1.1. INTRODUCTION

The pharmaceutical industry is one of the fastest growing sectors in terms of its size and revenue and has continued to exercise its tremendous sphere of influence for as long as mankind continue to exist. In spite of this apparent certainty, the industry is facing unprecedented level of challenges like, waning pipeline, looming patent expiries, increased generic competition, stringent regulations, amplified development costs, slashed public-health care budgets etc. which are threatening to crumble even the largest pharmaceutical industries (www.bioassociate.com; www.luxresearchinc.com).

To accommodate these dynamic challenges the pharmaceutical industry is continuously retooling their research efforts by adopting new delivery systems to rescue failed compounds due to their poor biopharmaceutical properties, and to extend patent lives through innovative repositioning and reformulation (www.luxresearchinc.com).

Colloidal drug delivery systems in the last few decades have provided the pharmaceutical industry with alternative formulation approaches for exigent molecules. Various colloids including nanoparticles, polymeric micelles, lipid based deliveries (liposomes, solid lipid nanoparticles, proliposomes and microemulsions) have been developed as carriers for encapsulation of both hydrophilic and hydrophobic drugs (Boyd, 2008; Mitragotri and Vanbever, 2011).

Delivery of drugs through lipid based colloidal carriers is an exhilarating domain that assists to resolve problems associated with unsatisfactory therapeutic response of various pharmaceutically challenging molecules. Oral lipid deliveries have taken a leap forward by addressing several key issues like poor solubility, rapid metabolism, positive food effect, low and erratic bioavailability that are common consequences faced during drug discovery process (Thomas et al., 2006; Christopher et al., 2007; Beija et al. 2012).

Lipids owing to their biocompatibility, unique physicochemical properties and their ability to enhance oral bioavailability, make them suitable vehicles for drug delivery (Mehnert and Mader, 2001; Chakraborty et al., 2009). The use of lipidic systems has been shown to enhance the luminal solubility, permeability and also prone to increase the dissolution by reducing GI transit time of the drugs. Compounds incorporated in lipids gain direct access to systemic circulation via lymphatic transport thereby bypasses the first-pass metabolism and hence increase the bioavailability (Hauss et al., 1998; Porter and Charman, 2001; Nilsson et al., 2004; Hauss, 2007).

Liposomal formulations are the most widely used lipid-based deliveries (Buse and El-Aneed, 2010). Liposomes delivered through oral route may help to enhance lipophilicity as well as absorption of poorly soluble drugs through gastrointestinal (GI) tract (Chen et al. 2009; Chiang and Weiner et al., 1987). Despite of these obvious advantages, drawbacks associated with the physiological pH and pancreatic enzymes present in the GI tract destabilize the liposomes limiting their clinical applications (Lian and Ho, 2001). Besides, liposomes also possess physical and chemical stability concerns due to leakage, sedimentation, aggregation, fusion and also tend to undergo degradation reactions such as oxidation and hydrolysis from their native form (Zhang and Zhu, 1999; Hiremath et al., 2009). Thus, as a measure to minimize the limitations associated with the liposomes they were developed into proliposomes for effectual employment in the oral drug delivery.

Proliposomes are ushering a new frontier in drug delivery sector by providing a significant alternative to conventional liposomal delivery systems. These are dry, free-flowing, freeze dried powders which upon addition of aqueous phase generate liposomes with similar structural properties and more uniform lamellarity of liposomes (Payne et al., 1986; Janga et al., 2012). Over the dacade, proliposomes are finding increasing applications because of their several notable advantages like protection of drug molecules from GI degradation, improve drug solubilisation, permeation across the GI barrier and there by enhance the bioavailability of the compounds. Ideally, clinical performance of the proliposomes can be improved by presenting them in a nano-meter size range. The submicron particles not only increases the aqueous solubility and dissolution rate of the drug but may also facilitate delivery of the drugs directly through the M-cells of intestinal payer's patches thereby avoiding the first-pass effect and hence improves overall oral bioavailability (Desai et al., 1996; Ohshima et al., 2009; Sanjula et al., 2009).

Nanoparticles are another exciting class of colloidal carriers shown to offer great potential for peroral drug administration. By definition, nanoparticles are solid, colloidal particles ranging in the size between 10-1000 nano-meters. The therapeutic agent of interest can be dissolved, encapsulated, absorbed or conjugated onto the surface of nanoparticles (Kreuter, 1991; Labhateshwar, 1997; Soppimath et al., 2001; Parveen and Sahoo 2008). The nanoparticles also offer multifaceted advantages such as, 1) provides

high stability by protecting drug against pH and enzymatic degradation, 2) improves oral bioavailability of the poorly water soluble drugs, 3) reduction in particle size and increased surface area enhances the solubility of encapsulated drugs, 4) high carrier capacity, 5) feasibility of incorporation of both hydrophilic and hydrophobic drugs, 6) control/sustain the drug release from the matrix and, 7) avoids first-pass metabolism through specialized uptake mechanisms. Overall, these properties of the nanoparticles increase therapeutic efficacy, reduce toxicity and thereby improves the patient compliance and convenience (Lode et al., 2001; Lamprecht et al., 2001; Gelperina et al., 2005; Jia, 2005; Mohanraj and Chen, 2006; Nayak, 2011). Importantly nanoparticulate systems are utilized to overcome the limitations associated with poorly soluble compounds. These compounds have reasonable membrane permeability and possess dissolution as the rate-limiting step for their absorption, resulting in poor bioavailability. The nanoparticles because of their nano-meter size range exhibits high surface area to mass ratio which increases the dissolution velocity according to Noye's-Whitney equation (Torchillin, 2006; Stegemann et al., 2007; Zhang et al., 2008; Muller et al., 2011; Muller and Keck, 2012).

The uptake process of orally delivered nanoparticles is discussed first from a recent historical perspective, emphasising on size and nature of the particles (Florence 2005). Predominantly, GI absorption of nanoparticles delivered through oral route occurs with three possible mechanisms, 1) transcellular uptake, 2) paracellular uptake, and mainly 3) uptake via membranous epithelial cells (M-cells) of Payer's patches in the gut associated lymphoid tissue (GALT) (Kreuter, 1991; Chen and Langer, 1998; Rieux et al., 2006). Transcellular transport of nanoparticles occurs by transcytosis, in which particles are taken up by cells. Transport of nanoparticles by this pathway depends upon the physicochemical properties like, size, charge on particles and hydrophobicity of surface of the particles as well as the GI physiology (Giannasca et al., 1999; Shakweh et al., 2004; Florence, 2004; Rieux et al., 2006). Paracellular transport involves crossing of particles between the adjacent intestinal cells. Many of the surfactants enhance the absorption of poorly soluble drugs through this route (Carino and Mathiowitz, 1999; Rieux et al., 2006). Most of the literatures reiterate that nanoparticles uptake occurs by intestinal lymphatic tissues, i.e. membranous epithelial cells (M-cells) of Payer's patches. Here, particles localize through apical surfaces of M-cells and then gets

internalized through these cells (Chen and Langer, 1998). The nanoparticle uptake through this mechanism is also governed by influencing factors such as, particle size and balance between hydrophobicity and hydrophilicity of the particles. It is generally adopted that nanoparticles anywhere less than one micron size are taken up by the M-cells of payer's patches (Clark et al., 2001; Rieux et al., 2006).

Under the scope of this view, colloidal lipid based proliposomes and nanoparticles are well suited for oral delivery of drugs that have disappointing biopharmaceutical properties.

1.2. MOTIVATION FOR TAKING UP THE PRESENT RESEARCH WORK

Lercanidipine (LER) is a dihydropyridine calcium antagonist which selectively inhibits influx of calcium ions through L-type calcium channels present in the cardiac and vascular smooth muscle cells. Orally administered lercanidipine is erratically absorbed with peak plasma concentration occurring in 1.5 to 3 h after administration with a plasma half-life of about 2 to 5 h (Sica and Prisant, 2007). Lercanidipine exhibits absolute bioavailability of only 10% due to its extensive and saturable first-pass metabolism. Further administration of lercanidipine along with food increases the absorption and hence the bioavailability. The food dependence dosing of lercanidipine is highly undesirable and can result in dose fluctuations, ineffectiveness, larger inter-patient variability and consequent patient compliance problems (Barchielli et al., 1997; Nilsson et al., 2004; Dedhiya et al., 2007; Leonardi et al., 2010). Another major problem associated with lercanidipine is its poor aqueous solubility which often results in low dissolution velocity and hence exhibits low bioavailability. It also shows small concentration gradient across the intestinal mucosa which can result in variable absorption culminating into poor therapeutic response. These shortcomings of lercanidipine motivated the need to develop suitable drug delivery systems in the form of nanoproliposomes and polymeric nanoparticles. It was hypothesized that these novel drug deliveries would provide the effective means to improve the drug solubility, avoid first-pass metabolism and thereby ultimately improve the absorption and bioavailability of lercanidipine.

1.3. OBJECTIVES OF THE PRESENT RESEARCH WORK

Aim of the present study was to find the suitability of novel nanotechnological approaches to improve the poor biopharmaceutical properties of lercanidipine. In view of this, lercanidipine loaded nanoproliposomes and lercanidipine loaded nanoparticles were formulated and evaluated for their effectiveness in improving bioavailability of the encapsulated drug.

The specific objectives of the present research work were:

1. To develop and validate high performance liquid chromatographic method for the quantification of lercanidipine in novel nanoformulations and in rabbit plasma.
2. Formulation development of lercanidipine loaded nanoproliposomes.
3. Formulation development of lercanidipine loaded nanoparticles.
4. Physicochemical characterization and *in vitro* drug release study of the developed nanoformulations.
5. To carry out *in situ* absorption study in rats to investigate absorption behaviour of the developed nanoformulations.
6. To carry out pharmacokinetic study in rabbits to understand the *in vivo* performance of developed nanoformulations.
7. To evaluate antihypertensive potential of prepared nanoformulations in rats.
8. To carry out stability study of the optimized nanoformulations.

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