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Development of a poloxamer analogs/carbopol-based in situ gelling and mucoadhesive ophthalmic delivery system for puerarin

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Abstract

Conventional ophthalmic solutions often eliminate rapidly after administration and cannot provide and maintain an adequate concentration of the drug in the precorneal area. To solve these problems, we developed a thermosensitive in situ gelling and mucoadhesive ophthalmic drug delivery system containing puerarin based on poloxamer analogs (21% (w/v) poloxamer 407/5% (w/v) poloxamer 188) and carbopol (0.1% (w/v) or 0.2% (w/v) carbopol 1342P NF). The combined solutions would convert to firm gels under physiological condition and attach to the ocular mucosal surface for a relative long time. The incorporation of carbopol 1342P NF not only did not affect the pseudoplastic behavior with hysteresis of the poloxamer analogs solution and leaded to a higher shear stress at each shear rate, but also enhanced the mucoadhesive force significantly. In vitro release studies demonstrated diffusion-controlled release of puerarin from the combined solutions over a period of 8 h. In vivo evaluation (the elimination of puerarin in tear and intraocular pressure-lowering effect) indicated the combined solutions had better ability to retain drug than poloxamer analogs or carbopol alone. It appears that ocular bioavailability can be increased more readily by using the in situ gelling and mucoadhesive vehicle.

Keywords: Ophthalmic delivery system; In situ gelling; Mucoadhesive; Poloxamer; Carbopol; Puerarin

1. Introduction

Due to the special anatomic structure and efficient protective mechanisms, many challenges are presented in the development of effective ophthalmic dosage forms. Most ophthalmic drugs are administrated topically in the form of eye drops. However, the rapid turnover of lacrimal fluid and efficient drainage apparatus make the ophthalmic solutions eliminate rapidly, which causes a short precorneal residence time and a limitation of transcorneal absorption. All these lead to an ocular bioavailability that is commonly less than 10% (Patton and Robinson, 1976; Lee and Robinson, 1979). Moreover, nasolacrimal drainage is also the major route to enter the circulatory system for drugs that are applied through topical administration. For potent drugs, the systemic exposure through nasolacrimal drainage after topical administration can be sufficiently high to cause systemic toxicity (Urtti and Salminen, 1993). In order to enhance the ocular bioavailability, many ophthalmic drugs are applied in high concentrations or increased times of administration, but these increase the possibility of causing both ocular and systemic side-effects (Kyyronen and Urtti, 1990).

In order to overcome these drawbacks, many researchers have attempted to retain delivery systems in the front of the eye given the enormous loss of an instilled drug solution that typically occurs. Some retentive systems, such as inserts and ointments were considered. These dosage forms have proven to be of long duration and able to substantially modify drug bioavailability compared to their solution dosage form counterparts. However, these dosage forms present some disadvantages, such as blurred vision and noncompliance, which bring about some new problems to patients (Robinson and Mlynek, 1995; Edsman et al., 1998).

An ideal ophthalmic formulation should be one that: (1) can be delivered in a drop form without causing blurred vision

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or irritation; (2) has a suitable strength to endure the lacrimal fluid dilution without rapid precorneal elimination after administration; (3) has a suitable mucoadhesive force to improve the retention of the drug in the precorneal area, thereby facilitating the reservoir effect of the cornea for the drug and increasing the bioavailability.

Recently, a liquid dosage form based on an in situ gelling solution, which consists of some polymers undergoing sol–gel phase transitions as a result of a special physical/chemical change (for example, pH, temperature or a specific ion) induced by the physiological environment has been investigated as a more convenient dosage form of topical application (Edsman et al., 1998; Ridell et al., 1999; Srividya et al., 2001; El-Kamel, 2002; Hsiue et al., 2003). Poloxamer (trade name Pluronic®), a block copolymer that consists of polyethylene oxide (PEO) and polypropylene oxide (PPO) units, is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature (Gilbert et al., 1987; Lenarets et al., 1987; Edsman et al., 1998). At a concentration of 18% (w/w) or higher in aqueous solution, poloxamer 407 (P407), in which the ratio of PEO and PPO is 7:3, is transformed from a low viscosity solution to a gel under the ambient temperature. But this lower concentration solution will lose the gelation ability after diluted by lacrimal fluid. So 25% (w/w) P407 should be used to ensure the completion of the phase transition under physiological condition. However, in this case, the gelation temperature (GT) is lower than room temperature and the solution must be stored in refrigerator, which causes great inconvenience for the preparation and the use (Wei et al., 2002; Edsman et al., 1998). Therefore, poloxamer 188 (P188), which is an analog of P407, was added to P407 solution as a regulatory substance. It exhibits a good perspective to increase the GT of P407 (Wei et al., 2002; Kim et al., 2002). Although the poloxamer-based in situ gel can gel at physiological condition without rapid precorneal elimination after administration, it exhibits a relatively short contact time when compared to gellan gum or carbopol due to gradual dilution by lacrimal fluid (Edsman et al., 1998), which cannot promise a high bioavailability.

Meanwhile, a retentive dosage form based on a so-called muco/bioadhesive polymer, which is capable of attaching to mucosal surfaces, offers the prospects of prolonging the residence time of an ocular drug delivery system at the sites of drug absorption and ensures optimal contact between the formulation and the absorbing surface has been investigated to enhance the bioavailability of topical administration (Robinson and Mlynek, 1995; Ludwig, 2005). Carbopol resins, acrylic-acid based polymers which are available in a range of molecular weights and may be linear, branched or cross-linked (Robinson and Mlynek, 1995), have been investigated very frequently for the development of ocular drug delivery systems owing to their excellent mucoadhesive property. Four possible interactions to account for the adhesion of the cross-linked polyacrylic acid to mucin have been proposed by Leung and Robinson (1988).

In order to fortify the adhesion of administered drug onto the mucosal surface of rectum (Choi et al., 1998a,b; Ryu et al., 1999), vagina (Chang et al., 2002a,b; Bilensoy et al., 2006) or buccal cavity (Shin et al., 2000), mucoadhesive polymers, such as hydroxypropylcellulose (HPC), carbopol, polycarbophil and polyvinylpyrrolidone (PVP) have been added to the in situ-gelling liquid dosage forms. But few efforts on ophthalmic thermosensitive in situ gelling and mucoadhesive dosage form were available to date. Lin and Sung (2000) developed a phase change solution by using a combination of carbopol and pluronic based on the pH- and temperature-sensitive properties of these two polymers, respectively. The sol–gel transition of this solution occurred primarily due to the neutralization of the buffering action of lacrimal fluid. However, the buffer capacity of lacrimal fluid is relatively lower and the dilution by lacrimal fluid was not taken into consideration. Moreover, there was little information concerning the mucoadhesive force of this dosage form, although this may be the primary cause to ensure the effective performance of this dosage form.

The purpose of the present study is to develop not only thermosensitive in situ gelling but also mucoadhesive ophthalmic drug delivery systems containing puerarin (PU) using poloxamer analogs and carbopol. PU is an isoflavone extracted from the radix of *Pueraria lobata* (Willd.) Ohwi. It is frequently used as a therapeutic agent for cataracta glauca and ocular hypertension in China due to its ability to block β acceptors, lower intraocular pressure, and improves ocular blood flow. However, there also exist problems such as lower bioavailability and systemic absorption which may cause respiratory and gastrointestinal side-effects for PU eye drops (Xuan et al., 1999; Ren et al., 2000; Wu et al., 1998). To develop poloxamer analogs/carbopol based ophthalmic drug delivery systems which gel at physiological condition without rapid precorneal elimination after administration and remain mucoadhesive to the corneal mucin, the in vitro evaluation (rheological behaviors, mucoadhesive force and in vitro puerarin release) and in vivo evaluation (the elimination of PU in tear and the intraocular pressure (IOP)-lowering effect) were both performed in poloxamer, carbopol as well as the mixture of them.

2. Materials and methods

2.1. Materials and animals

2.1.1. Materials

Poloxamers (P407 and P188) obtained from BASF Corp. (Ludwigshafen, Germany) were used as received. Carbopol (carbopol 971P NF (CP971), carbopol 980 NF (CP980) and carbopol 1342P NF (CP1342)) were kindly gifted by BF Goodrich (Brecksville, OH, USA). PU was supplied by Sichuan Yuxin Pharmaceutical Co. Ltd. (Sichuan, China). Hydroxypropyl-β-cyclodextrin (HPCD) was purchased from Xi'an Deli Biology & Chemical Industry Co. Ltd. (Shanxi, China). All other chemicals and solvents were reagent grade.

2.1.2. Animals

New Zealand albino rabbits were obtained from the nursery of the Experimental Animal Professional Committee, Sichuan, China (License No.: SCXK (111) 2004-14). The experimental animals weighed between 2.5 and 3.0 kg, were individually housed in an air-conditioned and light-controlled room at

25 ± 1 °C and at 70 ± 5% relative humidity. They were given a standard pellet diet and were provided with water ad libitum. All animals were healthy and free of clinically observable ocular abnormalities. 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 local ethics committees for animal experimentation.

2.2. Preparation of formulations

The carbopol solutions were prepared by dispersing the required amount in a certain volume of bidistilled water with continuous stirring until completely dissolved and the bidistilled water was then added to make the volume up to the total amount. The poloxamer solutions were prepared using the cold method (Wei et al., 2002; Kim et al., 2002). A certain volume of bidistilled water was cooled down to 4 °C. P407 and P188 were then slowly added to the water with continuous agitation. The solutions were left at 4 °C until clear solutions were obtained. Bidistilled water was then added to make up the volume to the total amount. For preparation of poloxamer analogs/carbopol solutions, the already swelled carbopol solution was cooled down to 4 °C and the required amount of P407 and P188 were added. Then, the following procedures were the same as described above. For preparation of drug-containing polymer solutions, according to our former investigation, 1% (w/v) PU could be solubilized by 5% (w/v) HPCD, which is able to not only make the puerarin concentration reach the effective value, but also enhance its stability and transcorneal permeability (Wu et al., 2007). So the required amount of PU and HPCD were firstly dissolved in a certain volume of bidistilled water, and then the polymer solutions were prepared as above description. All the sample solutions were adjusted to required pH values by 0.5 M sodium hydroxide solution and then stored in the refrigerator.

In order to identify the compositions suitable for use as in situ gelling and mucoadhesive systems, aqueous solutions of different grades and various concentrations of carbopol and poloxamer analogs (formulation codes Fl, F2, . . ., F8) were prepared and evaluated for gelling capacity and transparency at physiological condition (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 then visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve (Srividya et al., 2001). The composition of artificial tear fluid (STF) was prepared according to previous report (Hagerstrom et al., 2000).

2.3. Measurement of GT

Ten milliliters of sample solution and a magnetic bar were put into a transparent vial that was placed in a low-temperature water bath. A thermometer with accuracy of 0.1 °C was immersed in the sample solution. The solution was heated at the rate of 1 °C/1–2 min with the continuous stirring of 100 rpm (Tachometer, Model RM1000, Taiwan TES Co. Ltd., China). The temperature was determined as GT, at which the magnetic bar stopped moving due to gelation (Choi et al., 1998a; Wei et al., 2002). Each sample was measured at least in triplicate.

2.4. Evaluation of formulations in vitro

2.4.1. Rheological studies

The rheological properties were determined using a cone-and-plate geometry rheometer with a diameter of 40 mm (cone angle 4°) (Gemini200, Malvern Instruments, UK). The shear stress of the sample solutions was measured at different shear rates at 25 ± 0.1 and 35 ± 0.1 °C, respectively. A typical run comprised of changing the shear rate from 0 to 200 s^{-1} at a controlled ramp speed (keeping a period of 6 s at each shear rate). Then, the hierarchy of shear rate was reversed (200–0 s^{-1}) for a similar period of 6 s. The average of two readings was used to calculate the shear stress. Evaluations were conducted in triplicate. Error bars have been omitted to retain clarity, however, in all cases the standard deviations of replicate analyses were less than 5% (Lin and Sung, 2000).

In order to simulate the physiological disposition of gels more literally, the polymer solutions were diluted by STF in a ratio of 40:7 (Wei et al., 2002) and then adjusted to physiological

Table 1
Combinations of 21% P407/5% P188 and different carbopol resins studied

Formulation	Carbopol added	Concentration (%, w/v)	pH[a]	Gelling capacity[b]	Transparency[c]
Fl	971P NF	0.1	5.5	+	++
F2	971P NF	0.2	5.0	++	++
F3	971P NF	0.3	–	++	+++
F4	980 NF	0.1	–	+++	++
F5	980 NF	0.2	–	+++	+++
F6	1342 NF	0.1	5.5	++	+
F7	1342 NF	0.2	5.0	++	+
F8	1342 NF	0.3	–	+++	+

[a] At which and 25 °C, the sample was easy to flow; –: the appropriate pH value at which the sample was easy to flow could not be found at the value range studied (pH 4.0–7.4).

[b] +: gels after a few minutes, dissolves rapidly; ++: gelation immediate, remains for few hours; +++: gelation immediate, remains for extended period. The pH values for Fl, F2, F6 and F7 were those shown in the column 4 and the values for F3, F4, F5 and F8 were all 5.0 in this study.

[c] All samples were evaluated at physiological condition (35 °C, pH 7.4 and STF dilution). +: transparent; ++: translucent; +++: turbid.

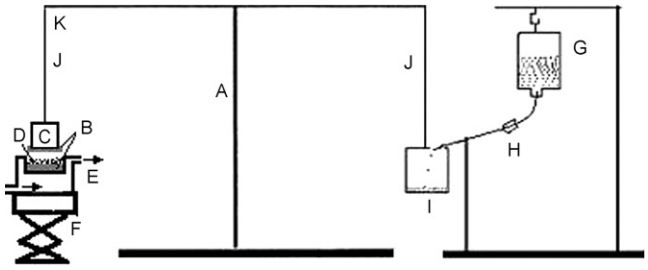

Fig. 1. Mucoadhesive force-measuring device: (A) modified balance; (B) corneal tissue; (C) Teflon cylinder; (D) gel; (E) thermostat; (F) height-adjustable pan; (G) dropping bottle; (H) infusion apparatus; (I) glass vial; (J) thin steel wire; (K) balance bar.

pH value (7.4 ± 0.1) by adding the required amount of sodium hydroxide before the rheological studies were conducted at $35 \pm 0.1\,^{\circ}C$.

2.4.2. Measurement of mucoadhesive force

The experimental technique used for determining the bioadhesive force has been derived from a previously published method (Choi et al., 1998a; Koffi et al., 2006). The experimental setup is presented in Fig. 1. Briefly, a section of tissue was cut from the cornea of the rabbit and washed with physiological saline, then immersed in newly prepared Glutathione Bicarbonate Ringer's solution at 35 °C for 10 min, which was prepared according to Montenegro et al. (2003). The corneal tissues were attached to the undersurfaces (0.785 cm^2) of the Teflon cylinder (C) and the sample cell of the thermostat (E) using a cyanoacrylate adhesive, respectively. The Teflon cylinder (C) was suspended by means of a thin steel wire (J) to the left of the balance (A). The balance (A) was made balanced. Forty microliters of polymer solution was added onto the sample cell of the thermostat (E) at 35 °C, which was placed on a height-adjustable pan (F). The height of the pan (F) was adjusted quickly to make the polymer solution just come into contact with the corneal tissue before the polymer solution shifted into gel. Then, the balance of the balance (A) was destroyed with a weight (5.0 g) put onto the left end of the balance bar (K), so that the contact was made with the force of the Teflon cylinder (C) (5.0 g). After 10 min contact, the weight was removed, so that the balance (A) could regain balance. Then, the switch (H) of the infusion apparatus was opened to make the water drop into the glass vial (I) with a constant flow rate of 5 mL/min. The weight of the water in the glass vial (I) kept increasing until the gel and the corneal tissue were detached. Bioadhesive force, the detachment stress (dyne/cm^2), was determined from the minimal weights that detached the gel and the corneal tissue. The corneal tissue pieces were changed for each measurement. To evaluate the bioadhesive force change after instillation and mixing with the tear fluid, the bioadhesive force measurements were taken at 35 °C, pH 7.4 and after diluting the formulations with STF.

2.4.3. In vitro release studies

According to previous reports (Kumar and Himmestein, 1995; Srividya et al., 2001), the in vitro release of PU from the formulations through a cellophane membrane fixed in a self-made polytef sample cell (4.0 cm i.d. and 0.9 cm in depth) using a dissolution testing apparatus (ZRS-8G, Tianjin University Precision Instrument Factory, Tianjin, China). A 1 mL volume of the formulation was accurately pipetted into this equipment. The container was immersed in 500 mL freshly prepared STF, which was used as the release medium. The temperature and rotating rate were maintained at $35 \pm 1\,^{\circ}C$ and 50 rpm, respectively. Aliquots (5 mL) were withdrawn from the release mediums at each sampling time and replaced by an equal volume of the release medium. The samples were filtered through 0.45 μm syringe filters, and were subjected to HPLC analysis to determine the PU concentrations.

2.5. Evaluation of formulations in vivo

2.5.1. Elimination of PU in tear

The Schimer tear strips technique (Small et al., 2000) was adopted as a sampling technique in this study. A Schirmer tear strip and a 2 mL polypropylene centrifuge tube and cap were weighed by an electronic balance (sartoriusBP211D, Germany). Both eyes of each rabbit were dosed by pipetting 40 μL of polymer solutions or STF, each with 1% PU, directly into the lower cul-de-sac. At each sampling time, the strip was gently inserted into the lower cul-de-sac of the eye and allowed to remain for 10 s. During sampling, contact between the strip and any visible gel lumps was avoided. It was then removed and immediately capped into the centrifuge tube, after which the tube and its contents were weighed. All capped vials and their contents were stored at $-20\,^{\circ}C$ until analysis.

The strip with tear absorbed was dried by ultra-high-purity nitrogen, and then 1 mL methanol was added to the tube. After soaked for 2 h in the methanol, each sample was centrifuged for 10 min (10,000 rpm). An 800 μL solution was shifted to another tube and evaporated to dryness with ultra-high-purity nitrogen. Then, the residue was dissolved with 80 μL mobile phase, which was then subjected to HPLC analysis to determine the PU concentration. The areas under the PU concentration versus time curves in 480 min ($AUC_{0\rightarrow 480\,min}$) were calculated using the trapezoidal rule.

2.5.2. IOP-lowering effect

IOP was measured using a Schiötz tonometer (YZ7A, 66 Vision-Tech Co. Ltd., Suzhou, China), by the same operator, using the same tonometer after instilling a drop of tetracaine (a local anaesthetic, 1% (w/v)). All measurement periods began during the same hour on each day. The left and right IOP values were alternatively measured four times within 30 min prior to giving the drug to establish baseline values for both eyes. For each pair of readings, the differences in IOP (control minus treatment eye) were determined. These predosing differences were averaged, and the mean was used to convert postadministration data to the baseline-corrected values.

Forty microliters of PU/HPCD-containing polymer solutions or STF were dosed from a micropipette. In order to avoid experimental bias, the left eye (treatment eye) of each rabbit was first administrated with drug-containing vehicle, followed

by the application of the control vehicle (formulation with no drug) to the right eye (control eye). All the solutions were instilled in the lower conjunctival sac, approximately midway between the inner and outer canthus. At regular intervals, the IOP was measured by the same method. A washout period of 5 days was allowed before reusing the same animal in the study. Change in IOP (ΔIOP) is expressed as follows: $\Delta IOP = IOP_{control\ eye} - IOP_{treatment\ eye}$. The efficiency of the different formulations was estimated by the time required to achieve peak ΔIOP (T_{max}), the peak ΔIOP (ΔIOP_{max}), and the area under the ΔIOP-time-curve ($AUC_{0\rightarrow 24\,h}$) after administration of the respective preparation.

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

3. Results and discussion

3.1. Selection of vehicle

The optimum ophthalmic thermosensitive in situ gels should have a GT higher than room temperature and a shift to gel at the conjunctival sac temperature (35.0 °C) after mixed with lacrimal fluid. In our previous study, a two-factor, five-level central composite design (CCD) was employed to optimize an ophthalmic thermosensitive gel based on P407 and P188 (Qi et al., 2006). According to the result of CCD, the formulation that contains 21% (w/v) P407 and 5% (w/v) P188 is the most suitable one, the GT before STF dilution of which is 27.3 °C and the one after STF dilution is 34.8 °C. Therefore, in this investigation, the concentrations of P407 and P188 were set to 21% (w/v) and 5% (w/v), respectively.

In order to enhance the mucoadhesive ability of 21% P407/5% P188 (abbreviated as P407/P188) based in situ gel, several carbopol resins were incorporated to the formulations. As shown in Table 1 and Table 2, the formulations F6 and F7, which contain 0.1% (w/v) and 0.2% (w/v) CP1342, respectively, not only have suitable gelling capacity and the best transparence, but also present a suitable GT. Therefore, these two formulations can be chosen as potential formulations for further investigation.

In the study of the transparence of gels under physiological condition (35 °C, pH 7.4 and STF dilution), we found that the P407/P188 solutions containing CP971 and CP980, respectively, had already become translucent or turbid before the temperature reached 35 °C. However, this phenomenon did not happen when CP1342 was incorporated into the P407/P188 solution. This can be assumed that carboxyl groups in the cross-linked poly(acrylic acid) of CP971 and CP980 molecules can form hydrogen bonds with the PEO blocks in the poloxamer molecules, which may cause the hydrophilicity of the poloxamer molecules becoming lower. When the temperature reached some extent (cloud point), the poloxamer molecules may separate out from the solution (Barreiro-Iglesias et al., 2001). According to our observation, the incorporation of CP971 or CP980 of different concentrations all had the cloud point of the P407/P188 solution lower than 35 °C, moreover, there existed a concentration-dependent property. CP1342 is a copolymer of cross-linked acrylic acid, modified by long chain (C10–C30) alkyl acrylates. This modification of the backbone increases the lipophilicity and the resistance to dissolved ions. The long chain (C10–C30) alkyl may interfere with the formulation of hydrogen bonds between the carboxyl groups of poly(acrylic acid) and the PEO blocks of poloxamer molecules. So the hydrophilicity of poloxamer molecules has a relatively smaller decrease. In our study, even when the concentration of CP1342 reached to 0.3% (w/v), the cloud point of the combined polymer solution was not observed and the solution was still clear and transparent after the autoclaving at 121 °C for 30 min. Therefore, it would not influence the normal vision after administrated in vivo.

Table 2
Gelation temperatures before and after STF dilution for different formulations

Sample	Gelation temperature (°C)[a]	
	Before STF dilution	After STF dilution
21% P407/5% P188	27.3 ± 0.3	34.8 ± 0.1
1% PU/5% HPCD + 21% P407/5% P188	28.7 ± 0.2	36.4 ± 0.2
F6	26.2 ± 0.4	33.7 ± 0.3
1% PU/5%HPCD + F6	27.1 ± 0.3	34.6 ± 0.2
F7	25.4 ± 0.1	32.8 ± 0.4
1% PU/5% HPCD + F7	26.3 ± 0.1	34.0 ± 0.3

[a] Each value represents the mean ± S.E. of four experiments.

3.2. Rheological studies

Fig. 2 shows the shear stress versus shear rate flow curves of different formulations. The steady shear behavior of different polymer solutions was influenced by the temperature and pH.

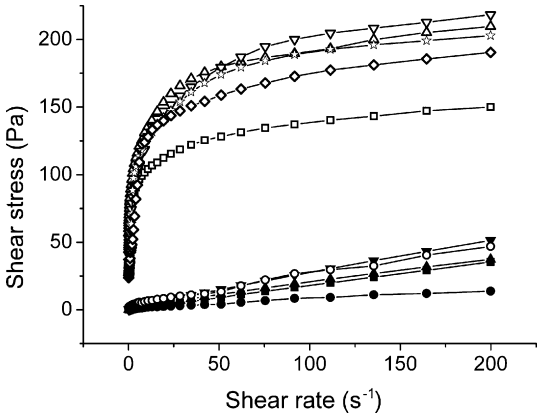

Fig. 2. Shear stress vs. shear rate flow curves of different polymer solutions. (■) 21% P407/5% P188 solution measured at 25 °C, pH 7.4; (□) 21% P407/5% P188 solution measured at 35 °C, pH 7.4 and after STF dilution; (●) 0.2% CP1342 solution measured at 25 °C, pH 4.0; (○) 0.2% CP1342 solution measured at 35 °C, pH 7.4 and after STF dilution; (▲) formulation F6 measured at 25 °C, pH 5.5; (△) formulation F6 measured at 35 °C, pH 7.4 and after STF dilution; (▼) formulation F7 measured at 25 °C, pH 5.0; (▽) formulation F7 measured at 35 °C, pH 7.4 and after STF dilution; (◊) PU/HPCD-containing formulation F6 measured at 35 °C, pH 7.4 and after STF dilution; (☆) PU/HPCD-containing formulation F7 measured at 35 °C, pH 7.4 and after STF dilution. All the measurements were conducted in triplicate and the standard deviations of replicate analyses were all less than 5%.

Therefore, the steady shear behavior of different formulations was examined at the non-physiological condition (25 °C, pH 7.4 for P407/P188, pH 4.0 for 0.2% CP1342, pH 5.5 for F6 and pH 5.0 for F7) and the physiological condition (35 °C, pH 7.4 and STF dilution), respectively. At non-physiological condition, the shear stress of all the polymer solutions increased linearly with an increase in shear rate, demonstrating a Newtonian flow behavior (Miller and Drabik, 1984). The reason why these polymer solutions exhibit this property is that under this condition, the molecules and micelles of these polymers did not entangle. All the polymer solutions did not undergo a phase change to turn into a gel and were still easily flowing liquid, similar to pure water, which displays a typical Newtonian flow behavior. The rheological behavior of 0.2% CP1342 under physiological condition also demonstrated a Newtonian flow behavior (Miller and Drabik, 1984). This may be due to its lower concentration used and the dilution by STF, which lead to that it cannot turn into a relatively hard gel. However, P407/P188, F6 and F7 at physiological condition resisted the initial rotary motion, and a sudden increase in the shear stress was observed at higher shear rates. The solutions began to flow after the shear stress reached its yield point. Therefore, the flow curve for these three solutions under the physiological condition exhibited pseudoplastic behavior with hysteresis (Patton and Robinson, 1975; Lin and Sung, 2000). This hysteresis phenomenon indicated the presence of an elastic component. Thus, the strong gels formed with these polymers can be characterized as viscoelastic materials. Viscoelastic material normally shows hysteresis under cyclic deformation (Kumar et al., 1994). Viscoelastic materials are preferable to viscous materials because they flow more easily and are less susceptible to deformation due to stress.

For all the polymer solutions except 0.2% CP1342, the shear stresses under physiological condition were much higher than those under non-physiological condition. For example, the shear stresses of P407/P188, F6 and F7 under physiological conditions were 4.24, 5.63 and 4.26 times greater than those under non-physiological condition at the shear rate of 200 s^{-1}, suggesting the occurrence of phase transition between these two conditions for these three systems. Under the physiological condition, the shear stresses of F6 and F7 were higher than those of P407/P188 and 0.2% CP1342. For instance, at the shear rate of 200 s^{-1}, the shear stress of F6 was 1.40 and 4.48 times greater than that of P407/P188 and 0.2% CP1342, respectively, and the shear stress of F7 was 1.46 and 4.66 times greater than that of P407/P188 and 0.2% CP1342, respectively. Meanwhile, the shear stress of F7 was a little higher than that of F6 at each shear rate. For the 0.2% CP1342 solution, by varying the shear rate from 0 to 200 s^{-1}, the shear stress at physiological condition was enhanced only a little compared with that at non-physiological condition.

When the non-physiological condition was changed into physiological condition with a temperature from 25 to 35 °C, the shear stress of P407/P188 solution had a significant increase. This can be owing to the thermosensitive in situ gelling property of poloxamer. When the concentration and temperature of the polymer are above a critical value, poloxamer molecules in aqueous solution will self-assemble to form spherical micelles with a dehydrated PPO core surrounded by hydrated swollen PEO chains (Wei et al., 2002). The thermoreversible gelation behavior is accepted as a result of micellar entanglement and packing with the increase of temperature. Furthermore, it is generally accepted that the PPO that is hydrophobic has the GT lowered and the PEO that is hydrophilic has the GT increased (Vadnere et al., 1984; Zhou and Chu, 1987; Wanka et al., 1990; Cabana et al., 1997; Bohorquez et al., 1999). Accordingly, a different PEO/PPO ratio will lead to a different GT. P407 and P188 possess the different PEO/PPO ratios. Thereby the optimal GT can be reached by mixing different amounts of P407 and P188 in aqueous solution. As a poly(acrylic acid), CP1342 not only has a mucoadhesive property, but also exhibits a pH-sensitive in situ gelling property. Its aqueous solutions can transform into stiff gels when the pH is raised. This is related to the increase in ionization as a result of increased pH, which leads to an increase in electrostatic repulsion between adjacent carboxyl groups and the subsequent expander polymer network (Kumar and Himmestein, 1995; Jeong et al., 2002). On the other hand, the hydrophobic nature of carbopol backbone may form hydrophobic interchain aggregation. This cross-linking phenomenon may result in the formation of more viscous gel at an acidic condition (Lin and Sung, 2000). However, in this investigation, the shear stresses of 0.2% CP1342 from non-physiological condition to physiological condition only had a slight increase. This can be attributed to the relatively lower CP1342 concentration used in this study in order to keep free flowing properties under non-physiological condition. On the other hand, the dilution by STF also had a great influence on the shear stress under the physiological condition, suggesting that the lower concentration CP1342 alone in vivo may not have enough strength to withstand the turnover and dilution of the lacrimal fluid, which may lead to a short precorneal residence time, although it has an excellent mucoadhesive property. For the formulations of F6 and F7, hydrogen bonds will form between the poloxamer molecules and CP1342 molecules through the carboxyl groups of poly(acrylic acid) and the PEO blocks of poloxamer molecules (Choi et al., 1998a,b). Although this combination will become weaker as the influence of the long chain alkyl existing in the backbone of CP1342 molecule, it can still leads to the formation of three-dimensional network and stronger gel (Jeong et al., 2002). Therefore, the shear stress of the combined polymer solution was significantly higher than that of individual poloxamer and CP1342 solutions at each shear rate, suggesting that the combined polymer solution may have enough strength to withstand the turnover and dilution by lacrimal fluid in vivo and a long precorneal residence time can be obtained.

In order to investigate the influence of PU/HPCD on the rheological behaviors of the combined polymer solutions, the rheological studies on the PU/HPCD-containing combined polymer solutions at physiological condition were performed. As shown in Fig. 2, the incorporation of PU/HPCD did not change the tendency of the flow curves of F6 and F7, which still demonstrated the pseudoplastic flow behaviors. This indicates that the incorporation of PU/HPCD did not disrupt the strong three-dimensional gel network formed at physiological condition. However, the shear stress of PU/HPCD-containing combined polymer solution was slightly lower than that of F6 and F7,

respectively, at each shear rate. This can be assumed that the hydrogen bonds may form between the hydroxyl groups on the surface of HPCD molecules and the PEO blocks of poloxamer molecules, which will interfere with the binding force between the poloxamer molecules and CP1342 molecules or within the poloxamers (Kim et al., 2002). On the other hand, the HPCD with a hydrophilic outer shell may mask the hydrophobic interaction between the backbone chains of PPO in poloxamer and the poly(acrylic acid) backbone chains modified by hydrophobic long chain (C10–C30) alkyls in CP1342 through selectively solvating the polymer chains. On the whole, the rheological behaviors of the combined polymer solutions were not significantly affected by the incorporation of PU/HPCD.

3.3. Determination of mucoadhesive force

Ocular mucoadhesion relies on the interaction of a polymer and the mucin coat covering the conjunctiva and corneal surfaces of the eye. This mucus layer is secreted by goblet cells of the conjunctiva. Structurally, mucin consists of a protein or polypeptide core with carbohydrate side chains branching off the core. The polymer with many hydrophilic functional groups (e.g. carboxyl group, hydroxyl group and sulfate) can establish electrostatic and hydrophobic interactions and hydrogen bond with the underlying surface. Of these noncovalent forces, hydrogen bonding appears to be the most important (Robinson and Mlynek, 1995; Lee et al., 2000). In this investigation, mucoadhesive force means the force with which polymers bind to corneal surface under physiological condition.

As shown in Fig. 3, in the absence of PU/HPCD, the incorporation of CP1342 could significantly enhance the mucoadhesive force of P407/P188 under physiological condition. The mucoadhesive forces of F6 and F7 were 3.0 and 3.7 times greater than that of the P407/P188, respectively. The mucoadhesive force of 0.2% CP1342 alone was 1.6 times greater than that of the P407/P188. These can be mainly attributed to the hydrogen bonds between the carboxyl groups of the CP1342 molecule and the carboxyl groups of the carbohydrate side chains of the mucin. Besides, electrostatic, hydrophobic interactions and interdiffusion of the mucin and the polymer also have some contribution. However, although CP1342 has a relatively better potentiality of mucoadhesion, the lower concentration of CP1342 alone after STF dilution fails to gelatinize completely and obtain sufficient gel strength, which leads to the fact that CP1342 cannot contact and interact with the mucin layer thoroughly. Therefore, in this investigation, 0.2% CP1342 alone did not exhibit a good mucoadhesive force. When CP1342 was combined with P407/P188, F6 and F7 exhibited excellent mucoadhesive ability based on the thermosensitive in situ gelling property of P407/P188 and the mucoadhesive property of CP1342. However, in the presence of PU/HPCD, the mucoadhesive force of P407/P188 exhibited a very significant ($P<0.01$) decrease of 62%. The mucoadhesive forces of F6 and F7 also had a significant ($P<0.05$) decrease, 6.5% and 7.8%, respectively. There was little influence on the mucoadhesive force of 0.2% CP1342 with the incorporation of PU/HPCD ($P>0.05$). The possible reason for these results is with the incorporation of PU/HPCD, the GT of the P407/P188 was a little higher than 35 °C, which leaded to the process of gelatinization not to thoroughly complete. On the other hand, the hydroxyl groups of the HPCD molecules can form hydrogen bonds with the PEO blocks of poloxamer molecules (Kim et al., 2002), which may interfere with the hydrogen bonds between the polymer and the mucin. Meanwhile, the HPCD with a hydrophilic outer shell may also interfere with the hydrophobic interaction between the polymer and the mucin.

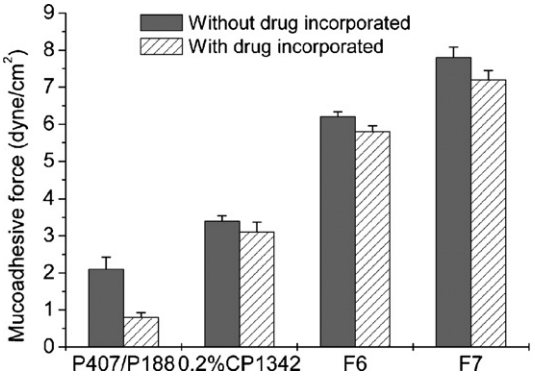

Fig. 3. Comparison of the mucoadhesive force of various polymer solutions with and without PU/HPCD incorporated at 35 °C, pH 7.4 and after STF dilution. Each bar represents the mean ± S.E. of three determinations.

3.4. In vitro release studies

The cumulative percent of PU released versus time profiles for PU/HPCD-containing polymer solutions or STF is shown in Fig. 4. For the PU/HPCD-containing STF, almost all the PU released immediately after the start of release study. Approximately 80% of PU already released after 15 min. For the PU/HPCD-containing 0.2% CP1342, about 70% of PU was released into the medium after 45 min, about 95% after 6 h. The PU/HPCD-containing P407/P188 had a similar release trend as the PU/HPCD-containing 0.2% CP1342 within 45 min, and then

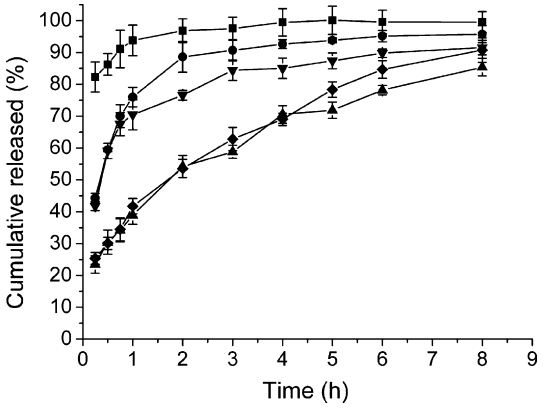

Fig. 4. Cumulative amount of PU released as a function of time from various PU/HPCD-containing solutions. (■) PU/HPCD-containing STF; (▼) PU/HPCD-containing 21% P407/5% P188 solution; (●) PU/HPCD-containing 0.2% CP1342 solution; (♦) PU/HPCD-containing formulation F6; (▲) PU/HPCD-containing formulation F7. Each value is the mean ± S.E. of three determinations.

the release profile became slow. There was a 90% of PU released after 6 h. In the case of formulation F6, only about 35.5% of PU was released into the medium after 45 min, approximately 84.7% after 6 h. The formulation F7 had a similar release trend as the formulation F6 in the main. Only at the time points of 5 and 6 h, the cumulative percents of formulation F7 were significantly lower than that of formulation F6 ($P < 0.05$). These results demonstrated that the formulations of F6 and F7 had relatively better sustained-release effect and can be used as an ophthalmic sustained release drug delivery system. The release data of the formulations of F6 and F7 over the whole time period were analyzed according to the treatment proposed by Higuchi (1962) for drug release from semisolid vehicles containing dissolved drug. It can be obtained that the cumulative amount is proportional to the square root of the time (up to 80% of total drug released for F6 and up to 90% for F7) and a linear relationship with a correlation coefficient is higher than 0.99. Similar results were also obtained by other researchers in other polymer systems (Kumar and Himmestein, 1995; Liu et al., 2006). The linear relationships in conjunction with the slow dissolution rate suggest that the in vitro drug release from formulations F6 and F7 under physiological conditions occurs primarily by diffusion.

3.5. Elimination of PU in tear

Fig. 5 shows the PU concentration in the tear fluid as a function of time. Although the general trend of all these PU concentration versus time curves was similar, the PU concentration of each PU/HPCD-containing polymer solution was higher than that of PU/HPCD-containing STF almost at each time point. For the individual P407/P188 and CP1342 formulations, the PU concentrations for the P407/P188 formulation were higher than those for the CP1342 formulation between 10 and 60 min of experimental times, and then after that, PU concentrations were lower than those for the CP1342 formulation. This indicates that at the initial time period, the P407/P188 formulation was suffered a smaller precorneal elimination owing to its thermosensitive in gelling property. However, the CP1342 exhibited a better ability to hold PU in tear afterwards due to its mucoadhesive property. For the formulations of F6 and F7, there was a similar trend for them between 10 and 120 min, at which the PU concentrations of them were roughly higher than those of the formulations of P407/P188 and CP1342. However, between 160 and 480 min, the PU concentrations of F7 were slightly higher than those of F6, which had a similar trend to the CP1342 formulation.

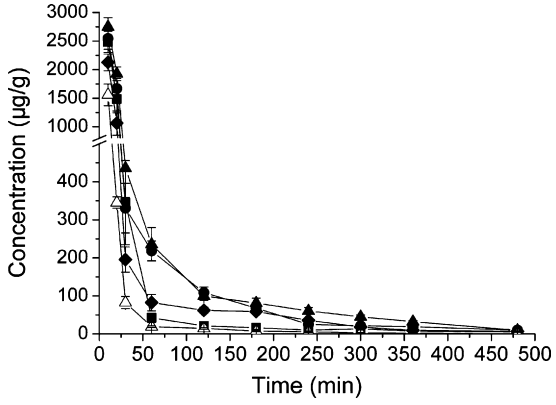

Fig. 5. PU concentrations in the tear fluid as a function of time following administration of various PU/HPCD-containing solutions. (△) PU/HPCD-containing STF; (■) PU/HPCD-containing 21% P407/5% P188 solution; (♦) PU/HPCD-containing 0.2% CP1342 solution; (●) PU/HPCD-containing formulation F6; (▲) PU/HPCD-containing formulation F7. Each value is the mean ± S.E. of four determinations.

Table 3
The area under the PU concentration in tear vs. time profiles in 480 min ($AUC_{0\rightarrow480\,min}$) for various formulations

Vehicles	$AUC_{0\rightarrow480\,min}$ (μg min/g)[a]	Ratio
STF	13883.9 ± 1523.9	
21% P407/5% P188	44689.9[b] ± 2107.0	3.22
0.2% CP1342	40647.0[b] ± 4595.5	2.93
F6	61526.9[b] ± 2161.1	4.43
F7	72978.6[b] ± 4159.3	5.26

[a] Each value represents the mean ± S.E. of four determinations.
[b] $P < 0.01$ vs. STF-based vehicle.

The $AUC_{0\rightarrow480\,min}$ values for various formulations are listed in Table 3. The differences between the $AUC_{0\rightarrow480\,min}$ following administration of the PU/HPCD-containing polymer solutions and the PU/HPCD-containing STF were all statistically significant ($P < 0.01$). The $AUC_{0\rightarrow480\,min}$ values of the formulations F6 and F7 were 4.43 times and 5.26 times greater than that of STF containing PU/HPCD, respectively. Less pronounced increases in $AUC_{0\rightarrow480\,min}$ value were observed for the P407/P188 (3.22-fold) and CP1342 (2.93-fold) formulations as compared to the STF containing PU/HPCD. These indicate that a greater amount of drug was present in the precorneal area during the 480 min time period when these polymer solutions were instilled in the eye, as compared to the STF containing PU/HPCD. The AUC serves as an indicator of the precorneal exposure to the drug and thus, for some drugs, the therapeutic efficacy of the formulation. For the polymer solutions, the formulations F6 and F7 had better performance than the formulations P407/P188 and CP1342 and the formulation F7 had the best retentive effect among them owing to the higher concentration of CP1342.

3.6. IOP-lowering effect

Fig. 6 shows the pharmacological response (the change in IOP, ΔIOP) versus time profiles for various PU/HPCD-containing polymer solutions and the PU/HPCD-containing STF. It can be seen from this figure that approximately before 2 h, the change of IOP was greater for the PU/HPCD-containing STF than for the PU/HPCD-containing polymer solutions and after that, the change of IOP became lower for the PU/HPCD-containing STF than for the PU/HPCD-containing polymer solutions, although the general shape of the profile was similar. The results of this experiment also demonstrated that F6 and F7 effectively prolonged the IOP-lowering effect to 24 h after administration, which was significantly better than STF (8 h) and relatively better than P407/P188 and CP1342 (>8 and <24 h).

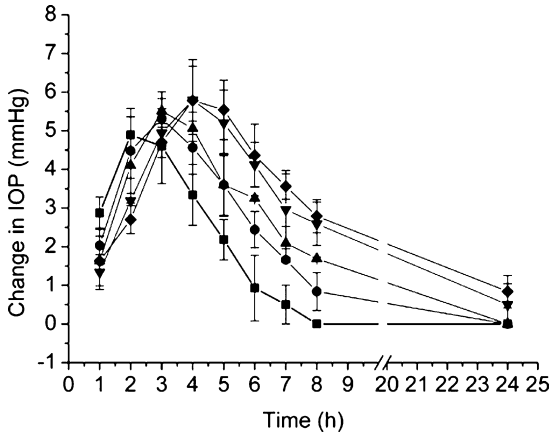

Fig. 6. Change in IOP vs. time for different formulations. (■) PU/HPCD-containing STF; (●) PU/HPCD-containing 21% P407/5% P188 solution; (▲) PU/HPCD-containing 0.2% CP1342 solution; (▼) PU/HPCD-containing formulation F6; (♦) PU/HPCD-containing formulation F7. Each value is the mean ± S.E. of five determinations.

Table 4
Pharmacokinetic parameters of PU from 21% P407/5% P188, 0.2% CP1342, F6, F7 and STF[a]

Vehicles	T_{max} (h)	ΔIOP_{max} (mmHg)	$AUC_{0\rightarrow 24\,h}$ (mmHg h)
STF	1.06 ± 0.27	4.94 ± 0.57	16.33 ± 2.09
21% P407/5% P188	1.81 ± 0.26	5.24 ± 1.17	27.04[b] ± 3.18
0.2% CP1342	1.71 ± 0.35	4.76 ± 0.29	27.19[b] ± 1.53
F6	3.47[b] ± 0.84	4.85 ± 0.23	50.51[b] ± 16.12
F7	3.93[b] ± 1.08	4.18 ± 0.34	58.90[b] ± 11.48

[a] Each value represents the mean ± S.E. of four determinations.
[b] $P < 0.05$ vs. STF-based vehicle.

The pharmacokinetic parameters are shown in Table 4. It can be seen that the T_{max} of PU from P407/P188 and CP1342 shows no significant differences at a 0.05 probability level compared with PU from STF. In contrast, F6 and F7 gave significantly faster T_{max} of PU than did STF ($P < 0.05$). However, the ΔIOP_{max} of PU from all the polymer solutions were not significantly different from that from STF. 3.09-fold and 3.61-fold increase ($P < 0.05$) in $AUC_{0\rightarrow 24\,h}$ were obtained for F6 and F7, respectively, relative to the STF. Less pronounced increases in ΔIOP were observed for P407/P188 (1.66-fold, $P < 0.05$) and CP1342 (1.67-fold, $P < 0.05$) solutions as compared to the STF.

4. Conclusion

In this study, a thermosensitive in situ gelling and mucoadhesive ophthalmic drug delivery system containing puerarin based on P407/P188 and CP1342 was developed. We have demonstrated that incorporating either 0.1% or 0.2% CP1342 into P407/P188 solution under physiological condition did not affect the rheological properties and would enhance the mucoadhesive force significantly. Both in vitro and in vivo results indicated that the combined poloxamer analogs and carbopol solutions performed better in retaining drugs than individual solution did. The combined solutions which were free flowing liquid below the room temperature would shift to firm gels after administration, which could ensure suitable gel strength and prevent rapid precorneal elimination, and attach to the ocular mucosal surface for a relative long time, which could improve the retention of the drug and promise a high bioavailability. Therefore, the combined systems can be used as the ocular in situ gelling and mucoadhesive vehicles to enhance bioavailability.

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