Rajiv Gandhi University of Health Sciences, Karnataka, Bangalore

Biopharmaceutics And pharmacokinetics

REZA RAHIMI

DOCTOR OF PHARMACY

kims hospital 2014-15

4.5 BIOPHARMACEUTICS AND PHARMACOKINETICS (THEORY)

Theory : 3 Hrs. /Week

1. Biopharmaceutics

1. Introduction to Biopharmaceutics
   a. Absorption of drugs from gastrointestinal tract.
   b. Drug Distribution.
   c. Drug Elimination.

2. Pharmacokinetics

2. Introduction to Pharmacokinetics.
   a. Mathematical model
   b. Drug levels in blood.
   c. Pharmacokinetic model
   d. Compartment models
   e. Pharmacokinetic study.
3. One compartment open model.
   a. Intravenous Injection (Bolus)
   b. Intravenous infusion.
4. Multicompartment models.
   a. Two compartment open model.
   b. IV bolus, IV infusion and oral administration
5. Multiple – Dosage Regimens.
   a. Repititive Intravenous injections – One Compartment Open Model
   b. Repititive Extravascular dosing – One Compartment Open model
   c. Multiple Dose Regimen – Two Compartment Open Model
6. Nonlinear Pharmacokinetics.
   a. Introduction
   b. Factors causing Non-linearity.
   c. Michaelis-menton method of estimating parameters.
7. Noncompartmental Pharmacokinetics.
   a. Statistical Moment Theory.
   b. MRT for various compartment models.
   c. Physiological Pharmacokinetic model.
8. Bioavailability and Bioequivalence.
   a. Introduction.
   b. Bioavailability study protocol.
   c. Methods of Assessment of Bioavailability

Introduction

Biopharmaceutics is defined as the study of 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.

-The process of movement of drug from its site of administration to the systemic circulation is called as absorption.

-The concentration of drug in plasma and hence the onset of action, and the intensity and duration of response depend upon the bioavailability of drug from its dosage form.

-Bioavailability is defined as the rate and extent (amount) of drug absorption.

-Any alteration in the drug's bioavailability is reflected in its pharmacological effects.

-Other processes that play a role in the therapeutic activity of a drug are distribution and elimination. Together, they are known as drug disposition.

-The movement of drug between one compartment and the other (generally blood and the extravascular tissues) is referred to as drug distribution.

-Elimination is defined as the process that tends to remove the drug from the body and terminate its action.

-Elimination occurs by two processes:

i- Biotransformation (metabolism): which usually inactivates the drug

ii- Excretion, which is responsible for the exit of drug/metabolites from the body.

Pharmacokinetics is the kinetics of ADME or KADME.

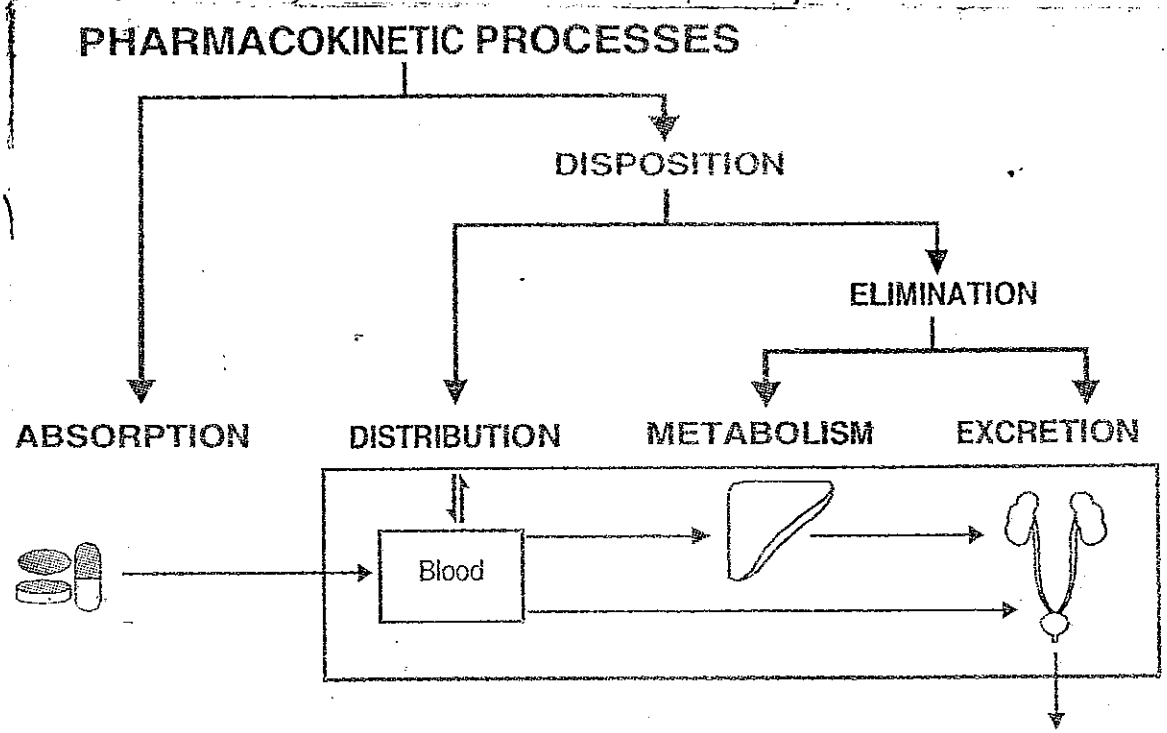


Fig. 1.1 Schematic illustration of pharmacokinetic processes

□ Phases of Drug administration and therapy:

1. The Pharmaceutical Phase:

It is concerned with:

a- Physicochemical properties of the drug

b- Design and manufacture of an effective drug product for administration by a suitable route.

2. The Pharmacokinetic Phase:

- It is the sum of all the processes inflicted by the body on the drug.

3. The pharmacodynamic Phase:

It is concerned with the biochemical and physiological effects of the drug and its mechanism of action.

4. The Therapeutic Phase:

It is concerned with the translation (تنزيل) of pharmacological effect into clinical benefit.

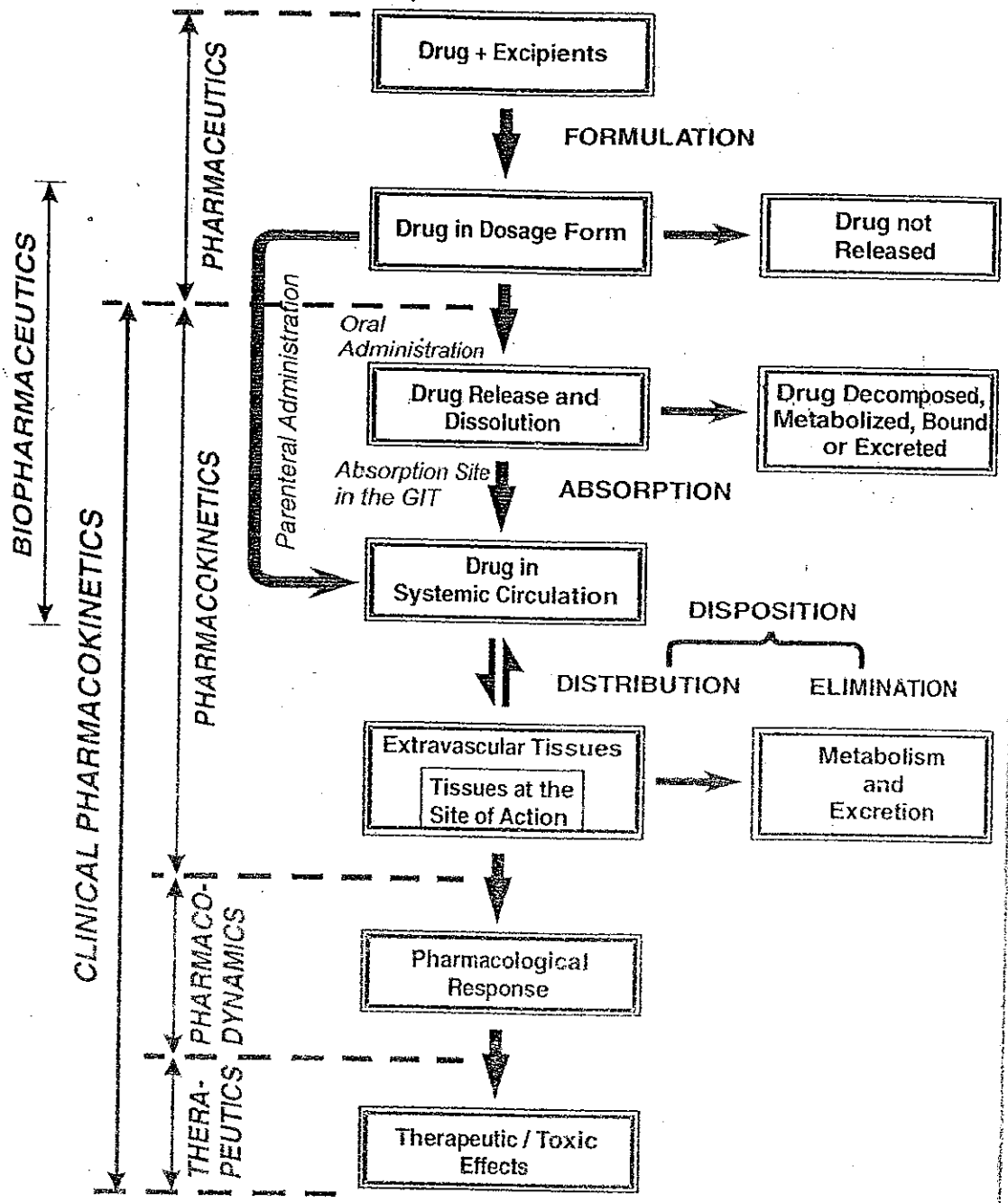
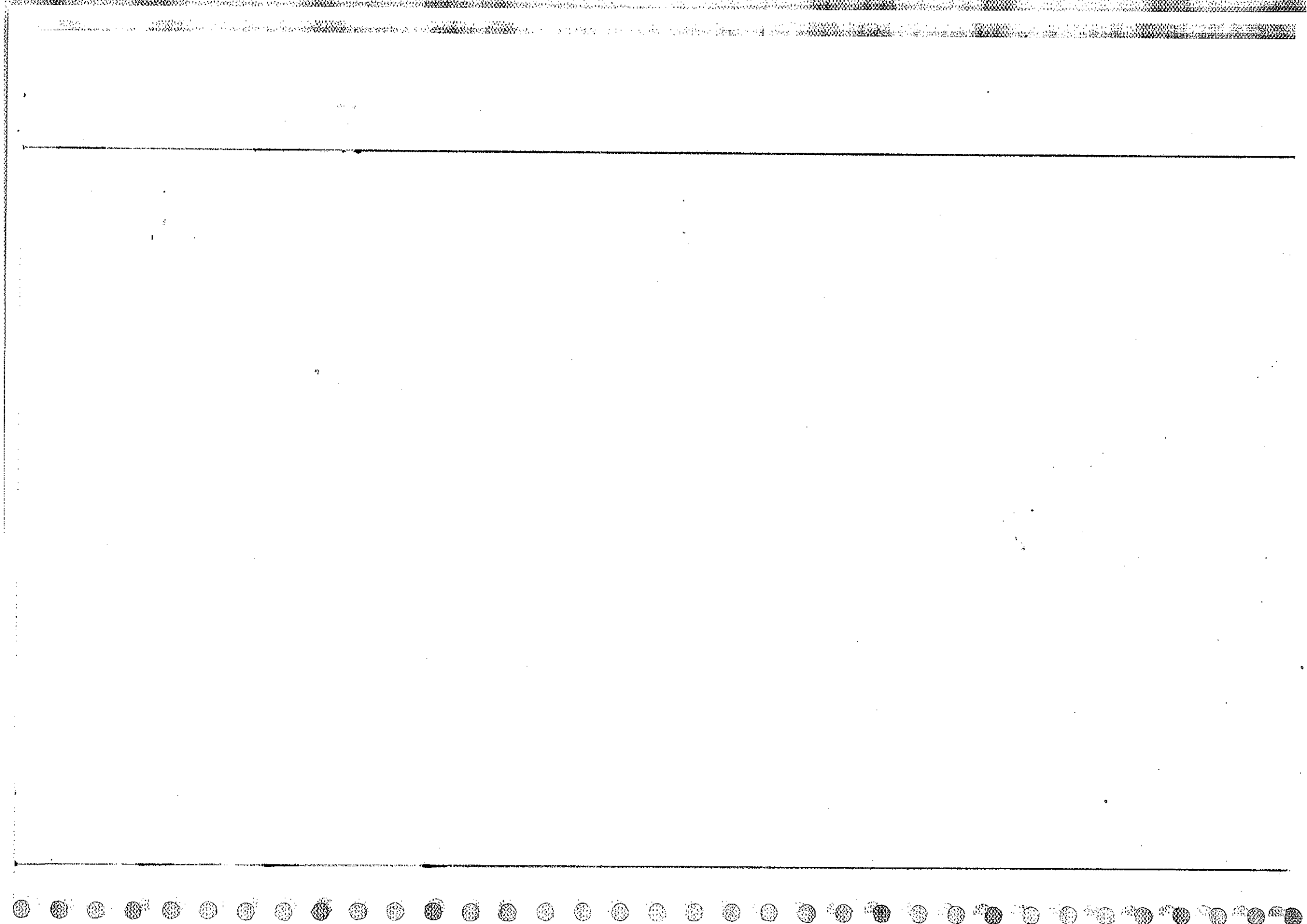


Fig. 1.2 Schematic representation of the processes involved in drug therapeutics

The knowledge and concepts of biopharmaceutics and pharmacokinetics have an integral role in the design and development of new drugs and their dosage forms and improvement of therapeutic efficacy of existing drugs.



Drug absorption is defined as the process of movement of unchanged drug from the site of administration to systemic circulation.

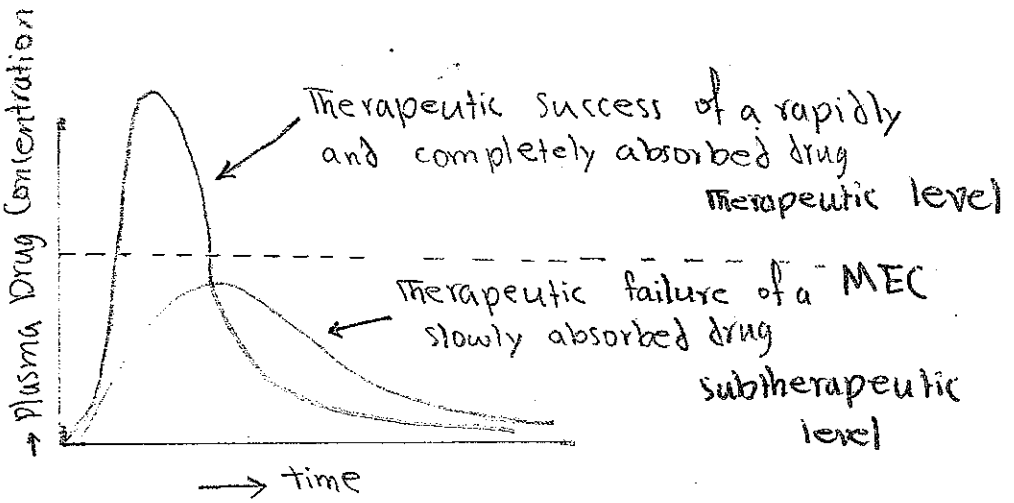


Fig: Plots showing significance of rate and extent of absorption in drug therapy

- A drug that is completely but slowly absorbed may fail to show therapeutic response as the plasma concentration for desired effect is never achieved. On the contrary, a rapidly absorbed drug attains the therapeutic level easily to elicit pharmacological effect. Thus, both the rate and the extent of drug absorption are important.

route of
ad
pharmacology

- Enteral Route:

- The mode of administration of drugs directly into any part of GIT is called enteral route of administration.

- Parenteral Route:

- Administration of drugs by injection or inhalation through lungs other than enteral route is called parenteral administration.

Topical Route:

Drugs administered in the form of creams, pastes, lotions etc are applied to the skin surface.

Bioavailability/Absorption of Drug from Common Routes of Drug Administration

Route	*Bioavailability*	*Advantages*	*Disadvantages*
Parenteral			
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)	• Rapid absorption from aqueous solutions. • Slow absorption from non-aqueous (oily) solutions.	• Easier to inject than intravenous injection. • Larger volumes may be used compared to subcutaneous solution.	• Irritating drugs may be very painful. • Variable rates of absorption depending upon muscle group injected and blood flow.
Subcutaneous injection (SC)	• 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 upon blood flow and injection volume.
Enteral Routes			
Buccal or sublingual (SL)	• Rapid absorption of lipid-soluble drugs.	• No presystemic metabolism.	• Some drug may be swallowed. Not for most drugs or drugs with high doses.

...continued

Route	*Bioavailability*	*Advantages*	*Disadvantages*
Oral (PO)	• Absorption may vary. Generally slower absorption rate compared to IV bolus or IM injection.	• Safest and easiest route of drug administration. • Suitable for both immediate-release and modified-release drug products.	• Some drugs are unstable in GIT, or undergo presystemic metabolism or show erratic absorption.
Rectal (PR)	• Absorption may vary from suppository. • More reliable absorption from enema (solution).	• Useful when patient cannot swallow medication. • Used for local and systemic effects.	• Absorption may be erratic. Suppository may migrate to different position. • Some patient discomfort.
Other Routes			
Transdermal	• 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 presystemic 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	• Rapid absorption. • Total dose absorbed is variable.	• May be used for local or systemic effects.	• Particle size of drug determines anatomic placement in respiratory tract. • May stimulate cough reflex. • Some drug may be swallowed.

#Gastrointestinal Absorption of Drugs

Cell Membrane: Structure and Physiology

For a drug to be absorbed and distributed into organs and tissues and eliminated from the body, it must pass through one or more biological membranes/barriers at various locations. Such a *movement of drug across the membrane* is called as **drug transport**.

The basic structure of cell membrane is shown in Fig. 2.2.

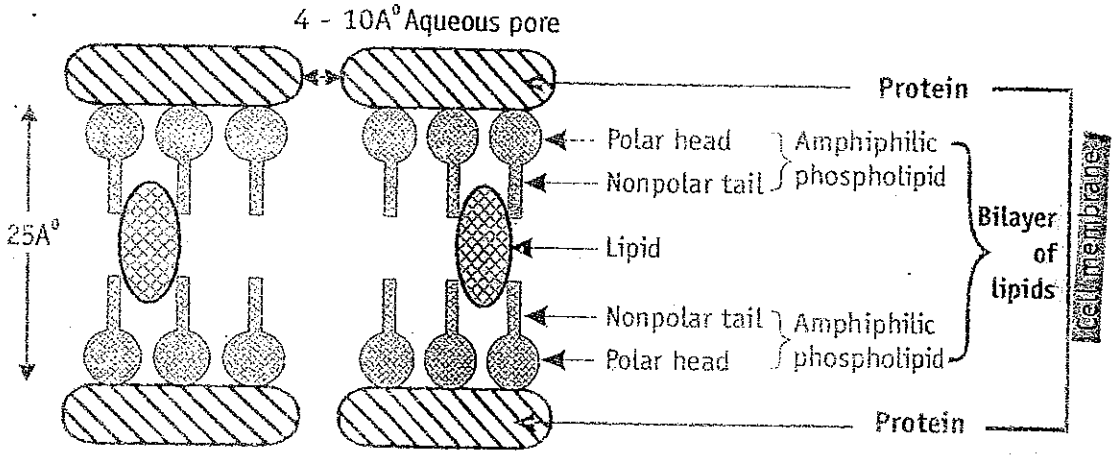


Fig. 2.2 Basic structure of functional cell membrane

The cellular membrane consists of a double layer of amphiphilic phospholipid molecules arranged in such a fashion that their hydrocarbon chains are oriented inwards to form the hydrophobic or lipophilic phase and their polar heads oriented to form the outer and inner hydrophilic boundaries of the cellular membrane that face the surrounding aqueous environment. Globular protein molecules are associated on either side of these hydrophilic boundaries and also interspersed within the membrane structure. In short, the membrane is a *mayonnaise sandwich* where a bimolecular layer of lipids is contained between two parallel monomolecular layers of proteins. The hydrophobic core of the membrane is responsible for the relative impermeability of polar molecules. Aqueous filled pores or perforations of 4 to 10 Å in diameter are also present in the membrane structure through which inorganic ions and small organic water-soluble molecules like urea can pass. In general, the biomembrane acts like a semipermeable barrier permitting rapid and limited passage of some compounds while restricting that of others.

The GI lining constituting the absorption barrier allows most nutrients like glucose, amino acids, fatty acids, vitamins, etc. to pass rapidly through it into the systemic circulation but prevents the entry of certain toxins and medicaments. Thus, for a drug to get absorbed after oral administration, it must first pass through this biological barrier.

Mechanisms of Drug Absorption

- A. Transcellular / Intracellular transport
- B. Paracellular / intercellular transport
- C. Vesicular transport

A. Transcellular / Intracellular Transport:

- is defined as the passage of drugs across the GI epithelium.
- It is the most common pathway for drug transport.

- 3 steps:

i- Permeation of GI epithelial cell membrane, a lipoidal barrier – this is the major obstacle to drug absorption.

ii- Movement across the intracellular space (cytosol)

iii- Permeation of the lateral or basolateral membrane - this is of secondary importance.

- The various transcellular transport processes involved in drug absorption are:

1. Passive Transport Processes:

- These transport processes do not require energy other than that of molecular motion (Brownian motion) to pass through the lipid bilayer.

#Classification:

- Passive diffusion
- Pore transport
- Ion-pair transport
- Facilitated- or mediated diffusion

2. Active Transport Processes:

- These transport processes require energy from ATP to move drug molecules from extracellular to intracellular milieu.

Types:

- Primary active transport
- Secondary active transport
  - Symport (co-transport)
  - Antiport (counter-transport)

B. Paracellular/Intercellular Transport:

- is defined as the transport of drugs through the junctions between the GI epithelial cells.

- This pathway is of minor importance in drug absorption.

Mechanisms:

1. Permeation through tight junctions of epithelial cells:

Ex: insulin, cardiac glycosides

2. Persorption: is permeation of drug through temporary openings.

C- Vesicular or Corpuscular Transport (Endocytosis):

- Like active transport, these are also energy dependent processes but involve transport of substances within vesciles into a cell.

Classification:

- i- Pinocytosis
- ii- Phagocytosis

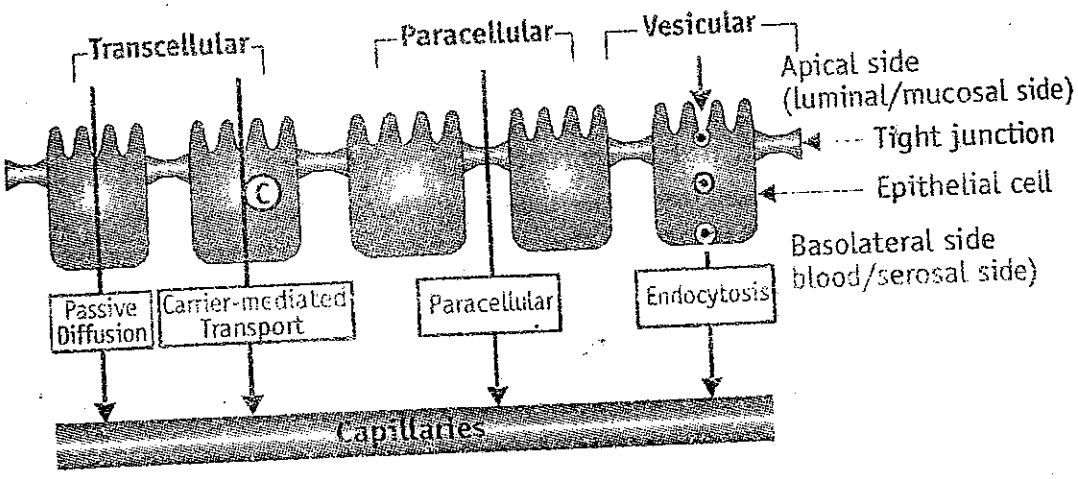


Fig. 2.3 Illustrative comparison of transcellular, paracellular and vesicular transport mechanisms.

-Passive Diffusion:

-Also called non-ionic diffusion.

-The driving force for this process is the concentration or electrochemical gradient. It is defined as the difference in the drug concentration on either side of the membrane.

-Passive diffusion is best expressed by Fick's first law of diffusion, which states that the drug molecules diffuse from a region of higher concentration to one of lower concentration until equilibrium is attained and that the rate of diffusion is directly proportional to the concentration gradient across the membrane.

-mathematically:

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

-where:

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

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 b/w the lipoidal membrane and the aqueous GI fluids (no units)

$(G_{GIT} - C)$ = difference in the concentration of drug in the GI fluids and the plasma, called as the concentration gradient (amount/volume)

h = thickness of the membrane (length)

#Characteristics of passive diffusion, based on the above equation:

1. The drug moves down the concentration

gradient indicating downhill transport.

2. The process is energy-independent and non-saturable.

3. The rate of drug transfer is directly proportional to the concentration gradient b/w GI fluids and the blood compartment.

4. Greater the area and lesser the thickness of the membrane, faster the diffusion; thus, more rapid is the rate of drug absorption from the intestine than from the stomach.

5. The process is rapid over short distances and slower over long distances.

6. Equilibrium is attained when the concentration on either side of the membrane become equal.

7. The rate of transfer of unionised species is 3 to 4 times the rate for ionised drugs.

8. Greater the membrane/water partition coefficient of drug, faster the absorption; since the membrane is lipoidal in nature, a lipophilic drug diffuses at a faster rate by solubilising in the lipid layer of the membrane.

9. The drug diffuses rapidly when the volume of GI fluid is low; conversely, dilution of GI fluids decreases the drug concentration in these fluids (C_{GIT}) and lower the concentration gradient ($C_{GIT} - C$). This phenomenon is, however, made use of in treating cases of oral overdose or poisioning.

10. The diffusion generally decreases with increase in the molecular weight of the compound.

□ Initially, when the drug is ingested, $C_{GIT} \gg C$ and a large concentration gradient exists thereby

acting as the driving force for absorption. As equilibrium approaches, the drug diffusion should stop and consequently a large fraction of drug may remain unabsorbed. But this is not the case; once the passively absorbed drug enters blood, it is rapidly swept away and distributed into a much larger volume of body fluids and hence, the concentration of drug at the absorption site, C_{GIT}, is maintained greater than the concentration of drug in plasma. Such a condition is called as sink condition for drug absorption.

□ Permeability refers to the ease with which a drug can penetrate or diffuse through a membrane.

- Moreover, due to sink conditions, the concentration of drug in plasma C is very small in comparison to C_{GIT}. As a result, last equation, may be simplified to:

$$\frac{dQ}{dt} = PC_{GIT}$$

- Equation is an expression for a first-order process. Thus, passive diffusion follows first-order kinetics. Since a large concentration gradient always exists at the absorption site for passive diffusion, the rate of drug absorption is usually more rapid than the rate of elimination.

Besides, dilution and distribution of the absorbed drug into a large pool of body fluids and its subsequent binding to various tissues are other reasons for elimination being slower than absorption.

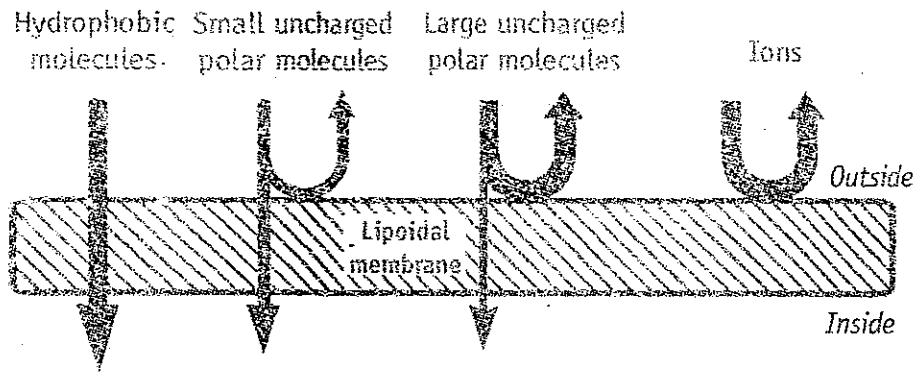


Fig. 2.4 Relative passive diffusion rate of different types of molecules

☐ Pore Transport:

- It is also called as convective transport, bulk flow or filtration.
- This mechanism is responsible for transport of molecules into the cell through the protein channels present in the cell membrane.

-Characteristics:

1. The driving force is constituted by the hydrostatic pressure or the osmotic differences across the membrane due to which bulk flow of water along with small solid molecules occurs through such aqueous channels. Water flux that promotes such a transport is called as solvent drag.

2. The process is important in the absorption of low molecular weight, low molecular size and generally water-soluble drugs through pores in the membrane structure - for ex urea, water and sugars.

- Drug permeation through water-filled channels is of particular importance in renal excretion, removal of drug from the cerebrospinal fluid and entry of drugs into the liver.

Ion-Pair Transport

Yet another mechanism that explains the absorption of drugs like quaternary ammonium compounds and sulphonic acids, which ionise under all pH conditions, is **ion-pair transport**. Despite their low o/w partition coefficient values, such agents penetrate the membrane by forming reversible neutral complexes with endogenous ions of the GIT like mucin. Such neutral complexes have both the required lipophilicity as well as aqueous solubility for passive diffusion. Such a phenomenon is called as ion-pair transport (Fig. 2.5). Propranolol, a basic drug that forms an ion pair with oleic acid, is absorbed by this mechanism.

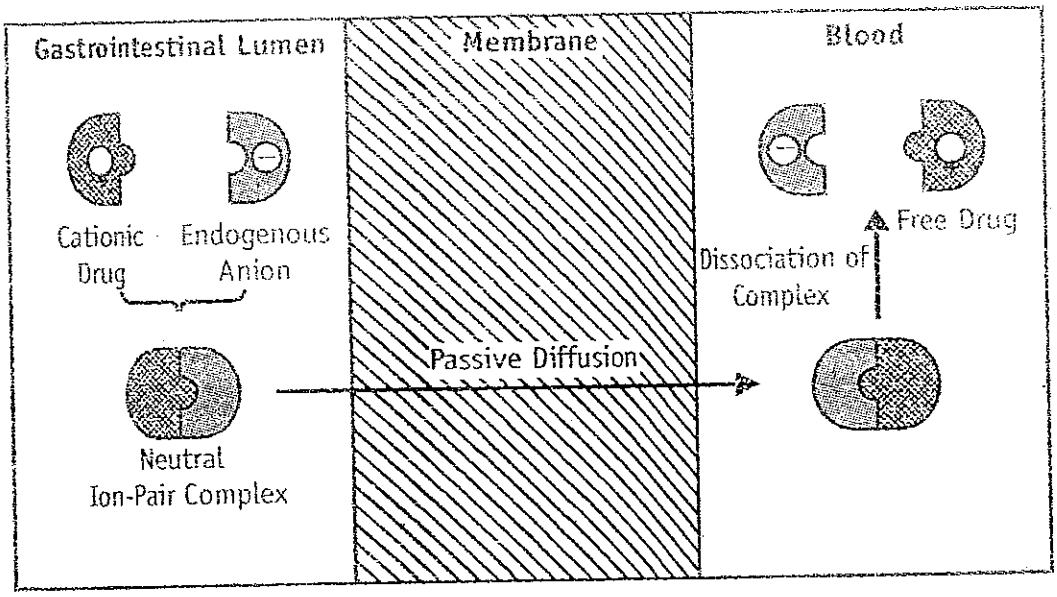


Fig. 2.5 Ion-pair transport of a cationic drug

☐ Carrier-Mediated Transport:

-A component of the membrane called as the carrier that binds reversibly or non-covalently with the solute molecules to be transported. This carrier-solute complex traverses across the membrane to the other side where it dissociates and discharges the solute molecule. The carrier then returns to its original site to complete the cycle by accepting a fresh molecule of solute.

- Characteristics:

1- A carrier protein always has an uncharged (non polar) outer surface which allows it to be soluble within the lipid of the membrane.

2. The carriers have no directionality; they work with same efficiency in both directions.

3. The transport process is structure-specific.

4. Since the system is structure-specific, drugs having structure similar to essential nutrients, called as false nutrients, are absorbed by the same carrier system.

5. As the number of carriers is limited, the transport system is subject to competition b/w agents having similar structure.

6. Since the number of carriers is limited, the system is capacity-limited i.e. at higher drug concentration, the system becomes saturated and approaches an asymptote.

It is important to note that for a drug absorbed by passive diffusion, the rate of absorption increases linearly with the concentration but in case of carrier-mediated processes, the drug absorption increases linearly with concentration until the carriers become saturated after which it becomes curvillinear and approach a constant value at higher doses (Fig 2.6.)

Such a capacity-limited process can be adequately described by **mixed-order kinetics**, also called as **Michaelis-Menten**, **saturation** or **non-linear kinetics**. The process is called mixed-order because it is first-order at sub-saturation drug concentrations and apparent zero-order at and above saturation levels. Moreover, the capacity-limited characteristics of such a system suggest that the bioavailability of a drug absorbed by such a system decrease with increasing dose—for example, vitamins like B_1, B_2 and B_{12}. Hence, administration of a large single oral dose of such vitamins is irrational.

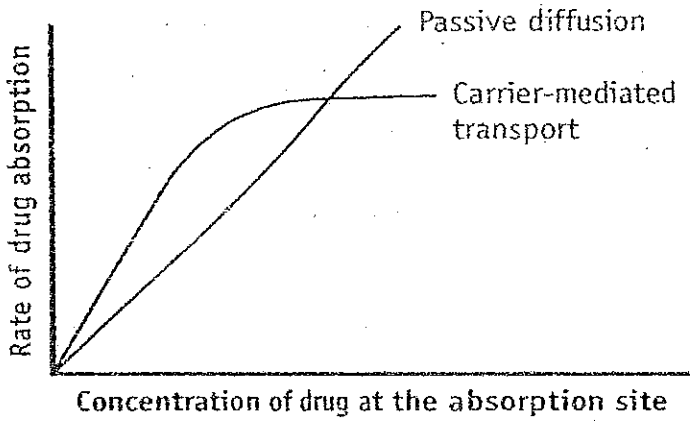


Fig. 2.6 Comparison of rate of absorption versus drug concentration plots for passive and carrier-mediated transport processes

7. Specialized absorption or carrier-mediated absorption generally occurs from specific sites of the intestinal tract which are rich in number of carriers. Such *an area in which the carrier system is most dense* is called as **absorption window**. Drugs absorbed through such absorption windows are poor candidates for controlled release formulations.

Two types of carrier-mediated transport systems have been identified. They are facilitated diffusion and active transport.

Facilitated Diffusion

It is a carrier-mediated transport system that operates down the concentration gradient (*downhill transport*) but at a much faster rate than can be accounted by simple passive diffusion. The driving force is concentration gradient (hence a passive process). Since no energy expenditure is involved, the process is not inhibited by metabolic poisons that interfere with energy production. Facilitated diffusion is of limited importance in the absorption of drugs. Examples of such a

transport system include entry of glucose into RBCs and intestinal absorption of vitamins B_1 and B_2. A classic example of passive facilitated diffusion is the GI absorption of vitamin B_{12}. An intrinsic factor (IF), a glycoprotein produced by the gastric parietal cells, forms a complex with vitamin B_{12} which is then transported across the intestinal membrane by a carrier system (Fig. 2.7).

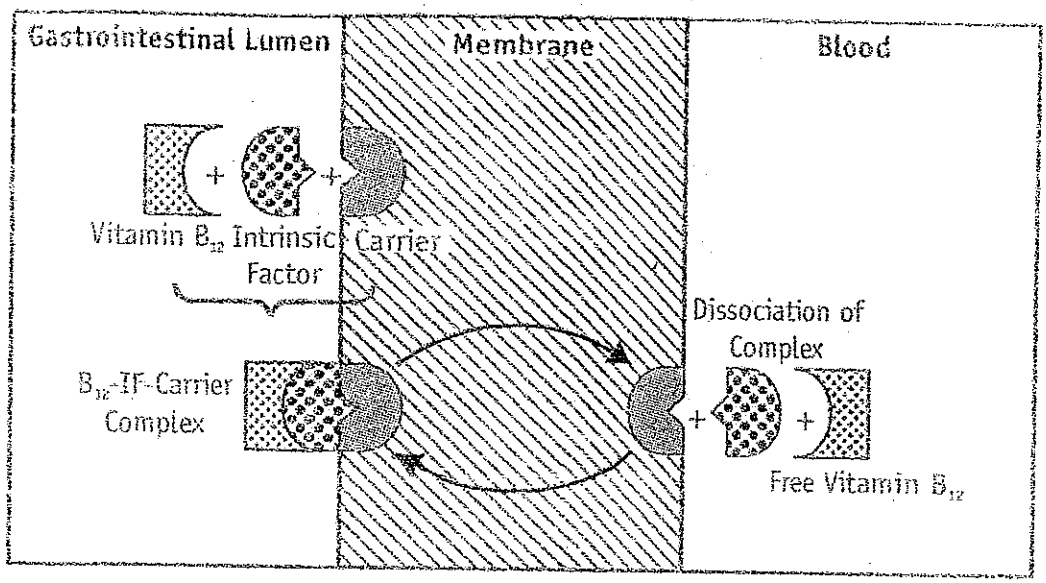


Fig. 2.7 Facilitated diffusion of vitamin B_{12}

- ☐ Active Transport:

-requires energy in the form of ATP.

a) Primary active transport:

-In this process, there is direct ATP requirement.

-Moreover, the process transfers only one ion or molecule and in only one direction, and hence called as uniporter e.g. absorption of Glucose.

-Carrier proteins involved in primary active transport are of two types:

i- Ion transporter:

-Are responsible for transporting ions in or out of cells.

Ex of ion transporter: proton pump

Ex of drug: absorption of diphenhydramine.

ii-ABC (ATP-binding cassette) transporters:

Ex: P-glycoprotein

-is responsible for pumping hydrophobic drugs especially anticancer drugs out of cells.

b. Secondary active transport:

-In these processes, there is no direct requirement of ATP i.e. it takes advantage of previously existing concentration gradient.

-The energy required in transporting an ion aids transport of another ion or molecule (co-transport or coupled transport) either in the same direction or in the opposite direction.

i- Symport (co-transport):

Involves movement of both the molecules in the same direction e.g. Na^+-glucose symporter uses the potential energy of the Na^+ concentration gradient to move glucose against its concentration gradient.

Ex: PEPT1 is implicated in the intestinal absorption of β-lactam antibiotics.

ii- Antiport (counter-transport)

involves movement of molecules in the opposite direction e.g. expulsion of H^+ ions using the Na^+ gradient in the kidneys.

- Active transport is a more important process than facilitated diffusion in the absorption of nutrients and drugs and differs from it in several respects:

1. The drug is transported from a region of lower to one of higher concentration i.e. against the concentration gradient (in the case of ions, against

an electrochemical gradient or uphill transport without any regard for equilibrium.

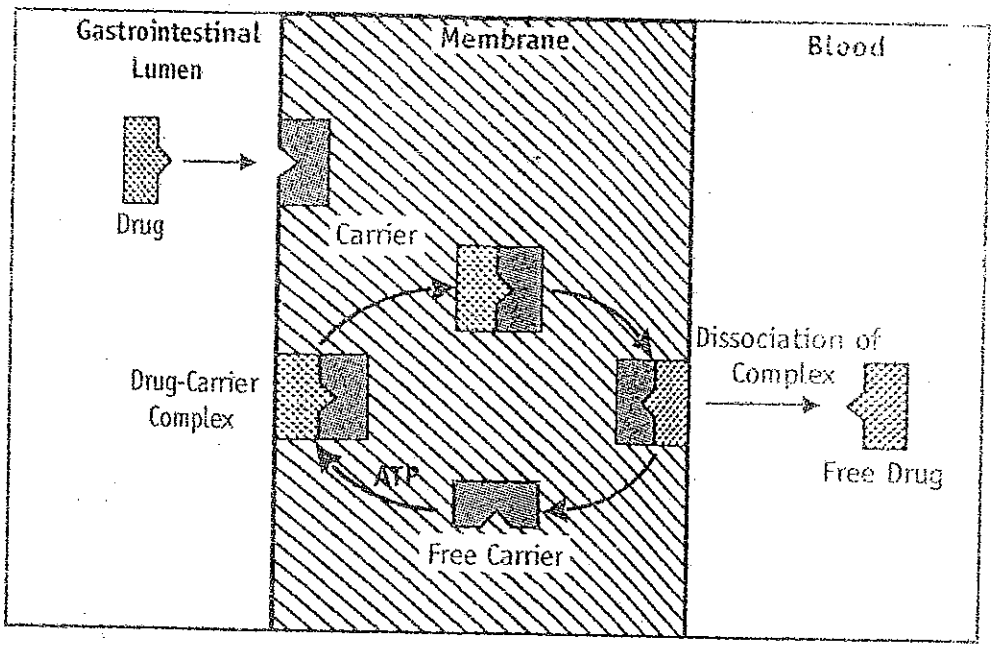


Fig. 2.8 Active absorption of a drug

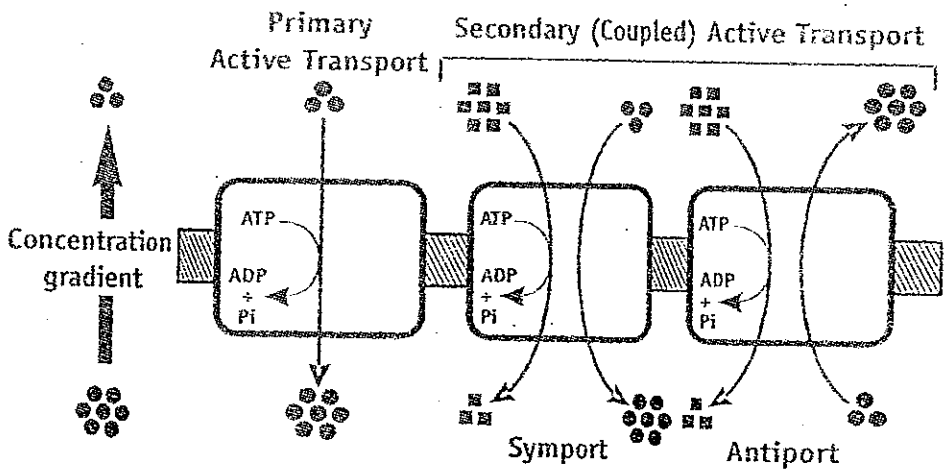


Fig. 2.9 Types of active transport

2. The process is faster than passive diffusion.
3. Since the process is uphill, energy is required in the work done by the carrier.
4. As the process requires expenditure of energy, it can be inhibited by metabolic poisons that interfere with energy production like fluorides, cyanide and dinitrophenol and lack of oxygen. Endogenous substances that are transported actively include sodium, potassium, calcium, iron, glucose, certain amino acids and vitamins like niacin, pyridoxin and ascorbic acid. Drugs having structural similarity to such agents are absorbed actively, particularly the agents useful in cancer chemotherapy. Examples

include absorption of methyl dopa and levo dopa via an L-amino acid transport system etc.

-A good example of competitive inhibition of drug absorption via active transport is the impaired absorption of levodopa when ingested with meals rich in proteins.

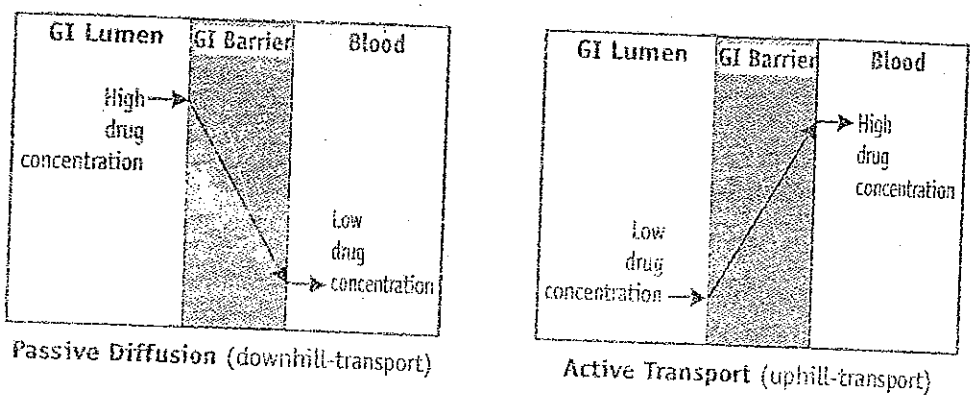


Fig. 2.10 Comparison between active and passive transport

□Endocytosis.

Engulfment of large and highly charged drug molecules by cell membrane.

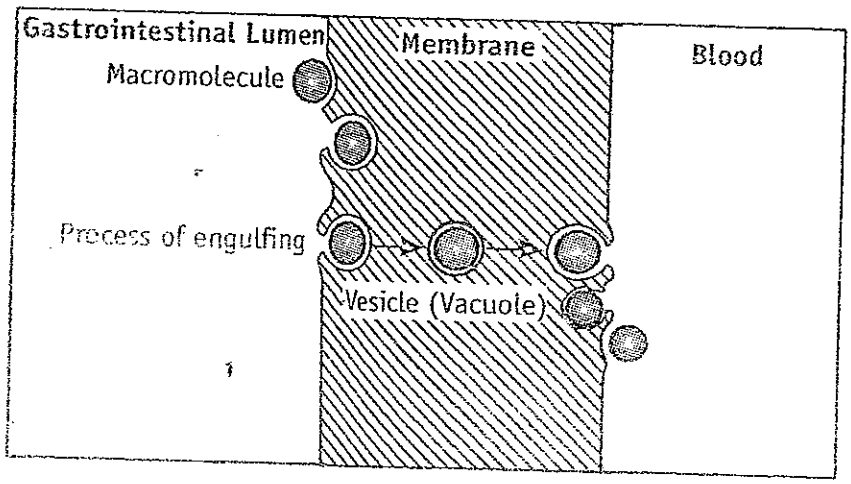


Fig. 2.11 Endocytic uptake of macromolecules.

Ex: Oil soluble vitamins like A, D, E, and K, water soluble vitamin like B_{12} etc.

-Significante:

1- This is the only transport mechanism whereby a drug or compound does not have to be in an aqueous solution in order to be absorbed.

ii- The drug is absorbed into lymphatic circulation thereby bypassing first-pass hepatic metabolism.

Types:

1. Phagocytosis (cell eating): adsorptive uptake of solid particulates.
2. Pinocytosis (cell drinking): uptake of fluid solute

Combined Absorption Mechanisms

A drug might be absorbed by more than just one mechanism—for example, cardiac glycosides are absorbed both passively as well as by

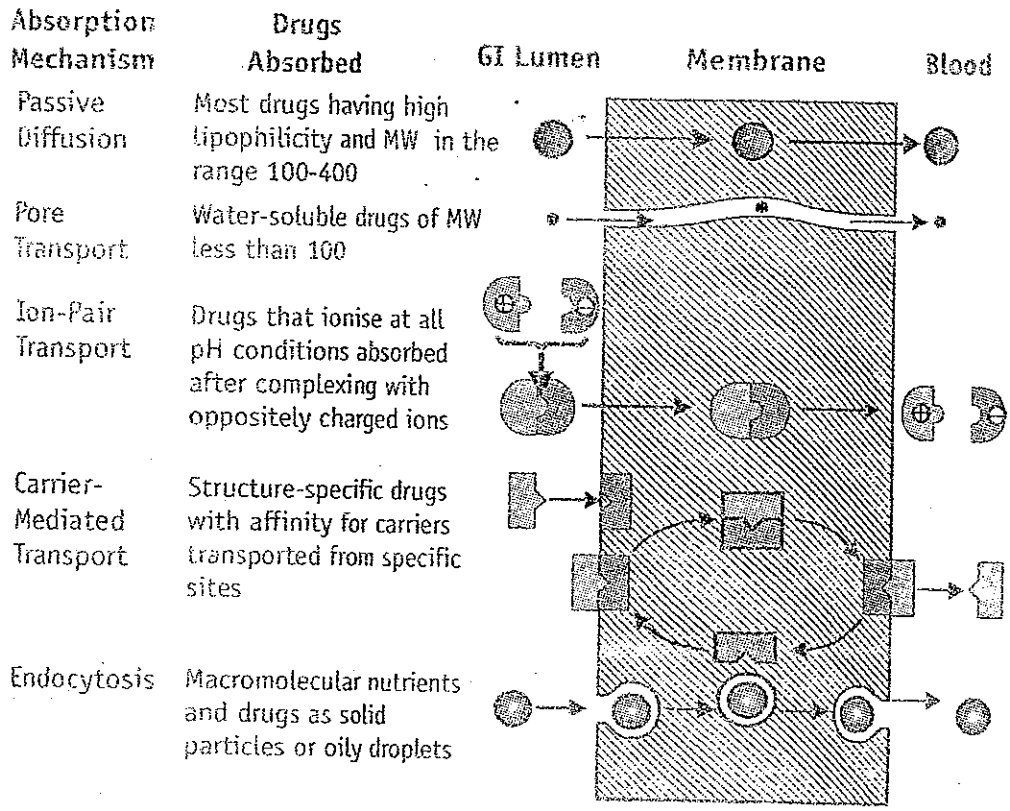


Fig. 2.12 Summary of important transport processes and drugs absorbed through them

active transport. Vitamin B_{12} is absorbed by passive diffusion, facilitated diffusion as well as endocytosis. The transport mechanism also depends upon the site of drug administration (*see* Table 2.10).

Absorption of drugs by various mechanisms is summarized in Figure 2.12.

Phases of Drug Transfer from GI Absorption Site (GI Epithelium) into Systemic Circulation

Absorption of drugs through the GI epithelium can be divided into three phases—

1. ***Pre-uptake phase*** : The two important pre-uptake processes are
   (a) Dissolution of drug in the GI fluids.
   (b) Metabolism of drug in the GI lumen—this can be affected by—
      (i) Digestive enzymes present in the GIT, and/or
      (ii) Bacterial enzymes in the colon.

2. ***Uptake phase*** : The three processes involved in drug uptake are
   (a) Delivery of drug to the absorption site in the GIT.
   (b) Metabolism of drug by enzymes in the GI epithelium (gut wall metabolism).
   (c) Passage of drug through the GI epithelium.
3. ***Post-uptake phase*** : The three important post-uptake processes are—
   (a) Metabolism of drug by the liver, *en route* to the systemic circulation (first-pass hepatic metabolism).
   (b) Enterohepatic circulation of drug – during the first-pass through the liver, the drug may be excreted in the bile, re-enter the GIT via gall bladder and gets reabsorbed.
   (c) Transfer of drug into the systemic circulation.

Routes of Drug Transfer from the Absorption Site in GIT into the Systemic Circulation

A drug is transferred from the absorption site into systemic circulation by one of the two routes—

1. ***Splanchnic circulation*** : is the network of blood vessels that supply the GIT. It is the major route for absorption of drug into the systemic circulation. A drug that enters splanchnic circulation goes to the liver first where it may undergo presystemic metabolism before finally arriving into the systemic circulation. A drug whose uptake is through stomach, small intestine or large intestine goes into the systemic circulation via splanchnic circulation. Rectally administered drugs have direct access to systemic circulation and thus circumvent first-pass effect.
2. ***Lymphatic circulation*** : is a path of minor importance in drug absorption into systemic circulation for two reasons—
   (a) The lymph vessels are less accessible than the capillaries
   (b) The lymph flow is exceptionally slow.

However, fats, fat-soluble vitamins and highly lipophilic drugs are absorbed through lymphatic circulation.

There are three advantages of lymphatic absorption of drugs—

(a) Avoidance of first-pass effect.

(b) Compounds of high molecular weight (above 16,000) can be absorbed by lymphatic transport.

(c) Targeted delivery of drugs to lymphatic system as in certain cases of cancer is possible.

Figure 2.13 represents the transfer of drug to splanchnic and lymphatic circulation after its uptake by the intestinal epithelium.

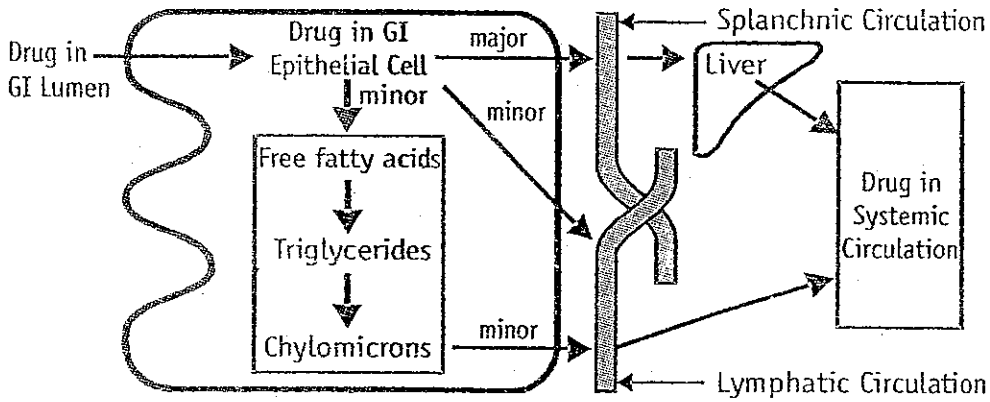


Fig. 2.13 Transfer of drug from intestinal epithelium to splanchnic and lymphatic circulation

Factors influencing Drug Absorption and Bioavailability

(dology

1- Pharmaceutical Factors/Physico-chemical Factors:

a- Physical Properties of Drug:

i- Physical State of Drug:

- It determine the rate and extent of absorption of a drug.

Ex:

Liquid forms of drugs are absorbed better than the solid forms.

ii- Solubility of drug:

-lipid or water solubility of a drug determines the absorption rate of the drug.

Ex:

lipid soluble drugs are absorbed more rapidly than water soluble drugs at cell surface because of lipophilic nature of the cell membrane.

b-Nature of dosage form:

-The order of absorption rate of different dosage form is:

Solution > Suspension > Capsules > Tablet > coated tablet

Solutions → Directly absorbed into Systemic circulation

Suspension and powders → Dissolution → Drug in solution → Absorption

Tablets and capsules → Disintegration → Fine particle of Drugs → Dissolution → Drug in solution → Absorption

C-Disintegration:

Conversion of dosage forms into fine particles before their absorption into systemic circulation is called disintegration.

- It is a rate limiting step for the absorption of lipid soluble drugs.
- The disintegration time determines the rate of break up of a solid dosage form into fine particles.

d. Dissolution:

- Conversion of an active drug from fine particles into drug solution is called dissolution.
- It is a rate limiting step for the release and absorption of drugs from their dosage form.

Theories:

1. Diffusion layer model / Film theory
2. Danckwert's model / Penetration th.
3. Interfacial barrier model

Theory of drug dissolution (10m, 5M)

□ Diffusion Layer Model/ Film theory:
- Simplest and most common theory for dissolution.

given solvent i.e. mass transfer from the solid surface to the liquid phase. Several theories to explain drug dissolution have been proposed. Some of the important ones are:

1. Diffusion layer model/Film theory,
2. Danckwert's model/Penetration or Surface renewal theory, and
3. Interfacial barrier model/Double-barrier or Limited solvation theory.

Diffusion Layer Model/Film Theory

This is the simplest and the most common theory for dissolution. Here, the process of dissolution of solid particles in a liquid, in the absence of reactive or chemical forces, consists of two consecutive steps:

1. Solution of the solid to form a thin film or layer at the solid/liquid interface called as the **stagnant film** or **diffusion layer** which is saturated with the drug. This step is usually rapid.
2. Diffusion of the soluble solute from the stagnant layer to the bulk of the solution. This step is slower and is therefore the rate-determining step in drug dissolution. The model is depicted in Figure 2.16.

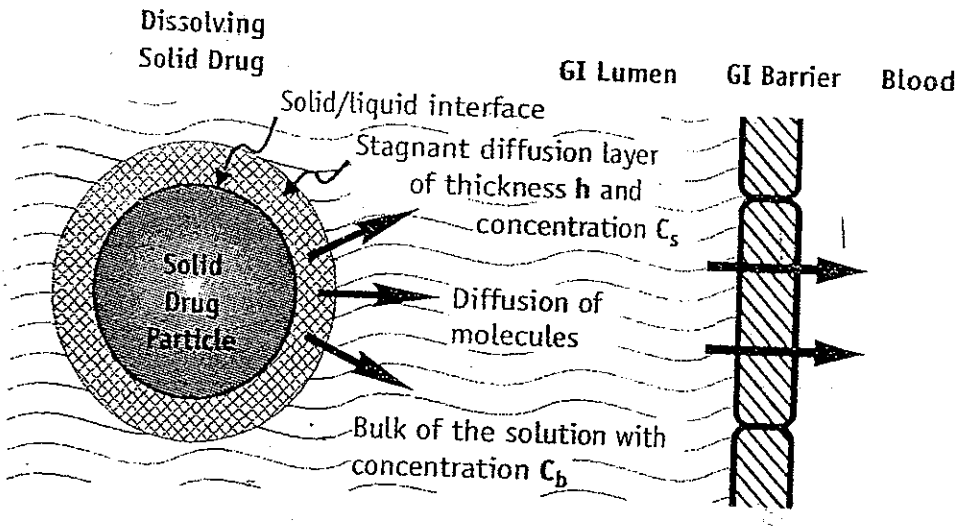


Fig. 2.16 Diffusion layer model for drug dissolution

The earliest equation to explain the rate of dissolution when the process is diffusion-controlled and involves no chemical reaction was given by **Noyes and Whitney**:

$$\frac{dC}{dt} = k\left(C_s - C_b\right) \qquad (2.3)$$

where,

dC/dt = dissolution rate of the drug,

k = dissolution rate constant (first order),

C_s = concentration of drug in the stagnant layer (also called as the **saturation** or **maximum drug solubility**), and

C_b = concentration of drug in the bulk of the solution at time t.

Equation 2.3 was based on Fick's second law of diffusion. **Nernst and Brunner** incorporated Fick's first law of diffusion and modified the Noyes-Whitney's equation to:

$$\frac{dC}{dt} = \frac{DAK_{w/o}(C_s - C_b)}{Vh} \qquad (2.4)$$

where,

D = diffusion coefficient (*diffusivity*) of the drug

A = surface area of the dissolving solid

$K_{w/o}$ = water/oil partition coefficient of the drug considering the fact that dissolution body fluids are aqueous. Since the rapidity with which a drug dissolves depends on the $K_{w/o}$. It is also called as the **intrinsic dissolution rate constant**. It is a characteristic of drugs.

V = volume of dissolution medium.

h = thickness of the stagnant layer.

$(C_s - C_b)$ = concentration gradient for diffusion of drug.

TABLE 2.4

Influence of Some Parameters on Dissolution Rate of Drug

Parameters	*Symbol*	*Influence on drug dissolution*
Diffusion coefficient	D	Greater the value, faster the dissolution of drug. Diffusion decreases as the viscosity of dissolution medium increases.
Surface area of solid	A	Greater the surface area, faster the drug dissolution; can be micronisation of drug.
Water/oil partition coefficient	$K_{w/o}$	Higher the value, more the coefficient of drug and its hydrophilicity and faster the dissolution in aqueous fluids.
Concentration gradient	$(C_s - C_b)$	Greater the concentration gradient, faster the diffusion and drug dissolution; can be increased by increasing drug solubility and the volume of dissolution medium.
Thickness of stagnant layer	h	More the thickness, lesser the diffusion layer and drug dissolution; can be decreased by increasing agitation.

The influence of various parameters in equation 2.4 on drug dissolution is depicted in Table 2.4.

Equation 2.4 represents first-order dissolution rate process, the driving force for which is the concentration gradient ($C_s - C_b$). Under such a situation, dissolution is said to be under non-sink conditions. This is true in case of *in vitro* dissolution in a limited dissolution medium. Dissolution in such a situation slows down after sometime due to build-up in the concentration of drug in the bulk of the solution. The *in vivo* dissolution is always rapid than *in vitro* dissolution because the moment the drug dissolves; it is absorbed into the systemic circulation. As a result, $C_b = 0$, and dissolution is at its maximum. Thus, under *in vivo* conditions, there is no concentration build-up in the bulk of the solution and hence no retarding effect on the dissolution rate of the drug i.e. $C_s >> C_b$ and *sink conditions* are maintained. Under sink conditions, if the volume and surface area of solid are kept constant, then equation 2.4 reduces to:

$$\frac{dC}{dt} = K \qquad (2.5)$$

where K incorporates all the constants in equation 2.4. Equation 2.5 represents that the dissolution rate is constant under sink conditions and follows zero-order kinetics i.e. yields a linear plot (Fig. 2.17).

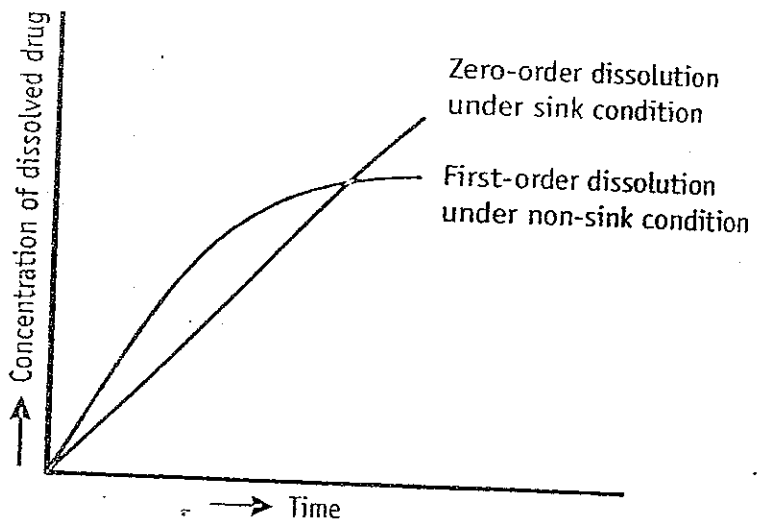


Fig. 2.17 Dissolution rate under non-sink and sink conditions.

To obtain good *in vitro-in vivo* dissolution rate correlation, the *in vitro* dissolution must always be performed under sink conditions. This can be achieved in one or more of the following ways:

1. Bathing the dissolving solid in fresh solvent from time to time.

2. Increasing the volume of dissolution fluid.
3. Removing the dissolved drug by partitioning it from the aqueous phase of the dissolution fluid into an organic phase placed either above or below the dissolution fluid—for example, hexane or chloroform.
4. Adding a water-smiscible solvent such as alcohol to the dissolution fluid.
5. Adding selected adsorbents to remove the dissolved drug.

The *in vitro* sink conditions are so maintained that C_b is always less than 10% of C_s.

The Noyes-Whitney's equation assumes that the surface area of the dissolving solid remains constant during dissolution, which is practically not possible for dissolving particles. Hence, dissolution methods that involve use of constant surface area discs are employed to determine the rate of dissolution.

To account for the particle size decrease and change in surface area accompanying dissolution, **Hixson and Crowell's cubic root law of dissolution** is used:

$$W_0^{1/3} - W^{1/3} = Kt \qquad (2.6)$$

where,

W_o = original mass of the drug
W = mass of the drug remaining to dissolve at time t
K = dissolution rate constant

Danckwert's Model (Penetration or Surface Renewal Theory)

Danckwert did not approve of the existence of a stagnant layer and suggested that turbulence in the dissolution medium exists at the solid/liquid interface. As a result, the agitated fluid consisting of macroscopic mass of eddies or packets reach the solid/liquid interface in a random fashion due to eddy currents, absorb the solute by diffusion and carry it to the bulk of the solution. Such solute containing packets are continuously replaced with new packets of fresh solvent due to which the drug concentration at the solid/liquid interface never reaches C_s and has a lower limiting value of C_i. *Since the solvent packets are exposed to new solid surface each time, the theory is called as* **surface renewal theory.**

The Danckwert's model is expressed by equation:

$$V\frac{dC}{dt} = \frac{dm}{dt} = A(C_s - C_b)\sqrt{\gamma D} \qquad (2.7)$$

where,

m = mass of solid dissolved, and

g = rate of surface renewal (or the interfacial tension).

The model is depicted in Figure 2.18.

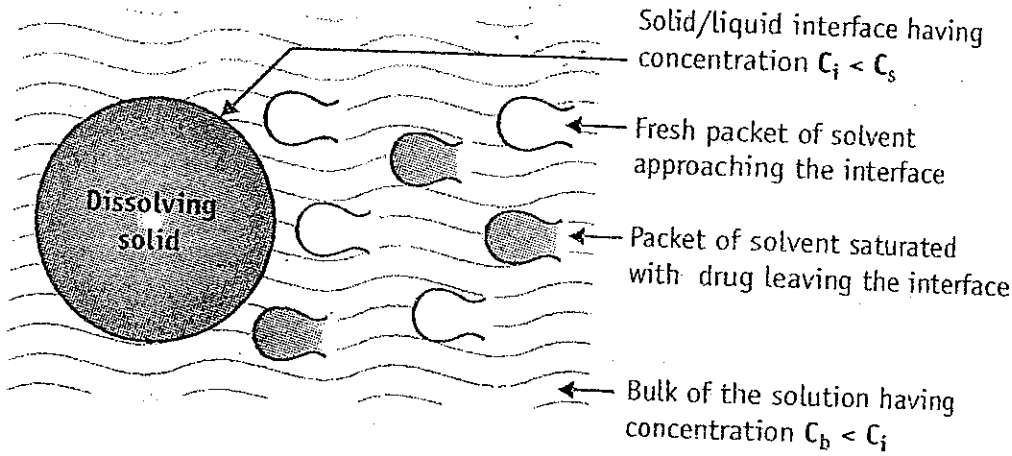


Fig. 2.18 Danckwert's model for drug dissolution

Interfacial Barrier Model (Double Barrier or Limited Solvation Theory)

The diffusion layer model and the Danckwert's model were based on two assumptions:

1. The rate-determining step that controls dissolution is the mass transport.
2. Solid-solution equilibrium is achieved at the solid/liquid interface.

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

$$G = K_i (C_s - C_b) \tag{2.8}$$

where,

G = dissolution rate per unit area, and

K_i = effective interfacial transport constant.

In this theory, the diffusivity D may not be independent of saturation concentration C_s. The interfacial barrier model can be extended to both diffusion layer model and the Danckwert's model

e- Particle size:

-The dissolution of a drug increases with an increase in surface area of the drug particles, which can be achieved by reducing the particle size of the drug.

Ex:
Digoxin.

-Bioavailability of certain drugs may be decreased due to reduction of particle size which leads to their extensive degradation (unstable in gastric fluids)

Ex: Pen G.

f- Crystal form:

-In general, the dissolution rate of amorphus form of a drug is faster than the crystalline form.

g) Salt form of the drug:

Most drugs are either weak acids or weak bases. One of the easiest approaches to enhance the solubility and dissolution rate of such drugs is to convert them into their salt forms. Generally, with weakly acidic drugs, a strong base salt is prepared such as the sodium and potassium salts of barbiturates and sulphonamides. In case of weakly basic drugs, a strong acid salt is prepared like the hydrochloride or sulphate salts of several alkaloidal drugs.

At a given pH, the solubility of a drug, whether acidic/basic or its salt form is a constant. The influence of salt formation on the drug solubility, rate of dissolution and absorption can be explained by considering the pH of the diffusion layer and not the pH of the bulk of the solution (refer diffusion layer theory of drug dissolution). Consider the case of a salt of a weak acid. At any given pH of the bulk of the solution, the pH of the diffusion layer (saturation solubility of the drug) of the salt form of a weak acid will be *higher* than that observable with the free acid form of the drug (can be practically observed in the laboratory). Owing to the increased pH of the diffusion layer, the solubility and dissolution rate of a weak acid in this layer is promoted; since it is a known fact that higher pH favours the dissolution of weak acids. Thus, if dissolution is faster, absorption is bound to be rapid. In case of salts of weak bases, the pH of the diffusion layer will be lower in comparison to that found with the free base form of the drug. Consequently, the solubility of a basic drug at this *lower* pH is enhanced. Thus, if:

$[H^+]_d$ = hydrogen ion concentration of the diffusion layer, and

$[H^+]_b$ = hydrogen ion concentration of the bulk of the solution, then,

for salts of weak acids, $[H^+]_d < [H^+]_b$

for salts of weak bases, $[H^+]_d > [H^+]_b$

The increase and decrease in pH of the diffusion layer by the salts of weak acids and bases have been attributed to the *buffering action* of strong base cation and strong acid anion respectively.

Yet another convincing reason for enhanced solubility of salts of weak acids is the precipitation of the drug as very fine particles. When the soluble ionic form of the drug diffuses from the stagnant diffusion layer into the bulk of the solution whose pH is low, it is transformed into its free acid form having lesser aqueous solubility at the lower pH of the bulk solution. Consequently, this free acidic form of the drug is precipitated in the form of fine particles. The resultant increase in the surface area is then responsible for the rapid dissolution and absorption

in comparison to the drug administered in just the acidic form (Fig. 2.20).

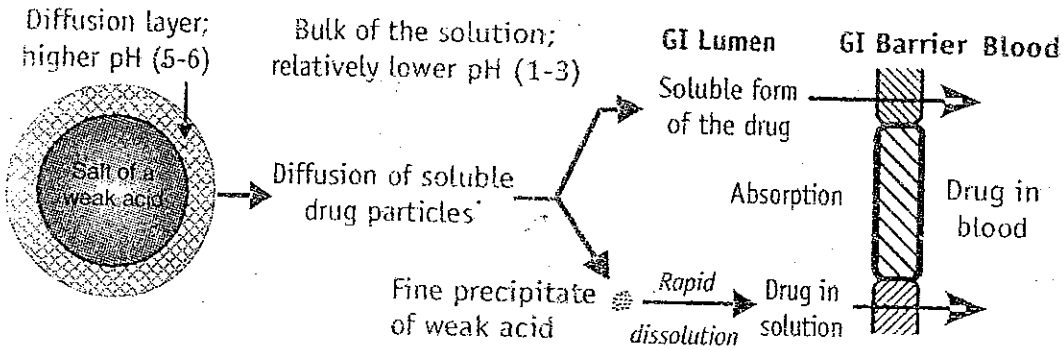


Fig. 2.20 Dissolution and absorption of an acidic drug administered in a salt form

The principle of *in situ salt formation* has been utilized to enhance the dissolution and absorption rate of certain drugs like aspirin and penicillin from buffered alkaline tablets. The approach is to increase the pH of the microenvironment of the drug by incorporating buffer agents and promote dissolution rate. Apart from the enhanced bioavailability, buffered aspirin tablets have two more advantages: firstly, the gastric irritation and ulcerogenic tendency of the drug is greatly reduced, and secondly, the problem with the use of sodium salt of aspirin (to enhance the solubility) which otherwise has poor hydrolytic stability, is overcome by *in situ* salt formation.

The selection of appropriate salt form for better dissolution rate is also important. It has been shown that the choline and the isopropanolamine salts of theophylline dissolve 3 to 4 times more rapidly than the ethylenediamine salt and show better bioavailability.

A factor that influences the solubility of salt forms of the drug is the *size of the counter ion.* Generally speaking, smaller the size of the counter ion, greater the solubility of salt—for example, the bioavailability of novobiocin from its sodium salt, calcium salt and free acid form was found to be in the ratio—50 : 25 : 1. Where the counter ion is very large in size and/or has poor ionic strength (as in the case of ester form of drugs), the solubility may be much lower than the free drug itself—for example, the pamoates, stearates and palmitates of weak bases have poor aqueous solubility. These forms are, however, useful in several ways such as to prolong the duration of action (steroidal salts), to overcome bad taste (chloramphenicol palmitate), to enhance GI stability (erythromycin estolate) or to decrease the side effects, local or systemic.

Drug pK_a and Lipophilicity and GI pH—pH Partition Hypothesis:

The pH partition theory (Brodie *et al*) explains in simple terms, the process of drug absorption from the GIT and its distribution across all biological membranes. *The theory states that for drug compounds of molecular weight greater than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by:*

1. The dissociation constant (pK_a) of the drug.
2. The lipid solubility of the unionised drug (a function of drug $K_{o/w}$).
3. The pH at the absorption site.

Since most drugs are weak electrolytes (weak acids or weak bases), their degree of ionisation depends upon the pH of the biological fluid. If the pH on either side on the membrane is different, then the compartment whose pH favours greater ionisation of the drug will contain greater amount of drug, and only the unionised or undissociated fraction of drug, if sufficiently lipid soluble, can permeate the membrane passively until the concentration of unionised drug on either side of the membrane becomes equal i.e. until equilibrium is attained.

The above statement of the hypothesis was based on the *assumptions* that:

1. The GIT is a simple lipoidal barrier to the transport of drug.
2. Larger the fraction of unionised drug, faster the absorption.
3. Greater the lipophilicity ($K_{o/w}$) of the unionised drug, better the absorption.

Drug pK_a and Gastrointestinal pH

The amount of drug that exists in unionised form is a function of dissociation constant (pK_a) of the drug and pH of the fluid at the absorption site.

It is customary to express the dissociation constants of both acidic and basic drugs by pK_a values. The lower the pK_a of an acidic drug, stronger the acid i.e. greater the proportion of ionised form at a particular pH. The higher the pK_a of a basic drug, the stronger the base. Thus, from the knowledge of pK_a of the drug and pH at the absorption site (or biological fluid), the relative amount of ionised and unionised drug in solution at a particular pH and the percent of drug ionised at this pH can be determined by *Henderson-Hasselbach equations*:

- pH - partition theory + limitation (SM)

for weak acids,

$$pH = pK_a + \log \frac{[\text{Ionised Drug}]}{[\text{Unionised Drug}]} \tag{2.10}$$

$$\% \text{ Drug Ionised} = \frac{10^{(pH - pK_a)}}{1 + 10^{(pH - pK_a)}} 100 \tag{2.11}$$

for weak bases,

$$pH = pK_a + \log \frac{[\text{Unionised Drug}]}{[\text{Ionised Drug}]} \tag{2.12}$$

$$\% \text{ Drug Ionised} = \frac{10^{(pK_a - pH)}}{1 + 10^{(pK_a - pH)}} 100 \tag{2.13}$$

When the concentration of ionised and unionised drug becomes equal, the second term of equations 2.10 and 2.12 reduces to zero (since log 1 = zero), and thus $pH = pK_a$. The pK_a is a characteristic of the drug.

If there is a membrane barrier that separates the aqueous solutions of different pH such as the GIT and the plasma, then the theoretical ratio R of drug concentration on either side of the membrane can be given by equations derived by *Shore et al*:

for weak acids,

$$R_a = \frac{C_{GIT}}{C_{plasma}} = \frac{1 + 10^{(pH_{GIT} - pK_a)}}{1 + 10^{(pH_{plasma} - pK_a)}} \tag{2.14}$$

for weak bases,

$$R_b = \frac{C_{GIT}}{C_{plasma}} = \frac{1 + 10^{(pK_a - pH_{GIT})}}{1 + 10^{(pK_a - pH_{plasma})}} \tag{2.15}$$

If one considers the pH range in the GIT from 1 to 8, that of the stomach from 1 to 3 and of the intestine (from duodenum to colon) 5 to 8, then certain *generalisations* regarding ionisation and absorption of drugs can be made, as predicted from the pH-partition hypothesis:

For Weak Acids:

1. Very weak acids ($pK_a > 8$) such as phenytoin, ethosuximide and several barbiturates are essentially unionised at all pH values and therefore their absorption is rapid and independent of GI pH.

2. Acids in the pK_a range 2.5 to 7.5 are greatly affected by changes in pH and therefore their absorption is pH-dependent; e.g. several NSAIDs like aspirin, ibuprofen, phenylbutazone, and a

TABLE 2.5

Influence of drug pK_a and GI pH on Drug Absorption

Drugs	*pK_a*	*pH/site of absorption*
Very weak acids (pK_a > 8.0)		
Pentobarbital	8.1	Unionised at all pH values; absorbed along the entire length of GIT
Hexobarbital	8.2	
Phenytoin	8.2	
Ethosuximide	9.3	
Moderately weak acids (pK_a 2.5 to 7.5)		
Cloxacillin	2.7	Unionised in gastric pH and ionised in intestinal pH; better absorbed from stomach
Aspirin	3.5	
Ibuprofen	4.4	
Phenylbutazone	4.5	
Stronger acids (pK_a < 2.5)		
Disodium cromoglycate	2.0	Ionised at all pH values; poorly absorbed from GIT.
Very weak bases (pK_a < 5.0)		
Theophylline	0.7	Unionised at all pH values; absorbed along the entire length of GIT
Caffeine	0.8	
Oxazepam	1.7	
Diazepam	3.7	
Moderately weak bases (pK_a 5 to 11.0)		
Reserpine	6.6	Ionised at gastric pH, relatively unionised at intestinal pH; better absorbed from intestine
Heroin	7.8	
Codeine	8.2	
Amitriptyline	9.4	
Stronger bases (pK_a > 11.0)		
Mecamylamine	11.2	Ionised at all pH values; poorly absorbed from GIT
Guanethidine	11.7	

number of penicillin analogs. Such drugs are better absorbed from acidic conditions of stomach ($pH < pK_a$) where they largely exist in unionised form.

3. Stronger acids with pK_a < 2.5 such as cromolyn sodium are ionised in the entire pH range of GIT and therefore remain poorly absorbed.

For Basic Drugs:

1. Very weak bases (pK_a < 5.0) such as caffeine, theophylline and a number of benzodiazepines like diazepam, oxazepam and nitrazepam are essentially unionised at all pH values and therefore their absorption is rapid and pH-independent.
2. Bases in the pK_a range 5 to 11.0 are greatly affected by changes in pH and hence their absorption is pH-dependent; e.g. several morphine analogs, chloroquine, imipramine and amitriptyline. Such drugs are better absorbed from the relatively alkaline conditions of the intestine where they largely exist in unionised form.
3. Stronger bases with pK_a > 11.0 like mecamylamine and guanethidine are ionised in the entire pH range of GIT and therefore poorly absorbed.

A summary of above discussion is given in Table 2.5.

By using equations from 2.10 to 2.15, one can calculate the relative amounts of unionised (absorbable) and ionised (unabsorbable) forms of the drug and predict the extent of absorption at a given pH of GIT. An example of this is illustrated in figure 2.21.

Drug	Stomach pH = 1.5	← Membrane Barrier → Plasma pH = 7.4	Intestine pH = 5.0
Weak acid e.g. Ibuprofen pKa = 4.4	[HA] = 100.00 ⇌ [A⁻] = 0.13 [Total] = 100.13	[HA] = 100 ⇌ [A⁻] = 100,000 [Total] = 100,100	[HA] = 100.00 ⇌ [A⁻] = 398.10 [Total] = 498.10
Weak base e.g. Nitrazepam pKa = 3.2	[BOH] = 100 ⇌ [B+] = 5012 [Total] = 5112	[BOH] = 100.000 ⇌ [B+] = 0.006 [Total] = 100.006	[BOH] = 100.60 ⇌ [B+] = 1.60 [Total] = 101.60

Fig. 2.21 Influence of pH on ionisation of drug. [HA] and [BOH] are concentration of unionised acid and base, and [A^-] and [B^+] are concentration of ionised acid and base respectively

Besides the dissociation constant pK_a, *total aqueous solubility*, S_T, of an ionisable drug is an important factor in the passive absorption of drugs. *It is defined as the sum of concentration of ionised drug in solution and concentration of unionised drug in solution. The solubility of unionised form of the drug is known as the* ***intrinsic solubility*** *of the drug.* If S_a is the intrinsic solubility of weakly acidic drugs and S_b that of weakly basic drugs, then—

for acidic drugs,

$$S_T = S_a\,[1 + 10^{(pH - pKa)}] \qquad (2.16)$$

for basic drugs,

$$S_T = S_b\,[1 + 10^{(pKa - pH)}] \qquad (2.17)$$

Figure 2.22 illustrates pH-solubility profile for a free acid and free base of weakly acidic and weakly basic drugs.

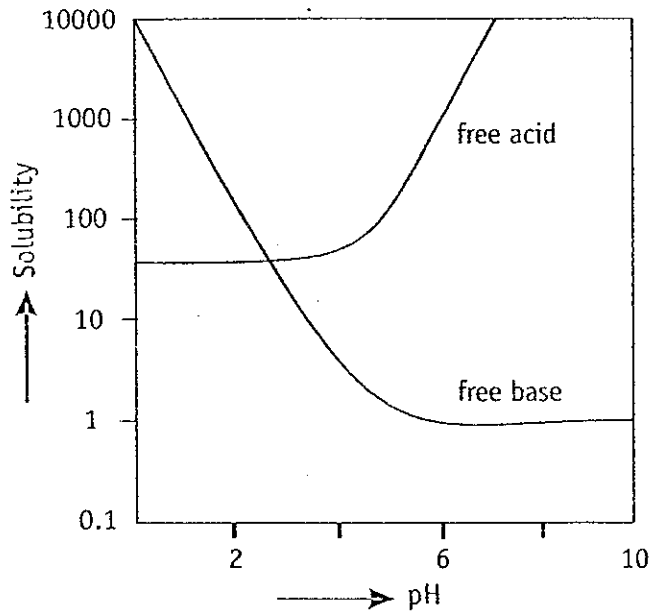


Fig. 2.22 pH-solubility curve for weakly acidic and weakly basic drugs

Certain conclusions and generalizations can now be stated—

For weakly acidic drugs,

1. When pH > pKa, $S_T >> S_a$ because ionisation of drug increases tremendously.
2. When pH = pKa, $S_T = 2S_a$, because the drug is 50% ionised.
3. When pH < pKa, $S_T \cong S_a$ since the drug exists predominantly in unionised form.

For weakly basic drugs,

1. When pH > pKa, $S_T \cong Sb$ since the drug exists predominantly in unionised form.
2. When pH = pKa, $S_T = 2S_b$, because the drug is 50% ionised.
3. When pH < pKa, $S_T >> S_b$ because ionisation of drug increases tremendously.

□ Lipophilicity and Drug Absorption:

- If the drug exists in the unionised form, it will be proorly absorbed if it has poor lipid solubility (or low $K_{o/w}$).
- Ideally, for optimum absorption, a drug should have sufficient aqueous solubility to dissolve in the fluids at the absorption site and lipid solubility ($K_{o/w}$) high enough to facilitate the partitioning of the drug in the lipoidal biomembrane and into the systemic circulation.
- In other words, a perfect hydrophilic-lipophilic balance (HLB) should be there in the structure of the drug for optimum bioavailability.
- The lipid solubility of a drug is measured by a parameter called as log P where P is oil/water partition coefficient ($K_{o/w}$ or simply P) value of the drug.
- This value is a measure of the degree of distribution of drug b/w solvents and an aqueous phase.

-For ionisable drugs where the ionised species does not partition into the aqueous phase, the apparent partition coefficient (D) can be calculated from following equations:

-for acidic drugs:

$$\log D = \log P - \log [1 + 10^{(pH - pK_a)}]$$

for basic drugs:

$$\log D = \log P - \log [1 + 10^{(pK_a - pH)}]$$

Limitations of pH-Partition Hypothesis

The pH-partition hypothesis over-simplified the otherwise complicated process of drug absorption and therefore has its own limitations. Some of the deviations from the theory are:

1. Presence of virtual membrane pH
2. Absorption of ionised drug
3. Influence of GI surface area and residence time of drug
4. Presence of aqueous unstirred diffusion layer

1. Presence of virtual membrane pH : The pH-partition hypothesis suggested that only the unionised drug at a given GI lumen pH is absorbed. An S-shaped curve, called as the **pH-absorption curve** denoting the dissociation of drug, is obtained when pH is plotted versus rate of drug absorption (Fig. 2.23).

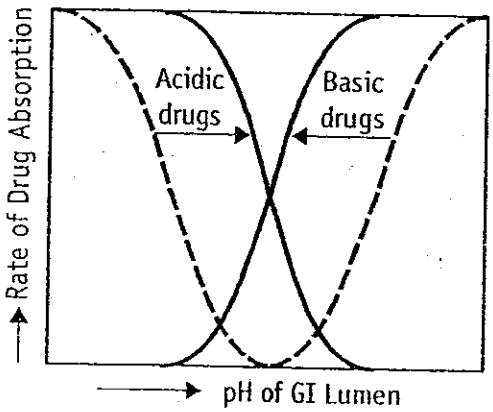


Fig. 2.23 pH-absorption curve for acidic and basic drugs. Dotted lines indicate curves predicted by pH-partition hypothesis and bold lines indicate the practical curves.

However, differences in the extent of absorption of salicylic acid have been observed at a given GI pH than that predicted by pH-partition hypothesis. The experimental pH-absorption curves are less steep and shift to the left (lower pH values) for a basic drug and to the right (higher pH values) for an acidic drug. This led to the suggestion that a virtual pH, also called as the microclimate pH, different from the luminal pH exists at the membrane surface. This virtual membrane pH actually determines the extent of drug ionisation and thus, drug absorption.

2. Absorption of ionised drugs : An important assumption of the theory was that only unionised form of the drug is absorbed and permeation of the ionised drug is negligible since its rate of absorption is 3 to 4 times less than that of unionised drug. This is called as ***principle of non-ionic diffusion***. The principle is true to a large extent as ionised drugs have low lipid solubility and relatively poor permeability. However, the pH-absorption curve shift suggested that ionised forms of some drugs also get absorbed to a considerable extent. If such drugs have a large lipophilic group in their structure, despite their ionisation, they will

be absorbed passively—for example, morphinan derivatives. Other mechanisms are also involved in the absorption of ionised drugs such as active transport, ion-pair transport and convective flow.

3. Influence of GI surface area and residence time of drug : According to the pH-partition theory, acidic drugs are best absorbed from stomach (acidic pH) and basic drugs from intestine (alkaline pH) in which conditions they are unionised to a large extent. This could be true under conditions where the surface area of stomach and intestine are same. It could also mean that once an acidic drug reaches the intestine, the remaining fraction will be poorly absorbed and that unless a basic drug reaches the intestine and gets absorbed considerably, it may not be able to attain its therapeutic level. But, irrespective of the GI pH and the degree of ionisation, both acidic and basic drugs are more rapidly absorbed from the intestine, primarily because of its large surface area and secondly, because of long residence time of the drug in the intestine.

4. Presence of aqueous unstirred diffusion layer : The pH-shift in the absorption of acidic and basic drugs, as discussed earlier, also accounts for the fact that the bulk of the luminal fluid is not in direct contact with the membrane but a barrier called as aqueous unstirred diffusion layer is interposed between them. Such a model is depicted in Fig. 2.24.

Such a layer has a real thickness and is a barrier to absorption of drugs. In the original pH-partition theory, the rate-limiting step in the absorption of drugs was the partitioning in the lipid barrier. With the incorporation of unstirred aqueous diffusion layer, a drug must diffuse first through this aqueous barrier and then through the lipoidal barrier. Thus, drugs having large partition coefficient can rapidly penetrate the lipid membrane but diffusion through unstirred water layer is the rate-limiting step in their absorption. This applies in particular to high molecular weight fatty acids and bile acids.

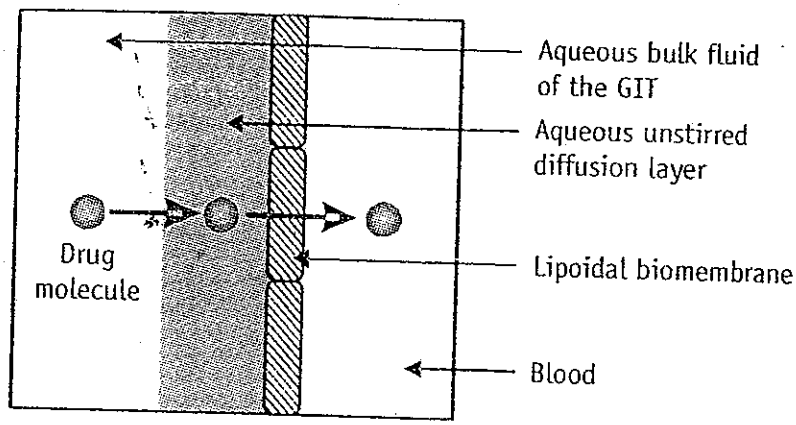


Fig. 2.24 Presence of aqueous unstirred diffusion layer on the membrane surface

Despite its limitations, the pH-partition theory is still useful in the basic understanding of drug absorption and movement of drug between various body compartments.

II) Dosage Form (pharmaco-technical) Factors:

-Disintegration Time (DT):

DT of a tablet is directly related to the amount of binder present and the compression force (hardness) of a tablet.

-A harder tablet with large amount of binder has a long DT.

-Disintegration can be aided by incorporating disintegrants in suitable amounts during formulation.

-Manufacturing / Processing Variables:

-The dosage form related factors that influence dissolution and hence absorption of a drug are:

1. Excipients
2. Manufacturing processes:
   - i- method of granulation
   - ii- compression force

-Method of Granulation:

The wet granulation process is the most conventional technique in the manufacture of tablets and was once thought to yield tablets that dissolve

faster than those made by other granulation methods.

-limitations:

i-The liquid may act as a medium for affecting chemical reactions such as hydrolysis.

ii-The drying step may harm the thermolabile drugs.

-APOC method: The process involves grinding of drugs in a ball mill for time long enough to affect spontaneous agglomeration. The tablets so produced were stronger and showed rapid rate of dissolution in comparison to tablets made by other methods.

-The reason attributed to it was an increase in the internal surface area of the granules prepared by APOC method.

Compression Force : The compression force employed in tabletting process influence density, porosity, hardness, disintegration time and dissolution of tablets. The curve obtained by plotting compression force versus rate of dissolution can take one of the 4 possible shapes shown in Fig. 2.25.

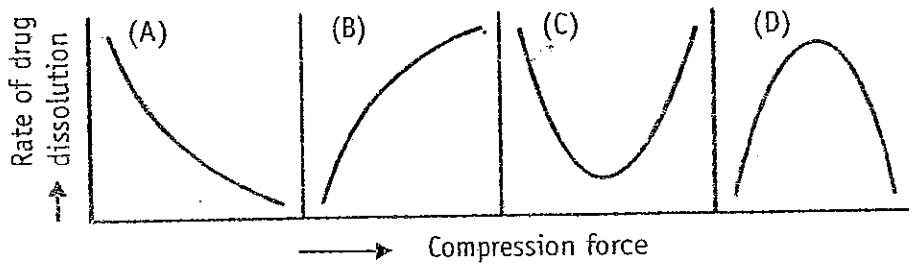


Fig. 2.25 Influence of compression force on dissolution rate of tablets

On the one hand, higher compression force increases the density and hardness of tablet, decreases porosity and hence penetrability of the solvent into the tablet, retards wettability by forming a firmer and more effective sealing layer by the lubricant, and in many cases, promotes tighter bonding between the particles, all of which result in slowing of the dissolution rate of tablets (curve A of Fig. 2.25). On the other hand, higher compression forces cause deformation, crushing or fracture of drug particles into smaller ones or convert a spherical granule into a disc shaped particle with a large increase in the effective surface area. This results in an increase in the dissolution rate of the tablet (curve B of Fig. 2.25). A combination of both the curves A and B is also possible as shown in curves C and D. In short, the influence of compression force on the dissolution rate is difficult to predict and a thorough study on each formulation should be made to ensure better dissolution and bioavailability.

Intensity of packing of capsule contents : Like the compression force for tablets, packing density in case of capsule dosage form can either inhibit or promote dissolution. Diffusion of GI fluids into the tightly filled capsules creates a high pressure within the capsule resulting in rapid bursting and dissolution of contents. Opposite is also possible. It has been shown that capsules with finer particles and intense packing have poor drug release and dissolution rate due to a decrease in pore size of the compact and poor penetrability by the GI fluids.

☐ Pharmaceutical Ingredients/Excipients:

- Excipients are added to ensure acceptability, physico-chemical stability during the shelf-life, uniformity of composition and dosage, and optimum bioavailability and functionality of the drug product.
- Despite their inertness and utility in the dosage form, excipients can influence absorption of drugs.

-Vehicle:

- The 3 categories of vehicles:

i- Aqueous vehicles (water, syrup etc).

ii- non-aqueous water-miscible V (sorbitol)

iii- Non-aqueous water-immiscible V (vegetable oil)

- Bioavailability of a drug from vehicles depends to a large extent on its miscibility with biological fluids.
- Aqueous and water miscible vehicles are miscible with the body fluids and drugs from them are rapidly absorbed.

- Quite often, a drug is more soluble in water miscible vehicles like propylene glycol (serving as a co-solvent) and show better bioavailability.

- Solubilisers such as polysorbate 80 are sometimes used to promote solubility of a drug in aqueous vehicles.

- In case of water immiscible vehicles, the rate of drug absorption depends upon it's partitioning from the oil phase to the aqueous body fluids, which could be a rate-limiting step.

- Viscosity of the vehicles is another factor in the absorption of drugs.

- Diluents (fillers):

→ Organic: Starch, lactose etc.)

These hydrophilic powders are very useful in promoting the dissolution of poorly water-soluble, hydrophobic drugs like

Spironolactone by forming a coat onto the hydrophobic surface of drug particles and render them hydrophilic.

ii - Inorganic : Dicalcium phosphate (DCP)

One classic example of drug - diluent interaction resulting in poor bioavailability is that of tetracycline and DCP. The cause is formation of divalent calcium - tetracycline complex which is poorly soluble and thus, unabsorbale.

- Binders and granulating agents:

- Are used to hold powders together to form granules.

Ex: Starch, acacia etc.

- In general, like fillers, the hydrophilic (aqueous) binders show better dissolution profile with poorly wettable drugs like phenacetin by imparting hydrophilic propertise to the granule surface.

-However, the proportion of strong binders in the tablet formulation is very critical. Large amounts of such binders increase hardness and decrease disintegration/dissolution rates of tablets.

▫Disintegrants:

-These agents overcome the cohesive strength of tablet and break them up on contact with water which is an important prerequisite to tablet dissolution.

-Almost all the disintegrants are hydrophilic in nature.

-A decrease in the amount of disintegrant can significantly lower bioavailability.

Ex:
Bentonite

▫Lubricants/antifrictional agents.

-Decreases the friction between the granules of and die wall.

Ex:
Talc
mineral oil.

-The commonly used lubricants are hydrophobic in nature and known to inhibit wettability, penetration of water into tablet and their disintegration and dissolution.

-The best alternative is use of soluble lubricants like SLS and carbowaxes which promote drug dissolution.

Coatings:

Enteric coat > Sugar coat > Non-enteric film coat

-The dissolution profile of certain coating materials change on aging; e.g. shellac coated tablets, on prolonged storage, dissolve more slowly in the intestine.

Suspending agents/viscosity imparters:

-Popular suspending agents are hydrophilic polymers like acacia, tragacanth etc. which primarily stabilize the solid drug particles by reducing their rate of settling through an increase in the viscosity of the medium.

-Mechanism:

1-The macromolecular gums often form unabsorbable complexes with drugs - for ex, sodium CMC forms a poorly soluble complex with amphetamine.

2. An increase in viscosity by these agents acts as a mechanical barrier to the diffusion of drug from the dosage form into the bulk of GI fluids and from GI fluids to the mucosal lining by forming a viscid layer on the GI mucosa. They also retard the GI transit of drugs.

-Surfactants:

Surfactants are widely used in formulations as wetting agents, solubilisers, emulsifiers, etc.

-They may enhance or retard drug absorption.

-Mechanisms involved in the increased absorption of drug by use of surfactants include:

1. Promotion of wetting and dissolution of drugs e.g. polysorbate 80 with phenacetin.

2. Better membrane contact of the drug for absorption.

3. Enhanced membrane permeability of the drug.

- Decreased absorption of drug in the presence of surfactants :

1. Formation of unabsorbable drug-micelle complex at surfactant concentrations above critical micelle concentration.

2. Laxative action induced by a large surfactant concentration.

Buffers : Buffers are sometimes useful in creating the right atmosphere for drug dissolution as was observed for buffered aspirin tablets. However, certain buffer systems containing potassium cations inhibit the drug absorption as seen with vitamin B_2 and sulphanilamide. The reason attributed to it was the uptake of fluids by the intestinal epithelial cells due to which the effective drug concentration in the tissue is reduced and the absorption rate is decreased. Such an inhibitory effect of the various buffer cations on the drug transfer rate is in the following order:

$$K^+ > NH_4^+ > Li^+ > Na^+ > TRIS^+$$

Hence, the buffer system for a salt of a drug should contain the same cation as the drug salt and introduce no additional cations.

Complexing agents : Complex formation has been used to alter the physicochemical and biopharmaceutical properties of a drug. A complexed drug may have altered stability, solubility, molecular siz partition coefficient and diffusion coefficient. Basically, such c

plexes are pharmacologically inert and must dissociate either at the absorption site or following absorption into the systemic circulation.

Several examples where *complexation has been used to enhance drug bioavailability* are:

1. Enhanced dissolution through formation of a soluble complex e.g. ergotamine tartarate-caffeine complex and hydroquinone-digoxin complex.
2. Enhanced lipophilicity for better membrane permeability e.g. caffeine-PABA complex.
3. Enhanced membrane permeability e.g. enhanced GI absorption of heparin (normally not absorbed from the GIT) in presence of EDTA which chelates calcium and magnesium ions of the membrane.

Complexation can be deleterious to drug absorption due to formation of poorly soluble or poorly absorbable complex e.g. complexation of tetracycline with divalent and trivalent cations like calcium (milk, antacids), iron (haematinics), magnesium (antacids) and aluminium (antacids).

Reasons for poor bioavailability of some complexes are—

1. Failure to dissociate at the absorption site, and
2. Large molecular size of the complex that cannot diffuse through the cell membrane—for example, drug-protein complex.

Colorants : Even a very low concentration of water-soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs. The dye molecules get adsorbed onto the crystal faces and inhibit drug dissolution—for example, brilliant blue retards dissolution of sulphathiazole. Dyes have also been found to inhibit micellar solubilisation effect of bile acids which may impair the absorption of hydrophobic drugs like steroids. Cationic dyes are more reactive than the anionic ones due to their greater power for adsorption on primary particles.

Precipitation/crystal growth inhibitors : When a significant increase in *free drug concentration* above saturation or equilibrium solubility occurs, it results in supersaturation which in turn leads to drug precipitation or crystallization. Precipitation or crystal growth inhibitors such as PVP, HPMC, PEG, PVA (polyvinylalcohol) and similar such hydrophilic polymers prevent or prolong supersaturation thus preventing precipitation or crystallization by—

1. Increasing the viscosity of vehicle.

2. Preventing conversion of a high-energy metastable polymorph into stable, less soluble polymorph.
3. Adsorbing on the faces of crystal thus reducing crystal growth.

Nature and Type of Dosage Form:

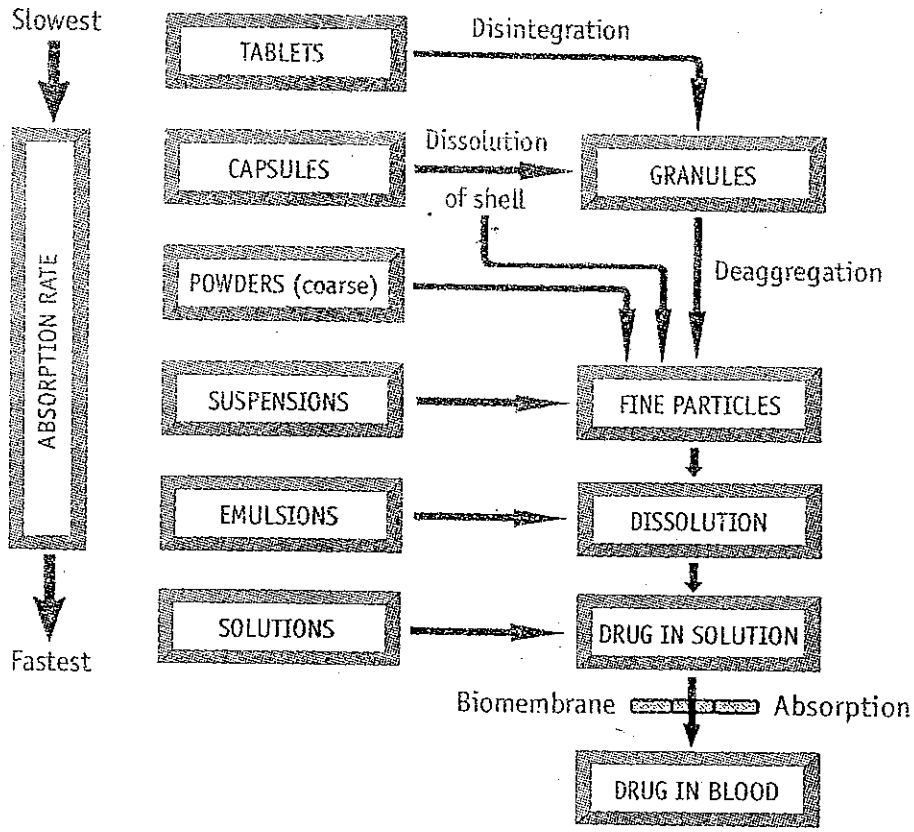


Fig. 2.26 Course of events that occur following oral administration of various dosage forms

-As a general rule, the bioavailability of a drug from various dosage forms decreases in the following order:

Solutions > Emulsions > Suspensions > Capsules > tablets > coated Tablets > Enteric-coated Tablets > Sustained-Release Products

As a general rule, the bioavailability of a drug from various dosage forms decreases in the following order:

Solutions > Emulsions > Suspensions > Capsules > Tablets > Coated Tablets > Enteric-Coated Tablets > Sustained-Release Products

Thus, absorption of a drug from solution is fastest with least potential for bioavailability problems whereas absorption from a sustained-release product is slowest with greatest bioavailability risk.

Several factors, especially the excipients which influence bioavailability of a drug from its dosage form, have been discussed earlier.

Solutions : A drug in a solution (syrups, elixirs, etc.) is most rapidly absorbed since the major rate-limiting step, drug dissolution, is absent. Factors that influence bioavailability of a drug from solution dosage form include—the nature of solvent (aqueous, water-miscible, etc.), viscosity, surfactants, solubilisers, stabilizers, etc. Quite often, dilution of a drug in solution with GI fluids results in precipitation of drug as fine particles which generally dissolve rapidly. Factors that limit the formulation of a drug in solution form include stability, solubility, taste, cost of the product, etc.

Emulsions : Emulsion dosage forms have been found to be superior to suspensions in administering poorly aqueous soluble lipophilic drugs. It was observed with indoxole (an NSAID) that when it is dissolved in a vegetable oil and emulsified in water, absorption increases 3-fold over its aqueous suspension. Emulsion dosage form presents a large surface area of oil to the GIT for absorption of a drug. Scientists have claimed that a drug administered in oily vehicle (emulsified and solubilised in the GIT by bile salts to form mixed micelles) can direct the distribution of drug directly into the lymphatic system thereby avoiding the hepatic portal vein and first-pass metabolism.

Suspensions : The major rate-limiting step in the absorption of a drug from suspension dosage form is drug dissolution which is generally rapid due to the large surface area of the particles. Important factors in the bioavailability of a drug from suspensions include particle size, polymorphism, wetting agents, viscosity of the medium, suspending agents, etc.

Powders : Though powders are superior to tablets and capsules, they are not in use nowadays due to handling and palatability problems. Major factors to be considered in the absorption of a drug from powders are particle size, polymorphism, wettability, etc.

Capsules : Powders and granules are popularly administered in hard gelatin capsules whereas viscous fluids and oils in soft elastic shells. Factors of importance in case of hard gelatin capsules include drug particle size, density, polymorphism, intensity of packing and influence of diluents and excipients. Hydrophilic diluents like lactose improve wettability, deaggregation and dispersion of poorly aqueous soluble drugs whereas inhibitory effect is observed with hydrophobic lubricants like magnesium stearate. A hydrophobic drug with a fine particle size in capsule results in a decrease in porosity of powder bed and thus, decreased penetrability by the solvent with the result that clumping of particle occurs. This can be overcome by incorporating a large amount of hydrophilic diluent (upto 50%), a small amount of wetting agent cum lubricant such as SLS (upto 1%) and/or by wet granulation to convert an impermeable powder bed to the one having good permeability. Other factors of importance include possible interaction between the drug and the diluent (e.g. tetracycline-DCP) and between drug and gelatin shell. The influence of capsule processing factors on drug dissolution and bioavailability has already been discussed.

Soft elastic capsules as such dissolve faster than hard gelatin capsules and tablets and show better drug availability from oily solutions, emulsions or suspensions of medicaments (especially hydrophobic drugs). One of the best examples of this is the faster dissolution of indoxole (equivalent to that of an emulsion dosage form) when formulated as soft gelatin capsule in comparison to hard gelatin capsule and aqueous suspension. Such poorly soluble drugs can be dissolved in PEG or other suitable solvent with the aid of surfactants and encapsulated without difficulty. Soft gelatin capsules are thus of particular use where the drug dose is low, drug is lipophilic or when oily or lipid based medicaments are to be administered. A problem with soft gelatin capsules is the high water content of the shell (above 20%). This moisture migrates into the shell content and crystallization of drug occurs during the drying stage resulting in altered drug dissolution characteristics.

Tablets : Compressed tablets are the most widely used convenient and cost effective dosage forms. A schematic representation of disintegration, deaggregation, dissolution and absorption of a drug from a tablet dosage form is shown in Fig. 2.27.

The bioavailability problems with tablets arise from the reduction in the effective surface area due to granulation and subsequent compression into a dosage form. Since dissolution is most rapid from primary drug particles due to their large surface area, disintegration of a tablet into granules and subsequent deaggregation of granules into fine particles is

very important. A number of formulation and processing factors influencing these steps and also the physicochemical properties of drug substance that influence bioavailability have already been discussed in the earlier sections of this chapter.

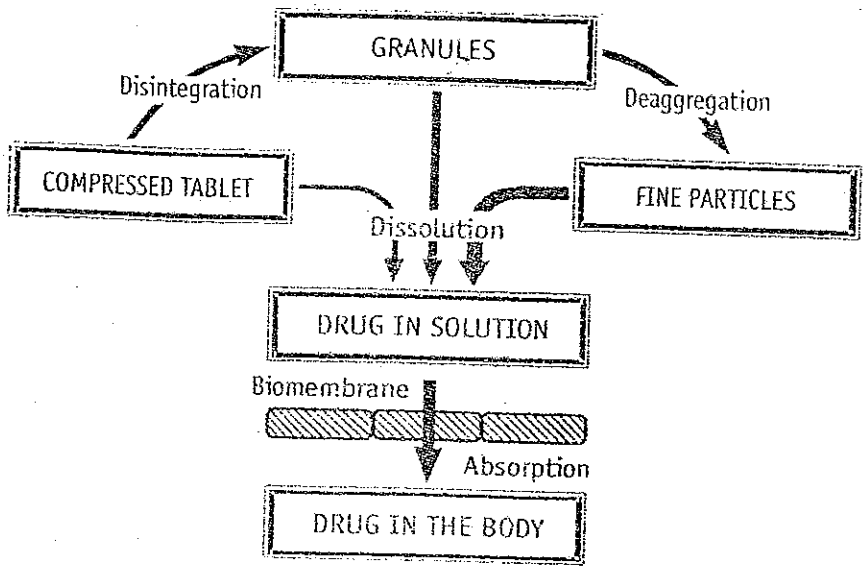


Fig. 2.27 Sequence of events in the absorption of a drug from tablet dosage form

Coated tablets : In addition to factors that influence drug release from compressed tablets, the coating acts as yet another barrier which must first dissolve or disrupt to give way to disintegration and dissolution of tablet. Of the two types of coatings, the film coat, which is thin, dissolves rapidly and does not significantly affect drug absorption. The sugar coat which though soluble, is generally tough and takes longer to dissolve. The sealing coat which is generally of shellac, is most deleterious. Press-coated tablets may be superior to sugar-coated tablets in such cases.

Enteric-coated tablets : Enteric coated tablets have great potential in creating bioavailability problems because the coat dissolves only in the alkaline pH of the intestine and it may take as long as 2 to 4 hours for such a tablet to empty from the stomach into the intestine depending upon the meals and the GI motility. Hence, the pharmacological response may eventually be delayed by as much as 6 to 8 hours. The problem of gastric emptying can, however, be overcome by enteric coating the granules or pellets and presenting them in a capsule or compressing into a tablet. The thickness of enteric coat is yet another determinant factor in drug dissolution, increasing thickness being more problematic. Aging of the dosage form also affects drug release, especially with shellac. In one of the studies, shellac coated tablets of

salicylic acid stored for 2 years showed a 60% decrease in the peak plasma level.

Sustained-release products : Drug release from such products is most unpredictable, the problems ranging from dose dumping to unsatisfactory or no drug release at all. However, with the development of newer innovations and technologies, it is becoming increasingly reliable and the results reproducible with little inter-subject variations.

Product Age and Storage Conditions

A number of changes, especially in the physicochemical properties of a drug in dosage form, can result due to aging and alterations in storage conditions which can adversely affect bioavailability. With solution dosage form, precipitation of drug due to altered solubility, especially due to conversion of metastable into poorly soluble, stable polymorph can occur during the shelf-life of the product. Changes in particle size distribution have been observed with a number of suspension dosage forms resulting in decreased rate of drug dissolution and absorption. In case of solid dosage forms, especially tablets, disintegration and dissolution rates are greatly affected due to aging and storage conditions. An increase in these parameters of tablets has been attributed to excipients that harden on storage (e.g. PVP, acacia, etc.) while the decrease is mainly due to softening/crumbling of the binder during storage (e.g. CMC).

Changes that occur during the shelf-life of a dosage form are affected mainly by large variations in temperature and humidity. In one of the studies conducted on prednisone tablets containing lactose as the filler, high temperature and high humidity resulted in harder tablets that disintegrated and dissolved slowly.

B- Patient-Related Factors Affecting Drug Absorption:

-The gastrointestinal tract (GIT) comprises of a number of components, their primary function being secretion, digestion and absorption.

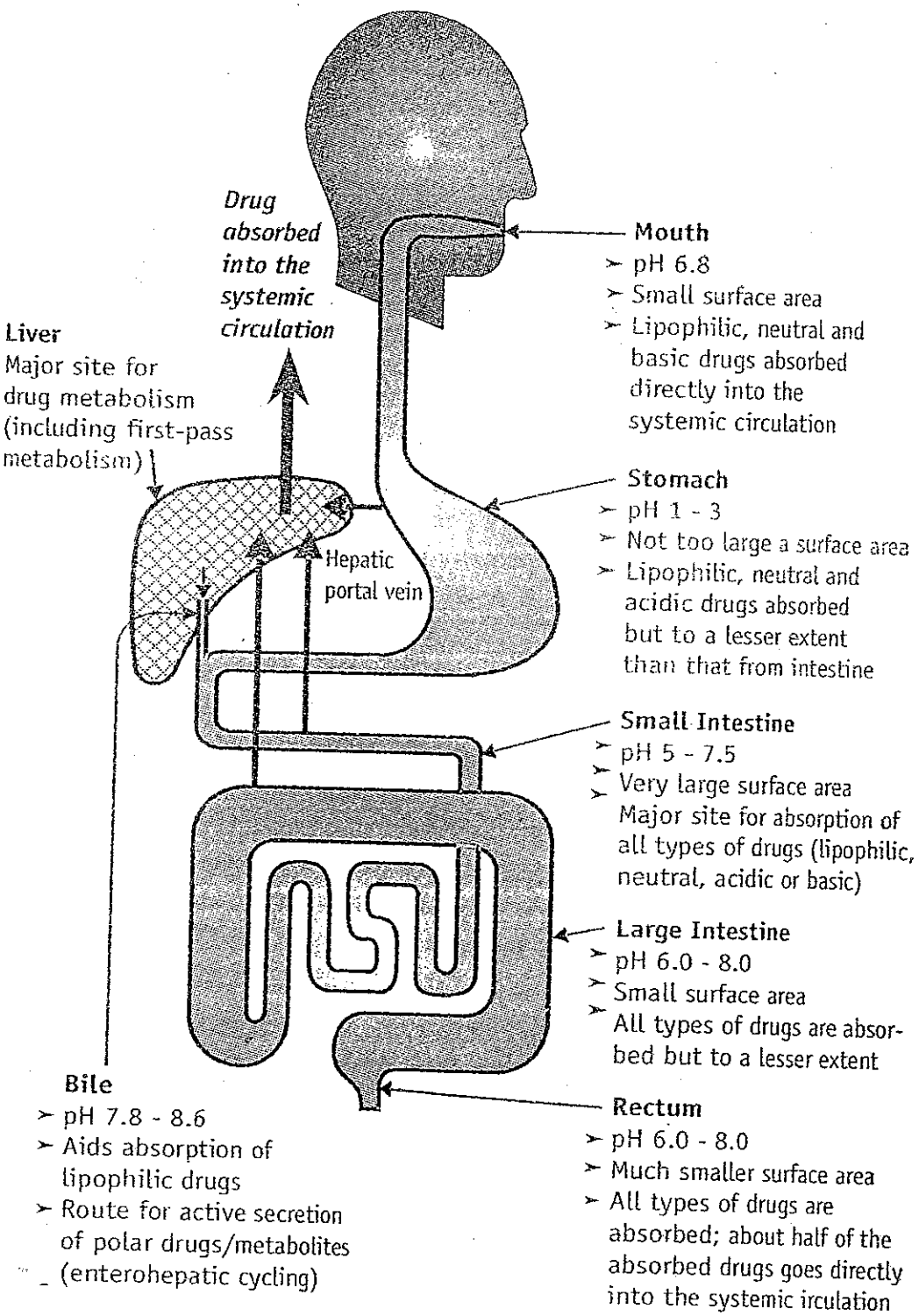


Fig. 2.28 Schematic representation of the GIT and different sites of drug absorption

-The mean length of the entire GIT is 450cm.
-The major functional components of the GIT are stomach, small intestine (duodenum, jejunum and ileum) and large intestine (colon) which grossly differ from each other in terms of anatomy, function, secretions and pH.

Anatomical and Functional Differences Between the Important Regions of the GIT

	Stomach	*Small Intestine*	*Large Intestine*	*Rectum*
pH range	1-3	5-7.5	6.0-8.0	6.0-8.0
Length (cms)	20	300-500	110	20
Diameter (cms)	15	2.5	5	2.5
Surface area (sq.M)	0.1-0.2	200	0.15	0.02
Blood flow (L/min)	0.15	1.0	0.02	—
Transit time (hours)	1-5	3-6	6-12	6-12
Absorptive role	Lipophilic, acidic and neutral drugs	All types of drugs	Some drugs, water and electrolytes	All types of drugs
Absorptive mechanisms	Passive diffusion. convective transport	All absorption mechanisms	Passive diffusion, convective transport	Passive diffusion, convective transport, endocytosis

The entire length of the GI mucosa from stomach to large intestine is lined by a thin layer of mucopolysaccharides (mucus/mucin) which normally acts as an impermeable barrier to the particulates such as bacteria, cells or food particles.

Stomach : The stomach is a bag like structure having a smooth mucosa and thus small surface area. Its acidic pH, due to secretion of HCl, favours absorption of acidic drugs if they are soluble in the gastric fluids since they are unionised to a large extent in such a pH. The gastric pH aids dissolution of basic drugs due to salt formation and subsequent ionisation which are therefore absorbed to a lesser extent from stomach because of the same reason.

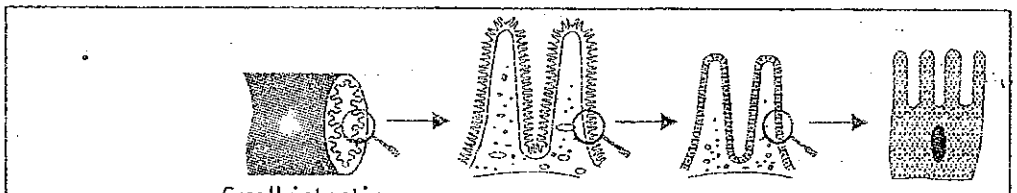
The *stomach is not the principal region for drug absorption* because—

1. The total mucosal area is small.
2. The epithelium is dominated by mucus-secreting cells rather than absorptive cells.
3. The gastric residence time is limited due to which there is limited opportunity for gastric uptake of drug.

Small Intestine : *It is the major site for absorption of most drugs due to its special characteristics*—

1. *Large surface area* : the folds in the intestinal mucosa, called as the folds of Kerckring, result in 3 fold increase in the surface area. The surface of these folds possesses finger like projections called as villi which increase the surface area 30 times. From the surface of villi protrude several microvilli (about 600 from each absorptive cell that lines the villi) resulting in 600 times increase in the surface area (Fig. 2.29).
2. *Great length of small intestine* : results in more than 200 square meters of surface which is several times that of stomach.
3. *Greater blood flow* : the blood flow to the small intestine is 6 to 10 times that of stomach.
4. *Favourable pH range* : the pH range of small intestine is 5 to 7.5 which is favourable for most drugs to remain unionised.
5. *Slow peristaltic movement* : prolongs the residence time of drug in the intestine
6. *High permeability* : the intestinal epithelium is dominated by absorptive cells.

A contribution of all the above factors thus make *intestine the best site for absorption of most drugs.*



	Small intestine as a cylinder	Folds of Kerckring	Villi	Microvilli
Surface area (m^2)	0.33	1	10	200
Relative increase in surface area	1	3	30	600

Fig. 2.29 Representation of the components of intestinal epithelium that accounts for its large surface area

Large intestine : Its length and mucosal surface area is very small (villi and microvilli are absent) in comparison to small intestine and thus absorption of drugs from this region is insignificant. Its contents are neutral or alkaline. The main role of large intestine is in the absorption of water and electrolytes. However, because of the long residence time (6 to 12 hours), colonic transit may be important in the absorption of some poorly soluble drugs and sustained release dosage forms.

(B) PATIENT-RELATED FACTORS

Age

In infants, the gastric pH is high and intestinal surface and blood flow to the GIT is low resulting in altered absorption pattern in comparison to adults. In elderly persons, causes of impaired drug absorption include altered gastric emptying, decreased intestinal surface area and GI blood flow, higher incidents of achlorhydria and bacterial overgrowth in small intestine.

Gastric Emptying

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

Rapid gastric emptying is advisable where:

1. A rapid onset of action is desired e.g. sedatives.
2. Dissolution of drug occurs in the intestine e.g. enteric-coated dosage forms.
3. The drugs are not stable in the gastric fluids e.g. penicillin G and erythromycin.
4. The drug is best absorbed from the distal part of the small intestine e.g. vitamin B_{12}.

For better dissolution and absorption, the gastric emptying can be promoted by taking the drug on empty stomach. Since gastric emptying is altered by several factors due to which large intersubject variations are observed, all biopharmaceutic studies that require the drug to be taken orally are performed in volunteers on empty stomach.

Gastric emptying of a drug is delayed by co-administering food because unless the gastric contents are fluid enough or the size of the solid particles is reduced below 2 mm, its passage through the pylorus into the intestine is not possible.

Delay in gastric emptying is recommended in particular where:

1. The food promotes drug dissolution and absorption e.g. griseofulvin.

2. Disintegration and dissolution of dosage form is promoted by gastric fluids.
3. The drugs dissolve slowly e.g. griseofulvin.
4. The drugs irritate the gastric mucosa e.g. aspirin, phenylbutazone and nitrofurantoin.
5. The drugs are absorbed from the proximal part of the small intestine and prolonged drug-absorption site contact is desired e.g. vitamin B_2 and vitamin C

Gastric emptying is a first-order process. Several parameters are used to quantify gastric emptying:

1. **Gastric emptying rate** *is the speed at which the stomach contents empty into the intestine.*
2. **Gastric emptying time** *is the time required for the gastric contents to empty into the small intestine.* Longer the gastric emptying time, lesser the gastric emptying rate.
3. **Gastric emptying** $t_{1/2}$ *is the time taken for half the stomach contents to empty.*

In vivo gastric emptying of a drug (so also the disintegration of dosage form and drug release) can be studied by using radio-opaque contrast materials like barium sulphate or tagging the drug with a radioisotope and scanning the stomach at regular intervals of time.

A large number of *factors influence gastric emptying* as discussed below.

1. **Volume of meal :** Larger the bulk of the meals, longer the gastric emptying time. However, an initial rapid rate of emptying is observed with a large meal volume and an initial lag phase in emptying of a small volume meal. Since gastric emptying is a first-order process, a plot of log of volume of contents remaining in the stomach versus time yields a straight line.
2. **Composition of meal :** Predictably, the rate of gastric emptying for various food materials is in the following order: *carbohydrates > proteins > fats.* Fats promote secretion of bile which too has an inhibitory effect on gastric emptying. Delayed gastric emptying as observed with fatty meals, is beneficial for the absorption of poorly soluble drugs like griseofulvin.
3. **Physical state and viscosity of meal :** Liquid meals take less than an hour to empty whereas a solid meal may take as long as 6 to 7 hours. Viscous materials empty at a slow rate in comparison to less viscous materials.

4. **Temperature of the meal :** High or low temperature of the ingested fluid (in comparison to body temperature) reduce the gastric emptying rate.

5. **Gastrointestinal pH :** Gastric emptying is retarded at low stomach pH and promoted at higher or alkaline pH. Chemicals that affect gastrointestinal pH also alter gastric emptying. The inhibitory effect of various acids on gastric emptying decreases with increase in molecular weight and is in the following order—

$$HCl > acetic > lactic > tartaric > citric$$

With alkaline solutions, a low base concentration (1% $NaHCO_3$) increases the gastric emptying rate more than the one of higher concentration (5%).

6 **Electrolytes and osmotic pressure :** Water, isotonic solutions, and solutions of low salt concentration empty the stomach rapidly whereas a higher electrolyte concentration decreases gastric emptying rate.

7. **Body posture :** Gastric emptying is favoured while standing and by lying on the right side since the normal curvature of the stomach provides a *downhill* path whereas lying on the left side or in supine position retards it.

8. **Emotional state :** Stress and anxiety promote gastric motility whereas depression retards it.

9. **Exercise :** Vigorous physical training retards gastric emptying.

10. **Disease states :** Diseases like gastroenteritis, gastric ulcer, pyloric stenosis, diabetes and hypothyroidism retard gastric emptying. Partial or total gastrectomy, duodenal ulcer and hyperthyroidism promote gastric emptying rate.

11. **Drugs :** Drugs that retard gastric emptying include poorly soluble antacids (aluminium hydroxide), anticholinergics (atropine, propantheline), narcotic analgesics (morphine) and tricyclic antidepressants (imipramine, amitriptyline). Metoclopramide, domperidone and cisapride (prokinetic-agents) stimulate gastric emptying.

The passage of drug through the oesophagus, called as **oesophageal transit**, is important in persons who swallow the solid dosage form lying down in supine position or with little or no water. In such cases, the dosage form remains lodged in the oesophagus and disintegrates slowly which may result in delayed absorption or local damage to the mucosa from drugs like NSAIDs.

Intestinal Transit

Since small intestine is the major site for absorption of most drugs, long intestinal transit time is desirable for complete drug absorption (*see* Table 2.8).

TABLE 2.8
Transit Time for Contents from Different Regions of Intestine

Intestinal region	*Transit time*
Duodenum	5 minutes
Jejunum	2 hours
Ileum	3 to 6 hours
Caecum	0.5 to 1 hour
Colon	6 to 12 hours

The residence time depends upon the intestinal motility or contractions. The *mixing movement* of the intestine that occurs due to peristaltic contractions promote drug absorption, firstly, by increasing the drug-intestinal membrane contact, and secondly, by enhancing drug dissolution especially of poorly soluble drugs, through induced agitation.

Delayed intestinal transit is desirable for:

1. Drugs that dissolve or release slowly from their dosage form (sustained-release products) or when the ratio of dose to solubility is high e.g. chlorothiazide.
2. Drugs that dissolve only in the intestine (enteric-coated formulations).
3. Drugs which are absorbed from specific sites in the intestine (several B vitamins, lithium carbonate, etc.).
4. When the drug penetrates the intestinal mucosa very slowly e.g. acyclovir.
5. When absorption of drug from the colon is minimal.

However, as the contents move down the intestine into the colon, its viscosity gradually increases due to absorption of water and electrolytes which limits the design of sustained-release products of drugs having short biological half-lives.

Like gastric emptying, intestinal transit is influenced by several factors like food, drugs and diseases. Food, decreased digestive secretions and pregnancy retard intestinal transit whereas diarrhoea promotes

it. Drugs like metoclopramide that promote gastric emptying and intestinal transit enhance absorption of rapidly soluble drugs. Laxatives also promote the rate of intestinal transit. Drugs such as anticholinergics that retard gastric and intestinal transit promote absorption of poorly soluble drugs—for example, propantheline shows a 100%, 50% and 30% rise in the absorption of vitamin B_2, nitrofurantoin and hydrochlorothiazide respectively.

Gastrointestinal pH

A tremendous 10^7 fold difference in the hydrogen ion concentration is observed between the gastric and colon fluids. The GI pH generally increases gradually as one move down the stomach to the colon and rectum (*see* Fig. 2.22). GI fluid pH influence drug absorption in several ways:

1. **Disintegration :** The disintegration of some dosage forms is pH sensitive. With enteric-coated formulations, the coat dissolves only in the intestine followed by disintegration of the tablet.
2. **Dissolution :** A large number of drugs are either weak acids or weak bases whose solubility is greatly affected by pH. A pH that favours formation of salt of the drug enhances the dissolution of that drug. Since drug dissolution is one of the important rate-determining steps in drug absorption, GI pH is of great significance in the oral bioavailability of drugs. Weakly acidic drugs dissolve rapidly in the alkaline pH of the intestine whereas basic drugs dissolve in the acidic pH of the stomach. Since the primary site for absorption of most drugs is small intestine, the poorly water-soluble basic drugs must first dissolve in the acidic pH of stomach before moving into the intestine.
3. **Absorption :** Depending upon the drug pK_a and whether its an acidic or a basic drug, the GI pH influences drug absorption by determining the amount of drug that would exist in the unionised form at the site of absorption. This topic has already been dealt with in sufficient details under pH-partition hypothesis.
4. **Stability :** GI pH also influences the chemical stability of drugs. The acidic stomach pH is known to affect degradation of penicillin G and erythromycin. This can be overcome by preparing prodrugs of such drugs that do not degrade or dissolve in acidic pH e.g. carindacillin and erythromycin estolate. With basic drugs, formation of insoluble drug hydroxide in the alkaline pH of the intestine has been observed.

Disease States

Several disease states can influence the rate and extent of drug absorption. The 3 major classes of disease states that can influence the bioavailability of a drug are:

1. Gastrointestinal diseases,
2. Cardiovascular diseases, and
3. Hepatic diseases.

1. Gastrointestinal diseases : A number of pathologic conditions of the GIT can influence changes in drug absorption pattern, namely:

(*a*) *Altered GI motility*: (discussed earlier)

(*b*) *Gastrointestinal diseases and infections* : The influence of achlorhydria (decreased gastric acid secretion and increased stomach pH) on gastric emptying and drug absorption, especially that of acidic drugs (decreased absorption, e.g. aspirin) has been studied. Two of the intestinal disorders related with *malabsorption syndrome* that influence drug availability are *celiac disease* (characterized by destruction of villi and microvilli) and *Crohn's disease*. Abnormalities associated with celiac disease include increased gastric emptying rate and GI permeability, altered intestinal drug metabolism, steatorrhea (impaired secretion of bile thus affecting absorption of lipophilic drugs and vitamins) and reduced enterohepatic cycling of bile salts, all of which can significantly impair drug absorption. Conditions associated with Crohn's disease that can alter absorption pattern are altered gut wall microbial flora, decreased gut surface area and intestinal transit rate. Malabsorption is also induced by drugs such as antineoplastics and alcohol which increase permeability of agents not normally absorbed. GI infections like shigellosis, gastroenteritis, cholera and food poisoning also result in malabsorption. Colonic diseases such as colitis, amoebiasis and constipation can also alter drug absorption.

(*c*) *Gastrointestinal surgery* Gastrectomy can result in drug dumping in the intestine, osmotic diarrhoea and reduced intestinal transit time. Intestinal surgery also influences drug absorption for predictable reasons.

2. Cardiovascular diseases : Several changes associated with congestive cardiac failure influence bioavailability of a drug viz. oedema of the intestine, decreased blood flow to the GIT and gastric emptying rate and altered GI pH, secretions and microbial flora.

3. Hepatic diseases : Disorders such as hepatic cirrhosis influence bioavailability mainly of drugs that undergo considerable first-pass

hepatic metabolism e.g. propranolol. Enhanced bioavailability is observed in such cases.

Blood Flow to the GIT

The GIT is extensively supplied by blood capillary network and the lymphatic system. The absorbed drug can thus be taken up by the blood or the lymph. Since the blood flow rate to the GIT (splanchnic circulation) is 500 to 1000 times (28% of cardiac output) more than the lymph flow, most drugs reach the systemic circulation via blood whereas only a few drugs, especially low molecular weight, lipid soluble compounds are removed by lymphatic system.

The high perfusion rate of GIT ensures that once the drug has crossed the membrane, it is rapidly removed from the absorption site thus maintaining the *sink conditions* and concentration gradient for continued drug absorption.

For drugs that have high permeation rates, e.g. highly lipid soluble drugs or drugs absorbed through pores, the GI perfusion rate could be a rate-limiting step in the absorption, e.g. tritiated water. This is not so in the case of drugs having poor permeability coefficient, e.g. ribitol. Blood flow is also important for actively absorbed drugs since oxygen and energy is required for transportation.

Food influences blood flow to the GIT. The perfusion rate increases after meals and persists for few hours but drug absorption is not influenced significantly.

Gastrointestinal Contents

A number of GI contents can influence drug absorption as discussed below:

1. Food-drug interactions : Presence of food may either delay, reduce, increase or may not affect drug absorption (Table 2.9).

TABLE 2.9
Influence of Food on Drug Absorption

Delayed	*Decreased*	*Increased*	*Unaffected*
Aspirin	Penicillins	Griseofulvin	Methyldopa
Paracetamol	Erythromycin	Nitrofurantoin	Propylthiouracil
Diclofenac	Ethanol	Diazepam	Sulphasomidine
Nitrofurantoin	Tetracyclines	Actively absorbed	
Digoxin	Levodopa	water soluble	
	Iron	vitamins	

Food-drug interactions may be due to the influence of food on physiological functions (alterations in the GI emptying rate, GI. fluid secretions, pH, blood flow and absorptive processes) and/or a consequence of physicochemical interaction with the drug (alteration in drug dissolution profile, complexation and adsorption).

As a general rule, *drugs are better absorbed under fasting conditions and presence of food retards or prevents it.* Food does not significantly influence absorption of a drug taken half an hour or more before meals and two hours or more after meals.

Delayed or decreased drug absorption by the food could be due to one or more of the several mechanisms:

(a) Delayed gastric emptying, affecting drugs unstable in the stomach e.g. penicillins, or preventing the transit of enteric-coated tablets into the intestine which may be as long as 6 to 8 hours.

(b) Formation of a poorly soluble, unabsorbable complex e.g. tetracycline-calcium.

(c) Increased viscosity due to food thereby preventing drug dissolution and/or diffusion towards the absorption site.

Increased drug absorption following a meal could be due to one or more of the under mentioned reasons—

(a) Increased time for dissolution of a poorly soluble drug.

(b) Enhanced solubility due to GI secretions like bile.

(c) Prolonged residence time and absorption site contact of the drug e.g. water-soluble vitamins.

(d) Increased lymphatic absorption e.g. acitretin.

The specific meal components also have an influence on drug absorption. Meals high in fat aid solubilisation of poorly aqueous soluble drugs e.g. isotretinoin. Food high in proteins inhibits absorption of levodopa. An interesting example of influence of high protein meal is that of increased oral availability of propranolol to which two reasons were attributed—firstly, such a meal increases the hepatic blood flow due to which the drug can bypass first-pass hepatic metabolism (propranolol is a drug with high hepatic extraction), and secondly, it promotes blood flow to the GIT thus aiding drug absorption.

2. Fluid volume : Administration of a drug with large fluid volume results in better dissolution, rapid gastric emptying and enhanced absorption—for example, erythromycin is better absorbed when taken with a glass of water under fasting condition than when taken with meals.

3. Interaction of drug with normal GI constituents : The GIT contains a number of normal constituents such as mucin, bile salts and enzymes which influence drug absorption. Mucin, a protective mucopolysaccharide that lines the GI mucosa, interacts with streptomycin and certain quaternary ammonium compounds and retards their absorption. It also acts as a barrier to diffusion of drugs. The bile salts aid solubilisation and absorption of lipid soluble drugs like griseofulvin and vitamins A, D, E and K on one hand and on the other, decreases absorption of neomycin and kanamycin by forming water-insoluble complexes.

The influence of GI enzymes on absorption will be discussed later.

4. Drug-drug interactions in the GIT : Like food-drug interactions, drug-drug interactions can either be physicochemical or physiological.

(a) *Physicochemical drug-drug interactions* can be due to—

Adsorption : Antidiarrhoeal preparations containing adsorbents like attapulgite or kaolin-pectin retard/prevent absorption of a number of drugs co-administered with them e.g. promazine and lincomycin.

Complexation : Antacids and/or mineral substitutes containing heavy metals such as aluminium, calcium, iron, magnesium or zinc retard absorption of tetracyclines through formation of unabsorbable complexes. The anion-exchange resins, cholestyramine and colestipol, bind cholesterol metabolites, bile salts and a number of drugs in the intestine and prevent their absorption.

pH change : Basic drugs dissolve in gastric pH; co-administration of such drugs, e.g. tetracycline with antacids such as sodium bicarbonate results in elevation of stomach pH and hence decreases dissolution rate or causes precipitation of drug.

(b) *Physiological drug-drug interactions* can be due to following mechanisms—

Decreased GI transit : Anticholinergics such as propantheline retard GI motility and promote absorption of drugs like ranitidine and digoxin, whereas delay absorption of paracetamol and sulphamethoxazole.

Increased gastric emptying : Metoclopramide promotes GI motility and enhances absorption of tetracycline, pivampicillin and levodopa.

Altered GI metabolism : Antibiotics inhibit bacterial metabolism of drugs, e.g. erythromycin enhances efficacy of digoxin by this mechanism. Co-administration of antibiotics with oral contraceptives like ethinyl oestradiol decreases the efficacy of latter by decreasing enterohepatic cycling of steroid conjugates which otherwise are hydrolysed by gut bacteria after biliary excretion.

Presystemic Metabolism/First-Pass Effects

For a drug administered orally, the 3 main reasons for its decreased bioavailability are:

1. Decreased absorption (owing to adsorption, precipitation, complexation and poor solubility).
2. Destabilisation or destruction of drug.
3. First-pass/presystemic metabolism (see figure 2.30)

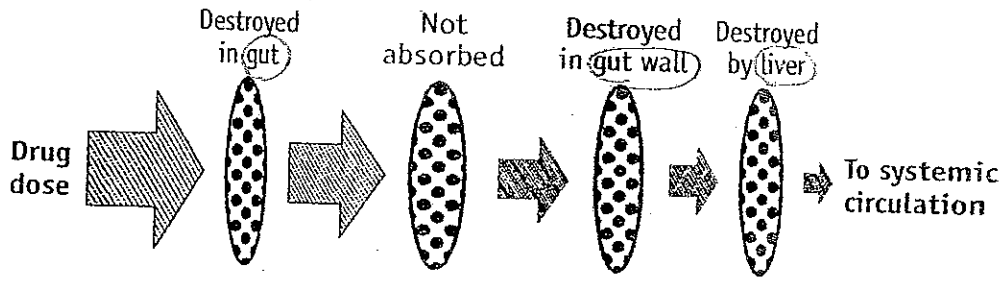


Fig. 2.30 Processes that reduce the availability of orally administered drugs

Before a drug reaches blood circulation, it has to pass for the first time through organs of elimination namely the GIT and the liver. *The loss of drug through biotransformation by such eliminating organs during its passage to systemic circulation is called as* **first-pass or presystemic metabolism.** The diminished drug concentration or rarely, complete absence of the drug in plasma after oral administration is indicative of first-pass effects. The 3 primary systems which affect presystemic metabolism of a drug are (Fig. 2.31):

1. Luminal enzymes : the metabolism by these enzymes are further categorised into two—
   (b) Digestive enzymes, and
   (c) Bacterial enzymes.
2. Gut wall enzymes/mucosal enzymes.
3. Hepatic enzymes.

1. Digestive enzymes : These are the enzymes present in the gut fluids and include enzymes from intestinal and pancreatic secretions. The latter contains hydrolases which hydrolyse ester drugs like chloram-

phenicol palmitate into active chloramphenicol, and peptidases which split amide linkages and inactivate protein/polypeptide drugs. Thus, one of the approaches to effect oral absorption of peptides is to deliver them to colon which lack peptidases.

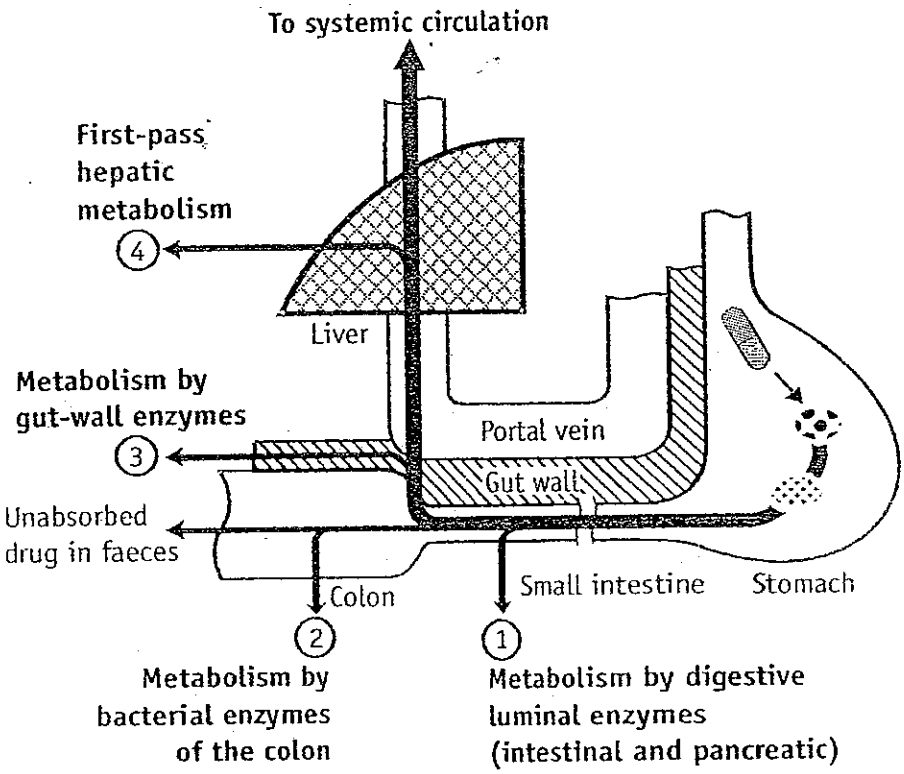


Fig. 2.31 Different sites of presystemic metabolism

2. Bacterial enzymes : The GI microflora is scantily present in stomach and small intestine and is rich in colon. Hence, most orally administered drugs remain unaffected by them. The colonic microbes generally render a drug more active or toxic on biotransformation—for example sulphasalazine, a drug used in ulcerative colitis, is hydrolysed to sulphapyridine and 5-amino salicylic acid by the microbial enzymes of the colon. An important role of intestinal microflora is that in enterohepatic cycling. Their enzymes hydrolyse the conjugates of drugs actively secreted via bile such as glucuronides of digoxin and oral contraceptives. The free drugs are reabsorbed into the systemic circulation.

3. Gut wall enzymes : Also called as mucosal enzymes, they are present in stomach, intestine and colon. Alcohol dehydrogenase (ADH) is an enzyme of stomach mucosa that inactivates ethanol. Intestinal mucosa contains both phase I and phase II (predominant) enzymes, e.g. sulphation of ethinyl oestradiol and isoprenaline. The colonic mucosa also contain both phase I and phase II enzymes. However, it is only the enzymes of the proximal small intestine that are most active.

4. **Hepatic enzymes :** Several drugs undergo first-pass hepatic metabolism, the highly extracted ones being isoprenaline, propranolol, alprenolol, pentoxifylline, nitroglycerine, diltiazem, nifedipine, lidocaine, morphine, etc.

METHODS FOR STUDYING DRUG UPTAKE

The experimental methods for studying absorption of drugs can be classified as *in vitro* and *in situ.*

1. ***In vitro experiments*** : are used to study the transport of drugs through different types of membranes or biological materials. Such experiments may utilize—

(a) Diffusion cells

(b) Segments of GIT of laboratory animals – Two well known established techniques are— (i) Everted sac technique, and (ii) Everted ring technique.

(c) Cell cultures of gut epithelium e.g. Caco-2 cells.

2. ***In situ experiments*** : simulates the *in vivo* conditions for drug absorption and are based on perfusion of a segment of GIT by drug solution and determination of amount of drug diffused through it. The two perfusion methods used in laboratory animals are—

(a) Doluisio method

(b) Single pass perfusion.

Diffusion Cell Method

Diffusion cells consist of two compartments—

1. *Donor compartment* which contains the drug solution and the lower end of which contains the synthetic or natural GI membrane that interfaces with the receptor compartment.
2. *Receptor compartment* which contains the buffer solution.

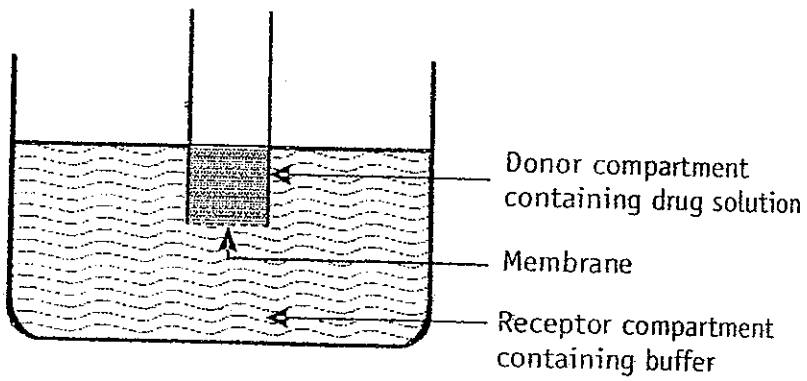


Fig. 2.32 Diffusion cell for studying drug uptake from GIT.

methods to determine absorption (10M, 5M)

The procedure of uptake study using this technique involves measurement of rate of arrival of drug in the receptor compartment (see figure 2.32).

Everted Sac Technique

This technique involves eversion of a segment (about 3 cm) of small intestine, conversion into a sac filled with buffer solution and its immersion into a bath containing oxygenated solution of drug. The whole preparation is maintained at 37°C and shaken mildly. At predetermined time intervals, the sac is removed and the concentration of drug in the serosal (internal) liquid is determined (see figure 2.33).

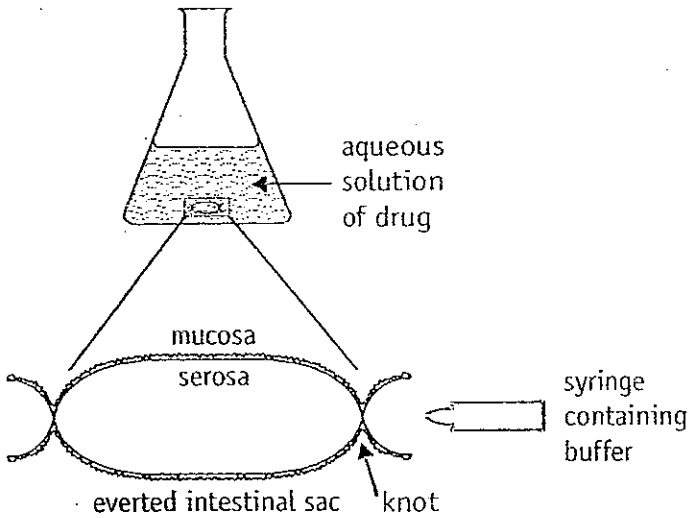


Fig. 2.33 Everted sac technique for studying drug uptake from GIT.

Everted Ring Technique

In this technique, appropriate section of intestine of rat is isolated, inverted and dissected in slices or rings of thickness between 1 and 3 mm. The slices are then placed in an oxygenated isotonic drug solution at 37°C and incubated for a predetermined period under gentle shaking.. After incubation, the rings are washed, dried and placed in a preweighed scintillation vials. Each vial is reweighed to determine the wet tissue weight, and then the sample is analysed for drug.

Cell Culture Technique

In this technique, differentiated cells of the intestine, originating from Caco-2 cells (cells of carcinoma of colon) are placed on synthetic polycarbonate membrane previously treated with an appropriate material such as collagen which on incubation aids reproduction of cells while not retarding drug permeation characteristics. Solution of drug is placed

on this layer of cultured cells and the system is placed in a bath (receptor compartment) of buffer solution. The drug that reaches the latter compartment is sampled and analysed periodically.

Doluisio Method

In this method, the upper and lower parts of the small intestine of anaesthetised and dissected rat are connected by means of tubing to syringes of capacity 10-30 ml (*see* figure 2.34). After washing the intestinal segment with normal saline, the syringe is filled with a solution of radiolabelled drug and a non-absorbable marker which is used as an indicator of water-flux during perfusion. Part of the content of the syringe containing drug is delivered to the intestinal segment which is then collected in the second syringe and analysed for drug.

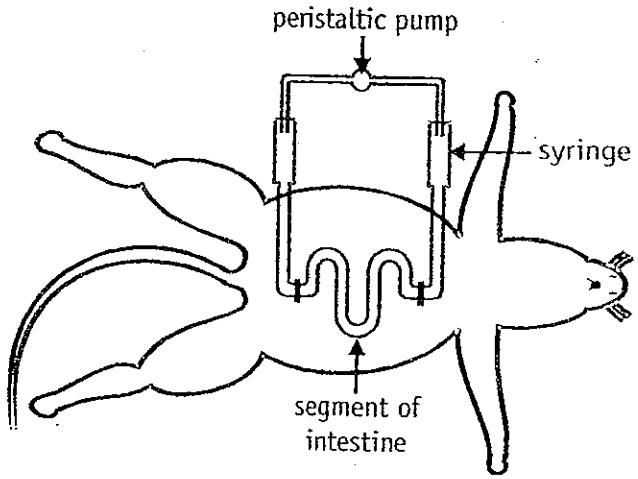


Fig. 2.34 Doluisio method for drug uptake study through rat intestine.

Single-Pass Perfusion

In this technique the drug solution passes through the intestinal segment just once (*see* figure 2.35). This technique is superior to

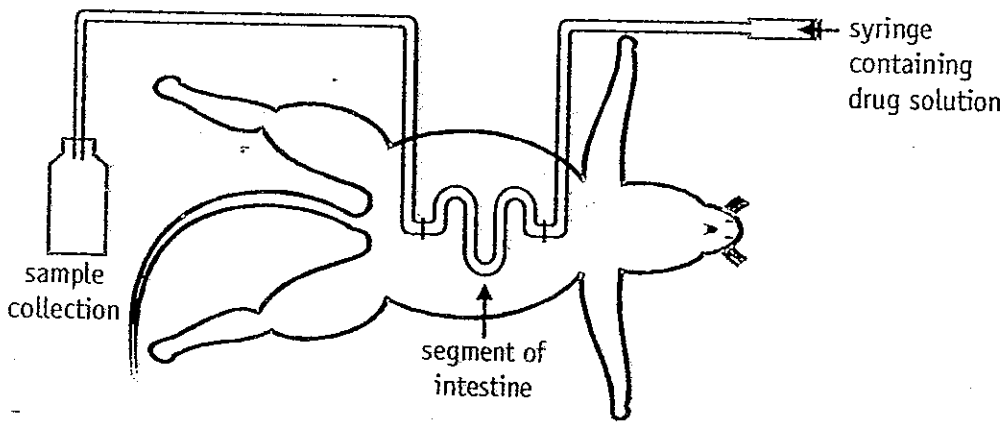


Fig. 2.35 Single-pass perfusion technique for studying drug uptake.

Doluisio method in that precise adjustment of hydrodynamic conditions that can influence blood circulation and puts stress on intestinal wall can be controlled.

Distribution of Drugs

- Disposition:

is defined as the processes that tend to lower the plasma concentration of drug.

- The two major drug disposition processe are:

1. Distribution which involves reversible transfer of a drug between compartments.
2. Elimination: which causes irreversible loss of drug from the body
   - a- Biotransformation (metabolism)
   - b- Excretion

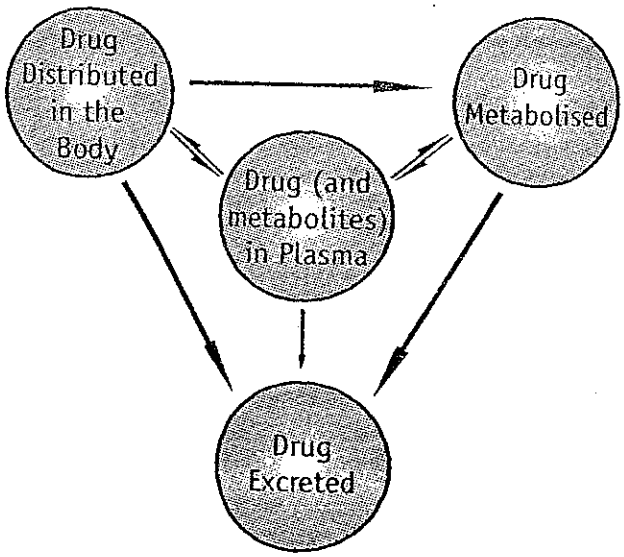


Fig. 3.1 Interrelationship between different processes of drug disposition

- Distribution is a passive process, for which, the driving force is concentration gradient between the blood and the extravascular tissues.

Steps in Drug Distribution:

1. Permeation of free or unbound drug present in the blood through the capillary wall (occurs rapidly) and entry into the interstitial/extracellular fluid (ECF).

2. Permeation of drug present in the ECF through the membrane of tissue cells and into the intracellular fluid.

- This step is rate-limiting and depends upon two major factors:

a - Rate of perfusion to the extracellular tissue

b - Membrane permeability of the drug.

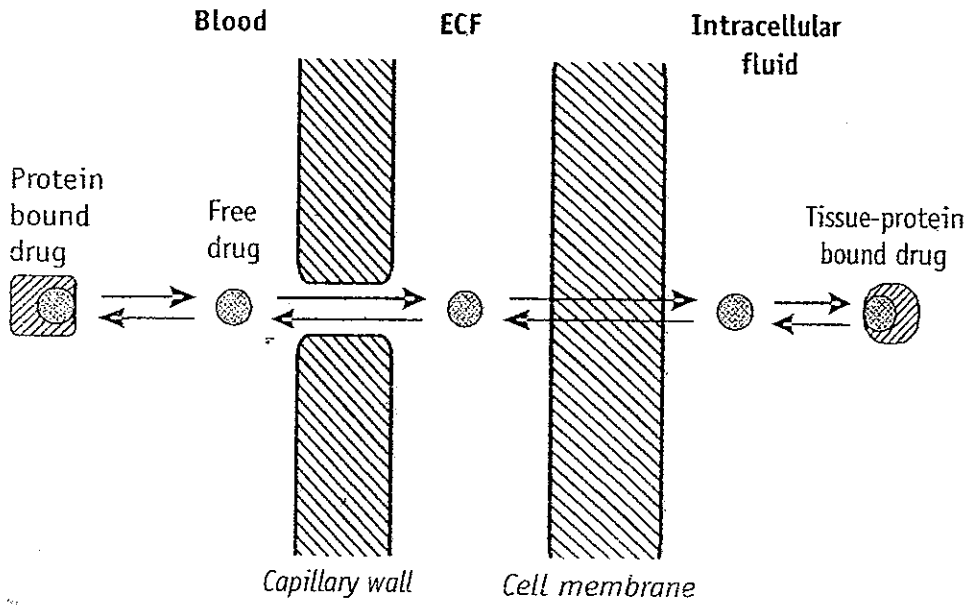


Fig. 3.2 Schematic of the steps involved in drug distribution

Factors Affecting Distribution of Drugs:

1. Tissue permeability of the drug:
   (a) Physicochemical properties of the drug like molecular size, pK_a and o/w partition coefficient
   (b) Physiological barriers to diffusion of drugs
2. Organ/tissue size and perfusion rate
3. Binding of drugs to tissue components:
   (a) Binding of drugs to blood components
   (b) Binding of drugs to extravascular tissue proteins
4. Miscellaneous factors:
   (a) Age
   (b) Pregnancy
   (c) Obesity
   (d) Diet
   (e) Disease states
   (f) Drug interactions

Tissue Permeability of Drugs:

- Two major rate-determining steps in the distribution of drugs are:

1. Rate of tissue permeation
2. Rate of blood perfusion

[Tissue permeability of a drug depends upon the physicochemical properties of the drug as well as the physiological barriers that restrict diffusion of drug into tissues]

a- Physicochemical Properties of the Drug:

- Molecular size:

Only small, water-soluble molecules and ions of size below 50 daltons enter the cell through aqueous filled channels whereas those of larger size are restricted unless a specialized transport system exists for them.

- Degree of ionization:

- The pH of the blood and the extravascular fluid also play a role in the ionisation and diffusion of drugs into cells.
- A drug that remains unionised at these pH values can permeate the cells relatively more rapidly.
- Most drugs are either weak acids or weak bases and their degree of ionisation at plasma or ECF pH depends upon their pK_a.
- Only unionised drugs which are generally lipophilic, rapidly cross the cell membrane.

- Since the blood and the ECF pH normally remain constant at 7.4, they do not have much of an influence on drug diffusion unless altered in conditions such as systemic acidosis or alkalosis.

- Among the drugs that have same O/W partition coefficient but differ in the extent of ionisation at blood pH, the one that ionises to a larger extent;

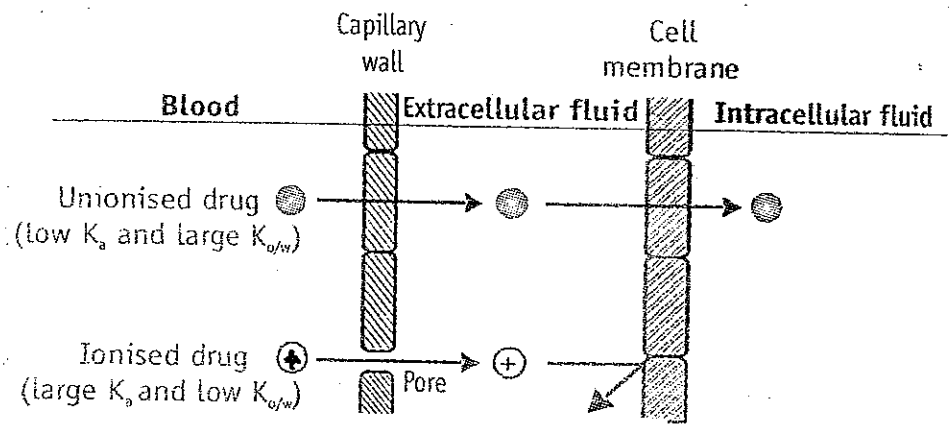


Fig. 3.3 Permeation of unionised and ionised drugs across the capillary and the cell membrane

- In case of polar drugs where permeability is the rate-limiting step in the distribution, the driving force is the effective partition coefficient of drug.

Effective $K_{o/w}$ = (Fraction unionised) ($K_{o/w}$ of unionised drug) at pH 7.4

b- Physiological Barriers to Distribution of Drugs:

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

1. Simple capillary endothelial barrier:

- All drugs, ionised or unionised, with a molecular size less than 600 Daltons, diffuse through the capillary endothelium and into the interstitial fluid.

Only drugs bound to the blood components are restricted because of the large molecular size of the complex.

2. The Simple Cell membrane barrier:

Once a drug diffuses from the capillary wall into the extracellular fluid, its further entry into cells of most tissues is limited by its permeability through the membrane that lines such cells.

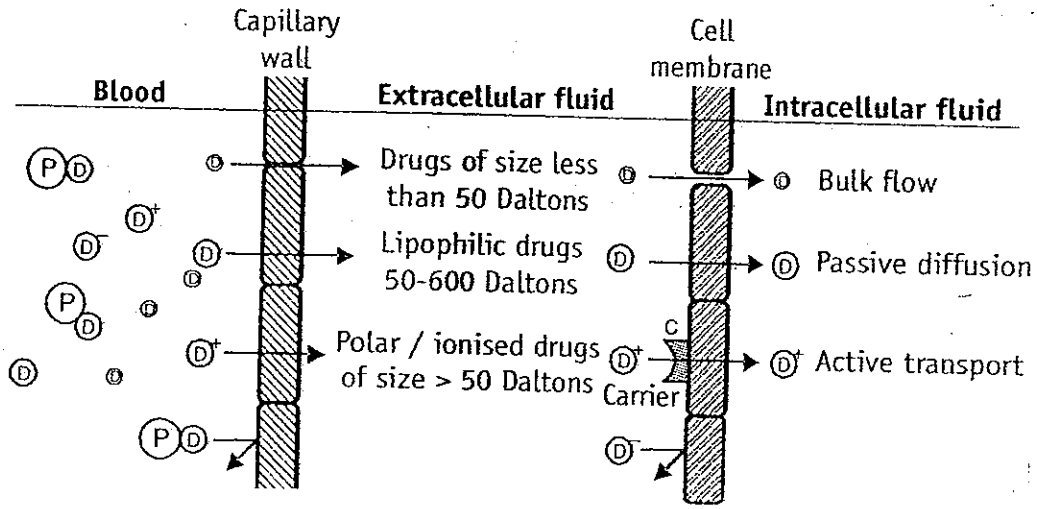


Fig. 3.4 Plasma membrane barrier and drug diffusion across it

3. Blood-brain barrier (BBB):

- The brain capillaries consist of endothelial cells which are joined to one another by continuous tight intercellular junctions comprising what is called as the blood-brain barrier.

- Moreover, the presence of special cells called as pericytes and astrocytes, which are the elements of

the supporting tissue found at the base of endothelial membrane, form a solid envelope around the brain capillaries. As a result, the intercellular passage is blocked and for a drug to gain access from the capillary circulation into the brain, it has to pass through the cells rather than between them.

-A solute may thus gain access to brain via only one of the two pathways:

1- Passive diffusion through the lipoidal barrier. which is restricted to small molecules having high o/w partition coefficient.

2. Active transport of essential nutrients such as

Sugars and amino acids. Thus, structurally similar foreign molecules can also penetrate the BBB by the same mechanism.

-The effective partition coefficient of thiopental, a highly lipid soluble drug is 50 times that of pentobarbital and crosses the BBB much more rapidly.

-Most antibiotics such as penicillin which are polar, water-soluble and ionised at plasma pH, do not cross the BBB under normal circumstances.

#Methods to promote crossing the BBB:

i- Use of permeation enhancers [Ex: DMSO]

ii- Osmotic disruption of the BBB by infusing internal carotid artery with mannitol.

iii- Use of dihydropyridine redox system as drug carriers to the brain.

Blood-cerebrospinal fluid barrier : The cerebrospinal fluid is formed mainly by the choroid plexus of the lateral, third and ventricles and is similar in composition to the ECF of brain. capillary endothelium that lines the choroid plexus have open junc or gaps and drugs can flow freely into the extracellular space betw the capillary wall and the choroidal cells. However, the choroidal cel are joined to each other by tight junctions forming the blood-CSF barrier which has permeability characteristics similar to that of the BBB (Fig. 3.6).

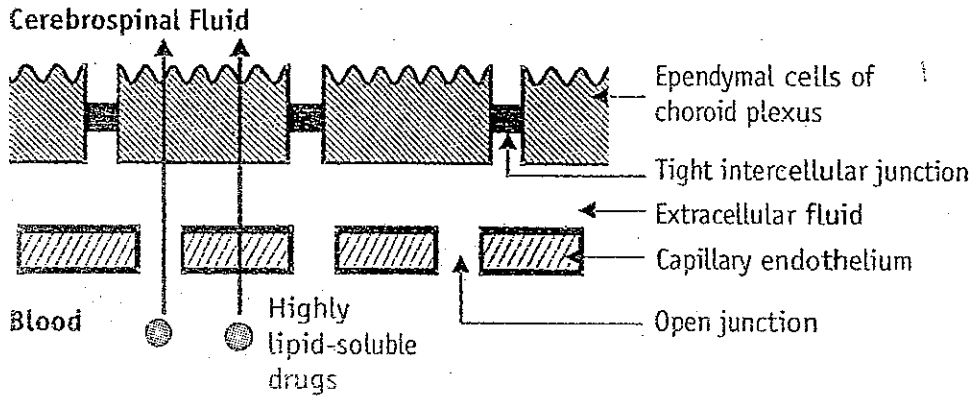


Fig. 3.6 The blood-CSF barrier

As in the case of BBB, only highly lipid soluble drugs can cross the blood-CSF barrier with relative ease whereas moderately lipid soluble and partially ionised drugs permeate slowly. A drug that enters the CSF slowly cannot achieve a high concentration as the bulk flow of CSF continuously removes the drug. For any given drug, its concentration in the brain will always be higher than in the CSF.

Although the mechanisms for diffusion of drugs into the CNS and CSF are similar, the degree of uptake may vary significantly. In some cases, CSF drug concentration may be higher than its cerebral concentration e.g. sulphamethoxazole and trimethoprim, and vice versa in other cases, e.g. certain β-blockers.

Blood-placental barrier : The maternal and the foetal blood vessels are separated by a number of tissue layers made of foetal trophoblast basement membrane and the endothelium which together constitute the placental barrier. The flow of blood in the maternal and the foetal blood vessels is shown in Fig. 3.7.

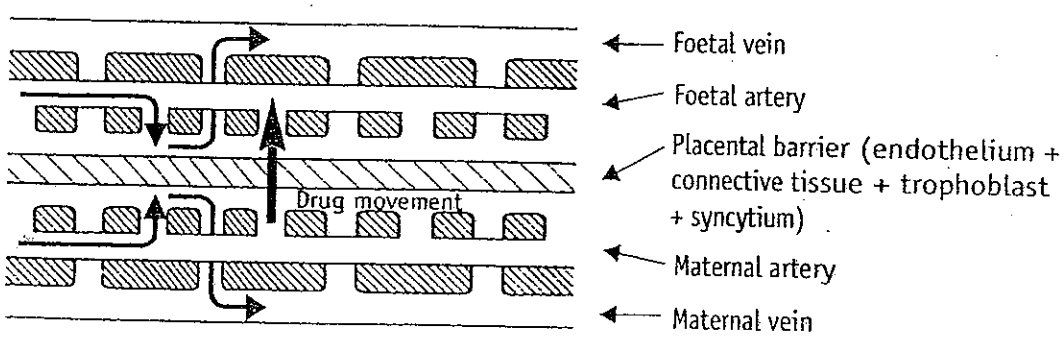


Fig. 3.7 Placental barrier and blood flow across it

The human placental barrier has a mean thickness of 25 microns in early pregnancy that reduces to 2 microns at full term which however does not reduce its effectiveness. Many drugs having molecular weight less than 1000 Daltons and moderate to high lipid solubility e.g. ethanol, sulphonamides, barbiturates, gaseous anaesthetics, steroids, narcotic analgesics, anticonvulsants and some antibiotics, cross the barrier by simple diffusion quite rapidly. This shows that the placental barrier is not as effective a barrier as BBB. Nutrients essential for the foetal growth are transported by carrier-mediated processes. Immunoglobulins are transported by endocytosis.

An agent that causes toxic effects on foetus is called as ***teratogen.*** ***Teratogenecity*** *is defined as foetal abnormalities caused by administration of drugs during pregnancy.*

Drugs can affect the foetus at 3 stages as shown in Table 3.2.

TABLE 3.2

Stages during which Teratogens Show Foetal Abnormalities

Period	*Significance*	*Harmful effects*
First 2 weeks	Fertilization and implantation stage	Miscarriage
2-8 weeks	Period of organogenesis	Cleft palate, optic atrophy, mental retardation, neural tube defects, etc.
8 weeks onwards	Growth and development	Development and functional abnormalities

It is always better to restrict all drugs during pregnancy because of the uncertainty of their hazardous effects.

Blood-testis barrier : This barrier is located not at the capillary endothelium level but at sertoli-sertoli cell junction. It is the tight junctions between the neighbouring sertoli cells that act as the blood-testis barrier. This barrier restricts the passage of drugs to spermatocytes and spermatids.

Organ/Tissue Size And Perfusion Rate:

- Distribution is permeability rate-limited in the following cases:

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

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

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

i- The drug is highly lipophilic

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

Perfusion rate: is defined as the volume of blood that flows per unit time per unit volume of the tissue.

- expressed in ml/min/ml of the tissue.

In Table 3.3, the various tissues are listed in decreasing order of their perfusion rate which indicates the rapidity with which the drug will be distributed to the tissues. Highly perfused tissues such as lungs, kidneys, adrenal, liver, heart and brain are rapidly equilibrated with lipid soluble drugs.

TABLE 3.3

Relative Volume of Different Organs, Blood Flow and Perfusion Rate under Basal Conditions Assuming the Total Body Volume to be 70 litres

Organ / tissue	*% of Body Volume*	*Blood Flow (ml/min)*	*% of Cardiac Output*	*Perfusion Rate (ml/min/ml)*
I. Highly Perfused				
1. Lungs	0.7	5000	100.0	10.2
2. Kidneys	0.4	1250	25.0	4.5
3. Adrenals	0.03	25	0.5	1.2
4. Liver	2.3	1350	27.0	0.8
5. Heart	0.5	200	4.0	0.6
6. Brain	2.0	700	14.0	0.5
II. Moderately Perfused				
7. Muscles	42.0	1000	20.0	0.034
8. Skin	15.0	350	7.0	0.033
III. Poorly Perfused				
9. Fat (adipose)	10.0	200	4.0	0.03
10. Bone (skeleton)	16.0	250	5.0	0.02

If $K_{t/b}$ is the tissue/blood partition coefficient of drug then the first-order distribution rate constant, K_t, is given by following equation:

$$K_t = \frac{\text{Perfusion rate}}{K_{t/b}} \qquad (3.2)$$

The tissue distribution half-life is given by equation:

$$\text{Distribution half-life} = \frac{0.693}{K_t} = \frac{0.693\, K_{t/b}}{\text{Perfusion rate}} \qquad (3.3)$$

- The extent to which a drug is distributed in a particular tissue or organ depends upon the size

of the tissue (i.e. tissue volume) and the tissue/blood partition coefficient of the drug.

Ex:

thiopental

→This lipophilic drug has a high tissue/blood partition coefficient towards the brain and still higher for adipose tissue. Since the brain (site of action) is a highly perfused organ, following i.v. injection, thiopental readily diffuses into the brain showing a rapid onset of action. Adipose tissue being poorly perfused, takes longer to get distributed with the same drug. But as the concentration of thiopental in the adipose proceeds towards equilibrium, the drug rapidly diffuses out of the brain and localizes in the adipose tissue whose volume is more than 5 times that of brain and has greater affinity for the drug. The result is rapid termination of action of thiopental due to such a tissue redistribution.

◻ Binding of Drugs To Tissue Components:

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

⊞ Miscellaneous factors Affecting Drug Distribution

1-Age:

a- Total body water (both intracellular and extracellular): is much greater in infants.

b- Fat content: is also higher in infants and elderly

c- Skeletal muscles: are lesser " " " "

d- Organ composition: the BBB is poorly developed in infants, the myelin content is low and cerebral blood flow is high, hence greater penetration of drugs in the brain.

e. Plasma protein content:

low albumin content in both infants and in elderly.

2. Pregnancy:

- During pregnancy, the growth of uterus, placenta and foetus increases the volume available for distribution of drugs.
- The foetus represents a separate compartment in which a drug can distribute. The plasma and the ECF volume also increase but there is a fall in albumin content.

3. Obesity:

- In obese persons, the high adipose tissue content can take up a large fraction of lipophilic drugs despite the fact that perfusion through it is low.
- The high fatty acid levels in obese persons alter the binding characteristics of acidic drugs.

4. Diet:

- A diet high in fats will increase the free fatty acid levels in circulation thereby affecting

binding of acidic drugs such as NSAIDs to albumin.

5. Disease States:

Ex:

In meningitis and encephalitis, the BBB becomes more permeable and thus polar antibiotics such as penicillin G and ampicillin which do not normally cross it, gain access to the brain.

- In a patient suffering from CCF, the perfusion rate to the entire body decreases affecting distribution of all drugs.

6. Drug interactions:

- Drug interactions that affect distribution are mainly due to differences in plasma protein or tissue binding of drugs.

Volume of Distribution:

Apparent Volume of Distribution $= \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}}$

$$V_d = \frac{X}{C}$$

- It is defined as the hypothetical volume of body fluid into which a drug is dissolved or distributed. It is called as apparent volume because all parts of the body equilibrated with the drug do not have equal concentration.

- The apparent volume of distribution bears no direct relationship with the real volume of distribution.

- The real volume of distribution has direct physiological meaning and is related to the body water.

-The body water is made up of 3 distinct compartments as shown in the table:

Fluid Compartments of a 70 Kg Adult

Body Fluid	*Volume (litres)*	*% of Body Weight*	*% of TBW*
1. Vascular fluid/blood (Plasma)	6 (3)	9 (4.5)	15 (7.5)
2. Extracellular fluid (excluding plasma)	12	17	28
3. Intracellular fluid (excluding blood cells)	24	34	57
Total Body Water (TBW)	**42**	**60**	**100**

-The volume of each of these real physiological compartments can be determined by use of specific tracers or markers.

Markers used to Measure the Volume of Real Physiological Compartments

Physiological Fluid Compartment	*Markers Used*	*Approximate Volume (litres)*
Plasma	Evans blue, indocyanine green, I-131, albumin	3
Erythrocytes	Cr-51	2
Extracellular fluid	Non-metabolisable saccharides like raffinose, inulin, mannitol and radioisotopes of selected ions: Na^+, Cl^-, Br^-, SO_4^{2-}	15
Total body water	D_2O, HTO, antipyrine	42

- The plasma volume can be determined by use of substances of high molecular weight or substances that are totally bound to plasma albumin.
- When given i.v., these remain confined to the plasma. The total blood volume can also be determined if the haematocrit is known.
- The extracellular fluid (ECF) volume can be determined by substances that easily penetrate the capillary membrane and rapidly distribute throughout the ECF but do not cross the cell membranes, for e.g: the Na^+, Cl^-, Br^- ions etc. However, none of these substances are completely kept out of the cells.
- The ECF volume, excluding plasma is approximately 15 litres.
- The total body water (TBW) volume can be determined by use of substances that distribute equally in all water compartments of the body for e.g. heavy water etc.
- The intracellular fluid volume is determined as the difference between the TBW and ECF volume.

- The intracellular fluid volume including those of blood cells is approximately 27 litres.

- Since the tracers are not bound or negligibly bound to plasma or tissue proteins, their apparent volume of distribution is same as their true volume of distribution.

- Some points:

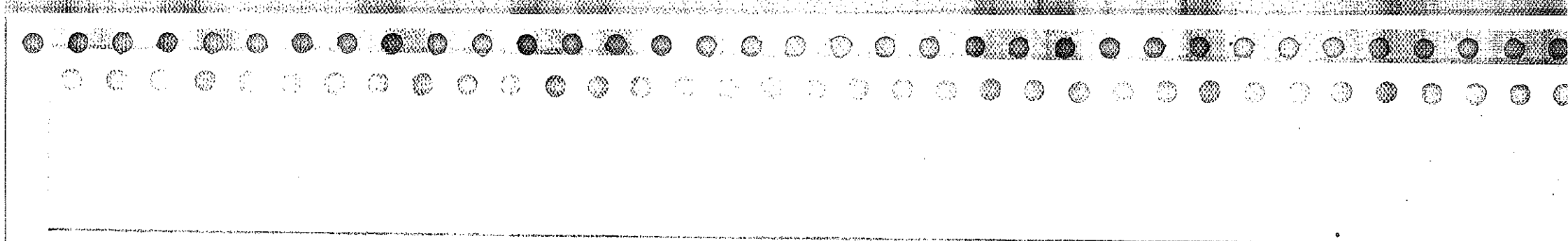
1- Drugs which bind selectively to plasma proteins or other blood components, e.g. warfarin (i.e. those that are less bound to extravascular tissues), have apparent volume of distribution smaller than their true volume of distribution.

- The V_d of such drugs lies b/w blood volume and TBW volume (i.e b/w 6 to 42 lit), for ex, warfarin has a V_d of about 10 litres.

2. Drugs which bind selectively to extravascular tissues, e.g. chloroquine (i.e. those that are less bound to blood components), have apparent volume of distribution larger than their real 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 drugs that do not distribute deeply into body tissues.

Thus, factors that produce an alteration in binding of drug to blood components, result in an *increase in* V_d and those that influence drug binding to extravascular components result in a *decrease in* V_d. Other factors that may influence V_d are changes in tissue perfusion and permeability, changes in the physicochemical characteristics of the drug e.g. ionisation, changes in the body weight and age and several disease states.

Apparent volume of distribution is expressed in litres and sometimes in litres/Kg body weight. The V_d of various drugs ranges from as low as 3 litres (plasma volume) to as high as 40,000 litres (much above the total body size). Many drugs have V_d greater than 30 litres. The V_d is a characteristic of each drug under normal conditions and is altered under conditions that affect distribution pattern of the drug.



Protein Binding of Drugs

- A drug in the body can interact with several tissue components of which the two major categories:

1. Blood — a. Plasma proteins
   b. Blood cells
2. Extravascular tissues. fats, bone etc

- The interacting molecules are generally the macromolecules such as proteins, DNA or adipose.
- The phenomenon of complex formation with proteins is called as protein binding of drugs.

- Protein binding may be divided into:

1. Intracellular binding:

Where the drug is bound to a cell protein which may be the drug receptor; if so, binding elicits a pharmacological response. These receptors with which drug interact to show response are called as primary receptors.

2. Extracellular binding:

Where the drug binds to an extracellular protein but the binding does not usually elicit a

pharmacological response. These receptors are called secondary or silent receptors.

#Mechanisms:

- Binding of drugs to proteins is generally reversible which suggests that it generally involves weak chemical bonds such as:

1. Hydrogen bonds
2. Hydrophobic bonds
3. Ionic bonds
4. Van der Waal's forces

- Irreversible drug binding, though rare, arises as a result of covalent binding and is often a reason for the carcinogenicity or tissue toxicity of the drug; for ex, covalent binding of chloroform and paracetamol metabolites to liver results in hepatotoxicity.

Binding of Drugs To Blood Components

- The extent or order of binding of drugs to various plasma proteins is:

Basic drugs: lidocaine

chlorpromazine

Albumin > α_1-Acid Glycoprotein > Lipoproteins > Globulins

Large variety of all types of drugs

α_1-G: corticosterone

α_2-G: Vit A, D, E and K

Binding of Drugs to Human Serum Albumin:

- Four different sites on HSA:

Site I:

- Also called as warfarin and azapropazone binding site, it represents the region to which large number of drugs are bound, e.g: several NSAIDs (indomethacin), phenytoin etc.

Site II:

- It is also called as the diazepam binding site.

Ex: benzodiazepines, ibuprofen

Site I and II are responsible for the binding of most drugs.

Site III: also called as digitoxin binding site.
Site IV: " " " tamoxifen " ".

- A drug can bind to more than one site in which case the main binding site is called as the primary site and the other as the secondary site; for ex, Site I is the primary site for dicoumarol and site II the secondary site.

- Groups of drugs that bind to the same site compete with each other for binding, but drugs that bind to the one site do not competitively inhibit binding of drugs to other sites.

However, they may either promote or retard binding of a drug to another site by energetic coupling mechanisms.

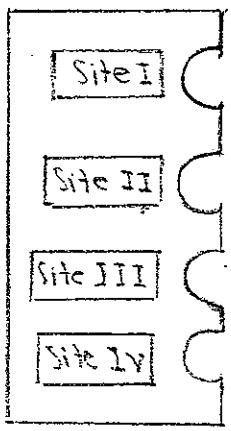


Fig: Four major drug-binding sites on human serum albumin

- Binding of Drugs to α_1- Acid Glycoprotein (α_1-AGP or AAG)

Ex:

lidocaine

propranolol

- Binding of Drugs to Lipoproteins:

- A drug that binds to lipoproteins does so by dissolving in the lipid core of the protein and thus its capacity to bind depends upon its lipid content.

- They are classified on the basis of their density into 4 categories:

1. Chylomicrons
2. Very low density lipoprotein (VLDL)
3. Low-density lipoproteins (LDL)
4. High-density lipoproteins (HDL)

- Binding of drugs to lipoproteins is non-competitive i.e. there are no specific or non-specific binding sites and binding is not depend on drug concentration.

-Binding rather reflects partitioning of drugs in hydrophobic core of lipoprotein molecule.

Ex:
- diclofenac (acidic drug)
- cyclosporin A (neutral)
- chlorpromazine (basic)

- Binding of Drugs to Globulins:

α_1-globulin, α_2 G, β_1-globulin, β_2 globulin,

γ-globulin: binds specifically to antigens

II Binding of Drugs to Blood Cells:

-The RBC comprises 3 components each of which can bind to drugs:

1. Haemoglobin:

Ex: phenytoin, pentobarbital

2. Carbonic Anhydrase: Ex: acetazolamide

3. Cell membrane (RBC membrane): Ex: chlorpromazine

Tissue Binding of Drugs (Tissue localization of Drugs)

- A drug can bind to one or more of the several tissue components.

- Tissue - drug binding is important in distribution from two viewpoints:

1. It increases the apparent volume of distribution of drugs in contrast to plasma protein binding which decreases it.

2. Tissue - drug binding results in localization of a drug at a specific site in the body

- This is more so because a number of drugs bind irreversibly with the tissues (contrast to plasma protein drug binding); for example oxidation products of paracetamol, carbon tetrachloride etc bind covalently to hepatic tissues.

- Factors influencing localization of drugs in tissues include lipophilicity and structural features of the drug, perfusion rate, pH differences etc.

heavy metal : lead, mercury

Liver > kidney > lung > Muscles

Paracetamol

antihistamine

Comparison Between Plasma Protein-Drug Binding and Tissue-Drug Binding

Plasma protein-drug binding	*Tissue-drug binding*
1. Binding involves weak bonds and thus reversible.	Binding generally involves strong and covalent bonds and thus irreversible.
2. Drugs that bind to plasma proteins have small apparent volume of distribution.	Drugs that bind to extravascular tissues have large apparent volume of distribution.
3. Half-life of plasma protein bound drug is relatively short.	Half-life of extravascular tissue bou drug is relatively long.
4. Does not result in toxicity.	Tissue toxicity is common.
5. Displacement from binding sites is possible by other drugs.	Displacement by other drugs does not occur.
6. Competition between drugs for binding to plasma proteins can occur.	Tissue-drug binding is ger competitive.

HR TV CD

DETERMINATION OF PROTEIN-DRUG BINDING

The analytical techniques used in protein binding studies can be divided into two classes:

1. ***Indirect techniques*** : are based on the separation of bound form from the free micromolecule. These techniques are usually applied in biological samples (blood, serum, plasma) for the determination of the percentage of binding. Indirect analytical methods used in protein binding studies are equilibrium dialysis, dynamic dialysis, ultrafiltration, diafiltration, ultracentrifugation, gel filtration, etc.
2. ***Direct techniques*** : do not require the separation of bound form of drug from the free micromolecule. These methods are used for the estimation of the number and the elucidation of the character of binding sites in pure aqueous solutions of proteins. Direct techniques used in protein binding studies are UV spectroscopy, fluorimetry, and ion-selective electrodes.

FACTORS AFFECTING PROTEIN-DRUG BINDING

Factors affecting protein-drug binding can be broadly categorized as—

1. Drug-related factors

(a) Physicochemical characteristics of the drug
(b) Concentration of drug in the body
(c) Affinity of a drug for a particular binding component

2. Protein/tissue related factors

(a) Physicochemical characteristics of the protein or binding agent
(b) Concentration of protein or binding component
(c) Number of binding sites on the binding agent

3. Drug interactions

(a) Competition between drugs for the binding site (displacement interactions)
(b) Competition between the drug and normal body constituents
(c) Allosteric changes in protein molecule

4. Patient-related factors

(a) Age
(b) Intersubject variations
(c) Disease states

Drug-related factors:

a- Physicochemical characteristics of the drug:

- An increase in lipophilicity increases the extent of binding.

Ex: Highly lipophilic drugs such as thiopental tend to localize in adipose tissues.

b- Concentration of Drug in the body:

c- Drug-Protein/Tissue Affinity:

- Lidocaine has greater affinity for AAG than for HSA.

- Digoxin has more affinity for proteins of cardiac muscles than those of skeletal muscles or plasma.

Protein/tissue-related Factors:

a- Physicochemical Properties of Protein/Binding Component:

- Lipoproteins and adipose tissue tend to bind lipophilic drugs by dissolving them in their lipid core.

b- Concentration of Protein/Binding Component:

- The amount of several proteins and tissue components available for binding, changes during disease states.

- Among the plasma proteins, binding predominantly occurs with albumin, as it is present in a higher concentration in comparison to other plasma proteins.

C- Number of binding sites on the Protein:

- Albumin has a large number of binding sites as compared to other proteins and is a high capacity binding component.

- Several drugs are capable of binding at more than one site on albumin. e.g dicoumarol

3. Drug interactions:

a- Competition b/w Drugs for the binding sites (Displacement Interactions)

- When two or more drugs can bind to the same site, competition between them for interaction

with the binding site results. If one of the drugs (drug A) is bound to such a site, then administration of another drug (drug B) having affinity for the same site results in displacement of drug A from its binding site.

Such a drug-drug interaction for the common binding site is called as. displacement interaction. The drug A here is called as the displaced drug and drug B as the displacer.

-Warfarin and phenylbutazone have same degree of affinity for HSA.

Administration of phenylbutazone to a patient on warfarin therapy results in displacement of latter from its binding site. The free warfarin may cause adverse hemorrhagic reactions which may be lethal.

-Displacement interactions can result in unexpected rise in free concentration of the displaced drug which may enhance clinical response.

- If the drug is easily metabolisable or excretable, its displacement results in significant reduction in elimination half-life.

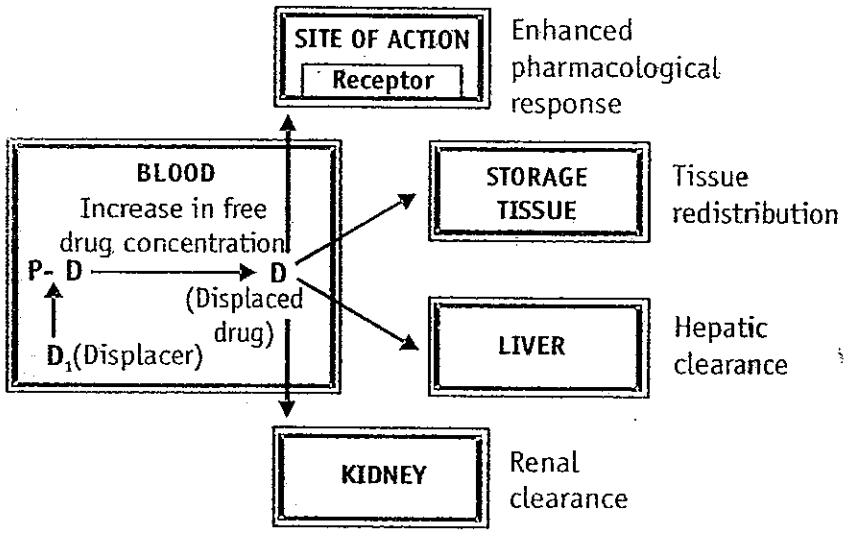


Fig. 4.3 Fate of a drug after displacement interaction

- Besides the direct displacement interaction, indirect interactions are also possible.

For example, the use of heparin as an anticoagulant activates lipoprotein lipase, an enzyme which metabolises triglycerides to free fatty acids. Heparin co-administration with drugs has also been shown to result in decreased protein binding of propranolol, etc via its effects on fatty acid levels.

b- Competition between drugs and normal body constituents:

- Among the various normal body constituents, the free fatty acids are known to interact with a number of drugs that bind primarily to HSA.
- The free fatty acid level is increased in several conditions : fasting, diabetes, etc.
- The fatty acids, which also bind to albumin, influence binding of several benzodiazepines and propranolol (decreased binding) and warfarin (increased binding).

c- Allosteric changes in Protein Molecule:

- The process involves alteration of the protein structure by the drug or its metabolite thereby modifying its binding capacity.
- The agent that produces such an effect is called as allosteric effector : Ex: Aspirin → modifying albumin capacity → phenylbutazone (increase affinity).

4- Patient-Related Factors:

a- Age:

- Modification in protein-drug binding as influenced by age of the patient is mainly due to differences in the protein content in various age group.

i- Neonates: Albumin content is low in newborn; as a result, the unbound concentration of drug that primarily binds to albumin, for example phenytoin and diazepam, is increased.

ii- Elderly: In old age, the albumin content is lowered and free concentration of drugs that bind primarily to it is increased.

- old age is also characterized by an increase in the levels of AAG and thus decreased free concentration is observed for drugs that bind to it.

b- Disease States:

- Since albumin is the major drug binding protein, hypo-albuminaemia can severely impair protein-drug binding.

- Hypoalbuminaemia is caused by aging, CCF, burns, pregnancy, renal and hepatic disorders, cancer etc.

- Hyperlipoproteinaemia, caused by hypothyroidism, obstructive liver disease, alcoholism, etc, affects binding of lipophilic drugs.

SIGNIFICANCE OF PROTEIN/TISSUE BINDING OF DRUGS

Absorption

The absorption equilibrium is attained by transfer of free drug from the site of administration into the systemic circulation and when the concentration in these two compartments becomes equal. Following equilibrium, the process may stop. However, binding of the absorbed drug to plasma proteins decreases free drug concentration and disturbs such an equilibrium. Thus, sink conditions and the concentration gradient are re-established which now act as the driving force for further absorption. This is particularly useful in case of ionised drugs which are transported with difficulty.

Systemic Solubility of Drugs

Water insoluble drugs, neutral endogenous macromolecules such as heparin and several steroids and oil soluble vitamins are circulated and distributed to tissues by binding especially to lipoproteins which act as a vehicle for such hydrophobic compounds.

Distribution

Plasma protein binding restricts the entry of drugs that have specific affinity for certain tissues. This prevents accumulation of a large fraction of drug in such tissues and thus, subsequent toxic reactions. Plasma protein-drug binding thus favours uniform distribution of drugs throughout the body by its buffer function (maintains equilibrium between the free and the bound drug). A protein bound drug in particular does not cross the BBB, the placental barrier and the glomerulus.

Tissue Binding, Apparent Volume of Distribution and Drug Storage

A drug that is extensively bound to blood components remains confined to blood. Such a drug has a small volume of distribution. A drug that shows extravascular tissue binding has a large volume of distribution. A tissue or blood component that has great affinity for a particular drug acts as a *depot* or *storage site* for that drug; for example, RBC is a storage site for the lipophilic compound tetrahydrocannabinol.

The relationship between tissue-drug binding and apparent volume of distribution can be established as follows:

$$V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}} = \frac{X}{C} \qquad (4.1)$$

or, the amount of drug in the body, $X = V_d\, C$ (4.2)

Similarly, we can write,

$$\text{Amount of drug in plasma} = V_p C \tag{4.3}$$

and, $$\text{Amount of drug in extravascular tissues} = V_t C_t \tag{4.4}$$

The total amount of drug in the body is the sum of amount of drug in plasma and the amount of drug in extravascular tissues. Thus,

$$V_d C = V_p C + V_t C_t \tag{4.5}$$

where, V_d = apparent volume of distribution of drug
V_p = volume of plasma
V_t = volume of extravascular tissues
C_t = tissue drug concentration

Dividing equation 4.5 with C we get:

$$V_d = V_p + \frac{V_t C_t}{C} \tag{4.6}$$

The fraction of drug unbound (f_u) in plasma is given as:

$$f_u = \frac{\text{Concentration of unbound drug in plasma}}{\text{Total plasma drug concentration}} = \frac{C_u}{C} \tag{4.7}$$

Similarly, fraction of drug unbound to tissues is:

$$f_{ut} = \frac{C_{ut}}{C_t} \tag{4.8}$$

Assuming that at distribution equilibrium, the unbound or free drug concentration in plasma equals that in extravascular tissues i.e. $C_u = C_{ut}$, equations 4.7 and 4.8 can be combined to give:

$$\frac{C_t}{C} = \frac{f_u}{f_{ut}} \tag{4.9}$$

Substitution of equation 4.9 in 4.6 yields:

$$V_d = V_p + \frac{V_t f_u}{f_{ut}} \tag{4.10}$$

From equation 4.10 it is clear that greater the unbound or free concentration of drug in plasma, larger its V_d.

Elimination

Only the unbound or free drug is capable of being eliminated. This is because the drug-protein complex cannot penetrate into the metabolising organ (liver). The large molecular size of the complex also prevents it from getting filtered through the glomerulus. Thus, drugs which are more than 95% bound are eliminated slowly i.e. they have long elimination half-lives; for example, tetracycline, which is only 65% bound, has an elimination half-life of 8.5 hours in comparison to 15.1 hours of doxycycline which is 93% bound to plasma proteins. However, penicillins have short elimination half-lives despite being extensively bound to plasma proteins. This is because rapid equilibration occurs between the free and the bound drug and the free drug is equally rapidly excreted by active secretion in renal tubules.

Displacement Interactions and Toxicity

As stated earlier, displacement interactions are significant in case of drugs which are more than 95% bound. This is explained from the example given in Table 4.4. A displacement of just 1% of a 99% bound drug results in doubling of the free drug concentration i.e. a 100% rise. For a drug that is bound to a lesser extent e.g. 90%, displacement of 1% results in only a 10% rise in free drug concentration which may be insignificant clinically.

Kernicterus in infants is an example of a disorder caused by displacement of bilirubin from albumin binding sites by the NSAIDs and sulphonamides. Another example discussed earlier was that of interaction between warfarin and phenylbutazone. Yet another example of displacement is that of digoxin with quinidine. Digoxin represents a drug with a large volume of distribution (i.e. shows extensive extravas-

Influence of Percent Binding and Displacement on Change in Free Concentration of Drugs

	Drug A	*Drug B*
% drug before displacement		
bound	99	90
free	1	10
% drug after displacement		
bound	98	89
free	2	11
% increase in free drug concentration	100	10

cular tissue binding). Since displacement interactions may precipitate toxicity of displaced drug, a reduction in its dose may be called for. This may become necessary for a drug having a small V_d such as warfarin since displacement can result in a large increase in free drug concentration in plasma. With a drug of large V_d such as digoxin, even a substantial increase in the degree of displacement of drug in plasma may not effect a large increase in free drug concentration and dose adjustment may not be required. This is for two reasons—one, only a small fraction of such a drug is present in plasma whereas most of it is localized in extravascular tissues, and secondly, following displacement, the free drug, because of its large V_d, redistributes in a large pool of extravascular tissues. The extent to which the free plasma drug concentration of drugs with different V_d values will change when displaced, can be computed from Equation 4.10.

Diagnosis

The chlorine atom of chloroquine when replaced with radiolabelled I-131 can be used to visualize melanomas of the eye since chloroquine has a tendency to interact with the melanin of eyes. The thyroid gland has great affinity for iodine containing compounds; hence any disorder of the same can be detected by tagging such a compound with a radioisotope of iodine.

Therapy and Drug Targeting

The binding of drugs to lipoproteins can be used for site-specific delivery of hydrophilic moieties. This is particularly useful in cancer therapies since certain tumour cells have greater affinity for LDL than normal tissues. Thus, binding of a suitable antineoplastic to it can be used as a therapeutic tool. HDL is similarly transported more to adrenal and testes. An example of site-specific drug delivery in cancer treatment is that of oestramustine. Oestradiol binds selectively and strongly to prostrate and thus prostrate cancer can be treated by attaching nitrogen mustard to oestradiol for targeting of prostrate glands. Drug targeting prevents normal cells from getting destroyed.

KINETICS OF PROTEIN-DRUG BINDING

If P represents proteins and D the drug, then applying **law of mass** action to reversible protein-drug binding, we can write:

$$P + D \rightleftharpoons PD \tag{4.11}$$

At equilibrium,

$$K_a = \frac{[PD]}{[P][D]} \tag{4.12}$$

$$[PD] = K_a [P][D] \tag{4.13}$$

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 protein present, bound and unbound, then:

$$P_T = [PD] + [P] \tag{4.14}$$

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

$$r = \frac{[PD]}{[P_T]} = \frac{[PD]}{[PD] + [P]} \tag{4.15}$$

Substituting the value of [PD] from equation 4.13 in equation 4.15 we get:

$$r = \frac{K_a [P][D]}{K_a [P][D] + [P]} = \frac{K_a [D]}{K_a [D] + 1} \tag{4.16}$$

Equation 4.16 holds when there is only one binding site on the protein and the protein-drug complex is a 1:1 complex. If more than one or N number of binding sites are available per mole of the protein then:

$$r = \frac{N K_a [D]}{K_a [D] + 1} \qquad (4.17)$$

The value of association constant, K_a and the number of binding sites N can be obtained by plotting equation 4.17 in four different ways as shown below (*see* Fig. 4.4.).

1. **Direct Plot** is made by plotting r versus [D] as shown in Fig. 4.4a. Note that when all the binding sites are occupied by the drug, the protein is saturated and plateau is reached. At the plateau, r = N. When r = N/2, [D] = $1/K_a$.

2. **Scatchard Plot** is made by transforming equation 4.17 into a linear form. Thus,

$$r = \frac{N K_a [D]}{K_a [D] + 1} \qquad (4.17)$$

$$r + r K_a [D] = N K_a [D]$$

$$r = N K_a [D] - r K_a [D]$$

Therefore,

$$\frac{r}{[D]} = N K_a - r K_a \qquad (4.18)$$

A plot of r/[D] versus r yields a straight line (Fig. 4.4b). Slope of the line = $- K_a$, *y*-intercept = NK_a and *x*-intercept = N.

3. **Klotz Plot/Lineweaver-Burke Plot (Double Reciprocal Plot) :** The reciprocal of equation 4.17 yields:

$$\frac{1}{r} = \frac{1}{N K_a [D]} + \frac{1}{N} \qquad (4.19)$$

A plot of 1/r versus 1/[D] yields a straight line with slope $1/NK_a$ and *y*-intercept 1/N (Fig. 4.4c).

4. **Hitchcock Plot** is made by rewriting equation 4.19 as—

$$\frac{N K_a [D]}{r} = 1 + Ka [D] \qquad (4.20)$$

Dividing both sides by NK_a gives—

$$\frac{[D]}{r} = \frac{1}{N K_a} + \frac{[D]}{N} \qquad (4.21)$$

Equation 4.21 is Hitchcock equation according to which a plot of [D]/r versus [D] yields a straight line with slope 1/N and y-intercept $1/NK_a$ (*see* Fig. 4.4d).

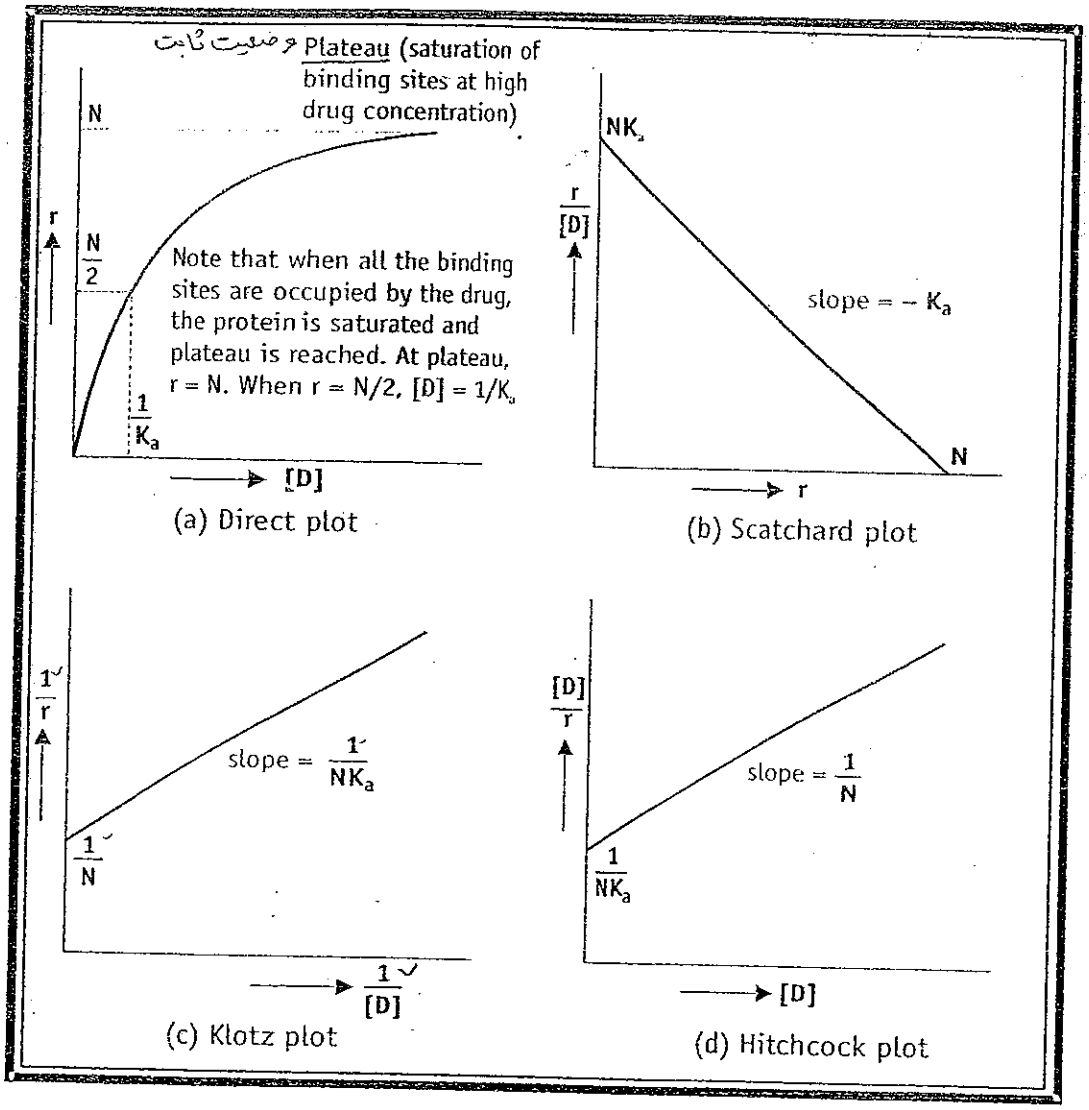
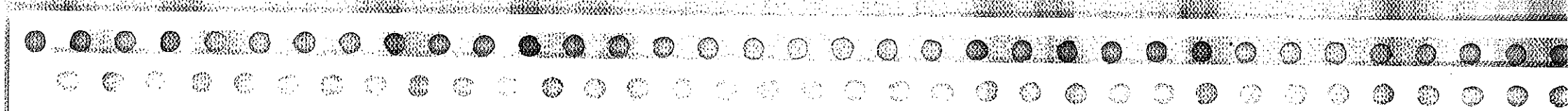
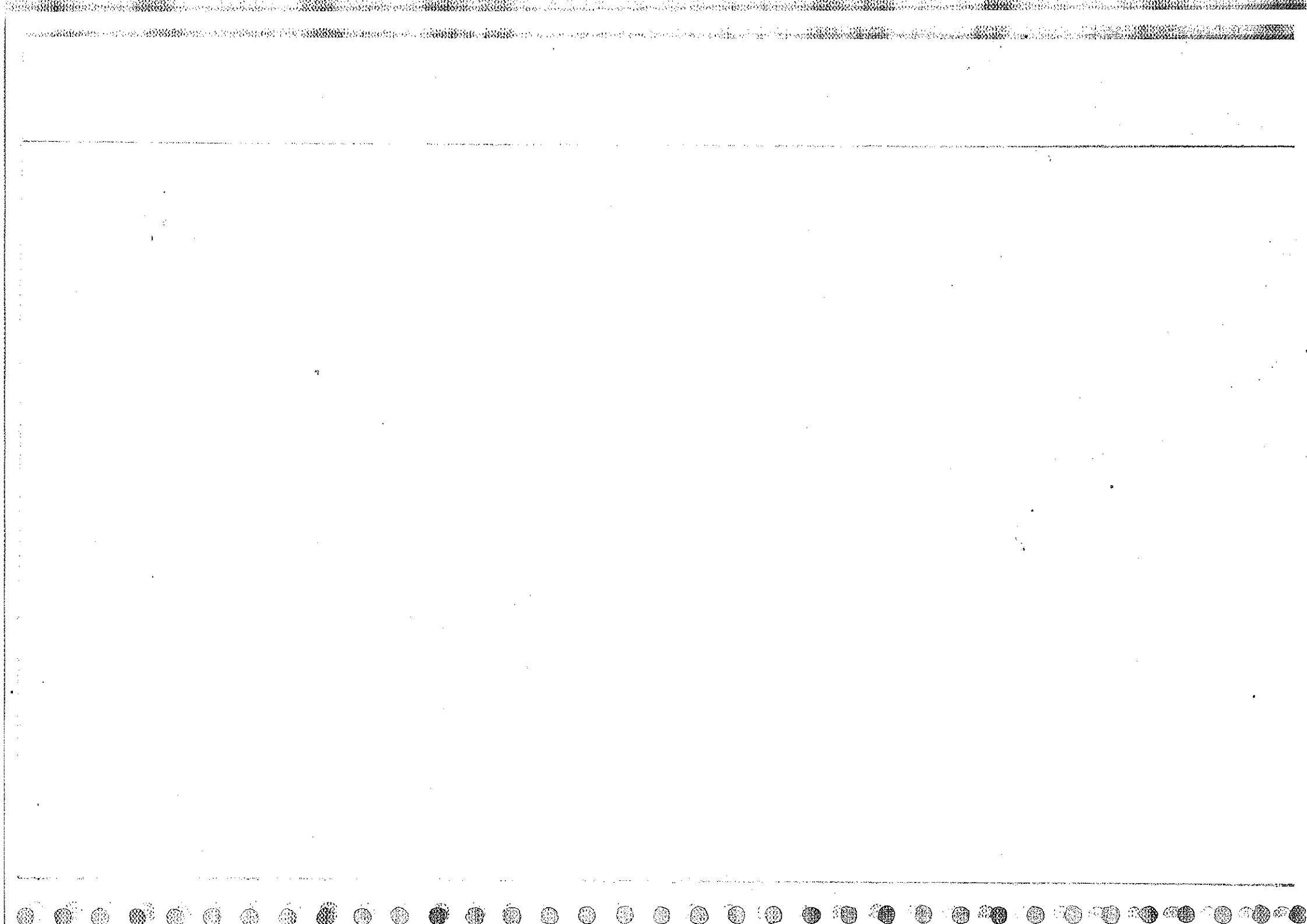
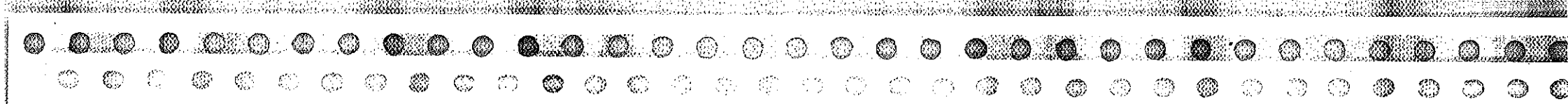


Fig. 4.4 Plots used for the study of protein-drug binding. (a) Direct plot; (b) Scatchard plot; (c) Klotz plot; and (d) Hitchcock plot.







Biotransformation of Drugs

Elimination is the major process for removal of a drug from the body and termination of its action. It is defined as the irreversible loss of drug from the body.

- Elimination occurs by two processes viz, biotransformation and excretion.

- Drug metabolism or biotransformation is the transformation of one chemical entity to another chemical entity inside the living body by specific enzymes.

- Drug metabolism facilitates in the quick elimination of the formed metabolites.

- The main purpose of drug metabolism is to eliminate xenobiotics. Xenobiotics are the substances other than nutrients, that enter the body by absorption, inhalation or ingestion.

Ex:
cosmetics, processed food, drugs etc.

- These xenobiotics accumulate inside the body,

are not excreted easily due to their lipophilic nature and hence result in toxicity.

- To avoid such conditions these lipophilic compounds are transformed into water soluble and polar compounds for their easy excretion. This is because polar drugs are eliminated easily than the non-polar drugs.

Hence drug metabolism comes to the rescue as detoxification system.

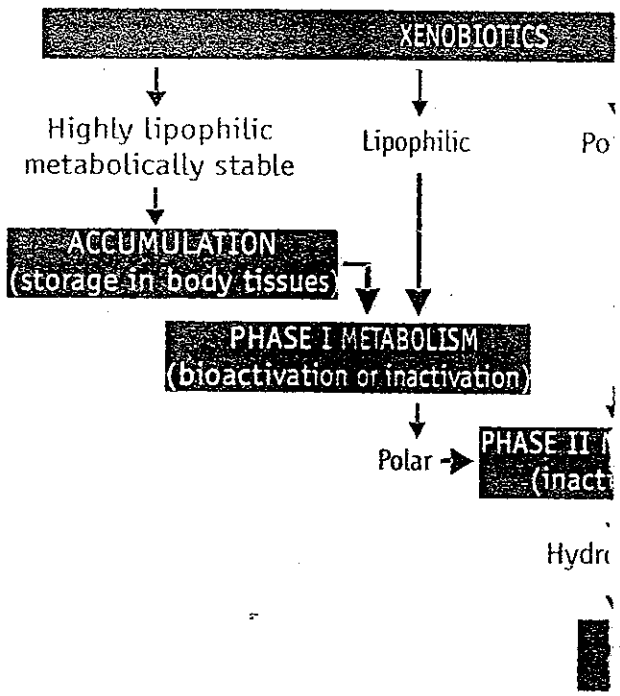


Fig. 5.1 Disposition of drug in the body as a c

1- Write a note on drug elimination [Jan-2014. 5M]

-Drug Metabolising Organs:

-Liver is the primary site for metabolism of almost all drugs (and other xenobiotics).

-Metabolism by organs other than liver called as extrahepatic metabolism.

Liver > Lungs > kidneys > Intestine > Placenta > Adrenals > skin

-Drug metabolising Enzymes:

-The enzymes that biotransform xenobiotics differ from those that metabolise food materials.

- Microsomal enzymes
- Non-microsomal enzymes

Chemical pathways of Drug Biotransformation:

- Phase I reactions
- Phase II reactions

Phase I Reactions:

- These reactions generally precede phase II reactions.

- Oxidation
- reduction
- Hydrolysis

-Objectives:

1. Increase in hydrophilicity: By way of these reactions, a polar functional group is either introduced or unmasked if already present on the otherwise lipid-soluble substrate, e.g. -OH, -COOH etc.

- Thus, phase I reactions are also called as functionalisation reactions.

- These transformations are also called as asynthetic reactions, opposite to the synthetic phase II reactions.

2. reduction in stability

3. Facilitation of conjugation: The resulting product of phase I reaction is susceptible to phase II reactions.

Phase II Reactions

These reactions generally involve covalent attachment of small polar endogenous molecules such as glucuronic acid, sulphate, glycine, etc. to either unchanged drugs or phase I products having suitable functional groups *viz.* -OH, -COOH, $-NH_2$ and -SH and form highly water-soluble *conjugates* which are readily excretable by the kidneys (or bile). Thus, these reactions are called as **conjugation reactions**. Since the outcome of such processes are generally products with increased molecular size (and altered physicochemical properties), they are also called as **synthetic reactions**. Quite often, a phase I reaction may not yield a metabolite that is sufficiently hydrophilic or pharmacologically inert but conjugation reactions generally result in products with total loss of pharmacological activity and high polarity. Hence, *phase II reactions are better known as* **true detoxification reactions**. Since these reactions generally involve transfer of moieties to the substrate to be conjugated, the enzymes responsible are called as *transferases*.

Phase III Reactions

Failure to remove the products of Phase II reactions can lead to:

1. Toxicity of conjugates to various cell components.
2. Hydrolysis of conjugates back to the original reactive species.
3. Inhibition of Phase II enzymes.

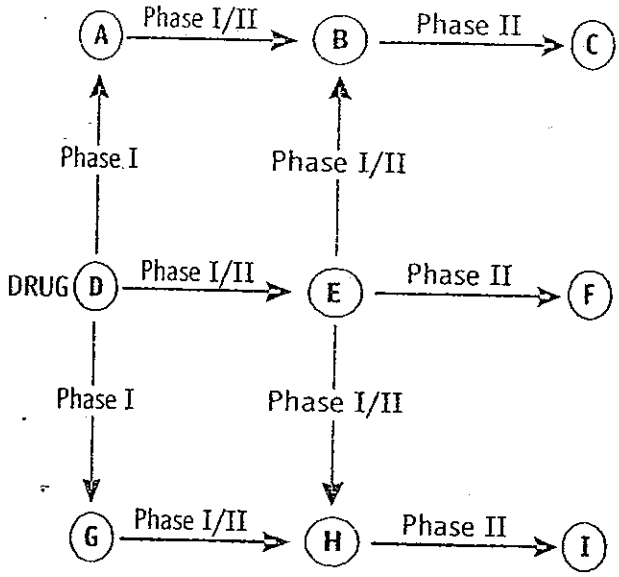


Fig. 5.2 Sequence of phase I and phase II reactions yielding a range of products

-Consequently, an impressive array of multi-purpose membrane bound transport carrier systems called as efflux pump transporters (P-glycoproteins or P-gp) have evolved which can actively remove hydrophilic metabolites and many other low molecular weight unmetabolised drugs and toxins from the cells.

-This essential arm of the detoxification process has recently been referred to as phase III biotransformation.

Chemical Pathways of Drug Biotransformation — (M) and (N) Indicate Reactions Catalysed by Microsomal and Non-microsomal Enzymes

PHASE I REACTIONS	
A. Oxidative Reactions	
1. Oxidation of aromatic carbon atoms	(M)
2. Oxidation of olefins (C=C bonds)	(M)
3. Oxidation of benzylic, allylic carbon atoms and carbon atoms alpha to carbonyl and imines	(M)
4. Oxidation of aliphatic carbon atoms	(M)
5. Oxidation of alicyclic carbon atoms	(M)
6. Oxidation of carbon-heteroatom systems:	
a. Carbon-Nitrogen systems (aliphatic and aromatic amines):	
i. N-Dealkylation	(M)
ii. Oxidative deamination	(M), (N)
iii. N-Oxide formation	(M)
iv. N-Hydroxylation	(M)
b. Carbon-Sulphur systems:	
i. S-Dealkylation	(M)
ii. Desulphuration	(M)
iii. S-oxidation	(M)
c. Carbon-Oxygen systems (O-dealkylation)	(M)
7. Oxidation of alcohol, carbonyl and acid functions	(M)
8. Miscellaneous oxidative reactions	(M), (N)

...continued

B. Reductive Reactions	
1. Reduction of carbonyl functions (aldehydes/ketones)	(M), (N)
2. Reduction of alcohols and C=C bonds	(M)
3. Reduction of N-compounds (nitro, azo and N-oxide)	(M), (N)
4. Miscellaneous reductive reactions	
C. Hydrolytic Reactions	
1. Hydrolysis of esters and ethers	(M),(N)
2. Hydrolysis of amides	(M),(N)
3. Hydrolytic cleavage of non-aromatic heterocycles	(M),(N)
4. Hydrolytic dehalogenation	
5. Miscellaneous hydrolytic reactions	

PHASE II REACTIONS

1. Conjugation with glucuronic acid	(M)
2. Conjugation with sulphate moieties	(N)
3. Conjugation with alpha amino acids	(N)
4. Conjugation with glutathione and mercapturic acid formation	(N)
5. Acetylation reactions	(N)
6. Methylation reactions	(N)
7. Miscellaneous conjugation reactions	(N)

#Phase I Reactions:

Oxidative reactions:

- Oxidation of xenobiotics is non-specifically catalysed by a number of enzymes located in the microsomes. Such enzymes require both molecular oxygen (O_2) and the reducing agent NADPH to effect reaction. They are therefore to as the mixed-function oxidases.

$$RH + O_2 + NADPH + H^+ \longrightarrow ROH + H_2O + NADP^+$$

- Since only one oxygen atom from the molecular oxygen is incorporated in the product formed, the mixed-function oxidases are also called as monooxygenases.

- The multienzyme mixed-function oxidase system, located in the endoplasmic reticulum of hepatic cells, is composed of an electron transfer chain consisting of 3 components:

1. Cytochrome P-450 (Cyp-450) (the most important component)
2. Cytochrome P-450 reductase
3. Phosphatidyl choline

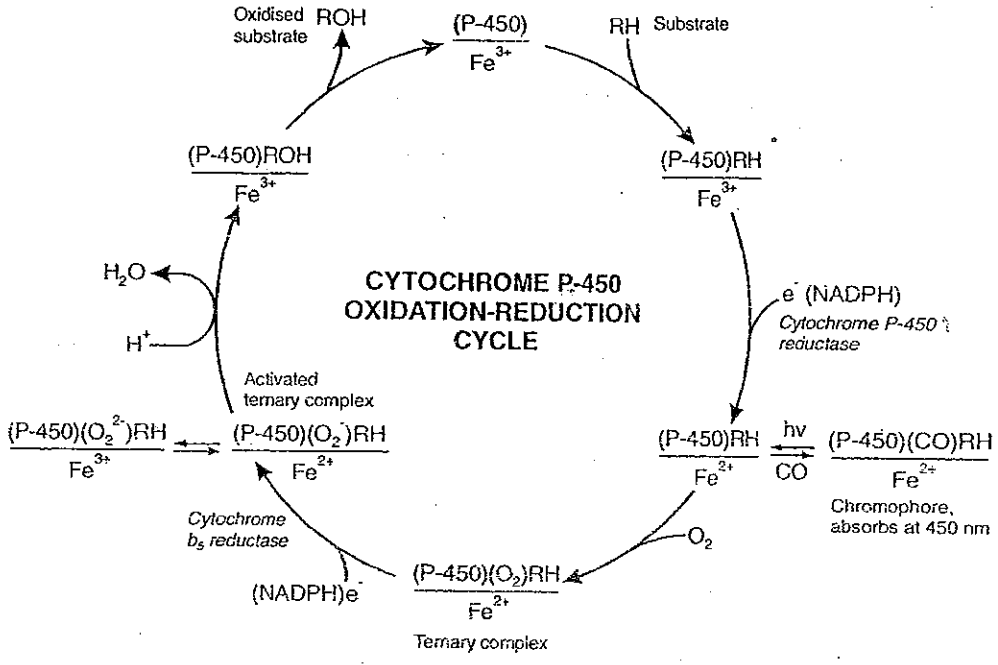
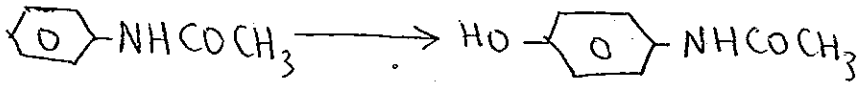


Fig. 5.3 Cytochrome P-450 oxidation-reduction cycle

The various *steps in the oxidation of xenobiotics* are:

1. Binding of the substrate (RH) to the oxidised form of the cytochrome P-450 (Fe^{+++}) to form a complex.
2. Transfer of one electron from NADPH to the complex by cytochrome P-450 reductase to form reduced (Fe^{++}) P-450—substrate complex. This step is considered as the rate-limiting step in the overall oxidation of xenobiotics.
3. Combination of reduced enzyme-substrate complex with a molecule of oxygen to form a *ternary complex.*
4. Combination of ternary complex with a second electron supplied by NADH in presence of enzyme cytochrome b_5 reductase to form a ternary activated *oxygen—P-450—substrate complex.*
5. Transfer of one atom of oxygen from the activated oxygen complex to the substrate to yield the oxidised product. The other atom forms water. The free oxidised form of cytochrome P-450 is now ready to attach to yet another molecule of substrate.

1-oxidation of Aromatic Carbon Atoms:

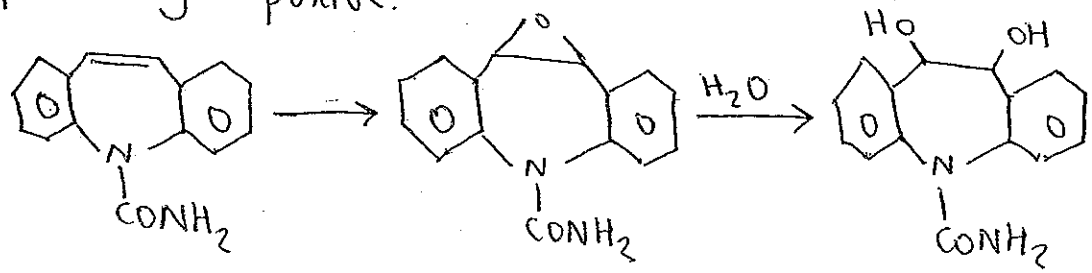


Acetanilide

Paracetamol

2- Oxidation of olefine:

-Metabolic oxidation of olefine (C=C) lead to corresponding epoxide:



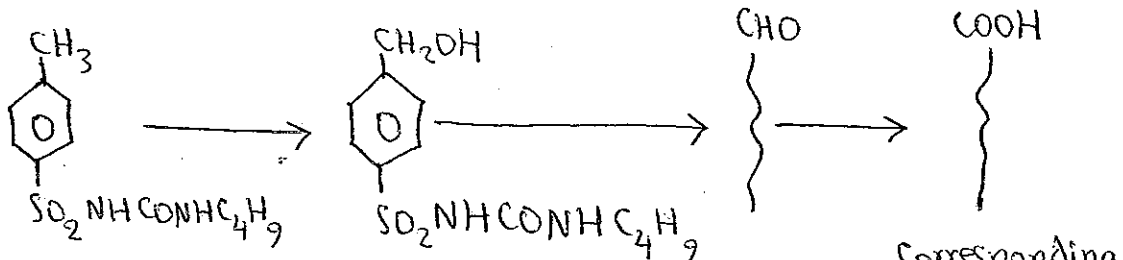
Carbamazepine

Carbamazepine-10,11-epoxide

Trans-10,11-dihydroxy carbamazepine

3- Oxidation of Benzylic Carbon Atoms:

-The carbon atom attached to benzen ring is called as benzylic carbon atom.

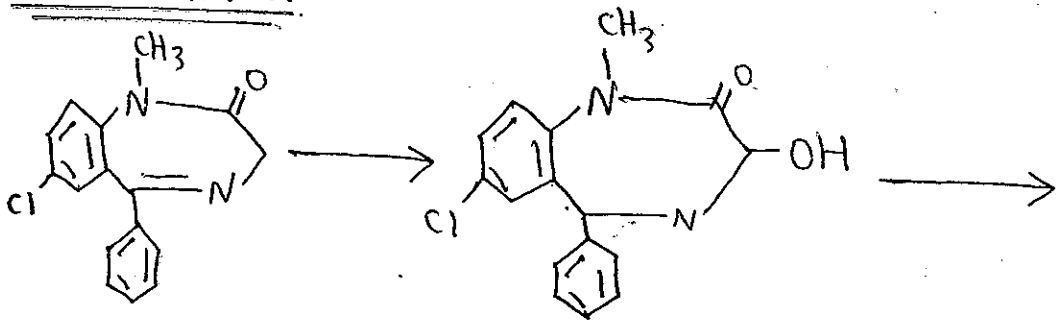


Tolbutamide

corresponding aldehyde

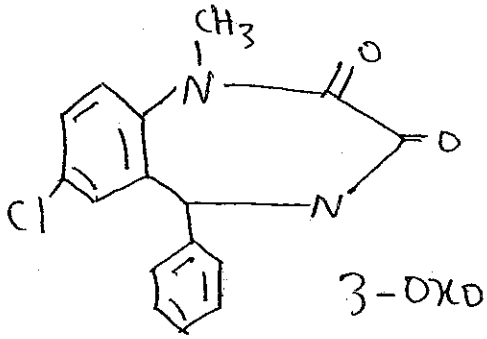
Corresponding Carboxylic acid

4- Oxidation of carbon atoms α to carbonyls and Imines:



Diazepam

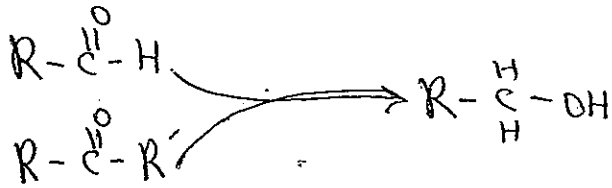
3-hydroxy diazepam



3-oxozepam

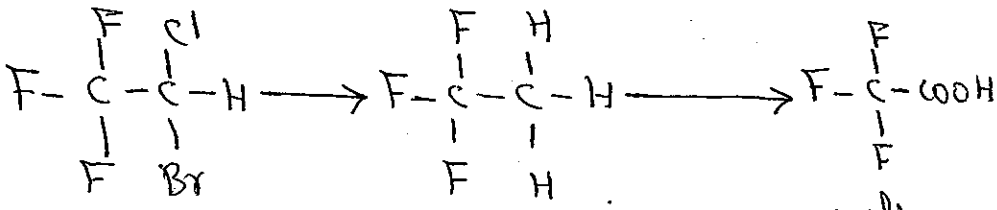
□ Reductive Reactions:

- A reaction which proceeds by the addition of hydrogen and removal of oxygen like reduction aldehyde and ketone to alcohol.



i- Redactive dehallogenation:

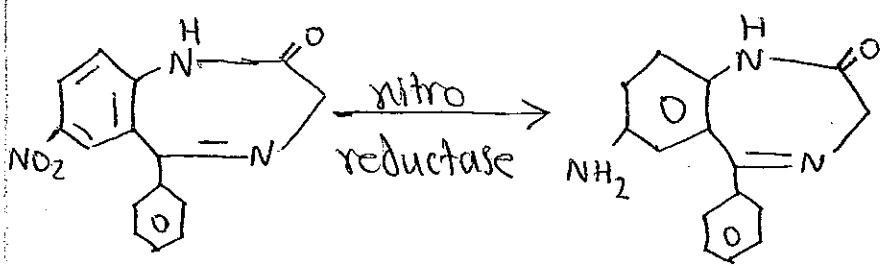
Ex: Hallothane



Hallothane 1,1,1-trifluro ethane trifluoro aceticacid

2. Reductive nitro and azo compounds:

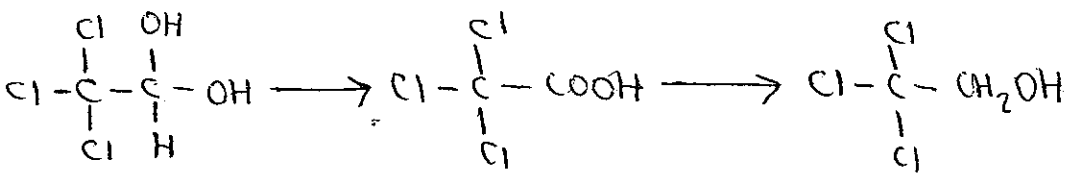
Ex: Nitrozepam



Nitrozepam 7-amino metabolite of Nitrozepam

3. Reduction of aldehyde and ketones:

Eg: Chloral Hydrate



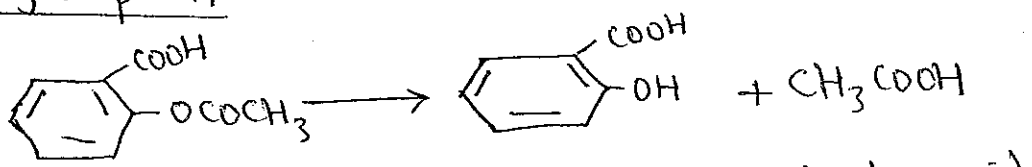
Chloral hydrate Trichloro aceticacid Trichloro ethanol

H Hydrolytic reactions:

- lysis of compound using a water molecule is called Hydrolysis.
- The metabolism of ester and amide linkage in many drug is catalysed by hydrolytic enzyme..
- Esters on hydrolysis yield alcohol and carboxylic acid. The reaction is catalysed by esterases.

1- Hydrolysis of ester:

Eg: Aspirin

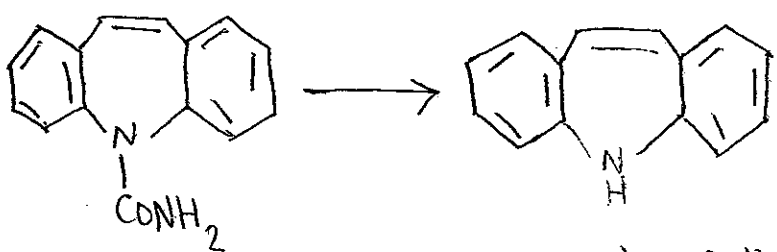


Aspirin — Salicylic acid — Acetic acid

2. Hydrolysis of Amide:

- Amides are hydrolysed slowly when compared with esters.

Eg: Carbamazepine



Imide Stilbene

Phase II Reactions:

-Phase II reactions involve transfer of a suitable endogenous moiety such as glucuronic acid, sulphate, glycine, etc. in presence of enzyme transferase to drugs or metabolites of phase I reactions having suitable functional groups to form highly polar, readily excretable and pharmacologically inert conjugates.

-Phase II reactions are the real drug detoxication pathways because:

1. The conjugates/products of phase II reactions are absolutely free of pharmacological activity.

2. The conjugates/products of phase II reactions are highly polar and thus easily excretable either in bile or urine.

3. Tissue-reactive and carcinogenic metabolites formed as a result of phase I reaction are rendered harmless by conjugation with moieties such as glutathione.

2. Discuss the Glucuronidation in phase II reactions? [5m, 13]
- Describe glucuronic acid conjugation and its importance in drug metabolism? [5m, 2017]

- The order of capacities of important conjugation reaction is:

Glucuronidation > Amino Acid conjugation > Sulphation and Glutathione Conjugation

Phase II Reactions and their Characteristics

Conjugation Reaction	*Conjugating Agent*	*Conjugating Agent Transferring Enzyme*	*Activated Inter-mediate*	*Functional Groups Combined with*
Glucuronidation	Glucuronic acid	UDP-glucuronyl transferase	UDPGA	-OH, -COOH, $-NH_2$, -SH
Sulphation	Sulphate	Sulphotransferase	PAPS	-OH, $-NH_2$
Amino acid conjugation	Glycine	Acyl transferase	Acyl CoA	-COOH, $-NH_2$
Glutathione	Glutathione	Gluthaione-S-transferase	—	Alkyl halides, alkyl nitrates, epoxides, lactones, etc.
Acetylation	Acetyl CoA	N-acetyl transferase	Acetyl CoA	$-NH_2$, $-SO_2NH_2$, hydrazines
Methylation	L-methio-nine	Methyl transferase	S-adenosyl methionine	-OH, $-NH_2$, -SH

#Glucuronic acid conjugation:

- Also called as glucuronidation, it is the most common and most important phase II reaction for several reasons:

1. Readily available source of conjugating

moiety, D-glucuronic acid which is derived from D-glucose.

2. Several functional groups viz, alcohols, acids, amines, etc. can combine easily with D-glucuronic acid.

3. All mammals have the common ability to produce glucuronides.

4. Glucuronidation can take place in most body tissues since the glucuronic acid donor, UDPGA is produced in processes related to glycogen synthesis and thus, will never be deficient unlike those involved in other phase II reactions.

Glucuronide formation occurs in 2 steps –

1. *Synthesis* of an activated coenzyme uridine-5'-diphospho-α-D-glucuronic acid (UDPGA) from UDP-glucose (UDPG). The coenzyme UDPGA acts as the donor of glucuronic acid. UDPG is synthesized by interaction of α-D-glucose-1-phosphate with uridine triphosphate (UTP).
2. *Transfer* of the glucuronyl moiety from UDPGA to the substrate RXH in presence of enzyme UDP-glucuronyl transferase to form the conjugate. In this step, the α-configuration of glucuronic acid undergoes *inversion* and thus, the resulting product is **β-D-glucuronide** (also called as **glucosiduronic acid** or **glucopyranosiduronic acid** conjugate).

The steps involved in glucuronide synthesis are depicted below:

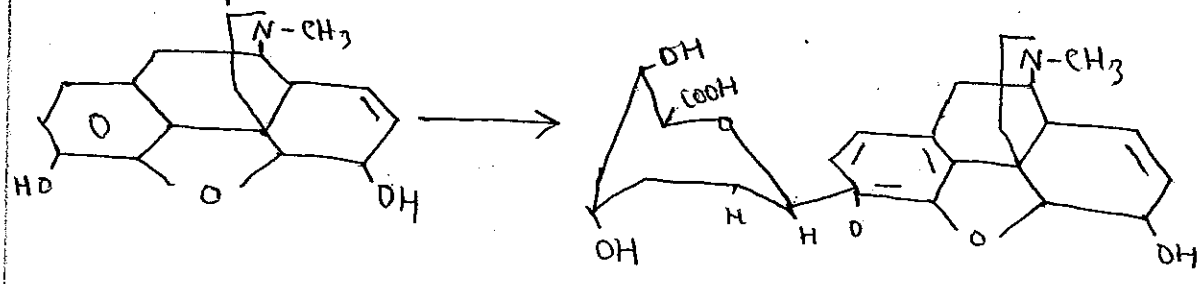
$$\alpha\text{-D-glucose-1-phosphate} + UTP \xrightarrow{\textit{Pyrophosphorylase}} UDPG + PPi$$

$$UDPG + 2NAD^{+} + H_2O \xrightarrow{\textit{UDPG-dehydrogenase}} UDPGA + 2NADH + 2H^{+}$$

$$UDPGA + RXH \xrightarrow{\textit{UDP-glucuronyl tranferase}} RX\text{-glucuronic acid} + UDP$$

where X = O, COO, NH or S.

Ex: Morphine



morphine Glucoronide

- In case of infats the enzyme for glucuronide conjugation are not develop, hence drug will be accumulate and lead to toxicity.

Ex:

Chloramphenicol

Sulphate Conjugation:

Like glucuronidation, sulphation also occurs in 2 steps:

1. *Synthesis* of an activated coenzyme 3'-phosphoadenosine-5'-phosphosulphate (PAPS) which acts as a donor of sulphate to the substrate. This also occurs in two steps —
   (a) An initial interaction between the sulphate and the adenosine triphosphate (ATP) to yield adenosine-5'-phosphosulphate (APS), followed by
   (b) Activation of APS to PAPS.
2. *Transfer* of sulphate group from PAPS to the substrate RXH in presence of enzyme sulphotransferase (sulphokinase) and subsequent liberation of 3'-phosphoadenosine-5'-phosphate (PAP).

The steps are summarized in the equations below:

$$ATP + SO_4^{2-} \xrightarrow{ATP\text{-}sulphurylase/Mg^{++}} APS + PPi$$

$$APS + ATP \xrightarrow{ATP\text{-}phosphokinase/Mg^{++}} PAPS + ADP$$

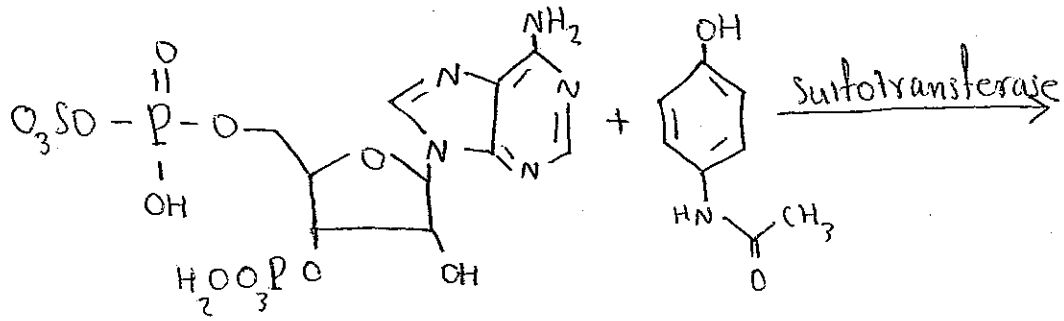
$$PAPS + RXH \xrightarrow{Sulphotransferase} RX\text{-}SO_3 + PAP$$

where X = O, NH

Functional groups capable of forming sulphate conjugates include phenols, alcohols, arylamines, N-hydroxylamines and N-hydroxyamides. The reaction product is a sulphate ester, also called as *ethereal sulphate*.

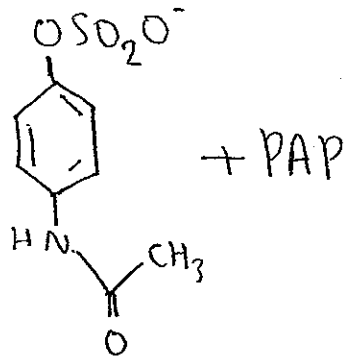
-Ex: Acetaminophen is mainly metabolised by sulphate conjugation.

$SO_4^{2-} + ATP \xrightarrow[2.\ APS\ phosphokinase]{1.\ ATP\ sulfurylase}$ 3-phosphoadenosine 5'phosphosulfate (PAPS) + ADP + PP_i



Acetaminophen

PAPS



+ PAP

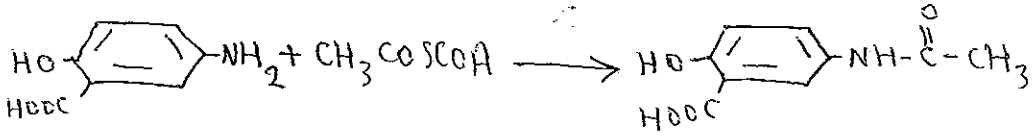
-Acetylation:

-The acetyl groups involved in acetylation is

- Discuss the methylation of substrates in drug elimination [EMQ, SAQ]

supplied by acetyl CoA. Transfer of acetyl group from acetyl CoA to the substrate is carried out by N-acetyl transferases present in liver.

Ex:



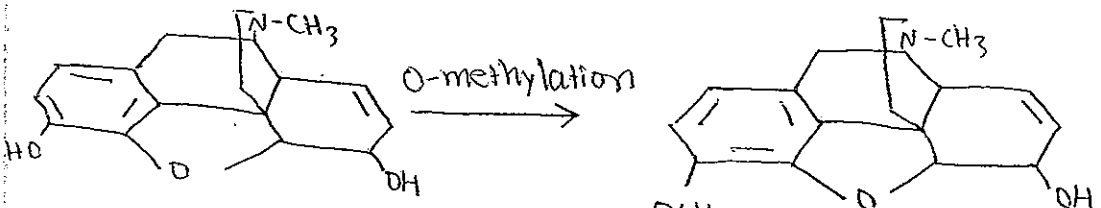
P-amino Salicylic acid

N-acetylated P-amino Salicylic acid

-Methylation:

- Transfer of methyl group to substrate is catalysed by microsomal methyl transferase.

Ex:



morphine

codeine

MISCELLANEOUS CONJUGATION REACTIONS

Some of the rare conjugation reactions are mentioned below.

Conjugation of Cyanide : The toxicity of cyanide ion is due to its ability to arrest enzymes involved in cellular respiration and convert haemoglobin to cyanomethemoglobin which lacks the ability to transport oxygen to tissues. Conjugation of cyanide ion involves transfer of sulphur atom from thiosulphate to the cyanide ion in presence of enzyme rhodanese to form inactive thiocyanate.

$$S_2O_3^{2-} + CN^- \xrightarrow{Rhodanese} SCN^- + So_3^{2-}$$

Conjugation with Ribose : Endogenous purine and pyrimidine bases conjugate with ribose to form nucleotides.

Conjugation with Taurine : Taurine, a β-amino sulphonic acid, conjugates the endogenous bile acids to produce components of bile.

METHODS FOR THE STUDY OF DRUG BIOTRANSFORMATION

***In Vitro* Methods :** There are several approaches to *in vitro* drug metabolism studies. The enzyme sources in these studies are human-derived systems currently under rapid development and evaluation. These systems consist of –

- Liver microsomes
- Hepatocytes and cell lines heterologously expressing drug-metabolising enzymes
- Liver slices, and
- Individually cDNA-expressed enzymes in host cell microsomes.

In these studies, the extent of uptake of drug is determined from the steady-state concentration in tissue and free concentration in the perfusate. The composition of the perfusate and the rate of perfusion are important experimental determinants of the rate of uptake of drug by the liver.

***In Vivo* Methods :** Investigations of biotransformation pathways *in vivo* require the collection and analysis of appropriate biological samples. The type of sample collected include urine, faeces, expired air, blood/plasma, bile, milk, saliva, synovial fluid and tissues. These samples can be divided into two groups –

1. Those requiring complete collection e.g. urine and faeces.
2. Those which are sampled at regular intervals of time e.g. blood.

The two *approaches to identification of metabolites* in the collected samples are –

1. Identification of metabolites in the biological samples when reference compounds are available.
2. Isolation and identification of all major metabolites regardless of the availability of reference compounds. This approach requires a method of detecting metabolites such as use of radiolabelled form of the drug. Radiolabelling is done at a metabolically stable position. HPLC and TLC techniques are employed to detect and measure the radiolabelled metabolites.

FACTORS AFFECTING BIOTRANSFORMATION OF DRUGS

The therapeutic efficacy, toxicity and biological half-life of a drug greatly depend upon its metabolic rate. A number of factors may influence the rate of drug metabolism. They are:

1. Physicochemical properties of the drug
2. Chemical factors:
   a. Induction of drug metabolising enzymes
   b. Inhibition of drug metabolising enzymes
   c. Environmental chemicals
3. Biological factors:
   a. Species differences
   b. Strain differences
   c. Sex differences
   d. Age
   e. Diet
   f. Altered physiological factors:
      i. Pregnancy
      ii. Hormonal imbalance
      iii. Disease states
   g. Temporal factors:
      i. Circadian rhythm
      ii. Circannual rhythm

PHYSICOCHEMICAL PROPERTIES OF THE DRUG

Just as the absorption and distribution of a drug are influenced by its physicochemical properties, so is its interaction with the drug metabolis-

ing enzymes. Molecular size and shape, pK_a, acidity/basicity, lipophilicity and steric and electronic characteristics of a drug influence its interaction with the active sites of enzymes and the biotransformation processes to which it is subjected. However, such an interrelationship is not clearly understood.

Stereochemical nature of drug also influences its metabolism. Biotransformation requires interaction of a drug with an enzyme, which is an interaction where spatial arrangement is critical. Many drugs that undergo metabolism exhibit stereoselective hepatic clearance, for example, the oral clearance of verapamil, a drug with high intrinsic clearance, displays profound stereoselectivity such that clearance ratio of +/- isomers is approximately 4.

CHEMICAL FACTORS

Induction of Drug Metabolising Enzymes

The phenomenon of increased drug metabolising ability of the enzymes (especially of microsomal monooxygenase system) by several drugs and chemicals is called as **enzyme induction** *and the agents which bring about such an effect are known as* **enzyme inducers.**

Most enzyme inducers have following properties –

1. They are lipophilic compounds.
2. They are substrate for the inducted enzyme system.
3. They have long elimination half-lives.

Mechanisms involved in enzyme induction are –

1. Increase in both liver size and liver blood flow.
2. Increase in both total and microsomal protein content.
3. Increased stability of enzymes.
4. Increased synthesis of cytochrome P-450.
5. Decreased degradation of cytochrome P-450.
6. Proliferation of smooth endoplasmic reticulum.

Two categories of inducers have been defined –

1. **Phenobarbital type inducers :** include several drugs and pesticides which increase the rate of metabolism of a large number of drugs. The most thoroughly studied enzyme inducer is phenobarbital which can increase enzyme activity up to 4 times.

2. **Polycyclic hydrocarbon type inducers :** such as 3-methyl cholanthrene and cigarette smoke which stimulate the metabolic rate of few drugs.

Some drugs such as carbamazepine, meprobamate, cyclophosphamide, rifampicin, etc. stimulate their own metabolism, the phenomenon being called as **auto-induction** or **self-induction**.

The most thoroughly studied enzyme inducer is phenobarbital which can increase enzyme activity up to 4 times. An example which shows that enzyme induction can have serious consequences in clinical practice is the inducing effect of phenobarbital on dicoumarol levels. Extreme caution must be exercised when phenobarbital and dicoumarol are co-administered to avoid either failure of the anticoagulant therapy or haemorrhagic crises.

Consequences of enzyme induction includes –

- Decrease in pharmacological activity of drugs
- Increased activity where the metabolites are active, and
- Altered physiological status due to enhanced metabolism of endogenous compounds such as sex hormones.

Some examples of inducers and drugs affected by them are given in Table 5.4.

TABLE 5.4
Inducers of Drug Metabolising Enzyme System and Drugs Commonly Affected by Them

Inducers	*Drugs with Enhanced Metabolism*
Barbiturates	Coumarins, phenytoin, cortisol, testosterone, oral contraceptives
Alcohol	Pentobarbital, coumarins, phenytoin
Phenytoin	Cortisol, coumarins, oral contraceptives, tolbutamide
Rifampicin	Coumarins, oral contraceptives, tolbutamide, rifampicin
Cigarette Smoke	Nicotine, amino azo dyes

Inhibition of Drug Metabolising Enzymes

A decrease in the drug metabolising ability of an enzyme is called as **enzyme inhibition**. The process of inhibition may be direct or indirect.

1. Direct Inhibition : may result from interaction at the enzymic site, the net outcome being a change in enzyme activity.

Direct enzyme inhibition can occur by one of the 3 mechanisms –

(a) ***Competitive Inhibition*** : results when structurally similar compounds compete for the same site on an enzyme. Such an inhibition due to substrate competition is *reversible* and can be overcome by high concentration of one of the substrates, e.g. methacholine inhibits metabolism of acetylcholine by competing with it for cholinesterase.

(b) ***Non-competitive Inhibition*** : results when a structurally unrelated agent interacts with the enzyme and prevents the metabolism of drugs. Since the interaction is not structure-specific, metals like lead, mercury and arsenic and organophosphorus insecticides inhibit the enzymes non-competitively. Isoniazid inhibits the metabolism of phenytoin by the same mechanism.

(c) ***Product Inhibition*** : results when the metabolic product competes with the substrate for the same enzyme. The phenomenon is also called as *autoinhibition*.

Certain specific inhibitors such as xanthine oxidase inhibitors (e.g. allopurinol) and MAO inhibitors (e.g. phenelzine) also inhibit the enzyme activity directly. Direct enzyme inhibition is usually rapid; a single dose of inhibitor may be sufficient to demonstrate enzyme inhibition.

2. **Indirect Inhibition** : is brought about by one of the two mechanisms.

(a) ***Repression*** : is defined as the decrease in enzyme content. It may be due to a fall in the rate of enzyme synthesis as affected by ethionine, puromycin and actinomycin D or because of rise in the rate of enzyme degradation such as by carbon tetrachloride, carbon disulphide, disulphiram, etc.

(b) ***Altered Physiology*** : due to nutritional deficiency or hormonal imbalance.

Enzyme inhibition is more important clinically than enzyme induction, especially for drugs with narrow therapeutic index, e.g. anticoagulants, antiepileptics, hypoglycaemics, since it results in prolonged pharmacological action with increased possibility of precipitation of toxic-effects.

Some examples of inhibitors and drugs affected by them are given in Table 5.5.

TABLE 5.5
Enzyme Inhibitors and Drugs Affected by Them

Inhibitors	*Drugs with Decreased Metabolism*
MAO inhibitors	Barbiturates, tyramine
Coumarins	Phenytoin
Allopurinol	6-Mercaptopurine
PAS	Phenytoin, hexobarbital

An important example of clinically significant enzyme inhibition is the effect of phenylbutazone on warfarin plasma levels. Warfarin is used as racemic mixture, with the *S*-isomer being 5 times more active than the *R*-isomer. Moreover, warfarin is eliminated from the body almost exclusively by metabolism and the *S*-isomer is metabolised more rapidly that the *R*-isomer. The effect of phenylbutazone on increased levels of warfarin can thus be concluded as –

- Increase in *R*-isomer levels is due to its displacement from the plasma proteins (little increase in anticoagulant activity).
- Increase in *S*-isomer levels is due to inhibition of metabolism, with tremendous increase in anticoagulant activity and tendency to cause haemorrhage.

Environmental Chemicals

Several environmental agents influence the drug metabolising ability of enzymes.

- Halogenated pesticides such as DDT and polycyclic aromatic hydrocarbons contained in cigarette smoke have enzyme induction effect.
- Organophosphate insecticides and heavy metals such as mercury, tin, nickel, cobalt and arsenic inhibit drug metabolising ability of enzymes.

Other environmental factors that may influence drug metabolism are temperature, altitude, pressure, atmosphere, etc.

BIOLOGICAL FACTORS

Species Differences

Screening of new therapeutic molecules to ascertain their activity and toxicity requires study in several laboratory animal species. Differences

in drug response due to species differences are taken into account while extrapolating the data to man.

Species differences have been observed in both phase I and phase II reactions. In phase I reactions, both qualitative and quantitative variations in the enzyme and their activity have been observed. An example of this is the metabolism of amphetamine and ephedrine. In men and rabbit, these drugs are predominantly metabolised by oxidative deamination whereas in rats the aromatic oxidation is the major route. In phase II reactions, the variations are mainly qualitative and characterized either by the presence of, or complete lack of certain conjugating enzymes; for example, in pigs, the phenol is excreted mainly as glucuronide whereas its sulphate conjugate dominates in cats. Certain birds utilize ornithine for conjugating aromatic acids instead of glycine.

Strain Differences/Pharmacogenetics

Enzymes influencing metabolic reactions are under the genetic control. Just as the differences in drug metabolising ability between different species are attributed to genetics, so also are the differences observed between strains of the same animal species. *A study of inter-subject variability in drug response (due to differences in, for example, rate of biotransformation) is called as* **pharmacogenetics**. The inter-subject variations in drug biotransformation may either be monogenically or polygenically controlled. A *polygenic control* has been observed in studies in twins. In identical twins (monozygotic), very little or no difference in the metabolism of phenylbutazone, dicoumarol and antipyrine was detected but large variations were apparent in fraternal twins (dizygotic; twins developed from two different eggs) for the same drugs.

Differences observed in the metabolism of a drug among different races are called as **ethnic variations**. Such a variation may be ***monomorphic*** or ***polymorphic***. *When a unimodal frequency distribution is observed in the entire population, the variations are called as continuous* or *monomorphic*; for example, the entire human race acetylate PABA and PAS to only a small extent. *A polymodal distribution is indicative of* ***discontinuous variation*** (***polymorphism***). An example of polymorphism is the acetylation of isoniazid (INH) in humans. A bimodal population distribution was observed comprising of slow acetylator or inactivator phenotypes (metabolise INH slowly) and rapid acetylator or inactivator phenotypes (metabolise INH rapidly) (*see* Table 5.6.).

TABLE 5.6

Ethnic Variations in the N-Acetylation of Isoniazid

Ethnic Group	*% Slow Acetylators*	*% Rapid Acetylators*
Whites (USA and Canada)	45	55
Blacks (USA)	48	52
Latin Americans	67	33
American Indians	79	21
Japanese	87	13
Eskimos	95	05

Source : Kalow, W.: *Pharmacogenetics: Heredity and the Response to Drugs*, Saunders, Philadelphia, 1962.

Approximately equal percent of slow and rapid acetylators are found among whites and blacks whereas the slow acetylators dominate Japanese and Eskimo populations. Dose adjustments are therefore necessary in the latter groups since high levels of INH may cause peripheral neuritis. Other drugs known to exhibit pharmacogenetic differences in metabolism are debrisoquine, succinyl choline, phenytoin, dapsone and sulphadimidine.

Sex Differences

Sex related differences in the rate of metabolism could be attributed to regulation of such processes by sex hormones since variations between male and female are generally observed following puberty. Such sex differences are widely studied in rats; the male rats have greater drug metabolising capacity. In humans, women metabolise benzodiazepines slowly than men and several studies show that women on contraceptive pills metabolise a number of drugs at a slow rate.

Age

Differences in the drug metabolic rate in different age groups are mainly due to variations in the enzyme content, enzyme activity and haemodynamics.

- In neonates (upto 2 months), the microsomal enzyme system is not fully developed and many drugs are biotransformed slowly; for example, caffeine has a half-life of 4 days in neonates in comparison to 4 hours in adults. A major portion of this drug is excreted unchanged in urine by the neonates. Conjugation with sulphate is well developed (paracetamol is excreted mainly as sulphate) but glucuronidation occurs to a very small extent. As

a result, hyperbilirubinaemia precipitates kernicterus and chloramphenicol leads to cyanosis or Gray baby syndrome in new born. Similarly, sulphonamides cause renal toxicity and paracetamol causes hepatotoxicity.

- Infants (between 2 months and one year) show almost a similar profile as neonates in metabolising drugs with improvement in the capacity as age advances and enzyme activity increases.
- Children (between one year and 12 years) and older infants metabolise several drugs much more rapidly than adults as the rate of metabolism reaches a maximum somewhere between 6 months and 12 years of age. As a result, they require large mg/kg doses in comparison to adults; for example, the theophylline half-life in children is two-third of that in adults.
- In very elderly persons, the liver size is reduced, the microsomal enzyme activity is decreased and hepatic blood flow also declines as a result of reduced cardiac output all of which contribute to decreased metabolism of drugs. Drug conjugation, however, remains unaffected.

Diet

The enzyme content and activity is altered by a number of dietary components. In general –

- Low protein diet decreases and high protein diet increases the drug metabolising ability. This is because the enzyme synthesis is promoted by protein diet which also raises the level of amino acids for conjugation with drugs.
- The protein-carbohydrate ratio in the diet is also important; a high ratio increases the microsomal mixed-function oxidase activity.
- Fat free diet depresses cytochrome P-450 levels since phospholipids, which are important components of microsomes, become deficient.
- Dietary deficiency of vitamins (e.g. vitamin A, B_2, B_3, C and E) and minerals such as Fe, Ca, Mg, Cu and Zn retard the metabolic activity of enzymes.
- Grapefruit inhibits metabolism of many drugs and improve their oral availability.
- Starvation results in decreased amount of glucuronides formed than under normal conditions.

- Malnutrition in women results in enhanced metabolism of sex hormones.
- Alcohol ingestion results in a short-term decrease followed by an increase in the enzyme activity.

Altered Physiological Factors

Pregnancy : Studies in animals have shown that the maternal drug metabolising ability (of both phase I and phase II reactions) is reduced during the later stages of pregnancy. This was suggested as due to high levels of steroid hormones in circulation during pregnancy. In women the metabolism of promazine and pethidine is reduced during pregnancy or when receiving oral contraceptives. Higher rate of hepatic metabolism of anticonvulsants during pregnancy is thought to be due to induction of drug metabolising enzymes by the circulating progesterone.

Hormonal Imbalance : The influence of sex hormones on drug metabolism has already been discussed. The effect of other hormones is equally complex. Higher levels of one hormone may inhibit the activity of few enzymes while inducing that of others. Adrenolectomy, thyroidectomy and alloxan-induced diabetes in animals showed impairment in the enzyme activity with a subsequent fall in the rate of metabolism. A similar effect was observed with pituitary growth hormone. Stress related changes in ACTH levels also influence drug biotransformation.

Disease States : As liver is the primary site for metabolism of most drugs, all pathological conditions associated with it result in enhanced half-lives of almost all drugs. Thus, a reduction in hepatic drug metabolising ability is apparent in conditions such as hepatic carcinoma, hepatitis, cirrhosis, obstructive jaundice, etc.[2] Biotransformations such as glycine conjugation of salicylates, oxidation of vitamin D and hydrolysis of procaine which occur in kidney, are impaired in renal diseases.[3] Congestive cardiac failure and myocardial infarction which result in a decrease in the blood flow to the liver, impair metabolism of drugs having high hepatic extraction ratio e.g. propranolol and lidocaine.[4] In diabetes, glucuronidation is reduced due to decreased availability of UDPGA.

Temporal Factors

Circadian Rhythm : *Diurnal variations or variations in the enzyme activity with light cycle are called as* **circadian rhythm** *in drug metabolism.* It has been observed that the enzyme activity is maximum during early morning (6 to 9 a.m.) and minimum in late afternoon (2 to 5 p.m.) which was suggested to correspond with the high and low serum levels of corticosterone (the serum corticosterone level is dependent upon the

light-dark sequence of the day). Clinical variation in therapeutic effect of a drug at different times of the day is therefore apparent. *The study of variations in drug response as influenced by time is called as* **chronopharmacology**. *Time-dependent change in drug kinetics is known as* **chronokinetics**. Drugs such as aminopyrine, hexobarbital and imipramine showed diurnal variations in rats. The half-life of metyrapone was shown to be 2.5 times longer during the night than in the day, in rats.

□Bioactivation And Tissue Toxicity:

-Formation of highly reactive metabolites (from relatively inert chemical compounds) which interact with the tissues to precipitate one or more of the several forms of toxicities such as carcinogenesis and tetragenesis is called as bioactivation or toxicological activation.

-The reactive, chemically unstable species, capable of toxications, are broadly divided into two categories.

→ Electrophiles

→ Free radicals

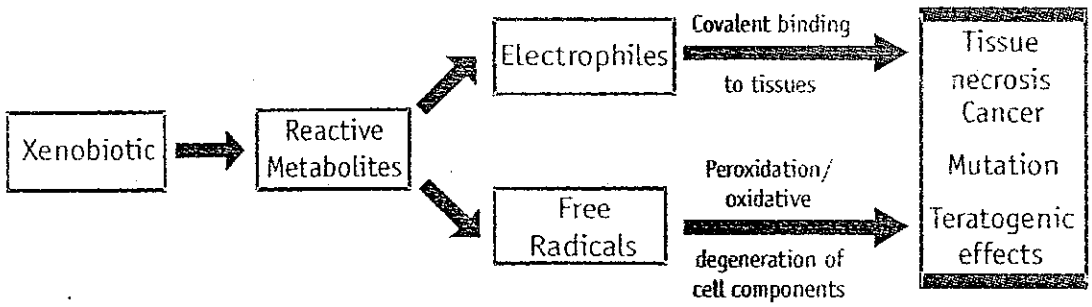


Fig. 5.4 Mechanisms of tissue toxicity by bioactivation of drugs

Electrophiles are species deficient in electron pair.

-The enzyme system through which they are genera-

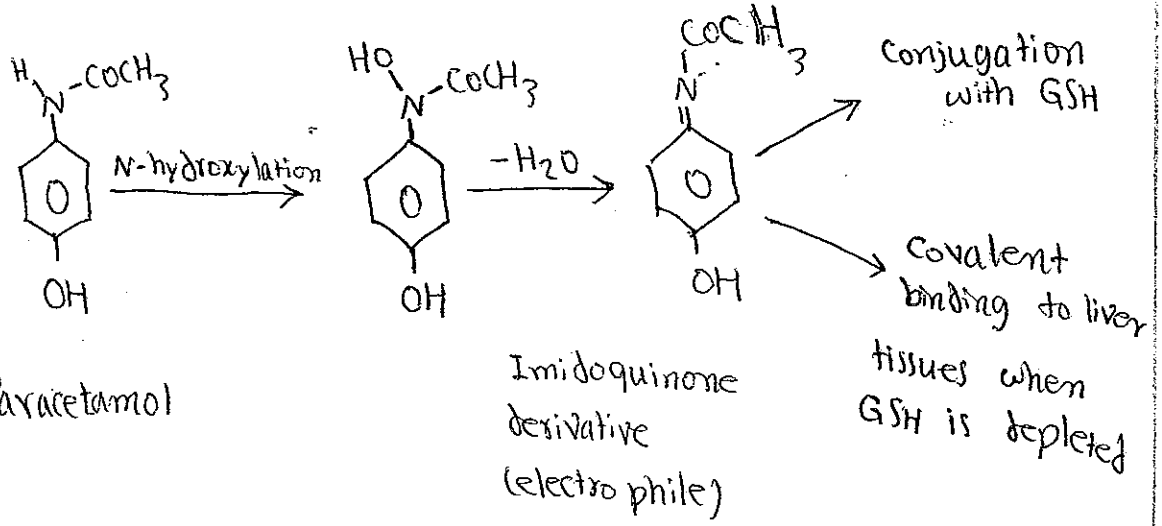
ted is Cytochrome P-450. Carbon, nitrogen or Sulphur-containing compounds can be metabolically activated to yield electrophiles.

- The mechanism by which electrophiles precipitate toxicity is through covalent binding to nucleophilic tissue components such as macromolecules (proteins, nucleic acids and lipids) or low molecular weight cellular constituents.

- Covalent binding to DNA is responsible for carcinogenicity and tumour formation.

- The body's defence against electrophiles is their inactivation by conjugation with glutathione, the most abundant cellular nucleophile with -SH gp.

- An example of tissue toxicity due to electrophiles is hepatotoxicity of paracetamol metabolites.



Free Radicals: are species containing an odd number of electrons.

- They may be positively charged (cation radicals), negatively charged (anion radical) or neutral (neutral radicals)

$R_{\cdot}^{+}$ $R_{\cdot}^{-}$ $R_{\cdot}$

Cation radical Anion radical Neutral Radical

- Xenobiotics that on metabolic activation yield free radicals are quinones, carbon tetrachloride etc.
- They produce toxicity by peroxidation of cellular components.
- The potential toxicity of free radicals is far greater than that of the electrophiles.

Cellular defence mechanisms against free radicals include control imposed by membrane structure, neutralization by glutathione, control exerted by non-enzymatic antioxidant scavengers such as Vitamins A, E and C and enzymatic inactivation of oxygen derived free radicals.

- Generation of reactive metabolites is indicated by modification in enzyme activities, formation of glutathione conjugates (or mercapturic acids) and depletion in tissue levels of glutathione. Since the availability of glutathione in the body determines the threshold for toxic response, thiols (e.g. N-acetyl cysteine) can be used to treat poisoning by drugs such as paracetamol that yield reactive metabolites.

Compounds and their Metabolic Reaction that Generate Toxic Intermediates

Compounds	*Metabolic Pathway*	*Toxicity*
Benzo(a)pyrene	Aromatic epoxidation	Lung cancer
Aflatoxin B_1	Olefin epoxidation	Hepatic cancer
Thalidomide	Hydrolytic cleavage of lactam	Teratogenesis
Chlorinated hydrocarbons e.g. $CHCl_3$	Oxidative dehalogenation	Nephrotoxicity

Figure 5.5 summarizes the impact of reactive species generated as a result of xenobiotic metabolism.

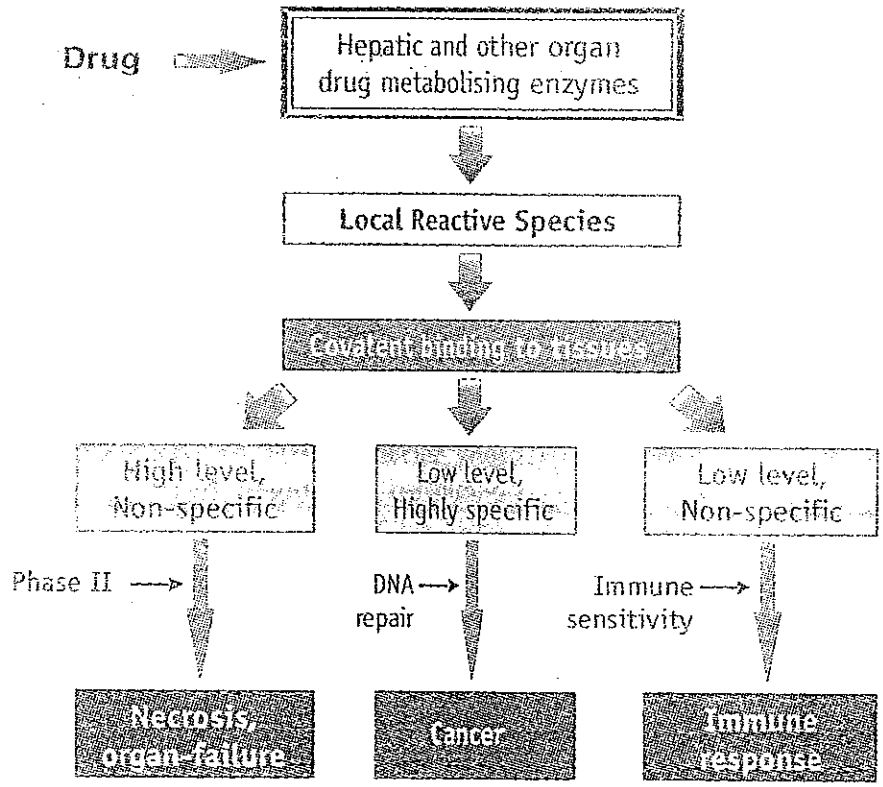


Fig. 5.5 The links between drug metabolism, formation of active species, covalent binding and irreversible damage to the organism. Phase II defences are crucial to prevent organ failure from high levels of covalent binding, whilst Phase II defence mechanisms as well as DNA repair are crucial to avoid carcinogenicity as a result of specific DNA binding of reactive species. For an immune response, the sensitivity of the individuals' immune system may be the major determinant of whether a response occurs.

BIOPHARMACEUTICS DRUG DISPOSITION CLASSIFICATION SYSTEM (BDDCS)

The *Biopharmaceutics Classification System* (BCS) was developed to allow prediction of *in vivo* pharmacokinetic performance of drug products from measurements of permeability and solubility. Recently, a modification of such a classification system, designated as the ***Biopharmaceutics Drug Disposition Classification System*** **(BDDCS)**, has been developed that may be useful in predicting overall drug disposition including: routes of drug elimination; the effects of efflux and absorptive transporters on oral drug absorption; when transporter-enzyme interplay will yield clinically significant effects (e.g., low bioavailability and drug-drug interactions); the direction, mechanism and importance of food effects; and transporter effects on post-absorptive systemic drug concentrations following oral and i.v. dosing.

TABLE 5.8
The Biopharmaceutics Drug Disposition Classification System (BDDCS)

Class	*Solubility*	*Metabolism*	*Elimination Pattern*	*Drug Examples*
I	High	Extensive	Predominantly eliminated by liver	Diltiazem
II	Low	Extensive	Predominantly eliminated by liver	Itraconazole
III	High	Poor	Poor metabolism, eliminated unchanged by renal and biliary routes	Doxycycline
IV	Low	Poor	Poor metabolism, eliminated unchanged by renal and biliary routes	Ofloxacin

In BDDCS it was proposed that it may be more useful to replace the permeability criterion with the major route of drug elimination in assigning drugs to BDDCS classes. As given in table 5.8, according to BDDCS—

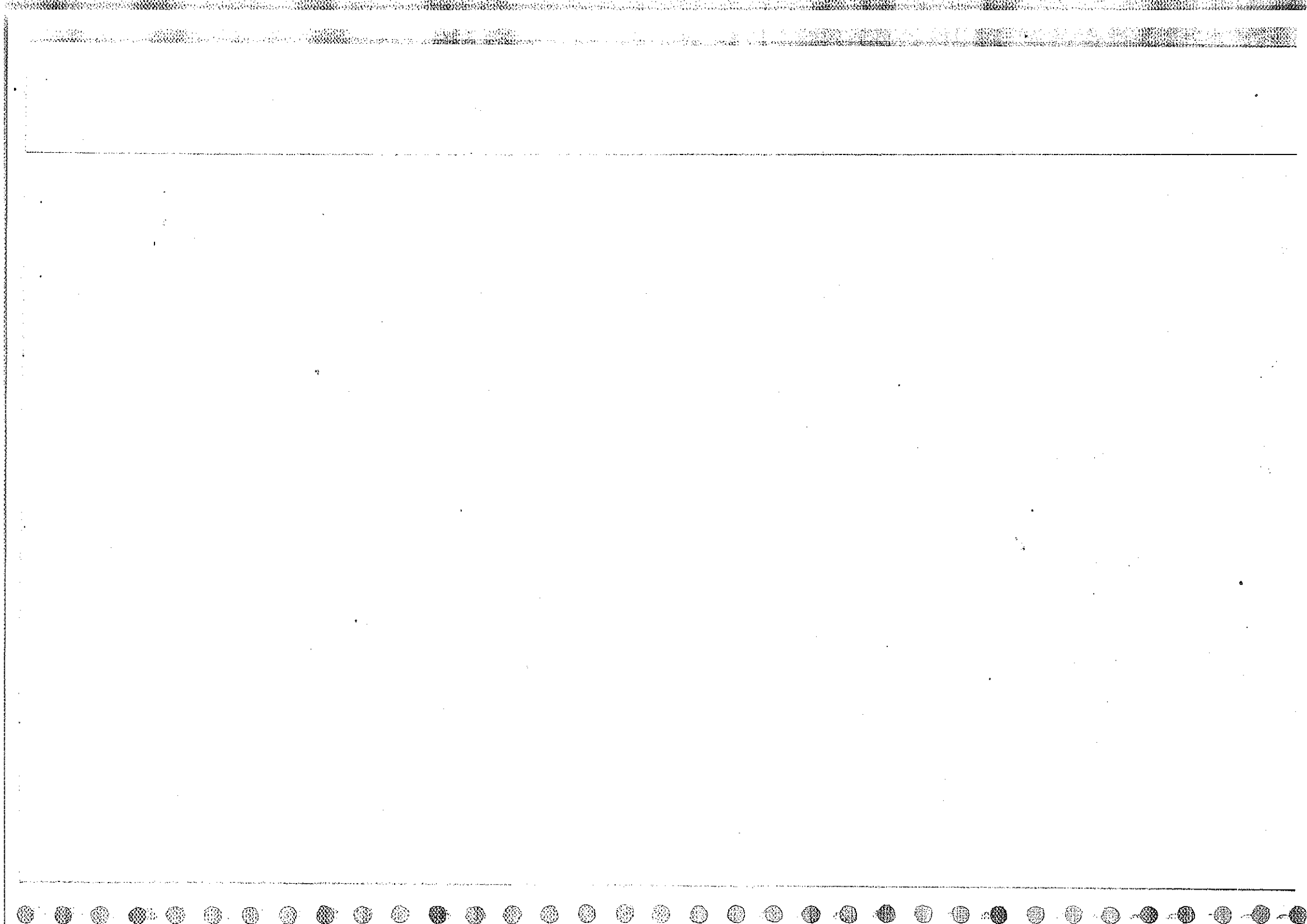
Class 1 compounds are designated as "*high solubility, extensive metabolism*" drugs

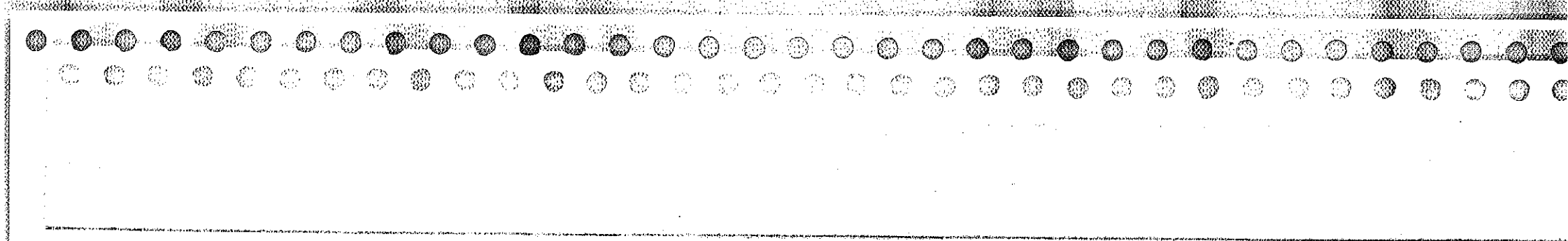
Class 2 compounds are designated as "*poor solubility, extensive metabolism*" drugs

Class 3 compounds are designated as "*high solubility, poor metabolism*" drugs, and

Class 4 compounds are designated as "*low solubility, poor metabolism*" drugs.

- What do you understand by Biopharmaceutics classification system for drugs? [5M, March '17]





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

- The principal organs of excretion are kidneys.
- Excretion of drug by kidneys is called as renal excretion.
- Excretion by organs other than kidneys such as lungs, biliary system, intestine, salivary glands and sweat glands is known as nonrenal excretion.

#Renal Excretion of Drugs:

- Agents that are excreted in urine are:

1- Water- soluble
2- Non-volatile
3. Small in molecular size (less than 500 Daltons)
4- The ones that are metabolised slowly.

- The basic functional unit of kidney involved in excretion is the nephron.
- Each kidney comprises of one million nephrons.

Excretion of Drugs

-Each nephron is made up of the glomerulus, the proximal tubule, the loop of Henle, the distal tubule and the collecting tubule.

- The principal processes that determine the urinary excretion of a drug are:

1- Glomerular filtration

2- Active tubular secretion

3. Active or passive tubular reabsorption

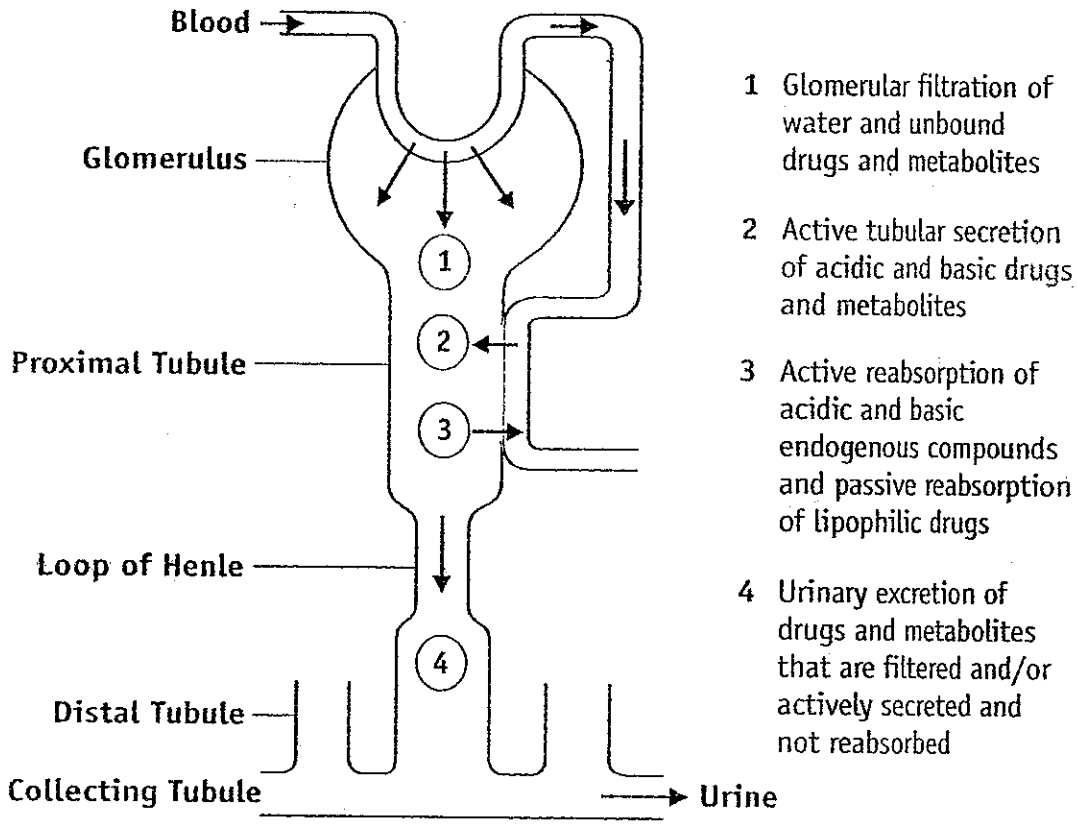


Fig. 6.1 A simplified diagram illustrating processes involved in the urinary excretion of drugs

Glomerular filtration and active tubular secretion tend to increase the concentration of drugs in lumen and hence facilitate excretion whereas tubular reabsorption decreases it and prevents the movement of drug out of the body. Thus, the rate of excretion can be given by equation:

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

#Glomerular Filtration:

- Glomerular filtration is a non-selective, unidirectional process whereby most compounds, ionised or unionised, are filtered except those that are bound to plasma proteins or blood cells and thus behave as macromolecules.

-The driving force for filtration through the glomerulus is the hydrostatic pressure of the blood flowing in the capillaries.

#Glomerular filtration rate (GFR):

1- The volume of water filtered out of the plasma through glomerular capillary walls into Bowman's capsules per unit of time.

2. is a test used to check how well the kidneys are working. Specifically, it estimates how much blood passes through the glomeruli each minute.

- The GFR can be determined by an agent that is excreted exclusively by filtration and is

neither secreted nor reabsorbed in the tubules. The excretion rate value of such an agent is 120 to 130 ml/min.

Creatinine, inulin, mannitol and sodium thiosulphate are used to estimate GFR..

-Active Tubular Secretion:

-It is a carrier-mediated process which requires energy for transportation of compounds against the concentration gradient.

-Two Systems:

1- System for secretion of organic acids/anions (like penicillins etc)

2- System for secretion of organic bases/cations (like morphine)

-Both the systems are relatively non-selective and independent of each other.

-Active secretion is unaffected by changes in pH and protein binding.

-But in contrast to glomerular filtration, it is dependent upon renal blood flow.

- Agents that are used to measure active tubular secretion are the ones that are filtered as well as secreted to such an extent that they are removed from the blood in a single pass through the kidneys.
- Para amino hippuric acid (PAH), a highly polar agent and iodopyracet are used to determine active secretion.
- Active secretion occurs predominantly in the proximal tubule region of the nephron.
- Two structurally similar drugs having similar ionic charge and employing the same carrier-mediated process for excretion enter into competition.
A drug with greater rate of clearance will retard the excretion of the other drug with which it competes.
The half-life of both the drugs is increased since the total sites for active secretion are limited.
This may result in accumulation of drugs and thus, precipitation of toxicity.
- However, the principle of competition can be exploited for therapeutic benefits.
- An interesting example of this is the anionic

agent probenecid. Probenecid inhibits the active tubular secretion of organic acids such as penicillins, PAS etc.

-Cimetidine is a competitive inhibitor of organic cation transport.

Tubular Reabsorption:

-Tubular reabsorption occurs after the glomerular filtration of drugs. It takes place all along the renal tubule.

-Contrary to tubular secretion, reabsorption results in an increase in the half-life of a drug.

-Tubular reabsorption can either be an:

1. Active process Ex: Uric acid, Oxopurinol
2. Passive process

↳ The driving force for such a process i.e. the concentration gradient is established by the back diffusion or reabsorption of water along with sodium and other inorganic ions.

- Lipophilic substances are extensively reabsorbed while polar molecules are not.

Since a majority of drugs are weak electrolytes (weak acids or weak bases), diffusion of such agents through the lipoidal tubular membrane depend upon the degree of ionisation which in turn depends on three factors:

1. pH of the urine
2. pK_a of the drug
3. Urine flow rate

Urine pH:

- Varies b/w 4.5 to 7.5
- The pH of the urine is dependent upon diet, drug intake and pathophysiology of the patient.

Food rich in carbohydrates results in higher urinary pH whereas proteins lower it.

- Antacids such as sodium bicarbonate produce alkaline urine, while ascorbic acid make it acidic.
- More significan alteration in urine pH is

brought about by i.v. infusion of solutions of sodium bicarbonate and ammonium chloride which are used in the treatment of acid-base imbalance.

-Respiratory and metabolic acidosis and alkalosis result in acidification and alkalinisation of the urine respectively.

-The relative amount of ionised and unionised drug in the urine at a particular pH and the present of drug ionised at this pH can be computed from the Henderson-Hasselbach equations:

-For weak acids:

$$pH = pK_a + \log \frac{[\text{Ionised Drug}]}{[\text{Unionised Drug}]}$$

$$\%\text{Drug Ionised} = \frac{10^{(pH - pK_a)}}{1 + 10^{(pH - pK_a)}} 100$$

For weak bases,

$$pH = pK_a + \log \frac{[\text{Unionised Drug}]}{[\text{Ionised Drug}]} \tag{6.4}$$

$$\% \text{ Drug Ionised} = \frac{10^{(pK_a - pH)}}{1 + 10^{(pK_a - pH)}} 100 \tag{6.5}$$

The concentration ratio R of the drug in urine to that in plasma (U : P) can be given by equations derived by *Shore et al*:

For weak acids,

$$R_a = \frac{U}{P} = \frac{1 + 10^{(pH_{urine} - pK_a)}}{1 + 10^{(pH_{plasma} - pK_a)}} \tag{6.6}$$

For weak bases,

$$R_b = \frac{U}{P} = \frac{1 + 10^{(pK_a - pH_{urine})}}{1 + 10^{(pK_a - pH_{plasma})}} \tag{6.7}$$

Note : The above equations from 6.2 to 6.7 are identical to equations 2.10 to 2.15 of chapter 2.

The relationship between drug pK_a, urine pH, degree of ionisation and renal clearance is illustrated in Table 6.1. Table 6.1 shows percent

TABLE 6.1

Percent Drug Ionised and Renal Clearance Values (in ml/min)

Drugs	*pK_a*	*Nature*	*Urine pH Values*					
			4.5		*6.3*		*7.5*	
			% Ionised	*Cl_R*	*% Ionised*	*Cl_R*	*% Ionised*	*Cl_R*
ACIDS								
A	2.0	Strong	99.7	0.001	99.99	0.8	100.0	1.26
B	6.0	Weak	3.0	0.04	66.6	0.115	97.0	1.25
C	10.0	V. Weak	0.0	0.99	0.02	0.99	0.3	1.0
BASES								
D	12.0	Strong	100.0	794.3	100.0	12.6	99.99	0.79
E	8.0	Weak	99.9	635.2	98.0	10.26	76.0	0.83
F	4.0	V. Weak	24.0	1.32	0.0	1.0	0.0	1.0

drug ionised and renal clearance values (in ml/min) of several acidic and basic drugs at various values of urine pH, assuming that the drug does not bind to plasma proteins, urine flow of 1 ml/min, plasma pH 7.4 and that equilibrium is achieved by diffusion of unionised drug only. The renal clearance values Cl_R are computed by use of equation 6.8.

$$Cl_R = \frac{U}{P} \text{ Urine flow rate} \qquad (6.8)$$

The significance of pH-dependent excretion for any particular compound is greatly dependent upon its pK_a and lipid solubility. A characteristic of drugs, pK_a values govern the degree of ionisation at a particular pH. A polar and ionised drug will be poorly reabsorbed passively and excreted rapidly (*see* Table 6.1). Reabsorption is also affected by the lipid solubility of drug; an ionised but lipophilic drug will be reabsorbed while an unionised but polar one will be excreted.

The combined effect of urine pH and drug pK_a and lipid solubility on reabsorption of drugs is *summarized* as follows:

1. An acidic drug such as penicillin or a basic drug such as gentamicin which is polar in its unionised form, is not reabsorbed passively, irrespective of the extent of ionisation in urine. Excretion of such drugs is independent of pH of urine and its flow rate. Their rate of excretion is the sum of rate of filtration and rate of active secretion.
2. Very weakly acidic, nonpolar drugs ($pK_a > 8.0$) such as phenytoin or very weakly basic, nonpolar drugs ($pK_a < 6.0$) such as propoxyphene are mostly unionised throughout the entire range of urine pH and are therefore extensively reabsorbed passively at all values of urine pH. The rate of excretion of such drugs is always low and insensitive to urine pH.
3. A strongly acidic drug ($pK_a \leq 2.0$) such as cromoglycic acid or a strongly basic drug ($pK_a \geq 12.0$) such as guanethidine, is completely ionised at all values of urine pH and are, therefore, not reabsorbed. Their rate of excretion is always high and insensitive to pH of urine.
4. Only for an acidic drug in the pK_a range 3.0 to 8.0 (e.g. several NSAIDs) and for a basic drug in the pK_a range 6.0 to 12.0 (e.g. morphine analogs, tricyclic antidepressants, etc.) the extent of reabsorption is greatly dependent upon urine pH and varies from negligible to almost complete; for example, the amount of dexamphetamine excreted in the urine varies from 3 to 55% of

the administered dose as the urine pH varies from 8.0 to 5.0. Fig. 6.2 illustrates the influence of urine pH on drug excretion.

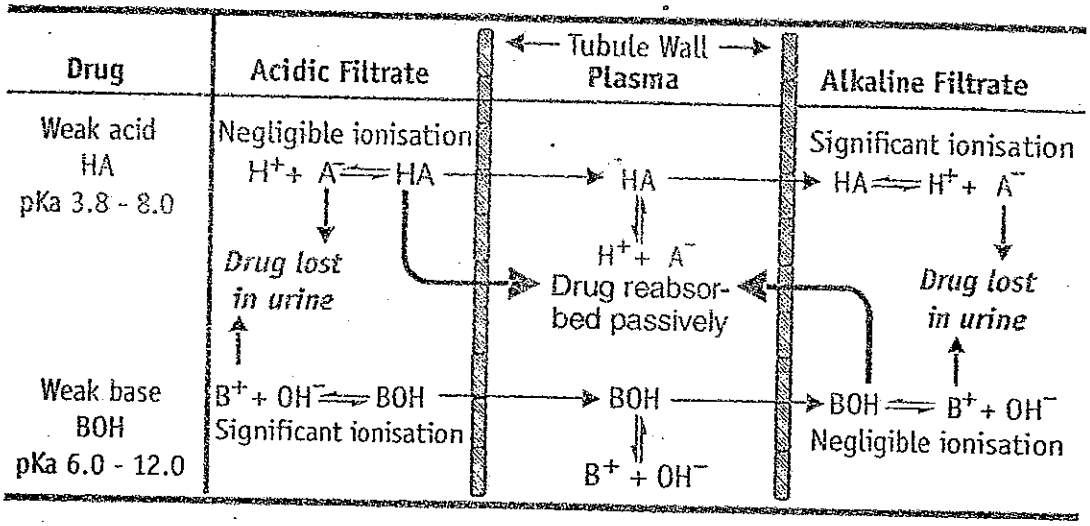


Fig. 6.2 Influence of urinary pH on excretion of weakly acidic and weakly basic drugs. Bold arrows indicate that the process is predominant.

The toxicity due to overdosage of drugs whose excretion is sensitive to pH change can be treated by acidification or alkalinisation of the urine with ammonium chloride or sodium bicarbonate respectively. Thus, crystalluria caused by precipitation of sulphonamides in the renal tubules and subsequent kidney damage can be overcome by alkalinising the urine. Excretion of basic drugs can be promoted by acidification of urine. The therapeutic activity of the urinary antiseptic hexamine also depends on the urine pH. It is not converted to active form i.e. formaldehyde unless the urine is acidic.

Urine Flow Rate : In addition to urine pH and drug pK_a, the rate of urine flow also influences the extent of reabsorption. Polar drugs whose excretion is independent of urine pH and are not reabsorbed, are unaffected by urine flow rate. An increase in urine flow in case of such drugs will only produce more dilute urine. Only those drugs whose reabsorption is pH-sensitive, for example, weak acids and weak bases, show dependence on urine flow rate. For such agents, reabsorption is inversely proportional to the urinary flow. These compounds can be divided into two types based on their extent of reabsorption in relation to that of water:

1. Drugs which are reabsorbed to an extent equal to or greater than the reabsorption of water e.g. phenobarbital. In such cases, the relationship between renal clearance and urinary excretion is linear.
2. Drugs which are reabsorbed to an extent lower than the reabsorption of water e.g. theophylline and many more drugs. In

these cases, the relationship between renal clearance and urinary excretion is convex curvilinear.

Urine flow rate can be increased by forced diuresis. **Forced diuresis** *is the increase in urine flow induced by large fluid intake or administration of mannitol or other diuretics.* The principle can be used in an intoxicated person to remove excessive drug by promoting its excretion and decreasing the time for reabsorption.

Both urine pH control and forced diuresis can be used to treat toxicity with drug overdose when –

1. Urinary excretion is the major route for elimination of drug.
2. The drug is extensively reabsorbed passively from the renal tubules.
3. The reabsorption is sensitive to urine pH (and urine flow rate).

Apart from the foregoing discussion on the passive reabsorption of drugs, the process is also important in the reabsorption of low threshold substances such as urea, certain phosphates and sulphates, etc.

CONCEPT OF CLEARANCE

The clearance concept was first introduced to describe renal excretion of endogenous compounds in order to measure the kidney function. The term is now applied to all organs involved in drug elimination such as liver, lungs, the biliary system, etc. and referred to as hepatic clearance, pulmonary clearance, biliary clearance and so on. *The sum of individual clearances by all eliminating organs is called as* **total body clearance** or **total systemic clearance.** It is sometimes expressed as a sum of renal clearance and nonrenal clearance.

Clearance *is defined as the hypothetical volume of body fluids containing drug from which the drug is removed or cleared completely in a specific period of time.* It is expressed in ml/min and is a constant for any given plasma drug concentration. In comparison to apparent volume of distribution which relates plasma drug concentration to the amount of drug in the body, clearance relates plasma concentration to the rate of drug elimination.

$$\text{Clearance (Cl)} = \frac{\text{Elimination rate}}{\text{Plasma drug concentration}} \qquad (6.9)$$

Renal Clearance (Cl_R) : *It can be defined as the volume of blood or plasma which is completely cleared of the unchanged drug by the kidney*

per unit time. It is expressed mathematically as:

$$Cl_R = \frac{\text{Rate of urinary excretion}}{\text{Plasma drug concentration}} \qquad (6.10)$$

Physiologically speaking, **renal clearance** *is the ratio of "sum of rate of glomerular filtration and active secretion minus rate of reabsorption"* to *"plasma drug concentration C"*.

$$Cl_R = \frac{\text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}}{C} \qquad (6.11)$$

TABLE 6.2
Relationship between Renal Clearance Values and Mechanism of Clearance

Renal Clearance (ml/min)	*Renal Clearance Ratio*	*Mechanism of Renal Clearance*	*Example(s)*
0 (least value)	0	Drug filtered and reabsorbed completely	Glucose
< 130	Above 0, Below 1	Drug filtered and reabsorbed partially	Lipophilic drugs
130 (GFR)	1	Drug is filtered only	Creatinine, Inulin
> 130	> 1	Drug filtered as well as secreted actively	Polar, ionic drugs
650 (Highest value)	5	Clearance equal to renal plasma flow rate	Iodopyracet, PAH

The contribution of each of the above physiological processes in clearing a drug cannot be determined by direct measurement. It can, however, be determined by comparing the clearance values obtained for a drug with that of an agent such as creatinine or inulin which is cleared by glomerular filtration only. The ratio of these two values is called as **renal clearance ratio** or **excretion ratio**.

$$\text{Renal Clearance Ratio} = \frac{Cl_R \text{ of drug}}{Cl_R \text{ of creatinine}} \qquad (6.12)$$

Thus, depending upon whether the drug is only filtered, filtered and secreted or filtered and reabsorbed, the clearance ratio will vary (Table 6.2.). The renal clearance values range from zero to 650 ml/min and the clearance ratio from zero to five.

Factors Affecting Renal Excretion or renal clearance:

- Apart from the three physiological processes that govern the urinary excretion, other factors:

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

1- Physicochemical Properties of the Drug:

- An agent of small molecular size can be easily filtered through the glomerulus.
- Compounds of weights below 300 Daltons, if water-soluble, are readily excreted by the kidneys.

300-500 Daltons can be excreted both in urine and bile.

- Greater than 500 are excreted in urine to a lesser extent.

- Urinary excretion of an unchanged drug is inversely related to its lipophilicity. This is because, a liphophilic drug is passively reabsorbed to a large extent.

2- plasma Concentration of the Drug:

- Glomerular filtration and reabsorption are directly affected by plasma drug concentration since both are passive processes.

- A drug that is not bound to plasma proteins and excreted by filtration only, shows a linear relationship between rate of excretion and plasma drug concentration.

- In case of drugs which are secreted or reabsorbed actively, the rate process increases with an increase in plasma concentration to a point when saturation of carrier occurs.

- In case of actively reabsorbed drugs, excretion is negligible at low plasma concentrations. Such agents are excreted in urine only when their concentration in the glomerular filtrate exceeds

the active reabsorption capacity, e.g. glucose. With drugs that are actively secreted, the rate of excretion increases with increase in plasma concentration up to a saturation level. These situations are depicted in Fig:

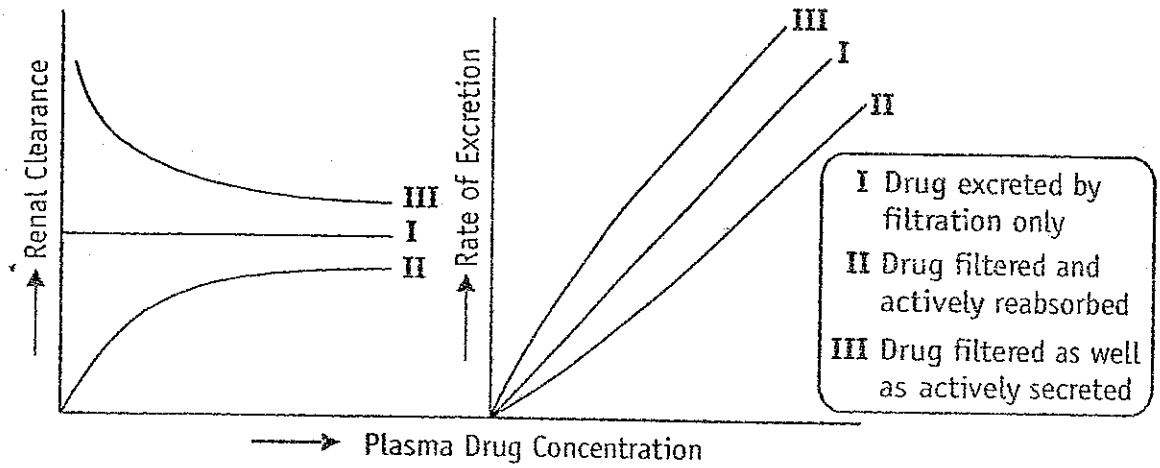


Fig. 6.3 Renal clearance and rate of excretion of a drug in relation to its plasma concentration as affected by the physiological processes — filtration, active reabsorption and active secretion

3- Distribution and Binding Characteristics of the drug

- clearance is inversely related to apparent volume of distribution of drugs.

A drug with large V_d is poorly excreted in urine

Drugs restricted to blood compartment have higher excretion rates.

- Drugs that are bound to plasma proteins behave as macromolecules and thus cannot be filtered through the glomerulus.

Only unbound or free drug appear in the glomerul filtrate.

- An earlier equation given for renal clearance is:

$$CI_R = \frac{\text{Urine drug concentration}}{\text{Plasma drug concentration}} \text{ Urine flow rate}$$

- Since only free drug can be excreted in the urine, the fraction of drug bound to plasma proteins is important and can be computed from equation:

$$f_u = \frac{C_u}{C}$$

where, f_u = fraction of unbound drug in plasma

C_u = concentration of unbound drug in plasma

C = total plasma concentration of drug

- Thus the CI_R equation can be written as:

$$CI_R = f_u \cdot \text{Urine flow rate}$$

-Drugs extensively bound to proteins have long half-lives because the renal clearance is small and urine flow rate is just 1 to 2 ml/min.

-The renal clearance of oxytetracycline which is 66% unbound is 99 ml/min while that of doxycycline (7% unbound) is just 16 ml/min.

4. Blood Flow to the kidneys:

-The renal blood flow is important in case of drugs excreted by glomerular filtration only and those that are actively secreted. In the latter case, increased perfusion increases the contact of drug with the secretory sites and enhances their elimination.

5. Biological Factors:

-Renal excretion is approximately 10% lower in females than in males.

-The renal function of newborns is 30 to 40% less in comparison to adults.

-In old age, the GFR is reduced and tubular

function is altered, the excretion of drugs is thus slowed down and half-life is prolonged.

6- Drug Interactions:

-Any drug interaction that results in alteration of protein-drug binding characteristics, renal flood flow, active secretion, urine pH and intrinsic clearance and forced diuresis would alter renal clearance of a drug.

-Alteration in P-D binding:

The renal clearance of a drug extensively bound to plasma proteins is increased after displacement with another drug.

Ex:

Gentamicin induced nephrotoxicity by furosemide. The increased free antibiotic concentration accelerates its renal clearance.

-Alteration of urin pH:

-Acidification of urine with ammonium chloride, methionine or ascorbic acid enhances excretion of basic drugs.

-Alkalinisation of urine with citrates, bicarbonates etc excretion of acidic drugs.

7. Disease States - Renal impairment:

-Renal dysfunction greatly impairs the elimination of drugs especially those that are primarily excreted by the kidneys.

-Some of the causes of renal failure are hypertension, diabetes mellitus etc.

Renal Function And Renal Failure

Renal function can be determined by measuring the GFR.

- Both endogenous and exogenous substances have been used as markers to measure GFR.
- In order to be useful as a marker, the agent should entirely get excreted in unchanged form by glomerular filtration only and should be physiologically and pharmacologically inert.
- The rate at which these markers are excreted in urine reflects the GFR and changes in GFR reflects renal dysfunction.

Ex: Inulin

Inulin clearance provides an accurate measure of GFR but has the disadvantage of being a tedious method.

Clinically, Creatinine clearance is widely used to assess renal function.

- Since creatinine production varies with age, weight and gender, different formulae are used to

Calculate creatinine clearance from the serum creatinine values.

Ex: For adults (above 20 years)

Male $Cl_{Cr} = \frac{(140 - Age)W}{72 S_{Cr}}$

Females $Cl_{Cr} = \frac{(140 - Age)W}{85 S_{Cr}}$

where, Cl_{Cr} = creatinine clearance in ml/min

S_{Cr} = serum creatinine in mg%

H = height in cms, and

W = weight in Kg.

- A direct method for determining creatinine clearance is determination of the amount of creatinine excreted in urine in 24 hours (to calculate the rate of creatinine excretion) and the mean of serum creatinine from blood samples taken just before and immediately after the urine collection period.

$$Cl_R = \frac{\text{Rate of creatinine excretion}}{\text{Serum creatinine in mg\%}}$$

The normal creatinine clearance value is 120 to 130 ml/min. A value of 20 to 50 ml/min denotes moderate renal failure and values below 10 ml/min indicate severe renal impairment.

The renal function, RF is calculated by equation 6.19.

$$RF = \frac{Cl_{cr} \text{ of patient}}{Cl_{cr} \text{ of a normal person}} \qquad (6.19)$$

Dose Adjustment in Renal Failure

Generally speaking, drugs in patients with renal impairment have altered pharmacokinetic profile. Their renal clearance and elimination rate are reduced, the elimination half-life is increased and the apparent volume of distribution is altered. Thus, dose must be altered depending upon the renal function in such patients. However, except for drugs having low therapeutic indices, the therapeutic range of others is sufficiently large and dosage adjustment is not essential.

Dosage regimen need not be changed when

- The fraction of drug excreted unchanged, f_u is ≤ 0.3, and
- The renal function RF is ≥ 0.7 of normal.

The above generalization is based on the assumption that the metabolites are inactive and binding characteristics and drug availability are unaltered and so is the renal function in kidney failure conditions. When the f_u value approaches unity and RF approaches zero, elimination is extremely slowed down and dosing should be reduced drastically. The significance of nonrenal clearance increases in such conditions.

The required dose in patients with renal impairment can be calculated by the simple formula:

$$\text{Drug dose in renal impairment} = \text{Normal dose} \times RF \qquad (6.20)$$

The dosing interval in hours can be computed from the following equation:

$$\text{Dosing interval} = \frac{\text{Normal interval in hours}}{RF} \qquad (6.21)$$

When the drug is eliminated both by renal and nonrenal mechanisms, the dose to be administered in patients with renal failure is obtained from equation 6.22.

$$\text{Drug dose} = \text{Normal dose} \left[\begin{matrix} RF \times \text{Fraction excreted in urine} + \\ \text{Fraction eliminated nonrenally} \end{matrix} \right] \qquad (6.22)$$

Dialysis and Haemoperfusion:

-In severe renal failure, the patients are put on dialysis to remove toxic waste products and drugs and their metabolites which accumulate in the body.

Dialysis is a process in which easily diffusible substances are separated from poorly diffusible ones by the use of semipermeable membrane.

types:

1. Peritoneal dialysis
2. Haemodialysis

- Factors that govern the removal of substances by haemodialysis are:

Water-Solubility: Only water-soluble substances are dialyzed; lipid soluble drugs such as glutethimide cannot be removed by dialysis.

Molecular weight: Molecules with size less than 500 Daltons are dialyzed easily. e.g. many

unbound drugs.

-Drugs having large molecular weight such as vancomycin cannot be dialyzed.

Protein Binding:

Drugs bound to plasma proteins or blood cells cannot be dialyzed since dialysis is a passive diffusion process.

Volume of Distribution:

Drugs with large volume of distribution are extensively distributed throughout the body and therefore less easily removed by dialysis, e.g. digoxin

The rate at which a drug is removed by the dialyser depends upon the flow rate of blood to the machine and its performance. The term **dialysance**, also called as **dialysis clearance**, is used to express the ability of machine to clear the drug from blood. It is defined in a manner similar to clearance by equation:

$$Cl_d = \frac{Q(C_{in} - C_{out})}{C_{in}} \qquad (6.23)$$

where, Cl_d = dialysance or dialysis clearance
Q = blood flow rate to dialyser
C_{in} = concentration of drug in blood entering the dialyser
C_{out} = concentration of drug in blood leaving the dialyser

In **haemoperfusion**, the blood is passed through a bed of adsorbent such as charcoal or resin; as a result, drugs and other unwanted molecules are adsorbed while plasma proteins are not. The method is also useful in treating severe drug intoxication. The limitation of haemoperfusion is that it also removes the blood platelets, white cells and endogenous steroids.

NON-RENAL ROUTES OF DRUG EXCRETION

Drugs and their metabolites may also be excreted by routes other than the renal route, called as the **extrarenal** or **nonrenal routes of drug excretion**. The various such excretion processes are:

1. Biliary excretion
2. Pulmonary excretion
3. Salivary excretion
4. Mammary excretion
5. Skin/dermal excretion
6. Gastrointestinal excretion
7. Genital excretion

Biliary Excretion of Drugs—Enterohepatic Cycling

The hepatic cells lining the bile canaliculi produce bile. The production and secretion of bile are active processes. The bile secreted from liver, after storage in the gall bladder, is secreted in the duodenum. In humans, the bile flow rate is a steady 0.5 to 1 ml/min. Bile is important in the digestion and absorption of fats. Almost 90% of the secreted bile acids are reabsorbed from the intestine and transported back to the liver for re-secretion. The rest is excreted in faeces.

Being an active process, bile secretion is capacity-limited and subject to saturation. The process is exactly analogous to active renal secretion. Different transport mechanisms exist for the secretion of organic anions, cations and neutral polar compounds. A drug, whose biliary concentration is less than that in plasma, has a small biliary clearance and *vice versa*. In some instances, the bile to plasma concentration ratio of drug can approach 1000 in which cases, the biliary clearance can be as high as 500 ml/min or more.

Compounds that are excreted in bile have been classified into three categories on the basis of their bile/plasma concentration ratios:

Group A compounds whose ratio is approximately 1, e.g. sodium, potassium and chloride ions and glucose.

Group B compounds whose ratio is >1, usually from 10 to 1000, e.g. bile salts, bilirubin glucuronide, creatinine, sulphobromophthalein conjugates, etc.

Group C compounds with ratio < 1, e.g. sucrose, inulin, phosphates, phospholipids and mucoproteins.

Drugs can fall in any of the above three categories.

Several *factors* influence secretion of drugs in bile –

1. Physicochemical Properties of the Drug : The most important factor governing the excretion of drugs in bile is their **molecular weight.** Its influence on biliary excretion is summarized in Table 6.3.

Polarity is the other physicochemical property of drug influencing biliary excretion. Greater the polarity, better is the excretion. Thus, metabolites are more excreted in bile than the parent drug because of their increased polarity. The molecular weight threshold for biliary excretion of drugs is also dependent upon its polarity. A threshold of 300 Daltons and greater than 300 Daltons is necessary for organic cations (e.g. quaternaries) and organic anions respectively. Nonionic compounds should also be highly polar for biliary excretion, e.g. cardiac glycosides.

TABLE 6.3
Influence of Molecular Weight on Excretion Behaviour of Drugs

Molecular Weight of Drug/Metabolite	*Excretion Pattern*
Below 300 Daltons	Excreted mainly in urine; less than 5% is excreted in bile
Above 500 Daltons	Excreted mainly in bile; less than 5% is excreted in urine
Between 300 to 500 Daltons	Excreted both in urine and in bile; a decrease in one excretory route is compensated by excretion through the other route

2. Nature of Biotransformation Process : A metabolic reaction that greatly increases the polarity as well as the molecular weight of drug favours biliary excretion of the metabolite. Thus, phase II reactions, mainly glucuronidation and conjugation with glutathione, result in metabolites with increased tendency for biliary excretion (increase the molecular weight by 176 and 300 Daltons respectively). Examples of drugs excreted in the bile as glucuronides are morphine, chloramphenicol and indomethacin. Stilbestrol glucuronide is almost entirely excreted in bile. Glutathione conjugates are exclusively excreted via bile and are not observable in the urine because of their large molecular size. Conjugation with amino acids and acetylation and methylation reactions do not result in metabolites with greatly increased molecular weight and therefore have little influence on biliary excretion of xenobiotics. For a drug to be excreted unchanged in the bile, it must have a highly polar functional group such as -COOH (cromoglycic acid), $-SO_3H$ (amaranth), $-NH_4^+$ (oxyphenonium), etc. Clomiphene citrate, an ovulation inducer, is almost completely removed from the body via biliary excretion.

3. **Other Factors :** Miscellaneous factors influencing biliary e- tion of drugs include sex and species differences, protein-drug bi- g, disease states, drug interactions, etc.

Substances having high molecular weight show good excretion in bile in case of rats, dogs, and hen and poor excretion in rabbits, guinea pigs and monkeys. The route is more important for the excretion of drugs in laboratory animals than in man. Protein bound drugs can also be excreted in the bile since the secretion is an active process. In cholestasis, the bile flow rate is reduced thereby decreasing biliary excretion of drugs. Agents such as phenobarbital stimulate biliary excretion of drugs, firstly, by enhancing the rate of glucuronidation, and secondly, by promoting bile flow. The route of drug administration also influences biliary drug excretion. Orally administered drugs which during absorption process go to the liver, are excreted more in bile in comparison to parenterally administered drugs. Food also has a direct influence on biliary excretion of drugs. Protein and fat rich food increase bile flow.

The efficacy of drug excretion by the biliary system and hepatic function can be tested by an agent that is exclusively and completely eliminated unchanged in the bile, e.g. sulphobromophthalein. This marker is excreted within half an hour in the intestine when the hepatic function is normal. A delay in its excretion is indicative of hepatic and biliary malfunction. The marker is also useful in determining hepatic blood flow rate.

The ability of liver to excrete the drug in the bile is expressed by **biliary clearance** (equation 6.24).

$$\text{Biliary clearance} = \frac{\text{Biliary clearance rate}}{\text{Plasma drug concentration}} \qquad (6.24a)$$

$$= \frac{\text{Bile flow} \times \text{Biliary drug clearance}}{\text{Plasma drug concentration}} \qquad (6.24b)$$

Just as the major portion of bile salts excreted in intestine is reabsorbed, several drugs which are excreted unchanged in bile are also absorbed back into the circulation. Some drugs which are excreted as glucuronides or as glutathione conjugates are hydrolysed by the intestinal or bacterial enzymes to the parent drugs which are then reabsorbed. The reabsorbed drugs are again carried to the liver for resecretion via bile into the intestine. *This phenomenon of drug cycling between the intestine and the liver is called as* **enterohepatic cycling** or **enterohepatic circulation of drugs** (Fig. 6.5.).

Such a recycling process continues until the drug is biotransforr in the liver or is excreted in the urine or both. The drugs which secreted via bile in the intestine but not reabsorbed, are finally ex in the faeces.

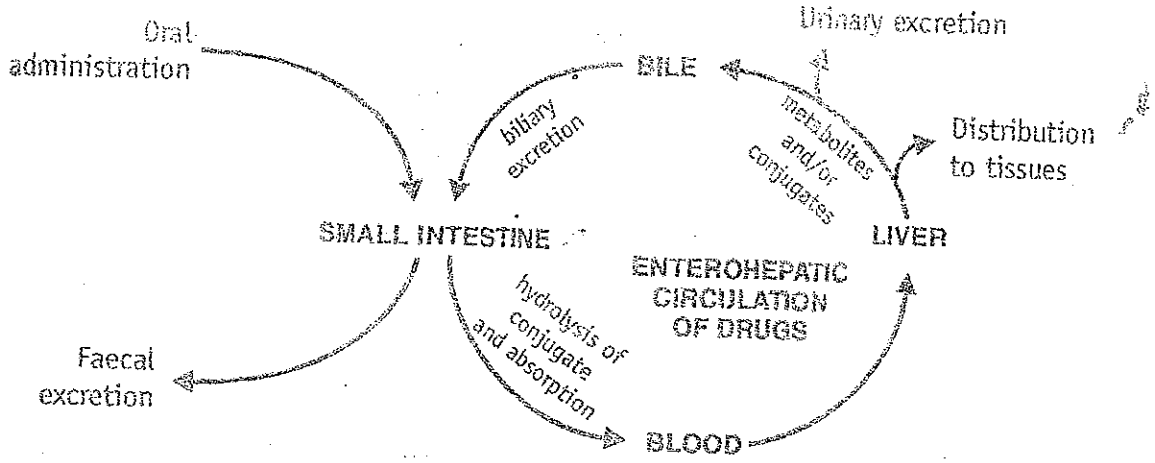


Fig. 6.5 Enterohepatic cycling of drugs

Enterohepatic circulation is important in the conservation of important endogenous substances such as vitamin B_{12}, vitamin D_3, folic acid, several steroid hormones and bile salts. The process results in prolongation of half-lives of several drugs (e.g. carbenoxolone) which are extensively excreted in bile. The half-life of agents such as DDT, which are resistant to biotransformation and are highly lipophilic, may increase to several days due to such a recycling phenomenon. The prolonged therapeutic activity of oral contraceptives (upto 12 hours) is also due to such a recirculation. Other examples of drugs undergoing enterohepatic circulation are cardiac glycosides, rifampicin, chlorpromazine and indomethacin. Drug interactions affecting enterohepatic cycling occur when agents such as antibiotics kill the intestinal microflora and thus retard hydrolysis of drug conjugates and their subsequent reabsorption, or the unabsorbable ion exchange resins such as cholestyramine which bind strongly to the acidic and neutral drugs (e.g. digitoxin) and thus prevent their reabsorption. The principle of adsorption onto the resins in the GIT can, however, be used to treat pesticide poisoning by promoting their faecal excretion.

Biliary excretion of drugs can be assessed by giving the drugs parenterally and detecting their presence in faeces. This also rules out the doubt about the incomplete absorption of such drugs when given orally and observed in faeces.

Pulmonary Excretion

Gaseous and volatile substances such as the general anaesthetics (e.g. halothane) are absorbed through the lungs by simple diffusion. Similarly, their excretion by diffusion into the expired air is possible. Factors influencing pulmonary excretion of a drug include pulmonary blood flow, rate of respiration, solubility of the volatile substance, etc. Gase-

ous anaesthetics such as nitrous oxide which are not very soluble in blood are excreted rapidly. Generally intact gaseous drugs are excreted but metabolites are not. Compounds like alcohol which have high solubility in blood and tissues are excreted slowly by the lungs. The principle involved in the pulmonary excretion of benzene and halobenzenes is analogous to that of steam distillation.

Salivary Excretion

Excretion of drugs in saliva is also a passive diffusion process and therefore predictable on the basis of pH-partition hypothesis. The pH of saliva varies from 5.8 to 8.4. The mean salivary pH in man is 6.4. Unionised, lipid-soluble drugs at this pH are excreted passively in the saliva. Equations analogous to 6.3, 6.5, 6.6 and 6.7 can be written for drugs with known pK_a at the salivary pH and percent ionisation and saliva/plasma drug concentration ratio (S/P) can be computed.

For weak acids,

$$R_a = \frac{S}{P} = \frac{1+10^{(pH_{saliva} - pK_a)}}{1+10^{(pH_{plasma} - pK_a)}} \times \frac{f_{plasma}}{f_{saliva}} \qquad (6.25)$$

For weak bases,

$$R_b = \frac{S}{P} = \frac{1+10^{(pK_a + pH_{saliva})}}{1+10^{(pK_a - pH_{plasma})}} \times \frac{f_{plasma}}{f_{saliva}} \qquad (6.26)$$

where, f_{plasma} and f_{saliva} are free drug fractions in plasma and in saliva respectively.

The S/P ratios have been found to be less than 1 for weak acids and greater than 1 for weak bases i.e. basic drugs are excreted more in saliva as compared to acidic drugs. The salivary concentration of some drugs reaches as high as 0.1%. Since the S/P ratio is fairly constant for several drugs, their blood concentration can be determined by detecting the amount of drug excreted in saliva, e.g. caffeine, theophylline, phenytoin, carbamazepine, etc. Some drugs are actively secreted in saliva, e.g. lithium, the concentration of which is sometimes 2 to 3 times that in plasma. Penicillin and phenytoin are also actively secreted in saliva.

The bitter after-taste in the mouth of a patient on medication is an indication of drug excretion in saliva. In few instances, the process is responsible for side effects such as black hairy tongue in patients receiving antibiotics, gingival hyperplasia due to phenytoin, etc. Some basic

drugs inhibit saliva secretion and are responsible for dryness of mouth.

Drugs excreted in saliva can undergo cycling in a fashion similar to enterohepatic cycling, e.g. sulphonamides, antibiotics, clonidine, etc. (Fig. 6.6.).

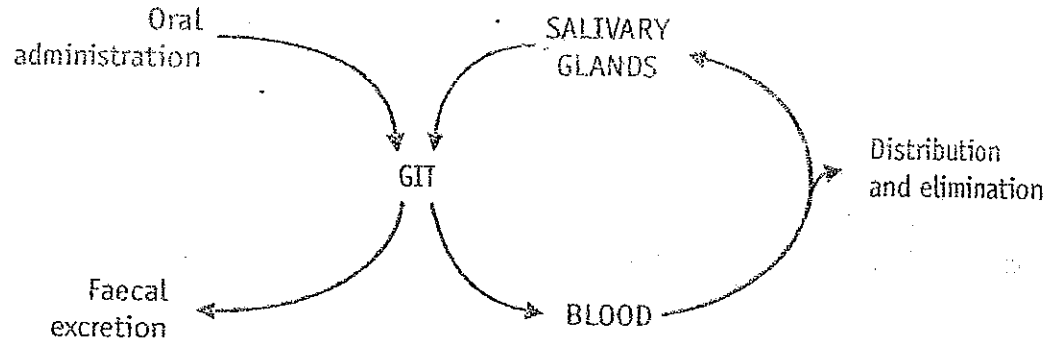


Fig. 6.6 Salivary cycling of drugs

Mammary Excretion

Excretion of a drug in milk is important since it can gain entry into the breast-feeding infant.

Milk consists of lactic secretions originating from the extracellular fluid and is rich in fats and proteins. About 0.5 to 1 litre/day of milk is secreted in lactating mothers

Excretion of drugs in milk is a passive process and is dependent upon pH-partition behaviour, molecular weight, lipid solubility and degree of ionisation. The pH of milk varies from 6.4 to 7.6 with a mean pH of 7.0. Free, unionised, lipid-soluble drugs diffuse into the mammary alveolar cells passively. The extent of drug excretion in milk can be determined from milk/plasma drug concentration ratio (M/P). Since milk is acidic in comparison to plasma, as in the case of saliva, weakly basic drugs concentrate more in milk and have M/P ratio greater than 1. The opposite is true for weakly acidic drugs. It has been shown that for acidic drugs, excretion in milk is inversely related to the molecular weight and partition coefficient and that for basic drugs, is inversely related to the degree of ionisation and partition coefficient. Drugs extensively bound to plasma proteins, e.g. diazepam, are less secreted in milk. Since milk contains proteins, drugs excreted in milk can bind to it. The amount of drug excreted in milk is generally less than 1% and the fraction consumed by the infant is too less to reach therapeutic or toxic levels. But some potent drugs such as barbiturates, morphine and ergotamine may induce toxicity in infants. Some examples of toxicity to breast-fed infants owing to excretion of drug in milk are –

- Chloramphenicol : Possible bone marrow suppression.
- Diazepam : Accumulation and sedation.
- Heroin : Prolonged neonatal dependence.
- Methadone : Possible withdrawal syndrome if breast-feeding is stopped suddenly.
- Propylthiouracil : Suppression of thyroid function.
- Tetracycline : Permanent staining of infant teeth.

Wherever possible, nursing mothers should avoid drugs.

If medication is unavoidable, the infant should be bottle-fed.

Skin Excretion

Drugs excreted through the skin via sweat also follow pH-partition hypothesis. Passive excretion of drugs and their metabolites through skin is responsible to some extent for the urticaria and dermatitis and other hypersensitivity reactions. Compounds such as benzoic acid, salicylic acid, alcohol and antipyrine and heavy metals like lead, mercury and arsenic are excreted in sweat.

TABLE 6.4

Excretion Pathways, Transport Mechanisms and Drugs Excreted

Excretory Route	*Mechanism*	*Drugs Excreted*
Urine	Glomerular filtration, active secretion, active/ passive reabsorption	Free, hydrophilic, unchanged drugs/ metabolites/conjugates of MW < 500
Bile	Active secretion	Hydrophilic, unchanged drugs/ metabolites/conjugates of MW ≥ 500
Lung	Passive diffusion	Gaseous and volatile, blood and tissue insoluble drugs
Saliva	Passive diffusion, active transport	Free, unionised, lipophilic drugs, some polar drugs
Milk	Passive diffusion	Free, unionised, lipophilic drugs (generally basic)
Sweat	Passive diffusion	Free, unionised, lipophilic drugs
Intestine	Passive diffusion	Water-soluble, ionised drugs

Gastrointestinal Excretion

Excretion of drugs into the GIT usually occurs after parenteral administration when the concentration gradient for passive diffusion is favourable. The process is reverse of GI absorption of drugs. Water-soluble and ionised form of weakly acidic and basic drugs is excreted in the GIT, e.g. nicotine and quinine are excreted in stomach. Orally administered drugs can also be absorbed and excreted in the GIT. Drugs excreted in the GIT are reabsorbed into the systemic circulation and undergo recycling.

Genital Excretion

Reproductive tract and genital secretions may contain the excreted drugs. Some drugs have been detected in semen.

Drugs can also get excreted via the lachrymal fluid.

- The frequency of administration of a drug in a particular dose is called as dosage regimen.
- Rational and optimal therapy with a drug depend upon:
  1. Choice of a suitable drug
  2. Balance between the therapeutic and the toxic effects

- The drug fails to elicit a therapeutic response when the concentration is below the effective level and precipitates adverse reactions when above the toxic level. The plasma drug concentration between these two limits is called as the <u>therapeutic concentration range</u> or <u>therapeutic window</u> (the ratio of maximum safe concentration to minimum effective concentration of the drug is called as the <u>therapeutic index</u>.)

- Thus, in order to achieve therapeu success, plasma concentration of the drug should be maintained within the therapeutic window.
- For this, knowledge is needed not only of the

mechanisms of drug absorption, distribution, metabolism and excretion, but also of the kinetics of these processes i.e. pharmacokinetics.

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

- Pharmacokinetic studies:

1. Theoretical aspect:

which involves development of pharmacokinetic models to predict drug disposition after its administration. Statistical methods are commonly applied to interpret data and assess various parameters.

2. Experimental aspect:

which involves development of biological sampling techniques, analytical methods for

measurement of drug (and metabolites) concentration in biological samples and data collection and evaluation.

Terms:

2m
- Clinical Pharmacokinetics: is defined as the application of pharmacokinetic principles in the safe and effective management of individual patient.

2m
- Population Pharmacokinetics: is defined as the study of pharmacokinetic differences of drugs in various population groups.

2m
- Toxicokinetics is defined as the application of pharmacokinetic principles to the design, conduct and interpretation of drug safety evaluation studies.

Plasma Drug Concentration-Time Profile

A direct relationship exists between the concentration of drug at the biophase (site of action) and the concentration of drug in plasma. Two

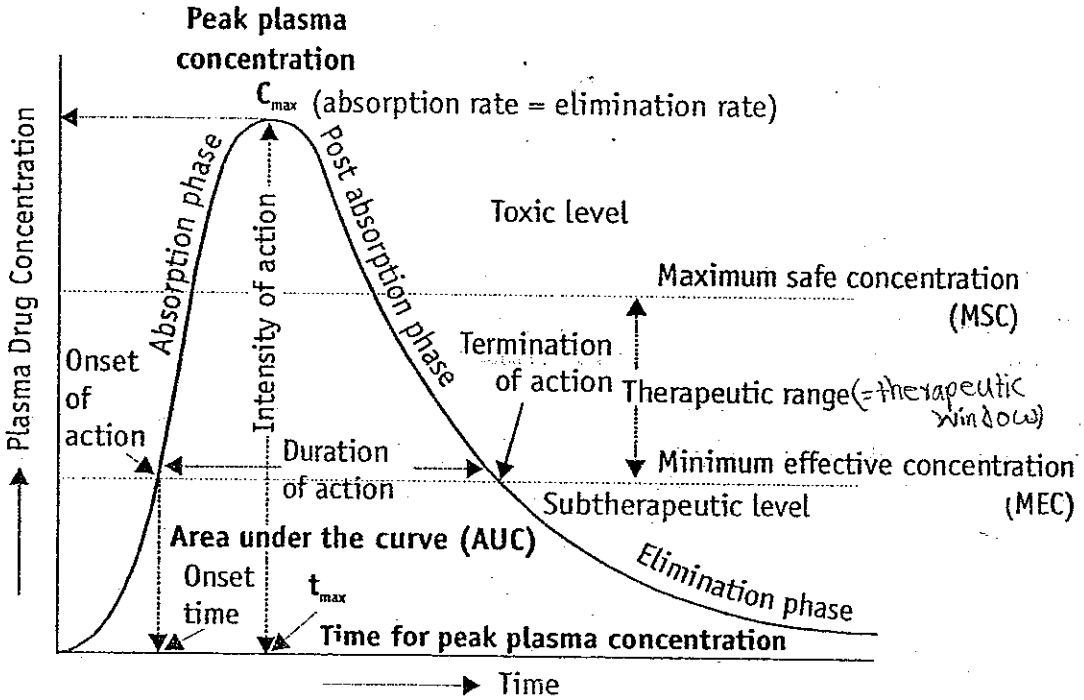


Fig. 8.1 A typical plasma concentration-time profile showing pharmacokinetic and pharmacodynamic parameters, obtained after oral administration of single dose of a drug.

Categories of parameters can be evaluated from a plasma concentration time profile:

- Pharmacokinetic parameters
- Pharmacodynamic parameters

Pharmacokinetic Parameters:

1. Peak Plasma Concentration (C_{max}):

The point of maximum concentration of drug in plasma is called as the peak and the concentration of drug at peak is known as peak plasma concentration.

- It is also called as peak height concentration and maximum drug concentration.
- C_{max} is expressed in mcg/ml
- The peak plasma level depends upon:
  - i- Dose administered
  - ii - Rate of absorption
  - iii - Rate of elimination

- The peak represents the point of time when absorption rate equals elimination rate of drug.
- The portion of curve to the left of peak represents absorption phase i.e. when the rate of absorption is greater than the rate of elimination.
- The section of curve to the right of peak generally represents elimination phase i.e. when the

rate of elimination exceeds rate of absorption.

- Peak concentration should ideally be above minimum effective concentration (MEC) but less than the maximum safe concentration (MSC).

2. Time of peak concentration (t_{max}):

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

- It is expressed in hours and is useful in estimating the rate of absorption.
- Onset time and onset of action are dependent upon t_{max}.
- This parameter is of particular importance in assessing the efficacy of drugs used to treat acute conditions like pain and insomnia which can be treated by a single dose.

3. Area Under the Curve (AUC):

- It represents the total integrated area under the plasma level-time profile and expresses

the total amount of drug that comes into the systemic circulation after its administration.

- AUC is expressed in mcg/ml X hours.

- It is the most important parameter in evaluating the bioavailability of a drug from its dosage form as it represents the extent of absorption.

- AUC is also important for drugs that are administered repetitively for the treatment of chronic conditions like asthma or epilepsy.

□ Pharmacodynamic Parameters:

1. Minimum Effective Concentration (MEC):

- It is defined as the minimum concentration of drug in plasma required to produce the therapeutic effect.

- It reflects the minimum concentration of drug at the receptor site to elicit the desired pharmacological response.

- The concentration of drug below MEC is said to be in the sub-therapeutic level.

- In case of antibiotics, the term minimum inhibitory concentration (MIC) is used. It describes the minimum concentration of antibiotic in plasma required to kill or inhibit the growth of microorganisms.

2. Maximum Safe Concentration (MSC): Also called as minimum toxic concentration (MTC), it is the concentration of drug in plasma above which adverse or unwanted effects are precipitated.

concentration of drug above MSC is said to be in the toxic level.

3. Onset of Action:

The beginning of pharmacological response is called as onset of action.

It occurs when the plasma drug concentration just exceeds the required MEC.

4. Onset Time:

It is the time required for the drug to start producing pharmacological response.

It corresponds to the time for the plasma concentration to reach MEC after administration of drug.

5. Duration of Action:

The time period for which the plasma concentration of drug remains above the MEC level is called as duration of drug action.

- It is also defined as the difference between onset time and time for the drug to decline back to MEC.

6. Intensity of Action:

It is the maximum pharmacological response produced by the peak plasma concentration of drug.

-It is also called as peak response.

7. Therapeutic Range:

The drug concentration between MEC and MSC represents the therapeutic range.

-It is also known as therapeutic window.

8. Therapeutic Index:

The ratio of MSC to MEC is called as therapeutic index.

It is also defined as the ratio of dose required to produce toxic or lethal effects to dose required to produce therapeutic effect.

□ Rate, Rate Constants and Orders of Reactions:

- Pharmacokinetics is the mathematical analysis of processes of ADME.

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

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

$$\text{Drug A} \longrightarrow \text{Drug B} \qquad (8.1)$$

The rate of forward reaction is expressed as—

$$\frac{-dA}{dt} \qquad (8.2)$$

Negative sign indicates that the concentration of drug A decreases with time t. As the reaction proceeds, the concentration of drug B increases and the rate of reaction can also be expressed as:

$$\frac{dB}{dt} \qquad (8.3)$$

Experimentally, the rate of reaction is determined by measuring the decrease in concentration of drug A with time t.

If C is the concentration of drug A, the rate of decrease in C of drug A as it is changed to B can be described by a general expression as a function of time t.

$$\frac{dC}{dt} = -K\,C^n \qquad (8.4)$$

where, K = rate constant
n = order of reaction

If n = 0, its a zero-order process, if n = 1, it is a first-order process and so on. The three commonly encountered rate processes in a physiological system are—

- Zero-order process
- First-order process
- Mixed-order process.

The pharmacokinetics of most drugs can be adequately described by zero- and first-order processes of which the latter are more important.

Zero-Order Kinetics (Constant Rate Processes)

If n = 0, equation 8.4 becomes:

$$\frac{dC}{dt} = -K_0 C^0 = -K_0 \qquad (8.5)$$

where K_0 = zero-order rate constant (in mg/min)

From equation 8.5, the zero-order process *can be defined as the one whose rate is independent of the concentration of drug undergoing reaction i.e. the rate of reaction cannot be increased further by increasing the concentration of reactants.*

Rearrangement of equation 8.5 yields:

$$dC = -K_0\, dt \qquad (8.6)$$

Integration of equation 8.6 gives:

$$C - C_0 = -K_0\, t \qquad (8.7)$$

$$\text{or simply,} \quad C = C_0 - K_0\, t$$

where C_0 = concentration of drug at t = 0, and
C = concentration of drug yet to undergo reaction at time t.

Equation 8.7 is that of a straight line and states that the concentration of reactant decreases linearly with time. A plot of C versus t yields such a straight line having slope – K_0 and y-intercept C_0 (Fig.8.2).

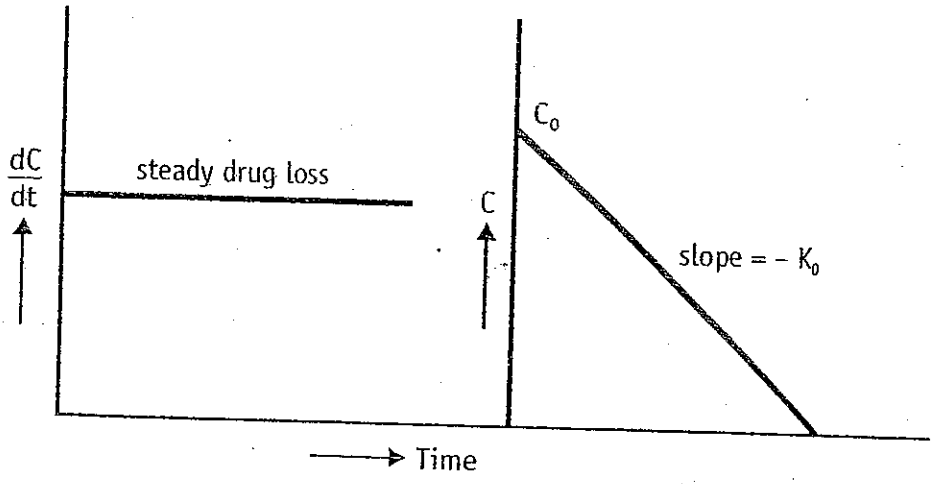


Fig. 8.2 Graphs of zero-order kinetics (equations 8.5 and 8.7)

Zero-Order Half-Life

Half-life ($t_{½}$) or half-time *is defined as the time period required for the concentration of drug to decrease by one-half.* When $t = t_{½}$, $C = C_0/2$ and the equation 8.7 becomes:

$$\frac{C_0}{2} = C_0 - K_0\, t_{1/2} \tag{8.8}$$

Solving 8.8, we get:

$$t_{1/2} = \frac{C_0}{2K_0} = \frac{0.5\,C_0}{K_0} \tag{8.9}$$

Equation 8.9 shows that the $t_{½}$ of a **zero-order process** is not constant but proportional to the initial concentration of drug C_0 and inversely proportional to the zero-order rate constant K_0. Since the zero-order $t_{½}$ changes with the decline in drug concentration, it is of little practical importance. Zero-order equations do not require logarithmic transformations.

Examples of zero-order processes are—

1. Metabolism/protein-drug binding and enzyme or carrier-mediated transport under saturated conditions. The rate of metabolism, binding or transport of drug remains constant as long as its concentration is in excess of saturating concentration.
2. Administration of a drug as a constant rate i.v. infusion.
3. Controlled drug delivery such as that from i.m. implants or osmotic pumps.

First-Order Kinetics (Linear Kinetics)

If n = 1, equation 8.4 becomes:

$$\frac{dC}{dt} = -K\,C \tag{8.10}$$

where K = first-order rate constant (in time^{-1} or per hour)

From equation 8.10, it is clear that a **first-order process** *is the one whose rate is directly proportional to the concentration of drug undergoing reaction i.e. greater the concentration, faster the reaction.* It is because of such proportionality between rate of reaction and the concentration of drug that a first-order process is said to follow **linear kinetics** (Fig. 8.3.).

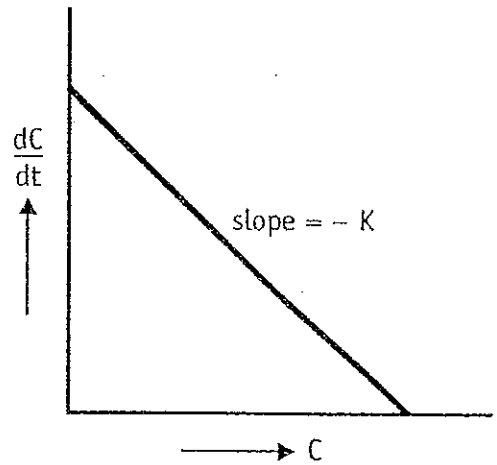


Fig. 8.3 Graph of first-order kinetics showing linear relationship between rate of reaction and concentration of drug (equation 8.10).

Rearrangement of equation 8.10 yields:

$$\frac{dC}{C} = -K\,dt \tag{8.11}$$

Integration of equation 8.11 gives:

$$\ln C = \ln C_0 - Kt \tag{8.12}$$

Equation 8.12 can also be written in exponential form as:

$$C = C_0\,e^{-Kt} \tag{8.13}$$

where e = natural (Naperian) log base.

Since equation 8.13 has only one exponent, the first-order process is also called as **monoexponential rate process**. Thus, a first-order process is characterized by **logarithmic** or **exponential** kinetics i.e. *a constant fraction of drug undergoes reaction per unit time.*

Since ln = 2.303 log, equation 8.12 can be written as:

$$\log C = \log C_0 - \frac{Kt}{2.303} \tag{8.14}$$

or

$$\log C = \log C_0 - 0.434\,Kt \tag{8.15}$$

A semilogarithmic plot of equation 8.14 yields a straight line with slope = –K/2.303 and y-intercept = log C_o (Fig. 8.4.).

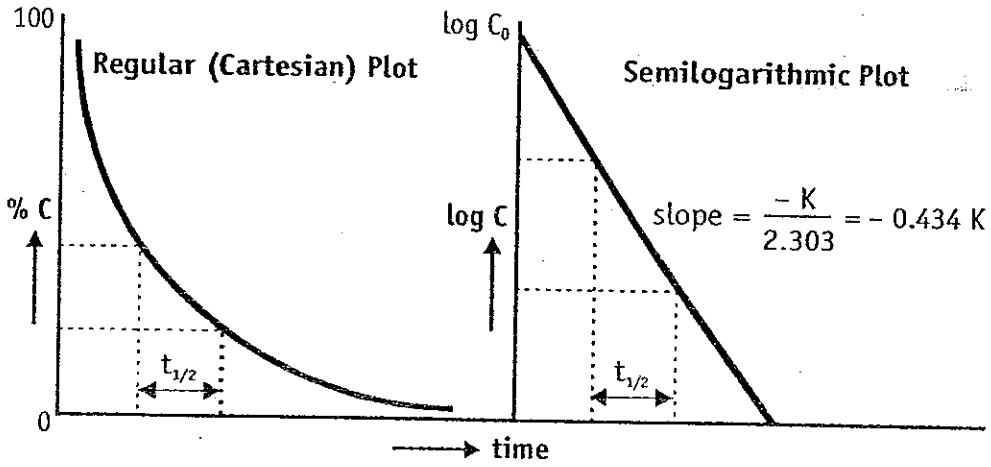


Fig. 8.4 Regular and semilog graphs of first-order kinetics

First-Order Half-Life

Substituting the value of $C = C_0/2$ at $t_{½}$ in equation 8.14 and solving it yields:

$$t_{1/2} = \frac{0.693}{K} \tag{8.16}$$

Equation 8.16 shows that, in contrast to zero-order process, the half-life of a **first-order process** is a constant and independent of initial drug concentration i.e. irrespective of what the initial drug concentration is, the time required for the concentration to decrease by one-half remains the same (see Fig. 8.4.). The $t_{½}$ of a first-order process is an important pharmacokinetic parameter.

Most pharmacokinetic processes *viz.* absorption, distribution and elimination follow first-order kinetics.

Mixed-Order Kinetics (Nonlinear Kinetics)

In some instances, the kinetics of a pharmacokinetic process changes from predominantly first-order to predominantly zero-order with increasing dose or chronic medication. A *mixture* of both first-order and zero-order kinetics is observed in such cases and therefore the process is

said to follow **mixed-order kinetics**. Since deviations from an originally linear pharmacokinetic profile are observed, the rate process of such a drug is called as **nonlinear kinetics**. Mixed-order kinetics is also termed as **dose-dependent kinetics** as it is observed at increased or multiple doses of some drugs. Nonlinearities in pharmacokinetics have been observed in—

- Drug absorption (e.g. vitamin C)
- Drug distribution (e.g. naproxen), and
- Drug elimination (e.g. riboflavin).

The nonlinearity phenomenon is seen when a particular pharmacokinetic process involves presence of carriers or enzymes which are substrate-specific and have definite capacities and can get saturated at high drug concentrations (i.e. capacity-limited). The kinetics of such capacity-limited processes can be described by the **Michaelis-Menten kinetics.**

Pharmacokinetic Parameters

The predictive capability of a pharmacokinetic model lies in the proper selection and development of *mathematical functions called parameters* that govern a pharmacokinetic process. In practice, pharmacokinetic parameters are determined experimentally from a *set of drug concentrations collected over various times known as data*. Parameters are also called as *variables*. Variables are of two types:

1. ***Independent variables*** which are not affected by any other parameter, for example *time.*
2. ***Dependent variables***, which change as the independent variables change, for example, *plasma drug concentration.*

Certain points, which are important to note regarding application of parameters in pharmacokinetic studies, include—

- The number of parameters needed to describe the pharmacokinetic model depends upon the complexity of the pharmacokinetic process and on the route of drug administration.
- More the number of parameters more are the difficulties in accurate estimation of these parameters.
- For the pharmacokinetic parameters to be valid, the number of data points should always exceed the number of parameters in the pharmacokinetic model.

PHARMACOKINETIC ANALYSIS OF MATHEMATICAL DATA : PHARMACOKINETIC MODELS

Drug movement within the body is a complex process. The major objective is therefore to develop a generalized and simple approach to describe, analyse and interpret the data obtained during *in vivo* drug disposition studies. The two major approaches in the quantitative study of various kinetic processes of drug disposition in the body are (see Fig. 8.5)—

- Model approach, and
- Model-independent approach (also called as non-compartmental analysis).

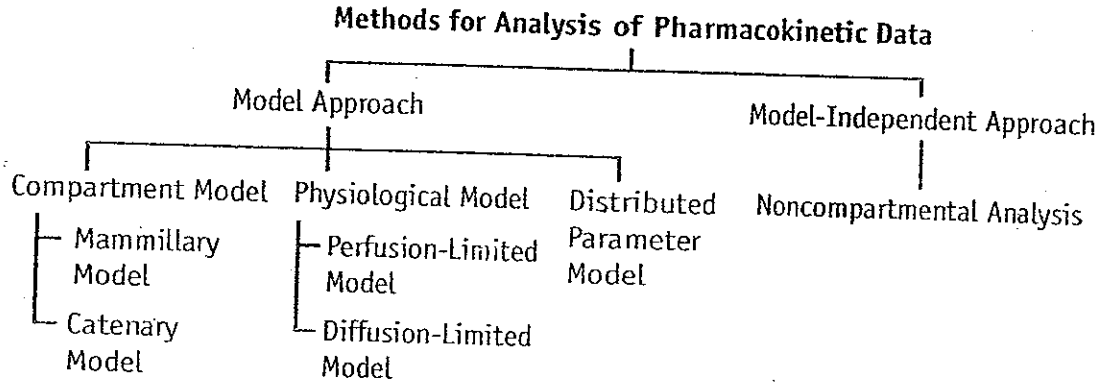


Fig. 8.5 Various approaches used for quantitative study of kinetic processes

Pharmacokinetic Model Approach

In this approach, models are used to describe changes in drug concentration in the body with time. *A **model** is a hypothesis that employs mathematical terms to concisely describe quantitative relationships.* **Pharmacokinetic models** provide concise means of expressing mathematically or quantitatively, the time course of drug(s) throughout the body and compute meaningful **pharmacokinetic parameters.**

Applications of Pharmacokinetic Models

Pharmacokinetic models are useful in—

1. Characterizing the behaviour of drugs in patients.
2. Predicting the concentration of drug in various body fluids with any dosage regimen.
3. Predicting the multiple-dose concentration curves from single dose experiments.
4. Calculating the optimum dosage regimen for individual patients.

5. Evaluating the risk of toxicity with certain dosage regimens.
6. Correlating plasma drug concentration with pharmacological response.
7. Evaluating the bioequivalence/bioinequivalence between different formulations of the same drug.
8. Estimating the possibility of drug and/or metabolite(s) accumulation in the body.
9. Determining the influence of altered physiology/disease state on drug ADME.
10. Explaining drug interactions.

Caution must, however, be exercised in ensuring that the model fits the experimental data; otherwise, a new, more complex and suitable model may be proposed and tested.

Types of Pharmacokinetic Models

Pharmacokinetic models are of three different types—

1. ***Compartment models*** – are also called as ***empirical models***.
2. ***Physiological models*** – are *realistic models*.
3. ***Distributed parameter models*** – are also *realistic models*.

Compartment Models

Compartmental analysis is the traditional and most commonly used approach to pharmacokinetic characterization of a drug. These models simply interpolate the experimental data and allow an *empirical formula* to estimate the drug concentration with time.

Since compartments are hypothetical in nature, compartment models are based on certain ***assumptions***—

1. The body is represented as a series of compartments arranged either in series or parallel to each other, which communicate reversibly with each other.
2. Each compartment is not a real physiological or anatomical region but a fictitious or virtual one and considered as a tissue or group of tissues that have similar drug distribution characteristics (similar blood flow and affinity). This assumption is necessary because if every organ, tissue or body fluid that can get equilibrated with the drug is considered as a separate compartment, the body will comprise of infinite number of

compartments and mathematical description of such a model will be too complex.

3. Within each compartment, the drug is considered to be rapidly and uniformly distributed i.e. the compartment is *well-stirred.*
4. The rate of drug movement between compartments (i.e. entry and exit) is described by first-order kinetics.
5. Rate constants are used to represent rate of entry into and exit from the compartment.

Depending upon whether the compartments are arranged parallel or in a series, compartment models are divided into two categories—

- *Mammillary model*
- *Catenary model.*

Mammillary Model

This model is the most common compartment model used in pharmacokinetics. It consists of one or more peripheral compartments connected to the central compartment in a manner similar to connection of satellites to a planet (i.e. they are joined parallel to the central compartment). The **central compartment** (or **compartment 1**) *comprises of plasma and highly perfused tissues* such as lungs, liver, kidneys, etc. which rapidly equilibrate with the drug. The drug is directly absorbed into this compartment (i.e. blood). Elimination too occurs from this compartment since the chief organs involved in drug elimination are liver and kidneys, the highly perfused tissues and therefore presumed to be rapidly accessible to drug in the systemic circulation. The **peripheral compartments** or **tissue compartments** (denoted by numbers 2, 3, etc.) *are those with low vascularity and poor perfusion.* Distribution of drugs to these compartments is through blood. Movement of drug between compartments is defined by characteristic first-order rate constants denoted by letter K (*see* Fig. 8.6). The subscript indicates the direction of drug movement; thus, K_{12} (K-one-two) refers to drug movement from compartment 1 to compartment 2 and reverse for K_{21}.

The number of rate constants which will appear in a particular compartment model is given by R.

For intravenous administration, $R = 2n - 1$ (8.17)

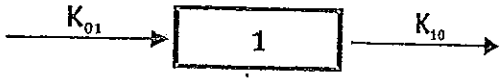
For extravascular administration, $R = 2n$ (8.18)

where n = number of compartments.

Model 1

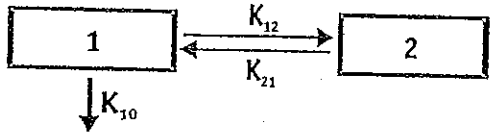
One-compartment open model, intravenous administration

Model 2



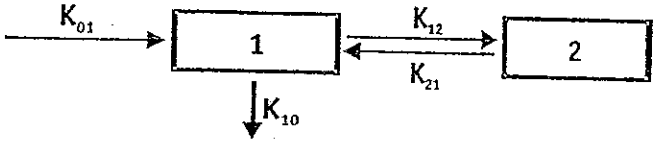
One-compartment open model, extravascular (oral, rectal, etc.) administration

Model 3



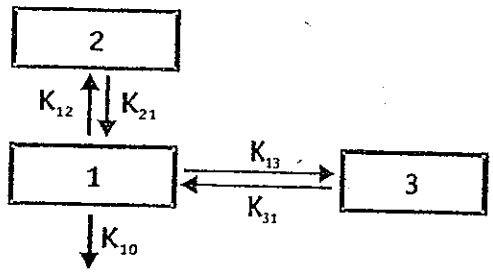
Two-compartment open model, intravenous administration

Model 4



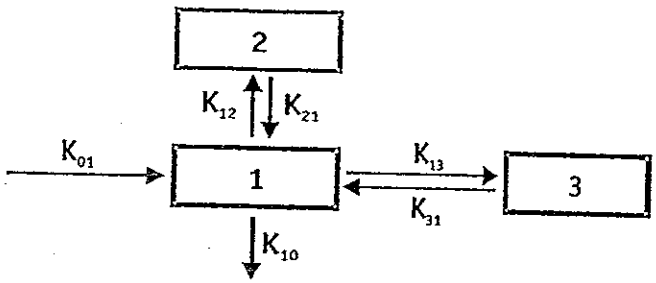
Two-compartment open model, extravascular administration

Model 5



Three-compartment open model, intravenous administration

Model 6



Three-compartment open model, extravascular administration

Fig. 8.6 Various mammillary compartment models. The rate constant K_{01} is basically K_a, the first-order absorption rate constant and K_{10} is K_E, the first-order elimination rate constant.

Catenary Model

In this model, the compartments are joined to one another in a series like compartments of a train (Fig. 8.7). This is however not observable physiologically/anatomically as the various organs are directly linked to the blood compartment. Hence, this model is rarely used.

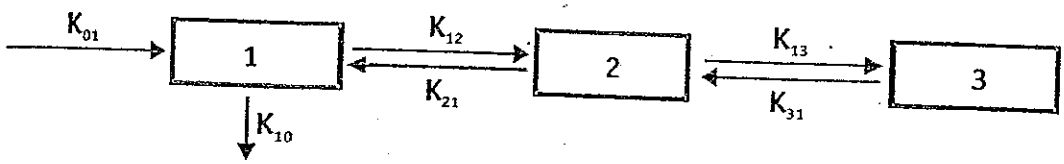


Fig. 8.7 A catenary model

The compartment modelling approach has several **advantages** and **applications**—

1. It is a simple and flexible approach and thus widely used. Fundamentally, the principal use of this approach is to account for the mass balance of drug in plasma, drug in extravascular tissues and the amount of drug eliminated after its administration. It often serves as a "first-model".
2. It gives a visual representation of various rate processes involved in drug disposition.
3. It shows how many rate constants are necessary to describe these processes.
4. It enables the pharmacokineticist to write differential equations for each of the rate processes in order to describe drug-concentration changes in each compartment.
5. It enables monitoring of drug concentration change with time with a limited amount of data. Only plasma concentration data or urinary excretion data is sufficient.
6. It is useful in predicting drug concentration-time profile in both normal physiological and in pathological conditions.
7. It is important in the development of dosage regimens.
8. It is useful in relating plasma drug levels to therapeutic and toxic effects in the body.
9. It is particularly useful when several therapeutic agents are compared. Clinically, drug data comparisons are based on compartment models.
10. Its simplicity allows for easy tabulation of parameters such as V_d, $t_{1/2}$, etc.

Disadvantages of compartment modelling include—

1. The compartments and parameters bear no relationship with the physiological functions or the anatomical structure of the species; several assumptions have to be made to facilitate data interpretation.
2. Extensive efforts are required in the development of an exact model that predicts and describes correctly the ADME of a certain drug.
3. The model is based on curve fitting of plasma concentration with complex multiexponential mathematical equations.
4. The model may vary within a study population.
5. The approach can be applied only to a specific drug under study.
6. The drug behaviour within the body may fit different compartmental models depending upon the route of administration.
7. Difficulties generally arise when using models to interpret the differences between results from human and animal experiments.
8. Owing to their simplicity, compartmental models are often misunderstood, overstretched or even abused.

Because of the several drawbacks of and difficulties with the classical compartment modelling, newer approaches have been devised to study the time course of drugs in the body. They are—physiological models and noncompartmental methods.

Physiological Models

These models are also known as *physiologically-based pharmacokinetic models* (***PB-PK models***). They are drawn on the basis of known anatomic and physiological data and thus present a more realistic picture of drug disposition in various organs and tissues. The number of compartments to be included in the model depends upon the disposition characteristics of the drug. Organs or tissues such as bones that have no drug penetration are excluded. Since describing each organ/tissue with mathematical equations makes the model complex, tissues with similar perfusion properties are grouped into a single compartment. For example, lungs, liver, brain and kidney are grouped as ***rapidly equilibrating tissues*** (RET) while muscles and adipose as ***slowly equilibrating tissues*** (SET). Fig. 8.8 shows such a physiological model where the compartments are arranged in a series in a flow diagram.

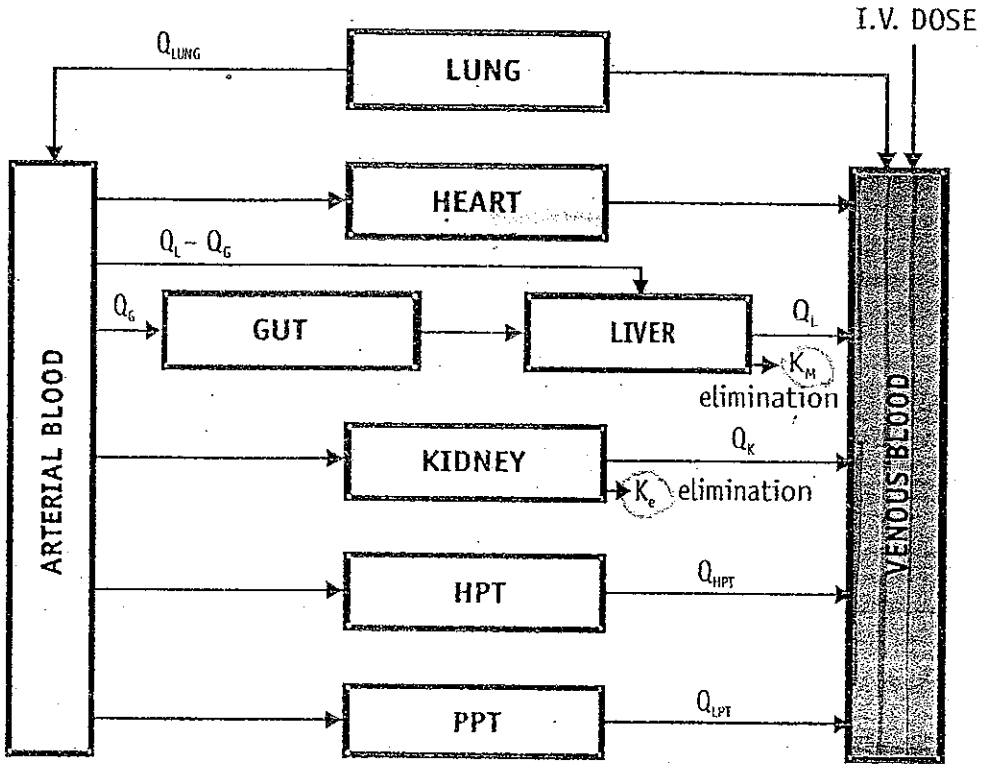


Fig. 8.8 Schematic representation of a physiological pharmacokinetic model. The term Q indicates blood flow rate to a body region. HPT stands for other highly perfused tissues and PPT for poorly perfused tissues. K_m is rate constant for hepatic elimination and K_e is first-order rate constant for urinary excretion.

The rate of drug carried to a tissue/organ or tissue drug uptake is dependent upon two major factors—

- Rate of blood flow to the organ, and
- Tissue/blood partition coefficient or diffusion coefficient of drug that governs its tissue permeability,

Accordingly the physiological models are further categorized into two types—

1. Blood flow rate-limited models : These models are more popular and commonly used than the second type, and are based on the assumption that the drug movement within a body region is much more rapid than its rate of delivery to that region by the perfusing blood. These models are therefore also called as ***perfusion rate-limited models***. This assumption is, however, applicable only to the highly membrane permeable drugs i.e. low molecular weight, poorly-ionised and highly lipophilic drugs, for example, thiopental, lidocaine, etc.

2. Membrane permeation rate-limited models : These models are more complex and applicable to highly polar, ionised and charged drugs, in which case the cell membrane acts as a barrier for the drug that

gradually permeates by diffusion. These models are therefore also called as ***diffusion-limited models***. Owing to the time lag in equilibration between the blood and the tissue, equations for these models are very complicated.

Physiological modelling has several **advantages** over the conventional compartment modelling—

1. Mathematical treatment is straightforward.
2. Since it is a realistic approach, the model is suitable where tissue drug concentration and binding are known.
3. Data fitting is not required since drug concentration in various body regions can be predicted on the basis of organ or tissue size, perfusion rate and experimentally determined tissue-to-plasma partition coefficient.
4. The model gives exact description of drug concentration-time profile in any organ or tissue and thus better picture of drug distribution characteristics in the body.
5. The influence of altered physiology or pathology on drug disposition can be easily predicted from changes in the various pharmacokinetic parameters since the parameters correspond to actual physiological and anatomic measures.
6. The method is frequently used in animals because invasive methods can be used to collect tissue samples.
7. Correlation of data in several animal species is possible and with some drugs, can be extrapolated to humans since tissue concentration of drugs is known.
8. Mechanism of ADME of drug can be easily explained by this model.

Disadvantages of physiological modelling include—

1. Obtaining the experimental data is a very exhaustive process.
2. Most physiological models assume an average blood flow for individual subjects and hence prediction of individualized dosing is difficult.
3. The number of data points is less than the pharmacokinetic parameters to be assessed.
4. Monitoring of drug concentration in body is difficult since exhaustive data is required

Table 8.1 briefly compares features of compartment and physiological models.

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TABLE 8.1.

Comparison of Features of Compartment and Physiological Models

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

Distributed Parameter Model

This model is analogous to physiological model but has been designed to take into account—

- Variations in blood flow to an organ, and
- Variations in drug diffusion in an organ.

Such a model is thus specifically useful for assessing regional differences in drug concentrations in tumours or necrotic tissues.

The distributed parameter model differs from physiological models in that the mathematical equations are more complex and collection of drug concentration data is more difficult.

Noncompartmental Analysis

The ***noncompartmental analysis***, also called as the **model-independent method**, does not require the assumption of specific compartment model. This method is, however, *based on the assumption that the drugs or metabolites follow linear kinetics*, and on this basis, this technique can be applied to any compartment model.

The noncompartmental approach, based on the **statistical moments theory**, involves collection of experimental data following a single dose of drug. If one considers the time course of drug concentration in plasma as a statistical distribution curve, then:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \tag{8.19}$$

where MRT = mean residence time
AUMC = area under the *first-moment curve*
AUC = area under the *zero-moment curve*

AUMC is obtained from a plot of product of plasma drug concentration and time (i.e. C.t) versus time t from zero to infinity (Fig. 8.9). Mathematically, it is expressed by equation:

$$\text{AUMC} = \int_0^\infty C\,t\,dt \tag{8.20}$$

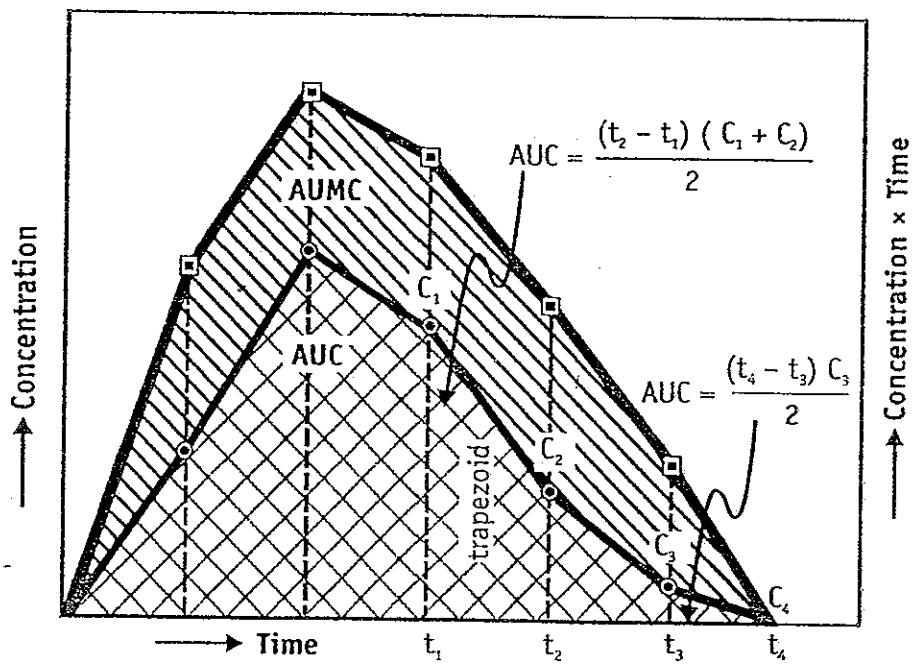


Fig. AUC and AUMC plots

AUC is obtained from a plot of plasma drug concentration versus time from zero to infinity. Mathematically, it is expressed by equation:

$$AUC = \int_0^\infty C\ dt \qquad (8.21)$$

Practically, the AUMC and AUC can be calculated from the respective graphs by the **trapezoidal rule** (the method involves dividing the curve by a series of vertical lines into a number of trapezoids, calculating separately the area of each trapezoid and adding them together).

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

Applications of noncompartmental technique include—

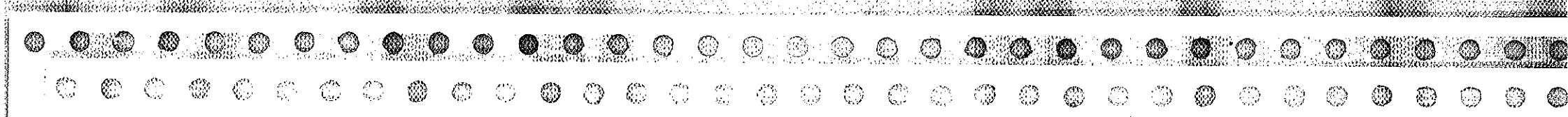
1. It is widely used to estimate the important pharmacokinetic parameters like bioavailability, clearance and apparent volume of distribution.
2. The method is also useful in determining half-life, rate of absorption and first-order absorption rate constant of the drug.

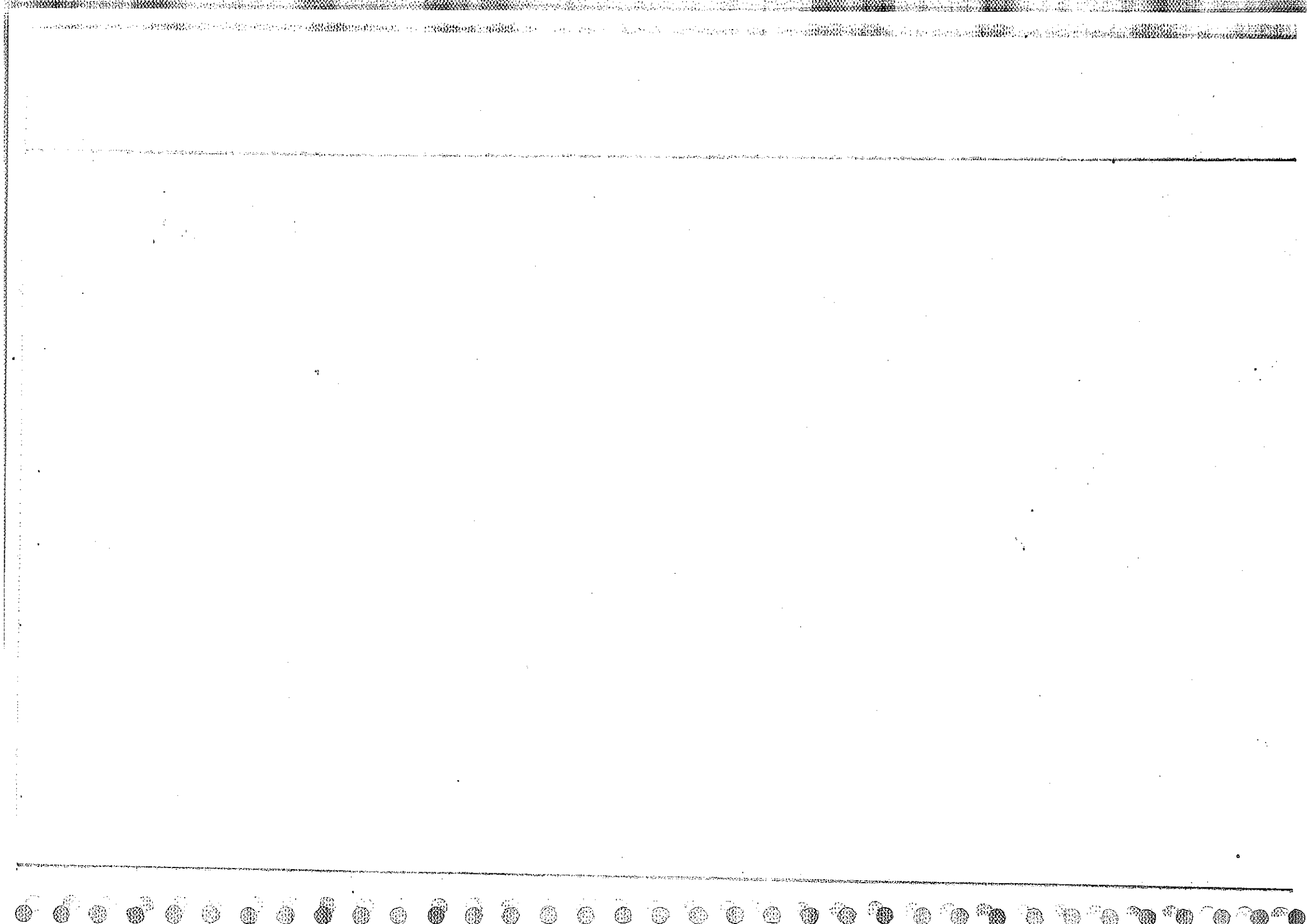
Advantages of noncompartmental method include—

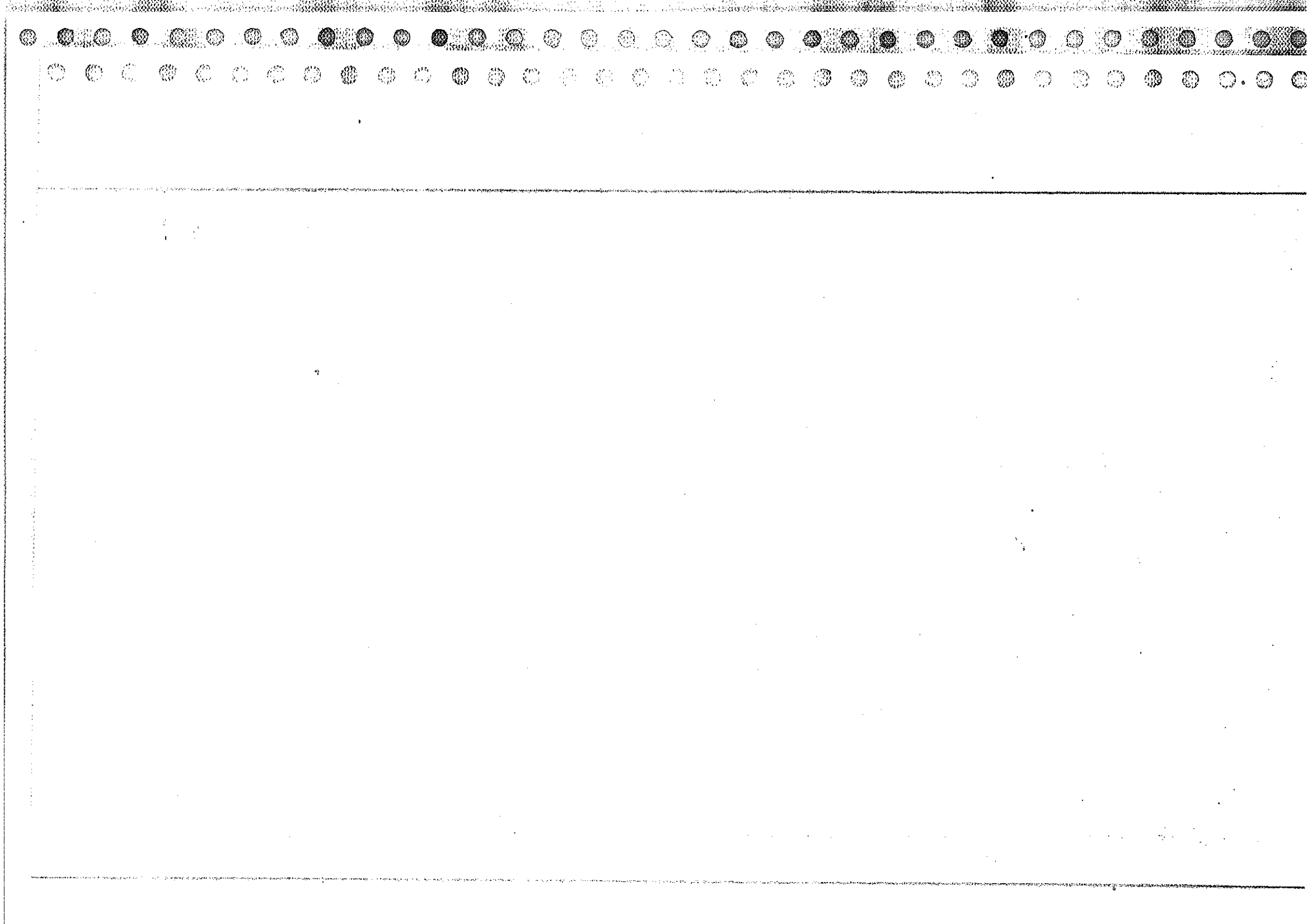
1. Ease of derivation of pharmacokinetic parameters by simple algebraic equations.
2. The same mathematical treatment can be applied to almost any drug or metabolite provided they follow first-order kinetics.
3. A detailed description of drug disposition characteristics is not required.

Disadvantages of this method include—

1. It provides limited information regarding the plasma drug concentration-time profile. More often, it deals with averages.
2. The method does not adequately treat non-linear cases.







One Compartment Open Model

(instantaneous Distribution Model)

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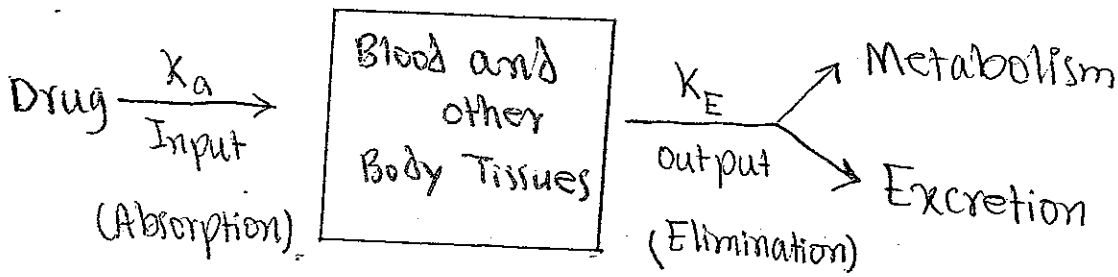
- Is the simplest model.

1. The body is considered as a single, kinetically homogenous unit that has no barriers to the movement of drug.
2. Final distribution equilibrium between the drug in plasma and other body fluids (i.e. mixing) is attained instantaneously and maintained at all times. This model thus applies only to those drugs that distribute rapidly throughout the body.
3. Drugs move dynamically (پرنمرکی), in (absorption) and out (elimination) of this compartment.
4. Elimination is a first-order (monoexponential) process with first-order rate constant.
5. Rate of input (absorption) > rate of output (elimination)
6. The anatomical reference compartment is plasma and concentration of drug in plasma is represen-

Compartment Modelling

tative of drug concentration in all body tissues i.e. any change in plasma drug concentration reflects a proportional change in drug concentration throughout the body.

- However, the model does not assume that the drug concentration in plasma is equal to that in other body tissues.
- The term open indicates that the input (availability) and output (elimination) are unidirectional and that the drug can be eliminated from the body.
- One-compartment open model is generally used to describe plasma levels following administration of a single dose of a drug.



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

— One-compartment open model, i.v. bolus administration

— One-compartment open model, continuous i.v. infusion

— One-compartment open model, e.v. administration, zero-order absorption

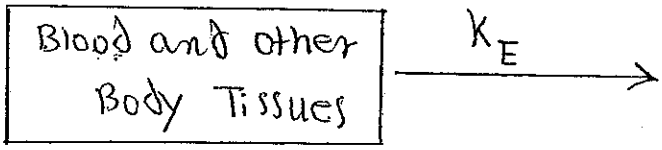
— One-compartment open model, e.v. administration, first-order absorption.

One-Compartment Open Model

Intravenous Bolus Administration

-When a drug that distributes rapidly in the body is given in the form of a rapid intravenous injection, it takes about one to three minutes for complete circulation and therefore the rate of absorption is neglected (نادیده گرفته شده) in circulations.

-The model can be depicted as follows:



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

$$\frac{dX}{dt} = \text{Rate in (availability)} - \text{Rate out (elimination)}$$

Since rate in or absorption is absent, the equation becomes:

$$\frac{dX}{dt} = -\text{Rate out}$$

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

$$\frac{dX}{dt} = -K_E X$$

Where

K_E = first-order elimination rate constant

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

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

Estimation of Pharmacokinetic Parameters – IV Bolus Administration

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

1. Elimination rate constant
2. Elimination half-life
3. Clearance

Elimination Rate Constant : Integration of equation 9.3 yields:

$$\ln X = \ln X_o - K_E t \qquad (9.4)$$

where, X_o = amount of drug at time t = zero
i.e. the initial amount of drug injected.

Equation 9.4 can also be written in the exponential form as:

$$X = X_o e^{-K_E t} \qquad (9.5)$$

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

Transforming equation 9.4 into common logarithms (log base 10), we get:

$$\log X = \log X_0 - \frac{K_E t}{2.303} \qquad (9.6)$$

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

$$X = V_d C \qquad (9.7)$$

where, V_d = proportionality constant popularly known as the *apparent volume of distribution.*

It is a pharmacokinetic parameter that permits the use of plasma drug concentration in place of amount of drug in the body. The equation 9.6 therefore becomes:

$$\log C = \log C_0 - \frac{K_E\, t}{2.303} \qquad (9.8)$$

where, C_o = plasma drug concentration immediately after i.v. injection.

Equation 9.8 is that of a straight line and indicates that a semilogarithmic plot of log C versus t will be linear with *y*-intercept log C_o. The elimination rate constant is directly obtained from the slope of the line (Fig. 9.2*b*). It has units of min^{-1}. Thus, a linear plot is easier to handle mathematically than a curve which in this case will be obtained from a plot of C versus t on regular (Cartesian) graph paper (Fig. 9.2*a*).

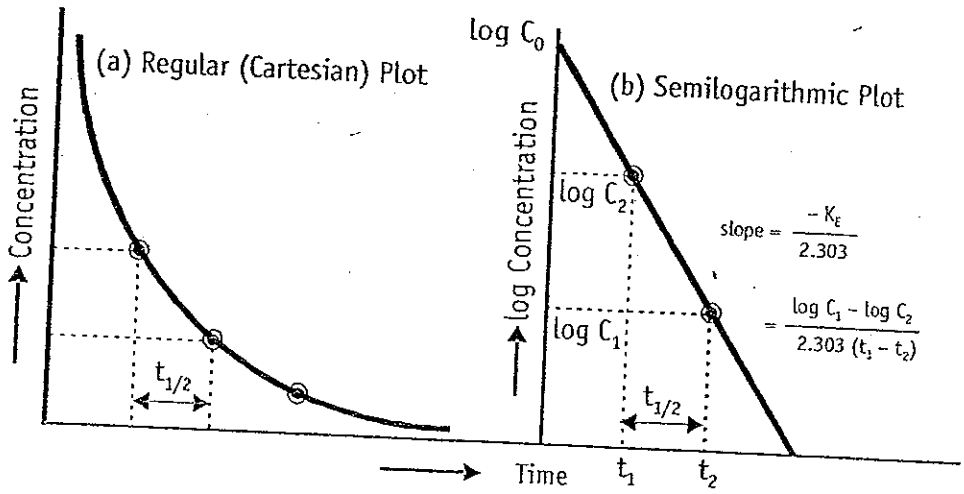


Fig. 9.2 (*a*) Cartesian plot of a drug that follows one-compartment kinetics and given by rapid i.v. injection, and (*b*) Semilogarithmic plot for the rate of elimination in a one-compartment model.

Thus, C_o, K_E (and $t_{½}$) can be readily obtained from log C versus t graph. The elimination or removal of drug from the body is the sum of urinary excretion, metabolism, biliary excretion, pulmonary excretion, and other mechanisms involved therein. Thus, K_E is an additive property of rate constants for each of these processes and better called as **overall elimination rate constant**.

$$K_E = K_e + K_m + K_b + K_l + \ldots\ldots$$

The fraction of drug eliminated by a particular route can be evaluated if the number of rate constants involved and their values are known. For example, if a drug is eliminated by urinary excretion and metabolism only, then, the fraction of drug excreted unchanged in urine F_e and fraction of drug metabolized F_m can be given as:

$$F_e = \frac{K_e}{K_E} \tag{9.10a}$$

$$F_m = \frac{K_m}{K_E} \tag{9.10b}$$

Elimination Half-Life : Also called as **biological half-life**, it is the oldest and the best known of all pharmacokinetic parameters and was once considered as the most important characteristic of a drug. *It is defined as the time taken for the amount of drug in the body as well as plasma concentration to decline by one-half or 50% its initial value.* It is expressed in hours or minutes. Half-life is related to elimination rate constant by the following equation:

$$t_{1/2} = \frac{0.693}{K_E} \tag{9.11}$$

Elimination half-life can be readily obtained from the graph of log C versus t as shown in Fig 9.2.

Today, increased physiological understanding of pharmacokinetics shows that *half-life is a secondary parameter that depends upon the primary parameters* clearance and apparent volume of distribution, according to following equation:

$$t_{1/2} = \frac{0.693\, V_d}{Cl_T} \tag{9.12}$$

Apparent Volume of Distribution : The two separate and independent pharmacokinetic characteristics of a drug are—

1. Apparent volume of distribution, and
2. Clearance.

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

Modification of equation 9.7 defines apparent volume of distribution:

$$V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma drug concentration}} = \frac{X}{C} \tag{9.13}$$

V_d is a measure of the extent of distribution of drug and is expressed in litres. The best and the simplest way of estimating V_d of a drug is administering it by rapid i.v. injection, determining the resulting plasma concentration immediately and using the following equation:

$$V_d = \frac{X_0}{C_0} = \frac{\text{i.v. bolus dose}}{C_0} \qquad (9.14)$$

Equation 9.14 can only be used for drugs that obey one-compartment kinetics. This is because the V_d can only be estimated when distribution equilibrium is achieved between drug in plasma and that in tissues and such an equilibrium is established instantaneously for a drug that follows one-compartment kinetics. A more general, more useful noncompartmental method that can be applied to many compartment models for estimating the V_d is:

For drugs given as i.v. bolus,

$$V_{d\,(area)} = \frac{X_0}{K_E\,AUC} \qquad (9.15a)$$

For drugs administered extravascularly (e.v.),

$$V_{d\,(area)} = \frac{F\,X_0}{K_E\,AUC} \qquad (9.15b)$$

where, X_o = dose administered, and F = fraction of drug absorbed into the systemic circulation. F is equal to *one* i.e. complete availability when the drug is administered intravenously.

Clearance : Difficulties arise when one applies elimination rate constant and half-life as pharmacokinetic parameters in an anatomical/ physiological context and as a measure of drug elimination mechanisms. A much more valuable alternative approach for such applications is use of clearance parameters to characterize drug disposition. *Clearance is the most important parameter in clinical drug applications and is useful in evaluating the mechanism by which a drug is eliminated by the whole organism or by a particular organ.*

Just as V_d is needed to relate plasma drug concentration with amount of drug in the body, clearance is a parameter that relates plasma drug concentration with the rate of drug elimination according to following equation:

$$\text{Clearance} = \frac{\text{Rate of elimination}}{\text{Plasma drug concentration}} \qquad (9.16)$$

$$\text{or} \qquad Cl = \frac{dX/dt}{C} \qquad (9.17)$$

Clearance *is defined as the theoretical volume of body fluid containing drug* (i.e. that fraction of apparent volume of distribution) *from which the drug is completely removed in a given period of time.* It is expressed in ml/min or litres/hour. Clearance is usually further defined as **blood clearance** (Cl_b), **plasma clearance** (Cl_p) or clearance based on unbound or free drug concentration (Cl_u), depending upon the concentration C measured for the right side of the equation 9.17.

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

Renal Clearance

$$Cl_R = \frac{\text{Rate of elimination by kidney}}{C} \qquad (9.18a)$$

Hepatic Clearance

$$Cl_H = \frac{\text{Rate of elimination by liver}}{C} \qquad (9.18b)$$

Other Organ Clearance

$$Cl_{Others} = \frac{\text{Rate of elimination by other organs}}{C} \qquad (9.18c)$$

The total body clearance, Cl_T, *also called as* **total systemic clearance**, *is an additive property of individual organ clearances.* Hence,

Total Systemic Clearance

$$Cl_T = Cl_R + Cl_H + Cl_{Others} \qquad (9.18d)$$

Because of the additivity of clearance, the relative contribution by any organ in eliminating a drug can be easily calculated. *Clearance by all organs other than kidneys is sometimes known as* **nonrenal clearance** Cl_{NR}. It is the difference between total clearance and renal clearance.

According to an earlier definition (equation 9.17),

$$Cl_T = \frac{dX/dt}{C} \qquad (9.17)$$

Substituting $dX/dt = K_E X$ from equation 9.3 in above equation, we

get:

$$Cl_T = \frac{K_E X}{C} \qquad (9.19)$$

Since $X/C = V_d$ (from equation 9.13), the equation 9.19 can be written as:

$$Cl_T = K_E V_d \qquad (9.20a)$$

Parallel equations can be written for renal and hepatic clearances as:

$$Cl_R = K_e V_d \qquad (9.20b)$$

$$Cl_H = K_m V_d \qquad (9.20c)$$

Since $K_E = 0.693/t_{½}$ (from equation 9.11), clearance can be related to half-life by the following equation:

$$\boxed{Cl_T = \frac{0.693\, V_d}{t_{1/2}}} \qquad (9.21)$$

Identical equations can be written for Cl_R and Cl_H in which cases the $t_{½}$ will be urinary excretion half-life for unchanged drug and metabolism half-life respectively. Equation 9.21 shows that as Cl_T decreases, as in renal insufficiency, $t_{½}$ of the drug increases. As the Cl_T takes into account V_d, changes in V_d as in obesity or oedematous condition will reflect changes in Cl_T.

The noncompartmental method of computing total clearance for a drug that follows one-compartment kinetics is:

For drugs given as i.v. bolus

$$\boxed{Cl_T = \frac{X_0}{AUC}} \qquad (9.22a)$$

For drugs given e.v.

$$\boxed{Cl_T = \frac{FX_0}{AUC}} \qquad (9.22b)$$

For a drug given by i.v. bolus, the renal clearance Cl_R may be estimated by determining the total amount of unchanged drug excreted in urine, X_u^∞ and AUC.

$$Cl_R = \frac{X_u^\infty}{t_{1/2}} \qquad (9.23)$$

Organ Clearance : The best way of understanding clearance is at individual organ level. Such a physiological approach is advantageous in predicting and evaluating the influence of pathology, blood flow, P-D binding, enzyme activity, etc. on drug elimination. At an organ level, the rate of elimination can be written as:

$$\text{Rate of elimination by an organ} = \text{Rate of presentation to the organ} - \text{Rate of exit from the organ} \quad (9.24)$$

$$\text{Rate of presentation (input)} = \text{Organ blood flow} \times \text{Entering concentration} = Q\,C_{in} \quad (9.25)$$

$$\text{Rate of exit (output)} = \text{Organ blood flow} \times \text{Exiting concentration} = Q\,C_{out} \quad (9.26)$$

Substitution of equations 9.25 and 9.26 in equation 9.24 yields:

$$\text{Rate of elimination} = Q\,C_{in} - Q\,C_{out}$$

$$\text{(also called as Rate of extraction)} = Q\,(C_{in} - Q\,C_{out}) \quad (9.27)$$

Division of above equation by concentration of drug that enters the organ of elimination C_{in} yields an expression for clearance of drug by the organ under consideration. Thus:

$$\frac{\text{Rate of extraction}}{C_{in}} = Cl_{organ} = \frac{Q(C_{in} - C_{out})}{C_{in}} = Q \quad (9.28)$$

where, $ER = (C_{in} - C_{out})/C_{in}$ is called as **extraction ratio.** It has no units and its value ranges from zero (no elimination) to one (complete elimination). Based on ER values, drugs can be classified into 3 groups:

1. Drugs with **high ER** (above 0.7),
2. Drugs with **intermediate ER** (between 0.7 to 0.3), and
3. Drugs with **low ER** (below 0.3).

ER *is an index of how efficiently the eliminating organ clears the blood flowing through it of drug.* For example, an ER of 0.6 tells that 60% of the blood flowing through the organ will be completely cleared of drug. The fraction of drug that *escapes removal* by the organ is expressed as:

$$F = 1 - ER \quad (9.29)$$

where, **F = systemic availability** when the eliminating organ is liver.

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

$$Cl_H = Cl_T - Cl_R \tag{9.30}$$

An equation parallel to equation 9.28 can also be written for hepatic clearance:

$$Cl_H = Q_H \, ER_H \tag{9.31}$$

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

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

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

1. Hepatic Blood Flow Rate-Limited Clearance : When ER_H is one, Cl_H approaches its maximum value i.e. hepatic blood flow. In such a situation, hepatic clearance is said to be **perfusion rate-limited** or **flow-dependent**. Alteration in hepatic blood flow significantly affects the elimination of drugs with high ER_H e.g. propranolol, lidocaine, etc. Such drugs are removed from the blood as rapidly as they are presented to the liver (high first-pass hepatic metabolism). Indocyanine green is so rapidly eliminated by the human liver that its clearance is often used as an indicator of hepatic blood flow rate. First-pass hepatic extraction is suspected when there is lack of unchanged drug in systemic circulation after oral administration. **Maximum oral availability** F for such drugs can be computed from equation 9.29. An extension of the same equation is the noncompartmental method of estimating F:

$$F = 1 - ER_H = \frac{AUC_{oral}}{AUC_{i.v.}} \tag{9.32}$$

TABLE 9.1

Influence of Blood Flow Rate and Protein Binding on Total Clearance of Drugs with High and with Low ER Values

Drugs with	*Changes in Total Clearance due to*			
	↑ Blood Flow	*↓ Blood Flow*	*↑ Binding*	*↓ Binding*
High ER (above 0.7)	↑	↓	No change	No change
Low ER (below 0.3)	No change	No change	↓	↑

where, ↑ = increase, and ↓ = decrease

On the contrary, hepatic blood flow has very little or no effect on drugs with low ER_H e.g. theophylline. For such drugs, whatever concentration of drug present in the blood perfuses liver, is more than what the liver can eliminate (low first-pass hepatic metabolism). Similar discussion can be extended to the influence of blood flow on renal clearance of drugs. This is illustrated in Table 9.1. Hepatic clearance of a drug with high ER is independent of protein binding.

2. Intrinsic Capacity Clearance : *Denoted as* Cl_{int}, *it is defined as the inherent ability of an organ to irreversibly remove a drug in the absence of any flow limitation.* It depends, in this case, upon the hepatic enzyme activity. Drugs with low ER_H and with elimination primarily by metabolism are greatly affected by changes in enzyme activity. Hepatic clearance of such drugs is said to be **capacity-limited**, e.g. theophylline. The $t_{½}$ of such drugs shows great intersubject variability. Hepatic clearance of drugs with low ER is independent of blood flow rate but sensitive to changes in protein binding.

The hepatic and renal extraction ratios of some drugs and metabolites are given in Table 9.2.

TABLE 9.2
Hepatic and Renal Extraction Ratio of Some Drugs and Metabolites

	Extraction Ratio		
	High	*Intermediate*	*Low*
Hepatic Extraction	Propranolol Lidocaine Nitroglycerine Morphine Isoprenaline	Aspirin Codeine Nortriptyline Quinidine	Diazepam Phenobarbital Phenytoin Procainamide Theophylline
Renal Extraction	Some penicillins Hippuric acid Several sulphates Several glucuronides	Some penicillins Procainamide Cimetidine	Digoxin Furosemide Atenolol Tetracycline

□ One-Compartment Open Model:

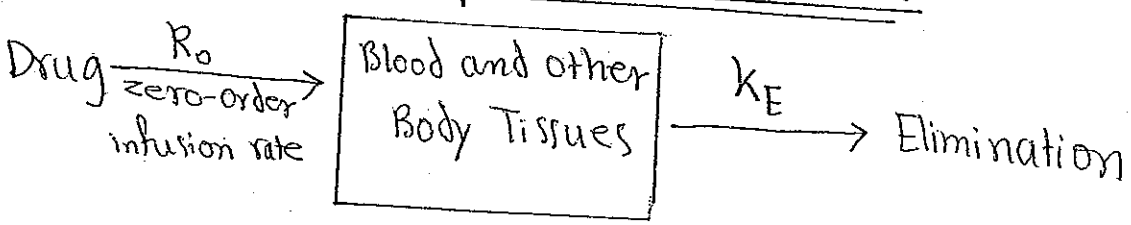
Intravenous Infusion

- Rapid i.v. injection is unsuitable when the drug has potential to precipitate toxicity or when maintenance of a stable concentration or amount of drug in the body is desired. In such a situation, the drug (eg: several antibiotics etc) is administrated at a constant rate (zero-order) by i.v. infusion.

-Advantages of zero-order infusion of drugs include:

1. Ease of control of rate of infusion to fit individual patient needs.
2. Prevents fluctuating maxima and minima (peak and valley) plasma level.
This is desired especially when the drug has a narrow therapeutic index.
3. Other drugs, electrolytes and nutrients can be conveniently administered simultaneously by the same infusion line in critically ill patients.

The model can be represented as follows:



-At any time during infusion, the rate of change in the amount of drug in the body, $\frac{dX}{dt}$ is the difference between the zero-order rate of drug infusion R_0 and first-order rate of elimination, $-K_E X$:

$$\frac{dX}{dt} = R_0 - K_E X \qquad 9.33$$

Integration and rearrangement of above equation yields:

$$X = \frac{R_0}{K_E}(1 - e^{-K_E t}) \qquad (9.34)$$

Since $X = V_d C$, the equation 9.34 can be transformed into concentration terms as follows:

$$C = \frac{R_0}{K_E V_d}(1 - e^{-K_E t}) = \frac{R_0}{Cl_T}(1 - e^{-K_E t}) \qquad (9.35)$$

At the start of constant rate infusion, the amount of drug in the body is zero and hence, there is no elimination. As time passes, the amount of drug in the body rises gradually (elimination rate less than the rate of infusion) until a point after which the rate of elimination equals the rate of infusion i.e. the concentration of drug in plasma approaches a constant value called as **steady-state, plateau** or **infusion equilibrium** (Fig. 9.3.).

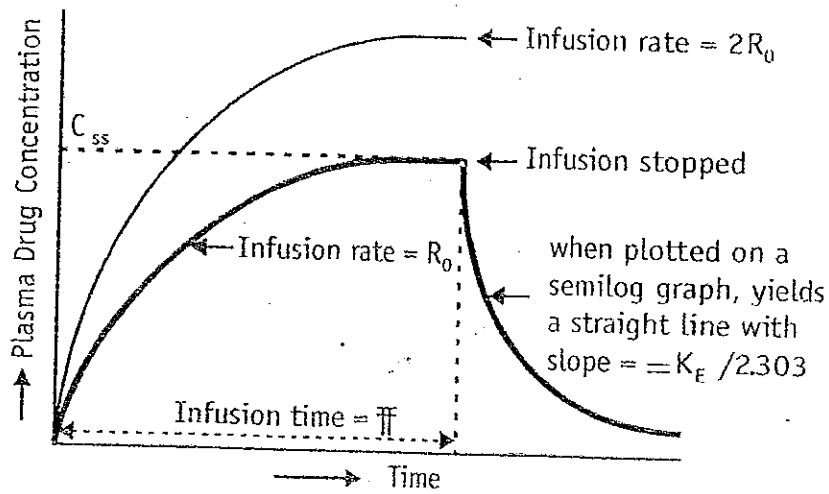


Fig. 9.3 Plasma concentration-time profile for a drug given by constant rate i.v. infusion (the two curves indicate different infusion rates R_0 and $2R_0$ for the same drug)

At steady-state, the rate of change in amount of drug in the body is zero, hence, the equation 9.33 becomes:

$$\text{Zero} = R_0 - K_E X_{ss}$$

or
$$K_E X_{ss} = R_0 \qquad (9.36)$$

Transforming to concentration terms and rearranging the equation:

$$C_{ss} = \frac{R_0}{K_E V_d} = \frac{R_0}{Cl_T} \text{ i.e. } \frac{\text{Infusion rate}}{\text{Clearance}} \qquad (9.37)$$

where, X_{ss} and C_{ss} are amount of drug in the body and concentration of drug in plasma at steady-state respectively. The value of K_E (and hence $t_{½}$) can be obtained from the slope of straight line obtained after a semilogarithmic plot (log C versus t) of the plasma concentration-time data gathered from the time when infusion is stopped (Fig. 9.3). Alternatively, K_E can be calculated from the data collected during infusion to steady-state as follows:

Substituting $R_0/Cl_T = C_{ss}$ from equation 9.37 in equation 9.35 we get:

$$C = C_{ss}(1 - e^{-K_E t}) \qquad (9.38)$$

Rearrangement yields:

$$\left[\frac{C_{ss} - C}{C_{ss}}\right] = e^{-K_E t} \qquad (9.39)$$

Transforming into log form, the equation becomes:

$$\log\left[\frac{C_{ss}-C}{C_{ss}}\right]=\frac{-K_E t}{2.303} \qquad (9.40)$$

A semilog plot of $(C_{ss} - C)/C_{ss}$ versus t results in a straight line with slope $-K_E/2.303$ (Fig. 9.4).

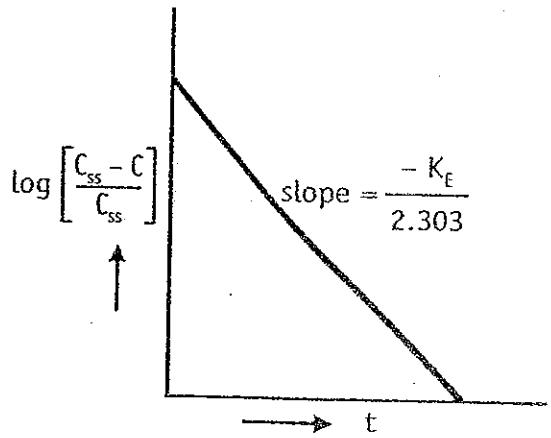


Fig. 9.4 Semilog plot to compute K_E from infusion data upto steady-state

The time to reach steady-state concentration is dependent upon the elimination half-life and not infusion rate. An increase in infusion rate will merely increase the plasma concentration attained at steady-state (Fig. 9.3). If n is the number of half-lives passed since the start of infusion $(t/t_{½})$, equation 9.38 can be written as:

$$C=C_{ss}\left[1-(1/2)^n\right] \qquad (9.41)$$

The percent of C_{ss} achieved at the end of each $t_{½}$ is the sum of C_{ss} at previous $t_{½}$ and the concentration of drug remaining after a given $t_{½}$ (Table 9.3).

TABLE 9.3

Per cent of C_{ss} Attained at the End of a given $t_{½}$

Half-life	*% Remaining*	*% Css Achieved*				
1	50	50				
2	25	50	+	25	=	75
3	12.5	75	+	12.5	=	87.5
4	6.25	87.5	+	6.25	=	93.75
5	3.125	93.75	+	3.125	=	96.875
6	1.562	96.875	+	1.562	=	98.437
7	0.781	98.437	+	0.781	=	99.218

For therapeutic purpose, more than 90% of the steady-state drug concentration in the blood is desired which is reached in 3.3 half-lives. It takes 6.6 half-lives for the concentration to reach 99% of the steady-state. Thus, the shorter the half-life (e.g. penicillin G, 30 min), sooner is the steady-state reached.

Infusion plus Loading Dose : It takes a very long time for the drugs having longer half-lives before the plateau concentration is reached (e.g. phenobarbital, 5 days). Thus, initially, such drugs have subtherapeutic concentrations. This can be overcome by administering an i.v. **loading dose** large enough to yield the desired steady-state immediately upon injection prior to starting the infusion. It should then be followed immediately by i.v. infusion at a rate enough to maintain this concentration (Fig. 9.5).

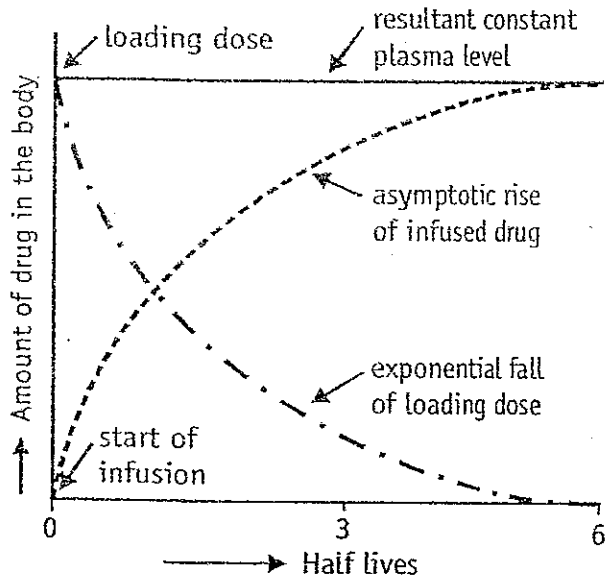


Fig. 9.5 Intravenous infusion with loading dose. As the amount of bolus dose remaining in the body falls, there is a complementary rise resulting from the infusion

Recalling once again the relationship $X = V_dC$, the equation for computing the loading dose $X_{o,L}$ can be given:

$$X_{0,L} = C_{ss}\ V_d \qquad (9.42)$$

Substitution of $C_{ss} = R_o/K_EV_d$ from equation 9.37 in above equation yields another expression for loading dose in terms of infusion rate:

$$X_{0,L} = \frac{R_0}{K_E} \qquad (9.43)$$

The equation describing the plasma concentration-time profile following simultaneous i.v. loading dose (i.v. bolus) and constant rate i.v. infusion is the sum of two equations describing each process (i.e. modified equation 9.5 and 9.35):

$$C = \frac{X_{0,L}}{V_d} e^{-K_E t} + \frac{R_0}{K_E V_d}(1 - e^{-K_E t}) \qquad (9.44)$$

If we substitute $C_{ss}V_d$ for $X_{o,L}$ (from equation 9.42) and $C_{ss}K_EV_d$ for R_o (from equation 9.37) in above equation and simplify, it reduces to $C = C_{ss}$ indicating that the concentration of drug in plasma remains constant (steady) throughout the infusion time.

Assessment of Pharmacokinetic Parameters – IV Infusion

The first-order elimination rate constant and elimination half-life can be computed from a semilog plot of post-infusion concentration-time data. Equation 9.40 can also be used for the same purpose. Apparent volume of distribution and total systemic clearance can be estimated from steady-state concentration and infusion rate (equation 9.37). These two parameters can also be computed from the total area under the curve (Fig. 9.3) till the end of infusion:

$$AUC = \frac{R_0 T}{K_E V_d} = \frac{R_0 T}{Cl_T} = C_{ss} T \qquad (9.45)$$

where, T= infusion time.

The above equation is a general expression which can be applied to several pharmacokinetic models.

Multicompartment Models

(Delayed Distribution Models)

- Are based on following assumptions:

1. Blood/plasma and the highly perfused tissues such as brain, heart, lung, liver and kidneys constitute the central compartment.

2. Other tissues with similar distribution characteristics are pooled together to constitute peripheral compartment tissues on the basis of similarity in their distribution characteristics.

3. Intravenously administered medications are introduced directly into the central compartment.
4. Irreversible drug elimination, either by hepatic biotransformation or renal excretion, takes place only from the central compartment.
5. Reversible distribution occurs between central and peripheral compartments, with a finite time required for distribution equilibrium to be attained.
6. After drug equilibration between central and the peripheral compartments, elimination of drug follows first-order kinetics.
7. All rate processes involving passage of drug in and out of individual compartment are first-order processes and plasma level-time curve is best described by sum of series of exponential terms each corresponding to first-order rate processes associated with a given compartment.
8. The peripheral compartment is usually inaccessible to direct measurement and is not a site of drug elimination or clearance.

Two-Compartment Open Model

-In such a model, the body tissues are broadly classified into 2 categories:

1. Central Compartment or Compartment 1 comprising of blood and highly perfused tissues like liver, lungs, kidneys etc. that equilibrate with the drug rapidly. Elimination usually occurs from this compartment.

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

Classification of a particular tissue, for example brain, into central or peripheral compartment depends upon the physicochemical properties of the drug. A highly lipophilic drug can cross the BBB and brain would then be included in the central compartment. In contrast, a polar drug cannot penetrate the BBB and brain in this case will be a part of peripheral compartment despite the fact that it is a highly perfused organ.

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

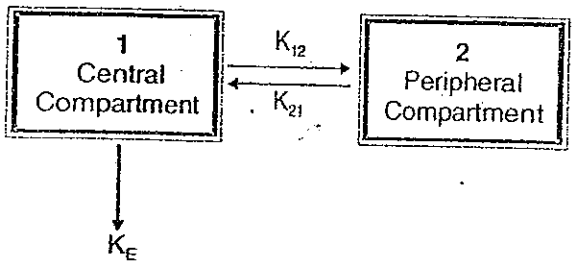
Depending upon the compartment from which the drug is eliminated, the two-compartment model can be categorized into 3 types:

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

In the absence of information, elimination is assumed to occur exclusively from central compartment.

Two-Compartment Open Model
Intravenous Bolus Administration

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



After the i.v. bolus of a drug that follows two-compartment kinetics, the decline in plasma concentration is biexponential indicating the presence of *two disposition processes viz. distribution and elimination.* These two processes are not evident to the eyes in a regular arithmetic plot but when a semilog plot of C versus t is made, they can be identified (Fig. 9.12). Initially, the concentration of drug in the central compartment *declines rapidly;* this is due to the distribution of drug from the central compartment to the peripheral compartment. The phase during which this occurs is therefore called as the **distributive phase.** After sometime, a *pseudo-distribution equilibrium* is achieved between the two compartments following which the subsequent loss of drug from the central compartment is slow and mainly due to elimination. This *second, slower rate-process is called as the* **post-distributive** or **elimination phase.** In contrast to the central compartment, the drug

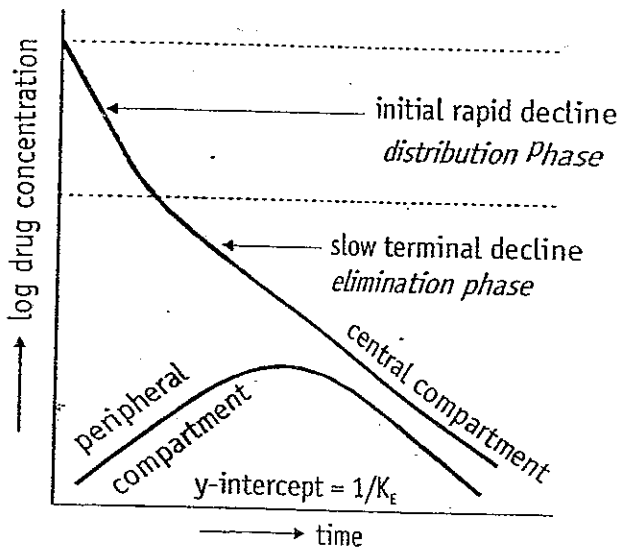


Fig. 9.12 Changes in drug concentration in the central (plasma) and the peripheral compartment after i.v. bolus of a drug that fits two-compartment model.

concentration in the peripheral compartment first increases and reaches a maximum. This corresponds with the distribution phase. Following peak, the drug concentration declines which corresponds to the post-distributive phase (Fig. 9.12).

Let K_{12} and K_{21} be the first-order distribution rate constants depicting drug transfer between the central and the peripheral compartments and let subscript c and p define central and peripheral compartment respectively. The rate of change in drug concentration in the central compartment is given by:

$$\frac{dC_c}{dt} = K_{21}C_p - K_{12}C_c - K_E C_c \tag{9.84}$$

Extending the relationship $X = V_d C$ to the above equation, we have

$$\frac{dC_c}{dt} = \frac{K_{21}X_p}{V_p} - \frac{K_{12}X_c}{V_c} - \frac{K_E X_c}{V_c} \tag{9.85}$$

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

$$\frac{dCp}{dt} = K_{12}C_c - K_{21}C_p \tag{9.86}$$

$$= \frac{K_{12}X_c}{V_c} - \frac{K_{21}X_p}{V_p} \tag{9.87}$$

Integration of equations 9.85 and 9.87 yields equations that describe the concentration of drug in the central and peripheral compartments at any given time t:

$$C_c = \frac{X_0}{V_c}\left[\left(\frac{K_{21}-\alpha}{\beta-\alpha}\right)e^{-\alpha t} + \left(\frac{K_{21}-\beta}{\alpha-\beta}\right)e^{-\beta t}\right] \tag{9.88}$$

$$C_p = \frac{X_0}{V_p}\left[\left(\frac{K_{12}}{\beta-\alpha}\right)e^{-\alpha t} + \left(\frac{K_{12}}{\alpha-\beta}\right)e^{-\beta t}\right] \tag{9.89}$$

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

بتصویر کشیدن

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

2M

$$\alpha+\beta = K_{12}+K_{21}+K_E \tag{9.90}$$

$$\alpha\beta = K_{21}K_E \tag{9.91}$$

Equation 9.88 can be written in simplified form as:

$$C_c = Ae^{-\alpha t} + Be^{-\beta t} \tag{9.92}$$

C_c = Distribution exponent + Elimination exponent

where A and B are also hybrid constants for the two exponents and can be resolved graphically by the method of residuals.

$$A = \frac{X_0}{V_c}\left[\frac{K_{21}-\alpha}{\beta-\alpha}\right] = C_0\left[\frac{K_{21}-\alpha}{\beta-\alpha}\right] \tag{9.93}$$

$$B = \frac{X_0}{V_c}\left[\frac{K_{21}-\beta}{\alpha-\beta}\right] = C_0\left[\frac{K_{21}-\beta}{\alpha-\beta}\right] \tag{9.94}$$

where C_0 = plasma drug concentration immediately after i.v. injection.

5M

Method of Residuals : The biexponential disposition curve obtained after i.v. bolus of a drug that fits two compartment model can be resolved into its individual exponents by the method of residuals. Re-writing the equation 9.92:

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$$C_c = Ae^{-\alpha t} + Be^{-\beta t} \tag{9.92}$$

As apparent from the biexponential curve given in Fig. 9.12, the initial decline due to distribution is more rapid than the terminal decline due to elimination i.e. the rate constant $\alpha >> \beta$ and hence the term $e^{-\alpha t}$ approaches zero much faster than does $e^{-\beta t}$. Thus, equation 9.92 reduces to:

$$\overleftarrow{C} = Be^{-\beta t} \tag{9.95}$$

In log form, the equation becomes:

$$\log \overleftarrow{C} = \log B - \frac{\beta t}{2.303} \tag{9.96}$$

where $\overleftarrow{C}$ = back extrapolated plasma concentration values. A semilog

plot of C versus t yields the terminal linear phase of the curve having slope $-\beta/2.303$ and when back extrapolated to time zero, yields y-intercept log B (Fig. 9.13.). The $t_{½}$ for the elimination phase can be obtained from equation $t_{½} = 0.693/\beta$.

Subtraction of extrapolated plasma concentration values of the elimination phase (equation 9.95) from the corresponding true plasma concentration values (equation 9.92) yields a series of residual concentration values C_r.

$$C_r = C - \overleftarrow{C} = Ae^{-\alpha t} \tag{9.97}$$

In log form, the equation becomes:

$$\log C_r = \log A - \frac{\alpha t}{2.303} \tag{9.98}$$

A semilog plot of C_r versus t yields a straight line with slope $-\alpha/2.303$ and y-intercept log A (Fig. 9.13).

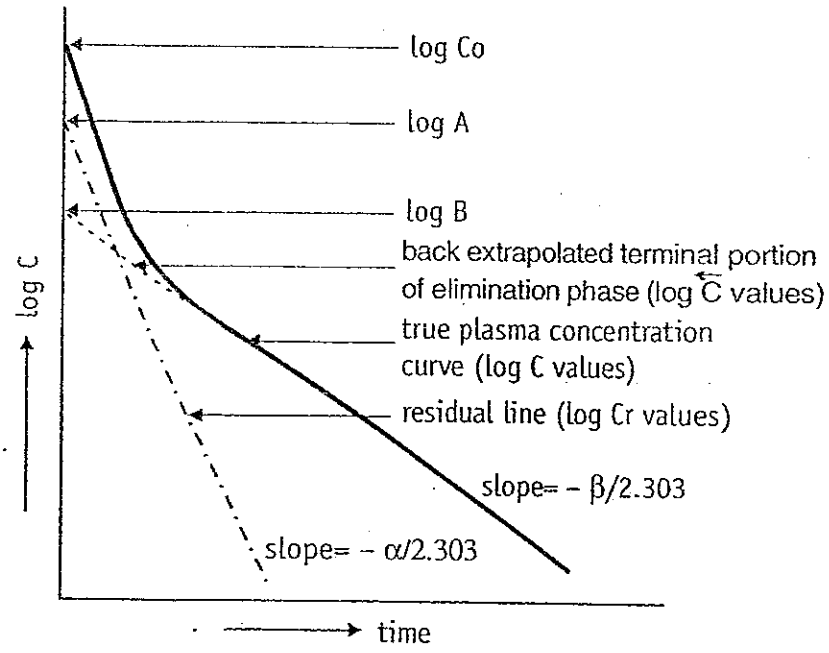


Fig. 9.13 Resolution of biexponential plasma concentration-time curve by the method of residuals for a drug that follows two-compartment kinetics on i.v. bolus administration.

Assessment of Pharmacokinetic Parameters – IV Bolus Administration

All the parameters of equation 9.92 can be resolved by the method of residuals as described above. Other parameters of the model viz. K_{12}, K_{21}, K_E, etc. can now be derived by proper substitution of these values.

$$C_0 = A + B \tag{9.99}$$

$$K_E = \frac{\alpha\beta C_0}{A\beta + B\alpha} \tag{9.100}$$

$$K_{12} = \frac{A\,B\,(\beta - \alpha)^2}{C_0\,(A\,\beta + B\,\alpha)} \tag{9.101}$$

$$K_{21} = \frac{A\beta + B\alpha}{C_0} \tag{9.102}$$

It must be noted that for two-compartment model, K_E is the rate constant for elimination of drug from the central compartment and β *is the rate constant for elimination from the entire body.* Overall elimination $t_{½}$ should therefore be calculated from β.

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

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \tag{9.103}$$

The apparent volume of central compartment V_c is given as:

$$V_d = \frac{X_0}{C_0} = \frac{X_0}{K_E\ AUC} \tag{9.104}$$

Apparent volume of peripheral compartment can be obtained from equation:

$$V_p = \frac{V_c K_{12}}{K_{21}} \tag{9.105}$$

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

$$V_{d,ss} = V_c + V_p \tag{9.106}$$

It is also given as:

$$V_{d,area} = \frac{X_0}{\beta\ AUC} \tag{9.107}$$

Total systemic clearance is given as:

$$Cl_T = \beta V_d \tag{9.108}$$

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

$$\frac{dX_u}{dt} = K_e V_c \qquad (9.109)$$

An equation identical to equation 9.92 can be derived for rate of excretion of unchanged drug in urine:

$$\frac{dX_u}{dt} = K_e A e^{-\alpha t} + K_e B e^{-\beta t} \qquad (9.110)$$

The above equation can be resolved into individual exponents by the method of residuals as described for plasma concentration-time data.

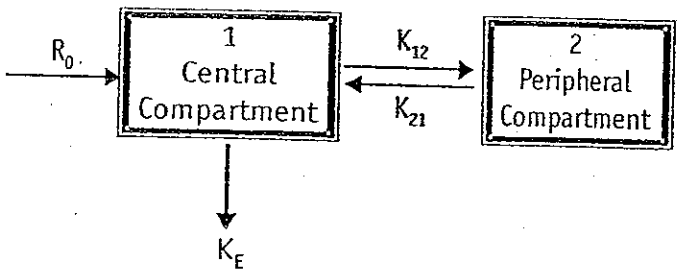
Renal clearance is given as:

$$Cl_R = K_e V_c \qquad (9.111)$$

Two-Compartment Open Model

Intravenous Infusion

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



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

$$C = \frac{R_0}{V_c K_E}\left[1 + \left(\frac{K_E - \beta}{\beta - \alpha}\right)e^{-\alpha t} + \left(\frac{K_E - \alpha}{\alpha - \beta}\right)e^{-\beta t}\right] \qquad (9.112)$$

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

$$C_{ss} = \frac{R_0}{V_c K_E} \qquad (9.113)$$

Now $V_c K_E = V_d ß$. Substituting this in equation 9.113, we get:

$$C_{ss} = \frac{R_0}{V_d \beta} = \frac{R_0}{Cl_T} \qquad (9.114)$$

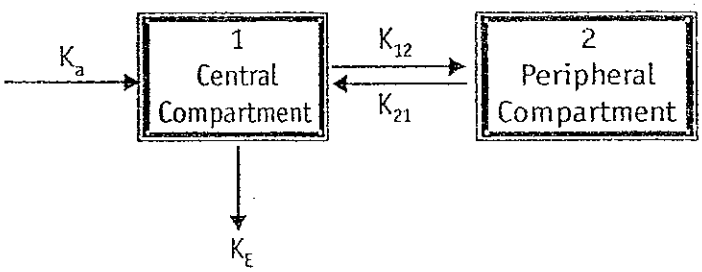
The loading dose $X_{0,L}$ to obtain C_{ss} immediately at the start of infusion can be calculated from equation:

$$X_{0,L} = C_{ss} V_c = \frac{R_0}{K_E} \qquad (9.115)$$

Two-Compartment Open Model

Extravascular Administration—First-Order Absorption

The model can be depicted as follows:



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

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

$$C = N e^{-K_a t} + L e^{-\alpha t} + M e^{-\beta t} \qquad (9.116)$$

C = Absorption exponent + Distribution exponent + Elimination exponent

where K_a, α and β have usual meanings. L, M and N are coefficients.

The 3 exponents can be resolved by stepwise application of method of residuals assuming $K_a > \alpha > \beta$ as shown in Fig. 9.14. The various pharmacokinetic parameters can then be estimated.

Besides the method of residuals, K_a can also be estimated by **Loo-Riegelman** method for a drug that follows two-compartment characteristics. This method is in contrast to the Wagner-Nelson method for determination of K_a of a drug with one-compartment characteristics. The Loo-Riegelman method requires plasma concentration-time data both after oral and i.v. administration of the drug to the same subject at

different times in order to obtain all the necessary kinetic constants. Despite its complexity, the method can be applied to drugs that distribute in any number of compartments.

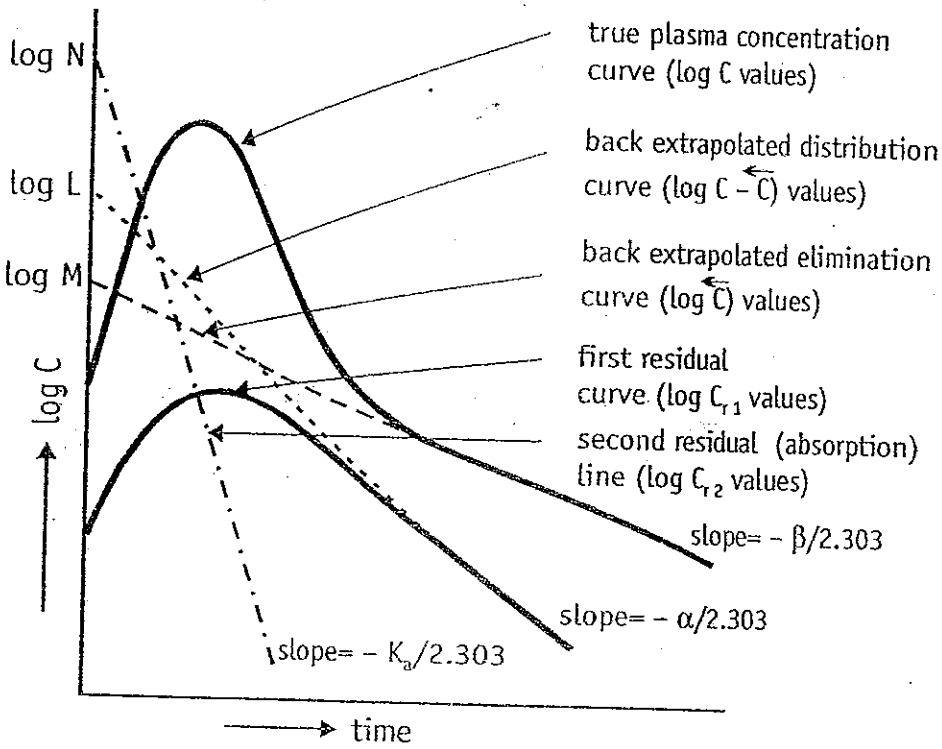


Fig. 9.14 Semilog plot of C versus t of a drug with two-compartment characteristics when administered extravascularly. The various exponents have been resolved by the method of residuals.

Nonlinear Pharmacokinetics

-In most cases, at therapeutic doses, the change in the amount of drug in the body or the change in its plasma concentration due to absorption, distribution, binding, metabolism or excretion, is proportional to its dose, whether administered as a single dose or as multiple doses.

In such situations, the rate processes are said to follow first-order or linear kinetics and all semilog plots of C versus t for different doses, when corrected for dose administered, are superimposable. This is called as principle of superposition.

-The important pharmacokinetic parameters viz. F, K_a, K_E, V_d, Cl_R and Cl_H which describe the time-course of a drug in the body remain unaffected by the dose i.e. the pharmacokinetics is dose-independent.

-In some instances, the rate process of a drug's ADME are dependent upon carrier or enzymes that are substrate-specific, have definite capacities, and susceptible to saturation at high drug concentration.

- Why are first-order processes said to follow linear kinetics? Explain 5m

In such cases, an essentially first-order kinetics transform into a mixture of first-order and zero order rate processes and the pharmacokinetic parameters change with the size of the administered dose. The pharmacokinetics of such drugs are said to be dose-dependent.

-Other terms synonymous with it are mixed-order, nonlinear and capacity-limited kinetics. Drugs exhibiting such a kinetic profile are sources of variability in pharmacological response.

-There are several tests to detect nonlinearity in pharmacokinetics: 2m

1. Determination of steady-state plasma concentration at different doses.

If the steady-state concentrations are directly proportional to the dose, then linearity in the kinetics exists. Such proportionality is not observable when there is nonlinearity.

2. Determination of some of the important pharmacokinetic parameters, such as fraction bioavailable, elimination half-life or total systemic clearance at different doses of the drug. Any change in these parameters which are usually constant, is indicative of nonlinearity.

□Causes of Nonlinearity:

-Nonlinearities can occur in drug absorption, distribution, metabolism and excretion.

□Drug Absorption:

Nonlinearity in drug absorption can arise from 3 important sources:

1. When absorption is solubility or dissolution rate-limited e.g. griseofulvin. At higher doses, a saturated solution of the drug is formed in the GIT or at any other extravascular site and the rate of absorption attains a constant value.

2. When absorption involves carrier-mediated transport systems e.g. absorption of riboflavin, ascorbic acid etc. Saturation of the transport system at higher doses of these vitamins results in nonlinearity.

3. When presystemic gut wall or hepatic metabolism attains saturation e.g. propranolol, verapamil etc. Saturation of presystemic metabolism of these

drugs at high doses leads to increased bioavailability.

-The parameters affected will be F, K_a, C_{max} and AUC. A decrease in these parameters is observed in the former two cases and an increase in the latter case.

-Other causes of nonlinearity in drug absorption are change in gastric emptying and GI blood flow and other physiological factors. Nonlinearity in drug absorption is of little consequence unless availability is drastically affected.

Drug Distribution:

Nonlinearity in distribution of drugs administered at high doses may be due to:

1. Saturation of binding sites on plasma proteins e.g. naproxen. There is a finite number of binding sites for a particular drug on plasma proteins and, theoretically, as the concentration is raised, so too is the fraction unbound.

2. Saturation of tissue binding sites e.g. thiopental. With large single bolus doses or multiple dosing, saturation

of tissue storage sites can occur.

In both cases, the free plasma drug concentration increases but V_d increases only in the former case whereas it decreases in the latter. Clearance is also altered depending upon the extraction ratio of the drug. Clearance of a drug with high ER is greatly increased due to saturation of binding sites. Unbound clearance of drugs with low ER is unaffected and one can expect an increase in pharmacological response.

Drug Metabolism

The nonlinear kinetics of most clinical importance is capacity-limited metabolism since small changes in dose administered can produce large variations in plasma concentration at steady-state. It is a major source of large intersubject variability in pharmacological response.

Two important causes of nonlinearity in metabolism are—

1. *Capacity-limited metabolism due to enzyme and/or cofactor saturation.* Typical examples include phenytoin, alcohol, theophylline, etc.
2. *Enzyme induction* e.g. carbamazepine, where a decrease in peak plasma concentration has been observed on repetitive administration over a period of time. Autoinduction characterized in this case is also dose-dependent. Thus, enzyme induction is a common cause of both dose- and time-dependent kinetics.

Saturation of enzyme results in decreased Cl_H and therefore increased C_{ss}. Reverse is true for enzyme induction. Other causes of nonlinearity in biotransformation include 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 drug that are saturable are –

1. *Active tubular secretion* e.g. penicillin G. After saturation of the carrier-system, a decrease in renal clearance occurs.
2. *Active tubular reabsorption* e.g. water-soluble vitamins and glucose. After saturation of the carrier-system, an increase in renal clearance occurs.

Other sources of nonlinearity in renal excretion include forced diuresis, changes in urine pH, nephrotoxicity and saturation of binding sites.

Biliary secretion, which is also an active process, is also subject to saturation e.g. tetracycline and indomethacin.

MICHAELIS MENTEN EQUATION

The kinetics of capacity-limited or saturable processes is best described by Michaelis-Menten equation:

$$-\frac{dC}{dt} = \frac{V_{max}\, C}{K_m + C} \qquad (10.1)$$

where, $-dC/dt$ = rate of decline of drug concentration with time,
V_{max} = theoretical maximum rate of the process, and
K_m = Michaelis constant.

Three situations can now be considered depending upon the values of K_m and C:

1. When $K_m = C$

Under this situation, the equation 10.1 reduces to:

$$-\frac{dC}{dt} = \frac{V_{max}}{2} \qquad (10.2)$$

i.e. the rate of process is equal to one-half its maximum rate (Fig. 10.1).

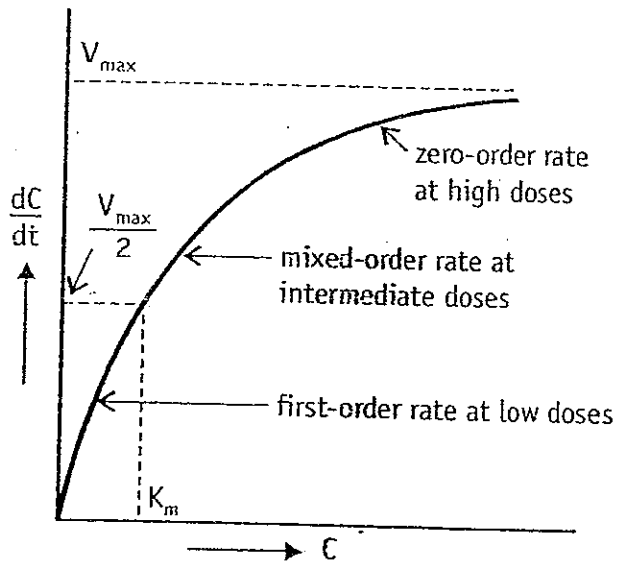


Fig. 10.1 A plot of Michaelis-Menten equation (elimination rate dC/dt versus concentration C). Initially, the rate increases linearly (first-order) with concentration, becomes mixed-order at higher concentration and then reaches maximum (V_{max}) beyond which it proceeds at a constant rate (zero-order).

2. When $K_m >> C$

Here, $K_m + C \equiv K_m$ and the equation 10.1 reduces to:

$$-\frac{dC}{dt} = \frac{V_{max}\, C}{K_m} \qquad (10.3)$$

The above equation is identical to the one that describes first-order elimination of a drug where $V_{max}/K_m = K_E$. This means that the drug concentration in the body that results from usual dosage regimens of most drugs is well below the K_m of the elimination process with certain exceptions such as phenytoin and alcohol.

3. When $K_m << C$

Under this condition, $K_m + C \equiv C$ and the equation 10.1 will become:

$$-\frac{dC}{dt} = V_{max} \qquad (10.4)$$

The above equation is identical to the one that describes 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 parameters of capacity-limited processes like metabolism, renal tubular secretion and biliary excretion can be easily defined by assuming one-compartment kinetics for the drug and that elimination involves only a single capacity-limited process.

The parameters K_m and V_{max} can be assessed from the plasma concentration-time data collected after i.v. bolus administration of a drug with nonlinear elimination characteristics.

Rewriting equation 10.1.

$$-\frac{dC}{dt} = \frac{V_{max}\, C}{K_m + C} \qquad (10.1)$$

Integration of above equation followed by conversion to log base 10 yields:

$$\log C = \log C_0 + \frac{(C_0 - C)}{2.303\, K_m} - \frac{V_{max}}{2.303\, K_m} \qquad (10.5)$$

A semilog plot of C versus t yields a curve with a terminal linear portion having slope $-V_{max}/2.303K_m$ and when back extrapolated to time zero gives *y*-intercept log $\overleftarrow{C_0}$ (*see* Fig. 10.2). The equation that describes this line is:

$$\log C = \log \overleftarrow{C_0} - \frac{V_{max}}{2.303\, K_m} \qquad (10.6)$$

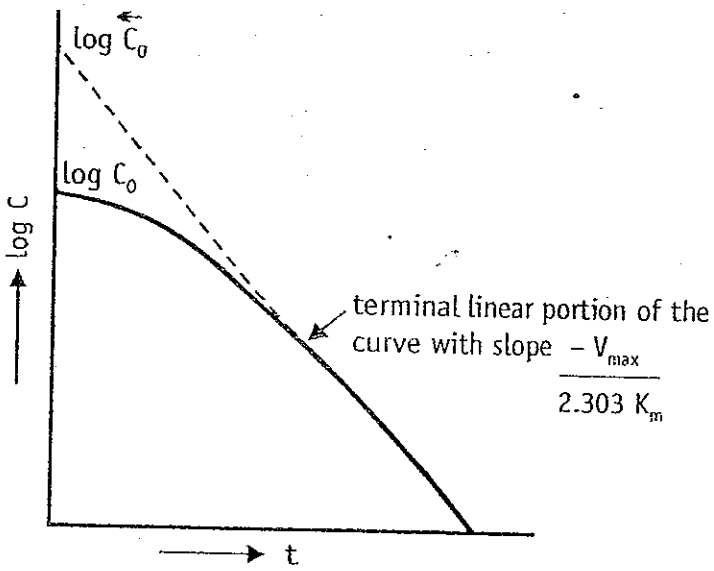


Fig. 10.2 Semilog plot of a drug given as i.v. bolus with nonlinear elimination and that fits one-compartment kinetics.

At low plasma concentrations, equations 10.5 and 10.6 are identical. Equating the two and simplifying further, we get:

$$\frac{(C_0 - C)}{2.303\ K_m} = \log \frac{\overleftarrow{C_0}}{C_0} \qquad (10.7)$$

K_m can thus be obtained from above equation. V_{max} can be computed by substituting the value of K_m in the slope value.

An alternative approach of estimating V_{max} and K_m is determining the rate of change of plasma drug concentration at different times and using the reciprocal of the equation 10.1. Thus:

$$\frac{1}{dC/dt} = \frac{K_m}{V_{max}\ C_m} + \frac{1}{V_{max}} \qquad (10.8)$$

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 the above equation yields a straight line with slope = K_m/V_{max} and y-intercept = $1/V_{max}$.

A *disadvantage* of **Lineweaver-Burke plot** is that the points are clustered. More reliable plots in which the points are uniformly scattered are **Hanes-Woolf plot** (equation 10.9) and **Woolf-Augustinsson-Hofstee plot** (equation 10.10).

$$\frac{C_m}{dC/dt} = \frac{K_m}{V_{max}} + \frac{C_m}{V_{max}} \qquad (10.9)$$

$$\frac{dC}{dt} = V_{max} - \frac{dC/dt\ K_m}{C_m} \qquad (10.10)$$

The above equations are rearrangements of equation 10.8. Equation 10.9 is used to plot $C_m/(dC/dt)$ versus C_m and equation 10.10 to plot dC/dt versus $(dC/dt)/C_m$. The parameters K_m and V_{max} can be computed 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 i.v. infusion or in a multiple dose regimen, the steady-state concentration C_{ss} is given in terms of **dosing rate** DR as:

$$DR = C_{ss}\, Cl_T \qquad (10.11)$$

where $DR = R_o$ when the drug is administered as zero-order i.v. infusion and it is equal to FX_o/τ when administered as multiple oral dosage regimen (F is fraction bioavailable, X_o is oral dose and τ is dosing interval).

At steady-state, the dosing rate equals rate of decline in plasma drug concentration and if the decline (elimination) is due to a single capacity-limited process (for e.g. metabolism), then;

$$DR = \frac{V_{max}\, C_{ss}}{K_m + C_{ss}} \qquad (10.12)$$

A plot of C_{ss} versus DR yields a typical *hockey-stick shaped curve* as shown in Fig. 10.3.

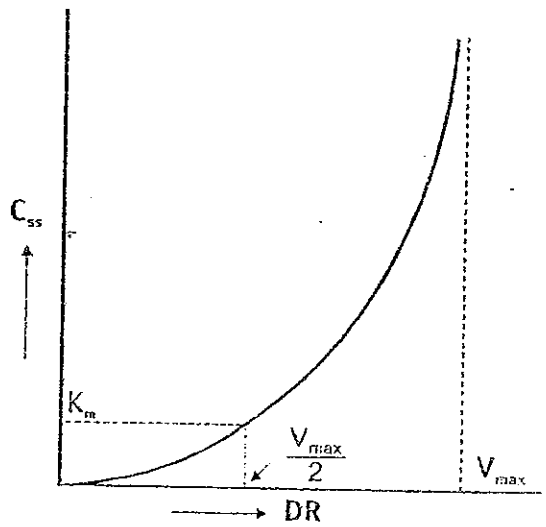


Fig. 10.3 Curve for a drug with nonlinear kinetics obtained by plotting the steady-state concentration versus dosing rates.

To define the characteristics of a curve with reasonable degree of accuracy, several measurements must be made at steady-state during dosage with different doses.

Practically, one can graphically compute K_m and V_{max} in 3 ways:

1. Lineweaver-Burke Plot/Klotz Plot

Taking reciprocal of equation 10.12, we get:

$$\frac{1}{DR} = \frac{K_m}{V_{max}\, C_{ss}} + \frac{1}{V_{max}} \qquad (10.13)$$

Equation 10.13 is identical to equation 10.8 given earlier. A plot of 1/DR versus $1/C_{ss}$ yields a straight line with slope K_m/V_{max} and *y*-intercept $1/V_{max}$ (see Fig. 10.4).

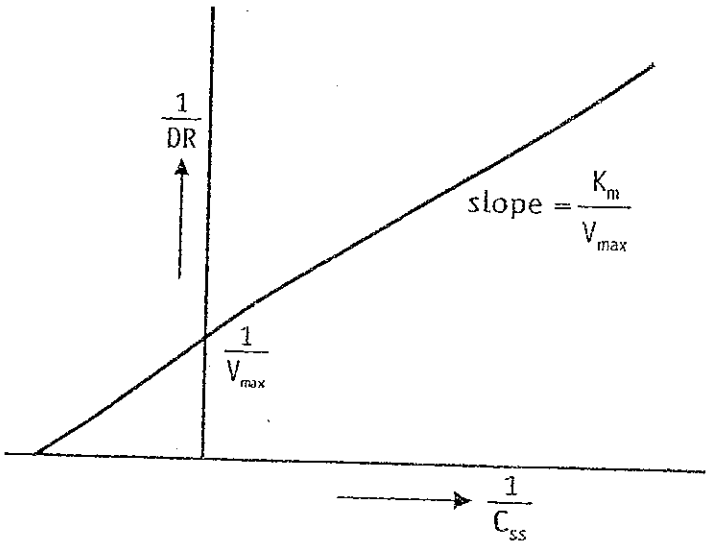


Fig. 10.4 Lineweaver-Burke/Klotz plot for estimation of K_m and V_{max} at steady-state concentration of drug.

2. Direct Linear Plot

Here, the graph is considered as shown in Fig. 10.5. 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. The points $C_{ss,1}$ and DR_1 are joined to form a line and a second line is obtained similarly by joining $C_{ss,2}$ and DR_2. The point where these two lines intersect each other is extrapolated on DR axis to obtain V_{max} and on x-axis to get K_m.

3. The third graphical method of estimating K_m and V_{max} involves rearranging equation 10.12 to yield:

$$DR = V_{max} - \frac{K_m\, DR}{C_{ss}} \qquad (10.14)$$

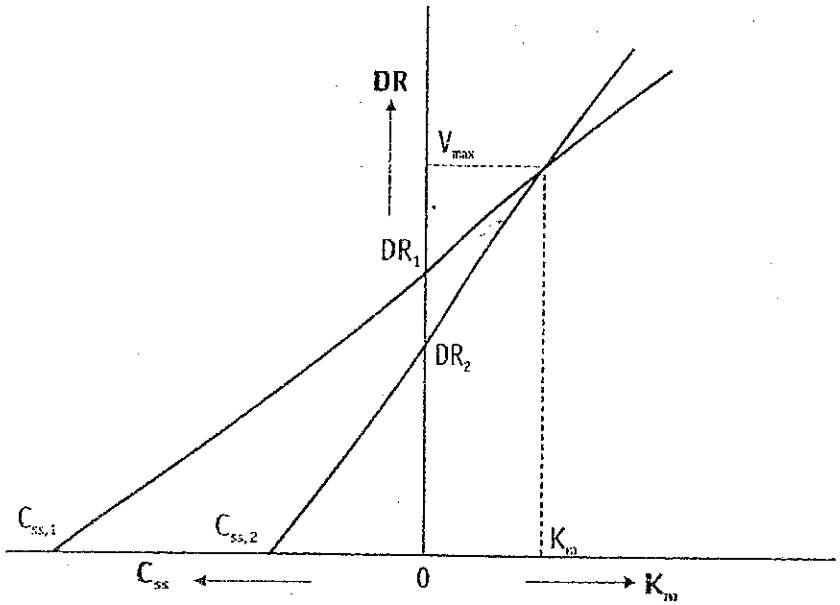


Fig. 10.5 Direct linear plot for estimation of K_m and V_{max} at steady-state concentrations of a drug given at different dosing rates.

A plot of DR versus DR/C_{ss} yields a straight line with slope $-K_m$ and *y*-intercept V_{max}.

K_m and V_{max} can also be calculated numerically by setting up simultaneous equations as shown below:

$$DR_1 = \frac{V_{max}\, C_{ss,1}}{K_m + C_{ss,1}} \tag{10.15}$$

$$DR_2 = \frac{V_{max}\, C_{ss,2}}{K_m + C_{ss,2}} \tag{10.16}$$

Combination of the above two equations yields:

$$K_m = \frac{DR_2 - DR_1}{\frac{DR_1}{C_{ss,1}} - \frac{DR_2}{C_{ss,2}}} \tag{10.17}$$

After having computed K_m, its subsequent substitution in any one of the two simultaneous equations will yield V_{max}.

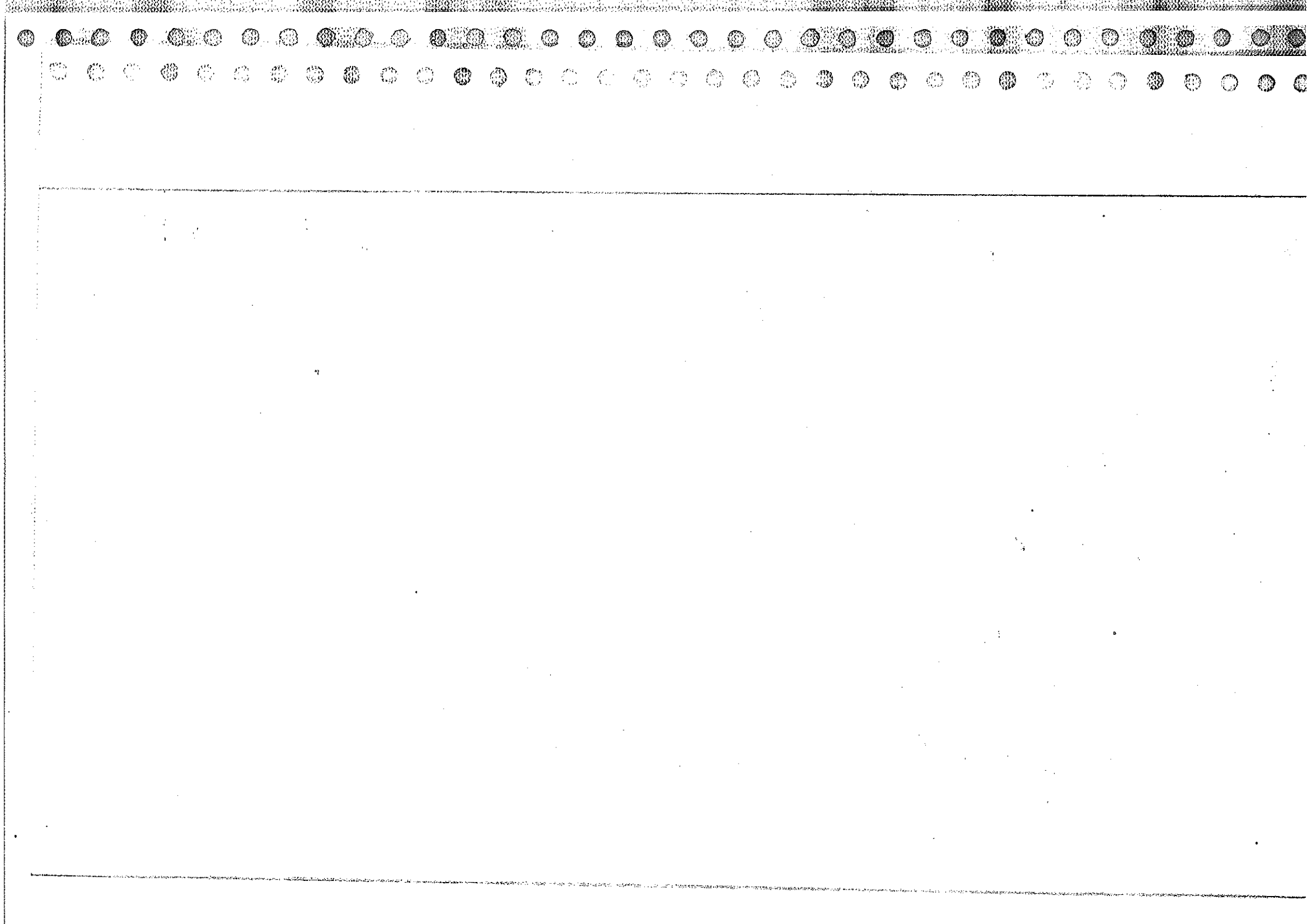
It has been observed that K_m is much less variable than V_{max}. Hence, if mean K_m for a drug is known from an earlier study, then instead of two, a single measurement of C_{ss} at any given dosing rate is sufficient to compute V_{max}.

There are several limitations of K_m and V_{max} estimated by assuming one-compartment system and a single capacity-limited process. More complex equations will result and the computed K_m and V_{max} will usually 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 multicompartment kinetics.

However, K_m and V_{max} obtained under such circumstances have little practical applications in dosage calculations.

Drugs that behave nonlinearly within the therapeutic range (for example, phenytoin shows saturable metabolism) yield less predictable results in drug therapy and possess greater potential in precipitating toxic effects.



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

- The rate or rapidity with which a drug is absorbed is an important consideration when a rapid onset of action is desired as in the treatment of acute conditions such as asthma attack, pain, etc.

- A slower absorption rate is, however, desired when the aim is to prolong the duration of action or to avoid the adverse-effects.

- If the size of the dose to be administered is same, then bioavailability of a drug from its dosage form depends upon 3 major factors:

1. Pharmaceutical factors related to physicochemical properties of the drug and characteristics of the dosage form.
2. Patient-related factors
3. Route of administration

- The influence of route of administration on drug's bioavailability is generally in the following

order with few exceptions:

Parenteral > Oral > Rectal > Topical

- Within the parenteral route, intravenous injection of a drug results in 100% bioavailability as the absorption process is bypassed.

However, for reasons of stability and convenience, most drugs are administered orally. In such cases, the dose available to the patient, called as the bioavailable dose, is often less than the administered dose.

- The amounts of drug that reaches the systemic circulation (i.e. extent of absorption) is called as systemic availability or simply availability.

- The term bioavailable fraction F, refers to the fraction of administered dose that enters the systemic circulation.

$$F = \frac{\text{Bioavailable dose}}{\text{Administered dose}}$$

Objectives of Bioavailability Studies

Bioavailability studies are important in the —

1. Primary stages of development of a suitable dosage form for a new drug entity to obtain evidence of its therapeutic utility.
2. Determination of influence of excipients, patient-related factors and possible interaction with other drugs on the efficiency of absorption.
3. Development of new formulations of the existing drugs.
4. Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage and stability on drug absorption.
5. Comparison of availability of a drug substance from different dosage forms or from the same dosage form produced by different manufacturers.

in vitro: مصنوعی

CONSIDERATIONS IN *IN-VIVO* BIOAVAILABILITY STUDY DESIGN

Bioavailability—Absolute *versus* Relative

When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration, it is called as **absolute bioavailability**. It is denoted by symbol **F**. Its determination is used to characterize a drug's inherent absorption properties from the e.v. site. Intravenous dose is selected as a standard because the drug is administered directly into the systemic circulation (100% bioavailability) and avoids absorption step. Intramuscular dose can also be taken as a standard if the drug is poorly water-soluble. An oral solution as reference standard has also been used in certain cases. However, there are several *drawbacks of using oral solution as a standard* instead of an i.v. dose — مشکل ایراد

1. Limits the pharmacokinetic treatment to one-compartment model only; one cannot apply the most applicable two-compartment kinetics to the data and all pharmacokinetic parameters cannot be assessed.
2. Differentiation between the fraction of dose unabsorbed and that metabolised is difficult.
3. If the rate of oral absorption is not sufficiently greater than the rate of elimination, the true elimination rate constant cannot be computed.

At best, when oral solution is used in conjunction with i.v. route, one can distinguish the dissolution rate limitation in drug absorption from solid dosage forms.

When the systemic availability of a drug after oral administration is compared with that of an oral standard of the same drug (such as an aqueous or non-aqueous solution or a suspension), *it is referred to as* **relative** or **comparative bioavailability**. It is denoted by symbol F_r. In contrast to absolute bioavailability, it is used to characterize absorption of a drug from its formulation. F and F_r are generally expressed in percentages.

Single Dose *versus* Multiple Dose Studies

The dose to be administered for a bioavailability study is determined from preliminary clinical experiments. *Single dose bioavailability studies* are very common. They are easy, offer less exposure to drugs and are less tedious. However, it is difficult to predict the steady-state characteristics of a drug and inter-subject variability with such studies. Moreover, sufficiently long sampling periods are necessary in order to get reliable estimates of terminal half-life, which is needed for correct calculation of the total AUC. The better alternative is thus, *multiple dose study* which offers several ***advantages*** —

1. More accurately reflects the manner in which the drug will be used clinically.
2. Allows blood levels to be measured at the same concentrations encountered therapeutically.
3. Easy to predict the peak and valley characteristics of drug since the bioavailability is determined at steady-state.
4. Requires collection of fewer blood samples.
5. The drug blood levels are higher due to cumulative effect which makes its determination possible even by the less sensitive analytical methods.
6. Can be ethically performed in patients because of the therapeutic benefit to the patient.
7. Small inter-subject variability is observed in such a study which allows use of fewer subjects.
8. Better evaluation of the performance of a controlled-release formulation is possible.
9. Nonlinearity in pharmacokinetics, if present, can be easily detected.
10. Eliminates the need for a long wash-out period between doses. Moreover, the switch-over from one formulation to the other is possible at steady state.

Limitations of multiple-dose studies include –

1. Tedious, requires more time to complete.
2. More difficult and costly to conduct (requires prolonged monitoring of subjects).
3. Poor compliance by subjects.
4. Greater exposure of subjects to the test drug, increasing the potential for adverse reactions.

In multiple dose study, one must ensure that the steady-state has been reached. For this, the drug should be administered for 5 to 6 elimination half-lives before collecting the blood samples.

Human Volunteers—Healthy Subjects *versus* Patients

Ideally, the bioavailability study should be carried out in *patients* for whom the drug is intended to be used because of apparent *advantages*—

1. The patient will be benefited from the study.
2. Reflects better the therapeutic efficacy of a drug.
3. Drug absorption pattern in disease states can be evaluated.
4. Avoids the ethical quandary of administering drugs to healthy subjects.

Patients are generally preferred in multiple dose bioavailability studies. The drawbacks of using patients as volunteers are equally large—disease, other drugs, physiological changes, etc. may modify the drug absorption pattern. Stringent study conditions such as fasting state required to be followed by the subject is also difficult. In short, establishing a standard set of conditions necessary for a bioavailability study is difficult with patients as volunteers. Such studies are therefore usually performed in young (20 to 40 years), healthy, male adult volunteers (body weight within a narrow range; ±10%), under restricted dietary and fixed-activity conditions. Female volunteers are used only when drugs such as oral contraceptives are to be tested. The number of subjects to be selected depends upon the extent of inter-subject variability but should be kept to a minimum required to obtain a reliable data. The consent of volunteers must be obtained and they must be informed about the importance of the study, conditions to be followed during the study and possible hazards if any, prior to starting the study. Medical examination should be performed in order to exclude subjects with any kind of abnormality or disease. The volunteers must be instructed to abstain from any medication for at least a week and to fast overnight prior to and for a minimum of 4 hours after dosing. The volume and type of fluid and the standard diet to be taken must also be specified. Drug washout period for a minimum of ten biological half-lives must be allowed for between any two studies in the same subject.

Measurement Of Bioavailability

Quantitatively:

I. Pharmacokinetic Methods:

- These are indirect methods.

- two major methods:

1. Plasma level-time studies
2. Urinary excretion studies

II. Pharmacodynamic Methods:

- These methods are complementary to pharmacokinetic approaches and involve direct measurement of drug effect on a (patho) physiological process as a function of time.

- Methods:

1- Acute pharmacological response
2. Therapeutic response.

Plasma Level - Time Studies:

- The method is based on the assumption that two dosage forms that exhibit superimposable plasma

level-time profiles in a group of subjects should result in identical therapeutic activity.

-With single dose study, the method requires collection of serial blood samples for a period of 2 to 3 biological half-lives after drug administration, their analysis for drug concentration and making a plot of concentration versus corresponding time of sample collection to obtain the plasma level-time profile.

-With i.v. dose, sampling should start within 5 minutes of drug administration and subsequent samples taken at 15 minute intervals.

To adequately describe the disposition phase, at least 3 sample points should be taken if the drug follows one-compartment kinetics and 5 to 6 points if it fits two-compartment model.

-For oral dose, at least 3 points should be taken on the ascending part of the curve for accurate determination of K_a.

The points for disposition or descending phase of the curve must be taken in a manner similar to that for i.v. dose.

The 3 parameters of plasma level-time studies which are considered important for determing bioavailability are:

1. C_{max}: The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.

- It is a function of both the rate and extent of absorption.

- C_{max} will increase with an increase in the dose, as well as with an increase in the absorption rate.

2. t_{max}:

The peak time that gives an indication of the rate of absorption.

It decreases as the rate of absorption increases.

3. AUC:

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:

$$F = \frac{[AUC]_{oral}\ D_{iv}}{[AUC]_{iv}\ D_{oral}}$$

$$F_r = \frac{[AUC]_{test}\ D_{std}}{[AUC]_{std}\ D_{test}}$$

D = dose administered

- With multiple dose study, the method involves drug administration for at least 5 biological half-lives with a dosing interval 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 taken at the end of previous dosing interval and 8 to 10 samples after the administration of next dose.

-The extent of bioavailability is given as:

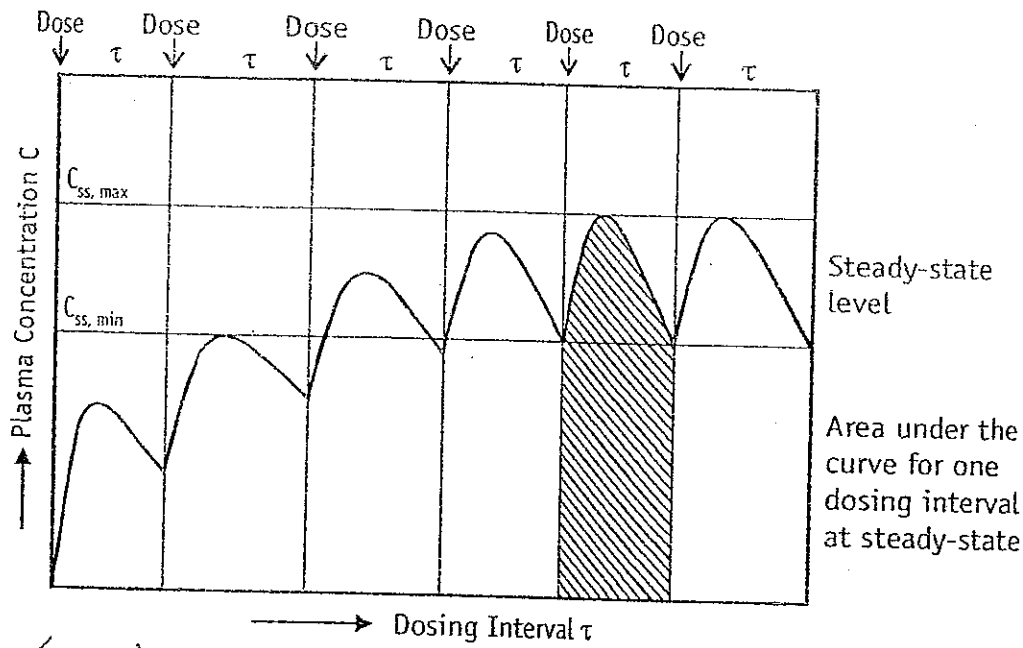
$$F_r = \frac{[AUC]_{test}\, D_{std}\, \tau_{test}}{[AUC]_{std}\, D_{test}\, \tau_{std}} \qquad (11.4)$$

where [AUC] values are area under the plasma level-time curve of one dosing interval in a multiple dosage regimen, after reaching the steady-state (Fig. 11.1) and τ is the dosing interval.

Bioavailability can also be determined from the peak plasma concentration at steady-state $C_{ss,max}$ according to following equation:

$$F_r = \frac{(C_{ss,max})_{test}\, D_{std}\, \tau_{test}}{(C_{ss,max})_{std}\, D_{test}\, \tau_{std}} \qquad (11.5)$$

The rate of absorption is not important in the multiple dosing methods.



assess:

Fig. 11.1 Determination of AUC and $C_{ss,max}$ on multiple dosing upto steady-state

Urinary Excretion Studies

This method of assessing bioavailability is *based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug*. As a rule of thumb, determination of bioavailability using urinary excretion data should be conducted only if

at least 20% of administered dose is excreted unchanged in the urine. The study is particularly useful for –

- Drugs extensively excreted unchanged in the urine – for example, certain thiazide diuretics and sulphonamides.
- Drugs that have urine as the site of action – for example, urinary antiseptics such as nitrofurantoin and hexamine.

The method has several advantages and disadvantages as discussed in *Chapter 9*. Concentration of metabolites excreted in urine is never taken into account in calculations since a drug may undergo presystemic metabolism at different stages before being absorbed. The method involves –

- Collection of urine at regular intervals for a time-span equal to 7 biological half-lives.
- Analysis of unchanged drug in the collected sample.
- Determination of the amount of drug excreted in each interval and cumulative amount excreted.

For obtaining valid results, following criteria must be met further –

- At each sample collection, total emptying of the bladder is necessary to avoid errors resulting from addition of residual amount to the next urine sample.
- Frequent sampling of urine is also essential in the beginning in order to compute correctly the rate of absorption.
- The fraction excreted unchanged in urine must remain constant.

The three major parameters examined in urinary excretion data obtained with a single dose study are:

1. $(dX_u/dt)_{max}$: The **maximum urinary excretion rate**, it is obtained from the peak of plot between rate of excretion *versus* midpoint time of urine collection period. It is analogous to the C_{max} derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in systemic circulation. Its value increases as the rate of and/or extent of absorption increases (*see* Fig. 11.2).
2. $(t_u)_{max}$: The **time for maximum excretion rate**, it is analogous to the t_{max} of plasma level data. Its value decreases as the absorption rate increases.
3. X_u^{∞} : The **cumulative amount of drug excreted in the urine**, it is related to the *AUC* of plasma level data and increases as the extent of absorption increases.

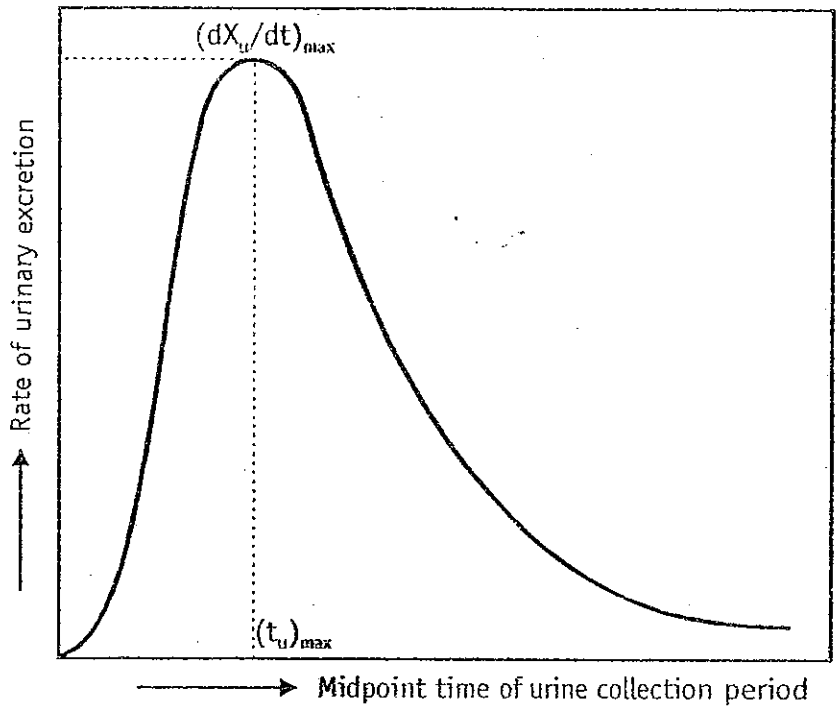


Fig. 11.2 Plot of urinary excretion rate *versus* time. Note that the curve is analogous to a typical plasma level-time profile obtained after oral administration of a single dose of drug.

The extent of bioavailability is calculated from equations given below:

$$F = \frac{\left(X_u^{\infty}\right)_{oral} D_{iv}}{\left(X_u^{\infty}\right)_{iv} D_{oral}} \tag{11.6}$$

$$F_r = \frac{\left(X_u^{\infty}\right)_{test} D_{std}}{\left(X_u^{\infty}\right)_{std} D_{test}} \tag{11.7}$$

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

$$F_r = \frac{\left(X_{u,ss}\right)_{test} D_{std}\, \tau_{test}}{\left(X_{u,ss}\right)_{std} D_{test}\, \tau_{std}} \tag{11.8}$$

where $(X_{u,ss})$ is the amount of drug excreted unchanged during a single dosing interval at steady-state.

In practice, estimation of bioavailability by urinary excretion method is subject to a high degree of variability, and is less reliable than those obtained from plasma concentration-time profiles. It is thus not recommended as a substitute for blood concentration data; rather, it should be used in conjunction with blood level data for confirmatory purposes.

Bioavailability can also be determined for a few drugs by assay of biological fluids other than plasma and urine. In case of theophylline, salivary excretion can be used whereas for cephalosporin antibiotics, appearance of drug in CSF and bile can be determined. Caution must, however, be exercised to account for salivary and enterohepatic cycling of the drugs.

Acute Pharmacological Response Method

When bioavailability measurement by pharmacokinetic methods is difficult, inaccurate or non-reproducible, an acute pharmacological effect such as a change in ECG or EEG readings, pupil diameter, etc. is related to the time course of a given drug. Bioavailability can then be determined by construction of pharmacological effect-time curve as well as dose-response graphs. The method requires measurement of responses for at least 3 biological half-lives of the drug in order to obtain a good estimate of AUC.

Disadvantages of this method include –

1. The pharmacological response tends to be more variable and accurate correlation between measured response and drug available from the formulation is difficult.
2. The observed response may be due to an active metabolite whose concentration is not proportional to the concentration of parent drug responsible for the pharmacological effect.

Therapeutic Response Method

Theoretically the most definite, this method is based on observing the clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used. However, the method has several ***drawbacks*** –

1. Quantitation of observed response is too improper to allow for reasonable assessment of relative bioavailability between two dosage forms of the same drug.
2. Bioequivalence studies are usually conducted using a crossover design in which each subject receives each of the test dosage forms, and it is assumed that the physiological status of the subject does not change significantly over the duration of the study.
3. Unless multiple-dose protocols are employed, a patient who required the drug for a disease would be able to receive only a single dose of the drug every few days or perhaps each week.

4. Many patients receive more than one drug, and the results obtained from a bioavailability study could be compromised because of a drug–drug interaction.

Because of the above considerations, the general conclusion is that most bioavailability/bioequivalence studies should be carried out in healthy subjects. However, for drugs which are not designed to be absorbed into the systemic circulation and are active at the site of administration, clinical studies in patients are the only means to determine bioequivalence. Such studies are usually conducted using a parallel, rather than a crossover design. Examples include studies of topical antifungal agents, drugs used in the treatment of acne and agents such as sucralfate used in ulcer therapy.

In Vitro Drug Dissolution Rate and Bioavailability

The physicochemical property of most drugs that has greatest influence on their absorption characteristics from the GIT is dissolution rate. The best way of assessing therapeutic efficacy of drugs with a slow dissolution rate is *in vivo* determination of bioavailability which is usually done whenever a new formulation is to be introduced into the market. However, monitoring batch-to-batch consistency through use of such *in vivo* tests is extremely costly, tedious and time consuming besides exposing the healthy subjects to hazards of drugs. It would therefore be always desirable to substitute the *in vivo* bioavailability tests with inexpensive *in vitro* methods. The simple *in vitro* disintegration test is unreliable. *The best available tool today which can at least quantitatively assure about the biological availability of a drug from its formulation is its* ***in vitro dissolution test.***

In Vitro Drug Dissolution Testing Models

For an *in vitro* test to be useful, it must predict the *in vivo* behaviour to such an extent that *in vivo* bioavailability test need not be performed. Despite attempts to standardize 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.

There are several factors that must be considered in the design of a dissolution test. They are –

1. ***Factors relating to the dissolution apparatus*** such as—the design, the size of the container (several ml to several litres), the shape of the container (round bottomed or flat), nature of agita-

tion (stirring, rotating or oscillating methods), speed of agitation, performance precision of the apparatus, etc.

2. ***Factors relating to the dissolution fluid*** such as—composition (water, 0.1N HCl, phosphate buffer, simulated gastric fluid, simulated intestinal fluid, etc.), viscosity, volume (generally larger than that needed to completely dissolve the drug under test), temperature (generally 37°C) and maintenance of sink (drug concentration in solution maintained constant at a low level) or non-sink conditions (gradual increase in the drug concentration in the dissolution medium).

3. ***Process parameters*** such as method of introduction of dosage form, sampling techniques, changing the dissolution fluid, etc.

The *ideal features* of a dissolution apparatus are:

1. Simple in design, easy to operate and usable under a variety of conditions.
2. Fabrication dimensions and positioning of all components are precisely specified and reproducible, run-to-run.
3. Provides an easy way of introducing the dosage form into the dissolution medium and once immersed, holding it in a regular and reliable fashion.
4. Permits controlled variable intensity of mild, uniform, non-turbulent liquid agitation.
5. Provides minimum mechanical abrasion to the dosage form during the test period to avoid disruption of the microenvironment surrounding the dissolving form.
6. Maintains nearly perfect sink conditions.
7. Prevents/eliminates evaporation of the dissolution medium and maintains it at a fixed temperature within a specified narrow range. Most apparatuses are thermostatically controlled at around 37°C.
8. Ease of drawing samples for automatic or manual analysis without interrupting the flow characteristics of the liquid.
9. Facilitates good inter-laboratory agreement.
10. Sensitive enough to reveal process changes and formulation differences but still yield repeatable results under identical conditions.
11. Permits evaluation of disintegrating, non-disintegrating, dense or floating tablets or capsules, and finely powdered drugs.

The dissolution apparatus has evolved gradually and considerably from a simple beaker type to a highly versatile and fully automated instrument. The devices can be classified in a number of ways. Based on the absence or presence of sink conditions, there are *two principal types of dissolution apparatuses*:

1. Closed-compartment apparatus : It is basically a limited-volume apparatus operating under non-sink conditions. The dissolution fluid is restrained to the size of the container, e.g. beaker type apparatuses such as the rotating basket and the rotating paddle apparatus.

2. Open-compartment (continuous flow-through) apparatus : It is the one in which 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 called as ***dialysis systems*** are used for very poorly aqueous soluble drugs for which maintenance of sink conditions would otherwise require large volume of dissolution fluid. Only the official or compendial methods (USP methods) will be discussed here briefly.

Rotating Basket Apparatus (Apparatus 1) : First described by Pernarowski *et al*, it is basically a closed-compartment, beaker type apparatus comprising of a cylindrical glass vessel with hemispherical bottom of one litre capacity partially immersed in a water bath to maintain the temperature at 37°C. A cylindrical basket made of 22 mesh to hold the dosage form is located centrally in the vessel at a distance of 2 cm from the bottom and rotated by a variable speed motor through a shaft (Fig. 11.3*a*). The basket should remain in motion during drawing of samples. All metal parts like basket and shaft are made of SS 316.

Rotating Paddle Apparatus (Apparatus 2) : The assembly is same as that for apparatus 1 except that the rotating basket is replaced with a paddle which acts as a stirrer (Fig. 11.3*b*). The method was first described by Levy and Hayes. The dosage form is allowed to sink to the bottom of the vessel. Sinkers are recommended to prevent floating of capsules and other floatable forms. A small, loose, wire helix may be attached to such preparations to prevent them from floating.

Reciprocating Cylinder Apparatus (Apparatus 3) : This apparatus consists of a set of cylindrical flat-bottomed glass vessels equipped with reciprocating cylinders (Fig. 11.3*c*). The apparatus is particularly used for dissolution testing of controlled-release bead-type (pellet) formulations.

Flow-Through Cell Apparatus (Apparatus 4) : The flow-through apparatus consists of a reservoir for the dissolution medium and a pump

that forces dissolution medium through the cell holding the test sample. (Fig. 11.3*d*) It may be used in either –

- Closed-mode where the fluid is recirculated and, by necessity, is of fixed volume, or
- Open-mode when there is continuous replenishment of the fluids.

The material under test (tablet, capsules, or granules) is placed in the vertically mounted dissolution cell, which permits fresh solvent to be pumped in (between 240 and 960 ml/h) from the bottom (Fig. 11.3*d*). **Advantages** of this apparatus include –

1. Ease of maintaining of sink conditions during dissolution which is often required for drugs having limited aqueous solubility.
2. Feasibility of using large volume of dissolution fluid.
3. Feasibility for automation of apparatus.

Paddle Over Disc Apparatus (Apparatus 5) : This apparatus is used for evaluation of transdermal products and consists of a sample holder or disc that holds the product. The disc is placed at the bottom of apparatus 2 (rotating paddle apparatus; *see* Fig. 11.3*e*) and the apparatus operated in the usual way.

Cylinder Apparatus (Apparatus 6) : This apparatus is also used for evaluation of transdermal products and is similar to apparatus 1 (Fig. 11.3*f*). Instead of basket, a stainless steel cylinder is used to hold the sample. The sample is mounted on an inert porous cellulosic material and adhered to the cylinder.

Reciprocating Disc Apparatus (Apparatus 7) : This apparatus is used for evaluation of transdermal products as well as non-disintegrating controlled-release oral preparations. The samples are placed on disc-shaped holders (Fig. 11.3*g*) using inert porous cellulosic support which reciprocates vertically by means of a drive inside a glass container containing dissolution medium. The test is carried out at 32°C and reciprocating frequency of 30 cycles/min.

Table 11.1 lists the various types of dissolution apparatus and their applications, and Table 11.2 summarises the dissolution methodology to be adopted for immediate-release products on the basis of BCS.

Dissolution Acceptance Criteria

On the basis of dissolution profile data, criteria for acceptance/passing of test results are based on Q values as given in Table 11.3. The value of Q *is defined as percentage of drug content dissolved in a given*

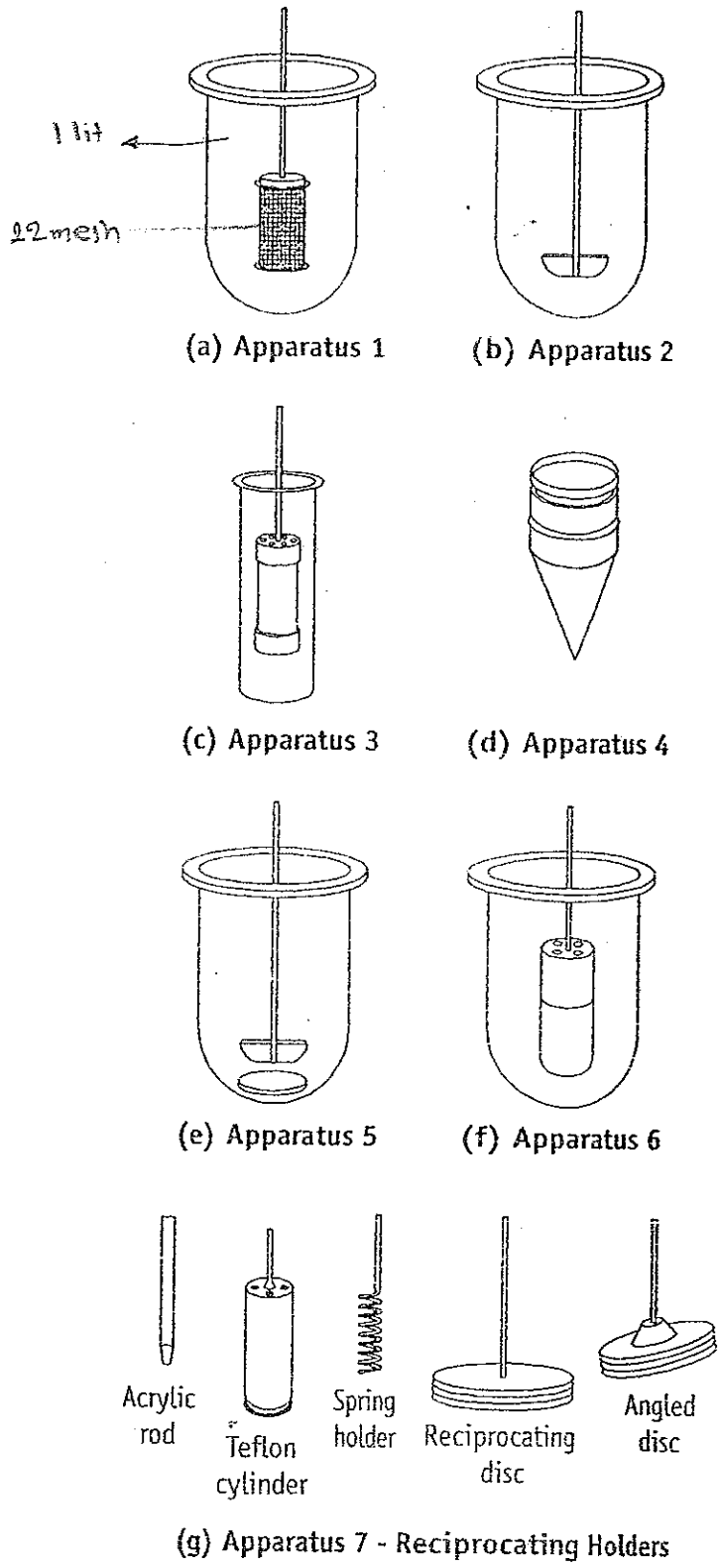


Fig. 11.3 Schematic representation of official USP dissolution apparatus - (*a*) Apparatus 1 - rotating basket apparatus, (*b*) Apparatus 2 – rotating paddle apparatus, (*c*) Apparatus 3 – reciprocating cylinder apparatus, (*d*) Apparatus 4 – flow through cell apparatus, (*e*) Apparatus 5 – paddle over disc apparatus, (*f*) Apparatus 6 – cylinder apparatus, and (*g*) Apparatus 7 – reciprocating disc apparatus

TABLE 11.1
Compendial Dissolution Apparatus Types and Their Applications

Apparatus	*Name*	*Drug Formulation Tested*
Apparatus 1	Rotating basket	Conventional tablets, chewable tablets, 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)

TABLE 11.2
Dissolution Methodology for Immediate-Release Products Based on BCS

BCS Class	*Dissolution Methodology*
I	Single point if NLT 85% Q in 15 minutes Multiple point if Q < 85% in 15 minutes
II	Multiple point
III	Same as class I
IV	Same as class II

time period. This value is generally specified in USP monograph of a given drug product. Three stages *viz.* S_1, S_2 and S_3 of dissolution testing are allowed as given in Table 11.3.

In the first stage, the dissolution test consists of testing six dosage units. If all of the dosage units are greater than or equal to Q+5%, then the dissolution test criteria are met and the test is passed. However, if this criterion is not met, six additional dosage units are tested and compared to the acceptance criteria for the twelve dosage units. To pass at the second stage, the average of twelve dosage units must be equal to

or greater than Q and no dosage unit can be less than Q-15%. If both of the above criteria are not met at the second stage, the final stage of testing is performed. Twelve additional dosage units are evaluated, providing a total of twenty four results. To pass at this final stage of testing, the average of the twenty four dosage units must be equal to or greater than Q, not more than two dosage units can be less than Q-15 %, and no dosage unit can be less than Q-25%.

TABLE 11.3
Dissolution Acceptance Criteria

Stage	*Number of Dosage Units Tested*	*Acceptance Criteria*
S_1	6	No dosage unit is less than Q+5%
S_2	6	Average of the twelve dosage units ($S_1 + S_2$) $\geq$ Q% and no dosage unit is less than Q-15%
S_3	12	Average of the twenty four dosage units ($S_1 + S_2 + S_3$) $\geq$ Q% and not more than two dosage units are less than Q-15% and no dosage unit is less than Q-25%

Objectives of Dissolution Profile Comparison

Comparison of *in vitro* dissolution profiles of test drug product and approved drug product are useful for –

- Development of bioequivalent drug products.
- Demonstrating equivalence after change in formulation of drug product.
- Biowaiver of drug product of lower dose strength in proportion to higher dose strength drug product containing same active ingredient and excipients.

Method for Comparison of Dissolution Profile

A *model-independent method* for comparison of two dissolution profiles is based on determination of ***difference factor*** f_1 and ***similarity factor*** f_2 which are calculated using following formulae –

$$f_1 = \frac{\sum_{t=1}^{n} (R_t - T_t)}{\sum_{t=1}^{n} R_t} 100 \qquad (11.9)$$

$$f_2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} 100 \right\} \qquad (11.10)$$

where n = number of dissolution time points
R_t = dissolution value of the reference drug product at time t
T_t = dissolution value of the test drug product at time t

The guidelines adopted for ***interpreting f_1*** and ***f_2 values*** are given in table 11.4.

TABLE 11.4
Comparison of Dissolution Profile

Difference factor f_1	*Similarity factor f_2*	*Inference*
0	100	Dissolution profiles are identical
≤ 15	≥ 50	Similarity or equivalence of two profiles

The evaluation of similarity between dissolution profiles is based on following ***conditions*** –

- Minimum of three dissolution time points are measured.
- Number of drug products tested for dissolution is 12 for both test and reference.
- Not more than one mean value of > 85% dissolved for each product.
- Standard deviation of mean of any product should not be more than 10% from second to last dissolution time point.

In Vitro—In Vivo Correlation (IVIVC)

A simple *in vitro* dissolution test on the drug product will be insufficient to predict its therapeutic efficacy. Convincing correlation between *in vitro* dissolution behaviour of a drug and its *in vivo* bioavailability must be experimentally demonstrated to guarantee reproducibility of biological response. ***In vitro-in vivo correlation*** *is defined as the predictive mathematical model that describes the relationship between an in-vitro property (such as the rate and extent of dissolution) of a dosage form and an in-vivo response (such as the plasma drug concentration or amount of drug absorbed).*

The main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a ***surrogate*** (*alternate*) for *in vivo* bioavailability studies in human beings.

The ***applications*** of developing such an IVIVC are —

1. To ensure batch-to-batch consistency in the physiological performance of a drug product by use of such *in vitro* values.
2. To serve as a tool in the development of a new dosage form with desired *in vivo* performance.
3. To assist in validating or setting dissolution specifications (i.e. the dissolution specifications are based on the performance of product *in vivo*).

There are ***two basic approaches*** by which a correlation between dissolution testing and bioavailability can be developed:

1. By establishing a relationship, usually linear, between the *in vitro* dissolution and the *in vivo* bioavailability parameters.
2. By using the data from previous bioavailability studies to modify the dissolution methodology in order to arrive at meaningful *in vitro-in vivo* correlation.

Though the former approach is widely used, the latter still holds substance, since to date, there is no single dissolution rate test methodology that can be applied to all drugs.

Some of the often used quantitative linear *in vitro-in vivo* correlations are –

1. **Correlations Based on the Plasma Level Data :** Here linear relationships between dissolution parameters and plasma level data are established (see Table 11.5).

TABLE 11.5
Parameters used for Correlating *In Vitro* Dissolution with Plasma Data

In vitro dissolution parameters	*In vivo plasma data parameters*
Time for specific amount of drug to dissolve (e.g., 50% of the dose)	AUC, C_{max}
Amount dissolved at a specific time point	Fraction absorbed, absorption rate constant K_a
Mean dissolution time	Mean residence time, mean dissolution time, mean absorption time
Parameter estimated after modelling the dissolution process	Concentration at time *t*, amount absorbed at time *t*

2. **Correlation Based on the Urinary Excretion Data :** Here, dissolution parameters are correlated to the amount of drug

excreted unchanged in the urine, cumulative amount of drug excreted as a function of time, etc.

3. **Correlation Based on the Pharmacological Response :** An acute pharmacological effect such as LD_{50} in animals is related to any of the dissolution parameters.

Statistical moments theory can also be used to determine the relationship such as mean dissolution time (*in vitro*) *versus* mean residence time (*in vivo*).

Though examples of good correlations are many, there are instances when positive correlation is difficult or impossible; for example, in case of corticosteroids, the systemic availability may not depend upon the dissolution characteristics of the drug. Several factors that limit such a correlation include variables pertaining to the drug such as dissolution methodology, physicochemical properties of the drug such as particle size, physiological variables like presystemic metabolism, etc.

In vitro-In vivo Correlation Levels

Three IVIVC levels have been defined and categorised in descending order of usefulness.

Level A – The highest category of correlation, it represents a point-to-point relationship between *in vitro* dissolution and the *in vivo* rate of absorption (or *in vivo* dissolution) i.e. the *in vitro* dissolution and *in vivo* absorption rate curves are superimposable and the mathematical description for both curves is the same.

Advantages of level A correlation are as follows –

1. A point-to-point correlation is developed. The *in vitro* dissolution curve serves as a surrogate for *in vivo* performance. Any change in manufacturing procedure or modification in formula can be justified without the need for additional human studies.
2. The *in vivo* dissolution serves as *in vivo* indicating quality control procedure for predicting dosage form's performance.

Level B – Utilises the principles of statistical moment analysis. The mean *in vitro* dissolution time is compared to either the mean residence time or the mean *in vivo* dissolution time. However, such a correlation is not a point-to-point correlation since there are a number of *in vivo* curves that will produce similar mean residence time values. It is for this reason that one cannot rely upon level B correlation to justify changes in manufacturing or modification in formula. Moreover, the *in vitro* data cannot be used for quality control standards.

Level C – It is a single point correlation. It relates one dissolution time point (e.g. $t_{50\%}$) to one pharmacokinetic parameter such as AUC, t_{max} or C_{max}. This level is generally useful only as a guide in formulation development or quality control owing to its obvious limitations.

Multiple Level C – It is correlation involving one or several pharmacokinetic parameters to the amount of drug dissolved at various time points.

Biopharmaceutics Classification System (BCS) and *In vitro-In vivo* Correlation (IVIVC)

The Biopharmaceutics Classification System (BCS) is a fundamental guideline for determining the conditions under which *in-vitro in-vivo* correlations are expected. Table 11.6.A indicates IVIVC expectations for immediate-release products and table 11.6.B indicates whether IVIVC is expected or possible for various drug categories when formulated as

TABLE 11.6.A
Biopharmaceutics Drug Classification System for Immediate-Release Drug Products and IVIVC Expectations

Class	*Solubility*	*Permeability*	*IVIVC expectations for immediate-release product*	*Possibility of predicting IVIVC from dissolution data*
I	High	High	IVIVC expected, if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlation.	Yes
II	Low	High	IVIVC expected, if *in vitro* dissolution rate is similar to *in vivo* dissolution rate, unless dose is very high.	Yes
III	High	Low	Absorption (permeability) is rate determining and limited or no IVIVC with dissolution.	No
IV	Low	Low	Limited or no IVIVC is expected.	No

Biopharmaceutics Drug Classification System for Extended-Release Drug Products and IVIVC Expectations

Class	*Solubility*	*Permeability*	*IVIVC*
Ia	High and site independent	High and site independent	IVIVC Level A expected
Ib	High and site independent	Dependent on site and narrow absorption window	IVIVC Level C expected
IIa	Low and site independent	High and site independent	IVIVC Level A expected
IIb	Low and site independent	Dependent on site and narrow absorption window	Little or no IVIVC
Va: Acidic	Variable	Variable	Little or no IVIVC
Vb: Basic	Variable	Variable	IVIVC Level A expected

controlled-release preparations. The importance of BCS in formulation design and drug delivery is further highlighted in Table 11.9.

BCS-Based Biowaiver to *In Vivo* Bioavailability/Bioequivalence Studies

According to BCS, *in vivo* bioavailability and bioequivalence studies need not be conducted for drug products under following circumstances -

- Rapid and similar dissolution.
- High solubility.
- High permeability.
- Wide therapeutic window.
- Excipients used in dosage form are same as those present in approved drug product.

□ Bioequivalence Studies

Need / objectives for Bioequivalence Studies

- Bioequivalence studies are conducted if there is:
  - A risk of bio-inequivalence and/or
  - A risk of pharmacotherapeutic failure or diminished clinical safety.

Important terms:

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.

Types:

Chemical Equivalence:

It indicates that two or more drug products contain the same labelled chemical substance as an active ingredient in the same amount.

Pharmaceutical Equivalence:

- This term implies that two or more drug products

are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics. They may, however, differ in containing different excipients.

□ Bioequivalence:

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

When statistically significant differences are observed in the bioavailability of two or more drug products, bio-inequivalence is indicated.

Therapeutic Equivalence:

This term indicates that two or more drug products that contain the same therapeutically active ingredient elicit identical pharmacological effects and can control the disease to the same extent.

Types of Bioequivalence Studies

- In vivo
- In vitro

In Vivo Bioequivalence Studies:

The following sequence of criteria is useful in assessing the need for in vivo studies:

1. Oral immediate-release products with systemic action
   - Indicated for serious conditions requiring assured response.
   - Narrow therapeutic margin
   - Pharmacokinetics complicated by absorption <70% or absorption window, nonlinear kinetics, presystemic elimination >70%.
   - Unfavourable physiochemical properties, e.g. low solubility, metastable modifications, instability etc.
   - Documented evidence for bioavailability problems
   - No relevant data available, unless justification by applicant that in vivo study is not necessary.

2. Non-oral immediate-release products
3. Modified-release products with systemic action.

-In vivo bioequivalence studies are conducted in the usual manner as discussed for bioavailability studies i.e. the pharmacokinetic and the pharmacodynamic methods.

In vitro Bioequivalence Studies

If none of the above criteria is applicable, comparative *in vitro* dissolution studies will suffice. *In vitro* studies, i.e. dissolution studies can be used in lieu of *in vivo* bioequivalence under certain circumstances, called as ***biowaivers*** (***exemptions***) –

1. The drug product differs only in strength of the active substance it contains, provided all the following conditions hold –
   - Pharmacokinetics are linear.
   - The qualitative composition is the same.
   - The ratio between active substance and the excipients is the same, or (in the case of small strengths) the ratio between the excipients is the same.
   - Both products are produced by the same manufacturer at the same production site.
   - A bioavailability or bioequivalence study has been performed with the original product.
   - Under the same test conditions, the *in vitro* dissolution rate is the same.

2. The drug product has been slightly reformulated or the manufacturing method has been slightly modified by the original manufacturer in ways that can convincingly be argued to be irrelevant for the bioavailability.

3. The drug product meets all of the following requirements –
   - The product is in the form of solution or solubilised form (elixir, syrup, tincture, etc.).

- The product contains active ingredient in the same concentration as the approved drug product.
- The product contains no excipients known to significantly affect absorption of the active ingredient.

4. An acceptable IVIVC and the *in vitro* dissolution rate of the new product is equivalent with that of the already approved medicinal product.

Moreover,

- The product is intended for topical administration (cream, ointment, gel, etc.) for local effect.
- The product is for oral administration but not intended to be absorbed (antacid or radio-opaque medium).
- The product is administered by inhalation as a gas or vapour.

The criteria for drug products listed above indicate that ***bioavailability and bioequivalence are self-evident.***

Bioequivalence Experimental Study Design

The various types of test designs that are usually employed in clinical trials, bioavailability and bioequivalence studies are discussed below.

1. Completely randomised designs

In a completely randomised design, all treatments (factor levels) are randomly allocated among all experimental subjects.

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

Advantages

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

Disadvantages

1. Although the design can be used for any number of treatments, it is best suited for situations in which there are relatively few treatments.
2. All subjects must be as homogeneous as possible. Any extraneous sources of variability will tend to inflate the random error

term, making it difficult to detect differences among the treatment (or factor level) mean responses.

2. Randomised block designs

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

Method of Randomisation : Subjects having similar background characteristics are formed as blocks. Then treatments are randomised within each block, just like the simple randomisation. Randomisations for different blocks are done independent of each other.

Advantages

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

Disadvantages

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

3. Repeated measures, cross-over and carry-over designs

This is essentially a randomised block design in which the same subject serves as a block. The same subject is utilized for each of the treatments under study. Since we take repeated measures on each subject we get the design name "repeated measures design". The study may involve several treatments or a single treatment evaluated at different points of time. *The administration of two or more treatments one after the other in a specified or random order to the same group of patients is called a* ***crossover design*** *or* ***change-over design.*** The drawback of crossover studies is the potential for distortion due to carry-

over, that is, residual effects from preceding treatments. To prevent ***carry-over effects***, one must always allow for a wash-out period during which most of the drug is eliminated from the body – generally about 10 elimination half-lives.

Example **:** clinical trials to monitor safety and side-effects.

Method of Randomisation **:** Complete randomisation is used to randomise the order of treatments for each subject. Randomisations for different subjects are independent of each other.

Advantages

1. They provide good precision for comparing treatments because all sources of variability between subjects are excluded from the experimental error.
2. It is economic on subjects. This is particularly important when only a few subjects can be utilized for the experiments.
3. When the interest is in the effects of a treatment over time, it is usually desirable to observe the same subject at different points of time rather than observing different subjects at the specified points of time.

Disadvantages

1. There may be an order-effect, which is connected with the position in the treatment order.
2. There may be a carry-over effect, which is connected with the preceding treatment or treatments.

4. Latin square designs

Completely randomised design, randomised block design and repeated measures design are experiments where the person/subject/volunteer remains on the treatment from the start of the experiment until the end and thus are called as ***continuous trial***. In a Latin square, however, each subject receives each treatment during the course of the experiment. *A Latin square design is a two-factor design (subjects and treatments are the two factors) with one observation in each cell.* Such a design is useful compared to the earlier ones when three or more treatments are to be compared and carry-over effects are balanced. In a Latin square design, ***rows represent subjects***, and ***columns represent treatments***. A $r \times r$ Latin square design is a square with r rows and r columns such that each of the r^2 cells contain one and only one of the r letters representing the treatments, and each letter appears once and only once in every row and every column. *A Latin square is called* ***standard*** *if the first row and the first column consist of the r letters in alphabetical order.*

Randomised, balanced, cross-over Latin square designs are commonly used for bioequivalence studies.

Advantages

1. It minimizes the inter-subject variability in plasma drug levels.
2. Minimizes the carry-over effects which could occur when a given dosage form influences the bioavailability of a subsequently administered product (intra-subject variability).
3. Minimizes the variations due to time effect.
4. Treatment effects can be studied from a small-scale experiment. This is particularly helpful in preliminary or pilot studies.
5. Makes it possible to focus more on the formulation variables which is the key to success for any bioequivalence study.

Disadvantages

1. The use of Latin square design will lead to a very small number of degrees of freedom for experimental error when only a few treatments are studied. On the other hand, when many treatments are studied, the degrees of freedom for experimental error maybe larger than necessary.
2. The randomisation required is somewhat more complex than that for earlier designs considered.
3. The study takes a long time since an appropriate washout period between two administrations is essential which may be very long if the drug has a long $t_{1/2}$.
4. When the number of formulations to be tested is more, the study becomes more difficult and subject dropout rates are also high.

TABLE 11.7

Latin Square Cross-over Design for 6 (or 12) Subjects to Compare Three Different Formulations, A, B and C

Subject number	*Study period I*	*Washout period*	*Study period II*	*Washout period*	*Study period III*
1, 7	A		B		C
2, 8	B		C		A
3, 9	C		A		B
4, 10	A		C		B
5, 11	C		B		A
6, 12	B		A		C

This can be overcome by use of a balanced incomplete block design in which a subject receives no more than 2 formulations.

An example of a typical Latin square design is given in Table 11.7.

Bioequivalence Study Protocol

The elements of *in vivo* bioequivalence study protocol are listed in table 11.8.

TABLE 11.8

Elements of Bioequivalence Study Protocol

1. Title
   a. Principal investigator
   b. Project number and date
2. Study objective
3. Study design
   a. Design
   b. Drug Products
      i. Test product(s)
      ii. Reference product
   c. Dosage regimen
   d. Sample collection schedule
   e. Housing
   f. Fasting/meals schedule
   g. Analytical methods
4. Study population
   a. Subjects
   b. Subject selection
      i. Medical history
      ii. Physical examination
      iii. Laboratory tests
   c. Inclusion/exclusion criteria
      i. Inclusion criteria
      ii. Exclusion criteria
   d. Restrictions/prohibitions
5. Clinical procedures
   a. Dosage and drug administration
   b. Biological sampling schedule
   c. Activity of subjects
6. Ethical considerations
   a. Basic principles
   b. Institutional review board
   c. Informed consent
   d. Indications for subject withdrawal
   e. Adverse reactions and emergency procedures
7. Facilities
8. Data analysis
   a. Analytical validation procedure
   b. Statistical treatment of data
9. Drug accountability
10. Appendix

The *in vivo* bioequivalence study requires determination of relative bioavailability after administration of a single dose of test and reference formulations by the same route, in equal doses, but at different times. The reference product is generally a previously approved product, usually the innovator's product or some suitable reference standard. The study is performed in fasting, young, healthy, adult male volunteers to assure homogeneity in the population and to spare the patients, elderly or pregnant women from rigors of such a clinical investigation. Homogeneity in the study population permits focus on formulation factors.

As for bioavailability studies, either plasma level or urinary excretion studies may be performed to assess bioequivalence between drug products. *In vitro-in vivo* correlation can also be established for the formulations.

It is always easier to establish bioequivalence between existing drug products than determination of pharmacokinetics of a new drug or bioavailability of a new dosage form since —

1. The human volunteers used for the study of both products are same and all pharmacokinetic parameters can be assumed to be same for both drug formulations and there is no need to investigate nonlinearity.
2. The study protocol for all subjects is uniform, the efficiency of drug absorption from both formulations can be considered as same and thus differences in absorption pattern can be ascribed to differences in drug release from the two dosage form.

Statistical Interpretation of Bioequivalence Data

After the data has been collected, statistical methods must be applied to determine the level of significance of any observed difference in the rate and/or extent of absorption in order to establish bioequivalence between two or more drug products. The commonly adopted approaches to determine statistical differences are —

1. **Analysis of variance** (ANOVA) is a statistical procedure used to test the data for differences within and between treatment and control groups. A statistical difference between the pharmacokinetic parameters obtained from two or more drug products is considered statistically significant if there is a probability of less than 1 in 20 or 0.05 ($p \leq 0.05$). The probability p is used to indicate the level of statistical significance. If $p \leq 0.05$, the differences between the two drug products are not considered statistically significant.
2. **Confidence interval approach** – Also called as ***two one-sided test procedure***, it is used to demonstrate if the bioavailability from the test product is too low or high in comparison to the reference product. The 90% confidence limits are estimated for the sample means based on ***Student's t distribution*** of data. A 90% confidence interval about the ratio of means of the two drug products must be within ± 20% for bioavailability parameters such as AUC or C_{max} i.e. the difference between the

bioavailabilities of the test product should not be greater than ± 20% of the average of reference product (between 80 and 120%). When log transformed data are used, the 90% confidence interval is set at 80-125%. These confidence limits are also termed as ***bioequivalence interval.***

METHODS FOR ENHANCEMENT OF BIOAVAILABILITY

As far as the definition of bioavailability is concerned, a drug with poor bioavailability is the one with —

- ***Poor aqueous solubility*** and/or ***slow dissolution rate*** in biological fluids.
- ***Poor permeability*** through the biomembrane owing to inadequate partition coefficient or lipophilicity or large molecular size such as that of protein or peptide drugs like insulin.

Both solubility as well as permeability of a drug depends upon its physicochemical characteristics as discussed in *Chapter 2.*

Based on the intestinal permeability and solubility of drugs, Amidon *et al* developed ***Biopharmaceutics Classification System*** (BCS) which classifies the drugs into one of the 4 groups as shown in the Table 11.9. The table also shows the approaches employed to overcome formulation challenges in each class of drugs.

Class I drugs (*high solubility/high permeability*) are well absorbed orally since they have neither solubility nor permeability limitation.

Class II drugs (*low solubility/high permeability*) show variable absorption owing to solubility limitation.

Class III drugs (*high solubility/low permeability*) also show variable absorption owing to permeability limitation.

Class IV drugs (*low solubility/low permeability*) are poorly absorbed orally owing to both solubility and permeability limitations.

Class V drugs – are the ones that do not come under the purview of BCS classification but includes drugs whose absorption is limited owing to their poor stability in GI milieu —

- Gastric instability (omeprazole).
- Complexation in GI lumen.
- First pass metabolism – by intestinal enzymes (peptide drugs), hepatic enzymes, microbial enzymes, etc.

TABLE 11.9

The Biopharmaceutics Classification System for Drugs

Class	*Solubility*	*Permeability*	*Absorption Pattern*	*Examples*	*Challenges in Drug Delivery*
I	High	High	Well absorbed	Diltiazem Propranolol Metoprolol	No major challenges for immediate release forms but CR forms need to limit drug release or dissolution since absorption of released drug is rapid.
II	Low	High	Variable	Nifedipine Carbamazepine Naproxen	Formulations are designed to overcome solubility or dissolution problems by various means (see later sections of this chapter).
III	High	Low	Variable	Insulin Metformin Cimetidine	Approaches are employed to enhance permeability (see later sections of this chapter).
IV	Low	Low	Poorly absorbed	Taxol Chlorthiazide Furosemide	Combination of strategies used for Class II and Class III drugs are employed to improve both dissolution and permeability.

Class V drugs : are those that are metabolically or chemically unstable thus limiting their bioavailability. The various approaches to overcome these problems are aimed at enhancing their stability by use of methods such as –

- § Prodrug design.
- § Enteric coating (protection from stomach acid).
- § Enzyme inhibition or lymphatic delivery (to prevent presystemic metabolism).
- § Lipid technologies.

The BCS classification of a drug depends upon its three key parameters that control absorption – solubility, dissolution rate and permeability that correlate with three respective dimensionless parameters – dose number, dissolution number and absorption number (see Table 11.10 and Fig. 11.4).

TABLE 11.10

Drug Properties that Determine BCS Classification

Drug property influencing absorption	*Corresponding dimensionless parameter*	*Significance*
Solubility : A *drug with high solubility* is the one whose largest dosage strength is soluble in 250 ml or less of water over a pH range of 1-8.	***Dose number*** : It is the mass of drug divided by an uptake volume of 250 ml and the drug's solubility.	Ideally, dose ratio should be below 1 if full dissolution is to be possible in principle. Obviously, higher doses will raise the ratio and absorption less likely.
Dissolution rate : A *drug product with rapid dissolution* is the one when ≥ 85% of the labelled amount of drug substance dissolves within 30 minutes using USP apparatus 1 or 2 in a volume of ≤ 900 ml buffer solutions.	***Dissolution number*** : It is the ratio of mean residence time to mean dissolution time.	Ideally, dissolution number should exceed 1. In the case of solid dosage forms, a combination of inadequate solubility or diffusivity, or excessive particle size or density can increase the time needed for full dissolution and reduce this ratio.
Permeability : A *drug with high permeability* is the one having extent of absorption greater than 90% of the administered dose given that the drug is stable in the gastrointestinal environment	*Absorption number* : It is the ratio of the mean residence time of drug in the GIT to the absorption time.	Ideally, absorption number should exceed 1. Longer absorption times resulting from lower permeability will reduce this ratio.

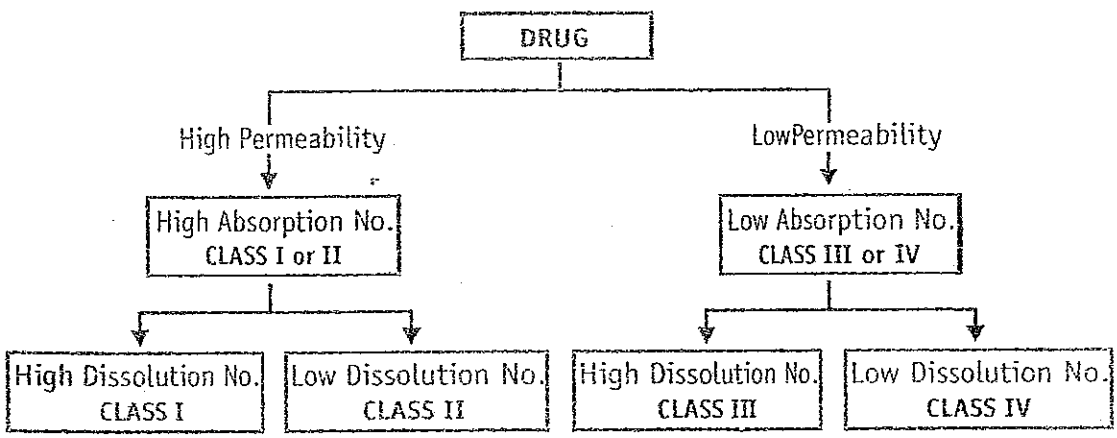


Fig. 11.4 Classification of drugs on BCS basis

Solubility Determination – Methods for determining drug solubility are –

- pH-solubility profile of test drug in aqueous media within a pH range of 1 to 7.5.
- Shake-flask or titration method.

Permeability Determination – The methods are further classified as–

Determination of extent of absorption in humans:

- Mass-balance pharmacokinetic studies.
- Absolute bioavailability studies.

Intestinal permeability methods:

- *In vivo intestinal perfusion studies in humans.*
- *In vivo or in situ intestinal perfusion studies in animals.*
- *In vitro permeation experiments with excised human or animal intestinal tissue.*
- *In vitro permeation experiments across epithelial cell monolayers.*

Dissolution Determination – Methods for determining drug product dissolution are –

- USP apparatus 1 (basket) at 100 rpm or USP apparatus 2 (paddle) at 50 rpm.
- Dissolution media (900 ml) : 0.1 N HCl or simulated gastric fluid, pH 4.5 buffer, and pH 6.8 buffer or simulated intestinal fluid.
- Compare dissolution profiles of test and reference products using a similarity factor (f_2).

Besides identifying the challenges in formulation design, the BCS is designed to guide decisions with respect to *in vitro* and *in vivo* correlations (IVIVC).

The three ***conceptual approaches in overcoming the bioavailability problems*** of drugs are:

1. The Pharmaceutical Approach which involves modification of formulation, manufacturing process or the physicochemical properties of the drug without changing the chemical structure.

2. The Pharmacokinetic Approach in which the pharmacokinetics of a drug is altered by modifying its chemical structure. This approach is further divided into two categories –

- Development of new chemical entity (NCE) with desirable features.
- Prodrug design.

3. The Biological Approach whereby the route of drug administration may be changed such as changing from oral to parenteral route.

The second approach of chemical structure modification has a number of drawbacks very expensive and time consuming, requires repetition of clinical studies and a long time for regulatory approval. Moreover, the new chemical entity may suffer from another pharmacokinetic disorder or bear the risk of precipitating adverse-effects. Only the pharmaceutical approach will be dealt herewith.

The ***pharmaceutical attempts***, whether optimising the formulation, manufacturing process or physicochemical properties of the drug, are mainly aimed at *altering the biopharmaceutic properties* of drug in one of the several ways –

A. ***Enhancement of drug solubility or dissolution rate***, as it is the major rate-limiting step in the absorption of most drugs. This approach applies to class II drugs according to BCS.

B. ***Enhancement of drug permeability***. This approach applies to class III drugs according to BCS.

C. ***Enhancement of drug stability***. This approach applies to class V drugs according to BCS.

D. ***Enhancement of gastrointestinal retention***. This approach can apply to class II, III or V drugs.

(A) BIOAVAILABILITY ENHANCEMENT THROUGH ENHANCEMENT OF DRUG SOLUBILITY OR DISSOLUTION RATE

There are several ways by which drug solubility or the dissolution rate can be enhanced. Some of the widely used methods are discussed briefly.

1. Micronization : The process involves reducing the size of the solid drug particles to 1 to 10 microns commonly by spray drying or by use of air attrition methods (fluid energy or jet mill). The process is also called as ***micro-milling***. Examples of drugs whose bioavailability have been increased by micronization include griseofulvin and several steroidal and sulpha drugs.

2. Nanonisation : It's a process whereby the drug powder is converted to nanocrystals of sizes 200-600 nm, e.g. amphotericin B. The main production technologies currently in use to produce drug nanocrystals yield as a product a dispersion of drug nanocrystals in a liquid, typically water (called ***nanosuspension***). There are three basic technologies currently in use to prepare nanoparticles:

i. Pearl milling

ii. Homogenisation in water (wet milling as in a colloid mill)

iii. Homogenisation in non-aqueous media or in water with water-miscible liquids.

3. Supercritical Fluid Recrystallization : Another novel nanosizing and solubilisation technology whose application has increased in recent years is particle size reduction *via* supercritical fluid (SCF) processes. Supercritical fluids (e.g. carbon dioxide) are fluids whose temperature and pressure are greater than its critical temperature (Tc) and critical pressure (Tp), allowing it to assume the properties of both a liquid and a gas. At near-critical temperatures, SCFs are highly compressible, allowing moderate changes in pressure to greatly alter the density and mass transport characteristics of a fluid that largely determine its solvent power. Once the drug particles are solubilised within SCF, they may be recrystallised at greatly reduced particle sizes.

4. Spray Freezing into Liquid (SFL) : This technique involves atomizing an aqueous, organic, aqueous-organic cosolvent solution, aqueous-organic emulsion or suspension containing a drug and pharmaceutical excipients directly into a compressed gas (i.e. CO_2, helium, propane, ethane), or the cryogenic liquids (i.e. nitrogen, argon or hydrofluoroethers). The frozen particles are then lyophilized to obtain dry and free-flowing micronized powders Use of acetonitrile as the solvent increases drug loading and decreases the drying time for lyophilization. The dissolution rate is remarkably enhanced from the SFL powder containing amorphous nanostructured aggregates with high surface area and excellent wettability.

5. Evaporative Precipitation into Aqueous Solution (EPAS) : The EPAS process utilizes rapid phase separation to nucleate and grow nanoparticles and microparticles of lipophilic drugs. The drug is first dissolved in a low boiling point organic solvent. This solution is pumped through a tube where it is heated under pressure to a temperature above the solvent's boiling point and then sprayed through a fine atomizing nozzle into a heated aqueous solution. Surfactants are added to the organic solution and the aqueous solution to optimize particle formation

and stabilization. In EPAS, the surfactant migrates to the drug-water interface during particle formation, and the hydrophilic segment is oriented towards the aqueous continuous phase. The hydrophilic stabilizer on the surface inhibits crystallization of the growing particles and therefore facilitates dissolution rates.

6. Use of Surfactants : Surfactants are very useful as absorption enhancers and enhance both dissolution rate as well as permeability of drug. They enhance dissolution rate primarily by promoting wetting and penetration of dissolution fluid into the solid drug particles. They are generally used in concentration below their critical micelle concentration (CMC) values since above CMC, the drug entrapped in the micelle structure fails to partition in the dissolution fluid. Nonionic surfactants like polysorbates are widely used. Examples of drugs whose bioavailability have been increased by use of surfactants in the formulation include steroids like spironolactone.

7. Use of Salt Forms : Salts have improved solubility and dissolution characteristics in comparison to the original drug. It is generally accepted that a minimum differenee of 3 units between the pKa value of the group and that of its counterion is required to form stable salts. Alkali metal salts of acidic drugs like penicillins and strong acid salts of basic drugs like atropine are more water-soluble than the parent drug. Factors that influence salt selection are physical and chemical properties of the salt, safety of counterion, therapeutic indications and route of administration.

Salt formation does have its ***limitations*** –

- It is not feasible to form salts of neutral compounds.
- It may be difficult to form salts of very weak bases or acids.
- The salt may be hygroscopic, exhibit polymorphism or has poor processing characteristics.
- Conversion of salt to free acid or base form of the drug on surface of solid dosage form that prevents or retards drug release.
- Precipitation of unionised drug in the GI milieu that has poor solubility.

8. Use of Precipitation Inhibitors : A significant increase in free drug concentration above equilibrium solubility results in supersaturation, which can lead to drug precipitation or crystallization. This can be prevented by use of inert polymers such HPMC, PVP, PVA, PEG, etc. which act by one or more of the following mechanisms –

- Increase the viscosity of crystallization medium thereby reducing the crystallization rate of drugs.
- Provide a steric barrier to drug molecules and inhibit crystallization through specific intermolecular interactions on growing crystal surfaces.
- Adsorb onto faces of host crystals, reduce the crystal growth rate of the host and produce smaller crystals.

9. Alteration of pH of the Drug Microenvironment : This can be achieved in two ways—*in situ* salt formation, and addition of buffers to the formulation e.g. buffered aspirin tablets.

10. Use of Amorphs, Anhydrates, Solvates and Metastable Polymorphs : Depending upon the internal structure of the solid drug, selection of proper form of drug with greater solubility is important. In general, amorphs are more soluble than metastable polymorphs, anhydrates are more soluble than hydrates and solvates are more soluble than non-solvates.

11. Solvent Deposition : In this method, the poorly aqueous soluble drug such as nifedipine is dissolved in an organic solvent like alcohol and deposited on an inert, hydrophilic, solid matrix such as starch or microcrystalline cellulose by evaporation of solvent.

12. Precipitation : In this method, the poorly aqueous soluble drug such as cyclosporine is dissolved in a suitable organic solvent followed by its rapid mixing with a non-solvent to effect precipitation of drug in nanosize particles. The product so prepared is also called as *hydrosol*.

13. Selective Adsorption on Insoluble Carriers : A highly active adsorbent such as the inorganic clays like bentonite can enhance the dissolution rate of poorly water-soluble drugs such as griseofulvin, indomethacin and prednisone by maintaining the concentration gradient at its maximum. The two reasons suggested for the rapid release of drugs from the surface of clays are—the weak physical bonding between the adsorbate and the adsorbent, and hydration and swelling of the clay in the aqueous media.

14. Solid Solutions : The three means by which the particle size of a drug can be reduced to submicron level are—

- Use of solid solutions,
- Use of eutectic mixtures, and
- Use of solid dispersions.

In all these cases, the solute is frequently a poorly water-soluble drug acting as the **guest** and the solvent is a highly water-soluble compound or polymer acting as a **host** or **carrier.**

A **solid solution** *is a binary system comprising of a solid solute molecularly dispersed in a solid solvent.* Since the two components crystallize together in a homogeneous one phase system, solid solutions are also called as **molecular dispersions** or **mixed crystals**. Because of reduction in particle size to the molecular level, solid solutions show greater aqueous solubility and faster dissolution than eutectics and solid dispersions. They are generally prepared by fusion method whereby a physical mixture of solute and solvent are melted together followed by rapid solidification. *Such systems, prepared by fusion, are often called as* **melts** e.g. griseofulvin-succinic acid (Fig. 11.5). The griseofulvin from such solid solution dissolves 6 to 7 times faster than pure griseofulvin.

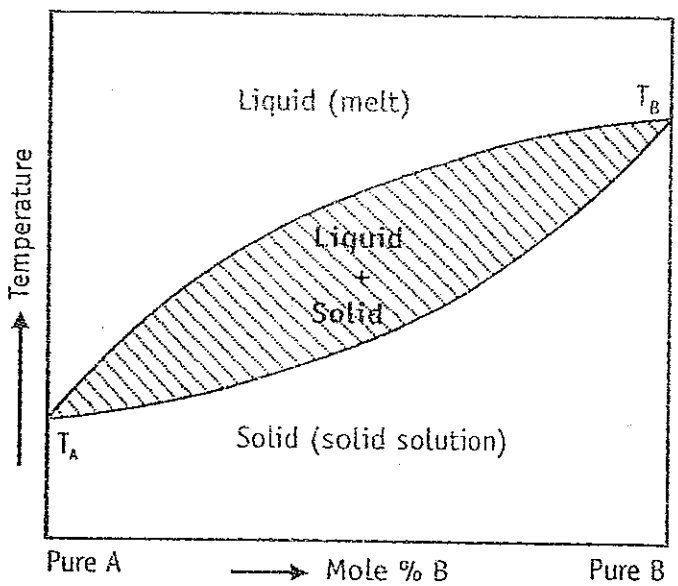


Fig. 11.5 Binary phase diagram for continuous solid solution of A and B. T_A and T_B are melting points of pure A and pure B respectively.

If the diameter of solute molecules is less than 60% of diameter of solvent molecules or its volume less than 20% of volume of solvent molecule, the solute molecule can be accommodated within the intermolecular spaces of solvent molecules e.g. digitoxin-PEG 6000 solid solution. Such systems show faster dissolution. [*When the resultant solid solution is a homogeneous transparent and brittle system, it is called as* **glass solution**.] Carriers that form glassy structure are citric acid, urea, PVP and PEG and sugars such as dextrose, sucrose and galactose.

acid, urea, PVP and PEG and sugars such as dextrose, sucrose and galactose.

Solid solutions can be classified on two basis –

A. Miscibility between the drug and the carrier – on this basis the solid solutions are divided into two categories –

1. ***Continuous solid solution*** *is the one in which both the drug and the carrier are miscible in all proportions.* Such a solid solution is not reported in pharmaceutical literature.
2. ***Discontinuous solid solution*** *is the one where solubility of each of the component in the other is limited* (*see* fig. 11.5).

B. Distribution of drug in carrier structure – on this basis the solid solutions are divided into two categories –

1. ***Substitutional crystalline solid solution*** *is the one in which the drug molecules substitute for the carrier molecules in its crystal lattice.* This happens when the drug and carrier molecules are almost of same size.
2. ***Interstitial crystalline solid solution*** *is the one in which the drug molecules occupy the interstitial spaces in the crystal lattice of carrier molecules.* This happens when the size of drug molecule is 40% or less than the size of carrier molecules (Fig. 11.6).

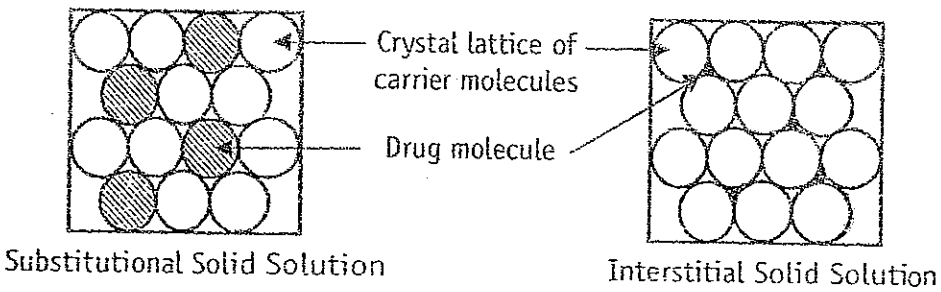


Fig. 11.6 Types of crystalline solid solution

The two mechanisms suggested for enhanced solubility and rapid dissolution of molecular dispersions are:

- When the binary mixture is exposed to water, the soluble carrier dissolves rapidly leaving the insoluble drug in a state of microcrystalline dispersion of very fine particles,
- When the solid solution, which is said to be in a state of randomly arranged solute and solvent molecules in the crystal lattice, is exposed to the dissolution fluid, the soluble carrier

Fig. 11.7 compares dissolution rates of different forms of griseofulvin.

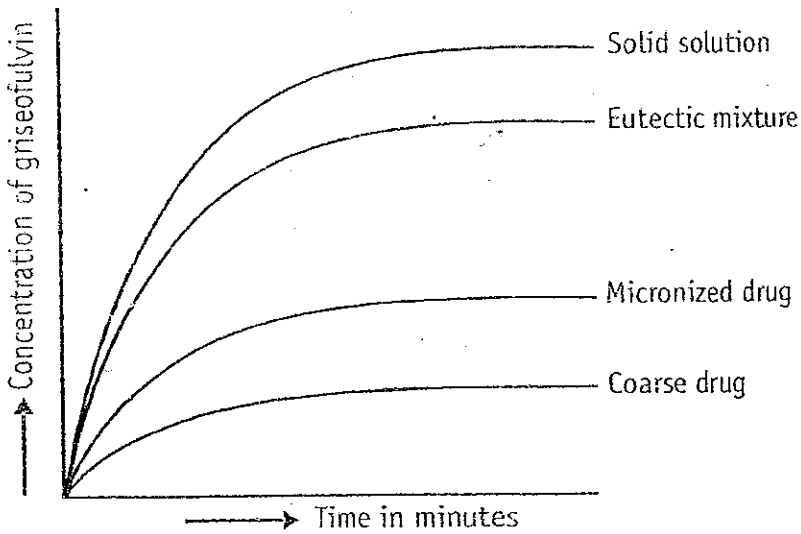


Fig. 11.7 Dissolution rates of griseofulvin as coarse particles, as micronized particles and as eutectic and solid solution with succinic acid.

15. Eutectic Mixtures : These systems are also prepared by fusion method. Eutectic melts differ from solid solutions in that the fused melt of solute-solvent show complete miscibility but negligible solid-solid solubility i.e. *such systems are basically intimately blended physical mixture of two crystalline components.* A phase diagram of two-compo-

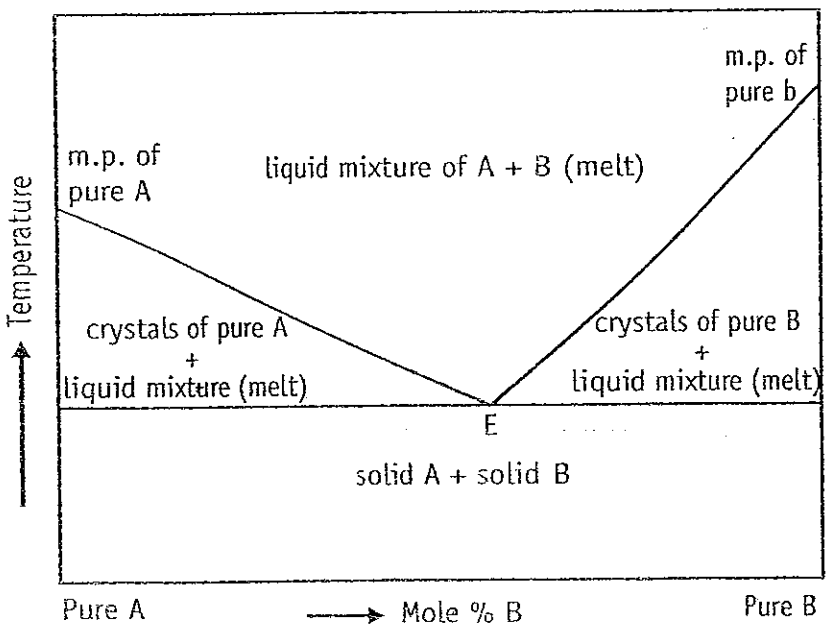


Fig. 11.8 Simple binary phase diagram showing eutectic point E. The eutectic composition at point E of substances A and B represents the one having lowest melting point.

nent system is shown in Fig. 11.8. When the eutectic mixture is exposed to water, the soluble carrier dissolves leaving the drug in a microcrystalline state which solubilises rapidly.

Examples of eutectics include paracetamol-urea, griseofulvin-urea, griseofulvin-succinic acid, etc. Solid solutions and eutectics, which are basically melts, are easy to prepare and economical with no solvents involved. The method, however, cannot be applied to:

- Drugs which fail to crystallize from the mixed melt.
- Drugs which are thermolabile.
- Carriers such as succinic acid that decompose at their melting point. The eutectic product is often tacky, intractable or irregular crystal.

16. Solid Dispersions : These are generally prepared by **solvent** or **co-precipitation** method whereby both the guest solute and the solid carrier solvent are dissolved in a common volatile liquid solvent such as alcohol. The liquid solvent is removed by evaporation under reduced pressure or by freeze-drying which results in amorphous precipitation of guest in a crystalline carrier. Thus, *the basic difference between solid dispersions and solid solutions/eutectics is that the drug is precipitated out in an amorphous form in the former as opposed to crystalline form in the latter*; e.g. amorphous sulphathiazole in crystalline urea. Such dispersions are often called as **co-evaporates** or **co-precipitates.** The method is suitable for thermolabile substances but has a number of

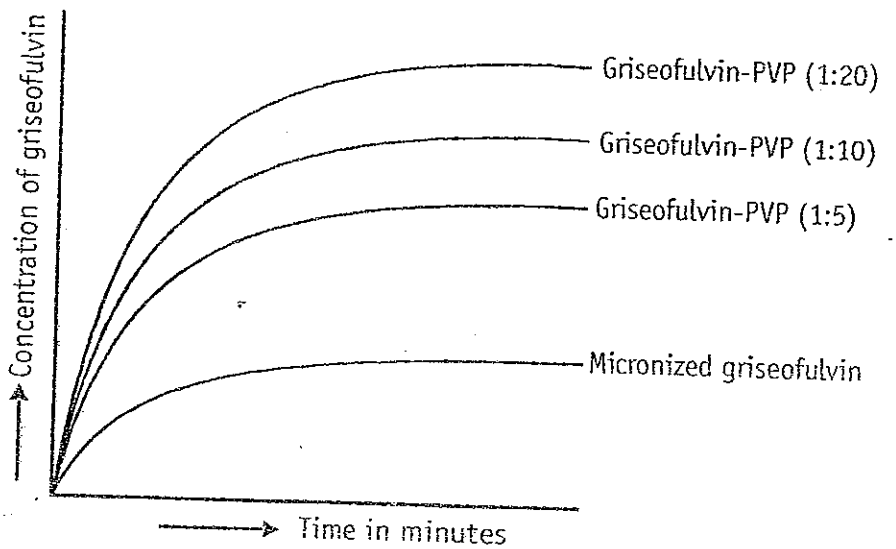


Fig. 11.9 Dissolution rate enhancement of griseofulvin by solid dispersion technique.

disadvantages like higher cost of processing, use of large quantities of solvent, difficulty in complete removal of solvent, etc. The carriers used are same as for eutectics or solid solutions. With glassy materials, the dispersions formed are called as **glass dispersions** or **glass suspensions.** Fig. 11.9 shows comparative dissolution rates of griseofulvin from PVP dispersions. Other polymers such as PEG and HPMC are also employed to prepare solid dispersions of poorly water-soluble drugs such as nifedipine and itraconazole.

Preparation of solid dispersions also presents several ***limitations*** –

- Since the carrier is hydrophilic and the drug is hydrophobic, it is difficult to find a common solvent to dissolve both components.
- The product is often soft, waxy and possesses poor compressibility and flowability.
- Physical instability of the solid dispersion.
- Difficulty in preparation of a reproducible product.

17. Molecular Encapsulation with Cyclodextrins : The beta- and gamma-cyclodextrins and several of their derivatives are unique in having the ability to form ***molecular inclusion complexes*** with hydrophobic drugs having poor aqueous solubility. These bucket-shaped oligosaccharides produced from starch are versatile in having a hydrophobic cavity of size suitable enough to accommodate the lipophilic drugs as guests; the outside of the host molecule is relatively hydrophilic (Fig. 11.10). Thus, the molecularly encapsulated drug has greatly improved aqueous solubility and dissolution rate. There are several examples of drugs with improved bioavailability due to such a phenomenon — thiazide diuretics, barbiturates, benzodiazepines and a number of NSAIDs.

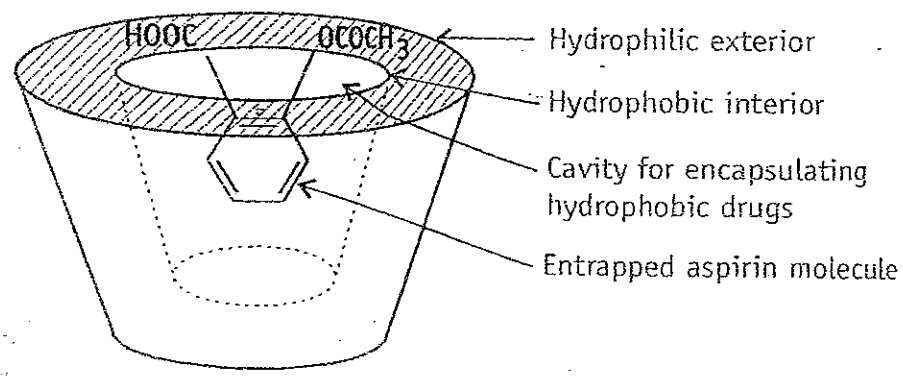


Fig. 11.10 Functional and structural feature of a cyclodextrin molecule showing an encapsulated drug.

(B) BIOAVAILABILITY ENHANCEMENT THROUGH ENHANCEMENT OF DRUG PERMEABILITY ACROSS BIOMEMBRANE

On several occasions, the rate-limiting step in drug absorption is transport through the intestinal epithelium owing to poor permeability. Several approaches besides the use of lipophilic prodrugs that increase the drug permeation rate are discussed below.

1. Lipid Technologies : With an increase in the number of emerging hydrophobic drugs, several lipid-based formulations have been designed to improve their bioavailability by a combination of various mechanisms briefly summarized as follows:

(*a*) ***Physicochemical***—Enhanced dissolution and solubility.

(*b*) ***Physiological***—potential mechanisms include—

- Enhancement of effective luminal solubility by stimulation of secretion of bile salts, endogenous biliary lipids including phospholipids and cholesterol which together form mixed micelles and facilitate GI solubilization of drug.
- Reduction in gastric emptying rate thereby increasing the time available for dissolution and absorption.
- Increased intestinal membrane permeability.
- Increased intestinal blood flow.
- Decreased luminal degradation.
- Increased uptake from the intestinal lumen into the lymphatic system (and a reduction in first-pass hepatic and GI metabolism).

The various ***lipid-based dosage forms*** include – lipid solutions and suspensions, micellar-solubilization, coarse emulsions, microemulsions, multiple emulsions, self-emulsifying drug delivery systems (SEDDS), self-microemulsifying drug delivery systems (SMEDDS), nanoparticles and liposomes.

The reasons for the increasing interest in lipid-based systems are due to the several ***advantages*** they offer and include:

(*i*) ***Physicochemical advantages*** : such as

- Solubilisation of drugs with low aqueous solubility.
- Stabilisation of labile drugs against hydrolysis or oxidation.

(*ii*) ***Pharmaceutical advantages*** : such as

- Better characterization of lipidic excipients.
- Formulation versatility and the choice of different drug delivery systems.
- Opportunity for formulation as sustained-release product.

(*iii*) ***Pharmacokinetic advantages*** : such as

- Improved understanding of the manner in which lipids enhance oral bioavailability.
- Reduced plasma profile variability.
- Potential for drug targeting applications.

(*iv*) ***Pharmacodynamic advantages*** : such as

- Reduced toxicity.
- Consistency in drug response.

*a. **Lipid solutions and suspensions*** : Some lipophilic drugs such as steroids have appreciable solubility in triacylglycerols alone. It is therefore comparatively straightforward to administer the drug in an oily liquid (e.g. encapsulated) and thereby achieve satisfactory absorption. One disadvantage of this formulation approach, however, is that oil alone rarely provides the solubilizing power to dissolve the required dose in a reasonable quantity of oil. This limits the option of using a simple drug/oil formulation system.

*b. **Coarse emulsions, microemulsions, SEDDS and SMEDDS*** : The ability of oil to accommodate a hydrophobic drug in solution can be improved by the addition of surfactants. The surfactants also perform the function of dispersing the liquid vehicle on dilution in gastrointestinal fluid. Hence, the drug is present in fine droplets of the oil/surfactant mixture which spread readily in the gastro-intestinal tract. Self-emulsifying/microemulsifying systems are formed using an oily vehicle (or a mixture of a hydrophilic phase and a lipophilic phase), a surfactant with a high HLB and if required, a co-surfactant. Unlike emulsions, the resultant liquid is almost clear. These pre-concentrates form spontaneously an emulsion/microemulsion in aqueous media (e.g. gastro-intestinal tract).

*c. **Solid lipid nanoparticles*** : To overcome the disadvantages associated with the liquid state of the oil droplets, the liquid lipid is replaced by a solid lipid leading to the formation of solid lipid nanoparticles. In contrast to emulsions, the particles consist of a solid core made from solid lipids. They are characterized by a mean diameter between

approximately 100 to 1000 nm. There are two basic production techniques for solid lipid nanoparticles –

- Homogenization of melted lipids at elevated temperature, and
- Homogenization of a suspension of solid lipids at or below room temperature.

d. Nanostructured lipid carriers (NLC) : are oil-loaded solid-lipid nanoparticles but offer the advantage of greater drug loading.

e. Lipid-drug conjugate (LDC) nanoparticles : involve covalent bonding or salt formation of a hydrophilic drug with lipid to enhance membrane permeability.

f. Liposomes : Liposomes are broadly defined as lipid bilayers surrounding an aqueous space. Liposoluble drugs can be embedded in the "fatty" regions, while hydrophilic substances are held in the aqueous internal spaces of these globular vesicles.

2. Ion Pairing : The ion pairing approach involves co-administration of a hydrophilic or polar drug with a suitable lipophilic counterion, which consequently improves the partitioning of the resultant ion-pair (relatively more lipophilic) into the intestinal membrane. In fact, the approach seems to increase the oral bioavailability of ionisable drugs, such as atenolol, by approximately 2-fold. However, it is important that a counterion possesses high lipophilicity, sufficient aqueous solubility, physiological compatibility, and metabolic stability.

3. Penetration Enhancers : *Compounds which facilitate the transport of drugs across the biomembrane are called as penetration/ permeation enhancers or promoters*. This method is used mainly in cases of hydrophilic drugs which are expected to have difficulty in penetrating the lipid structure of the biomembrane. Penetration enhancers act by interaction of its lipid part with the polar component of membrane phospholipids.

Penetration enhancers can be divided into three categories –

1. Substances that act very quickly, have a strong effect and cause injury to the membrane (which is reversible), e.g. fatty acids such as oleic, linoleic and arachidonic and their monoglycerides.
2. Substances that act quickly, cause temporary injury but have average activity, e.g. salicylates and certain bile salts.
3. Substances having average to strong activity but cause sustained histological changes, e.g. SLS, EDTA and citric acid.

(C) BIOAVAILABILITY ENHANCEMENT THROUGH ENHANCEMENT OF DRUG STABILITY

The various ways by which improvement of stability of a drug in the GIT has a positive impact on bioavailability are discussed below.

1. Enteric Coating : Enteric-coated systems utilize polymeric coatings that are insoluble in the gastric media and therefore, prevent or retard drug release in the stomach. Such systems release the drug in the alkaline milieu of intestine. Bioavailability of drugs that are unstable in the gastric milieu, for e.g. erythromycin, penicillin V, pancreatin and benzimidizoles such as omeprazole can be improved by enteric coating.

2. Complexation : Complexation, in certain instances, can be used to increase the stability of drug in the GI milieu, particularly those of ester drugs and thus enhance their oral availability. Generally speaking, β-cyclodextrins are potential carriers for achieving such objectives but other complexing agents, such as caffeine, sodium salicylate, sodium benzoate, and nicotinamide, may also be used.

3. Use of Metabolism Inhibitors : Co-administration of a drug (with low bioavailability) and its metabolism inhibitor, which can selectively inhibit any of the contributing processes, would result in increased fractional absorption and hence a higher bioavailability. In fact, this approach seems to be a promising alternative to overcome the enzymatic barriers to oral delivery of metabolically labile drugs such as peptides and proteins. Current novel approaches in this area include:

- ***Bioadhesive delivery systems*** that can reduce the drug degradation between the delivery system and absorbing membrane by providing intimate contact with GI mucosa.
- ***Controlled-release microencapsulated systems*** that can provide simultaneous delivery of a drug and its specific enzyme inhibitor at the desired site for required period of time.
- ***Immobilization of enzyme inhibitors*** on mucoadhesive delivery systems.

Interestingly, the intestinal wall metabolism (*prehepatic metabolism*) may also be inhibited by co-administration of certain drugs and diet, which act by selectively inhibiting an enzyme present in enterocytes. An illustrative example is that of cyclosporin, which undergoes extensive intestinal metabolism, resulting in low bioavailability ranging between 30–40%. Studies have shown that co-administration of ketoconazole and **grapefruit juice**, which contains the inhibitory components, can significantly decrease the presystemic metabolism (both act *via* selective

inhibition of intestinal, not hepatic, CYP3A4) and consequently increase the oral bioavailability of cyclosporin. In a differential manner, however, ketoconazole moderately inhibits *P*-gp, whereas grapefruit juice activates *P*-gp-mediated efflux of cyclosporin, which is a well-characterized substrate of *P*-gp, thereby partially counteracting the CYP3A4-inhibitory effects of grapefruit juice.

Grapefruit juice is reported to be a powerful inhibitor of enzyme CYP3A4 and is known to enhance the bioavailability of several drugs. It could be argued that extraction of such components from grapefruit juice (thought to be flavonoids) and their inclusion as excipient in the dosage form would lead to not only more complete but also more consistent systemic levels by counteracting inconsistencies brought about by enzyme inhibitors in food and drink.

Co-administration of a drug that can selectively inhibit an enzyme in the liver may lead to increased bioavailability of another drug. For example, co-administration of erythromycin can result in inhibition of hepatic metabolism and thereby significantly increase the oral bioavailability of cyclosporin. As a matter of fact, this attribute of erythromycin appears to be selective, which permits a noninvasive measurement of the *in vivo* hepatic CYP3A4 activity, popularly known as *erythromycin breath test*. Many examples also exist related to the inhibitory effects of diet on hepatic first-pass metabolism.

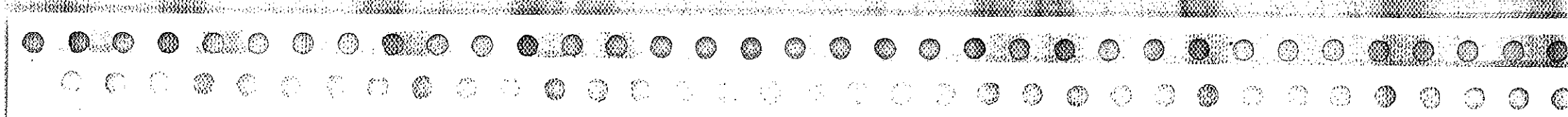
(D) BIOAVAILABILITY ENHANCEMENT THROUGH GASTROINTESTINAL RETENTION

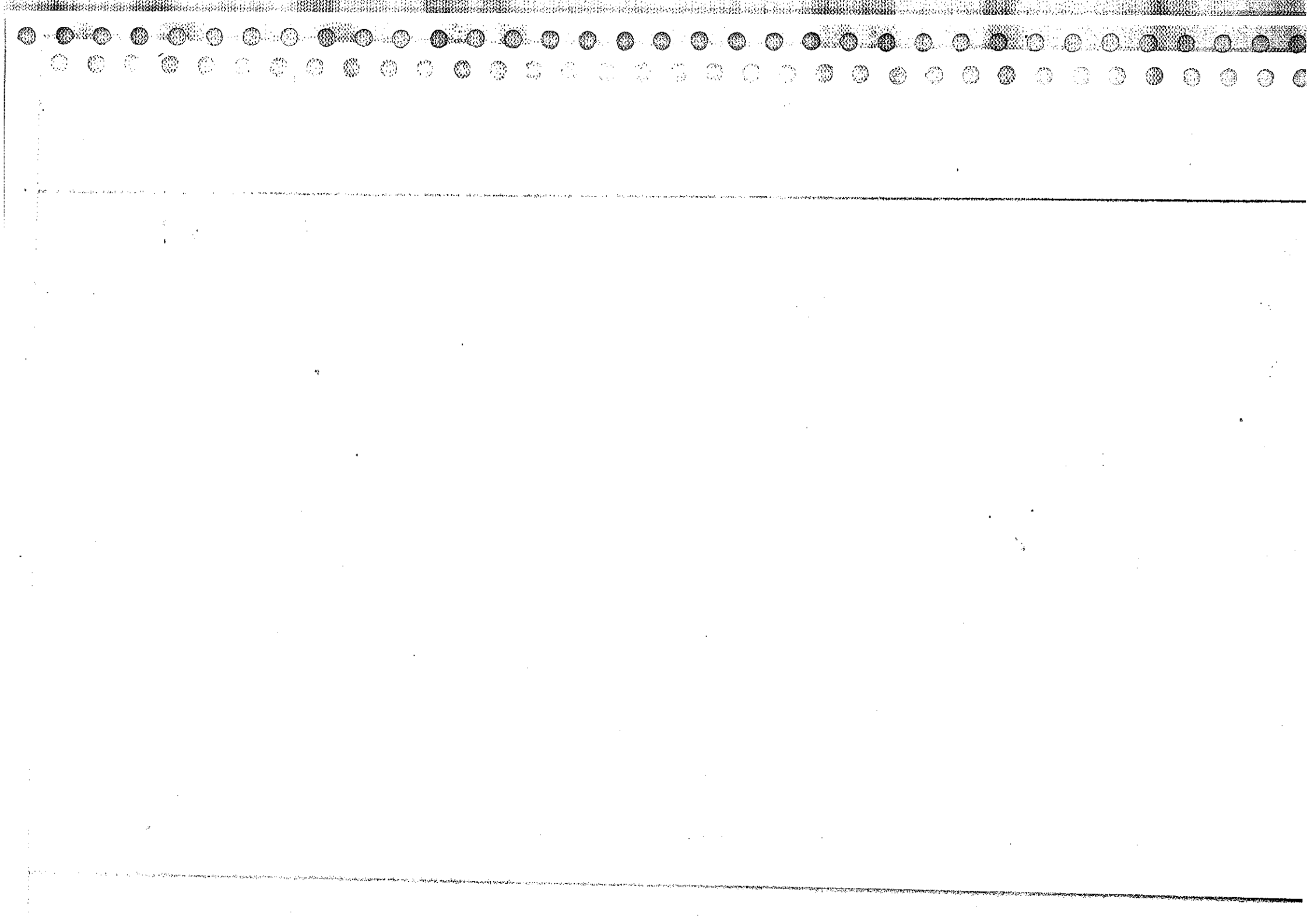
Gastro-retentive drug delivery systems (GRDDS) are designed on the basis of delayed gastric emptying and CR principles, and are intended to restrain and localize the drug delivery device in the stomach or within the upper part of the small intestine until the entire drug is released. Excipients that are bioadhesive or that swell on hydration when incorporated in an oral dosage form, can promote gastro-retention and absorption by –

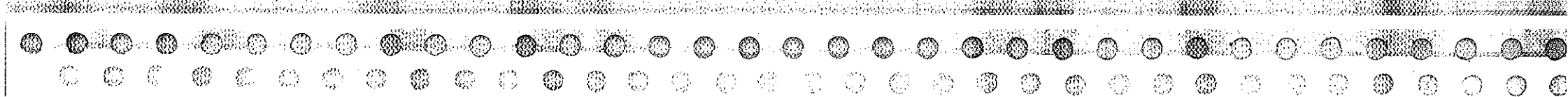
- Increased contact with epithelial surfaces.
- Prolonging residence time in the stomach.
- Delaying intestinal transit.

Cellulose ethers, gums of natural origin, and synthetic acrylic acid polymers have been evaluated for such purposes. The range of materials available and their differing viscoelastic and rheological behaviours mean that it is possible, by judicious admixture, to develop delivery

units with balanced properties so that adhesion, density, hydration, drug release rate, etc. can be tailored to the drug in question and the physiological characteristics of the target delivery site.







Applications of Pharmacokinetic Principles

1. Design and development of new drugs with greatly improved therapeutic effectiveness and fewer or no toxic-effects.
2. Design and development of an optimum formulation for better use of the drug.
3. Design and development of controlled/targeted release formulation.
4. Design an appropriate multiple dosage regimen
5. Select the appropriate route for drug administration.
6. Select the right drug for a particular illness
7. Predict and explain drug-food and drug-drug interactions.
8. Therapeutic drug monitoring in individual patients
9. Dosage adjustment in situations of altered physiology and drug interactions.

- The applications of pharmacokinetic principles are mainly aimed at achieving the therapeutic objective.
- The therapeutic objective is often control or cure

- Clinical applications of pharmacokinetic principles [2M]

of the condition in shortest possible time with minimum side-effects by the use of least amount of drug.

-Drug product design is aimed at optimising bioavailability or better control/cure of illness through controlled- or targeted-release.

-Proper choice of route of administration is necessary to ensure that the drug moves to the site of action at a sufficiently rapid rate and amount.

-Selection of a suitable drug is based on achieving optimal therapy by balancing the desirable and undersirable effects. Co-administration of several drugs to a patient may lead to changes in the pharmacokinetic profile of a drug which is indicative of interactions between drugs.

Such an understanding of interaction makes possible more rational use of drugs that have to be co-administered.

-clinically, the two most important applications of pharmacokinetic principles are:

1- Design of an optimal dosage regimen

2. Clinical management of individual patient and therapeutic drug monitoring.

/multiple dosage by iv infusion or extravascular dosing)

Design of dosage Regimens:

- Dosage regimen is defined as the manner in which a drug is taken.

- For some drugs like analgesics, hypnotics, anti-emetics, etc, a single dose may provide effective treatment.

- However, the duration of most illnesses is longer than than the therapeutic effect produced by a single dose. In such cases, drugs are required to be taken on a repetitive basis over a period of time depending upon the nature of illness. Thus, for sucessful therapy, design of an optimal multiple dosage regimen is necessary.

- An optimal multiple dosage regimen is the one

in which the drug is administered in suitable doses (by a suitable route), with sufficient frequency that ensures maintenance of plasma concentration within the therapeutic window (without excessive fluctuations and drug accumulation) for the entire duration of therapy.

For some drugs like antibiotics, a minimum effective concentration should be maintained at all times and for drugs with narrow therapeutic indices like phenytoin, attempt should be made not to exceed toxic concentration.

-Approaches to Design of Dosage Regimen:

1. Empirical Dosage Regimen:

-is designed by the physician based on empirical clinical data, personal experience and clinical observations.

2. Individualized Dosage regimen:

-is the most accurate approach and is based

on the pharmacokinetics of drug in the individual patient.

- The approach is suitable for hospitalised patients but is quite expensive.

3. Dosage Regimen on Population Averages:

- This is the most often used approach.
- The method is based on one of the two models:

a- Fixed model:

Here, population average pharmacokinetic parameters are used directly to calculate the dosage regimen.

b- Adaptive model:

It is based on both population average pharmacokinetic parameters of the drug as well as patient variables such as weight, age, sex, body surface area and known patient pathophysiology such as renal disease.

- In designing a dosage regimen based on population averages:

1. It is assumed that all pharmacokinetic parameters of the drug remain constant during the course of therapy once a dosage regimen is established. The same becomes invalid if any change is observed.

2. The calculations are based on open one-compartment model which can also be applied to two compartment model if β is used instead of K_E, and $V_{d,ss}$ instead of V_d while calculating the regimen.

- صرف نظر از
Irrespective of the route of administration and complexity of pharmacokinetic equations, the two major parameters that can be adjusted in developing a dosage regimen are: (2m)

1. The dose size – the quantity of drug administered each time.
2. The dosing frequency – the time interval between doses.

Both parameters govern the amount of drug in the body at any given time.

Dose Size

The magnitude of both therapeutic and toxic responses depends upon dose size. Dose size calculation also requires the knowledge of amount of drug absorbed after administration of each dose. Greater the dose size, greater the fluctuations between $C_{ss,max}$ and $C_{ss,min}$ during each dosing interval and greater the chances of toxicity (Fig. 12.1). For drugs administered chronically, dose size calculation is based on average steady state blood levels and is computed from equation 12.8.

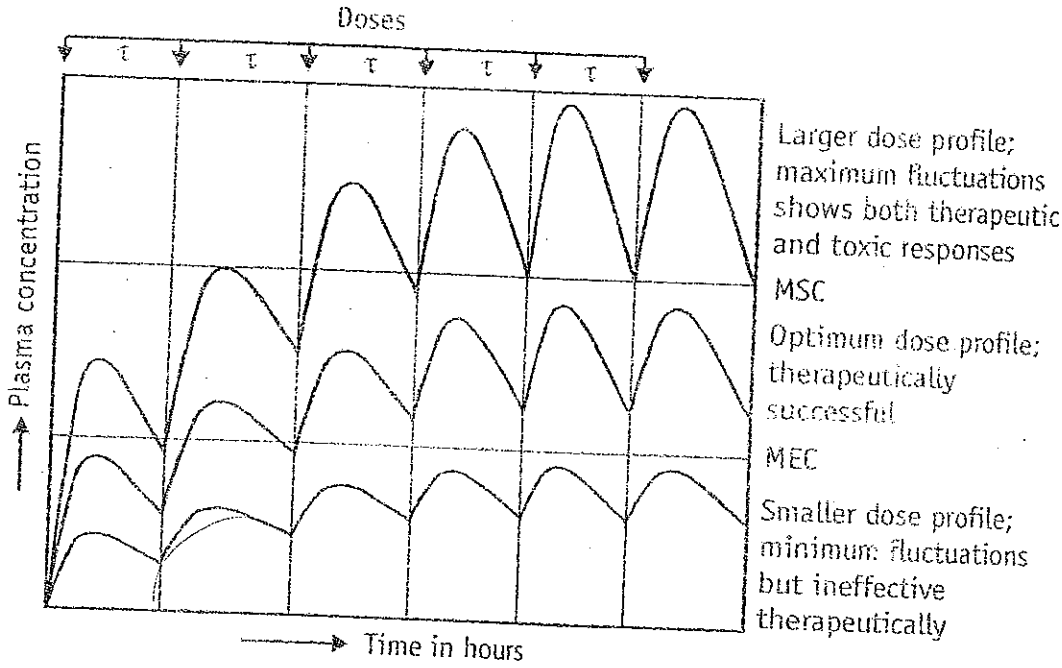


Fig. 12.1 Schematic representation of influence of dose size on plasma concentration-time profile after oral administration of a drug at fixed intervals of time.

Dosing Frequency

The *dose interval* (inverse of *dosing frequency*) is calculated on the basis of half-life of the drug. If the interval is increased and the dose is unchanged, C_{max}, C_{min} and C_{av} decrease but the ratio C_{max}/C_{min} increases. Opposite is observed when dosing interval is reduced or dosing frequency increased. It also results in greater drug accumulation in the body and toxicity (Fig. 12.2).

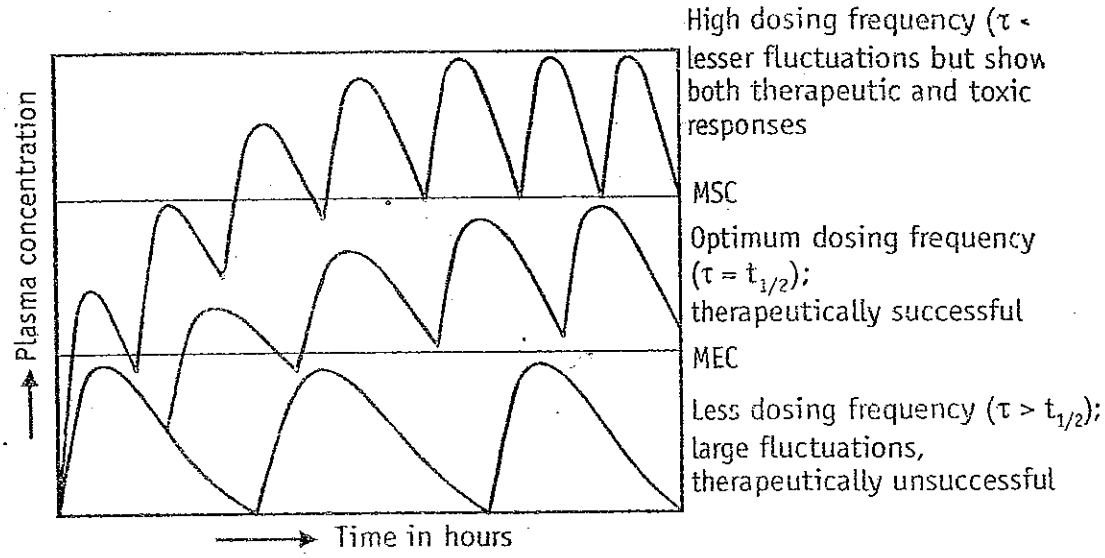


Fig. 12.2 Schematic representation of the influence of dosing frequency on plasma concentration-time profile obtained after oral administration of fixed doses of a drug.

A proper balance between both dose size and dosing frequency is often desired to attain steady-state concentration with minimum fluctuations and to ensure therapeutic efficacy and safety. The same cannot be obtained by giving larger doses less frequently. However, administering smaller doses more frequently results in smaller fluctuations.

Generally speaking, every subsequent dose should be administered at an interval equal to half-life of the drug. A rule of thumb is that—

- For drugs with *wide therapeutic index* such as penicillin, *larger doses* may be administered at relatively *longer intervals* (more than the half-life of drug) without any toxicity problem.
- For drugs with *narrow therapeutic index* such as digoxin, *small doses* at *frequent intervals* (usually less than the half-life of the drug) is better to obtain a profile with least fluctuations which is similar to that observed with constant rate infusion or controlled-release system.

Accumulation

In case of multiple dosage regimen (drugs are frequently administered) in such a case the 1st drug concentration remaining in the body after a certain time, is added to the next dose this condition is known as accumulation.

-Accumulation index (R_{ac}):

-The extent to which a drug will accumulate with any dosing interval in a patient can be derived from information obtained with a single dose and is given by accumulation index R_{ac} as:

$$R_{ac} = \frac{1}{1 - e^{-K_e \tau}} \qquad (12.1)$$

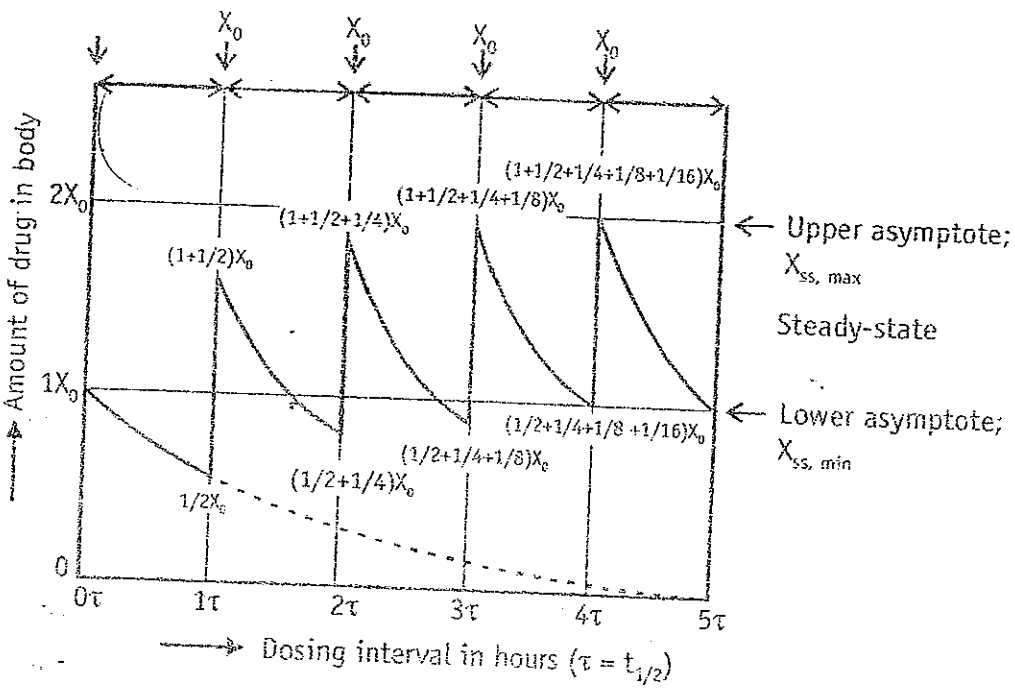


Fig.12.3 Accumulation of drug in the body during multiple dose regimen of i.v. bolus with dosing interval equal to one half-life of the drug. Approximately 5 half-lives are required for attainment of steady-state.

Time to Reach Steady-State during Multiple Dosing

The time required to reach steady-state depends primarily upon the half-life of the drug. Provided $K_a >> K_E$, *the plateau is reached in approximately 5 half-lives. This is called as plateau principle.* It also means that the rate at which the multiple dose steady-state is reached is determined only by K_E. The time taken to reach steady-state is independent of dose size, dosing interval and number of doses.

Maximum and Minimum Concentration during Multiple Dosing

If n is the number of doses administered, the C_{max} and C_{min} obtained on multiple dosing after the nth dose is given as:

$$C_{n,\,max} = C_0\left[\frac{1-e^{-nK_E\tau}}{1-e^{-K_E\tau}}\right] \qquad (12.2)$$

$$C_{n,\,min} = C_0\left[\frac{1-e^{-nK_E\tau}}{1-e^{-K_E\tau}}\right]e^{-K_E\tau} = C_{n,\,max}\,e^{-K_E\tau} \qquad (12.3)$$

The maximum and minimum concentrations of drug in plasma at steady-state are found by following equations:

$$C_{ss,\,max} = \frac{C_0}{1-e^{-K_E\tau}} \qquad (12.4)$$

$$C_{ss,\,min} = \frac{C_0 e^{-K_E\tau}}{1-e^{-K_E\tau}} = C_{ss,\,max}\,e^{-K_E\tau} \qquad (12.5)$$

where C_0 = concentration that would be attained from instantaneous absorption and distribution (obtained by extrapolation of elimination curve to time zero). Equations 12.2 to 12.5 can also be written in terms of amount of drug in the body. Fraction of dose absorbed, F, should be taken into account in such equations.

***Fluctuation** is defined as the ratio C_{max}/C_{min}.* Greater the ratio, greater the fluctuation. Like accumulation, it depends upon dosing frequency and half-life of the drug. It also depends upon the rate of absorption. The greatest fluctuation is observed when the drug is given as i.v. bolus. Fluctuations are small when the drug is given extravascularly because of continuous absorption.

Average Concentration and Body Content on Multiple Dosing to Steady-State

The average drug concentration at steady-state $C_{ss,av}$ is a function of—

- Maintenance dose X_0,
- Fraction of dose absorbed F,
- Dosing interval τ, and
- Clearance Cl_T (or V_d and K_E or $t_{1/2}$) of the drug.

$$C_{ss,\,av} = \frac{F X_0}{Cl_T \tau} \tag{12.6}$$

$$= \frac{1.44\, F X_0}{V_d \tau} = \frac{AUC\,(\text{single dose})}{\tau} \tag{12.7}$$

where the coefficient 1.44 is the reciprocal of 0.693 in equation 12.7. AUC is the area under the curve following a single maintenance dose. Equation 12.7 can be used to calculate maintenance dose of a drug to achieve a desired concentration. Since $X = V_d\, C$, the body content at steady-state is given as:

$$X_{ss,\,av} = \frac{1.44\, F X_0 t_{1/2}}{\tau} \tag{12.8}$$

These average values are not arithmetic mean of $C_{ss,max}$ and $C_{ss,min}$ since the plasma drug concentration declines exponentially.

Loading and Maintenance Doses

A drug does not show therapeutic activity unless it reaches the desired steady-state. It takes about 5 half-lives to attain it and therefore the time taken will be too long if the drug has a long half-life. Plateau can be reached immediately by administering a dose that gives the desired steady-state instantaneously before the commencement of maintenance doses X_0. *Such an initial or first dose intended to be therapeutic is called as* **priming dose** or **loading dose** $X_{0,L}$. A simple equation for calculating loading dose is:

$$X_{0,L} = \frac{C_{ss,\,av} V_d}{F} \tag{12.9}$$

After e.v. administration, C_{max} is always smaller than that after i.v. administration and hence loading dose is proportionally smaller. For drugs having low therapeutic indices, the loading dose may be divided into smaller doses to be given at various intervals before the first maintenance dose. When V_d is not known, loading dose may be calculated by the following equation:

$$\frac{X_{0,L}}{X_0} = \frac{1}{\left(1 - e^{-K_a \tau}\right)\left(1 - e^{-K_E \tau}\right)} \qquad (12.10)$$

The above equation applies when $K_a >> K_E$ and drug is distributed rapidly. When the drug is given i.v. or when absorption is extremely rapid, the absorption phase is neglected and the above equation reduces to **accumulation index**:

$$\frac{X_{0,L}}{X_0} = \frac{1}{\left(1 - e^{-K_E \tau}\right)} = R_{ac} \qquad (12.11)$$

The ratio of loading dose to maintenance dose $X_{0,L}/X_0$ *is called as* **dose ratio.** As a rule, when

- $\tau = t_{1/2}$, dose ratio equals 2.0
- $\tau > t_{1/2}$, dose ratio is smaller than 2.0
- $\tau < t_{1/2}$, dose ratio is greater than 2.0.

Fig. 12.4. shows that if loading dose is not optimum, either too low or too high, the steady-state is attained within approximately 5 half-lives in a manner similar to when no loading dose is given.

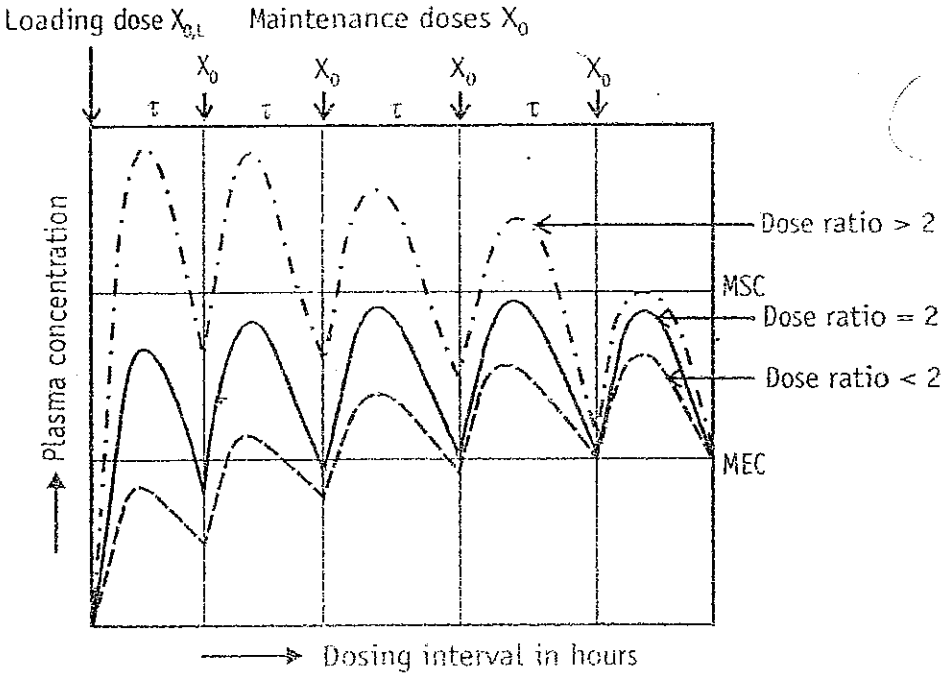


Fig. 12.4 Schematic representation of plasma concentration-time profiles that result when dose ratio is greater than 2.0, equal to 2.0 and smaller than 2.0.

→After loading dose (X0,2), another dose is give to maintain the steady-state drug concentration i.e. maintainan of drug within the therapeutic range is called as maintenance dose.

The foregoing discussion and the equations expressed until now apply only to drugs that follow one-compartment kinetics with first-order disposition. Equations will be complex for multicompartment models.

Maintenance of Drug within the Therapeutic Range

The ease or difficulty in maintaining drug concentration within the therapeutic window depends upon —

1. The therapeutic index of the drug.
2. The half-life of the drug.
3. Convenience of dosing.

Certain generalizations with regards to maintenance of drug within the therapeutic range can be stated –

- For a drug with short half-life (less than 3 hours) and narrow therapeutic index e.g. heparin, dosing frequency has to be essentially less than $t_{1/2}$.
- For a drug with short half-life (e.g. penicillin, $t_{1/2}$ = 0.9 hours) and high therapeutic index, dosing frequency can be several times that ofo $t_{1/2}$ (every 4 to 6 hours) but the maintenance dose has to be larger so that the plasma concentration persists above the minimum inhibitory level.
- A drug with intermediate $t_{1/2}$ (3 to 8 hours) may be given at intervals $\tau \leq t_{1/2}$ if therapeutic index is low and those with high indices can be given at intervals between 1 to 3 half-lives.
- Drugs with half-lives greater than 8 hours are more convenient to dose. Such drugs are usually administered once every half-life. Steady-state in such cases can be attained rapidly by administering a loading dose.
- For drugs with very long half-lives (above 24 hours) e.g. amlodipine, once daily dose is very convenient.

Design of Dosage Regimen from Plasma Concentrations

If the therapeutic range, apparent V_d and clearance or half-life of a drug is known, then dosage regimen can be designed to maintain drug concentration within the specified therapeutic range. The latter is defined by lower limit (C_{lower}) and an upper limit (C_{upper}). The maximum dosing interval, which ideally depends upon the therapeutic index (can be defined as a ratio of C_{upper} to C_{lower}) and elimination half-life of the drug, can be expressed by equation 12.12, a modification of equation 12.5.

$$\tau_{max} = \frac{2.303 \log \left(C_{upper} / C_{lower}\right)}{K_E} \qquad (12.12)$$

Since $K_E = 0.693/t_{½}$, the above equation can also be written as:

$$\tau_{max} = 3.32\, t_{1/2} \log\left(C_{upper} / C_{lower}\right) \qquad (12.13)$$

Understandably, the dosing interval selected is always smaller than τ_{max}. The maximum maintenance dose $X_{o,max}$ that can be given every τ_{max} is expressed as:

$$X_{0,\,max} = \frac{V_d \left(C_{upper} - C_{lower}\right)}{F} \qquad (12.14)$$

After a convenient dosing interval τ, smaller than τ_{max} has been selected, the maintenance dose is given as:

$$X_0 = \left[\frac{X_{0,max}}{\tau_{max}}\right]\tau \qquad (12.15)$$

Administration of X_o every τ produces an average steady-state concentration defined by equation 12.7. The value of $C_{ss,av}$ can also be calculated from the therapeutic range according to equation 12.16.

$$C_{ss,\,av} = \frac{\left(C_{upper} - C_{lower}\right)}{2.303 \log\left(C_{upper} / C_{lower}\right)} \qquad (12.16)$$

Keya Kabiraj

DOCTOR OF PHARMACY

Reza Rahimi
DOCTOR OF PHARMACY