Available online on 15.09.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

FORMULATION AND EVALUATION OF *IN SITU* OCULAR GEL OF LEVOFLOXACIN

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ABSTRACT

Total of 8 formulations of *in situ* gels of Levofloxacin hydrochloride were prepared by pH triggered *in situ* gelling system using different polymers like sodium alginate as gelling agent, Noveon AA-1 polycarbophil and HPMC E50LV as viscosity enhancing agent and benzalkonium chloride as preservative. All the prepared formulations were clear and the visual appearance was found to be transparent. The pH of the prepared formulations was ranged between 6.50and 7.00. The drug content varied between 96.84 ± 0.396 and 99.65±0.489 % which indicated that the uniform distribution of drug was found in all the prepared formulations. Among all the formulations, the formulations A4 and A8 showed better gelling capacity. The shear rate on the preparation was large during the blinking stage. From the in vitro drug release profile the formulations A4 and A8 was selected as the best formulations and these formulations were used for further studies such as mechanism of drug release, sterility, antimicrobial efficacy, ocular irritation and accelerated stability. Both formulations provided good fit to the Higuchi model. According to this model, the drug release from these gels may be controlled by diffusion through the micro-pores. The selected formulations showed good anti-microbial action against the organisms and ocular irritation studies revealed that the selected formulations were good with non-irritation and there were no ocular damage or abnormal clinical signs. During and at the end of the accelerated stability study, the selected formulations did not undergo any chemical changes/interaction and remained stable during the study period and showed almost similar physical stability and drug content.

Key Words: Levofloxacin hydrochloride, *in situ* gels, *in vitro* drug release, Higuchi model, accelerated stability study.

Article Info: Received 10 July, 2017; Review Completed 30 Aug, 2017; Accepted 31 Aug, 2017; Available online 15 Sep, 2017

Cite this article as:

Prasanth VV, Parambi DGT, Ranjan S, Formulation and evaluation of in situ ocular gel of levofloxacin, Journal of Drug Delivery and Therapeutics. 2017; 7(5):68-73

DOI: http://dx.doi.org/10.22270/jddt.v7i5.1489

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INTRODUCTION

Conventional liquid ophthalmic preparations reveal low bioavailability because of a steady lacrimal drainage in the eye. The average drainage of an instilled drug quantity commences instantly upon instillation and is basically accomplished within 5 min. Typical ophthalmic bioavailability (1–10%) are achieved owing to the short pre-corneal residence time of ophthalmic solutions. As a result there is a need for recurrent instillation of concentrated solutions to attain the preferred curative effect[1].

Ocular drug delivery is one of the major challenging and motivating activities which the pharmaceutical scientists are facing now a day, the main difficulty encountered to

the pharmaceutical scientists is fast pre-corneal removal of the drug, ensuing in deprived bioavailability and healing response because of high lacrimal fluid yield. *In situ* formed gels are liquid which upon insertion, experience phase conversion in the *cul de sac* of eye to form a visco-elastic gel and these formed gels tender a reaction to environmental changes. In the last few years, a notable number of pH, novel temperature, and ion induced *in situ* gel forming systems have been reported for constant ocular delivery of drugs[2]. The reduced bioavailability and curative response showed by conventional ocular solutions due to quick pre-corneal drug elimination may be sweeped over by the use of a gel system which are instilled into the *cul de sac* as a drop and which undergoes a sol-gel conversion in eye[3].

LEV, Biopharmaceutical Classification System I, is a broad spectrum anti-infective agent, under the third generation fluoroquinolone derivative mainly used in the infection of the eye such as acute conjunctivitis. The recommended dosage of LEV for the treatment of bacterial conjunctivitis is 1 or 2 drops of 0.5% solution in the affected eyes for every 2 hours upto 8 times for 2 days, then 1 or 2 drops every 4 hours up to 4 times for next 5 days[4]. LEV is quickly and fully absorbed subsequent to oral administration. Peak plasma concentrations are typically attained one to two hours subsequent to oral dose. The normal terminative plasma elimination half-life of levofloxacin is ranging from around 6 to 8 hours consequent to single or multiple doses of levofloxacin administered either intravenously or orally[5].

In the present study an endeavor was prepared to develop an *in situ* ocular gel of LEV to increase ocular contact time, enhance the corneal permeability and site specificity for the better treatment of conjunctivitis and corneal ulceration with reduced adverse effects and better patient compliance.

MATERIALS AND METHODS

Materials

Levofloxacin (LEV) was obtained as a gift sample from Caplin Point Laboratories Ltd, Chennai, India. Hydroxypropyl methylcellulose (HPMC E50LV), Noveon AA-1polycarbophil, Sodium alginate, Benzalkonium chloride, Sodium chloride and Sodium hydroxide were purchased from SD Fine Chemicals, Bangalore, India. All the chemicals and reagents used were of analytical grade.

Methods

Formulation of *in situ* gels of LEV

The composition of different formulations of LEV *in situ* ocular gels is shown in **Table 1**. The sodium chloride (0.9% w/v) was dissolved in 50 mL of distilled water and viscosity enhancifier was added to the above solution and stirred slowly with a magnetic stirrer. Care was taken that no lumps were formed during stirring. The polymers were sprinkled over this solution and allowed to hydrate overnight and stirred using a magnetic stirrer. LEV was dissolved in phosphate buffer (pH 7.4) and benzalkonium chloride was added and the solution was filtered through 0.2 µm cellulose acetate membrane filter. The drug solution was added to the polymeric solution under constant stirring until a uniform solution was obtained. The pH of the formulation was then adjusted to 6.50 using 0.1 N NaOH. The developed formulations were filled in glass vials, closed with gray butyl rubber closures and sealed with aluminium caps. The formulations in their final pack were subjected to terminal sterilization by autoclaving at 121° C at 15 psi for 20 min[6].

Table 1: Formulation of *in situ* ocular gel of LEV

Compositions	Concentration (% w/v)							
	A1	A2	A3	A4	A5	A6	A7	A8
Levofloxacin	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
HPMC E50LV	0.2	0.3	0.4	0.5	0.2	0.3	0.4	0.5
Noveon AA-1 polycarbophil	0.5	0.5	0.5	0.5	-	-	-	-
Sodium alginate	-	-	-	-	0.5	0.5	0.5	0.5
Benzalkonium chloride	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Sodium chloride	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Sodium hydroxide	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Phosphate buffer pH 7.4 (mL)	100	100	100	100	100	100	100	100

Evaluations

Clarity, visual appearance, pH and Drug content

The prepared formulations were observed for appearance and clarity by visually adjacent to black and white surroundings. The surface pH of the in situ ocular gels were determined by keeping the digisun digital pH meter on the formulations and allowed equilibrates for one minutes[7]. For drug content, sample (1 mL) was allowed to dissolve in 10 mLof STF solution. Aliquot of 1 mL was withdrawn and further diluted to 10 mL with STF. The resultant solution was filtered through filter paper and the amounts of LEV present in the in situ ocular gels are determined using UV Spectrophotometer at 287nm (Shimadzu 1800, Japan).

In vitro gelation studies

The gelling capability of the prepared formulation was determined by introducing a drop of the formulation in a vial containing 2 mL of freshly prepared STF and

visually observed. The time taken for its gelling was noted[8,9].

Rheological studies

The Brookfield viscometer LVDV-E was used to measure the viscosity of the LEV in situ ocular gels. The in situ gel formulations were placed in the sampler tube. The samples were analyzed by a circulating shower at 37 °C ± 0.5 °C linked to the viscometer adaptor prior to every determinations. The angular velocity of the spindle was increased from 1 to 4 rpm and the viscosity of the formulation was measured [10, 11].

***In vitro* drug release studies**

Modified diffusion apparatus was used to carry out the *in vitro* release of LEV from developed in situ ocular gels and STF (pH7.4) was used as a diffusion medium[12]. The soaked cellophane membrane in the diffusion medium for the night, was fixed to one end of a specially designed glass cylinder which was opened at both ends. The LEV ocular gel was accurately placed into the glass cylinder (donor compartment) and this cylinder was immersed in a beaker (receptor compartment) containing 50 mL of diffusion medium at a 37 ± 2 °C with 50 rpm, so that the membrane touches the surface of the medium. Sample (1 mL) was withdrawn at a predetermined time intervals (1, 2, 3, 4, 5, 6, 7 and 8 hours) and replaced with an equal volume of fresh diffusion medium. The aliquots were diluted with the diffusion medium and assayed at 287 nm using UV Spectrophotometer (Shimadzu 1800, Japan). Parallel 2 mL of marketed formulation of Levofloxacin 0.5 % (5mg/ml) - Quixin were studied in the similar manner. The mechanism of drug release from the ocular gel was determined by finding the best fit of the release data to Higuchi and Korsmeyer-Peppas plots. The release rate constants 'k' and 'n' of each model were calculated by linear regression analysis using Microsoft Excel 2010 software. Coefficients of determination (r^2) were used to evaluate the accuracy of the fit[13, 14].

Sterility

Direct inoculation method was used to perform the sterility of the prepared formulations. From the test solution (2 mL) was withdrawn using a sterile pipette and aseptically transferred to fluid thioglycolate medium (20 mL) and soyabean-casein digest medium (20 mL) separately. After inoculation the media was incubated for not less than 14 days at 30-35 °C in the case of fluid thioglycolate medium and 20-25 °C in the case of soyabean-casein digest medium[15].

Antimicrobial efficacy studies

The drug was allowed to diffuse through a solid agar medium. The standard minimum inhibitory concentration (MIC 2 µg/mL) of LEV and developed formulations containing LEV were prepared. Antimicrobial activity was determined by agar diffusion test employing cup plate technique. *Staphylococcus aureus*, *Pseudomonas aeruginosa* were used as the test organisms to study the antimicrobial efficacy (biological activity). The standard minimum inhibitory concentration (MIC 2 µg/mL) of control and developed formulations (10 µg/mL) containing LEV were prepared. The solutions were poured in to cups bored into sterile nutrient agar previously seeded with test organisms (*Pseudomonas aeruginosa*, and *Staphylococcus aureus*). The drug was allowed to diffuse through a solid agar medium. After the diffusion of the solutions for 2 hours, the agar plates were incubated at 37 ºC for 24 hours. The Zone of inhibition (ZOI) was measured around each cup and compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit. Both positive and negative controls were maintained during the study[15].

Ocular irritation studies

Ocular irritation study was performed for selected formulations using male albino rabbits (four), each weighing about 2 to 3 kg. 0.1 mL of the selected sterile LEV formulation was instilled in to cul-de-sac twice a day for a period of 14 days. The rabbits were monitored periodically for redness, swelling, watering of the eye[16].

Accelerated stability studies

Selected sterile LEV formulations were filled in glass vials, closed with gray butyl rubber closures and sealed with an aluminium caps. The vials contain optimized formulation were kept in stability chamber, maintained at 40 ± 2°C and 75 ± 5 % RH for one month. Samples were withdrawn weekly and estimated for drug content, pH, visual appearance, gelling capacity and in vitro drug release[17].

RESULTS AND DISCUSSION

Evaluation of *in situ* gels of LEV Hydrochloride

Total of 8 formulations of *in situ* gels of Levofloxacin hydrochloride were prepared by pH triggered *in situ* gelling system using different polymers like sodium alginate as gelling agent, Noveon AA-1 polycarbophil and HPMC E50LV as viscosity enhancing agent and benzalkonium chloride as preservative.

Appearance, Clarity, Determination of pH and Drug content

The visual appearance, clarity and the pH of the prepared formulations are shown in **Table 2.** All the prepared formulations were clear and the visual appearance was found to be transparent. Terminal sterilization by autoclaving had no effect on physicochemical properties and the clarity of the formulations. The pH, solubility and stability is one of the most significant factor concerned in the ophthalmic preparation. The two areas of considerable significance are the effect of pH and stability of the prepared formulation. During the formulation of in situ gels, care has been taken that there should not be any irritation while administration. The pHof the prepared formulations was ranged between 6.50 and 7.00. The drug content varied between 96.84 ± 0.396 % and 99.65±0.489 % which indicated that the uniform distribution of drug was found in all the prepared formulations. Among all the formulations, formulation code A4 showed maximum drug content.

Table 2: Appearance, Clarity, pH and Drug content of *in situ* ocular gels of LEV

Formulation Code	Appearance	Clarity	pH*	Drug content (%)*
A1	Transparent	Clear	6.70	97.57±0.561
A2	Transparent	Clear	6.62	97.96±0.624
A3	Transparent	Clear	6.93	98.64±0.341
A4	Transparent	Clear	6.98	99.65±0.489
A5	Transparent	Clear	6.50	96.84±0.396
A6	Transparent	Clear	6.82	98.12±0.474
A7	Transparent	Clear	6.78	98.61±0.547
A8	Transparent	Clear	7.00	99.09±0.647

*Mean ± S.D, n=3

In vitro gelation studies

Among all the formulations, the formulations A4 and A8 showed better gelling capacity **Table 3.** This may be due to the higher uptake capacity of the polymers.

Rheological studies

The rheological properties (pre gelation and post gelation viscosity studies) of the prepared *in situ* ocular gels of LEVare shown in **Figure 1- 2.** The results showed that the viscosity of all the prepared formulations reduced as the shear rate increased, which indicated the character of pseudo plastic fluid. The shear rate on the preparation was large during the blinking stage. If the viscosity is too high, this will result in irritation in the eye and it is too low, it may produce increased drainage. Therefore, the formulation should have optimum viscosity for easy instillation into the eye as liquid, which will go through a rapid sol-to-gel transition, hence the good gelling capacity.

Table 3: Gelling capacity of *in situ* ocular gels of LEV

Formulation code	Gelling Capacity
A1	+
A2	+
A3	++
A4	+++
A5	+
A6	+
A7	++
A8	+++

+Gels after few minutes and dissolves rapidly
++ Gels immediately and remains for few hours
+++ Gels immediately and remains for extended period of time

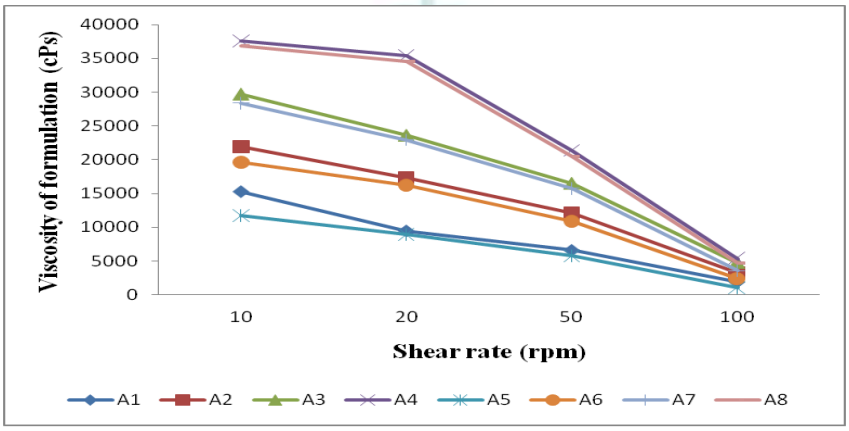

Figure 1: Pre-gelation viscosity studies of *in situ* ocular gels of LEV

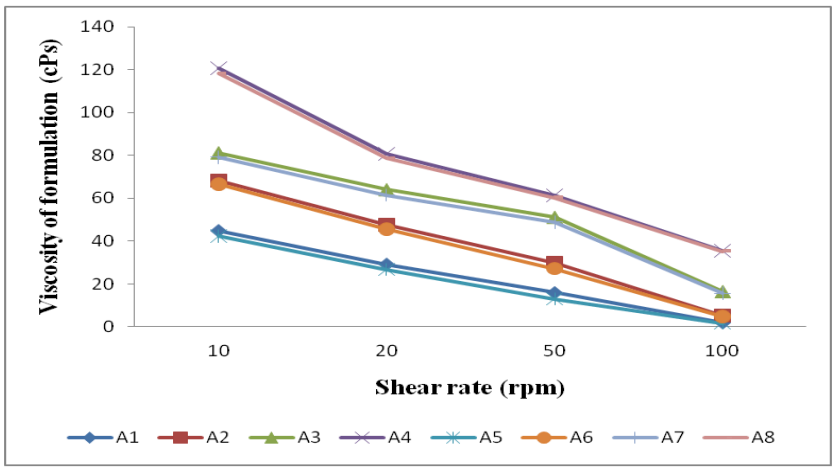

Figure 2: Post-gelation viscosity studies of *in situ* ocular gels of LEV

In vitro drug release studies

The *in vitro* release of LEV from the *in situ* ocular gelsis shown in **Figure 3.** The prolonged drug release was showed by the formulation A8 98.72% at a period of 8 h and followed by the formulation A4 96.19%. The formulations A3 showed 95.1% and A7 showed 97.7% maximum drug release for a period of 7 hours. The formulations A1 showed the maximum drug of release 97.95%, A2 showed 96.34%, A5 showed 97.13% and A6 showed 96.54% after 6 hours. The extended period of drug release may be due to relaxed diffusion of drug from the formulations. The combinations of polymers might have played an important role in diffusion and thereby exhibit controlled drug delivery. Moreover formation of gel matrix between drug and polymers might have helped to attain the rate controlled release. From the in vitro drug release profile the formulations A4 and A8 was selected as the best formulations and these formulations were used for further studies such as mechanism of drug release, sterility, antimicrobial efficacy, ocular irritation and accelerated stability.

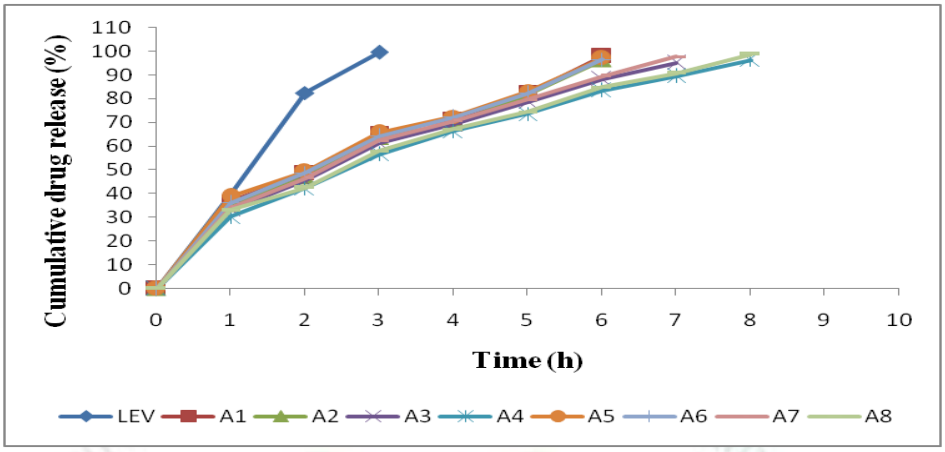

Figure 3: *In vitro* drug release of the formulations A1 – A8

Mechanism of drug release

The mechanisms of drug release from the selected in situ ocular gels were determined by finding the best fit of release data to Higuchi and Korsmeyer-Peppas plots. The release rate constant '*k*' and '*n*' of each model were calculated by linear regression analysis. Co-efficient of determination (r^2) was used to evaluate the accuracy of the fit. The r^2, k and n values are shown in **Table 4.**

Table 4: The r^2, k and n values of selected formulations

Formulations	Higuchi		Korsmeyer–Peppas		Mechanism of drug release
	r^2	k (h^{-1})	r^2	n	
A4	0.9984	0.2552	0.9976	0.4788	Diffusion
A8	0.9982	0.2883	0.9971	0.4979	Diffusion

Both formulations provided good fit to the Higuchi model. According to this model, the drug release from these gels may be controlled by diffusion through the micropores.

Sterility and Antimicrobial efficacy and Ocular irritation studies

The sterility studies indicated that the selected formulations were sterile when incubated for a minimum of 14 days at 30-35 ºC in case of fluid thioglycolate medium and at 20-25 ºC in the case of soya bean-casein digest medium. The antimicrobial efficiency of the selected formulation is shown in **Table 5.** After incubation, the result indicated that the selected formulations showed good anti-microbial action against the organisms. The ocular irritation studies revealed that the selected formulations were good with non-irritation and there were no ocular damage or abnormal clinical signs.

Table 5: Antimicrobial studies of the selected formulations

Test micro organisms	Diameter of the zone of inhibition produced by *in situ* gels (mm)		
	LEV	A4	A8
Staphylococcus Aureus	26	26	25
Pseudomonas Aeruginosa	33	33	33

Accelerated stability studies

During and at the end of the accelerated stability study, the selected formulations did not undergo any chemical changes/interaction and remained stable during the study period and showed almost similar physical stability and drug content.

CONCLUSION

LEV is a broad spectrum anti bacterial agent used in the treatment of various ocular infections. *In situ* ocular gels of LEV were prepared and *in vitro* drug release indicated that it is potential drug delivery of LEV. The optimized formulations (A4 and A8) showed good antibacterial efficacy with non irritant character. *In vivo* studies are warranted to confirm these results in future.

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