Syllabus

Module 01 **10Hours**

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.

Module 02 **10Hours**

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 Object ives 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.

Module 03 **10Hours**

Pharmacokinetics

- Definition and introduction to Pharmacokinetics, Compartment models, Non compartment models, physiological models, One compartment open model. Intravenous Injection (Bolus). Intravenous infusion. Extra vascular administrations.
- Pharmacokinetics parameters – KE ,t1/2,Vd,AUC,Ka, Clt and CLR - definitions methods of eliminations, understanding of their significance and application.

Module 04 **08 Hours**

Multicompartment Models

- Two compartment open model. IV bolus.
- Kinetics of multiple dosing, steady state drug levels, calculation of loading and mainetnance doses and their significance in clinical settins.

Module 05 **07 Hours**

Nonlinear Pharmacokinetics

- Introduction.
- Factors causing Non-linearity.
- Michaelis-menton method of estimating parameters, Explanation with example of drugs.

CHAPTER 1

Introduction to Biopharmaceutics and Absorption

1.1. BIOPHARMACEUTICS

1.1.1. Introduction

Drugs are used in the diagnosis, cure, mitigation, treatment, or prevention of diseases. They are given in various dosage forms, such as solids (tablets, capsules, etc.), semisolids (ointments, creams, etc.), liquids, suspensions, emulsions, etc., for systemic or local therapeutic activity. Drug products are drug delivery systems that deliver and release drug at the action site so that they produce the desired therapeutic effect. These products are also designed to ful fil the patient's requirements including palatability, convenience, and safety.

Release of drug substance from the drug product either for local drug action or for plasma drug absorption for systemic therapeutic action defines the drug product performance . Advancements in pharmaceutical technology and manufacturing have led to the development of safer, more effective, and more patient convenient quality drug products.

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

Biopharmaceutics is also defined as "**study of the factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimise the therapeutic efficacy of drug products**."

1.1.2. Applications of Biopharmaceutics

The field of biopharmaceutics has the following applications:

1) When a newly developed dosage form by a company is given to human beings, sometimes the drug is released slowly and sometimes the entire drug is released at a time; both these situations are not useful. Thus, the principles of biopharmaceutics are utilised to obtain the required action from the formulation.
2) When a company wishes to change the ingredients of tablet dosage forms, the altered ingredients will be approved by the FDA only if the bioavailability is equal to the initial formulation. Thus, the principles of biopharmaceutics are utilised to study the bioavailability of the new ingredients.
3) When a company wishes to change a drug's administration route from oral to transdermal, bioavailability of the drug is compared from both the routes and will be released only if the bioavailability is similar. Thus, the principles of biopharmaceutics are utilised to study the bioavailability of the drug.

1.2. ABSORPTION

1.2.1. Introduction

The desired therapeutic objective can be achieved if the drug product delivers the active drug at an optimal rate and extent. If a proper biopharmaceutical design is maintained, the rate and extent of drug absorption (or bioavailability) or the systemic delivery of drug into the body can be altered from rapid and complete absorption to slow and sustained absorption; however, this depends on the desired therapeutic action. The events that occur after a solid dosage form (a tablet or a capsule) is administered until its absorption in the systemic circulation are shown in the **figure 1.1**.

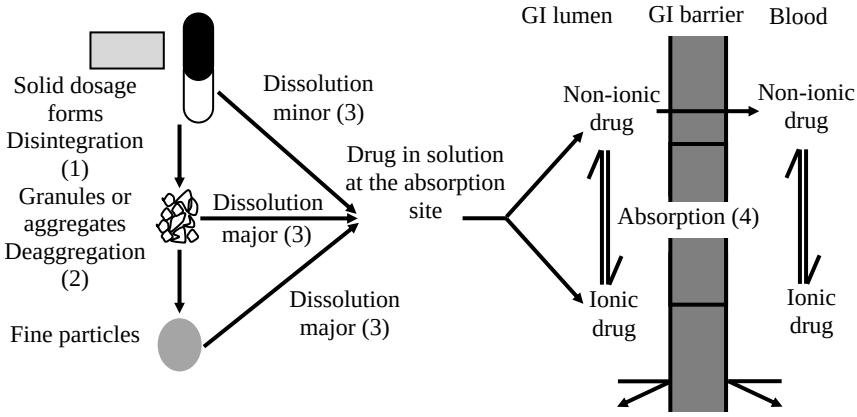


Figure 1.1: Sequence of Events in the Absorption of Drugs after Orally Administered Solid Dosage Forms

These events comprise of the following **four steps**:

1) **Disintegration** of the drug product,
2) **De-aggregation** and release of the drug,
3) **Dissolution** of the drug in aqueous fluids at the absorption site, and
4) **Movement** of the dissolved drug through the gastrointestinal membrane into the systemic circulation and away from the absorption site.

A drug molecule gets absorbed from the GIT and enters the systemic circulation only if it effectively penetrates all the intestinal regions. Once the drug enters the solution, its absorption is governed by the following three important factors:

1) The physicochemical properties of the drug molecule,
2) The properties and components of the GI fluids, and
3) The nature of the absorbing membrane.

1.2.2. Mechanisms of Drug Absorption Through GIT

The drug after entering the GI fluids exists in the form of a solution and can get absorbed. The drug's physicochemical properties (its inherent absorbability) and the environmental properties around it (such as pH, presence of interfering materials, and local properties of the absorbing membrane) decide whether or not the drug is in absorbable form. If there are no interfering materials (that affect

drug absorption), the drug molecule diffuses from the GI fluids to enter the absorbing membrane surface. A drug molecule gets absorbed from the GIT and enters the systemic circulation only if it effectively penetrates all the intestinal regions.

The pathways of drug transport show that drug absorption from the GI lumen into the systemic circulation involves the passage of drug molecules across several cellular membranes and fluid regions in the mucosa, i.e., the gastrointestinal-blood barrier. The GIT epithelium lining is the major cellular barrier to the absorption of drugs from the GIT. The drug molecules in GI fluids should cross the unstirred aqueous layer, mucus layer, and glycocalyx to reach the apical cell membrane.

The following **mechanisms** are involved in the transport of drug molecules across the cell membrane:

1) Passive diffusion,
2) Carrier-mediated transport:
   i) Facilitated diffusion, and
   ii) Active transport,
3) Pore transport,
4) Ionic or electrochemical diffusion,
5) Ion-pair transport, and
6) Endocytosis.

1.2.2.1. Passive Diffusion

Passive diffusion (or **non-ionic diffusion**) is the major process for absorption of more than 90% of drugs. It is defined as the difference in the drug concentration on either side of the membrane. **Concentration** or **electrochemical gradient** is the driving force for this process.

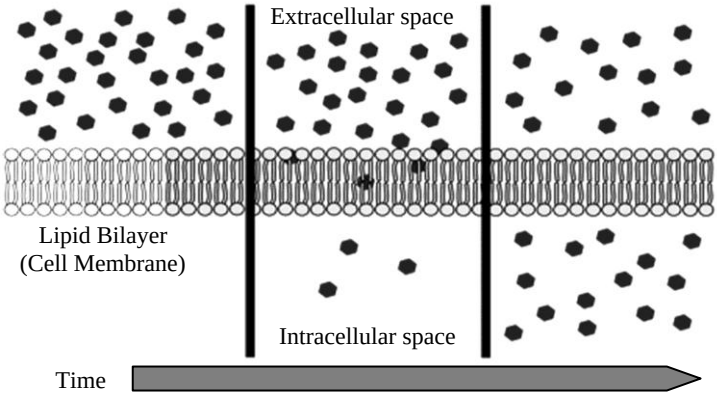


Figure 1.2: Passive Diffusion of a Drug

The kinetic energy of drug molecules is responsible for the movement of drug. Since no energy source is required, the process is called passive diffusion, during which the drug in the aqueous solution at the absorption site, partitions and dissolves in the lipid material of the membrane and leaves it by dissolving in an aqueous medium at the inside of the membrane.

Passive diffusion can be expressed by **Fick's first law of diffusion**, according to which the drug molecules diffuse from a region of higher concentration to lower concentration until equilibrium is achieved, and the rate of diffusion is directly proportional to the concentration gradient across the membrane. Fick's first law of diffusion is mathematically expressed as:

$$\frac{dQ}{dt} = \frac{DAK_{m/w}}{h}(C_{GIT} - C) \quad \text{.....(1)}$$

Where,

dQ/dt = Rate of drug diffusion (amount/time) or the rate of appearance of drug in blood.

D = Diffusion coefficient of the drug through the membrane (area/time).

A = Surface area of the absorbing membrane for drug diffusion (area).

$K_{m/w}$ = Partition coefficient of the drug between the lipid membrane and the aqueous GI fluids (no units).

$(C_{GIT} - C)$ = Concentration gradient (amount/volume), i.e., difference in the concentration of drug in the GI fluids and plasma.

h = Membrane thickness (length).

1.2.2.2. Carrier-Mediated Transport

Some polar drugs pass through the membrane more rapidly than can be predicted from their concentration gradient and p artition coefficient values. This is due to the presence of a specialised transport mechanism, without which monosaccharides, amino acids, and vitamins (essential water -soluble nutrients) will undergo poor absorption.

The mechanism involves a membrane component, called the **carrier** that reversibly or non -covalently binds to the solute molecules to be transported. This carrier -solute complex crosses the membrane to reach the other side, where it dissociates and releases the solute molecule; after which, th e carrier returns to its original site and accepts a fresh solute molecule, thereby completing the cycle.

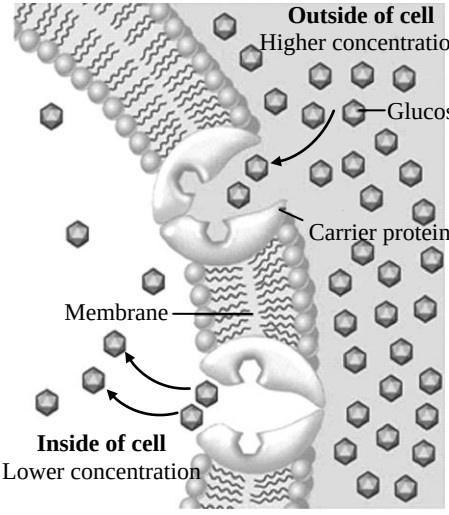


Figure 1.3: Carrier-Mediated Transport

Carrier-mediated transport system is of **two types**:

1) Facilitated diffusion, and
2) Active transport.

1.2.2.3. Facilitated Diffusion

Facilitated diffusion is a carrier -mediated transport system that works at a much faster rate than the passive diffusion. Concentration gradient is the driving force for this process that operates down the hill and thus is a passive process.

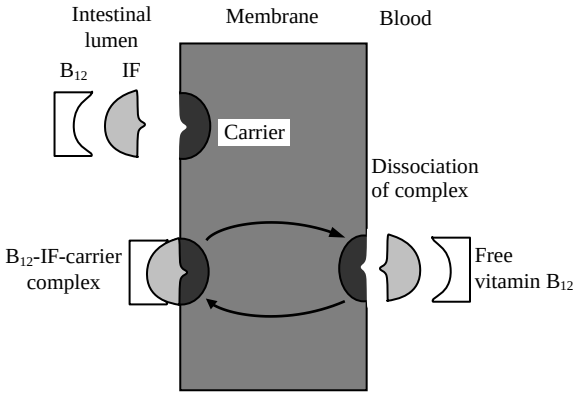


Figure 1.4: Facilitated Diffusion of Vitamin B_{12}

Since the process involves down-hill transport, there is no expenditure of energy, and the system is not inhibited by metabolic poisons. Some **applications** of facilitated diffusion system in drug absorption are:

1) Its major application involves entry of glucose into RBCs.
2) Another application involves intestinal absorption of vitamins B_1 and B_2.
3) The most significant application involves gastrointestinal absorption of vitamin B_{12} **(figure 1. 4)**, which forms a complex with glycopro tein [an Intrinsic Factor (IF) produced by gastric parietal cells]; and then this complex is transported across the intestinal membrane through a carrier system.

1.2.2.4. Active Transport

Active transport i nvolves movement of a substance from a region of low concentration to high concentration, i.e., against its concentration gradient. In all the cells, this is concerned with accumulating high concentrations of ions, glucose, and amino acids that the cell requires (**figure 1.5**).

Active transport utilises energy, unlike passive transport that does not use any type of energy. If it uses chemical energy from ATP, it is termed as **primary active transport**. If it uses an electrochemical gradient, it is termed as **secondary active transport**.

The significance of active transport pro cess in the absorption of nutrients and drugs is more than the facilitated diffusion, and both the processes differ in the following aspects:

1) In active transport, drug is transported from a region of lower concentration to a region of higher concentration, i.e., against the concentration gradient or uphill transport.
2) Active transport is an uphill process, thus energy is required in the work done by the carrier.
3) Active transport process requires energy, thus it can be inhibited by metabolic poisons (like flu orides, cyanides, di -nitrophenol, lack of oxygen, etc.) that interfere with energy production.

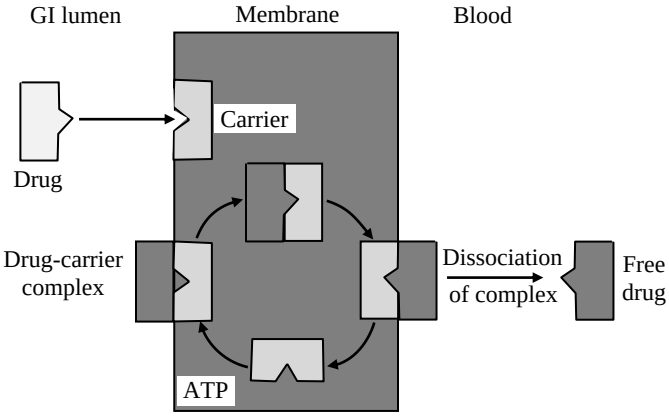


Figure 1.5: Active Absorption of a Drug

Sodium, potassium, calcium, iron, glucose, certain amino acids, and vitamins like niacin, pyridoxine and ascorbic acid are the endogenous substances that are actively transported. Drugs that are structurally similar to such agents (mainly the agents used in cancer chemotherapy) are actively absorbed. **For example,** absorption of 5-fluorouracil and 5-bromouracil through pyrimidine transport system; absorption of methyldopa and levodopa through L-amino acid transport system; and absorption of enalapril (ACE inhibitor) through small peptide carrier system.

An **example** of competitive inhibition of drug absorption through active transport is the impaired absorption of levodopa when taken with protein-rich meals. Active transport is also important in renal and biliary excretion of many drugs and their metabolites and secretion of certain acids out of the CNS.

1.2.2.5. Pore Transport

Pore transport (or **connective transport**, **bulk flow** or **filtration**) involves the absorption of low molecular weight, low molecular size, and water-soluble drugs (**e.g.,** urea, water, and sugar) through narrow, aqueous filled channels or pores in the membrane structure. This mechanism facilitates the transport of molecules into the cell through protein channels present in the cell membrane. Chain-like or linear compounds of 400 Daltons molecular weight can be observed by filtration. **Hydrostatic pressure** or the **osmotic differences** across the membrane is the **driving force** for this process. Due to this, bulk flow of water along with small solid molecules occurs through such aqueous channels. Water flux promoting such a transport is called as **solvent drag**. Drug permeation through water-filled channels is significant in renal excretion, removal of drug from the cerebrospinal fluid, and entry of drugs into the liver.

1.2.2.6. Ionic or Electrochemical Diffusion

Ionic or electrochemical diffusion involves the diffusion of ionic molecules across the membrane as a function of potential difference or electrical gradient; while **non-ionic diffusion** involves the diffusion of uncharged molecules. No matter ionic drug molecules diffuse through the membrane at a slower rate than the lipid-soluble, uncharged molecules, still they undergo significant absorption. The outer surface of membrane is positively charged, while its intracellular

surface carries negative charge. Cationic drugs, due to the repulsion between similarly charged molecules, show electrostatic repulsion on the outer surface of the membrane. Thus, the molecules with a high kinetic energy can only cross the membrane barrier. On t he other hand, cationic drugs inside the membrane undergo significant interaction with the negatively charged intracellular membrane, and create the electrical gradient, causing electrical diffusion. Electrochemical diffusion is the function of electrical field as well as the concentration difference across the membrane, and this process lasts till equilibrium is achieved.

1.2.2.7. Ion-Pair Transport

Drugs with a Zwitter ion undergo ionisation over the enti re pH range of the GIT, **e.g.,** ampicillin, amoxicillin, and tetracycline. Smaller ionic drugs travel through the water -filled pores; but, the Zwitter ionic drugs are large enough to pass through the water -filled pores and are also highly lipid insoluble to partition through lipoidal membrane. Thus, these drugs utilise their charge to pair with the endogenous ions of the GIT and cross the membrane. The resulting paired molecule partitions into the lipoidal membrane (**figure 1. 6**). These drugs are ionic, but sho w passive absorption and maximum partition coefficient when the net charge on the molecule is minimum.

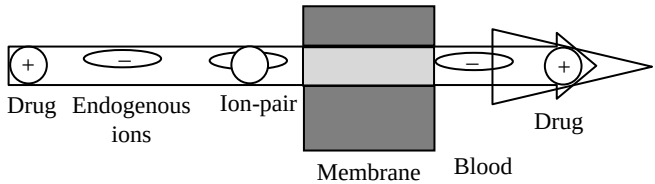


Figure 1.6: Ion Pair Transport of Cationic Drug

1.2.2.8. Endocytosis

Endocytosis is a minor transport mechanism in which the extracellular materials are engulfed within a segment of the cell membrane to form a saccule or a vesicle, which is then pinched -off intracellularly (**figure 1.7**). Thus, this process is also called as **corpuscular** or **vesicular transport**.

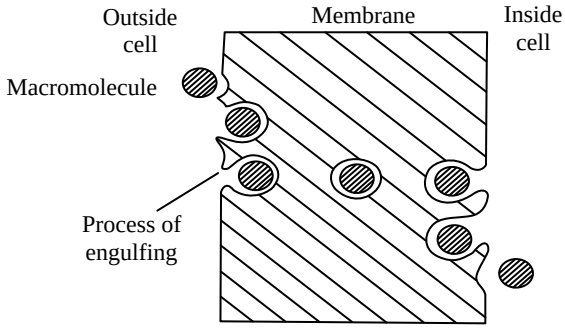


Figure 1.7: Endocytic Uptake of Macromolecules

The process of endocytosis helps in the cellular uptake of macromolecular nutrients (like fats and starch), oil -soluble vitamins (like A, D, E, and K), and drugs (like insulin). This process is also significant because since the drug is absorbed into the lymphatic circulation, it bypasse s first-pass hepatic metabolism.

Types of Endocytosis

The process of endocytosis is of the following **two types**:

1) **Phagocytosis (Cell Eating):** This process involves adsorptive uptake of solid particulates.
2) **Pinocytosis (Cell Drinking):** This process i nvolves uptake of fluid solute; **for example** , orally administered Sabin polio vaccine and large protein molecules are absorbed by pinocytosis.

At times, **transcytosis** occurs in which an endocytic vesicle is transferred from one extracellular compartment to another.

1.3. FACTORS INFLUENCING DRUG ABSORPTION THROUGH GIT

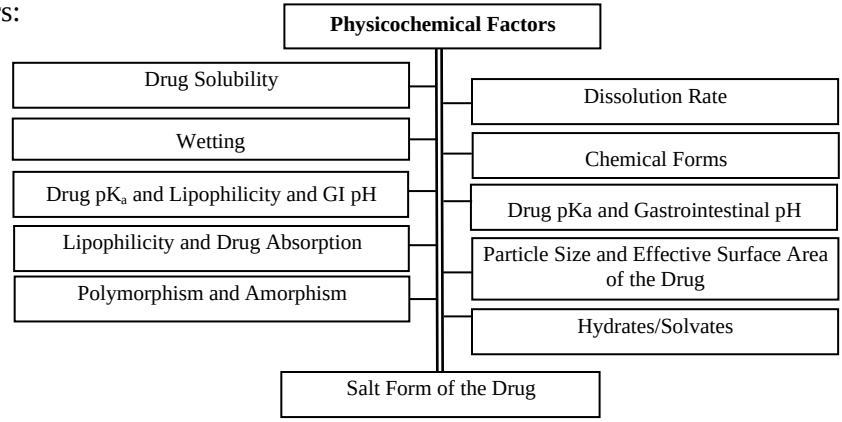
1.3.1. Introduction

The desired therapeutic objective is achieved when the drug product delivers the active drug at an optimal rate and extent, a nd at the target site. If a proper biopharmaceutical design is adopted, the rate and extent of drug absorption (i.e., bioavailability) or the systemic delivery of drug to the body can be varied from rapid and complete absorption to slow and sustained absorption depending on the desired therapeutic activity. The following factors influence drug absorption:

1) Physicochemical factors, and 2) Pharmaceutical factors.

1.3.2. Physicochemical Factors

Drug absorption through GIT is influenced by the following physicochemical factors:



1) **Drug Solubility:** Almost all the factors that influence dissolution rate, also affect the drug solubility either directly or indirectly. The dissolution rate is considered to be directly related to drug solubility. An empirical relation used for predicting the dissolution rate of a drug from its solubility is:

$$R = \frac{dc}{dt} = 2.24\ C_s$$ Where R = Dissolution rate of the drug.

Bioavailability problems can be avoided if the dr ug exhibits a minimum aqueous solubility of 1%.

An orally administered drug undergoes degradation due to the acidic or alkaline nature of gastrointestinal content, and the presence of enzymes and bacteria. **For example,** antibiotics are relatively stable be tween pH 6 -8 but get rapidly destroyed at gastric pH (1.5 -3.5). Longer is the gastric emptying time, more the drug is at risk to get degraded by acidic pH of the stomach. This problem of degradation can be avoided by enteric coating, but it delays drug rel ease and absorption. As an alter native, the drug is administered via other routes such as sublingual, transdermal, or rectal.

Due to degradation of drug, the following consequences may occur:

i) Little drug is absorbed, so reduced bioavailability.
ii) Toxicity increases upon degradation, **e.g.,** salicylic acid is more irritant than aspirin.
iii) Degradation process is essential for pro -drugs, because they release the therapeutically active molecule after degradation in the GIT.

The compromise between solubility and s tability of drug in gastrointestinal content is a key factor to determine the bioavailability of the orally administered drug. **For example,** Progabide is a weak base with pK a value of 3.41; it is highly soluble below pH 3 and poorly soluble above pH 4; it undergoes hydrolysis at acidic pH to release GABA and benzophenone; it has maximum solubility and undergoes rapid absorption from small intestine at pH 6.3. In such cases, the dosage form design and bioavailability is decided by considering the following **drug characteristics**:

i) Drug micronisation increases the drug solubility in small intestine (a better absorption site), but also increases the drug degradation rate in stomach due to increased surface area.
ii) Enteric coating protects the drug from acidic enviro nment, but the insoluble drug reaches the small intestine.
iii) The rapid gastric emptying of micronised drug ensures faster drug dissolution and minimum drug degradation.

2) **Dissolution Rate:** For the absorption of drug, it should be in solution state at the absorption site. Therefore, the factors influencing dissolution rate of the drug in gastrointestinal fluid also influence the drug bioavailability.

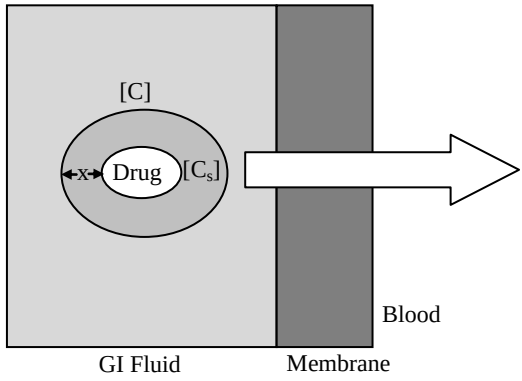


Figure 1.8: Dissolution Process of Drug in GIT

Dissolution rate is the amount of substance that goes into the solution per unit time under standard conditions of temperature, pressure, and solvent composition. Dissolution is a dynamic process and involves mass transfer.

The following **theories** explain the process of drug dissolution:

i) **Noyes-Whitney Theory or Stagnant Film Theory:** Noyes-Whitney equation describes the process of dissolution, and states that the dissolution rate of drug (d C/dt) is directly proportional to the diffusion coefficient of the drug in solution state in gastrointestinal fluid (D), effective surface area of the drug particle ava ilable for drug dissolution (A), difference in the saturation solubility of the drug in the diffusion layer (C_s), and the concentration of drug in solution state in the bulk of gastrointestinal fluid (C); while the dissolution rate of drug is inversely proportional to the thickness of diffusion layer (x) **(equation 2)**:

$$\frac{dC}{dt} = \frac{DA(C_s - C)}{x} \quad \text{..... (2)}$$

This equation is based on diffusion controlled dissolution process. The driving force for this first -order process is ($C_s - C$), but the *in vivo* conditions are different. Gastrointestinal motility affects the diffusion layer thickness by disturbing it. Rapid and continuous absorption of drug in blood circulation increases the concentration gradient. **Equation (2)** may be expressed as:

$$\frac{d_c}{dt} = \frac{D \cdot A \cdot C_S}{x} \quad \text{..... (3)}$$

This theory assumes that the surface area of the dissolving solute remains constant during dissolution; however, this is practically not possible. **Hixson** and **Crowell** modified the Noyes -Whitney equation to explain the effects of change in the surface area:

$$W_0^{1/3} - W^{1/3} = K \cdot t \quad \text{..... (4)}$$

Where,

W_0 = Initial powder weight.

W = Powder weight at time t.

K = Constant which is function of particle density, viscosity, diffusion coefficient, C_s and x.

Equation (4) is also termed as **Hixson and Crowell's Cubic Root Law**.

ii) **Danckwert's Model:** This model of dissolution does not consider the formation of diffusion layer and the solid surface is constantly exposed to fresh solvent causing mass transfer. It is assumed that the dissolution medium and the solid surface are under turbulence. T he agitated dissolution medium comprises of a mass of eddies or pockets, which are continuously exposed to the solid surface, absorb the solute by diffusion, and carry it to the bulk of the solution. The contact of fresh pockets with the solid surface tran sfers the mass to the bulk, and does not allow the formation of diffusion layer.

Since this dissolution process involves the contact of fresh pockets of solvent with new solid surface, it is termed as **surface renewal process**.

$$\frac{VdC}{dt} = \frac{dm}{dt} = A\sqrt{\gamma D\,(C_S - C)} \qquad \ldots\ldots (5)$$

Where,

V = Volume of dissolution medium.
m = Mass of solid dissolved.
γ = Rate of surface renewal or interfacial tension.

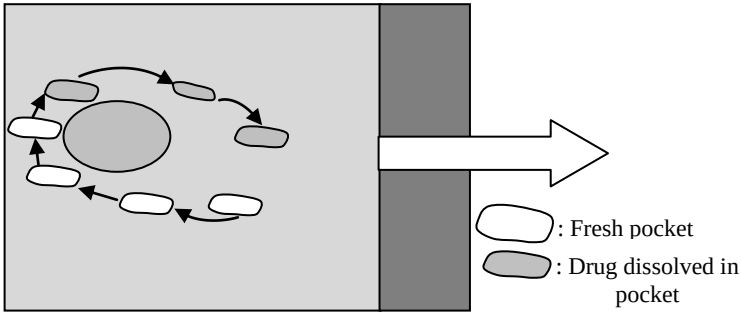


Figure 1.9: Surface Renewal Process of Dissolution

iii) **Limited So lvation Theory:** This theory states that an intermediate concentration less than saturation exist at the interface due to salvation mechanism, which is the function of solubility than that of the diffusion. In the dissolution of crystals, the different faces of a crystal have different interfacial barrier. Limited solvation theory is demonstrated by the following equation:

$$G = K_i \cdot (C_s - C) \qquad \ldots\ldots (6)$$

Were,

G = Dissolution rate per unit area.
K_i = Effective interfacial transport constant.

3) **Wetting:** Good wettability results in particle size reduction. Aggregation of powder and air adsorption on powder surface is minimised by adding a wetting agent. Surfactants and hydrophilic polymers have been added in dosage forms for enhancing drug dissolution and bioavailability.

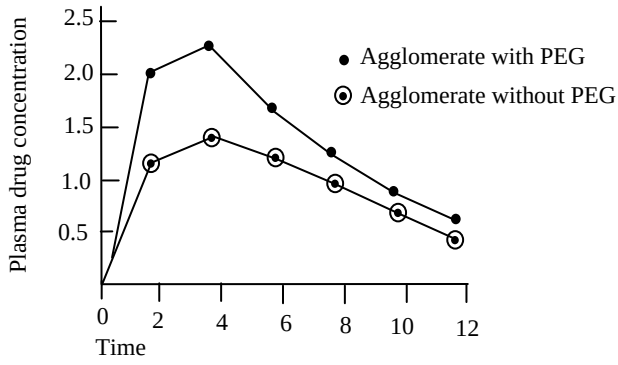


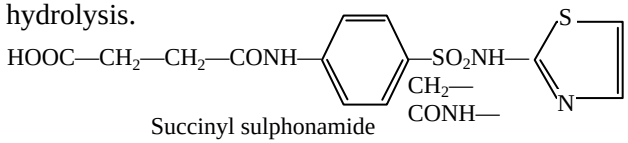
Figure 1.10: Effect of PEG on Bioavailability of Phenytoin

When phenytoin is agglomerated by spherical crystallisation, the formed agglomerates containing PEG show higher Area Under the blood concentration-time Curve (AUC) and C_{max}. A drug with poor wettability in water or in conventional dissolution media may have good wetting by gastrointestinal fluid. The native surfactants in GIT, such as bile salts, may also help in the wetting of drug.

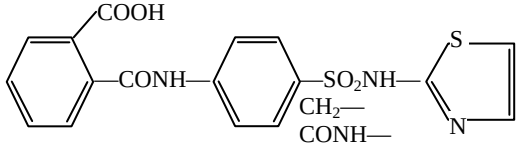
4) **Chemical Forms:** The desired effect can be achieved by selecting an appropriate chemical form of drug. Sometimes, chemical modifications are made in the structure of drugs to have better therapeutic response or effect than the parent drugs. These modified drugs exhibit the same therapeutic value as they breakdown in the body into active forms of the parent drugs. The **ideal conditions** for a **chemical form to act as a drug** are:
   i) It should have sufficient water solubility for dissolution.
   ii) It should have optimum o/w partition coefficient for diffusion through lipid layers.
   iii) Chemical modifications are made in the part of drug molecule that obstructs absorption.

Better therapeutic response can be achieved by implementing the following **chemical modifications** in the chemical forms:

i) **Specific Chemical Modifications:** These involve chemical modifications of drugs, **e.g.,** sulphonamides to succinyl sulphonamide or phthalyl sulphonamide. These chemical modifications in sulphonamides can ionise in the gut. Therefore, their lipid/water partition coefficient decreases, thus reducing the absorption of these chemically modified drugs. The antibacterial activity begins when the amide links are broken down by hydrolysis.

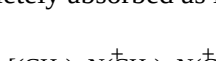


Succinyl sulphonamide

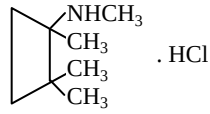


Phthalyl sulphonamide

In contrast, decreased ionisation results in better absorption; **for example**, hexa -methonium chloride (a quaternary amine) and mecamylamine hydrochloride (a tertiary amine) are steadily and completely absorbed as ionic species.



Hexamethonium chloride



Mecamylamine hydrochloride

ii) **Chemical Modifications to Increase Lipid Solubility:** In some drugs, chemical modifications are done to enhance their lipid solubility. **For example**, increase in lipid solubility of barbiturates is directly

proportional to their absorption from colon; doxycycline is better absorbed than its parent drug tetracycline; erythromycin is better absorbed than its estolate form.

Drugs	Partition Coefficient $CHCl_3/H_2O$	% of Absorption
Barbital	0.69	12.2
Apobarbital	4.88	17.2
Phenobarbital	4.78	20.1
Butabarbital	10.48	23.1
Butenal	11.67	23.9
Pentobarbital	28.02	29.9
Secobarbital	50.68	39.9
Hexabarbital	100.0	44.1

iii) **Salt Formation for Increasing Absorption:** Drugs are mostly weak acids or bases. The salts of such drugs have different solubility than their parent drugs. The solubilit y of such drugs can be easily enhanced by converting them into their salt forms. Some **examples** are:

Drugs	Salts of the Drug
Chloramphenicol	Chloramphenicol succinate
Menadiol	Menadiol phosphate
Oxazepam	Oxazepam semisuccinate
Testosterone	Testosterone phosphate
Tetracycline	Tetralysine
Theophylline	Diprophylline

a) The aluminium salt of aspirin undergoes very slow dissolution in the GIT due to the deposition of insoluble aluminium on the surface of solid particles. Due to this reason, the drug is only partially absorbed.

b) Dissolution rate of phenobarbital tablets is better than that of the tablets of sodium salt of phenobarbital because the former undergoes rapid disintegration while the latter swell and dissolve slowly from the surface.

c) Heptabarbital in tablet form attains the peak plasma level (C_{max}) in 1.5-5 hours (t_{max}). However, its sodium salt attains C_{max} at 0.4 -2 hours (t_{max}). Bioavailability of heptabarbital is up to 17% more than that of its sodium salt due to the precipitation of sodium salt of the drug in crystalline form.

5) **Drug pKa and Lipophilicity and GI pH (pH Partition Theory): Brodie** gave the pH partition theory to explain the absorption process of drug from GIT and its distribution across the biological membranes. This theory states that the absorption of drug compounds with molecular weight more than 100 Daltons involves transportation across the biomembrane by passive diffusion, and this process depends on:

i) The dissociation constant (pKa) of the drug,

ii) The lipid solubility ($K_{o/w}$) of unionised drug, and

iii) The pH at absorption site.

Drugs are mostly either weak acids or weak bases; thus, their ionisation degree is influenced by the pH of biological fluid. If the pH on either side of the membrane is different, the compartment whose pH favours greater ionisation of drug will have greater amount of drug. Also, the unionised or un-dissociated fraction of the drug, if is sufficiently lipid-soluble, will permeate the membrane by passive diffusion until equilibrium is attained between the concentrations of unionised drug on either side of the membrane.

The pH partition theory lies on the following **assumptions**:

i) GIT acts as a lipoidal barrier to the transport of drug.
ii) The absorption rate of a drug is directly proportional to the fraction of unionised drug.
iii) Higher the lipophilicity ($K_{o/w}$) of the unionised drug, better is its absorption.

Limitations of pH-Partition Theory

i) The pH-partition theory is based on the assumption that equilibrium is achieved by the distribution between the ionised and unionised forms of a drug. However, drug is continuously swept away due to blood circulation, thus maintaining sink condition. This theory therefore failed in calculating the absorption from equilibrium distribution.
ii) This theory describes absorption of weak acids and weak bases more suitably.
iii) Ionisation of drug in the lumen occurs similarly as in blood, but ion trapping may also occur.
iv) Absorption from GIT depends on pH of all sites, drug solubility in lipid, gastric residence, absorption surface area, drug degradation, etc.
v) Weak acidic drugs are better absorbed from small intestine, because:
   a) Poorly soluble weakly acidic drug has a high dissolution rate in an alkaline environment. This results in availability of higher surface area for absorption of the dissolved drug.
   b) The weakly acidic pH (pH 5.3) at surface of the intestinal mucosa is responsible for effective absorption of weakly acidic drug that exists in unionised state. Thus, this hypothesis raises questions regarding the absorption of weakly basic drug that exists in unionised state. These weak bases can interact with organic cations and are secreted from blood into the intestinal lumen.

   A poorly water-soluble, weakly basic drug,which dissociates and dissolves in stomach to a greater extent, also shows better absorption, when it reaches to small intestine. The delayed gastric emptying rate would permit a longer time for dissolution of weakly basic drug in acidic fluid and in turn would increase its absorption when it reaches the small intestine.**For example,** absorption of nitrofurantoin is increased in the presence of food.
vi) Solubility of unionised form of drugs is the rate determining step in the absorption of a drug, but presence of unionised drug is not the sole

concept, **e.g.,** derivatives of barbituric acid h ave almost same pK a, but have different lipid solubilities, which can be ranked as barbital < apobarbital < pentobarbital.

vii) Few drugs show good solubility in all parts of GI T, despite of good ionisation, **e.g.,** theophylline and tetracycline.

6) **Drug pKa and Gas trointestinal pH:** The dissociation constant (pKa) of drug and the pH of body fluid at the absorption site influence the amount of unionised drug. The unionised form of drug is also most suitable for absorption in GIT. The relative amounts of ionised and un ionised drug in a solution at a particular pH can be obtained from the pKa value of drug and pH of body fluid at the absorption site, by using the Henderson and Hasselbalch equation:

For Weak Acids

$$pH = pKa + \log \frac{\text{Ionized drug concentration}}{\text{Unionized drug concentration}} \quad \text{.....(7)}$$

$$\% \text{ of drug ionised} = \frac{10^{pH - pK_a}}{1+10^{pH - pK_a}} \times 100 \quad \text{.....(8)}$$

$$\% \text{ of drug unionised} = 100 - \frac{10^{pH-pK_a}}{1+10^{pH-pK_a}} \times 100 \quad \text{..... (9)}$$

For Weak Bases

$$pH = pK_a + \log \frac{\text{Unionized drug concentration}}{\text{Ionized drug concentration}} \quad \text{..... (10)}$$

$$\% \text{ of drug ionised} = \frac{10^{pK_a - pH}}{1+10^{pk_a - pH}} \times 100 \quad \text{..... (11)}$$

$$\% \text{ of drug unionised} = 100 - \frac{10^{pK_a - pH}}{1+10^{pK_a - pH}} \times 100 \quad \text{..... (12)}$$

If the concentrations of ionised and unionised drug are equal, the second term of **equations (8)** and **(11)** becomes zero ($\because \log 1 = 0$), and therefore pKa = pH. Thus, pKa is the characteristic of the drug. If a membrane barrier separates the aqueous solution of different pH (such as the GIT and plasma), the theoretica l ratio of drug concentration (R_a or R_b) on either side of the membrane can be obtained by the equations developed by **Shore *et al***.

For Weak Acids

$$R_a = \frac{C_{GIT}}{C_{Plasma}} = \frac{1+10^{pH_{Git} - pK_a}}{1+10^{pH_{Plasma} - pK_a}} \quad \text{..... (13)}$$

For Weak Bases

$$R_b = \frac{C_{GIT}}{C_{Plasma}} = \frac{1+10^{pK_a - pH_{Git}}}{1+10^{pK_a - pH_{Plasma}}} \quad \text{..... (14)}$$

If the pH ranges from 1 to 8 in the GIT, from 1 to 3 in the stomach, and from 5 to 8 in the intestine (duodenum to colon), certain generalisations about ionisation and absorption of drugs can be made from the pH -partition hypothesis.

For Weak Acids

i) Weakly acidic drugs with pKa > 8, **e.g.,** phenytoin, ethosuximide, and several barbiturates, are unionised at all pH values and hence their absorption rate is rapid and independent of gastrointestinal pH.
ii) Weakly acidic drugs with pKa ranging from 2.5 to 7.5, **e.g.,** aspirin, ibuprofen, ph enylbutazone, and penicillin analogues, undergo pH - dependent absorption. At acidic conditions of stomach (pH > pKa), they exist in unionised form and undergo better absorption.
iii) Strongly acidic drugs with pKa < 2.5, **e.g.,** cromolyn sodium, are ionised in the entire pH range of GIT and hence are poorly absorbed.

For Basic Drugs

i) Weakly basic drugs with pKa < 5.0, **e.g.,** caffeine, theophylline, diazepam, oxazepam, and nitrazepam, are ionised at all pH values and hence undergo rapid and pH-independent absorption.
ii) Weakly basic drugs with pKa ranging from 5 to 11 , **e.g.,** morphine analogues, chloroquine, imipramine, and amitriptyline, undergo pH - dependent absorption. At alkaline conditions of the intestine, they exist in unionised form and undergo better absorption.
iii) Strongly basic drugs with pKa > 11, **e.g.,** mecamylamine and guanethidine, remain ionised in the entire pH range of GIT and hence are poorly absorbed.

7) **Lipophilicity and Drug Absorption:** If a drug exists in unionised form, it will still undergo poor absorption , provided its lipid solubility is low, i.e., it has a low value of $K_{o/w}$. Thus, for a drug to undergo optimum absorption, it should be sufficiently soluble in aqueous medium at the absorption site and also highly lipid-soluble to bring about partitioning of the drug in the lipoidal biomembrane and into systemic circulation. Thus, for optimum bioavailability a perfect hydrophilic -lipophilic balance should exist in the drug structure.

The lipid solubility of a drug can be determined from its oil/water partit ion coefficient value ($K_{o/w}$).

8) **Particle Size and Effective Surface Area of the Drug:** A solid drug's particle size and surface area are inversely proportional. Smaller the drug particle, greater is its surface area. Two types of surface area are:
i) **Absolute Surface Area:** Total area of solid surface of any particle, and
ii) **Effective Surface Area:** Area of solid surface exposed to the dissolution medium.

With micronisation, the dose of certain drugs can be decreased because of increased absorption efficiency, **for example,** griseofulvin dose was reduced to half and spironolactone dose was reduced 20 times by micronisation.

However in hydrophobic drugs (like aspirin, phenacetin , and phenobarbital), micronisation reduces the effective surface area and hence the dissoluti on rate due to the following **three reasons**:

i) The hydrophobic drugs adsorb air on th eir surface, and thus inhibit their wettability and allow them to float on the dissolution medium.

ii) The particles, due to their high surface free energy, re -aggregate into larger particles, which either float on the surface or settle at the bottom of the dissolution medium.

iii) Extreme particle size reduction imparts surface charges that prevent wetting; electrically-induced agglomeration prevents intimate contact of the drug with the dissolution medium.

9) **Polymorphism and Amorphism:** A solid can exist either in a crystalline or amorphous form depending on its internal structure. The phenomenon in which a substance exists in more than one crystalline form is termed as polymorphism and the different forms are termed polymorphs. Polymorphs are of **two types**:

i) **Enantiotropic polymorphs** can be reversibly changed into another form by altering the temperature or pressure, **e.g.,** sulphur, and

ii) **Monotropic polymorphs** are unstable at all tem peratures and pressures, **e.g.,** glyceryl stearates.

Polymorphs differ from each other on the basis of their physical properties, such as solubility, melting point, density, hardness, and compression characteristics. They can be prepared by crystallising the drug f rom different solvents under varied conditions. Existence of polymorphs can be determined by techniques of optical crys tallography, X -ray diffraction, differential scanning calorimetry, etc.

10) **Hydrates/Solvates (Pseudopolymorphism):** The crystalline form of a drug can be a polymorph or a molecu lar adduct or both. Stoichiometric type of adducts in which the solvent molecules are incorporated in the crystal lattice of the solid are termed **solvates**, and the trapped solvent is termed **solvent of crystallisation**. Solvates can exist in different crystalline forms, which are known as **pseudopolymorphs**, and the phenomenon is termed **pseudopolymorphism**. If the solvent in association with the drug is water, the solvates are termed **hydrates**, which are the most common solva te forms of drugs.

Aqueous solubility of the anhydrous form of a drug , i.e., anhydrates, is more than that of the hydrates. This is because the latter are in interaction with water and have less energy for crystal break -up for further interaction with water in compari son to the former that are in thermodynamically higher energy state.

Theophylline and ampicillin in anhydrous form have higher aqueous solubility, dissolve at a faster rate, and show better bioavailability in comparison to when they are in mo nohydrate and trihydrate forms, respectively. In contrast, the aqueous solubility of organic (non -aqueous)

solvates is more than the non -solvates, **for example,** n-pentanol solvate of fluorocortisone and succinyl sulfathiazole and the chloroform solvate of griseofulvin have greater water solubility than their non -solvated forms like polymorphs. Solvates differ from each other in terms of their physical properties.

11) **Salt Form of the Drug:** Drugs are mostly weak acids or bases. The salts of such drugs have different solubility than their parent drugs. The solubility of such drugs can be easily enhanced by converting them into their salt forms. With weakly acidic drugs, a strong base salt is prepared (**e.g.,** sodium and potassium salts of barbiturates and sulphonamides). With weakly basic drugs, a strong acid salt is prepared (**e.g.,** hydrochloride or sulphate salts of several alkaloidal drugs).

1.3.3. Pharmaceutical Factors

Various processing variables can affect dissolution by altering effective surface area of drug particles. Drug dissolution is the single most important factor in the absorption of drugs.

The dosage form factors that influence dissolution and hence absorption of a drug are discussed below:

1) **Dosage Form Considerations:** The dosage form and its properties greatly affect the absorption rate and bioavailability, which also depend on the rate of drug release from dosage form , i.e., the rate of availability of the drug to the biological fluids. The decreasing order of drug availability is as follows:

   Solutions > Emulsions > Suspensions > Capsules > Compressed Tablets > Coated Tablets > Enteric-coated tablets.

   i) **Solutions:** Aqueous solutions of drugs are absorbed by a faster rate through GIT, but s ometimes drug solution in the gastric fluids may precipitate, where the extremely fine precipitate permits rapid re - dissolution.

   ii) **Emulsions:** An emulsion provides large surface area of oil to GIT that elevates rate of partitioning. Few drugs are absorbed fas t in an emulsion dosage form as compared to aqueous suspension (viscosity of the emulsion should not be limiting factor). If oils are digestible , absorption rate may increase further.

   iii) **Suspensions:** An aqueous suspension is one of most efficient dosage form. Mostly, dissolution rate limits the absorption of drug from a suspension; however, large surface area is immediately presented to the fluids at the absorption sites.

   iv) **Capsules:** Release of drug from hard gelatin capsule mainly depends on the formulation. The fine particles present in capsule are not compressed and may be fused, resulting in a reduced effective surface area. A large effective surface area will be available for di ssolution if particles in a capsule are intimately wet by the biological fluid s. Absorption from capsules is affected by particle size, selection of diluents and fillers.

v) **Compressed Tablets:** These are the most common dosage form, but simultaneously, the most difficult problem is with respect to availability of a drug for absorption. The main problems arise during the transfer of a solid drug from a compressed tablet to a solution in the GIT fluid as the effective surface area of drug is reduced to a greater extent during the tablet manufacturing process.

In an intact tablet, the sur face area is very limited, i.e., the dissolution rate is negligible, except for very water -soluble drugs. The absorption of drug is affected by primary disintegrat ion that ultimately influences the dissolution process. Tablet fragmentation increases the su rface area in contact with fluids at the absorption site, resulting in an increased rate of dissolution. In granular form , the effective surface area of drug is more than in the intact tablet, however, it is never less than the effective surface area of the primary drug particles. Compression force also affects the bioavailability of drug from compressed tablets.

vi) **Coated Tablets:** Commonly used coating technique for tablets are sugar coating, film coating, and press coating. Coating forms a physical barrier between the GI fluid and the tablet (drug). For proper drug dissolution , it is important that the film of coating dissolves before tablet disintegration. The disintegration is rate limiting process of drug absorption in coated tablets, because *in vitro* disintegration time affects bioavailability.

Film-coated tablets are compressed tablets coated with water -soluble material as a thin layer or film providing rapid solubility of the film. Coatings of the tablet have almost negligible effect on the drug availability as compared to the availability of drug from uncoated core tablet. Independent of GIT pH, the film coating should dissolve quickly in the GI fluids.

vii) **Enteric-Coated Tablets:** An enteric -coat is a special type of film coating designed to protect the tablet from gastric fluid and to promote or allow the dissolution of drug in the intes tine. The most important factor for enteric-coated tablet is the high liability of the coated tablet passing through the GIT. This problem mainly arises when enteric -coated products descend to release the drug at some finite time after administration. Lack of availability of drug can be decreased by the use of formulations that are based on pH difference between the stomach and intestine (a realistic assessment of intestina l pH should be provided). High intra- and inter-subject variability is observed in the enteric-coated preparation due to the variability in gastric emptying.

2) **Manufacturing Processes:** Processes of tablet manufacturing include:

i) **Granulation Method:** Conventional method for preparing granules is wet granulation process. Limitations of granulation are:

a) The liquid used may cause chemical reactions like hydrolysis.
b) Formation of crystal bridge due to the presence of liquid.
c) Harmful effects on thermo degradable drugs due to the drying process.

In granulation, many steps are involved that influence drug dissolution:

a) Method and duration of blending, and
b) Method, time and temperature of drying.

The method of direct compression is used to yield tablets that dissolve at a faster rate.

ii) **Compression Force:** This force is required in the tableting process and affects density, hardness, porosity, disintegration time , and dissolution rate. The dissolution rate is affected in the ways shown in **figure 4.14** when compression force is applied on drug-excipient mixture to form tablets.

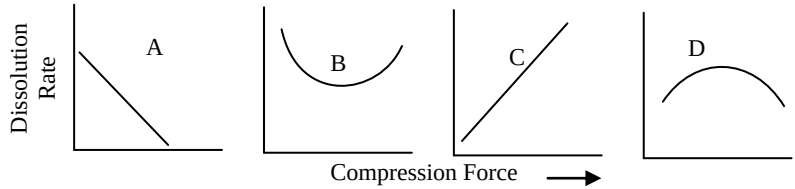


Figure 1.11: Effect of Compression Force on Dissolution Rate

On increasing the compression force, particles bind more tightly to each other (Curve A). High pressures may also break the particles and form smaller particles (Curve C). Sometime, a sum of an y of these two may result. So, it can be concluded that the effect of the compression force on the dissolution rate of a tablet would appear to be unpredictable. Therefore, a proper study should be performed on each formulation to ensure better dissolution as well as bioavailability.

3) **Pharmaceutical Ingredients/Excipients (Formulation Factors):** Many excipients (non-drug components) are added in a formulation. Excipients are used to enhance acceptability and physicochemical stability during shelf-life, uniform composition and dosage, optimum bioavailability and function - ability drug product. Important excipients are:

i) **Vehicles:** These are mainly:
   a) Aqueous vehicles (**e.g.,** water and syrup),
   b) Non-aqueous water miscible vehicles (**e.g.,** propylene glycol, glycerol, and sorbitol), and
   c) Non-aqueous water immiscible vehicles (**e.g.,** vegetable oils).

Miscibility of vehicles with biological fluids is an important factor for bioavailability. Aqueous and water mis cible vehicles are miscible with the body fluids imparting quick absorption of drug.

ii) **Diluents (Fillers):** Hydrophilic diluents (**e.g.,** starch, lactose, and microcrystalline cellulose) are used to enhance the dissolution of poorly water-soluble, hydrophobic drugs (**e.g.,** steroids). Hydrophobic diluents (**e.g.,** dicalcium phosphate) are used in the formulation of tablets.

iii) **Binders and Granulating Agents:** Poor wettable drugs (**e.g.,** phenacetin) can improve dissolution by imparting hydrophilic properties to the granule surface through hydrophilic binders. Proportion of stro ng binders during tablet formulation is very critical. If binders are used in

greater amount, it may increase hardness and decrease disintegration or dissolution rates of tablets. **For example,** PEG-6000 forms a poorly soluble complex with phenobarbital, and non-aqueous binders like ethyl cellulose also decrease drug dissolution.

iv) **Disintegrants:** These are mainly hydrophilic in nature. Amount of disintegrant directly affect the bioavailability of drug, **e.g.,** lower amount of disintegrant significantly decrease s bioavailability. Microcrystalline cellulose is a good disintegrant, but when compression force is increased, it may decrease the drug dissolution.

v) **Lubricants/Antifriction Agents:** These agents are mainly hydrophobic and form a coating over the drug particle. So, they should be used in such a way that they do not hinder or reduce drug dissolution rate and bioavailability. Increasing hydrophilicity of formulation also increases its dissolution rate in an aqueous medium, but, increase in hydrophobicity may decrease the rate of dissolution.

vi) **Suspending Agents/Viscosity Imparters:** These agents stabilise solid drug particles by decreasing the settling rate by increasing viscosity of the medium. Commonly used hydrophilic polymers are vegetable gums (**e.g.,** acacia and tragacanth), semi-synthetic gums (**e.g.,** CMC and MC), and synthetic gums. Increased viscosity functions as mechanical barrier to drug diffusion process from the dosage form into GI fluids as well as from GI fluids to mucosal lining (forms a viscid layer on the GI mucosa). These drugs also reduce the GI transit of drugs.

vii) **Surfactants:** In any formulation, surfactants are used as wetting agents, solubilisers, emulsifiers, etc. Surfactants involve the given mechanisms to increase drug absorption:

a) Drug dissolution and wetting are enhanced by increasing effective surface area, **e.g.,** Tween-80 with phenacetin.
b) Drug and membrane contactis modified for increasing drugabsorption.
c) Membrane permeability of drug is enhanced.

viii) **Buffers:** Potassium cations containing buffer systems can inhibit drug absorption, **e.g.,** as in sulfanilamide and vitamin B_2. It is because of uptake of fluids by the intestinal epithelial cells due to which the effective drug concentration reduced in the tissues decreases the rate of absorption.

Example of order of an inhibitory effect of various buffer catio ns on the drug transfer rate is $K^+ > NH_4^+ > Li^+ > Na^+ > TRIS^+$. Therefore, a buffer system for a salt of a drug should have the same cation as the drug salt and there should not be any additional cations.

ix) **Complexing Agents:** The physicochemical and biopharmaceutical properties of drug can be altered by complexing agents. Few **examples** where drug bioavailability is enhanced by complexation are:

a) Formation of a soluble complex (**e.g.,** ergotamine tartrate-caffeine complex and hydroquinone-digoxin complex) increases dissolution.

b) Membrane permeability is enhanced by increasing lipophilicity (**e.g.,** caffeine-PABA complex).
c) Enhanced membrane permeability (**e.g.,** GI absorption of heparin, which normally is not absorbed from the GIT is enhanced) by using EDTA that chelates magnesium and calcium ions of the membrane.

x) **Colourants:** An inhibitory effect is produced on dissolution rate of crystalline drugs by water-soluble dyes, even when used in little amount. This is because dyes are adsorbed on the crystal faces, and inhibit drug dissolution, **for example**, brilliant blue inhibits dissolution of sulfathiazole. Dyes also inhibit micellar solubilisation effect of bile acids and retard the absorption of hydrophobic drugs (**e.g.,** steroids). Cationic dyes have greater adsorption on particles, hence are more reactive than the anionic ones.

xi) **Crystal Growth Inhibitors:** These are used to maintain the initial physical properties of drug in suspension. Few crystal growth inhibitors (**e.g.,** PVP and PEG) hinder the conversion of high energy metastable polymorph into less soluble and stable polymorph.

xii) **Disintegration Rate:** Disintegration time for solid dosage form (**e.g.,** tablets and capsules) is significant. *In vitro* disintegration test relates to bioavailability because if the drug is not disintegrating, it will not dissolve, and there will not be any possibility of absorption.

If any drug does not confirm for the disintegration time, its bioavailability is directly affected, as the subsequent process of dissolution will be much slower and absorption may be insufficient. A solid dosage form disintegrate into fine particles or granules, which further deaggregate into fine particles; and dissolution of these fine particles is faster than that of granules.

Disintegration time of coated tablets is more than that of the compressed tablets. Disintegration can be enhanced by incorporating suitable amounts of disintegrants during formulation. Disintegration time directly depends on the concentration of disintegrant, fillers, and binders.

4) **Dissolution Time of Drug in Dosage Form:** Prediction of bioavailability can be done more preciously by dissolution test as compared to disintegration test. Bioavailability can be pre-determined by dissolution if these two conditions are met:
i) In GIT, the dissolved drug should remain free and intact. If the dissolved drug forms a complex with any GIT component or if drug decomposes in the GIT, dissolution test will not give a clear estimation of bioavailability.
ii) If absorption is not the rate limiting step, the drug solution formed in the GIT will be absorbed quickly, and the amount absorbed can be correlated with *in vitro* dissolution rate. However, if the absorption is limited or slow, bioavailability may not be proportional to the dissolution rate.

Drug dissolution is directly dependent on the particle size of drug. Dissolution rates of hydrophobic drugs can be increased by adding surfactants as they remove the air pockets around the particles that facilitate the contact of the dissolution medium with the drug. In gastric fluids, the

primary cause of surface activity is the reflux of intestinal contents into the stomach. Dissolution of drug is also affected by fillers and diluents used in formulation.

5) **Product Age and Storage Conditions:** Any changes in physicochemical properties of a drug in dosage form affect the drug absorption. Changes in physicochemical properties of drug can result due to ageing and alterations in storage conditions that adversely affect the b ioavailability of drug. In solutions, drug is precipitated due to alteration in solubility, conversion of metastable into poorly soluble, stable polym orph during the shelf -life of the product. In suspensions, changes in particle size distribution decrease the rate of drug dissolution and absorption.

   Ageing and storage conditions greatly affect the disintegration and dissolution rates of solid dosage forms, especially in tablets. It occurs due to the hardening of excipients (**e.g.,** polyvinyl pyrrolidone, aca cia etc.) on storage, whereas decrease in these parameters is due to the softening of tablet binder (**e.g.,** carboxymethyl cellulose) during storage. Alterations that occur during the shelf -life of a dosage form are mainly affected by variations in humidity and temperature.

1.4. ABSORPTION OF DRUGS FROM NON PER ORAL EXTRA-VASCULAR ROUTES

1.4.1. Introduction

Drugs are most often administered extravascularly and are mainly intended to produce systemic action; for this reason, absorption is a prerequisite for pharmacological effects. Delays or drug loss during absorption contribute to variability in drug response, and sometimes may result in drug therapy failure. The absorption site is separated from blo od via gastrointestinal membrane. Therefore, a drug to get absorbed have to pass through the membrane, and this is possible only when the drug is in solution form and dissolution becomes very critical for the absorption of a drug.

Non per Os or Oral indicates drug administration routes other than the oral route, which bypasses the GIT and enter the systemic circulation. A major **advantage** of drug administration through non-invasive transmucosal and transdermal routes (like nasal, buccal, rectal, etc.) is that a greater systemic availability is attained.

1.4.2. Sublingual/Buccal Route

Drug (small size d tablet) is kept beneath the tongue (without water) to disintegrate and absorb in mouth, **e.g.,** nitroglycerine tablets. The drug enters the systemic circulation through diffusion into the capillary network. In buccal route, the drug is kept within the mouth around the cheeks or buccal cavity, where it disintegrates and absorbs. The following factors should be considered in the oral mucosal delivery of drugs:

1) **Lipophilicity of Drug:** For passive permeation, slightly higher lipid solubility than that required for gastrointestinal absorption is necessary.

2) **Salivary Secretion:** The drug should be highly lipid-soluble, and should also be soluble in aqueous buccal fluids. Biphasic solubility of drug is required for absorption, because absorption will be delayed if the mouth is dry.
3) **Saliva pH:** Buccal pH of 6 assists the absorption of unionised drugs.
4) **Binding to Oral Mucosa:** Drugs that bind to oral mucosa have a poor systemic availability.
5) **Storage Compartment:** A storage compartment exists in the buccal mucosa for the slow absorption of drugs, like buprenorphine.
6) **Thickness of Oral Epithelium :** Sublingual absorption is faster than buccal absorption since the epithelium region of the former is thinner and immersed in a larger volume of saliva.

Merits of Drug Administration by Sublingual/Buccal Route

1) Rapid absorption of drugs due to highly vascu larised site, therefore fast onset of action.
2) Stomach enzymes and acids are not involved so the drug remains stable.
3) Drugs do not undergo first-pass metabolism.
4) Portal circulation is by-passed.
5) In case of any side effects, drugs can be withdrawn.
6) Drugs can be administered easily.
7) Less chances of infection.
8) No involvement of harsh gastrointestinal environment.

Demerits of Drug Administration by Sublingual/Buccal Route

1) It is sometimes inconvenient to keep drugs in mouth.
2) Small doses of drugs are required to keep in mouth.
3) Drugs having high molecular weight cannot be absorbed (**e.g.,** insulin).
4) Unpleasant, distasteful, irritant drugs cannot be administered.

1.4.3. Intravenous (IV) Route

Injections are preferred for orally unabsorbed drugs like atrac urium (neuromuscular blocker). First pass metabolism by the liver can be avoided by IV route because drugs do not enter into GI T. Intravenous route show s rapid effect and maintains level of drug in circulation.

Bacterial or other microbial infections may occur due to the use of syringe and contamination at the site of injection. Injected drugs cannot be removed from circulation through emesis or using activated charcoal like in oral delivery.

Merits of Drug Administration by Intravenous (IV) Route

1) 100% bioavailability is achieved.
2) Desired blood concentrations are achieved.
3) Large quantities.
4) Helpful in emergency situations.
5) No first-pass metabolism occurs.
6) Prevent gastric manipulation.

Demerits of Drug Administration by Intravenous (IV) Route

1) Inconvenient, painful and cause irritation, cellulitis and thrombophlebitis.
2) Repeated injections are not suitable.
3) Safety level is very low.
4) Technical and trained person are required to inject drugs.
5) Infection may occur.
6) Costly.

1.4.4. Intramuscular (IM) Route

In intramuscular (IM) route, drug is delivered in the form of aqueous solutions or depot prepara tions, i.e., drug suspension in non -aqueous vehicle (polyethylene glycol). By this route, aqueous preparation gets rapidly absorbed. Depots are used when slow release of drug is needed. Diffusion of vehicle from muscle and subsequent precipitation of drug at the site of injection occur providing a sustained release of drug. **For example**, sustained-release haloperidol decanoate slowly diffuses from the muscle and gives prolonged neuroleptic effect.

The following factors determine the absorption rate of drugs from intramuscular sites:

1) **Vascularity of the Injection Site:** The blood flow rate to muscular tissues in which drugs are injected is arranged in its decreasing order as - Arms (deltoid) > Thighs (vastus lateralis) > Buttocks (gluteus maximus). Blood flow rate is the rate-limiting step in drug absorption from intramuscular sites. The most rapid absorption occurs from deltoid muscles and slowest absorption occurs from gluteal region.

2) **Lipid Solubility and Drug Ionisation:** Highly lipophilic drugs undergo rapid absorption by passive diffusion, while the hydrophilic and ionised drugs are slowly absorbed through the capillary pores.

3) **Drug Molecular Size:** Small molecules and ions directly enter the capillaries through pores, while the macromolecules enter the lympha tic system. Small peptides and fluids undergo **cytopemphis**, in which they cross the endothelial tissue of blood capillaries and lymph vessels by getting transported in small vesicles that cross the membrane.

4) **Injection Volume and Drug Concentration:** A drug i n concentrated form and large volume injection undergoes faster absorption in comparison to the drug given in dilute form and small volume injection.

5) **pH, Composition and Viscosity of Injection Vehicle:** When a drug solution in acidic or alkaline pH (**e.g.,** phenytoin, pH 12) or in a non -aqueous solvent such as propylene glycol or alcohol (**e.g.,** digoxin) is injected intramuscularly, the drug precipitates at the injection site after a slow and prolonged absorption. Viscous vehicles such as vegetable oils also sl ow down the absorption of drug.

Merits of Drug Administration by Intramuscular (IM) Route

1) Uniform absorption occurs.
2) Onset of action is fast.

3) Mild irritants can be given.
4) Prevent first pass metabolism.
5) No GIT-related factors.

Demerits of Drug Administration by Intramuscular (IM) Route
1) Only small quantities (10ml) of drug can be administered.
2) Local pain, abscess, and infection may occur.
3) Expensive.

1.4.5. Subcutaneous (SC) Route

Subcutaneous (SC) route of drug administration is similar to IM injections, but the drug absorption in this route is slower than the IV route. The risk associated with IV route can be reduced by using subcutaneous route. **For example,** solid contraceptives (**e.g.,** a single rod with etonogestrel) is implanted for prolonged effect; Implanted programmable mechanical pumps are implanted to deliver insulin in diabetic patients.

Merits of Drug Administration by Subcutaneous (SC) Route
1) Can be self-administered.
2) Complete but slow absorption.
3) Massage/ heat: vasoconstriction.

Demerits of Drug Administration by Subcutaneous (SC) Route
1) Painful.
2) Irritant drugs cause tissue damage.
3) Only small quantities of dose (2ml) can be injected.

1.4.6. Inhalational Route

This route delivers drug throughout the respiratory tract, mucous membranes and pulmonary epithelium, as well as give fast effect as intravenous injections. Gases or aerosol forms of drugs (like anaesthetics) are administered through inhalational route. This route is effective in treatment of patients with respiratory complications such as asthma or chronic obstructive pulmonary disease. Systemic side effects related to some drugs (**e.g.,** albuterol and corticosteroids, and fluticasone) can be minimised in this route.

Merits of Drug Administration by Inhalational Route
1) Surface area of the respiratory endothelium is large and cause rapid absorption.
2) Bronchodilators and inhaled steroids affect lungs with less systemic absorption and minimum side effects.
3) Instant absorption of drug and rapid onset of action.
4) No hepatic first-pass metabolism of drug.

Demerits of Drug Administration by Inhalational Route
1) Specialised equipment required for drug delivery, **e.g.,** inhalers.
2) Bioavailability of drug depends on the patient's inhaler technique and drug particle size.
3) Due to the use of inhaler, dose regulation is difficult.

1.4.7. Transdermal Route

Transdermal patches are employed to deliver systemic effect of drug through skin. The rate of absorption depends on physical characteristics of the skin and application site. Transdermal patch es provide sustained de livery of drugs, **e.g.,** anti-anginal drug (nitroglycerine), antiemetic (scopolamine), and contraceptives.

Merits of Drug Administration by Transdermal Route

1) Sustained effect.
2) No hepatic first-pass metabolism.
3) Convenient and good patient compliance.

Demerits of Drug Administration by Transdermal Route

1) Relatively slow onset of action.
2) Excessive absorption may cause inflamed, rough, abraded or burning effects on skin.
3) Preferred for highly lipophilic drugs.

1.4.8. Topical Route

In topical route , the drug is applied on the skin surface (epidermis) or mucous membrane by means of special formulations, **e.g.,** creams, ointments, gels, lotions, sprays, powders, and aerosols. By this route, local (affecting a small area) to systematic (affecting the entire body) effects can be obtained. The drug is absorbed through the pores in the skin (**e.g.,** sweat glands, hair follicles, etc.). These dosage forms are used for treating skin infections, minim ising inflammation, and protecting skin.

There are three pathways via which solutes can diffuse through the skin:

1) Transcellular (passive diffusion),
2) Intercellular (paracellular),
3) Transappendageal in which drug diffusion occurs through:
   i) Hair follicles,
   ii) Sweat glands, and
   iii) Sebaceous glands.

The following factors influence passive percutaneous absorption of drugs:

1) **Skin Conditions**
   i) **Thickness of Stratum Corneum:** Drug absorption is slow from foot and palm as the skin in these regions has thick stratum corneum.
   ii) **Presence of Hair Follicles:** Drug absorption is rapid from scalp as the skin in this region has abundant hair follicles.
   iii) **Trauma:** Drug absorption is facilitated when the stratum corneum is destroyed by cuts, rashes, inflammation, mild burns, or other traumatic conditions.
   iv) **Hydration of Skin:** Drug absorption is facilitated when skin hydration is promoted by soaking the skin in water or occluding it by using emollients, plastic film, or dressing.

v) **Age:** With the ageing of skin, gross histological changes occur. Aged skin has hardened blood vessels and thus is more prone to allergic and irritant effects of topically contacted chemicals. Infants (just like adults) efficiently absorb drug through skin. Their ratio of surface area to body weight is 3 times that of adults , hence, systemic toxicity of topically applied drugs is of particular concern in infants.

vi) **Skin Microflora:** Skin surface holds a microbial population that promote the biotransformation of topically applied therapeutic agents.

vii) **Skin pH:** The surface pH of nor mal human skin is 4 -6. A pH gradient exists within the skin. Permeation of drugs prone to ionisation at skin pH can be influenced.

viii) **Skin Surface Lipids:** Skin surface exhibits sebaceous glands that secrete a mixture of lipids, which form an irregular film on the skin surface (0.4-4.0mm thick) that can solubilise drug in it and influence its permeation.

ix) **Anatomical Site:** Different skin regions have differences in thickness of stratum corneum, presence of appendages, blood circulation, and overall skin thickness. All these factors have a direct influence on drug permeation.

2) **Composition of Topical Vehicle**

i) **Vehicle or Base:** The vehicle incorporating the drug influences drug absorption. The vehicle in which the drug is dissolved promotes absorption and not the one in which the drug is dispersed.

ii) **Permeation Enhancers:** Incorporation of certain chemicals such as DMSO, propylene glycol, azone, etc., in topical formulations aid drug penetration.

3) **Application Conditions**

i) **Rubbing:** On rubbing the area of drug application, blood circulation to that area and thus drug penetration is influenced.

ii) **Occlusion:** Topical preparations produce an occlusive effect that prevents moisture loss to the atmosphere from the skin and aqueous delivery vehicles. This trapped endogenous or exogenous m oisture hydrates the stratum corneum, makes it swell, and thus influences drug permeation across it.

iii) **Loss of Vehicle:** Loss of vehicle from the application site or translocation of the applied dose from treated to untreated sites also influences transdermal penetration. Evaporation of solvents decrease or increase the drug solubility in the residual phase and thus the drug's thermodynamic activity and permeation, depending on the polarities of the volatile solvents(s) and the drug.

4) **External Factors**

i) **Environment Humidity and Temperature:** Higher humidity and temperature increase the hydration rate, local blood flow, and thus drug absorption.

ii) **Grooming:** Frequency and vigour with which one bathes and the type of soap one uses also contribute to variability in drug absorption.

iii) **Exposure to Chemicals:** Occupational exposure to solvents accelerates shedding of epidermal cells and thus enhances drug absorption.

iv) **Chronic Use of Certain Drugs:** Long-term use of cortisol or keratolytic (like salicylic acid) enhances drug penetration.

Nitroglycerine, lidocaine, betamethasone, oestradiol, testosterone, etc. are administered percutaneously. This route is useful for drugs having low oral availability and short duration of action; effect of the latter category of drugs is prolonged because percutaneous absorption is a slow process.

Merits of Drug Administration by Topical Route

1) Drug can be applied easily.
2) Less complication than oral delivery as drugs poorly absorb systemically.
3) Fast action on application site.
4) Creams and gels are less greasy and more convenient than ointments.
5) Lotions are best on hairy parts of the body.

Demerits of Drug Administration by Topical Route

1) Skin irritation occurs.
2) Improper absorption of certain drugs.
3) Ointments have longer duration of action due to sticky and oily texture.

1.4.9. Rectal Route

Suppositories are administered through rectal route. Drug is formulated with waxy additives in wh ich the drug is dissolved or liquefie d on insertion into the rectum. Drug absorbance occurs directly through thin, highly vascularised wall of rectum. Around 50% of rectal drainage bypasses the portal circulation, i.e., less biotransformation of drugs by liver. This route is used to avoid the destruction of drug by intestinal enzymes or by low pH of stomach. Moreover, this rou te is useful in preventing drug-induced vomiting due to oral administration as well as in unconscious patient. The drug is administered in the form of suppositories, through rectal route, when patient is not able to take drug orally (due to vomiting, in consciousness) or have restrictions on eating (mostly after surgery).

Merits of Drug Administration by Rectal Route

1) Useful in patients suffering from nausea and vomiting.
2) Bypasses first pass metabolism, since absorption occurs from external haemorrhoidal veins.
3) Gastric irritant drugs can be administered through this route.

Demerits of Drug Administration by Rectal Route

1) Rectal inflammation may occur.
2) Irregular absorption occurs.

1.4.10. Vaginal Route

Vagina is considered the best administration route for contraceptives, antifungals, and antimicrobials for best achievement of local or systemic abs orption. Vaginal wall contains a vast network of blood vessels, and thus is suitable for absorption of

drugs for systemic use. Drug administration via vaginal route avoids the gut and hepatic first pass metabolism, reduces gastrointestinal and hepatic sideeffects, and enables local targeting of drugs to the reproductive organs.

Drug transport across vaginal membrane takes place in three major ways:
1) Transcellularly via concentration-dependent diffusion through cells,
2) Paracellularly mediated via tight junctions, and
3) Vesicular- or receptor-mediated transport.

Drug absorption from vaginal delivery system takes place in two main steps:
1) Drug dissolution in vaginal lumen, and
2) Membrane penetration.

Merits of Drug Administration by Vaginal Route
1) Enables prolonged drug release.
2) Minimises systemic side effects.
3) Increases bioavailability.
4) Utilises less amount of drug than utilised via oral route.
5) Avoids first-pass metabolism.
6) Eases self-medication.
7) Avoids contact with digestive fluid and minimises drug degradation.
8) Inhibits the occurrence of nausea, vomiting, and emesis , generally induced after oral administration of drugs.
9) Enables a quick onset of action.

Demerits of Drug Administration by Vaginal Route
1) Gender specific.
2) Results in patient non-compliance.
3) Only a few drugs can be administered.
4) Drug absorption varies due to menstrual cycle, menopause, and pregnancy.
5) Affects the intercourse.
6) Demands maintenance of personal hygiene.
7) Some drugs are sensitive at vaginal pH.

1.4.11. Intraocular Administration

Drugs are topically applied to the eyes for local effects such as mydriasis, meiosis, anaesthesia, treatment of infections, glaucoma, etc. Sterile aqueous solutions of drugs are us ed as ophthalmic formulations and administered in the conjunctival *cul-de-sac*. Cornea possessing hydrophilic and lipophilic characteristics acts as a barrier to intraocular drug penetration. Thus, for optimum intraocular permeation of drugs, they should po ssess biphasic solubility. Lachrymal fluid pH influences absorption of weak electrolytes such as pilocarpine. On the other hand, formulation pH influences lachrymal output; ophthalmic solutions of higher pH decreases tear flow and promotes drug absorption while ophthalmic solutions of lower pH increase lachrymation and subsequent drug loss due to drainage. Rate of blinking also influences drainage loss. Volume of fluid instilled into the eyes also affects drug bioavailability and subsequent effectiveness.

Human eye can usually hold around 10 µl of fluid; hence, instilling concentrated form of drug solution in small volume increases its effectiveness than when administered in dilute form in large volume. Viscosity enhancers added in the formulation prolong the drug's contact time with the eye, thus increase bioavailability. Due to the same reason, oily solutions and ointments show sustained drug action. Sometimes systemic absorption of a drug having low therapeutic index (such as timolol) precipitates undesirable toxic effects. Systemic entry of drugs occurs by the absorption route into lachrymal duct that drains lachrymal fluid into the nasal cavity and GIT. This can be prevented by simply closing the eyelid or by nasolacrimal occlusion in which the fingertip is pressed on the inside corner of the eye after drug instillation.

1.4.12. Absorption of Drugs from Common Non per Oral Routes of Drug Administration

Absorption, advantages and disadvantages of common routes of drug administration are given in **table 1.1**:

Table 1.1: Absorption of Drugs from Common Routes of Drug Administration

Routes	Bioavailability/ Absorption	Advantages	Disadvantages
Intravenous (IV)	Complete (100%) systemic drug absorption.	Drug is given for immediate or controlled effect; Can inject large fluid volumes; Suitable for irritating drugs.	Increased chance for adverse reactions; Possible anaphylaxis; Requires skill in insertion of infusion set; Tissue damage at site of injection (infiltration, necrosis, or sterile abscess).
Intramuscular Injection (IM)	Passive diffusion, endocytosis, pore transport; Rapid absorption from aqueous solutions; Slow absorption from non-aqueous (oily) solutions.	Easier to inject than intravenous injection; Larger volumes can be used compared to subcutaneous solutions.	Irritating drugs can be painful; Variable rates of absorption depending on muscle injected and blood flow.
Subcutaneous Injection (SC)	Passive diffusion; Rapid absorption from aqueous solution; Slow absorption from depot formulations.	Generally used for vaccines and drugs not absorbed orally, **e.g.,** insulin.	Rate of drug absorption depends on blood flow and injection volume.
Buccal or Sublingual (SL)	Passive diffusion, carrier mediated transport; Rapid absorption of lipid-soluble drugs.	No pre-systemic metabolism.	Some drug can be swallowed; Not for most drugs or drugs with high doses.
Rectal (PR)	Passive diffusion; Absorption may vary from suppository; More reliable absorption from enema (solution).	Useful when patient cannot swallow medication; Used for local and systemic effects.	Absorption can be erratic; Suppository can migrate to different position; Some patients may feel discomfort.

Vaginal	Passive diffusion	Increases bioavailability; Minimises systemic side effects; Quick onset of action.	Gender-specific; Patient non-compliance.
Transdermal	Passive diffusion; Slow absorption, rate may vary; Increased absorption with occlusive dressings.	Transdermal delivery system (patch) is easy to use and withdraw; Continuous release for a specified period; Used for lipid - soluble drugs with low dose and low MW; Low pre - systemic metabolism.	Some irritation by patch or drug; Permeability of skin variable with condition, anatomic site, age and gender; Type of cream or ointment base affects drug release and absorption.
Inhalation	Passive diffusion, pore transport; Rapid absorption; Total dose absorbed is variable.	Can be used for local or systemic effects.	Particle size of drug determines anatomic placement in respiratory tract; Can stimulate cough reflex; Some drugs can be swallowed.

1.5. SUMMARY

The details given in the chapter can be summarised as follows:

1) **Biopharmaceutics** is defined as, "study of the interrelationship of physicochemical properties of the drug, dosage form in which the drug is given and the administration route on the rate and extent of systemic drug absorption."
2) **Biopharmaceutics** is also defined as "study of the factors influencing the rate and amount of drug that reaches the systemic circulation and the use of this information to optimise the therapeutic efficacy of drug products."
3) The GIT epithelium lining is the major cellular barrier to the absorption of drugs from the GIT.
4) **Passive diffusion** (or **non-ionic diffusion**) is defined as the difference in the drug concentration on either side of the membrane. Concentration or electrochemical gradient is the driving force for this process.
5) The kinetic energy of drug molecules is responsible for the movement of drug.
6) Passive diffusion can be expressed by **Fick's first law of diffusion**, according to which the drug molecules diffuse from a region of higher concentration to lower concentration until equilibrium is achieved, and the rate of diffusion is directly proportional to the concentration gradient across the membrane.

7) **Carrier-mediated transport** involves a membrane component, called the carrier that reversibly or non-covalently binds to the solute molecules to be transported.
8) **Facilitated diffusion** is a carrier-mediated transport system that works at a much faster rate than the passive diffusion. Concentration gradient is the driving force for this process that operates down the hill and thus is a passive process.
9) **Active transport** involves movement of a substance from a region of low concentration to high concentration, i.e., against its concentration gradient.
10) Active transport utilises energy, unlike passive transport that does not use any type of energy. If it uses chemical energy from ATP, it is termed as **primary active transport**. If it uses an electrochemical gradient, it is termed as **secondary active transport**.
11) **Pore transport** (or **connective transport**, **bulk flow** or **filtration**) involves the absorption of low molecular weight, low molecular size, and water-soluble drugs (**e.g.,** urea, water, and sugar) through narrow, aqueous filled channels or pores in the membrane structure.
12) **Ionic** or **electrochemical diffusion** involves the diffusion of ionic molecules across the membrane as a function of potential difference or electrical gradient.
13) **Endocytosis** is a minor transport mechanism in which the extracellular materials are engulfed within a segment of the cell membrane to form a saccule or a vesicle, which is then pinched-off intracellularly.
14) The process of endocytosis helps in the cellular uptake of macromolecular nutrients (like fats and starch), oil soluble vitamins (like A, D, E, and K), and drugs (like insulin).
15) **Phagocytosis** (cell eating) involves adsorptive uptake of solid particulates.
16) **Pinocytosis** (cell drinking) involves uptake of fluid solute.
17) At times, **transcytosis** occurs in which an endocytic vesicle is transferred from one extracellular compartment to another.
18) **Drug micronisation** increases the drug solubility in small intestine (a better absorption site), but also increases the drug degradation rate in stomach due to increased surface area.
19) **Dissolution rate** is the amount of substance that goes into the solution per unit time under standard conditions of temperature, pressure, and solvent composition.
20) **Noyes-Whitney equation** describes the process of dissolution, and states that the dissolution rate of drug (dc/dt) is directly proportional to the diffusion coefficient of the drug in solution state in gastrointestinal fluid (D), effective surface area of the drug particle available for drug dissolution (A), difference in the saturation solubility of the drug in the diffusion layer (C_s), and the concentration of drug in solution state in the bulk of gastrointestinal fluid (C).
21) **Danckwert's model** of dissolution does not consider the formation of diffusion layer and the solid surface is constantly exposed to fresh solvent causing mass transfer.

22) **Limited solvation theory** states that an intermediate concentration less than saturation exist at the interface due to s olvation mechanism, which is the function of solubility than that of the diffusion.
23) Good **wettability** results in particle size reduction.
24) The solubility of drugs can be easily enhanced by converting them into their salt forms.
25) **Brodie** gave the **pH partition theory** to explain the absorption process of drug from GIT and its distribution across the biological membranes.
26) The dissociation constant (pKa) of drug and the pH of body fluid at the absorption site influence the amount of unionised drug.
27) **Weakly acidic drugs with pKa > 8** are unionised at all pH values and hence their absorption rate is rapid and independent of gastrointestinal pH.
28) **Weakly acidic drugs with pKa ranging from 2.5 to 7.5** undergo pH - dependent absorption. At acidic conditions of stomach (pH > pKa), they exist in unionised form and undergo better absorption.
29) **Strongly acidic drugs with pKa < 2.5** are ionised in the entire pH range of GIT and hence are poorly absorbed.
30) **Weakly basic drugs with pKa < 5.0** are ionised at all pH values and hence undergo rapid and pH-independent absorption.
31) **Weakly basic drugs with pKa ranging from 5 to 11** undergo pH-dependent absorption. At alkaline conditions of the intestine, they exist in unionised form and undergo better absorption.
32) **Strongly basic drugs with pKa > 11** remain ionised in the entire pH range of GIT and hence are poorly absorbed.
33) If a drug exists in unionised form, it will still undergo poor absorption, provided its lipid solubility is low, i.e., it has a low value of $K_{o/w}$.
34) The lipid solubility of a drug can be determined from its oil/water partition coefficient value ($K_{o/w}$).
35) A solid drug's particle size and surface area are inversely proportional. Smaller the drug particle, greater is its surface area.
36) **Enantiotropic polymorphs** can be reversibly changed into another form by altering the temperature or pressure.
37) **Monotropic polymorphs** are unstable at all temperatures and pressures.
38) Stoichiometric type of adducts in which the solvent molecules are incorporated in the crystal lattice of the solid are termed **solvates**, and the trapped solvent is termed **solvent of crystallisation**.
39) Solvates can exist in different crystalline forms, which are known as **pseudopolymorphs**, and the phenomenon is termed **pseudopolymorphism**.
40) If the solvent in association with the drug is water, the solvates are termed **hydrates**, which are the most common solvate forms of drugs.
41) Aqueous solubility of the anhydrous form of a drug, i.e., anhydrates, is more than that of the hydrates.
42) **Non per Os** or **Oral** indicates drug administration routes other than the oral route, which bypasses the GIT and enter the systemic circulation.

43) A major advantage of drug administration through non -invasive transmucosal and transdermal routes (like nasal, buccal, rectal, etc.) is that a greater systemic availability is attained.

44) **Injections** are preferred for orally unabsorbed drugs like atracuriu m (neuromuscular blocker).

45) In **intramuscular (IM) route** , drug is delivered in the form of aqueous solutions or depot preparations, i.e., drug suspension in non -aqueous vehicle (polyethylene glycol).

46) **Subcutaneous (SC) route** of drug administration is simila r to IM injections, but the drug absorption in this route is slower than the IV route.

47) In **topical route** , the drug is applied on the skin surface (epidermis) or mucous membrane by means of special formulations.

48) Drug transport across vaginal membrane takes place transcellularly via concentration-dependent diffusion through cells, paracellularly mediated via tight junctions, and vesicular- or receptor-mediated transport.

1.6. EXERCISE

1.6.1. True or False

1) Endocytosis involves the absorption of low molecular weight dru gs through narrow, aqueous filled channels or pores in the membrane structure.
2) If active transport utilises chemical energy from ATP, it is termed as primary active transport.
3) Passive diffusion can be expressed by Fick's first law of diffusion.
4) Passive diffusion is defined as the difference in the drug concentration on either side of the membrane.
5) Pore transport is a minor transport mechanism in which the extracellular materials are engulfed within a segment of the cell membrane.
6) Solubility is the amount of substance that goes into the solution per unit time under standard conditions of temperature, pressure, and solvent composition.
7) Good wettability increases particle size.
8) Weakly basic drugs with pKa < 5.0 are io nised at all pH values and hence undergo rapid and pH-independent absorption.
9) Enantiotropic polymorphs are unstable at all tem peratures and pressures.

1.6.2. Fill in the Blanks

10) If active transport utilises _____, it is termed as secondary active transport.
11) _____ is the driving force for facilitated diffusion.
12) _____ involves uptake of fluid solute.
13) _____ states that an intermediate concentration less than saturation exist at the interface due to salvation mechanism.
14) Weakly acidic drugs with pKa _____ _ are unionised at all pH values and hence their absorption rate is rapid and independent of gastrointestinal pH.
15) The lipid solubility of a drug can be determined from its _____.
16) Smaller the _____, greater is its surface area.

17) Aqueous solubility of the _____ is more than that of the _____ .

18) Drug transport across vaginal membrane takes place _____ , _____ , and vesicular- or receptor-mediated transport.

Answers

1) False 2) True 3) True
4) True 5) False 6) False
7) False 8) True 9) False
10) Electrochemical gradient 11) Concentration gradient 12) Pinocytosis
13) Limited solvation theory 14) > 8
15) Oil/water partition coefficient value 16) Drug particle
17) Anhydrates and hydrates 18) Transcellularly and paracellularly

1.6.3. Very Short Answer Type Questions

1) Define biopharmaceutics.
2) Give the applications of biopharmaceutics.
3) Write about endocytosis.
4) What do you understand by active transport?
5) State the pH-partition theory.
6) Give the merits and demerits of sublingual route of drug administration.

1.6.4. Short Answer Type Questions

1) Enlist the different mechanisms of drug absorption and explain any two in detail.
2) Discuss how absorption of drug is affected by dissolution rate.
3) How pharmaceutical ingredients influence the absorption process?
4) Discuss the topical route of drug administration.
5) Write a note on intravenous and intramuscular routes of drug administration.

1.6.5. Long Answer Type Questions

1) Give a detailed review on mechanism of absorption through GIT.
2) Discuss the factors influencing drug absorption through GIT.
3) Write an exhaustive note on absorption of drugs from n on per oral extra -vascular routes.

CHAPTER 2

Introduction to Distribution

2.1. DISTRIBUTION

2.1.1. Introduction

The processes which lower the plasma drug concentration are termed **disposition**. For the disposition of drugs, mainly two processes are involved:

1) **Distribution:** This process involves the reversible transfer of a drug between ompartments. Drug distribution is also defined as the reversible transfer of a drug between one compartment and another.

2) **Elimination:** This process involves irreversible loss of drug from the body. Elimination is further divided into two processes, namely **biotransformation** (**metabolism**) and **excretion**.

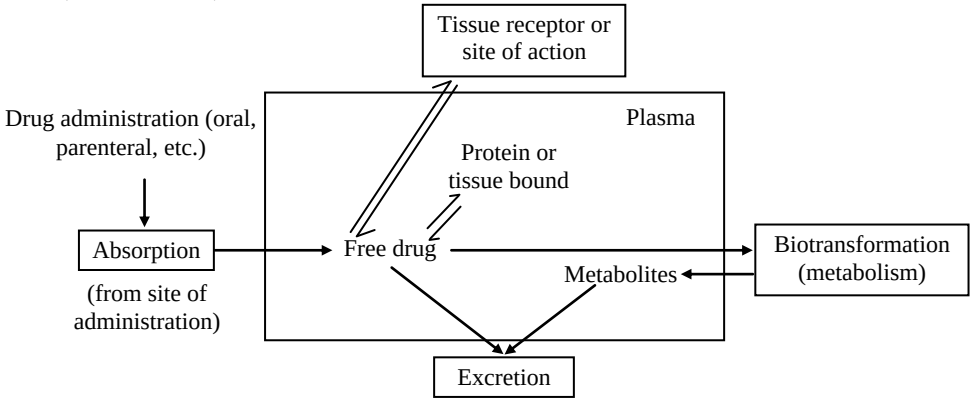


Figure 2.1: Inter-Relationships among Various Process of Drug Disposition

Distribution of drugs occurs through the circulatory system (by the circulation of blood). Therefore, blood or plasma si gnifies one of the compartments, while extravascular fluids and other body tissues signify the other compartments. In other words, distribution of drug is a reversible transfer of drug between the blood and the extravascular fluids and tissues.

Distribution is a passive transport process and the driving fo rce for this process is obtained from the difference of concentration gradient between the blood and extravascular tissues. By the diffusion process, the drug concentration increases in tissues until it reaches equilibrium where the amount of drug entering the tissues becomes equal to the amount of drug draining out from the tissues. At equilibrium state, the drug concentration in the tissues depends on the rate at which the drug is distributed in the tissues and on the drug concentration in the plasma.

Plasma concentration $\rightleftharpoons$ Tissue concentration

Drug distribution does not occur uniformly throughout the body because different tissues get the drug from the plasma at different rates and in different concentrations.

2.1.2. Tissue Permeability of Drugs

If the blood flows rapidly and uniformly to the entire body tissues, differences in the degree of distribution between tissues will indicatethe differences in the tissue penetrability of the drug. This process will be tissue permeability rate -limited. In distribution of drugs, following are the**two** main **rate-determining steps**

1) **Rate of Tissue Permeability:** Tissue permeability of a drug mainly depends on two factors, i.e., the physicochemical properties of the drug and the physiological barriers restricting diff usion of drug into tissues. Molecular size, degree of ionisation, and partition coefficient are t he main physicochemical properties influencing drug distribution.

   Most of the drugs having **molecular weight** less than 500 -600 Daltons can feasibly diffuse int o the extracellular interstitial fluids by crossing the capillary membrane . But, penetration of drugs from the extracellular fluid into the cells is determined by the molecular size, ionis ation constant , and drug lipophilicity. Only small, water -soluble molecules and ions of size less than 50 Daltons enter the cell via aqueous filled channels , while the larger - sized particles can be passed by a specialised transport system.

   The tissue permeability of a drug is mainly determined by its **degree of ionisation**. The ionisation and diffusion of drugs into cells decide the pH of blood and extravascular flu id. A drug which remains unionis ed at these pH values permeates the cells with a faster rate.

   The pH of blood and ECF generally remain constant at pH 7.4, therefore they do not affect dr ug diffusion unless conditions like systemic acidosis or alkalosis are static or remain unaltered.

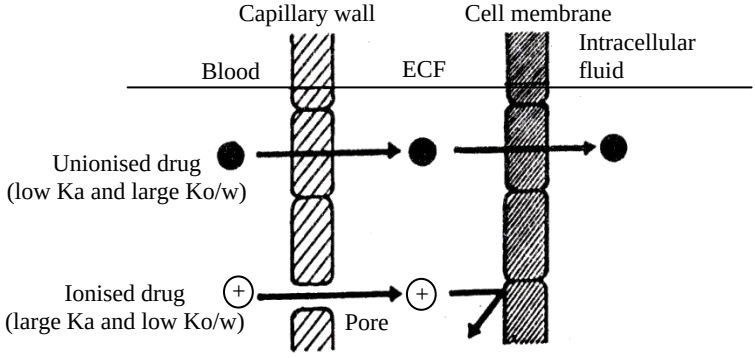


Figure 2.2: Permeation of Unionised and Ionised Drugs across the Capillary and Cell Membrane

Drugs are mostly weak acids or weak bases , and their degree of ionis ation at plasma or ECF pH depends on their pK a value. All polar and hydrophilic drugs get ionised at plasma pH, cannot penet rate the lipoidal cell membrane, and tissue permeability is the rate -limiting step in the ir distribution. Only lipophilic unionised drugs rapidly cross the cell membrane.

In case of polar drugs in which permeability is the rate-limiting step in their distribution, **effective partition coefficient** of drug is the driving force and it is calculated as follows:

$$\text{Effective } K_{o/w} = \text{Fraction unionised at pH 7.4} \times K_{o/w} \text{ of unionised drug} \quad \text{.....(1)}$$

The degree up to which effective partition coefficient influences rapidity of drug distribution can be explained by the following example (**table 2.1**):

Table 2.1: Distribution of Acidic Drugs in CSF

Drugs	Relative Acidity	Effective $K_{o/w}$ at pH 7.4	Relative Rate of Distribution
Thiopental	Weaker acid	2.0	80
Salicylic acid	Stronger acid	0.0005	1

Permeability is the rate-limiting step in drug distribution:

i) If the drug under consideration is ionic, polar, or water-soluble.
ii) If the highly selective physiological barriers restrict such drugs to diffuse into the cell.

On the other hand, **perfusion is the rate-limiting step** in drug distribution:

i) If the drug is highly lipophilic.
ii) If the membrane across which the drug is to diffuse is highly permeable (such as those of the capillaries and muscles).

Only highly lipophilic drugs (like thiopental) can cross the most selective barriers such as the blood-brain barrier (BBB), while highly permeable capillary wall allows almost all the drugs (except those bound to plasma proteins) to pass. In both the cases, the rate of blood flow or perfusion to the tissue is the rate-limiting step. Thus, greater the blood flow, faster the distribution.

2) **Rate of Blood Perfusion:** Perfusion rate is the **volume of blood that flows per unit time per unit volume of the tissue** Its unit is **ml/min/ml of the tissue**

If $K_{t/b}$ represents tissue/blood partition coefficient of drug, the first-order distribution rate constant (K_t) is expressed as:

$$K_t = \frac{\text{Perfusion Rate}}{K_{t/b}} \quad \text{..... (2)}$$

The tissue distribution half-life is given as:

$$\text{Distribution on half-life} = \frac{0.693}{K_t} = \frac{0.693 K_{t/b}}{\text{Perfusion Rate}} \quad \text{..... (3)}$$

The extent up to which a drug is distributed in a particular tissue or organ depends on the size of tissue (i.e., tissue volume) and the tissue/blood partition coefficient of the drug. This can be explained by taking the **example** of thiopental (a lipophilic drug), which has a high tissue/blood partition coefficient towards the brain and higher for adipose tissue. Because brain (site of action) is a highly perfused organ, thiopental diffuses very rapidly into the brain and shows a rapid onset of action when given intravenously.

Adipose tissue s are poorly perfused, therefore , distribution occurs very slowly with the same drug. If thiopental concentration in the adipose tissue shifts towards equilibrium, the drug rapidly diffuses out of the brain and localises in the adipose tissue whose volume is 5 times more than brain and has greater affinity for the drug. Such tissue redistribution results in rapid termination of action of thiopental.

2.1.3. Physiological Barriers

Different types of barrier s are found in the body that restrict the distribution of various compounds that enter the blood to different tissues. These barriers are present in the body for protecting the sensitive tissues from the effect of various chemicals that enter the blood via different routes.

The physiological barriers are discussed below:

1) **Blood Capillary Membrane :** Drug passes the capillary membrane t hrough passive diffusion an d hydrostatic pressure. By passive diffusion , drug molecules travel across the region of high concentration to low concentration; it can be described by **Fick's law of diffusion**.

$$\text{Rate of Diffusion} = \frac{dQ}{dt} = \frac{-DKA(C_p - C_t)}{h}$$

Where,

D = Drug diffusion coefficient in the membrane.

K = Lipid/water partition coefficient.

A = Membrane surface area.

C_p = Plasma drug concentration.

C_t = Tissue drug concentration.

h = Membrane thickness.

The negative sign indicates drug movement from inside the blood capillary into the tissues.

2) **Simple Capillary Endothelial Barrier :** Capillaries supply blood to most of the tissues. Capillary endothelium allows passage of drugs (ionised or unionised of molecular size < 600 Daltons) int o the interstitial fluid. Only drugs bound to the blood components are restricted because of the large molecular size of the complex.

3) **Cell Membrane :** Drug present in ECF is transported by passive diffusion into the cell. Factors influencing the penetration of drugs into cells are same as those observed in the gastrointestinal absorption of drugs.

For the transport of drugs, cell membrane acts like a lipid barrier. Permeability of drugs through cell membrane chec ks the entry into the cell.

The physicochemical properties that influence permeation of drugs across such a barrier are illustrated in **figure 2.3**.

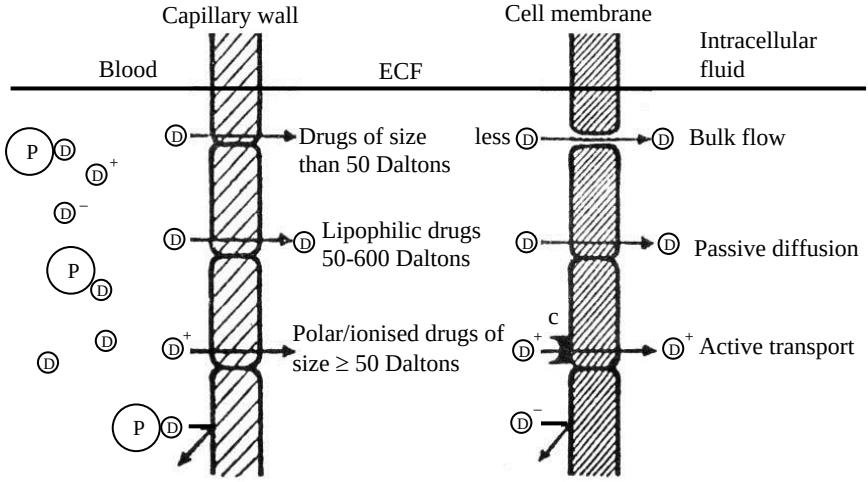


Figure 2.3: Cell Membrane Barrier and Drug Diffusion across it

4) **Blood-Brain Barrier (BBB):** Permeability of capillaries present in the brain and spinal cord is different from that of the capillaries of rest of the body. Endothelial cells of capillaries are covered by a layer of glial cells that have tight intercellular junctions providing thicker lipid barrier. This layer of glial cells reduces diffusion and penetration of water-soluble and polar drugs into the brain and spinal cord. **Figure 2.4** represents this lipid barrier (BBB).

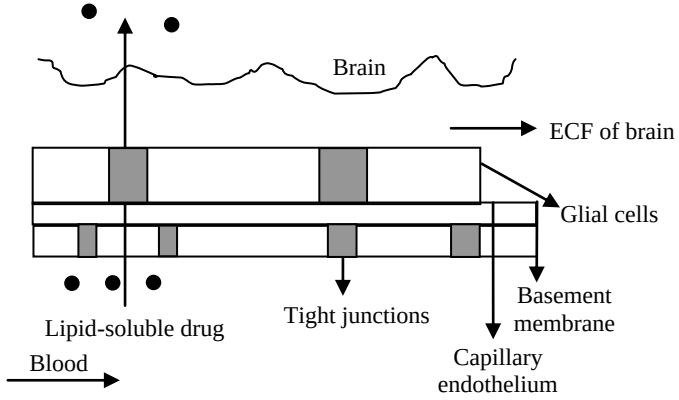


Figure 2.4: Transport of Lipid-Soluble Drug across BBB

Normally, only lipid-soluble drugs can penetrate the interstitial fluid of the brain and spinal cord, while water-soluble compounds need specific carriers to cross the endothelial lining. In diffusion process, many transport mechanisms are involved. Degree of ionisation in plasma and drug lipid solubility determines the penetration rate of a drug into the brain. Highly lipid-soluble drugs (**e.g.,** thiopental) cross BBB immediately, and reach the brain from plasma. Polar drugs (**e.g.,** barbital) penetrate the CNS slowly. Weak organic acids (**e.g.,** penicillin G having pKa 2.6) are found in completely ionised form in plasma, but penetrate the brain at a low rate due to poor lipid solubility.

Approaches to Promote Crossing the BBB

i) Permeation enhancers (**e.g.,** Dimethyl Sulfoxide or DMSO) are used for increasing penetration.

ii) Mannitol infused in internal carotid artery result in osmotic disruption of BBB.

iii) Carriers (**e.g.,** dihydropyridine redox system) are used to transport drug into brain.

5) **Blood-Cerebrospinal Fluid Barrier :** Cerebrospinal fluid forms in choroid plexus, found in the roof of the fourth ventricle and projects between the cerebellum and pons on the lower brain stem. At anterior brain stem in the roof of the diencephalon, two extensive folds of choroid plexus originate and extend through inter-ventricular foramina. Floors of the lateral ventricles are covered by folds of the choroid plexus.

Drug diffuses easily through the blood-CSF barrier because the junctions between the endothelial cells of blood capillaries are open; so the drug molecules easily reach the extracellular fluid (ECF) from the blood at the barrier. But at the region of choroid plexus, tight junctions are present between the cells that obstruct the penetration of polar drugs. So, only lipid-soluble drugs can diffuse through the lipoidal barrier (**figure 2.5**).

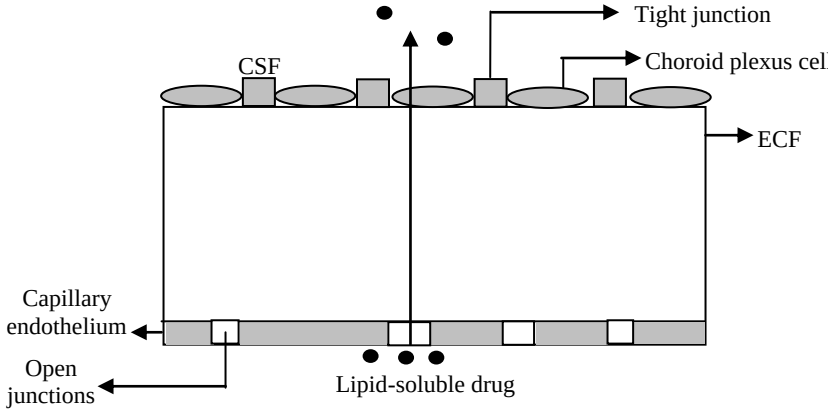


Figure 2.5: Transport of Lipid-Soluble Drug across Blood Cerebrospinal Fluid Barrier

As the CSF is almost devoid of protein, the CSF concentration of lipid-soluble drugs represents the free drug concentration in plasma. Concentration of drugs is greater in brain tissues as compared to the CSF, **e.g.,** in epileptic patients concentration of phenytoin is 6 times higher in the temporal lobe than in the CSF.

6) **Placental Barrier:** Maternal and the foetal blood vessels are separated by placental barrier, which is made up of endothelium and many layers of tissue of foetal trophoblast basement membrane.**Figure 2.6** shows the blood flow in maternal and foetal blood vessels. Placental barrier is less effective than BBB because drugs having molecular weight less than 1000 Daltons and with moderate to high lipid solubility (**e.g.,** ethanol, sulphonamides, barbiturates, gaseous anaesthetics, steroids, narcotic analgesics, anticonvulsants, and antibiotics) can cross the placental barrier through simple diffusion. Immunoglobulins are transferred by endocytosis, whereas, nutrients essential for foetal growth are transported by carrier-mediated process.

Drugs may give rise to lethal effects on the following two critical stages during foetal development:

i) **First Trimester:** It is the duration when the foetal organs are developing. At this stage , most of the drugs produce teratogenic effects (congenital defects), **e.g.,** thalidomide, phenytoin, isotretinoin, etc.

ii) **Latter Stages:** In the l atter stages of pregnancy, drugs may affect the physiological functions of body, **e.g.,** respiratory depression by morphine.

Therefore, it is better to avoid using any drug during the pregnancy period due to the uncertainty of harmful effects.

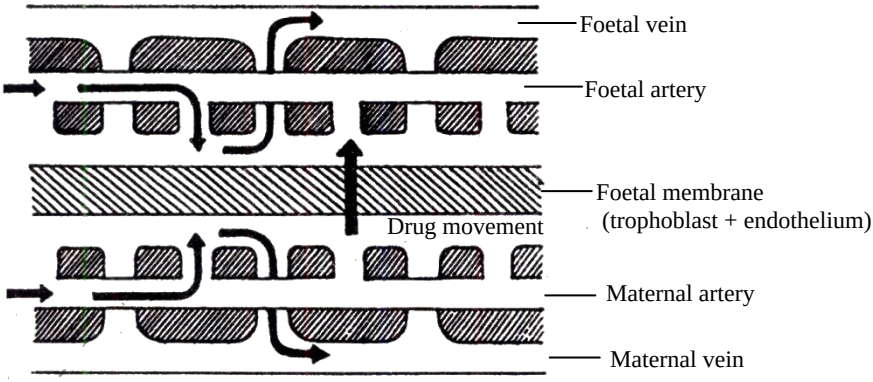


Figure 2.6: Placental Barrier and Blood Flow across Membrane

7) **Blood-Testes Barrier:** A layer is formed by the extensions of sustentacular cells (sertoli cells) that surround the seminiferous tubule beneath the spermatogonia. To maintain stable conditions, diffusion from interstitial fluid to the seminiferous tubule is prevented by the tight junctions present between the sustentacular cells.

2.1.4. Factors Affecting Drug Distribution

Drugs distribution is affected by the following factors:

1) **Age:** Distribution varies with difference in:
   i) **Total Body Content (Intracellular and Extracellular):** It is maximum in infants.
   ii) **Fat Content:** It is greater in infants and in elderly people.
   iii) **Skeletal Muscles:** It is less in infants and elderly people.
   iv) **Organic Composition:** Poorly developed BBB, low myelin content, and high cerebral blood flow in infants cause greater drug penetration in the brain.
   v) **Plasma Protein Content:** Albumin content is low in infants and elderly people.

2) **Pregnancy:** Growth of uterus, placenta and foetus rais es volume for drug distribution in pregnancy. Drug may also distribute in foetus which acts as a separate compartment. Plasma and ECF volume also increases , but albumin content is reduced.

3) **Obesity:** High adipose ti ssue content results in low drug distribution and perfusion. High fatty acid content alters the binding property of acidic drugs.
4) **Diet:** Fat-rich diet increases free fatty acid concentr ation in the blood that affects the binding of acidic drugs, **e.g.,** NSAIDs, albumin, etc.
5) **Disease States:** Drug distribution is severely affected in diseased conditions:
   i) Alteration in albumin and other drug-protein concentration,
   ii) Reduced or altered perfusion to organs and tissues, and
   iii) Alteration in tissue pH.

   In case of enceph alitis and meningitis, the BBB becomes more permeable, therefore, concentration of ionic antibiotics (**e.g.,** penicillin G and ampicillin) increases in brain tissues.
6) **Drug Interaction:** Two or more drugs administered together compete for the binding site, tend to replace each other, and the free drug may produce lethal effects, **e.g.,** phenylbutazone and warfarin.

2.1.5. Volume of Distribution

Varying concentrations of drug reaches different organs and tissues of the body. The process o f distribution is considered to be complete at distribution equilibrium. At this stage, different tissues and organs contain varying concentrations of drug that can be determined by the volume of tissues in which the drug is present. So, different body tis sues and organs have different concentrations of drug. The physiological meaning of volume of distribution is not clear. But, a constant relationship is seen between the amount of drug in body (X) and the concentration of drug in plasma (C):

$$X \propto C$$

Or, $X = V_d$

Where, V_d = Apparent volume of distribution, which is a proportionality constant having the unit of volume.

Determination of Volume of Distribution

1) The drug dose is administered by a rapid intravenous bolus injection , and then blood samples are taken at specific time intervals.
2) A suitable assay method is used to calculate the plasma concentration of each drug sample.
3) The obtained data is plotted on a graph paper so that the plasma profile of the drug can be obtained.
4) The drug level in plasma immediately after the administration of drug dose is calculated by back-extrapolating the plasma concentration *versus* time profile of the drug to time zero.

2.1.6. Apparent Volume of Distribution

Apparent volume of distribution is the **hypothetical volume of body fluid into which a drug is dissolved or distributed** . It is named as **apparent volume** because each part of the body equilibrated with the drug does not have equal concentration.

Thus, apparent volume of distribution can be represented as:

$$\text{Apparent Volume of Distribution} = \frac{\text{Amount of Drug in the Body}}{\text{Plasma Drug Concentration}}$$

There is no direct relationship between the apparent volume of distribution and true volume of distribution; while the real volume of distribution has direct physiological meaning and is related to the body water. By using specific tracers or markers, the volume of each of these real physiological compartments can be determined. High molecular weight substances that can totally bind to plasma albumin (**e.g.,** high molecular weight dyes such as Evans blue, indocyanine green and I-131 albumin) are used to determine the plasma protein. In case of intravascular route, these remain intact to the plasma. If the concentration of haematocrit is known, total blood volume can also be estimated.

The ECF volume can also be estimated by substances that can easily penetrate the capillary membrane and rapidly distribute throughout the ECF but do not cross the cell membranes (**e.g.,** the Na^+, Cl^-, Br, SCN and SO_4^{2} ions, insulin, mannitol, and raffinose). The volume of ECF is approximately 15 litres, excluding the plasma.

Tracer elements either negligibly bound or do not bound to plasma or tissue proteins, thus their apparent volume of distribution remain same to their true volume of distribution. These conditions differ for most of drugs that bind to extravascular tissues or plasma proteins or to both. A general concept can be made in respect of apparent volume of distribution of these drugs:

1) **Apparent Volume of Distribution Smaller than True Volume of Distribution:** Drugs that selectively bind to plasma proteins or other blood components (i.e., those that are less bound to extravascular tissues, **e.g.,** warfarin) have apparent volume of distribution smaller than their true volume of distribution. The V_d of such drugs are found between blood volume and Total Body Water (TBW) volume (i.e., between 6-42 litres); **for example,** warfarin has a V_d of about 10 litres.

2) **Apparent Volume of Distribution Larger than True Volume of Distribution:** Drugs that selectively bind to extravascular tissues, (i.e., those that are less bound to blood components, **e.g.,** chloroquine) have apparent volume of distribution larger than their true volume of distribution. The V_d of such drugs is always greater than 42 litres or TBW volume. **For example,** chloroquine has a V_d of approximately 15,000 litres. Such drugs leave the body slowly and are generally more toxic than the drugs that do not distribute deeply into body tissues.

2.1.7. Binding of Drugs

In general, protein binding is defined as the **binding of a drug to blood plasma proteins**. This binding can be between the drug and tissue membranes, RBCs, and other blood components. The effectiveness of a drug on the body depends on the amount of drug bound to protein. The bound drug remains in the blood and the unbound drug metabolises into the active part of the drug. Therefore, if a drug is 95% bound to a binding protein and 5% is free, it indicates that 5% of the drug is active in the system and gives rise to pharmacological effects.

Generally, protein binding is reversible, and therefore a chemical equilibrium develops, in which the chemical reaction occurs in backward and forward direction without any net change in reactants and products. This indicates that while achieving equilibrium, a cell which is effective at extracting the unbound drug may extract more drug as it disassociates. Reversible protein binding can be expressed as:

Protein + Drug ⇌ Protein-Drug Complex

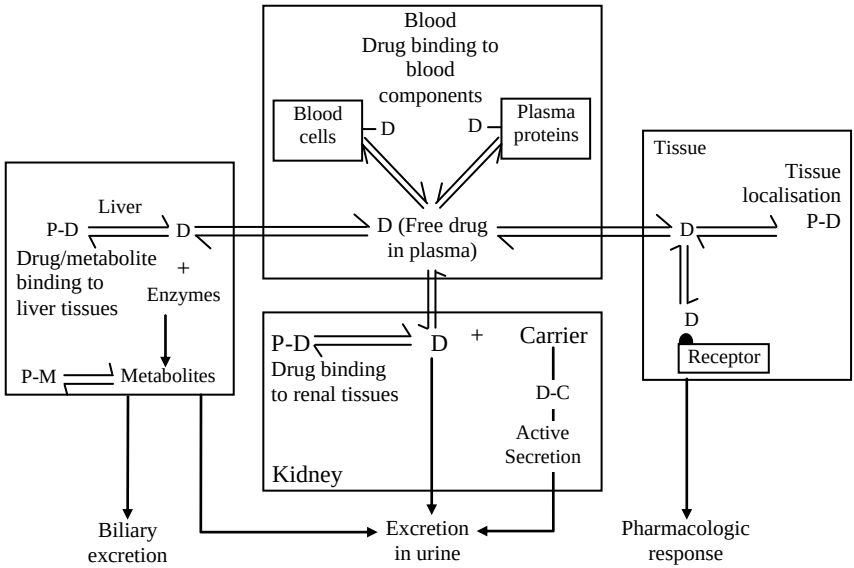


Figure 2.7: Protein-Drug Binding: Binding of Drugs to Various Tissue Components and its Influence on Disposition and Clinical Response. Only the Unbound Drug Moves Reversibly Between the Compartments

In the body, a drug interacts with several tissue components, of which blood and extravascular tissues are the two major classes. Generally, macromolecules such as proteins, DNA or adipose molecules, interact with body tissues. Protein molecules interact by forming complex molecules, and this phenomenon of complex formation is called **protein binding of drugs**. The importance of such protein binding is that the bound drug is pharmacokinetically as well as pharmacodynamically inert, thus indicating that a protein-bound drug is neither metabolised nor excreted nor is pharmacologically active.

A bound drug is also restricted so that it remains confined to a particular tissue for which it has greater affinity. However, a bound drug cannot undergo membrane transport because of its enormous size and therefore its half-life is enhanced. Binding of drugs falls into two classes:

1) Binding of drugs to blood components, like:
   i) Plasma proteins, and
   ii) Blood cells.
2) Binding of drugs to the extravascular tissues, proteins, fats, bones, etc.

2.1.7.1. Plasma Protein Binding of Drugs

When drug molecules reach systemic circulation, they interact with blood components, i.e., plasma proteins, blood cells, and haemoglobin (**table 2.2**). But, the drug molecules majorly interact with the plasma proteins present on cell

membrane of various blood components in abundant amounts and large variety. Binding of drugs to plasma proteins is a reversible proc ess and the extent of binding of drugs to various plasma proteins occurs as albumin > α_1-acid glycoprotein > lipoproteins > globulins.

Table 2.2: Blood Proteins to which Drugs Bind

Proteins	Molecular Weight	Concentration (g%)	Drugs that Bind
Human Serum Albumin	65,000	3.5-5.0	Large variety of all types of drugs.
α_1-Acid Glycoprotein	44,000	0.04-0.1	Basic drugs such as imipramine, lidocaine, quinidine, etc.
Lipoproteins	2,00,000 to 3,400,000	Variable	Basic, lipophilic drugs like chlorpromazine.
α_1-Globulin	59,000	0.003-0.007	Steroids like corticosterone and thyroxine, and cyanocobalamin.
α_2-Globulin	1,34,000	0.015-0.06	Vitamins A, D, E and K, and cupric ions.
Haemoglobin	64,500	11-16	Phenytoin, p entobarbital, and phenothiazines.

Binding of drugs to different plasma proteins is discussed below:

1) **Binding of Drugs to Human Serum Albumin (HAS):** Human Serum Albumin (molecular weight 65,000) is the most **abundant plasma protein** (59% of total plasma and 3.5 -5.0%) having high drug binding capacity. The therapeutic doses of most of the drugs are relatively much smaller and their plasma concentration does not normally achieve equimolar concentration with HSA. It has the property of binding to several compounds of varied structures of endogenous origin (**e.g.,** fatty acids, bilirubin , and tryptophan) as well as of exogenous origin (**e.g.,** drugs, weak acids, neutral compounds to weak bases, etc.). Drugs bind to the following four binding site s on the HSA (**figure 2.8**):

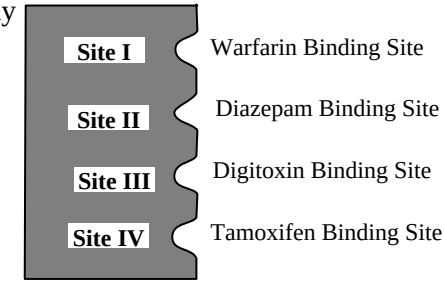


Figure 2.8: Four Major Drug Binding Sites on Human Serum Albumin

   i) **Site-I or Warfarin and Azathioprine Binding Site:** Maximum number of drugs bin d to this region, **e.g.,** several NSAIDs (phenylbutazone, naproxen, and indomethacin), sulfonamides (sulfamethizole and sulfadimethoxine), bilirubin sodium, phenytoin, and valproate.

   ii) **Site-II or Diazepam Binding Site:** Benzodiazepines, medium chain fatty acid s, ibuprofen, ketoprofen, tryptophan, cloxacillin, probenecid, etc. bind to this region.

   iii) **Site-III:** This site is also known as **digitoxin binding site**.

   iv) **Site-IV:** This site is also known as **tamoxifen binding site**.

2) **Binding of Drugs t o α_1-Acid Glycoprotein (α_1-AGP or AAG):** The molecular weight of α_1-acid glycoprotein is about 44,000 and its plasma

concentration ranges between 0.04-0.1g%. Many basic drugs bind to it, **e.g.,** propranolol, quinidine, disopyramide, imipramine, amitriptyline,nortriptyline, and lidocaine.

3) **Binding of Drugs to Lipoproteins:** Depending on the chemical composition, the molecular weight of lipoproteins varies between 0.2 to 3.4 million. On the basis of their density, lipoproteins are classified into:
   i) Chylomicrons,
   ii) Very Low Density Lipoproteins (VLDL),
   iii) Low Density Lipoproteins (LDL, predominant in humans), and
   iv) High Density Lipoproteins (HDL, most dense and smallest in size).

   A drug binds to lipoproteins by dissolving the lipid core of the lipoproteins. The binding capacity of drug depends on the lipid content of their lipid core. This process of binding of drugs to lipoproteins is non-competitive. Many acidic (**e.g.,** diclofenac), neutral (**e.g.,** cyclosporine A) and basic drugs (**e.g.,** chlorpromazine) bind to lipoproteins, of which the basic lipophilic drugs have maximum affinity.

4) **Binding of Drugs to Globulins:** Many plasma globulins are recognised and are labelled as follows:
   i) **α_1-Globulin or Transcortin or α_1-CBG (Corticosteroid Binding Globulin):** It binds to number of steroidal drugs, **e.g.,** cortisone, prednisone, thyroxin, and cyanocobalamin.
   ii) **α_2-Globulin or Ceruloplasmin:** It binds to vitamins A D, E and K and cupric ions.
   iii) **β_1-Globulin or Transferrin:** It binds to ferrous ions.
   iv) **β_2-Globulin:** It binds to carotenoids.
   v) **γ-Globulin:** It binds to antigens.

2.1.7.2. Binding of Drugs to Blood Cells

In blood, more than 40% are blood cells, in which RBCs constitute 95% of the total blood cells. Therefore, binding of drug to RBCs is important. The diameter of RBCs is 500 times more than the major plasma protein binding component, albumin. All the three components of RBC can bind to drugs:

1) **Haemoglobin:** Molecular weight of haemoglobin is 64,500 (almost equal to HSA), but in blood its concentration is 7-8 times of albumin. Phenytoin, pentobarbital, phenothiazines, etc. bind to haemoglobin.
2) **Carbonic Anhydrase:** Acetazolamide and chlorthalidone (carbonic anhydrase inhibitors) bind to it.
3) **Cell Membrane:** Imipramine and chlorpromazine bind to RBC membrane. Lipophilic drugs (**e.g.,** phenytoin) have a greater rate and extent of entry into RBCs. Hydrophilic drugs, **e.g.,** ampicillin, do not enter RBCs.

2.1.7.3. Tissue Binding of Drugs

Almost all body tissues, except HSA, consist of 40% of the body weight which is 100 times that of HSA. Therefore, tissue-drug binding is very vital in the study of drug distribution. A drug has the capability to bind to one or more of the several tissue components.

The importance of tissue -drug binding in distribution can be explained by the following two points:

1) Tissue-drug binding helps in increasing the apparent volume of distributi on of drugs in contrast to plasma protein binding which decreases it. This phenomenon occurs because the parameter is related to the ratio of drug amount in the body to free drug plasma concentration, and the latter decreases under extensive conditions of tissue-drug binding.
2) Due to tissue -drug binding, the drug gets distributed at some specific site in the body (with a subsequent increase in biological half -life). This is so because many drugs irreversibly bind to the tissues (opposite to the plasma protein-drug binding). **Examples** of tissue -drug binding are oxidation products of paracetamol, phenacetin, chloroform, carbon tetrachloride, and bromobenzene, which covalently bind to hepatic tissues.

Distribution of drugs in tissuesis affected by lipophilicity and structural features of the drug, perfusion rate, pH differences, etc. Drugs bind to extravascular tissues in the following order: Liver > Kidney > Lung > Muscle.

Several **examples** of extravascular tissue-drug binding are:

1) **Liver:** Epoxides of various h alogenated hydrocarbons and paracetamol bind irreversibly to liver tissues and cause hepatotoxicity.
2) **Lungs:** Basic drugs like imipramine, chlorpromazine and antihistamines accumulate in the lungs.
3) **Kidneys:** Metallothionein (a protein present in kidneys) bind s to heavy metals (such as lead, mercury, and cadmium) and causes their renal accumulation and thus toxicity.
4) **Skin:** Chloroquine and phenothiazines interact with melanin and accumulate in the skin.
5) **Eyes:** Retinal pigments of the eye contain melanin that bind s with chloroquine and phenothiazines, thus resulting in retinopathy.
6) **Hairs:** Arsenicals, chloroquine, and phenothiazines deposit in hair shafts.
7) **Bones:** Tetracycline binds to bones and teeth. If this antibiotic is administered to infants or children during odontogenesis, they will have permanent brown-yellow discolouration of teeth. Lead replaces calcium from bones and makes them brittle.
8) **Fats:** Lipophilic drugs (**e.g.,** thiopental) and DDT (pesticide) accumulate in adipose tissues by partitioning into it. Howe ver, high o/w partition coefficient is not the only criteria for adipose distribution of drugs since more lipophilic basic drugs (like imipramine and chlorpromazine) are not localised in fats. Poor perfusion of adipose tissues could be the reason for such an ambiguity. Adipose localisation of drugs occurs due to binding competition between adipose and non -adipose tissues (lean tissues like muscles, skin and viscera) and not due to partitioning.
9) **Nucleic Acids:** Molecular components of cells, like DNA, strongly interact with chloroquine and quinacrine, and result in distortion of its double helical structure.

2.1.8. Factors Affecting Protein-Drug Binding

Factors affecting protein-drug binding are categorised as follows:

1) **Drug-Related Factors**
   i) Physicochemical characteristics of drug,
   ii) Concentration of drug in body, and
   iii) Affinity of a drug for a particular binding component.

2) **Protein/Tissue Binding-Related Factors**
   i) Physicochemical properties of the protein or binding agent,
   ii) Concentration of protein or binding agent, and
   iii) Number of binding sites on binding agent.

3) **Drug Interactions**
   i) Competition between drugs fora binding site (displacement interactions),
   ii) Competition between drugs and normal body constituents, and
   iii) Allosteric changes in protein molecule.

4) **Patient-Related Factors**
   i) Age,
   ii) Intersubject variations, and
   iii) Disease states.

2.1.8.1. Drug-Related Factors

The drug-related factors which affect protein binding are discussed below:

1) **Physicochemical Characteristics of Drug:** Protein binding directly depends on the lipophilicity of drug; therefore an increase in lipophilicity increases the extent of binding. Highly lipophilic drugs (**e.g.,** thiopental) localise in adipose tissues, anionic or acidic drugs (**e.g.,** penicillins and sulphonamides) bind to HAS, cationic or basic drugs (**e.g.,** imipramine and alprenolol) bind to AAG, and the neutral or unionised drugs bind to lipoproteins.

2) **Concentration of Drug in Body:** The extent of protein -drug binding can change with the changes that occur in drug and protein concentration. The concentration of drugs that bind to HSA shows a very little influence as the therapeutic concentration of any drug is not sufficient to saturate it. But , the therapeutic concentration of lidocaine can saturate AAG with which it binds as the concentration of AAG is less than that of HSA in blood.

3) **Drug-Protein/Tissue Affinity:** The affinity of drug -protein/tissue binding varies according to the drug molecule. **For example,** lidocaine shows more affinity for AAG than for HAS; Digoxin has more affinity for proteins of cardiac muscles than those of skeletal muscles or plasma; Iophenoxic acid (a radio-opaque medium) has a great affinity for plasma proteins.

2.1.8.2. Protein/Tissue Binding-Related Factors

The protein/tissue binding-related factors affecting the protein -drug binding are discussed below:

1) **Physicochemical Properties of Protein or Binding Agent:** Lipophilic drugs mainly bind to lipoproteins and adipose tissues as they get easily

dissolved in their lipid core. The presence of active anionic and cationic groups on the albumin molecules to bind a variety of drugs depends on the physiological pH (pH of blood, plasma, ECF, etc.).

2) **Concentration of Protein or Binding Agent:** Among the plasma proteins, binding mainly occurs with albu min because its concentration is high er than other plasma proteins. During diseased states, the amount of several protei ns and tissue components available for binding gets changed.

3) **Number of Binding Sites on Binding Agent:** Albumin has numerous binding sites as well as high capacity binding component as compared to other proteins. Many drugs are capable of binding at more th an one site on albumin. **For example,** flucloxacillin, flur biprofen, ketoprofen, tamoxifen, and dicoumarol bind to both primary and secondary sites on albumin.

2.1.8.3. Drug Interactions

Drug-drug interaction affect drug-protein binding in the following ways:

1) **Competition between Drugs for a Binding Site (Displacement Interactions):** A competition occurs between two or more drugs for interacting with a binding site if the drugs can bind to the same site . If one drug (drug A) binds to a specific site, administration of the other drug (drug B) that has affinity for the same site causes dislocation or displacement of drug A from its binding site. This type of drug -drug interaction for the common binding site is known as **displacement interaction**.

   In this interaction, drug A is called the **displaced drug** while the drug B is called the **displacer**. In dis placement interactions , an unexpected rise is observed in free concentration of the displaced drug , which ma y increase clinical response or toxicity of the particular drug molecule. Displacement interaction can be affected by a drug metabolite also. The **clinical importance** of this type of interaction can be given as:

   i) The **displaced drug** (**e.g.,** warfarin) has a binding capacity of more than 95%, has a small volume of distribution (< 0.15 L/k g), shows a rapid onset of therapeutic or adverse effects, and has a narrow therapeutic index.
   ii) The **displacer drug** (**e.g.,** phenylbutazone) has a high affinity degree because the drug to be displaced competes for the same binding site. The drug/protein concentration ratio is high (> 0.10) and shows a rapid and large increase in plasma drug concentration.

   The extent of displace ment depends on the concentration of displacer drug and also on its affinity for the binding site with respect to that of the drug to be displaced. If a drug has 95% binding capacity , a displacement of just 5% of the bound drug results in a 100% rise in free drug concentration. If the displaced drug has a small volume of distribution, it remains in the blood compartment and gives rise to some toxic effects.

   If the displaced drug has a large V_d, it redistributes into a large volume of body fluids and gives rise to negligible or insignificant clinical effects. Increase in free drug concentration accompanying displacement also makes it

more available for elimination by the liver and kidneys (**figure 2.9**). If drug gets metabolised or excreted easily, its displacement causes a significant reduction in elimination half-life.

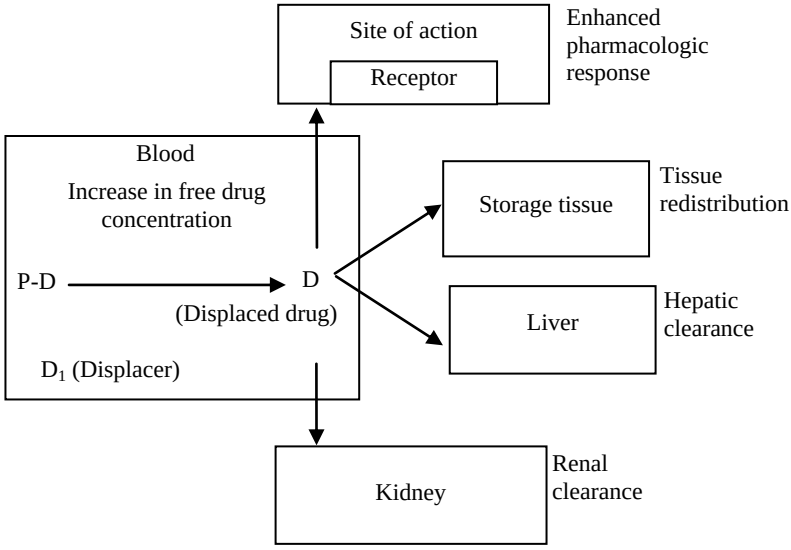


Figure 2.9: Fate of a Drug after Displacement Interaction

Displacement is almost insignificant if more selective, potent, and low dose drugs are used.

2) **Competition between Drugs and Normal Body Constituents:** Among the normal body constituents, the free fatty acids are the major ones that interact with a number of drugs that bind primarily to HSA. The level of free fatty acid increases in several physiologic al (fasting), pathologic al (diabetes, myocardial infarction, and alcohol abstinence) and pharmacologically induced conditions (after heparin and caffeine administration).

   The fatty acids binding to albumin influence the binding of several benzodiazepines and propranolol (decreased binding) and warfarin (increased binding). Binding of b ilirubin to HSA can be compromised by some drugs, and this is important for neonates who lack efficient BBB and bilirubin metabolising capacity.

   Acidic drugs (**e.g.,** sodium salicylate, sodium benzoate , and sulphonamides) have the ability to displace bilirubin from its albumin binding site. T he free bilirubin is not conjugated by the liver of neonates, and thus crosses the BBB and precipitates the condition of **kernicterus** (degeneration of brain and mental retardation).

3) **Allosteric Changes in Protein Molecule:** This mechanism is also used to explain that how the drugs affect protein binding interactions. This process includes alteration of protein structure by the drug or its metabolite to modify its binding capacity. The agents producing such an effect are known as **allosteric effectors**, **e.g.,** aspirin acetylates the lysine fraction of albumin and changes its capacity to bind NSAIDs , like phenylbutazone (increased affinity) and flufenamic acid (decreased affinity).

2.1.8.4. Patient-Related Factors

The patient-related factors that affect protein-drug binding are discussed below:

1) **Age:** A patient's age modifies protein-drug binding due to the differences in the protein content in various age groups:
   i) **Neonates:** In new-borns, albumin content is very low, therefore the unbound concentration of drug that primarily binds to albumin, **e.g.,** phenytoin and diazepam, is increased.
   ii) **Young Infants:** Binding of protein-drug in infants can be explained by an **example** of digoxin. It has been observed that infants suffering from congestive cardiac failure are given a digitalising dose 4 to 6 times the adult dose on body weight basis. It is opposite to the fact that infants should be given low doses considering their poorly developed drug eliminating system. The reason behind using large doses of digoxin is more binding of the drug in infants (the other reason is large renal clearance of digoxin in infants).
   iii) **Elderly:** In geriatric patients, the albumin content is lowered and free concentration of drugs that bind primarily to it is increased. It has been also seen that the levels of AAG increases in old age, and therefore decreased free concentration is observed for drugs that bind to it.
2) **Intersubject Variations:** Intersubject variability in drug binding is only studied in few drugs which show that the difference is small and not more than two times. These differences result in genetic and environmental factors.
3) **Disease States:** Protein-drug interaction gets altered in several pathologic conditions due to the alteration in protein content. Because albumin is the major drug-binding protein, hypoalbuminemia can severely impair protein-drug binding. It may occur in almost all pathological conditions such as ageing, CCF, trauma, burns, inflammatory states, renal and hepatic disorders, pregnancy, surgery, cancer, etc. Hyperlipoproteinemia may occur due to hypothyroidism, obstructive liver disease, alcoholism, etc., that affects binding of lipophilic drugs. All factors, mainly drug interactions and patient-related factors, affecting protein or tissue binding of drugs influence the following:
   i) **Pharmacokinetics of Drugs:** A decrease in plasma protein-drug binding, i.e., an increase in unbound drug concentration, favours tissue redistribution and/or clearance of drugs from the body (enhanced biotransformation and excretion).
   ii) **Pharmacodynamics of Drugs:** An increase in concentration of free or unbound drug increases the intensity of action (therapeutic/toxic).

2.1.9. Kinetics of Protein Binding

If P = proteins and D = the drug, then on applying law of mass action to reversible protein drug binding:

$$P + D \rightleftharpoons PD \qquad \text{.....(4)}$$

At equilibrium:

$$K_a = \frac{[PD]}{[P][D]} \qquad \text{.....(5)}$$

$$[PD] = K_a [P] [D] \qquad \text{.....(6)}$$

Where, [P] = Concentration of free protein.
[D] = Concentration of free drug.
[PD] = Concentration of protein-drug complex.
K_a = Association rate constant.
K_d = Dissociation rate constant.

$K_a > K_d$ indicates forward reaction, i.e., protein-drug binding is favoured. If P_T is the total concentration of bound and unbound protein:

$$P_T = [PD] + [P] \quad \text{.....(7)}$$

If r = number of moles of drug bound to total moles of protein:

$$r = \frac{[PD]}{[P_T]} = \frac{[PD]}{[PD]+[P]} \quad \text{.....(8)}$$

On substituting the value of [PD] from **equation (6)** in **equation (8)**:

$$r = \frac{K_a[P][D]}{K_a[P][D]+[P]} = \frac{K_a[D]}{K_a[D]+1} \quad \text{.....(9)}$$

Equation (9) holds when there is a single binding site on the protein and the protein-drug complex is a 1:1 complex. If more than one or N number of binding sites are available per mole of the protein:

$$r = \frac{NK_a[D]}{K_a[D]+1} \quad \text{.....(10)}$$

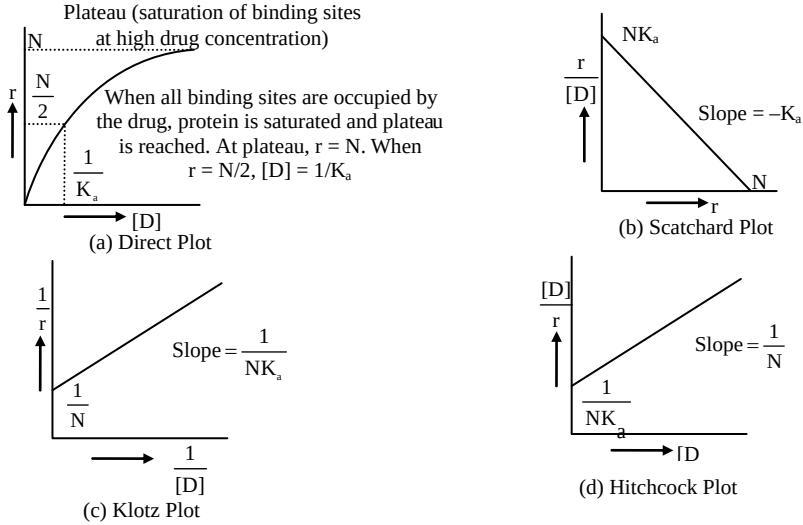


Figure 2.10: Plots Used for the Study of Protein-Drug Binding. (a) Direct Plot, (b) Scatchard Plot, (c) Klotz Plot, and (d) Hitchcock Plot

The value of association constant (K_a) and the number of binding sites (N) can be obtained by plotting **equation (10)** in four different ways (**figure 2.10**):

1) **Direct Plot:** It is made by plot ting r against (D) [**figure 2.10 (a)**]. 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] = $1/K_a$.

2) **Scatchard Plot:** It is made by transforming **equation (10)** into a linear form.

Thus, $$r = \frac{NK_a[D]}{K_a[D]+1} \quad \text{.....(11)}$$

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

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

Therefore,

$$\frac{r}{[D]} = NK_a - rK_a \quad \text{.....(12)}$$

A plot of r/[D] *versus* r yields a straight line **[figure 2.10 (b)]** with slope as $-K_a$, y-intercept as NK_a, and x-intercept as N.

3) **Klotz Plot/Lineweaver -Burke Plot (Double Reciprocal Plot):** The reciprocal of **equation (10)** yields:

$$\frac{1}{r} = \frac{1}{NK_a[D]} + \frac{1}{N} \quad \text{.....(13)}$$

A plot of 1/r *versus* 1/[D] yields a straight line with slope as $1/NK_a$ and y-intercept as 1/N **[figure 2.10 (c)]**.

4) **Hitchcock Plot:** It is made by rewriting **equation (13)** as:

$$\frac{NK_a[D]}{r} = 1 + K_a[D] \quad \text{.....(14)}$$

On dividing both sides by NK_a:

$$\frac{[D]}{r} = \frac{1}{NK_a} + \frac{[D]}{N} \quad \text{.....(15)}$$

Equation (15) is **Hitchcock equation** as per which a plot of [D]/r *versus* [D] yields a straight line with slope 1/N and y-intercept $1/NK_a$ **[figure 2.10 (d)]**.

2.1.10. Clinical Significance of Protein Binding of Drugs

Protein-drug binding has the following clinical applications:

1) In a few cases, drug -protein interaction may cause a prolonged residence of drug in the body. Plasma proteins are not a normal component of the glomerular filtrate, and drugs bound to the proteins will not be filtered. Therefore, a drug which gets eliminated by glomerular filtration will have a long biological half-life if it were subjected to protein binding.
2) Due to protein binding, the fraction of diffusible drug reduces; drug concentration is generally reduced at the sites of biotransformation when a drug bounds in a greater extent. This reduces the rate of drug elimination.
3) Plasma proteins act as physiological solubilisers. **For example,** bishydroxycoumarin is bound in the blood to the extent of 98%. It is because the drug is much more soluble in blood than in simple aqueous solutions. Bishydroxycoumarin remains insoluble at physiological pH without binding. Therefore, a therapeutic dose of the drug cannot be given intravenously without the precipitation of microcrystals in the blood vessels.
4) High blood levels and low volumes of distribution of the drugs results due to excessive plasma protein binding. This indicates that the ratio of drug in the blood to drug at the site of biological membrane may be long.

5) Bacterial infections generally affect the organ cells. The efficiency of a drug against a given pathogen can be evaluated by the drug's intrinsic antibacterial activity and its concentration in the extracellular spaces of the tissues, i.e., the site of infections. An antibiotic which is found in greater concentration in the blood show extensive binding in its compartment and is found in low concentration at the site of infection.

   Another antibiotic with same potency, unbound to plasma proteins, and free to be distributed may be present in high concentration at the site of infection. This antibiotic is more clinically effective, despite the fact that it would produce much lower blood levels than the first antibiotic.

6) The protein binding may also stimulate the probability of competition for the binding sites on a protein molecule, and the amount of bound drug might be decreased by another drug bound to plasma proteins. Protein binding may influence the activity, distribution, and elimination of a drug. This competitive phenomenon may have important clinical effects.

   When two drugs are simultaneously given, displacement of drugs from proteins occurs and increases the rate of biotransformation and elimination. Due to this property, the compounds that are effective **displacers** may be used potentially in case of drug intoxication to decrease the body drug content.

7) Another significant source of variation of free drug concentration in plasma is the competitive binding between drugs and endogenous substances.

8) In diseased states, the electrolytic balance in the blood is changed, which further changes the binding of drugs since the activity coefficient of drugs also change. Some alterations are also observed in the 3D structures of proteins when the electrolytic balance is disturbed. The effect of age on the binding of drugs is also an important factor because the plasma volume and its composition vary with age.

9) Physicochemical properties of drugs are also an important factor which plays a significant role in the binding of drugs to blood components.

   For example, tetracycline analogues show a correlation between their physical properties and disposition characteristics. When the drug molecules become more lipid-soluble, their interaction with proteins increases and their elimination from the body decreases.

2.2. SUMMARY

The details given in the chapter can be summarised as follows:

1) The processes which lower the plasma drug concentration are termed **disposition**.
2) **Distribution** involves the reversible transfer of a drug between compartments.
3) **Drug distribution** is also defined as the reversible transfer of a drug between one compartment and another.
4) **Elimination** involves irreversible loss of drug from the body.
5) Distribution is a passive transport process and the driving force for this process is obtained from the difference of concentration gradient between the blood and extravascular tissues.

6) **Tissue permeability** of a drug mainly depends on the physicochemical properties of the drug and the physiological barriers restricting diffusion of drug into tissues.
7) The tissue permeability of a drug is mainly determined by its degree of ionisation.
8) **Perfusion rate** is the volume of blood that flows per unit time per unit volume of the tissue. Its unit is ml/min/ml of the tissue.
9) Total body content (intracellular and extracellular) is maximum in infants.
10) Albumin content is low in infants and elderly people.
11) High adipose tissue content results in low drug distribution and perfusion.
12) **Apparent volume of distribution** is the hypothetical volume of body fluid into which a drug is dissolved or distributed.
13) Drugs that selectively bind to plasma proteins or other blood components have apparent volume of distribution smaller than their true volume of distribution.
14) Drugs that selectively bind to extravascular tissues have apparent volume of distribution larger than their true volume of distribution.
15) **Human Serum Albumin** (HSA, molecular weight 65,000) is the most abundant plasma protein (59% of total plasma and 3.5-5.0%) having high drug binding capacity.
16) Site-I binding site of HSA is known as **warfarin** and **azathioprine binding site**.
17) Site-II binding site of HSA is known as **diazepam binding site**.
18) Site-III binding site of HSA is known as **digitoxin binding site**.
19) Site-IV binding site of HSA is known as **tamoxifen binding site**.
20) **α_1-Globulin** or **transcortin** or α_1-CBG (Corticosteroid Binding Globulin) binds to γ number of steroidal drugs.
21) **α_2-Globulin** or **ceruloplasmin** binds to vitamins A, D, E and K and cupric ions.
22) **β_1-Globulin** or **transferrin** binds to ferrous ions.
23) **β_2-Globulin** binds to carotenoids.
24) **γ-Globulin** binds to antigens.
25) Epoxides of various halogenated hydrocarbons and paracetamol bind irreversibly to liver tissues and cause hepatotoxicity.
26) Basic drugs like imipramine, chlorpromazine and antihistamines accumulate in the lungs.
27) Metallothionein binds to heavy metals (such as lead, mercury, and cadmium) and causes their renal accumulation and thus toxicity.
28) Chloroquine & phenothiazines interact with melanin and accumulate in the skin.
29) Arsenicals, chloroquine and phenothiazines deposit in hair shafts.
30) Tetracycline binds to bones and teeth.
31) Lipophilic drugs and DDT accumulate in adipose tissues by partitioning into it.
32) Protein binding directly depends on the lipophilicity of drug; therefore an increase in lipophilicity increases the extent of binding.
33) The **displaced drug** has a binding capacity of more than 95%, has a small volume of distribution (< 0.15 L/kg), shows a rapid onset of therapeutic or adverse effects, and has a narrow therapeutic index.
34) The **displacer drug** has a high affinity degree because the drug to be displaced competes for the same binding sites.
35) In new-borns, albumin content is very low, therefore the unbound concentration of drug that primarily binds to albumin is increased.

36) A decrease in plasma protein-drug binding, i.e., an increase in unbound drug concentration, favours tissue redistribution and/or clearance of drugs from the body (enhanced biotransformation and excretion).
37) An increase in concentration of free or unbound drug increases the intensity of action (therapeutic/toxic).

2.3. EXERCISE

2.3.1. True or False

1) The processes which lower the plasma drug concentration are termed elimination.
2) Disposition involves reversible transfer of a drug between compartments.
3) Drugs that selectively bind to extravascular tissues have a pparent volume of distribution larger than their true volume of distribution.
4) Site-I binding site of HSA is known as digitoxin binding site.
5) α_2-Globulin binds to vitamins A D, E and K and cupric ions.
6) Basic drugs like imipramine, chlorpromazine and antihi stamines accumulate in the hair shafts.
7) Metallothionein binds to heavy metals and causes their hepatic accumulation and thus toxicity.

2.3.2. Fill in the Blanks

8) The tissue permeability of a drug is mainly determined by its _____.
9) The unit of perfusion rate is _____.
10) Site-IV binding site of HSA is known as _____.
11) β_2-Globulin binds to _____.
12) _____ binds to antigens.
13) Chloroquine and phenothiazines interact with melanin and accumulate in the _____.
14) _____ directly depends on the lipophilicity of drug.

Answers

1) False 2) False 3) True
4) False 5) True 6) False
7) False 8) Degree of ionisation 9) ml/min/ml of the tissue
10) Tamoxifen binding site 11) Carotenoids 12) γ-Globulin
13) Skin 14) Protein binding

2.3.3. Very Short Answer Type Questions

1) Define drug distribution.
2) Write about blood capillary membrane.
3) How drug distribution is affected by age?
4) What is volume of distribution?
5) Enlist the factors affecting protein-drug binding.

2.3.4. Short Answer Type Questions

1) Discuss the factors affecting drug distribution.
2) Explain plasma protein binding of drugs.
3) How distribution is affected by patient- and drug-related factors?
4) Discuss the kinetics of protein binding.

2.3.5. Long Answer Type Questions

1) Give a detailed review on different physiological barriers.
2) Discuss the factors affecting protein-drug binding.
3) Write an exhaustive note on tissue permeability of drugs.

CHAPTER 3

Introduction to Elimination

3.1. DRUG METABOLISM

3.1.1. Introduction

Elimination is the major process for the removal of a drug from the body and the termination of its action. It is defined as the **irreversible loss of drug from the body**. Elimination occurs by t wo processes, i.e., metabolism and excretion. The body metabolises some drugs chemically. Metabolism may either result in the formation of inactive substances (metabolites) or substances that may resemble to the original drug in terms of therapeutic activi ty or toxicity or substances that may differ from the original drug.

Liver is the main **site of metabolism** for most drugs, and hence, almost every drug passes through the liver. For the conversion of prodrug to active metabolites or for the conversion of active drugs to inactive forms, the drugs should enter the liver to be acted upon by many enzymes. A specific group of **cytochrome P-450 enzymes** involves in **liver's primary mechanism for metabolising drugs**. The metabolism rate of many drugs is controlled by the level of these cytochrome P - 450 enzymes. Since the enzymes possess a limited capacity of metabolism, they are burdened, when the levels of drug present in the blood are high.

It is **difficult** for **an infant** to **metabolise certain drugs**, because at the t ime of birth, the metabolic enzyme systems are not developed completely. Also, the activity of the enzymes reduces with an increase in the age of an individual. This is the reason why aged individuals and infants are unable to metabolise drugs as efficiently as the younger adults and children. As a result, often smaller doses per pound of body weight are dispensed for infants and aged individuals as compared to young individuals or middle-aged adults.

The **biochemical modification of a drug in the body** is t ermed **drug metabolism or biotransformation** . Once a drug enters the body it undergoes absorption, distribution, metabolism, and finally the metabolites (resultant products formed after metabolism) undergo excretion.

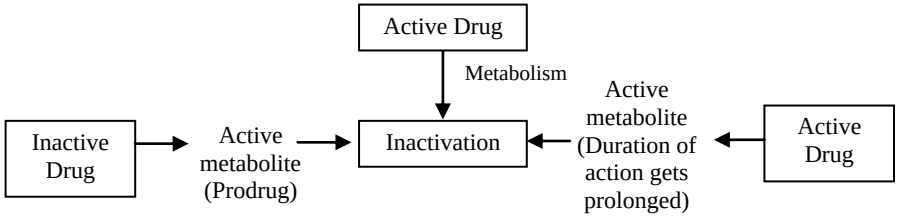


Figure 3.1: Metabolism of Drug

3.1.2. Organs Involved in Drug Metabolism

Though each biological tissue possesses a certain capability to metabolise drugs, but the principal site of drug metabolism is the smooth endoplasmic reticulum of the liver cell. Due to the given factors, liver functions as the major organ for metabolism:

1) Size of the liver is quite large.
2) Chemicals absorbed by the gut perfuse the liver first.
3) Most of the drug -metabolising enzymes are present in very high concentrations in the liver as compared to other organs.

After being swallowed, a drug is absorbed by the digestive system and the portal vein carries it to the hepatic portal system, where it undergoes maximum metabolism. Thus, the drug is said to exhibit first -pass effect. Epithelial cells of the GIT, lungs, kidneys, and skin are the other possible sites where the drug can undergo metabolism. However, localised side effects are often seen at these sites.

3.1.3. Enzymes Involved in Drug Metabolism

Metabolism of drugs involves many important enzymes and pathways. Based on the type of reaction catalysed by them, they can be categorised as:

1) **Enzymes involved in Phase I Metabolism** include:
   i) **In Oxidation:** Cytochrome P -450 monooxygenase system, flavin-containing monooxygenase system, alcohol dehydrogenase, aldehyde dehydrogenase, monoamine oxidase, and co-oxidation by peroxidase.
   ii) **In Reduction:** NADPH-cytochrome P -450 reductase and reduced (ferrous) cytochrome P -450. It is important to note that a chemical can enter substrate cycle during reduction reactions. In this cycle, a free - radical electron is gained by the chemical which is also quickly lost towards oxygen to form a superoxide anion.
   iii) **In Hydrolysis:** Esterase, amidase, and epoxide hydrolase.
2) **Enzymes involved in Phase II Metabolism** include:
   i) **In Methylation:** Methyltransferase.
   ii) **In Sulphation:** Glutathione S-transferases and sulfotransferases.
   iii) **In Acetylation:** N-acetyltransferases and amino acid N-acyl transferases.
   iv) **In Glucuronidation:** UDP-glucuronosyltransferases.

3.1.4. Factors Affecting Drug Metabolism

The following factors affect the biotransformation of a drug:

1) **Inhibitors:** Certain drugs, **e.g.,** cimetidine, omeprazole, and ciprofloxacin, can inhibit enzymes that metabolise a drug. Since the metabolising enzymes are inhibited, metabolism of the administered drug decreases, which in turn leads to an increase in the duration of its action.
2) **Stimulators:** Certain drugs like phenobarbitone and rifampic in can increase the activity of enzymes that metabolise a drug. Hence, it proves advantageous when drugs like phenytoin and warfarin are administered, as it increases their metabolism.

3) **Age:** Young children show poor drug metabolism as metabolic enzyme systems are not developed completely. **For example**, grey baby syndrome is seen in infants on administration of chloramphenicol as they lack glucuronyl transferase required for the inactivation of chloramphenicol.

4) **Sex:** In comparison to males, the females possess lesser ability for drug metabolism.

5) **Species:** Some enzymes may be species -specific. **For example** , rabbits possess atropinase enzyme and hence are able to metabolise atropine (therefore, atropine is non -toxic for rabbits); however, atropinase enzyme is absent in humans and hence, atropine proves toxic for humans.

6) **Genetics:** Drug metabolising enzymes show hereditary patterns; deficiencies of either of the enzymes belonging to the enzyme system can be inherited from one generation to the other. **For example,** an individual in whom Glucose-6-Phosphate Dehydrogenase (G-6-PD) enzyme is genetically deficient shows haemolysis when primaquine is administered to them.

7) **Body Temperature:** Temperature of the body is directly proportional to drug metabolism. Drug metabolism h as been found to increase with an increase in body temperature and *vice versa.*

3.2. BASIC UNDERSTANDING OF METABOLIC PATHWAYS

3.2.1. Introduction

Drug biotransformation involves enzymatic reactions that are divided into Phase I and Phase II reactions (**table 3.1**). Phase I reactions include hydrolysis, reduction, and oxidation. These reactions slightly increase the hydrophilicity. Phase II reactions include glucuronidation, sulfonation (or sulfation), acetylation, methylation, conjugation with glutathione, and conjugat ion with amino acids. These reactions increase the hydrophilicity by a greater extent.

Phase I reactions may or may not go before the Phase II reactions. **For example**, heroin forms morphine -3-glucuronide by undergoing hydrolysis (Phase I) and then conjuga tion with glucuronic acid (Phase II). However, morphine forms morphine-3-glucuronide by direct conjugation with glucuronic acid (Phase II).

Table 3.1: Reactions Involved in Drug Metabolism and their Subcellular Location

Type of Reactions	Phase-I	
	Enzymes Involved	**Subcellular Locations**
Hydrolysis	Esterase	Microsomes, cytosol, lysosomes, and blood
	Peptidase	Blood lysosomes
	Epoxidase and hydrolase	Microsomes and cytosol
Reduction	Azo- and nitro-reduction	Microsomes, microflora, and cytosol
	Carbonyl reduction	Cytosol, microsomes, and blood
	Disulphide reduction	Cytosol
	Sulphoxide reduction	Cytosol
	Quinone reduction	Cytosol and microsomes
	Reductive dehalogenation	Microsomes

Oxidation	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Cytosol and mitochondria
	Aldehyde oxidase	Cytosol
	Xanthine oxidase	Cytosol
	Monoamine oxidase	Mitochondria
	Diamine oxidase	Cytosol
	Prostaglandins H synthase	Microsomes
	Flavin monooxygenases	Microsomes
	Cytochrome P450	Microsomes
	Glucuronide conjugation	Microsomes
	Sulphate conjugation	Cytosol
	Glutathione conjugation	Cytosol and microsomes
	Amino acid conjugation	Mitochondria and microsomes
	Acylation	Mitochondria and cytosol
	Methylation	Cytosol, microsomes, and blood

3.2.2. Phase I Metabolic Pathways

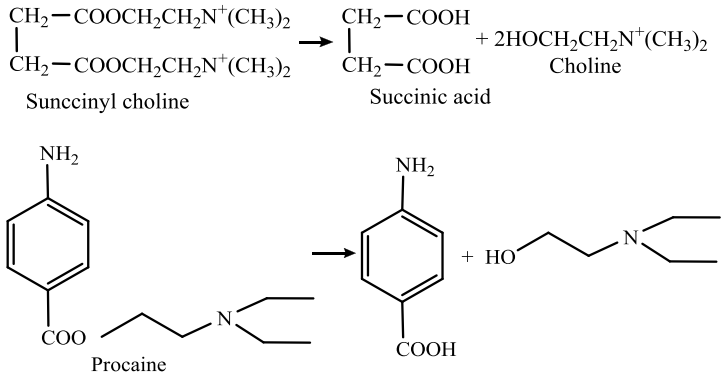
A molecule of drug initially enters phase I metabolism, where it undergoes a sequence of reactions, and at the end of this phase, the molecule shows the following changes:

1) **Forms a reactive site** or a functional group, like –OH, –SH, or $–NH_2$. During phase II, they successively conjugate with molecules, such as glucuronic acid, acetyl CoA, etc.
2) **Converts itself into forms that show reduced solubility in lipids as well as in water** so that its excretion is facilitated.

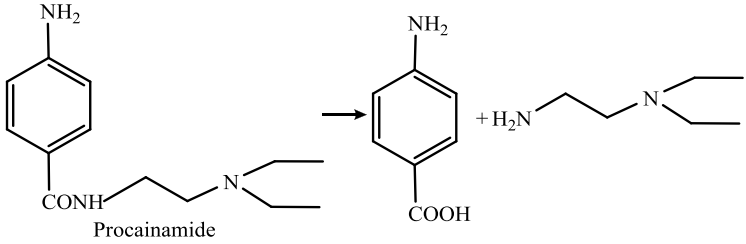
3.2.2.1. Hydrolysis Reaction

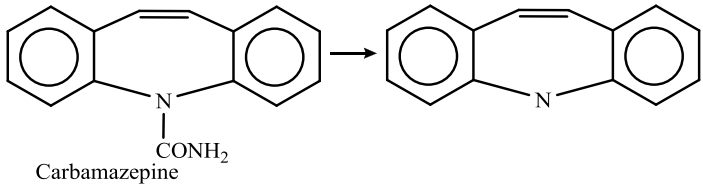
Drugs containing carboxylic acid (esterprocaine), amide (procainamide), thioesters (spironolactone), phosphoric acid ester (paraoxon), and aci d anhydride (diisopropylfluoro-phosphate) functional groups undergo hydrolysis. Hydrolysis of carboxylic acid esters, amides, and thioesters is catalysed by carboxylesterases located in various tissues and serum. Hydrolysis of phosphoric acid esters is catalysed by paraoxonase (or organophosphatase), which is a serum enzyme. Hydrolysis of phosphoric acid anhydrides is catalysed by diisopropylflurophosphatase. Carboxylesterases catalyse the trans -esterification of drugs in the presence of alcohol, **e.g.,** conversion of cocaine to ethylcocaine.

1) **Carboxylic Acid Esters**

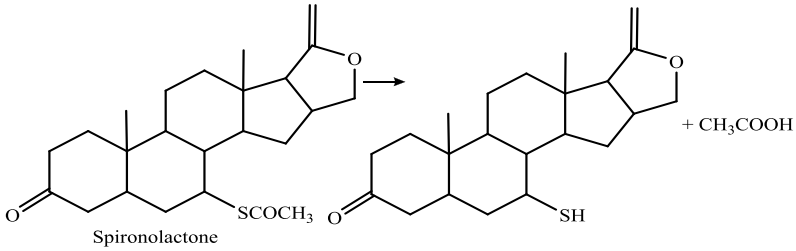


2) **Amides**

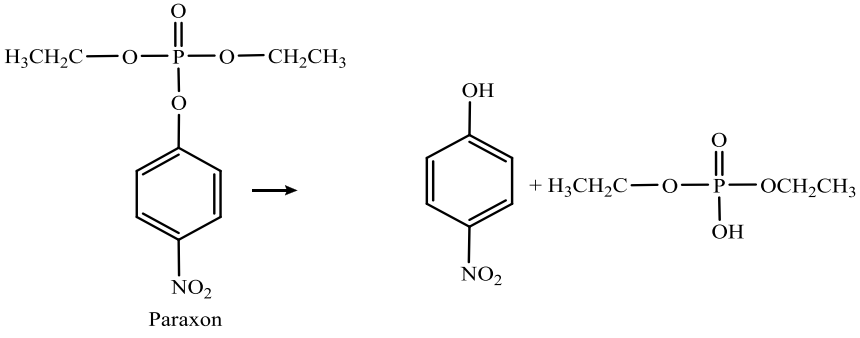




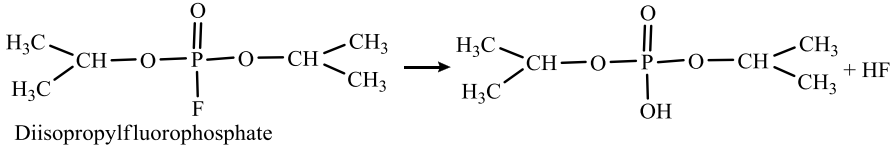
3) **Thioesters**



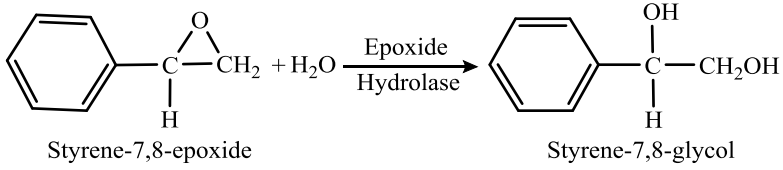
4) **Phosphoric Acid Esters**



5) **Acid Anhydride**

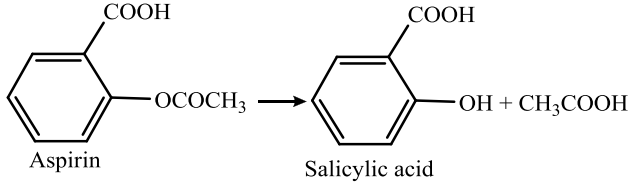


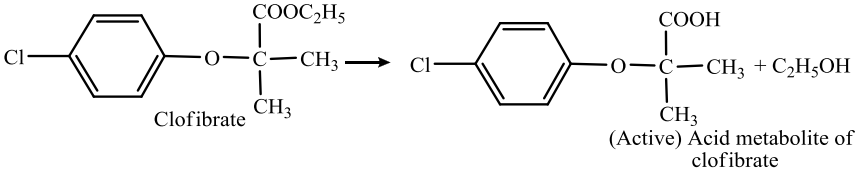
6) **Epoxides are Hydrolysed by Epoxide Hydrolase**



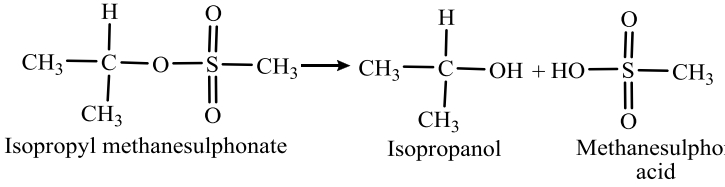
Another **example** of epoxide hydrolysis is conversion of leukotriene A_4 to leukotriene B_4. Peptidases that cleave th e amide linkage between adjacent amino acids act as amidases (hydrolysis reaction).

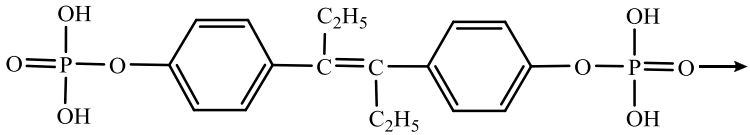
7) **Organic Acid Esters**



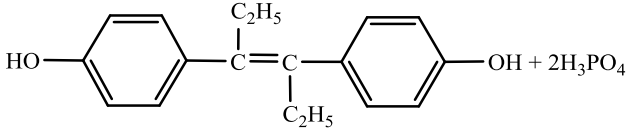


8) **Inorganic Acid Esters**



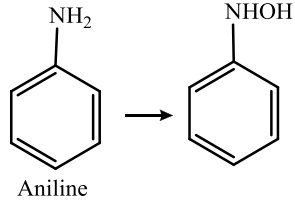


Stilbesterol diphosphate

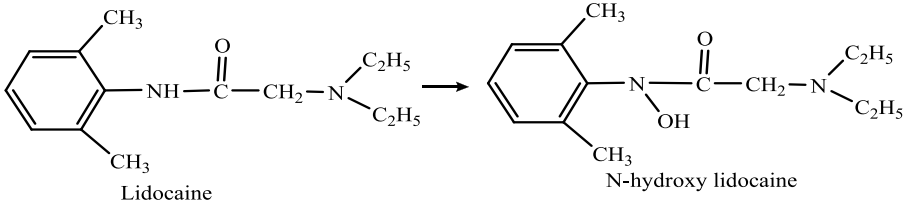


Stilbesterol

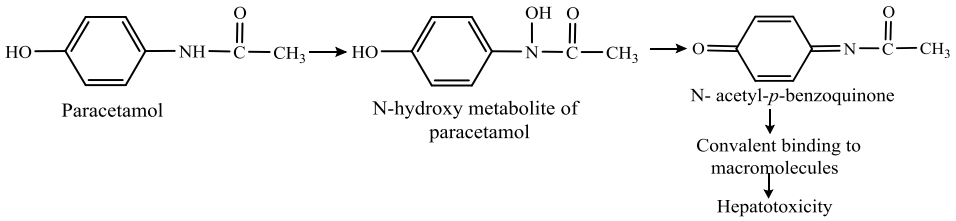
9) **N-Hydroxylation**



Aniline



N-hydroxylation of amides generates reactive intermediates that covalently bind to macromolecules, **e.g.,** paracetamol, and cause toxicity. Prolonge d usage or overdosage of paracetamol causes liver damage. Hepatotoxicity is caused by N - acetyl-*p*-benzoquinone metabolite, which is inactivated by glutathione conjugation. Prolonged use or overdose of paracetamol causes depletion of glutathione and the toxic metabolite of paracetamol causes liver damage.

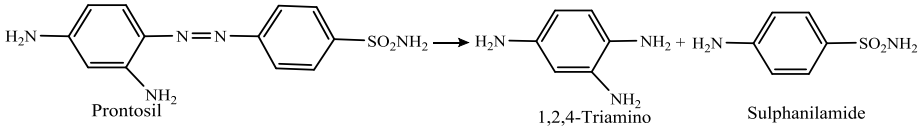


3.2.2.2. Reduction Reaction

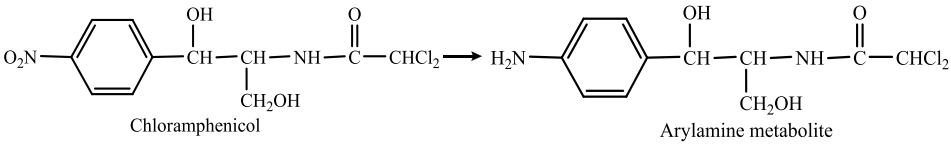
Drugs containing an aldehyde, ketone, disulphide, sulphoxide, quinine, N -oxide, alkene, azo or nitro group undergo *in vivo* reduction, **e.g.,** aldehyde can be reduced to alcohol or sulphoxide can be reduced to sulphide. Aldehyde oxidase is an enzyme involved in bioreductions.

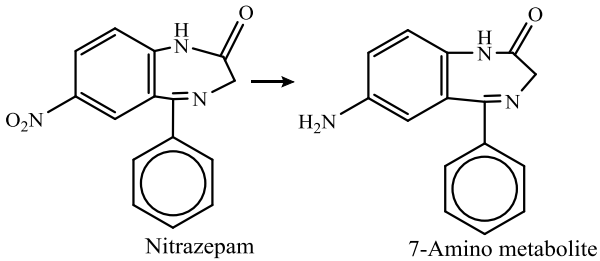
1) **Azo and Nitro Reduction:** This reaction is catalysed by intestinal microflora, cytochrome P-450 and NADPH-quinone oxidoreductase.

   i) **Azo Reduction**



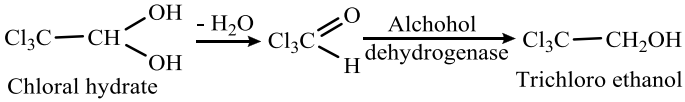
   ii) **Nitro Reduction**



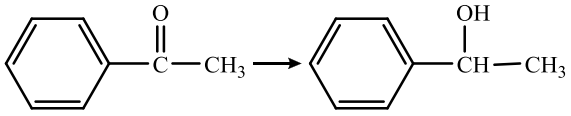


During azo reduction, N=N undergoes sequential reduction and gets cleaved into two primary amines. Four reducing equivalents are required for this reaction; whereas, six reducing equivalents are required for nitro reduction.

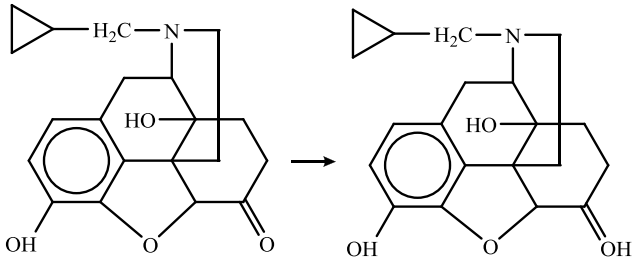
2) **Carbonyl Reduction:** This reaction is catalysed by carbonyl reductases present in blood and cytosolic fraction of the liver, kidney, brain, and other tissues. Reduction of aldehydes i nto primary alcohols and reduction of ketones into secondary alcohols are **examples** of carbonyl reduction.



Chloral hydrate — Trichloro ethanol



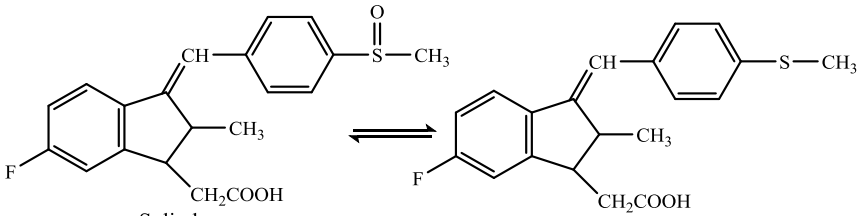
Acetophenone — Methyl phenyl carbinol



Naltrexone — Isomorphine derivative

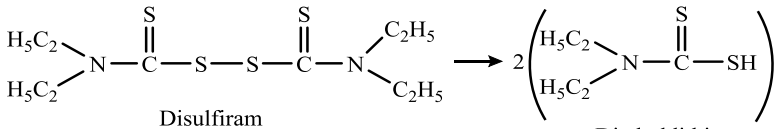
Acetohexamide, daunorubicin, ethacrynic ac id, warfarin, and menadione are other drugs that are reduced by carbonyl reductase.

3) **Sulphoxide and N -Oxide Reduction:** This reaction is catalysed by thioredoxin-dependent enzymes present in liver and kidney cytosol.



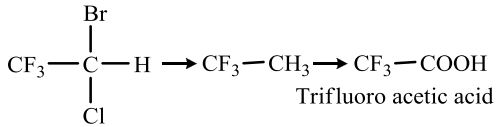
Sulindac — Sulindac sulphide

4) **Disulphide Reduction:** Disulphides undergo reduction and get cleaved into their respective sulphydryl components.



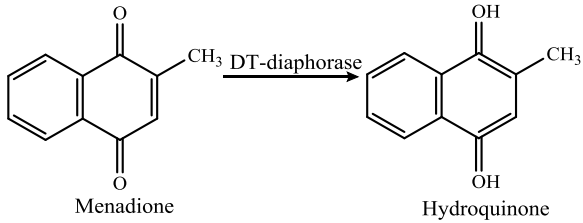
Disulfiram — Diethyldithio carbamic acid

5) **Reductive Halogenation:** This reaction involves replacement of halogen with hydrogen, and is catalysed by cytochrome P -450 and glutathione -S-reductase. The C-F bond is resistant to reduction.



Halothane — Trifluoro acetic acid

6) **Quinone Reduction:** Reduction of quinone into hydroxyquinones is catalysed by DT-diaphorase (NADPH-quinone oxidoreductase).



3.2.2.3. Oxidation Reaction

Liver cells (or **hepatocytes**) are the most common site for oxidation of a drug molecule and **microsomes** form the site of oxidation within the hepatocytes. **Mixed Function Oxidase** (MFO) is the enzyme system that causes oxidation of drug. The following components make up the enzyme system:

1) **Cytochrome Oxidase Enzyme:** It is commonly known as **Cytochrome P-450 (CYP-450)** and is chemically a haemoprotein. It is the terminal enzyme of the enzyme system that plays a role in drug oxidation. Oxidation of drug is caused by reduction of CYP -450 (as it exhibits maximum absorption at 450nm), hence, extra electrons are transferred to molecular oxygen, and so oxygen is reduced (reduced form of oxygen is known as **activated oxygen**). Once the electrons have been transferred, CYP -450 is free to again accept another electron, i.e., it is recovered.
2) **NADPH:** A co-enzyme that forms one more significant member of this system of oxidation.
3) **Oxygen**.
4) **NADPH Cytochrome Reductase:** It is chemically a flavoprotein, and another enzyme involved in the process.

Steps of Oxidation

1) The drug along with oxidised cytochrome P -450 (Fe^{+++}) enzyme forms a binary complex.
2) The oxidised CYP-450 now receives one electron from the reduced flavoprotein, and becomes reduced P-450 (Fe^{++}) but still remains attached to the drug.
3) Subsequently, activation of an oxygen molecule is seen. Oxygen attaches to the binary complex [obtained in step (1)], and forms a drug-reduced P-450 (Fe^{++})-activated oxygen complex, generally, abbreviated as drug-Fe^{++}-oxygen complex.
4) Lastly, the oxidised drug splits and CYP -450 again converts into oxidised (Fe^{+++}) form; thus the enzyme is recovered.

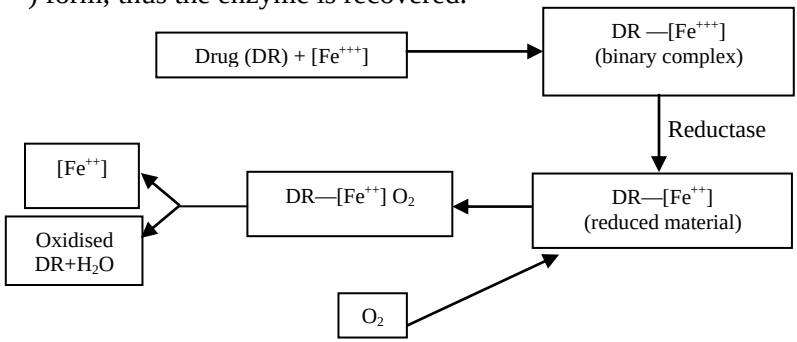
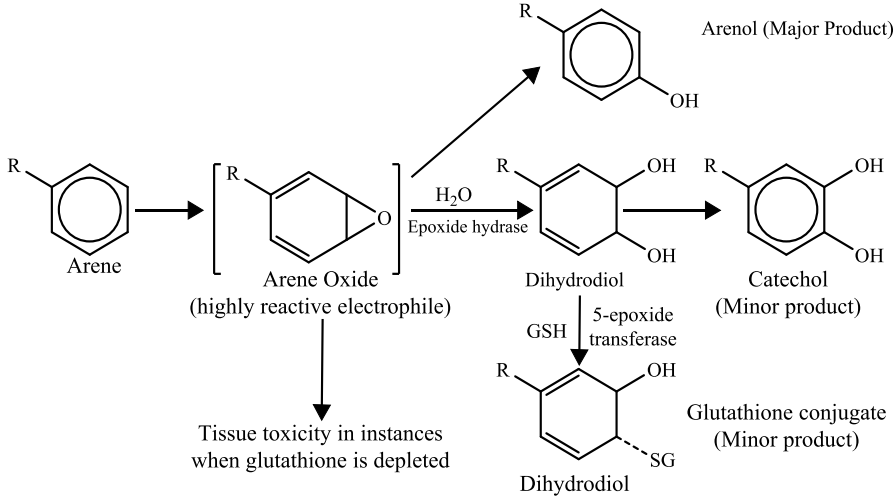


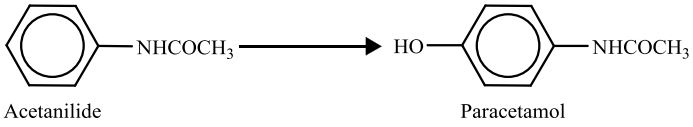
Figure 3.2: Oxidation by MFO System

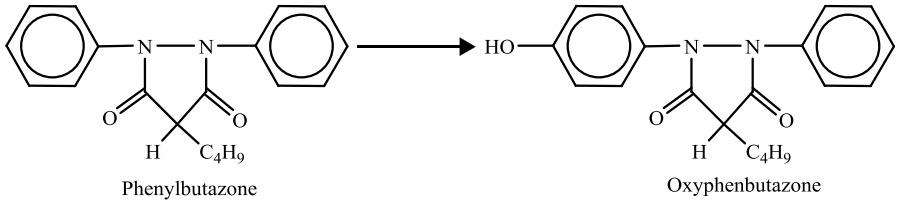
Oxidation Reactions

1) **Oxidation of Aromatic Carbon Atoms (Aromatic Hyd roxylation):** In this reaction, arene oxide (epoxide) is formed. It is a reactive intermediate that undergoes rearrangement to yield arenols, and sometimes catechols and glutathione conjugates.



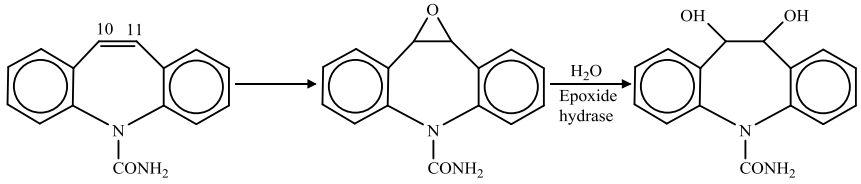
The arene oxide intermediate is highly reactive and carcinogenic or cytotoxic in some cases, **e.g.,** epoxides of bromobenzene and benzo(a)pyrene. Monosubstituted benzene derivatives undergo hydroxylation at *ortho*-, *meta*- or *para*-positions, and most commonly the *para*-hydroxylated produc t is formed, **e.g.,** acetanilide is converted to paracetamol and phenylbutazone is converted to oxyphenbutazone.





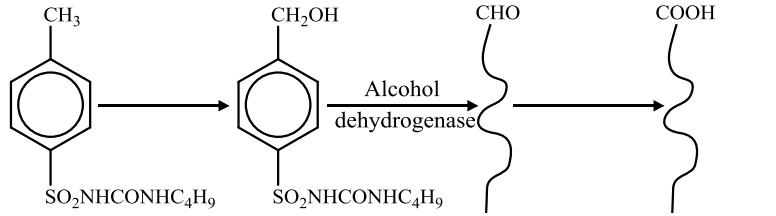
This reaction is favoured if the substituent is an activating or electron -rich group, like amino group. Deact ivating or electron -withdrawing groups (carboxyl and sulphonamide) retard or prevent aromatic hydroxylation, **e.g.,** probenecid.

2) **Oxidation of Olefins:** Oxidation of non -aromatic carbon -carbon double bonds is similar to aromatic hydroxylation, i.e., it forms epoxides to yield 1,2-dihydrodiols. Olefinic oxidation involving conversion of carbamazepine to carbamazepine -10,11-epoxide that further converts into corresponding *trans*-10,11-dihydrodiol is a well-known **example**.



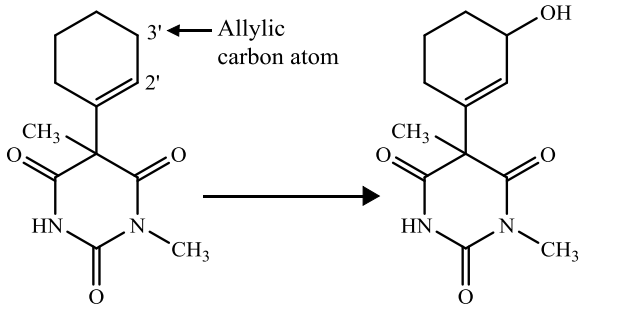
Carbamazepine | Carbamazepine-10,11-epoxide | *trans*-10,11-Dihydroxy carbamazepine

3) **Oxidation of Benzylic Carbon Atoms:** Carbon atoms attached to aromatic rings (benzylic carbon atoms) undergo hydroxylation to yield corresponding carbinols. If a primary carbinol is obtained, **e.g.,** tolbutamide, it gets further oxidised into aldehydes and then to c arboxylic acids. If a secondary carbinol is obtained, it gets converted into ketone.



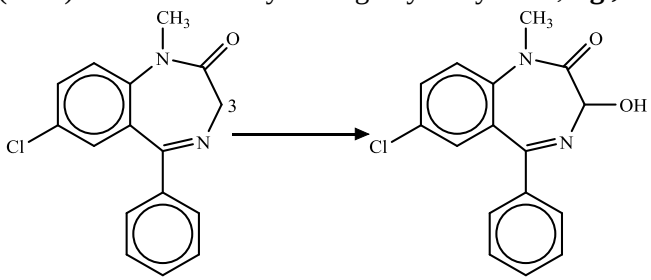
Tolbutamide | Primary carbinol | Corresponding aldehyde | Corresponding carboxylic acid

4) **Oxidation of Allylic Carbon Atoms:** Allylic carbon atoms lie adjacent to olefinic double bonds. They undergo hydroxylation similar to benz ylic carbons. Hydroxylation of hexobarbital into 3 ′-hydroxy hexobarbital is an **example** of this reaction.



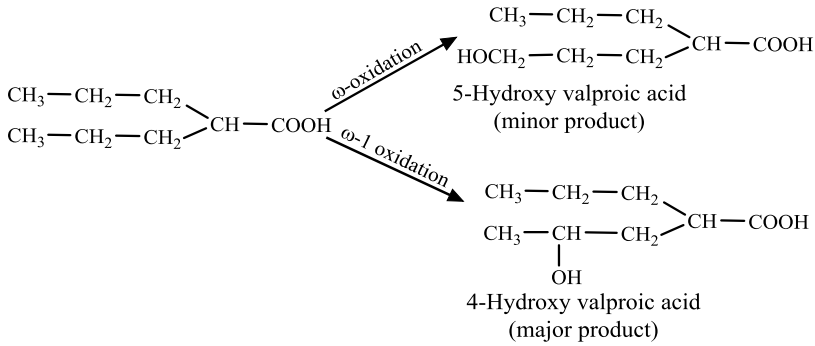
Hexobarbital | 3'-Hydroxy hexobarbital

5) **Oxidation of Carbon Atoms Alpha to Carbonyls and Imines:** Several benzodiazepines that contain a carbon atom (C -3) al pha to carbonyl (C=O) and imino (C=N) functions readily undergo hydroxylation, **e.g.,** diazepam.

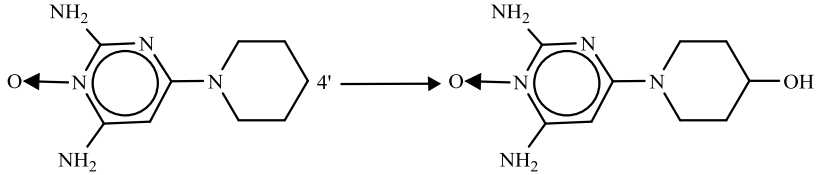


Diazepam | 3-Hydroxy diazepam (N-methyl oxazepam)

6) **Oxidation of Aliphatic Carbon Atoms (Aliphatic Hydroxylation):** Alkyl or aliphatic carbon atoms undergo hydroxylation at the te rminal methyl group (called as ω-oxidation) and the penultimate carbon atom (called as ω-1 oxidation). The latter oxidation reaction yields the major product, **e.g.,** valproic acid.

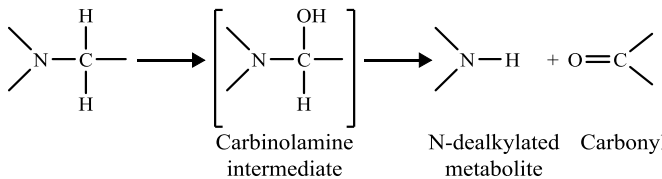


7) **Oxidation of Alicyclic Carbon Atoms (Alic yclic Hydroxylation):** Cyclohexane (alicyclic) and piperidine (non -aromatic heterocyclic) rings are found in various molecules, **e.g.,** acetohexamide and minoxidil, respectively. Such rings undergo hydroxylation at C-3 or C-4 positions.



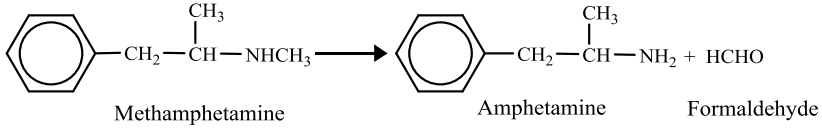
8) **Oxidation of Carbon-Nitrogen Systems**

i) **N-Dealkylation:** Alkyl groups attached to the nitrogen atom in nitrogen -bearing compounds undergo N-dealkylation reactions, **e.g.,** secondary and tertiary aliphatic and aromatic amines, tertiary alicyclic amin es, and N -substituted amides and hydrazines. Since N-dealkylation of amines yields amines and that of amides yields amides, the reaction does not undergo any change in the oxidation state. However, the removed alkyl group gets oxidised. Mechanism of N-dealkylation involves oxidation of α-carbon to generate an intermediate, carbinolamine that undergoes rearrangement by cleavage of C -N bond to yield the N -dealkylated product and the corresponding carbonyl of the alkyl group (a primary alkyl and a secondary alkyl are converted to aldehyde and ketone, respectively).

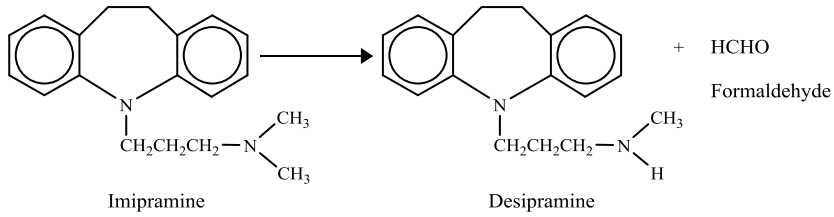


Tertiary nitrogen attached to different alkyl groups undergoes dealkylation by removal of smaller alkyl group. A representative **example** of each of the chemical cla sses of compounds capable of undergoing N-dealkylation is given below.

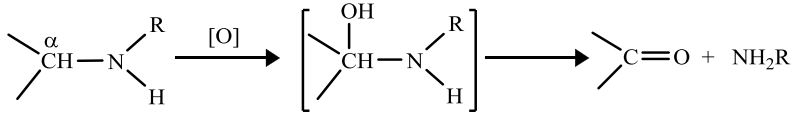
a) Secondary aliphatic amines, **e.g.,** methamphetamine.



b) Tertiary aliphatic amines, **e.g.,** imipramine.

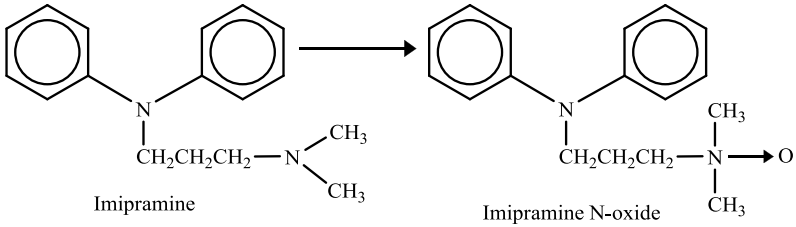


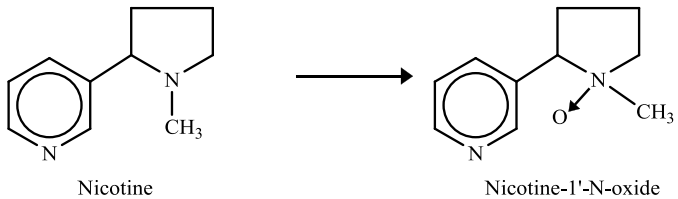
ii) **Oxidative Deaminat ion:** This reaction, similar to N -dealkylation, proceeds via carbinolamine pathway but the C-N bond cleavage occurs at the bond linking the amino group to the larger portion of the drug molecule. Thus, oxidative deamination is the reverse of N-dealkylation in terms of the product formed, the carbonyl product retains a large portion of the parent structure and the amines formed are simple **e.g.,** NH_3.

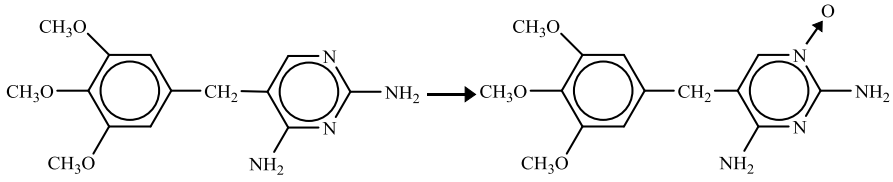


iii) **N-Oxide Formation:** N-oxides are formed by the nitrogen atoms exhibiting basic properties. Due to this reason, N -oxides can be formed from amines but not from amides. The tertiary amines yield N -oxides. The four categories of tertiary amines yielding N-oxides are:

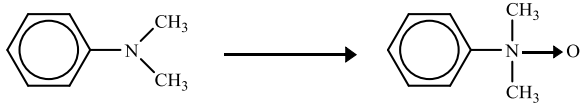
a) Aliphatic amines, **e.g.,** imipramine.
b) Alicyclic amines, **e.g.,** nicotine.
c) Nitrogen atoms of aromatic heterocyclics, **e.g.,** trimethoprim.
d) Amines attached to aromatic rings, **e.g.,** N, N-dimethyl aniline.







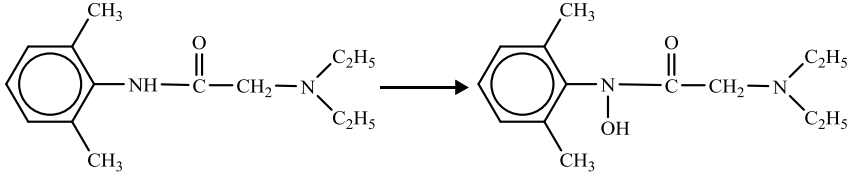
Trimethoprim — Trimethoprim-1-N-oxide



N, N-Dimethyl aniline — N-Oxide metabolite

The N-oxide products are highly water-soluble and get excreted in urine. But, they are prone to get reduced into the corresponding amine.

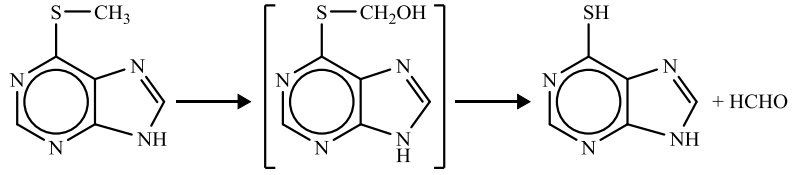
iv) **N-Hydroxylation:** Opposing the basic compounds that form N-oxides, N-hydroxy formation is displayed by non-basic nitrogen atoms such as amide nitrogen, **e.g.,** lidocaine.



Lidocaine — N-Hydroxy lidocaine

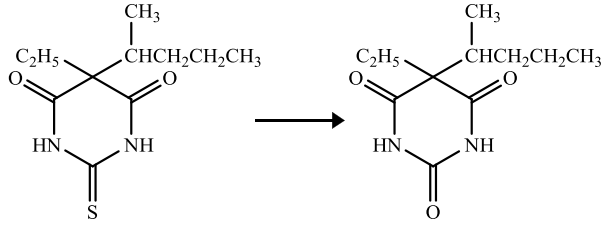
9) **Oxidation of Carbon-Sulphur Systems**

i) **S-Dealkylation:** Mechanism of S-dealkylation of thioethers (RSR′) is similar to that of N-dealkylation, i.e., it proceeds via α-carbon hydroxylation. The C-S bond cleavage forms a thiol (RSH) and a carbonyl product, **e.g.,** 6-methyl mercaptopurine.



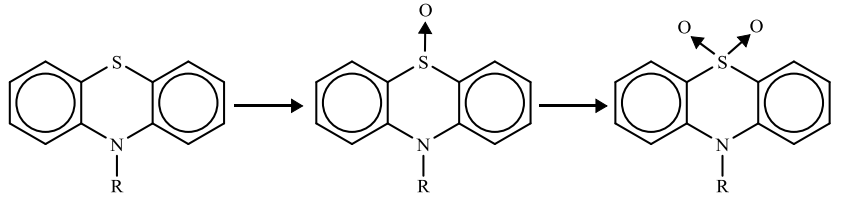
6-Methyl mercaptopurine — Hydroxylated intermediate — 6-Mercaptopurine

ii) **Desulphuration:** This reaction involves cleavage of carbon-sulphur bond (C=S or thiono) and formation of a product with C=O bond. Such a desulphuration reaction is observed in thioamides (RCSNHR'), **e.g.,** thiopental.

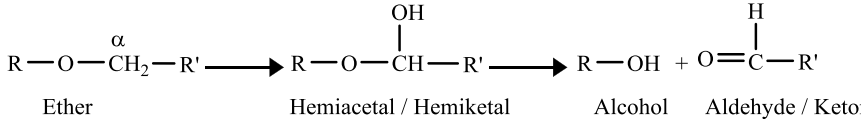


Thiopental — Pentobarbital

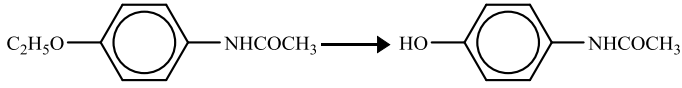
iii) **S-Oxidation:** Thioethers also undergo S -oxidation reaction to yield sulphoxides that further oxidise into sulphones (RSO $_2$R). Several phenothiazines, **e.g.,** chlorpromazine, also undergo S-oxidation.

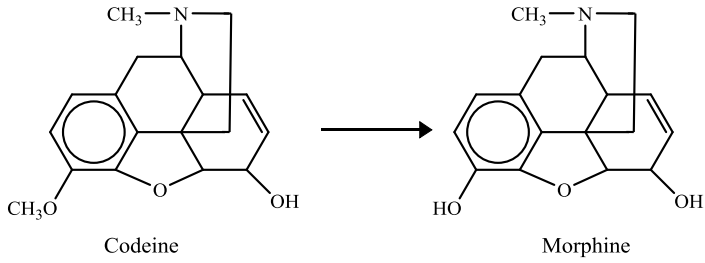


10) **Oxidation of Carbon -Oxygen Systems (O-Dealkylation):** This reaction, similar to N-dealkylation, proceeds via α-carbon hydroxylation and yield an unstable hemiacetal or hemiketal intermediate that undergoes spontaneous C-O bond cleavage to form alcohol (arenol or alkanol) and a carbonyl moiety.

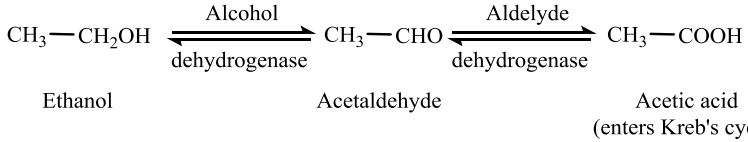


Methyl ethers get rapidly dealkylated than the longer chain ethers, such as the ones containing n-butyl group. The reaction results in active metabolites, **e.g.,** conversion of phenacetin to paracetamol and codeine to morphine.





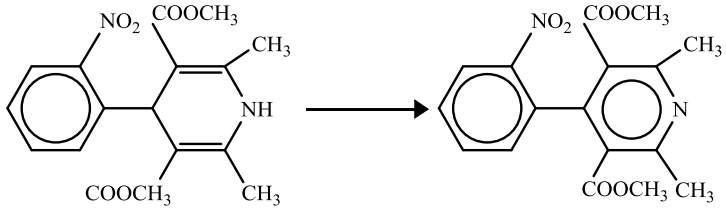
11) **Oxidation of Alcohol, Carbonyl and Carboxylic Acid:** This reaction is catalysed by non-microsomal dehydrogenase enzymes. Primary alcohols get rapidly metabolised into aldehydes that further metabolise into carboxylic acids; however, oxidation of secondary alcohols to ketones continues slowly.



In ethanol, reversible oxidation to acetaldehyd e occurs that further rapidly oxidises into acetic acid since acetaldehyde is highly toxic and should not accumulate in the body. With secondary alcohols, the oxidation rate increases with an increase in alkyl chain length. Compounds with primary and secondary alcohol groups are oxidised at the primary group.

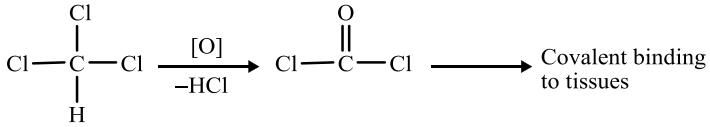
12) **Miscellaneous Oxidative Reactions**

i) **Oxidative Aromatisation/Dehydrogenation:** An **example** of metabolic aromatisation of drugs is nifedipine.



Nifedipine Pyridine metabolite

ii) **Oxidative Dehalogenation:** This reaction commonly occurs in drugs containing halogen, **e.g.,** chloroform. Dehalogenation of this drug yields phosgene that forms electrophiles capable of covalent binding to tissues.



Chloroform Phosgene

3.2.3. Phase II Metabolic Pathways

In Phase II reactions, drugs (or their metabolites) are combined with hydrophilic endogenous compounds to form complexes exhibiting adequate hydrophilic character to allow rapid excretion.

These conjugation reactions include gl ucuronidation, sulphonation (or sulphation), acetylation, methylation, conjugation with glutathione, and conjugation with amino acids such as glycine, glutamic acid and taurine (**table 3.2**). Phase II reactions are much faster than phase I reactions.

Table 3.2: Conjugation Reactions and Respective Functional Groups

Functional Groups	Conjugation Reactions
–OH, –COOH, $–NH_2$, –NH, –SH, and –CH	Glucuronidation
Aromatic –OH, aromatic $–NH_2$, and alcohols	Sulphonation (sulphation)
Aromatic $–NH_2$, aliphatic NH_2, hydrazines, and $–SO_2NH_2$	Acetylation
Aromatic –OH, $–NH_2$, –NH, and –SH	Methylation
Epoxides and organic halides	Glutathione conjugation
Aromatic $–NH_2$ and –COOH	Glycine conjugation

3.2.3.1. Glucuronidation

Glucuronidation is quantitatively the most significant phase II reaction. Glucuronic acid conjugation occurs only when glucuronic acid gets activated. The cofactor, Uridine Diphosphate -glucuronic acid (UDP -glucuronic acid) is required during glucuro nidation, which is also catalysed by UDP -Glucuronyl Transferases (UGTs, located in the endoplasmic reticulum of liver, kidney, intestine, skin, brain, spleen, and nasal mucosa) **(figure 3.3)**.

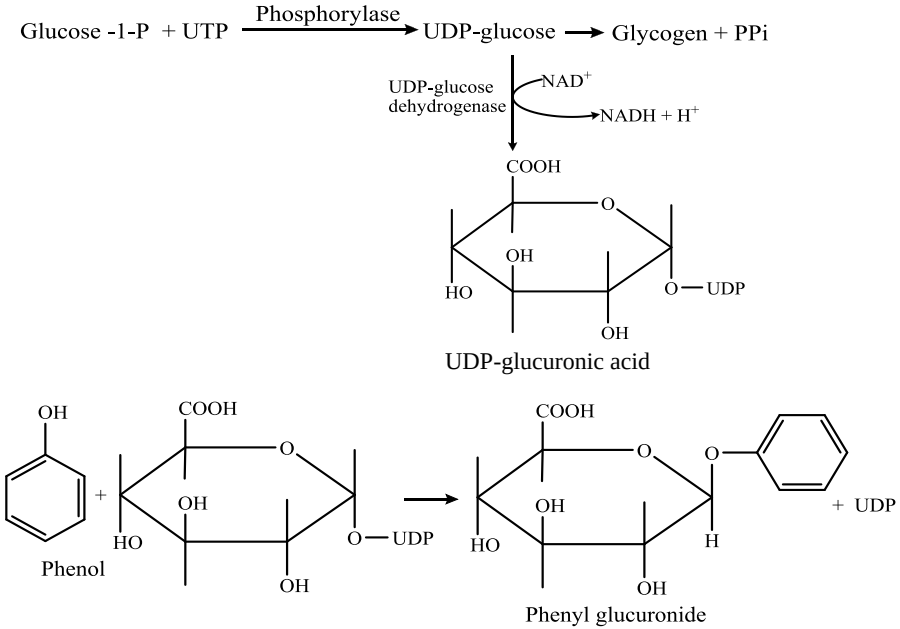
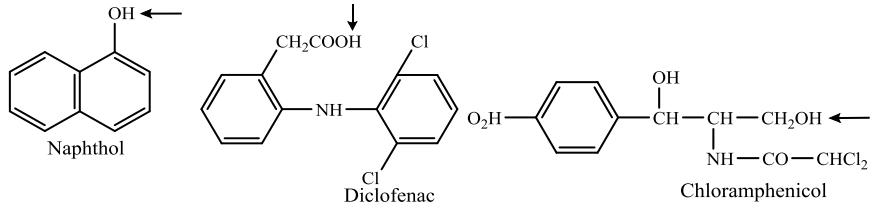


Figure 3.3: Synthesis of UDP-Glucuronic Acid

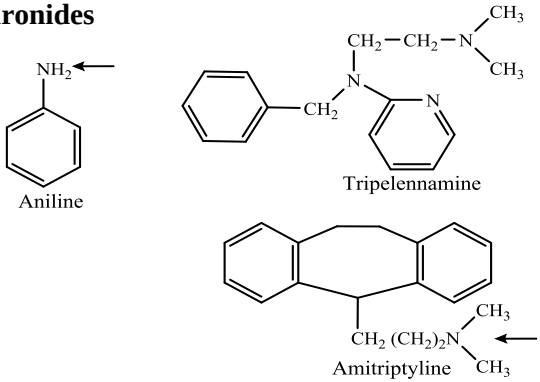
An electron -rich nucleophilic heteroatom, i. e., O, N , or S, forms the site of glucuronidation. Thus, aliphatic alcohols, phenols and carboxylic acids form O -glucuronidation, primary and secondary amines form N -glucuronides, and free sulphhydryl groups form S-glucuronides.

1) **O-Glucuronides**



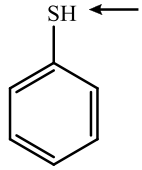
Other **examples** include acetaminophen, codeine, morphine, naloxone, oxazepam, propofol, propranolol, ketoprofen, naproxen, valproic acid, fenoprofen, chloramphenicol, and trichloroethanol.

2) **N-Glucuronides**

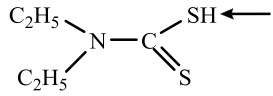


Other **examples** include benzidine, cyproheptadine, imipramine, lamotrigine, meprobamate, sulphisoxazole, and tripelennamine.

3) **S-Glucuronides**



Thiophenol



Diethyldithio carbamate

4) **C-Glucuronides:** Steroids, bilirubin, catechols, and phenylbutazone undergo C-glucuronidation. Glucuronide conjugates are polar, water -soluble, and are eliminated from the body through urine or bile. The size of aglycone (parent compound) decides whether glucuronides will be eliminat ed through bile or urine. Glucuronic acid is ionised at physiological pH and enhances elimination because:
   i) It increases the aqueous solubility of drug or its metabolites.
   ii) It is recognised by the biliary and renal organic transport system, thus glucuronides are secreted in urine and bile.

3.2.3.2. Sulphation

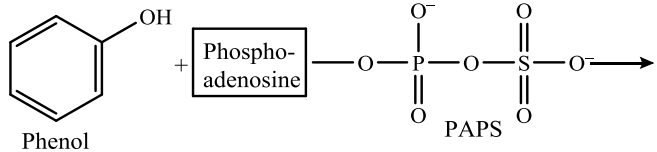
Sulphate conjugation forms a highly water -soluble sulphuric acid ester. This reaction is catalysed by sulphotransferases (sulphokinases), which are cytosolic enzymes found in liver, kidney, intestinal tract, lung, platelets, and brain. The cofactor required for sulphation is 3 '-Phosphoadenosine-5'-Phosphosulphate (PAPS). Many drugs undergoing O -glucuronidation also undergo sulphation. In sulphation reaction, just as in glucuronidation, the sulphate is activated before the reaction with substrate.

$$SH_4^{2-} + ATP \xrightarrow{\text{Sulphurylase}} APS + PPi$$

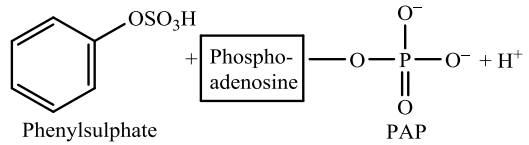
$$APS + ATP \xrightarrow{\text{APS-Phosphokinase}} PAPS + ADP$$

$$PAPS + \text{Substrate} \longrightarrow PAP^{+} + \text{Sulphate}$$

The sulphate is first converted into Adenosine -5'-Phosphosulphate (APS), which is then metabolised to 3 '-Phosphoadenosine-5'-Phosphosulphate (PAPS). This activated sulphate is then made to react with a substrate.



Phenol



Phenylsulphate PAP

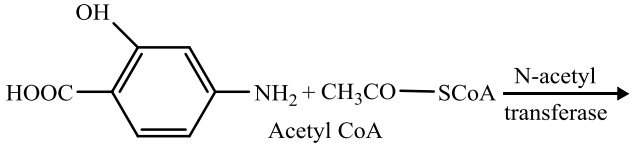
3.2.3.3. Acetylation

N-acetylation is a major biotransformation route for drugs with an aromatic amine or a hydrazine group. This reaction requires acetyl coenzyme A (acetyl CoA) cofactor and is catalysed by N-acetyltransferases.

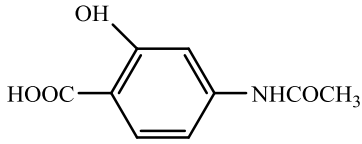
N-acetylation reaction involves the following **two steps**:

1) The acetyl group from acetyl -CoA is transferred to an active site cysteine residue in an N-acetyltransferase with release of coenzyme A.
2) The acetyl group is transferred from the acylated enzyme to the amino group of the substrate. The enzyme is regenerated.

N-acetyltransferases are cytosolic enzymes found in liver.



para-amino salicylic acid



N-acetylated *para*-amino salicylic acid

3.2.3.4. Methylation

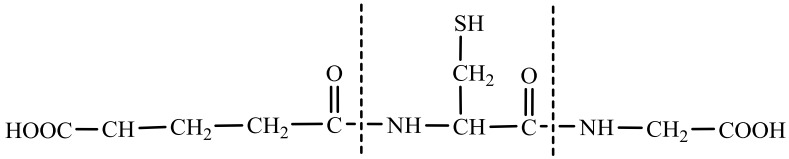
The metabolites resulting from methylation are not more polar or water-soluble. They possess equal or higher pharmacological activity than the parent drug. This reaction is catalysed by methyl transferase.

Methylation reaction involves the synthesis of an activated coenzyme, i.e., S -Adenosyl Methionine (SAM, donor of m ethyl group), from L -methionine and ATP. The methyl group is then transferred to a substrate.

Examples of methyl transferases are Catechol -O-Methyl Transferase (COMT), Phenyl Ethanolamine-N-Methyl Transferase (PNMT), etc.

3.2.3.5. Glutathione Conjugation

Glutathione (GSH) is a tripeptide comprised of glycine, cysteine, and glutamic acid. Its formation is catalysed by γ-glutamylcysteine synthetase.



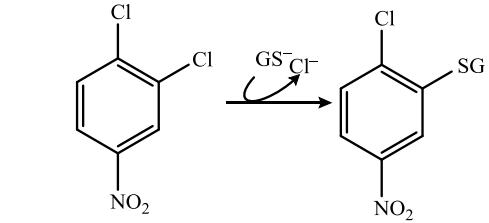
Glutamic acid Cysteine Glycine

Glutathione

Glutathione conjugates are thioesters formed by nucleophilic attack of glutathione thiolate anion (GS^-) on electrophilic carbon atom or electrophilic heteroatoms (O, N, or S). Glutathione conjugation reactions are of the following **two types**:

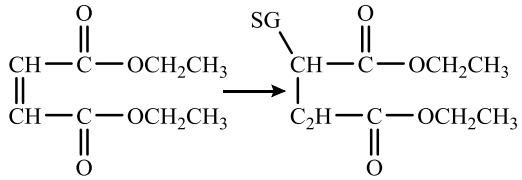
1) **Displacement Reactions:** In these reactions, glutathione displaces an electron-withdrawing group.
2) **Addition Reactions:** In these reactions, glutathione is added to an activated double bond.

Displacement Reaction



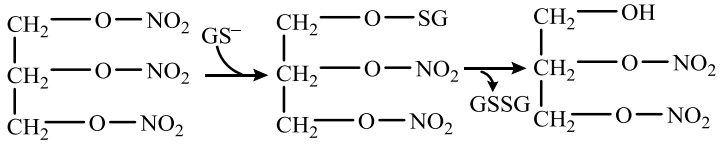
1,2-Dichloro-4-nitrobenzene — Glutathione conjugate

Addition Reaction



Diethylmaleate — Glutathione conjugate

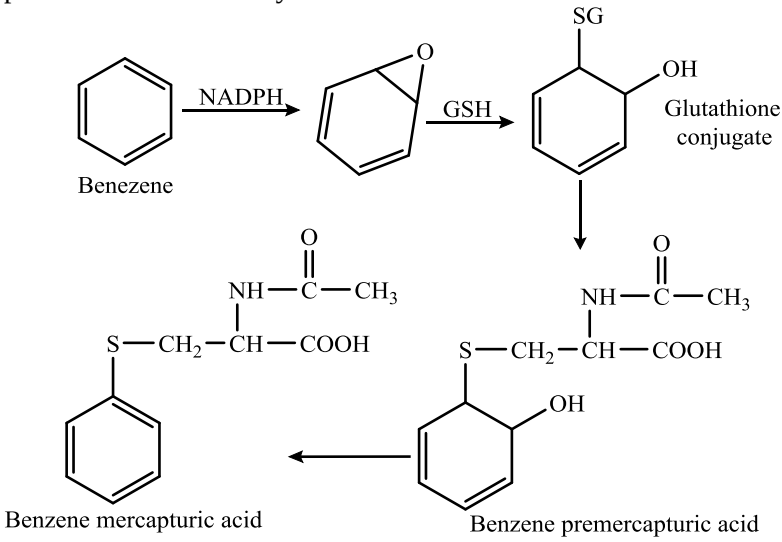
Conjugation of Heteroatom



Trinitroglycerine — Glutathione conjugate — Dinitroglycerine

Electrophiles are very toxic as they have the ability of binding to macromolecules (proteins and DNA) and causing cellular damage and mutations. Hence, conjugation of electrophiles with glutathione is an important detoxification reaction.

Glutathione conjugates are either eliminated in bile or first they are converted to mercapturic acid in the kidney and then excreted in urine.



Benzene mercapturic acid — Benzene premercapturic acid

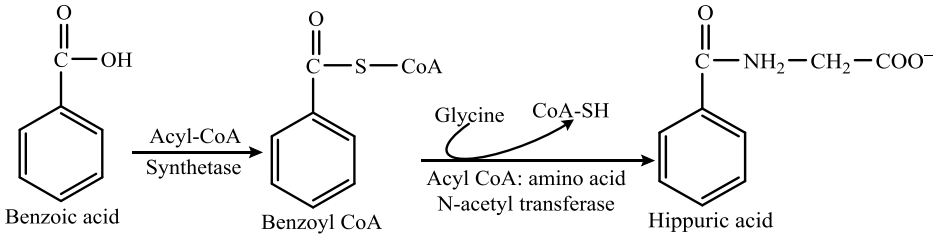
3.2.3.6. Amino Acid Conjugation

Drugs are conjugated with amino acids by the following **two pathways**:

1) Conjugation of drugs containing a carboxylic acid group with the amino group of amino acids, **e.g.,** glycine, glutamate, and taurine. This pathway involves initial activation of drugs with CoA. Thus, the acyl-CoA thioether formed reacts with amino group of amino acids.
2) Conjugation of drugs containing aromatic hydroxylamine with carboxylic acid groups of amino acids, **e.g.,** serine and proline.

The acceptor amino acid used for conjugation depends on the species and drug. Apart from glycine, glutamine and taurine, other amino acids, **e.g.,** ornithine, arginine, histidine, serine, and several dipeptides, **e.g.,** glycylglycine, glycyltaurine and glycylvaline, are also used for conjugation.

Conjugation with amino acids is generally a detoxification reaction.



3.3. DRUG EXCRETION

3.3.1. Introduction

Excretion removes drugs and/or their metabolites from the body. For cessation of the pharmacological action of a drug, its excretion, in intact or unchanged form is important. Kidneys are principal organs for excretion; and excretion through kidneys is termed as renal excretion. Non-renal excretion is the term used to describe excretion by other organs except the kidneys. The other organs include lungs, biliary system, intestine, salivary glands, and sweat glands.

All lipid-soluble drugs are converted into water-soluble compounds by the metabolic processes. This conversion enables their excretion (from the body). In case, the drug or its metabolites are water-soluble, it is excreted unchanged. Therefore, it can be said that for a drug to be eliminated from the body, both metabolism and excretion are important.

3.3.2. Clearance and Renal Clearance

Clearance may be defined as the complete removal of a drug in a specified time period from the hypothetical volume of body fluids containing the drug. A unit of **ml/min** expresses clearance of a drug and its value remains constant for any particular plasma drug concentration. **Clearance** describes the relationship between plasma drug concentration and the rate of drug elimination.

$$\text{Clearance(Cl)} = \frac{\text{Elimination Rate}}{\text{Plasma Drug Concentration}} \quad \text{..... (1)}$$

Renal Clearance (Cl_R)

Blood volume or plasma volume, completely cleared of the drug in its unchanged form, by the kidneys per unit time, defines the term renal clearance (Cl_R). Mathematically, it can be expressed as:

$$Cl_R = \frac{\text{Rate of Urinary Excretion}}{\text{Plasma Drug Concentration}} \quad \text{..... (2)}$$

Renal clearance can be physiologically expressed as the ratio of rate of reabsorption subtracted from the sum total of the rate of filtration and rate of secretion to plasma drug concentration (C):

$$Cl_R = \frac{\text{Rate of Filtration} + \text{Rate of Secretion} - \text{Rate of Reabsorption}}{C} \quad \text{..... (3)}$$

Clearance of a drug by an organ is not more than the blood flow to that organ because the rate of overall clearance is limited by the drug delivery to that organ via blood. If the volume of distribution and elimination rate constants are known, clearance can be calculated for a one-compartment model. Thus,

$$Cl = V_d \,.\, K_{el} \quad \text{.....(4)}$$

Clearance is a measure of renal function. Creatinine clearance is widely used for this purpose. Creatinine is a by-product of muscle catabolism and is chemically amine. Serum creatinine is measured in clinical practice for this purpose. As muscle catabolism and creatinine levels differ with age, weight, and sex, different formulas are used to calculate creatinine clearance from serum creatinine concentration.

For children between 1 to 20 years:

$$Cl_{cr} = \frac{0.48 \cdot H}{S_{cr}} . \left[\frac{W}{70}\right]^{0.7} \quad \text{.....(5)}$$

For adults above 20 years:

Males:

$$Cl_{cr} = \frac{(140 - Age) \cdot W}{72 \cdot S_{cr}} \quad \text{.....(6)}$$

Females:

$$Cl_{cr} = \frac{(140 - Age) \cdot W}{85 \cdot S_{cr}} \quad \text{.....(7)}$$

Where, Cl_{cr} = Creatinine clearance (ml/min).
S_{cr} = Serum creatinine (mg%)
H = Height (cm).
W = Weight (kg).
Age = in years.

Normal creatinine clearance is **120-130ml/min.** Creatinine clearance < 10ml/min indicates severe renal impairment. Renal function can be expressed as follows:

$$\text{Renal Function (RF)} = \frac{Cl_{cr} \text{ of patient}}{Cl_{cr} \text{ of normal subject}} \quad \text{.....(8)}$$

Hence, dose in patients with renal impairment = (Normal dose)·(RF)

Given below are some drawbacks and precautions when c alculating Cl_{cr} from serum creatinine:

1) Liver dysfunction is associated with a significant over -prediction of Cl_{cr}. These equations should be used with caution in patients with liver disease.
2) Thin individuals have low serum creatinine concentrations secon dary to decreased muscle mass, resulting in a significant over-prediction of Cl_{cr}.
3) Elderly patients have low serum creatinine concentrations secondary to decreased muscle mass, leading to a possible over-prediction of Cl_{cr}.

3.3.3. Renal Excretion of Drugs

Kidneys are responsible for the excretion of nearly all drugs as well as their metabolites, to a certain extent. Renal route exclusively is responsible for the excretion of some drugs, like gentamicin. Water -soluble, non -volatile, small molecular size (< 500 Daltons) agents that undergo slow metabolism, undergo excretion via urine.

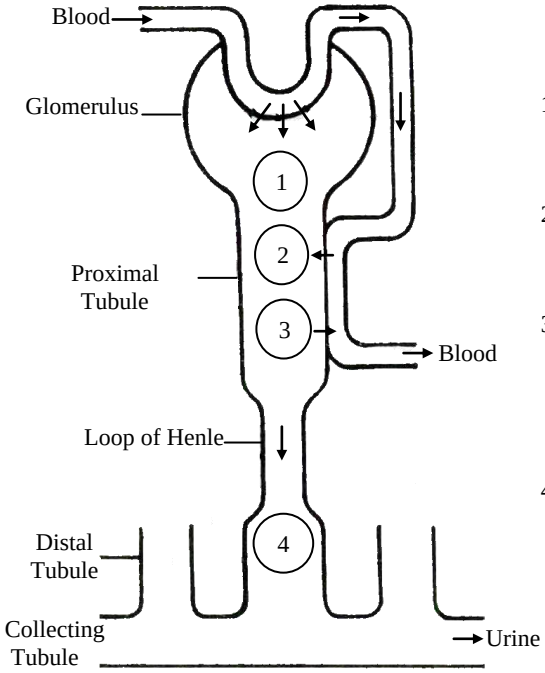


Figure 3.4: Simplified Diagram of the Processes involved in Urinary Excretion of Drugs

Nephron is the basic functional unit of kidney, which is involved in excretion. One million nephrons are present in each of t he kidney. Each nephron has the following parts:

1) Glomerulus, 2) Proximal tubule, 3) Loop of Henle,
4) Distal tubule, and 5) Collecting tubule.

Drug excretion via urine is determined by the following processes namely glomerular filtration, active tubular secretion, and active or passive tubular re - absorption (**figure 3.4**).

Concentration of drugs in the lumen tends to show an increase by the glomerular filtration and active tubular secretion, thus they are said to assist the process of excretion. However, tubular re -absorption reduces the rate of excretion besides preventing the drug from moving out of the body. Accordingly, the following equation can be used to denote the rate of excretion:

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

3.3.3.1. Glomerular Filtration

Glomerular filtratio n is a process involving the filtration of most compounds irrespective of whether they are ionised or unionised, with an exception of macromolecules (bounded to plasma proteins or blood cells). It is a non -selective process which occurs only in one directi on. Glomerulus also behaves as a negatively charged selective barrier, which promotes the retention of negatively charged compounds. The hydrostatic pressure of blood flowing in the capillaries acts as the driving force for filtration by the glomerulus. Only 10% (or 120 -130ml/min) of cardiac output is filtered through the glomeruli, out of the 25% (or 1.2 litres) of blood/min that reaches the kidneys through the renal artery. The rate of filtration is known as **Glomerular Filtration Rate (GFR)**. Although, aro und 180 litres of protein and free ultra -filtrate of cells pass through the glomeruli every day, yet, nearly 1.5 litres only is excreted as urine. The remaining filtrate undergoes reabsorption from the tubules.

An agent, excreted solely by filtration without being either secreted or reabsorbed in the tubules helps to determine the GFR. The rate of excretion of such an agent ranges from 120 -130ml/min. Estimation of GFR employs the use of creatinine, inulin, mannitol, and sodium thiosulfate. Also, creatinine and inulin are extensively used for the estimation of renal function.

3.3.3.2. Active Tubular Secretion

Active tubular secretion is a carrier-mediated process. Compounds are transported in a direction opposite to that of the concentration gradient during active t ubular secretion by utilising energy. The capacity of this system is limited and it gets saturated. Two mechanisms of active tubular secretion have been identified:

1) A system that secretes organic acids or anions (**e.g.,** penicillin, salicylates, glucuronides, sulphates, etc.) This system is similar to the one which secretes endogenous acids, like uric acid.
2) A system that secretes organic bases or cations (**e.g.,** morphine, mecamylamine, hexamethonium, endogenous amines like catecholamine, choline, histamine, etc.).

Both the mechanisms are comparatively non-selective and do not depend on each other. However, both of them can be bidirectional, i.e., the substances can undergo active secretion as well as reabsorption, **e.g.,** uric acid.

Active secretion remains un changed by alterations in pH and protein binding, because the bound drug rapidly dissociates at the moment when unbound drug gets excreted. However, it is influenced by renal blood flow opposite to the

process of glomerular filtration. The values of rate of excretion of drugs undergoing active secretion are greater than the normal GFR value of 130ml/min, **e.g.,** penicillin exhibits a renal clearance value of 500ml/min. Both glomerular filtration and tubular secretion are indicated by such a high value.

3.3.3.3. Tubular Re-Absorption

Tubular re-absorption of drugs follows the process of glomerular filtration. The entire length of the renal tubule shows tubular reabsorption. When the values obtained for the rate of excretion of a drug do not exceed that of GFR of 130ml/min, re-absorption of that drug is indicated. Agents that are completely re-absorbed after filtration, (**e.g.,** glucose) possess a clearance value of zero. Re-absorption increases the half-life of a drug, in contrast to tubular secretion.

Tubular re-absorption can take place in either of the **two ways:**

1) **Active Tubular Re-Absorption:** High threshold endogenous substances or nutrients like electrolytes, glucose, vitamins, amino acids, etc., which need to be conserved by the body, usually undergo active tubular re-absorption. Active re-absorption is shown by very few drugs, **e.g.,** oxopurinol.

2) **Passive Tubular Re-Absorption:** Numerous exogenous substances, including drugs, commonly exhibit passive tubular re-absorption. The **back diffusion** or **re-absorption** of water along with sodium and other inorganic ions establishes the concentration gradient, i.e., the driving force for such a process. Less than 1% of glomerular filtrate is excreted as urine; hence, if a drug does not undergo either secretion or re-absorption, its concentration in the urine will be 100 times more than that of free drug in plasma owing to water re-absorption.

3.3.4. Factors Affecting Renal Excretion of Drugs

Factors affecting drug excretion are:

1) **Physicochemical Properties of Drugs:** Various factors affect the physicochemical properties of drugs:
   i) **Molecular Weight (MW):** The excretion of drugs with large molecular weights is difficult, compared to that of drugs with smaller molecular weights. This is especially seen in case of glomerular filtration.
   ii) **Lipid Solubility:** The solubility of a drug in lipids is inversely proportional to its urinary excretion. **For example**, if drug A is more soluble in lipids compared to drug B, then drug A will show increased volume of distribution and a decreased renal excretion as compared to that exhibited by drug B (increased lipid solubility of a drug increases its volume of distribution and decreases renal excretion).
   iii) **Volume of Distribution:** The volume of distribution of drugs (V_d) is inversely proportional to their clearance. A drug that possesses a large V_d shows poor excretion in urine, while drugs possessing a smaller V_d (restricted to blood) show sufficient excretion (higher rate of excretion).

2) **Renal Blood Flow:** Drugs that show increased perfusion also show an increase in their excretion; this is especially significant for drugs that undergo excretion by glomerular filtration.

3) **Binding Characteristics of Drugs:** Drugs that are bounded to plasma proteins act as macromolecules, and their filtration by the glomerulus is not possible. Glomerular filtrate contains only those drugs that are unbound or free. Drugs that are protein-bound possess extended half-lives.

4) **Drug Renal Clearance:** It is the rate at which a drug is excreted by the kidney into urine comparative to the plasma drug concentration. Adequate renal function determines the renal clearance of several drugs and their metabolites.

   In cases of impaired renal clearance, $t_{½}$ of a drug may increase and the body may show the presence of toxic levels of the drug. Renal clearance is significant particularly for certain drugs which are:
   i) Excreted mainly by the kidneys, and
   ii) Possess limited therapeutic index (**e.g**. lithium, digoxin, and warfarin).

   Diseases decrease renal clearance in the following two patterns:
   i) **Diseases that Decrease Renal Blood Flow: For example,** congestive heart failure, haemorrhage, and cardiogenic shock.

   ii) **Diseases that Decrease Renal Excretion: For example,** renal disease (**e.g.,** glomerulonephritis) that may increase the half-life ($t_{½}$) of drugs.

5) **Plasma Drug Concentration:** Glomerular filtration and renal reabsorption are affected by the concentration of drug in plasma. If the drug is not protein bound, its glomerular filtration is directly proportional to its plasma concentration. In drugs showing renal reabsorption, excretion occurs only when concentration in glomerular filtrate is higher than reabsorption capacity.

6) **Urine pH:** For weakly acidic (pKa 3 -7) or weakly basic (pKa 6 -12) drugs, the degree of ionisation in tubular fluid depends on pH, **for example**:
   i) **Methamphetamine (Weak Base, pKa 10):** Renal excretion is 4 times faster in acidic urine than in alkaline urine because at lower pH, the ionisation is lower, reabsorption is less, and excretion of methamphetamine is more.

   ii) **Phenobarbital (Weak Acid, pKa 7.4):** Renal excretion is 7 times faster in alkaline urine. However, renal clearance is only a small fraction of total clearance.

   iii) **Salicylic Acid (Weak Acid, pKa 3.5):** This acid is mainly ionised at physiological pH. At pH 5.0, the amount of non-ionised form is 25 times of that present at pH 7.4. In over dosage of aspirin, systemic alkali and tubular acidosis occurs that enhances tubular reabsorption and prolongs half-life of elimination. This can be reversed by giving systemic alkali and fluids and producing an alkaline urine with high flow rate.

7) **Biological Factors:** Various biological factors, like age, sex and species, influence the drug excretion. Circadian rhythm also plays an important role. Females show 10% lower renal excretion than males; in neonates, renal function is only 60 -70% in comparison to adults; in elderly, renal function and hence renal excretion is reduced.

8) **Pregnancy:** The volume and composition of body fluids undergo significant changes in pregnant women. The plasma volume, the red cell mass, and the albumin mass increases. The plasma volume shows greatest increase (up to 40%), and the hematocrit and plasma albumin concentration also increase. Total body volume also increases between 6-8 lines.

   Pregnancy significantly increases renal blood flow and GFR in very early pregnancy. GFR increases up to 50% by the end of first trimester. Hence, plasma concentration of components handled by filtration falls; **e.g.,** normal serum creatinine in pregnancy is about 40-50mmol/l.

9) **Drug Interactions:** Drug excretion is influenced by many drug interactions and involve the following effects:
   i) Forced diuresis,
   ii) Alteration in urine pH,
   iii) Alteration in intrinsic clearance,
   iv) Alteration in renal blood flow,
   v) Alteration in binding characteristics, and
   vi) Alteration in active secretion.

   If the drug is highly protein bound and if any other drug displaces it, excretion of such highly protein bound drug increases. **For example,** furosemide enhances the excretion of gentamicin by displacing it from its protein binding sites; the excretion of basic drugs is enhanced by the acidification of urine, (**e.g.,** by drugs like ammonium chloride) and the excretion of acidic drugs is enhanced by the alkalinisation of urine (**e.g.,** by drugs like bicarbonates).

10) **Disease States:** Renal excretion is an important route of excretion, and hence the excretion of drugs will be affected by renal impairment or renal dysfunction. Renal impairment can be caused by hypertension, diabetes, nephrotoxicity (by aminoglycosides, lead, or mercury), polynephritis (inflammation of kidneys), and hypovolemia.

    For example, in uremia associated with decreased glomerular filtration and accumulation of fluids, renal excretion of drugs is reduced. Thus, half -life of the drug increases, thereby increasing its accumulation in body.

3.3.5. Non-Renal Routes of Drug Excretion

Non-renal or extra -renal routes for drug excretion are the terms used to describe excretion of drugs and their metabolites by all other routes except the renal route. The several routes of non-renal excretion are as follows:

1) **Biliary Excretion of Drugs- Enterohepatic Cycling:** Bile is produced by the hepatic cells present along the bile canaliculi. The production and secretion of bile is an active process. Bile is secreted in the liver, stored in the gall bladder, and released in the duodenum (as and when required). Bile flows at a steady rate of 0.5 -1ml/mm in humans. Bile significantly aids the digestion and absorption of fats. Re-absorption of nearly 90% of the secreted bile acids takes place in the intestine, and they are carried back to the liver for re -secretion. Faeces form the mode of excretion for the rest (10%) of the secreted bile acids.

The secretion of bile is a capacity -limited process and gets saturated, since it is an active process. The process of bile secretion is similar to that of active renal secretion. The secretion of organic anions, cations, and neutral polar compounds are linked to the existence of varying transpo rt mechanisms for the same. If a drug concentration in bile pigments is less than drug concentration in plasma, the drug is said to have a small biliary clearance and *vice versa*. The ratio of concentration of bile to the concentration of plasma in some cas es can give a value approximating 1000. The biliary clearance in such cases can be 500ml/min or more in value.

Based on their bile/plasma concentration ratios, the compounds excreted in bile can be categorised into the following three groups:

i) **Group A:** Compounds with bile/plasma concentration ratio of about 1 are categorised in Group A, **e.g.,** sodium, potassium and chloride ions and glucose.

ii) **Group B:** Compounds with bile/plasma concentration ratio of more than 1 (generally ranging from 10 -1000) are categorise d in Group B, **e.g.,** bile salts, bilirubin glucuronide, creatinine, sulfobromophthalein conjugates, etc.

iii) **Group C:** Compounds with bile/plasma concentration ratio of less than 1 are categorised in Group C, **e.g.,** sucrose, inulin, phosphates, phospholipids, and mucoproteins.

2) **Pulmonary Excretion:** The process of simple diffusion through lungs aids in the absorption of gaseous and volatile substances (like general anaesthetics, **e.g.,** halothane). In the same way, diffusion can also help in their excretion (they can be excreted into the expired air by diffusion). Pulmonary blood flow, rate of respiration, solubility of the volatile substance, etc., constitute the factors that influence the pulmonary excretion of a drug.

Those gaseous anaesthetics that are not very soluble in blood, **e.g.,** nitrous oxide, show rapid excretion. It is usually seen that gaseous drugs which are intact undergo excretion; however, their metabolites do not undergo excretion. Lungs are the organ of excretion for those compounds that are highly soluble in blood and tissues like alcohol.

3) **Salivary Excretion:** It involves excretion of drugs through saliva by the process of passive diffusion. The pH-partition hypothesis forms the basis for the prediction of salivary excretion of drugs. Though salivary pH ranges from 5.8-8.4, its mean value in human is 6.4. Drugs that exist in unionised forms and that are soluble in lipids, at the mean pH undergo passive salivary excretion.

For weak acids,

$$R_a = \frac{S}{P} = \frac{1+10^{(pH_{saliva} - pK_a)}}{1+10^{(pH_{plasma} - pK_a)}} \times \frac{f_{plasma}}{f_{saliva}} \quad \text{..... (9)}$$

For weak bases,

$$R_b = \frac{S}{P} = \frac{1+10^{(pK_a - pH_{saliva})}}{1+10^{(pK_a - pH_{plasma})}} \times \frac{f_{plasma}}{f_{saliva}} \quad \text{..... (10)}$$

Where,

f_{plasma} and f_{saliva} = Free drug fractions in plasma and in saliva, respectively.

For weak acids, the S/P ratios are found to be < 1, while for weak bases they are found to be > 1. This means that basic drugs are excreted in saliva in more amounts as compared to drugs that are acidic in nature. Some drugs show a high (as high as 0.1%) salivary concentration value. Several drugs show a fairly constant S/P ratio. Therefore, by detecting their quantity in salivary excretion, their concentration in blood can be determined, **e.g.,** caffeine, theophylline, phenytoin, carbamazepine, etc. Some drugs (**e.g.,** lithium) are secreted in saliva actively, and sometimes, their concentration in saliva is found to be 2-3 times more than that in the plasma. Saliva also actively secretes penicillin and phenytoin.

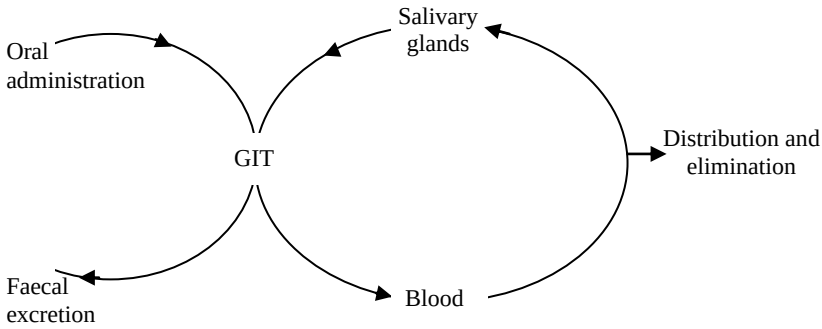


Figure 3.5: Salivary Cycling of Drugs

The unpleasant after taste in the mouth of a patient, who is on drug treatment, indicates salivary excretion of the drug. A few cases have been reported where this process results in adverse effects like black hairy tongue in patients on antibiotic therapy, gingival hyperplasia in patients on phenytoin, etc. Salivary secretion is inhibited by some basic drugs, and hence results in dryness of mouth. A cycling process (**figure 3.5**), resembling enterohepatic cycling can be seen in drugs undergoing salivary excretion, **e.g.,** sulphonamides, antibiotics, clonidine, etc.

4) **Mammary Excretion:** A drug that is excreted in milk can enter in infants feeding on breast, and therefore, it is significant. Milk contains lactic secretions synthesised in the extracellular fluid. It also contains rich amounts of fats and proteins. Lactating mothers secrete nearly 0.5-1 litres of milk per day.

Drug excretion in milk is not a passive process. It depends on:

i) pH-partition behaviour,
ii) Molecular weight,
iii) Lipid solubility, and
iv) Degree of ionisation.

The pH of milk ranges from 6.4-7.6 and possesses a mean pH of 7.0. Free, unionised, lipid-soluble drugs show passive diffusion in the alveolar cells of the mammary gland. The **ratio of concentration of drug in Milk to that in Plasma (M/P)** determines the **amount of drug excreted in milk**. Since milk bears an acidic nature as compared to plasma, drugs that are weakly basic in

nature show a greater concentration in milk , just like in case of saliva, and they possess an M/P ratio of more than 1. For drugs weakly acidic in nature, the reverse is true. Studies have revealed that the excretion of acidic drugs in milk is inversely correlated with its molecular weight and par tition coefficient; while in basic drugs, it is inversely correlated to the degree of ionisation and partition coefficient.

Drugs that show extensive binding with plasma proteins (**e.g.,** diazepam) are secreted in milk in lesser amounts. Drugs that undergo excretion via milk can bind to the proteins that are present in milk. Excretion of drugs in milk is usually in amounts less than 1% and the portion consumed by infants are in amounts that are too less to accomplish either beneficial or toxic levels. However, certain powerful drugs like barbiturates, morphine, and ergotamine may induce toxic effects in infants.Interaction of bilirubin with sulphonamides resulting in jaundice and discoloration of teeth with tetracycline are some instances showing that excretion of a drug in milk may result in adverse effects.

5) **Skin Excretion:** The pH-partition hypothesis also regulates the excretion of drugs through skin via sweat. Drugs and their metabolites that are passively excreted through the skin are accountable to some extent for urticaria and dermatitis in addition to other hypersensitivity reactions. Sweat shows the excretion of compounds like benzoic acid, salicylic acid, alcohol and antipyrine along with heavy metals such as lead, mercury, and arsenic.

6) **Gastrointestinal Excretion:** Generally, drugs are seen to be excreted in the GIT when they have been administered through parenteral route and their concentration gradient favours passive diffusion. The process of GI excretion of drugs is opposite to that of GI absorpti on. In GIT, stomach is a specific site that shows the excretion of water-soluble and ionised forms of drugs that are either weak acids or bases, **e.g.,** nicotine and quinine. GIT also shows the absorption and excretion of drugs that are administered orally. Excretions of drugs in GIT are followed by their re -absorption (into the systemic circulation) and hence are recycled.

7) **Genital Excretion:** Drugs may also be excreted in the reproductive tract and genital secretions. Semen has shown the presence of some drugs.

3.4. SUMMARY

The details given in the chapter can be summarised as follows:

1) **Elimination** is defined as the irreversible loss of drug from the body.
2) **Liver** is the main **site of metabolism** for most drugs.
3) A specific group of **cytochrome P-450 enzymes** involves in liver's primary mechanism for metabolising drugs.
4) The biochemical modification of a drug in the body is termed **drug metabolism or biotransformation**.
5) Young children show poor drug metabolism as metabolic enzyme systems are not developed completely.
6) In comparison to males, the females possess lesser ability for drug metabolism.

7) Temperature of the body is directly proportional to drug metabolism.
8) Drugs containing carboxylic acid (esterprocaine), amide (procainamide), thioesters (spironolactone), phosphor ic acid ester (paraoxon), and acid anhydride (diisopropylfluoro -phosphate) functional groups undergo **hydrolysis**.
9) Drugs containing an aldehyde, ketone, disulphide, sulphoxide, quinine, N -oxide, alkene, azo or nitro group undergo *in vivo* **reduction**.
10) **Carbonyl reduction** is catalysed by carbonyl reductases present in blood and cytosolic fraction of the liver, kidney, brain, and other tissues.
11) **Sulphoxide and N -oxide reduction** is catalysed by thioredoxin -dependent enzymes present in liver and kidney cytosol.
12) **Reductive halogenation** involves replacement of halogen with hydrogen, and is catalysed by cytochrome P450 and glutathione-S-reductase.
13) **Quinone reduction** of quinone into hydroxyquinones is catalysed by DT -diaphorase (NADPH-quinone oxidoreductase).
14) Liver cells (or **hepatocytes**) are the most common site for oxidation of a drug molecule and **microsomes** form the site of oxidation within the hepatocytes.
15) **Mixed Function Oxidase** (MFO) is the enzyme system that causes oxidation of drug.
16) **Cytochrome oxidase enzyme** , commonly known as **Cytochrome P-450 (CYP-450)** and is chemically a haemoprotein.
17) Alkyl groups attached to the nitrogen atom in nitrogen -bearing compounds undergo **N-dealkylation reactions**.
18) **Oxidative deamination** proceeds via carbinolamine pathway but the C -N bond cleavage occurs at the bond linking the amino group to the larger portion of the drug molecule.
19) **Desulphuration** reaction involves cleavage of carbon -sulphur bond (C=S or thiono) and formation of a product with C=O bond.
20) Thioethers undergo **S-oxidation reaction** to yield sulphoxides that further oxidise into sulphones (RSO_2R).
21) **Oxidative dehalogenation** reaction occurs in drugs containing halogen, **e.g.,** chloroform.
22) In **Phase II reactions** , drugs (or their metabolites) are combined with hydrophilic endogenous co mpounds to form complexes exhibiting adequate hydrophilic character to allow rapid excretion.
23) **Glucuronic acid conjugation** occurs only when glucuronic acid gets activated.
24) The cofactor, **Uridine Diphosphate -glucuronic acid (UDP -glucuronic acid)** is required during glucuronidation, which is also catalysed by UDP -Glucuronyl Transferases.
25) An electron -rich nucleophilic heteroatom, i.e., O, N or S, forms the site of glucuronidation.
26) Steroids, bilirubin, catechols, and phenylbutazone undergo **C-glucuronidation**.
27) **Sulphate conjugation** reaction is catalysed by sulphotransferases (sulphokinases), which are cytosolic enzymes found in liver, kidney, intestinal tract, lung, platelets, and brain.

28) **N-acetylation** reaction requires acetyl coenzyme A (acetyl CoA) cofactor and is catalysed by N-acetyltransferases.
29) **Methylation reaction** involves the synthesis of an activated coenzyme, i.e., S-Adenosyl Methionine (SAM, donor of methyl group), from L -methionine and ATP.
30) **Glutathione** (GSH) is a tripeptide comprised of glycine, cystei ne, and glutamic acid. Its formation is catalysed by γ-glutamylcysteine synthetase.
31) **Glutathione conjugates** are thioesters formed by nucleophilic attack of glutathione thiolate anion (GS^-) on electrophilic carbon atom or electrophilic heteroatoms (O, N, orS).
32) **Clearance** may be defined as the complete removal of a drug in a specified time period from the hypothetical volume of body fluids containing the drug.
33) **Renal clearance** can be physiologically expressed as the ratio of rate of reabsorption subtracted from the sum total of the rate of filtration and that of secretion to plasma drug concentration (C).
34) **Normal creatinine clearance** is 120-130ml/min.
35) **Glomerular filtration** is a process involving the filtration of most compounds irrespective of whether they are ionised or unionised, with an exception of macromolecules (bounded to plasma proteins or blood cells).
36) The rate of filtration is known as **Glomerular Filtration Rate (GFR)**.
37) **Active tubular secretion** is a carrier-mediated process.
38) High threshold endogenous substances or nutrients like electrolytes, glucose, vitamins, amino acids, etc., which need to be conserved by the body, usually undergo **active tubular re-absorption**.
39) Numerous exogenous substances, including drugs, commonly exhibit **passive tubular re-absorption**.
40) The excretion of drugs with large molecular weights is difficult, compared to that of drugs with smaller molecular weights.
41) The solubility of a drug in lipids is inversely proportional to its urinary excretion.
42) The volume of distribution of dru gs (V_d) is inversely proportional to their clearance.
43) Drugs that show increased perfusion also show an increase in their excretion.
44) Drugs that are bounded to plasma proteins act as macromolecules, and their filtration by the glomerulus is not possible.

3.5. EXERCISE

3.5.1. True or False

1) Liver is the main site of metabolism for most drugs.
2) Temperature of the body is inversely proportional to drug metabolism.
3) Drugs containing an aldehyde, ketone, disulphide, sulphoxide, quinine, N -oxide, alkene, azo or nitro group undergo hydrolysis.
4) S-methylation is catalysed by cytochrome P450 and glutathione-S-reductase.
5) Cytochrome oxidase enzyme, commonly known as Cytochrome P-450 (CYP-450) and is chemically a protein.
6) Desulphuration reaction involves cleavage of carbon -sulphur bond and formation of a product with C=O bond.

7) Oxidative dehalogenation reaction occurs in drugs containing oxygen.
8) N-acetylation reaction requires acetyl coenzyme A cofactor and is catalysed by N - acetyltransferases.
9) Glutathione is a tripeptide comprised of glycine, cysteine, and glutamic acid.
10) Numerous exogenous substances exhibit active tubular re-absorption.

3.5.2. Fill in the Blanks

11) The biochemical modification of a drug in the body is termed drug _____.
12) Carbonyl reduction is catalysed by _____ pres ent in blood and cytosolic fraction of the liver, kidney, brain, and other tissues.
13) _____ are the most common site for oxidation of a drug molecule.
14) _____ is the enzyme system that causes oxidation of drug.
15) Uridine Diphosphate -glucuronic acid is requ ired during glucuronidation, which is also catalysed by _____.
16) Normal creatinine clearance is _____.
17) The rate of filtration is known as _____.
18) The solubility of a drug in lipids is inversely proportional to its _____.
19) The volume of distribution of drugs is _____ proportional to their clearance.
20) Drugs that show increased perfusion also show an increase in their _____.

Answers

1) True	2) False	3) False
4) False	5) False	6) True
7) False	8) True	9) True
10) False	11) Biotransformation	12) Carbonyl reductases
13) Liver cells	14) Mixed function oxidase	15) UDP-Glucuronyl Transferases
16) 120-130ml/min	17) Glomerular filtration rate	18) Urinary excretion
19) Inversely	20) Excretion	

3.5.3. Very Short Answer Type Questions

1) Define drug metabolism.
2) Enlist the enzymes involved in drug metabolism.
3) How drug metabolism is affected by genetics?
4) Give two examples of hydrolysis reaction.
5) What is glucuronidation?
6) Define clearance.
7) How renal excretion of drugs is affected by their physicochemical properties?

3.5.4. Short Answer Type Questions

1) Discuss the factors affecting drug metabolism.
2) Write about oxidation reaction of phase I metabolic pathway.
3) Discuss the glutathione conjugation reaction.
4) Discuss the factors affecting renal excretion of drugs.
5) Explain any two non-renal routes of drug excretion.

3.5.5. Long Answer Type Questions

1) Give a detailed review on phase II metabolic pathways.
2) Discuss the non-renal routes of drug excretion.
3) Write an exhaustive note on renal excretion of drugs.

CHAPTER 4

Bioavailability and Bioequivalence

4.1. BIOAVAILABILITY

4.1.1. Introduction and Definition

Bioavailability is the **rate and extent of an administered dose of drug that reaches the systemic circulation in unchanged form**. It is a major pharmacokinetic property of drug. Bioavailability of a drug administered intravenously is 100%. A drug's therapeutic effectiveness depends on its ability to provide the medicament at a sufficient rate and in sufficient amount to its site of action in order to achieve the desired pharmacological response. This effectiveness of a drug dosage form is known as **bioavailability** or **physiologic availability** or **biologic availability**. The pharmacological response of many drugs can be directly related to their plasma levels; therefore, bioavailability can also be defined as the **rate and extent of absorption of unchanged drug from its dosage form**.

Bioavailability of a drug from its dosage form depends on:
1) The administration route,
2) Patient-related factors,
3) Physicochemical properties of the drug, and
4) Dosage form characteristics.

The drug dose given to the patient is the **administered dose**. The dose available to the patient is the **bioavailable dose** (usually less than the administered dose). The effect of administration route on bioavailable dose is in the following order:

Parenteral > Oral > Rectal > Topical

4.1.2. Objectives of Bioavailability Studies

The important objectives of bioavailability studies are:
1) It involves the primary development stages for a suitable dosage form of a new drug entity.
2) It helps in determining how efficiency of absorption is influenced by the excipients, patient-related factors, and possible interactions with other drugs.
3) It helps in the development of new formulations of the existing drugs.
4) It controls the quality of a drug product during the early stages of marketing to determine how drug absorption is affected by processing factors, storage, and stability conditions.

4.1.3. Types of Bioavailability

Bioavailability of drugs is of two types:
1) Absolute bioavailability, and
2) Relative bioavailability.

4.1.3.1. Absolute Bioavailability

The absolute bioavailability of a given drug from a dosage form is the **fraction (or percentage) of the administered dose absorbed int o the systemic circulation in unchanged form** . It is calculated by comparing the total amount of unchanged drug reaching the systemic circulation after administering a known dose of the dosage form via any route with the total amount of unchanged drug reaching the systemic circulation after administering an equivalent dose of the drug in the form of an intravenous bolus injection.

When a drug is administered via intravenous route, the administered dose is introduced directly into the systemic circulation, i.e., it does not have to pass through any absorption barriers, and therefore gives 100% bioavailability. Due to this reason, an intravenous bolus injection serves as a reference to compare the systemic availability of the drug administered via different routes.

Absolute bioavailability of a drug can be calculated from plasma data by comparing the total areas under the plasma concentration -time curves obtained after administering equivalent doses of the drug via absorption site and via intravenous route i n the same subject at different times. **Figure 4.1** shows the plasma concentration -time curves obtained after administering equivalent doses of the drug via intravenous bolus injection and gastrointestinal route.

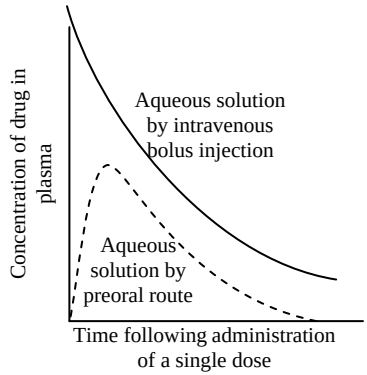


Figure 4.1: Typical Plasma Concentration-Time Curves obtained by Administering Equivalent Doses of the Same Drug by Intravenous Bolus Injection and by the Oral Route

For equivalent doses of administered drug:

$$\text{Absolute bioavailability} = \frac{(AUC_T)_{abs}}{(AUC_T)_{iv}} \quad \ldots. (1)$$

Where, $(AUC_T)_{abs}$ = Total area under the plasma con centration-time curve after administering a single dose via absorption site.

$(AUC_T)_{iv}$ = Total area under the plasma concentration -time curve after administering a single dose by rapid intravenous injection.

If different drug doses are administered by both the routes, the dose sizes can be corrected as follows:

$$\text{Absolute bioavailability} = \frac{(AUC_T)_{abs}/D_{abs}}{(AUC_T)_{iv}/D_{iv}} \quad \ldots. (2)$$

Where, D_{abs} = Size of the single dose of drug administered via absorption site.

D_{iv} = Size of the drug dose administered as an intravenous bolus injection.

Sometimes, different dosages of drugs should be administered via different routes. The dose administered via intravenous route is often kept low to avoid toxic si de effects and also for ease of formulation. Bioavailability data for

different dosages should be calculated carefully as sometimes the drug pharmacokinetics are non -linear; due to which different doses will result in an incorrect figure for absolute bioav ailability if calculated using a simple ratio, as in **equation (2)**. Absolute bioavailability can also be determined using urinary excretion data by comparing the total cumulative amounts of unchanged drug excreted in the urine after administering the drug v ia absorption site and intravenous route (bolus injection) to the same subject at different times.

For equivalent doses of administered drug:

$$\text{Absolute bioavailability}' = \frac{(X_u)_{abs}}{(X_u)_{iv}} \quad \text{..... (3)}$$

Where, $(X_u)_{abs}$ and $(X_u)_{iv}$ = Total cumulative amounts of unchanged dru g excreted in the urine after administering equivalent single doses of drug via absorption site and intravenous bolus injection, respectively.

If different doses of drug are administered:

$$\text{Absolute bioavailability}' = \frac{(X_u)_{abs}/D_{abs}}{(X_u)_{iv}/D_{iv}} \quad \text{..... (4)}$$

Absolute bioavailability of a drug from a par ticular type of dosage form may be expressed as a fraction or a percentage.

Factors related to oral route of drug administration may affect bioavailability, and these effects can be studied by measuring absolute bioavailability obta ined by administering a drug as a simple aqueous solution (that does not precipitate on contact with or dilution by gastrointestinal fluids) by oral and intravenous routes, **e.g.**, pre -systemic metabolism by the intes tine or liver, formation of complexes between the drug and endogenous substances (**e.g.,** mucin) at the site of absorption, and drug stability in the gastrointestinal fluids.

4.1.3.2. Relative Bioavailability

For drugs that cannot be administered by intra venous bolus injection, their relative (or comparative) bioavailability is determined instead of the absolute bioavailability. Relative bioavailability can be determined by comparing the bioavailability of a drug from a test dosage form with the same drug administered in a standard dosage form. The latter is either an orally administered solution (from which the drug is known to be well -absorbed) or an established commercial preparation of proven clinical effectiveness.

Hence, relative bioavailability is the fraction (or percentage) of a drug that is absorbed in unchanged form into the systemic circulation from a dosage form of the relative to a recognised (or clinically proven) standard dosage form of the same drug. Plasma concentration-time curves are used for calculating relative bioavailability of a drug administered as equal doses of a test dosage form and a recognised standard dosage form by the same administration route to the same subject at different times:

$$\text{Relative bioavailability} = \frac{(AUC_T)_{test}}{(AUC_T)_{standard}} \quad \text{.....(5)}$$

Where, $(AUC_T)_{test}$ and $(AUC_T)_{standard}$ = Total areas under the plasma concentration-time curves after administering a single dose of the test dosage form and of the standard dosage form, respectively.

If test and standard dosage forms are administered in different doses, the dose size can be corrected as follows:

$$\text{Relative bioavailability } r = \frac{(AUC_T)_{abs}/D_{test}}{(AUC_T)_{standard}/D_{standard}} \quad \ldots\ldots (6)$$

Where, D_{test} and $D_{standard}$ = Sizes of the single doses of test and standard dosage forms, respectively.

Relative bioavailability (alike absolute bioavailability) can be expressed as a fraction or a percentage, and can also be determined from urinary excretion data as follows:

$$\text{Relative bioavailability } r = \frac{(X_u)_{test}}{(X_u)_{standard}} \quad \ldots\ldots (7)$$

Where, $(X_u)_{test}$ and $(X_u)_{standard}$ = Total cumulative amounts of unchanged drug excreted in the urine after administering single doses of the test dosage form and standard dosage form, respectively.

If the test and standard dosage forms are administered in different doses at different times, the total amount of unchanged drug excreted in the urine per unit dose of drug should be used in this equation.

Relative bioavailability is measured to determine the effects of dosage form differences on systemic bioavailability of a drug. Many dosage form factors can influence the drug bioavailability, like the type of dosage form (**e.g.,** tablet, solution, suspension, and hard gelatin capsule), differences in the formulation of a particular type of dosage form, and manufacturing variables employed in the production of a particular type of dosage form.

4.1.4. Measurement of Bioavailability

The methods employed in the quantitative evaluation of bioavailability are broadly divided into:

1) **Pharmacokinetic Methods:** These are indirect methods as they are based on the assumption that the pharmacokinetic properties of a drug reveal its therapeutic efficacy. The two major pharmacokinetic methods are:
   i) Plasma level-time studies, and
   ii) Urinary excretion studies.

2) **Pharmacodynamic Methods:** These methods are complementary to pharmacokinetic approaches and involve direct measurement of drug effect on a pathophysiologic process as a function of time. The two pharmacodynamic methods for determination of bioavailability from:
   i) Acute pharmacologic response, and
   ii) Therapeutic response.

4.1.4.1. Plasma Level-Time Studies

The method of determining plasma drug concentration is based on the assumption that two dosage forms exhibiting superimposable plasma level-time profiles in a group of subjects should show identical therapeutic activity. In a single dose study, serial blood samples are collected for 2-3 biological half-lives after drug administration. Their drug concentration is analysed and is plotted against the corresponding time of sample collection to obtain the plasma level-time profile. Sampling should be started within 5 minutes of drug administration via intravenous route, and is continued at every 15 minutes. The disposition phase can be properly described only if not less than 3 sample points are taken for the drug following one-compartment model and 5 to 6 points are taken for the drug following two-compartment model. For oral dose, 3 points should be taken on the ascending part of the curve to accurately determine K_a. The points for disposition or descending phase of the curve should be taken in a manner similar to that for intravenous dose.

Given below are the **three parameters of plasma level-time studies** that are significant in the determination of bioavailability:

1) **C_{max}:** It is the peak that indicates the point at which the drug concentration in plasma is maximum. **Peak plasma concentration** (or **peak height concentration** or **maximum drug concentration**) is the drug concentration at peak. C_{max} is usually expressed in **mcg/ml**.

   Peak level depends on the administered dose and the absorption and elimination rate of drug. Peak is the point of time when the absorption rate and the elimination rate of drug becomes equal. The portion of the curve to the left of peak indicates the absorption phase where the drug's absorption rate is greater than its elimination rate. The portion of the curve to the right of peak indicates the elimination phase where the drug's elimination rate is greater than its absorption rate. Peak concentration is related to pharmacological response and should be more than the Minimum Effective Concentration (MEC) and less than the Maximum Safe Concentration (MSC).

2) **t_{max}:** It is the time the drug requires to reach peak concentration in plasma after extravascular administration. t_{max} is expressed in hours. **Time of peak concentration** is used in estimating the absorption rate of drug. It influences the onset time and onset of action. t_{max} is used for evaluating the efficacy of drugs employed in treating acute conditions (pain and insomnia) that can be treated with a single dose of a drug.

3) **AUC:** It is the **area under the plasma level-time curve** that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation. The extent of bioavailability can be determined by following equations:

$$F = \frac{[AUC]_{oral}\, D_{iv}}{[AUC]_{iv}\, D_{oral}} \qquad \text{..... (8)}$$

$$F_r = \frac{[AUC]_{test}\, D_{std}}{[AUC]_{std}\, D_{test}} \qquad \text{..... (9)}$$

Where, D = Dose administered.
Subscripts iv and oral = Administration route.
Subscripts test and standard = Test a nd standard doses of the same drug to determine relative availability.

With multiple dose study, the method involves drug administration for 5 biological half-lives with a dosing interval either equal to or greater than the biological half-life (i.e., administration of at least 5 doses) to reach the steady-state. A blood sample should be collected at the end of previous dosing interval and 8 to 10 samples should be collected after administering the next dose.

The extent of bioavailability is given as:

$$F_r = \frac{[AUC]_{test}\, D_{std}\, \tau_{test}}{[AUC]_{std}\, D_{testl}\, \tau_{std}} \quad \text{..... (10)}$$

Where, [AUC] values = Area under the plasma level-time curve of one dosing interval in a multiple dosage regimen, after reaching the steady-state (**figure 4.2**).
τ = Dosing interval.

Bioavailability can also be determined from the peak plasma concentration at steady-state ($C_{ss,max}$) as follows:

$$F_r = \frac{(C_{ss,max})_{test}\, D_{std}\, \tau_{test}}{(C_{ss,max})_{std}\, D_{testl}\, \tau_{std}} \quad \text{..... (11)}$$

The absorption rate is not important in multiple dosing methods.

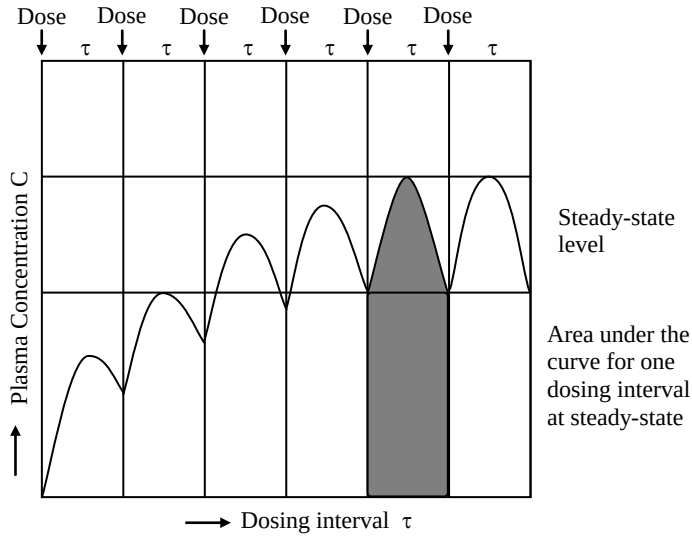


Figure 4.2: Determination of AUC and on Multiple Dosing upto Steady-States

4.1.4.2. Urinary Excretion Studies

Urinary excretion studies, performed to assess bioavailability, rely on the principle that the urinary excretion of unchanged drug and the plasma concentration of drug are directly proportional. Thus, even if 10 -20% of a drug gets excreted in the urine, its bioavailability can be determined. Urinary excretion

study is used for drugs that get excreted in urine in unchanged form (**e.g.**, certain thiazide diuretics and sulphonamides) and for drugs that have urine as the site of action (**e.g.**, urinary antiseptics such as nitrofurantoin and hexamine). The concentration of drug metabolites that get excreted in urine is never considered for calculations, because a drug before getting absorbed may undergo pre - systemic metabolism at different stages.

The urinary excretion method involves collecting urine samples at regular intervals for 7 biological half-lives, analysing the unchanged drug in the collected sample, and determining the amount of drug excreted at each interval and cumulative amount ex creted. Each time of sampling, the subject should completely empty the bladder to avoid errors in the next urine sample that may result from the residual amount. In the beginning of the study, frequent sampling is essential to correctly calculate the absorption rate.

Given below are the major parameters that are examined in urinary excretion data obtained with a single dose study:

1) **$(dX_u/dt)_{max}$:** It is the maximum urinary excretion rate, obtained from the peak of plot between excretion rate and midpoint time of urine collection period. It is analogous to the C_{max} obtained from plasma level studies since the rate of appearance of drug in urine and its concentration in systemic circulation are proportional. The value of $(dX_u/dt)_{max}$ increases with the increase in rate of and/or extent of absorption.

2) **$(t_u)_{max}$:** It is the time for maximum excretion rate. It is analogous to t_{max} of plasma level data. The value of $(t_u)_{max}$ decreases with increase in absorption rate.

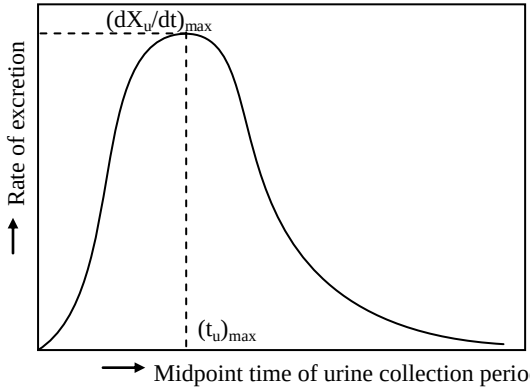


Figure 4.3: Plot of Excretion Rate *versus* Time. The curve is analogous to a typical plasma level-time profile obtained after oral administration of a single dose of drug

3) **Xu:** It is the cumulative amount of drug excreted in urine. It is related to the AUC of plasma level data. The value of Xu increases with the increase in extent of absorption. The extent of bioavailability is calculated from the following equations:

$$F = \frac{\left(X_u^{\infty}\right)_{oral} D_{iv}}{\left(X_u^{\infty}\right)_{iv} D_{oral}} \quad \text{..... (12)}$$

$$F_r = \frac{\left(X_u^{\infty}\right)_{test} D_{std}}{\left(X_u^{\infty}\right)_{std} D_{testl}} \quad \text{..... (13)}$$

With multiple dose study to steady -state, the equation for computing bioavailability is:

$$F_r = \frac{(X_{u,ss})_{test}\, D_{std}\, \tau_{test}}{(X_{u,ss})_{std}\, D_{testl}\, \tau_{test}} \quad \text{….. (14)}$$

Where, $(X_{u,ss})$ = Amount of drug excreted unchanged during a single dosing interval at steady-state.

4.1.4.3. Acute Pharmacologic Response

When pharmacokinetic methods cannot be used successfully and easily to measure bioavailability, an inaccurate, non -reproducible, or an acute pharmacologic effect such as change in ECG or E EG readings, pupil diameter, etc., is related to the time course of a given drug. Then bioavailability can be determined by making of pharmacologic effect -time curve and dose -response graphs. For this method, responses of the drug for not less than 3 biolo gical half-lives should be measured to obtain a good estimate of AUC.

The method of acute pharmacologic response has a **disadvantage** that the pharmacologic response may vary and thus an accurate correlation between the measured response and drug available from the formulation cannot be established. Also, the observed response may be due to an active metabolite whose concen tration is not proportional to the concentration of parent drug inducing the pharmacologic effect.

4.1.4.4. Therapeutic Response

The therapeutic response method involves observing the clinical response of a drug formulation in patients who are suffering from the disease for which th e drug is intended to be used.The therapeutic responsemethod has several **disadvantages**:

1) Quantitation of observed response is so inappropriate that reasonable assessment of relative bioavailability between two dosage forms of the same drug cannot be done.
2) Bioequivalence studies are conducted using a crossover design in which each subject is given each test dosage form, and it is anticipated that the subject's physiological status does not change during the entire study.
3) If multiple -dose protocols for a drug are not employed, a patient who requires the drug for a disease will be administered only a single dose of the drug in every few days or each week.
4) Many patients receive more than one drug, and the bioavailability study results obtained could be compromised because of a drug-drug interaction.

4.1.5. Significance of Bioavailability Studies

Bioavailability studies have the following significant features:

1) They help in estimating the fraction of the orally administered dose that is absorbed into the systemic circulation when compa red to the bioavailability for a solution, suspension, or intravenous dosage form that is completely available.

2) They provide useful information that is required for establishing dosage regimen and for drug label ling, such as distribution and elimination characteristics of the drug.
3) They provide information regarding the performance of a formulation.
4) They help in the determination of influence of excipient on absorption.
5) They help in the development of new formulations of existing drug.
6) They control the qua lity of drug product by determining the influence of processing factors, storage and stability on absorption.
7) They help in making comparison of drug in different dosage form or same dosage form of different manufacturer.

4.2. METHODS TO ENHANCE THE DISSOLUTION RATES AND BIOAVAILABILITY OF POORLY SOLUBLE DRUGS

4.2.1. Introduction

Various techniques are available for enhancing the solubility of poorly soluble drugs. Some of these approaches are as follows:

1) **Chemical Modifications**:
   - i) Use of salt forms
   - ii) Co-crystallisation
   - iii) Co-solvency
   - iv) Hydrotrophy
   - v) Solubilising agents
   - vi) Nanotechnology

2) **Physical Modifications:**
   - i) Particle size reduction
     - a) Micronisation
     - b) Nanosuspension
     - c) Sonocrystallisation
     - d) Supercritical fluid process
   - ii) Modification of the crystal habit
     - a) Polymorphs
     - b) Pseudopolymorphs
   - iii) Complexation - Use of complexing agents
   - iv) Solubilisation by surfactants
     - a) Microemulsions
     - b) Self microemulsifying drug delivery system
   - v) Drug dispersion in carriers

4.2.2. Use of Salt Forms

Salts have improved solubility and dissolution characteristics than the original drug. A minimum difference of 3 units between the pKa value of the group and that of its counter -ion is required to form stable salts. The solubility of alkali metal salts of acidic drugs (like penicillins) and strong acid salts of basic drugs (like atropine) in water is more than that of the parent drug. Factors influencing salt selection are physical and chemical properties of the salt, safety of counter -ion, therapeutic indications, and administration route.

Salt formation has the following **limitations**:
1) Salts of neutral compounds cannot be formed.
2) Salts of very weak bases or acids can be formed with much difficulty.
3) The salt may be hygroscopic, exhibit polymorphism, or has poor processing characteristics.
4) Conversion of salt to free acid or base form of the drug on surface of solid dosage form prevents or retards drug release.
5) Precipitation of unionised drug in the gastrointestinal milieu that has poor solubility.

4.2.3. Co-Crystallisation

With the application of co-crystals or molecular complexes, drug solubility can be improved. If the solvent forms an integral part of the network structure and at least two component crystal, it is termed as **co-crystal**. If the solvent does not directly involve itself in the network (as in open framework structures), it is termed as **clathrate** (or **inclusion complex**). A co-crystal is a crystalline material consisting of two or more molecular and electrically neutral species bound by non-covalent forces.

Co-crystals are more stable as the co-crystallising agents are solids at room temperature. Only three co-crystallising agents, i.e., saccharin, nicotinamide and acetic acid, are recognised as safe (GRAS), thus limiting their pharmaceutical applications. Co-crystallisation may also occur between two active pharmaceutical ingredients by using sub-therapeutic amounts of drug substances (such as aspirin or acetaminophen). Co-crystals can be prepared by evaporating a heteromeric solution or by grinding the components together. Another technique of preparing co-crystals includes sublimation, growth from the melt, and slurry preparation. Formation of molecular complexes and co-crystals and their importance is increasing day-by-day as an alternative to salt formation, mainly for neutral compounds or those having weakly ionisable groups.

4.2.4. Cosolvency

Solubilising the drugs in co-solvents is another technique of solubility enhancement of poorly soluble drugs. Adding an organic cosolvent to water can change the drug solubility. Water solubility of weak electrolytes and non-polar molecules is poor and this can be improved by adding another solvent that alters the solvent polarity. This process is termed **cosolvency**, and the solvent used for increasing solubility is termed a **cosolvent**.

The system of cosolvent involves **solvent blending** in which the interfacial tension between the aqueous solution and hydrophobic solute is reduced. Cosolvents mostly have hydrogen bond donor and/or acceptor groups and small hydrocarbon regions. Their hydrophilic hydrogen bonding groups ensure water miscibility, and their hydrophobic hydrocarbon regions interfere with water's hydrogen bonding network, thus reducing the intermolecular attraction of water. Cosolvents disrupt the water's self-association, reduce water's ability to squeeze out non-polar, hydrophobic compounds, and thus increase solubility. A different

perception is that cosolvents facilitate solubilisation by making the polar water environment more non -polar like the so lute. Solubility enhancement as high as 500-fold is achieved by using 20% of 2-pyrrolidone.

4.2.5. Hydrotrophy

Hydrotrophy is the increase in water solubility due to the presence of large amounts of additives. Its mechanism of improving solubility is closely rel ated to complexation that involves a weak interaction between the hydrotrophic agents (**e.g.,** sodium benzoate, sodium acetate, sodium alginate, and urea) and the solute. An **example** of hydrotrophy is solubilisation of theophylline with sodium acetate and sodium alginate.

4.2.6. Solubilising Agents

Solubility of poorly soluble drugs can also be improved by using solubilising materials. **For example** , PEG 400 is used for improving the solubility of hydrochlorothiazide; Modified Gum Karaya (MGK) was evaluated as a carri er for dissolution enhancement of nimodipine; addition of caffeine and nicotinamide for improving the aqueous solubility of halofantrine (antimalarial).

4.2.7. Nanotechnology Approaches

Nanotechnology is the study and use of materials and structures at the nanos cale level of approximately 100 nanometers (nm) or less. For many new chemical entities of low solubility, enhancement of oral bioavailability by micronisation is not sufficient because the micronised product may undergo agglomeration, which decreases the effective surface area for dissolution.

4.2.8. Particle Size Reduction

Particle size reduction techniques by various milling processes are well - established and are a standard part of formulation development. Particle size of the drug is reduced by micronisation, nanosuspension, sonocrystallisation, etc. techniques. As particle size decreases, surface area of particle increases, and this increases the solubility.

Micronisation

Drug solubility is often intrinsically related to drug particle size. By reducing the particle size of the drug, its surface area is increased and its dissolution properties are improved. The conventional methods of particle size reduction (such as comminution and spray drying) rely on mechanical stress to disaggregate the active compound. M icronisation is used to increase the surface area for dissolution.

Nanosuspension

Nanosuspensions are sub -micron colloidal dispersion of pure drug particles, which are stabilised by adding surfactants. They offer the advantages of **increased dissolution ra te** due to larger surface area exposed, and **absence of Ostwald ripening** due to the uniform and narrow particle size range obtained that eliminates the concentration gradient factor.

Sonocrystallisation
Recrystallisation of poorly soluble materials with liquid solvents and anti-solvents has been successfully employed for particle size reduction. Sonocrystallisation is a novel approach for particle size reduction that involves inducing crystallisation using ultrasound waves at a frequency range of 20 -100kHz. T his technique enhances the nucleation rate, is an effective means of size reduction, and also controls size distribution of the active pharmaceutical ingredients. In most cases, ultrasound waves are used in the range of 20 kHz–5 MHz.

Supercritical Fluid Process
Application of novel nanosizing and solubilisation technology has increased the use of Supercritical Fluid (SCF) processes for particle size reduction. SCF is a dense non -condensable fluid with temperature and pressure greater than its critical temperature (Tc) and critical pressure (Tp). By manipulating the pressure of SCFs, the favourable properties of gases, like high diffusivity, low viscosity, and low surface tension, may be imparted upon liquids to control drug solubilisation with a supercritical fluid. SCFs are highly compressible, and allow moderate changes in pressure to alter the density and mass transport characteristics of fluid, which determine its solvent power.

Once the drug particles solubilise in SFs, they may be recrystallised at r educed particle sizes. SCF process allows micronisation of drug particles within a narrow range of particle size, often to sub-micron levels. The current SCF processes have the ability to create nanoparticulate suspensions of particles 5 -2,000nm in diameter. The most widely employed SCF processing methods for micronised particles are Rapid Expansion of Supercritical Soluti ons (RESS) and gas anti - solvent recrystallisation (GAS). These methods are used by the pharmaceutical industries using carbon dioxide (CO_2) as the SCF due to its favourable processing characteristics like low critical temperature (Tc = 31.10°C) and pressure (Pc = 73.8 bar).

In the method of RESS, a drug or a mixture of drug and polymer is solubilised in SCF and this solution is sprayed thr ough a conventional nozzle or capillary tube into a lower pressure environment. Rapid expansion the solution undergoes reduces CO_2 density and its solvent power, thus supersaturating the lower pressure solution. This supersaturation results in the recrysta llisation and precipitation of pure drug or drug -polymer particles of greatly reduced size, narrow size distribution, and high purity. **For example**, solubility of nifedipine has been improved by using RESS. In the GAS process, the drug or drug - polymer mixtu re is solubilised into a solvent via conventional means and then sprayed into a SCF; the drug should be insoluble in the SCF, and the SCF should be miscible with the organic solvent. The SCF diffuses into the spray droplets, causing expansion of the present solvent, and precipitation of the drug particles.

4.2.9. Modification of the Crystal Habit

Polymorphism is the element's or compound's ability to crystallise in more than one crystalline form. Different drug polymorphs are chemically same, but differ in their physicochemical properties like solubility, melting point, density, texture,

and stability. In the same way, amorphous form of drug is more suitable than the crystalline form as higher energy is associated with the former and increase in surface area. The dissolution of different solid forms of drug follows the given order: **Amorphous >Metastable polymorph >Stable polymorph**

4.2.10. Complexation

Complexation involves association between two or more molecules to form a non-bonded entity with a well-defined stoichiometry. Complexation relies on weak forces such as London forces, hydrogen bonding, and hydrophobic interactions. The complexing agents commonly used in improving bioavailability of poorly soluble drugs are given in the table below:

Table 4.1: List of Complexing Agents

Types	Examples
1) Inorganic	I_B
2) Coordination	Hexamine cobalt(III) chloride
3) Chelates	EDTA and EGTA
4) Metal-Olefin	Ferrocene
5) Inclusion	Cyclodextrins and Cholic acid
6) Molecular Complexes	Polymers

4.2.11. Solubilisation by Surfactants

Surfactant molecules have separate polar and non-polar regions. They mostly consist of a hydrocarbon segment that is linked to an anionic, cationic, Zwitterionic or non-ionic polar group. When small polar molecules are added they accumulate in the hydrophobic core of the micelles. This solubilisation process is important in industrial and biological field. Presence of surfactants decreases the surface tension but increases the solubility of drug in an organic solvent.

Microemulsion

Jack H. Shulman first used the term **microemulsion** in **1959**. It is a four-component system comprising of an external phase, an internal phase, a surfactant, and a co-surfactant. On adding a surfactant that is soluble in the internal phase (unlike the co-surfactant), an optically clear, isotropic, thermodynamically stable emulsion is formed, which is termed microemulsion because the internal or dispersed phase is < 0.1µ droplet diameter. Microemulsion is formed spontaneously and does not involve the input of external energy as in case of coarse emulsions.

The surfactant and co-surfactant alternate each other and a mixed film is formed at the interface, which contributes to the stability of microemulsion. Non-ionic surfactants, such as Tweens (polysorbates) and Labrafil (polyoxyethylated oleic glycerides), having high HLB values are used so that o/w droplets are immediately formed during production. Microemulsion is **advantageous over coarse emulsion** due to its ease of preparation involving spontaneous formation, thermodynamic stability, transparent and elegant appearance, increased drug loading, enhanced penetration through biological membranes, increased bioavailability, and less inter- and intra-individual variability in drug pharmacokinetics.

4.2.12. Drug Dispersion in Carriers

The approach of solid dispersion for particle size reduction and dissolution and absorption rate enhancement of drugs was first introduced in 1961. The term **solid dispersion** refers to the dispersion of one or more active ingredients in an inert carrier in a solid state, prepared by the melting (fusion) method, solvent method, or fusion solvent method.

The novel preparation techniques include rapid precipitation by freeze drying, spray drying in the presence of amorphous hydrophilic polymers, supercritical fluid technology, and melt extrusion method. Polyvinyl pyrrolidone, polyethylene glycols, Plasdone -S63020, a nd surfactants (Tween -80, docusate sodium, Myrj -52, Pluronic -F68, and SLS) are the most commonly used hydrophilic carriers for solid dispersions.

With the approach of solid dispersion using suitable hydrophilic carriers, the solubility of etoposide, glyb uride, itraconazole, ampelopsin, valdecoxib, celecoxib, and halofantrine can be improved. The eutectic combination of chloramphenicol/urea and sulphathiazole/urea are the **examples** for the preparation of a poorly soluble drug in a highly water soluble carrier.

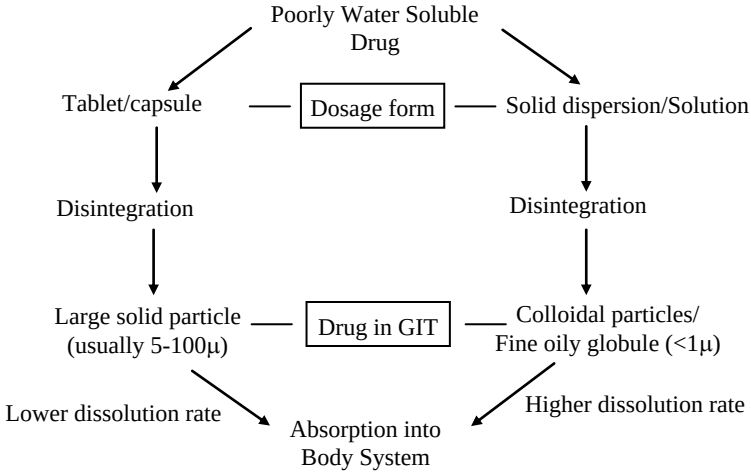


Figure 4.4: Schematic Representation of Bioavailability Enhancement of a Poorly Water-Soluble Drug by Solid Dispersion

Simple Eutectic Mixture

A eutectic mixture of a sparingly water -soluble drug and a highly water -soluble carrier is thermodynamically regarded as an intimately blended physical mixture of two crystalline components. Increased surface a rea increases the dissolution rate, thus concluding that the increase in dissolution was due to decreased particle size.

Solid Solutions

Solid solution is a binary system comprising of a solid solute molecularly dispersed in a solid solvent. Solid solutions are also termed **molecular dispersions** or **mixed crystals** as the two components crystallise together in a homogeneous one phase system. Reduction of particle size up to the molecular level increases the aqueous solubility of solid solutions, thus they undergo faster dissolution than eutectics and

solid dispersions. Solid solutions are prepared by fusion method in which a physical mixture of solute and solvent are melted together and then rapidly solidified. Such systems, prepared by fusion, are termed **melts**.

Solid solutions are classified on the following two bases:

1) **Miscibility between the Drug and the Carrier:** Solid solutions are divided into two categories on this basis:
   i) **Continuous Solid Solution:** In this, the drug and the carrier are miscible in all proportions.
   ii) **Discontinuous Solid Solution:** In this, solubility of each com ponent in the other is limited.
2) **Distribution of Drug in Carrier Structure:** Solid solutions are divided into two categories on this basis:
   i) **Substitutional Crystalline Solid Solution:** In this, if the drug and carrier molecules are almost of same size, the drug molecule substitutes for the carrier molecules in its crystal lattice.
   ii) **Interstitial Crystalline Solid Solution:** In this, if the size of drug molecule is 40% or less than the size of carrier molecules, the drug molecules occupy the interstitial spaces in the crystal lattice of carrier molecules (**figure 4.5**).

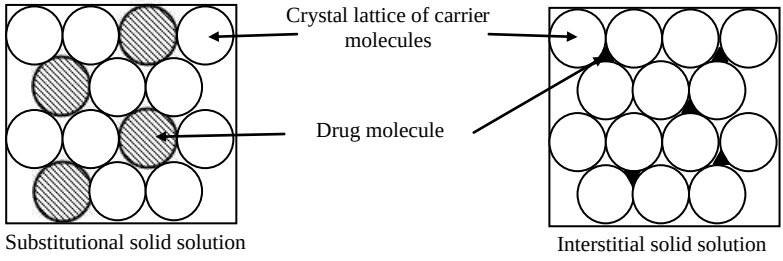


Figure 4.5: Types of Crystalline Solid Solution

Glass Solution of Suspension

A glass solution is a homogenous system in which a glassy or a vitreous of the carrier solubilises drug molecules in its matrix. PVP dissolved in organic solvents undergoes a transition to a glassy state on solvent evaporation.

Compound or Complex Formation

This system is characterised by complexation of two components in a binary system during solid dispersion preparation. Availability of the drug from the complex depends on the solubility dissociation constant and the intrinsic absorption rate of the complex.

Amorphous Precipitation

When drug precipitates as an amorphous form in the ine rt carrier, amorphous precipitation occurs. Higher energy state of the drug in this system produces much greater dissolution rates than the corresponding crystalline forms of the drug.

4.3. *IN VITRO-IN VIVO* CORRELATIONS (IVIVC)

4.3.1. Introduction and Definition

Development of a drug and optimisation of a formulation are very tedious, time taking and expensive process, and during these processes *in vitro -in vivo* correlations (IVIVC) play an important role. Optimisation of a formulation includes alteration in formulation, composition, equipment, batch sizes, and manufacturing processes. If any such types of one or more alterations are made to the formulation, the new formulation demands carrying out bioequivalence studies to prove its similarity with the previous formulation and thus increases the cost of optimisation process. As a result of these alterations, the market cost of the new formulation increases. These problems can be resolved by developing *in vitro* tests that reflect bioavailability data. IVIVC is the technique which can be used to develop new pharmaceuticals so that the number of human studies during the development of formulation can be reduced. So IVIVC acts as a surrogate for *in vivo* bioavailability and supports bio waivers.

United State Pharmacopoeia (USP) defined IVIVC as "**the establishment of a rational relationship between a biological property or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form**".

Food and Drug Administration (FDA) defined IVIVC as "**a predictive mathematical model describing the relationship between an *in-vitro* property of a dosage form and an *in-vivo* response**".

The *in vitro* studies are performed to analyse the rate or extent of drug dissolution or release, while the *in vivo* studies help to study the plasma drug concentration or amount of drug absorbed. IVIVC is performed to obtain drug dissolution results from two or more products so that the similarity or dissimilarity of expected plasma drug concentration profiles can be determined. It is important to know how to establish similarity or dissimilarity of *in vivo* response, i.e., plasma drug concentration profiles before deducing a relationship between the results obtained from *in vitro* and *in vivo* studies. The method used to establish similarity or dissimilarity of plasma drug concentration profiles is called **bioequivalence testing**. There are well-defined guidelines and standards for establishing bioequivalence between drug profiles and products.

4.3.2. Purpose of IVIVC

The purposes or objectives of IVIVC can be given as:

1) **To Reduce Regulatory Burden:** Under some specific circumstances, IVIVC serves as a substitute for additional *in vivo* experiments.
2) **In Optimisation of Formulation:** The optimisation of formulations includes alterations in composition, manufacturing process, equipment, and batch sizes. Therefore, an exhaustive study of bioequivalence (BE)/bioavailability (BA) is necessary to prove the validity of a new formulation that is bioequivalent with the target formulation.

3) **To Justify the Product's Therapeutic Quality:** IVIVC technique is used to prove the therapeutic efficiency of the formulation.

4) **To Scale -up Post-Approval Changes (Time and Cost Saving during Product Development):** Validated IVIVC process is used as approval tool for biowaivers during the filings of a Level 3 (or Type II in Europe) variation, either during scale-up or post-approval, and also for line extensions (**e.g.,** different dosage strengths).

5) **Used as Surrogate for *in vivo* Bioequivalence and Support Biowaivers (Time and Cost Saving):** The main aim of an IVIVC process is to utilise *in vitro* dissolution profiles as a surrogate for *in vivo* bioequivalence studies and to support biowaivers.

4.3.3. Levels of IVIVC

As per the FDA guidelines, there are four levels of IVIVC, which include levels A, B, C, and multiple C. The correlation level concept is based on the ability of the correlation to reflect the entire plasma drug level -time profile which is the result of administration of the given dosage form.

4.3.3.1. Level A Correlation

The level A correlation of IVIVC has regulatory relevance and correlates the entire *in vitro* and *in vivo* profiles. It is the highest category of correlation and represents a point -to-point relationship between *in vitro* dissolution rate and *in vivo* input rate of the drug from dosage form.

It is important to achieve level A correlation because it allows bio waiver for changes in manufacturing site, raw material suppliers, and minor changes in formulation. Level A correlation defines a direct relationship between *in vivo* data such as the measurement of *in vitro* dissolution rate is required to determine the biopharmaceutical rate of the dosage form.

4.3.3.2. Level B Correlation

The level B correlation of IVIVC relies on the principles of statistical moment analysis. This level includes comparison of *in vitro* dissolution time (MDT vitro) of the product to either *in vivo* residence time (MRT) or *in vivo* dissolution time (MDT vivo). Level B correlation is not so useful for regulatory purposes because it does not reflect the actual *in vivo* plasma level curves, and also *in vitro* data from such a correlation cannot be used to justify the extremes of quality control standards.

4.3.3.3. Level C Correlation

The level C correlation relates one dissolution time point ($t_{50\%}$, $t_{90\%}$, etc.) to one mean pharmacokinetic parameter (AUC, t_{max}, or C_{max}). It is the weakest level of correlation as partial relationship between absorption and dissolution is established because it does not represent the complete shape of plasma drug concentration-time curve (the critical factor that defines the performance of a drug product). Therefore, the usefulness of level C correlation is restricted during the prediction of *in vivo* drug performance.

In the initial phase of formulation deve lopment, l evel C correlations can be useful when pilot formulations are being selected while waiver of an *in vivo* bioequivalence study (biowaiver) is generally not possible.

4.3.3.4. Multiple Level C Correlations

Mutliple level represents the relationship between one or more desirable pharmacokinetic parameters (C_{max}, AUC, etc.) and concentration of drug dissolved at different time point s of dissolution profile. If the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest, the m ultiple level C correlation can be used to justify biowaivers.

It is important that a multiple l evel C correlation should be based on minimum three dissolution time points including the early, middle, and late stages of the dissolution profile. Also the development of a level A correlation occurs, when multiple level C correlation is achieved at each time point at the same parameter so that the effect on *in vivo* performance of any change in dissolution can be evaluated.

4.3.3.5. Level D Correlation

The level D correlation is a semi -quantitative (qualitative analysis) and rank order correlation. It is not a formal correlation and is not useful for regulatory purpose, but can be used during the development or processing of a formulation.

4.3.4. *In vitro* Drug Dissolution Models

An *in vitro* test can be useful if it predicts the *in vivo* behaviour to such an extent that there is no need to conduct an *in vivo* bioavailability test. Despite attempts to standardise the test performance, the *in vitro* dissolution technique is still by no means a perfect approach. The efforts are mainly aimed at mimicking the environment offered by the biological system.

An **ideal dissolution apparatus** should have the following **features**:

1) It should be simple in design, easy to operate, and functional under variable conditions.
2) The fabrication dimensions and positioning of all components should be precisely specified and reproducible.
3) It should provide an easy way of introducing the dosage form into the dissolution medium and once immersed, it should hold it in a regular and reliable manner.
4) It should permit controlled variab le intensity of mild, uniform, and non - turbulent liquid agitation.
5) It should provide minimum mechanical abrasion to the dosage form during the test period so that the microenvironment surrounding the dissolving form does not get disrupted.
6) It should maintain perfect sink conditions.
7) It should prevent or eliminate the evaporation of dissolution medium and maintain it at a fixed temperature within a specified narrow range. The dissolution apparatuses are mainly thermostatically controlled at 37°C.

8) It should provide ease of drawing samples for automatic or manual analysis without interrupting the flow characteristics of the liquid.
9) It should facilitate good inter-laboratory agreement.
10) It should be sensitive enough to reveal the process changes and formul ation differences, but still should yield repeatable results under identical conditions.
11) It should permit evaluation of disintegrating, non -disintegrating, dense or floating tablets or capsules, and finely powdered drugs.

The dissolution apparatus has g radually evolved from a simple beaker type to a highly versatile and fully automated instrument. The dissolution apparatuses can be classified into **two principal types** based on the absence or presence of sink conditions:

1) **Closed-Compartment Apparatus:** It i s a limited -volume apparatus that operate under non -sink conditions. The dissolution fluid is restrained to the container size. **Example**, beaker type apparatuses such as the rotating basket and the rotating paddle apparatus.

2) **Open-Compartment (Continuous Flow-Through) Apparatus:** In this, the dosage form is contained in a column which is brought in continuous contact with fresh, flowing dissolution medium (perfect sink condition).

   A third type of apparatus, called **dialysis system**, is used for very poorly aqueous soluble drugs that require large volume of dissolution fluid for maintenance of sink conditions.

3) **Rotating Basket Apparatus (Apparatus 1):** It was first described by **Pernarowski *et al***. It is a closed -compartment, beaker type apparatus containing a cyl indrical glass vessel (one litre capacity) with hemispherical bottom. This vessel is partially immersed in a water bath to maintain 37 °C temperature. A cylindrical basket of 22 mesh (meant to hold the dosage form) is centrally placed in the vessel such tha t it is 2cm above the bottom of the vessel. The basket is rotated by a variable speed motor through a shaft (**figure 4.6a**). While withdrawing samples, the basket should remain in motion.

4) **Rotating Paddle Apparatus (Apparatus 2):** It was first described by **Levy and Hayes** . Its assembly is same as that for Apparatus 1, with the only difference that instead of the rotating basket , a paddle is used as a stirrer (**figure 4.6b**). The dosage form is sunk to the bottom of the vessel. Sinkers prevent the floating of ca psules and other floatable forms. Such preparations are attached with a small, loose, wire helix to prevent them from floating.

5) **Reciprocating Cylinder Apparatus (Apparatus 3):** It comprises of a set of cylindrical flat -bottomed glass vessels fitted with rec iprocating cylinders (**figure 4.6c**). It is used for dissolution testing of controlled-release bead-type (pellet) formulations.

6) **Flow-Through Cell Apparatus (Apparatus 4):** It comprises of a reservoir for the dissolution medium and a pump that forces the diss olution medium through the cell holding the test sample (**figure 4.6d**). It is used in either:
   i) Closed-mode where the fluid is re-circulated and is of fixed volume, or
   ii) Open-mode where the fluids are continuously replenished.

The test sample (table, capsule s, or granules) is placed in the vertically mounted dissolution cell that allows the fresh solvent to be pumped in (between 240 and 960ml/h) from the bottom (**figure 4.6d**). This apparatus has the following **benefits**:

i) It provides ease of maintaining sink cond itions during dissolution which is required for drugs of limited aqueous solubility,
ii) It allows using large volume of dissolution fluid, and
iii) It provides feasibility for automation of apparatus.

7) **Paddle Over Disc Apparatus (Apparatus 5):** It comprises of a s ample holder or disc that holds the product. The disc is placed at the bottom of Apparatus 2 (rotating paddle apparatus; **figure 4.6e**) and the apparatus is operated in the usual way. It is used for evaluating transdermal products.

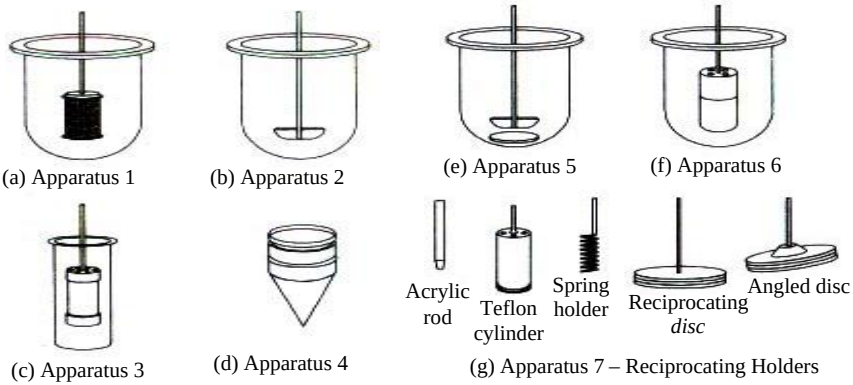


Figure 4.6: Official USP Dissolution Apparatus – (a) Apparatus 1 – Rotating Basket Apparatus, (b) Apparatus 2 – Rotating Paddle Apparatus, (c) Apparatus 3 – Reciprocating Cylinder Apparatus, (d) Apparatus 4 – Flow Through Cell Apparatus, (e) Apparatus 5 – Paddle Over Disc Apparatus, (f) Apparatus 6 – Cylinder Apparatus, and (g) Apparatus 7 – Reciprocating Disc Apparatus

8) **Cylinder Apparatus (Apparatus 6):** It is similar to Apparatus 1 (**figure 4.6f**) with the only difference that the basket is replaced with a stainless steel cylinder to hold the sample. The sample is mounted on an inert porous cellulosic material and adhered to the c ylinder. It is used for evaluating transdermal products.

9) **Reciprocating Disc Apparatus (Apparatus 7):** In this, the samples are placed on disc-shaped holders (**figure 4.6g**) using inert porous cellulosic support that vertically reciprocates via drive inside a glass container containing dissolution medium. The test is carried out at 32 °C temperature and at 30 cycles/min reciprocating frequency. It is used for evaluating transdermal products and non-disintegrating controlled-release oral preparations.

Table 4.2 lists the various types of dissolution apparatus and their applications:

Table 4.2: Compendial Dissolution Apparatus Types and Their Applications

Apparatus	Name	Drug Formulation Tested
Apparatus 1	Rotating basket	Conventional tablets, chewable tablet s, controlled-release formulations.
Apparatus 2	Rotating paddle	Tablets, orally disintegrating tablets, chewable tablets, capsules, controlled -release products, suspensions.

Apparatus 3	Reciprocating cylinder	Controlled-release formulations, chewable tablets.
Apparatus 4	Flow-through cell	Formulations containing poorly soluble drugs, powders and granules, microparticles, implants.
Apparatus 5	Paddle over disc	Transdermal formulations.
Apparatus 6	Cylinder	Transdermal formulations.
Apparatus 7	Reciprocating disc	Controlled-release formulations (non - disintegrating oral formulations and transdermal formulations).

4.3.5. Applications of an IVIVC

IVIVC has the following applications:

1) **In Drug Delivery Sys tem:** Many rate controlling technologies (**e.g.,** diffusion-dissolution, matrix retardation, osmosis, etc.) are used as modified release dosage forms. Such technologies are used for control ling and prolonging the release of drugs when the drug is administered either orally or parenterally. As a result , novel drug delivery systems (such as OROS, liposomes, niosomes, pharmacosomes, microspheres, nanoparticles, implants, in situ gelling system, organogels, transdermal drug delivery systems, parenteral depots, etc .) have been developed as a substitute to conventional dosage forms. The primary objective of these dosage forms is to achieve zero-order, long term, pulsatile, or "on demand" delivery.

2) **In Early Stages of Drug Delivery Technology Development:** During drug development, the most important stage is drug candidate selection, which is based on the drug developability criteria that include physicochemical properties of drug, results obtained from preformulation, and the p reliminary studies (that involve several *in vitro* systems and *in vivo* animal models) addressing efficacy and toxicity.

   Since IVIVC explores the relationship between *in vitro* and *in vivo* properties, IVIVC studies of the drug in animal models during drug candidate selection represents the feasibili ty of drug delivery system for given drug candidates. Such correlations should incorporate study designs that include the study of several formulations of the modified-release dosage forms and a rank order of release (fast/slow) of the formulations. At thi s stage, better design and development efforts are expected in the future, even though the formulations and methods used are not up to the mark.

3) **In F ormulation Assessment – *In vitro* Dissolution:** In product development, a suitable dissolution method that can distinguish the performance of formulations with different release rates *in vitro* and *in vivo* is an important tool. On the basis of the nature of correlation, the dissolution method can be changed . If the discriminatory *in vitro* method is validated, further development in formulation relies on *in vitro* dissolution only.

4) **Dissolution Specifications:** IVIVC plays an important role in setting specifications like modified-release dosage forms require dissolution testing over multiple time points. Specificationtime points are generally selected in the early, middle, and late stages of dissolution profiles. The range of dissolution

specification in very rare cases exceeds 10% of the dissolution of the pivotal clinical batch, if IVIV correlations are not present. But in the presence of IVIVC, a range of specifications are applicable based on the prediction of concentration-time profiles of test batches bioequivalent to the reference batch.

The dissolution specifications can be established in the presence of an IVIVC that starts after obtaining the reference (pivotal clinical batch) dissolution profile. Dissolution of batches with different dissolution properties (including slowest and fastest batches) are used along with the IVIVC model, and the concentration-time profiles should be deduced using a suitable convolution method. Specifications should be established in such a way that all batches with dissolution profiles between the fastest and slowest batches are bioequivalent and less optimally bioequivalent to the reference batch.

5) **Future Biowaivers:** During drug development, various changes are made in the formulations due to various reasons, **for example,** unexpected problems in stability, development, availability of better materials, better processing results, etc. If an established IVIVC is present, bioequivalence studies can be avoided by using the dissolution profile from the changed formulation, and predicting the *in vivo* concentration-time profile. Such a type of established profile can be used as a surrogate for the *in vivo* bioequivalence study. This is a cost-saving approach as it reduces drug development spending and speedy implementation of post-approval changes. The post-approval changes may range from minor (**e.g.,** change in non-release-controlling excipient) to major (**e.g.,** site change, equipment change, change in manufacture method, etc.).

6) **IVIVC in Parenteral Drug Delivery:** IVIVC is also applicable to parenteral dosage forms that are either injected or implanted (such as controlled-release particulate systems, depot system, implants, etc.); but, the success rate of such development of IVIVC for parenteral dosage forms is very low due to multiple reasons. For establishing a correlation between the *in vitro* and *in vivo* data, sophisticated modelling techniques are needed which are unpredictable and unavoidable.

7) **Biowaivers:** A validated IVIVC procedure can be used for justification of a biowaiver in filings of a Level 3 (or Type II in Europe) variation, either during scale-up or during post-approval, and also for line extensions (**e.g.,** different dosage strengths). The primary condition before granting a biowaiver is that the prediction of *in vivo* performance of the product with the modified *in vitro* release rate should remain bioequivalent with the originally tested product (i.e., the new dissolution rate remains within the IVIVC based biorelevant corridor). The FDA guidance puts forward the following categories of biowaivers:
   i) Biowaivers without an IVIVC,
   ii) Biowaivers using an IVIVC: Non-narrow therapeutic index drugs,
   iii) Biowaivers using an IVIVC: Narrow therapeutic index drugs,
   iv) Biowaivers when *in vitro* dissolution is independent of dissolution test conditions, and
   v) Situations for which an IVIVC is not recommended for biowaivers.

Biowaivers is granted for changes in manufacturing site, equipment, manufacturing process, and formulation composition according to a predictive and reliable IVIVC. These changes range from minor ones (that are not important to change product performance) to major ones where an IVIVC fails to validate the change for regulatory judgment.

8) **Mapping:** This process establishes a relation between Critical Manufacturing Variables (CMV, including formulation processes) and equipment variables that can affect drug release from the product. The process of mapping specifies boundaries of *in vitro* dissolution profiles according to the acceptable bioequivalent criteria.

   The main goal is to develop such product specifications, which ensure bioequivalence of future batches prepared within the limits of acceptable dissolution specifications. Mapping-based dissolution specifications increase the credibility of dissolution as a bioequivalent surrogate marker as well as provide continuous assurance and predictability of product performance.

4.3.6. Limitations in the IVIVC Arising from *In Vivo* Data

Following are the limitations of IVIVC:

1) In IVIVC, more than one dosage form is essential and sometimes intravenous or solution becomes compulsory to calculate deconvolution.
2) In IVIVC, pharmacokinetics and absorption of drug should be linear. If the pharmacokinetic processes depend on the fraction of dose reaching the systemic circulation (or of the dose administered) or on the rate of absorption, the formulation and simulation cannot be compared. Such non-linearity is due to saturable absorption processes (active absorption), induction or inhibition of metabolism, the rate/absorption dependent first past effect, etc. These points should be analysed before establishing an IVIVC.
3) The limiting factor for IVIVC should not be absorption, because it is convenient to attempt an IVIVC if solubility is not the limiting factor in comparison to drug release. Drug release should depend on the formulation, and should be slower than dissolution and absorption.

4.4. BIOEQUIVALENCE STUDIES

4.4.1. Introduction and Definition

Several formulations of the same drug are designed in the same dose, in a similar dosage form, and to be given by the same route. Substitution of one product for another can be done if their therapeutic efficacy is same as the standard accepted. The clinical performance of such drug products can be ensured by performing bioequivalence studies.

Some **important terms** applicable to bioequivalence studies are defined below:

1) **Equivalence:** It is a relative term that compares drug products with respect to a specific characteristic or function or to a defined set of standards.
2) **Chemical Equivalence:** It indicates that two or more drug products contain the same amount of the same labelled chemical substance as an active ingredient.

3) **Pharmaceutical Equivalence:** It indicates that two or more drug products are identical in strength, quality, purity, content uniformity, and disintegration and dissolution characteristics; however, they differ in their excipients.

4) **Bioequivalence:** It is a relative term indicating that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and extent, i.e., their plasma concentration -time profile s are identical without any signifi cant statistical differences. Bioequivalence is indicated if any statistically significant differences are observed in the bioavailability of two or more drug products.

5) **Therapeutic Equivalence:** It indicates that two or m ore drug products containing the same therapeutically active ingredient produce identical pharmacological effects and can control the disease to the same extent.

4.4.2. Objectives for Bioequivalence Studies

If a new product is designed as a pharmaceutical equivalent or alternative to be a substitute for an approved medicinal product, the equivalence with this product should be shown or justified. Bioequivalence stud ies should be performed to ensure the clinical performance of such drug products. Bioequivalence studies are conducted if there is:

1) A risk of bio-inequivalence, and/or
2) A risk of pharmacotherapeutic failure or diminished clinical safety.

4.4.3. Types of Bioequivalence Studies

The two types of bioequivalence studies are:

1) ***In vivo* Bioequivalence Studies:** The sequence of criteria used for evaluating the need for *in vivo* studies is as follows:
   i) Oral immediate release products with systemic action:
      a) These are indicated for serious conditions requiring a ssured response,
      b) They have a narrow therapeutic margin,
      c) Pharmacokinetics complicated by absorption <70% or absorp tion window, non-linear kinetics, pre-systemic elimination >70%,
      d) They have unfavourable physi cochemical properties, **e.g.,** low solubility, metastable modifications, instability, etc.,
      e) They have documented evidence for bioavailability problems, and
      f) No relevant data is available, unless the applicant justifies that *in vivo* study is not necessary.
   ii) Non-oral immediate release products.
   iii) Modified release products with systemic action.

   In vivo bioequivalence studies are conducted as the bioavailability studies, i.e., by the pharmacokinetic and pharmacodynamic methods.

2) ***In vitro* Bioequivalence Studies:** If the above criteria are not applicable, comparative *in vitro* dissolution studies are sufficient. *In vitro* dissolution

studies can be used instead of *in vivo* bioequivalence studies under the following circumstances, which are termed **biowaivers** (or exemptions):

i) The drug product and the active substance it contains differs only in strength, provided that all the following conditions hold:
   a) Pharmacokinetics are linear,
   b) Qualitative composition is same,
   c) Ratio between the active substance and the excipients is same, or (in case of small strengths) the ratio between the excipients is same,
   d) Both the products are produced by the same manufacturer at the same production site,
   e) A bioavailability or bioequivalence study has been conducted with the original product, and
   f) The *in vitro* dissolution rate is same under the same test conditions.

ii) The original manufacturer of the drug product has slightly reformulated or has slightly changed the manufacturing method in ways that can be argued to be inappropriate for bioavailability.

iii) The drug product meets all the following requirements:
   a) The product is in the form of a solution or solubilised form (elixir, syrup, tincture, etc.),
   b) The product contains active ingredient in the same concentration as the approved drug product, and
   c) The product contains no such excipients that affect the absorption of active ingredient.

iv) An acceptable IVIVC and the *in vitro* dissolution rate of the new product is equivalent to that of the already approved medicinal product. Furthermore
   a) The product is intended for topical administration (cream, ointment, gel, etc.) to exert local effect,
   b) The product is intended to be for oral administration but not to be absorbed (antacid or radio-opaque medium), and
   c) The product is inhaled as a gas or vapour.

It can be concluded from the above-mentioned criteria for drug products that bioavailability and bioequivalence are self-evident.

4.4.4. Bioequivalence Study Parameters

If two products show similar rate and extent of drug release, they are said to be bioequivalent, i.e., they will release the drug molecules in the same amount and rate (speed). These characteristics of two drug products inside the body (*in vivo* situation) can be studied by the following parameters:

1) **AUC:** It is the area under plasma drug concentration-time curve. It provides information on the amount of drug in plasma, i.e., extent of release.

2) **C_{max}:** It is the maximum plasma drug concentration. It partially depends on the rate of drug release from the formulation.

3) **t_{max}:** It is the time required to reach maximum plasma drug concentration. It also depends on the rate of drug release from the formulation.

4) **$t_{1/2}$:** It is the elimination half-life. It provides information on the elimination of the drug from the body.

Given below are some other parameters that are used in the bioequivalence study:

1) **Normalised C_{max}:** C_{max} and t_{max} show significant intra-subject variability, and hence normalised C_{max} is used in some cases. It is calculated by the following equation:

$$\text{Normalised } C_{max} = \frac{C_{max}}{AUC} \quad \text{.....(15)}$$

Studies indicate that normalised C_{max} in comparison to C_{max} shows less intra-subject variability.

2) **Mean Residence Time (MRT):** It is the time a drug molecule spends in the body before getting excreted. MRT can be calculated by first determining the Area Under the Moment Curve (AUMC) by the following equation:

$$AUMC_{0\text{-}t} = \sum_{i=1}^{t}\left(\frac{t_i C_i + t_{i-1} \cdot C_{i-1}}{2}\right)\left[t_i - t_{i-1}\right] \quad \text{..... (16)}$$

Equation (16) indicates that AUMC can be calculated in the same manner as AUC, with the only difference that in the latter drug plasma concentration is multiplied by time. $AUMC_{0\text{-}t}$ is calculated from $AUMC_{0\text{-}\infty}$, and then MRT is calculated by the following equation:

$$MRT = \frac{AUMC_{0\text{-}\infty}}{AUC_{0\text{-}\infty}} \quad \text{..... (17)}$$

3) **Plasma Trough Fluctuation (%):** It is used in the bioequivalence study of sustained release formulations, which are designed such that they maintain steady-state plasma drug concentration for extended time periods. Hence, in bioequivalence study of sustained release formulations, the steady-state plasma drug concentrations obtained from two drug products are compared.

C_{min} is the lowest plasma drug concentration just before the next dose, and % PTF is the % change in plasma drug concentration between two administered doses. It is calculated by the following equation:

$$\%PTF = \frac{C_{max} - C_{min}}{C_{average}} \quad \text{..... (18)}$$

Where, $C_{average}$ = Average plasma drug concentration during the dosing period.

4.4.5. Evaluation of Bioequivalence Data

The bioequivalence data obtained is evaluated by the following methods:

1) **Analytical Method:** This method for drug measurement should be validated for accuracy, precision, sensitivity, and specificity. Using multiple analytical methods during a bioequivalence study does not give valid results as different methods yield different values. Data should be presented in tabulated as well as graphical form to ease evaluation. The plasma drug concentration-time curve should be available for each drug product and each subject.

2) **Pharmacokinetic Evaluation of the Data:** For single-dose studies, including a fasting study or a food intervention study, the pharmacokinetic

analyses include calcu lating AUC to the last quantifiable concentration and to infinity, t_{max}, C_{max}, elimination rate constant (k), elimination half-life ($t_{1/2}$), and other parameters for each subject. For multiple -dose studies, pharmacokinetic analyses include calculating the s teady-state AUC, t_{max}, C_{min}, C_{max}, and % fluctuation for each subject. Proper statistical evaluation should be performed on the estimated pharmacokinetic parameters.

3) **Statistical Evaluation of the Data:** Bioequivalence is determined by comparing population averages of a bioequivalence metric, such as AUC and C_{max}. This approach is termed **average bioequivalence** , and involves calculating a 90% confidence interval for the ratio of averages (population geometric means) of the bioequivalence metrics for the test a nd reference drug products. Bioequivalence can be established if the calculated confidence interval falls within a prescribed bioequivalence limit (usually 80 -125% for the ratio of the product averages).

Another approach is termed **individual bioequivalenc e** (proposed by the FDA) that requires a replicate crossover design for estimating within -subject variability for the test and reference drug products, and subject -by formulation interaction.

Bioequivalence can be proved only if there is no statistical dif ference between the bioavailability of the test product and the reference product. There are many statistical approaches (or parametric tests) that are used for comparing the bioavailability of drug from the test dosage form and from the reference dosage f orm. Many of these approaches assume that the data are distributed according to a normal distribution or “bell-shaped curve”.

The distribution of many biological parameters (C_{max} and AUC) has a longer right tail than observed in a normal distribution. The true distribution of these biological parameters may also be difficult to establish because small number of subjects are used in a bioequivalence study. The distribution of data that has been transformed to log values resembles a normal distribution compared to the distribution of non -log-transformed data. Therefore, for determining bioequivalence, log transformation of the bioavailability data (**e.g.,** C_{max}, AUC) is performed before statistical data evaluation.

The obtained bioequivalence data is statisti cally evaluated by the following two methods:

i) **Analysis of Variance (ANOVA):** This is a statistical procedure in which the data is tested for differences within and between treatment and control groups. A bioequivalent product should not produce any signific ant differences in the tested pharmacokinetic parameters.

$AUC_{(0-t)}$, $AUC_{(0-\infty)}$, t_{max}, and C_{max} obtained for each treatment or dosage form are usually tested. Other metrics of bioavailability have also been used for comparing the bioequivalence of two or more formulations. The ANOVA may evaluate variability in subjects, treatmen t groups, study period, formulation, and other variables, depending on the study design. If the data shows large variability, the mean difference for each

pharmacokinetic parameter (such as AUC) may be masked, and the investigator might mistakenly conclude the two drug products to be bioequivalent.

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

ii) **Two One -Sided Test s Procedure:** This method is also termed as **confidence interval approach** . This statistical method validates if the bioavailability of the drug from the test formulation is too low or high than that of the reference product. This approach aims to determine i f there are large differences (i.e., > 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, in which a 90% confidence interval about the ra tio of means of the two drug products should be within ±20% for measuring the rate and extent of drug bioavailability. For most drugs, a 20% difference in AUC or C_{max} between the two formulations would have no clinical significance. The lower 90% confidence interval for the ratio of means cannot be less than 0.80, and the upper 90% confidence interval for the ratio of the means cannot be more than 1.20. The 90% confidence interval is set at 80-125% when log-transformed data are used. These confidence limits are termed as the **bioequivalence interval**.

The 90% confidence interval is a function of sample size and study of inter- and intra -subject variability. For analysing a single -dose, fasting study, ANOVA is performed on the log -transformed AUC and C_{max} values. No statistical differences should be present between the mean AUC and C_{max} parameters for the test (generic) and reference drug products. The 90% confidence intervals about the ratio of the means for AUC and C_{max} values of the test drug product should neither be less than 0.80 (80%) nor greater than 1.25 (125%) of that of the reference product based on log-transformed data.

4.4.6. Significance of Bioequivalence Studies

Bioequivalence studies have the following significant features:

1) They provide a link between the pivotal and early clinical trial formulation.
2) They assist in the determination of therapeutic equivalence between the pharmaceutical equivalence generic drug product and a corresponding reference listed drug.
3) They provide information on product quality and performance when there are changes in components, composition, and manufacture method after approval of the drug product.

4) They evaluate the absolute bioavailability of dosage form compared with reference dosage forms.
5) They perform the dose proportionality study to determine if bioavailability parameters are linear over the proposed dosage range.
6) They involve an intervention study to examine the effect of food and concomitant medication.
7) They involve a dosage form p roportionality study to determine if equipotent drug treatments administered at different dose strength of the market form produce equivalent drug bioavailability.
8) They are needed as a result of changes in the formulation or manufacturing processes.

4.5. SUMMARY

The details given in the chapter can be summarised as follows:

1) **Bioavailability** is the rate and extent of an administered dose of drug that reaches the systemic circulation in unchanged form.
2) The drug dose given to the patient i s the **administered dose** . The dose available to the patient is the **bioavailable dose**.
3) The **absolute bioavailability** of a given drug from a dosage form is the fraction (or percentage) of the administered dose absorbed into the systemic circulation in unchanged form.
4) **Relative bioavailability** can be determined by comparing the bioavailability of a drug from a test dosage form with the same drug administered in a standard dosage form.
5) $\mathbf{C_{max}}$ is the peak that indicates the point at which the drug concentration in plasma is maximum. It is usually expressed in **mcg/ml**.
6) $\mathbf{t_{max}}$ is the time the drug requires to reach peak concentration in plasma after extravascular administration. It is expressed in **hours**.
7) **AUC** is the area under the plasma level -time curve that gives a me asure of the extent of absorption or the amount of drug that reaches the systemic circulation.
8) **Urinary excretion studies** , performed to assess bioavailability, rely on the principle that the urinary excretion of unchanged drug and the plasma concentration of drug are directly proportional.
9) $\mathbf{(dX_u/dt)_{max}}$ is the maximum urinary excretion rate, obtained from the peak of plot between excretion rate and midpoint time of urine collection period.
10) $\mathbf{(t_u)_{max}}$ is the time for maximum excretion rate.
11) $\mathbf{X_u}$ is the cumulative amount of drug excreted in urine.
12) The method of **acute pharmacologic response** has a disadvantage that the pharmacologic response may vary and thus an accurate correlation between the measured response and drug available from the formulation cannot be established.

13) The **therapeutic response method** involves observing the clinical response of a drug formulation in patients who are suffering from the disease for which the drug is intended to be used.

14) If the solvent forms an integral part of the network structure and at least two component crystal, it is termed as **co-crystal**.

15) If the solvent does not directly involve itself in the network (as in open framework structures), it is termed as **clathrate** (or inclusion complex).

16) Water solubility of weak electrolytes and non-polar molecules is poor and this can be improved by adding another solvent that alters the solvent polarity. This process is termed **cosolvency**, and the solvent used for increasing solubility is termed a **cosolvent**.

17) The system of cosolvent involves **solvent blending** in which the interfacial tension between the aqueous solution and hydrophobic solute is reduced.

18) **Hydrotrophy** is the increase in water solubility due to the presence of large amounts of additives.

19) **Jack H. Shulman** first used the term microemulsion in 1959.

20) The term **solid dispersion** refers to the dispersion of one or more active ingredients in an inert carrier in a solid state, prepared by the melting (fusion) method, solvent method, or fusion solvent method.

21) A **eutectic mixture** of a sparingly water-soluble drug and a highly water-soluble carrier is thermodynamically regarded as an intimately blended physical mixture of two crystalline components.

22) **Solid solution** is a binary system comprising of a solid solute molecularly dispersed in a solid solvent. Solid solutions are also termed **molecular dispersions** or **mixed crystals** as the two components crystallise together in a homogeneous one phase system.

23) In **continuous solid solution**, the drug and the carrier are miscible in all proportions.

24) In **discontinuous solid solution**, solubility of each component in the other is limited.

25) In **substitutional crystalline solid solution**, if the drug and carrier molecules are almost of same size, the drug molecule substitutes for the carrier molecules in its crystal lattice.

26) In **interstitial crystalline solid solution**, if the size of drug molecule is 40% or less than the size of carrier molecules, the drug molecules occupy the interstitial spaces in the crystal lattice of carrier molecules.

27) A **glass solution** is a homogenous system in which a glassy or a vitreous of the carrier solubilises drug molecules in its matrix.

28) United State Pharmacopoeia (USP) defined **IVIVC** as “the establishment of a rational relationship between a biological property or a parameter derived from a biological property produced by a dosage form, and a physicochemical property or characteristic of the same dosage form”.

29) Food and Drug Administration (FDA) defined **IVIVC** as "a predictive mathematical model describing the relationship between an *in-vitro* property of a dosage form and an *in-vivo* response".

30) The **level A correlation** of IVIVC has regulatory relevance and correlates the entire *in vitro* and *in vivo* profiles.

31) The **level B correlation** of IVIVC relies on the principles of statistical moment analysis.

32) The **level C correlation** relates one dissolution time point ($t_{50\%}$, $t_{90\%}$, etc.) to one mean pharmacokinetic parameter (AUC, t_{max}, or C_{max}).

33) **Multiple level C correlations** represent the relationship between one or more desirable pharmacokinetic parameters (C_{max}, AUC, etc.) and concentration of drug dissolved at different time points of dissolution profile.

34) The **level D correlation** is a semi-quantitative (qualitative analysis) and rank order correlation.

35) **Equivalence** is a relative term that compares drug products with respect to a specific characteristic or function or to a defined set of standards.

36) **Chemical equivalence** indicates that two or more drug products contain the same amount of the same labelled chemical substance as an active ingredient.

37) **Pharmaceutical equivalence** indicates that two or more drug products are identical in strength, quality, purity, content uniformity, and disintegration and dissolution characteristics; however, they differ in their excipients.

38) **Bioequivalence** is a relative term indicating that the drug substance in two or more identical dosage forms, reaches the systemic circulation at the same relative rate and extent, i.e., their plasma concentration-time profiles are identical without any significant statistical differences.

39) **Therapeutic equivalence** indicates that two or more drug products containing the same therapeutically active ingredient produce identical pharmacological effects and can control the disease to the same extent.

40) **Mean Residence Time (MRT)** is the time a drug molecule spends in the body before getting excreted.

41) **Plasma Trough Fluctuation (%)** is used in the bioequivalence study of sustained release formulations, which are designed such that they maintain steady-state plasma drug concentration for extended time periods.

42) **Analytical method** for drug measurement should be validated for accuracy, precision, sensitivity, and specificity.

43) **Average bioequivalence** involves calculating a 90% confidence interval for the ratio of averages (population geometric means) of the bioequivalence metrics for the test and reference drug products.

44) **Individual bioequivalence** requires a replicate crossover design for estimating within-subject variability for the test and reference drug products, and subject-by formulation interaction.

45) **Analysis of Variance (ANOVA)** is a statistical procedure in which the data is tested for differences within and between treatment and control groups.

46) **Two one-sided tests procedure** is also termed as **confidence interval approach**

4.6. EXERCISE

4.6.1. True or False

1) The relative bioavailability of a given drug from a dosage form is the fraction of the administered dose absorbed into the systemic circulation in unchanged form.
2) $(dX_u/dt)_{max}$ is the m aximum urinary excretion rate, obtained from the peak of plot between excretion rate and midpoint time of urine collection period.
3) The acute pharmacological response method involves observing the clinical response of a drug formulation in patients who are suffering from the disease for which the drug is intended to be used.
4) In continuous solid solution, solubility of each component in the other is limited.
5) In substitutional crystalline solid solution , if the drug and carrier molecules are almost of same size, the drug molecule substitutes for the carrier molecules in its crystal lattice.
6) A glass solution is a homogenous system in which a glassy or a vitreous of the carrier solubilises drug molecules in its matrix.
7) The level C correlation of IVIVC relie s on the principles of statistical moment analysis.
8) Chemical equivalence indicates that two or more drug products contain the same amount of the same labelled chemical substance as an active ingredient.
9) Statistical method for drug measurement should be va lidated for accuracy, precision, sensitivity, and specificity.

4.6.2. Fill in the Blanks

10) The drug dose given to the patient is the _____ and the dose available to the patient is the _____.
11) C_{max} is usually expressed in _____.
12) _____ is the cumulative amount of drug excreted in urine.
13) Solid solutions are also termed _____ or _____ .
14) The level _____ correlation is a semi -quantitative (qualitative analysis) and rank order correlation.
15) _____ indicates that two or more drug products containing the s ame therapeutically active ingredient produce identical pharmacological effects and can control the disease to the same extent.
16) _____ is the time a drug molecule spends in the body before getting excreted.
17) _____ requires a replicate crossover design f or estimating within -subject variability for the test and reference drug products, and subject -by formulation interaction.
18) _____ is a statistical procedure in which the data is tested for differences within and between treatment and control groups.

Answers

1) False 2) True 3) False
4) False 5) True 6) True
7) False 8) True 9) False
10) Administered dose and bioavailable dose 11) mcg/ml
12) X_u 13) Molecular dispersions & mixed crystal
14) D 15) Therapeutic equivalence
16) Mean residence time 17) Individual bioequivalence
18) ANOVA

4.6.3. Very Short Answer Type Questions

1) Define bioavailability.
2) What is relative bioavailability?
3) Give the objectives of bioavailability studies.
4) Define t_{max} and C_{max}.
5) How bioavailability can be enhanced by using salt form of drugs?
6) Give the level D correlation of *In Vitro-In Vivo* Correlation.
7) Enlist the dissolution models.
8) Give any one limitation of *In Vitro-In Vivo* Correlation.
9) Define bioequivalence.
10) What is MRT?

4.6.4. Short Answer Type Questions

1) Discuss in detail about absolute bioavailability.
2) Give the significance of bioavailability studies.
3) Write about urinary excretion studies.
4) Explain the levels of *In Vitro-In Vivo* Correlation.
5) Give the applications of *In Vitro-In Vivo* Correlation.
6) Write in detail the objectives and types of bioequivalence studies.
7) Give the significance of bioequivalence studies.

4.6.5. Long Answer Type Questions

1) Discuss about the measurement of bioavailability in detail.
2) Explain briefly the *in vitro* drug dissolution models.
3) Write an exhaustive note on evaluation of bioequivalence data.

CHAPTER 5

Pharmacokinetics

5.1. PHARMACOKINETICS

5.1.1. Definition and Introduction

Pharmacokinetics is the **study of rate processes involved in absorption, distribution, metabolism, and excretion of a drug**. It includes the study of biological, physiological and physicochemical factors that influence the transfer processes of drugs in the body, and also influence the rate and extent of ADME of those drugs in the body. In most of the cases, both pharmacologic al and toxicological actions are considered with respect to the plasma concentration of drugs. The study of pharmacokinetics helps the pharmacists in individualising therapy for the patients.

The word **pharmacokinetic** has been originated from the Greek word ***pharmakon*** which means **drug** and ***kinesis*** which means **motion** or **change of rate**. Thus, **pharmacokinetics** can also be defined as "**the kinetics of drug absorption, distribution, metabolism and excretion (KADME) and their relationship with the pharmacological, therapeutic or toxicological response in humans**".

Absorption is the **process of movement of unchanged drug from the site of administration to systemic circulation or to the site of measurement (i.e., plasma)**.

Distribution is the **reversible transfer of a drug between the blood and the extravascular fluids and tissues**.

Elimination is the major process of drug removal from the body and termination of its action. It is defined as **the irreversible loss of drug from the body**. Elimination is a two-step process which involves biotransformation and excretion.

Metabolism (or **biotransformation**) of drugs is the **chemical conversion of one form to another**.

Excretion is the process by which **drugs and/or their metabolites are irreversibly transferred from internal to external environment**.

5.1.2. Objectives

The objectives of pharmacokinetics are as follows:

1) The main objective of pharmacokinetics is to measure the extent of drug absorption, distribution, biotransformation, and excretion in intact, living animals or humans. The information obtained from such studies is used to determine the effect of alterations in dose, dosage regimen, administration route, and physiologic state on drug accumulation and disposition.

2) Pharmacokinetic profile of a drug can be inferred by analysing the changes in concentration of a drug or its metabolite in body fluids in the given time period.
3) At any time after drug administration in known dose or dosage regimen, its plasma or urine concentration is the net result of its absorption, distribution, metabolism, and excretion. Therefore, the main purpose is to resolve the observed kinetic profiles into their components.
4) By using appropriate experimental design, various types of models, and kinetic data analysis, the contribution of absorption, distribution, metabolism, and excretion can be individually isolated. After this, quantisation can be achieved by maintaining material balance at all times.
5) It helps to understand the basics of a therapeutic drug monitoring service.
6) It is used to describe and understand how physiological changes affect the pharmacokinetic profile of a drug in very young and elderly individuals.
7) In drug disposition, subsequent absorption of drug occurs, while in elimination, an irreversible loss of drug occurs from the body by metabolism and excretion.

5.1.3. Applications

Following are the applications of pharmacokinetics:

1) Equations of pharmacokinetics are used to determine the drug bioavailability.
2) Using the principles of pharmacokinetics the dosing pattern of any drug can be fixed.
3) The principles of pharmacokinetics are also used to determine the dosing pattern of controlled release dosage forms.
4) In case of kidney failure, the drug dose that should be given can be measured by using pharmacokinetic principles.

5.2. PHARMACOKINETIC MODELS

5.2.1. Introduction

Many mathematical models are used to describe the rate processes of drug absorption, distribution, metabolism, and excretion. These models are also used to develop various equations which analyse drug concentrations in body with respect to time. Since drug concentrations depend on time, the drug concentration and time variables are termed as **dependent** and **independent variables**, respectively. Direct measurement of pharmacokinetic parameters is not possible; thus, can be determined through *in vitro* experiments using the given set of dependent and independent variables, which are collectively termed as **data**. These data are used to design a pharmacokinetic model and test its validity.

Selection of pharmacokinetic model for data analysis relies on a hypothesis and set of assumptions that describe the biological events mathematically. The pharmacokineticist should be careful while depending on a pharmacokinetic model for determining drug action. For data analysis, often a simplest pharmacokinetic model and statistical methods are used to select the best model that fits the data.

If it is found that the model does not appropriately fit all the experimental observations, a new and a more complex model (hypothesis) is devised and tested for validity. It should always be known that the pharmacokinetic data should not replace the clinical observations in patient and sound judgment by the clinician.

5.2.2. Compartment Models

The most traditional and common approach used for pharmacokinetic characterisation of a drug is **compartment analysis**. In this approach, the body is considered to be composed of various compartments that reversibly communicate with each other. If every organ, tissue or body fluid that can be equilibrated with the drug is considered as a compartment, a body can have numerous compartments and mathematical description of such a model will be highly complex. Therefore, tissues with similar drug distribution properties are pooled to form a hypothetical compartment that is kinetically homogeneous.

These compartments are not real physiological or anatomic regions, but are hypothetical or virtual. These compartments represent the bio system and are assumed to comprise of tissues with similar blood flow and affinity for drug. A hypothetical model comprising of one, two or maximum three functional compartments (arranged serially or parallelly to each other) is used to analyse the kinetics of most of the drugs. It is also considered that the rate of drug movement between compartments (i.e., entry and exit) follow first-order kinetics.

5.2.2.1. Types

Compartment models are divided into the following two categories based on their arrangement, i.e., whether parallel to each other or in a series:

1) **Mammillary Model:** This is the most common compartment model to be used in pharmacokinetic studies. In this model, one or more peripheral compartments are connected to a central compartment, consisting of plasma and highly perfused tissues in which the drug undergoes rapid distribution. The administered drug firstly reaches the central compartment and then distributes to all the other compartments connected to the central compartment. Since the major organs involved in drug elimination (i.e., kidney and liver) are found in the central compartment, drug elimination is assumed to occur from there.

   Several types of compartment models have been shown in **figure 5.1**. Compartment 1 is the **plasma** or **central compartment**, and compartments 2, 3 and 4 are the **tissue** or **peripheral compartments**. The **letter K** indicates pharmacokinetic rate constants, and the numbers describe the movement direction of drug between the compartments. **For example,** K_{12} indicates the rate constant with respect to drug movement from compartment 1 to compartment 2.

   Parameters of Mammillary Model

   Model 1 (**figure 5.1**) can be described by two parameters, i.e., the **volume of compartment** and the **elimination rate constant** (K). In model 4, the pharmacokinetic parameters include the volume of compartment 1 and 2 and the rate constants K_a, K_{13}, K_{12} and K_{21} for total six parameters.

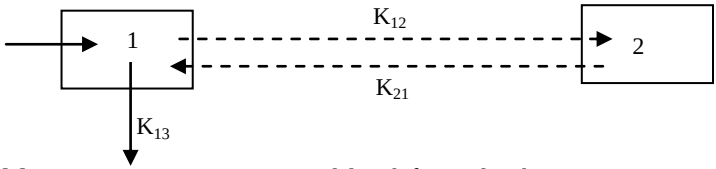
During the study of these models, it is necessary to know whether drug concentration data can be directly obtained from each compartment. For models 3 and 4 (**figure 5.1**) the data obtained from compartment 2 cannot be obtained easily since tissue sampling is a tedious task and drug concentration may not be homogenous in different tissues.

The amount of drug in the tissue compartments can be mathematically calculated if the data regarding the amount of drug absorbed and eliminated per unit time is obtained by the sampling of compartment 1. Various mathematical equations are used to describe these models and evaluate various pharmacokinetic parameters.

Model 1: One-compartment open model I.V. injection

Model 2: One-compartment open model with first-order absorption

Model 3: Two-compartment open model I.V. injection



Model 4: Two-compartment open model with first order absorption

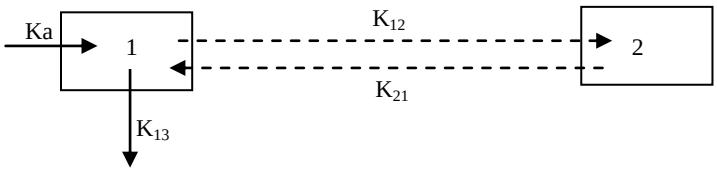


Figure 5.1: Various Compartment Models

2) **Catenary Model:** In this model, the compartments are joined to each other in a chain or series (**figure 5.2**). However, this model is rarely preferred owing to the drawback that it is not physiologically/anatomically comparable because the various organs are directly linked to the blood compartment.

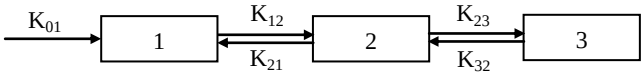


Figure 5.2: Catenary Model

5.2.2.2. Advantages

Following are the advantages of compartment models:

1) They give a visual representation of variou s rate processes involved in drug disposition.
2) They provide various rate constants required to describe these processes.
3) They help the pharmacokineticist to derive differential equations for various rate processes so that changes in drug concentration in various compartments can be described.

4) They help in determining drug concentration -time profile under normal physiological as well as pathological conditions.
5) They are also used in the development of dosage regimens.

5.2.2.3. Disadvantages

Following are the disadvantages of compartment models:

1) All the physiologic al functions or the anatomic structure of the species cannot relate to the compartment model. Therefore, many assumptions have to be made to interpret the data obtained from compartment models.
2) Exhaustive effort is required to find an exact model that can be used to predict and describe the ADME of a drug under consideration.
3) Compartment modelling is based on curve fitting of plasma concentration with multi-exponential mathematical equations.
4) This approach is not a common procedure and is applicable only for some specific drugs.
5) The behaviour of drug in body can fit more than one c ompartmental model depending on the drug administration route.
6) Sometimes, it becomes difficult to explain the differences between results obtained from human and animal experiments using compartment models.
7) A variation in the study model can be observed.

5.2.3. Non-Compartmental Models

In non-compartmental analysis, assumption of specific compartment model is not required; therefore it is also called as the **model-independent method** This method of analysis can be used for any compartment model in which the drugs or metabolites follow linear kinetics. This model is based on statistical moment theory in which experimental data is collected after the administration of a single dose of drug.

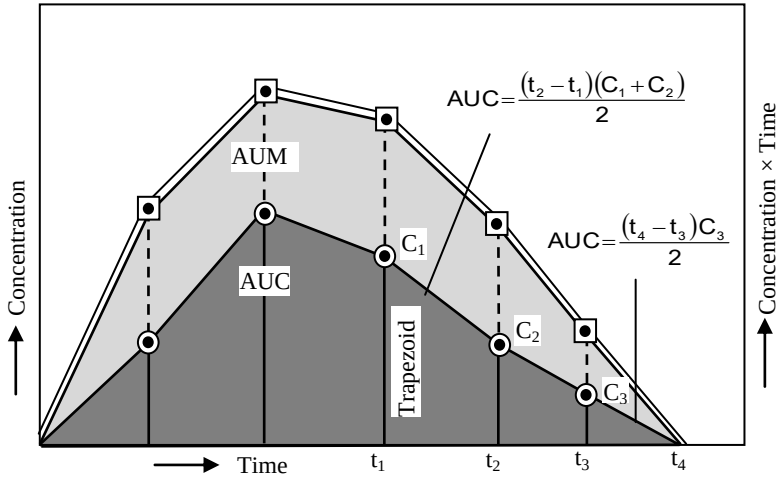


Figure 5.3: AUC and AUMC Plots

If the time course of drug concentration in plasma is considered as a statistical distribution curve:

$$MRT = \frac{AUMC}{AUC} \quad \text{..... (1)}$$

Where MRT = Mean residence time.
AUMC = Area under the first-moment curve.
AUC = Area under the zero-moment curve.

After plotting a graph between the product of plasma drug concentration and time (C.t) *versus* time (t) from zero to infinity (**figure 5.3**), the value of AUMC is obtained, that can be mathematically represented as:

$$AUMC = \int_0^\infty C\,t\,dt \qquad \text{..... (2)}$$

From a plot of plasma drug concentration *versus* time from zero to infinity, the value of AUC is obtained, which is mathematically represented as:

$$AUC = \int_0^\infty C\,dt \qquad \text{……(3)}$$

The values of AUMC and AUC can be calculated from their respective graphs using the **trapezoidal rule** in which the curve is divided by a series of vertical lines int o a number of trapezoids, followed by calculating the area of each trapezoid separately and then adding them together.

MRT is the **average amount of time a drug spends in the body before being eliminated**. Thus, it can be said that MRT is the statistical mo ment analogy of half-life ($t_{1/2}$). Practically, MRT defines the time for the elimination of 63.2% of the intravenous bolus dose. The values will always be greater when the drug is administered in any form or through any route other than the intravenous bolus.

The non -compartmental model is mostly used to analyse the important pharmacokinetic parameters , such as bioavailability, clearance, and apparent volume of distribution. This method is also used to determine half -life, rate of absorption, and first-order absorption rate constant of the drug.

5.2.3.1. Statistical Moment Theory

Compartment models can be used in pharmacokinetic analysis of drug concentration in blood *versus* time data with the application of various mathematical equ ations devised on the basis of certain assumptions. In non -compartmental methods , such assumptions and mathematical equations are not required because the non-compartmental models used for predicting absorption, distribution, and elimination parameters rely on the statistical moment theory.

The statistical moment theory helps in studying the changes in drug concentration in plasma and/or tissues with time . The **total area under the curve from time zero to infinity (AUC_0^∞)** is the zero momen t of a drug concentration in plasma *versus* time curve.

The **total area under the curve obtained from a plot of drug concentration *versus* time** is the zero moment of a drug concentration in a plasma -time profile. The **total area under the curve obtained from a plot of product of drug concentration and time *versus* time ($AUMC_0^\infty$)** is the first moment of a plasma concentration-time profile.

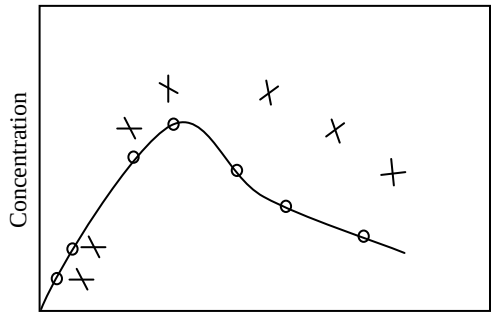


Figure 5.4: Plots of Drug Concentration (μg/ml) (○) and Drug Concentration-Time (μg-hr/ml) (X) *versus* Time, during and after an Hour Constant Rate Intravenous Infusion. The Area under the Drug Conc. *versus* Time Plot to Infinity is AUC_0^{∞}, the Area under the Drug Conc. Time *versus* Time Plot to Infinity is $AUMC_0^{\infty}$.

During the calculation of various pharmacokinetic parameters, the plasma concentration *versus* time data is plotted (**figure 5.4**) and the AUC from t = 0 to the last sampling time (t*) is calculated using the trapezoidal rule. The values of (C) × (t) (i.e., product of concentration of drug and time) are plotted against time (**figure 5.4**). The area under the (C) × (t) *versus* t plot from t = 0 to the last sampling time (t*) is called the **first moment of drug concentration with respect to time (AUMC)**.

The area under the curve from t* to ∝ for both the curves can be calculated using appropriate equations to obtain $AUC_{t^*}^{\infty}$ and $AUMC_{t^*}^{\infty}$. By adding these areas to $AUC_0^{t^*}$ and $AUMC_0^{t^*}$, the total areas under zero moment and first moment curves, i.e., AUC_0^{∞} and $AUMC_0^{\infty}$, respectively are obtained. The above obt ained values are used to calculate various pharmacokinetic parameters.

5.2.3.2. Advantages

Following are the advantages of non-compartmental analysis:

1) The simple algebraic equations are used for easy derivation of the pharmacokinetic parameters.
2) Similar mathematical treatment can be used for any drug or metabolite, provided that they follow first-order kinetics.
3) A detailed description of drug disposition is not needed.

5.2.3.3. Disadvantages

The main drawback of non-compartmental analysis is that it offers limited information about the plasma drug concentration -time profile and most of the time it deals with averages.

5.2.4. Physiological Models

Physiological pharmacokinetic models (or blood flow or perfusion models) rely on known anatomic and physiological data. These models describe the data kinetically considering that the blood flow distributes a drug to various body

parts. Drug uptake into organs is determined by binding the drug in these tissues. In contradiction of the tissue volume of distribution, the actual tissue volume is used. There are many tissue organs in the body, and each tissue volume and its drug concentration should be estimated. The physiological model potentially predicts the accurate tissue drug concentration, which the compartment model fails to do.

A physiological pharmacokinetic model, despite the limitation, provides a better understanding of the changes in drug distribution from one animal species to another by the physiological factors. Other major differences are:

1) Data fitting is not required in perfusion model. Drug concentrations in various tissues depend on the organ tissue size, blood flow, and experimentally determined drug tissue-blood ratios (i.e., drug partition between tissue and blood).
2) Due to some pathophysiological conditions, blood flow, tissue size, and the drug tissue-blood ratio may vary. Effect of these variations on drug distribution should be considered in physiological pharmacokinetic models.
3) Physiological pharmacokinetic models can be applied to several species, and human data can be extrapolated with some drugs. In compartment models, the volume of distribution is a mathematical concept with no relation to the blood volume and blood flow, thus extrapolation is not possible. So far, many drugs (like digoxin, lidocaine, methotrexate, and thiopental) have been described using perfusion models. It is not possible to estimate the tissue levels of these drugs using compartment models; however, they describe blood levels well. A perfusion model is shown in **figure 5.5**.

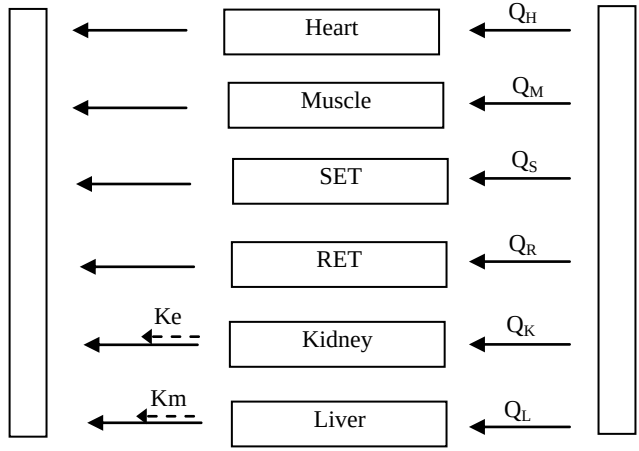


Figure 5.5: Pharmacokinetic Model of Drug Perfusion

In a perfusion model, the number of tissue compartments varies with drug-to-drug. The tissues or organs in which the drugs have little or no penetration (brain, bones, and other parts of CNS) are not considered. If each organ is described separately with a differential equation, the model will turn out to be very complex and mathematically difficult. A simpler and equally good approach is that the tissues with similar blood perfusion properties are

grouped into a single compartment. **For example**, distribution of lidocaine in blood and various organs have been successfully described using perfusion model; organs like lungs, liver, brain, and muscles were individually described by differential equations, while other tissues were grouped as RET (Rapidly Equilibrating Tissue) and SET (Slowly Equilibrating Tissue).

5.2.4.1. Types

The physiological models are categorised into the following two types:

1) **Blood Flow Rate -Limited Models:** These models are more popular and common in use. They rely on the assumption that drug movement in a body region is more rapid than its delivery rate to that region by the perfusing blood. Ho wever, this assumption is applicable to low molecular weighed, poorly-ionised and highly lipophilic drugs (**e.g.,** thiopental, lidocaine, etc.) having high membrane permeability.

2) **Membrane Permeation Rate -Limited Models:** These models are more complex. They are used for highly polar, ionised and charged drugs. In these models, the cell membrane acts as a barrier for the drug that gradually permeates by diffusion; thus, these models are also called **diffusion-limited models**. Equations for these models are very co mplicated due to the time lag in equilibration between the blood and the tissue.

5.2.4.2. Advantages

The physiological pharmacokinetic models have the following advantages over the conventional compartment models:

1) Their mathematical treatment is direct.
2) They do not require data fitting; drug concentration in various body regions can be estimated based on the organ or tissue volume, perfusion rate and experimentally determined tissue-to-plasma partition coefficient.
3) They provide accurate description of drug concentration -time profile in any organ or tissue, and thus, provide a better insight of drug distribution characteristics in the body.
4) They can easily predict the effect of altered physiology or pathology on drug distribution from the changes in various pharmacokinetic parameters that correspond to actual physiologic and anatomic measures.
5) They can correlate data in several animal species and with some drugs, and then can extrapolate to humans.

5.2.4.3. Disadvantages

The physiological pharmacokinetic models have the following disadvantages:

1) Obtaining the experimental data using these models is a very exhaustive process.
2) Most of them assume an average blood flow for individual subjects, h ence making the prediction of individualised dosing is difficult.
3) The number of data points in these models is less than the pharmacokinetic parameters to be assessed.
4) They can monitor drug concentration in body with much difficulty since exhaustive data is required.

5.3. ONE COMPARTMENT OPEN MODEL

5.3.1. Introduction

One-compartment open model is the simplest model that describes the body as a single, kinetically homogeneous unit having no barriers to drug movement. Also in this model, final distribution equilibrium between the drug in plasma and other body fluids is rapidly attained and maintained all the times. This model applies only to those drugs that rapidly distribute throughout the body.

The anatomical reference compartment is the plasma and the drug concentration in plasma represents the drug concentration in all body tissues, i.e., any change in plasma drug concentration indicates a proportional change in drug concentration throughout the body. However, the model does not assume that the plasma drug concentration is equal to drug concentration in other body tissues. The term **open** indicates that the input (absorption) and output (elimination) are unidirectional and the drug can be eliminated from the body. **Figure 5.6** shows such a one - compartment model.

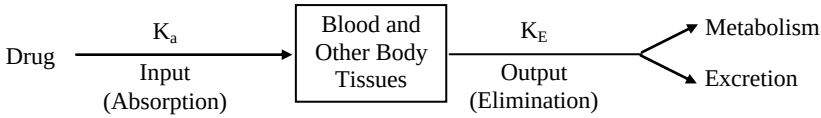


Figure 5.6 Representation of One-Compartment Open Model showing Input and Output Processes

The following **assumptions** help in deriving mathematical equations for one - compartment open model:

1) Drug absorption from the absorption site may be explained by the first -order kinetics.

   Explanation: Most drugs are absorbed by passive diffusion governed by the first-order process, i.e., the drug absorption rate and the drug concentration at absorption site are proportional. Drugs which are absorbed by active transport, facilitated diffusion, etc., do not follow this assumption.

2) A drug on entering the systemic circulation rapidly distributes to other body fluids and tissue, thus a dynamic equilibrium is rapidly achieved between the drug in the blood and the drug in other tissues.

   Explanation: The drug distributing to highly perfused tissues, like heart, lungs, liver, kidney, etc., can distribute instantaneously and attain equilibrium between the drug levels in plasma and other body fluids and tissues. If the drug is distributed to poorly perfused tissues, the time required for its distribution and equilibrium is considered. Hence, this simple model is not used for pharmacokinetic analysis of the data obtained with such drugs.

3) Any changes that occur in the drug plasma levels indicate proportional changes in the tissue drug levels.

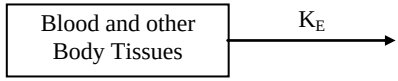
   Explanation: A dynamic equilibrium exists between the drug concentration in plasma and drug concentration in tissues. Thus, a change in plasma drug level reflects a proportional change in the drug levels in tissues.

4) Drug elimination from the body follo ws apparent first-order kinetics and its rate constant (K) is known as an **apparent first-order rate constant**.

   Explanation: Drug elimination from the body occurs by different processes, like renal, biliary, biotransformation, excretion in the expired air, etc.

5.3.2. Intravenous Injection (Bolus)

If a drug that rapidly distributes in the body is given as a rapid intravenous injection (i.e., I.V. bolus or slug), it takes 1 -3 minutes for complete circulation , and therefore the absorption rate is neglected in calculations. This model can be represented as follows:



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

$$\frac{dX}{dt} = \text{Rate in (absorption)} - \text{Rate out (elimination)} \quad \text{..... (4)}$$

Since the rate in or absorption is absent, **equation (4)** becomes:

$$\frac{dX}{dt} = -\text{Rate out} \quad \text{..... (5)}$$

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

$$\frac{dX}{dt} = -K_E X \quad \text{..... (6)}$$

Where, K_E = First-order elimination rate constant.
X = Amount of drug in the body remaining to be eliminated at time (t).

The negative sign indicates that the drug is lost from the body. Now, the various related pharmacokinetic parameters can be estimated.

Determination of Pharmacokinetic Parameters from Plasma Data after Intravenous Injection (Bolus)

The different pharmacokinetic parameters that can be determined after intravenous bolus administration are:

1) Elimination rate constant,
2) Elimination half-life,
3) Apparent volume of distribution,
4) Clearance,
5) Total clearance, and
6) Area under curve.

5.3.2.1. Elimination Rate Constant (K_E)

A drug that follows one-compartment kinetics and is administered as a rapid I.V. injection experiences decline in plasma drug concentration due to elimination of drug from the body (and not due to distribution); this phase is called as **elimination phase**.

On integrating **equation 6)**:

$$\ln X = \ln X_o - K_E t \quad \text{..... (7)}$$

Where, X_o = Amount of drug at time (t) = 0, i.e., the initial amount of drug injected.

Equation (7) can also be written in the exponential form as:

$$X = X_0 e^{-K_E t} \quad \text{..... (8)}$$

Equation (8) indicates that disposition of a drug following one -compartment kinetics is mono-exponential.

On transforming **equation (8)** into common logarithms (log base 10):

$$\log X = \log X_o = \frac{K_E t}{2.303} \quad \text{..... (9)}$$

Direct determination of the amount of drug in the body (X) is difficult, thus advantage is taken of the fact that a constant relationship exists between the plasma drug concentration (C, easily measurable) and X; thus:

$$X = V_d C \quad \text{..... (10)}$$

Where, V_d = Apparent volume of distribution (a proportionality constant). It is a pharmacokinetic parameter that allows the use of plasma drug concentration instead of amount of drug in the body. Therefore, **equation (6)** becomes:

$$\log C = \log C_o = - \frac{K_E t}{2.303} \quad \text{..... (11)}$$

Where, C_o = Plasma drug concentration immediately after I.V. injection.

Equation (11) is of a straight line and indicates that a semi -logarithmic plot of log C *versus* t will be linear with y -intercept log C_o. Elimination rate constant is directly obtained from the slope of the line **(figure 5.7b)**. It has units of min^{-1}. Thus, a linear plot is easily handled mathematically than a cur ve which will be obtained from C *versus* t plot on a regular (Cartesian) graph paper **(figure 5.7a)**.

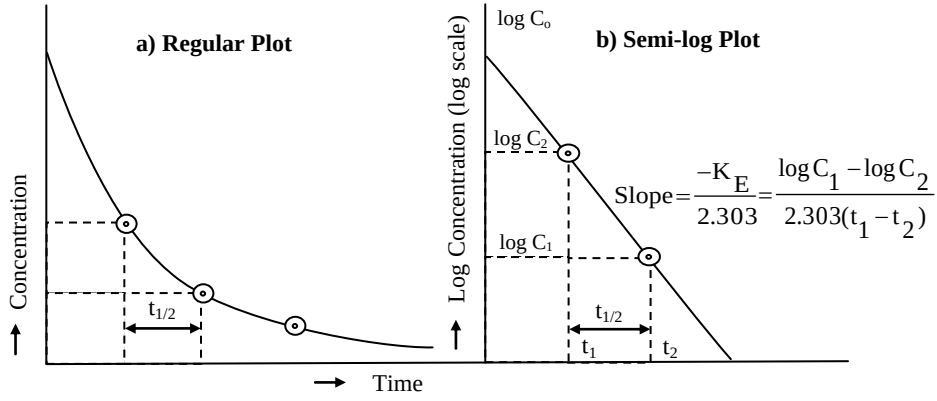


Figure 5.7 (a) Cartesian Plot of a Drug that follows One-Compartment Kinetics and given by Rapid I.V. Injection, and (b) Semi-logarithmic Plot for the Rate of Elimination in a One-Compartment Model

Thus, C_o, K_E, and $t_{1/2}$ can be readily obtained from log C *versus* t graph. Elimination of drug from the body is the sum of urinary excretion, metabolism, biliary excretion, pulmonary excretion, and other involved mechanisms. Thus, **K_E** is an additive property of rate constants for each of these processes and is called the **overall elimination rate constant**.

$$K_E = K_e + K_m + K_b + K_l + \ldots\ldots \quad \text{..... (12)}$$

If the number of rate constants involved and their values are known, evaluating the fraction of drug eliminated by a particular route becomes easier. **For example,** if a drug is eliminated by urinary excretion and metabolism, the fraction of drug excreted unchanged in urine (F_e) and the fraction of drug metabolised (F_m) is given as:

$$F_e = \frac{K_e}{K_E} \quad \text{..... (13)}$$

$$F_m = \frac{K_m}{K_E} \quad \text{..... (14)}$$

5.3.2.2. Elimination Half-Life ($t_{1/2}$)

Elimination half-life (or **biological half-life**) is the oldest, best known pharmacokinetic parameter, and was once considered the most important drug characteristic. It is **the time taken for the amount of drug in the body and the concentration of drug in plasma to decline by one-half or 50% of its initial value**. It is expressed in **hours** or **minutes**. Half-life is related to elimination rate constant as follows:

$$t_{1/2} = \frac{0.693}{K_E} \quad \text{..... (15)}$$

Increased physiologic understanding of pharmacokinetics signifies that half-life is a secondary parameter that depends on the primary parameters, like clearance and apparent volume of distribution as follows:

$$t_{1/2} = \frac{0.693 V_d}{Cl_T} \quad \text{..... (16)}$$

5.3.2.3. Apparent Volume of Distribution (V_d)

Apparent volume of distribution is an independent pharmacokinetic drug parameter. It is closely related to the physiological mechanisms of the body, thus is considered a **primary parameter**.

On modifying **equation (7)**:

$$V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}} = \frac{X}{C} \quad \text{..... (17)}$$

V_d is the **measure of the extent of drug distribution** and is expressed in **litres**. For evaluating the V_d of a drug in a best and simplest way, it is administered by rapid I.V. injection and the following equation is used:

$$V_d = \frac{X_o}{C_o} = \frac{\text{I.V. bolus dose}}{C_o} \quad \text{..... (18)}$$

Equation (1 8) can be used for drugs following one -compartment kinetics because V_d can be estimated when distribution equilibrium is achieved between drug in plasma and drug in tissues. Such equilibrium is established for a drug that follows one -compartment k inetics. A more useful non -compartmental method that can be applied to many compartment models for estimating V_d is:

For drugs given as I.V. bolus:

$$V_{d(area)} = \frac{X_o}{K_E.AUC} \quad \text{..... (19)}$$

For drugs administered extravascularly (e.v.):

$$V_{d(area)} = \frac{F\ X_o}{K_E.AUC} \quad \text{..... (20)}$$

Where, X_o = Dose administered and F = Fraction of drug absorbed in the systemic circulation. F = 1, i.e., the drug achieves complete availability when the drug is administered via intravenous route.

5.3.2.4. Clearance (Cl_R)

Many difficulties occur when elimination rate constant and half -life are applied as pharmacokinetic parameters in an anatomical physiological context and as a measure of drug elimination mechanisms. Clearance is the most impor tant 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.

$$\text{Clearance} = \frac{\text{Rate of elimination}}{\text{Plasma drug concentration}} \quad \text{..... (21)}$$

Or, $$Cl = \frac{dX/dt}{C} \quad \text{..... (22)}$$

Clearance is the **theoretical volume of body fluid contain ing the drug (i.e., that fraction of apparent volume of distribution) from which the drug has been completely removed in a given period of time**.

5.3.2.5. Total Body Clearance (Cl_T)

Drug elimination from the body involves several processes that occur in kidneys, liver, lungs, and other eliminating organs. Clearance at an individual organ level is termed **organ clearance**. It can be estimated by dividing the elimination rate by each organ with the drug concentration presented to it. Thus,

$$\text{Renal Clearance } Cl_R = \frac{\text{Rate of elimination by kidney}}{C} \quad \text{..... (23)}$$

$$\text{Hepatic Clearance } Cl_H = \frac{\text{Rate of elimination by liver}}{C} \quad \text{.....(24)}$$

$$\text{Other Organ Clearance } Cl_{others} = \frac{\text{Rate of elimination by other organs}}{C} \quad \text{..... (25)}$$

Total body clearance (Cl_T or **total systemic clearance**) is an additive property of individual organ clearances. Hence,

$$Cl_T = Cl_R + Cl_H + Cl_{others} \quad \text{..... (26)}$$

5.3.2.6. Area Under Curve (AUC)

AUC can be determined by various methods. It can be accurately determined by collecting adequate num ber of blood samples. For first -order kinetics, blood collection up to 6 half -lives provides information about 98% of the process or reaction. The following methods are used for determining AUC:

1) **Planimeter:** It is an instrument used for me chanically measuring the area of plane figures drawn on rectilinear graph paper.
2) **Cut and Weigh Method:** In this method, the total area under the curve on rectilinear graph paper is cut and accurately weighed on an analytical balance. The obtained total weight is divided by the weight of unit area of the same paper.
3) **Trapezoidal Rule:** In this method, the plasma drug concentration-time curve is expressed as a series of straight lines, and thus AUC is divided into trapezoids. Area of each trapezoid can be easi ly calculated, and sum of all trapezoidal areas yield the true area under the curve (**figure 5.8**).

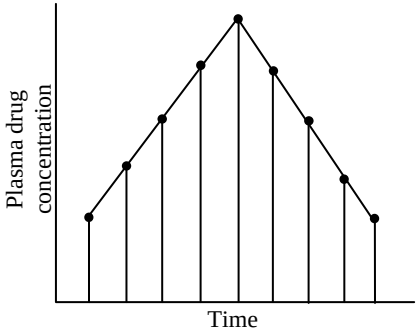


Figure 5.8: Graphical Representation for Linear Trapezoidal Method to Estimate

Let us assume that f(t) is a function that describes plasm a drug concentration - time curve, and θ(t) is a function that is linear between two su ccessive plasma drug level-time points.

The AUC expressed by θ(t) $\left(\int_{t_0}^{t_n}\theta(t)\cdot dt\right)$ will be approximately $\int_{t_0}^{t_n}(t)\cdot dt$

The integral $\int_{t_0}^{t_n}\theta(t)$ is expressed as the sum of total number of trapezoids into which the curve is divided (n).

$$\int_{t_0}^{t_n}\theta(t)\cdot dt=\int_{t_0}^{t_1}\theta(t)\cdot dt+\int_{t_0}^{t_2}\theta(t)\cdot dt...+\int_{t_{n-1}}^{t_n}(t)\cdot dt \quad .....(27)$$

Since it is an area of trapezoid:

$$\int_{t_0}^{t_1}\theta(t)\cdot dt=\frac{t_1-t_0}{2}(C_0+C_1) \quad .....(28)$$

Where, C_0 and C_1 = Plasma drug concentration at time, t_0 and t_1.

Similarly,

$$\int_{t_{n-1}}^{t_n}\theta(t)\cdot dt=\frac{t_n-t_{n-1}}{2}(C_{n-1}+C_n) \quad .....(29)$$

Based on equation s (28) and (29), equation (27) can be expressed as:

$$\int_{t_0}^{t_n}\theta(t)\cdot dt = \frac{t_1 - t_0}{2}(C_0 + C_1) + \frac{t_2 - t_1}{2}(C_1 + C_2) \ldots + \frac{t_n - t_{n-1}}{2}(C_{n-1} + C_n) \quad \ldots..(30)$$

Thus, $$\int_{t_0}^{t_n}\theta(t)\cdot dt = \sum_{i=0}^{n-1}\frac{t_{1+1} - t_i}{2}(C_i + C_{i+1}) \quad \ldots..(31)$$

The true representation of AUC depends on t he number of blood samples collected, i.e., more the number of blood samples collected better is the accuracy in determining AUC.

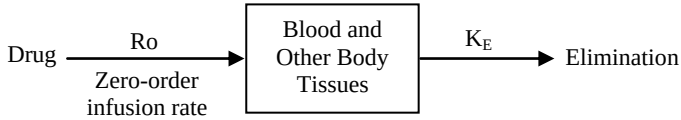
5.3.3. Intravenous Infusion

Rapid I.V. injection is not suitable if the dr ug precipitates toxicity or if maintenance of a stable concentration or amount of drug in the body is required. In such a case, the drug (**e.g.**, several antibiotics, theophylline, procainamide, etc.) is administered as I.V. infusion at a constant rate (zero -order). The duration of constant rate infusion is much longer than the half -life of the drug; this is contrary to the short duration of infusion of an I.V. bolus (few seconds).

Advantages of such a zero-order infusion of drugs are:

1) The rate of infusion can be easily controlled to fit individual patient's needs.
2) Fluctuation in maxima and minima (peak and valley) plasma level is prevented; this is desired when the drug has a narrow therapeutic index.
3) Other drugs, electrolytes and nutrients can be admin istered simultaneously by the same infusion line in critically ill patients.

The model can be represented as follows:



The rate of change in the amount of drug in the body (dX/dt) at any time during infusion, is the difference between the zero -order r ate of drug infusion (R_0) and the first-order rate of elimination ($-K_EX$):

$$\frac{dX}{dt} = R_o - K_E X \quad \ldots.. (32)$$

On integrating and rearranging **equation (32)**:

$$X = \frac{R_o}{K_E}(1 - e^{-K_E t}) \quad \ldots.. (33)$$

Since $X = V_d C$, **equation (33)** can be trans formed into concen tration terms as follows:

$$C = \frac{R_o}{K_E V_d}(1 - e^{-K_E t}) \quad \ldots.. (34)$$

$$C = \frac{R_o}{Cl_T}(1 - e^{-K_E t}) \quad \ldots.. (35)$$

Since the drug amount in body is zero at the beginning of constant rate infusion, no elimination occurs. With time, the amount of drug in body gradually increases (elimination rate < infusion rate) up to a point after which the plasma drug concentration reaches a constant value, i.e., **steady-state**, **plateau** or **infusion equilibrium** (elimination rate = infusion rate) (**figure 5.9**).

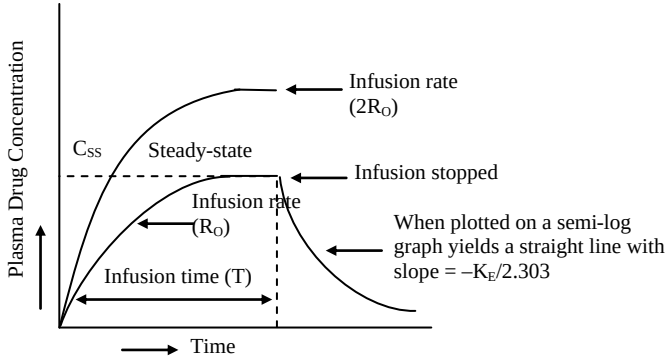


Figure 5.9: Plasma Concentration -Time Profile for a Drug given by Constant Rate I.V. Infusion (the two curves indicate different infusion rates R_o and $2R_o$ for the same drug)

At steady -state, the rate of change of amount of drug in the body is zero, so **equation (38)** becomes:

$$\text{Zero} = R_o - K_E X_{ss}$$

Or $$K_E X_{ss} = R_o \quad \text{..... (36)}$$

On transforming and rearranging **equation (36)** to concentration terms:

$$C_{ss} = \frac{R_o}{K_E V_d} = \frac{R_o}{Cl_T}, \text{ i.e., } \frac{\text{Infusion rate}}{\text{Clearance}} \quad \text{..... (37)}$$

Where, X_{SS} and C_{SS} = Amount of drug in the body and plasma drug concentration at steady-state, respectively.

The value of K_E (and $t_{1/2}$) can be obtained from the slope of straight line obtained from a semi-log plot (log C *versus* t) of plasma concentration-time data collected at the time when the drug is no more infused. Alternatively, K_E can be calculated from the data collected during infusion to steady-state.

On substituting $R_0/Cl_T = C_{SS}$ from **equation (37)** in **equation (35)**:

$$C = C_{SS}(1 - e^{-K_E t}) \quad \text{..... (38)}$$

On rearranging **equation (38)**:

$$\left[\frac{C_{SS} - C}{C_{SS}}\right] = e^{-K_E t} \quad \text{..... (39)}$$

On transforming **equation (39)** into log form:

$$\text{Log}\left[\frac{C_{SS} - C}{C_{SS}}\right] = \frac{-K_E t}{2.303} \quad \text{..... (40)}$$

A semi -log plot of $(C_{SS} - C)/C_{SS}$ *versus* t yields a straight line with slope of $-K_E/2.303$ (**figure 5.10**).

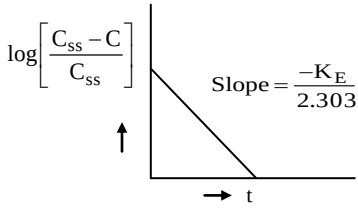


Figure 5.10: Semi-log Plot to Compute K_E from Infusion Data Up to Steady-State

The time to reach steady-state concentration depends on the elimination half-life. An increase in infusion rate will increase the plasma concentration attained at steady-state. If since the beginning of infusion ($t_{1/2}$), n is the number of half-lives passed, **equation (38)** can be written as:

$$C = C_{SS}\,[1 - (t_{1/2})^n] \qquad \text{..... (41)}$$

The percent of C_{SS} achieved at the end of each $t_{1/2}$ is the sum of C_{SS} at previous $t_{1/2}$ and the concentration of drug remaining after a given $t_{1/2}$.

Determination of Pharmacokinetic Parameters from Plasma Data after Intravenous Infusion

The first-order elimination rate constant and elimination half-life can be obtained from a semi-log plot of post-infusion concentration-time data. **Equation (40)** can also be used for this purpose. Apparent volume of distribution and total systemic clearance can be computed from steady-state concentration and infusion rate [**equation (36)**].

These two parameters can also be computed from the total area under the curve till the end of infusion:

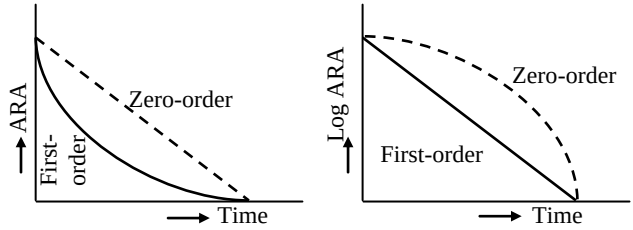
$$AUC = \frac{R_o T}{K_E V_d} = \frac{R_o T}{Cl_T} = C_{ss} T \qquad \text{….. (42)}$$

Where, T = Infusion time

5.3.4. Extravascular Administration

On administering a drug via extravascular route (oral, intramuscular, rectal, etc.), it should get absorbed to exert its therapeutic activity. Absorption rate can be mathematically described as a zero-order or first-order process. A large number of plasma concentration-time profiles can be described by one-compartment model with first-order absorption and elimination. Under certain conditions, absorption of some drugs is also described by zero-order (constant rate) kinetics. Differences between zero-order and first-order kinetics are shown in **figure 5.11**.

Zero-order absorption process has a constant absorption rate, and is independent of Amount Remaining to be Absorbed (ARA). Its regular ARA *versus* t plot is linear with slope equal to absorption rate while its semi-log plot shows an ever-increasing gradient with time. On the other hand, first-order absorption process shows a decline in the rate with ARA, i.e., absorption rate depend on ARA; its regular plot is curviline ar and semi-log plot is a straight line with slope equal to absorption rate constant.



a) Cartesian Plot **b) Semi-log Plot**

Figure 5.11: Distinction between Zero-Order and First-Order Absorption Processes. (a) Regular Plot and (b) Semi-log Plot of Amount of Drug Remaining to be Absorbed (ARA) *versus* Time (t)

After extravascular administration, the rate of change in the drug amount in 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 = Rate of absorption – Rate of elimination

$$\frac{dX}{dt} = \frac{dX_{ev}}{dt} - \frac{dX_E}{dt} \quad \text{..... (43)}$$

The plasma concentration -time profile of a drug following one -compartment kinetics shows the absorption phase, post -absorption phase, and elimination phase (**figure 5.12**).

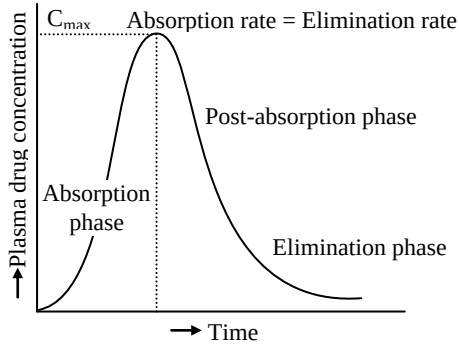


Figure 5.12: Absorption and Elimination Phases of Plasma Concentration-Time Profile Obtained After Extravascular Administration of a Single Dose of Drug

During the **absorption phase**, the absorption rate > the elimination rate.

$$\frac{dX_{ev}}{dt} > \frac{dX_E}{dt} \quad \text{..... (44)}$$

At **peak plasma concentration**, the absorption rate = the elimination r ate, and the change in amount of drug in the body is zero.

$$\frac{dX_{ev}}{dt} = \frac{dX_E}{dt} \quad \text{..... (45)}$$

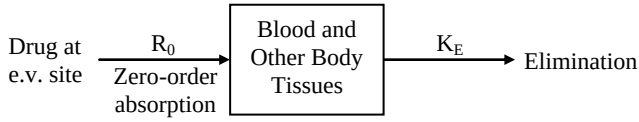
During the **post-absorption phase**, some drug still remains to be absorbed at the extravascular site, and the elimination rate > the absorption rate.

$$\frac{dX_{ev}}{dt} < \frac{dX_E}{dt} \quad \text{..... (46)}$$

After the **completion of drug absorption**, the absorption rate be comes zero and the plasma level-time curve shows only the elimination phase.

Zero-Order Absorption Model

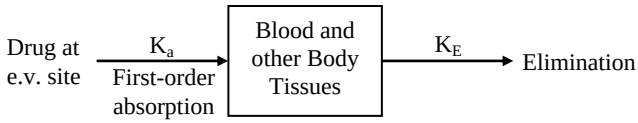
This model is similar to the model for constant rate infusion.



As in the case of several controlled drug delivery systems, the drug absorption rate is constant and continues till the amount of drug at the absorption site (**e.g.,** GIT) gets depleted. The equations explaining the plasma concen tration-time profile for constant rate intravenous infusion are also applicable to this model.

First-Order Absorption Model

A drug that enters the body by first -order absorption process gets distributed according to one-compartment kinetics and gets eliminated by first-order process. This model is depicted as follows:



The differential form of the **equation (43)** is:

$$\frac{dX}{dt} = K_a X_a - K_E X \quad \text{..... (47)}$$

Where,

K_a = First-order absorption rate constant

X_a = Amount of drug at the absorption site remaining to be absorbed (i.e., ARA).

On integrating **equation (47)**:

$$X = \frac{K_a F X_o}{(K_a - K_E)}\left[e^{-K_E t} - e^{-K_a t}\right] \quad \text{..... (48)}$$

On transforming **equation (48)** into concentration terms:

$$C = \frac{K_a F X_o}{V_d (K_a - K_E)}\left[e^{-K_E t} - e^{-K_a t}\right] \quad \text{..... (49)}$$

Where, F = Fraction of drug absorbed systemically after e.v. administration.

5.3.4.1. Determination of Pharmacokinetic Parameters - C_{max} and t_{max}

At peak plasma concentration, th e absorption rate becomes equal to the elimination rate, i.e., $K_a X_a = K_E X$, and the rate of change in plasma drug concentration (dC/dt) becomes zero. This rate can be obtained by differentiating **equation (49)**.

$$\frac{dC}{dt} = \frac{K_a F X_0}{V_d (K_a - K_E)}\left[-K_E e^{-K_E t} + K_a e^{-K_a t}\right] = \text{zero} \quad \text{..... (50)}$$

On simplifying **equation (50)**:

$$K_E e^{-K_E t} = K_a e^{t^{-K_a t}} \quad \text{..... (51)}$$

On converting **equation (51)** to logarithmic form:

$$\log K_E - \frac{K_E t}{2.303} = \log K_a - \frac{K_a t}{2.303} \quad \text{..... (52)}$$

Where, $t = t_{max}$

On rearranging **equation (52)**:

$$t_{max} = \frac{2.303 \log (K_a / K_E)}{K_a - K_E} \quad \text{..... (53)}$$

Equation (53) shows that as K_a becomes greater than K_E, t_{max} becomes smaller since $(K_a - K_E)$ increases faster than $\log K_a/K_E$.

By substituting **equation (53)** in **equation (49)**, the value of C_{max} can be obtained. However, this can be expressed in a simpler way as follows:

$$C_{max} = \frac{F X_o}{V_d} e^{-K_E t_{max}} \quad \text{..... (54)}$$

It is shown that at C_{max}, when $K_a = K_E$, $t_{max} = 1/K_E$. Hence, **equation (54)** reduces to:

$$C_{max} = \frac{F X_o}{V_d} e^{-1} = \frac{0.37 F X_o}{V_d} \quad \text{..... (55)}$$

Since, FX_o/V_d represents C_o after I.V. bolus, the maximum plasma concentration that can be attained after extravascular administration is 37% of the maximum level that can be attained with I.V. bolus in the same dose. If bioavailability is <100%, lower concentration will be attained.

5.3.4.2. Elimination Rate Constant

Elimination rate constant can be obtained from the elimination phase of the plasma level-time profile. For the drugs administered via extravascular route, the absorption rate is much greater than the elimination rate ($K_a t >> K_E t$). Hence, $e^{-Ka}t$ approaches zero faster than $e^{-KE}t$. At this stage absorption completes, and the change in plasma drug concentration depends only on elimination rate and **equation (49)** reduces to:

$$C = \frac{K_a F X_0}{V_d (K_a - K_E)} e^{-K_E t} \quad \text{.....(56)}$$

On transforming **equation (56)** into log form:

$$\log C = \frac{K_a F X_0}{V_d (K_a - K_E)} - \frac{K_E t}{2.303} \quad \text{.....(57)}$$

A plot of log C *versus* t yields a straight line with slope $-K_E/2.303$ (half-life can be computed from K_E). Elimination rate constant can also be deduced from urinary excretion data.

5.3.4.3. Absorption Rate Constant (K_a)

Absorption rate constant can be computed by the **method of residuals** (or **feathering**, **peeling**, and **stripping**). This method is used in pharmacokinetics to resolve a multi-exponential curve into its individual components. For a drug

that follows one -compartment kinetics and is administered via extravascular route, the plasma drug concentration is expressed by a bi -exponential **equation (58)**.

$$C = \frac{K_a FX_0}{V_d (K_a - K_E)} \left[e^{-K_E t} - e^{-K_a t} \right] \quad \text{.....(58)}$$

If $K_aFX_0/V_d(K_a–K_E) = A$, a hybrid constant:

$$C = A e^{-K_E t} - A e^{-K_a t} \quad \text{.....(59)}$$

During the elimination phase, when absorption is almost over, $K_a >> K_E$ and the value of second exponential $e^{-K_a t}$, approaches zero; whereas the first exponential $e^{-K_a t}$, approaches a finite value. Hence, **equation (59)** reduces to:

$$\overleftarrow{C} = A e^{-K_E t} \quad \text{.....(60)}$$

On taking logarithm of **equation (60)**:

$$\log \overleftarrow{C} = \log A - \frac{K_E t}{2.303} \quad \text{.....(61)}$$

Where, $\overleftarrow{C}$ = the back extrapolated plasma concentration value. A plot of log C *versus* t yields a bi -exponential curve having a terminal linear phase with slope $–K_E/2.303$ (**figure 5.13**). Back extrapolation of the straight line to time zero yields y-intercept log A.

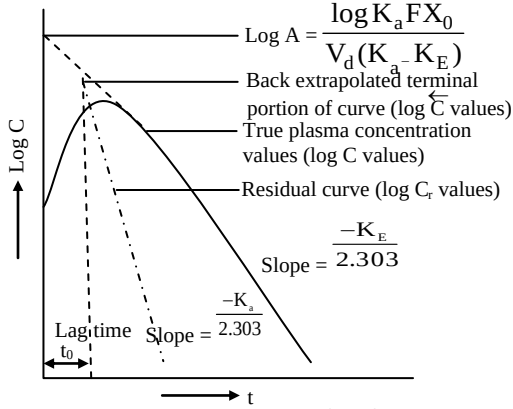


Figure 5.13: Plasma Concentration-time Profile after Oral Administration of a Single Dose of a Drug. The Biexponential Curve has been Resolved into its Two Components, i.e., Absorption and Elimination

By deducting true plasma concentration values, i.e., **equation (59)** from the extrapolated plasma concentration values, i.e., **equation (60)**, a series of residual concentration values (C_τ) is obtained:

$$(\overleftarrow{C} - C) = C\tau = A e^{-Kat} \quad \text{.....(62)}$$

On taking logarithm of **equation (62)**:

$$\log C\tau = \log A - \frac{K_a t}{2.303} \quad \text{.....(63)}$$

A plot of log C_τ *versus* t yields a straight line with slope $-K_a/2.303$ and y-intercept log A (**figure 5.13**). Absorption half-life can be obtained from K_a using the relation $0.693/K_a$. Thus, the method of residuals allows resolution of the bi-exponential plasma level-time curve into its two exponential components. This method gives best results if the difference between K_a and K_E is large ($K_a/K_E \geq 3$). In some cases, K_E obtained after I.V. bolus of the same drug is much larger than the K_a obtained by the method of residuals (**e.g.,** isoprenaline). If $K_E/K_a \geq 3$, the terminal slope gives the value of K_a (and not K_E), while the slope of residual line gives the value of K_E (and not K_a). This is called **flip-flop phenomenon**, since the slopes of the two lines exchange their meanings.

Ideally, the extrapolated and the residual lines intersect each other on y-axis, i.e., at time t = zero, and there is no lag in absorption. However, if such an intersection occurs at time > zero, it indicates **time lag**, which is the time difference between drug administration and start of absorption. It is denoted by t_0 and represents the beginning of absorption.

The above method for K_a estimation is a **curve-fitting method** that is suitable for drugs that get rapidly and completely absorbed and follow one-compartment kinetics even when given intravenously. However, if the drug absorption is affected by gastrointestinal motility or enzymatic degradation, and if the drug shows multi-compartment characteristics after intravenous administration (which is true for virtually all drugs), the value of K_a computed by curve-fitting method is invalid even if the drug has undergone absorption by first-order kinetics. The so obtained K_a is the best estimate of first-order disappearance of drug from the GIT instead of the first-order appearance in the systemic circulation.

Wagner-Nelson Method for Estimation of K_a

In the method of residuals, absorption process is assumed to be of the first-order kinetics. This assumption is valid for dosage forms in which absorption process is the rate-determining step, i.e., in solutions and rapidly dissolving dosage forms. In cases where drug release from the dosage form is the rate-limiting step, the absorption process follows zero-order, mixed zero, and first-order kinetics, or even more complex processes.

Assumptions

The Wagner-Nelson method of calculation does not require a model assumption concerning the absorption process. It does require the assumption that:

1) The body behaves as a single homogeneous compartment, and
2) The elimination process of drug follows first-order kinetics.

Wagner-Nelson method is a better alternative to curve-fitting method in the estimation of K_a. It involves determination of K_a from % unabsorbed-time plots and does not require the assumption of zero- or first-order absorption kinetics. When a single dose of drug is administered via oral route, at any given time, the amount of drug absorbed into the systemic circulation (X_A) is the sum of the amount of drug in body (X) and the amount of drug eliminated from the body (X_E). Thus:

$$X_A = X + X_E \quad \text{..... (64)}$$

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:

$$X_E = K_E V_d [AUC]_o^t \quad \text{..... (65)}$$

On substituting the values of X and X_E in **equation (64)**:

$$X_A = V_d C + K_E V_d [AUC]_o^t \quad \text{..... (66)}$$

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

$$X_A^\infty = V_d C^\infty + K_E V_d [AUC]_o^\infty \quad \text{..... (67)}$$

Since at $t = \infty$, $C^\infty = 0$, **equation (67)** reduces to:

$$X_A^\infty = K_E V_d [AUC]_o^\infty \quad \text{..... (68)}$$

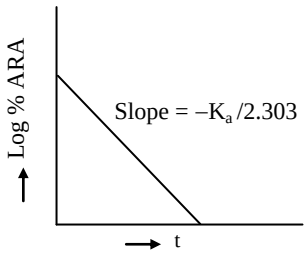


Figure 5.14: Semi-log Plot of %ARA *versus* t according to Wagner-Nelson Method

The fraction of drug absorbed at time t is given as:

$$\frac{X_A}{X_A^\infty} = \frac{V_d C + K_E V_d [A \cup C]_o^t}{K_E V_d [A \cup C]_o^\infty} = \frac{C + K_E [A \cup C]_o^t}{K_E [A \cup C]_o^\infty} \quad \text{..... (69)}$$

% drug unabsorbed at any time is:

$$\%ARA = \left[1 - \frac{X_A}{X_A^\infty}\right] 100 = \left[1 - \frac{C + K_E [A \cup C]_o^t}{K_E [A \cup C]_o^\infty}\right] 100 \quad \text{..... (70)}$$

The Wagner-Nelson method involves collecting blood samples after a single oral dose at regular intervals of time till the entire amount of drug is eliminated from the body. The value of K_E is obtained from a plot of log C *versus* t and $[AUC]_o^t$ and $[AUC]_o^\infty$ are obtained from a plot of C *versus* t. A semi-log plot of % unabsorbed (i.e., %ARA) *versus* t yields a straight line with slope $–K_a/2.303$ (**figure 5.14**). If a regular plot of the same is a straight line, the absorption is assumed to be following zero-order kinetics.

Advantages of Wagner-Nelson Method

1) Absorption and elimination processes give accurate determinations of K_a.
2) Absorption process does not have to follow first-order kinetics.
3) The method can be used for investigating the absorption process.

Disadvantages of Wagner-Nelson Method

1) The method is only applicable to drugs having one-compartment characteristics.
2) Problem arises when a drug that obeys one-compartment model after extravascular administration shows multicompartment characteristics after intravenous injection.

Loo–Riegelman Method for Estimation of K_a

Loo-Reigelman method is used for determining the absorption rate constant (K_a) from plasma concentration -time profile of a drug that obeys two -compartment model. This method utilises the % drug unabsorbed *versus* time plot. The amount of drug (that obeys two-compartment model) absorbed after oral administration is the sum of the amount of drug in the central compartment (X_c), tissue compartment (X_t), and the amount of drug eliminated:

$$Ab = X_c + X_t + X_3 \quad \text{..... (71)}$$

The terms in **equation (71)** can also be expressed as:

$$X_c = V_c.C \quad \text{..... (72)}$$

$$X_t = V_t.C_t \quad \text{..... (73)}$$

$$X_3 = V_c.K_{13}\int_0^t C.dt = V_cK_{13}[AUC]_0^t \quad \text{..... (74)}$$

On substituting the values of X_c and X_3 in **equation (71)**:

$$Ab = V_c.C + X_t + V_cK_{13}[AUC]_0^t \quad \text{..... (75)}$$

On dividing **equation (75)** by V_c:

$$\frac{Ab}{V_c} = C + \frac{X_t}{V_c} + K_{13}[AUC]_0^t \quad \text{..... (76)}$$

On putting $t = \alpha$, **equation (76)** becomes:

$$\frac{Ab^\alpha}{V_c} = 0 + 0 + K_{13}[AUC]_0^t = K_{13}[AUC]_0^\alpha \quad \text{..... (77)}$$

Where, Ab^α = Amount of drug absorbed from the dosage form. The r atio of Ab^α to the dose is the fraction of the dose absorbed (F).

$$F = Ab^\alpha/X_0 \quad \text{..... (78)}$$

The fraction of dose absorbed at any time in comparison to Ab^α can be obtained by dividing **equation (76)** by **equation (77)**:

$$\frac{Ab}{Ab^\alpha} = \frac{C + X_t/V_c + K_{13}[AUC]_0^t}{K_{13}[AUC]_0^\alpha} \quad \text{..... (79)}$$

Or, $$\frac{Ab}{Ab^\alpha} = \frac{C + X_t/V_c + K_{13}[AUC]_0^t}{K_{13}[AUC]_0^\alpha} \quad \text{..... (80)}$$

Where, $C_t = X_t/V_c$ = Apparent tissue concentration.

On plotting the fraction of dose unabsorbed [1 – (Ab/Ab^α)] against time, a straight line with slope $-K_\alpha/2.303$ is obtained. The value of absorption rate constant can be obtained from the slope (**figure 5.15**). The C and $K[AUC]_0^t$ values can be obtained from the plot of plasma drug level *versus* time. The values for C_t can be computed by the Loo-Riegelman method using equation:

$$(C_t)_{tn} = K_{12}\Delta C_{\Delta t} + \frac{K_{12}}{K_{21}}(C)t_{n-1}\left(1 - e^{-K_{21}\Delta t}\right) + (C_t)t_{n-1}\, e^{-K_{21}\Delta t} \quad \text{..... (81)}$$

Where,

C_t = Apparent tissue concentration

t_n = Time of sampling for sample n

t_{n-1} = Time of sampling for the sampling point proceeding sample n

$(C)t_{n-1}$ = Concentration of drug at central compartment for sample n –1

ΔC = Concentration difference at central compartment between two sampling times

Δt = Time difference between two sampling times

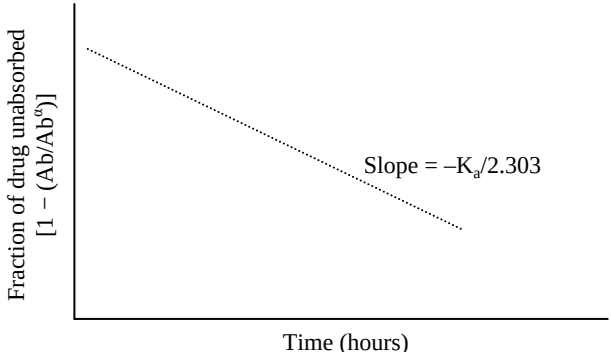


Figure 5.15: Plot of the Fraction of Drug Unabsorbed [1 – (Ab/Ab$^{\infty}$)] *versus* Time used to determine the Absorption Rate Constant by Loo-Riegelman Method for a Drug that Follows a Two-Compartment Model

For example, plasma is sampled at times t = 0 and 0.5 hours, and corresponding concentrations of the drug in the central compartment are 0 and 2μg/ml.

Then, $(C_t)_{tn}$ = 2μg/ml and $(C)_{tn-1}$ = 0, ΔC = 2μg/ml, Δt = 0.5 hours

If the drug is administered via intravenous route for estimating the distribution and elimination rate constants, only then the absorption rate can be estimated by Loo-Riegelman method. For drugs that cannot be given intravenously, K_a cannot be calculated by the Loo-Riegelman method.

Advantage of Loo Riegelman

The method has no limitation on the order of the absorption process.

Disadvantage of Loo Riegelman

The method requires the plasma concentration -time data after intravenous bolus and oral administration to obtain all the necessary kinetic constants.

5.3.5. Methods of Elimination

If plasma level-time data is not availabl e, necessary information can be obtained from urine data regarding elimination kinetics of a drug. The **urine excretion method has some advantages** over other methods in the analysis of a pharmacokinetic system:

1) This method is used when no accurate analytica l techniques are available for accurate measurement of plasma drug concentration.
2) Urine samples can be conveniently collected while withdrawing blood samples periodically cannot be conveniently collected.

3) Urine drug concentration can be determined using a less sensitive analytical method, while plasma drug concentration is determined using a highly sensitive analytical technique. If urine drug concentrations are low, large urine drug samples are readily assayed.
4) First order elimination, excretion and absorption rate constants and fraction excreted unchanged are calculated from the data obtained from urine drug analysis. This data also helps in determining the first-order metabolism or extra-renal excretion rate constant from the difference, $K_E - K_e = K_M$.
5) Urine drug data can be used for measuring absolute and relative bioavailability without fitting the data to a complex mathematical model.
6) This method is non-invasive, therefore, assures better compliance.
7) When urine drug-time data is linked with plasma level-time data, renal clearance of unchanged drug can be deduced from the following relation:

$$Cl_R = \frac{\text{Total amount of drug which gets excreted unchanged}}{\text{Area under the plasma level-time curve}}$$

Total systemic clearance and non-renal clearance can also be estimated if V_d is known.

Disadvantages of Urine Method

1) V_d and Cl_T cannot be calculated only using urine data.
2) The method is less accurate than the plasma drug method because it gives only a rough estimate of pharmacokinetic parameters.
3) If the drug release is very slow, the resulting urinary drug concentration will be too low to be assessed with accuracy.
4) If the drug has a very long biological half-life, urine samples should be collected for several days till the entire drug has excreted.

Criteria for Getting Valid Urinary Excretion Data

1) At least 10% of the drug administered should be excreted unchanged in the urine.
2) A specific analytic method should be used for determining unchanged drugs. Further, metabolites should not interfere in this method.
3) After fasting overnight, the patient should administer 400ml water to promote diuresis so that sufficient urine samples can be collected.
4) After administering 400ml water, the patient's bladder should be completely emptied after an hour and the urine sample is collected as a blank. The drug should be first administered with 200ml water, followed by 200ml at hourly intervals for the next 4 hours.
5) While collecting urine samples, the patients are advised to empty their bladders completely.
6) Frequent sample should be collected to get a good curve.
7) While sampling, the exact time and volume of urine excreted should be recorded.
8) The individual collection period should be less than the biological half-life of the drug.
9) To collect more than 99% of the excreted drug, urine samples should be collected for at least 7 half-lives of the drug.
10) Urine excretion rate is also influenced by changes in urine pH and urine volume.

5.3.5.1. Determination of Pharmacokinetic Parameters from Urine Data after Intravenous Bolus Administration

The urine data is shown in **table 5.1**:

Table 5.1: Urinary Excretion Data Following I.V. Bolus of 100mg of a Drug

Observation				Treatment of Data					
Sample	Time of Urine Collection (t) (Hours)	Volume of Urine Collected (ml)	Concentration of Unchanged Drug in Urine (mcg/ml)	Urine Collection Interval dt (or Δt)	Mid-Point of Urine Collection (t*)	Amount Excreted in Time Interval (mg) dXu (or ΔXu)	Excretion Rate (mg/H) dXu/dt	Cumulative Amount Excreted (mg) Xu^t	Amount Remaining to be Excreted $Xu^\infty - Xu^t$
0	0	–	–	–	–	–	–	0	66.7
1	0-2	140	250	2	1	35.0	17.5	35.0	31.7
2	2-4	150	100	2	3	15.0	7.5	50.0	16.7
3	4-6	90	80	2	5	7.2	3.6	57.2	9.5
4	6-8	200	20	2	7	4.0	2.0	61.2	5.5
5	8-12	310	10	4	10	3.1	0.8	64.3	2.4
6	12-24	600	04	12	18	2.4	0.2	66.7 ↓ Xu^∞	–

From ur inary excretion data, the first -order elimination (and excretion) rate constants can be determined by using the following two methods:

Rate of Excretion Method

In the rate of excretion metho d, the rate of urinary drug excretion (dX_u/dt) is directly proportional to the amount of drug in the body (X):

$$\frac{dX_u}{dt} \propto X$$

Or, $$\frac{dX_u}{dt} = K_e X \quad \text{..... (82)}$$

Where, K_e = first -order urinary excretion rate constant (a proportionality constant). According to the first-order disposition kinetics:

$$X = X_O e^{-K_E t} \quad \text{..... (83)}$$

On substituting **equation (83)** in **equation (82)**:

$$\frac{dX_u}{dt} = K_e X_O e^{-K_E t} \quad \text{..... (84)}$$

Where, X_O = Dose administered (intravenous bolus).

On taking logarithm of **equation (84)**:

$$\log \frac{dX_u}{dt} = \log K_e X_O - K_E t \log e$$

But, $\log e = \log 2.718 = 0.4343 = \frac{1}{2.303}$

$$\therefore \quad \log \frac{dX_u}{dt} = \log K_e X_O - \frac{K_E t}{2.303} \quad \text{..... (85)}$$

Equation (85) is used to draw a semi -log plot of rate of excretion (log dX_u/dt) *versus* time (t) that gives a straight line with slope $-K_E/2.303$ **(figure 5.16)**. The slope of such an excretion rate *versus* time plot is re lated to elimination rate constant (K_E), and not to excretion rate constant (K_e). However, the excretion rate constant can be obtained from the intercept on y -axis (log $K_e X_o$). From K_E and K_e, the elimination half -life and non -renal elimination rate consta nts can be computed.

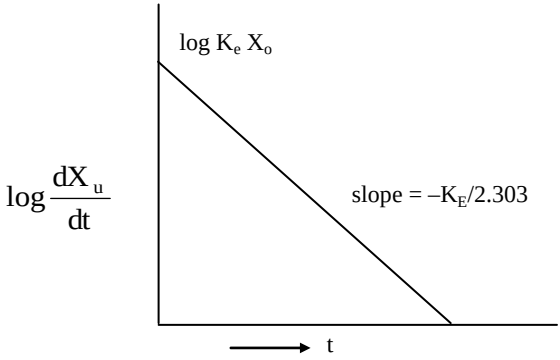


Figure 5.16: Semi-log Plot of Excretion Rate versus Time of Urine Collection Period for Computing Elimination Rate Constant after I.V. Bolus Administration

This method has an **advantage** that it can be used for drugs with long half -lives. For such drugs, urine samples are collected only for 3 -4 half -lives. Another **advantage** is that it is not essential to collect all the urine samples because collection of any two urine samples at two times yield points on the rate plot through which a straight line can be constructed.

The **disadvantage** of this method is that while estimating K_E a high degree of fluctuation occurs in the drug e limination rate and the data obtained are so scattered that estimation of drug half -life becomes difficult. These problems, however, can be overcome by **sigma-minus method**.

Sigma-Minus Method

In sigma-minus method, from **equation (84)**:

$$\frac{dX_u}{dt} = K_e X_O e^{-K_E t} \qquad \ldots\ldots(86)$$

Or, $$\frac{dX_u}{dt} = K_e X_O e^{-K_E t} dt$$

On integrating **equation (86)**:

$$\int dX_u = K_e X_O \int e^{-K_E t} dt$$

$$X_u = K_e X_O \frac{e^{-K_E t}}{-K_E} + I \qquad \ldots\ldots (87)$$

Where, I = Integration constant whose value is obtained from initial conditions , i.e., t = 0, $X_u = 0$.

Thus, **equation (87)** becomes:

$$0 = K_e X_O \frac{e^o}{-K_E} + I$$

Or, $I = \frac{K_e X_O}{K_E} \quad [\because e^0 = 1]$ ..... (88)

On substituting **equation (88)** in **equation (87)**:

$$X_u \; K_e X_O \frac{e^{-K_E t}}{-K_E} + \frac{K_e X_O}{K_E}$$

$$X_u = \frac{K_e X_O}{K_E}(1 - e^{-K_E t}) \quad \text{..... (89)}$$

In **equation (89)**, X_u = cumulative amount of drug excreted unchanged in urine at any time (t). The time, after 6 -7 half -lives, approaches infinity and $e^{-K_E t}$ becomes $e^{-K_E \infty}$ that further becomes zero, and hence the cumulative amou nt excreted at infinity time ($X_u^{\propto}$) can be given by the equation:

$$X_u^{\propto} = \frac{K_e X_O}{K_E} \quad \text{..... (90)}$$

On substituting **equation (90)** in **equation (89)**:

$$X_u - X_u^{\propto}(1 - e^{-K_E t})$$

$$X_u = X_u^{\propto} - X_u^{\propto} \; e^{-K_E t}$$

Or, $X_u^{\propto} - X_u = X_u^{\propto} \; e^{-K_E t}$ ..... (91)

On taking logarithm of **equation (91)**:

$$\log(X_u^{\propto} - X_u) = \log X_u^{\propto} - \frac{K_E t}{2.303} \quad \text{..... (92)}$$

Where, $X_u^{\infty} - X_u$ = ARE at any given time = Amount remaining to be excreted at the given time.

By using **equation (92)**, K_E can be calculated from the urinary excretion data; but this data is applicable to a drug that follows one -compartment model and is administered as I.V. bolus.

5.3.5.2. Determination of Pharmacokinetic Parameters from Urine Data after Intravenous Infusion

The data obta ined during constant rate I.V. infusion is used for determining the elimination rate constant. The equation describing the urinary excretion rate of unchanged drug, when administered as I.V. bolus, is also applicable when it is administered at constant rate as I.V. infusion. Thus:

$$\frac{dX_u}{dt} = K_e X \quad \text{..... (93)}$$

If a drug is given as I.V. infusion, the amount of drug in the body (X) is given as:

$$X = \frac{R_0}{K_E}(1 - e^{-K_E t}) \quad \text{..... (94)}$$

On substituting **equation (94)** in **equation (93)**:

$$\frac{dX_u}{dt} = \frac{K_e R_0}{K_E}(1 - e^{-K_E t}) \quad \text{..... (95)}$$

On integrating **equation (95)**:

$$X_u = \frac{K_e R_0 t}{K_E} - \frac{K_e R_0}{K_E^2}(1 - e^{-K_E t}) \quad \text{..... (96)}$$

If a drug is infused for a long period to attain steady -state in the plasma, the term $e^{-K_E t}$ approaches zero and **equation (96)** becomes:

$$X_u = \frac{K_e R_0 t}{K_E} - \frac{K_e R_0}{K_E^2} \quad \text{..... (97)}$$

On plotting the cumulative amount of drug excreted (X_u) against time (t), a curvilinear plot is obtained (**figure 5.17**). The linear portion of this plot has a slope $K_e R_0/K_E$. On extrapolatin g the linear portion to time axis, x -intercept is obtained that is equal to $1/K_E$ because when $X_u = 0$, $t = 1/K_E$.

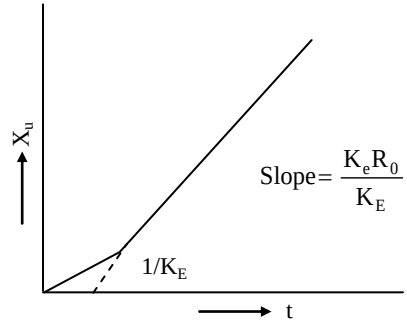


Figure 5.17: Regular Plot of X_u *versus* t during Constant Rate I.V. Infusion

5.3.5.3. Determination of Pharmacokinetic Parameters from Urine Data after Extravascular Administration

The equation for rate of excretion when the drug is administered via extravascular route can be written as:

$$\frac{dX_u}{dt} = K_e X \quad \text{..... (98)}$$

If a drug is administered intr avenously and absorbed by first -order process, X is given as:

$$X = \frac{K_a F X_O}{(K_a - K_E)}\left[e^{-K_E t} - e^{-K_a t}\right] \quad \text{..... (99)}$$

On substituting **equation (99)** in **equation (98)**:

$$\frac{dX_u}{dt} = \frac{K_e K_a F X_O}{(K_a - K_E)}\left[e^{-K_E t} - e^{-K_a t}\right] \quad \text{…..(100)}$$

On integrating **equation (100)**:

$$X_u = \frac{K_e K_a F X_O}{K_E}\left[\frac{1}{K_a} + \frac{e^{-K_E t}}{(K_E - K_a)} - \frac{K_E e^{-K_a t}}{K_a (K_E - K_a)}\right] \quad \text{..... (101)}$$

At t = ∞, **equation (101)** becomes:

$$X_u^{\infty} = \frac{K_e F X_O}{K_E} \quad \text{..... (102)}$$

On substituting **equation (102)** in **equation (101)**:

$$\text{ARE} = (X_u^{\infty} - X_O) = \frac{X_u^{\infty}}{(K_a - K_E)} = \left(K_a e^{-K_E t} - K_E e^{-K_a t}\right) \quad \text{..... (103)}$$

A semi-log graph of $X_u^{\infty} - X_O$ *versus* time (t) yields a bi-exponential curve. If $K_a > K_E$, the slope of the terminal linear portion of the curve gives the value of K_E for the drug. **Equation (104)** is also used to estimate the absorption rate constant (K_a) by the method of residuals.

The urinary excretion data obtained after administering a drug orally can be analysed by the Wagner-Nelson method to evaluate K_a by drawing %ARA plots. In this method, urine samples are collected for sufficient time intervals to ensure accurate estimation of K_E (but should not be collected to time infinity). The equation obtained relating % ARA with urine excretion rate is as follows:

$$\%\,\text{ARA} = \left[1 = \frac{X_A}{X_u^{\infty}}\right]100 = \left[1 - \frac{dX_u/dt + K_E X_u}{K_E X_u^{\infty}}\right]100 \quad \text{..... (104)}$$

A semi-log plot of %ARA *versus* t yields a straight line with slope $-K_a/2.303$.

Accurate values of K_a are obtained from urine excretion data of drugs having slow rate of absorption. Collection of urine samples at very short intervals of time is difficult for drugs having rapid absorption rate.

5.4. SUMMARY

The details given in the chapter can be summarised as follows:

1) **Pharmacokinetics** is the study of rate processes involved in absorption, distribution, metabolism, and excretion of a drug.
2) The word pharmacokinetic has been originated from the Greek words ***pharmakon*** which means drug and ***kinesis*** which means motion or change of rate.
3) **Absorption** is the process of movement of unchanged drug from the site of administration to systemic circulation or to the site of measurement (i.e., plasma).
4) **Distribution** is the reversible transfer of a drug between the blood and the extravascular fluids and tissues.
5) **Elimination** is the major process of drug removal from the body and termination of its action. It is defined as the irreversible loss of drug from the body.

6) **Metabolism** (or biotransformation) of drugs is the chemical conversion of one form to another.
7) **Excretion** is the process by which drugs and/or their metabolites are irreversibly transferred from internal to external environment.
8) The most traditional and common approach used for pharmacokinetic characterisation of a drug is **compartment analysis**.
9) In **mammillary model**, one or more peripheral compartments are connected to a central compartment, consisting of plasma and highly perfused tissues in which the drug undergoes rapid distribution.
10) In **catenary model**, the compartments are joined to each other in a chain or series
11) In **non-compartmental analysis**, assumption of specific compartment model is not required; therefore it is also called as the model-independent method.
12) **MRT** is the average amount of ti me a drug spends in the body before being eliminated.
13) The values of AUMC and AUC can be calculated from their respective graphs using the trapezoidal rule in which the curve is divided by a series of vertical lines into a number of trapezoids, followed by calculating the area of each trapezoid separately and then adding them together.
14) The total area under the curve from time zero to infinity (AUC_0^∞) is the zero moment of a drug concentration in plasma *versus* time curve.
15) The total area under the curve obtai ned from a plot of drug concentration *versus* time is the zero moment of a drug concentration in a plasma -time profile.
16) The total area under the curve obtained from a plot of product of drug concentration and time *versus* time ($AUMC_0^\infty$) is the first moment of a plasma concentration-time profile.
17) **Physiological pharmacokinetic models** (or blood flow or perfusion models) rely on known anatomic and physiological data.
18) **Blood flow rate-limited models** rely on the assumption that drug movement in a body region is more rapid than its delivery rate to that region by the perfusing blood.
19) **Membrane permeation rate -limited models** are used for highly polar, ionised and charged drugs.
20) **One-compartment open model** is the simplest model that de scribes the body as a single, kinetically homogeneous unit having no barriers to drug movement.
21) A drug that follows one -compartment kinetics and is administered as a rapid I.V. injection experiences decline in plasma drug concentration due to elimination of drug from the body (and not due to distribution); this phase is called as **elimination phase**.
22) **Elimination half-life** (or biological half-life) is the time taken for the amount of drug in the body and the concentration of drug in plasma to decline by one-half or 50% of its initial value. It is expressed in **hours** or **minutes**.

23) V_d is the measure of the extent of drug distribution and is expressed in litres.
24) **Clearance** is the theoretical volume of body fluid containing the drug (i.e., that fraction of apparent volume of distribution) from which the drug has been completely removed in a given period of time.
25) Clearance at an individual organ level is termed **organ clearance**.
26) **Planimeter** is an instrument used for mechanically measuring the area of plane figures drawn on rectilinear graph paper.
27) **Absorption rate constant** can be computed by the method of residuals (or feathering, peeling, and stripping).
28) **Wagner-Nelson method** involves determination of K_a from % unabsorbed-time plots and does not require the assumption of zero- or first-order absorption kinetics.
29) **Loo-Reigelman method** is used for determining the absorption rate constant (K_a) from plasma concentration-time profile of a drug that obeys two-compartment model.
30) In the **rate of excretion method**, the rate of urinary drug excretion (dX_u/dt) is directly proportional to the amount of drug in the body (X).

5.5. EXERCISE

5.5.1. True or False

1) The most traditional and common approach used for pharmacokinetic characterisation of a drug is non-compartment analysis.
2) In mammillary model, the compartments are joined to each other in a chain or series
3) The total area under the curve from time zero to infinity is the zero moment of a drug concentration in plasma *versus* time curve.
4) The total area under the curve obtained from a plot of product of drug concentration and time *versus* time is the first moment of a plasma concentration-time profile.
5) Membrane permeation models rely on known anatomic and physiological data.
6) V_d is the measure of the extent of drug distribution and is expressed in hours.

5.5.2. Fill in the Blanks

7) The word pharmacokinetic has been originated from the Greek word _____ and _____ .
8) Non-compartmental analysis is also called as the _____ .
9) _____ are used for highly polar, ionised and charged drugs.
10) _____ is an instrument used for mechanically measuring the area of plane figures drawn on rectilinear graph paper.
11) _____ can be computed by the method of residuals.
12) In the _____, the rate of urinary drug excretion is directly proportional to the amount of drug in the body.

Answers

1) False 2) False 3) True
4) True 5) False 6) False
7) *pharmakon* and *kinesis* 8) Model-independent method
9) Membrane permeation rate-limited models 10) Planimeter
11) Absorption rate constant 12) Rate of excretion method

5.5.3. Very Short Answer Type Questions

1) Define pharmacokinetics.
2) What are the applications of pharmacokinetics?
3) Give the advantages of pharmacokinetic models.
4) What are catenary model?
5) What is trapezoidal rule?
6) Give the advantages and disadvantages of non-compartment models.
7) Give the types of physiological models.
8) What is elimination half-life?
9) Give the advantages and disadvantages of Wagner Nelson method.
10) Define clearance.
11) What is flip-flop phenomenon?
12) Give the criteria for getting valid urinary excretion data.

5.5.4. Short Answer Type Questions

1) Discuss the applications of pharmacokinetics.
2) Write about the types of compartment models.
3) Give the statistical moment theory.
4) Explain the physiological models.
5) Write in detail the calculation of elimination rate constant, elimination half-life, AUC, and clearance for one compartment open model for IV bolus.
6) Give the method of residuals.
7) Briefly explain the sigma minus method.
8) Write a note on extravascular administration of one compartment open model.

5.5.5. Long Answer Type Questions

1) Discuss about compartment models.
2) Explain briefly about non-compartment models.
3) Write an exhaustive note on methods of elimination.
4) How pharmacokinetic parameters can be evaluated from one compartment open model for IV bolus?

CHAPTER 6

Multicompartment Models

6.1. MULTI-COMPARTMENT MODELS

6.1.1. Introduction

One-compartment model describes the pharmacokinetic profile of many drugs. In such case, instantaneous dist ribution equilibrium is assumed, and decline in the amount of drug in the body with time is expressed as elimination by an equation with a mono -exponential term. Instantaneous distribution, however, is not possible for larger number of drugs and drug disposition is not mono-exponential but bi- or multi-exponential. The reason for this is that the body is composed of a heterogeneous group of tissues, each with different degree of blood flow, drug affinity, and therefore different equilibration rates.

An ideal pharmacokinetic model should have a rate constant for each tissue undergoing equilibrium (which is difficult mathematically). The best approach is to combine the tissues together based on the similarity in their distribution characteristics. Just like one -compartment models, drug disposition in multi -compartment models is also assumed to follow first-order kinetics.

Multi-compartment drug characteristics can be understood by giving it as intravenous bolus and observing the decline in plasma drug concentration with time. The number of exponentials required to describe such a plasma level -time profile d etermines the number of kinetically homogeneous compartments into which a drug will distribute.

Multi-compartment models are based on the following **assumptions**:

1) Blood/plasma and the highly perfused tissues (like brain, hea rt, lung, liver, and kidneys) form the central compartment.
2) Other tissues with similar distribution characteristics are combined together to constitute peripheral compartment tissues based on the similarity in their distribution characteristics.
3) Drugs admi nistered via intravenous route are directly introduced into the central compartment.
4) Irreversible drug elimination by hepatic biotransformation or renal excretion occurs from the central compartment.
5) Reversible distribution occurs between central and perip heral compartments, and a finite time is required to attain distribution equilibrium.
6) After drug equilibration between central and peripheral compartments, drug elimination follows first-order kinetics.
7) The rate processes involving drug passage in and out of individual compartments follow first-order kinetics, and the plasma level -time curve is

described by the sum of series of exponential terms , each of which corresponds to the first -order rate processes associated with a given compartment.

8) Peripheral compartment cannot be accessed for direct measurement, and it is not a site of drug elimination or clearance.

6.1.2. Two Compartment Open Model

Two-compartment models are the most common multi -compartment model. In these models, the **body tissues** are classified into the following **two categories**:

1) **Central Compartment or Compartment 1:** This comprises of blood and highly perfused tissues (like liver, lungs, kid neys, etc.) that rapidly attain equilibrium with the drug. Elimination occurs from this compartment.
2) **Peripheral or Tissue Compartment or Compartment 2:** This comprises of poorly perfused and slow equilibrating tissues (like muscles, skin, adipose, etc.), and is a hybrid of several functional physiologic units.

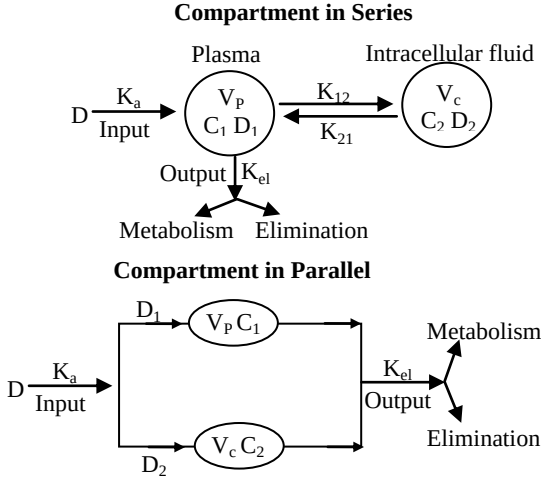


Figure 6.1: Open Two-Compartment Pharmacokinetic Model. (C_1 -Concentration in Compartment 1; D_1 - Amount of Drug in Compartment 1; C_2 -Concentration in Compartment 2; D_2 - Amount of Drug in Compartment 2).

Classification of a particular tissue (like brain) into central or peripheral compartment depends on the drug 's physicochemical properties. A drug that is highly lipophilic can cross the BBB, and in this case brain will be included in the central compartment. On the other hand, a polar drug ca nnot cross the BBB and in this case brain in spite of being a highly perfused organ will be included in the peripheral compartment.

The **two-compartment model**, depending on the compartment from whic h drug elimination has occurred, is categorised into the following **three types**:

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

If suitable information is no t present, elimination occurs from the central compartment.

Through one- and two-compartment models, the processes of pharmacokinetics can be understood. But, these models are crude approximation of complex body processes, and for being more precise and accurate, the body is sub-divided into a larger number of compartments.

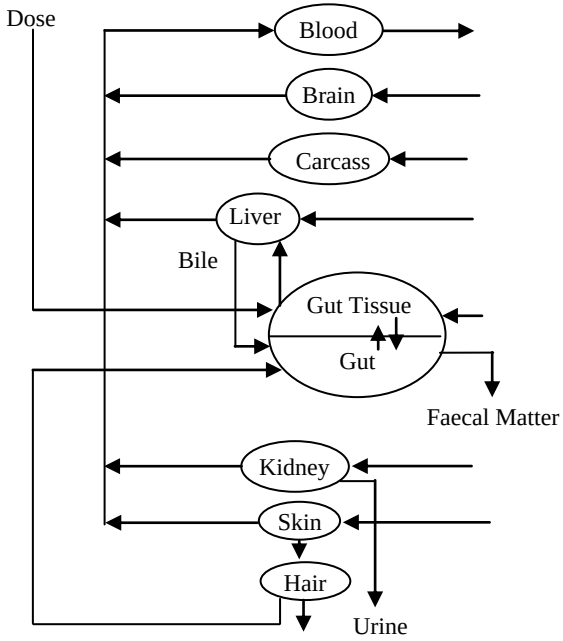
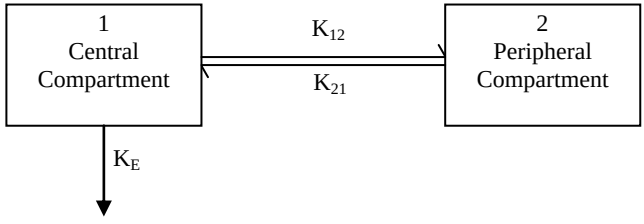


Figure 6.2: Nine-Compartment Model of Mercury

In **figure 6.2**, a nine-compartment research model for mercury is shown. Separate compartments are included for major organ systems. Input to the model is oral (into the gut) and excretion occurs through hair, urine, and faeces. The model studied had 18 compartments, nine for each of two chemical forms of mercury. With the help of computers, the mathematical equations for 9 and 18 compartment models can be easily so lved. However, these large models behave in a more complex manner than the one- or two-compartment models.

6.1.3. Two Compartment Open Model – I.V. Bolus

Two-compartment open model - I.V. bolus is shown below with elimination from the central compartment:



After administering a drug (that follows two -compartment kinetics) as intravenous bolus, the decline in plasma concentration is bi -exponential. This indicates that there are two d isposition processes, i.e., **distribution** and **elimination**, which are not obvious in a regular arithmetic plot but can be identified in a semi-log plot of C *versus* t **(figure 6.3)**.

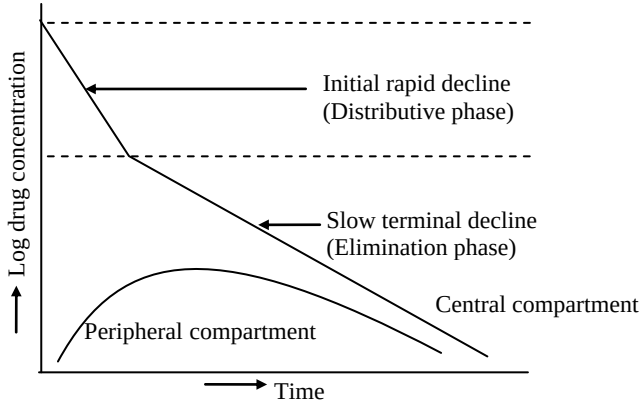


Figure 6.3: Changes in Drug Concentration in the Central (Plasma) and the Peripheral Compartment after I.V. Bolus of a Drug that Fits Two-Compartment Model

Drug concentration in the central compartment initially undergoes a rapid decline due to drug distribution from the central to the peripheral compartment. This phase is therefore termed the **distributive phase**. After a while, pseudo-distribution equilibrium is attained between the two compartments, and then the subsequent loss of drug from the central compartment becomes slow due to elimination. This phase of slower rate process is termed the **post-distributive** or **elimination phase**. Contrary to the central compartment, drug concentration in the peripheral compartment first increases and reaches maximum in the distribution phase. Thereafter, the drug concentration declines in the post-distributive phase **(figure 6.3)**.

Let K_{12} and K_{21} be the first-order distribution rate constants representing reversible drug transfer between the central and peripheral compartments; they are termed **microconstants** or **transfer constants**. Let subscript c and p define central and peripheral compartments, respectively. The rate of change in drug concentration in the central compartment is expressed as:

$$\frac{dC_c}{dt} = K_{21}C_p - K_{12}C_c - K_E C_c \qquad \text{.....(1)}$$

On extending the relationship $X = V_d C$ to **equation (1)**:

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

Where,
X_c and X_p = Amounts of drug in the central and peripheral compartments, respectively.
V_c and V_p = Apparent volumes of the central and peripheral compartments, respectively.

The rate of change in drug concentration in the peripheral compartment is expressed as:

$$\frac{dC_p}{dt} = K_{12}C_c - K_{21}C_p \qquad \text{.....(3)}$$

$$= \frac{K_{12}X_c}{V_c} - \frac{K_{21}X_p}{V_p} \qquad \text{.....(4)}$$

On integratin g **equations (2) and (4)**, an equation forms that represent drug concentration in the central and peripheral compartments at any given time (t):

$$C_c = \frac{X_0}{V_c}\left[\left(\frac{K_{21}-\alpha}{\beta-\alpha}\right)e^{-\alpha t} + \left(\frac{K_{21}-\beta}{\alpha-\beta}\right)e^{-\beta t}\right] \quad \text{.....(5)}$$

$$C_p = \frac{X_0}{V_p}\left[\left(\frac{K_{12}}{\beta-\alpha}\right)e^{-\alpha t} + \left(\frac{K_{12}}{\alpha-\beta}\right)e^{-\beta t}\right] \quad \text{.....(6)}$$

Where, X_0 = Intravenous bolus dose.

α and β = Hybrid first-order constants for the rapid distribution phase and the slow elimination phase, respectively.

The hybrid first -order constant values (α and β) depend on the first -order ra te constant values (K_{12}, K_{21} and K_E). The mathematical relation ships between the former and latter are given as:

$$\alpha + \beta = K_{12} + K_{21} + K_E \quad \text{.....(7)}$$

$$\alpha\beta = K_{21}K_E \quad \text{.....(8)}$$

Equation (6) can be simplified as:

$$C_c = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{.....(9)}$$

C_c = Distribution exponent + Elimination exponent

Where, A and B = Hybrid constants for the two exponents (i.e., distribution and elimination), and can be resolved graphically by the method of residuals.

$$A = \frac{X_0}{V_c}\left[\frac{K_{21}-\alpha}{\beta-\alpha}\right] = C_0\left[\frac{K_{21}-\alpha}{\beta-\alpha}\right] \quad \text{.....(10)}$$

$$B = \frac{X_0}{V_c}\left[\frac{K_{21}-\beta}{\alpha-\beta}\right] = C_0\left[\frac{K_{21}-\beta}{\alpha-\beta}\right] \quad \text{.....(11)}$$

Where, C_0 = Plasma drug concentration after intravenous injection.

Determination of Pharmacokinetic Parameters after I.V. Bolus

Method of residuals can be used to determine all the parameters of **equation (9)**. Other parameters of the model, i.e., K_{12}, K_{21}, K_E, etc., can be derived by proper substitution of these values.

$$C_0 = A + B \quad \text{.....(12)}$$

$$K_E = \frac{\alpha\beta C_0}{A\beta + B\alpha} \quad \text{.....(13)}$$

$$K_{12} = \frac{AB(\beta-\alpha)^2}{C_0(A\beta + B\alpha)} \quad \text{.....(14)}$$

$$K_{21} = \frac{A\beta + B\alpha}{C_0} \quad \text{.....(15)}$$

For two -compartment model, K_E = rate constant for drug elimination from the central compartment and β = rate constant for drug elimination from the body. Overall elimination $t_{1/2}$ should be calculated from the value of β.

Area under the plasma concentration-time curve (AUC) can be obtained as:

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad \text{.....(16)}$$

The apparent volume of central compartment (V_c) is given as:

$$V_d = \frac{X_0}{C_0} = \frac{X_0}{K_E AUC} \quad \text{.....(17)}$$

The apparent volume of peripheral compartment (V_p) is given as:

$$V_p = \frac{V_c K_{12}}{K_{21}} \quad \text{.....(18)}$$

The apparent volume of distribution at steady-state or equilibrium ($V_{d,ss}$) is given as:

$$V_{d,ss} = V_c + V_p \quad \text{.....(19)}$$

It is also given as:

$$V_{d,area} = \frac{X_0}{\beta AUC} \quad \text{.....(20)}$$

Total systemic clearance is given as:

$$Cl_T = \beta V_d \quad \text{.....(21)}$$

The pharmacokinetic parameters can be calculated from the urinary excretion data as follows:

$$\frac{dX_u}{dt} = K_e V_c \quad \text{.....(22)}$$

An equation identical to **equation (6)** can be derived for rate of excretion of unchanged drug in urine:

$$\frac{dX_u}{dt} = K_e A e^{-\alpha t} + K_e B e^{-\beta t} \quad \text{.....(23)}$$

Equation (23) can be resolved into individual exponents by the method of residuals as described for plasma concentration-time data.

Renal clearance is given as:

$$Cl_R = K_e V_c \quad \text{.....(24)}$$

Methods of Residuals

This method aids in resolving the bi-exponential disposition curve, obtained after intravenous bolus of a drug that follows two -compartment model, into its individual exponents. On rewriting **equation (9)**:

$$C_c = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{.....(25)}$$

The bi -exponential curve in **figure 6.4** shows that 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 more rapidly than the does $e^{-\beta t}$. Thus, **equation (25)** reduces to:

$$\overleftarrow{C} - Be^{-\beta t} \quad \text{.....(26)}$$

On taking logarithm of **equation (26)**:

$$\log \overleftarrow{C} = \log B - \frac{\beta t}{2.303} \quad \text{.....(27)}$$

Where, $\overleftarrow{C}$ = Back extrapolated plasma concentration values.

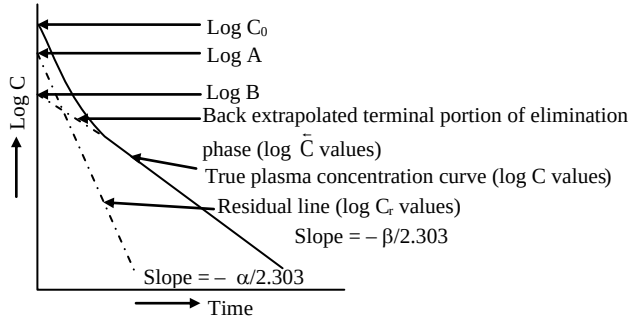


Figure 6.4: Resolution of Bi-exponential Plasma Concentration-Time Curve by the Method of Residuals for a Drug that Follows Two-Compartment Kinetics on I.V. Bolus Administration

A semi-log plot of C *versus* t yields the terminal linear phase of the curve with slope –β/2.303. On back extrapolating to time zero, y -intercept log B is yielded **(figure 6.4)**. The $t_{½}$ for the elimination phase can be obtained from $t_{½} = 0.693/\beta$. On subtracting extrapolated plasma concentration values of the elimination phase [**equation (26)**] from the corresponding true plasma concentration values [**equation (25)**], a series of residual concentration values (C_r) are obtained.

$$Cr = C - \overleftarrow{C} = Ae^{-\alpha t} \quad \text{.....(28)}$$

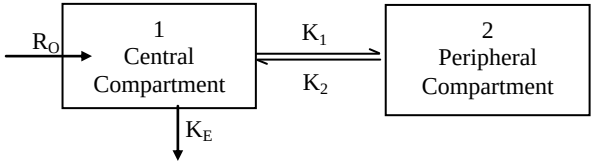
On taking logarithm of **equation (28)**:

$$\log C_r = \log A - \frac{at}{2.303} \quad \text{.....(29)}$$

A semi-log plot of C_r *versus* t yields a straight line with slope –α/2.303 and y -intercept log A (**figure 6.4)**.

6.1.4. Two Compartment Open Model – I.V. Infusion

Two-compartment open model – I.V. infusion is shown below with elimination from the central compartment:



The plasma or central compartment concentration of a drug that follows two -compartment model when administered at a constant rate (zero -order) intravenous infusion is expressed as:

$$C = \frac{R_0}{V_c K_E}\left[1 + \left(\frac{K_E - \beta}{\beta - \alpha}\right)e^{-\alpha t} + \left(\frac{K_E - \alpha}{\alpha - \beta}\right)e^{-\beta t}\right] \quad \text{.....(30)}$$

The second and the third term in the bracket becomes zero at steady -state (i.e., at time infinity), and **equation (30)** reduces to:

$$C_{ss} = \frac{R_0}{V_c K_E} \quad \text{..... (31)}$$

On substituting $V_C K_E = V_d \beta$ in **equation (31)**:

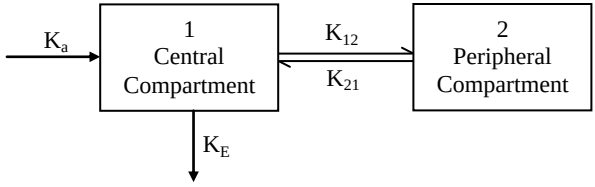
$$C_{ss} = \frac{R_0}{V_d \beta} = \frac{R_0}{Cl_T} \quad \text{..... (32)}$$

The loading dose ($X_{0,L}$) to obtain C_{ss} immediately at the start of infusion can be calculated from **equation (33)**:

$$X_{0,L} = C_{ss} V_c = \frac{R_0}{K_E} \quad \text{..... (33)}$$

6.1.5. Two Compartment Open Model - Extravascular Administration

The two-compartment open model - extravascular administration is shown below with elimination from the central compartment:



The rate of change in concentration of a drug that enters the body by first -order absorption process and distributes according to the two-compartment model in the central compartment is described by an absorption exponent, and the two usual exponents describing drug disposition. The plasma concentration at any time (t) is given by **equation (34)**:

$$C = Ne^{-Kat} + Le^{-\alpha t} + Me^{-\beta t} \quad \text{..... (34)}$$

C = Absorption exponent + Distribution exponent + Elimination exponent

Where, K_a, α and β have usual meanings; while L, M and N are coefficients.

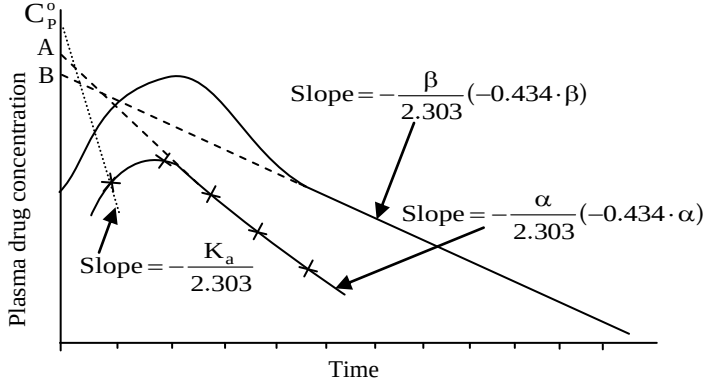


Figure 6.5: Semi-log Plot of Plasma Drug Concentration *versus* Time for Two Compartment Model after Extravascular Administration

The above multi-exponentials can be determined by the method of residuals. In the first step, elimination line (**figure 6.5** solid line, $\text{Slope} = -\frac{\beta}{2.303}$) is subtracted from the curve to result in a two -compartment residual plot having a post -absorptive linear segment (**figure 6.5**, $\text{Slope} = -\frac{\alpha}{2.303}$). On subtracting the residuals from this plot, a line with slope $-\frac{K_a}{2.303}$ is produced (**figure 6.5**).

The intercepts A and B are same. C_p^0 is the y-axis intercept of absorption residual line and is theoretically equals to A + B.

6.1.6. Kinetics of Multiple Dosing

A single dose of drug is not sufficient for treating certain diseases. So drugs are repeated at specific time intervals for maintaining the therapeutic plasma drug concentration throughout the treatment period. The objective of drug treatment is to achieve and maintain plasma drug concentration within the therapeutic range with minimum fluctuations. Thus, the objective for antibiotics is to maintain plasma drug concentration above minimum effective concentration; the objective for drugs with narrow therapeutic range is to maintain the plasma drug concentrations below toxic levels.

When the first dose is administered in an oral multiple dosing regimen, the plasma drug concentration increases and reaches the peak and then declines. When the second d ose is administered, plasma drug concentration again increases to reach a level higher than the first dose, and continues to increase till a steady state plasma drug concentration is achieved (**figure 6.6**).

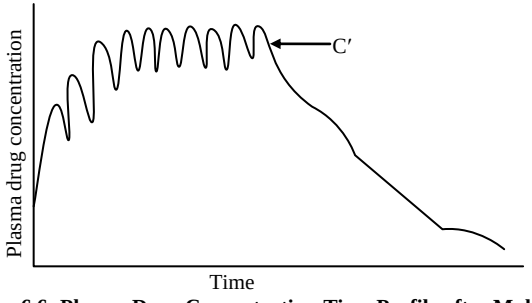


Figure 6.6: Plasma Drug Concentration-Time Profile after Multiple Dosing Regimen (C′ – Average Steady State Plasma Drug Concentration)

At steady state, the drug input and o utput will be equal. The amount of drug that accumulates in the body relative to the first dose is calculated as follows:

$$R = \frac{1}{1 - e^{(-0.693\tau)/t_{1/2}}} \qquad \text{.....(35)}$$

Where, R = Accumulation factor (depends on dosing interval and half-life).
τ = Dosing interval.
Thus, smaller the $\tau/t_{1/2}$ ratio, greater will be the accumulation.

From first-order kinetics, approximately 90% of steady state will be reached within four half-lives. However, the time required to reach the steady state depends on the drug's half-life. Average steady state plasma drug concentration (C') depends on:

1) Maintenance dos (X_0),
2) Fraction of the dose absorbed (F),
3) Dosing interval (τ), and
4) Clearance (Cl).

$$C' = \frac{F.X_0}{Cl \cdot \tau} \qquad \text{.....(36)}$$

$$C' = \frac{1.44 \cdot F \cdot X_0 \cdot t_{1/2}}{V_d \cdot \tau}$$

$$= \frac{AUC}{\tau} \qquad \text{.....(37)}$$

Where, V_d = Volume of distribution.

ACU = Area under the plasma drug concentration-time curve after single maintenance dose.

From **equations (36)** and **(37)** maintenance dose can be calculated as:

$$X_0 = \frac{C' \cdot Cl \cdot \tau}{F}$$

$$= \frac{C' \cdot V \cdot \tau}{1.44 \cdot F \cdot t_{1/2}} \qquad \text{.....(38)}$$

6.1.7. Steady State Drug Levels

The time required to reach steady state depends on the drug's half -life. If $K_a >> K_E$, the drug reaches the plateau in approximately five half-lives; this is called the **plateau principle**, which also means that K_E determines the rate at which the multiple dose steady state is reached. The time taken to reach steady state is not influenced by the dose size, dosing interval, and number of doses.

Maximum and Minimum Concentration During Multiple Dosing

If n is the number of doses administered, C_{max} and C_{min} obtained o n multiple dosing after the n^{th} dose is given as:

$$C_{n,max} = C_0 \left[\frac{1 - e^{-nK_e\tau}}{1 - e^{-K_E\tau}} \right] \qquad \text{....(39)}$$

$$C_{n,min} = C_0 \left[\frac{1 - e^{-nK_e\tau}}{1 - e^{-K_E\tau}} \right] e^{-K_E\tau} = C_{n,max} e^{-K_E\tau} \qquad \text{....(40)}$$

The maximum and minimum concentrations of drug in plasma at steady-state are given by the following equations:

$$C_{ss,max} = \frac{C_0}{1 - e^{-K_n\tau}} \qquad \text{....(41)}$$

$$C_{ss,min} = \frac{C_0 e^{-K_E\tau}}{1 - e^{-K_E\tau}} C_{ss,max} e^{-K_E\tau} \qquad \text{....(42)}$$

Where, C_0 = concentration attained from instantaneous absorption and distribution (obtained by extrapolating the elimination curve to time zero). **Equations (39) to (42)** can be written in terms of amount of drug in the body, and fraction of dose absorbed (F) should be considered.

Fluctuation is the **ratio of C_{max} to C_{min}. Greater the ratio, greater is the fluctuation**. Like accumulation, fluctuation depends on dosing frequency, half-life, and the absorption rate of drug. Greatest fluctuation is observed when the drug is given as intravenous bolus; while small fluctuations are observed when the drug is given extravascularly and it undergoes continuous absorption. The average drug concentration at steady-state ($C_{ss,av}$) depends on:

1) Maintenance dose (X_o),
2) Fraction of dose absorbed (F),
3) Dosing interval (τ), and
4) Clearance (Cl_T) (or V_d and K_E or $t_{1/2}$) of the drug.

Average concentration and drug content on multiple dosing to steady state:

$$C_{ss,av} = \frac{FX_0}{Cl_T \tau} \quad \text{....(43)}$$

$$= \frac{1.44FX_0}{V_d \tau} = \frac{AUC(\text{single dose})}{\tau} \quad \text{....(44)}$$

Where, the coefficient 1.44 is the reciprocal of 0.693, while AUC is the area under the curve after a single maintenance dose. **Equation (44)** is used for determining the maintenance dose of a drug to achieve a desired concentration.

Since $X = V_dC$, the body content at steady state is given as:

$$X_{ss,av} = \frac{1.44FX_0 t_{1/2}}{\tau} \quad \text{....(45)}$$

These average values are not arithmetic mean of $C_{ss,max}$ and $C_{ss,min}$ as the plasma drug concentration undergoes exponential decline.

6.1.8. Calculation of Loading and Maintenance Doses and their Significance in Clinical Settings

A drug is therapeutically active when it attains the desired steady state in five half-lives. However if the drug is having a long half-life, it will take a longer time. The plateau can be reached immediately after administering a dose that yields the desired steady state before starting the maintenance doses. Such an **initial dose** or **first dose intended to be therapeutic** is termed **priming** or **loading dose** ($X_{0,L}$). The equation used for evaluating loading dose is given as:

$$X_{0,L} = \frac{C_{ss,av} V_d}{F} \quad \text{..... (46)}$$

The value of C_{max} after extravascular administration is always smaller than that after intravenous administration. Therefore, the loading dose is proportionally small. If drugs have low therapeutic indices, the loading dose can be divided into smaller doses which are to be given at various intervals before the first maintenance dose.

If V_d is not known, the loading dose can be determined as:

$$\frac{X_{0,L}}{X_0} = \frac{1}{\left(1-e^{-K_a\tau}\right)\left(1-e^{-K_E\tau}\right)} \quad \text{..... (47)}$$

Equation (47) is true when $K_a >> K_E$ and the drug is distributed rapidly. If the drug is given in travenously or if drug is absorbed rapidly, the absorption phase can be neglected and **equation (47)** becomes:

$$\frac{X_{0,L}}{X_0} = \frac{1}{\left(1-e^{-K_E\tau}\right)} = R_{ac} \quad \text{..... (48)}$$

Loading Dose

The ratio of loading dose to maintenance dose ($X_{0,L}/X_0$) is termed **dose ratio**. As a rule, when

1) $\tau = t_{1/2}$, dose ratio = 2.0.
2) $\tau > t_{1/2}$, dose ratio < 2.0.
3) $\tau < t_{1/2}$, dose ratio > 2.0.

Figure 6.7 shows that if loading dose is not optimum, and is either too low or too high, the steady state is attained in five half -lives just as when no loading dose is given.

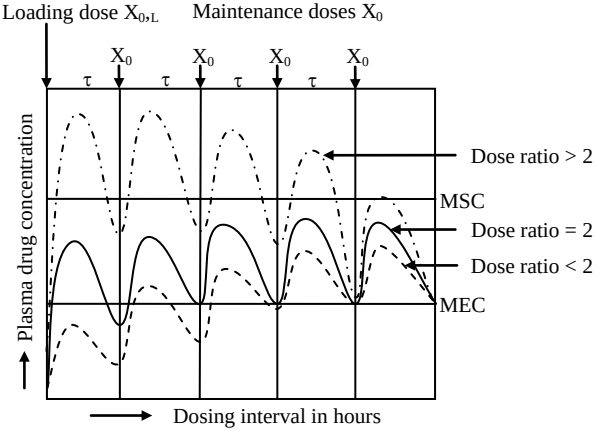


Figure 6.7: Schematic Representation of Plasma Concentration-Time Profiles that Result when dose Ratio is Greater than 2.0, Equal to 2.0 and Smaller than 2.0

Maintenance of Drug within Therapeutic Range

Maintenance of drug concentration within the therapeutic window depends on:

1) Drug therapeutic index,
2) Drug half-life, and
3) Drug convenience.

In drugs with short half -life (< 2.5 hours) and narrow therapeutic i ndex, **e.g.,** heparin, it is not easy to maintain such a level as the dosing frequency must be less than the half -life. However, some drugs like penicillin with half -life = 0.9 hours may be given less frequently (in every 4 -6 hours) but the maintenance dose should be larger so that the plasma concentration is maintained above the minimum inhibitory level.

A drug with intermediate half-life (3-8 hours) but low therapeutic index should be given at intervals less than or equal to half -life. A drug having high therapeutic index should be given at intervals between 1 to 3 half -lives. A drug with long half-life (> 8 hours) should be giv en once every half-life. Steady state in such a case can be rapidly attained by administering a loading dose.

A drug with very lon g half-life (> 24 hours), **e.g.,** amlodipine, should be given once in 24 hours.

6.2. SUMMARY

The details given in the chapter can be summarised as follows:

1) In multi-compartment models, blood/plasma and the highly perfused tissues (like brain, heart, lung, liver, and kidneys) form the central compartment.
2) In multi-compartment models, drugs administered via intravenous route are directly introduced into the central compartment.
3) In multi -compartment models, irreversible drug elimination by hepatic biotransformation or renal excretion occurs from the central compartment.
4) In multi-compartment models, after drug equilibration between central and peripheral compartments, drug elimination follows first-order kinetics.
5) In multi-compartment models, peripheral compartment c annot be accessed for direct measurement, and it is not a site of drug elimination or clearance.
6) **Central compartment or compartment 1** comprises of blood and highly perfused tissues (like liver, lungs, kidneys, etc.) that rapidly attain equilibrium with the drug. Elimination occurs from this compartment.
7) **Peripheral or tissue compartment or compartment 2** comprises of poorly perfused and slow equilibrating tissues (like muscles, skin, adipose, etc.), and is a hybrid of several functional physiologic units.
8) **Methods of residuals** aids in resolving the bi-exponential disposition curve, obtained after intravenous bolus of a drug that follows two -compartment model, into its individual exponents.
9) The time required to reach steady state depends on the drug's half-life.
10) If $K_a >> K_E$, the drug reaches the plateau in approximately five half -lives; this is called the **plateau principle**, which also means that K_E determines the rate at which the multiple dose steady state is reached.
11) **Fluctuation** is the ratio of C_{max} to C_{min}. Greater the ratio, greater is the fluctuation.
12) The ratio of loading dose to maintenance dose ($X_{0,L}/X_0$) is termed **dose ratio**.
13) A drug with intermediate half-life (3-8 hours) but low therapeutic index should be given at intervals less than or equal to half-life.
14) A drug having high therapeutic index should be given at intervals between 1 to 3 half-lives.
15) A drug with long half-life (> 8 hours) should be given once every half-life.
16) A drug with very long half -life (> 24 hours), **e.g.,** amlodipine, should be given once in 24 hours.

6.3. EXERCISE

6.3.1. True or False

1) In multi-compartment models, blood/plasma and the highly perfused tissues form the peripheral compartment.
2) In multi -compartment models, after drug equilibration between central and peripheral compartments, drug elimination follows zero-order kinetics.
3) Fluctuation is the ratio of C_{max} to C_{min}.
4) A drug with intermediate half-life (3-8 hours) but low therapeutic index should be given once every half-life.
5) A drug with very long half-life (> 24 hours) should be given once in 24 hours.

6.3.2. Fill in the Blanks

6) In multi -compartment models, irreversible drug elimination by hepatic biotransformation or renal excretion occurs from the _____.
7) _____ comprises of poorly perfused and slow equilibrating tissues, and is a hybrid of several functional physiologic units.
8) The time required to reach steady state depends on the drug's _____.
9) The ratio of loading dose to maintenance dose is termed _____.
10) A drug with long half-life, i.e., _____, should be given once every half-life.

Answers

1) False 2) False 3) True
4) False 5) True 6) Central compartment
7) Peripheral compartment 8) Half-life 9) Dose ratio
10) > 8 hours

6.3.3. Very Short Answer Type Questions

1) What are loading and maintenance doses?
2) Give the advantages of pharmacokinetic models.
3) Give any two assumptions of multi-compartment models.
4) Draw the graph for method of residuals.

6.3.4. Short Answer Type Questions

1) Discuss the steady state drug levels.
2) Write a short note on multi-compartment models.
3) Write about two compartment open model.

6.3.5. Long Answer Type Questions

1) Discuss about kinetics of multiple dosing.
2) Write an exhaustive note on two compartment open model for IV bolus.

CHAPTER 7

Non-Linear Pharmacokinetics

7.1. NON-LINEAR PHARMACOKINETICS

7.1.1. Introduction

At therapeutic doses, the change in amount of drug in the body or the change in plasma drug concentration due to absorption, distribution, binding, metabolism, or excretion is proportional to the administered dose (whether a single dose or multiple doses). In such a case, the rate processes follow first-order or linear kinetics and the semi-log plots of C *versus* t for different doses are superimposable when corrected for dose administered. This is the **principle of superposition**. Pharmacokinetic parameters, like F, K_a, K_E, V_d, Cl_R and Cl_H describe that the time-course of a drug in the body remains unaffected by the dose, i.e., their pharmacokinetic is dose-independent.

In some cases, the rate process of a drug's ADME depends on carrier or enzymes that are substrate-specific, have definite capacities, and are susceptible to saturation at high drug concentration. In these cases, first-order kinetics transform into a mixture of first-order and zero-order rate processes and the pharmacokinetic parameters alter with the administered dose. Pharmacokinetics of such drugs is dose-dependent. Other synonymous terms are mixed-order, non-linear, and capacity-limited kinetics. Drugs with such a pharmacokinetic profile give rise to variability in pharmacological response.

7.1.2. Factors Causing Non-Linearity

Non-linearity can occur in drug absorption, distribution, metabolism and excretion.

Drug Absorption

Non-linearity in drug absorption occurs due to the following reasons:

1) **When Absorption is Solubility or Dissolution Rate-Limited:** When a drug, **e.g.,** griseofulvin, is administered at higher doses, a saturated solution of the drug is formed in the GIT or at any other extravascular site and the absorption rate attains a constant value.

2) **When Absorption Involves Carrier-Mediated Transport Systems:** Saturation of the transport system at higher doses of vitamins, **e.g.,** riboflavin, ascorbic acid, cyanocobalamin, etc., causes non-linearity.

3) **When Pre-Systemic Gut Wall or Hepatic Metabolism Attains Saturation:** Saturation of pre-systemic metabolism of drugs, **e.g.,** propranolol, hydralazine and verapamil, at high doses increases bioavailability.

The parameters, F, K_a, C_{max} and AUC, undergo a decrease in the first two cases and undergo an increase in the last case. Changes in gastric emptying and gastrointestinal blood flow and other physiological factors are some other causes of non-linearity in drug absorption. Non-linearity in drug absorption is of little consequence unless availability is significantly affected.

Drug Distribution

Non-linearity in distribution of drugs administered at high doses occurs due to the following reasons:

1) **Saturation of Binding Sites on Plasma Proteins:** A fixed number of binding sites are present on plasma proteins for a particular drug, **e.g.,** phenylbutazone and naproxen; therefore, with increase in drug concentration, the fraction of unbound drug also increases.

2) **Saturation of Tissue Binding Sites:** With large single bolus doses or multiple dosing of drugs, **e.g.,** thiopental and fentanyl, the tissue storage sites undergo saturation.

In both the cases, the free plasma drug concentration increases; however, V_d increases in the first case and decreases in the last one. Clearance also alters depending on the extraction ratio of the drug. Clearance of a drug with high Extraction Ratio (ER) is increased due to saturation of binding sites. Unbound clearance of drugs with low ER is unaffected and the pharmacological response increases.

Drug Metabolism

Non-linear kinetics of clinical importance is capacity-limited metabolism as small changes in dose administered produce large variations in plasma drug concentration at steady state. It is a major source of large inter-subject variability in pharmacological response.

Non-linearity in drug metabolism occurs due to the following reasons:

1) **Capacity-Limited Metabolism due to Enzyme and/or Cofactor Saturation:** This occurs in phenytoin, alcohol, theophylline, etc.

2) **Enzyme Induction:** The peak plasma concentration of carbamazepine decreases on repetitive administration over a time period. Auto-induction characterised in this case is also dose-dependent. Thus, enzyme induction is a common cause of dose- and time-dependent kinetics.

Saturation of enzyme decreases Cl_H and increases C_{ss}; while, the reverse is true for enzyme induction. Other causes of non-linearity in metabolism are saturation of binding sites, inhibitory effect of the metabolite on enzyme, and pathological situations such as hepatotoxicity and changes in hepatic blood flow.

Drug Excretion

The two active processes in renal excretion of a saturable drug are:

1) **Active Tubular Secretion:** After the saturation of carrier system, a decrease in renal clearance of drug, **e.g.,** penicillin G, occurs.

2) **Active Tubular Reabsorption:** After the saturation of carrier system, an increase in renal clearance of drug, **e.g.,** water-soluble vitamins and glucose, occurs.

Other sources of non-linearity in renal excretion are forced diuresis, changes in urine pH, nephrotoxicity, and saturation of binding sites.

7.1.3. Michaelis-Menten Method of Estimating Parameters

Michaelis-Menten equation describes the kinetics of capacity-limited or saturable processes:

$$-\frac{dC}{dt}=\frac{V_{max}\,C}{K_m+C} \qquad \text{..... (1)}$$

Where, –dC/dt = Rate of decline of drug concentration with time.
V_{max} = Theoretical maximum rate of the process.
K_m = Michaelis constant.

Three situations can be considered depending on the values of K_m and C:

1) **When Km = C:** Under this situation, **equation (1)** reduces to:

$$-\frac{dC}{dt}=\frac{V_{max}}{2} \qquad \text{..... (2)}$$

The rate of process is one-half of its maximum rate **(figure 7.1)**:

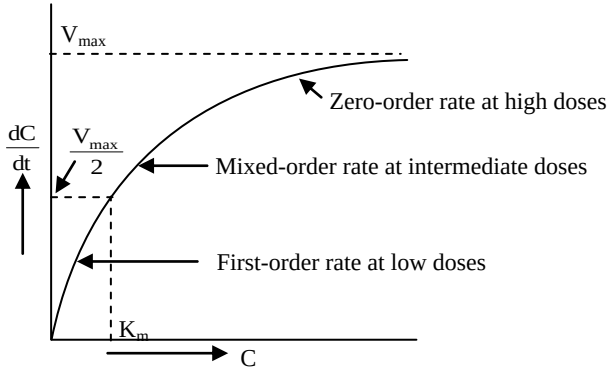


Figure 7.1: Plot of Michaelis-Menten Equation [Elimination Rate (dC/dt) *versus* Concentration (C)]. Initially, the rate increases linearly (first-order) with concentration, becomes mixed-order at higher concentration and then reaches maximum (V_{max}) beyond which it proceeds at a constant rate (zero-order)

2) **When K_m >> C:** Under this condition, $K_m + C = K_m$ and **equation (1)** reduce to:

$$-\frac{dC}{dt}=\frac{V_{max}\,C}{K_m} \qquad \text{..... (3)}$$

Equation (3) is similar to the one describing first-order elimination of a drug, where $V_{max}/K_m = K_E$. This indicates that the drug concentration in body resulting from usual dosage regimens of most drugs is below the K_m value of elimination process (however, phenytoin and alcohol are certain exceptions).

3) **When K_m << C:** Under this condition, $K_m + C = C$ and **equation (1)** reduces to

$$-\frac{dC}{dt}=V_{max} \qquad \text{..... (4)}$$

Equation (4) is similar to the one describing a zero -order process, i.e., the rate-process occurs at a constant rate (V_{max}) and is independent of drug concentration, **e.g.,** metabolism of ethanol.

Estimation of K_m and V_{max}

The parame ters of capacity -limited processes, like metabolism, renal tubular secretion, and biliary excretion, can be defined by assuming one -compartment kinetics for the drug and that elimination involves a single capacity -limited process. The K_m and V_{max} parameters can be evaluated from the plasma concentration-time data obtained after intravenous bolus administration of a drug with non-linear elimination characteristics. On re-writing **equation (1)**:

$$-\frac{dC}{dt}=\frac{V_{max}\,C}{K_m+C} \qquad \text{..... (5)}$$

On integrating **equation (5)** followed by conversion to log base 10:

$$\log C=\log C_0+\frac{(C_0-C)}{2.303K_m}-\frac{V_{max}}{2.303K_m} \qquad \text{..... (6)}$$

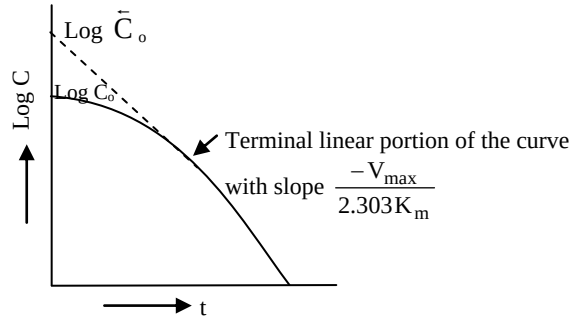


Figure 7.2: Semi-log Plot of a Drug given as I.V. Bolus with Non-Linear Elimination and that Fits One-Compartment Kinetics

A semi-log plot of C *versus* t yields a curve with a terminal linear portion having slope $-V_{max}/2.303K_m$. Back extrapolation to time zero gives y -intercept of log $\bar{C}_0$ (**figure 7.2**). This line can be described by the following equation:

$$\log C=\log \bar{C}_0-\frac{V_{max}}{2.303K_m} \qquad \text{..... (7)}$$

Equations (6) and **(7)** are similar at low plasma concentrations. On equating and simplifying both the equations:

$$\frac{(C_0-C)}{2.303K_m}=\log\frac{\bar{C}_0}{C_0} \qquad \text{..... (8)}$$

The value of K_m can be obtained from **equation (8)**. V_{max} can be calculated by substituting the value of K_m in the slope value.

V_{max} and K_m can also be obtained by determining the rate of change of plasma drug concentration at different times and by using the reciprocal **equation (1)** Thus:

$$\frac{1}{dC/dt}=\frac{K_m}{V_{max}\,C_m}+\frac{1}{V_{max}} \qquad \text{..... (9)}$$

Where, C_m = plasma concentration at midpoint of the sampling interval. A double reciprocal plot or the **Lineweaver-Burke plot** of $1/(dC/dt)$ *versus* $1/C_m$ of **equation (9)** yields a straight line with slope K_m/V_{max} and y-intercept $1/V_{max}$.

Lineweaver-Burke plot has a **disadvantage** that its points are clustered. Thus, **Hanes-Woolf plot** (**equation 10**) and **Woolf-Augustinsson-Hofstee plot** (**equation 11**) are used that give more reliable plots and uniformly scattered points.

$$\frac{C_m}{dC/dt} = \frac{K_m}{V_{max}} + \frac{C_m}{V_{max}} \quad \text{..... (10)}$$

$$\frac{dC}{dt} = V_{max} - \frac{dC/dt\, K_m}{C_m} \quad \text{..... (11)}$$

Equations (10) and **(11)** are rearrangements of **equation (9)**. Equation (10) is used to plot $C_m/(dC/dt)$ *versus* C_m. Equation (11) is used to plot dC/dt *versus* $(dC/dt)/C_m$. The K_m and V_{max} parameters can be determined from the slopes and y-intercepts of the two plots.

K_m and V_{max} from Steady State Concentration

When a drug is administered as a constant rate intravenous infusion or as a multiple dose regimen, the steady state concentration (C_{ss}) is given in terms of Dosing Rate (DR):

$$DR = C_{SS}C1_T \quad \text{.....(12)}$$

Where, $DR = R_o$ when the drug is administered as zero-order intravenous infusion; and $DR = FX_o/\tau$ when the drug is administered as multiple oral dosage regimen (F = fraction bioavailable, X_o = oral dose, and τ = dosing interval).

At steady state, the dosing rate and the decline rate of plasma drug concentration are the same. If the decline (elimination) is due to a single capacity-limited process (**e.g.,** metabolism):

$$DR = \frac{V_{max}\, C_{ss}}{K_m + C_{ss}} \quad \text{.....(13)}$$

A plot of C_{ss} *versus* DR yields a hockey-stick shaped curve (**figure 7.3**):

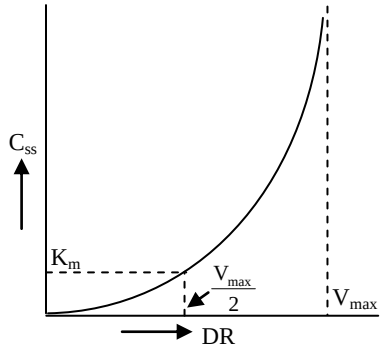


Figure 7.3: Curve for a Drug with Non-Linear Kinetics Obtained by Plotting the Steady State Concentration *versus* Dosing Rates

Several measurements should be made at steady state during dosage with different doses in order to accurately define the characteristics of a curve.

The parameters K_m and V_{max} can be graphically determined in the following ways:

1) **Lineweaver-Burke Plot/Klotz Plot:** On taking reciprocal of **equation (13)**:

$$\frac{1}{DR} = \frac{K_m}{V_{max}\, C_{ss}} + \frac{1}{V_{max}} \qquad \ldots\ldots (14)$$

Equation (14) is similar to **equation (9)**. A plot of 1/DR *versus* $1/C_{ss}$ yields a straight line with slope K_m/V_{max} and y-intercept $1/V_{max}$ (**figure 7.4**):

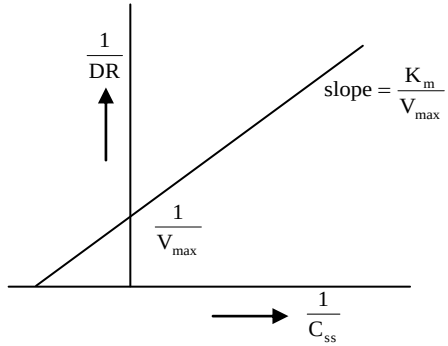


Figure 7.4: Lineweaver-Burke/Klotz Plot for Estimation of K_m and V_{max} at Steady State Concentration of Drug

2) **Direct Linear Plot :** A pair of C_{ss}, *viz.*, $C_{ss,1}$ and $C_{ss,2}$ obtained with two different dosing rates (DR_1 and DR_2) is plotted (**figure 7.5**). The points $C_{ss,1}$ and DR_1 are joined to form a line, and the points $C_{ss,2}$ and DR_2 are joined to form a second line. The intersection point of these two lines is extrapolated on DR axis to obtain V_{max} and on x-axis to obtain K_m.

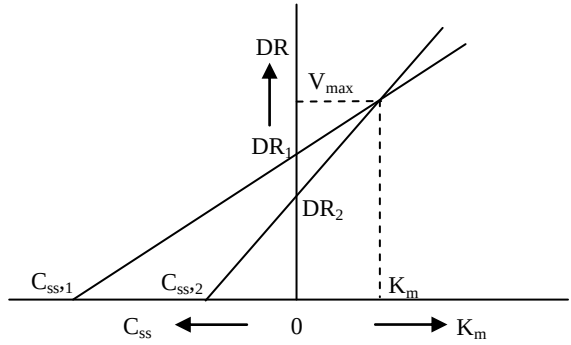


Figure 7.5: Direct Linear Plot for Estimation of K_m and V_{max} at Steady-State Concentrations of a Drug Given at Different Dosing Rates

3) **Graphical Method :** This method of estimating K_m and V_{max} involves re-arranging **equation (13)** to yield:

$$DR = V_{max} - \frac{K_m\, DR}{C_{ss}} \qquad \ldots\ldots (15)$$

A plot of DR *versus* DR/C_{ss} yields a straight line with slope $-K_m$ and y-intercept V_{max}. The parameters K_m and V_{max} can also be determined numerically by setting-up simultaneous equations:

$$DR_1 = \frac{V_{max}\, C_{ss,1}}{K_m + C_{ss,1}} \qquad \ldots\ldots (16)$$

$$DR_2 = \frac{V_{max}\, C_{ss,2}}{K_m + C_{ss,2}} \quad \text{..... (17)}$$

On combining **equations (16)** and **(17)**:

$$K_m = \frac{DR_2 - DR_1}{\frac{DR_1}{C_{ss,1}} - \frac{DR_2}{C_{ss,2}}} \quad \text{..... (18)}$$

After computing K_m, it is substituted in any one of the two simultaneous equations (i.e., 16 or 17) to obtain V_{max}.

K_m is much less variable than V_{max}. Hence, if mean K_m for a drug is known from an earlier study, V_{max} can be determined from a single measurement of C_{ss} at any given dosing rate.

The parameters K_m and V_{max} estimated by assuming one-compartment system and a single capacity-limited process have several **limitations**. More complex equations result and the computed K_m and V_{max} will be larger when:

1) The drug is eliminated by more than one capacity-limited process.
2) The drug exhibits parallel capacity-limited and first-order elimination processes.
3) The drug follows multi-compartment kinetics.

However, K_m and V_{max} obtained under such situations are little practically applicable in dosage calculations.

Significance of Michaelis-Menten Constant

1) If the value of K_m of a particular enzyme-substrate system is known, it can be predicted whether the cell needs more enzymes or more substrate to speed up the enzymatic reaction.
2) If a reaction with two similar substrates (**e.g.,** glucose and fructose) can be catalysed by an enzyme in the cell, it will prefer the substrate for which the enzyme has lower K_m value.
3) The value of K_m is an approximate measure of the concentration of substrate of the enzyme in the cell where reaction is taking place. The enzymes catalysing reactions with more concentrated substrates (**e.g.,** sucrose) have relatively high K_m value. In contrast, the enzymes that react with substrates present in very low concentrations (**e.g.,** hormones) have comparatively lower K_m values for the substrates.

The Time Required to Attain 90% of the True Steady State Plasma Concentration for Phenytoin

The time required to attain 90% of the true steady state plasma concentration for phenytoin administered at different rates, where V = 50 L, V_{max} = 500mg/day, and K_m = 4μg/ml (= 4mg/l) is to be determined. From **equation (19)**, the time required to attain 90% of the steady state concentration can be determined for various daily doses.

$$\frac{K_m V}{(V_{max} - R)^2}(2.303V_{max} - 0.9R) = t0.9. \quad \text{.....(19)}$$

For 100mg dose:

$$\frac{(4\text{mg L}^{-1})(50\text{L})}{(400\text{mg day}^{-1})^2}(2.303[500\text{mg day}^{-1}] - 0.9[100\text{mg day}^{-1}]) = 1.33 \text{ days.}$$

For 200mg dose:

$$\frac{(4\text{mg L}^{-1})(50\text{L})}{(300\text{mg day}^{-1})^2}(2.303[500\text{mg day}^{-1}] - 0.9[200\text{mg day}^{-1}]) = 2.16 \text{ days.}$$

For 300mg dose:

$$\frac{(4\text{mg L}^{-1})(50\text{L})}{(200\text{mg day}^{-1})^2}(2.303[500\text{mg day}^{-1}] - 0.9[300\text{mg day}^{-1}]) = 4.41 \text{ days.}$$

For 400mg dose:

$$\frac{(4\text{mg L}^{-1})(50\text{L})}{(100\text{mg day}^{-1})^2}(2.303[500\text{mg day}^{-1}] - 0.9[400\text{mg day}^{-1}]) = 15.8 \text{ days.}$$

At times and doses above plasma phenytoin concentrations can be achieved by making slight modifications in the steady state phenytoin concentration as follows:

$$0.9(C_p)_{ss} = 0.9K_mR/(V_{max}-R). \quad \text{.....(20)}$$

From this data table **7.1** can be constructed.

If the value of K_m was 5.7mg/l (and not 4mg/l), it can be said that since K_m is the numerator of **equation (19)**, K_m and the time to reach 90% of the steady state phenytoin concentration are directly proportional. Similarly, $0.9(C_p)_{ss}$ is also directly proportional to K_m. Therefore, each value can be multiplied by the factor (5.7/4.0) to obtain the figures given in **table 7.2**.

Table 7.1: Time ($t_{0.9}$) to 90% of Steady State Plasma Concentration $(C_p)_{ss}$ Level as a Function of Daily Dose (R); K_m = 4.0mg L^{-1}

R (mg day^{-1})	$t_{0.9}$ (days)	$0.9(C_p)_{ss}$ (mg L^{-1})
100	1.33	0.90
200	2.16	2.40
300	4.41	5.10
400	15.8	14.4

Table 7.2: Time ($t_{0.9}$) to 90% of Steady State Plasma Concentration $(C_p)_{ss}$ Level as a Function of Daily Dose (R); K_m = 5.7 mg L^{-1}

R (mg day^{-1})	$t_{0.9}$ (days)	$0.9(C_p)_{ss}$ (mg L^{-1})
100	1.90	1.28
200	3.08	3.42
300	6.28	7.27
400	22.5	20.5