Chapter 4

CONCLUSION AND FUTURE PERSPECTIVES

Transdermal delivery is always considered as an alternative to oral route but effective permeation is to be made possible to cross the skin barrier. Majority of the transdermal drug delivery systems which are approved by United States FDA are for controlled systemic delivery of drugs. But this route can be used for targeted delivery of drugs to various disease sites on the different layers of skin especially for skin cancers. The most serious form of skin cancer, the melanoma affects the melanocytes located at the lower part of epidermis. Design of carriers with proper size and surface property may prove effective to cross the stratum corneum barrier and get localized at these sites. Based on this assumption, we developed nanogels using the biodegradable cationically charged chitin.

- The pH responsive and biodegradable chitin nanogels (CNGs) was prepared by regeneration chemistry by optimizing the probing parameters. Two anticancer drugs, the lipophilic curcumin and hydrophilic 5- Fluorourcil were effectively loaded with chitin nanogels resulting in curucimin loaded chitin nanogels (CCNGs) and 5- Fluorouracil loaded chitin nanogels (FCNGs) respectively.

- The chitin nanogels as well as the two drug loaded chitin nanogels were characterized by DLS, SEM, FTIR and TG/DTA which confirmed the nanosized spherical particles with intermolecular hydrogen bonding and the drug loaded nanogels were thermally more stable compared to chitin nanogels.

- The swelling and degradation studies of the chitin nanogels indicated the faster biodegradation of the nanogels compared to control chitin and CNGS, CCNGS as well as FCNGs showed increased swelling at acidic pH ie. at pH below the pKa value of chitin (6.1).

- The in vitro drug release was found to be higher at acidic pH (pH 4.5) due to the protonation of free amine groups in the nanogel.
The blood compatibility studies showed that all the nanogels are free from hemolytic as well as thrombogenic property. The PT-APTT test results indicate that the blood coagulation time for all the nanogels lies within the normal range.

The *in vitro* cell uptake studies carried out by fluorescent microscopy indicated good uptake of CNGs, CCNGs and FCNGs by A375 as well as HDF cells. DAPI/actin staining showed cellular localization of Rhod-CNGs with no signs of change in morphology in either of these cells. The same with Rhod-CCNGs and Rhod-FCNGs were also studied. Both the cells showed cellular localization and in case of Rhod-CCNGs an obvious change in morphology was visible in case of A375 but not in HDF indicating the specificity towards cancer cells. Confocal images were taken to confirm the cellular internalization of Rhod-CCNGs and Rhod-FCNGs. In case of Rhod-CCNGs there was significant difference in fluorescent intensity as we go deeper into the cells and deeper internalization of Rhod-CCNGs was shown in A375 compared to HDF whereas such a difference in internalization was not found for Rhod-FCNGs.

The cytotoxicity studies of CNGs on various normal cell lines was done by MTT assay which proved the cytocompatibility of the prepared chitin nanogels. The cytotoxicity studies of CCNGs as well as FCNGs were carried out on HDF and A375 cells in which the CCNGs showed specific toxicity on A375 whereas the FCNGs were devoid of that specificity.

The cytotoxicity results were further confirmed by apoptosis assay by flow cytometry. The chitin nanogels loaded curcumin showed comparable toxicity as that of control curcumin on A375 cells whereas in case of HDF, the toxicity was found to be reduced in case of CCNGs as compared to control drug, due to the antioxidant effect of chitin. The FCNGs showed very less apoptosis on A375 cells and again the toxicity on HDF was found to be reduced in case of the nanogel loaded drug.

The *Ex vivo* skin permeation studies of CNGs as well as Rhod-CNGs was carried out using pig skin in a vertical Franz diffusion cell and the FTIR analysis of the acceptor fluid clearly showed the presence of CNGs. The acceptor fluid collected in case of
Conclusion and future perspectives

Rhod-CNGs, showed good fluorescence upon UV exposure confirming the penetration of the same through the skin.

- The *Ex vivo* skin permeation studies of CCNGs, FCNGs as well as Rhod- FCNGs were done in the same way and the steady state flux as well as permeability constant were calculated. The CCNGs was found to have 4 fold increase in transdermal flux compared to control curcumin whereas in case of FCNGs there was not much increase in permeation as that of control 5-FU. The fluorescent images of the skin at different depth and the concentration depth profile indicated a 4-5 times increased retention of the drug at the deeper layers of the skin in both the cases.

- The histopathological evaluation of the skin from the permeation studies of CNGs, CCNGS and FCNGS showed prominent loosening in the stratum corneum layer responsible for enhanced permeation without indications of edema or erythema.
Conclusion and future perspectives

Chapter 4

Future perspectives

Most of the enhancement techniques involved in transdermal delivery have limitations with regard to skin toxicity. The chitin nanogels prepared from the biocompatible chitin possessing nanosize and cationic charge as well as enhanced biodegradation properties has proved to be an ideal candidate for transdermal delivery of drugs. The *ex vivo* experimental data need to be further confirmed by *in vivo* studies for proving the enhanced permeation as well retention effects.

The study can further be extended for *in vivo* anticancer effect in melanoma model. Eventhough melanoma is the most serious type of skin cancer; the more prevalent types are basal cell carcinoma and squamous cell carcinoma. A transdermal formulation for the treatment of these less serious but more common cancers would be a novel approach. The limitation here is the nonavailability of corresponding cell lines.

Eventhough chitin nanogels is thought to have passive targeting to melanocytes because of size and surface properties, an active targeting method *via* the transdermal route would be more effective. Antibodies specific for receptors like VEGF or MC1R which are overexpressed in skin cancers can be conjugated to the drug loaded chitin nanogles for this. A study in this area is highly essential.

Further it is well known that curcumin is effective against melanoma and 5-FU is being used topically as a cream in the other two types of skin cancers. So the chitin nanogel loaded with both these drugs can be a prophylactic strategy in countries where people are at risk of skin cancers due to UV exposure.