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Research Article

SUSTAINED OPHTHALMIC DELIVERY OF LEVOFLOXACIN FROM ONCE A DAY OCUSERTS

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ABSTRACT

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 necessitates the controlled and sustained drug delivery to become a standard one in 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 , sodium alginate and ethyl cellulose. All the formulations were subjected to evaluation of thickness, weight variation, folding endurance and drug content uniformity, *In vitro* release study. On the basis of *in vitro* drug release studies, the formulation with PEO/EC (3:7) was found to be better than the other formulations and it was selected as an optimized formulation, which was further subjected to *in vivo* studies and ageing study. *In vitro* result revealed that formulation L6 followed perfect zero order kinetics release (n=1.03) and rest of formulations released the drug by super case II kinetics (n>1). It was also observed that increasing the proportion of PEO and SA in to EC increases the rate of release of Levofloxacin. On the basis of *In vitro*, *In vivo* correlation stability studies, it can be concluded that this ocular inserts formulation can be a promising once-a-day controlled release formulation.

Keywords: Levofloxacin, ocular inserts, *In vivo* study, sustained release, release kinetics.

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 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 to 8 hrs frequently used in ocular infections, and is sparingly soluble in water[5]. There are only a few ocular inserts available on the market, made of EVA as a rate controlling membrane[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

literature indicates that none are made of hydrophobic monolithic systems containing levofloxacin. Hence this investigation has been done to study the drug release kinetics of levofloxacin from a hydrophobic matrix system of EC cast with incorporating different proportions of PEO and SA with the addition of hydrophilic polymer to EC, the films of EC become resilient and 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. Sodium alginate and EC were purchased from Loba Chemie, Mumbai and SD Fine Chemicals respectively. PEO was purchased from Alfa Aesar Inc., USA.

Preparation of ocular inserts

The matrix type of films were prepared by film casting technique from EC (3% w/v) alone (i.e. L1, L2 and L3) and also in combination with PEO and SA. Three different proportions of EC:PEO were modeled, i.e. 9:1(L4), 8:2(L5) and 7:3(L6) ratios. Similarly, for EC:SA ratios were 9:1 (L7), 8:2(L8) and 7:3 (L9). Weighed quantities of the drug and polymers were solubilized in DCM (Dichloromethane) with continuous mixing. The solutions were then sonicated for few seconds to remove the air. Polymeric drug solutions were poured on to a Teflon coated Petridish. The matrix films were dried constantly under the ambient conditions. In all the films dibutyl phthalate (20% w/w) was incorporated as a palstizers[12].

Table 1: Composition of levofloxacin ocuserts

Formulation code	Drug (mg)	Film former (3%w/v)			Plasticizer
		EC(Parts)	PEO (parts)	SA(Parts)	DBP(%w/w)
L1	2	10	-	-	20
L2	2	-	10	-	20
L3	2	-	-	10	20
L4	2	9	1	-	20
L5	2	8	2	-	20
L6	2	7	3	-	20
L7	2	9	-	1	20
L8	2	8	-	2	20
L9	2	7	-	3	20

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 petridish at room temperature for 30 minutes in 0.1ml of distilled water. pH paper was kept on surface and after one minutes the colour developed was compared with the standard colour scale. Thickness was evaluated using a micro meter of sensitivity of 0.001mm (mitutoyo, japan), 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 mortar and pestle. The solution was filtered and one ml solution was withdrawn, diluted and measured by UV-Visible Spectrophotometer at 290 nm[13].

***In vitro* release study**

Since 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 open at both ends was used as an *in vitro* diffusion cell. Two fluted glass adopters were fused at both 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 Simulated Tear Fluid (STF) pH 7.4. The head of the reservoir was kept constant. Flexible 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 0.2ml/min. Taking 25 reading initially, the setup was validated and the standard deviation (0.2±0.08) and % co-efficient of variation were observed to be minimum; hence the setup was used throughout work.

STF pH 7.4 was put in to 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 in to the cell containing 2 ml of fluid. The temperature of the fluid was kept at 35 ± 1^0C constantly. At regular intervals diffusion fluid was taken to analyze for drug content using UV 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 Institutional Animal Ethics Committee (IAEC). On the day of experiments, the sterilized ocuserts 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 remaining drug content by HPLC analysis[15].

Ocular safety study

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

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

Note: 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	Non irritating
0.5 – 2.5	Practically non irritating
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

Ageing study

The optimized inserts (L6) were stored in amber colored glass bottles at 3 different temperatures 4^{o}C, Room temperature (R.T.) and 37^{o}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].

3. 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 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 formulations were ranging from 4.7±0.32 mg to 6±0.18 mg for matrix films. This indicates that there was no significant weight variation in all 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 matrix films. The minimum intrabatch variations revealed the suitability of the process used to prepare the ocuserts.

The folding endurance for all formulations was good. The maximum folding endurance of formulation L3 was 96.3±4.5 foldings and formulation L1 showed minimum folding endurance of 61±4.5 foldings (Table 4). This showed that as the concentration of polymer increased in the formulation, folding endurance was decreased.

The surface pH of the prepared inserts varied between 6.5 to 7.5, indicating that the inserts did not have an irritation potential as the pH is within the accepted ocular range.

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

S. No.	Evaluation tests	Formulations								
		L1	L2	L3	L4	L5	L6	L7	L8	L9
1	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
2	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
3	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
4	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	89± 5.8

All readings are in the form of Mean±SD

** Average of 3 determinations

- Average of 10 determinations

Formulation L1 showed 51% release within 24 hrs while L2 and L3 released nearly 100% of drug within just 10 hrs. Therefore, to get once-a-day delivery, films of EC were modeled by incorporating PEO and SA in different proportions.

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 SA. Zero order plots of L1 to L9 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 L6 was 101.35% at the end of 24 hrs and hence found to be suitable for once a day therapy. So it was taken as optimized formulation and subjected to further studies.

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**
L1	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
L2	26.54± 0.924	45.67± 1.512	61.96± 0.581	81.42± 0.348	92.65± 1.438	98.83± 0.682	-	-
L3	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 + PEO based matrix films

Formulations	**% CR at different time intervals (hr)***							
	1	**2**	**4**	**6**	**8**	**10**	**22**	**24**
L4	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
L5	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
L6	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

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

Formulations	% CR at different time intervals (hr)*							
	1	**2**	**4**	**6**	**8**	**10**	**22**	**24**
L7	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
L8	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
L9	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 8: Kinetic treatment of release study data of ocuserts

Formulations	Zero order plots		Higuchi's plots		Peppa's plots		
	r	**r^2**	**r**	**r^2**	**r**	**r^2**	**n**
L1	0.9938	0.9876	0.9225	0.8511	0.9999	0.9998	1.329
L2	0.9913	0.9886	0.9165	0.8402	0.9934	0.9868	1.216
L3	0.9843	0.9688	0.9356	0.8753	0.9954	0.9894	1.232
L4	0.9933	0.9847	0.9228	0.8515	0.9995	0.9991	1.297
L5	0.9980	0.9960	0.9397	0.8831	0.9998	0.9996	1.152
L6	0.9996	0.9991	0.9405	0.8864	0.9995	0.9990	1.036
L7	0.9888	0.9778	0.9032	0.8157	0.9996	0.9993	1.518
L8	0.9877	0.9755	0.9067	0.8221	0.9996	0.9993	1.477
L9	0.9943	0.9886	0.9241	0.8540	0.9999	0.9998	1.309

***In vivo* release study**

The results of *in vivo* release study of the ocusert L6 is shown in table-9 and figure-1. The ocusert showed 96.03% of drug release after 24 hours which was comparable to *in vitro* drug release (Table-9). Thus there was good *in vitro – in vivo* correlation for the ocusert L6 (Figure - 1) indicating the effectiveness of the formulation to be used *in vivo*.

Table 9. *In vivo* drug release data of optimized ocusert L6

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

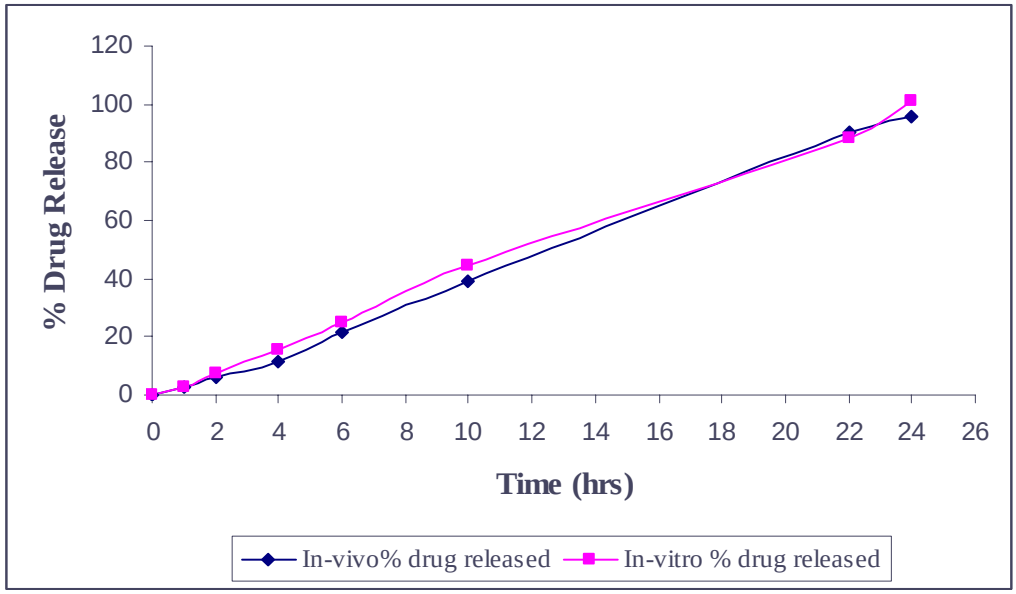

Fig. 1: *In vitro – in vivo* correlation for formulation F7 ocular safety studies

The ocular safety score of the formulation L6 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 the rate controlling membrane. Thus, it can be concluded that they were safe for ocular administration.

Ageing study

Ageing study of the ocusert L6 was performed at R.T., 4^{o}C and 37^{o}C for the period of 3 months. The results (Table-10) showed that there was no change in physical appearance of ocuserts. The drug content showed no marked change after two months and L6 passed the sterility test. These results concluded that ocusert L6 was chemically, physically and microbiologically stable at RT for 3 months. However, further studies at different temperatures and humidity conditions are needed to establish their shelf life.

Table 10: Ageing study data for the formulation L6

Time (days)	4 °C			R.T.			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±S.D.

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REFERENCES

1. Shoenwald RD. Ocular drug delivery: pharmacokinetic considerations. *Clin.* Pharmacokinet 1998; 18: 255-269.
2. Lee VHL. Drugs and pharmaceutical sciences. In: Swarbrick J editor. Precorneal, corneal and post corneal factors. New York: marcel Dekker Inc; 58, 1993. p. 59-81.
3. Attia MA, Kassem MA, Safwat S. *In-vivo* performance of dexomethasone ophthalmic film delivery system in the rabbit eye. International J Pharm 1988; 47: 21-30.
4. Barath S, Hiremath SR. Ocular delivery systems of pefloxacin mesylate. Pharmazie 1999; 54: 55-58.
5. www.medicinescomplete.com.
6. Li HY, Li FW, Ping QN. Determination of release rate, miotic activity and irritation of controlled release pilocarpine ophthalmic film. Nan Yao Xue 1985; 16: 21-27.
7. Kenawy R, Bowlin GL. Release of tetracycline hydrochloride from electrospun poly (ethylene-co-vinylacetate), Poly(Lactic acid) and a blend. J Controlled Release 2002; 81: 57-64.
8. Vemba T, Gillard J, Roland N. Influence of solvents and plasticizers on the permeability and the rupture force of ethyl cellulose film coating. Pharma Acta Helv 1980; 55: 65-71.
9. Chowdry KP, Naidu RA. Studies on permeability of ethyl cellulose films for tarnsdermal use. Eastern Pharmacist 1991; 34: 119-121.
10. Hyppola R, Husson I. Evaluation of physical properties of plasticized ethyl cellulose films cast from ethanol solution. International J Pharm 1996; 133: 161-170.
11. Yamne S, Takayama K, Nagai T. Effect of fractal dimension on drug permeation through porous ethyl cellulose films. J Controlled Release 1998; 50: 103-109.
12. Abhilash AS, Jayaprakash S, Nagarjan M, Dhachinamoorthi D. Design and

evaluation of timolol ocuserts. Indian J Pharma Sci 2005; 67 (3): 311-314.

13. Balasubramaniam J, Srinatha A, Pandit JK, Gopalnath. *In Vitro* microbiological evaluation of polyvinyl alcohol based ocular inserts of ciprofloxacin hydrochloride. Indian J Pharma Sci 2006; 68 (5): 626-630.
14. Langenbucher F. *In Vitro* assessment of dissolution kinetics: description and evaluation of a column-type method. J Pharma Sci 1969; 58: 1265-1272.
15. Dandagi PM, Manvi FV, Patil MB, Mastiholimath VS, Ratohd R. Development and evaluation of ocular films of cromolyn sodium. Indian J Pharm Sci 2004; 66 (3): 309-312.
16. Wilhelmus KR. The Draize Eye Test. Survey of Ophthalmology 2001; 45(6): 493 – 515.
17. Sankar V, Chandrasekaran AK, Durga S, Geetha G. Design and Evaluation of Diclofenac Sodium Ophthalmic Inserts. The Indian Pharmacist Nov-2005; 98-100.