Model Answer
B.Pharm. VII sem, Examination 2013
Biopharmaceutics and Pharmacokinetics
Paper code: AS-2532

Section A: Short Answer

1.

i) Objective of bioavailability studies
- Primary stages of development of a suitable dosage form for a new drug entity.
- Determination of influence of excipients, patient related factors and possible interaction with other drugs on the efficiency of absorption.
- Development of new formulations of the existing drugs
- 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.

ii) Pharmaceutic equivalence: this term implies that two or more drug products are identical in strength, quality, purity, content of uniformity and disintegration and dissolution characteristics they may however differ in containing different excipients.

iii) Advantage of urinary excretion data in pharmacokinetic analysis
- The method is useful when there is lack of sufficiently sensitive analytic techniques to measure concentration of drugs in plasma with accuracy.
- The method is noninvasive and therefore better subject compliance is assured.
- Convenience of collecting urine samples in comparison to drawing of blood periodically.
- Often a less sensitive analytic method is required for determining urine drug concentration as compared to plasma concentrations; if urine drug concentration are low, assaying of larger sample volumes is relatively easy.
- First order elimination, excretion and absorption rate constants and fraction excreted unchanged can be computed from such data; first order metabolism or extrarenal excretion rate constant can also be calculated subsequently from the difference.
- Direct measurement of bioavailability both absolute and relative is possible without the necessity of fitting the data to a mathematical model.
- When coupled with plasma level time data it can also be used to estimate renal clearance of unchanged drug according to following equation
  Cl_R = total amount of drug excreted unchanged/ area under the plasma level time curve

iv) Drugs retard gastric emptying: antacids (aluminum hydroxide), anticholinergics (atropine, propantheline), narcotic analgesics (morphine), tricyclic antidepressants (imipramine, amitryptyline).

v) Chemical equivalence: it indicates that two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

vi) Therapeutic window: the drug fails to elicit a therapeutic response when the concentration is below the effective level precipitates adverse reactions when above toxic level. The plasma drug concentration between these two limits is called as the therapeutic concentration or therapeutic window.

vii) 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.

viii) Renal clearance: 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.
ClR = rate of urinary excretion/ plasma drug concentration

ix) Healthy human volunteers are used to establishing a standard set of conditions necessary for a bioavailability study. Such studies are therefore usually performed in young (20-40 years) healthy male adult volunteers under restricted dietary and fixed activity conditions. Female volunteers are used only when drugs such as oral contraceptives are to be tested.

x) Apparent volume of distribution:

$$X \propto C$$

$$X = Vd\ C$$

Where, C concentration of drug in plasma, X amount of drug in the body and Vd= proportionality constant having the unit of volume and popularly called as **Apparent volume of distribution.** It is **defined** as the hypothetical volume of the body fluid into which a drug is dissolved or distributed.

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

xi) Sink condition- the continuous renewal of solvent carrying drugs with fresh solvent is called sink condition. In the sink condition the concentration gradient is always maintained as the concentration in bulk remains negligible. This sink condition thus helps in faster diffusion and dissolution and thus absorption of drug in the body. The sink condition is provided by continuous blood circulation in the body.

xii) Classify body tissues on the basis of perfusion rate

Highly perfused	**Moderately perfused**	**Poorly perfused**
Lungs	Muscles	Fat (adipose)
Kidney	Skin	Bone (skeleton)
Adrenals		
Liver		
Heart		
Brain		

Section B: Long Answer

2. Factors affecting drug absorption

i). Physicochemical properties of drug substances

1. Drug solubility & dissolution rate
2. Particles size & effective surface area
3. Polymorphism & amorphism
4. Solvates & hydrates
5. Salt form of drug
6. lipophilicity of the drug
7. pKa & pH of the drug
8. drug stability
9. Ionization state

ii). Formulation Factors

1. Disintegration time
2. Manufacturing variables
   a. Method of granulation b. Compression force
3. Nature & type of dosage form
4. Pharmaceutical ingredients
5. Product age & storage conditions

Discuss in detail above factors with suitable examples:

3. Factors affecting protein drug binding and significance of protein binding

DRUG RELATED FACTORS

(a) Physiochemical characteristics of the drug.

Lipophilicity is the most desirable physiochemical parameter that is perquisite for protein binding to occur. Also an increase in the lipid content of drug moiety eventually enhances the rate as well as extends of protein binding process. As observed in case of intramuscular injection of cloxacillin as attributed to greater lipophilicity displays 95% protein binding.

(b) Concentration of drug in the body.

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

(c) Drug's affinity towards protein/tissue.

This factor entirely depends upon the degree of attraction or affinity the protein molecule or tissues have towards drug moieties. For ex. Digoxin has more affinity for cardiac muscles proteins as compared to that of proteins of skeletal muscles or those in the plasma like HSA.

PROTEIN/TISSUE RELATED FACTORS

(a) Physicochemical characteristics of the protein or binding agent.

An increase in lipophilicity increases the extent of binding; for example, the slow absorption of cloxacillin in comparison to amoxicillin after i.m. injection is attributed to its higher lipophilicity and larger (95%) binding to proteins while the latter is less lipophilic and just 20% bound to proteins.

(b) Concentration of protein/binding component.

This is the most important tissue related parameter to be given priority. As the human serum plasma proteins constitute the major portion of the plasma proteins, a large number of drugs undergo an extensive binding with them as compared to the concentration of other protein molecule.

(c) Number of binding sites on the protein

In association to the concentration of proteins molecules available the number of binding sites available in the protein molecules is also significant. Albumin not only possesses large number of binding sites but also has greater potential of carrying out binding process. Numerous drug exhibit multiple site binding with albumin molecules in plasma like fluocloxacillin, ketoprofen, indomethacin etc.

DRUG INTERACTIONS

(a) Competitive binding of drugs.

Displacement interactions are predominant ones among these reactions. In case where two or more drugs have same or identical affinity for a same site then they struggle with one another to bind at the same site. Consider a drug I is bound to a specific site on the molecule and if a second drug called as Drug II is administered now, then the drug meaty having greater affinity towards the bound site would effectively displace the former drug. This phenomenon is said to be Displacement reaction. The drug which is been removed from its binding site is said to be displaced drug while the one that does the displacement is called as displacer.

The best example for such interactions is the competitive protein binding that occurs between Warfarin and phenylbutazone for HSA, as both are potent binders of HAS, where phenylbutazone is displacer while warfarin is displaced. Clinically such reaction acquire importance when the

displaced drug (any) is more than 95% bound to plasma proteins, or occupies small volume of distribution even less than that of 0.15 L/Kg. also when the active drug or the administered pharmacological agent possess narrow therapeutic index. Such situation may also develop in case the displacer drug has greater affinity or at the same time the drug/protein concentration ratio is very high and exhibits a very rapid and significant increase in the plasma concentration of drug.

(b) Competition between drugs and normal body constituents

Among the various normal body constituents, 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 physiologic (fasting), pathologic (diabetes, myocardial infarction, alcohol abstinence) and pharmacologically induced conditions (After heparin and caffeine administration).

(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.

PATIENT RELATED FACTORS

Patients related factors have their own importance after all the drug has to generate its response on to the administered patient. In this numerous parameters are taken into account like Age, diseased state, pharmacokinetic and Pharmacodynamic characteristics.

(a) Age: Protein content and its specific type greatly varies with the age factor. As observed the **neonates** or newly born babies have very low levels of albumins in the plasma thereby resulting in rebound concentration of drug that is primarily bind to albumin is a major shortcoming. As far as elderly patients are concerned the albumin levels goes down while the concentration of AAG is high enough.

(b) Disease states: the alterations in protein content and thereby the rate and extend of protein binding is greatly influenced by the albumin which is the major drug binding protein. This may ultimately lead to hypoalbuminemia which eventually with the pace of time completely impairs the entire protein drug binding process. For such situations the basic pathological conditions of diseases like trauma, burns, renal, cardiac or hepatic failure etc are largely responsible. Pharmacokinetics as well as Pharmacodynamic of drugs greatly influences the distribution,

clearance and thus the biotransformation of drugs to a greater extend. Usually an increased potential of toxicity is observed due to increased concentration of free or the unbound drug.

(c) Intersubject variations: These differences have been attributed to genetic and environmental factors.

Discuss in detail above factors with suitable examples.

Significance of protein binding of drugs - As a protein bound drug is neither metabolized nor excreted hence it is pharmacologically inactive due to its pharmacokinetic and Pharmacodynamic inertness. A bound drug always remains confined to a specific tissue or to a particular site with which it possess a greater affinity. The major benefit is the prolonged duration of action of drug as the protein bound-enormous size of drug complex cannot undergo membrane transport.

4. Assessment of bioavailability

There are several methods for bioavailability assessment in humans and the selection of a method depends upon the purpose of the study, nature of the dosage form and the analytical method of drug measurement.

Methods for bioavailability measurement

A. Pharmacokinetic methods (Indirect method)
i) Plasma level-time studies
ii) Urinary excretion studies
B. Pharmacodynamic methods (direct method)
i) Acute pharmacologic response
ii) Therapeutic response

Discuss in detail these Pharmacokinetic methods with suitable procedure, equations and schematics

i) Plasma level-time studies
   a) C_{max} b) t_{max} c) AUC
ii) Urinary excretion studies
   a) Maximum urinary excretion rate $(dXu/dt)_{max}$
   b) Time for maximum excretion rate $(tu)_{max}$
   c) Cumulative amount of drug excreted in the urine (X_u)

5. Mechanisms of drug transport

Discuss in detail these with suitable equations, examples and schematics

The principal mechanism for transport of drug molecules to cross the cell membranes in order to their importance is as follows:

a. Passive diffusion
b. Pore transport
c. Facilitated diffusion
d. Active transport
e. Ionic or electrochemical diffusion
f. Ion paired transport
g. Endocytosis

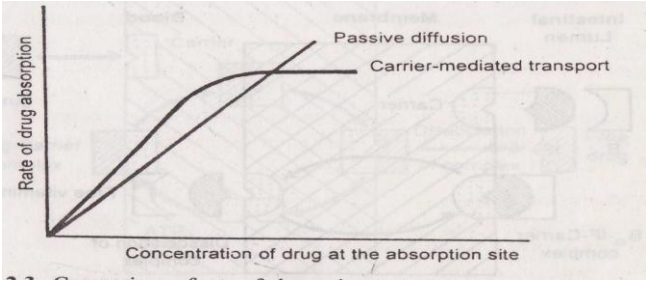
Passive diffusion: also called as non ionic diffusion. It is the major process for absorption of more than 90% of the dose. The driving force for this process is the concentration or electrochemical gradient. It is defined as the difference in the drug concentration on either side of the membrane. It is expressed by fick's first law of diffusion

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

It is energy independent and nonsaturable but depend to a lesser extent on the square root of the molecular size of the drug. The molecular sizes of the drugs lie between 100-400 D.

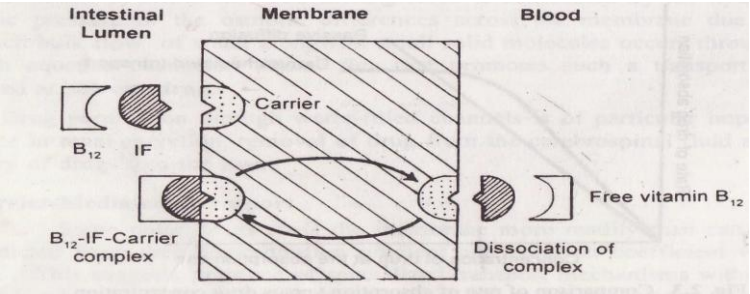
Pore transport: also called as convective transport, bulk flow or filtration. The process is important in the absorption of low molecular weight, low molecular size and generally water soluble drugs through narrow aqueous filled channels or pores in the membrane structure for example urea, water and sugar. Chain like or linear compounds of molecular weight upto 400 daltons can be absorbed by filtration.

Carrier mediated transport: the mechanism is thought to involve a component of the membrane called as carrier that binds reversibly or noncovalently with the solutes molecules to be transported. This carrier solute complex traverses across the membrane to the other side where it dissociates and discharge the solute molecules. The carrier then returns to the its original site to complete the cycle by accepting a fresh molecule of solute. The carrier may be an enzyme or some other component of the membrane.

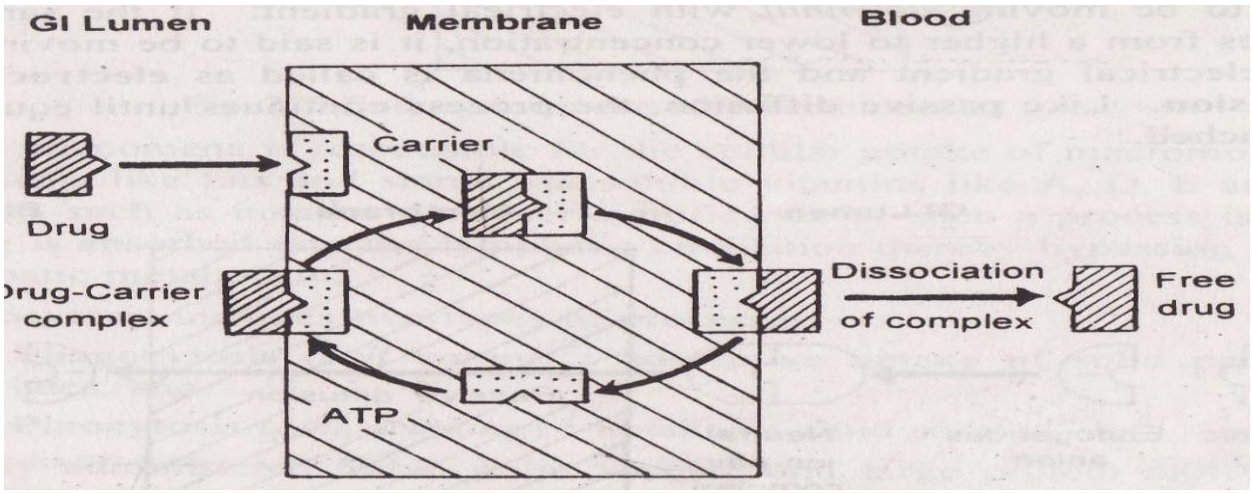


Facilitated diffusion: it is carrier mediated transport system that operates down the concentration gradient but at a much a faster rate than can be accounted by simple diffusion. No energy

expenditure is involved. The process is not inhibited by metabolic poisons that interfere with the energy production. Examples: entry of glucose into RBCs and intestinal absorption of vit. B_1 and B_2. A classic example of passive facilitated diffusion is GI absorption of vit B_{12}.

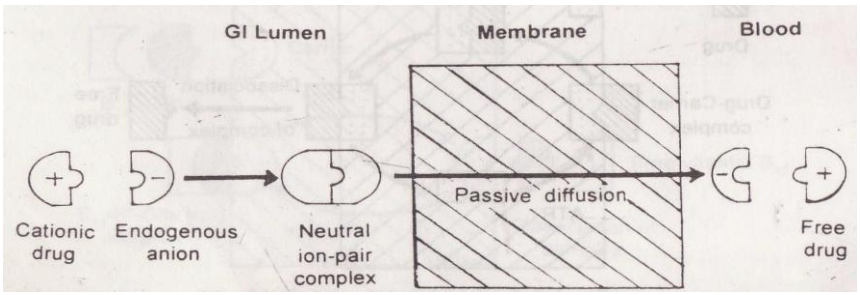


Active transport: the drug is transported from a region of lower to one of a region of higher concentration. Since the process is uphill energy is required in the work done by the carrier. Endogenous substances that are transported actively include Na, K, Ca, Fe, glucose, certain amino acids and vitamins like niacin, pyridoxine and ascorbic acid.



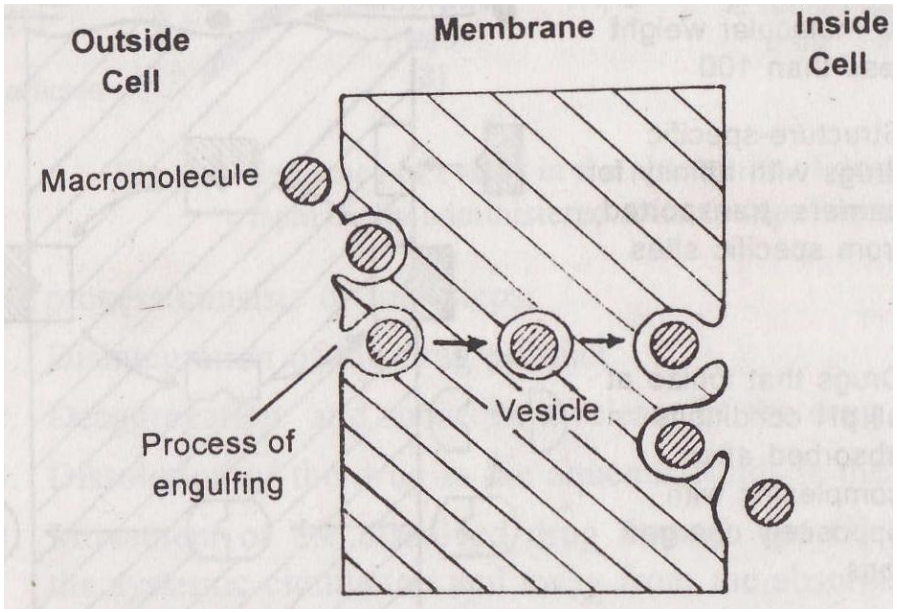
Ionic or electrochemical diffusion: the charge on the membrane influence the permeation of the drugs, molecular forms of the solute are unaffected by the membrane charge and permeate faster than the ionic forms. Of the ionic forms the anionic solute permeates faster than the cationic form. Thus at a given pH the rate of permeation is in the following order- unionized molecules > anions > cations. The permeation of ionized drugs particularly the cationic drugs depends on the potential difference or electrical gradient as the driving force across the membrane.

Ion paired transport: despite their low o/w partition coefficient value such agents penetrate the membranes 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 paired transport.

Endocytosis: it is a minor transport mechanism which involves engulfing extra cellular materials within a segment of a cell membrane to form a saccule or vesicle which is then pinched off intracellularly. This phenomenon is responsible for the cellular uptake of macromolecular nutrients like fats and starch, oil soluble vitamins like A, D, E, K and drugs such as insulin. It includes two types of process- phagocytosis (cell eating) and pinocytosis (cell drinking).



6. The schematic representation of drug movement in the body in case of IV bolus administration is shown by the equation

$$\frac{dX}{dt} = Rate\ in\ (availability) - Rate\ out\ (elimination)$$

Since rate in or absorption is absent, the equation can be written as:

$$\frac{dX}{dt} = -Rate\ out$$

If the elimination follows first order kinetics

$$\frac{dX}{dt} = -K_E X$$

Where, X = amount of drug present in the body at any time t

K_E = apparent overall first order elimination rate constant

Negative sign indicates that the drug is being lost from the body. This equation is used for the estimation of various pharmacokinetic parameters.

Elimination rate constant: integration of above equation results into

$$In\, X = In\, X_0 - K_E t$$

Where, X_0 = amount of drug at time 0

In exponential form the equation can be written as

$X = X_0\, e^{-K_E t}$ (Monoexponential)

In logarithmic form the equation can be written as

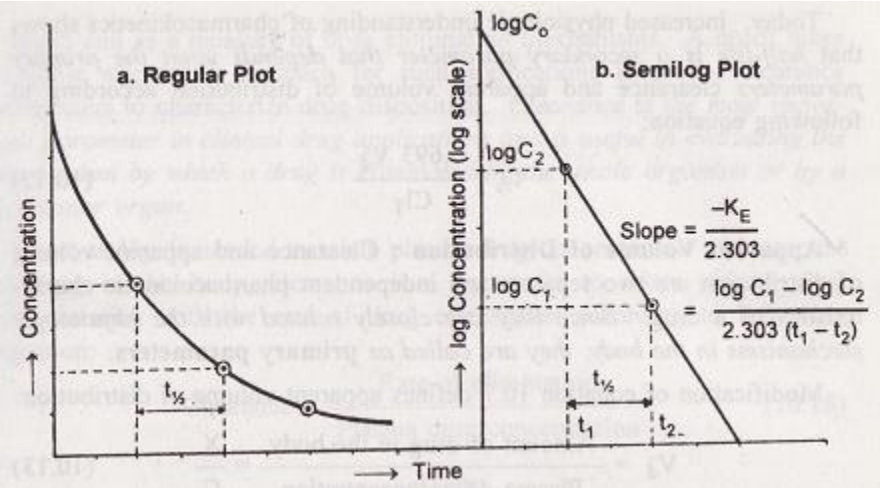
$$\log X = \log X_0 - \frac{K_E t}{2.303}$$

Since the estimation of amount of drug in the body is difficult, the equation is transformed in the terms of plasma drug concentration (C)

$\log C = \log C_0 - \frac{K_E t}{2.303}$(X = V_dC and V_d is constant)

Where, C_0 = plasma drug conc. immediately after I.V bolus administration.

A plot between log C and time on semi-logarithmic scale gives a straight line with a slope of –K/2.303.



Thus, C_0 and K_E can be readily obtained from log C versus t graph.

Biological Half-life ($t_{1/2}$): the time required for conc. to fall a half of its initial value.

$$\log C = \log C_0 - \frac{K_E t}{2.303}$$

At, $t_{1/2}$ $C = C_0/2$

$$\log \frac{C_0}{2} = \log C_0 - \frac{K_E t_{1/2}}{2.303}$$

$$\log C_0 - \log 2 = \log C_0 - \frac{K_E t_{1/2}}{2.303}$$

$$\log 2 = \frac{K_E t_{1/2}}{2.303}$$

$$0.3 = \frac{K_E t_{1/2}}{2.303}$$

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

Apparent volume of distribution (V_d):V_d can be expressed as

$$V_d = \frac{amount\ of\ drug in\ the body}{plasma\ drug\ concentration} = X/C$$

For I.V bolus administration

$$V_d = \frac{X_0}{C_0} = \frac{I.V\ bolus\ dose}{C_0}$$

Clearance: it is defined as the theoretical volume of body fluid containing drug from which the drug is completely removed in a given period of time. As only Vd is needed to relate plasma drug conc. with amount of drug in the body, clearance is a parameter to relate plasma drug conc. with the rate of drug elimination as per the equation given below:

$$Clearance = \frac{rate\ of\ elimination}{plasma\ drug\ conc.}$$

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

Total body clearance: elimination of a drug from body involves processes in kidney, liver, lungs and other eliminating organs. Hence,

Total body clearance = Renal clearance + hepatic clearance + other organs clearance

$$Cl_T = Cl_R + Cl_H + Cl_{others}$$

According to the definition of clearance

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

$$Cl_T = \frac{K_E\, X}{C}$$

$$Cl_T = K_E V_d$$

For renal clearance,

$$Cl_R = K_E V_d$$

For hepatic clearance,

$$Cl_H = K_m V_d$$

Since $K_E = 0.693/t_{1/2}$

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

Excretion rate method: urinary excretion of a drug follow first order kinetics, the rate of appearance of unchanged drug in urine is proportional to the amount of drug in the body.

$$\frac{dXu}{dt} \propto X$$

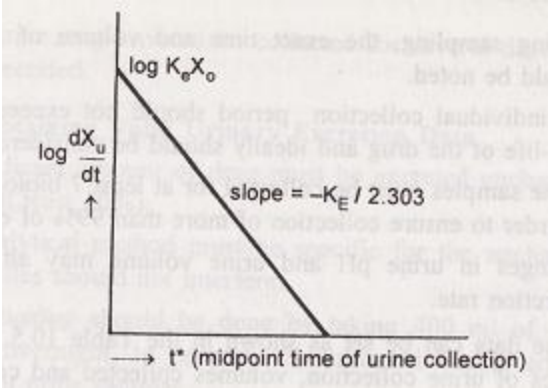
$$\frac{dXu}{dt} = K_e X$$

Since, $X = X_0\, e^{-K_E t}$

$$\frac{dXu}{dt} = K_e X_0\, e^{-K_E t}$$

In logarithmic form the equation can be written as

$$\log(\frac{dXu}{dt}) = \log K_e X_0 - \frac{K_E t}{2.303}$$



Sigma minus method: Which requires an accurate assessment of total amount of the drug or metabolite excreted in the urine.

$$\frac{dXu}{dt} = K_e X_0 \, e^{-K_E t}$$

On Integration

$$Xu = \frac{K_e X_0}{K_E}(1 - e^{-K_E t})$$

As time approaches infinity the value $e^{-K_E \infty}$becomes zero and therefore the cumulative amount excreted at infinite time Xu∞ can be given by equation:

$$Xu^{\infty} = \frac{K_e X_0}{K_E}$$

On substitution of this equation in previous equation and rearrangement

$$log\,(Xu^{\infty} - Xu) = logXu^{\infty} - \frac{K_E t}{2.303}$$

Where $(Xu^{\infty} - Xu)$ = amount remaining to be excreted (ARE) at any given time

7(i) Role of biopharmaceutics and pharmacokinetics in formulation development

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 optimize efficacy of the drug products. Pharmacokinetics is defined as the study of time course of drug ADME and their relationship with its therapeutic and toxic effect of the drug. Simply speaking pharmacokinetic principles in optimizing the drug dosage to suit individual patient needs and achieving maximum therapeutic utility is called clinical pharmacokinetics.

To achieve optimal therapy with a drug, the drug product must be designed to deliver the active principle at an optimum rate and amount depending upon the patients need. The factors affecting

the bioavailability of drug helps in designing such an optimum formulation and save many drugs that may be discarded as useless. On the other hand rational use of the drug or the therapeutic objective can only be achieved through a better understanding of pharmacokinetics, which helps in designing a proper dosage regimen. This obviates the use of the empirical approach where a considerable experimentation is needed to arrive at the balance between the desired therapeutic and the undesired toxic effects in order to define an appropriate dosage regimen. The knowledge and concepts of biopharmacetics and pharmacokinetic thus have an integral role in the designing and development of new drugs and their dosage forms an improvement of therapeutic efficacy of existing drugs.

7(ii). Factors affecting distribution of drug in body

Discuss in detail these factors with suitable examples

1. Tissue permeability of the drug.
   a. physiochemical properties of the drug like molecular size, Pka and oil-water 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 extra cellular tissue proteins.
4. Miscellaneous factors
   a. Age
   b. Pregnancy
   c. Obesity
   d. Diet
   e. Disease states
   f. Drug interaction

7(iii). Protocol for bioavailability measurement

The aim of bioavailability study is to find out the doses form influence on the biological performance of the drug. Therefore the bioavailability protocol used should be of sufficient sensitivity to detect difference in the rate and extant of absorption that are attributable only to dosage form variability and should avoid variabilities due to other factors.

List the element of the bioavailability study protocols and discuss in brief

1) Study objective
2) Study design
   a) Experimental design
   b) Washout period
   c) Drug products: test products and recognized standards.
   d) Route of administration
   e) Dosage regimen
   f) Frequency and duration of sampling

g) Randomization of drug administration
h) Single v/s multiple dose study design
i) Subjects
i) Healthy subject's v/s patients
ii) Subject selection
- Medical history
- Physical examination
- Laboratory tests

iii) Study conditions
j) Analysis of biological fluids

3. Method of bioavailability assessment
a) Plasma data
b) Urine data
c) Acute pharmacological effect
d) Clinical response

4. Analysis and presentation of data
a) Statistical treatment of data- analysis of variance (ANOVA)
b) Format of data