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Sustained ophthalmic delivery of Ciprofloxacin Hydrochloride from an ion-activated *in situ* gelling system

Tanvi P. Pandya*, Moin K. Modasiya and Vishnu M. Patel

A.P.M.C. College of Pharmaceutical Education and Research, Motipura, Himatnagar, North Gujarat, India

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ABSTRACT

Most eye diseases are treated with topical application of eye drops. The poor bioavailability and therapeutic response exhibited by these conventional eye drops due to rapid precorneal elimination of the drug may be overcome by the use of in situ gelling systems that are instilled as drops into the eye and undergo a sol-to-gel transition in the cul-de-sac. Hence, the purpose of the present work was to formulate and evaluate an ophthalmic delivery system for the ciprofloxacin hydrochloride, based on the concept of ionic interaction approach for in situ gelation. Sodium alginate was used as the gelling agent in combination with Hydroxy Propyl Methyl Cellulose (HPMC K4M) which acted as a viscosity enhancing agent. The prepared formulations were characterized for clarity, pH, drug content, viscosity, gelling capacity, in vitro drug release, antimicrobial efficacy, ocular irritation and stability. The clarity, pH viscosity and drug content of the developed formulation were found to be satisfactory. The developed formulation was therapeutically efficacious, stable, non-irritant, and provided sustained drug release over an 8-h period. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to produce sustained drug release. Also important factor is the ease of instillation and the reduced frequency of instillation resulting in better patient acceptance.

Key words: *In situ* gelling, sodium alginate, ciprofloxacin hydrochloride, antimicrobial efficacy.

INTRODUCTION

Ophthalmic drug delivery is one of the challenging endeavors facing the pharmaceutical scientists today. The structural and functional aspects of the eye render this organ highly impervious to foreign substances. A significant challenge to the formulator is to overcome the protective barriers of the eye without causing permanent tissue damage. The major problem

encountered with topical administration is the rapid pre-corneal loss caused by nasolacrimal drainage and high tear fluid turnover which leads to only 10% drug concentrations available at the site of actions. The approaches to enhance the ocular bioavailability aim at increasing the corneal permeability by using penetration enhancers or prodrugs, and prolonging the contact time with the ocular surface by using viscosity-enhancing or *in situ* gelling polymers.

The *in situ* gelling polymers undergo sol-to-gel phase transition on exposure to the physiological conditions present in the eye. *In situ* gels are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change in a specific physico-chemical parameter (ionic strength, temperature or pH) [1, 2]. *In situ* gelling systems can be classified as ion activated systems (e.g. Gelrite [3, 4], sodium alginate [5] temperature dependent systems (e.g Pluronics [6, 7], Tetronics and polymethacrylates [8]), pH triggered systems (e.g Carbopol [9, 10, 11] and cellulose acetate phthalate). The principal advantage of *in situ* gels is that they can be easily administered with accurate and reproducible dose compared to that of preformed gels and have an advantage over preformed gels that they can be easily instilled in liquid form, and are capable of prolonging the residence time of the formulation on the surface of the eye due to gelling.

The objective of the present study was to develop an ion activated *in situ* gelling for Ciprofloxacin hydrochloride, a fluoroquinolone derivative used to treat external infections of the eye such as acute and sub acute bacterial conjunctivitis, conjunctivitis, keratitis, keratoconjuctivitis and corneal ulcers which can prevent frequent drug administration and enhance patient compliance. Sodium alginate was used as the gelling agent in combination with Hydroxy Propyl Methyl Cellulose (HPMC) as the viscosity enhancer for the formulation of Ciprofloxacin Hydrochloride eye drops (0.3% w/v), which undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride was obtained as a gift sample from Montage Pharmaceutical Pvt. Ltd., Himatnagar, India. Sodium alginate and HPMC were purchased from Anilax Enterprises Inc. Columbia Turnpike, Florham Park USA. All other reagents were of analytical grade.

Table 1: Composition of developed formulations

Ingredients	F1	F2	F3	F4	F5
Ciprofloxacin hydrochloride (%w/v)	0.3	0.3	0.3	0.3	0.3
Sodium alginate (%w/v)	0.6	0.8	1.0	1.2	0.8
HPMC (%w/v)	0.5	0.5	0.5	0.5	-
Benzalkonium chloride (%w/v)	0.02	0.02	0.02	0.02	0.02
Distilled water (q.s. upto)	100	100	100	100	100

Preparation of formulation

The table 1 shows the composition of all the formulations. Sodium alginate and HPMC were dissolved in a beaker containing purified water, and this solution was heated about 85°C for 15 min, then beaker was cooled with stirring. After cooling benzalkonium chloride and drug

solution were added to the polymer solution and volume was made up to 100 ml and this solution was filtered through 0.2 mm filter paper. [12]

Characterization for Ion Activated Ocular Gels

Visual appearance and Clarity

The clarity of the formulations before and after gelling was determined by visual examination of the formulations under light alternatively against white and black backgrounds. [13]

pH

Formulation was taken in a beaker and pH was checked using pH meter (pH Systronics digital pH meter). [13]

Viscosity

The viscosity was measured using a Brookfield Synchrolectric viscometer (RVT model) in the small volume adapter. The viscosity measured at 20 rpm was used for purposes of comparative evaluation. **[**14, 15]

***In vitro* Release Studies**

The *in vitro* release of Ciprofloxacin hydrochloride from the formulations was studied through cellophane membrane using a modified USP XXIII dissolution testing apparatus. Freshly prepared artificial tear fluid (pH 7.4) was used as the dissolution medium. 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 placed 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. Aliquots, each of 1 ml volume, was withdrawn at hourly intervals and replaced by an equal volume of the receptor medium. The aliquots were diluted with the receptor medium and analyzed by UV-Vis spectrophotometer at 272 nm. [16]

Sterility

All ophthalmic preparations should be sterile therefore the test for sterility is very important evaluation parameter. The sterility test was performed according to Indian Pharmacopoeia. Direct inoculation method was used. 2 ml of liquid from test container was removed with a sterile pipette or with a sterile syringe or a needle. The test liquid was aseptically transferred to fluid thioglycolate medium (20 ml) and soyabean-casein digest medium (20 ml) separately. The liquid was mixed with the media. The inoculated media were incubated for not less than 14 days at 30˚C to 35˚C in the case of fluid thioglycolate medium and 20˚C to 25˚C in the case of soyabean-casein digest media. **[**17]

Anti microbial efficacy studies

This was determined by the agar diffusion test employing “Cup plate technique”. Sterile solutions of Ciprofloxacin hydrochloride (standard solution) and the developed formulations were diluted at different concentration (test solutions) these solutions were poured in to cups bored into sterile nutrient agar previously 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°C for 24 hrs. The zone of inhibition (ZOI) measured around each cup was compared with that of control. The entire operation except the incubation was carried out in a laminar airflow unit. Both positive and negative controls were maintained during the study. [18]

Ocular Irritation Studies

In vivo ocular irritation studies were performed according to the Draize technique (Organization for Economic Co-operation and Development (OECD). Assessment of ocular irritation potential of ophthalmic formulations was as per OECD guideline number 405, 1987. Thus, six female albino rabbits each weighing 2 to 3 kg were used for the study of the formulations. The sterile formulation was instilled once a day for a period of 21 days and the rabbits were observed periodically for redness, swelling, and watering of the eye, as mentioned in OECD guidelines. [19, 20]

Accelerated stability study

Selected sterilized formulations were stored at 4±1˚C, room temperature (25±1˚C), 37±1˚C and 45±1˚C for a period of three months. The formulations were evaluated at periodic intervals for drug content, clarity, pH, gelling capacity, viscosity, in vitro drug release and sterility.

RESULTS AND DISCUSSION

The color of the formulation is light yellow and Clarity of all formulations was found to be satisfactory. The pH was within acceptable range and hence would not cause any irritation upon administration of the formulation. Table 2 also shows the result of pH, gelling capacity, drug content and viscosity for all formulations. The drug content was found to be in acceptable range for all formulations. Percent drug content in all four formulations were in the range 98-99 %.The two main prerequisite of gelling system are viscosity and gelling capacity. The formulation should have an optimum viscosity which will allow its instillation into the eye as a liquid which will then undergo rapid sol-gel transition due to pH change. Moreover, to facilitate sustained release of drug to the ocular tissue them in situ formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time. All the formulations gelled instantaneously on contact with simulated tear fluid (STF). STF was prepared using NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2 \cdot 2H_2O$ 0.008 g and water up to 100.0 g. [21]

Table 2: Evaluation of *in situ* gelling systems of Ciprofloxacin hydrochloride

Parameter	F1	F2	F3	F4	F5
pH	6.8	6.8	6.7	6.9	6.6
Gelling capacity	+	+++	+++	+++	++
Drug content (%)	98.32	99.12	98.26	98.89	98.43
Viscosity (cps)	32	36	39	48	21

Note: + gels slowly and dissolves; ++ gelation immediate and remains for a few hours; +++ gelation immediate and remains for an extended period.

The formulations were liquid at room temperature and at the pH formulated (pH 6.0) and they underwent rapid transition to the gel phase at the pH of the tear fluid (pH 7.4). Terminal sterilization by autoclaving had no effect on clarity, pH, viscosity and gelling capacity of F1-F4. The haziness that was observed after autoclaving in F1-F4 (due to precipitation of HPMC at

elevated temperature) was found to disappear and the original clarity was regained after standing overnight.

In vitro drug release study

The cumulative percent of Ciprofloxacin hydrochloride released as a function of time is shown in Fig. 1. 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 Ciprofloxacin hydrochloride and that premature drug release will not occur. In the cul-de-sac, the gels will probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movements.

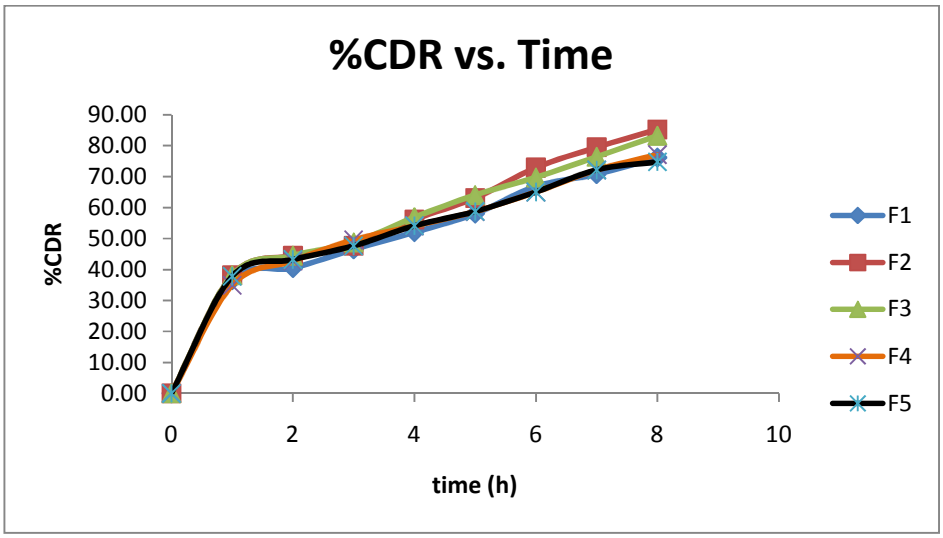

Figure 1. *In vitro* drug release profile

Sterility

The formulations passed the sterility test 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^{o}C in case of fluid thioglycollate medium and at 20-25^{o}C in the case of soyabean casein digest medium.

Table 3: Antimicrobial efficacy testing

Concentration (mg/ml)	Zone of inhibition (mm)		Percent efficacy (%)
	Standard	Test	
S. aureus			
1	1.7	1.5	88.23
10	2.9	2.8	96.55
100	3.8	3.5	92.10
500	5.8	5.2	89.65
P. aeruginosa			
1	0.8	0.7	87.5
10	1.4	1.3	92.85
100	3.5	3.3	94.28
500	4.9	4.6	93.87

Antimicrobial efficacy study

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

Ocular irritation study

The results of the ocular irritation studies indicated that the formulation F2 and F3 is non-irritant with excellent ocular tolerance. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible. Watering of eyes, redness or inflammation was not observed during the period of ocular irritation study.

Accelerated stability studies

Stability studies were carried out on F2 and F3. It was found that F3 turned viscous at 4±1˚C and the study was discontinued after 1 month. F2 was tested for 3 months and was found to be clear with no change in pH (6-6.5), drug content (96-98%), viscosity, *in vitro* release, gelling capacity and sterility.

CONCLUSION

Ciprofloxacin, a broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as pH-triggered *in situ* gel forming eye drops (0.3% w/v) using sodium alginate as a gelling agent in combination with HPMC as a viscosity enhancing agent. The formulation was liquid and underwent rapid gelation when it comes in contact with tear fluid due to ionic interactions. The gel formed *in situ* afforded sustained drug release over an 8-h period. The formulations were therapeutically efficacious. Stability data recorded over a 3-month period under accelerated temperature conditions indicated the formulation to be stable. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and 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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