5. SUMMARY AND CONCLUSIONS

Acquired immunodeficiency syndrome (AIDS), an immunological disorder, accounts for around 37 million sufferers worldwide. Early after infection, the human immunodeficiency virus (HIV) is also reported to enter the central nervous system, through the blood–cerebrospinal fluid barrier and/or through the blood-brain barrier, leading eventually to the HIV-associated neurocognitive disorder (HAND).

The current work was, therefore, embarked upon developing diverse novel lipid-based nanostructured drug delivery systems (DDS) with improved therapy for AIDS by enhanced bioavailability and biodistribution.

The drug candidates explored for the purpose included protease inhibitor antiretrovirals, viz. lopinavir and darunavir, widely employed against optimized the resistant strains of HIV. Rational use of systematic Quality by Design (QbD) paradigms helped to precisely predict the “best possible” formulations of both drug molecules leading to enhanced product and process understanding and saving vital resources of time, effort and cost.

Both the protease inhibitors drugs are poorly water soluble, undergoing cytochrome-mediated extensive hepatic first-pass metabolism and p-glycoprotein efflux, resulting ultimately in the poor oral bioavailability. Their inaccessibility in the viral sanctuary sites, i.e., gut-associated lymphatic tissue and brain also adds to its inefficacy in reducing the viral loads there. To overcome these, the conventional use of these drugs is boosted with ritonavir to augment their efficacy in peripheral blood and sanctuary sites. Nevertheless, the side effects associated with ritonavir like, glucose intolerance, gastrointestinal intolerance, lipid elevations and perioral paraesthesia, call for safe and effective therapy using ritonavir-free formulations. The SNEOFs of lopinavir and darunavir, thus, were developed not only to improve the bioavailability through circumvention of extensive metabolism by the cytochrome P450.
Summary and Conclusions

(CYP450) present in gut enterocytes and liver hepatocytes, along with inhibition of P-glycoprotein (P-gp) efflux but also for ameliorating the HIV by targeting the antiretroviral drugs to the GALT sanctuary sites.

The lipidic nanocarriers, i.e., SLNs of darunavir and the NLCs of the lopinavir were developed in order to improve drug permeation across blood-brain barrier (BBB) into CNS, in order to improve therapeutic efficacy of these molecules in HAND.

The salient outcomes of the research work carried out under the present project, “Development of Optimized Lipid-based Nanostructured Drug Delivery Systems of Darunavir and Lopinavir for Improved Bioavailability and Biodistribution” are enumerated as under:

SNEOFs of lopinavir

➢ Reversed phase HPLC procedure using PDA detector, and spectrophotometric method were developed to analyze lopinavir in various in vitro and biofluid samples. The developed was also validated, exhibiting excellent linearity, accuracy, intra- and inter-day precision and robustness with low values of limit of detection (LOD) and limit of quantification (LOQ).

➢ As a prelude to systematic formulation development and optimization, quality target product profile (QTPP) of DDS was defined, and critical quality attributes (CQAs) were identified. Initial quality risk management (QRM) studies were performed through risk estimation matrix and screening studies were conducted employing fractional factorial design. The studies indicated Maisine 35-1, Tween 80 and Transcutol HP as critical material attributes (CMAs) to be the most influential factors in the formulation of SNEOFs. Studies employing FTIR ruled out any plausibility of physicochemical incompatibility between the drug and the investigated excipients.

➢ The chosen experimental design for response surface methodology, i.e., a D-optimal mixture design, mathematical model for generation of
polynomials, i.e., multiple linear regression analysis, and the methods for location of optima i.e., overlay plots and desirability function, all successfully vouched the appropriate selection of the optimized formulation, comprising of Maisine 35-1 (M 35-1): 308.75 mg, Tween 80 (T 80): 432.57 and Transcutol HP (T HP): 258.68 mg, exhibited globule size (Dnm) of 53.14 nm, percent permeated in 45 minutes (Perm45 min) of 76.08 µg.mL⁻¹, dissolution efficiency in 15 minutes (DE15 min) of 32.33%, mean dissolution time (MDT) 21.49 min, percent drug release in 15 minutes (Q15 min) of 95.36% and emulsification time (Temul) of 74 s. Validation of QbD studies distinctly demonstrated validity, accuracy and prognostic ability of the proposed model in the prediction of studied response variables.

- The OPT-L-SNEOFs were then successfully adsorbed on to the microcrystalline cellulose (Aeroperl) and finally compressed into the tablets. No significant change in the globule size and shape ratified the similarity between the L-SNEOFs and solid SNEOFs. This was subsequently followed by ratification of similarity in the dissolution profiles of both the formulations too.

- Mammalian cell lines studies in Caco-2 and macrophages reveal the biosafety and significantly enhanced uptake of the formulation vis-à-vis the pure drug.

- *In situ* single pass intestinal perfusion (SPIP) technique in Wistar rats were carried out to determine the augmentation of the permeability and absorption potential of the drug. Highly significant increase in permeability and absorption parameters construe the sagacious use of such SNEOFs for augmenting the oral bioavailability of drugs.

- Stability analysis of the OPT-SNEOFs tablets indicated minimal degradation when stored at accelerated and long term stability conditions.

- *In vivo* pharmacokinetic studies carried out in rats indicated nearly 5.1 to 9.1-fold enhancement in bioavailability of lopinavir vis-à-vis the
pure drug. The chylomicron flow blocked *in vivo* pharmacokinetic studies in rats also vouches the increased distribution of the drug to the lymph from the SNEOFs as compared to the pure drug.

- *In vitro/in vivo* correlation (IVIVC) studies between the *in vitro* drug release and *in vivo* drug absorption parameters by exploration of through level A, B,C and multiple level C correlations indicated high degree of correlation in the data.

**NLCs of lopinavir**

- The systematic formulation development exercise for NLCs was also performed employing QRM strategy and factor screening studies, which facilitated the selection of CMAs, i.e., Compritol and oleic acid as the solid and liquid lipids, and Tween 80 as the emulsifier. Optimization of the NLCs was carried out using RSM design for optimizing the levels of the CMAs.

- Evaluation of particle size and entrapment efficiency of the formulations prepared as the experimental design for RSM, i.e. Box-Behnken design, indicated that intermediate levels of lipids and surfactant yielded low particle size and high drug entrapment efficiency. Analysis of drug release profile of the optimized formulation indicated drug release behaviour being governed by nearly zero-order release mechanism.

- Studies employing FTIR and DSC ruled out any plausibility of physicochemical incompatibility between the drug and the investigated excipients.

- The formulation containing 454 mg of Compritol, 123.0 mg of oleic acid and 5.5 g of Tween 80 was selected as the optimal NLC formulation. The said formulation fulfilled all the desired criteria of low particle size, high drug entrapment, adequate drug release and high zeta potential.
Stability analysis carried out for six months indicated refrigeration (i.e. 5 ± 2 °C) as the preferred storage condition for SLNs.

*In vivo* pharmacokinetic studies carried out in rats indicated nearly 4.1 to 9.52-fold enhancement in bioavailability of lopinavir vis-à-vis the pure drug. *In vivo* pharmacokinetic studies by blocking also revealed the increased distribution of lopinavir to the lymphatic system.

*In vivo* brain targeting studies depicted nearly 2.35-fold enhancement in the brain drug levels in animals treated orally with lopianvir-loaded NLCs vis-à-vis those treated parenterally with plain lopinavir.

**SNEOFs of darunavir**

- Reversed phase HPLC using PDA detector, and spectophotometric method were developed and validated for quantitative estimation of darunavir exhibiting excellent linearity, accuracy, precision and robustness with quite low values of LOD and LOQ.

- As a prelude to systematic formulation development and optimization, QTPP was defined and CQAs were identified. QRM was performed through FMEA and screening studies were conducted employing Taguchi design. The studies indicated Lauroglycol 90, Tween 80 and Transcutol HP as CMAs to be the most influential factors in formulation of SNEOFs. Studies employing FTIR ruled out any plausibility of physicochemical incompatibility between the drug and the investigated excipients.

- The chosen experimental design for RSM, i.e., an IV-optimal mixture design, mathematical model for generation of polynomials, i.e., MLRA, and the methods for location of optima i.e., overlay plots and desirability function, all successfully vouched appropriate selection of the optimized formulation, LG 90: 318.25 mg, T 80: 407.29 and T HP: 274.46 mg, exhibited $D_{nm}$ of 50.08 nm, $Perm_{45 \text{ min}}$ of 95.99 µg.mL$^{-1}$,
DE_{15} min of 90.94%, MDT 11.66 min, Q_{15} min of 88.37% and T_{emul} of 54.50 s.

- Validation of QbD studies distinctly demonstrated the accuracy, validity and finally high predictive ability of the proposed model.
- Cell lines studies in Caco 2 and macrophages unearth the safety and enhanced uptake of the darunavir lipidic nanoformulations.
- The SPIP studies in rats demonstrated significant augmentation in permeability and absorption parameters form the formulation vis-à-vis pure drug.
- Stability analysis of the optimized drug delivery formulation indicated minimal degradation from the formulation stored for long-term and accelerated stability studies.
- *In vivo* pharmacokinetic studies carried out in rats indicated predominant (nearly 6.33 to 7.53 fold) enhancement in bioavailability of darunavir vis-à-vis the pure drug. The chylomicron flow blocked studies ratified the mechanism of the drug absorption from the SNEOFs through the lymphatic system.

**SLNs of darunavir**

- Initially, QTPP was defined and CQAs were identified. The QRM were studies performed through REM and screening studies were conducted employing FFD. The studies indicated Compritol and Tween 80 as CMAs to be the most influential factors in formulation of SLNs.
- Studies employing FTIR and DSC ruled out any possibility of physicochemical incompatibility between the drug and the investigated excipients.
- The chosen experimental design for RSM, i.e., central composite design (CCD), indicated that high levels of lipid and intermediate levels of surfactant were counterproductive for low particle size and
high drug entrapment. Analysis of drug release profile of the optimized SLN formulation indicated non-Fickian release behaviour.

- The formulation containing 480 mg of Compritol, and 5.61 g of Tween 80 was selected as the optimal SLNs. The said formulation fulfilled all the desired criteria of low particle size, high drug entrapment and drug loading, adequate drug release and high zeta potential.

- Stability analysis carried out for six months indicated refrigeration (i.e. 5 ± 2 °C) as the preferred storage condition for SLNs.

- *In vivo* pharmacokinetic studies carried out in rats indicated nearly 6.81 to 7.39-fold enhancement in bioavailability of darunavir vis-à-vis the pure drug.

- *In vivo* brain targeting studies indicated nearly 21.4-fold enhancement in brain drug levels was observed in animals treated orally with coated and plain darunavir-loaded SLNs, respectively, vis-à-vis plain drug when administered parenterally at 6 h.

In a nutshell, the present work embodied in this thesis, successfully demonstrates improved bioavailability and biodistribution of the lopinavir and darunavir, potentially leading to the improved therapeutic success of the antiretroviral therapy. Also, the work documents enhanced biodistribution of lopinavir and the darunavir to the HIV sanctuary sites, i.e., lymph and brain, thus offering a complete and holistic solution for the management of the HIV. Successful use of QbD paradigms helped not only in the systematic development and characterization of diverse lipid-based nano DDS in an effectual and resource-effectual manner, but also unraveled minutiae of their working, leading to enhanced product and process understanding. The outcome of the work can also be rationally extrapolated to other BCS class II/IV antiretroviral posing similar problem of poor and inconsistent bioavailability and/or inadequate biodistribution to CNS.