Chapter – XI

Summary
11. SUMMARY

- This thesis was aimed at developing a novel ocular niosomal in-situ gel of Dorzolamide Hydrochloride for reduction in intra ocular pressure.

- Ophthalmic drug delivery is one of the most interesting and challenging endeavours faced by pharmaceutical scientists. The current available eye drops for glaucoma treatment has conventional disadvantages like poor bioavailability, lachrymation, frequent instillation, tear turn over and poor patient compliance and hence there is a need to develop a novel dosage form that could break through the barriers of conventional dosage forms.

- Literature review revealed that no work has been explored on Dorzolamide Hydrochloride niosomal in-situ gel. Hence an attempt has been made to develop a combination of two delivery systems with niosomes and in-situ gel as a novel niosomal in-situ gel drug delivery system to reduce the intra ocular pressure in order to overcome the problem associated with the conventional therapy.

- Solubility profile of Dorzolamide HCl was studied as per USP monograph and the drug was found to be freely soluble in methanol and ethanol. However solubility in water was found to be 0.699 mg/ml.

- UV spectral analysis of Dorzolamide HCl at various concentrations (2-12 µg/ml) in phosphate buffer pH7.4 showed linearity results. Hence it obeyed Beer’s Lambert law.
Drug and excipients interactions were studied by Fourier transform infra red (FT-IR) spectroscopy. The presence of functional groups was confirmed by the various stretching, bending and rocking vibrations based on the group present. Hence it was revealed from the studies there were no specific interactions found.

The physical state of drug in the formulation was examined through Differential scanning calorimetry (DSC) studies. Endothermic peak of pure Dorzolamide HCl was observed which was very close to its melting point. Hence the thermogram studies revealed physical nature of drug was found to be stable. Powder X-ray Diffraction (PXRD) analysis revealed crystalline nature of drug.

Niosomes of Dorzolamide HCl were prepared using span series (20,40,60,80) surfactants by thin film hydration method. Sixteen trial formulations were made by keeping drug and cholesterol as fixed ratio and varying the concentration of surfactant alone. The developed niosomal formulations were characterized for optical microscopy, scanning electron microscopy (SEM) studies, transmission electron microscopy (TEM) studies, zeta potential, entrapment Efficiency, drug content analysis and in vitro drug release studies.

Based on the entrapment efficiency and in vitro release studies, optimised formulation (F9) containing (Cholesterol/ Span 60 C/S 1:1) ratio was characterized for further studies.

Optical microscopic images of optimised formulation (F9) showed most of the vesicles were spherical in shape. SEM images of optimized niosomal
formulation (F9) revealed that vesicles were discrete, round and uniform. Smaller unilamellar vesicle of about 500 nm was observed. TEM studies revealed the internal morphology of niosomal vesicles and also it gives the structure and size of niosome vesicles. Optimized formulation (F9) was found to be spherical in shape, uniform and discrete and it was found to be in the range of 50-100 nm. Zeta potential measurement indicated long term stability of niosomes. It was found to be negative for the optimized formulation (F9) (i.e.) -50.4mv and size distributed by zeta sizer was found to be 568.8 nm. Hence these values suggested sufficient kinetic stability of niosomes.

- Entrapment Efficiency of niosomal formulations were measured by centrifugation method. Among all the formulation, F9 (Cholesterol/ Span 60 C/S 1:1) showed maximum entrapment efficiency compared with other formulations due to its low HLB value and high transition temperature. Drug content of niosomes was studied by lysing the vesicles and the drug content for all the developed formulation was found to be in the range of 96% - 98%

- In vitro drug release study of all niosomal formulation was carried out by diffusion method. The rate of drug release depends on the percentage of drug entrapment efficiency. Among all the developed formulation, optimized formulation F9 containing cholesterol and span 60 (C/S 1:1) showed sustained drug release of 50.16% in 12 hours than other span series. The optimized formulation F 9 was further developed into in-situ gelling system by utilizing phase transition property of HPMC K 15M (low & high viscosity) and Carbopol 940 polymer.
Summary

- Developed *in-situ* gel formulations were characterized for pH, visual appearance, drug content, gelation studies, viscosity, osmotic shock, kinetic study, sterility test, *In vitro* HETCAM test, ex vivo corneal study and stability studies.

- pH was found to be in the range of 6.22- 6.26. Visual appearance was found to be milky white dispersion. Drug content was found to be 99.82- 99.82 %. Gelation studies exhibited immediate and stiff gelation for NG4. The viscosity of all developed niosomal *in-situ* gel formulation was found to be satisfactory. Osmotic shock was determined by subjecting to various tonicity conditions and it revealed that no significance difference in vesicle diameter. *In vitro* release profile of niosomal *in-situ* gel formulations (NG4) revealed 68.72% drug release pattern in 24 hours study. The Kinetic studies revealed that optimized formulation NG4 obeyed first order release kinetics with $R^2=0.9965$ and followed non fickian release mechanism.

- Based on the gelling studies and *in vitro* drug release of niosomal *in-situ* gel, the optimised formulation (NG4) was selected and subjected for sterility test, stability studies, *in vitro* HET CAM test, *Ex vivo* corneal permeation studies and *in vivo* studies.

- Sterility test was performed for the optimized formulation (NG4). It was found to be sterile. *In vitro* HETCAM studies were done to assess the irritation potential of the developed formulation. It was found that sum of numerical scores for various irritant effects like hyperaemia, haemorrhage, clarity was found to be zero. *Ex vivo* trans corneal permeation study proved the optimized formulation (NG4) showed sustained release profile when compared with
marketed drops and niosomal formulation. Optimised niosomal *in-situ* gel (NG4) formulation were subjected to short term accelerated stability and refrigerator conditions. The results revealed that niosomal *in-situ* gel preparation retained good stability throughout study period when stored at refrigerator temperature.

An ocular irritation study was performed for the optimised formulation (NG4) by using male New Zealand white rabbits. The results revealed that developed niosomal *in-situ* gel exhibited excellent ocular tolerance and no abnormal clinical signs were noticed in coronary iris. Histopathology studies also revealed that there were no signs of abnormalities observed.

Aqueous Humour analysis was performed to determine the concentration of drug in the eye. The $C_{\text{max}}$ of Dorzolamide HCl was found to be 8.424 µg/ml, AUC-355.71 µg/ml.min, $t_{\text{max}}$ 240 minutes for developed Niosomal *in-situ* gel formulation whereas marketed formulation showed 3.793 µg/ml, $t_{\text{max}}$ 120 minutes and AUC 117.28 µg/ml.min. Hence from the results it was found that developed niosomal *in-situ* gel formulation was found to be effective and better than marketed formulation.

Intra ocular pressure lowering activity was determined for normotensive rabbits. It was observed that IOP lowering activity of marketed formulations (Dorzox drops) reached to maximum of 2.33 ± 0.14 mg at 2 hr and dropped within 5 hours whereas niosomal *in-situ* gel formulation reached of 2 ± 0.24 mg at 4 hours and pharmacological effect was sustained upto 8 hoursrs. Hence from this study niosomal *in-situ* gel formulation proved to be better formulation than reference product in terms of therapeutic efficacy.