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Research Article

DESIGN AND DEVELOPMENT OF pH-TRIGGERED IN SITU GELLING SYSTEM OF CIPROFLOXACIN

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ABSTRACT
The aim of the present work was formulation and evaluation of in situ gelling system of ciprofloxacin. The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid precorneal elimination of the drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and undergo a sol–gel transition in the cul-de-sac. Hence, the purpose of the present work was to formulate pH-triggered, temperature triggered & ion activated in situ Gelling system of ciprofloxacin to provide sustained release of drug based on polymeric carriers that undergo sol-to-gel transition upon change in pH. The ciprofloxacin in situ gelling system formulated by using poly acrylic acid (Carbopol 934) in combination with hydroxyl propylmethyl cellulose (HPMC) which acted as viscosity enhancing agent. The developed formulation was efficacious, stable, non-irritant and provided sustained release over 8-hour period and it is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time & ability to produced sustained drug release.
Keywords: In situ gel, Cabopol 934, pH, ciprofloxacin

INTRODUCTION

Eye has special attributes that make local drug delivery and non-invasive clinical assessment of the disease possible. There are a wide variety of ophthalmic drug delivery systems in the market.

Ophthalmic drug delivery is one of the most interesting and challenging drug delivery for pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the Ophthalmic drug delivery is one of the most interesting and challenging drug delivery for pharmaceutical scientist. The anatomy, physiology and biochemistry of the eye render this organ exquisitely impervious to foreign substances. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased. Several new preparations 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 drug elimination. However, these preparations have some disadvantages such as poor compliance, especially by elderly people and many patients sometimes lose the device without noticing it. From the point of view of patient acceptability, a liquid dosage form is preferable. This problem can be overcome by the use of polymeric solutions, which change to a gel as a result of exposure to the physiological temperature, pH or ionic composition of the lacrimal fluid. Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye, which upon exposure to physiological conditions, changes to the gel phase thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased [1, 2, 3].

These in situ gelling systems consist of polymer that exhibit sol-to-gel phase transitions due to change in specific physicochemical parameters (pH, Temperature, ionic strength) in the environment, cul-de-sac in the case of eye. The sol-to-gel phase transition on the eye surface depending on the different methods employed and they are: pH-triggered system (eg. Cellulose acetate hydrogen phthalate latex), temperature dependent system (eg. Pluronics and tetronics) and ion activated system (eg. Gelrite) [4].

The aim of the present work is to study the pH-triggered in situ gelling system of ciprofloxacin, a second generation fluoroquinolone derivative used in the infection of the eye such as acute conjunctivitis. A combination of carbopol and HPMC was investigated as a vehicle for the formulation of eye drops of ciprofloxacin (0.5%) to form gel when instilled into the eye to provide sustained release of the drug to improve the patient compliance by reducing the frequency of administration. with simulated tear fluid (in the proportion of 25:7 i.e. application volume 25µl and normal volume of tear fluid inthe eye is 7µl) to find out the gelling capacity of the ophthalmic product. The gelation was then assessed visually by noting the time for the gelation and the time taken for dissolution of the formed gel.

Various problems encountered in poor bioavailability of t he eye installed drugs are:

- Binding by the lachrymal proteins.
- Drainage of the instilled solutions;
- Lacrimation and tear turnover;
- Limited corneal area and poor corneal
- Metabolism;
- Non-productive absorption/adsorption;
- Tear evaporation and permeability;

VARIOUS APPROACHES OF IN-SITU GELATION:

Ideally, an in-situ gelling system should be a low viscous, free flowing liquid to allow for reproducible administration to the eye as drops, and the gel formed following phase transition sh-ould be strong enough to with stand the shear forces in the cul-de-sac and demonstrated long residence

times in the eye. In order to increase the effectiveness of the drug a dosage form should be chosen which increases the contact time of the drug in the eye. This may then prolonged residence time of the gel formed in situ along with its ability to release drugs in sustained manner will assist in enhancing the bioavailability, reduce systemic absorption and reduce the need for frequent administration leading to improved patient compliance.

Depending upon the method employed to cause sol to gel pha setransition on the ocular surface, the following types of syste ms are recognized [5, 6, 15]:

• **pH-triggered systems:** cellulose acetate phthalate (CAP) latex, carbopol, polymethacrilic acid (PMMA), polyethylene glycol (PEG), pseudolatexes.

•**Temperature dependent systems**: Chitosan, pluronics, tetronics, xyloglucans, hydroxypropylmethyl cellulose or hypromellose (HPMC).

•**Ionactivated systems (osmotically induced gelation):** Gelr ite, gellan, hyaluronic acid, and alginate [4, 7].

pH-triggered in-situ gelation: Polyacrylic acid (Carbopol 940) is used as the gelling agent in combination with hydroxypropyl-methylcellulose (Methocel E50LV) which acted as a viscosity enhancing agent. The formulation with pH-triggered in-situ gel is therapeutically efficacious, stable, non irritant and provided sustained release of the drug tear fluid[16].

MATERIALS AND METHODS

Materials: Ciprofloxacin was obtained as gift sample from chempure labs limited, Mumbai, Cabopol 934 (polyacrylic acid), Hydroxypropyl methyl cellulose was obtained as gift sample from colorcon Asia PVT. Ltd, Tween 20, Benzalkonium chloride was obtained as a gift from seemed Labs Limited, Hydrabad.

Preparation of formulations

Selection of vehicle

The solubility of ciprofloxacin was test in various buffers, such as acetate buffer I.P. (pH 4.6, 5.0 & 5.5), citrophosphate buffer B.P. (pH 5.0, 6.0, 7.2), phosphate buffer U.S.P.(pH 5.5, 6.5) in order to select a suitable vehicle. Solutions of ciprofloxacin (0.5% w/v) in buffer in which it was soluble were prepared & these were tested for stability to light, temperature & autoclaving using a stability indicating high-performance thin-layer chromatography [7, 8].

Preparation of in situ gelling system

To prepare the aqueous solutions of different concentration of carbopol 934 & different grades of HPMC (Grades HPMC K4M, E50LV, E15LV) used. Also prepared different formulation (formulation F1, F2…….F6) & evaluated there gelling capacity & viscosity in order to identify the compositions most suitable use as in situ gelling systems (Table 1) The gelling capacity was determined by placing 100 µl of the system in a vial containing 2 ml of artificial tear fluid freshly prepared & equilibrated at 37^0C & visually assessing the gel formulation & noting the time for gelation & the time taken for gel formed to dissolve [16, 17, 18].

Table 1: Composition of in situ gels

Ingredient % w/v	F1	F2	F3	F4	F5	F6
Ciprofloxacin	0.5	0.5	0.5	0.5	0.5	0.5
Carbopol 934	0.5	0.3	0.5	0.5	0.3	0.25
HPMC-K4M	0.7	0.6	-	-	-	-
HPMC-E15LV	-	-	-	-	1.5	1.5
HPMC-E50LV	-	-	1.5	1.5	-	-
Acetic acid	10ml	10ml	10ml	10ml	10ml	10ml
Di-sodiumhydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125
Citric acid	0.407	0.407	0.407	0.407	0.407	0.407
Tween 20	1.0	1.0	1.0	1.0	1.0	1.0
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02
Purified water	100ml	100ml	100ml	100ml	100ml	100ml
Gelling capacity	+++	++	+	+++	-	+

Methods of preparation

Different concentrations of polymers were used to prepare ophthalmic solutions as per the composition shown in table no.1.

1. The buffer salts were dissolved in 75ml purified water, then added the different grades of polymers & allow to hydrate. To this buffered polymeric solution tween 20 was added. Carbopol 934 was sprinkled over this solution & allow to hydrate overnight. The solution was stirred with an overhead stirrer.
2. Ciprofloxacin was dissolved in acetic acid (0.1N) separately and after adjusting the pH, benzalkonium chloride was added and the solution was passed through a 0.2 µm cellulose acetate filtered.
3. The drug solution was then added to the polymeric solution under constant stirring until a uniform solution was obtained. Distilled water was then added to make up the final volume (100ml). The formulations were filled in vials under aseptic conditions, sterilized in the autoclave (121^0 C and 15 p.s.i.) for 20 minutes and further evaluations were carried out [8, 9].

Composition of Simulated Tear Fluid (STF)

Sodium chloride	: 1.34 gm
Sodium bicarbonate	: 0.4 gm
Calcium chloride dihydrate	: 16 mg
Water upto	: 200ml

EVALUATION

Visual appearance, Clarity, pH, drug content:

The appearance and clarity were determined visually. The pH of the formulations was adjusted by using pH meter. The drug content of in situ gel was determined by taking sample (2ml) of in-situ gel in a 100 ml volumetric flask and diluted with simulated tear fluid of pH 7.4 to get the concentration of 10g/ml (approximately). Then the absorbance was measured at max (254nm) using UV-spectrophotometer (Simadzu UV 1800) to calculate the percentage of drug content [10, 11].

Gelling capacity

The prepared in situ gelling system was evaluated for gelling capacity in order to identify the composition suitable for use as in situ gelling system. The in situ gelling system was mixed with simulated tear fluid (in the proportion of 25:7 i.e.application volume 25µl and normal volume of tear fluid in the eye is 7µl) to find out the gelling capacity of the ophthalmic product. The gelation was then assessed visually by noting the time for the gelation and the time taken for dissolution of the formed gel [14].

Rheological studies

The relationship between contact time and the rheology was easily understood for viscosity enhanced ophthalmic solutions. It was noted from various literature that the formulations before gelling should have a viscosity of 5 to1000 mpa and after gelling in the eye will have a viscosity from about 50-50,000 mpa. Rheological studies of the prepared formulations were carried out by Brook field synchroelectric viscometer (LVDV Pro II) using spindle S18 (small sample adaptor). The viscosities of the formulations were determined at different speed conditions (10, 20, 50, 75 to 100 rpm) [12, 13].

In vitro release studies

The drug release from the prepared formulation was studied by placing the test solution in a circular Teflon cup of 2.5cm internal diameter and1.2cm depth. This was in turn placed on an inverted USP basket kept inside a 250-ml beaker containing 200 ml of simulated tear fluid (pH 7.4) as a dissolution medium and it was stirred by a magnetic stirrer (50 rpm) maintained at a temperature of 37±1 C. Samples (1ml) were withdrawn at regular intervals and replaced with an equal volume of fresh medium. The absorbance of the diluted samples was measured at max (254nm) by UV-spectrophotometer using simulated tear fluid as a blank to calculate amount of drug release from in situ gel. The percentage of drug release was plotted against time to find the drug release pattern of all in situ gel preparations. Then, the release of selected in situ gelling system was compared with that of marketed eye drops.

Sterility testing

IP method (1996) was followed for the sterility testing of eye drops. Sterility testing was carried out by incubating formulations for not less than 14 days at 30 to 35^{0}C in the fluid thioglycolate medium to find the growth of bacteria and at 20 to 25 C in the soyabean-casein digest medium to find the growth of fungi in the formulations [19, 20].

Ocular irritacy studies

Ocular irritation studies were performed on male albino rabbits weighing 1-2kg. The modified Draize technique was designed for the ocular irritation potential of the ophthalmic product. According to Draize test, the eye drops (100µl) was normally placed in the lower cul-de-sac and irritancy was tested at the time interval of 1hr, 24hrs, 48hrs, 72hrs, and 1week after administration. The rabbits were observed periodically for redness, swelling and watering of the eye [19].

FT-IR studies

The possibility of drug-excipient interactions were investigated by FTIR studies. The FTIR graph of pure drug and combination of drug with excipient were recorded using KBR pellet [20].

RESULTS & DISCUSSION

The various formulation were prepared the in situ gelling system by using a various concentration of carbopol 934 along with different grades of hydroxyl propyl methyl cellulose in different ratio as per formula given in table 1. The formulation was prepared six & all the formulations fixed drug concentration of (0.5%w/v) ciprofloxacin.

Appearance, clarity, pH and drug content

The appearances of all formulations were light yellow in colour and were clear except the formulation F-2 (cloudy). Terminal sterilization by autoclaving had no effect on the formulations. The haziness observed during autoclaving due to precipitation of HPMC at elevated temperature was found to disappear and the clarity was regained after overnight standing. The pH of all the formulations was found to be within the range of 6.0 to 6.4, which is desirable for the ophthalmic formulations. The drug content of all the formulations was within the range of 96.35% to 101.13%, showed the uniform distribution of drug in the ophthalmic formulations and the results are shown in Table-2.

Table 2: Evaluation of In situ gels

Formulations	Appearance	Clarity	pH	Percentage Drug content
F1	Light Yellow	Clear	6.1	97.74%
F2	Light Yellow	Cloudy	6.0	98.25%
F3	Light Yellow	Clear	6.1	98.33%
F4	Light Yellow	Clear	6.0	101.22%
F5	Light Yellow	Clear	6.2	96.53%
F6	Light Yellow	Clear	6.3	99.56%

Gelling capacity

The viscosity and gelling capacity plays important role for in situ gelling system. The formulation should have an optimum viscosity for easy instillation into the eye as a liquid which undergo sol-to-gel transition.

Formulations F-1 , F-4 prepared from Carbopol 934 (0.5 %) / HPMC (K4 M) , Carbopol 934 (0.5 %) / HPMC (E50 LV) and Carbopol 934 (0.5%) /HPMC (E15LV) respectively showed better gelling capacity & formulation F-2 showing the minimum gelling capacity. The other formulations were not having desirable gelling capacity. The grading for gelling capacity was shown in Table-1.

Rheological studies

The pseudo plastic character of precorneal tear film should be disturbed less by the administration of ophthalmic products. The ocular shear rate is about 0.03 s during inter-blinking periods and 4250 – 28500 s during blinking. So, the viscoelastic fluids having high viscosity under low shear rates and low viscosity under high shear rates which is called as pseudoplastic fluid is often preferred.

Moreover, the pseudoplastic property of these formulations may be in favor of sustaining the release of drug in the conjuctival sac of the eye, & also without blinking difficulty for undergoing shear thinning.

In vitro release studies

The release profile of the formulations shown in Figure 2. The results indicated that the formulation F-4 showed better sustaining effect amongst all formulations. This may be due to the presence of higher concentration of cabopol 934 along with HPMC (E50LV) in the formulation F-4. Results indicated that, the drug release was significantly prolonged by using the in situ gelling system due to the addition of the polymers carbopol 940 and HPMC (E50LV).

From the results it is concluded that the high viscosity plays important role in controlling the release of drug from the formulations. When the polymer concentration increases drug release decreases, and when polymer concentration decreases drug release from the formulation increases.

Table 3: Absorbance of different concentration of standard solution

Sr.no.	Concentration	Absorbance
1	2	0.0796
2	4	0.153
3	6	0.245
4	8	0.328
5	10	0.415
6	12	0.486
7	14	0.546

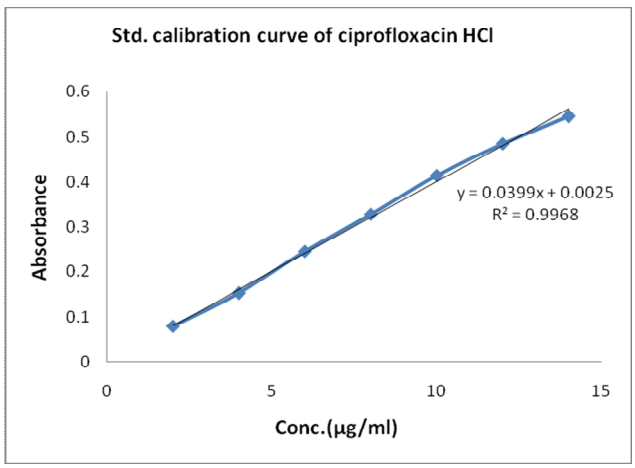

Fig 1: Standard calibration curve of ciprofloxacin HCl

Table 4: Comparison of in vitro release of in situ gel formulations

Time	F 1	F 2	F 3	F 4	F 5	F 6
1	14.77	13.88	16.11	12.88	14.33	14.88
2	23.88	29.44	30.55	28.44	25.66	28.44
3	35.77	38.33	41.22	38.33	43.22	41.33
4	50.22	51.33	51.66	48.33	51.33	45.77
5	62.66	66.44	59.11	59.55	62.66	64.22
6	72.66	75.33	71.33	72.33	72.33	69.44
7	84.33	84.33	87.11	86.88	84.88	82.77
8	93.88	96.11	97.33	90.11	95.11	93.55

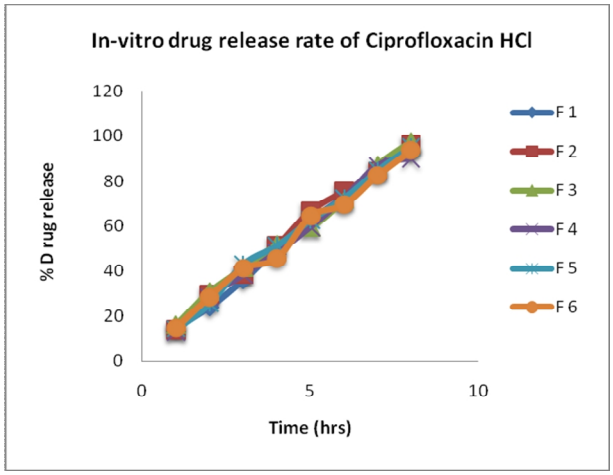

Fig 2: In-vitro drug release rate of Ciprofloxacin HCl

Sterility test

The formulation F-4 passed the test for sterility 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^{0}C in case of fluid thioglycolate medium and at 20-25^{0}C in the case of soyabean casein digest medium.

Ocular irritancy studies

The results of the ocular studies indicated that the formulation F-4 was non-irritant with excellent ocular tolerance. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctiva were visible.

Stability studies

From the results it has been observed that the formulations showed no change in appearance, clarity and pH. Further it was observed that the gelling capacity of the formulations was least affected.

FT-IR studies

FT-IR spectrum of pure drug and mixture of drug and polymers are shown in Figure-5, 6, 7, 8. From the spectral study it was observed that there was no significant change in the peaks of pure drug and drug polymer mixture. Hence, no specific interaction was observed between the drug and the polymers used in the formulations.

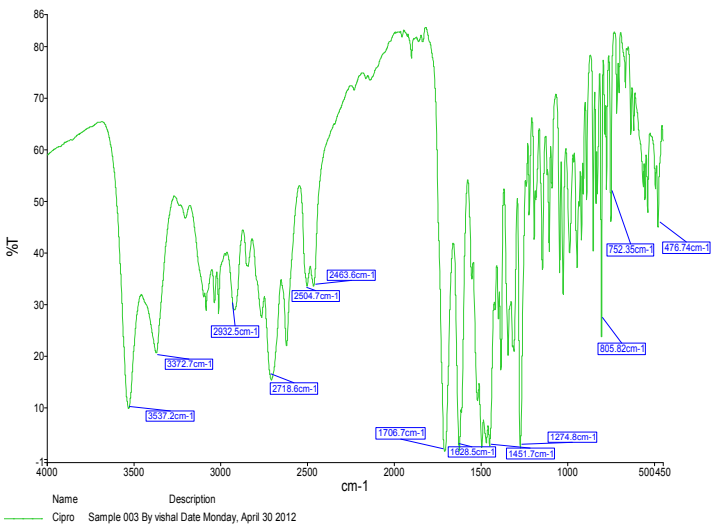

Fig 5: IR spectrum of Drug (Ciprofloxacin)

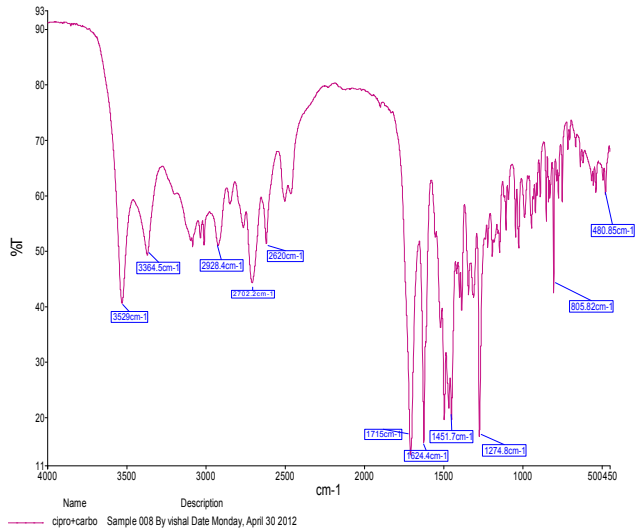

Fig 6: IR spectrum of Drug & Carbopol

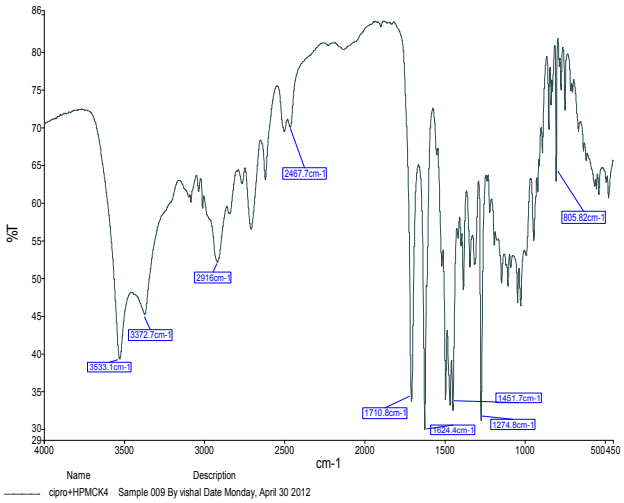

Fig 7: IR spectrum of Drug & polymer (HPMCK4M)

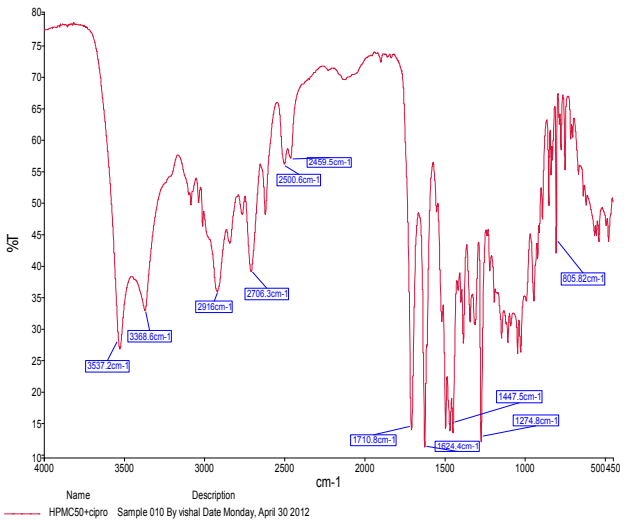

Fig 8: IR spectrum of Drug & polymer (HPMCE50)

CONCLUSION

In-situ gel offers-easy, accurate and reproducible administration of a dose, patient compliance, effective alternative to conventional dosage form, can easily be instilled in liquid form, but are capable of prolonging the residence time of the formulation on the surface of the eye ability to release drug in sustained manner, assist enhancing ocular bioavailability, flexibility in design system with desirable rheological properties and drug release rate.

The novel ophthalmic pH-triggered in situ gelling drug delivery was successfully formulated by using carbopol 940and HPMC (K4M, E50LV and E15LV). The formulated in situ gelling systems were characterized for appearance, clarity, pH, gelling capacity, rheological character, in vitro release in simulated tear fluid. The formulation was liquid at the formulated pH (6.0) and underwent rapid gelation upon raising the pH to 7.4. The pH-triggered in situ gelling system showed sustained drug release over 8-hr. period of time. So, this formulation is an alternate to conventional eye drops to improve the bioavailability through its longer precorneal residence time and ability to sustain drug release. The patient compliance may be improved due to the decrease in frequency of drug administration.

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