Design and Evaluation of a Brinzolamide Drug–Resin in Situ Thermosensitive Gelling System for Sustained Ophthalmic Drug Delivery

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In this study a brinzolamide drug–resin ophthalmic thermosensitive in situ gelling system was developed and evaluated. Brinzolamide was combined with ion exchange resins to prolong the retention time of drugs in the eye and to reduce ocular and systemic side effects. Poloxamer F127 was used as gelling vehicle in combination with carbopol 934P, which acted as a viscosity-enhancing agent. They were prepared using the cold method. The optimized formulation exhibited a sol–gel transition at 33.2±1.1°C with pseudoplastic flow behavior. This formulation was stable and nonirritant to rabbit eyes. In vitro release studies demonstrated diffusion-controlled release of brinzolamide from the combined solutions over a period of 8 h. In vivo evaluation (the elimination of brinzolamide through tears and absorption of brinzolamide in aqueous humor) indicated that the solution combination was better able to retain the drug than commercial preparations. Thus this formulation is safe for ophthalmic use and significantly increases brinzolamide bioavailability in aqueous humor.

Key words thermosensitive in situ gel; ion exchange resin; glaucoma; ophthalmic drug delivery; sustained release

Glaucoma is the most prevalent eye disease and a leading cause of irreversible blindness in the Western industrialized world.1) Due to the increase in intraocular pressure (IOP), glaucoma damages the optic disc gradually causing vision loss, usually without symptoms. It has been reported that glaucoma is the result of an imbalance between aqueous humor drainage and secretion.2)

Brinzolamide is a noncompetitive, effective and highly specific carbonic anhydrase inhibitor used in the treatment of glaucoma.3) A white powder insoluble in water, brinzolamide is commercially formulated as a 1% ophthalmic suspension and able to lower IOP by reducing aqueous humor formation in the eye.4,5) The commercially available preparation of brinzolamide is Azopt®, which can be used as a first-line antiglaucoma medication. However, its usage has been restricted by many factors such as, systemic adverse event (taste aversion), ocular adverse events (ocular burning and stinging), and the very expensive price.5,6)

The conventional ophthalmic drugs are administered topically in the form of eye drops. However, due to physiological constraints (such as the blinking reflex, lacrimal secretion and nasolacrimal drainage), most of the currently listed ophthalmic solutions are rapidly eliminated, which causes a short precorneal residence time and a limited transcorneal absorption. Hence, the absorption of the ophthalmic drug is severely limited, resulting in poor ocular bioavailability.6) Moreover, a small amount of the drug passes from the nasolacrimal duct into the gastrointestinal tract causing systemic toxicity.7,8) Thus, frequent application of the eye drops is necessary to maintain therapeutic ocular bioavailability; but the frequent use of high concentration solutions may induce both ocular and systemic side effects.

To overcome these problems, many methods for increasing the corneal permeability and prolonging the contact time on the ocular surface have been studied. Ion exchange resins are three-dimensional network structural polymers, which have been used extensively as effective vehicles for drug delivery in recent years.8,9) There are many reports on the applications of ion exchange resins in sustained and controlled release drug delivery. Most reports have concentrated on oral sustained-release suspensions.10) Only a few reports have described their application in the ophthalmic drug delivery system, which is one of the most interesting and challenging endeavors to the pharmaceutical scientist. Therefore, we attempted to develop a drug–resin complex that could be used for ophthalmic drug delivery. The complex was exchanged with the endogenous ocular ions and delivered drug at a controlled rate over a period of time.11) Because of the sustained release mechanism of the drug–resin complex, the major problem of the rapid drug elimination from the pericorneal area due to anatomical constraints was improved. In fact, the drug–resin complex could remain in the conjunctival sac for 1.5 h,12) which prolonged the retention time of the drug on the ocular surface and provided an enhancement in bioavailability. This allowed for a reduction in the dose and frequency of administration, so that the ocular and systemic side effects were reduced compared with conventional preparations. However, the drug–resin suspensions have some drawbacks such as, dosage heterogeneity of suspensions, low mucoadhesive activity on ocular surface, generating an unsatisfactory ocular bioavailability and the inability to reach the desired effective sustained release.13)

Current research efforts are focused on the design and evaluation of ophthalmic drugs that can be delivered in drop form without causing blurred vision or irritation. This would provide a suitable mucoadhesive force to improve the retention time and sustained and controlled drug release to increase therapeutic efficacy and patient compliance.14,15) Consequently, in situ gelling system has been developed as an ideal ophthalmic...
mic formulation, which undergoes sol-to-gel phase transition upon exposure to the physiological conditions present in the eye. In situ gels can be classified as pH-triggered, temperature-dependent and ion-activated systems.\textsuperscript{16,17} The oldest and most common system is the temperature sensitive type in situ gel. The aim of this study was to prepare and evaluate a brinzolamide drug–resin ophthalmic thermosensitive in situ gel. It was easily and accurately instilled in liquid form without causing blurred vision or irritation and formed a gel at the precorneal temperature (35°C) to endure the lachrymal fluid dilution without rapid precorneal elimination after administration.\textsuperscript{18} This formulation had a prolonged precorneal residence time and improved ocular bioavailability compared with eye drops and suspensions. In our present work, poloxamer 407 was studied as an in situ gelling vehicle for the ophthalmic drug delivery system, and carbopol 934P was added in order to fortify the adhesion of the administered drug onto the ocular surface.

Brinzolamide was selected as a model drug. First, it was combined with an Amberlite IRP-69 ion exchange resin, and then an in situ thermosensitive gel was developed for ophthalmic use. As far as we know, this is the first report to combine ion exchange with in situ gels in the treatment of patients with glaucoma. The objective of the present study was to decrease side effects, prolong the precorneal residence time and enhance ocular bioavailability.

### Experimental

#### Materials

The poloxamer 407 (Fl27) was obtained from BASF (Ludwigshafen, Germany). The carbopol 934P was purchased from Qingdao Tianliyuan Bio-technology Co., Ltd. (Shandong, China). Brinzolamide was purchased from Jinan Hongfangde Pharmatech Co., Ltd. (Shandong, China). Amberlite IRP-69 ion exchange resin (particle size: 19.07±1.07 μm) was purchased from Rohm and Haas Co. (Philadelphia, PA, U.S.A.). The brinzolamide 10mg/mL eye drops (AZOPT\textsuperscript{®}) were purchased from Alcon (Puurs, Belgium). All other chemicals and solvents used were of analytical grade, and purified water was used throughout the study.

#### Preparation of the Drug–Resin Complex

The ion exchange reaction itself may be described by the following equilibrium equation:

\[
\text{A}^{-}\text{NH}_2^+ + \text{R-SO}_3\text{Na} \leftrightarrow \text{R-SO}_3\text{H}_2\text{N-A} + \text{Na}^+
\]

In the equation, A–NH\textsubscript{2}\textsuperscript{+} and R-SO\textsubscript{3}Na represent brinzolamide and cations resin, respectively.

To prepare the drug–resin complex, the drug and resin at the ratio 1:1 were dispersed in a hydrochloric acid solution (0.01 mol/L) and then put into a constant temperature oscillator at shaking frequency 175 r/min. After 1 h, the samples were removed and centrifuged. The supernatant was analyzed for unconjugated drug using an ultraviolet spectrophotometer at 257 nm. The drug–resin complex obtained was washed, dried and stored in a desiccator for further use. The embedding ratio (ER) and drug loading (DL) of the drug–resin complex were calculated as follows:

\[
\text{ER\%} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}}} \times 100\%
\]

\[
\text{DL\%} = \frac{W_{\text{total}} - W_{\text{free}}}{W_{\text{total}} - W_{\text{free}} + W_{\text{resin}}} \times 100\%
\]

Where $W_{\text{total}}$ is the total amount of the brinzolamide added, $W_{\text{free}}$ is the amount of the unconjugated brinzolamide, and $W_{\text{resin}}$ is the amount of resin in the drug–resin complex.

#### Binding mechanism studies of the drug–resin complex (resinate) were carried out using differential scanning calorimetric (DSC). DSC was carried out by heating four substances (drug, resin, drug–resin complex and drug–resin physical mixture) separately from 40 to 270°C at the heating rate of 10°C/min under a nitrogen environment.\textsuperscript{19} The instrument used was a DSC-60A (Shimadzu Corp., Kyoto, Japan).

#### Preparation of the in Situ Gel

The formulation was prepared on a weight/volume basis using the cold method.\textsuperscript{20} Distilled water was cooled to 4°C and the polymers (0.2% Carbopol 934P and 22% P407) were slowly added with continuous agitation. The solution remained at 4°C until a clear solution was obtained. 2.5% drug–resin complex (equivalent to 1% w/v of brinzolamide) was added to the solution with constant stirring to ensure a uniform suspension of resinate. 0.01% (w/v) ethylparaben was incorporated as a bacterial inhibitor. The pH of the system was adjusted to 5.5–6.0 using a sodium hydroxide solution. The osmolality of the system was adjusted to 295 mosm/L using mannitol. The sample solution was stored at 4°C.

#### Measurement of the Gelation Temperature (GT)

The GT of the poloxamer solution was determined using the tube inversion method and the magnetic stirring method. Various concentrations of poloxamer 407 solutions were prepared and the poloxamer 407 solutions diluted with artificial tears (STF) at a ratio of 40:7 (v/v) were also prepared. First, 5mL of the sample solution was put into a transparent vial and placed in a 4°C refrigerator. A thermometer with an accuracy of 0.1°C was immersed in the sample solution, which was heated at a rate of 1°C/1–2 min.\textsuperscript{21} The temperature was determined as the GT when the magnetic bar completely stopped moving and the solution did not flow after being whirled at an angle of 180°. The influence of ingredients on the gelation temperature of poloxamer solutions was determined.

#### Rheological Studies

The rheological properties were determined using an NDJ-1 rotational viscometer (Shanghai Changji Geological Instrument Co., Ltd., Shanghai, China). The shear stress of the sample solutions was measured at different shear rates (6, 12, 20, 30, 40, 60 rpm) under physiological conditions (35±0.1°C) and non-physiological conditions (25±0.1°C). To simulate the physiological eye environment of the gels, the thermosensitive gels were diluted with artificial tears (STF) at a ratio of 40:7 before the rheological studies were conducted at 35±0.1°C.

#### In Vitro Release Studies

The dialysis bag method was used for in vitro studies. Half milliliter formulation was applied in dialysis bags (cellulose membrane, MWCO 8–14kDa). The release medium was 40mL of fresh artificial tears (sodium chloride 6.78 g, potassium chloride 1.38 g, sodium bicarbonate 2.18 g, calcium chloride dihydrate 0.084 g and purified water q.s. 1000mL). To simulate the eye temperature, the shaking temperature was 35±0.5°C and the stirring rate was maintained at 50 rpm. The samples (4mL) were withdrawn from the release media at intervals of 0.5, 1, 2, 4, 6, 8, 10 and 12h and replaced with an equal volume of fresh artificial isotonie tear solution. The concentration of brinzolamide was determined by UV Spectrophotometer (Shimadzu UV-2550) at 257 nm. The percentage of cumulative release was calculated.
and a graph of percent cumulative release against time was plotted. Then, the release medium was exchanged with purified water. The brinzolamide released from the drug–resin in situ gel and marketed eye drops into purified water were performed using the same method.

The correlation between drug release and gel dissolution was investigated. The drug–resin in situ gel solution was put in a centrifuge tube and then remained at 35±0.5°C until a gel was obtained. Two milliliters of 35°C artificial tears were slowly added to the gel. During the experiment, destruction of the gel surface was avoided. The centrifuge tube was put into a constant temperature oscillator at shaking frequency 50 c/min and a shaking temperature of 35°C. The release medium were poured out at intervals of 0.5, 1, 2, 3, 4, 5, 6 and 8h, after each interval the centrifuge tube was rubbed dry, weighed and weight difference between adjacent time was determined as the amount of gel dissolution during this period. Then the centrifuge tube was put back into the constant temperature oscillator for 10 min and then 2 mL of artificial tears were slowly added to the gel and continue to shake. The drug concentration of pour-out release medium was determined by UV at 257 nm. A graph of gel dissolution versus time was plotted.

Elimination of Brinzolamide through Tears New Zealand albino rabbits were obtained from the Laboratory Animal Center, Zhengzhou University, China (License No.: SCXK (YU) 2011-0003). The experimental animals (2.0 to 2.5 kg) were individually housed in an air-conditioned and light-controlled room at 25±1°C and 70±% relative humidity. They were given a standard pellet diet and provided 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.

In this study, the Schirmer strip method was used as a sampling technique. A Schirmer strip (approximately 8×2.2mm) and a 1.5 mL polypropylene centrifuge tube were weighed using an electronic balance. The self-contrasted method was used. Then, 40 µL of brinzolamide eye drops (marketed product) were administered into the left eye of each rabbit as the control group, and 40 µL of drug–resin in situ gel were administered into the right eye of each rabbit as the experimental group. At various sampling time points, the strip was gently placed into the conjunctival sac to draw tears and allowed to remain for 5 s. During the experiment, contact between the strip and any visible gel lumps was avoided. After the strip was removed, it was immediately placed into the centrifuge tube, and the tube was weighed. All samples were stored at −20°C until analysis.

The strips with absorbed tears were dried using ultra-high-purity nitrogen, and then 500 µL of methanol was added to precipitate the proteins. Each sample was vortexed for 3 min and centrifuged at 4000 rpm for 8 min, and then, the supernatants were diluted with methanol. Finally, 20 µL of the mixture was injected into the HPLC system to determine the drug concentration.

Absorption of Brinzolamide in the Aqueous Humor An aliquot of 100 µL of aqueous humor was withdrawn with 1 mL micro-syringe inserted through the corneoscleral junction and slightly upward into the anterior chamber. The collected aqueous humor was added to 500 µL of methanol to precipitate proteins, vortexed for 3 min and centrifuged at 4000 rpm for 8 min. The supernatant was dried using ultra-high-purity nitrogen and the residue was dissolved in 100 µL of methanol. Finally, the samples were subjected to HPLC analysis to determine the drug concentration.

Ocular Irritation Studies Based on the chemical drugs irritation, allergic and hemolytic technical research guidelines,22 rabbits were used as animal models. Before the start of the experiment, the cornea, conjunctiva and iris of each rabbit were checked for lesions or inflammation, which were grounds for removal from the study. In the experimental group, 40 µL of the brinzolamide drug–resin complex thermosensitive hydrogel was instilled into the right eye, while physiological brine was instilled into left eye as control.

Ocular irritation studies were performed according to the Draize test.23 The Draize technique was used to assess acute, intermediate and chronic exposure by applying compounds to the skin, penis and eyes of rabbits. A total of 6 New Zealand white rabbits were used for the irritation study. Irritation was tested after both a single dose and multiple doses. In the multiple doses test, the formulation was instilled two times a day for a period of 7 d. The condition of the cornea, iris and conjunctiva was observed at the time intervals of 0.5, 1, 2, 4, 6, 12, 24, 48 and 72 h after administration. The conjunctival congestion, swelling and discharge were graded on scales of 0 to 3, 0 to 4 and 0 to 3, respectively. Iris hyperemia and corneal opacity were graded on a scale of 0 to 4. The mean values from 6 treated eyes were calculated. The evaluation criteria used in accordance with the Draize technique were non-irritant from 0 to 3.9, slightly irritant from 4 to 8.9, moderately irritant from 9 to 12.9 and seriously irritant from 13 to 16.

Accelerated Stability Studies Selected ophthalmic formulations were stored at 4±1°C, room temperature (25±1°C) and 40±1°C for three months. The formulations were evaluated after one, two, three months for clarity, pH, gelling temperature and drug content.

Data Analysis All the experiments in the study were performed at least three times and the data were expressed as the mean±standard deviation (S.D.). Statistical analysis of data was performed using ANOVA.

Results and Discussion Preparation of Drug–Resin Complex Structural formula of brinzolamide and cation exchange resin IRP-69 are shown as following:

![](image)

The model drug of brinzolamide, containing a secondary amine in its structural formula, can have a positive charge in hydrochloric acid. Thus, it can be exchanged with the Na+ of resin. Additionally, the H+ in the HCl will compete with the
drug for the action sites resulting in a decline in drug loading and the encapsulation rate. Therefore, in suitable solubility conditions, the minimum amount of hydrochloric acid in which the drug can be dissolved was used to prepare the drug–resin complex.

Brinzolamide was combined with the cation exchange resin IRP-69. To obtain a desirable modified release of the drug–resin complex, a single factor experiment was used to optimize the cation exchange resins. The optimum drug–resin complex should have a good embedding ratio (ER) and drug loading (DL). Under similar conditions, the small particle size resin had an ER and DL that were slightly higher than the large particle size resin and had less stimulation to the ocular surface. Our preliminary experimental results showed that the preparation and characteristics of drug–resin complex were influenced by several factors, including the ratio of drug to resin, shaking temperature and shaking time. In our study, a three factors and three levels orthogonal experiment was used to optimize a drug–resin complex (data not shown). The results indicated that the ratio of drug to resin had the greatest impact on the release of the drug. The drug resin complex had the slowest release rate at the ratio of 1 : 1. Based on various trials, a shaking temperature of 60°C, a shaking frequency of 175 r/min and a shaking time of 1 h were selected as the optimized conditions.

The thermograms obtained are shown in Fig. 1. As shown in Fig. 1, the pure brinzolamide drug exhibited a sharp endotherm at 134.5°C. The melting point of brinzolamide is approximately 131°C.24) The disappearance of this peak in the Dotherm at 134.5°C. The melting point of brinzolamide is

![DSC Thermogram](https://via.placeholder.com/150)

**Fig. 1. DSC Thermogram**

|Ingredients Before diluted by STF/°C | After diluted by STF/°C |
|------------------------------------|------------------------|
|Poloxamer solutions                | 25.8±0.3               | 33.4±1.6               |
|Carbomer 934P                      | 25.7±1.3               | 33.5±0.5               |
|Drug–resin complex                 | 25.9±0.4               | 33.3±0.1               |
|Mannitol                            | 25.3±0.6               | 33.6±0.1               |
|Ethylparaben                        | 25.7±0.1               | 33.4±0.8               |

Results represent mean values±S.D., n=3.

**Table 1. Influence of Ingredients on the Gelation Temperatures of Poloxamer Solutions**

GT of Poloxamer Solutions The two main requirements of an in situ gelling system are viscosity and gelling capacity. The optimum ophthalmic thermosensitive in situ gel should have a GT higher than room temperature and a shift to gel at the conjunctival sac temperature (35.0°C) after being mixed with artificial tears. Based on the suitable range of gelation temperature (25–35°C), poloxamer 407 was chosen as the optimum gelling agent. The gelation of a poloxamer solution is a reversible process. Gels revert to free-flowing solutions when the temperature drops below the GT. Moreover, poloxamer 407 has been reported to be the least toxic of the commercially available poloxamers.25) Figure 2 shows that the GT is dependent on the polymer concentration. According to our results, the GT of the poloxamer decreased as the P407 concentration increased. When the concentration of poloxamer 407 was higher than 18%, it was possible to form a transparent gel. However, when the concentration was lower than that, even if it was heated to 50°C, it could not form a gel. After being mixed with artificial tears, the gelation temperature accordingly increased approximately 7°C because of the decreased concentration of poloxamer. When the concentration was lower than 21%, even at a temperature of 50°C, it would not form a gel after being diluted with artificial tears. As expected, poloxamer 407 was found to have unique thermo-reversible gelation properties in agreement with other studies.26) A reduction in the poloxamer concentration without compromising the gelling capacity and rheological properties of the delivery system may be achieved at 22%. The GT was 26.8°C before a STF dilution to 22% P407 and 33.2°C after STF dilution. Therefore, we chose a 22% poloxamer solution as the gel matrix.

**Fig. 2. Effect of Concentration on the Gelation Temperature of Poloxamer Solutions**

Results represent mean values±S.D., n=3.

**Influence of Ingredients on the Gelation Temperatures of Poloxamer Solutions** The influence of the drug–resin complex, mannitol, 0.2% carbomer 934p and ethylparaben on the GTs of poloxamer solutions is shown in Table 1. Brinzolamide was the active component of this pharmaceutical preparation, and its concentration in the eye drops was 1% (w/v). A certain amount of the drug–resin complex were added, according to the drug loading. Table 1 shows that the drug–resin complex did not significantly interfere with the gelation temperatures of poloxamer solutions. In addition, the drug content, pH, dispersibility and thermal reversibility of the delivery system were

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|------------------------------------|------------------------|
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|Drug–resin complex                 | 25.9±0.4               | 33.3±0.1               |
|Mannitol                            | 25.3±0.6               | 33.6±0.1               |
|Ethylparaben                        | 25.7±0.1               | 33.4±0.8               |

Results represent mean values±S.D., n=3.
found to be satisfactory for ophthalmic use. The drug–resin in situ gel could help prolong the drug retention time on the ocular surface, although it was not able to shift the GT of poloxamer.

Carbomers have been successfully used to enhance the mucoadhesive ability of poloxamer solutions. Therefore, a combination of this polymer with a poloxamer would be very promising for ocular administration, as the in situ viscosity would be higher than polymers alone. In our study, the formulation with carbomer 934p not only had a suitable gelling capacity and the best transparency but also had a suitable GT in agreement with previous work. In our study, the hydrogels were neutralised using NaOH, which affected pH and gelling capacity of carbomers. However, this increased pH only had a slight effect on the GT. In our study, mannitol was incorporated to adjust the osmotic pressure. Isotonicity and buffering play a crucial role in ophthalmic formulations. They contribute significantly to the chemical stability, clinical response and also influence the comfort and stability of the product. In addition, mannitol can not only maintain the ophthalmic osmotic pressure but also increase the viscosity of the aqueous poloxamer solution and reduce intraocular pressure. Ethylparaben (0.01%, w/v) was incorporated as a bacterial inhibitor. Typical side effects of the preservatives are epithelial erosions, conjunctival hyperaemia and subjective sensations such as dry eye, burning, stinging and ocular irritation. It has been reported that ethylparaben damaged the corneal epithelium the least, although it has a direct cytotoxicity on corneal epithelial cells. Table 1 shows that mannitol and ethylparaben had no significant effects on the gelation temperatures of the poloxamer solutions. This was possibly because the small amount of ethylparaben and mannitol had no obvious effect on the poloxamer concentration. They did not significantly affect the thermal reversibility, pH or rheological properties of the poloxamer solutions.

**Rheological Studies**  Previous papers have shown that a poloxamer solution under either non-physiological or physiological conditions demonstrated a Newtonian flow behavior and that the shear stress increased linearly with an increase in the shear rate. For a carbopol solution under physiological condition, the flow curve shows a pseudoplastic behavior and under non-physiological condition, it has a Newtonian flow behavior. Although the shear stress of the carbopol solution increased significantly at physiological condition, a stronger gel can be formed by combining the poloxamer with the carbopol solutions. They form a network structure via hydrogen bonds. The flow curve of the carbopol/poloxamer solution at non-physiological condition (25°C) showed a Newtonian flow behavior, while a pseudoplastic flow behavior with a hysteresis was observed for the carbopol/poloxamer solution at physiological condition (35°C).

Figure 3 illustrates the relationship of viscosity and shear rate under both non-physiological (25°C) and physiological conditions (35°C). Under non-physiological conditions, the viscosity of the drug–resin in situ gel solution was not significantly changed as the shear rate increased, which is Newtonian flow behavior. The reason for this is that the polymer solution under lower temperature did not turn into a gel but remained an easily flowing liquid similar to pure water, which displays Newtonian flow behavior. This formulation can easily be used to accurately control the dose for patient. When the drug–resin thermosensitive in situ gel solution was dropped into conjunctiva of the eye, it formed a gel under physiological condition (35°C) because of the phase transition, and the viscosity of the gel drastically decreased as the shear rate increased, which is a characteristic of a pseudoplastic fluid. Generally, the ocular shear rate during interblinking is quite low, about 0.03 s⁻¹, and becomes higher during blinking, about 4250–28000 s⁻¹. This suggests that the formulation had more strength to withstand the low shear forces and could release eye irritation after instillation. The pseudoplastic property of

![Fig. 3. The Rheological Profiles of Thermosensitive Hydrogel with and without Drug–Resin Complex at 25°C, before STF Dilution and 35°C, after STF Dilution](image)

Results represent mean values±S.D., n=3.
the drug–resin *in situ* gel under physiological conditions was allowed for the sustained drainage of the drug from the conjunctival sac of the eye, without the unwanted blinking pain caused by too high viscosity at a high shear rate.\(^{31}\)

In order to investigate the effects of drug–resin complex on the rheological behavior of the formulation, the rheological studies on formulation with and without drug–resin complex under non-physiological and physiological conditions were carried out. Figure 3 demonstrated that, under physiological conditions, the formulation without drug–resin complex (viscosity was from 6900 mPas to 3870 mPas at each shear rate) had similar flow behaviors as formulation with drug–resin complex (viscosity was from 7100 mPas to 4900 mPas at each shear rate). Under non-physiological, viscosity of formulation without drug–resin complex (about 280 mPas) was similar than those of formulation with drug–resin complex (about 350 mPas) at each shear rate. The pictures of blank gel solution, blank gel, drug–resin gel solution and drug–resin thermosensitive gel are shown in Fig. 4. The results suggest that the incorporation of drug–resin complex did not disrupt the strong three-dimensional gel network formed under non-physiological and physiological conditions. Although the viscosity of formulation at 35°C was markedly decreased when it was diluted with STF, its viscosity was still higher than that of low viscous formulation at 25°C. This indicated that the drug–resin *in situ* gel had a potential for increasing contact time of brinzolamide in the eye.

**In Vitro Release Studies**  Figure 5 shows the cumulative amount of brinzolamide released versus time profiles for the brinzolamide drug–resin *in situ* thermosensitive gel, marketed eye drop, drug–resin complex and drug *in situ* gel. The release of brinzolamide from marketed eye drop in purified water had no big difference with that in artificial tears, but brinzolamide released from the drug–resin *in situ* gel into purified water was significantly different compared with that in the artificial tear. The release of brinzolamide from the drug–resin *in situ* gel was based on an ion exchange reaction. In the case of the eye drop release in artificial tears, approximately 40% of the brinzolamide was released from the solution after 0.5 h. Approximately 80% of the drug was released into the medium after 2 h. The diffusion-controlled release of brinzolamide from the drug–resin complex in artificial tears occurred over a period of 4 h because of the sustained release mechanism of drug–resin complex. The complex was exchanged with the endogenous ocular ions and delivered drug at a controlled rate over a period of time. However, when a drug–resin complex thermosensitive hydrogel was formed, only approximately 13% was released into the artificial tears after 0.5 h, and approximately 80% was released after 8 h. The gelation low-

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**Fig. 4. The Picture of Blank Gel Solution, Blank Gel, Drug–Resin Gel Solution and Drug–Resin Thermosensitive Gel**

**Fig. 5. The Cumulative Amount of Brinzolamide Released from the Brinzolamide Drug–Resin *in Situ* Thermosensitive Gel, Marketed Eye Drop, Drug–Resin Complex and Drug *in Situ* Gel**

Each data point is represented as mean±S.D. (n=3).
order release properties. Figure 6(b) illustrates the correlation

\[ y = 11.53x + 6.3213 \quad \text{R}^2 = 0.9971 \]

between drug release and gel dissolution. The result shows the **in vitro** drug release from formulations under physiological conditions occurs by diffusion of ion exchange resin before gel forming. But the release of the drug is significantly affected by gel dissolution after gel forming. Similar results were also obtained by other researchers using other gelling systems.\(^{20}\)

**Elimination of Brinzolamide through Tears** The concentration–time profiles of brinzolamide in rabbit tears are shown in Fig. 7. The pharmacokinetic parameters were estimated using DAS analysis (Drug And Statistics 1.0) as shown in Table 3. The brinzolamide drug–resin thermosensitive **in situ** gel exhibited a 1.8-fold increase in the \( AUC_{0-60} \) compared with the eye drops \((p<0.05)\). The brinzolamide concentrations for the drug–resin thermosensitive **in situ** gel were higher than for the brinzolamide eye drops from 10 to 60 min following administration. This result indicates that in the initial time period, the drug–resin complex could remain in the conjunctival sac for a certain time, which prolonged the retention time of the drugs on the ocular surface. The drug–resin thermosensitive **in situ** gel suffered a smaller precorneal elimination owing to its thermosensitive gelling property, which provided an enhancement in bioavailability. We speculate that the formulation was able to withstand tear dilution and blinking without network disruption. During the experiment, a thin gel in the surface layer of the cornea was observed.

**Absorption of Brinzolamide in the Aqueous Humor** The concentration–time profiles of brinzolamide in the rabbit aqueous humor are shown in Fig. 8. The pharmacokinetic parameters were estimated using DAS analysis (Drug And Statistics 1.0) as shown in Table 4. The \( C_{\text{max}} \) and \( AUC_{0-6h} \) values of the drug–resin thermosensitive **in situ** gel were 1.4 and 1.8 times greater than brinzolamide eye drop, respectively \((p<0.05, p<0.05)\). From 10 to 120 min, the content of brinzolamide in the aqueous humor was significantly higher after administration of brinzolamide drug–resin **in situ** gel formulations compared with after the instillation of the eye drops. This result suggests that with the use of the drug–resin **in situ** gel, more brinzolamide was absorbed into the eye before being washed out of the conjunctival sac by tears when compared to the use of the brinzolamide eye drops. Furthermore, the drug–resin complex was firstly exchanged with the endogenous ocular ions before the drug permeate the cornea, which prolonged the retention time and delivered drug at a controlled rate over a period of time. The \( T_{\text{max}} \) value of the drug–resin **in situ** gel was 1.3-fold higher than that of the eye drops \((p<0.05)\). The delay in \( T_{\text{max}} \) was due to the sustained brinzolamide delivery from the **in situ** gel. These **in vivo** results further demonstrate that the drug–resin thermosensitive **in situ** gelling system because of its ability to sustain drug release, can increase ocular bioavailability, reduce the frequency of administration and thereby improve patient compliance.

**Ocular Irritation Studies** The results of the Draize eye irritation test of the brinzolamide drug–resin complex thermosensitive hydrogel in rabbits are shown in Table 5. The brinzolamide drug–resin complex thermosensitive hydrogel did not irritate the rabbits’ eyes as shown by the total score of an eye irritation assessment after both a single dose and multiple doses equaling 0 and 1.33, respectively. Therefore, the brinzolamide drug–resin complex thermosensitive hydrogel could be accepted as safe for ophthalmic use.

| Model       | Equation                  | \( R^2 \) |
|-------------|---------------------------|-----------|
| Zero-order  | \( y = 6.2287x + 35.978 \) | 0.755     |
| First-order | \( y = -0.2379x - 0.3001 \) | 0.933     |
| Higuchi     | \( y = 28.852x + 7.4846 \)  | 0.897     |
| Ritger–Peppas| \( y = 43.075e^{0.1005} \) | 0.890     |

Fig. 6. (a) Gel Dissolution vs. Time Profiles of Drug–Resin **in Situ** Gels; (b) Plot of Percent of Drug Released vs. Percent of Gel Dissolved

Each data point is represented as mean±S.D. \((n=3)\).
Fig. 7. Brinzolamide Concentrations in Rabbit Tears at Different Time after Topical Application of the Eye Drop and Brinzolamide Drug–Resin *in Situ* Gel in the Rabbit Eyes

Each point represents the means±S.D. (n=5).

Table 3. Pharmacokinetics Parameters of Brinzolamide in Tear after Topical Administration Rabbit

| Group         | $T_{\text{max}}$ (min) | $C_{\text{max}}$ (g/g) | MRTlast (min) | $AUC_{0-360\text{min}}$ (min·g·g$^{-1}$) | $AUC_{0-\infty}$ (min·g·g$^{-1}$) |
|---------------|------------------------|-------------------------|----------------|----------------------------------------|----------------------------------|
| Eye drop      | 10.0±0.00              | 540.00±9.37             | 36.67±3.02     | 18701.08±571.57                       | 19395.77±761.12                  |
| *In situ* gel | 10.0±0.00              | 710.36±16.06            | 57.73±3.42     | 34155.86±1097.03                      | 35115.49±1245.65                 |

Results represent mean values±S.D., n=5.

Fig. 8. Brinzolamide Concentrations in Rabbit Aqueous Humors at Different Time after Topical Application of the Eye Drop and Brinzolamide Drug–Resin *in Situ* Gel in the Rabbit Eyes

Each point represents the means±S.D. (n=5).
The drug–resin in situ gel performed better than brinzolamide eye drops (marketed product) in retaining drugs. Moreover, the eye irritation test confirmed that the formulation was nonirritating to rabbit eyes. The gel formation was a free flowing liquid below 25°C and shifts to a firm gel after administration. The in vitro release profile shows an extended release of the drug over a period of 8 h. Moreover, the eye irritation test confirmed that the formulation was nonirritating to rabbit eyes. The in vivo results indicate that the drug–resin in situ gel performed better than brinzolamide eye drops (marketed product) in retaining drugs and producing high bioavailability. Finally, the performance of the drug–resin in situ gel against glaucoma is to be investigated.

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Table 4. Pharmacokinetics Parameters of Brinzolamide in Aqueous Humor after Topical Administration Rabbit

| Group     | $T_{max}$ (min) | $C_{max}$ (µg/mL) | MRTlast (min) | $AUC_{0-\infty}$ (min·µg·mL$^{-1}$) | $AUC_{0-t}$ (min·µg·mL$^{-1}$) |
|-----------|----------------|------------------|---------------|------------------------------------|---------------------------------|
| Eye drop  | 45.0±0.00      | 1.19±0.14        | 118.11±5.36   | 124.71±4.60                        | 131.36±5.20                     |
| In situ gel | 60.0±0.00      | 1.61±0.07        | 123.18±5.16   | 223.90±10.15                       | 235.99±11.78                    |

Results represent mean values±S.D., n=5.

Table 5. The Result of Eye Irritation Test (n=6)

| No.       | 1 | 2 | 3 | 4 | 5 | 6 | Average |
|-----------|---|---|---|---|---|---|---------|
| Single dose | 0 | 0 | 0 | 0 | 0 | 0 |         |
| Multiple dose | 1 | 1 | 1 | 1 | 1 | 1 | 1.33     |

Table 6. The Drug Content (%) of Stability Studies for 3 Months

| °C | Months | 0 | 1 | 2 | 3 |
|----|--------|---|---|---|---|
| 4±1 | 100.0±0.0 | 100.3±0.2 | 99.7±0.4 | 99.1±0.5 |
| 25±1 | 100.0±0.0 | 100.1±0.1 | 99.4±0.5 | 98.9±0.6 |
| 40±1 | 100.0±0.0 | 99.8±0.4 | 99.2±0.6 | 98.5±0.7 |

Results represent mean±S.D., n=3.