2. RESEARCH ENVISAGED

AIDS is a pathological state wherein the human immune system begins to fail, leading to life-threatening opportunistic infections. HIV, the causative retrovirus for AIDS, primarily infects and destroys vital cells of the human immune system, precipitating an acute loss of cellular immunity (Johnson, 1998). HIV is also reported to disseminate to a number of diverse cellular (CD4+ lymphocytes, macrophages and dendritic cells) and anatomical (blood, lymph nodes, GALT, CNS, genital tract, spleen and lungs) sites. GALT has been reported to harbour a majority of body’s lymphoid tissue and acts as a reservoir for establishment and persistence of HIV infection (Ghosh et al., 2011; Hoetelmans, 1998). These anatomical and cellular reservoirs of HIV make it difficult to completely eradicate the virus by employing the conventional antiretroviral therapy. Also, the chances of viral mutations increase, leading ultimately to drug resistance (Alexaki et al., 2008; Bagasra, 2006). In CNS, the virus replicates actively to form a sanctuary site, resulting in HAND (Xia et al., 2011).

Several antiretroviral drugs exhibit low bioavailability owing to myriad causes including, poor aqueous solubility, and metabolism by CYP450 family, extensive hepatic first-pass effect and efflux by P-gp transporter. Besides, the issue of low oral bioavailability, antiretroviral drugs do not gain access into the sanctuary sites owing to a number of physico-chemical factors, like, molecular size, lipophilicity, degree of ionization and plasma protein binding and physiological factors, like presence of BBB, efflux pumps and expression of metabolizing enzymes (McGee et al., 2006; Ronaldson et al., 2008).

Considering the aforementioned challenges, therefore, the holistic management of the HIV requires lipid-based nanotechnology-enabled systems. Over the last one decade, the presence of lipid-based systems have revolutionized the field of drug delivery for enhancing the oral bioavailability and subsequent biodistribution of poorly soluble and permeable antiretroviral drugs (Date et al., 2010).

The SNEOFs, in this regard, have proved as effective delivery systems owing to ability to circumnavigate the hepatic portal route, reducing metabolism by CYP450 enzymes, inhibiting P-gp efflux and promoting the lymphatic transport of drug(s) (Date et al., 2010; Porter and Charman, 2001). Self-nanoemulsifying
systems have conferred a promising platform for ameliorating the HIV by targeting the antiretroviral drugs to the GALT sanctuary site. The SNEOFs also tend to exhibit stellar technological features like reduction in dose, consistent temporal profiles of drug absorption, selective targeting of drugs towards specific absorption window in the GI tract, and protection of drugs from degradation due to intestinal CYP450 in the gut environment (Pouton, 1997).

Apart from SNEOFs, SLNs and NLCs present themselves as attractive drug delivery vehicles for enhancing the biodistribution of drug to the brain owing to their nano size structure and excipients employed therein for the purpose. Further, surface modified and coated SLNs and/or NLCs will results in enhancement of the brain distribution of the drug. Moreover, the RES system is unable to recognize these coated nanoparticulates in the blood, thus prolonging the circulation of the drug (Alex et al., 2011).

### 2.1. SELECTION OF THE DRUGS

Darunavir is a new second-generation PI approved by US-FDA and EMEA as a viable therapeutic option for the treatment-experienced HIV-infected patients with resistance to other available PIs (Phung and Yeni, 2011). Darunavir is a poorly water soluble drug molecule (0.15 mg.mL\(^{-1}\)) with log P of 1.89, belonging to BCS class II (Patel and Lalwani, 2011). It exhibits low oral bioavailability of 37% owing to poor aqueous solubility, hepatic first-pass metabolism and it being a substrate of the efflux transporters \textit{viz.} P-gp and MRP2 (Inugala et al., 2015; Thommes et al., 2009). \(C_{\text{max}}\) is attained within 2.5 to 4 hours, with elimination half life (\(t_{1/2}\)) being 10 hours (Rittweger and Arasteh, 2007). Additionally, darunavir is not able to traverse the BBB, it being a large molecule, highly bound to plasma proteins and susceptible to active efflux from the CNS (Ruela Correa et al., 2012). Thus, it becomes less efficacious for the treatment of HAND.

Lopinavir is a potent PI used for the treatment of HIV infections (Maartens et al., 2009). Lopinavir also possesses a log P of 4.56, and exhibits low and inconsistent bioavailability (27%), plausibly attributed to the poor aqueous solubility, metabolism by CYP450 family, extensive hepatic first-pass effect and efflux by P-gp transporter (Alex et al., 2011; Pal et al., 2011).
2.2. SELECTION OF EXCIPIENTS

The SNEOFs employ components of diverse nature, viz. lipids, emulgents and cosolvents, the nature of these components affects the in vitro as well as in vivo performance of the formulation. The current work, therefore, would focus on the effect of lipidic and emulsifying excipients on the bioavailability of antiretroviral drugs. In this regard, the choice of the excipients for the formulation of the bioenhanced systems depends upon a number of considerations like solubilizing potential of the lipid, emulsifying capacity of the emulgent and the emulsion stability by preventing the drug precipitation on the dilution of the cosolvent. The selection of lipid would primarily depend on the carbon chain length and its drug solubilizing capacity. Emulgent selection would depend on its capability to form nano/microemulsion region and its stability. Further, some of the excipients like Maisine, Peceol, Labrasol, Cremophor, Tweens, etc., have also been reported to surmount the efflux transporters viz. P-gp and MRP2 (Sha et al., 2005; Singh et al., 2014a; Werle, 2008).

2.3. PLAN OF THE RESEARCH WORK

To formulate SNEOFs of both the drugs, systematic QbD-based approach will be adopted. First, the drug-excipient and excipient-excipient compatibilities would be examined using FTIR and DSC studies (Taha et al., 2004). Systematic optimization of the prepared formulation would be later carried out using QbD paradigms employing appropriate experimental designs. These techniques aid in choosing the best formulation under the given set of conditions using lesser experimentation, thus saving a great deal of time, effort and developmental cost.

Numerous previous experimental studies carried out in our laboratories employing QbD principles on diverse lipidic formulations including lipid-based DDS on diverse drugs, have vividly construed the precise prognosis of the optimized lipid-based drug delivery formulations. The current studies also aimed at extending the benefits of QbD approaches on various types of lipid-based nanostructured delivery systems of lopinavir and darunavir.

As per the QbD-based drug delivery development, QTPP would be defined along with the justification and the Ishikawa Fish-bone diagram would be constructed for establishing the cause-and-effect relationship among the material
attributes/process parameters with the CQAs. Prioritization exercise would be carried out employing initial risk assessment and QRM techniques for identifying the “prominent few” input variables, termed as CMAs and CPPs from the “plausible so many” through factor screening studies. Factor screening studies will be carried out for identifying the formulation and process related factors critically influencing the CQAs like, emulsion droplet size ($D_{nm}$), emulsification time ($T_{emul}$), and drug released in 15 minutes ($Q_{15\text{min}}$) employing designs like, FFD, PBD, and Taguchi design. For systematic optimization studies, experimental designs like, D-optimal and IV-optimal mixture would be employed, wherein the CMAs/CPPs will be studied and evaluated for various CQAs, viz. $Q_{15\text{min}}$, $Perm_{45\text{min}}$, $D_{nm}$, $T_{emul}$, MDT and $DE_{15\text{min}}$.

The *in vitro* dissolution studies would be preferably carried out in USP 31 Apparatus 2 (paddle type) using replacement sampling method and 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. Selection of optimum formulations would be conducted by two methods *viz.* graphical (i.e., overlay plots) and numerical (i.e., desirability function). The DoE optimization methodology was also planned to be validated by comparing the predicted values of the CQAs with their corresponding experimental values using linear correlation and residual plots.

Depending upon the initial pre-optimization studies various types of specialized SNEOFs like, solid-SNEOFs, supersaturable SNEOFs and cationic SNEOFs would be endeavored to surmount the problems associated with liquid SNEOFs. The drug release profile of the optimized formulation would be studied *vis-à-vis* the pure drug. The drug release data will be fitted into various drug release kinetic models in order to ascertain the mechanism of drug release. Rheological characterization would be planned to ascertain the flow behavior of the formulations, as viscosity is a crucial parameter which assists in determining the ability of the SNEOFs formulation to be filled in hard gelatin capsules. Subsequently, *in situ* perfusion studies of the selected optimum formulations would also be conducted *vis-à-vis* the pure drug.

Finally, it was envisaged to evaluate the *in vivo* pharmacokinetic behaviour of the optimized SNEOFs formulations *vis-à-vis* the pure drug in unisex rats after
obtaining the requisite approval from institutional Animal Ethics Committee of Panjab University. Also, the lymphatic uptake studies of the drugs from the optimized formulations via chylomicron flow block model have been planned in rats. Initially, an analytical procedure in both *in vitro* and *in vivo* conditions would be developed to estimate lopinavir and darunavir in rat plasma. The developed method would then be developed and validated for linearity, precision, accuracy, sensitivity, LOD and LOQ. Consequently, the drug plasma levels would be analyzed for various formulations, administered in rats, by reverse phase HPLC using this validated technique. Various compartmental and non-compartmental pharmacokinetic parameters were planned to be estimated using Win Nonlin or other software. It was also envisaged to establish various levels of IVIVC between the *in vitro* dissolution parameters and *in vivo* pharmacokinetic parameters. Besides, the safety and biocompatibility of the developed formulations would be carried out by histopathological studies by evaluation of the rat intestinal segments. Finally, the optimized formulations will be subjected to stability studies as per the ICH guidelines.

Since brain becomes a reservoir for HIV, the antiretroviral drugs are not effectively transported across the brain *per se*. Some patient-friendly, novel, biosafe, and easy technologies, with no risk to damage the brain tissue permanently are envisaged. Accordingly, the present investigation aimed at formulating SLNs of darunavir and NLCs of lopinavir for oral administration in order to improve its permeation across blood brain barrier into the CNS (Bondi *et al.*, 2010; Wong *et al.*, 2009), and leading eventually, to its improved therapeutic efficacy in HAND.

In addition, the influential factors grossly affecting the formulation characteristics of SLNs of darunavir and NLCs of lopinavir would be planned to be “screened” using screening experimental designs like FFD, PBD and Taguchi design. Microemulsification technique was planned to be adopted for formulating both of the systems employing response surface designs like, CCD, BBD, and optimal design.

All the formulations prepared employing the experimental design will be evaluated for particle size (crucial for entry of particles into the CNS), drug entrapment efficiency (imperative for adequate drug loading) drug release characteristics (necessary for complete release of drug) and zeta potential.
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(indispensable for preventing the particles from coalescence). Further the effect of the two influential factors on these CQAs will be envisioned using 3-D response surface plots and 2-D contour plots. Optimum search methods, like, brute-force methodology, overlay plots and desirability function will be implemented for accurately delineating the optimized formulations of darunavir-loaded SLNs and lopinavir-loaded NLCs. The DoE optimization methodology will be validated by comparing the predicted values of the response variables with their corresponding experimental values using linear correlation and residual plots. The optimized formulations will be subjected to transmission electron microscopic examination to ascertain the shape and size of SLNs and NLCs. The drug release data will be attempted for apt model fitting in order to ascertain the mechanism of drug release. In vitro cell lines (Caco 2 cell line) and macrophage toxicity and uptake studies will be carried out to ascertain the cytotoxicity and permeability of the prepared formulations.

*In vivo* pharmacokinetic studies for in suitable animals like rats will also be carried out, and various compartmental and non-compartmental pharmacokinetic parameters will be estimated using Win Nonlin or other software. The work will also encompass establishing various levels of IVIVC between the *in vitro* dissolution parameters and *in vivo* pharmacokinetic parameters. Stability studies on the optimized formulation will be conducted as per the ICH guidelines. Furthermore, it will be envisaged to compare the *in vivo* potential of SLNs to target darunavir and NLCs to target lopinavir to the CNS. Pure drugs and their respective carriers will be administered orally in rats and the subsequent brain levels of drugs will be quantified using HPLC analysis.