1.1 INTRODUCTION

Oral route is the most common and convenient route of administration. This choice is mainly related with its non-invasiveness and ease of administration, which increases patient's compliance and therapeutic success. At present, about 40% of the drugs in the development pipelines and approximately 60% of the drugs coming directly from synthesis are poorly soluble (1). These new chemical entities (NCEs) identified by pharmaceutical industry screening programs have failed to be developed because of poor water-solubility, which makes their formulation difficult or even impossible. As solubility and permeability of Biopharmaceutics Classification System (BCS) Class II and IV are considered prerequisites to oral absorption many of these drugs exhibit poor and variable bioavailability (2). Formulation of poorly aqueous soluble drugs is a challenging job to the pharmaceutical scientists and oral delivery of such drugs is frequently associated with low bioavailability, high inter subject variability and lack of dose proportionality (3,4).

Poor solubility and/or poor permeability of drugs are the main causes for their poor oral bioavailability (BA). Physicochemical and metabolic instability in both stomach and liver negatively influence the drug concentration in blood. Hepatic first-pass metabolism is another major cause of poor BA upon peroral administration. Poor solubility of the drugs not only affects oral BA but also encumbers the development of suitable delivery system. The trend in drug discovery toward lipophilic molecules has increased the need to develop alternative oral delivery systems for poorly soluble compounds. Nevertheless, oral formulations are being developed keeping in consideration the basic biological and pharmaceutical approaches of drug delivery via the oral route (5,6).

This increasing number of poorly soluble drugs, belonging to BCS class II and class IV, require alternative formulation approaches to achieve a sufficiently high bioavailability after oral administration. There are various drug delivery approaches which have been investigated to improve the aqueous solubility and poor dissolution rate of class II and IV drugs. Many recent advances have been made in the design of delivery technologies such as lipid-based formulations, solid dispersions, micro- and nano suspensions etc., and in many cases methodology and design of development of particular formulation remains empirical and uncertain. Interest in Lipid Based Drug Delivery Systems (LBDDS) is relatively recent and relates to the development in the

past 10 to 15 years, largely driven by the growing need for novel drug delivery systems to deal with the vast majority of the new chemical entities that have poor solubility or permeability, to improve the delivery of existing drugs, and for line extensions. The primary application for lipid based excipients is in bioavailability enhancement where the aim may be to increase solubility, targeting lymphatic transport, and modulation of enterocyte-based drug transport and disposition. Other applications include drug coating for either taste masking or protection of the active, and in sustained release dosage forms.

LBDDS are promising, since lipids are known oral drug absorption enhancers, can be prepared with low particle size and may be a promising strategy to improve the rate and extent of oral absorption (7). The availability of lipid excipients with acceptable regulatory and safety profiles with their bioactive property (ability to enhance oral bioavailability) has aided in the development of lipid based formulations as a means for drug delivery. In the recent years, LBDDS have gained much importance because of their ability to improve the solubility and bioavailability of poorly water-soluble drugs (8).

The inherent physicochemical properties of lipophilic drug molecules dictate that they become associated with endogenous lipidic microdomains, ranging from lipids and lipid digestion products within the gastrointestinal tract (GIT) to lymph and plasma lipoproteins in the systemic circulation. The affinity with which lipophilic drugs bind to, and interchange between, these carrier systems can have a significant impact on the free drug fraction available for absorption, distribution, metabolism, and excretion and can therefore play a major role in defining both drug pharmacokinetics and therapy. Importantly, the nature and behavior of these lipid microdomains changes dramatically in response to the ingestion of exogenous lipid (as either food or formulation excipients), thereby influencing the disposition of lipophilic drugs (9).

LBDDS can be explored as potential vehicles for site specific drug delivery to lymphatics. Many particulate systems such as emulsions, lipid solutions, microemulsions, micellar solutions, liposomes and lipid nanoparticles are investigated for targeting of drugs to the lymphatic system (10). There are some of the drugs which are successfully marketed as lipid based formulations such as ritonavir (Norvirs), clofazimine (Lamprenes), efavirenz (Sustivas) and saquinavir (Fortovases), etc. Success of any lipid based formulations basically depends on an appropriate selection of lipid vehicles, formulation strategies and rational delivery system design (11).

There are several possible complex mechanisms through which lipids exert their effects which in turn lead to alteration in the biopharmaceutical properties of the drug which includes increased dissolution rate of the drug and solubility in the intestinal fluid, protection of the drug from chemical as well as enzymatic degradation in the oil droplets, and the formation of lipoproteins promoting the lymphatic transport of lipophilic drugs (12-14).

In order to overcome the challenges associated with oral drug delivery, nanoparticles (NPs) are considered as alternatives to various conventional drug delivery techniques and often used to improve the oral BA of drugs. A number of nanoparticulate systems based on biocompatible polymers, lipids and oils have come to the fore, which can be efficiently used to improve the oral BA of drugs either by increasing the drug permeability or by overcoming the first-pass effect and/or Permeability glycoprotein (P-gp) efflux. Apart from these, NPs can also improve the stability of drugs in the GIT while modulating their physicochemical and biological properties (6).

Ahead of other nanoparticles, lipid nanoparticles are widely being investigated by pharmaceutical scientists because of their advantages such as higher degree of biocompatibility and versatility. Lipid nanoparticles can be formulated for topical, oral, pulmonary or parenteral delivery which are commercially feasible. Lipid based nano-formulations can be developed to fulfil product requirements such as route of administration, disease condition, cost of product, stability of formulation, its toxicity and efficacy. Because of the proven safety and efficacy of lipid based nano-formulations, SLNs are prime choice for the formulation of pharmaceuticals, vaccines, diagnostics and nutraceuticals (15).

Research on the nanoparticles depicts that there are rapid advances come in existence to produce nanoparticles of uniform size, shape, and composition which has given science a revolution. Among that the development of lipid-based drug carriers has attracted increased attention over the last decade. Lipid nanoparticles are one of the potential carriers for the controlled and site-specific drug delivery. Amongst various lipid nanoparticles, solid lipid nanoparticles (SLNs) are one of the rapidly growing in the field of nanotechnology with potential advantages in the drug delivery, research and clinical medicine. The following advantages among others, could be ascribed to lipid based nanocarriers (16):

- They can control and target drug release.
- Improve the stability of pharmaceuticals.

- They can encapsulate high drug content
- The feasibility of encapsulating both lipophilic and hydrophilic drugs.
- Most of the lipids used are biodegradable and they have excellent biocompatibility, also non-toxic, non-allergenic and non-irritating.
- They can be formulated by water-based technologies and thus can avoid organic solvents.
- They are easy to scale-up and sterilize.
- They are less expensive than polymeric/surfactant based carriers.
- They are easy to validate.

SLNs have attracted increasing attention as colloidal drug carriers during the last decade (17). SLNs have emerged as a potential alternative colloidal carrier because they do not just combine the advantages but also overcoming the drawbacks associated with other colloidal drug delivery systems like liposomes polymeric nanoparticles and fat emulsions etc. (18). SLNs are aqueous colloidal dispersions, the matrix of which comprises of solid biodegradable lipids. SLNs are preferred because it avoid the drawbacks of other colloidal carriers such as physical stability, protection of incorporated labile drugs from degradation, controlled release, and excellent tolerability (19). SLN formulations are developed and characterized for in vitro and in vivo studies by various routes like parenteral, oral, pulmonary, ocular, and dermal etc. SLNs offer an attractive means of drug delivery mainly for poorly water-soluble drugs. SLNs are in the submicron size range, and are composed of physiologically tolerated lipid components, which remains in the solid state at room temperature. Most orally administered drugs reach to systemic circulation via portal vein. But the co-administration of lipids with drugs also impact their absorption pathway because some lipophilic drugs are directly transported via intestinal lymphatic to the systemic circulation which improves its oral bioavailability (20). SLNs can effectively overcome the challenges associated with oral delivery of drugs which are poor water soluble, instable in the GIT, having poor permeability, P-gp efflux and presystemic drug metabolism (6).

Self-Micro Emulsifying Drug Delivery System (SMEDDS) are isotropic mixtures of oils, surfactants and co-surfactants/co-solvents and which are spontaneously emulsified to produce oil-in-water emulsions when introduced into aqueous phase under gentle agitation. This property renders SMEDDS a good candidate for oral delivery of hydrophobic drugs with adequate solubility in oils or oil/surfactant blends (21).

The SMEDDS gets emulsified in the stomach and drug is presented in the small droplets having size in the range of 10-200 nm which can lead to improve drug dissolution and also provide larger surface area for partitioning the drug between the oil and GI fluid. Other advantages of the SMEDDS include (i) stability of drug molecules get increased and (ii) final product can be administered as a gelatin capsule. SMEDDS can enhance both rate and extent of drug absorption in the drugs showing dissolution rate-limiting absorption and this type of drug delivery systems allow reproducibility of plasma concentration profile (22).

Schizophrenia is a complex neuropsychiatric disorder that produces severe symptoms and significant lifelong disability, causing massive personal and societal burden. It is a major psychotic disorder that frequently has devastating effects on various aspects of the patient's life and carries a high risk of suicide and other life-threatening behaviors. Pharmacological treatments are available for schizophrenia; yet, most of the currently used antipsychotic medications were discovered in the 1950s, or are a variation of those medications, and since then no new major drug class has been introduced to the clinic. In addition, efficacy of medication is poor, and only about 40% of schizophrenic patients respond effectively to initial treatment with antipsychotics. Unfortunately, comprehensive studies on molecular mechanisms of schizophrenia have been scant; hence, current treatments are only partly beneficial to a subset of symptoms (23). Patients with this condition frequently show resistance to medical treatments, especially because of the unpleasant side effects and dosing frequency.

The primary distinction between conventional and second-generation antipsychotics has been made on clinical basis. Conventional or "typical" antipsychotics are characterized by undesirable side effects such as extrapyramidal symptoms (EPS), hyperprolactinaemia, tardive dyskinesia and possible neuroleptic malignant syndrome. These symptoms are specific to the group as a whole and generally associated with high doses but in some cases also at clinically effective dosages. The

second-generation or "atypical" antipsychotic drugs can be differentiated from traditional antipsychotics by their low or negligible levels of these unwanted side effects, by effectiveness and in general supposed increased safety (24). Over the past decade, atypical (or second-generation) antipsychotics have been increasingly used in the treatment of schizophrenia in preference to conventional/typical (first-generation) drugs (25,26).

Asenapine maleate (AM) is an atypical antipsychotic agent which shows strong $5HT_2A$ (serotonin) and D_2 (dopamine) receptor antagonism. Asenapine maleate may improve cognitive function and negative symptoms in patients with schizophrenia. Asenapine maleate is a BCS class II, slightly water-soluble drug having log P of 4.9. The oral bioavailability of AM <2% due to its extensive first pass metabolism (27-28). It is mainly metabolized by CYP450 isozymes such as CYP3A4, CYP1A2, CYP2D6, CYP2B6 and CYP2C19. Initial phase half-life of around 5 hours with a terminal phase half-life of around 24 h. It has dose and time dependent hepatotoxicity, metabolite concentration dependent cardiac arrhythmias, as well as significant drug-drug-interactions (29).

Lurasidone Hydrochloride (LH) is an atypical antipsychotic that is a D_2 and 5-HT_2A receptor blocker**.** LH is a poorly water soluble having log P value of 5.6. The oral bioavailability of LH is 9-19% because it is metabolized mainly via CYP3A4. It is recommended that Lurasidone HCl should be administered with food. The adverse effects associated with LH are somnolence, akathisia, nausea, parkinsonism, and agitation (30,31).

Additionally, according to biopharmaceutical classification system (BCS) class II and IV drug which means that it has poor water solubility, permeability and consequently, a dissolution rate-limited absorption through the gastrointestinal tract. Therefore, the development of new drug delivery systems remains a challenge for technologists. If we join the advantages of controlled drug release and specific targeting with the well-known principle that lipids promote oral absorption of poorly water-soluble lipophilic drugs, the use of these systems for improving oral delivery could be beneficial (32). So, AM and LH were selected as drug candidates to improve their bioavailability by reducing first pass metabolism via lymphatic targeting.

The concept of **Quality by Design (QbD)** was first introduced in 2006 by the International Conference on Harmonization (ICH) Q8 guidance. QbD is defined as "a systematic approach to development that begins with predefined objectives and

emphasizes product and process understanding and process control, based on sound science and quality risk management". United States Food and Drug Administration (US FDA) has promoted QbD as an important tool to enhance pharmaceutical development through design efforts from product conceptualization to commercialization (33). Pharmaceutical formulation development involves complex procedure which includes various process and formulation variables that can affect quality of final product. The effect of these individual parameters and their interaction can affect the quality of critical quality attribute (CQA). Thus, QbD aids in understanding effect of these critical processing parameters (CPPs) by identifying risk identification (fishbone diagram), risk analysis (screening design) and optimization using Design of Experiment (DoE) on CQA of final product (34,35).

1.2 AIMS

The aims of present research work were,

- To develop and evaluate solid lipid nanoparticles and self microemulsifying drug delivery system of asenapine maleate and lurasidone hydrochloride.
- To improve the solubility of poorly soluble drugs using lipid excipients.
- To improve bioavailability of AM and LH by reducing first pass metabolism via intestinal lymphatic system.

1.3 OBJECTIVES

To develop lipid based nanoformulations of selected drugs for oral bioavailability improvement with following objectives:

- To formulate and optimize lipid based formulations viz. SLNs and SMEDDS of selected drugs using Design of Experiment (DoE).
- To characterize and evaluate the developed nanoformulations.
- To perform in vitro drug release study and ex vivo permeation study.
- To perform qualitative and quantitative cellular uptake study, cytotoxicity study, uptake mechanism elucidation study using specific inhibitors and permeability study using Caco-2 cell line.
- To perform in vivo pharmacokinetic, lymphatic uptake and pharmacodynamic study in rats. Thus, proving the utility of developed

nanoformulations in oral bioavailability enhancement through lymphatic uptake.

1.4 HYPOTHESIS

It was hypothesized that developed lipid based nanoformulations would:

- Improve the absorption from the gastrointestinal tract by
  - (i) reducing particle size and
  - (ii) increasing drug solubility in lipid excipients.
- Bypass the hepatic metabolism and increase plasma concentration of drug, through intestinal lymphatics.

1.5 PLAN OF WORK

- Literature review, procurement of Active Pharmaceutical Ingredients (APIs) and excipients.
- Pre-formulation studies: Screening of excipients for solid lipid nanoparticles and self microemulsifying drug delivery system.
- Analytical method development and validation.
- To prepare and optimize solid lipid nanoparticles (SLNs) and self microemulsifying drug delivery system (SMEDDS) for AM..
- To prepare and optimize Solid lipid nanoparticles (SLNs) and optimize self microemulsifying drug delivery system (SMEDDS) for LH.
- To characterize and evaluate optimized formulations.
- To perform in vitro release study using dialysis bag method.
- To perform ex vivo permeation study using rat intestine.
- To perform cellular uptake (Qualitative and quantitative uptake) study, cytotoxicity study, uptake mechanism elucidation and permeability study using Caco-2 cell line.
- To perform in vivo pharmacokinetic study, pharmacodynamic study and lymphatic uptake study using rats.
- To perform stability studies of optimized formulations.
- Paper publication and thesis write up.

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