Among the new chemical entities presently available in the healthcare market, more than one-half are identified as poorly water soluble drugs (PWSDs). Such PWSDs are either BCS class II or class IV compounds, which often present formulators with considerable technical challenges. Absorption of such compounds, when presented in the crystalline state to the GI tract, is typically dissolution rate-limited. The rate and extent of absorption of BCS class II compounds, in particular, are limited by their solubility and dissolution. Numerous efforts, therefore, have been undertaken to improve the solubility of BCS class II drugs. Some viable options amongst these involve reduction of particle size, formulation of solid dispersions using hydrophilic carriers, amorphous systems, micronization, use of cosolvents, micelle solubilization, use of salts & meta stable forms, supersaturated system, gastroretentive tablets, cyclodextrin inclusion complexes, nano-emulsion and suspension, and supercritical fluid technology (Serajuddin et al. 1990; Milhem et al. 2000; Baboota and Agarwal 2003; Kopecky et al. 2003).

The problem of bioavailability assumes more serious dimensions, when a BCS class II drug undergoes extensive hepatic first-pass effect, P-gp efflux and/or metabolism in gut wall. Since lipids have intrinsic properties of solubilizing the poorly soluble lipophilic drugs, lately there has been an increasing focus on the utility of self-emulsifying lipid-based formulations (Gershanik and Benita 2000; Gursoy and Benita 2004a). For lipophilic poorly soluble drug compounds exhibiting dissolution rate limited absorption, the SEDDS are known to offer a distinct improvement in bioavailability. This augmentation is probably due to the drug being administered in a pre-dissolved state and/or enhanced solubilization of the drug in the colloidal structures. This phenomenon occurs as a result of interactions between the SEDDS formulation and its digestion products, and endogenous biliary amphiphiles such as bile salts, phospholipids and dietary lipids (Lindstrom et al. 1981). Enhancement in bioavailability, for the drugs undergoing hepatic first-pass effect, through lymphatic pathways results in vital improvement in rate and extent of absorption (Porter et al. 2007). The SEDDS also tend to exhibit marked advantageous technological features like reduction in dose, more consistent temporal profiles of drug absorption, selective targeting

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of drugs towards specific absorption window in GI tract, and protection of drugs from degradation due to intestinal cytochrome P450 enzymes in the gut environment (Pouton 1997).

2.1 SELECTION OF THE DRUGS

**Ezetimibe** is a poorly water soluble drug molecule belonging to the BCS class II of drugs exhibiting a relative bioavailability of 30% vis-à-vis its suspension drug formulation. The marked reduction in relative oral bioavailability have already been reported in humans and animals like dogs, rats, etc (Simard and Turgeon 2003). The absolute bioavailability cannot be determined because the compound is virtually insoluble in aqueous media suitable for injection (Nutescu and Shapiro 2003). This reduced bioavailability of ezetimibe is primarily due to low aqueous solubility and rapid metabolism in intestine and liver (first-pass metabolism). Besides, it is also reported to be a substrate of the efflux transporters viz. P-gp and MRP2, which markedly influence the disposition and efficacy of the drug in man (Oswald *et al.* 2007). Further, It is primarily metabolized in the small intestine and liver via glucuronide conjugation (a phase II reaction) with subsequent biliary and renal excretion (Yamamoto *et al.* 2007). Although, concomitant food administration (high-fat or non-fat meals) had no effect on the extent of absorption of the drug when administered as 10 mg tablets yet Cmax value of the drug was increased by 38% with consumption of high-fat meals (Araujo *et al.* 2005).

Further, ezetimibe has a unique mechanism whereby it acts by decreasing cholesterol absorption in the intestine and hence lowers the serum cholesterol levels in the body. It is the only drug which can be administered alone, when other cholesterol-lowering medications are not tolerated, or when statins therapies are unable to control cholesterol levels (Bays 2002; Gupta and Ito 2002). Additionally, not only it is indicated in hypercholesterolaemia or hyperlipidemia, but has immense potential in homozygous familial hypercholesterolemia and homozygous sitosterolemia (phytosterolemia) (Farnier 2002; Marais *et al.* 2004; Lutjohann *et al.* 2008). Hence, it is highly desirable to overcome its problems of diminished oral bioavailability.
Although, **valsartan**, BCS class II, is partially water-soluble drug yet its absolute bioavailability is about 25% in man (capsule formulation) (Martin and Krum 2002). Also, it is absorbed to moderate extent as observed in rat, marmoset and man. Further, food decreases the exposure (as measured by AUC) of the drug by about 40% and Cmax by about 50%. Pgp and multidrug resistance associated protein 2 have been found to be the major efflux systems involved in disposition of valsartan (Yamashiro *et al.* 2006). Its elimination is largely mediated by biliary excretion of unchanged drug. Hepatobiliary elimination appears to be due partly to MRP2, but other transporters may contribute as well. The enzyme(s) responsible for its metabolism have been identified to be CYP2C9 (Nakashima *et al.* 2005). Hence, all the aforementioned problems necessitated the exploration suitable formulation approach to augment its oral bioavailability.

### 2.2 SELECTION OF EXCIPIENTS

The current work focuses on the effect of lipidic and hydrophilic excipients on the bioavailability of ezetimibe. Several examples are excerpted from literature suggesting that the chain length of lipids affect the route of absorption and distribution of the drug in the body (Gursoy and Benita 2004b; Porter *et al.* 2007). MCTs are directly absorbed in systemic circulation via portal vein, while LCTs being larger in size are unable to diffuse and are transported via lymphatic system (Caliph *et al.* 2000; Singh *et al.* 2009).

The number of lipids, surfactants and co-solvents available for SNEDDS formulations are unlimited. However, their choice depends on characteristics of drug and its interaction within the systems (i.e., solvent capacity of oil to retain drug within the system), characteristics of surfactant to form nanoglobules and stability of emulsion formed, and characteristics of co-solvents to prevent drug precipitation on dilution with water. In such complex systems, all components and their interaction play important roles in deciding formulation characteristics (Chistyakov *et al.* 2001; Hauss 2007; Chen 2008; Date *et al.* 2010).

Further, a few of the excipients like, Labrasol, Cremophor, Tweens, etc have been reported to surmount the efflux transporters viz. P-gp and MRP2 and metabolism in intestine and liver (first-pass metabolism) (Nerurkar *et al.* 1996; Lo *et al.* 1998;
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Sha et al. 2005).

2.3 SELECTION OF DELIVERY SYSTEM

Till date various types of dosage forms for ezetimibe have been developed for
surmounting the aforementioned problems. However, the formulations i.e., co-
crystals (Mulye et al. 2012), cyclodextrins inclusion complexes (Patel et al.
2008), liquisolid compacts (Khanfar et al. 2012), nanocrystal (Gulsun et al. 2011)
and nanoparticles (Pandya and Patel 2011) were only able to increase the
solubility and/or dissolution performance of ezetimibe. Also, two emulsion
based delivery systems were reported viz. nanocarrier (Bali et al. 2011)
nanoemulsion (Bali et al. 2010) and in both the cases, the authors even proved
the superiority of the formulation using pharmacodynamic and pharmacokinetic
studies. However, the studies lack in a few aspects like,

- an optimized formulation was selected employing the traditional COST
  methods instead of DoE
- only the effect of MCT's was explored
- pharmacodynamic studies were observed for the formulations containing
  drug but no studies were carried out to observe the effect of formulation
  excipients
- effect of permeation across the intestine was not observed employing
  non-everted gut sac technique
- in order to explore the effect of P-gp efflux no SPIP studies were reported
- the robustness and stability studies were not carried for accelerated and
  long term conditions as per ICH guidelines

In order to overcome all the above issues, a few SEDDS formulations i.e., self
nanoemulsifying granules (Dixit and Nagarsenker 2008b) and self
nanoemulsifying granules with simvastatin (Dixit and Nagarsenker 2008a) were
reported. However, in case of self-nanoemulsifying granules the dissolution
profile of ezetimibe showed decrease in the rate of dissolution whereas the
nanoemulsion were reported for their inability to bypass the systemic
circulation. As such neither of the two delivery systems could effectively address
the issue of improved oral bioavailability.

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Valsartan, have been intensely explored and formulated into various delivery systems in order to enhance its oral bioavailability. The formulations developed so far included β-Cyclodextrin complex (Jensen et al. 2010), dispersion granules (Shrivastava et al. 2009), monolithic adhesive patch (Nishida et al. 2010), nanotransfersomes (Ahad et al. 2012), orodispersible tablets (Ibrahim and El-Setouhy 2010), pulsatile capsule (Nayak et al. 2009), solid dispersion (Park et al. 2010) and proniosome powders (Gurrapu et al. 2011).

β-Cyclodextrin complex, dispersion granules, orodispersible tablets, solid dispersion and proniosome powder are the only orally administered immediate release systems. All this delivery systems are able to increase the solubility and/or dissolution performance of valsartan only. It can be observed that monolithic adhesive patch and nanotransfersomes were meant for sustained transdermal delivery whereas pulsatile capsule is the only oral controlled delivery formulation. The monolithic adhesive patch was mainly prepared to assess the effect various permeation enhancers and nanotransfersomes facilitated the penetration and permeation across the skin. This indicates that there are a few studies that needs to be addressed:

- an optimized formulation was selected employing the traditional COST methods instead of DoE (except nanotransfersomes and dispersion granules)
- no significant IVIVC was established (pulsatile capsule and other immediate release dosage forms)
- the effect of lipids and other excipients were not explored
- pharmacodynamic studies were observed for the formulations containing drug but no studies were carried out to observe the effect of formulation excipients
- no other studies to indicate that the factors influencing its reduced bioavailability have been overcome
- effect of permeation across the intestine was not observed employing non-everted gut sac technique
- in order to explore the effect of P-gp efflux no SPIP studies were reported
- the robustness and stability studies were not carried for accelerated and
In order to overcome all the above issues, only one SEDDS formulation i.e., self-microemulsifying drug delivery system (Dixit et al. 2010) was reported. However, only the effect of MCT was observed in various studies including pharmacokinetic studies. Also, the factors affecting its diminished oral bioavailability i.e., P-gp efflux, extensive hepatic first-pass metabolism, lymphatic transportation of the drug, etc. were not observed.

It can be concluded that none of the delivery systems for both the drugs were effective in surmounting the variegated problems associated with their reduced and inconsistent oral bioavailability. Hence, it was planned to develop SEDDS in order to study the effect of the factors causing decreased oral bioavailability. Additionally, as in most of the cases the optimized formulation for both the drugs were obtained using COST method, it was also decided to systematically optimized the formulations employing DoE.

2.4 PROPOSED PLAN OF THE WORK

To formulate SEDDS of both the drugs, the influential factors grossly affecting the formulation characteristics of films were planned to be “screened” using a screening design. Since such systems employ components of diverse nature, viz. lipids, emulgents and co-emulgents, the nature of these components especially lipids affects the *in vitro* as well as *in vivo* performance of the formulation. Hence, in the initial phase, it was planned to screen out various SEDDS constituents from the diverse range of commercially available excipients. The choice of lipid would primarily depend on the carbon chain length and its drug solublizing capacity. Emulgent selection would depend on its capability to form nano/microemulsion region, and the stability of microemulsion thus formed. Further, the drug-excipient and excipient-excipient compatibilities would be examined using FTIR studies (Taha et al. 2004). A 3² CCD was planned to be employed for formulation of SEDDS.

Since the last two decades, systematic optimization of various DDS using experimental designs has become a routine practice across the world both in industrial and academic milieu. These techniques aid in choosing the best...
formulation under the given set of restrictions using lesser experimentation, thus saving a great deal of time, effort and developmental cost. The previous experimental studies carried out in our laboratories on the hydrophilic matrices of diclofenac sodium (Singh and Gupta 1997), verapamil hydrochloride (Singh et al. 2006b) and lamivudine (Singh and Arora 2007), lipid matrices of captopril (Singh et al. 1998), microcapsules of diltiazem hydrochloride (Singh and Agarwal 2002), buccoadhesive dosage forms of diltiazem hydrochloride (Singh and Ahuja 2002), transdermal hydrogels of tenoxicam (Singh et al. 2010c), oral fast release supersaturated systems and inclusion complexes of flurbiprofen (Singh et al. 2005a), nimesulide (Ahuja et al. 2008) and etodolac (Singh et al. 2007), self nano-emulsifying oral DDS of carvedilol (Singh et al. 2011), lovastatin (Singh et al. 2008a), simvastatin (Singh et al. 2010a), candesartan (Singh et al. 2010e), mucoadhesive tablets of atenolol (Singh et al. 2006a), vesicular drug delivery systems of nimesulide (Singh et al. 2005b) and finasteride (Singh and Kumar 2007), solid lipid nanoparticles of quercetin (Dhawan et al. 2011), hydrodynamically balanced bioadhesives of tramadol hydrochloride (Singh et al. 2010d), trimetazidine (Singh et al. 2008b), oral gastroretentive in situ gelling DDS of acyclovir (Singh et al. 2010b), mucoadhesive nasal microspheres of lercanidipine (Singh et al. 2010f) and periodontal drug delivery system of ofloxacin and ornidazole (Singh et al. 2011) have all construed extremely precise prognosis of the optimized formulations. Hence, the current study aimed at extending the benefits of DoE optimization techniques on various types of self-nanoemulsifying formulation of ezetimibe and valsartan.

A CCD for two factors at three levels was planned to optimize varied response variables viz. percent drug release (Q), amount permeated in 45 min (Perma_{45mm}), globule size (D_{nm}), emulsification time (T_{emul}), percent dissolution efficiency (%DE) and mean dissolution time (MDT). The in vitro dissolution studies would be carried out on USP 31 Apparatus 2 (paddle type) apparatus using replacement sampling method to determining the drug concentration, spectrophotometrically. The raw dissolution data will be analyzed using in-house computer software, ZOREL (Singh et al. 1997). The ex-vivo permeation studies will be carried out by non-everted sac technique. The selection of optimum
formulations would be conducted by three methods *viz.* Brute-force methodology, overlay plots and desirability function. The DoE optimization methodology was also planned to be validated by comparing the predicted values of the response variables with their corresponding experimental values using linear correlation and residual plots. The dosage forms with the desired optimal characteristics (i.e., validation check points) would be formulated, evaluated for their release performance and the results will be critically compared with those predicted using RSM optimization studies. The drug release data was planned to be fitted into various drug release kinetic models in order to ascertain the mechanism of drug release.

Rheological characterization would be planned in order to observe the Newtonian flow behavior of the formulations. Also the calculation of viscosity is crucial parameters which ultimately assist in determining the ability of the SNEDDS formulation to be filled in hard gelatin capsules.

In order to assess the stability and robustness of the optimized formulations, the formulations would be subjected to various thermodynamic and stability studies (i.e., long term and accelerated). Depending upon the initial pre-optimization and stability studies various types of special SEDDS *viz.* supersaturated and cationic SEDDS would be endeavored to surmount the problems.

The current studies would aim at investigating the *in-vivo* pharmacodynamic studies (i.e., hypolipidemia) for ezetimibe in animals. The optimized formulations along with the specialized SEDDS system would be analyzed for efficacy in modifying the various plasma lipid levels *viz.* triglyceride, total cholesterol, HDL, LDL and VLDL. Finally statistical significance in the results of the difference in the lipid levels of control and treatment groups would be performed by two-way ANOVA using GraphPad Prism software ver 5.0 (M/s GraphPad Software Inc., California, USA). The results would be subsequently confirmed by Bonferroni’s multiple comparison as a post hoc test.

It was also endeavored to investigate the *in-vivo* pharmacokinetic studies for valsartan in animals. Initially, an analytical procedure in both *in vitro* and *in vivo* conditions would be analyzed to estimate valsartan in rat plasma. The developed
method would then be developed and validated for sensitivity, linearity, precision, accuracy, sensitivity, inter-intraday variability, LOD and LOQ. Consequently, the drug plasma levels would be analyzed for various formulations, administered in rats, by reverse phase HPLC using the validated technique. Various compartmental and non-compartmental pharmacokinetic parameters were planned to be estimated using Win Nonlin software. It was also envisaged to establish various levels of *in vitro*/*in vivo* correlations (IVIVC) between the *in vitro* dissolution parameters and *in vivo* pharmacokinetic parameters.

Lastly, in order to investigate the underlying biological mechanism(s) of bioavailability enhancement i.e., absorption and permeation potential of a drug, *in situ* single pass perfusion studies would be performed on the optimized SEDDS vis-à-vis other types of formulation. The role of P-gp efflux would also be calculated using the permeability parameters (i.e., effective and wall permeability). The absorptivity parameters would also be calculated to determine the pathway of drug absorption.