1 SUMMARY AND CONCLUSIONS

1.1 INTRODUCTION

The possibility of reaching the posterior segments of the eye through topical instillation of formulations is of great clinical interest as it provides various advantages for drug administration, including direct and localized delivery to the target tissue, better accessibility into the intraocular environment than can generally be achieved by systemic delivery, convenience, and relative painlessness (Davis, 2000; Maurice, 2002; Takashaki et al., 2003). However, this required specialized drug delivery systems, as conventional formulations like eyedrops are unable to achieve therapeutic drug levels in the retina because of the presence of corneal and conjunctival epithelia and tear film which serves as biological barriers to protect the eye from potentially harmful substances and drugs (Inokuchi et al., 2010).

The use of colloidal nanoparticulate drug delivery systems can be exciting new modalities of drug delivery that offer effective treatment of visually devastating diseases and as a way to enhance the bioavailability of drugs administered both systemically and topically (Dayle et al., 2000) Nanoparticulate drug delivery systems include nanorange carriers (100-1000 nm) like nanoparticles, nanospheres, nanoemulsion, polymeric micelles, nanogels etc. The biologically active agent can be dissolved or encapsulated in the macromolecular material composing the particles (Dobrovolskaia et al., 2008).

Ophthalmic drug delivery, more than any other route of administration, may benefit to a full extent from the characteristics of nanoparticulate systems (Tamilvanan et al., 2004). The nano-size of nanoparticles provides smaller particle size resulting in higher surface area available for mucoadhesion which ultimately leads to increased bioavailability and corneal penetration. It has been recommended that particles should be less than 10 μ m to minimize particle irritation to the eye, decrease tearing and drainage of instilled dose and therefore increase the efficacy of an ocular treatment (Yasukawa et al, 2004). Because of the nanosize, these carriers they can diffuse rapidly and got internalized in the ocular tissues and cells of the anterior and posterior segments (Meredio et al.,

2002). Therefore, these carriers may contribute to the preparation of a more efficacious and secure pharmaceutical dosage form which may improve the patient acceptance and compliance (Hosoya et al, 2005).

In the present investigation, nanoparticulate carriers (emulsomes and nanoparticles) of ganciclovir and triamcinolone acetonide have been developed, optimized and evaluated *in vivo* for their ability to reach the posterior segment of eye after topical administration. It was envisaged that the prepared emulsomes and nanoparticles will reach the posterior segment of eye after topical administration which will ultimately increase patient acceptance and compliance.

1.2 GANCICLOVIR EMULSOMES

Ganciclovir Emulsomes was prepared by thin film hydration method using glycerylmonostearate (GMS), dipalmitoylphosphatidylcholine (DPPC) and cholesterol. The process and formulation parameters were optimized systematically. After Preliminary experiments, critical parameters were identified and optimized. The important parameters identified were DPPC: GMS ratio, lipid: drug ratio and sonication time and hence Box- Behnken Design (BBD) was used to optimize and study the influence of independent variables (DPPC: GMS ratio, lipid: drug ratio and sonication time) on the dependent variables (size and entrapment efficiency).

The optimized batch was prepared evaluated for particle size, zeta potential, entrapment efficiency, surface morphology, DSC, *in vitro* drug release, *ex vivo* corneal permeation, and stability studies. The *in vitro* irritation potential of excipients and emulsomes was evaluated by performing short term exposure test (STE test) and cytotoxicity test on SIRC cell lines. Precorneal retention time of prepared formulation was studied in New Zealand rabbits. Ocular distribution study of dye loaded formulation was performed in mice.

1.3 TRIAMCINOLONE ACETONIDE EMULSOMES

Triamcinolone acetonide loaded emulsomes were prepared by thin film hydration method using glyceryl- monostearate (GMS), dipalmitoylphosphatidylcholine (DPPC)

and cholesterol. The process and formulation parameters were optimized systematically. After Preliminary experiments, critical parameters were identified and optimized. The important parameters identified were DPPC: GMS ratio, lipid: drug ratio and sonication time and hence Box- Behnken Design (BBD) was used to optimize and study the influence of independent variables (DPPC: GMS ratio, lipid: drug ratio and sonication time) on the dependent variables (size and entrapment efficiency).

The optimized batch was prepared and evaluated for particle size, zeta potential, entrapment efficiency, surface morphology, DSC, *in vitro* drug release, *ex vivo* corneal permeation, and stability studies. The *in vitro* irritation potential of excipients and emulsomes was evaluated by performing short term exposure test (STE test) and cytotoxicity test on SIRC cell lines. Precorneal retention time of prepared formulation was studied in New Zealand rabbits. Ocular distribution study of dye loaded formulation was performed in mice.

1.4 PREPARATION OF CHITOSAN HYDROCHLORIDE SALT

Chitosan hydrochloride salt (CS HCl) was prepared from commercial grade medium molecular weight Chitosan by slight modification in the reported method of Signini et al., 1999 as per the laboratory set up. The salt was then characterized for average degree of acetylation by ¹H NMR spectroscopy, by dissolving CS HCl in D₂O/ HCl (100:1 v/v) at 80 °C, by using a 200 MHz spectrometer. Molecular weight of chitosan hydrochloride salt was determined by Gel permeation chromatography.

Degree of acetylation was found to be %.

The number average molecular weight (Mn), weight average molecular weight (Mw) and molecular weight distribution (Mw/Mn) of chitosan hydrochloride salt was determined by GPC. The average molecular weight (Mn), weight average molecular weight (Mw) and molecular weight distribution (Mw/Mn) was found out to be 3528, 3756 and 1.064, respectively. The retention time of chitosan hydrochloride in GPC column was found out at 8.439 mins.

1.5 GANCICLOVIR LOADED CS/HA NANOPARTICLES

Ganciclovir loaded nanoparticles was prepared by ionotropic gelation method using chitosan hydrochloride salt, hyaluronic acid, sodium tri poly phosphate and ganciclovir. The process and formulation parameters were optimized systematically. The important parameters identified were HA/CS ratio and drug loading, hence 3² factorial design was used to optimize and study the influence of independent variables (HA/CS ratio and drug loading) on the dependent variables (size and entrapment efficiency).

The optimized batch was prepared and evaluated for particle size, zeta potential, entrapment efficiency, surface morphology, DSC, *in vitro* drug release, *ex vivo* corneal permeation, and stability studies. The *in vitro* irritation potential of excipients and emulsomes was evaluated by performing short term exposure test (STE test) and cytotoxicity test on SIRC cell lines. Precorneal retention time of prepared formulation was studied in New Zealand rabbits. Ocular distribution study of dye loaded formulation was performed in mice.

1.6 TRIAMCINOLONE ACETONIDE LOADED LECITHIN/ CS HCI NANOPARTICLES

Triamcinolone acetonide loaded nanoparticles were prepared by reported method of Sonvico et al., 2009 using chitosan hydrochloride salt, lipoid S100 and triamcinolone acetonide. The process and formulation parameters were optimized systematically. The important parameters identified were lecithin/CS ratio and lecithin: TA ratio. Hence 3² factorial design was used to optimize and study the influence of independent variables (lecithin/CS ratio and lecithin: TA ratio) on the dependent variables (size and entrapment efficiency).

The optimized batch was prepared and evaluated for particle size, zeta potential, entrapment efficiency, surface morphology, DSC, *in vitro* drug release, *ex vivo* corneal permeation, and stability studies. The *in vitro* irritation potential of excipients and emulsomes was evaluated by performing short term exposure test (STE test) and cytotoxicity test on SIRC cell lines. Precorneal retention time of prepared formulation

was studied in New Zealand rabbits. Ocular distribution study of Nile red loaded formulation was performed in mice.

1.7 CONCLUSIONS

The posterior eye segment is an important therapeutic target with unmet medical needs. There is an urgent requirement of focusing research interest in this area specially relating to the development of efficient drug delivery systems that can reach posterior portion of eye by topical route. The advances in the field of nanotechnology and biomaterial sciences will help in achieving these goals. In this research work, we evaluated the potential of ganciclovir and triamcinolone acetonide loaded emulsomes and chitosan hydrochloride nanoparticles for reaching the posterior segment of mice eye after topical administration. Following outcomes and inferences were withdrawn after aforementioned extensive experimentation:

Results of *in vivo* precorneal studies in rabbits after topical administration of ganciclovir loaded emulsomes and triamcinolone acetonide loaded emulsomes revealed that, these lipid based carriers remained present for a longer period of time on surface of eye by virtue of their nanometric size range and lipid composition which mimics the cell membrane composition. Also, results of the epifluorescence microscopy of the retinal flat-mount images revealed that the sodium fluorescein/ Nile red loaded emulsomes reached retina after topical administration, by either corneal/ conjuctival route or both.

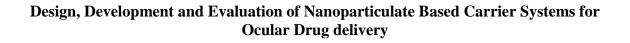
Similarly, *in vivo* precorneal studies of ganciclovir loaded nanoparticles and triamcinolone acetonide loaded nanoparticles in rabbit eye revealed that, these mucoadhesive carriers remained present for a longer period of time on surface of eye by virtue of their nanometric size range and bioadhesive properties of the polymers, which ultimately increases the precorneal retention and bioavailability of drugs. The epifluorescence microscopy of the retinal flat-mount images of these carriers revealed that the sodium fluorescein/ Nile red loaded nanoparticles does not show fluorescence in the retina section after topical administration, suggesting that these carriers cannot reach the retina after topical administration from either corneal or non- corneal route.

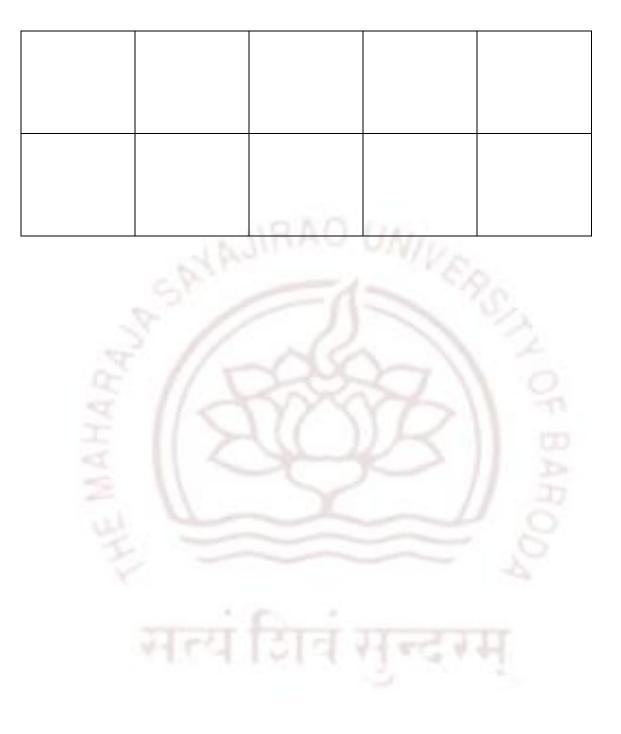
Multiple aspects like particle size, charge, composition and structure of nanocarrier altogether are attributable for difference in these observations. These properties altogether affects the retention time of these nanocarriers on corneal and conjuctival sac and thereby affecting the ocular bioavailability.

The results of the present investigations conclusively indicate the lipid based carriersemulsomes containing GCV/TA can reach the posterior segment of mice eye after topical administration by corneal as well as non- corneal route suggesting their suitability in delivering the drugs to the posterior segment of eye after topical administration. These initial investigations in mice eye indicate that developed nanosized emulsomal formulation of ganciclovir and triamcinolone acetonide can hold promise as better alternative to the conventional drug delivery techniques which uses invasive techniques like intraviteral injections or systemic administration of these drugs which causes severe side effects. However, further investigations in higher animals and human beings under clinical conditions are necessary before they can be commercially exploited.

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Chapter 10