

B.Pharm. VII Semester

BIOPHARMACEUTICS AND PHARMACOKINETICS

MODEL ANSWER

Section A

- 1. In bioavailability study, the volunteers should be instructed to completely empty their bladder while giving urine samples to avoid cumulative effect of drug concentration from the previous sample being added to the consecutive sample which would lead to erroneous result.**
- 2. When method of residuals is used to determine K_e and K_a , the terminal phase of oral Absorption curve is represented by elimination rate constant and absorption rate Constant is represented by residual curve ie $K_a \gg K_e$. In drugs showing fast elimination, $K_e \gg K_a$. For such drugs like salicylic acid, terminal phase of oral absorption Curve represents K_a and residual curve K_e . This is flip flop in pharmacokinetics.**
- 3. Binding sites in HAS are-**
 - Warfarin binding site**
 - Diazepam binding site**
 - Digitoxin binding site**
 - Tamoxifen binding site**
- 4. Significance of plasma conc. measurement-**
 - To determine dose size**
 - To determine dose frequency**
 - To study drug interaction**
 - To optimize therapeutic drug regimens**

To guide the progress of disease

To identify patients supersensitive to drug

5. Phenobarbital increases metabolism of warfarin and phenytoin, steroid hormones

Phenytoin increases metabolism of meperidine

Rifampine and phenytoin increase metabolism of propranolol

Disulfiram inhibits metabolism of alcohol

Isoniazid inhibits metabolism of phenytoin

6. In normal circumstances creatinine production is equal to creatinine excretion so

That serum creatinine levels remain constant. The clearance of creatinine is used as

A measure of GFR. Therefore creatinine clearance gives the corrected renal

Clearance of a drug.

7. Methods of determining area under curve-

Cut and weigh method

Planimetric method

Integration method

Trapezoidal method

Definite integral method

8. Dopamine cannot cross the Blood Brain Barrier so it is given as levodopa which is

A prodrug of dopamine and after crossing the brain it is converted into dopamine.

9. Pharmaceutical factors affecting drug absorption are-

Manufacturing process - method of granulation and compression force

Pharmaceutical excipients

Disintegration and dissolution time

Product age and storage conditions

10. Bioavailability is defined as the rate and extent to which the unchanged form of

Drug reaches the systemic circulation.

11. Aspirin, anticholinergics, ethanol, narcotic analgesics.

12. Active transport is energy dependant

Active transport is site specific

Active transport shows structure specificity

Active transport shows competitive inhibition.

SECTION 'B'

- **Discuss purpose of bioavailability studies. Elaborate the protocol for bioavailability studies.**

ANS: BIOAVAILABILITY: Bioavailability means the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. For drug products that are not intended to be absorbed into the bloodstream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or active moiety becomes available at the site of action.

PURPOSE OF BIOAVAILABILITY STUDIES: Bioavailability studies are performed for both approved active drug ingredients and therapeutic moieties not yet approved for marketing by the FDA.

- New formulations of active drug ingredients must be approved by the FDA before marketing.

- In approving a drug product for marketing, the FDA ensures that the drug product is safe and effective for its labeled indications for use.
- The drug product must meet all applicable standards of identity, strength, quality, and purity.
- To ensure that these standards are met, the FDA requires bioavailability/pharmacokinetic studies and, where necessary, bioequivalence studies for all drug products (FDA Guidance for Industry, 2003).
- Bioavailability may be considered as one aspect of drug product quality that links in-vivo performance of the drug product used in clinical trials to studies demonstrating evidence of safety and efficacy.
- For unmarketed drugs that do not have full NDA approval by the FDA, in-vitro and/or in-vivo bioequivalence studies must be performed on the drug formulation proposed for marketing as a generic drug product.
- The essential pharmacokinetics of the active drug ingredient or therapeutic moiety must be characterized.
- Essential pharmacokinetic parameters, including the rate and extent of systemic absorption, elimination half-life, and rates of excretion and metabolism, should be established after single- and multiple-dose administration. Data from these in-vivo bioavailability studies are important to establish recommended dosage regimens and to support drug labeling.
- In-vivo bioavailability studies are also performed for new formulations of active drug ingredients or therapeutic moieties that have full NDA approval and are approved for marketing. The purpose of these studies is to determine the bioavailability and to characterize the pharmacokinetics of the new formulation, new dosage form, or new salt or ester relative to a reference formulation.

- Clinical studies are useful in determining the safety and efficacy of drug products.
- Bioavailability studies are used to define the effect of changes in the physicochemical properties of the drug substance and the effect of the drug product (dosage form) on the pharmacokinetics of the drug.
- Bioequivalence studies are used to compare the bioavailability of the same drug (same salt or ester) from various drug products. Bioavailability and bioequivalence can also be considered as performance measures of the drug product in-vivo.
- If the drug products are bioequivalent and therapeutically equivalent (as defined above), then the clinical efficacy and the safety profile of these drug products are assumed to be similar and may be substituted for each other.

BIOAVAILABILITY STUDY PROTOCOL

- Title
 - Principle investigator (study director)
 - Project/protocol number and date
- Study objective
- Study design
- Experimental design:
- Wash out period
- Drug products
 - Test product(s)
 - Recognized product

- Rout of administration
- Dosage regimen
- Frequency and duration of sampling
- Randomization of drug administration
- Single-versus multiple-dose study design
- Subjects
- Healthy subjects versus patients
- Subject selection
 - Medical history,
 - Physical examination,
 - Laboratory tests
- Study conditions
- Analysis of biological fluids
- Method of assessment of Bioavailability
 - Plasma data
 - Urine data
 - Acute pharmacological effect
 - Clinical response

- Analysis and Presentation of data
 - Statistical treatment of data-Analysis of variance (ANOVA)
 - Formate of data

Q.No.2

What are the various mechanisms for absorption of drugs? Discuss Pharmaceutical factors affecting drug absorption.

ANSWER: DEFINITION OF ABSORPTION:

The amount of drug that enters the body from site of administration to the systemic circulation is known as absorption. The rate of absorption affects the onset, duration and intensity of drug action.

Absorption involves several phases. **First**, the drug needs to be introduced via some route of administration and in a specific dosage form such as a tablet, capsule, and so on. Absorption is a primary focus in drug development and medicinal chemistry, since the drug must be absorbed before any pharmacological effects can take place.

Various Mechanisms For Absorption Of Drugs

It is apparent from the pathways of drug transport that absorption of a drug from the lumen of GIT into blood

Processes involved in the absorption of drug:

Following are the processes involved in the transport of drugs:

1: Passive membrane transport

- a) Simple diffusion b) Filtration/ aqueous diffusion

c) Osmosis

d) Bulk flow

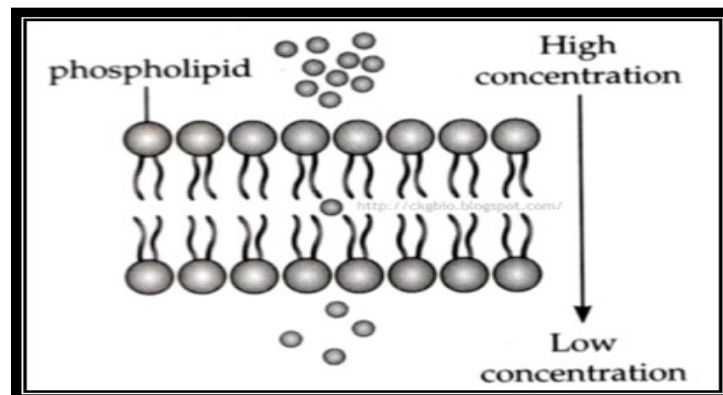
2: Specialized transport

a) Active membrane transport (primary/secondary) b) Facilitated diffusion

c) Endocytosis (phagocytosis/pinocytosis)

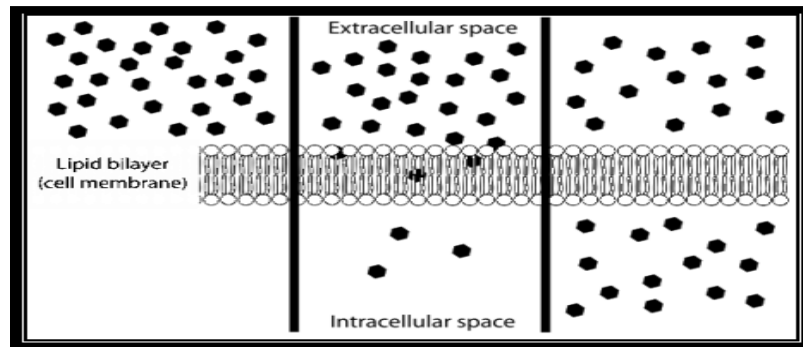
d) Exocytosis

1: Passive membrane transport: In passive transport, the drug molecule penetrates in the lipid bilayer membrane from higher concentration to the lower concentration of solutes along the concentration gradient without expenditure of energy. So passive transport mechanism involves following processes.



a) Simple diffusion:

It is characterized by the direct movement of a solute through the semi permeable cell membrane from a phase of higher concentration to the phase of lower concentration without expenditure of energy until the equilibrium is achieved.



Concentration gradient:

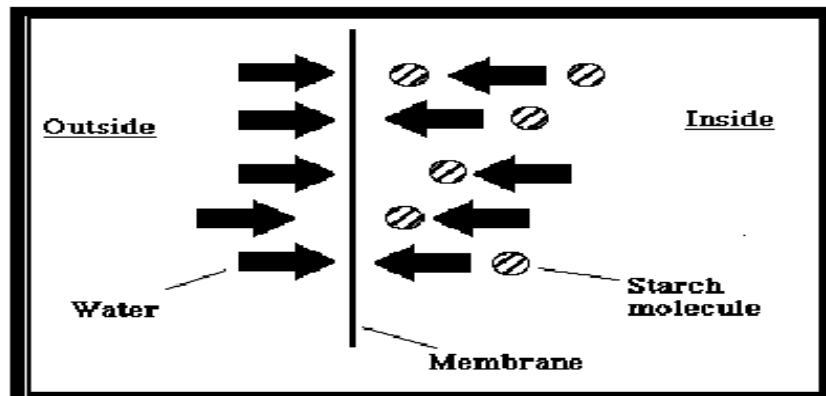
The force which directs the movement of solutes toward or against the gradient or the difference in concentration between both sides of membrane is known as concentration gradient. Transfer is directly proportional to the magnitude of the concentration gradient across the membrane.

Fick's law of diffusion: Rate of diffusion across an exchange surfaces depends upon

- Surface area across within diffusion occurs
- Thickness of cell membrane
- Difference in conc. gradient on both sides

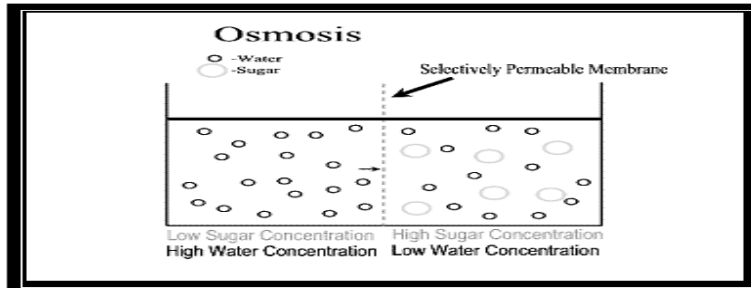
b) Filtration or aqueous diffusion:

It is the passage of a substance through pores in the cell membrane by means of their hydrostatic or osmotic pressure gradient. Water, ions and some polar and non polar molecules of low molecular weight diffuse through membrane indicating the existence of pores or channels. Glomerular membrane of kidney is a good example of filtration.



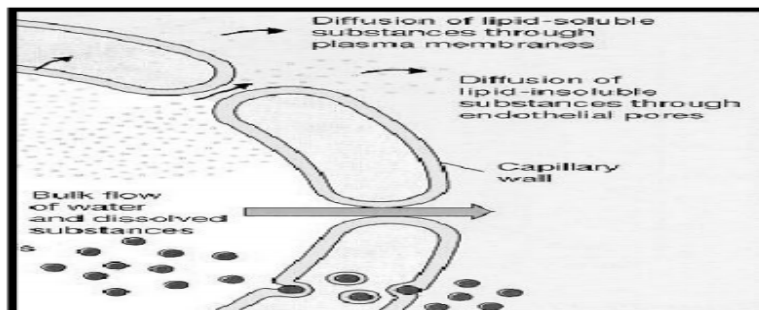
c) Osmosis:

Osmosis is a special case of diffusion. In this case, a large molecule of drug like starch is dissolved in water. The starch molecule is too large to pass through the pores in the cell membrane, so it cannot diffuse from one side of the membrane to the other. The water molecules can do, pass through the membrane. Hence the membrane is said to be semi permeable, since it allows some molecules to pass through but not others.



d) Bulk flow:

It is the movement of drug molecules across the membrane by pores between capillaries endothelial cells. It is important in regulating the distribution of fluids between the plasma and interstitial fluid, that is important in maintain the blood pressure



2: Specialized transport mechanism

Specialized transport of drug across the cell membrane requires transport mechanisms or carrier protein. It involves various processes of mechanisms. In these mechanism drug forms a complex with the carrier proteins or transporters at the outer surface of the cell membrane and then transported across the cell membrane to the inner surface when the drug is released from the

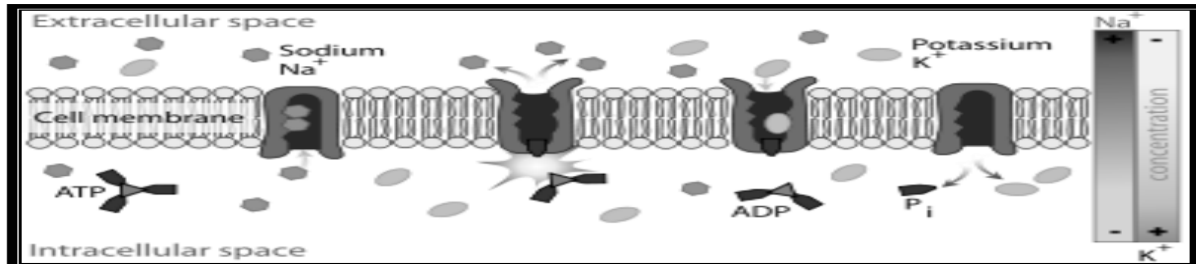
carrier complex.

a) Active transport mechanism

In active transport, the drug molecule penetrates in the lipid bilayer membrane from lower concentration to the higher concentration of solutes against the concentration gradient with the expenditure of energy and with the help of special carrier proteins. This process has two types of energy dependent mechanisms. i. Primary active transport, ii. Secondary active transport

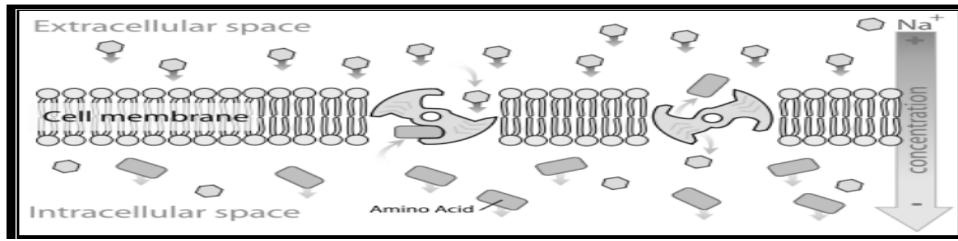
i. Primary active transport:

Primary active transport, also called direct active transport, directly uses energy to transport molecules across a membrane. The energy used in this type of active transport is ATP. The only substances transported by carriers that directly hydrolyze ATP. These include positively-charged ions - Na^+ , K^+ , Ca^{++} or H^+



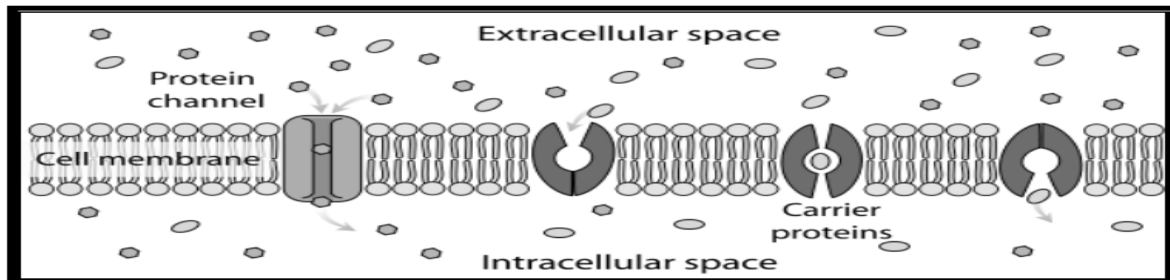
ii. Secondary active transport

Secondary active transport, also called co-transport, electrochemical potential difference created by pumping ions out of the cell is used to transport molecules across a membrane but there is no direct coupling of ATP. Sodium-proton or Sodium-calcium co transport mechanism involved in this type of absorption mechanism.



b) Facilitated diffusion:

It is a special form of carrier mediated transport in which the movement across cell membrane occurs along with the concentration gradient but with the help of special transporters or carrier proteins without the expenditure of energy.

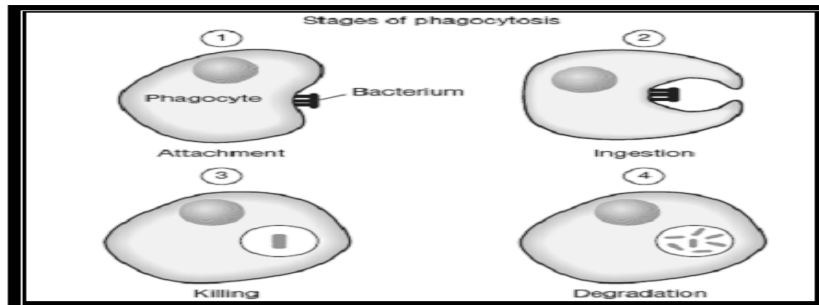


c) **Endocytosis:** Endocytosis is a process in which a substance gains entry into a cell by formation of intracellular vesicle by virtue of invagination of plasma membrane and membrane fusion takes place. i. Phagocytosis, ii. Pinocytosis

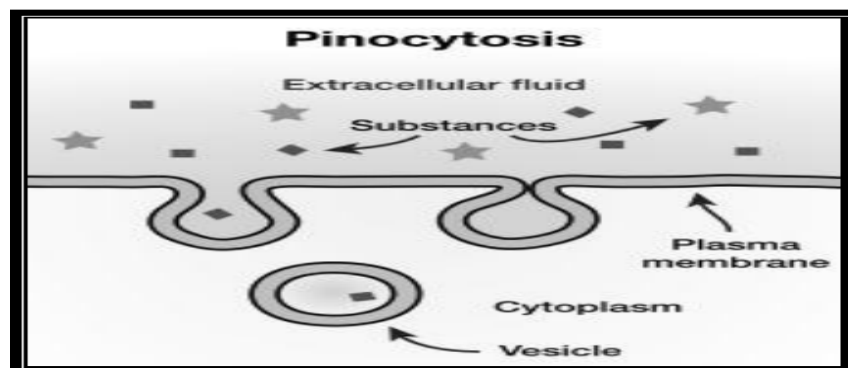
iii. Receptor mediated endocytosis

- **Phagocytosis:** The cellular process of engulfing the solid particles e.g bacterium by

vesicular internalization by the cell membrane itself to form the internal phagosome is known as phagocytosis.

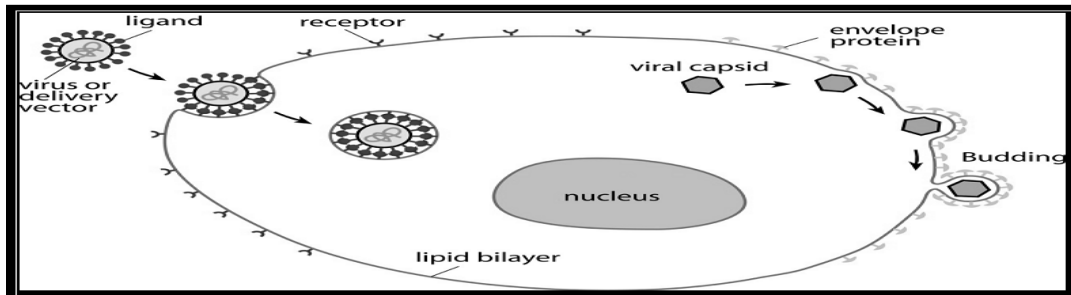


- **Pinocytosis:** The endocytosis in which small particle or liquid material is brought into the cell by forming an invagination or vesicle called lysosomes, is known as pinocytosis. This process requires a lot of energy in the form of ATP.

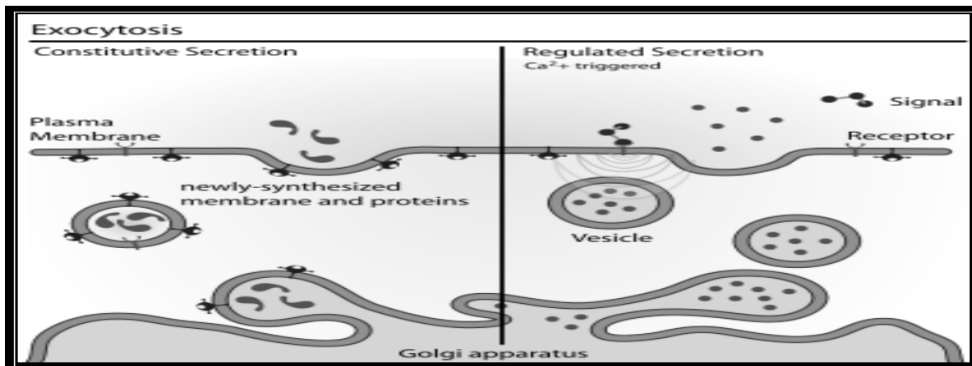


iii. **Receptor-mediated endocytosis:** Receptor-mediated endocytosis (RME), also called

clathrin-dependent endocytosis, is a process by which cells internalize molecules (endocytosis) by the inward budding of plasma membrane vesicles containing proteins with receptor sites specific to the molecules being internalized.



d) Exocytosis: Exocytosis is a process in which a substance removes from the cell by formation of extracellular vesicle by virtue of invagination of plasma membrane itself. Finally the vesicle is discharged with its contents into extracellular space.



FACTORS INFLUENCING THE ABSORPTION

MECHANISMS:

These are the factors influence the diffusion of the drug molecule across the membrane. The rate of absorption determines by

- Onset of absorption
- Duration of absorption
- Intensity of absorption

- Physiological factor
 - Membrane thickness
 - Membrane surface area
 - pH of gastrointestinal fluids
 - Functional integrity of GIT
 - Gastric emptying time
 - Blood flow

- Physiochemical factor
 - Lipid solubility
 - pH of the medium
 - Ionization of drug
 - Ionization coefficient

- Partition coefficient
- Protonated and Unprotonated form
- Pharmaceutical factor
 - Particle size of drug
 - Nature of the drug
 - Concentration of drug
 - Formulation
 - Physical state
 - Dosage form
 - Chemical nature of drug
- **Physiological factor**
 - **Membrane thickness:** Membrane thickness is inversely proportional to the absorption of the drug across the membrane. As the thickness of the lipid bi layer membrane increases the absorption of the drug decreases.
 - **Membrane surface area:** Drugs are better absorbs from large surface area such as pulmonary alveolar epithelium, intestinal mucosa etc. The area of absorbing surface depends to a greater extent on the route of administration.
 - **pH of gastrointestinal fluids:** In the stomach the weak acids e.g aspirin, phenobarbitone will be in the nonionized state hence these are lipid soluble and would be better absorbed than in the alkaline medium of the intestine. On the other hand weak bases e.g quinidine, ephedrine would be highly ionized in the stomach and poorly

absorbed from the stomach while it would be better absorbed in the intestine with alkaline medium.

- **Functional integrity of GIT:** Increased peristaltic movement as in diarrhea reduces the drug absorption. Gastro intestinal mucosal edema depresses the absorption of drug.
- **Gastric emptying time:** Enhance the gastric emptying time increase the rate of absorption in the GIT. E.g Metolopramide enhances the gastric emptying time.
- **Blood flow:** In shock, blood flow to intestine is decreased and absorption from theintestine is reduced.

- **Physiochemical factor**
- **Lipid solubility:** Drugs those are lipid soluble diffuse through the cell membrane more rapidly than the lipid insoluble drugs because the compatibility of the drug with the structure of lipid bi layer membrane.
- **pH of the medium:** In the stomach the weak acids e.g aspirin, phenobarbitone will be in the nonionized state hence these are lipid soluble and would be better absorbed than in the alkaline medium of the intestine. On the other hand weak bases e.g quinidine, ephedrine would be highly ionized in the stomach and poorly absorbed from the stomach while it would be better absorbed in the intestine with alkaline medium.
- **Ionization of drug:** Most drugs are weak acids or bases and are present in the solution in both ionized and unionized form. The nonionized being the lipid soluble diffuse across the cell membrane while ionized form being the lipid insoluble unable to penetrate the lipid membrane. The rate of diffusion of weak electrolytes is dependent on their degree of ionization. The greater the nonionized fraction greater will be the rate of diffusion.

For acid in acidic medium: $HA \leftrightarrow A^- + H^+$

For base in acidic medium: $BH^+ \leftrightarrow B + H^+$

- **Ionization coefficient:** The distribution of weak electrolyte is determined by its ionization coefficient pKa value and pH gradient across the membrane. When pKa of the drug is equal to pH of the medium then 50% of the drug will be ionized and 50% will be in the unionized state.

Dissociation of an acid: $(K_a = [A^-] [H^+] / [HA])$

Dissociation of an acid: $(K_a = [B] [H^+] / [BH^+])$

- **Partition coefficient:** Partition coefficient deals with motion of molecule across the cell membrane. Greater the ionization coefficient greater will be the partition coefficient and thus greater will be the diffusion. Relation between pH, pKa and ionized/unionized fractions can be depicted from Henderson

Hasselbalch equation:

For an acid:

For a base:

- **Protonated and Unprotonated form:** Protonated form of acid is well absorbed and in unionized form while protonated form of base is less absorbed because it is ionized form of base.

For acid:

Protonated form of acid and base: (HA or BH⁺) form

Unprotonated form of acid and base: (A or B) form

- **Pharmaceutical factor**
- **Particle size of drug:** Small water soluble molecules diffuse more efficiently across cell membrane through pores. The smaller the particle, the faster the rate of diffusion
- **Nature of the drug:** The drug administered in different dosage form having different rate of disintegration and dissolution those are limiting factor in absorption of drug particle.
- **Concentration of drug:** Drug ingested or injected in solution of high concentration are absorbed more rapidly than drug in low concentration.
- **Formulation:** Substances like sucrose, lactose, starch, are commonly used as excipients in formulating the drugs. These substances may effects the absorption.
- **Physical state:** liquids are better absorbed than solids and crystalloids are better absorbed than colloids.
- **Dosage form:** In case of tablet and capsule disintegration time and dissolution rate are important factor affecting the rate and extent of absorption.
- **Chemical nature of drug:** Inorganic iron preparation is better absorbed from GIT than organic preparations. Ferrous salts are better absorbed than ferric salts.

Q.no.3 Discuss determination of pharmacokinetic parameters from plasma data after drug administration by i.v. bolus and oral route.

ANSWER: 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 or three minutes for complete circulation.

The rate of drug presentation in body is expressed as

- In bolus injection absorption of drug is absent so available the equation is depicted as
- After applying integrations to the above it can be written

$$x = x_0 e^{-kt}$$

- Transforming the above equation

$$\log x = \log x_0 - K_{Et}/2.303$$

ESTIMATION OF PHARMACOKINE PARAMETERS BY I.V BOLUS

- From i.v bolus the elimination phase can be characterized three parameters they are:
 - Elimination rate constant.
 - Elimination half life.
 - Clearance.

- Elimination Rate Constant It can be expressed:

$$\log c = \log c_0 - K_{Et}/2.303$$

- The elimination rate constant is an additive property and they are said to be overall elimination constants

$$K_E = K_e + K_m + K_b + K_1 \dots$$

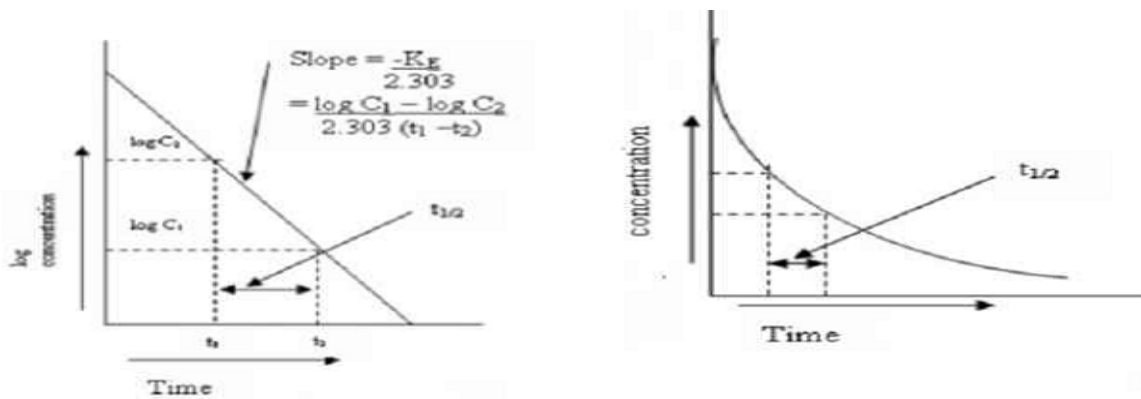
- The units of elimination rate constant are min^{-1} .
- Elimination half life can be also be called as biological half life. It can be defined as the time taken for the amount of drug in body to decline by one half or 50% of its initial

volume. It can be depicted as $t_{1/2}$.

$$t_{1/2} = 0.693/k$$

- A graph is plotted by taking log concentration versus time it gives a straight line and slope gives the elimination rate constant

$$\frac{-KE}{2.303} = \text{slope}$$



CLEARANCE:

- It is the most important parameter in drug clinical applications and is useful in evaluating the mechanism by which drug is eliminated.
- Clearance is a parameter that relates plasma drug concentration with rate of drug elimination from below equation

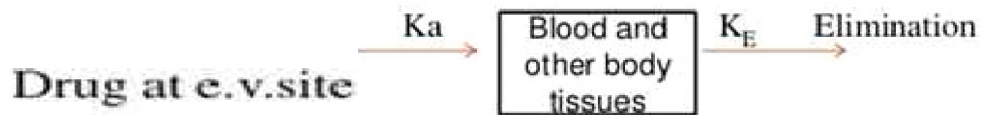
Clearance = rate of elimination / plasma drug concentration

$$\text{clearance} = \frac{dx/dt}{c}$$

- The clearance is expressed as ml/min or lit/hr.

FIRST ORDER ABSORPTION MODEL

- Whenever the drug enters into body it follows first order absorption process according to one compartment kinetics. The model can be represented as



first order absorption

The differential form of above equation can be

$$dx/dt = K_a X_a - K_e X$$

- Integrating the above equation

$$X = \frac{K_a F X_0}{(K_a - K_e)} [e^{-K_e t} - e^{-K_a t}]$$

- Transforming into c terms as $X = V_d C$

$$c = \frac{K_a F X_0}{(K_a - K_e) V_d} [e^{-K_e t} - e^{-K_a t}]$$

F=Fraction

of drug absorbed systematically after e.v. administration

- Here from the EV absorption study the t_{\max} and C_{\max} can be calculated

$$t_{\max} = \frac{2.303 \log\left(\frac{K_a}{K_e}\right)}{K_a - K_e} \quad C_{\max} = \frac{FX_0}{V_d} e^{-K_e t_{\max}}$$

METHOD OF RESIDUALS

- This method also called as feathering, peeling and stripping. For a drug that follows one compartment and it is a biexponential equation.

$$C = \frac{K_a FX_0}{V_d (K_a - K_e)} [e^{-K_e t} - e^{-K_a t}]$$

$$C = A e^{-K_e t} - A e^{-K_a t}$$

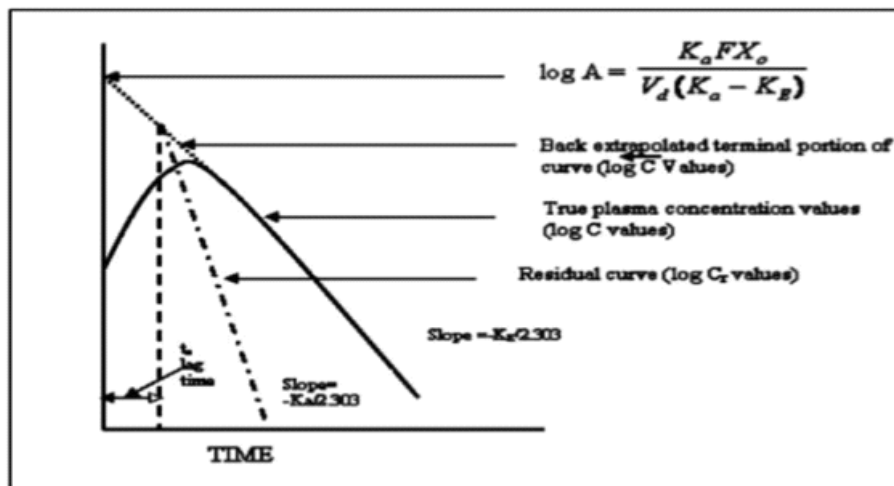
$$\frac{C}{A} = e^{-K_e t}$$

$$\log C = \log A - \frac{K_e t}{2.303}$$

DETERMINATION OF ABSORPTION RATE

- It is the important pharmacokinetic parameter when a drug follows first order absorption. It can be calculated by two methods in one compartment open model they are:

Graphical representation of method of residuals



- Subtraction of true values to extrapolated concentration that is residual concentration

$$C_r = A e^{-K_a t}$$

$$\log C_r = \log A - (-K_a t / 2.303)$$

- If $K_e/K_a \geq 3$ the terminal slope eliminates K_a and not K_e . The slope of residual lines gives K_e and not K_a
- This is also known as flip-flop kinetics as the slopes of two lines exchanged their meanings.

Extrapolated concentration (C_E)	Original concentration (C_0)	Residual concentration ($C_E - C_0$)
5.8mg/ml	2.5mg/ml	3.3mg/ml

Lag time:

- It is defined as the time difference between drug administration and start of absorption. It is denoted by t_0 .
- This method is best suited for drugs which are rapidly and completely absorbed and follow one compartment kinetics even when given i.v.

WAGNER NELSON METHOD

- One of the best alternatives to curve fitting method in the estimation of K_a is **Wagner Nelson method**.
- This method involves the determination of K_a from percent unabsorbed time plots and does not require the assumptions of zero or first order absorption.
- According to this the amount of drug in body is depicted as

$$X_A = X + XE$$

$$X_A^t = V_d C + V_d K [AUC]_0^t \quad (1)$$

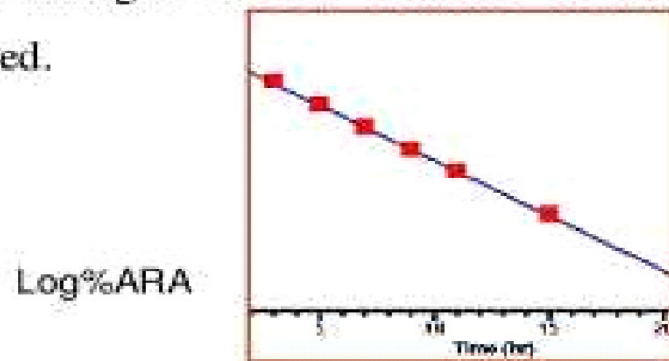
$$X_A^\infty = V_d C^\infty + V_d K [AUC]_0^\infty \quad (2)$$

$$\begin{aligned} \frac{(1)}{(2)} &= \frac{X_A^t}{X_A^\infty} = \frac{V_d C + V_d K [AUC]_0^t}{V_d K [AUC]_0^\infty} \\ &= \frac{X_A^t}{X_A^\infty} = \frac{C + K [AUC]_0^t}{K [AUC]_0^\infty} \end{aligned}$$

If the fraction of total amount of drug absorbed = 1

- Amount remaining to be absorbed = $1 - \frac{C + K[AUC]_0^t}{K[AUC]_0^\infty}$
- % amount remaining to be absorbed = $(1 - \frac{C + K[AUC]_0^t}{K[AUC]_0^\infty}) \cdot 100$
- A graph is plotted by taking log%ARA on Y-axis and time on

X-axis a straight line is obtained from that slope K_u is determined.



Q. No.5 Discuss the scope of clinical pharmacokinetics. How is dose adjusted in patients with renal failure?

Answer:

Clinically, the two most important applications of pharmacokinetic principles are:

- Design of an optimal dosage regimen, and
- Clinical management of individual patient and therapeutic drug monitoring.

Such applications permit the physician to use certain drugs more safely and sensibly.

DESIGN OF DOSAGE REGIMENS

Dosage regimen is defined as the manner in which a drug is taken. For some drugs like analgesics, hypnotics, antiemetics, etc., a single dose may provide effective treatment. However, the duration of most illnesses is longer 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 successful 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.

In designing a dosage regimen—

- 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.

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—

- I. The dose size—the quantity of drug administered each time, and
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_{ssm} and C_{mjn} during each dosing interval and greater the chances of toxicity (Fig.).

2. The calculations are based on open one-compartment model which can also be applied to two compartment model if B is used instead of KE , and V_5 instead of V_d while calculating the regimen.

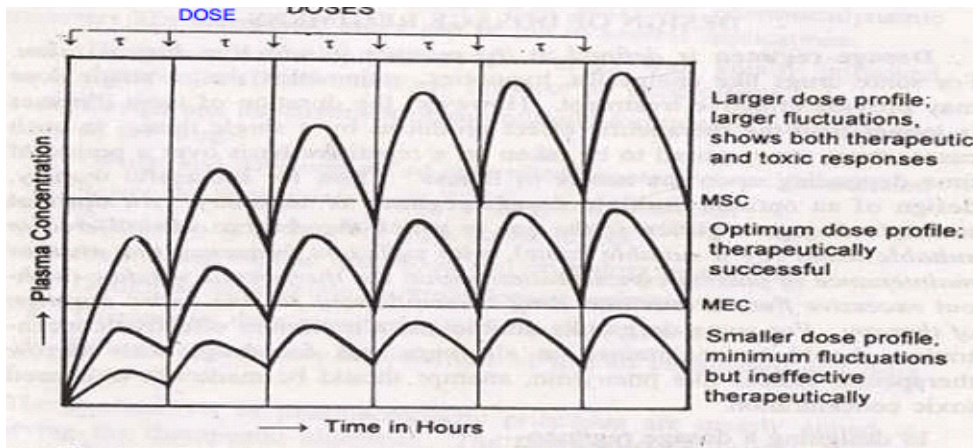


Fig. Schematic representation of influence of dose size on plasma concentration-time profile after oral administration of a drug

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_{ay} decrease but the ratio C_m/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 .

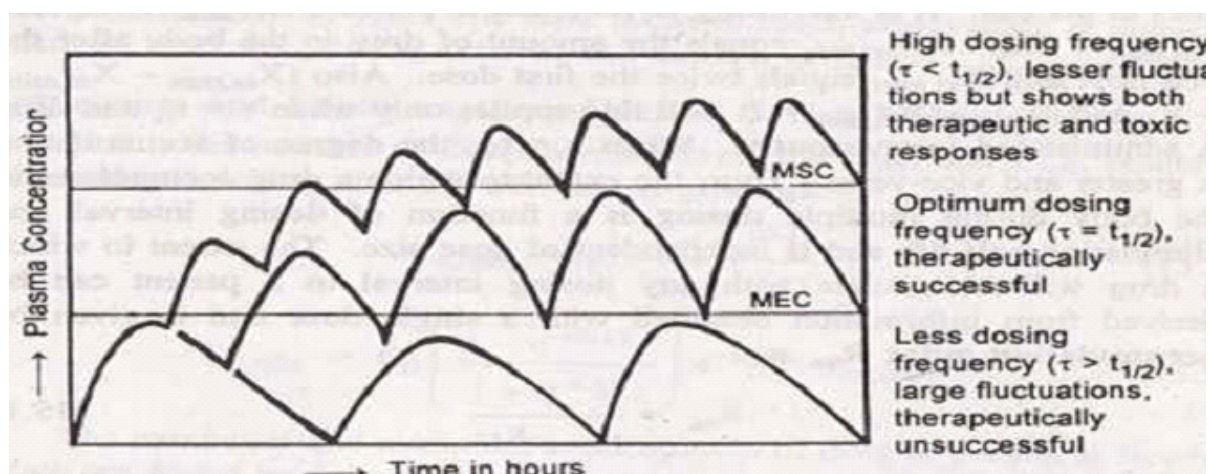


Fig. 13.2 Schematic representation of the influence of dosing frequency on plasma concentration-time profile obtained after oral administration of fixed doses of a drug.

MONITORING DRUG THERAPY

Management of drug therapy in individual patient often requires evaluation of response of the patient to the recommended dosage regimen. This is known as monitoring of drug therapy. It is necessary to ensure that the therapeutic objective is being attained and failure to do so requires readjustment of dosage regimen.

Depending upon the drug and the disease to be treated, management of drug therapy in individual patient can be accomplished by:

- I. Monitoring therapeutic effects—therapeutic monitoring
2. Monitoring pharmacologic actions—pharmacodynamic monitoring
3. Monitoring plasma drug concentration—pharmacokinetic monitoring.

Therapeutic Monitoring

In this approach, the management plan involves monitoring the incidence and intensity of both the desired therapeutic effects and the undesired adverse effects. A direct measure of the desired effect is considered as a therapeutic endpoint e.g. prevention of an anticipated attack of angina or shortening of duration of pain when attack occurs through the use of sublingual glyceryl

trinitrate. In certain cases, definition of therapeutic endpoint may not be clear and onset of toxicity is used as a dosing guide i.e. dosage regimen is titrated to a toxic endpoint e.g. excessive dryness of mouth with atropine when used as an antispasmodic agent.

Pharmacodynamic Monitoring

In some instances, the pharmacologic actions of a drug can be measured and used as a guide to therapeutic process. The response observed may or may not correlate exactly with the therapeutic effect e.g. blood glucose lowering with insulin, lowering of blood pressure with antihypertensives, enhancement of hemoglobin levels with hematinics, etc.

Pharmacokinetic Monitoring

This approach involves monitoring the plasma drug concentration within a target concentration range (called as target concentration strategy) and based on the principle that free drug at the site of action is in equilibrium with the drug in plasma. The strategy is particularly useful when:

1. Therapeutic endpoints are difficult to define or are lacking e.g. control of seizures with phenytoin.
2. The objective is to maintain the therapeutic effect in order to obtain optimum drug use.
3. The probability of therapeutic failure is high as with drugs having low therapeutic indices, erratic absorption, pharmacokinetic variability or when the drug is used in multiple drug therapy.

Successful application of this approach requires complete knowledge of pharmacokinetic parameters of the drug, the situation in which these parameters are likely to be altered and the extent to which they could be altered, and a sensitive, specific and accurate analytic method for determination of drug concentration. Examples of drugs monitored by this method include digoxin, phenytoin, gentamicin, theophylline, etc. The strategy is very useful in individualizing therapy in patients with hepatic or renal impairment when dose adjustments are necessary.

Dosing of Drugs in Renal Disease

In patients with renal failure, the half-life of a drug is increased and its clearance drastically

decreased if it is predominantly eliminated by way of excretion. Hence, dosage adjustment should take into account the renal function of the patient and the fraction of unchanged drug excreted in urine. One such method was discussed in chapter 7 on Excretion of Drugs.

There are two additional methods for dose adjustment in renal insufficiency if the Vd change is assumed to be negligible. These methods are based on maintaining the same average steady-state concentration during renal dysfunction as that achieved with the usual multiple dosage regimen and normal renal function.

1. Dose adjustment based on total body clearance : writing equation, the parameters to be adjusted in renal insufficiency are shown below:

$$C_{ss,av} = F \times \frac{1}{Cl_T} \times \left(\frac{X_0}{\tau} \right)$$

\uparrow \uparrow \uparrow \uparrow
 to be kept assumed decreased needs
 normal constant due to disease adjustment

If Cl_T' , X_0' and τ' represent the values for the renal failure patient, then the equation for dose adjustment is given as:

$$C_{ss,av} = \frac{X_0}{Cl_T \tau} = \frac{X_0'}{Cl_T' \tau'}$$

Rearranging in terms of dose and dose interval to be adjusted, the equation is:

$$\frac{X_0'}{\tau'} = \frac{Cl_T' X_0}{Cl_T \tau}$$

From above equation, the regimen may be adjusted by reduction in dosage or increase in dosing interval or a combination of both.

2. Dose adjustment based on elimination rate constant or half-life : writing equation, the

parameters to be adjusted in renal insufficiency are:

$$C_{ss,av} = \frac{1.44 F}{V_d} \times t_{1/2} \times \left(\frac{X_0}{\tau}\right)$$

\uparrow to be kept normal \uparrow assumed constant \uparrow increased due to disease \uparrow needs adjustment

If $t_{1/2}$, X_0' and τ' represent the values for the renal failure patient, then:

$$C_{ss,av} = \frac{t_{1/2} X_0}{\tau} = \frac{t_{1/2}' X_0'}{\tau'}$$

Rearranging the above equation in terms of dose and dose interval to be adjusted, we get:

$$\frac{X_0'}{\tau'} = \frac{t_{1/2} X_0}{t_{1/2}' \tau}$$

Because of prolongation of half-life of a drug due to reduction in renal function, the time taken to achieve the desired plateau takes longer the more severe the dysfunction. Hence, such patients sometimes need loading dose.

Q. no. 6. Write short notes on: (any two)

- Mechanism of renal clearance
- Protein binding of drug
- Biliary excretion

• **MECHANISM OF RENAL CLEARANCE**

Clearance :

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

particular organ. Just as V_d is needed to relate plasma drug concentration with amount of drug in the body, clearance is a parameter to relate plasma drug concentration with the rate of drug elimination according to following equation:

Clearance = Rate of elimination / Plasma drug concentration

$$Cl = \frac{dX/dt}{C}$$

Or

Clearance is defined as the theoretical volume of body fluid containing drug (i.e. that fraction of apparent volume of distribution) from which the drug is completely removed in a given period of time. It is expressed in ml/min or liters/hour. Clearance is usually further defined as blood clearance (Cl_b), plasma clearance (Cl_p) or clearance based on unbound or free drug concentration (C) depending upon the concentration C measured for the right side of the equation

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 $Cl_R = \text{Rate of elimination by kidney} / C$

Other Organ Clearance

$Cl_{\text{other}} = \text{Rate of elimination by other organs} / C$

The total body clearance, Cl_{total} , also called as total systemic clearance, is an additive property of individual organ clearances. Hence,

Total Systemic Clearance, $Cl_T = Cl_R + Cl_H + Cl_{\text{other}}$

Because of the additivity of clearance, the relative contributor by any organ in eliminating a drug can be easily calculated. Clearance by all organs other than kidney is sometimes known as nonrenal clearance Cl_{NR} . It is the difference between total clearance and renal clearance.

According to an earlier definition

$$Cl_T = \frac{dX/dt}{C}$$

Substituting $dX/dt = K_E X$ from equation $dX / dt = - K_E X$ in equation, we get:

$$Cl_T = K_E X / C$$

Since $X / C = V_d$ can be written as:

$$Cl_T = K_E V_d$$

Parallel equations can be written for renal and hepatic clearances as:

$$Cl_R = K_e V_d$$

$$Cl_H = K_m V_d$$

Since $K_E = 0.693/t_{1/2}$, clearance can be related to half-life by the following equation:

$$Cl_T = 0.693 V_d / t_{1/2}$$

The noncompartmental method of computing total clearance for a drug that follows one-compartment kinetics is:

For drugs given as i. v. bolus,

$$Cl_T = X_0 / AUC$$

For drugs administered e.v.

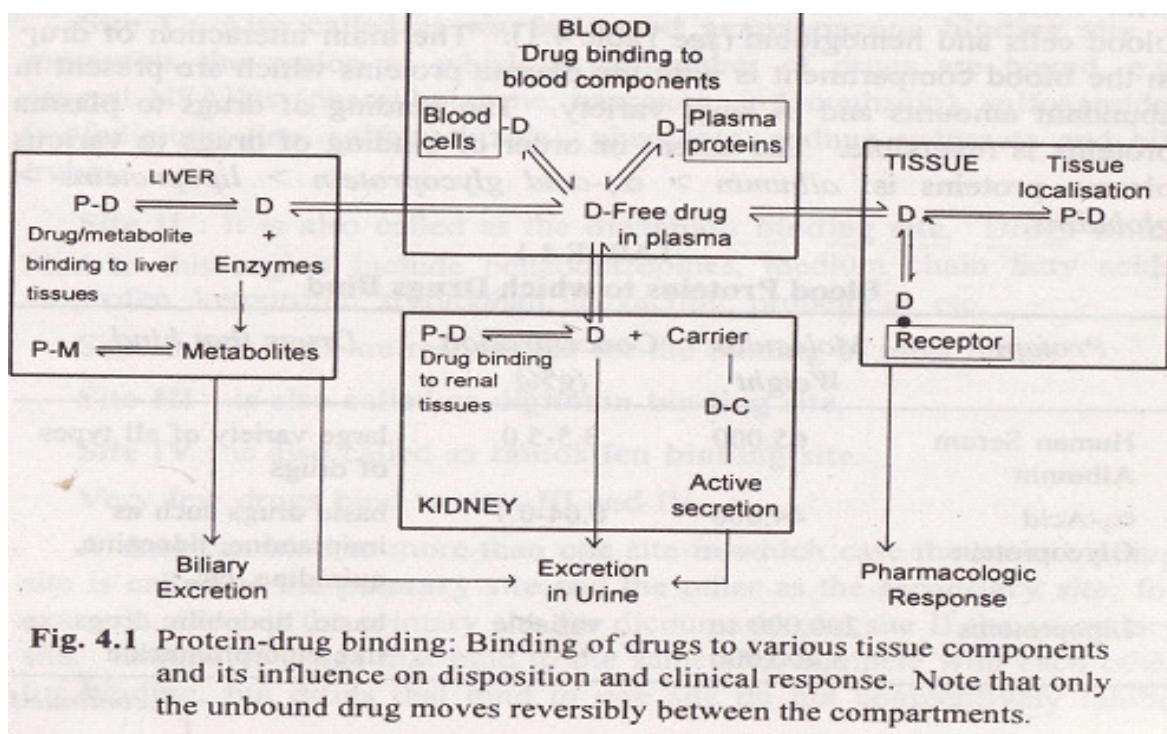
$$Cl_T = F X_0 / AUC$$

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.

$$Cl_R = X_u^\infty / AUC$$

- **PROTEIN BINDING OF DRUGS**

A drug in the body can interact with several tissue components of which the two major categories are blood and extra vascular tissues. The interacting molecules are generally the macromolecules such as proteins, DNA or adipose. The proteins are particularly responsible for such an interaction. The phenomena of complex formation with proteins are called as protein binding of drugs. The importance of such a binding derives from the fact that the bound drug is both pharmacokinetically as well as pharmacodynamically inert i.e. a protein bound drug is neither metabolized nor excreted nor is it pharmacologically active. A bound drug is also restricted since it remains confined to a particular tissue for which it has greater affinity. Moreover, such a bound drug, because of its enormous size, cannot undergo membrane transport and thus its half-life is



Plasma Protein-Drug Binding

Following entry of a drug into the systemic circulation, the first thing with which it can interact are blood components like plasma proteins, blood cells and hemoglobin (see Table 4.1). The main interaction of drug in the blood compartment is with the plasma proteins which are present in abundant amounts and in large variety. The binding of drugs to plasma proteins is reversible. The extent or order of binding of drugs to various plasma proteins is:

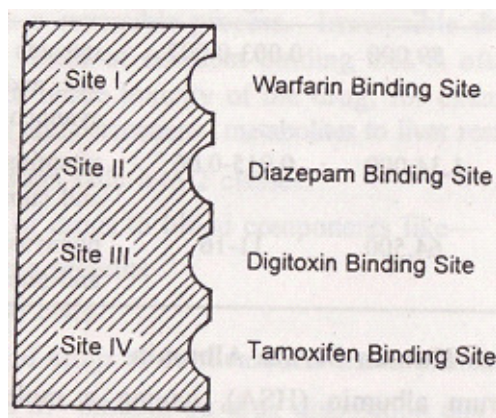
albumin > α_1 -acid glycoprotein > lipoproteins > globulins.

Blood Proteins to which Drugs Bind			
<i>Protein</i>	<i>Molecular Weight</i>	<i>Concentration (g%)</i>	<i>Drugs that bind</i>
Human Serum Albumin	65,000	3.5-5.0	large variety of all types of drugs
α_1 -Acid Glycoprotein	44,000	0.04-0.1	basic drugs such as imipramine, lidocaine, quinidine, etc.
Lipoproteins	200,000 to 3,400,000	variable	basic, lipophilic drugs like chlorpromazine

<i>Protein</i>	<i>Molecular Weight</i>	<i>Concentration (g%)</i>	<i>Drugs that bind</i>
α_1 -Globulin	59,000	0.003-0.007	steroids like corticosterone, and thyroxine and cyanocobalamin
α_2 -Globulin	1,34,000	0.015-0.06	vitamins A, D, E and K and cupric ions
Hemoglobin	64,500	11-16	phenytoin, pentobarbital, and phenothiazines

Binding of Drugs to Human Serum Albumin

The human serum albumin (HSA), having a molecular weight of 65,000, is the most abundant plasma protein (59% of total plasma and 3.5 to 5.0 g%) with a large drug binding capacity. The therapeutic doses of most drugs are relatively much smaller and their plasma concentrations do not normally reach equimolar concentration with HSA. The HSA can bind several compounds having varied structures. Both endogenous compounds such as fatty acids, bilirubin and tryptophan as well as drugs bind to HSA. A large variety of drugs ranging from weak acids, neutral compounds to weak bases bind to HSA. Four different sites on HSA have been identified for drug-binding. They are:



Different Sites on HSA Have Been Identified

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 (phenylbutazone, naproxen, indomethacin), sulfonamides (sulfadimethoxine, sulfamethizole), phenytoin, sodium valproate and bilirubin.

Site II: It is also called as the diazepam binding site. Drugs which bind to this region include benzodiazepines, medium chain fatty acids, ibuprofen, ketoprofen, tryptophan, cloxacillin, probenecid, etc.

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

Site III : is also called as digitoxin binding site.

Site IV : is also called as tamoxifen binding site.

Very few drugs bind to sites III and IV.

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

Also called as the **orosomucoid**, it has a molecular weight of 44,000 and a plasma concentration range of 0.04 to 0.1 g%. It binds to a number of basic drugs like imipramine, amitriptyline, nortriptyline, lidocaine, propranolol, quinidine and disopyramide.

Binding of Drugs to Lipoproteins

Binding of drugs to HSA and AAG involve hydrophobic bonds. Since only lipophilic drugs can undergo hydrophobic bonding, lipoproteins can also bind to such drugs because of their high lipid content. However, the plasma concentration of lipoproteins is much less in comparison to HSA and AAG.

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. The molecular weights of lipoproteins vary from 2 kDa to 34 kDa depending on their chemical composition. They are classified on the basis of their density.

The 4 classes of lipoproteins are:

1. Chylomicrons

2. Very low density lipoproteins (VLDL)
3. Low density lipoproteins (LDL) (predominant in humans)
4. High density lipoproteins (HDL)

The lipid core of these macromolecules consists of triglycerides and cholesterol esters and the outside is made of apoproteins (free cholesterol and protein)

Binding of Drugs to Globulins

Several plasma globulins have been identified and are labeled as α_1 -, α_2 -, Pr, P2- and γ -globulins.

1. α_1 -globulin : also called as transcortin or CBG (corticosteroid binding globulin), it binds a number of steroidal drugs such as cortisone and prednisone. It also binds to thyroxin and cyanocobalamin.
2. α_2 -globulin: • also called as ceruloplasmin, it binds vitamins A, D, E and K and cupric ions.
3. β_1 -globulin: also called as transferrin, it binds to ferrous ions.
4. β_2 -globulin: binds to carotinoids
5. γ -globulin: binds specifically to antigens.

• **BILIARY 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 1 to 1.5 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 resecretion. The rest is excreted in feces.

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 3 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, sulfobromophthalein 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 the Table 7.3.

Polarity is the other physicochemical property of drug influencing biliary excretion. Greater the polarity, better the excretion. Thus, metabolites are more excreted in bile than the parent drugs 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.

Influence of Molecular Weight on Excretion Behavior of Drugs	
<i>Molecular Weight of Drug/Metabolite</i>	<i>Excretion Pattern</i>
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

The ability of liver to excrete the drug in the bile is expressed by biliary clearance equation.

Biliary clearance = Biliary excretion rate / Plasma drug concentration

$$\frac{\text{Bile flow} \times \text{Biliary drug concentration}}{\text{Plasma drug concentration}}$$

Dr. ALPANA RAM

Assoc. Professor

Pharmacy