

PHARMACEUTICAL TECHNOLOGY

Biopharmaceutics

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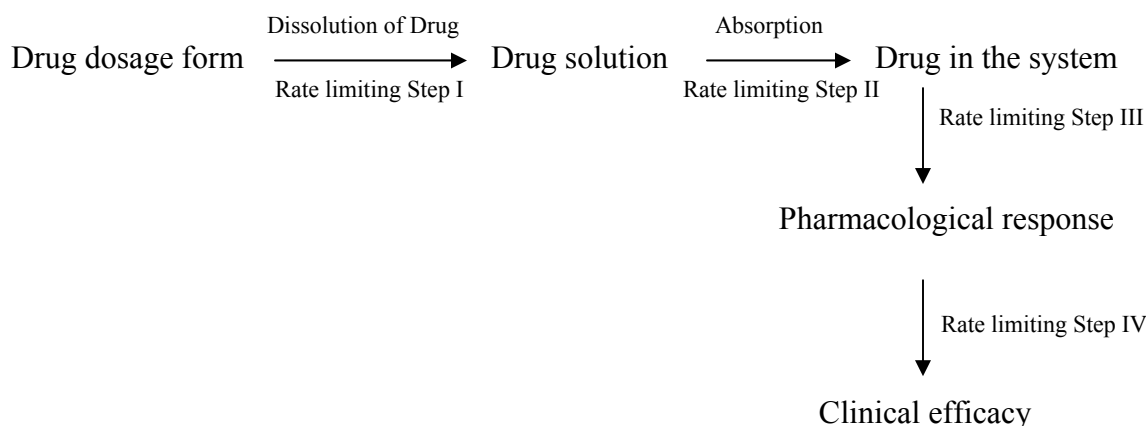
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Introduction

Biopharmaceutics is an area of pharmaceutical technology dealing with the biopharmaceutical aspects of drugs or any therapeutic moiety such as it deals with the absorption, distribution, metabolism and excretion of drugs, affecting the clinical response of the drug. Usually, we specify it as ADME of the drug, which discloses the pattern, which the drug faces when ingested as dosage form. This discipline involves the complete understanding of physical, chemical, pharmacokinetic, pharmacodynamic and toxic kinetic factors, which are likely to affect the ADME profile of drug molecules. All these factors when governed thoroughly leads to effective therapeutic concentration of the drug in the biological fluids thus a desirable action is seen. Therefore, biopharmaceutics involves the study of the relationship between the physicochemical and biological factors affecting the in-vivo behavior of the drug molecules. The complete knowledge of these variables not only help in designing the drug delivery systems but also helps in generating the sub-therapeutic, therapeutic and toxic concentration of any drug in biological fluids. Not only the conventional dosage forms are designed but the novel drug delivery systems are also based on the biopharmaceutical aspects of the known therapeutic drugs.

In general, for any drug molecule to be active pharmacologically, it should have sufficient aqueous solubility for dissolution, optimum oil / water partition coefficient to provide diffusion through lipids and protein layers and should have an active chemical group that could interact with the receptor site. But such ideal characteristics are not present in any drug molecule thus require chemical modifications. The overall process of clinical response of any drug moiety is expressed by the scheme given below:



From the above scheme, it is obvious that for any clinical response the drug moiety should bear certain physical characteristics like particle size, particle form, solubility, partition coefficient and rheology. Also, certain chemical characteristics like coefficient surface, effect of temperature, hydrolysis, oxidation etc. are to be explored. Once physical and chemical characteristics are expressed clearly, the biological factors like gastric emptying, gastric pH, diet and glomerular filtration are screened for clinical efficacy. Let us discuss the effect of these factors one by one.

Physicochemical Characters Affecting Absorption

Physical properties that are due to shape, size and physical form of any drug molecule, usually unaffected by chemical changes, affect the bioavailability of therapeutic moieties. These properties are important for dosage form design. These include particle size, solubility, surface –

interfacial tension, bulk density, flow characteristics, rheology, partition coefficient, compressibility and wettability.

1. Physical form: A large number of therapeutic moieties are associated with more than one physical form, usually the amorphous and crystalline. It has been found that one form is therapeutically more active than the other one or one form is more stable than the other. To understand the influence of this physical factor, here are a few examples:

- (i) A long-term storage of novobiocin suspension leads to change of suspended amorphous form to crystalline form of drug resulting in loss of pharmacological activity, as the latter is not absorbed through gut.
- (ii) Zinc insulin occurs both as amorphous and crystalline form and the change is triggered by change in pH. However, the crystalline form has greater biological half-life due to slower rate of absorption in contrast to amorphous form that is absorbed quickly and eliminated fast.
- (iii) The crystalline form of sodium and potassium penicillin G can withstand the dry heat for several hours without losing the pharmacological effect while the amorphous form is unstable.

2. Particle Size: Particle size plays a vital role in the design of conventional drug delivery systems like tablet, capsule, injection or biphasic drug delivery system like suspension and emulsion, or the controlled drug delivery systems like implants, transdermal drug delivery, microemulsions, nanoparticulate drug delivery. Moreover the spatial drug delivery like tissue targeting, brain drug delivery and lung drug delivery are based on controlling the particle size. In all drug delivery systems mentioned above, the particle size affects the rate of dissolution, which ultimately affects the rate of absorption and thereby affecting the pharmacological efficacy of the drug. Due to this reason griseofulvin in micronised form absorbs to a greater extent than in larger particle size.

Since it is known that drug absorption is dependent on the solubility of the drug in the respective dissolution medium. The solubility in turn is dependent on the particle size; smaller the particle size greater is the absorption (greater bioavailability). To understand this relation, the drug griseofulvin in different particle size (micro size, ultra micro size and ultra micro size of larger dose) was given to humans and plasma profile of the drug was generated. The figure for that is shown below.

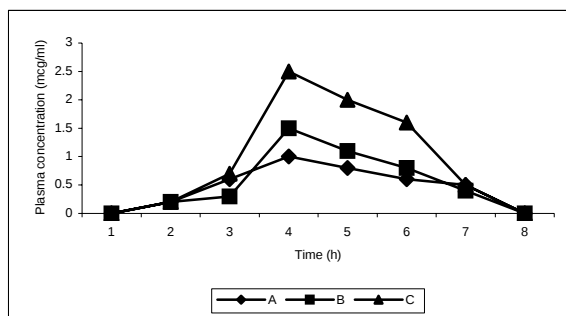


Fig. 1: Influence of particle size on drug absorption

A = Micro size griseofulvin 500 mg., B = Ultramicro size griseofulvin 250 mg ,C = Ultramicro size griseofulvin 500 mg.

However, there are drugs like nitrofurantoin, where the reduction in particle leads to gastric irritation due to increased gastrointestinal concentration of fine state drug than the coarser size. The other examples of the drugs showing enhanced bioavailability due to particle size reduction are chloramphenicol, reserpine, digitoxin, tolbutamide etc. Amongst all griseofulvin and digitoxin show remarkable increase in bioavailability after particle size reduction.

Dosage forms usually available are tablets, capsules, solutions, suspensions, and all these differ in bioavailability as appearance of drug as particle is different for these dosage forms, like drug in tablet form first disintegrates to granules, then comes in solution form which is absorbed through intestinal mucosa. The size reduction is achieved by milling, grinding or by dispersion technique where drug is dispersed in water-soluble carrier. The dispersion of griseofulvin in PEG 6000 results in increase in its bioavailability. Other examples are reserpine, chloramphenicol, prednisone. Thus variability in bioavailability is dependent on the dissolution of released particles from the dosage form.

3. Ionization constant: Since we know mostly the drug molecules are weak acidic or weak basic in nature, which in turn depends on the degree of ionization that gives the ratio of extent of ionized and unionized form of the drug thereby ultimately determines the solubility and dissolution of the drug. The total solubility of any drug moiety is the sum of ionized concentration and unionized concentration. The unionized concentration is thermodynamic parameter influenced by temperature and pressure, however the ionized concentration determines the aqueous solubility of the drug and is dependent on the pH of the solution. Therefore, the solubility of weak acids is given by the equation.

$$S_t = S_v \left[1 + \frac{K_a}{H^+} \right]$$

Where S_t = total solubility

S_v = Solubility of unionized concentration

K_a = Ionization constant for acids.

H^+ = Hydrogen ion Concentration

For weak base equation will be:

$$S_t = S_v \left[1 + \frac{H^+}{K_a} \right]$$

Thus from the above equations, it is observed that increasing the concentration of H^+ , will decrease the solubility of acidic drugs while the reverse happens with basic drugs. Therefore, to generalize this, acids have higher solubility in alkaline pH while bases have higher solubility in acidic pH ($-\log H^+$).

The ionization constant (dissociation constant) is expressed as pK_a , which is given by Henderson-Hasselbalch equation:

(a) For acidic drugs:

$$pH = pK_a + \log \left[\frac{\text{salt concentration (Ionized)}}{\text{acid concentration (Unionized)}} \right]$$

(b) For basic drugs:

$$pH = pK_a + \log \left[\frac{\text{base concentration (Unionized)}}{\text{salt concentration (Ionized)}} \right]$$

pK_a helps in determining the site of absorption of the drug i.e. whether the drug will be absorbed in stomach (pH-1), lumen of the intestine (pH 6.6) or blood plasma (pH 7.4), thereby gives the idea for selective dosage form design and the route of administration.

To understand the importance of Henderson–Hasselbalch equation consider an example of a drug with $pK_a = 3$ and $pH = 1$ (i.e, expected to absorb in the stomach). It indicates that the drug is a weak acid (ie. pK_a is low). Therefore considering the equation for weak acid

$$pH = pK_a + \log \left[\frac{\text{Ionized}}{\text{Unionized}} \right]$$

$$pH - pK_a = \log \left[\frac{\text{Ionized}}{\text{Unionized}} \right]$$

$$pK_a - pH = \log \left[\frac{\text{Unionized}}{\text{Ionized}} \right]$$

$$\text{or } 3 - 1 = \log \left[\frac{\text{Unionized}}{\text{Ionized}} \right]$$

$$2 = \log \left[\frac{\text{Unionized}}{\text{Ionized}} \right]$$

It indicates that the concentration of ionized part is high in the stomach, thus greater absorption occurs at stomach site when the drug is given by oral route thus a capsule or tablet can be formulated for such a drug.

Also, pK_a is defined as pH at which drug is half ionized, that is, the ratio of concentration of ionized Vs unionized is 1 i.e. $pH - pK_a = \log [1]$

$$pH - pK_a = 0$$

$$\text{or } pH = pK_a$$

Therefore, drugs like phenobarbital, a weakly acidic drug with pK_a 7.4, will be in ionized as well as unionized state in equal concentration in blood plasma (pH 7.4), provided the drug is directly in contact with the blood, eliminating the distribution. Also the drug phenobarbital will be in

high concentration as unionized in stomach at pH 1 and thus well absorbed through the gut wall. Table 1 showing the pK_a values of some acidic and basic drugs.

Table 1: Drugs with corresponding pK_a values

Drug	Nature	pK_a
Acetyl salicylic acid	Acid	3.49
Benzyl penicillin	Acid	2.76
Ethosunamide	Acid	9.3
Chlorpropamide	Acid	4.8
Sulfadrazine	Acid	6.48
Dephenghydantoin	Acid	8.3
Atropine	Base	9.65
Amphetamine	Base	9.8
Lignocaine	Base	7.9
Procaine	Base	8.8
Tetracycline	Base	3.3, 7.8, 9.7

Therefore seeing the Henderson-Hasselbach equation, it is seen that the dissolution rate of weakly acidic drug increases with increase in pH. However, with basic drug, it does not happen especially around neutral pH as dissolution depends on formation of diffusion layer formed by the drug particles. This can be well understood by an example of dissolution of salicylic acid. When the drug dissolves, the pH around the particle (i.e. diffusion layer) is less than the intestinal fluid, therefore incorporating sodium bicarbonate enhances the dissolution many folds. The ionization constant for any drug is determined either spectrophotometrically by estimating the drug-ionized concentration in 0.01N HCl or 0.01N NaOH provided there occurs difference in absorbance in acidic or basic solution. Also, potentiometrically, the ionization constant is determined based on the principle that pH is equal to pK_a of a drug at half ionization because the concentration of ionized and unionized fraction is equal.

4. Solubility: Solubility is the indicative of maximum concentration that can be dissolved in the solvent to form a saturated solution. Thus it is expressed as grams of solute dissolving in volume of solvent. This gives the exact solubility of any solute, however, Pharmacopoeias express the solubility of any therapeutic moiety as a relative expression like soluble, very soluble, slightly soluble etc. The terms use to express solubility are given below:

Relative Expressions	Parts of solvent required dissolving 1 part of solute.
Very soluble	Less than 1
Freely soluble	From 1 to 10
Soluble	From 10 to 30
Sparingly soluble	From 30 to 100
Slightly soluble	From 100 to 1000
Very slightly soluble	From 1000 to 10,000
Practically insoluble	More than 10,000

Based on the principle like dissolves like, organic compounds dissolve faster in organic solvent because of structural similarities. That is why solubility is determined mostly in aqueous vehicle and one or more organic solvents to know lipophilic or hydrophilic nature of the drug. In pharmacokinetic studies, solubility of drug is of paramount importance as it depicts the in vivo behavior of the drug that means, the absorption of the drug is dependent on the dissolution in the gastric milieu. Thus greater the dissolution in the gastric fluid greater will be the absorption. Generally, the drugs showing solubility greater than 1% w/v do not pose absorption related problem. Usually to generate the solubility profile of any new drug, the studies are carried out over a wide pH range from 1 to 8, the pH showing maximum stability (minimum degradation) is selected for product development. Solubility profile of any drug candidate can be generated by dissolving the slight excess of the drug in fixed volume of solvent to form a saturated solution. The solution is stirred constantly for hours at a constant temperature. The sample containing slight amount of undissolved solute is filtered and the clear solution is estimated by modern analytical techniques like UV, HPLC for drug content dissolved. The solvent can be aqueous vehicles like water or buffer or organic solvents like polyethylene glycol or alcohol.

The solubility of any solute is dependent on important parameters like temperature, solute state (crystal form), particle size, pH and type of solvent. All these factors govern the intrinsic solubility of the solute.

5. Effect of crystal form: Since it is known that certain drugs exhibit polymorphism, where more than one form of the drug exists and one form either amorphous or crystalline is more stable than the other. However, a less stable form i.e. metastable frequently changes to stable form. Each crystal form has a definite pattern i.e. lattice, the integrity of that affects the solubility of the drug. This lattice has its own enthalpy of crystal lattice (ΔH_{cl}). Therefore solubility of crystalline solute is dependent on the total enthalpy i.e.

$$\Delta H = \Delta H_{cl} + \Delta H_{solv.}$$

Where ΔH_{cl} is enthalpy of crystalline lattice

ΔH_{solv} is enthalpy of solvation.

ΔH = Total enthalpy

Usually the ΔH_{cl} is greater than ΔH_{solv} , thus ΔH is positive indicating the dissolution of the drug is endothermic process. Sometimes ΔH_{solv} , which is the heat absorbed when solute is in complete contact with the solvent, is greater, then the process of dissolution is exothermic. The influence of solubility on dissolution is dependent on the rate of conversion of metastable form to stable one, which is very slow, thus the metastable form is usually regarded as stable form. A few examples of drugs showing polymorphism are steroids, barbiturates and sulphonamides, which exhibit form dependent bioavailabilities. The prominent example of drug is insulin having amorphous and crystalline form; the mixture of two provides initial fast absorption due to amorphous form and a sustained effect due to crystalline form. Another example is novobiocin, which when administered as suspension shows greater bioavailability due to its amorphous form. However, upon storage amorphous form changes to crystalline one, thus decreasing the bioavailability, that is why methylcellulose is incorporated to the formulation containing novobiocin. Another example is chloramphenicol palmitate, the drug shows variable bioavailability because of different polymorphs. The form A of the drug is as suspension least

bioavailable than form B which is most bioavailable in the blood. Chlortetracycline also has two polymorphs α and β , β form is more bioavailable than α as bioavailability is dissolution rate limited.

6. Effect of pH: Since Henderson-Hasselbach equation gives the idea of ionization of drug with respect to pH of the dissolution medium, a modification of the equation gives the relationship between pH of the solution and the solubility.

For acidic drug,

$$PH = pK_a + \log \frac{S - S_0}{S_0}$$

Where S is total solubility of the drug

S_0 is the solubility of the unionized form.

Thus, if the pH of acidic drug is reduced then proportion of unionized drug will increase and precipitation of the drug from the medium occurs.

For basic drug,

$$PH = pK_a + \log \frac{S_0}{S - S_0}$$

Similarly, if the pH of the weakly basic drug is increased, then precipitation occurs due to greater concentration of unionized – lipophilic drug.

The above equations suggest that pH of the medium need to be maintained to prevent incompatibility like precipitation.

7. Partition coefficient : To predict the absorption of any drug, it is desirable to know its partition coefficient or distribution coefficient, which gives the idea of the concentration of unionized drug likely to pass through the biological membranes. Since we know biological membranes are consisting of lipoidal bilayers, which are impermeable to most of the drugs and favor the permeation of unionized portion of the drug. Therefore, to study the effect of partitioning, a number of organic solvents with a portion of aqueous medium were tried like chloroform, ether, isopropyl myristate, *n*-octanol etc. These solvents depict the lipid nature of the biological membranes. Amongst all the solvents, *n*-octanol simulated best with the body lipids. The drugs with good partition coefficient values show greater absorption as they penetrate the complex lipids of the biological membranes. The examples of few drugs with their partition coefficient value and percentage absorbed are given below:

Drugs (Unionized)	% Absorbed through colon membrane	Chloroform / water partition coefficient
Barbital	12 $\pm$ 2	0.7
Cyclobarbital	24 $\pm$ 3	18
Secobarbital	40 $\pm$ 3	50.7
Hexethal	44 $\pm$ 3	> 100.0

Also the rate and extent of absorption of drug decreases with the increase in the polarity of the drug, for example, rat jejunum absorption is high with thebaine than codeine and the least with morphine as thebaine has no hydroxyl group while codeine has one and morphine has three hydroxyl group. To determine partition coefficient of any drug, the drug is dissolved in organic or aqueous phase till saturation occurs, then the drug is separated from either of the vehicle by centrifugation. The drug in any of the phases is determined spectrophotometrically or by HPLC. The content in the other phase is the difference of the organic phase or aqueous phase.

$$\text{The partition coefficient (log P)} = \frac{\text{Concentration of drug in organic phase (D}_o\text{)}}{\text{Concentration of drug in aqueous phase (D}_a\text{)}}$$

8. Prodrugs: There are certain drugs, which become less bioavailable due to gut wall enzyme activation or pH of the gastric medium thus to be administered after some chemical modification. The modifications affect the localization of the drug or increase the lipid solubility so that absorption is enhanced. Therefore, prodrug concept is an alternative method of protecting the susceptible drug. To understand it clearly, consider an example of sulfonamide, it has greater absorption but to achieve higher concentration towards gut site for localized action, succinyl sulfathiazole or phthalyl sulfacetamide are formed which ionize at the gut site and are liberated as free drug as ionized one which has less partition coefficient and lesser absorption. Another example is of erythromycin estolate, the parent drug is less absorbed thus estolate ester is formed that has much better absorption. Sometimes, prodrug approach is used to enhance the solubility of weak acids or weak bases like formation of tetracycline hydrochloride or atropine sulphate. These salts dissolve rapidly than the parent drug. However, not always the salt formation leads to increased solubility or increased solubility (dissolution) dependent bioavailability for example aluminium salt of aspirin that dissolves very slowly in the gastrointestinal fluid, the incomplete bioavailability occurs due to retarded absorption. These prodrugs liberate the parent drug molecule depending upon the variation in the pH, for example, erythromycin stearate after passing through the stomach (pH 1-3), as an undissolved entity, liberates the free drug in the intestine for ready absorption.

9. Dissolution of the drugs: Since it is known that any therapeutic moiety can exert the effect only when the drug is in solution form as it easily gets transported across the biological membrane. Therefore, there are basically two steps for any drug molecule to be in dissolved form, first being the appearance of drug in particulate form (i.e., segregation of the particles from dosage form) and then diffusion of the particles in the vehicle after crossing static boundary layer is the second step. Any of these steps can be the rate-limiting step. Generally the rate of diffusion is the rate limiting step and is given by Noyes-Whitney equation:

$$\frac{dm}{dt} = KA (C_s - C)$$

where m = mass of solute transferred towards the vehicle in time 't' i.e.

$$\frac{dm}{dt} = \text{Rate of dissolution.}$$

A = Surface area of undissolved particle

C_s = Concentration of solute required to saturate the solvent.

C = Solute concentration at time t

K = Intrinsic dissolution rate constant.

Which is given by the equation

$$K = \frac{D}{Vh}$$

Where, D = Diffusion coefficient of the solute in the solvent

V = Volume of dissolution medium

h = Thickness of the boundary layer

Thus seeing the above equation, it is observed that if the dissolution medium is replaced constantly then sink conditions are maintained (i.e. $C_s > C$) and higher dissolution rate is expected as most of the solute dissolved in the medium is constantly removed and fresh medium provides better dissolution of the undissolved solute. However, if the solute or the segregated particles accumulate in the dissolution medium then no sink conditions are achieved (i.e. $C > C_s$) and a retarded dissolution is expected. Also, when $C = C_s$, it indicates dissolution is zero i.e. concentration of dissolved drug is equal to drug in concentrate form. Generally, the factors, which affect the dissolution rate of any drug, are as follows:

(a) Surface area of the undissolved drug or the particle size of the drug: Since surface area of the drug is inversely proportional to the drug particle size. Therefore, the smaller particles dissolve faster than the larger ones. This is exemplified by the dissolution profile of griseofulvin, the rate of dissolution was high when 2-5 μm particle size was used than the 10 μm particle size. Other examples are of phenothiazine, tolbutamide, aspirin, nitrofurantoin etc.

(b) Solubility of the drug in the dissolution medium: This is related to the pH of the dissolution medium. Simulated dissolution media resembling gastric fluid (pH 1-3) or intestinal fluid of (pH 5-6) is used to know the solubility pattern of drugs. The details have been discussed in the earlier in this chapter.

(c) Concentration of the solute: The concentration of the drug dissolved is dependent on the volume of dissolution medium and the rate of absorption of the drug in the systemic system. For example, if the rate of absorption is high for any drug, then a sink condition is maintained and higher dissolution is expected for such drugs. However, if any other solute is present in the gastric tract, it will retard the process of dissolution, like presence of food delays the absorption of some drugs as it increases the solute concentration in the gastric fluid. This is reason why acetaminophen shows better absorption when taken empty stomach.

(d) Intrinsic dissolution rate constant: Dissolution rate is dependent on the thickness of the diffusion layer formed round the particle as well as on the diffusion coefficient of the drug. Thickness of the diffusion layer is decreased by agitation that is why the gastric motility may increase the dissolution of the drug that is less soluble in the gastric fluid. Also the diffusion coefficient of the drug is decreased by the substances which increase the viscosity of the medium like food tends to retard the dissolution rate limited absorption of the drug. Dissolution rate of any drug candidate can be carried out with the help of various dissolution apparatus specified in Pharmacopoeias. As per USP/BP the pharmaceutical formulations are classified into categories discussed below and each category has different release specifications for the medicaments and

different dissolution apparatus are employed for such formulations. The formulations are categorized as:

1) **Conventional dosage forms** like tablets and capsules. They are also called as immediate release dosage forms.

2) **Non conventional dosage form** or modified release dosage form. These formulations are those where the rate and/or place of release of the medicament is intentionally modified by administration through the same route. With such formulations prolonged release is obtained. These dosage forms include *extended release formulations*-where a slow release of the active medicament occurs, *delayed release dosage form*-where the release of the medicament is delayed like enteric coated tablets and *pulsatile dosage form* – where a sequential release of the medicament takes place. The apparatus used for these different categories of dosage form are as follows:

1. Apparatus 1 (basket) – For capsules and is operated at 100 rpm
2. Apparatus 2 (Paddle) – For tablets and operated at 50 rpm
3. Apparatus 3 (reciprocating cylinder) – For bead type modified release dosage forms
4. Apparatus 4 (flow cell) – For modified release dosage forms with limited solubility
5. Apparatus 5 (Paddle over disc) and Apparatus 6 (cylinder) – for transdermal dosage forms.
6. Apparatus 7 (Reciprocating disc) – For non-disintegrating type oral modified release dosage forms.

Apart from the above-mentioned apparatus, there are certain modified dissolution apparatus like Levy's beaker type apparatus, basket-racks assembly, Pernarowski method and membrane filtration method for estimating the dissolution of drugs.

9. Influence of temperature on absorption: Atmospheric temperature affects the stability of the drug molecule. Usually it is assumed that every 10°C rise in temperature doubles the reaction kinetics leading to drug degradation. Arrhenius equation shown below gives the specific rate of reaction

$$\log K = \frac{\Delta H_a}{2.303 R} \times \frac{1}{T} + \log S.$$

where K = Rate of degradation

S = Frequency of collision between reactive molecules.

ΔH_a = Heat of activation

R = Gas constant

T = Absolute temperature.

A plot of 1/T verses log K gives a slope value of H_a/RT .

Thus greater the ΔH_a , greater is the stability of molecules to thermal degradation.

Temperature influences the solubility of any solute in the solvent and usually some solutes absorb heat when dissolved which is known as negative heat of solution, resulting in increased solubility with increase in temperature. A few solutes exhibit positive heat of solution indicating

a lower solubility with rise in temperature. Therefore rate of solution is influenced by temperature apart from other factors that will be discussed later.

From this graph it is obvious that solubility for compound $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ increases linearly with temperature till 30°C but then occurs an independent curve. It indicates that initially the compound is in a hydrated state ($\cdot 10\text{H}_2\text{O}$) and increase in temperature lead to endothermic process while after 30°C compound changes to anhydrous state (Na_2SO_4) Thus process becomes exothermic. In case of second compound, (NaCl) the solubility is independent of temperature indicating the solubility of supersaturated solution of the compound. Shown below is graph of solubility curve of various solutes.

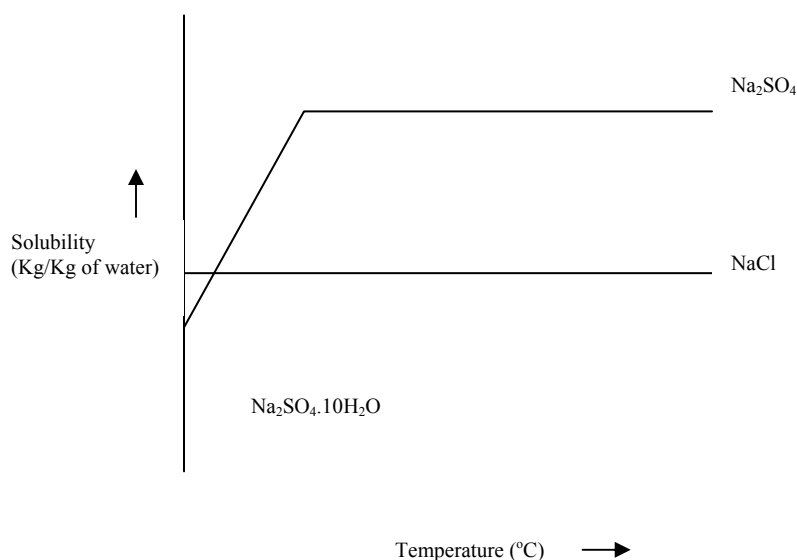


Fig. 3 Influence of temperature on solubility of $\text{Na}_2\text{SO}_4 \cdot 10 \text{H}_2\text{O}$

10. Influence of hydrolysis: Hydrolysis causes degradation of several formulations and drugs. It follows a second order reaction depending on the concentration of the drug undergoing hydrolytic degradation. Usually the catalyst for the reaction are OH^- or H^+ ions, otherwise the bacteria or enzymes also affect the reaction. Thus hydrolytic degradation can be prevented by any of the following exemplified methods:

- (i) Replacement of water with other solvent e.g., sorbitol in aspirin suspension in place of water as vehicle.
- (ii) Complexation as in case of benzocaine and procaine with caffeine.
- (iii) Use of micelle to provide steric stability (usually 5% sodium lauryl sulphate solution) for ester type of drugs.
- (iv) Control of pH to prevent degradation

11. Influence of oxidation: Oxidation is another important degradation reaction. It follows the first or second order reaction depending on the concentration of oxygen. Examples of drugs degraded by oxidation are ascorbic acid, morphine, neomycin, prednisolone, streptomycin etc. the oxidized drugs have different pattern of absorption thus have different bioavailabilities. To prevent oxidation:

- (a) Use inert environment for processing and storage of drugs.
- (b) Reducing the concentration of drug.
- (c) Removal of ions likely to catalyze the reaction.
- (d) Polymerization and isomerization.

Routes of Drug Administration and Their Effect on Drug Absorption

The drugs can be administered by oral route, non-oral routes like i.v, i.m, s.c, ocular, rectal or by inhalation. Amongst all, oral route is widely preferred mode of administration as it is easily acceptable by most of the patients, the dosage form designing is easy and the rapid and safe ADME of drug is expected. However various variable parameters like body elimination rate, body posture, type of food consumed etc. affect the drug's ADME profile thus limiting this route of administration. Moreover, this route of administration requires a mode of transportation of drug to the specific site either involving active or passive transport. In general, drugs tend to show different ADME profile with different mode of administration i.e. usually a prompt action occurs for any drug when given by i.v. route than by any other route of administration. This can be well understood by observing the examples that show that the same drug is needed in different doses when administered by different routes.

Drugs	Dose (Route I)	Dose (Route II)
Morphine	75 mg (po)	10 mg (im)
Isoproterenol	3-20 mg (po/Solution)	0.02 – 0.15 mg (iv)
Bethanechol	5 – 30 mg (po)	2.5 – 5 mg (sc)
Propranolol	80 mg (po)	1 – 3mg (iv)

Generally the drugs administered by oral route (po) show absorption based on various mechanisms like passive diffusion that is dependent on drug concentration across the gastro intestinal wall and is given by the equation.

$$\frac{dc}{dt} = -DA(C_{git} - C)$$

where $\frac{dc}{dt}$ = rate of absorption of drug

D = Diffusion coefficient of drug dependent on partition coefficient of drug

A = Area of absorption surface

C_{git} = Concentration in gastrointestinal tract

C = concentration across the gut wall (that is in the plasma)

Usually the plasma concentration is lower than the gastrointestinal (GI) concentration as the drug is constantly removed from that site thus a sink condition is always maintained. Drugs are also absorbed into circulating blood through active diffusion mechanism which involves a carrier for transportation usually the enzymes. These carrier mediated drug absorption can also occur against the concentration gradient i.e. drug from lower concentration site are transported to higher concentration site but this mechanism of drug absorption is highly specific therefore is effective when drug concentration is low.

Another mechanism by oral route of absorption is facilitated drug absorption which is also carrier-mediated. The carrier is non-specific thus a competition with the structurally similar groups occurs. It does not take place against the concentration gradient. The example of facilitated drug absorption is of drug vitamin B₁₂, it forms complex with intrinsic factor produced by stomach wall. The drug is transported across the stomach wall as a complex with intrinsic factor.

Drug absorption also takes place by ion pairing, which is favourable for highly ionisable drugs like sulfuric acid; these drugs form complex with ions present in the gut wall like mucin, which are then absorbed. Pinocytosis is other mechanism where the gut wall itself engulfs the drug molecule as tiny droplet or particle and it does not require that drug should be in solution state. Examples of drugs absorbed by this mechanism are vitamins A, D, E and K. Usually this mechanism is shown by nutrients.

Thus in general, for any drug to elicit pharmacological effect it must be present in sufficient concentration and then it is distributed via blood to the site of action. This drug concentration in the blood is called as “drug input”. However to have site specific drug delivery like the oral administration of antacid for stomach as site of absorption or bronchodilators administered as inhalators or aerosol formulation, the drug delivery design becomes difficult as an unpredictable drug concentration is achieved leading to variable bioavailability. This is the problem even with modified dosage form design leading to little clinical efficacy.

Further, after oral administration, the majorities of drugs are absorbed through the GI membrane into liver and get metabolized. After metabolism, the amount of drug available in the blood is sampleable. This is first pass liver metabolism of linear system. However, the available fraction of drug (F) after oral dose is not only dependent on the extent of drug absorbed from GIT but also on the fraction metabolized by GI wall enzymes and by the liver enzymes. Thus if it is assumed that the drug is not at all metabolized by liver and GI wall enzymes, then the oral dose administered will be equal to an i.v. injection into the hepatic portal vein. This is linear system. Examples of drugs showing hepatic metabolism are aspirin, morphine, lidocaine, alprenolol, propranolol, carvedilol, losortan and metaclopramide.

The non-oral routes like subcutaneous route, pulmonary route, sublingual route, rectal route, ocular and intravenous route have advantages over the other like if immediate drug availability in blood is required then i.v. route is preferred provided drug should have sufficient solubility in the aqueous vehicle and is precipitated at the site of administration due to change in pH, as it will lead to embolism. If site specific drug delivery is required than pulmonary route is preferred provided drug has particle size less than 10 µm as larger particles show nasal impaction while the smaller ones traverse the nasal tract and get deposited in the alveolar sac e.g., salbutamol spray. Sublingual route promises greater absorption for lipophilic drugs like lidocaine, chlorpheniramine and barbiturates, as buccal mucosa is rich with blood supply. Therefore choice of route of administration is dependent on the intrinsic characteristic of the drug molecule, for example, the drug phenytoin, is insoluble in water and is given as hydro alcoholic injection in pH 12 but when given as i.v. injection, it precipitates as it comes across pH 7.4 but the lungs do have the capacity to retain these particles till they are dissolved by the circulating blood because of the smaller particle size administered.

Physiological and Biological Factors Affecting Drug Absorption:

When the drug traverses the gastrointestinal tract, it faces many physiological and biological variables like different pH of the system, different surface area and thickness of the gastric membrane, different gastric emptying and gastric motility patterns. The gastric emptying is in turn affected by food, the contents of the food and the physical state of the food as well as pathological conditions of the patient. The drugs also show different absorption pattern depending on the presence/absence of food and the type of food. For example, the capsule dosage form of cephadrine when given empty stomach showed higher serum concentration (almost 890 folds higher with 500 mg capsule) than when given with meal. Thus food decreases the rate of absorption (as t_{max} shifted) while extent of bioavailability was same (the AUC was same for both). Another example is of oral administration of erythromycin 250 mg tablet when given with different contents of the food as well as variable volume of liquid consumed. The variable bioavailability is depicted in figure below where the drug is given with protein diet, carbohydrate diet and fat diet and in a fasted state with low (20ml) water and high (250 ml) volume of water.

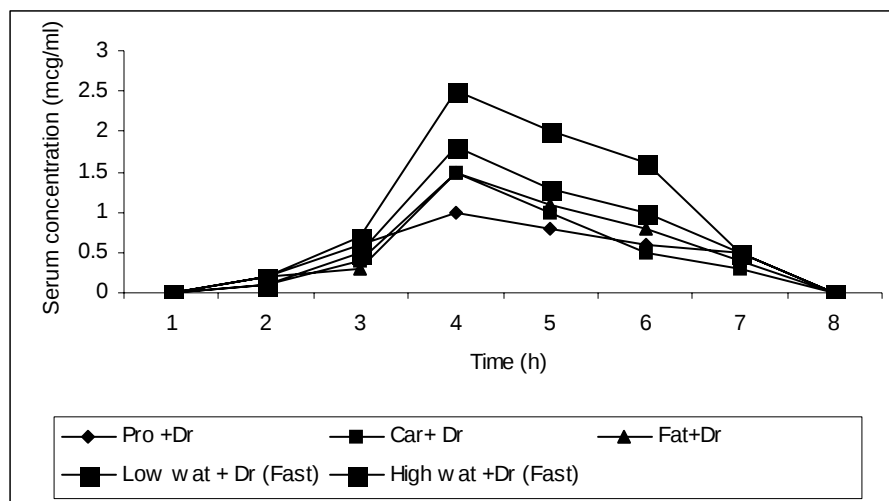


Fig. 4: Serum concentration of erythromycin with various contents of meal

Another factor affecting the availability of the drug in the biological fluid is gastric emptying which delivers the contents of the stomach and small intestine, that is the site of absorption for maximum drugs because of greater surface area due to presence of villi and microvilli. The drug then encounters two types of movement in the intestine i.e. propulsive and mixing. The propulsive movements push the drug into the intestinal fluid for intimate mixing while the mixing movement resulting out of contraction of intestinal mucosa causes thorough mixing of drug with the intestinal secretions. These movements affect the residence time of the drug. Therefore drugs increasing the gastric motility ultimately increase the rate of absorption of other drugs, for example, metaclopramide, (10 mg tablet) an antiemetic drug, when given with digoxin 0.375 mg tablet, the GI motility increases, thus the drug digoxin is propelled into the intestine without getting released and absorbed into the GI fluid, leading to decrease, in extent of drug availability. However, with propantheline, the drug that retards the gastrointestinal motility, the digoxin level was observed to be high in serum indicating the high extent of drug availability. Therefore, drugs that alter GI motility may increase or decrease the rate of availability of another

drug. In addition, concurrent therapy may increase, decrease or not affect the extent of availability of a drug.

Another factor is the blood flow surrounding the absorption site. Since we know that drug after disintegrating into the stomach, gets absorbed through the gastric mucosa into the blood, which is then transported to liver for metabolism before appearing into the systemic circulation from where the drug is then transported to the desired sites via blood. This leads to existence of a sink condition towards blood side favoring the unidirectional concentration gradient dependent drug absorption, which is independent of blood flow. Further, the drugs, which are absorbed by active mechanism, show dependency on the blood flow as the blood is the carrier for such molecules as well as provides the sufficient energy in a form of oxygen for transportation for example the absorption of riboflavin tablet. The drugs, which penetrate through the mucosa of stomach or intestine, require blood flow for partitioning into that affects the rate of absorption. The absorption rate is independent of blood flow for drugs with low partition coefficient like ribitol.

Drug absorption is also dependent on the various enzymes present in the gut, intestine or pancreas. These enzymes hydrolyze the drug into the parent compound, which exerts the pharmacological response like pancreatic enzymes hydrolyze chloramphenicol palmitate to Chloramphenicol, mucosal esterases appear to affect various esters of penicillin pancreatin and trypsin deacetylate N-acetylated drugs. These drugs after enzymatic action get released and absorbed from the GIT. The enzymes are also helpful for controlled drug delivery. In GIT, mucin-a viscous mucopolysaccharide, which lines the intestinal membrane, is known to bend drugs nonspecifically thus reducing their absorption. It acts as a barrier for drug diffusion across the membrane.

Disposition

Since pharmacokinetics of any drug involves its absorption, distribution, metabolism and excretion profile this ADME for any drug is studied broadly under two categories:

- (a) Pharmacokinetic of drug elimination
- (b) Pharmacokinetic of drug absorption

Both categories involve i.v. as well as oral administration. The pharmacokinetic profile is different for these two routes of administration and follows different kinetic models.

(a) Pharmacokinetics of drug elimination: Blood estimation for drug concentration and urine analysis is done to generate ADME profile of any drug. Usually the drug follows pseudo first order kinetics, not just they are simple but because everything except the drug concentration is constant, e.g. elimination process after oral administration of any drug includes influence of factors like enzymes, pH and protein binding, which affect the excretion of the drug. It is represented as:

Drug in body + enzymes, pH, protein binding $\longrightarrow$ metabolism or excretion of drug

It means enzymes, pH and protein binding, all these factors are constant and drug metabolism or excretion is dependent on drug in body only.

Therefore equation for kinetics is given as:

$$\log \frac{C_0}{C} = \frac{Kt}{2.303}$$

Where, C_0 = initial concentration

C = final concentration

K = elimination rate constant

t = time

The half-life of the drug in the system is given as

$$t_{1/2} = \frac{0.693}{K}$$

However, pharmacokinetic after i.v. injection follows first order elimination profile, that is, it assumes that after i.v. injection (bolus), drug comes into blood, mixes with the body fluid and is eliminated. The rate of elimination is proportional to amount remaining in the body. Therefore the plot of serum concentration vs time on semi log scale reveals slope and initial plasma concentration. Thus, this approach assumes:

1. Drug is eliminated by pseudo-first order process
2. The drug is rapidly distributed
3. That a single homogeneous compartment or one compartment model has “apparent volume of distribution” parameter as characteristic feature because it gives plasma concentration and the amount of the drug in the body. This V_d is given as:

$$V_d = \frac{\text{Amount of drug}}{\text{Concentration of drug}}$$

$$\text{or } V_d = \frac{\text{Dose}}{C^0_p}$$

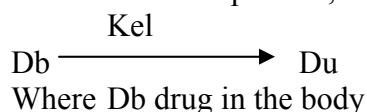
But the apparent volume of distribution of a drug very rarely corresponds to any physiologic volume, and if it at all indicates then it never depicts that the drug has or has not entered body spaces. Therefore volume of distribution is a physiological volume, it is an apparent volume and should not be linked with lymph, which is 2% of body weight, plasma volume (4.5% of body weight), total body water (50 to 70% of body weight) or any other body fluid volume.

Thus, V_d can be easily understood by considering an analogy where a beaker containing 100 ml of water has 100 mg drug dissolved in it. When aliquots are estimated for percent dissolved, it assumes each ml has 1 mg drug. However, if it assumed that bottom of the beaker has soft masses (depicting as receptors), having affinity for the drug, then it is expected that each aliquot will have less than 1 mg/ml drug concentration thus indicating that some drug has bound to tissues like receptors. Therefore when drugs have high affinity for a tissue binding sites, plasma concentration of the drug is low and high volume of distribution is there. However, for low affinity drugs, the reverse happens. But this does not give indication for the type of tissues responsible for binding. Usually the plasma volume is 3 liters and whole blood volume is 6 liters in a standard 70 kg man. The extracellular fluid is 12 liters and total body water is 41 liters for such a man. Given below is the table showing drugs of varying volume of distribution.

Table 2: Drug with corresponding percent protein binding and Vd values

Drug	Percent bound to plasma proteins	V _d (L / Kg)
Warfarin	99	0.14
Tolbutamide	96	0.10
Flurbeprofen	> 99.5	0.15
Ketoprofen	99.2	0.15
Phenylbutazone	96	0.097
Naltrexone	20	19
Haloperidol	92	18
Labetalol	50	9.4

Pharmacokinetic analysis by urine analysis for one compartment model involves the assumption that drug when not metabolized extensively binds with body proteins and appears in the urine after elimination process, which involves different kinetics i.e.



Du = drug in the urine

Kel = Elimination rate constant

It indicates that the drug appearing in urine (Du) is same as the drug remaining in the body (Db). That is why their concentration Vs time plots are mirror images. The total amount of drug recovered in urine throughout the study (D_u^0) is equal to dose (D_B^0).

Therefore, the drug in body (Db) + drug in urine (Du) = D_B^0

Solving this equation we have:

$$\log (D_u^0 - Du) = \frac{-\text{kel } t}{2.3} + \log D_u^0$$

Where D_u^0 is total amount excreted

Du = amount excreted at any time t.

$D_u^0 - Du$ = amount remaining to be excreted (ARE). The Renal Clearance Rate (RCR) for any drug is given by the equation

$$\text{RCR} = \frac{\text{Amount excreted in urine per unit time}}{\text{Plasma concentration}}$$

The drugs can be cleared solely by glomerular filtration (GF) mechanism whose rate is 125 ml/min. If renal clearance rate is equal to 125 ml/min then GF is the mechanism, but if it is less than 125 ml/min then tubular reabsorption is the mechanism of drug elimination. If it is greater than 125 ml/min, then tubular secretion is the mechanism of renal clearance.

Usually drugs are eliminated either by excretion or by metabolism from the body, therefore we calculate total clearance rate (TCR), which is

$$TCR = K_{el} V_d$$

Also the metabolic clearance rate (MCR) is given as

$$MCR = TCR - RCR$$

Or $RCR = f_e \times TCR$

Where f_e = fraction of dose excreted unchanged into urine.

Or $MCR = (1 - f_e) \times TCR$

Further, pharmacokinetic of drugs, which are eliminated by simultaneous metabolism and excretion, the rate constant for elimination (k_{el}) and for metabolism (K_m) is calculated. The equation deduced after derivation is same as the previous one i.e.

$$\log (D_u^0 - D_u) = \frac{-k_{el} t}{2.303} + \log D_u^0$$

Therefore elimination kinetic for any drug assumes that

- (i) The process of elimination is first order
- (ii) The urine collection is accurately and very timely carried out and complete urine specimens are collected at every time
- (iii) The drugs not appearing unchanged in urine are metabolized and with i.v. route dosing, complete absorption is there.

Therefore, excretion of drug and their metabolites occurs primarily through three important routes, that is glomerular filtration, tubular reabsorption and active secretion. However, the other routes of excretion such as biliary (shown by drugs like sullindac and vincristine), pulmonary (like with nitrous oxide and paraldehyde), salivary and mammary routes are not the main routes as there occurs variation in drug concentration estimated in the respective fluids.

Since the arterial blood carrying drug enters the glomerulus at a rate of 1200 ml/min where as serum filters across the capillary wall at a rate of 130 ml/min and enters the Bowmans capsule, so if the drug is not bound it appears in the filtrate while the protein bound drug is not filtered and remains in the blood. With active secretion, which is carrier mediated mechanism occurring in proximal tubule and Loop of Henle, is shown by polar drugs following first order process of kinetics. Thus at low concentration it appears linear. Tubular reabsorption is a passive process usually shown by water and electrolytes. Approximately 99% of water is filtered and reabsorbed and only 1% appears as urine. For most physiological substances, reabsorption process is active or carrier mediated but for drug molecules it is passive and pH dependent. It means the extent of reabsorption is dependent on the concentration of ionized and unionized drug. Like, for example, if weak acidic drug is given, then, if the pH of urine is decreased (for example co-administration of ammonium chloride tablet), the drug will be more in unionized concentration thus favoring the transport through semi permeable membrane of renal canal. However, if the pH of the urine is increased the drug will be in ionized concentration favoring urine side than the blood thus equilibrium shifts from blood side towards urine side.

With weak basic drugs, an increase in pH results in decrease in unionized drug and increase in ionized drug concentration, therefore raising pH promotes slower elimination of drug. With lowering of pH the reverse happens.

Therefore, clearance of the drug from the body includes blood clearance and plasma clearance of bound or unbound drug. This body clearance of any drug includes renal clearance, hepatic clearance and other organ clearance. The total clearance for any drug is the sum of all the three clearance rates. The renal clearance is usually depicted by creatinine clearance, which is usually 80-140 ml/min for healthy person of age 1 to 70 years. The creatinine is a metabolic end product of muscle cleared by glomerular filtration and its value in the urine is indicative of renal function. Table below shows the level of creatinine clearance and renal function.

Table 3: The level of creatinine clearance depending on renal function

Renal Function	Creatinine clearance (ml/min)
Normal	Above 80
Slightly reduced	50-80
Mild renal failure	30-50
Moderate renal failure	10-30
Severe renal failure	5-10

The total clearance is affected mainly by hepatic clearance, which is given as

$$Cl_H = Cl_T - Cl_R$$

Or $Cl_H = Q_H \times ER_H$

Where Q_H = Hepatic blood flow (about 1.5 liters/min)

E R_H = Hepatic extraction ratio.

The table below shows the drugs with variable renal and hepatic extraction ratios.

Table 4: Drugs with respective renal and hepatic extraction ratios

Extraction Ratio			
Hepatic Extraction	High	Intermediate	Low
	Propranolol	Asprin	Diazepam
	Lidocaine	Codeine	Phenobarbital
	Nitroglycerine	Nortriptyline	Phenytoin
	Morphine	Quinidine	Procainamide
Renal Extraction	Some penicillins	Procainamide	Digoxin
	Several sulfates	Cimetidine	Furosemide
	Several Glucuronides		Atenolol
			Tetracycline

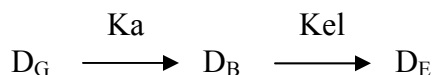
Usually the extraction ratio of above 0.7 is considered high while below 0.3 is considered as low. Change in protein binding (either increase or decrease) does not affect the total clearance rate for drugs with high extraction ratio. While drugs with low extraction ratio show decreased total clearance rate with increased protein binding and increased total clearance rate with decreased protein binding.

(b) Pharmacokinetics of drug absorption: The pharmacokinetic of drug absorption is determined by three methods

- (a) Residual (Feathering) curve method
- (b) Wagner – Nelson method.
- (c) Cumulative urine excretion analysis.
- (d)

We know that the drug given by iv route shows complete absorption as the drug is directly dissolved in the fluid and diffuses through one or more membranes. Apart from intravenous route, other routes are classified as extravascular route and exhibits rate limiting absorption, dependent on the appearance of drug in plasma.

Extravascular route (usually we consider the oral route) shows first order kinetics given as:



Where D_G = drug at a absorption site.

D_B = drug in the body

D_E = eliminated drug

K_a = first order absorption rate constant

K_{el} = Overall elimination rate constant

Such route of administration shows bi exponential nature that is initially the rate is dependent on absorption and elimination, later the elimination predominates the plasma concentration. The equation following this kinetic is given as:

$$C_p^\circ = \frac{D_G^\circ K_a [\exp(-k_{el} t) - \exp(-k_a t)]}{V_d (K_a - K_{el})}$$

This equation assumes that D_G° is equal to dose administered i.e. absorption is 100% complete, thus V_d is calculated.

Further, the elimination rate is calculated from the tail of the curve between concentration Vs time on semi log scale keeping the assumption that K_a is at least five times larger than k_{el} .

Therefore, this method assumes that

- (a) K_a is at least five times larger than K_{el} to determine the constants (K_a and K_{el}) accurately.

- (b) Absorption and elimination processes are first order that is why residual lines and elimination lines are straight.
- (c) It assumes absorption is complete that's why V_d is non erroneously determined.

Wagner – Nelson Method

With the previous method, the kinetic determination has a drawback as it assumes absorption is complete but it is possible with oral solutions or fast dissolving tablets but with other types of dosage forms this cannot be assumed as mixed order kinetics is followed. Therefore, Wagner – Nelson method assumes

- (a) Human body as a single homogeneous compartment
- (b) Drug elimination obeys first order kinetics

Therefore, equation following Wagner – Nelson model is as follows

$$A = W + E$$

Where, A = amount of drug absorbed

W= amount of drug in the body

E = amount of drug eliminated from the body

$$\frac{A_t}{V_d} = C_p^t + K_{el} \int_0^t C_p dt.$$

Where A_t = Amount of drug absorbed upto time t, divided by volume of distribution V_d

C_p^t = Plasma concentration at time t.

$\int_0^t C_p dt$ = the area under the plasma concentration vs time curve upto time t

Also, further the equation is simplified as

$$\frac{A_{max}}{V_d} = k_{el} \int_0^t C_p dt.$$

For comparing the bioavailability of two drugs with different plasma concentration time curve

$$\frac{A_{max 1}}{A_{max 2}} = \frac{\int_0^\infty (C_p dt)_1}{\int_0^\infty (C_p dt)_2}$$

Therefore extent of absorption usually the absolute bioavailability is given as

$$\text{Absolute bioavailability (for extravascular dosage form)} = \frac{AUC_{\text{extravascular}}}{AUC_{\text{iv}}} \times 100$$

Cumulative urinary excretion method

The pharmacokinetic parameters for extravascular dosage forms can also be determined by the equation given below which gives the idea of fraction of drug recovered in urine after absorption.

$$\frac{D_u^0}{D_B^0} = f_e$$

where D_u^0 = drug in urine

D_B^0 = drug in body

f_e = fraction absorbed drug recovered in urine as unchanged.

The relative bioavailability for extravascular dosage forms is given as

$$\frac{D_{u1}^o}{D_{u2}^o} = \frac{D_{B1}^o}{D_{B2}^o}$$

Similarly, the absolute bioavailability is given as

$$\text{Absolute bioavailability} = \frac{D_{Bu}^o (\text{Extravascular})}{D_u^o (\text{iv})}$$

Sometimes multiple dosing is done to have drug accumulation in the system to reach the some plateau as that of single dose so that minimum effective concentration (MEC) is attained and $C_{\max}$ is below the minimum toxic concentration (MTC)

Therefore after repetitive intravenous dosing 'R' factor which is the factor of initial plasma concentration that remains at the end of any dosing interval is given as

$$R = \exp(-K_{el} T) = 10^{-K_{el} \cdot T / 2.303}$$

$$C_{\max} = \frac{C_{p1}^o}{1 - R}$$

$$C_{\min} = C_{\max} R = \frac{C_{p1}^o R}{1 - R}$$

Where T = dosing interval

K_{el} = elimination rate constant

C_p^o = initial plasma concentration

$C_{\max}$ = initial plasma concentration

$C_{\min}$ = end plasma concentration

With repetitive extravascular dosing, the equation will be

$$C_{\max} = \frac{FD}{Vd} \frac{Ka}{ka - Kel} \left(\frac{\exp(-kel t_{\max})}{1 - \exp(-kel T)} - \frac{\exp(-ka t_{\max})}{1 - \exp(-ka T)} \right)$$

$$C_{\min} = \frac{FD}{Vd} \frac{Ka}{ka - Kel} \left(\frac{1}{1 - \exp(-kel T)} - \frac{1}{1 - \exp(-ka T)} \right)$$

Bioavailability

The ADME (Absorption, Deposition, Metabolism and Excretion) and pharmacokinetic profile of any drug in its dosage form is dependent on the important characteristics like particle size, salt form, the dissolution rate and lipophilic-hydrophilic profile. These factors are of paramount importance to the effectiveness of any dosage form. Such factors help in developing biopharmaceutical activity of the drug. To understand biopharmaceutics of the dosage form, the important parameter is to assess the relative amount of drug present in-vivo from an administered dosage form that enters the systemic circulation i.e., to assess bioavailability of the formulation.

Bioavailability is the measure of rate and extent to which the drug is able to perform its intended function. To understand the relationship of the drug product and its bioavailability, there are certain terminologies that should be clarified before calculating the ADME profile of the drug candidate.

1. **Drug product:** A finished dosage form that contains the active ingredient with pharmaceutically inactive components.
2. **Pharmaceutical equivalents:** Drug products that contain identical amounts of the same active drug ingredient that is, the same salt in the identical dosage form but not necessarily containing the same inactive component.
3. **Pharmaceutical alternatives:** Drug products that contain an identical active component but not necessarily in the same amount or dosage form. Both product forms meet the official compendium for evaluation.
4. **Bioequivalent drug products:** Bioequivalent drug products are pharmaceutical equivalent products having insignificant difference in rate and extent of drug absorption either in single or multiple doses. Some pharmaceutical equivalents or pharmaceutical alternatives may be equivalent in the extent not the rate of their absorption are considered bioequivalent.
5. **Drug absorption:** Drug absorption is the process of uptake of the pharmaceutical active component from the site of administration into the systemic circulation.
6. **Drug distribution:** Drug distribution is the process of distributing the drug to the site of action through body fluid.
7. **Drug metabolism.** It is also called as biotransformation and includes the biochemical changes in the drug molecule upon action of pH or enzymes leading to formation of active or inactive metabolites within the system. For example procaine upon hydrolysis produces inactive metabolite p-aminobenzoic acid and diethylethanolamine with loss of anesthetic activity. However, imipramine is demethylated to active metabolite desipramine as antidepressant.
8. **Drug excretion:** Drug excretion involves elimination of active drug molecules from the body or from body tissues. This process involves de-equilibrium of tissues or back diffusion of drug molecules from tissue to the general circulation.
9. **Absolute Bioavailability:** It is a fraction of systemic availability of the drug from the dosage form. It is calculated as the ratio of AUC of drug given orally and intravenously. The absolute bioavailability (F) value is 1 for 100% systemically absorbed drugs that is for injection formulations.
10. **Relative Bioavailability:** It is the bioavailability of drug from a dosage form in comparison to the reference dosage form given by the same route and in the same strength. The relative bioavailability of value 1 indicates that both test product and reference product are having similar bioavailability but their systemic absorption may not be identical.
11. **Essentially similar products:** A proprietary medicinal product is regarded as essentially similar to another product if it has the same qualitative and quantitative composition in terms of active principles and the pharmaceutical form is the same and where necessary,

bioequivalence with the first product has been demonstrated by appropriate bioavailability studies.

12. **Therapeutic equivalents:** A medicinal product is therapeutically equivalent with another product if it contains the same active substance or therapeutic moiety and clinically shows the same efficacy and safety as that product whose efficacy and safety have been established.
13. **Comparative bioavailability:** The comparative bioavailability studies involve determination of the relative bioavailability of an active drug in two or more formulations, without regard for the actual amount absorbed from each formulation.

Therefore, the calculations based on pharmacokinetic of any drug molecule involve the following important formulas for easy understanding and accurate results.

1. Calculations for Oral bioavailability by graphical method:

- a) Trapezoidal rule is followed to estimate the bioavailability of a dosage form. It involves, the plot of plasma concentration of drug ($\mu\text{g/ml}$) verses time (hrs). The curve is sectioned into trapezoids; the area under each trapezoid is calculated using the formula given below. The total area is calculated by adding up the individual areas.

$$\text{Area under the curve (AUC)} = \frac{(C_0 + C_1)}{2} (t_1 - t_0) + \frac{(C_1 + C_2)}{2} (t_2 - t_1)$$

for two trapezoids.

Where C_0 = Initial drug plasma concentration.

C_1 = Drug plasma concentration at time t_1 , and

C_2 = Drug plasma concentration at time t_2

- b) Absolute bioavailability is calculated from blood level data using the given formula.

$$F = \frac{\text{Dose}_{iv} \times \text{AUC}_{po}}{\text{Dose}_{po} \times \text{AUC}_{iv}}$$

Where F = Absolute bioavailability and indicates the absorption efficiency or the fraction of orally administered dose which is absorbed.

Dose_{iv} is the dose of the drug given intravenously. AUC_{iv} is the area under the curve of the iv dose. Dose_{po} is the dose of the drug given orally. AUC_{po} is the area under the curve of the orally administered drug.

The absolute bioavailability is also calculated using another formula which is not based on intravenous data. In that case, the renal clearance data will be considered to know the bioavailability-

$$F = \frac{\Delta Cl_g (\text{AUC}_1) (\text{AUC}_2)}{\text{Dose}_{po} (\text{AUC}_1 - \text{AUC}_2)}$$

Where, ΔCl_g is the change in renal clearance. AUC_1 and AUC_2 are the area under the curves of plasma profile of any drug under two conditions i.e. alkaline and acidic conditions. Dose_{po} is the oral dose of the drug formulation.

The renal clearance in the above formula is defined as the ratio between the urinary excretion rate and the plasma concentration and it has units of volume/time i.e. ml/min or liters/hour.

(c) There are other ways of calculating the percent pharmacological availability using the given formula

$$\text{Percent Pharmacological Availability} = \frac{\text{AUC}_{\text{oral}} \times \text{Dose}_{\text{sc}}}{\text{AUC}_{\text{sc}} \times \text{Dose}_{\text{oral}}} \times 100$$

Where AUC_{oral} and AUC_{sc} are the area under the curves of plasma profile of oral and subcutaneous doses, respectively.

2. Calculations for volume of distribution (V_d) to assess pharmacokinetic profile of the drug: The volume of distribution estimates the extent and intensity of the exposure of the body to a given dose of a drug. It is related to the plasma concentration to the total amount of drug in the body.

$$V_d = \frac{\text{Amount of drug in the body}}{\text{Plasma concentration}}$$

The volume of distribution is hypothetically volume of body fluid in which the drug is dissolved. This value is not true and is dependent on the amount of drug in the body and the percent bound drug whether to proteins (albumin) or fats.

$$V_D \times C_p = D_B$$

Where, V_D = Apparent volume of distribution,

C_p = Concentration in plasma

D_B = Drug in the body.

However, if the drug is given as intravenous bolus injection, then the equation is

$$V_D = \frac{D_B^0}{C_p^0}$$

Where D_B^0 = dose (D_0) given as i.v. bolus

C_p^0 = Concentration at zero time.

Also, when volume of distribution for any drug is known, and the rate of infusion is to be calculated, then the following formula is applied.

$$R = C_{ss} V_D K$$

Here, C_{ss} is the steady state plasma concentration indicating that the amount of drug eliminated is equal to the amount of drug systemically absorbed.

R is the rate of infusion (mg/hr)

K is the elimination half-life (hr^{-1})

The factor $V_D \times K$ also indicates the total body clearance (Cl_T).

Since, it is known that the intravenous bolus injection shows complete absorption, therefore in such dose calculations, the loading dose (D_L) is same as the bolus injection. However, if the drug is given by route other than IV, then the dose calculations are dependent on the elimination rate of the drug. Usually the steady state concentration of 90%, 95%, or 99%, without loading dose, takes around 3.32, 4.32 or 6.65 half-lives respectively.

Therefore, when no loading dose is given,

Then, $3.32 \times t_{1/2}$ = Time to reach 90% of C_{ss} concentration.

Thus, D_L is calculated using following formula.

$$D_L = C_{ss} V_D$$

$$\text{Or } D_L = R/K$$

Similarly, the frequency of dosing is also calculated as τ

$$\text{Dosing rate} = \frac{D_o}{\tau}$$

To know the steady state peak concentration of multiple intravenous injection, the formula to be used is,

$$C = \frac{D_o / V_D}{1 - e^{-K\tau}}$$

or

$$C = \frac{D_o F}{K V_D \tau}$$

Where D_o = Dose size

F = Bioavailability factor, and for intravenous injection $F = 1$.

For immediate-release dosage forms, by multiple dosing regime, the formula for calculating the concentration at steady state peak plasma concentration is:

$$C = \frac{F D_o}{V_D} \times \frac{1}{1 - e^{-K\tau}}$$

The factor $(1 - e^{-K\tau})$ is known as accumulation rate. To calculate the loading dose for oral multiple dosage formulations, the equation used is

$$D_L = D_M \frac{1}{1 - e^{-K\tau}}$$

Where D_M = Maintenance dose

If Maintenance dose is given at a rate of the drug's elimination half life, then $D_L = 2 D_M$

Similarly, drugs following non-dependent first order kinetics, the equation to calculate the peak plasma concentration is given by Michaelis-Menten equation

$$- \frac{dC_p}{dt} = \frac{V_{max} C_p}{K_M + C_p}$$

Where, V_{max} = Maximum velocity of the reaction

C_p = Plasma drug concentration

K_M = Rate constant equal to the C_p at $0.5 V_{max}$

Since, the drugs showing non-linear kinetics follow carrier mediated drug absorption, there is non-linearity in area under the curve in proportion to the dose.

The volume of distribution is high for drugs which are readily diffused into the body fluid, however, drugs having affinity for tissue binding or protein binding are less distributed thus have low values of volume of distribution. V_d value of 10 to 20 liters or between 15 and 27% of bodyweight, it indicates that the drug has distributed in intracellular fluids. If it is between 25 and 30 liters or between 35 and 42% of body weight, then its distribution is mainly in intracellular fluids. If the distribution is around 40 liters or 60% of the body weight, the assumption is that the drug has distributed in the whole body fluid. If the volume of distribution exceeds the body weight, then it is assumed that the drug is stored in body fat, bound to body tissues or distributed in peripheral compartments.

3. Elimination half-life of drug dosage form: Elimination phase of any drug within the biological system indicates the decline in the plasma concentration. The elimination half life is designated as $t_{1/2}$ that is the time taken by the plasma to reduce the concentration of the drug to its half. Mostly the drug given for systemic action follows first order kinetics that is the concentration of the drug eliminated per unit time is proportional to the amount present at the initial time. The elimination rate constant (K_{el}) for first order kinetic is calculated using the following formula

$$K_{el} = \frac{0.693}{t_{1/2}}$$

Where K_{el} is the elimination rate constant

$t_{1/2}$ = Plasma half life of the drug.

4. Creatinine clearance: It is known that the elimination of drugs and other compounds involves the estimation of the excretion profile of the drug, which is known by the glomerulus filtration rate. Since inulin and creatinine are used to estimate the excretion rate, the excretion is proportional to the plasma concentration of this polymeric carbohydrate (inulin) and an endogenous product of body metabolism (creatinine). Therefore, the excretion ratio of drug using inulin as an index is calculated as

$$\text{Excretion Ratio} = \frac{\text{Renal clearance of drug (ml/min)}}{\text{Normal inulin clearance (ml/min)}}$$

The normal glomerular filtration rate is 125 to 130 ml/min and if the value of excretion ratio is less than 1 then the drug is perhaps filtered or is partially reabsorbed. A value greater than 1 indicates secretion in addition to filtration and reabsorption. The value of zero for the above ratio is for compounds that are not excreted through kidney. However, the highest value for this ratio is 5 indicating that the renal clearance of the drug is equal to the total volume of plasma flowing per minute.

Another way of estimating the excretion profile of any drug is by creatinine clearance. Kidneys through glomerular filtration excrete creatinine. The normal range of serum creatinine

concentration is about 1 to 2 mg/ 100ml and this clearance is dependent on the body surface area of an adult (1.72 m² BSA). This creatinine clearance is given by the formula

$$\text{Creatinine clearance} = \frac{(140 - \text{age in years}) \times (\text{Body weight in kgs})}{72 \times \text{serum creatinine (mg/100ml)}} \quad (\text{ml/min})$$

The creatinine clearance as is known represents the volume of blood plasma that has cleared the creatinine by kidney filtration per minute, is also calculated by using two most important equations.

(c) Jelliffe Equation

For male

$$\text{Creatinine clearance rate} = \frac{98 - 0.8 \times (\text{patients age in years} - 20)}{\text{Serum creatinine (as mg/dL)}}$$

For females, the equation is

$$\text{Creatinine clearance rate} = 0.9 \times \text{CrCl determined for males.}$$

(a) Cockcroft and Gault Equation

$$\text{Creatinine clearance rate} = \frac{(140 - \text{patient's age in years}) \times \text{Body weight in kg}}{72 \times \text{serum Creatinine in mg/dL}}$$

For females, the Equation is

$$\text{Creatinine clearance Rate} = 0.85 \times \text{CrCl for males.}$$

Since normal creatinine clearance rate is considered 100 ml/ minute, also this rate along with body weight of the drug is helpful in calculating the loading dose (L_D) and maintenance dose of the drug. The loading dose is calculated using the formula:

$$L_D = \text{Ideal body weight in Kg or lb} \times \text{drug dose per kg or pound.}$$

Maintenance dose (M_D) is calculated as

$$M_D = \text{Ideal body weight (kg)} \times \text{dose per kg per dosing interval.}$$

This maintenance dose is for normal patient (creatinine clearance 100ml / min).

The maintenance dose for patients with renal impairment

$$M_D = \frac{\text{CrCl (Patient)}}{\text{CrCl (Normal)}} \times \text{dose for normal patient}$$

Numericals

Q. 1. If the bioavailability factor (F) for a 200 mg tablet of a drug is 0.70 compared to the bioavailability factor of 1.0 for an injection of the same drug, how many milliliters of the injection containing 40 mg/ml would be considered bioequivalent to the tablet?

Ans: The amount of the tablet that will be bioavailable is:

$$\text{Bioavailability Factor} \times \text{Dose (strength)}$$

Thus, for tablet, $0.70 \times 200 = 140 \text{ mg}$

Therefore out of 200 mg tablet, only 140 mg will be bioavailable.

For injection formulation, F is 1 and the strength is 40 mg / ml

For injection, amount bioavailable will be

$$= 1.0 \times 40 \text{ mg/ml}$$

$$= 40 \text{ mg/ml}$$

Therefore, by proportion equation

$$x = 140/40 = 3.5 \text{ ml}$$

Therefore, the quantity of injection that will provide 140 ml of bioavailable tablet is 3.5 ml

Q. 2. A patient received an intravenous dose of 10 mg of a drug. A blood sample was drawn and it contained 40 $\mu\text{g}/100 \text{ ml}$. Calculate the apparent volume of distribution for the drug.

Ans: Since $V_d = \frac{D}{C_p}$

Therefore, $C_p = 40 \mu\text{g} / 100 \text{ ml}$

or $0.04 \text{ mg} / 100 \text{ ml}$

or $0.4 \text{ mg} / \text{liter}$

$$V_d = \frac{10 \text{ mg}}{0.4 \text{ mg} / \text{liter}} \\ = 25 \text{ liters}$$

Q. 3. The alpha (α) value for a drug in the blood is 0.90, equating to 0.55 mg/ml. What is the concentration of total drug in the blood?

Ans: Since, the fraction of unbound drug in the plasma compared to the total plasma drug concentration, bound and unbound is α , is given or

$$\alpha = \frac{\text{Concentration (Unbound)}}{\text{Concentration in Plasma}}$$

Since $\alpha = 0.90$ and concentration in plasma (Unbound) = 0.55 ng

$$\alpha = \frac{\text{Concentration Unbound}}{\text{Concentration Unbound} + \text{Concentration bound}}$$

$$0.90 = \frac{0.55}{0.55 + X}$$

$$0.90 (0.55 + x) = 0.55$$

$$0.90 (0.55) + 0.90 X = 0.55$$

$$X = \frac{0.055}{0.90} = 0.6011 \text{ ng/ml}$$

Therefore, total drug in blood will be;

Concentration total = concentration unbound + concentration bound

$$= 0.55 + 0.0611$$

$$= 0.611 \text{ ng/ml}$$

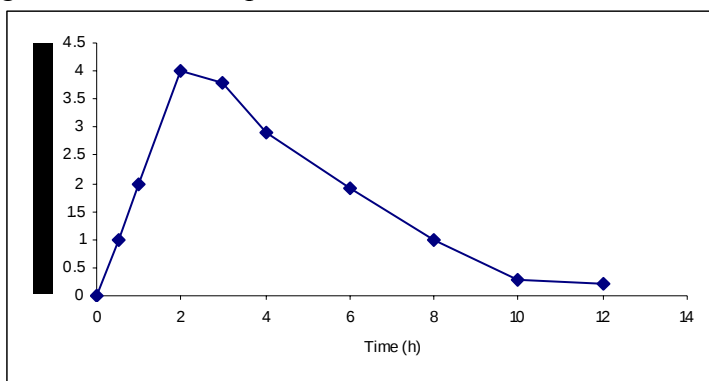
Q. 4. From the following data, plot a serum concentration time-curve and determine

(a) the peak height concentration ($C_{\max}$) and

(b) the time of the peak height concentration ($T_{\max}$)

Time Period (hours)	Serum Drug Concentration ($\mu\text{g/ml}$)
0.5	1
1.0	2
2.0	4
3.0	3.8
4.0	2.9
6.0	1.9
8.0	1.0
10.0	0.3
12.0	0.2

Ans: After plotting the data and interpretation of the curve;



$$C_{\max} = 4.0 \mu\text{g/ml}$$

$$T_{\max} = 2 \text{ hrs}$$

Q. 5. Calculate the elimination rate constant for a drug having an elimination half-life of 1.7 hours.

Ans: Elimination rate constant (K_{el}) for a drug is given by the formula

$$K_{el} = \frac{0.693}{t_{1/2}}$$

$$= \frac{0.693}{1.7}$$

$$K_{el} = 0.407 \text{ hr}^{-1}$$

Q. 6. If the half-life of a drug is 4 hours, approximately what percent of the drug administered (20mg) would remain in the body 16 hours after administration?

Ans: Since half-life indicates the time when the drug is reduced to its half concentration after administration, therefore to know concentration of drug after administration method given below is followed.

Concentration of drug (mg).	Time (hours)
20	T ₀
10	T ₄
5	T ₈
2.5	T ₁₂
1.25	T ₁₆

Therefore, after sixteen hours of administration, the concentration of drug remaining in the body is 1.25 mg.

The percent drug remaining after administration-

$$\begin{aligned}
 &= (1.25/20) \times 100 \\
 &= 0.0625 \times 100 \\
 &= 6.25\%
 \end{aligned}$$

Q. 7. If 150 mg of a drug is administered intravenously and the resultant drug plasma concentration is determined to be 2.5 µg/ml. Calculate the apparent volume of distribution.

Ans: Apparent volume of distribution (V_d) = $\frac{D}{C_p}$

$$\begin{aligned}
 \text{Dose} &= 150 \text{ mg} \\
 C_p &= 2.5 \text{ µg/ml} \\
 V_d &= \frac{150}{2.5 \text{ µg/ml}} \\
 &= 150/2.5 \text{ mg / litre} \\
 &= 60 \text{ litres}
 \end{aligned}$$

Q. 8. Calculate the creatinine clearance rate for a 20 year old male patient weighing 70 kg with a serum creatinine of 1 mg/dL. If a patient is 5 ft, 8 inches tall, adjust the creatinine clearance based on body surface area.

Ans: The formula for calculating the creatinine clearance is.

$$\begin{aligned}
 CrCl &= \frac{(140 - \text{age in years}) \times \text{Bodyweight (kg)}}{72 \times \text{serum creatinine (mg / 100 ml)}} \\
 &= \frac{(140 - 20) \times 70}{72 \times 1 \text{ mg/100 ml}} \\
 &= \frac{120 \times 70}{72 \times 1 \text{ mg / 100 ml}} \\
 &= 116.66 \text{ ml/min}
 \end{aligned}$$

Q. 9. A female patient weighing 70 kg and 65 year old, has serum creatinine level 2 mg/dL, what is the expected creatinine clearance rate. Calculate the CrCl for a female patient.

Ans: For female Patient, the creatinine clearance rate is 0.9 x CrCl for males.

$$\text{CrCl for males} = \frac{98 - 0.8 \times (\text{patients age in years} - 20)}{\text{Serum creatinine (mg/dL)}}$$

$$= \frac{98 - 0.8 \times (65 - 20)}{2 \text{ mg/dL}}$$

$$= \frac{98 - 0.8 \times 45}{2}$$

$$= \frac{98 - 36}{2}$$

$$= 31 \text{ ml/min}$$

$$\text{CrCl for females} = 0.9 \times 31 \text{ ml / min}$$

$$= 27.9 \text{ ml/min}$$

Q. 10. A new antibiotic is given at a dose 10 mg / kg by a single intravenous bolus injection to a 58 year old man who weighed 75 kg. The antibiotic follows the pharmacokinetic of a one-compartment model and has elimination half-life of 1.5 hours. The apparent volume of distribution is 0.18 L/kg and the drug is 35% bound to plasma protein. Calculate the following:

(a) Initial Plasma concentration

(b) How long after the dose is exactly 75% of the drug eliminated from the patient's body?

Ans: The initial plasma concentration is given by the following formula.

$$(a) \quad C^{\circ}_P = \frac{D_0}{V_D}$$

$$C^{\circ}_P = \frac{10 \text{ mg/kg}}{0.18 \text{ L/kg}}$$

$$C^{\circ}_P = 55.55 \text{ mg/L}$$

(c) Since the elimination half life of the drug is 1.5 hours, it means that the drug is 50% remained in the system after 1.5 hours, then to have 75% drug eliminated, exactly 2 half lives are required i.e 1.5 hours x 2 = 3 hours.

Exercises

Q. 1 Calculate the infusion rate in mg/min that will maintain an average plasma procainamide concentration of 6 mg / L. The drug is showing half-life of 1.5 hrs and volume of distribution 6 L.

Ans: 0.2772 mg/min.

Q. 2. A 35 year old man who weighs 70 kg and has normal renal function needs an intravenous infusion of the antibiotic. The desired steady state plasma drug concentration is 12.85 mg/dl. The

physician wants the antibiotic to be infused into the patient for 10 hours. The antibiotic has an elimination half life ($t_{1/2}$) of 2 hour and an apparent volume of distribution (V_D) of 9L in this Patient. Calculate.

(a) Rate of intravenous infusion.

(b) How long after initiation of the extravenous infusion would be plasma drug concentration reach 95% of the theoretic steady state concentration.

Ans: (a) $R = 40.072 \text{ mg/hr}$; (b) $T = 8.64 \text{ hours}$

Q.3. Show the pharmacokinetic parameters for the following serum concentration profile.

Time (hrs)	Serum Concentration (ng/ml)
1	0.081
1.5	0.142
2.0	0.213
2.5	0.308
3.0	0.411
3.5	0.318
4.0	0.299
4.5	0.216
5.0	0.188
5.5	0.103
6.0	0.0981