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Development and Evaluation of Polymeric Sustained Release Levofloxacin Ocuserts

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ABSTRACT

The main aim of this study is to develop ocular drug delivery system for Levofloxacin; fluoroquinolone (or quinolone) anti-infective. The ocuserts were prepared by the solvent casting technique in aluminum coated Petri dishes using different polymers such as Poly vinyl pyrrolidone K30 and Chitosan at various proportions and Hydroxy Propyl Methyl Cellulose (15 cps) combinations using PEG-400 as plasticizer. The prepared ocuserts were evaluated for their physicochemical parameters. The *in vitro* drug release from the formulations was studied using commercial semi permeable membranes which were following zero order kinetics. It was also observed that increasing the proportion of PVP increases the rate of release of Levofloxacin. On the basis of In vitro release studies and stability studies, it can be concluded that this ocular inserts formulation can be a promising once-a-day controlled release formulation.

Keywords: Ocular Insert, Sustained Ocular Delivery, Levofloxacin.

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INTRODUCTION

Ophthalmic dosage forms are required to be manufactured sterile and to maintain sterility during multiple applications or administration. A number of approaches to the delivery of drugs for ocular treatment has been investigated and proposed. The residence time, has been increased by cellulosic polymers to complex systems such as penetration enhancers, external devices (collagen shields, iontophoresis, pumps), ion exchange resins, liposomes, microspheres/microparticles, polymeric films, inserts, prodrugs, mucoadhesives, and metabolism based drug design. The most common dosage forms for topical veterinary ophthalmic medications are solutions, suspensions, ointments, and gels [24]. Ocular drug delivery is one of the most fascinating and challenging tasks facing the Pharmaceutical researchers. One of the major barriers of ocular medication is to obtain and maintain a therapeutic level at the site of action for prolonged period of time.

The ocular inserts maintain an effective drug concentration in the target tissues and yet minimize the number of applications constant with the function of controlled release systems. Limited popularity of ocular inserts has been attributed to psychological factors, such as reluctance of patients to abandon the traditional liquid and semisolid medications, and to occasional therapeutic failures. A number of ocular inserts were prepared utilizing different techniques to make soluble, erodible, nonerodible, and hydrogel inserts [25].

Levofloxacin is a fluoroquinolone antibacterial active against a broad spectrum of Gram-positive and Gram-negative ocular pathogens. Levofloxacin is the pure (-)-(S)-enantiomer of the racemic drug substance, ofloxacin. It is more soluble in water at neutral pH than Ofloxacin [26]. Dose of Levofloxacin in bacterial conjunctivitis is 1-2 drops and dosing frequency is 7-8 times in a day. This drug is available in dosage forms such as in gels the dosing frequency is 3-4 times in a day. Only a few ocular inserts made of (EVA) as rate controlling membranes are available on the market (13-14). Likewise, Chitosan, PVP and HPMC are also an excellent film-forming polymer, but the films of HPMC and PVP alone are brittle. The current literatures indicate that no inserts are made of Levofloxacin using HPMC, PVP and Chitosan. Hence, this investigation has been performed to study the drug release kinetics of Levofloxacin cast with incorporating different proportions of PVP, HPMC and Chitosan.

The main objective of the ophthalmic inserts is to increase the contact time between the preparation and the conjunctival tissue to ensure a sustained release suited to topical or systemic treatment. In comparison with the traditional ophthalmic preparation i.e., eye drops, the solid ophthalmic devices presents some advantages such as

- Increasing contact time and thus improving bioavailability.
- Possibility of providing a prolong drug release and thus a better efficacy.
- Reduction of systemic side effects and thus reduced adverse effects.
- Reduction of the number of administrations and thus better patient compliance.

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MATERIALS AND METHOD

Levofloxacin was received as a gift sample from Ranbaxy (Dewas). Water soluble Chitosan was procured from Indian Sea Foods (Cochin). Hydroxy propyl methyl Cellulose (15 cps), Poly vinyl pyrrolidine (K30) and Poly Ethylene Glycol-400 was acquired from Central Drug House (CDH) (Mumbai). All other solvents and reagents (like Ethanol, Methanol, NaOH, NaCl, Na_2HPO_4, and KH2PO4 etc.) were of analytical grade and procured from MERCK (Mumbai) and SDFCL (Mumbai).

Preparation of Ocular Insert

Table 1: Formulation compositions of Ocusert of Levofloxacin

Formulation Code	Drug	Chitosan	HPMC	PVP	PEG-400
LI01	200mg	300mg.	-	-	0.2ml
LI02	200mg	-	300mg.	-	0.2ml
LI03	200mg	-	-	300mg	0.2ml
LI04	200mg	200mg	100mg.	-	0.2ml
LI05	200mg	100mg.	200mg.	-	0.2ml
LI06	200mg	150mg	150mg	-	0.2ml
LI07	200mg	200mg	-	100mg.	0.2ml
LI08	200mg	100mg.	-	200mg	0.2ml
LI09	200mg	150mg	-	150mg	0.2ml
LI10	200mg		200mg	100mg	0.2ml
LI11	200mg		100mg	200mg	0.2ml
LI12	200mg		150mg	150mg	0.2ml
LI13	200mg	100mg	100mg	100mg	0.2ml

The Levofloxacin ocular inserts based on chitosan, HPMC and PVP were prepared by solvent casting technique [6]. Polymeric solutions were prepared by dissolving HPMC, PVP and chitosan at distinct compositions (Table 1 Insert codes: LI01, LI02, LI03, LI04, LI05, Li06, LI07, LI08, LI09, LI10, LI11, LI12, LI13) along with 4% (m/V) of Levofloxacin (LVX), and PEG-400 (20%m/m) in distilled water. PVP and chitosan was added in aqueous solution of HPMC and LVX with constant stirring. The plasticizer was added thereafter and the drug polymer solutions were stirred for 5-6 h and allowed to stand overnight to remove any entrapped air bubbles. The pH range of the solutions was found to be 5-8. The solutions were then poured into glass rings (4 cm diameter and 12ml volume) placed over mercury in the glass Petri dishes. Solvent was allowed to evaporate. Dried films were carefully removed from the Petri dish and then cut into oval shaped inserts with the help of a sharp edged die (13.2mm in length and 5.4 mm in width).

Amount of Drug Loaded on Single Ring

Area of circle = πr^2 .... (1)
Diameter of the ring = 98 mm

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$$\text{Radius} = \frac{98}{2} = 49 \text{ mm} \quad \text{.... (2)}$$

$$\text{Area of the ring} = 3.14 \times 49 \times 49 = 7539.14 \text{ mm}^2 \quad \text{.... (3)}$$

$$\text{Area of single insert} = 3.14 \times 10 \times 10 = 314 \text{ mm}^2 \quad \text{.... (4)}$$

$$\text{Total number of inserts to be formulated casted} = \frac{7539.14}{314} = 24 \quad \text{.... (5)}$$

$$\text{Amount of drug loaded on single ring} = \frac{200}{24} = 8.32 \text{ mg.} \quad \text{.... (6)}$$

Evaluation Parameters

Weight uniformity

Each film was weighed individually and then the average weight of films taken as the weight of the film [20].

Thickness

The above films were evaluated for the thickness of each film using a micrometer of sensitivity of 0.001 mm (Mitutoyo, Japan). The average of 10 readings was taken. The mean thickness, standard deviation and percent coefficient of variation were calculated [1].

Folding Endurance

The folding endurance is expressed as the number of folds (number of times the insert is folded at the same place, either to break the specimen or to develop visible cracks. This test is important to check the ability of the sample to withstand folding. This also gives an indication of brittleness. The specimen was folded in the center, between the fingers and the thumb and then opened. This was termed as one folding. The process was repeated till the insert showed breakage or cracks in center of insert. The total folding operations were named as folding endurance value [2].

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Percentage Moisture Loss

The ocuserts were weighed accurately and kept in a desiccators containing anhydrous calcium chloride. After 3 days, the films were taken out and weighed [7].

$$\text{Percentage moisture loss} = \frac{\text{Initial weight- Final weight}}{\text{Initial weight}} \times 100 \quad \ldots. (7)$$

Percentage Moisture Absorption

The ocuserts were pre weighed accurately and kept in desiccators containing 100ml of saturated solution of aluminum chloride. After 3 days, the films were taken out and weighed [7].

$$\text{Percentage moisture absorption} = \frac{\text{Final weight- Initial weight}}{\text{Initial weight}} \times 100 \quad \ldots. (8)$$

Water vapor transmission

The vials of equal diameter were used as transmission cells were washed and dried. About 1gm of fused calcium chloride was taken in the cells and the films were fixed over the brim with the help of solvent. Then, the cells were weighed accurately and kept in a closed desiccators containing saturated solution of potassium chloride (200 ml) and the cells taken out and weighed after 3rd day of storage. Then, the water vapors transmitted were calculated by the following formula [7].

$$\text{WVT Rate} = \frac{WL}{S} \quad \ldots.. (9)$$

W- Gm of water transmitted
L- Thickness of film
S- Exposed surface area of film

Surface pH Determination

Inserts were left to swell for 5 hours on agar plate prepared by dissolving 2% (m/v) agar in warm simulated tear fluid (STF; sodium chloride: 0.670 g, sodium bicarbonate: 0.200 g, calcium chloride. $2H_2O$: 0.008 g, and purified water q.s. 100 g (3)) of pH 7.2 under stirring and then pouring the solution into Petri dish till gelling at room temperature. The surface pH was measured by means of a pH paper placed on the surface of swollen patch.

ISSN: 0975-8585

Swelling index

Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared ocular inserts, initial weight of insert was taken, and then placed in agar gel plate (2% *m/v* agar in STF, pH 7.2) and incubated at 37±1°C. For five hours, insert was removed from plate after every one hour, surface water was removed with help of filter paper, and insert was reweighed. Swelling index was calculated [4].

$$\text{Swelling Index } (S_w)\ \% = [w_t - w_0/w_t] \times 100 \qquad \ldots\ldots (10)$$

(S_w) % = Equilibrium percent swelling.
W_t = Weight of swollen insert after time t.
W_0 = Original weight of insert at zero time.

Drug content uniformity

Drug content was estimated by triturating ocular inserts in 20 ml of phosphate buffer pH. 7.2 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 298 nm [19].

In vitro drug release studies

In vitro drug release study was carried out by using biochemical donor- receptor compartment model [5]. The commercial semi permeable egg membrane, presoaked overnight in the freshly prepared dissolution medium (STF pH7.2), and was tied to one end of a cylinder (open at both the sides) which acted as donor compartment. The ocular insert was placed inside the donor compartment in contact with the semi-permeable membrane. The donor compartment was attached to a stand and suspended in 25 ml of the dissolution medium maintained at 37±1°C in the way that touches the receptor medium surface. The dissolution medium was stirred at a low speed using magnetic stirrer. The aliquots of 5 ml were withdrawn at regular intervals for 12h and replaced by an equal volume of dissolution medium every time. The samples were analyzed on UV spectrophotometer at 294 nm. (UV Spectrophotometer-1800)

Stability studies

The inserts were stored in amber colored glass bottles at 3 different temperatures 4^{o}C, Room temperature (R.T.) and 40^{o}C for a period of 2 months. The samples were withdrawn after 7, 15, 30 and 60 days and analyzed for physical appearance, drug content and sterility (21). The optimized formulation was packed in aluminum foil. It was then stored at 40°C / 75 % RH

according to ICH (22). Samples were withdrawn after three month and evaluated for change in drug release pattern.

Sterility testing

The sterility test [23] was carried out using by direct inoculation of the culture media with the product to be examined. Sealed package was opened using aseptic precautions and the inserts were placed in the culture medium. Then the inserts were incubated in soya-bean casein digest medium (pH 7.3) at 35 ±0.5 0C for 14 days. The experiments were performed in duplicate.

RESULTS AND DISCUSSION

Table 2: Physicochemical parameter of Ocusert of Levofloxacin

Formulation Code	Weight Uniformity (grams)	Thickness (mm.)	Surface pH	*Folding Endurance (no. of folds	% *Swelling Index
LI01	0.0151±0.0004	0.05±0.002	6.0±0.2	98.52	127.36
LI02	0.0381±0.0005	0.10±0.001	6.5±0.3	75	105.61
LI03	0.0171±0.0007	0.09±0.02	6.0±0.2	50.66	113.79
LI04	0.0351±0.0008	0.08±0.0005	6.5±0.3	77.66	117.51
LI05	0.0161±0.0004	0.15±0.003	6.5±0.3	64	124.23
LI06	0.0211±0.0008	0.08±0.002	6.0±0.25	55	113.40
LI07	0.0552±0.0006	0.13±0.002	6.0±0.2	52.66	58.70
LI08	0.0251±0.0006	0.16±0.006	6.5±0.3	65.52	55.46
LI09	0.0351±0.0007	0.15±0.003	6.0±0.2	59.33	59.65
LI10	0.0151±0.0003	0.07±0.004	6.5±0.3	60	109.27
LI11	0.0381±0.0009	0.12±0.015	7.0±0.5	57.63	95.08
LI12	0.0403±0.0009	0.11±0.01	7.0±0.5	63.25	116.42
LI13	0.0231±0.0006	0.10±0.012	5.5±0.4	53.33	124.15

The prepared inserts were translucent, light yellow to colorless and smooth in texture, uniform in appearance and show no visible crack or imperfection. The inserts had a thickness varying from 0.05 ± 0.02 to 0.20 ± 0.05 mm and weight varying from 0.0151±0.0004 to 0.0552±0.0006 g. The drug content was consistent in all batches and varied from 97.6 ± 0.10 % to 99.8 ± 0.42 %.The folding endurance of inserts ranges from 95±10. The percentage moisture absorption and moisture loss was also influenced by polymer used in the insert preparation. The moisture loss and gain was less in case of HPMC (15 cps) formulations as compared to PVP and chitosan. The equilibrium swelling % varied from 35.61± 136.42. Increase in amount of HPMC in formulation decreased swelling, which may be attributed to its relatively poor water solubility. In case of chitosan increasing amount in formulation increased swelling, which may be due to its solubility in water. The release of the drug from the insert follows zero order kinetics and up to 24 hours. There was no any change in physical appearance. The drug content and drug release of the formulations were showing no significant change in their values. The sterility of the inserts was maintained till the formulations were evaluated. The formulations

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were sterile and do not show any change physically as well as chemically upto two months at two different temperatures. The results of evaluation of inserts are shown in table II, III & IV. In vitro release studies of inserts are shown in table IV and fig. 1&2, which shows the formulations LI01, LI07, LI08, LI09 & LI12 release drug till the end of 24 hours.

Table 3: Physicochemical parameter of Ocusert of Levofloxacin

Formulation Code	% *Drug Content	% *Moisture Absorption	% *Moisture Loss	*Water Vapour Transmission (mg. /mm.)
LI01	99.34±0.97	65.55±0.25	44.00±0.23	0.0144±0.0044
LI02	83.47±0.39	63.33±0.21	32.81±0.35	0.0045±0.0005
LI03	79.56±1.77	78.15±0.15	47.09±0.42	0.0068±0.0006
LI04	83.83±1.77	71.05±0.57	34.28±0.65	0.0044±0.0007
LI05	86.55±0.48	92.23±0.28	30.81±0.43	0.0056±0.0011
LI06	85.49±3.25	86.66±0.66	46.66±0.40	0.001±0.0012
LI07	92.34±0.87	70.52±0.75	26.31±0.55	0.0058±0.0028
LI08	84.44±0.36	80.79±0.29	42.06±0.34	0.0061±0.0009
LI09	92.44±1.36	94.28±0.56	40.01±0.41	0.0091±0.0015
LI10	91.94±0.25	65.06±0.50	26.66±0.67	0.0025±0.0004
LI11	79.56±1.77	78.15±0.15	47.09±0.42	0.0068±0.0006
LI12	86.55±0.48	92.23±0.28	30.81±0.43	0.0056±0.0011
LI13	87.60±0.77	95.23±0.25	26.81±0.42	0.0049±0.0008

Table 4: % Cumulative Drug Release of Drug from Ocular insert

Time (hrs)	LI 01	LI 02	LI 03	LI 04	LI 05	LI 06	LI 07	LI 08	LI 09	LI 10	LI 11	LI 12	LI 13
0	0.29	0.45	0.11	0.17	1.26	0.91	0.98	0.68	0.86	0.22	0.39	0.59	0.75
2	5.40	2.11	7.52	3.86	8.82	7.08	6.95	4.46	5.59	1.41	5.77	3.27	5.70
4	14.64	11.98	11.68	7.12	17.63	13.23	13.19	11.08	11.26	7.69	9.86	8.22	11.26
6	24.71	35.24	18.82	11.92	29.35	22.18	22.67	19.37	23.15	19.87	17.27	12.66	22.16
8	32.74	51.62	29.22	19.65	41.47	31.23	31.21	28.44	30.21	26.67	25.11	18.97	53.86
10	39.57	75.21	36.35	25.17	54.72	40.96	39.96	36.63	39.35	38.27	31.33	23.16	75.82
20	90.96	-	91.66	83.29	-	99.38	76.53	73.39	79.22	-	98.62	75.89	-
22	92.65	-	98.25	99.69	-		88.61	86.51	87.62	-		87.55	-
24	99.48	-	-	-	-		96.28	97.89	95.67	-		99.87	-

The prepared films were evaluated for the thickness; average of five readings was taken. The mean thickness, standard deviation was calculated. All the formulations, measured thickness with low standard deviation values ensured the uniformity of the films prepared by solvent casting technique. The estimation of drug content was found to be almost same with their low standard deviation value. Cumulative percentage drug release of each film in the *in vitro* release studies was based on the mean content of the drug present in the respective films. The weight of all the films was found to be uniform indicating good distribution of drug, polymers and plasticizer. The percentage moisture absorption was calculated for all the formulations. According to the results obtained, the moisture absorption is more in the formulations where hydrophilic polymers are present. Formulation LI01 has shown the

maximum percentage moisture absorption as the film contains chitosan as polymer; due to their hydrophilic nature. Formulation LI02 has shown the minimum percentage moisture absorption. In general, it can be concluded that, the chitosan have more tendency to absorb moisture as compared to polyvinyl pyrrolidone and hydroxyl propyl methyl cellulose. At humid condition, there was more moisture absorption but there was no change in the integrity; which was observed by its physical appearance. The percentage moisture loss was calculated for all the formulations. It was observed that when the formulations were kept at very dry condition the maximum moisture loss has been occurred. Formulation LI03 & LI11 showed the maximum amount of moisture loss and formulation LI13 had shown a minimum loss of moisture. Presence of poly vinyl pyrrolidone increases the percentage of moisture loss. At different time interval sample was withdrawn and cumulative percentage drug released in mg was calculated, on the basis of mean amount of Levofloxacin present in the respective films. Formulation LI01, LI07, LI08, LI09, LI12 showed a maximum cumulative percentage drug release at the end of 24 hours. The insert containing PVP (200 mg) and HPMC (100 mg) showed a release of 99.87 % at the end of 24 hours which indicated that, the polymer combination with same quantities can be used for the formulation of ocular film for therapeutic drug management in the systemic circulation. LI13 is a combination of hydrophilic polymers. The programmed release is due to the formation of hydrogen bonds between the drug and polymers which have helped in rate control release of drug. Chitosan also has good adhesive property which is helpful, when the ocular film is inserted in the cul-de-sac.

After the 2 month stability studies at both temperatures (4^{o}C & 40^{o}C) of the insert were shows good results in their physical appearance, drug content, sterility and drug release.

CONCLUSION

It was observed that increasing the proportion of PVP in to HPMC and Chitosan increases the rate of release of Levofloxacin. On the basis of In vitro release studies, it can be concluded that ocular insert formulation LI01, LI07, LI08, LI09, LI12 can be a promising once-a-day controlled release formulation.

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