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Ion-Activated In Situ Gelling Systems for Sustained Ophthalmic Delivery of Ciprofloxacin Hydrochloride

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The poor bioavailability and therapeutic response exhibited by the conventional ophthalmic solutions due to 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. Our present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, CPH, based on the concept of ion-activated in situ gelation. Gelrite gellan gum, a novel ophthalmic vehicle that gels in the presence of mono or divalent cations, present in the lacrimal fluid was used alone and in combinations with sodium alginate as the gelling agent. The developed formulations were therapeutically efficacious and provided sustained release of the drug over an 8-hr period in vitro.

Keywords Ciprofloxacin Hydrochloride, Gellan, Gelling Systems, In Situ

Upon instillation of an ophthalmic solution, most of the instilled volume is lost from the precorneal area (Chrai et al. 1974; Schoenwald 1990), mainly due to the drainage of the excess fluid by the naso lacrimal duct and dilution and elimination of the solution by tear turnover. An increase in the dosing frequency or the use of highly concentrated solutions to compensate for the short ocular residence time is undersirable because of poor patient compliance and the risk of systemic toxicity due to absorption via the nasolacrimal duct (Middleton et al. 1990).

To increase the ocular bioavailability and duration of drug action, various ophthalmic vehicles such as viscous solutions (Schoenwald et al. 1978), ointments/gels (Maurice and Srinivas 1992), or polymeric inserts (Sasaki et al. 1993; Baeyens et al. 1998) have been used. The corneal contact time increases to varying degrees by these vehicles, but because of blurred vision (ointments), lack of patient compliance (inserts), and sticking of lids (gels), they have not been widely accepted. These shortcomings have led researchers to seek other systems that would combine ease of administration of liquid dosage forms with the prolonged residence time of the inserts.

From the point of view of patient acceptability, a liquid dosage form that can sustain drug release and remain in contact with the cornea for extended periods of time is ideal. If the precorneal residence time of a drug could be improved from a few minutes to, say, a few hours, then improved local bioavailability, reduced drug concentration, less dosing frequency, and improved patient acceptability may be achieved. Drug delivery systems based on the concept of in situ gel formation should provide these benefits. These systems consist of polymers that exhibit sol-to-gel phase transition due to a change in specific physicochemical parameter (pH, temperature, or ionic interaction) in their environment, the cul-de-sac in this case (Cohen et al. 1997).

Depending on the method employed to cause sol-to-gel phase transition on the ocular surface, the following three types of systems are recognized: pH triggered systems—cellulose acetate hydrogen phthalate latex (Gurny 1981; Gurny et al. 1985) and Carbopol (Srividya et al. 2001); temperature-dependent system—pluronics (Miller and Donovan 1982; Desai and Blanchard 1998) and tetronics (Vadnere et al. 1984; Spancake et al. 1989); and ion activated systems—Gelrite (Rozier et al. 1989) and gellan (Sanzgiri et al. 1993).

The objective of the present work was to develop an ion-activated in situ gelling system of ciprofloxacin hydrochloride (CPH), a fluoroquinolone derivative used in external infections of the eye. Gellan, alone and in combinations with sodium alginate, was investigated as vehicle for the formulation of eye drops of CPH (0.3% w/v), which would undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug during treatment of ocular infections.

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MATERIALS AND METHODS

CPH was generously gifted by Ranbaxy Labs (India). Gellan was obtained commerically from Sigma (USA). The microbiological medium was purchased from Hi Media (India) and bovine serum albumin (BSA), lysozyme, and γ-globulin were purchased from CDH Ltd (India). All other reagents used were of analytical grade.

Preparation of Formulations

Gellan alone and its combinations with sodium alginate, with or without sodium citrate (20% w/w, w.r.t gellan), were dissolved in hot acetate buffer (70°C, prepared in fresh water for injection under laminar flow) pH 5.0 by continuous stirring at 40°C. The required quantity of CPH to give a final drug concentration of 0.3% w/v was added to the polymeric solution and stirred until dissolved. The formulations were filled in 10-ml amber-colored 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 p.s.i. for 20 min. The sterilized formulations were stored in a refrigerator (4–8°C) until further use.

Evaluation of the Formulations

Drug Content Uniformity

The vials ($n = 3$) containing the preparation were shaken for 2–3 min and 100 μl of the preparation were transferred aseptically to sterile 25-ml volumetric flasks with a micropipette and the final volume made up with acetate buffer pH 5.0. The concentration of CPH was determined at 276 nm (Shimadzu, UV-1601, Japan).

Gelation Studies

The gelation studies were carried out in gelation cells, fabricated locally using Teflon®. The cells were cylindrical reservoirs capable of holding 3 ml of the gelation solution (simulated tear fluid [STF]). Within the cells located at the bottom was a 250-μl transparent plastic cup to hold the gel sample in place after its formation. The studies were carried out using STF of composition 1 (sodium chloride-0.670 g, sodium bicarbonate-0.200 g, calcium chloride.$2H_2O$-0.008 g, and purified water q.s. to 100 g) (Srividya et al. 2001) and of composition 2 (BSA-0.268 g, lysozyme-0.268 g, gamma-globulin-0.134 g, calcium cholride $2H_2O$-0.008 g, D-Glucose-0.15 g, sodium chloride-0.65 g, and distilled water q.s. to 100 g) (Van Haeringen 1981), which simulate the divalent cation content and both the protein and divalent cation content of the tear fluid, respectively.

Then, 100 μl of the preparation were carefully placed into the cavity of the cup using a micropipette, and 2 ml of the gelation solution (composition 1 or 2) were added slowly. Gelation was detected by visual examination.

Rheological Studies

Viscosity determinations of the prepared formulations were carried out on a cone (0.8°) and plate geometry viscometer (Brookfield) using spindle cp 40. The viscosity of the sample solutions was measured at different angular velocities at a temperature of 37 ± 1°C. A typical run comprised of changing the angular velocity from 0.5 to 100 rpm at a controlled ramp speed. After a wait of 0.1 min (6 sec) at 0.5 rpm, the velocities were increased to 100 at the same controlled ramp speed with similar wait at each rpm. The hierarchy of the angular velocity was reversed at the same ramp speed with a similar wait of 0.1 min. The average of the two readings was used to calculate the viscosity. The evaluations were conducted in triplicate.

In Vitro Release Studies

The drug release kinetics from the prepared formulations was studied using a modified method reported earlier (Lin and Sung 2000). First, 2 ml of the test solution was placed in circular plastic cup (2.5-cm internal diameter and 1.2 cm in depth). This was in turn placed on an inverted USP basket kept inside a 250-ml beaker. Then, 200 ml of dissolution medium (STF of composition 1) was added and stirred with a star headed magnetic bead. Temperature of 37 ± 1°C was maintained throughout the study. Next, 5-ml samples were withdrawn at regular time intervals and replaced with an equal volume of the prewarmed medium. The samples were analyzed for CPH content at 276 nm using an ultraviolet spectrophotometer (UV-1601, Shimadzu, Japan).

Antimicrobial Efficacy Studies

This efficacy was determined by the agar diffusion test employing the cup-plate technique. Marketed eyedrops of CPH (standard preparation) and the developed formulations, diluted suitably with acetate buffer pH 5.0 (test preparations), were poured into cups bored into sterile nutrient agar previously seeded with *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*). After allowing diffusion of the solutions for 2 hr, the agar plates were incubated at 37 ± 0.5°C for 24 hr. 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 flow unit. Each solution was tested in triplicate. Both positive and negative controls were maintained throughout the study.

In Vivo Rabbit Eye Study

Albino rabbits of either sex weighing 1.5 to 1.8 kgs were used for the single dose study. The animals were placed in restraining boxes during the experiment, which allowed their heads to move freely and their eye movements were not restricted. Between experiments, the rabbits were housed singly and allowed food and water ad libitum.

Suspensions of *S. aureus* and *P. aeruginosa* were prepared to give 0.5 McFarland standard. The standard is said to have been achieved when the absorbances of the prepared suspensions of the microorganisms matched with that of a barium sulphate 0.5 McFarland standard at 625 nm. Ideally the absorbances should be between 0.08 to 0.1.

Three rabbits were used for each of 10, 25, and 40 μl of 0.5 McFarland suspensions of *S. aureus* and *P. aeruginosa* instilled in both the eyes of each rabbit. After allowing the growth to proceed to the log phase (~18 hr) swabs from both eyes were taken at periodic intervals up to 24 hr and streaked on sterile nutrient agar plates and incubated at $37 \pm 0.5^{\circ}$C for 24 hr and checked for growth. This study helped to arrive at an inoculum volume capable of maintaining a growth up to 24 hr for conducting the in vivo study. The selected inoculum dose was then instilled in the right eye of each of 5 rabbits and the left eye served as the positive control. The formulations (2 drops each of GC_3 and GC_7) were then instilled in the right eye of the rabbit and cotton swabs from both eyes were taken at 2, 4, 8 and 24 hr.

RESULTS AND DISCUSSION

The composition of the various batches of the fabricated eyedrops are shown in Table 1. In the case of gellan-containing preparations, increasing the concentration of gellan beyond 0.0625% caused gelation upon cooling to 40°C (during stirring). This observation was quite interesting since gellan at a concentration of 0.6% was used earlier (Rozier et al. 1989; Sanzgiri et al. 1993) to prepare eyedrops of timolol maleate and methylprednisolone. The ionic content of the vehicle used, in this case acetate buffer pH 5.0, and the presence of HCl in CPH could have contributed to the gelation of gellan when used beyond 0.0625% (used in this study). In the combination systems with sodium alginate, the concentration of gellan was kept constant at 0.03% and the concentration of sodium alginate was varied to give a maximum of 1% polymer concentration, since an increase in total polymer concentration beyond 1% resulted in gelation during formulation.

Evaluation of Formulations

The physicochemical properties of the prepared formulations are shown in Table 2. The drug content, clarity, and pH of the formulations were found to be satisfactory and the formulations were liquid at both room temperature and when refrigerated.

TABLE 1
Composition of the prepared in situ gelling formulations

Batch code	Gellan (% w/v)	Sodium citrate (% w/w)	Sodium alginate (% w/v)
GC_1	0.015	—	—
GC_2	0.032	—	—
GC_3	0.0625	—	—
GC_4	0.0625	20	—
GC_5	0.032	20	—
GC_6	0.03	—	0.22
GC_7	0.03	—	0.47
GC_8	0.03	—	0.72
GC_9	0.03	—	0.97

TABLE 2
Physicochemical properties of the prepared gelling systems

Batch code	Drug content uniformity (% ± SD)	Gelling capacity	
		STF 1	STF 2
GC_1	98.48 ± 1.14	+	+
GC_2	99.64 ± 0.38	++	++
GC_3	99.18 ± 0.66	+++	+++
GC_4	98.81 ± 0.41	++	++
GC_5	99.08 ± 0.63	++	++
GC_6	98.64 ± 0.34	+++	+++
GC_7	98.11 ± 0.51	+++	+++
GC_8	100.08 ± 0.18	+++	+++
GC_9	100.11 ± 0.71	+++	+++

+ = gels after few minutes; ++ = gelation immediate but remains for a few hours (less stiffer); +++ = gelation immediate and remains for extended periods and formed gels are stiffer.

The two main prerequisites of an in situ gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that allows easy instillation into the eye as a liquid (drops), which then undergo a rapid sol-to-gel transition due to ionic interaction. Moreover, the in situ formed gel should preserve its integrity without dissolving or eroding for a prolonged period of time to facilitate sustained release of the drug to the ocular tissues. All the formulations showed instantaneous gelation when contacted with the gelation fluids (STF 1 and 2). However, the nature of the gel formed depended upon the polymer concentration. Of the formulations, batch GC_1 showed the weakest gelation, which could be due to the presence of a minimal amount of gellan (0.01%). The nature of the components of the gelation medium did not seem to influence the nature of gel formed presuming that the gelation occurred primarily due to the presence of cation in the fluid.

Rheological Studies

The formulations exhibited pseudoplastic rheology as evidenced by shear thinning and an increase in the shear stress with increase in the angular velocity. The viscosity directly depended on the polymeric content of the formulations. The viscosity of the formulations containing sodium alginate showed around 10–15% reduction on autoclaving, whereas the formulations containing gellan alone did not show any change in viscosity after autoclaving. The concentration of sodium alginate was adjusted in such a way that the difference in the viscosity before and after sterilization was compensated. Addition of sodium citrate in gellan formulations reducted the viscosity significantly in comparison to the corresponding batch without sodium citrate (Figures 1 and 2).

The administration of ophthalmic preparations should influence as little as possible the pseudoplastic character of the

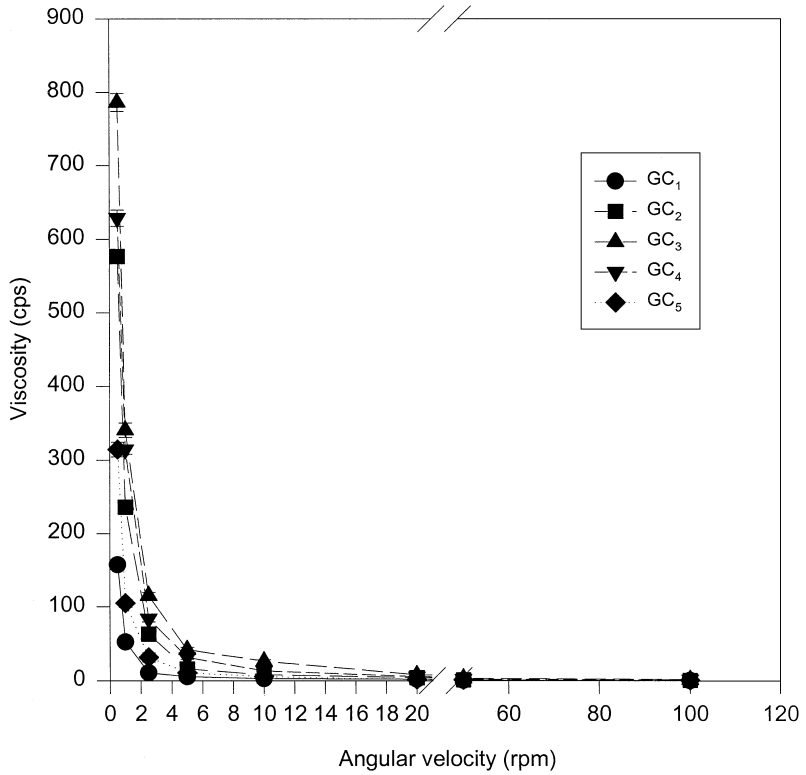

FIG. 1. Viscosity of gellan based in situ gelling system.

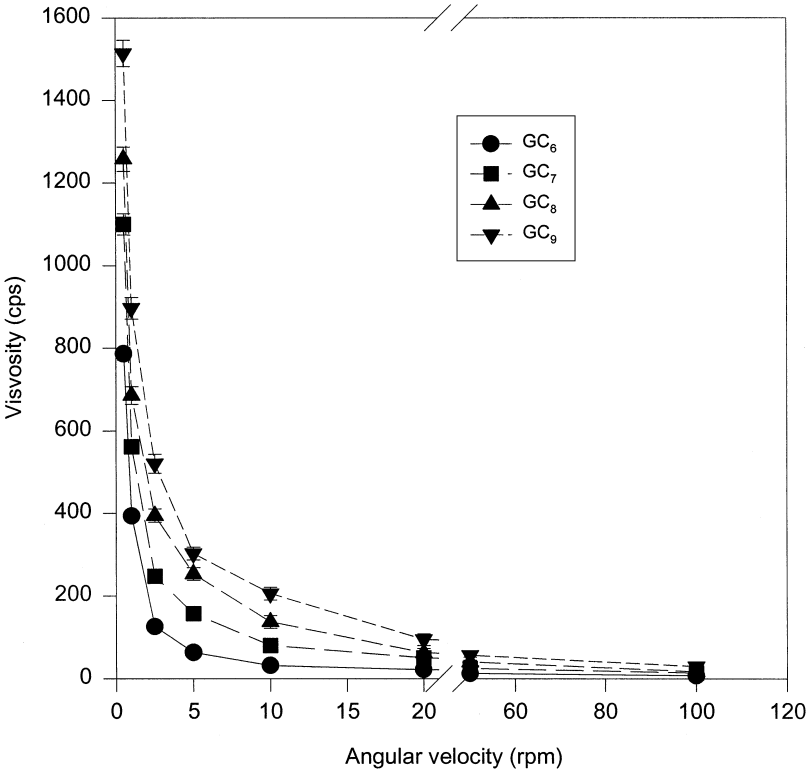

FIG. 2. Viscosity of formulations containing combinations of gellan and sodium alginate.

precorneal film (Van Ootegham 1993). Since the ocular shear rate is very large, ranging from $0.03s^{-1}$ during interblinking periods to $4250–28500s^{-1}$ during blinking (Bothner et al. 1990), viscoelastic fluids with a viscosity that is high under conditions of low shear rate and low under conditions of high shear rate are often preferred.

In Vitro Release Studies

The gelling studies showed that the nature of gelation of the formulations with STF of either composition 1 or 2 was similar. But STF 1 was selected as the dissolution medium to avoid interference by the protein components used in STF 2 during spectrophotometric analysis of the release study samples for CPH content. First sampling was done 1 min after the gelling system came in contact with the dissolution medium to account for the drug released before the complete formation of the gel and also to evaluate the effect of increasing polymer concentration on the nature of the gel formed. The results showed that the amount of drug released in the first minute decreased with increasing polymer concentration (Figures 3 and 4) and this trend continued for the entire duration of the study.

The initial fast release of CPH from the prepared systems could be explained by the fact that these systems were formulated in aqueous vehicle. The matrix formed on gelation was already hydrated and hence hydration and water permeation would no longer limit the drug release. A similar release pattern for pilocarpine, wherein the initial fast release (burst effect) decreased with an increase in polymer concentration, is reported (Cohen et al. 1997) from alginate systems. These results also correlated well with the results of the gelation study. The formulations containing sodium citrate (GC_4 and GC_5) showed significantly higher release than the corresponding batches without sodium citrate, presumably because a less stiffer gel results in faster diffusion of CPH from the gel to the dissolution medium.

Gellan at a concentration of 0.03% w/v was present in the formulations containing varying proportions of sodium alginate (GC_6–GC_9). Comparison of the release profile of GC_2 (containing only gellan) with those of GC_6–GC_9 indicate that the burst effect was considerably reduced, thus indicating the additive effect of the formed calcium alginate on gel formation and consequently on drug release. The release indices (n) of the formulations studied ranged from 0.48 to 0.57 indicating square root of time release kinetics.

The eyedrops formed an opaque matrix immediately on addition to the dissolution medium, due to the cation interaction in the STF (composition 1). Hence the release of CPH from this matrix could be influenced by diffusion and/or by erosion of the matrix. The combination of these processes seemed to result in the overall diffusion-controlled release kinetics as indicated by the n values. These results are in accordance with that reported earlier (Sanzgiri et al. 1993) for methylprednisolone from gellan eyedrops.

The in vitro release study conditions may be very different from those likely to be encountered when instilled into the eye.

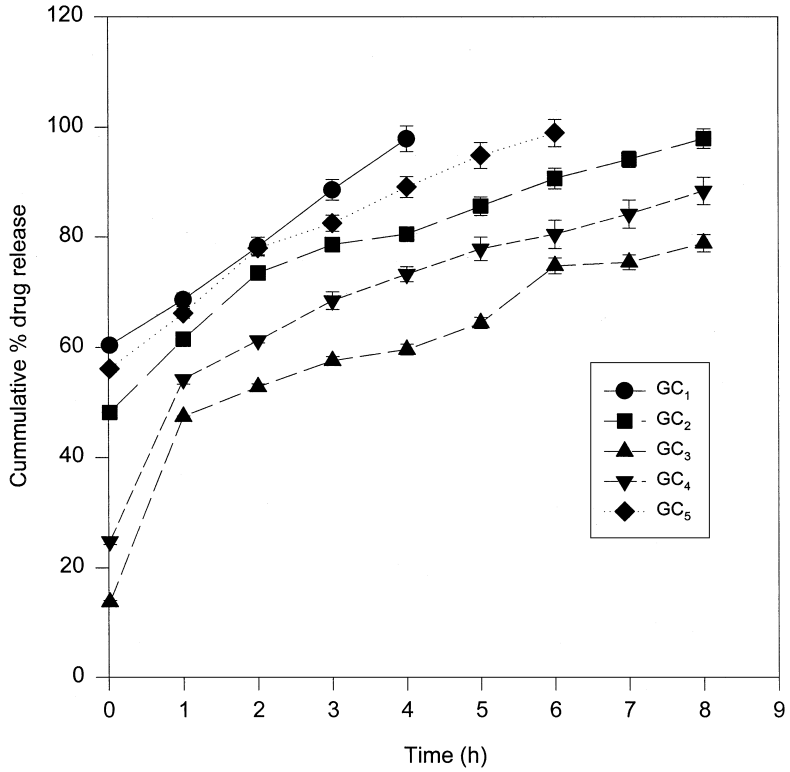

FIG. 3. In vitro drug release from gellan-based formulations.

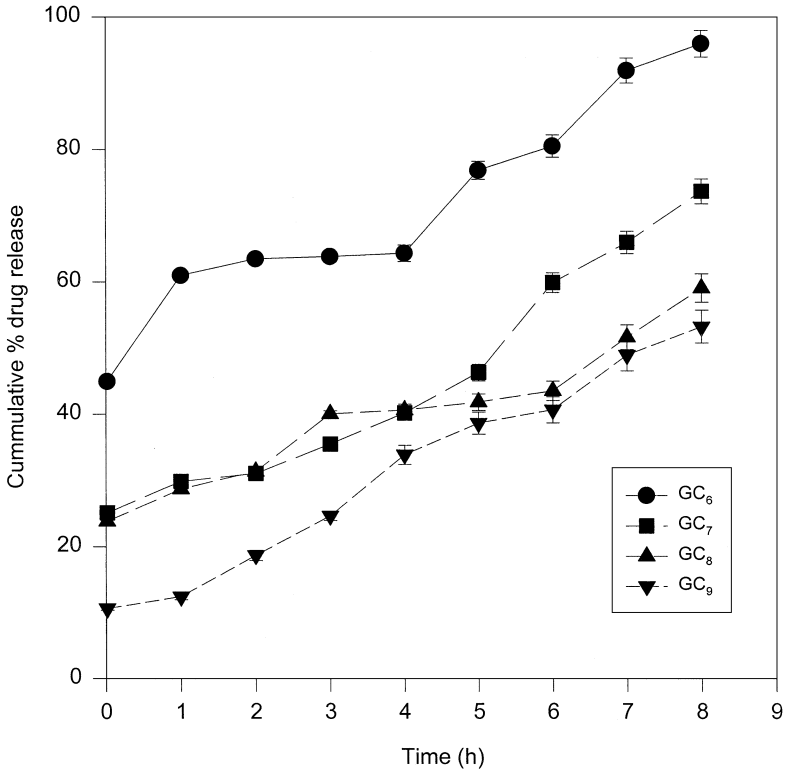

FIG. 4. In vitro release from gellan-sodium alginate based formulations.

However, the results showed that the formed gels had the ability to retain CPH for the duration of the study (8 hr). In the cul-de-sac, the gels would probably undergo faster dissolution due to the shearing action of the eyelid and eyeball movements.

Antimicrobial Efficacy Studies

Formulations were selected to study the effect of increasing gellan concentration (GC_2, GC_3), sodium citrate (GC_2, GC_5), and sodium alginate concentration (GC_7, GC_9). The ZOI values for the prepared formulations were either on par or higher than the ZOI values of the standard preparation in most of the cases (Table 3). Overall the ZOI values against *P. aeruginosa* was higher than that against *S. aureus*. The higher ZOI values obtained for the formulations in comparison to the standard could be attributed to the slow and prolonged diffusion of the drug from the polymeric solution due to its higher viscosity.

TABLE 3
Antimicrobial efficacy of the prepared gelling systems

Concentration (μg/ml)	Zone of inhibition (cm) (% efficiency)					
	Std	GC_2	GC_3	GC_5	GC_7	GC_9
			S. aureus			
3	1.4	1.4 (100)	1.4 (100)	1.2 (85.7)	1.2 (85.7)	1.4 (100)
30	2.0	2.0 (100)	2.2 (110)	1.8 (90)	1.8 (90)	2.2 (110)
60	2.6	3.2 (123.07)	3.0 (115.38)	2.8 (107.69)	2.8 (107.69)	2.6 (100)
			P. aeruginosa			
3	1.8	2.0 (111.11)	2.2 (122.22)	1.8 (100)	1.8 (100)	2.0 (111.11)
30	2.4	2.6 (108.33)	3.0 (125)	2.4 (100)	2.4 (100)	2.6 (108.33)
60	3.4	3.6 (105.88)	3.6 (105.38)	3.4 (100)	3.2 (94.1)	3.4 (100)

Std = standard (marketed eye drop of CPH); GC_2, GC_3, GC_5, GC_7, GC_9 = prepared formulations whose compositions are shown in Table 1.

Values in parenthesis indicate the percent efficiency; percent efficiency was calculated by (ZOI of test/ZOI of standard) $\times 100$.

TABLE 4
In vivo rabbit eye study of the prepared formulations ($n = 5$ rabbits)

	S. aureus				*P. aeruginosa*			
Formulation	2 hr	4 hr	8 hr	24 hr	2 hr	4 hr	8 hr	24 hr
Std	−5	+5	+5	+5	−5	−5	+5	+5
GC_3	+5	−5	−5	−5	−5	−5	−5	−5
GC_7	+5	−5	−5	−5	−5	−5	−5	−5

+5 = indicates growth of microorganisms in all the animals.
−5 = indicates absence of growth of microorganisms in all the animals.

In Vivo Rabbit Eye Study

Varying aliquots of the 0.5 McFarland standard suspension (10, 25, and 40 μl) were instilled in both eyes of the rabbit and checked for growth over 24 hr. We observed that the time period of growth was directly proportional to the inoculum volume of the organisms. For 10 μl of the inoculum, growth occurred over 8 hr; for 25 and 40 μl it occurred over 16 and 24 hr, respectively. Therefore, 40 μl was selected as the inoculum dose for further studies.

The tested formulations showed a markedly improved effect when compared with the marketed (standard) eyedrop. The developed formulations were able to prevent growth of both *S. aureus* and *P. aeruginosa* until 24 hr (Table 4). Growth was observed in all the animals after 2-hr postinstillation of the formulations, when infected with *S. aureus*, whereas the formulations were successful in inhibiting the growth for the entire duration of the study in all the animals infected with *P. aeruginosa*. Repeated-dose study was not attempted since the aim of the study was to develop a suitable formulation for once daily application. The formulations used for the in vivo study formed a translucent gel immediately after instillation into the eye. Gross examination of the ocular tissues showed that the formulations did not cause undue irritation and no leakage of the gelled material was seen from any part of the eye.

CONCLUSION

CPH was successfully formulated as an in situ gelling system using gellan alone and in combination with sodium alginate. Combining gellan with sodium alginate did not offer any advantage (as in therapeutic efficacy) over the formulations based on gellan alone. The formulated systems provided sustained release of the drug over an 8-hr period in vitro, and the developed formulations were devoid of any deleterious effect on the ocular tissues. The formulations demonstrated better therapeutic efficacy as they were successful in inhibiting the growth of the microorganisms for the entire duration of the study (24 hr) when compared with the marketed eyedrop. Thus, this formulation can be viewed as a viable alternative to conventional eyedrops by virtue of its ability to enhance precorneal residence time and consequently ocular bioavailability. The ease of administration coupled with its ability to provide sustained release could result in less frequent administration, thus enhancing patient compliance.

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