

# In vivo and in vitro Evaluation of in situ Gel Formulation of Pemirolast Potassium in Allergic Conjunctivitis

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**Background:** To establish a novel delivery system of pemirolast potassium-loaded gellan gum in situ gel in allergic conjunctivitis therapy.

**Methods:** The prepared in situ gels were studied in the following aspects: in vitro gelation, in vitro release, stability, viscosity measurement, in vivo tear kinetics and pharmacodynamics.

**Results:** In this study, the results showed that the viscosity of the in situ gels significantly increased when the preparation was in contact with simulated tear fluid and it also exhibited good stability in a period of three months. In vitro release showed that the release of pemirolast potassium from in situ gels had a good sustained release ability. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible. Consistent with the in vitro studies, pemirolast potassium in situ gels were highly efficient in suppressing the inflammatory symptoms and improving the ocular bioavailability.

**Conclusion:** Pemirolast potassium ocular in situ gels are safe and promising therapeutic alternatives to the existing medications for allergic conjunctivitis therapy.

**Keywords:** pemirolast potassium, gellan gum, in situ gel, allergic conjunctivitis, pharmacodynamic

## Introduction

Allergic conjunctivitis, including seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis (PAC), are a common immunologically mediated disease of the eye.<sup>1</sup> The symptoms of patients with SAC are related to the appearance of pollen from trees, weeds, and grass. Patients with PAC exhibit a chronic sensitivity to common household allergens, such as dust mites, molds, or animal dander.<sup>2</sup> SAC and PAC always induce aggravating and sometimes debilitating symptoms, including severe ocular itching and discomfort, erythema, eyelid edema, chemosis, and tearing.<sup>3</sup>

Many clinical trials have verified pemirolast potassium (PP) efficiently prevents and relieves allergic conjunctivitis.<sup>4–6</sup> PP is one of the mast cell stabilizers that can inhibit the antigen-induced release of chemical mediators from mast cells.<sup>7,8</sup> In addition, when combined with levocabastine, it can enhance the therapeutic effect in experimental allergic conjunctivitis in rats.<sup>9</sup> However, PP eye drops cannot control the drug release and will drain rapidly, which leads to poor bioavailability and a reduction of therapeutic efficacy. Therefore, what is important is to prepare a more efficient PP formula, in order to control stable drug release.

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In situ gelling system, which is a solution at the time of administration but undergoes sol-to-gel transformation when contact is made with physiological fluids or mucosa, can control drug release in a sustained manner.<sup>10</sup> This inimitable property of sol-to-gel conversion provides various advantages to these systems, such as easy administration, like a traditional eye drop formulation, reproducible and precise dosing, easy fabrication, prolonged retentivity at the site of application, and sustained drug release due to gel network formation after being influenced by the physiological stimulation.<sup>11</sup> Based on these advantages, the in situ gel system is suitable for ocular drug delivery.

The ocular region can provide three types of biological stimulus via temperature, pH, and the presence of ions. Temperature-sensitive in situ gelling systems respond to changes in temperature as an external stimulus. The difference in solubility at different temperatures is assumed to be the main reason for sol-to-gel conversion.<sup>12</sup> In a pH-sensitive gelling system, gel forms instantaneously upon interacting with bio-stimuli. Due to the presence of ionizable groups present on the polymer surface, this system can exhibit a sharp change in the form of ionization and water solubility at specific pH levels.<sup>13</sup> The presence of ions in the ocular environment, like  $\text{Ca}^{2+}$ , and other ions present in tear fluid, induces phase transitions of certain polymers. Therefore, based on this property, ion-sensitive gelling systems, using gellan gum,<sup>14</sup> alginates,<sup>15</sup> or  $\beta$ -carrageenan,<sup>16</sup> for ocular delivery have been investigated as well.

Gellan gum (GLG), isolated from *Pseudomonas elodea*, is a natural, biocompatible, nontoxic and biodegradable biopolymer.<sup>17</sup> The mechanism of gelation involves the formation of double-helical junction zones followed by the aggregation of the double-helical segments to form a 3D network by the complexation with cations and hydrogen bonding with water.<sup>18–20</sup> Numerous publications show GLG is suitable for ocular drug delivery.<sup>21–23</sup> Therefore, we fabricated the PP-loaded GLG in situ gel according to intraocular environment to investigate the therapeutic effect of allergic conjunctivitis in vitro and in vivo.

## Materials and Methods

### Materials

PP was gifted by the Yuanye Biopharmaceutics, Co., Ltd (Shanghai, China). GLG was purchased from ZhongWei Biochemical Ltd (Shanghai, China). The PP eye drop (1 mg/mL) was obtained from Shentian Pharmaceutical Co., Ltd. Simulated tear fluid (STF, composition: NaCl

0.68 g,  $\text{NaHCO}_3$  0.22 g,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  0.008 g, KCl 0.14 g, and distilled deionized water to 100 mL).<sup>24</sup> All other reagents were of commercially analytical grade.

New Zealand White rabbits (2.0–3.0 kg) and BALB/c mice (20–25 g) were provided by the Animal Experimental Center of Shanghai Jiao Tong University. The experimental animals were individually housed in an air-conditioned and light-controlled room at  $24 \pm 1^\circ\text{C}$  and at  $65 \pm 5\%$  relative humidity. They were given a standard pellet diet and provided with water ad libitum. All animals were healthy and free of clinically observable ocular abnormalities. All animal experiments were performed in accordance with institutional guidelines, following a protocol approved by the Ethics Committees of Shanghai Jiao Tong University (SMP-VET-008-A). The guide of the National Institutes of Health for the care and use of laboratory animals was strictly followed.

### Preparation of PP in situ Gel<sup>25</sup>

F1–F5 formulations of GLG between 0.1 and 1% (w/w) was prepared by slowly adding a weighed amount of GLG to cold ultrapure water with continuous stirring for 10 min. The partially dissolved mixture was stored in the refrigerator until the entire polymer was completely dissolved (approximately 24 h). A pre-weighed amount of PP was added to the abovementioned homogeneous solution and dissolved completely to obtain a homogeneous phase of polymer, solvent, and drug. Such homogenization was performed using a lab stirrer at 1300 rpm. For the preparation of drug-containing polymer solutions, an appropriate amount of PP was then dissolved in the resulting solution to produce a final drug concentration of 0.1% (w/v). A 0.1% methyl paraben (w/w, as preservative) was added to the preparation. Preparations were made isotonic by the addition of mannitol (5%, w/v), and adjusted to 7.0 using hydrochloric acid.

### Viscosity Measurement<sup>26</sup>

The viscosity of the F1–F5 formulations (0.1% GLG F1, 0.25% GLG F2, 0.5% GLG F3, 0.75% GLG F4, 1% GLG F5), either in solution or in gel made with STF, was determined with a rotational viscometer (NDJ-5S, Shanghai, China) using a 20 mL aliquot of the sample. Measurements were performed using suitable spindle number at 60 r/min, and the temperature was maintained at  $37^\circ\text{C}$ . The viscosity was read directly from the viscometer display. The variation of viscosity is defined as

below, dividing the viscosity with STF by the viscosity without STF. All measurements were made in triplicate.

$$\text{Variation of viscosity} = \frac{[\text{viscosity with STF}]}{[\text{viscosity without STF}]}$$

## Stability Studies<sup>26</sup>

The optimized formulation was stored at room temperature for three months. After the first, second, and third months, the appearance, pH, gelling capacity, and drug content of the formulations were evaluated.

## In vitro Release Studies

The in vitro release of PP from the formulations was through a dynamic dialysis membrane method.<sup>27</sup> Briefly, 0.5 mL volume of the formulation and 0.5 mL STF were accurately pipetted into the dialysis bag; each container was placed in the bottom of a 500 mL beaker. The experiments were performed in a dissolution tester. The beaker was then filled with 200 mL dissolution medium and placed in a circulating water bath equipped with stirring rods with paddles to stir the release medium. The temperature and stirring rate were maintained at 37±1°C and 75 rpm, respectively. The dissolution medium was the freshly prepared STF. At each sampling time, an equal sample (2 mL) was extracted and replaced by the same amount of release medium. The collected samples were directly injected into the HPLC system for analysis (wavelength=360 nm). The same amount of PP eye drops was used for comparison.

## Irritation Studies

According to the Draize technique,<sup>28</sup> the eye irritation of New Zealand White rabbits was studied. Each rabbit weighed 2.0–3.0 kg. 50 µL of formulation was applied to the left eye of the model rabbit. The right eye was untreated as a control. To prevent the loss of the test material, the upper and lower eyelids will be gently closed together for about five seconds. The formulations were instilled three times a day for a period of 10 days, and the rabbits were observed periodically for ocular redness, swelling, and watering. The Draize technique was used for evaluation. The assessment was conducted in three sessions.

## In vivo Tear Kinetics<sup>29</sup>

In vivo ocular tear pharmacokinetic studies of PP formulations, in comparison with PP eye drops, were carried out in rabbits. Fifty microliters of the different PP formulations,

and PP eye drops were instilled into the cul-de-sac of the right eye while the left eye served as the control. About 5 µL of tear was collected from the cul-de-sac of the test eye, using a micropipette, at intervals of 15, 30, 60, 90, 120, 180, 240 and 300 min. The tear samples were extracted in methanol and analyzed using the HPLC method. All experiments were performed in triplicate.

## Pharmacodynamic Evaluation<sup>32</sup>

BALB/c mice were used to investigate the pharmacodynamic effect of PP in situ gels in this study. BALB/c mice were subcutaneously injected with short ragweed pollen (SRW) (25 mg/kg with 10 µL aluminum hydroxide) on immun-day one and on immun-days seven and eight, and the eyes were challenged with SRW in PBS (10 mg/kg with 10 µL aluminum hydroxide per eye) eye drops. Then the eyes were examined under a microscope, scoring was performed at the same time every day and done once daily from day one to at least day 14. As shown in Table 1, mice were examined biomicroscopically based on four independent parameters, which include redness, chemosis, discharge, and tearing. Each parameter was ascribed 0 (none) to 4+ points (severe) and was summed to yield a maximum score of 20+. Changes in the symptom scores were calculated and graphed. In addition, animals were sacrificed after the pharmacodynamic study. Blood samples were collected by drawing blood from the heart under ether anesthesia. The concentration of total IgE was measured using a mouse IgE EIA kit (Yamasa, Tokyo, Japan), according to the manufacturer's protocol. And the pathological sections of corneal tissue of different treatment groups in mice were also determined.

## Statistical Analysis

All data were presented as mean ±SD and analyzed with SPSS19.0. One-way ANOVA was used to compare differences and  $P < 0.05$  was considered statistically significant.

## Results

### Physical Characterization

Viscosity and gelling capacity are the main prerequisites of an in situ gelling system.<sup>29</sup> Aqueous solutions of various concentrations of GLG were prepared and evaluated for viscosity and gelling capacity in order to identify the optimal formulation. The results indicated that the variation of viscosity of formulation 3 was the most significant (Figure 1A, variation=4.9 fold). Therefore, the optimized prescription is

**Table 1** Scoring of Allergic Conjunctivitis Signs and Symptoms

Conjunctivitis Symptoms (Score)		None (0)	Minor (1)	Mild (2)	Moderate (3)	Severe (4)
Conjunctiva	Redness	(-)	Several of vasodilatation	Numerous vasodilatation	All vasodilatation	White of the eye is hard to distinguish
	Chemosis	(-)	Partial swelling	Diffuse slight swelling	Foamed swelling	Ballooning of overall conjunctiva
	Lid swelling	(-)	Partial swelling	Diffuse slight swelling	Foamed swelling	Swelling with diffuse opacities
Discharge		(-)	Filamentous and sticky mucous discharge	Mucus concentrated	Explicit mucus secretion	Severe mucus secretion
Tearing		(-)	Eye feels slightly watery	Easily determined tears	Blows nose occasionally	Tears overflow

F3 (Table 2). Besides, the gel formed obviously after the reaction between F3 and STF (Figure 1B).

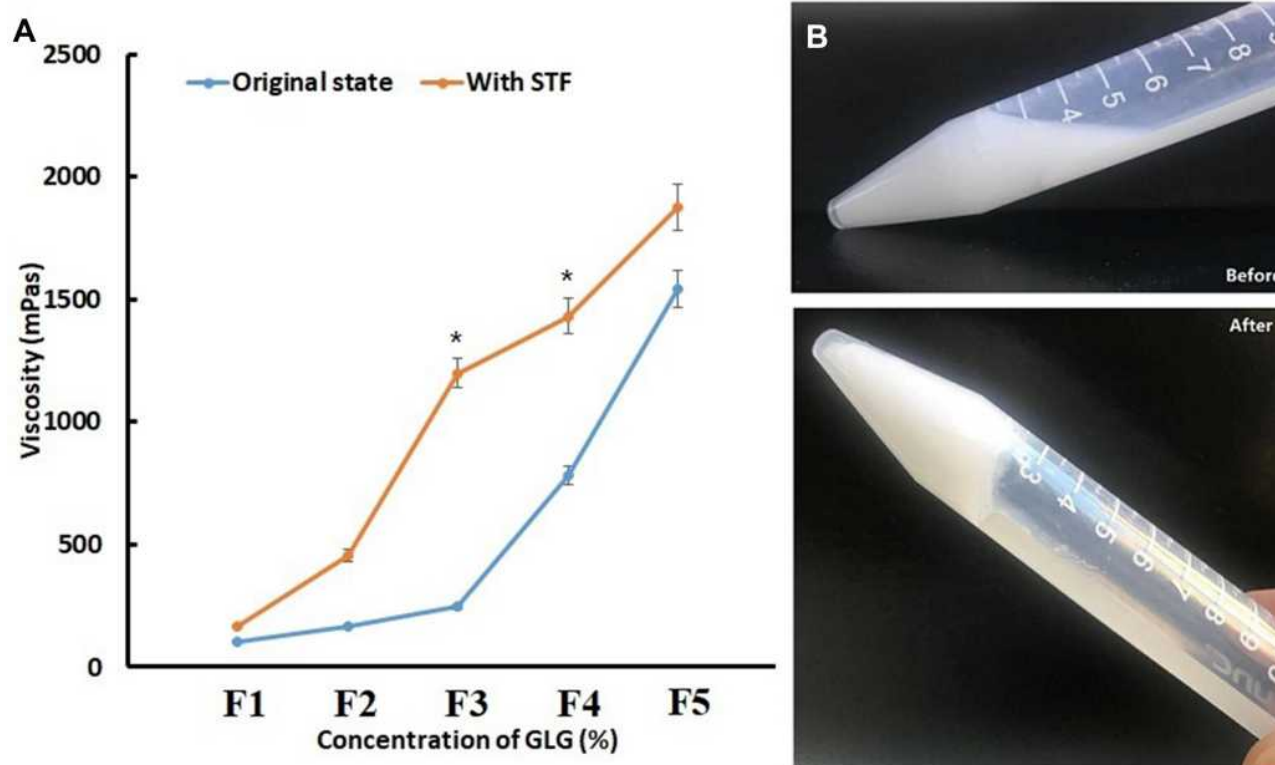
## Stability Studies

PP in situ gels exhibited good stability within three months. During storage, the initial viscosity of the formula has little change. HPLC was used to analyze the drug content of the

samples. The degradation of the drug to 5% is negligible. No significant changes in index and phase separation were observed during the observation period (Table 3).

## In vitro Release Studies

The cumulative amount of PP released over time was shown in Figure 2. Free drug released quickly and almost released



**Figure 1** (A) Viscosity for the various GLG formulations (0.1% F1, 0.25% F2, 0.5% F3, 0.75% F4, 1% F5) and for the GLG formulations with STF, simulating the in vivo gelation. (B) Photographs of in situ gels formed before and after (with STF) gelation. \* $P < 0.05$ . GLG formulations with STF vs GLG formulations without STF.

**Table 2** The Final Optimized Prescription of PP in situ Gel

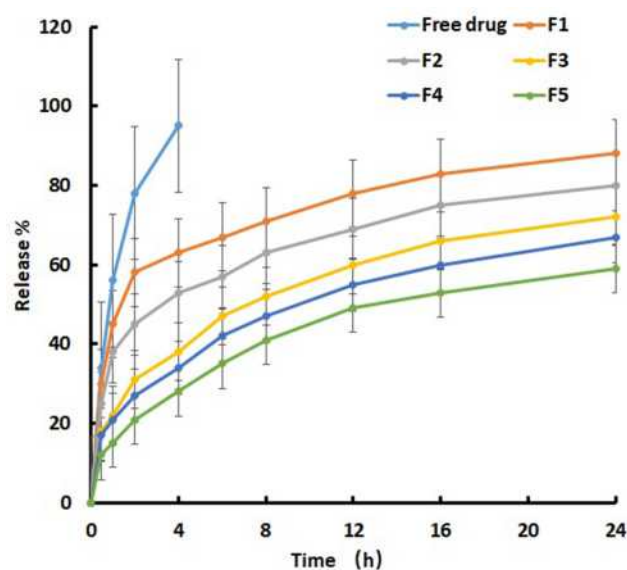
Name	Concentration (% w/v)
PP	0.1
GLG	0.5
Methyl paraben	0.1
Mannitol	5
pH	Adjusted to 7.0 using hydrochloric acid

entirely in four hours, whereas F3, F4, and F5 of drug-loaded GLG released slowly and released less than 40% in four hours. Due to the low viscosity, F1 and F2 released abruptly at the beginning of drug release. The remaining drug was released at a slower rate followed by a second phase of moderate release. Thus, 0.5% GLG has a better delayed release effect. In addition, the release rate also depended on the polymer concentration. The F3, F4, and F5 exhibited a good capacity to retain drugs, which means these three formulations are able to control PP release as the in situ gelling vehicle for drug delivery. However, with the increase of polymer concentration, the viscosity of drug delivery system will increase, and the drug release will slow down, which is not the result that researchers expect.

According to the zero order, first-order and diffusion controlled release mechanisms, the in vitro release kinetics was analyzed. Through analyzing the amount of drug released (F3) vs the square root of time, a relatively high correlation coefficient was obtained, indicating that the release followed the Higuchi kinetic model ( $r=0.994$ ). The results showed that PP-loaded GLG in situ gel had a good sustained release effect.

## Irritation Studies

Figure 3 showed the ocular irritation studies according to the Draize technique. The results of the studies indicate that PP in situ gel was non-irritant (Figure 3). For the F3 formulation, the average irritation scores were 0.4, close to the negative control (0.3, 0.9% NaCl solution). Dioctyl sodium sulfosuccinate (1%, w/w) had the highest average score of 8.2 for ocular lesions. Therefore, the optimized formulation

**Figure 2** The in vitro drug release profiles of PP in situ gel and PP eye drop (free PP). Number represents the percentage of release. (n=6).

may be considered as the least irritating to rabbit eyes. Excellent ocular tolerance was noted. No ocular injury or corneal, iris or conjunctival abnormalities were found.

## In vivo Tear Kinetics

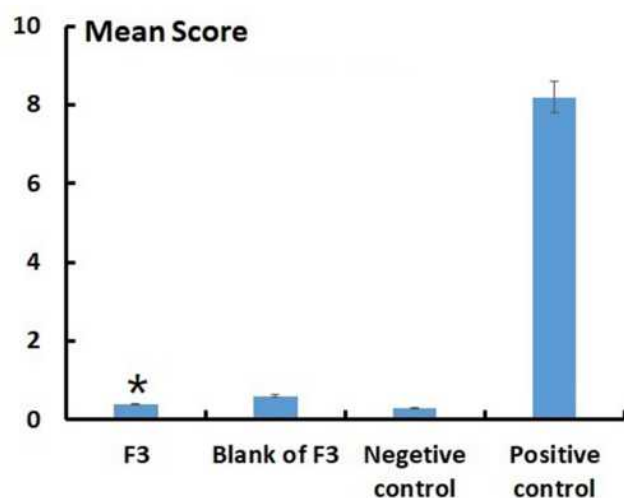
The concentration in tear-time plots, in rabbits after ophthalmic administration of test formulations (F1~F5 and PP eye drops) are shown in Figure 4 and the AUC parameters are tabulated in Table 4. The T<sub>max</sub> was 15 min and the C<sub>max</sub> was 4123.3 ng/mL after ophthalmic administration of PP eye drops. However, the time to achieve maximum concentration of PP was delayed in the form of in situ gel. The drug clearance of F1~F5 was slower with the increase of viscosity. The C<sub>max</sub> of F3 was 2787.6 ng/mL, which was significantly ( $P<0.05$ ) lower than that obtained with the PP eye drops. Meanwhile, after 30 min the concentration in tear of F3 was remarkably higher than that administered with PP eye drops. Three hundred minutes after ophthalmic administration, the concentration of PP in

**Table 3** The Stability of PP in situ Gel During the Observation Period at the Room Temperature

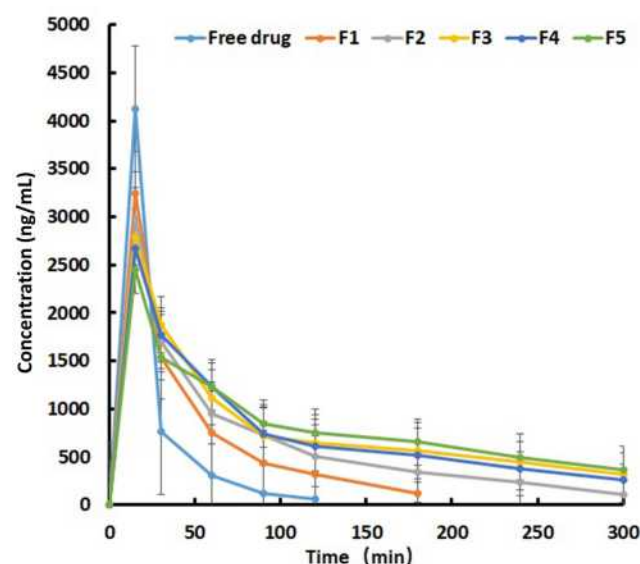
Date	Appearance	pH	Gelling Capacity	% Drug Content	Clarity	Osmolality (mOsmol/kg)
0	Milk	6.96	+++	93.5	Opaque	290
1st month	Milk	7.01	+++	92.1	Opaque	292
2nd month	Milk	6.98	+++	90.4	Opaque	293
3rd month	Milk	7.02	+++	88.2	Opaque	295

**Note:** +++ Represents the ability of stronger gel formation.





**Figure 3** Ocular irritation studies according to the Draize technique (n=3). \*P<0.05. F3 vs positive control.



**Figure 4** Concentration–time curve of PP in different formulations (n=5).

gels was still 100~300 ng/mL, whereas the concentration of PP in eye drops was undetectable. The  $AUC_{0-t}$  of F3 was 238530.6 ng·min/mL, that was 2.51 folds higher than  $AUC_{0-t}$  of 94995.2 ng·min/mL for PP eye drops, clearly defining performance superiority of in situ gels over drops. Lower values of  $V_d$  for gels in comparison to drops are indicative of a larger fraction of formulation being retained in the central compartment. According to tear kinetic data, the application of in situ gels greatly improves the bioavailability of drugs.

**Table 4** Area Under Concentration of PP in Tear vs Time Profiles in 300 Min ( $AUC_{0-t}$ ) for Various Formulations. Each Value Represents the Mean  $\pm$ SD of Three Determinations. (n=5).

Formulation	$AUC_{0-t}$ (ng min/mL)	Ratio
PP eye drops	94995.2 $\pm$ 8721.3	–
F1	140917.5 $\pm$ 12817.2*	1.48
F2	194565.3 $\pm$ 17281.4*	2.05
F3	238530.6 $\pm$ 21342.5*	2.51
F4	228285.3 $\pm$ 20192.3*	2.40
F5	248092.3 $\pm$ 22091.4*	2.61

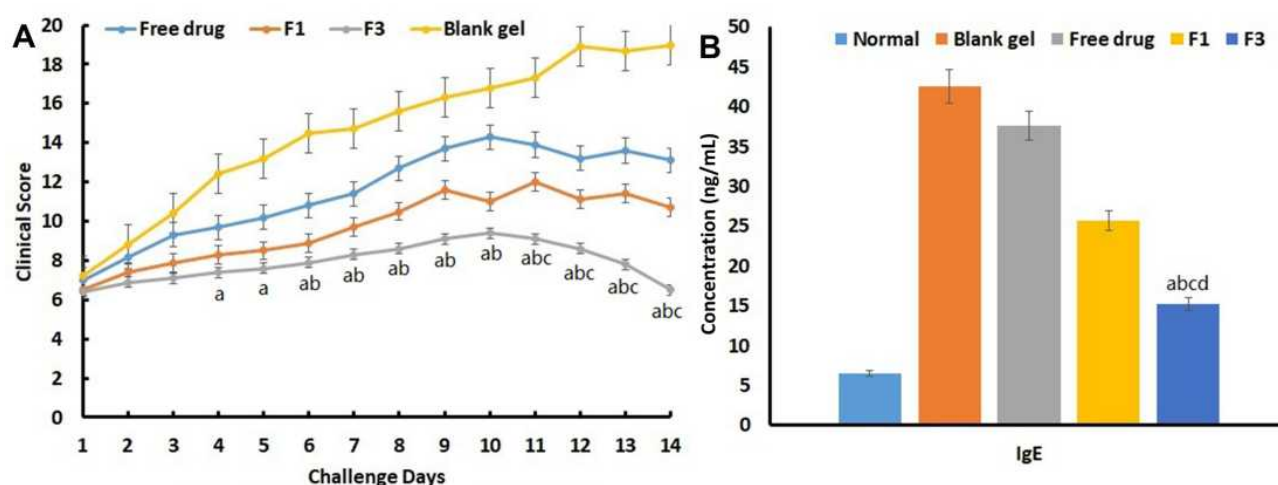
**Note:** \*P<0.05 (compared to PP eye drops).

## Pharmacodynamic Evaluation

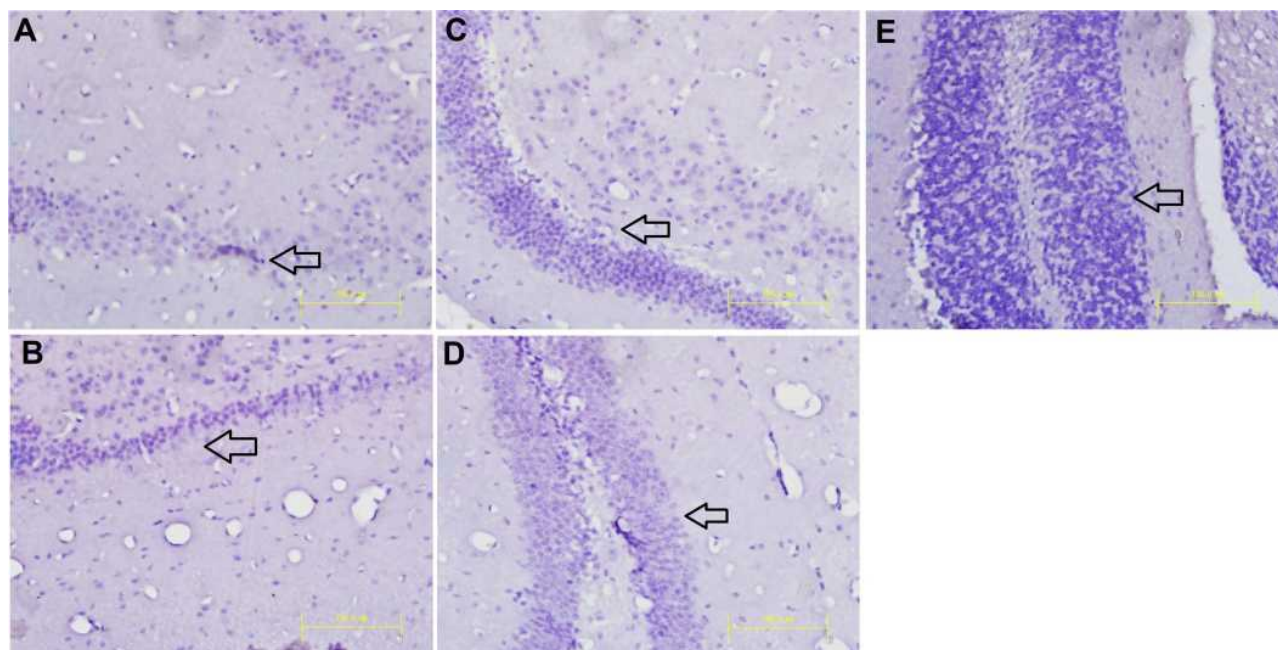
The pharmacodynamic effect of PP in situ gels was tested in BALB/c mice allergic conjunctivitis model (Figure 5). As shown in Figure 5A, The clinical score of allergic conjunctivitis constantly increased in the case of blank gel group. The free drug (eye drops), though inhibited the progression of inflammatory symptoms to an extent, was far behind the reasonable response. Consistent with the in vitro studies, PP in situ gels (both F1 and F3) were highly efficient in suppressing inflammatory symptoms. At the same time, from the curve, the F3 group also had a significant anti-inflammatory advantage with a lasting effect. IgE concentration of different treatment groups in mice were also checked and the result stated that IgE in the mice treated with F3 was significantly lower than in other treatment groups (Figure 5B). The histopathological studies of corneal tissue of different groups were shown in Figure 6. The pathological section of corneal tissue showed the PP-loaded GLG in situ gel is safe for corneal use.

## Discussion

In situ gel systems have been developed well and showed beneficial effect over other traditional dosage forms. These profits contain sustained and prolonged release of the drug, biocompatibility, easy instillation, minimum chances of irritation, etc. However, additional studies are required to determine the innovative and alternative ocular dosage forms, like the combination of different types of in situ gel systems, to improve patient care. GLG was composed of 1 $\beta$ -L-rhamnose, 1 $\beta$ -D-glucuronic acid and 2 $\beta$ -D-glucose and has ion-activated properties. In addition, we found that the optimum concentration of GLG for delivery PP was 0.5% (F3). Under this concentration, GLG can best balance the speed of drug release and therapeutic effect, which means F3 can control drug



**Figure 5 (A)** The pharmacodynamic effect of PP in situ gels was tested in BALB/c mice allergic conjunctivitis model. (n=6) a  $P < 0.05$ . F3 vs Blank gel; b  $P < 0.05$ . F3 vs free drug; c  $P < 0.05$ . F3 vs F1. **(B)** IgE concentration of different treatment group in mice after pharmacodynamic study. a  $P < 0.05$ . F3 vs normal; b  $P < 0.05$ . F3 vs blank gel; c  $P < 0.05$ . F3 vs free drug; d  $P < 0.05$ . F3 vs F1.



**Figure 6** Histopathological studies of corneal tissue of different groups. **(A)** normal; **(B)** F1; **(C)** F3; **(D)** free drug (eye drops); **(E)** blank gel. The black arrow indicates the area of inflammation.

release stably and achieve an optimal therapeutic effect more than the free drug.<sup>30</sup>

Proteins, pollens and dust mites are not only common allergens in patients with allergic conjunctivitis, but also common antigens for constructing animal models of allergic conjunctivitis.<sup>31–33</sup> It can establish a stable and reliable animal model of allergic conjunctivitis. Our previous results showed that the expression of CCL5 mRNA and IL-17 mRNA in conjunctiva tissue and the percentage of

IL-17 in spleen single cell suspension were the highest in mice after they were challenged with SRW, suggesting that SRW had the best sensitization effect under the experimental conditions. Subsequently, BALB/c mice were challenged with SRW by gavage, aerosol inhalation and subcutaneous injection. The results showed that the expression of CCL5 mRNA and IL-17 mRNA in conjunctiva tissue and the percentage of IL-17 in spleen single cell suspension of mice in the subcutaneous injection group

and the positive control group (eye drop stimulation) bore a striking resemblance, suggesting that subcutaneous injection stimulation can more effectively induce allergic conjunctivitis in mice.<sup>34–36</sup>

## Conclusions

In this study, we fabricated the PP-loaded GLG in situ gels successfully. The results indicated that the variation of viscosity of formulation 3 was the most significant (variation=4.9 fold). Therefore, the optimized prescription is F3. The release study showed that the release of PP from in situ gels had a good sustained release ability. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible. Consistent with the in vitro studies, PP in situ gels were highly efficient in suppressing the inflammatory symptoms and improving the ocular bioavailability. PP ocular in situ gels are safe and promising therapeutic alternatives to existing medications for allergic conjunctivitis therapy.

## Disclosure

The authors report no conflicts of interest in this work.

## References

- Alfonso SA, Fawley JD, Lu XA. Conjunctivitis. *Prim Care*. 2015;42(3):325–345. doi:10.1016/j.pop.2015.05.001
- Dinowitz M, Rescigno R, Bielory L. Ocular allergic diseases: differential diagnosis, examination techniques, and testing. *Clin Allergy Immunol*. 2000;15:127–150.
- Balyeat RM, Bowen R. Allergic conjunctivitis. *South Med J*. 1935;28(11):1005–1011. doi:10.1097/00007611-193511000-00009
- Gous P, Ropo A. A comparative trial of the safety and efficacy of 0.1 percent pemirolast potassium ophthalmic solution dosed twice or four times a day in patients with seasonal allergic conjunctivitis. *J Ocul Pharmacol Ther*. 2004;20(2):139–150. doi:10.1089/108076804773710812
- Shulman DG. Two mast cell stabilizers, pemirolast potassium 0.1% and nedocromil sodium 2%, in the treatment of seasonal allergic conjunctivitis: a comparative study. *Adv Ther*. 2003;20(1):31–40. doi:10.1007/BF02850117
- Abelson MB, Berdy GJ, Mundorf T, Amdahl LD, Graves AL. Pemirolast potassium 0.1% ophthalmic solution is an effective treatment for allergic conjunctivitis: a pooled analysis of two prospective, randomized, double-masked, placebo-controlled, Phase III Studies. *J Ocul Pharmacol Ther*. 2002;18(5):475–488. doi:10.1089/10807680260362759
- Kawashima T, Iwamoto I, Nakagawa N, Tomioka H, Yoshida S. Inhibitory effect of pemirolast, a novel antiallergic drug, on leukotriene C4 and granule protein release from human eosinophils. *Int Arch Allergy Immunol*. 1994;103(4):405–409. doi:10.1159/000236662
- Fujimiya H, Nakashima S, Miyata H, Nozawa Y. Effect of a novel antiallergic drug, pemirolast, on activation of rat peritoneal mast cells: inhibition of exocytotic response and membrane phospholipid turnover. *Int Arch Allergy Immunol*. 1991;96(1):62–67. doi:10.1159/000235536
- Minami K, Hossen MA, Kamei C. Increasing effect by simultaneous use of levocabastine and pemirolast on experimental allergic conjunctivitis in rats. *Biol Pharm Bull*. 2005;28(3):473–476. doi:10.1248/bpb.28.473
- Üstündağ Okur N, Eş Ç, Sifaka PI, et al. Novel ocular drug delivery systems: an update on microemulsions. *J Ocul Pharmacol Ther*. 2020;36(6):342–354. doi:10.1089/jop.2019.0135
- Agrawal AK, Das M, Jain S. In situ gel systems as ‘smart’ carriers for sustained ocular drug delivery. *Expert Opin Drug Deliv*. 2012;9(4):383–402. doi:10.1517/17425247.2012.665367
- Bromberg L. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. *Adv Drug Deliv Rev*. 1998;31(3):197–221. doi:10.1016/S0169-409X(97)00121-X
- Gil ES, Hudson SM. Stimuli-responsive polymers and their bioconjugates. *Prog Polym Sci*. 2004;29:1173–1222. doi:10.1016/j.progpolymsci.2004.08.003
- Kang KS, Veeder GT, Mirrasoul PJ, Kaneko T, Cottrell IW. Agar-like polysaccharide produced by a *Pseudomonas* species: production and basic properties. *Appl Environ Microbiol*. 1982;43(5):1086–1091. doi:10.1128/AEM.43.5.1086-1091.1982
- Korgel BA, Rotem A, Monbouquette HG. Effective diffusivity of galactose in calcium alginate gels containing immobilized *zymomonas mobilis*. *Biotechnol Prog*. 1992;8(2):111–117. doi:10.1021/bp00014a004
- Stellwagen J, Stellwagen NC. The effect of gel structure on matrix orientation. *Electrophoresis*. 1992;13(9–10):595–600. doi:10.1002/elps.11501301120
- Palumbo FS, Federico S, Pitarresi G, et al. Gellan gum-based delivery systems of therapeutic agents and cells. *Carbohydr Polym*. 2020;229:115430
- Grasdalen H, Smidsroed O. Gelation of gellan gum. *Carbohydr Polym*. 1987;7:371–393. doi:10.1016/0144-8617(87)90004-X
- Chanrasekaran R, Puigjaner LC, Joyce KL, Arnott S. Cation interaction in gellan: an X-ray study of the potassium salt. *Carbohydr Res*. 1988;181:23–40. doi:10.1016/0008-6215(88)84020-5
- 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. doi:10.1016/0144-8617(90)90092-7
- Chowhan A, Giri TK. Polysaccharide as renewable responsive biopolymer for in situ gel in the delivery of drug through ocular route. *Int J Biol Macromol*. 2020;150:559–572. doi:10.1016/j.ijbiomac.2020.02.097
- Janga KY, Tatke A, Dudhipala N, et al. Gellan gum based sol-to-gel transforming system of natamycin transfersomes improves topical ocular delivery. *J Pharmacol Exp Ther*. 2019;370(3):814–822. doi:10.1124/jpet.119.256446
- Thrimawithana TR, Young SA, Bunt CR, Green CR, Alany RG. In-vitro and in-vivo evaluation of carrageenan/methylcellulose polymeric systems for transscleral delivery of macromolecules. *Eur J Pharm Sci*. 2011;44:399–409. doi:10.1016/j.ejps.2011.08.026
- Paulsson M, Hägerström H, Edsman K. Rheological studies of the gelation of deacetylated gellan gum (Gelrite) in physiological conditions. *Eur J Pharm Sci*. 1999;9(1):99–105. doi:10.1016/S0928-0987(99)00051-2
- Srividya B, Cardoza RM, Amin PD. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. *J Contr Rel*. 2001;73:205–211. doi:10.1016/S0168-3659(01)00279-6
- 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. doi:10.1016/j.ijpharm.2008.08.042
- Manjappa AS, Nanjwade BK, Manvi FV, Murthy RSR. Sustained ophthalmic in situ gel of ketorolac tromethamine: rheology and in vivo studies. *Drug Dev Res*. 2009;70:417–424. doi:10.1002/ddr.20317
- Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances. *J Pharmacol Exp Ther*. 1944;82:377–390.



29. Lin HR, Sung KC. Carbopol/pluronic phase change solutions for ophthalmic drug delivery. *J Contr Rel.* 2000;69(3):379–388. doi:10.1016/S0168-3659(00)00329-1
30. Buket N, Aksu V, Yozgatli ME. Preparation and evaluation of QbD based fusidic acid loaded in situ gel formulations for burn wound treatment. *J Drug Deliv Sci Tech.* 2019.
31. Kuo CH, Collins AM, Boettner DR, Yang TF, Ono SJ. Role of CCL7 in type I hypersensitivity reactions in murine experimental allergic conjunctivitis. *J Immunol.* 2017;198(2):645–656. doi:10.4049/jimmunol.1502416
32. Sun W, Sheng Y, Chen J, Dong X, Yangshun G. Down-regulation of miR-146a expression induces allergic conjunctivitis in mice by increasing TSLP level. *Med Sci Monit.* 2015;21:2000–2007. doi:10.12659/MSM.894563
33. Yasue M, Nakamura S, Yokota T, Okudaira H, Okumura Y. Experimental monkey model sensitized with mite antigen. *Int Arch Allergy Immunol.* 1998;115(4):303–311. doi:10.1159/000069461
34. Üstündagokur N, Yozgatli V, Okur ME, et al. Improving therapeutic efficacy of voriconazole against fungal keratitis: Thermo-sensitive in situ gels as ophthalmic drug carriers. *J Drug Deliv Sci Technol.* 2019;49:323–333. doi:10.1016/j.jddst.2018.12.005
35. Okur NÜ, Yozgatli V, Okur ME. In vitro-in vivo evaluation of tetrahydrozoline-loaded ocular in situ gels on rabbits for allergic conjunctivitis management. *Drug Dev Res.* 2020;81(6):716–727. doi:10.1002/ddr.21677
36. Makwana SB, Patel VA, Parmar SJ. Development and characterization of in-situ gel for ophthalmic formulation containing ciprofloxacin hydrochloride. *Results in Pharma Sci.* 2015.

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