Chapter 6

Conclusion

CONCLUSION

Phenytoin is an excellent anticonvulsant and because of its putative mechanisms of action, it masters three different conditions like Grandmal, Partial & status epileptics. (Aaron et al., 1999). However, it is also a good candidate of P-glycoprotein (P-gp), Mrp1, Mrp2, and BCRP (Van vliet et al., 2005, Van vliet et al., 2006, Prasad et al., 2011).

Now, it is obvious that unless the molecules do not imitate the structure of substrate of the endogenous receptor expressed at BBB, it will not be transported into the brain. Otherwise it should not be recognized by or should not be the substrate of brain-to-blood efflux transport system (Ohtsuki, S. and Terasaki, 2007).

Folic acid is a notable endogenous substrate. Further, its receptor has wildly been expressed in the cells of brain and CP. Folate is relatively easy to ligate to any therapeutics, and retains its ability to bind to its receptor with normal affinity when attached via its γ-carboxylate, and thereby enter receptor-bearing cell by endocytosis.

In this study, FA was attached to gelatin through amide bond, between γ -carboxyl group of FA and primary amino groups of gelatin, using EDC chemistry. Gelatin-folate was precipitated using acetone. It was characterized by using modern analytical techniques such as IR, NMR (1H NMR, 13C NMR COSY and HSQC) for chemical characterization and DSC and XRD for solid state characterization and SEM for morphological characterization.

Gelatin nanoparticles were formulated by two step desolvation method and the formulated nanoparticles were optimized for formulation variables such as percentage of

polymer, degree of cross-linking and duration of crosslinking. Further, nanoparticles were studied for particle size, PDI, zeta potential, total drug content and drug loading (%DL), *in vitro* release and drug release mechanism. Formulation GT3 showed better loading efficiency and release profile. According to the composition of GT3, gelatin-folate Nanoparticles (GF1) were formulated and the above characterizations were done. Further, nanoparticles were studied by DSC and XRD for solid state characterization and SEM for morphological characterization.

Residual solvents present in synthesized Gelatin folate and nanoparticles formulation GT3 and GF1 were recorded on Gas chromatography head space using flame-ionization detector. All the tested compounds were free from residual solvents.

Pharmacodynamics (*in vivo)* was studied through Maximal electroshock induced seizure model (corneal electroshock method) in rat using simple phenytoin solution (DPH Soln), its gelatin nanoparticles (DPH-NP), and gelatin-folate nanoparticles (DPH-NP-FA). All the formulations showed significant decrease in duration of hind limb extension as compared to vehicle treated group. DPH-NP-FA significantly potentiated the anticonvulsant action of phenytoin by reducing its ED50 value from 12 mg/kg to 7 mg/kg against Maximal electroshock induced seizure.

Pharmacokinetic profiles were studied by administration of single dose of three different phenytoin formulation viz. DPH Soln, DPH-NP, and DPH-NP-FA, equivalent to 30mg/kg of phenytoin per oral in rats and comparing their plasma pharmacokinetics to brain neuropharmacokinetics. The brain Cmax and AUC values of phenytoin were substantially lower to those in plasma and the brain/plasma AUC ratio was 0.1. After

DPH-NP-FA administration, the brain Cmax and AUC were increased from 1.2 ±0.33 to 4.1±0.26 μmol/l and 3.5 ±0.12 to 14.5±0.2 h*μmol/l, respectively and the brain/plasma ratio was improved from 0.1 to 0.4, whereas the ratio was 0.1 and 0.16 after DPH soln, DPH-NP administration, respectively. The order of the ratio suggests that equilibrium between the blood and brain compartments was not observed for all the 3 formulations at the evaluated dose.

In this study, folic acid was covalently attached to gelatin, and nanoparticles of gelatin-folate using DPH were prepared. The formulated nanoparticles were studied for drug loading (%DL), *in vitro* release and drug release mechanism along with other physiochemical properties. Pharmacodynamic and Pharmacokinetic profiles of formulated nanoparticles were studied. From the above study, it can be concluded that gelatin-folate nanoparticles showed better pharmacokinetics profile in both compartment. Nanoparticles of Gelatin folate could be a promising carrier for CNS targeting.