Research Envisages
2. RESEARCH ENVISAGED

AD inflicts about 3 percent of world’s population between the age of 65 to 74 years, and nearly half of the population with age 85 years and older (Pietrzik & Behl 2005; Behl 2006). Rivastigmine, a widely prescribed ChEI, has been employed since 1997 for the treatment of mild to moderate Alzheimer disease and, more recently, for mild to moderate dementia associated with Parkinson disease. The efficacy of rivastigmine is dose-related, with oral daily doses ranging from 6 mg to 12 mg (b.d. or t.i.d.).

However, the drug undergoes extensive first-pass metabolism leading to its low oral bioavailability (~ 40%). Apart from this, rivastigmine is associated with severe central cholinergic GI side effects (Inglis 2002; Jhee et al. 2002). A rapid increase in brain acetylcholine levels after effective inhibition of target enzymes is believed to cause these side effects (Darvesh et al. 2003; Grossberg 2003). The side effects of rivastigmine are most likely related to high $C_{\text{max}}$, short $t_{\text{max}}$, and large fluctuations in plasma levels (Jann et al. 2002). Thus, with the rivastigmine, strategies that prolong $t_{\text{max}}$ and reduce the fluctuations of plasma drug concentration have been shown to reduce the incidence of GI adverse events of rivastigmine. Besides, the twice- or thrice-a-day dosage regimen associated with rivastigmine also reduces its patient compliance.

Thus, it was envisaged to formulate buccal-adhesive films of rivastigmine which, besides providing a sustained release of the drug, would circumnavigate the hepatic first-pass. Also, as oral route, being the natural route of administration, is the most sought-after route, it was envisaged to formulate once-a-day CR tablets of rivastigmine to reduce fluctuations in plasma drug concentration. However, the conventional CR formulations are dislodged from stomach and intestine within a short span of time. On being dislodged from these two sites of maximal drug absorption, the absorption of drug becomes erratic and even more susceptible to hepatic first pass, as only a small amount of drug is presented to this saturable system. GI therapeutic systems are the formulations of choice in such conditions, which besides localizing the drug in stomach and intestine, provide a sustained release of drug, thus reducing the fluctuations in plasma drug levels largely.

To formulate buccoadhesive films of rivastigmine, the influential factors grossly
Research Envisaged

affecting the formulation characteristics of films were planned to be “screened” using a screening design. A $3^2$ CCD was planned to be employed for formulation of buccoadhesive films. Further, it was planned to design an in-house assembly in order to obtain thin films of uniform thickness.

Since the last two decades, systematic FbD optimization of various DDS using experimental designs has become a routine practice across the world both in industrial and academic milieu. These techniques aid not only in choosing “the best” formulation under the given set of restrictions using lesser experimentation, but also in saving a great deal of developmental time, effort and cost. The previous experimental studies carried out in our laboratories on the hydrophilic matrices of diclofenac sodium (Singh & Gupta 1997), verapamil hydrochloride (Singh et al. 2006c), lipid matrices of captopril (Singh et al. 1998), microcapsules of diltiazem hydrochloride (Singh & Agarwal 2002), transdermal hydrogels of tenoxicam (Singh et al. 2010b), vesicular drug delivery systems of nimesulide (Singh et al. 2005d) and finasteride (Kumar et al. 2007b), oral fast release supersaturated systems and inclusion complexes of flurbiprofen (Singh et al. 2005b), nimesulide (Ahuja et al. 2008) and etodolac (Singh et al. 2007), self nano-emulsifying oral DDS of carvedilol (Singh et al. 2011b) and lovastatin (Singh et al. 2008e), mucoadhesive tablets of atenolol (Singh et al. 2006b), buccoadhesive dosage forms of diltiazem hydrochloride (Singh & Ahuja 2002), hydrodynamically balanced bioadhesive tablets of tramadol hydrochloride (Singh et al. 2010f), trimetazidine (Singh et al. 2008c), lamivudine (Singh et al. 2011c) and hydralazine hydrochloride (Singh et al. 2009a), oral gastroretentive in situ gelling DDS of acyclovir (Singh et al. 2010d) and nasal mucoadhesive microspheres of lercanidipine (Singh et al. 2010c) have all construed extremely precise prognosis of these systematically “optimized” formulations.

All the formulations prepared employing the experimental design were planned to be evaluated for their drug release characteristics (imperative for sustained, extended and complete drug release), buccal permeation characteristics (crucial for permeation of drug through buccal mucosa) and buccoadhesive potential (necessary for localization of the film in buccal cavity). Further, the effect of the two influential factors on these response variables was envisioned to be evaluated using 3-D response surface plots and 2-D contour plots. Three optimum search
Research Envisaged

methods (Brute-force methodology, overlay plots and desirability function) were planned to be implemented for correctly selecting the optimized formulation. The FbD optimization methodology was also planned to be validated by comparing the predicted values of the response variables with their corresponding experimental values using linear correlation and residual plots. The optimized formulation was planned to be subjected to stability studies as per the ICH guidelines. The drug release of profile the optimized formulation was planned to be studied vis-à-vis the marketed formulation to compare its sustained release potential. The drug release data was planned to be fitted into various drug release kinetic models in order to ascertain the mechanism of drug release. Finally, it was envisaged to evaluate the \textit{in vivo} pharmacokinetics of the optimized buccoadhesive film in rabbits. Various compartmental and non-compartmental pharmacokinetic parameters were planned to be estimated using Win Nonlin software. It was also envisaged to establish various levels of \textit{in vitro}/\textit{in vivo} correlations (IVIVC) between the \textit{in vitro} dissolution parameters and \textit{in vivo} pharmacokinetic parameters.

As with buccoadhesive films, the influential factors grossly affecting the formulation characteristics of gastroretentive tablets of rivastigmine were planned to be “screened” using a screening design. A $3^2$ CCD was planned to be employed for formulation of buccoadhesive films. All the formulations prepared employing the experimental design were planned to be evaluated for their drug release characteristics (imperative for sustained, extended and complete drug release), and floatation and bioadhesive characteristics (necessary for localization of the formulation in GI milieu). Further, as with the previous formulation, the effect of the two influential factors on these response variables was envisioned to be evaluated using 3-D response surface plots and 2-D contour plots. Three optimum search methods (Brute-force methodology, overlay plots and desirability function) were planned to be implemented for correctly selecting the optimized formulation. The FbD optimization methodology was also planned to be validated as in the case buccal films. The optimized formulation was planned to be subjected to stability studies as per the ICH guidelines. The drug release of profile the optimized formulation was planned to be studied vis-à-vis the marketed formulation to compare its sustained release potential. The drug release data was planned to be fitted into various drug release kinetic models in order to ascertain
the mechanism of drug release. Finally, it was envisaged to evaluate the in vivo pharmacokinetics of the optimized tablet formulation, as in the case of buccal films.

Despite the medical need for an effective therapeutic treatment of AD, the pace of progress towards this goal has been painstakingly slow. Current therapies for AD such as the cholinesterase inhibitors and NMDA receptor antagonists, provide moderate symptomatic delay of the disease without arresting the disease progression. Accordingly, newer approaches for the disease management are the acute need of hour.

Flavonoids, a class of secondary plant metabolites, have recently gained wide attention because of their antioxidant, anti-inflammatory, antiplatelet and other beneficial properties (Dhawan et al. 2003; Dhawan et al. 2004; Dhawan & Dhawan 2006; Lee et al. 2012). Quercetin, a natural flavonoid molecule, has a long history of consumption as a part of human diet, such as fruits, vegetables, wine and tea. It has been postulated to act as a novel neuroprotectant by mitigating the increased levels of reactive oxygen species produced by normal mitochondrial activity, which accelerate the neurodegenerative processes of AD (Behl & Moosmann 2002; Huber et al. 2006). In this regard, quercetin has been documented to be more potent antioxidant and radical scavenger than vitamin C, vitamin E and β-carotene (Rice-Evans et al. 1995). Besides, this flavonoid has been shown to improve spatial learning and memory in D-galactose-treated aging in mice (Hu et al. 2008). These valuable effects of quercetin, however, get thwarted because of its limited penetration into the CNS. Accordingly, the present investigation aimed at formulating SLNs of quercetin for intravenous administration in order to improve its permeation across blood brain barrier into the CNS (Wong et al. 2009; Bondi et al. 2010), and eventually, to its improved therapeutic efficacy in AD. The potential of SLNs in targeting both hydrophilic and hydrophobic molecules to the CNS has been widely reported.

The influential factors grossly affecting the formulation characteristics of SLNs were planned to be "screened" using a screening design. Microemulsification technique was planned to be adopted for formulating SLNs using different ratios of lipid and surfactant as dictated by the experimental design, a CCD for two factors at three levels.
All the formulations prepared employing the experimental design were planned to be evaluated for their 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 (indispensable for preventing the particles from coalescence). Further the effect of the two influential factors on these response variables was envisioned to be evaluated using 3-D response surface plots and 2-D contour plots. Three optimum search methods (Brute-force methodology, overlay plots and desirability function) were planned to be implemented for correctly selecting the optimized formulation. The DoE optimization methodology was also planned to be validated by comparing the predicted values of the response variables with their corresponding experimental values using linear correlation and residual plots. The optimized formulation was planned to be subjected to electron microscopic examination to ascertain the shape and size of SLNs. The drug release data was planned to be fitted into various drug release kinetic models in order to ascertain the mechanism of drug release. Stability studies on the optimized formulation were planned to be conducted as per the ICH guidelines. Furthermore, it was envisaged to compare the in vivo potential of SLNs to target quercetin to the CNS. Pure quercetin and the flavonoid entrapped in the SLNs were planned to be injected in rats and the subsequent brain levels of quercetin were planned to be quantified using HPLC analysis. Conclusively, the in vivo pharmacodynamic evaluation of quercetin loaded SLNs vis-à-vis pure flavonoid was envisaged to be conducted using an animal model of Alzheimer’s dementia. The memory-enhancement potential of quercetin loaded SLNs was planned to be evaluated in elevated plus maze and Morris water maze models. Also, biochemical investigations (i.e., malondialdehyde, glutathione and nitrite levels) were planned to be conducted to ascertain the anti-oxidant potential of quercetin transported into the CNS by SLNs.