Abstract

The treatment of neurological disorders associated with the central nervous system alignment has been a noteworthy challenge over the decades. The effectiveness of a drug delivery system is majorly dependent on the ability to deliver the therapeutic agent at specific site of action with minimal adverse effects. The major problem associated with the brain delivery is the hindrance of the biological barriers. Blood brain barrier (BBB) is one of the important barriers which not only hinders the entry of therapeutic agents but also protects the brain from the entry of foreign bodies. Various biomaterial-based novel approaches have been explored by the investigators for the transport of drugs to the brain including, polymeric nanoparticles, liposomes, polymer-lipid hybrid nanoparticles, nanoemulsions, solid lipid nanoparticles etc. Donepezil (DNP) is an acetyl cholinesterase inhibitor approved by the USFDA for the symptomatic treatment of Alzheimer's disease. Higher doses of DNP need to be administered to exhibit its pharmacological action as it undergoes degradation and unable to reach sufficient dose to the brain. Moreover, it also exhibits several adverse effects due to administration of higher doses. The objective of the current research work was to design and characterize the polymeric nanoparticulate drug delivery system for DNP with preferential enhancement in brain availability and reduced adverse effects.

Analytical methods play a key role for the successful development and characterization of any sort of drug delivery system. Thus, we have developed and validated an in-house RP-HPLC analytical and bio-analytical methods which are suitable to carryout routine work. The developed analytical method was applied for the quantification of DNP to conduct routine analysis like preformulation, formulation characterization and cellular uptake studies. Further, the developed bio-analytical method was found to be selective and specific for the detection of DNP in rat plasma and various tissues. This method was successfully applied for the pharmacokinetic and biodistribution studies in rats.

Preformulation studies revealed that, DNP exhibited a pH dependent solubility in various buffers with high solubility in acidic pH buffers and low solubility in alkaline pH buffers. This kind of phenomenon exhibited because DNP in solution form exists as a free base at higher pH (alkaline conditions). Solution and solid state solubility studies demonstrated that, DNP was found to be more stable in acidic environment and exhibited moisture mediated degradation. The results obtained from the drug-excipient compatibility studies by DSC and ATR-FTIR revealed that, there was no significant interaction with the selected excipients.

Amphiphilic di-block co-polymer(mPEG-PCL) was successfully synthesized by using ring opening polymerization reaction and was thoroughly characterized by NMR, GPC, ATR-FTIR and DSC. DNP loaded nanoparticles were successfully developed using nanoprecipitation method. The preparation procedure was simple, reproducible and able to reproduce nanoparticles with narrow size distribution range, better percent entrapment efficiency and percent drug loading. The role of various process parameters like drug:polymer ratio (w/w) and solvent:non-solvent ratio (v/v) on response factors like particle size, polydispersity index and percent drug loading were studies in detail by using design of experiments. These formulations showed high redispersibility after lyophilization process and the organic content was determined using GC-HS. Further, polymeric nanoparticles were surface modified with polysorbate 80 and ApoE3 to enhance the brain uptake. The data obtained from SEM and TEM analysis revealed that the particles were isometric in shape and with regular surface. Extended release of these nano-formulations has been observed in *in vitro* studies. The *in vitro* data in simulated gastric fluids suggested that polysorbate 80 coating was found to be stable in ApoE3 coated nanoparticles and the concentration of polysorbate 80 remaining on the surface was in agreement with the initial coating amount. Moreover, it has been observed that, ApoE3 conjugated polymeric nanoparticles (ApoE3-NPs) efficiently interferes at all steps of Aβ aggregation kinetics *in vitro*, which was confirmed by thioflavin t assay. All the formulations were found to be non-cytotoxic and biocompatible in neuroblastoma and liver cell lines and ApoE3 conjugated polymeric nanoparticles were able to attenuate $A\beta_{1-42}$ induced cytotoxicity in SHSY-5Y cells.

Pharmacokinetic and biodistribution studies in rat represented that as compared to pure drug, polymeric nanoparticulate formulations exhibited higher AUC values along with prolonged residence time of DNP in the rat blood circulation. Further, the distribution of DNP to the brain was significantly improved with increased mean residence times for surface modified nanoparticulate formulations as compared to pure drug alone. DNP has been reported for excess accumulation at liver, ApoE3-NPs showed significant decrease in the liver deposition might be because of bypassing of liver and targeting moieties attached over the surface of nanoparticles. On the other hand, significantly reduced concentrations were noticed in the other undesired organs also. The therapeutic efficacy of the developed formulation has been assessed in ICV- $A\beta_{1-42}$ induced cognitive rats. From the pharmacodynamic data it is also evident that these nanoparticles have significantly attenuated the behavior and cognitive function in rats.