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Thermosensitive *in-situ* forming gels for ophthalmic delivery of tea polyphenols

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ABSTRACT

This study aimed to develop a thermosensitive *in situ* gelling vehicle containing tea polyphenols (TPs) for ophthalmic delivery. Based on the different experiments of appropriate gel strength and gelling capacity under physiological conditions, the optimized concentrations of poloxamer 407/poloxamer 188 and carbopol 940 in the formulation were established to be 25% (w/v)/5% (w/v) and 0.1% (w/v), respectively. The combined solutions could be easily dropped as a fluid into the eye and further converted to gels under physiological conditions. Furthermore, using rabbit cornea, the effects of various formulation factors on the permeation of TPs were investigated both *in vitro* and *in vivo*. According to the outcome of transcorneal penetration experiment through the excised rabbit cornea *in vitro*, the formulation of F4 including 0.1% Azone showed the highest apparent permeability coefficient (Papp). Meanwhile, the result of ocular pharmacokinetic studies showed the area under the curve of the aqueous humor concentration-time profiles of TPs gel including 0.1% Azone was 2.83 times higher than that of TPs solution in the presence of Azone. These results demonstrate the *in situ* gel-forming delivery system may hold some promise in ocular delivery and can be a viable alternative to enhance the ocular bioavailability.

1. Introduction

Due to the special structure of the eye, the most common agents, e.g., eye drops, are eliminated rapidly within 5–6 min after administration and thus have a low bioavailability. It was found that the normal tear dynamics, nonproductive absorption via the conjunctiva, the nasolacrimal drainage system, the barriers of the exclusive tight junctions of corneal epithelial and stromal layer can lead to a short precorneal residence time and a limitation of absorption [1]. As a result, ophthalmic drugs are often used in high concentrations or frequent instillation to improve the ocular bioavailability. However, these methods may cause both partial and systemic side-effects and have a low patient compliance [2]. Therefore, a dosage form of ocular drug should be chosen which could efficiently enhance the drug bioavailability by prolonged exposure of the cornea.

Many ocular delivery systems have been utilized to improve the bioavailability of topically applied drugs such as hydrogel, contact lenses, nanowafer, nanoemulsions, ion-sensitive *in situ* hydrogel [3–6]. However, they have not been completely accepted because the delivery systems suffer from some drawbacks including the blurred vision or low compliance. Hence, prolonging topically applied drug precorneal residence time and enhancing corneal drug absorption remain a critical challenge and still need to be resolved. An ideal ophthalmic formulation should be administrated in liquid form without blurred vision or irritation and possess a suitable strength to resist the lacrimal fluid dilution and can also remain the drug in the precorneal area for extended periods of time. Recently, *in situ* gel delivery systems have been developed because of achieving these purposes. The *in situ* gel delivery systems are consisted with some polymers that undergo sol-to-gel phase transition because of a special physicochemical parameter change. Based on different mechanisms caused a sol-to-gel phase transition on the ocular surface, three types of delivery systems were investigated: pH-triggered, temperature-dependent and ion-activated [7–11]. These *in situ* gel-forming systems could efficiently improve the drug bioavailability, reduce the dose and frequency, and improve the patient compliance by means of prolonging the precorneal residence time of

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the drug because it can reduce the number of administrations.

Poloxamer is composed of polyethylene oxide (PEO) and polyproylene oxide (PPO), which exhibits a sol-to-gel conversion, resulting in the temperature change induced by the physiological environment under a certain concentration. In the systems, Poloxamer188 was added to obtain a temperature-dependent gel with a suitable gelation temperature (GT) of ophthalmic delivery system. Due to its relatively short contact cornea time in comparison with gellan, carbopol 940 can be used as a mucoadhesive polymer which is added into the system to prolong the precorneal residence time of TP. The drug delivery systems of poloxamer combined with mucoadhesive polymer could overcome the drawbacks of conventional eye drops. The *in situ* gelling liquid dosage form has low viscosity at room temperature. When a polymeric eye drop is instilled into eyes, a sol-to-gel transition occurs due to simultaneous variations in pH and temperature.

Tea polyphenols (TPs) are a natural polyphenolic compound extracted from tea. In the aspect of physical and chemical properties, TPs are light yellow or light green powder at room temperature. The dissolubility of TPs in water and organic solvents is prosperous. The essential constituents of TPs are (−)-epigallocatechin gallate (EGCG), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (+)-catechin (C) and gallocatechin gallate (GCG). The percentage of EGCG in TPs can be up to 59% [12]. The major active component EGCG in TPs provides the potential for therapeutic and preventive application. The beneficial functions of anti-oxidant, anti-inflammatory, anti-cancer and nutraceutical have already been studied [13–16]. The absorption of ophthalmic topical drug not only depends on the precorneal residence time but also relies on the chemical nature of the drug. The corneal permeability of the drug was influenced by the molecular weight and the hydrophobicity. It has been reported that the lipophilic drugs and hydrophilic drugs cross the cornea through transcellular and paracellular pathway, respectively [17,18], and hydrophilic drugs absorbed through the cornea were less than lipophilic drugs.

Laurocapram (Azone) is external used as a powerful penetration enhancer and non-toxic and irritating [19,20]. It also can increase the intraocular penetration of hydrophilic drugs due to its lipophilicity [21,22]. It was reported that Azone had no damage to corneal endothelial cells even up to 0.9% [23,24]. Therefore, Azone has been widely used in drug delivery vehicles.

An ophthalmic delivery system based on poloxamer analogs/carbopol *in situ* gelling and mucoadhesive had been developed [25]. The thermosensitive *in situ* gelling and mucoadhesive ophthalmic drug delivery system possesses sol-to-gel transition of this solution primarily due to the temperature increase and the neutralization of the buffering action of lacrimal fluid. The combined application of mucoadhesive could attach to the ocular mucosal surface for a relative long time to have a high bioavailability. Due to their hydrophilicity, TPs possess a poor corneal penetration. Thus, Azone can be used in *in situ* gel system to improve the penetration of TPs which acts as a penetration enhancer.

The present study aimed to develop and evaluated the thermosensitive *in situ* gelling vehicle of TPs for ophthalmic delivery system we fabricated. The polymers analogue and carbopol were used to form a solution which undergo sol-to-gel transition due to the simultaneous variations in pH and temperature. For the preparation of the *in situ* thermosensitive hydrogel, Azone was used as a penetration enhancer. The effect of the addition of Azone on gelation temperature and the release *in vitro* were evaluated. *In vitro* transcorneal permeation and *in vivo* pharmacokinetics were further carried out to investigate the permeation properties of TPs *in situ* gel system formulation.

2. Materials and methods

2.1. Materials and reagents

TPs were supplied by ShanXi Sciphar Hitech Industry Co., Ltd (Xian, China). Azone was purchased from Shanghai Kayon Biological Technology Co., Ltd (Shanghai, China). Poloxamers (P407 and P188) were purchased from Sigma-Aldrich (St. Louis, MO, U.S.A.). Carbopol (940P NF, B. F. Goodrich), was kindly gifted by Colorcon (UK). Acetonitrile and methanol were obtained from Tedia Co., Ltd, USA and were high performance liquid chromatography (HPLC) grade. All other chemicals were analytical grade or higher.

2.2. Animals

All New Zealand white rabbits (2.5–3.0 kg) were obtained from the Animal Experimental Center of Shandong University (Jinan, China). The rabbits were fed according to the Guidelines of Care and Use of Laboratory Animals approved by the China National Institute of Health. All experiments were strictly followed by the Association for Research in Vision and Ophthalmology Statement for the use of animals in ophthalmic and vision research. In the present study, the protocol of the study was approved by Eye Institute of Shandong University of Traditional Chinese Medicine (approval number 2013-003), and the European Community guidelines as accepted principles for the use of experimental animals, were adhered to.

2.3. Preparation of test formulations

2.3.1. Preparation of temperature-dependent in situ gel-forming formulations

The preparation of the temperature-dependent *in situ* gel-forming system was as follows: the carbopol solution was prepared by sprinkling a definite amount over a certain volume distilled water and allowed to hydrate overnight at 4 °C. The poloxamer solution was prepared by dispersing P188 in distilled water until completely dissolved and P407 were then added with continuous stirring in cold condition. The solution was stored at 4 °C until the clarified poloxamer solution was obtained. The required amounts of P407 and P188 were added into the carbopol solution in order to obtain the poloxamer analogs/carbopol solution. The following procedures were identical to the description mentioned above. For the preparation of TPs gel (5%, w/v), the required amount of the drug was dissolved in a certain distilled water and then prepared the solutions as above. The pH value was adjusted with 0.1 mol/L of triethanolamine and the solution was stirred with an overhead stirrer constantly until a uniform solution was obtained. Finally, distilled water was added to make the volume to the total amount.

2.3.2. TPs ophthalmic in situ gel containing different viscolizing agents

Aqueous solutions of different concentrations of poloxamer analogs and carbopol 940 were prepared with the method described in “Preparation of temperature-dependent *in situ* gel-forming formulations”. The formulations were listed in Table 1.

2.3.3. Preparation of TPs ophthalmic in situ gel containing Azone

Azone was added to the TPs ophthalmic *in situ* gel as a permeation enhancer. The concentrations of Azone were 0.05% and 0.1% (v/v), respectively.

2.3.4. The in situ gel system of different TPs concentrations

The required TPs in the gel system were dissolved to obtain 1%, 5% and 10% (w/v) solutions, respectively.

2.4. Viscosity measurement

In order to confirm whether the various concentrations of poloxamer analogs and carbopol used were suitable for TPs ophthalmic *in situ* gel systems, the viscosity of *in situ* gelling system was measured using a rotating viscometer (Shanghai Changji Instruments Co., Ltd, China). The gelling capacity was determined by placing a drop of the system in a vial containing 2 mL of simulated tear fluid (STF) and equilibrated at