Importance of *in -vitro in -vivo* studies in pharmaceutical formulation development

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ABSTRACT

Bioavailability of drug product can be altered by drug and excipients properties in the formulation and manufacturing process. Sccessful Pharmaceutical development is the perfect understanding of the in vivo and in vitro performance of the dosage form. In-vitro dissolution methods are developed with correlation of In-vivo parameters. In-vitro specifications are set to maintain the consistency and reproducibility of the in vivo characteristics (bioavailability) of the dosage forms.
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INTRODUCTION

A key goal in development of oral dosage forms [1] is a good understanding of the in vivo and in vitro performance of the dosage form importantly for poorly soluble drugs and controlled release dosage forms.

This article is intended to explain in detail about In vivo studies includes bioavailability and bioequivalence, Physicochemical properties of drug, In vitro dissolution studies, IVIVC correlation, Biowaiver and Manufacturing process to improve the bioavailability.
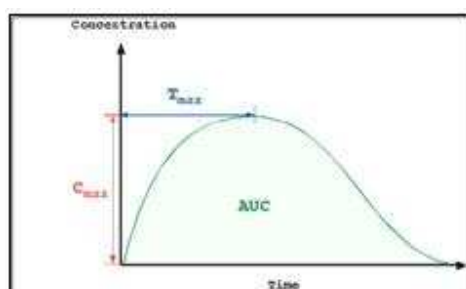
Bioavailability:
Bioavailability is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action [2]. Absorption is the process of movement of unchanged drug from the site of administration to systemic circulation or site of measurement i.e. plasma. The extent of intestinal absorption is dependent on drug stability, aqueous solubility and intestinal permeability. Any alteration in the drug's bioavailability is reflected in its pharmacological effect. Other processes that play a role in the therapeutic activity of a drug are distribution and elimination. The movement of drug between one compartment and the other (generally blood and the extra vascular tissues) is referred to as

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drug distribution. Elimination is defined as the process that tends to remove the drug from the body and terminate its action.

Absolute bioavailability is defined as the dose normalized area under the plasma concentration time curve after oral administration divided by that after intravenous administration.

Absolute bioavailability $F = (AUCoral / Doral) / (AUCiv / Div)$

Measurement of Bioavailability:

The two major pharmacokinetic methods used measure bioavailability are:

(i)Plasma level-time studies
(ii)Urinary excretion studies

The 3 parameters of plasma level-time studies which are considered important for determining bioavailability are (i). The peak plasma concentration (C_{max}) that gives an indication whether the drug is sufficiently absorbed systemically to provide a therapeutic response, (ii). The peak time (t_{max}) that gives an indication of the rate of absorption and (iii) The area under the plasma level-time curve (AUC) that gives a measure of the extent of absorption or the amount of drug that reaches the systemic circulation. The 3 major parameters examined in urinary excretion data obtained with single dose study are: The maximum urinary excretion rate, the time for maximum excretion rate and cumulative amount of drug excreted in the urine.

A systematic approach to ensure bioavailability of pharmaceutical products:

Bioequivalence:
Bioequivalence is defined as the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in pharmaceutical equivalents or pharmaceutical alternatives becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

The three major pharmacokinetic parameters to asses bioequivalence are:

• AUC is the principal criterion to characterize the extent of absorption and to assess bioequivalence.
• Cmax is the rate and extent of absorption (wider acceptance criteria)

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• Tmax is the rate of absorption (considered only when clinically relevant)

Relative bioavailability   F' = $\dfrac{AUC_{Test}/ D_{Test}}{AUC_{Ref} / D_{Ref}}$
(Bioequivalence)

Relative bioavailability   F'' = $\dfrac{Cmax_{Test} / D_{Test}}{Cmax_{Ref} / D_{Ref}}$
(Rate of absorption)

Pharmaceutical equivalence implies that two or more drug products are identical in strength, quality, purity, content uniformity and disintegration and dissolution characteristics; they may however differ in containing different excipients. Pharmaceutical alternatives if drug products contain the same active moiety but differ in chemical form of that moiety or in the dosage form or strength (salt, ester, complex, dosage form). Therapeutic equivalence is the pharmaceutical equivalents whose bioavailability or dissolution profiles, after the same molar doses, are similar to such an extent that their safety and efficacy can be assumed to be substantially equal. Therapeutic equivalents are interchangeable. Essential similar products: if it has the same qualitative and quantitative composition in terms of active substances and the pharmaceutical form is the same and, where necessary, bioequivalence with the first product has been demonstrated by appropriate bioavailability studies carried out.

Generic Product [3]:
Generic name product is a copy that is the same as brand name (Innovator) product. A generic drug is an identical, or bioequivalent to a brand name drug in dosage form, safety, strength, route of administration, quality, performance characteristics and intended use. Once innovator patents and exclusivity periods of the innovator expires, generic company can market their product by proving equivalence of bioavailability (bioequivalence/relative bioavailability) with innovator to market the product.

The possible difference between generic and innovator product is drug particle size, polymorphic form, excipients, Manufacturing process equipment, site of manufacturing, batch size etc. Preformulation studies include drug-excipient compatibility, polymorphic studies to be conducted to ensure that the generic product possesses equivalent and sometimes even superior stability characteristic to the innovator brand. Dissolution specification should be same between generic and innovator. Generic products must be prepared in accordance with Goods Manufacturing Practices, should usually originate from a batch of at least 1/10 of production scale or 100,000 units whichever is greater, unless otherwise justified.

The regulatory requirement of new and generic product:

New drug	Generic drug
1. Chemistry	1. Chemistry
2. Manufacturing	2. Manufacturing
3. Controls	3. Controls
4. Labeling	4. Labeling
5. Testing	5. Testing
6. Animal Studies	6. Bioequivalence
Clinical Studies	
Bioavailability	

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Bioequivalence studies are particularly needed for:
Immediate release
i) Documented evidence for bioavailability problems related to the drug, ii) Narrow therapeutic window, iii) Complicated pharmacokinetics, iv) Lower water soluble drugs, v) Where a high ratio of excipients to active ingredients exists Modified release & Fixed combination

Bioequivalence studies are not needed for a powder for reconstitution as a solution / solution for oral or parenteral use and an aqueous solution of ophthalmic, nasal spray and topical application, these products must be similar to reference product.

Factors to consider in the design of a study[4]
• Protocol must state *a* priori, the study objectives and methods to be used
• Study formulation should be representative of formulation to be marketed
• Subjects: Number, health status, age, weight, height, ethnicity, gender, special characteristics e.g. poor metabolizers, smoking, inclusion/exclusion criteria specified in protocol.
• Randomization, Blinding, Sampling protocol,
• Adequate Washout period (> 5 time's drug half-life).
• Administration of food and beverages during study, Recording of adverse events

Studies should be carried out in accordance with provisions of guidelines on Good Clinical Practice, Good Manufacturing Practice, and Good Laboratory Practice. In general, single dose two-way crossover, fasted & fed studies will sufficient but there are situation, steady-state studies may be required. Alternatives studies includes Single-dose, parallel, fasted (Long Half-Life drugs), Single-dose, replicate design (Highly Variable Drugs ie.CV>30%), Multiple-dose, two-way crossover, fasted, Chemotherapy Trials and Clinical endpoint study (Topical Nasal Suspensions).   The number of subjects required is determined by the error variance (%CV) of the primary characteristic (AUC or Cmax). Healthy volunteers (male and female) of age 18-55 years old, BMI = 18-30 kg/m2 (Asians = 18-25), should be screened for clinical laboratory tests, medical history prefer non-smokers / without a history of alcohol or drug abuse.

Statistical Interpretation of BE Data:
Bioequivalence problem is relatively new area. Pharmaceutical companies are pretty much interested and statistical knowledge is demanding in this field.

· BA parameters used for BE assessment are AUC, C_{max} and t_{max}
· AUC, C_{max} should be log transformed before statistical analysis
· logAUC or C_{max} of both products will be compared using ANOVA
· Construct a 90% CI for comparison between both products
· T_{max}, non-parametric statistics used on untransformed data
· Summary statistics such as median, min, max should be given
· Bioequivalence criteria: (i).Two one-sided tests procedure, (ii).Test (T) is not significantly less than reference, (iii).Reference (R) is not significantly less than test, (iv).Significant difference is 20% (a = 0.05 significance level), (v).If test is 80, reference is 100 then T/R ratio is 80 and If test is 100, reference is 80 then T/R ratio is 125.

Acceptance range for PK parameters of average BE is evaluated

- AUC-ratio   90% CI = 80-125%
- C_{max}-ratio   90% CI = 80-125%
    90% CI = 75-133% (high variable drugs)
- t_{max} 90% CI must fall within clinically relevant interval

Biopharmaceutical Classification Of Drugs:
Based on aqueous solubility and intestinal permeability biopharmaceutical classification system classifies the drugs as
Class 1: High soluble, highly permeable
Class 2: Low soluble, highly permeable
Class 3: High soluble, low permeable
Class 4: Low soluble, low permeable

High solubility:
A drug substance is considered highly soluble when the highest dose strength is soluble in 250 ml or less of aqueous media over the pH range of 1-7.5. The pH solubility profile of the drug substance is determined at $37 \pm 10°C$ in aqueous medium with pH in the range of 1-7.5. A sufficient number of pH conditions should be evaluated to accurately define the pH-solubility profile.

High permeability:
A drug substance is considered to be highly permeable when the extent of absorption in humans is determined to be 90% or more of an administered dose based on a mass balance determination or in comparison to an intravenous reference dose. (e.g., when the absolute bioavailability is 90% or more, or when 90% or more of the administered drug is recovered in urine). The methods used for determination of permeability include:

a. Mass balance studies, Absolute bioavailability studies and Intestinal perfusion methods in human
b. In vivo or in situ intestinal perfusion in a suitable animal model
c. In vitro permeability methods using excised intestinal tissues
d. Monolayers of suitable epithelial cells e.g. Caco-2 cells or TC-7 cells

Class I drugs exhibit a high dissolution and absorption. The rate limiting step is drug dissolution and if dissolution is very rapid then gastric emptying rate becomes the rate determining step.

Examples: Metoprolol, Diltiazem, Verapamil, Propranolol, Abacavir, Acetaminophen, Acyclovir, Amitryptyline, Antipyrine, Atropine, Bucspirone, Caffeine, Captopril, Chloroquine, Chlorpheniramine, Cyclophosphmide, Desipramine, Diazepam, Dilitiazem, Diphenhydramine, Disopyramide, Doxepin, Doxycycline, Enlapril, Ephedrine, Ergonovine, Ethambutol, fluoxetine, Glucose, Imipramine, Ketorolac, Ketoprofen, Labetolol, Levodopa, Levofloxacin, Meperidine, Metoprolol, Metronidazole, Midazolam, Minocycline, Misoprostol, Nifedipine, Phenobarbital, Phenylalamine, Prednisolone, Primaquine, Promazine, Propranolol, Quinidine, Rosiglitazone, Salicylic acid, Theophylline, Verapamil, Zidovudine. Risperidone.

Class 2 drugs have a high absorption but a low dissolution therefore absorption is limited primarily by drug dissolution in the gastrointestinal tract[31]. In vivo drug dissolution is then a rate limiting step for absorption except at a very high dose.

Examples: Phenytoin, Danazol, Ketoconazole, Mefenamic acid, Nifedinpine. Amiodarone, Atorvastatin, Azithromycine, Carbamazepine, Carvedilol, Chlorpromazine, Ciprofloxancin, Cyclosporine, Danazol, Dapsone, Diclofenac, Digoxine, Erythromycin, Flurbiprofen, Glyburide, Griseofulvin, Ibuprofen, Indinavir, Indomethacin, Itraconazole, Ketoconazole, Lansoprazole, Lovastatin, Mebendazole, Naproxen, Nelfinavir, Ofloxacin, Oxaprozin, Phenazopyridine, Phenytoin, Piroxicam, Raloxifene, Ritonavir, Saquinavir, Sirolimus, Spironolactone, Tacrolimus, Talmolol, Tamoxifen, Terfenadine, Warfarin

Class 3 drugs, have high dissolution, low absorption. In vivo permeability is rate limiting step for drug absorption. 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.

Examples: Cimetidine, Acyclovir, Neomycin B, Captopril. Ranitidine, Acyclovir, Amiloride, Amoxicillin, Atenolol, Atropine, Bisphosphonates, Captopril, Cefazolin, Cetrizine, Cimetidine, Ciprofloxacin, Cloxacillin, Dicloxacillin, Erythromycin, Famotidine, Fexofenadine, Ganciclovir, Lisinopril, Metformin, Methotrexate, Nadolol, Pravastatin, Penicillins, Tetracyline, Trimethoprim, Valsartan, Zalcitabinne.

Class 4 drugs exhibit a lot of problems for effective oral administration. The route of choice for administering is parenteral with the formulation containing solubility enhancers.

Examples: Taxol. Amphotericin B, Chlorthalidone, Chlorothiazide, Colistin, Ciprofloxacin, Furosemide, Hydrochlorothiazide, Mebandazole, Methotrexate, Neomycin.

Factors Influencing Bioavailability:
A. Pharmaceutical factors
1. Physicochemical attributes of Drug substances
- Drug solubility and dissolution rate
- Particle size and effective surface area
- Bulk and tapped density, Powder flow characterization
- Polymorphism, amorphism and hygroscopicity
- Pseudopolymorphism (hydrates/solvates)
- Salt form of the drug
- Lipophilicity
- pKa of the drug and pH
- Drug stability (% volatile, LOD, moisture content)
2. Dosage Form Characteristics and Pharmaceutical Ingredients
- Disintegration time (tablets/capsules)
- Dissolution time
- Manufacturing variables (method of granulation, compression force, intensity of packing of capsule contents)
- Pharmaceutical ingredient (excipients/adjuvants)

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- Nature and type of dosage form
- Product age and storage condition

B. Barrier functions of the organism:
- Age
- Gastric emptying time & Intestinal transit time
- Gastrointestinal pH
- Diseases states
- Blood flow through the GIT
- Gastrointestinal contents:
  a.Other drugs, b. Food, c. Fluids, d. Other normal GI contents
- Presystemic metabolism by:
  a. Lumenal enzymes, b. Gut wall enzymes, c. Bacterial enzymes, d. Hepatic enzymes.

Physicochemical Attributes and Drug Permeability:
The bioavailability of oral drug administration is a function of Molecular characteristics, Dosage form design, and Barrier function of the organism. Barrier to oral delivery of a compound can involve both stability and transport. GIT is challenging environment for stability and transport of drug. Permeation depends on molecular size, aqueous solubility and liphophilicity. Drug of large molecular size transported through receptor mediated endocytosis (Transcytosis), and low molecular size (<400-500) through paracellular route (passive diffusion across the intestinal membrane). The Gastro intestinal tract pH from 1-8 reacts may cause acid or base catalysis hydrolysis or non-specific hydrolysis by enzymes includes pepsin, pancreatic enzyme, cytoplasm to drug and Gastric transit time. Drug bioavailability can be altered by the nature of food present.

Physiological factors [8]

Parameters	Stomach	Small intestine	Large intestine	Rectum
pH range	1-3	5-7.5	7.9-8.0	7.5-8.0
Length(cms)	20	285	110	20
Diameter (cms)	0.1-0.2	2.5	5	2.5
Surface area (m^2)	15	200	0.15	0.02
Transit time (hrs)	1-5	3-6	6-12	6-12
Absorption role	Lipophilic acidic and neutral drugs	All type of drugs	Some drugs water and electrolytes	All type of drugs
Absorptive mechanisms	Passive diffusion convective transport	All absorption mechanism	Passive diffusion convective transport	Passive diffusion convective transport endocytosis

A drug with poor bioavailability is the one with
- Poor aqueous solubility and/or slow dissolution rate in the biologic fluids.
- Inadequate partition coefficient and thus poor permeation through the biomembrane
- Poor stability of the dissolved drug at the physiologic pH
- Extensive presystemic metabolism

Particle size [9]:
Dissolution rate is typically influenced by particle size and wettability. The influence of wettability on the dissolution rate of pharmaceutical powder was studied by Lippold and ohm. Example: wetting agent: Polysorbate 80.

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Polymorphism [10]:
New drug substances exist in different crystalline forms which differ in their physical properties. Polymorphism may also include salvation or hydration products (also know as pseudopolymorphs) and amorphous forms. Polymorphism has direct impact on solubility. Particle shape and powder density is depended on polymorphism (crystal forms). These two physical parameters can affect manufacturability resulting in poor flowability and compaction.

Example: Ibuprofen, acetaminophen because of crystal habit of drug tendency of poor flow and sticking. Bioequivalent product manufactured with control of the polymorphic form of the drug substance and the dissolution behaviour of the drug product.

Ionization:
An acid in acid solution will not ionize; an acid in basic solution will ionize, A base in a basic solution will not ionize; a base in acid solution will ionize. The amount of drug that exists in unionized form is a function of dissociation constant (pK_a) of the drug and pH of the fluid at the absorption site.

The negative log of the acid ionization constant (pka) is defined as the ability of an ionizable group of an organic compound to donate a proton (H^+) in aqueous media normally at 25°C. Henderson–Hasselbach equations are used to identify the percent of drug ionized at gastrointestinal pH. i.e.,

Acids: $pH = pK_a + \log$ ionized drug concentration/Unionized drug concentration
Bases: $pH = pK_a + \log$ unionized drug concentration/ionized drug concentration
When the concentration of ionized and unionized drug becomes equal, and thus $pH = pK_a$.
When the concentration of ionized and unionized drugs are not equal, the percent of ionization calculated by following formula:

Percent of Ionization[21] $= 100 \; 1 + 10^{X(pH-Pka)}$ Where x = -1 for acid drug, 1 for basic drug.

Thumb rule for estimating % of ionization for acid and bases for easy to remember[12]:

pH-pKa	Percentage of Ionization	
	Acids	**Bases**
≥ -3	0.1	99.9
-2 to -3	1	99
-1 to 2	10	90
0	50	50
≥ 1	90	10
2 to 3	99	1
≥ 3	99.9	0.1

Influence of drug pK_a and GI pH on Drug Absorption

S.No	Drugs	pK_a	pH/Site of absorption
1.Stronger acid (pK_a<2.5)			
	Disodium cromoglycate	2.0	Ionized at all pH values; Poorly absorbed from GIT
2.Moderately weak acids (pK_a 2.5 to 7.5)			

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	Cloxacilin	2.7	Unionized in gastric pH and ionized in intestinal pH; Better absorbed from stomach
	Aspirin	3.5	
	Ibuprofen	4.4	
3.Very weak acids (pk$_a$>8.0)			
	Pentobarbital	8.1	Unionized at all pH values; Absorbed along the entire length of GIT
	Ethosuximide	9.3	
4.Stronger base (pk$_a$>11.0)			
	Mecamylamine	11.2	Ionized at all pH values; Poorly absorbed from GIT
	Guanethidine	11.7	
5.Moderately weak bases (pK$_a$ 5 to 11.0)			
	Reserpine	6.6	Ionized at gastric pH; relatively unionized at intestinal pH. Better absorbed from intestine.
	Amitriptyline	9.4	
6.Very weak bases (pK$_a$<5.0)			
	Theophylline	0.7	Unionized at all pH values; absorbed along the entire length of GIT.
	Diazepam	3.7	

Partition coefficient:

partition coefficient of a drug substance can provide useful information about its permeability characteristics. The partition coefficient is the ratio of concentrations of un-ionized compound between the two solutions. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is called log P.

LogP is the octanol-water partition coefficient, P, is a measure of the differential solubility of a neutral substance between these immiscible liquids and thereby, a descriptor of hydrophobicity (or the lipophilicity) of a neutral substance. It is typically used in its logarithmic form, logP. Higher the value, more the hydrophilic and faster the dissolution in aqueous fluids. If log p>4 then the drug is very lipophilic, which is practically estimated by shake flask method. Usually intestinal permeability increases with liphophilicity but decreases with molecular weight or H-bonding properties. The formula to calculate log P is given below.

$$log \ P_{oct/wat} = log\left(\frac{[solute]_{octanol}}{[solute]_{water}^{un-ionized}}\right)$$

Ideally, for optimum absorption, a drug should have sufficient aqueous solubility to dissolve in the fluids at the absorption site and lipid solubility high enough to facilitate the partitioning of the drug in the lipoidal biomembrane and into systemic circulation. In other words, a perfect hydrophilic-lipophilic balance (HLB) should be there in the structure of the drug for optimum bioavailability. Gastro intestinal tract is a simple lipoidal barrier to the transport of drug, Larger the fraction of unionized drug, faster the absorption and greater the lipophilicity ($K_{o/w}$) of the unionized drug, better the absorption.
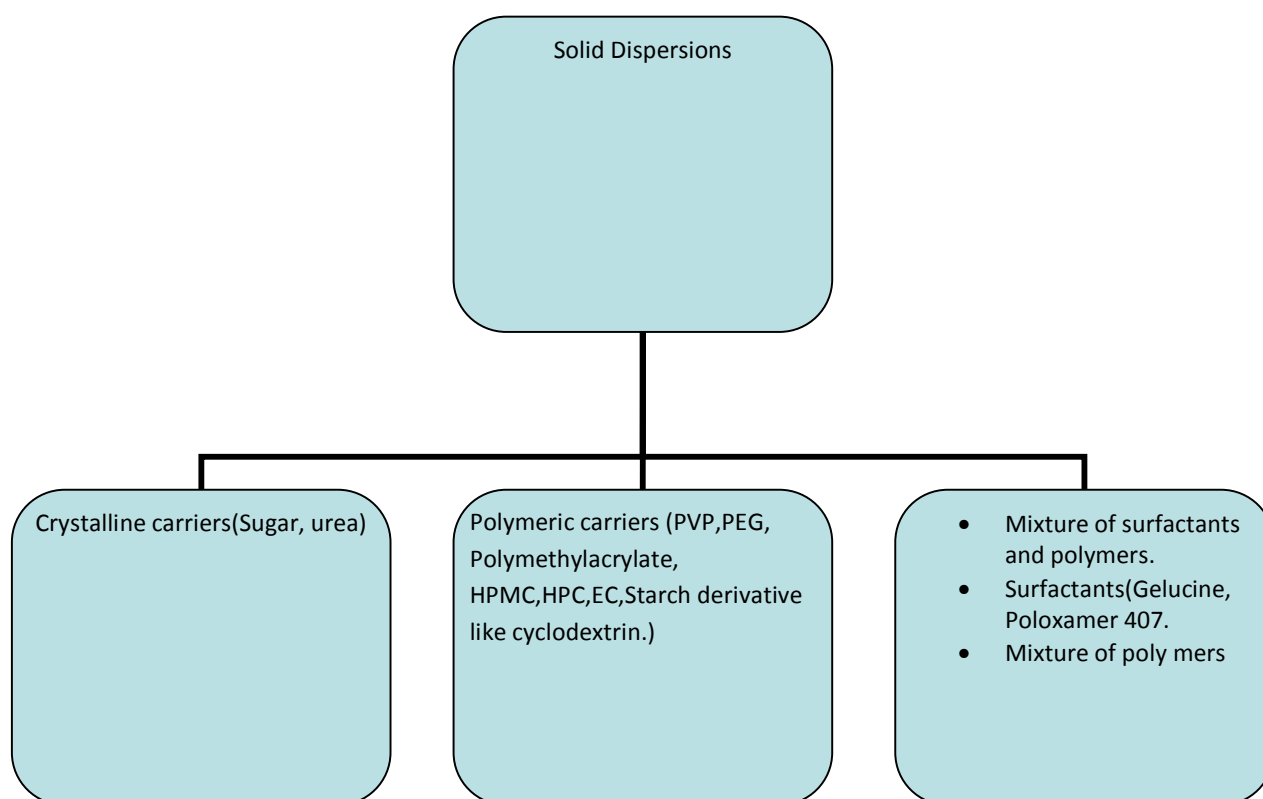
Improving Bioavailability by Manufacturing Process:
The three major approaches in overcoming the bioavailability problem are:
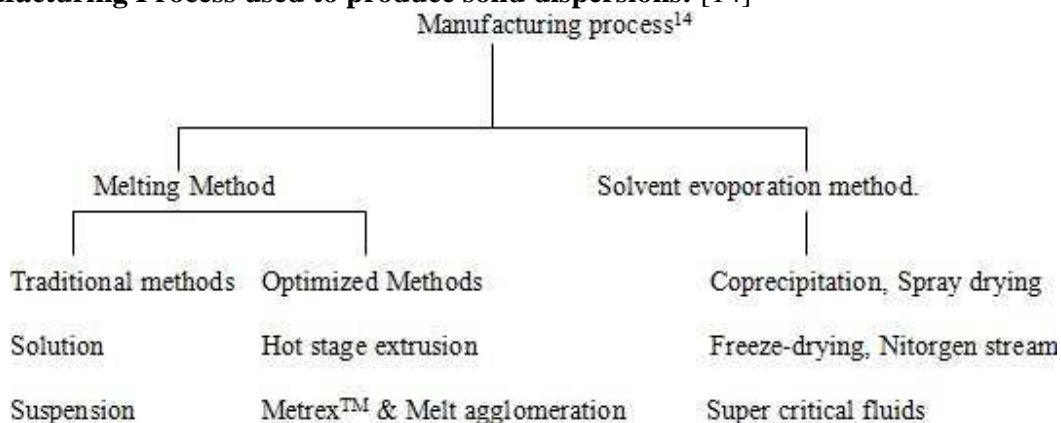- The Pharmaceutical approach, which involves modification of formulation, manufacturing process or the physicochemical properties of the drug without changing the chemical structure.

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- The chemical approach in which the pharmacokinetics of the drug is altered by modifying its chemical structure. This approach includes salt formation or incorporating polar or ionizable groups in the main drug structure resulting in the formation of prodrug.
- The Biologic approach whereby the route of drug administration may be changed such as changing from oral to parentral route.

The attempts, whether optimizing the formulation, manufacturing process or physicochemical properties of the drug, are mainly aimed at enhancement of dissolution rate, as it is the major rate-limiting step in the absorption of most drugs. Increasing the effective surface are of the drugs will be discussed briefly.

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                          │  Solid Dispersions  │
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│ Crystalline carriers │ │ Polymeric carriers   │ │ • Mixture of         │
│ (Sugar, urea)        │ │ (PVP,PEG,            │ │   surfactants and    │
│                      │ │ Polymethylacrylate,  │ │   polymers.          │
│                      │ │ HPMC,HPC,EC,Starch   │ │ • Surfactants        │
│                      │ │ derivative like      │ │   (Gelucine,         │
│                      │ │ cyclodextrin.)       │ │   Poloxamer 407.     │
│                      │ │                      │ │ • Mixture of poly    │
│                      │ │                      │ │   mers               │
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Manufacturing Process used to produce solid dispersions: [14]

Manufacturing process[14]		
Melting Method		**Solvent evoporation method.**
Traditional methods	Optimized Methods	Coprecipitation, Spray drying
Solution	Hot stage extrusion	Freeze-drying, Nitorgen stream
Suspension	Metrex™ & Melt agglomeration	Super critical fluids

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Solid Dispersions[13]:

Solid dispersion is drug dispersed in a biologically inert matrix. Drug in soluble hydrophilic carrier improves the dissolution rate by reducing particle size, higher porosity, drug is in amorphous state, improving wettability and hence possibly bioavailability for poorly water soluble drugs**.** Polymers used are Polyethylene glycol, polyvinyl pyrollidone of low molecular weight material such as sugars. The mechanism of improving dissolution was not yet understood. Recently surfactants have been included to stabilize the formulations, this avoiding drug recrystallisation and potentiating their solubility.

Co-precipitation method:

Solute and solid carrier solvent are dissolving in a common volatile liquid solvent such as alcohol. The liquid solvent is removed by evaporation under reduced pressure or by freeze-drying which result in amorphous precipitation of solute in a crystalline carrier. Example: amorphous sulfathiazole in crystalline urea.

Such dispersions are often called as co-evaporates or co-precipitates. The method is suitable for thermolabile substances but has a number of disadvantages like higher cost of processing, use of large quantities of solvent, difficulty in complete removal of solvent, etc

The key problem areas in solid dispersions are:
1. The solid state structure
2. The mechanism by which dissolution enhancement occurs
3. The stability of the dispersions on storage
4. Poor understanding of IVIVC.

The methods of preparation of solid dispersion: (i) Melting (fusion) (ii) Solvent or melting solvent method. [14]

Nanosizing[15]:

Reducing drug particle size to below submicron level i.e., 100-200nm. This reduction of particle size leads to significant increase in the dissolution rate of drug. Elans nanomilling technology is utilized, which works on two approaches 'Top down' (Wet milling technology) and 'bottom up' (Precipitation, crystallization). Stabilizers are used to stabilize the nanosupension against inter-particle forces between particles due to dispersion or Vander walls forces. The inter-particle forces are needed to overcome by repulsive forces. Polymer stabilizers (Examples:. Hydroxy propyl cellulose, Hydroxy propyl methyl cellulose, PVP K30,) and Surfactant stabilizers (Examples: Tween 80, Sodiumlauryl sulphate, Docusate sodium) are used. There are two modes of imparting repulsive forces or energetic barriers to colloidal system. Steric stabilization and electrostatic stabilization. Steric stablisation is achieved by adsorbing polymers in to particle surface, Electrostatic stablization is obtained by adsorbing changed molecules, which can be ionic surfactants or charged polymers, on to the particle surface. Nanosupension are typically converted to a solid dosage form by spray drying process.

Micronization:

The process involves reducing the size of the solid drug particles to 1 to10 microns commonly by spray drying or by use of air attrition methods (fluid energy mill). Examples of drugs whose

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bioavailability have been increased by micronization include griseofulvin and several steroidal and sulfa drugs. Greater the surface area, faster the dissolution, can be increased by micronization of drug

Cogrinding of drug with Excipients[16]:
Particle size reduction is performed by milling in jet miller for poorly soluble compound to increase the bioavailability after micronisation of drugs. In cogrinding method the large quantities of water soluble polymers are used as an excipient. Markus Vogt et.al[17] describes the rate of dissolution of poorly soluble drugs albendazole, felodipine was improved by cogrinding them with various excipients like lactose monohydrate, cornstarch, polyvinylpyrolidone, hydroxypropyl methyl cellulose and sodiumlauryl sulphate .

Lyophilization:
The material to be dried is first frozen and then subjected under a high vacuum to heat (supplied by conduction or radiation or by both), so that the frozen liquid sublimed leaving only the solid, dried components of the original liquid. The four components of freeze driers are vacum chamber for drying, vacum source, heat source and vapor removal system. Gole et al[32] and Lawrence et al[33] describes the inventive preparation of lyophilized matrix with gelatin, pectin, soy fibre protein and mannitol. The low soluble and bitter actives like risperidone and ibuprofen are coated by particulate coating with natural or synthetic polymer and organic solvents and dried by vapor removal system. The Matrix disperses rapidly with in 10 seconds in water and thus improves dissolution.

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

Use of Salt forms:
Salts have improved solubility and dissolution characteristics in comparison to the original drug. Alkali metals salts of acidic drug like penicillin's and strong acid salts of basic drugs like atropine are more water-soluble than the parent drug.

Alteration of pH of the Drug Microenvironment: Buffered aspirin tablets
Use of more soluble metastable polymorphs: the B form of chlorampheical palmitate is more water-soluble than the A and the C forms.

Solute-solvent complexation: solvates of drugs with organic solvents (also called as pseudopolymorphs) generally have higher aqueous solubility than their respective hydrates or the original drug. Much higher solubility can be

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

Selective adsorption on insoluble carriers:
The weak physical bonding between the adsorbate and the adsorbent, and hydration and swelling of the clay in the aqueous media. Bentonite can enhance the dissolution rate of poorly water-soluble drugs such as griseofulvin, indomethacin and prednisolone by maintaining the concentration gradient at its maximum.

Solid solutions :
A solid solution is a binary system comprising of a solid solute molecularly dispersed in a solid solvent by fusion method whereby physical mixture of solute and solvent are melted together followed by rapid solidification. Griseofulvin-succinic acid

Eutetic Mixtures :
Eutetic melts differ from solid solution in that the fused melt of solute-solvent show complete miscibility but negligible solid-solid solubility i.e. such systems are basically intimately blended physical mixture of two crystalline components. Paracetamol-urea, grisefulvin-urea. The methods cannot be applied for drugs which fail to crystallize from the mixed melt, thermolabile drugs and carriers such as succininc acid that decompose at their melting point. The eutectic product is often tacky, intractable or irregular crystal.

Molecular encapsulation and cyclodextrins:
The beta and gamma cyclodextrins and several of their derivatives are unique in having the ability to form molecular inclusions complexes with hydrophobic drug having poor aqueous solubility. These cyclodextrin molecules are versatile in having a hydrophobic cavity of size suitable enough to accommodate the lipophilic drugs as guest; the outside of the host molecule is relatively hydrophilic. Thiazide diuretics, barbiturates, benzodiazepines

Invitro Dissolution Studies:
In the pharmaceutical industry, dissolutin is defined as the amount of drug substance that goes into solution per unit time under standardized conditions of liquid/solid interface, temperature and solvent composition. ie. mass transfer from the solid surface to the liquid phase. Intrinsic dissolution rate can be defined as rate of dissolution pure pharmaceutical active when conditions such a pH, surface area, temperature, agitation, rate and ionic strength of dissolution media kept constant.

Dissolution as a Process

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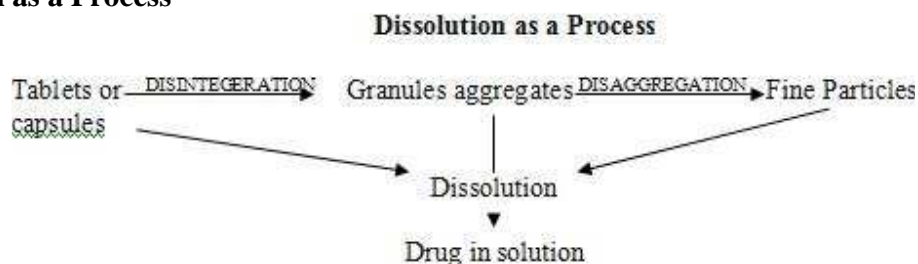
Important dissolution factors can be identified by Noyes Whitney equation[32]:

$$\frac{dC}{dt} = D.A \times \frac{(C_s - C_b)}{h}$$

Where dC/dt is a rate of drug dissolution at time 't'

A = surface area of the particle, h=thickness of the stagnant film layer

Cs=Saturated solubility of compound at the particle media interface

Cb= Concentration of compound in the bulk medium Cb<<<Cs dependency is only on Cs

D=1/N X (VA); D=diffusion coefficient of compound in the medium

   Where N= solvent viscosity; VA = Solute molecular volume;

In vivo tests are extremely costly, tedious and time consuming moreover exposing the healthy subjects to hazards of drugs. So need to reduce our reliance on in vivo studies. Dissolution is a prerequisite for bioequivalence since the drug must first dissolve before it can be absorbed by the gastrointestinal tract[19]. Dissolution tests are now designed to mimic the general conditions encountered in the physiological environment of the GIT. The dissolution of drugs from orally administered solid dosage forms in vivo and in vitro is influenced by variations in the natural or simulated gastrointestinal fluid (and physical variables such as hydrodynamic flow, and mechanical stress. The physiological conditions that can affect drug release include the following: Intestinal transit time, gastric emptying and variable pH.

The dissolution test methods are also now designed to mimic the general conditions encountered in the physiological environment of the GIT and they are desirable alternate for in vivo tests as well as quality control tests.

Dissolution Method Development:

There are several factors that must be considered in the design of a dissolution test. Selection of apparatus, Nature of agitation, Speed of agitation (50/75/100 rpm), Performance precision of the apparatus, media composition, Viscosity, Volume (500/900/1000/2000ml), Temperature and 'sink conditions' to be maintained, since in vivo 'sink condition' created due to intestinal permeability and in vivo dissolution is a complex process, method of introduction of the dosage form, location of dosage unit, sampling techniques, changing the dissolution fluid, pH of the media and Time points to get discrimination etc.

Systematic Approach involves:

• Literature information: Reference listed drugs, summary basis of approval, Physicians desk reference, Pharmacokinetic data, BCS class, food affects, particle size, crystal form, bulk density of API.

• **Sink condition:** Maintenance of large volumes of solution defined as 'sink conditions' (drug concentration in solution maintained constant at a low level) 3 times the unit dose to be taken for studies, NLT 1.5 times the unit dose is the acceptance criteria; at 25°C using drug, at 37±0.5°C using drug; At 25°C using drug + process placebo; at 37±0.5°C using drug + process placebo.

• Dissolution media selection based on physiological conditions, pH solubility profile, pH stability profile, pKa of the drug substance, partition coefficient.

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- Medias:0.1N HCl, 0.01N HCl, pH 4.5 Acetate/Phosphate buffer, pH 6.8 Phosphate buffer, pH 7.2 /7.4 Phosphate buffer.

- **Biorelevant dissolution medias[20].:** Medias used for studying fast and fed effects on release from dosage forms.

FaSSIF	Fasted state simulated intestinal fluid	pH6.5
FeSSIF	Fed state simulated intestinal fluid	pH 5.0
SGF	Simulated Gastric fluid	pH 1.2
FaSSGF	Fasted state simulated Gastric fluid	pH1.8

Composition of FeSSIF (pH 6.5)

Sodium taurocholate	3m/M
Lecithin	0.75m/M
NaH_2PO_4	3.438g
NaCl	6.186g
NaOH pellets	qs to adjust pH 6.5
Deionized water	qs to adjust 1 liter

Composition of FaSSIF (pH 5.0)

Sodium taurocholate	3m/M
Lecithin	0.75m/M
Acetic acid	8.65g
NaCl	11.874g
NaOH pellets	4.04g
Deionized water	qs adjust to 1liter

- **Temperatures:** Body temperature $37\pm0.5°C$
- **Sinkers** for low weight formulations
- If the drug is highly hydrophobic and insoluble, buffers with added surfactants can be used. Surfactant of 1% is preferable, beyond 2% shall be justified.
- If the Tmax is less than 2 hours- acidic media is preferred for the release testing. Discrimination pH should be determined
- If food affects bio-availability, then simulated media study is very important and pure grade of reagents should be used for simulated media preparation.
- Selection of apparatus[21-22].

United Sates of Pharmacopiea:

-Apparatus 1	Rotating basket	Tablets/Capsules
-Apparatus 2	Paddle assembly	Tablets/Capsules
-Apparatus 3	Reciprocating cylinder	Escalating pH media
-Apparatus 4	Flow-through cell	Low soluble drugs
-Apparatus 5	Paddle over disk	Semisolids and transdermal
-Apparatus 6	Cylinder	Transdermal patches
-Apparatus 7	Reciprocating holder	Transdermal patches

European Pharmacopiea:

-For solid dosage forms	Paddle/basket/flow through cell
-For transdermal patches	Disk assembly method/cell method/rotating cylinder
-For special dosage forms	Chewing apparatus/Flow through apparatus.

- Crescent shaped spindle can be used to improve poor hydrodynamics of paddle apparatus and thus improved and bio-relevant dissolution characteristics[31] .

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- **Physical observation:** Disintegration pattern, floating particle of drug or excipients, Heap formation, Cone effect.
- **Investigation during analysis:** If drug is degrading during dissolution, inject dissolution sample in RS method and find out the possible degradant.
- **Influencing Parameters of dissolution**
  - Wetting speed   - Surface tension - Contact angle
  - Addition of surfactant – Air bubble trapping
  - Hydrophobic lubricant like talc, magnesium stearate in formulations
  - For capsule gelation is hydrophilic
  - Wetability of powder bed inside capsules
  - Disaggregatrion – Compactibility
  - Tablets – pore volume is mall – addition of disintegrates – strain and rupture

Limitations of dissolution testing:

Invitro dissolution testing can be non–discriminate: Example; Mebandazole polymers A, B, and C dissolution profiles met the specification of 75% dissolved in 120 minutes although they exhibited different therapeutic effects.

Invitro dissolution testing can be over –discriminate: Example; FDA sponsored studies with manufactured fast, medium and slow dissolving tablets of metoprolol and propranolol. Slow dissolving tablets of metoprolol failed in USP dissolution test. However the in vivo pharmacokinetic a study demonstrates the bioequivalence of fast, medium and slow dissolving tablets with their corresponding formulations. The IVIVC suggests that in vivo dissolutions are not rate limiting step for this formulation so that difference in dissolution rate does not make any difference.

Formulation specific IVIVC: IVIVC is only valid for one particular type of dosage form containing certain rate controlling excipients with the same release mechanism. If a drug is formulated in the same type of a solid dosage form, such as tablets, formulations with different drug release mechanisms would require the development of separate IVIVC with different *in vitro* dissolution methodology.

Setting of Dissolution Specifications[24]:

A specification[25] is defined as a list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. When a specification is first proposed, justification should be presented for each procedure and each acceptance criterion included.

The justification should refer to relevant development data, pharmacopoeial standards, test data for drug substances and drug products used in toxicology and clinical studies, and results from accelerated and long term stability studies, as appropriate. Additionally, a reasonable range of expected analytical and manufacturing variability should be considered. It is important to consider all of this information.

Immediate release : 3 categories of dissolution test specification are described

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(i) For BCS class 1 and 3 drugs of rapidly dissolving products single point dissolution in 0.1N HCl, 85% released at 15 mints. If dissolution is rapid then multi point and multimedia dissolution profile to be studied.

(ii)   For BCS class 2 drugs; Multi point (15,30,45 and 60 mints) and multimedia dissolution profile to be studied.

(iii)   **Dissolution profile Comparison[11]:**
Moore and Flanner proposed a model independent mathematical approach to compare the dissolution profiles using two factors, f_1(difference factors) and f_2(similarity factors). The formula used to calculate f_1 and f_2 are

$$f_1 = \{[\textstyle\sum_{t=1}^{n} |R_t - T_t|]/[\sum_{t=1}^{n} R_t]\} \cdot 100$$
$$f_2 = 50 \cdot \log\{[1+(1/n)\textstyle\sum_{t=1}^{n}(R_t - T_t)^2]^{-0.5} \cdot 100\}$$

Where log = logarithm base 10, n= number of sampling time points, $\sum$=summation over all time points, R_t= dissolution time point t of the reference (pre-change batch), T_t=dissolution at time point t of the test(post change)

The f_1 value should be between 0-15 to indicate difference between two dissolution profiles. The f2 value should be between 50-100 to indicate similarity between two dissolution profiles. When the two profiles are identical, f_2=100. If an average of 10% difference at all measured time points results then f_2 = 50. When both test and reference products dissolve 85% or more of the label amount of the drug in ≤15 minutes using all three dissolution media recommended above, the profile comparison with an f_2 test is unnecessary.

Conditions for a Dissolution profile comparison:
* At least 12 units should be used for each profile determination. Mean dissolution values can be used to estimate the similarity factor, f2. To use mean data, the % coefficient of variation at the earlier point should not be more than 20% and at other time points should not be more than 10%.
* For circumstances where wide variability is observed, or a statistical evaluation of f2 metric is desired, a bootstrap approach to calculate a confidence interval can be performed.
* The dissolution measurements of the two products (test and reference, pre- and post-change, two strengths) should be made under the same test conditions. The dissolution time points for both the profiles should be the same, e.g., for immediate release products 15, 30, 45 and 60 minutes, for extended release products 1, 2, 3, 5 and 8 hours.
* Because f2 values are sensitive to the number of dissolution time points, only one measurement should be considered after 85% dissolution of the product.
* For products which are rapidly dissolving, i.e., more than 85% in 15 minutes or less, a profile comparison is not necessary.
* A f2 value of 50 or greater (50-100) ensures sameness or equivalence of the two curves and, thus, the performance of the two products.

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Delayed release :
A modified release product in which the release of active substance is delayed for a finite "lag time", after which release is unhindered [e.g. enteric coated or "Gastro resistant" (Ph.Eur.) oral tablets or capsules which remain intact in the stomach and only disintegrate in the higher pH of the small intestine]. Delayed release results in a longer Tmax but with Tmax and elimination half life unchanged.

In delayed release component, the drug may not be sufficiently protected for residence time greater than 2 hours in the gastric pH of 1.2. Low pH may also alter the performance by causing chemical reactions of the materials used in the dosage for modifying the release of drug. Therefore, while the final dissolution test may only require a 1-2 hour presoak at gastric pH, the dosage form should be thoroughly evaluated at gastric pH if there is potential for long gastric residence times. If the goal of the dosage form is to release the drug in the duodenum, e.g., target transport through tight junctions, then the dissolution test should reflect the possibility of a short residence time. This is especially true if the mechanism for targeting the release is enteric coating. Further hampering of drug release can occur if the enteric coating erodes at pH 6.5, since the pH at the proximal duodenum is closer to 5.5 than 6.5. Therefore, an appropriate dissolution test for pH sensitive release mechanism such as enteric-coated dosage forms may require several pHs simultaneously taking into consideration the potential in vivo residence time at each pH.

Coated particles/beads currently used in both extended release and delayed release dosage forms offer advantages over larger, non-disintegrating delivery systems. Depending on the design of the delivery system, dissolution tests for bead formulations may consist of 2-3 hours in simulated gastric fluid at pH 1.2, followed by 15-30 minutes in simulated intestinal fluid at pH 5.5, and then simulated intestinal fluid at pH 6.8 or pH 8.0.

Extended Release:
The FIP -Guideline and European Pharmacopeia demand at least 3 specification points, the first after 1-2 hours (around 20-30% drug release) to provide assurance against premature drug release. The second specification point has to be around 50 % drug release to define the dissolution pattern. At the last point, the dissolution limit should be at least 80 % drug release to ensure almost quantitative release. Alternatively, a dissolution of <80% has to be justified and should be supported by a test duration of at least 24 hours[26]. As can be seen, there are slight differences with regard to the United States Pharmacopeia, where only > 2 test points are demanded considering the individual monograph.

***In-vitro-In-vivo* Correlation:**
Invitro-invivo correlation is the demonstration of the direct relationship of *in vitro* dissolution rate of drugs and their in vivo bioavailability. Generally, the in vitro property is the rate or extent of drug dissolution or release while the in vivo response is the plasma drug concentration or amount of drug absorbed[28]. Correlation is used to ensure batch-to-batch consistency in the physiologic performance of a drug product by use of such in vitro values and to serve as a tool in the development of a new dosage form with desired in vivo performance.

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There are two basic approaches by which a correlation between dissolution testing and bioavailability can be developed.

1. By establishing a relationship, between the in vitro dissolution and the in vivo bioavailability parameters. If this relationship becomes linear with a slope of 1, then curves are super imposable, and there is a 1:1 relationship which is defined as point-to-point or level A correlation.
2. By using the data from previous bioavailability studies to modify the dissolution methodology in order to arrive at meaningful in vitro-invivo correlation.

Levels of IVIVC :
Five correlation levels have been defined in IVIVC FDA guidance.
Level A: Represents a point to point relationship between in vitro dissolution rate and in vivo input rate of the drug from the dosage forms. Usually estimated by a two stage procedure (Example: deconvolution followed by comparison of the fraction absorbed to the fraction dissolved). Generally linear, but non-linear are also acceptable.
Level B: Correlation based on statistical moment analysis. Example: in vitro MDT vs. in vivo MRT or MAT
Level C: In this level of correlation, one dissolution time point (t50%, t90%, etc.) is compared to one mean pharmacokinetic parameter such as AUC, t_{max} or C_{max}. Therefore, it represents a single point correlation. Example: in vitro T50% vs. in vivo Tmax
Multiple C :   Relationship between one or several pharmacokinetic parameters and amount of drug dissolved at several time points
Level D: It is a rank order and qualitative analysis and is not considered useful for regulatory purposes.

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Establishment of Invitro and Invivo data

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Level	Invitro	Invivo
A	Dissolution curve	Input (absorption) curves
B	Statistical moments (MDT)	Statistical moments (MRT, MAT, etc.)
C	Disintegration time, time to have 10, 50, 90% dissolved in dissolution rate, dissolution efficiency	Cmax – Tmax – Ka time to have 10, 50, 90% absorbed AUC (total or cumulative)

Systematic Development:
Assumed IVIVR: Is assume model, developed for prototype formulation, this assumed model can be the subject of as prototype formulations are developed and characterized in vivo, with the results often leading to a further cycle of prototype formulation and in vivo characterization.

Retrospective IVIVR. With a defined formulation that meets the in vivo specification, Stage 2 commences. At this stage based on a greater understanding and appreciation of defined formulation and its characteristics,

Prospective IVIVR is established through a well defined prospective IVIVR study. Once the IVIVR is established and defined it can be then used to guide the final cycle of formulation and process optimization.

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IVIVC in the product development process for extended-release products:
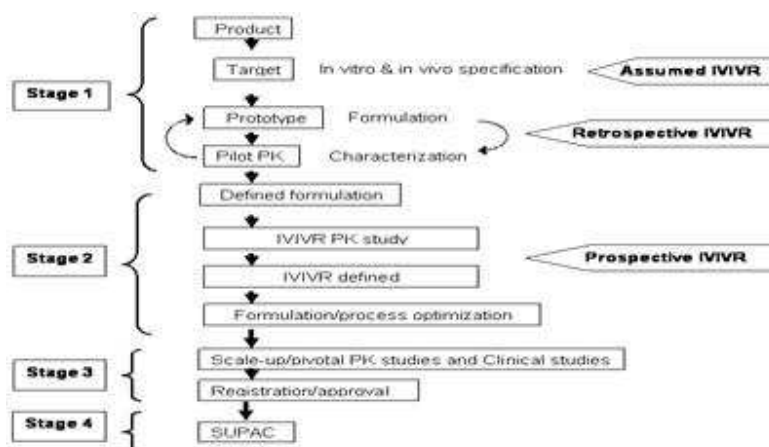
IVIVC expectations for immediate release products based on BCS :

Class	Absorption rate control	IVIVC expectation for immediate release product
1	Gastric emptying	IVIVC expected, if dissolution rate is slower than gastric emptying rate, otherwise limited or no correlations
2	Dissolution	IVIVC expected. If in vitro dissolution rate is similar to in vivo dissolution rate, unless very high dose.
3	Permeability	Absorption (permeability) is rate determining and limited or no IVIVC with dissolution
4	Case by case	Limited or no IVIVC is expected

IVIVC expectations for extended release products based on BCS:
For Controlled/Modified Release formulations (independent drug release) at least 1 batch has to be tested. All other cases need at least 3 batches to be tested with the following conditions: Profiles of at least 12 individual dosage units from each lot with a coefficient of variation of not more than 10 % are required by the FDA[30]. The number of volunteers to be included in the bioavailability study should be at least 12 according to the FIP guidelines, whereas 6 to 36 are accepted by the FDA

	Solubility	Permeability	IVIVC
1	High & Site independent	High & Site Independent	IVIVC Level A expected
1	High & Site Independent	Dependent on site and Narrow absorption window	IVIVC Level C expected
2	Low & Site Independent	High & Site Independent	IVIVC Level A expected
2	Low & Site Independent	Dependent on site and Narrow absorption window	Little or no IVIVC
Acidic	Variable	Variable	Little or no IVIVC
Basic	Variable	Variable	IVIVC Level A expected

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Application of An IVIVC

Biowaivers: Recent research has lead to the use of in-vitro tests to waive additional in vivo bioequivalency studies for some pharmaceutical products. The use of in vitro testing to achieve a waiver of in vivo studies is commonly referred to as a biowaiver.

The FDA guidance outlines five categories of biowaivers: 1) biowaivers without an IVIVC, 2) biowaivers using an IVIVC: non-narrow therapeutic index drugs, 3) biowaivers using an IVIVC: narrow therapeutic index drugs, 4) biowaivers when in vitro dissolution is independent of dissolution test conditions and 5) situations for which an IVIVC is not recommended for biowaivers.

Biowaivers for new drug :

Biowaivers of a higher strength will be determined to be appropriate based on (i) clinical safety and/or efficacy studies including data on the dose and the desirability of the higher strength, (ii) linear elimination kinetics over the therapeutic dose range, (iii) the higher strength being proportionally similar to the lower strength, and (iv) the same dissolution procedures being used for both strengths and similar dissolution results obtained. A dissolution profile should be generated for all strengths.

Biowaiver of Generic drug:

(i)Waiver of in vivo BE studies based on BCS: Recommended for a solid oral test product that exhibit rapid (85% in 30 mints) and similar in vitro dissolution under specified conditions to an approved reference product when the following conditions are satisfied:

· Products are pharmaceutical equivalent
· Drug substance is highly soluble and highly permeable and is not considered have a narrow therapeutic range
· Excipients used are not likely to effect drug absorption;
Examples: Excipient Effect for a BCS Class-3 drug(ranitidine) in bioavailability (Hussain et al. AAPS , 2000).
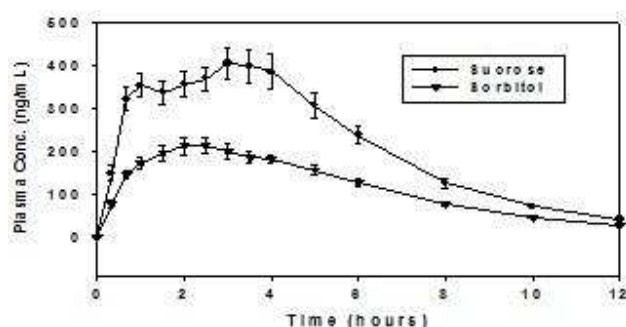
Ranitidine 150mg
Sucrose 5g
Sorbitol 5g.

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(ii). Waiver of invivo BE for IR oral dosage form: bioequivalence studies may be waived for compositionally similar strengths when one strength in a range has been studied, under these conditions the following conditions are satisfied

–   product are manufactured by the same manufacturer and process

–   linear pharmacokinetics

–   the qualitative composition of the different strengths is the same; except in the case of flavours/colours

–   the ratio between amounts of drug and excipients is the same or in case of preparations containing a low concentration of the drug (less than 5%), the ratio between the amounts of excipients is similar

–   the dissolution profile should be similar for additional strengths and the strength of the batch used in BE study

Waivers for Scale-up and Post approval changes: Biowaivers may be granted for manufacturing site changes, equipment changes, manufacturing process changes, and formulation composition changes according to a predictive and reliable IVIVC.

Summary:
Bioavailability of drug product can be altered by drug and excipients properties in the formulation and manufacturing process. Sccessful Pharmaceutical development is the perfect understanding of the in vivo and in vitro performance of the dosage form. In-vitro dissolution methods are developed with correlation of In-vivo parameters. In-vitro specifications are set to maintain the consistency and reproducibility of the in vivo characteristics (bioavailability) of the dosage forms.

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