Optimization and Physicochemical Characterization of Thermosensitive Poloxamer Gel Containing Puerarin for Ophthalmic Use

Hongyi Qi, Li Li, Chunyan Huang, Wenmin Li, and Chunjie Wu

The purpose of this study was to systematically optimize an ophthalmic thermosensitive poloxamer analog gel containing puerarin that was a free flowing liquid below the room temperature and could shift to a gel with an eligible gel strength and bioadhesive force in physiological condition (dilution by the simulated tear fluid and at 35.0°C). A two-factor, five-level central composite design (CCD) was employed to the optimization procedure. The effect of formulation variables (the w/v concentration of poloxamer 407 (X1) and poloxamer 188 (X2)) on a number of response variables (the gelation temperature before (Y1) and after (Y2) the simulated tear fluid diluted, the difference between them (Y3)) was systemically investigated. A second order polynomial equation was fitted to the data. The resulting equation and response surface plots were used to predict the responses in the optimal region. Finally, 21.0% (w/v) poloxamer 407 and 5.0% (w/v) poloxamer 188 were chosen as the optimal poloxamer gel matrix. The influence of the other ingredients on the physicochemical properties of the formulation was also investigated. Hydroxypropyl-β-cyclodextrin (HPCD) enhanced the gelation temperature and reduced the gel strength and the bioadhesive force, while puerarin and benzalkonium chloride (BC) had a comparatively smaller influence. All the isotonicity agents studied had the gelation temperatures lowered, and the gel strengths and the bioadhesive forces enhanced. But only sodium chloride appears to be a promising isotonicity agent for the poloxamer gel containing puerarin, HPCD and BC.

Key words thermosensitive gel; puerarin; central composite design (CCD); poloxamer; physicochemical property; ophthalmic delivery system

The eye is a very sensitive organ, which presents many challenges to develop effective ophthalmic dosage forms. Due to the lachrymation, the normal tear turnover and the drainage from the nasolacrimal duct, the ophthalmic solutions eliminate rapidly, which causes a short precorneal residence time and a limitation of transcorneal absorption. These lead to an ocular bioavailability that is commonly less than 10%. Meanwhile, after draining from the nasolacrimal duct into the gastrointestinal tract, some drugs may cause systemic side-effects.1-3) Although these drawbacks can be overcome in some degree by using several new preparations, such as ointments and inserts, these preparations present some disadvantages, such as blurred vision and noncompliance, which bring about some new problems to patients.4)

Compared to these preparations mentioned above, in situ gels have more advantages in these aspects. These systems consisting of polymers undergo sol-to-gel phase transitions as a result of a special physical/chemical change (for example, pH or temperature) induced by the physiological environment.4,5) According to the different factors that cause sol-to-gel phase transitions on the eye surface, the ophthalmic in situ gels can be divided into the following three types: pH triggered (e.g. cellulose acetate hydrogen phthalate latex5) and acrylic polymer6), temperature-dependent (e.g. poloxamer,8 and Ethyl hydroxyl ethylcellulose9) and ion-activated (e.g. Gelrite10) and alginate11).

Poloxamer is a block copolymer that consists of polyethylene oxide (PEO) units and polypropylene oxide (PPO) blocks and is known for exhibiting the phenomenon of reverse thermal gelation under a certain concentration and temperature.5,11-13) 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 semisolid gel under the ambient temperature.14) Depending on this character, it is possible to develop a new preparation that is a liquid form allowing a comfortable and precise delivery and shift to gel phase with a long precorneal residence time and high bioavailability after being triggered by the temperature of conjunctival sac (35°C). But the dilution by tear fluid is a factor that can’t be disregarded, as the P407 solution of lower concentration will lose the gelation ability after diluted by tear fluid.4) Considering this factor, 25.0% P407 (w/w) is essential to form gel in situ. In this case, the gelation temperature is lower than room temperature and the solution must be stored in refrigerator, which causes great inconvenience for the preparation and the use. Therefore, some regulatory substances were added to P407 solutions. El-Kamel attempted to reduce the poloxamer concentration without compromising the in situ gelling capacity by adding various viscosity enhancing agents such as methylcellulose, hydroxypropylmethylcellulose and sodium carboxymethylcellulose.5) However, there was little information concerning how these additives affected the gelation temperature. Although obviously increasing the gelation temperature of P407, the addition of polyethylene glycol made the gel system more sensitive to dilution occurring in the eye.4) Poloxamer 188 (P188), which is a homologen of P407, not only increased the gelation temperature of P407, but also enhanced the bioadhesive force and the ocular bioavailability to some extent,14,15) so it may be a regulatory substance that has a good perspective for application. However, the dilution by tear fluid should be taken into consideration in the time of the formulation optimization, which was often neglected. Therefore, it deserves further optimization and investigation.

For effective optimization of poloxamer in situ gel formulation, a systemic approach is required. Univariate approach, sequential techniques and simultaneous techniques are three kinds of optimization strategies. The univariate approach,
which is the simplest one, is typically used in such cases when the effect of a limited number of factors without mutual interactions is examined. It has mainly been used for the optimization of in situ gel formulation.\textsuperscript{14–17} However, the poloxamer analogs may be interdependent to form the optimum in situ gel and the effect of multiple variables should be studied simultaneously. Therefore, we employed central composite design (CCD) to optimize the poloxamer in situ gel formulation.

Central composite design (CCD), an experimental design method of Response Surface Methodology, is composed of two-level factorial design with axial point and central point. It can derive a functional relationship between an experimental response and a set of factors through experiments and statistics. Then the response surface of a certain response to a set of factors can be plotted. Furthermore, the optimum level of experimental factors required for a given response can be determined. CCD enables the simultaneous investigation of the effect of each factor and their interaction over the experimental responses and reduces the number of experimental runs that are necessary to establish a mathematical functional relationship in the experimental design region.\textsuperscript{18–20} Therefore, it is a systematic and efficient method to simultaneously study the effect of multiple variables and to find an optimum formulation.

Puerarin is an isoflavone extracted from the radix of Pueraria lobata (Willd.) Ohwi. It can block β acceptors, lower intraocular pressure, and improve ocular blood flow and be used as a therapeutic agent for cataracta glauca, ocular hypertension.\textsuperscript{21,22} However, there also exists the systemic absorption and gastrointestinal side effects, so it is necessary to develop new dosage forms to minimize the systemic absorption and enhance ocular bioavailability of puerarin.

The purpose of the present study is to reveal the functional relationship between the gelation temperatures before and after the simulated tear fluid (STF) dilution and the concentrations of poloxamer analogs through CCD, and to systematically optimize a thermosensitive gel of poloxamer analogs that has a gelation temperature higher than room temperature before STF dilution and can still complete the phase transition in physiological condition (dilution by STF and at 35 °C). Moreover, based on this result, the influence of puerarin which may cause respiratory and gastrointestinal side effects, so it is necessary to develop new dosage forms to minimize the systemic absorption and enhance ocular bioavailability of puerarin.

Table 1. Independent Variables and Their Levels in Coded and Physical Form

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Rang and levels</th>
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<tbody>
<tr>
<td>$X_{1,0}$</td>
<td>$-1.414$</td>
</tr>
<tr>
<td>$X_{1,0}$</td>
<td>$12.5$</td>
</tr>
<tr>
<td>$X_{2,0}$</td>
<td>$3.66$</td>
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\(a: X_1\) the concentration of P407 (%, w/v). \(b: X_2\) the concentration of P188 (%, w/v).

Measurement of Gelation Temperature

Ten milliliters poloxamer 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 poloxamer 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 the gelation temperature, at which the magnetic bar stopped moving due to gelation.\textsuperscript{14,16,17} Each sample was measured at least in triplicate. In order to simulate the in vivo phase transition process of thermosensitive gels more literally, the gelation temperatures were measured after the poloxamer formulations were diluted by STF in a ratio of 40 : 7.\textsuperscript{14} STF according to the electrolyte composition of tear fluid was prepared as previously reported.\textsuperscript{24}

**Central Composite Design**

The concentrations of P407 ($X_1$) and P188 ($X_2$) were chosen as factors in this experimental design. According to our preliminary experimental results, the principle of CCD and the feasibility of preparing the thermosensitive gel under the highest or the lowest level, five levels of each factor were determined as shown in Table 1.

Usually, $2^f+2f+1$ experiments are required according to the principle of CCD, where $f$ represents the number of factors to be studied. Therefore, 9 experimental points are required in a two-factor CCD, each of which being a result of different formulations. In order to estimate the pure experimental uncertainty of CCD, it is important to measure repeatedly the response function to the conditions determined by the central points. In this case, five repeated experiments were performed.\textsuperscript{18–20} Experimental runs are shown in Table 2. The observed responses were determined according to the method mentioned above. Each experimental response can be represented by the following quadratic equation of the response surface.

$$Y=b_0+b_1X_1+b_2X_2+b_3X_1^2+b_4X_2^2+b_5X_1X_2$$

In this equation, $Y$ is the measured response associated with each factor level combination; $b_0$ is an intercept; $b_1$, $b_2$, $b_3$, $b_4$, $b_5$ are the regression coefficients; $X_1$ and $X_2$ are the factors studied. The statistical package Statistica (version 5.0, Tulsa, OK) was used to calculate the constant and the regression coefficients. With the purpose of checking the reliability of the model, analysis of variance was applied. The resulting equations were subjected to lack-of-fit and model simplification at 95% significance level. Response surfaces that demonstrate the relationship between the response variables and the formulation variables were generated from the fitting equations. The optimal surfaces for individual response variables were located by superimposing the contour plots for all the response variables. Finally, a series of experiments were made in order to check the reliability of the response surface model, by comparing the predicted values with the experimental data.

Measurement of Gel Strength

The experiment was carried out according to the previously published methods.\textsuperscript{16,21} Twenty-five milliliters poloxamer solution was put into a 50 ml glass cylinder, which was placed into a thermostat at 35.0 °C for 10 min to make the solution gel. A piston with eye-holes in the undersurface was put into the cylinder; meanwhile, a weight (10 g) was placed onto the piston to make it go down. The gel strength was determined by time(s) it took to move the piston 5 cm down through the poloxamer gel. To evaluate the gel strength change after instillation and mixing with the tear fluid, the gel strength measurements were also taken after diluting the formulations with STF. The gel strength of sample solutions was measured as described above.

Measurement of Bioadhesive Force

The experimental technique used for determining the bioadhesive force has been derived from a previously published method.\textsuperscript{25–27} The experimental setup is presented in Fig. 1. Briefly, a section of tissue was cut from the cornea of New Zealand albino rabbit (2.5±0.2 kg, ¥C, the nursery of the Experimental Animal Professional Committee, Sichuan, China) 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.\textsuperscript{28} The corneal tissue was attached to the undersurface of the teflon cylinder (C) (0.785 cm\(^2\)) using a cyanoacrylate adhesive, and 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. 40 μl poloxamer solution was added onto the sample cell of the thermostat (E) at 35.0 °C, which was placed on a height-adjustable pan (F), and the height of the pan (F) was adjusted quickly to make the poloxamer solution just come into contact with the corneal tissue before the poloxamer 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 of 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 poloxamer solution and the corneal tissue were detached. Bioadhesive force, the detachment stress (dyne/cm\(^2\)), was determined from the minimal weights that detached the corneal tissue before the poloxamer 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 also taken after diluting the formulations with STF. The bioadhesive force of sample solutions was measured as described above. This experiment was approved by the local ethics committees for animal experimentation.

Results and Discussion

Observed Responses and Model Fitting

The dilution by tear fluid must be taken into consideration in the design of ophthalmic formulations. The optimum ophthalmic thermosensitive gels should have a gelation temperature higher than room temperature (according to the previous reports\textsuperscript{4,14}) 25.0 °C was chosen as a representative temperature for the room temperature in this study) and form gel at the conjunctival sac temperature (35.0 °C) after mixed with tear fluid. Therefore, the gelation temperatures before and after STF dilution were selected as responses of CCD, which were sensitive gel to endure the dilution by STF. When meeting the precondition that the gelation temperature after STF dilution is lower than 35.0 °C, although a lower DGT doesn’t imply a higher gelation temperature before STF dilution, the formulation with a higher gelation temperature before STF dilution usually has a comparatively lower DGT. So DGT (Y\(_1\)) was also selected as one of the responses. The observed values of the responses were shown in Table 2. A second-order polynomial model was individually fitted to all the response variables. The results of the applied statistical tests indicated that all three response variables measured in this study showed good fitting to the second-order polynomial model. The fitting equations that resulted after model simplification are given below and the corresponding \(R^2\) values and significance levels of individual responses are also exhibited.

\[
Y_1 = 83.2183 - 2.9792X_1 + 0.0708X_2 - 0.0724X_1^2 \\
\text{(2)} \quad (R^2=0.9923; \ p<0.0001)
\]

\[
Y_2 = 93.2488 - 3.2313X_1 + 0.0923X_2 - 0.0746X_1^2 \\
\text{(2)} \quad (R^2=0.9839; \ p<0.0001)
\]

\[
Y_3 = 4.8208 + 0.4107X_2 \\
\text{(1)} \quad (R^2=0.9815; \ p<0.0001)
\]

Coefficients with one factor represent the effect of that particular factor on responses while the coefficients with more than one factor and those with second order terms represent the interaction between those factors and the quadratic nature of the phenomena, respectively. Positive sign in front of the terms indicates synergistic effect while negative sign indicates antagonistic effect upon the responses.\textsuperscript{18–20} Therefore, from the Eqs. 1 and 2, it can be qualitatively concluded that \(X_1\) had the largest antagonistic effect on the responses of \(Y_1\) and \(Y_2\), which indicated that \(X_1\) was a more important parameter to regulate gelation temperature, while the antagonistic effect of the quadratic term of \(X_2\) was comparatively smaller. On the contrary, the interaction between \(X_1\) and \(X_2\) had synergistic effect on the responses of \(Y_1\) and \(Y_2\). Although the effect was also small compared to that of \(X_1\), it couldn’t be disregarded, as its coefficients in both Eqs. 1 and 2 were significant at \(p<0.0001\). In Eq. 3, it is shown that there was a positive correlation between \(X_2\) and \(Y_3\), which meant DGT was only relevant to the concentration of P188. From the constant in Eq. 3, we can see that theoretically the gelation temperature of the poloxamer solutions at least increased approximately 4.8 °C after STF dilution.

Analysis of the Response Surfaces

According to the second-order polynomial equations, the three-dimensional response surface curves and two-dimensional contour curves for \(Y_1\), \(Y_2\) and \(Y_3\) were shown in Figs. 2—4, respectively.

Various investigators have reported the possible mechanism of the gelation of poloxamer: When the concentration and the 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. The thermoresversible 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 gelation temperature lowered and the PEO that is hydrophilic has the gelation temperature increased.\cite{29-33} Therefore, a different PEO/PPO ratio will lead to a different gelation temperature. Poloxamer analogs possess the different PEO/PPO ratio. Therefore, the aim of the modulation of gelation temperature can be reached by mixing various amounts of P407 and P188 in aqueous solution accordingly. Figure 2 showed that the gelation temperature of the mixed poloxamer formulations increased before STF dilution. This can be explained that both the quantity of micelles and the probability that micelles entangled and packed with each other increased as the P407 concentration increased, which made the gelation temperatures lowered proportionally. However, as the P188 concentration increased gradually with constant P407 content, the gelation temperature initially increased to maximum, and then decreased. The same result was reported by Wei et al.\cite{14} The possible reason is the incorporation of slight amount of P188 can only change the PEO/PPO ratio, which causes the increase of gelation temperature. While increasing the P188 concentration sequentially, not only the PEO/PPO ratio changes, but also the micellization P188 molecules can participate in the construction of the gel, which lead to the decrease of the gelation temperature finally.

As illustrated in Fig. 3, owing to the fact that the total concentration of the polymer lowered after STF dilution, the gelation temperature all increased. However, this didn’t alter the tendency of the change of the gelation temperature caused by the change of P407 and P188 concentrations.

As shown in Fig. 4, $D_{GT}$ was only relevant to the P188 concentration, and had nothing to do with P407 concentrations. When the P188 concentration was 25% (w/v), according to the fitting model, the gelation temperature would increase approximately 15.0°C after STF dilution. This demonstrated that the ability of the thermosensitive gels to endure the dilution by STF lowered after the incorporation of P188. As a possible mechanism by which P188 affected $D_{GT}$, it is conceivable that the ratio of PEO and PPO is 7:3 in P407, whereas that is 8:2 in P188. When a slight amount of P188 is incorporated into the P407 solution, the proportion of the PEO will increase, which will lead to the increase of the gelation temperature. However, the original balance between PEO and PPO will also change at this time, which may disturb the formation of the P407 micelles, so the ability to endure the STF dilution will also become weaker compared with P407 only. As when the P188 concentration exceeds 30% (w/w) and the temperature is above 50.0°C, P188 alone will also form gel through the entanglement and packing of the micelles.\cite{25} Therefore, when the amount of P188 incorporated into the P407 solution increases further, although the P188 concentration and the temperature don’t reach the requirement by which it can form gel alone, the P188 micelles can participate into the entanglement and packing with the P407 micelles in a certain extent, which may lead to P407 gelation under a comparatively lower temperature. So when the P188 concentration increases to a certain value, the gelation temperature of the mixture will decrease. However, under this condition, the ratio of PEO and PPO changes even bigger, the original balance between PEO and PPO is also destroyed even bigger and the regularity of the entanglement and packing of the micelles may become much lower. Therefore, although the micelles can entangle and pack with each other under a comparatively lower temperature at this time, the regularity and the fastness of the entanglement and packing are lower than that when P407 is used alone and the ability to endure the STF dilution will also become lower. Taking all these together, we may conclude that when P188 is incor-
Optimized into the P407 solution, the gelation temperature will increase firstly and then decrease, whereas the DcOT will increase and the ability to endure the STF will decrease constantly. Therefore, when P188 is used as a regulatory substance, the two aspects, enhancing the gelation temperature and enduring the STF dilution, must be taken into consideration.

Optimization of the Formulation The optimization was performed by superimposing the contour plots of the response Y1 and Y2 and locating the region of optimal surface common to both the plots. As the optimal formulation should have a gelation temperature higher than 25.0 °C before mixed with STF, and lower than 35.0 °C, the region of optimal surface is located to the region that is surrounded by the 25.0 °C isothermal before STF dilution and the 35.0 °C isothermal after STF dilution. As shown in Fig. 5, at the point of these two isothermals intersecting with each other, Y1 is just 10.0 °C. According to the relationship between Y1 and X2, which is shown in Eq. 3 and Fig. 4, Y1 of the formulations in region B (Fig. 5) exceed 10.0 °C, which means that Y1 should not exceed 10.0 °C, therefore, the region of optimal surface is located to the region that is surrounded by the 25.0 °C isothermal before STF dilution and the 35.0 °C isothermal after STF dilution. As shown in Fig. 5, at the point of these two isothermals intersecting with each other, Y1 is just 10.0 °C. According to the relationship between Y1 and X2, which is shown in Eq. 3 and Fig. 4, Y1 of the formulations in region B (Fig. 5) exceed 10.0 °C, which means that Y1 should not exceed 10.0 °C, therefore, the region of optimal surface is located to the region that is surrounded by the 25.0 °C isothermal before STF dilution and the 35.0 °C isothermal after STF dilution. As shown in Fig. 5, at the point of these two isothermals intersecting with each other, Y1 is just 10.0 °C. According to the relationship between Y1 and X2, which is shown in Eq. 3 and Fig. 4, Y1 of the formulations in region B (Fig. 5) exceed 10.0 °C, which means that Y1 should not exceed 10.0 °C, therefore, the region of optimal surface is located to the region that is surrounded by the 25.0 °C isothermal before STF dilution and the 35.0 °C isothermal after STF dilution.

So the region B isn’t the region of optimal surface. The region B in which all the DGT values exceed 10.0 °C isn’t the region of optimal surface. From the region A, the optimal range of Y1 we can get is 19.0—22.0% (w/v) and that of Y2 is 0—12.0% (w/v).

Comparing the observed Y1 and Y2 values of the formulations prepared in the optimum region with the model predicted values (Table 3) shows that the observed values are satisfactorily close to the predicted values, with a low percentage of bias, and it is concluded that the optimal surface was chosen correctly and that the model has satisfactory predictive power. From the observed values, we can see that the formulations marked 1, 4, 5, 7, 8 and 11 can satisfy the requirement. Among these formulations, even though Y1 of the formulations 1, 4, 5, 7, 8 and 11 can be comparatively lower, Y2 of them are also lower than that of the formulation 8. The formulation 8 that contains 21.0% (w/v) P407 and 5.0% (w/v) P188 (abbreviated as poloxamer gel (21/5)) had the highest Y1 (27.3 °C), meanwhile, the Y2 was 34.8 °C which is within 35.0 °C. So the formulation 8 was chosen as the formulation for further investigation.

Influence of Puerarin, HPCD and Benzalkonium Chloride (BC) on the Physicochemical Properties of the Poloxamer Gels (21/5) Puerarin is the active component of this pharmaceutical preparation, and usually, its concentration in ophthalmic preparations is 1.0% (w/v). In these preparations, solubilizing agents have to be added to make puerarin reach the effective concentration due to its poor water solubility. Our former investigation demonstrated that 5.0% (w/v) HPCD is able to not only make the puerarin concentration reach the effective value, but also increase its stability and transcorneal permeability (effect of hydroxypropyl-β-cyclodextrin on aqueous solubility, stability and corneal permeation of puerarin, submitted for publication). So 5.0% (w/v) HPCD was added into the formulation. 0.02% (w/v) benzalkonium chloride (BC) was incorporated as a bacterial inhibitor. In this part, the influence of puerarin, HPCD, BC on physicochemical properties of poloxamer gels (21/5) was further investigated.

As shown in Fig. 6, HPCD when added to the poloxamer gel (21/5) obviously increased the gelation temperature and greatly reduced the gel strength and the bioadhesive force compared with the poloxamer gel (21/5) alone. Previous study has shown the similar results.15) This can be assumed that the binding force (hydrogen bond) of cross-linked reticular poloxamer gel became weaker by replacing coniform HPCD molecules in the gel matrix, and the entanglement and packing of the poloxamer micelles might be disturbed. However, in the presence of puerarin, the gelation temperatures decreased 2.5 °C and the gel strength and the bioadhesive force increased 20.5% and 2.7%, respectively, compared with those of the poloxamer gel (21/5) containing HPCD only. It is concluded that when puerarin and HPCD were used together, puerarin may be included inside the HPCD ring. Meanwhile, as it contains hydroxyl groups, it may form hy-
Hdrogen bonding with the hydroxyl groups of the HPCD ring, which will affect the hydrogen bonding between HPCD and poloxamer gel and reduce the interference of HPCD to the entanglement and packing of the poloxamer micelles.

**Influence of Various Isotonicity Agents on the Physicochemical Properties of the Poloxamer Gels (21/5)**

Isotonic ophthalmic preparation was preferred by the majority of the patients as it could make eyes more comfortable. Therefore, the incorporation of isotonicity agents was taken into consideration. Propylene glycol (PG), glycerol, sorbitol, mannitol, sodium chloride (NaCl) and glucose have been used to prepare isotonic vehicles for eyes and were added to the poloxamer gels to examine their effects on the gelation temperature, the gel strength, and the bioadhesive force.

As can be seen in Fig. 7, glycerol, PG and glucose, the concentrations of which were 2.6% (w/v), 2.0% (w/v) and 5.6% (w/v), respectively, had slight influence on the gelation temperatures. Under the conditions of A and B, $D_{GT}$ values were 8.1—9.1 °C. However, all the gelation temperatures of the poloxamer gels (21/5) containing these three isotonicity agents respectively were higher than 35.0 °C under the condition of B; Sorbitol and mannitol, the concentrations of which were 5.6% (w/v) and 5.0% (w/v), both had a comparatively larger influence on the gelation temperatures. The gelation temperatures before STF dilution were both lower than 25.0 °C under the conditions of A and B, and $D_{GT}$ increased to 10.1 °C and 10.9 °C, respectively, under the condition of B; 0.9% (w/v) NaCl had a comparatively smaller influence on the gelation temperature. Under the conditions of A and B, $D_{GT}$ values were 7.8 °C and 8.5 °C, respectively, and the gelation temperatures before and after STF dilution were within the temperature range of 25.0—35.0 °C under the condition of B.

As shown in Fig. 8, of the isotonicity agents studied, glycerol, PG and glucose slightly increased the gel strengths and the bioadhesive forces compared with those of the poloxamer gel (21/5) without puerarin, HPCD and BC. Contrary to this, sorbitol, mannitol and NaCl had larger influence on the gel strengths, which enhanced 57.4%, 82.5% and 76.8%, respectively, and the bioadhesive forces, which increased 38.0%, 32.2% and 28.5%, respectively.

In the presence of puerarin, HPCD and BC, the influence of all these isotonicity agents on the gel strengths and the bioadhesive forces of the poloxamer gel (21/5) was similar to that of the poloxamer gel (21/5) in the absence of puerarin, HPCD and BC. However, with the addition of puerarin, HPCD and BC, the gel strengths and the bioadhesive forces of the poloxamer gels (21/5) containing glycerol, PG and glucose, respectively, greatly decreased compared with those of the poloxamer gels (21/5) containing puerarin, HPCD and BC, whereas the addition of puerarin, HPCD and BC caused smaller decreases in both the gel strengths and the bioadhesive forces of the poloxamer gel (21/5) added with sorbitol,
mannitol and NaCl, respectively.

It is of great importance that the ophthalmic gels complete the phase transition and have suitable gel strength and bioadhesive force in physiological condition considering that the gels will be drained continually by tear fluid as a result of lachrymation and normal tear turnover,\(^6\) which causes a shortened precorneal retention time and lower bioavailability. The polymer that has a stronger effect of bioadhesion usually contains a certain quantity of hydrophilic functional groups (e.g. hydroxyl group) that can form hydrogen bonds, electrostatic attraction, or hydrophobic interaction with mucopolymers of the corneal surface. Moreover, its molecular chains often possess enough flexibility and can tangle with mucopolymers to form a fluffy network structure, which may delay the drug’s elimination.\(^3,^4\) As a possible mechanism by which these isotonicity agents except NaCl affected the gel strengths and the bioadhesive forces of poloxamer gel base as observed in this study, it is speculated that the differences existing in the number of the hydrogen bonds and stereoc- hemical structures of the isotonicity agents cause the different extent bonding with poloxamer micelles in the gel matrix or with mucopolymers in the corneal surface. Sorbitol and mannitol both have chain structures with six hydroxyl groups, obtaining stronger hydrogen bonds with chainging poloxamer micelles or mucopolymers, so the gel strength and the bioadhesive force of the poloxamer gel (21/5) with sorbitol or mannitol as the isotonicity agent is even bigger. As glycerol and PG contain less hydroxyl groups, two and three, respectively, their ability to form the hydrogen bond is weaker than that of sorbitol and mannitol. Even though glucose contains five hydroxyl groups, its stereo circular structure makes it difficult for its hydroxyl groups to form hydrogen bonds and entanglements with the linear backbones of poloxamer analogs or mucopolymers. Therefore, it causes a slight influence on the gel strength and the bioadhesive force.

The effect of NaCl on the gelation temperature and the gel strength consists with Choi et al. and Yong et al.,\(^16,^17\) who investigated that the influence of NaCl on the gel strength of the liquid suppository. They supposed this might be attributed to the fact that NaCl could bind strongly with the cross-linked reticular poloxamer gel by the strong cross-linking bonding of sodium salt with poloxamer. But they didn’t point out what is the driving force of strong cross-linking bonding of sodium salt with poloxamer. Malmsten et al. and Pandit et al. who investigated the change of the gelation ability of poloxamer in the presence of some salts found that NaCl could lower the gelation temperature of poloxamer and ascribe this to salting-out effects. Some inorganic salts have the ability to reduce the water activity of the polymer system, which can influence the temperature-depending dissolving behavior of the PEO in the poloxamer molecule. That is to say the salts can lead to the salting-out of PEO from aqueous solutions with the temperature increases, which leads to the decrease of the gelation temperature of the poloxamer. They pointed out the critical micelle concentration and the critical micelle temperature might decrease with some salts and the micelle would entangle and pack more tightly in the presence of some salts under the identical temperature.\(^3,^7\) These seem more reasonable to explain why NaCl can reduce the gelation temperature and enhance the gel strength of the poloxamer gels (21/5). Since the poloxamer with hydrophilic oxide group could bind to mucopolymers. However, NaCl has no capacity of binding to them. The bioadhesive force-enhancing effect of NaCl seemed to be due to its gel strength-enhancing effect of poloxamer gel, resulting in the more increased binding of poloxamer gels with the mucopolymers of cornea.

Taking the experimental results together, it can be seen that sorbitol and mannitol had a strong effect on the gel strengths and the bioadhesive forces, but they both made DGT values exceed 10.0 °C. Therefore, the poloxamer gels with the optimal gelation temperatures couldn’t be obtained when sorbitol and mannitol were used as the isotonicity agents. Even though the poloxamer gels with the glycerol, PG and glucose as the isotonicity agents, respectively, can obtain the eligible gelation temperatures, the gel strengths and the bioadhesive forces of these three gels were comparatively smaller. When NaCl was used as the isotonicity agent, the poloxamer gel (21/5) had not only an eligible gelation temperature, but also a comparatively higher gel strength and bioadhesive force. Although the poloxamer gel containing puerarin, HPCD and BC had a gelation temperature higher than 35.0 °C after mixed with STF, which caused the gel strength and the bioadhesive force to decrease, the gelation temperature became lower than 35.0 °C after the incorporation of NaCl, and then the gel strength and the bioadhesive force significantly increased.

**Conclusions**

In this study, a thermosensitive ophthalmic gel of puerarin was optimized and developed with 21.0% (w/v) P407 and 5.0% (w/v) P188 as the gel matrix, HPCD as the solubilizing agent, NaCl as the isotonicity agent, and BC as bacterial inhibitor. This in situ gelling formulation was a free flowing liquid below the room temperature and could convert to a gel that had an eligible gel strength and bioadhesive force after instilled into conjunctival sac. Therefore, it is a viable alternative to conventional eye drops by virtue of its abilities that it can not only be readily administered and decrease the frequency of administration, thus resulting in better patient acceptance, but also prolong the precorneal residence time to get higher bioavailability and reduce the systemic side-effects caused by the drainage from the nasolacrimal duct. The further study is to be performed on the in vitro release, pharmacokinetics and pharmacodynamics of this poloxamer gel in rabbits.

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**References**
