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THERMOSENSITIVE AND pH INDUCED *IN SITU* OPHTHALMIC GELLING SYSTEM FOR CIPROFLOXACIN HYDROCHLORIDE: HYDROXYPROPYL-β-CYCLODEXTRIN COMPLEX

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Abstract: The prolonged residence of drug formulation in the ocular cavity is important for ocular drug delivery. The purpose of the present study was to develop ophthalmic *in situ* gelling systems of ciprofloxacin hydrochloride with reduced pre-corneal elimination in order to improve the bioavailability and therapeutic response. Hydroxypropyl-β-cyclodextrin was used in order to increase the stability of ciprofloxacin hydrochloride. *In situ* gels were prepared based on the concept of thermosensitive and pH induced *in situ* gelation. The inclusion complex of ciprofloxacin hydrochloride with hydroxypropyl-β-cyclodextrin was prepared at a 1:1 molar ratio. The complex formation was thoroughly confirmed using various techniques, including ^{1}H NMR spectroscopy, FTIR spectrophotometry and differential scanning calorimetry. Both pure ciprofloxacin HCl and the inclusion complex were individually used in the formulations. Formulations were successfully prepared which were liquid at room temperature and exhibited viscosity increase and gelation at ophthalmic temperature. As a result of antimicrobial efficacy and *in vitro* release experiments, the developed formulations were found therapeutically efficient and provided sustained release of the drug over an 8 h period. These systems can be more advantageous than conventional eye drops.

Keywords: ciprofloxacin hydrochloride, hydroxypropyl-β-cyclodextrin, ophthalmic *in situ* gel, Poloxamer, Carbopol

Topical administration of antibacterial medication to the conjunctival sac is considered to be a preferred way for treating some ocular diseases (1). A fluoroquinolone antibacterial agent – ciprofloxacin hydrochloride (CPH) (Fig. 1) is active against a broad spectrum of aerobic Gram-positive and Gram-negative bacteria. Resistance to this drug develops slowly and a minimal toxicity is associated with its use. It is currently the drug of choice as an anti-infective agent for the eye (2). Because of its short elimination half-life, it must be applied as 3–4 drops at least three times a day (3). It is sensitive to the sunlight and loss of antibacterial activity has been reported (4).

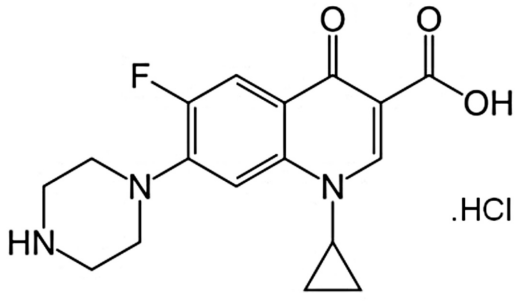

Figure 1. Structure of ciprofloxacin hydrochloride

Cyclodextrins (CD) are groups of cyclic oligosaccharides which have been shown to increase aqueous solubility, to enhance aqueous stability or photostability, to overcome unwanted characteristics, or to reduce side effects of many drugs achieved by formation of inclusion complexes (5). In particular, hydroxypropyl-β-cyclodextrin (HP-β-CD) is most commonly used agent in aqueous eye drop formulations because of lower toxicity compared to parent CDs (6, 7).

Upon application of an ophthalmic solution, most of the applied volume is lost from the precorneal area (8). The precorneal constraints responsible for the poor ocular bioavailability of conventional ophthalmic dosage forms are solution

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drainage, lacrimation, tear dilution, tear turnover and conjunctival absorption (9). To increase the ocular bioavailability and duration of drug at the action site, various ophthalmic vehicles such as viscous solutions, ointments, gels and polymeric inserts have been used. The basic disadvantage associated with the use of ocular formulation is the rapid loss of solutions. Ophthalmic ointments give blurred vision, leading to poor patient acceptance (1). Due to difficulty with self insertation and foreign body sensation, only few insert products are developed for commercialization (9). A significant increase in the precorneal residence time of drugs and consequent ocular bioavailability can be achieved by using delivery systems based on the concept of *in situ* gel formation. *In situ* forming gels are formulations which are applied as solutions, sols, or suspensions and undergo gelation in the ocular cul-de-sac due to physicochemical changes inherent to the eye (10). Depending on the method employed to cause sol to gel phase transition on the ocular surface, following three types of systems are recognized; pH triggered systems (e.g., Carbopol (11)); temperature dependent systems (e.g., pluronics (6)) and ion activated systems (e.g., gelrite gellan gum (8)).

Poloxamer is nonionic polyoxyethylene-polyoxypropylene-polyoxyethylene triblock copolymer molecules that form non-chemically cross-linked hydrogel upon warming to ambient temperature due to the dehydration of the polymer blocks with temperature. The gel formation is a result of micellar enlargements, they can not seperate easily from each other, which accounts for the rigidity and high viscosity of gel containing high concentrations of Poloxamer (12, 13).

Table 1. Combinations of Carbopols and HPMC studied.

Concentration (% w/v)			Gelling capacity
C934	C940	HPMC	
0.5	–	1.5	++
0.5	–	1	++
0.3	–	1.5	++
0.2	–	0.5	–
-	0.5	1.5	++
-	0.5	1	++
-	0.3	1.5	++
-	0.2	0.5	–

–, no gelation; +, gels after a few minutes, dissolves rapidly;
++, gelation immediate, remained for extended period

Table 2. Ingredients of the developed formulations with Carbopols.

Ingredient (% w/v)	Formulations					
	F1	F2	F3	F4	F5	F6
Carbopol 934	0.5	0.3	0.5	0.5	0.3	0.5
HPMC	1.5	1.5	1	1.5	1.5	1
CPH	0.35	0.35	0.35	–	–	–
Inclusion complex	–	–	–	1.675	1.675	1.675
Tween 20	–	–	–	–	–	–
Benzalkonium chloride	0.02	0.02	0.02	0.02	0.02	0.02
Sodium hydroxide	0.16	0.16	0.16	0.16	0.16	0.16
Citric acid	0.407	0.407	0.407	0.407	0.407	0.407
Disodium hydrogen phosphate	1.125	1.125	1.125	1.125	1.125	1.125

Table 3. Gelation temperatures of Poloxamer solutions.

P407 (% w/w)	P188 (% w/w)	Gelation temperature (°C)
15	–	> 37
15.5	–	32
16	–	28
16	18	36
18	10	36
18	12	32
20	10	32

Carbopol polymers are manufactured by cross-linking process. Depending upon cross-linking density and degree of branching, they are differed from member to member. The cross-link network enables the entrapment of drugs in the hydrogel domains. These polymers swell when dispersed in water forming a colloidal, mucilage like dispersion. Increasing the amount of polymer does an increase in swelling degree and decreases the size of channels which form between the polymer hydrogels (4, 14).

In this paper, CPH was complexed with HP-β-CD to enhance photostability and the inclusion complex was characterized using various techniques. The effect of HP-β-CD on the photostability of CPH was investigated. A pH triggered *in situ* gel for CPH was developed and different Carbopol types were used as a gel forming agent in combination with hydroxypropylmethylcellulose (HPMC) which acted as a viscosity enhancing agent. A temperature dependent *in situ* gelling system of CPH was developed and Poloxamers were used as a gel forming agent. The rheological behaviors of the formulations were evaluated. In addition, the *in vitro* CPH release and antimicrobial efficacy of the selected formulations were determined.

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl was kindly provided by Bayer (Germany). Carbopol 934 (C934) and Carbopol 940 (C940) were kindly gifted by BF Goodrich (USA). HP-β-CD was purchased from Aldrich (Germany) (molar substitution 0.8, MW 1460). Poloxamer 407, Poloxamer 188 and hydroxypropylmethylcellulose were purchased from Sigma-Aldrich (USA). All other reagents were of analytical grade.

Table 4. Compositions of the developed formulations with Poloxamers.

Ingredient (% w/w)	F7	F8
Poloxamer 407	15.5	15.5
Ciprofloxacin HCl	0.35	–
Inclusion complex	–	1.675
Benzalkonium chloride	0.02	0.02
Sodium chloride	0.72	0.72

Preparation and characterization of CPH : HP-β-CD inclusion complex

The inclusion complex of CPH with HP-β-CD in a 1:1 molar ratio was prepared by freeze-drying technique. Briefly, CPH and HP-β-CD were dissolved in distilled water and filtered through a 0.45 µm filter. The filtrate was frozen at –42°C for 24 h and then freeze-dried using a Christ Gamma 2-16 LSC Freeze Dryer at –90 ± 1°C for 24 h.

Fourier transform infrared (FTIR) spectra of CPH, HP-β-CD and CPH : HP-β-CD complex were taken with a Jasco 420 FTIR spectrophotometer using discs of each sample previously prepared with potassium bromide between wavelengths of 400 and 4000 cm^{-1}.

Differential scanning calorimetry (DSC) analyses were carried out in the temperature range from 25 up to 400°C on TA Instruments DSC. During experiments, aluminium crucibles were used. Sample weights were 5 mg. The heating rate was 15°C/min.

Nuclear magnetic resonance (^{1}H NMR) spectra of CPH and CPH : HP-β-CD complex were taken by Varian Mercury 400 High Performance Digital FT-NMR spectrophotometer. Five to ten mg weighed CPH or CPH : HP-β-CD complex were dissolved in deuterated water and their ^{1}H NMR were done.

Effect of HP-β-CD on the stability of CPH

Solutions of pure CPH and CPH : HP-β-CD complex in pH 7.4 phosphate buffer were prepared. Solutions were stored at 25°C in daylight, 25°C in dark place and at 2–8°C for 2 months. CPH concentration was determined at specified time intervals.

Preparation of Carbopol/HPMC i*n situ* gelling system

Solutions of with different concentrations of Carbopols types (C934, C940) and HPMC were prepared and evaluated for gelling capacity in order to identify the compositions suitable for the use as *in situ* gelling systems (Table 1). The gel forming capacity was determined by placing a drop of the

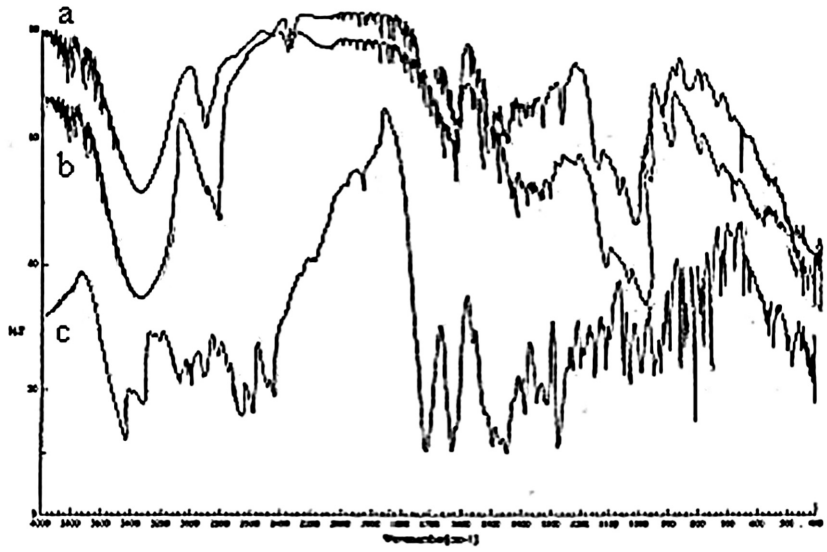

Figure 2. IR spectra of ciprofloxacin HCl / HP-β-CD complex (a), HP-β-CD (b) and ciprofloxacin HCl (c)

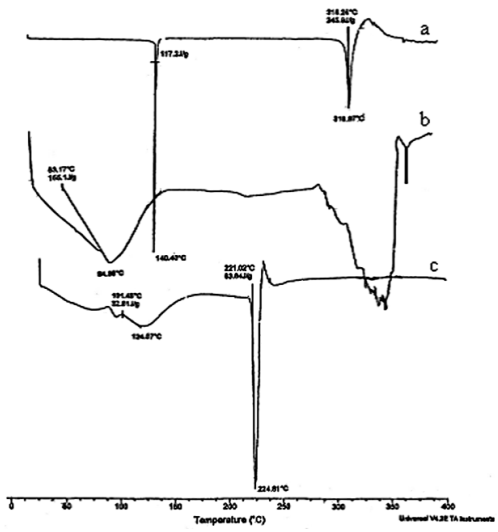

Figure 3. DSC curves of ciprofloxacin HCl (a), HP-β-CD (b) and ciprofloxacin HCl / HP-β-CD complex (c)

system in a vial containing 2 mL of artificial tear fluid which was freshly prepared and equilibrated at 34°C and visually assessing the gel formation, noting the time for gelation and the time taken for the gel formed to dissolve. The composition of artificial tears fluid (pH 7.4) used was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride×2 H_2O 0.008 g, purified water q.s. 100 g.

Initially, formulations containing Carbopol and HPMC were tried to prepare with acetate buffer pH 4.9 because CPH was more stable in acidic medium. When Carbopol was dispersed in an acetate buffer pH 4.9 and CPH was added to the mixture, the formation of an incompatible lumpy mass occurred. This problem was solved as preparing the formulation with citrophosphate buffer pH 6.0 and dissolving the CPH in sodium hydroxide solution (0.5 M) prior to mixing with Carbopol dispersion.

The detailed procedure for preparing the Carbopol/HPMC *in situ* gel forming system of CPH is outlined in Table 2. Buffer salts were dissolved in 75 mL of purified water, HPMC was added and allowed to hydrate. Carbopol was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overheaded stirrer. CPH or inclusion complex was dissolved in sodium hydroxide solution (0.5 M) and benzalkonium chloride was added. The drug solution was filtered through 0.2 µm cellulose acetate membrane filter and added to the Carbopol-HPMC solution under constant stirring. Then the volume was made up to 100 mL with purified water.

Preparation of Poloxamer *in situ* gel

Solutions of varying concentrations of Poloxamer 407 (P407) and Poloxamer 188 (P188) were prepared and evaluated for gelling temperature in order to determine suitable compositions for *in situ* gelling (Table 3). The gelation temperature was measured using the method reported by El-Kamel and El-Khatib (15). Two grams of the prepared solution was transferred to a 5 mL transparent vial containing a magnetic stirring bar. The vial was heated with a constant stirring rate at 125 rpm. The temper-

Table 5. Chemical shift δ and Δδ of protons in ciprofloxacin under the existence of HP-β-CD.

	δ (ppm)		Δδ
	CPH	CPH : HP-β-CD 1:1 complex	
2'-H	8.333	8.490	0.157
5'-H	7.240	7.449	0.209
8'-H	7.145	7.367	0.222

Table 6. Remained CPH content after 60 days (n = 3).

Days	Ciprofloxacin HCl			Ciprofloxacin HCl:HP-β-CD complex		
	Daylight (25°C)	Dark place (25°C)	2–8°C	Daylight (25°C)	Dark place (25°C)	2–8°C
0	100 ± 2.40	100 ± 1.72	100 ± 0.40	100 ± 0.42	100 ± 0.41	100 ± 0.41
6	88 ± 0.69	100 ± 0.40	100 ± 0.79	98 ± 0.42	100 ± 0.81	100 ± 0.70
10	86 ± 0.69	100 ± 0.40	100 ± 1.04	95 ± 0.83	100 ± 0.81	100 ± 0.41
20	78 ± 0.69	102.7 ± 1.19	100 ± 0.79	93 ± 1.82	102 ± 0.41	102.5 ± 0.70
30	71 ± 0.69	102.4 ± 1.19	100 ± 0.40	85 ± 0.42	102.5 ± 0.41	100 ± 0.70
40	63 ± 0.40	102.8 ± 0.40	102.5 ± 0.40	76 ± 0.00	102 ± 0.41	100 ± 0.00
50	55 ± 0.79	102.7 ± 0.69	102.5 ± 0.69	75 ± 0.42	102 ± 0.00	100 ± 0.41
60	49 ± 1.37	102.7 ± 0.69	100 ± 1.4	72 ± 0.72	100 ± 0.70	102.5 ± 0.41

Data are presented as the means ± S.D.

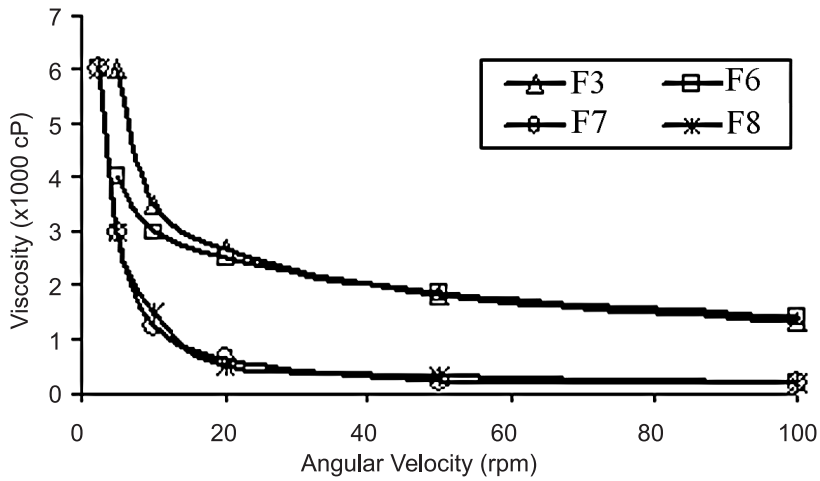

Figure 4. Rheological profile of formulations before gelation

ature at which the rotation of the bar stopped was taken as gelation temperature.

Thermosensitive Poloxamer gels were prepared according to the cold method (16). Appropriate amounts of CPH or CPH : HP-β-CD complex, sodium chloride and benzalkonium chloride were slowly added to pH 4.9 acetate buffer and dissolved. The solution was cooled down to 4°C. P407 and P188 were then slowly added to the solution with continuous agitation and the dispersion was stored at 4°C. After an overnight period, a clear viscous solution was obtained (Table 4).

Content uniformity of the drug and pH

Vials containing prepared formulations were shaken for 2–3 min and 100 µL of the solution was transferred to volumetric flasks and the final volume (100 mL) was made up with purified water. The concentration of CPH was determined at 271 nm (Shimadzu, UV Mini-1240, Japan). pH of the formulations were measured with SenTix 82 pH electrode.

Rheological studies

Viscosity determinations of prepared formulations were carried out on a Brookfield RVTDV-II viscometer using spindle T-E. Angular velocity increased gradually from 0.5 to 100 rpm. The average of three readings was used to calculate viscosity. Initially, viscosities were measured before gelation. Then, the pH was raised to 7.4 by adding 0.5 M NaOH for formulations containing Carbopol and HPMC and temperature raised to 34°C for formulations containing Poloxamers. Rheological alternations of resultant gels were studied.

***In vitro* release studies**

The *in vitro* release of CPH from formulations was studied using a dialysis membrane (cut of size: 12000 Da). The release medium was 50 mL of pH 7.4 phosphate buffer. *In vitro* release studies were done in a water bath at 34 ± 1°C, stirring rate was 80 rpm. The 100 µL of the formulation was kept in a dialysis membrane which was previously hydrated with dissolution medium for an hour. Two mL samples were withdrawn at predetermined time intervals and replaced with an equal volume of the prewarmed medium. The samples were analyzed for CPH content at 271 nm using an ultraviolet spectrophotometer (UV Mini-1240, Shimadzu, Japan).

Analysis of drug release data

The data obtained from the *in vitro* release experiments were analyzed considering zero order, first order, Higuchi kinetics and commonly used Peppas equation (17):

$$\frac{M_t}{M} = kt^n \qquad \log \frac{M_t}{M} = \log k + n \log t \quad [1]$$

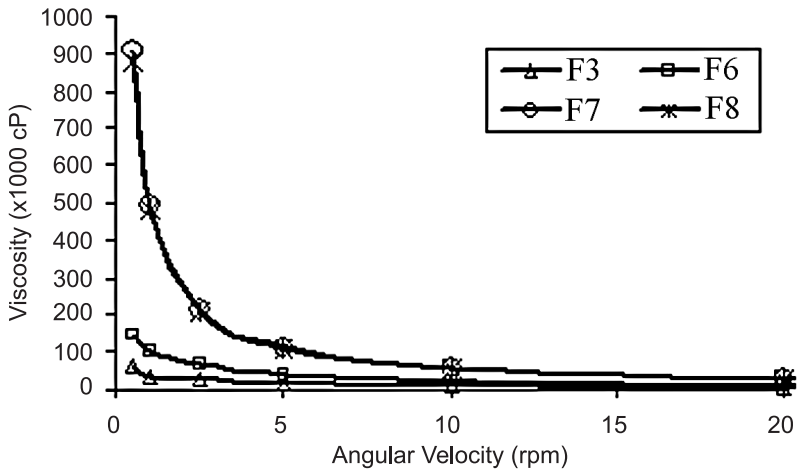

Figure 5. Rheological profile of formulations after gelation

Table 7. Evaluation of formulations (n = 3).

Formulation	Drug content (%w/v)	pH	Viscosity (cP) (25°C, 10 rpm)	Viscosity (cP) (34°C, pH 7.4)	Viscosity (cP × 10^3) (34°C, 10 rpm)	After a month (25°C)		
						Drug content (%w/v)	pH	Viscosity (cP)
F3	98.51 ± 0.97	6.06	3500 ± 1250	9670 ± 1443	–	87.33 ± 0.167	6.01	3500 ± 0
F6	99.55 ± 0.65	6.07	3000 ± 0	20333 ± 1443	–	91.57 ± 0.242	6.06	3000 ± 0
F7	96.39 ± 0.33	4.95	1250 ± 625	–	58.17 ± 0.72	96.61 ± 0.179	5.03	1250 ± 620
F8	95.85 ± 0.50	4.98	1500 ± 625	–	56.67 ± 0.72	96.17 ± 0.196	5.05	1750 ± 620

Data are presented as the means ± S.D.

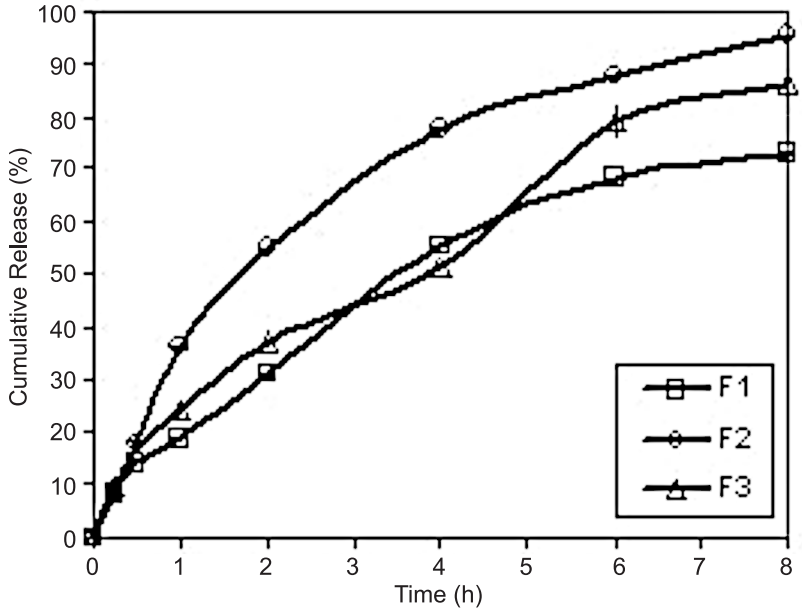

Figure 6. Release of CPH from F1, F2 and F3 formulations (Error bars are S.D. with n = 3)

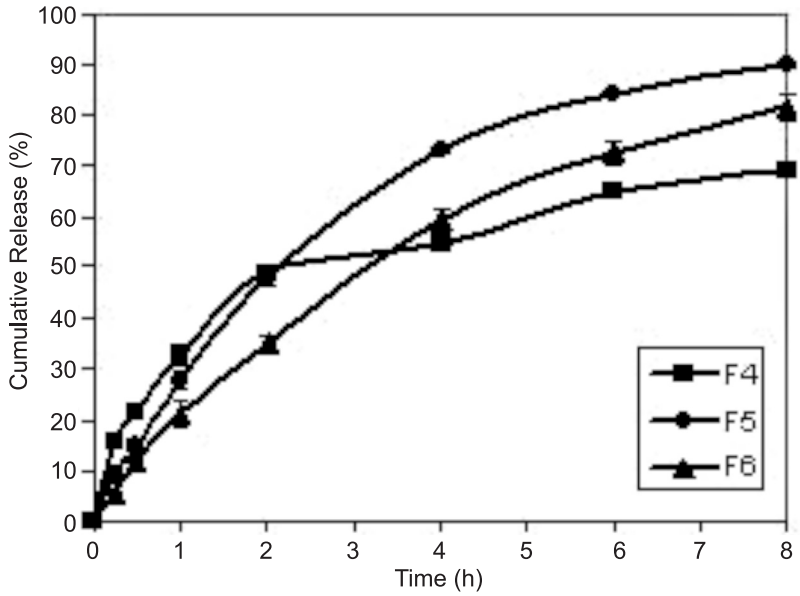

Figure 7. Release of CPH from F4, F5 and F6 formulations (Error bars are S.D. with n = 3)

where Mt/M: the fraction of released drug at time t; k: release constant; n: release exponent indicates the release mechanism. When n is equal to 0.5, the drug is released from the polymer with a fickian diffusion mechanism, if $0.5 < n < 1$ this indicates anomalous or non-fickian release (17).

Antimicrobial efficacy studies

Antimicrobial efficacy of selected formulations were determined by agar diffusion test employing the cup plate technique (1). Appropiate dilutions of formulations were prepared with purified water and poured into the cups bored into the

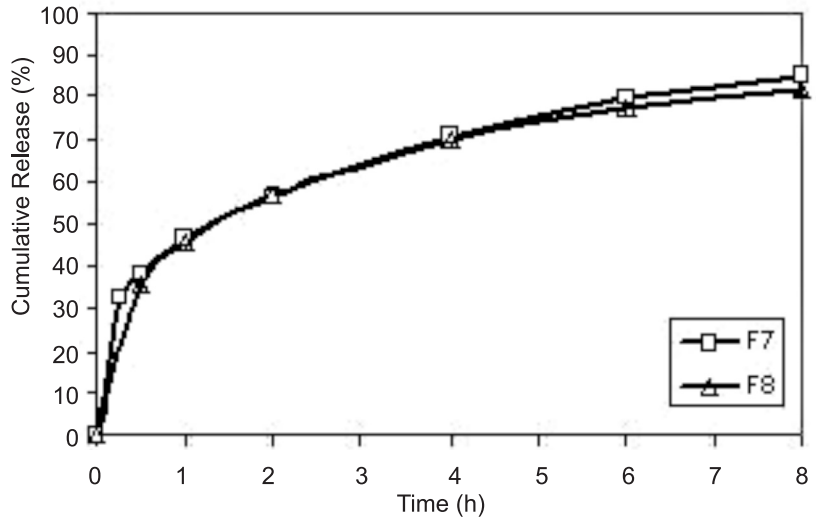

Figure 8. Release of CPH from F7 and F8 formulations (Error bars are S.D. with n = 3)

Table 8. Release data of CPH ophthalmic in situ gels.

		F3	F6	F7	F8
0 order	k_0	10.47	10.36	8.32	8.10
	r^2	0.963	0.953	0.776	0.743
1 order	k_1	0.24	0.21	0.21	0.19
	r^2	0.978	0.999	0.955	0.925
Higuchi	k_h	31.73	31.61	27.49	27.47
	r^2	0.982	0.985	0.941	0.932
Peppas	n	0.594	0.838	0.266	0.334
	r^2	0.993	0.993	0.998	1.000

sterile nutrient agar seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). After allowing diffusion of the solutions for 2 h, the agar plates were incubated at 37°C for 24 h. The zone of inhibition (ZOI) measured around each cup was compared with the control. Each solution was tested in triplicate.

Short term stability tests

Selected formulations were stored at 25°C for a month. The formulations were evaluated for drug content, pH and viscosity.

RESULTS AND DISCUSSION

Characterization of CPH : HP-β-CD complex

Formation of the inclusion complex was confirmed using FTIR spectrophotometry, DSC analysis and ^{1}H NMR spectroscopy.

Comparing IR spectra of CPH, HP-β-CD and CPH : HP-β-CD complex, just as in Figure 2, it was shown that the absorption intensity of the CN group appearing in 1624 cm^{-1} gave rise to changes, the absorption intensity of CN in inclusion complex was weaker than on CPH, so it can be deduced that CN in CPH was included into the cavity of HP-β-CD (18, 19). According to the DSC results presented in Figure 3, it appears that the DSC curves of the inclusion complex, CPH and HP-β-CD are different. The DSC results demonstrated an endothermic peak for CPH and HP-β-CD at 140.40°C and 94.98°C, respectively. The inclusion complex shows a wide endothermic peak around 124°C. The disappearance of endothermic peaks of CPH and HP-β-CD and the appearance of another endothermic peak might indicate formation of inclusion complex between CPH and HP-β-CD. Melting points of CPH and inclusion complex were found as 317 ± 0.577°C and 222 ± 2°C, respectively.

Most of H atoms of CPH were more or less influenced by the presence of HP-β-CD, but 2^1-H, 5^1-H and 8^1-H experienced great downfield shift attributable to diminished freedom of rotation caused by the penetration into HP-β-CD cavity (Table 5) (18).

Effect of HP-β-CD on the stability of CPH

In solutions which were stored at daylight at 25°C, a decrease on the remaining CPH concentration of the solution containing plain CPH was observed at the 10th day, whereas it was observed at 30th day with the solution containing the CPH : HP-β-CD complex. At the end of two months, the CPH concentration of the solution containing plain CPH was 49% and the solution containing the CPH : HP-β-CD complex was 72% (Table 6). These results indicate that HP-β-CD increased the stability of CPH against daylight.

Gel forming capacity of formulations containing Carbopol and HPMC

The gel forming capacity of formulations prepared with different Carbopol types and HPMC are shown in Table 1. The two main prerequisites of an *in situ* gelling system are viscosity and gel forming capacity. The formulation should have an optimum viscosity that will allow for an easy application into the eye as a liquid, which then undergoes a rapid sol-to-gel transition. Additionally, the gel formed *in situ* should preserve its integrity without dissolving or eroding for prolonged period. Formulations prepared with 0.5% C934 or C940 with 1% or 1.5 % HPMC and 0.3% C934 or C940 with 1.5% HPMC were shown to have the best gel forming capacities and these polymer rates were consistent with previous studies (1, 11, 20).

Effect of Poloxamer composition on gelation temperature

A gelation temperature suitable for *in situ* gel formulations would be 30–36°C. In order to investigate the optimum concentration ratio of P407 and P188, various compositions of Poloxamers were prepared (Table 3). According to the results, the compositions that exhibited gelation temperature between 30–36°C were used for preparing *in situ* gel formulations. Precipitation was observed with both P407 and P188 containing formulations several days after preparation, so P407 (15.5 w/w) was found to be suitable for further experiments (Table 4).

Drug content and pH

Formulations were evaluated for drug content and pH (n = 3) (Table 7). Drug content values of F3 and F6 formulations ranged from 98.5–100% and the pH of these formulations was found about 6 as in the study of Al-Kassas and El-Khatib (20). Formulations F7 and F8, were found in the pH of about 5. The drug content and pH of all formulations were satisfactory for ophthalmic delivery.

Rheological studies

When shear rate was increased, viscosity of the formulations was decreased. Viscoelastic fluids with low viscosity under high shear rate and high viscosity under low shear rate are often preferred, because the administration of ophthalmic preparations should influence as little as possible the pseudoplastic character of the precorneal film (1, 8). Decreases in viscosity were observed with increases in angular velocity (pseudoplastic rheology) (Figs. 4, 5).

The formulations were liquid at room temperature and at the pH formulated and underwent rapid transition into the gel phase at the pH of the tear fluid (pH 7.4). The type of *in situ* gelling polymer, its concentration, and the type of cellulose derivative had a significant effect on the gelling capacity of ciprofloxacin formulations. Formulations containing C934 exhibited lower viscosity than formulations containing C940 at room temperature. It was considered that low viscosity before gelling was suitable for application. C934 was found preferable because of low viscosity before gelation. Al-Kassas

Table 9.Antimicrobial efficacy of the prepared gelling systems.

Concentration (µg/mL)	Zone of inhibition (mm) (% efficiency)				
	Std*	F3	F6	F7	F8
	S. aureus				
100	42	28 (66.67)	28 (66.67)	30 (71.43)	29 (69.05)
500	45	32 (71.11)	33 (73.33)	35 (77.78)	34 (75.56)
	P. aeruginosa				
500	37	33 (89.19)	35 (94.60)	37 (100)	37 (100)

* Standard – the CPH solution.

and El-Khatib (20) reported that in formulations based on HPMC and Carbopol, increasing in the concentration of each polymeric component significantly increased the formulation viscosity. In this study, it was shown that the HPMC concentration was more effective on formulation viscosity than Carbopol concentration. F3 and F6 coded formulations containing 1% HPMC exhibited lower viscosity values than other formulations before gelation. After gelation of F3 and F6 coded formulations, viscosity values increased 3-fold and 7-fold, respectively (Table 7). So, F3 and F6 coded formulations were selected in terms of both applying convenience and viscosity increase after gelation. Viscosity increase provides sustained precorneal residence time of the drug.

Formulations containing Poloxamers were found to have lower viscosity values at room temperature and exhibited higher viscosity values at 34°C than Carbopol formulations (Table 7). Formulations prepared with Poloxamers were considered more convenient in terms of applying easiness and viscosity.

***In vitro* release studies**

The developed formulations provided sustained release of the drug (Figs. 6–8). Formed gels had the ability to retain CPH over an 8-h period similar to previous studies (8). Increased Carbopol concentration in the formulation decreased the drug release rate for F1, F2, F3 coded formulations. While Carbopol concentration was constant, an increase in HPMC concentration decreased the drug release rate as in the study of Jain et al. (1). The slowest releasing drug was observed with F1 and F4 formulations, but these formulations were found to have higher viscosity before gelation and application was not found easy. So, these formulations weren't preferred. F3 and F6 formulations showed slower drug release than F2 and F5 coded formulations and their viscosity values were covenient for application, so they were selected among the Carbopol containing formulations. The F7 and F8 formulations containing Poloxamers showed faster drug release than carbopol containing formulations. An incomplete release was shown in the release profiles of formulations. Increasing the amount of polymer, decreases the size of channels which form between the polymer hydrogels. These results indicate that the structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration of polymer increased.

In the case of F3 and F7 formulations, *in vitro* CPH release was shown significantly greater than for formulations complexed with CD (F6 and F8) ($p < 0.05$).

Release kinetics of the drug

The release kinetics of CPH from F3, F6, F7 and F8 coded formulations were investigated and drug release rates were calculated for zero order, first order, Higuchi kinetics and Peppas equation (Table 8). Higher r^2 values were obtained for Peppas and first order kinetics. Calculated n values from Peppas equation for F3 and F6 coded formulations were 0.594 and 0.838, respectively. The non-fickian (anomalous) release kinetics, revealed according to n values for F3 and F6 formulations, might indicate that the release of ciprofloxacin HCl followed coupled erosion-difusion mechanism (9, 21). n values calculated for the F7 and F8 formulations were 0.266 and 0.334, respectively. These were less than 0.5, which indicated that formulations showed drug release by the fickian diffusion mechanism (22).

Based on the mechanical properties and release characteristics of the investigated formulations, F3 and F6 containing C934 and HPMC, F7 and F8 containing P407 and P188 were selected for microbiological studies.

Antimicrobial efficacy studies

The results of antimicrobial efficacy tests are shown in Table 9. The zone of inhibition values were found similar with previous studies and likewise, values against *Pseudomonas aeruginosa* were higher than those against *Staphylococcus aureus* (8, 11, 20). This study indicates that ciprofloxacin retained its antimicrobial efficacy when incorporated in the *in situ* gelling system.

There was correlation with *in vitro* release of F7 and F8 formulations and antimicrobial efficacy studies. *In vitro* drug release from F7 formulation was shown higher than from F8 formulation. Correlated with *in vitro* release studies, the zone of inhibition values of F7 was shown higher than F8. On the contrary, there was no correlation with *in vitro* release of F3 and F6 formulations and antimicrobial efficacy studies.

Short term stability studies

Stability studies were carried out at 25°C for a month. Drug contents of F3 and F6 formulations decreased after a month from 98.51% and 99.55% to 87.33% and 91.57% respectively. It was found that F6 formulation containing CPH : HP-β-CD complex was more stable than F3 formulation. The drug contents of F7 and F8 formulations were found

stable after a month. There wasn't any change shown on pH and viscosity of the selected formulations (Table 7).

CONCLUSION

The developed Poloxamer and Carbopol based ophthalmic *in situ* gel formulations of CPH are shown to have favorable gelation, rheological and release properties *in vitro*. The inclusion complex also increased the drug stability. The most prominent advantage of these *in situ* gels are fluid like behavior prior to contact with the ocular mucosa and this provides convenience and decreased frequency of administration for patients and accuracy of drug dosing. Assuming that the results of *in vitro* release studies are in the preliminary stage for the *in vivo* studies, developed formulations are thought to increase the bioavailability of CPH depending on the longer residence time and ability to sustain drug release. These formulations appear to be promising for an opthalmic delivery system for CPH.

Declaration of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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