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In Situ Ophthalmic Gel of Ciprofloxacin Hydrochloride for Once a Day Sustained Delivery

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This article focuses on preparation and evaluation of a once a day ophthalmic delivery system for ciprofloxacin hydrochloride based on the concept of pH-triggered in situ gelation. The in situ gelling system involves the use of polyacrylic acid (Carbopol® 980NF) as a phase transition polymer, hydroxypropyl methylcellulose (Methocel® K100LV) as a release retardant, and ion exchange resin as a complexing agent. Ciprofloxacin hydrochloride was complexed with ion exchange resin to avoid incompatibility between drug and polyacrylic acid. The developed formulation was stable, and nonirritant to rabbit eyes and in vitro drug release was found to be around 98% over a period of 24 hours.

Keywords pH-triggered in situ gel; ciprofloxacin hydrochloride; Carbopol®; hydroxypropyl methylcellulose; once a day ocular delivery system

INTRODUCTION

Today, topical ophthalmic application is considered the preferred way to achieve therapeutic levels of active medicament used to treat ocular diseases. Solutions, suspensions, and semisolids like ointments and gels are conventionally available as ophthalmic delivery systems. From a biopharmaceutical standpoint, their use has met some criticism over their efficiency as drug delivery systems. Bioavailability, particularly for ocular solutions, ranges from 1% to 10% of the total administered dose. This could be due to the rapid precorneal kinetics resulting from reflex tearing and blinking. The basic disadvantage associated with the use of ocular formulation is rapid loss of both solutions and suspended solid. Ophthalmic ointments give blurred vision, leading to poor patient acceptance (Olejnic, 1993).

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A high frequency of eye drop instillation is associated with patient noncompliance. Inclusion of excess drug in the formulation in an attempt to overcome bioavailability problems is potentially dangerous if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct (Middleton, Leung, & Robinson, 1990). A significant increase in the precorneal residence time of drugs and, consequently, bioavailability can be achieved by using delivery systems based on the concept of in situ gel formation. These systems consist of polymers that exhibit sol-to-gel phase transitions due to a change in a specific physicochemical parameter (pH, temperature, ionic concentration) in their environment; the cul-de-sac in this case (Sechoy, Tissie, Sebastian, Maurin, & Driot, 2000).

In situ gel–forming systems can be classified as pH-triggered systems (e.g., cellulose acetate phthalate; [Gurny, Boye, & Ibrahim, 1985], Carbopol® [Aggarwal & Ibrahim, 2005; Sultana, Aqil, Ali, & Zafar, 2006; Wu et al., 2007]), temperature-dependent system (e.g., Pluronics® [El-Kamel, 2002; Cho et al., 2003; Cho et al., 2005; Qi et al., 2007], tetronics [Spancake, Mitra, & Kildsig, 1991], and polymethacrylates [Hsiue, Chang, Wang, & Lee, 2003]), and ion-activated systems (e.g., Gelrite® [Balasubramaniam, Kant, & Pandit, 2003; Balasubramaniam & Pandit, 2003] and sodium alginate [Liu et al., 2006]).

Ciprofloxacin hydrochloride monohydrate is a synthetic fluoroquinolone antibacterial agent. It is rapidly active against Gram-negative aerobic bacteria including *Enterobacteriaceae, Pseudomonas aeruginosa, Haemophilus*, and *Neisseriae*. It is also active against many Gram-positive aerobic pathogens including penicillinase-producing and methicillin-resistant *Staphylococci* (Reynolds & Martindale, 2002). Ciprofloxacin acts by inhibiting the DNA synthesis of the microorganism (Mandell & Sande, 1996).

The objective of the present work involved preparation and evaluation of a once a day delivery system based on the concept of pH-triggered in situ gelation. Because of the short elimination half-life of ciprofloxacin hydrochloride, three to four drops

must be instilled three to four times a day (Kamath, Singh, & Udupa, 1993; Tsai, Tseng, Chang, & Hu, 1995).

A combination of Carbopol® 980NF and hydroxypropyl methylcellulose (Methocel® K100LV) was used for the preparation of a pH-triggered system. The above-mentioned combination was used for the preparation of eye drops of ciprofloxacin hydrochloride (0.3% w/v as equivalent to ciprofloxacin) for once a day instillation, which releases about 98% of the drug over a period of 24 hours.

MATERIALS AND METHODS

Materials

Ciprofloxacin hydrochloride I.P. was obtained as a gift sample from Cipla Limited (Mumbai, India). Carbopol® 980NF and hydroxypropyl methylcellulose (Methocel® K100LV) were gifted by Noveon and Colorcon Asia Pvt. Ltd., respectively, (Mumbai, India). Ion exchange resins (particle size: 19.07 ± 1.07 μ) were gifted by Ion Exchange (India) Ltd., (Mumbai, India). All other chemicals and solvents used were of analytical grade and purified water was used throughout the study.

Methods

Pretreatment of the Drug

An ion exchange resin, Indion® 254F (chemically: polystyrene cross-linked with divinylbenzene), possessing ion exchange capacity of 110 to 135 mg/dry gm, was dispersed uniformly in water using an overhead stirrer for 15 minutes. The drug was added to the dispersion while stirring and this continued until complexation was complete. Periodically, the samples were withdrawn and centrifuged. Supernatant was analyzed for uncomplexed drug by ultraviolet (UV) spectrophotometer at 273 nm. Resinate (drug-resin complex) so obtained was filtered, washed, dried, sieved through 100# (ASTM), and stored in desiccator until further use. Differential scanning calorimetric (DSC) studies were carried out by heating drug, resin, and the resinate separately from 37°C to 425°C at the heating rate of 10°C/min in a nitrogen environment. The instrument used was Perkin-Elmer differential scanning calorimeter with Pyris 6 software. Thermograms obtained were as shown in Figure 4.

Method of Preparation

Optimization of Carbopol® for In Situ Gelling Capacity. The level of Carbopol® 980NF was optimized by determining gelling capacity of the system. The gelling capacity was assessed by placing a drop of the system in a vial containing 2 ml of artificial tear fluid (ATF), freshly prepared and maintained at 37 ± 0.5°C. The time taken by the system to form gel and dissolution of gel was recorded (Table 1).

Carbopol® 980NF was uniformly dispersed in water and resinate (equivalent to 0.3% ciprofloxacin) and was incorporated into the solution. Then the formulation was subjected to in vitro release studies using ATF pH 7.4 as dissolution medium (maintained at 37 ± 0.5°C), which is equivalent to the pH of the eye (Desai & Blanchard, 1995). The composition of the ATF used was: sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride dihydrate 0.008 g, and purified water q.s. 100 ml (Bottari, Dicolo, Nannipieri, Saettone, & Serafini, 1974).

TABLE 1
Gelling Capacity Studies of In Situ Gelling System at 37 ± 0.5°C

Formulations	Cabopol® 980NF (%)	Methocel® K100LV (%)	Gelling Capacity
CP1	0.1	-----	−
CP2	0.2	-----	−
CP3	0.3	-----	+
CP4	0.4	-----	++
CP5	0.5	-----	++
HCP1	0.5	0.5	+++
HCP2	0.5	1.0	+++
HCP3	0.5	1.5	+++
HCP4	0.5	2.0	+++

− = no gel;
+ = gels after 1 to 2 minutes and dissolve soon;
++ = gels after 1 to 2 minutes and remains for 4 to 6 hours;
+++ = gels within a minute and remains for 10 to 12 hours.

Optimization of Methocel® K100LV for Once a Day In Situ Gelling System. Methocel® K100LV as a release retardant was incorporated into the optimized Carbopol® formulation (CP5), as mentioned in Table 1, for once a day instillation. Different concentrations of Methocel® K100LV were used in order to achieve the desired release profile. The prepared solutions were evaluated for in vitro release studies so as to optimize the concentration of the Methocel® K100LV.

The detailed procedure for preparing the in situ gel forming system is as follows:

Disodium edetate (0.01% w/v), mannitol (5% w/v) and benzalkonium chloride (0.02% w/v) were dissolved in 75 ml of water. Methocel® K100LV (2% w/v) was added to the above solution and allowed to hydrate for 20 minutes. Then Carbopol® (0.5% w/v) was dispersed in this solution and allowed to hydrate with stirring. The solution was filtered through a cellulose acetate membrane filter (pore size 0.45 μ). Resinate (equivalent to 0.3% w/v of ciprofloxacin) was added to the filtered solution under stirring so as to ensure uniform suspension of resinate. The pH of the system was adjusted to 4.0 ± 0.2 using 0.5 N sodium hydroxide solution. Finally, the volume was increased to 100 ml with water.

The above formulation was dispensed in 5 ml capacity amber-colored glass vials, closed with grey butyl rubber closures and sealed with aluminium caps. The formulations were

subjected to terminal sterilization by autoclaving at 121°C, 15 psi for 20 minutes.

Evaluation of Formulation

The developed in situ gel formulation was evaluated for sol-gel transformation studies by changing the appropriate physicochemical parameter, drug content by UV spectrophotometry at 273 nm (JASCO V530 UV spectrophotometer), in vitro drug release, pH (equiptronics digital pH meter), sterility, and effect of sterilization.

Rheological Studies. The developed formulation was poured into the small sample adaptor of the Brookfield synchrolectric viscometer and the angular velocity was increased gradually from 0.5 to 100 rpm. The hierarchy of the angular velocity was reversed. The average of two readings was used to calculate the viscosity. The developed system was poured into an ointment jar, and the pH of the formulation was increased to 7.4 by adding 0.5 N sodium hydroxide solution. The rheology of the resultant gel was studied using the T-bar spindle (E).

In Vitro Release Studies. A modified USP XXII dissolution testing apparatus (apparatus 1; Figure 5; Bottari et al., 1974; Dotegham, 1993) was used for evaluating the in vitro release profile using cellophane membrane (pore size: 0.45 μ), which does not hamper the diffusion of the drug. This phenomenon was confirmed by placing the plain drug on the membrane. Within 15 minutes, 100% dissolution of the drug was obtained. As the particle size of the resin is larger than the pore size of the membrane, the resin does not pass through the membrane. The dissolution medium used was ATF.

A glass cylinder of 2.5 cm in diameter open at both ends was designed for the purpose of our study. Cellophane membrane previously soaked overnight in ATF was taken, patted dry, and tied on to one end of the cylinder. One ml of the formulation was accurately pipetted and poured on the cellophane membrane. Further, the glass cylinder was attached to the shaft of USP apparatus 1, in place of basket as shown in Figure 5. The cylinder was then suspended in the dissolution medium maintained at 37 ± 0.5°C such that the membrane just touched the dissolution medium. The speed of the metallic device shaft was set at 50 rpm. Aliquots were withdrawn at intervals of 1, 2, 4, 6, 8, 10, 12, 14, 16, and 24 hours and replaced by equal volumes of dissolution medium. Aliquots were suitably diluted with ATF and analyzed by JASCO V530 UV Spectrophotometer at 270 nm. The percent cumulative release of the drug was computed and the graph of percent cumulative release versus time was plotted. Each experiment was repeated six times.

Antimicrobial Efficacy Studies. This was determined by agar diffusion test employing the cup plate technique. Dilutions of ciprofloxacin hydrochloride in purified water (standard solutions) were prepared. The formulation was diluted suitably with purified water. All the above solutions were sterilized and poured into the cups bored into the sterile nutrient agar seeded with test organisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*). After allowing diffusion of the solutions for 2 hours, the agar plates were incubated at 37°C for 24 hours. The zone of inhibition (ZOI) measured around each cup was compared with the control. The entire operation except the incubation was carried out in a laminar airflow unit. Each solution was tested in triplicate. Both positive and negative controls were maintained throughout the study.

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

Accelerated Stability Studies. Selected sterilized formulations were stored at 4 ± 1°C room temperature (27 ± 1°C), 37 ± 1°C, and 45 ± 1°C for a period of 3 months. The formulations were evaluated at periodic intervals for pH, sol gel transition, rheology, in vitro drug release, sterility, and drug content by developed high-performance liquid chromatography (HPLC) method using a Spherisorb® ODS 2 (5 μ) column (250×4.6 mm). The mobile phase was 25:75:0.6 of acetonitrile:water:triethylamine and the pH was adjusted to 4.0 by using phosphoric acid at a flow rate of 1.00 ml/minute. The detection was carried out at λmax of 276 nm. The shelf life of the developed formulations was calculated using the Arrhenius plot.

RESULTS AND DISCUSSION

Pretreatment of the Drug

Ciprofloxacin hydrochloride exhibited instantaneous incompatibility with Carbopol®, resulting in a lumpy precipitate. Hence, various methods were tried for separating the two incompatible compounds.

The modifications tried to overcome incompatibility were:

(a) Carbopol® was dispersed in an acetate buffer I.P. pH 4.0 and drug was added to the above mixture, but this too led to the formation of an incompatible lumpy mass.
(b) Dispersion of Carbopol® in water decreases the pH of the system to 3.0, and this could be the reason for drug precipitation. Hence, 0.5 N sodium hydroxide was added to the Carbopol® dispersion to adjust the pH of the system to pH 4.0. However, drug separation was observed.
(c) Hence, the next approach involved the use of surfactants and cosolvents. Pluronic® F127 (18%), tween-20 (1% and 2%), propylene glycol (1%, 2%, and 3%), and glycerin (1%, 2% and 3%) were tried; here, too, drug separation was observed.

All the above-mentioned difficulties in formulating in situ gelling system using Carbopol® led to the need for complexation of drug with a complexing agent to avoid interaction of the drug with Carbopol®.

Several grades of strong and weak cation exchange resins were tried for complexation with ciprofloxacin hydrochloride. Weak cation exchange resins, namely Indion® 234S (containing -COOH group,) exhibited burst release, whereas strong cation exchange resin Indion® 244F (containing a $-SO_3H$ group) held brown color, which would affect the vision.

Hence, off-white coloured cation exchange resin (Indion® 254F) was selected for the complexation. Based on various trials, the ratio of 1:2 of drug to the ion exchange resin (Indion® 254F) was optimized.

As shown in Figure 4, thermogram C represents the DSC thermogram of pure drug ciprofloxacin hydrochloride, exhibiting a sharp endotherm at 318.6°C. Disappearance of this peak in the thermogram A indicates complete complexation of the drug with the resin. Thermogram B is of pure resin.

Method of Preparation

Carbopol® is a cross-linked acrylic polymer that shows pH-mediated phase transitions. Carbopol® was used as an in situ gel forming polymer as it forms acidic, low viscosity, aqueous solutions that transform to a stiff gel when the pH is raised to 7.4.

Amongst the various grades of Carbopol® used for pharmaceutical applications, only Carbopol® 980 NF is used for ophthalmic preparations, as the other grades as per Noveon product information literature, namely Carbopol® 934P NF, Carbopol® 941 NF, Carbopol® 940 NF, contain benzene as the residual solvent, which may be irritating to eyes. However, Carbopol® 981 NF and Carbopol® 980 NF were the only grades that were benzene free. Carbopol® 980 NF exhibited higher viscosity at a lower concentration range and gave a clear sparkling gel as compared with Carbopol® 981 NF. Hence, Carbopol® 980 NF was selected for further studies.

Optimization of Carbopol® for In Situ Gelling Capacity

The optimization of the concentration of Carbopol® 980NF was necessary as it decreases the pH of the formulation to a level that is difficult for the human eye to neutralize. The concentration of Carbopol® 980 NF was optimized by studying in vitro gelling capacity. The initial optimization was carried out between 0.1% to 0.5% w/v of Carbopol®, 980 NF. The results are shown in Table 1. Formulation CP5, containing 0.5% Carbopol® gave better results. However, the viscosity of the gel starts reducing after 4 to 6 hours.

Resinate (equivalent to 0.3% of ciprofloxacin) was incorporated into formulation CP5. In vitro release studies were carried out for CP5 ($n = 6$) as shown in Table 1, and it retarded the drug release up to 10 hours, hence further modifications were made in the formulations to retard the release of the drug for 24 hours.

Optimization of Methocel® K100LV for Once a Day In Situ Gelling System

Methocel® K100LV was incorporated in the formulation at the concentration of 0.5% to 2.0% w/v. The various combinations were as shown in Table 1. All the above-mentioned formulations exhibited acceptable flowability at pH 4.0. Hence in vitro release studies were carried out for formulationS HCP4 ($n = 6$) and HCP2 ($n = 6$). Formulation HCP4 retarded the release of the drug for up to 24 hours, while in the case of formulation HCP2, around 90% of the drug release was obtained at the end of 12 hours.

Mannitol (5% w/v) was added as a tonicity adjusting agent. Benzalkonium chloride (0.02% v/v) was added as a preservative. Disodium edetate was added at a concentration of 0.01% w/v to enhance the efficiency of the benzalkonium chloride. 0.5 N sodium hydroxide solution was used to adjust the pH of the formulation to 4.0. Finally the formulation was sterilized by autoclaving at 121°C, 15 psi for 20 minutes. Then the final formulation was subjected to in vitro release studies ($n = 6$) and the release profile was found to be similar to the release of HCP4 with a similarity factor of 69.

Evaluation of the Formulation

The formulation was evaluated for drug content ($n = 3$), pH, and clarity. The results are shown in Table 2. The developed in situ gelling system was liquid at nonphysiological conditions and underwent rapid gelation at a pH of cul-de-sac.

Terminal sterilization by autoclaving had no effect on the pH, drug content, and viscosity of the formulation. Figure 1 shows that autoclaving did not affect the viscosity of the preparation. Thus, the formulation could withstand terminal autoclaving, a method of sterilization used widely in the pharmaceutical industry, without undergoing any major change in their properties.

Rheological Studies

The rheological behavior of the formulation was investigated as a function of pH. All measurements were performed

TABLE 2
Evaluation of the Marketed and Developed Formulation

Formulation	Drug Content (% w/v)	pH	Clarity
HCP4 (Before autoclaving)	100.86 ± 0.75	4.00	Clear
HCP4 (After autoclaving)	100.27 ± 0.42	3.97	Clear
Marketed eye drop (Ciplox™)	101.08 ± 0.41	4.00	Clear
Marketed eye drop (Cefran™)	100.62 ± 0.23	4.70	Clear

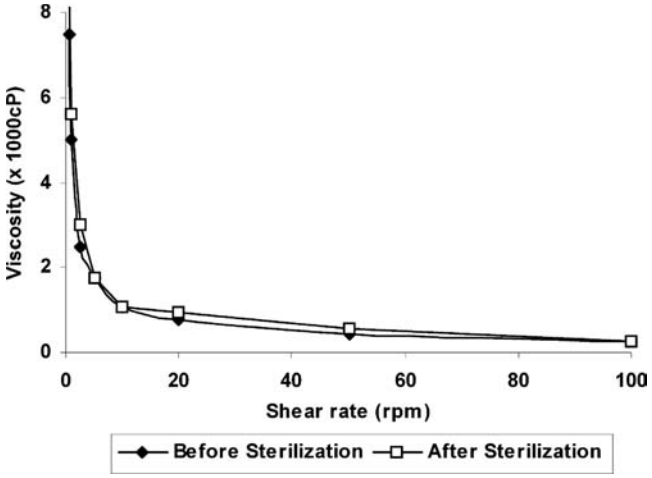

FIGURE 1. Viscosity versus shear rate of HCP4 at pH 4.0 ± 0.05 before and after sterilization.

in triplicate with good reproducibility. The standard deviation was found to be below 2.5%. The formulation exhibited pseudoplastic behavior, that is, a decrease in viscosity was observed with increase in shear rate. Such viscoelastic fluids with low viscosity under conditions of high shear rate and high viscosity under the conditions of low shear are generally preferred. Figure 2 demonstrates increase in viscosity of formulation HCP4 with increase in pH from 4.0 to 7.4, confirming that HCP4 formulation is a pH dependent gelling system. At pH 4.0, the formulation HCP4 was in a liquid state and exhibited low viscosity. An increase in pH to 7.0 caused the HCP4 solution to transform into gels with high viscosity (Figure 2).

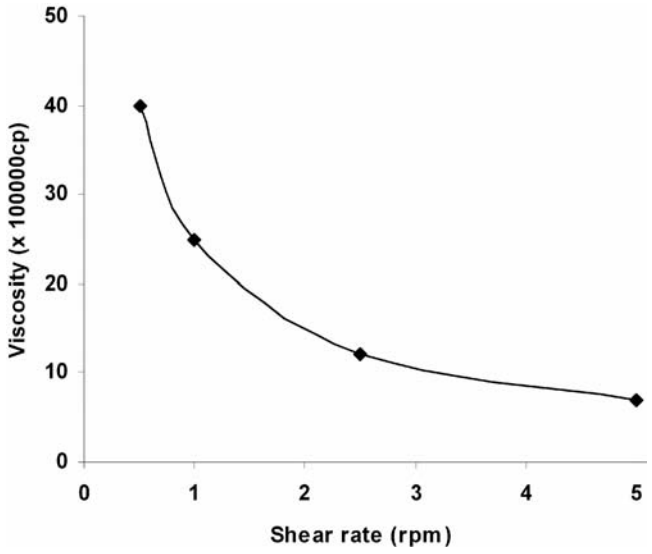

FIGURE 2. Viscosity versus shear rate of HCP4 at pH 7.4 ± 0.05.

In Vitro Release Studies

The in vitro drug release studies ($n = 6$) were carried out for optimizing the in situ gelling systems, namely HCP4, HCP2, and CP5. The release profile is shown in Figure 3. Formulation CP5 containing only Carbopol® 980 NF released around 90% of the drug in 10 hours, thus necessitating use of additional release retardant to obtain the desired release profile. Further studies were carried out using Methocel® K100LV as a release retarding polymer. Formulations HCP2 and HCP4 contain 1% and 2% Methocel® K100LV, respectively. HCP2 released 90% of the drug in 12 hours, whereas HCP4 released 90% of the drug in around 16 hours.

The release of drug depends not only on the nature of the matrix, but also upon the polymer concentration. This may be due to structural reorganization of the hydrophilic polymer, Methocel® K100LV. Increase in concentration of Methocel® K100LV may result in increase in the tortuosity or gel strength of the polymer. When Methocel® K100LV is exposed to an aqueous medium, it undergoes rapid hydration and chain relaxation to form a viscose gelatinous layer (gel layer). Failure to generate a uniform and coherent gel may cause rapid drug release (Basak, Jayakumar, & Lucas, 2006). Formulation HCP4 was found to be the optimized once a day formulation of ciprofloxacin hydrochloride.

Release Kinetics of the Drug

Upon least squares linear regression analysis of the percent cumulative release as a function of time, r-values of 0.99723, 0.9743, and 0.9744 were obtained for first-order, zero-order, and Higuchi kinetics, respectively (Table 4). This clearly indicates that the overall drug release from formulation HCP4 follows first-order release kinetics. However, the drug release from the formulation followed zero-order release kinetics up to 10 hours ($r = 0.9991$), releasing approximately 75% of the drug. Also, drug release from the formulation followed first-order release kinetics from 10 to 24 hours ($r = 0.9999$).

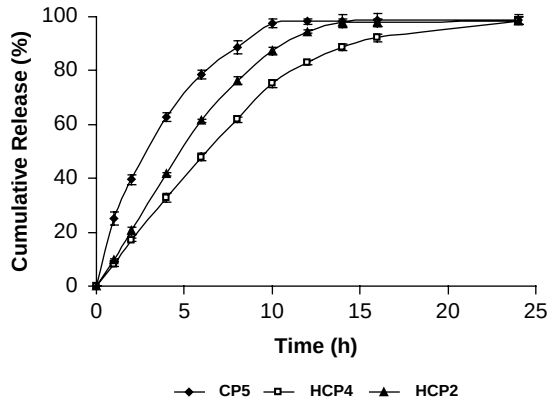

FIGURE 3. In vitro release of CP5, HCP2, and HCP4 (n = 6).

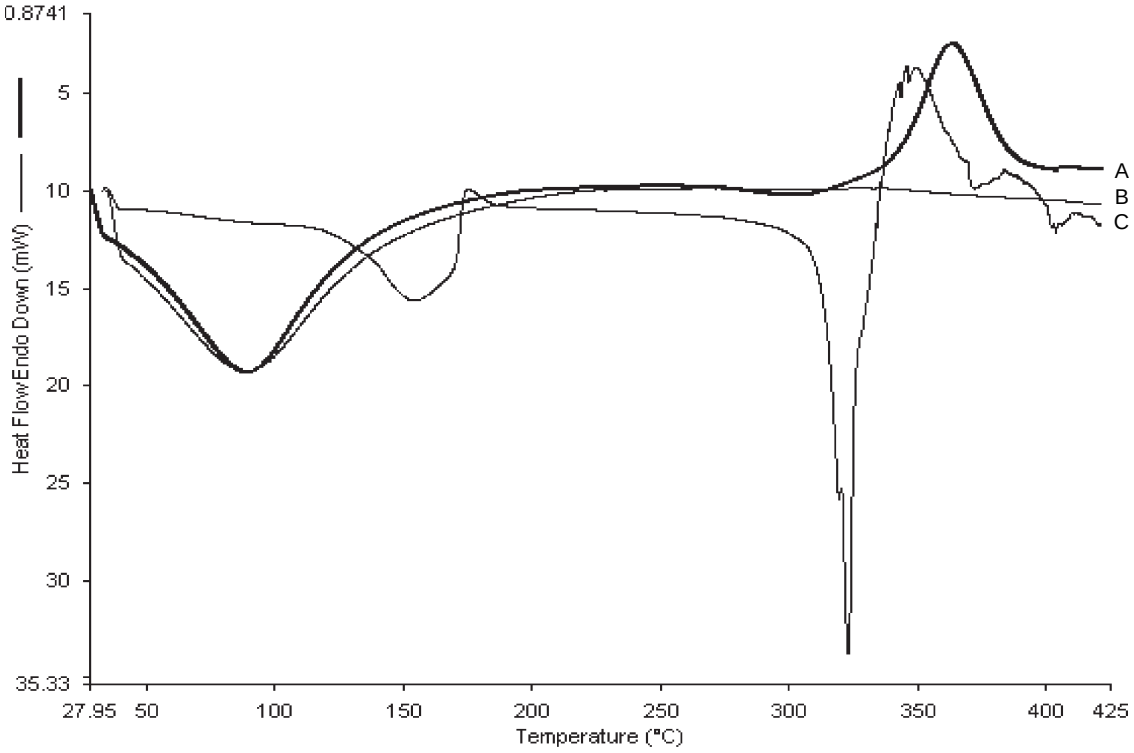

FIGURE 4. DSC thermogram of the (A) drug, (B) resin, and (C) resinate.

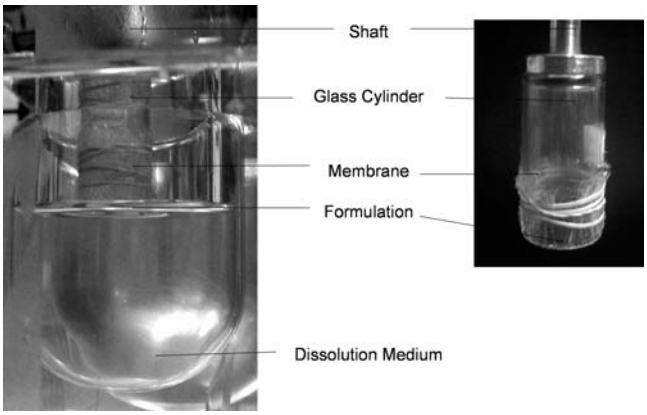

FIGURE 5. Modified in vitro dissolution apparatus.

Release of the drug from a formulation containing hydrophilic polymers generally involves factors of diffusion. Diffusion is related to transport of drug from the formulation into the in vitro study fluid, depending upon the concentration. As gradient varies, the drug is released, and the distance for diffusion increases (Reddy, Mutalik, & Reddy, 2003). This could explain why the drug diffuses at a comparatively slower rate after 10 hours as the distance for diffusion increases, which is observed in all formulations, namely CP5, HCP2, and HCP4, as shown in Figure 3. To confirm the diffusion mechanism, the data were fit into Korsmeyer's equation. (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983);

$$Qt / Q\alpha = K t^n$$

where Q is the amount released at time t, $Q\alpha$ is overall released amount, K is a constant incorporating the properties of macromolecular polymeric system and the drug, and n is a kinetic constant that depends on the transport mechanism. The exponent n gives information about the release mechanism; $n = 0.5$ characterizes diffusion controlled release, $0.5 < n < 1.0$ indicates anomalous (non-Fickian transport), and $n = 1.0$ indicates swelling controlled release (zero-order kinetics). Drug diffusion and polymer erosion control the release process in equal parts if $n = 0.66$ (Mockel & Lippold, 1993).

Formulation HCP4 showed a diffusion controlled-release mechanism as reflected by its n value of 0.4999. However, the release up to 10 hours was found to follow swelling controlled release, reflected by $n = 0.9997$, releasing almost 75% of the drug. The relative complexity of this formulation may indicate that the drug release is controlled by more than one process. In formulation HCP4, two components play a role in controlling release of the drug, that is, Carbopol® 980NF and Methocel® K100LV. For up to 10 hours, both the polymers controlled the release of the drug, giving a zero-order release profile. After 6 hours, Carbopol® 980NF starts dissolving, as indicated in Table 1, and does not play any role in controlling release after

TABLE 3
Antimicrobial Efficacy Testing

Microbial Strain	Concentration (μg/ml)	Zone of Inhibition (mm) Standard	Zone of Inhibition (mm) HCP4	Efficacy (%)
Staphylococcus aureus	1	–	–	–
	10	2.0 ± 0.057	1.9 ± 0.057	95 ± 1.02
	100	3.0 ± 0.10	2.9 ± 0.10	96.67 ± 0.75
	500	3.5 ± 0.10	3.4 ± 0.15	97.14 ± 1.20
Pseudomonas aeruginosa	1	–	–	–
	10	–	–	–
	100	2.9 ± 0.10	2.8 ± 0.057	96.55 ± 1.25
	500	3.8 ± 0.10	3.8 ± 0.10	100 ± 0.00

10 hours. From 10 to 24 hours, Methocel® K100LV is the only release-controlling polymer.

Antimicrobial Efficacy Studies

The results of the antimicrobial efficacy studies are depicted in Table 3. The study indicates that ciprofloxacin retained its antimicrobial efficacy when incorporated in an in situ gelling system.

Ocular Irritation Studies

Ocular irritation studies (Table 5) indicate that HCP4 was a nonirritant. The formulation was very well tolerated by the eye. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible.

TABLE 4
Release Kinetics of the Formulation HCP4

Order	Correlation coefficient	T25 (h)	T50 (h)	T75 (h)	T90 (h)
First	–0.99723	2.79	5.57	10.28	16.54
Zero	0.9743	2.21	7.71	13.21	16.50
Higuchi	0.9744	1.95	5.88	11.93	16.57

TABLE 5
Ocular Irritation Testing (As Per the OECD Guidelines)

Formulations	Average Score
Blank of HCP4	0
Formulation HCP4	0
Dioctyl sodium sulphosuccinate (positive control)	11 ± 0.599
0.9% w/v sodium chloride solution (negative control)	0

Accelerated Stability Studies

Stability studies were carried out at various temperature conditions, at 4 ± 1°C, ambient temperature (25 ± 1°C), 37 ± 1°C, and 45 ± 1°C for a period of 3 months. The formulation was found to be stable as indicated by clarity, no change in pH, viscosity, in vitro release, gelling capacity, and drug content (100%–101%). Stability indicating HPLC method was used for assay. The formulation was found to be sterile at the end of 3 months. Based on the stability data, shelf life of 2.00 years could be assigned for HCP4.

CONCLUSION

There is no literature available that reports the incompatibility of ciprofloxacin hydrochloride with Carbopol®. This incompatibility was successfully overcome in the present work by complexing ciprofloxacin hydrochloride with ion exchange resin, and a pH-dependent in situ gel of ciprofloxacin was successfully formulated. The in vitro release profile showed extended release of drug over a period of 24 hours. The formulation was subjected to various physicochemical studies and was found to be satisfactory. The rheological profile showed the gel formation at pH 7.4, pH of cul-de-sac. Tests for antimicrobial efficacy proved the formulation to be therapeutically efficacious. Also, eye irritation test confirmed that the formulation was nonirritating to eyes. Further, the stability data recorded over a 3-month period under accelerated conditions of temperature ensure the stability of the formulation.

The in situ gel forming system could have good patient acceptance because it is easy to instil and gradually erodes by dissolution of the gel, avoiding the need for removal. The long residence time of the gel formed in situ along with its ability to release drugs in a sustained manner would enhance bioavailability. It is also possible to design in situ gels with desirable rheological properties and drug release rates. Hence, it can be concluded that in situ gels are a viable alternative to

conventional eye drops by providing sustained release of medicaments to the eye.

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