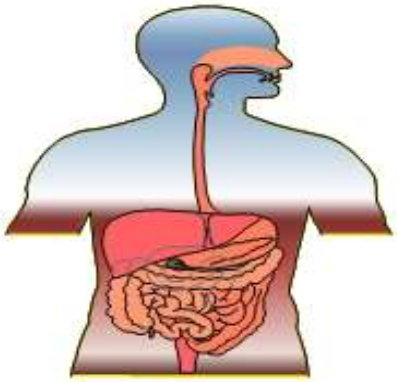
https://www.slideshare.net/jaspreetguraya/in-vitro-in-vivo-correlation-ivivc

IN VITRO IN VIVO CORRELATION (IVIVC)

In vitro

IVIVC

In vivo



Presented By: Harneet Kaur, Jaspreet Singh

Contents

- Introduction to BCS
- Classification
- Determination of Solubility, Permeability & Dissolution
- Comparison of Dissolution Profile
- Limitations of BCS
- Extensions To BCS
- IVIVC and its Levels
- Predictability
- IVIVR, IVIVM and IVIVP
- IVIVC for parenterals
- Applications of IVIVC
- Softwares used for IVIVC

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,

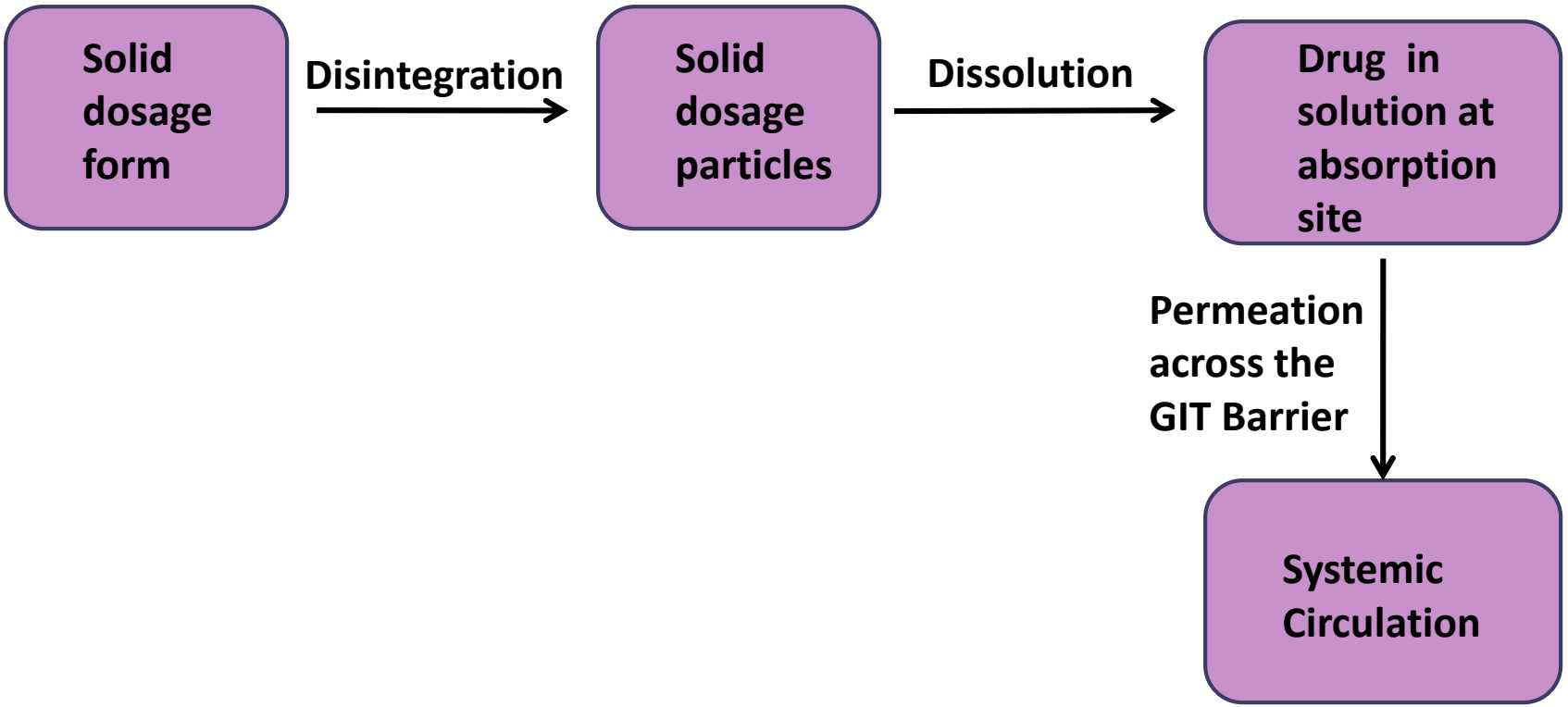
J_w = Drug flux (mass/area/time) through the intestinal wall at any position and time.

P_w = Permeability of membrane

C_w = Drug conc. 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

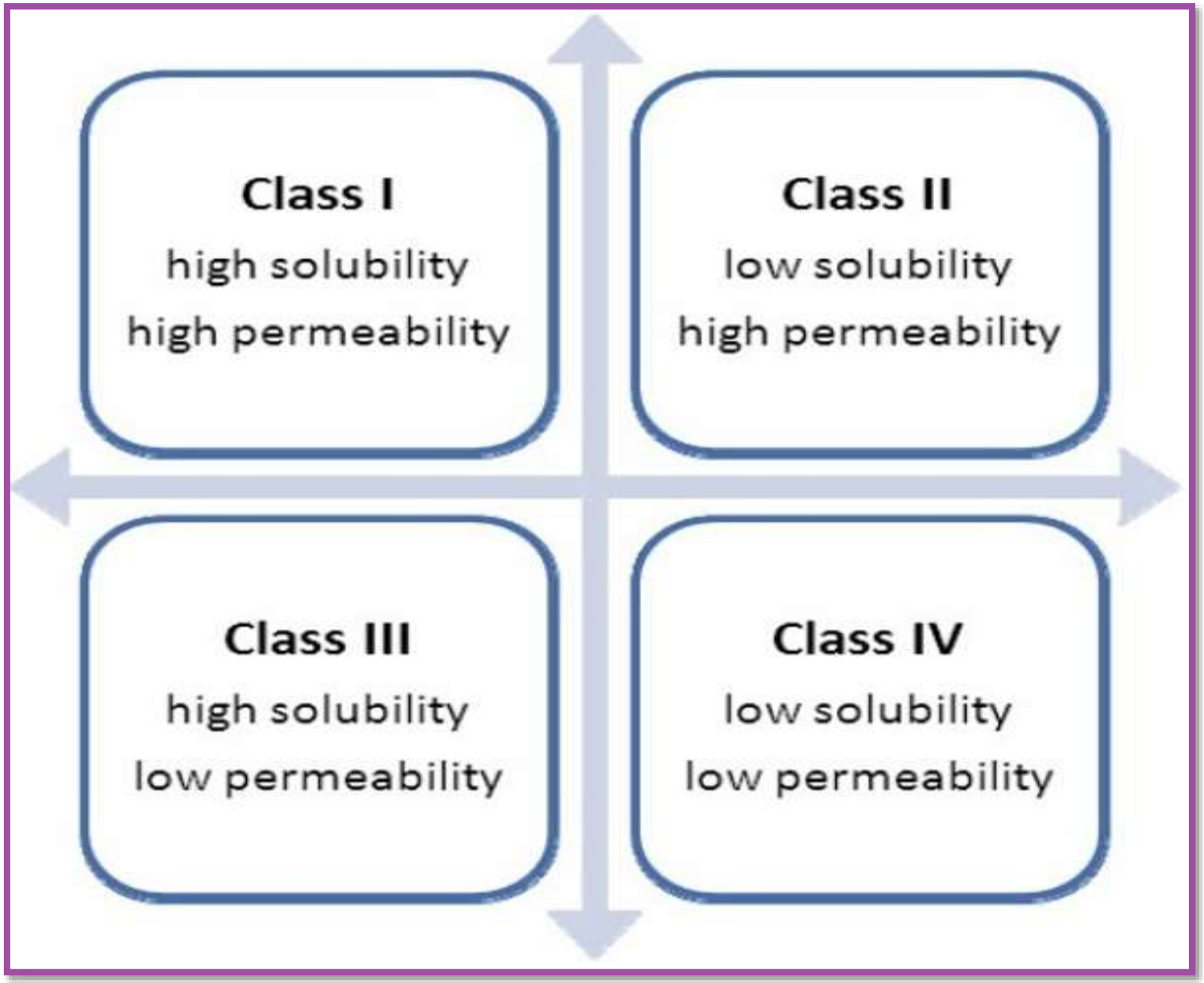
A drug substance is considered *highly soluble* when the highest dose strength is soluble in 250mL water over a pH range 1 to 7.5.

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.


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.

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

where,

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 an of uptake volume (250 mL) and solubility of drug.
- Ideally D_0 <1 for full dissolution in principle .
- 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).

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 assist in QC in SUPAC.
- 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 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.

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.

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.

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

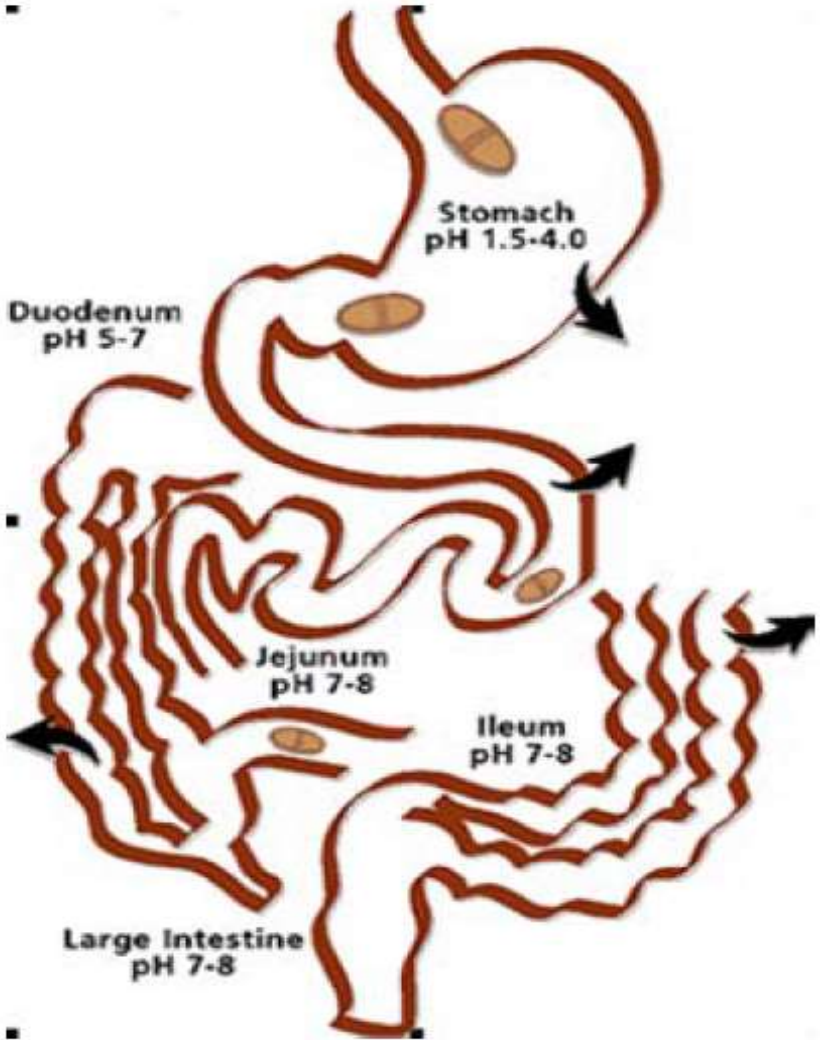
Sub Classes of BCS Class II Drugs

- **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

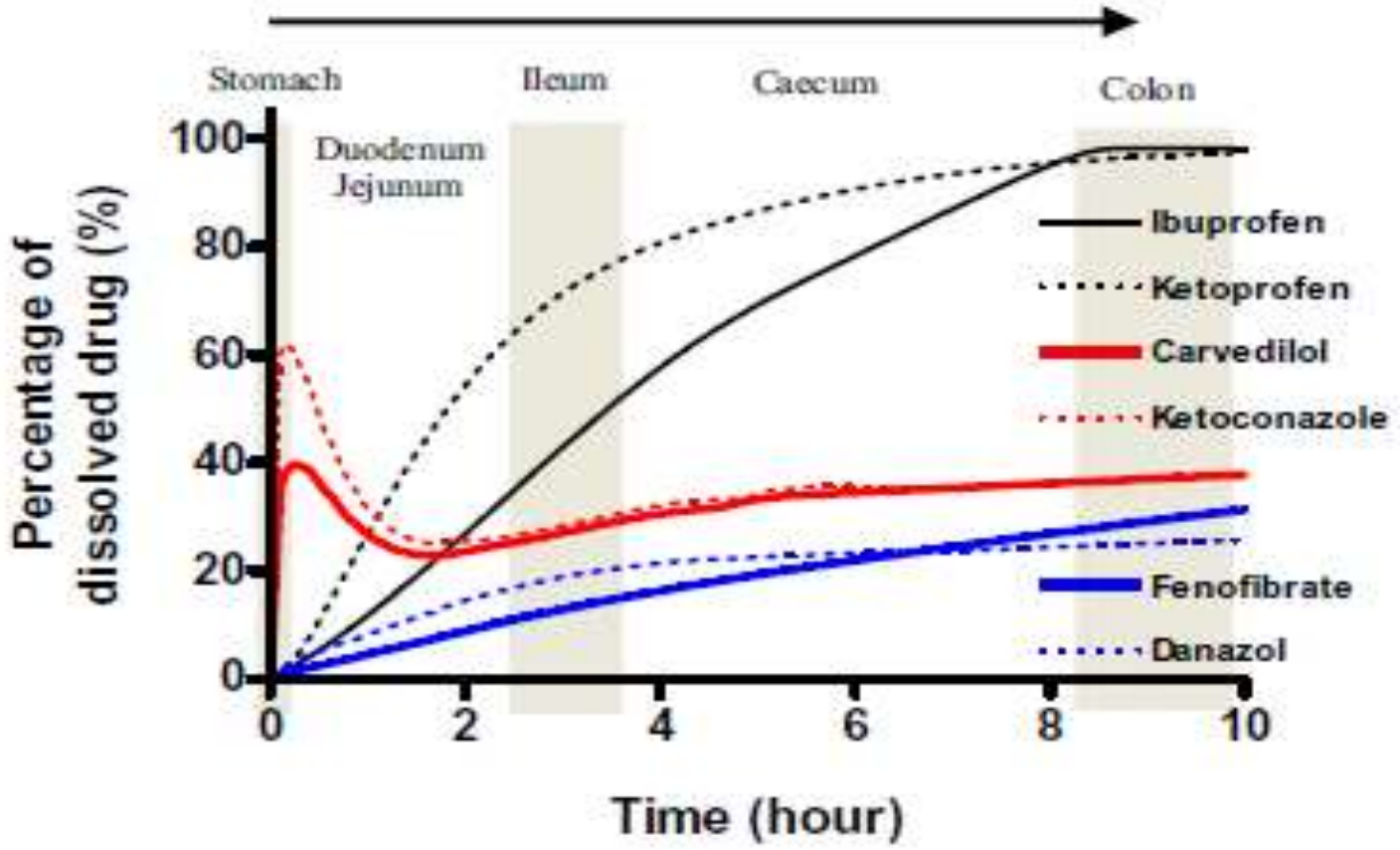
- **Class IIa Drugs**
  - Drugs are insoluble at gastric pH & soluble at intestinal pH
  - At intestinal pH (~6.5), the dissolution would increase upto 100 times
  - Hence, dissolution rate would be faster than gastric emptying rate
  - Thus, these drugs **reflect gastric emptying** and **luminal pH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

Sub Classes of BCS Class II Drugs

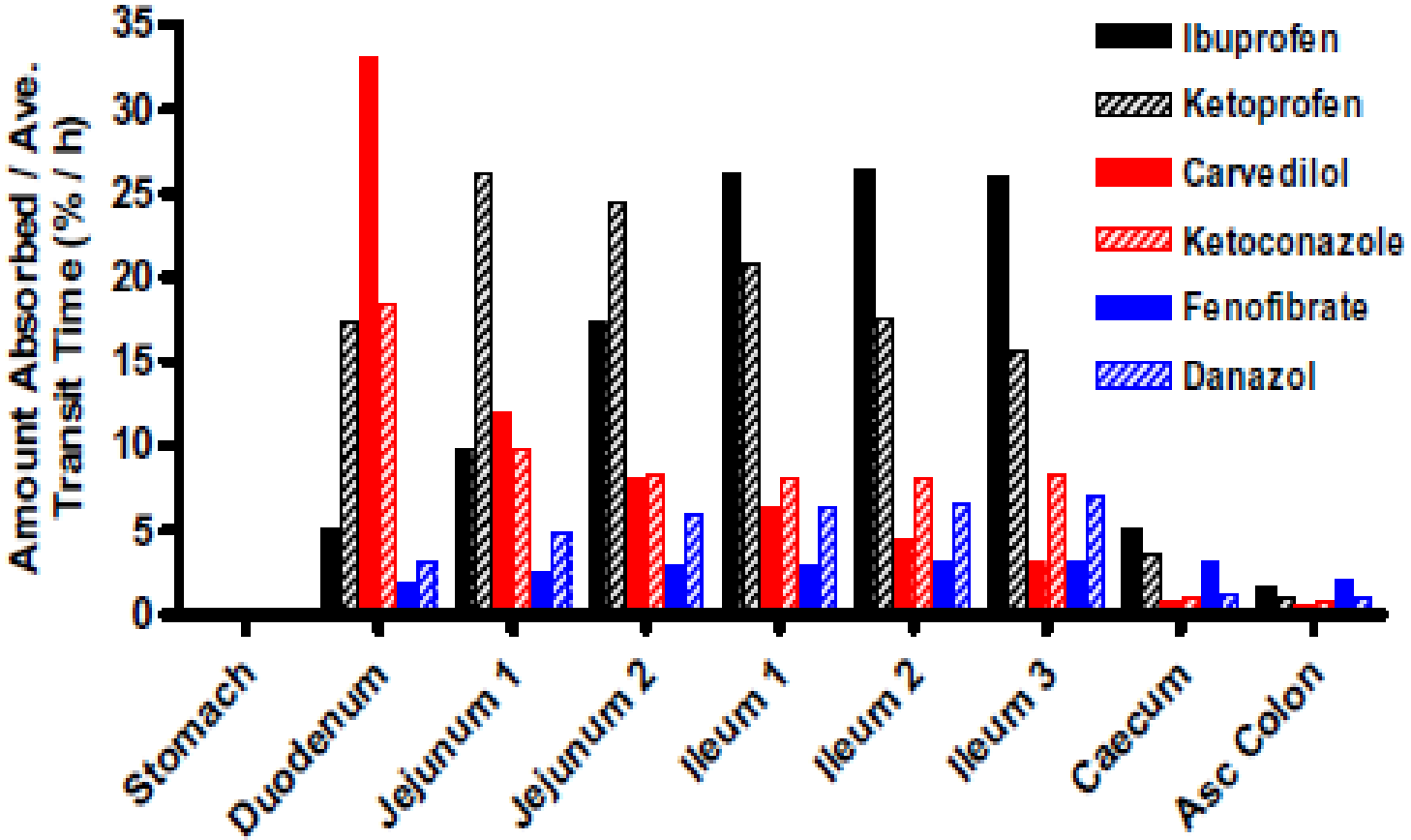
- **Class IIb Drugs**
  - Exhibit high solubility and dissolution rates at acidic pH in stomach
  - May precipitate in intestinal pH
  - Examples- carvedilol and ketoconazole

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

Sub Classes of BCS Class II Drugs



Sub Classes of BCS Class II Drugs



Sub Classes of BCS Class II Drugs

Results:

- Ibuprofen and ketoprofen are absorbed more in the distal small intestine than in the proximal small intestine due to the environmental pH in the GI tract and their pH-dependent solubility/dissolution.

- Major absorption of ketoconazole and carvedilol is at lower pH environment in duodenum and proximal jejunum due to higher solubility at this region of intestine.

- Fenofibrate and danazole show constant dissolution profile throughout the dissolution profile, hence show slow and prolonged absorption.

Determination of Solubility

Determination of Permeability

- Effective permeability (*P) is generally* described in terms of units of molecular movement distance per unit time (e.g. 10 cm/ s).
- High permeability drugs- with an extent of absorption greater than or equal to 90% and are not associated with any documented instability in the gastrointestinal tract.
- The permeability is based directly on the extent of intestinal absorption of a drug substance in humans or indirectly on the measurements of the rate of mass transfer across the human intestinal membrane.
- The methods range from simple oil/water (O/W) partition coefficient to absolute bioavailability studies.

Determination of Permeability

Human Studies

- Mass balance pharmacokinetic studies
- Absolute bioavailability studies, intestinal perfusion Methods

Intestinal Permeability Methods

- *In vivo* intestinal perfusions studies in humans
- *In vivo* or in situ intestinal perfusion studies in animals
- *In vitro* permeation experiments with excised human or animal intestinal tissue

In Vitro Permeation Experiments

Across epithelial cell monolayers (e.g., Caco-2 cells or TC-7 cells)

Determination of Dissolution

- **A powerful and a useful method for determining:**
  - ✓ The product quality
  - ✓ Clinical performance of dosage form
  - ✓ Batch to batch consistency
  - ✓ Bioequivalence/ bioinequivalence
  - ✓ Provides an insight to *in vivo* behavior

- **Dissolution performance is influenced by:**
  - ✓ Physicochemical properties of the substance
  - ✓ Physiological conditions in the GIT tract

  However these can vary between fasted- and fed-states as well as within and among subjects.

Factors Affecting Dissolution

Factor	Physicochemical Properties	Physiological Properties
Surface area of drug	Particle size, wettability	Surfactants in gastric juice and bile
Diffusivity of drugs	Molecular size	Viscosity of luminal contents
Boundary layer thickness	Concentration of the drug	Motility patterns and flow rate
Solubility	Hydrophilicity, crystal structure, solubilization	pH, buffer capacity, bile and food composition
Amount of drug already dissolved	Hydrophilic, lipophilic nature of the drug	Permeability
Volume of solvent available	Depends upon type of body fluid	Secretion, coadminis-tered fluids

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	

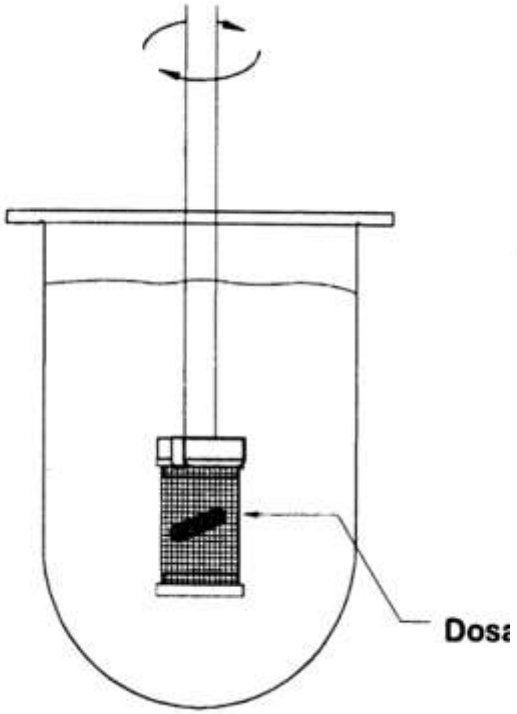
Official Dissolution Apparatus

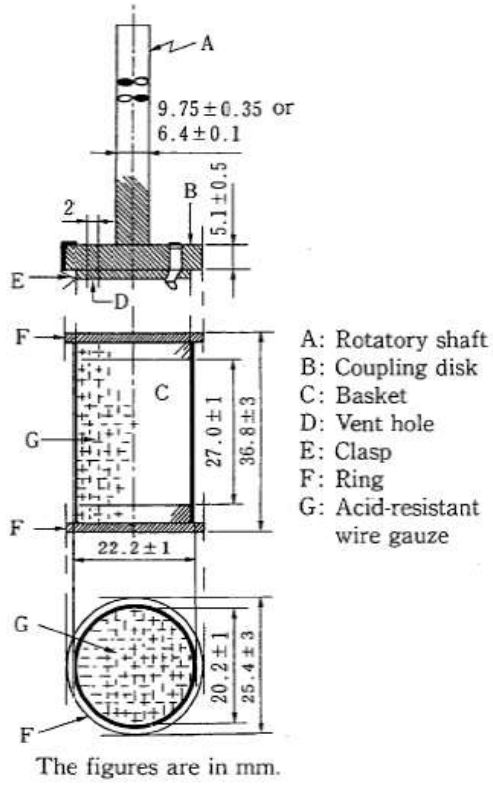
USP 30 classification

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 / enteric 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)
- full pH change during the test
- can be easily automated which is important for routine work

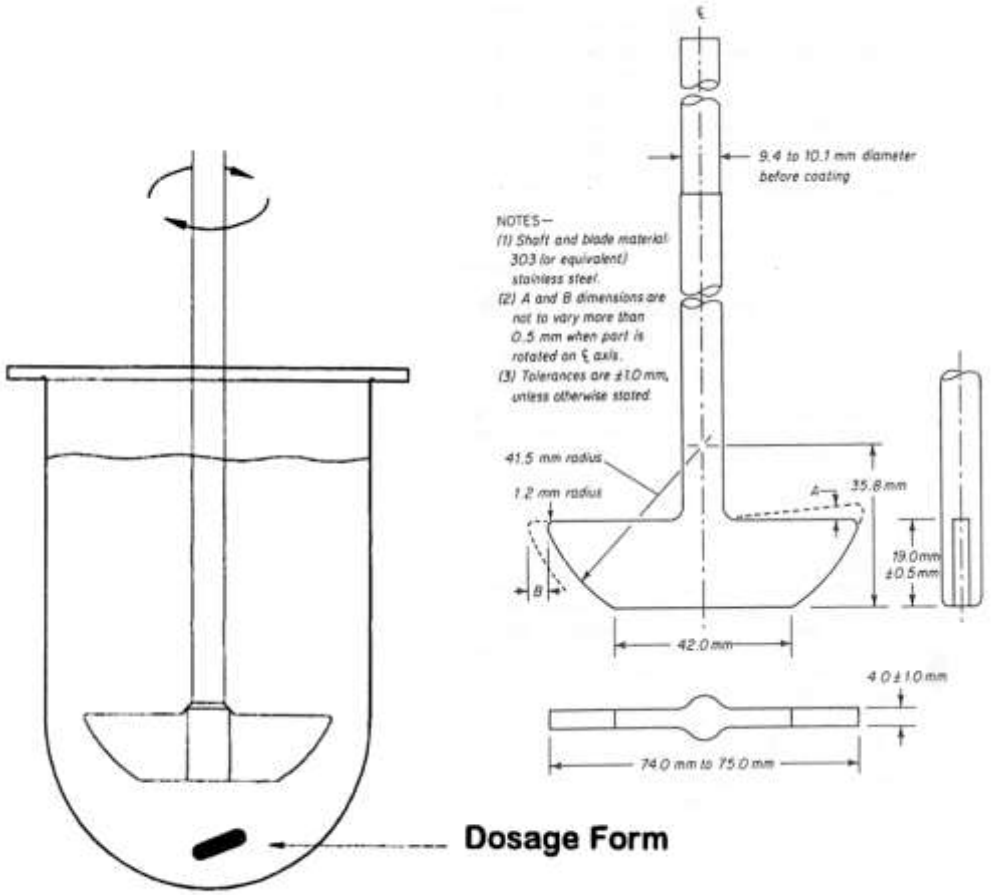
Disadvantages

- disintegration-dissolution interaction
- hydrodynamic „dead zone“ under the basket
- 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

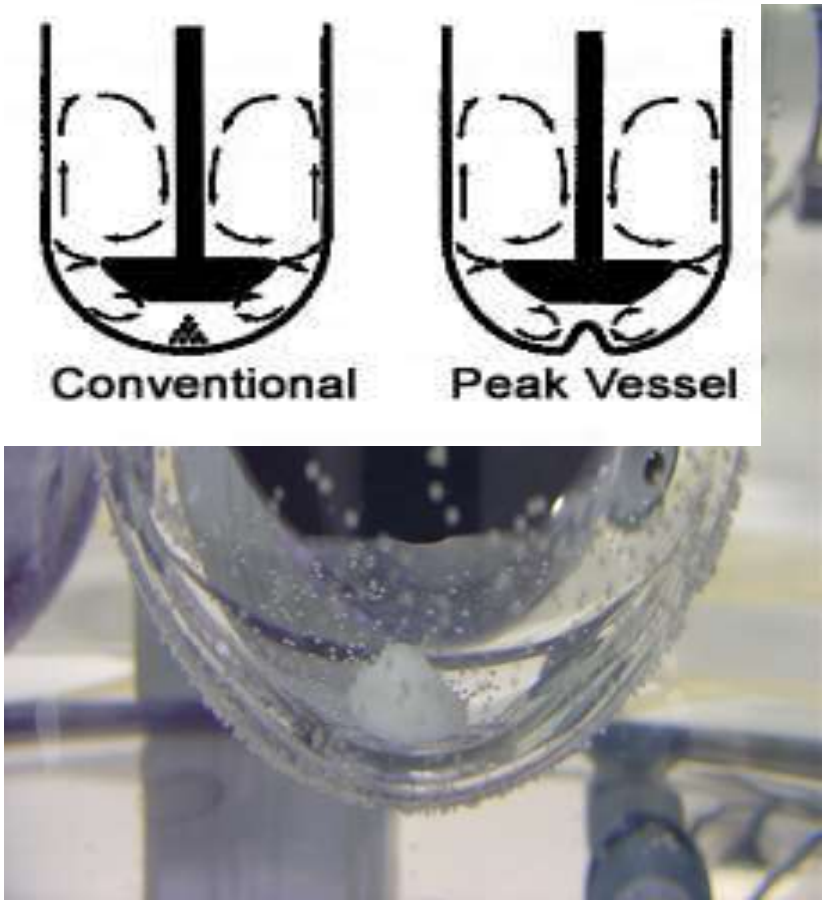
- pH/media change is difficult
- limited volume → sink conditions for poorly soluble drugs
- hydrodynamics are complex
- coning
- sinkers for floating dosage forms

Apparatus 2 - Paddle

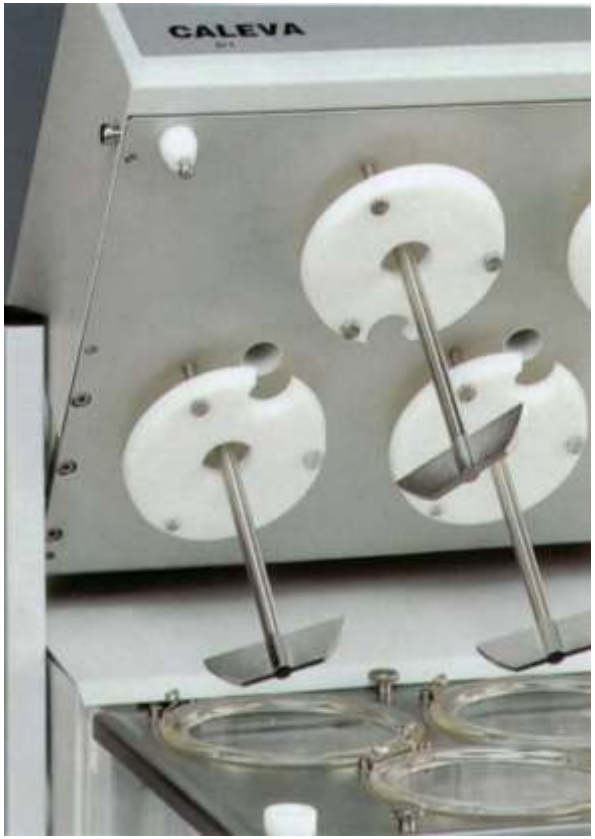
Sinkers

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

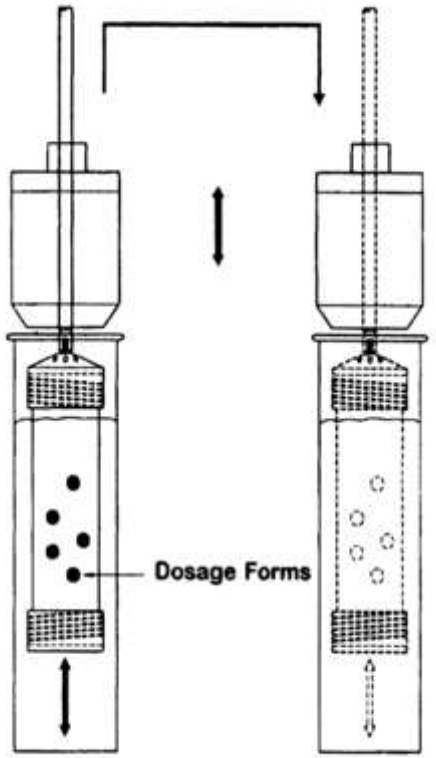


Apparatus 2 - Paddle



Apparatus 3 – Reciprocating Cylinder

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



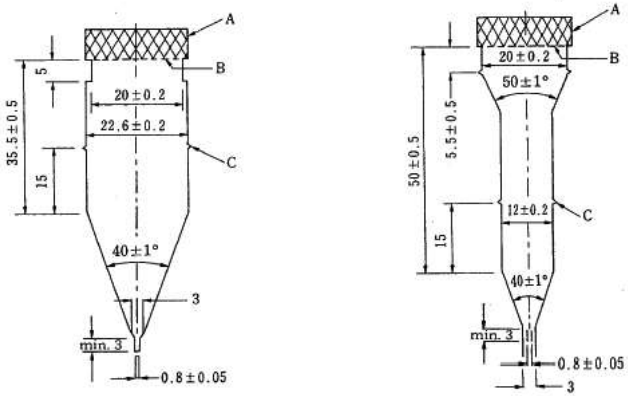
Apparatus 3 – Reciprocating Cylinder

- **Advantages**
  - easy to change the pH
  - hydrodynamics can be directly influenced by varying the dip rate

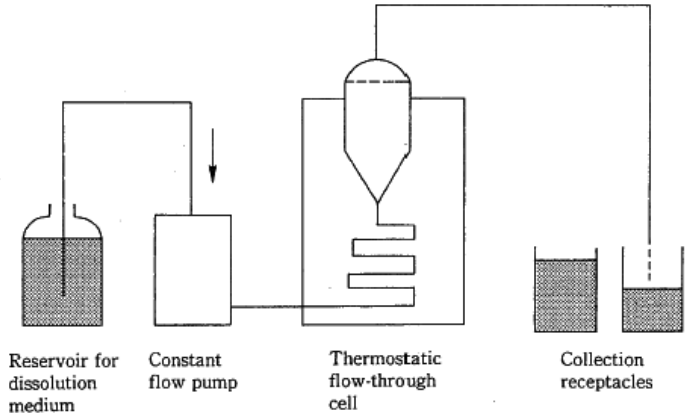
- **Disadvantages**
  - small volume (max. 250 mL)
  - little experience
  - limited data

Apparatus 4 – Flow-Through Cell

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

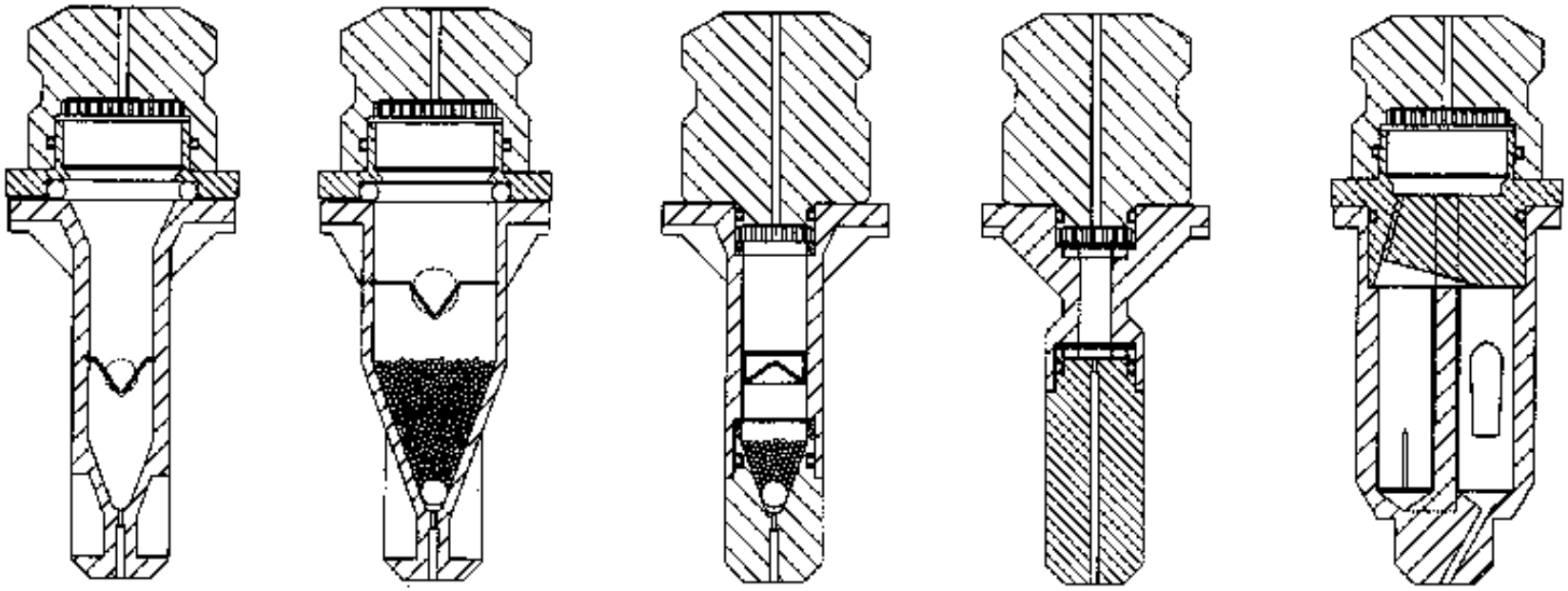


- **Variations**
  - open system
  - closed system



Apparatus 4 – Flow-Through Cell

CELL TYPES



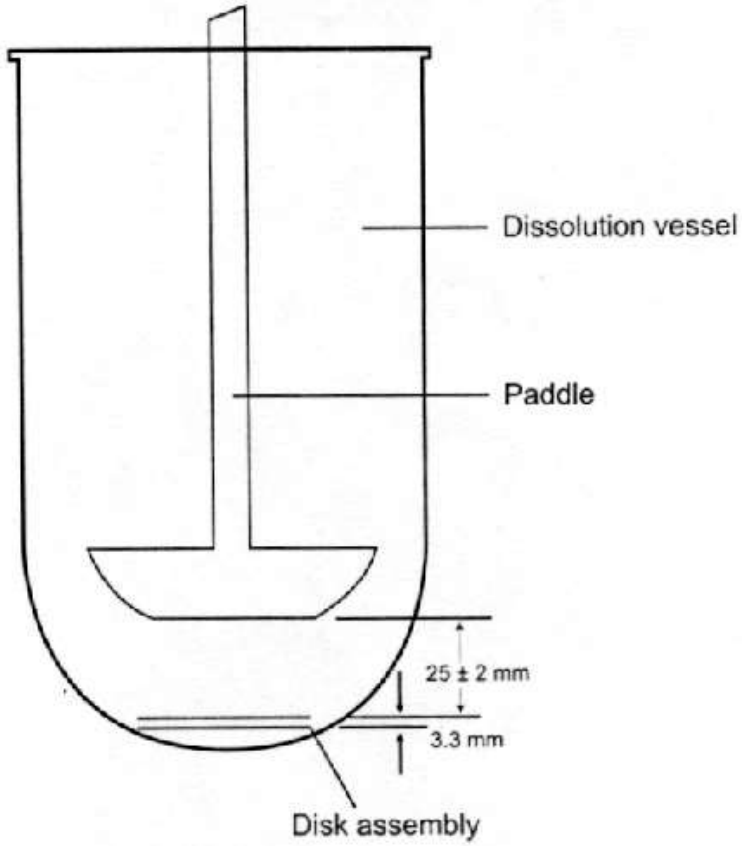
Apparatus 4 – Flow-Through Cell

- **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

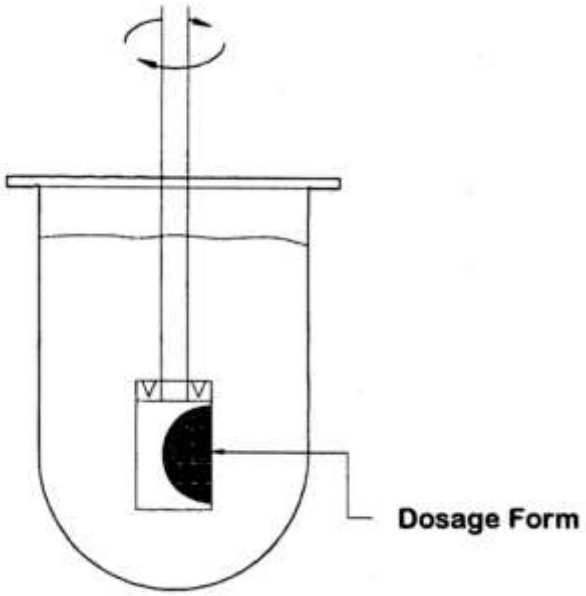
- **Useful for**
  - transdermal patches
- **Standard Volume**
  - 900 mL
- **Advantages**
  - standard equipment (paddle) can be used, only add a stainless steel disk assembly
- **Disadvantages**
  - disk assembly restricts patch size



Apparatus 6 – Rotating Cylinder

- **Useful for**
  - transdermal patches

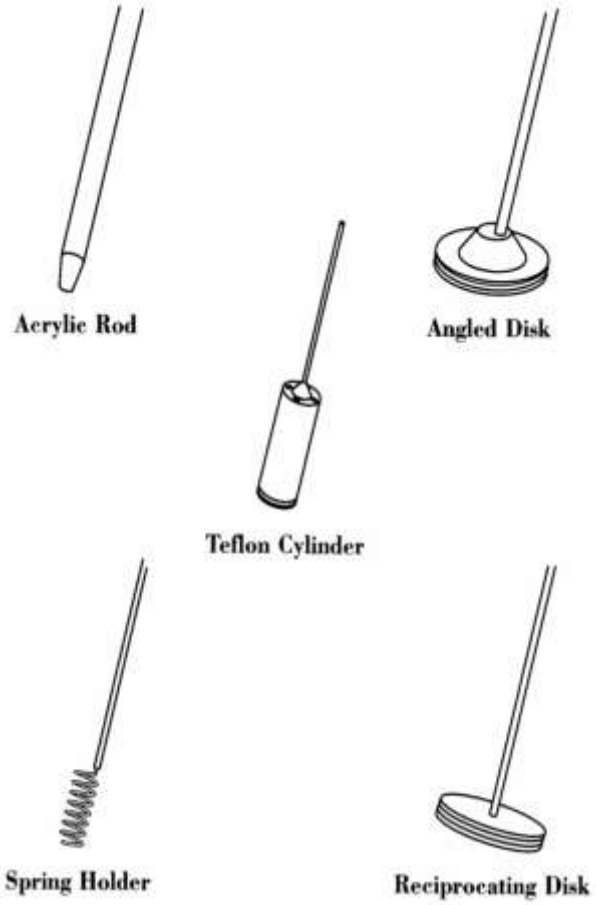
- Similar to apparatus 1
- Instead of basket, a stainless steel cylinder holds the sample



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.



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

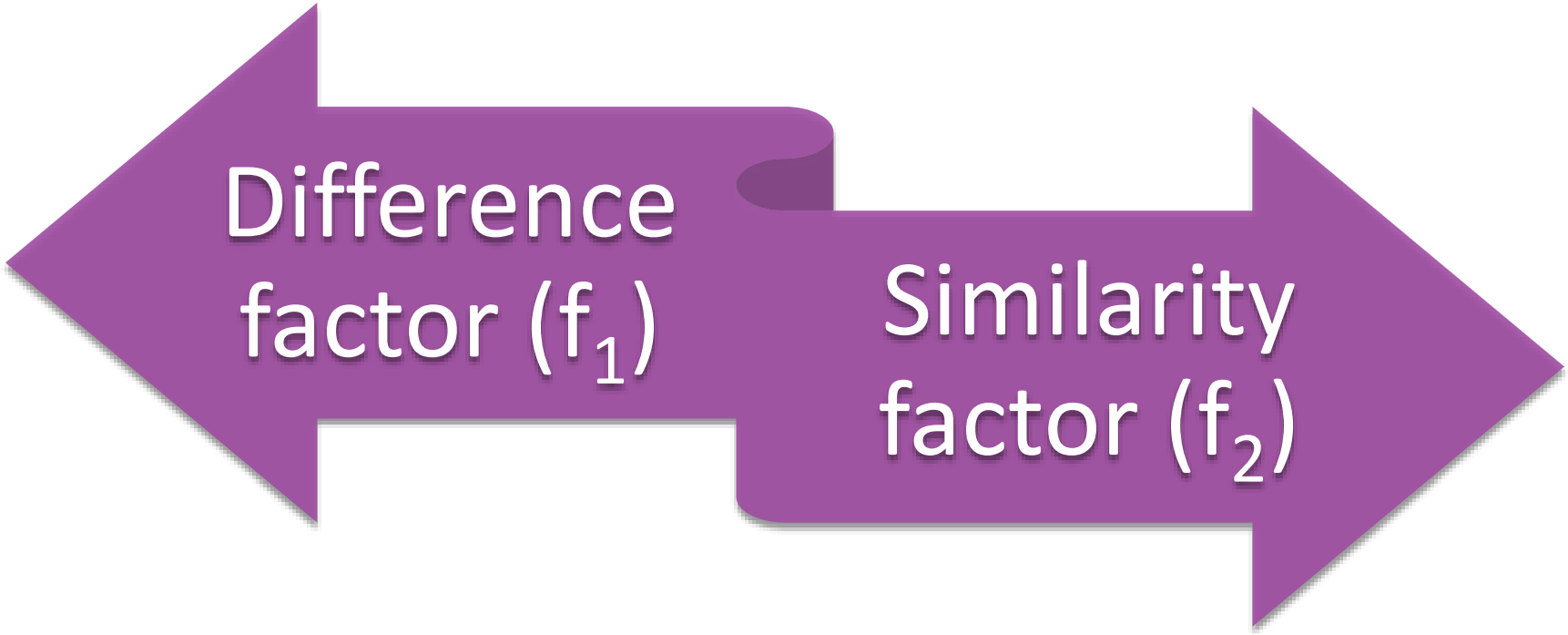
Comparison of Dissolution Profile

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 2 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

Limitations of BCS

- Effects of ***Food***, ***Absorptive transporters***, ***Efflux transporters*** & ***Routes of elimination (renal/biliary)*** are important determinants of BA for immediate release oral dosage forms, which are not considered in BCS.

- **BCS based biowaivers are not applicable for the following:**
  - Narrow therapeutic range drug products.
  - Limited application for the class II drugs and not applicable for class III.
  - Dosage form meant for absorption in the oral cavity e.g. sublingual or buccal tablets.

Extensions to BCS

1. Six class BCS:

- The drugs are classified into six classes.
- The solubility was classified as "**low**" or "**high**" and the permeability was allotted as "**low**" "**intermediate**" or "**high**".

2. Quantitative BCS (QBCS):

- Quantitative BCS (QBCS) was developed using the dose: solubility ratio as core parameter for classification.
- States that solubility is a static equilibrium parameter and cannot describe the dynamic character of the dissolution process for the entire dose administered.

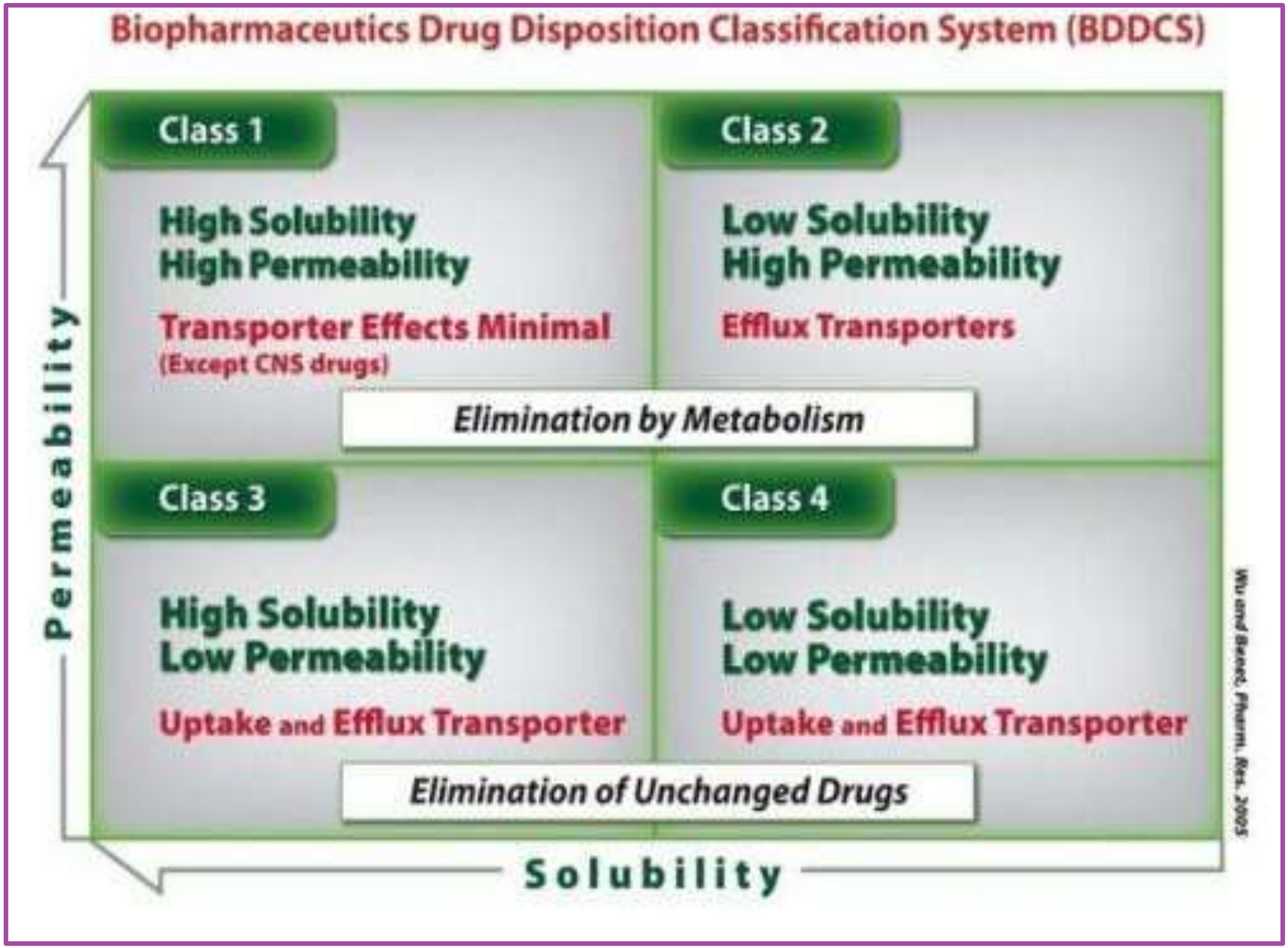
Extensions to BCS

3. Pulmonary BCS

- The BCS is limited to the gastrointestinal tract.
- The pulmonary BCS (PBCS) consider the specific biology of the lung as well as particle deposition, aerosol physics, and the subsequent processes of drug absorption and solubility

4. BDDCS CLASSIFICATION

- BDDCS (Biopharmaceutical Drug Disposition and Classification System) divides compounds into four classes based on their permeability and solubility.
- This classification system is useful in predicting effects of efflux and uptake transporters on oral absorption as well as on post absorption systemic levels following oral and intravenous dosing.

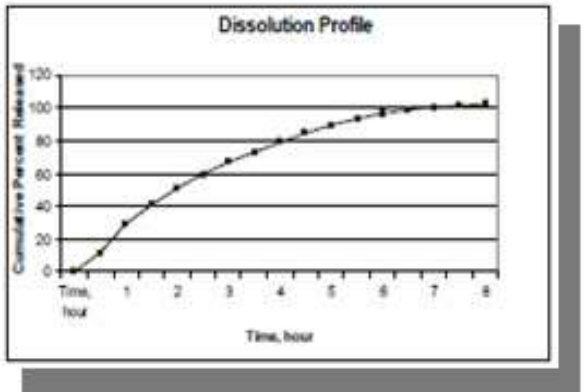

Biopharmaceutics Drug Disposition Classification System (BDDCS)
Class 1
High Solubility
High Permeability
Transporter Effects Minimal
(Except CNS drugs)
Class 2
Low Solubility
High Permeability
Efflux Transporters
Elimination by Metabolism
Class 3
High Solubility
Low Permeability
Uptake and Efflux Transporter
Class 4
Low Solubility
Low Permeability
Uptake and Efflux Transporter
Elimination of Unchanged Drugs
Permeability
Solubility
Wu and Benet, Pharm. Res. 2005

Difference Between BCS & BDDCS

BCS	BDDCS
It takes into account solubility and permeability criteria to classify drugs.	It takes into account solubility and metabolism criteria.
It is more ambiguous.	It is less ambiguous.
Less number of drugs are available for biowaiver.	More number of drugs are available for biowaiver.
It is not applicable in condition where food and transporter interaction occur.	It is applicable in condition where food and transporter interaction occur.

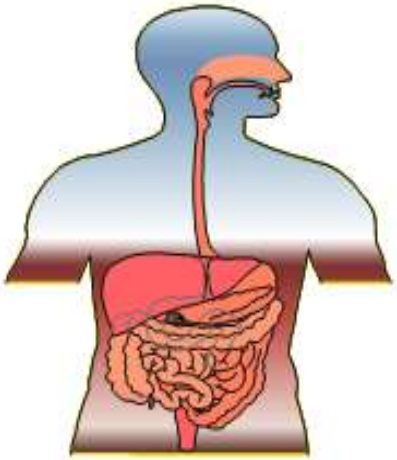
IVIVC

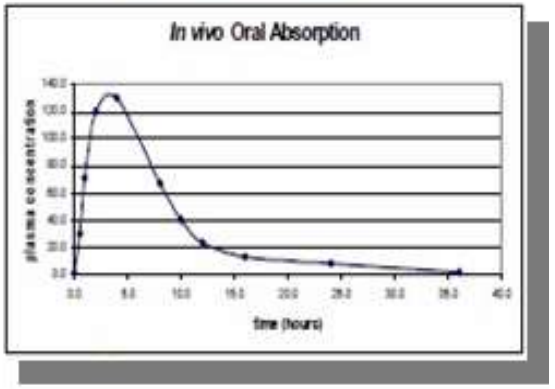
In vitro



IVIVC

In vivo





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.
- Conduct of such biostudies is a Herculean task involving myriad technical, economical and ethical issues.
- Also, development and optimization of a formulation is an time consuming and costly process.

Concept of IVIVC

- This may require alteration in formulation composition manufacturing process, equipment and batch size.
- These type of changes call for the need of BA studies to prove that new formulation is bioequivalent with the old one.
- Implementation of these requirements :
  - halt the marketing of new formulation
  - increase the cost of optimization process
  - demand for strict regulatory guidelines to be followed
- Thus, it would be very convenient if inexpensive *in vitro* experiments could be substituted for *in vivo* bioavailability tests.

Concept of IVIVC

- For *in vitro* test to be useful in this context, it must predict in vivo behavior to such an extent that in vivo bioavailability test becomes redundant.
- *In vitro* dissolution is one such test that can predict the in vivo performance of a drug.
- For *in vitro* dissolution to act as surrogate for bioavailability studies an accurately validated correlation needs to be established between *in vitro* and *in vivo* performance of drug.
- Thus, by establishing IVIVC , *in vitro* dissolution can act as surrogate for bioequivalence studies.
- This would circumnavigate the hiccups caused by the biostudies by seeking for requisite biowaivers.

Concept of IVIVC

- The concept of IVIVC has been extensively discussed for modified release dosage forms.
- This is because the dissolution behavior of of the drug from ER or MR product is the rate limiting factor for absorption in GIT.
- This is why IVIVC are expected more generally for ER formulations than with IR products especially when the latter releases the drug rapidly (>80 % in < 20 minutes)
- But, it does not mean that IVIVC cannot be applied for IR products.
- In recent times, IVIVC for parenterals, transdermals, pulmonary formulations etc. are also coming.

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.

Dissolution as Surrogate for BA Studies

- The purpose of in vitro dissolution studies in the early stages of drug development is to:
  - Select the ***optimum formulation***
  - Evaluate the ***active ingredient*** and ***excipients.***
  - Assess any ***minor changes*** in the drug products

- From IVIVC point of view in vitro dissolution is proposed to be a surrogate of drug bioavailability studies.

- This is possible only if an accurately validated **IVIVC** is established.

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

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.

Need of IVIVC

- Setting up of an in vitro release test that would serve as a ***surrogate*** for *in vivo* plasma profiles ***(bioequivalence testing***).
- To **minimize** unnecessary **human testing**;
- To set up biopharmaceutically meaningful *in vitro* release specifications.
- **Decreased regulatory burdens.**
- **Minimization of cost** and **time** required in additional bioavailability studies

History

1987

- FDA-sponsored workshop entitled *Report on CR Dosage Forms: Issues and Controversies (1987)* - **did not permit consistently meaningful IVIVC for ER dosage forms**

1988

- A USP PF Stimuli article established different IVIVC Levels

1990

- FDA-sponsored workshop entitled *In vitro/In vivo Testing and Correlation for Oral Controlled/Modified Release Dosage Forms* (1990) concluded that the **development of an IVIVC was an important objective** on a product-by-product basis.

1993

- FDA-sponsored workshop entitled *Scale-up of Oral Extended Release Dosage Forms (1993)* identified **dissolution as a surrogate for bioequivalency testing.**

History

1995

- Amidon and Lennernäs et al. proposed BCS to utilise *in vitro* dissolution tests as a surrogate for *in vivo* bioequivalence studies .

1997

- FDA published regulatory guidances for ***in vitro-in vivo*** **correlations (IVIVC)**
- EMEA followed suit in 2000.

2000

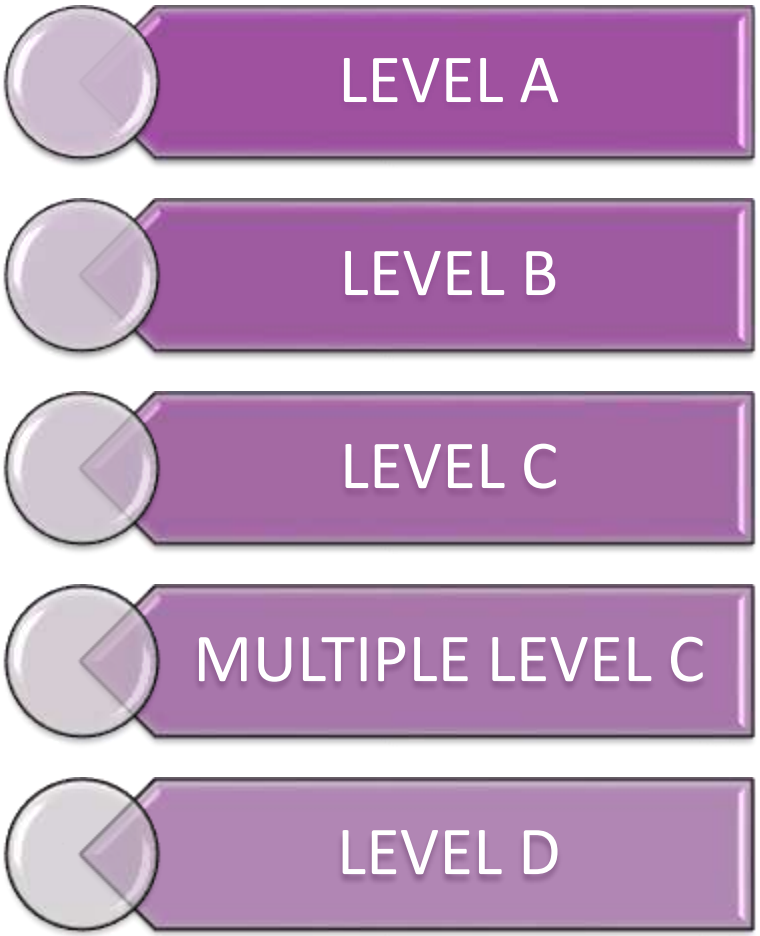
- FDA introduced **regulatory guidlines for BCS biowaivers .**
- EMEA guidelines came in 2002.

2005

- IVIVC and IVIVR established tools

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
- Or can be made superimposable by use of a constant offset value
- 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

Developing Level A Correlation

- **Deconvolution:** it is the process where **output** (plasma concentration profile) is converted into **input** (*in vivo* dissolution of dosage form)
- The plasma or urinary excretion data obtained in the definitive bioavailability study of MR dosage form are treated by deconvolution.
- The resulting data represent the in vivo input rate of the dosage form.
- It can also be called in vivo dissolution when the rate controlling step is dissolution rate.
- Any deconvolution procedure will produce the acceptable results.

Developing Level A Correlation

Deconvolution methods

Model Dependent

- WN Method
- Loo- Riegelman Method

Model Independent

- Numeric Deconvolution

Developing Level A Correlation

WAGNER NELSON

- Used for a one compartment model
- Less complicated
- The cumulative fraction of drug absorbed at time t is calculated as:

$$F_T = \frac{C_T + K_E \int_0^T Cdt}{K_E \int_0^\infty Cdt}$$

C_T is plasma conc. at time T
K_E is elimination rate constant

LOO- RIEGELMAN METHOD

- Used for multi compartment system
- More complicated
- Fraction absorbed at any time t is given by:

$$F_T = \frac{C_T + K_{10} \int_0^T Cdt + (X_p)_T / V_C}{K_{10} \int_0^\infty Cdt}$$

$(X_p)_T$ is amount of drug in peripheral compartment as a function of time
V_c is apparent volume of distribution
K_{10} is apparent first order elimination rate constant

Developing Level A Correlation

Numeric Deconvolution Approach

- Alternative approach requiring in vivo plasma data from an oral solution or iv dose
- Based on convolution integral equation
- The absorption rate r_{abs} that results isn plasma concentration c(t) may be estimated by solving following eq.

$$c(t) = \int_0^t c_\delta(t-u) r_{abs}(u) du$$

C_δ is the concentration time profile resulting from instantaneous absorption of a unit amount of drug which is typically absorbed from bolus IV injection or reference oral solution data

c(t) is plasma conc. versus time profiles of tested formulation

r_{abs} is the input rate of the oral solid dosage form in to the body

u is the variable of integration

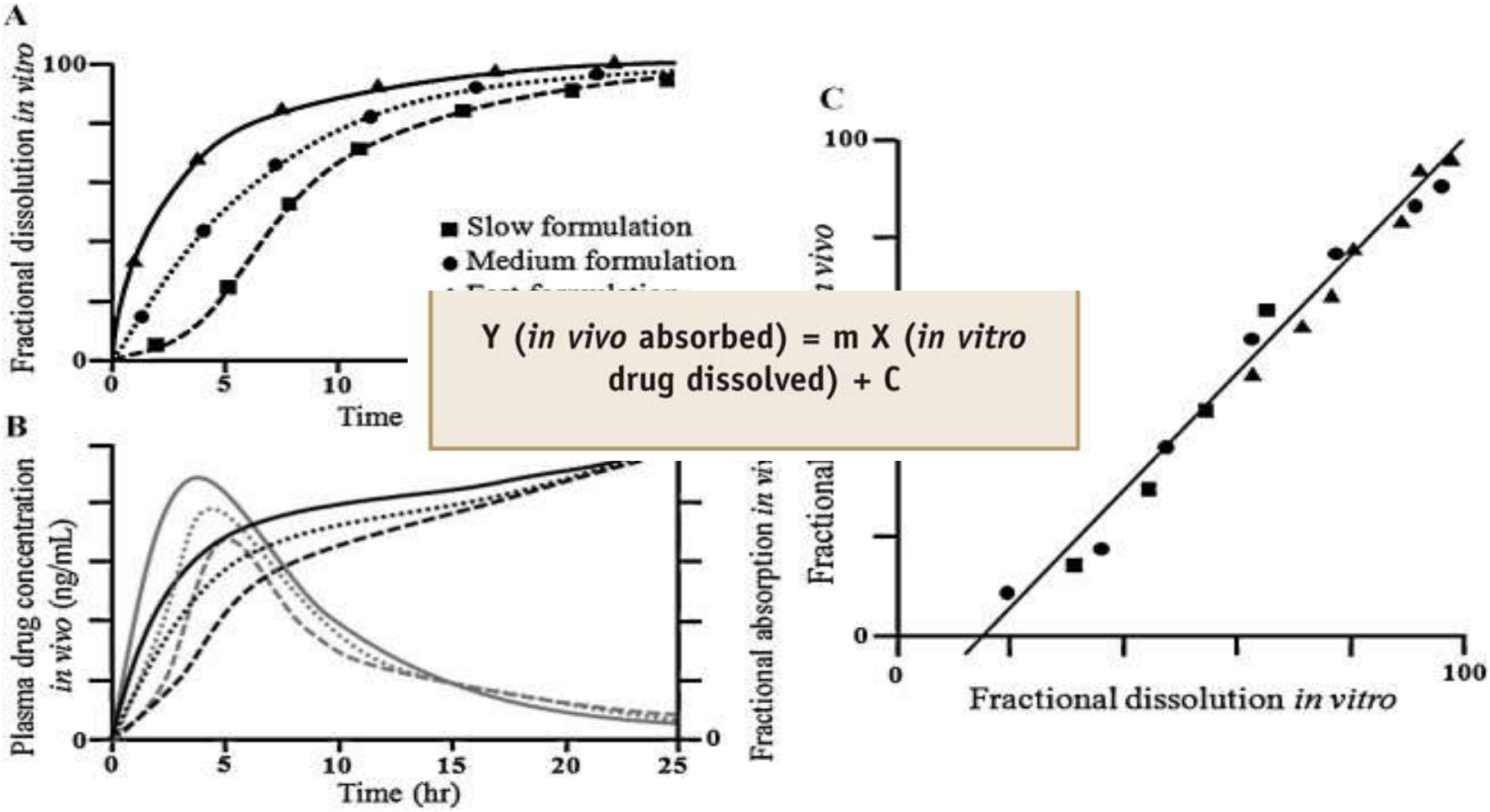
Developing Level A Correlation

- $Y(t) = G(t)\ X(t)$
  - Where Y(t) is the function describing plasma C-t profile following extravascular administration,
  - G(t) is the function describing the Concentration-time profile following bolus iv dose
  - X(t) is the function describing input i.e. dissolution from dosage form
- Deconvolution method requires no assumptions regarding number of compartments.
- It requires data obtained after both oral and intravenous administration in the same subject.
- It assumes no differences in PK of drug distribution and elimination from one study to the other.
- Drug concentrations must be measured at same times following both oral and iv administration.

Developing Level A Correlation

- Biobatch is then subjected to in vitro dissolution evaluation.
- In vitro dissolution curve is then compared to drug input rate curve i.e. in vivo dissolution curve.
- To compare Graph b/w Fraction of drug absorbed (FRA) and Fraction of Drug Dissolved (FRD)is plotted.
- Mathematically scale *in vivo* profile to match with *in vitro* profile
- Linear correlations – superimposable curves
- If not superimposable then can be made by use of scaling factor.
- Nonlinear correlations, though uncommon, are also possible.

Developing Level A Correlation

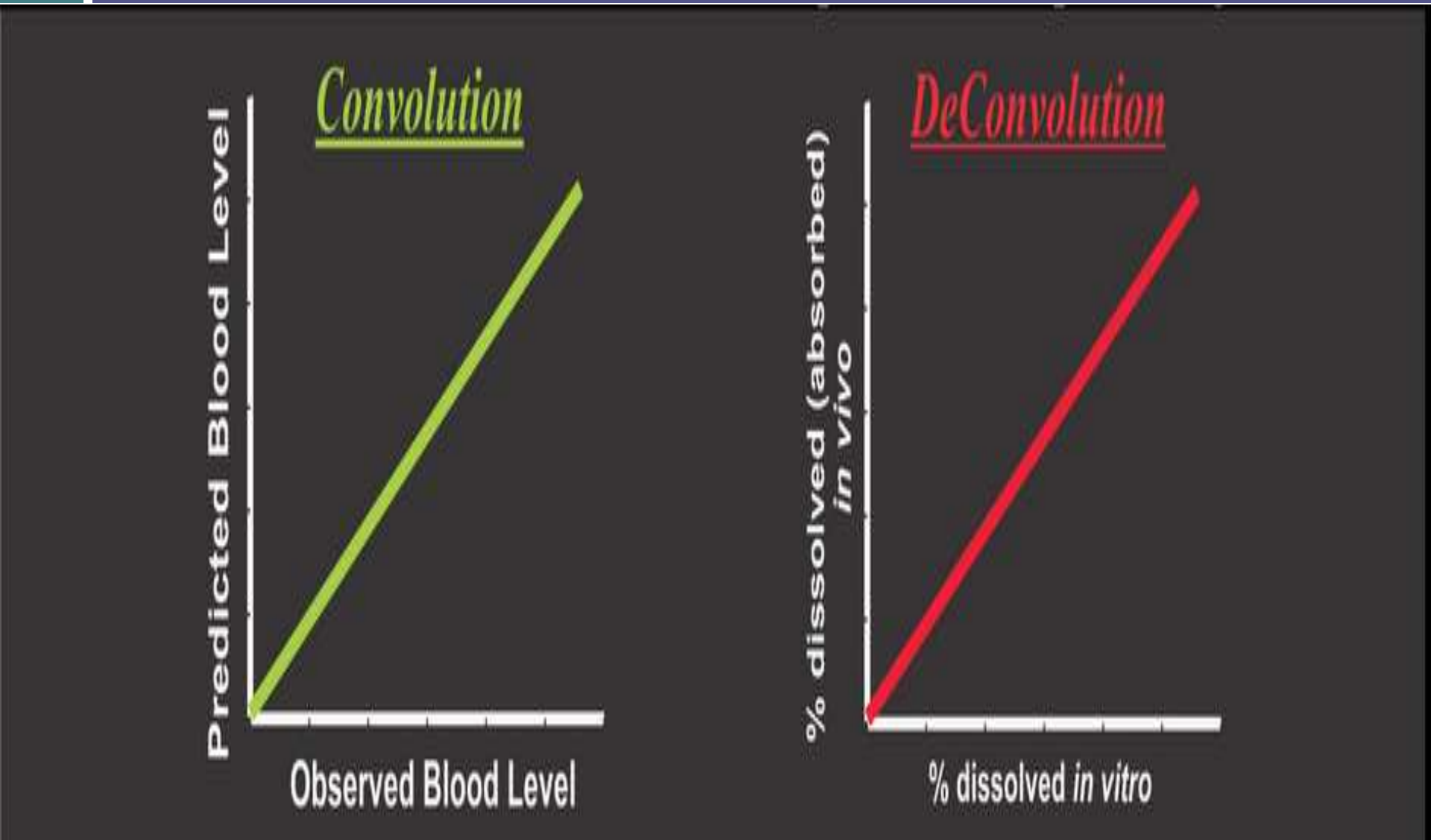


Developing Level A Correlation

Convolution Approach

- Input is converted into output.
- Single step approach
- Here in vitro dissolution profile is converted into plasma concentration time profile.
- It can be done by model independent or model dependent approaches, physiology based softwares and simulation can be applied.
- Then predicted plasma profile is compared with the real plasma profile.

Developing Level A Correlation



Developing Level A Correlation

Scaling of Data

- Since, significant difference exists between in vitro and in vivo dissolution conditions, it is not uncommon to see time scale difference while comparison.
- The introduction of time scale factor is acceptable as long as the same factor is being used for all formulations
- In addition to time scale factor, other approaches like lag time and cut-off factor can be used.
- Lag time is used to account for gastric emptying .
- Cut off factor is used to account for lack of colon absorption.

Developing Level A Correlation

1.Types of Formulations used

Condition dependent dissolution	Condition independent dissolution
• Formulations with different release rates	• Single release formulation

2.Number of formulations

- Minimum 2 formulations with different release rates
- But 3 or more formulations with different release rates (slow medium or fast) recommended
- **EXCEPTION–** conditions independent dissolution where only one formulation

Developing Level A Correlation

3. Design

- Single study cross over design

4. Dissolution conditions

- Should adequately discriminate among different formulations
- Once a discriminating condition is established, the conditions should be same for all the formulations.
- During the early stages, dissolution conditions can be altered to develop point-to-point correlation.

5. Time scaling

- Should be same for all the formulations

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.

Evaluating Predictability

- An IVIVC should be evaluated to demonstrate that predictability of the *in vivo* performance of a drug product, from the *in vitro* dissolution characteristics of the drug product formulations, is maintained over a range of *in vitro* release rates
- Evaluation approaches focus on estimation of predictive performance or prediction error.

$$\%PE = \left(\frac{observed - predicted}{observed}\right) * 100$$

Internal predictability

- Evaluates how well model describes the data used to define IVIVC
- based on the initial data sets used to define the IVIVC
- Used for wide therapeutic range drugs
- Used if formulations with 3 or more release rates were used

External predictability

- Relates how well the model predicts when one or more additional data sets are used
- based on additional data sets obtained from a different (new) formulation
- Used for narrow therapeutic range drugs
- Used if formulations with only 2 release rates were used

Internal predictability

- **Acceptance Criteria**

- Average %PE of 10% or less for C_{max} and AUC
- %PE for each formulation should not exceed 15%
- If these criteria are not met external predictability should be performed.

External predictability

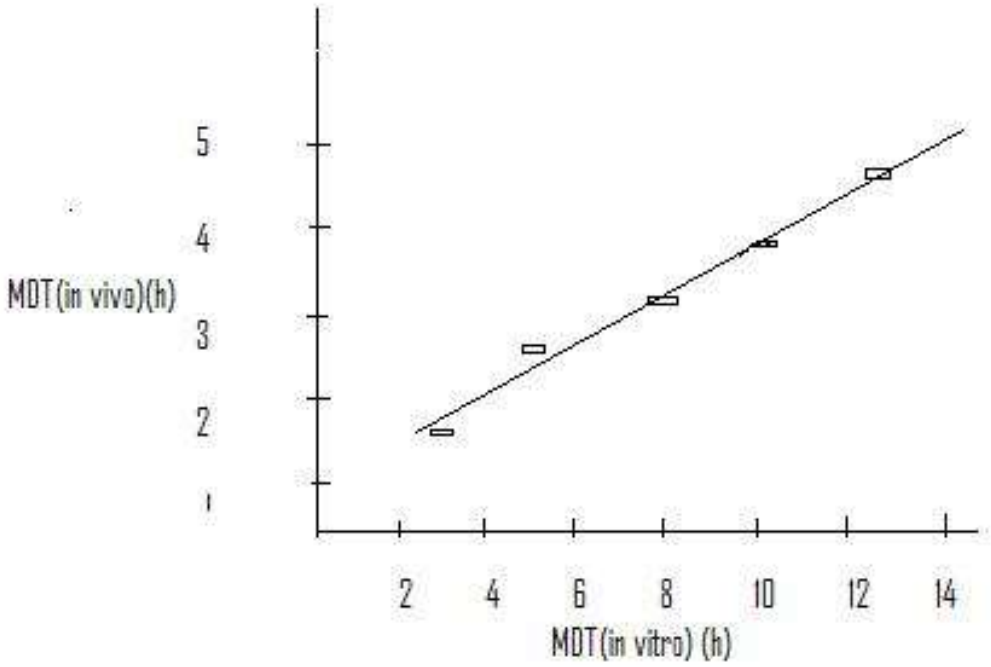
- **Acceptance Criteria**

- Average % PE of 10% or less for C_{max} and AUC
- %PE between 10-20% demands for additional data sets.
- %PE greater than 20% indicates inadequate predictiability

LEVEL B CORRELATION

- Uses the principles of statistical moment analysis
- 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.
- Level B correlations are rarely seen in NDAs

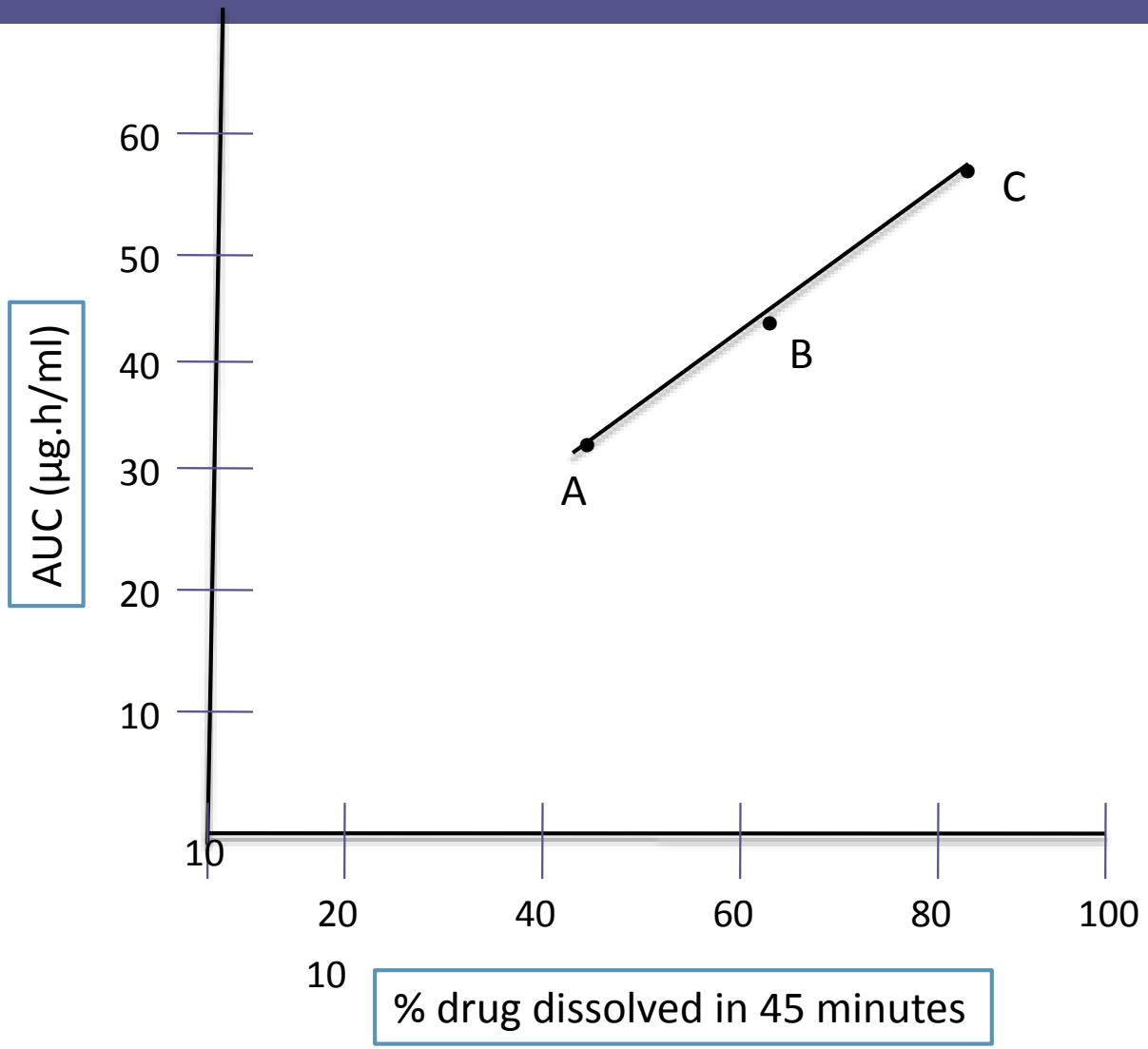
LEVEL B CORRELATION



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.
- Weakest level of correlation
- Can be useful in early stages of formulation development when pilot formulations are being selected
- Biowaiver not possible

LEVEL C CORRELATION



MULTIPLE LEVEL C CORRELATION

- Relates one or several pharmacokinetic parameters of interest (C_{max}, AUC etc.) to the amount of drug dissolved at several time points of the dissolution profile

May be used to justify biowaiver, provided that the correlation has been established over the entire dissolution profile with one or more pharmacokinetic parameters of interest

If such correlation is achievable; then development of level A is likely and preferred

- It should be based on atleast 3 dissolution time points covering early, middle and late stages of dissolution profile.

LEVEL D CORRELATION

- Level D correlation is a rank order and qualitative analysis and is not considered useful for regulatory purposes.

- It is not a formal correlation but serves as an aid in the development of a formulation or processing procedure.

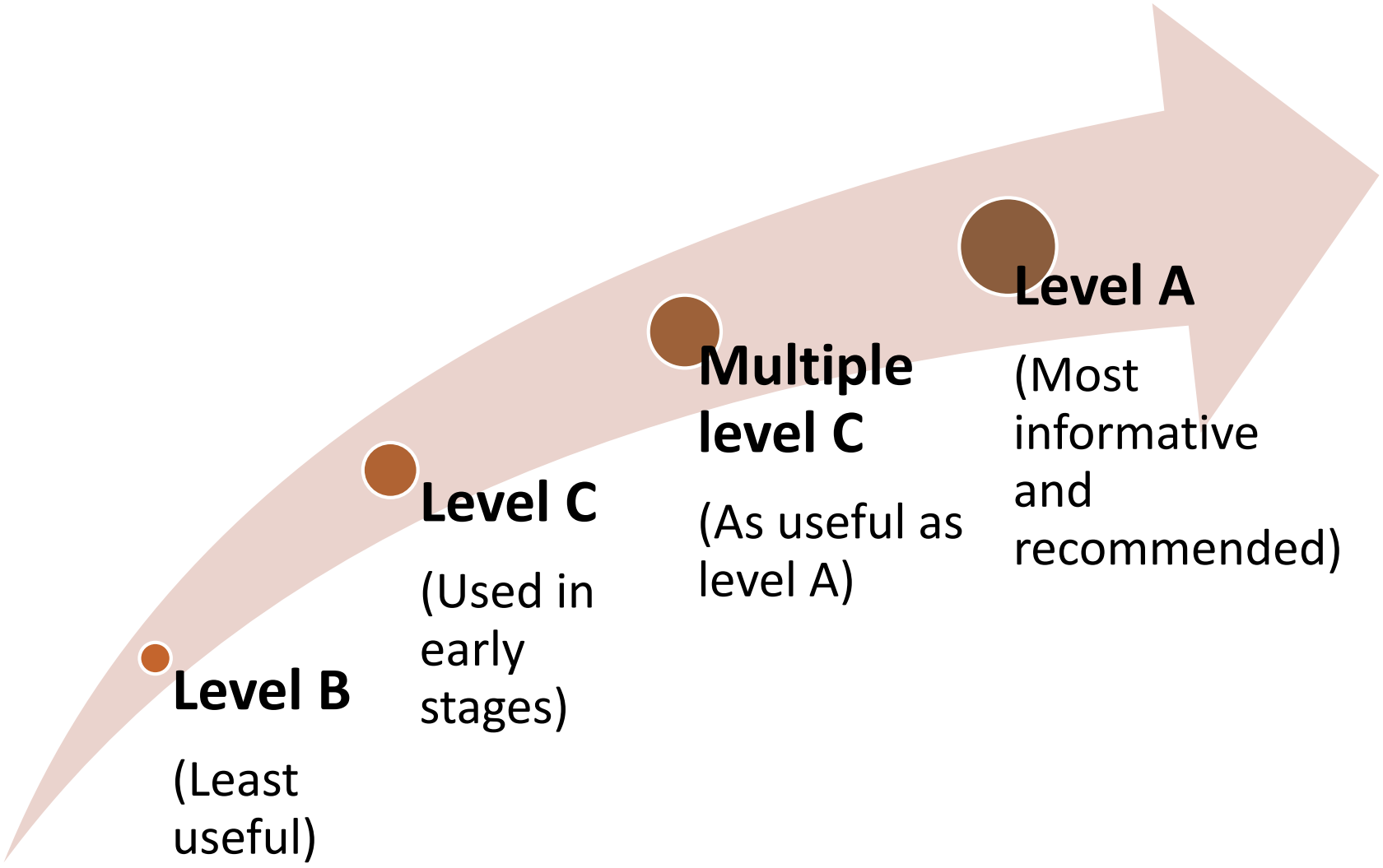
General Considerations

- Number of subjects = 6-36
- Cross over studies preferred, but parallel or cross studies also possible
- The reference product may be iv solution, an aqueous oral solution or an immediate release product.
- IVIVCs are developed in fasted state unless the drug is not tolerated in fasted state.
- The preferred dissolution apparatus is USP basket type or paddle type at compendial rotation speeds.
- The same dissolution method should be used for different formulations.

General Considerations

- An aqueous medium, either water or a buffered solution preferably not exceeding pH 6.8 is recommended.
- Sufficient data should be submitted to justify pH greater than 6.8
- Non aqueous and hydroalcohlic systems are discouraged unless all attempts with aqueous media are unsuccessful.
- For poorly soluble drugs addition of surfactants may be appropriate.
- The dissolution profile of at least 12 individual dosage units from each lot should be determined.

FDA Ranks


Level B
(Least useful)
Level C
(Used in early stages)
Multiple level C
(As useful as level A)
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.

IVIVC for Parenterals

- IVIVC has been successfully applied to solid oral dosage forms
- IVIVC can be applied to parenteral Modified Release (MR) dosage forms as well.
- To obtain such a correlation following steps are followed:
  - Obtain *in vivo* data
  - Identify *in vivo* drug release mechanism
  - Identify factors affecting *in vivo* release
  - Design *in vitro* release method based on *in vivo* release mechanism
  - Correlate the in *vitro* and *in vivo* data
- For MR release dosage forms, it is often necessary to use an in vitro method of release testing that exceeds the in vivo rate of drug release.

Development of *in vitro* Release Tests

- Since these dosage forms are typically designed to release their contents over periods of weeks, months or even years, it becomes impractical to wait for a real-time test for batch release of product.
- Therefore, accelerated methods are often developed to assist in batch release of the product.
- Accelerated tests, by their nature, (e.g. elevated temperature or use of solvents)can change not only the rate of drug release but also the mechanism of release.
- Therefore, it is very important to understand the accelerated release mechanism.

Development of *in vitro* Release Tests

- When dealing with MR systems, it is the mechanism of release that should dictate the science of the in vitro test method.
- Following test methods have been successfully employed:
  - Modified rotating paddle for suspensions
  - Franz diffusion cell for gels
  - Flow through cell for implants
  - Floatable dialysis bag for nanoparticles or microspheres
  - USP apparatus for with glass beads for microspheres
- Release medium, flow rate, agitation characteristics etc. are important.

Developing an IVIVC for Parenteral Products

- Real-time data for drug release is essential to correlate to *in vivo* bioavailability, accelerated testing can also be used.
- For tests intended to support an IVIVC, the release profile from an accelerated test should correlate with the *in vivo* release profile.
- Where it is not possible to achieve such a correlation with an accelerated release test, such a test may still be useful for batch release of the product.
- However, the development of an additional real-time test will still be needed if the intent is to develop an in vitro test that is predictive of in vivo product performance.

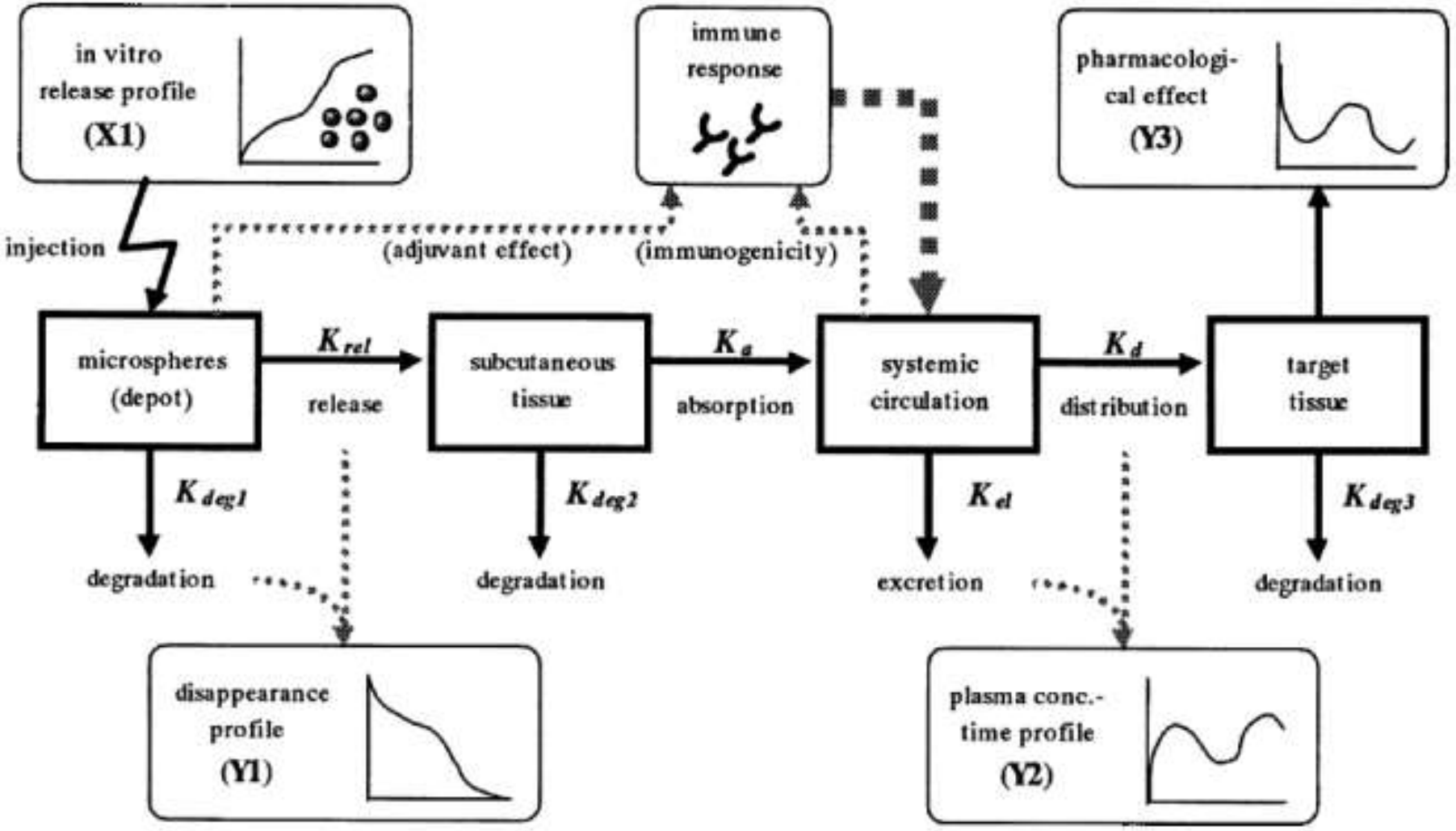
Developing an IVIVC for Parenteral Products

- Accelerated testing will often result in a change in the mechanism of release.
- Nevertheless, accelerated conditions can still serve as a discriminatory tool so long as all formulations experience similar changes and continue to exhibit performance characteristics that can be differentiated from each other.
- In some cases, a correlation between in vivo data and accelerated in vitro data may be obtained, regardless of a change in the mechanism of release.
- However, there are numerous other situations where the use of accelerated test conditions maybe problematic.

Developing an IVIVC for Parenteral Products

- For example, some MR dosage forms are associated with multiphasic release characteristics, such as an initial burst release followed by a secondary release phase.
- It is often impossible to separate these different phases in an accelerated test.
- For that reason, “real-time” test is often needed to characterize the initial burst phase.
- The initial burst release phase is usually diffusion controlled, whereas the later phases tend to be controlled by erosion and diffusion.

Developing an IVIVC for Parenteral Products



Developing an IVIVC for Parenteral Products

- In this scheme, there are three output functions which are used to establish IVIVC,
  - X1 *in vitro* release profile correlated to either Y1 (defined as disappearance profile from the administration site,
  - X1 related to or plasma concentration time profile as Y2,
  - X1 to the pharmacological effects of drugs at the target tissue Y3.
- If Y2 is used, convolution procedure or any other modeling technique can be used to relate plasma concentration time profile to in vivo absorption or release rate.
- If a linear relationship between the in vitro and release data does not occur then, IVIVC can be achieved by mathematical modeling (e.g.time variant nonlinear modeling) of the *in vitro* and *in vivo* data

In vitro- in silico- in vivo Correlation

- This approach is used in drug discovery and early preclinical phases where PK data is not available.
- IVIVC at this time is usually conducted through in silico simulation of structural properties of a molecule or high throughput experimental data generated.
- Although simulation is not a replacement for definitive scientific experiments, it provides in sight what one would expect in vivo based on physicochemical properties.
- There are two in silico approaches for prediction of *in vivo* oral absorption:
  - Statistical models
  - Mechanism-based models

In vitro- in silico- in vivo Correlation

- One mechanism based model that has gained popularity in recent times is GastroPlus™.

- Inputs to software include:
  - Oral dose
  - Physiochemical properties (pH-solubility profile, permeability etc.)
  - Physiological properties (species, GI transit, GI pH, food status etc.)
  - Formulation properties (release profile, particle size etc.)
  - PK parameters (optional)

- The output includes:
  - Fraction of oral dose absorbed
  - Plasma Concentration time profiles (if PK parameters are given)

In vitro- in silico- in vivo Correlation

CASE STUDY

- In one relatively simple application of GastroPlus ™, it was asked whether or not the mean particle size requirement of Compound I (aqueous solubility>100 mg/mL) may be relaxed from 35 µm to approximately 100 µm without affecting its oral bioavailability.

- A simulation suggested that the extent of absorption is not sensitive to changes in particle size in the range of 35–250 µm.

- This helps in decision making with respect to dosage form design.

Failure of Level A IVIVC for IR Products

- For Level A analysis, Fa is plotted against Fd and requires linear regression of Fa vs Fd.
- IVIVC for IR products is less successful as they do not show dissolution limited absorption.
- A reason for this lack of success and acceptance may be the general failure of the Level A method to immediate release products.
- Controlled release products, rather than immediate release products, are the focuses in the IVIVC literature.
- But it does not indicate that dissolution from such products fails as a surrogate for bioavailability.

Slippery Slope of Correlation

- Since dissolution is perhaps not rate-limiting in an IR product, the Fa against Fd profile will be non-linear.

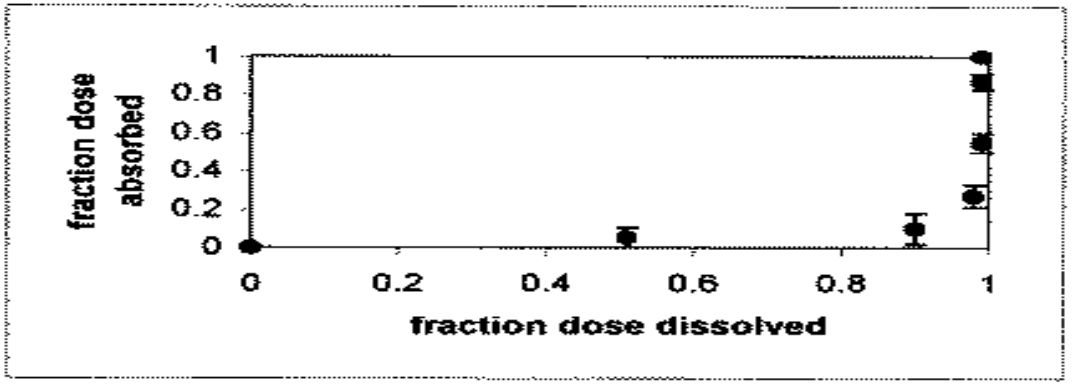


Fig 2

- In practice, a correlation is often taken to imply a linear relationship which is problematic for IR products.
- Thus, avoidance of term “correlation” and use of more general term that would allow for non-linear relationships may aid in development of IVIVC-type analysis of IR products.

IVIVR

- One possible substitution for IVIVC is IVIVR, with "R" denoting "relationship
- IVIVR need not be limited to straight-line relationships, which appear to be generally incorrect for IR products.

$$F_a = \frac{1}{f_a}\left(1 - \frac{\alpha}{\alpha - 1}\left(1 - F_d\right) + \frac{1}{\alpha - 1}\left(1 - F_d\right)^{\alpha}\right)$$

Where,

F_a =fraction of the total amount of drug absorbed at time t,

f_a =fraction of the dose absorbed at t = #,

a =ratio of the apparent first-order permeation rate constant (kpaap) to the first-order dissolution rate constant (kd), and

F_d =fraction of drug dose dissolved at time t.

IVIVR

- Level A method is a special (linear) case of eq If fa = 1.0 (i.e. complete absorption) and a>>1 (i.e. strongly dissolution rate-limited absorption), then Fa = Fd.

- This IVIVR analysis has been applied to several formulations of metoprolol, piroxicam, and ranitidine .

- The use of the term IVIVR rather than IVIVC is preferred.

IVIVM

- The objective of dissolution testing is to achieve predictability of testing based on (co)relationship.
- Differences/similarity in vitro should be reflected in vivo and vice versa under the same testing conditions/ environment whether products are from same lot, different formulation, or different products.
- If one set of experimental conditions provides a matched ranking between dissolution and in vivo profiles, then it is considered as achieving IVIVC. The dissolution test would be called as bio-relevant.
- If none of the prior dissolution methods provide such matching, then a new set of experimental conditions may also be developed to match the ranking.

IVIVM

- This approach is considered as *In Vitro-In Vivo* Matching (IVIVM).
- It is clear to see that this approach seeks to match, thus would NOT reflect a relationship or predictability aspect, which is the requirement of an IVIVC.
- Thus, it is of limited use as compared to IVIVC.

IVIVP

- The objective of IVIVC is to **link or relate** the in vitro (dissolution) and in vivo (C-t) profiles.
- A dissolution test is performed and then C-t profile from it is predicted.
- Therefore, it can be said that in reality the purpose of commonly referred practices of IVIVC is to transfer a dissolution (in vitro) to a C-t (in vivo) profile, or simply in vitro-to-in vivo profiling.
- The mathematical technique to transfer in vitro profile to in vivo profile is known as convolution.
- Convolution is relatively simpler than de-convolution as the former can be applied using simple spreadsheet software, e.g., MS Excel.

Applications of IVIVC

IVIVC plays an important role in product development:-

- serves as a surrogate of *in vivo* and assists in supporting biowaivers;
- supports and / or validates the use of dissolution methods and specifications; and
- assists in quality control during manufacturing and selecting appropriate formulations.

Applications of IVIVC

1. Biowaivers

- The first and main role of establishing IVIVC is to use dissolution test as a surrogate for human studies.

- The benefit of this is to minimize the number of bioequivalence studies performed during the initial approval process and during the scaling-up and post-approval changes.

Applications of IVIVC

The FDA guidance outlines five categories of biowaivers:

- biowaivers without an IVIVC
- biowaivers using an IVIVC: non-narrow therapeutic index drugs
- biowaivers using an IVIVC: narrow therapeutic index drugs
- biowaivers when *in vitro* dissolution is independent of dissolution test conditions
- situations for which an IVIVC is not recommended for biowaivers

Applications of IVIVC

Biowaivers Without IVIVC

Biowaivers for the changes made on lower strengths are possible without an IVIVC if -

- all strengths are compositionally proportional or qualitatively the same,
- in vitro dissolution profiles of all strengths are similar,
- all strengths have the same release mechanism,
- bioequivalence has been demonstrated on the highest strength (comparing changed and unchanged drug product), and
- dose proportionality has been demonstrated for this ER drug product.

Applications of IVIVC

For these situations, waivers can be granted without an IVIVC if dissolution data are submitted in the application/compendial medium and in three other media (e.g., water, 0.1N HCl, and USP buffer at pH 6.8).

Applications of IVIVC

Biowaivers based on IVIVC

For generic products to qualify for biowaiver, based on IVIVC , one of the following situations should exist:

- Bioequivalence has been established for all strengths of the reference listed product.

- Dose proportionality has been established for the reference listed product, and all reference product strengths are compositionally proportional or qualitatively the same, have the same release mechanism, and the *in vitro* dissolution profiles of all strengths are similar.

Applications of IVIVC

- Bioequivalence is established between the generic product and the reference listed product at the highest and lowest strengths and, for the reference listed product, all strengths are compositionally proportional or qualitatively the same, have the same release mechanism, and the *in vitro* dissolution profiles are similar.

Applications of IVIVC

2. Establishment of dissolution specifications

- In vitro dissolution specifications should generally be based on the bioavailability performance of the lots. This approach is based on the use of the in vitro dissolution test as a quality control test.
- An IVIVC adds in vivo relevance to in vitro dissolution specifications, beyond batch-to-batch quality control.
- In this approach, the *in vitro* dissolution test becomes a meaningful predictor of in vivo performance of the formulation, and dissolution specifications may be used to minimize the possibility of releasing lots that would be different in in vivo performance.

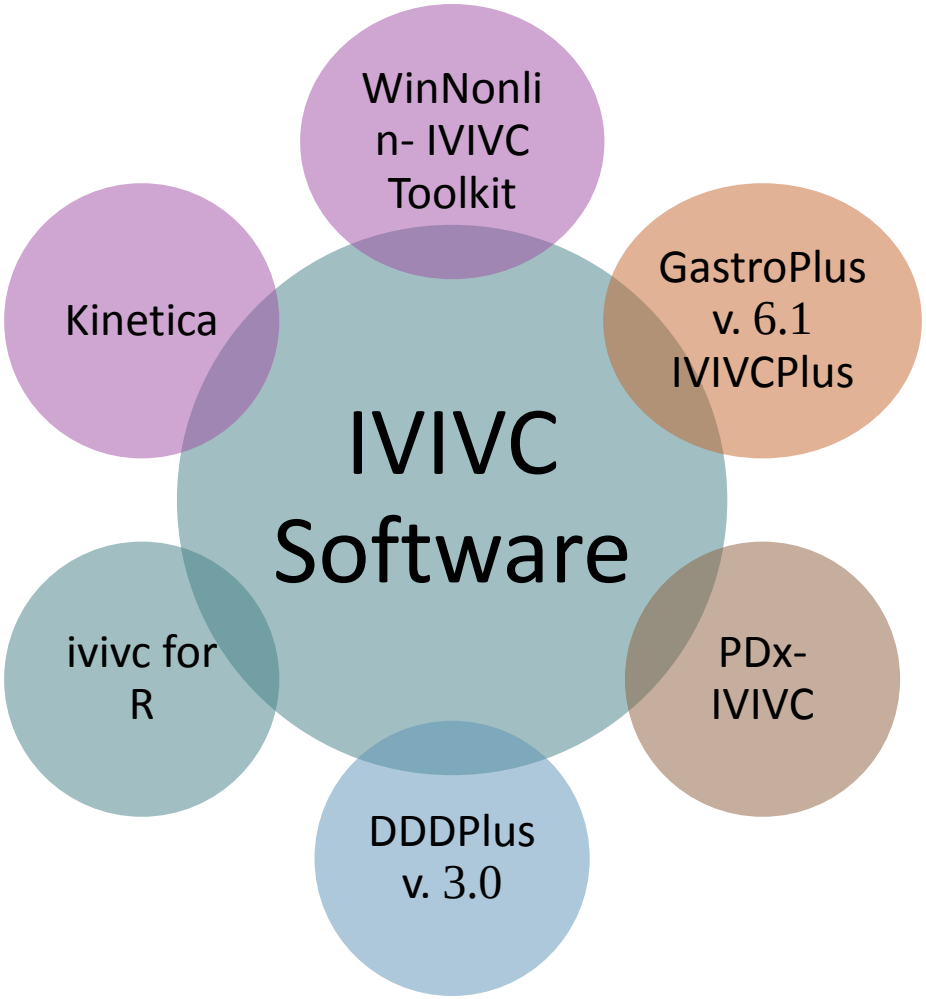
Applications of IVIVC

- Major drawback in the widespread use of IVIVC is that this approach is **product dependent.**

- The IVIVC cannot be used across the products, especially drug product with different release mechanisms .

  E.g. in the case of controlled release drug delivery systems .

Softwares Used in IVIVC



Survey Results for IVIVC

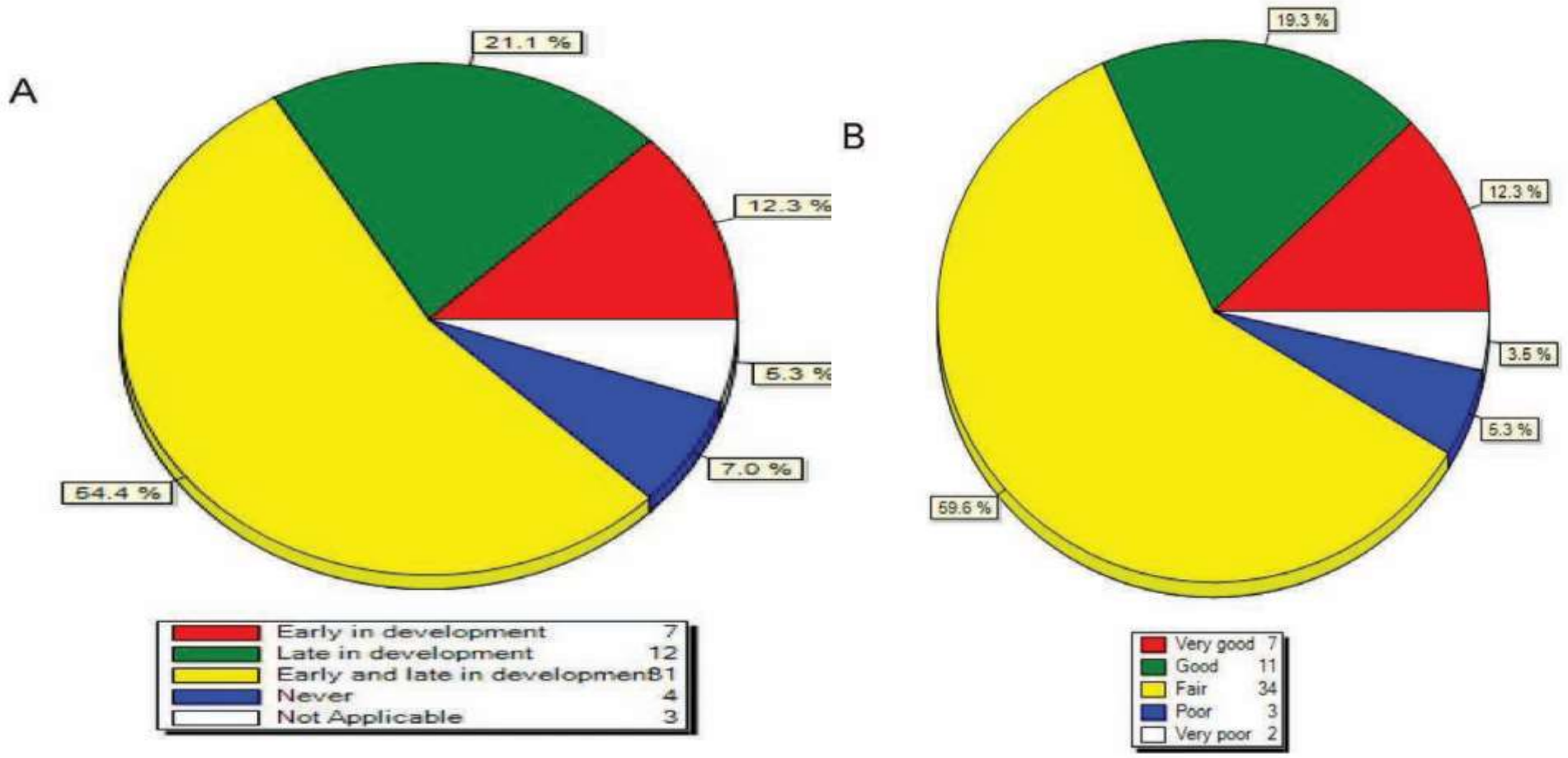


Figure 1. (A) IVIVC use (Question 2) and (B) success rates (Question 3).

Survey Results for IVIVC

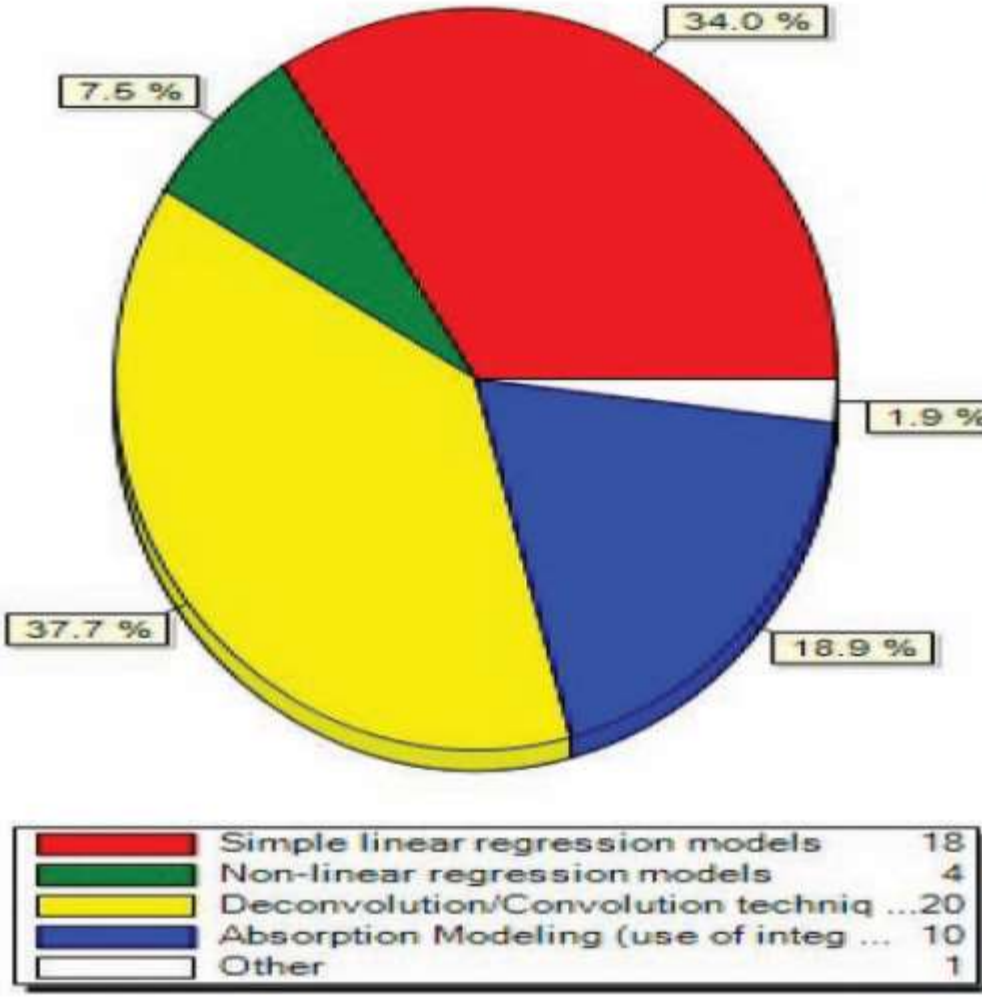


Figure 3. Development of Level A correlations (Question 10).

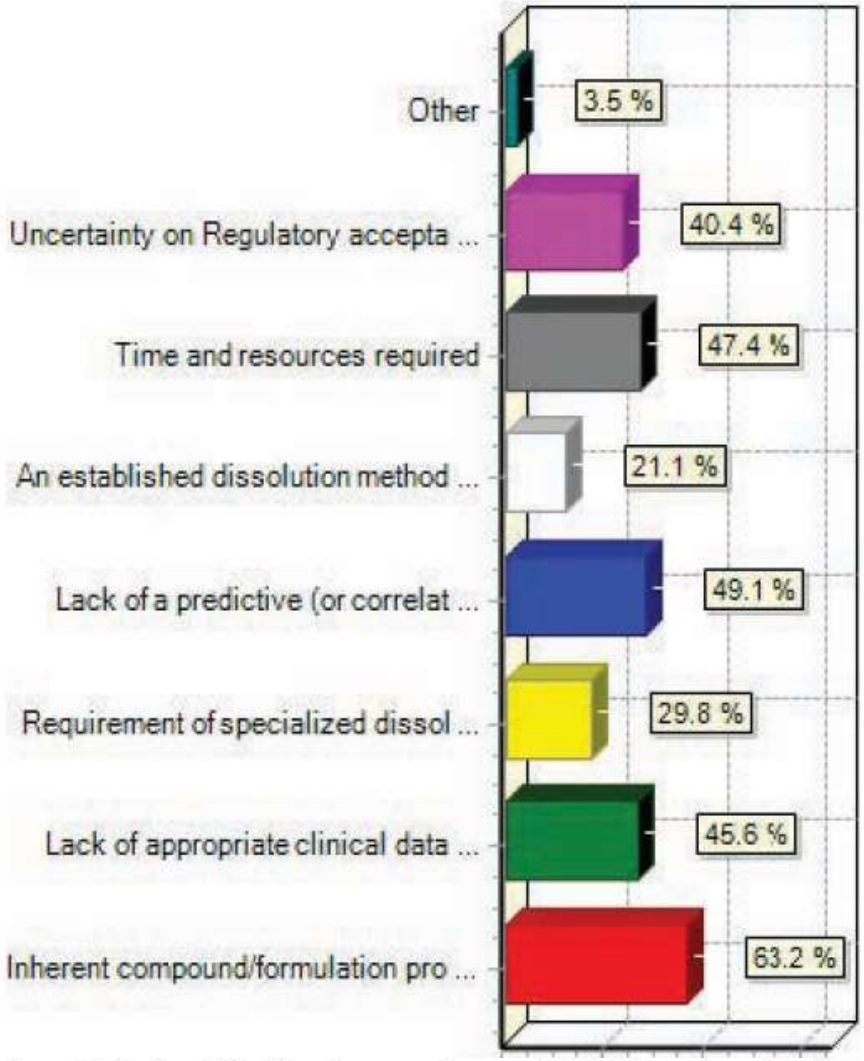


Figure 2. Main difficulties for pursuing an IVIVC (Question 4).

Thank you!

for participating in our presentation