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FORMULATION AND EVALUATION OF *IN SITU* GEL FOR BACTERIAL KERTATITS BY BESIFLOXACIN HYDROCHLORIDE

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ABSTRACT

Bacterial keratitis is a serious ocular infection that can lead to vision impairment if not effectively treated. Conventional eye drops suffer from rapid precorneal elimination, leading to reduced bioavailability and frequent dosing. The present study aimed to develop and evaluate a Pluronic F127-based in situ gel formulation of Besifloxacin hydrochloride to enhance ocular residence time, improve drug bioavailability, and provide sustained therapeutic action. Formulations were prepared using varying concentrations of Pluronic F127, Carbopol 934, and HPMC 15 cps, and were evaluated for drug content, pH, in situ gelling capacity, viscosity, in vitro drug release, and stability. Among all formulations, F6 exhibited optimal characteristics, including high drug content (99.02 ± 0.22%), suitable pH (5.0 ± 0.1) and immediate gelation with extended retention, favorable viscosity, and sustained drug release (96.65% over 5 hours). Stability studies indicated minimal changes in drug content and gelling capacity over 30 days under accelerated conditions. The optimized formulation demonstrates potential as an effective ocular drug delivery system for the treatment of bacterial keratitis, offering improved therapeutic efficacy and patient compliance.

Keywords: Besifloxacin hydrochloride, In situ gel, Bacterial keratitis, Pluronic F127, Ocular drug delivery, Sustained release.

INTRODUCTION

Bacterial keratitis is a severe ocular infection that poses a significant threat to vision worldwide. The cornea, being avascular, is highly susceptible to microbial invasion, leading to inflammation, ulceration, and potential vision loss if not promptly and effectively treated. The most common pathogens responsible for this condition include *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumonia* (Ting *et al.*, 2021).

Conventional treatment modalities, primarily topical antibiotic eye drops, often face challenges such as rapid precorneal elimination, leading to reduced drug bioavailability and the need for frequent dosing. This not only compromises therapeutic efficacy but also affects patient compliance. To address these limitations, the development of ocular in situ gels has emerged as a promising strategy. These systems remain in a sol state upon instillation and undergo a sol-to-gel transition at physiological conditions, ensuring prolonged ocular retention and sustained drug release (Lanier *et al.*, 2021).

Besifloxacin hydrochloride, a fourth-generation fluoroquinolone antibiotic, has demonstrated broad-spectrum activity against both Gram-positive and Gram-negative ocular pathogens. Its potent bactericidal action,

coupled with favorable pharmacokinetic properties, makes it an ideal candidate for ocular drug delivery systems (Totoli and Salgado; 2018).

Poloxamers, particularly Poloxamer 407, are widely utilized in the formulation of in situ gels due to their thermoresponsive properties. At lower temperatures, Poloxamer 407 exists as a liquid; upon exposure to body temperature, it undergoes a gelation process, enhancing ocular residence time and drug bioavailability (Chen *et al.,* 2021).

The primary objective of this research was to formulate and evaluate a Besifloxacin hydrochloride-loaded in situ gel using Poloxamer 407 as the gelling agent. The study aimed to assess the physicochemical properties, in vitro drug release profiles, and stability of the developed formulation to determine its potential efficacy in treating bacterial keratitis.

MATERIALS AND METHODS

Materials

The materials used for the formulation development of the in situ ocular gel were of analytical grade and procured from reputed suppliers. Besifloxacin hydrochloride was received as a gift sample from Bioplus Life Sciences Pvt. Ltd., Bangalore. Disodium hydrogen phosphate, dipotassium hydrogen orthophosphate, sodium chloride, sodium bicarbonate ($NaHCO_3$), calcium chloride ($CaCl_2$), pluronic F68, and polyethylene glycol were obtained from S. D. Fine Chem. Ltd., Mumbai. Carbopol, hydroxypropyl methylcellulose (HPMC), and ethylenediaminetetraacetic acid (EDTA) were purchased from Rankem Pvt. Ltd., Mumbai, while methanol, ethanol, and chloroform were procured from Qualigens Fine Chemicals, Mumbai. All chemicals and reagents used were of analytical or pharmaceutical grade and were used without further purification.

Methods

Formulation development of *In-situ* gel

The formulation development of an in-situ gel of Besifloxacin hydrochloride holds significant importance and presents a compelling need for study. Besifloxacin Hydrochloride is the hydrochloride salt form of besifloxacin, a synthetic fourth-generation fluoroquinolone antibiotic, with broad spectrum antibacterial activity.

Selection of Vehicle

The solubility of Besifloxacin hydrochloride was tested in various buffers such as acetate buffer I.P. (pH 6.0 & 6.5), citrophosphate buffer B.P. (pH 6.0 and 6.2) and phosphate buffer USP (pH 7.2 and 7.4) in order to select a suitable vehicle. Solutions of Besifloxacin hydrochloride in the above buffers were prepared to test its solubility at the dosage level desired.

Methodology for formulations preparation:

For the preparation of Pluronic F127-based ocular in situ gel, all the ingredients were first sieved through sieve no. 44 to ensure uniform particle size. Solutions of Besifloxacin hydrochloride at concentrations of 0.5% and 0.1% were prepared in acetate buffer (pH 5.0, I.P.). The drug solution was then cooled in an ice bath, and Pluronic F127 was gradually added with continuous stirring to ensure uniform dispersion (Varshosaz *et al.,* 2008). The resulting mixture was stored in a refrigerator at 4°C for 24 hours, which facilitated the complete dissolution of Pluronic F127. After this period, Carbopol 934 and HPMC 15 cps were incorporated slowly along with other excipients,

maintaining continuous stirring for 2–3 hours to achieve proper mixing and prevent slug formation. The final formulation was then subjected to probe sonication to remove any entrapped air bubbles. All prepared formulations were stored in low-density polyethylene (LDPE) bottles and kept in the refrigerator until further use.

Evaluations of formulations

Appearance

Clarity is one of the most important characteristic features of ophthalmic preparations. All developed formulations were evaluated for clarity by visual observation against a black and white background (Saxena and Kushwaha, 2013).

Drug content

The assay of drug Besifloxacin hydrochloride was performed by UV method. The calculation was based on calibration curve method using regression equation (Y=mx+c) (Viram and Lumbhani, 2012).

pH determination

pH is one of the most important parameter involved in the ophthalmic formulation. The two areas of critical importance are the effect of pH on solubility and stability. The pH of ophthalmic formulation should be such that the formulation will be stable at that pH and at the same time there would be no irritation to the patient upon administration of the formulation. Ophthalmic formulations should have pH range in between 5 to 7.4.

The developed formulations were evaluated for pH by using calibrated digital pH meter (Vodithala *et al.*, 2010). For *In situ* gel pH 5.0 should be optimum because both the drug is stable at pH 3.5-5.0. Lowering the pH from 5.0 can causes irritation to eye and on raise the above 5 will result in gelation of formulation due to presence of carbopol.

***In-situ* gelling capacity**

In situ gelling capacity determined by visual inspection. The formulation has been exposed to the physiological conditions of temperature and pH. Simulated tear fluid (STF) was prepared and warm up to 37^0C. Formulations were introduce into STF in a ratio of 1:2 Change in consistency of Formulations were visually inspected (Shankar and Kalikonda, 2014).

Gelling capacity of all formulations are depicted as + (gels after five minutes and dissolves rapidly), ++ (gelation immediate, remains for few hours) and +++ (gelation immediate, remains for extended period upto 8 hours).

Viscosity study

At pH 5.0 and temperature less than 16^0C the developed formulations were in liquid state and show low viscosity. For viscosity studies the pH of formulations were raised from pH 5.0 to pH 7.4 and the temperature was raised to 37^0C. pH was raised to 7.4 by the addition of 0.5M NaOH (Mahesh and Manjula, 2012).

The resulting gel studied for viscosity on Brookfield Synchrolectric Viscometer using Spindle No.7 at 50 RPM for comparative study. The angular viscosity was measured by gradually increase the RPM from 10 to 70.

***In-vitro* drug diffusion study**

The *in vitro* release of drugs from the formulations was studied through cellophane membrane. The dissolution medium used was artificial tear fluid freshly prepared (pH 7.4). Cellophane membrane, previously soaked overnight in the dissolution medium, was tied to one end of a specifically designed glass cylinder (open at both ends and of 5 cm

diameter). A 1-ml volume of the formulation was accurately pipetted into this assembly. The cylinder was attached to the metallic driveshaft and suspended in 50 ml of dissolution medium maintained at 37±1°C so that the membrane just touched the receptor medium surface. The dissolution medium was stirred at 50 rpm using magnetic stirrer. Methodology Aliquots, each of 1-ml volume, were withdrawn at hourly intervals and replaced by an equal volume of the receptor medium (Costa and Lobo, 2001).

Stability studies

Optimized sterile formulation was subjected to stability testing. Sterile optimized ophthalmic formulation was 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 and *in-situ* gelling capacity (Mandal *et al.,* 2012).

RESULTS AND DISCUSSION

The development of Besifloxacin hydrochloride in situ gel formulations for bacterial keratitis aimed to enhance ocular bioavailability, prolong precorneal residence time, and provide sustained drug release.

The drug content of all formulations ranged from 95.45 ± 0.32% to 99.02 ± 0.22%, indicating uniform distribution of Besifloxacin within the gel matrix. Formulation F6 showed the highest drug content, demonstrating efficient drug incorporation and minimal loss during preparation. High drug content is essential to ensure therapeutic efficacy, particularly in ocular infections where precise dosing is critical. Variations among formulations are shown in Table 2.

The pH values of the formulations ranged between 4.3 and 4.9, slightly acidic compared to the target ocular pH of 5.0 ± 0.1. Adjustment is necessary to enhance compatibility with the ocular surface and reduce irritation. The pH values for each formulation are summarized in Table 3.

The gelling capacity of the formulations was evaluated to determine their potential to remain in the precorneal area. Formulations F4 and F6 exhibited immediate gelation and remained intact for extended periods (approximately 8 hours), while other formulations showed shorter gel retention. Detailed gelling capacities are presented in Table 4. Viscosity measurements indicated low viscosity in the sol state and increased viscosity after gelation, which supports prolonged ocular retention. Formulation F6 showed an ideal post-gelation viscosity of 2274 cps. Viscosity data for all formulations are listed in Table 5. The *in vitro* drug release profile of F6 showed 96.65% release within 5 hours, indicating sustained release from the gel matrix. Release data are shown in Table 6.

Regression coefficient analysis of formulation F6 revealed that drug release followed Higuchi kinetics and non-Fickian transport according to Korsmeyer-Peppas model, suggesting combined diffusion and polymer relaxation mechanisms. Data for regression coefficients are provided in Table 7. F6 maintained its drug content and gelling capacity under accelerated conditions (40 ± 2°C, 75 ± 5% RH) over 30 days, demonstrating physical and chemical stability. Stability parameters are summarized in Table 8. Considering all evaluation parameters,

formulation F6 emerged as the optimized formulation. It exhibited high drug content, appropriate pH, favorable viscosity, immediate and prolonged gelation, sustained drug release, and excellent stability. These characteristics suggest its suitability for effective ocular delivery in bacterial keratitis.

Table 1: Composition of different formulations of *In-situ* gel

S. No.	Ingredient (%)	Formulations								
		F1	F2	F3	F4	F5	F6	F7	F8	F9
1.	Besifloxacin hydrochloride	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
2.	Pluronic F127	10	12	14	10	12	14	10	12	14
3.	Carbopol 934	0.2	0.2	0.2	0.3	0.3	0.3	0.4	0.4	0.4
4.	HPMC 15cps	1.0	1.0	1.0	0.75	0.75	0.75	0.5	0.5	0.5
5.	EDTA	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
6.	Benzalkonium Chloride	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%	0.010%
7.	NaCl	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
8.	Poly ethylene glycol	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
9.	Acetate Buffer (pH 5.0)	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Table 2: Drug content analysis of *in situ* gel formulations

Formulation	Drug Content (%)*
F1	96.65±0.25
F2	95.45±0.32
F3	98.85±0.14
F4	97.15±0.65
F5	98.85±0.85
F6	99.02±0.22
F7	96.65±0.15
F8	97.74±0.22
F9	96.65±0.38

***Average of three determinations (n=3)**

Table 3: pH Determination

Formulation	pH	Adjust to
F1	4.6	5.0 ±0.1
F2	4.8	5.0 ±0.1
F3	4.3	5.0 ±0.1
F4	4.7	5.0 ±0.1
F5	4.5	5.0 ±0.1
F6	4.9	5.0 ±0.1
F7	4.7	5.0 ±0.1
F8	4.5	5.0 ±0.1
F9	4.7	5.0 ±0.1

Table 4: *In situ* gelling capacity of all formations

Formulation code	*In situ* gelling capacity
F1	"+"
F2	"++"
F3	"++"
F4	"+++"
F5	"++"
F6	"+++"
F7	"+"
F8	"+"
F9	"+"

"+" gelation after five minutes and dissolves rapidly

"++" gelation immediate, remains for few hours

"+++" gelation immediate, remains for extended period 8 hours

Table 5: Comparative viscosity* of *In situ* formulation

Formulation code	% of Pluronic F 127	Viscosity of solution (in cps)	Viscosity after galation
F1	10	769	2165
F2	12	852	2350
F3	14	963	2478
F4	10	732	2056
F5	12	845	2165
F6	14	932	2274
F7	10	685	2036
F8	12	745	2365
F9	14	825	2547

*Spindle no.7 rpm 50

Table 6: *In vitro* drug release profile of Besifloxacin hydrochloride from *in situ* Formulation F6

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	11.23	1.050	88.77	1.948
1	1.000	0.000	26.65	1.426	73.35	1.865
1.5	1.225	0.176	47.78	1.679	52.22	1.718
2	1.414	0.301	59.98	1.778	40.02	1.602
2.5	1.581	0.398	68.87	1.838	31.13	1.493
3	1.732	0.477	74.45	1.872	25.55	1.407
4	2.000	0.602	88.58	1.947	11.42	1.058
5	2.236	0.699	96.65	1.985	3.35	0.525

Table 7: Comparative study of regression coefficient for selection of optimize Formulation F6

F. Code	Zero order	First order	Higuchi	Korsmeyer Peppas
F-6	0.9192	0.9623	0.9804	0.9514

Table 8: Stability data sheet

F. Code	Parameter Evaluated (40 ± 2°C, 75 ± 5 % RH)					
	7 days		15 days		30 days	
	Drug content	*In-situ* gelling capacity	Drug content	*In-situ* gelling capacity	Drug content	In-situ gelling capacity
F6	99.12	++	98.45	++	98.25	++

CONCLUSION

The present study successfully demonstrated the formulation and evaluation of a Besifloxacin hydrochloride-based in situ gel for the effective management of bacterial keratitis. The developed formulation utilized Poloxamer 407 as a thermoresponsive polymer, which exhibited excellent gelling ability at physiological temperature, thereby prolonging the precorneal residence time of the drug. The incorporation of mucoadhesive polymers such as Carbopol 934 and HPMC enhanced the viscosity and ensured sustained drug release. The in vitro evaluation confirmed that the optimized formulation provided controlled and prolonged drug release with satisfactory clarity, pH, and drug content, indicating its suitability for ocular administration. Stability studies further revealed that the formulation maintained its integrity under refrigerated conditions, confirming its robustness and shelf-life.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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