Development of a Poloxamer Analogs/Bioadhesive Polymers-Based *In Situ* Gelling Ophthalmic Delivery System for Tiopronin

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ABSTRACT: The purpose of this study was to develop a poloxamer analogs/bioadhesive polymers-based *in situ* gelling ophthalmic delivery system aiming at enhancing bioavailability and anticataract effect. The effect of poloxamer 407 (P407), poloxamer 188 (P188), carbopol 934P (C934), and sodium hyaluronate (NaHA) concentration on the gelation temperature (GT) was examined. The GT of P407 based *in situ* gel increased with an increase in the P188 concentration. NaHA and C934 lowered the GT of poloxamer analogs based *in situ* gel. Correlation analysis demonstrated that *in vitro* drug release from *in situ* gel was controlled by gel dissolution and followed zero-order kinetics. Tiopronin *in vitro* transcorneal transit accorded with zero-order kinetics. Twenty-two percent P407 and 6% P188 containing 0.2% NaHA based formulation can be chosen as *in situ* gel matrix of tiopronin because of proper GT and sustained releasing ability. *In vivo* study showed that the area under the aqueous humor–concentration time curve of tiopronin increased by 1.6 folds for *in situ* gel, compared with tiopronin aqueous solution. High-dose tiopronin *in situ* gel and solution delayed the development of selenite cataract 6 d and 4 d, respectively. The results showed that tiopronin *in situ* gel exhibits higher bioavailability and therapeutical effect. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 114: 775–783, 2009

Key words: *in situ* gel; tiopronin; ophthalmic drug delivery system; permeability; bioavailability; cataract

INTRODUCTION

When a traditional eye drop is dropped into the eye, the effective tear drainage and blinking action of the eye result in a 10-fold reduction in the drug concentration within 4–20 min.[1] Because of tear drainage, most of the administered dose passes *via* the nasolacrimal duct into the gastrointestinal tract, leading to side effects. Rapid elimination of the traditional eye drops administered often results in a short duration of the therapeutic effect making a frequent dosing regimen necessary. Ocular therapy would be significantly improved if the precorneal residence time of drugs could be increased. Several new preparations have been developed for ophthalmic use, not only to prolong the contact time of the vehicle on the ocular surface but also to slow down drug elimination.[2–4] For example, some inserts and collagen shields are successful preparations, but they present some disadvantages of noncompliance, especially by elderly people, and many patients lose the device sometimes without becoming aware of it.[5] From the point of view of patient acceptability, a liquid dosage form is preferable.

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The problem can be overcome by using *in situ* gel-forming ophthalmic drug delivery systems prepared from polymers that exhibit reversible phase transitions (sol–gel) and pseudoplastic behavior to minimize interference with blinking.[5] Such a system can be formulated as a liquid dosage form suitable to be administered by instillation into the eye, which, on exposure to physiological conditions, changes to the gel phase, thus, increasing the precorneal residence time of the delivery system and enhancing ocular bioavailability.

Tiopronin is a kind of glycine derivative with thiol group. Its chemical name is *N*-(2-mercaptopropionyl) glycine. Because of the sulfhydryl on side chain, tiopronin has various pharmacologic actions, and was always clinically used to treat hepatitis, hepatic injury, and cystinuria. In addition, tiopronin also has curative effect on senium cataract through cleaning excess free radicals and inhibiting crystallin agglomeration.

The overall objective of the present study was to develop and evaluate an *in situ* gel containing poloxamer analogs and bioadhesive material to improve the ocular bioavailability and anticataract effect of tiopronin. To achieve this objective, the concentration of poloxamer 407 and poloxamer 188 was examined. The influence of concentration of various polymers on gelation temperature (GT) was also

investigated. In an attempt to improve the ability of bioadhesion, various bioadhesive polymers were added to poloxamer solution containing tiopronin. A membraneless model was used to study the gel dissolution and drug release simultaneously. The pharmacokinetics of *in situ* gel was studied compared with tiopronin solution and pharmacodynamics of *in situ* gel and tiopronin solution were studied using the model of selenite-induced cataract rats.

MATERIALS AND EXPERIMENTAL

Materials

Poloxamers 407 and Poloxamers 188 (P407 and P188) obtained from Badishe Anihn & Soda Fabrik (BASF) (Ludwigshafen, Germany) were used as received. Carbopol® 934P (C934) was obtained from Goodrich (USA). Sodium hyaluronate (NaHA) was obtained from Shanghai Bioengineering (China). Sodium selenite (Na_2SeO_3) was supplied by Wako Pure Chemical Industries (Japan). Bromophenacyl bromide (*p*-BPB) was purchased from Santen Pharmaceutical (Japan). All the other chemicals were of analytical grade and commercially available.

Animals

Male and female Wistar rats, 13 days old, and New Zealand adult albino rabbits, weighing 2.5–3.0 kg, were used in this study. They were housed under standard conditions (12 h/d fluorescent light at 7:00–19:00, room temperature) and given a commercial diet (CE-2, Clea Japan) and water ad libitum (rat pups were kept with their mother). All procedures were performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) resolution on the use of animals in research.

Preparation of formulations

The preparation of the tiopronin solution was as the following procedure. A total of 0.025 g ethyl hydroxybenzoate and 0.1 g hydroxypropyl methyl cellulose (HPMC) were dissolved in 40°C water. The other ingredients (0.1 g tiopronin, 0.05 g sodium bisulfite, 0.05 g EDTA, 0.075 g azone, and 6 g mannitol) were added to the solution, completely stirred and pH was adjusted to 5.8 with 0.5 M NaOH.

The preparation of the tiopronin *in situ* gel was as the following procedure. The carbopol solutions were prepared by dispersing the given amount in a certain volume of bidistilled water with 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.[6,7] A certain volume of bidistilled water was cooled down to 4°C. P407 and P188 were then slowly added to the bidistilled water with continuous stirring. The solutions were kept at 4°C until clear solutions were obtained. Bidistilled water was then added to make the volume up to the total amount. For preparation of poloxamer analogs/carbopol or NaHA solutions, the already dissolved carbopol or NaHA 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, tiopronin was first dissolved in a certain volume of bidistilled water, and then the polymer solutions were prepared as described earlier. All the sample solutions were adjusted to required pH values by 0.5 M sodium hydroxide solution and then stored in a refrigerator. To develop the compositions suitable for use as *in situ* gel and mucoadhesive systems, poloxamer analogs solutions with various concentrations of C934 and NaHA (formulation codes F_1, F_2, ..., F_8) were prepared and evaluated for gelling capacity at physiological condition (Table I). The gelling capacity was determined by placing 100 μL of the system in a vial containing 2 mL of simulated tear fluid (STF) freshly prepared and equilibrated at 35°C and then visually assessing the gel formation and noting the time for gelation. The composition of STF was prepared according to previous report.[8]

Measurement of the GT

Ten milliliters of sample solution and a magnetic bar were put in 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/min with continuous agitation (100 r/m). The temperature was determined as GT, at which the magnetic bar stopped due to gelation.[8–10] Each sample was measured in triplicate.

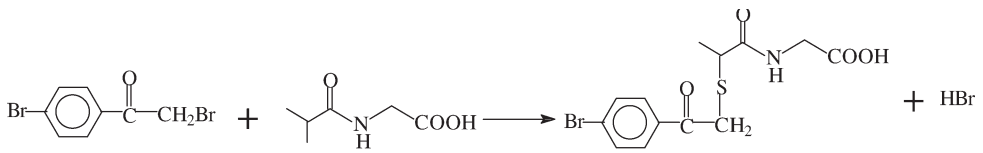
TABLE I
GT Before and After STF Dilution for the Formulations Containing 22% P407/6%P188 and Different Bioadhesive Polymers

Formula	Concentration of bioadhesive polymers (w/v)	GT before STF dilution (°C)	GT after STF dilution (°C)
F_0	0	28.0 ± 0.4	34.9 ± 0.2
F_1	0.1% Carbopol	26.7 ± 0.2	34.1 ± 0.3
F_2	0.2% Carbopol	25.9 ± 0.4	33.2 ± 0.2
F_3	0.3% Carbopol	24.0 ± 0.3	32.0 ± 0.5
F_4	0.4% Carbopol	22.2 ± 0.5	30.8 ± 0.2
F_5	0.1% NaHA	27.2 ± 0.2	35.1 ± 0.3
F_6	0.2% NaHA	26.8 ± 0.5	35.3 ± 0.2
F_7	0.3% NaHA	25.5 ± 0.4	34.0 ± 0.4
F_8	0.4% NaHA	24.1 ± 0.6	33.6 ± 0.3
F_9	0.5% NaHA	23.2 ± 0.4	32.7 ± 0.5

Precolumn derivation HPLC assay of tiopronin

High-performance liquid chromotography (HPLC) was used to determine the tiopronin contents *in vivo* cornea-transit experiment. The HPLC apparatus (LC-10AD)(Shimadzu, Japan) consisted of a liquid chromatographic system equipped with a UV SPD-10A (Shimadzu, Japan) and the analysis was performed on a Diamonsil RP-18 column (200 mm × 4.6 mm, 5 μm) (Diamonsil, Japan) with a mobile phase composed of methanol, acetonitrile, and 0.1% acetic acid (40 : 35 : 25, V : V : V). Quantitative detection of tiopronin was performed at a wavelength 263 nm with 1.0 mL/min flow rate at room temperature.

The tiopronin, a sulfhydryl compound, is unstable in aqueous humor. *p*-BPB is used as a derivatizing reagent to determine the concentration of tiopronin precisely. The reaction of *p*-BPB (A) with tiopronin (B) to form the derivative (C) is shown by following equation:



(A) (B) (C)

10 μL aqueous humor obtained in the in vivo transcorneal transit experiment of tiopronin was completely mixed with 5 μL NaOH solution (0.2 M) and kept for 5 min at room temperature. To this mixture was added 40 μL *p*-BPB solution (100 μg/mL). To achieve complete tiopronin derivatization, the mixture was stirred for 30 min. Then, 5 μL 0.2 mol/L HCl was added, mixed and centrifuged (1.6×10^4 g) for 1min. The supernatant (10 μL) was subjected to chromatographic separation.

Gel dissolution dynamics and *in vitro* drug release

A membraneless model was used to study the gel dissolution and drug release simultaneously. Three grams of eye drop was put in a test tube weighed previously. After preheating for 10 min at 35°C, the *in situ* gel eye drops completely gelated. Two milliliters STF at 35°C was added to the gelating *in situ* gel as release medium. The test tube was kept thermostatically agitating for 20 min at 35°C, all release medium was decanted, and the *in situ* gel was weighed and then preheated for 10 min at 35°C. Two milliliters fresh STF was added again and used as release medium again. Then, the following procedures were the same as described above until the 20% gel was remained. Weight variation corresponding to consecutive time points was the amount of gel dissolution. The taken release medium was properly diluted, filtered through 0.45-μm microporous membrane and analyzed by HPLC. Accumulative release amount of tiopronin was calculated. The curves of accumulative release amount of tiopronin versus time were drawn.

In vitro transcorneal transit of tiopronin

The *in vitro* transcorneal transit of tiopronin was examined using the method of Iwata et al.[11] Rabbits were killed by injection of a lethal dose of pentobarbital into the marginal ear vein. The eyes were removed and the corneas were carefully separated from other ocular tissues. The individual corneas were placed in a methacrylate cell designed for transcorneal transit studies. The side of the chamber (donor) with the exterior surface of the cornea was filled with 2 mL tiopronin solution or *in situ* gel eye drops and 0.5 mL STF. The other side of the chamber (acceptor) containing 2.5 mL 10 mM N-2-hydroxyethylpiperazine-N-ethane-sulphonic acid (HEPES) buffer (pH 7.4) with 136 mM NaCl, 5.3 mM KCl, 1 mM K_2HPO_4, and 1.7 mM $CaCl_2$ and 5.5 mM glucose that was preheated at 35°C and saturated with O_2—CO_2 (95 : 5) mixture. The temperature was maintained at 35°C by an external water bath and the transit studies lasted for 3 h. Three hundred microliters of the sample solution was withdrawn from the acceptor chamber at the indicated times. The content of tiopronin in the sample was directly determined by common HPLC. A mobile phase composed of methanol and water (15 : 85, V : V) adjusted by phosphoric acid to pH 2.5. Quantitative detection of tiopronin was performed at a wavelength 210 nm with 1.0 mL/min flow rate at room temperature. The viability of the corneas was monitored by measurement of the thickness and hydration level (no significant changes in thickness or hydration level were observed over the 3-h period).

In vivo transcorneal transit of tiopronin

The experiment was performed by a previous method.[12] Six rabbits were divided randomly into two groups, after the rabbits were anesthetized by an abdominal injection of 0.45 mL Nembutal Sodium (50 mg/mL). Three rabbits in each group were instilled with 50 μL *in situ* gel or solution eye drops

(containing 1 mg/mL tiopronin), respectively. Ten microliters of aqueous humor samples were removed from the anterior chamber of the eye at specific times in the same rabbit using a 29-guage needle with silicon tubing (inner diameter: 0.5 mm) connected to a 25-μL microsyringe. The tiopronin content of each sample was determined by precolumn derivatization HPLC as described earlier. The area under the aqueous humor–concentration time curve (AUC), elimination rate constant (K_e) and half-life ($t_{1/2}$) for transcorneal tiopronin transit were calculated by applying a one-compartment model with a first-order absorption process.[13,14]

Administration of eye drops in anticataract studies

The eyelids of rat pups, 13 days old, were opened gently and carefully with blunt tweezers. Five microliters of 1 mg/mL, 2 mg/mL, and 4 mg/mL tiopronin solution and *in situ* gel eye drops were instilled into the eyes of different groups of animals separately. The eyes were kept open for 1 min to prevent the eye drops from overflowing. Eye drops were instilled four times per day for 1 week. At 30 min after the first instillation, except the normal control group, a single subcutaneous injection of selenite (19 μmol/kg body weight) was administered to induce of selenite cataract. The normal control rats were only instilled with normal saline, and model control group rats were instilled with normal saline within 30 min before sodium selenite injection.

Image analysis of cataract development

The experiment was performed as described by Ito et al.[15] All animals were alive during opacification monitoring. The pupils were dilated with 0.1% pivalephrine 5 min before taking slit photographs using a photo slit lamp microscope and an anterior eye segment analysis systetriom (EAS-1000, Nidek, Japan) at selected time points from 1 to 8 d. The lens images were obtained using an EAS-1000 equipped with a CCD camera. The flash level was 100 Watt-second and the slit length was 4.4 mm. The area of opacity, in pixels, was analyzed by a computer and image analysis software connected to EAS-1000.[16,17] The outline of the slit lens image was determined by selecting points on the image. The transparent area within the outline and threshold level was set automatically by the computer software. There are approximately 100 threshold levels both for a normal lens and a lens with cataracts. The total area of opacity of the lenses, expressed as pixels, was calculated by the following equation:

$$\text{Pixels within opacity} = \text{pixels within outline} - \text{pixels within transparent area}$$

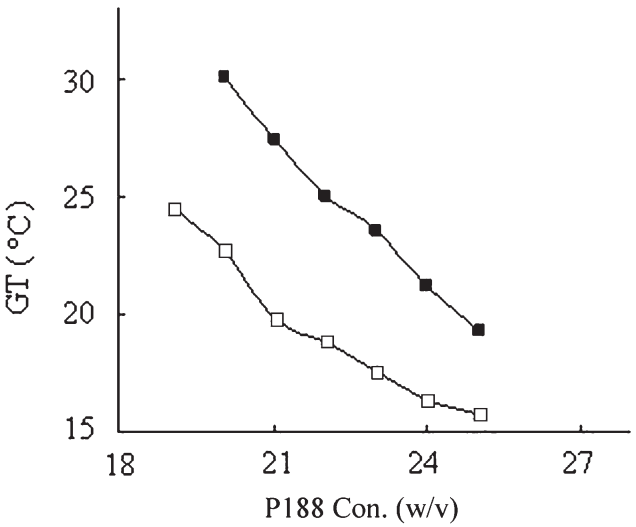


Figure 1 The gelation temperature (GT) as a function of P407 concentrations in pure water. □, before STF dilution; ■, after STF dilution.

RESULT AND DISCUSSION

The gelation temperature

The effect of P407 and P188 concentration on GT

The optimum ophthalmic thermosensitive *in situ* gels should have a higher GT than room temperature (25.0°C) and a shift to gel at the conjunctival sac temperature (35.0°C) after mixing with tear fluid. The GTs of 19, 20, 21, 22, 23, 24, and 25% (w/v) P407 solutions were measured (Fig. 1). The GT decreased with an increase in the P407 concentration and expressed evident concentration dependence. The GT of each solution heighten after STF dilution; moreover, the heightening extent decreased with the increasing P407 concentration.

To adjust the ratio of the polyethylene oxide block and polypropylene oxide block, P188 solutions with various concentrations were added to the 20, 22, and 24% (w/v) P407 solutions. P188 exhibited a good perspective to increase the GT of P407.[6,7] The GT heightened with an increase in the concentration of P188 added (Fig. 2). The formulations containing 20% (w/v) P407 and P188 having higher GT possessed the weaker capability against the STF dilution. The formulations containing 24% (w/v) P407 and P188 gelated at room temperature. The formulations containing 22% (w/v) P407 and 6% (w/v) P188 was chosen as the matrix of *in situ* gel, because its GT before STF dilution is 29°C and the one after STF dilution is 34.9°C.

The effect of bioadhesive polymers on GT

Poloxamer analogs are widely used as the matrix of thermosensitive gel, but exhibit a relatively short contact time when compared with gellan gum or carbo-

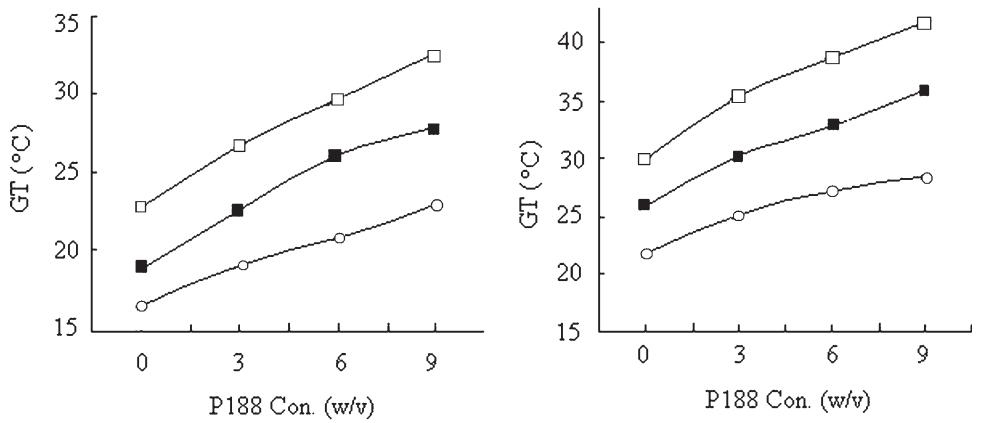


Figure 2 The GT of mixed P407 and P188 solutions before (left) and after STF dilution (right). □, 20% P407; ■, 22% P407; ○, 24% P407.

pol because of gradual dilution by lacrimal fluid[18] and too quick dissolution, which cannot promise a high bioavailability. Therefore, bioadhesive polymers, which are capable of attaching to mucosal surfaces, offer the prospects of prolonging the residence time of an ocular drug delivery system at the sites of drug absorption and ensure optimal contact between the formulation and the absorbing surface, were needed to use to improve the bioadhesion to tunica mucosa and prolong the corneal retention time. Additionally, they are useful to adjust the GT and drug release. To enhance the mucoadhesive ability of *in situ* gel containing 22% P407 and 6% P188, C934 and NaHA were incorporated to the formulations (Table I). The formulations F_1–F_4 contain 0.1, 0.2, 0.3, and 0.4% (w/v) C934, and the formulations F_5–F_9 contain 0.1, 0.2, 0.3, 0.4, and 0.5% (w/v) NaHA, respectively. The GT decreased with an increase in the concentration of C934 or NaHA. It could be reasoned that hydrogen bonds between carboxyl groups of C934 or NaHA and poly(ethylene oxide) (PEO) blocks of the poloxamer molecules may lower the hydrophilicity and solubility of the poloxamer molecules. The similar record that hydrogen bonds between carboxyl groups of alginate and PEO blocks lower the solubility of poloxamer was reported previously.[10,19] With an increase in the concentration of C934 or NaHA, the amount of hydrogen bonds increased and the GT decreased. F_1, F_2, F_5, F_6, and F_7 not only have the suitable gelling capacity and the best transparence, but also present suitable GTs. Therefore, these formulations can be chosen as potential formulations for further investigation. Gatifloxacin was successfully formulated as ion-activated *in situ* gel-forming ophthalmic solutions (0.3% (w/v)) using sodium alginate (Kelton®) as a gelling agent in combination with HPMC as a viscosity-enhancing agent.[20] In the previous study, a certain amount of sodium alginate was also incorporated to the formulations to strengthen the gelling sensitivity and mucoadhesive ability in physiological condition. However, we found that sodium alginate not only lowered the transparency of solution, but also lowered the GT too much after STF dilution.

Gel dissolution and *in vitro* release studies

Effect of release areas on the gel dissolution and *in vitro* release

Figure 3 shows the curves of gel dissolution and tiopronin release versus release areas. The rate of gel dissolution and tiopronin release increased with an

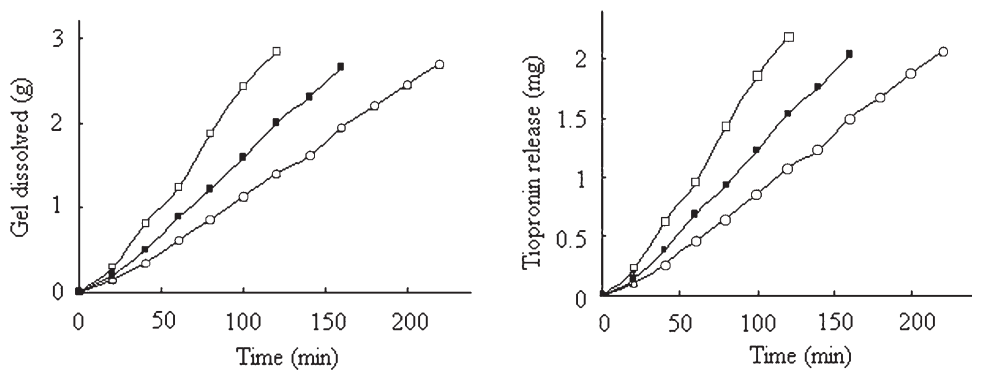


Figure 3 The effect of surface area on gel dissolution (left) and tiopronin release (right). Surface area: ○, 1.16 cm^2; ■, 1.64 cm^2; □, 2.48 cm^2.

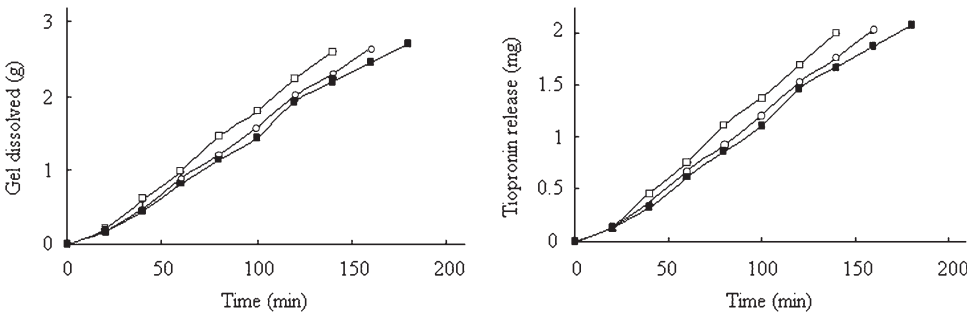


Figure 4 The effect of Carbopol 934P (C934) concentration on gel dissolution (left) and tiopronin release (right). ○, 0%; □, 0.1%; ■, 0.2%.

increase in the release areas. Accumulative amount of gel dissolution and drug release versus time all exhibited well linear correlation ($r > 0.993$). Moreover, the change of release areas did not affect the characteristic. Under various release areas, correlation between tiopronin release and gel dissolution is good. Tiopronin release and gel dissolution possessed the same rate, because the slope rate of the curves of tiopronin release versus gel dissolution were all close to 1 (figure not given). Thus, it is presumed that gel dissolution is a major factor affecting tiopronin release.

Effect of carbopol concentration on gel dissolution and tiopronin release

The gel dissolution and tiopronin release from 22% P407 and 6% P188–based *in situ* gel containing 0.1%, 0.2% (w/v) C934 (corresponding to formulations F_1, F_2), were examined at 35°C and compared with that from the formulations F_0 without any additives. For various concentrations of C934, gel dissolution and tiopronin releases both exhibited zero-order kinetics characteristic (Fig. 4). F_1 significantly accelerated gel dissolution and tiopronin release ($P < 0.05$). For 0.1% C934 in F_1, the amount of the metal ions in STF was enough to generate an electrostatic shielding effect on the ionizing carboxyl group that weakened the electrostatic repulsion of C934 molecule backbone, which may result in the curling conformation of C934 molecule and the decrease of gel viscosity. Thus, 0.1% C934 quickened gel dissolution and tiopronin release. For 0.2% C934, the similar electrostatic shielding effect was just counteracted by increased gel intensity, so the gel dissolution and tiopronin release of F_2 had no insignificant difference from ones of F_0 ($P > 0.05$). C934 with higher concentration incorporated would make 22% P407 and 6% P188 solution generate gelatination.

Effect of NaHA concentration on gel dissolution and tiopronin release

The gel dissolution and tiopronin release from 22% P407 and 6% P188 based *in situ* gel containing 0.1%, 0.2%, 0.3% (w/v) NaHA (corresponding to F_5, F_6, F_7) were also examined at 35°C. For various concentrations of NaHA, gel dissolution and tiopronin release both exhibited zero-order kinetics characteristic (Fig. 5). The F_5 in gel dissolution and tiopronin release had no insignificant difference from the F_0 ($P >$

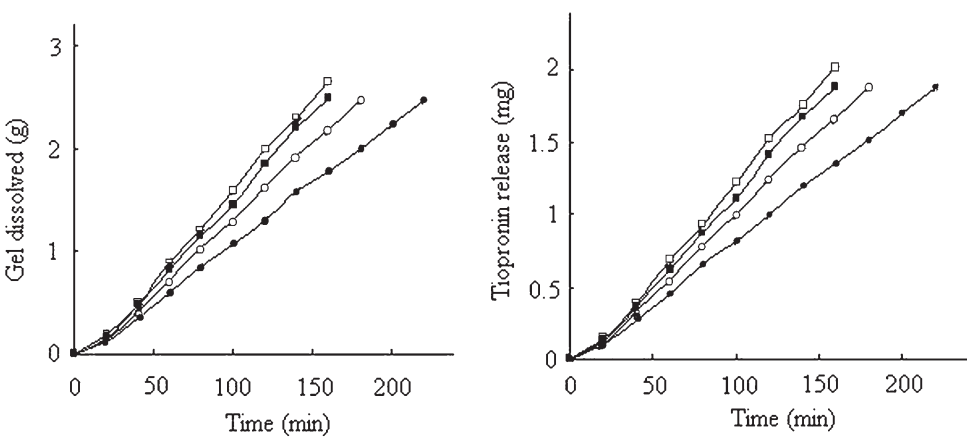


Figure 5 The effect of NaHA concentration on gel dissolution (left) and tiopronin release (right). □, 0%; ■, 0.1%; ○, 0.2%; ●, 0.3%.

TABLE II
***In Vitro* Transcorneal Transit of Tiopronin of Different Formulations**

	Accumulative amount of drug (μg/cm^2)								
Time (min)	30	60	90	120	150	180	Correlation coefficient	$P_{app} \times 10^6$ (cm·s^{-1})	$J_{ss} \times 10^3$ (μg·s^{-1}·cm^{-2})
Solution	24.15 ± 2.17	53.27 ± 4.86	82.40 ± 13.25	110.50 ± 9.820	140.7 ± 19.34	159.76 ± 23.31	0.9991	17.39 ± 1.29	16.18 ± 1.21
F_0	16.78 ± 1.35	41.75 ± 11.44	63.78 ± 13.65	91.23 ± 11.85	116.59 ± 19.93	243.08 ± 20.31	0.9993	15.90 ± 2.41	13.88 ± 2.10
F_6	16.38 ± 1.07	38.34 ± 8.99	62.65 ± 20.95	85.87 ± 2.20	108.90 ± 43.30	131.03 ± 31.57	0.9989	15.43 ± 0.94	12.85 ± 0.87
F_7	13.36 ± 1.90	33.45 ± 4.61	55.63 ± 7.80	76.80 ± 14.93	96.43 ± 12.17	118.19 ± 14.14	0.9985	14.56 ± 1.02	12.13 ± 0.92

0.05). F_6 and F_7 both evidently delayed gel dissolution and tiopronin release ($P < 0.05$). Similar results were obtained by Paavola et al.[19], who reported that cellulose additives significantly prolonged ibuprofen release. The slope rate of the straight line of tiopronin release versus gel dissolution is 1.1393, which shows tiopronin release precedes gel dissolution. NaHA is of high molecular weight, dissolve in water and yield much more viscous solutions compared with gel without additives. The increased viscosity might have contribution to the decreased rate of drug release from these formulations compared with F_0. Poloxamer are polyethyloxylene–polypropyloxylene block copolymers (Pluronic),[21,22] and the chemical structure can form micelles in aqueous medium. Micelles continue to grow in size and number at higher temperatures, leading eventually to gel structure. NaHA contained in gel increased the viscosity and entanglement of polymer chains that prevented poloxamer micelle diffusing into release medium. As a result, the gel dissolution decreased with an increase the NaHA concentration. The size of drug molecule is much smaller than that of micelle, so tiopronin is easier to diffuse through surface layer and tiopronin release precedes gel dissolution. The increased NaHA concentration decreased the rate of gel dissolution and tiopronin release. Thus, F_5, F_6 and F_7 were chosen as potential formulations to investigate the *in vitro* transcorneal transit.

In vitro transcorneal transit of tiopronin

F_5, F_6, and F_7 were investigated in the effect of various concentrations of NaHA on the transcorneal permeation of tiopronin. Table II summarizes the accumulative release of tiopronin and the pharmacokinetic parameters for *in vitro* transcorneal, respectively. For tiopronin solution, F_0, F_6, and F_7, the curves of accumulative release of tiopronin *in vitro* transcorneal transit versus time all exhibit good linearity, which indicates that tiopronin *in vitro* transcorneal transit accord with zero-order kinetics

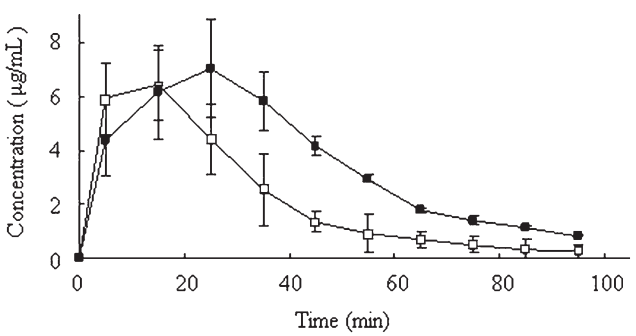


Figure 6 The mean aqueous humor–concentration versus time curves of tiopronin after instilling eye drops. □, solution, ■, F_6 based *in situ* gel.

TABLE III
The Intraocular Pharmacokinetic Parameters of Tiopronin Solution and *In Situ* Gel

Samples	AUC ($\mu g \cdot min \cdot ml^{-1}$)	C_{max} ($\mu g \cdot ml^{-1}$)	T_{max} (min)	K_e (min^{-1})	$t_{1/2}$ (min)
Solution	217.26 ± 52.68	6.78 ± 1.15	11.67 ± 5.77	0.0338 ± 0.0009	18.49 ± 0.55
In situ gel	344.81 ± 50.47	7.32 ± 1.26	21.67 ± 5.77	0.0253 ± 0.0045	27.86 ± 4.71

characteristic. Within the experimental period (3 h), 8.94, 7.76, and 7.02% tiopronin permeated through cornea for tiopronin solution, F_6 and F_7, respectively. Moreover, the apparent permeability coefficients (P_{app}) and steady-state fluxes (J_{ss}) decreased with an increase in the concentration of NaHA. It was attributed to the reason that NaHA increased the tortuosity and mini-viscosity of gel, and decreased the diffusing capacity of tiopronin in aqueous phase. Finally, F_6 was chosen to undertake *in vivo* study considering the cornea permeability and drug sustained release.

Ocular bioavailability of tiopronin

Figure 6 shows the level of tiopronin in aqueous humor after instillation of 50 μL of 0.1% tiopronin formulated as aqueous solution and as F_6. Table III displays the pharmacokinetic parameter. The maximum level of tiopronin in aqueous humor (C_{max}) was 6.78 and 7.32 $\mu g \cdot ml^{-1}$ for tiopronin solution and F_6, respectively. The time required to C_{max} for tiopronin solution was 11.7 min, and then the drug concentration decreased rapidly due to conjunctival absorption and drainage of drug induced by lachrymation and normal tear turnover. The time required to C_{max} for F_6 was 21.7 min due to the sustained release of gel and the prolonging residence time of drug on cornea. AUC (0–95 min) for the concentration/time profiles of various formulations showed that the ocular bioavailability increased in aqueous humor by 1.6 fold for *in situ* gel compared with tiopronin solution.

Effect of tiopronin eye drops on selenite-injected rat pups

Figure 7 shows the slit images of the rat pups that had been injected selenite injections and then instilled with low, middle, and high dose of tiopronin solution and tiopronin *in situ* gel eye drops, respectively. Figure 8 displays curves of percent of opacity of lenses versus time after injection selenite that can directly reflect the effect of middle- and high-dose tiopronin solution and *in situ* gel eye drops on the lenses opacification of selenite cataract rats. In age-matched normal rats (blank control), the lenses (both right and left) remained clear throughout the study period. In the model control, cataracts in the nuclei of crystalline lenses, which were depicted by the opacity of lenses, were observed clearly at 48 h and increased rapidly

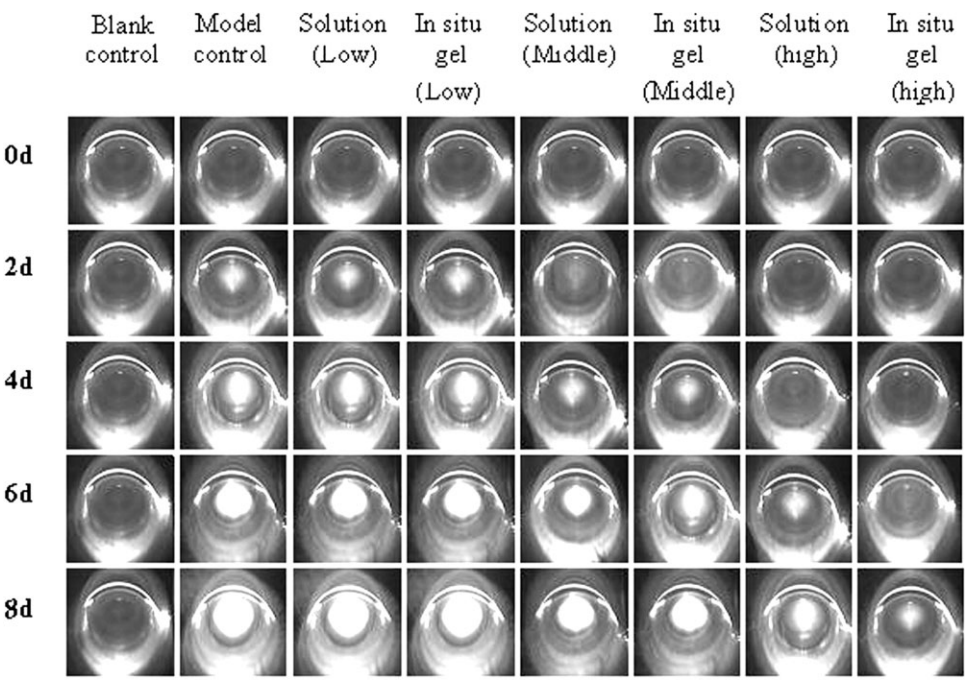


Figure 7 The slit images of eyes from selenite cataract rats with or without treatment of tiopronin.

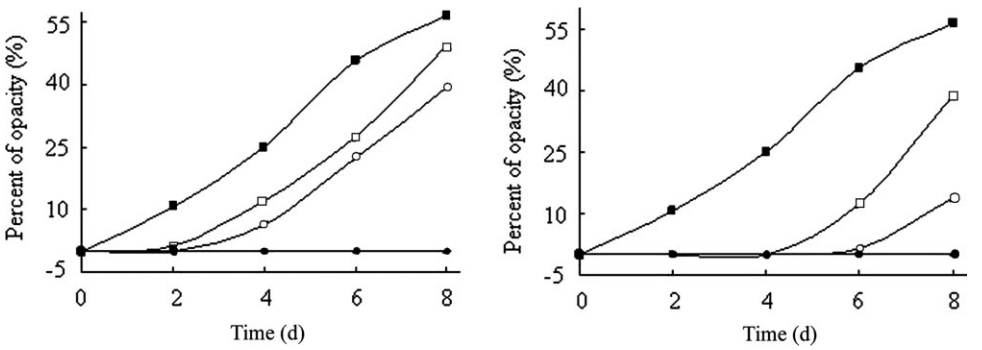


Figure 8 The effect of middle-dose (left) and high-dose (right) tiopronin on the lenses opacification of selenite cataract rats. ●, blank control; ■, model control; □, tiorpronin solution; ○, tiorpronin *in situ* gel.

(closed squares in Fig. 7) beyond 48 h after sodium selenite injection in 13-day-old rats. Compared with model control, the opacity of the lenses has no evident difference at 48 h in low-dose tiopronin solution group and *in situ* gel-treated group. This confirms that the low-dose (1 mg/mL) tiopronin hardly exhibits therapeutic effect. Lenses opacity of middle dose tiopronin solution group and *in situ* gel group is lower than that of model control and low-dose groups. Moreover, it should be worth noticing that the lenses opacity of tiopronin solution group is more serious than that of *in situ* gel group. High-dose tiopronin solution and tiopronin *in situ* gel delay the appearing of lenses opacity to 4 d and 6 d, respectively, and display the best anticataract effect. These results not only show that the therapeutical effect of tiopronin possesses dose dependent, but also the *in situ* gel can increase the anticataract effect and is an optimal dosage form. The tiopronin *in situ* gel eye drops prolonged the contact period of tiopronin and cornea due to the gelation and bioadhesion, which enhances the total amount of tiopronin permeating through cornea. Therefore, tiopronin *in situ* gel eye drops exhibited the better therapeutic effect against selenite cataract.

In conclusion, 22% P407 and 6% P188 containing 0.2% NaHA–based formulations of *in situ* gel can be used as liquid for administration by instillation into the eye, which on exposure to eye temperature will shift to the gel phase. On the basis of *in vitro* and in vivo results, the *in situ* gel containing 3 mg/mL tiopronin showed potential for use as delivery system with improved ocular bioavailability and anticataract effect.

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