1.1 BIOPHARMACEUTICAL CLASSIFICATION SYSTEM

Drug solubility enhancement is an important challenge in the field of pharmaceutical formulation development. Among the five key physicochemical screens in early compound screening viz. pKa, solubility, permeability, stability and lipophilicity, poor solubility tops the list of undesirable compound properties [Jochem A; 2007]. Advances in combinatorial chemistry and high throughput screening have led to the development of large number of molecules with requisite pharmacological activity. However these immobilized receptor techniques may lead to the selection of compounds with undesirable physicochemical attributes like high lipophilicity, high molecular weights and poor aqueous solubility [Lipinsky C; 1997, Lipinsky C; 2001]. Retrospective analysis completed in the late 1980s revealed that almost 40% of drug failures were related to the poor biopharmaceutical properties [Prentis R; 1988]. Molecules of this type can provide a number of challenges in pharmaceutical development and may potentially lead to slow dissolution in biological fluids, insufficient and inconsistent systemic exposure and route [Blagden N; 2007]. Permeability and solubility are thus, key parameters for controlling drug absorption. The biopharmaceutical classification system (BCS) is the scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. It is a drug development tool that allows estimation of the contributions of three major factors: dissolution, solubility and intestinal permeability that affect oral absorption of drugs. The interest in this classification system is largely because of its application in early drug development and then in the management of product change through its life cycle [US-FDA Draft Guidance; 1999]. Knowledge of the BCS can also help the formulation scientist to develop a dosage form based on mechanistic, rather than empirical approaches [Meyer M; 1992]. The BCS defines four classes depending on drug’s solubility and permeability through the intestinal cell layer. The classification is given in Table 1.1.

<table>
<thead>
<tr>
<th>Class</th>
<th>Solubility</th>
<th>Permeability</th>
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<tbody>
<tr>
<td>I</td>
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<td>II</td>
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<tr>
<td>IV</td>
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</table>
1.1.1. Class boundaries:
A drug substance is said to be highly soluble when the highest dose strength is soluble in 250 ml water over a pH range of 1-7.5. A drug substance is considered highly permeable when extent of absorption in humans is determined to be 90% of an administered dose based on mass-balance or in comparison to an intravenous reference dose. A drug product is rapidly dissolving when 85% of the labeled amount dissolves in 30 min using USP apparatus I or II in 900 ml of buffer solutions.

The BCS defines three dimensionless numbers, viz. dose number (Do), dissolution number (Dn) and absorption number (An) to characterize drug substances. These numbers are combinations of physicochemical and physiological parameters and represent the most fundamental view of GI drug absorption [Löbenberg R;2000].

Absorption number is the ratio of permeability (Peff) and the gut radius (R) times the residence time (Tsi) in the small intestine.

$$\text{An} = \frac{\text{Peff} \times (\text{Tsi})}{\text{R}}$$  \hspace{1cm} \text{Equation 1.1}

Dissolution number is the ratio of residence time to the dissolution time which includes solubility (Cs), diffusivity (D), density ($\rho$) and initial particle radius (r) of a compound and the intestinal transit time (Tsi).

$$\text{Dn} = \frac{3D}{r^2} \frac{\text{Cs} \times \text{Tsi}}{\rho}$$  \hspace{1cm} \text{Equation 1.2}

Dose number is defined as the ratio of dose concentration to drug solubility.

$$\text{Do} = \frac{\text{M/V}_0 \text{Cs}}{\text{Cs}}$$  \hspace{1cm} \text{Equation 1.3}

Where Cs is the solubility, M is the dose and V_0 is the volume of water taken with the dose, which is generally set to be 250 ml.

BCS class I drugs exhibit a high absorption number and high dissolution number. These drugs dissolve rapidly in an aqueous environment and are rapidly transported over the absorbing membrane. No strategies are required to increase their absorption. Class II drugs have high absorption number (An) and dose number (Do) but a low dissolution number (Dn < 1). In vivo drug dissolution is then a rate limiting step for absorption expected at a very high dose number and it is affected by several physiological fluctuations, like the volume and pH of the intestinal juices, presence of bile salts, food,
enzymes, bacteria, gut motility and viscosity of fluids in gut lumen. Class III drugs have low An but high Dn. Permeability is then the rate limiting step. These drugs exhibit a high variation in the rate and extent of absorption. Since the dissolution is rapid, the variation is attributed to alteration of physiology and membrane permeability rather than dosage form factors. Class IV drugs exhibit a lot of problems for effective oral administration due to low solubility and low permeability. For this category of drugs little IVIVC is expected and it is upto the formulation scientist to not only increase the extent of absorption but also to improve the IVIVC. This will reduce the patient-to-patient variability and improve the bioavailability and predictability of pharmacokinetic parameters. It is clear that, depending on the classification of the drug, different strategies can be applied to increase or accelerate the absorption of a drug: either increasing the permeability of the absorbing membrane or increasing the amount of dissolved drug that is in contact with the absorbing membrane [Löbenberg R;2000].

1.1.2. Determination of solubility:

The solubility of a substance is the amount of solute that has passed into solution when equilibrium is attained between the solution and excess i.e. undissolved substance at a given temperature and pressure.

Methods other than shake flask method are used with justification to support the ability for predicting equilibrium solubility of test drug substance e.g. acid or base titration methods. The concentration of drug substance in selected buffers or pH conditions should be determined using a validated solubility indicating assay that can distinguish between the drug substance and degradation products [Rawlinson C;2007].

1.1.3. Determination of permeability:

Fundamental to understanding of the nature of GI permeability limitations are methods and techniques used to screen and grade these characteristics. These methods range from simple oil/water partition coefficient to absolute bioavailability studies. The methods that are routinely used for permeability studies include:

   b. Absolute bioavailability studies
   c. Intestinal permeability studies
2. *In vivo* or in situ intestinal perfusion in a suitable animal model
3. *In vitro* permeability methods using excised intestinal tissues
4. Monolayers of suitable epithelial cells e.g. Caco-2 cells or TC-7 cells

In mass balance studies, unlabelled, stable isotopes or radiolabelled drug substances are used to determine the extent of drug absorption. Further oral bioavailability is determined and compared against the intravenous bioavailability as reference. Intestinal perfusion models and in vitro methods are recommended for passively transported drugs. The observed low permeability of some drug substances in humans could be attributed to the efflux of drug by various membrane transporters like p-glycoprotein. This leads to misinterpretation of the permeability of drug substance. An interesting alternative to intestinal tissue models is the use of well established in vitro systems based on the human adenocarcinoma cell line Caco-2. These cells serve as model of small intestinal tissue. The differentiated cells exhibit the micro villi typical of the small intestinal mucosa and the integral membrane proteins of the brush border enzymes. In addition they also form the fluid filled domes typical of a permeable epithelium. Recent investigations of Caco-2 cell lines have indicated their ability to transport ions, sugars and peptides [Amidon G;1995].

### 1.1.4. Theory of dissolution of pure solids:

The rate at which a solid dissolves in a solvent was proposed in quantitative terms [Noyes A;1897] and elaborated subsequently by other workers. They rotated cylinders of benzoic acid and lead chloride with constant surface area and analyzed the resultant solution at various time intervals. They found that rate of change of concentration of dissolved substance (dc/dt) was proportional to difference between the saturation solubility of that substance (Cs) and the concentration existing at any time (Ct).

Using k as a proportionality constant this can be expressed as:

\[
\frac{dc}{dt} = k (Cs - Ct)
\]

**Equation 1.4**

The dissolution of solid drug in an agitated liquid was discussed in terms of film theory [Nernst W;1904]. It is assumed that there is a thin stagnant film of liquid of thickness (h) on surface of dissolving solid. The solid is believed to be dissolved at an infinitely rapid
rate maintaining a saturated layer at solid liquid interface (at x = 0) within bulk medium (at a distance of x >h from solid surface). It is thought that the agitation of medium maintains uniform concentration of dissolved drug. This sets up concentration gradient throughout the stagnant film. Dissolved solid is transported through this film to bulk medium totally by Brownian motion diffusion. Therefore the rate of dissolution of solid depends on the rate of solute diffusion through this layer. Mathematically this can be described by following equation:

\[ \frac{dw}{dt} = \frac{D \cdot S}{h} (C_s - C_t) \]  \hspace{1cm} \text{Equation 1.5}

where

- D = diffusion coefficient of the solute molecules in the medium
- S = surface area of solid
- h = thickness of diffusion layer
- \( \frac{dw}{dt} = \) rate at which solid is dissolved where ‘w’ is mass of solid

The Dankwerts’ theory suggests that turbulence within the bulk medium extends to surface of solid and there is no stagnant film or diffusion layer, surrounding the solid. The rate-limiting step in this surface renewal theory is diffusion of solvent from the bulk surface.

Dankwerts’ theory can be expressed mathematically as:

\[ \frac{dc}{dt} = P^{1/2} [C_s - C_t] \]  \hspace{1cm} \text{Equation 1.6}

where P = mean rate of production of fresh surface [Higuchi W;1967].

These two dissolution theories i.e. film theory and surface renewal theories are relevant only for single non-disintegrating particles. Disintegration or deaggregation of the solid dosage form results in large number of smaller granules with distribution of sizes. The total surface area changes during dissolution process owing to dissolution of the smaller particles with time.

The dissolution process in an agitated system where the surface area was changing was further studied by Hixson A, et.al., [1931]. They derived a relationship known as the cube root equation based on the Noyes-Whitney equation. This expression assumes that the dissolution is less than about 10-20% saturated at any time during the test. In a monodispersed system the equation can be written as:
\[ W_0^{1/3} - W^{1/3} = (\pi N \rho / 6)^{1/3} 2D (Cs) t/h \]  

Equation 1.7

Where, \( W_0 \) = initial weight of particles  
\( N \) = number of particles  
\( W \) = weight of particles at time \( t \)  
\( \rho \) = particle density

**1.2. TECHNIQUES OF SOLUBILITY ENHANCEMENT**

Numerous methodologies have been suggested and practically applied to improve the marketability of drug candidates whose development is limited by drug solubility, dissolution rate and absorbability. These include the use of particle size manipulation via micronization and nanonization, use of complexing agents such as cyclodextrins, the preparation of high energy drug states related to polymorphic or amorphous transformations [Kim C;2004, Merisko E;2003, Forster A;2001, Humberstone A;1997, Davis M;2004] use of co-solvents, micellar solutions and lipid based systems for lipophilic drugs [Amin K;2004, Torchillin V;2007]. The strategies that can be used for this purpose can be broadly classified into: formulation based approach and structure based approach. The former involves inclusion of various solubility enhancers such as hydrophilic polymers, cyclodextrins, surfactants, oils and liposomes which produce an apparent increase in solubility. The second approach involves modification either in the physical structure or chemical structure. The various techniques used for solubility enhancement are further classified as follows:

1.2.1 **Physical modifications**

A] Particle size reduction  
Micronization  
Sonocrystallization  
Supercritical fluid processing  
Spray drying  

B] Crystal engineering  
Modification of crystal habit  
Polymorphs  
Pseudopolymorphs  

C] Drug dispersion in carriers
Eutectic mixtures
Solid dispersions
Solid solutions
D] Complexation
Use of complexing agents like cyclodextrins
E] Lipid-based systems
Microemulsions
Self emulsifying drug delivery systems

1.2.2. Chemical modifications
A] Formation of salts and prodrugs

1.2.3. Miscellaneous
A] Cosolvency
B] Co-crystallization
C] Hydrotrophy
D] Solubilizing agents
E] Liquisolid technology

1.2.4. Nanotechnology based approaches
A] Nanospheres
B] Nanocrystals
C] Nanosuspensions

1.2.1 Physical modifications:

A] Particle size reduction

According to Noyes-Whitney equation, the dissolution rate of drugs could be increased by reducing the size at the micro- or nano-scale to increase the surface area of drug particles [Drooge D;2004, Mosharraf M;1995,. Müller R;1998]. The principle is to increase the dissolution velocity by enlarging the surface area of the drug powder. The conventional approaches to produce ultrafine drug particles can be divided into top-down and bottom-up technologies [Gupta R;2006, Keck C;2006, Rabinow B;2004]. Top-down technologies includes jet-milling, pearl/ball milling and high pressure homogenization. In
this the bulk drugs with the size of several hundred microns are comminuted into micro- or nano-sized range by the use of mechanical force [Rasenack N;2004]. However milling technologies such as wet milling can cause contamination of products because of the abrasion between the grinding beads, leading to broad size distribution with only a fraction of the particles below 1μm [Qiao P;2008]. The major disadvantage of high pressure homogenization is that the crystal structure of drugs may vary in some cases due to the high pressure, which may result in instability and cause quality control problems [Kharb V;2006].

In case of bottom-up technologies such as precipitation, spray freezing into liquid, supercritical fluid (SCF) technology and so on, the ultrafine particles can be built from the molecular state. These technologies have been employed to prepare several drugs in micro- or nano-scale, such as cephradine [Zhong J; 2005], cefuroxime axetil [Zhang J;2006], danazol [Rogers T;2002] and ibuprofen [Rasenack N;2004]. Examples for precipitation techniques are the hydrosols developed by Novartis [List M; 1988, Gassmann P; 1994] and the product Nanomorph by the company Soliqs/Abbott differing in precipitation details such as stabilizers used [Violante M; 1991, Thies J; 1998, Kipp J; 2003]. Basically, the drug is dissolved in a solvent and this solution is added to a nonsolvent. Addition of non-solvent is necessary to yield a very fine product [Keck C; 2006].

For spironolactone, submicron particles have been produced by high pressure homogenization [Langguth P;2005], emulsion solvent diffusion method [El-Shabouri M; 2002] and antisolvent precipitation technique [Dong Y;2009]. Cyclodextrins were employed as protective stabilizers for the preparation of surfactant-free nanocrystals of indomethacin by emulsion solvent diffusion method. However the hazards of organic solvent residuals has led to the emergence of supercritical fluid-based technologies as novel methods for preparation of nanocrystals [Makhlof A 2008]. The commonly known processes are supercritical anti-solvent precipitation [Chattopadhyay P; 2001] and rapid expansion of supercritical solutions [Pathak P; 2006] both of which have been employed to reduce the particle sizes of drugs like salbutamol, insulin and peptides. For every solvent there exists a temperature, its critical temperature (Tc), above which no pressure
can force the solvent into its liquid phase. Above its Tc and corresponding critical pressure (Pc), the solvent is in its supercritical state and is termed as a supercritical fluid (SCF). SCF’s are highly compressible and have densities that are liquid –like and transport properties that are gas-like. Small changes in the temperature or pressure near the critical point result in large changes in the fluid’s density and hence it’s solubilizing power [Phillips E;1993]. Once the drug particles are solubilized within SCF’s they can be recrystallized at greatly reduced particle sizes. A SCF process allows micronization within narrow particle size range, often to sub micron levels [Charoenchaitrakool M; 2000].

Nanoparticles of cefuroxime axetil have also been produced by sonocrystallization method for enhancing oral bioavailability [Dhumal R;2008]. This method utilizes ultrasound power in the frequency range of 20-100kHz for inducing crystallization. It not only enhances the nucleation rate but also is an effective means of size reduction and controlling size distribution of API’s like budesonide [Ting-Ting Hu; 2008]. Spray drying is another commonly used method for drying liquid feed through a hot gas or air. 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 which may be solution, suspension or a colloidal dispersion. This process of drying and particle size reduction for enhanced solubilization is a one step rapid process and eliminates additional processing [Karavas E;2007]. Spray drying of salicylic acid dispersed in acacia solution resulted in 50% increase in its solubility [Modi A; 2007].

B] Crystal engineering

Crystal engineering approaches, which can potentially be applied to a wide range of crystalline materials, offer an alternative and potentially fruitful method for improving the solubility, dissolution rate and subsequent bioavailability of poorly soluble drugs. An in depth understanding of the crystallization process is necessary to confer habit modification in crystalline materials. Studies of crystallization and communnition have revealed that exposure of different crystal faces determines the nature of those faces which in turn will influence the wettability and subsequent dissolution of an API [Heng
A number of examples in literature demonstrate the effects of changing crystal morphology on in vitro dissolution rate, with potential for improving bioavailability. The habit modification of dipyridamole by crystallization using different solvents, additives and crystallization conditions has been reported [Adhiyaman R; 2006]. The dissolution rate of rod shaped particles crystallized from benzene was significantly more rapid compared to rectangular needle shaped crystals produced using methanol. Needle shaped and rhombic crystals of phenytoin were produced when recrystallization was done using ethanol and acetone, respectively [Nokhodchi A; 2003]. Differences in dissolution rate was observed between the two crystals. Although crystal engineering approach appears promising for dissolution rate enhancement, there are only a limited number of examples reported where habit modification has resulted in notable enhancement of systemic exposure in human subjects or in suitable animal models [Blagden N; 2007].

The impact of crystal form on pharmaceutical development has been reviewed by Singhal D; 2004. The influence of crystalline modification on drug dissolution and bioavailability was first highlighted for chloramphenicol palmitate in the late 1960s, in which metastable polymorph B was shown to provide greater absorption in humans than polymorph A [Aguiar A; 1967]. It was suggested that the greater solubility of the metastable form could be exploited to enhance absorption and bioavailability. Different additives or solvents can be employed which have the ability to inhibit or interfere with formation of a stable polymorph. However conversion to more stable crystalline forms during processing and storage was a major concern. The metastable crystalline forms of phenobarbital [Kato Y; 1984], spironolactone [Salole E; 1985] and carbamazepine [Kobayashi Y; 2000] have been reported to provide enhanced dissolution. Difference in dissolution rate, area under plasma concentration time curve (AUC) and maximum plasma concentration (Cmax) for two polymorphs of phenylbutazone in beagle dogs was reported [Pandit J; 1984]

C] Drug dispersion in carriers

Molecular or near molecular dispersions of poorly soluble drugs in carriers combines the benefits of a local increase in the solubility and maximizing the surface area of the compound that comes in contact with the dissolution medium as the carrier dissolves
Chapter 1

Introduction

[Leuner C;2000]. These dispersions are classified into the following: a] Simple eutectic mixtures b] Solid solutions that may be latter further classified into i] Continuous solid solutions or ii] Discontinuous solid solutions, depending on their miscibility.

A simple eutectic mixture consists of two compounds which are completely miscible in the liquid state but only to a very limited extent in the solid state. When a mixture of A and B with composition E is cooled, A and B crystallize out simultaneously. Solid eutectic mixtures are usually prepared by rapid cooling of a comelt of the two compounds in order to obtain a physical mixture of very fine crystals of the two components. When a mixture with composition E, consisting of a slightly soluble drug and an inert, highly water soluble carrier, is dissolved in an aqueous medium, the carrier will dissolve rapidly, releasing very fine crystals of the drug [Sekiguchi K;1961, Goldberg A;1966]. The large surface area of the resulting suspension should result in an enhanced dissolution rate and thereby improved bioavailability.

Solid solutions are comparable to liquid solutions, consisting of just one phase irrespective of the number of components. They consist of the drug dispersed molecularly in a highly water soluble carrier. By judicious selection of the carrier, the dissolution rate of the drug can be increased by up to several orders of magnitude. Discontinuous solid solutions are those in which the solubility of each of the component in the other is limited. Depending on the location of the solvate molecules in the solvent, solid solutions can be substitutional crystalline, interstitial crystalline or amorphous solid solutions. In substitutional solid solutions, solvent molecules in the crystal lattice are substituted by solute molecules. This is possible when the size of the solute molecules differs by less than 15% from that of solvent molecules. In interstitial solid solutions, the dissolved molecules occupy the interstitial spaces between the solvent molecules in the crystal lattice. The solute molecules should have a molecular diameter that is not greater than 0.59 of the solvent’s molecular diameter [Leuner C; 2000]. In amorphous solid solutions, the solute molecules are dispersed molecularly but irregularly within the amorphous solvent. With griseofulvin in citric acid, it was reported that the formation of an amorphous solid solution led to improvement in drug’s dissolution properties [Chiou W;1969]. Carriers that were used include urea and sugars such as sucrose, dextrose and galactose, organic polymers such as polyvinylpyrrolidone (PVP), polyethylene glycol.
(PEG) and various cellulose derivatives. The improvement in dissolution characteristics is attributed to factors like: a) presence of drug in molecular form [Goldberg A; 1966], b) low energy required to break up the crystalline structure [Taylor L; 1997], c) inhibition of precipitation of the drug from supersaturated solution due to presence of carrier [Simonelli A 1976], d) improvement in wettability of the drug [Chiou W; 1971].

Two commonly used methods for preparing solid solutions/dispersions are hot melt and solvent evaporation method. Hot melt method was used to prepare simple eutectic mixtures [Sekiguchi K; 1961]. Numerous modifications were introduced to improve the process such as injection molding [Wacker S; 1991], spraying the hot melt onto a cold surface [Kanig J; 1964], hot melt extrusion [Adel M; 1971] and hot spin melting [Dittgen M; 1995 Part 1, Dittgen M; 1995 Part 3]. Solvent evaporation method was used for preparing solid dispersions of β carotene in polyvinyl pyrrolidone [Tachibani T; 1965].

D) Complexation

Complexation is an association between two or more molecules to form a nonbonded entity with a well defined stoichiometry. It relies on relatively weak forces such as London forces, hydrogen bonding and hydrophobic interactions [Shin S; 2003]. Caffeine forms an absorbable complex with ergotamine tartrate thereby enhancing its dissolution rate by a factor of three and at intestinal pH caffeine has been found to increase the in vitro partitioning of ergotamine into lipid phase [Zoglio M; 1969]. Besides caffeine, other complexing agents include theobromine, gentisic acid, salicylic acid, ferrulic acid and nicotinamide. Higuchi T; [1954] investigated the complexing of caffeine with a number of acidic drugs. They attributed the interaction between caffeine and a drug such as sulfonamide or a barbiturate to a dipole-dipole force or hydrogen bonding between the polarized carbonyl groups of caffeine and the hydrogen atom of the acid. The effect of sodium salicylate on the release of benzocaine from topical vehicles was studied [York P; 1976]. Complexation between drug and complexing agents can improve or impair drug absorption and bioavailability. The authors found that the presence of sodium salicylate significantly influenced the release of benzocaine, depending on the type of vehicle involved. The largest increase in absorption was observed for water-miscible polyethylene glycol base.
Chapter 1

Introduction

The class of inclusion complexes known as inclusion or occlusion compounds result more from the architecture of molecules than from their chemical affinity. One of the constituents of the complex is trapped in the open lattice or cage-like crystal structure of the other to yield a stable arrangement [Sinko P; 2006]. α-, β- and γ-cyclodextrins (cyclic D-glucose oligomers) and their derivatives are such complexing agents which have a hydrophobic interior and have the ability to bind hydrophobic portion of the guest molecule, usually forming a 1:1 complex. Appropriately sized lipophilic molecules can be accommodated wholly or partially in the complex. Lach and associates [Lach J; 1963] used cyclodextrins to trap, stabilize and solubilize sulfonamides, tetracyclines, morphine, aspirin, benzocaine, ephedrine, reserpine and testosterone. The aqueous solubility of retinoic acid (0.5mg/l), is increased to 160 mg/l by complexation with β-CD [Amdidouche D; 1989]. The dissolution rate of famotidine [Hassan M; 1990], a potent drug in the treatment of gastric and duodenal ulcers, and that of tolbutamide, an oral antidiabetic drug are both increased by complexation with β-CD [Kedzierewicz F; 1990]. However, β-CD is often associated with nephrotoxicity when administered by parenteral route. Derivatives of natural crystalline CD have been developed to improve the water solubility and to avoid toxicity. Amorphous derivatives of β-CD and γ-CD are more effective as solubilizing agents for sex hormones than the parent CD. Complexes of testosterone with amorphous hydroxypropyl β-CD allow an efficient transport of the hormone when given sublingually [Pitha J; 1987].

E] Lipid based systems

The use of natural and synthetic lipids has generated much interest as a potential formulation strategy for improving the oral bioavailability of poorly water soluble drugs [Humberstone A; 1997]. Lipid-based formulations not only improve but normalize drug absorption, which is particularly beneficial for low therapeutic index drugs [Vonderscher J; 1994]. These formulations can also enhance drug absorption by a number of ancillary mechanisms, including inhibition of P-glycoprotein-mediated drug efflux and preabsorptive metabolism by gut membrane-bound cytochrome enzymes [Cornaire G; 2004, Wandel C; 2003] promotion of lymphatic transport, which delivers the drug.
directly to the systemic circulation while avoiding hepatic first-pass metabolism [Hauss D; 1998] and by increasing GI membrane permeability [Rege B; 2002].

The most frequently chosen excipients for preparing oral lipid-based formulations are dietary oils composed of medium- or long chain triglycerides such as coconut, palmseed, corn, olive, peanut, rapeseed, sesame or soyabean oils, lipid soluble solvents (e.g. PEG 400, ethanol, propylene glycol, glycerine) and pharmaceutically acceptable surfactants like Cremophor, polysorbate 20/80, TPGS, spans, Labrafils, Labrasols and Gelucires. These formulations comprise simple solutions of drug in dietary oil or multi-excipient, self-emulsifying drug delivery systems.

Self-emulsifying formulations are physically stable, isotropic mixtures of oil, surfactant, co-surfactant and solubilized drug that are suitable for oral delivery in soft or hard gelatin or HPMC capsules [Hauss D; 2007]. Depending on the type and concentration of excipients used, aqueous dilution results in spontaneous emulsification having droplet size ranging from 100 nm (SEDDS) to > 50 nm (SMEDDS) [Gursoy R; 2004]. The optimum concentration ranges of oil, surfactant and co-surfactant necessary to promote self-emulsification are determined by construction of pseudo-ternary phase diagrams. Pouton C (2006) first described the lipid formulation classification system which is presented in Table 1.2.

This classification helps to rationalize development of lipid based drug delivery systems and provides framework to guide regulatory agencies. Type I systems contain oil-based systems that are non-dispersing and require digestion to release the drug. They are applicable for highly lipophilic drugs. Type II systems are self emulsifying in nature and form turbid o/w dispersions. Type IIIA systems are self microemulsifying and form clear dispersions. Digestion of oils is not a pre requisite for drug absorption in these systems. Type IIIB systems are also self microemulsifying but differ from IIIA systems in that they are likely to lose solvent capacity due to presence of hydrophilic co solvents. Type IV systems are oil-free formulations and disperse to form micellar solutions but they may not be digestible.
Many theories have been proposed to explain the principle of self emulsification. Some of the more prominent ones include:

- Mixed Film Theory
- Solubilization Theory
- Thermodynamic Theory

### 1. Mixed film theory:

The interfacial tension between the oil and water is lowered by the addition and adsorption of surfactant. When surfactant concentration is increased further, it lowers interfacial tension till CMC. This ultra low or negative interfacial tension leads to a simultaneous and spontaneous increase in the area of the interface. Due to this large number of closed shells around small droplets of oil in water or water in oil are formed and further decrease the free energy of the system. In many cases, the interfacial tension is not ultra low when the CMC is reached, so addition of co surfactant results in virtually zero interfacial tension and further negative interfacial tension. Thus spontaneous formation of micro emulsion is due to the formation of a complex film at the o/w interface by the surfactant and co surfactant.

\[
\gamma_i = \gamma_{0/w} - \pi_i
\]

**Equation 1.8**

where, $\gamma_{0/w}$ = oil-water interfacial tension without the film present

$\pi_i$ = Spreading pressure

$\gamma_i$ = Interfacial tension in presence of surfactant and co surfactant.
This equation indicates that when interfacial tension is low spreading pressure is high which favors formation of a curvature resulting in formation of large number of small droplets.

2. Solubilization theory:
As per this theory micro emulsions are assumed to be thermodynamically stable monophasic solutions of water swollen or oil swollen micelles.

3. Thermodynamic theory:
This theory explains the formation of micro emulsion even in the absence of co surfactant. For micro emulsion to form spontaneously the free energy is:

\[ \Delta G_f = \gamma \Delta A - T \Delta S \]

where \( \Delta G_f \) = Free energy of formation
\( \gamma \) = Interfacial tension
\( \Delta A \) = increase in surface area
\( \Delta S \) = change in entropy of system
\( T \) = absolute temperature

It should be noted that when micro emulsion is formed, change in \( \Delta A \) is very large as a large number of very small droplets are formed. The free energy of formation can be zero or negative if the interfacial tension is very low. Entropy relates to the spontaneity of a process. An increase in entropy is always spontaneous in nature and self emulsification process is also spontaneous [Rosen M; 2004, Calderon F; 2007].

Cyclosporin A (CsA) is a potent immunosuppressive drug used in organ transplantation. It is a cyclic undecapeptide with very poor aqueous solubility. Sandimmun Neoral® is a microemulsion formulation of CsA with improved bioavailability [Gursoy R; 2004]. Tipranavir(TPV), a potent anti-HIV drug showed a two-fold higher bioavailability when formulated as SEDDS [Yeni P;2003]. Self-microemulsifying formulations of simvastatin were prepared by for oral bioavailability enhancement. Various combinations of oils, surfactants and co surfactants were investigated and efficient self-emulsification region was identified by constructing pseudo-ternary phase diagrams. Optimized formulations were evaluated for in vitro dissolution and bioavailability in beagle dogs and compared with conventional marketed tablet. A 1.5 fold increase in bioavailability was found with SMEDDS than the conventional tablet [Kang K; 2004]. Kommuru T; [2001] developed self emulsifying formulations of Coenzyme Q10 using polyglycolized glycerides as emulsifiers. Four types of formulations were prepared using Myvacet 9-45 and Captex...
200 as oils, Labrafac CM 10 and Labrasol as surfactants and lauroglycol as co surfactants. Various parameters such as in vitro self emulsification and droplet size were studied as well as pseudo ternary phase diagrams were constructed to identify the self emulsification region. Effect of co surfactants on self emulsification and and chain length of the oils on solubility were studied. A two fold increase in bioavailability was observed in comparison with a powder formulation.

A self emulsifying formulation of indomethacin was prepared to increase its in vitro dissolution and in vivo absorption. Oral administration of the formulation led to a 57% increase in bioavailability whereas rectal administration of the self emulsifying system in hollow gelatin shells led to a 41% increase in the bioavailability of indomethacin [Ku S; 2000].

Julianto T; [2000] conducted a single dose study to evaluate the bioavailability of a novel self emulsifying Vitamin E preparation, in comparison to a commercial product available as soft gelatin capsules. The self emulsifying formulation achieved a faster rate and higher extent of absorption. A statistically significant difference was observed between the two preparations in the parameters of AUC, Cmax and Tmax. Pang X; [2007] developed a stable formulation for self microemulsifying drug delivery systems in order to enhance the solubility, release rate, and oral absorption of the poorly water soluble drug silymarin. They showed that differences in release medium significantly influenced the drug release from SMEDDS, and the release profiles of silymarin from SMEDDS was higher than that for commercial capsules, and significantly higher than that for commercial tablets.

1.2.2. Chemical modifications:

A] Formation of salts and prodrugs

For organic solutes that are ionizable, changing the pH of the system is the simplest & most effective means of increasing aqueous solubility. Under proper conditions, the solubility of an ionizable drug can increase exponentially by adjusting the pH of the solution. A drug that can be efficiently solubilized by pH control should be either weak acid with a low pKa or a weak base with a high pKa. There is little effect of pH on non-ionizable substances. Non-ionizable, hydrophobic substances exhibit improved solubility
by changing the dielectric constant of the solvent by the use of cosolvents rather than changing the pH of the solvent [Veiga M; 1993]. The use of salt forms is a well-known technique to enhance dissolution profiles and is the most common and effective method to enhance the solubility and dissolution rates of acidic and basic drugs [Nawa M; 2007]. An alkaloid base is slightly soluble in water, but if the pH of the medium is reduced by addition of acid, the solubility of base is increased. The reason for this increase is that the base is converted to a salt, which is relatively more soluble in water. The solubility of slightly soluble acid can be increased by increasing the pH by addition of alkali. Solubility of aspirin and theophylline has been enhanced by salt formation.

Scientists are directing their efforts on prodrug strategy as a chemical/biochemical approach to overcome various barriers which can hinder drug delivery, including solubility. Placing a polar functional group in the structure of a molecule with limited aqueous solubility should enhance solubility. In the case of a prodrug, that functionality would have to be removed/modified, either chemically or enzymatically, to regenerate the parent drug. An excellent example of a non-ionizable drug used to achieve better oral delivery is the drug sulindac as a more water-soluble delivery form of its sulfide metabolite, which is an effective NSAID [Duggan D; 1980]. The sulfoxide group is more polar and therefore has better interaction with the solvent than the reduced sulfide.

![Figure 1.1. Structure of Sulindac](image)

Many prodrugs designed to increase water solubility involve the addition of an ionizable promoiety to the parent molecule. Because charged molecules have greater difficulty in crossing biological barriers, one must balance increased water solubility with the potential for decreased permeability. Many recent examples have focused on the use of the phosphate group either directly linked to the parent drug, where possible, or through a linker group such as formaldehyde [Stella V; 1996]. Unlike some other promoieties, many phosphate esters show good chemical stability while undergoing rapid and often
quantitative cleavage \textit{in vivo} via alkaline phosphatases. Forsamprenavir, a phosphate prodrug of the HIV protease inhibitor, amprenavir in the form of a calcium salt is 10 times more soluble than amprenavir [Sohma Y; 2003, Vierling P 2003, Brouwers J 2007]. Another example is fosphenytoin, a prodrug of phenytoin, an erratically absorbed, poorly water-soluble drug [Fischer J; 2003] and which in the form of prodrug has excellent oral availability [Burstein A; 1999].

1.2.3. Miscellaneous:

A] Co-crystallization

A co-crystal is a crystalline material that consists of two or more (electrically neutral) species held together by non-covalent forces [Aakeroy C; 1997]. The term encompasses a whole range of complexes such as molecular complexes, solvates, inclusion compounds, chanel compounds and clathrates [Dunitz J; 2006]. Pharmaceutical co-crystallisation is emerging as an attractive alternative to polymorphs, salts and solvates in the modification of an API during dosage form design. Co-crystals primarily consist of two substances that are solids at room temperature. They are a product of more rational design than solvates and are more stable [Track A; 2004]. While co-crystals are defined by a single phase (miscible) multi-component system in the crystalline state, in the amorphous state they are referred to as molecular dispersions [Tong P; 2001] with interactions between the components distinguishing them from solid dispersions. Co-crystals are not referred to as solid dispersions but solid dispersions may occur whilst preparing co-crystals from solutions.

A number of co-crystals have been prepared with co-crystallising agents classified as GRAS (generally recognized a safe) [Remenar J; 2003]. Co-crystallisation between two APIs has also been proposed. This may require the use of sub-therapeutic amounts of drug substances such as aspirin or acetaminophen [Almarsson A; 2004] or the APIs to have similar levels of therapeutic active concentration. Co-crystals can be prepared by evaporation of a heteromeric solution or by grinding the components together. Sublimation, growth from melt and slurry preparation has also been reported [Zaworotko
Chapter 1

Introduction

M; 2005]. Addition of small amounts of solvents during the grinding process has been shown to enhance the kinetics of co-crystal formation [Shan N; 2002].

The dissolution of co-crystals of itraconazole with succinic acid, malic acid and tartaric acid was compared to that of pure crystalline and amorphous drug and it was found that there was a 4-20-fold increase in dissolution parameters [Remenar J; 2003]. Co-crystal of a development candidate API formed with glutaric acid increased its aqueous dissolution rate by 18 times over that of the homomeric crystalline form and studies in beagle dogs showed that the co-crystal form also gave notably increased plasma AUC than the parent crystal form [McNamara D; 2006]. Despite lack of precedence in marketed products and concerns about safety and toxicity of co-crystal agents, there is growing activity on this area.

B] Co-solvency

The addition of co-solvent to water can produce a dramatic increase in the solubility of drugs particularly weak electrolytes and non polar molecules [Kawakami K; 2006]. Most co-solvents have hydrogen bond donor and acceptor groups as well as small hydrophobic regions [Maheshwari R; 2008]. Their hydrophilic hydrogen bonding groups ensure water miscibility, while their hydrophobic regions interfere with water’s hydrogen bonding network, reducing overall intermolecular attraction of water. By disrupting water’s self association, co-solvents reduce its ability to squeeze out non polar, hydrophobic compounds, thus increasing solubility.

The water soluble organic solvents used in commercially available solubilized oral formulations are polyethylene glycol 400, ethanol, propylene glycol and glycerine. Etoposide, a sparingly water soluble antineoplastic agent is solubilized in a cosolvent mixture of PEG 400, glycerine, citric acid and water in 50 mg VePesid soft gelatin capsules. Digoxin is solubilized in a cosolvent mixture of propylene glycol, PEG 400 and 8% ethanol in 200 µg soft gelatin capsules. The oral bioavailability was observed to be 90-100% in comparison to solid tablet formulations which have a bioavailability of 60-80% [Strickley R; 2004]. Ritonavir, an HIV protease inhibitor with peptide-like structure, has an intrinsic water solubility of 1µg/ml and two weakly basic thiazole groups with
pKas of 1.8 and 2.6 [Law D; 2001]. It is solubilized in a cosolvent mixture of propylene glycol, ethanol, water, the surfactant Cremophor EL and peppermint oil to 80 mg/ml in Norvir oral solution [Strickley R; 2004].

**C] Liquisolid Tablet technology**

A recent entrant into this arena of techniques for enhancing solubility is the liquisolid tablet technology [Javadzadeh Y; 2007]. These systems are acceptably flowing and compressible powdered forms of liquid medications that imply liquid lipophilic (oily) drugs or water-insoluble solid drugs dissolved in a suitable water-miscible solvent system. Such liquid medication may be converted into a dry-looking, nonadherent, free-flowing and readily compressible powder by a simple admixture with selected powder excipients referred to as carriers and coating materials. However, even though in the liquisolid and powdered solution systems the drug might be in a solid dosage form, it is held within the powder substrate in solution or in a solubilized, almost molecularly dispersed state. Therefore, due to their significantly increased wetting properties and increased surface of drug available for dissolution, liquisolid compacts of water-insoluble substances may be expected to display enhanced drug release properties and therefore improved bioavailability [Tayel S; 2008, Khaled K; 2001].

**1.2.4. Nanotechnology based approaches:**

**A] Nanosponges**

Different names have been used to refer to CDs. These include Schardinger dextrins, cycloamyloses and cycloglucans, with cyclodextrins being the most common usage. The CDs are designated as α, β and γ depending on whether 6,7 or 8 glucopyranose units are present in the cyclic structure. CDs containing glucose units are also known however the most commonly studies are the α, β and γ CDs. The structures were deciphered in 1942 with the development of X-ray crystallography [Szejtli J; 1998].
An important structural feature that is evident in these compounds is their toroidal shape with a well-defined cylindrical cone defining a cavity of about 8Å deep and 5-10 Å in diameter, depending on the number of glucose units. The CD ring contains primary hydroxyl groups lying on the narrow end of the cone and secondary hydroxyls on the wider side making their extension hydrophilic, with cavities that are hydrophobic in nature.

This feature plays a role in trapping hydrophobic molecules of different sizes into their cavities and have been exploited for various applications such as chemical processing agents, chromatographic separations and also in the pharmaceutical industry [Jiang Z; 2004].

The Table 1.3 summarizes important physical properties of α, β and γ CDs.
Table 1.3. Physical properties and molecular dimension of Cyclodextrins

<table>
<thead>
<tr>
<th>Property</th>
<th>α-CD</th>
<th>β-CD</th>
<th>γ-CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of glucose units</td>
<td>6</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>972</td>
<td>1135</td>
<td>1297</td>
</tr>
<tr>
<td>Water solubility (g/100 cm³) at 25°C</td>
<td>14.5</td>
<td>1.85</td>
<td>23.2</td>
</tr>
<tr>
<td>Internal diameter (Å)</td>
<td>4.9</td>
<td>6.2</td>
<td>7.9</td>
</tr>
<tr>
<td>External diameter (Å)</td>
<td>14.6</td>
<td>15.4</td>
<td>17.5</td>
</tr>
<tr>
<td>Height cone (Å)</td>
<td>7.9</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td>Cavity volume (Å)</td>
<td>176</td>
<td>346</td>
<td>510</td>
</tr>
</tbody>
</table>

**Host-Guest interaction of CDs:**

CD can form inclusion compounds as well as crystalline clathrates with a large variety of molecules and exhibit size specificity in binding organic compounds. They are known as molecular hosts capable of including, with a degree of selectivity, a range of guest molecules via a non covalent interaction in their hydrophobic cavities. This phenomenon is known as host-guest interaction. Although the interaction between CDs and organic molecules has been used as a basis for absorption or separation of various organic agents, the solubility of these CDs in water and other organic solvents impose limitations to their application in such media.

**CD modifications:**

Several reviews on the chemical modification of CD to effect the introduction of functional groups onto the CD backbone by different chemical mechanisms have been reported [Croft A; 1983]. The modifications can be effected at the primary and secondary alcohol positions [Rong D; 1990, Yu L; 2000]. CD polymers can also be prepared by covalently linking two or more CDs. The CDs fixed into polymeric structures behave differently from their monomeric derivatives. They can be water-soluble or water insoluble depending on the reaction conditions [Mocamu G; 2001]. Water soluble polymers have a wide variety of potential applications such as controlling the release of soluble substances across a membrane and partitioning of organic compounds in an aqueous two-phase system. As with other CD-based compounds, the usefulness of these
polymers lies mainly in their ability to form inclusion complexes with lipophilic guests [Harada A; 1976].

CDs and their derivatives have been used as solubilizers to enhance the loading capacity of liposomes, microparticles and nanoparticles. CD inclusion complexes have been found to increase the entrapment efficiency of liposomes [Jiang Z; 2004]. β-CD complexes have been incorporated into solid lipid nanoparticles to improve the loading capacity and give a slower drug release [Harada A, 1984]. Modified CDs such as amphiphilic CDs, have been used as matrices for preparing nanoparticulate systems [Harada A; 1990, Xinyuan Z; 2004, Tomoki O; 2003].

Nanosponges

Nanosponges (NS) are nanoporous colloidal systems which can be used as carriers for drug delivery. They can be used to solubilize poorly water-soluble drugs and provide prolonged release as well as improve drug’s bioavailability [Modi A; 2007]. Nanosponges are β-CDs cross-linked with carbonate bonds. These are solid, water-insoluble and crystalline in nature. They form opalescent suspension in water. They are used as carriers for active ingredients. Their unique features include range of dimensions possible (1µ or more), tunable polarity of the cavities and ability to be linked with different functional groups [Trotta F.2006].

Advantages of NSs:

- Capable of carrying both hydrophilic and lipophilic drugs
- Can improve the solubility of poorly water-soluble molecules
- Can decrease the side effects of drugs
- Can mask unpleasant flavours
- Adsorbents to convert liquids to solids
- Can protect drug from degradation

The use of NSs based on CD obtained using organic carbonates as cross-linkers was initially aimed with success at eliminating the chlorinated aromatic compounds present in traces in water as well as in soil or air. The cross linkers investigated included diisocyanates, epichlorhydrin and diphenyl carbonate. Following the process of synthesis developed for their preparation, the nanosponges solidify into spherical particles. The
nanosponges contain β-CD as building blocks, which are cross-linked by carbonate groups to form a highly cross-linked network. The final structure contains a network of more hydrophilic channels which contains both CD lipophilic cavities and carbonate bridges [Trotta F:2009 ]. Nanosponge structural characterization showed that the carbonate linkage was added to the primary hydroxyl groups of the parent β-CD unit [Swaminathan S; 2010]. Thus, drug molecules could be included inside the nanocavities of β-CD and due to cross-linking further interactions of the guest molecule with more CD might be occurring. Cyclodextrin nanosponges have been used as a vehicle for antitumoral drugs such as paclitaxel, camptothecin and tamoxifen which present problems of bioavailability because their solubility in water is low or non-existent. The drugs were incorporated into nanosponges and experiments were carried out on various cell-lines to study their antiproliferative effect. The complexes showed greater effect than that of drug alone [Trotta F;2009].

\[
\begin{align*}
\text{Diphenyl carbonate} & + \text{β- Cyclodextrine} \\
\rightarrow \text{β- Cyclodextrine based Nanospone}
\end{align*}
\]

**Figure 1.4. Crosslinking of β-CD with diphenyl carbonate**

Cavalli R [2006] prepared CD based NSs for drug delivery. The structure of β-CD based NSs were principally investigated by FTIR, DSC and PXRD analysis. Sizes, morphology and toxicity was also studied. The capacity of NSs to incorporate molecules within their structure was evaluated using drug with different structures and solubilities. The NSs were found capable of carrying both lipophilic and hydrophilic drugs and improve the solubility of poorly water soluble molecules. Swaminathan S [2007] formulated β-CD based NSs for itraconazole with rational to enhance the solubility of itraconazole, so that the bioavailability problems are solved. Solid dispersion technique had been used for drug incorporation. They studied effect of ternary component copolyvidonum on solubility of itraconazole. Phase solubility studies had been carried out to compare the
solubilization efficiency of NSs, copolyvidonum and combination. Result showed that the solubility of itraconazole enhanced more than 50 folds with ternary solid dispersion system. Phase solubility studies showed that use of copolyvidonum in conjunction with NSs helps to increase the solubilization efficiency of NSs.

Hyper-cross-linked CD polymers nanostructured to form 3-dimensional networks were developed [Trotta F;2009]; they were obtained by reacting CD with a cross-linker such as carbonyldiimidazole. NSs were synthesized in neutral or acidic forms, depending on the agent used as cross-linker. They were found to be solid nanoparticles and were prepared in crystalline form with spherical shape using an ultrasound-assisted preparation method. The average diameter of a NS is below 1 µm but fractions below 500 nm can be selected. NSs can encapsulate various types of molecules by forming inclusion and non-inclusion complexes. In the study dexamethasone was used as model molecule; up to 35% could be incorporated.

Three types of β-CD based NSs obtained with different cross-linking ratio (viz. 1:2, 1:4 and 1:8 on molar basis with the cross-linker) were formulated to protect the lactone ring from hydrolysis and to prolong the release kinetics of camptothecin [Swaminathan S;2010]. PXRD, DSC and FTIR studies confirmed the interactions of camptothecin with NS. PXRD showed that the crystallinity of camptothecin decreased after loading. The particle sizes of the loaded NS formulations were between 450 and 600 nm with low polydispersity indices. The zeta potentials were sufficiently high (-20 to -25 mV) to obtain a stable colloidal nanosuspension. The in vitro studies indicated a slow and prolonged release over a period of 24 h. The NS formulations protected the lactone ring of camptothecin after their incubation in physiological conditions at 37°C for 24 h with an 80% w/w of intact lactone ring when compared to only around 20% w/w of plain camptothecin. The cytotoxicity studies on HT-29 cells showed that the camptothecin formulations were more cytotoxic than plain camptothecin after 24 h of incubation.

B & C] Nanocrystals and nanosuspensions:

Low saturation solubility combined with a low dissolution velocity of the drug is the obstacle preventing sufficiently high blood levels. A general approach used for many years is the micronization of poorly soluble drugs by colloid mills or jet mills.
Micronization increases the dissolution velocity of the drug but does not change the saturation solubility. At very low saturation solubility, the achieved increase in dissolution velocity does not lead to a sufficiently high bioavailability [Müller R; 2001]. Products of nanotechnology are revolutionizing modern medicine as evident from the global initiatives to support nanotechnology and nanomedicine research. The field of drug delivery is a direct beneficiary of these initiatives. Due to their versatility, nanoparticles are helping to address the challenges of modern as well as conventional drugs. Although any particle of a size < 1 µm diameter is a nanoparticle, the present trend is for developing particles < 100nm as they might exhibit some unique physical properties and hence potentially different and useful biological properties. However, achieving sizes <100 nm is more feasible with hard materials compared to drug and polymer molecules, which are soft materials. For drugs that are soft materials with melting point below 300 °C particles in the size range of 1-100 nm are more difficult to prepare. Hence a reasonable goal is 300nm for drugs and polymer materials [Gupta R; 2006]. Comminuting or milling is the oldest mechanical unit operation for size reduction of solids. With decreasing particle size, materials exhibit increasing plastic behavior making it more difficult to break small particles than large particles. An empirical index, known as Bond work index (\( W_i \)) has been developed, which represents energy required for grinding [Bond F; 1954].

\[
W_i = 10 \left( d_p^{-1/2} - d_f^{-1/2} \right) \quad \text{Equation 1.10}
\]

\( d_p \) = diameter of product \hspace{1cm} \( d_f \) = diameter of feed

To reduce the size of a 1mm particle, the energy required in terms of Bond index is given in Table IV for various product sizes [Gupta R; 2006].

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Product diameter(µm)</th>
<th>Energy required (Bond work index)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>100</td>
<td>0.68</td>
</tr>
<tr>
<td>02</td>
<td>10</td>
<td>2.85</td>
</tr>
<tr>
<td>03</td>
<td>1</td>
<td>9.68</td>
</tr>
<tr>
<td>04</td>
<td>0.5</td>
<td>13.83</td>
</tr>
<tr>
<td>05</td>
<td>0.1</td>
<td>31.31</td>
</tr>
</tbody>
</table>
Hence, it is a very energy intensive to go down to nanoparticle size range. Besides size, other critical parameters are i) toughness/brittleness (in tough materials, stress causes plastic deformation, whereas in brittle materials cracks are propogated; hence size reduction of brittle materials is easier than for tough materials) and ii) hardness, abrasiveness, particle shape and structure, heat sensitivity and explodability. 

In building-up process, drug is dissolved in a solvent to achieve molecular solution. Then, the nanoparticle precipitate is obtained by removing the solvent rapidly or by mixing an antisolvent to the solution, reducing its solubilizing strength. Initially, nuclei are formed, which grow because of condensation and coagulation giving the final particles. If the rate of desolubilization is slow, then sticky nuclei/particles are formed that have a higher tendency of agglomeration, giving large-size particles. To obtain nanoparticles, a high desolubilization rate or a surfactant is required, which can isolate the particles. Based on these requirements, general methods for nanoparticle production are available which include supercritical fluid process and emulsification-diffusion process. Relative to mechanical micronization processes, precipitation from solution can offer greater flexibility for controlling the amorphous or crystalline nature of the active pharmaceutical ingredient as well as for achieving high drug loadings [Matteucci M; 2006]. Hydrophilic groups in the surfactants lead to rapid wetting of the high surface area particles in aqueous media, as in case of oral delivery [Gassman P; 1994, Johnson B; 2003]. During mixing, the nucleation rate depends on the degree of supersaturation (S). The degree of supersaturation, prior to precipitation varies locally as a function of the mixing process until the solution is fully mixed [Matteucci M; 2006].

The rate of primary nucleation is given by

\[
B^\circ \propto \exp \left( \frac{16\pi \gamma^3 V_m^2 N_A}{3(RT)^3(\ln(1+S))^2} \right)
\]

Equation 1.11

Where \( B^\circ = \) nucleation rate, \( \gamma = \) interfacial tension, \( V_m = \) molar volume,
\( N_A = \) Avogadro’s number, \( R = \) ideal gas law constant, \( T = \) temperature.
Once nucleation occurs, particles grow by condensation as molecules diffuse to the particle surface and are incorporated into the solid phase and by coagulation [Matteucci, M; 2006]. Condensation decreases supersaturation by reducing the mass of solute in the mixture and therefore competes with nucleation. The nucleation rate depends more strongly on $S$ as seen in above equation, than does the rate of condensation [McCabe W; 2001]. High nucleation rates offer the potential to produce a large number of submicrometer particles in the final suspension, if the growth can be arrested. Stabilizers can be used to slow growth by condensation and coagulation. The hydrophobic portion of an amphilic stabilizer may be adsorbed on the precipitating hydrophobic drug surface, while the hydrophilic portion provides steric and/or electrostatic stabilization in the aqueous medium [Matteucci M; 2006].

**Mechanism:**

The increase in saturation solubility due to nanonisation can be explained by the Kelvin and the Ostwald-Freundlich equation. Kelvin equation describes the change in vapour pressure due to a curved liquid/vapor interface (meniscus) with radius $r$ (for example, in a capillary or over a droplet). The Kelvin equation is used for determination of pore size distribution of a porous medium using adsorption porosimetry.

The Kelvin equation may be written in the form:

$$\ln \frac{p}{p_0} = \frac{2\gamma V_m}{rRT}$$  \hspace{1cm} \text{Equation 1.12}$$

where $p$ is the actual vapor pressure, $p_0$ is the saturated vapor pressure, $\gamma$ is the surface tension, $V_m$ is the molar volume, $R$ is the universal gas constant, $r$ is the radius of the droplet, and $T$ is absolute temperature.

Equilibrium vapor pressure depends on droplet size. If $p_0 < p$, then liquid evaporates from the droplets. If $p_0 > p$, then the gas condenses onto the droplets increasing their volumes. As $r$ increases, $p$ decreases and the droplets grow into bulk liquid.

This process is termed "Ostwald ripening".
If we now cool the vapor, then $T$ decreases, but so does $p_0$. This means $p / p_0$ increases as the liquid is cooled. We can treat $\gamma$ and $V$ as approximately fixed, which means that the critical radius $r$ must also decrease. The further a vapor is supercooled, the smaller the critical radius becomes. Ultimately it gets as small as a few molecules and the liquid undergoes homogeneous nucleation and growth.

The above equation was extended to Ostwald Freundlich relationship to consider solubility terms [Melgardt de Villiers; 2009]:

$$\ln \frac{S}{S_0} = \frac{2\gamma V_m}{rRT}$$

\[ \text{Equation 1.13} \]

Where $S=$solubility of small particle $S_0=$solubility at equilibrium

The Kelvin equation describing the transition of molecules from a liquid phase to a gas phase can also be applied to the transition of molecules from a solid phase (drug particle) to a liquid phase. The vapor pressure is then replaced by dissolution pressure. The equilibrium between dissolving molecules and molecules recrystallizing on particle surfaces (determining the extent of saturation solubility) is shifted in favor of the dissolution process. As a result of this increased dissolution tendency, the saturation solubility increases.

The Noyes-Whitney equation describes the dissolution velocity $dc/dt$ which depends on the surface area $A$ and difference $(c_s - c_x)/h$ [$c_x=$equilibrium concentration in the bulk phase, $h=$diffusional distance]. According to the Prandtl equation, the diffusional distance $h$ is reduced for small particles. The simultaneous increase in saturation solubility ($C_s$) and decrease in $h$ leads to an increased concentration gradient, thus enhancing the dissolution velocity [Müller R; 2001].

Another pronounced property of nanoparticles is their adhesiveness. There is a distinct increase in adhesiveness of ultrafine powders as compared to coarse powders. Based on physics this can be explained by the larger surface area providing more interactive forces between the particles and the surface [Peters K; 1996]. The adhesiveness of the particles to the gut wall after oral administration further enhances the bioavailability. The drug dissolves exactly at the place of its absorption. This process was found to be very
reproducible as there was very little influence of the nutritional state of the patients i.e. between fed state and fasted state [Müller R; 2000].

A special feature of nanosuspensions is the absence of Ostwald ripening [Müller R; 2001], meaning physical long term stability of the aqueous suspension. Ostwald ripening has been described as a reduction in size of the finest particle fraction and their final disappearance combined with simultaneously growth of the largest particles. Reasons for this phenomenon are the different saturation solubilities in the vicinity of differently sized particles and the concentration gradient existing between them. Molecules from the higher concentration solution around very small particles diffuse to the vicinity of larger particles where a lower concentration is present. This leads to supersaturation and drug crystallization which means growth of the larger particles. Simultaneously the vicinity of smaller particles will be below the saturation concentration, thus the new drug will be dissolved that is fine particles will become smaller and smaller and ultimately disappear. The lack of Ostwald ripening in nanosuspensions is majorly attributed to the uniform particle size.

Preparation and stabilisation of nanosuspensions/nanoparticles:

Surfactants have to be added for the physical stability of the nanosuspensions. The choice of surfactants and stabilizers depends not only on the properties of the particles to be suspended (eg: affinity of the surfactant/stabilizer to the crystal surface) but also on the physical principles (electrostatic vs steric stabilization) and the route of administration. In general, steric stabilization is recommended as the first choice because it is less susceptible to electrolytes in the gut or blood. Electrolytes reduce the zeta potential and subsequently impair the physical stability, especially ionic surfactants. In many cases an optimal approach is the combination of a steric stabilizer with an ionic surfactant. There is a wide choice of various charged surfactants available for drug nanocrystals for oral administration [Gupta R; 2006].

In the 80s, drug nanoparticles were produced by Sucker and co-workers [Gabman P; 1994] using a precipitation technique. Precipitation was performed by dissolving the drug in a solvent and adding this to a non solvent. The basic challenge in this technique is that during precipitation procedure the growing of the drug crystals needs to be limited by
addition of surfactant to avoid formation of microparticles. Addition of the solvent to the antisolvent is necessary to yield a very fine product by passing the Ostwald Mier area fast [Keck C;2006]. The first generation nanoparticles were produced by disintegration technique involving the use of pearl mill leading to the product Nanocrystals® [Liversidge G;1991]. The second generation products are drug nanoparticles produced by high pressure homogenization leading to nanosuspensions (Dissocubes®)[Müller R;1994]. 

The disintegration principle for obtaining nanosuspensions are the cavitation forces created in high pressure homogenizers (HPH). The drug powder is dispersed in an aqueous surfactant solution by high speed stirring. The obtained macro suspension is passed through a HPH applying pressures upto 1500 bar and 3 to 10 and maximum upto 20 cycles. The suspension passes through a very small homogenization gap, typically having a width of 25 µm. Due to narrowness of the gap the streaming velocity of the suspension increases drastically, that means the dynamic fluid pressure increases. Simultaneously the static pressure on the fluid decreases below the boiling point of water at room temperature [Müller R; 2001]. Consequently, water starts boiling at room temperature due to high pressure and gas bubbles are formed which implode when the fluid leaves the homogenization gap. These cavitation forces are strong enough to break the drug microparticles to nanoparticles. The mean particle size in the nanometer range obtained by this procedure depends on the pressure and the number of cycles applied, in addition to the hardness of the drug itself. For example, for paclitaxel nanosuspension a mean diameter of 330 nm was reported [Böhm ;1999], and for clofazemine 600 nm [Peters K;1999]. Besides the above factors, the diameters also depend on the production conditions chosen like pressure and number of cycles [Müller R; 2001].

In case of bottom up technology precipitated drug nanoparticles exhibit very often the tendency to continue crystal growth to the micrometer size. Besides this, depending on the conditions of precipitation the particles are completely amorphous, partially amorphous or completely crystalline. Amorphous or partially amorphous particles bear the risk of re-crystallization followed by decrease in bioavailability. Both problems that is avoidance of crystal growth and uncertainty of crystalline/amorphous state could be solved by combining the precipitation with a second high energy step [Kipp J; 2003]. In the patent, it is shown that precipitated particles continued crystal growth if they are not
undergoing a step of high energy shearing. This can preserve the size range of the particles obtained after precipitation step. In addition, this annealing process converts all precipitated particles to crystalline state and removes any concern about physical; stability of amorphous material.

The melt emulsification method traditionally used to prepare solid lipid nanoparticles was adapted to produce drug nanosuspensions. The method was compared with the solvent diffusion process for ibuprofen as model drug. The effect of preparation variables such as stabilizers, drug content, homogenization procedure and cooling conditions on particle size were studied. The nanosuspension of ibuprofen in the form of lyophilized powder as well as granules, was found to be successful in enhancing the dissolution rate [Kocbeck P; 2006]. Amorphous nanoparticles of cefuroxime axetil were prepared by sonoprecipitation method for enhancing its oral bioavailability. Sonoprecipitation was compared with spray drying and precipitation without sonication. Though all three techniques yielded amorphous drug, sonoprecipitation resulted in uniform sized nanoparticles and also showed enhanced dissolution rate and oral bioavailability [Dhumal R; 2008]. Margulis-Goshen [2010] prepared nanoparticles of simvastatin by evaporation of all solvents from spontaneously formed oil-in water microemulsions. In this method, microemulsions containing a volatile solvent as an oil phase was converted into nanoparticles in the form of dry non-oily flakes by freeze-drying. It was found that > 95% of the drug was present in amorphous form, smaller than 100 nm. Tablets containing the flakes of simvastatin nanoparticles showed a tremendous enhancement in dissolution profile compared with conventional tablets.

Microcrystals of ECU-01, a low molecular weight enzyme-inhibitor with anti-inflammatory properties for oral administration, were prepared by precipitation technique using stabilizers such as gelatin, chitosan and cellulose ethers followed by spray-drying of the formed dispersion. The dissolution rate was found to be significantly enhanced due to large surface area which was hydrophilized by the adsorbed stabilizers as evident from the decreased contact angle [Rasenack N; 2003].
1.3. RATIONALE

Poorly water soluble drugs present challenges during dosage form design due to their inadequate solubilization in digestive fluids.

- Drugs with poor water solubility can show performance limitations such as incomplete or erratic absorption, poor bioavailability and slow onset of action.
- Effectiveness can vary from patient to patient and there can be a strong effect of food on drug absorption.
- Inter and intra individual variability leads to inadequate therapy and or safety concern.

In order to overcome problems associated with poorly water soluble drugs there is need to improve solubility. Aqueous solubility of any therapeutically active substance is a key property as it governs dissolution, absorption and thus the efficacy in vivo. To improve the dissolution and bioavailability of poorly water soluble drugs, researchers have employed various techniques, such as micronization, solubilization, salt formation, complexation with polymers, change in physical form, use of prodrug and drug derivatization, alteration in pH, addition of surfactants etc.

Enhancement in dissolution rate and solubility of poorly water soluble drugs can be achieved by formulating supersaturated systems (i.e. solid dispersions) of the drug employing diverse types of carriers, ranging widely from water soluble to amphiphilic to lipid soluble ones. Some of these carriers are PEGs, gelucires, poloxamers, PVP, sugars and urea.

Nanotechnology based and lipid based approaches are being widely investigated to improve the solubility profile of BCS class II/IV drugs [Müller R; 2001]. Nanosizing is a classical approach based on Noyes-Whitney equation wherein the dissolution rate and saturation solubility of drugs could be increased by reducing size at the micro- or nano-scale to increase the surface area of drug particles. The conventional approaches to produce ultrafine drug particles can be divided into top-down and bottom-up technologies [Merisko-Liversidge E; 2003].

The use of natural and synthetic lipids has generated much academic and commercial interest as a potential formulation strategy for improving the oral bioavailability of poorly water soluble drugs [Hauss DJ; 2007]. These formulations can also enhance drug
absorption by a number of ancillary mechanisms, including inhibition of P-glycoprotein-mediated drug efflux and preabsorptive metabolism by gut membrane-bound cytochrome enzymes, promotion of lymphatic transport, which delivers the drug directly to the systemic circulation while avoiding hepatic first-pass metabolism and by increasing GI membrane permeability. These formulations comprise simple solutions of drug in dietary oil and multi-excipient, self-emulsifying drug delivery systems. Self-emulsifying formulations are physically stable, isotropic mixtures of oil, surfactant, co-surfactant and solubilized drug that are suitable for oral delivery in soft or hard gelatin or HPMC capsules.

Nanosponges are nanoporous colloidal systems which can be used as carriers for drug delivery. They can be used to solubilize poorly water-soluble drugs and provide prolonged release as well as improve a drug’s bioavailability. Nanosponges may be prepared by cross-linking β-cyclodextrins with carbonate bonds. They are used as carriers for active ingredients. Their unique features include possibility of fabrication of particles with a range of dimensions (1µ or more), tunable polarity of the cavities and ability to be linked with different functional groups [Trotta F;2009].

1.4. AIMS & OBJECTIVES

The objectives of the present work are application of various nanotechnology based strategies to achieve the objectives of improved solubility, dissolution and oral bioavailability.

- To select suitable drugs belonging to BCS Class II and IV
- To identify various nanotechnology-based approaches for solubility enhancement
- To improve the saturation solubility of the BCS Class II/IV drugs
- To improve dissolution rate of the BCS Class II/IV drugs
- To investigate the effect of selected approach on permeability of chosen BCS Class IV drug
- To select appropriate approach for in vivo studies
- To investigate the effect on the oral bioavailability of the drugs
- To study effect on relevant pharmacodynamic parameters
- To investigate changes in distribution to key organs of animals
To carry out sub acute oral toxicity studies
To carry out statistical studies to identify effect of some variables

1.5. PLAN OF WORK

- Literature review
- Selection and procurement of BCS class II/IV drugs
- Selection of carrier systems/excipients
- Preformulation studies of drugs and excipients

A. Characterization of drugs
B. Analytical method development [UV spectrophotometric, HPLC and HPLC (bioanalytical)]
C. Saturation solubility studies (in distilled water, buffers pH 1.2 and pH 6.8 and biorelevant media)
D. Compatibility studies (Drug & Excipients)

- Selection of approaches for solubility enhancement

A. Development of Nanosponges by cross linking of β cyclodextrin

   i] Synthesis of nanosponges
   ii] Preparation of inclusion complexes of drugs with nanosponges & β CD
   iii] Phase solubility and solution state interaction studies
   iv] Evaluation of NS and complexes based on
      - Drug content
      - Saturation solubility studies (in Distilled Water, buffers pH 1.2 and pH 6.8, FaSSIF & FeSSIF)
      - *In vitro* dissolution studies
      - Porosity determination
      - FT-IR spectra
      - PXRD spectra
      - DSC thermograms
      - C^{13} NMR spectra
      - Particle size and zeta potential determination
B. **Nanosizing by bottom-up technology**

i] Determination of solubility of TEL

ii] Preparation of nanosuspensions

iii] Particle size analysis and zeta potential measurement

iv] Ultracentrifugation and freeze drying

v] Evaluation of freeze dried product by

  o Saturation solubility
  o FT-IR
  o DSC
  o PXRD
  o Wettability
  o Specific surface area
  o SEM
  o Gas chromatography
  o Atomic absorption spectroscopy
  o Pelletization of nanosuspension
  o *In vitro* dissolution studies
  o Stability studies (ambient conditions, 40°C & 75%RH and under refrigeration i.e. 4°C)
  o Effect of electrolytes and pH on physical stability

C. **Self nanoemulsifying drug delivery systems**

i] Determination of solubility of CP in various oils, surfactants and co surfactants

ii] Construction of ternary phase diagrams

iii] Drug loading into SNEDDS mixture

iv] Evaluation of SNEDDS

  o Percent transmission studies
  o Measurement of globule size and zeta potential
Chapter 1

Introduction

- Cloud point determination
- Determination of self emulsification time
- Saturation solubility studies
- *In vitro* dissolution studies
- *Ex vivo* permeability studies
- Preparation of solid SNEDDS and their evaluation

v] Selection of methodology for *in vivo* studies for both drugs

vi] Statistical evaluation of some critical parameters influencing the approach that is selected for *in vivo* studies

vii] *In vivo* studies for determination of relevant pharmacokinetic parameters, pharmacodynamic studies organ distribution studied by scintigraphy and oral toxicity studies
1.6. DRUG PROFILES

Telmisartan:

Telmisartan is a potent, long-lasting, nonpeptide antagonist of the angiotensin II type-1 (AT1) receptor that is indicated for the treatment of essential hypertension and is developed by Boehringer Ingelheim, Germany. It selectively and insurmountably inhibits stimulation of the AT1 receptor by angiotensin II without affecting other receptor systems involved in cardiovascular regulation. It belongs to BCS class II with an aqueous solubility of 9.9 µg/ml and log P of 7.7. Its oral bioavailability is dose dependent with a 40 mg dose reportedly having an oral bioavailability of 42%.

It is a BCS Class II drug that has a dissolution rate limited poor bioavailability. Therapy with this drug offers a good quality of life for hypertensive patients due to the minimal side effects. According to the chemical structure of TEL, it is readily ionizable and subsequently the solubility is also pH-dependent.

It is a white crystalline powder with a molecular weight of 514.6 and a melting point of 261 to 263°C (47). Telmisartan has no chiral centres and exhibits no stereoisomerism. The solubility of telmisartan in aqueous solutions is strongly pH-dependent, with maximum solubility observed at high and low pH. In the range of pH 3–9 it is only poorly soluble. Telmisartan is active as such: it is not a prodrug. The molecule is unusually stable. No Phase I-type metabolism has been observed. Among the AII antagonists, telmisartan is the most lipophilic compound with a partition coefficient log P = 3.2 (n-octanol_buffer at pH 7.4). [Wolfgang W; 2000]

Telmisartan is marketed in dose strengths of 20, 40 and 80 mg once daily. It is marketed under the trade name of Micardis (Boehringer Ingelheim, Germany), Telpres (Abbott Healthcare Pvt Ltd, India), Targit (Pfizer India), Axeten (Organon, India), Temax (Wockhardt) and Telma (Glenmark Pharmaceuticals Ltd.).
**Cefpodoxime proxetil** : Cefpodoxime proxetil (CP) is a prodrug, third generation cephem type broad-spectrum antibiotic developed by Sankyo Co Ltd., Japan and administered orally. CP is a non-crystalline, slightly basic compound and possesses an asymmetric carbon atom in the ester group and is supplied as a racemic mixture of R-isomer and S-isomer.

It is white, slightly brownish powder with a bitter taste or no odour. It is very slightly soluble in water (~ 400 μg/ml). CP is known to exhibit a pH dependent solubility behavior, with highest solubility in acidic pH conditions, and the solubility falls as the pH increases. Its octanol-water partition coefficient at pH values of 1.2, 5 and 9 are 0.08, 1.53 and 1.50, respectively.

CP is absorbed from the intestinal tract after oral administration and hydrolyzed to its parent moiety cefpodoxime acid (CA) by nonspecific esterases in the intestinal wall/plasma. Although CP is designed to improve the permeability and thus bioavailability of the parent molecule CA, it still has only 50% oral bioavailability, when administered orally. The reasons for low oral bioavailability of CP remain poorly investigated. Reported studies have pointed possible reasons of low bioavailability as—the low solubility, typical gelation behavior of CP particularly in acidic environments [Kakumanu V;2006], and pre-absorption luminal metabolism into CA by the action of digestive enzymes [Crauste-Manciet; 1997].

It is widely used in the treatment of respiratory and urinary tract infections and its utility has also been demonstrated in the treatment of skin structure infections, acute otitis media, pharyngitis, tonsillitis and sexually transmitted diseases. It is marketed in dose strengths of 100 and 200mg as tablets and suspensions under the trade name of Vantin (Torrent Pharmaceuticals, USA), Opox FC tablets (Hetero HC, India), Cefoprox (Cipla, India), Cepocor Suspension (Ranbaxy, India).
The preliminary studies including selection of excipients and compatibility studies were initiated based on available resources. Procurement of drug and excipients was carried out by contacting the companies manufacturing or marketing the said ingredient for gift samples. Telmisartan and Cefpodoxime proxetil were provided by Glenmark Generics and Maxim Pharmaceuticals, Pune, respectively. TPGS was a generous gift sample by Isochem, France sourced locally through Chemet India and Capmul MCM was provided by Abitec Corporation, US. Espheres were provided by Ideal Cures Pvt.Ltd., Mumbai as gift sample. All other ingredients and excipients were sourced locally.