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Research Article

In Situ Gelling Gelrite/Alginate Formulations as Vehicles for Ophthalmic Drug Delivery

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Abstract. The objective of this study was to develop an ion-activated *in situ* gelling vehicle for ophthalmic delivery of matrine. The rheological properties of polymer solutions, including Gelrite, alginate, and Gelrite/alginate solution, were evaluated. In addition, the effect of formulation characteristics on *in vitro* release and *in vivo* precorneal drug kinetic of matrine was investigated. It was found that the optimum concentration of Gelrite solution for the *in situ* gel-forming delivery systems was 0.3% (*w*/*w*) and that for alginate solution was 1.4% (*w*/*w*). The mixture of 0.2% Gelrite and 0.6% alginate solutions showed a significant enhancement in gel strength at physiological condition. On the basis of the *in vitro* results, the Gelrite formulations of matrine-containing alginate released the drug most slowly. For each tested polymer solution, the concentration of matrine in the precorneal area was higher than that of matrine-containing simulated tear fluid (STF) almost at each time point ($p<0.05$). The area under the curve of formulation 16 (0.2%Gelrite/0.6%alginate) was 4.65 times greater than that of containing matrine STF. Both the *in vitro* release and *in vivo* pharmacological studies indicated that the Gelrite/alginate solution had the better ability to retain drug than the Gelrite or alginate solutions alone. The tested formulation was found to be almost non-irritant in the ocular irritancy test. The overall results of this study revealed that the Gelrite/alginate mixture can be used as an *in situ* gelling vehicle to enhance ocular retention.

KEY WORDS: alginate; Gelrite®; *in situ* gelling; matrine; ophthalmic delivery.

INTRODUCTION

The extent of absorption of an ophthalmic drug is severely limited by physiological constraints. The conventional liquid ocular formulation is eliminated from the precorneal area immediately upon instillation because of lachrymation and effective nasolacrimal drainage (1). Various preparations, such as ointments (2), suspensions (3), inserts (4), and hydrogels, have been developed for ophthalmic delivery system not only to slow down the drug elimination but also to lengthen the residence time of vehicle on ocular surface (5). However, they have not been used extensively because of some drawbacks, such as blurred vision with ointments or low patient compliance with inserts (6).

An ideal ophthalmic formulation should be administrated in eye drop form, without causing blurred vision or irritation. This problem can be overcome using *in situ* gel-forming drug delivery systems prepared from polymers that exhibit sol-to-gel phase transitions due to a change in a specific physicochemical parameter in the cul-de-sac (7). In the past few years, an impressive number of pH (e.g., cellulose acetate phthalate and Carbopol), temperature (e.g., Poloxamer), and ion (e.g., gellan gum and alginate) induced *in situ* forming systems have been reported to sustain ophthalmic drug delivery (8–11). These *in situ* gel-forming systems could prolong the precorneal residence time of a drug (12–16) and improve ocular bioavailability (17). The choice of a special hydrogel depends on its intrinsic properties and envisaged therapeutic use.

Deacetylated gellan gum (an exocellular polysaccharide of microbial origin, commercially available as Gelrite®) is an interesting *in situ* gelling polymer that has been tested since it seems to perform very well in humans (10,18). Preparations of Gelrite are dropped into eyes; gel formation takes place, induced by the electrolytes of the tear fluid (19). The other *in situ* gelling compound examined, sodium alginate, is widely used in pharmaceutical preparation (20,21). Similarly, aqueous solutions of alginate (a natural polysaccharide extracted from brown sea algae) also form gels when instilled into the eye. It was previously reported that Joshi *et al.* (22) used a combination of polymers in the delivery system to reduce total polymer content and improve gelling properties. They demonstrated that aqueous compositions reversibly gelled in response to simultaneous variations in at least two physical parameters (e.g., pH, temperature, and ionic strength) can be formed by using a combination of polymers that exhibit reversible gelation properties. Many authors, on the basis of this finding, have developed the similar delivery system to improve patient compliance and therapeutic activity (23–25).

Matrine is an alkaloid isolated from the root of *Sophora subprostrata* (Leguminosae); it has obvious anti-inflammatory, immune suppressive, hepatic protective, and anti-tumor effects. Hence, matrine was used widely in clinical treatment in China (26). Aqueous solution of matrine (1%, *w*/*w*) showed significant

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inhibition of ocular inflammation induced by lens proteins (27). Matrine eye drops can be used to treat the bacterial keratitis and bacterial conjunctivitis (28). However, due to the lachrymation, the normal tear turnover, and the drainage from the nasolacrymal duct, matrine eye drops eliminate rapidly, which causes a short precorneal residence time and a limitation of transcorneal absorption. It has been reported that there existed a systemic absorption of matrine which might cause some side effects such as general malaise, nausea, chest distress, abdominal distension, and headache (29,30). Therefore, it is necessary to develop new dosage forms to minimize the systemic absorption and enhance ocular bioavailability of matrine. According to previous reports (19,31), the Gelrite-based ocular delivery system for indomethacin demonstrated better therapeutic efficacy compared to conventional eye drops, and the *in situ* gelling alginate system was an excellent drug carrier for the prolonged delivery of pilocarpine. As clarified by Joshi *et al.* (22), the combined Gelrite/alginate system could improve the gelling properties and reduce the total polymer to be introduced into the eye. In this study, we describe an alternative *in situ* gelling system prepared by the combination of Gelrite and alginate. The rheological behaviors of various polymer solutions under different conditions were analyzed. In addition, the *in vitro* matrine release and *in vivo* precorneal retention of different drug-containing formulations were characterized to evaluate the use of *in situ* gelling polymer solutions for ocular drug delivery.

MATERIALS AND METHODS

Materials

Gelrite® (Deacetylated gellan gum) was obtained from Kelco division of Shanghai, China. Sodium alginate (Manucol DMF®, which is composed of 37% mannuronic acid and 63% guluronic acid) was kindly gifted by ISP (USA). Matrine was supplied by Xi'an Tianze Biotechnology Co., Ltd. (Shanxi, China). Sodium fluorescein was provided by Shanghai Reagent Factory, China. Methanol was chromatographic grade (Fisher Chemicals). Sodium chloride, sodium bicarbonate, calcium chloride dihydrate, potassium chloride, and hydrochloric acid were purchased from Kelong Chemical Reagent Factory (Chengdu, China) and were of analytical grade.

New Zealand White rabbits, weighing 2.0–3.0 kg, were provided by the Animal Experimental Center of Chengdu University of Traditional Chinese Medicine. The experimental animals were individually housed in an air-conditioned and light-controlled room at 24±1°C and at 65±5% relative humidity. They were given a standard pellet diet and were provided with water *ad libitum*. All animals were healthy and free of clinically observable ocular abnormalities. The approval of an animal ethics committee was obtained before starting the study. The procedures involving animals were reviewed and approved by the Animal Ethical Committee at Chengdu University of Traditional Chinese Medicine.

Methods

Preparation of Formulations

Gelrite gels of concentrations between 0.1 and 0.5% (*w*/*w*) were prepared by slowly adding a weighed amount of the deacetylated gellan gum to cold ultrapure water with continuous stirring for 10 min. The partially dissolved mixture was stored in the refrigerator until the entire polymer was completely dissolved (approximately 24 h). Alginate solutions (0.1–1.5%, *w*/*w*) were prepared in a similar manner and stored overnight at 5°C to ensure complete dissolution before the evaluation of their rheological properties.

The Gelrite/alginate solutions were prepared by dispersing the required amount of alginate in the desired concentration of Gelrite with continuous stirring for 10 min. The partially dissolved solutions were then refrigerated until solutions were thoroughly mixed (approximately 24 h). For the preparation of drug-containing polymer solutions, an appropriate amount of matrine was then dissolved in the resulting solution to produce a final drug concentration of 1% (*w*/*v*). Preparations were made isotonic by the addition of mannitol (5%, *w*/*v*), and the pH was adjusted to 8.0 using hydrochloric acid. Simulated tear fluid (STF, composition: NaCl 0.68 g, $NaHCO_3$ 0.22 g, $CaCl_2{\cdot}2H_2O$ 0.008 g, KCl 0.14 g, and distilled deionized water to 100 mL) was used as a dispersion medium in the *in vitro* release study (32). The gelling capacity was determined by placing 100 μL of the preparation in a vial containing 2 mL of simulated tear fluid freshly prepared and equilibrated at 34°C and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve (33). The concentrated simulated tear fluid (composition: NaCl 6.8 g, $NaHCO_3$ 2.2 g, $CaCl_2{\cdot}2H_2O$ 0.08 g, KCl 1.4 g, and water to 100 mL) was made with ultrapure water as a solvent and mixed with the preparation in order to investigate the effects of ions in tear fluid on the gel strength and the consequences of dilution due to the ocular protective mechanisms.

Rheological Studies

The rheological studies were carried out on a rotating cylinder viscometer (Shanghai Precision Instrumentation Co., Ltd., China). The viscosity of the sample solutions was measured at various shear rates at 25°C and 34°C. The temperature was maintained within ±0.2°C by a recirculating bath connected to the sample cup of the viscometer. To evaluate the viscosity change after instillation and mixing with the tear fluid, rheological measurements were taken after diluting the formulations with the concentrated simulated tear fluid. The average of two readings was used to calculate the viscosity. All measurements were performed in triplicate.

In Vitro Release Studies

The *in vitro* release of matrine from the formulations through a 0.45-μm cellophane membrane was measured at 34°C using a plastic sample cell (4.0-cm inside diameter and 0.9 cm in depth). A 1-mL volume of the formulation was accurately pipetted into this equipment; each container was placed in the bottom of a 1,000-mL beaker. Care was taken to make sure that no air bubbles were inside the polymer solutions. The experiments were performed in a dissolution tester (ZRS-8G, Tianjin University Precision Instrument Factory, Tianjin, China). The beaker was then filled with 500 mL dissolution medium and placed in a circulating water bath equipped with stirring rods with paddles to stir the release medium (11). The

temperature and stirring rate were maintained at 34±1°C and 60 rpm, respectively. The dissolution medium was freshly prepared simulated tear fluid (32). Aliquots (5 mL) were withdrawn at each sampling time and replaced with an equal volume of the release medium. The release of matrine was analyzed by UV spectrophotometry (Shimadzu 1600 UV spectrophotometer) at 220 nm (no interference was observed for matrine with other ingredients under this wavelength). Evaluations were conducted in triplicate.

In Vivo Studies of Contact Time

The contact times of the gels were measured in the eyes of three human volunteers. The experiments were carried out after approval of the protocol by the scientific ethics committee of Chengdu University of Traditional Chinese Medicine. Informed consent was obtained from volunteers for this study. Twenty-five microlitres of the gel was added into the lower conjunctival sac, and the presence of the gel was detected using a slit lamp (KJ5D, Kangjie Medical Equipment Company Limited, Suzhou, China). The human subject remained in upright position during the study, and both eyes of the volunteer were treated. To facilitate the visualization of the gel, all preparations contained fluorescein (34). Ocular inspection was performed at selected time intervals; when only a minute amount or none of the gel remained, it was considered as lost from the eye. The foregoing time for inspection was defined as the contact time of the gel.

Ocular Irritation Studies

Ocular irritation studies were performed according to Draize technique on New Zealand White rabbits, each weighing 2.0–3.0 kg. Fifty microliters of formulation was applied into the left eye of the model rabbit. The right eye, which remained untreated, served as control. To prevent loss of test material, the upper and lower lids were gently held together for approximately 5 s. The formulations were instilled thrice a day for a period of 10 days, and the rabbits were observed periodically for ocular redness, swelling, and watering. Evaluation was done as per Draize technique (35). Evaluations were conducted in triplicate.

In Vivo Studies of Precorneal Drug Kinetics

Male, New Zealand albino rabbits of 2.0–2.5 kg were used. A volume of 50 μL of matrine-containing polymer solution was instilled into the lower conjunctival sac of both eyes, with care to avoid spillage. Tear fluid samples (1 μL) were collected without anesthesia using microcapillaries (M290181, Beijing West Instrument and Equipment Company, China) at appropriate intervals over a 5-h period. During the experiments, the rabbits were placed in restraining boxes where they could move their heads and eyes freely. The samples were transferred into centrifuge tubes and the microcapillaries were flushed with 10 μL methanol. After further dilution with 90 μL methanol, the samples were centrifuged at 10,000 rpm for 5 min. Then, 20 μL of supernatant was injected into the HPLC system to determine the matrine concentration (36). Kinetic data were obtained from six eyes. The $AUC_{0-300\,min}$ of the tested formulation were calculated using the trapezoidal method.

The chromatographic system consisted of LC-2010A automatic HPLC (Shimadzu, Japan) and a SPD-M10Avp diode array detector (Shimadzu). Samples were injected into a 20-μL sample loop. The HPLC separation was performed on a reversed phase C_{18} column (5 mm, 250×4.6 mm, Kromasil, Sweden). The mobile phase consisted of a mixture of methanol and 0.05% triethylamine (85:15, *v*/*v*). The flow rate was 1.0 mL/min, and detection was monitored at 220 nm.

Results are expressed as mean ± SD. Student's *t* test was used to identify differences which were considered to be statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Rheology

The two main prerequisites of an *in situ* gelling system are viscosity and gelling capacity (37,38). Aqueous solutions of

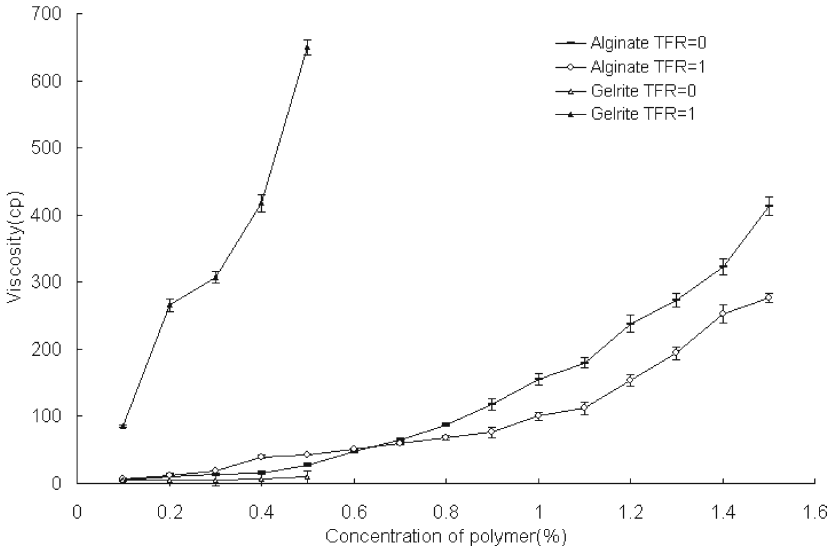

Fig. 1. Effect of concentration on the viscosity of different aqueous polymer solutions (without matrine). Solutions tested at 60 rpm. The data represent mean ± SD ($n=3$)

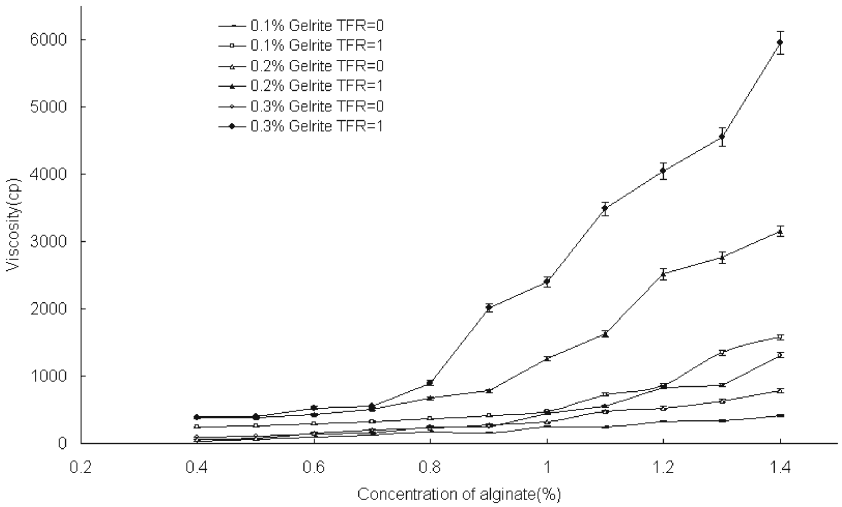

Fig. 2. Effect of concentration on the viscosity of various Gelrite/alginate solutions (without matrine). Solutions tested at 60 rpm. The data represent mean ± SD (n=3)

varying concentrations of Gelrite and alginate were prepared and evaluated for viscosity and gelling capacity in order to identify the compositions suitable for use as *in situ* gelling systems.

Gelrite® (deacetylated gellan gum) is a linear, anionic heteropolysaccharide produced by the microbe *Sphingomonas elodea*. The polymer backbone consists of glucose, glucuronic acid, and methyl pentose in the molar ratio 2:1:1 (39). Gelrite gels in the presence of monovalent or divalent cations (40,41). The mechanism of gelation involves the formation of double-helical junction zones followed by aggregation of double-helical segments to form a 3D network by complexation with cations and hydrogen bonding with water (42). Sodium alginate, the sodium salt of alginic acid, is a natural hydrophilic polysaccharide containing two types of monomers, mannuronic acid (M units) and guluronic acid (G units) (43,44). Alginate forms stable hydrogels in the presence of certain divalent cations (e.g., Ca^{2+} and Sr^{2+}) at low concentrations through the ionic interaction between the cation and the carboxyl functional group of G moieties located on the polymer chain (31,45).

The tear fluid ratio (TFR) is defined as the quotient, i.e., in a solution with TFR=1, all ions have the same concentration that they have in tear fluid (32).

$$\text{TFR} = \frac{[\text{ions present in sample}]}{[\text{ions present in tear fluid}]}.$$

To mimic the situation where no dilution occurs upon ocular instillation and the electrolytes from the tear fluid are freely supplied for sol-to-gel phase transition, some polymer solutions were prepared in the ionic environment of simu-

Table I. Gelling Capacity and Viscosity of Different Formulations Without Matrine

Formulation	Concentration (%, *w*/*w*)		Viscosity (cp)[a] (TFR=1.34°C)	Gelling capacity[b]
	Gelrite	Alginate		
1	0.1	–	85±1.3	+
2	0.2	–	266±9.4	+
3	0.3	–	308±8.5	++
4	0.4	–	417±12.8	++
5	0.5	–	652±11.2	+++
6	–	0.2	12.5±0.2	+
7	–	0.4	41±1.9	+
8	–	0.6	51±0.8	+
9	–	0.8	67±2.7	+
10	–	1.0	103±7.5	+
11	–	1.2	154±9.1	+
12	–	1.4	252±1.7	++
13	–	1.5	276±13.8	++
14	0.2	0.2	287±14.5	+
15	0.2	0.4	395±4.7	++
16	0.2	0.6	433±9.6	++
17	0.2	0.8	671±8.1	+++

Each value represents the mean ± SD (n=3). The standard deviations were all within 3%

[a] Solutions tested at 60 rpm

[b] +: Gels after a few minutes, dissolves rapidly, ++: Gelation immediate, remains for few hours, +++: Gelation immediate, remains for extended period

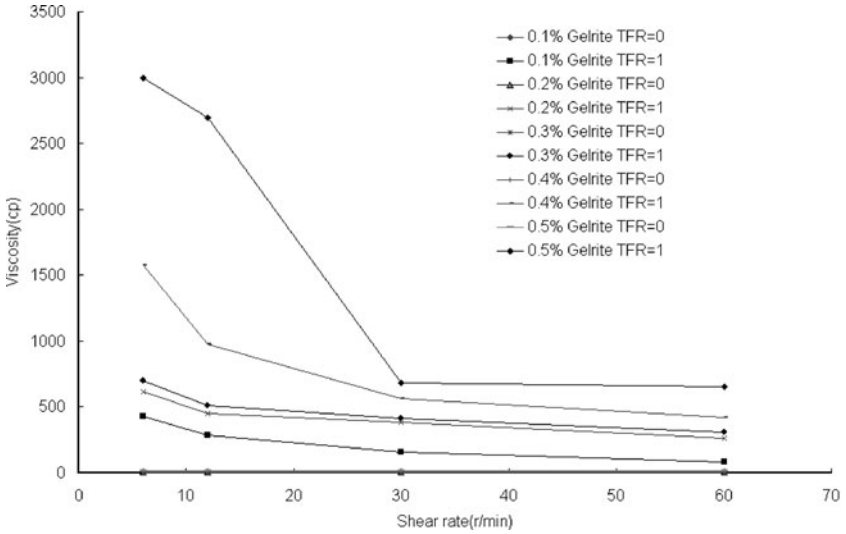

Fig. 3. Viscosity *versus* shear rate of different Gelrite solutions with 1% (*w*/*v*) matrine. Mannitol 5% (*w*/*v*) was used as the isotonic agent. All the measurements were performed in triplicate and the standard deviations were all within 3%

lated tear fluid, TFR=1 (32). The detailed procedure was as follows. The required amount of polymer powder was dispersed in ultrapure water with continuous stirring for 10 min. The partially dissolved polymer solution was stored in the refrigerator until the entire polymer was completely dissolved. Then, the polymer solution was thoroughly mixed with concentrated simulated tear fluid in a ratio of 90:10 by volume. Thus, junction zones which were due to spontaneous nucleation (46) would not be formed in the gel. Some measurements were performed in the presence of ions simulating the physiological situation in the eye.

Viscosity measurements were made at 25°C and 34°C, respectively, with shear rates between 6 and 60 rpm. As shown in Fig. 1, the concentration dependency of the viscosity was compared with 0.1–0.5% (*w*/*w*) solutions of Gelrite and 0.1–1.5% (*w*/*w*) solutions of alginate. All of the Gelrite solutions have higher viscosity than that of alginate solutions upon salt uptake. This characterization is advantageous for their proposed usage as the leakage of solution from the eye during instillation would be minimized. Above the concentration of 0.6% (*w*/*w*), the alginate solutions show a decrease in viscosity upon increasing the ion content compared with their initial values. The decrease may be explained by the contribution of Ca^{2+} to the gel strength being small due to its low content in tear fluid. In addition, other salts (e.g., NaCl and KCl) present in simulated tear fluid probably induce the reduction.

From the preparation procedures of alginate solution, it was found that for alginate concentrations ≤1.3% (*w*/*w*), the

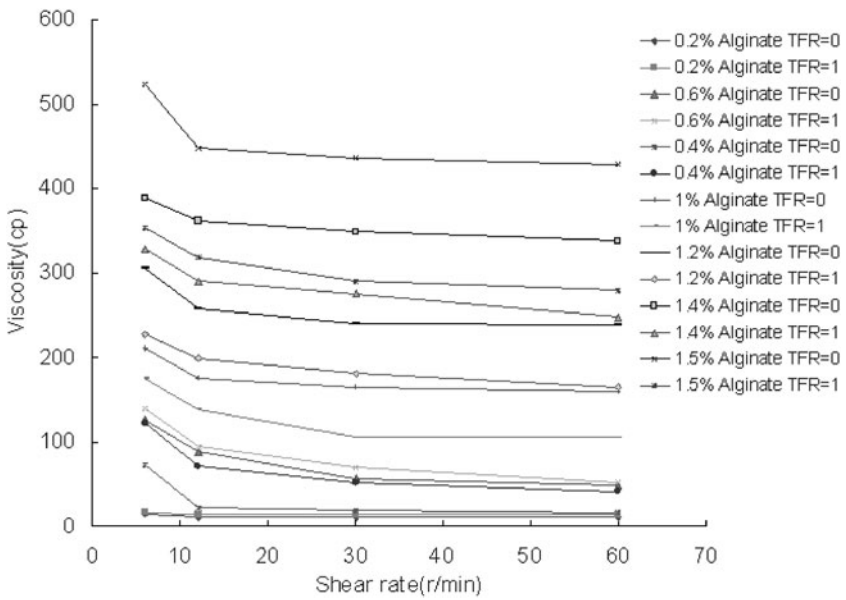

Fig. 4. Viscosity *versus* shear rate of different alginate solutions with 1% (*w*/*v*) matrine. Mannitol 5% (*w*/*v*) was used as the isotonic agent. All the measurements were performed in triplicate and the standard deviations were all within 3%

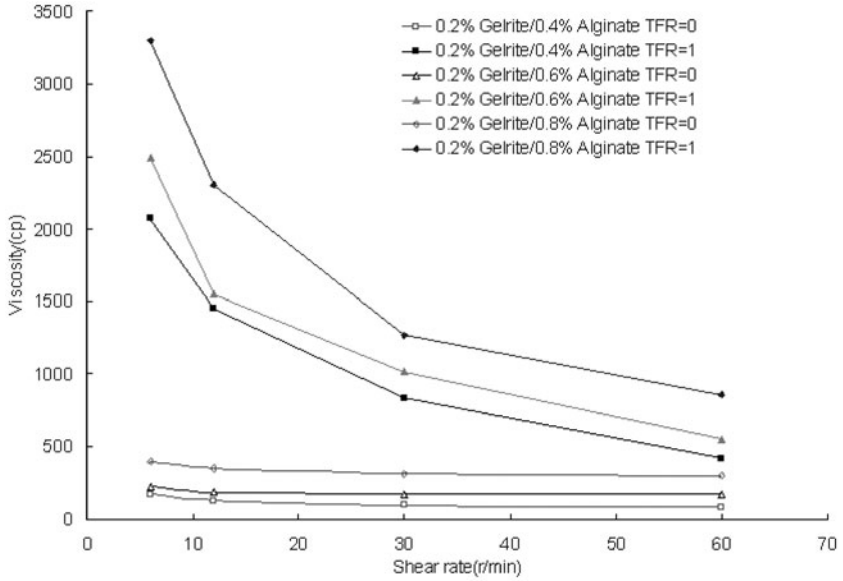

Fig. 5. Viscosity *versus* shear rate of various Gelrite/alginate solutions with 1% (*w*/*v*) matrine. Mannitol 5% (*w*/*v*) was used as the isotonic agent. All the measurements were performed in triplicate and the standard deviations were all within 3%

solution exhibited a liquid state in physiological condition due to the low viscosity. For the high concentration of alginate (1.5%, *w*/*w*) was accompanied by viscosity close to 450 cp at non-physiological condition, a gel may have formed before administration, making dropping impossible. Hence, the optimum concentration of alginate to be used as the *in situ* gel-forming agent is 1.4% (*w*/*w*). The Gelrite solution with a concentration ≤0.2% (*w*/*w*) had free-flowing property under non-physiological condition; however, these compositions could not form strong gel under physiological condition. On the other hand, when the Gelrite concentration is ≥0.5% (*w*/*w*), the solution became a stiff gel under physiological condition because of its high viscosity. The Gelrite solutions had similar rheological behavior in this 0.3–0.4% (*w*/*w*) concentration range. Accordingly, the optimum concentration for Gelrite solution used as *in situ* gel-forming system was 0.3% (*w*/*w*).

The viscosity of the polymer solution in the physiological condition can be enhanced significantly by combining the two individual solutions (see Fig. 2). This comparative study of the rheological properties of Gelrite and alginate gels has shown that similar gel strengths can be achieved using much lower concentrations of alginate with the added advantage of more viscous sols that would minimize the leakage of preparation from the eye during instillation. This phenomenon could be explained by the formation of cross-links between the two polymers (11). Therefore, it may be a

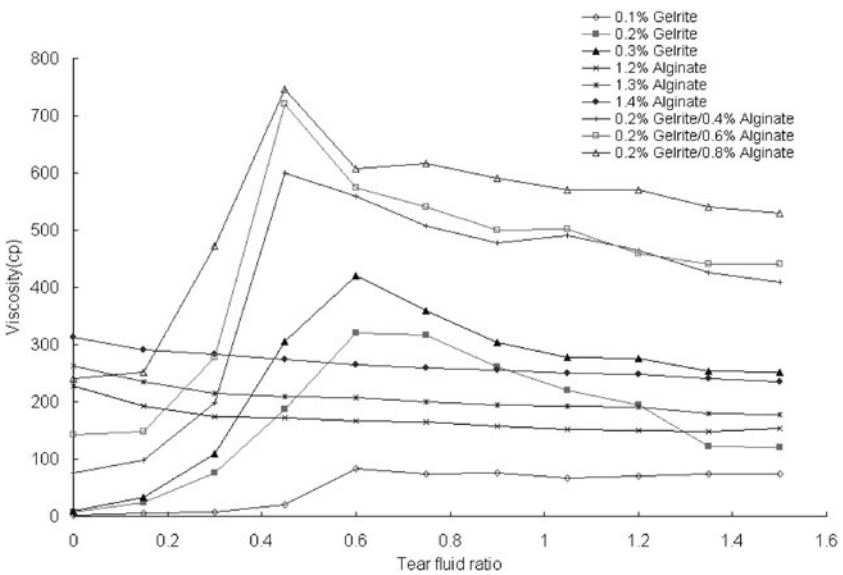

Fig. 6. Viscosity *versus* tear fluid ratio of different polymer solutions with 1% (*w*/*v*) matrine. Mannitol 5% (*w*/*v*) was used as the isotonic agent. All the measurements were performed in triplicate and the standard deviations were all within 3%

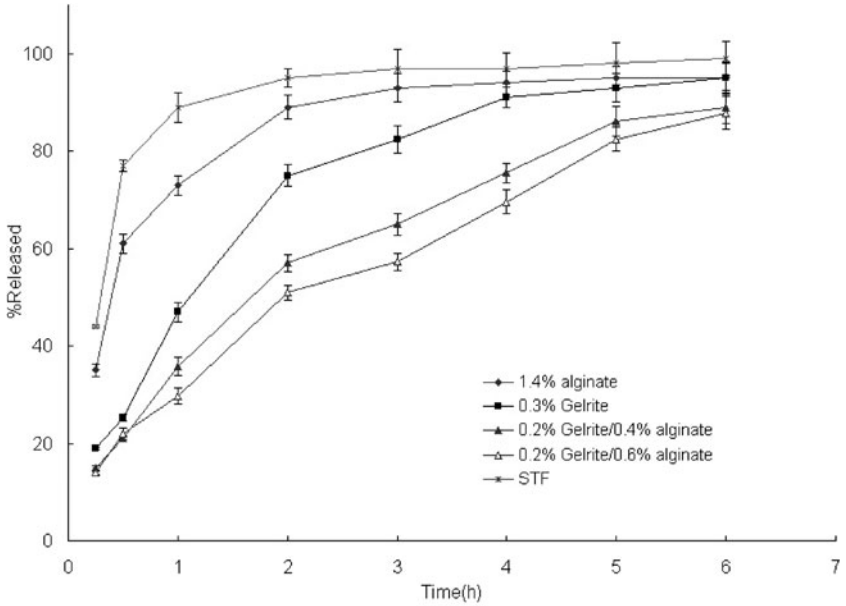

Fig. 7. Cumulative amount of matrine released as a function of time from various matrine-containing solutions. All the measurements were performed in triplicate and the standard deviations were all within 3%

feasible approach to decrease the amount of alginate required for gelation by incorporating Gelrite in the preparation.

As can be seen from Table I, formulations 3, 4, 12, 15, and 16 had more suitable gelling capacity, which completed the gelation immediately and remained for a few hours, compared with formulations 5 and 17, which also completed the gelation immediately, but remained for an extended period. However, formulation 13 was accompanied by high viscosity under non-physiological condition; a gel had already formed before administration. Due to the higher viscosity (>650 cp), formulations 5 and 17 may cause blurred vision and may be difficult to spread out on the cornea. Formulation 16 (0.2%Gelrite/0.6%alginate) was selected as it had satisfactory attributes of viscosity and gelling capacity.

The viscosities of polymer solutions at different concentrations were measured at varying shear rates for the relevance of administration by dropping. The Gelrite solution was shear thinning, and a decrease in viscosity was observed with an increase in shear rate (Fig. 3). The rheological behaviors of all formulations were not significantly affected by the addition of matrine. With TFR=1, it can be seen from Fig. 4 that for alginate concentrations ≥0.6% (*w*/*w*), the solution exhibited a decrease in viscosity at all shear rates. As shown by Fig. 5, the viscosity of Gelrite/alginate solutions all decreased as the shear rate increased, which showed the characteristic of pseudoplastic fluid. Despite the shearing force on the preparation being large during blinking, if the viscosity at high shear rate is too high, it may induce irritation. But then, if that is too low, it will give rise to increased drainage. The pseudoplastic property of these formulations is in favor of sustaining drainage of drugs from the cul-de-sac of the eye, simultaneously without blinking difficulty.

Since the volume instilled from a commercial eye dropper is between 20 and 50 μL, the volume of lacrimal fluid in the eye is 7 μL and that the ions present in tear fluid mix with the preparation at the time of application, then the ionic content may be between 12.2% and 25.9% of the normal value. The polymer and ionic contents were varied to examine the effect of dilution upon instillation, and the contributions of electrolytes in the simulated tear fluid to the gel strength were studied. To change the ionic environment, the required amount of the concentrated salting liquid was added to the preparation under constant stirring until uniform solution was obtained.

Between a tear fluid ratio of 0.3 and 0.6, it can be seen from Fig. 6 that the viscosity is >320 cp for 0.3% Gelrite, indicating a strong gel with a salt content-independent viscosity, compared to the TFR=0.1 gel. Thus, at this concentration of Gelrite, there is a sufficient amount of cations present for the immediate formation of a gel in the *in vivo* case. At higher ionic contents, e.g., TFR=1.5, the gel strength decreases and the system becomes brittle. With increasing salt content, the viscosity of alginate solution is

Table II. Contact Time for Different Formulations in Humans

Formulation	Concentration (%, *w*/*w*)		Contact time (min)
	Gelrite	Alginate	
1	0.1	–	8.7±1.5
2	0.2	–	13.0±2.0
3	0.3	–	18.3±5.1
4	0.4	–	24.7±6.0
5	0.5	–	26.7±5.5
7	–	0.4	11.7±4.0
8	–	0.6	14.7±2.5
9	–	0.8	17.0±5.6
10	–	1.0	23.0±2.0
11	–	1.2	27.7±3.5
12	–	1.4	30.0±4.6
13	–	1.5	31.3±3.2
15	0.2	0.4	40.7±2.5
16	0.2	0.6	51.7±6.1
17	0.2	0.8	46.0±7.0

Each value represents the mean ± SD (n=3)

Table III. Ocular Irritation Testing (n=3)

Formulation	Average score
Blank of 3	0
3	0
Blank of 12	0
12	0
Blank of 15	0
15	0
Blank of 16	0
16	0
0.9% Sodium chloride (negative control)	0
Dioctyl sodium sulfosuccinate (positive control)	8

then continuously decreased. In addition, a reduction in gel strength for all formulations is observed with TFR=1. These results may suggest that the gel strength was heavily influenced by the salts (e.g., NaCl, KCl, and $NaHCO_3$) present in tear fluid. The critical point of tear fluid ratio was defined as the tear fluid ratio when the viscosity of formulation reached the maximum. The viscosity increased at first, and above the critical point of tear fluid ratio, it began to decrease.

Similar trends were observed which contribute to understanding the relevance of the rheological properties for the Gelrite solution and Gelrite/alginate solution in Fig. 6. For the Gelrite/alginate formulation, the viscosity reached a maximum with TFR=0.45. A similar phenomenon was observed for the

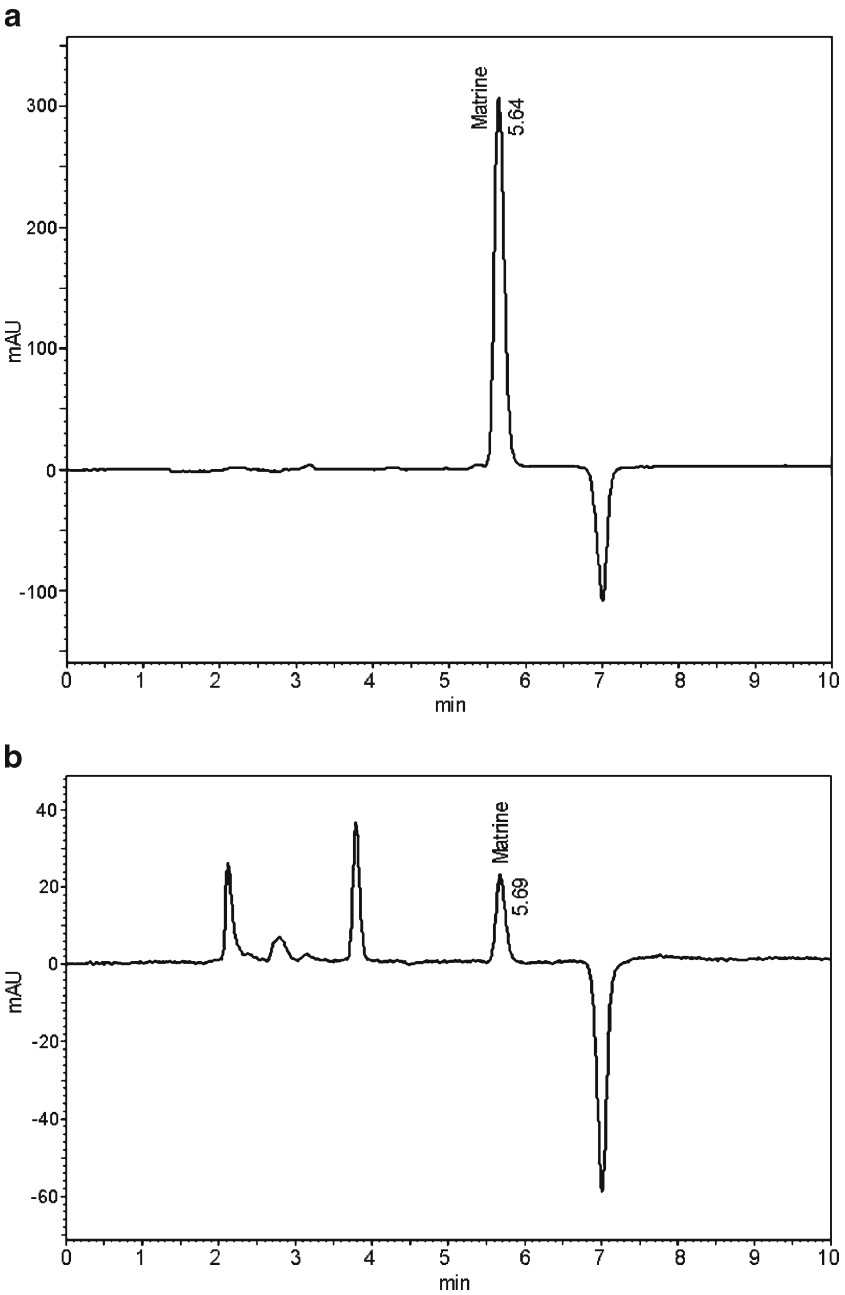

Fig. 8. HPLC chromatogram of standard and tear sample. **a** Standard (0.33 mg/mL). **b** Tear sample at the time point of 30 min (0.02 mg/mL)

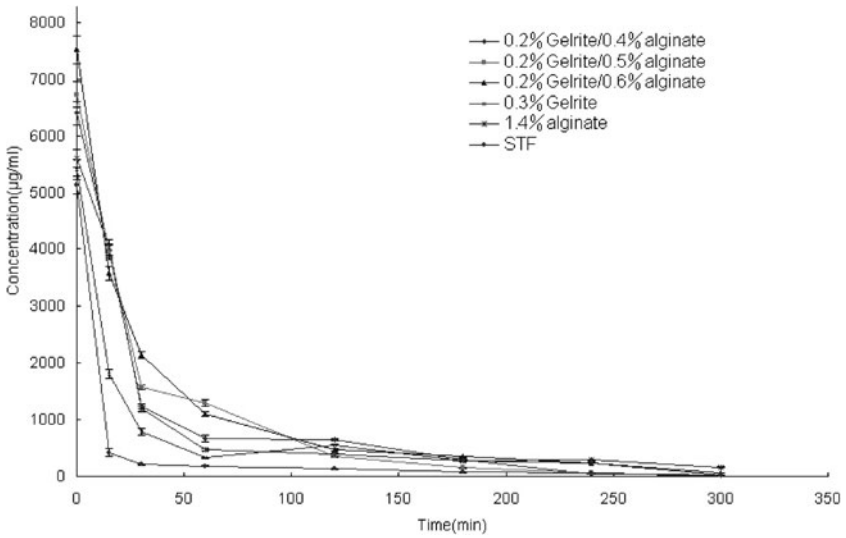

Fig. 9. Matrine concentrations in tears after ocular administration of different matrine-containing solutions. Each data point is the mean ± SE of six eyes

Gelrite solution; however, the peak viscosity was lower than that of the Gelrite/alginate formulation with TFR=0.6. Since the viscosity of alginate solution was continuously decreased with increasing ionic concentration, Gelrite content may play an important role in the gel strength of Gelrite/alginate formulation. Furthermore, the untoward effect may be minimized by combining those two individual polymer solutions. However, there is an experimental error in the determination of viscosity induced by the dilution of concentrated simulated tear fluid; further study is needed to minimize the error.

In Vitro Drug Release

The cumulative amount of matrine released as function of time is shown in Fig. 7. All solutions contained 1% (*w*/*v*) matrine. For the eye drops, almost all of the matrine released immediately at the beginning of experiment. The drug released about 89.0% to the medium after 1 h. In the case of alginate solution, the drug released 73.6% to the medium after 1 h and approximately 95.1% released after 6 h. It obviously exhibited some extent of delayed release effect. There was a 47.2% of matrine released from Gelrite solution after 1 h and approximately 94.6% after 6 h. Thus, Gelrite solution has a better delayed release effect. The Gelrite/alginate solution had a better ability to retain drugs than the individual polymer solution. Formulation 16 (0.2% Gelrite/0.6% alginate) exhibited a significantly improved release effect than Gelrite solution as the drug released about 29.8% to the medium after 1 h and approximately 87.8% of matrine released after 6 h. The *in vitro* drug release conditions may be different from those likely to be encountered in the eye. However, the results clearly show that the Gelrite/alginate aqueous system has the ability to retain matrine and can be used as the *in situ* gelling vehicle for ophthalmic drug delivery.

Human Contact Time

The results from the contact time measurements are given in Table II. The values are averages of at least three measurements. There was no obvious irritation and other serious adverse effects observed. The only untoward effect was a temporary blurring of vision of the eye treated with polymer preparations. The human ocular contact times for Gelrite solutions showed a concentration dependence, indicating that gels were formed with low ion contents. This could be explained and correlated to the rheology of Gelrite sols mixed with simulated tear fluid. Within the interval studied, the contact time of alginate solutions was also dependent on the concentration and varied among the individuals. The difference among subjects was probably due to physiological differences such as the lacrimation response and blinking frequency. It was found that the contact time of the polymer solution in the physiological condition could be enhanced significantly by combining the two individual solutions; the maximum contact time obtained is around 1 h. The trends were consistent in this study and could contribute to the discussion of the rheological results.

Eye Irritation

The results of the ocular irritation studies indicate that all preparations are non-irritant (Table III). For all the polymer systems studied, the average irritation scores were zero, including the negative control. The maximum mean score for ocular lesions observed was 8.0 for dioctyl sodium sulfosuccinate (1%, *w*/*w*). Hence, all tested polymer solutions

Table IV. Area Under the Matrine Concentration in Tear *Versus* Time Profiles in 300 min ($AUC_{0-300\,min}$) for Various Formulations

Formulation	$AUC_{0-300\,min}$ (μg min/mL)	Ratio
STF	80,325.7±1,365.53	–
0.3% Gelrite (3)	227,523.5*±9,502.76	2.83
1.4% Alginate (12)	276,945.1*±8,938.62	3.44
0.2% Gelrite/0.4% alginate (15)	320,685.6*±14,168.91	3.99
0.2% Gelrite/0.5% alginate	342,705.3*±10,084.35	4.26
0.2% Gelrite/0.6% alginate (16)	373,515.5*±7,988.12	4.65

Each value represents the mean ± SD of three determinations
*$p<0.05$ (compared to STF)

might be considered as minimally irritating to the eye of the rabbits. Excellent ocular tolerance was noted. No ocular damage or abnormal clinical signs to the cornea, iris, or conjunctivae were visible.

In Vivo Precorneal Retention

The chromatogram of tear sample using the HPLC system was shown in Fig. 8. A symmetrical peak was observed for matrine with a retention time of 5.6–5.7 min. There was no interference with the matrine peak, and the overall chromatographic run time was 10 min. The determination of matrine was processed by peak area, and the external standard method was used for quantification. Figure 9 illustrates the matrine concentration in the tear fluid as a function of time. Although the general trend of all these matrine concentration *versus* time curves was similar, the matrine concentration of each matrine-containing polymer solution was higher than that of matrine-containing STF almost at each time point ($p<0.05$). For the individual Gelrite and alginate solution, the matrine concentrations for the alginate formulation were higher than those for the Gelrite formulation between 10 and 60 min of experimental times. And then matrine concentrations were lower than those for the Gelrite formulation. Therefore, the alginate formulation exhibited a better ability to hold matrine in tear probably due to the mucoadhesive property. For formulations 15 and 16, there was a similar trend between 15 and 60 min at which their matrine concentrations were roughly higher than those of the solutions of Gelrite and alginate. However, between 120 and 180 min, the matrine concentrations of 15 were slightly higher than those of 16, which had a similar trend to the Gelrite formulation.

The area under the curve (AUC) for various formulations is listed in Table IV. The differences between the AUC following administration of the matrine-containing polymer solutions and the matrine- containing STF were statistically significant ($p<0.05$). The AUC of the formulations 15 and 16 were 3.99 times and 4.65 times greater than that of matrine-containing STF, respectively. Less pronounced increases in AUC were observed for the Gelrite (2.83-fold) and alginate (3.44-fold) formulations as compared to the STF containing matrine. These results indicated that a greater amount of drug was retained in the precorneal area for a prolonged period following instillation compared to the STF containing matrine. The AUC serves as an indicator of the precorneal exposure to the drug and the therapeutic efficacy of formulation for some drugs. For the polymer solutions, formulations 15 and 16 had better performance than the formulation with Gelrite and alginate; formulation 16 (0.2%Gelrite/0.6%alginate) had the best retentive effect among them owing to the higher content of alginate.

CONCLUSION

In the present study, we found that the optimum concentrations of Gelrite and alginate solutions for ocular drug delivery system were 0.3% and 1.4% (*w*/*w*), respectively. When 0.2% Gelrite and 0.6% alginate solutions were combined, the gel strength under physiological conditions was significantly increased and the combined solution was easy to administer during ocular instillation. Both *in vitro* and *in vivo* results indicated that the combined polymer systems performed better in retaining matrine than the individual solutions. Therefore, the Gelrite/alginate system can be used as the *in situ* gelling vehicle for ophthalmic drug delivery.

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