1. Introduction

In recent decade, central nervous system (CNS) disorders have been increasing enormously (Kuehn BM, 2010; Nutt DJ and Attridge J, 2014). When global burden of neuropsychiatric disorders was measured by years lived with disability and years lost due to premature death in disability adjusted life years, psychiatric and neurological conditions accounts for 13% of the global burden of disease (WHO). In CNS disorders, schizophrenia is severe chronic debilitating psychological disease affecting approximately 1-2% of the world population, more prevalent in urban population than rural population albeit without any sexual disposition. It is reduces the patient's life span by an average of ten years. (Rossler W *et al.*, 2005; WHO S). This debilitating condition predisposes more treatment challenges in low and middle income counties due to poor healthcare sector, limited accessibility to psychiatrists and delay in diagnosis. The age of onset is generally between 20-35 year and it is characterized by positive symptoms (change in behavior or thoughts, such as hallucinations or delusions), negative symptoms (lack of social interaction, emotional expression and motivation) and cognitive dysfunction (disorganized thoughts and memory problems). Among several factors, suicide tendency is most prevalent cause of early death in schizophrenia patients. Patient non-adherence is another major cause of morbidity in schizophrenia treatment. During first 4-6 weeks of treatment, more than 34% of patients demonstrated adherence problems while it goes to 74% within 2 years resulting in significantly high rate of relapse,

risk and length of hospitalization (Gilmer TP *et al.*, 2004; Lieberman JA *et al.*, 2005; Valenstein M *et al.*, 2004; Weiden PJ and Olfson M, 1995). The most common outcomes of non-adherence are usually a remitting course with one or multiple relapses in 50-92% of cases (Tollefson GD *et al.*, 1998). Reasons behind this patient non-adherence are associated with significant personal, social and economic impacts due to side effects associated with drug and frequent dosing regimen. In treatment of schizophrenia, atypical antipsychotic drugs are more prominently used as compared to typical drugs due to lower incidence of extrapyramidal symptoms, less tardive dyskinesia, less dysphoria and better cognition (Tandon R, 2006).

Asenapine maleate (ASM) is a newer antipsychotic drug with high affinity towards dopamine, serotonin, α-adrenergic and histamine receptors, and appreciable activity at muscarinic cholinergic receptors. Like other atypical antipsychotic, asenapine mode of action is mediated through a combination of antagonist activity at 5-HT_{2A} and D_2 receptors. It is approved for treatment of schizophrenia in adults and as an adjunctive therapy with lithium or valproate for acute treatment of manic or mixed episodes associated with bipolar I disorder (Tarazi FI and Shahid M, 2009). It is slightly soluble in water and classified as BCS class II drug (Bartlett JA and van der Voort Maarschalk K, 2012). Asenapine has been developed as a structural modification of the atypical antidepressant mianserin (Minassian A and Young JW, 2010). It is the first antipsychotic drug to be administered through a sublingual route of administration. ASM is available in 2.5, 5 and 10 mg sublingual tablet dosage form with maximum administered daily dose up to 20 mg and administered

twice daily. The patients are advised to place the tablet sublingually and it should not be swallowed and allowed to dissolve completely in mouth without eating and drinking for 10 minute. The bioavailability of ASM is around 35% by sublingual route while it is <2% via oral due to its high gastro-hepatic metabolism (Saphris; Stoner SC and Pace HA, 2012). Despite its therapeutic potential in schizophrenia treatment, the limiting factors with current dosage forms of ASM such as low bioavailability, drinking and eating restriction, twice a day dosing regimen and extra pyramidal side effects are still a very challenging task for pharmaceutical researchers. Thus, new formulation strategies have to be adopted to overcome the problem of asenapine delivery (Citrome L, 2014). At the conceptualization of these problems, literature search revealed three methods for the estimation of ASM in human plasma by liquid chromatography mass spectroscopy technique (de Boer T *et al.*, 2012a; b; van de Wetering-Krebbers SF *et al.*, 2011). These three methods were reported for estimation of ASM in plasma and urine samples. These methods were not suitable for analysis of drug in bulk and pharmaceutical dosage form. Thus, there was need to develop a sensitive, specific and validated analytical method for the routine analysis of the drug.

Treatment of CNS related disorders is very challenging due to limited assess of therapeutic agents to the brain. Blood brain barrier, whilst trying to protect brain from toxic substances also prevents entry of sometimes intended therapeutic agents. This restricted access of drug molecules reflected in drug development processes as well, where many CNS acting candidates exhibit better *in-vitro* efficacy

but fail *in-vivo* due to tight junctions between capillary endothelial cells of blood brain barriers (Pardridge WM, 2012; Patel M *et al.*, 2013).

Different strategies have been investigated for successful delivery of drug to the brain (Alam MI et al., 2010; Chen Y and Liu L, 2012). Among them, it has been reported that intranasal (i.n.) route opens a new possibility of non-invasive delivery of drugs. The nasal mucosa is highly vascularized with porous endothelium membrane which gives a broad surface area for rapid drug absorption that results in prompt pharmacological action (Kozlovskaya L and Stepensky D, 2013; Patel S et al., 2011). Apart from these advantages, nasal route provides unique anatomical connections where the olfactory and trigeminal nerves extend directly from brain to the upper posterior segments of the nose and gives quick and easy access to the CNS for therapeutic substances (Djupesland PG *et al.*, 2014). In fact, this route is helpful in delivery of drug which has high gastric or hepatic metabolism (in case of oral delivery) and also it does not require sterility of product (in case of parental formulation) (Carlotta M *et al.*, 2015). This route is also superior over the intravenous route in aspects of invasiveness and patient compliance and also does not require skilled person for administration.

In general, the conventional intranasal liquid formulations are limited by large dose volume, low drug loading, low solubility of drug, physico-chemical instability and low residence time (Turker S *et al.*, 2004). However, the above problems of dosage form have been successfully addressed by use of nano colloidal drug delivery system. The inherent properties such as nano size, tailored surface, solubility

improvement, release modification and multi-functionality facilitate the enhancement of bioavailability, efficacy and targetability. Different research groups have explored various nano carriers such as liposome, polymeric micelles, nanoemulsion, nanosuspensions, polymeric nanoparticles, solid lipid nanoparticles and nanostructured lipid carriers for intranasal delivery of CNS acting drug (Chiappetta DA *et al.*, 2012; Gartziandia O *et al.*, 2015; Kumar M *et al.*, 2008; Migliore MM *et al.*, 2010; Seju U *et al.*, 2011). The polymeric and/or lipid nanoparticles in size less than 200 nm are preferable carriers for brain delivery (Kozlovskaya L *et al.*, 2014; Mistry A *et al.*, 2009). Lipid based nanoparticles show advantages in brain targeted drug delivery over polymeric nanoparticles due to its rapid uptake by the brain, biocompatibility, biodegradability and less toxicity. The avoidance of organic solvent in production of lipid nanoparticles is one of the unique features associated with them. Among lipid nanoparticles, nanostructured lipid carriers (NLC) are alternative to the solid lipid nanoparticle (SLN), composed of both solid lipid and liquid lipid. NLC are superior to SLN in respect to smaller particle size, higher drug loading due to more imperfection in the solid lipid matrix and no drug leakage during storage by lipid polymorphism (Blasi P *et al.*, 2011; Eskandari S *et al.*, 2011; Tiwari R and Pathak K, 2011). In spite of the presence of liquid lipid, NLC is solid at room and body temperature. Furthermore, the particles size and drug loading and its release can also be modulated by changing liquid to solid lipid ratio of the matrix (Das S *et al.*, 2012; Yang X-y *et al.*, 2013). However, when it comes to delivery via intranasal route, NLC major drawbacks are low residence time in the nasal cavity

and incomplete drug absorption due to mucociliary clearance (Gartziandia O *et al.*, 2015). The use of penetration enhancer, mucoadhesive polymer and cationic charge development at the surface of particles are some of the approaches that could be used to overcome challenges of NLC in intranasal delivery.

The surface modification by cationic polysaccharides such as chitosan or its derivative shows excellent bio-adhesive properties. This cationic charge interacts with anionic part of mucous layer, mainly sialic acid and increases the epithelial permeability by transient opening of tight junction between apical cells (Casettari L and Illum L, 2014; Chirio D *et al.*, 2014). The chitosan is insoluble in physiological pH and only dissolves in acidic pH. Therefore, numerous derivatives have been introduced by functional group modification of chitosan to overcome this problem (Lee SJ *et al.*, 2014). Glycol chitosan (GC) is a conjugated product of chitosan and ethylene glycol. It exhibit complete water solubility at all physiological pH range. In glycol chitosan, glycol branches on the polymer increase aqueous solubility of the chitosan as well as provide steric stabilization to nanoparticles (Trapani A *et al.*, 2009).

Despite numerous benefits of targeted drug delivery via nanocarriers, there have been several reports which implicate the materials employed in their preparation as possible perpetrators of adverse effect in reproductive organs and embryo due to sometimes unintended and/or unaccounted accumulation (Ema M *et al.*, 2010; Yu WJ *et al.*, 2014). Physical properties of carriers such as size, charge, surface area, nature of material, concentration, and pH of formulation play a significant role in in-

vivo toxicity and biocompatibility with organelles (Cho WS et al., 2014; Yu WJ et al., 2014). These properties are especially worrisome when long term dosage regimen is recommended for chronic condition or for prenatal ailments where even mild distresses are accentuated. Since asenapine possesses inherent dose dependent teratogenic potential, embryo-fetal assessment of any novel carrier professing delivery of asenapine is a prerequisite to justify its long term applicability over a large population. Most of the embryo-fetal toxicity studies are reported for metallic nanoparticles only, which cannot be correlated with lipid nanoparticles due to distinct nature of material (Austin CA *et al.*, 2012; Philbrook NA *et al.*, 2011). To the best of our knowledge, very limited literatures are available on embryo-fetal effect of lipid nanoparticles. It is still unclear and unexplored. For asenapine incorporated nanostructured lipid carriers, its assessment is essential before recommending of the drug during pregnancy.

Novel dosage forms are complex and require manipulation of intricate process variables. Thus, its development requires critical process control to obtain desired output. In development of novel dosage form, a crucial issue is to design and optimize a formulation with define therapeutic benefit. Historically, optimization process involved trial and error method in which one variable was changed at a time while keeping others constant. Outcome from these methods overlook the interaction among different factors and may not give optimum values. Hence, regulatory agencies like FDA (USA) and EMA (European Union) have espoused a paradigm shift from trial and error estimation of variables to quality by design

(QbD) approach, emphasizing on systemic development of pharmaceutical product based on computational and material science backed by mathematical modeling. QbD is defined as a systematic development approach that begins with predefined objectives and emphasizes product and process, based on sound science and quality risk management (FDA, 2009; Verma S *et al.*, 2009). Response surface methodology (RSM) is one of the QbD based approach used for pharmaceutical product development. It shows the relationship between the factors response and interaction between independent variables. Among RSM, box-behnken design (BBD) is more cost-effective than other techniques such as central composite design, three-level factorial design and D-optimal design, as it requires lesser experimental runs for optimization of a process at set independent variables (Li GY *et al.*, 2011; Mujtaba A *et al.*, 2014).

In connection with above discussion, the objective of this research work was to develop asenapine loaded nanostructure lipid carriers (ANLC) and further effect of glycol chitosan coating over asenapine loaded nanostructured lipid carriers (GC-ANL) for targeted brain delivery after nasal administration. The BBD was used to analyze effect of critical parameters of composition and process variables in optimization process of ANLC. The ionic interaction technique was used for GC - ANLC preparation. Both ANLC and GC-ANLC were characterized for particle size, shape, *in-vitro* release, stability and solid state characteristics. Both ANCL and GC-ANLC, the systemic and brain bioavailability were determined by pharmacokinetic study in Charles foster (CF) rats. In addition, potential of these carriers in

therapeutic efficacy and extra pyramidal symptoms were assessed pharmacologically on CF rats upto three weeks. Further, *in-vitro* cell viability and nasal toxicity studies were carried out to exclude any harmful effect on nasal epithelium. The present work also evaluates the potential effects of optimized carriers on embryo-fetal development in CF female rats.

Overall this research work was aimed to provide an entirely safe and efficient delivery system for asenapine via intranasal route with brain-targeting potential to overcome the limitation related to drug and current dosage form.