CHAPTER 5

SUMMARY

&

FUTURE PERSPECTIVES

5.1. Summary

The thesis demonstrates the significance of developing a pharmaceutically acceptable parenteral nanoformulation of diclofenac sodium to overcome the current limitations of the drug. The polymer selected for the synthesis of nanocarrier to entrap diclofenac sodium was hydroxyethyl starch (HES), which is an FDA approved polymer for injectables. This approach helped to address the limitations of the drug in terms of its solubility, stability, bioavailability, pharmacokinetics and toxicity. Entrapment of diclofenac sodium at its clinical dose in hydroxyethyl starch nanocarrier resulted in significant improvement in the bioavailability of the drug with minimal toxicity. Following are the highlights of the work presented in this thesis:

- ✓ HES nanoparticles were prepared through a facile crosslinking precipitation route with a hydrodynamic size of 160±15 nm and a polydispersity index (PDI) of 0.162±0.025. AFM and SEM analysis showed spherical particles of average size 150 nm, which correlated well with its hydrodynamic diameter. The surface charge of HES nanoparticles deciphered from zeta potential analysis was found to be -41±2.6 mV, which imparted good colloidal stability to the nanoformulation. No significant aggreggation was noticed for particles tested in different media, implying their stability.

- ✓ Diclofenac sodium at clinical dose (100mg) was incorporated into the HES matrix via precipitation, yielding stable diclofenac sodium loaded hydroxyethyl starch nanoparticles (Nanodiclo) of size ~170nm and entrapment efficiency ~72%. Influence of processing parameters on size and entrapment efficiency of the nanoformulation was evaluated. Nanodiclo exhibited a sustained release pattern with negligible burst, and the kinetics following Higuchi model and mechanism, non-fickian diffusion. Scaleup ability of the nanoformulation was tested by preparing 5 ampoules of drug loaded HES nanoparticles, without any significant change in particle size and entrapment efficiency.

- ✓ Nanodiclo was subjected to a detailed cyto/hemocompatibility evaluation *in vitro* since this was proposed for intravenous delivery. Results demonstrated excellent biocompatibility of the developed HES nanoformulation for parenteral use. Findings were reaffirmed by the lack of any *in vitro* or *in vivo* inflammatory potential induced by HES nanoparticles. Nitric oxide assay proved enhanced *in vitro* efficacy for the nanoformulation over the free drug owing to the controlled drug releasing property of the developed nanocarrier.

- ✓ Acute toxicity analysis of Nanodiclo was carried out for three different dosage groups, i.e., 2.5, 25 and 50 mg/kg b.w., all in comparison with bare diclofenac sodium as per OECD guidelines. Neither at gross level, nor at cellular level was there any significant behavioural, body weight, haematological or biochemical changes observed for Nanodiclo. The study suggested that the drug induced hepatic and renal toxicity can be alleviated upon its entrapment within nanoparticles, which was also confirmed by histopathological analysis.

- ✓ A detailed pharmacokinetic study of bare and nano forms of diclofenac sodium in comparison to a marketed formulation, voveran, was carried out at two different dosages, 2.5 mg/kg b.w. and 15.4 mg/kg b.w. The influence of PEGylation and unreacted excipients on the pharmacokinetics of nanodiclo was studied. It was observed that administration of excipient removed PEGylated nanodiclo at lower dosage (2.5 mg/kg b.w.) exhibited better pharmacokinetics with significant improvement in AUC and MRT. In a therapeutic view point, the study indicates a likelihood of reducing the current dosage of diclofenac sodium with the help of nanocarrier mediated drug delivery.

- ✓ Biodistribution study was carried out with bare DS and excipient removed PEGylated nanodiclo as test samples at 2.5 and 15.4 mg/kg b.w. doses in order to comprehend the discrepancy observed in the pharmacokinetics results. A higher accumulation of drug was observed in the tissues when the higher dose of 15.4 mg/kg b.w. was injected, which may be the reason

for its lesser plasma concentration, implying that increasing the drug concentration alone will not help in improving the plasma concentration.

- ✓ Finally, the pharmacodynamic study was conducted in carrageenan induced rat paw edema (acute inflammation) model. The result showed significant reduction in paw edema for the lower dosage, i.e., 2.5 mg/kg b.w. than the higher dosage, 15.4 mg/kg b.w. Lesser efficacy despite higher concentration may be due to the higher tissue accumulation that correlates with the results of biodistribution. On the whole, pharmacodynamic study substantiated the findings of pharmacokinetics and point towards the possibility of lowering the current clinical dosages by exploiting the platform of nanotechnology.

5.2. Future Perspectives

With the successful culmination of the thesis work, a proof of concept regarding the use of nanoformulations to improve the kinetics and dynamics of the drug as well as alleviation of its toxicity, thereby improving the quality of life has been laid out. However, there are certain limitations that need to be addressed before the formulation is considered for a clinical translation. The following section lists the limitations of the study and suggestions to improve it.

- ➢ The formulation exhibits low drug loading efficiency despite its good entrapment efficiency. Considering the nature of the polymer and drug selected for the study, the possible option to improve drug loading would be to prepare polymer-drug conjugates or modify the polymer to better accommodate the drug. However, the performance of the newly developed system would entail a detailed pharmacokinetic/dynamic study to decipher information about its efficacy.

- ➢ Though, a detailed *in vitro* evaluation was carried out to study the interaction of nanoformulation with cell and blood interactions, a comprehensive molecular investigation on the downstream signalling pathways pertaining to inflammation is lacking in our study. Hence the

future work might focus on the different signalling pathways to assess the inflammatory potential of nanodiclo.

- Pharmacodynamic study in our thesis lacks molecular evidence for the therapeutic effect elicited by nanodiclo. The analysis of the inflammatory markers such as C-reactive protein could be included to validate the results. Pharmacodynamic study needs to be demonstrated on chronic inflammation model also to prove the efficacy of the nanoformulation as well as to establish the efficacy at lower dosage.