



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Ion – Activated In Situ Gelling System of Levofloxacin for Sustained Ophthalmic Delivery

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ABSTRACT

Ophthalmic drug delivery is one of the most interesting and challenging endeavours facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. Various ophthalmic vehicles, such as inserts, ointments, suspensions and aqueous gels, have been developed to lengthen the residence times of instilled dose and enhance ophthalmic bioavailability¹. The objective of the present work was to develop an ion activated in situ gelling system of Levofloxacin, a fluoroquinolone derivative used in external infections of the eye. Gellan, alone, was investigated as vehicle for the formulation of eye drops of Levofloxacin (0.5%), which would undergo gelation when instilled into cul-de-sac of the eye and provide sustained release during treatment of ocular infections. The developed formulations were therapeutically efficacious and provide sustained release of the drug during over a 8-hrs period in vitro².

Keywords: Levofloxacin, Gellan Gum, In situ gelling system

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Received 28 September 2020, Accepted 17 October 2020

Please cite this article as: Suchita G *et al.*, Ion – Activated In Situ Gelling System of Levofloxacin for Sustained Ophthalmic Delivery. American Journal of PharmTech Research 2020.

INTRODUCTION

Ophthalmic drug delivery is one of the most interesting and challenging endeavours facing the pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ highly impervious to foreign substances. In clinical practice the anterior segment of the eye (cornea, conjunctiva and sclera) can be treated with many conventional topical drug delivery systems like solutions, suspensions which show poor bioavailability and therapeutic response. Whenever an ophthalmic drug is applied topically to the anterior segment of the eye, only a small amount (5%) actually penetrates the cornea and reaches the internal anterior tissue of the eyes. Rapid and efficient drainage by the nasolacrimal apparatus, noncorneal absorption and the relative impermeability of the cornea to both hydrophilic and hydrophobic molecules, all account for such poor ocular bioavailability¹. The goal of pharmacotherapeutics is to treat a disease in a consistent and predictable fashion. An assumption is made that a correlation exists between the concentration of a drug at its intended site of action and the resulting pharmacological effect. Thus to increase the ocular bioavailability of drug, we need to increase the ocular residence time of the drug. Various ophthalmic vehicles, such as inserts, ointments, suspensions, and aqueous gels, have been developed to lengthen the residence times of instilled dose and enhance ophthalmic bioavailability. These ocular drug delivery systems, however, have not been used extensively because of some drawbacks, such as blurred vision from ointments or low patient compliance from inserts. Several in situ gelling systems have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and consequently enhance ocular bioavailability². These systems exhibit sol-to-gel phase transitions due to a change in a specific physicochemical parameter (e.g: pH, temperature, and ions) in the cul-de-sac. Gelrite (deacetylated gum), one of the most interesting environmentally responsive gelling polymers, is a polysaccharide composed of tetra saccharide repeating units. It is gelled in the presence of cations in the tear fluid. Once gelled, the formulations resist the natural elimination process of drainage from the precorneal area; their residence time at the site of drug absorption is prolonged and subsequently the amount of drug absorbed is increased. The objective of the present work was to develop an ion activated in situ gelling system of Levofloxacin, a fluoroquinolone derivative used in external infections of the eye. Gellan, alone, was investigated as vehicle for the formulation of eye drops of Levofloxacin (0.5% w/v), which undergoes gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug during treatment of ocular infections³.

MATERIALS AND METHOD

Materials

Levofloxacin was purchased from Yarrow chem. Mumbai, Gellan gum (Gelrite® CP Kelco, USA) was obtained as a gift sample from Applied Biosciences, Mumbai, India. All other solvents used were purchased from local suppliers and of analytical grade unless mention⁴.

Preparation of the Formulation

Appropriate quantities of Gellan were dispersed in ultrapure deionized water containing mannitol (isotonicity agent) to obtain 0.1 to 0.5% w/w polymer concentrations (Table 1). The dispersions were heated to 90⁰C for 20 min while stirring. The solutions were allowed to cool at room temperature, and then Levofloxacin was added to obtain drug concentration of 0.5 % w/w. The pH was adjusted to 7.4 ± 0.1 using drops of 0.5M NaOH, and the dispersion was equilibrated at 4⁰C overnight. Methyl paraben was added a preservative⁵.

Ingredients	Quantity (%w/v)				
	G1	G2	G3	G4	G5
Levofloxacin	0.5	0.5	0.5	0.5	0.5
Gellan gum	0.1	0.2	0.3	0.4	0.5
Mannitol	5	5	5	5	5
Methyl paraben	0.1	0.1	0.1	0.1	0.1
Deionised water	q.s to 100 ml				

EVALUATION OF FORMULATIONS

Drug Content Uniformity

The drug content was determined by diluting 1 ml of the formulation to 50 ml freshly prepared simulated tear fluid (pH 7.4). The formed gel was completely crushed with the help of a glass rod, followed by vigorous shaking until the formed gel got completely dispersed to give a clear solution¹¹. The volume was adjusted to 100 ml with simulated tear fluid. The solution was filtered through a 0.45-mm filter membrane and Levofloxacin concentration was then determined at 288 nm by using UV-Vis spectrophotometer. The results were the means of three runs.

Gelation Studies

Table 2: Physicochemical properties of the prepared gelling systems

Formulation code	Drug content uniformity (%±SD)	Gelling capacity
G1	98.31±1.12	+
G2	98.7±0.41	++
G3	99.15±0.63	++
G4	99±0.46	++
G5	99.4±0.26	+++
G6	99.2±0.63	+++

Note: (+) Phase transition within 60 sec, collapse of gel structure within 1-2 hrs,

(++), Phase transition within 60 sec, collapse of gel structure within 3-4 hrs,

(+++), Phase transition within 60 sec and gel structure stable for more than 6 hrs.

In vitro Drug Release Studies

The in vitro release studies were carried out on formulation codes G1 to G6 using a modified USP dissolution testing apparatus. The dissolution medium used was freshly prepared simulated tear fluid (pH 7.4). Cellulose membrane (spectra / Por dialysis membrane 12,000 14,000 MW cut off), previously soaked overnight in the dissolution medium, was tied to one end of specifically designed glass cylinder (open at both ends and of 2.0 cm diameter). An accurately weighed amount of the formulation (1g) was transferred to the glass tube. Then the glass cylinders were attached to the metallic driveshaft to the dissolution apparatus and suspended in 100ml of dissolution medium maintained at temperature of $37 \pm 1^{\circ}\text{C}$. The shafts were allowed to rotate at a constant speed (50 rpm). At predetermined time intervals for 8 hrs, aliquots were withdrawn and replaced by an equal volume of the receptor medium. The drug content in the withdrawn samples was determined at 288 nm using UV-visible double beam spectrophotometer. The results were the means of three runs. The results of in vitro data were analyzed by statistical software PCP Disso, version 2.04, to obtain the best fit kinetic model for in vitro drug release. The formulations were optimized on the basis of viscosity and in vitro release studies. The optimized formulation was subjected to permeation studies across a sheep's corneal membrane, antimicrobial efficacy and ocular irritancy studies⁶.

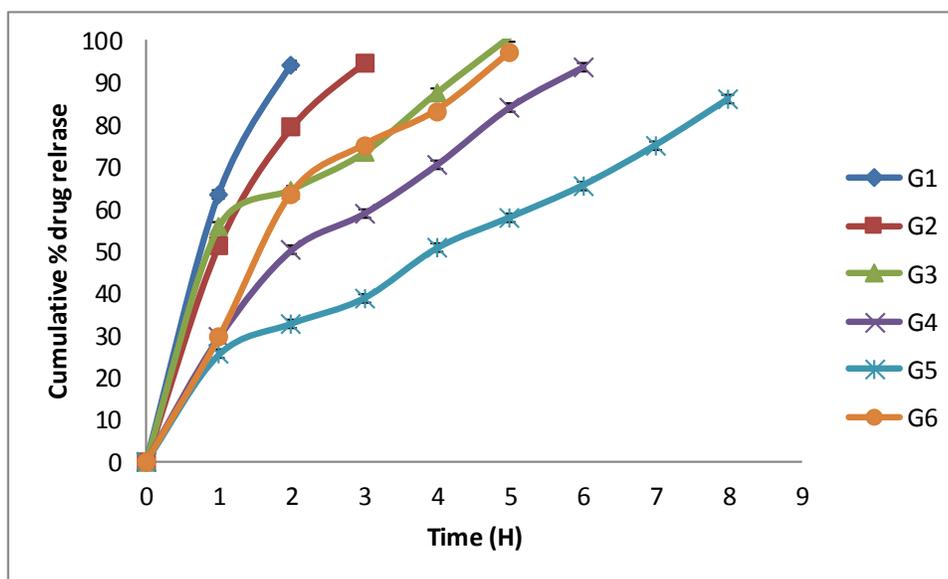


Figure 1: In Vitro Drug Release of Levofloxacin from in situ gelling systems G1-G6 (average of three experiments).

PERMEATION STUDIES ACROSS A SHEEP'S CORNEAL MEMBRANE

A device designed by Gonjari *et al.*¹² was used to evaluate drug permeation through a sheep's corneal membrane. Whole eyeballs of goat were procured from slaughter house and transported to laboratory in cold condition in normal saline maintained at 4°C. The corneas were carefully removed along with a 5-6 mm of surrounding scleral tissue and washed with cold saline. The washed corneas were kept in cold freshly prepared simulated tear fluid (pH 7.4). This membrane was sandwiched between donor and receptor chamber. Simulated tear fluid was used as a diffusion medium. The optimized formulation (G5) to be tested was added to the donor chamber with the help of a micropipette. The donor surface of the membrane was constantly in contact with simulated tear fluid. A temperature of $37 \pm 0.5^\circ\text{C}$ was maintained throughout the study. A magnetic stirrer in the cell provided continuous agitation. At regular time intervals, 1 ml of sample was withdrawn and replaced with fresh simulated tear fluid in order to maintain sink conditions. The samples were appropriately diluted and the absorbance was measured at 288nm using a Shimadzu 1700UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). The results were the means of three runs. Drug release data was fitted to different kinetic models like zero-order, first-order, Higuchi and Korsmeyer-Peppas⁷.

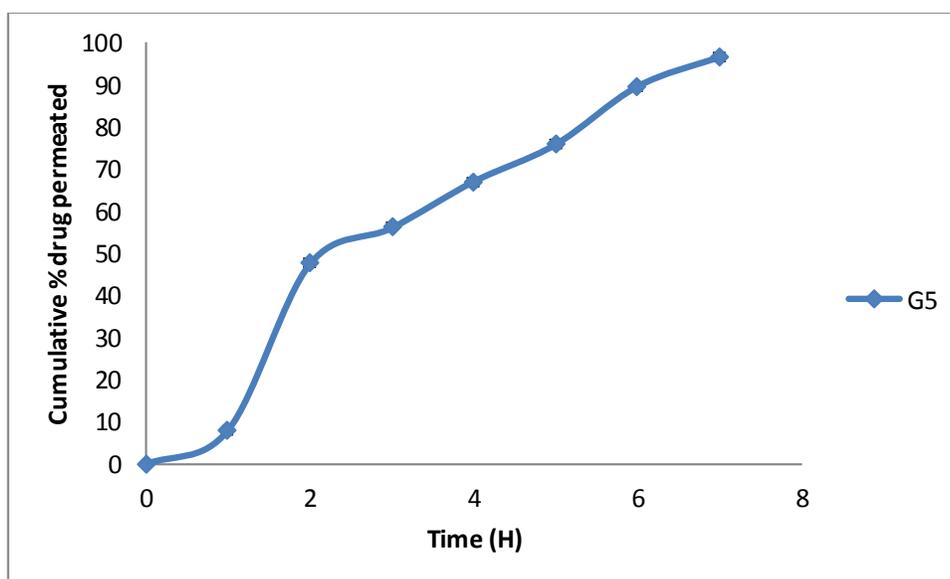


Figure 2: *In Vitro* Drug transcorneal permeation profile from in situ gelling system G5 (average of three experiments).

Antimicrobial efficacy studies

Antimicrobial efficacy was determined by the agar diffusion test employing 'Boar well method'. Sterile solutions of Levofloxacin (marketed eye drop solution as standard solution) and the developed formulations (test solutions)were poured in to wells bored into sterile nutrient agar previously seeded with test organisms (*Staphylococcus aureus*, and *Pseudomonas aeruginosa*) after

allowing diffusion of the solutions for 2 hr. agar plates were incubated at 37°C for 24 hr. The zone of inhibition (ZOI) measured around each well was compared with that of control. The entire operation except the incubation was carried out laminar airflow unit. Each solution was tested intriplicate. Both positive and negative controls were maintained through the study⁸.

Table 3: Antimicrobial efficacy testing

Sr. no	Formulation	<i>Pseudomonas aeruginosa</i>		<i>Staphylococcus aureus</i>	
		Zone of inhibition (mm)	% Efficacy	Zone of inhibition (mm)	% Efficacy
1	Standard	34	100	37	100
2	G5	33	97.05	31	83.78

Ocular Irritation Studies

The Ocular irritation was performed according to Draize technique on New Zealand white albino rabbits, each weighing 2–3 kg. 100 ul of formulation was instilled into the lower cul-de-sac the left eye of the rabbit. The right eye, which remained untreated, served as a control. To prevent loss of test material, the lower eye lid was gently held together for app. 5 sec. The sterile formulations were instilled twice a day and the rabbits were observed after 1h, 4 h, 24 h, 48h, and 72 h for redness, Excessive Tearing, Inflammation of the eye (Table4)⁹.

Table 4: Ocular irritation testing

Parameter	Duration			
	1hrs	2-4hrs	48hrs	72hrs
Redness	0	0	0	0
Excessive Tearing	0	0	0	0
Inflammation	0	0	0	0

(0 - No redness, no inflammation or excessive tearing, 1 - Mild redness with inflammation & slight tearing, 2 - Moderate redness with moderate inflammation and excessive tearing, 3 - Severe redness with severe inflammation and excessive tearing)

RESULTS AND DISCUSSION

Gelation and In Vitro Release Studies

The in situ gelling formulations of Levofloxacin were prepared and characterized on the basis of gelation studies, and in vitro drug release. The physicochemical properties of the prepared formulations are shown in Table 2. The drug content, clarity, and pH of the formulations were found to be satisfactory. We found that on addition of STF of pH 7.4, all the formulations showed instantaneous gelation. However, the nature of the gel formed depended on the polymer concentration. The formulation G1 and G2 showed the weakest gelation, which could be due to the presence of a lesser amount of gellan (0.1% and 0.2%), and formulations G3 to G6 produced a stiff gel; the desired and optimum gelation was produced by formulation G5 (0.5%). All formulations

exhibited and the viscosity was directly dependent on the polymeric content of the formulations (Figure 1). The interaction studies were carried out to check any possible physiochemical interaction among the formulation ingredients. No new bands were detected in the IR spectra of physical mixtures, indicating no interaction between the drug and polymer mixture. As the concentration of gellan gum was increased from 1 to 6 % w/v (G1-G6), drug release was found to be decreased. For formulation G1 and G2 (1% and 2%), drug release after 1 hour was 98.31 ± 1.12 and after 2 hours was $98.7 \pm 0.41\%$. While for formulations G3, G4, G5 and G6 drug release after 1 hour were 55.73 ± 1.105 , 28.53 ± 0.7611 , 26 ± 1.17 and 29.24 ± 1.16 % resp. (Fig.2). Increase in the concentration of gellan gum, decreases the burst release of the drug from formulation to greater extent and also sustained the drug release for extended period, because increase in the concentration of gellan gum increased the gel strength which is due to increase in chain interaction¹⁰.

Permeation studies across a sheep's corneal membrane

Transcorneal permeation studies of optimized formulation, G5 was conducted and a higher permeation across goat cornea was observed after 7 hrs. Initially rapid release was observed, gradually approaching constant values for the rest of the time (Fig.3). Thus conforming to the controlled release behaviour of the formulations. The initial quick release (burst effect) 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. Burst effect might be due to initial migration of the drug toward the surface of the matrix¹¹.

Antimicrobial efficacy studies

The results of the antimicrobial efficacy tests are shown in Table 3. The study indicates that Levofloxacin retained its antimicrobial efficacy when formulated as an in situ gelling system.

Ocular irritation studies

The results of the ocular irritation studies (Table 4) indicate that optimized formulation was non-irritant. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible¹².

CONCLUSION

Levofloxacin, a broad spectrum antibacterial against in the treatment of ocular infections, was successfully formulated as an in situ gelling system (0.5% w/ v) using gellan alone. The formulations were liquid at the formulated pH (6- 5-7.0) and underwent rapid gelation in contact with STF due to ionic interactions. The formulated system provided sustained release of the drug over 8- hrs. in vitro, and the developed formulations were devoid of any deleterious effect on the

ocular tissues. The formulation demonstrated better therapeutic efficacy as it were successful in inhibiting the growth of the microorganisms for the entire duration of the study (24 hr) when compared with the marketed eye drop, thus developed formulations are viable alternative to conventional eye drops by virtue of its ability to sustain drug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

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