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Research Article

Sustained Ophthalmic In Situ Gel of Ketorolac Tromethamine: Rheology and In Vivo Studies

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ABSTRACT Most ocular diseases are treated with topical eye drops. The poor bioavailability and therapeutic response exhibited by these conventional eye drops due to rapid precorneal elimination of the drug may be overcome by the use of in situ gelling systems that are instilled as drops into the eye and undergo a sol-to-gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of the nonsteroidal anti-inflammatory drug (NSAID), ketorolac tromethamine, based on the concept of pH-triggered in situ gelation. Polyacrylic acid (Carbopol® 934) was used as the gelling agent in combination with hydroxypropylmethylcellulose (Methocel E15LV), which acted as a viscosity enhancer. The prepared formulations were characterized for clarity, pH, drug content, rheology, and in vivo drug release. Clarity, pH, and drug content of the developed formulations were found to be satisfactory. The developed formulation showed pseudo-plastic rheology. The formulation with benzalkonium chloride and edetate disodium improved the rate of corneal absorption but not the extent. The developed formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its longer precorneal residence time and ability to sustain drug release. Also importantly is the ease of instillation afforded and decreased frequency of instillation resulting in better patient acceptance. Drug Dev Res 70 : 417–424, 2009.

Key words: in situ gelation; ophthalmic sustained delivery; Carbopol® 934; differential scanning calorimetry (DSC); rheology

INTRODUCTION

Delivering drugs to the front of the eye is an exceedingly complicated issue because of the numerous protective mechanisms that are present in the eye to shield the visual pathway from foreign substances [Worakul and Robinson, 1997]. Topical delivery of eye drops into the lower cul-de-sac is the most common method of drug treatment in ocular diseases and diagnostics [Jarvinen et al., 1995]. Eye drops that are conventional ophthalmic delivery systems often result in poor bioavailability and therapeutic response due to high tear fluid turnover and dynamics that cause rapid precorneal elimination of the drug [Schoenwald, 1990]. A high frequency of eye drop instillation is also associated with patient non-compliance. Inclusion of excess drug in the formulation to overcome

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bioavailability problems is associated with side effects issues if the drug solution drained from the eye is systemically absorbed from the nasolacrimal duct [Middleton et al., 1990]. One way of prolonging the presence of drug in the precorneal area involves increasing the viscosity of the dosage form by adding water-soluble polymers [Thermes et al., 1992; Felt et al., 1999]. An alternative approach aimed at increasing precorneal residence time of drugs and consequently bioavailability is the use of polymeric solutions that change to gel as a result of exposure to the physiological temperature, pH or ionic composition of the lachrymal fluid [Sechoy et al., 2000].

Many NSAIDs have been tested as ocular anti-inflammatory agents so as to diminish the well-documented ocular side effects caused by corticosteroids [Cooper et al., 1980; Searle et al., 1990]. Ketorolac tromethamine (KT) is a potent and effective aryl-acetic acid NSAID and is nonirritating to the eye at 0.5% w/v concentration [Mahoney and Waterbury, 1983]. Aqueous ocular drops of KT are effective and safe for topical use following cataract surgery and intraocular lens implantation [Flach et al., 1988; Heier et al., 1999]. KT is also a viable alternative to corticosteroids in treating ocular inflammation in the presence of pathogens [Fraser-Smith and Mathews, 1988]. Ophthalmic solutions of KT (0.5%) are effective in the treatment of chronic aphakic and pseudoaphakic macular edema [Flach et al., 1987] and a beneficial effect of KT (0.5%) topical solution in reducing postoperative pain after laser in situ keratomileusis has been reported [Price et al., 2002]. The topical ophthalmic dose of KT is 1 drop qid in allergic conjunctivitis and in cystoid macular edema.

The objective of the present research was to develop a pH-triggered in situ gelling system for sustained ophthalmic delivery of KT and to determine the rheology and ocular availability of KT following ocular instillation of in situ formulations to normal corneas of rabbits. A combination of Carbopol and hydroxypropylmethylcellulose (HPMC) was investigated as vehicle for the formulation of eye drops of KT (0.5%, w/v) that would gel when instilled into the eye, and provide sustained release of KT during treatment of seasonal allergic conjunctivitis and in some ocular inflammation situations.

MATERIALS AND METHODS

Materials

KT was a gift from Symed Labs Limited, Hyderabad. Carbopol® 934 and HPMC (Methocel) were gifts from Loba Chamie Pvt. Ltd., Mumbai, and Colorcon Asia Pvt. Ltd., Goa, respectively. Benzalkonium chloride was purchased from Ranbaxy Fine Chemicals Limited, New Delhi. All other reagents used were of analytical reagent grade.

Methods

Drug-excipient compatibility studies

Differential scanning calorimetry (DSC) characterization. Calorimetric characterization of KT, Carbopol® 934, and HPMC (E4M and E15 LV) alone and their physical mixtures were carried out using a DSC 822^e (Mettler Toledo Star^e System, Switzerland). Argon was used as purging gas at a rate of 80 ml min^{-1}. The calorimeter was calibrated for baseline using no pans, for cell constant and temperature using indium. All experiments were performed using non-hermetic aluminum pans, in which samples were accurately weighed, and then just covered with the lid. The samples were loaded on an auto sampler tray. Samples for the DSC study were program-heated from 25 to 200°C, then cooled to 0°C using liquid nitrogen, and finally heated to 200°C again, at a rate of 10°C min^{-1} [Ho et al., 1996; Gomez-Carracedo et al., 2004].

Preparation of formulations

Selection of vehicle. KT solubility was evaluated in various buffers, e.g., acetate buffer I.P. (pH 4.6, 4.8, 5.0, 5.5, and 6.0), citrophosphate buffer B.P. (pH 5.0. 6.0. 6.2 and 7.0) and phosphate buffer USP (pH 5.5, 6.0, 6.5, and 7.2) to select a suitable vehicle. Solutions of KT (0.5%, w/v) in the buffers in which it was soluble were prepared and tested for stability to light, temperature, and autoclaving, using a stability indicating high-performance thin-layer chromatographic (HPTLC) method [Devarajan et al., 2000].

Preparation of in situ gelling systems. Aqueous solutions of varying concentrations of Carbopol®934 (CP) and HPMC of different grades (formulation codes F 1, F 2,…,F 7) were prepared and evaluated for gelling capacity and viscosity in order to identify the compositions suitable for use as in situ gelling systems (Table 1). The gelling capacity was determined by placing 100 µl of the system in a vial containing 2 ml of artificial tear fluid (NaCl 0.670 g, sodium bicarbonate 0.200 g, calcium chloride · 2 H_2O 0.008 g, purified water q.s. 100.0 g [V'Ooteghem, 1993]) freshly prepared and equilibrated at 37°C and visually assessing gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. Viscosity at 20 rpm was measured using a Brookfield Synchrolectric viscometer (RVT model) in a small volume adapter used for purposes of comparative evaluation.

The detailed procedure for preparing the in situ gel-forming system of ketorolac tromethamine is

TABLE 1. Combinations of Carbopol and HPMC Studied*

Formulation	HPMC grade	Concentration (% w/v)		Gelling capacity	Viscosity (cP) (20 rpm)
		HPMC	Carbopol® 934		
F 1	E4 M	1.0	0.2	–	408.5
F 2	E4 M	1.0	0.4	+	1595.2
F 3	E4 M	1.5	0.3	+++	2963.2
F 4	E4 M	1.0	0.5	++	1782.6
F 5	E15 LV	1.5	0.3	–	202.5 (very low)
F 6	E15 LV	1.5	0.4	+	782.0
F 7	E15 LV	1.5	0.5	++	915.4

*–, No gelation; +, Gels after a few a minutes, dissolves rapidly; ++, Gelation immediate, remains for few hours; +++, Gelation immediate, remains for extended period.

TABLE 2. Ingredients of the Developed Formulations

Ingredient	Quantity (g)	
	F 7.1	F 7.2
Ketorolac tromethamine	0.5	0.5
Carbopol® 934	0.5	0.5
HPMC E15LV	1.5	1.5
Edetate disodium	–	0.01
Benzalkonium chloride	0.01	0.01
Citric acid IP	0.407	0.407
Disodium hydrogen phosphate IP	1.125	1.125
Purified water IP	100 ml	100 ml

outlined in Table 2. Buffer salts were dissolved in 75 ml of purified water. Methocel E15LV was added and allowed to hydrate. Carbopol®934 was sprinkled over this solution and allowed to hydrate overnight. The solution was stirred with an overhead stirrer, edetate disodium (EDTA) solution was added while stirring. KT was dissolved in purified water; benzalkonium chloride (BKC) was then added and the solution was filtered through 0.2-μm cellulose acetate membrane filter. The drug solution was added to the Carbopol-HPMC solution under constant stirring until a uniform solution was obtained. Purified water was then added to make up the volume to 100 ml. The developed formulations were filled in 5-ml capacity amber glass vials, closed with gray butyl rubber closures and sealed with aluminum caps. The formulations, in their final pack were subjected to terminal sterilization by autoclaving at 121°C and 15 psi for 20 min.

Evaluation of Formulations

The developed formulations were evaluated for drug content by UV spectrophotometry at 245 nm (Cecil 2021 UV spectrometer), clarity by visual observation against a black and white background in a well-lit cabinet, pH (Equiptronics digital pH meter), sol-to-gel transition, and sterility.

Rheological Studies

The developed formulation (pH 6.0) was poured into the small sample adaptor of the Brookfield Synchrolectric viscometer, and the angular velocity was increased gradually from 0.5 to 50 rpm. The hierarchy of the angular velocity was reversed. The average of the two readings was used to calculate the viscosity. The formulation was then poured into an ointment jar and the pH raised to 7.4 by adding 0.5 M NaOH. The rheology of the resultant gel was studied using T bar F.

In Vivo Drug Release Studies

In vivo release of KT from the prepared in situ gelling formulations was assessed in six male New Zealand albino rabbits each weighing 2.5–3.0 kg and with no signs of ocular inflammation or gross abnormalities. Animal procedures conformed to the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research. All animals were maintained according to Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) guidelines.

An aliquot (50 μl) of in situ gelling formulations and marketed eye drop of KT was instilled in the lower cul-de-sac of each eye, and the upper and lower eyelids were gently held closed for 2 min to maximize drug cornea contact. At 0.5, 1, 2, 4, 6, and 8 h postdose, eyes were anesthetized using 4% xylocaine solution topically, and aqueous humor was sampled from 6 eyes for each formulation using a 28-gauge needle. Aqueous humor samples (100 μL) were mixed with 100 μl of methanol and kept in a refrigerator for 1 h. The mixture was then centrifuged at 3000 rpm for 15 min and 20 μl of the supernatant, thus obtained, was analyzed for

ketorolac content by HPLC. The remaining supernatant was again mixed with 100 µl of methanol, kept in the refrigerator for 1 h, and centrifuged at 3,000 rpm. With all samples, this repeat exercise resulted in no protein precipitation [Malhotra and Mujumdar, 2005].

HPLC Analysis

Quantitative estimation of ketorolac was done by HPLC. A filtered and degassed mixture of methanol, water, and acetic acid (60:39.9:0.1) was used as the mobile phase. The equipment included the following: Shimadzu UV-Vis detector (SPD-10 Avp); Spinchrome data module integrator; Shimadzu LC-10 ATvp HPLC pump; Luna-Phenomenex C-18, 5 µ reverse-phase column (250 × 4.6 mm) and 7725 i Rheodyne injector fitted with a 20-µl sample loop. The mobile phase was delivered at a flow rate of 1.5 ml/min, single injection volume was 20 µl, and the effluent was monitored at 317 nm. Three solutions of known KT concentration were used as external standards and the 3 standards were run after every 10–15 samples in order to ensure reliable and accurate quantification. An aliquot (1 ml) of a 4-µg ml^{-1} concentration of KT was added to all samples as standard addition [Martinez et al., 2003; Nagaraj et al., 2007].

Data Analysis

The maximum concentration of drug in the aqueous humor (C_{max}) and the time required to reach the maximum concentration (T_{max}) were obtained from the aqueous humor drug concentration versus time curves. The area under the aqueous humor concentration versus time curve (AUC) was calculated by trapezoidal rule. The rate constant (k) of ketorolac was calculated by log linear regression of the last data points (terminal portion) of the aqueous humor concentration versus time curve. The half-life of ketorolac was calculated from the following equation:

$$t_{1/2} = 0.693/\mathrm{K}.$$

Statistical analysis was done by Student's *t*-test. A *P*-value of <0.05 was considered significant.

RESULTS AND DISCUSSION

Drug-Excipient Compatibility Studies

Differential scanning calorimetric characterization

Differential scanning calorimetry (DSC) can be used to investigate and predict any physicochemical interactions between components in a formulation and therefore can be applied to the selection of suitable chemically compatible excipients. In the absence of any interaction, the thermograms of mixtures show patterns corresponding to those of the individual components. In the event that interaction occurs, this is indicated in the thermogram of a mixture by the appearance of one or more new peaks or the disappearance of one or more peaks corresponding to those of the components. Polymorphism is the capability of a substance to crystallize into two or more different crystalline forms. Any polymorphic changes in the drug may change its melting point, bioavailability, and release kinetics. The polymorphic change in the drug, KT, was also studied by using differential scanning calorimetry (DSC) by testing the melting characteristics of the drug in the presence and absence of other additives [Dash et al., 1999].

Figure 1 compares the DSC thermograms of KT, Carbopol® 934, and HPMC (E4M, and E15LV) alone. Ketorolac tromethamine showed a long and sharp characteristic endothermic peak at 170°C due to its phase transition. DSC thermogram of Carbopol® 934 showed endotherms between 50–100°C corresponding to the evaporation of moisture content and a poor resolved change in the base line of Carbopol® 934 at 129–139°C, as previously reported [Kanis et al., 2000]. Additional phase transition at lower temperatures, detected by DSC, may be related to the residual solvents that act as plasticizers but evaporate easily

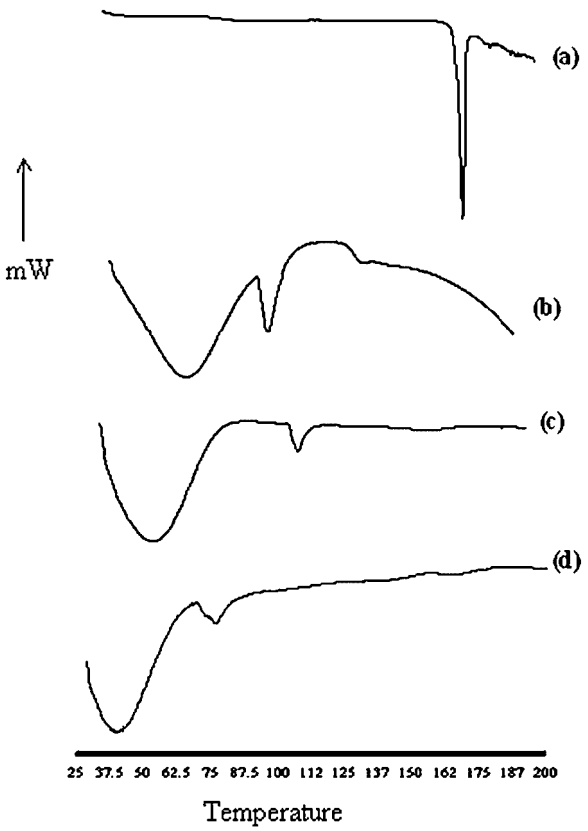

Fig. 1. DSC thermogram of (**a**) Ketorolac tromethamine, (**b**) Carbopol® 934, (**c**) HPMC-E4M, and (**d**) HPMC-E15LV.

when temperature increases; the polymer then showed the characteristic phase transition at 133°C. The occurrence of several phase transitions was also seen in linear poly (acrylic acids) using DSC [Park et al., 1991]. The lower phase transitions were related to the plasticizing effect of the remaining solvent used for synthesis. The remnants of residual solvents, which alter the hydrogen bonding interactions among the carboxylic acids groups, may be responsible for the apparition of a secondary transition at lower temperatures in Carbopol® 934 [Gomez-Carracedo et al., 2004]. DSC thermograms of different HPMC grades, E4M, and E15LV, showed a small and sharp characteristic endothermic peak at 106°C, and 75°C, respectively. Additional phase transitions were also found in HPMC grades at temperature between 40°C and 50°C. These secondary transitions may also be related to the residual solvents, which evaporate easily when temperature increases.

Figure 2 compares the DSC thermograms of KT with physical mixtures of KT with Carbopol® 934 and different grades of HPMC (E4M, and E15LV). The DSC thermograms of physical mixtures showed characteristic endothermic peaks corresponding to those of the individual components and there is no appearance of one or more new peak or disappearance of one or more peak corresponding to those of the individual components. This indicates that no interaction occurs between ketorolac, HPMC (E4M, and E15LV), and Carbopol® 934. Therefore, HPMC and Carbopol® 934 can be used, as excipients, in the formulation of ketorolac in situ gelling systems. There were no additional peaks to demonstrate the different crystalline or amorphous forms of KT or significant changes in the melting characteristics of ketorolac in the presence and absence of other additives, indicating no polymorphic changes in the KT.

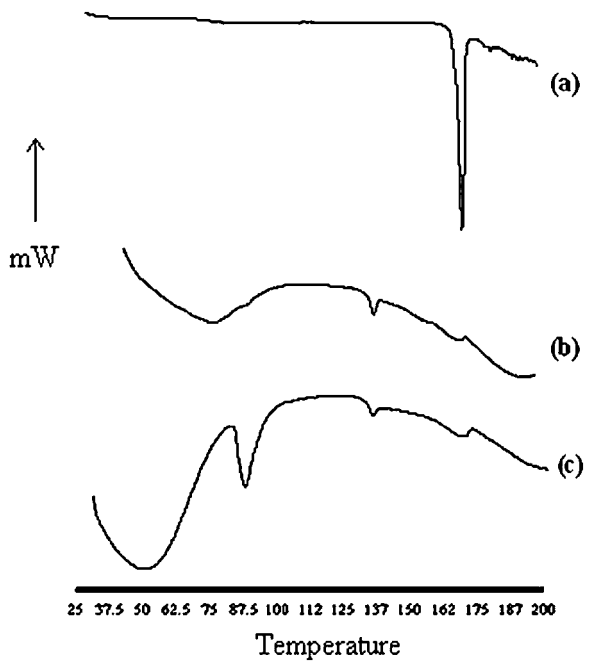

Fig. 2. Comparison of DSC thermogram of (**a**) KT with physical mixture, (**b**) KT+HPMC-E4M+CP, and (**c**) KT+HPMC-E15LV+CP.

Preparation of Formulations

Selection of vehicle

Buffers play a pivotal role in formulating ophthalmic drops contributing significantly to chemical stability and clinical response and also influencing the comfort and safety of the product, hence the importance of selecting a suitable buffer ensures product stability and desired drug solubility. The studies in various buffer solutions indicated the drug was soluble in acetate buffers of pH 5.0, 5.5, and 6.0, citrophosphate buffers of pH 5.0, 6.0, 6.2, and 7.0 and phosphate buffers of pH 5.5, 6.0, 6.5 and 7.2 at the dosage level desired (0.5%, w/v). The solutions were stable to elevated temperatures and autoclaving. However, their instability to light as evidenced by discoloration of the exposed solutions necessitated their packing in amber vials. Citrophosphate buffer, pH 6.0, was selected as a vehicle for the formulated in situ gelling systems as the ketorolac tromethamine precipitates at a pH value of <5.0, at the dosage level desired; it is easily neutralized by the buffering action of the tear fluid.

Preparation of formulations

The use of carbopol (polyacrylic acid, PAA) in in situ gel-forming systems is substantiated by the property of its aqueous solutions to transform into stiff gels when the pH is raised [Schoenwald et al., 1978]. However, the concentration of PAA required to form stiff gels results in highly acidic solutions that are not easily neutralized by the buffering action of the tear fluid. A reduction in PAA concentration without compromising the gelling capacity and rheological properties of the delivery system may be achieved by the addition of viscosity-enhancing polymers such as HPMC.

The two main prerequisites of an in situ gelling system are viscosity and gelling capacity (speed and extent of gelation). The formulation should have an optimum viscosity that will allow easy instillation into the eye as a liquid (drops), which would undergo a rapid sol-to-gel transition (triggered by a rise in pH from 6.0 to 7.4). Additionally, to facilitate sustained release of drug to the ocular tissue, the gel formed in situ should preserve its integrity without dissolving or eroding for a prolonged period of time. Table 1 shows the gelling capacity and viscosity of formulations

F1–F7. A concentration of 1.5% Methocel E15LV and 0.5% Carbopol® 934 (code F 7) was selected as it had satisfactory attributes of viscosity and gelling capacity. To study the effect of benzalkonium chloride (0.01%, w/v), as preservative, alone and in combination with edetate disodium (0.01%, w/v), as permeation enhancer, on the in vivo drug release the final formulations F 7.1 and F 7.2 were selected. The formulae for F 7.1 and F 7.2 are listed in Table 2. Carbopol, being acidic in nature, with a pH of 1% solution, is 2.5–3 [Kumar et al., 2005], lowered the pH of the formulation prepared with citrophosphate buffer B.P. of pH 6.0–5.2. As the KT precipitates at the pH below 5.0 at the dosage level desired, the pH of the final formulation was adjusted to 6.0 with 0.5 M sodium hydroxide solution. The contribution of each ingredient to the osmotic pressure of the formulation was calculated in the concentration used in the terms equivalent to sodium chloride. Since the ingredients themselves contributed to the tonicity, no tonicity adjusting agents were added. BKC (0.01%, w/v) was incorporated as a preservative in both formulation code F 7.1 and F 7.2. Edetate disodium (0.01%, w/v) was incorporated, as permeation enhancer, in formulation code F 7.2 but formulation code F 7.1 is free of permeation enhancer.

Evaluation of formulations

The clarity, pH and drug content of the formulations were found to be satisfactory (Table 3). The formulations were liquid at room temperature and at the pH formulated (pH 6.0) and underwent rapid transition in to the gel phase at the pH of the tear fluid (pH 7.4). Terminal sterilization by autoclaving had no effect on clarity, pH, viscosity, and gelling capacity of F 7.1 and F 7.2. The haziness observed after autoclaving (due to precipitation of HPMC at elevated temperature) was found to disappear, and the original clarity was regained after overnight standing, as previously reported [Srividya et al., 2001].

Rheological Study

Formulations were shear thinning and an increase in shear stress was observed with increase in angular velocity (pseudo-plastic rheology) (Fig. 3a,b). At pH 6.0, the formulations were in a liquid state and exhibited low viscosity. An increase in pH to 7.4 (the pH of the tear fluid) caused the solutions to transform into gels with high viscosity. The administration of ophthalmic preparations should influence as little as possible the pseudo-plastic character of the precorneal tear film [Bothner et al., 1990]. Since the ocular shear rate is very high, ranging from 0.03 s^{-1} during inter-blinking periods to 4250–28,500 s^{-1} during blinking [Kumar and Himmestein, 1995], viscoelastic fluids with a viscosity that is high under low shear rate conditions and low under the high shear rate conditions are often preferred.

TABLE 3. Drug Content, pH, and Clarity of the Formulations; n = 3

Formulation	Drug content (%, w/v)	pH	Clarity
F 7.1	98.83±72	6.08	Clear
F 7.2	99.42±88	6.05	Clear
Marketed eye drop	100.08±52	7.21	Clear

In Vivo Drug Release Studies

The in vitro release studies were completely devoid of complications by variability in precorneal factors such as blinking, lachrymation, tear turnover, and dug washout. The in vitro studies provided relative permeation characteristics of ketorolac from different formulations, but these could not simulate in vivo conditions. It was therefore necessary to study the in vivo ocular absorption

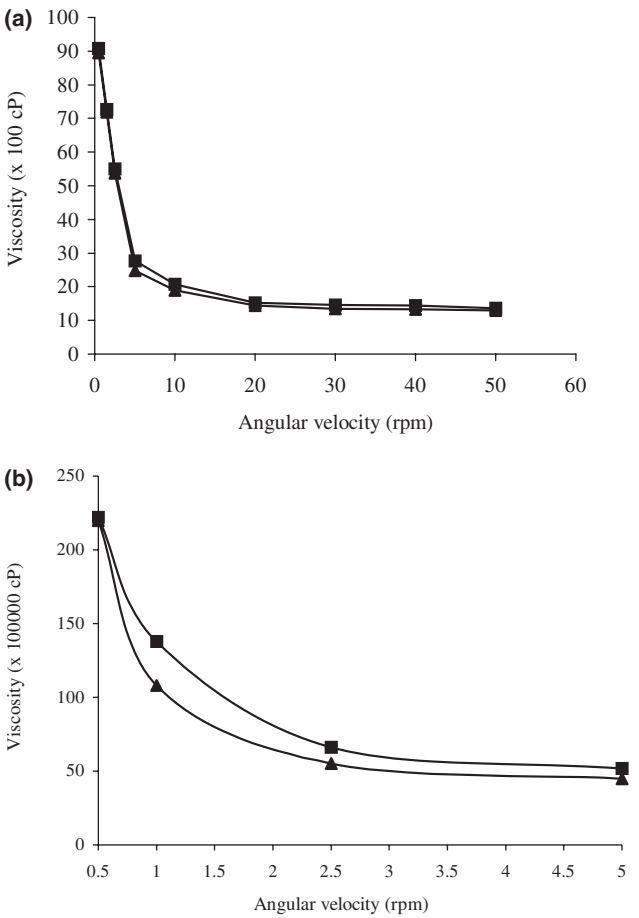

Fig. 3. Rheological profile of pH-triggered in situ gelling systems (**a**) at pH 6.0 and (**b**) at pH 7.4, n = 3. ▲, F 7.1; ■, F 7.2.

TABLE 4. Pharmacokinetic Parameters of Ketorolac (as Free Acid) in Aqueous Humor of Rabbit's Eye

Formulation	C_{max} (μg/ml)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{(0-8\,h)}$ (μg · h/ml)
F 7.1	3.933 ± 0.067	2	1.663 ± 0.122	16.477 ± 0.467
F 7.2	4.100 ± 0.058	1	1.888 ± 0.251	16.577 ± 0.738
Marketed eye drop, 0.5% w/v	1.744 ± 0.048	1	2.566 ± 0.221	3.795 ± 0.662

Values are mean ± SE, n = 6.

of drug from the formulation (Table 4, Fig. 4). On topical instillation of KT in situ gelling formulations F 7.1 and F 7.2 into rabbit eye, the maximum concentration of ketorolac in aqueous humor (C_{max}) was achieved slowly from F 7.1 with BKC with the time to reach maximum aqueous humor concentration of ketorolac (T_{max}) being 2 h. F 7.2, with BKC and EDTA, reduced the T_{max} to 1 h and increased C_{max} (statistically insignificant). Thus BKC and EDTA increased the rate of absorption of the ketorolac into the eye. The AUC obtained with F 7.1 was however smaller but not statistically significant than F 7.2 with BKC and EDTA. Mean concentrations of KT obtained from 0.5 to 8 h for formulations F 7.1 and F 7.2 were not significant ($P < 0.41$).

BKC, a cationic surfactant, increases in vitro permeation of ketorolac (an anionic drug) through rabbit cornea [Fu and Lidgate, 1986] with a mechanism suggested to be: (1) formation of more lipid soluble ion pair, and (2) disruption of corneal epithelium. EDTA, a known calcium-chelating agent, acts on cell junctions by interfering with calcium ions and altering intercellular integrity. EDTA also disrupts plasma membrane and consequently increases intercellular permeability [Grass et al., 1985]. Thus, it seems reasonable to expect that BKC and EDTA combination would also increase in vivo penetration of KT. But formulation F 7.2, with BKC and EDTA, increased the rate of absorption of KT but not the extent. One explanation could be that BKC being a surfactant reduces the interfacial tension between the formulation and corneal epithelium resulting in spreading of the formulation over the cornea. Furthermore, BKC may emulsify corneal epithelium resulting in quicker saturation of epithelium with the drug. Since only the drug present in the epithelium can partition through the stroma and epithelium to aqueous humor, quicker saturation of epithelium could possibly explain faster absorption. But BKC (being a surfactant) can cause ocular irritation [Kennah et al., 1989], as does EDTA. Thus formulation F 7.2 with BKC and EDTA could cause ocular irritation, resulting in increased lachrymation and loss of drug from conjunctival sac leading to reduced extent of absorption of ketorolac. However, further studies are needed to ascertain the fact.

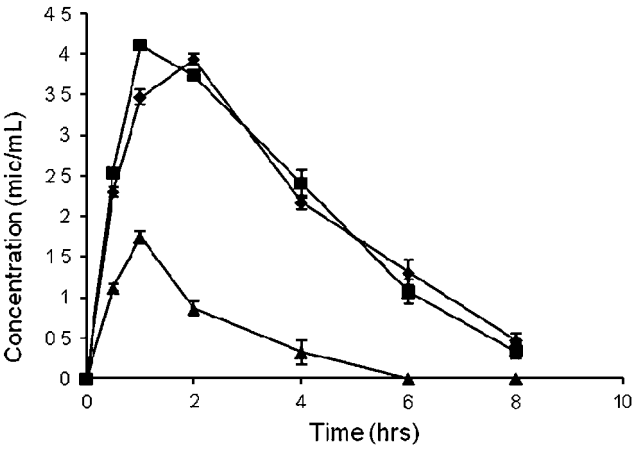

Fig. 4. Ketorolac concentrations in aqueous humor of rabbit's eye. Values are Mean ± SE, n = 6. MF: Marketed formulation of ketorolac tromethamine. ♦, F 7.1; ■, F 7.2; ▲, MF.

In contrast, in the case of marketed eye drops, a sharp increase in aqueous humor drug concentration was observed at 0.5, 1 h. The drug concentration was decreased later at 2 and 4 h. At 6 and 8 h, drug was not detected in aqueous humor from marketed eye drops. Thus, in situ gelling systems can overcome the seesaw pattern of aqueous humor drug concentration level versus time profile of the conventional marketed eye drops. Because of the low ketorolac levels in the aqueous humor samples, the amount of standard addition for these samples was determined with a final concentration within the calibration linear range. Also, the amount of standard addition was kept as low as possible to minimize error prediction for the actual ketorolac levels [Martinez et al., 2003]. Consequently, 1 ml of a 4-μg ml^{-1} concentration of ketorolac was added to all samples as a standard.

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