CHAPTER-1

INTRODUCTION

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1.1 Introduction

The aim of this introduction is to place the research work of this Ph.D. project in the perspective of current pharmaceutical industry strategy to develop some useful pharmaceutical products, particularly, drug-cyclodextrin molecular inclusion complex and drug nanosuspension formulations.

The concepts of high throughput screening (HTS) approaches and combinatorial chemistry for a new age of potential drug discovery in the pharmaceutical industry, has resulted to increase in number of promising pharmacological effective substances in the preclinical research area[1,2]. With modern techniques drugs are optimized with respects to target protein binding which is usually attained by introduction of additional hydrophobic functional groups into drug molecule, leading to higher *in-vitro* pharmacodynamic efficacies. This was based on the fact, that research interest in the past few years was mainly focused on targets like kinases or nuclear receptors, which required more hydrophobic substrates[3,4].

Recent drug discoveries, as well as predictive combinatorial chemistry, have shown that 75% of current drug development candidates are poorly water soluble[5,6] and thus belong to the classes II (high permeability) and IV (low permeability) of the Biopharmaceutical Classification System (BCS)[7]. Nowadays, a large portion of new drug candidates have high molecular mass and high Log P, which leads to insolubility and low bioavailability of the emerging compounds. The poor water solubility and low permeability subsequently low bioavailability of the lead compound are the major obstacles responsible for the low turnout in the development of new chemical entities as effective drug formulations.

According to the European Medicines Evaluation Agency (EMEA, human guidelines) 'bioavailability means the rate and extent to which the active substance or active moiety is absorbed from a pharmaceutical form, and becomes available at the site of action[8]. From a pharmacokinetic perspective, bioavailability data for a given formulation provide an estimate of the relative fraction of the orally administered dose that is absorbed into the systemic circulation when compared to the bioavailability data for a solution, suspension or intravenous dosage form. In addition, bioavailability studies provide other useful pharmacokinetic information related to distribution, elimination, effects of nutrients on absorption of the drug, dose proportionality and linearity in

pharmacokinetics of the active and inactive moieties. Bioavailability data can also provide information indirectly about the physico-chemical properties of a drug substance before entry into the systemic circulation, such as solubility or permeability and the influence of pre-systemic enzymes and/or transporters.

Thus, depending on the physico-chemical properties of the drugs, either solubility or the permeation rate across the intestinal epithelium may be the rate limiting factor for the drug to enter systematic circulation. In case of drugs belonging to BCS class II and IV, solubility is a rate limiting factor. These drugs possess low oral bioavailability. As a result, small amount of drug is available in the systemic circulation requiring frequent dose in a day and thus resulting in increased dose dependent systemic side effects into the body. This bleak outlook has helped to drive the development of some advance, efficient and novel techniques to administer poorly soluble compounds at safe and effective therapeutic drug levels. It is well known that the oral route has been considered as the most convenient route for drug administration. Drug formulations intended for oral administration are preferred over their non-oral alternatives mainly for reasons such as lower production cost, better suitability for self-medication, higher level of patient safety and better patient compliance[9]. In order to be efficient, an orally administered drug must meet some special criteria. For instance, it must possess sufficient solubility in the gastrointestinal (GI) fluids, should withstand acidic and enzymatic degradation in the GI tract, and must be able to permeate the intestinal barrier so as to reach the systemic circulation in sufficient amounts[10]. Hence, development of suitable oral formulations for poorly water soluble drug is the major challenge for formulation scientists because the first step in the oral absorption process is dissolution of the drug compound in the gastrointestinal (GI) lumen contents but poor aqueous solubility is rapidly becoming the leading hurdle for formulation scientists working on oral delivery of drug compounds[11]. For such specific kind of drugs, several physical and chemical approaches (i.e. reduction in particle size of the drugs, modification of the crystal habit, polymorphs, pseudo-polymorphs, inclusion complex with cyclodextrins, microemulsifying and self-microemulsifying systems, amorphous solid dispersions, soluble prodrugs and salt formation) are used to overcome the low solubility and bioavailability problems[12]. There has been plenty of prevailing literature on polymorphs[13], amorphous form of the drug[14], reduction in particle size of drugs

(nanoparticle/nanosuspension/nanocrystals)[15-19], inclusion into cyclodextrins[20-24], use of cosolvents[25,26] and salt formation[27].

In modern drug delivery research, nanotechnology based approaches are widely used for poorly water soluble drugs in order to improve their therapeutic performance. Nanoparticles acted as ferries for medicinal compound with poor aqueous solubility and/or undesired pharmacokinetic properties including organ toxicity, bear several advantages in comparison to the use of conventional formulations such as solutions[15,28]. Nanoparticulate technology has proven its competency for numerous drugs for large number of applications. The flexibility, versatility and adaptability of the nanoparticulate delivery systems have proven its importance to fulfill the need for improved health care and better patient compliance. Nanoparticulate drug delivery systems include polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, liposomes, nanostructured lipid carriers, nanogels and drug nanoparticles. In recent years, researchers focused their research on nanoparticulate engineering to enhance the oral bioavailability of drugs possessing low aqueous solubility. Nano-particulate drug delivery system proposes enormous assurance to increase drug absorption in intestine. There are several articles and investigation reports available which showed that these strategies are very effective for sufficient increase in bioavailability of poor water soluble drugs[29,30].

These days, pure drug nanoparticles may help to provide a feasible and viable formulation route for the oral administration of drugs having a poor dissolution rate and/or aqueous solubility[31]. Nanosizing technology (nanonization) is one of the most promising approaches to improve the solubility of drugs by an increase in surface area via reduction of the particle size below 1 μm, typically a few hundred nanometers[32,33]. The ability to formulate poorly-water soluble compounds as nanometer sized particles can have a dramatic effect on performance, such as enhancing bioavailability, eliminating food effects, allowing for dose escalation and hence improving efficacy and safety. The potential of nanosized particles to alter tissue distribution after intravenous dosing should always be a consideration. Nanonization has also been applied to reduce variability in pharmacokinetic behavior of oral dosage forms[34]. Nanosizing drug or formulating drug as a nanoparticulate system results in better dissolution and solubilization of drug due to increase in surface area and saturation solubility. Since this approach has been adapted to handle milligram quantities of drug substance, this

technology provides an opportunity for the research scientist to improve screening efforts without having to deal with solubility-related performance issues. The utility of this technology has been proven from the number of marketed/available products based on these techniques. Additionally, the marketed products that have performance issues related to poor solubility of the active pharmaceutical ingredient, redevelopment into nanoparticulate formulations can propose the possibility of adding new life to old compounds with improved efficacy and patient compliance.

Another successful and widely used method to increase drug solubility is by complexation with cyclodextrins is advantageous over the above mentioned techniques because of low hygroscopicity, less toxicity (as compared with solid dispersions) high fluidity, excellent compatibility and compressibility of cyclodextrin complexation improves the stability of drugs in a formulation, resulting in longer shelf life[35]. Presenting the compound as a molecular dispersion combines the benefits of a local increase in the solubility (within the solid solution) and maximizing the surface area of the compound that comes in contact with the dissolution medium as the carrier dissolves. The progression of these useful derivatives from "toys to tools" is a direct result of their availability in highly pure form, their extreme usefulness as pharmaceutical excipients for increasing solubility, bioavailability, stability and general acceptance by various regulating bodies[36,37].

The present study has been undertaken to explore following Nanotechnology based Drug Delivery System (i.e. Nanosuspension) and Molecular Inclusion Complex of drug in Cyclodextrins as these approaches are having wide importance as tools for bioavailability improvement due to their process simplicity and chance of getting market in short period.

1.1.1 Nanosuspension

1.1.2 Molecular Inclusion Complex of drug in Cyclodextrins

1.1.1 Nanosuspension

Nanosuspensions is one of the most potential approaches that can be used for enhancing the dissolution of poorly water soluble/water insoluble compounds as well as low bioavailability by an increase in surface area and saturation solubility via particle size reduction of drug molecule less than 1 μm.

The term "nanosuspension" indicates, strictly spoken, a submicron colloidal dispersion of drug particles in fluid which are stabilized by surfactants/polymers. A

pharmaceutical nanosuspension is defined as very finely dispersed solid drug particles in an aqueous vehicle for either oral and topical use or parenteral and pulmonary administration. The particle size distribution of the solid particles in nanosuspensions is usually less than one micron with an average particle size ranging between 200 nm and 600 nm[38,39]. In nanosuspension technology, the drug is maintained in the required crystalline state with reduced particle size, leading to an increased dissolution rate and therefore improved bioavailability. An increase in the dissolution rate of micronized particles (particle size < 10 μm) is related to an increase in the surface area and consequently the dissolution velocity. Nanosized particles can increase solution velocity and saturation solubility because of the vapour pressure effect[38]. In addition, the diffusion distance on the surface of drug nanoparticles is decreased, thus leading to an increased concentration gradient. The increases in surface area and concentration gradient lead to a much more pronounced increase in the dissolution velocity as compared to a micronized product. Furthermore, the saturation solubility is increased as well. Another possible explanation for the increased saturation solubility is the creation of high energy surfaces when disrupting the more or less ideal drug microcrystals to nanoparticles. Dissolution experiments can be performed to quantify the increase in the saturation solubility of a drug when formulated into a nanosuspension[40].

The stability of the particles obtained in the nanosuspension is attributed to their uniform particle size which is created by various manufacturing processes. The absence of particles with large differences in their size in nanosuspensions prevents the existence of different saturation solubility and concentration gradients, consequently preventing the Oswald ripening effect[41]. Ostwald ripening is responsible for crystal growth and subsequently formation of microparticles. It is caused by a difference in dissolution pressure/saturation solubility between small and large particles.

The oral administration of drugs in form of stabilized pure drug nanoparticles has been reported to have a number of encouraging effects[42]:

a) Bioavailability enhancement
b) Better dose proportionality
c) Reducing fed/fasted variability
d) Reducing inter-subject variability
e) Improved absorption rate (both human and animal data)

Above to all, the most desirable characteristic of any innovation/research is its commercial adaptability and applicability. The pharmaceutical industry has increasingly implemented a systematic pattern of research in an effort to reduce cost and deploy resources in an efficient way. Considering this strategy, industries have centered their research on various nanotechnology based drug-delivery systems. The key focus of pharmaceutical industries is a system that has a greater chance of getting into the market within a short period of time; hence, nanosuspension technology is expected to receive much focus in the near future. Table 1.1 represents the current marketed nanosuspension based formulations manufactured by various pharmaceutical companies[43-45].

Table 1.1 Marketed pharmaceutical nanosuspension formulations manufactured by top down approaches.

Product	Drug/Indication	Company	Manufacturing Technique	Nanoparticle Technology
Rapamune®	Sirolimus / Immunosuppressant	Wyeth	Media Milling	Elan Nanocrystals®
Emend®	Aprepitant / Antiemetic	Merck	Media Milling	Elan Nanocrystals®
TriCor®	Fenifibrate / Treatment of Hypercholesterolemia	Abbott	Media Milling	Elan Nanocrystals®
Megace® ES	Megestrol acetate / Appetite stimulant	PAR Pharm.	Media Milling	Elan Nanocrystals®
Avinza®	Morphine Sulphate/ Severe pain management	King Pharm.	Media Milling	Elan Nanocrystals®
Focalin®XR	Dexmethylphenidate HCl / Treatment of ADHD*	Novartis	Media Milling	Elan Nanocrystals®
Ritalin®LA	Methylphenidate HCl / Treatment of ADHD	Novartis	Media Milling	Elan Nanocrystals®
Zanaflex Capsule™	Tizanidine HCl / Management of Spasticity	Acorda	Media Milling	Elan Nanocrystals®
Trigilide™	Fenofibrate / Treatment of hypercholesterolemia	Horizon Pharm.	High Pressure Homogenization	Skye Pharma IDD®-P Tech.

*ADHD: Attention Deficit Hyperactivity Disorder

Aspects of formulation theory and stabilization of Nanosuspension

Nanoparticles can be formed by building particles up from the molecular state, as in precipitation, or by breaking larger micron-sized particles down, as in milling. In either case, a new surface area, ΔA, is formed, which necessitates a free-energy (ΔG) defined by

$$\Delta G = (\gamma_{S/L})\Delta A \quad (1)$$

Where, $\gamma_{S/L}$ is the interfacial tension between the solid particles and liquid medium. This arises because water molecules incur fewer attractive forces with other water molecules when located at a free surface. The system prefers to reduce this increase in

surface area by either dissolving incipient crystalline nuclei, in the case of precipitation, or by agglomerating small particles, regardless of their formation mechanism. This tendency is resisted by the formulator through the addition of surface-active agents, which reduce the $\gamma_{S/L}$ and therefore the free energy of the system (Figure 1.1).

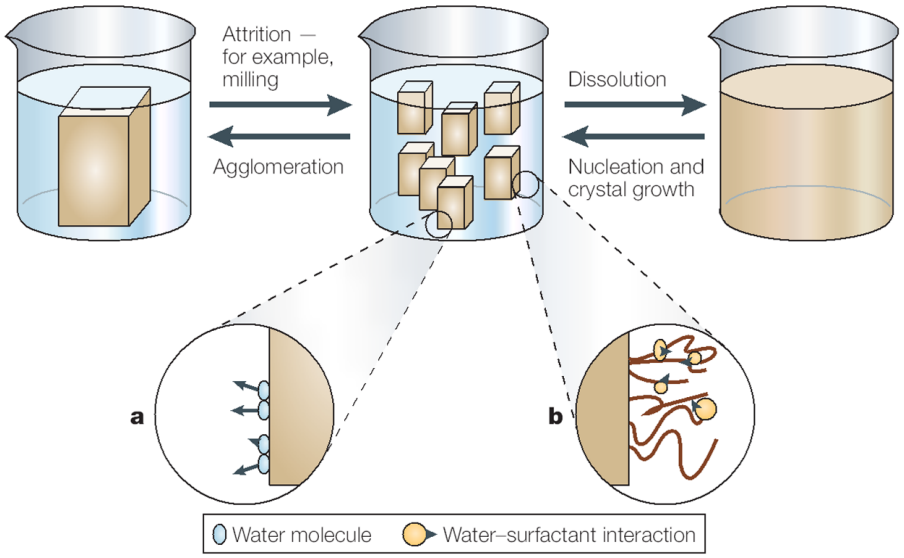


Fig. 1.1 Creation and stabilization of nanoparticles, from the perspective of surface energetics.

These agents confer immediate protection and are more effective when present at the time of creation of the new, fresh surface than if added afterwards. By virtue of their complementary properties, surfactants of two classes are utilized: charged or ionic surfactants, which effect an electrostatic repulsion among the particles; and non-ionic polymers, which confer a steric repulsion that is, they resist compression. If the particles approach each other too closely, they will agglomerate. This must be prevented to ensure a stable system. Energetically, this requires the placement of a sufficiently high energy barrier at relatively long separation distances, to prevent the particles coming too close together. Therefore, a non-ionic polymeric surfactant is also used that coats the surface with a hydrophobic chain, and permits a hydrophilic tail to project into the water. Compression of the polymeric coating, as by the approach of a similarly coated particle, causes loss of entropy and is therefore unfavorable. This provides the necessary repulsive barrier between two neighboring particles. The polymeric coating performs a dual role (i).inhibiting crystal growth and (ii).reducing particle size[46]. The repulsive energy of two similarly charged particles is given by the

equation

$$V_R = \left(\frac{\varepsilon a \psi_0}{\kappa^2}\right) \ln[1 + \exp(-\kappa H_0)] \quad (2)$$

Where, a is the particle radius, H_0 is the distance of separation between the two particles, ε is the dielectric constant of the medium, ψ_0 is the electrostatic surface potential, and κ is related to the thickness of the diffuse electric double layer .

The net repulsive energy decreases with separation of the particles. At shorter distances of separation, there is an attractive force between the two particles due to van der Waals forces. The superposition of both these forces results in an attractive potential, provided the particles can overcome an energy barrier[47]. Therefore, regardless of the nature of the bulk particles, colloidal stability is determined primarily by the choice of surfactants, which affects the repulsive potentials.

Stabilizers (surfactants or polymers) are essential to produce a stable nanosuspension. The selection of stabilizers is based on the route of administration. A full range of surfactants and polymers can be used for production of nanosuspension from GRAS (Generally Regarded As Safe) listed stabilizer[48,49].

The stabilizer's nature and concentration play a significant role in creating a stable formulation. Too small quantity of stabilizer provokes agglomeration/aggregation and too much stabilizer helps in Ostwald ripening. Ostwald ripening is nothing but the crystal growth in colloidal suspension which is responsible for variation in particle size and size distribution. Ostwald ripening is directly proportional to the size dependent solubility of the particles. According to Ostwald-Freundlich equation[50], small particles have higher saturation solubility than the larger particles, developing a concentration gradient between small and larger particles. As a consequence, molecules diffuse from the higher concentration surrounding small particles towards the space around larger particles with lower drug concentration. This generates supersaturated solution around the larger particles, resulting to drug deposition and crystallization onto the large particles. This diffusion process leaves an unsaturated solution surrounding the small particles, causing dissolution of the drug molecules from the small particles to the bulk medium. This diffusion process continues until all the small particles are dissolved. The Ostwald ripening is essentially a process where large particles grow at the expense of smaller particles[51-53]. (Figure 1.2)

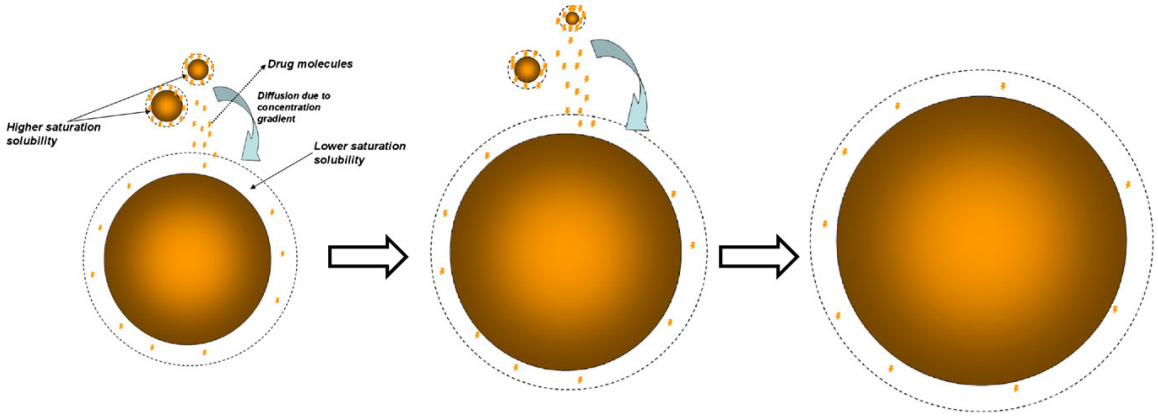


Fig. 1.2 Schematic illustration of Ostwald ripening.

Stabilizers may also lessen the Ostwald ripening as long as they do not enhance the drug solubility[54,55]. Being absorbed on the nanoparticle surface, the stabilizers can reduce the interfacial tension between the solid particles and liquid medium, and thus preventing the Ostwald ripening. The uniform size of particles in nanosuspension can help to restrain the Ostwald ripening, as a narrow particle size distribution minimize the saturation solubility variation and drug concentration gradient with in the medium, and thus reduce the chances of Ostwald ripening[52].

Steric stabilization is attained by adsorbing polymers onto the particle surface and provides effective steric blockade to aggregation of nanoparticles. In pharmaceutical industry, commonly used steric stabilizers include non-ionic polymers such as hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), povidone (PVP), polyvinyl alcohol (PVA), and poloxamers/pluronics. Stabilizing polymers must possess a strong surface affinity to the solid-liquid interface, and their polymer chains should be long enough to provide enough steric barriers at the interface, but not too large to slow down dissolution. Non-ionic nonpolymeric surfactants such as polysorbate 80 (Tween 80) have also been widely used as stabilizers. In addition to stabilizing the system, these can also help as wetting and dispersant agents for very hydrophobic drugs[53-55].

Electrostatic stabilization is gained by adsorbing charged molecules onto the particle surface, and offers efficient electrostatic barriers to agglomeration. Generally used electrostatic stabilizers in pharmaceutical field include anionic surfactants or polymers such as sodium lauryl sulfate (SLS), and sodium di-(2-ethylhexyl)-sulfosuccinate (DOSS)[53-55] The negatively charged ions or molecules on the particle surface provide an electrical barrier to the particles. The combination of steric and electrostatic

stabilization is often used to obtain long-term stabilization.

This technology can be highly beneficial to the pharmaceutical industry and can efficiently meet the drug development industry requirements, such as increasing solubility of poorly water soluble drugs; easy technology transfer to the production scale; cost effective and with little or no regulatory obstacles[31].

Mechanism of bioavailability enhancement of a drug by improving solubility via nanonization

The poor bioavailability of the drug may be due to poor solubility, poor permeability or poor stability in gastrointestinal tract (GIT). Nanonization resolve the problem of poor bioavailability by solving the twin problems of poor solubility and poor permeability across the membrane[42,56,57]. The improved *in vivo* performance of nanoparticles is mainly attributed to-

a) An increase in dissolution velocity and saturation solubility due to increase in surface area.
b) Adherence of nanoparticle to the GIT-wall after peroral administration due to generally adhesive properties of small particles.
c) Changed pharmacokinetic and organ distribution after intravenous administration of nanoparticles compared to solutions[58].

Now, nanonization of drug particles leads to an increase in the surface area, resulting in increased dissolution velocity and saturation solubility. Dissolution velocity is an important parameter affecting the oral bioavailability. Poor water solubility correlates with slow dissolution rate, and inherently lower bioavailability.

According to Noyes-Whitney Equation (Eq. 3), an increase in surface area leads to an improvement of dissolution rate[59].

$$\frac{dc}{dt} = \frac{\mathrm{DA}}{h}(\mathrm{C_S} - \mathrm{C_X}) \qquad (3)$$

Where, dc/dt is dissolution rate, D is diffusion coefficient, A is particle surface area, C_s is saturation solubility, C_x is bulk concentration, and h is diffusional distance over which the concentration gradient occurs (effective boundary layer thickness). The equation shows that the dissolution rate of drug is proportional to the surface area available for dissolution. This principle has been extensively used in micronization/nanonization of drugs for improving their oral bioavailability. Obviously, decrease in particle size to nanometer range will further increase the dissolution rate due to significant increase in

effective particle surface area. Figure 1.3 illustrates the relationship of the parameters of Noyes-Whitney equation.

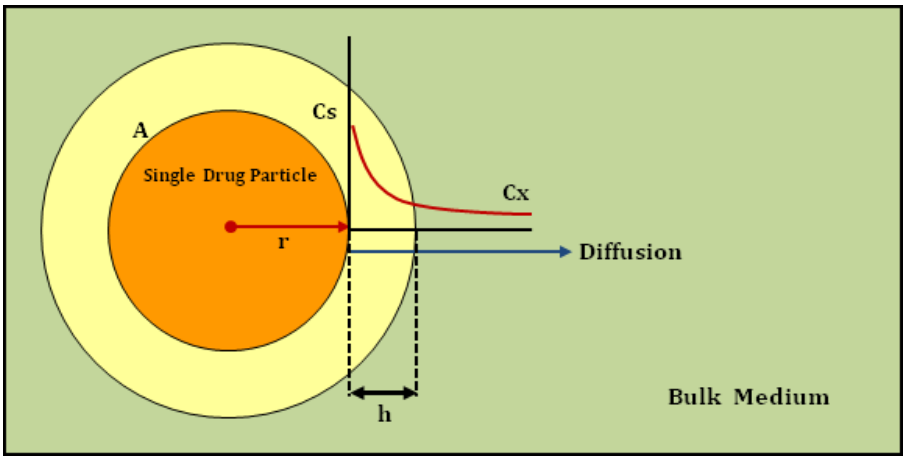


Fig. 1.3 Schematic representation of the parameters of Noyes-Whitney equation.

Further, as per Prandtl equation (Eq. 4), nanonization results in the decrease of the diffusion layer thickness surrounding the particles and an increased concentration gradient between the surface of the particle and bulk solution, which facilitates particle dissolution by increasing dissolution velocity[60].

$$h_H = k\left(\frac{L^{\frac{1}{2}}}{V^{\frac{1}{2}}}\right) \quad (4)$$

Where, h_H is hydrodynamic boundary layer thickness, k is constant, L is length of the surface in the direction of flow and V is relative velocity of the flowing liquid against a flat surface.

It is evident from Eq. 3 & 4 that nanonization is a suitable technique for enhancing bioavailability of poorly soluble drugs, where dissolution is the rate limiting step in systemic absorption[61].

Besides the modifications in dissolution rates, nanosizing can also be capable to improve the saturation solubility of drug particle, which can be explained by the Kelvin-Gibbs equation (Eq. 5) and Ostwald-Freundlich equation (Eq. 6).

As per Kelvin equation, the vapor pressure increases with increasing curvature of the droplet of a liquid in gas. If this is extended to a solid, it implies that the dissolution pressure increases with decrease in particle size[62].

$$\ln\frac{P_r}{P_\infty} = \frac{2\gamma M_r}{rRT\rho} \quad (5)$$

Where, P_r is the dissolution pressure of a particle with the radius r, P_∞ is the dissolution

pressure of infinitely large particles, γ is the surface tension, M_r is the molecular weight of compound, r is the radius of particle, ρ is the density, R is a gas constant and T is the absolute temperature.

In addition to this, according to Ostwald-Freundlich equation, the increased saturation solubility is due to creation of high energy surfaces when disrupting the more or less ideal drug microcrystal to nanoparticle[40].

$$S = S_\infty \, exp\left(\frac{2\gamma M_r}{rRT\rho}\right) \quad (6)$$

Where, S is the saturation solubility of nanoparticle with the radius r, S_∞ is the saturation solubility of infinitely large particles, γ is the surface tension, M_r is the molecular weight of compound, r is the radius of particle, ρ is the density, R is a gas constant and T is the absolute temperature.

The theoretical backgrounds of Kelvin, Ostwald-Freundlich and Prandtl equations support the fact that below a size of approximately 1-2μm, the saturation solubility is a function of the particle size.

The bioavailability of low solubility drugs is directly proportional to drug particle size. Therefore, by reduction in the drug particle size in the nanometer range leads to increase in surface area which furthers results in improvement of solubility as well as the dissolution velocity. Both are very important factors with regard to the aim of enhancing the bioavailability of poorly soluble drugs.

Preparation and fabrication of nanosuspensions

There is several production technologies used to prepare drug nanosuspension. The existing approaches are based on mainly two basic techniques of nanonization; viz "Top down" and "Bottom up". A schematic diagram of top down and bottom up techniques has been shown in Figure 1.4.

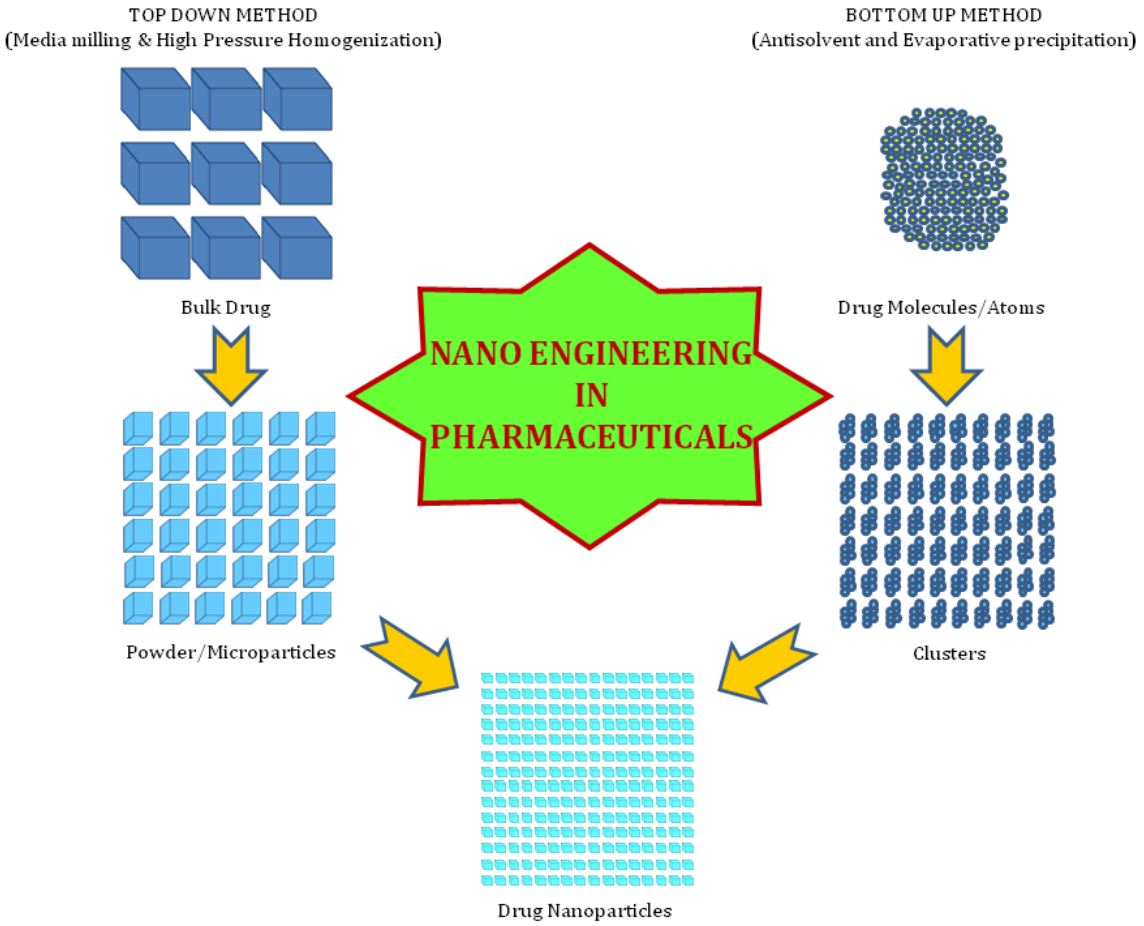


Fig. 1.4 Schematic diagram of "Top Down" and "Bottom Up" methods for preparation of Nanoparticles (Nano Engineering in Pharmaceuticals).

The top down techniques are disintegration methods that basically rely on mechanical communition of large crystalline drug particles into nanoparticles that means various types of wet milling[40,41,63-70]. On the other hand, the bottom up technologies starts from the drug molecules which are dissolved in suitable solvent and precipitate them by adding solvent to the anti-solvent/non-solvent[71-77].

It must be taken in consideration that developed nano-formulation should be compatible with appropriate biological fluid for effective biological activity of API. For nanosuspension, intended to use for oral administration, compatibility of the formulation in simulated gastric and intestinal fluid is recommended[78] and for parenteral administration, compatibility test of nano-formulation in plasma should be carried out[79-81]. Figure 1.5 summarizes the various technologies of production of nanosuspension.

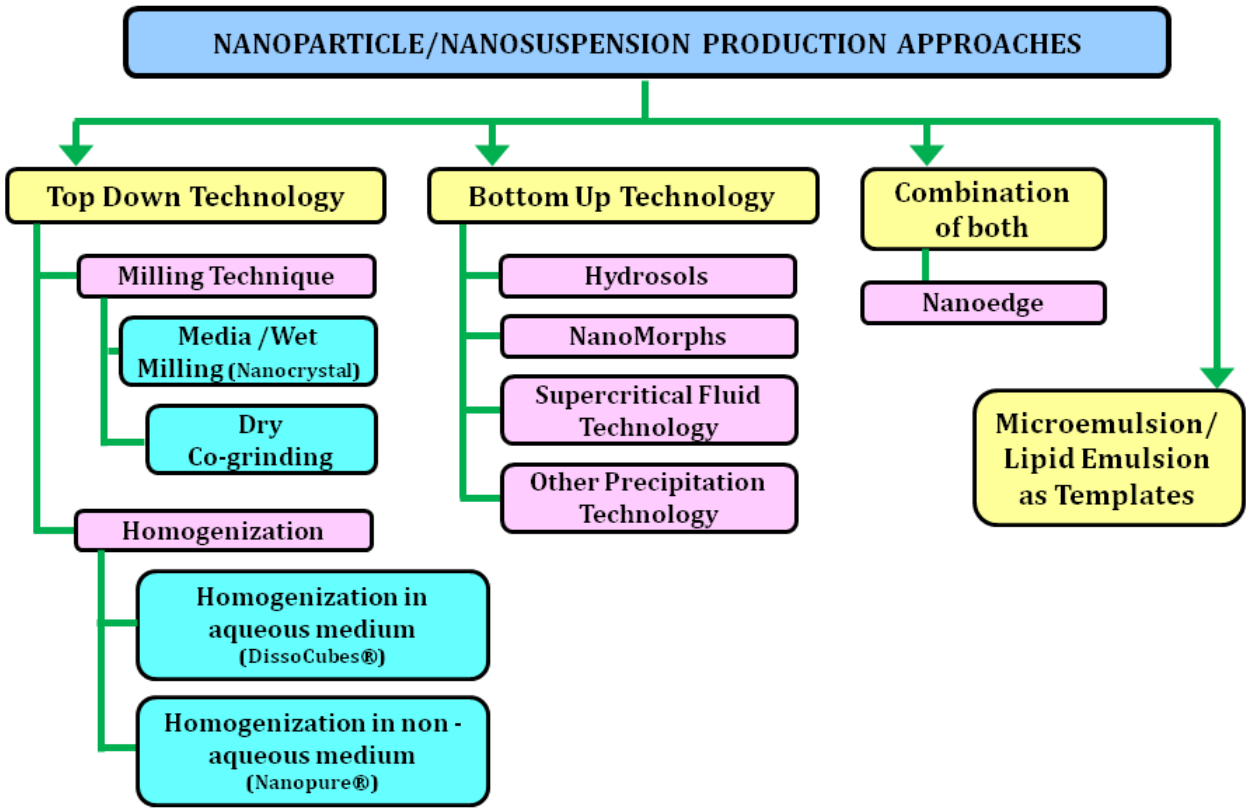


Fig. 1.5 Various approaches of production of Nanoparticle/Nanosuspension.

Top down technology

Top down approach is based on use of mechanical force to convert large crystalline drug particles to drug nanoparticles and performing particle size diminution. Top down technology includes:

- Milling Technique and,
- High Pressure Homogenization (HPH)

Milling Technique

Wet Media Milling (Nanocrystal®)

This is the extensively used top down approach for size reduction of drug particles to the nanometer range. Nanocrystal® is a patent protected technology by Liversidge et al. in 1992[82]. Previously, the technology was possessed by the company NanoSystems but in recent time it has been acquired by Elan Drug Discovery, PA, USA. Among all methods reported for nanosizing drug particles in pharmaceutical industry, media milling technique is considered to be the leader with the uppermost commercial applicability. In this method the drug nanosuspension is prepared by subjecting the drug particles to media milling in presence of grinding media, surface stabilizer(s) and dispersion media, wherein the high energy shear forces generated as a result of collision of hard milling

media with drug particles provide sufficient energy to disintegrate drug microparticles to nanosized drug particles[56,63,64,83]. The grinding media consist of stiff milling media, may be composed of glass, zirconium oxide, ceramics and plastics (e.g., cross-linked polystyrene resin). During manufacturing of nanosuspension through wet media milling technique, the typical process temperature should be kept less than 40°C to prevent thermal degradation. In general, the technology involves pre-dispersing drug in aqueous solution containing hydrophilic stabilizers and then the slurry is wet milled with a grinding media over a specified time period. Necessary milling time mainly depends on the rigidity of the drug particles, viscosity of the drug suspension, temperature, size and density of the milling medium and total energy input during milling[84]. Milling time can last from about 30 minutes to several hours or even days[85].

Dry co-grinding

As per the literature, drug nanosuspension can also be prepared by dry milling/dry co-grinding technique[86,87]. In this method, the milling chamber is charged with drug, additives (polymer/surfactant) and grinding media (beads/pearls) without any aqueous/organic solvent and the mixture is ground at very high speed. Formation of drug nanoparticle occurs when ground mixture of drug and additives is dispersed in water. Improvement in physicochemical properties and dissolution of poorly water soluble drugs due to co-grinding can be attributed to an improvement in the surface polarity and transformation from a crystalline to an amorphous form[88].

High Pressure Homogenization (HPH)

High Pressure Homogenization (HPH) is another widely used technique based on top down disintegration theory for production of drug nanosuspension[33,66,70,83,89-96]. In the process of development in nano-engineering, HPH established itself as a promising technology which offers outstanding option for production of high quality drug nanoparticle on small/large scale. Effective nanosizing of drug particle essentially requires high energy input, resulting in huge cloud of impact forces. When a suspension is homogenized, the critical high energy parameters such as particle collision and cavitation play an important role.

High pressure homogenization basically includes two techniques;

(a) Homogenization of drug in an aqueous medium (surfactant solution in water) i.e. DissoCubes®.

(b) Homogenization of drug in a non-aqueous medium or medium with reduced water

content (mixtures of water with reduced water content) i.e. Nanopure® technology[49].

The advantages of HPH method include, applicability for drugs having poor solubility in water as well as organic solvents, capacity to handle very dilute to highly concentrated suspensions, industrial scale up feasibility, allows aseptic production, low risk of product contamination etc. The limitations of this method include, prerequisite for drug to be in micronized state before homogenization, high cost of instrument, high number of homogenization cycles and possible contamination by metal ions from homogenizer.

Bottom up Approaches

Bottom up approaches means that one starts from the molecular level, and goes via molecular association to the formation of solid particles, using classical precipitation techniques. Examples of precipitation techniques are:

- Hydrosols,
- NanoMorph,
- Supercritical Fluid Technology,
- Other precipitation techniques

Hydrosols

The hydrosols were developed by Sucker and his team at the Sandoz Company (at present Novartis Inc.)[97]. In this technique the drug is dissolved in a solvent (organic solvent) and added at slow and constant flow to a non-solvent /anti-solvent (usually water and surfactant solution) in a high shearing environment to initiate rapid precipitation of a finely dispersed product. To attain this it is essential to cross the so called Ostwald-Mier region very fast, which means reducing the solvent quantity very quickly[98]. This is achieved by adding the solvent to a non-solvent; doing it the other way would lead to generation of larger drug crystals. The produced nanoparticle/nanocrystals require to be stabilized by surfactants or polymers prevent the growth of macro-crystals from nanocrystals.

Nanomorph

The hydrosols developed by Sucker and team were crystalline in nature but contrary to this, the company Knoll (currently owned by Abbott Inc.) develop a new method based on precipitation technique which produce amorphous articles[99]. The product is called Nanomorph. The unique characteristic of Nanomorph is an improvement in dissolution velocity due to amorphous nature of the product. The precipitation in the amorphous

form is achieved by an aqueous polymer solution. However, for getting into the market with Nanomorph, it is necessary to preserve the amorphous characteristic of product during shelf life, as any polymorphic change will result in variation in bioavailability.

Supercritical fluid (SCF) Technology

Nanoparticles can be prepared by supercritical fluid (SCF) technology using drug solution with supercritical fluids. SCF technology has been used to manufacture fine particles of medicinal substances by a build-up process i.e. in contrast to conventional bottom up technique; this involves growing of the particles in controlled fashion to attain desired morphology. A SCF is defined as a substance that is at a pressure and temperature greater than its critical poin. Supercritical fluids are gases or liquids at temperatures and pressures above their critical points (Tc–critical temperature; Pc–critical pressure). Above these points, the SCF exists as a single phase with several advantageous properties of both liquids and gases. The most widely used SCF for pharmaceutical applications is carbon dioxide because of its low critical temperature (31.18 °C), attractiveness for heat sensitive materials including products sourced from biologicals, as well as being non-flammable, non-toxic, GRAS status and inexpensive. Supercritical Carbon dioxide (CO_2), due to its excellent thermodynamic and transport properties, creates very rapid, uniform, and extremely high supersaturation in the solution which leads to formation of nanoparticles/microparticles with narrow particle size distribution[100].

The various methods attempted are rapid expansion of supercritical solution process (RESS), gas antisolvent process (GAS), and supercritical antisolvent process (SAS).

Other precipitation techniques

Recently, some precipitation techniques have been reported for preparing nanosized particles. These include high gravity controlled precipitation (HGCP), sonoprecipitation, the aerosol flow reactor method, evaporative precipitation in aqueous solutions (EPAS) and spray drying.

One of the most promising nano-precipitation techniques available at the commercial production scale is HGCP. This technology uses a rotating packed bed to intensify mass and heat transfer by several orders in a multiphased system. The technique has subsequently been applied to the production of nanoparticulate drugs like cefuroxime axetil, azithromycin, danazol, cephadrine, and salbutamol sulphate[100]. Recently, sonoprecipitation or sonocrystallization, a crystallization process assisted by

ultrasound, has been developed. Its principle involves creation of bubbles (cavitation) followed by collapse, which releases shock waves, thereby promoting nucleation assisted by changes in temperature and pressure. The setup can be relatively simple, comprising an ultrasound probe in a mechanically stirred reaction tank where the anti-solvent is mixed with the drug solution to precipitate the fine drug particles.

Aerosol flow reactor method is a simple and efficient one-step continuous process for engineering drug nanoparticles with narrow particle size distribution. In this method, drug is dissolved in a suitable biocompatible volatile solvent and atomized with the help of pressurized inert carrier gas into nanodroplets by means of an atomizer. Then the nanodroplets suspended in the inert carrier gas are passed through a heated tubular laminar flow reactor maintained at a temperature sufficient to evaporate the solvent. The drug nanoparticles are formed due to instantaneous evaporation of the solvent which induces supersaturation of the drug in the inert gas.

Another precipitation method reported for nanoparticle preparation is EPAS. In this method, an organic solution of drug is preheated through a coil and injected under the surface of a heated aqueous solution with surfactant(s) added to stabilize the particles. Intensive atomization occurs below the liquid surface, which produces a large interface between the organic and aqueous solutions, causing rapid evaporation of the organic solvent and precipitation of particles. Cyclosporine A nanoparticles have been produced using this technique[43]. In spray drying, a drug solution (aqueous or organic) is atomized to fine droplets, which are evaporated in a warm air current to form dry particles. This method is not suitable for production of nanoparticles because spray drying has low cyclone collection efficiency for nanoparticles. With an electrostatic collector, it is now possible to collect spray-dried nanoparticles. The applicability of this approach has been demonstrated using bovine serum albumin solution[101].

Combination of "Top down" and "Bottom up" Technology (NANOEDGE®®)

The previous nanosuspension methods are combined in some cases to gain better size reduction and improved stability of the system. The combination of these methods has resulted in improved advantages associated with nanosuspension technology. The NANOEDGE® is a registered trademark of Baxter International Inc. and its subsidiaries and is patented technology which relies on the precipitation of friable materials for subsequent fragmentation under conditions of high shear and/or thermal energy[75]. The basic principles of NANOEDGE® are the same as those of precipitation and

homogenization nanoprocesses. A combination of these techniques results in smaller particle size and better stability in a shorter time. The success of drug nanosuspension prepared by combination of two technique has been reported[102].

Lipid Emulsion/Microemulsion as template

Apart from the above stated approaches, drug nanosuspension can also be prepared by using lipid emulsion/microemulsion as template. The use of these drug delivery systems as template is applicable for the drugs that are soluble in either volatile organic solvent or partially water miscible solvent.

Drug nanosuspension can be produced by two methods using emulsion/microemulsion as template. In the first method, an organic solvent or mixture of solvents loaded with the drug is added slowly in the aqueous phase containing suitable surfactants to form an emulsion. The organic phase is then evaporated under reduced pressure so that the drug particles precipitate instantaneously to form a nanosuspension stabilized by surfactants. Successful application of these approaches for the production of drug nanosuspension have been reported[74,94].

Characterization of Nanosuspension

The exceptional characteristics and role of nanosuspension as a unique drug delivery system is directly related to their physicochemical properties. Hence, assessment and understanding of such properties is very much necessary to understand their *in vitro* and *in vivo* performance. A good understanding allows prediction of *in vivo* performance as well as allowing particle design, formulation development, and process troubleshooting to be carried out in a rational fashion. The essential characterization parameters for nanosuspensions are as follows:

- Mean particle size and particle size distribution
- Particle surface charge (Zeta potential)
- Crystalline state and particle morphology
- Saturation solubility and dissolution velocity
- Surface morphology
- Stability

Mean particle size and particle size distribution

The mean particle size and the width of particle size distribution (poly dispersity index, PI or PDI) are the basic and central properties of a nanoparticulate system especially

nanosuspension. These important properties directly govern the other characteristics of a nanosuspension such as saturation solubility, dissolution velocity, physical stability and *in vivo* performance of the system also. It has been reported that saturation solubility and dissolution velocity vary significantly with the alteration in the particle size of drug in nano-formulation[40]. A polydispersity index value in between 0.1 to 0.25 indicates a fairly narrow particle size distribution whereas a PI value greater than 0.5 point out a very broad distribution. The generally used techniques for particle size determination of a nanoparticulate system are dynamic light scattering technique, static light scattering technique and microscopy. Photon correlation spectroscopy (PCS) is used for rapid and accurate determination of particle size and polydispersity index[103].

Particle surface charge (Zeta potential)

Particle charge/zeta potential is an indication of physical stability of an aqueous nanosuspension. Particle surface charge is usually determined by Laser Doppler electrophoresis technique and expressed as electrophoretic mobility [(mm/S)/(V/cm)] or zeta potential (mV) of suspended particles in medium. Surface charge can arise from ionization of the particle surface or adsorption of ions (such as surfactants) on to the particle surface. Zeta potential is used as surrogate for surface charge and is often measured by observing the oscillations in signal that results from light scattered by particles located in an electric field[104,105]. The zeta potential of a nanosuspension is governed by both the surfactant and drug itself. In order to obtain a nanosuspension exhibiting good physical stability (stabilized by electrostatic repulsion), a minimum zeta potential of ±30 mV is required whereas in case of combined electrostatic and steric stabilization, a minimum zeta potential of ±20 mV is desirable[96].

Crystalline state and particle morphology

The study and investigation of crystalline state and particle morphology of a drug nanosuspension is essential to predict and understand the polymorphic or morphological changes that occurred due to nanosizing of drug. Drug particles in amorphous form are likely to be generated when nanosuspensions are prepared. Hence, it is essential to investigate the extent of amorphous drug particles generated during production of nanosuspensions. The crystallinity of drug nanoparticles can be assessed by Differential Scanning Calorimetry (DSC)[15]. This is especially important when a drug exists in different polymorphic forms. X-Ray Diffraction (XRD) differentiates amorphous and crystalline nanoparticles as well as different polymorphic phases of the particles,

while DSC is often used as a supplementary tool to XRD. Crystalline particles usually have a sharp melting peak which is absent in amorphous materials. The melting point can also be utilized to differentiate different polymorphs[51].

Saturation solubility and dissolution velocity

The increase in saturation solubility and consequently an increase in dissolution velocity of a drug decide its applications. Although saturation solubility is defined as a compound specific, temperature dependent constant, it also depends on particle size. The determination of saturation solubility and dissolution velocity is very important as these two parameters together help to anticipate any changes in the *in vivo* performance (blood profiles, plasma peaks and bioavailability). To determine the saturation solubility, shaking experiments in various physiological buffers at different temperatures (i.e. 4°C, 20°C and 40°C) need to be performed until equilibrium has been reached. The dissolution velocity of drug nanosuspension should be assessed in various physiological buffers of different pH as per the method described in pharmacopoeia. Literature showed that nanosizing of drugs leads to increase in saturation solubility, further results in enhanced bioavailability of drugs[83].

Surface morphology

Atomic force microscopy (AFM), Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) offer a way for direct morphological examination of developed nanosuspension[106-108]. TEM has a smaller size limit of detection and acquire structural information via electron diffraction but sometimes staining is also required. Experimenter must be aware of the statistically small sample size and effect of applied vacuum on the nanoparticles during investigation of sample. An illustrated image can be produced using freeze-fracture approaches in which a cast is made of the original sample[109].

Stability

Physical and chemical stability of the drug in nanosuspension is an important issue to be analyzed. Physical stability of drug nanoparticle in nanosuspension may alter due to Ostwald ripening whereas chemical stability of the active pharmaceutical ingredient (API) in the nanosuspension is affected in some cases due to possible hydrolysis of the compound. Thus, drug content of the formulation must be studied immediately after preparation to check the chemical stability of the drug. Formation of impurities due to process and formulation parameters must be studied. HPLC is the most common

characterization technique used to evaluate chemical stability that provides precise quantitative analysis of process related degradation impurities.

***In vitro* dissolution study**

Dissolution rate may be defined as amount of drug substance that goes to the solution per unit time under standard conditions of liquid-solid interface, temperature and solvent composition. It can be considered as a specific type of heterogeneous reaction in which a mass transfer results as a net effect between escape and deposition of solute molecules at a solid interface[110].

In vitro dissolution screening should be the first line of biopharmaceutical evaluation of nano-formulations. Since oral nano-formulations are designed to disperse in the stomach contents, dissolution in simulated gastric fluid (SGF) should provide an initial estimate of dissolution rate enhancement. For insoluble compounds, where dissolution is expected to mainly occur in the intestinal region, further *in vitro* testing in simulated intestinal media will provide additional insight on expected bio-performance . Several research articles in literature report an increased *in vitro* dissolution rate for nanosized drugs[29,111-114].

***In vivo* biological performance**

The establishment of an in vitro/in vivo correlation and the monitoring of the *in vivo* performance of the drug is essential part of study, irrespective of the route and the delivery system employed. The bioavailability of a nanosuspension given by any route of administration depends on the dissolution of the drug. *In vitro* dissolution testing in a bio-relevant medium is very significant to predict the *in vivo* performance (bioavailability and pharmacokinetics) of the drug in nanosuspension[78,115]. Dissolution velocity of the nanoparticle can be affected by pH of biological fluids and the characteristics of polymorph, which can vary the pharmacokinetics of drug[48].

There is another special feature of drug nanocrystals, the potential adhesiveness of nanoparticles, which largely affect the *in vivo* performance of drug. The nanoparticles are likely to be stick to a surface because of their enlarged surface area. This can be understood by the principles of physics that the larger surface area providing more interactive forces between the particles and the surface. So according to this theory, the orally administered nanoparticles adhere to the GIT wall and significantly improve the absorption of drug from the site which in turn results in bioavailability enhancement.

Post production processing of nanosuspension

Post-production processing of nanosuspensions becomes essential when the drug candidate is highly susceptible to hydrolytic cleavage or chemical degradation. Processing may also be required when the best possible stabilizer is not able to stabilize the nanosuspension for a longer period of time or there are acceptability restrictions with respect to the desired route. Considering these aspects, techniques such as lyophilization or spray drying may be employed to produce a dry powder of nano-sized drug particles. Rational selection has to be made in these unit operations considering the drug properties and economic aspects[41].

Lyophilization or freeze drying is preferred over spray drying for reasons of convenience of converting nanosuspension to a solid powder. The obtained dry product requires reconstitution prior to administration but it is quite simple to handle and storage and also minimizes the risks caused by inadequate handling (e.g. exposure to high temperature)[62]. Freeze drying process consists of the nucleation and propagation of ice crystals (freezing) and a subsequent sublimation process. Freeze drying has been successfully used for conversion of a nanosuspension to a dry form for various drugs like 2-methoxyestradiol, naproxen, and loviride[116-118].

Spray drying is another method used for drying of a drug nanosuspension in solid state. This technique is widely used due to its simplicity and cost-effectiveness. Spray drying has been successfully used for conversion of nanoparticulate suspension to dry form for various drugs like celecoxib and itraconazole[119,120].

In order to improve patient compliance, dried powder of nanosuspension can be filled directly in capsules or converted to tablets.

Drug nanosuspension administration

Drug nanosuspension can be formulated in variety of dosage forms depending on its application. Their major advantage remains on the nanosize, consequent increase in surface area and low toxicity. Particle size engineering can be used to achieve the intended dissolution rate, in case the drug substance adsorption is dependent on the solubility or both solubility and permeability. The nanosize and increase in the specific surface area of nanoparticle constitute their major advantage and inputs in manufacturability and stability issues. Therefore, one of their obvious critical to quality attributes is the particle size distribution as well as re-dispersion both in the gastric intestinal fluids or plasma.

Nanosized drug particles in liquid or dried nanosuspension formulations offer several therapeutic benefits. Firstly, drug nanoparticles exhibit enhanced dissolution rate, which is due to the greatly increased specific surface area, as described by the Noyes–Whitney equations. Secondly, drug nanoparticles also show increased solubility, which is due to the vapour and dissolution pressure of solid particles that increases when particles sizes decrease below 1000 nm, as described by the Freundlich–Ostwald equation. On the other hand their particulate nature can lead to targeting of monocyte phagocytic systems (MPSs), with unusual pharmacokinetic consequences. From an industrial perspective, nanosuspensions are advantageous, because they can be applied through variety of routes, most importantly the oral and parenteral routes, including intravenous administration, topical, pulmonary, ocular, transdermal, topical and other targeted drug delivery systems.

Felodipine nanosuspension developed by Sahu et al. showed a significant improvement in its oral bioavailability in comparison to bulk drug[29]. Nanosuspension of BMS-488043 (an HIV-attachment inhibitor) exhibited ~5 fold enhancement in oral bioavailability in comparison with conventional dosage forms prepared by utilizing micronized crystalline drug substance[111].

A successful long-acting nanosuspension is Invega® Sustenna® (Jansen), a once-a-month extended release nanosuspension of paliperidone palmitate for intramuscular (deltoid or gluteal muscle) injection. Paliperidone palmitate nanosuspension is obtained by nano-grinding in presence of a surfactant and marketed as a single-use prefilled syringe[121,122].

Nanosuspension of some glucocorticoid drugs as an ophthalmic delivery system has been investigated by Kaseem et al[123]. Pignatello et al. has developed and reported nanosuspensions as ocular drug delivery system for a range of drugs[124-127].

Diclofenac sodium nanosuspension was dispersed into isopropyl myristate as a nanosized suspension via complex formation with sucrose erucate and successfully used for transdermal application. The resultant nanosuspension increased the permeability flux of diclofenac sodium across the skin by up to 3.8-fold compared to the control. The optimal weight ratio for the highest diclofenac sodium permeation was 8.8, at which point the mean diameter of the nanosuspension was 14.4 nm[128].

Targeting of *Crytosporidium parvum,* the organism responsible for crytosporidiosis, was achieved by using surface modified mucoadhesive nanosuspension of

bupravaquone[96,129]. Amphotericin B was used as pulmonary nanosuspension to target pulmonary aspergillosis[130].

1.1.2 Molecular inclusion complex of drug in Cyclodextrins

Inclusion compounds

An inclusion compound is a unique type of inclusion chemical complex, in which one molecule, "the guest", is enclosed within another molecular structure, "the host". In this process of inclusion, covalent or ionic bond formation is not essential. The interaction between the molecules mainly depends on weak Vander Waals forces or hydrogen bonds. The essential criteria for inclusion compound is that the enclosed molecule or guest should be of a suitable size and shape to fit into a cavity within a solid structure formed by a host molecule. The stereochemical structure and polarity of host and guest molecules play an important role in inclusion complex formation. The resulting close fit of the two components produce a combination of significant strength due to total dispersion force between interacting components. This type of spatial complex does not occur by means of ionic, covalent or co-ordinate covalent bond but is dependent upon dispersion forces and possibly highly oriented dipoles for stability differing greatly from chemical complexation. Frank proposed the following classification for the hosts of inclusion compounds:

- Polymolecular inclusion compounds
  - Channel like spaces
  - Cage like spaces
- Monomolecular inclusion compounds
- Macromolecular inclusion compounds
- Products of blue-iodine reaction

Polymolecular inclusion compounds

Polymolecular inclusion compounds can be divided in two classes, viz compounds forming channel like spaces and compounds forming cage like spaces.

Compounds forming channel like spaces

This class of hosts can be understood by the mechanism that they form channel like structure in which a succession of guest molecules fits.

The commonly known compounds of this group are urea, thiourea, selenurea and choleic acids[131-133]. Urea form long-hexagonal prism shaped crystals in presence of

straight chain hydrocarbons (C_6 or long). These crystals combine and form a hollow channel like structure with a diameter of about 5.25 Å. Thiourea also forms the similar channel like spaces of larger diameter with branched or cyclic hydrocarbon. Vitamin A could be protected from oxidation catalyzed by unsaturated fatty esters by using urea-ester inclusion compounds[134].

Compounds forming cage-like spaces

In this type of inclusion complexes, the guest molecule is completely covered by host molecules and forms a cage like structure. This class of inclusion compounds is generally referred as clathrates. This can be understood by gas hydrates, in which water crystal trap a number of gases or liquids[132,135,136]. A typical example of a cage like inclusion compound used in pharmacy is clathrate of warfarin sodium with isopropyl alcohol and water[137].

Monomolecular inclusion compounds

Monomolecular inclusion compounds can be defined as the complexes in which every guest molecules included by one host molecule. However, compounds consisting of two, three or four host molecules for every single guest, are also considered to belong to this class of inclusion compounds, when the host is known to form 1:1 inclusion compounds with other guests. The host molecules in this group are relatively large and contain cavities of molecular dimensions. The main examples of host molecules of this class are cyclodextrins[135], crown ethers[138] and cryptates[139].

Cyclodextrin complexes are certainly one of the major representatives of the monomolecular inclusion compounds and they will be described in detail later in this introduction.

Macromolecular inclusion compounds

This category consists of those hosts molecules which are of macromolecular size. They generally create three dimensional network structures in which cavities are formed.

The classical example of this class is zeolites. The zeolites are hydrated aluminum silicates that may contain sodium, potassium, calcium or barium[83,132]. Zeolites can be imagined as a precisely arranged number of cavities interconnected by channels. Depending on the size of channels and cavities a variety of molecules can be included in the cavities.

Products of the blue-iodine reaction

Iodine is having ability to form long linear inclusion compounds with many molecules. Theses complexes can be identified by their blue black colour. The iodine atoms are polymerized into a linear template around which the host molecules are ordered. The system is stabilized by the electron donor capacity of the host (oxygen atoms or carbonyl groups), which leads to the formation of loose charge transfer complexes with the iodine[83,132].

Cyclodextrins (CDs)

Cyclodextrins were discovered approximately 100 years ago when a research article on the formation of some unidentified crystalline substance at fermentation of starch was published by Villiers, the French author, in 1891[140]. About 15 years later, an Austrian microbiologist, Franz Schardinger reported the production of α-, and β-dextrins[141-143].

In the second half of the 1930s, Freudenberg and his group elucidated the cyclic structure of these two dextrins. These 45 years, from 1891–1936, can be considered the "discovery stage" in the history of CDs. Afterwards Freudenberg and his group[144-146], Karrel[147], Miekley[148] and others, came to the conclusion that reported dextrins are comprising of maltose units and contain only α,1,4-glycosidic linkages and postulated the cyclic structure of these crystalline dextrins[149]. In 1948, the discovery and structural elucidation of γ-CD was published[150].

The primary purpose of drug delivery systems is to deliver the necessary amount of drug to the targeted site for a necessary period of time, both efficiently and precisely[151-153]. To design advanced dosage forms, suitable carrier materials are used to overcome the undesirable properties of drug molecules. Cyclodextrins (CDs) are potential candidates for such a role, because of their ability to alter physical, chemical, and biological properties of guest molecules through the formation of inclusion complexes.

Physical and chemical aspects of cyclodextrin

Cyclodextrins (CDs) are crystalline, cyclic oligosaccharides with a bucket-like structure having a hydrophobic internal cavity and a hydrophilic exterior wall. The interior of the toroid is hydrophobic as a result of the electron rich environment provided in large part by the glycosidic oxygen atoms. This structure allows the formation of inclusion complexes in which lipophilic compounds are non-covalently bound within the cavity. It is the interplay of atomic (Van der Waals), thermodynamic (hydrogen bonding), and solvent (hydrophobic) forces that accounts for the stable complexes that may be formed

with chemical substances while in the apolar environment of the cyclodextrin cavity. The complex exists in an equilibrium dependent upon the concentrations of the cyclodextrin, the guest chemical nature and water.

During the inclusion complex formation, the water molecules located within the lipophilic central cavity of cyclodextrin are replaced by a lipophilic guest molecule. However, the hydroxyl groups on the outer surface of the cyclodextrin molecule are able to form hydrogen bonds with other molecules and cyclodextrins can form water soluble complexes with lipophilic water-insoluble compounds[154-157]. **Figure** 1.6 represents the molecular dimensions, shape and chemical structure, of α-,β- and γ-cyclodextrins[158].

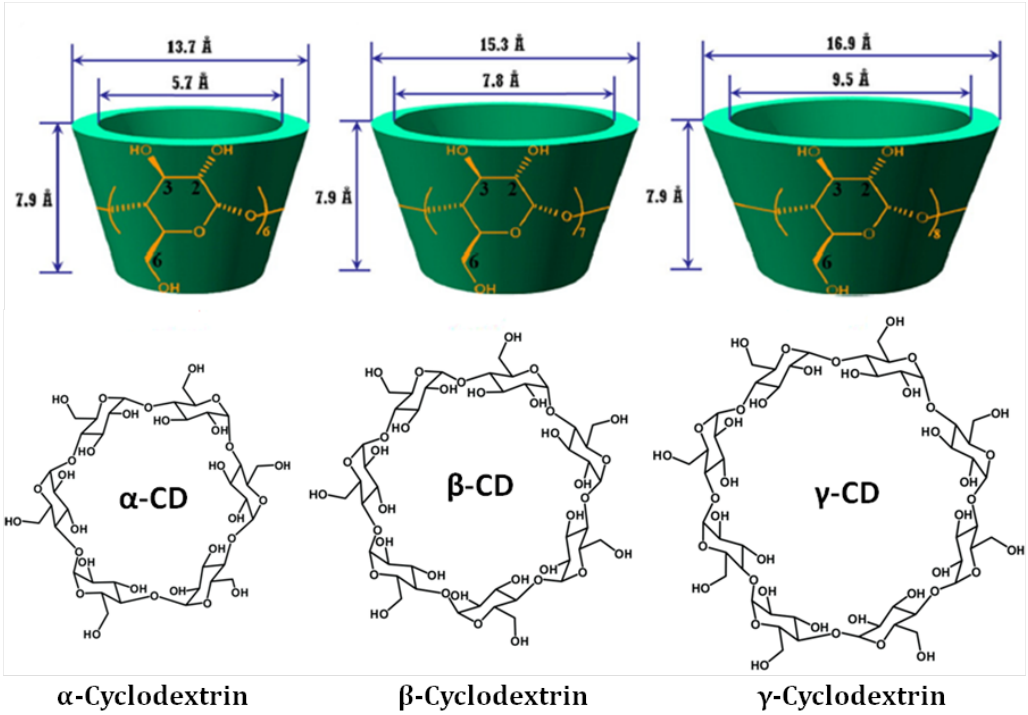


Figure 1.6 Molecular dimensions, shape and chemical structure, of α-,β- and γ-cyclodextrins.

The fundamental properties of α-,β- and γ-CDs are summarized in Table 1.2[157,159].

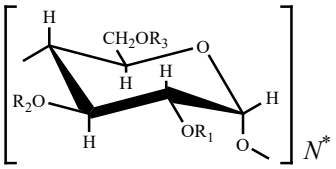
Table 1.2 The fundamental properties of α-,β- and γ-CDs.

Property	α-cyclodextrin	β-cyclodextrin	γ-cyclodextrin
Number of glucopyranose units	6	7	8
Molecular weight (g/mol)	972	1135	1297
Outer diameter (Å)	13.4	15.3	16.9
Cavity diameter (Å)	5.7	7.8	9.5
Torus height (Å)	7.9	7.9	7.9
Cavity volume ($Å^3$)	174	263	427
Aqueous solubility at 25ºC (%w/v)	14.5	1.85	23.2
Surface tension (Mn/m) (0.1 mM)	71	71	71
Water of crystallization (%)	10.2	13.2-14.5	8.1-17.7
Diffusion coefficient (40°C)(m^2/sec)(x 10^{10})	3.4	3.2	3.0
Melting Temperature range (ºC)	255-260	255-265	240-245

Recently, various kinds of cyclodextrin derivatives such as hydrophilic, hydrophobic and ionic derivatives have been successfully utilized to extend physicochemical properties and inclusion capacity of natural Cyclodextrins[160-166]. The desirable attribute for the drug carrier is the ability to control the rate and/or time profile of drug release[167-169]. Hydrophilic Cyclodextrins can modify the rate of drug release, which can be used for the enhancement of drug absorption across biological barriers, serving a potent drug carrier in the immediate release formulations. Because of the molecular dimensions (more exactly the cavity diameter), its availability, low price and the better complexion efficiency the β-cyclodextrin have significant practical importance in pharmacy but the low aqueous solubility and nephrotoxicity limited the use of β-CD especially in parenteral drug delivery[170].

In cyclodextrins, every glucopyranose unit has a three free hydroxyl groups(C-2,C-3 and C-6) which can be modified by substituting the hydrogen atoms by variety of groups such as alkyl-, hydroxylalkyl-, carboxyalkyl-, sulphoalkyl, amino-, thio-, tosyl-, glucosyl-, maltosyl- etc. groups to form derivatives of cyclodextrins like, methylated cyclodextrins, hydroxypropylated cyclodextrins, hydroxyethylated cyclodextrins, carboxymethylated cyclodextrins, ethylated cyclodextrins and branched cyclodextrins. These derivatives can be prepared by chemical or enzymatic reactions. Table 1.3 illustrating the chemical structures of various cyclodextrin derivatives[161].

Table 1.3 Chemical structures of various cyclodextrin derivatives.



Compounds	R_1	R_2	R_3
Hydrophilic Derivatives			
Methylated cyclodextrin			
3-mono-*O*-methylcyclodextrin	H	CH_3	H
2,6-di-*O*-methylcyclodextrin	CH_3	H	CH_3
2,3,6-tri-*O*-methylcyclodextrin	CH_3	CH_3	CH_3
Randomly methylated cyclodextrin	R_1, R_2, R_3=H or CH_3		
Hydroxylalkylated cyclodextrin			
2-hydroxyethylcyclodextrin	R_1, R_2, R_3=H or CH_2CH_2OH		
2-hydroxypropylcyclodextrin	R_1, R_2, R_3=H or $CH_2CH(OH)CH_3$		
3-hydroxyethylcyclodextrin	R_1, R_2, R_3=H or $CH_2CH_2CH_2OH$		
2,3-di-hydroxyethylcyclodextrin	R_1, R_2, R_3=H or $CH_2CH(OH)CH_2OH$		
Branched cyclodextrin			
6-*O*-glucosylcyclodextrin	H	H	H or glucose
6-*O*-maltosylcyclodextrin	H	H	H or maltose
6-*O*-di-maltosylcyclodextrin	H	H	H or $(maltose)_z$
Hydrophobic Derivatives			
Alkylated cyclodextrin			
2,6-di-*O*-ethylcyclodextrin	C_2H_5	H	C_2H_5
2,3,6-tri-*O*-ethylcyclodextrin	C_2H_5	C_2H_5	C_2H_5
Acylated cyclodextrin			
2,3-di-*O*-hexanoylcyclodextrin	COC_5H_{11}	COC_5H_{11}	H
2,3,6-tri-*O*-acetylcyclodextrin	$COCH_3$	$COCH_3$	$COCH_3$
2,3,6-tri-*O*-propanoylcyclodextrin	COC_2H_5	COC_2H_5	COC_2H_5
2,3,6-tri-*O*-butanoylcyclodextrin	COC_3H_7	COC_3H_7	COC_3H_7
2,3,6-tri-*O*-valerylcyclodextrin	COC_4H_9	COC_4H_9	COC_4H_9
2,3,6-tri-*O*-hexanoylcyclodextrin	COC_5H_{11}	COC_5H_{11}	COC_5H_{11}
2,3,6-tri-*O*-octanoylcyclodextrin	COC_7H_{15}	COC_7H_{15}	COC_7H_{15}
Ionizable Derivatives			
Anionic cyclodextrin			
6-*O*-(carboxymethyl)cyclodextrin	H	H	H or CH_2COONa
6-*O*-(carboxymethyl)-*O*-cyclodextrin	C_2H_5	C_2H_5	H or C_2H_5 or CH_2COONa
cyclodextrin sulfates	R_1, R_2, R_3=H or SO_3Na		
Sulfobutylcyclodextrins	R_1, R_2, R_3=H or $(CH_2)_4SO_3Na$		

* *N* = 6, α-CDs; *N*=7, β-CDs; *N*=8, γ-CDs

There are a number of cyclodextrin derivatives reported in various research reports, scientific papers and patents but only few can be used at commercial level. Among several commercialized β-CD derivatives, the most important ones are the heterogeneous, amorphous, highly water soluble methylated-β-CDs and 2-hydroxypropyle-β-CDs (Fig. 6). Due to their heterogeneity, these products cannot be crystallized, which is an important advantage (e.g., at producing liquid drug formulations). It is much more important, however, that these derivatives cannot form

crystalline cholesterol complexes, because the unmodified β-CD has a particularly high affinity to cholesterol. If administered parenterally, it is not metabolized in the organism, but forms insoluble cholesterol complex crystals in the kidneys, resulting in nephrotoxicity. A methylated-β-CD is more hydrophobic than the β-CD itself, therefore, it forms a more stable (but soluble) complex with cholesterol. Its affinity to cholesterol is so strong that it extracts cholesterol from the blood cell membranes, resulting in hemolysis already in around 1 mg/cm3 concentration[157,171]. An another methylated-β-CD (2,6-di-*O*-methyl-β-CD) is a crystalline product. It is highly soluble in cold water, but insoluble in hot water; therefore, its purification, and also the isolation of its complexes, is technically very simple. Up to now, no better solubilizer has been found among the CDs. It is available in more than 95 % isomeric purity for injectable drug formulation, but for widespread industrial application the cheaper randomly methylated β-CD is produced and marketed. The heptakis-(sulfobutyl)-β-CD is very soluble in water, non-crystallizable, and even at extremely high doses seems to be free from any toxic side-effects. It can be used as chiral separating agent in capillary zone electrophoresis, but the aim of the intensive research is to develop it as a parenteral drug carrier, for preparation of aqueous injectable solutions of poorly soluble drugs. Table 1.4 represents the solubility and regulatory status of CDs that are used as excipients in pharmaceutical formulations[172].

Table 1.4 Solubility and Pharmacopoeial monographs of CDs that are used as excipients in pharmaceutical formulations.

Cyclodextrins[a]	MW (Da)[b]	Solubility in water (mg/ml)[c]	Pharmacopoeia[d]		
			Ph. Eur.	USP/NF	JPC
α-CD	972	145	Yes	No	Yes
β-CD	1135	18.5	Yes	Yes	Yes
HP-β-CD	1400	>600	Yes	Yes	No
RM-β-CD	1312	>500	No	No	No
SBE-β-CD	2163	>500	No	No	No
γ-CD	1297	23.2	Yes	Yes	Yes
HP-γ-CD	1576	>500	No	No	No

[a] α-CD: α-cyclodextrin, β-CD: β-cyclodextrin, HP-β-CD: 2-hydroxypropyl-β-cyclodextrin, RM-β-CD: randomly methylated-β-cyclodextrin, SBE-β-CD: sulfobutyl ether-β-cyclodextrin, γ-CD: γ-cyclodextrin, HP-γ-CD: 2-hydroxypropyl-γ-cyclodextrin.

[b] MW: molecular weight in Dalton.

[c] Solubility in pure water at 25°C.

[d] Ph. Eur: European Pharmacopoeia 5th Edition (2005), USP/NF: United States Pharmacopoeia 28th Edition/ National Formulary 23rd Edition (2005), JPC: Japanese Pharmaceutical Codex.

Toxicological considerations of cyclodextrins

The safety profiles of the three most common natural cyclodextrins and some of their derivatives have recently been reviewed[173,174]. In general, the natural cyclodextrins are not practically absorbed under normal physiological conditions and the metabolites produced are not unusual, and they are therefore considered safe for oral use. In the U.S. since 1997, β-cyclodextrin have been considered GRAS for certain food uses. The natural cyclodextrins and their hydrophilic derivatives are only able to permeate lipophilic biological membranes, such as the eye cornea, with considerable difficulty. Even the somewhat lipophilic randomly methylated β-cyclodextrin does not readily permeate lipophilic membranes, although it interacts more readily with membranes than the hydrophilic cyclodextrin derivatives[175]. All toxicity studies have demonstrated that orally administered cyclodextrins are practically non-toxic, due to lack of absorption from the gastrointestinal tract[173]. However, β-cyclodextrin is not safe for parenteral use since it forms insoluble complexes in the kidney, causing nephrotoxicity[176]. Chemically modified cyclodextrins were introduced partially in order to improve the parenteral safety profile of β-cyclodextrin. Randomly methylated β-cyclodextrin has been shown to have very good complexing ability, in addition to being very water soluble. However its lipophilicity can create irritation and hemolysis. Hydroxypropyl-β-cyclodextrin has been shown to be safe at very high oral dose and observed to have no irreversible adverse effects in i.v. dose upto 400 mg/Kg[176].

Aspects of inclusion complex formation and mechanisms of drug release from cyclodextrin complexes

The interior of the cyclodextrin cavity is relatively hydrophobic because of the presence of skeletal carbon and ethereal oxygen, which comprise the cavity, where as the cavity entrances are hydrophilic owing to the presence of primary and secondary hydroxyl groups. As the water molecule located inside the cavity cannot satisfy their hydrogen bonding potential. They are having high enthalpy than the bulk water molecules located in the solution. Water inside the cavity tends to squeezed out and to be replaced by more hydrophobic species. Thus, molecules of appropriate size and stereochemistry can be included in the cyclodextrin by hydrophobic interactions. Figure 1.7 illustrates the conventional model of drug-cyclodextrin complex formation and equilibrium.

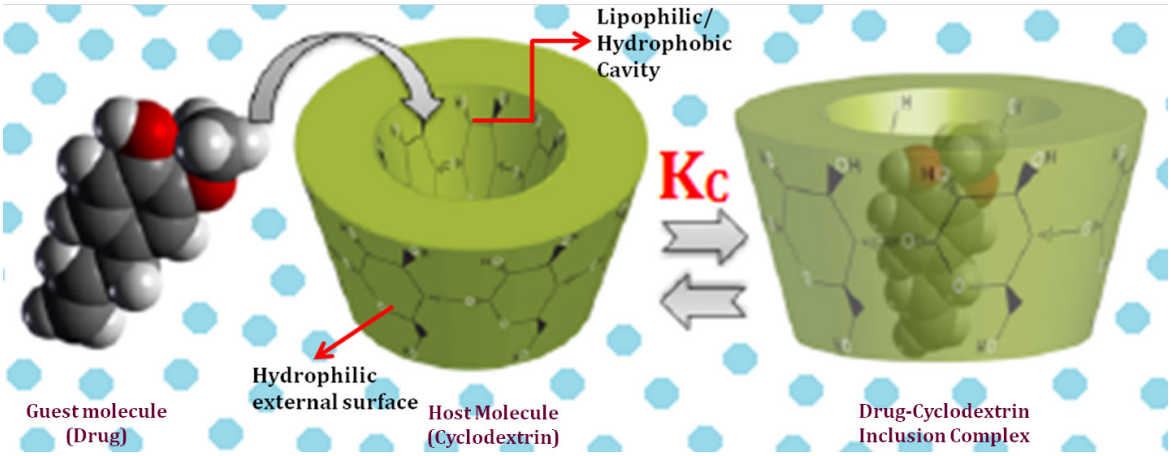


Fig. 1.7 Conventional models of drug-cyclodextrin complex formation and equilibrium.

In general, the mechanism of complexation can be described in following six energetically favorable steps:

a) Approach of the guest or substrate molecules to the cyclodextrin molecules.
b) Loss of the water structure within the cyclodextrin cavity with removal of some water molecules.
c) Breakdown of the water structure around the portion of the substrate that will be included and transport of some water molecules in the solution.
d) Interaction of the substituent groups of the substrate with groups on the rim or inside the cyclodextrin ring.
e) Possible formation of the bonds between the cyclodextrin and the substrate.
f) Re-establishment of the water structure around the exposed parts of the substrate after the inclusion has occurred.

While the initial equilibrium to form complex is very rapid, the final equilibrium can take much longer to reach. Once inside the cyclodextrin cavity, the guest molecule makes conformational adjustment to take maximum advantage of the weak Vanderwaal forces that exists[160,177-179].

There are diverse mechanisms which participate significantly in drug release from drug-cyclodextrin inclusion complex such as dilution, competitive displacement, protein binding, drug uptake by tissue, and change in ionic strength and temperature. Inclusion of drug (D) in cyclodextrin (CD) takes place through non-covalent bonds between the drug and CD cavity. This is a reversible reaction whereby the drug molecule persistently associates and dissociates from the host CD. The dynamic process will be as follows:

$$D_{free} + CD_{free} \overset{K_C}{\Leftrightarrow} DCD_{complex}$$

The complexation constant (K_C) and stability of complex are the key parameters in drug release mechanism.

Liquid state (Phase solubility) study of cyclodextrin complexation

The most widely used approach to study cyclodextrin inclusion complexation is the phase solubility method described by Higuchi and Connors,[180] which examines the effect of complexing agents on the compound being solubilized. Experimentally, an excess of a poorly water-soluble drug (i.e. a substrate or drug, D) is introduced into several vials to which a constant volume of an aqueous vehicle containing successively larger concentrations of the CD are added. The need for excess drug is based on the desired to maintain as high a thermodynamic activity of the drug as possible. The vials are shaken or otherwise agitated at constant temperature until equilibrium is established. The suspensions are then filtered and the total concentration of the drug determined based on appropriate analytical techniques (UV spectrophotometry, HPLC, etc). The phase–solubility profile is then constructed by assessing the effect of the CD on the apparent solubility of the drug (D).

The practical and phenomenological implications of phase–solubility analysis were developed by Higuchi and Connors in their pioneering work published in 1964[180] and as later reviewed by Connors[181]. Based on the shape of the generated phase–solubility relationships, several types of behaviors can be identified[182]. Phase solubility diagrams are categorized into A and B types; A type curves indicate the formation of soluble inclusion complexes while B type suggest the formation of inclusion complexes with poor solubility. A B_S type response denotes complexes of limited solubility and a B_I curve indicates insoluble complexes. A-type curves are subdivided into A_L (linear increases of drug solubility as a function of CD concentration), A_P (positively deviating isotherms), and A_N (negatively deviating isotherms) subtypes. Less soluble natural cyclodextrin (e.g. β-CD) often gives rise to B-type curves due to their poor water solubility whereas the more soluble chemically modified CDs (like HP-β-CD and SBE-β-CD) usually produce soluble complexes and thus give A-type systems. The most common type of cyclodextrin complex is the 1:1 drug-cyclodextrin complex (D-CD) where one drug molecule (D) forms a complex with one cyclodextrin molecule. Figure 1.8 represents the dynamics of drug-cyclodextrin complex (1:1) formation and phase

solubility diagram[183].

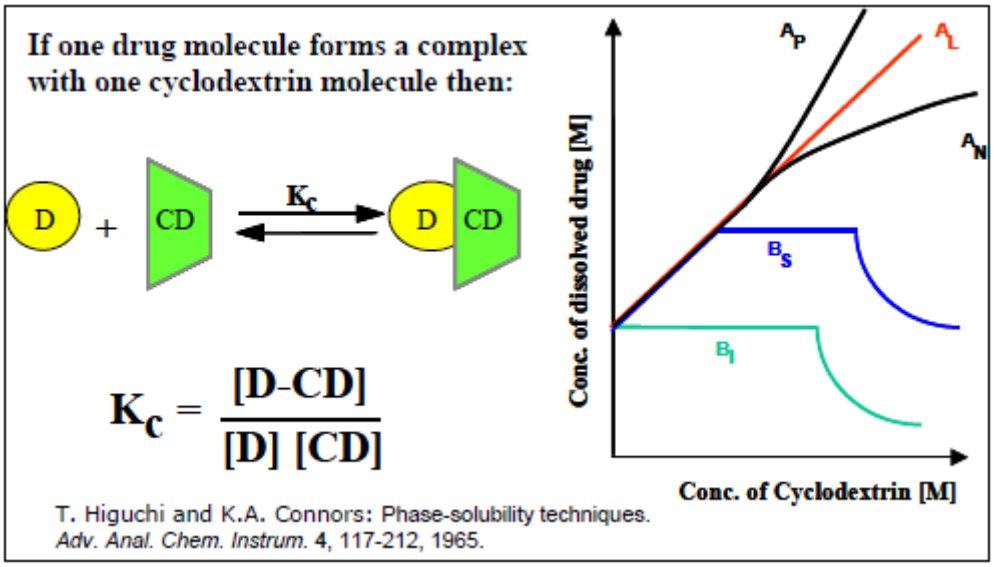


Fig. 1.8 Phase solubility relationships.

Under such conditions an A_L-type phase-solubility diagram, with slope less than unity, would be observed and the equilibrium/binding/association/stability constant ($K_{1:1}$) of the complex can be calculated from the slope of the linear portion of curve and the intrinsic solubility (S_0) of the drug in the aqueous complexation media (i.e. drug solubility when no cyclodextrin is present):

$$\text{Stability Constant } (K_{1:1}) = \frac{Slope}{S_0(1 - Slope)}$$

The value of stability constant ($K_{1:1}$) is most often between 50 and 2000 M^{-1} with a mean value of 129, 490 and 355 M^{-1} for α-, β- and γ-cyclodextrin, respectively[184]. For 1:1 drug/cyclodextrin complexes the complexation efficiency (CE) can be calculated from the slope of the phase-solubility diagram:

$$CE = \frac{[D - CD]}{[CD]} = S_0 . K_{1:1} = \frac{Slope}{(1 - Slope)}$$

It is more convenient to compare CE values instead of $K_{1:1}$ values while selecting cyclodextrin or complexation conditions during formulation work. The most common stoichiometry of higher order drug/cyclodextrin complexes is the 1:2 drug-cyclodextrin complex resulting in A_P-type phase-solubility diagram. Consecutive complexation is assumed where the 1:2 complex is formed when one additional cyclodextrin molecule forms a complex with an existing 1:1 complex:

$$[D - CD] + [CD] \overset{K_{1:2}}{\Longleftrightarrow} [D - [CD]_2]$$

The stoichiometry of the system can be probed by curve fitting of the diagram with a quadratic model:

$$S_{TOTAL} = S_0 + K_{1:1}.S_0[CD] + K_{1:1}K_{1:2}S_0[CD]_2$$

Here [CD] represents the concentration of free cyclodextrin but it is customary to plot the total amount of dissolved drug (S_{TOTAL}) against the total amount of cyclodextrin in solution ($[CD]_{TOTAL}$) assuming that the extent of complexation is low (i.e. $[CD] \sim [CD]_{TOTAL}$). The value of $K_{1:2}$ is frequently between 10 and 500 M^{-1}, or significantly lower than that of $K_{1:1}$.

Approaches for drug-cyclodextrin complexation

Various techniques can be applied to prepare drug-cyclodextrin complexes including

- Physical blending method
- Kneading method
- Co-precipitation technique
- Solution/solvent evaporation method
- Neutralization precipitation technique
- Milling/co-grinding method
- Slurry complexation
- Microwave irradiation method
- Supercritical anti-solvent technique

Physical blending method (Physical mixture)

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 appropriate sieve to get the desired particle size in the final product. In industry scale, the preparation of physical mixtures is based on 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 little amount of water or hydro-alcoholic solutions to converted into a paste, using mortar and pestle. The drug is then added to the above paste and kneaded for a specified time. The kneaded mixture is then dried and passed through sieve if required[185]. Parik et al. have reported the dissolution enhancement of nimesulide in nimesulide-cyclodextrin complex prepared by kneading method[186].

Co-precipitation technique

This method involves the co-precipitation of drug and CDs in a complex. In this method, required amount of drug is added to the solution of CDs. The system is kept under magnetic agitation with controlled process parameters and the content is protected from the light. The formed precipitate is separated by vacuum filtration and dried at room temperature in order to avoid the loss of the structure water from the inclusion complex. Moyano et al. have studied the solid-state characterization and dissolution characteristics of gliclazide-β-cyclodextrin inclusion complexes[187].

Solution/solvent evaporation method

This method involves dissolving of the drug and CDs separately in to 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[188]. 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°C. The dried mass was pulverized and passed through a 60-mess sieve. Figueiras et al. studied and reported the effect of evaporation method for improved dissolution profiles of the inclusion complexes of omeprazole with native and modified β-cyclodextrin[189].

Neutralization precipitation method

This method is based on the precipitation of inclusion compounds by neutralization technique and consists of dissolving the drug in alkaline solutions like sodium/ammonium hydroxide and mixing with an aqueous solution of CDs. The resultant clear solution is then neutralized under agitation using hydrochloric acid solution till reaching the equivalence point. A white precipitate is being formed at this moment, corresponding to the formation of the inclusion compound. This precipitate is filtered and dried. Doijad et al. have studied the enhancement of solubility of piroxicam

by complexation with beta-cyclodextrin using this method[190].

Milling/Co-grinding technique

A solid binary inclusion compounds can be prepared by grinding and milling of the drug and CDs with the help of mechanical devices. Drug and CDs are mixed intimately and the physical mixture is introduced in an oscillatory mill and grinded for suitable time. Alternatively, the ball milling process can also be utilized for preparation of the drug-CD binary system. This method differs from the physical mixture method where simple blending is sufficient and in co-grinding it requires to achieve extensive combined attrition and impact effect on powder blend.

Slurry complexation

In this method, first the cyclodextrin is dissolved in water (as much as 50-60% solids) and then guest molecule (drug) was added to the solution. The resultant mixture was stirred at optimum speed for sufficient duration of time. The guest molecule will complex with the cyclodextrin present in solution. Obtained drug-cyclodextrin complex can be further dried using spray drying or freeze drying techniques. The amount of time required to complete the complexation is variable, and depends on the guest. Assays must be done to determine the amount of time required.

Microwave irradiation method

This technique involves the microwave irradiation reaction between drug and complexing agent using a microwave oven. The drug and CD in 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 short time of about one to two minutes at 60°C in the microwave oven. The precipitate so obtained is separated using Whatmann filter paper, and dried in vacuum oven at 40°C for 48 hrs. Deshmukh et al. have developed inclusion complexes of ziprasidone hydrochloride with beta-cyclodextrin and hydroxypropyl beta-cyclodextrin to design the fast dissolving formulation using various super-disintegrants[191].

Supercritical anti-solvent 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 makes it attractive for processing heat-labile pharmaceuticals. Supercritical carbon dioxide is suggested as a new complexation medium due to its properties of

improved mass transfer and increased solvating power[192-196]. 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). 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[197,198].

Techniques for solid-state characterization of drug-cyclodextrin inclusion complex

Solid-state characterization of an inclusion complex can be carried out by the following techniques-

- Thermo-analytical methods
- X-ray Diffraction analysis
- Infra-Red (IR) spectroscopy
- Scanning electron microscopy
- Wettability and dissolution test

Thermo-analytical methods

Thermo-analytical methods determine whether the guest substance undergoes some change before the thermal degradation of cyclodextrin[199-207]. The change of the guest substance may be melting, evaporation, decomposition, oxidation or polymorphic transition. The change of the guest substance indicates the complex formation. The effect of cyclodextrins on the thermogram obtained by DTA and DSC were observed for broadening, shifting and appearance of new peaks or disappearance of certain peaks. Changes in the weight loss were evaluated to provide supporting evidence for the formation of inclusion complexes. The nature of the drug and cyclodextrins used and method of preparation of complex have been found to influence the above finding considerably. If the interaction between the drug and the excipient is weak, the shift in the endothermic peak is very small. The formation of inclusion complex of Salbutamol with cyclodextrins by various methods was evaluated using DSC. The DSC endotherm of Salbutamol at 158°C was shifted to 150°C in the physical mixture showing a weak interaction. But the freeze dried complex showed no peak around 157°C indicating the formation of a true inclusion complex[208].

X-ray Diffraction analysis

Powder X-ray diffractometry may be used to detect inclusion complexation in the solid state[200,201]. When the guest molecules are liquid since liquid have no diffraction pattern of their own, then the diffraction pattern of a newly formed substance clearly differs from that of un-complexed cyclodextrin. This difference of diffraction pattern indicates the complex formation[185,209-211]. When the guest compound is a solid substance, a comparison has to be made between the diffractogram of the assumed complex and that of the mechanical mixture of the guest and cyclodextrin molecules[212-214]. Comparison of the diffractogram is only possible if the cyclodextrin as well as the guest molecules are treated under identical conditions as that of the assumed complex because cyclodextrin inclusion complex preparation processes such as freeze drying and grinding, may change the crystallinity of the pure substances and this may lead to different diffraction patterns[199]. On the other hand, formation of amorphous complexes leads to the disappearance of certain peaks or the peaks become less sharpen than those of the pure compound or physical mixture. For example, the spray dried complexes of Acetaminophen, Indomethacin, Piroxicam and Warfarin with β-CD and the freeze dried complexes of Naproxen with β-CD[207,215].

Infra-Red (IR) spectroscopy

Infra-Red spectroscopy is used to estimate the interaction between cyclodextrin and the guest molecules in the solid state[202,216]. Infra-red spectral studies give information regarding the involvement of hydrogen in various functional groups. This generally shifts the absorbance bands to the lower frequency, increases the intensity and widens the band caused by stretching vibration of the group involved in the formation of the hydrogen bonds. Hydrogen bond at the hydroxyl group causes the largest shift of the stretching vibration band. It has been observed that cleavage of the hydrogen bonding due to inclusion complexation results in the shift of absorbance bands to higher frequency. For example, a shift of the aromatic carbon stretching at 1272 to 1296 cm^{-1} in case of β-CD complex, and the stretch of the ester function from 1183 to 1206 cm^{-1} in case of dimethyl-β-cyclodextrin was reported.[305] Formation of hydrogen bond has resulted in lengthening of bond O-H, N-H etc due to reduction of elasticity. Accordingly, the frequencies of the stretching vibrations were decreased. For example, when Piroxicam was complexed with β-CD, the band at 1180 per cm got shifted to 1154 per cm[217].

Scanning electron microscopy

Scanning Electron Microscopy[203,204,216,218] is used to study the microscopic aspects of the raw material (cyclodextrin and the guest substances, respectively) and the product obtained by co-precipitation /evaporation[208,209,214]. The difference in crystallization state of the raw material and the product seen under electron microscope indicates the formation of the inclusion complexes[210,211] ,even if there is a clear difference in crystallization state of the raw material and the product obtained by co-precipitation. This method is inadequate to affirm inclusion complex formation[200,202].

Wettability and dissolution test

The wetting of the solid phase by a solvent is always the first step of any dissolution process. Cyclodextrin complexation of the lipophilic drug often improves the wettability in water considerably, but also simple addition of β-cyclodextrin to non-wettable solid enhances their wettability. Three methods to characterize the wettability of solid cyclodextrin formulations include the measurement of the contact angles, powder sedimentation studies which may be carried out by layering equal amounts of the samples onto the surface of water, following their sedimentation photographically and the last method demonstrates the upward migration of a colored front of three open tubes containing the guest compound, a mixture of the guest compound with cyclodextrin and the inclusion complex, respectively, as function of the time. When an assumed complex is dispersed in water, very rapid dissolution rate tests are based on this observation. The most often used dissolution tests are the rotating disk method[207] and the dispersed amount technique[219-222].

Effects of drug-cyclodextrin inclusion complex on important drug properties in formulation

The complexation of drug in cyclodextrin cavity leads to variation in a range of important drug properties in formulation. Here we discuss about the effects of drug-cyclodextrin inclusion complex on

- Drug solubility and dissolution,
- Permeability of drug through biological membranes,
- Bioavailability of the drug,
- Drug safety and stability

Effect on drug solubility and dissolution

The cyclodextrin has been playing a very important role in formulation of poorly water soluble drugs by improving the apparent drug solubility and dissolution through inclusion complexation. It act as hydrophilic carriers for drugs with inadequate molecular characteristics for complexation, or as tablet dissolution enhancers for drugs with high dose[223]. Among the various commercially available cyclodextrin, methylated cyclodextrin with a relatively low molecular substitution appears to be the most powerful solubilizer.

Investigation reports revealed that solid dispersions of Celecoxib with β-CD or HP-β-CD exhibited enhanced and faster rates of dissolution compared to that of celecoxib alone[224,225].

Effect on drug permeability through biological membranes

In spite, the solubility enhancement application, CDs can also be used as membrane permeability enhancer and stabilizing agents[226,227]. The permeability through biological membrane is enhanced by the presence of cyclodextrins. Masson reported about the permeation enhancement property of poorly water soluble drugs in presence of the CDs[228]. These act as permeation enhancers by carrying the drug through the aqueous barrier which exists before the lipophilic surface of biological membranes[229]. Rifampicin is a so- called concentration-dependent antibiotic, the rate and extent of bacterial kill is related to the attainment of high maximum concentration relative to the minimal inhibitory concentration. The rifampicin-CD inclusion compound can improve the lung transport of drug when nebulized with compatible pulmonary deposition and achieve required concentration of drug in broncho-alveolar epithelium lining-fluid when administered as aerosolized solution[230-233].

Effect on Bioavailability

The cyclodextrin enhances the bioavailability of insoluble drugs by increasing its drug solubility, dissolution and drug permeability. This is achieved by making the drug available at the surface of the biological barrier, e.g., skin, mucosa, or the eye cornea, from where it partitions into the membrane without disrupting the lipid layers of the barrier[234]. In such cases, it is important to use just enough cyclodextrin to solubilize the drug in the aqueous vehicle since excess may decrease the drug availability[235,236]. It was found that the addition of polymers can further enhance the drug permeability from aqueous cyclodextrin solutions. In the case of water-soluble drugs, cyclodextrins

increase the drug permeability by direct action on mucosal membranes and enhance drug absorption and bioavailability[237].

Labile drug stabilization by cyclodextrins[238,239] and their ability to ameliorate drug irritation, and thus improve drug contact time at the absorption site in nasal, ocular, rectal and transdermal delivery are some other important factors that contribute to the cyclodextrin-improved bioavailability. α-CD improved the rectal bioavailability of morphine by inhibiting the drug's upward movement from areas impacted by first pass metabolism.

Effect on drug stability

Cyclodextrins can improve the stability of several labile drugs against dehydration, hydrolysis, oxidation and photodecomposition and thus increase the shelf life of drugs. It was reported that cyclodextrin-induced enhancement of drug stability may be a result of inhibition of drug interaction with vehicles and inhibition of drug bioconversion at the absorption site. By providing a molecular shield, cyclodextrin complexation encapsulates labile drug molecules at the molecular level and thus insulates them against various degradation processes. SBE-β-CD showed greater stability enhancement of many chemically unstable drugs than other cyclodextrins[240]. The cyclodextrins were reported to have improved the photo stability of trimeprazine[241] and promethazine[242]. They also enhanced the solid state stability and shelf life of drugs[243]. The physical stability of viral vectors for gene therapy and the formulations containing sucrose and cyclodextrins were stable for 2 years when stored at 20°C has been reported[244].

Effect on drug safety

Cyclodextrins have been used to ameliorate the irritation caused by drugs[160]. Effective drug-cyclodextrin inclusion complex results in improved drug efficacy and potency, caused by increased drug solubility, may reduce drug toxicity by making the drug effective at lower doses. The studies stated that the β-CD enhances the antiviral activity of ganciclovir on human cytomegalo virus clinical strains and the resultant increase in the drug potency has reduced the drug toxicity[245]. The toxicities associated with crystallization of poorly water-soluble drugs in parenteral formulations can often be reduced by formation of soluble D-CD complexes. Further cyclodextrin entrapment of drugs at the molecular level prevents their direct contact with biological membranes and thus reduces their side effects and local irritation with no drastic loss of therapeutic benefits[161].

1.2 Hypothesis

It was hypothesized that nanosuspension and cyclodextrin inclusion complex of selected drugs might lead to increased solubility, improved dissolution and absorption which subsequently results in enhanced oral bioavailability.

It was also hypothesized that these formulations would help to improve the clinical convenience, reduced dose and dosing frequency, decreased dose related side effects and improved therapeutic efficacy of selected drugs.

1.3 Selection of drugs and drugs profiles

As per the hypothesis, this project was centred on solubility improvement and bioavailability enhancement of poorly water soluble drugs. Hence, two poor water soluble drugs with low bioavailability, Diacerein (anti-osteoarthritic class) and Febuxostat (anti-hyperuricemic / anti-gout class) have been selected for the present study depending on current need for their new formulations.

Osteoarthritis (OA); is the most prevalent and costly form of musculoskeletal diseases (arthritis) that can affect the hands, hips, shoulders and knees. In OA, the cartilage that protects the ends of the bones breaks down and causes pain and swelling. Drug and non-drug treatments are used to relieve pain and/or swelling. In the USA, it is second only to ischemic heart disease as a cause of work disability in men over 50 years of age, and accounts for more hospitalizations than rheumatoid arthritis (RA) each year[246]. The definition for OA according to the American College of Rheumatology summarizes this idea: "a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone at the joint margins"[247].

There may be mono, oligo or polyarticular OA and, in each joint , according to the part affected: lateral, medial or patellofemoral compartments in the knee; pole superior, pole medial or concentric in the hip, interphalangeal and/or thumb base and in the spine, apophyseal joints or intervertebral disc disease. According to specific features we can have: inflammatory osteoarthrosis, erosive osteoarthrosis, atrophic or destructive osteoarthrosis, and OA with chondrocalcinosis. Established individual risk factors include:

- Heredity (mainly for poly-articular forms with hands involvement)
- Obesity (mainly for knee OA),

- Hyper-mobility
- Mechanical factors such as trauma
- Joint shape
- Occupation, sports and leisure physical activity

Diacerein (DAR) is a drug used in the treatment of osteoarthritis by inhibiting interleukin-1. It is a slow-acting drug that may slow down the breakdown of cartilage and relieve pain and swelling. In animal and in vitro human experiments, diacerein demonstrated properties of inhibition of interleukin-1 beta, inhibition of collagenase expression, reduced fibrinolytic activity in synovial fluid and synovial fibroblasts, inhibition of superoxide anion production, lysosomal enzyme release and chemotaxis[248-252]. There was improvement of OA induced in animal models when treated with Diacerein[253,254]. Unlike non-steroidal anti-inflammatory drugs (NSAIDs) which inhibit prostaglandin synthesis, diacerein stimulates or does not affect prostaglandin synthesis, with no prejudice to gastric mucosal[255] or renal function[256].

Since 1982, trials have been done to show the effects of diacerein in the OA. In 1992, diacerein received licensing approval in France for the symptomatic treatment of osteoarthritis, and the drug has been marketed since September 1994. On the strength of these trials, diacerein has been proposed as most effective, slow-acting, symptom modifying and perhaps disease/structure modifying drug for OA[257].

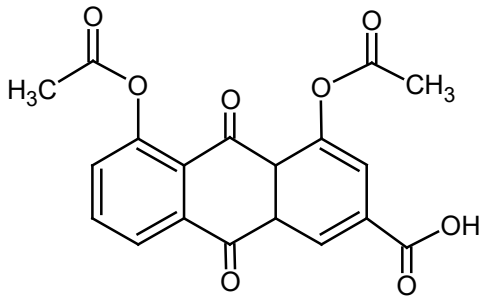


Fig. 1.9 Molecular structure of Diacerein (DAR).

Systematic name of DAR is 4,-diacetyloxy-9,10-dioxo-anthracene-2-carboxylic acid. The chemical formula, molecular mass, Log P and melting point of DAR are $C_{19}H_{12}O_8$, 368.294 g/mol, 2.47 and 242°C-246°C respectively. The oral bioavailability of DAR is about 30-55%[258]. Diacerein is non-hygroscopic and yellow colored crystalline powder. It is practically insoluble in solvents such as water, alcohols, acetone, dichloromethane and chloroform, which are generally used in pharmaceutical preparations. It is freely soluble in dimethylsulfoxide and tetrahydrofuran.

Diacerein is well absorbed following oral dosing. Oral administered diacerein undergoes complete deacetylation to its active metabolite rhein. It exerts its pharmacologic action through its active metabolite-rhein[259]. The optimum time (t_{max}) to reach peak plasma concentration of rhein was 2.4 to 5 hours and the maximum plasma concentration (C_{max}) of rhein was 3.2 mg/ lit, after administration of a single oral dose of diacerein 50 mg. Area under the plasma rhein concentration – time curve (AUC_0) was 21.3 mg/lit/ hr, apparent volume of distribution was 13.2 litre, terminal elimination half life ($t_{1/2}$) was 4-4.5 hrs, apparent total plasma clearance was 1.6lit /hrs and renal clearance (CL_r) was 0.13 lit/hr[260].

Gout is the most common form of inflammatory arthritis in men and is caused by the deposition of monosodium urate crystals in tissues[261]. The condition generally occurs after years of sustained high uric acid concentrations and it is estimated to affect approximately 5.1 million people in the United States, according to the National Health and Nutrition Examination Survey (NHANES III)[262]. Prevalence of gout and/or Hyperuricemia during the past 10 years has been increasing, possibly because of an increase in the prevalence of two important risk factors for Hyperuricemia, namely obesity and aging[261,263]. Acute and chronic arthritis, tophi and renal diseases are manifestations of gout that reflect the magnitude and duration of Hyperuricemia, which is the biological hallmark of gout[264].

Febuxostat (FBX); is urate lowering drug, an inhibitor of xanthine oxidase that is indicated for the treatment of Hyperuricemia and chronic gout. FBX was approved in February 2009 by the USFDA for the management of Hyperuricemia and gout. The introduction of FBX provided clinicians with an additional hope for the treatment of of Hyperuricemia and gout in patients who are non responsive to allopurinol[265].

FBX is an orally active drug found to be effective in the dosage of 40-120 mg/day. The pharmacokinetics of the drug allows it to be suitable once a day dosing. The recommended starting dosage of Febuxostat is 40 mg once daily, if the patients do not achieve the target serum uric acid concentration (<6 mg/dl) after 2 weeks then the dose is increased to 80 mg once daily.

Systematic name of FBX is 2-(3-cyano-4-isobutoxyphenyl)-4-methyl-1,3-thiazole-5-carboxylic acid. The chemical formula, molecular mass, Log P and melting point of FBX are $C_{16}H_{16}N_2O_3S$, 316.374 g/mol, 3.5-3.8 and 205°C-208°C respectively. FBX is non-hygroscopic, white crystalline powder that is freely soluble in dimethylformamide,

soluble in dimethylsulfoxide, sparingly soluble in ethanol, slightly soluble in methanol and Acetonitrile; and practically insoluble in water. The oral bioavailability of FBX is about ~49%[266].

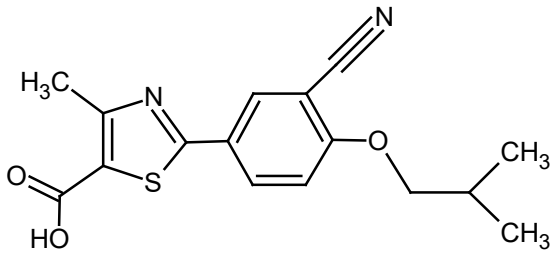


Fig. 1.10 Molecular structure of Febuxostat (FBX).

Febuxostat is rapidly absorbed after oral administration with a time to reach peak plasma concentration (t_{max}) of approximately 1 hour. After multiple oral 40 mg and 80 mg once daily doses, C_{max} is approximately 1.6 ± 0.6 µg/mL (N=30), and 2.6 ± 1.7 µg/Ml (N=227), respectively.The drug is highly bound to albumin in blood (~99%) and appears to have a low to apparent volume of distribution of approximately 0.71/kg. Less than 6% of the administered dose is excreted in the urine as unchanged drug. The mean half life ($t_{1/2}$) is 5-8 hours[267].

1.4 Aims & Objectives

The prime objective of the study was to formulate stable Oral formulations (i.e. Nanosuspension and Molecular inclusion complex with cyclodextrin) of Diacerein (DAR) and Febuxostat (FBX) for improvement of oral bioavailability by improving solubility, dissolution and absorption properties. The main aims of the study were:

- The study was aimed at formulating a drug delivery system capable of enhanced bioavailability resulting in improving the potency and efficacy of selected drugs (i.e. DAR and FBX) via increasing solubility, dissolution rate and systemic absorption.
- The study was aimed at developing an effective oral formulation (Nanosuspension and Cyclodextrin complexes) for administration of selected drugs and to minimize their dose associated site effects.
- The study was aimed at developing a suitable formulation for DAR and FBX and to provide better alternative to currently available commercial formulations of both, respectively.

Considering the aims of this project, the specific objectives of the study were to reduce dose and dosing frequency of selected drugs and thereby reducing cost of therapy and

improving patient compliance.

1.5 Plan of work

- To procure the APIs and excipients and perform pre-formulation studies for screening of excipients.
- To formulate Nanosuspensions of DAR & FBX using suitable technique.
- To formulate Molecular Inclusion Complexes of DAR & FBX with various cyclodextrins and their derivatives using suitable technique.
- To optimize formulation and process variables parameters of these formulations.
- To characterize the prepared formulations for its physicochemical properties.
- To carry out the stability studies
- To carryout *in vitro* dissolution study of optimized formulations.
- To carryout *in vitro* Cytotoxicity studies of optimized formulations, standard drugs and excipients using Caco-2 cell lines (MTT Assay).
- To carryout *in vitro* permeability studies of optimized formulations and standard drugs using Caco-2 cell lines.
- To evaluate the in vivo pharmacokinetic study (Bioavailability study) in rabbits from prepared formulations with respect to bulk drug and marketed formulations.
- To provide cost effective therapy with higher bioavailability for the treatment of Osteoarthritis and Hyperuricemia / Chronic Gout.

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