RESEARCH ARTICLE

Once a day ocular inserts for sustained delivery of levofloxacin: Design, formulation and evaluation

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Levofloxacin is a fluoroquinolone antibacterial drug effective in the treatment of bacterial conjunctivitis. The objective of the present work was to develop ocular inserts of levofloxacin and evaluate their potential for sustained ocular delivery. Conventional ophthalmic solution shows the poor bioavailability and therapeutic response due to many pre-corneal constraints. These constrains necessitate the controlled and sustained drug delivery to become a standard one in the modern pharmaceutical era. Matrix-type ocular inserts were prepared by the film-casting technique in Teflon-coated Petri dishes and characterized *in vitro* by drug release studies using a flow-through apparatus that simulated the eye conditions. Nine formulations were developed, which differed in the ratio of polymers polyethylene oxide (PEO), hydroxypropyl cellulose (HPC) and ethyl cellulose (EC). All the formulations were subjected to evaluation of thickness, weight variation, folding endurance and drug content uniformity and *in vitro* release study. On the basis of *in vitro* drug release studies, the formulation with PEO/EC (F9) was found to be better than the other formulations (release of 101.35% within 24 hrs) and it was selected as an optimized formulation, which was further subjected to *in vivo* studies and ageing studies. The *in vitro* result revealed that formulation F9 followed a perfect zero-order kinetics release ($n = 1.03$) and the rest of the formulations released the drug by super case II kinetics ($n > 1$). It was also observed that increasing the proportion of PEO and HPC to EC increases the rate of release of Levofloxacin. On the basis of *in vitro and in vivo* correlation stability studies, it can be concluded that this ocular inserts formulation can be a promising once-a-day controlled release formulation.

Key words: *In vivo study, levofloxacin, ocular inserts, release kinetics, sustained release*

INTRODUCTION

Continuous delivery of drugs to the eye offers major advantages over conventional therapies that involve administration of drug solutions or suspensions as eye drops. Eye drop administration often results in poor bioavailability and therapeutic response due to rapid precorneal elimination of the drug and is also associated with patient compliance problems.[1,2]

A basic concept in ophthalmic research and development is that the therapeutic efficacy of an ophthalmic drug can be greatly improved by prolonging its contact with the corneal surface. Ophthalmic inserts offer many advantages over conventional dosages forms, like increased ocular residence, possibility of releasing drug at a slow and constant rate, accurate dosing, exclusion of preservatives and increased shelf-life. Design, construction and technology of the ocular insert in a controlled and sustained ocular delivery device are gaining rapid improvement to overcome these constraints.[3,4]

Levofloxacin is a broad-spectrum antibacterial with a half-life of 6-8 hrs, frequently used in ocular infections, and is sparingly soluble in water.[5] Only a few ocular inserts made of (EVA) as a rate controlling membrane are available on the market.[6,7] Likewise, ethyl cellulose (EC) is also an excellent film-forming polymer, but the films of EC alone are brittle. It offers more resistance to the diffusion of drug molecules and is less explored as a polymer for ocular delivery of drugs. The current literatures indicate that no inserts are made of hydrophobic monolithic systems using levofloxacin. Hence, this investigation has been performed to study the drug release kinetics of levofloxacin from a hydrophobic matrix system of EC cast with incorporating different proportions of PEO and HPC with the addition of hydrophilic polymer to EC, because of which the films of EC become resilient and

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DOI: 10.4103/0973-8398.59954

do not break easily and it was ascertained that the diffusion might improve.[8-11]

MATERIALS AND METHODS

Levofloxacin was obtained as a complimentary sample from Alkem Labs, Mumbai, India. HPC and EC were purchased from Loba Chemie, Mumbai, India and SD Fine Chemicals, Mumbai, India respectively. PEO was purchased from Alfa Aesar Inc., Heysham, Lancashire LA3 2XY United Kingdom.

Preparation of ocular inserts

The matrix type of films were prepared by the film casting technique from EC (3% w/v) alone (i.e., F1, F2 and F3) and also in combination with PEO and HPC [Table 1]. Three different proportions of EC: HPC were modeled, i.e. the 9:1 (F4), 8:2 (F5) and 7:3 (F6) ratios. Similarly, for EC: PEO, the ratios were 9:1 (F7), 8:2 (F8) and 7:3 (F9). Weighed quantities of the drug (2 mg) and polymers were solubilized in dichloromethane (DCM), with continuous mixing using a magnetic stirrer at 100 rpm. The solutions were then sonicated for a few seconds to remove the air. Polymeric drug solutions were poured on to a Teflon-coated Petri dish. The matrix films were dried constantly under ambient conditions. In all the films, dibutyl phthalate (20% w/w) was incorporated as a plasticizer.[12] Inserts were sterilized under UV for 1 min and individual inserts were packed in sterilized aluminum foils, which were further stored in amber colored glass bottles at room temperature.

Physicochemical evaluation of ocular inserts

Prepared inserts were evaluated for surface pH, thickness, weight variations, folding endurance and drug content uniformity. Surface pH was determined by allowing them to swell in a closed petri dish at room temperature for 30 min in 0.1 ml of distilled water. pH paper was kept on the surface and after one min. the color that developed was compared with the standard color scale. Thickness was evaluated using a micrometer of sensitivity 0.001 mm (Mitutoyo, Japan) and the average of ten readings was taken. Folding endurance was determined by repeatedly folding a small strip of ocular film at the same place till it broke. Drug content was estimated by triturating ocular inserts in 20 ml of phosphate buffer pH. 6.8 with the help of a mortar and pestle. The solution was filtered and one ml of the solution was withdrawn, diluted and measured by a UV-Visible Spectrophotometer (Model-1700, Shimadzu, japan) at 290 nm.[13]

In vitro release study

Because there was no specific official method prescribed for *in vitro* studies of ocular inserts, we fabricated an open flow through assembly, simulating the condition of the ocular cavity. A 2 ml glass tube that was open at both ends was used as an *in vitro* diffusion cell. Two fluted glass adopters were fused at both the open ends so that one formed the inlet and the other fluted end was used to withdraw the sample. The inlet end of this tube was connected to a reservoir containing

Table 1: Composition of levofloxacin ocular inserts

Formulation code	Film former (3%w/v)		
	EC (parts)	PEO (parts)	HPC (parts)
F1	10	-	-
F2	-	10	-
F3	-	-	10
F4	9	-	1
F5	8	-	2
F6	7	-	3
F7	9	1	-
F8	8	2	-
F9	7	3	-

Simulated Tear Fluid (STF), pH 7.4. The head of the reservoir was kept constant. Flexible Polyvinyl Chloride (PVC) tubing was connected from this reservoir to the cell, in which 2 ml of buffer was maintained constant. The rate of flow of buffer was controlled with a valve and adjusted to 0.2 ml/min. Taking a 25 reading initially, the setup was validated and the standard deviation (0.2 ± 0.08) and % coefficient of variation were observed to be minimum, hence the setup was used throughout the work.

STF pH 7.4 was put into the reservoir. A small volume of fluid was allowed to drain away so as to remove any entrapped air bubbles in the cell. An ocular insert was stuck on to a thin, small, circular, teflon disc so 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 at 35 ± 1°C constantly. At regular intervals, the diffusion fluid was taken to analyze for drug content using UV-Visible Spectrophotometer. Simultaneously, a blank was performed under similar conditions as described with a drug-devoid film. Triplicate readings were taken and the average was calculated and tabulated.[14]

In vivo release study

Approval for the use of animals in the study was obtained from the Institutional Animal Ethics Committee (949/a/06/CPCSEA). On the day of the experiments, the sterilized ocular inserts were inserted into one eye of seven rabbits at the same time and another eye served as control. After 1, 2, 4, 6, 10, 22 and 24 hrs, the inserts were carefully removed and analyzed for the remaining drug content by high performance liquid chromatography (HPLC) analysis.[15]

High performance liquid chromatography condition

Mobile phase: 82% of 0.4% triethylamine (pH 3.0) and 18% of acetonitrile.
Flow rate: 1.0 ml/min.
Column: Phenomenex C18 Column.
Detector: SPD-M20A Prominence Diode array detector.
Retention time: 4.08 min.
Injection volume: 20 μl by Rheodyne 7725*i* injector.
Standard solution: 2 μg/ml of Levofloxacin in HPLC-grade water.

Ocular safety study

The ocular safety of the administered delivery system is based on the Draize Irritancy Test [Tables 2 and 3]. The observations based on the scoring approach established the safety of the developed ocular inserts in the rabbit eye.[16]

Stability study

The optimized inserts (F9) were stored in amber-colored glass bottles at 3 different temperatures: 4°C, Room temperature (RT) and 37°C, for a period of 3 months. The samples were withdrawn after 30, 60 and 90 days and analyzed for physical appearance, drug content and sterility.[17,18]

Table 2: Draize irritancy test for ocular safety

Ocular tissue	Scoring scale	Calculations	Total
Cornea			
Opacity (O)	0, 1, 2, 3, 4	O × A × 5	80
Area involved (A)	0, 1, 2, 3, 4		
Iris			
Values for congestion and hemorrhage (I)	0, 1, 2	I × 5	10
Conjunctiva			
Redness (R)	0, 1, 2, 3		
Chemosis (C)	0, 1, 2, 3, 4	(R + C + D) × 2	20
Discharge (D)	0, 1, 2, 3		
Total maximum			110

Score of 0 is normal, 3 and 4 is severe in case of O, R, C and D. Score of 0 is none, 1, 2, 3, 4 is the extent of cornea covered for A. Score of 0 is normal and 2 is severe in case of I

Table 3: Safety evaluation chart

Score	Rating
0.0-0.5	Nonirritating
0.5-2.5	Practically nonirritating
2.5-15	Minimally irritating
15.0-25.0	Mildly irritating
25.0-50.0	Moderately irritating
50.0-80.0	Severely irritating
80.0-110.0	Extremely irritating

RESULTS AND DISCUSSION

The physicochemical evaluation data presented in Table 4 indicates that the thickness of the matrix films varies from 0.20 ± 0.01 mm to 0.25 ± 0.09 mm. All the formulations exhibited uniform thickness with low standard deviation values, ensuring the uniformity of the films prepared by the film casting method. Hence, formulations were not thick enough to produce any irritation while placing and being in *cul-de-sac*.

The results showed that weights of the formulations ranged from 4.7 ± 0.32 mg to 6 ± 0.18 mg for matrix films. This indicates that there was not much variation in weight for all the formulations [Table 4].

The drug content of all the formulations was found to be within the range of 1.95 ± 0.04 mg to 2.03 ± 0.02 mg for the matrix films. The minimum intrabatch variations revealed the suitability of the process used to prepare the ocular inserts [Table 4].

The folding endurance for all the formulations was good. The maximum folding endurance of formulation F3 was 96.3 ± 4.5 foldings and formulation F1 showed a minimum folding endurance of 61 ± 4.5 foldings [Table 4]. This showed that as the concentration of the polymer increased in the formulation, the folding endurance was decreased.

The surface pH of the prepared inserts varied between 6.5 and 7.5, indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range (7.3-7.7).

Formulation F1 showed 51% release within 24 hrs while F2 and F3 released nearly 100% of drug within just 10 hrs [Table 5]. Therefore, to get once-a-day delivery, films of EC were modeled by incorporating PEO and HPC in different proportions [Tables 6 and 7].

In controlled drug delivery, zero order is the most preferred kinetics of drug release. Therefore, inserts of EC were modeled to release the drug in zero-order modes by incorporating hydrophilic polymers PEO and HPC. Zero-order

Table 4: Physicochemical evaluation data of different batches of matrix films

Evaluation tests	Formulations								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Thickness ± SD (mm)*	0.20 ± 0.01	0.22 ± 0.007	0.25 ± 0.009	0.19 ± 0.01	0.20 ± 0.004	0.22 ± 0.002	0.20 ± 0.009	0.21 ± 0.005	0.23 ± 0.01
Weight variation ± SD (mg)*	4.9 ± 0.21	5.3 ± 0.28	5.9 ± 0.26	4.7 ± 0.32	4.9 ± 0.29	5.5 ± 0.16	4.8 ± 0.33	5.2 ± 0.14	6.0 ± 0.18
Drug content ± SD (mg)**	2.01 ± 0.04	2.02 ± 0.01	1.96 ± 0.04	2.02 ± 0.02	1.98 ± 0.05	2.00 ± 0.05	1.95 ± 0.04	1.99 ± 0.03	2.03 ± 0.02
Folding endurance ± SD**	61 ± 4.5	85 ± 2.9	96.3 ± 4.5	70.5 ± 2.9	78.2 ± 5.2	92.2 ± 3.5	72 ± 5.5	81.35 ± 6.5	91 ± 5.8

All readings are in the form of Mean ± SD; *Average of 3 determinations; **Average of 10 determinations

Table 5: *In vitro* drug release profile of ocular inserts

Formulations	%CR at different time intervals (hr)*							
	1	2	4	6	8	10	22	24
F1	0.9 ± 0.091	1.92 ± 0.073	5.06 ± 0.068	8.23 ± 0.112	12.16 ± 0.142	15.34 ± 0.108	45.23 ± 0.114	51.24 ± 0.215
F2	26.54 ± 0.924	45.67 ± 1.512	61.96 ± 0.581	81.42 ± 0.348	92.65 ± 1.438	98.83 ± 0.682	-	-
F3	30.28 ± 0.658	37.24 ± 2.042	67.86 ± 0.452	87.48 ± 0.241	99.36 ± 1.105	-	-	-

*Average of 3 determinations ± SEM

Table 6: *In vitro* drug release profile of EC + HPC-based matrix films

Formulations	% CR at different time intervals (hr)*							
	1	2	4	6	8	10	22	24
F4	1.17 ± 0.081	2.88 ± 0.063	5.36 ± 0.036	8.45 ± 0.190	13.24 ± 0.063	17.41 ± 0.205	61.53 ± 0.219	69.30 ± 0.219
F5	1.46 ± 0.057	3.47 ± 0.068	6.27 ± 0.053	10.26 ± 0.019	14.56 ± 0.054	20.55 ± 0.078	67.20 ± 0.047	77.26 ± 0.126
F6	1.96 ± 0.104	3.91 ± 0.069	9.81 ± 0.076	15.42 ± 0.045	21.50 ± 0.123	30.40 ± 0.128	84.43 ± 0.354	98.81 ± 0.396

*Average of 3 determinations ± SEM

Table 7: *In vitro* drug release profile of EC + PEO based matrix films

Formulations	% CR at different time intervals (hr)*							
	1	2	4	6	8	10	22	24
F7	2.36 ± 0.083	3.39 ± 0.183	6.13 ± 0.188	10.03 ± 0.065	14.33 ± 0.190	18.68 ± 0.247	53.63 ± 0.141	60.40 ± 0.091
F8	2.68 ± 0.114	6.24 ± 1.183	11.18 ± 0.056	16.81 ± 0.466	24.21 ± 0.176	31.37 ± 0.120	78.77 ± 0.355	86.08 ± 0.206
F9	2.87 ± 0.084	7.55 ± 0.055	15.41 ± 0.119	24.87 ± 0.174	33.90 ± 0.114	44.82 ± 0.218	88.43 ± 0.354	101.35 ± 0.362

*Average of 3 determinations ± SEM

plots of F1-F9 were found to be fairly linear, as indicated by their high regression values [Table 8].

As results indicated that the % cumulative release (%CR) for ocular insert F9 was 101.35% at the end of 24 hrs, it was found to be suitable for once-a-day therapy. The *in vitro* result revealed that formulation F9 followed perfect zero-order kinetics release ($n = 1.03$) and that the remaining formulations released the drug by super case II kinetics ($n > 1$). Thus, it was taken as the optimized formulation and subjected to further studies.

The results of the *in vivo* release study of ocular insert F9 is shown in Table 9 and Figure 1. The ocular insert showed 96.03% of drug release after 24 hrs, which was comparable to *in vitro* drug release [Table 9]. Thus, there was good *in vitro-in vivo* correlation for ocular insert F9 [Figure 1], indicating the effectiveness of the formulation to be used *in vivo*.

The ocular safety score of formulation F9 was found to be 4 at the end of 24 hours and therefore, considered as minimally irritating. This irritation might be due to the organic solvent used in the preparation of inserts. Thus, it can be concluded that they were safe for ocular administration.

Table 8: Kinetic treatment of release study data of ocular inserts

Formulations	Zero-order plots	Higuchi's plots	Peppa's plots	
	r^2	r^2	r^2	*n*
F1	0.9876	0.8511	0.9998	1.329
F2	0.9886	0.8402	0.9868	1.216
F3	0.9688	0.8753	0.9894	1.232
F4	0.9778	0.8157	0.9993	1.518
F5	0.9755	0.8221	0.9993	1.477
F6	0.9886	0.8540	0.9998	1.309
F7	0.9847	0.8515	0.9991	1.297
F8	0.9960	0.8831	0.9996	1.152
F9	0.9991	0.8864	0.9990	1.036

Ageing study of the ocular insert F9 was performed at RT, 4°C and 37°C for a period of 3 months. The results [Table 10] showed that there was no change in the physical appearance of ocular inserts. The drug content showed no marked change after three months and F9 passed the sterility test. These results concluded that ocular insert F9 was chemically, physically and microbiologically stable

Table 9: *In vivo* drug release data of optimized ocular insert F9

Time (hrs)	Remaining drug content (mg)	Amount of drug released (mg)	% drug released	*In vitro* % drug released
1	1.9484	0.0496	2.48	2.87
2	1.8775	0.1205	6.03	7.55
4	1.7682	0.2298	11.50	15.41
6	1.5724	0.4256	21.24	24.87
10	1.2159	0.7821	39.14	44.82
22	0.1924	1.8056	90.37	88.43
24	0.0792	1.9188	96.03	101.35

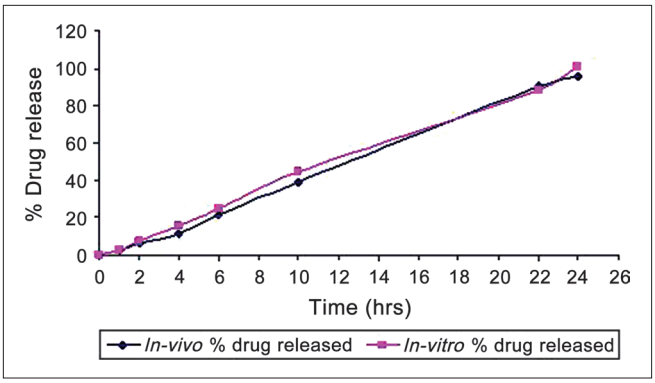

Figure 1: *In vitro-in vivo* correlation of F9

Table 10: Ageing study data for the formulation F9

Time (days)	4°C			RT			37°C		
	P.A.	RDC*	SRT	P.A.	RDC*	SRT	P.A	RDC*	SRT
0	+	1.998 ± 0.016	√	+	1.998 ± 0.016	√	+	1.998 ± 0.016	√
30	+	1.997 ± 0.036	√	+	1.998 ± 0.029	√	+	1.995 ± 0.063	√
60	+	1.984 ± 0.028	√	+	1.982 ± 0.042	√	+	1.985 ± 0.058	√
90	+	1.981 ± 0.032	√	+	1.984 ± 0.038	√	+	1.986 ± 0.045	√

√ = Passes the sterility test (SRT): + - = Good. *Average of 3 determinations ± SD; PA = Physical appearance; RDC = Remaining drug content

at RT for 3 months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf-life.

CONCLUSION

The present work showed that incorporation of hydrophilic polymers into a hydrophobic matrix system was successful in order to model ocular inserts having perfect zero-order release, proving a promising controlled release delivery system. It was observed that increasing the proportion of PEO and HPC to EC increases the rate of release of Levofloxacin. On the basis of *in vitro-in vivo* correlation and stability studies, it can be concluded that this levofloxacin ocular insert can be a promising once-a-day controlled release formulation after due considerations of *in vivo* antibacterial activity studies.

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Source of Support: Nil, **Conflict of Interest:** None declared.