PROTEIN BINDING OF DRUGS

For Class- B.Pharmacy 6th Semester

Subject- BIOPHARMACEUTICS AND PHARMACOKINETICS (BP604T)

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INTRODUCTION

- The interacting molecules are generally the macromolecules such as protein, DNA or adipose. The protein are particularly responsible for such an interaction.
- The phenomenon of complex formation of drug with protein is called *as **protein binding of drug***
- As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to its pharmacokinetic and Pharmacodynamic inertness.

  - **Protein + drug ⇌Protein-drug complex**
  - Protein binding may be divided into:
  - 1. Intracellular binding.
  - 2. Extracellular binding.

MECHANISMS OF PROTEIN DRUG BINDING

- Binding of drugs to proteins is generally of reversible &irreversible.
- Reversible generally involves weak chemical bond such as:
  1. Hydrogen bonds
  2. Hydrophobic bonds
  3. Ionic bonds
  4. Van der waal's forces.
- Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug.

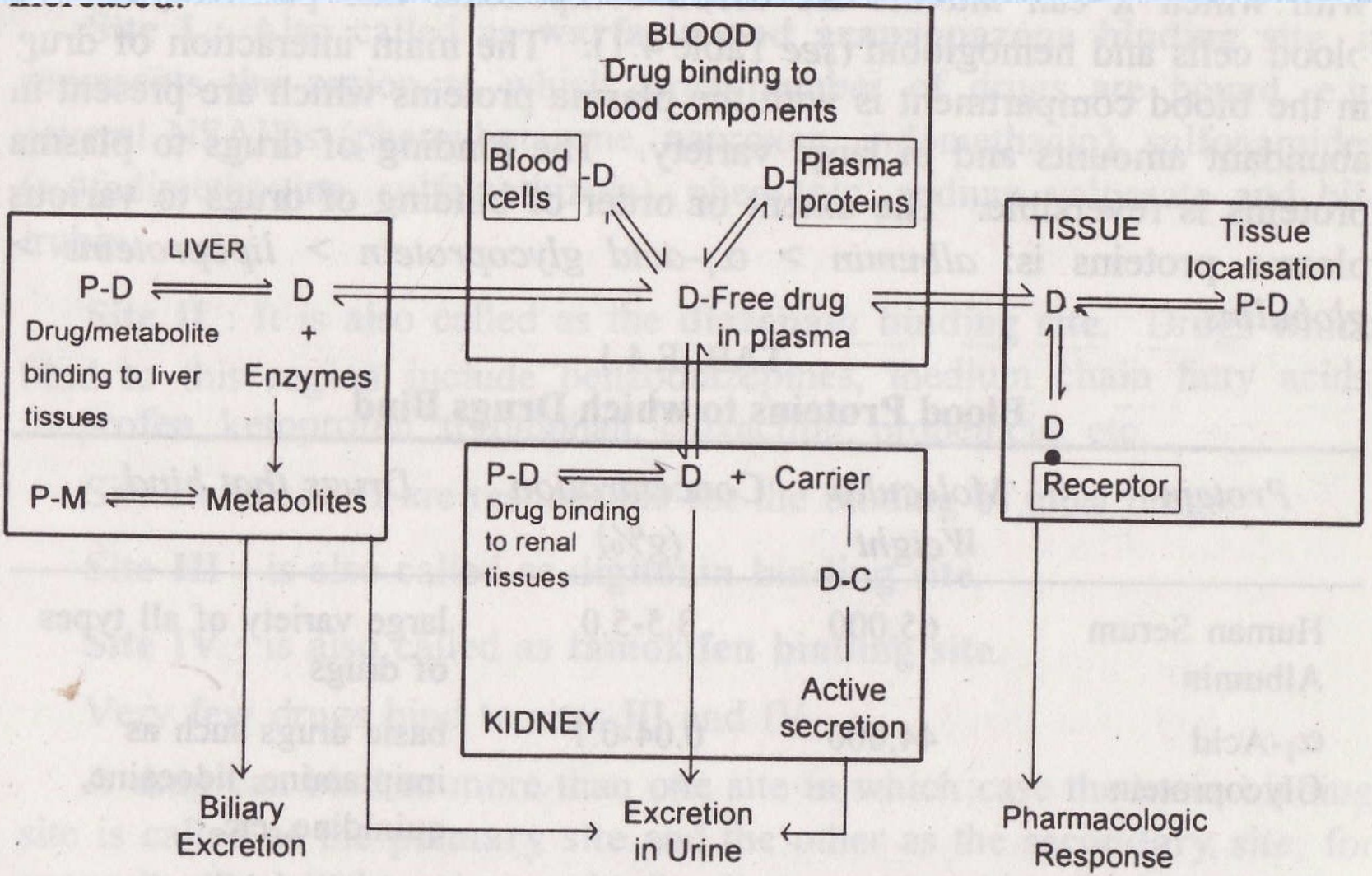


Fig. 4.1 Protein-drug binding: Binding of drugs to various tissue components and its influence on disposition and clinical response. Note that only the unbound drug moves reversibly between the compartments.

1. BINDING OF DRUG TO BLOOD COMPONENTS

A. Plasma protein-drug binding:-

- The binding of drugs to plasma proteins is reversible.
- The extent or order of binding of drug to plasma proteins is:

  Albumin ›à1-Acid glycoprotein ›Lipoproteins ›Globulins.

1. Binding of drug to human serum Albumin.

- It is the most abundant plasma protein (59%), having M.W. of 65,000 with large drug binding capacity.
- Both endogenous compounds such as fatty acid, bilirubin as well as drug binds to HSA.
- Four diff. sites on HSA for drug binding.

  Site I: warfarin & azapropazone binding site.

  Site II: diazepam binding site.

  Site III: digitoxin binding site.

  Site IV: tamoxifen binding site.

2. **Binding of drug to α1-Acid glycoprotein:** (orosomucoid)

It has a M.W. 44,000 and plasma conc. range of 0.04 to 0.1 g%. It binds to no. of basic drugs like imipramine, lidocaine, propranolol, quinidine.

3. **Binding of drug to Lipoproteins:**

Binding by: Hydrophobic Bonds, Non-competative.

Mol wt: 2-34 Lacks dalton.

Lipid core composed of:

Inside: triglyceride & cholesteryl esters.

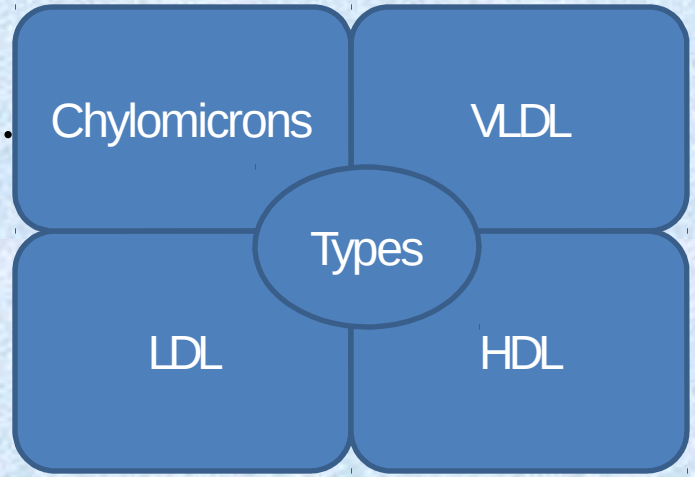
Outside: Apoprotein.

e.g.

Acidic: Diclofenac.

Neutral: Cyclosporin A.

Basic: Chlorpromazine.



4. **Binding of drug to Globulins**

Globulin	Synonym	Binds to
1. α1 Globulin	Transcortine /Corticosteroid globulin Binding	Steroidal drugs, Thyroxin & Cyanocobalamine.
2. α2 Globulin	Ceruloplasmine	Vitamin A,D,E,K.
3. β1Globulin	Transferin	Ferrous ions
4. β2Globulin	---	Carotinoids
5. γ Globulin	---	Antigens

B. BINDING OF DRUG TO BLOOD CELLS

- In blood 40% of blood cells of which major component is RBC (95%). The RBC is 500 times in diameter as the albumin. The rate & extent of entry into RBC is more for lipophilic drugs.
- The RBC comprises of 3 components.

a) **Haemoglobin:** It has a M.W. of 64,500 Dal. Drugs like phenytoin, pentobarbital bind to haemoglobin.

b) **Carbonic anhydrase:** Carbonic anhydrase inhibitors drugs are bind to it like acetazolamide & chlorthalidone.

c) **Cell membrane:** Imipramine & chlorpromazine are reported to bind with the RBC membrane.

2. BINDING OF DRUG TO EXTRAVASCULAR TISSUE PROTEIN

- Importance: 1. It increases apparent volume of distribution of drug.

  2. localization of a drug at a specific site in body.

- Factor affecting: lipophilicity, structural feature of drug, perfusion rate, pH differences.

- Binding order: ***Liver › Kidney › Lung › Muscles***

Tissue	Binding of
1.Liver	Irreversible binding of Epoxides of Halogenated Hydrocarbon & Paracetamol.
2.Lungs	Basic drugs: Imipramine, Chlorpromazine, & AntiHistaminics.

Cont

Tissue	Binding of
3.Kidney	Metallothionin protein binds to Heavy metals & results in Renal accumulation and toxicity.
4.Skin	Chloroquine & Phenothiazine binds to Melanin.
5.Eye	Chloroquine & Phenothiazine also binds to Eye Melanin & results in Retinopathy.
6.Hairs	Arsenicals, Chloroquine, & Phenothiazine.
7.Bones	Tetracycline(yellow discoloration of teeth), Lead(replaces Ca & cause brittleness)
8.Fats	Lipophilic drugs (thiopental), Pesticides (DDT)
9.Nucleic Acid	Chloroquine & Quinacrine.

FACTORS AFFECTING PROTEIN DRUG BINDING

1. Drug-related factors

a. Physicochemical characteristics of the drug:-

- Protein binding is directly related to the lopophilicity of drug. An increase in lipophilicity increases the extent of binding.

b. Concentration of drug in the body:-

- Alteration in the concentration of drug substance as well as the protein molecules or surfaces subsequently brings alteration in the protein binding process.

c. Affinity of a drug for a particular binding component:-

- This factor entirely depends upon the degree of attraction or affinity the protein molecule or tissues have towards drug moieties.

- For Digoxin has more affinity for cardiac muscles proteins as compared to that of proteins of skeletal muscles or those in the plasma like HSA.

2. Protein/ tissue related factors:

a. Physicochemical characteristics of protein or binding agent:

- Lipoproteins & adipose tissue tend to bind lipophilic drug by dissolving them in their lipid core.
- The physiological pH determines the presence of active anionic & cationic groups on the albumin to bind a variety of drug.

b. Concentration of protein or binding component:

- Among the plasma protein , binding predominantly occurs with albumin, as it is present in high concentration in comparision to other plasma protein.
- The amount of several proteins and tissue components available for binding, changes during disease state.

3. Drug interactions

a. Competition between drugs for the binding sites[Displacement interactions]:-

D1: Displaced drug. D2: Displacer drug.

e.g. Administration of phenylbutazone to a patient on Warfarin therapy results in Hemorrhagic reaction.

b. Competition between drug & normal body constituents:-

The free fatty acids are known to interact with a no. of drugs that binds primarily to HSA. the free fatty acid level increase in physiological, pathological condition.

c. Allosteric changes in protein molecule:-

- The process involves alteration of the protein structure by the drug or it's metabolite thereby modifying its binding capacity.
- e.g. aspirin acetylates lysine fraction of albumin thereby modifying its capacity to bind NSAIDs like phenylbutazone.

4. Patient-related factors

a. Age:

1. Neonates: Low albumin content: More free drug.
2. Young infants: High dose of Digoxin due to large renal clearance.
3. Elderly:Low albumin: So more free drug.

b. Intersubject variability: Due to genetics & environmental factors.

c. Disease states:-

Disease	Influence on plasma protein	Influence on protein drug binding
Renal failure	↓ Albumin content	↓ binding of acidic drugs; neutral and basic drugs are un affected
Hepatic failure	↓ Albumin synthesis	↓ binding of acidic drugs; and binding of basic drugs is normal or ↓ depending on AAG levels
Inflamatory states i.e,truama surgery etc…	↑AAG levels	↑ binding of basic drugs; neutral and acidic drugs are un affected

KINETICS OF PROTEIN-BINDING

If "P" represents protein and "D" the drug then applying law of mass action to reversible protein-binding binding

$$P + D \rightleftharpoons PD$$

At equilibrium,

$$Ka = \frac{[PD]}{[P][D]}$$

$$[PD] = Ka\,[P]\,[D]$$

Where, [P] – concentration of free protein

[D] – concentration of free drug

[PD] – concentration of free - drug complex

Ka – association rate constant

If "P_T" is the total concentration of protein present, unbound and bound, then:

$$P_T = [PD] + [P]$$

If "**r**" is the number of moles of drug bound to total moles of protein, then,

$$r = \frac{[PD]}{P_T}$$

$$= \frac{[PD]}{[PD] + [P]}$$

$$r = \frac{Ka\,[P]\,[D]}{Ka\,[P]\,[D] + [P]} = \frac{Ka\,[D]}{Ka\,[D] + 1}$$

The above equation holds when there is only one binding site on the protein and the protein – drug complex is a 1:1 complex

If more than one or N number of binding sites are available per molecule of protein then :

$$r = \frac{N\,Ka\,[D]}{Ka\,[D] + 1}$$

The value of association constant, Ka and the number of binding sites N can be obtained by plotting the above equation in four different ways

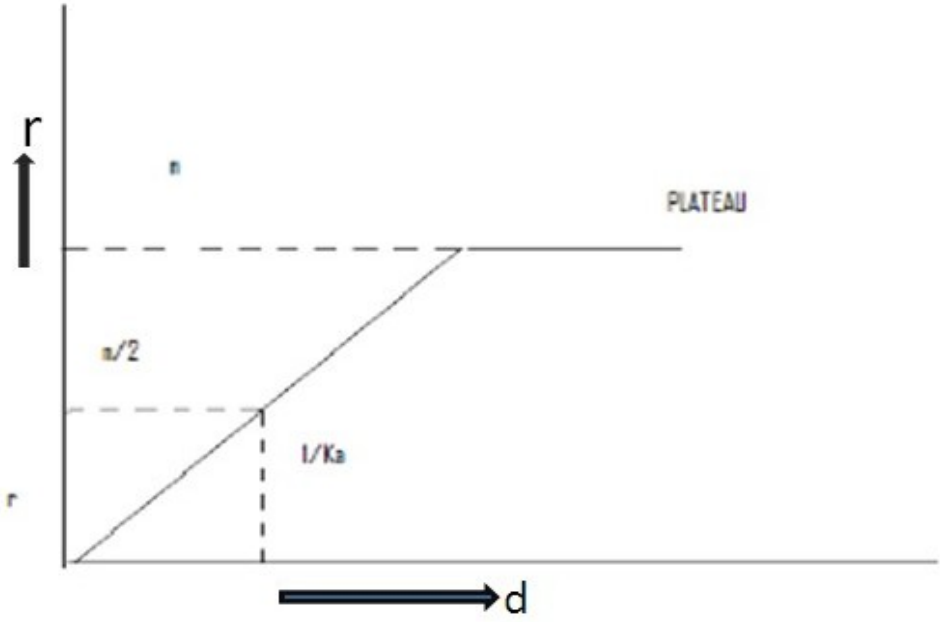
1. Direct plot
2. Scatchard plot
3. Klotz plot
4. Hitchcock plot

PLOTS OF DRUG DISTRIBUTION

1) DIRECT PLOT METHOD:

A direct plot of "r" Vs [D] can be used to find out the no of binding sites on protein 'n' (plateau value).

Ka is obtained by finding drug conc required to saturate the half of the total binding sites available (i.e; n/2).



2) SCATCHARD PLOT:

Obtained by rearranging an equation into linear form.

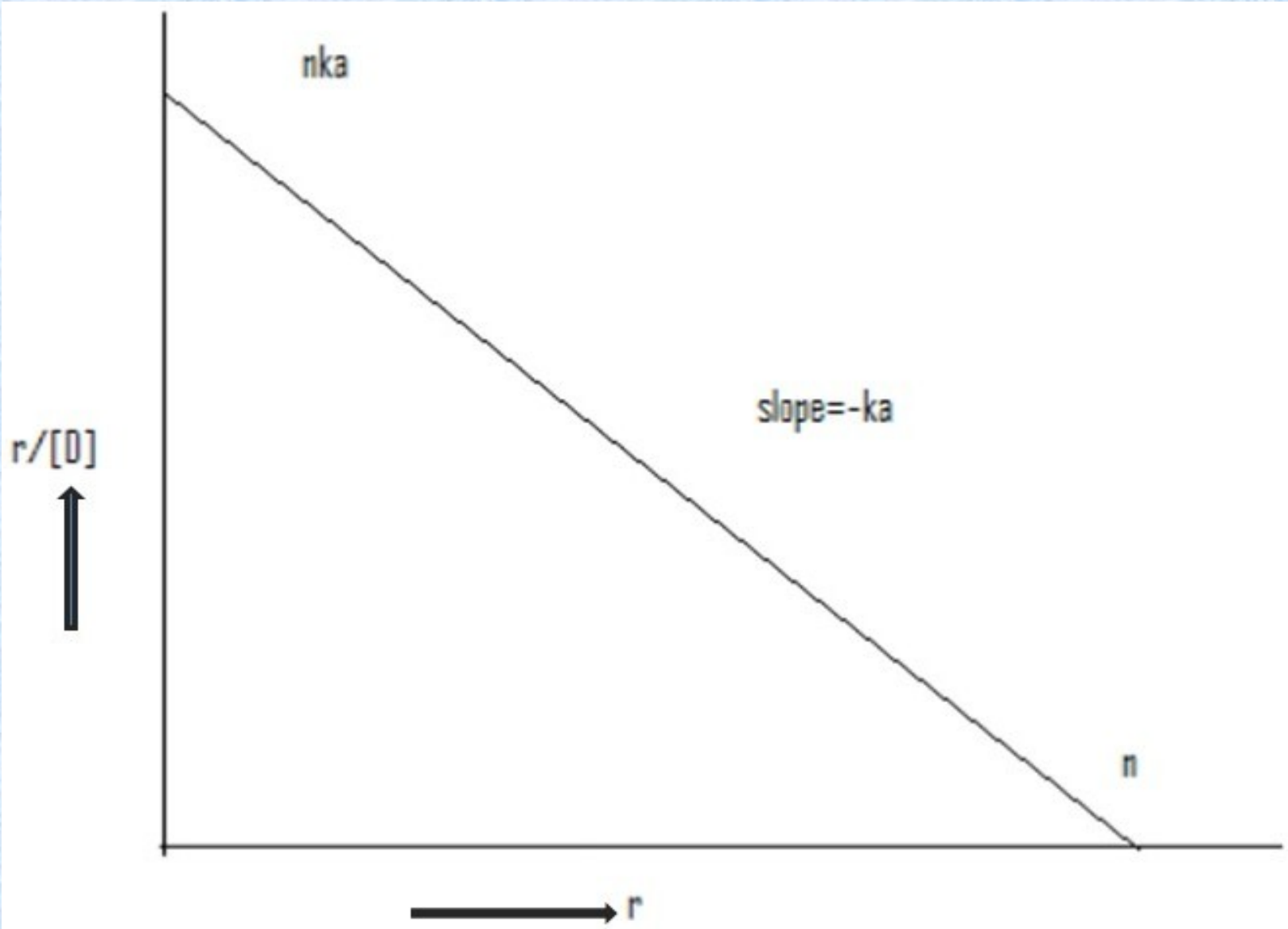
$$r = \frac{nK_a[D]}{K_a[D] + 1}$$

$$r + rK_a[D] = nK_a[D]$$

$$r = nK_a[D] - rK_a[D]$$

$$\frac{r}{[D]} = nK_a - rK_a$$

A plot of r/[D] Vs r yields a st.line with X & Y intercepts equal to 'n' & 'nKa' & the slope is equal to Ka.



Scatchard plot

3) DOUBLE RECIPROCAL PLOT OR KLOTZ PLOT:

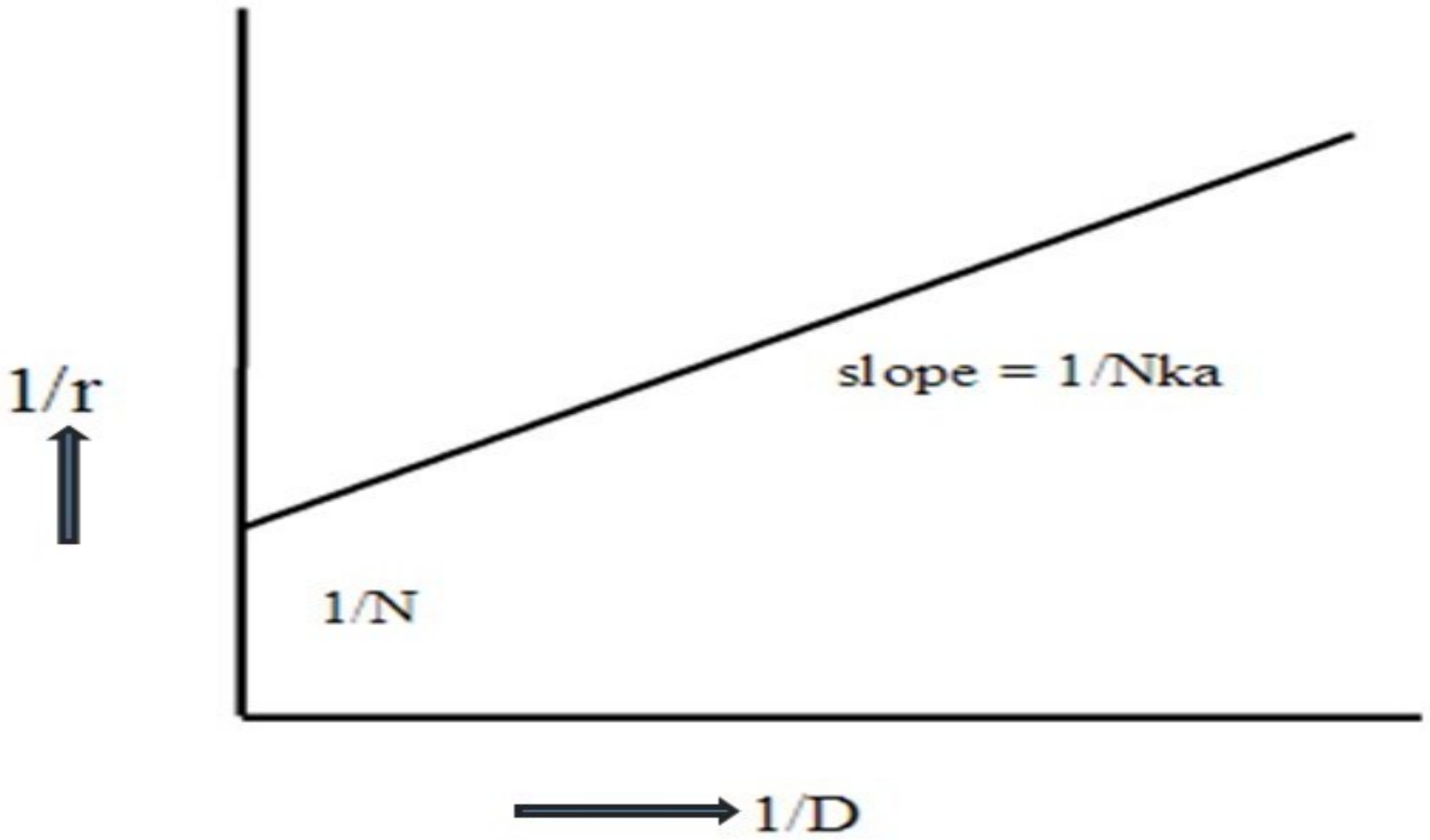
(*LINE WEAVER -BURK PLOT*)

Reciprocal of equation gives-

$$1/r = 1/nka(D) + 1/n$$

A plot of 1/r Vs 1/D yields a double reciprocal plot.

It is straight line with slope 1/Nka and Y-intercept 1/N



Klotz plot

4) HITCHCOCK PLOT

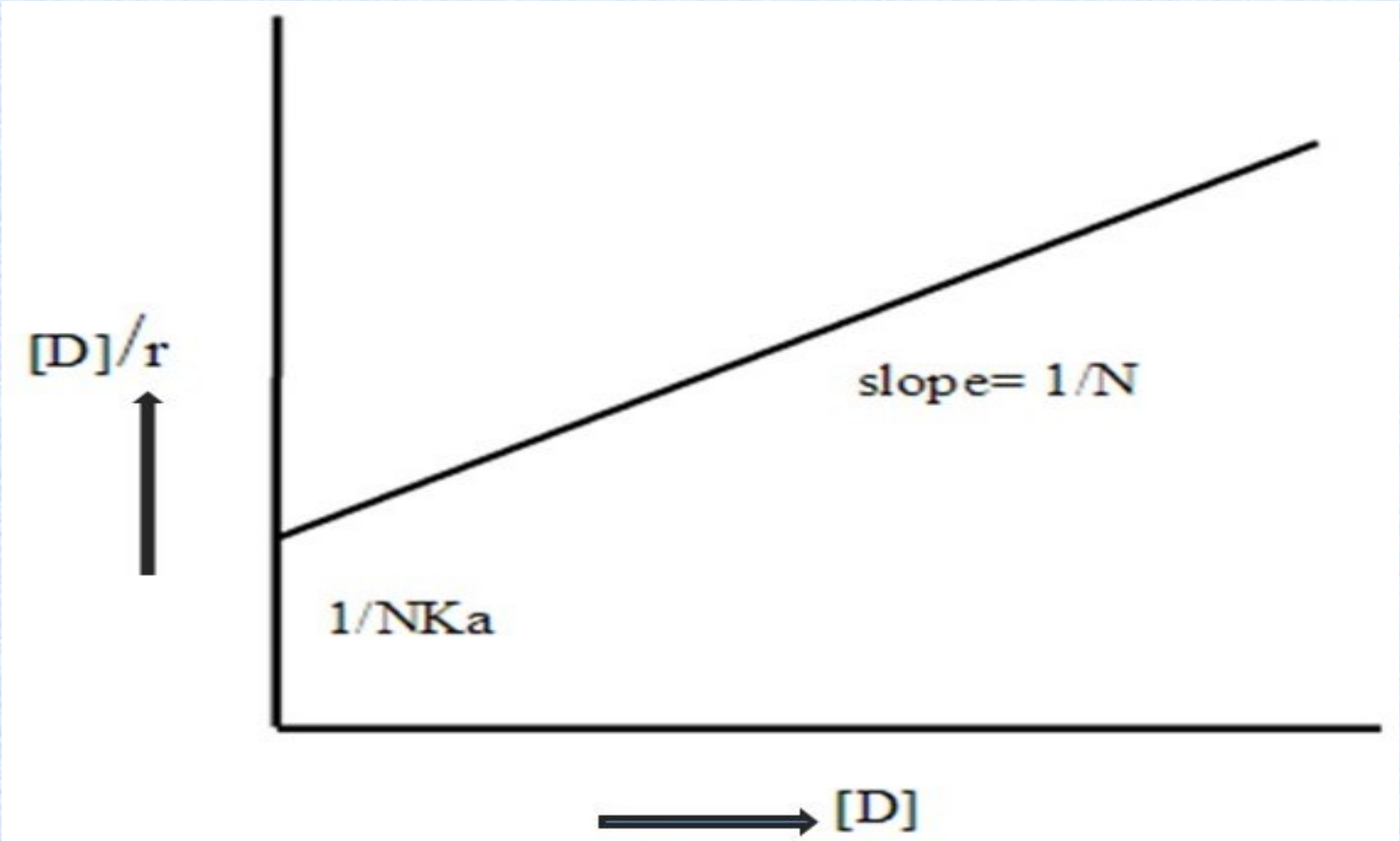
It is made by rearranging the equation as –

$$N\,Ka\,[D]/r = 1 + Ka$$

dividing both sides by Nka gives –

$$[D]/r = 1/NKa + [D]/N$$

A plot of $[D]/r$ Vs [D] yields a straight line with slope 1/N and intercept 1/NKa



Hitchcock plot

SIGNIFICANCE OF PROTEIN/TISSUE BINDING OF DRUG

a. **Absorption-**

- As we know the conventional dosage form follow first order kinetics. So when there is more protein binding then it disturbs the absorption equilibrium.

b. **Distribution-**

- A protein bound drug in particular does not cross the BBB, the placental barrier, the glomerulus.
- Thus protein binding decreases the distribution of drugs.

c. **Metabolism-**

- Protein binding decreases the metabolism of drugs & enhances the biological half life.
- Only unbound fraction get metabolized.
- e.g. Phenylbutazone & Sulfonamide.

d. Elimination

- Only the unbound drug is capable of being eliminated.
- Protein binding prevent the entry of drug to the metabolizing organ (liver) & to glomerulus filtration.
- e.g. Tetracycline is eliminated mainly by glomerular filtration.

e. **Systemic solubility of drug**

- Lipoprotein act as vehicle for hydrophobic drugs like steroids, heparin, oil soluble vit.

f**. Drug action-**

- Protein binding inactivates the drugs because sufficient concentration of drug can not be build up in the receptor site for action.
- e.g. Naphthoquinone

g. **Sustain release-**

- The complex of drug protein in the blood act as a reservoir & continuously supply the free drug.
- e.g. Suramin sodium-protein binding for antitrypanosomal action.

h. **Diagnosis-**

- The chlorine atom of chloroquine replaced with radiolabeled I-131 can be used to visualize-melanomas of eye & disorders of thyroid gland.