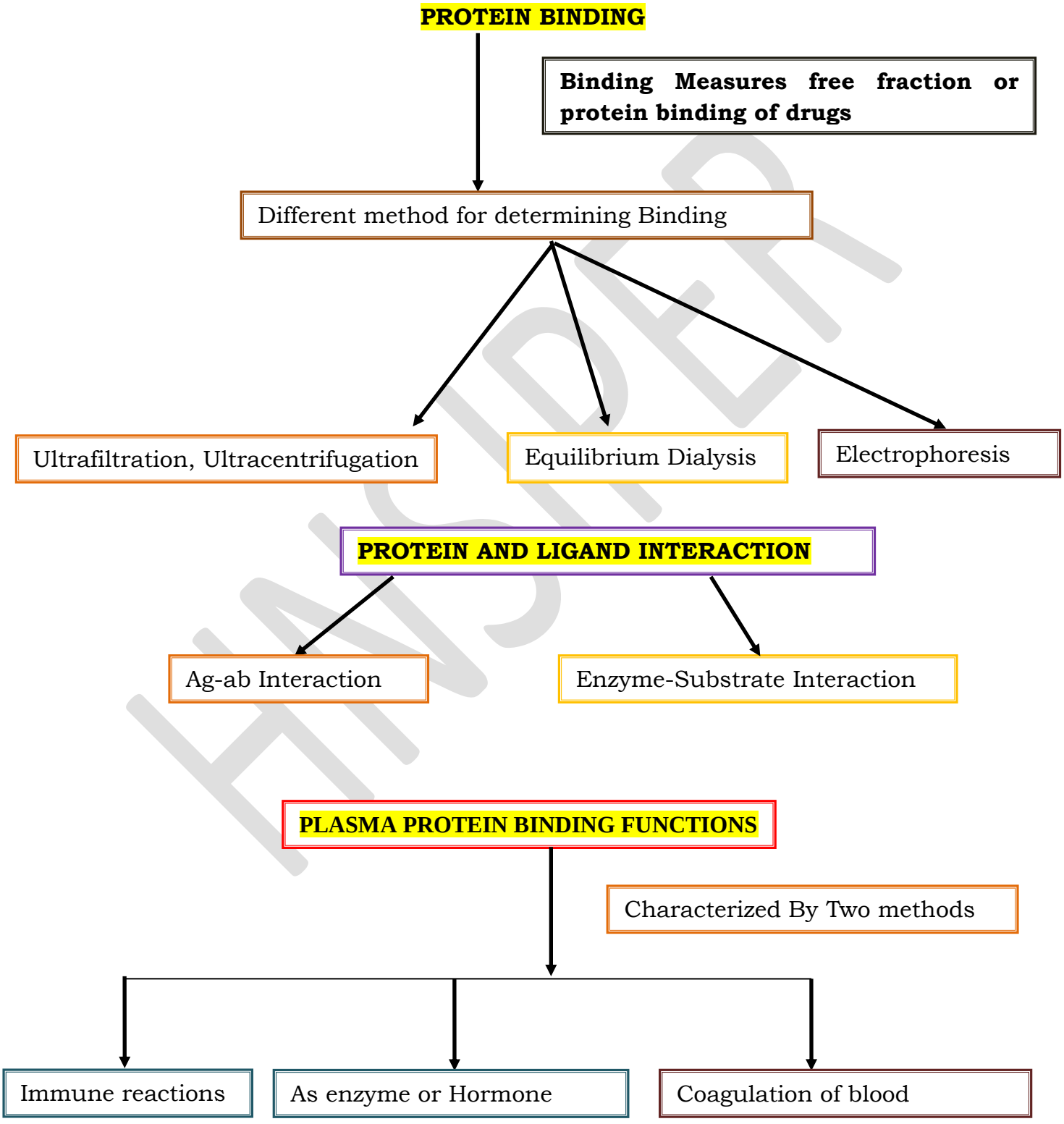


Fig. 10-5. (*a*) Representation of cyclodextrin as a truncated cone. (*b*) Mitomycin C partly enclosed in cyclodextrin to form an inclusion complex.

- Cyclodextrins are studied as solubilizing and stabilizing agents in pharmaceutical dosage forms. and used to trap, stabilize, and solubilize sulfonamides, tetracyclines, morphine, aspirin, benzocaine, ephedrine, reserpine, and testosterone.
- The aqueous solubility of retinoic acid (0.5 mg/liter), a drug used topically in the treatment of acne, is increased to 160 mg/liter by complexation with β-CD. Dissolution rate plays an important role in bioavailability of drugs, fast dissolution usually favoring absorption.
- Thus, the dissolution rates of famotidine,a potent drug in the treatment of gastric and duodenal ulcers, and that of tolbutamide, an oral antidiabetic drug, are both increased by complexation with β-cyclodextrin.

TOPIC: What is Protein Binding?

Ans



Detailing:

- 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** by Ultrafiltration, Ultracentrifugation, Equilibrium dialysis, Chromatography, Spectrophotometry, electrophoresis.

Protein and 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] [PL] **2. K_a= [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)

Mechanisms of Interaction

- 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.

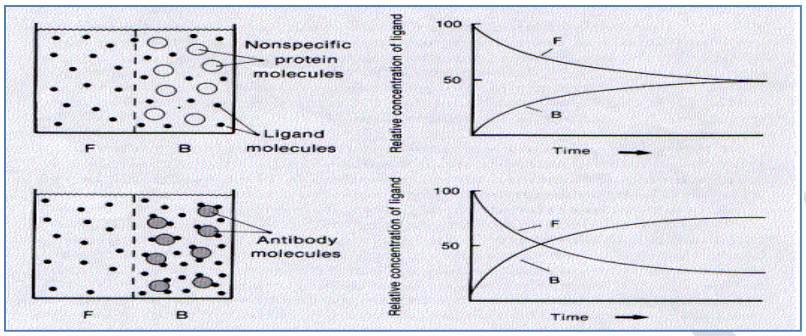
Experimental Methods

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 (Intracellular Fluid) and ECF (Extracellular Fluid.
- 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.
- Interactions between plasma protein and drugs after systemic administration can have profound implications for the therapeutic outcomes.

Analysis of Protein Binding

- Mathematical analysis of protein drug interactions is performed to evaluate the binding activity or association constant (Ka) 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]

One Word Question Answer

SR NO.	QUESTION	ANSWER
1	Binding of drugs into proteins may-Facilitate	distribution of drugs into the body
2	Free fraction or protein binding of drugs	Plasma protein binding
3	Which bond form between donor and acceptor functional group in amino acid sequence?	H-Bond
4	Antibody- antigen recognition are which type of interaction?	Antigen –Antibody recognisation
5	Human plasma is composed of no of protein?	200
6	Which type of protein bind basic drugs?	alpha-1 acid glycoprotein & Albumin

Noncovalent bond	chemical bonding that does not involve the sharing of pairs of electrons; typically bond between macromolecules Van der Waals Forces Dipole- Dipole Dipole-Induced Dipole Induced Dipole Ion Dipole interaction, Hydrogen Bonds
Covalent bond	chemical bonding that is characterized by the sharing of pairs of electrons between atoms
Metal complexes	(covalent bonds)
Organic molecular complexes	(held together by weak forces of the donor-acceptor type or by hydrogen bonds
Inclusion compounds	(result more from the architecture of molecules rather than from their chemical affinity, one chemical is trapped in another open cage-like molecule)
A metal complex	consists of a central metal atom or ion that is bonded to one or more ligands This type of interaction leads to the formation of coordination bonds between the species.
Classification of metal complex	Inorganic types Chelates Olefin type
Chelates	ligand provides only one site for binding with metal.
Olefin types	used as catalysts in the manufacture of bulk drugs, intermediates and in the analysis of drugs
EDTA	ethylenediaminetetraacetic acid has six points for attachment to the metal ion Colorless, water-soluble solid Widely used to “sequester” metal ions, such as Ca+2 and Fe+3

	used to sequester and remove calcium ions from hard water used in biomedical research (to improve the stability of reagents in solutions) adding EDTA to drug formulations can prevent many types of chemical oxidation

Chelation therapy	a treatment that involves oral or intravenous administration of EDTA to remove heavy metals (lead, mercury, etc.) or other harmful toxins from the body EDTA forms complexes with toxins → immune system recognizes the complex as a "foreign body" → the toxins will be destroyed and excreted in the urine (within 1-3 hours) EDTA chelation therapy is approved by the FDA for the treatment of lead and heavy metal poisoning (500,000 - 1,000,000 people in the US are treated with EDTA chelation therapy every year
Problems Associated with Chelation Therapy	EDTA may form complexes with important elements (e.g., proteins, calcium, vitamins, minerals) in the body It is possible that these elements may also be removed from the body, leading to health problems EDTA chelation therapy is often administered together with essential nutrients.
Inclusion Complex	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
Classifications of Inclusion complex	Channel types Layer types Clathrates Monomoleculartypes
Cyclodextrin	Monomolecular inclusion compounds involve the entrapment of a single guest molecule in the cavity of one host molecule (can form water-soluble inclusion complexes with small molecules or portions of large molecules) Cyclic oligomer of glucose containing 6-

	glucopyranoside units Interior is relatively hydrophobic (CH2 groups) Cavity entrances are hydrophilic (hydroxyl groups

Cyclodextrins (CDs)	No formation of covalent bonds Biocompatible, low toxicity, low immunogenicity Simple production from starch by means of enzymatic conversion The inner cavities are able to interact with hydrophobic molecules . (increase solubility Dynamic equilibrium
Applications of Cyclodextrins "Food application	Remove cholesterol Weight loss food supplement Dryer sheet (complexed fragrances are released during heating) Environmental protection (remove toxic compounds)
Pharmaceutical sciences	increase drug solubility Increase drug stability Mask undesirable taste of drugs in solutions Controlled drug delivery (colon-specific drug delivery) Peptide/protein/gene delivery Used in combination with liposomes, microspheres, or nanoparticles
β-Cyclodextrin (β-CD)	Limitations in parenteral formulations (low aqueous solubility and nephrotoxicity) Formation of the internal hydrogen bonds accounts for low solubility Hydroxypropyl-β-CD improved solubility (2% - >60%, w/w)
Reversible: Noncovalent interaction (weak chemical bonds)	Reversible Noncovalent interaction (weak chemical bonds) Hydrogen bonds Electrostatic attractions Van der Waals interactions Hydrophobic forces

Types of Drug-Protein Binding	Reversible Covalent interaction Irreversible Covalent interaction
Irreversible: ***Covalent interaction***	Accounts for certain toxicities of drugs and carcinogens E.g. high dose of acetaminophen causes hepatotoxicity
Protein Binding Affects	Distribution, Tissue penetration, Clearance, Interaction
Effect of Protein Binding	The pharmacokinetic/pharmacodynamic (PK/PD) profiles of a drug are largely affected by the reversible binding to plasma proteins The binding of small molecules to plasma proteins is a very important parameter in drug metabolism and pharmacokinetic studies. Generally, only free drugs are available for diffusion and transport across cell membranes to its target sites. Protein binding inactivates the drug, because sufficient concentration of drug cannot be built up at the receptor site for action. High degree of protein binding in the blood circulation prevents urinary excretion Drug/protein complexes may act as a "storage depot" in the blood circulation due to its prolonged circulation time Determining the level of protein binding is critical and directly correlates with the in vivo efficacy of a drug
Factors Affecting Drug-Protein Binding	Pathophysiological conditions of the patients Hepatic failure and renal failure present a decrease in albumin Inflammatory conditions and Trauma cause an increase in AAG

Albumin Binding Sites	Albumin has a large number of binding site as compare to other protein and is a high capacity binding component Several drugs are capable of binding to more than one binding site
Albumin Binding Sites (Several drugs are capable of binding to more than one binding site.)	e.g.- flucoxacillin , flurbiprofen , ketoprofen , tamoxifen and dicoumarol bind to both primary and secondary site of albumin Indomethacin binds to three different sites
(Albumin Binding Sites) AAG is a protein with limited binding capacity because of it low -conc. and molecular size	The AAG has only one binding site for lidocaine
Albumin Binding Sites	Warfarin Binding Site -->Site 1 Diazepam binding Site -----> Site 2 Digitoxin binding Site ---> Site 3 Tamoxifen ----> binding Site
Binding of Selected Drugs to Albumin (& Compete) Site 1	Chlorothiazide Furosemide Indomethacin Naproxen Phenytoin
Problems Associated with High Protein- Binding Drug (Disease may change the protein binding)	Patients with hypoalbuminemia (low albumin levels in the blood) The protein binding of drug is reduced → increase free drug in the blood stream → toxicity ??