1.1 Introduction

An important fraction (~40%) of the new drug candidates emerging from drug discovery programs has poor water solubility and this trend is not expected to change in the future [Stegemann et al; 2007]. Nowadays, large portion of new molecules come from combinatorial chemistry which focuses on target-receptor geometry, target identification and lead candidate generation. However, candidates emerging from these screens invariably have high molecular mass and high Log P, which contribute to insolubility. Also, high affinity and high specificity binding to molecular targets generally entails some degree of hydrophobic interactions which leads to solubility constraints. The major problem of many newly developed pharmaceutical drugs is their poor solubility in water and simultaneously in organic media. The basic challenges associated with poorly soluble drugs are low bioavailability and/or erratic absorption. In case of poor bioavailability after oral administration, many times parenteral administration cannot solve this problem. Available strategies for poorly water soluble drugs include; aqueous mixtures with an organic solvent (e.g. water-ethanol), solubilization, formation of complexes e.g. using β-cyclodextrin, solid dispersions, co crystallization, exploiting the effects of pH or salt form. But, these approaches have certain limitations such as side effects associated with co-solvents, need for sufficient ionizing groups for salt formation, need for possessing sufficient solubility in oils or other hydrophobic media, need for having a suitable molecular size and shape to incorporate in the cyclodextrin ring etc. Hence, identification of some novel formulation approaches for drugs having poor water solubility is the mainstay of drug delivery research throughout the world.

The oral route is by far the most convenient one for drug administration. However, for oral administration, the low concentration gradient between the gut and blood vessel due to the poor solubility of the drug leads to a limited transport, consequently influencing its oral absorption. The pharmacological effect for any orally-administered drug relies on involved mechanism of transport from the site of entry into the body to the site of action [Klueglish et al; 2005]. Recent drug delivery research mainly focuses on nanotechnology based strategies for poorly water soluble drugs in order to improve their therapeutic performance. Nanoparticulate technology has proven its competence for numerous drugs.
for large number of applications. The versatility, flexibility and adaptability of the nanoparticulate delivery systems have proven their potential to fulfill the need for improved health care and better patient compliance. Nanoparticulate delivery systems include polymeric nanoparticles, solid lipid nanoparticles, nanoemulsions, liposomes, nanostructured lipid carriers, nanogels and drug nanoparticles. The oral delivery of poorly water soluble drugs presents a major challenge because of their low aqueous solubility. For such compounds, the absorption rate from the gastrointestinal (GI) lumen is controlled by dissolution [Amidon et al; 1995]. In recent years, much attention has focused on particle size engineering and lipid based formulations to improve the oral bioavailability of poorly water soluble drug compounds. Nicolaos et al reported that the bioavailability of cefpodoxime proxetil increased from 50 to 98 % when using submicronic emulsions for oral administration [Nicolaos et al; 2003]. Some investigations show that the dissolution rate of griseofulvin particles with sizes in the range of 200 nm was about two-fold higher than for the conventional micronized material [Turk et al; 2002]. There are many reports available which prove that these approaches are very successful for drugs having low bioavailability. Particulate drug delivery systems offer great promise to increase drug absorption at intestine.

Pure drug nanoparticles are nowadays considered as a viable formulation route for the oral administration of drugs having poor dissolution rate and/or aqueous solubility [Kessisoglou et al; 2007]. 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. Nanosizing technology (nanonization) has also been applied to reduce variability in pharmacokinetic behavior of oral dosage forms [Shono et al; 2010]. 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 adopted to handle milligram quantities of drug substance, this technology provides an avenue 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. Also, for marketed products that have performance issues related to poor solubility of the active, reformulation into nanosized dosage forms can offer the possibility of adding new life to old compounds while improving efficacy and patient compliance.

The two main approaches used for nanosizing drug or formulating drug nanoparticles are top down and bottom up approaches. Top down approach is widely used and generally referred to as nanosizing. This approach is based on use of mechanical force to convert large crystalline particles to nanosized drug particles. Bottom up approach involves controlled precipitation i.e. drug is dissolved in one solvent and then it is precipitated by addition of antisolvent in a controlled manner. One top down (media milling) and one bottom up (supercritical technique) are gaining wide acceptability now days. Media milling is a widely used top down approach for nanonization of the drug. Among all methods reported for nanosizing drug particles in pharmaceutical industry, media milling technique is considered to be the leader with highest commercial applicability. In this technique, the drug particles are subjected to media milling wherein the high-energy shear forces generated as a result of impaction of the milling media with drug provide energy to disintegrate drug micro-particles to nanosized drug particles. In this method, the milling chamber is charged with milling pearls, dispersion medium (e.g. water), drug powder and stabilizer. The pearls are rotated at a very high speed to generate strong shear forces which disintegrate the drug powder into nanoparticles [Merisko-Liversidge et al; 2003].

Supercritical fluid (SCF) technologies have revealed great potential in particle engineering and have emerged as an alternate to most of the existing techniques. 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. For particle size reduction of neat drug particles, two SCF technologies are generally used, Rapid Expansion of Supercritical Solutions (RESS) and Supercritical Anti Solvent (SAS) process. In the SAS method, the solid material (drug) is dissolved in an organic solvent and a supercritical fluid is then forced by means of pressure to dissolve in it. In this way, the volume of the system is expanded, thus
lowering the density, and therefore the solubility of the material of interest is also decreased. As a result, the material precipitates out of the solution as a solid with a very small particle diameter. In the case of **RESS**, the supercritical fluid is used to dissolve the solid material (drug) under high pressure and temperature, thus forming a homogeneous supercritical phase. Thereafter, the solution is expanded through a nozzle and drug nanoparticles are formed. Recently, the SAS process has been proposed as an alternative for formulating coprecipitates that may be smaller in particle size and lower in residual organic solvent. Solid dispersion particles (SDP) of felodipine with enhanced solubility and dissolution rate were prepared by SAS method using HPMC (Won et al; 2005).

Nanoemulsion drug delivery system is one of the promising technologies, which is being applied to enhance the oral bioavailability of the poorly soluble drugs. Nanoemulsions are a class of emulsions with a droplet size between 20 and 500 nm [Tadros; 1983]. Nanoemulsions are transparent or translucent systems mostly covering the size range 50–200 nm [Nakajima; 1997]. A nanoemulsion has fundamental difference from microemulsions. Microemulsions are equilibrium systems (i.e. thermodynamically stable), while nanoemulsions are non-equilibrium systems with a spontaneous tendency to separate into the constituent phases. Nevertheless, nanoemulsions may possess a relatively high kinetic stability [Gutierrez et al; 2008]. In addition, high kinetic stability, low viscosity and optical transparency make them very attractive systems for industrial applications in the pharmaceutical field as drug delivery systems [Taha et al; 2004].

Many deadly diseases are treated with first line drugs that are having problem of poor bioavailability. There is need of suitable delivery systems for such drugs. **Cardiovascular diseases (CVD)** are the most prevalent cause of death and disability in both developed as well as developing countries. CVD is usually due to atherosclerosis of large and medium sized arteries and dyslipidemia has been found to be one of the most important contributing factors. Dyslipidemias, including hyperlipidemia (hypercholesterolemia) and low levels of high-density-lipoprotein cholesterol (HDL-C), are major causes of increased atherogenic risk. Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis-associated conditions, such as coronary heart disease (CHD), ischemic cerebrovascular disease, and peripheral vascular disease. The statin class of drugs that lower cholesterol
levels are among the most commercially successful drugs. Simvastatin is a first line option for treatment of hyperlipidemia but suffers from problem of low solubility and poor bioavailability.

Simvastatin (SIM) is a lipid-lowering agent that is derived synthetically from a fermentation product of Aspergillus terreus. SIM, an inactive lactone, is hydrolyzed to the corresponding hydroxyacid form after oral administration. It is a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol. SIM, a crystalline powder, is practically insoluble in water and poorly absorbed from the gastro-intestinal (GI) tract. It is a BCS class II drug (Graeser et al; 2008) and shows poor bioavailability due to limited dissolution rate [Jun et al; 2007]. Therefore, improvements in solubility and/or dissolution rate of this poorly water-soluble drug may lead to enhancement in their bioavailability [Yamamura and Rogers; 1996].

**Parkinson's disease** (PD) is major neurodegenerative disease characterized by progressive degeneration of the dopaminergic nigrostriatal pathways, which results in marked loss of cerebral dopamine. Worldwide there are likely to be more than 6 million people with PD. The prevalence of PD is about 0.3% of the whole population in industrialized countries. PD is more common in the elderly and prevalence rises from 1% in those over 60 years of age to 4% in the population over 80. The mean age of onset is around 60 years, although 5–10% of cases, classified as young onset, begin between the ages of 20 and 50. However, due to so many people with Parkinson's disease remaining undiagnosed, there may be millions more. There is no cure for Parkinson's disease, but medications, surgery and multidisciplinary management can provide relief from the symptoms.

**Entacapone (ENT)** is an inhibitor of catechol-O-methyltransferase (COMT), used in the treatment of Parkinson's disease as an adjunct to levodopa/carbidopa therapy. The aqueous solubility of Entacapone increases with increased pH. Its bioavailability after oral administration is low (29–46%) and is characterized by large inter individual variation [Keranen et al; 1994]. The reason for poor bioavailability may be poor aqueous solubility in GI fluids, poor membrane permeation and first pass effect. Entacapone as per BCS
classification is a class IV drug (Kalantri; 2010). Nanosized formulations are reported to improve aqueous solubility, permeability and avoiding first pass effects. Thus, improving the solubility and dissolution rate could lead to improved bioavailability and therapeutic activity of Entacapone.

1.2 Aims & objectives:
The present investigation was aimed at the development of nanoparticulate delivery system for oral administration of Simvastatin and Entacapone with following objectives:

- To formulate nanoparticulate delivery systems such as drug nanoparticles and nanoemulsions with improved solubility, dissolution rate and improved permeability which ultimately will increase absorption and hence bioavailability of the poorly water soluble drugs
- To evaluate the prepared formulation for in vitro and in vivo parameters
- To prove the utility of nanosizing approaches in improving oral bioavailability of drugs with poor water solubility
- To compare the prepared nanoparticulate systems with respect to ease of formulation, improvement in solubility and dissolution rate, in vitro characteristics, stability and in vivo performance viz. pharmacokinetic and pharmacodynamic studies.

1.3 Plan of Work:

- Literature survey, procurement of APIs and excipients
- Preformulation studies – Screening of excipients and characterization of API
- Formulation of Nanoparticulate systems for Simvastatin and Entacapone: Nanosuspension, nanoemulsion and nanoparticles by SAS method
- Optimization of process and formulation variables for each formulation by factorial design.
- In vitro characterization and drug release studies of these formulation in comparison with plain drug suspension
- Stability studies – Short term stability studies
- In vivo pharmacokinetic and pharmacodynamic studies of Simvastatin formulations.
- In vivo pharmacokinetic studies of Entacapone formulations.
1.4 References


