Summary & Conclusions
5. SUMMARY AND CONCLUSIONS

Alzheimer’s disease, a rampant cognitive disorder, accounts for around 30 million sufferers worldwide. The current work embarked upon developing diverse novel drug delivery systems employing Formulation by Design (FbD) for improved therapy of Alzheimer’s. The drug candidates explored for the purpose included a potent anti-Alzheimer’s drug, rivastigmine, and a potential bioactive flavonoid with immense anti-Alzheimer’s potential, quercetin. Rational use of systematic FbD optimization methodology helped to precisely predict the “best possible” formulations of both drug molecules.

Rivastigmine, a widely prescribed cholinesterase inhibitor, suffers from some major hiccups like low bioavailability owing to high hepatic first-pass metabolism, frequent dosing and severe gastrointestinal adverse effects primarily attributed to sharp peaks and troughs in the plasma drug level profiles. Buccoadhesive films of rivastigmine, thus, were developed not only to improve its bioavailability by circumventing the hepatic portal route, but also to provide a sustained release of drug in vivo to avoid GI adverse effects due to saw-tooth pharmacokinetics. Oral route, being the most popular, patient-compliant and natural route for drug administration, CR gastroretentive tablets of rivastigmine, were also developed to avoid the cholinergic adverse effects by releasing the drug in a sustained manner at the preferred site of drug absorption.

Out of the four available FDA approved drugs; none is proven to cure the underlying cause of AD, i.e., oxidative stress in the brain. Quercetin, a potent flavonoid molecule with established anti-oxidant properties, was accordingly selected for developing an effective therapeutic alternative for AD. However, like a majority of other bioactives, this phytochemical also suffers form the limitation of restricted entry into the CNS. SLNs of quercetin, for that reason, were developed in order to facilitate the ingestion of quercetin into the CNS, and subsequently, block the ROS-mediated oxidative pathways leading to AD.
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The salient outcomes of the present research work carried out under the project, “Formulation Development and Optimization of Novel Drug Delivery Systems for the Treatment of Alzheimer's Disease” are enumerated as under:

**Buccoadhesive films of rivastigmine for sustained drug release and improved bioavailability**

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

- As a prelude to systematic formulation development and optimization, screening studies were conducted employing Plackett-Burman design. The studies indicated HPMC K15M CR and Carbopol 971P to be the most influential factors in formulation of buccoadhesive films. Studies employing DSC and FTIR ruled out any plausibility of physicochemical incompatibility between the drug and the investigated excipients. Preliminary *ex vivo* buccoadhesive studies indicated the prominent role of Carbopol in attainment of high buccoadhesion. Drug release, on the other hand, was majorly governed by HPMC content. Hence, FbD optimization employing these two factors was considered imperative for attaining the optimized buccoadhesive film formulation.

- The chosen experimental design for response surface methodology (RSM), i.e., FCCD, mathematical model for generation of polynomials, i.e., multiple linear regression analysis (MLRA), and the methods for location of optima i.e., Brute-force, overlay plots and desirability function, all successfully vouched the appropriate selection of the optimized formulation, i.e. the buccoadhesive film containing 110 mg of HPMC and 217 mg of Carbopol. The said formulation fulfilled all the desired criteria of high buccoadhesive strength, high $P_{cof}$, adequate $n$, and high $T_{ho}$ and $Q_{io}$. Validation of FbD studies distinctly demonstrated the accuracy, validity
and finally high prognostic ability of the proposed model in the prediction of studied response variables.

- Stability analysis of the optimized drug delivery formulation indicated minimal degradation from the formulation stored at refrigerated conditions (i.e., 2 - 8 °C).

- *In vivo* pharmacokinetic studies carried out in rabbits indicated nearly 1.6-fold enhancement in bioavailability of rivastigmine vis-à-vis the conventional marketed formulation. Also, the plasma drug profiles of animals administered with buccal films were far more sustained than those with OD and BD administration of the marketed formulation. No sharp peaks or troughs were observed in the profile of the optimized formulation.

Effervescent floating bioadhesive CR tablets of rivastigmine for localized sustained drug release

- Preliminary and screening studies conducted employing Taguchi design indicated that Carbopol 971P and HPMC K 15M CR exhibited excellent promise for controlled release and mucoadhesion.

- *Ex vivo* bioadhesive strength of all the formulations prepared as per FCCD, as observed with texture analyzer, increased with an increase in either polymer, the contribution of Carbopol being more prominent than HPMC. A positive interaction (synergism) between HPMC and Carbopol was apparent especially at high levels of carbomer. Buoyancy time tended to show increasing trends with increasing the levels of HPMC, while a somewhat decreasing trend was observed with increase in the levels of Carbopol. The influence of HPMC was found to be more prominent in attaining sustained drug release. All formulations prepared as per the experimental design exhibited non-Fickian drug release.

- The formulation containing 30.6 mg of Carbopol and 16.4 mg of HPMC was selected as the optimal floating-bioadhesive tablet formulation. The said
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formulation fulfilled all the desired criteria of high bioadhesive strength, and high $T_{10}$ and $Q_{20}$ and floatation time. Drug release of optimized formulations was found to be far more regulated than that of the conventional marketed formulation. Validation of FbD studies demonstrated high prognostic ability of the proposed experimental design.

- Stability analysis indicated that the optimized gastroretentive tablet was stable both at room temperature and accelerated stability conditions.

- *In vivo* gamma scintigraphic studies showed gastroretention of the optimized formulation for 6 h. The control formulation, on the other hand, disintegrated within 30 min.

- *In vivo* pharmacokinetic studies carried out in rabbits indicated that the plasma drug profile of animals administered with the optimized floating-bioadhesive tablets was far more sustained (i.e., without any sharp peaks or troughs in the profile) than once-a-day and twice-a-day administration of the marketed immediate release formulation.

Solid Lipid Nanoparticles of quercetin for improved brain delivery

- Reversed phase HPLC and UV spectrophotometric method developed for quantitative estimation of quercetin exhibited excellent linearity, accuracy, precision and robustness with low values of LOD and LOQ.

- Following selection of Compritol as the lipidic component and Tween 80 as the emulsifier during screening studies employing an fractional factorial design, analysis of particle size and drug entrapment efficiency of all formulations prepared as the experimental design for RSM, i.e. FCCD, indicated that extreme levels of lipid and surfactant were counterproductive for low particle size and high drug entrapment. Analysis of drug release profile of all formulations indicated both Fickian and non-Fickian release behaviors for SLNs.
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- The formulation containing 384 mg of Compritol and 5.76 g of Tween 80 was selected as the optimal SLN 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. 2 - 8 °C) as the preferred storage condition for SLNs.

- In vivo pharmacodynamic behavioral studies in rats, employing elevated plus maze paradigm and Morris water maze demonstrated markedly better memory enhancement in animals treated with quercetin loaded SLNs vis-à-vis those treated with pure quercetin. Biochemical estimations of rat brain homogenates indicated better regulation of MDA, glutathione and nitrite levels in animals treated with quercetin loaded SLNs vis-à-vis those treated with pure quercetin. In vivo brain targeting studies depicted nearly four-fold enhancement in the brain drug levels was observed in animals treated quercetin-loaded SLNs vis-à-vis those treated with plain quercetin.

In a nutshell, the present work embodied in this thesis successfully demonstrates improved bioavailability and sustained release potential of the optimized buccoadhesive and gastroretentive formulations, thus ameliorating the therapeutic success of the anti-Alzheimer’s therapy using rivastigmine. Also, the work documents with fruition the improved biodistribution of the antioxidant flavonoid, quercetin, across blood-brain-barrier to the nervous tissue, thus paving the way to prove its anti-Alzheimer’s potential.