UNIT-2

Elimination

The onset of pharmacological response depends upon two pharmacokinetic processes:

- Drug absorption
- Drug distribution

The duration and intensity of action depend upon:

- Tissue redistribution of drug
- Rate of elimination

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

Elimination involves two processes:

- Biotransformation (Metabolism)
- Excretion

Biotransformation is defined as the chemical conversion of one form to another.

Need for Drug Biotransformation

All chemical substances that are not nutritious for the body and enter the body through, ingestion, inhalation or absorption are called as xenobiotics (xenos = foreign). Drugs are also xenobiotics for the body. They needs to be eliminated from the body, otherwise they will cause toxicity in the body. That is why, biotransformation is a detoxification process.

→ water-soluble drugs undergo renal excretion (major route for exit of drugs) whereas lipid-soluble substances are passively reabsorbed from the renal tubules into the blood after glomerular filtration.

→ Lipophilic drugs are first transformed into polar and water-soluble products by various metabolic systems so that they can be easily excreted by the kidneys.

→ Biotransformation normally results in pharmacological inactivation of drugs, i.e., it results in formation of metabolites with little or no pharmacological activity.

Drug Metabolising Organs

Liver is the primary site for metabolism of almost all drugs because it possesses a large variety of enzymes in large amounts.

Metabolism by organs other than liver is called extrahepatic metabolism.

Decreasing order of their metabolizing activity is:

Liver > Lungs > Kidneys > Intestine > Placenta > Adrenals > skin

Brain, testes, muscles, spleen, etc. also metabolise drugs but to a small extent.

Drug Metabolising Enzymes

The enzymes that transform xenobiotics differ from those which metabolise food materials.

They are non-specific in metabolising a large number of drugs.

These enzymes are divided into 2 categories:

1) Microsomal enzymes → The endoplasmic reticulum specially SER of liver and other tissues contain a large variety of enzymes, together called microsomal enzymes.

They catalyse glucuronide conjugation, most oxidative reactions and some reductive and hydrolytic reactions. The monooxygenases, glucuronyl transferase, etc. are important microsomal enzymes.

2) Non-microsomal enzymes → Enzymes occurring in organelles / sites other than endoplasmic reticulum are called non-microsomal enzymes. These are usually present in cytoplasm or mitochondria, etc.

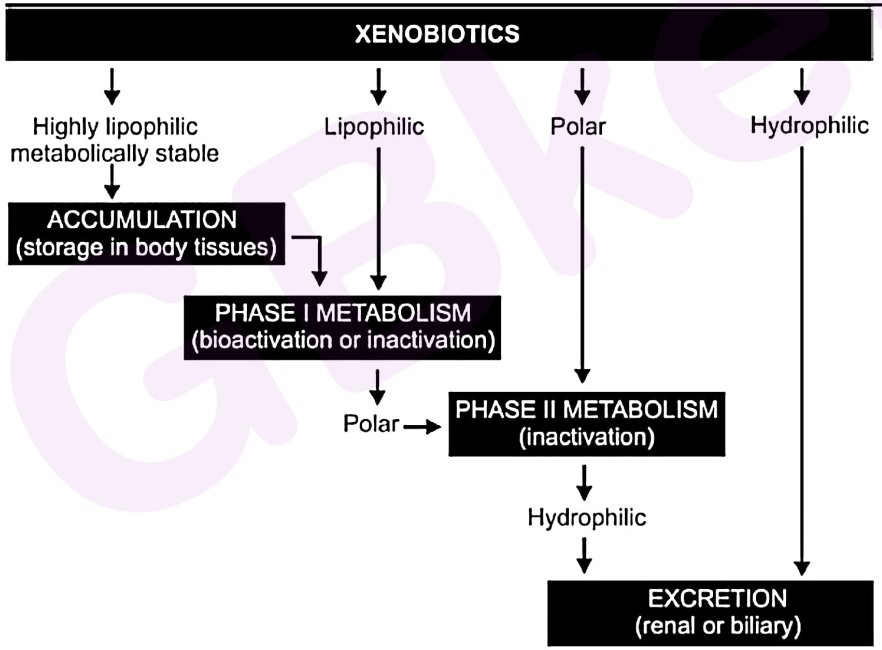
They catalyse few oxidative reactions, a number of reductive and hydrolytic reactions and all conjugative reactions other than glucuronidation.

Metabolic Pathways

Pathways of drug metabolism reactions are divided into 2 general categories:

1) Phase-I reactions
2) Phase-II reactions

The metabolism of different types of xenobiotics can be understood by following chart:



Phase-I reactions

Phase-I reactions include oxidative, reductive and hydrolytic reactions. The primary objectives of phase-I reactions are-

1) Increase in hydrophilicity :- By these reactions, a polar functional group is introduced into a lipophilic drug, e.g., -OH, -COOH, $-NH_2$ and -SH. Thus, phase-I reactions are also called as functionalisation reactions. These transformations are called as asynthetic reactions whereas phase-II reactions are synthetic.
2) Facilitation of conjugation: The resulting product of phase-I reaction is susceptible to phase-II reactions that make the xenobiotics highly water-soluble which can be easily excreted out of the body.

Phase-II reactions (Synthetic Phase)

This step almost always results in loss of biological activity of a compound.

→ These reactions generally involve covalent attachment of small polar endogenous molecules such as glucuronic acid, sulfate, glycine, etc. to either unchanged drugs or phase-I products to form highly water-soluble conjugates which are readily excretable by the kidneys. Thus, these reactions are also called as conjugation reactions.

metabolites → These are the products formed after biotransformation of drugs in phase-I and phase-II reactions.

Renal Excretion of Drugs

Excretion is the irreversible transfer of drugs or their metabolites from internal to external environment. The principal organ of excretion is kidney, thus, renal excretion.

Almost all drugs and their metabolites are excreted by kidneys.

Agents that are excreted in urine by kidneys are:

1) water-soluble agents
2) non-volatile agents
3) small in molecular size (<500 Daltons)
4) The ones that are metabolised slowly.

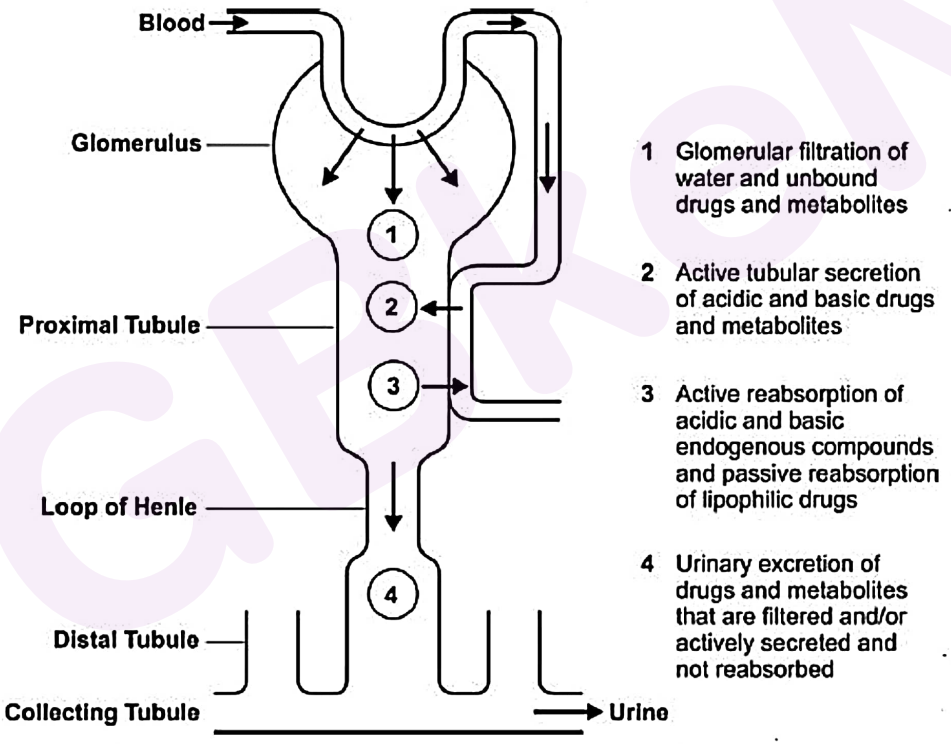
The basic functional unit of kidney involved in excretion is the nephron. Each kidney comprises of about one million nephrons. Each nephron is made up of the:

- glomerulus
- proximal tubule (PCT)
- loop of Henle
- distal convoluted tubule (DCT)

- collecting tubule

The principal processes that are involved in the urinary excretion of drugs are:

1) Glomerular filteration
2) Active tubular secretion
3) Active or passive tubular reabsorption.



Glomerular filteration and active tubular secretion increase the concentration of drugs in lumen and hence facilitate excretion whereas tubular reabsorption decreases it and prevents the drug movement out of the body.

Thus,

- Rate of excretion = Rate of filteration + Rate of excretion − Reabsorption Rate

Glomerular Filteration

Glomerular filtration is a non-selective, unidirectional process whereby most compounds, whether ionised or unionised are filtered.

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

→ Out of ~25% of cardiac output (or ~1.2 litres of blood/min that goes to the kidneys via renal artery, only 10% or 120 to 130 ml/min is filtered through the glomeruli.

This rate of filtration is called glomerular filtration rate (GFR).

→ Though some 180 litres of blood is filtered by glomeruli each day, only about 1.5 litres is excreted as urine. The remainder is reabsorbed from the tubules.

Active Tubular Secretion

It is a carrier-mediated process which requires energy for transportation of compounds against the concentration gradient. The system is capacity-limited and saturable. There are two active tubular secretion mechanisms:

1) System for secretion of organic acids/anions: like penicillins salicylates, glucuronides, sulphates. It is the same system by which endogenous acids such as uric acid are secreted.

2) System for secretion of organic bases/cations like morphine, mecamylamine, hexamethonium and endogenous amines such as catecholamines, choline.

Tubular Reabsorption

Tubular reabsorption occurs after the glomerular filtration of drugs. It takes place in the renal tubule. Tubular reabsorption delays the excretion of drugs. It can either be an:

1) Active process, or a
2) Passive process

The reabsorption of drugs that are acids or weak bases is influenced by

1. The pH of the fluid in the renal tubule
2. pKa of the drug.

Renal Clearance

It can be defined as the volume of blood or plasma which is completely cleared from unchanged drug by the kidney per unit time.

$$\text{Clearance} = \frac{\text{Rate of urinary excretion}}{\text{Plasma drug concentration}}$$

Physiologically, renal clearance is the ratio of "sum of rate of glomerular filtration and active secretion minus rate of reabsorption" to "plasma drug concentration".

$$Cl. = \frac{\text{Rate of filtration} + \text{Rate of secretion} - \text{Reabsorption rate}}{\text{Plasma drug concentration}}$$

Note: Total body clearance is the sum of individual clearances by all eliminating organs.

$$\text{Total body clearance} = \frac{\text{Elimination rate}}{\text{Plasma drug concentration}}$$

Factors affecting renal excretion of drugs

1) Physicochemical properties of drug → Important physicochemical factors are:

→ molecular size: compounds of weights < 300 Daltons, if water-soluble get easily excreted.

→ Drug pKa govern the degree of ionization at the particular pH.

→ A polar and ionized drugs are poorly reabsorbed and excreted rapidly.

2) Plasma concentration of drug → It directly affects glomerular filtration and reabsorption since both are passive processes

3) Distribution and Binding characteristics of drug clearance is inversely related to apparent volume of distribution of drugs.

→ A drug with large V_d is poorly excreted in urine. Drugs that remain in blood compartment have higher excretion rates.

→ Plasma protein bound drugs behave as macromolecules (large size) and thus cannot be filtered through the glomerulus.

→ only unbound or free drug appear in the glomerular filtrate.

4) Blood flow to the kidneys → A good flow of blood increases the contact of drug with the secretory sites (e.g. glomerulus) and enhances their elimination.

5) Biological factors → Age, sex, species, differences in the genetic make-up, etc. alter drug excretion. Renal excretion is 10% lower in females than in males. In old age, GFR is reduced, the excretion of drugs is thus slowed.

6) Drug interactions → Any drug interaction that results in alteration of protein-drug binding characteristics, renal blood flow, active secretion, urine pH would alter renal clearance of a drug.

7) Disease states - Renal Impairment

Disorders like renal dysfunction and uraemia affects drug clearance.

Non-renal routes of drug excretion

Routes of excretion other than renal route are called as non-renal routes.
The various such routes are:

1) Biliary excretion

The hepatic cells produce bile and is secreted into the duodenum after storage in the gall bladder. Bile is important in the digestion and absorption of fats. Almost 90% of bile acids are reabsorbed from the intestine and transported back to the liver for re-secretion. The rest is excreted in faeces. Various drugs are secreted in bile and excreted out of the body like sodium, insulin, phosphates.

2) Pulmonary Excretion

Gaseous and volatile substances such as the general anaesthetics (e.g. halothane) are absorbed through the lungs by simple diffusion and are excreted by diffusion into the expired air.

Factors influencing pulmonary excretion of a drug include pulmonary blood flow, rate of respiration, solubility of the volatile substance, etc.

3) Salivary excretion

Unionised lipid soluble drugs are excreted passively. The bitter taste in the mouth of a patient is the indication of drug excreted. compounds excreted in saliva are caffeine, phenytoin, Theophylline.

4) Mammary Excretion

Excretion of drug in milk is important as it can enter into the breast feeding infant. Excretion of drug in milk is a passive process. Free, unionised, lipid-soluble drugs diffuse into the mammary alveolar cells passively.

5) Skin excretion

Drugs are excreted through the skin via sweat. compounds such as benzoic acid, salicylic acid, alcohol and heavy metals like Lead, mercury excreted.

6) Gastrointestinal excretion

Excretion of drugs into the GIT usually occurs after parenteral administration of drugs. water-soluble and ionised form of weakly acidic and basic drugs are excreted in the GIT, e.g., nicotine and quinine are excreted in the stomach.

7) Genital Excretion

Reproductive tract and genital secretions may contain the excreted drug. Some drugs have been detected in semen.

Bioavailability and Bioequivalence

Bioavailability is defined as the rate and extent (amount) of absorption of unchanged drug from its dosage form.

In simplest way, bioavailability is the measure of how much drug is available at the site of action to effect a pharmacological response.

If the size of 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 → Bioavailability from different routes in the order given:

Parenteral > oral > Rectal > Topical

→ In parenteral route, intravenous injection of a drug gives 100% bioavailability as there is no absorption of drug take place here.

→ For reasons of stability and convenience, most drugs are administered orally. In this case, the dose available to the patient, called as the bioavailable dose, is often less than the administered dose.

→ The term bioavailable fraction F, refers to the fraction of administered dose that enters the systemic circulation.

$$F = \frac{\text{Bioavailable dose}}{\text{Administered dose}}$$

objectives of bioavailability studies

Bioavailability studies are important in the:-

1) Primary stages of development of a suitable dosage form to obtain evidence of therapeutic utility of 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 the new formulations of the existing drugs.
4) Comparison of availability of a drug substance from different dosage forms or from the dosage form produced by different manufacturers.

Bioavailability – absolute versus relative

→ when systemic availability of a drug (in blood) of a drug administered orally is determined in comparison to its intravenous administration, it is called as absolute bioavailability (F).

→ when the systemic availability of a drug after oral administration is compared with that of an oral standard of that drug, it is referred to as relative bioavailability (F_r).

Measurement of Bioavailability

There are two categories of measurement methods:

1) Pharmacokinetic Methods → These methods are based on the assumption that the pharmacokinetic profile reflects the therapeutic effectiveness of a drug. These are indirect methods.
   a) Plasma level- time studies
   b) Urinary excretion studies
2) Pharmacodynamic Methods → These methods are complimentary to pharmaco-kinetic methods and involve direct measurement of drug effect on a physiological process as a function of time.
   a) Acute pharmacological response
   b) Therapeutic response

Plasma level-time studies

→ Most common type of bioavailability studies.

→ Based on the assumption that there is a direct relationship between the concentration of drug in blood and concentration of drug at the site of action.

For single-dose study

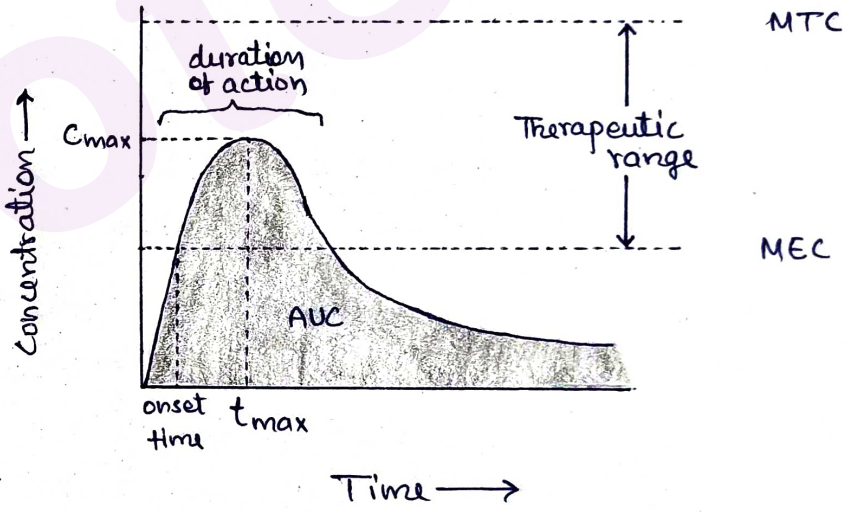
- Collection of serial blood samples for a period of 2-3 biological half-lives after drug administration.
- Plot of concentration vs time to obtain the plasma level time profile.
- Sampling should start within 5 minutes of drug administration and subsequent samples at 15 min time.

3 parameters considered for plasma level-time studies:

1) **Cmax** :- This is peak plasma concentration. Cmax will increase with an increase in the dose as well as an increase in absorption rate.

2) **tmax** :- The peak time that gives an indication of the rate of absorption. It decreases with the rate of absorption increases.

3) **AUC** :- The area under plasma level-time curve which 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 equation,

$$F = \frac{[AUC]_{oral} \cdot D_{iv}}{[AUC]_{iv} \cdot D_{oral}}$$

where, D stands for dose administered and subscripts iv and oral are routes of administration.

$$F_r = \frac{[AUC]_{test} \cdot D_{std}}{[AUC]_{std} \cdot D_{test}}$$

subscripts 'test' and 'std' indicate the test and the standard doses of the same drug to determine relative bioavailability.

For multiple dose study

The extent of bioavailability is given as:

$$F_r = \frac{[AUC]_{test}\ D_{std}\ \tau_{test}}{[AUC]_{std}\ D_{test}\ \tau_{std}}$$

where, [AUC] is area under the plasma level-time curve of one dosing interval in a multiple dosage regimen, after reaching the steady-state level.

τ is the dosing interval.

In-vitro Drug Dissolution Models

Dissolution → is a process in which a solid substance solubilises in a given solvent.

Dissolution rate → is the amount of drug substance that goes in solution per unit time under standardized conditions.

Factors to be considered while designing of a dissolution test

A) Factors relating to the dissolution apparatus:
- design of the container
- size and shape of the container
- nature and speed of agitation
- performance precision of the apparatus.

B) Factors relating to the dissolution fluid:
- volume
- temperature
- deaeration of dissolution medium
- pH

C) Process parameters such as method of introduction of dosage form, sampling techniques, etc.

Classification

Based on the absence or presence of sink conditions, there are two principal types of dissolution apparatus: closed and open.

sink condition → It is the ability of the dissolution media to dissolve at least 3 times the amount of drug that is in your dosage form.

1) **Closed-compartment apparatus**

It is a limited-volume apparatus operating under non-sink conditions.

2) **Open-compartment (continuous flow-through) apparatus**

In this apparatus, the dosage form is contained in a column which is brought in continuous contact with fresh, flowing dissolution medium (perfect sink condition).

- A third type called as dialysis systems are used for very poorly aqueous soluble drugs for which maintenance of sink conditions would otherwise require large amount of dissolution fluid.

Rotating Basket Apparatus (Apparatus-1)

→ First described by Pernarowski.
→ It is basically a closed-compartment, beaker type apparatus.
→ It comprises of a cylindrical glass vessel of one litre capacity partially immersed in a water bath.
→ A cylindrical basket made of 22 mesh is located centrally in the vessel at a distance of 2cm from the bottom and rotated by a motor.

Rotating Paddle Apparatus (Apparatus-2)

→ First described by Levy and Hayes.
→ Same as apparatus-1 but basket is replaced with a paddle which acts as a stirrer.
- A small, loose, wire helix may be attached to the dosage form that would otherwise float.

Reciprocating Cylinder Apparatus (Apparatus-3)

This apparatus consists of a set of cylindrical flat-bottomed glass vessels equipped with reciprocating cylinders. The apparatus is particularly used for dissolution testing of controlled-release bead-type (pellet) formulations.

Flow-Through Cell Apparatus (Apparatus-4)

It consists of a reservoir for the dissolution medium and a pump that forces dissolution medium through the cell holding the test sample.

Paddle over disc Apparatus (Apparatus-5)

This apparatus is used for evaluation of transdermal products and consists of a sample holder that holds the product. The disc is placed at the bottom of apparatus-2 and apparatus is operated in the usual way.

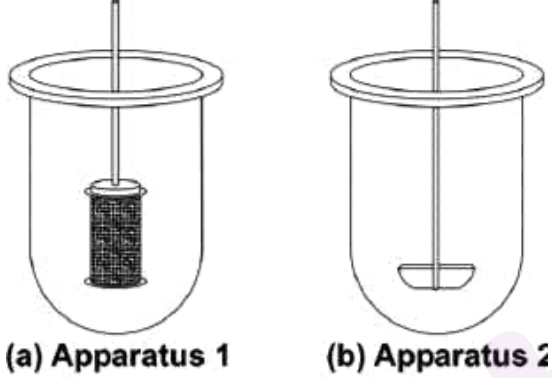
Cylinder Apparatus (Apparatus-6)

This apparatus is also used for evaluation of transdermal products and is similar to apparatus-1. Instead of basket, a stainless steel cylinder is used to hold the sample.

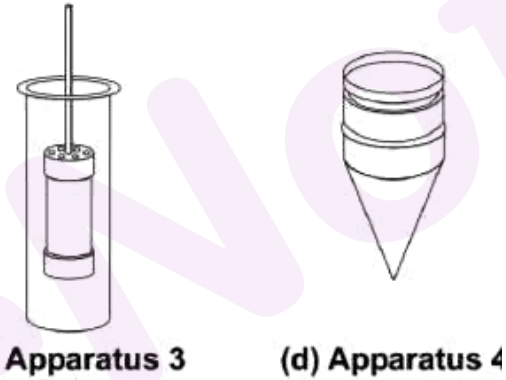
Reciprocating Disc Apparatus (Apparatus-7)

→ The assembly consists of a set of calibrated solution containers, a motor and drive assembly to reciprocate the system vertically.

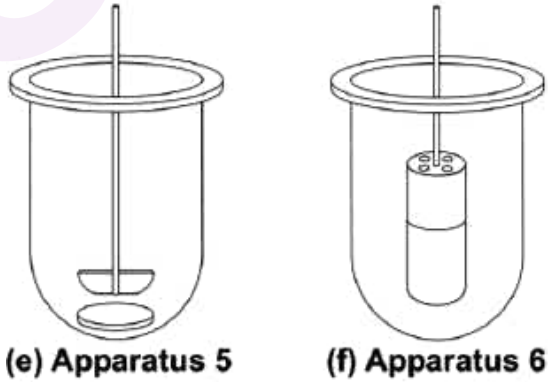
→ Used for the evaluation of transdermal products as well as non-disintegrating controlled-release oral preparations.



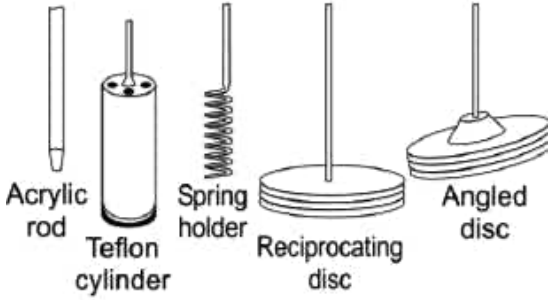
(a) Apparatus 1 (b) Apparatus 2



(c) Apparatus 3 (d) Apparatus 4



(e) Apparatus 5 (f) Apparatus 6



(g) Apparatus 7 - Reciprocating Holders

In vitro- In vivo Correlation (IVIVC)

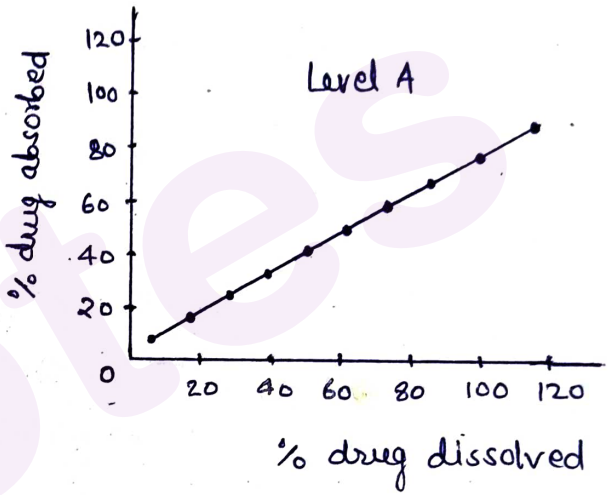
IVIVC is defined as the predictive mathematical model that describes the relationship between an in-vitro property (such as the rate and extent of dissolution) of a dosage form and an in-vivo response (such as the plasma drug concentration or amount of drug absorbed).

IVIVC Levels

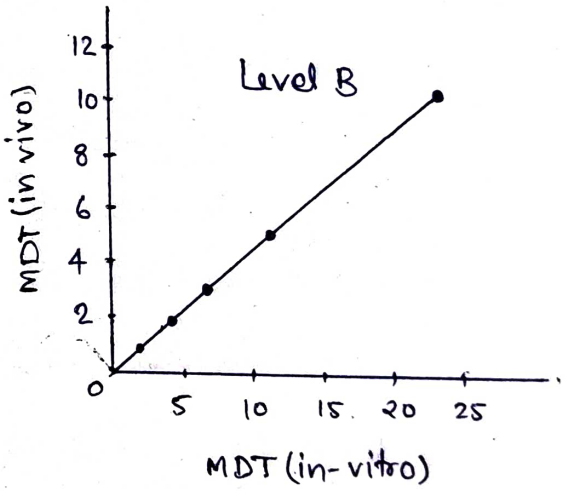
Three IVIVC levels have been defined and categorised in descending order of usefulness.

1) Level-A correlation → It represents a point-to-point relationship between in-vitro dissolution and the in-vivo rate of absorption (or in-vivo dissolution).

→ The in-vitro dissolution and in-vivo absorption rate curves are superimposable and the mathematical description for both curves is the same.

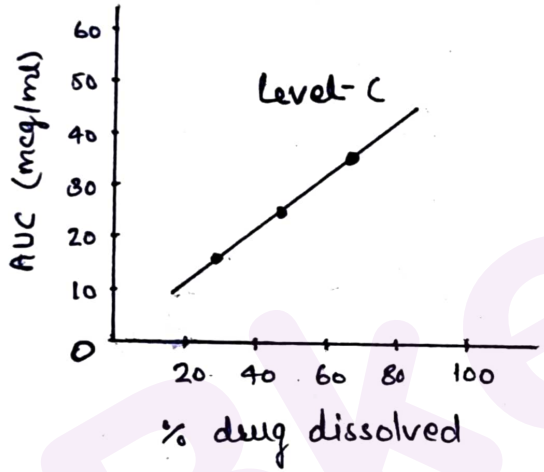


2) Level-B correlation → Mean absorption time is plotted against mean dissolution time for at least 3 different preparations.



MDT → Mean Dissolution Time

3) **Level-C correlation** → It is a single point correlation. It relates one dissolution time point (e.g. $t_{50\%}$) to one pharmacokinetic parameter such as AUC, t_{max} or C_{max}.



BCS and IVIVC

The Biopharmaceutics Classification System (BCS) is a fundamental guideline for determining the conditions under which IVIVC correlation are expected.

Class	Solubility	Permeability	Absorption rate control	IVIVC expectations for Immediate release product
I	High	High	Gastric emptying	IVIVC expected, if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlations
II	Low	High	Dissolution	IVIVC expected, if in vitro dissolution rate is similar to *in-vivo* dissolution rate, unless dose is very high.
III	High	Low	Permeability	Absorption (permeability) is rate determining and limited or no IVIVC with dissolution.
IV	Low	Low	Dissolution and permeability, Case by case	Limited or no IVIVC is expected

According to BCS, in vivo bioavailability and bioequivalence studies need not be conducted for drug products under following circumstances:

- Rapid and similar dissolution
- High solubility
- High permeability
- Wide therapeutic window
- Excipients used in dosage form are same as those present in approved drug product.

Bioequivalence studies

Bioequivalence is the property of two dosage forms with similar blood concentration levels that produce the same effect at the site of physiologic activity.

Chemical equivalence → It indicates that two or more drug products contain same chemical substance as an active ingredient in the amount.

Pharmaceutical equivalence → Two or more drugs products are identical in strength, quantity, purity, content uniformity and disintegration and dissolution characteristics.

Bioequivalence studies are the studies used to compare the bioavailability of a drug from various drug products. These studies are required during the course of development of new drugs.

Bioequivalence studies are conducted if there is:

→ a risk of bio-inequivalence, and
→ a risk of pharmacotherapeutic failure or reduced clinical safety.

Types of bioequivalence studies

→ In-vivo
→ In-vitro

A) In vivo bioequivalence studies

The following sequence of criteria is useful in assessing the need for in-vivo studies:

1) Oral-immediate-release products with systemic action—
2) Non-oral immediate-release products.
3) Modified-release products with systemic action

B) In-vitro bioequivalence studies

In vitro studies, i.e., dissolution studies can be used in lieu of In vivo bioequivalence under certain circumstances, called as biowaivers.

Methods for enhancement of bioavailability

A drug with poor bioavailability is the one with-

→ poor aqueous solubility or slow dissolution rate in biological fluids.

→ poor permeability through the biomembrane

The three conceptual approaches in overcoming the bioavailability problems:

1) The pharmaceutical Approach → which involves modification of formulation, manufacturing process or the physicochemical properties of the drug without changing the chemical structure.

2) Pharmacokinetic approach → Alteration of pharmacokinetic parameters by modifying its chemical structure.

3) Biological approach → The route of drug administration is changed such as changing from oral to parenteral route.

Methods of enhancing bioavailability

1) Micronisation → The process involves reducing the size of the solid drug particles to 1 to 10 microns commonly by spray drying. Examples of drugs- griseofulvin, several steroidal and sulpha drugs.

2) Use of surfactants → surfactants enhance dissolution rate by promoting wetting and penetration of fluid into the solid drug particles.

3) Use of salt forms → salts have improved solubility and dissolution characteristics in comparison to the original drug.

4) Alteration of pH of the drug microenvironment:

This can be achieved in two ways

- in situ salt formation
- addition of buffers to the formulation

5) Solvent Deposition → In this method, the poorly acqueous 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 micro-crystalline cellulose by evaporation of solvent

6) Solid Solutions → Because of reduction in particle size to the molecular level, solid solutions show greater acqueous solubility and faster dissolution.

7) Solid dispersions → This 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.

8) 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 acqueous solubility.

→ These cyclodextrin molecules are versatile in having a hydrophobic cavity of size suitable enough to accomodate the lipophilic drugs as guests; the outside of the host molecule is relatively hydrophilic.

→ 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 phenomina- thiazide diuretics, barbuturates, benzodiazepines and a number of NSAIDs.