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Original Article

FORMULATION AND EVALUATION OF IN SITU MUCOADHESIVE OPHTHALMIC HYDROGEL FOR SUSTAINED DELIVERY OF PEFLOXACIN MESYLATE

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ABSTRACT

Objective: The drawback associated with conventional eye drops needs development of novel ophthalmic delivery system with improved bioavailability and patient compliances. The objective of present work was to develop and evaluate mucoadhesive *in situ* ocular gel of Pefloxacin mesylate based on the concept of ion activated *in situ* gelation for the prolonged precorneal residence time.

Methods: Formulations were prepared using Sodium Alginate as gelling agent and Hydroxy ethyl cellulose as mucoadhesive agent. Gels were evaluated for gelling capacity, bio adhesion force, rheological property, *ex vivo* and *in vitro* release profile for selection of optimized formulation.

Results: Developed formulations with satisfactory clarity, pH, drug content, gelling capacity, viscosity and bio adhesion were selected for *ex vivo* and *in vitro* release studies. An optimum formulation which could sustain the release for 12hours was finalized for further evaluations like; antimicrobial efficacy and irritation test. Formulations were found to be non irritant, effective against the test organisms and exhibited Zero order release pattern and pseudoplastic rheology.

Conclusion: The developed system with sodium alginate and hydroxyl ethyl cellulose can be a feasible alternative to conventional eye drops.

Keywords: Ocular drug delivery, Sodium alginate, Hydroxyethyl cellulose, Sustained delivery, Flouroquinolone antibiotic.

INTRODUCTION

Conventional ophthalmic eye drop often results in poor bioavailability and therapeutic response due to rapid pre-corneal loss caused by nasolacrimal drainage and high tear fluid turnover. Several new preparations like ointments, inserts and collagen shields 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 the drug elimination. Even though successful results were obtained with these preparations it involves some disadvantages, such as blurred vision or patient non-compliances [1, 2].

An alternative approach that significantly increases the precorneal residence time and bioavailability of the drug can be achieved by using the novel delivery system based on the concept of *in situ* gel formation. *In situ* gel forming system can be described as liquids dosage form that can be delivered in a drop form, and they undergo a phase transition in the ocular cul-de-sac to form a viscoelastic gel. In contrast to pre formed gelled formulations the *in situ* gelling system has an advantage of reproducible and accurate administration of drug and creates little to no problem of vision [3].

An *in situ* gelling system consists of polymers that exhibit sol-to-gel phase transition due to change in a specific physicochemical parameter; the pH [4] temperature [5] and electrolyte composition [6] in the lacrimal fluid of the eye. *In situ* gel forming solution has been studies extensively to enhance ocular bioavailability and prolonged duration of action. A natural hydrophilic polysaccharide SA (SA) the sodium salt of alginic acid, is used in preparation of in situ gelling system as it forms polymeric dispersion in buffer that exhibit low viscosity up to pH 5 and converted to gel in contact with lacrimal fluid [3-7]**.** Despite the fact that, *in situ* gelling polymers can be applied to the conjunctival sac where they undergo sol to gel and facilitate drug delivery continuously, its viscosity alone cannot significantly prolong the precorneal residence time. Consequently the need of mucoadhesive polymers was emphasized to enhance pre corneal residence of *in situ* gel for improved bioavailability of drug. Muco adhesive polymers are macromolecular hydrocolloids with numerous hydrophilic functional groups and they may be natural, synthetic, or semi-synthetic in nature [8, 9]. In present study Hydroxy ethylcellulose (HEC) was included as mucoadhesive polymer and SA was chosen as *in situ* gelling agent since it exhibits several favorable biological properties such as biodegradability and non-toxicity [10].

Pefloxacin is a flouro quinolone bactericidal antibiotic, which inhibits bacterial DNA replication as other flouroquinolones. Compared to other antibiotics it has wider antibacterial spectrum, better bioavailability, better ocular penetration, and has least chances of developing resistance to antibiotic [11, 12].

Hence the aim of this study was to examine the potential use of SA and HEC in designing a long acting mucoadhesive *in situ* ophthalmic delivery system of pefloxacin mesylate.

MATERIALS AND METHODS

Perfloxacin mesylate was obtained from Smruthi Pharma Pvt Ltd., Kolhapur [India]. SA and Hydrox ethyl cellulose were purchased from S. D. Fine chemicals Ltd., Mumbai [India].

Instruments used were UV-Vis Spectrophotometer [Shimadzu UV-1700 PC, Shimadzu corporation, Japan], Brookfield Viscometer [LV-DVE] Brookfield Engineering Labouratories INC USA], Dissolution Tester [USP XXXIII dissolution Tester, Electrolab, Mumbai, India]

Preparation of *In situ* gelling systems of Pefloxacin mesylate

SA in combination with hydroxyl ethyl cellulose was dissolved in Acetate buffer pH 5.0 by continuous stirring at 40 °C. Required quantity of Pefloxacin mesylate was added to polymeric solution and stirred to get a final drug concentration of 0.3% w/v. Mannitol [5% w/v] and Benzalkonium chloride [0.01 % w/v] was added later which acts as isotonicity adjusting agent and preservative, respectively. The complete formulas of the formulations are given in the [table1]. The formulations were filled in sterile 20 ml glass vials, capped with rubber closures and sealed with aluminum caps. The formulations in their final pack were terminally sterilized by autoclaving at 121 °C and 15 psi for 20 min. The sterilized formulations were stored in refrigerator [4 °C – 8 °C] until further use.

Table 1: Composition of *In situ* gelling formulations

Formulation ingredients (% w/v)	Formulation codes								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
Drug	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
SA	1	1	1	1.5	1.5	1.5	2	2	2
Hydroxy ethyl cellulose	1	2	3	1	2	3	1	2	3
Mannitol	5	5	5	5	5	5	5	5	5
Benzalkonium chloride	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Acetate buffer	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s	q. s

In vitro gelation studies

Formulations were evaluated for gelling capacity by placing a drop of the formulation in a vial containing 2 ml of freshly prepared STF and equilibrated at 37 °C. The gel formed, gelation time and the time taken for the gel to dissolve was assessed visually. The composition of simulated tear fluid (STF) was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride 0.008g and purified water q. s 100 g [9].

Rheological studies

In order to evaluate the rheological behavior, viscosity of the formulations before and after addition of simulated lacrimal fluid was evaluated using Brookfield viscometer [RVE Model]. Studies were carried out at room temperature. Sample was placed in a suitable beaker and the instrument continuously sheared the material at various angular velocities from 10 to 100 rpm. The hierarchy of angular velocity was reversed and the average viscosity was determined.

Measurement of bio adhesion force

In this method excised sheep corneal membrane was immediately fixed with the mucosal side outwards onto a glass vial using a rubber band. Vials with the corneal membrane were stored at 37 °C for 5 min. Then, the next vial with a section of the membrane was connected to a balance in an inverted position while the first vial was placed on a height adjustable pan. The gel was placed onto the corneal membrane of the first vial. Then, the height of the second vial was adjusted so that the membrane surfaces of both the vials came in close contact. A ten minute contact time was chosen. Then, the weight was allowed to increase in the pan by adding weights until the vials get detached. The bioadhesive force was the minimum weight required to detach two vials.

The bioadhesive force, expressed as detachment stress in dynes/cm^2, was determined from minimal weights needed to detach the tissues from the surface of each formulation, using following equation.

$$\text{Detachment stress [dynes}/cm^2] = \frac{\text{m x g}}{\text{A}}$$

Where m is the weight added to the balance in grams; g is the acceleration due to gravity taken as 980 cm/s2; and A is the area of tissue exposed. Measurements' were repeated three times for each of the gel preparations [13].

In vitro release studies

The in-vitro release studies of all the formulations were carried out through cellophane membrane. Overnight soaked cellophane membrane was tied to one end of the specially designed glass cylinder which is open at both sides. With the help of metallic shaft the cylinder is suspended such that the membrane just touches the 100 ml of STF medium in the flask of a USP rotating paddle apparatus. For release studies, the temperature of the medium was maintained at 37.2 °C and 10 ml of formulation was kept in the well of the diffusion cell. The paddle was lowered into the flask and rotated at 50 rpm. Aliquots of the medium were withdrawn everyone hour up to 12 h and each time equal volume of STF was replaced to the receptor medium. The absorbances were read by UV spectrophotometer at 272 nm and cumulative percentage drug releases were calculated [14].

Ex vivo corneal permeation studies using goat's cornea

Whole eye balls of goat were procured from a local slaughter house and corneas were used to study the trans corneal permeability. A diffusion cell was fabricated to evaluate drug permeation through a sheep's corneal membrane. The modified diffusion cell consists of an upper and lower chamber and it was separated by goat cornea. Simulated tear fluid was used as a diffusion medium. The formulation 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±0.5 °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 STF in order to maintain sink conditions. The samples were appropriately diluted and the absorbance was measured at 272 nm using a Shimadzu 1700UV-VIS spectrophotometer [15].

Isotonicity evaluation

Isotonicity is an important characteristic of the ophthalmic preparations. Isotonicity has to be maintained to prevent tissue damage or irritation of eye. Formulations were mixed with few drops of blood and observed under the microscope at 45 X magnification and compared with the standard solution containing pefloxacin. The shape of blood cell was compared with the standard solution containing pefloxacin [16].

Hemolysis test

In vitro eye irritancy screening method described by *Dharmendra Jain et al.* was performed to predict the eye irritation potential of formulations. According to procedure 5 ml of whole blood was collected from healthy individual and EDTA was used as an anticoagulant. Blood was centrifuged at 1500 × g for 20 min. The buffy coat was removed and the packed cells [RBCs] were washed thrice with normal saline. Normal saline was added to the cells to get 40–50% hematocrit. Positive and negative controls were prepared in two different test tubes by diluting 100 µl of RBC to 3 ml with double distilled water in positive control and normal saline in the negative control. In positive control RBC will be lysed and in negative control there will be absence of lyses. Formulations were incubated at 37 °C for1 hours with 100 µl RBCs in test tubes and volume was made up to 3 ml with normal saline. All the formulations were tested for haemolysis in triplicates. The blood samples were then centrifuged at 795 × g for 20 minutes and the absorbance of the supernatant was measured at 540 nm by UV spectrometer [17, 18]. Percentage hemolysis was calculated using the following formula:

$$\%\ \text{Hemolysis} = \frac{\text{Aborbance of sample}}{\text{Absorance of 100\% haemolysis}} \text{x } 100$$

Antimicrobial studies

To ascertain the biological activity of the optimized formulation, the antimicrobial activity was carried out against *Pseudomonas aeruginosa* [MTCC 1934] and *Staphylococcus aureus* [MTCC 737]. A layer of nutrient agar [20 ml] seeded with test microorganism [0.2 ml] was allowed to solidify in the petriplate. Cups were made on the solidified agar layer with the help of sterile borer with 4 mm diameter. The volume of the formulations [optimized formulation and standard drug solution] containing equal amount of the drug is poured into the cups. After keeping the Petri plates at room temperature for 4 h, the plates were incubated at 37 °C for 24 h. The

zone of inhibition obtained was measured by an antibiotic zone reader [19].

RESULTS AND DISCUSSION

Preparation of formulations

The use of SA in *in situ* gel-forming systems is substantiated by the property of SA showing low viscosity up to pH 5 and which coacervate in contact with tear fluid pH 7.4 [20]. However, from preliminary studies it was confirmed that SA concentrations 1 to 2% w/v does not exhibit the desired bioadhesive force and hence polymer HEC in the concentration range of 1 to 3 % w/v was added as bioadhesive polymer. The dose of the drug in the *in situ* gel formulation was calculated with respect to the dose of drug in marketed eye drops i. e 0.3 % w/v.

Evaluation of formulations

Interaction studies were carried out by FTIR spectra to check possible interaction among the ingredients in the formulations. No new bands were detected in spectra of physical mixtures when compared with drug and individual polymers. This revealed that the ingredients are compatible with each other and no interaction took place between drug and polymer mixture. The drug content, clarity and pH of the formulations were found to be satisfactory [table 2].

Rheological studies of in situ gels

Viscosity is one of the prerequisites of an in situ gelling system. Formulation should have an optimum viscosity that will allow easy instillation into the eye as a liquid drops and must undergo a rapid sol-to-gel transition in contact with tear fluid [6]. Rheological study of formulations [fig. 1] indicated that in situ gels exhibited pseudo-plastic rheology i.e., viscosity is high under low shear rate and low under high shear rate conditions. This property offered the advantage of low viscosity during blinking and stability of tear film during inter-blinking periods [8]**.** An increase in pH to 7.4 [STF] caused the formulations to transform to gels with high viscosity.

Bioadhesion force

Direct relation was observed between polymer concentrations and detachment stress. As the concentration of polymers HEC and SA increased the Bioadhesion force is increased, [table 2]. Increase in bioadhesive force of formulations may be due to presence of HEC, which has an abundance of hydroxyl and ether groups along their length, which are responsible for the mucoadhesive properties [21].

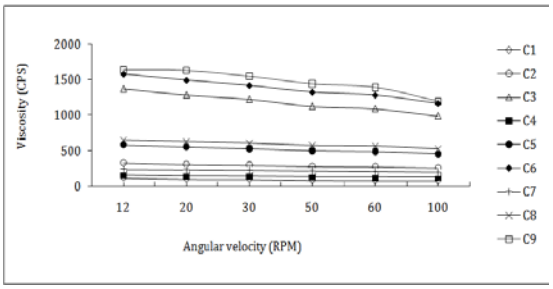

Fig. 1: Rheological profile of prepared *in situ* formulations

It can also be substantiated due to the presence of SA a polymer with high glucuronic acid content which forms 3 dimensional ionotropic hydrogel matrices, generally by preferential interaction of G moieties resulting in formation of inhomogenous gels [22] and also due to the low surface tension [31.5 mN/m] of the alginate, which is lower than the critical surface tension of the mucin coated cornea [38 mN/m], resulting in good spreading and adhesion [23]. Thus, the increase in the bioadhesive force will lead to increased retention time and therefore increased bioavailability.

From the *in vitro* gelation studies [table 2] it was found that, all the formulations showed instantaneous gelation when contacted by tissue fluid [STF] and remained for extended hours after gelation. However, the nature of gel formed depends on the concentration of SA and HEC. Formulations C1 and C2 showed immediate gelation but remained for few hours, which may be due to lesser concentration of SA. Formulations C6, C8 and C9 produced very stiff gel; this may be attributed to higher concentration of HEC and SA. The desired gel was produced by C3, C4, C5 and C7which remained for an extended period. These four formulations were selected for further evaluation.

Table 2: Physicochemical properties of prepared *in situ* gel

Formulations	Drug Content (%w/v)	pH	Clarity	Gelling Capacity	Biadhesion force (dyne/cm2)	Viscosity (cps) at 60rpm		Selected (S)/Rejected(R)
						Before gelling	After gelling	
C1	99.37	5.23	Clear	+	78.4	82	450	R
C2	98.23	5.25	Clear	++	117.6	274	1230	R
C3	97.56	5.24	Clear	+++	196	1092	1950	S
C4	98.52	5.20	Clear	+++	313.6	140	920	S
C5	98.12	5.22	Clear	+++	392	486	940	S
C6	95.76	5.25	Clear	+++	509.6	1286	2090	R
C7	98.72	5.21	Clear	+++	470.4	208	1360	S
C8	96.36	5.20	Clear	+++	588	566	1880	R
C9	95.22	5.24	Clear	+++	862.4	1396	2700	R

+Gels after few minutes, dissolves rapidly,**++**Gelation immediate, remains for few hours and**+++**Immediate gelation, remains for extended period

In vitro release studies

The drug release data obtained for formulations C3, C4, C5, C7 and Pure drug is depicted in fig. 2. The release profile of a drug predicts how a delivery system might function and gives valuable insight into its *in vivo* behavior. The *in vitro* release studies of *in situ gelling* formulations of Pefloxacin mesylate were carried out using STF of pH 7.4 as the dissolution medium and 93.9%, 91.62%, 86.8 %, and 89.6% of the drug was released in 12 h from the formulation C3, C4, C5 and C7 respectively.

Ex vivo corneal permeation studies

Corneal permeation studies were performed using isolated goat's cornea using STF [pH 7.4] at 34±0.5 °C. Compared to pure drug the formulations C3, C4, C5 and C7 have sustained the release up to 6, 8, 10 and 8hr respectively [fig. 3]. Viscosity and release profiles of the four formulations were considered for selecting an optimum formulation for further evaluation. Normally viscosity values in the range of 5-1000 cps significantly improve the contact time in the eye which is fruitful for ophthalmic use due to the fact that the ocular shear rate is very high particularly 0.03 s^{-1} during inter blinking periods to 4250–28,500s-1during the blinking period [24, 25]. At higher shear rate the formulations C4 and C5 exhibited viscosity [after gelation] 920cps and 940cps respectively [table 2], which was within the reported range. Simultaneously from *in vitro* and *ex vivo* studies it was found that formulation C5 showed better sustained release compared to C4 formulation. Hence the formulation C5 was selected as an optimized formulation

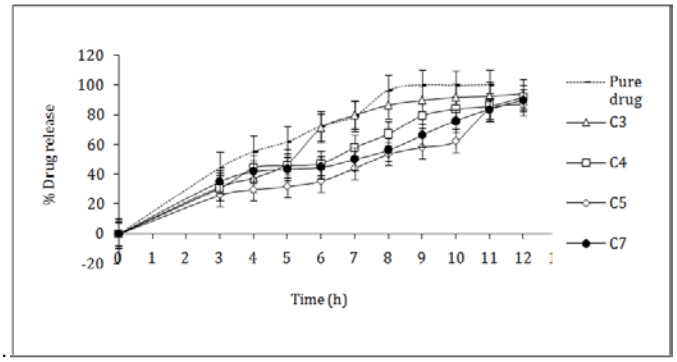

Fig. 2: Comparison of *In vitro* release profile of selected formulations (C3, C4, C5 and C7) with Pure drug, The error bars indicates standard deviations of three tests.

With the aim of increasing the cumulative drug release, Tween 20 a permeation enhancer was added to formulation in a concentration of 2% w/v and it was coded as C5a. Formulation C5a showed release of 94.01% [fig. 4] in comparison with that of formulation C5 with 86.88% after 12 h. Thus Tween 20 in the C5 formulation has increased the release profile of the C5a formulation. Both the formulations C5 and C5a showed an initial burst release in 1 to 2 h. The initial burst release of the drug can be explained by the fact that, the *in situ* gelling system is formulated in water and hence the polymer was completely hydrated. When they come in contact with STF, the alginate forms 3-dimensional ionotropic hydrogel matrices, by the interaction of calcium ions with glucouronic acid residues of alginates resulting in the formation of inhomogeneous gel which can entrap the drug and sustain their release [3].

The *in vitro* release data's of formulations C5 and C5a were subjected to various kinetic models and the regression coefficient [r] values were found to be 0.9794 and 0.9841 respectively for Zero order plot [table 3]. Hence the best fit model for the optimized formulations was found to be zero order kinetic models.

The *in vitro* drug release conditions may be very different from those likely to be encountered in the eye. However, the results clearly show that the gels have the ability to retain drug for the prolonged period of time [12 h] and premature drug release will not occur.

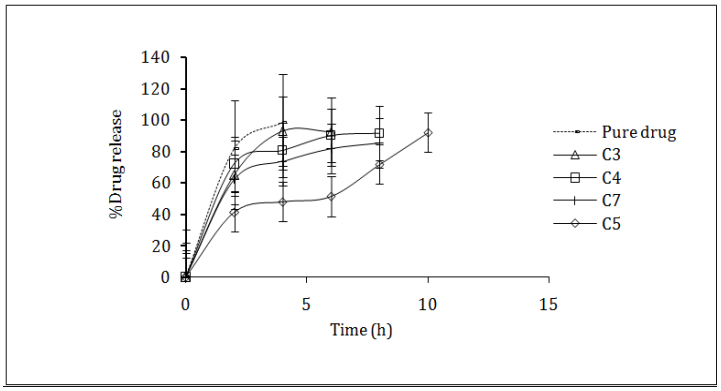

Fig. 3: Comparison of *Ex vivo* release profile of selected formulations (C3, C4, C5 and C7) with Pure drug, The error bars indicates standard deviations of three tests

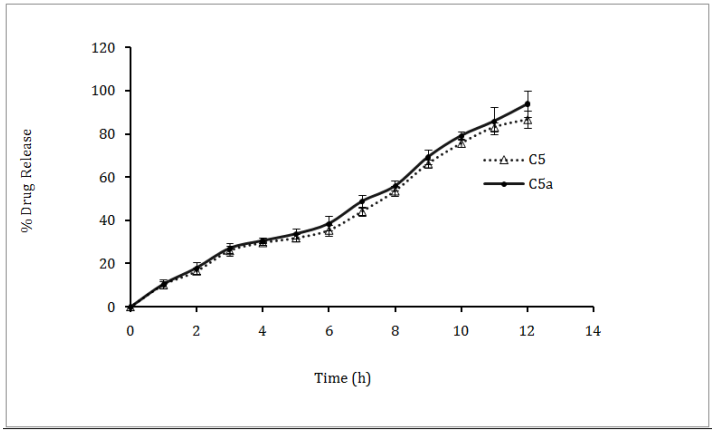

Fig. 4: *In vitro* Drug release profile of optimized formulation C5 and C5$_a$, The error bars indicates standard deviations of three tests

Table 3: Correlation coefficient values of various kinetic models for optimized formulations

Formulation	Regression Coefficient(r)			
	Higuchi Matrix	Peppas plot	First order plot	Zero order plot
C5	0.8764	0.9711	0.8874	0.9794
C5a	0.8817	0.9767	0.8329	0.9841

Antimicrobial efficacy study

Pefloxacin has been proved to exhibit superior antibacterial activity against *Pseudomonas aeruginosa which is a causative* organism of bacterial conjunctivitis. Hence *in vitro* antimicrobial activity was done, using strain of gram negative bacteria, *pseudomonas aeruginosa* [26]. Microbiological studies were carried out to ascertain the biological activity of the optimized formulation against microorganisms *Staphylococcus aureus* and *Pseudomonas aeruginosa***.** Results of Antimicrobial studies are as shown in the table 4**.** The formulations C5 and C5a showed good antimicrobial efficacy which was comparable to that of pure drug. This study indicated that the Pefloxacin retained its antimicrobial efficacy even after formulated as an *in situ* gelling system.

Table 4: Comparison of antimicrobial activity of pure drug and optimum formulations

Formulation	Pseudomonas aeruginosa (MTCC 1934)		Staphylococcus aureus (MTCC 737)	
	ZOI(mm)	% Efficacy	ZOI(mm)	%Efficacy
Standard (Pure Drug)	46	100	47	100
C5	45	97.82	44	95.65
C5a	41	89.13	44	95.65

Hemolysis studies

To check the hemo compatibility of the formulations hemolysis study was performed using red blood cells [RBCs]. Results of hemolysis study are shown in table 5**.** Ocular irritation generally results from cytotoxic reactions of the applied substances with ocular tissues. Hemolysis test is considered as a simple, rapid, and reproducible technique to assess ocular irritation based on damage to RBC membranes. Also, hemolysis assay offers good sensitivity and specificity and shows minimum difference with the Draize test among the different *in vitro* tests available [27]. Formulations C5 and C5a showed negligible hemolysis of 3.6 and 3.8% respectively which was in the range of 0-4 %, similar to that of normal saline-treated erythrocytes.

Table 5: Results of Hemolysis studies of optimum formulations

Formulations/control	Absorbance at 540 nm	Percentage hemolysis
Positive control	0.442	100 %
Negative control	000	0%
C5	0.0161	3.642%
C5a	0.0172	3.89%

CONCLUSION

An optimized ion activated *in situ* opthalmic gel of Pefloxacin mesylate can be formulated by using the combination of SA and HEC. Formulation was liquid at non physiological pH [pH 5] and underwent rapid gelation at physiological pH [pH 7.4]. The formulation was found to be clear, having good bioadhesion, and viscosity, and the release kinetic study showed sustained release for a period of 12hr that followed Zero order kinetics. Optimized formulation was isotonic, non irritant and exhibited good antimicrobial efficacy. Hence from all the above results we can conclude that prepared formulation can be conveniently administer in drop form that undergo a phase transition in the ocular cul-de-sac to form a viscoelastic gel, which increases pre corneal residence time and sustain the drug for the longer period of time.

CONFLICT OF INTERESTS

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper

REFERENCES

1. Gupta H, Velpandian T, Jain S. Ion and pH activated novel in-situ gel system for sustained ocular drug delivery. J Drug Target 2010;18:499-505.
2. Balasubramaniam J, Kant S, Pandit J. K.-*In vitro* and *in vivo* evaluation of the Gelrite gellan gum-based ocular delivery system for indomethacin. Acta Pharm 2003;53:251-61.
3. Nanjawade BK, Manvi FV, Manjappa AS. In situ-forming hydrogels for sustained ophthalmic drug delivery. J Controlled Release 2007;122:119-34.
4. Manjappa AS, Nanjwade BK, Manvi FV, Murthy RSR. Sustained ophthalmic in situ gel of ketorolac tromethamine: rheology and *in vivo* studies. Drug Dev Res 2009;70:417-24.
5. Shastri DH, Prajapati ST, Patel LD. Thermoreversible mucoadhesive ophthalmic in situ hydrogel: Design and optimization using a combination of polymers. Acta Pharm 2010;60:349-60.
6. Paulsson M, Hägerström H, Edsman K. Rheological studies of the gelation of deacetylated gellan gum [Gelrite] in physiological conditions. Eur J Pharm Sci 1999;9:99-105.
7. Gonjari ID, Karmarkar AB, Khade TS, Hosmani AH, Navale RB. Use of factorial design in formulation and evaluation of ophthalmic gels of gatifloxacin: Comparison of different mucoadhesive polymers. Drug Discoveries Ther 2010;4:423-34.
8. Hagerstrom H, Paulsson M, Edsman K. Evaluation of mucoadhesion for two polyelectrolyte gels in simulated physiological conditions using a rheological method. Eur J Pharm Sci 2000;9:301–9.
9. Mohammed MM, Hoda AE, Magda W. Mucoadhesive liposomes as ocular delivery system: physical, microbiological, and *in vivo* assessment. Drug Dev Ind Pharm 2010;36:108-18.
10. Séchoy O, Tissié G, Sébastian C, Maurin F, Driot JY, Trinquand C. A new long acting ophthalmic formulation of Carteolol containing alginic acid. Int J Pharm 2000;207:109-16.

11. Gonzalez JP, Henwood JM. Pefloxacin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic uses. Drugs 1989;37:628-68.
12. Blondeau JM. Fluoroquinolones: mechanism of action, classification, and development of resistance. Surv Ophthalmol 2004;49:73-8.
13. Choi HG, Jung JH, Ryu JM, Yoon SJ, Oh YK, Kim CK. Development of in situ gelling and mucoadhesive acetaminophen liquid suppository. Int J Pharm 1998;165:33-44.
14. Sultana Y, Aqil M, Ali A, Zafar S. Evaluation of carbopol-methylcellulose based sustained-release ocular delivery system for pefloxacin mesylate using rabbit eye model. Pharm Dev Technol 2006;11:313-9.
15. Gonjari ID, Hosmani AH, Karmarkar AB, Godage AS, Kadam SB, Dhabale PN. Formulation and evaluation of in situ gelling thermoreversible mucoadhesive gel of Fluconazole. Drug Discoveries Ther 2009;3:6-9.
16. Doijad RC, Manvi FV, Malleswara Rao VSN, Prajakta, Alsae. Sustained ophthalmic delivery of gatifloxacin from in situ gelling system. Indian J Pharm Sci 2006;68:814-8.
17. Mitjans M, Infante MR, Vinardell MP. Human hemoglobin denaturation as an alternative to the Draize test for predicting eye irritancy of surfactants. Regul Toxicol Pharmacol 2008;52:89-93.
18. Dharmendra J, Edmund C, Ajit KB, Rinti B. Development of polyvinyl alcohol–gelatin membranes for antibiotic delivery in the eye drug delivery in the eye. Drug Dev Ind Pharm 2011;37:167–77.
19. Desi HA, Bhalla HL. Preparation and evaluation of new eye drops containing a combination of ciprofloxacin and dexamethasone. Indian Drugs 2000;37:112.
20. Pandit J, Bharathi D, Srinatha A, Ridhurkar D, Singh S. Long acting ophthalmic formulation of indomethacin: evaluation of alginate gel systems. Indian J Pharm Sci 2007;69:37-40.
21. Sechoy O, Tissié G, Sébastian C, Maurin F, Driot JY, Trinquand C. A new long acting ophthalmic formulation of Carteolol containing alginic acid. Int J Pharm 2000;207:109-16.
22. Larsen B, Smidsrød O, Painter TJ, Haug A. Calculation of the nearest-neighbour frequencies in fragments of alginate from the yields of free monomers after partial hydrolysis; Acta Chem Scand 1970;24:726–8.
23. Mohanambal E, Arun K, Abdul Hasan Sathali A. Formulation and Evaluation of pH-Triggered in situ Gelling System of Levofloxacin. Indian J Pharm Educ Res 2011;45:58-64.
24. Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a PH triggered in situ gelling system. J Controlled Release 2001;73:205-11.
25. Patel PB, shastri DH, shelat PK. Development and evaluation pH triggered in situ gelling ophthalmic gel formulations of ofloxacin. AJPTR 2011;1:430-45.
26. Suman Y, Nayyar P. A comparative evaluation of 0.3% eye drops and in situ forming gel of pefloxacin mesylate in experimentally induced pseudomonas conjunctivitis. Continental J Pharmacol Toxicol Res 2008;2:1-5.
27. Sharma P, Sharma JD. *In vitro* hemolysis of human erythrocytes by plant extracts with antiplasmodial activity. J Ethnopharmacol 2001;74:239–43.