

***In vitro* and *in vivo* evaluation of ocular inserts of ofloxacin**

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ABSTRACT

Ocular inserts of ofloxacin were prepared with objectives of reducing the frequency of administration, obtaining controlled release and greater therapeutic efficacy in the treatment of eye infections such as conjunctivitis, keratitis, corneal ulcers, etc. Polyvinyl alcohol (PVA) ofloxacin films were prepared by mercury substrate method. The ocular inserts were evaluated for drug-excipient interaction, physico-chemical characteristics, microbiological and *in vitro* and *in vivo* release studies. There was no interaction between drug and excipients as revealed by UV absorption and IR spectra of the pure drug, medicated and placebo formulations. The weight and thickness of the inserts were in the range of 57.3-126.0 mg and 55.6-99.3 microns, respectively for different formulations; casting of rate-controlling membrane increased the weight and thickness of the inserts. Tensile strength and percent elongation at break varied with the nature of rate-controlling membrane and film thickness. Moisture vapour transmission through films followed zero-order kinetics and decreased with increase in film thickness. The drug content varied from 99.53-99.86%. The method of exposure to UV radiation was used for sterilization of ocular inserts and no microbial growth was observed in any formulation during sterility testing by direct inoculation method. The influence of rate-controlling membrane of different polymers of ethyl cellulose (EC) and polymethacrylates (Eudragit RL100, Eudragit RS100) on release kinetics was studied. The drug release for prepared formulations with rate-controlling membrane of EC, Eudragit RS100 (ERS), Eudragit RL 100 (ERL) was found to be 85.80, 93.85 and 98.71%, respectively and followed zero-order kinetics. Ocular insert F3 with rate-controlling membrane of Eudragit RS100, when inserted into the eye of rabbit showed controlled release up to 24 hours. There was a good correlation between *in vitro* and *in vivo* release data. The developed formulation was effective against selected microorganism during *in vitro* antimicrobial efficacy studies. PVA-ofloxacin ocular inserts with rate-controlling membrane of Eudragit RS100 are promising for controlled ocular delivery of ofloxacin.

Keywords: Ofloxacin, ocular insert

INTRODUCTION

Fluoroquinolones are one of the promising group of antibiotics currently being used topically to treat conjunctivitis and corneal ulcers. Ofloxacin has proved to possess superior antibacterial activity *in vivo* and has better pharmacokinetic properties as compared with ciprofloxacin and norfloxacin. Ofloxacin is a broad-spectrum antibacterial agent with activities against gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Serratia* species, *Proteus* species, *Pseudomonas aerogenosa* and *H. influenzae*) and gram-positive bacteria (*Staphylococcus* species, *Streptococcus enterococci*). It is used in the treatment of keratoconjunctivitis, blepharo-conjunctivitis, corneal ulcer, preoperative prophylaxis and other ocular infections. It has a plasma half-life of 5.7±1 hours (1,2).

Topical application of ophthalmically active drugs is the most prescribed route of administration for treatment of various ocular disorders. It is generally agreed that the intraocular bioavailability of topically applied drugs is extremely poor. This is mainly due to drainage of the excess fluid by the nasolacrimal duct as well as dilution and elimination of the solution by tear turnover. Ocular bioavailability of drugs is an important parameter influencing the efficacy of ophthalmic preparations (3,4).

The aim of the present work was to design polymeric ocular drug delivery system of ofloxacin to overcome the disadvantages associated with conventional ophthalmic dosage forms (eye drops and suspensions), to achieve long duration of action and to improve ocular bioavailability. Poly (ethylene oxide), Hydroxy

propyl methyl cellulose, Methyl cellulose, Chitosan, Eudragit NE 30D and Eudragit L 100 based inserts for ocular controlled delivery of ofloxacin have been reported (5,6,7). The current literature indicates that no attempt has yet been made on development of ofloxacin-PVA ocular inserts with rate-controlling membranes of ethyl cellulose, Eudragit RS100 and Eudragit RL100. Therefore this study investigated the microbiological efficacy and drug release kinetics of ofloxacin from a hydrophilic, monolithic reservoir system of polyvinyl alcohol cast with rate-controlling membranes of ethyl cellulose and polymethacrylates.

MATERIALS AND METHODS

Intas Pharmaceutical Ltd., Ahmedabad, India provided Ofloxacin and Eudragit RS100 while Eudragit RL100 was a gift sample from Torrent Research Centre, Ahmedabad, India. Ethyl cellulose (18-22cp) was procured from Loba Chemie Pvt. Ltd., Mumbai, India. Polyvinyl alcohol (M.W. 14,000) was purchased from C.D.H. (P) Ltd., New Delhi, India. Polyvinyl pyrrolidone K30 was purchased from Otto Kemi Ltd., Mumbai, India. Polyethylene glycol 400 (PEG-400) was obtained from E. Merck Ltd., Mumbai, India. The microbiological media were purchased from Hi-Media Ltd., Mumbai, India. All other reagents used were of analytical grade.

Preparation of ocular inserts

The monolithic drug reservoir films were prepared from aqueous solutions of polyvinyl alcohol (PVA, 2% w/v) by a solvent casting technique employing mercury as a substrate (8). A teflon ring was placed in a pool of mercury and then the matrix solution containing the drug was loaded onto this ring and was allowed to dry uniformly under ambient conditions. An area of 0.50 cm² containing 1.5 mg of ofloxacin was used in all studies.

The rate-controlling membranes (1%w/v) were prepared from ethyl cellulose (EC), Eudragit RL100 (ERL) and Eudragit RS100 (ERS) in combination with polyvinyl pyrrolidone K30 (PVP) in a proportion of 3:1 by the mercury substrate technique described above. In all films, polyethylene glycol (PEG-400) (30%w/w of total polymer) was incorporated as a plasticizer. Formulation code F1 stands for ocular inserts without rate-controlling membrane whereas F2, F3 and F4 correspond to inserts with rate-controlling membranes of EC, ERS and ERL respectively.

These formulations were sterilized separately by exposing to UV radiation for 90 minutes in a cabinet under aseptic conditions and were finally packaged in pre-sterilized aluminum foil.

Interaction Studies

Drug-excipient interaction studies were carried out by ultraviolet scanning assay and infrared spectroscopy. The sterilized formulations were dissolved in artificial tear fluid (ATF), pH 7.4 (sodium chloride 0.670 gr, sodium bicarbonate 0.200 gr, calcium chloride.2H₂O 0.008 gr, purified water q.s. 100 ml). After filtration through 0.45μ membrane, the solutions were scanned for UV absorption between 200 and 400 nm. UV scans of the placebo formulations (without drug) also were run and were compared with those of medicated formulations. An accurate amount of ofloxacin was dissolved in ATF of pH 7.4 and the absorbance of the resulting solutions were determined at 290 nm. Drug content was calculated to estimate the percentage recovery of loaded drug. The IR absorption spectra of the pure drug, medicated and placebo formulations were taken in the range of 40-4000 cm⁻¹ by potassium bromide disc method using IR spectrophotometer (Hitachi, Japan).

Physico-chemical characterization

The ocular inserts of ofloxacin were evaluated for physico-chemical characteristics such as thickness, weight variations, percentage elongation at break, tensile strength, moisture vapor transmission and test for sterility.

The thickness was measured using a micrometer (Mitutoyo Co., Kanagawa, Japan). The inserts were subjected to weight variation by individual weighing of 10 randomly selected inserts. Such determinations were carried out for each formulation (8,9).

Percentage of elongation at break and tensile strength of films (4x1cm) were measured using a pulley based tensile strength apparatus fabricated in the laboratory, as reported previously (10):

$$\text{Percent elongation at break} = 100 \times (I_b - I_0) / I_0$$

$$\text{Tensile strength} = \text{break force} \cdot (1 + \Delta L/L) / (a \cdot b)$$

where I₀ is original length of film, I_b is length of film at break when stress was applied, a is width of the film, b is thickness of the film, L is length of the film, ΔL is elongation at break and break force is the weight required to break the film.

Moisture vapour transmission (MVT) is defined as the quantity of moisture transmitted through unit area of film in unit time. Glass cells were filled with 2g of anhydrous calcium chloride and a film of specified area (0.50 cm²) was affixed on the rim of cell. The assembly was accurately weighed and placed in a humidity chamber (80±5% RH) at 25±2°C for 24 h. The glass cells were weighed at intervals times and MVT was calculated (9).

Direct inoculation method as described in Indian Pharmacopoeia (11), was used to test sterility of ocular inserts. A sterilized ocular insert was placed aseptically in a culture tube containing 10 ml of sterile soya bean-casein digest media. The mouth of the tube was closed tightly with a cotton plug which was wrapped with aluminum foil. It was incubated at $25\pm 2^\circ\text{C}$ for 7 days. The tubes were examined visually for sign of any microbial growth during the incubation period. Positive and negative controls were also employed in order to support the test.

Five film units of each formulation were dissolved in 10 ml of ATF of pH 7.4 in separate volumetric flasks. The resulting solutions were filtered through a 0.45μ membrane, diluted suitably and analyzed for ofloxacin spectrophotometrically at 290 nm.

In vitro release studies

To simulate the actual physiological conditions prevailing in the eye, an *in vitro* 'open flow through' assembly was designed for release studies as described by Rao *et al* (12). A 2 ml glass tube open at both ends was used as an *in vitro* diffusion cell. Two-fluted glass adapters were fused at both open ends so that one formed the other fluted end which was used to withdraw samples. The inlet of this tube was ATF of pH 7.4. The head of the reservoir was kept constant. Flexible PVC tubing was connected from this reservoir to the cell which had 2 ml of ATF and the rate of flow of ATF was controlled with a valve. A small volume of fluid was allowed to drain away in order to remove any entrapped air bubbles in the cell. An ocular patch was stuck onto a thin small circular teflon disc, in such a way that only one surface was exposed to the diffusion fluid. This disc was steadily inserted into the cell containing 2 ml of fluid. The temperature of the fluid was kept constant at $37 \pm 1^\circ\text{C}$. At regular intervals the diffusion fluid was withdrawn to analyze drug content at 290 nm using a Thermospectronic-1 UV/Vis double-beam spectrophotometer. Cumulative percentage of the drug release was calculated using an equation obtained from a standard curve.

In vivo release study

The *in vivo* study was performed on male albino rabbits (n=5), weighing about 2.0 kg and 24 months old. They were housed in cages in animal house under controlled conditions of temperature ($27\pm 2^\circ\text{C}$) and light. They were fed with standard laboratory diet; and water was provided *ad libitum*. Ethical clearance for the handling of experimental animals was obtained from the

institutional animal ethical committee (IAEC) constituted for the purpose.

The ophthalmic inserts (F3) were placed in the cul-de-sac of the right eye and similarly a blank film was placed in the left eye to serve as control. At regular time intervals, the ocular inserts were removed carefully and analyzed for the drug content spectrophotometrically at 290 nm. Whenever an insert was removed from the rabbit's eye a new one replaced it. The drug content obtained was subtracted from the initial drug content in the ocusert, to give the amount of drug released in the rabbit's eye (13,14).

Statistical analysis

Statistical analysis of release data was carried out by using a one-way ANOVA for repeated measures followed by a Student-Newman-Keuls multiple-comparison test to determine whether type of rate-controlling membrane affected the release of ofloxacin from ocular inserts. All results are reported as means \pm SD (n=5); $p < 0.05$ was considered to be of statistical significance.

Microbiological studies

Suspension of the test organism *Staphylococcus aureus* was prepared so as to give 0.5 McFarland standard (10^6 organisms). McFarland standard (0.5) is said to have been achieved when the absorbances of the prepared suspensions of the microorganisms matched with that of a barium sulphate 0.5 McFarland standard at 625 nm. Aliquots of 1, 2 and 3 ml of the 0.5 McFarland equivalent suspensions of *S. aureus* were inoculated into sterile peptone water, and the volume was made up to 5 ml with peptone water. The inserts were introduced into these solutions, and the plugged tubes were incubated at $37\pm 1^\circ\text{C}$. At periodic time intervals, standard loopfuls from individual tubes were streaked on sterile nutrient agar plates and incubated at $37\pm 1^\circ\text{C}$ for 24 h and observed for growth. To account for the growing number of organisms in the media at the initial times of drug release from the inserts, challenging was done at 3 h from the start of the study by further inoculation with 20% v/v of the initial inoculum of *S. aureus*. Positive and negative controls were maintained throughout the study (15).

RESULTS AND DISCUSSION

The ocular inserts of ofloxacin were prepared by solvent casting technique employing mercury as substrate and characterized on the basis of interaction studies, physico-chemical characteristics, microbiological studies, *in vitro* and *in vivo* release studies.

Table 1. Characteristics of ofloxacin ocular inserts.

Code	Rate-controlling membrane	Thickness (μm)	Weight of ocular insert (mg)	Elongation at break (%)	Tensile strength (kg/mm^2)	MVT in 24 h ($\text{g}\cdot\text{cm}^{-2}\cdot\text{h}^{-1}$)	Drug content (mg)
F1	--	55.6 \pm 0.005	57.3 \pm 0.009	17.51 \pm 0.14	0.170 \pm 0.003	0.406 \pm 0.008	1.496 \pm 0.010
F2	EC	87.4 \pm 0.004	115.0 \pm 0.01	18.07 \pm 0.17	0.190 \pm 0.005	0.354 \pm 0.002	1.493 \pm 0.015
F3	ERS	80.5 \pm 0.009	126.0 \pm 0.03	21.67 \pm 0.20	0.214 \pm 0.002	0.364 \pm 0.004	1.497 \pm 0.013
F4	ERL	99.3 \pm 0.002	109.5 \pm 0.007	18.73 \pm 0.11	0.194 \pm 0.005	0.381 \pm 0.007	1.498 \pm 0.010

Values are expressed as mean \pm S.D (n=5)

Table 2. Kinetics of *in vitro* ofloxacin release from ocular inserts.

Code	Zero-order		First-order		Korsmeyer-Peppas	
	k_0 ($\text{mg}\cdot\text{h}^{-1}$)	R^2	k_1 (h^{-1})	R^2	n	R^2
F1	0.309	0.959	-0.305	0.996	0.972	0.968
F2	0.051	0.996	-0.029	0.924	1.000	0.998
F3	0.060	0.998	-0.044	0.910	1.001	0.999
F4	0.072	0.997	-0.060	0.887	1.000	0.998

Interaction studies were carried out to ascertain any kind of interaction of the drug with the excipients used in the formulations of ocular inserts. The UV and IR spectra of the formulations exhibited absorption peaks similar to those of the pure drug sample. The spectra were found to be in sharp contrast to UV and IR spectra of the placebo formulations. The UV absorption spectra for pure drug and its formulations were quantitatively similar regarding λ_{max} at 290 nm. Result of the assay showed that 98.03 to 98.72% of the loaded drug from different formulations were recovered unchanged. The IR spectra of the pure drug showed characteristic peaks at wavenumber at 1459, 1621, 1714, and 1085 cm^{-1} which were similar to those of the pure drug. It could thus be concluded that there was no chemical interaction between the drug and the excipients in the ocular inserts.

The characteristics of different formulations are shown in Table 1. The nature of rate-controlling membrane had influences on the physico-chemical characteristics of the ocular films. The plasticizer is the most important component which may affects mechanical properties of the films by lowering the glass-transition temperature of the polymer. In this study, PEG-400 at concentration of 30%w/w of total polymer was selected since it gave sufficiently pliable films to allow for uniform subdivision into inserts without breaking the film. Ocular films of PVA with rate-controlling membranes of EC and polymethacrylates were flexible and elastic. Thickness and weights were uniform in the all batches. Tensile strength measures the ability of film to withstand rupture. The percent elongation at break and tensile strength were measured using the instrument as described by Seth *et al* (10). The maximum elongation at break was observed with F3 whereas the least value was found with F1. The ocular films in the order of tensile strength

were: F3 > F4 > F2 > F1. By addition of PVP to EC, ERS and ERL membranes the films do not break easily and it was ascertained that the drug diffusion might improves, PVA films cast with ERS as rate-controlling membrane exhibited maximum elongation and tensile strength whereas films without rate-controlling membrane showed the least value. Hence, the properties of the inserts was influenced by the molecular weight distribution or viscosity of the polymer employed for casting the rate-controlling membrane.

Moisture vapour transmission (MVT) study was performed at 25 \pm 2 $^{\circ}\text{C}$ (80 \pm 5% RH) for a period of 24 h. Moisture vapour transmission through inserts followed a pattern close to zero-order kinetics (Figure 1) and decreased by increase in film thickness. F1 (without rate-controlling membrane) was found to be appreciably permeable to water vapour. It was observed that MVT was reduced by increase in insert thickness due to increase in diffusion path length. F2, F3, F4 films had greater thickness than F1; therefore transmission was least for F1.

For various formulations, drug content was between 1.493 to 1.498 mg per film. The low coefficient of variation (C.V.<1.0%) indicates content uniformity of each batch.

The ocular inserts were sterilized by UV radiation and sterility testing was carried out under aseptic conditions. The growth of microorganism (*Bacillus subtilis*) was observed in positive control, showing that the media was suitable for test conditions. No growth of microorganisms was observed in the negative control test, which confirmed that all the apparatus used for the test were sterile and aseptic conditions were maintained. There was no growth of microorganisms in the samples under test, confirming the sterility of ocular inserts; therefore, the sterilized inserts were considered suitable for *in vivo* studies.

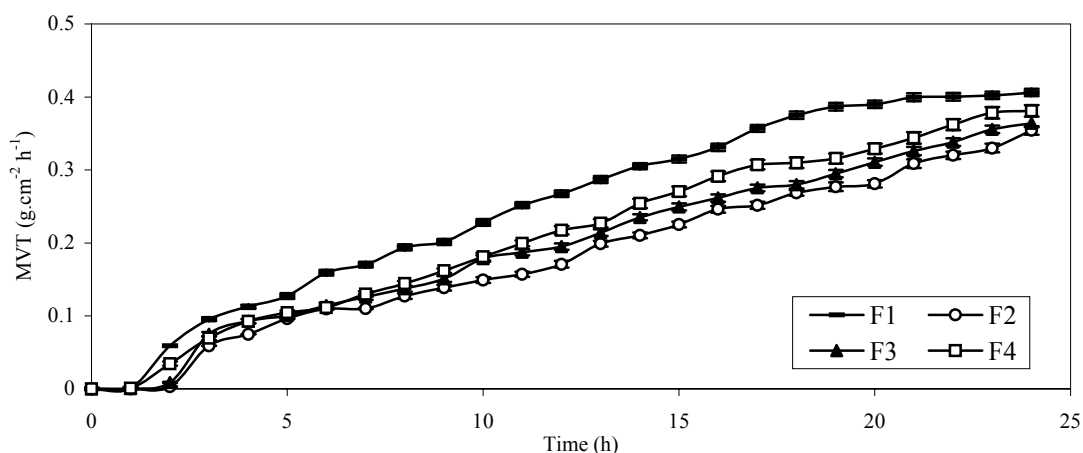


Figure 1. Moisture transmission study of ocular films. Values are mean \pm S.D of five determinations.

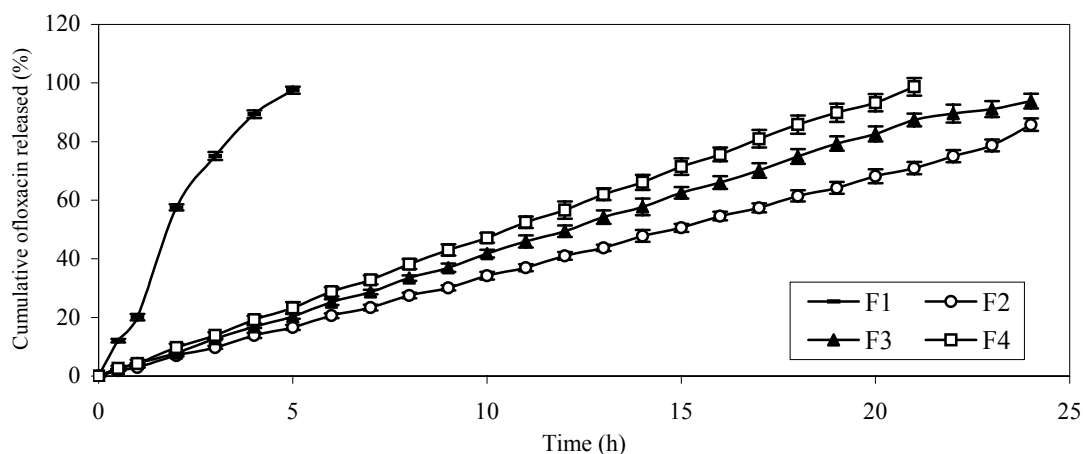


Figure 2. *In vitro* release profile of ofloxacin from ocular inserts. Values are mean \pm S.D of five determinations.

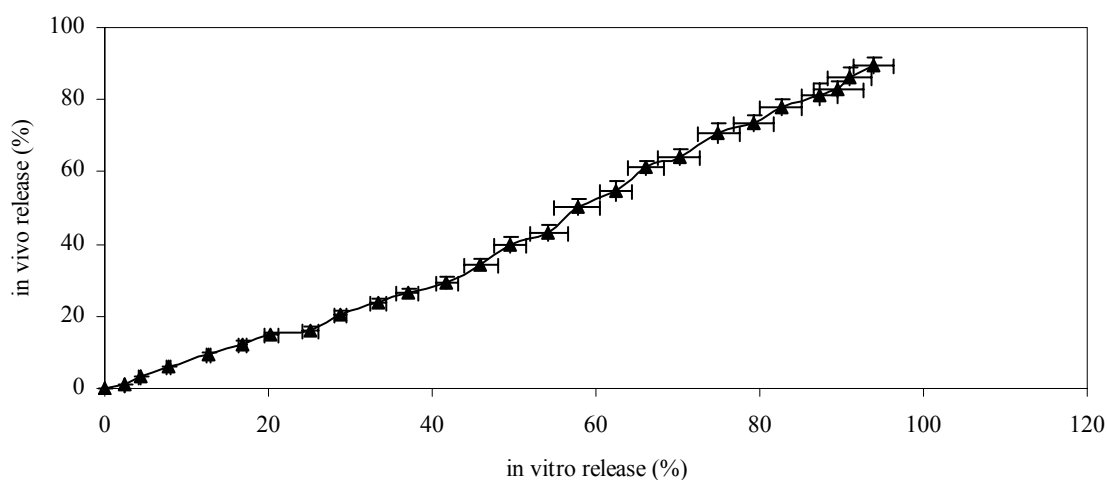


Figure 3. *In vitro* and *in vivo* correlation for ocular insert, F3. Values are mean \pm S.D of five determinations

The *in vitro* release data were fitted to different models, viz.-zero-order, first-order and Korsmeyer-Peppas (Table 2). It was found that 97.50% of the drug was released within 5 hrs from F1 and followed first-order kinetics. This means that it was required the film to be applied several times a day for achieving therapeutic coverage. The rate-controlling membranes of EC, ERS and ERL in combination with PVP were cast with the objective of achieving controlled release of ofloxacin from ocular drug reservoirs of PVA.

The drug release at the end of the 24 hrs were found to be 85.80 and 93.85%, for F2 and F3 respectively, whereas 98.71% of ofloxacin was released from F4 at the end of 21 hrs. The zero-order plots of F2, F3 and F4 were found to be fairly linear (Figure 2) as indicated by their coefficient of determination values. Therefore, it is probable that drug release from F2, F3 and F4 followed either near zero or zero-order kinetics. Consequently, in order to confirm the exact mechanism of drug release from these films, the data were fitted according to Korsmeyer *et al.* equation (16,17) which is a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$m_t/m_\infty = k \cdot t^n$$

where m_t/m_∞ = fraction of drug released, k = kinetic constant, t = release time and n = the diffusional exponent for drug release.

It has been stated that the above equation could adequately describes the release of solutes from slabs, spheres, cylinders and discs regardless of the release mechanism. The value of 'n' gives an indication of the release mechanism. When $n = 1$, the release rate is independent of time (zero-order) (case II transport); $n = 0.5$ is for fickian diffusion; and $0.5 < n < 1.0$, means diffusion and non-fickian transport are implicated. Lastly, when $n > 1.0$ super case II transport is apparent 'n' is the slope value of $\log m_t/m_\infty$ versus \log time curve (12).

The drug release data were computed and graphed according Korsmeyer *et al.* equation (16,17) and the curves were linear. The coefficients of determination, 'R²' were found to be 0.998, 0.999 and 0.998 for F2, F3 and F4, respectively. Slope values ($n=1.0$) suggest that F2, F3 and F4 followed perfect zero-order kinetics. When the release rate constants of the formulations were compared, it was found to follow the order of: F4 > F3 > F2. ANOVA test indicated that the type of rate-controlling membrane is significant in terms of ofloxacin release properties ($p < 0.05$, F-value is 63.051). The order of release rate was: ERL > ERS > EC. The higher proportion of quaternary ammonium groups in ERL resulted in rapid hydration and drug release, whereas the lower

proportion of ammonium groups in ERS is responsible for prolonged release of ofloxacin (18).

Formulation F3 was subjected to *in vivo* studies in the rabbit eye. The drug release at the end of the 24 hrs was found to be 89.47% and the release characteristics were similar to those obtained from *in vitro* studies. The *in vitro* and *in vivo* release profiles of the insert, F3 containing ofloxacin indicated that there were no significant differences between the amounts of drug released at the end of 24 h. The difference between the ofloxacin released from ocular inserts during *in vitro* and *in vivo* studies for formulation F3 was found to be insignificant ($p > 0.05$, F-value is 0.557). Polymeric ocular inserts appeared to be devoid of any irritant effect on cornea, iris, and conjunctiva up to 24 h after application, which probably suggest its suitability for ophthalmic drug delivery. An attempt was made to correlate *in vitro* and *in vivo* release (Figure 3). A linear correlation was obtained as it was evident from the regression value of 0.990 for F3 with an ERS rate-controlling membrane. Hence, ocular drug reservoir of PVA with rate-controlling membrane of Eudragit RS100 appears promising for controlled and prolonged delivery of ofloxacin.

The 0.5 McFarland standard suspensions of *S. aureus* revealed a count of 1.04×10^6 sub organisms/ml respectively. The antimicrobial effectiveness of the insert (F3) was tested by varying the volume of the inoculum. Challenging was made with 20%v/v of the initial inoculum size after 3 hrs to simulate the number of growing organisms. Ocular insert F3 containing 1.5 mg of ofloxacin was effective in inhibition of the growth in all the tubes for 24 hrs, beyond which growth was observed in tubes at 48 h at an inoculum volumes of 2 and 3 ml. Overall, the inserts were able to inhibit the growth of *S. aureus* for the entire period of the study (48 hrs) when the inoculum volume was 1 ml. At higher inoculum volumes (2 and 3 ml), the inserts were unable to inhibit the growth of microorganism in all tubes, showing an efficiency of $83.25 \pm 0.72\%$ from the 24th hour onwards. Introduction of one more insert at the 24th hour would have resulted in the inhibition of the growth in all the tubes beyond the 24th hour. In other words, the developed insert (F3) has the potential of once-a-day application in treatment of ocular infections such as bacterial conjunctivitis.

CONCLUSION

Ocular inserts of ofloxacin prepared in PVA matrix and cast with rate-controlling membranes prepared from ethyl cellulose, Eudragit RL100 and Eudragit RS 100 in combination with PVP

were smooth, flexible and transparent. *In vitro* release studies revealed that the ocular inserts followed zero-order release kinetics. There was a good correlation between *in vitro* and *in vivo* release data. PVA ocular inserts with rate-controlling membrane of Eudragit RS100 achieved controlled release of ofloxacin for a

period of 24 hours and was effective against selected microorganism in antimicrobial studies. These inserts have the potential to form the basis of a once-daily therapy of ocular infections. However, their potential to improve ofloxacin ocular bioavailability in humans need to be investigated in further studies.

REFERENCES

1. Drew RH, Gallis HA. Ofloxacin, its pharmacology, pharmacokinetics and potential for clinical application. *Pharmacotherapy* 1988; 8(1): 35-46.
2. Hardman JG, Limbirel LE, Gilman AG. Goodman and Gilman's-The pharmacological basis of therapeutics. New York: Mc. Graw Hill; 2001. p.1990.
3. Chien YW. Novel drug delivery systems. New York: Marcel Dekker Inc.; 1996.
4. Chrai SS, Makoid MC, Erikson SP, Robinson JR. Drop size and initial dosing frequency problems of topically applied ophthalmic drugs. *J Pharm Sci* 1974; 64: 333.
5. Di CG, Burgalassi S, Chetoni P, Fiaschi MP, Zambito Y, Saettone F. Gel-forming erodible inserts for ocular controlled delivery of ofloxacin. *Int J Pharm* 2001; 215(1-2): 101-111.
6. Domb AJ. Ocular inserts. US Patent 5660851, Aug 26, 1997.
7. Saettone MF, Salminen L. Ocular inserts for topical delivery. *Adv Drug Del Rev* 1995; 16: 94-106.
8. Rao PR, Ramakrishna S, Diwan PV. Drug release from polymeric films containing propranolol hydrochloride for transdermal use. *Pharm Dev Technol* 2000; 5: 465-472.
9. Dandagi PM, Manvi FV, Patil MB, Mastiholimath VS, Rathod R. Development and evaluation of ocular films of cromolyn Sodium. *Indian J Pharm Sci* 2004; 66(3): 309-312.
10. Seth AK, Agarwal GP, Saini TR. Evaluation of free films. *Indian Drugs*. 1985; 23(1): 45-46.
11. Indian Pharmacopoeia. Delhi: The Controller of Publications. 1996; p. A-123.
12. Rao V, Shyale S. Preparation and evaluation of ocular inserts containing norfloxacin. *Turk J Med Sci* 2004; 34: 239-246.
13. Attia MA, Kassem MA, Safwat SM. *In vivo* performance of [³H] dexamethasone ophthalmic film delivery systems in the rabbit eye. *Int J Pharm* 1988; 47: 21-30.
14. Charoo NA, Kohli K, Ali A, Anwer A. Ophthalmic delivery of ciprofloxacin hydrochloride from different polymer formulations: *in vitro* and *in vivo* studies. *Drug Dev Ind Pharm* 2003; 29(2): 215-221.
15. Balasubramaniam J, Srinatha A, Pandit JK, Nath G. *In vitro* microbiological evaluation of polyvinyl alcohol-based ocular inserts of ciprofloxacin hydrochloride. *Indian J Pharm Sci* 2006; 68(5): 626-630.
16. Ritger PL, Peppas NA Simple equation for solute release Part 1. Fickian and nonfickian release from nonswellable devices in the form of slabs, spheres, cylinders or disks. *J Control Release* 1987; 5: 37-42.
17. Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. *Int J Pharm* 1983; 15: 25-35.
18. Sahoo SK, Mallick AA, Barik BB, Senapati P Ch. Formulation and *in vitro* evaluation of eudragit microspheres of stavudine. *Trop J Pharm Res* 2005; 4(1): 369-375.