SUSTAINED RELEASED OCULAR TEMPERATURE AND P_H TRIGGERED LEVOFLOXACIN IN-SITU GEL SYSTEM

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Abstract

The work present the develop formulation and caharacterisation of an ophthalmic in situ hydrogels of an antibacterial agent, levofloxacin, which is used in the treatment of eye infection such as dacrocystitis, bacterial conjunctivitis, corneal ulceration, with concept of both temperature and pH triggered *in-situ* gelation. Pluronic F-127 and Pluronic F-188 (thermo sensitive polymer) combining with chitosan (pH sensitive mucoadhesive polymer also act as permeation enhancer) was responsible for gel formation and hydroxy propyl methylcellulose, was acted as a viscosity-enhancing agent. The developed formulae were evaluated regarding their gelation temperature, gelling capacity, viscosity and *in vitro* release behavior. Among different formulae tested, P7 showed optimum gelation temperature of 33.0±0.5°C after dilution with simulated tear fluid (STF) and gel form that remains for few hours respectively. The selected formulation P7 was evaluated for drug contents, bioadhesion potential, *in vitro* transcorneal permeation study, antimicrobial efficacy studies and ocular irritation studies. The formulation was therapeutically efficacious, non irritant and has sustained release of the drug over a 7 hours period. The developed system can overcome the disadvantages of conventional eye drops.

Keywords: Levofloxacin, Poloxamers, Chitosan, *In-situ* gelling system

Introduction

Ophthalmic drug delivery is one of the most interesting and challenging endeavors facing the pharmaceutical scientist [1, 2]. 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 bioavailability [3, 4]. 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 [4,5].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 [6]. Several *in-situ* gelling systems have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and consequently enhance ocular bioavailability [7]. 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 [8]. Common topical antibacterial used in the treatment of ocular infectious diseases include sulfonamides, aminoglycosides, polymyxin-based combinations and fluoroquinolones. The fluoroquinolones represents an expanding class of broad-spectrum antibacterial which are effective against most of the gram negative and anaerobic species responsible for ocular infections. Levofloxacin is third generation fluoroquinolone derivative used to treat acute and sub acute infections of the eye viz. conjunctivitis, bacterial keratities and keratoconjunctivitis. A thermosensitive polymer, Poloxamer, a surface-active block copolymer made of polyoxyethylene and polyoxypropylene, is known for its excellent compatibility with other chemicals, least toxicity, high solubility capacity for different drugs, and good drug-release characteristics [9, 10]. Poloxamer, changes from low viscosity solutions at any temperature or below room temperature (25°C) to semisolid gels at the corneal surface temperature (37°C). Bioadhesion can be used as a means to improve intimacy of contact, as well as a way to increase dosage form residence time to various administration routes [11-13]. To fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers such as carbopol, hydroxyl ethyl cellulose (HEC), and hydroxypropylmethyl cellulose (HPMC) have been added to the *in-situ* gelling liquids [12, 14, 15]. Our present work describes the formulation and evaluation of an ocular delivery system and based on concept of both temperature and pH triggered *in-situ* gelation. Pluronic F-127 and Pluronic F-188 (thermo sensitive polymer) in combination with chitosan (pH sensitive polymer also act as permeation enhancer) was used as gelling agent. Levofloxacin, the drug frequently used for conjunctivitis was used as model drug to check the efficacy of the formulation.

Materials and Methods

Materials

Levofloxacin was procured from Yarrow chem. ltd, Mumbai. Pluronic F-127 was supplied by ICPA Ankaleshwar Gujarat; Chitosan (practical grade, 75-85 % deacetylated, molecular weight 150kDa) chitosan was given as gift from Indian Sea Industries, Thiruvanthpuram. All other solvents used were purchased from local suppliers and of analytical grade unless mention.

Development of In-Situ *Forming Gels*

A combination of placebo in situ were developed and evaluated for gelling capacity to identify the composition suitable for use as in- situ gelling system (Table 1).Solution of Chitosan with glacial acetic acid and distilled water. Mucoadhesive polymer, namely HPMC K4M was dispersed in chitosan solution. Pluronic F-127 and Pluronic F-188 were slowly added to above cold mixture with continuous mixing using a thermostatically controlled magnetic stirrer. The partially dissolved poloxamer solutions were stored in a refrigerator and stirred periodically until clear homogenous solutions were obtained (approximately 24 h). Then it is evaluated for gelling capacity using vial containing 2 ml of artificial tear fluid freshly prepared and equilibrated at 37ºC and put a drop of prepared formulation and visually assessing the gel formation, noting the time of gelation and the time taken for the gel formed to dissolve. The composition of the artificial tear fluid used was sodium chloride 0.670g, sodium bicarbonate 0.200g, calcium chloride 0.008g and purified water q.s. 100g.

Table1. Combination of chitosan / Pluronic F-127 and F-188 studied

Formulation code	Pluronic F-127 (% w/v)	Pluronic F-68 (% w/v)	Chitosan (% w/v)	HPMC K4M (% w/v)	HPMC 100 LV	Gelation Temp. (ºC)	Gelling capacity
P1	15	4	-	-	-	55.0±0.58	+
P2	16	4	-	-	-	48.3±0.40	+
P3	17	4	-	-	-	42.0±0.50	++
P4	18	4	-	-	-	38.24±0.36	++
P5	18	4	-	0.2	-	37.40±0.25	+++
P6	18	4	-	0.4	-	36.0±0.58	+++
P7	18	4	0.25	0.2	-	33.0±0.5	+++
P8	18	4	0.25	-	0.2	34.0±0.5	+++

Note: (+) Gel formation within 60 sec, collapse of gel structure within 1-2 hrs, (++) Gel formation within 60 sec, collapse of gel structure within 3-4 hrs, (+++) Gel formation within 60 sec and gel structure stable for more than 6 hrs.

Medicated Formulation

Medicated *in-situ* forming gels were prepared on a weight basis by using the modified cold method [16]. The ingredients of the prepared formulations is shown in Table 1. Drug solution was prepared by dissolving the appropriate amount of Levofloxacin, 0.3% (wt/wt), in required quantity of distilled water and 0.1 N HCL was added during the mixing step to get a clear solution and mixed with previously optimized *in-situ* gelling system containing chitosan, Pluronics and Mucoadhesive polymer, namely HPMC K4M and HPMC 100 LV separately in optimum ratio. Mannitol and benzalkonium chloride were added to all formulations as isotonicity agent and preservative, respectively. All glassware used should be sterilized by autoclaving, and the entire procedure was carried out in a laminar flow hood.

Table 2. Formula of the developed *in-situ* gel formulation (P7)

Ingredients	Concentration (w / v)
Levofloxacin	0.3%
Pluronic F-127	18%
Pluronic F-68	4%
Chitosan	0.25%
HPMC K4M	0.2%
HPMC 100 LV	0.2%
Mannitol	5%
Benzalkonium Chloride	0.01%
Purified Water (q.s)	100

Evaluation of Formulation

The prepared formulations were evaluated for drug content by UV spectrophotometer at 272 nm, clarity by visual observation against a black and white background in a well lit cabinet, pH, sol–gel transition, bioadhesion potential and sterility (Table 3).

Clarity and Viscosity

The clarity of the prepared formulations after and before gelling was determined by visual examination under light alternatively against white and black backgrounds. Viscosity of formulation was determined before and after gelation by Brookfield's LVDV-II+ Pro model viscometer. The gel under study was placed in a small sample holder to measure viscosity at 20 rpm. Gelation was induced in formulation by raising temperature to 37ºC.

Gel pH and Gelation Temperature

1M NaOH was added drop wise with continuous stirring to the beaker containing formulation. pH was checked using pH meter. Gelation temperature was measured by heating the formulation in a 15-ml glass test tube [17, 18]. In each test tube, 2 ml of formulation solution was placed and heated with gentle stirring until the formulation solution got gelled. Gel formation was considered as the point where there was no flow when the test tubes were tilted more than 90º.

Table 3. Physicochemical properties of the developed *in- situ* gel formulation

Parameter	Inference
Clarity	Clear solution
Solution pH	6.0-6.2
Gelation pH	6.8-7.4
Gelation temperature (ºC)	33.0±0.5
Viscosity (at 25 ºC and pH 6.0-6.2)	170 cps
Viscosity (at 37 ºC and pH 7.2-7.4)	28700 cps
Drug Content (%)	96.00±1.22
Bioadhesion potential (dynes/cm^2)	6805.76 ± 17.9

Values are expressed as mean ± SD (*n*=3).

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 solution [19]. 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 271 nm by using UV-Vis spectrophotometer.

Mucoadhesion test (Bioadhesion potential)

Gels were evaluated for bioadhesive force by the method described by Choi *et al*.[20] The mucoadhesive strength of ocular gel was determined by means of the mucoadhesive force-measuring device, using corneal membrane of sheep. The corneal membranes were stored, frozen in normal saline solution and thawed to room temperature before use. At the time of testing a section of tissue was secured, keeping the mucosal side out, onto each glass vial using a rubber band and an aluminum cap. The diameter of each exposed mucosal membrane was 1.1cm. The vials with the mucosal membrane were stored at 37^{o}C for 10 min. Next, one vial with a section of tissue was connected to the balance and the other vial was fixed on a height-adjustable pan. The gel was placed onto the corneal membrane from the first vial. Then, the height of the second vial was adjusted so that the membrane surfaces of both vials would come in close contact. A contact time of 10 minutes was allotted. Then, the weight was allowed to increase in the pan until the vials detached. The bioadhesive force was the minimum weight required to detach two vials.

In Vitro *Drug Release Studies*

In vitro release of levofloxacin from *in-situ* gelling formula was studied using a modified USP dissolution testing apparatus [8]. 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 formulations (1 g) with and without chitosan, a permeation enhancer was transferred to the glass tubes (P5 and P7) and suspended in 100 ml of dissolution medium maintained at temperature of 37±1^{o}C. The dissolution testing apparatus starts and shaft were allowed to rotate at a constant speed (50 rpm). At time intervals for 10 h, aliquots were withdrawn every after one hour and replaced by an equal volume of the receptor medium. The drug content in the withdrawn samples was determined at 271 nm using UV-visible double beam spectrophotometer. The results were the means of three runs (Fig.1).

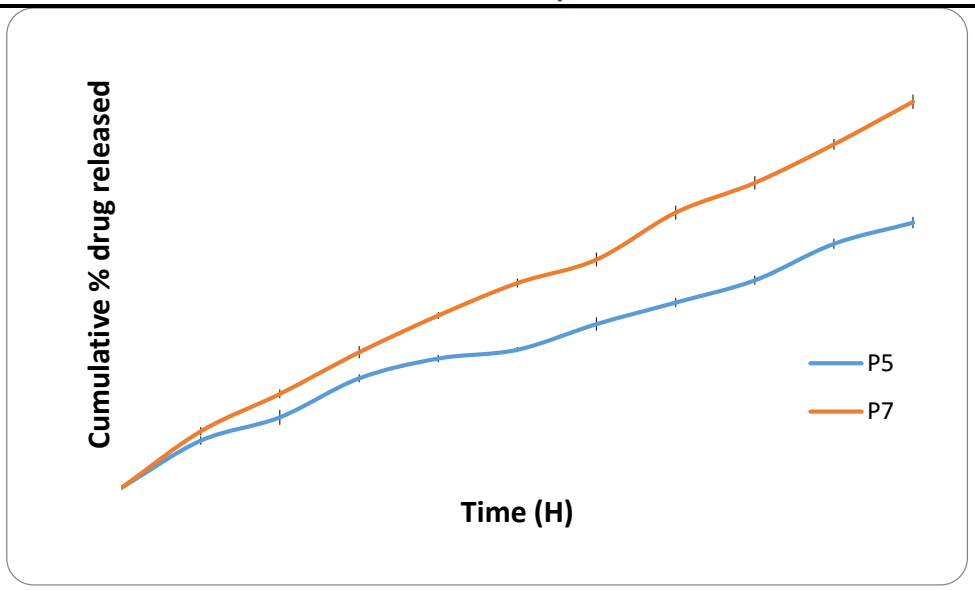

Figure 1. *In Vitro* Drug Release of levofloxacin from *in-situ* gelling system (average of three experiments).

Permeation studies across a sheep's corneal membrane

A device designed by Gonjari *et al.* [21] 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^{o}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) and mounted on by sandwiching between the clamped donor and receptor compartment. Simulated tear fluid was used as a diffusion medium. The optimized formulation 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 ± 0.5^{o}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 271 nm using a Shimadzu UV-VIS spectrophotometer. The results were the means of three runs (Fig 2). Drug release data was fitted to different kinetic models like zero-order, first-order, Higuchi and Korsmeyer-Peppas.

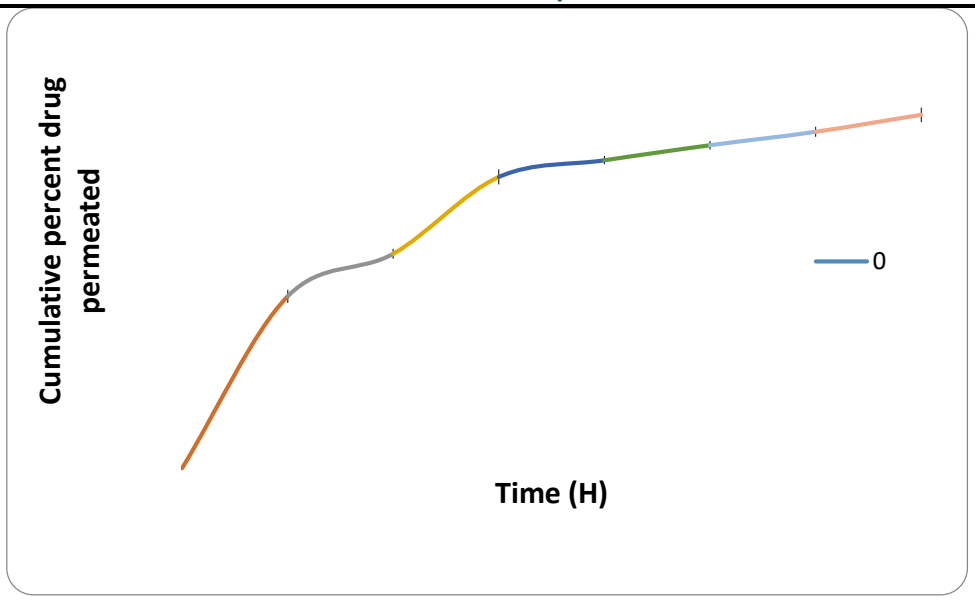

Figure 2. *In Vitro* Drug transcorneal permeation profile from *in-situ* gelling system (average of three experiments).

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^{o}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 in triplicate. Both positive and negative controls were maintained through the study.

Table 4. Antimicrobial efficacy testing

S.No	Formulation	Pseudomonas aeruginosa		Staphylococcus aureus	
		Zone of inhibition (mm)	% Efficacy	Zone of inhibition (mm)	% Efficacy
1	**Standard**	34	100	37	100
2	**P7**	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, and inflammation of the eye (Table 5).

Table5. Ocular irritation testing

Parameter	Duration			
	1hrs.	24hrs.	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

Measurement of the Sol–Gel Transition Temperature

The sol–gel transition temperatures of ophthalmic thermoreversible gels have been considered to be suitable for ocular delivery if they were in the range of 25–34°C. If the gelation temperature of thermosensitive formulation is lower than 25°C, a gel might be formed at room temperature, and if the gelation temperature is higher than 34°C, a liquid dosage form still exists at corneal surface temperature, resulting in the drainage of the formula from the eyes. Poloxamer solutions are known to exhibit thermoreversible gelation, depending on the polymer grade, concentration, and other included formulation components. Poloxamers were previously proven to undergo thermal gelation or sol-gel transition at a temperature of about 25 to 35°C. Below the transition temperature, poloxomer solutions allow a comfortable and precise delivery by the patient to the cul-de-sac, where thermogelation occurs. Immediate gelling increases a drug's residence time and enhances its bioavailability. Gels containing poloxamer 407 had good gelation properties in that the gelation temperature of the gel decreased as the concentration of poloxamer increased (Table 1). The formulations containing poloxamer 407 at concentration 18%w/v (P4) was found to be forming gels quickly within 60 sec. and gel structure stable for more than 3 hrs. Hence for further study, 18%w/v concentration of poloxamer 407 was considered as optimum. When HPMC K4M was incorporated in 18% w/v of poloxamer 407 formulations (P5 and P6), the gel formulation was quick and gel structure retained for longer time ie more than 6 hrs and when HPMC 100LV was added formulation was stiff gel as compared to HPMC K4M. This suggests increase in gel strength with addition of this mucoadhesive polymer. Also addition of mucoadhesive polymer such as HPMC K4M and Chitosan lowered the

gelation temperature of the *in- situ* forming gels. The gelation temperature-lowering effect of such mucoadhesive polymers could be explained by their ability to bind to polyoxyethylene chains present in the poloxamer molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing intermolecular hydrogen bonding which will lead to gelation at lower temperature [22, 23].

Formulation and* in vitro *Characterization

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. The present study used a formulation of chitosan and Pluronic F-127 for the development of sustained ocular drug delivery system. Chitosan is reported to act as penetration enhancers that increase transcorneal permeation of drug. Besides this, other properties of chitosan such as bioadhesiveness, viscous nature, and ability to convert into hydrogel at ocular pH (PH 7.4) make it the best suitable candidate for the development of this type of delivery systems [24, 25, 26]. Poloxomers, commercially available as pluronics also possess all the necessary characteristics such as good thermal gelling, no irritation to eye, and tolerance that make them suitable for ocular administration. Different combinations of placebo formulations of Pluronic F -127 and F-188 were prepared and evaluated for the gelation temperature and gelling capacity (Table1).Gelling capacity and viscosity are the two main prerequisites of an *in-situ* gelling system. The formulation should have an optimum viscosity that will allow easy instillation into the eye as a liquid (drops) that would undergo a rapid sol-to-gel transition. Additionally, the gel formed *in-situ* should preserve its integrity without dissolving or eroding for prolonged period of time. A concentration of 0.25% chitosan and 18.0% Pluronic F-127 was selected as it had satisfactory attributes (Table 1). The developed formulation was further characterized for various physicochemical parameters (Table 3). Formulation was clear solution having pH between 6.0-6.2 and gelation temperature was 35-37^{o}C. Results clearly indicated that the formulation is converted into gel when the pH of the formulation is raised and resulted in sudden increases in the viscosity. Results confirm that the formulation was liquid room temperature and at the pH formulated (pH 6.0-6.2) it underwent rapid transition into the gel phase at the pH of the tear fluid (pH7.4) and physiological temperature (37^{o}C).Terminal sterilization by autoclaving had no effect on pH, gelling capacity, and viscosity of the formulation. The formulation that converted into stiff gel due to exposure to elevated temperature during autoclaving was converted into liquid again after cooling. *In vitro* drug release profile of the formulation was determined in simulated tear fluid (pH 7.4) and the formulation displayed a slow release profile. A cumulative drug release was 86.402± 1.62% after 10

hr. with developed *in-situ* gelling system (P7). While formulation (P5) without chitosan showed cumulative drug release of 59.35±1.31%. This can be attributed to well known transmucosal enhancer property of chitosan (Figure 1).

In vitro *bioadhesion evaluation*

Bioadhesive force means the force with which gels bind to ocular mucosa. Greater bioadhesion is indicative of a prolonged residence time of a gel and thus prevents its drainage from the cul-de-sac. The addition of bioadhesive polymers HPMC K4M increased the bioadhesive force (Table 3) but using HPMC 100LV has increased viscosity it shows HPMC K4M has better gel bioadhesion force than HPMC 100 LV.

Permeation studies across a sheep's corneal membrane

Transcorneal permeation studies of optimized formulation, P7 was conducted and a higher permeation across goat cornea was observed after 7 hrs. (Fig.2). *In vitro* permeation across a sheep's corneal membrane was fitted to various release kinetic models. All of the batches indicated that a Peppas model of permeation kinetics was the best-fit model. Initial faster release indicates that initially the drug in the solution quickly diffused in the space outside the gel. Release of the drug within the gel is controlled by the nature and the concentration of the polymer used. The initial fast release of the drug from the formulations of levofloxacin containing poloxamer 407 may be due to the rapid leaching of extramicellar ionized drug. Entrapped in the micelles, the drug may be released rather slowly.

Antimicrobial efficacy studies

The results of the antimicrobial efficacy tests are shown in Table 4. The study indicates that levofloxacin retained its antimicrobial efficacy when formulated as an *in-situ* gelling system [27].

Ocular irritation studies

The results of the ocular irritation studies (Table 5) indicate that optimized formulation was non-irritant. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible.

Conclusion

Levofloxacin , a broad spectrum antibacterial against in the treatment of ocular infections, was successfully formulated as both temperature and pH triggered in-situ gel forming eye drops (0.3% w/v) using Pluronic F-127 and Pluronic F-188 in combination with chitosan. The formulations were liquid at the formulated pH (6.0-6.2) and underwent rapid gelation on raising the pH (7.4) and temperature (37^{o}C). The developed formulation was therapeutically efficacious, stable, non irritant and provided sustained release of the drug over a 7 hours period. The developed formulations are

viable alternative to conventional eye drops by virtue of its ability to sustain dug release. Also important is the ease of administration afforded and decreased frequency of administration resulting in better patient acceptance.

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References

1. Mitra AK, Ophthalmic Drug Delivery System. Vol. 58, Marcel Dekker, New York, **1993,** pp. 105–110.

2. Indu P, Kaur AG, Anil KS, Deepika A, *Int J Pharm.*, **2004,** 269, 1-14.

3. Le Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R, *Prog. in Ret. and Eye Res.*, **1998,** 17, 1, 33-58.

4. Le Bourlais CA, Treupel-Acar L, Rhodes CT, Sado PA, Leverge R, *Drug Dev Ind Pharm.*, **1995,** 21, 1, 19-59.

5. Lee VHL, Robinson JR, J. *Ocul. Pharmacol.*, **1986,** 2, 67–108.

6. Lee VHL, *J. Ocul. Pharmacol.*, **1999,** 6, 157–164.

7. Lin H, Sung KC, *J. Control. Rel.*, **2000,** 69, 379–388.

8. Srividya B, Cardoza RM, Amin PD, *J. Control. Rel.*, **2001,** 73, 205–211.

9. Miyazaki S, Yokouchi C, Nakamura T, Hashiguchi N, Hou WM, Takada M, *Chem. Pharm. Bull.*, **1986,** 34, 1801–1808.

10. Morishita M, Borichello JM, Takayama K, Chiba Y, Tokiwa S, Nagai T, *Int. J. Pharm.*, **2001,** 212, 289–293.

11. Lee JW, Park JH, Robinson JR, *J. Pharm. Sci.*, **2000,** 89, 850–866.

12. Park H, Robinson JR, *J. Control. Rel.*, **1985,** 2, 47–57.

13. Robinson JR, Longer MA, Veillard M, *Ann. NY Acad. Sci.*, **1987,** 507, 307–314.

14. Chu JS, Amidon GL, Weiner ND, Goldberg AH, *Pharm. Res.*, **1991,** 8, 1401–1407.

15. Jones DS, Woolfson AD, Djokic, J, Coulter WA, *Pharm. Res.*, **1996,** 13, 1734–1738.

16. Schmolka, IR, *J. Biomed. Mater. Res.*, **1972,** 6, 571–582.

17. Khan S, Singhara DS, Yeole PG, *Indian Drug*, **2005,** 42 519–524.

18. Gilbert JC, Richardson JL, Davies MC, Palin KJ, Hadgraft J, *J. Control. Rel.*, **1987,** 5 113–118.

19. Balasubramaniam J, Kant S, Pandit JK, *Acta Pharm.*, **2003,** 53 251–261.

20. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK, *Int. J. Pharm.*, **1998,** 165,33–44.

21. Gonjari ID, Hosmani AH, Karmarkar AB, Godage AS, Kadam SB, Dhabale PN, *Drug Discov Ther.*, **2009,** 3:6-9.

22.Gilbert JC, Richardson JL, Davies MC, Palin KJ, Hadgraft J, *J. Control. Rel.*, **1987,** 5, 113–118.

23. Ryu JM, Chung SJ, Lee MH, Kim CK, Shim CK, *J. Control. Rel.*, **1999,** 59, 163–172.

24. Lehr CM, Bouwstra JA, Schacht EH, Junginger HE, *Int. J. Pharm.*, **1992,** 78, 43–48.

25. Felt O, Buri P, Gurny R, *Drug Dev Ind Pharm.*, **1998,** 24: 979–993.

26. Felt O, Furrer P, Mayer JM, Plazonnet B, Buri P, Gurny R, *Int J Pharm.*, **1999,** 180: 185–193.

27. Pandya TP, Modasiya MK, and Patel VM, *Der Pharmacia Lettre*, **2011,** 3(3): 404-410

28. Rathore KS, Nema RK, Ishibashi Tejraj, Yokoi N, International Journal of Pharm Tech Research 1(2); 2009: 164-169.

29. Nanjawade BK, Manvi FV, Manjappa AS. J Control Release 2007;122:119–34.

30. Gupta P, Vermani K and Garg S. Drug Discov Today 2002;7:569-79.

31. He C, Kim SW, Lee DS. J Control Release 2008;127:189–207.

32. Khurana AK. Comprehensive ophthalmology; 4th ed. Age International (P) Ltd Pub. 2007.