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# Preparation, pharmacokinetics and pharmacodynamics of ophthalmic thermosensitive in situ hydrogel of betaxolol hydrochloride

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ABSTRACT

Conventional ophthalmic formulations often eliminate rapidly after administration and cannot provide and maintain an adequate concentration of the drug in the precorneal area. To solve those problems, a thermosensitive in situ gelling and mucoadhesive ophthalmic drug delivery system was prepared and evaluated, the system was composed of poloxamer analogs and polycarbophil (PCP) and betaxolol hydrochloride (BH) was selected as model drug. The concentrations of poloxamer 407 (P407) (22% (w/v)) and poloxamer 188 (P188) (3.5% (w/v)) were identified through central composite design-response surface methodology (CCD-RSM). The BH in situ hydrogel (BH-HG) was liquid solution at low temperature and turned to semisolid at eye temperature. BH-HG showed good stability and biocompatibility, which fulfilled the requirements of ocular application. In vitro studies indicated that addition of PCP enhanced the viscosity of BH-HG and the release results of BH from BH-HG demonstrated a sustained release behavior of BH because of the gel dissolution. In vivo pharmacokinetics and pharmacodynamics studies indicated that the BH-HG formulation resulted in an improved bioavailability and a significantly lower intraocular pressure (IOP). The results suggested BH-HG could be potentially used as an in situ gelling system for ophthalmic delivery to enhance the bioavailability and efficacy.


## 1. Introduction

In market, ninety percent ocular medicines are aqueous or oily liquids that are instilled into the eye. However, such therapies are hard to enter the posterior of the eye due to the special anatomic structure and efficient protective mechanisms of the eye, such as dosage spill-over, nasolacrimal drainage, blinking, tear film and mucin, and low corneal permeability [1]. Despite of the improvement by local therapies, ocular drug delivery is still limited by short ocular contact time, low ocular bioavailability, high dosing frequency, limited drug penetration and invasiveness of treatments [2]. To overcome these drawbacks, attempts have been made to develop novel ophthalmic drug delivery systems, including colloidal systems [3], cyclodextrin inclusion compounds [4,5], gel systems [6], ocular inserts [7].

In situ gelling system is an ideal candidate for ophthalmic formulations because the gelling system can turn to gel from solution via stimuli-responsive phase transition of polymers [8]. In situ gelling can be triggered by pH, temperature and ions [9]. Ophthalmic thermosensitive in situ gelling systems can be stored and administered as liquid, but undergo the phase transition to semisolid gel upon exposure to physiological temperature [10,11]. Such transition can prolong the retention time and improve bioavailability of ocular formulations compared to eye drops and eye suspensions.

Thermosensitive hydrogels can undergo sol–gel transitions upon heating or cooling because of changes in the intermolecular interactions such as ionic, hydrogen bonding and hydrophobic forces. Poloxamer 407 (P407), a triblock copolymer with a central hydrophobic chain of polyoxypropylene (PPO) and two identical lateral hydrophilic chains of polyoxyethylene (PEO), is frequently used as a gelling agent in the development of bioadhesive, thermosensitive and controlled release formulations [12]. Gelling ability of P407 is concentration-dependent. At low concentration, P407 solution will lose gelation ability, and at high concentration,

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the gelling temperature (GT) of P407 is lower than room temperature [13]. To optimize GT, P407 solution is mixed with poloxamer 188 (P188). Additionally, polycarbophil (PCP) is a mucoadhesive biomaterials and is used to increase the gel viscosity in the study [14]. PCP has many superior characteristics for ophthalmic drug delivery system, such as low irritation and long resident time in the corneal surface, which can be used as gels or emulsions matrix and can enhance the bioavailability of drugs [15].

Betaxolol hydrochloride, a selective beta-adrenergic blocking agent, is frequently used as a model drug for open-angle glaucoma and ocular hypertension due to its ability to reduce aqueous humor production and intraocular pressure (IOP). BH eye drops are BH solutions and BH ophthalmic suspensions [16]. But BH solutions possess short ocular contact time and high dosing frequency. BH suspensions demonstrate poor stability and quality control.

In the present study, poloxamer analogs/polycarbophil based thermosensitive in situ hydrogel was designed and optimized by using central composite design- response surface methodology (CCD-RSM). The in vitro (rheological behaviors and BH release) and in vivo (the elimination of BH in tear, the absorption of BH in aqueous humor and the IOP-lowering effect) evaluation of the BH thermosensitive hydrogel (BH-HG) were performed and compared with commercial BH eye drops (BH suspensions, BH-SP).

## 2. Materials and methods

### 2.1. Materials

Betaxolol hydrochloride was prepared by School of Pharmaceutical Sciences, Zhengzhou University (Zhengzhou, China). Betaxolol hydrochloride ophthalmic suspensions were purchased from Alcon (Puurs, Belgium). Poloxamer 407 and poloxamer 188 were purchased from BASF (Ludwigshafen, Germany). Polycarbophil was obtained from BASF (Ludwigshafen, Germany). The purified water used in all the experiments was obtained from a MilliQ System. The organic solvents used in HPLC were of chromatographic grade (Tianjin, China). All the other chemicals and reagents used in the study were of analytical grade.

### 2.2. Animals

The animal experiments were reviewed and approved by the Institutional Animal Ethics Committee of Zhengzhou University. The experimental rabbits were provided by animal experiment center (Xingyang, China). Rabbits weighed between 2.0 and 3.0 kg, were individually housed in an air-conditioned and light-controlled room with water and normal food. All rabbits were healthy and free of clinically observable ocular abnormalities.

### 2.3. Preparation and optimization of BH-HG

The poloxamer solutions were prepared by using the cold method [17]. A volume of bi-distilled water was cooled down to 4 °C. P407 and P188 were then slowly added to the water under stirring. After the P188 and P407 solution was kept in the refrigerator until the polymers were completely dissolved (approximately 24 h), EDTA-2Na and mannitol were dispersed in P188 and P407 solution in 4 °C. 0.2% (w/v) PCP solution was prepared by dispersing the required amount of PCP in distilled and deionized water under continuous stirring for 1.5 h. BH, citric acid and sodium citrate were subsequently added to the PCP solution with continuous agitation at room temperature and cooled down to 4 °C. Finally, the mixture of P188 (0–5%, w/v) and P407 (19–25%, w/v) solution was added to the PCP solution with benzalkonium chloride (0.1%, w/v). The solution was adjusted to pH 6.5 ± 0.1 by 1 mol/L sodium hydroxide solution and then stored in the refrigerator.

The BH-HG formulation was optimized by using CCD-RSM, which saved time and resources with fewer experimental runs than industrial procedures [18]. A central composite design (CCD) was carried out on the two independent variables (variable $X_1$: the concentration of P407 ranging from 19% to 25% (w/v); variable $X_2$: the concentration of P188 ranging from 0% to 5% (w/v)) at five experimental levels: −1.414, −1, 0, 1, and 1.414, respectively. Responsive variables $T_1$ (°C) and $T_2$ (°C) selected in the study were GT of the BH-HG formulation before and after simulated tear fluid (STF) dilution (components (1 L): 6.78 g sodium chloride, 0.084 g calcium chloride dehydrate, 2.18 g sodium bicarbonate, and 1.38 g potassium chloride).

### 2.4. Rheological studies of BH-HG

The rheological studies were carried out on rotary viscometer (Brookfield TC-550) [19]. The viscosities of the BH-HG formulation were measured at various shear rates (from 5 $s^{-1}$ to 20 $s^{-1}$) at 25 °C and 27 °C, respectively. The BH-HG formulation before and after STF dilution was tested from 22 °C to 28 °C (keeping a shear rate of 5 $s^{-1}$). The formulations containing 0%, 0.1%, and 0.2% PCP were prepared, respectively. The viscosities of the formulations were measured at various temperatures. In addition, 0.9% (w/v) NaCl and 5% mannitol were added to the formulations as the osmotic pressure regulator. And the two formulations were also tested at different temperatures.

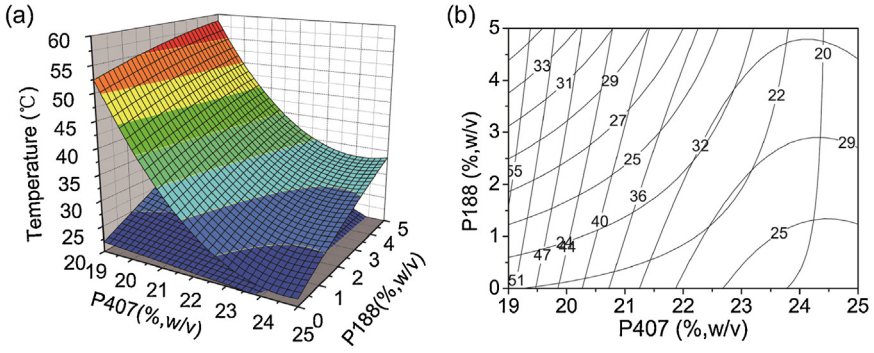


**Fig. 1.** Effect of P407 and P188 concentrations on the gelling temperature (n = 3). (a) Response surface results of gelling temperature before and after simulated tear fluid dilution. (b) Contour results of gelling temperature before and after simulated tear fluid dilution.