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Summary and Conclusions
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According to the World Health Organization (WHO), corneal diseases are major cause of vision loss and blindness, second only to cataract. NSAIDs have proven to be a safe and effective alternative to corticosteroids in the topical management of ocular inflammations. NSAIDs comprise of several chemically heterogeneous class of drugs which possess potent cyclooxygenase inhibitory activity. However, the topical use of NSAIDs in ophthalmology is limited to relatively water soluble salicylic acid, indole acetic acid, aryl acetic acid, aryl propionic acid and enolic acid derivatives.

There are four NSAIDs currently approved by the US Food and Drug Administration for the treatment of postoperative inflammation after cataract surgery, namely kerotolac, bromfenac, nepafenac and diclofenac. For ophthalmic use, diclofenac is commercially available as 0.1% w/v aqueous solution of its sodium salt. Diclofenac is applied topically in the eye for the management of pain in corneal epithelial defects following surgery or accidental trauma, treatment of postoperative ocular inflammations, chronic non-infectious inflammations, and prevention of intra-operative miosis during cataract surgery and for symptomatic relief of seasonal allergic conjunctivitis.

In this context, aceclofenac, 2-[[2-[2-[(2, 6-dichloro phenyl) amino] phenyl] acetyl] oxy] acetic acid, is a NSAID of the phenyl acetic acid group which is structurally related to diclofenac. It possesses good anti-inflammatory and analgesic activities, while maintaining better gastric tolerance in comparison with other NSAIDs such as indomethacin and diclofenac. Aceclofenac acts as such by inhibiting the secretion of tumor necrosis factor (TNF- alpha) and interleukin-1 along with preferential selective cyclooxygenase-2 (COX-2) inhibition after conversion into active metabolite. Taking above information in view,

In chapter 2, attempts were made to prepare aqueous formulations of aceclofenac which is slightly soluble in water. The different formulation factors have to be considered when drug delivery to the eye is attempted. So the purpose of this investigation was to evaluate the effect of formulation factors on in-vitro permeation of aceclofenac from aqueous drop through freshly excised goat, sheep, and buffalo corneas. The stability studies were
conducted as per ICH guidelines for different aqueous formulations of aceclofenac. Aqueous isotonic ophthalmic solutions of aceclofenac of different concentrations (pH 7.2) or 0.1% (w/v) solutions of different pH or 0.1% solutions (pH 7.2) containing different preservatives were made. Permeation characteristics of drug were evaluated by putting 1 mL formulation on freshly excised cornea (0.50 cm²) fixed between donor and receptor compartments of an all-glass modified Franz diffusion cell and measuring the drug permeated in the receptor (containing 10 mL bicarbonate ringer at 37°C under stirring) by spectrophotometry at 270 nm, after 120 min. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett’s test. Increase in drug concentration in the formulation resulted in an increase in the quantity permeated but a decrease in percentage permeation. Increase in pH of the solution from 5.5 to 7.2 decreased drug permeation, indicating pH-dependent transport. Compared with control formulation, aceclofenac 0.1% (w/v) solution (pH 7.2) containing sodium sulphite (0.2% w/v) produced significantly (p < 0.05) higher permeation with all the corneas. The results suggest that aceclofenac 0.1% ophthalmic solution (pH 7.2) containing sodium sulphite (SS) (0.2% w/v) provides increased in-vitro ocular availability through goat, sheep, and buffalo corneas. During stability study, aceclofenac showed instability in aqueous environment, therefore subsequent studies were attempted to prevent the hydrolytic degradation of aceclofenac either by replacing the aqueous environment with oily vehicles or encapsulating the drug within a polymer matrix. Aceclofenac is poorly soluble in water and gets hydrolyzed in aqueous environment. Thus, the oily vehicles could be a promising alternative to improve the drug stability. Further, the oil formulation would improve the bioavailability of aceclofenac by enhancing the residence time on ocular surface.

In chapter 3, oily formulations of aceclofenac were made in different food grade oily vehicles. For this purpose, studies were conducted to evaluate transcorneal permeation characteristics through freshly excised mammalian corneas. The stability studies were conducted as per ICH guidelines for different oily formulations of aceclofenac. The efficacy of the selected formulation was also evaluated against arachidonic acid-induced
ocular inflammation in rabbits. Ophthalmic solutions of aceclofenac with or without (0.5% v/v) benzyl alcohol were formulated in different vegetable oils based on saturation solubility and permeation studies were carried out. Post permeation corneal hydration was measured to assess corneal damage. The aceclofenac ophthalmic solution in linseed oil containing benzyl alcohol exhibited maximum permeation through all corneas under studies. The partition characteristics of drug reinforce the results of permeation studies. The optimized formulation (linseed oil containing benzyl alcohol) showed better stability profile along with higher transcorneal permeability. The optimized formulation has shown significant inhibitory effect on ocular inflammation induced by arachidonic acid ($p<0.05$). Aceclofenac (0.12%, w/v) ophthalmic solution in linseed oil containing benzyl alcohol appears ideal on the basis of all aspects of formulation strategies (corneal permeability, stability and pharmacodynamic study).

In chapter 4, attempts were made to prepare Eudragit RL 100-based nanoparticles of aceclofenac by nanoprecipitation and evaluate the particle size, zeta potential, drug entrapment, particle morphology, *in-vitro* drug release and *in-vivo* efficacy. Change in drug-polymer ratio from 1:5 to 1:20 increased the particle size and entrapment efficiency. The particles showed sustained *in-vitro* drug release which followed the Higuchi square-root kinetics. The results indicate that the nanoparticles release the drug by a combination of dissolution and diffusion. Based on the particle size (134.97 nm) and entrapment efficiency (95.73%), the formulation made with 1:10 drug-polymer ratio was selected for further studies. The particles were spherical with a polydispersity index of 0.186 and zeta potential of $+30.5$ mV. Powder X-ray diffraction and differential scanning calorimetry indicated decrease in crystallinity of drug in the nanoparticle formulation. In the *in-vitro* permeation study, the nanoparticle formulation showed 2-fold higher permeation of drug through excised cornea compared to an aqueous solution of drug with no signs of corneal damage. The *in-vivo* studies involving arachidonic acid-induced ocular inflammation in rabbits revealed significantly higher inhibition of polymorphonuclear leukocytes migration ($p < 0.05$) and lid closure scores by the nanoparticle formulation compared
with the aqueous solution. The formulation was quite stable to ensure two year shelf life at room temperature.

**In chapter 5**, attempt were made to prepare Eudragit RS 100-based nanoparticles of aceclofenac by nanoprecipitation and evaluate the particle size, zeta potential, drug entrapment, particle morphology, *in-vitro* drug release profile and *in-vivo* efficacy. Change in drug-polymer ratio from 1:2.5 to 1:10 increased the particle size and entrapment efficiency. The particles showed sustained *in-vitro* drug release following the Higuchi square-root kinetics. The results indicated that the nanoparticles released the drug by combination of dissolution and diffusion. Based on the particle size (238.9 nm) and entrapment efficiency (94.53%), the formulation made with 1:10 drug-polymer ratio was selected for further studies. The particles were spherical with a polydispersity index of 0.273 and zeta potential of +40.3 mV. Powder X-ray diffraction and Differential scanning calorimetry indicated decrease in crystallinity of drug in the nanoparticle formulation. In the *in-vitro* permeation study, the nanoparticle formulation showed 2-fold higher permeation of drug through excised cornea compared to an aqueous solution of drug with no signs of corneal damage. The *in-vivo* studies involving the arachidonic acid-induced ocular inflammation in rabbits revealed significantly higher inhibition of polymorphonuclear leukocytes migration (*p*<0.05) and lid closure scores by the nanoparticle formulation compared with the aqueous solution. The formulation was quite stable to ensure two year shelf life at room temperature.

It can be concluded from the present investigation that aceclofenac (0.12% w/v) ophthalmic solution in linseed oil containing benzyl alcohol (0.5% v/v) appears ideal taking stability, corneal permeability and therapeutic efficacy into consideration. Similarly, Eudragit RL 100 or Eudragit RS 100- based nanoparticles of aceclofenac appear promising. However, further studies are needed to comment more in this respect.
Publications