

# A novel in situ gel base of deacetylase gellan gum for sustained ophthalmic drug delivery of ketotifen: in vitro and in vivo evaluation

Lina Zhu<sup>1</sup>  
Junping Ao<sup>2</sup>  
Peiling Li<sup>1</sup>

<sup>1</sup>Department of Ophthalmology,

<sup>2</sup>State Key Laboratory of Oncogenes and Related Genes, Shanghai Cancer Institute, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, People's Republic of China

**Abstract:** In this study, an ion-activated ketotifen ophthalmic delivery system was developed by using a natural polysaccharide, deacetylase gellan gum. Its rheological characteristics, stability, in vitro gelation, release in vitro, and pharmacodynamic activity in vivo were investigated. The formulation had an optimum viscosity that will allow easy drop as a liquid, which then underwent a rapid sol–gel transition due to ionic interaction. There were negligible alterations in the initial values of viscosity of the formulations over a storage period of 180 days. The in vitro release profiles indicated that the release of ketotifen from in situ gels exhibited a sustained feature. Scintigraphic studies indicated that deacetylase gellan gum could increase the residence time of the formulation. At the same dose, in situ gels demonstrated a typical sustained and prolonged drug-effects behavior compared with the common drops.

**Keywords:** ophthalmic delivery, deacetylase gellan gum, sol–gel transition, scintigraphic studies, pharmacodynamic

## Introduction

In conventional ophthalmic delivery system, liquor solution was widely used because of the simple preparation process, accurate dosage, easiness to use, and high medication compliance among patients.<sup>1</sup> However, due to the tear secretion and blinking, liquor solution had very short residence time (~5 minutes) on the surface of the cornea. Once the liquid entered the corneal capsule, it was immediately diluted or caused corneal erosion and increased the need for frequent drug times to improve efficacy.<sup>2</sup> Although some bio-adhesive formulations such as ocular gel were able to extend the residence time of the drug in the conjunctiva of the eye, they had higher viscosity, inaccurate dosage, and higher risk of adverse reactions. Meanwhile, they may severely limit the development of the dosage form.<sup>3,4</sup> Therefore, it is important to improve the efficacy of ophthalmic preparations by selecting suitable polymeric materials as a thickener and for an anti-tear clearance of drugs.

Ketotifen fumarate (KF) is a drug for histamine H1 receptor antagonist and mast cells. It can inhibit type I allergic or immediate-type allergy-related cells (mast cells, eosinophils, basophils, and neutrophil cells). The release of inflammatory mediators can also inhibit the chemotaxis, activation, and degranulation of eosinophils.<sup>5</sup> Clinically, KF ophthalmic solution was mainly used for the treatment of seasonal allergic conjunctivitis following a dosage of one to two drops per time and four times a day (during the morning, noon, night, and before sleep). However, the discontinuity of drug use by many clinical patients often affected the final effects.<sup>6,7</sup> Therefore, our

Correspondence: Peiling Li  
Department of Ophthalmology, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, 160 Pu Jian Road, Shanghai 200127, People's Republic of China  
Tel/fax +86 21 5875 2345  
Email lipeiling2014@yeah.net

group intended to develop a suitable ophthalmic preparation to improve the efficacy of KF.

In situ gel is a polymer solution that changes in accordance with temperature of the applied place,<sup>8,9</sup> ionic strength,<sup>10</sup> or pH<sup>11</sup> and forms a viscoelastic semisolid gel system. The formation mechanism is to use polymer materials in response to external stimuli so that the polymer undergoes reversible changes in the dispersion state or conformation under physiological conditions, and then completes the transformation from solution to gel. During the preparation, gelation phenomenon occurs under appropriate physiological conditions and at the medication area, leading to extended residence time of the drug and improved bioavailability. Combining advantages of liquid and gel, the gel has a wide-ranging application prospect in drug delivery field, for instance, in ophthalmic administration, oral administration, implants, and nasal system. In recent years, the in situ gel delivery system has attracted great attention from scholars and become one of the focus areas of pharmacy.

In situ gel can be divided into three types according to its phase change performance: temperature sensitive, pH sensitive, and ionic strength sensitive. Temperature-sensitive materials mainly include block copolymer and poloxamer.<sup>12</sup> pH-sensitive materials include cellulose acetate phthalate and acrylic acid polymers, which, by changing the pH value of the environment, can facilitate phase transition.<sup>13</sup> Among ion-sensitive materials, sodium alginate is the most commonly used.<sup>14</sup> But in recent years, deacetylase gellan gum (DGG) starts to be considered more frequently.

DGG is an extracellular polysaccharide, produced from *Pseudomonas elodea*, a Gram-negative bacterium separated from water lily. It has a parallel double-helix structure, and the whole gel chain is formed by four basic units via repeat polymerization; when formulated into solution with a certain concentration, it exhibits the feature of cation-induced gelation.<sup>15</sup> Because of this unique nature, it has been used for oral,<sup>16,17</sup> ocular,<sup>18–20</sup> and nasal delivery system.<sup>21,22</sup> But there is no report about KF in ophthalmic drug delivery systems.

In this study, an ion-activated ketotifen ophthalmic delivery system was developed by using a natural polysaccharide, DGG. Its rheological characteristics, stability, in vitro gelation, release in vitro, and pharmacodynamic activity in vivo were investigated.

## Materials and methods

### Materials

KF was given by Xingyin Chemical Ltd. (Hubei, People's Republic of China; patch number: 10209). KF eye drops

(0.5 mg/mL) was purchased from Jiminkexin Group Co., Ltd. (Wuxi, People's Republic of China; patch number: 090515). DGG was obtained from Kelco Co., Ltd. (Atlanta, Georgia) (molecular weight 500 kDa, 95% deacetylation degree; USA; patch number: 20050929). Egg albumin (EA) was purchased from Sigma Aldrich Co., Ltd (Shanghai, People's Republic of China). Artificial tears (AT) including NaCl 6.78 g, CaCl<sub>2</sub>·2H<sub>2</sub>O 0.084 g, KCl 1.38 g, and NaHCO<sub>3</sub> 2.18 g in 1,000 mL of purified water was prepared according to previous report.<sup>23</sup> Purified water from Milli-Q system (Millipore, Bedford, MA, USA) was used throughout the experiment. All other reagents were of commercially analytical grade.

### Animal

This study was approved by an ethics committee of Shanghai Renji Hospital. Both male and female white rabbits from New Zealand were used in this study, each weighing approximately 2.5±0.5 kg. They were provided by the Experimental Animal Center of Shanghai Institute of Materia Medica of Chinese Academy of Sciences. Male Wistar rats weighing approximately 220±20 g were provided by Department of Laboratory Animal of Jiaotong University Medical School. All animals were housed in standard animal cages at a constant temperature of 22°C±2°C and a relative humidity of 50%±5%, with standard diet and access to water.

### Preparation of in situ gel

Briefly, a certain amount of DGG (0.25%, 0.6%, 1.25%; w/v) was added to deionized water and dissolved by heating to 60°C with moderate stirring. Once completely dissolved, the solution was cooled to a temperature below 30°C. Then, KF (0.05%, w/v), chlorhexidine acetate (0.01%, w/v), and glycerol (2%, w/v) were slowly added to the system and mixed well for at least 30 minutes. The pH of the formulation was between 6.0 and 8.0. In this formulation, DGG served as a gel base, and chlorhexidine acetate, a preservative.

### In vitro gelation and rheological characteristics

The three types of in situ gels prepared were mixed with AT at the ratio of 25:7, and the temperature was maintained at 34°C±0.5°C, simulating a real environment in vivo. Then, the viscosity of the different DGG formulations, either in solution or in gel made with AT, was determined with a rotational viscometer using a proper sample. Measurements were performed using suitable spindle number at different rotation rates. The viscosity was read directly from the viscometer display. All measurements were made in triplicate.

## HPLC analysis

High-performance liquid chromatography (HPLC)-variable wavelength detector was used in the determination of KF in vitro samples, and specific chromatographic conditions were as follows: column: Kromasil C18 column (150 mm×4.6 mm, 5  $\mu$ m); mobile phase: methanol and water (1,000 mL, 65:35, v/v), where triethylamine of 0.35 mL was added; column temperature: 30°C; flow rate: 1 mL/min; detection wavelength: 300 nm; and injection volume: 20  $\mu$ L.

## Stability studies

The proposal of stability study was modified mainly based on the guiding principles of the Chinese Pharmacopoeia. KF in situ gel (0.6% DGG) was placed in three stable boxes kept at 4°C, room temperature, and 40°C, and saturated sodium chloride solution was added and maintained at a relative humidity of 75%±5%. Then, at 0 day, 30 days, 60 days, 90 days, and 180 days of the test, they were observed to find if the gel content, appearance, gelation, and viscosity were changed or not.

## In vitro release behavior

### Preparation of release media

In order to maintain the biological activity of cornea in vitro, this study used glutathione media buffer for release. This buffer consisted of two parts: 1) NaCl 12.4 g/L, KCl 0.716 g/L, NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O 0.206 g/L, and NaHCO<sub>3</sub> 4.908 g/L; and 2) CaCl<sub>2</sub>·2H<sub>2</sub>O 0.23 g/L, MgCl<sub>2</sub>·6H<sub>2</sub>O 0.318 g/L, glucose 1.8 g/L, and glutathione (oxidized form) 0.184 g/L.<sup>23</sup> Solutions of the two parts are placed at 4°C in a refrigerator and equally mixed before use.

### Preparation of in vitro cornea

Air was injected into the ear vein of a number of New Zealand white rabbits, causing their death. Thirty minutes after their death, their corneas were separated using ophthalmic surgical blades and tweezers. The sclera, iris, ciliary body, and other excess tissues were removed and washed with distilled water for several times, and water attached to the stand was sucked using filter paper.

### In vitro drug release

The studies on the in vitro release of KF from in situ gels were carried out using the Franz diffusion cell method. The stirring speed was set at 50 rpm, and the temperature was maintained at 37°C±0.5°C. Aliquots of 200  $\mu$ L of KF in situ gels (or 200  $\mu$ L of KF eye drops) were directly placed into cornea and then mixed with 56  $\mu$ L of AT. Samples of 0.2 mL

were extracted from the tubes at 0.25 hour, 0.5 hour, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, 12 hours, and 24 hours after centrifugation at 4,000 rpm for 5 minutes. Then, the KF was analyzed by HPLC. The accumulative release was calculated, and the results were shown as mean ± SD (n=3).

## Scintigraphic studies

The ocular mucosal residence time of in situ gels was investigated using a modified scintigraphic method.<sup>24</sup> Briefly, 0.6% DGG KF in situ gels (prepared as described earlier) was incorporated with <sup>99m</sup>Tc-diethylene triamine pentaacetic acid (DTPA). A solution of <sup>99m</sup>Tc-DTPA dissolved in phosphate-buffered saline [PBS] was used as control. Then, six rabbits were divided into two groups, each receiving gels and solution formulations in a crossover design with at least 1 week of washout period. The rabbit was positioned 10 cm in front of the probe and 100  $\mu$ L of the radiolabeled gels or solutions, which had been stored at 20°C for 30 minutes before use, was instilled into the ocular mucosal surface. Single-photon emission computed tomography (ZLC 3700, Munich, Germany) was autotuned to detect the 140 keV radioactivity of <sup>99m</sup>Tc-DTPA. Recording started 2 seconds after administration and continued for 60 minutes using a 156×156 pixel matrix. A total of 84 frames of dynamic images were recorded and analyzed by medical system ICONP workstation (Siemens, Munich, Germany).

## Pharmacodynamic activity

### Immunization and challenge

All rats were injected intraperitoneally with 100  $\mu$ g of EA plus 20 mg of alum in 1 mL of PBS (pH 7.4) for immunization. Fourteen days later, rats were challenged topically with 10  $\mu$ L of 10% EA in PBS. To obtain a remarkable ocular reaction, 20  $\mu$ L of 1 mol/L dl-dithiothreitol and a mucolytic agent in PBS (freshly prepared before use) were applied to each eye 15 minutes before topical challenge as described by Calonge et al.<sup>25</sup> Evans Blue (EB; 2 mg/100 g) in 1 mL of PBS was intravenously injected immediately prior to challenge. Animals were anesthetized with intraperitoneal 5% chloral hydrate (approximately 0.2 mL/100 g).

## Drug administration

Animals were divided into two groups (gels and solutions), 30 in each. One eye of each animal received one drop of relevant drug (0.1% KF formulation), and the fellow eye was untreated as control. All rats were killed by exsanguination 1 hour after challenge. Globes and adnexa were taken at 5 minutes, 10 minutes, 15 minutes, 30 minutes, 45 minutes,

and 60 minutes after ocular administration. Then, the tissues were exenterated, weighed, immersed in an extracting solution of 5 mL of sodium sulfate (0.5%) plus acetone (3:7, v/v), shaken strongly, and kept at room temperature. Twenty-four hours later, solutions were centrifuged at 1,000 rpm for 10 minutes. The color intensity of supernatant was detected spectrophotometrically at 620 nm.

Standard curve was generated to transform absorbance unit in microgram of EB per milliliter of solution, and EB extravasation in ocular tissues was calculated. Drug efficacy was expressed as percent inhibition of the reaction on the vehicle-treated eye. In the equation,  $IR (\%) = (UCE - DTE) / UCE$ , where IR is the drug inhibitory rate, DTE is EB content in drug-treated eye, and UCE, in the untreated control eye. A *P*-value of  $\leq 0.05$  was considered significant.

## Statistical analysis

Results were expressed as the mean value  $\pm$  standard deviation. Statistical analysis was evaluated by two-tailed Student's *t*-test or by two-way analysis of variance followed by Dunnett's *t*-test, with a level of significance chosen at  $P < 0.05$ .

## Results and discussion

### In vitro gelation

To gain a better understanding of the existence of gel, hydrogel formation in vitro was observed. The two main prerequisites of in situ gelling systems are optimum viscosity and gelling capacity. The formulation should have an optimum viscosity that will allow easy drop as a liquid, which then undergoes a rapid sol–gel transition due to ionic interaction. Figure 1 shows the prepared gels formed under the conditions of AT. Because of the microscale of ions required for gel formation, rapid gelation in vivo can be expected. In the selection of the concentration of the gelling polymer, a compromise is sought between a satisfactory gel strength

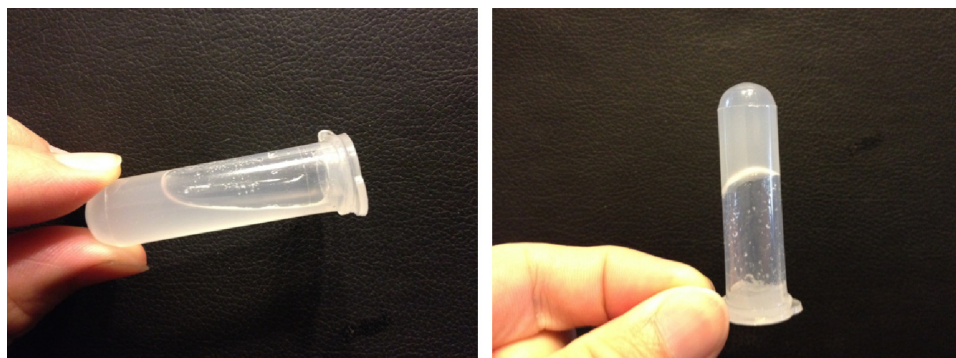
for use as a delivery vehicle and an acceptable viscosity for easiness of spraying. As shown in Figure 2, three different DGG formulations demonstrated pseudoplastic behavior either in solution or in gel. The viscosity of the tested gels grew with the increase in DGG concentrations, and a great viscosity change was found when DGG underwent sol–gel transition at lower concentrations (0.25% and 0.6%). By contrast, with a relatively high original viscous solution obtained with 1.25% DGG, viscosity change observed after gel formation was slight. It was proposed that as the concentration of DGG increased, the polymer chains approached closer and the number of interactions between the polymer chains increased, leading to a denser three-dimensional network structure.

### Stability studies

There were negligible alterations in the initial values of viscosity of the formulations over a storage period of 180 days. The samples were also analyzed for drug content by the HPLC method. Again, the drug degraded to a negligible extent, and the percentage of drug degradation is  $< 5\%$ . Many factors affected the stability of a pharmaceutical product, including the stability of the active ingredient(s), and the potential interaction between active and inactive ingredients. To calculate the shelf life of the formulation, extensive stability data are collected according to the International Conference on Harmonization guidelines. Because the overall degradation is  $< 5\%$ , a tentative shelf life of 2 years may be assigned to the optimized formulation.

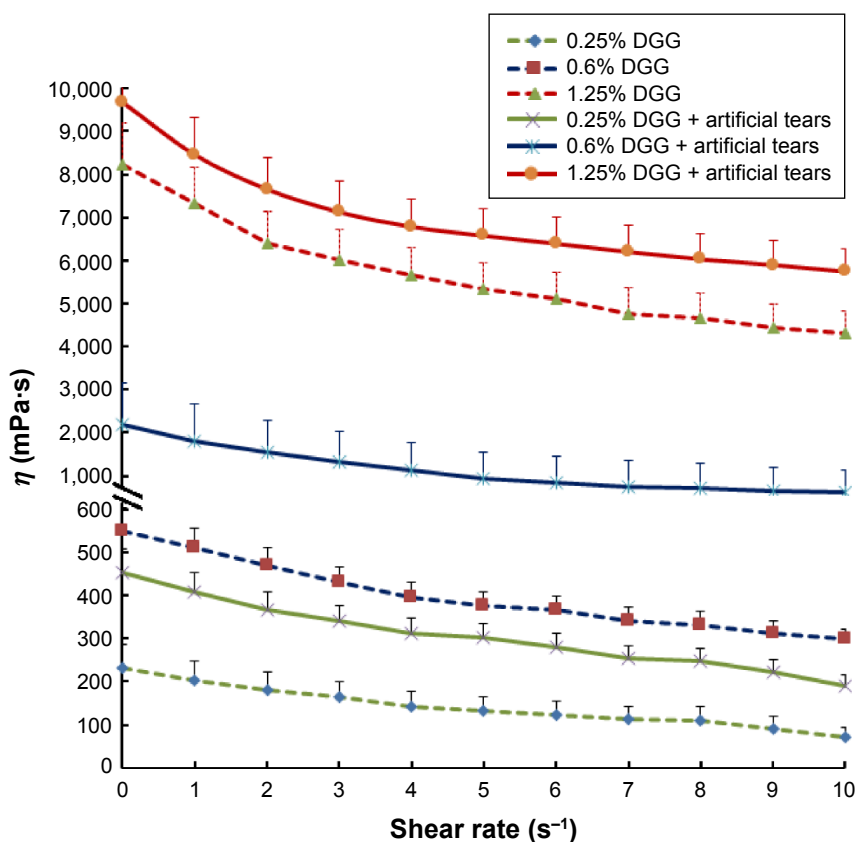
### In vitro release

In vitro release of KF from different formulations was studied using Franz diffusion cell method at  $37^\circ\text{C}$ . The plot of drug release percentage versus time was drawn to exhibit the release profile (Figure 3). As described in Figure 3,  $> 50\%$  of KF was dissolved in release medium from KF eye drop



**Figure 1** In vitro hydrogel formation with in situ gels (0.6% DGG, w/v) and artificial tears (25:7, v/v).  
**Abbreviation:** DGG, deacetylase gellan gum.

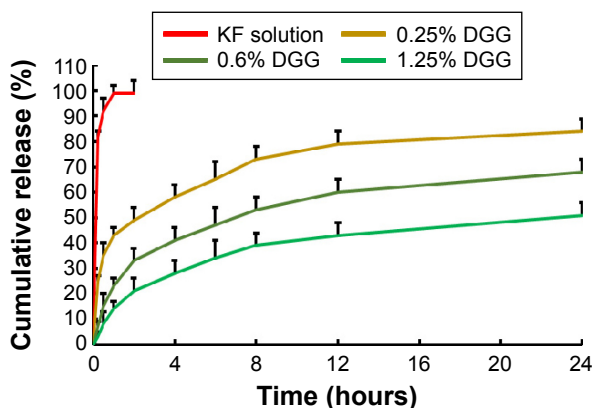




**Figure 2** Viscosity of the various DGG solutions and of the DGG preparation with artificial tears, simulating the in vivo gelation (n=3).

**Abbreviation:** DGG, deacetylase gellan gum.

within 30 minutes, and nearly 100% of drug was released after 2 hours. By contrast, KF in situ gels presented a sustained-release manner in glutathione media buffer. After incubated for 24 hours, approximately 78.4%, 66.2%, and 57% KF release from 0.25%, 0.6%, and 1.25% in situ gels were found, respectively. The in vitro release profiles indicated that KF was released from in situ gels in a sustained fashion. The phenomenon could be related to the fact that



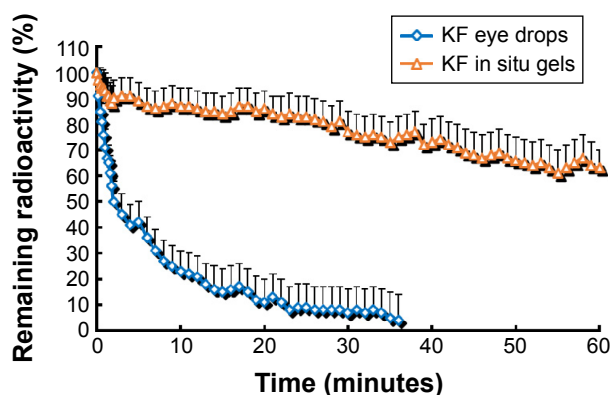
**Figure 3** Drug release profiles of different KF formulations (n=3).

**Abbreviations:** KF, ketotifen fumarate; DGG, deacetylase gellan gum.

DGG underwent a rapid sol–gel transition when exposed to AT, which was also confirmed by the viscosity experiment. During the hydrogel formation, a portion of KF might be loaded into the hydrogel phase to slow down drug release. In addition, the release rate and DGG concentration were shown to be negatively correlated. At fixed drug concentrations, the higher the DGG concentration was, the lower the rate of drug release was. The release from various DGG formulations could be ranked as follows: 0.25% > 0.6% > 1.25%. The in vitro release data were kinetically analyzed according to zero-order, first-order, and the diffusion-controlled release mechanism. The relatively high correlation coefficient values obtained from the analysis of the amount of drug released versus the square root of time indicated that the release followed the Higuchi kinetic model ( $r=0.9993$ – $0.9997$ ). Overall, opposite to the fast drug release in control group, KF in situ gels performed a controlled-release character which was beneficial to persistent ocular therapy.

## Scintigraphic studies

Gamma scintigraphy is a technique whereby the transit of a dosage form through its intended site of delivery can be



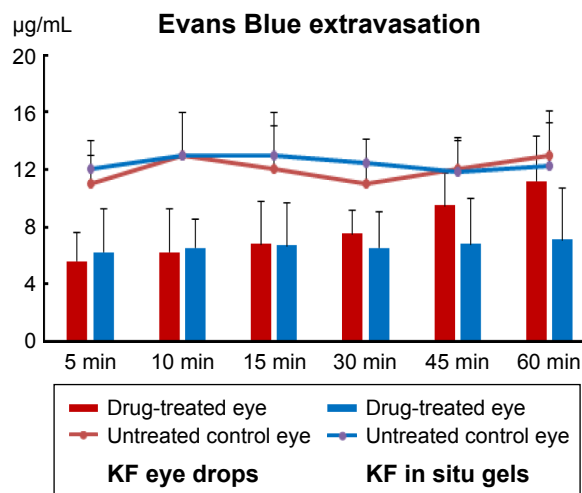
**Figure 4** Corneal surface clearance of two formulations incorporating  $^{99m}\text{Tc}$ -DTPA. **Abbreviations:** DTPA, diethylene triamine pentaacetic acid; KF, ketotifen fumarate.

noninvasively imaged in vivo via the judicious introduction of an appropriate short-lived gamma-emitting radioisotope. It was selected as it emits low-energy gamma rays which do not lead to serious health hazards. The observation of the acquired gamma camera images showed that for developed in situ gelling system, the formulation distributed more evenly over the entire precorneal area immediately after administration without spillage when compared with eye drop formulation.

The curve of the remaining activity on the corneal surface as a function of time (time–activity curve) was generated as shown in Figure 4. In the group of gels, there was approximately 80% of the gel deposited in the rabbit corneal surface 20 minutes after administration. More than 63% was in contact with the corneal surface after 60 minutes; however, it was only approximately 5% with the applied reference eye drops (at 35 minutes). These results indicated that DGG could increase the residence time of the formulation, which was further demonstrated by the pharmacodynamics.

## Pharmacodynamic activity

Rat models are used for studies on allergic conjunctivitis because they closely resemble human physiology and their ocular vascular permeability is comparable with the human counterpart.<sup>24</sup> KF not only blocks H1 receptors and



**Figure 5** Evans Blue extravasation in ocular tissues of two KF formulations (n=5). **Abbreviations:** KF, ketotifen fumarate; min, minutes.

stabilizes mast cells but also prevents the recruitment of eosinophils, which play a central role in the more chronic and severe forms of allergic inflammation processes, such as vernal keratoconjunctivitis and atopic keratoconjunctivitis. The multiple mechanisms of action of ketotifen would be expected to provide rapid and prolonged relief of symptoms in a patient.

The mean pharmacodynamic profiles of KF after ocular administration of eye drop and in situ gels are presented in Figure 5. As shown in Figure 5, the basal data of EB extravasation in ocular tissues were 10–12  $\mu\text{g/mL}\cdot\text{g}$  (untreated control eye). KF significantly decreased the EB extravasation in ocular tissues of both KF formulations. However, although the dose of KF eye drops and in situ gel was the same, the recovery of EB extravasation was obviously different (especially at 45 minutes and 60 minutes), suggesting that a better effect might be expected of the gel group. The inhibition rates of the drugs on EB leakage in eyes are shown in Table 1. During the observation period, the inhibition rates of the eye drop group slowly decreased to approximately 13% by the end of the observation (60 minutes). In contrast, those of in situ gels only reached 41.8% after 60 minutes and demonstrated a typical sustained and prolonged drug-effects behavior.

**Table 1** Inhibition rates of the drugs on Evans Blue leakage in eyes (n=10)

	Inhibition rates of the drugs (%)					
	5 minutes	10 minutes	15 minutes	30 minutes	45 minutes	60 minutes
KF eye drops	49.09±11.32	52.31±15.21	43.33±13.26	31.82±11.09	20.38±10.12	13.85±5.43
KF in situ gels	48.33±12.56	50.91±13.73	48.46±15.31	48.00±12.89*	42.37±13.29*	41.80±14.19*

**Note:** \* $P<0.05$ , KF in situ gels versus KF eye drops. Data are presented as mean  $\pm$  standard deviation.

**Abbreviation:** KF, ketotifen fumarate.

## Conclusion

In the present study, an ophthalmic delivery system of ion-activated in situ gel for ketotifen with DGG was developed, and its rheological characteristics, stability, in vitro gelation, release in vitro, and pharmacodynamic activity in vivo were investigated. The formulation had an optimum viscosity that will allow easy drop as a liquid, which then underwent a rapid sol–gel transition due to ionic interaction. There were negligible alterations in the initial values of viscosity of the formulations over a storage period of 180 days. In vitro release showed that the release of ketotifen from in situ gels was moderate without any burst effects. Scintigraphic studies indicated that DGG could increase the residence time of the formulation. At the same dose, in situ gels demonstrated a typical sustained and prolonged drug-effects behavior than the common drops.

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Ali J, Fazil M, Qumbar M, Khan N, Ali A. Colloidal drug delivery system: amplify the ocular delivery. *Drug Deliv*. 2014;1–17.
2. Achouri D, Alhanout K, Piccerelle P, Andrieu V. Recent advances in ocular drug delivery. *Drug Dev Ind Pharm*. 2013;39(11):1599–1617.
3. Chou TY, Hong BY. Ganciclovir ophthalmic gel 0.15% for the treatment of acute herpetic keratitis: background, effectiveness, tolerability, safety, and future applications. *Ther Clin Risk Manag*. 2014;10:665–681.
4. Liu B, Ding L, Xu X, Lin H, Sun C, You L. Ocular and systemic pharmacokinetics of lidocaine hydrochloride ophthalmic gel in rabbits after topical ocular administration. *Eur J Drug Metab Pharmacokinet*. 2014. Epub ahead of print.
5. Mortemousque B, Bourcier T, Khairallah M, et al; Ketotifen Study Group. Comparison of preservative-free ketotifen fumarate and preserved olopatadine hydrochloride eye drops in the treatment of moderate to severe seasonal allergic conjunctivitis. *J Fr Ophthalmol*. 2014;37(1):1–8.
6. Uchio E. Treatment of allergic conjunctivitis with olopatadine hydrochloride eye drops. *Clin Ophthalmol*. 2008;2(3):525–531.
7. Lee JS, Lee JE, Kim N, Oum BS. Comparison of the conjunctival toxicity of topical ocular antiallergic agents. *J Ocul Pharmacol Ther*. 2008;24(6):557–562.
8. Ruan H, Yu Y, Liu Y, Ding X, Guo X, Jiang Q. Preparation and characteristics of thermoresponsive gel of minocycline hydrochloride and evaluation of its effect on experimental periodontitis models. *Drug Deliv*. 2014;1–7.
9. Mishra GP, Kinser R, Wierzbicki IH, Alany RG, Alani AW. In situ gelling polyvalerolactone-based thermosensitive hydrogel for sustained drug delivery. *Eur J Pharm Biopharm*. 2014. Epub ahead of print.
10. Gupta H, Velpandian T, Jain S. Ion- and pH-activated novel in-situ gel system for sustained ocular drug delivery. *J Drug Target*. 2010;18(7):499–505.
11. Kassem AA, Ismail FA, Naggar VF, Aboulmagd E. Comparative study to investigate the effect of meloxicam or minocycline HCl in situ gel system on local treatment of periodontal pockets. *AAPS Pharm Sci Tech*. 2014;15(4):1021–1102.
12. Matanović MR, Kristl J, Grabnar PA. Thermoresponsive polymers: insights into decisive hydrogel characteristics, mechanisms of gelation, and promising biomedical applications. *Int J Pharm*. 2014;472(1–2):262–275.
13. Singh NK, Lee DS. In situ gelling pH- and temperature-sensitive biodegradable block copolymer hydrogels for drug delivery. *J Control Release*. 2014;193:214–227.
14. Sharma A, Sharma J, Kaur R, Saini V. Development and characterization of in situ oral gel of spiramycin. *Biomed Res Int*. 2014;2014:876182.
15. Chanrasekaran R, Thailambal VG. The influence of calcium ions, acetate and l-glycerate groups on the gellan double helix. *Carbohydr Polym*. 1990;12:431–432.
16. Mahdi MH, Conway BR, Smith AM. Evaluation of gellan gum fluid gels as modified release oral liquids. *Int J Pharm*. 2014;475(1–2):335–343.
17. Xu H, Shi M, Liu Y, Jiang J, Ma T. A novel in situ gel formulation of ranitidine for oral sustained delivery. *Biomol Ther (Seoul)*. 2014;22(2):161–165.
18. Chen Q, Zheng Y, Li Y, et al. The effect of deacetylated gellan gum on aesculin distribution in the posterior segment of the eye after topical administration. *Drug Deliv*. 2012;19(4):194–201.
19. Geethalakshmi A, Karki R, Jha SK, Venkatesh DP, Nikunj B. Sustained ocular delivery of brimonidine tartrate using ion activated in situ gelling system. *Curr Drug Deliv*. 2012;9(2):197–204.
20. Rupenthal ID, Alany RG, Green CR. Ion-activated in situ gelling systems for antisense oligodeoxynucleotide delivery to the ocular surface. *Mol Pharm*. 2011;8(6):2282–2290.
21. Galgatte UC, Kumbhar AB, Chaudhari PD. Development of in situ gel for nasal delivery: design, optimization, in vitro and in vivo evaluation. *Drug Deliv*. 2014;21(1):62–73.
22. Cao SL, Ren XW, Zhang QZ, et al. In situ gel based on gellan gum as new carrier for nasal administration of mometasone furoate. *Int J Pharm*. 2009;365(1–2):109–115.
23. Moshirfar M, Pierson K, Hanamaikai K, Santiago-Caban L, Muthappan V, Passi SF. Artificial tears potpourri: a literature review. *Clin Ophthalmol*. 2014;8:1419–1433.
24. Cao SL, Zhang QZ, Jiang XG. Preparation of ion-activated in situ gel systems of scopolamine hydrobromide and evaluation of its antinotion sickness efficacy. *Acta Pharmacol Sin*. 2007;28(4):584–590.
25. Calonge MC, Pastor JC, Herreras JM, González JL. Pharmacologic modulation of vascular permeability in ocular allergy in the rat. *Invest Ophthalmol Vis Sci*. 1990;31(1):176–180.

### Drug Design, Development and Therapy

### Publish your work in this journal

Drug Design, Development and Therapy is an international, peer-reviewed open-access journal that spans the spectrum of drug design and development through to clinical applications. Clinical outcomes, patient safety, and programs for the development and effective, safe, and sustained use of medicines are a feature of the journal, which

Submit your manuscript here: <http://www.dovepress.com/drug-design-development-and-therapy-journal>

### Dovepress

has also been accepted for indexing on PubMed Central. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.