Chapter II Literature review

II.1 Glibenclamide

II.1.1 Physicochemical properties

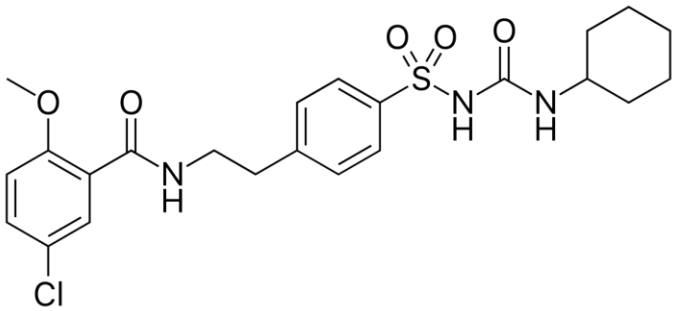


figure 1 chemical structure of glibenclamide

$C_{23}H_{28}ClN_3O_5S$

Glibenclamide is a white, crystalline, odourless powder; practically without taste. Molecular weight 494g/mol, its melting point has been reported as 172-1740, 169-170C° and 168-170C°. Its dose ranges between 2.5mg to 5 mg once a day. The solubility of Glibenclamide is virtually insoluble in water and ether; soluble in 330 parts of alcohol, in 36 parts of chloroform, and in 250 parts of methanol. It forms water-soluble salts with alkali hydroxides. Glibenclamide is a weak acid. It has been concluded that it has the same dissociation constant as tolbutamide PKA 5.3.(PubChem)

II.1.2 Pharmacology

Glibenclamide is a potent oral hypoglycemic agent from the second generation sulfonylureas used in the treatment of type II diabetes mellitus.

II.1.2.1 Sulfonylurea

Sulfonylureas have long been established in the treatment of diabetes and were the first oral glucose-lowering medications to be introduced into clinical practice. They account for around 20% of newly initiated oral diabetes medications. They are the mainstay of oral diabetes therapy in many parts of the world, including South Asia, either as first-line agents or in combination with other agents. They have helped provide symptomatic relief, better quality of life, and euglycaemia, to countless millions of people over the past half-century. Their economical cost, availability as fixed-dose combinations with metformin,

and comfortable acceptance by patients with diabetes, and physicians, alike implies that they cannot be whisked away'(Kalra, 2013).

II.1.2.2 Mechanism of action of sulfonylureas

The sulfonylureas produce their hypoglycemic actions via several mechanisms that can be broadly subclassified as **pancreatic** and **extra-pancreatic**

A. ***Pancreatic Mechanism***: All sulfonylurea hypoglycemics inhibit the efflux of K^+ (K^+ channel blockers) from pancreatic ß-cells via a sulfonylurea receptor which may be closely linked to an ATP-sensitive K^+channel. The inhibition of efflux of K^+ leads to depolarization of the ßcell membrane and, as a consequence, voltage-dependent Ca^{++}-channels on the ß-cell the membrane then opens to permit entry of Ca^{++}. The resultantly increased binding of Ca^{++} to calmodulin results in activation of kinases associated with endocrine secretory granules thereby promoting the exocytosis of insulin-containing secretory granules.

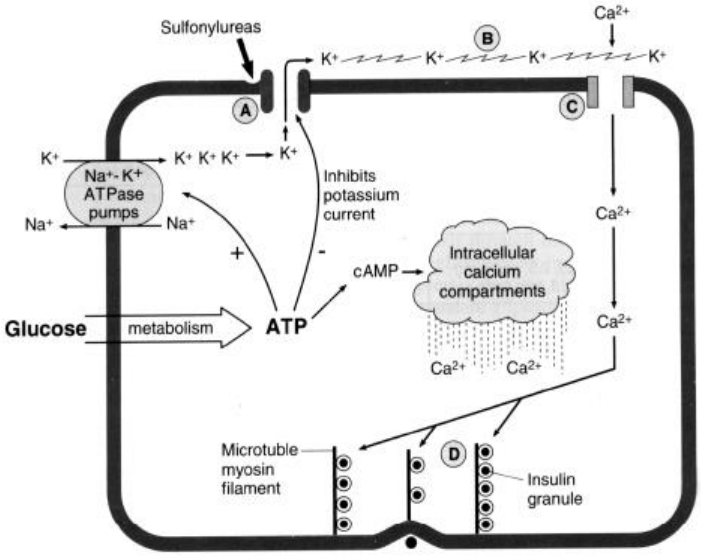


Figure 2 mechanism of action of sulfonylureas

B. ***Extra-Pancreatic Mechanisms***

The sulfonylureas also reduce serum glucagon levels possibly contributing to its hypoglycemic effects. The precise mechanism by which this occurs remains unclear but may result from indirect (secondary) inhibition due to enhanced release of both somatostatin and insulin.

Sulfonylureas may also potentiate insulin action at target tissues (drug-dependent characteristic).(Deruiter & Deruiter, 2003)

II.1.2.3 Insulin secretion

β-cells are excitable endocrine cells that secrete insulin. The essential secretory machinery is analogous to that for other peptide hormones such as GH and PRL, Although abnormalities in insulin secretion are not common, insulin secretion is of pharmacologic interest because oral hypoglycemic agents act by increasing insulin secretion.

Multiple mechanisms mediate insulin secretion, the most important being glucose-stimulated, K_{ATP} channel dependent. Other pathways either augment or complement that pathway.

Glucose-stimulated, K_{ATP} channel-dependent pathway starts with intracellular transport of extracellular glucose by a glucose transporter (GLUT2)

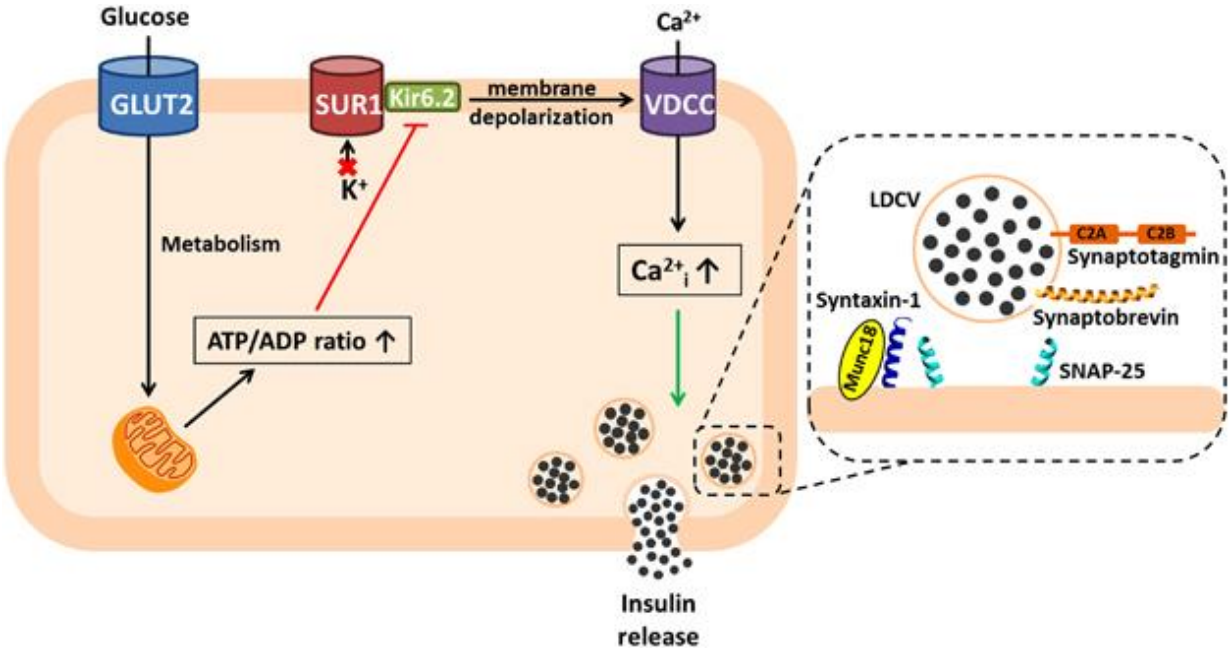


Figure 3 Schematic illustration of glucose-stimulated insulin

Intracellular glucose undergoes cytosolic glycolysis catalyzed by glucokinase (glucokinase gene mutations cause MODY2). Pyruvate, a product of glycolysis, is shuttled into mitochondria as a substrate of the tricarboxylic acid cycle with production of adenosine triphosphate (ATP). As a result, cytosolic ATP levels are elevated and adenosine 5´-diphosphate (ADP) levels reduced. Increased cytosolic ATP/ADP ratio closes an ATP-sensitive K^+ channel, K_{ATP}, which results in discontinued K^+ outflow, thus depolarizing the β-cell membrane.

Voltage-dependent calcium channels on the cell membrane are opened by depolarization, and calcium influx increases intracellular calcium (phase 1), which, in turn, activates

calcium-dependent calcium release from the endoplasmic reticulum (phase 2). The resultant biphasic increase in intracellular calcium triggers fusion of secretory vesicles with the plasma membrane and insulin is released.

The K_{ATP} channel plays the crucial role of converting metabolic to electric signals. All components of glucose- stimulated, K_{ATP} channel-dependent insulin secretion are found in several other cell types, except for the K_{ATP} channel, which is unique for β cells (a similar channel is also found in muscle and brain). Each β-cell posseses thousands of K_{ATP} channels, which is a high density considering the small size of β-cells. This channel has eight subunits; four identical smaller subunits, called Kir6.2 ("ir" stands for "inward rectifier"), which form the inner pore for K^+ passage; and four identical larger subunits, termed the sulfonylurea receptors (SUR1), which form the outer regulatory structure. ATP regulates K_{ATP} channel by binding to Kir6.2, and oral sulfonylurea hypoglycemic medications bind to SUR1 and close the K_{ATP} channel, thus stimulating insulin secretion. Not surprisingly, mutations in the K_{ATP} channel cause either hyperglycemia or hypoglycemia. Mutations in SUR1 and Kir6.2 that render the K_{ATP} channel less open cause insulin hypersecretion with neonatal hypoglycemia (nesidioblastosis). Conversely, other mutations in Kir6.2 that maintain the K_{ATP} channel in an open state result in neonatal diabetes. K_{ATP} channel mutations are also found in some patients with type 2 diabetes. Mitochondria produce ATP, the signal for the K_{ATP} channel, and they are implicated in a subtype of diabetes termed *mitochondrial diabetes* (1% of all diabetes). The most common is a mutation in the mitochondrial gene encoding transfer RNA for leucine, which decreases production of some mitochondrial proteins. Interestingly, this mutation causes two distinct syndromes:

(1) maternally inherited mitochondrial diabetes and deafness, and (2) mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes (MELAS syndrome), the latter often associated with diabetes. Uncoupling protein 2 (UCP2) is an anion carrier located on the mitochondrial inner membrane that uncouples proton gradient and ATP synthesis, thus decreasing ATP production and increasing heat generation. Mice deficient in β-cell UCP2 have increased ATP production and insulin hypersecretion. UCP2 is upregulated in obesity and may contribute to diabetes associated with obesity. Certain UCP2 gene polymorphisms are weakly associated with body mass index (measure of obesity) and type 2 diabetes.

Three other pathways are also known to stimulate insulin secretion. The augmentation pathway may facilitate the K_{ATP} channel–dependent pathway but is controversial. The

second pathway is also calcium dependent and works through Gq-coupled receptors that produce inositol triphosphate (IP3), which releases calcium from the endoplasmic reticulum through IP3 receptors. Acetylcholine releases insulin through this mechanism. GLP-1 and vasoactive intestinal peptide act through binding to Gs-coupled receptors that produce cyclic adenosine monophosphate, which activates PKA. PKA stimulates insulin secretion through a calcium- independent mechanism. GLP-1 is an ideal hormone candidate for diabetic treatment. Besides the stimulatory effects on β-cell mass growth, GLP-1- stimulated insulin release is regulated by serum glucose and is suppressed by hypoglycemia. In clinical trials, GLP-1 normalizes serum glucose in patients with type 2 diabetes without development of hypoglycemia.(Yu & Hui, n.d.)

II.1.3 Pharmacokinetics of Glibenclamide

- Onset is about 1.5 hrs. Administer about 30 minutes before a meal (breakfast)
- Glyburide is **extensively bound** by plasma proteins and is **recycled** hepatically. Both of these factors contribute to the prolonged duration of action. These are also a function of the complex Ar moiety.
- Metabolized in the liver. Glyburide has a **short plasma half-life** (2-10 hrs) but **prolonged biological effect** due to the formation of active metabolites! It is metabolized primarily by oxidation of the cyclohexyl ring (ω and ω-1 type oxidations), of the 4 possible isomeric metabolites, the *cis*-3-OH and *trans*-4-OH compounds are the major ones formed
- Elimination profile: 50% renal and 50% biliary.
- **Other than hypoglycemia**, fewer adverse effects than most first generation agents. It does not cause water retention (as does chlorpropamide). Hypoglycemia may be a problem due to the drug's prolonged therapeutic action.
- Contraindications based on clearance profile: **hepatic impairment** and **renal insufficiency.** Dose reduction is required in the elderly (from 2.5 mg/day to 1.25 mg/day).
- Combination formulations with metformin (Glucovance) (Deruiter & Deruiter, 2003)

II.2 Bioavailability and Bioequivalence

II.2.1 Bioavailability and Bioequivalence

Definition of Bioavailability

Bioavailability is a measurement of the extent of a therapeutically active medicine that reaches the systemic circulation and is therefore available at the site of action. For most medicines that are taken orally, the active ingredients are released in the gastrointestinal tract and arrive at their site of action via the systemic circulation. Blood concentrations of the active ingredients and/or their active metabolites thereby provide a marker for the concentration at the site of action and a valid measure of bioavailability. A blood concentration – time curve (achieved by serial measurements over time) reflects not just the release of the active ingredient from the medicine and its absorption from the GI tract, but also other factors including presystemic metabolism, distribution and elimination.

Bioavailability is assessed using three main pharmacokinetic variables

- the area under the blood drug concentration versus time curve (AUC)
- the maximum blood concentration (C_{max})
- the time to reach maximum concentration (T_{max})

Definition of Bioequivalence

two medicines are bioequivalent there is no clinically significant difference in their bioavailability. Although bioequivalence is most commonly discussed in relation to generic medicines, it is important to note that bioequivalence studies are also performed for innovator medicines in some situations such as:

- between early and late clinical trial formulations or between the formulations used in clinical trials and the product to be marketed for new medicines
- when changes in formulation have occurred after an innovator product has been approved, for example a change in one or more excipients (inactive ingredients)

Bioequivalence studies are a surrogate marker for clinical effectiveness and safety data as it would not normally be practical to repeat clinical studies for generic products. It is accepted that if plasma concentrations of the active ingredient of the generic and innovator medicines are the same, then their concentration at the site of action and therefore their safety and effectiveness will be the same. In addition to being bioequivalent, a generic medicine must conform to high quality standards in terms of the method of manufacture

and the purity of the final pharmaceutical form. There are internationally agreed standards for measuring and assessing bioequivalence.

Acceptance Criteria for Bioequivalence Bioequivalence is determined based on the relative bioavailability of the innovator medicine versus the generic medicine. It is measured by comparing the ratio of the pharmacokinetic variables for the innovator versus the generic medicine where equality is 1. The acceptance criteria are such that to be classified as bioequivalent, plasma concentrations of the generic medicine will not differ significantly compared with the innovator medicine. Studies have demonstrated that actual differences between observed mean plasma concentrations of generic and innovator medicines were no greater than 5%. In order to determine that two medicines are bioequivalent there must be no more than a 20% difference between the AUC and Cmax. This is based on international consensus that differences less than this are not clinically significant. In order to establish this, the AUC and Cmax for the generic medicine are compared to that for the innovator medicine.(Zealand, n.d.)

II.2.2 Biopharmaceutical Processes

There are five biopharmaceutical processes

II.2.2.1 Liberation

The mechanism of liberation of active substance from its dosage form depends on the nature of it in the dosage form .The active substance is physically mixed (solid dosage form: i.e: tablet) ,also active substance dissolves in the basis (suppository,ointment) and active substance is dispersed (suspension, emulsion)

Liberation from tablet dosage form

Swelling

The most widely accepted general mechanism of action for tablet disintegration is swelling. Swelling is believed to be a mechanism in which certain disintegrating agents (such as starch) impart the disintegrating effect. By swelling in contact with water, the adhesiveness of other ingredients in a tablet is overcome causing the tablet to fall apart. Tablets with high porosity show poor disintegration due to lack of adequate swelling force. On the other hand, sufficient swelling force is exerted in the tablet with low porosity. It is worthwhile to note that if the packing fraction is very high, fluid is unable to penetrate in the tablet and disintegration is again slows down.

Porosity and capillary action (Wicking)

Effective disintegrants that do not swell are believed to impart their disintegrating action through porosity and capillary action. When we put the tablet into suitable aqueous medium, the medium penetrates into the tablet and replaces the air adsorbed on the particles, which weakens the intermolecular bond and breaks the tablet into fine particles. Tablet porosity provides pathways for the penetration of fluid into tablets. The disintegrant particles (with low cohesiveness &compressibility) themselves act to enhance porosity and provide these pathways into the tablet. Water uptake by tablet depends upon hydrophilicity of the drug /excipient and on tableting conditions. For these types of disintegrants maintenance of porous structure and low interfacial tension towards aqueous fluid is necessary which helps in disintegration by creating a hydrophilic network around the drug particles.

Due to disintegrating particle/particle repulsive forces

Another mechanism of disintegration attempts to explain the swelling of tablet made with "non-swellable" disintegrants. Guyot-Hermann has proposed a particle repulsion theory based on the observation that non-swelling particle also cause disintegration of tablets. The electric repulsive forces between particles are the mechanism of disintegration and water is required for it. Researchers found that repulsion is secondary to wicking.

Due to deformation

During tablet compression, disintegrated particles get deformed and these deformed particles get into their normal structure when they come in contact with aqueous media or water. Occasionally, the swelling capacity of starch was improved when granules were extensively deformed during compression. This increase in size of the deformed particles produces a breakup of the tablet. Starch grains are generally thought to be "elastic" in nature meaning that grains that are deformed under pressure will return to their original shape when that pressure is removed. But, with the compression forces involved in tableting, these grains are believed to be deformed more permanently and are said to be "energy rich" with this energy being released upon exposure to water.

II.2.2.2 Dissolution

Dissolution process occurs when the active ingredient is not dissolved in the basis/excipients (i.e. Suspension). Dissolution process occurs in the basis (suspension in oil) or after undissolved active ingredient is released/liberated from the basis/excipients (i.e. tablet)

II.2.2.3 Diffusion

Occurs in a medium (basis of dosage form, body fluid) where the active ingredient is dissolved (in solution form).The molecules of active ingredient moves from higher concentration to lower concentration.

II.2.2.4 Transfer

Movement of molecules of a substance from a medium to another medium based on different concentrations and solubilities (partition coefficient).Occurs at intersurfaces of the two mediums.

II.2.2.5 Absorption

Drug absorption is the movement of the drug from its site of administration into the bloodstream.

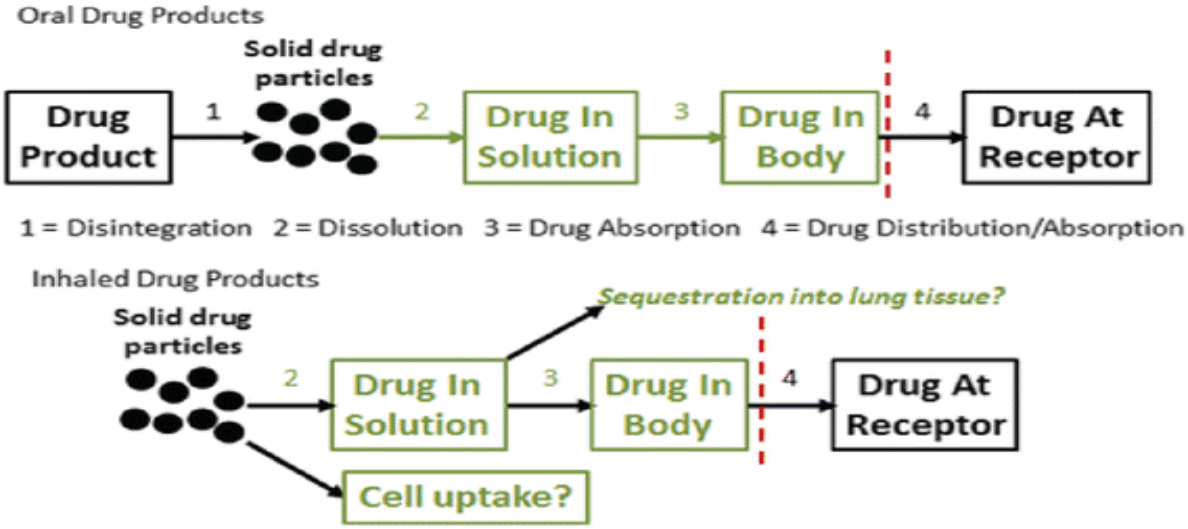
II.2.3 Factors affecting biopharmaceutical processes

II.2.3.1 Physicochemical properties

- **Drug solubility and dissolution rate**

The rate determining steps in absorption of orally administered drugs are**:**

1. Rate of dissolution
2. Rate of drug permeation through the biomembrane.



- **Particle size and effective surface area**

Smaller the particle size (by micronization) greater is the effective surface area more intimate contact b/w solid surface and aq solvent higher is the dissolution rate increase in absorption efficiency e.g. poorly aqueous soluble nonhydrophobic drugs like Griseofulvin,

chloramphenicol whose dissolution is rate limited. Particle size reduction has been used to increase the absorption of a large number of poorly soluble drugs, such as bishydroxycoumarin, digoxin, griseofulvin, nitrofurantoin,and tolbutamide.

Griseofulvin has extremely low aqueous solubility, and material of normal particle size gave rise to poor and erratic absorption.

Microsize particles improve absorption, but it is improved even more when it is formulated in ultramicrosize particles as a monomolecular dispersion in polyethylene glycol.

- **Polymorphism and amorphism**

When sub exist in different crystalline form i.e. in polymorphic form then diff forms are Many compounds form crystals with different molecular arrangements, or polymorphs. These polymorphs may have different physical properties, such as dissolution rate and solubility.

Tabel 1 comparsion between stable and metastable form

Stable form	Metastable form
• Lowest energy state • Highest melting point • Least aq solubility • Dissolution rate limited	• Less stable form • Highest energy state • Lowest melting point • Higher aq solubility • Better absorption and Bioavailability

The vitamin riboflavin exists in several polymorphic forms, and these have a 20-fold range in aqueous solubility.

- Polymorphs that have no crystal structure, or amorphic forms, have different physical properties from the crystalline forms.
  - Absorption of many orally administered drugs is controlled by dissolution rate.
  - Amorphous forms generally dissolve faster than crystalline forms because no energy is needed to break up the crystal lattice. For this reason, the amorphous form is often preferred over the crystalline form and several drugs, including

hydrocortisone and prednisolone, are marketed in the amorphic form.e.g. novobiocin

Tabel 2 cmparasion between amorphous and crystalline drug

Amorphous form	Crystalline form
• More soluble • Rapidly dissolving • Readily absorbed	• Less soluble • Slower dissolving • Not absorbed to significant extent

- **Solvates/hydrates**

During their preparation, drug crystals may incorporate one or more solvent molecules to form solvates. The most common solvate is water. If water molecules are already present in a crystal structure, the tendency of the crystal to attract additional water to initiate the dissolution process is reduced, and solvated (hydrated) crystals tend to dissolve more slowly than anhydrous forms. Significant differences have been reported in the dissolution rate of hydrated and anhydrous forms of ampicillin, caffeine, theophylline, glutethimide, and mercaptopurine The clinical significance of these differences has not been examined but is likely to be slight. Solvates have greater solubility than their nonsolvates.e.g. chloroform solvates of Griseofulvin, n-pentanol solvate of fludrocortisone.

- **Salt form of drug**

At given pH, the solubility of drug, whether acidic/basic or its salt, is a constant. While considering the salt form of drug, pH of the diffusion layer is iimportant not the pH of the bulk of the solution, e.g. of salt of weak acid. Which increases the pH of the diffusion layer, which promotes the solubility and dissolution of a weak acid and absorption is bound to be rapid.

Reverse in the case of salts of weak bases, it lowers the pH of diffusion layer and the promoted the absorption of basic drugs. Other approach to enhance the dissolution and absorption rate of certain drugs is by formation of in situ salt formation i.e. increasing in

pH of microenvironment of drug by incorporating buffer agent.e.g. aspirin, penicillin But sometimes more soluble salt form of drug may result in poor absorption.e.g. sodium salt of phenobarbitone and phenobarbitone, tablet of salt of phenobarbitone swelled, it did not get disintegrate thus dissolved slowly and results in poor absorption

- **Ionization state**

Unionized state is important for passive diffusion through membrane so important for absorption.whereas Ionized state is imp for solubility.

- **Drug pKa & lipophilicity & GI pH /pH partition hypothesis**

pH partition theory states that for drug compounds of molecular weight more than 100, which are primarily transported across the biomembrane by passive diffusion, the process of absorption is governed by pKa of drug, The lipid solubility of the unionized drug, pH at the absorption site

- **pKa of drug**

Amount of drug that exist in unionized form and in ionized form is a function of pKa of drug & pH of the fluid at the ***absorption site and it can be determined by Henderson-hesselbach equation***

$$pH = pka + log\frac{adionized\ form}{unionized\ form}$$ For, Acidic drugs

$$pH = pka + log\frac{unionized\ form}{ionized\ form}$$ For, Basic drugs

- ***Lipophilicity and drug absorption***

Ideally for optimum absorption, a drug should have sufficient aq solubility to dissolve in fluids at absorption site and lipid solubility (Ko/w) high enough to facilitate the partitioning of the rug in the lipoidal biomembrane i.e. drug should have perfect HLB for optimum Bioavailability

II.2.3.2 Formulation Factors

- **Disintegration time**

Rapid disintegration is important to have a rapid absorption so lower D.T is required.

Now D.T of tablet is directly proportional to amount o f binder&Compression force.

And one thing should be remembered that in vitro disintegration test gives no means of a guarantee of drugs B.A. because if the disintegrated drug particles do not dissolve then absorption is not possible.

- **Manufacturing variables**
  - ***Method of granulation***

Wet granulation yields a tablet that dissolves faster than those made by other granulating methods. But wet granulation has several limitations like formation of crystal bridge or chemical degradation.

Other superior recent method named APOC (agglomerative phase of communition) that involves grinding of drug till spontaneous agglomeration and granules are prepared with higher surface area. So tablet made up of this granules have higher dissolution rate.

- ***Compression force***

Higher compression force yields a tablet with greater hardness and reduced wettability & hence have a long D.T. but on other hand higher compression force cause crushing of drug particles into smaller ones with higher effective surface area which in decrease in D.T.So effect of compression force should be thoroughly studied on each formulation.

- **Nature and type of dosage form**

Drug formulations are designed to provide an attractive, stable, and convenient method to use products. Conventional dosage forms may be broadly characterized in order of decreasing dissolution rate as solutions, solid solutions, suspensions, capsules and tablets, coated capsules and tablets, and controlled release formulations.

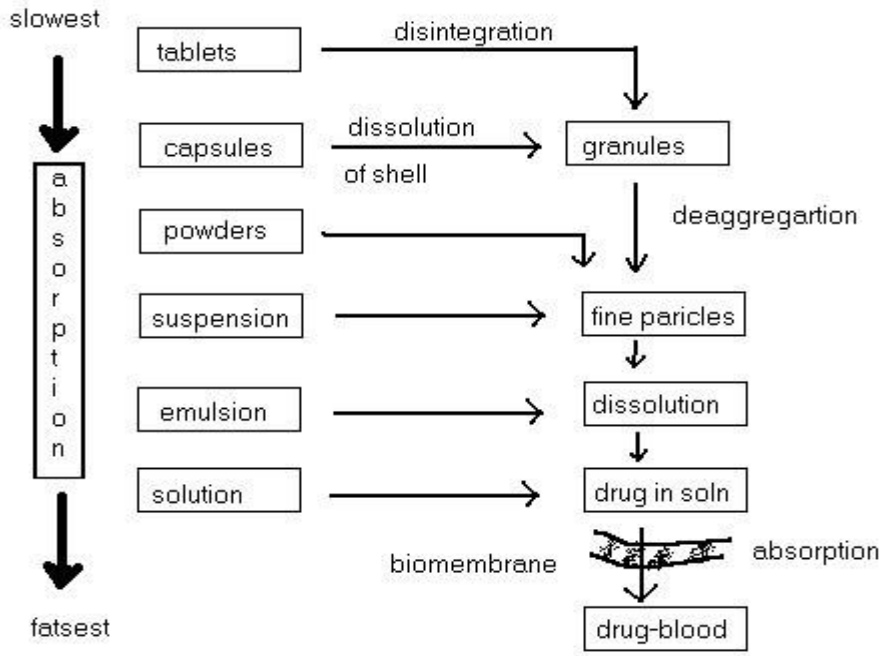


figure 4 effect of dosage form on drug absorption

- **Solutions**

Aqueous solutions, syrups, elixirs, and emulsions do not present a dissolution problem and generally result in fast and often complete absorption as compared to solid dosage forms. Due to their generally good systemic availability, solutions are frequently used as bioavailability standards against which other dosage forms are compared.

- **Solid solutions**

The solid solution is a formulation in which drug is trapped as a solid solution or monomolecular dispersion in a water-soluble matrix. Although the solid solution is an attractive approach to increase drug absorption, only one drug, griseofulvin, is currently marketed in this form.

- **Suspensions**

A drug in a suspension is in solid form, but is finely divided and has a large surface area. Drug particles can diffuse readily between the stomach and small intestine so that absorption is relatively insensitive to stomach emptying rate.

Adjusting the dose to a patient's needs is easier with solutions and suspensions than with solid dosage forms. Liquid dosage forms, therefore, have several practical advantages besides simple dissolution rate. However, they also have some disadvantages, including greater bulk, difficulty in handling, and perhaps reduced stability.

- **Capsules and tablets**

These formulations differ from each other in that material in capsules is less impacted than in compressed tablets. Once a capsule dissolves, the contents generally disperse quickly. The capsule material, Although water soluble, can impede drug dissolution by interacting with the drug, but this is uncommon. Tablets generally disintegrate in stages, first into granules and then into primary particles. As particle size decreases, dissolution rate increases due to of increased surface area. Tablet disintegration was once considered a sufficient criterion to predict in vivo absorption. As a general rule,the bio-availability of a drug from various dosage forms decrease in the following order

Solutions>Emulsions>Suspensions>Capsules>Tablets>Coated Tablets>Enteric coateds Tablets>Sustained Release Products.

- **Pharmaceutical ingredients/Excipients**

More the no. of excepients in dosage form, more complex it is & greater the potential for absorption and Bioavailability problems Changing an excipient from calcium sulfate to lactose and increasing the proportion of magnesium silicate, increases the activity of oral phenytoin. Systemic availability of thiamine and riboflavin is reduced by the presence of Fuller's earth.Absorption of tetracycline from capsules is reduced by calcium phosphate due to complexation. Most of these types of interactions were reported some time ago and are unlikely to occur in the current environment of rigorous testing of new dosage forms and formulations.

- **Vehicle**

Rate of absorption – depends on its miscibility with biological fluid. Miscible vehicles (aq or water miscible vehicle) rapid absorption e.g. propylene glycol. Immiscible vehicles absorption depends on its partitioning from oil phase to aqueous body fluid.

- **Diluents**

Hydrophilic diluents-form the hydrophilic coat around hydrophobic drug particles –thus promotes dissolution and absorption of poorly soluble hydrophobic drug.

- **Binders & granulating agent**

Hydrophilic binders – imparts hydrophilic properties to granule surface – better dissolution of poorly wettable drug. e.g. starch, gelatin, PVP. More amount of binder – increases hardness of tablet – decrease dissolution & disintegration rate.

- **Disintegrants**

Mostly hydrophilic in nature. Decrease in amount of disintegrants, significantly lowers B.A.

- **Lubricants**

Commonly hydrophobic in nature – therefore inhibits penetration of water into tablet and thus dissolution and disintegration.

- **Suspending agents/viscosity agent**

Stabilized the solid drug particles and thus affect drug absorption. Macromolecular gum forms unabsorbable complex with drug e.g. Na CMC. Viscosity imparters act as a mechanical barrier to diffusion of drug from its dosage form and retard GI transit of drug.

- **Surfactants**

May enhance or retards drug absorption by interacting with drug or membrane or both. Surfactants have been considered as absorption enhancers, again mostly in animals. Polyoxyethylene ethers have been shown to enhance gastric or rectal absorption of lincomycin,penicillin, cephalosporins, and fosfomycin in rats and rabbits.

However, in humans, oral polyoxyethylene-20-oleyl ether resulted in poor and variable insulin absorption. In general, unionic surfactants have little effect on membrane structure but cationic surfactants have been associated with reversible cell loss and loss of goblet cells. Physiologic surfactants – bile salts – promotes absorption – e.g. Griseofulvin, steroids. It may decrease absorption when it forms the unabsorbable complex with drug above CMC.

- **Bile salts**

Bile contains conjugates of cholic acid and chenodeoxycholic acid, which emulsify dietary fat, facilitate lipolysis, and transport lipid molecules through the unstirred layer of the

intestinal mucosa by micellar solubilization. The ability of bile salts to promote lipid absorption has prompted their investigation as absorption enhancers for drugs, with modest success.Absorption of insulin can be increased by bile salts, both in experimental animals and in humans.

- **Colourants**

Even a low concentration of water soluble dye can have an inhibitory effect on dissolution rate of several crystalline drugs. The dye molecules get absorbed onto the crystal faces and inhibit the drug dissolution. For example: Brilliant blue retards dissolution of sulfathiazole.

- **Product age and storage conditions**

Product aging and improper storage conditions adversely affect B.A. e.g. –precipitation of drug in solution decrease rate of Change in particle size of suspension drug dissolution & Hardening of tablet & absorption.

II.2.3.3 Patient factors

- **Physiologic Factors Related to Drug Absorption**

The systemic absorption of a drug is dependent on

(1) the physicochemical properties of the drug,

(2) the nature of the drug product, and

(3) the anatomy and physiology of the drug absorption site.

- ***Membrane Physiology***

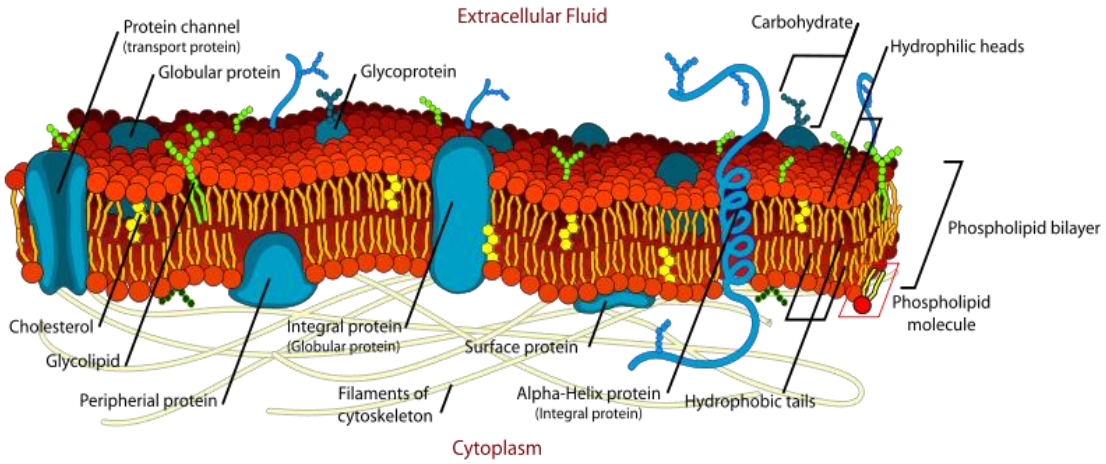


figure 5 nature of cell membrane

The fluid mosaic model, proposed by , explains the transcellular diffusion of polar molecules. According to this model, the cell membrane consists of globular proteins embedded in a dynamic fluid, lipid bilayer matrix. These proteins provide a pathway for the selective transfer of certain polar molecules and charged ions through the lipid barrier.
As shown in , transmembrane proteins are interdispersed throughout the membrane. Two types of pores of about 10 nm and 50 to 70 nm were inferred to be present in membranes based on capillary membrane transport studies. These small pores provide a channel through which water, ions, and dissolved solutes such as urea may move across the membrane.

- ***Trasport Processes***

Passive Diffusion

lipophilic drug may pass through the cell or go around it. If the drug has a low molecular weight and is lipophilic, the lipid cell membrane is not a barrier to drug diffusion and absorption. *Passive diffusion* is the process by which molecules spontaneously diffuse from a region of higher concentration to a region of lower concentration. This process is *passive* because no external energy is expended.

Carrier-Mediated Transport

Theoretically, a lipophilic drug may pass through the cell or go around it. If the drug has a low molecular weight and is lipophilic, the lipid cell membrane is not a barrier to drug diffusion and absorption.

In the intestine, drugs and other molecules can go through the intestinal epithelial cells by either diffusion or a carrier-mediated mechanism. Numerous specialized carrier-mediated transport systems are present in the body, especially in the intestine for the absorption of ions and nutrients required by the body.

Active Transport

Active transport is a carrier-mediated transmembrane process that plays an important role in the gastrointestinal absorption and in renal and biliary secretion of many drugs and metabolites.

A few lipid-insoluble drugs that resemble natural physiologic metabolites (such as 5-fluorouracil) are absorbed from the gastrointestinal tract by this process. Active transport is characterized by the transport of drug against a concentration gradient that is, from regions of low drug concentrations to regions of high concentrations.

Therefore, this is an energy-consuming system. In addition, active transport is a specialized process requiring a carrier that binds the drug to form a carrier–drug complex that shuttles the drug across the membrane and then dissociates the drug on the other side of the membrane

Vesicular Transport

An example of exocytosis is the transport of a protein such as insulin from insulin producing cells of the pancreas into the extracellular space. The insulin molecules are first packaged into intracellular vesicles, which then fuse with the plasma membrane to release the insulin outside the cell.

Pore (Convective) Transport

Very small molecules (such as urea, water, and sugars) are able to cross cell membranes rapidly, as if the membrane contained channels or pores. Although such pores have never been directly observed by microscopy, the model of drug permeation through aqueous pores is used to explain renal excretion of drugs and the uptake of drugs into the liver.

Ion-Pair Formation

Strong electrolyte drugs are highly ionized or charged molecules, such as quaternary nitrogen compounds with extreme pKa values. Strong electrolyte drugs maintain their charge at all physiologic pH values and penetrate membranes poorly. When the ionized drug is linked up with an oppositely charged ion, an *ion pair* is formed in which the overall charge of the pair is neutral. This neutral drug complex diffuses more easily across the membrane.For example, the formation of ion pairs to facilitate drug absorption has been

demonstrated for propranolol, a basic drug that forms an ion pair with oleic acid, and quinine, which forms ion pair with hexylsalicylate

- **Gstero-Intestinal Physiology**
- **Gastric emptying rate**

Anatomically, a swallowed drug rapidly reaches the stomach. Eventually, the stomach empties its contents into the small intestine. Because the duodenum has the greatest capacity for the absorption of drugs from the GI tract, a delay in the gastric emptying time for the drug to reach the duodenum will slow the rate and possibly the extent of drug absorption, thereby prolonging the onset time for the drug.

Some drugs, such as penicillin, are unstable in acid and decompose if stomach emptying is delayed. Other drugs, such as aspirin, may irritate the gastric mucosa during prolonged contact.

- Gastric emptying rate is faster in case of solution & suspensions than solid & nondisintegrating dosage forms.

Factors that influence gastric emptying rate are

- ✓ Volume of meal
- ✓ Composition of meal
- ✓ Physical state and viscosity of meal
- ✓ Temperature of meal
- ✓ Gastrointestinal pH
- ✓ Electrolyte and osmotic pressure
- ✓ Body posture
- ✓ Emotional state
- ✓ Disease state.

- **Intestinal motility**

Normal peristaltic movements mix the contents of the duodenum, bringing the drug particles into intimate contact with the intestinal mucosal cells. The drug must have a sufficient time (*residence time*) at the absorption site for optimum absorption. In the case of high motility in the intestinal tract, as in diarrhea, the drug has a very brief residence time and less opportunity for adequate absorption.

- **Drug stability in GIT**

  Metabolism or degradation by enzymes or chemical hydrolysis may adversely affect the drug absorption and thus reduces B.A. Destruction in gastric acid. Generally a problem with orally administered drugs.

- **Intestinal transit**

Long intestinal transit time is desirable for complete absorption of drug e.g. for enteric coated formulation & for drugs absorbed from specific sites in the intestine.

Peristaltic contraction promotes drug absorption by increasing the drug membrane contact and by enhancing dissolution especially of poorly soluble drugs.

Influenced by food, disease and drugs. e.g. metoclopramide which promotes intestinal transit &thus enhance absorption of rapidly soluble drugs while anticholinergic retards intestinal transit and promotes the absorption of poorly soluble drugs.

- **Blood flow to GIT**

Once the drug is absorbed from the small intestine, it enters via the mesenteric vessels to the hepatic-portal vein and the liver prior to reaching the systemic circulation. Any decrease in mesenteric blood flow, as in the case of congestive heart failure, will decrease the rate of drug removal from the intestinal tract, thereby reducing the rate of drug bioavailability

GIT has higher perfusion rate because it is extensively supplied by blood capillary network. Therefore help in maintaining sink conditions &concentration gradient for drug absorption by rapidly removing of drug from site of action. Blood flow is important for actively absorption of drugs. *Highly permeable drugs or drugs that absorbed through pores –GI perfusion is rate limiting while the drugs with poor permeability GI perfusion is not important* Perfusion increases after meals & persist for few hours but absorption is not affected.

- **Effect of Food**

The presence of food in the GI tract can affect the bioavailability of the drug from an oral drug product. Digested foods contain amino acids, fatty acids, and many nutrients that may affect intestinal pH and solubility of drugs. The effects of food are not always predictable

and can have clinically significant consequences. Some effects of food on the bioavailability of a drug from a drug product include:

Delay in gastric emptying, Stimulation of bile flow, A change in the pH of the GI tract, An increase in splanchnic blood flow, A change luminal metabolism of the drug substance, Physical or chemical interaction of the meal with the drug product or drug substance

The absorption of some antibiotics, such as penicillin and tetracycline, is decreased with food; whereas other drugs, particularly lipid-soluble drugs such as griseofulvin and metazalone, are better absorbed when given with food containing a high fat content

- **Age**

In infants, the gastric pH is high and intestinal surface and blood flow to the GIT is low resulting in altered absorption pattern in comparison to adults.

In elderly persons, causes of impaired drug absorption include altered gastric emptying, decreased intestinal surface area and GI blood flow, higher incidents of achlorhydria and bacterial over growth in small intestine.

- **Clinical Factors**
- **Diseases**

Parkinson's disease may have difficulty swallowing and greatly diminished gastrointestinal motility. A case was reported in which the patient could not be controlled with regular oral levodopa medication because of poor absorption. Infusion of oral levodopa solution using a j-tube gave adequate control of his symptom.

Patients on tricyclic antidepressants (imiprimine, amitriptyline, and nortriptyline) and **antipsychotic drugs** (phenothiazines) with anticholinergic side effects may have reduced gastrointestinal motility or even intestinal obstructions. Delays in drug absorption, especially with slow-release products, have occurred.

Achlorhydricpatients may not have adequate production of acids in the stomach; stomach HCl is essential for solubilizing insoluble free bases. Many weak-base drugs that cannot form soluble salts will remain undissolved in the stomach when there is no hydrochloric acid present and are therefore unabsorbed. Salt forms of these drugs cannot be prepared because the free base readily precipitates out due to the weak basicity.

Dapsone, itraconazole, and ketoconazole may also be less well absorbed in the presence of achlorhydria. In patients with acid reflux disorders, proton pump inhibitors, such as omeprazole, render the stomach achlorhydric, which may also affect drug absorption. Co-administering orange juice, colas, or other acidic beverages can facilitate the absorption of some medications requiring an acidic environment.

HIV-AIDSpatients are prone to a number of gastrointestinal (GI) disturbances, such as increased gastric transit time, diarrhea, and achlorhydria. Rapid gastric transit time and diarrhea can alter the absorption of orally administered drugs. Achlorhydria may or may not decrease absorption, depending on the acidity needed for absorption of a specific drug. Indinavir,
for example, requires a normal acidic environment for absorption. The therapeutic window of indinavir is extremely narrow, so optimal serum concentrations are critical for this drug to be efficacious.

Congestive heart failure (CHF) patients with persistent edema have reduced splanchnic blood flow and develop edema in the bowel wall. In addition, intestinal motility is slowed. The reduced blood flow to the intestine and reduced intestinal motility results in a decrease in drug absorption. For example, furosemide (Lasix), a commonly used loop diuretic, has erratic and reduced oral absorption in patients with CHF and a delay in the onset of action.

Crohn's disease is an inflammatory disease of the distal small intestine and colon. The disease is accompanied by regions of thickening of the bowel wall, overgrowth of anaerobic bacteria, and sometimes obstruction and deterioration of the bowel. The effect on drug absorption is unpredictable, although impaired absorption may potentially occur because of reduced surface area and thicker gut wall for diffusion.

- **Drugs**

Anticholinergic drugs, in general, may reduce stomach acid secretion. Propantheline bromide is an anticholinergic drug that may slow stomach emptying and motility of the small intestine. Tricyclic antidepressants and phenothiazines also have anticholinergic side effects that may cause slower peristalsis in the GI tract. Slower stomach emptying may cause a delay in drug absorption. (Factors, Patient, & Factors, n.d.)

II.2.4 Biopharmaceutics Classification System (BCS)

Oral route is the most desirable route of administering the dosage form. The major problem faced during the oral administration of an active agent is the bioavailability. The solubility is defined as a maximum quantity of solute that can dissolve in a certain quantity of solvent or quantity of solution at a specified temperature (Qiu, Chen, Zhang, Yu, & Mantri, 2016) as the solubility increase bioavailability increases. Solubility defines as:

Tabel 3:Table 1definition of solubility

Indian Pharmacopoeia, 1996

Definition	Parts of solvent required for one part of solute
Very Soluble	< 1
Freely soluble	1 – 10
Soluble	10 – 30
Sparingly soluble	30 – 100
Slightly	100 – 1000
Very slightly soluble	1000 - 10,000
Insoluble	> 10,000

Biopharmaceutics Classification System guidelines are provided by U.S. Food and Drug Administration (FDA), World Health Organization (WHO) and the European Medicines Evaluation Agency (EMEA).

The objective of BCS is to predict the in vivo pharmacokinetic performances of drugs from measurements of permeability and solubility. It allows estimation of the contributions of three major factors, dissolution, solubility and intestinal permeability.

Dissolution and gastrointestinal permeability are the fundamental parameters controlling rate and extension of drug absorption thus BCS becomes a fundamental tool in drug development. (Löbenberg & Amidon, 2000).

II.2.4.1 Based on drug solubility and permeability, the following BCS is recommended in the literature

BCS-based biowaivers are applicable to drug products where the drug substance exhibits high solubility and, either high permeability (BCS Class I) or low permeability (BCS Class III).

A biowaiver is only applicable when the drug substance in test and reference products are identical. For example, a biowaiver is not applicable when the drug substance in the test product is a different salt, ester, isomer, or mixture of isomers from that in the reference product. Pro-drugs may be considered for a BCS-based biowaiver when absorbed as thepro-drug.

Solubility

A drug substance is classified as highly soluble if the highest single therapeutic dose is completely soluble in 250 ml or less of aqueous media over the pH range of 1.2 – 6.8 at 37 ± 1°C. In cases where the highest single therapeutic dose does not meet this criterion but the highest strength of the reference product is soluble under the aforementioned conditions, additional data should be submitted to justify the BCS based biowaiver approach.

The applicant is expected to establish experimentally the equilibrium saturated solubility of the drug substance over the pH range of 1.2 – 6.8 at 37 ± 1ºC using a shake-flask technique or an alternative method if justified. At least three buffers within this range, including buffers at pH 1.2, 4.5 and 6.8, should be evaluated. In addition, solubility at the pKa of the drug substance should be evaluated if it is within the specified pH range. The pH for each test solution should be measured after the addition of the drug substance and at the end of the equilibrium solubility study to ensure the solubility measurement is conducted under the specified pH. The pH should be adjusted if necessary. The lowest measured solubility over the pH range of 1.2 – 6.8 will be used to classify the drug substance.

A minimum of three replicate determinations at each solubility condition/pH is necessary to demonstrate solubility using a validated stability-indicating method, with appropriate compendial references for the media employed.

In addition, adequate stability of the drug substance in the solubility media should be demonstrated. In cases where the drug substance is not stable with >10% degradation over the extent of the solubility assessment, solubility cannot be adequately determined and thus the drug substance cannot be classified. In this case, a BCS-based biowaiver cannot be applied. In addition to experimental data, literature data may be provided to substantiate and support solubility determinations, keeping in mind that peer-reviewed articles may not contain the necessary details of the testing to make a judgement regarding the quality of the studies.

Permeability

The assessment of permeability should preferentially be based on the extent of absorption derived from human pharmacokinetic studies, e.g., absolute bioavailability or mass balance.

High permeability can be concluded when the absolute bioavailability is ≥ 85%. High permeability can also be concluded if ≥ 85% of the administered dose is recovered in urine as unchanged (parent drug), or as the sum of the parent drug, Phase 1 oxidative and Phase 2 conjugative metabolites. Regarding metabolites, in faeces, only oxidative and conjugative metabolites can be considered. Metabolites produced through reduction or hydrolysis should not be included unless it can be demonstrated that they are not produced by microbial action within the gastrointestinal tract. Unchanged drug in faeces cannot be counted toward the extent of absorption, unless appropriate data support that the amount of parent drug in faeces to be accounted for absorbed drug material is from biliary excretion, intestinal secretion or originates from an unstable metabolite, e.g., glucuronide, sulphate, N-oxide that has been converted back to the parent by the action of microbial organisms.

Human *in vivo* data derived from published literature (for example, product knowledge and previously published bioavailability studies) may be acceptable, keeping in mind that peer-reviewed articles may not contain the necessary details of the testing to make a judgement regarding the quality of the results.

Permeability can be also assessed by validated and standardized *in vitro* methods using Caco-2 cells. The results from Caco-2 permeability assays should be discussed in the context of available data on human pharmacokinetics. *In vitro* cell permeability assays (Caco-2) used in support of high permeability should be appropriately validated and

standardized as outlined in Annex 1. If high permeability is inferred by means of an *in the vitro* cell system, permeability independent of active transport should be proven as outlined in Annex I, "Assay Considerations".

If high permeability is not demonstrated, the drug substance is considered to have low permeability (e.g. BCS class III). (Council et al., 2018).

Table 4: Biopharmaceutical classification system

Class 1	High solubility	High permeability Drugs
Class 2	Low solubility	High permeability Drugs
Class 3	High solubility	Low permeability Drugs
Class 4	Low solubility	Low permeability Drugs

Class I (High Permeability, High Solubility)

These compounds are well absorbed and their absorption rate is usually higher than excretion. Drugs exhibit a high absorption number and a high dissolution number. The rate-limiting step is drug dissolution and if dissolution is very rapid then gastric emptying rate becomes the rate determining step.

Class II (High permeability, Low solubility)

The bioavailability of these products is limited by their solvation rate. Drugs have a high absorption number but a low dissolution number. The absorption for class II drugs is usually slower than class I and occurs over a longer period of time.

Class III (Low permeability, High solubility)

The absorption is limited by the permeation rate but the drug is solvated very fast. If the formulation does not change the permeability or gastro-intestinal duration time then class I criteria can be applied. These drugs exhibit a high variation in the rate and extent of drug absorption. Since the dissolution is rapid, the variation is an aspect to alteration of physiology and membrane permeability rather than the dosage form factors.

Class IV (Low permeability, Low solubility)

These compounds have poor bioavailability and not good absorbed over the intestinal mucosa properly. Such drugs show evidence of a lot of problems for effective oral administration(Ku, 2008),(Lawrence et al., 2002).

Table 5: Examples of some drugs as biopharmaceutical

Class 1	Class 2	Class 3	Class 4
Chloroquine	Carbamazepine	Acyclovir	Coenzyme Q10
Diltiazem	Danazol	Atenolol	Cyclosporin A
Metoprolol	Glibenclamide	Captopril	Ellagic acid
Paracetamol	Ketoconazole	Cimetidine	Furosemide
Propranolol	Nifedipine	Metformin	Ritonavir
Theophylline	Phenytoin	Neomycin B	Saquinavir
Verapamil	Troglitazone	Ranitidine	Taxol

II.2.5 Dissolution test

II.2.5.1 Compendial dissolution test

Apparatus

The most commonly employed dissolution test methods are the basket method (Apparatus 1) and, the paddle method (Apparatus 2). The basket and the paddle methods are simple, robust, well standardized, and used worldwide. These methods are flexible enough to allow dissolution testing for a variety of drug products. For this reason, the official in vitro dissolution methods described in *U.S. Pharmacopeia* (USP), Apparatus 1 and Apparatus 2 should be used unless shown to be unsatisfactory. The in vitro dissolution procedures, such as the reciprocating cylinder (Apparatus 3) and a flow-through cell system (Apparatus 4) described in the USP, may be considered if needed. These methodologies or other alternatives/modifications should be considered on the basis of their proven superiority for a particular product. Because of the diversity of biological and formulation variables and the evolving nature of understanding in this area, different experimental modifications may need to be carried out to obtain a suitable in vivo correlation with in vitro release data.

Dissolution methodologies and apparatus described in the USP can generally be used either with manual sampling or with automated procedures.

Dissolution Medium

Dissolution testing should be carried out under physiological conditions, if possible. This allows interpretation of dissolution data with regard to in vivo performance of the product. However, strict adherence to the gastrointestinal environment need not be used in routine dissolution testing. The testing conditions should be based on physicochemical characteristics of the drug substance and the environmental conditions the dosage form might be exposed to after oral administration.

The volume of the dissolution medium is generally 500, 900, or 1000 mL. Sink conditions are desirable but not mandatory. An aqueous medium with pH range 1.2 to 6.8 (ionic strength of buffers the same as in USP) should be used. To simulate intestinal fluid (SIF), a dissolution medium of pH 6.8 should be employed. A higher pH should be justified on a case-by-case basis and, in general, should not exceed pH 8.0. To simulate gastric fluid (SGF), a dissolution medium of pH 1.2 should be employed without enzymes. The need for enzymes in SGF and SIF should be evaluated on a case-by-case basis and should be justified. Recent experience with gelatin capsule products indicates the possible need for enzymes (pepsin with SGF and pancreatin with SIF) to dissolve pellicles, if formed, to permit the dissolution of the drug. Use of water as a dissolution medium also is discouraged because test conditions such as pH and surface tension can vary depending on the source of water and may change during the dissolution test itself, due to the influence of the active and inactive ingredients. For water insoluble or sparingly water soluble drug products, use of a surfactant such as sodium lauryl sulfate is recommended.The need for and the amount of the surfactant should be justified. Use of a hydro alcoholic medium is discouraged.

All dissolution tests for IR dosage forms should be conducted at 37±0.5°C. The basket and paddle method can be used for performing dissolution tests under multimedia conditions (e.g., the initial dissolution test can be carried out at pH 1.2, and, after a suitable time interval, a small amount of buffer can be added to raise pH to 6.8). Alternatively, if addition of an enzyme is desired, it can be added after initial studies (without enzymes).

Use of Apparatus 3 allows easy change of the medium. Apparatus 4 can also be adopted for a change in dissolution medium during the dissolution run.

Certain drug products and formulations are sensitive to dissolved air in the dissolution medium and will need deaeration. In general, capsule dosage forms tend to float during dissolution testing with the paddle method. In such cases, it is recommended that a few turns of a wire helix (USP) around the capsule be used. The apparatus suitability tests should be carried out with a performance standard (i.e., calibrators) at least twice a year and after any significant equipment change or movement. However, a change from basket to paddle or vice versa may need recalibration. The equipment and dissolution methodology should include the product related operating instructions such as deaeration of the dissolution medium and use of a wire helix for capsules. Validation of automated procedures compared to the manual procedures should be well documented. Validation of determinative steps in the dissolution testing process should comply with the set standards for analytical methodology.

Agitation

In general, mild agitation conditions should be maintained during dissolution testing to allow maximum discriminating power and to detect products with poor in vivo performance. Using the basket method, the common agitation (or stirring speed) is 50-100 rpm; with the paddle method, it is 50-75 rpm. Apparatus 3 and 4 are seldom used to assess the dissolution of immediate release drug products.

Validation

Validation of the dissolution apparatus/methodology should include

- the system suitability test using calibrators;
- deaeration, if necessary;
- validation between manual and automated procedures;
- validation of a determinative step (i.e., analytical methods employed in the quantitative analysis of dissolution samples). This should include all appropriate steps and procedures of analytical methods validation. (Solid & Dosage, 1997)

II.2.5.2 Dissolution testing and Similarity of Dissolution Profiles

General aspects of dissolution testing as related to bioavailability

During the development of a medicinal product, a dissolution test is used as a tool to identify formulation factors that are influencing and may have a crucial effect on the bioavailability of the drug. As soon as the composition and the manufacturing process are defined a dissolution test is used in the quality control of scale-up and of production batches to ensure both batch-to-batch consistencies and that the dissolution profiles remain similar to those of pivotal clinical trial batches. Furthermore, in certain instances a dissolution test can be used to waive a bioequivalence study. Therefore, ***dissolution studies can serve several purposes:***

- ***Testing on product quality***

To get information on the test batches used in bioavailability/bioequivalence studies and pivotal clinical studies to support specifications for quality control, also To be used as a tool in quality control to demonstrate consistency in manufacture and to get information on the reference product used in bioavailability/bioequivalence studies and pivotal clinical studies.

- ***Bioequivalence surrogate inference***

To demonstrate in certain cases similarity between different formulations of an active substance and the reference medicinal product (biowaivers e.g., variations, formulation changes during development and generic medicinal products; and to investigate batch to batch consistency of the products (test and reference) to be used as basis for the selection of appropriate batches for the *in vivo* study.

Test methods should be developed product related based on general and/or specific pharmacopoeial requirements. In case those requirements are shown to be unsatisfactory and/or do not reflect the *invivo* dissolution (i.e. biorelevance) alternative methods can be considered when justified that these are discriminatory and able to differentiate between batches with acceptable and non-acceptable performance of the product *in vivo*. Current state-of-the-art information including the interplay of characteristics derived from the BCS classification and the dosage form must always be considered.

Sampling time points should be sufficient to obtain meaningful dissolution profiles, and at least every 15 minutes. More frequent sampling during the period of greatest change in the dissolution profile is recommended. For rapidly dissolving products, where complete dissolution is within 30 minutes, generation of an adequate profile by sampling at 5 or 10 minute intervals may be necessary. If an active substance is considered highly soluble, it is reasonable to expect that it will not cause any bioavailability problems if, in addition, the dosage system is rapidly dissolved in the physiological pHrange and the excipients are known not to affect bioavailability. In contrast, if an active substance is considered to have a limited or low solubility, the rate limiting step for absorption may be dosage form dissolution. This is also the case when excipients are controlling the release and subsequent dissolution of the active substance. In those cases a variety of test conditions is recommended and adequate sampling should be performed.

- ***Comparative dissolution test***

When dissolution profiles or a similar term is used in this guidance, data should be generated in a comparative manner as follows:

At least 12 dosage units (e.g. tablets, capsules) of each batch must be tested individually, and mean and individual results reported.

The percentage of nominal content released are measured at a minimum of three suitably spaced time points (excluding zero time point) to provide a profile for each batch (e.g. at 5, 15, 30 and 45 minutes, or as appropriate to achieve virtually complete dissolution).

The batches are tested using the same apparatus and, if possible, on the same day.

The stirrer used is normally a paddle at 50 rpm for tablets and a basket at 100 rpm for capsules. However, other systems or speeds may be used if adequately justified and validated.

Test conditions are those used in routine quality control or, if dissolution is not part of routine quality control, any reasonable, validated method.

The similarity factor, f2, is calculated using the equation and conditions stated in Appendix I of the European Medicines Agency (EMA) Guideline on the investigation of bioequivalence

to demonstrate the similarity of two dissolution profiles. The f2 value must be between 50 and 100.

If more than 85 per cent of the active substance is dissolved within 15 minutes in all tested batches, dissolution profiles are considered to be similar without the need to calculate the similarity factor.

If there are insufficient quantities of recently manufactured batches available to meet this requirement, then both test retention batches,and explain in the test report why this was done, stating the age and storage history of the samples.(Hussain et al., 1999)

- ***Similarity of dissolution profiles***

Dissolution profile similarity testing and any conclusions drawn from the results (e.g. justification for a biowaiver) can be considered valid only if the dissolution profile has been satisfactorily characterised using a sufficient number of time points.

For immediate release formulations, further to the guidance given in section 1 above, comparison at 15 min is essential to know if complete dissolution is reached before gastric emptying. Where more than 85% of the drug is dissolved within 15 minutes, dissolution profiles may be accepted as similar without further mathematical evaluation.

In case more than 85% is not dissolved at 15 minutes but within 30 minutes, at least three time points are required: the first time point before 15 minutes, the second one at 15 minutes and the third time point when the release is close to 85%.

For modified release products, the advice given in the relevant guidance should be followed.

Dissolution similarity may be determined using the $f2$ statistic as follows:

$$f_2 = 50 \cdot \log\left[\frac{100}{\sqrt{1+\frac{\sum_{t=1}^{t=n}\left[\overline{R}(t)-\overline{T}(t)\right]^2}{n}}}\right]$$

In this equation $f2$ is the similarity factor, n is the number of time points, R(t) is the mean percent reference drug dissolved at time t after initiation of the study; T(t) is the mean percent

test drug dissolved at time t after initiation of the study. For both the reference and test formulations, percent dissolution should be determined.

The evaluation of the similarity factor is based on the following conditions:

- A minimum of three time points (zero excluded)
- The time points should be the same for the two formulations
- Twelve individual values for every time point for each formulation
- Not more than one mean value of > 85% dissolved for any of the formulations.
- The relative standard deviation or coefficient of variation of any product should be less than 20% for the first point and less than 10% from second to last time point.

An *f2* value between 50 and 100 suggests that the two dissolution profiles are similar. When the $f2$ statistic is not suitable, then the similarity may be compared using model dependent or model-independent methods e.g. by statistical multivariate comparison of the parameters of the Weibull function or the percentage dissolved at different time points.

Alternative methods to the $f2$ statistic to demonstrate dissolution similarity are considered acceptable, if statistically valid and satisfactorily justified.

The similarity acceptance limits should be pre-defined and justified and not be greater than a 10% difference. In addition, the dissolution variability of the test and reference product data should also be similar, however, a lower variability of the test product may be acceptable.

Evidence that the statistical software has been validated should also be provided. A clear description and explanation of the steps taken in the application of the procedure should be provided, with appropriate summary tables.(Agency, 2010)

II.3 Methods to increase the solubility and dissolution rate

II.3.1 Methods to increase the dissolution rate

The dissolution rate can be expressed via Noyes &whitney equation:

$$\frac{dc}{dt} = \frac{D.A}{h}(Cs - Cb)$$

where dC/dt = rate of drug dissolution at time t, D = diffusion rate constant, A = surface area of the particle, Cs = concentration of drug (equal to solubility of drug) in the stagnant layer, C = concentration of drug in the bulk solvent, and h = thickness of the stagnant layer.

The rate of dissolution, *dC*/*dt*, is the rate of drug dissolved per time expressed as concentration change in the dissolution fluid.

The Noyes–Whitney equation shows that dissolution in a flask may be influenced by the physicochemical characteristics of the drug, the formulation, and the solvent. The dissolution of drug in the body, particularly in the gastrointestinal tract, is considered to be dissolving in an aqueous environment. Permeation of drug across the gut wall (a model lipid membrane) is affected by the ability of the drug to diffuse (*D*) and to partition between the lipid membranes. A favorable partition coefficient (*K*oil/water) will facilitate drug absorption).

In addition to these factors, the temperature of the medium and the agitation rate also affect the rate of drug dissolution. *In vivo*, body temperature is maintained at a constant 37°C, and the agitation (primarily peristaltic movements in the gastrointestinal tract) is reasonably constant. In contrast, *in vitro* studies of dissolution kinetics require maintenance of constant temperature and agitation. Temperature is generally kept at 37°C, and the agitation or stirring rate is held to a specified agitation rate such as 75 rpm (revolutions per minute). An increase in temperature will increase the kinetic energy of the molecules and increase the diffusion constant, *D*. Moreover, an increase in agitation of the solvent medium will reduce the thickness, *h*, of the stagnant layer, allowing for more rapid drug dissolution.

Factors that affect drug dissolution of a solid oral dosage form include

- the physical and chemical nature of the active drug substance,
- the nature of the excipients,
- the method of manufacture,
- the dissolution test conditions.(Shargel, Andrew, & Wu-Pong, 1999)

the interpretation of dissolution from tablet follows this creteria:(USP 711, 40AD)

Tabel 6 dissolution acceptance Criteria

Stage	Number Tested	Acceptance Criteria
S1	6	Average amount dissolved is not less than Q + 10%.
S2	6	Average amount dissolved (S 1 + S 2) is equal to or greater than Q + 5%.
S3	12	Average amount dissolved (S 1 + S 2 + S 3) is equal to or greater than Q.

II.3.2 Methods to increase the solubility

II.3.2.1 Physical Modifications

Particle size reduction

Particle size reduction can be achieved by Micronization and Nanosuspension. Each technique utilizes different equipments for reduction of the particle size.

Micronization

The solubility of drug is often intrinsically related to drug particle size. By reducing the particle size, the increased surface area improves the dissolution properties of the drug. Conventional methods of particle size reduction, such as comminution and spray drying, rely upon mechanical stress to disaggregate the active compound. The Micronization is used to increased surface area for dissolution. Micronisation increases the dissolution rate of drugs through increased surface area, it does not increase equilibrium solubility. Micronization of drugs is done by milling techniques using jet mill, rotor stator colloid mills etc. Micronization is not suitable for drugs having a high dose number because it does not change the saturation solubility of the drug.

Nanosuspension

Nanosuspensions are sub-micron colloidal dispersion of pure particles of drug, which are stabilised by surfactants. The advantages offered by Nanosuspension is increased dissolution rate is due to larger surface area exposed, while absence of Ostwald ripening is due to the uniform and narrow particle size range obtained, which eliminates the concentration gradient factor. Techniques for the production of nanosuspensions

Homogenization

The homogenizers are commonly used for particle size reduction in the pharmaceutical and biotechnology industries: conventional homogenizers, sonicators, and high shear fluid processors. The suspension is forced under pressure through a valve that has nanoparticle. This causes bubbles of water to form which collapses as they come out of valves. This mechanism cracks the particles.

Wet milling

Other technique involves the spraying of a drug solution in a volatile organic solvent into a heated aqueous solution. Rapid solvent evaporation produces drug precipitation in the

presence of surfactants. The nanosuspension approach has been employed for drugs including tarazepide, atovaquone, amphotericin B, paclitaxel and bupravaquone. All the formulations are in the research stage.

Other techniques for reduction of the particle size

Sonocrystallisation

Recrystallization of poorly soluble materials using liquid solvents and antisolvents has also been employed successfully to reduce particle size. The novel approach for particle size reduction on the basis of crystallisation by using ultrasound is Sonocrystallisation. Sonocrystallisation utilizes ultrasound power characterised by a frequency range of 20–100 kHz for inducing crystallisation. It's not only enhances the nucleation rate but also an effective means of size reduction and controlling size distribution of the active pharmaceutical ingredients18 (API). Most applications use ultrasound in the range 20 kHz - 5 MHz.

Spray drying

Spray drying is a commonly used method of drying a liquid feed through a hot gas. Typically, this hot gas is air but sensitive materials such as pharmaceuticals and solvents like ethanol require oxygen-free drying and nitrogen gas is used instead. The liquid feed varies depending on the material being dried and is not limited to food or pharmaceutical products and may be a solution, colloid or a suspension. This process of drying is a one step rapid process and eliminates additional processing19. Spray drying of the acid dispersed in acacia solutions resulted in as much as a 50% improvement in the solubility of poorly water soluble salicylic acid.

Modification of the crystal habit

Polymorphism is the ability of an element or compound to crystallize in more than one crystalline form. Different polymorphs of drugs are chemically identical, but they exhibit different physicochemical properties including solubility, melting point, density, texture, stability etc. Broadly polymorphs can be classified as **enantiotropes** and **monotropes** based on thermodynamic properties. In the case of an enantiotropic system, one polymorphs form can change reversibly into another at a definite transition temperature below the melting point, while no reversible transition is possible for monotropes. Once the drug has been

characterized under one of this category,further study involves the detection of metastable form of crystal. Metastable forms are associated with higher energy and thus higher solubility. Similarly the amorphous form of drug is always more suited than crystalline form due to higher energy associated and increase surface area. Generally, the anhydrous form of a drug has greater solubility than the hydrates. This is because the hydrates are already in interaction with water and therefore have less energy for crystal breakup in comparison to the anhydrates (i.e. thermodynamically higher energy state) for further interaction with water. On the other hand, the organic (nonaqueous) solvates have greater solubility than the nonsolvates. Thus, the order for dissolution of different solid forms of drug is **Amorphous >Metastable polymorph >Stable polymorph.** Melting followed by a rapid cooling or recrystallization from different solvents can be produce metastable forms of a drug.

Drug dispersion in carriers

The solid dispersion approach to reduce particle size and therefore increase the dissolution rate and absorption of drugs was first recognised in 1961. The term "solid dispersions" refers to the dispersion of one or more active ingredients in an inert carrier in a solid state, frequently prepared by the melting (fusion) method, solvent method, or fusion solvent method. Novel additional preparation techniques have included rapid precipitation by freeze drying and using supercritical fluids and spray drying, often in the presence of amorphous hydrophilic polymers and also using methods such as melt extrusion. The most commonly used hydrophilic carriers for solid dispersions include polyvinylpyrrolidonepolyethylene glycols, Plasdone- S630. Many times surfactants may also used in the formation of solid dispersion. Surfactants like Tween-80, Docusate sodium, Myrj-52, Pluronic-F68 and Sodium Lauryl Sulphate used. The solubility of etoposide, glyburide, itraconazole, ampelopsin, valdecoxib, can be improved by solid dispersion using suitable
hydrophilic carriers. The eutectic combination of chloramphenicol/urea and sulphathiazole/ urea served as examples for the preparation of a poorly soluble drug in a highly water soluble carrier.

Hot Melt method

Sekiguchi and Obi21 used a hot melt method to prepare solid dispersion. Sulphathiazole and urea were melted together and then cooled in an ice bath. The resultant solid mass was then milled to reduce the particle size. Cooling leads to supersaturation, but due to solidification

the dispersed drug becomes trapped within the carrier matrix. A molecular dispersion can be achieved or not, depends on the degree of supersaturation and rate of cooling used in the process. An important requisite for the formation of solid dispersion by the hot melt method is the miscibility of the drug and the carrier in the molten form. When there are miscibility gaps in the phase diagram, this usually leads to a product that is not molecularly dispersed. Another important requisite is the thermostability of the drug and carrier.

Solvent Evaporation Method

Tachibana and Nakumara were the first to dissolve both the drug and the carrier in a common solvent and then evaporate the solvent under vacuum to produce a solid solution. This enabled them to produce a solid solution of the highly lipophilic β-carotene in the highly water soluble carrier polyvinylpyrrolidone. An important prerequisite for the manufacture of a solid dispersion using the solvent method is that both the drug and the carrier are sufficiently soluble in the solvent. The solvent can be removed by various methods like by spray-drying or by freeze-drying. Temperatures used for solvent evaporation generally lie in the range 23-65 C, The solid dispersion of the 5- lipoxygenase/cyclooxygenase inhibitor ER-34122 shown improved in vitro dissolution rate compared to the crystalline drug substance which was prepared by solvent evaporation. These techniques have problems such as negative effects of the solvents on the environment and high cost of production due to extra facility for removal of solvents. Due to the toxicity potential of organic solvents employed in the solvent evaporation method, hot melt extrusion method is preferred in preparing solid solutions.

Hot-melt Extrusion

Melt extrusion was used as a manufacturing tool in the pharmaceutical industry as early as 1971. It has been reported that melt extrusion of miscible components results in amorphous solid solution formation, whereas extrusion of an immiscible component leads to amorphous drug dispersed in crystalline excipient. The process has been useful in the preparation of solid dispersions in a single step.

Melting –solvent method

A drug is first dissolved in a suitable liquid solvent and then this solution is incorporated into the melt of polyethylene glycol, obtainable below 70C without removing the liquid solvent. The selected solvent or dissolved drug may not be miscible with the melt of the polyethylene

glycol. Also polymorphic form of the drug precipitated in the solid dispersion may get affected by the liquid solvent used

Complexation

Complexation is the association between two or more molecules to form a non bonded entity with a well defined stochiometry. Complexation relies on relatively weak forces such as London forces, hydrogen bonding and hydrophobic interactions. Examples of complexing agents are; chelates- EDTA, EGTA, molecular complexes- polymers, inclusion complexes cyclodextrins.

Complexes are two categories

Stacking complexes

is driven by association of non polar area of drug and complexes agent this results in exclusion of the non polar area from contact with water, thereby reducing total energy of the system. Stacking canbe homogeneous or mixed, but results in clear solution.

Inclusion complexes

are formed due to the ability of a compound to enclose in another complex. There are no forces involved between them and therefore there are no bond is also called as no-bond complexes.

II.3.2.2 Chemical modification

Salt Formation

is the most common and effective method of increasing solubility and dissolution rates of acidic and basic drugs. Acidic or basic drug converted into salt having more solubility than respective drug. Ex. Aspirin**,** Theophylline, Barbiturates.

Co-crystallisation

new approach available for the enhancement of drug solubility is through the application of the co-crystals, it is also referred as molecular complexes. A Co-crystals may be defined as crystalline material that consist of two or more molecular (&electrical neutral) species held together by non-covalent forces. It can be prepared by evaporation of a heteromeric solution or by grinding the components together or by sublimation, growth from the melt & slurry

preparation. It is increasingly important as an alternative to salt formation, particularly for neutral compounds.

Co-solvent

It is well-known that the addition of an organic cosolvent to water can dramatically change the solubility of drugs. Weak electrolytes and nonpolar molecules have poor water solubility and it can be improved by altering polarity of the solvent. Solvent used to increase solubility known as cosolvent. It is also commonly referred to as solvent blending. Most cosolvents have hydrogen bond donor and/or acceptor groups as well as small hydrocarbon regions. Their hydrophilic hydrogen bonding groups ensure water miscibility, while their hydrophobic hydrocarbon regions interfere with waters hydrogen bonding network, reducing the overall intermolecular attraction of water. By disrupting waters self-association, cosolvents reduce waters ability to squeeze out non-polar, hydrophobic compounds, thus increasing solubility. A different perspective is that by simply making the polar water environment more non-polar like the solute, cosolvents facilitate solubilization. Solubility enhancement as high as 500-fold is achieved using 20% 2-pyrrolidone.

Hydrotropy

It designate to increase in solubility in water due to presence of large amount of additives. It improves solubility by Complexation involving weak interaction between hydrophobic agents (Sodiumbenzoate, sodium alginate, urea) & solute. Ex.Sublimation of Theophylline with Sodium acetate & Sodium alginate.

Solubilising Agents

The solubility of poorly soluble drug can also be improved by various solubilizing materials. Ex. PEG 400 is improving the solubility of hydrochlorothiazide. Modified gum karaya (MGK), a recently developed excipient was evaluated as carrier for dissolution enhancement of poorly soluble drug, nimodipine. The aqueous solubility of the antimalarial agent halofantrine is increased by the addition of caffeine and nicotinamide.

Nanotechnology Approaches:

Nanotechnology will be used to improve drugs that currently have poor solubility. Nanotechnology refers broadly to the study and use of materials and structures at the nanoscale level of approximately 100 nanometres (nm) or less. For many new chemical

entities of very low solubility, oral bioavailability enhancement by micronisation is not sufficient because micronised product has very low effective surface area for dissolution and next step taken was nanonisation.(S. K. Patil, Wagh, Parik, Akarte, & Baviskar, 2011),(Arun et al., 2012).

II.4 Inclusion complex

II.4.1 Inclusion complex

Cyclodextrins have an internal non-polar hole and hydroxyl groups placed on the surface, the inclusion of hydrophobic compounds takes place mainly by hydrophobic interactions between guest molecules and the walls of the cyclodextrin cavity. However, other forces, such as van der Walls and dipole-dipole interactions, may be involved in the binding of the guest. Despite the number of factors and different forces involved in the complexation with cyclodextrins, the production of complexes is a rather simple process. There are several methods to obtain cyclodextrin–guest complexes depending on the properties of the guest and the nature of the chosen cyclodextrin.(Cheirsilp & Rakmai, 2017).

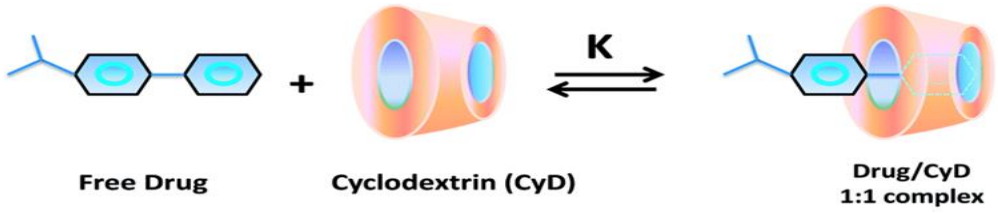


Figure 6 inclusion complex of drug with Cyclodextrins

II.4.2 Cyclodextrins

II.4.2.1 History of Cyclodextrin

Cyclodextrins (CDs) belong to the category of carbohydrates and are cyclic oligosaccharides discovered just over 100 years ago. They are called “Cellulosine” when first discovered by A.Villiers in 1891. F. Schardinger identified the three naturally occurring Cyclodextrins α, β and γ. And these were referred to as “Schardinger Sugars”.

For 25 years, between 1911 and 1935, Pringsheim in Germany was the leading researcher in this area, demonstrated that CDs formed stable aqueous complexes with many other chemicals. CDs are produced from starch by means of enzymatic conversion. Over the last

few years, an application of CDs is expanded into food, pharmaceutical, chemical, agricultural, and environmental engineering fields.

Due to the specific structure and the orientation of the hydroxyl groups made the CDs capable of solubilizate in an aqueous medium and to encapsulate the lipophilic molecules into their interior cavity(J. S. Patil, Kadam, Marapur, & Kamalapur, 2010)

II.4.2.2 Cyclodextrins and their derivatives

Various approaches of complexation with cyclodextrins have gained good acceptance in recent years in the industry for enhancing the solubility and dissolution rate of poorly soluble drugs. Cyclodextrins are cyclic torus-shaped molecules with a hydrophilic outer surface and a lipophilic central cavity which can accommodate a variety of lipophilic drugs. As a consequence of the inclusion process, many physicochemical properties such as solubility, dissolution rate, stability and bioavailability can be favourably enhanced. Cyclodextrins are being increasingly applied in various pharmaceutical formulations in recent years due to their approval by various regulatory agencies. Cyclodextrins are produced from starch by means of enzymatic conversion (Saravana Kumar, Sushma, & Prasanna Raju, 2013)

Cyclodextrin (CD) are cyclic oligosaccharides consisting of glucopyranosyl units linked by α-(1,4) bonds. The widely used natural cyclodextrins are α-, β- and γ-cyclodextrin consisting of 6, 7 and 8 glucopyranose units, respectively.

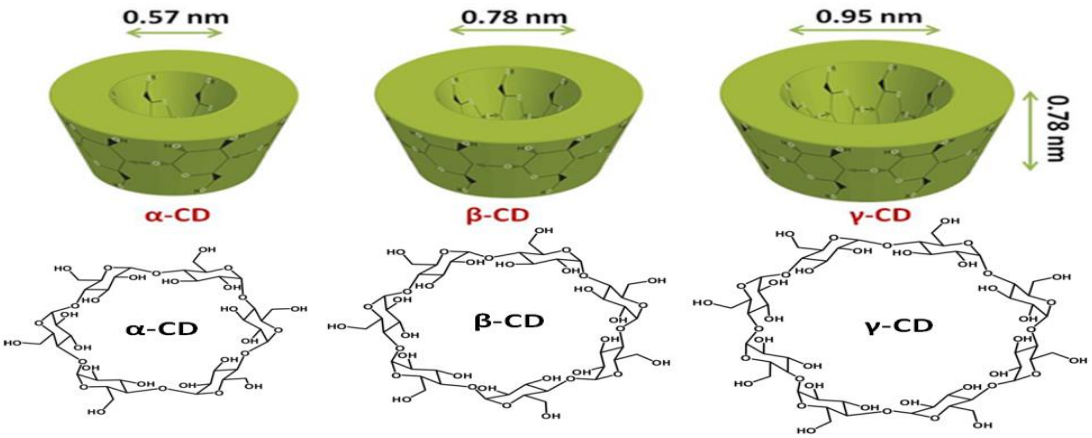


Figure 7: chemical structure of Cyclodextrins

The cyclodextrin molecules have a unique structure with a hydrophobic cavity and a hydrophilic surface which can form an inclusion complex with a wide variety of guests. The use of cyclodextrins and their derivatives for the encapsulation of bioactive compounds can protect the compounds from environmental conditions and improve the aqueous solubility for increasing their capacity to functionalize the products.

In some cases, there is a need to enhance water solubility of β-cyclodextrin by adding the hydroxyalkyl groups on the β-cyclodextrin surface. (Cheirsilp & Rakmai, 2017).

Table 7 : properties of Cyclodextrins

(Del Valle, 2004)

properties	αCyclodextrin	βCyclodextrin	γCyclodextrin
Number of glucopyranose units	***6***	***7***	***8***
Molecular weight (g/mol)	***972***	***1135***	***1297***
Solubility in water at 25 ◦C (%, w/v)	***14.5***	***1.85***	***23.2***
Outer diameter (Å)	***14.6***	***15.4***	***17.5***
Cavity diameter (Å)	***4.7-5.3***	***6-6.5***	***7.5-8.3***
The height of torus (Å)	***7.9***	***7.9***	***7.9***
Cavity volume (Å3)	***174***	***262***	***427***

II.4.3 Methods to prepare inclusion complex

Physical blending method

A solid physical mixture of drug and CDs are prepared simply by mechanical trituration. *In laboratory scale,* CDs and drug are mixed together thoroughly by trituration in a mortar and passes through an appropriate sieve to get the desired particle size in the final product. *In industrial scale,* the preparation of physical mixtures is based on an extensive blending of the drug with CDs in a rapid mass granulator usually for 30 minutes. These powdered physical mixtures are then stored in the room at controlled temperatures and humidity conditions

Kneading method

This method is based on impregnating the CDs with a little amount of water or hydroalcoholic solutions to converted into a paste. The drug is then added to the above paste and kneaded for a specified time. The kneaded mixture is then dried and passed through a sieve if required have reported the dissolution enhancement of nimesulide using complexation method.

In the laboratory, scale kneading can be achieved by using a mortar and pestle. *In a large scale,* the kneading can be done by utilizing the extruders and other machines. This is the **most common and simple method** used to prepare the inclusion complexes and it presents the **very low cost of production**.(J. S. Patil et al., 2010).

Co-precipitation method

Co-precipitation technique is useful for non-water-soluble substances. Poor yields are obtained from this method because of the competitive inhibition from organic solvents used as the precipitant.

The guest is dissolved in organic solvents (such as chloroform, benzene and diethyl ether, etc), and an appropriate amount of cyclodextrin dissolved in water is added with agitation. The solution is cooled and complex crystals occur. The crystals are washed with organic solvent and then dried at 50°C. The co-precipitation technique has previously applied for encapsulation of drugs such as oxaprozin and trans-anethole (a major component of anise and fennel essential oils)

Freeze-drying or lyophilization

The freeze-drying technique is suitable for thermolabile or water-soluble guests. The required proportion of cyclodextrin and the guest molecule are dissolved in water with stirring. The solution is freeze-dried and the obtained powder is washed with organic solvent and then dried under vacuum. This method can produce a very good yield of inclusion complex and it is possible to scale up.

Comparing with other available techniques, the freeze-drying technique has been wildly applied for cyclodextrin inclusion complex formation, especially water-soluble

hydroxypropyl-β-cyclodextrin. Several essential oils and their pure major active compounds have been encapsulated in hydroxypropyl-β-cyclodextrin. These include cinnamon and clove, estragole (a major component of basil and tarragon essential oils), black pepper essential oil, thymol and thyme essential, multitarget drug and chloramphenicol.

Spray drying

Cyclodextrin and guest molecule is dissolved in deionized water and then the solution is dried by the spray-dryer. The spray dryer is operated under the most appropriate conditions such as inlet temperature and sample feeding speed. As temperatures of 50–70°C are used this technique is only used for thermostable molecules. Recently, the spray-drying technique has been used for encapsulation of folic acid in cyclodextrin(Cheirsilp & Rakmai, 2017).

Neutralization method

Drug and CD are separately dissolved in 0.1 N NaOH mixed and stirred for about half an hour, pH is recorded and 0.1 N HCl is added dropwise with stirring until pH reaches 7.5, whereupon complexes precipitate. The residue is filtered and washed until free from chlorine; it is dried at 2500 C for 24 hrs and stored in a desiccator. (A & K, 1970).

Solvent evaporation method

This method involves dissolving of the drug and CDs separately into two mutually miscible solvents, mixing of both solutions to get molecular dispersion of drug and complexing agents and finally evaporating the solvent under vacuum to obtain solid powdered inclusion compound. Generally, the aqueous solution of CDs is simply added to the alcoholic solution of drugs.

The resulting mixture is stirred for 24 hours and evaporated under vacuum at 45 ºc. The dried mass was pulverized and passed through a 60-mesh sieve. This method is quite simple and economic both on laboratory and large-scale production and is considered an alternative to the spray drying technique. (J. S. Patil et al., 2010)

Microwave irradiation method

In this technique, the microwave irradiation reaction between the drug and the complexing agent takes place using a microwave oven. The drug and CD in the definite molar ratio are dissolved in a mixture of water and organic solvent in a specified proportion into a round bottom flask. The mixture is reacted for a short time of about one to two minutes at 60ºC in the microwave oven. After the reaction completes, an adequate amount of solvent mixture is added to the above reaction mixture to remove the residual, uncomplexed free drug and CD. The precipitate so obtained is separated using Whatman filter paper, and dried in vacuum oven at 40ºC for 48 hrs. Microwave irradiation method is a novel method for industrial-scale preparation due to its major advantage of shorter reaction time and higher yield of the product.

Supercritical antisolvent technique

In the supercritical fluid anti-solvent technique, carbon dioxide is used as anti-solvent for the solute but as a solvent with respect to the organic solvent. The use of supercritical carbon dioxide is advantageous as its low critical temperature and pressure make it attractive for processing heat-labile pharmaceuticals. This method is important for improving the bioavailability of pharmaceutically active compounds. Supercritical carbon dioxide due to its properties of improved mass transfer and increased solvating power it proved as a new complexation medium. In this technique, first, drug and CD are dissolved in a good solvent then the solution is fed into a pressure vessel under supercritical conditions, through a nozzle (i.e. sprayed into supercritical fluid anti-solvent). When the solution is sprayed into supercritical fluid anti-solvent, the anti-solvent rapidly diffuses into that liquid solvent as the carrier liquid solvent counter diffuses into the anti-solvent. Because of the supercritical fluid expanded solvent has lower solvent power than the pure solvent, the mixture becomes supersaturated resulting in the precipitation of the solute and the solvent is carried away with the supercritical fluid flow.

Milling/Co-grinding technique

By using this method a solid binary inclusion compound of drug and CD is prepared. In this method, Drug and CDs are mixed intimately and the physical mixture is introduced in an oscillatory mill and ground for a suitable time. Ball mill is also used for the preparation of binary complex. (Kadam, Shinkar, & Saudagar, 2013).

II.4.4 Charetirization of inclusion complex

The complexation depends largely on the dimensions of the cyclodextrins and the particular sterical arrangement of the functional groups of the molecules, which leads to a relatively hydrophilic outside and a hydrophobic inside cavity of the molecule. Inclusion complexes formed between the guest and cyclodextrin molecules can be characterized both in the solid and solution state by the following techniques(Badr-Eldin, Ahmed, & Ismail, 2013).

differential scanning calorimeter (DSC)

The thermal behaviour of drugs such as melting point and heat change formulation was studied by DSC. Changes in the melting point during the formation of inclusion complexes indicated the entrapment of the guest molecules into host molecules. The thermogram obtained from DSC during thermal analysis also explained the crystal behaviour endothermic or exothermic reaction and formation of a new compound(Xu, Zhang, Li, & Zheng, 2017)

X-ray diffractometry (XRD)

XRD technique performs structural analysis based on the spreading of X-rays on the samples. XRD allowed the examination of the solid state structure of material and more advanced technique confirmed the formation of inclusion complexes. Change in intensity and shifting of the peaks indicated the formation of new solid structure(Vikas, Sandeep, Braham, Manjusha, & Budhwar, 2018).

Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra indicate the type of intermolecular or intermolecular interaction between drug and polymer. Drug, polymer, physical mixture, and formulation were evaluated in the range of 4000–400 cm−1. Shifting in the peaks toward higher or lower wavenumber in inclusion complexes indicated the formation of H-bonding between drug and Cyclodextrin(Menezes et al., 2012) and also indicated that drug was covered into the cavity of the polymer of inclusion complex formation(Wei, Zhang, Memon, & Liang, 2017).