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ResearchArticle

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FORMULATION AND EVALUATION OF GELRITEBASED IN SITU OPHTHALMIC GEL FOR CONTROLLED RELEASE OF DRUG USING RABBIT EYE MODEL.

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ABSTRACT

The purpose of our work to formulate and evaluate an ophthalmic delivery system of a fluoroquinolone antibiotic, levofloxacin. Levofloxacin is an antibacterial agent which exhibits rapid precorneal elimination and poor ocular bioavailability, when given in the form of conventional ophthalmic solutions. To overcome this, an attempt has been made to formulate in situ gelling system of levofloxacin, based on the concept of ion-activated in situ gelation, which upon instillation as drops into the eye undergo a sol-gel transition in the cul-de-sac. This may result in better ocular bioavailability and provide sustained release of the drug. Gelrite®gellan gum, a novel ophthalmic vehicle, which gels in the presence of mono or divalent cations present in the lacrimal fluid, was used as the gelling agent in combination with HPMC (Methocel E50LV) which acted as a viscosity enhancing agent. The formulations were evaluated for rheological characteristics, in vitro release behavior, antimicrobial efficacy and ocular irritancy studies. We found that in situ gelling formulations passed the test for sterility. These results demonstrate that the gellan gum-HPMC mixture can be used as an in situ gelling vehicle to enhance ocular bioavailability and patient compliance. The developed formulation was stable, non-irritant and provided sustained release over 8-hour period and it can be considered as a viable alternative to conventional eye drops

Keywords :Ion activated- in situ gel, ophthalmic delivery, Gelrite, Controlled release.

INTRODUCTION

Various applications like solutions, suspensions, and semisolids (ointments and gels) are conventionally available as ophthalmic drug delivery systems for treatment of ocular diseases. When a drug solution is dropped into the eye, it results in rapid precorneal kinetics with a 10-fold reduction in the drug concentration within 4–20 min, due to the effective tear drainage and blinking action [1]. The limited permeability of the cornea contributes to the low absorption of drugs. Due to tear drainage, most of the administered dose passes via the nasolacrimal duct into the GI tract, leading to side-effects [2].

Rapid elimination of the both solutions and suspensions administered often results in a blurred vision, poor patient acceptance, short duration of the therapeutic effect making a frequent dosing regimen necessary [3].

Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination. Successful results have been obtained with inserts [4],[5], nanoparticles [6], in situ gel [7], [8] and microemulsion [9] etc. A significant increase in the precorneal residence time of drug and consequently better bioavailability can be achieved by using delivery system based on the concept of *in situ* gel formation.These types of systems prepared from polymers that exhibit reversible phase transitions (sol–gel–sol) and pseudoplastic behavior to minimize interference with blinking [10]. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye which, upon exposure to physiological conditions, changes to the gel phase [11], thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability.

Depending on the method employed to produce the sol to gel phase transition on the ocular surface, the following three types of systems have been used: pH-triggered systems including cellulose acetate hydrogen phthalate latex [12],[13], carbopol [14], [15], [16], [17], [18], temperature dependent systems including pluronics [19], [20], [21], [22], [23], tetronics [24], [25], and polymethacrylates [26], and ion-activated systems including Gelrite® [27], [28], [29], gellan [30], [31]and sodium alginate [32].

Levofloxacin, a fourth generation fluoroquinolone antiinfective agent, optically active L-isomer of ofloxacin and two fold more potent than ofloxacin and reported to be more effective in the treatment of external infections of the eye, such as acute and subacute conjunctivitis, bacterial keratitis and keratoconjunctivitis. Levofloxacin is favoured by simple pharmacokinetics with very high bioavailability and no metabolism resulting in high concentration and in low risks for drug-drug interaction. Levofloxacin is available in the market as a conventional dosage form such as tablets, capsules, and parenterals for the treatment of bacterial infections but no suitable means for the treatment of infection locally.

The objective of the present study was to develop an ophthalmic controlled delivery of an ion activated in situ gelling system of Levofloxacin with rate controlling polymers, which has a prolonged action, and show the antibacterial activity against Gram-positive and Gram-negative bacteria directly at the site of infection without loss of dosage. The various combinations of Gelrite® gellan gum with sodium alginate and HPMC were used for the preparation of eye drops of

levofloxacin (0.5%w/v) which undergo gelation when instilled into the cul-de-sac of the eye and provide controlled release of the drug during treatment of ocular infections.

MATERIALS & METHODS

Levofloxacin was obtained from Zydus Healthcare, Ahmedabad. Gelrite® was a gift sample from CP Kelco, USA. HPMC (Methocel®E50LV) was kindly gifted by Colorcon Asia Pvt. Ltd., Goa, India. All other reagents, chemicals and solvents were of analytical grade.

Method of preparation

Gelrite based in situ gel systems were prepared by dissolving gellan alone and its combination with sodium alginate and/or HPMC in hot phosphate buffer (70˚C, prepared in fresh water for injection under laminar flow) pH 7.4, by continuous stirring at 40-45˚C (Table 1). Then the weighed quantities of levofloxacin added to give final drug concentration of 0.5% w/v. Mannitol (5% w/v), methyl paraben (0.05% w/v) and propyl paraben (0.01% w/v)were added to the polymeric solution and stirred until dissolved. The solutions were transferred into previously sterilized amber-colored glass vials, capped with rubber bungs and sealed with aluminium caps. The formulations were sterilized by terminal autoclaving at 121˚C, and 15 PSI pressure for 20 min. The sterilized formulations were stored in a refrigerator at 4-8˚C until use.

Evaluation of formulation

The prepared formulations were evaluated for sol-gel transformation studies by gelation studies, viscosity studies, gel strength studies by QTS 25 Texture analyzer (Brookefield), identification of drug and drug – polymer interaction studies by FTIR spectroscopy (IR – 8400 S, Shimadzu), drug content, and in vitro release by UV spectrophotometer (Shimadzu UV-2450) at 287nm, bioadhesive strength measurement, antimicrobial efficacy studies, in vivo ocular irritation studies, sterility and stability studies.

Gelation Studies

The gelation studies were carried out in a vial containing simulated tear fluid (STF solution) as gelation solution. The composition of STF was sodium chloride 0.670 g, sodium bicarbonate 0.200g, calcium chloride dihydrate 0.008g and purified water q.s. to 100 g, which stimulate either the divalent cation content or both the divalent cation content and

protein of the tear fluid. The preparation (100 µl) was carefully placed into the vial using a micropipette, and 2 ml of gelation solution (STF) was added slowly. Gelation was assessed by visual examination.

Rheological Studies

The prepared formulations were poured into the small volume adaptor of the Brookfield synchrolectric viscometer (LVDVI prime). Viscosity was measured at different angular velocities at a temperature of 37±1˚C. The angular velocity was increased from 0.5 to 100rpm with 6sec between two speeds. The hierarchy of the angular velocity was reversed. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate.

Drug Content Uniformity

The vials (n=3) containing preparations were shaken for 2-3 min and 100 µl of the preparations were transferred aseptically to sterile 25-ml volumetric flasks with a micropipette and the final volume was made up with phosphate buffer pH 7.4. The concentration of levofloxacin was determined at 287 nm.

In Vitro Release Studies

The studies were carried out using Franz diffusion cell with STF (pH 7.4) as dissolution medium. The cell consisted of glass donor and receptor compartment (30 ml). The prepared formulations were placed in donor compartment and freshly prepared STF in receptor compartment. Between donor and receptor compartment cellophane membrane (pore size – 0.45µm) was placed. The whole assembly was placed in the thermostatically controlled shaker water bath. The temperature of the medium was maintained at 37˚C± 0.5˚C. Aliquots were withdrawn at predetermined time intervals of 1, 2, 4, 6, 8, 10, and 12 hours and replaced by equal volumes of dissolution medium. The withdrawn aliquots were diluted with STF and analyzed by UV spectrophotometer at 287nm.

Infrared Spectoscopy studies

The identification of drug and drug-polmer interactions were studied by FTIR studies (Shimadzu 8400S). The FTIR graph of pure drug and combination of drug with polymer were recorded using KBR pellets.

Antimicrobial Efficacy Studies

This was determined by agar diffusion test employing the cup plate technique for marketed eyedrops of levofloxacin Quixin® (standard preparation) and the developed formulation. All the above solutions were sterilized and poured into the cups bored into the sterile nutrient agar seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37±0.5 ˚C for 24 hours and zone of inhibition (ZOI) was measured around each cup and compared with the control. Both positive and negative controls were maintained throughout the study.

Sterility testing

Sterility testing was carried out by incubating formulation for not less than 14 days at 30-35˚C in the fluid thioglycolate medium to find the growth of bacteria and at 20-25 ˚C in the soyabean-casein digest medium to find the growth of fungi in the formulation.

In Vivo Ocular Irritation Studies

In vivo ocular irritation studies were performed according to the Draize technique [33]. Six albino rabbits each weighing 2-3 kg were used for the study of the formulations. The sterile formulation was instilled twice a day for a period of 21 days and the rabbits were observed periodically for redness, swelling, and watering of the eye.

Stability Studies

Stability studies were carried out on optimized formulation according to ICH guidelines. A sufficient quantity of formulation in amber-colored vials was stored in stability chamber at 40±0.5˚C and 75±5% RH for period of 6 months. The samples were withdrawn at 0, 30, 60, 90, and 180 days and evaluated for appearance, pH, gelling capacity and drug content during the study period.

Results and discussion

Various trial batches have been prepared to optimize the concentration of gellan gum, sodium alginate and HPMC and evaluated for gelling capacity, drug content and rheology behaviour. Six formulations of levofloxacin hemihydrates in situ gelling systems were prepared by using various concentrations of gellan gum along with sodium alginate and HPMC in different ratios as shown in Table 1. The drug content, gelling capacity of the formulations were mentioned in Table 1. All the formulations showed instantaneous gelation when contacted with the gelation fluids. The drug content of all the

formulations was within the range of 98% to 100%, showed the uniform distribution of drug in the ophthalmic formulations. The pH of all the formulations was found to be within the range of 6.2 to 6.4, which is desirable for the ophthalmic formulations.

Rheological studies

Viscosities of prepared formulations were determined and shown in Figure 1. All formulations exhibited psuedoplastic property, as evidenced by shear thinning and an increase in the shear stress with increased angular velocity. Such viscoelastic fluids with low viscosity under conditions of high shear rate and high viscosity under the conditions of low shear are generally preferred. No change in the viscosity of the formulations was observed after autoclaving. All measurements were performed in triplicate with good reproducibility.

In vitro release studies

Figure 2 shows the release profiles of prepared formulations. The results indicated that the formulation LV6 showed better sustaining effect amongst all formulations due to the presence of higher concentration of gellan gum with HPMC.The initial fast release would be beneficial as it would help to achieve the therapeutic concentration of drug in a minimum time, and the constant release later on would then provide a sustained and controlled release of the drug. The fast release might be due to initial migration of the drug toward the surface of the matrix.

The *in vitro* release profile of LV6 was compared with marketed formulation of levofloxacin in figure 3. It was found that the drug release was about 19.34% and 4.63% for marketed product and LV6 respectively after initial 15min. And at the end of two hours the drug release was 100% and 15.30% for marketed product and LV6, respectively. The comparative release was shown in figure 3. Results indicated that, the drug release was significantly controlled by using the *in situ* gelling system with polymers gellan gum and HPMC.

Goodness of fit test for optimized batch was conducted using various models like Zero order, First order, Higuchi, Korsmeyer-Peppas, Weibull and Hixon-Crowell. Release of formulation LV6fitted to Higuchian matrix equation showing high R square value (0.982), least SSR value (186.25) and F value (20.7) as compared to other formulations. Thus, it can be concluded that release of Levofloxacin from in situ gelling formulation was based on Higuchian matrix diffusion

controlled mechanism. From the Higuchi matrix diffusion model equation, it was found that entire drug was released within 12 hrs.

From the results it is concluded that the high viscosity plays important role in controlling the release of drug from the formulations. When the polymer concentration increases drug release decreases, and when polymer concentration decreases drug release from the formulation increases.

Infrared studies

The IR spectrum of the pure Levofloxacin sample recorded by FTIR spectrometer, which was compared with standard functional group frequencies of Levofloxacin. It wasshown that, functional group frequencies of Levofloxacin were in the reported range which indicates that the obtained sample was of Pure Levofloxacin.The individual IR spectra of the drug and polymers as well as the combination spectra of the drug and polymer (figure 4) indicate no specific interaction between levofloxacin and polymers when compared with infrared spectrum of pure drug as all functional group frequencies were present.

Antimicrobial efficacy studies

The in situ gelling formulation (LV6) showed antimicrobial activity when tested microbiologically by the cup plate technique. Clear zones of inhibition were obtained in the case of LV6 formulation and marketed eye drops. The diameter of the zone of inhibitions produced by formulation against both test organisms were either on par or higher than that produced by marketed eye drops in most of the cases (Table 2). Overall the ZOI values against *P. aeruginosa* were higher than that against *S. aureus*. The antimicrobial effect of levofloxacin in situ gel formulation is probably due to a fairly rapid initial release of drug into the viscous solution formed by dissolution of gel, followed by formation of a drug reservoir that attributed to the slow and prolonged diffusion of the drug from the polymeric solution due to its higher viscosity.

Sterility test

The formulation LV6 passed the test for sterility as there was no appearance of turbidity and hence no evidence of microbial growth when incubated for not less than 14 days at 30-35 ˚C in case of fluid thioglycolate medium and at 20-25 ˚C in the case of soyabean casein digest medium.

Ocular irritation studies

Local Irritation test was performed to provide estimation of human ocular response to the tested products. Griffith and his team [34] have reported a good correlation between rabbit eye response and human eye response. The normal average of blinking counts in rabbits was documented to be 2-5 times/min [35]. The counted blinking rates obtained after instillation of optimized formulation was within the normal range (3-5 blinks/min). Ocular irritation studies indicated that formulation was nonirritant. The formulation was very well tolerated by the eye of rabbit. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible. We observed no redness, swelling, or excessive lachrymation after instillation of formulation LV6. The results are shown in Table 3.

Stability studies

From the result, it has been observed that the formulation was sterile at the end of 6 months and no change in appearance, clarity and pH. Further it was observed that drug content and gelling capacity of the formulation was least affected.

Conclusions

Levofloxacin, a broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as ion-activated in situ gel-forming ophthalmic solutions using Gelrite® gellan gum as a gelling agent in combination with HPMC as a viscosity-enhancing agent. Combining gellan with sodium alginate did not offer any advantage over other formulations without sodium alginate. The formulation underwent gelation in the cul-de-sac upon instillation as drops into the eye. The gel formed in vitro produced sustained drug release over 8 hours period and the developed formulations were devoid of any deleterious effect on the ocular tissues. Stability data recorded over a 6-month period under accelerated temperature conditions indicated that the formulation is stable. This new formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its sustained drug release, higher viscosity, longer pre-corneal residence time, and better miscibility with the lacrimal fluid. Also important is its ease of administration and reduced frequency of administration resulting in better patient acceptance and compliance.

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TABLES WITH CAPTIONS

Table 1. Composition of prepared in situ gelling systems

Table 2. Antimicrobial efficacy of best formulation

Table 3. Ocular irritation testing of LV6 (OECD guidelines)

Table 1

Batch	**Gellan (%w/v)**	**SA (%w/v)**	**HPMC (%w/v)**	**Gelling capacity**	**Drug content %**
LV1	0.3	0.15	-	+++	98.13±0.51
LV2	0.3	0.15	0.5	+++	99.05±0.25
LV3	0.3	-	0.5	++	98.69±0.17
LV4	0.5	0.15	-	+++	98.46±0.22
LV5	0.5	0.15	0.5	+++	99.78±0.38
LV6	0.5	-	0.5	+++	98.88±0.12
++ gelation immediate and remains for a few hours; +++ gelation immediate and remains for an extended period.					

Table 2

Concentration (µg/ml)	**Zone of Inhibition (cm) (% efficiency)**	
	STD	**LV6**
	S. aureus	
1	1.4	1.4 (100)
10	2.0	2.2 (110)
100	3.2	3.8 (118.75)
	P. aeruginosa	
1	1.4	1.8 (128.57)

10	2.2	2.5 (113.63)
100	3.8	4.2 (110.52)
STD = standard (marketed eye drops); LV6 = best formulation. Values in parenthesis indicate the percent efficiency; percent efficiency was calculated by (ZOI of test/ZOI of standard) x 100.		

Table 3

Formulations	Average Score
Blank of LV6	0
Formulation LV6	0
Positive control	9 ± 0.233
Negative control (0.9% w/v sodium chloride solution)	0

LEGEND TO FIGURES

Figure 1. Rheological profiles of prepared formulations

Figure 2. In vitro release profile of the prepared formulations

Figure 3. Comparison of in vitro release of LV6 and marketed formulation

Figure 4. Infrared spectroscopy studies of (a) IR spectrum of drug, (b) Ir spectrum of drug and polymer

Figure 1

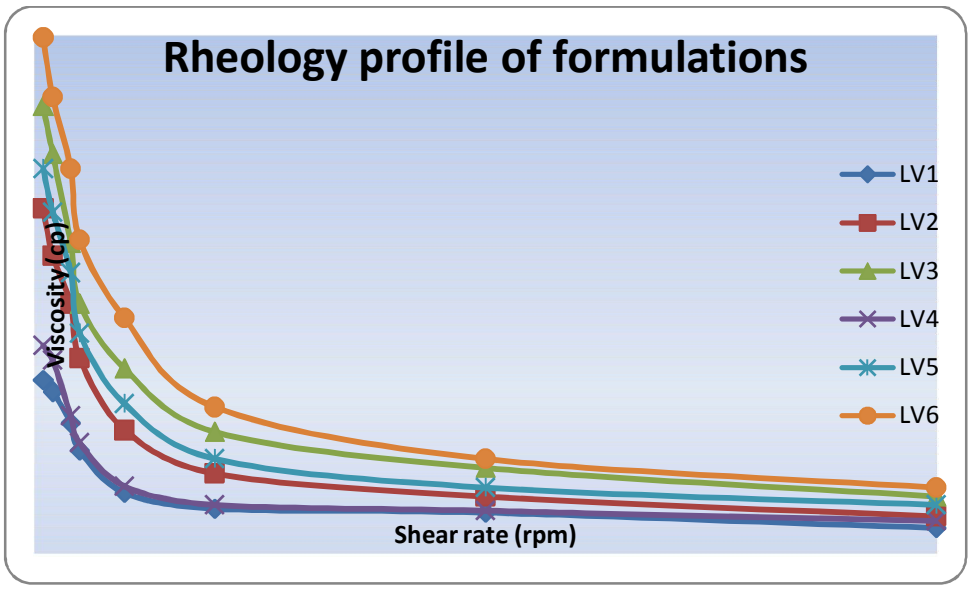
Rheology profile of formulations
Viscosity (cp)
Shear rate (rpm)
LV1
LV2
LV3
LV4
LV5
LV6

Figure 2

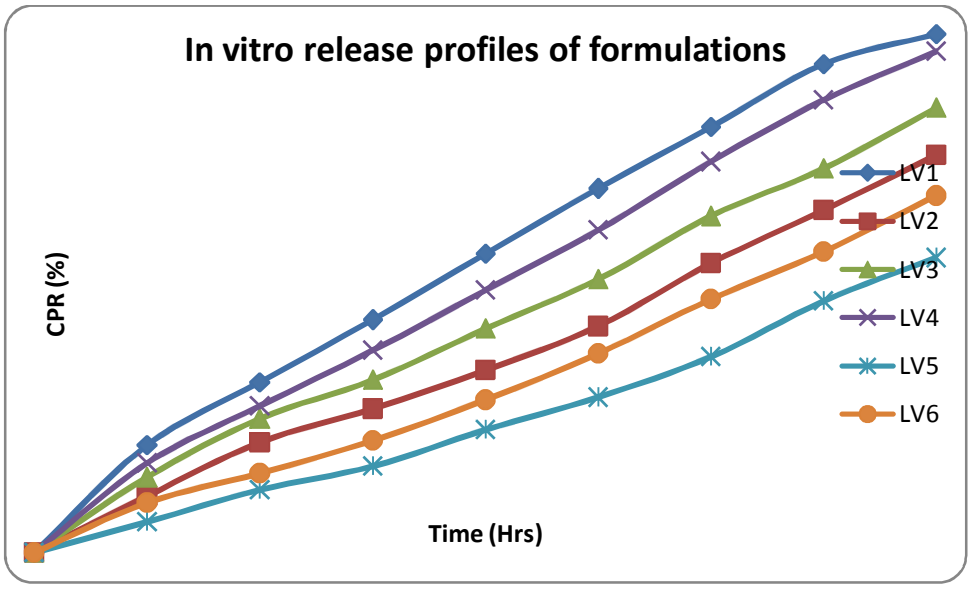
In vitro release profiles of formulations
CPR (%)
Time (Hrs)
LV1
LV2
LV3
LV4
LV5
LV6

Figure 3

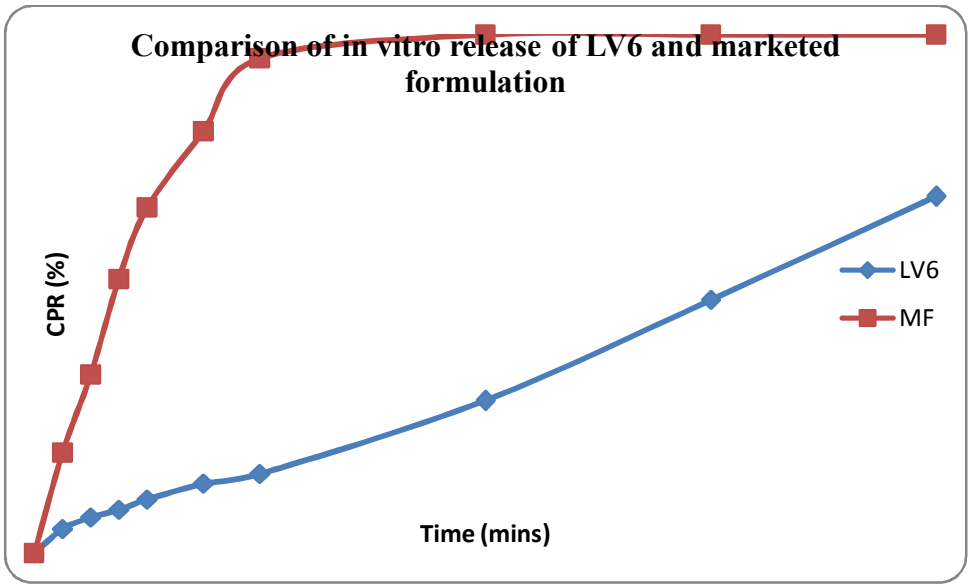

Figure 4

Figure 4a

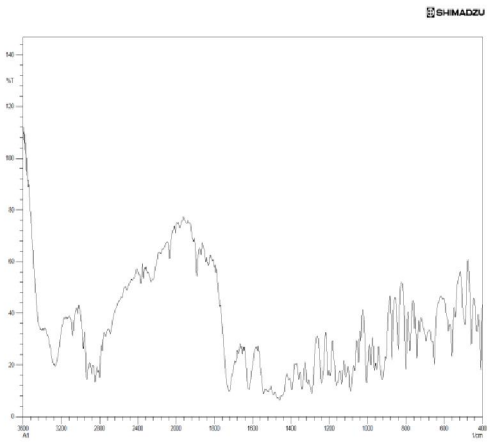

Figure 4b