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Ocular Poloxamer-Based Ciprofloxacin Hydrochloride In Situ Forming Gels

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The purpose of this study was to develop poloxamer-based in situ gelling formulations of ciprofloxacin hydrochloride (HCl) aiming at prolonging corneal contact time, controlling drug release, enhancing ocular bioavailability, and increasing patient compliance. The in situ forming gels were prepared using different concentrations of poloxamer 407 (P407) and poloxamer 188 (P188). Mucoadhesives such as hydroxypropylmethyl cellulose (HPMC) or hydroxyethyl cellulose (HEC) were added to the formulations to enhance the gel bioadhesion properties. The prepared formulations were evaluated for their in vitro drug release, sol–gel transition temperature, rheological behavior, and mucoadhesion force. The in vivo antimicrobial efficacy of selected ciprofloxacin HCl in situ gelling formulations was studied on infected rabbit's eyes and compared with that of the marketed conventional eye drops. The gelation temperature of the prepared formulations ranged from 28.00 to 34.03°C. Increasing the concentrations of P407, HPMC, and HEC increased the viscosity and mucoadhesion force of the preparations and decreased the in vitro drug release. Ciprofloxacin HCl in situ forming gel formulae composed of P407/P188/HPMC (18/13/1.5%, wt/wt), and P407/P188/HEC (18/13/0.5%, wt/wt) showed optimum release and mucoadhesion properties and improved ocular bioavailability as evidenced by an enhanced therapeutic response compared with the marketed conventional eye drops.

Keywords poloxamer; ciprofloxacin hydrochloride; ophthalmic drug delivery; in situ forming gels; bacterial infections

INTRODUCTION

The conventional liquid ophthalmic delivery systems exhibit short precorneal residence time and poor bioavailability due to rapid elimination induced by lachrymal flow, blinking, normal tear turnover, and solution drainage by gravity. As a result, frequent instillation of solutions is needed in order to achieve the desired therapeutic effect (Lee & Robinson, 1979; Lin & Sung, 2000).

Topical administration of antibacterial medication to the conjunctival sac is usually an effective avenue for treating bacterial conjunctivitis. Ciprofloxacin hydrochloride (HCl), a broad spectrum fluoroquinolone derivative, is a very widely used antibiotic for the treatment of infectious types of conjunctivitis caused mainly by gram-negative bacteria such as *Pseudomonas aeuroginosa* (Kamath, Singh, & Udopa, 1993; Tsai, Tseng, Chang, & Hufr, 1995). The topical dosage of ciprofloxacin HCl eye drops is one to two drops of 0.3% solution in the affected eye(s) every 4 h or hourly once in the case of severe infection (Srividya, Cardoza, & Amin, 2001). One of the major drawbacks of an antibiotic eye drops is the pulsatile drug level, with a transient period of overdose followed by an extended period of subtherapeutic levels before the next dose is administered. This means that the infectious agent will be exposed to lower (sub-MIC) concentrations of the antibiotic leading to bacterial resistance (Alonso, Campanario, & Martinez, 1999).

Various ophthalmic vehicles, such as inserts, ointments, suspensions, and aqueous gels, have been developed to lengthen the residence times of instilled dose and enhance ophthalmic bioavailability (Lee & Robinson, 1986). These ocular drug delivery systems, however, have not been used extensively because of some drawbacks, such as blurred vision from ointments or low patient compliance from inserts (Lee, 1999).

Several in situ gelling systems have been developed to prolong the precorneal residence time of a drug, improve patient compliance, and consequently enhance ocular bioavailability (Lin & Sung, 2000). These systems exhibit sol-to-gel phase transitions due to a change in a specific physicochemical parameter (e.g.,: pH, temperature, and ions) in the cul-de-sac (Srividya et al., 2001).

Poloxamer, a surface-active block copolymer made of polyoxyethylene and polyoxypropylene, is known for its excellent compatibility with other chemicals, least toxicity, high

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solubility capacity for different drugs, and good drug-release characteristics (Miyazaki et al., 1986; Morishita et al., 2001). Poloxamer, a thermosensitive polymer, changes from low viscosity solutions at any temperature or below room temperature (25°C) to semisolid gels at the corneal surface temperature (34°C).

Bioadhesion can be used as a means to improve intimacy of contact, as well as a way to increase dosage form residence time to various administration routes (Lee, Park, & Robinson, 2000; Park & Robinson, 1985; Robinson, Longer, & Veillard, 1987). To fortify the adhesion of administered drugs onto the mucosal surfaces, mucoadhesive polymers such as carbopol, hydroxyethyl cellulose (HEC), and hydroxypropylmethyl cellulose (HPMC) have been added to the in situ gelling liquids (Chu, Amidon, Weiner, & Goldberg, 1991; Jones, Woolfson, Djokic, & Coulter, 1996; Park & Robinson, 1985).

The evaluation of rheological properties for the gel type dosage forms would be important for predicting their behavior in vivo. The rheological properties of eye gels were reported to affect the ocular residence time of the gels (Carlfors, Edsman, Petersson, & Jornving, 1998; Desai & Blanchard, 2000; Edsman, Carlfors, & Petersson, 1998).

The objective of our study was to develop a temperature-dependant in situ gelling ophthalmic delivery system of 0.3% ciprofloxacin HCl, using combinations of poloxamer 407 (P407) and poloxamer 188 (P188). In an attempt to optimize the gel bioadhesion properties, two mucoadhesives (HPMC or HEC) were adjuncted with poloxamer at different concentrations. The prepared formulations were evaluated for their in vitro drug release, sol–gel transition temperature, rheological behavior, and mucoadhesion force. The in vivo antimicrobial efficacy of selected formulae was studied on infected rabbit's eyes and compared with that of the marketed conventional eye drops.

MATERIALS AND METHODS

Materials

Ciprofloxacin HCl was kindly supplied by MEMPHIS Pharmaceutical Company, Cairo, Egypt. P407, P188, benzalkonium chloride, and crude porcine gastric mucin were purchased from Sigma Chemical Co., St. Louis, MO, USA. HPMC (Methocel E5, low viscosity, 3.6 cp of 2% solution) was kindly supplied by E.I.P.I.Co. Pharmaceutical Company, Cairo, Egypt. HEC (high viscosity, 640 cp of 2% solution) was purchased from Tama, Japan. Spectra/Por dialysis membrane, 12,000–14,000 molecular weight cut off, was purchased from Spectrum Laboratories Inc., Rancho Dominguez, Canada. α-Cyanoacrylate glue was purchased from Amir, China. Sodium acetate, glacial acetic acid, glycerin, sodium bicarbonate, sodium chloride, and calcium chloride dihydrate were purchased from ADWIC, El-Nasr Chemical Co., Cairo, Egypt. All other chemicals were of analytical grade.

Preparation of the In Situ Forming Gels

Different concentrations of P407 and P188 with or without mucoadhesives such as HPMC and HEC were used for the preparation of in situ forming gels of ciprofloxacin HCl. Medicated in situ forming gels were prepared on a weight basis by using the modified cold method (Schmolka, 1972). The composition of the prepared formulations is shown in Table 1.

TABLE 1
Composition of Ophthalmic In Situ Forming Gels of Ciprofloxacin Hydrochloride

	Ingredient (%, wt/wt)							
Formulations	Ciprofloxacin Hydrochloride	Poloxamer 407	Poloxamer 188	HPMC	HEC	Glycerin[a]	Benzalkonium Chloride	Acetate Buffer (pH 4)
F1	0.3	18	13	–	–	2.154	0.004	66.54
F2	0.3	17	13	–	–	2.160	0.004	67.53
F3	0.3	18	13	0.5	–	2.154	0.004	66.04
F4	0.3	18	13	1	–	2.154	0.004	65.54
F5	0.3	18	13	1.5	–	2.154	0.004	65.04
F6	0.3	18	13	–	0.5	2.154	0.004	66.04
F7	0.3	18	13	–	1	2.154	0.004	65.54
F8	0.3	18	13	–	1.5	2.154	0.004	65.04
F9	0.3	17	13	0.5	–	2.160	0.004	67.03
F10	0.3	17	13	1	–	2.160	0.004	66.53
F11	0.3	17	13	1.5	–	2.160	0.004	66.03
F12	0.3	17	13	–	0.5	2.160	0.004	67.03
F13	0.3	17	13	–	1	2.160	0.004	66.53
F14	0.3	17	13	–	1.5	2.160	0.004	66.03

[a]Isotonic agent.

Briefly for formulations F1 and F2, P407 and P188 were slowly added to the calculated amount of cold acetate buffer (pH 4) in a vial containing a magnetic bar with continuous mixing using a thermostatically controlled magnetic stirrer (Labinco, model BV, The Netherlands). The partially dissolved poloxamer solutions were stored in a refrigerator and stirred periodically until clear homogenous solutions were obtained (approximately 24 h). The medicated formulations were prepared by dissolving the appropriate amount of ciprofloxacin HCl, 0.3% (wt/wt), in the calculated amount of acetate buffer during the mixing step. For formulations (3–14), the total poloxamer content was mixed during preparation with additional amount of mucoadhesive polymers, namely HPMC and HEC, each in concentrations of 0.5, 1, and 1.5% (wt/wt). Glycerin and benzalkonium chloride were added to all formulations as isotonicity agent and preservative, respectively. The concentration of the isotonicity adjustment agent that rendered the formulations isotonic with eye fluid was calculated using the molecular concentration percent method (Stoklosa & Ansel, 1997). All glassware used during the preparation of the in situ forming gels was sterilized by autoclaving, acetate buffer was passed through 0.2-μm membrane filter, and the entire procedure was carried out in a laminar flow hood (Esco, Singapore).

Measurement of the Sol–Gel Transition Temperature

The sol-to-gel phase transition temperature (gelation temperature) was measured for all the prepared ciprofloxacin HCl formulations according to the technique described by Vadnere, Amidon, Lindenbaum, and Haslam (1984) and Gilbert, Richardson, Davies, Palin, and Hadgraft (1987). An aliquot of 2 mL refrigerated tested formulation was transferred to a test tube and sealed with a parafilm. The tube was maintained in a thermostatically controlled digital water bath (GFL, model mbH, Germany) at 4°C. The temperature of the water bath was increased gradually in increments of 3°C in the beginning of the experiment and then 1°C increments in the region of sol–gel transition temperature (25–34°C) (Desai & Blanchard, 1998b) and 0.1°C when it approaches gelation. The tested formulation was left to equilibrate for 10 min at each new setting (or for 2 min when temperature was increased with an increment 0.1°C). The gelation is considered to be occurred when the meniscus of the formula would no longer move upon tilting through angle 90°. The maximum accepted gelation temperature tested was 34°C, which represents the corneal surface temperature. Results were average of three determinations.

Determination of the Mucoadhesive Force

The mucoadhesive forces of all the prepared formulae were determined using the mucoadhesive force measuring device

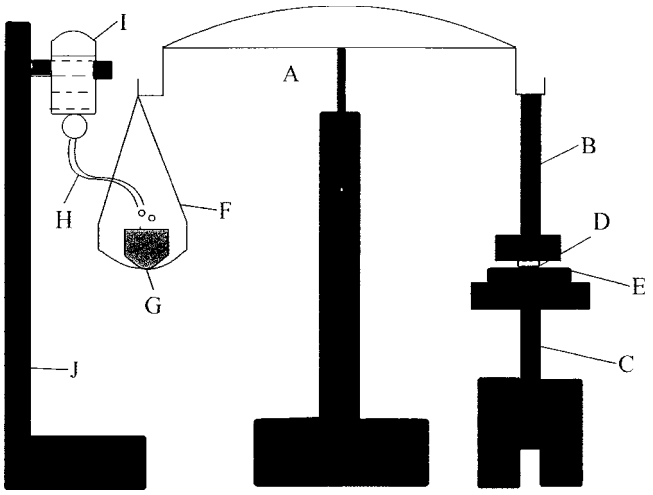

FIGURE 1. Diagrammatic representation of the mucoadhesive force-measuring device. (A) modified balance; (B) upper stage; (C) lower stage moving on screw; (D) mucin disc; (E) gel preparation; (F) balance pan; (G) plastic jar; (H) IV infusion set; (I) glass bottle containing water; (J) stand-holder.

(Figure 1), which is a modified balance that was developed in our laboratory according to previously reported methods (Desai & Kumar, 2004; Mikos & Peppas, 1990; Yong et al, 2001). The mucoadhesive force of the formulae under examination was determined by measuring the force required to detach the formulation from a mucin disc using the measuring device. Initially, the mucin discs were prepared by compression of crude porcine mucin (250 mg) by a single-punch tablet machine (Karishma Pharma machines, Mumbai, India) using a flat-faced punch of 10 mm diameter. At the right arm of the balance, a mucin disc was horizontally glued to the upper stage of the modified balance by α-cyanoacrylate adhesive. The mucin disc was hydrated with distilled water prior to mucoadhesion testing. Three drops of each formula were placed on the lower vertically movable stage of the balance. The in situ forming gel sample was exposed to an electric lamp as a source of heat to allow its gelation. The lower stage was then elevated till the surface of the sample came in contact with the mucin disc. Both the in situ gel and the hydrated mucin disc were left in contact for 1 min using a preload of 10 g to establish the contact between them and allow the formation of an adhesive bond. The preload time and force were kept constant for all the tested formulations. After completion of the preload time, water was allowed to drip from a glass bottle through an infusion set into a preweighed plastic jar placed on the left pan of the balance at a constant rate of 30 drops per minute. The addition of water was stopped when the mucin disc was detached from the tested sample, the filled plastic jar was reweighed, and the weight of water required to detach the tested sample from the mucin disc was calculated by difference. The results were the mean of three runs. The detachment force (dyne/cm^2)

was determined using the following equation stated by Ch'ng, Park, Kelly, and Robinson (1985):

$$\text{Detachment force}\,(\text{dyne/cm}^2) = \frac{\text{m.g}}{\text{A}}$$

where *m* is the weight of water in grams; *g* is acceleration due to gravity taken as 980 cm/sec^2; and *A* is the area of the mucin disc (area of contact) and is equal to πr^2 (r is the radius of the mucin disc).

Rheological Studies

Rheological properties of the prepared ciprofloxacin HCl in situ forming gels were measured using cone and plate programmable viscometer (Brookfield Engineering Laboratories Inc., Model HADV-II, USA) connected to a digital thermostatically controlled circulating water bath (Polyscience, Model 9101, USA) with spindle 52. All the samples were equilibrated at 34°C for 5 min prior to each experiment. The samples were also thermostated at 34 ± 0.1°C during the experiment using circulating water bath connected to the viscometer. The measurements were taken over the whole range of speed settings of shear rate ranging from 15 to 180 s^{-1} in the case of formulations prepared using 17% P407 and from 0.06 to 1s^{-1} in the case of formulations prepared using 18% P407 with 20 s between each two successive speeds in ascending and then in descending order. The viscosity was determined for each sample at different values of shear rate. All measurements were made in triplicate.

In Vitro Drug Release Studies

In vitro release of ciprofloxacin HCl from in situ gelling formulae was studied using a modified USP dissolution testing apparatus (Pharma test, type PTW, Hainburg, Germany) (Srividya et al., 2001). The dissolution medium used was freshly prepared simulated tear fluid (STF), pH 7.4. The STF is composed of NaCl (0.67 g), $NaHCO_3$ (0.2 g), $CaCl_2.H_2O$ (0.008 g), and distilled water up to 100 g (Lin & Sung, 2000).

Cellulose membrane (Spectra/Por dialysis membrane, 12,000–14,000 MW cut off), previously soaked overnight in the dissolution medium, was tied to one end of specifically designed glass cylinder (open at both ends and of 2.5 cm diameter). An accurately weighed amount of the formula (1 g) was transferred to the glass tube. Then the glass cylinder was attached to the metallic driveshaft of the dissolution apparatus and suspended in 500 mL of dissolution medium maintained at temperature of 34°C. The shaft was allowed to rotate at a constant speed (50 rpm). At predetermined time intervals for 8 h, aliquots were withdrawn and replaced by an equal volume of the receptor medium. The drug content in the withdrawn samples was determined spectrophotometrically at λ 272 nm using UV-visible double beam spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan). The results were the means of three runs.

The release mechanism of ciprofloxacin HCl from the in situ gel formulations was investigated using the following equations (Peppas, 1985):

$$\frac{M_t}{M_\infty} = Kt^n \tag{1}$$

$$\log\left(\frac{M_t}{M_\infty}\right) = \log K + n\log t \tag{2}$$

where M_t/M_∞ is the fraction of the drug released at time *t*, *K* is a constant incorporating structural and geometric characteristics of the drug/polymer system, and *n* is the release exponent, which is indicative of the drug-release mechanism. When *n* is equal to 0.5, the drug is released from the polymer with Fickian diffusion mechanism. If $0.5 < n < 1$, it indicates anomalous or non-Fickian release, whereas if $n = 1$, it indicates zero-order release.

In Vivo Study

Ciprofloxacin HCl in situ forming gel formulae (F5 and F6) composed of P407/P188/HPMC (18/13/1.5%, wt/wt) and P407/P188/HEC (18/13/0.5%, wt/wt), respectively, were selected for the in vivo study as they showed optimum release rate and mucoadhesion properties. The in vivo antimicrobial efficacy of the selected formulae against induced bacterial conjunctivitis on infected rabbit's eyes were studied and compared with that of the marketed conventional eye drops (Ciprocin®, E.I.P.I.Co. Pharmaceutical Company, Cairo, Egypt). The experimental procedures conform to the ethical principles of the Experiments and Advanced Pharmaceutical Research Unit (EAPRU), Faculty of Pharmacy, Ain Shams University, Cairo (Egypt) on the use of animals.

Eighteen adult rabbits each weighing 1.5–2 kg were used in the experiment. The animals were divided into three groups (I–III) each of six groups: group (I) was treated after infection with the in situ forming gel formulae (F5), group (II) was treated with F6, and group (III) was treated with the marketed ciprofloxacin HCl eye drops. The rabbits were kept in individual cages. Bacterial conjunctivitis was induced in the rabbit's right eyes by exposing them to standard bacterial strain of *P. aeuroginosa* ATCC 27853. Treatment was initiated 24 h later. Using a calibrated dropper, one drop of ciprofloxacin HCl in situ forming gel formulae containing 0.15 mg of drug per dose was instilled twice daily for 5 days for groups I and II, whereas group III was treated with two to three drops of the marketed eye drops four times a day for the same period of time. Eyes of each animal were observed every day until the end of the study using a scoring system that was previously described by Charoo, Kohli, and Ali (2003) for the following

parameters: (a) redness of the mucous membrane of the eye—observed visually and graded from 0 to 4 (0 absent; 1 mild; 2 moderate; 3 severe; 4 very severe); (b) lacrimal secretion—graded from 0 to 3 (0 normal; 1 slightly more than normal; 2 more than normal; 3 abnormally more than normal); (c) mucoidal discharge—yellowish to yellowish-green discharge if any was noted and graded from 0 to 3 (0 absent; 1 little; 2 more; 3 much more); (d) response to ocular stimulus—assessed by shining torch light on the eye from a particular distance, observing the response to this stimulus, and grading the response from 0 to 2 (0 normal; 1 faster; 2 very fast); (e) swelling of eyelid—graded from 0 to 2 (0 absent; 1 slight; 2 more).

Significance of treatment effects for the in situ forming gel formulae was determined by applying Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple comparison test at significance levels ($p < 0.05$).

RESULTS AND DISCUSSION

Measurement of the Sol–Gel Transition Temperature

The sol–gel transition temperatures of ophthalmic thermoreversible gels have been considered to be suitable for ocular delivery if they were in the range of 25–34°C. If the gelation temperature of thermosensitive formulation is lower than 25°C, a gel might be formed at room temperature, and if the gelation temperature is higher than 34°C, a liquid dosage form still exists at corneal surface temperature, resulting in the drainage of the formula from the eyes. Poloxamer solutions are known to exhibit thermoreversible gelation, depending on the polymer grade, concentration, and other included formulation components.

Previous findings indicated that neither P407 (up to 20%) nor P188 (up to 30%) alone could provide gelation at the physiological temperature (Abd ElHady, Mortada, Awad, Zaki, & Taha, 2003). A modulation of the gelation temperature to reach the desired range (25–34°C) could be achieved through the use of a combination of the two poloxamer grades.

Several combinations of the two polymers grades were tested and used in the formulation of the in situ forming gels in order to select formulations having suitable sol–gel transition temperature and representing the least total poloxamer concentrations. Ciprofloxacin HCl in situ forming gel formulations composed of P407/P188 (18/13 and 17/13%, wt/wt) showed satisfactory results. Data in Table 2 reveal that all the prepared ciprofloxacin HCl in situ forming gel formulations were found to gel between 28.00 and 34.03°C, which are considered to be suitable for ophthalmic application. Decreasing the concentration of P407 from 18 to 17% causes significant increase in the gelation temperature (ANOVA at $p < 0.05$).

Drainage of ophthalmic formulations from the precorneal surface would be considerably reduced by addition of mucoadhesive polymers, which allow attachment of the formulae to the corneal mucin. Table 2 shows that addition of mucoadhesive polymers such as HPMC and HEC lowered the gelation temperature of the in situ forming gels; increasing the concentration of the polymers from 0.5 to 1 and 1.5% produced a gradual decrease in the transition temperature of the in situ forming gels. The gelation temperature-lowering effect of such mucoadhesive polymers could be explained by their ability to bind to polyoxyethylene chains present in the poloxamer molecules. This will promote dehydration, causing an increase in entanglement of adjacent molecules and extensively increasing

TABLE 2
Gelation Temperature, Mucoadhesive Force, and Cumulative % Released After 8 h ($T_{8\,h}$) of Ophthalmic Ciprofloxacin Hydrochloride Formulations

Formulations	Mean Gelation Temperature (°C) ± *SD*	Mean Mucoadhesive Force (dyne/cm^2) • 1,000 ± *SD*	Cumulative % Released after 8 h ($T_{8\,h}$) ± *SD*
F1	31.50 ± 0.11	19.41 ± 0.79	88.18 ± 2.64
F2	34.03 ± 0.06	19.16 ± 0.84	97.78 ± 0.91
F3	31.07 ± 0.06	23.57 ± 1.54	76.58 ± 3.53
F4	29.47 ± 0.06	27.09 ± 2.76	74.82 ± 4.70
F5	29.00 ± 0.00	39.57 ± 0.61	67.33 ± 0.09
F6	30.47 ± 0.06	40.91 ± 3.18	71.57 ± 0.50
F7	29.60 ± 0.00	43.41 ± 1.44	60.12 ± 0.29
F8	28.00 ± 0.00	55.92 ± 0.98	54.56 ± 0.41
F9	33.70 ± 0.00	19.93 ± 2.92	78.73 ± 0.19
F10	32.73 ± 0.12	20.29 ± 0.25	75.20 ± 3.67
F11	31.73 ± 0.06	33.37 ± 1.20	68.05 ± 0.29
F12	33.00 ± 0.00	34.20 ± 2.47	80.49 ± 2.98
F13	32.10 ± 0.00	36.01 ± 1.10	69.04 ± 1.16
F14	31.07 ± 0.06	53.76 ± 2.39	57.81 ± 1.84

intermolecular hydrogen bonding which will lead to gelation at lower temperature (Gilbert et al., 1987; Ryu, Chung, Lee, Kim, & Shim, 1999; Abd ElHady et al., 2003).

Determination of the Mucoadhesive Force

The mucoadhesive force is an important physicochemical parameter for in situ forming ophthalmic gels because it prevents the formulation from rapid drainage and hence lengthens its precorneal residence time. Results of the determination of mucoadhesive forces of all the prepared formulae are tabulated in Table 2.

The mucoadhesive force of the prepared ciprofloxacin HCl in situ forming gels formulations significantly increased in the formulations containing mucoadhesive polymers such as HPMC and HEC. Also, increasing the concentration of the mucoadhesive polymer in the formulations significantly increased the mucoadhesive force (ANOVA at $p < 0.05$). This could be explained by the fact that secondary bond forming groups (e.g., carboxyl, hydroxyl, ether, oxygen, and amine) are the principle source of mucoadhesion, and the cellulosic polymers used during the formulation of the in situ forming gels have an abundance of hydroxyl and ether groups along their length, which are responsible for the mucoadhesive properties. Increasing the concentration of the cellulosic derivatives in the in situ forming gels increased the bonds-forming groups, thus increasing the mucoadhesive force of the formulations.

By further inspection of the mucoadhesive forces in Table 2, it could be concluded that the mucoadhesive forces of formulations prepared using HEC is higher than that of formulations containing the same concentration of HPMC. This could be attributed to the high viscosity and molecular weight of the used HEC than HPMC. High molecular weight is important to maximize adhesion through entanglements and van der Waals forces (Hunt, Kearney, & Kellaway, 1987).

Rheological Studies

The results of the viscosity measurements for each ciprofloxacin HCl in situ gelling formulations at different values of shear rate were plotted in Figures 2–5. All the prepared formulations exhibited shear thinning flow behavior at 34 ± 0.1°C, at which condition the viscosity of the tested formulations decreased by increasing the shear rate. The viscosity increased by increasing the concentration of P407 from 17 to 18% (wt/wt). This may be attributed to the fact that poloxamer is nonionic polyoxythylene–polyoxypropylene–polyoxyethylene triblock copolymer molecules that aggregate into micelles at 34°C due to the dehydration of the polymer blocks with temperature. The gel formation is a result of micellar enlargements and packing, and the gel is more entangled at higher poloxamer concentrations. As a result of these micelle entanglements, they cannot separate easily from each other, which accounts for the rigidity and high viscosity of gel containing high concentrations of poloxamer (Cabana & Ait-kadiJuhasz, 1997; Jain, Aswal, Goyal, & Bahadur, 1998).

By studying the effect of different concentrations of mucoadhesives, such as HPMC and HEC, on viscosity of

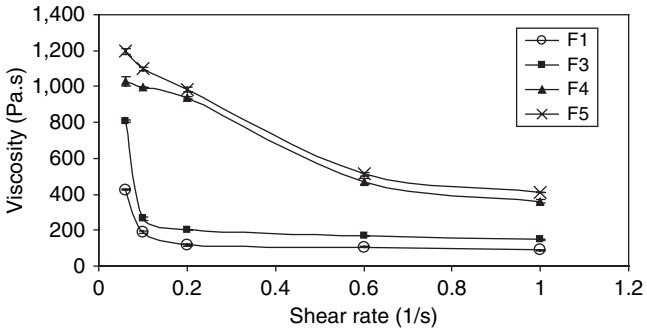

FIGURE 2. Viscosity versus shear rate flow curves of different ciprofloxacin hydrochloride in situ gelling formulations composed of P407/P188 (18/13%, wt/wt) with or without hydroxypropylmethyl cellulose (HPMC).

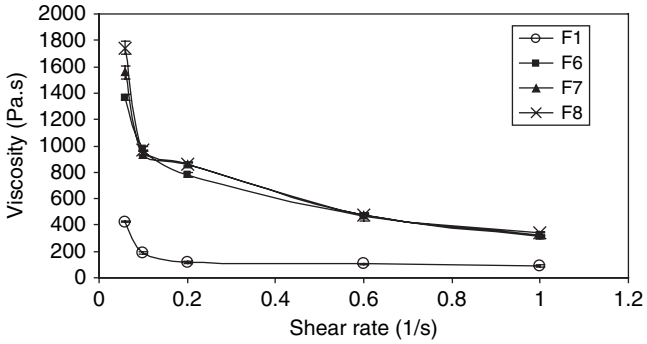

FIGURE 3. Viscosity versus shear rate flow curves of different ciprofloxacin hydrochloride in situ gelling formulations composed of P407/P188 (18/13%, wt/wt) with or without hydroxyethyl cellulose (HEC).

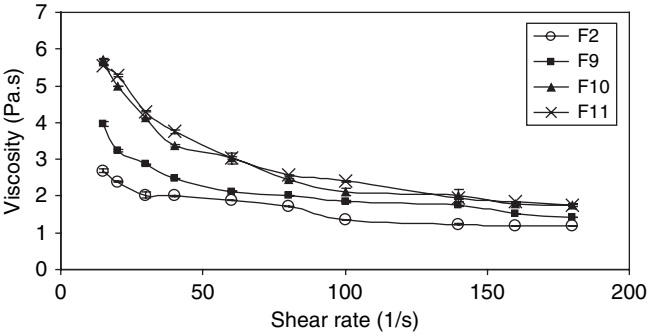

FIGURE 4. Viscosity versus shear rate flow curves of different ciprofloxacin hydrochloride in situ gelling formulations composed of P407/P188 (17/13%, wt/wt) with or without hydroxypropylmethyl cellulose (HPMC).

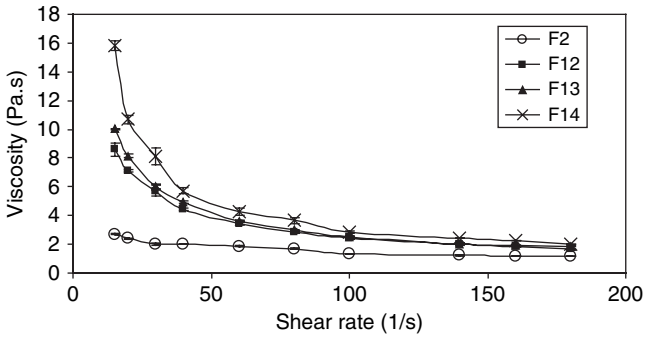

FIGURE 5. Viscosity versus shear rate flow curves of different ciprofloxacin hydrochloride in situ gelling formulations composed of P407/P188 (17/13%, wt/wt) with or without hydroxyethyl cellulose (HEC).

poloxamer in situ forming gels, we can deduce that increasing concentrations of mucoadhesives increases the viscosity of the formulations. This may be attributed to the fact that block copolymer P407 thermosensitive gels are thought to be formed by hydrogen bonding in aqueous systems, caused by the attraction of the poloxamer ether oxygen atom with protons of water. The number of hydrogen bonds is expected to increase by adding compounds with hydroxyl groups such as the examined cellulose derivatives, thus leading to increase the measured viscosity of the prepared formulations.

In Vitro Drug Release Studies

The cumulative percentages of drug released from the in situ forming gels after 8 h ($T_{8\,h}$) are shown in Table 2.

From the results, it is obvious that as the concentration of poloxamer P407 increased from 17 to 18%, the amount of the drug released was decreased. These results indicate that the structure of the gel functioned as an increasingly resistant barrier to drug release as the concentration of P407 increased. The mechanism for such enhanced resistance may be due to reduction in the number and dimension of water channels and due to the increase in the number and size of micelles within the gel structure (Schmolka, 1991). The shorter intermicellar distance leads to greater numbers of cross-links between neighboring micelles leading to higher viscosity and lower rate of drug release (Alexandridis & Hatton, 1995; Bhardwaj & Blanchard, 1996). This assumption may be potentiated by the rheology study that indicates direct proportionality between gel concentration and viscosity (El-Kamel, 2002).

The retarding effect of the cellulosic mucoadhesive polymers could be attributed to their ability to increase the overall product viscosity (Desai & Blanchard, 1998a) as well as their ability to distort or squeeze the extra-micellar aqueous channels of poloxamer micelles through which the drug diffuses, thereby delaying the release process (Choi, Oh, & Kim, 1998). Similar results were obtained by Paavola, Yliruusi, and Rosenberg (1998), who reported that cellulose additives significantly prolonged ibuprofen release. Data of the cumulative percentages of drug released from the in situ forming gels after 8 h ($T_{8\,h}$) shown in Table 2 reveal that the release-retarding effect of HEC is greater than HPMC. This result correlated well with the viscosity of the two polymers HPMC (Methocel E5, low viscosity, 3.6 cp of 2% solution) and HEC (high viscosity, 640 cp of 2% solution). The slowest release rate was observed with formulation F8 containing 1.5% HEC, in which only 54% of the drug was released after 8 h.

By reviewing the kinetic data in Table 3, it could be deduced that all the prepared formulations exhibited n values between 0.506 and 0.769 indicating an anomalous or non-Fickian release suggesting a coupled erosion–diffusion mechanism for the tested ciprofloxacin HCl in situ forming gels.

In Vivo Study

F5 and F6 were chosen for further in vivo studies as they showed retardation in drug release rate and high mucoadhesive force. The antimicrobial efficacy of ciprofloxacin HCl formulations was tested using the scoring system that provides an important index for monitoring the severity of redness of the eye, lacrimal secretion, mucoid discharge, response to ocular stimulus, and swelling of eyelid. The observed results for the antimicrobial efficacy of ciprofloxacin HCl in situ forming gel formulations and marketed eye

TABLE 3
Estimated Values of K and n by Regression of log M_t/M_∞ on log t for the In Situ Forming Gels of Ciprofloxacin Hydrochloride

Formulations	Release Exponent (n)	Kinetic Constant (K)	Correlation Coefficient
F1	0.506	0.544	.996
F2	0.723	0.118	.993
F3	0.609	0.276	.991
F4	0.675	0.111	.986
F5	0.685	0.057	.996
F6	0.532	0.442	.999
F7	0.590	0.228	.992
F8	0.544	0.265	.995
F9	0.614	0.118	.993
F10	0.691	0.314	.994
F11	0.628	0.109	.996
F12	0.769	0.217	.995
F13	0.573	0.009	.991
F14	0.698	0.345	.997

$M_t/M_\infty = Kt^n$.

drops for each rabbit were recorded each day during the experiment, and the median for the readings were tabulated in Table 4. Significance of the in vivo antimicrobial efficacy results using Kruskal–Wallis nonparametric ANOVA test followed by Dunn's multiple comparison test is shown in Table 5.

Significant improvement in the observed parameters was detected earlier in the case of the in situ forming gels than the marketed eye drops, where significant difference in the measured parameters between the in situ forming gels (F5 and F6) and the marketed eye drops appeared from the fourth day of the treatment although they are less frequently instilled. Also, F6 showed very significant results in decreasing lacrimal secretions and mucoid discharge. These results could be explained by the better precorneal residence time of F5 and F6 due to their high mucoadhesive power than the eye drops that were rapidly eliminated by lachrymal flow, blinking, and normal tear turnover.

By comparing the median results for both F5 and F6 using the Kruskal–Wallis test, no significant differences between all the results of both formulae throughout the whole period of the test were detected at $p < 0.05$.

CONCLUSION

From the previous study, it could be concluded that ciprofloxacin HCl would be successfully formulated as mucoadhesive thermoreversible system for the treatment of eye infections. Ciprofloxacin HCl in situ forming gel formulae composed of P407/P188/HPMC (18/13/1.5%, wt/wt), and P407/P188/HEC (18/13/0.5%, wt/wt) showed optimum mucoadhesion properties, prolonged the precorneal residence time and drug release, improved ocular bioavailability, with a decreased frequency of administration, and hence increased the patient compliance compared with the marketed conventional eye drops.

TABLE 4
Median for the Score Testing of the Severity of Redness, Lacrimal Secretion, Mucoid Discharge, Response to Ocular Stimulus, and Swelling of Eyelid for Different Ciprofloxacin Hydrochloride Formulations

No. of Days	Redness			Lacrimal Secretion			Mucoid Discharge			Response to Ocular Stimulus			Swelling of Eyelid		
	F5	F6	M	F5	F6	M	F5	F6	M	F5	F6	M	F5	F6	M
1	3.5	3.5	3.5	2.5	2.5	2.5	3	3	3	2	2	2	2	2	2
2	3	3	3	2	2	2	2	2	3	1	1	2	2	1.5	2
3	2	2	2	1	1	1.5	1	1	2	1	0.5	1	1	1	1
4	1	1	2	1	0.5	1	1	1	2	0	0	1	1	0.5	1
5	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1

M, marketed ciprofloxacin hydrochloride eye drops.

TABLE 5
Significance In Vivo Antimicrobial Efficacy Results for Different Ciprofloxacin Hydrochloride Formulations for ANOVA Testing

No. of Days	Reddness			Lacrimal Secretion			Mucoid Discharge			Response to Ocular Stimulus			Swelling of Eyelid		
	F5 vs. M	F6 vs. M	F5 vs. F6	F5 vs. M	F6 vs. M	F5 vs. F6	F5 vs. M	F6 vs. M	F5 vs. F6	F5 vs. M	F6 vs .M	F5 vs. F6	F5 vs. M	F6 vs. M	F5 vs. F6
1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
3	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
4	NS	NS	NS	NS	NS	NS	S	VS	NS	NS	S	NS	NS	NS	NS
5	S	S	NS	NS	S	NS	S	VS	NS	NS	NS	NS	S	S	NS

M, marketed ciprofloxacin hydrochloride eye drops; NS, nonsignificant; S, significant; VS, very significant.

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