5. SUMMARY

Acetazolamide and timolol maleate are two commonly used agents for treatment of glaucoma. Antiglaucoma therapy requires a continuous and chronic administration of the drug, but many patients cannot tolerate treatment with these drugs because of the systemic side effects associated with the oral (acetazolamide) or topical administration (timolol maleate) of these agents. Repeated administration can lead to an inadequate compliance, such that, a less frequent or once a day dosing schedule will be preferred provided a sufficient concentration of the drug is maintained in the ocular tissues for sufficient time. Improved topical absorption through the cornea, by an increased contact time; improvement in permeation; reduction in the dosage and a less frequent dosing can all help to alleviate the systemic side effects.

Further, development of a topically effective formulation of acetazolamide has not been possible because of its unfavourable partition coefficient, solubility and a permeability coefficient [Michael and Kass, 1989]. Other reasons could be its poor stability, lesser activity against carbonic anhydrase enzyme and a lesser time of residence at the active sites in the ciliary epithelium, metabolism in the eye and/or binding to pigment or protein in the eye. Hence, in the present study niosomes were considered as a suitable delivery system considering that they will help in a better permeability and hence a low incidence of side effects. The study indicates them to be highly efficient carriers. Out of various types of spans tried (span 40, 60 and 85), span 60 was found to be most suitable based on aggregation behaviour and sustainability. Different methods (film hydration, ether evaporation and reverse phase evaporation (REV)) of preparing niosomes were tried. REV was selected as the method of choice because the vesicles (REV’s) prepared by this method were observed to show maximum entrapment efficiency and corneal permeability. Literature reports also indicate it to be a method of choice for hydrophilic drugs because of the large aqueous space present within the large unilamellar vesicles (LUV’s) prepared by REV method [Gould-Fogerite and Mannino, 1992]. Timolol and acetazolamide being hydrophilic and amphiphilic in nature, respectively, seem to be suitable for entrapment within this aqueous space. Further, the niosomes were coated with suitable bioadhesive polymer (Carbopol or
chitosan). The pH of acetazolamide formulations was adjusted between 4-5 (the pH of maximum stability of acetazolamide being 4) using a 2% boric acid solution. The characterization of the formed vesicles was done by transmission electron microscopy (TEM) and particle size analysis. Viscosity of the final formulation(s) was also determined.

Niosomes of acetazolamide were prepared successfully using different methods and REV method was found to be the most suitable, both in terms of entrapment efficiency and corneal permeability. The study shows the incorporation of acetazolamide in niosomes can be of a considerable value as a means of reducing the side effects of the drug encountered with oral therapy and for development of effective topical delivery. Moreover, it was found that positively charged niosomes produced higher entrapment efficiency compared to neutral and negatively charged niosomes; but at the same time induction of charge reduced corneal permeability and increased toxicity. Similar effects with charged vesicles have been reported by other workers [Taniguchi, 1988]. Hence, the use of bioadhesive polymer to achieve an intimate contact at the corneal surface can be considered a better approach. Moreover, the bioadhesive formulation (ACZREVbio) showed a high IOP lowering effect, which compared well with that achieved by positively charged niosomes. However, a high corneal toxicity expected with ACZREV+ preparation establishes the usefulness of bioadhesives in ocular drug delivery systems. The results indicate that using niosomes as an ocular drug carrier system for topical delivery of acetazolamide (0.5%w/v, ACZREVbio) can produce a peak effect, which compares well with that of a 2% dorzolamide solution, having a longer duration of action (6h with ACZREVbio vis a vis 3h with Dorzox®). Hence, the bioadhesive coated formulation is presented as the most promising formulation. It was then subjected to challenge test and test for safety and its ocular pharmacokinetics were determined in terms of the aqueous humor concentration.

In the case of timolol maleate, chitosan or Carbopol coated niosomal timolol maleate (0.25%) formulation were prepared and compared to the timolol solution (TMS; 0.25%) and a marketed in situ gel forming solution (Timolet® GFS; 0.5%; Sun Pharma) with respect to corneal permeability and IOP lowering effect. Carbopols were also evaluated for their usefulness. Chitosan coated TMREVbio1
formulation showed a more sustained effect of up to 8h (vis a vis 6h for Carbopol coated niosomes). TMS in comparison showed effect for only 2h though the peak effect was slightly more (14%). Lowering of IOP in the contralateral eye (20-40% as compared to 100% in case of solution), considerably reduces with REV and REVbio formulations indicating lesser systemic side effects. Moreover, the results of chitosan coated niosomal formulation containing 0.25% of timolol compared well with the 0.5% marketed gel formulation, indicating our formulation to be significantly better considering that similar effect is obtained at half the concentration. The later becomes especially important in context of the cardiovascular side effects associated with ocular timolol maleate therapy.

It may be added that these studies were performed only in the normotensive rabbits. It has however, been reported that the basal IOP in contrast to the elevated IOP of α-chymotrypsinized rabbit eye, is more resistant to change, after the instillation of topical CAI’s and other ocular hypotensive drugs, e.g. timolol maleate [Vareilles et al., 1977].

In vitro corneal cytotoxicity was also carried out for the final formulations. Approximately 80% of cells were found to be viable even after 24h and 48h exposure of cells to the formulation of both the agents. Bioadhesive coated formulations were comparatively less (p<0.05) toxic than the REV niosomal formulations indicating a cytoprotective role of both chitosan (for timolol maleate) and Carbopol (for acetazolamide). Eventhough the viability of niosomal formulations was significantly less than that of the plain suspension/solution, but considering the fact that the formulation(s) were in contact with the corneal cells for a fairly long time (24h and 48h), the 80% viability observed, seems to be sufficiently safe considering that the result obtained even with the 24h study are highly exaggerated in comparison to the actual use condition (where only 1% of the instilled dose remains in contact with the eye surface for not more than 20-30 min).

Further, the concentration of both acetazolamide and timolol maleate absorbed in the aqueous humor at various times from the control solution or suspension and from the developed formulations was determined by microdialysis in male albino rabbits. Microdialysis provides a complete concentration-vs-time profile and hence is an important advance to the regional sampling of tissues. The
peak concentration of drug absorbed in the aqueous humor from niosomal formulation (Figure 29, 45) was much more than the respective suspension and solution forms (P<0.01). The peak concentration obtained with acetazolamide suspension was 6.93 µg/ml compared to 14.94 µg/ml in case of ACZREVbio. Similarly in case of TMS the concentration obtained was 7.2 µg/ml compared to 12.46 µg/ml in case of TMREVbio1.

The niosomal formulations were sterilized by autoclaving and sterility test was also carried out to confirm the successfuleness of the sterility process. Further, the sodium perborate was selected as a preservative of choice over benzalkonium chloride based on the in vitro toxicity data (Figure 36). Challenge test (B.P.,1999) using a set of standard bacterial and fungal strains was also carried out to check the efficacy of the preservative added. The stability of the final formulations (ACZREVbio and TMREVbio) was carried out at 4°C, ambient temperature and at 40°C (75%RH) and it was found that the formulations were stable at 4°C and showed high degree of drug leakage and aggregation at high temperature.

The present study can thus be considered a successful attempt at developing topical niosomal formulation(s) of acetazolamide and timolol maleate with a controlled and sustained ocular effect and a low, systemic/ocular toxicity. Establishing the safety, stability and sterility of the developed formulations indicates the scope of attaining economically viable preparations, once their clinical effectiveness is established in humans.