SEMESTER VII

BIOPHARMACEUTICS & PHARMACOKINETICS

BP604TT
SYLLABUS

UNIT- 1
Introduction to Biopharmaceutics
Absorption:  Mechanisms of drug absorption through GIT, factors influencing drug absorption though GIT, absorption of drug from Non per oral extra-vascular routes
Distribution:  Tissue permeability of drugs, binding of drugs, apparent, volume of drug distribution, plasma and tissue protein binding of drugs, factors affecting protein-drug binding. Kinetics of protein binding, Clinical significance of protein binding of drugs

UNIT- 2
Elimination:  Drug metabolism and basic understanding metabolic pathways renal excretion of drugs, factors affecting renal excretion of drugs, renal clearance, Non renal routes of drug excretion of drugs
Bioavailability and Bioequivalence:  Definition and Objectives of bioavailability, absolute and relative bioavailability, measurement of bioavailability, in-vitro drug dissolution models, in-vitro-in-vivo correlations, bioequivalence studies, methods to enhance the dissolution rates and bioavailability of poorly soluble drugs.

UNIT- 3
Pharmacokinetics:  Definition and introduction to Pharmacokinetics, Compartment models, Non compartment models, physiological models, One compartment open model. (a). Intravenous Injection (Bolus) (b). Intravenous infusion and (c) Extra vascular administrations. Pharmacokinetics parameters - K$_E$, t1/2, V$_d$, AUC, Ka, Cl, and CL$_R$ definitions methods of eliminations, understanding of their significance and application.

UNIT- 4
Multicompartment Models:
Two compartment open model. IV bolus Kinetics of multiple dosing, steady state drug levels, calculation of loading and maintenance doses and their significance in clinical settins.

UNIT- 5
Nonlinear Pharmacokinetics:
## CONTENTS

<table>
<thead>
<tr>
<th>Sr No.</th>
<th>Topic</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absorption</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>Distribution</td>
<td>36</td>
</tr>
<tr>
<td>3</td>
<td>Protein binding</td>
<td>44</td>
</tr>
<tr>
<td>4</td>
<td>Excretion</td>
<td>56</td>
</tr>
<tr>
<td>5</td>
<td>Bioavailability</td>
<td>64</td>
</tr>
<tr>
<td>6</td>
<td>Bioequivalence</td>
<td>74</td>
</tr>
<tr>
<td>7</td>
<td>Dissolution</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td>Pharmacokinetics</td>
<td>96</td>
</tr>
<tr>
<td>9</td>
<td>Nonlinear Pharmacokinetics</td>
<td>131</td>
</tr>
</tbody>
</table>
**Question bank**

**BIOPHARMACEUTICS & PHARMACOKINETICS (BP604TT)**

**BIOPHARMACEUTICS**

- What are the physiologic barriers to distribution of drugs?
- What are the physiologic properties of drug that affect the absorption of drug?
- Discuss the absorption of drugs from non-oral extravascular routes.
- Enlist various barriers to drug absorption. Describe active transport and passive diffusion mechanism for absorption.
- Discuss the physiological factor influencing drug absorption.
- What is biopharmaceutics? Explain its role in formulation development.
- Explain renal clearance. Explain the factors affecting renal excretion.
- What is Gastric emptying? Explain influence of food on drug absorption.
- Write note on Plasma protein binding (kinetics). Or Describe the influence of protein binding on drug disposition.
- Discuss the significance of protein binding of drugs.
- Describe the importance of volume of distribution in detail.
- Explain the half life and shelf life of a formulation product. How are both determined and expressed?
- Enumerate the factors affecting drug absorption from GIT. Discuss the effect of gastric emptying, drug pKa and GI pH on drug absorption.
- Enlist the various mechanism of transport of drug across biological barriers. Discuss any one mechanism in details.
- Write a note on: Biotransformation
- Differentiate between drug elimination and drug excretion.
- Difference between active transport and passive transport across the gastro intestinal tract.
- Describe the role of dosage form in drug absorption.
How does particle size influence GI absorption of drugs? Explain with suitable example.

Writes the importance of physical forms in drug absorption. Explain with suitable example.

Explain the characteristics of Active transport process. Write their application in the development of formulation and administration of drugs.

Discuss pH –Partition hypothesis briefly with suitable examples. Enumerate the assumptions of this hypothesis.

Explain the role of biopharmaceutics in formulation development.

Discuss the role of active tubular secretion and tubular reabsorption on renal drug excretion.

**BIOAVAILABILITY & BIOEQUIVALENCE**

- Differentiate absolute and relative bioavailability. Discuss various methods used for the measurement of bioavailability.
- How the bioavailability of drug can be improved? Or Explain various methods used for enhancement of bioavailability.
- Define bioequivalence. How bioequivalence study can be performed by Latin Square Cross over Design?
- Discuss the regulatory requirements for conduction of bio-equivalent studies.
- Volunteer selection for bioavailability studies is a critical issue. Discuss the statement with examples.
- Explain various designs used to perform bioequivalence study.
- Briefly describe the interpretation of results of bioequivalence studies.
- Explain AUC. What is its significance? How will you measure it?

**PHARMACOKINETICS**

- What do you mean by pharmacokinetic models? Classify the pharmacokinetic Model and Discuss its importance (advantages and disadvantages of compartment model & physiological model) and application of it...
- Enlist different pharmacokinetic models. What is compartment model? Mention advantages and disadvantages of the same.
- What is apparent volume of distribution? Write an equation to calculate it. State its significance...
Explain the term “renal clearance”. Describe graphical method for determination of renal clearance.

Explain pharmacokinetics. Explain a typical plasma level time curve after single oral dose.

What are pharmacokinetic models? Explain in detail compartment models.....

What do you mean by method of residuals? Describe the method of residuals for determination of absorption rate constant Draw an illustrative diagram for that. (Wagner Nelson method and Loo-Riegelman method)..........

What is non-linear pharmacokinetic? Describe the equation that governs the non-linear Pharmacokinetics (Discuss on Michaeles Menten Equation)....

Discuss one compartment open model - i.v. infusion model and discuss the effect of loading i.v. injection dose. Describe the derivation of various pharmacokinetic parameters for the model.

Define and explain extraction ratio and discuss hepatic clearance in detail...

Enlist different methods for determination of pharmacokinetics parameters from urinary excretion data. What are the merits and demerits of using urinary excretion data for pharmacokinetic parameters? Give the criteria for obtaining valid urinary excretion method and explain any one method in detail...

What process of drug ADME are known to show non linearity Explain giving suitable examples...

Derive equations for first and zero order kinetics.

Write merits of non compartmental analysis. Explain AUC & AUMC plot.

What is extraction ratio? Define clearance, total body clearance and organ clearance.

Discuss one compartment open model, I.V. infusion model and discuss the effect of loading IV injection dose. Describe the derivation of various pharmacokinetic parameters for the model
Glossary or Terminology

- **Biopharmaceutics**: Biopharmaceutics involves the study of the interrelationship of the physicochemical properties of the drug, the dosage form in which the drug is given and the route of administration on the rate and extent of systemic drug absorption.

- **Absorption**: it is the process of movement of unchanged drug from the site of administration to the systemic circulation.

- ** Passive Diffusion**: it comprises the transport of a drug against concentration gradient from areas of low drug concentrations to higher concentrations until the equilibrium.

- **Absolute Solubility or intrinsic solubility**: it is the maximum amount of solute dissolved in the given solvent under standard conditions of temperature, pressure and pH.

- **Endocytosis**: It is the process of capturing a substance or particle from outside the cell by engulfing it with the cell membrane, and bringing it into the cell. Or It includes consuming extracellular materials within a segment of the cell membrane to form a saccule or a vesicle.

- **Phagocytosis** involves engulfment of larger particles or macromolecules.

- **Pinocytosis** involves the engulfment of small molecules or fluid.

- **Gastric emptying time** is the time required for the stomach contents empty into the intestine.

- **Dissolution** is a process in which solid substances solubilize in a given solvent i.e. mass transfer from the solid surface to the liquid phase.

- **Polymorphism**: When a substance exists in more than one crystalline form, the different forms are designated as polymorphs and the phenomenon as polymorphism.

- **Drug distribution**: It refers the reversible movement of the drug to and from the blood and various tissue of the body.

- **Apparent volume of distribution**: It is defined as the hypothetical volume of body fluid into which a drug is dissolved or distributed.

- **Protein binding of drugs**: The phenomenon of complex formation of drugs with proteins.

- **Elimination**: It is the process of removal of a medication from the body.

- **Clearance**: It is defined as the hypothetical volume of body fluids containing drug from which the drug is removed or cleared completely in a specific period of time.

- **Renal Clearance (Cl_R)**: It is the volume of plasma that is completely cleared of a unchanged drug by kidney per unit time.
Renal Clearance ratio: It is measured by comparing the ratio of clearance value of a drug with clearance value of an agent (Creatinine or inulin) that is cleared by glomerular filtration only.

Non renal route of excretion: When the drug is excreted by organs other than kidney or renal route, this is called non renal route of excretion.

Biotransformation or metabolism: It is the process by which a substance changes from one chemical form to another within the body.

First pass metabolism: The loss of drug through biotransformation by eliminating organ during its passage to blood is called first pass metabolism.

Pharmacokinetics is defined as the kinetics of drug absorption, distribution, metabolism and excretion (ADME) and their relationship with the pharmacological, therapeutic or toxicological response in man and animals.

Peak Plasma Concentration (Cmax): The point of maximum concentration of drug in plasma is called as the peak and the concentration of drug at peak is known as peak plasma concentration.

Time of Peak Concentration (tmax): The time for drug to reach peak concentration in plasma (after extravascular administration) is called as the time of peak concentration.

Area under the Curve (AUC): It represents the total integrated area under the plasma level-time profile and expresses the total amount of drug that comes into the systemic circulation after its administration.

Rate: The velocity with which a reaction or a process occurs is called as its rate.

Order of reaction: The manner in which the concentration of drug (or reactants) influences the rate of reaction or process is called as the order of reaction or order of process.

MRT is defined as the average amount of time spent by the drug in the body before being eliminated.

Biological half-life: It is defined as the time taken for the amount of drug in the body as well as plasma concentration to decline by one-half or 50% of its initial value.

Extraction ratio (ER) is an index of how efficiently the eliminating organ clears the blood flowing through it of drug.

Bioavailability is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form.

Absolute bioavailability: When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration, it is called as absolute bioavailability.

Relative bioavailability: When the systemic availability of a drug after oral administration is compared with that of an oral standard of the same drug (such as an aqueous or non-aqueous solution or a suspension), it is referred to as relative or comparative bioavailability.
Bioequivalence: It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and to the same relative extent i.e. their plasma concentration-time profiles will be identical without significant statistical differences.

**SIGNIFICANCE AND APPLICATIONS OF BIOPHARMACEUTICAL STUDIES**

(a) The aim of biopharmaceutics is to adjust the delivery of a drug to the site of action to provide optimal therapeutic activity for the patient. Biopharmaceutics links the physical and chemical properties of a drug and its dosage form to their clinical performance, in vivo. The design, formulation and the manufacturing process of a dosage form require a thorough understanding of the biopharmaceutics principles of drug delivery.

(b) Biopharmaceutical considerations in the design of a dosage form to deliver the active drug with the desired bioavailability characteristics and therapeutic objectives include (1) the physicochemical properties of the drug molecule, (2) the finished dosage form (e.g. tablet, capsule, etc.), (3) the nature of excipients in the drug product, (4) the manufacturing method, and (5) the route of drug administration.

(c) Biopharmaceutical considerations often determine the ultimate dose of a drug in a dosage form. For example, the quantity of a locally acting drug in a topical dosage form such as an ointment is often expressed in concentration or as a percentage of the active drug in the formulation (e.g., 0.5% w/w hydrocortisone ointment). The amount of drug applied is not specified because the concentration of the drug at the active site relates to the pharmacodynamic action. In contrast, the quantity of a systemically active drug in a dosage form such as a tablet is expressed as milligrams. In this case, the dose is based on the amount of drug that is absorbed systemically and dissolved in an apparent volume of distribution to produce a desired drug concentration at the target site.

(d) Each route of drug administration has special biopharmaceutical considerations in dosage form design. For ex., an ophthalmic preparation may require special biopharmaceutical considerations such as appropriate pH, isotonicity, local irritation to the cornea, draining by tears, and concern for systemic drug absorption.

(e) Biopharmaceutical studies must be performed to ensure that the dosage form does not irritate, cause an allergic response or allow systemic drug absorption from a topical dosage form.

(f) Biopharmaceutics has an important role in establishing a link between the in vivo dosage form performance (such as bioavailability, onset of action, safety, and efficacy of the drug to be released from the dosage form) to the dosage form manufacturing process parameters and drug-excipients properties (such as tablet hardness, disintegrants, etc.). Both in vitro (e.g. dissolution) and in vivo methods (bioavailability) are used to evaluate a dosage forms quality and its performance.
Chapter 1

**ABSORPTION**

**Question:** Enlist the various mechanisms of transport of drug across biological barriers. Discuss any one mechanism in details.

Enlist various mechanisms of drug transport. Discuss active transport in detail.

Enumerate the drug transport mechanisms. Discuss passive diffusion in detail.

Describe active transport and passive diffusion mechanism for absorption.

**Mechanism of drug absorption**

The main mechanisms by which absorption occurs include:

(a) Transcellular or intracellular transport

(b) Paracellular or intercellular transport

(c) Vesicular transport or endocytosis

**Transcellular/Intracellular Transport** is defined as the passage of drugs across the GI epithelium. It is the most common pathway for drug transport.

**Paracellular/Intercellular Transport** – is defined as the transport of drugs through the junctions between the GI epithelial cells. This pathway is of minor importance in drug absorption.

**Vesicular** or **Corpuscular Transport (Endocytosis)** – Like active transport, these are also energy dependent processes but involve transport of substances within vesicles into a cell. Since the mechanism involves transport across the cell membrane, the process can also be classified as transcellular.
**Mechanism of drug absorption**

- Passive diffusion
- Pore transport
- Facilitated diffusion
- Active transport
- Ionic diffusion
- Ion pair transport
- Endocytosis

**PASSIVE DIFFUSION**

Drug molecule diffuse from a region of higher concentration to region of lower concentration until equilibrium is attained.

- Major process for absorption of more than 90% of drugs
- Non ionic diffusion
- Driving force – concentration or electrochemical gradient
- Difference in the drug concentration on either side of the membrane
- Drug movement is a result of kinetic energy of molecules

**Fick’s First law of diffusion**

\[
\frac{dQ}{dt} = \frac{DA K_{m/w} (C_{GIT} - C)}{h}
\]

Where, \( \frac{dQ}{dt} \) = rate of drug diffusion (amount/time)

- \( D \) = diffusion coefficient of the drug
- \( A \) = surface area of the absorbing membrane for drug diffusion
- \( K_{m/w} \) = partition coefficient of drug between the lipoidal membrane & the aqueous GI fluids
- \( (C_{GIT} – C) \) = difference in the concentration of drug in the GI fluids & the plasma (Concentration Gradient)
- \( h \) = thickness of the membrane
Characteristics of Passive diffusion:

- Energy independent
- Greater the area & lesser the thickness of the membrane, faster the diffusion
- The process rapid over for short distances
- Concentration equal on both the sides of the membrane - Equilibrium is attained
- Greater the PC of the drug faster the absorption

The passively absorbed drug enters blood, rapidly swept away & distributed into a larger volume of body fluids

Hence, the concentration of drug at absorption site CGIT is maintained greater than the concentration in the plasma. Such a condition is called as sink condition for drug absorption.

PORE TRANSPORT

- It is also called as convective transport, bulk flow or filtration.
- Mechanism – through the protein channel present in the cell membrane.
- Drug permeation through pore transport – renal excretion, removal of drug from CSF & entry of drug into the liver
- The driving force – hydrostatic or osmotic pressure differences across the membrane. Thus, bulk flow of water along with the small solid molecules through aqueous channels. Water flux that promotes such a transport is called as solvent drag
- The process is important in the absorption of low molecular weight (<100D), low molecular size (smaller than the diameter of the pore) & generally water soluble drugs through narrow, aqueous filled channels or pores e.g. urea, water & sugars.
- Chain like or linear compounds (upto 400D)- filtration
IONIC OR ELECTROCHEMICAL DIFFUSION

- Charge on membrane influences the permeation of drugs.
- Molecular forms of solutes are unaffected by the membrane charge & permeate faster than ionic forms.
- The permeation of anions & cations is also influenced by pH.
- Once inside the membrane, the cations are attached to negatively charge intracellular membrane, thus giving rise to an electrical gradient.
- If the same drug is moving from a higher to lower concentration, i.e., moving down the electrical gradient, the phenomenon is known as electrochemical diffusion
- Thus, at a given pH, the rate of permeation may be as follows:
  
  Unionized molecule > anions > Cations

ION-PAIR TRANSPORT

- Responsible for absorption of compounds which ionizes at all pH values. e.g. quaternary ammonium, sulphonic acids
- Ionized moieties forms neutral complexes with endogenous ions which have both the required lipophilicity & aqueous solubility for passive diffusion.
- E.g. Propranolol, a basic drug that forms an ion pair with oleic acid & is absorbed by this mechanism
CARRIER MEDIATED TRANSPORT

- Involves a carrier which reversibly binds to the solute molecules and forms a solute-carrier complex.
- This molecule transverse across the membrane to the other side and dissociates, yielding the solute molecule.
- The carrier then returns to the original site to accept a new molecule.
- There are two type of carrier mediated transport system
  1) Facilitated diffusion
  2) Active transport

FACILITATED DIFFUSION

- Facilitated diffusion is a form of carrier transport that does not require the expenditure of cellular energy.
- Carriers are numerous in number & are found dissolved in cell membrane.
- The driving force is concentration gradient, particles move from a region of high concentration to low conc.
- The transport is aided by integral membrane proteins.
- Facilitated diffusion mediates the absorption of some simple sugars, steroids, amino acids and pyrimidines from the small intestine and their subsequent transfer across cell membranes.
ACTIVE TRANSPORT

- It is characterized by the transport of drug against concentration gradient with using energy. Requires energy, which is provided by hydrolysis of ATP for transportation.
- More commonly, metabolic energy is provided by the active transport of Na+, or is dependent on the electrochemical gradient produced by the sodium pump, Na+/K+ ATPase (secondary active transport).

Primary Active Transport

- Direct ATP requirement
- The process transfers only one ion or molecule & only in one direction. Hence, called as UNIPORT
- E.g. absorption of glucose
- ABC (ATP binding Cassette) transporters

Secondary Active Transport

- No direct requirement of ATP
- The energy required in transporting an ion aids transport of another ion or molecule (co-transport or coupled transport) either in the same direction or opposite direction.

2 types:
- Symport (co-transport)
- Antiport (counter transport)
ENDOCYTOSIS
It is a process in which cell absorbs molecules by engulfing them.

- Also termed as vesicular transport
- Minor transport mechanism involving engulfing extracellular materials within segment of cell membrane to form a saccule or vesicle then pinched of intracellularly.
- It occurs by 3 mechanisms:
  - Phagocytosis
  - Pinocytosis
  - Transcytosis

**Phagocytosis** (*cell eating*): adsorptive uptake of solid particulates
PINOCYTOSIS

- It is a form of endocytosis in which small particles are brought to the cell, forming an invagination.
- These small particles are suspended in small vesicles.
- It requires energy in the form of ATP.
- It works as phagocytosis, the only difference being, it is non specific in the substances it transports.
- This process is important in the absorption of oil soluble vitamins & in the uptake of nutrients

TRANSCYTOSIS

- It is the process through which various macromolecules are transferred across the cell membrane.
- They are captured in vesicles, on one side of the cell and the endocytic vesicle is transferred from one extracellular compartment to another.
- Generally used for the transfer of IgA and insulin
ABSORPTION FACTORS

FACTORS INFLUENCING DRUG ABSORPTION

1. Physicochemical factors:
   a. Drug solubility and dissolution rate
   b. Particle size and effective surface area
   c. Polymorphism and amorphism
   d. Pseudopolymorphism (hydrates or solvates)
   e. Salt form of the drug
   f. Lipophilicity of the drug
   g. Drug stability
   h. Stereochmical nature of the drug

2. Pharmaceutical or Formulation or Dosage form related factors:
   a. Disintegration time
   b. Manufacturing variables
   c. Nature and type of dosage form
   d. Pharmaceutical ingredients (excipients)
   e. Product age and storage conditions

3. Patient related or physiological or biological factors:
   a. Age
   b. Gastric emptying time
   c. Intestinal transit time
   d. Gastrointestinal pH
   e. Diseased states
   f. Blood flow through the GIT
   g. Gastrointestinal contents
   h. Presystemic metabolism
A. Physicochemical properties of drug substances

1. Drug solubility and dissolution rate:
   - The rate determining steps in absorption of orally administered drugs are:
     - Rate of dissolution
     - Rate of drug permeation through the biomembrane.

   ![Drug dissolution process diagram]

   - Imp prerequisite for the absorption of a drug is that it must be present in aq solution & this depends on drug’s aq solubility & its dissolution rate.

2. Particle size and effective surface area:
   - Smaller the particle size (by micronization) greater is the effective surface area more intimate contact b/w solid surface and aq solvent higher is the dissolution rate increase in absorption efficiency
   - E.g. poorly aqsoluble nonhydrophobic drugs like Griseofulvin, chloramphenicol whose dissolution is rate limited.
   - Particle size reduction has been used to increase the absorption of a large number of poorly soluble drugs, such as bishydroxycoumarin, digoxin, griseofulvin, nitrofurantoin, and tolbutamide.
   - Griseofulvin has extremely low aqueous solubility, and material of normal particle size gave rise to poor and erratic absorption.
   - Microsize particles improve absorption, but it is improved even more when it is formulated in ultramicrosize particles as a monomolecular dispersion in polyethylene glycol.

3. Polymorphism and amorphism:
When sub exist in different crystalline form i.e. in polymorphic form then diff forms are many compounds form crystals with different molecular arrangements, or polymorphs. These polymorphs may have different physical properties, such as dissolution rate and solubility.

   Stable form
   - Lowest energy state - Highest m.pt.
- Least aq solubility
- Dissolution rate limited

Metastable form
- Less stable form - Highest energy state - Lowest m.pt.
- Higher aq solubility
- Better absorption and Bioavailability
- E.g The vitamin riboflavin exists in several polymorphic forms, and these have a 20-fold range in aqueous solubility.
- Polymorphs that have no crystal structure, or amorphic forms, have different physical properties from the crystalline forms.
- Absorption of many orally administered drugs is controlled by dissolution rate.
- Amorphous forms generally dissolve faster than crystalline forms because no energy is needed to break up the crystal lattice. For this reason, the amorphous form is often preferred over the crystalline form and several drugs, including hydrocortisone and prednisolone, are marketed in the amorphic form. E.g. novobiocin

Amorphous form
- More soluble
- Rapidly dissolving
- Readily absorbed

Crystalline form
- Less soluble
- Slower dissolving
- Not absorbed to significant extent

4. Solvates/hydrates:
- During their preparation, drug crystals may incorporate one or more solvent molecules to form solvates.
- The most common solvate is water. If water molecules are already present in a crystal structure, the tendency of the crystal to attract additional water to initiate the dissolution process is reduced, and solvated (hydrated) crystals tend to dissolve more slowly than anhydrous forms.
- Significant differences have been reported in the dissolution rate of hydrated and anhydrous forms of ampicillin, caffeine, theophylline, glutethimide, and mercaptopurine.
- The clinical significance of these differences has not been examined but is likely to be slight.
5. Salt form of drug:

- At given pH, the solubility of drug, whether acidic/basic or its salt, is a constant.
- While considering the salt form of drug, pH of the diffusion layer is imp not the pH of the bulk of the solution.
- E.g. of salt of weak acid. ---Which increases the pH of the diffusion layer, which promotes the solubility and dissolution of a weak acid and absorption is bound to be rapid.

- Reverse in the case of salts of weak bases, it lowers the pH of diffusion layer and the promoted the absorption of basic drugs.
- Other approach to enhance the dissolution and absorption rate of certain drugs is by formation of in-situ salt formation i.e. increasing in pH of microenvironment of drug by incorporating buffer agent e.g. aspirin, penicillin
- But sometimes more soluble salt form of drug may result in poor absorption e.g. sodium salt of phenobarbitone and phenobarbitone, tablet of salt of phenobarbitone swelled, it did not get disintegrate thus dissolved slowly and results in poor absorption.

6. Ionization state:

- Unionized state is imp for passive diffusion through membrane so imp for absorption.
- Ionized state is imp for solubility.
7. **Drug pKa & lipophilicity & GI pH --- pH partition hypothesis:**
   - pH – partition theory states that for drug compounds of molecular weight more than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by
   - pKa of drug
   - The lipid solubility of the unionized drug
   - pH at the absorption site.
   - pKa of drug: Amount of drug that exist in unionized form and in ionized form is a function of pKa of drug & pH of the fluid at the absorption site and it can be determined by Henderson-hesselbach equation:

\[
\text{pH} = \text{pKa} + \log \left( \frac{\text{ionized form}}{\text{Unionized form}} \right) \\
\text{For, Acidic drugs}
\]

\[
\text{pH} = \text{pKa} + \log \left( \frac{\text{unionized form}}{\text{Ionized form}} \right) \\
\text{For, Basic drugs}
\]

**Lipophilicity and drug absorption:**
- Ideally for optimum absorption, a drug should have sufficient aq solubility to dissolve in fluids at absorption site and lipid solubility (Ko/w) high enough to facilitate the partitioning of the rug in the lipoidal biomembrane i.e. drug should have perfect HLB for optimum Bioavailability.
- And \( \text{Ko/w} = \text{Distribution of drug in organic phase (octanol)} / \text{Distribution of drug in aq phase} \)
- As Ko/w i.e. lipid solubility i.e. partition coefficient increases percentage drug absorbed increases.
Pharmaceutical Factors

1. Disintegration time:
   - Rapid disintegration is important to have a rapid absorption so lower D.T is required.
   - Now D.T of tablet is directly proportional to:
     - amount of binder
     - Compression force.

And one thing should be remembered that in vitro disintegration test gives no means of a guarantee of drugs B.A. because if the disintegrated drug particles do not dissolve then absorption is not possible.

2. Manufacturing variables:

a). Method of granulation
   - Wet granulation yields a tablet that dissolves faster than those made by other granulating methods. But wet granulation has several limitations like formation of crystal bridge or chemical degradation.
   - Other superior recent method named APOC (agglomerative phase of comminution) that involves grinding of drug till spontaneous agglomeration and granules are prepared with higher surface area. So tablet made up of this granules have higher dissolution rate.

b) Compression force:
   - Higher compression force yields a tablet with greater hardness and reduced wettability & hence have a long D.T. but on other hand higher compression force cause crushing of drug particles into smaller ones with higher effective surface area which in decrease in D.T.
   - So effect of compression force should be thoroughly studied on each formulation.

3. Nature and type of dosage form –

![Diagram showing pharmaceutical factors](image-url)
Drug formulations are designed to provide an attractive, stable, and convenient method to use products. Conventional dosage forms may be broadly characterized in order of decreasing dissolution rate as solutions, solid solutions, suspensions, capsules and tablets, coated capsules and tablets, and controlled release formulations.

A. Solutions

- Aqueous solutions, syrups, elixirs, and emulsions do not present a dissolution problem and generally result in fast and often complete absorption as compared to solid dosage forms. Due to their generally good systemic availability, solutions are frequently used as bioavailability standards against which other dosage forms are compared.

B. Solid solutions

- The solid solution is a formulation in which drug is trapped as a solid solution or monomolecular dispersion in a water-soluble matrix. Although the solid solution is an attractive approach to increase drug absorption, only one drug, griseofulvin, is currently marketed in this form.

C. Suspensions

- A drug in a suspension is in solid form, but is finely divided and has a large surface area. Drug particles can diffuse readily between the stomach and small intestine so that absorption is relatively insensitive to stomach emptying rate.

- Adjusting the dose to a patient’s needs is easier with solutions and suspensions than with solid dosage forms. Liquid dosage forms, therefore, have several practical advantages besides simple dissolution rate.

- However, they also have some disadvantages, including greater bulk, difficulty in handling, and perhaps reduced stability.

D. Capsules and tablets

- These formulations differ from each other in that material in capsules is less impacted than in compressed tablets. Once a capsule dissolves, the contents generally disperse quickly. The capsule material,

- Although water soluble, can impede drug dissolution by interacting with the drug, but this is uncommon.

- Tablets generally disintegrate in stages, first into granules and then into primary particles. As particle size decreases, dissolution rate increases due to of increased surface area.

- Tablet disintegration was once considered a sufficient criterion to predict in vivo absorption.
As a general rule, the bio-availability of a drug from various dosage forms decrease in the following order: Solutions > Emulsions > Suspensions > Capsules > Tablets > Coated Tablets > Enteric coated Tablets > Sustained Release Products.

4. Pharmaceutical ingredients/Excipients: -

- More the no. of excepients in dosage form, more complex it is & greater the potential for absorption and Bioavailability problems.
- Changing an excipient from calcium sulfate to lactose and increasing the proportion of magnesium silicate, increases the activity of oral phenytoin.
- Systemic availability of thiamine and riboflavin is reduced by the presence of Fuller’s earth.
- Absorption of tetracycline from capsules is reduced by calcium phosphate due to complexation.
- Most of these types of interactions were reported some time ago and are unlikely to occur in the current environment of rigorous testing of new dosage forms and formulations.

a) Vehicle-

- Rate of absorption – depends on its miscibility with biological fluid.
- Miscible vehicles (aq or water miscible vehicle) –rapid absorption e.g. propylene glycol.
- Immiscible vehicles - absorption –depends on its partitioning from oil phase to aq body fluid.

b) Diluents-

- Hydrophilic diluents-form the hydrophilic coat around hydrophobic drug particles –thus promotes dissolution and absorption of poorly soluble hydrophobic drug.

c) Binders & granulating agent -

- Hydrophilic binders – imparts hydrophilic properties to granule surface – better dissolution of poorly wettable drug. e.g. starch, gelatin, PVP.
- More amount of binder – increases hardness of tablet – decrease dissolution & disintegration rate.

d) Disintegrants -

- Mostly hydrophilic in nature.
- Decrease in amount of disintegrants – significantly lowers B.A.

e) Lubricants -

- Commonly hydrophobic in nature – therefore inhibits penetration of water into tablet and thus dissolution and disintegration.

f) Suspending agents/viscosity agent –

- Stabilized the solid drug particles and thus affect drug absorption.
- Macromolecular gum forms unabsorbable complex with drug e.g. Na CMC.
Viscosity impartets – act as a mechanical barrier to diffusion of drug from its dosage form and retard GI transit of drug.

g) Surfactants –
- May enhance or retards drug absorption by interacting with drug or membrane or both.
- Surfactants have been considered as absorption enhancers, again mostly in animals. Polyoxyethylene ethers have been shown to enhance gastric or rectal absorption of lincomycin, penicillin, cephalosporins, and fosfomycin in rats and rabbits.
- However, in humans, oral polyoxyethylene-20-oleyl ether resulted in poor and variable insulin absorption.
- In general, unionic surfactants have little effect on membrane structure but cationic surfactants have been associated with reversible cell loss and loss of goblet cells.
- Physiologic surfactants – bile salts – promotes absorption – e.g. Grisofulvin, steroids.
- It may decrease absorption when it forms the unabsorbable complex with drug above CMC.

h) Bile salts-
- Bile contains conjugates of cholic acid and chenodeoxycholic acid, which emulsify dietary fat, facilitate lipolysis, and transport lipid molecules through the unstirred layer of the intestinal mucosa by micellar solubilization. The ability of bile salts to promote lipid absorption has prompted their investigation as absorption enhancers for drugs, with modest success.
- Absorption of insulin can be increased by bile salts, both in experimental animals and in humans.

i) Colourants
- Even a low concentration of water soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs.
- The dye molecules get absorbed onto the crystal faces and inhibit the drug dissolution. For example: Brilliant blue retards dissolution of sulfathiazole.

5. Product age and storage conditions –
- Product aging and improper storage conditions adversely affect B.A.
**Question:** Discuss the physiological factor influencing drug absorption.

What is gastric emptying? Describe its role in drug absorption.

Enlist factors affecting gastrointestinal absorption. Discuss in detail effect of gastric emptying time on drug absorption.

**PATIENT RELATED FACTORS AFFECTING DRUG ABSORPTION**

**Gastrointestinal tract**

The gastrointestinal tract (GIT) comprises of a number of components, their primary function being secretion, digestion and absorption. The mean length of the entire GIT is 450 cm. The major functional components of the GIT are stomach, small intestine (duodenum, jejunum and ileum) and large intestine (colon) which grossly differ from each other in terms of anatomy, function, secretions and pH.

**Gastric emptying**

Apart from dissolution of a drug and its permeation through the biomembrane, *the passage from stomach to the small intestine, called as gastric emptying*, can also be a rate-limiting step in drug absorption because the major site of drug absorption is intestine. Thus, generally speaking, rapid gastric emptying increases bioavailability of a drug.

Rapid gastric emptying is advisable
- 1. Rapid onset of action is desired – Sedatives
- 2. Dissolution of drug occurs in intestine- Enteric coated tablets
- 3. Drugs not stable in gastric fluid – Penicillin G & erythromycin
- 4. Drugs absorbed from distal part of intestine- Vitamin B 12

Delay in gastric emptying is recommended
- 1. Food promotes drug dissolution & absorption – Griseofulvin
- 2. Disintegration & dissolution of dosage form is promoted by gastric fluids
- 3. Drugs dissolve slowly- Griseofulvin
- 4. Drugs irritate gastric mucosa- Aspirin, phenylbutazone & nitrofurantoin
- 5. Drugs absorbed from proximal part of small intestine – Vitamin B & C
Factors influencing gastric emptying

1. **Volume of meal** – Larger bulk longer gastric emptying time
2. **Composition of meal** – Rate of gastric emptying: Carbohydrates > Proteins > Fats
3. **Physical state & viscosity** – Liquid meals (hour to empty) > solid meals (6 to 7 hrs)
4. **Temperature of meal** - high or low temperature of ingested (in comparison with to body temperature)
   reduce gastric emptying
5. **Gastrointestinal pH** - Less acidic pH of stomach promotes gastric emptying while more acidic pH retards
6. **Electrolytes** – Water isotonic solutions, solutions empty stomach rapidly whereas a higher electrolyte concentration decreases gastric emptying
7. **Body posture** – Gastric emptying favoured while standing and lying on right side and vice versa
8. **Emotional state** – Stress & anxiety promotes while depression retards it
9. **Exercise** - Vigorous physical activity retards
10. **Disease states:** Diseases like gastroenteritis, gastric ulcer, pyloric stenosis, diabetes and hypothyroidism retard gastric emptying. Partial or total gastrectomy, duodenal ulcer and hyperthyroidism promote gastric emptying rate.
11. **Drugs:** Drugs that retard gastric emptying include poorly soluble antacids (aluminium hydroxide), anticholinergics (atropine, propantheline), narcotic analgesics (morphine) and tricyclic antidepressants (imipramine, amitriptyline). Metoclopramide, domperidone and cisapride (prokinetic agents) stimulate gastric emptying.

**Intestinal transit**

- Small intestine is major site for drug absorption: Long intestinal transit time is desired for complete drug absorption.
- Residence time depends upon intestinal motility or contraction.
- Peristaltic contraction promotes drug absorption by increasing the drug intestinal membrane contact, by enhancing drug dissolution.

Delayed intestinal transit is desirable for:

- Drugs that release slowly (sustained release)
- When the ratio of dose to solubility is high. (chlorthiazide)
- Drugs that dissolve only in intestine (enteric coated)
- Drugs which are absorbed from specific site in the intestine (Lithium carbonate, Vitamin B)
- When drug penetrate the intestinal mucosa very slowly (e.g. acyclovir)
- When absorption of drug from colon is minimal.
Gastro intestinal PH:

- A difference in PH is observed between gastric and colon fluids. The GI PH increases gradually from stomach to the colon and rectum.
- The PH of GI fluids influence the drug absorption in several ways:
  i) Disintegration: some dosage forms is Ph sensitive, with enteric coating the coat dissolves only in intestine.
  ii) Dissolution: A large no. of drugs whose solubility is affected by pH are weak acidic and weak basic drugs.
  Weak acidic drugs dissolve rapidly in the alkaline medium whereas
  Weak basic drugs dissolve in acidic medium.
  iii) Absorption: Depending on drug pKa and whether it is acidic or basic, absorption depends on the amount of unionized form at site of absorption.
  iv) Stability: GI pH affects chemical stability of drug. Eg. Acidic pH of stomach degrades Penicillin G and erythromycin. Hence they are administered as prodrugs namely carindacillin and erythromycin estolate.

Blood flow to GIT

GIT is extensively supplied by blood capillary, about 28% of cardiac output is supplied to GIT portion, most drug reach the systemic circulation via blood only.

Any factor which affects blood flow to GIT may also affect absorption.

Disease state

- Several disease states may influence the rate and extent of drug absorption.
- Three major classes of disease may influence bioavailability of drug.

GI diseases

Achlorhydria: Decreased gastric emptying and absorption of acidic drugs like aspirin
Malabsorption syndrome and celiac disease: decreased absorption
Gastrectomy may cause drug dumping in intestine, osmotic diarrhea and reduce intestinal transit time.

CVS disease

In CVS diseases blood flow to GIT decrease, causes decreased drug absorption.

Hepatic disease

Disorders like hepatic cirrhosis influences bioavailability of drugs which under goes first pass metabolism.
  - E.g. propranalol
Gastro intestinal contents

1. **Food- drug interaction:** In general presences of food delay, reduce, increase or may not affect absorption.

2. **Fluid volume:** high vol • better absorption e.g. erythromycin

3. **Interaction of drug with normal GI contents:**
   - Bile salts: increases lipid soluble drugs e.g. gresiofulvin
   - Decreased: neomycin, kanamycin

4. **Drug-Drug interaction in the GIT:**
   - (A) Physico chemical drug- drug interaction:
     - Adsorption: Eg; anti diarrheal preparations contains adsorbents like kaolin, prevents a absorption of many drugs co-administered with them.
     - Complexation: Eg; calcium, aluminium salts decreases tetracycline
     - pH changes: Basic drugs changes gastric pH
     - E.g.; tetracycline with antacids
   - (B) Physiological interaction:
     - Decreased GI transit: Anticholinergics like propanthelin decrease GI transit and increased absorption of ranitidine and digoxin
     - Increase GI emptying: Metoclopramide increases GI motility and increased GI absorption of tetracycline, levodopa etc.
     - Altered GI metabolism: Antibiotics decrease bacterial metabolism of drug e.g. erythromycin increases efficacy of digoxin.

**Presystemic or First pass metabolism**

- The loss of drug as it passes through GIT membrane, liver for the first time during the absorption process.
- The main reason for the decrease in bioavailability of a drug is decreased absorption or first pass metabolism.

Four primary systems which affect pre systemic metabolism of a drugs

1. Luminal enzymes.
2. Gut wall enzymes or mucosal enzymes.
**Luminal enzymes:** These are enzymes present in gut fluids and include enzymes from intestinal and pancreatic secretions. E.g. hydrolases

**Gut wall enzymes:** Also called mucosal enzymes they are present in gut and intestine, colon. E.g. alcohol dehydrogenase

**Bacterial enzymes:** GI microfloras scantily present in stomach and small intestine and are rich in colon. e.g. sulphasalazine • sulphapyridine + 5 ASA

**Hepatic enzyme:** several drug undergo firstpass hepatic metabolism, highly extracted ones being isoprenaline, nitroglycerin, morphine etc.

**AGE:**

- In children & Infants Gastric pH is high and intestinal surface and flow to git is low.
- while in adults altered gastric emptying, decrease intestinal surface area, decrease gastric blood flow & higher incidence of achlorhydria cause impaired drug absorption.
Question: Discuss the absorption of drugs from non-oral extravascular routes.

❖ Absorption of drug from Non-per oral route

- Buccal/Sublingual Administration
- Rectal Administration
- Topical Administration
- Inhalation Administration
- Intramuscular Administration
- Subcutaneous Administration
- Intranasal Administration
- Intraocular Administration
- Vaginal Administration

Buccal/Sublingual Administration

In buccal route the medicament is placed between the cheek and the gum.

In sublingual the drug is placed under the tongue.
- Barrier to drug absorption from these route is epithelium of oral mucosa.
- Absorption of drug is by passive diffusion.

Eg; lozenges, nitrates and nitrites,

Advantages-

- Rapid absorption and higher blood levels
- No first pass metabolism
- No degradation of drugs such as that encountered in the GIT
- Presence of saliva facilitates both drug dissolution and permeation.

Disadvantages:-

- Concern for taste and discomfort
- Limited mucosal surface- small doses are administered.

Rectal Administration

- An important route for children and old patients.
- The drug may be administered as solution or suppositories.
- Irritating suppositories bases such as PEG promotes defecation and drug loss, and presence of fecal matter retards drug absorption.
• By passes the presystemic hepatic metabolism.
• Drug administered by this route includes Aspirin, paracetamol, few barbiturates.

Advantages-
• Alternative route for administration of unpleasant drugs
• Avoids nausea, vomiting
• Can be used in case of unconscious patients
• Bypasses presystemic hepatic metabolism from lower half of rectum.

Disadvantages-
• Absorption is sometimes irregular and incomplete and many drugs cause irritation of rectal mucosa.

Topical Administration
• Skin is the largest organ in the body weighing around 2kg and 2mtsq in area and receives about 1/3rd of total blood circulating through the body.
• Topical mode of administration is called as percutaneous or transdermal delivery.
• The drug act either locally or systemically.
• Drug that administered precutaneously include lidocaine, testosterone, estradiol, etc.

Transdermal route-
• This route of administration achieves systemic effects by application of drugs to the skin, usually via a transdermal medicated adhesive patch.
• The rate of absorption can vary markedly, depending on the physical characteristics of the drug (lipid soluble) and skin at the site of application.
• This route is most often used for the sustained delivery of drugs, such as the antianginal drug nitroglycerin, the antiemetic scopolamine.

INJECTIONS

Intravenous (IV) Injection.
Drug is directly goes into blood stream

Intramuscular (IM) Injection.
Absorption of drugs from I.M. sites is relatively rapid but much slower than I.V. injection.

Subcutaneous (SC) Injection.
Absorption is slower than I.M. site due to poor perfusion
Intraperitoneal (IP) Injection.
I.P.route is rarely employed in human beings but most widely used in laboratory animals

Inhalations Administration
- All drugs intended for systemic effect can be administered by inhalation since the larger surface area of alveoli, higher permeability to the alveolar epithelium & rapid absorption just exchange of gases in blood.
- Route has been limited for drugs such as bronchodilators, anti-inflammatory steroids and antiallergics.
- Drug do not undergo first pass metabolism.
- lipid soluble drugs absorption rapid by passive diffusion and polar drug by pore transport.
- Generally administered by inhalation either as gases or aerosols

Intranasal administration
- Drug absorption by this route is as rapid as parenteral administered because of its high permeability and rich vasculature.
- Popular for administration of peptides and protein drugs.
- Route treat local symptoms like nasal congestion, rhinitis.
- Absorption depends upon drug lipophilicity and molecular weight.
- Rapid absorption by diffusion is observed up to 400 -1000 dalt.

Intraocular Administration
- Mainly for the treatment of local effects such as mydriasis, meiosis, anesthesia and glaucoma.
- The barrier in the ocular membrane is called cornea which contains both hydrophilic and lipophilic characters.
- Thus for optimum intra ocular permeation drug should possess biphasic solubility.
- pH of formulation influences lacrimal output.
- Rate of blinking.
- Volume of fluid.
- The addition of viscosity increasing agents in the ophthalmic solution will increases ocular bio availability.
- Ex: pilocarpine, timmolol, atropine.
Vaginal Administration

- Available in various forms tablets, creams, ointments, douches and suppositories.
- Used for systemic delivery of contraceptive and other steroids.
- By passes first pass metabolism.
- Factors effecting drug absorption are:
  - pH of the lumen fluid 4-5.
  - Vaginal secretions.
  - Microbes at vaginal lumen.
- Bio availability of vaginal product was about 20% more compared with oral.
- Ex: Steroidal drugs and contraceptives.

**SUMMARY OF MECHANISM AND DRUGS ABSORBED FROM VARIOUS NON INVASIVE ROUTES**

<table>
<thead>
<tr>
<th>ROUTES</th>
<th>ABSORPTION MECHANISM</th>
<th>DRUG DELIVERED</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Buccal/Sublingual</td>
<td>Passive diffusion, carrier mediated transport</td>
<td>Nitrites, antianginal, morphine, etc.</td>
</tr>
<tr>
<td>2. Rectal</td>
<td>Passive diffusion</td>
<td>Aspirin, Paracetamol, barbiturates, etc.</td>
</tr>
<tr>
<td>3. Transdermal</td>
<td>Passive diffusion</td>
<td>Nitroglycerine, lidocaine, etc.</td>
</tr>
<tr>
<td>4. Intramuscular</td>
<td>Passive diffusion, endocytosis, pore transport</td>
<td>Phenytoin, digitoxine</td>
</tr>
<tr>
<td>5. Subcutaneous</td>
<td>Passive diffusion</td>
<td>Insuline, heparin, etc.</td>
</tr>
<tr>
<td>6. Inhalation</td>
<td>Passive diffusion, Pore transport</td>
<td>Salbutamol, cromolyn</td>
</tr>
<tr>
<td>7. Intranasal</td>
<td>Passive diffusion, Pore transport</td>
<td>Phenylpropanolamine, antihistaminics</td>
</tr>
<tr>
<td>8. Intraocular</td>
<td>Passive diffusion</td>
<td>Atropine, pilocarpine</td>
</tr>
<tr>
<td>9. Vaginal</td>
<td>Passive diffusion</td>
<td>Steroidal drugs &amp; contraceptives</td>
</tr>
</tbody>
</table>
Chapter 2

DRUG DISTRIBUTION

- **Drug distribution**: refers to the reversible transfer of a drug between the blood and the extra vascular fluids and tissues of the body
- Drugs come into the circulation after absorption. From plasma, drugs have to cross the capillary membrane to come to interstitial space. And then need cross the cell-membrane to enter into the intracellular fluid.

FACTORS AFFECTING DISTRIBUTION OF DRUGS
- **Tissue Permeability of the Drug**
  a. Physiochemical Properties of the drug like Molecular size, pKa and o/w Partition coefficient.
  b. Physiological Barriers to Diffusion of Drugs.
- **Organ / Tissue Size and Perfusion Rate**
- **Binding of Drugs to Tissue Components**
  - binding of drug to blood components
  - binding of drug to extra cellular components
- **Miscellaneous Factors**
  - Age, Pregnancy, Obesity, Diet, Disease states, and Drug Interactions...

❖ **Tissue Permeability of the Drugs depends upon:**
  1. Rate of Tissue Permeability, and
  2. Rate of Blood Perfusion.

The Rate of Tissue Permeability depends upon Physiochemical Properties of the drug as well as Physiological Barriers that restrict the diffusion of drug into tissues.

A. **Physiochemical Properties** that influence drug distribution are:
  i. Molecular size,
  ii. pKa, and
  iii. o/w Partition coefficient.

I) **Molecular Size:**
  - Mol wt less than 500 to 600 Dalton easily pass capillary membrane to extra cellular fluid.
- Penetration of drug from ECF to cells is function of Mol size, ionization constant & lipophilicity of drug.
- From extra cellular fluid to cross cell membrane through aqueous filled channels need particle size less than 50 Dalton (small) with hydrophilic property.
- Large mol size restricted or require specialized transport system.

ii) Degree of Ionization (pKa):
- The pH at which half of a drug is unionized is called pKa.
- Most of the drugs are weak acids or bases & their degree of ionization depends upon pKa.
- The PH of Blood plasma, extra cellular fluid and CSF is 7.4(constant) except in acidosis and alkalosis.
- All the drugs ionize at plasma pH (i.e. Polar, Hydrophilic Drugs) Can not penetrate the Lipoidal cell membrane hence the distribution is limited for these type of drugs.

iii) o/w permeability:
- Polar and hydrophilic drugs are less likely to cross the cell membrane.
- Nonpolar and hydrophobic drugs are more likely to cross the cell membrane.
- Only unionized drugs that are generally lipophilic can cross the cell membrane. Among the drugs having same o/w partition coefficient but differ in extent of drug Ionization, the drug which is less ionized is absorbed or have greater permeability than that of more ionized form.
- Ex- Salicylic acid & phenobarbitone have same o/w Partition coefficient but phenobarbitone is more unionized and hence distributed rapidly.

Fig. Permeation of unionized drugs across the capillary and cell membrane
Question: Discuss physiological barriers for distribution of drugs.

B. PHYSIOLOGICAL BARRIERS

1) Simple capillary endothelial barrier
2) Simple cell membrane barrier
3) Blood brain barrier
4) Blood CSF barrier
5) Blood placental barrier
6) Blood testis barrier

1. The simple capillary endothelial barrier

Capillary supply the blood to the most inner tissue

All drugs ionized or unionized molecular size less than 600 dalton diffuse through the capillary endothelium to interstitial fluid

Only drugs that bound to that blood component can’t pass through this barrier Because of larger size of complex

2. Simple cell membrane barrier

Once the drug diffuse through capillary to extracellular fluid, its further entry in to cells of most tissue is limited

Simple cell Membrane is similar to the lipoidal barrier (absorption)

Non polar & hydrophillic drugs will passes through it (passively).

Lipophilic drugs with 50-600 dalton mol size & Hydrophilic, Polar drugs with <50 dalton will pass this membrane

![Diagram of Plasma membrane barrier and drug diffusion across it](image-url)
3. BLOOD-BRAIN BARRIER (BBB):

- Unlike the capillary found in other parts of body, the brain capillaries made of tight junctions of capillary cells.
- The brain capillaries consist of endothelial cells which are joined to one another by continuous tight intercellular junctions comprising what is called as the **blood-brain barrier**.
- As a result the intercellular passage is blocked and for a drug to enter from capillary it has to pass THROUGH the cells rather BETWEEN them.
- Since BBB is lipoidal barrier, it allows only the drugs having high o/w partition coefficient to diffuse passively.
- 3 different approaches have been utilized successfully to promote crossing the BBB by drugs:
  a. use of **permeation enhancers** (dimethyl sulfoxide)
  b. **osmotic disruption** of the BBB by infusing internal carotid artery with mannitol
  c. using **dihydropyridine redox system** as drug carrier (active transport).

4. Blood-cerebrospinal fluid barrier:

- The cerebrospinal fluid (c.s.f) is formed mainly by the choroid plexus of the LATERAL, THIRD, AND FOURTH VENTRICLES and is similar in composition to the ECF of brain.
- Here the capillary endothelium that lines the c.s.f has open junctions, and drug can flow freely in to the extracellular spaces between capillary wall and choroidal cells.
- But the choroidal cells are joined to each other by tight junctions forming the blood-csf barrier which has permeability characters similar to that of BBB.
- Although the mechanism for diffusion of drugs in to CNS ans CSF is similar, the degree of uptake may vary significantly.
5. PLACENTAL BARRIER:

- The maternal and fetal blood vessels are separated by a number of tissue layers made of fetal trophoblast basement membrane and the endothelium which together constitutes the placental barrier.
- Many drugs having molecular weight of less than 1000 Daltons and moderate to high lipid solubility cross the barrier by simple diffusion process.
- This shows that placental barrier is not as effective a barrier as BBB.

6. BLOOD-TESTIS BARRIER:

This barrier is located not at the capillary endothelium level but at sertoli-sertoli junction, it is the tight junction between the neighboring sertoli cells that act as the blood-testis barrier. This barrier restricts the passage of drugs to spermatocytes and spermatids.
ORGAN/TISSUE SIZE AND PERFUSION RATE

Distribution is permeability rate-limited in the following cases:

a. When the drug under consideration is ionic, polar or water-soluble.

b. Where the highly selective physiologic barriers restrict the diffusion of such drugs to the inside of the cell.

In contrast, distribution will be perfusion rate-limited when:

i. The drug is highly lipophilic.

ii. The membrane across which the drug is supposed to diffuse is highly permeable such as those of the capillaries and the muscles.

Perfusion rate is defined as the volume of blood that flows per unit time per unit volume of the tissue. It is expressed in ml/min/ml of the tissue.

BINDING OF DRUGS TO TISSUE COMPONENTS

A drug in the body can bind to several components such as the plasma proteins, blood cells and haemoglobin (i.e. blood components) and extravascular proteins and other tissues.

This topic is dealt comprehensively in chapter 4 on Protein Binding of Drugs.

Miscellaneous Factors:

**Age:** Differences in distribution pattern of a drug in different age groups are mainly due to differences in:

   a) Total body water-which is greater in infants.
   b) Fat content-is also higher in infants and elderly.
   c) Skeletal muscles-are lesser in infants and elderly.
   d) Plasma protein content-low albumin content in both infants and elderly.

**Diet:** A Diet high in fats will increase the free fatty acid levels in circulation thereby affecting binding of acidic drugs such as NSAIDS to Albumin.

**Obesity:** In Obese persons, high adipose tissue content can take up a large fraction of lipophilic drugs.

**Pregnancy:** During pregnancy the growth of the uterus, placenta and fetus increases the volume available for distribution of drugs.

**Disease States:** Altered albumin or drug – binding protein conc.

   Altered or reduced perfusion to organs /tissues
   Altered Tissue pH

**Drug Interactions:** Drug interactions that affect distribution are mainly due to differences in plasma protein or tissue binding of drugs.
Volume of Distribution

Definition: A hypothetical volume of body fluid into which a drug is distributed

- The Volume of distribution (VD), also known as apparent volume of distribution, is used to quantify the distribution of a drug between plasma and the rest of the body after oral or parenteral dosing.
- It is called as Apparent Volume because all parts of the body equilibrated with the drug do not have equal concentration.
- It is defined as the volume in which the amount of drug would be uniformly distributed to produce the observed blood concentration.
- The apparent volume of distribution is a proportionality constant relating the plasma concentration to the total amount of drug in the body.

\[ X \propto C \]
\[ X = V_d C \]
\[ V_d = X / C \]

**Apparent volume of distribution = amount of drug in the body/ plasma drug concentration**

- It is expressed in liters or liters /kg body weight.
- Apparent volume of distribution is dependent on concentration of drug in plasma.
- Drugs with a large apparent volume are more concentrated in extra vascular tissues and less concentrated intravascular.

<table>
<thead>
<tr>
<th>Volume of Distribution of Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Empirical nomenclature</strong></td>
</tr>
<tr>
<td><strong>( V_{\mu} )</strong> (L/70 kg)</td>
</tr>
<tr>
<td><strong>Examples and ( V_{\mu} )</strong></td>
</tr>
<tr>
<td>(L/70 kg body weight)</td>
</tr>
<tr>
<td>Low</td>
</tr>
<tr>
<td>3 to 20</td>
</tr>
<tr>
<td>Warfarin – 7</td>
</tr>
<tr>
<td>Ibuprofen - 10</td>
</tr>
<tr>
<td>Medium</td>
</tr>
<tr>
<td>50 to 500</td>
</tr>
<tr>
<td>Theophylline – 50</td>
</tr>
<tr>
<td>Ranitidine – 500</td>
</tr>
<tr>
<td>High</td>
</tr>
<tr>
<td>1,000 to 5,000</td>
</tr>
<tr>
<td>Nortriptylline – 1,000</td>
</tr>
<tr>
<td>Desipramine – 3,000</td>
</tr>
<tr>
<td>Very high</td>
</tr>
<tr>
<td>10,000 to 40,000</td>
</tr>
<tr>
<td>Chloroquine – 15,000</td>
</tr>
<tr>
<td>Quinacrine – 40,000</td>
</tr>
</tbody>
</table>
Applications of volume of distribution

1. $V_d$ provides the qualitative information
2. Amount of drug in the body, $D_B$ can be determined
   In case of target $C_1$ is known, dosage regimen can be fixed for therapy
3. Loading dose required to achieve steady stage $C_1$ can be estimated
4. Dose required for individual patients can be calculated
5. Drug – drug interaction can be explained
6. Total body clearance can be calculated
Chapter 3

PROTEIN BINDING OF DRUGS

Question: Write note on Plasma protein binding.

- A drug in the body can interact with several tissue components of which the two major categories are blood and extravascular tissues. The interacting molecules are generally the macromolecules such as proteins, DNA, and adipose tissue.
- 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.
- It remains confined to a particular tissue for which it has greater affinity. Binding of drugs to proteins is generally of reversible & irreversible in nature.

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.
Binding of drugs falls into two classes:

1. Binding of drugs to blood components like:
   (a) Plasma proteins
   (b) Blood cells

2. Binding of drugs to extravascular tissue proteins, fats, bones, etc.

**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:
     
     \[ \text{Albumin} \gg 1\text{-Acid gl \( \alpha \) ycoprotein} \gg \text{Lipoproteins} \gg \text{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 bind 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:**
   - Lipoproteins are amphiphilic in nature. It contains combination of lipid & apoproteins. The lipophilic lipid consist of triglycerides & cholesteryl esters and hydrophilic apoprotein consists of free cholesterol & proteins.
   - The M.W. of lipoproteins from 2 lakhs to 34 lakhs depends on their chemical composition. They are classify on the basis of their density:

```
Chylomicrones   VLDL
```
```
LDL   HDL
```

- e.g. Acidic: Diclofenac. Neutral: Cyclosporin A. Basic: Chlorpromazine.
4. Binding of drug to Globulins:

- It mainly binds to endogenous substances. In plasma several globulins have been identified.

<table>
<thead>
<tr>
<th>Globulin</th>
<th>Synonym</th>
<th>Binds to:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. $\alpha_1$ Globulin</td>
<td>Transcortine / Corticosteroid Binding globulin</td>
<td>Steroidal drugs, Thyroxin &amp; Cyanocobalamin.</td>
</tr>
<tr>
<td>2. $\alpha_2$ Globulin</td>
<td>Ceruloplasmine</td>
<td>Vitamin A, D, E, K.</td>
</tr>
<tr>
<td>3. $\beta_1$ Globulin</td>
<td>Transferine</td>
<td>Ferrous ions</td>
</tr>
<tr>
<td>4. $\beta_2$ Globulin</td>
<td>---</td>
<td>Carotinoids</td>
</tr>
<tr>
<td>5. $\gamma$ Globulin</td>
<td>---</td>
<td>Antigens</td>
</tr>
</tbody>
</table>

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.

➢ BINDING OF DRUG TO EXTRAVASCULAR TISSUE PROTEIN

- The tissue-drug binding is much more significant because the body tissues comprise 40% of the body wt which is 100 times that of HAS.

- A tissue can act as the storage site for drugs.

- Importance:
  1. It increases apparent volume of distribution of drug.
  2. Localization of a drug at a specific site in body.

- Factors that influence localization of drug in tissues are lipophillicity & structural features of the drug, perfusion rate, pH differences etc.

- The order of binding of drug to extravascular tissue is: Liver › Kidney › Lung › Muscles
<table>
<thead>
<tr>
<th>Organ</th>
<th>Binding of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Liver</td>
<td>Irreversible binding of Epoxides of Halogenated Hydrocarbon &amp; Paracetamol.</td>
</tr>
<tr>
<td>2. Lungs</td>
<td>Basic drugs: Imipramine, Chlorpromazine, &amp; AntiHistaminics.</td>
</tr>
<tr>
<td>3. Kidney</td>
<td>Metallothione protein binds to Heavy metals &amp; results in Renal accumulation toxicity.</td>
</tr>
<tr>
<td>4. Skin</td>
<td>Chloroquine &amp; Phenothiazine binds to Melanin.</td>
</tr>
<tr>
<td>5. Eye</td>
<td>Chloroquine &amp; Phenothiazine also binds to Eye Melanin &amp; results in Retinopathy.</td>
</tr>
<tr>
<td>7. Bones</td>
<td>Tetracycline (yellow discoloration of teeth),</td>
</tr>
<tr>
<td></td>
<td>Lead (replaces Ca &amp; cause brittleness)</td>
</tr>
<tr>
<td>8. Fats</td>
<td>Lipophilic drugs (thiopental),</td>
</tr>
<tr>
<td></td>
<td>Pesticides (DDT)</td>
</tr>
</tbody>
</table>

- Several examples of extravascular tissue-drug binding are: Liver, Lungs, Kidneys, skin, eyes, hairs, etc.
- It is also seen in hairs, bones, fats & nucleic acids etc.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Mol.wt</th>
<th>Concentration (g%)</th>
<th>Drug that bind</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Serum Albumin</td>
<td>65,000</td>
<td>3.5 – 5.0</td>
<td>Large variety of all type of drug</td>
</tr>
<tr>
<td>α1-Acid glycoprotein</td>
<td>44,000</td>
<td>0.04-0.1</td>
<td>Basic drug such as imipramine</td>
</tr>
<tr>
<td>Lipoprotein</td>
<td>200,000 to 3,400,000</td>
<td>variable</td>
<td>Basic lipophilic drug like chlorpromazine</td>
</tr>
<tr>
<td>α1-Globulin</td>
<td>59,000</td>
<td>0.003-0.007</td>
<td>Steroid like corticosterone, and thyroxine</td>
</tr>
<tr>
<td>α2-Globulin</td>
<td>1,34,000</td>
<td>0.015-0.06</td>
<td>Vitamin A, D, E, and cupric ions</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>64,500</td>
<td>11-16</td>
<td>Phenytoin, phenobarbital, phenothiazines</td>
</tr>
</tbody>
</table>
Question: Write brief note on factors affecting plasma protein binding.

**FACTORS AFFECTING PROTEIN DRUG BINDING**

1. **Drug-related factors**
   a) Physicochemical characteristics of the drugs
   b) Concentration of drugs in the body
   c) Affinity of drug for particular binding components

2. **Protein / Tissue related factors**
   d) Physicochemical characteristics of the protein or binding agents
   e) Concentration of protein or binding components
   f) Number of binding sites on the binding agents

3. **Drug interactions**
   g) Competition between drugs for the binding site
   h) Competition between the drug and normal body constituents
   i) Allosteric changes in protein molecule

4. **Patient-related factors**
   j) Age
   k) Intersubject variations
   l) Disease states

1. **Drug-related factors**
   a. **Physicochemical characteristics of the drug:**
      - Protein binding is directly related to the lipophilicity 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 comparison to other plasma protein.
   - The amount of several proteins and tissue components available for binding, changes during disease state.

c. Number of binding sites:
   - Albumin has a large number of binding sites as compared to other proteins. Indomethacin binds to 3 sites on albumin.

3. Drug interactions

a. Competition between drugs for the binding sites [Displacement interactions]:
   - A drug-drug interaction for the common binding site is called as displacement interaction. D.I. can result in unexpected rise in free conc. of the displaced drug which may enhance clinical response or toxicity. Even a drug metabolite can affect D.I.
   - If the drug is easily metabolisable or excretable, it’s displacement results in significant reduction in elimination half-life.
   - 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:
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:-

<table>
<thead>
<tr>
<th>Disease</th>
<th>Influence on plasma protein</th>
<th>Influence on protein drug binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal failure</td>
<td>↓ Albumin content</td>
<td>↓ binding of acidic drugs; neutral and basic drugs are unaffected</td>
</tr>
<tr>
<td>Hepatic failure</td>
<td>↓ Albumin synthesis</td>
<td>↓ binding of acidic drugs; and binding of basic drugs is normal or ↓ depending on AAG levels</td>
</tr>
<tr>
<td>Inflammatory states i.e. trauma surgery etc…</td>
<td>↑AAG levels</td>
<td>↑ binding of basic drugs; neutral and acidic drugs are unaffected</td>
</tr>
</tbody>
</table>

**Question:** Discuss the significance of protein binding of drugs.

**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 fractions 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

- Lipoproteins act as vehicle for hydrophobic drugs like steroids, heparin, oil soluble vitamins.

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 acts as a reservoir & continuously supplies 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.

➢ Determination of Protein-drug Binding

1. Indirect technique: Based on separation of bound form.
   - Equilibrium dialysis
   - Dynamic dialysis
   - Ultrafiltration
   - Diafiltration
   - Gel filtration
   - Ultracentrifugation

2. Direct technique: Do not required separation of bound form.
   - UV Spectroscopy
   - Fluorimetry
   - Ion selective electrodes
Question: Explain kinetic involved in protein drug binding with description of plots.

**Kinetics of protein binding**

*Assumptions:* The drug-protein binding is reversible.

On the protein molecule one binding site is present

Under this condition the protein binding of drug may be described as follows:

\[
\text{Protein (P) + Drug (D) } \xrightarrow{} \text{Drug-Protein complex (D-P )}
\]

or,

\[
\text{P + D } \xrightarrow{} \text{PD}
\]

From the law of mass action

\[
K_a = \frac{[PD]}{[P][D]} \quad \text{eqn (i)}
\]

Where, \( K_a \) is the association constant. Drugs strongly bound to protein have a very large \( K_a \).

[ ] this symbol denotes molar concentration

To study the binding behavior of drugs, a ratio ‘r’ is defined as follows:

\[
r = \frac{\text{moles of drug bound}}{\text{total moles of protein}}
\]

hence,

\[
r = \frac{[PD]}{[PD] + [P]} \quad \text{eqn. (ii)}
\]

Substituting, \([PD] = K_a [P][D]\) from eqn (i) into eqn (ii) we get:

\[
r = \frac{K_a [P][D]}{K_a[P][D] + [P]} = \frac{K_a[D]}{1 + K_a[D]} \quad \text{eq. (iii)}
\]

Eqn. (iii) describes the situation where 1 mole of drug binds to one mole of protein in a 1 : 1 complex.

If drug molecules can bind independently to ‘n’ number of identical sites per protein molecule then the following equation may be used:

\[
r = \frac{nK_a[D]}{1 + K_a[D]} \quad \text{... (i)}
\]

The value of association constant, \( K_a \) and the number of binding sites \( N \) can be obtained by plotting equation 1 in four different ways as shown below.
Direct Plot:
- It is made by plotting \( r \) versus \([D]\) as shown in Fig. 1. Note that when all the binding sites are occupied by the drug, the protein is saturated and plateau is reached. At the plateau, \( r = N \). When \( r = N/2 \), \([D] = 1/Ka\).

Scatchard Plot:
- It is made by transforming equation 1 into a linear form. Thus,

\[
r + rKa[D] = N Ka[D]
\]

\[
r = N Ka[D] - rKa[D]
\]

\[
\frac{r}{[D]} = N Ka - rKa \quad \text{............... (2)}
\]

- A plot of \( \frac{r}{[D]} \) versus \( r \) yields a straight line (Fig. 2). Slope of the line = – Ka, y intercept = NKa and x-intercept = N.
Klotz Plot/ Lineweaver-Burke Plot (Double Reciprocal Plot):

- The reciprocal of equation 1 yields:

\[
\frac{1}{r} = \frac{1}{n} + \frac{n}{k_{a}[D]} \quad \text{……………….. (3)}
\]

- A plot of \(\frac{1}{r}\) versus \(\frac{1}{[D]}\) yields a straight line with slope \(\frac{1}{Nk_{a}}\) and y-intercept \(\frac{1}{N}\) (Fig.3).

Hitchcock Plot:

- It is made by rewriting equation 2 as –

\[
\frac{Nk_{a}[D]}{r} = 1 + k_{a}[D]
\]

\[
[D]/r = \frac{1}{Nk_{a}} + [D]/N \quad \text{………………………(4)}
\]

- Equation 4 is Hitchcock equation according to which a plot of \([D]/r\) versus \([D]\) yields a straight line with slope \(1/N\) and y-intercept \(1/Nk_{a}\) (see Fig. 4).
Chapter 4
Excretion of drugs

Excretion is defined as the process whereby drugs and/or their metabolites are irreversibly transferred from internal to external environment.

Kidney is the most important organ involved in excretion of most drugs especially which are water soluble or having low molecular weight. Nephron is the functional unit of kidney consisting of various parts viz glomerulus, proximal tubule, loop of Henle, distal tubule and the collecting tubule.

Renal excretion refers to the process by which drugs are excreted through kidney.

The major excretory processes that determine the excretion are:

I. Glomerular filtration
2. Active tubular secretion
3. Tubular reabsorption

Rate of Excretion = Rate of Filtration + Rate of Secretion – Rate of Reabsoprtion

- Glomerular filtration:
It is the first step in formation of urine. This is the process by which the kidneys filter excess fluid and the waste produced from the blood into the urine collection tubes that are removed from the body. Glomerular filtration is unidirectional process which permits a high degree of filtration of water soluble compounds while restricting the gross particulate matter having large molecular weight. The kidney filters both metabolized and unmetabolized drugs. The filtrate contains low molecular weight drugs (<500 daltons), unionized and ionized drugs and which are water soluble. Protein bound drugs are not filtered due to their high molecular weight. Hence, the availability of drug in filtrate depends on the concentration of free form of drug in plasma. The driving force required for glomerular filtration is obtained from hydrostatic pressure of blood flowing in capillaries.

The rate at which kidneys filter blood is called the glomerular filtration rate (GFR).

The normal GFR value is 125 ml/minute or per day. GFR is also an indicator of urine production, increased GFR will increase urine production, and vice versa.

- Tubular Reabsorption:
Tubular reabsorption is the process which takes place in renal tubule to essential constituents like Glucose, amino acids etc. Here, the drug is transferred from renal tubule into blood by passive or active transport. Drug reabsorption is indicated the excretion rate values are less than GFR. Tubular reabsorption is the process that moves solutes and water out of the filtrate and back into your bloodstream. Reabsorption is a two-step process:
a. The first step is the passive or active movement of water and dissolved substances from the fluid inside the tubule through the tubule wall into the space outside.

b. The second step is for water and these substances to move through the capillary walls back into your bloodstream, again, either by passive or active transport.

The fluid passes through the components of the nephron (the proximal/distal convoluted tubules, loop of Henle, the collecting duct) as water and ions are removed as the fluid osmolarity (ion concentration) changes.

- **Tubular secretion:**
This is an active carrier mediated process occurs in proximal tubule. Here, drug diffuses against the concentration gradient from the blood capillaries across the renal tubular membrane. Active tubular secretion is not affected by changes in pH and protein binding. Some substance such as hydrogen ions, creatinine, and drugs will be removed from the blood through the peritubular capillary network into the collecting duct. The end product of all these processes is urine, which is collection of substances that has not been reabsorbed. The mechanisms by which secretion occurs are similar to those of reabsorption; however these processes occur in the opposite direction.

**Question:** Explain the factors affecting renal excretion.

**FACTORS AFFECTING RENAL EXCRETION OF DRUGS**

Different factors that affect renal excretion of drugs are

- Physicochemical properties of the drug
- Plasma concentration of the drug
- Distribution and binding characteristics of the drug
- Urine pH
- Blood flow to the kidneys
- Biological factors
- Drug interactions
- Disease states

**Physiochemical properties of drug:** It include

a. **Molecular weight:** The molecular weight of drug is important consideration during elimination. Larger molecular Weight drugs are difficult to be excreted than smaller molecular Weight especially by glomerular filtration.

b. **Drug lipid solubility:** The urinary excretion is inversely related to lipophillicity. Higher the lipid solubility cause increases the volume of distribution of drug and reduces renal excretion.
Plasma concentration of drug:
Increase in plasma concentration of the drug, increases the rate of urinary drug excretion. It affects both glomerular filtration and renal reabsorption. Drugs that show renal reabsorption, they excreted only when concentration in glomerular filtrate is more than reabsorption capacity.

Binding characteristics of the drugs:
Drugs that are bound to plasma proteins behave as macromolecules and cannot be filtered through glomerulus. Only the free fraction of the drug is filtered. Protein bound drug has long half lives.

Volume of distribution:
Clearance is inversely related to volume of distribution of drugs (vd). A drug with large vd is poorly excreted in urine. While the drugs have low Vd have higher excretion rates.

Urine pH:
Most drugs are weak acids or weak bases thus by changing pH of urine via chemicals can inhibit the passive tubular re-absorption of drugs. Urine is normally slightly acidic and favours excretion of basic drugs.

Blood Flow to Kidney:
Increased perfusion leads to increased excretion. Renal blood flow play important role for drugs excreted by glomerular filtration.

Biological factor:
Age, sex, species and strain differences, differences in the genetic make-up, circadian rhythm, etc. can affect renal excretion. Renal excretion is reduced in neonates and elderly. Renal excretion is approximately 10% lower in females than in males. The renal function of newborns is 30 to 40% less in comparison to adults and attains maturity between 2.5 to 5 months of age. In old age, the GFR is reduced and tubular function is altered, the excretion of drugs is thus slowed down and half-life is prolonged.

Disease states:
Disease state impairs the elimination of drugs. The various causes of renal impairment are hypertension, Diabetes, pyelonephritis etc. These diseases greatly impair elimination of drugs primarily excreted by kidneys and causes accumulation of drugs lead to toxicity.

Drug Interaction:
It refers to the process of interaction of drugs when administered simultaneously into the body.
The drug interactions affect drug excretion and cause forced diuresis, alteration in urine pH, Alteration in renal blood flow, and also alteration in binding Characteristic. Example: Furosemide increases the excretion of gentamicin by displacing gentamicin from its protein binding sites. Another example is the urinary excretion of digoxin is decreased by diazepam.
NON RENAL ROUTES OF DRUG EXCRETION

When the drugs are excreted by organs other than kidney, this is called non renal route of excretion. The various routes of excretion other than kidney are

1. Biliary excretion
2. Pulmonary excretion
3. Salivary excretion
4. Mammary excretion
5. Skin excretion
6. Gastro-intestinal excretion
7. Genital Excretion

Biliary Excretion:

- This excretion is also referred as enterohepatic excretion as the excretion is done by the hepatic cells of the liver. The unchanged drugs which are passes through liver are excreted in bile. Other drugs get converted into metabolites in liver before excreted in bile. The secreted drug may be reabsorbed in the small intestine and undergoes enterohepatic cycling. The rest is excreted in the faeces. The cycle in which the drug is absorbed, secreted in bile and reabsorbed is known as **enterohepatic cycling**.
- Hepatic cells produce bile which is important in digestion and absorption of fat. It is a active process. Bile secreted in liver and stored in gall bladder. The flow rate of bile is 0.5 to 1ml/min. Drugs are carried from the plasma into the bile through the hepatocytes.
- Different transport mechanisms exist for the secretion of organic anions, cations and neutral polar compounds. On the basis of their bile/plasma concentration ratio, the compounds that are excreted in bile have been classified into 3 categories.
  - **Group A**: Whose ratio 1 e.g. sodium, potassium and chloride ions and glucose.
  - **Group B**: Whose ratio >1 usually from 10 to 1000 e.g. bile salt, bilirubin glucuronide, creatinine, sulfobromophthalein conjugates etc
  - **Group C**: Whose ratio <1 e.g. sucrose, insulin, phosphates, phospholipids and mucoproteins.

**Enterohepatic circulation** of bile salt

- Enterohepatic circulation is a continuous process of secretion of bile salt into bile, then pass into duodenum (where some are converted into bile acid), then absorption in ileum and then returns to liver as mixture of bile acid and salt.
Bile salt secreted into intestine where they are effectively reabsorbed and thus reused. Primary and secondary bile acids are converted into bile salt in the liver by conjugating them with glycine/taurine and then secreting them into bile.

The bile salts present in bile are reabsorbed by sodium- bile salt cotransporter that occurs mainly in ileum. This is active transport process. They are actively transported out of ileal mucosal cell into portal blood and then taken up by hepatocytes via an isoform of the co-transporter. Since bile salts are hydrophobic, for them to travel in portal blood, they are bound to protein called albumin, which carries them in non covalent complex.

Pulmonary Excretion:
For the excretion of gaseous and volatile substances, lung is considered as important organ. It is a simple diffusion process. The rate of respiration and solubility of volatile substances affects the pulmonary blood flow. For example: Gaseous anaesthetics, nitrous oxide not very soluble in blood are excreted rapidly by the lungs in the expired air. Compounds like alcohol which have high solubility in blood and tissue are excreted slowly by the lungs.

Salivary Excretion:
It is a passive diffusion process and based on pH-partition hypothesis. The pH of saliva varies from 5.8 to 8.4 (i.e pH 6.4). Hence at this pH unionized lipid soluble drugs are excreted through saliva passively. Basic drugs are excreted more in saliva than acidic drugs. Drugs and their metabolites that are excreted through saliva and again swallowed and thus the whole process of absorption of drugs again begin. The drugs blood concentration can be determined by detecting the amount of drug excreted in saliva. Example: caffeine, theophylline, phenytoin
**Mammary Excretion:**
The excretion of drug and their metabolites into milk is called mammary excretion. It is a passive process. The pH partition molecular weight, lipid solubility, degree of ionization all affects excretion rate. The agents that easily cross Blood Brain Barrier (BBB) usually enter breast milk more rapidly. The Mammillary epithelium allows lipid soluble drugs to penetrate into milk. The extent of drug excretion in milk can be determined from milk/plasma drug concentration ratio (M/P). The basic drugs concentrate more in milk and have M/P ratio greater than 1. Drugs bound to plasma protein and are less secreted by milk.

**Skin/dermal Excretion:**
Drugs excreted through skin via sweat also follow pH partition hypothesis. The excretion of drugs depends on unionized and lipid soluble form of drug across epithelial gland of cell, therefore pKa of drug and pH of individual secretion formed in gland are important determination of total quality of drugs appear in sweat. Passive excretion of drugs and their metabolites through skin is responsible to some extent for the urticaria and dermatitis and other hypersensitivity reactions. Compounds such as benzoic acid, salicylic acid, alcohol and antipyrine and heavy metals like lead, mercury and arsenic are excreted in sweat.

**Gastrointestinal Excretion:**
Excretion of drugs into the Gastro Intestinal Tract (GIT) occurs after parenteral administration when the concentration gradient for passive diffusion is favourable. The process is reverse of G.I. absorption of drugs. Water soluble and ionized form of weakly acidic and basic drugs is excreted in the G.I.T. Example: Nicotine and quinine are excreted in stomach. Orally administered drugs can also be absorbed and excreted in GIT. Drugs excreted in GIT are reabsorbed into the systemic circulation and undergo recycling.

**Genital Excretion:**
The drugs are also eliminated via Reproductive tract and genital route. Some drugs have been detected in semen. The drugs can also get eliminated via the lachrymal fluid.
CONCEPT OF CLEARANCE

Clearance is defined as “the hypothetical volume of body fluids containing drug from which the drug is removed or cleared completely in a specific period of time”.

\[ \text{Clearance (Cl)} = \frac{\text{Elimination rate}}{\text{Plasma drug concentration}} \]

It is expressed in ml/min.

Applied to all organs involved in drug elimination and referred to as renal clearance, hepatic clearance, pulmonary clearance, biliary clearance etc.

**Total body clearance** is the sum of individual clearances by all eliminating organs.

**Question:** Explain Renal Clearance.

Renal clearance \((C_{LR})\) can be defined as “the volume of blood or plasma which is completely cleared of the unchanged drug by the kidney per unit time”. OR renal clearance is defined as the urinary drug excretion rate \((\frac{dD_u}{dt})\) divided by the plasma drug concentration \((C_p)\).

\[ \text{Renal clearance } C_{LR} = \frac{\text{Rate of Elimination by kidney}}{\text{plasma drug concentration}} \]

Renal clearance may be considered the ratio of the sum of the glomerular filtration and active secretion rates less the reabsorption rate divided by the plasma drug concentration:

\[ C_{LR} = \frac{\text{Rate of (filtration + secretion – reabsorption)}}{\text{plasma drug concentration}} \]

The renal clearance of a drug is often related to the renal glomerular filtration rate, GFR, when reabsorption is negligible and the drug is not actively secreted.

The renal clearance value for the drug is compared to that of a standard reference, such as inulin, which is cleared completely through the kidney by glomerular filtration only.

The **clearance ratio**, which is the ratio of drug clearance to inulin clearance, may give an indication for the mechanism of renal excretion of the drug.

**Renal clearance ratio** or excretion ratio may be defined as the ratio of renal clearance of a drug to the renal clearance of creatinine. It has no units.

\[ \text{Renal clearance ratio} = \frac{\text{renal clearance of drug}}{\text{renal clearance of creatinine}} \]
DETERMINATION OF RENAL CLEARANCE

- Graphical Methods
- Model Independent Methods

**Graphical Methods**

Clearance is given by the slope of the curve obtained by plotting the graph of rate of drug excretion in urine \( \frac{dD_u}{dt} \) against \( C_p \).

For a drug that is excreted rapidly, \( \frac{dD_u}{dt} \) is large, the slope is steeper, and clearance is greater.

For a drug that is excreted slowly through the kidney, the slope is smaller.

\[
Cl_r = \frac{\text{Urinary drug excretion rate}}{\text{Plasma drug concentration}}
\]

**Model Independent Methods**

If total amount of drug excreted in urine \( D_u^\infty \) has been obtained, then clearance is calculated by

\[
Cl_r = \frac{D_u^\infty}{[AUC]_0^\infty}
\]

Where, \( D_u^\infty = \) total amount of drug excreted in urine

\( [AUC]_0^\infty = \) area under the curve (total amount of drug absorbed)

**FACTORS AFFECTING RENAL EXCRETION OR RENAL CLEARANCE**

1. Physicochemical properties of drug
2. Plasma concentration of drug
3. Distribution and binding characteristics of drug
4. Urine pH
5. Blood flow to the kidney
6. Biological factors
7. Drug interactions
8. Disease state
Chapter 5
BIOAVAILABILITY

DEFINITION
Bioavailability refers to the extent and speed with which the active part (drug or metabolite) enters the systemic circulation, accessing the site of action.

OBJECTIVES OF BIOAVAILABILITY STUDIES
1. Bioavailability studies are one of the factors that link in-vivo performance of the drug product used in clinical studies (for determination of evidence of safety and efficacy).
2. Bioavailability studies provide useful information important to establish dosage regimen and to support drug labeling.
3. These studies help in determining the influence of excipient, patient related factors and possible interaction with other drugs on the efficiency of absorption.
4. Bioavailability studies estimates the quality control of a drug product during the early stages of marketing so that the influence of processing factors, storage and stability on drug absorption can be determined.

Bioavailable fraction (F) is a term which refers to the fraction of administered dose that enters the systemic circulation and can be calculated as:

\[ F = \frac{\text{Bioavailable dose}}{\text{Administered dose}} \]

Bioavailability of a drug depends upon mainly three major factors.
1. Pharmaceutical factors including physiochemical factors and formulation factors.
2. Patient related factors.
3. Route of drug administration.

**Question:** Differentiate absolute and relative bioavailability. Discuss the pharmacokinetic methods for the bioavailability measurement. Explain measurement of bioavailability by Plasma level-time study.

**Absolute bioavailability**
Absolute bioavailability is a measure of the given drug absorbed systematically administered compared to its intravenous administration.

\[
\text{Absolute Bioavailability (F, %)} = \frac{\text{AUC(Oral)} \times \text{Dose (IV)}}{\text{AUC(IV)} \times \text{Dose (Oral)}} \times 100
\]
Where dose (IV) is the dose administered intravenously, dose (oral) is the dose administered orally and AUC (Oral) and AUC (IV) are the area under the plasma drug concentration-time curve after oral and IV administration respectively.

Significance of absolute bioavailability
The drug is completely Bioavailable when F=1, for the drugs given by intravenous injection. F<1 is for the drugs which are poorly absorbed or metabolized by first pass effect. E.g. Insulin solution shows zero bioavailability as it is extensively degraded in GI tract.

Relative bioavailability
Relative bioavailability is a measure of the given drug absorbed systematically after oral administration compared with that of an oral standard of the same drug. E.g. comparison between Amoxicillin capsule and Amoxicillin suspension.

\[
\text{Relative Bioavailability (Fr, \%) = } \frac{\text{AUC(Test)} \times \text{Dose (Std)}}{\text{AUC (Std)} \times \text{Dose (Test)}} \times 100
\]

Significance of relative bioavailability
In comparison to absolute bioavailability, it is used to characterize absorption of a drug from its formulation. Both absolute and relative bioavailabilities are expressed in percentages.

MEASUREMENT OF BIOAVAILABILITY

Pharmacokinetics methods: These are indirect methods based on the assumption that pharmacokinetics profile reflects the therapeutic effectiveness of a drug. The two major pharmacokinetics methods are:

- Plasma level time profile
  - Time for peak plasma concentration (tmax)
  - Peak plasma drug concentration (Cmax)
  - Area under the plasma drug concentration time curve (AUC)

- Urinary excretion data
  - Cumulative amount of drug excreted in the urine (Dt)
  - Rate of drug excretion in the urine — Du dt
  - Time for maximum urinary excretion (t)

Pharmacodynamic methods: These involve direct measurement of pharmacologic or therapeutic end point. The two pharmacodynamic methods involve determination of bioavailability from:
Acute pharmacologic response

i. Maximum pharmacodynamic effect (Emax)

ii. Time for maximum pharmacodynamic effect

iii. Area under the pharmacodynamic effect time curve

iv. Onset time for pharmacodynamic effect

Pharmacokinetic Method

1. Plasma drug concentration (Plasma Level- Time Studies)
   - Most common type of human bioavailability studies.
   - Based on the assumption that there is a direct relationship between the concentration of drug in blood or plasma and the concentration of drug at the site of action.
   - Following the administration of a single dose of a medication, blood samples are drawn at specific time intervals and analyzed for drug content.
   - The method requires collection of serial blood sample after drug administration plasma drug concentration is plotted against time.

![Diagram showing plasma concentration-time profile](image)
a. Time for peak plasma concentration (tmax)
It is the time at which the maximum plasma concentration, (Cp) max is achieved after drug administration. At tmax, rate of absorption is equal to the rate of elimination. After tmax is reached, drug absorption still continue but at slow rate. It is expressed in hours, tmax indicates drug absorption rate.

b. Peak plasma drug concentration (Cmax)
It is the maximum plasma drug concentration achieved after drug administration. It indicates that drug is absorbed systemically and therapeutic response is obtained. It is expressed in μg/ml or mg/ml.

c. Area under Curve (AUC)
Area under plasma level time curve is measurement the extent of drug absorption. The AUC indicates the entire amount of active drug that reaches systemic circulation. AUC not depends on route of administration.
If the drug is following linear kinetics, and then AUC is directly proportional to the dose AUC is inversely proportional to the clearance of the drug.

Methods to determine Area under Curve (AUC)
Different methods are available to determine Area under Curve
(a) CUT AND WEIGH METHOD: In this method the plasma concentration profile are plotted on smooth texture paper. The Area under curve is cut out carefully and weighed on an analytical balance. The weight obtained of these cut out plot will be proportional to AUC.
(b) By using Planimeter: Planimeter is an instrument which calculates areas by tracing their outerlines.
(c) Trapezoidal method:
In this method the plasma concentration versus time profile is divided into several trapezoids. The area of individual trapezoids is calculated and then these areas are added to obtain cumulative AUC.
(d) Counting the Square:

Total no. Of squares enclosed in the curve is counted.

- Area of each square determined using relationship:

2. Urinary drug excretion data

Sometimes it is not possible to collect blood (plasma) samples but one may be able to estimate the amount of drug excreted unchanged into the urine. Under these conditions, urinary excretion data become more appropriate for pharmacokinetics studies.

- Urinary excretion of unchanged drug is directly proportional to plasma concentration of drug.
- Thus, even if a drug is excreted to some extent (at least 10 to 20%) in the urine, bioavailability can be determined. eg: Thiazide diuretics, Sulphonamides.
- Method is useful when there is lack of sufficiently sensitive analytical technique to measure drug concentration.
- Non-invasive method, so better patient compliance.

a. Du∞: It is the cumulative amount of drug excreted in urine. It is related directly to amount of drug absorbed. The urine samples are collected at regular interval after drug administration. Each urine specimen is analyzed for unchanged drug by assay method.

b. dDu / dt (Rate of drug excretion): It is related to first order elimination rate constant (K) and concentration of drug in plasma (Cp).

c. t∞ (Total time for drug to be excreted): Its value decreases as increases.
PHARMACODYNAMIC METHODS:
These methods are used when bioavailability measurement by pharmacokinetic method is difficult or giving non-reproducible data.

**Acute pharmacological response:** It includes various parameters such as change in ECG or EEG reading, pupil diameter, Blood Pressure. These are useful parameters to determine bioavailability. Bioavailability can be predicted by plotting Pharmacological effect time curve as dose response graph. At least three biological half life of drug is measured to get accurate estimate of AUC.

**Therapeutic response:** It includes measurement of clinical response of drug. For example: an Anti-inflammatory drug is given for the reduction in inflammation is determined.
Question: Explain various methods used for enhancement of bioavailability. OR How the bioavailability of drug can be improved?

❖ BIOAVAILABILITY ENHANCEMENT OF POORLY SOLUBLE DRUGS

There are different methods which are used to overcome the bioavailability problem:

ENHANCEMENT OF DRUG SOLUBILITY
- Micronization
- Nanonisation
- Super critical fluid recrystallization
- Spray freezing into liquid (SFL)
- Evaporative Precipitation into aqueous solution (EPAS)
- Use of surfactant
- Use of salt form
- Eutectic mixtures
- Use of precipitation inhibitors
- Solid solution
- Solid dispersion
- Molecular encapsulation with Cyclodextrins

ENHANCEMENT OF DRUG PERMEABILITY
- Lipid technologies
- Ion pairing
- Penetration enhancers

ENHANCEMENT OF DRUG STABILITY
- Enteric coating
- Complexation
- Use of metabolic inhibitors

Enhancement of drug solubility

Particle size reduction: There is a direct relationship between the surface area of the drug and its rate of dissolution. As the surface area increases with decreasing particle size, higher dissolution rates can be achieved by reducing the particle size. The Micronization of a sparingly soluble drug to reduce particle size results better dissolution and bioavailability. Conventional methods of particle size reduction, such as
comminution and spray drying. Nowadays Particle size reduction can be achieved by micronization and nano-suspension.

**Micronization**: This technique is called a micro milling. The process involves reducing the size of the solid drug particles 10-100 microns by spray drying or by use of air attrition methods, fluid energy or jet mill. E.g. Griseofulvin and several steroidal of sulpha drugs.

**Nanonisation**: In this technique the drug powder is converted to nano Crystals of sizes 200-600 nm. E.g. amphotericin B. There are three technologies which are used to nano particles.

  i. Pearl milling.

  ii. Homogenization in water.

  iii. Homogenization in non aqueous media or in water with water miscible liquid.

**Super critical fluid process**: Super critical fluids are fluids whose temperature and pressure are greater than its critical temperature (Tc) and critical pressure (Tp) allowing it to assume the properties of both are liquid and a gas. Once the drug are solubilize within SCF, they may be recrystallize at greatly reduced particle size.

**Spray freezing into liquid (SFL)**: This technique involves atomizing an aqueous solution or suspension containing a drug and pharmaceutical excipients directly in compressed gas or the cryogenic liquid (nitrogen). The frozen particles are then lyophilized to obtain dry and free flowing micronized powders.

**Evaporative Precipitation into aqueous solution (EPAS)**: This process utilizes rapid phase separation to nucleate and grow nano particles and microparticles of lipophilic drugs. The drug is first dissolved in a low boiling point organic solvent which is pumped through a tube where it is heated above the solvent boiling point and then spread through a fine atomizing nozzle into a heated aqueous solution. Surfactant are added to the, this process facilitates dissolution rate.

**Use of surfactant**: Surfactant enhances both dissolution rate and permeability of drug. They enhance dissolution rate primarily by promoting, wetting and penetration of dissolution fluid into the solid drug particle. Nonionic surfactants like polysorbate are widely used. Bioavailability of steroids like spironolactone drugs have been increased by use of surfactant.

**Use of cosolvents**: The solubility of a poorly water soluble drug can be enhanced by the addition of a water miscible solvent or cosolvents. Cosolvents are mixtures of water and one or more water miscible solvents used to increase the solubility for poorly soluble compounds.

**Use of Salt forms**: Salt has improved solubility and dissolution characteristics in comparison to the original drug. It is generally accepted that a minimum difference of three units between the pKa value of the group and that of the counter ion is required to form stable salt. Alkyl metal salt of acidic drugs like Penicillin and strong acid salts of basic drugs like atropine are more water soluble than the parent drug.
Eutectic Mixture: These systems are prepared by fusion method. When the eutectic is exposed to water, the soluble carrier dissolves leaving the drug in a microcrystalline state which solubilize rapidly. Such systems are basically intimately blended physical mixture of two crystalline components.

Use of precipitation inhibitors: Increase in free drug concentration above equilibrium solubility results in super absorption, which can lead to drug precipitation or crystallization. This can be prevented by use of inert polymers such as HPMC, PVP, PEG etc. Precipitation inhibitors act by one or more mechanism:

- Inhibit crystallization three specific intermolecular interactions on growing crystal surface.
- Adsorbs onto faces of host crystals, reduce the crystal growth rate of the host.

Solid Solution: These are generally prepared by fusion method where by a physical mixture of solute and solvent are melted together followed by rapid solidification. Solid solutions show together followed by rapid solidification. Solid solution show greater aqueous solubility and faster dissolution than eutectic and solid dispersion because of reduction in particle size to the molecular level. The griseofulvin form such solid solution dissolves 6-7 times faster than pure griseofulvin.

Solid Dispersion: Solid dispersion refers to the dispersion of one or more active ingredients in an inert carrier in a solid state frequently prepared by hot melt method, solvent evaporation method, hot melt extrusion method and co-precipitation techniques where both the gas solute and the solid carrier solvent are dissolved in a common volatile liquid which is removed and results in amorphous precipitation of guest in a crystalline carrier. Such dispersion are often called co-evaporates.

Molecular encapsulation with Cyclodextrins: Molecularly encapsulated drug has greatly improved aqueous solubility and dissolution rate. These Cyclodextrins molecules are versatile in having a hydrophobic cavity of size suitable enough to accommodate the lipophilic drug as guest; the outside of the host molecules is relatively hydrophilic. Thiazide diuretics, barbiturates, and number of NSAIDs are the examples of drugs with improved bioavailability by this method.

ENHANCEMENT OF DRUG PERMEABILITY

Lipid Technologies: Lipid base formulations have been designed to improve the bioavailability by various mechanisms like:

- Reduction in gastric emptying rate by increasing the time available for dissolution and absorption.
- Increased intestinal membrane permeability.
- Increased intestinal blood flow.
- Increased luminal degradation.
- Increased uptake from the intestinal lumen in to the lymphatic system.
**Ion pairing:** This approach involves co-administration of a hydrophilic or polar drug with a suitable lipophilic counter ion, which improves the partitioning of the resultant ion pair into the intestinal membrane. Ion pairing is increase the oral bioavailability of the ionizable drug by two folds.

**Penetration enhancer:** It acts by interaction its lipid part with the polar component of membrane phospholipids. These facilitate the transport of drug across the biomemebrane. This method is used for hydrophilic drugs which have difficulty in penetrating the liquid structure of the biomemebrane. E.g.: Fatty acids, salicylates, EDTA etc.

**ENHANCEMENT OF DRUG STABILITY**

**Enteric Coating:** This technique retards the release of drug in stomach example Erythromycin, Penicillin-V etc.

**Complexation:** This technique is used to increase the stability of drug in GIT. Examples ester drugs thus enhance their bioavailability.

**Use of metabolism Inhibitor:** Metabolism inhibitor selectively inhibits any Of the contributing processes which would result in increased fractional absorption and enhance the bioavailability.
Chapter 6
Bioequivalence Studies

Definition:
“‘It is a relative term which denotes that the drug substance in two or more identical dosage forms, reaches the circulation at the same relative rate & to same relative extent i.e. their plasma concentration-time profiles will be identical without significant statistical differences.”

Bioequivalence studies are the studies used to compare the bioavailability of the same drug from various drug products. These studies are required during the course of development of new drugs or when a patent of innovator’s drug product expires.

It’s commonly observed that there are several formulations of the same drug, in the same dose, in similar dosage form and meant to be given by the same route. In order to ensure clinical performance of such drug products, bioequivalence studies should be performed.

Need for bioequivalence studies

- Bioequivalence studies provide a link between the pivotal and early clinical trial formulation.
- Bioequivalence studies are made mandatory for all drug products by FDA before approving them for marketing.
- Bioequivalence studies provide assurance given by the manufacturers to the patients that their product gives the labeled therapeutic effect and safety.

Applications of bioequivalence studies

- Bioequivalence studies allow substitution of one product by another product which is equally effective.
- Efficiency and safety of product from batch to batch produced by same company are reduced.
- Bioequivalence studies also reduce the formulation variables.

Limitations of bioequivalence studies

- It is very difficult, time consuming and expensive process.
- Therapeutically equivalent drug products may not be equally suitable for a particular patient.
- Bioequivalence studies are not necessary for the following cases:
  a. An aqueous solution for parenteral use.
  b. A solution for oral use.
  c. A powder for reconstitution as a solution for oral or parenteral use.
  d. An ophthalmic solution.
  e. An inhalation product or nasal spray as an aqueous solution.
Types of equivalence

**Equivalence:** It is a relative term that compares one drug product with another or with a set of established standards.

**Chemical equivalence:** Chemical equivalence indicates that two or more drug products are containing the same labeled quantities of drug in the same amount.

**Pharmaceutical equivalence:** It indicates that two or more drug products are identical in strength, quality, disintegration time and dissolution rate characteristics, but may differ in excipients.

**Therapeutic equivalence:** It indicates that two or more drug products containing same therapeutic ingredients, elicit identical pharmacological effects. FDA considers drug products to be therapeutically equivalent if they meet the following criteria:

- a. Approved as safe and effective.
- b. Pharmaceutically equivalent.
- c. Adequately labeled.
- d. Manufactured in compliance with cGMP.

**Bioequivalence:** Bioequivalence indicates that drug in two or more similar dosage forms reach the systemic circulation to the same relative rate and extent.

**Clinical equivalence:** When the same drug from two or more dosage forms give identical \textit{in-vivo} effect (measured by pharmacological response) are said to be clinically equivalent.

---

7.15 ELEMENTS OF BIOEQUIVALENCE STUDY PROTOCOL

1. Title
2. Study objective
3. Study design Title
   a. Drug product
   b. Dosage regimen
   c. Sample collection schedule
   d. Fasting/meal schedule
   e. Analytical method
4. Study population
   a. Subjective
   b. Subject selection
   Medical history
   Physical examination
5. Clinical procedures
   a. Dosage and drug administration
   b. Biological sampling schedule
   c. Activity of subject
6. Ethical consideration
   a. Basic principle
   b. Informed consent
   c. Indication for subject withdrawal
   d. Adverse reaction and emergency procedure
7. Facilities
8. Data analysis
9. Drug accountability
10. Appendix
**METHODS TO STUDY BIOEQUIVALENCE**

1. **In vivo bioequivalence study**

This study requires determination of relative bioavailability. The reference product may be previously approved product or usually an innovator's product. The study is performed in fasting, young, healthy, adult male volunteers to assure homogeneity in the population. This study comprises of different types of design and then evaluation of data by differ methods.

**Subjects**
- Performed with healthy volunteers.
- Include subjects of both the sexes.
- Between 18-55 years old.
- Administered after the over night fasting.
- Meals and water taken during the course of the study should be standardized.
- Should not be taking any medications.
- Should not be allowed to have alcoholic or xanthine containing beverages. e.g. coffee, tea, soft drinks.
- Non smoker.

**Reference and Test product**
- Generic or test product, which is pharmaceutical equivalent, is compared with reference product.
- *Reference listed drug* (RLD), which is listed in *Approved Drug Products with Therapeutic Equivalence Evaluations*—the *Orange Book*
- Reference product is generally the “Innovator product”.
- In some cases another well established product available in the market could be used.
- But choice of reference product should be justified by the principal investigator.

**STUDY DESIGN**
- FDA provide guidelines for in-vitro dissolution and in-vivo bioequivalence study. Similar guidelines appear in the United States Pharmacopeia NF
- Currently 3 diff. studies which include:
  1. A fasting study method
  2. A food intervention study
  3. A multiple dose (steady state) study
Fasting study
1. Both male and female subjects are used in study.
2. Blood sampling is performed just before adm. Of dose and also at particular time interval after dose.
3. Subjects should be in fasting state before drug adm. (at least 10 hrs) & should continue to fast up for 4 hrs after dosing.
4. No other medication is given for at least 1 week prior.

Food intervention study
- Co-admn. of food with an oral drug product.
- The test meal is a high-calorie and high fat meal.
- A typical test meal is two eggs fried in butter, two strips of bacon, two slices of toast with butter, 4 ounce of brown potatoes, and 8 ounce of milk.
- Test meal derives approximately 150, 250, and 500–600 calories from protein, carbohydrate, and fat, respectively.
- Both test and ref. drugs should be affected similarly by food.

Multiple-dose (Steady state) study
- In few cases a Multiple-dose, steady state, two way cross-over study comparing equal doses of test and ref. drug in healthy adult subject.
- For these studies, 3 consecutive trough conc. \( (C_{min}) \) on 3 consecutive days should be determined to ascertain that the subject are at steady state.
- Last morning dose is given after overnight fast, with continual fasting for 2hrs after dose
- Blood sampling is performed similarly to single-dose study.

Question: Discuss latin-square cross-over design.

Types of design
**Parallel group design:** In a parallel group design, subjects are divided randomly into groups, each group receiving one treatment randomly.

**Cross over design:** In a cross over design each subject receives two or more different treatments on successive occasions.
1. Completely randomized designs (CRD)
2. Randomized complete block design (RCBD)
3. Latin square design (LSD)
4. Balanced incomplete block design (BIBD)
5. Partially incomplete block design (PIBD)

1) LATIN SQUARE CROSS OVER DESIGN: In which
1. Each formulation is administered just once to each subject & once in each study period, &
2. Unlike parallel design, all the subjects do not receive the same formulation at the same time; in a given study period, they are administered different formulations.

An example of the Latin square cross-over design for a bioequivalence study in human volunteers is given in following table:

- Examples of Latin-square crossover designs for a bioequivalence study in human volunteers, comparing three different drug formulations (A, B, C).
- The Latin-square design plans the clinical trial so that each subject receives each drug product only once, with adequate time between medications for the elimination of the drug from the body.
- In this design, each subject is his own control, and subject-to-subject variation is reduced. Moreover, variation due to sequence, period, and treatment (formulation) are reduced, so that all patients do not receive the same drug product on the same day and in the same order.
- Possible carryover effects from any particular drug product are minimized by changing the sequence or order in which the drug products are given to the subject.
- Thus, drug product B may be followed by drug product A, D, or C. After each subject receives a drug product, blood samples are collected at appropriate time intervals so that a valid blood drug level–time curve is obtained. The time intervals should be spaced so that the peak blood concentration, the total area under the curve, and the absorption and elimination phases of the curve may be well described.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Study Period 1</th>
<th>Study Period 2</th>
<th>Study Period 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>4</td>
<td>A</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>5</td>
<td>C</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>6</td>
<td>B</td>
<td>A</td>
<td>C</td>
</tr>
</tbody>
</table>

Period refers to the time period in which a study is performed. A two-period study is a study that is performed on two different days (time periods) separated by a washout period during which most of the drug is eliminated from the body—generally about 10 elimination half-lives. A sequence refers to the number of
different orders in the treatment groups in a study. For example, a two-sequence, two-period study would be designed as follows:

<table>
<thead>
<tr>
<th></th>
<th>Period 1</th>
<th>Period 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 1</td>
<td>T</td>
<td>R</td>
</tr>
<tr>
<td>Sequence 2</td>
<td>R</td>
<td>T</td>
</tr>
</tbody>
</table>

where R = reference and T = treatment.

Shows a design for three different drug treatment groups given in a three-period study with six different sequences. The order in which the drug treatments are given should not stay the same in order to prevent any bias in the data due to a residual effect from the previous treatment.

✔ **REPLICATED CROSS OVER DESIGN**

Replicated crossover designs are used for the determination of individual bioequivalence, to estimate within-subject variance for both the Test and Reference drug products, and to provide an estimate of the subject-by-formulation interaction variance. Generally, a four-period, two-sequence, two-formulation design is recommended by the FDA.

<table>
<thead>
<tr>
<th></th>
<th>Period 1</th>
<th>Period 2</th>
<th>Period 3</th>
<th>Period 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequence 1</td>
<td>T</td>
<td>R</td>
<td>T</td>
<td>R</td>
</tr>
<tr>
<td>Sequence 2</td>
<td>R</td>
<td>T</td>
<td>R</td>
<td>T</td>
</tr>
</tbody>
</table>

where R = reference and T = treatment.

The same reference and the same test are each given twice to the same subject. Other sequences are possible. In this design, Reference-to-Reference and Test-to-Test comparisons may also be made.

**Advantages of Cross over design:**

- Minimize intersubject variability in plasma drug level.
- Minimize intrasubject variability → affecting bioavailability of a subsequently administered product.
- Minimize variation due to time effect.
- Make it more possible to focus more on formulation variables which is the key to success for any bioequivalence study.

**Drawbacks of cross-over design:**

- Takes long time since appropriate washout period between 2 administrations is essential.
- Time may be longer if the drug has t1/2 long.
When the no. of formulations to be tested are more, the study becomes more difficult and subject dropout rate may increase.
This can be overcome by use of a balanced incomplete design in which a subject receives no more that two formulations.

2) **Balanced Incomplete Block Design (BIBD)**
- Each subject receives n.m.t 2 formulations
- Each formulation is administered same no. of times
- Each pair of formulations occurs together in the same number of subjects
- Four formulations :A,B,C,D
- Each formulation administered: 6 times
- Each subject receives: 2 formulations
- Each pair of formulation: AB,AC,AD,BC,BD,CD occurs together in 2 subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>3</td>
<td>A</td>
<td>C</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>5</td>
<td>A</td>
<td>D</td>
</tr>
<tr>
<td>6</td>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>7</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>8</td>
<td>C</td>
<td>B</td>
</tr>
<tr>
<td>9</td>
<td>B</td>
<td>D</td>
</tr>
<tr>
<td>10</td>
<td>D</td>
<td>B</td>
</tr>
<tr>
<td>11</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>12</td>
<td>D</td>
<td>C</td>
</tr>
</tbody>
</table>

3. **Completely randomized designs**
In a completely randomized design, all treatments (factor levels) are randomly allocated among all experimental subjects.

**Method of randomization**
Label all subjects with the same number of digits, for e.g., if there are 20 subjects, number them from 1 to 20. Randomly select non-repeating random numbers (like simple randomization) with among these labels for the first treatment, and then repeat for all other treatments.

**Advantages**
1) The design is extremely easy to construct.
2) It can accommodate any number of treatments and subjects.
3) The design is easy and simple to analyze even though the sample sizes might not be the same for each treatment.

Disadvantages
1) Although the design can be used for any number of treatments, it is best suited for situations in which there are relatively few treatments.
2) All subjects must be as homogeneous as possible. Any extraneous sources of variability will tend to inflate the random error term, making it difficult to detect differences among the treatment (or factor level) mean responses.

4. Randomized block designs
First, subjects are sorted into homogeneous groups, called blocks and the treatments are then assigned at random within the blocks.

Method of Randomization
Subjects having similar background characteristics are formed as blocks. Then treatments are randomized within each block, just like the simple randomization. Randomizations for different blocks are done independent of each other.

Advantages
1) With effective and systematic way of grouping, it can provide substantially more precise results than a completely randomized design of comparable size.
2) It can accommodate any number of treatments or replications.
3) Different treatments need not have equal sample size.
4) The statistical analysis is relatively simple. The design is easy to construct.
5) If an entire treatment or block needs to be dropped from the analysis for some reason, such as spoiled results, the analysis is not thereby complicated.
6) Variability in experimental units can be deliberately introduced to widen the range of validity of the experimental results without sacrificing the precision of results.

Disadvantages
1) Missing observations within a block require more complex analysis.
2) The degrees of freedom of experimental error are not as large as with a completely randomized design.

Evaluation of the data
The data is evaluated by following methods:
- By using analytical method
- Pharmacokinetics evaluation of the data
Statistical evaluation of the data
Analysis of variance (ANOVA)
One or two sided tests

The data has been collected; statistical methods must be applied to determine the level of significance of any observed difference in the rate and/or extent of absorption in order to establish bioequivalence between two or more drug products.

**Analysis of Variance (ANOVA)**

An analysis of variance (ANOVA) is a statistical procedure used to test the data for differences within and between treatment and control groups. A bioequivalent product should produce no significant difference in all pharmacokinetic parameters tested.

Typically, an Analysis of Variance (ANOVA) method is applied to determine statistical differences. If a statistically significant difference is observed, it is important to determine if it is clinically significant.

A statistical difference between the pharmacokinetic parameters obtained from two or more drug products is considered statistically significant if there is a probability of less than 1 in 20 times or 0.05 probability ($p \leq 0.05$) that these results would have happened on the basis of chance alone. The probability, $p$, is used to indicate the level of statistical significance. If $p < 0.05$, the differences between the two drug products are not considered statistically significant.

**Two One-Sided Tests Procedure**

The two one-sided tests procedure is also referred to as the *confidence interval approach*. This statistical method is used to demonstrate if the bioavailability of the drug from the Test formulation is too low or high in comparison to that of the Reference product. The objective of the approach is to determine if there are large differences (ie, greater than 20%) between the mean parameters.

The 90% confidence limits are estimated for the sample means. The interval estimate is based on a Student’s $t$ distribution of the data. The 90% confidence intervals about the ratio of the means for AUC and $C_{\text{max}}$ values of the Test drug product should not be less than 0.80 (80%) nor greater than 1.25 (125%) of that of the Reference product based on log-transformed data.

**2. In vitro bioequivalence study**

Equivalence may be assessed by the use of in vitro dissolution testing to confirm unchanged product quality and performance characteristics with minor formulation or manufacturing changes after approval.
3. Pharmacodynamic studies

Pharmacodynamic parameters may be used for establishing equivalence between two pharmaceutical products.

These studies may become necessary
1. If quantitative analysis of the drug and/or metabolite(s) in plasma or urine cannot be made with sufficient accuracy and sensitivity.
2. if measurement of drug concentrations cannot be used as surrogate endpoints for the demonstration of efficacy and safety of the particular pharmaceutical product e.g. topical products without an intended absorption of the drug into the systemic circulation.

4. Comparative clinical trials

These trials are carried out in following conditions:

- Plasma drug concentration time profile data may not be suitable to assess equivalence between two formulations.
- Pharmacodynamic studies cannot be performed.
- Pharmacodynamic and pharmacokinetics studies are not feasible.

Question: Discuss the regulatory requirements for conduction of bio-equivalence studies.

- Regulatory Requirements for Conduction of Bio-Equivalent Studies

Bioequivalence requirements may be imposed by the FDA on the basis of the following:

- Evidence from well-controlled clinical trials or controlled observations in patients that various drug products do not give comparable therapeutic effects.
- Evidence from well-controlled bioequivalence studies that such products are not bioequivalent drug products.
- Evidence that the drug products exhibit a narrow therapeutic ratio and minimum effective concentrations in the blood, and that safe and effective use of drug products requires careful dosage titration and patient monitoring.
- Competent medical determination that a lack of bioequivalence would have a serious adverse effect in the treatment or prevention of a serious disease or condition.
- Physicochemical evidence of the following:
  - The active drug ingredient has a low solubility in water, e.g., less than 5.0mg/ml.
  - The dissolution rate of one or more such products is slow, e.g., less than 50% in 30min when tested with a general method specified by the FDA.
  - The particle size and/or surface area of the active drug ingredient are critical in determining its bioavailability.
Certain structural forms of the active drug ingredient (e.g., polymorphic forms, solvates, complexes and crystal modifications) dissolve poorly, thus affecting absorption.

Such drug products have a high ratio of excipients to active ingredients, e.g., greater than 5 to 1.

Specific inactive ingredients (e.g., hydrophilic or hydrophobic excipients and lubricants) either may be required for absorption of the active drug ingredient or therapeutic moiety or may interfere with such absorption.

Pharmacokinetic evidence of the following:

- The active drug ingredient, therapeutic moiety, or its precursor is absorbed in large part in a particular segment of the GI tract or is absorbed from a localized site.
- The degree of absorption of the active drug ingredient, therapeutic moiety, or its precursor is poor (e.g., less than 50%, ordinarily in comparison to intravenous dose) even when it is administered in pure form.
- There is rapid metabolism of the therapeutic moiety in the intestinal wall or liver during the absorption process (first-order metabolism), so that the rate of absorption is unusually important in the therapeutic effect and/or toxicity of the drug product.
- The therapeutic moiety is rapidly metabolized or excreted so that rapid dissolution and absorption are required for effectiveness.
- The active drug ingredient or therapeutic moiety is unstable in specific portions of the GI tract and requires special coatings or formulations (e.g., buffers, enteric coating and film coatings) to ensure adequate absorption.
- The drug product is subject to dose-dependent kinetics in or near the therapeutic range, and the rate and extent of absorption are important to bioequivalence.

A summary of regulatory requirements of the American (U.S. Food and Drug Administration, USFDA) and European (the European Agency for the Evaluation of Medicinal Products, EMEA) is given in table.
Chapter 7

Dissolution

**Definition:** Dissolution rate may be defined as amount of drug substance that goes in the solution per unit time under standard conditions of liquid/solid interface, temperature and solvent composition. It can be considered as a specific type of certain heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid surface.

**FACTORS AFFECTING DISSOLUTION RATE**

1. Physicochemical Properties of Drug
2. Drug Product Formulation Factors
3. Processing Factors
4. Factors Relating Dissolution Apparatus
5. Factors Relating Dissolution Test Parameters

**PHYSICOCHEMICAL PROPERTIES OF DRUG**

**DRUG SOLUBILITY:** The solubility of the drug plays a major role in controlling its dissolution from the dosage form. The Minimum aqueous solubility of 1% is needed to avoid potential solubility limited absorption problems.

**PARTICLE SIZE:** There is a direct relationship between the surface area of the drug and its rate of dissolution. As the surface area increases with decreasing particle size, higher dissolution rates can be achieved by reducing the particle size. The micronization of sparingly soluble drug to reduce particle size results better dissolution and bioavailability.

**SALT FORMATION:** This is one of the common approaches used to increase the solubility and rate of dissolution of the drug. It has always been assumed that sodium salts dissolve more rapidly than their corresponding insoluble acids. For example, Sodium and potassium salts of Pencillin-G, sulfonamides, phenytoin, barbiturates, etc.

**SOLVATES AND HYDRATES:** A solvate is a molecular complex that has incorporated the crystallizing solvent molecules into specific sites in the crystal lattice. If the solvent is water, it is called a hydrate. The anhydrous compounds are highly soluble as hydrate. eg: anhydrous and hydrate forms of ampicillin.

**pH EFFECT:** The solubility of a weak acid drug or a weak basic drug is influenced by the pH of the fluid. Therefore, differences are expected in the solubility and rate of dissolution of these drugs in different regions of the GIT.
POLYMORPHISM AND AMORPHISM: When a substance exists in more than one crystalline form, the different forms are designated as polymorphs and the phenomenon as Polymorphism. Stable polymorph has lower energy state, and least aqueous solubility. Metastable polymorphs have higher energy state, and higher aqueous solubility. The dissolution rate is as such Amorphous > metastable > stable.

CO-PRECIPITATION: The rate of dissolution of sulfathiazole could be significantly increased by co-precipitating the drug with povidone.

COMPLEXATION: Complexation of a drug in GIT fluids can also alter the rate and the extent of absorption.

WETTING: Wettability of hydrophobic drugs measure by contact angle. High contact angle means poor Wettability and vice versa. A bile salt decrease contact angle of poorly soluble drugs in GIT (gastrointestinal tract) and further increase dissolution rate.

FACTORS RELATING DISSOLUTION APPARATUS

AGITATION: Relationship between intensity of agitation and rate of dissolution varies considerably according to type of agitation used; Speed of agitation generates a flow at liquid/solid interface between solvent and drug. In order to prevent turbulence, agitation should be maintained at a relatively low rate.

STIRRING ELEMENT ALIGNMENT: The USP / NP XV states that the axis of the stirring element must not deviate more than 0.2 mm from the axis of the dissolution vessel which defines centering of stirring shaft to within ± 2 mm, Tilt in excess of 1.5 may increase dissolution rate from 2 to 25%.

SAMPLING PROBE POSITION: Sampling probe can affect the hydrodynamic of the system & so that change in dissolution rate. For position of sampling, USP states that sample should be removed at approximately half the distance from the basket or paddle to the dissolution medium and not closer than 1 cm to the side of the flask.

FACTORS RELATED TO DISSOLUTION MEDIA

TEMPERATURE: Drug solubility is temperature dependent, therefore careful temperature control during dissolution process is extremely important. Generally, temp of 37°C ± 0.5 is maintained during dissolution of oral dosage forms and suppositories. However, for topical preparations temp as low as 30°C and 25°C have been used.

DISSOLUTION MEDIUM

pH of medium: pH varies with different locations of GIT and further influence saturation solubility of ionizable drugs. Specific gravity decrease leads to floating of powder which leads to wetting and penetration problem.
Dissolution media composition: For example additions of sodium sulfate decrease the dissolution rate. And addition of urea increase dissolution rate.

Volume of dissolution medium and sink conditions: Volume generally 500, 900 or 1,000 ml. and simulated gastric fluid (SGF) pH 1.2. Simulated intestinal fluid (SIF) pH 6.8 (not exceed pH 8.0) is generally used media.

FACTORS RELATED TO DRUG PRODUCT

DISINTEGRANTS: Disintegrating agent added before & after the granulation affects the dissolution rate. For example microcrystalline cellulose is a very good disintegrating agent but at high compression force, it may retard drug dissolution.

BINDERS: The hydrophilic binder increase dissolution rate of poorly wettable drug. But large amount of binder increase hardness & decrease disintegration/dissolution rate of tablet.

Lubricants: Lubricants are hydrophobic in nature (several metallic stearate & which inhibit wettability, penetration of water into tablet so decrease in disintegration and dissolution. The use of soluble lubricants like Sodium Lauryl Sulfate promote drug dissolution,

SURFACTANTS: They enhance the dissolution rate of poorly soluble drug. This is due to lowering of interfacial tension, increasing effective surface area, which in turn results in faster dissolution rate.

EFFECT OF COATING COMPONENT ON TABLET DISSOLUTION: Coating ingredients especially shellac & CAP etc. They also have significant effect on the dissolution rate of coated tablet.

PROCESSING FACTORS

METHOD OF GRANULATION: Granulation process in general enhances dissolution rate of poorly soluble drug.

COMPRESSION FORCE: The compression process influence density, porosity hardness, disintegration time & dissolution of tablet.

DRUG EXCIPIENT INTERACTION: These interactions occur during any unit operation such as mixing, milling, blending, drying, and/or granulating change in dissolution.
THEORIES OF DISSOLUTION

1) Diffusion Layer Model (Film Theory)
2) Danckwert’s Model (Penetration or Surface Renewal Theory)
3) Interfacial Barrier Model (Double Barrier Mechanism OR Limited Solvation Theory)

DIFFUSION LAYER MODEL (FILM THEORY):
- It is a simplest model where dissolution of crystal, immersed in liquid takes place without involving reactive or electrical forces. Consist of two consecutive steps:
- Solution of the solid to form a thin film or layer at the solid / liquid interface called as stagnant film or diffusion layer which is saturated with the drug this step is usually rapid (instantaneous).
- Diffusion of the soluble solute from the stagnant layer to the bulk of the solution this step is slower and is therefore the rate determining step in the drug dissolution.

DANCKWERT’S MODEL (PENETRATION OR SURFACE RENEWAL THEORY)
- This theory assumes that solid-soln equilibrium is achieved at interface and mass transport is slow step in dissoln process.
- The model could be visualized as a very thin film having a conc. Ci which is less than saturation, as it is constantly being exposed to fresh surfaces of liquid having a conc. much less than Ci. Acc. to model, the agitated fluid consist of mass of eddies or packets that are continuously being exposed to new surfaces of solid and then carried back to bulk of liquid.
- Diffusion occurs into each of these packets during short time in which the packet is in contact with surface of solid.
- Since turbulence actually extends to surface, there is no laminar boundary layer and so no stagnant film exists. Instead, surface continually being replaced with fresh liquid.
INTERFACIAL BARRIER MODEL (DOUBLE BARRIER OR LIMITED SOLVATION THEORY)

The Diffusion layer model and the Dankwert’s model were based on two assumptions:

1) The rate determining step that controls dissolution is the mass transport.

2) Solid solution equilibrium is achieved at the solid/liquid interface.

- According to interfacial barrier model, an intermediate conc. can exist at the interface as a result of solvation mechanism and is a function of solubility rather than diffusion.
- When considering the dissolution of the crystal will have a different interfacial barrier given by following equation,

\[ G = k_i (C_s - C_b) \]

Where \( G \) = dissolution per unit area \( k_i \) = effective interfacial transport constant

- In this theory, the diffusivity \( D \) may not be independent of saturation conc. \( C_s \).
- The interfacial barrier model can be extended to both Diffusion layer model and the Dankwert’s model.
IN-VITRO DRUG DISSOLUTION MODELS

Dissolution performance is influenced by physiochemical properties of the Substance and physiological conditions in the GIT. The technique which assure about the biologic availability of a drug is its in vitro dissolution test.

<table>
<thead>
<tr>
<th>Apparatus type</th>
<th>USP</th>
<th>BP</th>
<th>IP</th>
<th>Drug products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparatus 1</td>
<td>Rotating basket type</td>
<td>Rotating basket type</td>
<td>Paddle type</td>
<td>Beads, capsules, floating dosage forms</td>
</tr>
<tr>
<td>Apparatus 2</td>
<td>Paddle type</td>
<td>Paddle type</td>
<td>Rotating basket type</td>
<td>Tablet, capsules, enteric coated dosage forms</td>
</tr>
<tr>
<td>Apparatus 3</td>
<td>Reciprocating cylinder</td>
<td>Flow through cell</td>
<td></td>
<td>Controlled release drug products</td>
</tr>
<tr>
<td>Apparatus 4</td>
<td>Flow through cell</td>
<td></td>
<td></td>
<td>Low solubility drugs</td>
</tr>
<tr>
<td>Apparatus 5</td>
<td>Paddle over disk</td>
<td></td>
<td></td>
<td>Transdermal drug products</td>
</tr>
<tr>
<td>Apparatus 6</td>
<td>Cylinder type</td>
<td></td>
<td></td>
<td>Transdermal drug products</td>
</tr>
<tr>
<td>Apparatus 7</td>
<td>Reciprocating disk</td>
<td></td>
<td></td>
<td>Non disintegrating controlled release drug products</td>
</tr>
</tbody>
</table>

**Apparatus I (Rotating basket type):** In this apparatus rotating basket acts as a stirrer located cylindrical glass vessel with hemispherical bottom. This assembly is immersed in a water bath to maintain the temperature at 37°C. The most common rotating speed for the basket method is 100 rpm.

**Advantages**
- Full pH change can be made during the test.
- It can be easily automated which is important for routine work.

**Disadvantages**
- Hydrodynamic dead zone is created under the basket.
- Disintegration-dissolution interaction occurs.
- Sink conditions for poorly soluble drugs.
**Apparatus-2 (Paddle type):** In this apparatus rotating basket is replaced with a paddle which acts as a stirrer. The paddle is attached vertically to a speed motor that rotates at a controlled speed. This method is very sensitive to tilting. Improper alignment may drastically affect the dissolution results. The most common operating speed for apparatus 2 is 50 rpm for solid oral dosage forms and 25 rpm for suspension.

**Advantages**
- It is easy to use and robust.
- It can be easily adapted to apparatus 5.
- It can be easily automated which is important for routine investigation.

**Disadvantages**
- pH/media changes is difficult.
- Hydrodynamics are complex.

**USP apparatus 3 (Reciprocating cylinder):** It is considered as the first line apparatus product development of controlled release preparations. The assembly consists of cylindrical flat bottomed glass vessels, a set of glass reciprocating cylinder, stainless steel fitting screen and a motor. Dosage form unit is placed in each of the six reciprocating cylinders. Sample withdrawn from the surface of the dissolution medium and the bottom of each vessel.

**Advantages**
- It exposes the products to mechanical as well as a variety of physiochemical conditions which may influence the release of products in the GI tract.
- This apparatus is the technically easy and problem free use of test solutions with different pH values for each time interval. It also avoids cone formation for disintegrating (immediate release) products, which can be encountered with the USP apparatus 2.
- Feasibility of drug release testing of chewable tablets.
Apparatus 4 (Flow through cell): It consists of a reservoir for the dissolution medium and a pump that force dissolution medium through the cell holding the test sample. This apparatus is of two types:

1. Open flow cell: This system has a configuration where fresh medium is pumped through the cell and the fractions are collected every 30 to 60 minutes which result in rather high fraction volume. This is not practicable for the laboratory and therefore a volume splitting device (splitter) is used.

2. Close flow cell: This system has a configuration in which medium is pumped in circle and not replaced by fresh medium. Elute is collected in a beaker which is stirred by a magnetic stirrer. Readings can be taken online with spectrophotometer which measures the cumulative drug release.

Advantages

- Open type flow cell offers the advantages of ability to change the pH conveniently during the test.
- Closed flow cell is used for drug products having very low dosage strength and essentially it can be performed with very small volumes.

Apparatus 5 (Paddle over disk): This apparatus consists of disk assembly that hold the product in such a way that release surface is parallel with paddle. Paddle is directly attached over disk assembly for holding transdermal dosage form. Samples are withdrawn away from the surface of the medium and top of the paddle blade. The disk assembly is designed to minimize any dead volume between the disk assembly and the bottom of the vessel.

Apparatus 6 (Cylinder type): This apparatus is a modified form of apparatus 1. In place of the basket, a stainless steel cylinder is used to hold the sample. Transdermal patches can be studied but cannot be cut into small size.

Apparatus 7 (Reciprocating disk): In the reciprocating disk method for testing transdermal products a motor driven assembly is used to reciprocate the system vertically and the samples are placed on disk-shaped holders using cuprophan supports. The solution containers are partially immersed in a suitable water bath and maintained at 32±05°C.
<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Name</th>
<th>Apparatus design</th>
<th>Suitable for</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparatus 1</td>
<td>Rotating basket (In this Rotating stainless steel basket is used)</td>
<td>Basket</td>
<td>Tablets</td>
</tr>
<tr>
<td>Apparatus 2</td>
<td>Paddle (In this Rotating stainless steel metallic blade attached to shaft is used)</td>
<td>Paddle</td>
<td>Tablets, capsules, modified drug products, suspensions</td>
</tr>
<tr>
<td>Apparatus 3</td>
<td>Reciprocating cylinder (In this a set of glass reciprocating cylinders in Cylindrical flat bottomed glass vessel is used)</td>
<td>Drug</td>
<td>Extended-release drug products</td>
</tr>
<tr>
<td>Apparatus 4</td>
<td>Flow-through-cell</td>
<td></td>
<td>Drug products containing low-water-soluble drugs</td>
</tr>
<tr>
<td>Apparatus 5</td>
<td>Paddle over disk (it consist of rotating paddle over a disk)</td>
<td></td>
<td>Transdermal drug products</td>
</tr>
</tbody>
</table>
In Vitro In Vivo Correlation (IVIVC)

The in vitro in vivo correlation (IVIVC) is a scientific approach to describe the relationship between an in vitro property of a dosage form (e.g., the rate or extent of drug release) and a relevant in vivo response (e.g., plasma drug concentration or amount of drug absorbed). The IVIVC is needed to establish dissolution specifications and to support and/or validate the use of dissolution methods.

CORRELATION LEVELS

The concept of correlation has been decided based on their ability to reflect the plasma concentration time profile upon the administration of the drug. They are:

1. Level A
2. Level B
3. Level C

1. Level A correlation: This level of correlation is the highest category of correlation. It represents a point to point linear relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage forms. An in vitro dissolution curve can serve as a surrogate for in vivo performance. Therefore a change in manufacturing site, method of manufacturing, raw material supplies, minor formulation modifications. This is excellent quality control procedure.
2. **Level b correlation**: Level B IVIVC utilizes the principles of statistical moment analysis. In this level of correlation, the mean in vitro dissolution time (MDT vitro) (or) the mean in vivo dissolution time (MDT vivo). This is not considered a point to point correlation. Level B correlation does not uniquely reflect the actual in vivo plasma level curves therefore; one cannot rely upon a level B correlation alone to justify formulation modification, manufacturing sit, etc.

3. **Level C correlation**: In this level of correlation, one dissolution time point (t50%, t90%, etc) is compared to one mean pharmacokinetic parameter such as AUC, Tmax, (or) Cmax. Therefore it represents a single point correlation. It describes a relationship between the amount of drug dissolved at one time point and one pharmacokinetic parameter. This is the lowest level of correlation as partial relationship between the absorption and dissolution is established.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Level</th>
<th>In vitro</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A</td>
<td>Dissolution curve</td>
<td>Input (absorption curves)</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>Statistical moment, MDT</td>
<td>Statistical moments, MRT, MAT etc.</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>Disintegration time, Dissolution rate</td>
<td>C&lt;sub&gt;max&lt;/sub&gt;, t&lt;sub&gt;max&lt;/sub&gt;, k&lt;sub&gt;a&lt;/sub&gt;, AUC, time to achieve 10%, 50% and 90% drug absorbed</td>
</tr>
</tbody>
</table>
Chapter 8

Pharmacokinetics

Definition:
Pharmacokinetic is defined as the kinetics of drug absorption, distribution, metabolism and excretion (ADME) and their relationship with the pharmacologic, therapeutic or toxicologic response in human and animals.

PHARMACOKINETICS MODELS
A model is a hypothesis using mathematical terms to describe quantitative relationships. The mathematical model used to calculate absorption, distribution and elimination are known as pharmacokinetic model. The pharmacokinetic models express the time course of drug throughout the body. It is also used to predict concentration of drugs in body fluid after administering dose. It is also used to predict the behaviors of drugs in patients.

Applications of Pharmacokinetic Models –
Pharmacokinetic models are useful in —
1. Characterizing the behaviour of drugs in patients.
2. Predicting the concentration of drug in various body fluids with any dosage regimen.
3. Predicting the multiple-dose concentration curves from single dose experiments.
4. Calculating the optimum dosage regimen for individual patients.
5. Evaluating the risk of toxicity with certain dosage regimens.
6. Correlating plasma drug concentration with pharmacological response.
7. Evaluating the bioequivalence/bioinequivalence between different formulations of the same drug.
8. Estimating the possibility of drug and/or metabolite(s) accumulation in the body.
9. Determining the influence of altered physiology/disease state on drug ADME.
10. Explaining drug interactions.
Question: Enlist different pharmacokinetic models. What is compartment model? Mention advantages and disadvantages of the same.

What are pharmacokinetic models? Explain in detail non-compartmental approach.

❖ TYPE OF PHARMACOKINETICS MODELS

They are of three different types
1. Compartment models
2. Physiological models
3. Non-Compartment models

1. Compartment models:
It is assumed that the body consists of series of compartments. Each compartment may exchange material with other compartments. A compartment is not real physiologic or anatomic region but it is considered as tissues have same blood flow and affinity for drugs. The compartment is "well stirred" and distribution of drug is uniform and rapid within each Compartment. The rate of movement of drug within compartments follows first order kinetic. Rate constant are used to express the overall rate process of drugs come in and out from the Compartment. Compartment models are divided into Mammillary and Caternary Model.

a. Mammillary Model:
This model is the most common compartment model used in pharmacokinetics. The model consists of central compartment and peripheral compartments. The peripheral Compartments are connected to central compartment.

- Central Compartment: It comprise of blood and highly perfuse tissues like liver, lungs, kidneys, etc. that equilibrate with the drug rapidly. Elimination usually occurs from this compartment.
- peripheral compartment: It comprise of poorly perfuse and slow equilibrating tissues such as muscles, skin, adipose tissues etc. and considered as a hybrid of several functional physiological units.
2. **Caternary Model:** It consists of compartments which are joined to one another like compartments of a train. This model is rarely used.

**Advantages of compartment model:**

- The compartment model provides visual presentation of rate processes involved in drug disposition.
- This model enables to estimate drug concentration time profile in normal and pathological condition.
- It is also helpful in dosage form development of dosage regimen.
- It helps pharmacokineticist to write differential equation for each of the rate process to explain drug concentration changes in individual compartment.
Disadvantages of compartment model:

✓ The model may differ within a study population.
✓ The model is applicable to specific drug under observation.
✓ The model depends on curve fitting of plasma concentration with multiexponential mathematical equation.

2. Physiological models

In this model, absorption, distribution and elimination of drug is represented as series of organs or tissue spaces. The drug concentration profile has drawn from the uptake and elimination capacity of organs composing the body. The distribution of the drug to an organ based on the blood flow to the organ, the organ size and the partition coefficient of the drug between blood and the organ. The elimination capacities are based on the drug and the organ involved. The overall drug concentration profile results from the sum of the Processing of the drug by different organs. Lungs, liver, brain and kidney are rapidly equilibrating tissue (RET) and muscles and adipose are considered as slowly equilibrating tissue (SET).

Physiologic models are either blood flow rate limited model or membrane permeation rate limited model. The blood flow rate limited model are also called perfusion rate limited model. These models are applicable to low molecular weight, highly lipophilic and poorly ionized drugs such as lidocaine and thiopental etc. While Membrane permeation rate limited model are also called diffusion limited models. These models are applicable to highly polar, ionized and charged drugs.
Advantages of Physiological models

✓ It is more realistic model.
✓ The mathematical treatment is straightforward.
✓ No data fitting is required in this model.
✓ The model provides a better picture of drug concentration time profile in an organ or tissue.
✓ The extrapolation of animal data in the prediction of human pharmacokinetics is simple by using this model.

Disadvantages of Physiological models

It is very exhaustive process and monitoring of drug concentration in the body is difficult.

### TABLE 8.1. Comparison of features of compartment and physiological models

<table>
<thead>
<tr>
<th>Compartment Modelling</th>
<th>Physiological Modelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hypothetical/empirical approach – no relation with real physiology or anatomy</td>
<td>Realistic approach since it is based on physiological and anatomical information.</td>
</tr>
<tr>
<td>2. Experimentally simple and flexible approach as far as data collection is concerned</td>
<td>Difficult experimentally since exhaustive data collection is required.</td>
</tr>
<tr>
<td>3. Owing to its simplicity, it is widely used and is often the “first model”.</td>
<td>Less commonly used owing to complexity.</td>
</tr>
<tr>
<td>4. Complex multiexponential mathematical treatment is necessary for curve fitting.</td>
<td>Mathematical treatment is straightforward.</td>
</tr>
<tr>
<td>5. Data fitting is required for predicting drug concentration in a particular compartment.</td>
<td>Data fitting is not necessary since drug concentration in various tissues is practically determined.</td>
</tr>
<tr>
<td>6. Used when there is little information about the tissues.</td>
<td>Used where tissue drug concentration and binding are known.</td>
</tr>
<tr>
<td>7. Easy to monitor time course of drug in body with limited data.</td>
<td>Exhaustive data is required to monitor time course of drug in body.</td>
</tr>
<tr>
<td>8. Extrapolation from data to humans and vice versa is not possible.</td>
<td>Extrapolation of animal data to humans is easy on the basis of tissue concentration of drugs.</td>
</tr>
<tr>
<td>9. Mechanism of drug’s ADME cannot be explained.</td>
<td>Easy to explain drug’s ADME mechanisms.</td>
</tr>
<tr>
<td>10. Effect of pathological condition on drug ADME cannot be determined.</td>
<td>Effect of pathology on drug ADME can be easily determined.</td>
</tr>
</tbody>
</table>

3. Non Compartment Analysis

They are also considered as model-independent method because they do not rely upon assumptions about body compartments. It relies upon algebraic equations to detect Pharmacokinetic parameters and therefore making the analysis less complex. The blood or plasma samples are collected from study subjects and
analyzed. The noncompartmental approach, based on the statistical moments theory, involves collection of experimental data following a single dose of drug. The amount of drug in the body is proportional to the concentration in plasma at all time points. By making these substitutions, we can calculate MRT

\[
MRT = \frac{AUMC}{AUC}
\]

MRT = Mean Residence Time
AUMC = Area under first moment curve
AUC = Area under zero moment curve

MRT is defined as the average time spent by drug in the body before elimination. AUMC is obtained from plot of product of plasma drug concentration and time vs time (t) from zero to infinity. AUC is obtained from a plot of plasma drug concentration versus time from zero to infinity. Practically, the AUMC and AUC can be calculated from the respective graphs by the trapezoidal rule

MRT is defined as the average amount of time spent by the drug in the body before being eliminated. In this sense, it is the statistical moment analogy of half-life, \( t_{\frac{1}{2}} \). In effect, MRT represents the time for 63.2% of the intravenous bolus dose to be eliminated. The values will always be greater when the drug is administered in a fashion other than i.v. bolus.

Applications of noncompartmental technique includes —
1. It is widely used to estimate the important pharmacokinetic parameters like bioavailability, clearance and apparent volume of distribution.
2. The method is also useful in determining half-life, rate of absorption and first order absorption rate constant of the drug.

Advantages of noncompartmental method include —
1. The calculations involved simple algebraic equations.
2. The same mathematical expression can be applied to all drugs or metabolites that follow first-order kinetics.
3. A detailed description of drug disposition characteristics is not required.

Disadvantages of this method include —
1. It provides limited information regarding the plasma drug concentration-time profile. More often, it deals with averages.
2. The method does not adequately treat non-linear cases.
ONE COMPARTMENT OPEN MODEL

Question: Explain in detail one compartment model.

- The one-compartment open model is the simplest model which depicts the body as a single, kinetically homogeneous unit that has no barriers to the movement of drug and final distribution equilibrium between the drug in plasma and other body fluids is attained instantaneously and maintained at all the time.
- This model thus applies only to those drugs that distribute rapidly though out the body.
- The concentration of drug in plasma represents the drug concentration in all body tissues.
- The term “open” indicates that the input (availability) and output (elimination) are unidirectional and that the drug can be eliminated from the body.

![Diagram of One Compartment Open Model]

Depending upon the rate of input, several one-compartment open models can be defined:

- One-compartment open model, i.v. bolus administration
- One-compartment open model, continuous i.v. infusion
- One-compartment open model, e.v. administration, zero-order absorption
- One-compartment open model, e.v. administration, first-order absorption

❖ Intravenous bolus administration

- When a drug that distributes rapidly in the body is given in the form of a rapid intravenous injection (i.e IV bolus dose), it takes about 2 to 3 minutes for complete circulation and therefore the rate of absorption is neglected in calculations. The model can be depicted as follows:

![Diagram of Intravenous Bolus Administration]

- The general expression for rate of drug presentation to the body is:

\[
dX/dt = \text{Rate in (availability)} - \text{Rate out (elimination)} \ldots \ldots \ldots (1)
\]

- Since rate in or absorption is absent, the equation becomes:
\[ \frac{dX}{dt} = -\text{Rate out} \quad \text{(2)} \]

- If the rate out or elimination follows first-order kinetics, then:

\[ \frac{dX}{dt} = -K_E X \quad \text{(3)} \]

- where, \( K_E \) = first-order elimination rate constant, and

\( X = \) amount of drug in the body at any time \( t \) remaining to be eliminated.

- Negative sign indicates that the drug is being lost from the body.

**Estimation of Pharmacokinetic Parameters**

- For a drug that follows one-compartment kinetics and administered as rapid i.v. injection, the decline in plasma drug concentration is only due to elimination of drug from the body (and not due to distribution), the phase being called as elimination phase. **Elimination phase** can be characterized by 3 parameters—
  1. Elimination rate constant
  2. Elimination half-life
  3. Clearance.

- **Elimination Rate Constant**: Integration of equation 3 yields:

\[ \ln X = \ln X_0 - K_E t \quad \text{(4)} \]

- Where, \( X_0 = \) amount of drug at time \( t = 0 \) i.e. the initial amount of drug injected.

- Equation 4 can also be written in the exponential form as:

\[ X = X_0 e^{-K_E t} \quad \text{(5)} \]

- The above equation shows that **disposition of a drug that follows one-compartment kinetics is monoexponential**.

- Transforming equation 4 into common logarithms (log base 10), we get:

\[ \log X = \log X_0 - \frac{K_E t}{2.303} \quad \text{(6)} \]

- Since it is difficult to determine directly the amount of drug in the body \( X \), advantage is taken of the fact that a constant relationship exists between drug concentration in plasma \( C \) (easily measurable) and \( X \); thus:

\[ X = \frac{V_d}{C} \quad \text{(7)} \]

- where, \( V_d = \) proportionality constant popularly known as the **apparent volume of distribution**. It is a pharmacokinetic parameter that permits the use of plasma drug concentration in place of amount of drug in the body. The equation 6 therefore becomes:

\[ \log C = \log C_0 - \frac{K_E t}{2.303} \quad \text{(8)} \]
where, Co = plasma drug concentration immediately after i.v. injection.

Equation 8 is that of a straight line and indicates that a semilogarithmic plot of log C versus t will be linear with Y-intercept log Co. The elimination rate constant is directly obtained from the slope of the line (Fig. 1). It has units of min\(^{-1}\).

Elimination Half-Life (biological half-life):

- It is defined as the time taken for the amount of drug in the body as well as plasma concentration to decline by one-half or 50% its initial value. It is expressed in hours or minutes. Half-life is related to elimination rate constant by the following equation:

\[
\text{i.e. at time } \ t = 0 \quad C = C_0. \\
\text{and at time } \ t = t_{1/2} \quad C = \frac{C_0}{2}
\]

- Substituting the values of t and C in the logarithmic form of eqn. (iii) yields:

\[
\log (C_0/2) = \log C_0 - (k_E / 2.303) t_{1/2}.
\]

or,

\[
t_{1/2} = \frac{\log 2 \times 2.303}{k_E} = 0.301 \times 2.303 = 0.693
\]

\[
t_{1/2} = \frac{0.693}{k_E}
\]

- Elimination half-life can be readily obtained from the graph of log C versus t.
**Apparent Volume of Distribution:**

- Since these parameters are closely related with the physiologic mechanisms in the body, they are called as **primary parameters**.

  \[ V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}} = \frac{X}{C} \]

- It is determined by administering it by rapid i.v. injection and using the following equation:

  \[ V_d = \frac{X_0}{C_0} = \frac{i.v. \text{ bolus dose}}{\text{plasma concentration at time } t=0} \]

- The \( C_0 \) value is obtained by extrapolation of the plot of log(plasma conc) vs time.

- A more general, more useful non-compartmental method that can be applied to many compartment models for estimating the \( V_d \). \( V_d \) can be determined by another way if the AUC and the *first order elimination rate constant*, \( k_E \) is known.

- For drug given as IV bolus,

  \[ V_d = \frac{X_0}{k_E \cdot \text{AUC}} \]

- For drug given as extravascularly

  \[ V_d = \frac{FX_0}{k_E \cdot \text{AUC}} \]

- Where \( X_0 = \text{dose administered} \) and \( F = \text{fraction of drug absorbed into systemic circulation} \).

- The calculation of \( V_d \) by means of the above equation is *model independent* because no pharmacokinetic model is considered while calculating and the AUC is determined by the trapezoidal rule.

**Clearance:**

- Clearance is the most important parameter in clinical drug applications and is useful in evaluating the mechanism by which a drug is eliminated by the whole organism or by a particular organ.

- **Clearance** is defined as the theoretical volume of body fluid containing drug (i.e. that fraction of apparent volume of distribution) from which the drug is completely removed in a given period of time. It is expressed in ml/min or liters/hour.

  \[ \text{Clearance} = \frac{\text{Rate of elimination}}{\text{Plasma concentration}} = \frac{[\text{mg} / \text{min}]}{[\text{mg} / \text{ml}]} = [\text{ml} / \text{min}] \]

  \[ \text{CL} = \frac{(dX/ \text{dt})}{C} = \frac{-k_E X}{C} = \frac{-k_E V_d C}{C} = -k_E V_d \]

- The negative sign refers to the drug exiting from the body.
Total Body Clearance: (total systemic clearance) Elimination of a drug from the body involves processes occurring in kidney, liver, lungs and other eliminating organs. Clearance at an individual organ level is called as organ clearance. It can be estimated by dividing the rate of elimination by each organ with the concentration of drug presented to it. Thus,

**Renal clearance CIR** = Rate of Elimination by kidney/C

**Hepatic clearance CIH** = Rate of Elimination by liver/C

**Other organ clearance Clothers** = Rate of elimination by other organs/C

**Total Systemic Clearance, CL_T** = CL_R + CL_H + CL_Others

Clearance by all organs other than kidney is sometimes known as nonrenal clearance CI NR. It is the difference between total clearance and renal clearance.

\[ \text{CL}_T = \text{K}_E.X/C \]

Since \( X/C = V_d \) equation can be written as

\[ \text{CL}_T = \text{K}_E.V_d \]

Since \( K_E = 0.693 / t\frac{1}{2} \), clearance can be related to half-life by the following equation:

\[ \text{CL}_T = 0.693 \ V_d / t\frac{1}{2} \]

The non compartmental method of computing total clearance of a drug that follows one compartment kinetic is:

For drugs given as IV bolus,

\[ \text{CL}_T = X_0/AUC \]

For drugs administered extravascularly

\[ \text{CL}_T = F(0)/AUC \]

**ORGAN CLEARANCE**

Rate of elimination by organ= rate of presentation to the organ – rate of exit from the organ.

Rate of elimination = Q. C_in - Q. C_out

(Rate of extraction) = Q (C_in - C_out)

\[ \text{Cl} \ \text{organ} = \text{rate of extraction} / C_{in} \]

\[ = Q \ (C_{in} - C_{out}) / C_{in} \]

\[ = Q \ . \ Er \ \ldots \ldots \ldots \ldots \ldots \ldots \ (eq \ 1) \]
**Question:** Discuss extraction ratio and hepatic clearance in detail.

**Extraction ratio:**

$$ER = \frac{C_{in} - C_{out}}{C_{in}}$$

ER is an index of how efficiently the eliminating organs clear the blood flowing through it of drug.

It has no units and its value ranges from zero (no elimination) to one (complete elimination). Based on ER values, drugs can be classified into 3 groups:

- Drugs with high ER (above 0.7)
- Drugs with intermediate ER (between 0.7-0.3)
- Drugs with low ER (below 0.3)

The fraction of drug that escapes removal by organ is expressed as

$$F = 1 - ER$$

Where $F$ = systemic availability when the eliminating organ is liver.

**Hepatic Clearance:** For certain drugs, the nonrenal clearance can be assumed as equal to hepatic clearance $Cl_H$. It is given as:

$$Cl_H = Cl_T - Cl_R$$

An equation can also be written for hepatic clearance:

$$Cl_H = Q_H \cdot ER_H$$

where, $Q_H$ = hepatic blood flow (about 1.5 liters/min), and $ER_H$ = hepatic extraction ratio.

The hepatic clearance of drugs can be divided into two groups:

1. Drugs with hepatic blood flow rate-limited clearance, and
2. Drugs with intrinsic capacity-limited clearance.

**1. Hepatic blood flow**

- When $ER_H$ is one, $Cl_H$ approaches its maximum value. In such a situation, hepatic clearance is said to be perfusion rate limited or flow dependent.
- Alteration in hepatic blood flow significantly affects the elimination of drugs with high $ER_H$ example propanol, lidocaine, etc.
- First pass hepatic extraction is suspected when there is lack of unchanged drug in systemic circulation after oral administration.
- Maximum oral availability $F$ for such drugs can be computed from equation. An extension of the same equation is the non compartmental method of estimating $F$:

$$F = 1 - ER_H$$
2. Intrinsic Capacity Clearance

- It is defined as the inherent ability of an organ to irreversibly remove a drug in the absence of any flow limitation.
- It depends in this case upon the enzyme activity.
- Drugs with low ERH and drugs with elimination primarily by metabolism are greatly affected by enzyme activity.
- Hepatic clearance of such drugs is said to be capacity limited example theophylline.
- Hepatic clearance of drugs with low ER is independent of blood flow rate but sensitive to changes in protein binding.
Question: Discuss one compartment open model, I.V. infusion model and discuss the effect of loading IV injection dose. Describe the derivation of various pharmacokinetic parameters for the model.

One-Compartment Open Model

- **Intravenous Infusion**
  - Rapid IV injection is unsuitable when the drug has potential to precipitate toxicity or when maintenance of a stable concentration or amount of drug in the body is desired.
  - In such a situation, the drug is administered at a constant rate (zero order) by IV infusion.

Advantages of such a zero order infusion of drugs include-
- Ease of control of rate of infusion to fit individual patient needs.
- Prevents fluctuating plasma level (maxima and minima), desired especially when the drug has a narrow therapeutic index.
- Other drugs, electrolytes and nutrients can be conveniently administered simultaneously by the same infusion line in critically ill patients.

![Diagram of One-Compartment Open Model]

\[ \frac{dX}{dt} = R_0 - K_E X \]

\[ X = \frac{R_0}{K_E} (1 - e^{-K_E t}) \]

Since \( X = V_d C \), the equation 2 can be transformed into concentration terms as follows:

\[ C = \frac{R_0}{K_E V_d} (1 - e^{-K_E t}) = \frac{R_0}{Cl_t} (1 - e^{-K_E t}) \]
After infusion, as time passes, amount of drug rises gradually (elimination rate less than the rate of infusion) until a point after which the rate of elimination equals the rate of infusion i.e. the concentration of drug in plasma approaches a constant value called as **steady state, plateau** or **infusion equilibrium**.

At steady-state, the rate of change of amount of drug in the body is zero hence the **equation 3** becomes:

\[ \text{Zero} = R_0 - K_E X_{SS} \]

Therefore, \( K_E X_{SS} = R_0 \)

\[ C_{ss} = \frac{R_0}{K_E V_d} = \frac{R_0}{C_{IT}} \quad \text{i.e.} \quad \frac{\text{Infusion rate}}{\text{Clearance}} \]

\[ \text{(5)} \]

Where, \( X_{SS} \) and \( C_{SS} \) are amount of drug in the body and concentration of drug in plasma at steady state respectively.

The value of \( K_E \) (and hence \( t1/2 \)) can be obtained from the slope of straight line obtained after a semi logarithmic plot (log \( C \) versus \( T \)) of plasma concentration-time data gathered from the time when infusion is stopped.

Alternatively \( K_E \) can be calculated from the data collected during infusion to steady state as follows: Substituting \( R_0/C_{IT} = C_{SS} \) from equation 5 in equation 3 we get:

\[ C = C_{SS} (1-e^{-K_E t}) \quad \text{..........(6)} \]

Rearrangement yields:

\[ \frac{C_{SS} - C}{C_{SS}} = e^{-K_E t} \quad \text{..........(7)} \]
• Transforming to log form the equation becomes:

\[
\log \left[ \frac{C_{ss} - C}{C_{ss}} \right] = -\frac{K_E t}{2.303} \]  \hspace{1cm} (8)

• A plot of log (Css – C) / Css versus t results in a straight line with slope –Ke/2.303

\[
\log \left[ \frac{C_{ss} - C}{C_{ss}} \right] = \text{slope} = -\frac{K_E}{2.303}
\]

• The time to reach steady state concentration is dependent upon the elimination half life and not infusion rate.

• An increase in infusion rate will merely increase the plasma concentration attained at steady state.

• If n is the number of half-lives passed since the start of infusion (t/t1/2), equation 6 can be written as

\[
C = C_{ss} [1 - (1/2)^n] \]  \hspace{1cm} (9)

**Infusion plus Loading Dose**

• It takes a very long time for the drugs having longer half-lives before the plateau concentration is reached (e.g. Phenobarbital, 5 days).

• This can be overcome by administering an IV loading dose large enough to yield the desired steady state immediately upon injection prior to starting the infusion.

• It should then be followed immediately by IV infusion at a rate enough to maintain this concentration.
Recalling once again the relationship $X = V_d C$, the equation for computing the loading dose $X_{O,L}$ can be given:

$$X_{O,L} = C_{SS} V_d$$

Substitution of $C_{SS} = \frac{R_o}{K_E}$ from equation 37 in above equation yields another expression for loading dose in terms of infusion rate:

$$X_{O,L} = \frac{R_o}{K_E}$$

The equation describing the plasma concentration-time profile following simultaneous i.v. loading dose (i.v. bolus) and constant rate i.v. infusion is the sum of two equations describing each process.

$$C = \frac{X_{O,L}}{V_d} e^{-K_E t} + \frac{R_o}{K_E V_d} (1 - e^{-K_E t})$$

One-Compartment Open Model

- Extravascular Administration
  - When a drug is administered by extra vascular route (e.g. oral, rectal, etc.) absorption is a prerequisite for its therapeutic activity.
  - Absorption kinetics of drug may be first order or it may be zero order kinetics in rare cases.

- Zero order absorption is characterized by a constant rate of absorption. It is independent of amount of drug remaining to be absorbed (ARA), and its regular ARA versus t plot is linear with slope equal to rate of absorption while the semilog plot is described by an ever increasing gradient with time.
• In contrast, the first order absorption process is distinguished by a decline in the rate with ARA i.e. absorption rate is dependent upon ARA; its regular plot is curvilinear and semilog plot of a straight line with absorption rate constant as its slope.

• After extravascular administration, the rate of change in amount of drug in the body $dX/dt$ is the difference between the rate of input (absorption) $dX_{ev}/dt$ and rate of output (elimination) $dX_{e}/dt$

$$dX/dt = \text{Rate of absorption} - \text{Rate of elimination}$$

$$dx / dt = dx_a / dt - dx_e / dt \ldots \ldots \ldots (1)$$

• During the absorption phase, the rate of absorption is greater than the rate of elimination

$$dx_a / dt > dx_e / dt \ldots \ldots \ldots (a)$$

• At peak plasma concentration, the rate of absorption equals the rate of elimination and the change in amount of drug in the body is zero

$$dx_a / dt = dx_e / dt \ldots \ldots \ldots (b)$$

• During the post absorption phase, there is some drug at the extravascular site still remaining to be absorbed and the rate of elimination at this stage is greater than the absorption rate.

$$dx_a / dt < dx_e / dt \ldots \ldots \ldots (c)$$

• After completion of drug absorption, its rate becomes zero and the plasma level time curve is characterized only by the elimination phase.

Zero-Order Absorption Model

• This model is similar to that for constant rate infusion.

• Example of zero order absorption, rate of drug absorption for controlled drug delivery systems.
All equations that explain the plasma concentration-time profile for IV infusion are also applicable to this model.

First-Order Absorption Model

- For a drug that enters the body by a first-order absorption process, gets distributed in the body according to one-compartment kinetics and is eliminated by a first-order process, the model can be depicted as follows:

\[
\begin{align*}
\text{Drug at e.v. site} & \xrightarrow{K_a} \text{Blood and Other Body Tissues} & \xrightarrow{K_E} \text{Elimination} \\
\text{first-order absorption} & & \\
\end{align*}
\]

- The differential form of the equation

\[
\frac{dX}{dt} = K_a X_a - K_E X 
\]

\[
\text{…………(2)}
\]

where, \(K_a\) = first-order absorption rate constant, and \(X_a\) = amount of drug at the absorption site remaining to be absorbed i.e. ARA.

- Integration of equation 1 yields:

\[
X = \frac{K_a F X_0}{(K_a - K_E)} [e^{-K_E t} - e^{-K_a t}] \quad \text{…………(3)}
\]

- Transforming into concentration terms, the equation becomes:

\[
C = \frac{K_a F X_0}{V_d (K_a - K_E)} [e^{-K_E t} - e^{-K_a t}] \quad \text{…………(4)}
\]

Assessment of Pharmacokinetic Parameters

Cmax and Tmax:

- At peak plasma concentration \(K_a X_a = K_E X\) and the rate of change in plasma drug concentration \(dC/dt = 0\).

- On simplifying, the above equation becomes:

\[
K_E e^{-K_E t} = K_a e^{K_a t} \quad \text{…………(5)}
\]

- Converting to logarithmic form,
where \( t \) is \( t_{\text{max}} \). Rearrangement of above equation yields:

\[
t_{\text{max}} = \frac{2.303 \log \left( \frac{K_a}{K_E} \right)}{K_a - K_E}
\]

\[
\text{...........(7)}
\]

C\(_{\text{max}}\) can be obtained by substituting equation 7 in equation 4.

\[
C_{\text{max}} = \frac{F X_0}{V_d} e^{-K_E t_{\text{max}}}
\]

\[
\text{...........(8)}
\]

It has been shown that at \( C_{\text{max}} \), when \( K_a = K_E \), \( t_{\text{max}} = 1/K_E \). Hence, the above equation further reduces to:

\[
C_{\text{max}} = \frac{F X_0}{V_d} e^{-\frac{1}{K_E}} = \frac{0.37 F X_0}{V_d}
\]

\[
\text{...........(9)}
\]

**Elimination Rate Constant:**

- This parameter can be computed from the elimination phase of the plasma level time profile.
- For most drugs administered extravascularly, absorption rate is significantly greater than the elimination rate i.e. \( K_a \gg K_E \).
- Hence one can say \( e^{-K_A t} \) approaches zero must faster than does \( e^{-K_E t} \).
- The stage at which absorption is complete, change in plasma concentration is dependent on elimination rate and equation 4 reduces to:

\[
C = \frac{K_a F X_0}{V_d(K_a - K_E)} e^{-K_E t}
\]

\[
\text{...........(10)}
\]

Transforming to log form the equation becomes:

\[
\log C = \frac{K_a F X_0 e^{-K_E t}}{V_d(K_a - K_E)} - \frac{K_E t}{2.303}
\]

\[
\text{...........(11)}
\]

A plot of \( \log C \) versus \( t \) yields a straight line with slope \(-K_E/2.303\) (therefore, \( t_1/2 = 0.693/K_E \)).
Question: Describe the method of residuals for determination of absorption rate constant. Draw an illustrative diagram for that.

**Determination of Absorption Rate Constant (Ka):**

- It can be calculated by **method of residuals**.
- This technique is also known as **feathering, peeling** and **stripping**.
- It is commonly used in pharmacokinetics to resolve a multiexponential curve into its individual components.
- For a drug, that follows one compartment kinetic and administered extravascularly, the concentration of drug in plasma is expressed by a biexponential equation 4:

\[
C = \frac{K_a F X_0}{V_d (K_a - K_E)} \left[ e^{-K_E t} - e^{-K_{at}} \right]
\]

\[\text{...........(12)}\]

- IF \( K_a F X_0 / V_d (K_a - K_E) = A \) a hybrid constant, then:

\[
C = A \left[ e^{-K_E t} - e^{-K_{at}} \right] \text{...........(13)}
\]

- During the elimination phase, when absorption is almost over \( K_a >>> K_E \) and the value of second exponential \( e^{-K_E t} \) approaches zero whereas the first exponential \( e^{-K_{at}} \) retains some finite value. At this time equation 13 reduces to:

\[
C = A e^{-K_E t}
\]

\[\text{...............(14)}\]

- In log form above equation can be written as:

\[\text{Log } C = \text{log } A - K_E t \]

\[2.303 \text{ ...........(15)}\]

- Where \( \text{log } C \) represents the back extrapolate plasma concentration values.
- A plot of \( \text{log } C \) versus \( t \) yields a biexponential curve with a terminal linear phase having slope – \( K_E / 2.303 \) (figure 1).
- Back extrapolation of this straight line to time zero yields \( y \)-intercept equal to \( \text{log } A \).
Subtraction of true plasma concentration value i.e. equation 13 from the extrapolated plasma concentration values i.e. equation 14 yields a series of residual concentration values $C_r$:

$$ (C - C) = C_r = A e^{-K_a t} $$

In log form the equation is:

$$ \log C_r = \log A - \frac{K_a t}{2.303} $$

A plot of $\log C_r$ versus $t$ yields a straight line with slope $-K_a/2.303$ and $Y$ intercept $\log A$.

(Question: Explain Wagner nelson method in detail.)

**Wagner-Nelson Method for Estimation of Absorption Rate Constant ($K_a$):**

- One of the better alternatives to curve fitting method in the estimation of $K_a$ is Wagner-Nelson method.
- The method involves the determination of $K_a$ from percent unabsorbed time plots and does not require assumption of zero or first order absorption.
- After oral administration of a single dose of a drug, at any given time, the amount of drug absorbed into the systemic circulation $X_A$, is the sum of amount of drug in the body $X$ and the amount of drug eliminated from the body $X_E$. Thus

$$ X_A = X + X_E \ldots \ldots \ldots \ldots (1) $$
• The amount of drug in the body is \( X = V_d C \). The amount of drug eliminated at any time \( t \) can be calculated as follows:

\[
X_E = K_E V_d [AUC]_0^t
\]  

(2)

• Substitution of values of \( X \) and \( X_E \) in equation 1 yields:

\[
X_A = V_d C + K_E V_d [AUC]_0^t
\]  

(3)

• The total amount of drug absorbed into systemic circulation from time zero to infinity \( X_A^\infty \) can be given as:

\[
X_A^\infty = V_d C^\infty + K_E V_d [AUC]_0^\infty
\]  

(4)

• Since at \( t = \infty \), \( C^\infty = 0 \), the above equation reduces to:

\[
X_A^\infty = K_E V_d [AUC]_0^\infty
\]  

(5)

• The fraction of drug absorbed at any time \( t \) is given as:

\[
\frac{X_A}{X_A^\infty} = \frac{V_d C + K_E V_d [AUC]_0^t}{K_E V_d [AUC]_0^\infty}
\]

\[
= \frac{C + K_E [AUC]_0^t}{K_E [AUC]_0^\infty}
\]  

(6)

• Percent drug unabsorbed at any time is therefore:

\[
% ARA = \left[ 1 - \frac{X_A}{X_A^\infty} \right] 100
\]

\[
= \left[ 1 - \frac{C + K_E [AUC]_0^t}{K_E [AUC]_0^\infty} \right] 100
\]  

(7)
This method requires collection of blood samples after a single oral dose at regular intervals of time till the entire amount of drug is eliminated from the body.

- $K_E$ is obtained from plot of log C versus t and $[\text{AUC}]_0^t$ and $[\text{AUC}]_0^\infty$ are obtained from plots of C versus t.
- A semi log plot of percent unabsorbed (i.e. percent ARA) versus t yields a straight line whose slope is $-K_a/2.303$ (figure 1). If a regular plot of the same is a straight line, the absorption is zero order. $K_a$ can similarly be estimated from urinary excretion data.
- The biggest disadvantage of Wagner-Nelson method is that it applies only to drugs with one-compartment characteristics.
- Problem arises when a drug that obeys one compartment model after extra vascular administration shows multicomartment characteristics on IV injection.

**Question:** Give the criteria for obtaining valid urinary excretion method. What are the merits and demerits of using urinary excretion data for pharmacokinetic parameters?

**URINARY EXCRETION DATA**

**Advantages**

- Useful when there is lack of sufficiently sensitive analytical techniques to measure concentration of drug in plasma.
- Noninvasive method therefore better subject compliance.
- Convenience of collecting urine samples in comparison to drawing of blood periodically.
- If any case the urine drug concentration is low, assaying of larger sample volume is relatively more.
• Direct measurement of bioavailability, both absolute & relative is possible without the necessity of fitting the data to the mathematical model.

Criteria for Obtaining Valid Urinary Excretion Data
1. A significant amount of drug must be excreted unchanged in the urine (at least 10%).
2. The analytical method must be specific for the unchanged drug; metabolites should not interfere.
3. Water-loading should be done by taking 400 ml of water after fasting overnight, to promote diuresis and enable collection of sufficient urine samples.
4. Before administration of drug, the bladder must be emptied completely after 1 hour from water-loading and the urine sample taken as blank. The drug should then be administered with 200 ml of water and should be followed by 200 ml given at hourly intervals for the next 4 hours.
5. Volunteers must be instructed to completely empty their bladder while collecting urine samples.
6. Frequent sampling should be done in order to obtain a good curve.
7. During sampling, the exact time and volume of urine excreted should be noted.
8. An individual collection period should not exceed one biological half-life of the drug and ideally should be considerably less.
9. Urine samples must be collected for at least 7 biological half-lives in order to ensure collection of more than 99% of excreted drug.
10. Changes in urine pH and urine volume may alter the urinary excretion rate.

Question: Explain Sigma – Minus Method for determination of elimination rate constant.

Determination of KE from Urinary Excretion Data
The first-order elimination (and excretion) rate constants can be computed from urine data by two methods:
1. Rate of excretion method, and
2. Sigma-minus method.

Rate of Excretion Method:
• The rate of urinary drug excretion dXu/dt is proportional to the amount of drug in the body X and written as:

\[ \frac{dX_u}{dt} = K_E X \ldots \ldots \ldots \ldots \ldots \ldots \ldots (1) \]

• Where, \( K_E \) = first-order urinary excretion rate constant. According to first-order disposition kinetics, \( X = X_0 e^{-K_E t} \). Substituting it in above equation yields:
\[ \frac{dX_u}{dt} = K_E X_0 e^{-K_E t} \] ............(2)

- Where, \( X_0 \) = dose administered (i.v. bolus). Transforming to log form the equation becomes:

\[ \log \frac{dX_u}{dt} = \log K_E X_0 - K_E t / 2.303 \] ............(3)

- The above equation states that a semilog plot of rate of excretion versus time yields a straight line with slope \(-K_E/2.303\) (Fig.1).

**Sigma-Minus Method:**

- A disadvantage of rate of excretion method in estimating \( K_E \) is that fluctuations in the rate of drug elimination are observed to a high degree and in most instances, the data are so scattered that an estimate of half-life is difficult. These problems can be minimized by using the alternative approach called as sigma-minus method.

\[ \frac{dX_u}{dt} = K_E X_0 e^{-K_E t} \] ............(1)

- Integration of equation 1 yields:

\[ X_u = \frac{K_E X_0 (1-e^{-K_E t})}{K_E} \] ............(2)

- Where, \( X_u \) = cumulative amount of drug excreted unchanged in urine at any time \( t \).

- As time approaches infinity i.e. after 6 to 7 half-lives, the value \( e^{-K_E \infty} \) becomes zero and therefore the cumulative amount excreted at infinite time \( X_u^{\infty} \) can be given by equation:

\[ X_u^{\infty} = \frac{K_E X_0}{K_E} \] ............(3)

- Substitution of equation 9.75 in equation 9.74 and rearrangement yields:

\[ X_u^{\infty} - X_u = X_u^{\infty} e^{-K_E t} \] ............(4)
Converting to logarithms, we get:

\[
\log (X_u - X_u) = \log X_u - K_E t / 2.303
\]

where, \((X_u - X_u) = \text{amount remaining to be excreted}\) i.e. ARE at any given time. A semilog plot of ARE versus \(t\) yields a straight line with slope \(-K_E/2.303\). The method is, therefore, also called as ARE plot method.

MULTI-COMPARTMENT MODELS

- Ideally a true pharmacokinetic model should be the one with a rate constant for each tissue undergoing equilibrium.
- Therefore best approach is to pool together tissues on the basis of similarity in their distribution characteristics.
- The drug disposition occurs by first order.
- The one compartment model adequately describes pharmacokinetics of many drugs.
- Instantaneous distribution is assumed in such cases and decline in the amount of drug in the body with time is expressed by an equation with mono-exponential term (i.e. elimination).
- However, instantaneous distribution is not truly possible for an even larger number of drugs and drug disposition is not mono exponential but bi- or multi-exponential.
- This is because the body is composed of a heterogeneous group of tissues each with different degree of blood flow and affinity for drug and therefore different rates of equilibration.
- Multi-compartment characteristics are best described by administration as i.v bolus and observing the manner in which the plasma concentration declines with time.
- The number of exponentials required to describe such a plasma level-time profile determines the no. of kinetically homogeneous compartments into which a drug will distribute.

**TWO-COMPARTMENT OPEN MODEL**
The simplest and commonest of all multi-compartment models is the two compartment model which classifies the body tissues in two categories:
- Central compartment or compartment 1
- Peripheral or tissue compartment or compartment 2.
1. **Central Compartment** or **Compartment 1** comprising of blood and highly perfuse tissues like liver, lungs, kidneys, etc. that equilibrate with the drug rapidly. Elimination usually occurs from this compartment.

2. **Peripheral** or **Tissue Compartment** or **Compartment 2** comprising of poorly perfuse and slow equilibrating tissues such as muscles, skin, adipose, etc. and considered as a hybrid of several functional physiologic units.

The plasma concentration for a drug that follows a two-compartment model declines biexponentially as the sum of two first-order processes – distribution and elimination.

Depending upon the compartment from which the drug is eliminated, the two compartment model can be categorized into 3 types:

1. Two-compartment model with elimination from central compartment.
2. Two-compartment model with elimination from peripheral compartment.
3. Two-compartment model with elimination from both the compartments.

In the absence of information, elimination is assumed to occur exclusively from central compartment.
Two-Compartment Open Model

Intravenous Bolus Administration

- After the i.v. bolus of a drug that follows two-compartment kinetics, the decline in plasma concentration is biexponential indicating the presence of two disposition processes viz. distribution and elimination. These two processes are not evident to the eyes in a regular arithmetic plot but when a semilog plot of C versus t is made, they can be identified (Fig. 1).

- Initially, the concentration of drug in the central compartment declines rapidly; this is due to the distribution of drug from the central compartment to the peripheral compartment. The phase during which this occurs is therefore called as the distributive phase.

- After sometime, a pseudo-distribution equilibrium is achieved between the two compartments following which the subsequent loss of drug from the central compartment is slow and mainly due to elimination. This second, slower rate process is called as the post distributive or elimination phase.

- In contrast to the central compartment, the drug concentration in the peripheral compartment first increases and reaches a maximum. This corresponds with the distribution phase. Following peak, the drug concentration declines which corresponds to the post-distributive phase.
Let $K_{12}$ and $K_{21}$ be the first-order distribution rate constants depicting drug transfer between the central and the peripheral compartments and let subscript $c$ and $p$ define central and peripheral compartment respectively. The rate of change in drug concentration in the central compartment is given by:

$$\frac{dC_c}{dt} = K_{21}C_p - K_{12}C_c - K_E C_c \quad \ldots \ldots (1)$$

Extending the relationship $X = \frac{Vd}{C}$ to the above equation, we have

$$\frac{dC_c}{dt} = \frac{K_{21}X_p}{V_p} - \frac{K_{12}X_c}{V_c} - \frac{K_E X_c}{V_c} \quad \ldots \ldots (2)$$

Where, $X_c$ and $X_p$ are the amounts of drug in the central and peripheral compartments respectively and $V_c$ and $V_p$ are the apparent volumes of the central and the peripheral compartment respectively. The rate of change in drug concentration in the peripheral compartment is given by:

$$\frac{dC_p}{dt} = K_{12}C_c - K_{21}C_p \quad \ldots \ldots (3)$$

$$\frac{dC_p}{dt} = K_{12}\frac{X_c}{V_c} - K_{21}\frac{X_p}{V_p} \quad \ldots \ldots (4)$$

Integration of equations 2 and 4 yields equations that describe the concentration of drug in the central and peripheral compartments at any given time $t$:

$$C_c = X_0 \left( K_{21} - \alpha \right) e^{-\alpha t} + \left( K_{21} - \beta \right) e^{-\beta t}$$

$$V_c \left( \beta - \alpha \right) \quad (\alpha - \beta)$$

$$C_p = X_0 \frac{K_{21}}{V_p} e^{-\alpha t} + \frac{K_{21}}{V_p} e^{-\beta t}$$

$$\ldots \ldots (5)$$

Where, $X_0 = i.v.$ bolus dose, and are **hybrid first-order constants** for the rapid distribution phase and the slow elimination phase respectively which depend entirely upon the first-order constants $K_{12}$, $K_{21}$ and $K_E$.

The constants $K_{12}$ and $K_{21}$ that depict reversible transfer of drug between compartments are called as **micro constants** or **transfer constants**. The mathematical relationships between hybrid and micro constants are given as:

$$\alpha + \beta = K_{12} + K_{21} + K_E \quad \ldots \ldots (6)$$

$$\alpha \beta = K_{21} K_E \quad \ldots \ldots (7)$$

Now equation 5 is simplified as follows,

$$C_c = A e^{-\alpha t} + B e^{-\beta t} \quad \ldots \ldots (8)$$
\[ C_c = \text{Distribution exponent} + \text{Elimination component} \]

**Method of Residuals:** The biexponential disposition curve obtained after i.v. bolus of a drug that fits two compartment models can be resolved into its individual exponents by the method of residuals.

\[ C_c = A e^{-\alpha t} + B e^{-\beta t} \quad \text{.........(1)} \]

As apparent from the biexponential curve given in Fig. 1, the initial decline due to distribution is more rapid than the terminal decline due to elimination i.e. the rate constant \( \alpha >> \beta \) and hence the term \( e^{-\alpha t} \) approaches zero much faster than does \( e^{-\beta t} \). Thus, equation 1 reduces to:

\[ C = B e^{-\beta t} \quad \text{...........(2)} \]

In log form, the equation becomes:

\[ \log C = B - \beta t/2.303 \quad \text{..................(3)} \]

Where, \( C \) = back extrapolated plasma concentration values. A semilog plot of \( C \) versus \( t \) yields the terminal linear phase of the curve having slope \( -\beta/2.303 \) and when back extrapolated to time zero, yields \( Y \)-intercept \( \log B \) (Fig. 2). The \( t\frac{1}{2} \) for the elimination phase can be obtained from equation \( t\frac{1}{2} = \frac{0.693}{\beta} \).

Subtraction of extrapolated plasma concentration values of the elimination phase (equation 2) from the corresponding true plasma concentration values (equation 1) yields a series of residual concentration values \( C_r \).

\[ C_r = C - C = A e^{-\alpha t} \quad \text{...............(4)} \]

In log form, the equation becomes:

\[ \log C_r = \log A - \frac{\alpha t}{2.303} \quad \text{.......(5)} \]

A semilog plot of \( C_r \) versus \( t \) yields a straight line with slope \( -\alpha/2.303 \) and \( Y \)-intercept \( \log A \) (Fig. 2).
Assessment of Pharmacokinetic Parameters:

All the parameters of equation 1 can be resolved by the method of residuals as described above. Other parameters of the model viz. $K_{12}$, $K_{21}$, $K_E$, etc. can now be derived by proper substitution of these values.

\[
C_0 = A + B
\]

\[
K_E = \alpha \beta \frac{C_0}{A \beta + B \alpha}
\]

\[
K_{12} = AB \left(\beta - \alpha\right)^2 / C_0(A \beta + B \alpha)
\]

\[
K_{21} = A \beta + B \alpha
\]

Area under the plasma concentration-time curve can be obtained by the following equation:

\[
AUC = A + B
\]

The apparent volume of central compartment $V_c$ is given as:

\[
V_c = \frac{X_0}{C_0} = \frac{X_0}{K_E AUC}
\]

Apparent volume of peripheral compartment can be obtained from equation:

\[
V_p = V_c K_{12}
\]

The apparent volume of distribution at steady-state or equilibrium can now be defined as:

\[
V_{d,ss} = V_c + V_p
\]

It is also given as:
Total systemic clearance is given as:

\[ \text{Cl}_T = \beta V_d \]

The pharmacokinetic parameters can also be calculated by using urinary excretion data:

\[ \frac{dx_u}{dt} = K_e V_c \]

An equation identical to equation 1 can be derived for rate of excretion of unchanged drug in urine:

\[ \frac{dx_u}{dt} = K_e^{-\alpha t} + K_e^{-\beta t} \]

Renal clearance is given as:

\[ \text{Cl}_R = K_e V_c \]

**Two-Compartment Open Model**

- **Intravenous Infusion**

The model can be depicted as shown below with elimination from the central compartment.

![Two-Compartment Open Model Diagram]

The plasma or central compartment concentration of a drug that fits two-compartment model when administered as constant rate (zero-order) i.v. infusion is given by equation:

\[
C = \frac{R_0}{V_c K_E} \left[ 1 + \left( \frac{K_e - \beta}{\beta - \alpha} \right) e^{-\alpha t} + \left( \frac{K_e - \alpha}{\alpha - \beta} \right) e^{-\beta t} \right]
\]

\[ ..........(1) \]

At steady-state (i.e. at time infinity), the second and the third term in the bracket becomes zero and the equation reduces to:

\[ C_{ss} = \frac{R_0}{V_c K_E} \quad ..........(2) \]

Now \( V_c K_E = V_d \beta \). Substituting this in above equation, we get:

\[ C_{ss} = \frac{R_0}{V_d \beta} \quad \text{or} \quad C_{ss} = \frac{R_0}{\text{Cl}_T} \quad ..........(3) \]

The loading dose \( X_{0L} \) to obtain \( C_{ss} \) immediately at the start of infusion can be calculated from equation:

\[ X_{0L} = C_{ss} V_c = \frac{R_0}{K_E} \quad ..........(4) \]
Two-Compartment Open Model

- Extravascular Administration – First-Order Absorption

The model can be depicted as follows:

For a drug that enters the body by a first-order absorption process and distributed according to two-compartment model, the rate of change in drug concentration in the central compartment is described by 3 exponents—an absorption exponent, and the two usual exponents that describe drug disposition.

The plasma concentration at any time t is given by equation:

\[ C = N e^{K_a t} + L e^{-\alpha t} + M e^{-\beta t} \ldots \ldots (1) \]

\( C = \text{Absorption exponent} + \text{Distribution exponent} + \text{Elimination exponent} \)

where \( K_a, \alpha, \) and \( \beta \) have usual meanings. \( L, M \) and \( N \) are coefficients.

The 3 exponents can be resolved by stepwise application of method of residuals assuming \( K_a >> \) as shown in Fig. 3. The various pharmacokinetic parameters can then be estimated.
**LOO – RIEGELMAN METHOD**

\[ Ab = D_p + D_t + D_u \]

Loo -Riegelman method is useful in determining the absorption rate constant for a drug follows a two compartment model.

It requires the plasma concentration time data after i.v. bolus and oral administration to obtain all necessary kinetic constants.

This method can be applied to drug that can distributed by any number of compartments.

Each of these terms may be expressed in terms of kinetics constants and plasma drug concentrations, as follows:

\[ D_p = V_p C_p \quad (7.48) \]
\[ D_t = V_t C_t \quad (7.49) \]
\[ \frac{dD_u}{dt} = kV_p [AUC]_0 \quad (7.50) \]
\[ D_u = kV_p [AUC]_0 \]

Substituting the above expression for \( D_p \) and \( D_u \) into Equation 7.46,

\[ Ab = V_p C_p + D_t + kV_p [AUC]_0 \quad (7.51) \]

By dividing this equation by \( V_p \) to express the equation on drug concentrations, we obtain

\[ \frac{Ab}{V_p} = C_p + \frac{D_t}{V_p} + k[AUC]_0 \quad (7.52) \]

At \( t \to \infty \) this equation becomes

\[ \frac{Ab}{V_p} = k[AUC]_0 \quad (7.53) \]

Equation 7.53 divided by Equation 7.54 gives the fraction of drug absorbed at any time.

\[ \frac{Ab}{Ab_{\infty}} = \frac{C_p + \left( \frac{D_t}{V_p} \right) + k[AUC]_0}{k[AUC]_0} \quad (7.54) \]

A plot of the fraction of drug unabsorbed, \( 1 - Ab/Ab_{\infty} \), versus time gives \(-k/2.3\) as the slope from which the value for the absorption rate constant is obtained (refer to Eq. 7.34).

\( C_p \) and \( k[AUC]_0 \) are calculated from a plot of \( C_p \) versus time. Values for \( D_t / V_p \) can be approximated by the Loo–Riegelman method, as follows:

\[ \left( C_{p_t} \right) = \frac{k_{12} \Delta C_p \Delta t}{2} + \frac{k_{12}}{k_{21}} \left( C_p \right)_{t-1} \left( 1 - e^{-k_{12} \Delta t} \right) + \left( C_p \right)_{t-1} e^{-k_{12} \Delta t} \quad (7.55) \]

where \( C_p \) is \( D_t / V_p \), or apparent tissue concentration; \( t = \) time of sampling for sample \( n \); \( t_{n-1} = \) time of sampling for the sampling point preceding sample \( n \); and \( \left( C_p \right)_{t-1} \) is concentration of drug at central compartment for sample \( n - 1 \).
Chapter 9

Nonlinear Pharmacokinetics

The rate process of drug’s ADME is depending upon carrier or enzymes that are substrate specific, have definite capacities and are susceptible to saturation at a high drug concentration.

In such cases, an essentially first-order kinetics transform into a mixture of first-order and zero-order rate processes and the pharmacokinetic parameters are changed with the size of the administered dose. Pharmacokinetics of such drugs are said to be dose dependent. Terms synonymous with it are mixed-order, nonlinear and capacity-limited kinetics.

Question: Discuss various causes for non-linearity of drug.

❖ CAUSES OF NONLINEARITY

Nonlinearities can occur in drug absorption, distribution, metabolism and excretion.

Drug absorption

Generally, Non-linearity within drug absorption arise from 3 important factors,

I) When absorption is solubility / dissolution of drug is rate-limited - at high concentration or dose in GIT saturated solution of drug is formed. Moreover, at any other extravascular site and the rate of absorption achieve a constant value. For ex: Griseofulvin.

II) When absorption includes Carrier - mediated transport system - the saturation of transport system at higher doses of such vitamins shows non-linearity. For ex: Riboflavin, ascorbic acid, cyanocobalamin, etc.

III) Saturation due to Presystemic gut wall / hepatic metabolism - saturation of presystemic metabolism of drugs at high doses leads to increase bioavailability. For ex: Propranolol, verapamil, Hydrazine, etc.

Drug distribution

In drug distribution, at high doses non-linearity occurs due to the two prime factors:

I) Saturation of binding sites occurring on plasma proteins - For example, Phenylbutazone and naproxen. Due to limited number of binding sites of specific drug on plasma protein saturation occurs.

II) Saturation of tissue binding sites - e.g. thiopental and fentanyl. Tissue binding sites get saturated due to large single bolus doses or multiple dosing.
Drug metabolism
Non-linearity occurs due to capacity limited metabolism, small changes in dose administration large variations in plasma concentration at steady state. Two major cause of non-linearity in metabolism are,
I) Capacity limited metabolism due to enzyme and cofactor saturation - Drugs such as Phenytoin, Alcohol.
II) Enzyme induction - decrease in plasma concentration occurs after repetitive administration over a period of time. Auto indication characterized in this is also dose dependent. Therefore, enzyme induction is common cause of both dose and time dependent kinetics. Ex: Carbamazepine.

Drug excretion
Two active processes which are Saturable in renal excretion of drug include
I) Active tubular secretion - For Ex: Penicillin G, a renal clearance decrease is occur due to saturation of carrier system.
II) Active tubular reabsorption - after saturation of carrier system, an increase in water soluble vitamins and glucose causes increase in renal excretion.

Drugs that demonstrate saturation kinetics usually show the following
1. Elimination of drug does not follow Simple first order kinetics that is, elimination kinetics are nonlinear.
2. The elimination half-life changes as dose is increased. Usually, the elimination half life increases with increased dose due to saturation of an enzyme system.
3. The area under the curve (AVC) is not proportional to the amount of bioavailability drug.
4. The saturation of capacity-limited process may be affected by other drugs that require the same enzyme or carrier-mediated system (ie. competition effects).

Question: Discuss Michaelis Menten equation for nonlinear pharmacokinetics.

❖ METHOD OF ESTIMATING PARAMETRS

Michaelis Menten Method of Estimating Parameters
Nonlinear pharmacokinetics can be best described by Michaelis Menten Equation.
The kinetics of capacity-limited or saturable processes is best described by Michaelis - Menten equation:

\[
\frac{dC}{dt} = \frac{V_{max} C}{K_m + C}
\]
Where,

$$-\frac{dC}{dt} = \text{rate of decline of drug concentration with time},$$

$$V_{\text{max}} = \text{theoretical maximum rate of the process},$$

and

$$K_m = \text{Michaelis constant}.$$ 

Fig. 1 A plot of Michaelis-Menten equation

Three situations can now be considered depending upon the values of $K_m$ and $C$:

**When $K_m = C$**

Under this situation, the above equation reduced to:

$$-\frac{dC}{dt} = \frac{V_{\text{max}}}{2}$$

i.e. the rate of process is equal to one-half its maximum rate

**When $K_m >> C$**

In this condition, $K_m + C = K_m$ and the equation reduces to:

$$-\frac{dC}{dt} = \frac{V_{\text{max}} C}{K_m}$$

The above equation shows first-order elimination of drug where $V_{\text{max}} / K_m = KE$. It means normal dosage a regimen of many drugs is well below the $K_m$ of the elimination process with certain exception of some drugs.

**When $K_m << C$**

Under this condition, $K_m + C = C$ and the equation will become:

$$-\frac{dC}{dt} = V_{\text{max}}$$
The above equation is identical to the one that describes a zero-order process i.e. the rate process occurs at a constant rate Vmax and is independent of drug concentration.

**Estimation of Km and Vmax**

The parameters such as Km and Vmax can be calculated from the plasma concentration time data collected after i.v. bolus administration of a drug with nonlinear elimination kinetics by integrating equation.

Integration of Michaelis-Menten Equation

\[
\log C = \log Co + \left(\frac{Co - C}{V_{\text{max}}} \right) - \frac{V_{\text{max}}}{2.303Km}
\]

Semilog plot of C vs t yields a curve with terminal linear portion, which on back extrapolation to time zero gives y intercept log Co.

\[
\log C = \log Co - \frac{V_{\text{max}}}{2.303Km}
\]

![Semilog plot of a drug given as i.v. bolus with nonlinear elimination](image)

**Fig. 2** Semilog plot of a drug given as i.v. bolus with nonlinear elimination

An alternating approach of estimating Vmax & Km is determining rate of change of plasma drug conc. at different times & using the reciprocal of Michaelis-Menten Equation

\[
\frac{1}{V} = \frac{Km}{V_{\text{max}}C + 1} + \frac{1}{V_{\text{max}}}
\]

This is known as **double reciprocal plot** or **Lineweaver-Burke plot**.
When $1/V$ is plotted against $1/C$, a straight line is obtained with a slope of $K_m/V_{max}$ and an intercept of $1/V_{max}$.

A disadvantage of Lineweaver-Burke plot is that the points are clustered. More reliable plots in which the points are uniformly scattered are

**Hanes-Woolf plot**

\[
\frac{C}{V} = \frac{K_m \cdot 1}{V_{max} \cdot C} + \frac{C}{V_{max}}
\]

A plot of $C/V$ vs $C$ gives a straight line with $1/V_{max}$ as the slope and $K_m/V_{max}$ as the intercept (shown in the fig.).
Woolf-Augustinsson-Hofstee plot

\[
V = \frac{V_{\text{max}}C}{K_m + C}
\]

\[
V(K_m + C) = V_{\text{max}}C
\]

\[
VC + K_mV = V_{\text{max}}C
\]

\[
VC = V_{\text{max}}C - K_mV
\]

\[
V = \frac{(V_{\text{max}}C - K_mV)}{C}
\]

\[
\frac{V}{C} = \frac{V_{\text{max}}}{K_m}
\]

A plot of \( V \) vs \( V/C \) gives a straight line with a slope of - \( K_m \) and an intercept of \( V_{\text{max}} \)

**Km & V_max From Steady-state Concentration**

If drug is administered for constant rate IV infusion/ in a multiple dosage regimen, the steady-state conc. is given in terms of dosing rate (DR):

\[
\text{DR} = \text{Css} \cdot \text{Cl} \cdot T
\]

If the steady-state is reached, then the dosing rate = the rate of decline in plasma drug conc. & if the decline occurs due to a single capacity-limited process then eq. I become as:

From a plot of Css vs. DR, a typical curve having a shape of hockey-stick is obtained

\[
\text{DR} = \frac{V_{\text{max}} \cdot \text{Css}}{K_m + \text{Css}}
\]
There are three methods which are used to define the KM & Vmax at steady-state with appreciable accuracy:

1) Lineweaver-Burk Plot:-

The reciprocal of eq. we get

\[
\frac{1}{DR} = \frac{K_M}{V_{max} C_{ss}} + \frac{1}{V_{max}}
\]

If 1/DR is plotted against 1/Css a straight line is obtained having slope KM/Vmax & y-intercept 1/Vmax.

2) Direct linear plot:-

3. The third graphical method of estimating $K_m$ and $V_{max}$ involves rearranging the equation to yield:

$$DR = V_{max} - \frac{K_M D_R}{C_{ss}}$$

A plot of $DR$ versus $DR/C_{ss}$ yields a straight line with slope $-K_m$ and $Y$-intercept $V_{max}$.

KM & $V_{max}$ can be estimated by simultaneous eq. as

$$DR_1 = V_{max} - \frac{K_M D_{R1}}{C_{ss1}}$$

$$DR_2 = V_{max} - \frac{K_M D_{R2}}{C_{ss2}}$$

$$K_M = \frac{DR_2 - DR_1}{\frac{DR_1}{C_{ss1}} - \frac{DR_2}{C_{ss2}}}$$
By substituting values of DR1, DR2, Css1 & Css2 we get value of KM & from KM we can found value of Vmax at steady-state concentration.

From experimental observations, it shows that KM is much less variable than Vmax.

**Difference between linear and non linear pharmacokinetics**

<table>
<thead>
<tr>
<th>Linear Pharmacokinetics</th>
<th>Non Linear Pharmacokinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pharmacokinetic parameters for a drug would not change with change in dose</td>
<td>Pharmacokinetic parameters for a drug can change with change in dose.</td>
</tr>
<tr>
<td>Dose Independent</td>
<td>Dose dependent</td>
</tr>
<tr>
<td>First Order kinetics</td>
<td>Also called as Mixed order, Saturated kinetics, capacity limited</td>
</tr>
<tr>
<td>All semilog plots of C vs t for diff. doses are superimposable</td>
<td>Not superimposable</td>
</tr>
</tbody>
</table>