BIOAVAILABILITY
&
BIOEQUIVALENCE

CHAPTER OUTLINE

- CONSIDERATIONS IN BIOAVAILABILITY STUDY DESIGN:

  - Absolute Vs Relative Bioavailability

  - Single dose Vs Multiple dose studies

  - Healthy Volunteers Vs Patients

  - Measurement of Bioavailability

  - *In vitro* drug dissolution rate and bioavailability (dissolution testing models)

  - *In vitro- In vivo correlation*

  - Biopharmaceutical Classification System

- CONSIDERATIONS IN BIOEQUIVALENCE STUDY DESIGN:

  - *In vitro Vs in vivo bioequivalence studies*

  - *Types:*

    Compppletely randomized

    Randomized block design

    Repeated measures, cross-over and carry over design

    Latin square design

  - Bioequivalence Study Design Protocol

  - Statistical Interpretation

Methods of enhancement of bioavailability

- through enhancement of drug solubility or dissolution rate
- through enhancement of drug permeability across bio-membrane
- through enhancement of drug stability
- through gastrointestinal retention

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DEF: BIOAVAILABILITY

"The term Bioavailability is defined as a rate & extent (amount) of absorption of unchanged drug from its dosage form."

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Brahmankar & Jaiswal

- ***"Bioavailability*** is a term used to indicate the fractional extent to which a dose of drug reaches its site of action or a biological fluid from which the drug has access to its site of action."

- Goodman & Gillman

Objectives of Bioavailability studies :

- During primary stages of development of suitable dosage forms of new drug entity .

- Determination of influence of excipients, patient related factors & possible interaction with other drugs on the efficiency of absorption .

Components

- **Rate of absorption** – the rapidity with which the drug is absorbed.

  - Rapid onset : conditions like acute attack of asthma, intense acute pain

  - Slower onset : To prolong duration of action

    To avoid adverse effects.

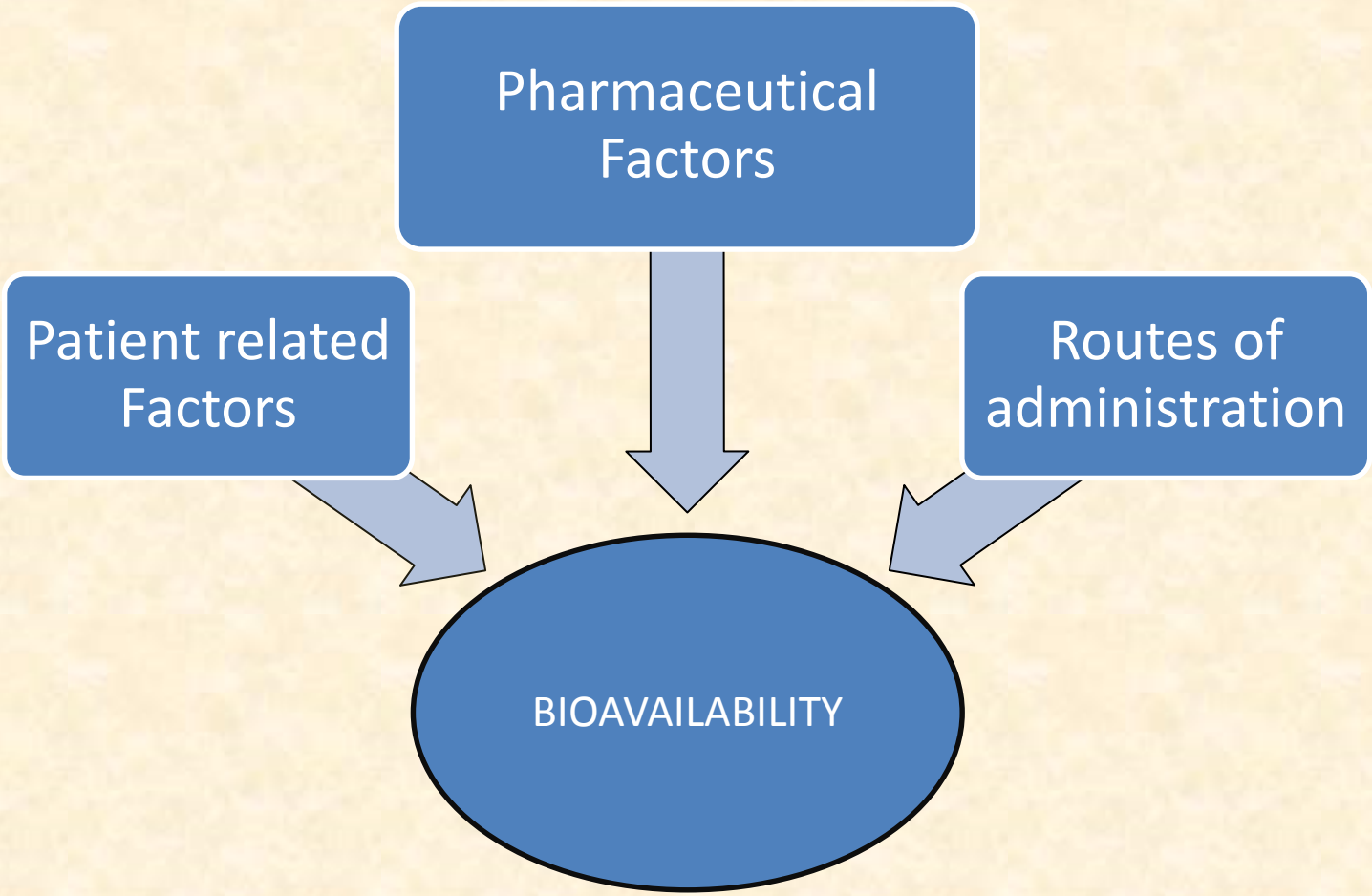
- **Extent of absorption** -chronic conditions like Epilepsy

Bioavailable fraction (F)

- It refers to the fraction of administered dose that enters the systemic circulation.

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

Factors affecting Bioavailability :



A) Pharmaceutic factors :

1) Physicochemical properties of drug :

1. Drug solubility & dissolution rate.
2. Particle size & effective surface area.
3. Polymorphism & Amorphism.
   - Amorphous > metastable > stable
4. Pseudopolymorphism (Hydrates / Solvates)
   - Anhydrates > hydrates e.g. Theophylline, Ampicillin
   - Organic solvates > non solvates e.g. fludrocortisone
5. Salt form of the drug.
   - Weakly acidic drugs – strong basic salt e.g.barbiturates , sulfonamides.
   - Weakly basic drugs – strong acid salt
6. Lipophilicity of the drug .
7. pKa of the drug & pH .
8. Drug stability.

2) Dosage form characteristics & Pharmaceutic Ingredients :

1. Disintegration time (tab/cap)
2. Dissolution time.
3. Manufacturing variables.
4. Pharmaceutic ingredients (excipients / adjuvants)
5. Nature & type of dosage form.
   - Solutions> Emulsions> Suspensions> Cap> Tab> Enteric Coated Tab > Sustained Release
6. Product age & storage conditions.

B) Patient related factors :

1. Age
2. Gastric emptying time .
3. Intestinal transit time .
4. Gastrointestinal pH .(HCL > Acetic > citric)
5. Disease States .
6. Blood flow through the gastrointestinal tract .
7. Gastrointestinal contents :
    a) Other drugs .
    b) Food .
    c) Fluids
    d) Other normal g.i. contents
8. Presystemic metabolism (First – Pass effect) by :
    a) Luminal enzymes .
    b) Gut wall enzymes .
    c) Bacterial enzymes .
    d) Hepatic enzymes .

C) Routes of administration :

Parenteral > **O**ral > **R**ectal > **T**opical

Route	Bioavailability (%)	Characteristics
Intravenous (IV)	100 (by definition)	Most rapid onset
Intramuscular (IM)	75 to ≤ 100	Large volumes often feasible; may be painful
Subcutaneous (SC)	75 to ≤ 100	Smaller volumes than IM; may be painful
Oral (PO)	5 to < 100	Most convenient; first pass effects may be significant
Rectal (PR)	30 to < 100	Less first-pass effects than oral
Inhalation	5 to < 100	Often very rapid onset
Transdermal	80 to ≤ 100	Usually very slow absorption; used for lack of first-pass effects; prolonged duration of action

Absolute Bioavailability (F)

- Def :

  "When the systemic availability of a drug administered ***orally*** is determined in comparison to its ***intravenous*** administration is called as Absolute Bioavailability"

$$\% \text{ Absorption} = \frac{\text{Dose (iv)} \times \text{AUC (oral)}}{\text{Dose (oral)} \times \text{AUC (iv)}} \times 100$$

  - Used to characterize drug's inherent absorption properties from extravascular site.
  - IM route may be used as standard for poorly water soluble drugs.

Relative Bioavailability (Fr)

- Def :

  " When the systemic availability of the drug after oral administration is compared with that of oral standard of same drug (such as aqueous or non aqueous solution or a suspension) is referred as Relative Bioavailability"

  e.g. comparison between ***capsule*** Amox and ***suspspension*** *A*mox.

  - Used to characterize absorption of a drug from its formulation.

Bioavailability study design

Single dose vs Multiple dose studies :

- ***Single dose study :***

  Advantages:

  - More common
  - Easy, less tedious
  - Less exposure to drug

  Disadvantages:

  - Difficult to predict steady state characteristics

Multiple dose study :

Advantages :

- Accurate
- Few blood samples required
- Ethical
- Small intersubject variability
- Better evaluation of controlled release formulations.
- Can detect non linearity in pharmacokinetics.
- Higher blood levels (d/t cumulative effect)

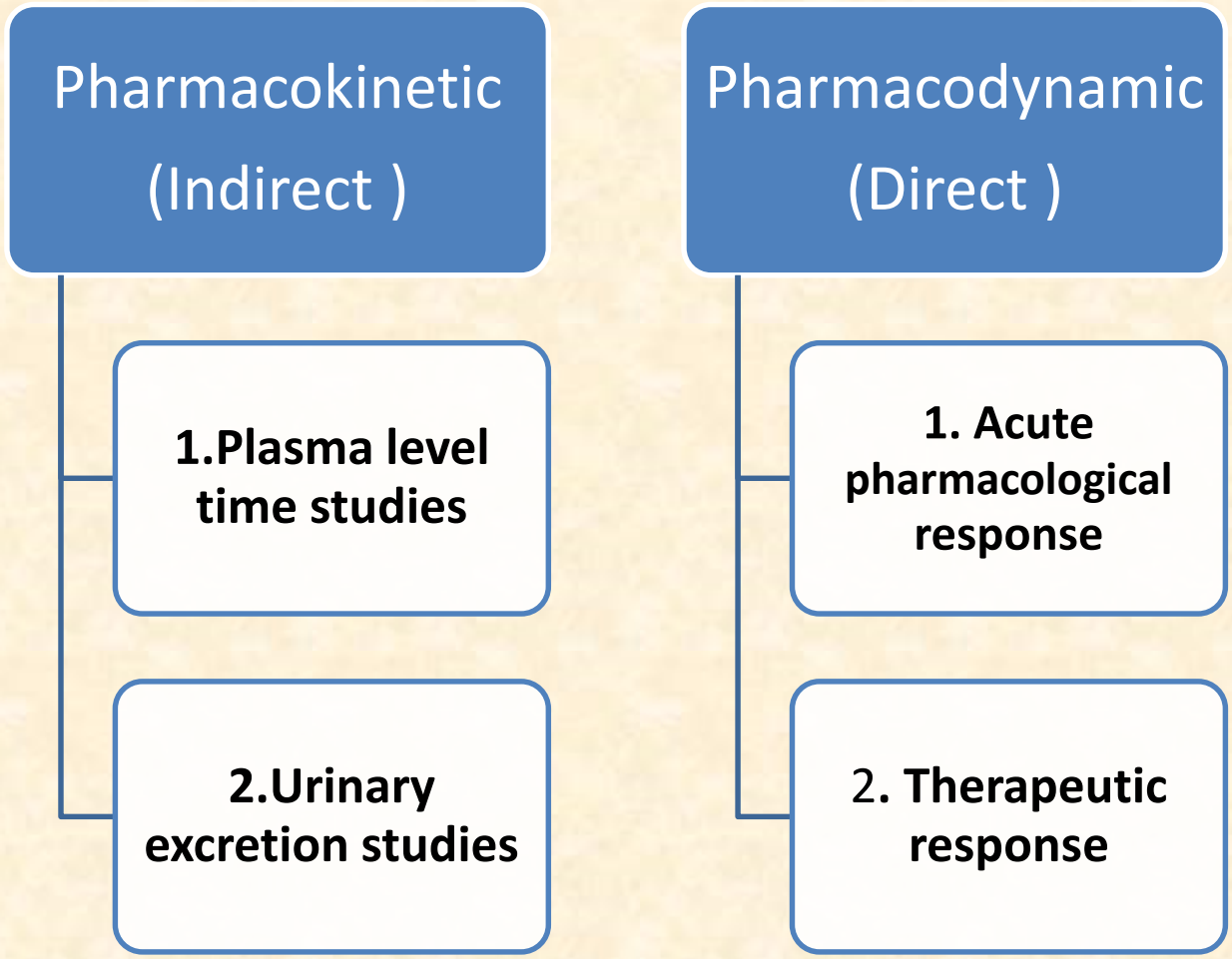
Disadvantages :

- Poor subject compliance .
- Tedious, time consuming
- More drug exposure.

Human volunteers : Healthy subjects vs. patients ?

- **Patients : used in multiple dose studies.**
- **Advantages :**
  1. Patient gets benefited from the study.
  2. Reflects better therapeutic efficacy.
  3. Drug absorption pattern in disease states evaluated.
  4. Avoids ethical quandary.
- **Disadvantages :**
  1. Disease states , other drugs affects study
  2. Difficult to follow stringent study conditions.

Measurement of Bioavailability

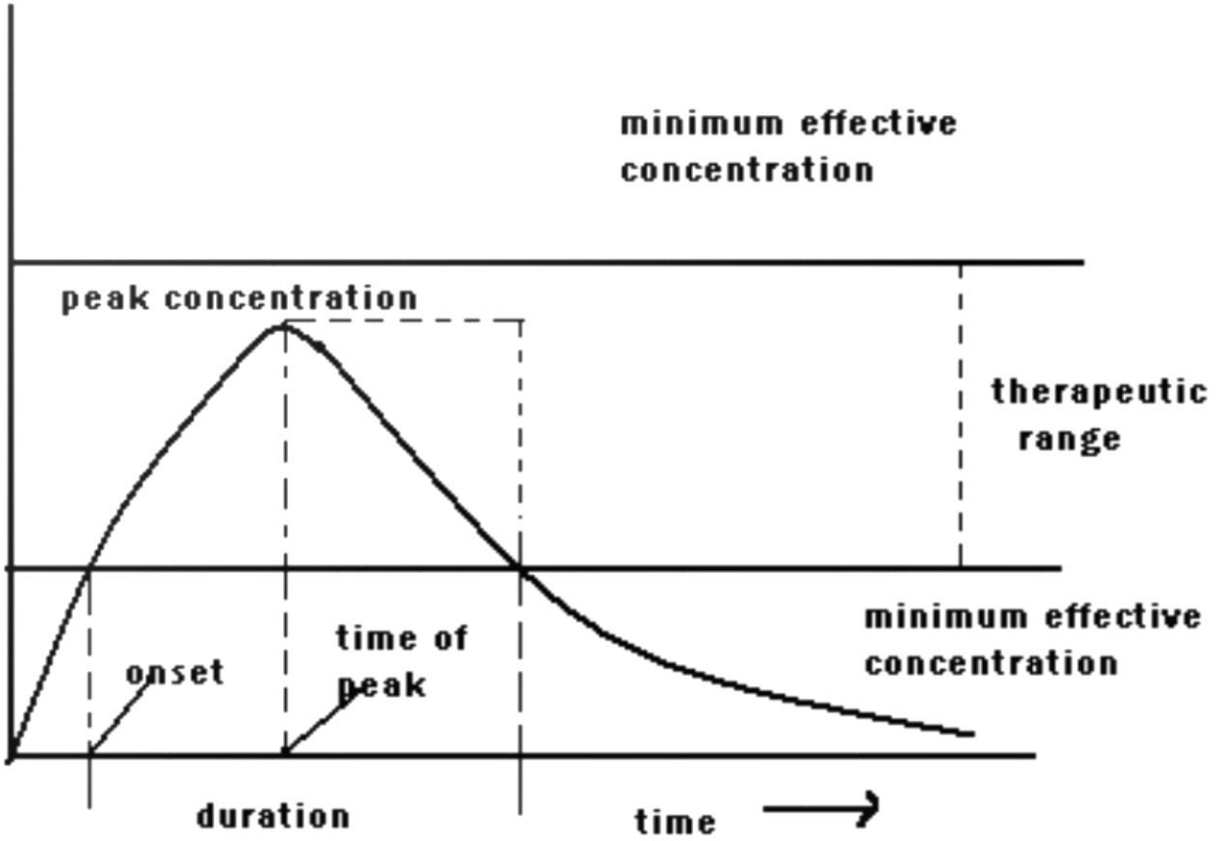


1) Plasma level-time studies:

- Two dosage forms that exhibit super imposable plasma level-time profiles should result in identical therapeutic response.

$$F = \frac{[AUC]oral \times [D]iv}{[AUC]iv \times [D]oral}$$

$$F = \frac{[AUC]test \times [D]std}{[AUC]std \times [D]test}$$



plasma concentration-time curve foll single oral dose
a-b absorption phase of curve
c-d elimination phase of curve

- With single dose study,

  - it requires collection of serial blood samples for a period of 2-3 biological half lives after drug administration, their analysis and plotting plasma-concentration time profile.

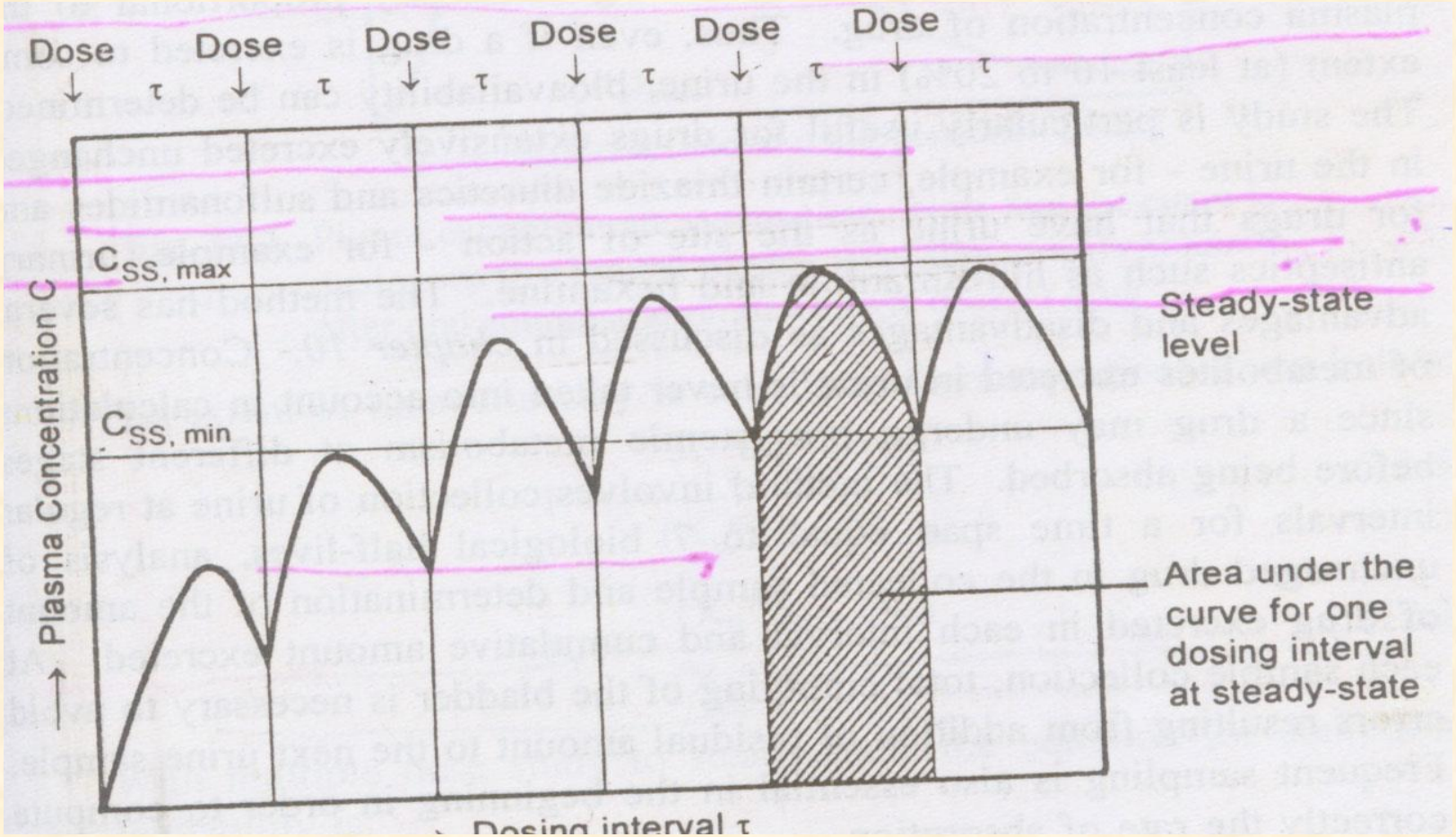
  - with IV dose, sampling should start within 5 minutes of drug administration and subsequent sampling at 15 minutes time interval.

  - at least, 3 sample points should be taken if it fits one compartment model and 5-6 points if it follows two compartment model.

- Important parameters
  - C_{max} - peak plasma concentration
  - t_{max} - time taken to reach peak concentration
    - it indicates rate of absorption
  - AUC - Area Under the plasma level time Curve

    give the measure of extent of absorption

In multiple dose study:



- the method involves drug administration for atleast 5 biological half lives with the dosing interval equal to or greater than the biological half life (i.e. administration of atleast five doses) to reach steady state.

- blood sample should be taken at the end of previous dosing interval and 8 to 10 samples after the administration of next dose.

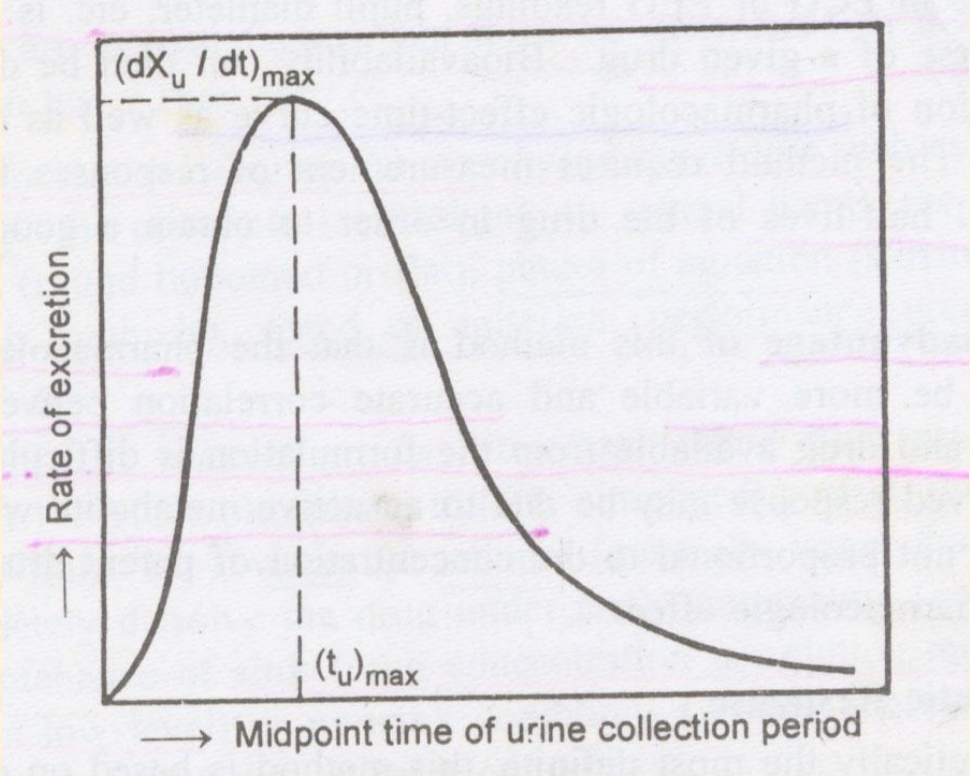
$$F_r = \frac{[AUC]_{test} \times [D]_{std} \times \zeta_{test}}{[AUC]_{std} \times [D]_{test} \times \zeta_{std}}$$

$$F = \frac{[C_{ss,max}]_{test} \times [D]_{std} \times \zeta_{test}}{[C_{ss,max}]_{std} \times [D]_{test} \times \zeta_{std}}$$

Urinary excretion studies :

- **Urinary excretion α plasma concentration of drug**
- Mainly used in drugs extensively excreted unchanged in urine.
  - E.g. Thiazide diuretics
    Sulfonamides
    Urinary antiseptics : nitrofurantoin , Hexamine.

$$F = \frac{[Xu\infty]oral \times D\ iv}{[Xu\infty]iv \times D\ oral}$$



a. **$(dX_u / dt)_{max}$** : Maximum urinary excretion rate

b. **$(t_u)_{max}$** : Time for maximum urinary excretion rate

c. **X_u** : Cumulative amount of drug excreted in the urine.

Biological fluids used for determination of Bioavailability

1. Plasma
2. Urine
3. Saliva
4. CSF
5. Bile

B. Pharmacodynamic methods

1) **Acute Pharmacological Response** :

- Used when pharmacokinetic methods are difficult , inaccurate & non reproducible.

- E.g. Change in ECG/EEG readings.

Pupil diameter

<u>Disadvantages</u> :

- More variable
- Active metabolite interferes with the result.

2) Therapeutic Response :

- measurement of clinical response to a drug formulation given to patients suffering from disease for which it is intended to be used.

Disadvantages :

- Improper quantification of observed response.

DISSOLUTION AND DRUG RELEASE TESTING

DISSOLUTION STUDIES

THEORIES OF DISSOLUTION TESTING

Dissolution is a process in which a solid substance solubilizes in a given solvent *i.e.* mass transfer from solid surface to liquid phase.

- Diffusion layer model/ film theory
- Danckwert's Model (Penetration/ Surface Renewal Theory)
- Interfacial Barrier Model (Double BARRIER OR Limited Solvation Theory)

Diffusion layer model/ film theory

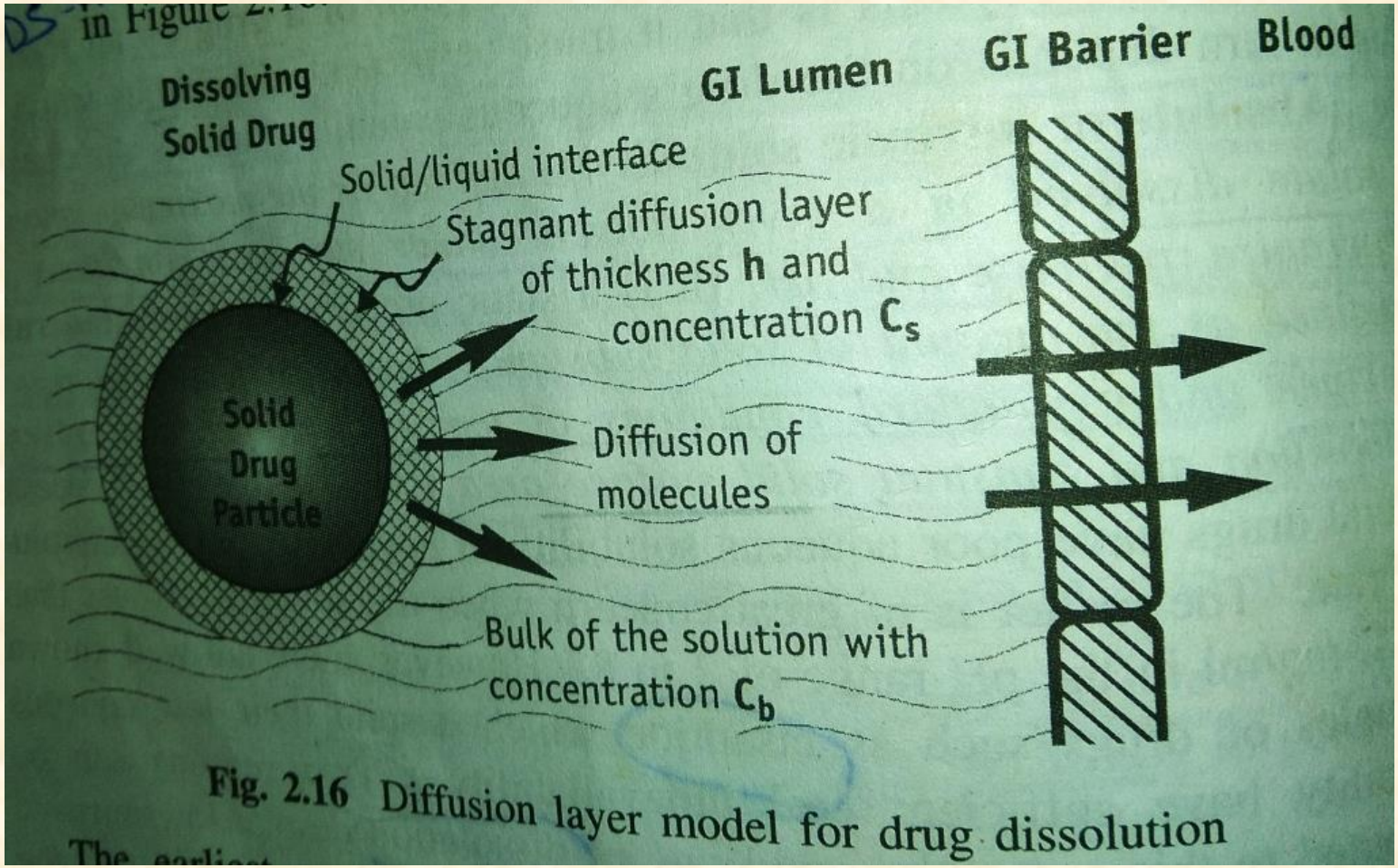


Fig. 2.16 Diffusion layer model for drug dissolution

Diffusion layer model/ film theory

and Brunner incorporated Fick's first law of diffusion and modified the Noyes-Whitney's equation to:

$$\frac{dC}{dt} = \frac{DAK_{w/o}(C_s - C_b)}{Vh} \qquad (2.4)$$

where,

D = diffusion coefficient (*diffusivity*) of the drug

A = surface area of the dissolving solid

$K_{w/o}$ = water/oil partition coefficient of the drug considering the fact that dissolution body fluids are aqueous. Since the rapidity with which a drug dissolves depends on the $K_{w/o}$. It is also called as the **intrinsic dissolution rate constant**. It is a characteristic of drugs.

V = volume of dissolution medium.

h = thickness of the stagnant layer.

$(C_s - C_b)$ = concentration gradient for diffusion of drug.

Danckwert's Model (Penetration/ Surface Renewal Theory)

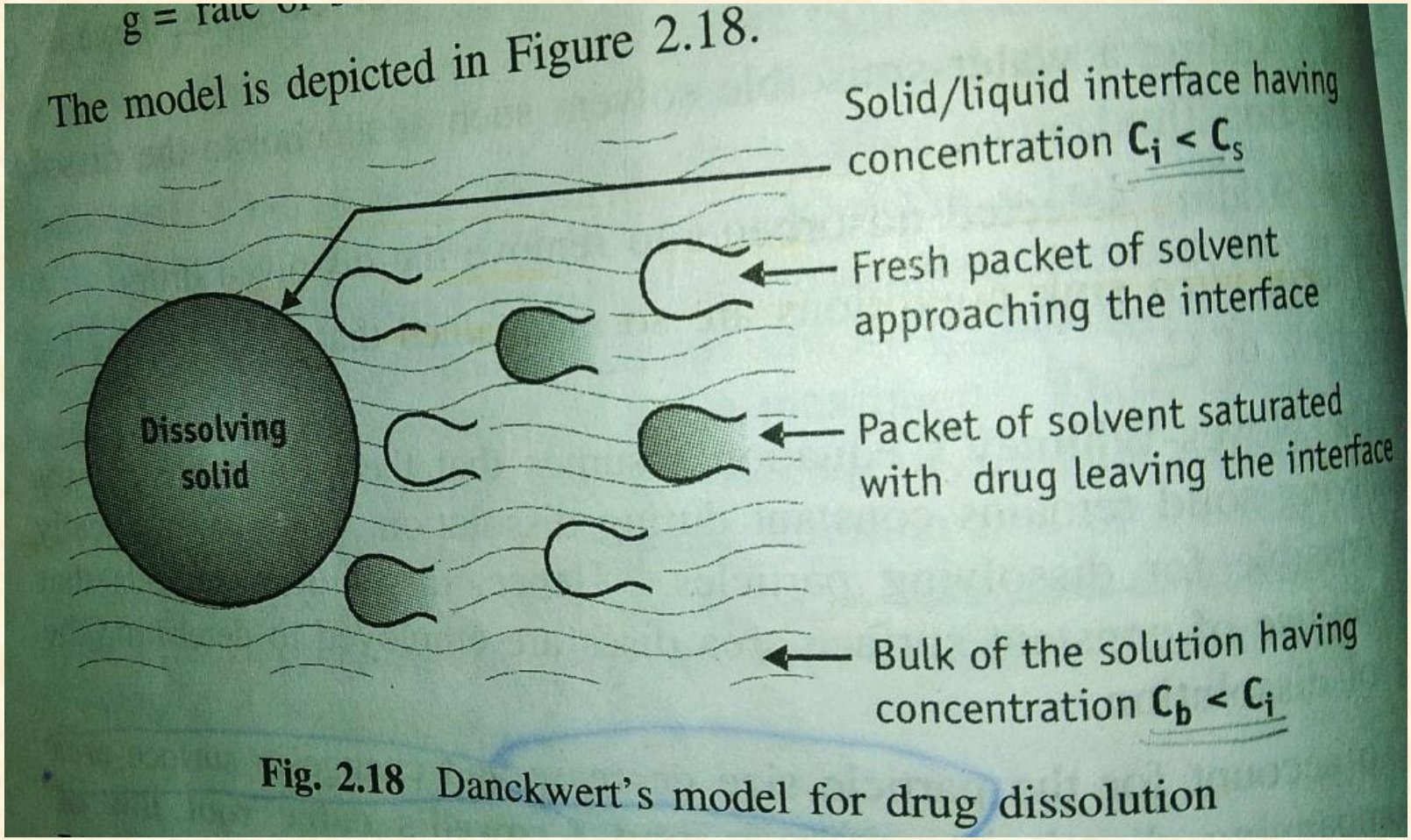


Fig. 2.18 Danckwert's model for drug dissolution

Interfacial Barrier Model (Double BARRIER OR Limited Solvation Theory)

- Accordingly, an intermediate concentration may exist at the interface as a result of solvation mechanism and is the function of solubility rather than diffusion.

  $G = K_I (C_s – C_b)$

Where, G = Dissolution rate per unit area,

K_I = effective interfacial transport constant

Drug dissolution rate & Bioavailability :

- Correlation between Dissolution testing and bioavailability

- **In vivo determination test :**
  - Tool in the development of new dosage form.

- **In vitro dissolution test** :
  - To ensure batch to batch consistency
  - Best available tool which can quantitatively assure about bioavailability.

Dissolution Apparatus

Type	USP	BP
Apparatus 1	rotating basket	Rotating basket
Apparatus 2	paddle	paddle
Apparatus 3	reciprocating cylinder	flow-through cell
Apparatus 4	flow-through cell	
Apparatus 5	paddle over disk	
Apparatus 6	cylinder	
Apparatus 7	reciprocating disk	

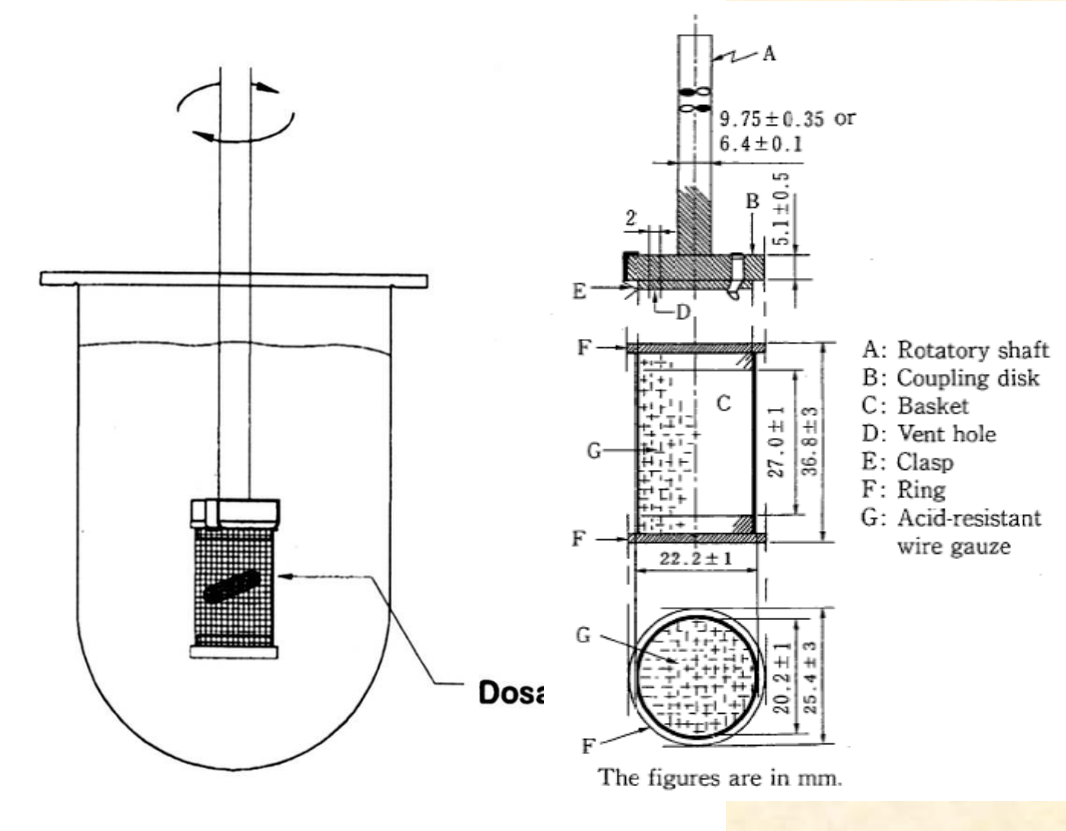
Official Dissolution Apparatus

USP 30 classification

1. Rotating Basket (Ph.Eur./BP/JP)
2. Paddle (Ph.Eur./BP/JP)
3. Reciprocating Cylinder (Ph.Eur.)
4. Flow Through Cell (Ph.Eur./BP/JP)
5. Paddle Over Disk (Ph.Eur.)
6. Rotating Cylinder (Ph.Eur.)
7. Reciprocating Holder

Apparatus 1 - Basket

- **Useful for**
  - capsules
  - beads
  - delayed release / enter coated dosage forms
  - floating dosage forms
  - surfactants in media
- **Standard volume**
  - 900/1000 mL
  - 1, 2, 4 L vessels



Apparatus 1 - Basket

Advantages

- breadth of experience (> 200 monographs)
- can be easily automated which is important for routine work

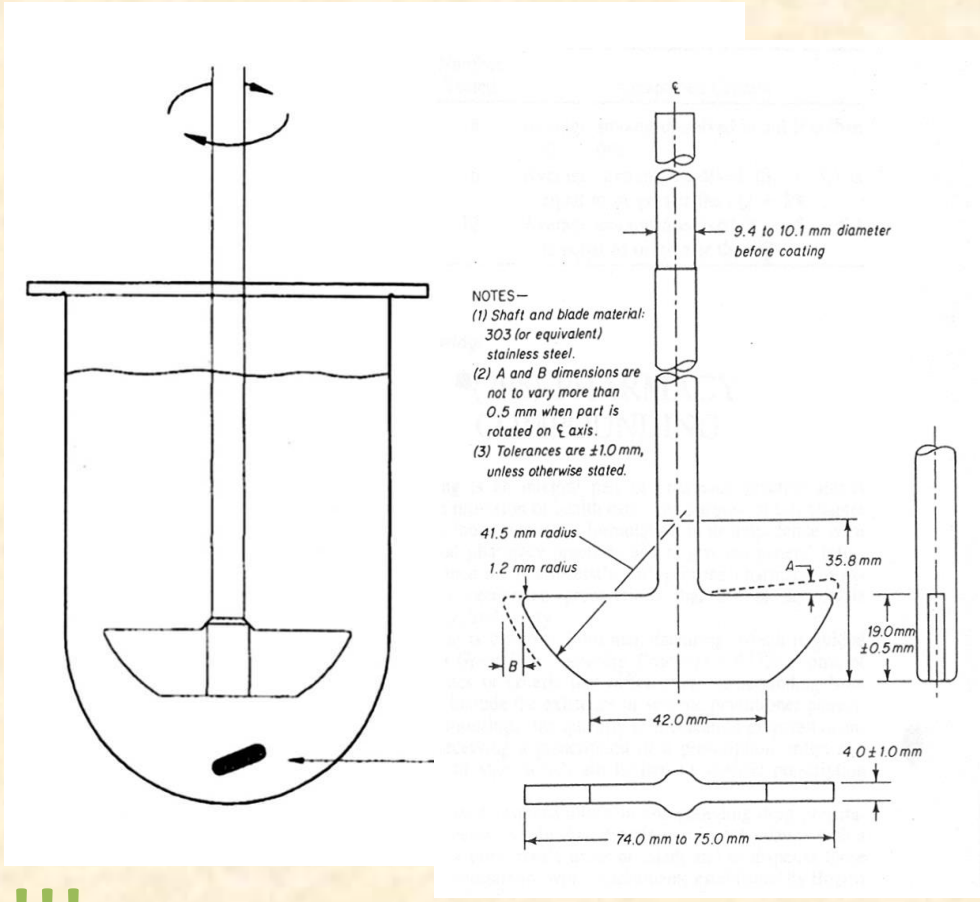
Disadvantages

- disintegration-dissolution interaction
- degassing is particularly important
- limited volume → sink conditions for poorly soluble drugs

Apparatus 1 - Basket

Apparatus 2 - Paddle

- **Useful for**
  - tablets
  - capsules
  - beads
  - delayed release / enteric coated dosage forms
- **Standard volume**
  - 900/1000 ml
- **Method of first choice !!!**



Apparatus 2 - Paddle

Advantages

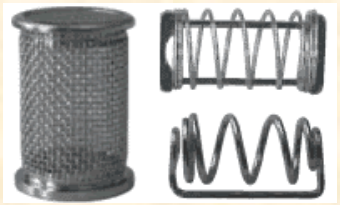
- easy to use
- robust
- can be easily adapted to apparatus 5
- long experience
- can be easily automated which is important for routine investigations

Disadvantages

- pH/media change is difficult
- limited volume → sink conditions for poorly soluble drugs
- hydrodynamics are complex
- coning
- sinkers for floating dosage forms

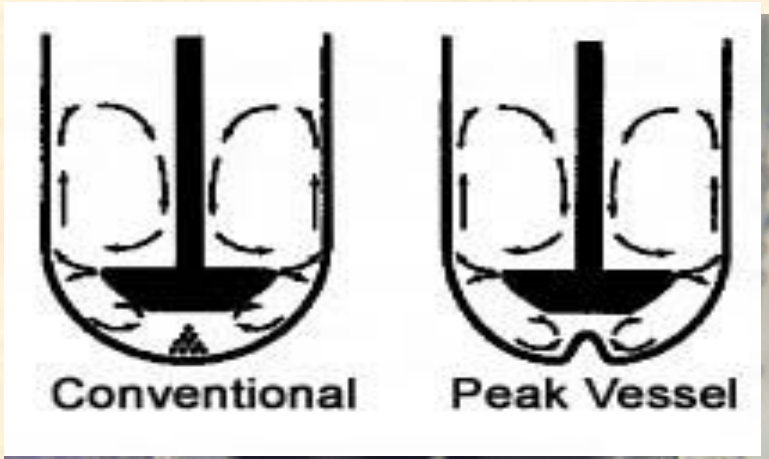
Apparatus 2 - Paddle

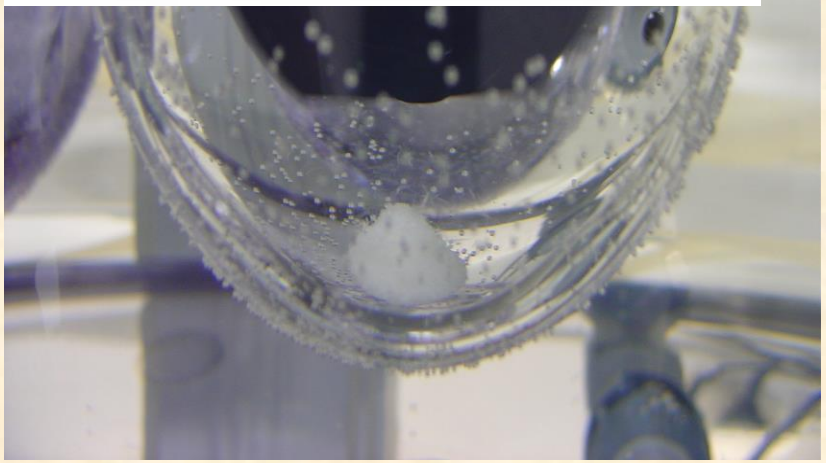
Sinkers



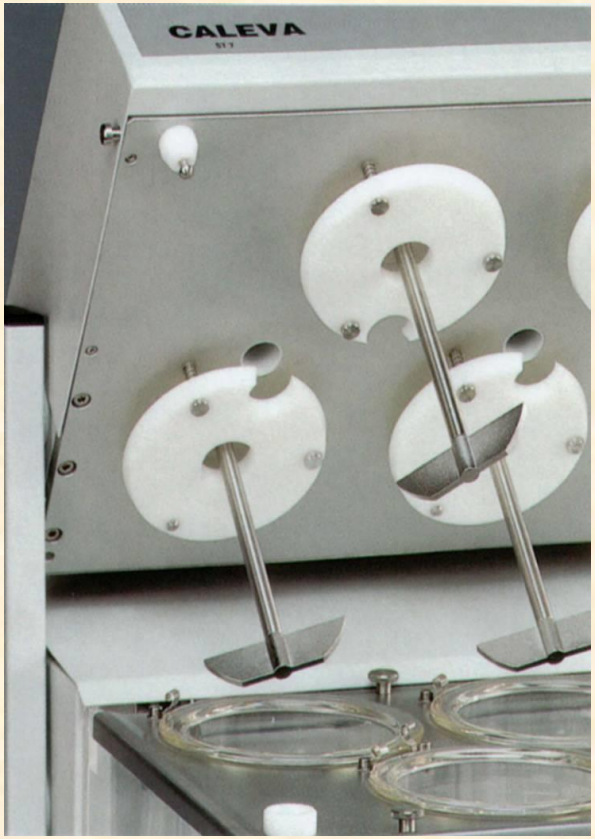
A small loose piece of nonreactive material such as, not more than a few turns of wire helix may be attached to dosage units that would otherwise float.

Coning



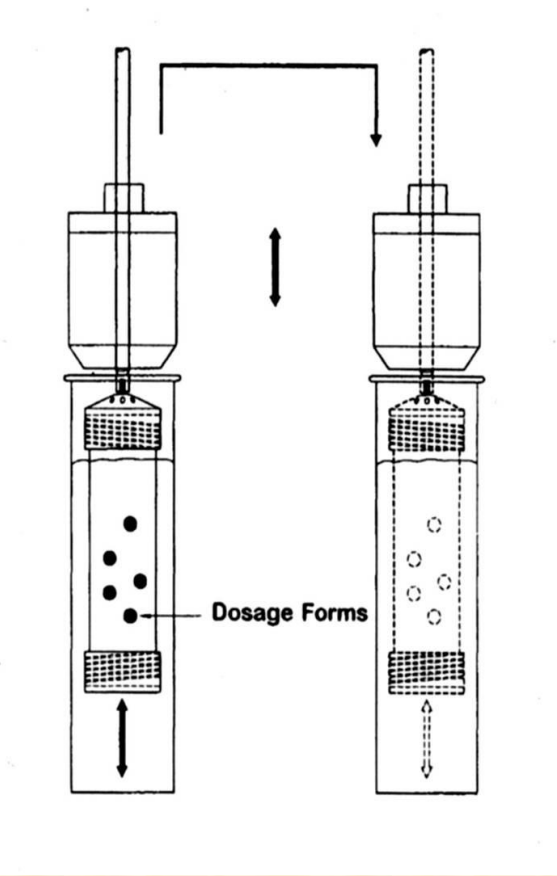


Apparatus 2 - Paddle



Apparatus 3 – Reciprocating Cylinder

- **Useful for**
  - tablets
  - beads
  - controlled release formulations
- **Standard Volume**
  - 200-250 mL per station

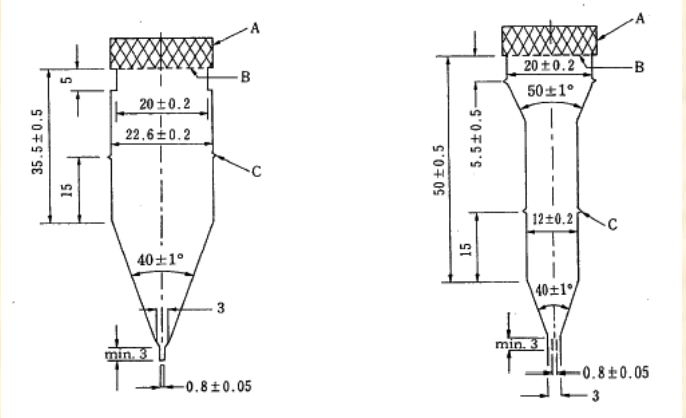


Apparatus 3 – Reciprocating Cylinder

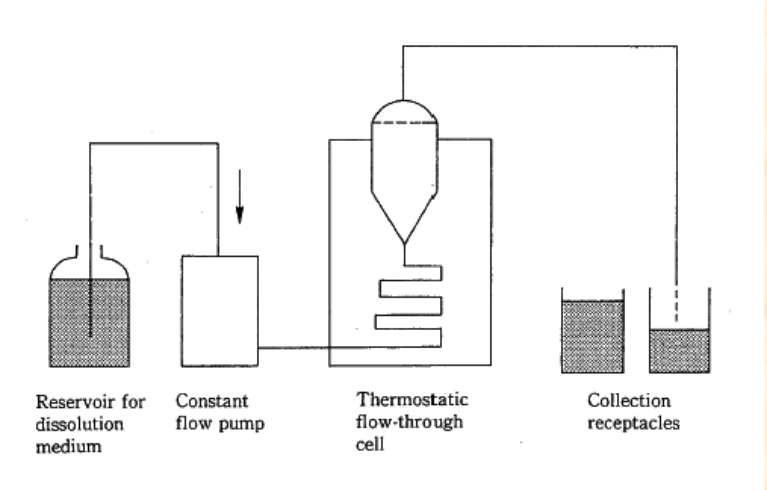
- **Advantages**
  - easy to change the pH
  - hydrodynamics can be directly influenced by varying the dip rate
- **Disadvantages**
  - small volume (max. 250 mL)
  - little experience
  - limited data

Apparatus 4 – Flow-Through Cell

- **Useful for**
  - low solubility drugs
  - microparticulates
  - implants
  - suppositories
  - controlled release formulations

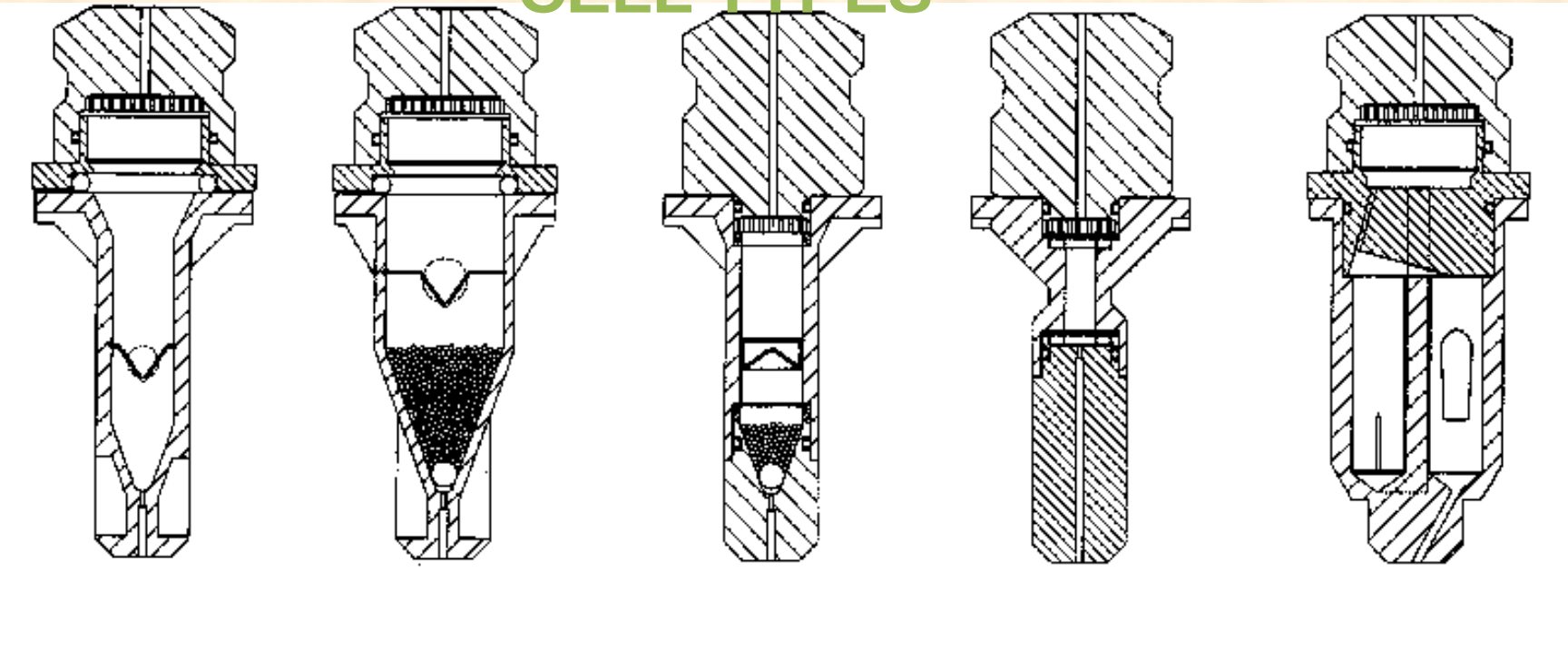


- **Variations**
  - open system
  - closed system



Apparatus 4 – Flow-Through Cell

CELL TYPES



Apparatus 4 – Flow-Through Cell

- **Advantages**
  - easy to change media pH
  - pH-profile possible
  - sink conditions
  - different modes
    a) open system
    b) closed system

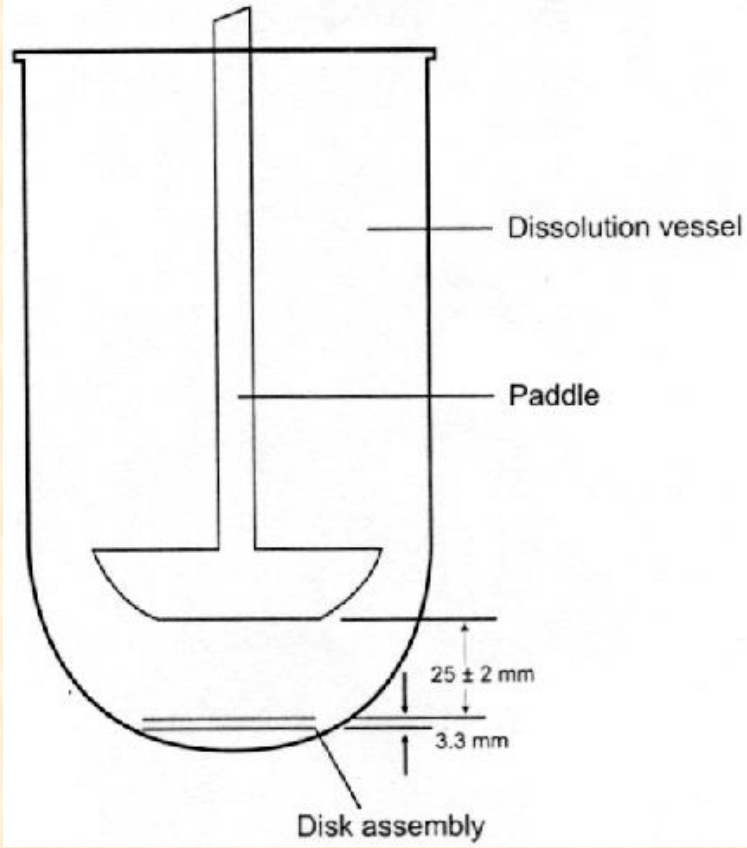
- **Disadvantages**
- Deaeration necessary
- high volumes of media
- labor intensive

Apparatus 5 – Paddle Over Disk

- **Useful for**
- transdermal patches
- **Standard Volume**
- 900 mL

- **Advantages**
- standard equipment (paddle) can be used, only add a stainless steel disk assembly

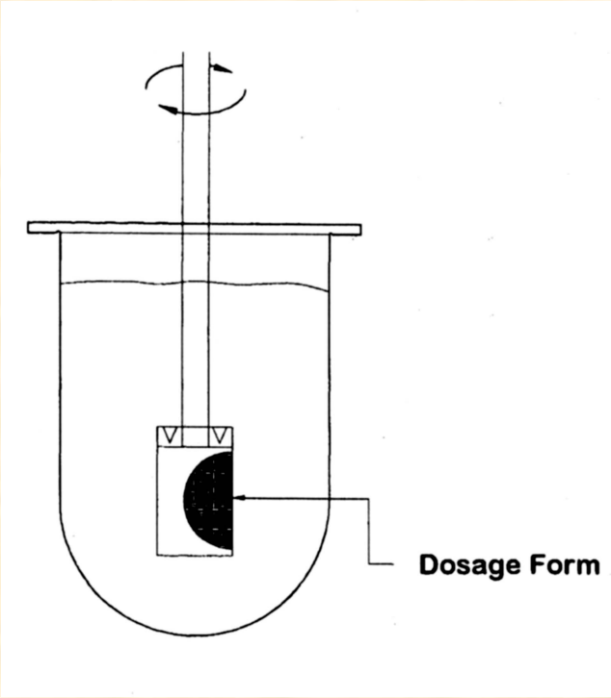
- **Disadvantages**
- disk assembly restricts patch size



Apparatus 6 – Rotating Cylinder

- **Useful for**
- transdermal patches

- Similar to apparatus 1
- Instead of basket, a stainless steel cylinder holds the sample



Apparatus 7 – Reciprocating Holder

- **Useful for**
- Transdermal products
- Non-disintegrating controlled release preparations
- Samples are placed on holders using inert porous cellulosic support.
- It reciprocates vertically at frequency of 30 cycles/sec.
- The test is carried out at 32°C.

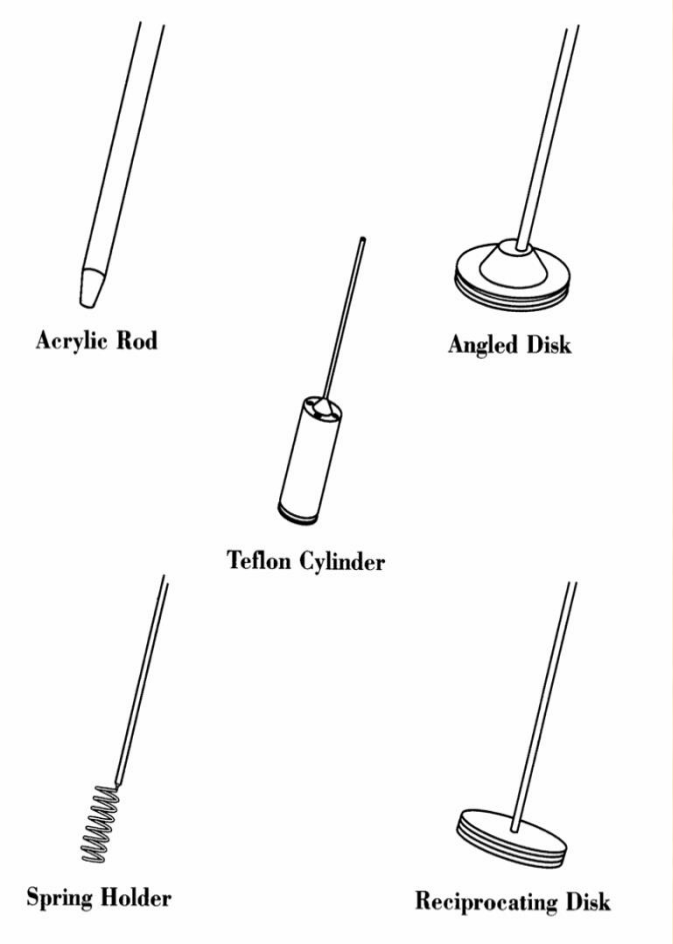


TABLE 11.1

Compendial Dissolution Apparatus Types and Their Applications

Apparatus	*Name*	*Drug Formulation Tested*
Apparatus 1	Rotating basket	Conventional tablets, chewable tablets, controlled-release formulations
Apparatus 2	Rotating paddle	Tablets, orally disintegrating tablets, chewable tablets, capsules, controlled-release products, suspensions
Apparatus 3	Reciprocating cylinder	Controlled-release formulations, chewable tablets
Apparatus 4	Flow-through cell	Formulations containing poorly soluble drugs, powders and granules, microparticles, implants
Apparatus 5	Paddle over disc	Transdermal formulations
Apparatus 6	Cylinder	Transdermal formulations
Apparatus 7	Reciprocating disc	Controlled-release formulations (non-disintegrating oral formulations and transdermal formulations)

TABLE 11.5

Dissolution Acceptance Criteria

Stage	Number 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 twenty four dosage units ($S_1 + S_2 + S_3$) $\geq$ Q% and not more than two dosage units are less than Q-15% and no dosage unit is less than Q-25%

TABLE 11.2

Dissolution Methodology for Immediate-Release Products Based on BCS

BCS Class	*Dissolution Methodology*
I	Single point if NLT 85% Q in 15 minutes Multiple point if Q < 85% in 15 minutes
II	Multiple point
III	Same as class I
IV	Same as class II

Dissolution Media

- Aqueous media is the most preferred.
- 0.1N HCl – to simulate gastric media
- Simulated Intestinal Fluid (SIF)
- Phosphate buffers of various pH
- Fasted State Simulated Intestinal Fluid (FaSSIF)
- Fed State Simulated Intestinal Fluid (FeSSIF)
- TRIS Buffered Saline (TBS)

Selection of Dissolution Media

Class I & Class III

- Simulated gastric fluid (without enzymes)
- Simulated intestinal fluid (without enzymes)

Class II & Class IV

- SGF plus surfactant
- Milk with 3.5%fat
- SIF

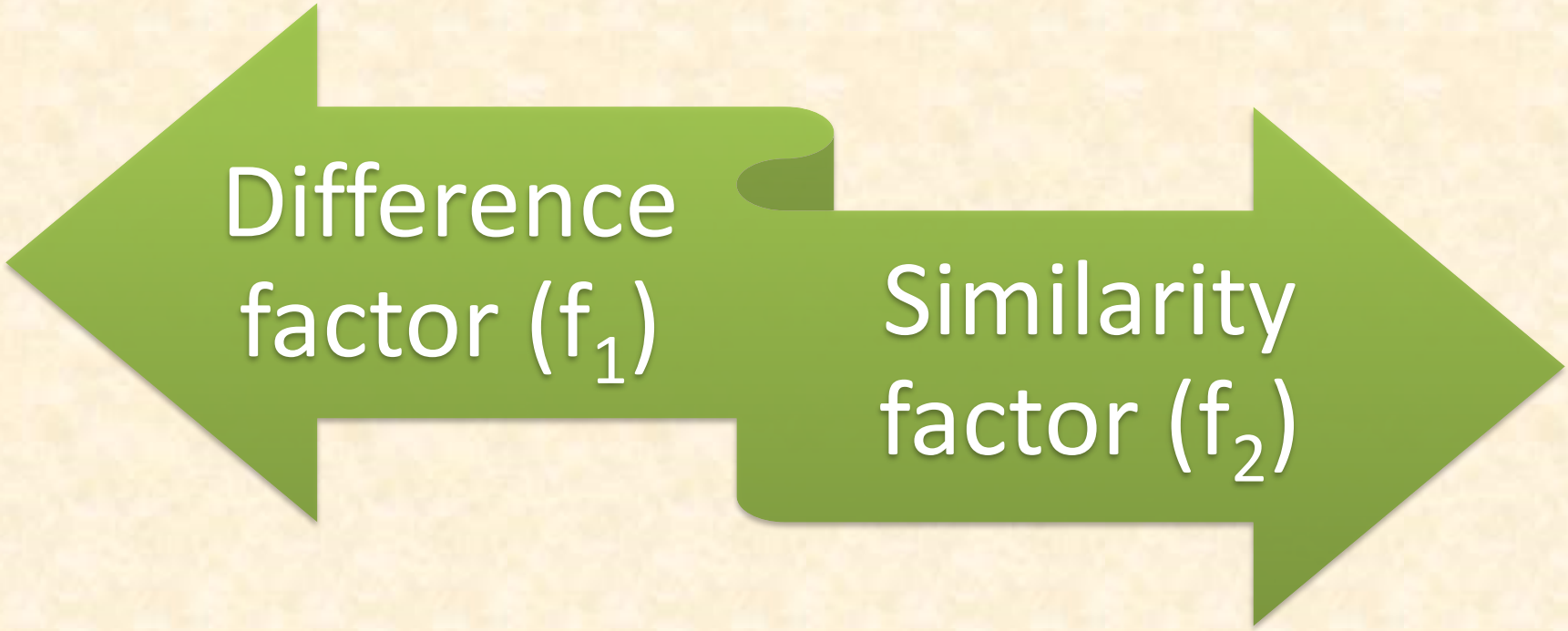
Comparison of Dissolution Profile

A *model-independent mathematical* approach is used to compare the dissolution profile of two products:

- To compare the dissolution profile between T (generic, multisource) product & R (comparator) product in biowaiver conditions
- To compare the dissolution profile between the two strengths of products from a given manufacturer
- For SUPAC after the product is approved

Comparison of Dissolution Profile

To compare the dissolution profile, two factors are determined:



Difference Factor

The difference factor calculates the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves.

$$f_1 = \left\{ \left[\sum_{t=1}^{n} |R_t - T_t| \right] \Big/ \left[\sum_{t=1}^{n} R_t \right] \right\} * 100$$

Where:

R_t: reference assay at time point t

T_t: test assay at time point t

n: is the number of dissolution time points

Difference Factor

f_1 Equation:

- approximates the error between two curves
- % Error is zero when the test & reference profiles are identical
- % Error increases as the dissimilarity between two profiles increases

Similarity Factor

The similarity factor is a logarithmic reciprocal square root transformation of the sum squared error and is a measurement of the similarity in the percent dissolution between the two curves.

$$f_2 = 50 * \log\left\{\left[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} * 100\right\}$$

Where:

R_t: reference assay at time point t

T_t: test assay at time point t

n : is the number of dissolution time points

Similarity Factor

f_2 Equation:

- takes the average sums of square of the difference between the test & reference profiles
- the results fit between 0 & 100
- fit factor is 100 when the profiles are identical
- fit factor approaches zero as the dissimilarity increases

Introduction to BCS

- The Biopharmaceutical Classification System (BCS) is a framework for classifying drug substances based on their aqueous **solubility** and intestinal **permeability.**
- ***G. L. Amidon, Vinod P. Shah, Hans Lennernas,*** and ***John R. Crison*** gave this concept in **1994.**
- The classification system is based on Fick's first law applied to a membrane:

$$J_w = P_w C_w$$

Where,

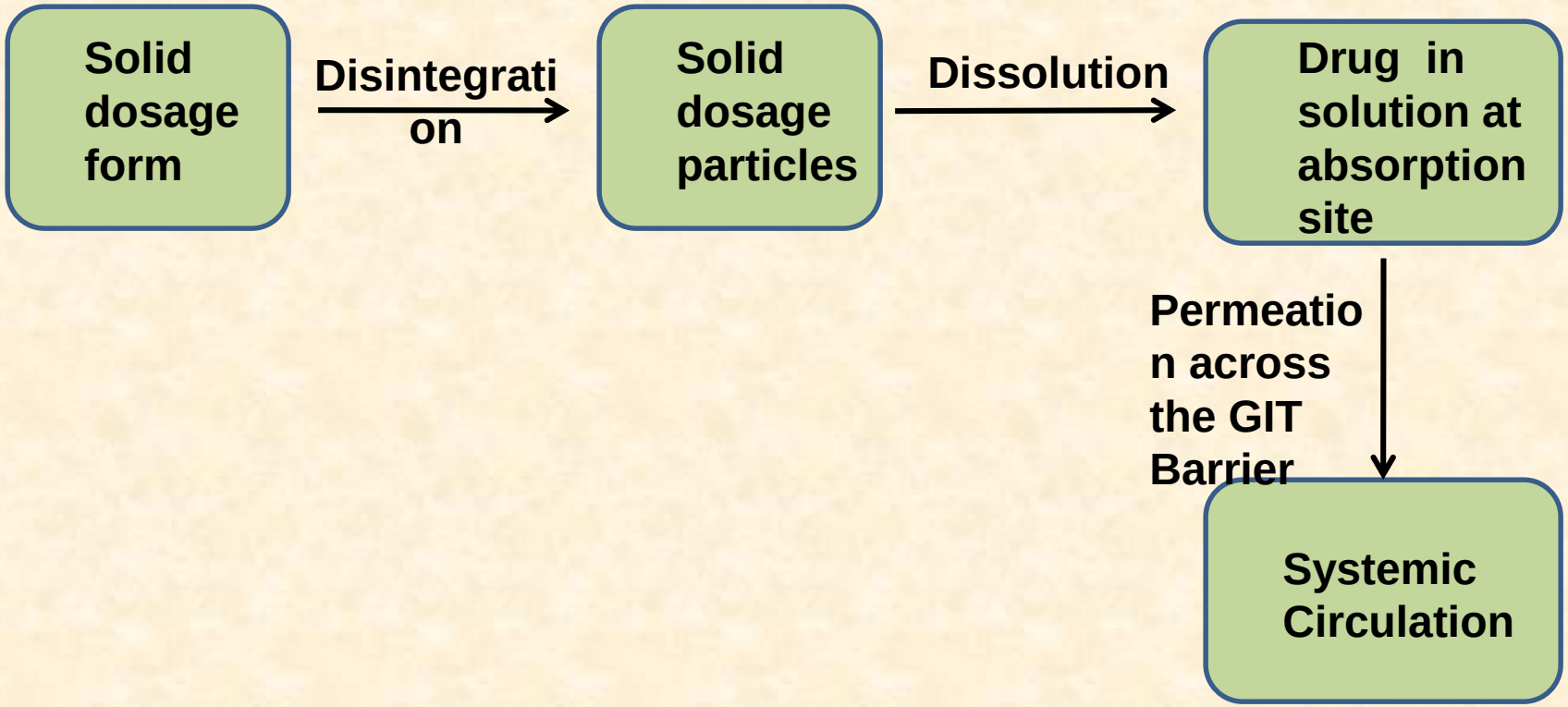
J_w = Drug flux (mass/area/time) through the intestinal wall at any position and time.

P_w = Permeability of membrane

C_w = Drug concentration at membrane

Introduction to BCS

Whenever a dosage form is administerd orally, the events that follow are:



Introduction to BCS

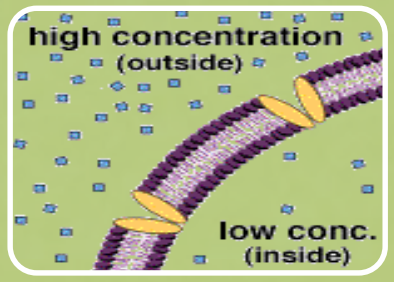
Drug absorbance from a solid dosage form following oral administration depends on:

- Release of drug substance from drug product
- Dissolution of drug under physiological conditions
- Permeability across the GI tract

Boundaries Used in BCS

Highly soluble

A drug substance is considered *highly soluble* when the highest dose strength is soluble in 250mL water over a pH range 1 to 7.5.



Highly permeable

A drug substance is considered *highly permeable* when the extent of absorption in humans is determined to be 90%of an administered dose, based on the mass balance or in comparison to intravenous dose.

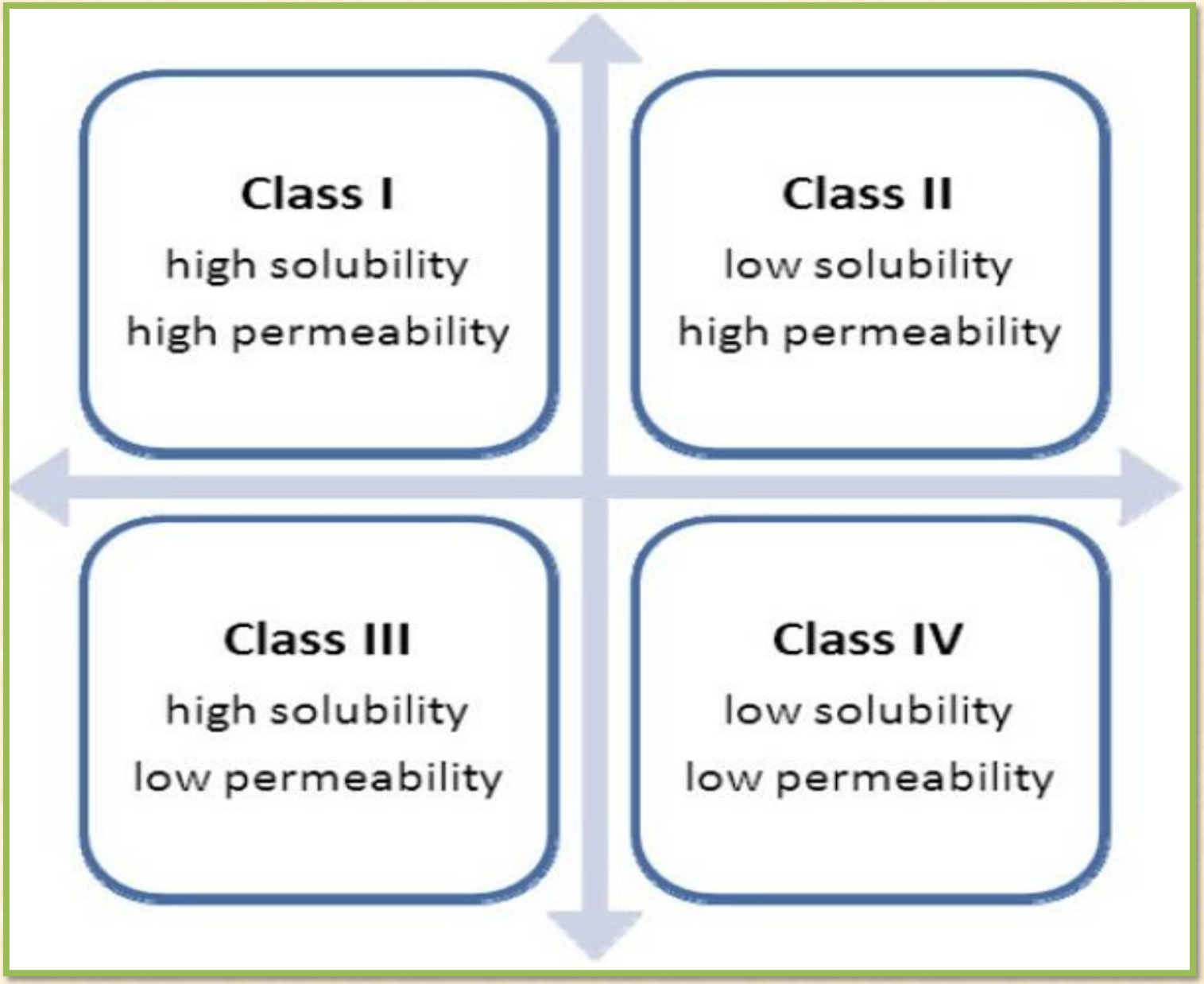
Rapidly dissolving

A drug product is considered to dissolve rapidly when 85% of the labeled amount of drug substance dissolves within 30 minutes, using USP apparatus I or II in a volume of 900mL buffer solution.

- A sufficient number of pH conditions should be evaluated to accurately define the pH solubility profile. These conditions are based on the ionization characteristics of drug. When pka of a drug is in the range of 3-5, solubility is determined as follows:

  pH = pka, pH = pka + 1, pH = pka – 1

  pH = 1, pH = 7.5


Class I
high solubility
high permeability
Class II
low solubility
high permeability
Class III
high solubility
low permeability
Class IV
low solubility
low permeability

Contd…

The BCS additionally proposes 3 dimensionless ratios to classify drug absorption:

- Absorption Number
- Dissolution Number
- Dose Number

Contd…

Absorption Number (A_n)

- Defined as the ratio of the mean residence time of the drug in GIT to the mean absorption time.
- A_n = MRT/MAT
- Ideally A_n>1
- It's the corresponding dimensionless parameter for permeability.
- Lower permeability decreases the ratio.

where,

$$A_n = P_{eff} \times t_{res} / R$$

P_{eff} is the effective permeability,

t_{res} is mean residence time and;

R is the radius of intestinal segment.

Contd…

Dissolution Number (D_n)

- Defined as the ratio of mean residence time to mean dissolution time

$$D_n = t_{res} / t_{Diss}$$

- Ideally, $D_n > 1$
- Inadequate solubility, diffusivity, excessive particle size reduce this ratio
- It's the corresponding dimensionless parameter for dissolution rate

Contd…

Dose Number (D_0)

- Defined as the mass of the drug divided by uptake volume (250 mL) and solubility of drug.
- Ideally D_0 <1 for full dissolution.
- It's the corresponding dimensionless parameter for solubility.

$$D_0 = M_0 / C_s V_0$$

where,

M_0 is dose,

C_s is saturation solubility and;

V_0 is initial gastric volume (≈250ml).

TABLE 11.10

Drug Properties that Determine BCS Classification

Drug property influencing absorption	*Corresponding dimensionless parameter*	*Significance*
Solubility **:** A *drug with high solubility* is the one whose largest dosage strength is soluble in 250 ml or less of water over a pH range of 1-8.	***Dose number*** **:** It is the mass of drug divided by an uptake volume of 250 ml and the drug's solubility.	Ideally, dose ratio should be below 1 if full dissolution is to be possible in principle. Obviously, higher doses will raise the ratio and absorption less likely.
Dissolution rate **:** A *drug product with rapid dissolution* is the one when ≥ 85% of the labelled amount of drug substance dissolves within 30 minutes using USP apparatus 1 or 2 in a volume of ≤ 900 ml buffer solutions.	***Dissolution number*** **:** It is the ratio of mean residence time to mean dissolution time.	Ideally, dissolution number should exceed 1. In the case of solid dosage forms, a combination of inadequate solubility or diffusivity, or excessive particle size or density can increase the time needed for full dissolution and reduce this ratio.
Permeability **:** A *drug with high permeability* is the one having extent of absorption greater than 90% of the administered dose given that the drug is stable in the gastrointestinal environment	***Absorption number*** **:** It is the ratio of the mean residence time of drug in the GIT to the absorption time.	Ideally, absorption number should exceed 1. Longer absorption times resulting from lower permeability will reduce this ratio.

Goals of BCS

- To identify the challenges of formulation Design.
- To guide decisions w.r.t IVIVC.
- To improve the efficiency of drug development and identifying expendable clinical bioequivalence tests.
- To explain when a waiver for *in vivo* bioavailability and bioequivalence may be requested.
- To recommend a class of immediate-release (IR) solid oral dosage forms for which bioequivalence may be assessed based on in vitro dissolution tests.

Class I drugs

- The required dissolution is 85% in 0.1 N hydrochloric acid in 15 minutes. (dissolution is not rate-limiting step).
- Apparatus I at 100 rpm (or apparatus II at 50 rpm) is specified.
- The volume is 900 ml (37 C) or less, in each of the following dissolution media:

  - 0.1 N hydrochoric acid solution or simulated gastric fluid USP without enzyme.

  - pH 4.5 buffer

  - pH 6.8 or simulated intestinal fluid USP without enzymes.

Class I drugs

- The drugs of this class exhibit high absorption number and high dissolution number.
- For those class 1 drugs formulated as IR products, dissolution rate generally exceeds gastric emptying.
- Behave like an oral solution in-vivo.
- The rate-limiting step is gastric emptying.
- These compounds are well absorbed.
- Absorption rate is usually higher than the excretion rate.
- The mean $T_{(50\%)}$ gastric residence time is 15 to 20 minutes under fasting conditions.

  - if dissolution time is lower than gastric emptying time, a drug solution profile with multi-point in several media is recommended.

Class II drugs

- The drugs of this class have a high absorption number but a low dissolution number.
- *In vivo* drug dissolution is then a rate-limiting step for absorption except at a very high dose number.
- The absorption for Class II drugs is usually slower than for Class I and occurs over a longer period of time.
- The bioavailability of these products is limited by their solvation rates.
- Hence, a correlation between the in vivo bioavailability and the in vitro solvation can be found.
- the dissolution profile in multiple media is recommended for drug products of this category.

Class III drugs

- Drug permeability is the rate-limiting step for drug absorption, but the drug is solvated very quickly.
- These drugs exhibit a high variation in the rate and extent of drug absorption.
- Since the dissolution is rapid, the variation is attributable to alteration of physiology and membrane permeability rather than the dosage form factors.
- Limited IVIVC may be possible as it depends on the relative rates of dissolution and intestinal transit.

Class IV drugs

- The drugs of this class are problematic for effective oral administration.
- These compounds have poor bioavailability.
- They are usually not well absorbed through the intestinal mucosa, and a high variability is expected.
- Fortunately, extreme examples of Class IV compounds are the exception rather than the rule, and these are rarely developed and marketed. Nevertheless, several Class IV drugs do exist.

Examples

	High Solubility	Low Solubility
High Permeability	**Class 1** Abacavir Acetaminophen *Acyclovir*[b] *Amiloride*[S,I] Amitryptyline[S,I] Antipyrine *Atropine* **Buspirone**[c] Caffeine *Captopril* Chloroquine[S,I] **Chlorpheniramine** Cyclophosphamide Desipramine **Diazepam** **Diltiazem**[S,I] **Diphenhydramine** Disopyramide **Doxepin** Doxycycline Enalapril Ephedrine Ergonovine Ethambutol Ethinyl Estradiol Fluoxetine[I] Glucose Imipramine[I] **Ketorolac** Ketoprofen Labetolol Levodopa[S] Levofloxacin[S] **Lidocaine**[I] Lomefloxacin **Meperidine** Metoprolol Metronidazole **Midazolam**[S,I] **Minocycline** Misoprostol **Nifedipine**[S] Phenobarbital Phenylalanine Prednisolone **Primaquine**[S] Promazine Propranolol[I] **Quinidine**[S,I] Rosiglitazone Salicylic acid Theophylline Valproic acid **Verapamil**[I] Zidovudine	**Class 2** **Amiodarone**[I] **Atorvastatin**[S, I] **Azithromycin**[S ,I] **Carbamazepine**[S,I] **Carvedilol** Chlorpromazine[I] **Cisapride**[S] *Ciprofloxacin*[S] **Cyclosporine**[S, I] **Danazol** **Dapsone** Diclofenac Diflunisal Digoxin[S] *Erythromycin*[S,I] Flurbiprofen **Glipizide** **Glyburide**[S,I] Griseofulvin Ibuprofen **Indinavir**[S] Indomethacin **Itraconazole**[S,I] **Ketoconazole**[I] **Lansoprazole**[I] **Lovastatin**[S,I] *Mebendazole* Naproxen Nelfinavir[S,I] Ofloxacin Oxaprozin Phenazopyridine Phenytoin[S] Piroxicam Raloxifene[S] **Ritonavir**[S,I] **Saquinavir**[S,I] **Sirolimus**[S] Spironolactone[I] **Tacrolimus**[S,I] Talinolol[S] **Tamoxifen**[I] **Terfenadine**[I] Warfarin

Examples

	High Solubility	Low Solubility
Low Permeability	**Class 3** *Acyclovir* *Amiloride* [S,I] Amoxicillin [S,I] Atenolol *Atropine* Bisphosphonates Bidisomide *Captopril* Cefazolin Cetirizine Cimetidine [S] *Ciprofloxacin* [S] Cloxacillin Dicloxacillin [S] *Erythromycin* [S,I] Famotidine Fexofenadine [S] Folinic acid *Furosemide* Ganciclovir *Hydrochlorothiazide* Lisinopril Metformin *Methotrexate* Nadolol Pravastatin [S] Penicillins Ranitidine [S] Tetracycline Trimethoprim [S] Valsartan Zalcitabine	**Class 4** Amphotericin B Chlorthalidone Chlorothiazide Colistin *Ciprofloxacin* [S] *Furosemide* *Hydrochlorothiazide* *Mebendazole* *Methotrexate* Neomycin

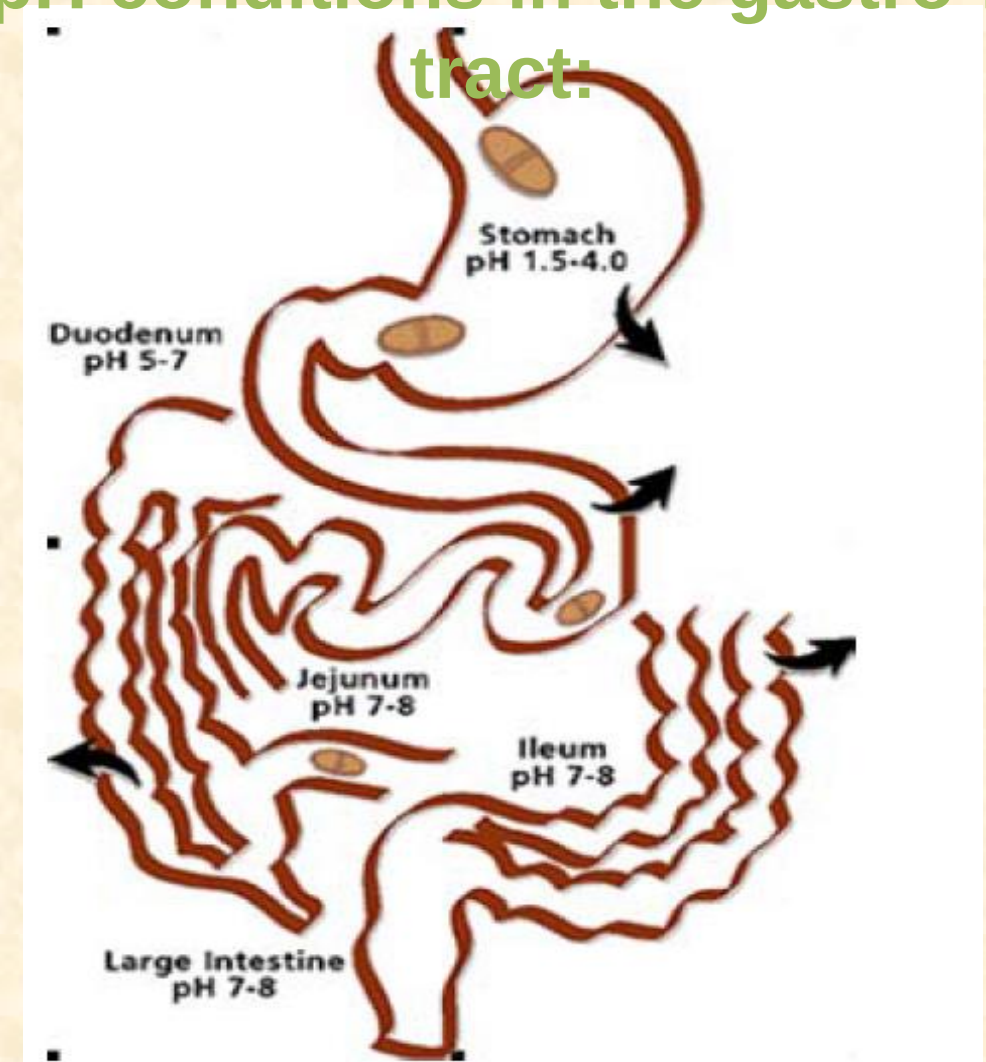
Sub Classes of BCS Class II Drugs

- **Basis-** significant impact of pka on the solubility and dissolution of drugs.
- BCS Class II drug product dissolution *in vitro* as well as *in vivo* is highly dependent on acidic or basic nature of drug.
- Hence, the class II drugs are subclassified as:

Class IIa drugs	Class IIb Drugs	Class IIc Drugs
• Weakly Acidic Drugs • pka ≤ 5	• Weakly Basic Drugs • pka ≥ 6	• Neutral Drugs

Sub Classes of BCS Class II Drugs

Various pH conditions in the gastro-intestinal tract:



Sub Classes of BCS Class II Drugs

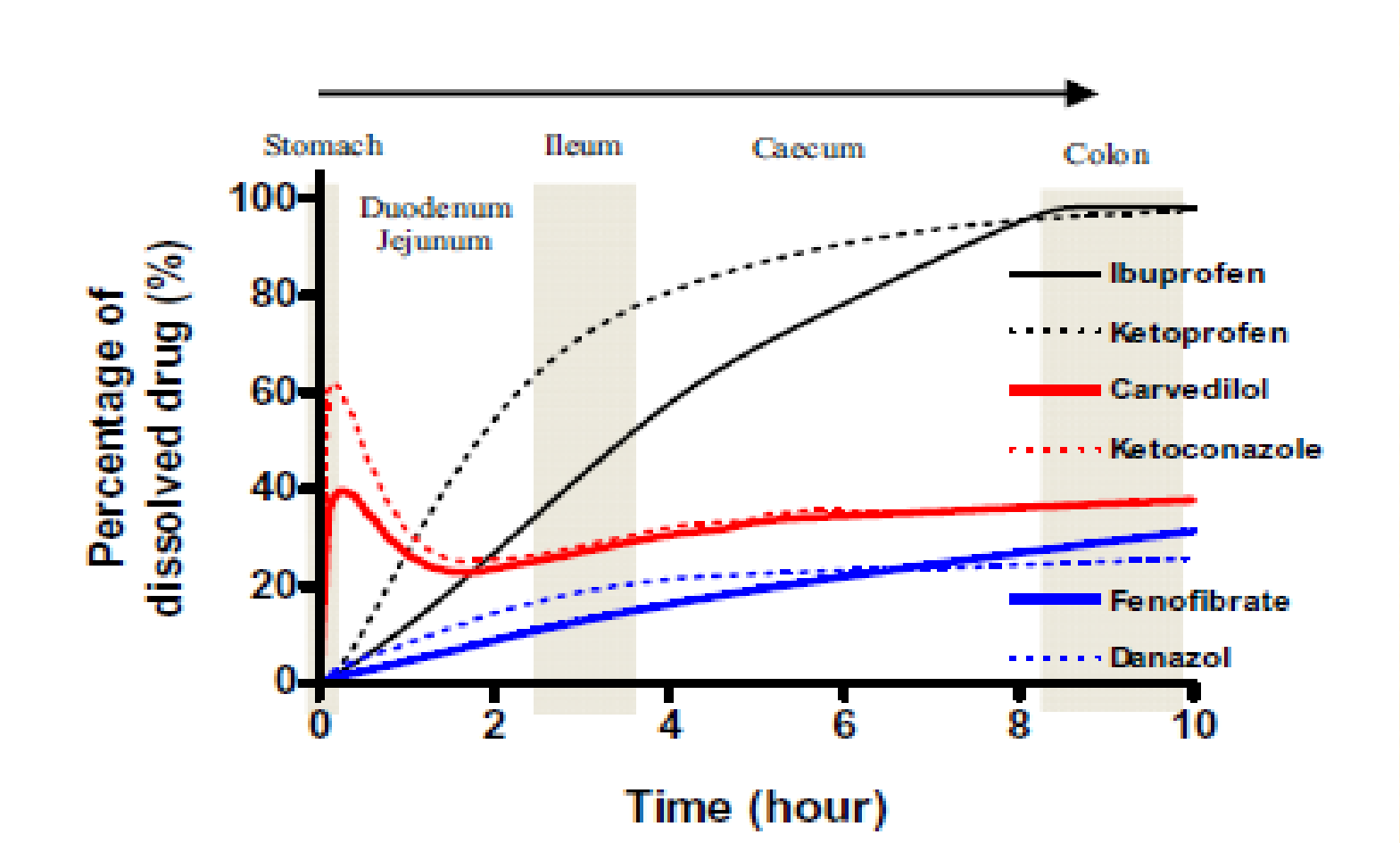
- **Class IIa Drugs**
  - Drugs are insoluble at gastric pH & soluble at intestinal pH
  - At intestinal pH (~6.5), the dissolution would increase upto 100 times
  - Hence, dissolution rate would be faster than gastric emptying rate
  - Thus, these drugs **reflect gastric emptying** and **luminal pH differences.**
  - Examples- ibuprofen and ketoprofen

Sub Classes of BCS Class II Drugs

- **Class IIb Drugs**
  - Exhibit high solubility and dissolution rates at acidic pH in stomach
  - May precipitate in intestinal pH
  - Examples- carvedilol and ketoconazole

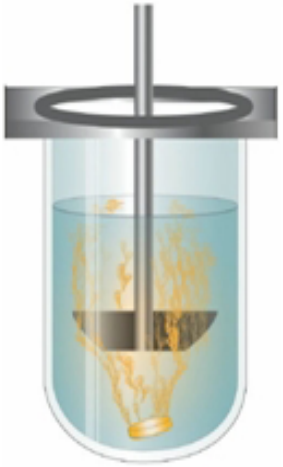
- **Class IIc Drugs**
  - Solubility is not affected by *in vivo* pH change
  - Example- fenofibrate and danazole

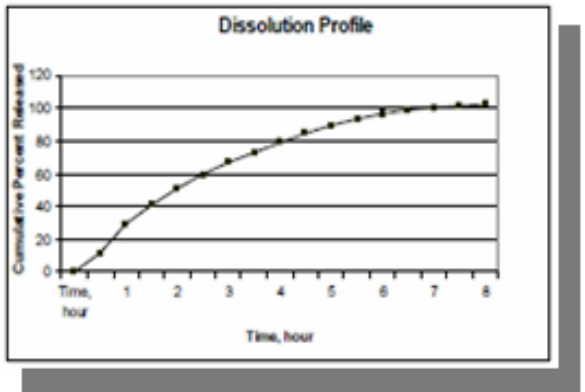
Sub Classes of BCS Class II Drugs



IVIVC

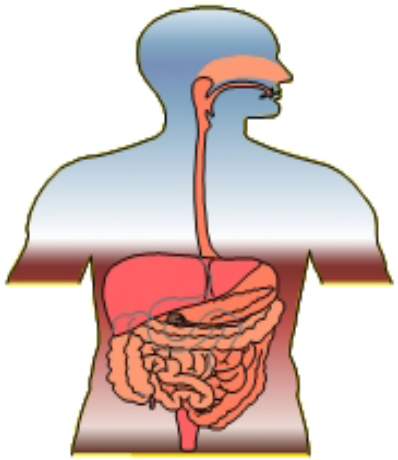
In vitro





IVIVC

In vivo



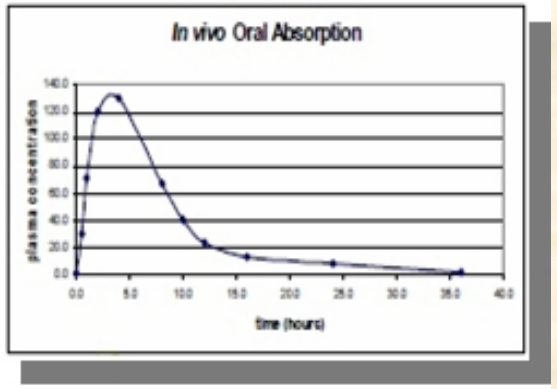


TABLE 11.5

Parameters used for Correlating *In Vitro* Dissolution with Plasma Data

In vitro dissolution parameters	*In vivo plasma data parameters*
Time for specific amount of drug to dissolve (e.g., 50% of the dose)	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	Concentration at time *t*, amount absorbed at time *t*

Concept of IVIVC

- Systemic absorption of drugs is a prerequisite for eliciting their therapeutic activity, whenever given non-instantaneously.
- As per federal guidelines, all the oral dosage forms have to be evaluated for their *in vivo* bioavailability.
- Thus, generic manufacturers must provide detailed bioequivalence evidence showing head-to-head comparative performance of their product against reference.
- Also, development and optimization of a formulation is an time consuming and costly process.

What is Correlation?

- The word Correlation has two different definitions:
  - Mathematical
  - Biopharmaceutical

Mathematically- the word correlation means interdependence between qualitative and quantitative data, or relationship between measurable variable or rank.

From **Biopharmaceutical** point of view, it simply means relationship between observed parameters derived from *in vitro* and *in vivo* studies.

IVIVC - Definition

FDA

- A predictive mathematical model describing the relationship between an in vitro property of dosage form (usually the rate or extent of drug dissolution or release) and a relevant in vivo response, e.g., plasma drug concentration or amount of drug .

USP

- The establishment of a relationship between a biological property or a parameter derived from a biological property (C_{max}, AUC) produced by a dosage form, and a physicochemical characteristic (in vitro release) of the same dosage form.

Establishment of Dissolution Standards

- Dissolution test results depend upon various dissolution test conditions such as pH, volume, ionic strength, deaeration, dissolution medium, surfactants, agitation and temperature.

- Dissolution results may vary with change in dissolution conditions.

- So, establishment of proper dissolution standards reflecting in vivo performance of a drug is important.

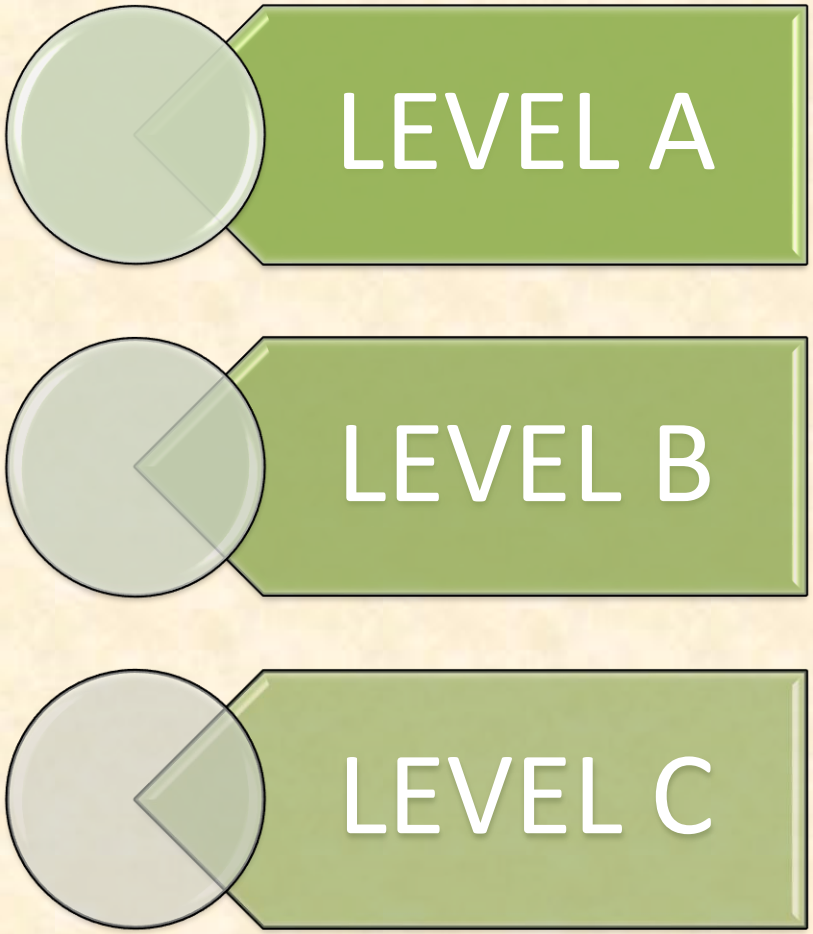
- No single dissolution test conditions can be applied to all drugs.

Dissolution as Surrogate for BA Studies

- If a valid correlation of in vitro dissolution is established with in vivo performance of the formulation then it can be used to:
  - Assess batch to batch consistency
  - Distinguish acceptable and unacceptable i.e. bioequivalent and bioinequivalent drug products
  - Ensure product quality i.e. ability to manufacture the product reproducibly and maintain its release properties throughout shelf-life
  - Provide insight to in vivo behavior of product
  - Guide development of new formulations

LEVELS OF CORRELATION

Based on the ability of the correlation to reflect the complete plasma level profile, which will result from administration of the given dosage form.



LEVEL A CORRELATION

- Highest category of correlation
- Linear correlation
- Superimposable in vitro and in vivo input curve
- Represents point to point correlation between in vitro dissolution time course and in vivo response time course
- Utilizes all the dissolution and plasma level data available to develop correlation
- Most informative and useful from a regulatory perspective

ADVANTAGES

They **reflect the whole curve** because all dissolution and plasma level data points are used.

They are **excellent quality control procedures.**

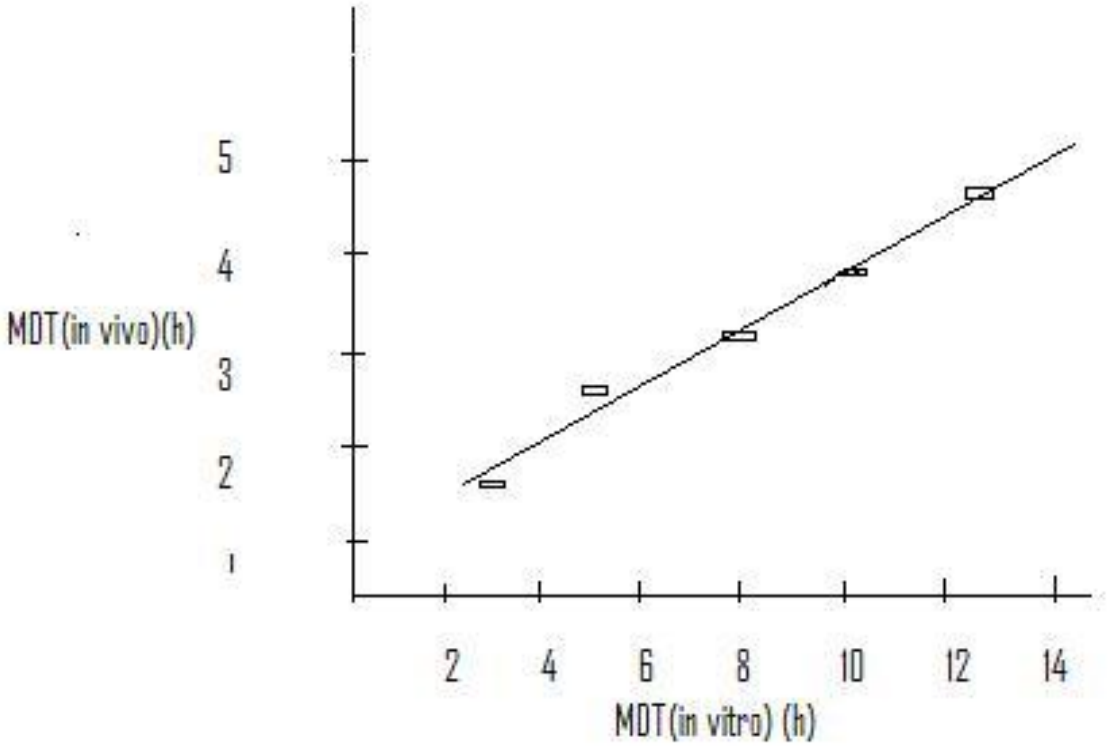
More informative

Very useful from regulatory point of view.

LEVEL B CORRELATION

- The mean in vitro dissolution time is compared either to the mean residence time (MRT) or to the mean in vivo dissolution time.
- Is not a point-to-point correlation
- **Reason** - because a number of different in vivo curves will produce similar mean residence time values.
- One cannot rely upon level B correlation to justify changes in manufacturing or modification in formula.
- Level B correlations are rarely seen in NDAs.

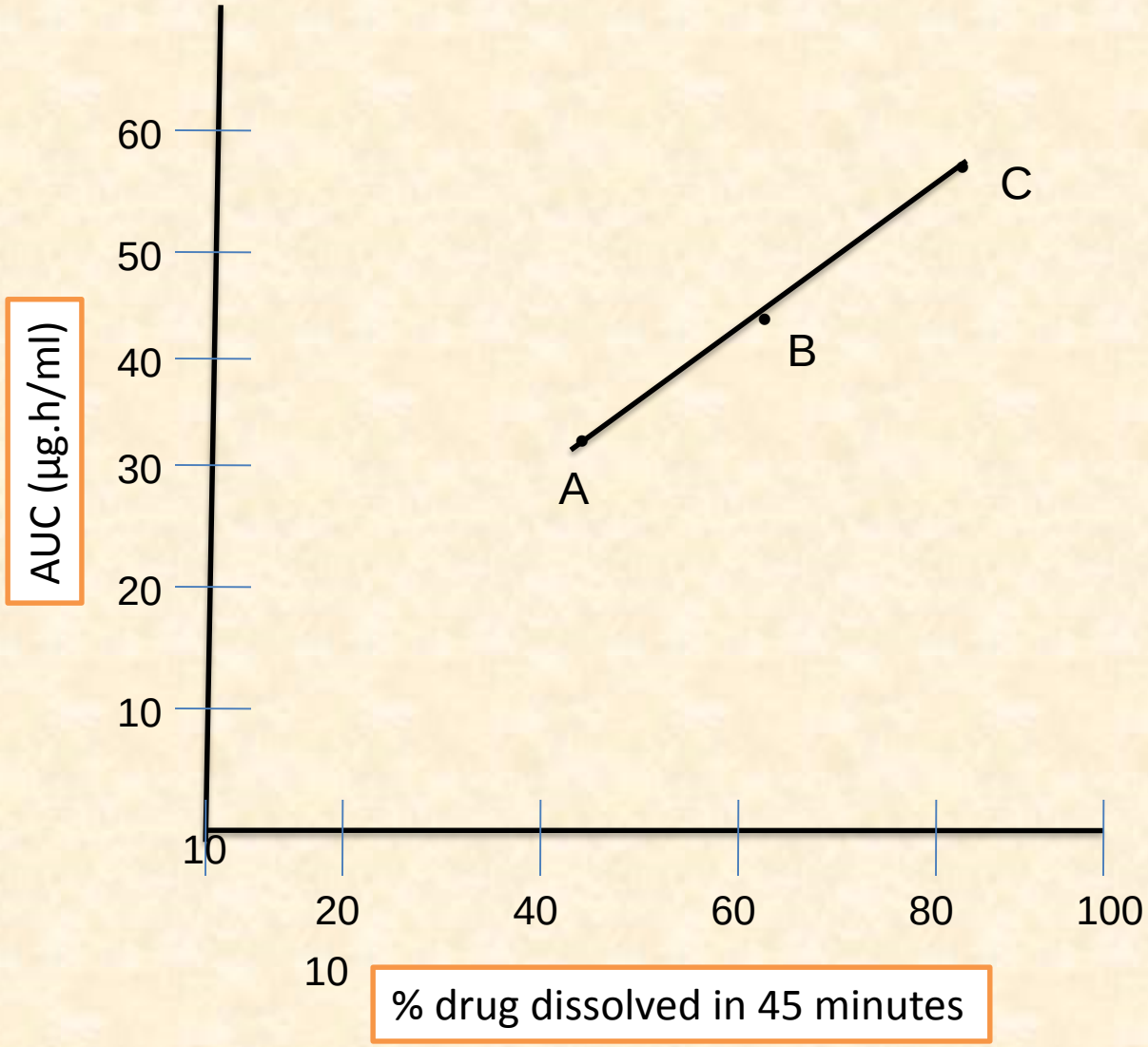
LEVEL B CORRELATION



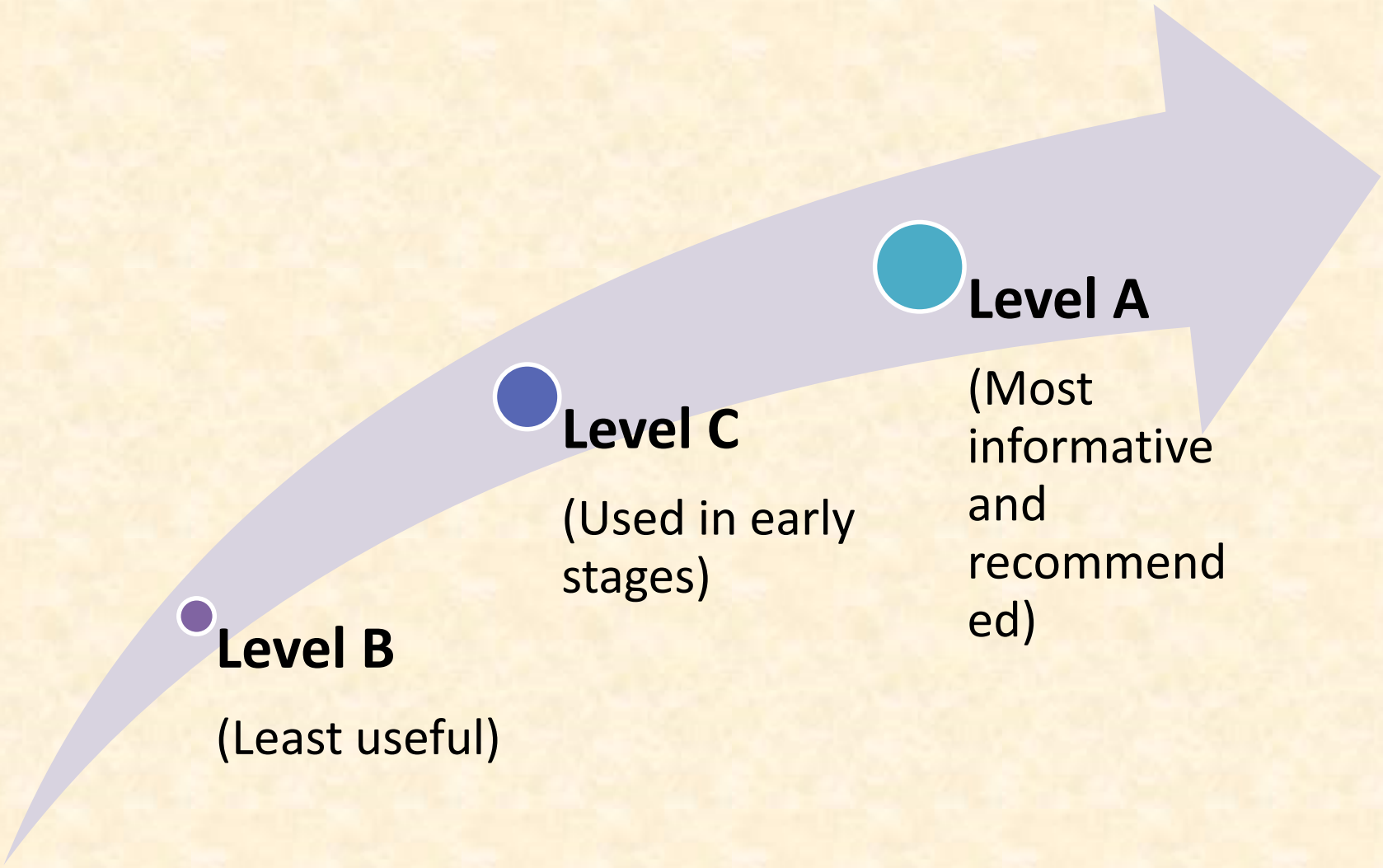
LEVEL C CORRELATION

- One dissolution time point ($t_{50\%}$ $t_{90\%}$ etc.) is compaired to one mean pharmacokinetic parameter such as AUC, T_{max} , C_{max}
- A single point estimation and does not reflect the entire shape of plasma drug concentration curve.
- Can be useful in early stages of formulation development when pilot formulations are being selected
- Biowaiver not possible

LEVEL C CORRELATION



FDA Ranks


Level B
(Least useful)
Level C
(Used in early stages)
Level A
(Most informative and recommended)

IVIVC expectations for immediate release products based on BCS				
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	Case by case	Limited or no IVIVC is expected.

- CONSIDERATIONS IN BIOEQUIVALENCE STUDY DESIGN:

  - *In vitro Vs in vivo* bioequivalence studies

  - Types:

    Completely randomized

    Randomized block design

    Repeated measures, cross-over and carry over design

    Latin square design

  - Bioequivalence Study Design Protocol

  - Statistical Interpretation

BIOEQUIVALENCE

- Definition :

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

- **Pharmaceutical equivalence :**

  "Drug products are considered to be pharmaceutical equivalents if they contain the same active ingredients and are identical in strength or concentration, dosage form, and route of administration."

- **Therapeutic equivalence :**

  "It indicates that two or more drug products that contain the same therapeutically active ingredient, elicit identical pharmacological effects & can control the disease to the same extent".

- **Clinical equivalence:**

  "when the same drug from two or more dosage forms gives identical in vivo effects as measured by a pharmacological response or by control of a symptom or a disease."

In vitro Vs in vivo bioequivalence studies

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

  1. Oral IR products with systemic action:

  - indicated for serious conditions requiring assured response.

  - narrow therapeutic margin.

  - PK complicated by absorption less than 70% or absorption window, nonlinear kinetics, pre-systemic elimination is greater than 70%.

- unfavorable physicochemical properties
- documented evidence for bioavailability problems
- no relevant data available.

2. Non-oral IR products.
3. Modified release products with systemic action.

The following sequence of criteria is useful in assessing the need for *in vitro* studies:

1. the drug product differs only in strength of the active substance it contain, provided all the following conditions hold-

- PK is linear.

- the qualitative composition is the same.

- the ratio between active substance and the excipients is the same, or (in the case of small strengths) the ratio between the excipients is the same.

- both products are produced by the same manufacturer at the same production site.

- a bioavailability or bioequivalence study has been performed with the original product.

- under the same test conditions, the *in vitro* dissolution rate is the same.

2. The drug product has been slightly reformulated or the manufacturing method has been slightly modified by the original manufacturer in ways that can convincingly be argued to be irrelevant for the bioavailability.
3. The drug product meets all the following requirements:

   - the product is in the form of solution or solubilized form.

   - the product contains active ingredient in the same concentration as the approved product.

   - the product contains no excipients known to significantly affect absorption of the active ingredient.

BIOEQUIVALENCE EXPERIMENTAL STUDY DESIGN

1. COMPLETELY RANDOMIZED DESIGNS

Advantages: 1. Easy,

2. it can accommodate any number of treatments and subjects.

Disadvantages: 1. it is best suited for situations in which there are relatively few treatments.

2. all subjects must be as homogenous as possible.

2. RANDOMIZED BLOCK DESIGN

Advantages

1. With effective and systematic way of grouping, it can provide substantially more precise results than a completely randomised design of comparable size.
2. It can accommodate any number of treatments or replications.
3. Different treatments need not have equal sample size.
4. The statistical analysis is relatively simple. The design is easy to construct.
5. If an entire treatment or block needs to be dropped from the analysis for some reason, such as spoiled results, the analysis is not thereby complicated.
6. Variability in experimental units can be deliberately introduced to widen the range of validity of the experimental results without sacrificing the precision of results.

Disadvantages

1. Missing observations within a block require more complex analysis.
2. The degrees of freedom of experimental error are not as large as with a completely randomised design.

3. Repeated mea

3. REPEATED MEASURES, CROSS-OVER AND CARRYOVER DESIGNS

dvantages

1. They provide good precision for comparing treatments because all sources of variability between subjects are excluded from the experimental error.
2. It is economic on subjects. This is particularly important when only a few subjects can be utilized for the experiments.
3. When the interest is in the effects of a treatment over time, it is usually desirable to observe the same subject at different points of time rather than observing different subjects at the specified points of time.

Disadvantages

1. There may be an order-effect, which is connected with the position in the treatment order.
2. There may be a carry-over effect, which is connected with the preceding treatment or treatments.

4. LATIN SQUARE DESIGNS

Advantages

1. It minimizes the inter-subject variability in plasma drug levels.
2. Minimizes the carry-over effects which could occur when a given dosage form influences the bioavailability of a subsequently administered product (intra-subject variability).
3. Minimizes the variations due to time effect.
4. Treatment effects can be studied from a small-scale experiment. This is particularly helpful in preliminary or pilot studies.
5. Makes it possible to focus more on the formulation variables which is the key to success for any bioequivalence study.

Disadvantages

1. The use of Latin square design will lead to a very small number of degrees of freedom for experimental error when only a few treatments are studied. On the other hand, when many treatments are studied, the degrees of freedom for experimental error maybe larger than necessary.
2. The randomisation required is somewhat more complex than that for earlier designs considered.
3. The study takes a long time since an appropriate washout period between two administrations is essential which may be very long if the drug has a long $t_{1/2}$.
4. When the number of formulations to be tested is more, the study becomes more difficult and subject dropout rates are also high.

Latin Square Cross-over Design for 6 (or 12) Subjects to Compare Three Different Formulations, A, B and C

Subject number	*Study period I*	*Washout period*	*Study period II*	*Washout period*	*Study period III*
1, 7	A		B		C
2, 8	B		C		A
3, 9	C		A		B
4, 10	A		C		B
5, 11	C		B		A
6, 12	B		A		C

Elements of Bioequivalence Study Protocol

1. Title
   a. Principal investigator
   b. Project number and date
2. Study objective
3. Study design
   a. Design
   b. Drug Products
      i. Test product(s)
      ii. Reference product
   c. Dosage regimen
   d. Sample collection schedule
   e. Housing
   f. Fasting/meals schedule
   g. Analytical methods
4. Study population
   a. Subjects
   b. Subject selection
      i. Medical history
      ii. Physical examination
      iii. Laboratory tests
   c. Inclusion/exclusion criteria
      i. Inclusion criteria
      ii. Exclusion criteria
   d. Restrictions/prohibitions
5. Clinical procedures
   a. Dosage and drug administration
   b. Biological sampling schedule
   c. Activity of subjects
6. Ethical considerations
   a. Basic principles
   b. Institutional review board
   c. Informed consent
   d. Indications for subject withdrawal
   e. Adverse reactions and emergency procedures
7. Facilities
8. Data analysis
   a. Analytical validation procedure
   b. Statistical treatment of data
9. Drug accountability
10. Appendix

Methods for enhancement of Bioavailability

- Pharmaceutic Approach
- Pharmacokinetic Approach :
- Biologic Approach

Pharmaceutical Approach:

- It involves modification of --formulations, manufacturing process or the physicochemical properties of drug without changing the chemical structure.

- Mainly aimed at **enhancement of dissolution rate (rate limiting step)**.

Pharmacokinetic approach :

- Modification of chemical structure .

Biologic approach :

- Changes in the routes of administration.

Methods to increase effective surface area :

- **Micronization** .

  Methods:

  - spray drying
  - air attrition methods.

  E.g. : Aspirin
  Griseofulvin
  Steroidal compounds
  Sulfa drugs

- **Use of surfactants** :
  1. ‘Surfactants promote wetting & penetration of fluids into solid drug particles.’
  2. Better membrane contact.
  3. Enhanced membrane permeability.
  - Surfactants are used below CMC(critical micelle concentration)
  - E.g. Spironolactone

- **Use of salt forms**:

  E.g. Alkali metal salts of acidic drugs like penicillins

  Strong Acid salt of basic drugs like atropine.

- **Alteration of pH of drug microenvironment:**
  i. In situ salt formation
  ii. Buffered formulation e.g. Aspirin

- **Use of Metastable Polymorphs :**
  - more stable than stable polymorph

  e.g. Chloramphenicol palmitate .

- **Solute-solvent complexation**:

  - Solvates of drugs with organic solvents (pseudo polymorphs) have higher aqueous solubility than their respective hydrates or original drug .

  E.g. 1:2 Griseofulvin – Benzene solvate.

- **Selective adsorption on insoluble carriers :**

  - A highly active adsorbent like inorganic clay e.g. Bentonite, enhance dissolution rate by maintaining concentration gradient at its maximum.

  E.g. Griseofulvin
  Indomethacin
  Prednisone.

- **Solid solution(Molecular dispersion/mixed crystals)** :

  - It is a binary system comprising of solid solute molecularly dispersed in a solid solvent.
  - Systems prepared by Fusion method : **Melts**
  - e.g. Griseofulvin-succinic acid

- **Solid dispersions (Co evaporators/co precipitates)** :

  - Both the solute and solid carrier solvent dissolved in common volatile liquid e.g. Alcohol
  - The drug is precipitated out in an amorphous form as compared to crystalline forms in solid solutions/eutectics.

  E.g. Amorphous sulfathiazole in crystalline urea.

- **<u>Molecular encapsulation with Cyclodextrins</u>** :

  -β and γ Cyclodextrins have ability to form inclusion complexes with hydrophobic drug having poor aqueous solubility.

  - These molecules have inside hydrophobic cavity to accommodate lipophilic drug , outside is hydrophilic.

  E.g. Thiazide diuretics

  Barbiturates

  Benzodiazepines

  NSAIDS.

Bioavailability enhancement through enhancement of drug permeability across membrane

- 1. Lipid Technologies

  a. Physicochemical approach

  b. Physiological approach

  - enhancement of luminal solubility by stimulation of secretions of bile salts, endogenous biliary lipids including phospholipids and cholesterol which together form mixed micelles and facilitated GI solubilization of drug.

  - Reduction in gastric emptying rate thereby increasing the time available for dissolution and absorption.

  - increased intestinal membrane permeability.

  - increased intestinal blood flow.

  - decreased luminal degradation.

- 2. Ion Pairing
- 3. Penetration Enhancers

Bioavailability enhancement through enhancement of drug stability

- Enteric Coating
- Complexation
- Use of metabolic inhibitors

Bioavailability enhancement through gastrointestinal retention

Significance of Bioavailability

- Drugs having low therapeutic index, e.g. cardiac glycosides, quinidine, phenytoin etc.and narrow margin of safety (e.g. antiarrythmics, antidiabetics, adrenal steroids, theophylline)

- Drugs whose peak levels are required for the effect of drugs, e.g. phenytoin, phenobarbitone, primidone, sodium valporate, anti-hypertensives,antidiabetics and antibiotics.

- Drugs that are absorbed by an active transport,e.g. amino acid analogues. Purine analogues etc.

- Drugs which are disintegrated in the alimentary canal and liver,e.g.chiorpromazine etc. or those which under go first pass metabolism.
- Formulations that give sustained release of drug.
- Any new formulation has to be tested for its bioavailability profile.
- Drugs with steep dose response relationship i.e. drugs obeying zero order kinetics / mixed order elimination kinetics (e.g. warfarin , phenytoin, digoxin, aspirin at high doses, phenylbutazone)

Limitations of BA/BE studies :

- Difficult for drugs with a long elimination half life.
- Highly variable drugs may require a far greater number of subjects
- Drugs that are administered by routes other than the oral route drugs/dosage forms that are intended for local effects have minimal systemic bioavailability.

  E.g. ophthalmic, dermal, intranasal and inhalation drug products.
- Biotransformation of drugs make it difficult to

  evaluate the bioequivalence of such drugs:e.g. stereoisomerism