NEW DELIVERY SYSTEMS OF CIPROFLOXACIN FOR OPHTHALMIC USE

SUMMARY & CONCLUSIONS
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SUMMARY AND CONCLUSIONS

Extensive literature survey provided useful information which assisted in the logical development and evaluation of new delivery systems of ciprofloxacin, a drug of choice for treating bacterial keratitis and conjunctivitis. Additionally, the feasibility of combining ciprofloxacin with an anti-inflammatory agent, such as dexamethasone was also evaluated.

The work was commenced by developing/standardizing analytical methods for assay of drug-s and preservative. After this, samples of ciprofloxacin HCl and dexamethasone were tested for compliance with their respective monographs. Further studies were designed to identify compatible additives, suitable methods of sterilization of bulk drug powders and solutions, selection of suitable containers/closures etc. Samples of ciprofloxacin HCl and dexamethasone which complied with their respective monographs were used for the study. The preformulation studies revealed that the following additives were compatible with ciprofloxacin HCl solution, viz., sodium chloride, mannitol, propylene glycol, glycerol, boric acid, dextrose, disodium EDTA, benzalkonium chloride, HPMC E4M and poloxamer 407, hence some of these were selected for being used in the preparation of formulations. The additives that proved to be incompatible with ciprofloxacin HCl solution were carbomer 940, carbomer 971, sodium metabisulphite and thiomersal, hence these were not used in the preparation of the formulations. Amber coloured vials provided adequate

protection to ciprofloxacin HCl solution from the harmful rays of light and hence were used for dispensing the preparations. Ciprofloxacin HCl solution was chemically stable to autoclaving in amber coloured glass vials, however, it crystallized out on cooling. In order to prevent this, membrane filtration method was used to sterilize ciprofloxacin HCl solution and additionally acetate buffer was incorporated in the formulation so as to resist fluctuations of pH. Addition of the buffer greatly minimized the precipitation of ciprofloxacin at the mouth of vials, which is a common problem with most of its marketed formulations. Ciprofloxacin HCl powder was sterilized successfully by exposure to EtO gas. Dexamethasone was sterilized *in vacuo* by heating it to 140°C for a period of 3 hours. Heating it at higher temperatures or in the presence of air caused considerable degradation with marked changes in colour. Preparations were then developed with the help of information generated form the preformulation studies as well as the literature survey.

The main objective of this work was to develop an improved delivery system of ciprofloxacin, which would decrease dosing frequency, and provide long lasting sustained levels of drug in the ocular tissues. Before developing a long acting formulation, it was decided to first develop and evaluate conventional formulations, namely, solution and ointment, and then utilize information from these to develop the long acting gel type formulation. Poloxamer 407 was identified as a suitable polymer,

since it forms clear transparent gels which exhibit the desirable pseudoplastic rheological behaviour, which is the same as that of natural tears. Additionally this gel has a unique thermoreversible property, i.e. it liquefies on cooling and gels at body temperature. Hence, it was decided to fill this gel in glass vials as well as lacquered aluminium tubes. Thus, the following three

formulations containing ciprofloxacin alone were prepared:

(i) 0.3%w/v ciprofloxacin ophthalmic solution

(ii) 0.3%w/w ciprofloxacin ophthalmic ointment and

(iii) 0.3%w/w ciprofloxacin ophthalmic gel (long-acting)

Furthermore, it is not only necessary to treat the infection but also the inflammation caused by it, since the inflammation when left untreated can lead to permanent corneal scarring or neovascularization. Many antibiotic-steroid combinations are available in the Indian as well as international markets, however no such combination incorporates ciprofloxacin HCl and dexamethasone, a potent antibacterial and a potent anti-inflammatory steroid, respectively. During preformulation studies, dexamethasone sodium phosphate was found to be incompatible with ciprofloxacin HCl solution and hence plain dexamethasone was used to prepare the ciprofloxacin and dexamethasone eye drops. It was decided to formulate the combination of ciprofloxacin and dexamethasone as a suspension as well as a clear solution. Hydroxypropyl-β-cyclodextrin has been reported to solubilize dexamethasone and is also non-toxic to the eye and was therefore selected in formulating the clear solution.

Hence the following two combination eye drops were prepared:

(i) 0.3% w/v ciprofloxacin and 0.1% w/v dexamethasone ophthalmic solution

(ii) 0.3% w/v ciprofloxacin and 0.1% w/v dexamethasone ophthalmic suspension

The formulations were prepared under aseptic conditions with just the essential additives and the simplest process, so as to make them cost effective. After estimating the initial drug content, pH, *in-vitro* drug release profile, clarity etc., these formulations were subjected to accelerated stability studies at 5°C, 25°C, 37°C, 45°C, 37°C/75%RH and also accelerated conditions of light for a period of 6 months. It was found that the content of ciprofloxacin HCl in the solution, ointment and the gel dispensed in the glass vial did not decrease appreciably with time and the percentage of Analog-A, a degradation product too remained unchanged. These formulations were thus stable and a shelf life of 2 years was assigned to them. However, considerable degradation was observed in case of the gel filled in ointment tube, which could have been due to the interaction between the aluminium metal and the acidic ciprofloxacin HCl solution. This container was thus considered unsuitable for dispensing ciprofloxacin HCl gel. The *in-vitro* release profile of the drug from the gel showed that drug release from the gel followed Higuchi kinetics, with non-Fickian diffusion ($n > 0.5$). The release profile of drug from the gel remained unaltered when subjected to accelerated stability studies. Except the gel dispensed in the ointment tube, no other formulation showed

discolouration and were also otherwise clear. The pH of all the formulations was close to its original value of 4.5.

The suspension containing ciprofloxacin HCl and dexamethasone was physically unstable at temperatures of 37°C and above. The settled dexamethasone particles aggregated to form a non-dispersible cake. However, at lower temperatures, the suspension remained easily resuspendable, and did not show any increase in particle size. In case of the clear solution containing ciprofloxacin HCl and dexamethasone, there was a 4-6% reduction in the dexamethasone content after a period of 2 months storage at 37°C and above. Hence, it was decided to store both of these preparations under refrigerated conditions only.

All the prepared formulations were tested for sterility by the USP XXIII membrane filtration method and were found to be sterile. The preservative efficacy test was conducted on all the prepared formulations, as per the procedure given in USP XXIII. It was found that in all the formulations growth of the test micro-organisms was not only arrested but their numbers decreased significantly over the 28 day test period. This proves that the preservative was effective in controlling the growth of microbes that may be inadvertently introduced into the formulations during use.

The preparations were evaluated *in-vivo* for their eye irritation potential according to the guidelines given in the Draize test and all of them were found to be non-irritating.

The preparations containing ciprofloxacin HCl alone were evaluated pharmacokinetically in rabbit eyes. The aqueous humour

drug concentration - time profiles were obtained after application of (i) a single dose of the conventional solution and compared to Ciplox^{R}, (ii) a single dose of the ointment, (iii) a single dose of the long acting gel and (iv) multiple doses of the solution. A reported HPLC method was used to assay drug in the aqueous humour. In case of the conventional solution, that the Cmax and AUC was comparable with that of Ciplox^{R}. The Tmax was marginally higher for the conventional solution as compared to Ciplox^{R}. After application of the ointment, very low aqueous humour drug concentrations were obtained, as compared to the solution. This was thought to be because of the extreme hydrophobicity of the ointment base and partial expulsion of the ointment from the eyes due to blinking. Hence it was decided to use the ointment only as adjunctive therapy with conventional solution or the gel. The gel formulation was retained in the eye for a longer duration as compared to the conventional solution and hence improved bioavailability of the drug. The magnitude of AUCs obtained after application of the various types of preparations were in the order: gel > conventional solution > ointment. Application of the gel resulted in high initial drug concentration in the cornea which is desirable in order to eradicate the infecting bacteria at a faster rate so as to minimize damage to the delicate ocular tissues. Multiple dosing of the conventional solution produced a gradual increase in the aqueous humour concentration of ciprofloxacin due to accumulation of the drug, which is desirable to eliminate the infecting bacteria.

A long-acting gel formulation of ciprofloxacin HCl was developed which could be conveniently instilled as a drop and yet improved drug absorption. A technology was developed for the manufacture of the ointment and the conventional solution as the same is not reported in the literature.

The efficacy of eye drops containing ciprofloxacin and dexamethasone was studied in a rabbit eye model of *S. aureus* induced keratitis leading to corneal neovascularization. The efficacy was studied in terms of the ability of this combination to prevent neovascularization and was compared to normal saline and ciprofloxacin alone. The eyes treated with the combination prevented neovascularization, where as those treated with ciprofloxacin alone or normal saline developed mild to severe neovascularization. The prevention of neovascularization was attributed to the combination of the antibacterial activity of ciprofloxacin and the anti-inflammatory as well as immunosuppressive activity of dexamethasone. Combining dexamethasone with ciprofloxacin greatly enhanced the clinical efficacy of ciprofloxacin.

The study provided an opportunity to develop and evaluate conventional and long-acting delivery systems of ciprofloxacin. Additionally, it also provided an insight into the rational use of antibiotic-steroid combinations, in sight-threatening bacterial ocular infections. The study also provided an opportunity to develop and standardize some new analytical methods, evaluation of techniques for ensuring stability, safety, efficacy and acceptability of the delivery systems.