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Study of an alginate/HPMC-based in situ gelling ophthalmic delivery system for gatifloxacin

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Abstract

The poor bioavailability and therapeutic response exhibited by conventional ophthalmic solutions due to rapid pre-corneal elimination of the drug may be overcome by the use of in situ gel-forming systems that are instilled as drops into the eye and then undergo a sol–gel transition in the cul-de-sac. The present work describes the formulation and evaluation of an ophthalmic delivery system of an antibacterial agent, gatifloxacin, based on the concept of ion-activated in situ gelation. Alginate (Kelton®) was used as the gelling agent in combination with HPMC (Methocel E50Lv) which acted as a viscosity-enhancing agent. The rheological behaviors of all formulations were not affected by the incorporation of gatifloxacin. Both in vitro release studies and in vivo pre-corneal retention studies indicated that the alginate/HPMC solution retained the drug better than the alginate or HPMC E50Lv solutions alone. These results demonstrate that the alginate/HPMC mixture can be used as an in situ gelling vehicle to enhance ocular bioavailability and patient compliance.

Keywords: Ophthalmic delivery system; In situ gelling; Alginate; HPMC; Gatifloxacin

1. Introduction

In ocular delivery, the physiological constraints imposed by the protective mechanisms of the eye lead to low absorption of drugs, resulting in a short duration of the therapeutic effect. When a drug solution is dropped into the eye, the effective tear drainage and blinking action of the eye result in a 10-fold reduction in the drug concentration within 4–20 min (Maurice, 1987). The limited permeability of the cornea contributes to the low absorption of ocular drugs. Due to tear drainage, most of the administered dose passes via the naso-lacrimal duct into the GI tract, leading to side-effects. Rapid elimination of the eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary.

Ocular therapy would be significantly improved if the pre-corneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface, but also to slow down drug elimination (Bourlais et al., 1998; Ding, 1998). Successful results have been obtained with inserts (Ding, 1998) and collagen shields (Hill et al., 1993). However, these preparations have some disadvantages, such as poor compliance, especially by elderly people, and many patients sometimes lose the device without noticing it. From the point of view of patient acceptability, a liquid dosage form is preferable.

This problem can be overcome by using in situ gel-forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol–gel–sol) and pseudo-plastic behavior to minimize interference with blinking (El-Kamel, 2002). Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye which, upon exposure to physiological conditions, changes to the gel phase, thus increasing the pre-corneal residence time of the delivery system and enhancing ocular bioavailability.

Depending on the method employed to produce the sol to gel phase transition on the ocular surface, the following three types of systems have been used: pH-triggered systems including cellulose acetate hydrogen phthalate latex (Gurny, 1981; Gurny et al., 1985), carbopol (Srividya et al., 2001), temperature-dependent systems including pluronics (Miller and Donovan,

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1982; Desai and Blanchard, 1998) and tetronics (Vadnere et al., 1984; Spancake et al., 1989), and ion-activated systems including Gelrite® (Rozier et al., 1989), gellan (Sanzgiri et al., 1993) and carbopol/pluronic (Lin and Sung, 2000). Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, b-D-mannuronic acid (M) and a-L-guluronic acid (G). The polymer forms three-dimensional hydrogel matrices and the high G content alginate forms a low viscosity, free-flowing liquid at concentrations suitable for gel formation in the lacrimal fluid. Alginate transforms into stable gel upon exposure to divalent cations, which is not easily eroded by tear fluid. Therefore, it might be a feasible approach to improve patient compliance to decrease the amount of alginate required for gelation by incorporating HPMC in the formulation. This point was not investigated by other authors (Smadar et al., 1997).

The objective of the present study was to develop an ion-activated in situ gelling system for gatifloxacin, a fourth-generation fluoroquinolone derivative used to treat external infections of the eye, such as acute and subacute conjunctivitis, bacterial keratitis and keratoconjunctivitis, and to enhance patient compliance. Alginate and HPMC were investigated as a vehicle for the formulation of gatifloxacin eye drops (0.3% (w/v)), which undergo gelation when instilled into the cul-de-sac of the eye and provide sustained release of the drug during the treatment of uveitis.

2. Materials and methods

2.1. Materials and animals

2.1.1. Materials

Gatifloxacin was purchased from HuBei Qianjiang Pharmaceutical Manufacture (Qianjiang, China). Sodium alginate (Kelton®, which composed of 60% mannuronic acid and 40% guluronic acid) was kindly gifted by ISP (USA). HPMC (Methocel E15LV & E50LV) was kindly gifted by Colorcon (UK). All other reagents were of analytical grade.

2.1.2. Animals

New Zealand White rabbits, weighing 2.5–3.0 kg, were provided by the Animal Experimental Center of Shenyang Pharmaceutical University. The animals, housed in standard cages in a light-controlled room at $19 \pm 1\,^{\circ}C$ and $50 \pm 5\%$ R.H., were given a standard pellet diet and water ad libitum. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no. 92-93, revised in 1985), and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. The procedures involving animals were reviewed and approved by the Animal Ethical Committee at Shenyang Pharmaceutical University.

2.2. Preparation of formulations

Aqueous solutions of different concentrations of alginate and HPMC of different grades (formulation codes A1, A2, . . ., A9) were prepared and evaluated for gelling capacity 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 freshly prepared and equilibrated at 35 °C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. The composition of artificial tear fluid was sodium chloride 0.670 g, sodium bicarbonate 0.200 g, calcium chloride·$2H_2O$ 0.008 g, and purified water q.s. 100 g (V'Ooteghem, 1993).

Table 1
Combinations of alginate and HPMC studied

Formulation	HPMC grade	Concentration (w/v%)		Gelling capacity
		HPMC	Alginate	
A1	–	–	1	+
A2	–	–	2	++
A3	–	–	3	+++
A4	E15LV	6	1	+
A5	E15LV	8	1	+
A6	E15LV	12	1	+
A7	E50Lv	1	1	+
A8	E50Lv	2	1	++
A9	E50Lv	3	1	+++

Notice: +, gels after a few minutes, dissolved rapidly; ++, gelation immediate, remains for a few hours; +++, gelation immediate, remains for an extended period.

The detailed procedure for preparing the in situ gel-forming system of gatifloxacin is as follows. The alginate solutions were prepared by dispersing the required amount in 75 mL distilled, deionized water with continuous stirring until completely dissolved. The alginate/HPMC solutions were prepared by dispersing the required amount of HPMC in the desired concentration of alginate with continuous stirring until completely dissolved. Gatifloxacin was dissolved in hydrochloric acid and the pH was adjusted to 6.3 using sodium hydroxide. Benzalkonium chloride (BKC) was then added to the above solution. The drug solution was added to the alginate or alginate/HPMC solution under constant stirring until uniform, clear solution was obtained. Distilled, deionized water was then added to make the volume up to 100 mL.

2.3. Evaluation of formulations in vitro

2.3.1. Rheological studies

Viscosity determinations of the prepared formulations (A1, A2, A8 and 2% HPMC E50Lv) were determined using a cone(CC17) and plate viscometer (PAAR PHYSICA MCR300, Germany) on a 5-mL aliquot of the sample. The viscosity of sample solutions was measured at different angular velocities at a temperature of $37 \pm 1\,^{\circ}C$. A typical run involved changing the angular velocity from 1 to 100 rpm at a controlled ramp speed. After 6 s at 1 rpm, the velocity was increased to 100 rpm with a similar period at each speed. The hierarchy of angular velocity was reversed (100–1 rpm) for a similar period of 6 s. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate (Jagdish et al., 2003).

To evaluate the viscosity change after instillation and mixing with artificial tear fluid, rheological measurements were taken after diluting the formulations containing drugs (A1, A2, A8 and 2% HPMC E50Lv) with artificial tear fluid (Vandamme and Brobeck, 2005). The viscosity of sample solutions was measured as described above.

2.3.2. *In vitro release studies of gatifloxacin*

The in vitro release of gatifloxacin from the formulations (gatifloxacin ophthalmic solutions, 2% HPMC E50Lv-solutions, A1, A2 and A8) through a 0.45 μm syringe membrane using a modified USP XXIII dissolution testing apparatus (27 mm i.d. and 5 mm in depth, diffusion surface 5.72 cm^2). The dissolution medium was freshly prepared artificial tear fluid (pH 7.4). A 2-mL volume of the formulation was accurately pipetted into this equipment. The container was attached to the metallic driveshaft and immersed in 500 mL dissolution medium maintained at 35 ± 1 °C with a rotating speed of 50 rpm. Samples, each 10-mL in volume, were withdrawn at hourly intervals and replaced by an equal volume of receptor medium. The release of gatifloxacin was analyzed by UV spectrophotometry at 293 nm.

2.4. *Evaluation of formulations in vivo*

2.4.1. *Pre-corneal retention time*

The in vivo pre-corneal drainage of each formulation was determined after instillation of 100 μL radiolabeled solution onto the left cornea using a gamma camera (Toshiba GCA 602A) adjusted to detect the radiation of ^{99m}Tc(MBp) and fitted with a 4-mm pinhole. The activity instilled ranged from 1 to 2 MBp per 100 μL dose. A small plastic vial containing a 100 μL aliquot of solution to be tested was placed near the eye of the rabbit, and used as a position tracer. After instillation, the eyelids were kept closed for 5 s to prevent loss of the instilled solution. The rabbit was kept on a table without a restraining box, its head being supported by the experimenter's hand with its left eye in front of the collimator aperture at a distance of 6 cm.

Recording was started 5 s after instillation and frames were recorded over a period of 10 min using a 128 × 128 pixel matrix. Each formulation was tested on three rabbits.

Individual 63 frames (36 × 5 s frames followed by 12 × 10 s frames and 15 × 20 s frames) were summed to obtain an overall picture of distribution of the label. The final images was divided into five regions of interest (ROIs), which were, respectively, (1) the background, (2) the position reference, (3) the pre-corneal surface, (4) the inner canthus, and (5) the lachrymal duct.

The parameters calculated were t_{10} (remaining activity on the corneal surface at the end of the study: 10 min), $AUC_{0–10\,min}$ (area under the curve of the percentage activity remaining in the pre-corneal ROI versus time) which represents the residence time of the formulation tested and, $t_{1/2}$ (half-life of elimination).

Results are expressed as mean ± S.D. Student's *t*-test was used to identify differences which were considered to be statistically significantly at $P < 0.05$.

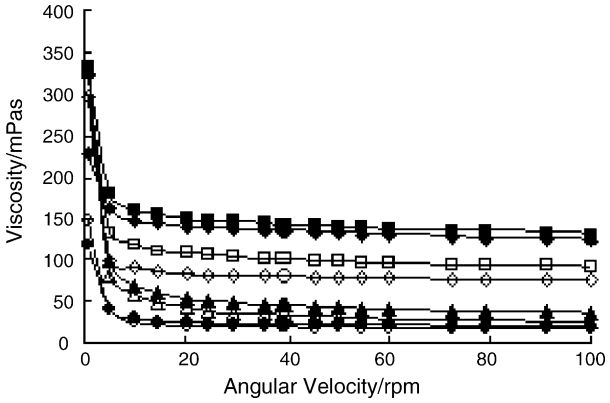

Fig. 1. Rheological profiles of the formulation A1, A2, A8 and 2% HPMC E50Lv with and without gatifloxacin. ▼: formulation A8 with drug; □: formulation A8 without drug; ♦: formulation A2 with drug; ◇: formulation A2 without drug; ▲: formulation A1 with drug; △: formulation A1 without drug; ●: 2% HPMC E50Lv with drug; ○: 2% HPMC E50Lv without drug.

2.4.2. *Ocular irritation studies*

Ocular irritation studies were performed according to the Draize technique (Draize et al., 1944) using 36 New Zealand white rabbits divided into six groups, each weighing 2.5–3.0 kg. The solutions (Blank of 2% HPMC E50Lv, A1 and A8; 2% HPMC E50Lv, A1 and A8) were instilled five times a day for a period of 7 days and the rabbits were observed periodically for ocular redness, swelling, and watering. Evaluation was carried out according to the Draize technique.

3. Results and discussion

3.1. *Rheological studies*

The formulations without drug exhibited pseudo-plastic rheology, as shown by shear thinning and a decrease in the viscosity with increased angular velocity (Fig. 1). The rheological behaviors of all formulations were not affected by addition of gatifloxacin. The order of viscosity of all formulations with or without drug was A8 > A2 > A1 ≈ 2% HPMC E50Lv, respectively.

As shown by Fig. 2, the viscosities of the formulations diluted by artificial tear fluid were significantly increased except for

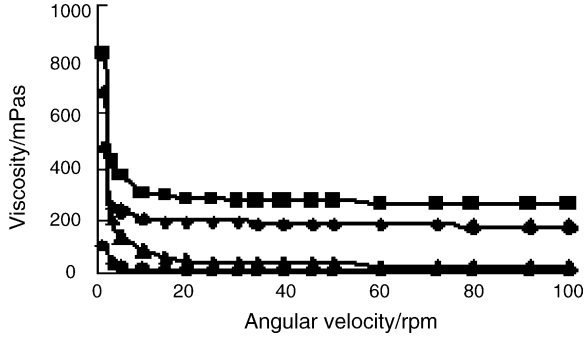

Fig. 2. Rheological profiles of the formulation A1, A2, A8 and 2% HPMC E50Lv with gatifloxacin diluted with artificial tear fluid. ■: formulation A8 with drug; ♦: formulation A2 with drug; ▲: formulation A1 with drug; ●: 2% HPMC E50Lv with drug.

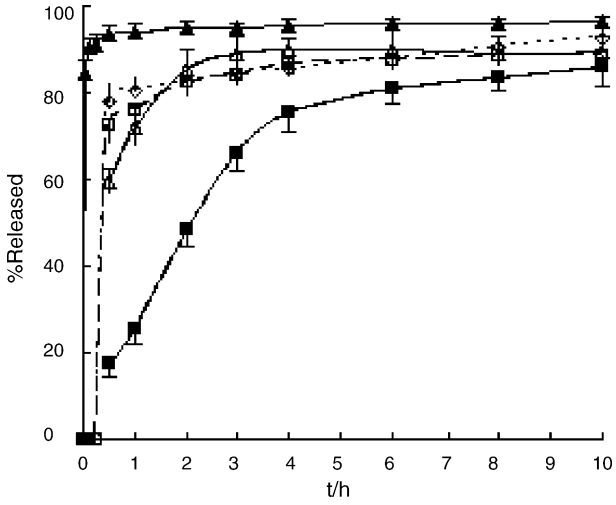

Fig. 3. Cumulative amount of gatifloxacin released as a function of time from formulation A1, A2, A8, 2% HPMC E50Lv and control (gatifloxacin ophthalmic solution). □: formulation A1; △: formulation A2; ■: formulation A8; ◇: 2% HPMC E50Lv; ▲: control.

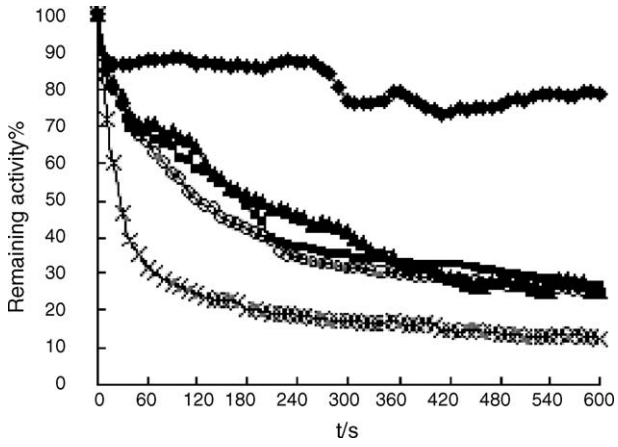

Fig. 4. Pre-corneal drainage of ^{99m}Tc-DTPA in preparations. ■: Formulation A1; ▲: formulation A2; ♦: formulation A8; ◇: 2% HPMC E50Lv; ×: control (gatifloxacin ophthalmic solution).

2% HPMC E50Lv solutions. These results suggest that alginate changed to the gel phase upon exposure to lacrimal fluid.

So HPMC E50Lv as the viscosity-enhancing agent can enhance the viscosity of the preparation and decrease the amount of alginate in the preparation. This might improve patient compliance.

The administration of ophthalmic preparations should have as little effect as possible on the pseudo-plastic character of the pre-corneal 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.

3.2. In vitro release studies

Fig. 3 shows the cumulative amount of gatifloxacin released versus time profiles for different drug-containing solutions. All the solutions contained 0.3% (w/v) gatifloxacin. For ophthalmic solutions, almost all the gatifloxacin released immediately after the start of release study. In the case of formulation A1, approximately 72% of gatifloxacin released from the alginate solution after 0.5 h. The HPMC containing-gatifloxacin solution had a faster release rate than the formulation A1 within 2 h, and then it had a similar release trend as the formulation A1. In formulation A2, about 60% of the drug was released into the medium after 0.5 h, and then the drug was gradually released thereafter. There was a 17.2% of gatifloxacin released from formulation A8 after 0.5 h, approximately 80% after 6 h, and the release profile continued to rise thereafter. These results indicate that formulation A8 (1% alginate/2% HPMC E50Lv) has a better ability to retain drugs than the individual polymer solution (formulation A2). These results also suggest that the alginate/HPMC aqueous system can be used as an in situ gel-forming system for ophthalmic drug delivery systems. Furthermore, by plotting cumulative amount versus the square root of the time curve for formulation A8 (up to 75% of total drug released) a linear relationship with a correlation coefficient higher than 0.99 can be obtained. This observation is in accordance with the carbopol/hydroxypropyl methylcellulose systems reported by Kumar and Himmestein (1995). The linear relationships in conjunction with the slow dissolution rate suggest that the in vitro drug release from formulation A8 under physiological conditions occurs primarily by diffusion.

3.3. Pre-corneal retention time

The curve of the remaining activity on the corneal surface as a function of time (10 min dynamic imagining) is shown in Fig. 4 and the parameters describing the pre-corneal drainage are summarized in Table 2.

Table 2
Clearance half-life ($t_{1/2}$), remaining activity after 10 min (t_{10}) and area under the curve value ($AUC_{0\rightarrow10}$) for formulations A1, A2, A8, 2% HPMC E50Lv and control ($n=3$)

Formulation	$t_{1/2}$ (min)	t_{10} (%)	$AUC_{0\rightarrow10\,min}$ (%)
A8	44.5* ± 22.95	78.66** ± 5.02	820.00** ± 38.50
A2	10.73 ± 6.33	24.49 ± 13.86	447.11** ± 39.05
A1	2.50 ± 1.25	26.2 ± 15.98	382.87** ± 27.99
2% HPMC E50Lv	2.00 ± 0.69	25.89 ± 15.02	322.30 ± 6.98
Control	2.13 ± 0.35	12.56 ± 2.16	229.33 ± 46.76

* $P<0.05$ vs. control (gatifloxacin ophthalmic solution).
** $P<0.01$ vs. control (gatifloxacin ophthalmic solution).

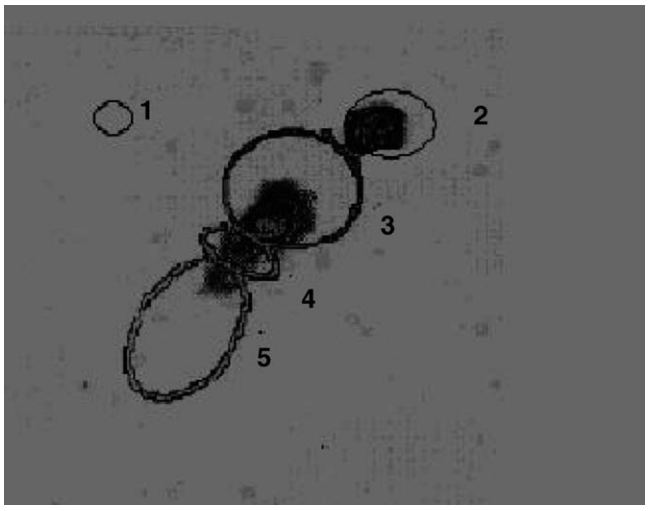

Fig. 5. Typical scintigraphic images divided into five regions of interest (ROIs): (1) the background, (2) the position reference, (3) the pre-corneal surface, (4) the inner canthus, and (5) the lachrymal duct.

As shown by the $AUC_{0\rightarrow 10\,min}$ values, the presence of alginate/HPMC (A8) or alginate (A1 and A2) alone in ophthalmic preparations resulted in a significant increase ($P < 0.01$) in the mean pre-corneal residence time of the formulation on the corneal surface, when compared with an ophthalmic solution (control) (Fig. 5). More precisely, a 2.6-fold improvement was achieved by adding alginate/HPMC (A8) to the control. Similarly, the $t_{1/2}$ values show that elimination of the formulations from the pre-corneal area was delayed in the presence of alginate (A2) or alginate/HPMC (A8), being 4.0- to 19.9-fold greater than the ophthalmic solution. Furthermore, the remaining activity of A8 after 10 min was 78.66%, which was almost 6.3-fold that of the eye drops ($P < 0.01$). However, the presence of 2% HPMC E50lv alone did not increase the $AUC_{0\rightarrow 10\,min}$ and $t_{1/2}$ values in the mean pre-corneal residence time of the formulation on the corneal surface than those of control. These results suggest that the pre-corneal retention activity of A8 is superior to the polymer alone systems (2% HPMC E50Lv, A1 and A2).

3.4. Ocular irritation studies

The results of the ocular irritation studies (Table 3) indicate that all formulations are non-irritant. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris or conjunctivae were visible.

Table 3
Ocular irritation test ($n = 6$)

Preparation	Average score
Blank of 2% HPMC E50Lv	0
2% HPMC E50Lv	0
Blank of A1	0
A1	0
Blank of A8	0
A8	0

4. Conclusion

Gatifloxacin, a broad-spectrum antibacterial agent used in the treatment of ocular infections, was successfully formulated as ion-activated in situ gel-forming ophthalmic solutions (0.3% (w/v)) using alginate (Kelton®) as a gelling agent in combination with HPMC E50Lv as a viscosity-enhancing agent. The formulation underwent gelation in the cul-de-sac upon instillation as drops into the eye. The gel formed in vitro produced sustained drug release over an 8-h period. This new formulation is a viable alternative to conventional eye drops by virtue of its ability to enhance bioavailability through its sustained drug release and longer pre-corneal residence time. Also important is its ease of administration and reduced frequency of administration resulting in better patient acceptance.

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