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RESEARCH PAPER

Ophthalmic Delivery of Ciprofloxacin Hydrochloride from Different Polymer Formulations: In Vitro and In Vivo Studies

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ABSTRACT

Reservoir-type ocular inserts were fabricated using sodium alginate containing ciprofloxacin hydrochloride as the core (drug reservoir) that was sandwiched between the Eudragit and/or polyvinylacetate films. Ocular inserts were packaged in aluminium foil and sterilized by gamma radiation. These were tested for sterility as per British Pharmacopoeia (BP). Ocular inserts were evaluated for in vitro release rate studies, microbial efficacy, in vivo release studies, efficacy against induced bacterial conjunctivitis in rabbit's eyes, concentration in the aqueous humor, and stability studies as per the International Conference on Harmonization (ICH) guidelines. Ocular inserts passed the test for sterility. They showed zero-order release of the drug in the in vitro and in vivo release studies over a period of 120 hr. The drug was found to be active against selected microorganisms as was proved by microbial efficacy studies. A high correlation coefficient was found between in vitro and in vivo release rate studies. Better improvement was observed in artificially induced bacterial conjunctivitis in rabbit's eyes, compared with marketed eye drops and placebo. Drug concentration in the aqueous humor was found above Minimum Inhibitory Concentration (MIC-90) against selected microorganisms. Shelf-life of the product was found to be more than 2 years.

Key Words: Ciprofloxacin hydrochloride; Bacterial conjunctivitis; Ocular inserts.

INTRODUCTION

A number of technical advancements have been made for making delivery systems by regulating the rate of drug delivery for obtaining the constant blood level of drug in therapeutic range for a particular time. Increased attention has been devoted to improving the oral, transdermal, ophthalmic, and

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topical dosage forms by which new and old drugs are administered. Major therapeutic advances are realized through the control of their delivery within the patient.[1]

The controlled drug delivery system has an upper edge over eye drops and ointments, because the drug is delivered at the site of action, a lesser quantity of it is required, and a constant drug supply is maintained over a predetermined time.[2]

Ciprofloxacin hydrochloride is very effective against bacterial keratitis and conjunctivitis. It effects bacterial DNA gyrase without affecting mammalian DNA activity. Because of its short plasma half-life, it must be instilled as 3–4 drops at least three times a day. Patient compliance and efficacy of ciprofloxacin hydrochloride could be improved by a drug delivery system promoting prolonged release of drug and thus increasing its application interval.[3–7]

In the present study, reservoir-type ocular inserts was fabricated and evaluated for in vitro release studies, microbial efficacy, in vivo release studies, efficacy against induced bacterial conjunctivitis in rabbit's eyes, concentration in the aqueous humor, and stability studies as per the ICH guidelines.

EXPERIMENTAL

Materials

Ciprofloxacin hydrochloride was a gift sample from Ranbaxy Research Laboratories (New Delhi, India). Pharmax India donated Eudragit RL and RS 100. Sodium alginate was a gift sample from Loba Chemie (Mumbai, India). All other chemicals and solvents were of analytical grade.

Fabrication of Ocular Inserts

Ciprofloxacin hydrochloride (0.75 mg per ocular insert), the plasticizer PEG 600 (20–55% of polymer concentration), and sodium alginate (0.120 g) were dissolved in simulated tear fluid of pH 7.4 to form the drug reservoir, and films were casted on a teflon surface. Films were evaluated for folding endurance, drug content, smoothness, uniformity, and flexibility. Different rate-controlling films of Eudragit RL and RS (C-1) were prepared in the ethanol–acetone co-solvent system (3:2); and diethylphthalate was used as the plasticizer. Polyvinylacetate rate-controlling films (C-2) were prepared in chloroform using diethylphthalate as the plasticizer. The drug reservoir was sandwiched between Eudragit RL and RS films using the ethanol–acetone co-solvent system (3:5) as the sealing agent. Similarly, drug reservoirs containing polyvinylacetate as the rate-controlling membrane were prepared using chloroform as the sealing agent.

In Vitro Release of Ciprofloxacin Hydrochloride

Ocular inserts were tested for in vitro release rate studies using flowthrough apparatus.[6] The apparatus consisted of the following parts: a jacketed 250-mL capacity conical flask, a jacketed flow-through cell, a magnetic stirrer, a pump, a flow-regulating device, and a constant temperature water bath. Different parts of the apparatus were joined by the delivery tubes obtained from the intravenous infusion set, the ocular inserts were placed in the jacketed flowthrough cell (artificial eye), and simulated tear fluid was placed in the jacketed conical flask. Simulated tear fluid was allowed to flow through the artificial eye with the help of the pump, and flow was regulated with the help of flow regulator. Contents of the conical flask were continuously mixed with the help of the magnetic stirrer. The whole assembly was maintained at $37 \pm 0.5^{\circ}C$ by regulating hot water through the jacket.

Fifty milliliters of simulated tear fluid of pH 7.4 were taken into the flask. Flow was regulated to 10 drops/min. The ocular insert was placed in the flowthrough apparatus cell. Sampling was done at different time intervals. About 3 mL of the sample was withdrawn and replaced by 3 mL of the fresh simulated tear fluid. Samples were filtered through a Whatmann no. 42 filter paper and estimated at 274 nm using simulated tear fluid of pH 7.4 as a blank. Drug concentration was determined using a standard curve, and a cumulative percentage of drug released was calculated.

Packaging, Sterilization, and Interaction Studies

Ocular inserts (C-1 and C-2) were packaged by a strip packaging machine using laminated aluminium foil. The package system was evaluated for leakage, a water—vapor transmission test, and a removability

test. Ocular inserts were sterilized by gamma radiation after packaging. The package was exposed to a total dose of 2.5 mega rads. The total dose was given in 24 hr. Sterilized ocular inserts were also evaluated for physical stability for color of packaging material, color of ocular inserts, and intactness of ocular inserts to see the effect of gamma radiation. No change in these physical properties was observed. A test for sterility on the sterilized ocular inserts was performed according to BP. Interaction studies were carried out to investigate any interaction between drug and polymer, as well as to study the effect of gamma radiation on ciprofloxacin hydrochloride. Interaction studies were carried out by UV scanning, infrared studies, and assay of ocular inserts.

Microbiological Studies

Controlled release of ciprofloxacin hydrochloride from ocular inserts was investigated bacteriologically in agar plates seeded with *Staphylococcus aureus* as a test microorganism. A layer of agar (10 mL) seeded with the test microorganism (0.5 mL) was allowed to solidify in the petri dish. Ocular inserts were embedded at a suitable distance on the petri dish. A second layer of nutrient agar seeded with the same microorganism was allowed to gel over the inserts. After 24-hr incubation at $37 \pm 0.5^{\circ}$C, the length and width of the zones of inhibition were measured around the inserts. After 24 hr, the insert was transferred on a fresh plate seeded with *S. aureus*. Again, a second layer was used to cover the insert. The petri dish was then incubated for 24 hr at $37 \pm 0.5^{\circ}$C. The study was carried out in the same way, and the zones were measured for 5 days.

In Vivo Studies

The in vivo release studies were performed in albino rabbits, because the rabbit's eye simulates an adult human eye with respect to size, shape, physiology, and composition of tears.[7] Six healthy rabbits were selected, and two groups containing three rabbits each were formed. Both eyes were used for the study (i.e., six eyes in each group). Ocular inserts were placed in the lower cul-de-sac of the eyes. Ocular inserts were removed one from each group at an interval of 24 hr. The amount of drug remaining in each ocular insert was determined. A cumulative percentage of drug released in vivo was calculated, and scatter diagrams were constructed to determine the in vitro and in vivo correlations.

Estimation of Ciprofloxacin Hydrochloride in the Aqueous Humor of the Rabbit's Eye

Twelve healthy albino rabbits with weight between 1.5–2 kg were selected and divided into two groups of six rabbits each. Ocular insets were placed in the right eye of the rabbits, and 0.3% w/v (marketed preparation) ciprofloxacin hydrochloride eye drops were placed in the left eyes of the rabbits in group 1, three times a day (3 drops per dose). After appropriate time intervals (i.e., 4, 8, 24, 48, 72, 96, and 120 hr), 200 μL aqueous humor was withdrawn with the help of a no. 26 syringe. After being vortexed for 30 sec and centrifuged for 15 min at 2,000 rpm, 20 μL of the sample was directly injected into the chromatograph. High pressure liquid chromatography analysis was performed by modifying the method of Baver et al. for HPLC analysis of the drug. Drug concentration in aqueous humor was calculated using the standard graph for ocular inserts and marketed eye drops.[8–11]

The entire experiment was repeated on the second group of rabbits with C-2 ocular inserts. Graphs were plotted for the concentration of drug in the aqueous humor (μg/mL) vs. time.

Animal Studies—Pharmacodynamics

Bacterial conjunctivitis was introduced in rabbit's eyes by exposing them to bacterial strains of *S. aureus* and *Escherichia coli*. Twenty-four hours later, the treatment was started. Animals were divided into three groups of five rabbits each. The left eye of each animal was treated as a control by instilling simulated tear fluid (placebo) of pH 7.4, 2–3 drops three times a day for the duration of the study. The right eye of each animal was treated: group 1 with 0.3% w/v eye drops of ciprofloxacin hydrochloride, group 2 animals with sterilized ocular insert C-1 and sterilized ocular insert C-2 were inserted in to the right eyes of group 3. The eyes of each animal were observed for redness, lacrimal secretion, mucoidal discharge, response to ocular stimulus, and swelling of the eyelids. Scores were given for the observed parameters (e.g., redness of the mucous membrane of the eye was

observed visually and grades were given from 0 to 4: 0 = absent; 1 = mild; 2 = moderate; 3 = severe; and 4 = very severe). Similarly, scores were given to other parameters. Significant levels were determined to compare results. For $p < 0.05$ (two-tail), the theoretical t' value was found to be 2.306. Treatment was given significance (s) if the t' value exceeded the table t' value. If it did not exceed the value, then treatment was considered nonsignificant.

STABILITY STUDIES

Stability studies were carried out on ocular inserts C-1 and C-2, according to ICH guidelines. A sufficient number of ocular inserts (packaged in aluminium foil) were stored in a humidity chamber, with a relative humidity of 75% and a temperature of $40 \pm 0.5^{\circ}$C. Samples were withdrawn at 0, 30, 60, and 90 days. Ocular inserts were also evaluated for their physical characteristics (viz. thickness, weight, and folding endurance). Samples were also analyzed for drug content. The degradation rate constant was determined from the plot of logarithms of the percentage of drug remaining vs. time in days.

$$\text{Slope} = -\text{K}/2.303$$

where K is the degradation rate constant.

RESULTS AND DISCUSSION

Ocular inserts were prepared by placing the drug containing sodium alginate reservoir in between two rate-controlling films of polyvinylacetate or Eudragits and sealing them with the help of vapors from the film solvent.

Ocular inserts C-1 and C-2 released 93% and 96% of the drug, respectively, over a period of 5 days into in vitro drug release studies. In the case of Eudragits films in ocular inserts, by increasing the amount of Eudragit RL 100 and reducing RS 100, the rate of release of the drug was increased. This might have occurred because of the greater permeability of the films by the presence of higher quantities of Eudragit RL 100. Almost constant and controlled release of the drug was observed in both types of ocular inserts. The release of the drug was by zero order, as shown in Fig. 1.

All the packs passed the test for sterility and were found stable. No interaction between drug and polymer and/or interaction because of gamma radiation was observed.

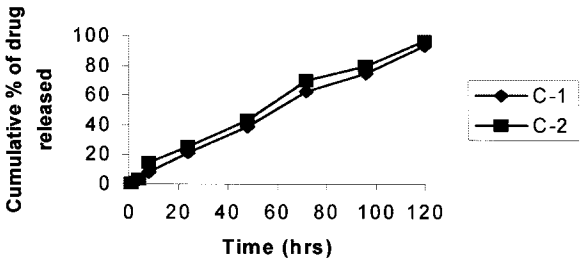

Figure 1. In vitro release rate of curves of ciprofloxacin hydrochloride from ocular inserts C-1 and C-2.

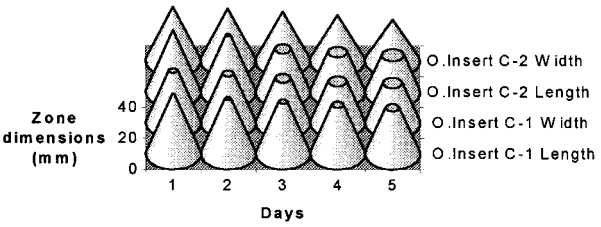

Figure 2. Cone chart showing controlled release of drug from ocular inserts C-1 and C-2 over a period of 5 days (microbiologically).

Formulations were found to be effective against *S. aureus*. Clear zones of inhibition observed each day showed that ciprofloxacin hydrochloride exhibited a constant controlled release from ocular inserts. Furthermore, the cone charts, as shown in Fig. 2, clearly show that the release of the drug occurred in a constant manner over a period of 5 days. As shown in Table 1, a lower value of coefficient of variation and percentage of the error of the mean indicated accuracy in the results of this study.

In vivo release of the drug from ocular inserts was studied in rabbit's eyes by measuring the content of the drug remaining in the ocular inserts at particular time intervals. For 5 days, total release observed was 88.8% and 87.2% for ocular inserts C-1 and C-2, respectively, as shown in Fig. 3. The release rate constants were observed for zero-order kinetics, which were found to be $0.6984\,\text{mol}\,\text{L}^{-1}\,\text{hr}^{-1}$ and $0.6430\,\text{mol}\,\text{L}^{-1}\,\text{hr}^{-1}$ for ocular inserts C-1 and C-2, respectively. Correlation coefficient (r) values for the cumulative percentage of drug released in vivo were found to be very high, and a positive correlation was found ($r = 0.9958$ for C-1 and $r = 0.9895$ for C-2). Scatter diagrams also showed high correlation between in vitro and in vivo release studies, as

Table 1. In vitro inhibition of growth of microorganisms (*Staphylococcus aureus*) by ocular insert C-1 and C-2 over 5 days.

	Zone dimensions (mm)			
	C-1		C-2	
No. of days	Length	Width	Length	Width
1	39	34	40	35
2	36	32	37	34
3	34	29	28	32
4	32	27	26	30
5	30	26	24	27
Coefficient of variation (%)	10.21	11.35	22.80	10.15
SEM	1.5650	1.5074	3.1708	1.4391

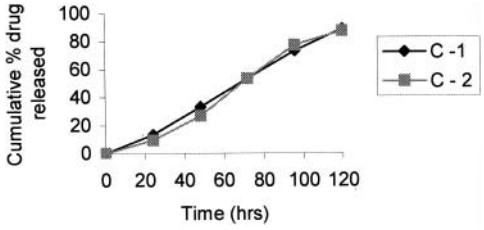

Figure 3. In vivo release rate curves of drug from ocular inserts C-1 and C-2.

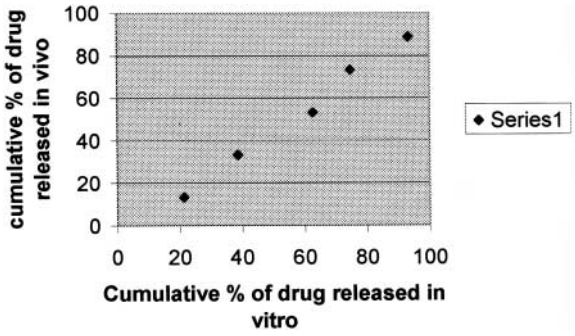

Figure 4. Scatter diagram showing in vitro and in vivo correlations of ocular insert C-1 ($r = 0.9958$).

shown in Figs. 4 and 5. Thus, ocular inserts showed good in vivo release behavior and good in vitro and in vivo correlations.

The amount of ciprofloxacin hydrochloride in aqueous humor was calculated after application of ocular inserts C-1 and C-2 in the rabbit's eye at

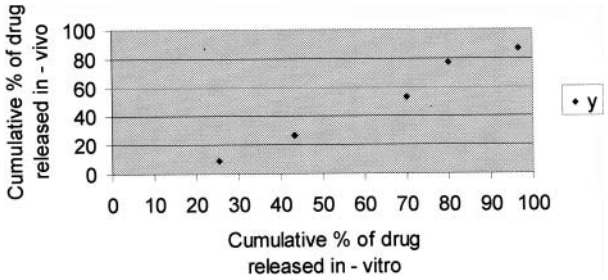

Figure 5. Scatter diagram showing in vitro and in vivo correlation of ocular insert C-2 ($r = 0.9895$).

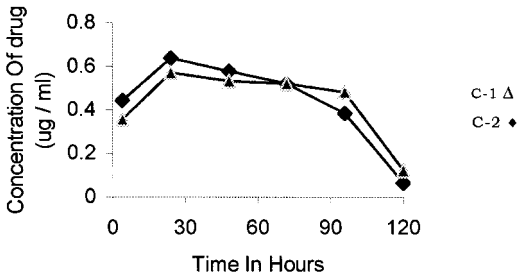

Figure 6. Concentration of drug in aqueous humor vs. time for ocular inserts C-1 and C-2.

different time intervals (i.e., 4, 24, 48, 72, 96, and 120 hr). As shown in Fig. 6, it was found that minimum inhibitory concentration of ciprofloxacin hydrochloride in the aqueous humor [i.e., approximately 0.380 (±0.328) μg/mL] was reached quickly and lasted for 4–4.5 days. Thus, ocular inserts overcame the disadvantage of the see-saw pattern of the eye drops by maintaining the constant amount of drug for longer periods of time.

Bacterial conjunctivitis was introduced by instilling mixed strains of *S. aureus* and *E. coli* in rabbit's eyes. Treatments were given with marketed eye drops and ocular inserts (C-1 and C-2). The eyes were observed for redness, lacrimal secretion, mucoidal, discharge, swelling of the eyelid, and response to ocular stimuli on a score basis up to 5 days. Improvement in the symptoms was observed effectively for the ocular inserts. The infection was successfully treated with ocular inserts in a much shorter time. Results were analyzed statistically by applying significance "t" test at $p < 0.05$ for two tails. It was observed that improvement in redness in the case of ocular inserts occurred after 1 day, compared with eyedrops, whereas significant values were observed on the second day of treatment. Similarly, in other cases, significant values for

Table 2. Significance t' test results of ocular insert C-1 vs. eyedrop and ocular insert C-2 vs. eyedrop for improving the symptoms of conjunctivitis in rabbit's eye (viz. redness, lacrimal secretion, mucoid dishcharge, response to ocular stimulus and swelling of eyelid).

	Calculated t' value and significance results											
	Redness		Lacrimal secretion		Mucoid discharge		Response to ocular		Swelling of eyelid			
No. of days	I Ocular insert (C-1) vs. eye drop	II Ocular insert (C-2) vs. eye drop	I Ocular insert (C-1) vs. eye drop	II Ocular insert (C-2) vs. eye drop	I Ocular insert (C-1) vs. eye drop	II Ocular insert (C-2) vs. eye drop	I Ocular insert (C-1) vs. eye drop	II Ocular insert (C-2) vs. eye drop	I Ocular insert (C-1) vs. eye drop	II Ocular insert (C-2) vs. eye drop
1	2.1198	2.1198	0.6320	1.8960	1.2640	1.2640	0	0	2.4483	3.9974
	NS	NS	NS	NS	NS	NS	NS	NS	S	S
2	1.8960	3.5330	2.5280	3.5330	2.4483	—	0	1.6322	2.4483	2.5280
	NS	S	S	S	S		NS	NS	S	S
3	2.4483	2.8847	1.2640	2.8847	2.8847	4.4240	0	0.9993	1.896	2.3078
	S	S	NS	S	S	S	NS	NS	NS	S
4	5.7128	5.1926	2.4483	3.9974	3.9974	—	0.632	0.5769	3.9974	—
	S	S	S	S	S		NS	NS	S	
5	7.0661	10.9930	1.6322	1.6322	0.9993	0	0	0	3.9974	3.9974
	S	S	NS	NS	NS	NS	NS	NS	S	S

Theoretical t' value at $p < 0.05$ for two-tailed = 2.306. S, significant (if calculated t' values are greater than theoretical t' values); NS, nonsignificant.

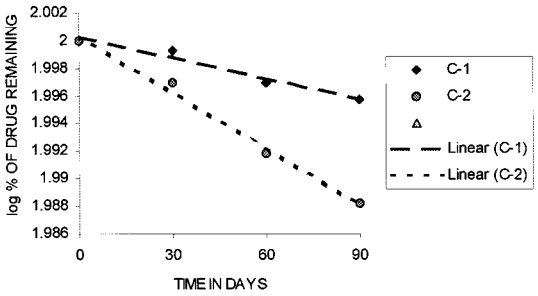

Figure 7. Plot of log% of drug remaining vs. time for ocular inserts C-1 and C-2.

ocular insert were observed earlier, compared with treatment with eye drops (as summarized in Table 2). Thus, ocular inserts gave better response in the treatment of bacterial conjunctivitis, compared with eye drops.

Finally, accelerated stability studies at elevated temperature and humidity revealed no significant changes in thickness, weight, or folding endurance. Ocular inserts could be stored safely at study storage conditions. However, storage temperature not in excess of 40°C and moisture-proof packing are recommended to ensure stability of formulation. The degradation rate constant for formulations C-1 and C-2 were found to be 1.3426×10^{-4} and 3.185×10^{-4} day^{-1}, respectively, as shown in Fig. 7. Because the overall degradation is less than 5%, a tentative shelf-life of 2 years may be assigned to the formulations as per the ICH guidelines.

ACKNOWLEDGMENT

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CONCLUSIONS

On the basis of in vitro, in vivo, and microbiological studies, it could be concluded that ciprofloxacin hydrochloride, a potent antibacterial agent, could be successfully administered through reservoir-type controlled release ocular inserts for the treatment of bacterial keratitis and conjunctivitis.

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