

Unit - 2Elimination -

Elimination is the major process for removal of drug from the body & termination of its action. It is defined as the irreversible loss of drug from the body.

Elimination occur by two process:

- (i) Biotransformation (metabolism)
- (ii) Excretion

Biotransformation :-

Biotransformation of drug is defined as the chemical conversion of one form to another form. It is called metabolism.

Xenobiotics :-

All chemical substances that are not nutrients for the body & enter the body through ingestion, inhalation or absorption are called as xenobiotics.

Biotransformation (metabolism) -

Normally results in pharmacological inactivation of drugs. It results in formation of metabolites. Occasionally it metabolites with equal activity. Rarely leads to toxicological activation of drug.

Drug Metabolising organ:

Liver is the primary site of metabolism almost all drugs.

Metabolism by organs other than liver called as extrahepatic metabolism.

Liver > lungs > Kidney > Intestine > Placenta > Adrenal > skin

Other are - Brain, testis, muscles, Spleen, etc.

Drug Metabolising enzyme:

The enzymes are divided into 2 category -

- (i) Microsomal Enzymes - They are first interact nature of lipoidal membrane bound enzyme of the microsome is essential for lipid soluble substance. A no. of lipid soluble substrates can interact non-specifically with the microsomal enzyme.
- (ii) Non-microsomal enzymes - It include those that are present in soluble form in the cytoplasm & those attach to the mitochondria but don't ER.

Metabolic Pathway -Phase I Reaction:

- Functionalization Reaction.
- Converts the parent drug to a more polar metabolite by introducing or unmasking a functional group (-OH, -NH₂, -SH).

Phase II Reaction :

- Conjugation Reaction.
- Subsequent reaction in which a covalent linkage is formed between a functional group on the parent compound or Phase I metabolite & an endogenous substrate such as glucuronic acid, sulfate, acetate or an amino acid.

Chemical Pathway of Drug Biotransformation :-(i) Phase I Reaction -
Types of ReactionEnzymes(A) Oxidative Reaction

(i) Oxidation of aromatic C-atoms	Microsomal (M)
(ii) Oxidation of olefins	M
(iii) Oxidation of benzylic, allylic C-atom & C-atom alpha to carbonyl & imine	M
(iv) Oxidation of aliphatic C-atoms	M
(v) Oxidation of alicyclic C-atom	M
(vi) Oxidation of C-heteroatom system:	
(a) Carbon-Nitrogen system	
(1) N-dealkylation	M
(2) Oxidative deamination	M, N
(3) N-oxide formation	M, N
(4) N-Hydroxylation	M
(b) Carbon-Sulfur system :	
(1) S-dealkylation	M
(2) Desulfuration	M
(3) S-oxidation	M
(c) Carbon-oxygen system :	
(1) O-dealkylation	M

(vii) Oxidation of alcohol, carbonyl & acid function

M, N

(viii) Miscellaneous oxidative reaction

(B) Reductive Reaction

(i) Reduction of carbonyl function

M, N

(ii) Reduction of alcohol & C=C bond

M

(iii) Reduction of N-Compounds

M, N

(iv) Miscellaneous reductive reaction

(C) Hydrolytic Reaction

(i) Hydrolysis of esters & ethers

M, N

(ii) Hydrolysis of amides

M, N

(iii) Hydrolytic cleavage of nonaromatic heterocycles

M, N

(iv) Hydrolytic dehalogenation

(v) Miscellaneous hydrolytic reaction

(D) Phase II Reaction-

Types of Reaction

Enzymes

(A) Conjugation with glucuronic acid

M

(B) Conjugation with Sulfate moieties

N (non-microsomal)

(C) Conjugation with α -amino acid

N

(D) Conjugation with glutathione & mercapturic acid formation

N

(E) Acetylation reaction

N

(F) Methylation reaction

N

(G) Miscellaneous conjugation reaction

N

∴ Renal Excretion of Drugs =

- Almost all drugs & their metabolites are excreted by the kidneys to some extent or the other.
- The basic functional unit of the kidney involved in excretion is the nephron.
- Each kidney comprises of 1 million nephron.
- Each nephron is made up of the glomerulus, the proximal tubule, the loop of henle, the distal tubule, the collecting tubule.
- The principal processes that determine the urinary excretion of a drug are:
 - (i) Glomerular filtration
 - (ii) Active tubular secretion
 - (iii) Tubular Reabsorption.
- Glomerular filtration & active tubular secretion tend to increase the concentration of drugs in lumen & hence facilitate excretion whereas tubular reabsorption decreases it & prevents the movement of drug out of the body.
- The rate of excretion can be given by eqⁿ -

$$\text{Rate of excretion} = \text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}$$

(i) Glomerular Filtration:

Glomerular filtration is a non-selective, unidirectional process, ionized or unionized are filtered except those that are bound to plasma proteins or blood cells.

The glomerulus also acts as a negatively charged selective barrier promoting retention of anionic compounds. The driving force for filtration 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 kidney via renal artery, only 10% or 120 to 130 ml/min is filtered through the glomeruli. The rate of filtration is called Glomerular Filtration rate (GFR)."

The GFR can be determined by an agent that is excreted exclusively by filtration & is neither secreted nor reabsorbed in the tubules.

The excretion value of such an agent is 120 to 130 ml/min.

Creatinine, inulin, mannitol, sodium thiosulfate are used to estimate GFR.

(ii) Active Tubular Secretion :

It is a carrier mediated process which requires energy for transportation of compounds against the concentration gradient.

Two active tubular secretion mechanism are :

(a) System of secretion of organic acids / anions -

Penicillins, Salicylates, glucuronides, sulfate, etc.

It is the same system by which endogenous acids such as uric acid are secreted.

(b) System of secretion of organic bases / cations - Morphine, metamylamine, hexamethonium & endogenous amines such as catecholamines, choline, histamine.

(iii) Tubular Reabsorption :

Tubular reabsorption occurs after the glomerular filtration of drugs. Reabsorption of a drug is indicated when the excretion rate values are less than GFR of 130 ml/min.

An agent such as glucose that is completely reabsorbed after filtration has a clearance value of zero.

Reabsorption increases the half-life of a drug in contrast to tubular secretion.

Tubular reabsorption can take place in either of the 2 ways :

(i) Active tubular reabsorption.

(ii) Passive tubular reabsorption.

∴ Concept of Clearance :

The clearance concept was first introduced to describe renal excretion of endogenous compounds in order to measure the kidney function.

The term is now applied to all organs involved in drug elimination such as liver, lungs, the biliary system, etc and are referred to as hepatic clearance, pulmonary clearance, Biliary clearance & so on.

"The sum of individual clearances by all eliminating organ is called as total body clearance or total systemic clearance."

"Clearance is defined as the hypothetical volume of body fluids containing drug from which the drug is removed or cleared completely in a specific period of time." It is expressed in ml/min .

$$\text{clearance (Cl)} = \frac{\text{Elimination rate}}{\text{Plasma drug concentration}}$$

"Renal clearance (Cl_r) is defined as the volume of blood or plasma which is completely cleared of the unchanged drug by the kidney per unit time."

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

"Renal clearance (Cl_r) is the ratio of sum of rate of glomerular filtration & active secretion minus rate of reabsorption to plasma drug concentration C ."

$$\text{Cl}_r = \frac{\text{Rate of filtration} + \text{Rate of secretion} - \text{Rate of reabsorption}}{C}$$

"Renal clearance ratio or excretion ratio is the ratio of Cl_r of drug to Cl_r of creatinine."

$$\text{Renal clearance ratio} = \frac{\text{Cl}_r \text{ of drug}}{\text{Cl}_r \text{ of creatinine}}$$

3. Factors Affecting Renal excretion or Renal clearance

(1) Physicochemical Properties of Drug -

Important physicochemical factors affecting renal excretion of a drug are molecular size, pK_a , lipid solubility.

Molecular Size

- Compounds of weight < 300 dalton, if water soluble, are readily excreted by the kidneys.
- Drugs in the molecular weight range 300 to 500 dalton can be excreted in both urine & bile.
- Drug wt > 500 dalton. They are excreted in urine.

pK_a

- Drug pK_a governs the degree of ionization at the particular pH.
- A polar and ionized drugs will be poorly reabsorbed passively & excreted rapidly.

Lipid Solubility

- Urinary excretion of an unchanged drug is inversely related to its lipophilicity. This is because a lipophilic drug is passively reabsorbed.

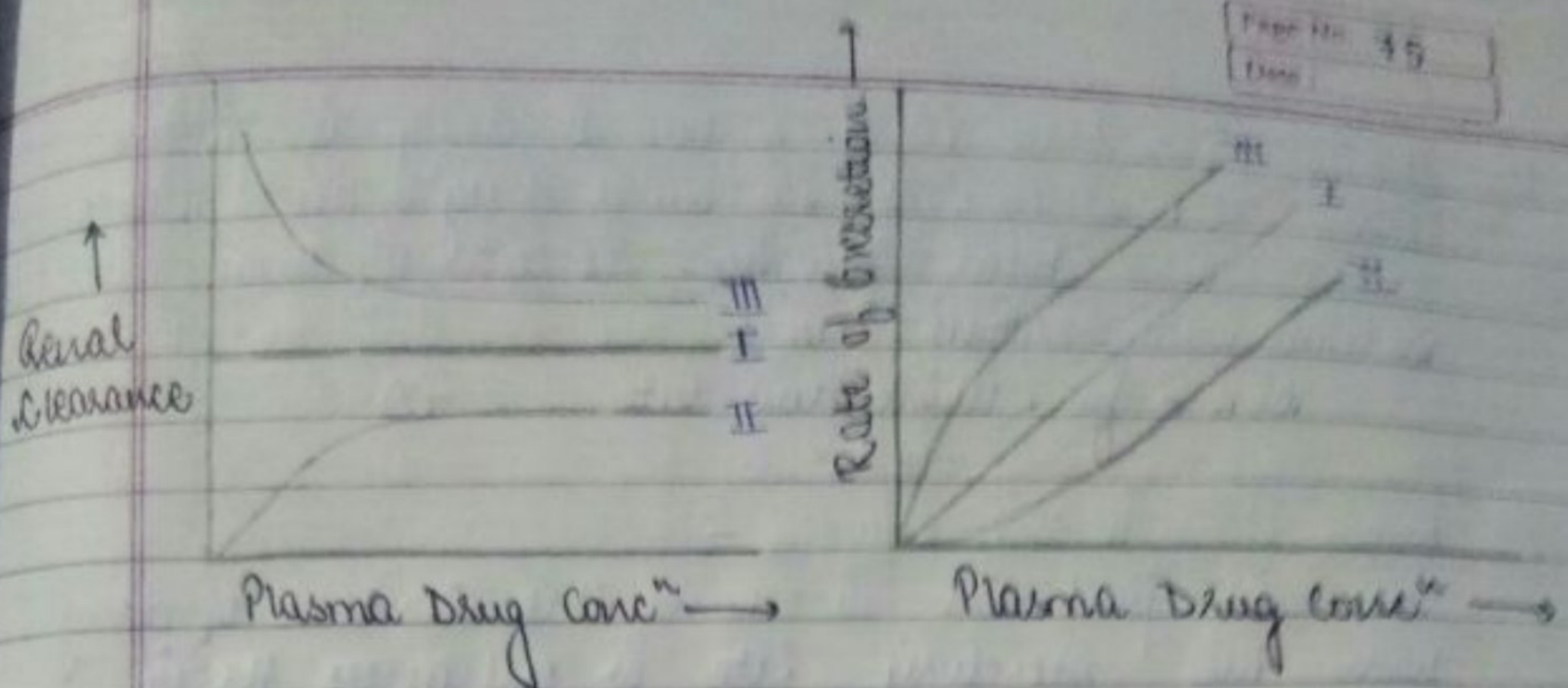
↑
Renal Clearance

(3)

(2) Plasma Concentration of the Drug -

A drug that is not bound to plasma proteins & excreted by filtration only shows a linear relationship between rate of excretion & plasma drug concentration.

In case of drug which are secreted or reabsorbed actively, the rate process increases with an increase in plasma concentration to a point where saturation of carrier occurs.



- I : Drug excreted by filtration only
- II : Drug filtered & actively reabsorbed
- III : Drug filtered & actively secreted.

(3) Distribution & Binding characteristics of the drug - Clearance is inversely related to apparent volume of distribution of drugs. A drug with large V_d is poorly excreted in urine. Drugs restricted to blood compartment have higher excretion rates. Drugs that are bound to plasma protein behave as macromolecules & thus cannot be filtered through the glomerulus. Only unbound or free drug appears in the glomerular filtrate.

$$Cl_r = \frac{U_{\text{urine}} \text{ Conc}^n}{\text{Plasma Conc}^n} \times \text{Urine flow rate} - (1)$$

The fraction of drug unbound to plasma protein is important & can be eqⁿ :

$$f_u = \frac{C_u}{C} - (2)$$

where, f_u = fraction of unbound drug in plasma
 C_u = concⁿ of unbound drug in plasma
 C = total plasma concⁿ of drug.

we can write eqⁿ (1) as:

$$CL_R = f_u \cdot \text{Urine flow rate} \quad - (3)$$

(4) Blood flow to the kidneys -

Increased perfusion increases the contact of drug with the secretory site & enhances their elimination. Renal clearance in such instances is said to be perfusion rate-limited.

(5) Biological Factors -

Age, sex, species & strain differences, differences in the genetic makeup, cardiac rhythm alter drug excretion.

Renal excretion is approximately 10% lower in females than in males.

The renal function of newborns is 30-40% less in comparison to adults.

In old age, the GFR is reduced & tubular function is altered, the excretion of drug is thus slowed down & half-life is prolonged.

(6) Drug Interactions -

Any drug interaction that results in alteration of protein-drug (P-D) binding characteristic, renal blood flow, active secretion & urine pH would alter renal clearance of a drug.

Alteration in P-D binding - The renal clearance of drug extensively bound to plasma protein is

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increased after displacement with another drug.

Alteration of urine pH - Acidification of urine with ammonium chloride, methionine or ascorbic acid enhances excretion of basic drugs. Alkalinization of urine with citrate, tartarates, bicarbonates & carbonic anhydrase inhibitors promote excretion of acidic drugs.

Forced Diuresis - A diuretic increases elimination of drugs whose renal clearance gets affected by urine flow rate.

(7) Disease State - Renal Impairment -

Renal dysfunction - greatly impairs the elimination of drugs especially those that are primarily excreted by the kidneys. Some of the causes of renal failure are Hypertension, diabetes mellitus, hypovolemia [↓ blood supply to the kidney], pyelonephritis [inflammation of kidney due to infection].

Uremia - characterized by impaired glomerular filtration & accumulation of fluids & protein metabolites also impairs renal clearance of drugs. In both these conditions, the half-lives of drugs are increased, drug accumulation & toxicity may result.

∴ Non-Renal Routes of drug excretion - Drugs & their metabolites may also be excreted by routes other than the renal route is called as extrarenal or non-renal routes of drug excretion.

The various such excretion processes are :

- (i) Biliary excretion
- (ii) Pulmonary excretion
- (iii) Salivary excretion
- (iv) Mammary excretion
- (v) Skin excretion
- (vi) Gastrointestinal excretion
- (vii) Genital excretion

→ Biliary Excretion of Drugs - Enterohepatic Cycling :

The production & secretion of bile are active processes. The bile secreted from liver, after storage in the gall bladder is secreted in the duodenum.

Bile is important in the digestion & absorption of fats. Almost 90% of the secreted bile acids are reabsorbed from the intestine & transported back to the liver for resecretion. The rest is secreted in feces.

Compounds that are excreted in bile have been classified into 3 categories on the basis of their bile / plasma concⁿ. ratios :

Group A - Compounds whose ratio is approx. 1.
eg - Sodium, potassium, chloride ions & Glucose

Group B -
eg - Bile

Group C -
eg - Sugar

Drugs can

⇒ Several factors

(i) Physicochemical

(a) Molecular

Molecular
Below 300

Above 500

Between

(b) Polymers
Gases
are
bec

Group B - Compounds whose ratio > 1
eg- Bile salts, Bilirubin, Creatinine, etc.

Group C - Compounds whose ratio < 1
eg- Sucrose, insulin, phosphates, phospholipids.

Drugs can fall in any of the above categories.

⇒ Several factors influence secretion of drugs in bile:

(1) Physicochemical properties of the drug -

(a) Molecular weight:

Molecular weight of Drug	Excretion Pattern
Below 500 dalton	Excreted mainly in urine, less than 5%. & Excreted in bile
Above 500 daltons	Excreted mainly in bile, less than 5%. is Excreted in urine.
Between 500 to 500 dalton	Excreted in both urine & bile.

(b) Polarity -

Greater the polarity, better the excretion. Thus, metabolites are more excreted in the bile than the parent drug because of their increased polarity.

(c) Other Factors -

It includes sex & species differences, protein-drug binding, disease states, drug interaction.

"Biliary clearance is defined as the ability of liver to excrete the drug in the bile."

$$\text{Biliary clearance} = \frac{\text{Biliary excretion rate}}{\text{Plasma drug conc.}}$$

$$= \frac{\text{Bile flow} \times \text{Biliary drug conc.}}{\text{Plasma drug conc.}}$$

"Enterohepatic cycle or enterohepatic circulation of drug is defined as the phenomenon of drug cycling between the intestine & the liver."

→ Pulmonary Excretion :

Gaseous & volatile substances are absorbed through lungs by simple diffusion.

Pulmonary blood flow, rate of respiration & solubility of substance effect pulmonary excretion.

Intact gaseous drugs are excreted but not metabolites. Alcohol which has high solubility in blood & tissues are excreted slowly by lungs.

eg - General anaesthetic (Halothane).

→ Salivary

the pH of lipid & the bitter medication saliva. & are resp compound.

Therapeutic

→ Mammary

milk fat & day in milk is feeding pH & lip

bound since to it. Amount fraction to

mol

Adverse

• disc

due

• nic

&

Salivary excretion :

The pH of saliva varies from 5.8 to 6.4. Unionized, lipid soluble drugs are excreted passively.

The bitter after taste in the mouth of a patient on medication is an indication of drug excretion in saliva. Some basic drugs inhibit saliva secretion & are responsible for mouth dryness.

Compounds excreted in saliva are **Caffeine, Phenytoin, Theophylline**.

Mammary excretion :

Milk consist of lactic secretion which is rich in fats & proteins. 0.5-1 litre of milk is secreted per day in lactating mothers. Excretion of drug in milk is important as it gains entry in breast feeding infants.

pH of milk varies from 6.4 to 7.6. Free, unionized & lipid soluble drug diffuse passively. Highly plasma bound drug like **Diazepam** is less secreted in milk. Since milk contains proteins; drugs excreted can bind to it.

Amount of drug excreted in milk is less than 1% & fraction consumed by infant is too less to produce toxic effects. Some potent drugs like **barbiturates, morphine** may induce toxicity.

Adverse effects -

- Discoloration of teeth with tetracycline & jaundice due to interaction of bilirubin with sulfonamides.
- Nicotine is secreted in the milk of mothers who smoke.

→ Skin Excretion:

Drugs excreted through the skin via sweat follow pH-partition hypothesis. Passive excretion of drugs through skin may lead to urticaria & dermatitis.

Compound such as benzoic acid, Salicylic acid, alcohol, antipyrine & heavy metals like lead, mercury & arsenic are excreted in sweat.

→ Gastrointestinal Excretion:

Excretion of drugs through GIT usually occurs after parenteral administration. Water soluble & ionized form of weakly acidic & basic drugs are excreted in the GIT.

eg - nicotine & quinine are excreted in stomach.

Drugs are excreted in the GIT are reabsorbed into the systemic circulation & undergo recycling.

→ Genital Excretion:

Drugs may be excreted in the reproductive tract & genital secretion. Semen has shown the presence of some drugs.

∴ Bioavailability - & Bioequivalence
"The term bioavailability is defined as the rate & extent (amount) of absorption of unchanged drug from its dosage form."
It is an absolute term.

Bioavailability of a drug from its dosage form depends upon 3 factors:

- (a) Pharmaceutical factor related to physicochemical properties
- (b) Patient related factor.
- (c) Route of Administration.

The influence of route of administration on drug's bioavailability is generally in the following order:

Parenteral > Oral > Rectal > Topical.

Parenteral route, intravenous injection of a drug results in 100% bioavailability as the absorption process is bypassed.

"The dose available to the patient called as bioavailable dose."

"The amount of drug that reaches the systemic circulation is called as systemic circulation or simply availability."

"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

- (a) Primary stages of development of a suitable dosage form for a new drug entity.
- (b) Determination of influence of excipients, patient related factors & possible interaction with other drugs on the efficiency of absorption.
- (c) Development of new formulation of the existing drugs.
- (d) Control of quality of a drug product during the early stages of marketing.
- (e) Comparison of availability of a drug substance from different dosage form.

Sub

Absolute & Relative Bioavailability

"When the systemic availability of a drug administered orally is determined in comparison to its intravenous administration, it is called as absolute bioavailability."

It is denoted by ' F '.

- Intravenous dose is selected as a standard because the drug is administered directly into the systemic circulation and avoids absorption steps.
- Intramuscular dose can also be taken as a standard if the drug is poorly water soluble.

"When the systemic availability of a drug after oral administration is compared with that of an oral standard of the same drug, it is referred to as relative bioavailability."

It is denoted by ' F_r '.

Measurements of Bioavailability -

They are 2 methods to measure the bioavailability :

- (1) Pharmacokinetic method.
- (2) Pharmacodynamic method.

→ Pharmacokinetic Method :- These are indirect method. Pharmacokinetic profile reflects therapeutic effectiveness of drug.

The two major pharmacokinetic methods are -

- (a) Plasma level - time studies.
- (b) Urinary excretion studies.

→ Pharmacodynamic Method :- These are direct method. Measurement of drug effect on physiological process as a function of time.

The two major pharmacodynamic method are -

- (a) Acute pharmacologic response.
- (b) Therapeutic response.

Plasma level - time studies -

Plasma drug concⁿ is difficult or impossible, it is the most reliable method & method of choice in comparison to urine data.

Two dosage forms that exhibit superimposable plasma level - time profiles in a group of patient should result in identical therapeutic activity.

- With single dose study, Serial blood samples for a period of 2 to 3 biological half-lives after drug administration; concⁿ 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 & subsequent samples taken at 15 minutes intervals.

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

- (i) C_{max} : [Peak Plasma Concⁿ] C_{max} will increase with an increase in the dose as well as with an increase in the absorption rate.
- (ii) t_{max} : [Peak time] t_{max} decreases as the rate of absorption increases.
- (iii) AUC : [Area under the plasma level-time curve]. The amount of drug that reaches the systemic circulation.

The extent of bioavailability can be determined by following eqⁿ :

$$F = \frac{[AUC]_{oral} D_{iv}}{[AUC]_{iv} D_{oral}} \quad \text{--- (1)}$$

$$F_r = \frac{[AUC]_{test} D_{std}}{[AUC]_{std} D_{test}} \quad \text{--- (2)}$$

Where, D - Dose administration

Subscripts iv & oral - Route of administration.

Subscripts std & test - test & std doses of the same drug to determine relative bioavailability.

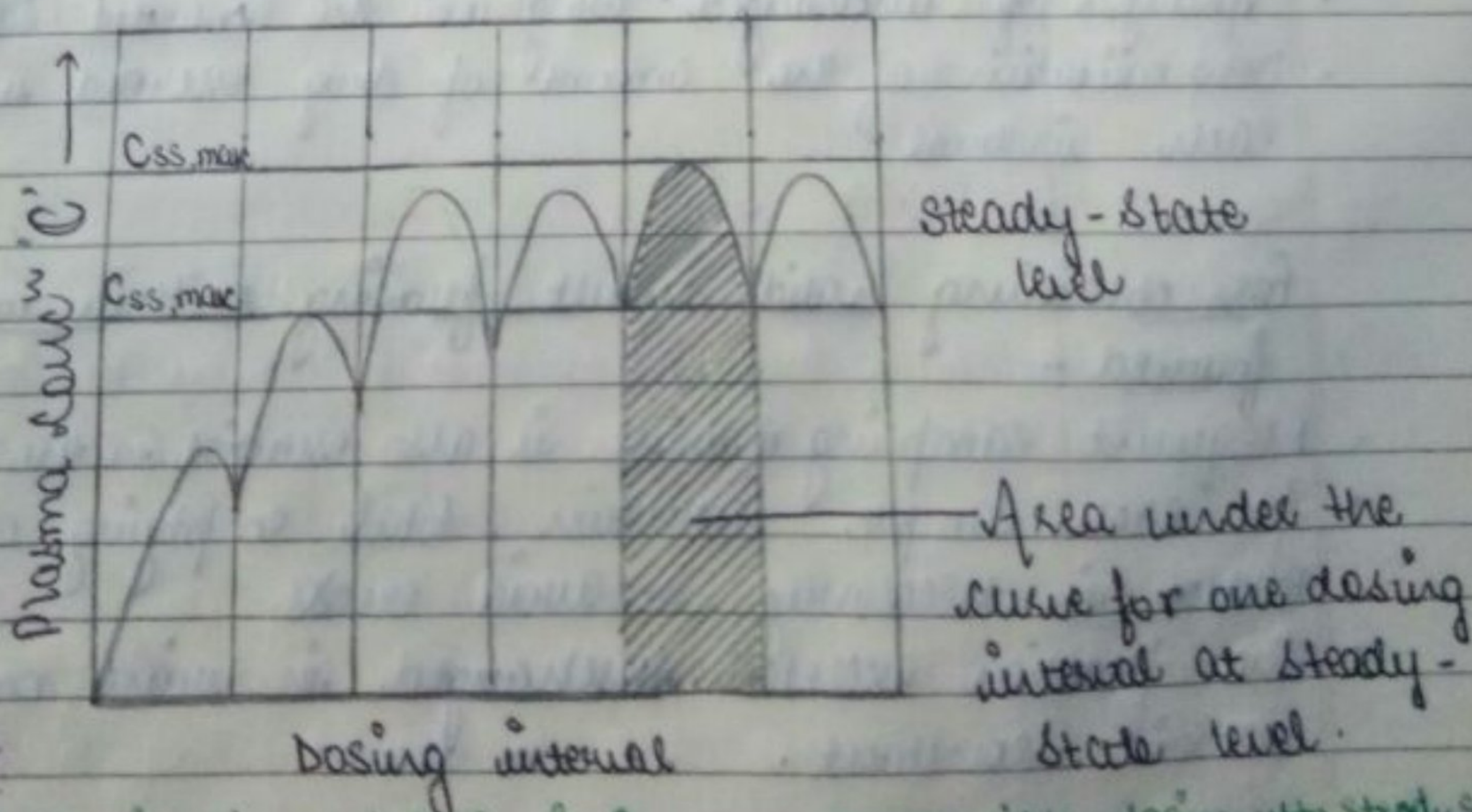
- With multiple dose study, the method involves drug administration for atleast 5 biological half-lives with a dosing interval equal to or greater than the biological half-life. A blood-sample should be taken at the end of previous dosing interval & 8 to 10 samples after administration of next dose.

The extent of bioavailability is given as :

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

Bioavailability can also be determined from the peak plasma concⁿ at steady-state $C_{ss, \text{max}}$ according to following eqⁿ :

$$F_r = \frac{(C_{ss, \text{max}})_{\text{test}} D_{\text{std}} T_{\text{std}}}{(C_{ss, \text{max}})_{\text{std}} D_{\text{test}} T_{\text{std}}}$$



Determination of AUC & $C_{ss, \text{max}}$ on multiple dosing upto steady state

Urinary Excretion Studies -

Bioavailability is based on the principle that the urinary excretion of unchanged drug is directly proportional to the plasma concⁿ of drug.

As a rule of thumb, determination of bioavailability using urinary excretion data should be conducted only if at least 20% of administered dose is excreted unchanged in the urine.

Advantages -

- (i) Noninvasive method.
- (ii) Convenience of collecting urine samples.
- (iii) Direct measurement of bioavailability.
- (iv) It is useful when there is lack of sufficiently sensitive analytical techniques to measure concⁿ of drug in plasma.

Methods involves -

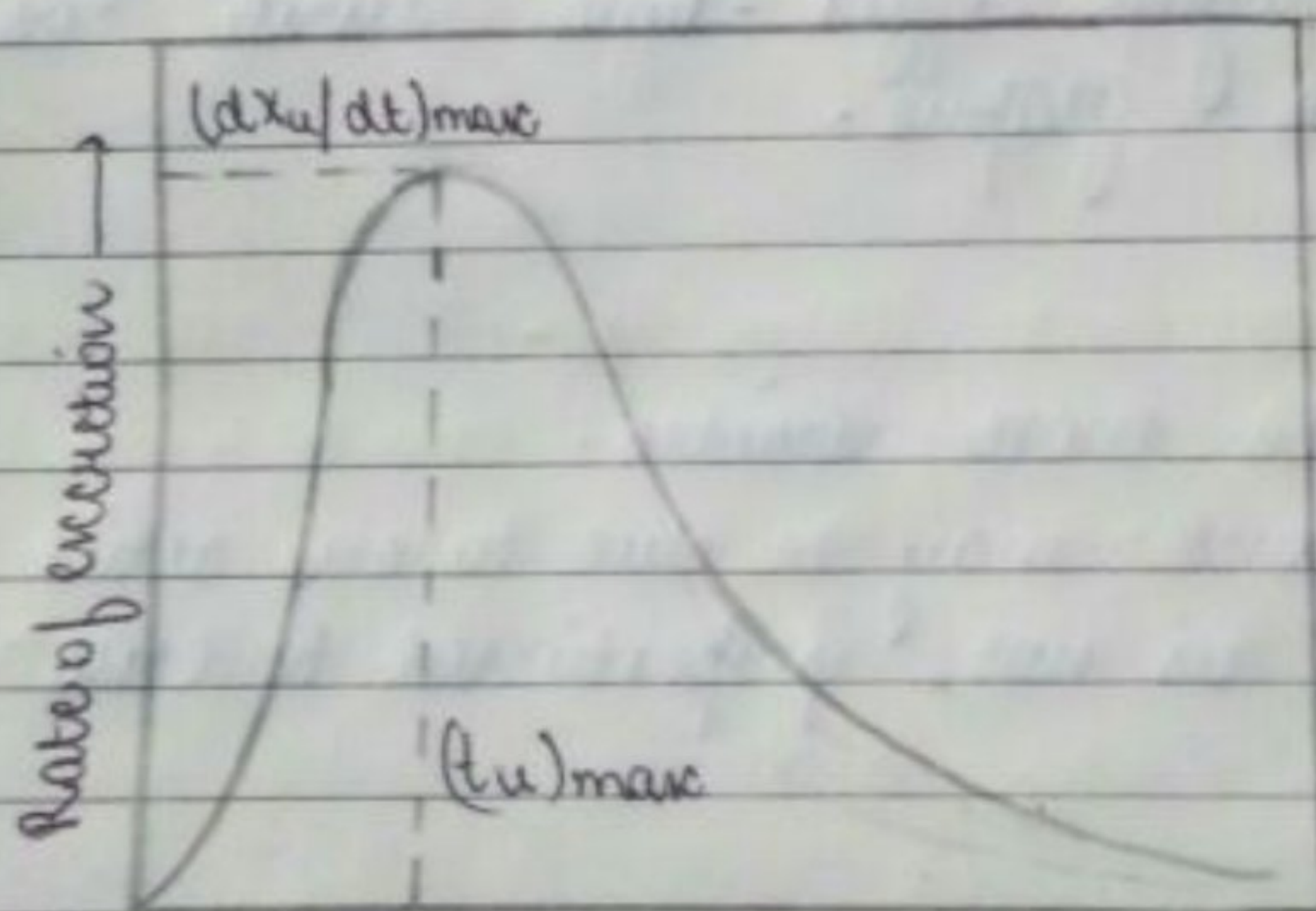
- Collection of urine at regular intervals for a time span equal to 7 biological half lives.
- Analysis of unchanged drug in the collected sample.
- Determination of the amount of drug excreted in each interval.

For obtaining valid result following criteria must be followed -

- Frequent sampling of urine is also essential in the beginning.
- At each sample collection, total emptying of the bladder is necessary to avoid errors.
- The fraction excreted unchanged in urine must remain constant.

The 3 major parameters examined in urinary excretion data obtained with a single dose study are:

- (i) $(dx_u/dt)_{max}$: [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} .
- (ii) $(t_u)_{max}$: [Time for maximum excretion rate]. Its value decreases as the absorption rate increases. It is analogous to the t_{max} of plasma level data.
- (iii) X_u : [Cumulative amount of drug excreted in the urine]. It is related to the AUC of plasma level data & increases as the extent of absorption increases.



Midpoint time of urine collection period $\rightarrow$
 Plot of excretion rate versus time

The extent of bioavailability is calculated from eqⁿ. given below :

$$F = \frac{(X_u^\infty)_{oral} \cdot D_{iv}}{(X_u^\infty)_{iv} \cdot D_{oral}} \quad \text{--- (1)}$$

$$F_R = \frac{(X_u^\infty)_{test} \cdot D_{std}}{(X_u^\infty)_{std} \cdot D_{test}} \quad \text{--- (2)}$$

With multiple dose study to steady-state, the eqn :

$$F_d = \frac{(X_{u,ss})_{\text{test}} \cdot D_{\text{std}} \cdot T_{\text{std}}}{(X_{u,ss})_{\text{std}} \cdot D_{\text{test}} \cdot T_{\text{std}}} \quad \text{--- (3)}$$

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

Acute Pharmacologic Response -

- (1) when bioavailability measurement by pharmacokinetic method is difficult, an acute pharmacologic effect such as effect on pupil diameter, ECG & ECG reading related to time course of drug.
- (2) Bioavailability can then be determined by construction of pharmacological effect-time curve as well as dose response graphs.

Disadvantage -

- It tends to be more variable.
- Observed response may be due to an active metabolite whose concⁿ is not proportional to concⁿ of parent drug.

Therapeutic Response -

This method is based on observing the clinical response to a drug formulation given to patients suffering from disease.

Drawbacks -

- Quantitation of observed response is too improper to allow for reasonable assessment of relative bioavailability between two dosage forms of the same drug.

- Unless multiple dose protocols are employed.
- Bioequivalence studies are usually conducted using a crossover design.

∴ In Vitro Drug Dissolution Testing Models -

In vitro test to be useful, it must predict the in vivo behaviour to such an extent that in vivo bioavailability test need not be performed. Despite attempts to standardize the test performance the in vivo dissolution technique is still by no means a perfect approach.

Several Factors of dissolution apparatus -

(i) Factors relating to the dissolution apparatus -

Design, the size of the container (several ml to l), the shape of the container (round bottomed or flat), nature of agitation (stirring, rotating or oscillating method), speed of agitation.

(ii) Factors relating to the dissolution fluid -

Composition (water, 0.1N HCl, phosphate buffer, simulated gastric fluid, simulated intestinal fluid, etc), viscosity, volume, temperature (generally 37°C).

(iii) Process method -

Introduction of dosage form, sampling technique, changing the dissolution fluid.

Ideal Features of dissolution apparatus -

- Simple in design, easy to operate & usable.
- Fabrication dimensions & positioning of all components.
- Permits controlled variable intensity of mild, uniform non-turbulent liquid agitation.
- Provides minimum mechanical abrasion to the dosage form.
- Maintains nearly perfect sink condition.
- Prevents / eliminates evaporation of the dissolution medium at 37°C .
- Ease of drawing samples.
- Facilitates good inter-laboratory agreement.

Based on the absence or presence of sink condition, there are two principal types of dissolution apparatus -

(1) Closed-Compartment Apparatus -

A limited volume apparatus operating under non-sink condition. The dissolution fluid is restrained to the size of the container.

eg - Beaker type apparatus.

(2) Open-Compartment Apparatus -

Dosage form is contained in a column which is brought in continuous contact with fresh, flowing dissolution medium (perfect sink condition).

Dialysis System are used for very poorly aqueous soluble drugs for which maintenance of sink condition would otherwise require large volume of dissolution fluid.

Dissolution Apparatus Type -

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Rotating Basket Apparatus (Apparatus 1) :

It is described by Burrowski et al, it is a closed-compartment, a cylindrical glass vessel which are partially immersed in a water bath at 37°C . A cylindrical basket made of 22 mesh & 2cm distance from the bottom & rotated by variable speed motor through a shaft.

All metal parts like basket & shaft are made of S.S. 316.

Rotating Paddle Apparatus (Apparatus 2) :

The rotating basket is replaced with a paddle which acts as a stirrer. They prevent floating of capsules & other floatable forms. A small, loose, wire helix may be attached to such prepⁿ to prevent them from floating.

Reciprocating Cylinder Apparatus (Apparatus 3) :

It consist a set of cylindrical flat-bottomed glass vessels equipped with reciprocating cylinders.

It is used for dissolution testing of controlled-release bead-type formulation.

Flow Through Cell Apparatus (Apparatus 4) :

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

It is used in close mode [where the fluid is in fixed volume] & open mode [where continuous replenishment of the fluids].

Advantage -

- Easy condition during dissolution which have limited aqueous solubility.
- Feasibility of using large volume of dissolving fluid.
- Feasibility for automation of apparatus.

→ Paddle over Disc Apparatus (Apparatus 5):

- It is used for evaluation of transdermal product.
- It consist of a sample holder or disc that holds the product.

→ Cylinder Apparatus (Apparatus 6):

- It is used for evaluation of transdermal product.
- An inert porous cellulosic material is adhered to the cylinder.

→ Reciprocating Disc Apparatus (Apparatus 7):

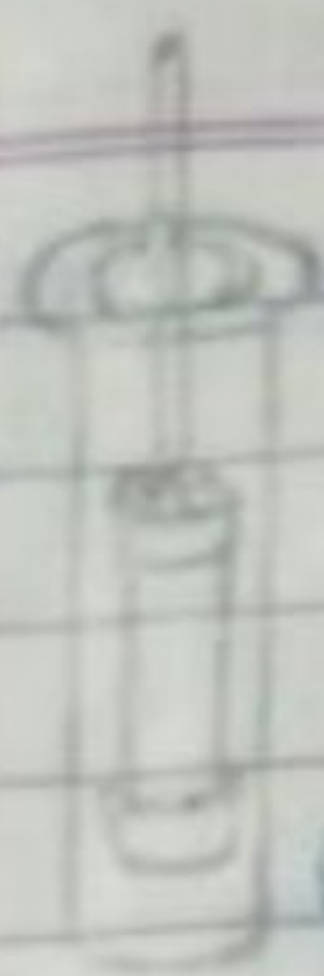
- It is used for evaluation of transdermal product as well as non-disintegrating controlled-released oral prepⁿ.
 - The sample are placed on disc-shaped holder using inert porous cellulosic support.
- Imp: The test is carried out at 32°C & reciprocating frequency of 30 cycles/min.



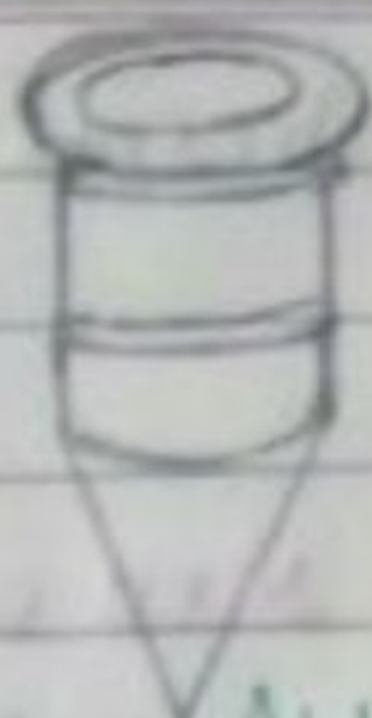
Apparatus 1



Apparatus 2



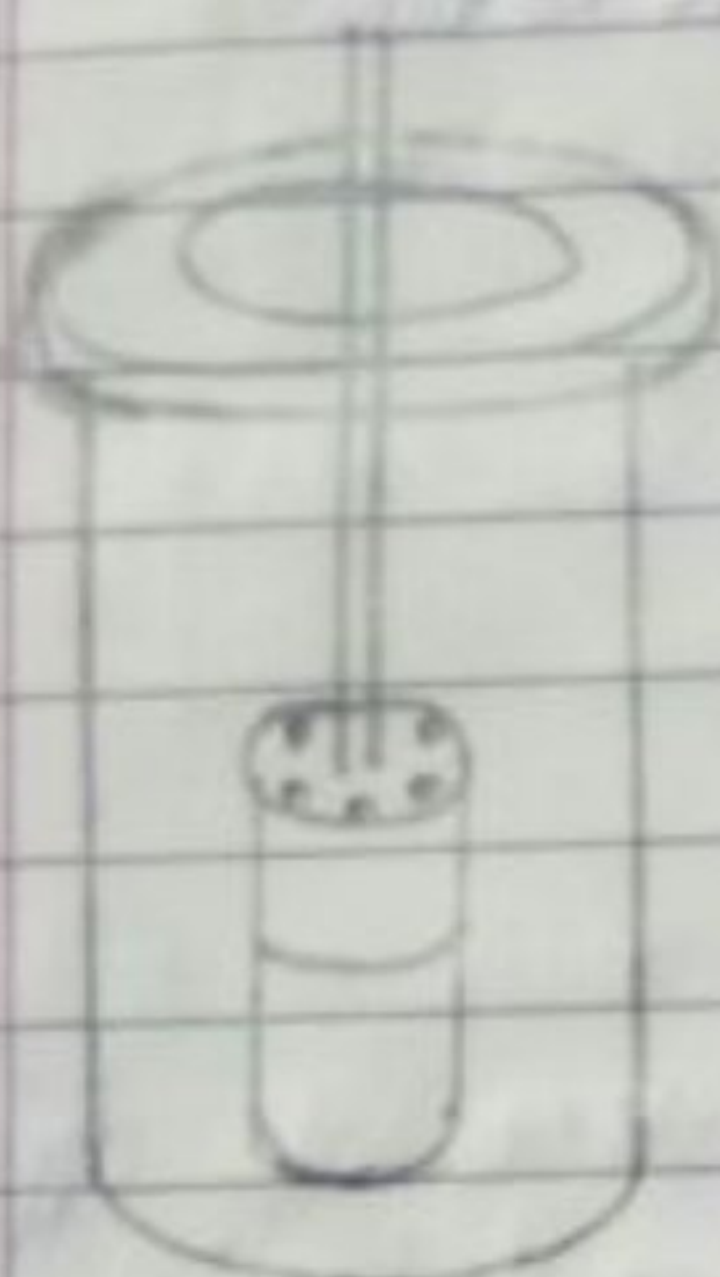
Apparatus 3



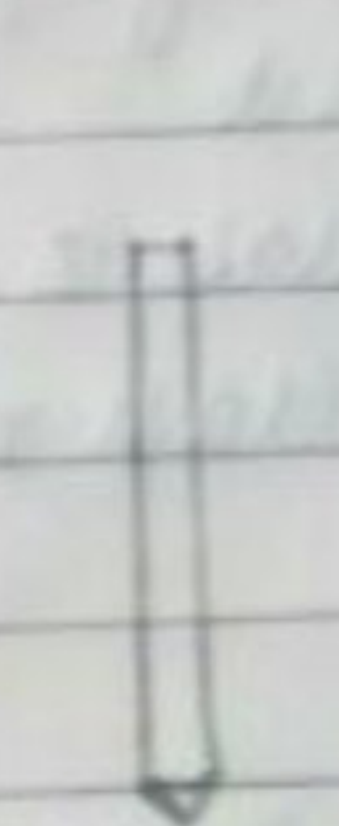
Apparatus 4



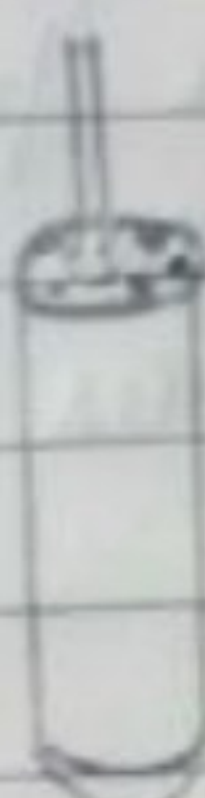
Apparatus 5



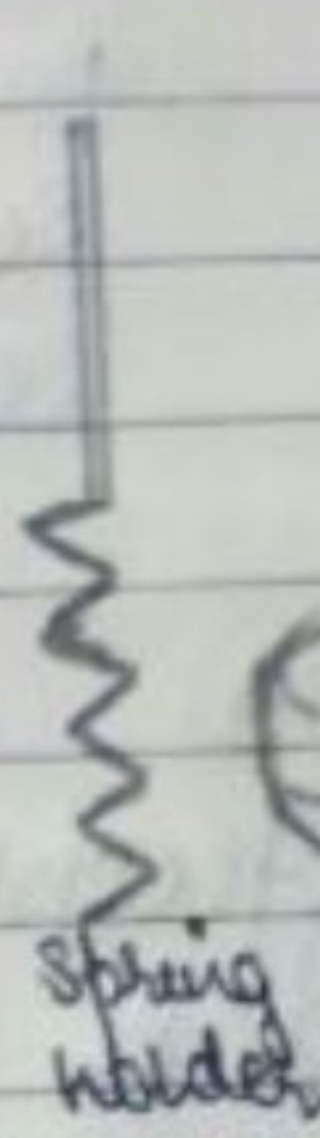
Apparatus 6



Acrylic rod

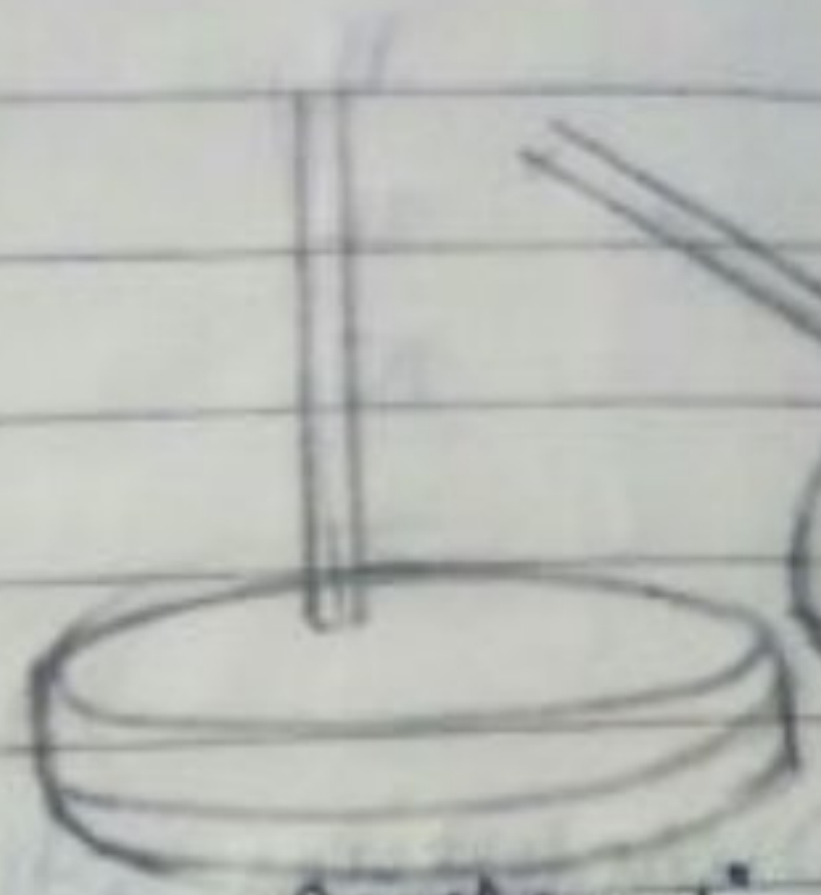


Teflon cylinder



Spring holder

Apparatus 7



Reciprocating disc



Angled disc

Compendial Dissolution Apparatus Types & Their Application

Apparatus	Name	Drug formulation Tested
1.	Rotating basket	Conventional tablets, chewable tablets, controlled-release formulation
2.	Rotating Paddle	Tablets, orally disintegrating tablets, chewable tablets, capsules, suspension, controlled-release formulation
3.	Reciprocating cylinder	Controlled-release formulation, Chewable tablets
4.	Flow-through cell	Formulation containing poorly soluble drugs, powders & granules, implants, microparticles
5.	Paddle over disc	Transdermal formulation
6.	Cylinder	Transdermal formulation
7.	Reciprocating disc	Controlled-release formulations

Dissolution Methodology for Immediate Release Products Based on BCS -

BCS class	Dissolution methodology
I	Single point if $Q \geq 85\%$ in 15 min multiple point if $Q < 85\%$ in 15 min
II	multiple point
III	Same as class I
IV	Same as class II

Dissolution Acceptance Criteria -

The value of Q is defined as percentage of drug content dissolved in a given time period.

Stage	no. of Dosage units tested	Acceptance Criteria
S_1	6	no dosage unit is less than $Q + 5\%$.
S_2	6	Average of the twelve dosage units $(S_1 + S_2) \geq Q\%$ and no dosage unit is less than $Q - 15\%$.
S_3	12	Average of the 24 dosage unit $(S_1 + S_2 + S_3) \geq Q\%$ & not more than 2 dosage units are less than $Q - 15\%$ & no dosage unit is less than $Q - 25\%$.

In Vitro-In Vivo Correlation (IVIVC)

In vitro dissolution test on the drug product will be insufficient to predict its therapeutic efficacy.

Consistent correlation between in vitro dissolution behaviour of a drug & its in vivo bioavailability must be experimentally demonstrated to guarantee reproducibility of biological response.

"In vitro-in vivo correlation is defined as the predictive mathematical model that describes the relationship between an in-vitro property of a dosage form & in-vivo response."

The main objective of developing & evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for in-vivo bioavailability studies in human beings.

Application -

- Batch-to-batch consistency.
- Development of a new dosage form with desired in vivo performance.
- To assist in validating or setting dissolution specification.

Two Basic Approaches -

- By establishing a relationship, usually linear, between the in-vitro dissolution & the in-vivo bioavailability parameters.
- By using the data from previous bioavailability studies to modify the dissolution methodology in order to arrive at meaningful IVIVC.

Quantitative linear in vivo :

(1) Correlation Based on the Plasma Level Data -

Here, linear relationship between dissolution parameters & plasma level data are established.

Parameters used for correlating in vitro dissolution with Plasma Data

In-vitro dissolution Parameter	In vivo Plasma Data Parameter
Time for specific amount of drug to dissolve	AUC, C_{max}
Amount dissolved at a specific time point	Fraction absorbed, absorption rate constant k_a
Mean dissolution time	Mean residence time, mean dissolution time, mean absorption time
Parameter estimated after modelling the dissolution process	C_{acc} , at time 't', amount absorbed at time 't'.

(2) Correlation Based on the Urinary Excretion data -

Here, dissolution parameters are correlated to the amount of drug excreted unchanged in the urine, cumulative amount of drug excreted as a function of time.

(3) Correlation Based on the Pharmacological Response -

An acute pharmacological effect such as LD_{50} in animals is related to any of the dissolution parameters.

Imp "Statistical Moments theory can used to determine the relationship such as mean dissolution (in vitro) versus mean residence (in vivo)".

Imp IVIVC levels:

- (1) Level A - The in-vitro dissolution & in-vivo absorption rate curves are superimposable & the mathematical description for both curves is the same.

Advantage -

- Point-to-Point correlation is developed.
- The in-vivo dissolution serves as in-vivo indicating quality.

- (2) Level B - The mean in-vitro dissolution time is compared to either the mean residence time or the mean in-vivo dissolution time.
- It is not a point-to-point correlation

- (3) Level C - It is a single point correlation. It relates one dissolution point to one pharmacokinetic parameter such as AUC, t_{max} or C_{max} .

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Bioequivalence Study

"Equivalence is a relative term that compares drug product with respect to a specific characteristic or function or to a defined set of standards."

Types of Equivalence

Chemical Equivalence is defined as two or more drug products contain the same labeled chemical substance as an active ingredient in the same amount.

Pharmaceutical Equivalence is defined as two or more drug products are identical in strength, quality, purity, content uniformity & disintegration & dissolution characteristics. They may however differ in containing different excipients.

Bioequivalence is a relative term which denotes that the drug substance in two or more identical dosage forms reaches the systemic circulation at the same relative rate & to the same relative extent, i.e. their plasma concⁿ-time profiles will be identical without significant statistical differences.

Therapeutic equivalence is defined as two or more drug products that contain the same therapeutically active ingredient elicit identical pharmacological effects & can control the disease to the same extent.

→ Objectives of Bioequivalence Studies:

- If a new product is intended to be a substitute for an approved medicinal product as a pharmaceutical equivalent, the equivalence with this product should be justified.
- To ensure clinical performance of such drug products bioequivalence studies should be performed.
- Bioequivalence studies are conducted if there is :-
 - (a) A risk of bio-inequivalence.
 - (b) A risk of pharmacotherapeutic failure or diminished clinical safety.

→ Types of Bioequivalence studies:

They are 2 types -

- (a) In-vivo
- (b) In-vitro

∴ Methods to enhance the dissolution rate & Bioavailability of poorly soluble drugs -

(1) Micronization:

- Reduce the size of solid drug particle to 1-10 microns.
- It is also called micromilling.
- Particle size is reduced by spray drying or by use of air attrition methods.

Eg - Cyrisofunin, several steroidal, supra drug.

(2) Nanonisation:

- The drug powder is converted to nano crystals of sizes 200-600 nm.

Eg - Amphotericin B.

- Dispersion of drug nano crystals in a liquid called as nanosuspension.
- These are 3 technologies use to prepare nano particles:
 - (a) Pearl milling
 - (b) Homogenisation in water
 - (c) Homogenisation in non-aqueous media

(3) Super Critical Fluids (SCF) Recrystallization :

- SCF are fluids whose temperature & pressure are greater than its critical temperature (T_c) & critical pressure (P_c), allowing it to assume the properties of both a liquid & gas.
- At near ' T_c ', SCF are highly compressible, allowing moderate changes in pressure to greatly alter the density & mass transport characteristics of a fluid that largely determine its solvent power.
- Once the drug particles are solubilised within SCF, they may be recrystallised at greatly reduced particle sizes.

(4) Spray Freezing Into Liquid (SFL) :

- This technique involve atomizing an aqueous, organic, aqueous-organic cosolvent solⁿ, aqueous-organic emulsion or suspension containing a drug & pharmaceutical excipients directly into a compressed gas (CO_2 , helium, propane, ethane) or the cryogenic liquids (nitrogen, oxygen, hydrofluorocarbon).
- use of Acetonitrile as the solvent increases drug loading & decreases the drying time for lyophilization.

(5) Evaporative Precipitation Into Aqueous Solution (EPAS):

- The EPAS process utilizes rapid phase separation to nucleate & grow nanoparticles & microparticles of lipophilic drugs.
- The drug is first dissolved in a low b.p. organic solvent.
- This solution is pumped through a tube where it is heated under pressure to a temperature above the solvent's b.p. & then sprayed through a fine atomizing nozzle into a heated aqueous solⁿ.
- Surfactants are added to the organic solⁿ & the aqueous solⁿ to optimize particle formation & stabilization.

(6) Use of Surfactant:

- Surfactants are very useful as absorption enhancers & enhance both dissolution rate & permeability of drug.
- They enhance dissolution rate primarily by promoting wetting & penetration of dissolution fluid into the solid drug particles.
- They are generally used in concⁿ below their Critical Micelle Concⁿ (CMC).
- Non-surfactant like polyoxabates
- Steroids like spiroanabolactones.

(7) Use of Salt form:

- Salts have improved solubility & dissolution characteristics in comparison to the original drug.
- Alkali metal salts of acidic drugs like penicillin, strong acid salts of basic drugs like Atropine are more water-soluble than the parent drug.

(8) Use of Precipitation Inhibitors :

- A significant increase in free drug concⁿ above equilibrium solubility results in supersaturation which can lead to drug ppt or crystallization.
- This can be prevented by use of inert polymers such as **HPMC, PVP, PVA, PEG, etc.**
- It act by one or more mechanism :
 - (i) ↑ the viscosity of crystallization medium thereby reducing the crystallization rate of drugs.
 - (ii) Provide a steric barrier to drug molecules & inhibit crystallization.
 - (iii) Adsorb onto faces of host crystal, reduce the crystal growth rate of the host & produce smaller crystals.

(9) Alteration of pH of the drug microenvironment :

- This can be achieved in 2 ways -
 - (a) In situ salt formation
 - (b) Addition of buffers to the formulation.
- **Eg - Buffered aspirin tablet.**

(10) Use of Amorphs, Anhydrides, Solvates, Metastable polymorphs

- Depending upon the internal structure of the solid drug, selection of proper form of drug with greater solubility is important.
- In general,
 - (a) Amorphs are more soluble than metastable polymorphs.
 - (b) Anhydride are more soluble than hydrates
 - (c) Solvates are more soluble than non-solvates.

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(11) Solvent Deposition:

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

(12) Precipitation:

The poorly aqueous soluble drugs such as cyclosporin is dissolved in a suitable organic solvent followed by its rapid mixing with a non-solvent to effect ppt of drug in nanosize particles. The product so prepared is also called as Hydrosol.

(13) Selective Adsorption on Insoluble Carriers:

- An highly active adsorbent such as inorganic clays like bentonite can enhance the dissolution rate of poorly water soluble drugs such as griseofulvin, indomethacin, prednisone by maintaining the concⁿ. gradient at its maximum.

- Two reasons for the rapid release of drugs from the surface of clay are -

- (a) The weak physical bonding b/w the adsorbate & the adsorbent.

- (b) Hydration & swelling of the clay in the aqueous media.

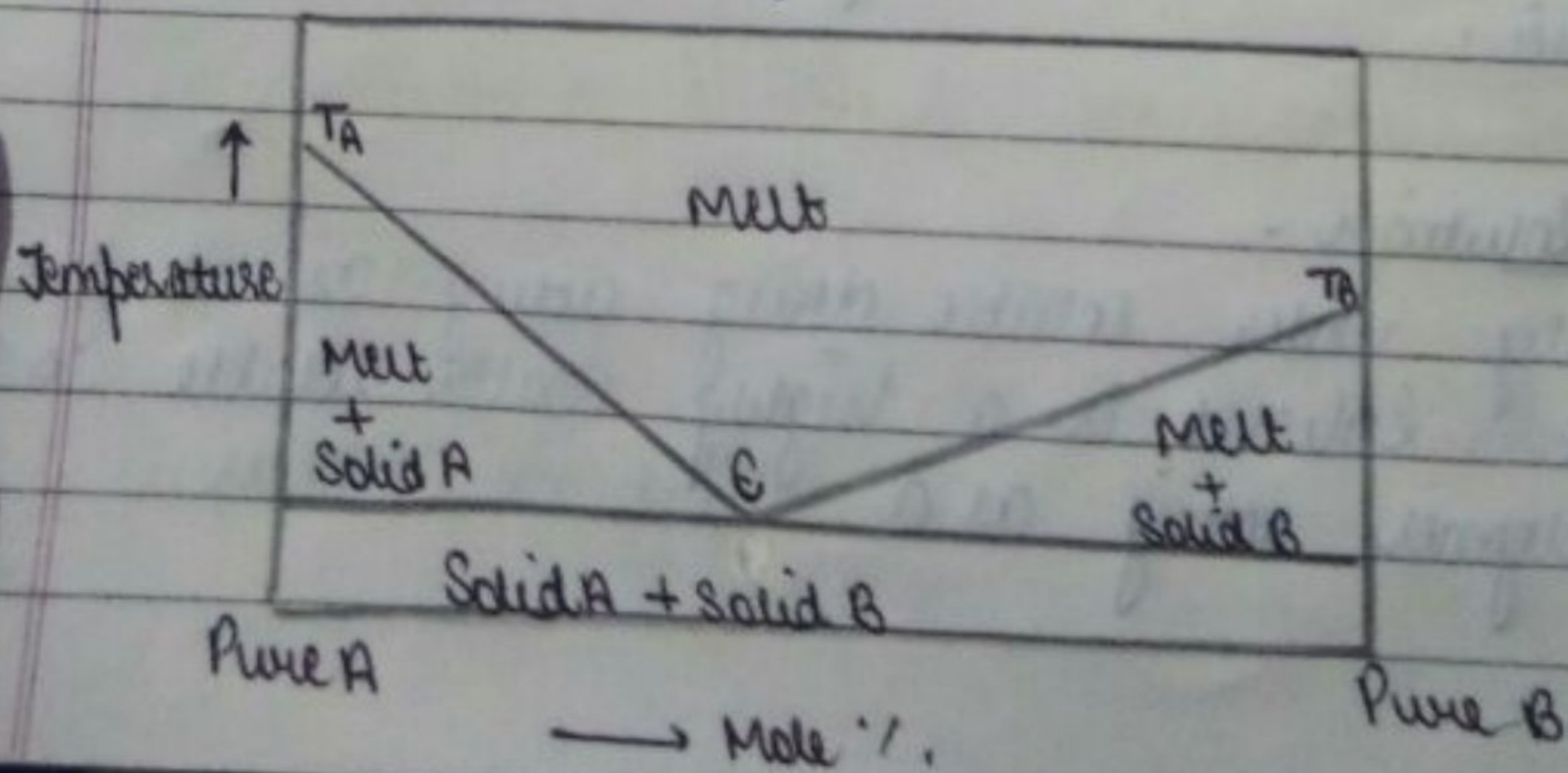
(14) Solid Solution -

- A poorly water soluble drug acting as the guest & the solvent is a highly water soluble compound or polymer acting as a host or carriers.

- "A solid solⁿ 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 solⁿ are also called as molecular dispersion or mixed crystals."
- Because of reduction in particle size to the molecular level, solid solⁿ show greater aqueous solubility & faster dissolution than eutectics & solid dispersion.
- They are generally prepared by fusion method.
- Eg. glibenclamide - succinic acid.
- When the resultant solid solⁿ is a homogeneous transparent & brittle system, it is called as glass solution.
- Carriers that form glassy structure are citric acid, urea, PVP, PEG & sugars (dextrose, sucrose, galactose).

(15) Eutectic Mixtures :

- These systems are also prepared by fusion method.
- A phase diagram of two-component system is shown in diagram.



- When the eutectic mixture is exposed to water, the soluble carrier dissolves leaving the drug in a microcrystalline state which solubilizes rapidly.

Eg - Paracetamol-urea, Griseofulvin-urea,
Griseofulvin-succinic acid

(16) Solid Dispersion :

- The term 'solid dispersion' refers to the dispersion of one or more active ingredients in an inert carrier in a solid state, frequently prepared by the :

(i) Hot melt method

(ii) Solvent evaporation method

(iii) Hot melt extrusion method

- The basic difference between the solid solⁿ/eutectics is that the drug is precipitated out in an amorphous form in the former as opposed to crystalline form in the latter.

Eg - Amorphous sulphathiazole in crystalline urea.

- Such dispersion are often called as co-evaporates or co-precipitates.

- With glassy materials, the dispersion formed are called as glass dispersion or glass suspension.

(17) Molecular Encapsulation with cyclodextrins :

- The β - & γ -cyclodextrins & several of their derivatives are unique in having the ability to form molecular inclusion complexes with hydrophobic drugs having poor aqueous solubility.

- These bucket shaped oligosaccharides produced from Starch are versatile in having a hydrophobic cavity of size suitable enough to accommodate the lipophilic drugs as guest, the outside of the host molecule is relatively hydrophilic.
- Eg. - Thiuride diuretics, barbiturates, benzodiazepines & no. of NSAIDs.

