7. SUMMARY AND CONCLUSIONS

The primary objectives of the project are listed below:

1. Formulation and optimization of DZP and CUR loaded lipid based colloidal drug delivery systems viz. NE and NLC.

2. Characterization and toxicity assessment of developed nanocarriers.

3. Comparative evaluation of nanocarriers with respect to brain uptake and pharmacokinetics.

4. Pharmacodynamic evaluation of effect of nanoformulations on memory functions, AChE activity and OS markers in STZ induced model of AD.

The selection of excipients for formulation of NE and NLC was done based on partition coefficient in various solid lipids and solubility studies in oils, surfactants and co-surfactants. These preliminary investigations helped to select excipients having highest solubility for the selected drugs, an important criterion to maintain the drug in solubilized state. Further, the compatibility of drugs and lipids was established using FT-IR and DSC studies. Stability studies of the drugs were performed in SNF to ascertain that drugs are stable at nasal pH.

To optimize the components of NE formulation, pseudoternary phase diagrams were constructed using aqueous titration technique. Phase diagrams are valuable in terms of identifying the NE region based on visual observations. The physical state of NE was marked on phase diagrams consisting of 3 axis representing oil, smix and aqueous phase. The results showed that wider NE regions were observed when mixture of surfactants and mixture of co-surfactants were used instead of surfactant or co-surfactant alone. Based on results obtained from phase diagrams, further optimization of NEs was carried out using BBD. The NEs were made mucoadhesive by addition of chitosan and optimization was carried out using full-factorial design.

Preliminary studies were carried out to optimize the formulation/process variables affecting particle size and PDI of lipid nanoparticles. Based on the results obtained, further optimization of drug loaded NLCs was carried out using BBD using microemulsion technique. This method has advantage of being “solvent less” technique.

The developed nanoformulations were characterized for various physicochemical parameters, *in vitro* release and *ex vivo* diffusion. The results indicate that formulated NE/MNE were clear and transparent which is attributed to their GS being less than 100nm. The NE/MNE showed significantly faster release compared to respective drug
solutions owing to small GS and presence of surfactants whereas NLC formulations showed slower release compared to drug solutions. This was attributed to barrier properties of lipid matrix. It can be concluded from these results that NEs are more preferable than NLCs when faster release is desired and vice versa. The results of diffusion studies confirmed the advantage of MNEs compared to NEs and NLCs in terms of achieving higher flux across sheep nasal mucosa. The higher diffusion is due to mucoadhesive nature and penetration enhancing effects of chitosan. The drug solutions can be rapidly cleared from nasal cavity due to mucociliary clearance, thus mucoadhesive formulations have an advantage over simple solutions as they significantly reduce the clearance from nasal cavity. The crystalline behavior of drug loaded NLCs was studied using DSC and PXRD. The results indicate that the drugs were present in amorphous form due to complete solubilization in liquid lipid (oil) and solid lipid.

All the formulations were subjected to toxicological analysis using in vitro cell line assay, hemolytic assay and nasal histopathological studies. The results demonstrated that NE/MNE and NLC were non-toxic and safe for nasal administration. The long term stability studies of NE/MNE revealed that formulations were highly stable both under refrigeration (4°C) and room temperature (25°C) with no significant changes in the evaluated parameters. In contrast, NLC formulations were stable at 4°C but appreciable increase in PS was observed at 25°C. However, it was still in nano-range and suitable for nasal administration. The stability studies of NLCs using DSC showed that rate of polymorphic transitions was very slow as there was no significant change in the endothermic peaks of respective drug loaded formulations which is ideal since it minimizes drug expulsion during storage.

The pharmacokinetic and brain uptake studies confirmed that nanoformulations had significantly higher DTE and DTP compared to drug solutions. Among all formulations mucoadhesive nanoformulations had highest brain uptake. The following observations were made: first, addition of chitosan to NE formulations significantly improved the brain targeting efficacy. Second, the size of nanoformulations was important in terms of achieving rapid concentration in brain. NE/MNE formulations having GS<50nm had lower t_{max} compared to NLC which had PS of 115-125nm. However, there was no significant difference in NE and NLC formulations w.r.t. DTE and DTP. Though, higher drug concentration was achieved in case of NE the clearance was much faster compared to NLC. This is due to the difference in the structure of these formulations. NEs comprise of liquid droplets stabilized with surfactants which permit faster release whereas NLCs are composed of solid lipid matrix in which oil is dispersed throughout the matrix.
or localized at the shell which act as a barrier for immediate release of drug. Also, Based on the results of pharmacokinetic studies MNEs were selected for pharmacodynamic studies.

The pharmacodynamic studies of MNEs were carried out in ICV-STZ model of AD. This model mimics most of the features of human SAD and is suitable for studying cognitive deficits and screening neuroprotective molecules since it involves generation of ROS. The ICV-STZ infused animals were treated with DMNE at dose of 0.3mg/kg which is reported as parenteral dose of DZP whereas for CMNE a dose of 1mg/kg was selected based on literature reports and compared with oral treatment. The behavioral studies were carried out using RAM task and biochemical parameters were evaluated.

The results indicate that DZP was effective even at 1/10th dose compared to oral treated group when formulated as MNE. Similarly, CMNE was as effective as positive control in ameliorating STZ induced cognitive deficits and its efficacy was significantly improved compared to oral CUR. Further estimation of AChE and OS markers revealed that cholinergic deficits were reversed upon IN administration of nanoformulations with significantly lower OS compared to STZ infused groups. Histopathological studies of hippocampus and frontal cortex confirmed neuronal density was restored in groups treated with nanoformulations. Both DMNE and CMNE showed significant neuroprotective activity. The study establishes the potential role of neuroprotective molecules in treatment of AD. The level of antioxidant enzymes GSH and CAT was significantly higher in groups treated with DZP and CUR combination. Thus, study indicates potential of CUR as an adjunctive therapy in ameliorating OS. However, there was no significant improvement in cognitive deficits in combination therapy, which may be due to dose selected for pharmacodynamic studies.

Based on these results following observations can be made: first, the same pharmacological response can be achieved at much lesser dose compared to oral treatment due to improved uptake and localization of drug at the target site. Second, IN route can be an alternative treatment strategy for AChEIs, which are associated with severe GIT disturbances upon oral administration, and neuroprotective molecules like CUR whose oral absorption and clinical efficacy is limited due to poor aqueous solubility. It is evident from these results nanotechnology offers promising approach for improving pharmacokinetics of the drugs at molecular level thus the dose of drugs can be reduced without affecting its pharmacological response.
Future perspective

The release of drug over olfactory epithelium allows direct nose to brain transport whereas release over respiratory epithelium results in systemic transport. To improve the targeting to olfactory region, promising approaches that can be used in future relate to selective targeting of olfactory epithelium through conjugation of olfactory selective biorecognitive ligands (e.g. lectins). Such a strategy would reduce the reliance of the drug delivery devices to target the olfactory epithelium. Olfactory epithelium has reduced metabolic activity compared to respiratory epithelium. From direct nose to brain delivery perspective and depending on drug to be delivered, this may be either detrimental (by causing slower uptake of drug into olfactory epithelial cells) or beneficial (slower drug degradation or efflux from apical cells). The relationship between epithelial metabolic activity and drug transport would need to be investigated in greater detail to establish this opinion. Further, in depth nasal toxicity studies involving measurement of ciliary beat frequency and release of marker enzymes (LDH and proteins) can be carried out. Immunohistochemistry studies can be carried out for quantification of various oxidative stress biomarkers. Adjunctive therapy could be a useful strategy using abundant neuroprotective molecules with potential in treatment in AD.