

Bioavailability and Bioequivalence

The therapeutic effectiveness of a drug depends upon the ability of the dosage form to deliver the medicament to its site of action at a rate and amount sufficient to elicit the desired pharmacologic response. This attribute of the dosage form is referred to as *physiologic availability*, *biologic availability* or simply *bioavailability*. For most drugs, the pharmacologic response can be related directly to the plasma levels. Thus, *the term bioavailability is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form.* It is an absolute term. The rate or rapidity with which a drug is absorbed is an important consideration when a rapid onset of action is desired as in the treatment of acute conditions such as asthma attack, pain, etc. A slower absorption rate is however desired when the aim is to prolong the duration of action or to avoid the adverse effects. On the other hand, extent of absorption is of special significance in the treatment of chronic conditions like hypertension, epilepsy, etc.

If the size of the dose to be administered is same, then *bioavailability of a drug from its dosage form depends upon 3 major factors:*

- ① Pharmaceutical factors related to physicochemical properties of the drug and characteristics of the dosage form
- ② Patient related factors
- ③ Route of administration

Objectives of Bioavailability Studies

Bioavailability studies are important in the---

- ① Primary stages of development of a suitable dosage form for a new drug entity.
- ② Determination of influence of excipients, patient related factors and possible interaction with other drugs on the efficiency of absorption.
- ③ Development of new formulations of the existing drugs.
- ④ Control of quality of a drug product during the early stages of marketing in order to determine the influence of processing factors, storage and stability on drug absorption.

METHODS FOR ENHANCEMENT OF BIOAVAILABILITY

As far as the definition of bioavailability is concerned, a drug with poor bioavailability is the one with -

- ① Poor aqueous solubility and/or slow dissolution rate in the biologic fluids.
- ② Poor stability of the dissolved drug at the physiologic pH.
- ③ Inadequate partition coefficient and thus poor permeation through the biomembrane.
- ④ Extensive presystemic metabolism.

The three major approaches in overcoming the bioavailability problems due to such causes are:

- 1) **The Pharmaceutical Approach** which involves modification of formulation, manufacturing process or the physicochemical properties of the drug without changing the chemical structure.
- 2) **The Pharmacokinetic Approach** in which the pharmacokinetics of the drug is altered by modifying its chemical structure.
- 3) **The Biologic Approach** whereby the route of drug administration may be changed such as changing from oral to parenteral route.

The second approach of chemical structure modification has a number of drawbacks of being very expensive and time consuming, requires repetition of clinical studies and a long time for regulatory approval. Moreover, the new chemical entity may suffer from another pharmacokinetic disorder or bear the risk of precipitating adverse effects. Only the pharmaceutical approach will be dealt herewith.

The attempts, whether optimizing the formulation, manufacturing process or physicochemical properties of the drug, are mainly aimed at enhancement of dissolution rate as it is the major rate-limiting step in the absorption of most drugs. There are several ways in which the dissolution rate of a drug can be enhanced. Some of the widely used methods, most of which are aimed at increasing the effective surface area of the drugs, will be discussed briefly.

1. Micronization : The process involves reducing the size of the solid drug particles to 1 to 10 microns commonly by spray drying or by use of air attrition methods (fluid energy mill). Examples of drugs whose bioavailability have been increased by micronization include griseofulvin and several steroidal and sulfa drugs.

2. Use of Surfactants : The surface-active agents enhance dissolution rate primarily by promoting wetting and penetration of dissolution fluid into the solid drug particles. They are generally used in concentration below their critical micelle concentration (CMC) values since above CMC, the drug entrapped in the micelle structure fails to partition in the dissolution fluid. Nonionic surfactants like polysorbates are widely used. Examples of drugs whose bioavailability have been increased by use of surfactants in the formulation include steroids like spironolactone.

3. Use of Salt Forms : Salts have improved solubility and dissolution characteristics in comparison to the original drug. Alkali metal salts of acidic drugs like penicillins and strong acid salts of basic drugs like atropine are more water-soluble than the parent drug.

4. Alteration of pH of the Drug Microenvironment : This can be achieved in two ways—*in situ* salt formation, and addition of buffers to the formulation e.g. buffered aspirin tablets.

5. Use of Metastable Polymorphs : A metastable polymorph is more soluble than the stable polymorph of a drug that exhibits polymorphism—for example, the B form of chloramphenicol palmitate is more water soluble than the A and the C forms.

6. Solute-Solvent Complexation : Solvates of drugs with organic solvents (also called as pseudopolymorphs) generally have higher aqueous solubility than their respective hydrates or the original drug. Much higher solubility can be attained by freeze drying such a solute in solution with an organic solvent with which it is known to form a solvate. Such a process results in a powder of particles of submicron size, e.g. 1:2 griseofulvin-benzene solvate. However, one should take care that the solvent is nontoxic.

7. Solvent Deposition : In this method, the poorly aqueous soluble drug such as nifedipine is dissolved in an organic solvent like alcohol and deposited on an inert, hydrophilic, solid matrix such as starch or microcrystalline cellulose by evaporation of solvent.

8. Selective Adsorption on Insoluble Carriers : A highly active adsorbent such as the inorganic clays like bentonite can enhance the dissolution rate of poorly water soluble drugs such as griseofulvin, indomethacin and prednisone by maintaining the concentration gradient at its maximum. The two reasons suggested for the rapid release of drugs from the surface of clays are—the weak physical bonding between the adsorbate and the adsorbent, and hydration and swelling of the clay in the aqueous media.

9. Solid Solutions : The three means by which the particle size of a drug can be reduced to submicron level are—

- (i) use of solid solutions,
- (ii) use of eutectic mixtures, and
- (iii) use of solid dispersions.

In all these cases, the solute is frequently a poorly water soluble drug acting as the guest and the solvent is a highly water-soluble compound or polymer acting as a host or carrier.

A solid solution is a binary system comprising of a solid solute molecularly dispersed in a solid solvent. Since the two components crystallize together in a homogeneous one phase system, solid solutions

are also called as **molecular dispersions** or **mixed crystals**. Because of reduction in particle size to the molecular level, solid solutions show greater aqueous solubility and faster dissolution than eutectics and solid dispersions. They are generally prepared by fusion method whereby a physical mixture of solute and solvent are melted together followed by rapid solidification. *Such systems, prepared by fusion, are often called as melts* e.g. griseofulvin-succinic acid (Fig. 12.4). The griseofulvin from such solid solution dissolves 6 to 7 times faster than pure griseofulvin.

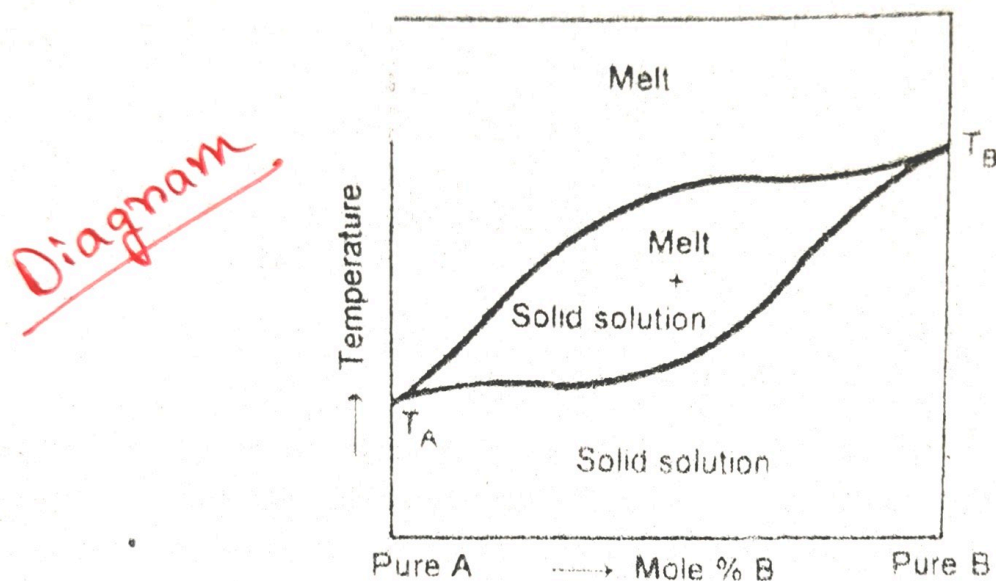


Fig. 12.4 Binary phase diagram for continuous solid solution of A and B
 T_A and T_B are melting points of pure A and pure B respectively.

If the diameter of solute molecules is less than 60% of diameter of solvent molecules or its volume less than 20% of volume of solvent molecule, the solute molecule can be accommodated within the intermolecular spaces of solvent molecules e.g. digitoxin-PEG 6000 solid solution. Such systems show faster dissolution. *When the resultant solid solution is a homogeneous transparent and brittle system, it is called as glass solution.* Carriers that form glassy structure are citric acid, urea, PVP and PEG and sugars such as dextrose, sucrose and galactose.

The two mechanisms suggested for enhanced solubility and rapid dissolution of molecular dispersions are:

1. When the binary mixture is exposed to water, the soluble carrier dissolves rapidly leaving the insoluble drug in a state of microcrystalline dispersion of very fine particles, and
2. When the solid solution, which is said to be in a state of randomly arranged solute and solvent molecules in the crystal lattice, is exposed to the dissolution fluid, the soluble carrier dissolves rapidly leaving the insoluble drug stranded at almost molecular level.

Fig. 12.5 shows a comparison between the dissolution rates of different forms of griseofulvin.

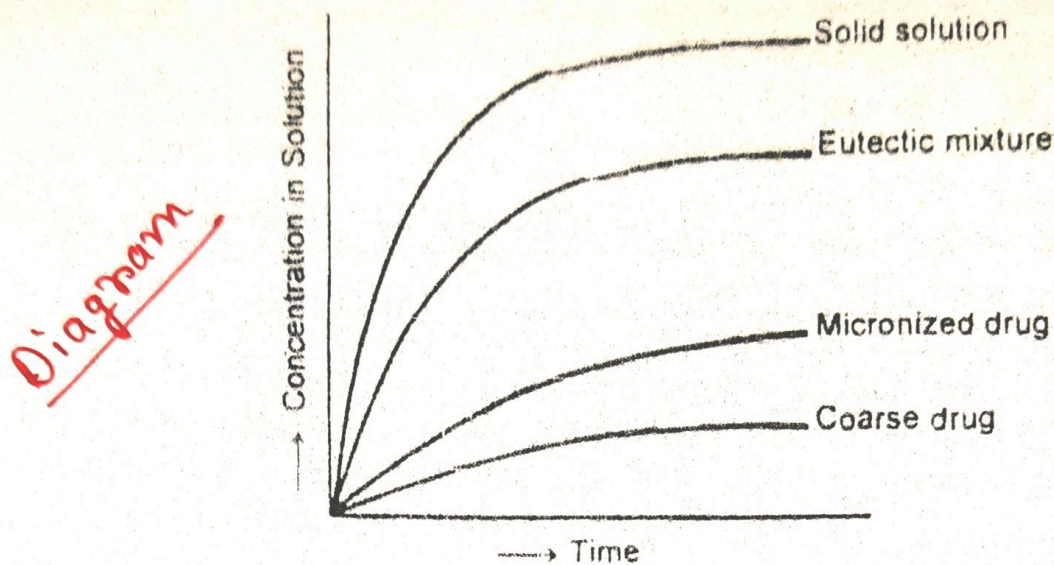


Fig. 12.5 Dissolution rates of griseofulvin as coarse particles, as micronized particles and as eutectic and solid solution with succinic acid.

10. Eutectic Mixtures : These systems are also prepared by fusion method. Eutectic melts differ from solid solutions in that the fused melt of solute-solvent show complete miscibility but negligible solid-solid solubility i.e. *such systems are basically intimately blended physical mixture of two crystalline components*. A phase diagram of two component system is shown in Fig. 12.6. When the eutectic mixture is exposed to water, the soluble carrier dissolves leaving the drug in a microcrystalline state which solubilizes rapidly.

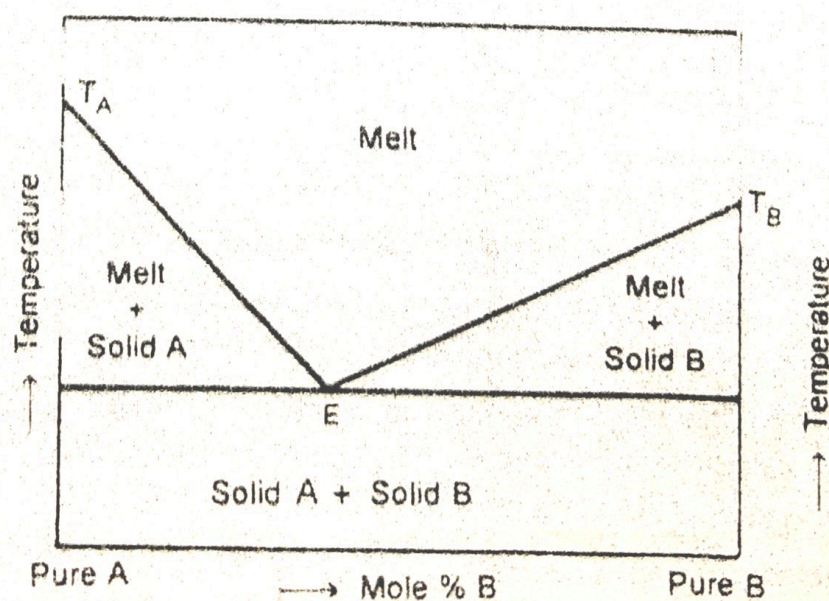


Fig. 12.6 Simple binary phase diagram showing eutectic point E. The eutectic composition at point E of substances A and B represents the one having lowest melting point. T_A and T_B are melting points of pure A and pure B respectively.

Examples of eutectics include paracetamol-urea, griseofulvin-urea, griseofulvin-succinic acid, etc. Solid solutions and eutectics, which are basically melts, are easy to prepare and economical with no solvents involved. The method however cannot be applied to:

- drugs which fail to crystallize from the mixed melt,
- thermolabile drugs, and
- carriers such as succinic acid that decompose at their melting point.

The eutectic product is often tacky, intractable or irregular crystals.

11. Solid Dispersions : These are generally prepared by solvent or co-precipitation method whereby both the guest solute and the solid carrier solvent are dissolved in a common volatile liquid solvent such as alcohol. The liquid solvent is removed by evaporation under reduced pressure or by freeze drying which results in amorphous precipitation of guest in a crystalline carrier. Thus, the basic difference between solid dispersions and solid solutions/eutectics is that the drug is precipitated out in an amorphous form in the former as opposed to crystalline form in the latter; e.g. amorphous sulfathiazole in crystalline urea. Such dispersions are often called as co-evaporates or co-precipitates. The method is suitable for thermolabile substances but has a number of disadvantages like higher cost of processing, use of large quantities of solvent, difficulty in complete removal of solvent, etc. The carriers used are same as for eutectics or solid solutions. With glassy materials, the dispersions formed are called as glass dispersions or glass suspensions. Fig. 12.7. shows comparative dissolution rates of griseofulvin from PVP dispersions.

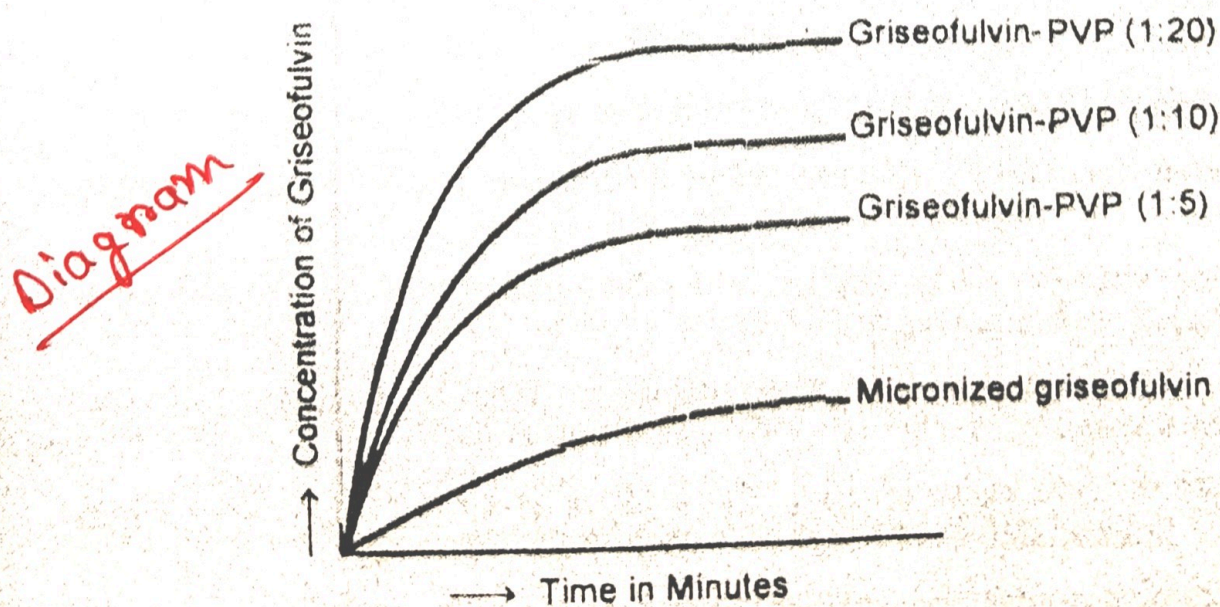


Fig. 12.7 Dissolution rate enhancement of griseofulvin by solid dispersion technique.

12. Molecular Encapsulation with Cyclodextrins : The beta- and gamma-cyclodextrins and several of their derivatives are unique in having the ability to form molecular inclusion complexes with hydrophobic drugs having poor aqueous solubility. These cyclodextrin molecules are versatile in having a hydrophobic cavity of size suitable enough to accommodate the lipophilic drugs as guests; the outside of the host molecule is relatively hydrophilic (Fig. 12.8). Thus, the molecularly encapsulated drug has greatly improved aqueous solubility and dissolution rate. There are several examples of drugs with improved bioavailability due to such a phenomena—thiazide diuretics, barbiturates, benzodiazepines and a number of NSAIDs.

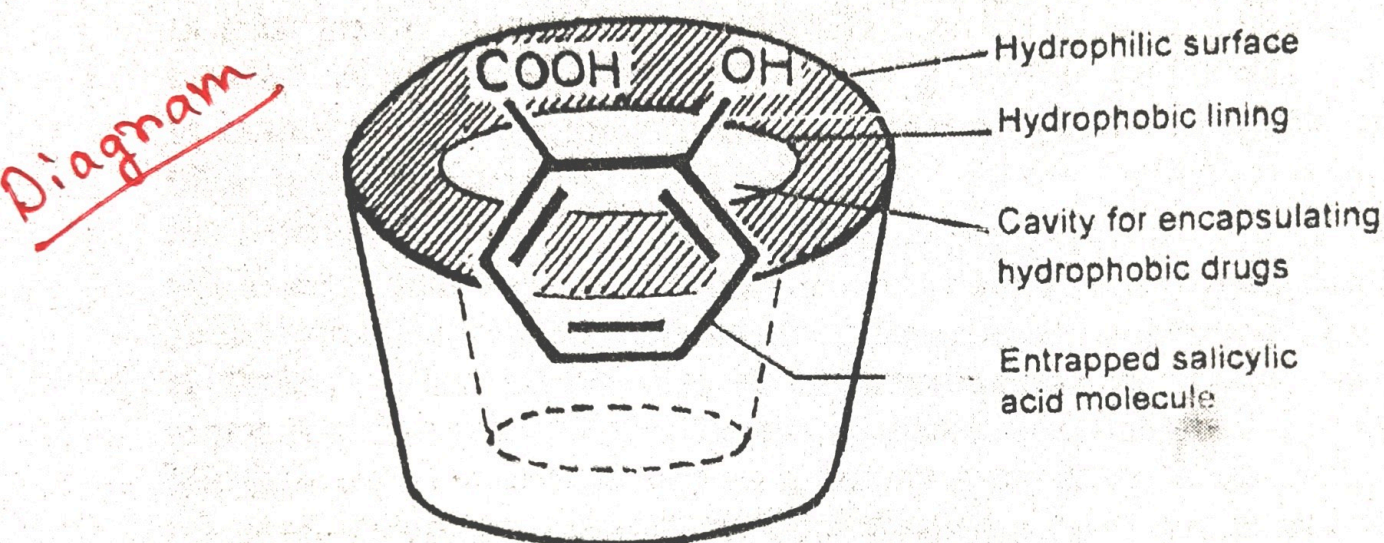


Fig. 12.8 Functional and structural feature of a cyclodextrin molecule showing an encapsulated drug.

QUESTIONS

1. Define bioavailability. What are the objectives of bioavailability studies?
2. Enumerate the factors affecting bioavailability of a drug from its dosage form.
3. In a bioavailability study, explain how determination of both rate and extent of absorption are important.
4. Define absolute and relative bioavailability. What is the basic difference between the two?
5. What are the limitations of using oral solution as a standard for determining absolute bioavailability?
6. Compare single dose with multiple dose bioavailability studies.
7. Discuss the merits and demerits of using healthy subjects and patients as volunteers for bioavailability studies.

Noncompartmental Analysis

Because of the several drawbacks of and difficulties with the classical compartment modeling, newer approaches have been devised to study the time course of drugs in the body. They are—physiologic models and noncompartmental methods.

The noncompartmental analysis, also called as the model independent method, does not require the assumption of specific compartment model. This method can however be applied to any compartment model provided the drugs or metabolites follow linear kinetics. The approach, based on the statistical moments theory, involves collection of experimental data following a single dose of drug. If one considers the time course of drug concentration in plasma as a statistical distribution curve, then:

$$\text{MRT} = \frac{\text{AUMC}}{\text{AUC}} \quad (9.19)$$

✓ where MRT = mean residence time

AUMC = area under the first-moment curve

AUC = area under the zero-moment curve

2 marks.

AUMC is obtained from a plot of product of plasma drug concentration and time (i.e. $C \cdot t$) versus time t from zero to infinity (Fig. 9.7). Mathematically, it is expressed by equation:

$$\text{AUMC} = \int_0^{\infty} C \cdot t \, dt \quad (9.20)$$

AUC is obtained from a plot of plasma drug concentration versus time from zero to infinity. Mathematically, it is expressed by equation:

$$\text{AUC} = \int_0^{\infty} C \, dt \quad (9.21)$$

Advantages of noncompartmental method include—

1. Ease of derivation of pharmacokinetic parameters by simple algebraic equations.
2. The same mathematical treatment can be applied to almost any drug or metabolite provided they follow first-order kinetics.
3. A detailed description of drug disposition characteristics is not required.

The **disadvantage** of this method is that it provides limited information regarding the plasma drug concentration-time profile; more often, it deals with averages.

Physiologic Models

These models are also termed as **blood flow rate-limited models** and **perfusion rate-limited models**. The model is drawn on the basis of known anatomic and physiologic data and thus presents a more realistic picture of drug disposition in various organs and tissues. The number of compartments to be included in the model depends upon the disposition characteristics of the drug. Organs or tissues such as bones that have no drug penetration are excluded. Fig. 9.8 shows such a physiologic model where the compartments are arranged in a series in a flow diagram.

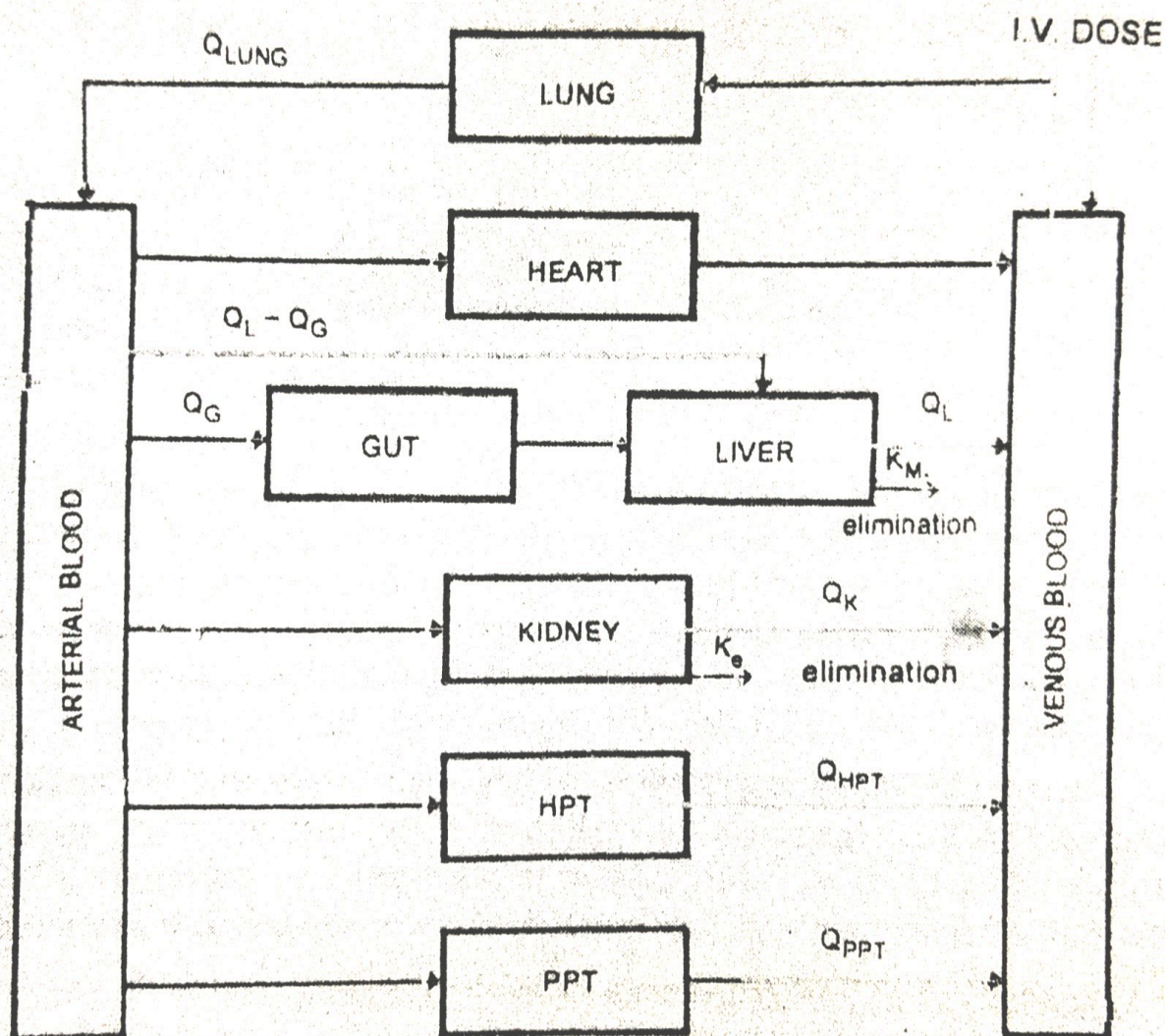


Fig. 9.8 Schematic representation of a physiologic pharmacokinetic model. The term Q indicates blood flow rate to a body region. HPT stands for other highly perfused tissues and PPT for poorly perfused tissues. K_m is rate constant for hepatic elimination and K_e is first-order rate constant for urinary excretion.

Most physiologic models are based on the assumption that the drug movement within a body region is much more rapid than its rate of delivery to that region by the perfusing blood and therefore, the model is said to be perfusion rate-limited. The assumption is however applicable

only to the highly membrane permeable drugs i.e. low molecular weight, poorly ionized and highly lipophilic drugs. For highly polar, ionized and charged drugs, the model is referred to as membrane permeation rate-limited.

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Practically, the AUMC and AUC can be calculated from the respective graphs by the **trapezoidal rule** (the method involves dividing the curve by a series of vertical lines into a number of trapezoids, calculating separately the area of each trapezoid and adding them together).

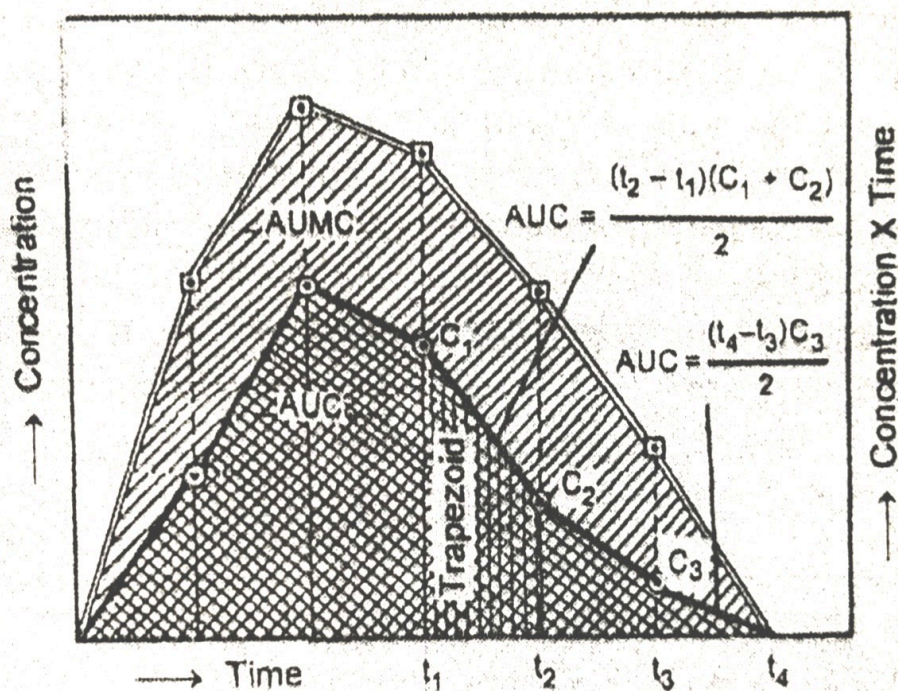


Fig. 9.7 AUC and AUMC plots

2 marks

MRT is defined as the average amount of time spent by the drug in the body before being eliminated. In this sense, it is the statistical moment analogy of half-life, $t_{1/2}$. In effect, MRT represents the time for 63.2% of the intravenous bolus dose to be eliminated. The values will always be greater when the drug is administered in a fashion other than i.v. bolus.)

CAUSES OF NONLINEARITY

Nonlinearities can occur in drug absorption, distribution, metabolism and excretion:

* Drug Absorption

Nonlinearity in drug absorption can arise from 3 important sources:

- ① *When absorption is solubility or dissolution rate-limited e.g. griseofulvin. At higher doses, a saturated solution of the drug is formed in the GIT or at any other extravascular site and the rate of absorption attains a constant value.*
- ② *When absorption involves carrier-mediated transport systems e.g. riboflavin, ascorbic acid, cyanocobalamin, etc. Saturation of the transport system at higher doses of these vitamins results in nonlinearity.*
- ③ *When presystemic gut wall or hepatic metabolism attains saturation e.g. propranolol.*

The parameters affected will be F , K_a , C_{max} and AUC. A decrease in these parameters is observed in the former two cases and an increase in the latter case. Other causes of nonlinearity in drug absorption are changes in gastric emptying and GI blood flow and other physiologic factors. Nonlinearity in drug absorption is of little consequence unless availability is drastically affected.

* Drug Distribution

Nonlinearity in distribution of drugs administered at high doses may be due to:

- ① *Saturation of binding sites on plasma proteins e.g. phenylbutazone.*
- ② *Saturation of tissue binding sites.*

In both cases, the free plasma drug concentration increases but V_d increases only in the former case whereas it decreases in the latter. Clearance is also altered depending upon the extraction ratio of the drug. Clearance of a drug with high ER is greatly increased due to saturation of binding sites. Unbound clearance of drugs with low ER is unaffected and one can expect an increase in pharmacologic response.

* Drug Metabolism

The nonlinear kinetics of most clinical importance is capacity-limited metabolism since small changes in dose administered can produce large variations in plasma concentration at steady-state. It is a major source of

large intersubject variability in pharmacologic response. Two important causes of nonlinearity in metabolism are:

- ① *Capacity-limited metabolism due to enzyme and/or cofactor saturation.* Typical examples include phenytoin, alcohol, theophylline, etc.
- ② *Enzyme induction* e.g. carbamazepine, where a decrease in peak plasma concentration has been observed on repetitive administration over a period of time. Autoinduction characterized in this case is also dose-dependent. Thus, enzyme induction is a common cause of both dose- and time-dependent kinetics.

Saturation of enzyme results in decreased Cl_H and therefore increased C_{ss} . Reverse is true for enzyme induction. Other causes of nonlinearity in biotransformation include saturation of binding sites, inhibitory effect of the metabolite on enzyme and pathologic situations such as hepatotoxicity and changes in hepatic blood flow.

* Drug Excretion

The two active processes in renal excretion of a drug that are saturable are:

- ① *Active tubular secretion* e.g. penicillin G, and
- ② *Active tubular reabsorption* e.g. water soluble vitamins and glucose.

After saturation of the carrier systems, a decrease in renal clearance in the former case and an increase in the latter situation is observed. Other sources of nonlinearity in renal excretion include forced diuresis, changes in urine pH, nephrotoxicity and saturation of binding sites.

Biliary secretion, which is also an active process, is also subject to saturation e.g. tetracycline and indomethacin.

MICHAELIS MENTEN EQUATION

The kinetics of capacity-limited or saturable processes is best described by Michaelis-Menten equation:

$$-\frac{dC}{dt} = \frac{V_{\max} C}{K_m + C} \quad (11.1)$$

✓ where $-dC/dt$ = rate of decline of drug concentration with time,

- $V_{\max}$ = theoretical maximum rate of the process, and
- K_m = Michaelis constant.

Three situations can now be considered depending upon the values of K_m and C :

1. When $K_m = C$: Under this situation, the equation 11.1. reduces to:

$$\frac{dC}{dt} = \frac{V_{\max}}{2} \quad (11.2)$$

i.e. the rate of process is equal to one-half its maximum rate (Fig. 11.1).

Diagram

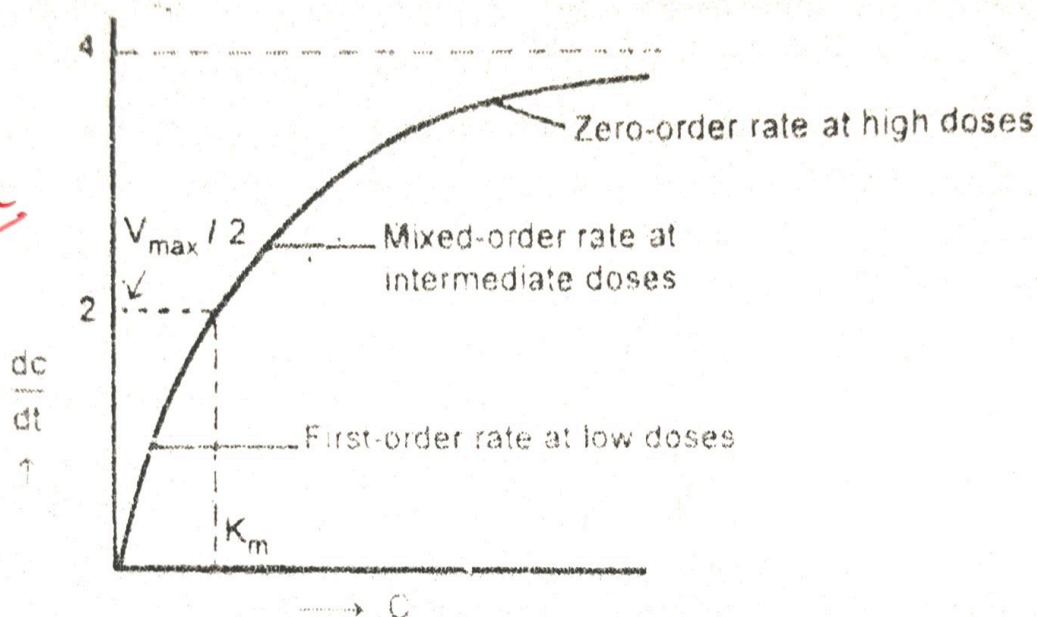


Fig. 11.1 A plot of Michaelis-Menten equation (elimination rate dC/dt versus concentration C). Initially, the rate increases linearly (first-order) with concentration, becomes mixed-order at higher concentration and then reaches maximum ($V_{\max}$) beyond which it proceeds at a constant rate (zero-order).

2. When $K_m \gg C$: Here, $K_m + C \approx K_m$ and the equation 11.1. reduces to:

$$\frac{dC}{dt} = \frac{V_{\max} C}{K_m} \quad (11.3)$$

The above equation is identical to the one that describes first-order elimination of a drug where $V_{\max}/K_m = K_d$. This means that the drug concentration in the body that results from usual dosage regimens of most drugs is well below the K_m of the elimination process with certain exceptions such as phenytoin and alcohol.

3. When $K_m \ll C$: Under this condition, $K_m + C \approx C$ and the equation 11.1 will become:

$$\frac{dC}{dt} = V_{\max} \quad (11.4)$$

The above equation is identical to the one that describes a zero-order process i.e. the rate process occurs at a constant rate $V_{\max}$ and is independent of drug concentration e.g. metabolism of ethanol.

* Estimation of K_m and $V_{\max}$

The parameters of capacity-limited processes like metabolism, renal tubular secretion and biliary excretion can be easily defined by assuming one-compartment kinetics for the drug and that elimination involves only a single capacity-limited process.

The parameters K_m and $V_{\max}$ can be assessed from the plasma concentration-time data collected after i.v. bolus administration of a drug with nonlinear elimination characteristics.

Rewriting equation 11.1.

$$-\frac{dC}{dt} = \frac{V_{\max} C}{K_m + C} \quad (11.1)$$

Integration of above equation followed by conversion to log base 10 yields:

$$\log C = \log C_0 + \frac{(C_0 - C)}{2.303 K_m} - \frac{V_{\max} t}{2.303 K_m} \quad (11.5)$$

A semilog plot of C versus t yields a curve with a terminal linear portion having slope $-V_{\max}/2.303K_m$ and when back extrapolated to time zero gives Y -intercept $\log \bar{C}_0$ (see Fig. 11.2). The equation that describes this line is:

$$\log C = \log \bar{C}_0 - \frac{V_{\max} t}{2.303 K_m} \quad (11.6)$$

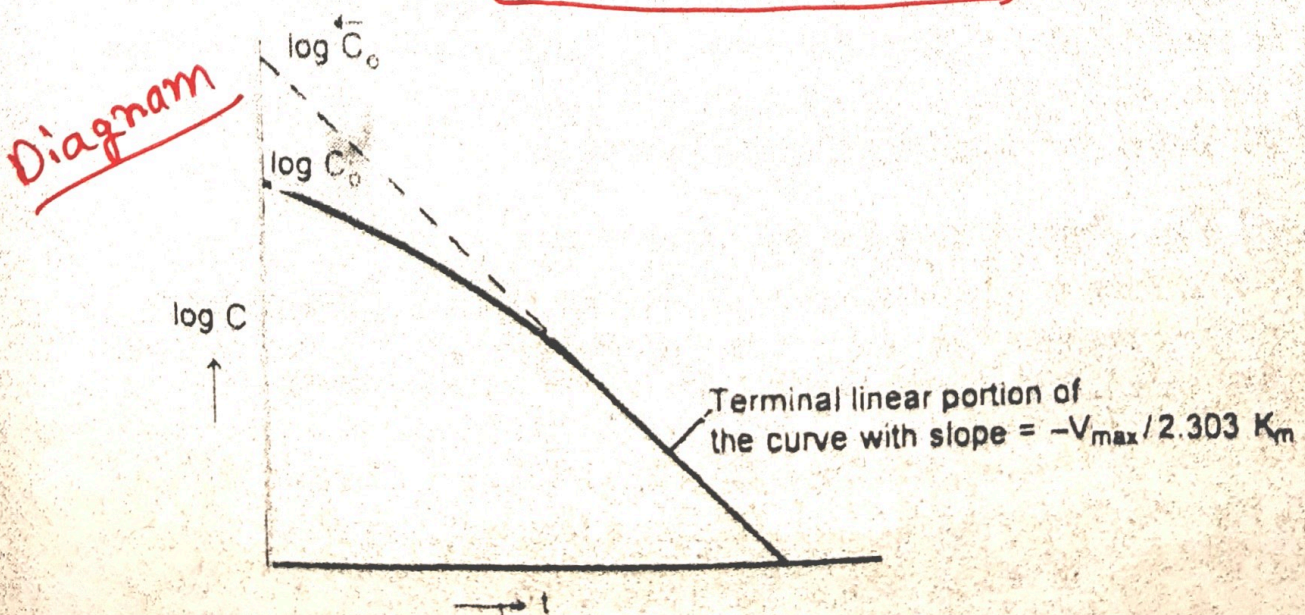


Fig. 11.2 Semilog plot of a drug given as i.v. bolus with nonlinear elimination and that fits one-compartment kinetics.

At low plasma concentrations, equations 11.5 and 11.6 are identical. Equating the two and simplifying further, we get:

$$\frac{(C_0 - C)}{2.303 K_m} = \log \frac{C_0}{C} \quad (11.7)$$

K_m can thus be obtained from above equation. V_{max} can be computed by substituting the value of K_m in the slope value.

An alternative approach of estimating V_{max} and K_m is determining the rate of change of plasma drug concentration at different times and using the reciprocal of the equation 11.1. Thus:

$$\frac{1}{dC/dt} = \frac{K_m}{V_{max} C_m} + \frac{1}{V_{max}} \quad (11.8)$$

where C_m = plasma concentration at midpoint of the sampling interval. A double reciprocal plot or the **Lineweaver-Burk plot** of $1/(dC/dt)$ versus $1/C_m$ of the above equation yields a straight line with slope = K_m/V_{max} and y-intercept = $1/V_{max}$.

A *disadvantage* of Lineweaver-Burk plot is that the points are clustered. More reliable plots in which the points are uniformly scattered are **Hanes-Woolf plot** (equation 11.9) and **Wolf-Augustinsson-Hofstee plot** (equation 11.10).

$$\frac{C_m}{dC/dt} = \frac{K_m}{V_{max}} + \frac{C_m}{V_{max}} \quad (11.9)$$

$$\frac{dC}{dt} = V_{max} - \frac{dC/dt K_m}{C_m} \quad (11.10)$$

The above equations are rearrangements of equation 11.8. Equation 11.9 is used to plot $C_m/(dC/dt)$ versus C_m and equation 11.10 to plot dC/dt versus $(dC/dt)/C_m$. The parameters K_m and V_{max} can be computed from the slopes and y-intercepts of the two plots.

* K_m and V_{max} from Steady-State Concentration

When a drug is administered as a constant rate i.v. infusion or in a multiple dose regimen, the steady-state concentration C_{ss} is given in terms of dosing rate DR as:

$$DR = C_{ss} Cl_T \quad (11.11)$$

where $DR = R_0$ when the drug is administered as zero-order i.v. infusion and it is equal to FX_0/τ when administered as multiple oral dosage regimen (F is fraction bioavailable, X_0 is oral dose and τ is dosing interval).

NONLINEAR PHARMACOKINETICS

At steady-state, the dosing rate equals rate of decline in plasma drug concentration and if the decline (elimination) is due to a single capacity-limited process (for e.g. metabolism), then;

$$DR = \frac{V_{\max} C_{ss}}{K_m + C_{ss}} \quad (11.12)$$

A plot of C_{ss} versus DR yields a typical *hockey-stick shaped curve* as shown in Fig. 11.3.

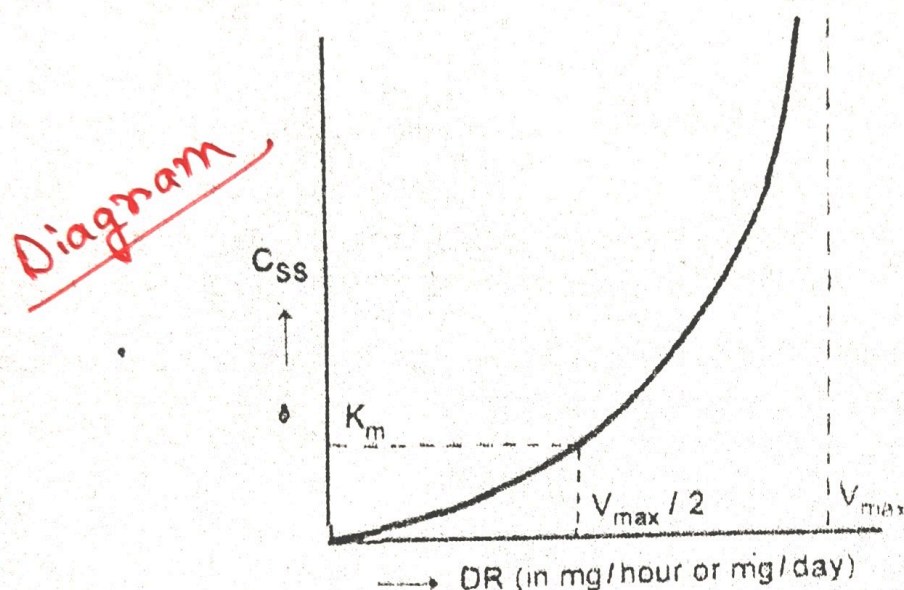


Fig. 11.3 Curve for a drug with nonlinear kinetics obtained by plotting the steady-state concentration versus dosing rates.

To define the characteristics of the curve with a reasonable degree of accuracy, several measurements must be made at steady-state during dosage with different doses.

Practically, one can graphically compute K_m and $V_{\max}$ in 3 ways:

1. **Lineweaver-Burk Plot** : Taking reciprocal of equation 11.12, we get:

$$\frac{1}{DR} = \frac{K_m}{V_{\max} C_{ss}} + \frac{1}{V_{\max}} \quad (11.13)$$

A plot of $1/DR$ versus $1/C_{ss}$ yields a straight line with slope $K_m/V_{\max}$ and y-intercept $1/V_{\max}$.

2. **Direct Linear Plot** : Here, the graph is considered as shown in Fig. 11.4. A pair of C_{ss} viz. $C_{ss,1}$ and $C_{ss,2}$ obtained with two different dosing rates DR_1 and DR_2 are plotted. The points $C_{ss,1}$ and DR_1 are joined to form a line and a second line is obtained similarly by joining $C_{ss,2}$ and DR_2 . The point where these two lines intersect each other is extrapolated on DR axis to obtain $V_{\max}$ and on x-axis to get K_m .

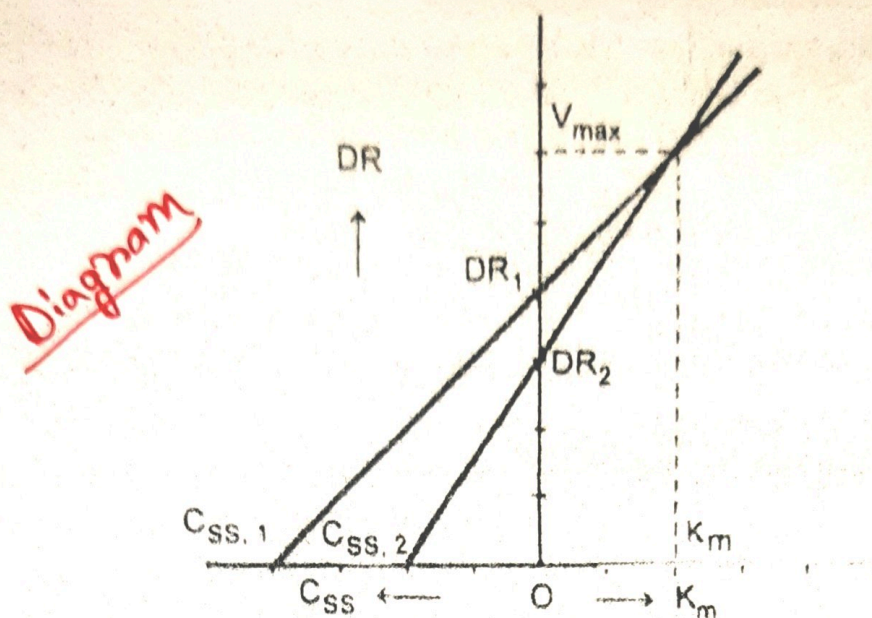


Fig. 11.4 Direct linear plot for estimation of K_m and $V_{\max}$ at steady-state concentrations of a drug given at different dosing rates.

3. The third graphical method of estimating K_m and $V_{\max}$ involves rearranging equation 11.12. to yield:

$$DR = V_{\max} - \frac{K_m DR}{C_{ss}} \quad (11.14)$$

A plot of DR versus DR/C_{ss} yields a straight line with slope $-K_m$ and Y-intercept $V_{\max}$.

K_m and $V_{\max}$ can also be calculated numerically by setting up simultaneous equations as shown below:

$$DR_1 = \frac{V_{\max} C_{ss,1}}{K_m + C_{ss,1}} \quad (11.15)$$

$$DR_2 = \frac{V_{\max} C_{ss,2}}{K_m + C_{ss,2}} \quad (11.16)$$

Combination of the above two equations yields

$$K_m = \frac{DR_1 - DR_2}{\frac{DR_1}{C_{ss,1}} - \frac{DR_2}{C_{ss,2}}} \quad (11.17)$$

After having computed K_m , its subsequent substitution in any one of the two simultaneous equations will yield $V_{\max}$.

It has been observed that K_m is much less variable than $V_{\max}$. Hence, if mean K_m for a drug is known from an earlier study, then instead of two, a single measurement of C_{ss} at any given dosing rate is sufficient to compute $V_{\max}$.

Urinary Excretion Studies

This method of assessing bioavailability is based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concentration of drug. Thus, even if a drug is excreted to some extent (at least 10 to 20%) in the urine, bioavailability can be determined. The study is particularly useful for drugs extensively excreted unchanged in the urine - for example, certain thiazide diuretics and sulfonamides and for drugs that have urine as the site of action - for example, urinary antiseptics such as nitrofurantoin and hexamine. The method has several advantages and disadvantages as discussed in *chapter 10*. Concentration of metabolites excreted in urine is never taken into account in calculations since a drug may undergo presystemic metabolism at different stages before being absorbed. The method involves collection of urine at regular intervals for a time span equal to 7 biological half-lives, analysis of unchanged drug in the collected sample and determination of the amount of drug excreted in each interval and cumulative amount excreted. At each sample collection, total emptying of the bladder is necessary to avoid errors resulting from addition of residual amount to the next urine sample. Frequent sampling is also essential in the beginning in order to compute correctly the rate of absorption.

The three major parameters examined in urinary excretion data obtained with a single dose study are:

1. $(dX_u/dt)_{\max}$: The maximum urinary excretion rate, it is obtained from the peak of plot between rate of excretion versus midpoint time of urine collection period. It is analogous to the $C_{\max}$ derived from plasma level studies since the rate of appearance of drug in the urine is proportional to its concentration in systemic circulation. Its value increases as the rate of and/or extent of absorption increases (see Fig. 12.2).

2. $(t_u)_{\max}$: The time for maximum excretion rate, it is analogous to the $t_{\max}$ of plasma level data. Its value decreases as the absorption rate increases.

3. X_u : The cumulative amount of drug excreted in the urine, it is related to the AUC of plasma level data and increases as the extent of absorption increases.

Diagram

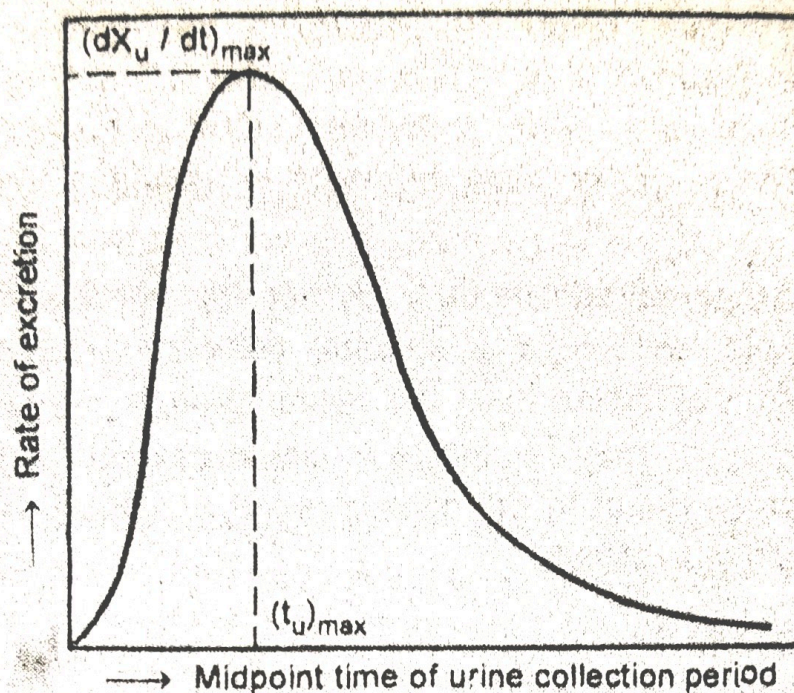


Fig. 12.2 Plot of excretion rate versus time. Note that the curve is analogous to a typical plasma level-time profile obtained after oral administration of a single dose of drug.

The extent of bioavailability is calculated from equations given below:

$$F = \frac{(X_u^\infty)_{\text{oral}} D_{\text{iv}}}{(X_u^\infty)_{\text{iv}} D_{\text{oral}}} \quad (12.6)$$

$$F_r = \frac{(X_u^\infty)_{\text{test}} D_{\text{std}}}{(X_u^\infty)_{\text{std}} D_{\text{test}}} \quad (12.7)$$

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

$$F_r = \frac{(X_{u,ss})_{\text{test}} D_{\text{std}} \tau_{\text{test}}}{(X_{u,ss})_{\text{std}} D_{\text{test}} \tau_{\text{std}}} \quad (12.8)$$

where $(X_{u,ss})$ is the amount of drug excreted unchanged during a single dosing interval at steady-state. ✓

Bioavailability can also be determined for a few drugs by assay of biologic fluids other than plasma and urine. In case of theophylline, salivary excretion can be used whereas for cephalosporin antibiotics, appearance of drug in CSF and bile can be determined. Caution must however be exercised to account for salivary and enterohepatic cycling of the drugs.

Plasma Level—Time Studies

Unless determination of plasma drug concentration is difficult or impossible, it is the most reliable method and method of choice in comparison to urine data. The method is based on the assumption that two dosage forms that exhibit superimposable plasma level-time profiles in a group of subjects should result in identical therapeutic activity.

With single dose study, the method requires collection of serial blood samples for a period of 2 to 3 biological half-lives after drug administration, their analysis for drug concentration and making a plot of concentration versus corresponding time of sample collection to obtain the plasma level-time profile. With i.v. dose, sampling should start within 5 minutes of drug administration and subsequent samples taken at 15 minute intervals. To adequately describe the disposition phase, at least 3 sample points should be taken if the drug follows one-compartment kinetics and 5 to 6 points if it fits two-compartment model. For oral dose, at least 3 points should be taken on the ascending part of the curve for accurate determination of K_a . The points for disposition or descending phase of the curve must be taken in a manner similar to that for i.v. dose.

The 3 parameters of plasma level-time studies which are considered important for determining bioavailability are:

1. **C_{max}** : The peak plasma concentration that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response.
2. **t_{max}** : The peak time that gives an indication of the rate of absorption.
3. **AUC** : The area under the plasma level-time curve that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation.

The extent of bioavailability can be determined by following equations:

Equation

$$F = \frac{[AUC]_{\text{oral}} D_{\text{iv}}}{[AUC]_{\text{iv}} D_{\text{oral}}} \quad (12.2)$$

$$F_r = \frac{[AUC]_{\text{test}} D_{\text{std}}}{[AUC]_{\text{std}} D_{\text{test}}} \quad (12.3)$$

where D stands for dose administered and subscripts iv and oral indicates the route of administration. Subscripts test and std indicate the test and the standard doses of the same drug to determine relative availability. The rate of absorption can be computed from one of the several methods discussed in chapter 10.

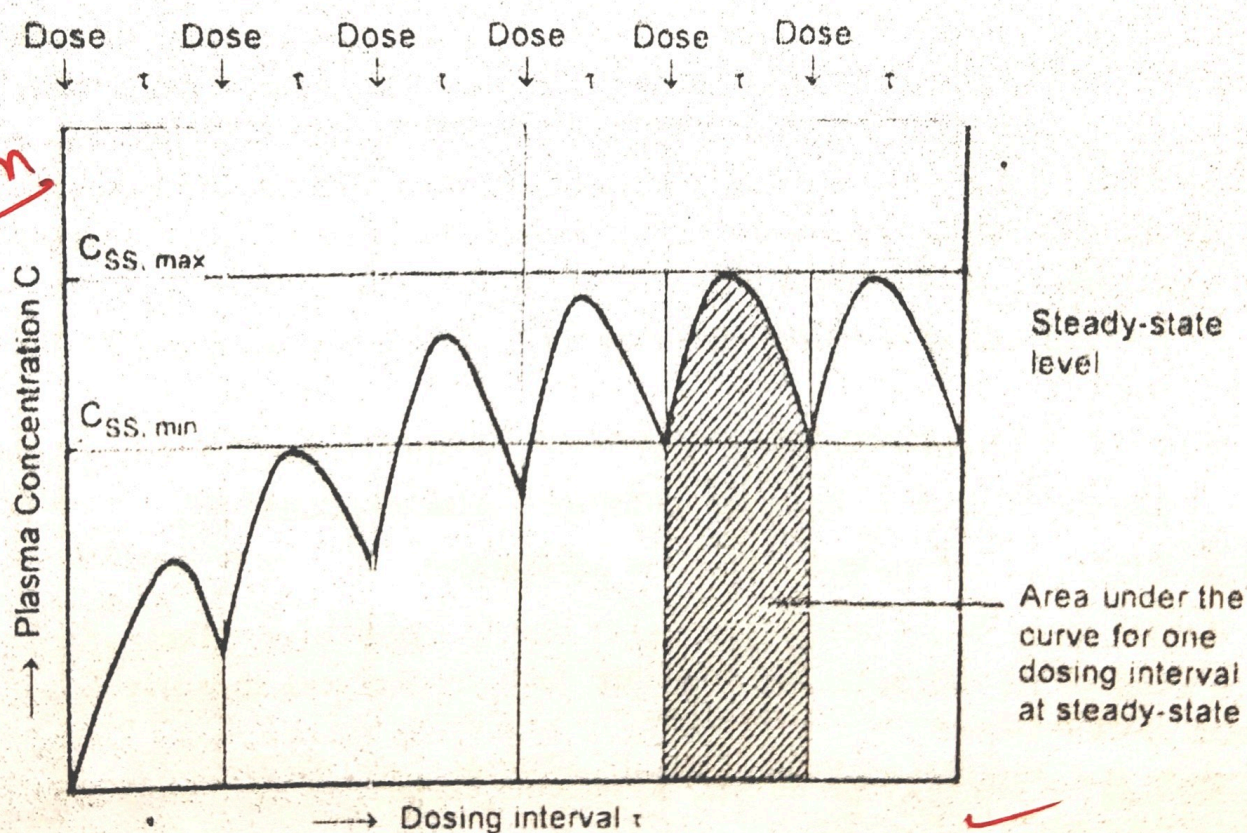


Fig. 12.1 Determination of AUC and $C_{ss, \max}$ on multiple dosing upto steady-state