International Journal of Pharmaceutics 365 (2009) 143–149

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of lipophilic cyclosporine A: In vitro and in vivo results

Li Gan [a,b], Yong Gan [b,*], Chunliu Zhu [b], Xinxin Zhang [b], Jiabi Zhu [a,**]

[a] School of Pharmacy, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China

ARTICLE INFO

Article history:
Received 6 June 2008
Received in revised form 31 July 2008
Accepted 11 August 2008
Available online 15 August 2008

Keywords:
Cyclosporine A
Microemulsion
In situ gelling system
Kelcogel®
Ophthalmic delivery

ABSTRACT

The objective of the present study was to design a novel microemulsion in situ electrolyte-triggered gelling system for ophthalmic delivery of a lipophilic drug, cyclosporine A (CsA). A CsA-loaded microemulsion was prepared using castor oil, Solutol HS 15 (surfactant), glycerol and water. This microemulsion was then dispersed in a Kelcogel® solution to form the final microemulsion in situ electrolyte-triggered gelling system. In vitro, the viscosity of the CsA microemulsion Kelcogel® system increased dramatically on dilution with artificial tear fluid and exhibited pseudo-plastic rheology. In vivo results revealed that the $AUC_{0\rightarrow 32h}$ of corneal CsA for the microemulsion Kelcogel® system was approximately three-fold greater than for a CsA emulsion. Moreover, at 32 h after administration, CsA concentrations delivered by the microemulsion Kelcogel® system remained at therapeutic levels in the cornea. This CsA microemulsion in situ electrolyte-triggered gelling system might provide an alternative approach to deliver prolonged precorneal residence time of CsA for preventing cornea allograft rejection.

Published by Elsevier B.V.

1. Introduction

Ocular diseases are usually treated with topical application of drug solutions (eye drops). These conventional dosage forms account for nearly 90% of the currently available marketed formulations owing to their simplicity and good acceptance by patients. However, one of the major drawbacks associated with topical ocular drug delivery is the rapid and extensive precorneal loss caused by drainage and high tear fluid turnover (Bourlais et al., 1998). Typically, less than 5% of the drug applied penetrates the cornea/sclera and reaches the intraocular tissue, with the major fraction of the dose applied often absorbed systemically through the conjunctiva and nasolacrimal duct. This can result in undesirable systemic side effects (Lang, 1995).

To overcome these disadvantages, many ophthalmic drug delivery systems have been investigated, such as hydrogels (Durrani et al., 1995; Robinson and Mlynek, 1995), micro- and nanoparticles (Zimmer and Kreuter, 1995), liposomes (Meisner and Mezei, 1995) and collagen shields (Reidy et al., 1990). On the other hand, oil/water microemulsions may provide a promising alternative. From the point of view of production and sterilization, microemulsions are relatively simple and inexpensive because they are thermodynamically stable. Microemulsions are also used to formulate poorly water-soluble drugs since their structure allows solubilization of lipophilic drugs in the oil phase. In situ gel-forming systems are viscous liquids that shift to a gel phase upon exposure to physiological conditions. The principal advantage of this formulation is the possibility of delivering accurate and reproducible quantities, in contrast to already gelled formulations, and promoting precorneal retention (Bourlais et al., 1998). To exploit the benefits of these two dosage forms, we propose a microemulsion in situ gelling system as a new vehicle for ophthalmic drug delivery. The essential idea is to encapsulate the drug in droplets that form a microemulsion, then disperse the drug-loaded droplets in a polymer solution that gels upon triggering by the electrolyte used.

Deacetylated gellan gum is a very interesting in situ gelling polymer that seems to perform very well in humans (Carlfors et al., 1998). Because of the presence of free carboxylate groups in deacetylated gellan gum, it is anionic in nature and thus undergoes ionic gelation in the presence of mono- and divalent cations. Cation-induced gelation has been widely used in the formulation of in situ gelling ophthalmic preparations (Sanzgiri et al., 1993; Rozier et al., 1997).

Cyclosporine A (CsA), a hydrophobic peptide with powerful immunosuppressive action, is effective in the treatment of extraoc-

* Corresponding author. Tel.: +86 21 50806600 2122; fax: +86 21 50806600 2122.
** Corresponding author. Tel.: +86 25 85338217; Fax: +86 25 83301606.
E-mail addresses: simm2122@vip.sina.com (Y. Gan), lgan@mail.shcnc.ac.cn (J. Zhu).

0378-5173/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijpharm.2008.08.004

ular disorders such as keratoconjunctivitis sicca and dry eye disease and for the prevention of corneal allograft rejection (Power et al., 1993). However, the poor aqueous solubility of CsA (6.6 μg/ml) is a limiting factor for the formulation of solutions intended for ocular administration (Lallemand et al., 2003). In 2002, a CsA 0.05% lipid emulsion (Restasis™, Allergan, Irvine, USA) received FDA approval as the first and only therapy for patients with keratoconjunctivitis sicca, whose lack of tear production is presumed to be due to ocular inflammation. However, as the corneal concentration achieved with dosing four times a day is insufficient to prevent immunologic graft reactions, Restasis™ is not effective in preventing rejection after corneal allograft (Price and Price, 2006).

The aim of the present study was thus to incorporate the lipophilic drug CsA into a novel ophthalmic microemulsion in situ gelling system with a higher drug load (2%), and to investigate its potential for delivering CsA to the ocular tissue as therapy to prevent corneal allograft rejection.

2. Materials and methods

2.1. Materials

Cyclosporine A was purchased from FuJian KeRui Pharmaceutical Co. Ltd. (Fujian, China). Castor oil was purchased from HuNan Erkang Pharmaceutical Manufacture (Hunan, China). Glycerol was purchased from Shanghai Minshi Chemical Company (Shanghai, China). Solutol HS 15 was kindly donated by BASF (Germany). Kelcogel® (deacylated gellan gum) was a kind gift from Shanghai Evephone Enterprise Co. Ltd. (Shanghai, China). Carbopol 980 and Pemulen TR_2 were donated by Noveon Inc. (USA). Deionized water was used as the aqueous component. All other reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of CsA microemulsion

The pseudoternary phase diagram of castor oil, Solutol HS 15 and glycerol is illustrated in Fig. 1. In the microemulsion region, a translucent emulsion with light blue opalescence formed on addition of water. Then systems following the sampling path marked by the arrow were prepared by adding water to the mixture of Solutol HS 15, glycerol and castor oil.

CsA microemulsions were prepared in two steps. In the initial step, Solutol HS 15 (2.4 g), glycerol (1.6 g) and castor oil (1.0 g) were placed in a mortar and heated to 60 °C. CsA (200 mg) was then added and manually ground with a pestle until it dissolved. Then 50 ml of water in a 100-ml glass beaker was heated to 60 °C. In the second step, the hot oil phase was then emulsified using a high-shear dispersing emulsifier (T25 basic, IKA Guangzhou, China) at 10,000 rpm for 30 min, with slow addition of the water. After treatment, the microemulsion was cooled at room temperature (25 °C).

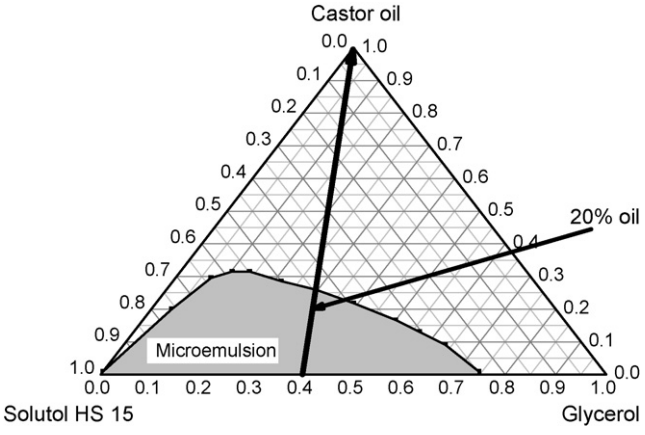

Fig. 1. Pseudoternary phase diagram for a system containing castor oil, glycerol and Solutol HS 15 (Solutol HS 15/glycerol/castor oil 0.48:0.32:0.2).

2.2.1.1. Particle size analysis. The particle size distribution of the oil droplets in the microemulsion was analyzed using a dynamic light-scattering particle size analyzer (Particle Sizing System, Nicomp388/ZetaPALS, Santa Barbara, USA) without dilution at 25 °C.

2.2.1.2. Drug encapsulation efficiency. The drug encapsulation efficiency of microemulsion was determined by ultrafiltration. A 500-μl aliquot of microemulsion was transferred to the upper chamber of a centrifuge tube fitted with an ultrafilter (Microcon YM-10, Millipore, MWCO 10 kDa), which was then centrifuged at 4000 rpm for 30 min. The amount of CsA loaded in the microemulsion was calculated as the difference between the total amount in the microemulsion and the amount in the filtrate as determined by HPLC. The drug encapsulation efficiency was calculated according to:

$$Q_w = \frac{W_{total} - W_{free}}{W_{total}} \times 100\% \approx \frac{C_{total} - C_{free}}{C_{total}} \times 100\%,$$

where Q_w is the drug encapsulation efficiency, W_{total} is the total amount of CsA in the microemulsion, W_{free} is the amount of CsA in the filtrate, C_{total} is the CsA concentration in the microemulsion, and C_{free} is the CsA concentration in the filtrate. The same method was used for the control CsA emulsion.

The recovery of this process was evaluated by adding low, medium and high amounts of CsA solution (solubilized by surfactant Solutol SH 15) to blank microemulsions (no drug loaded) and using the ultrafiltration process. The recovery was calculated according to $R = C_d/C_t \times 100\%$, where C_d is the CsA amount detected in the filtrate and C_t is the theoretical amount added.

The HPLC system (Agilent 1100 series) used comprised an autosampler (G1313A ALS), a pump (G1311A Quatpump), a column oven (G1316A Column), a UV detector (G1314A VWD) and data processing software (HP Chemstation Rev.A.10.01). A C18 column (SUPELCO Discovery 150 mm × 4.6 mm, 5 μm) was used for CsA analysis with acetonitrile–water–methanol (50:45:5) as mobile phase at a flow rate of 2.0 ml/min at 70 °C. Detection was performed at 210 nm.

2.2.2. Formulation of microemulsion in situ gelling system

The in situ gelling system was prepared by dispersing CsA microemulsion into a gel solution. Kelcogel® was dispersed with a stirrer (850 rpm) in 50 ml of deionized water until it completely dissolved. Then CsA microemulsion was added to obtain final Kelcogel® concentrations of 0.1%, 0.3% and 0.6%. Carbopol 980 (0.4%) was used to formulate a microemulsion-based gel system. The final formulations are listed in Table 1.

Table 1
Formulations of the microemulsion in situ gel studied

Formulation	Content (g/100 ml)				
	Solutol HS 15	Glycerol	Castor oil	Kelcogel®	Carbopol 980
F1	2.4	1.6	1.0	–	–
F2	2.4	1.6	1.0	–	0.4
F3	2.4	1.6	1.0	0.1	–
F4	2.4	1.6	1.0	0.3	–
F5	2.4	1.6	1.0	0.6	–

2.2.3. Transmission electron micrographic studies

Images were recorded with transmission electron microscope (EM-1230, JEOL, Japan). Analysis was performed at 25 ± 2 °C. Microemulsions before/after mixing with 0.3% Kelcogel® solution were suitably diluted and dyed with phosphotungstic acid for visualization.

2.2.4. Rheological studies

The viscosity of the prepared formulations was determined at different angular velocities at 34 ± 1 °C using a rotary viscometer (DV-III, Brookfield, USA). A typical run involved changing the angular velocity from 1 to 100 rpm at a controlled ramp speed. After 6 s at 1 rpm, the velocity was successively increased to 100 rpm, with a similar period at each speed. The angular velocity was then decreased (100–1 rpm) for a similar period of 6 s. The average of two readings was used to calculate the viscosity. Evaluations were conducted in triplicate.

To evaluate the viscosity change after administration, rheological measurements were taken after diluting the CsA formulations with artificial tear fluid (0.67% NaCl, 0.2% $NaHCO_3$, 0.008% $CaCl_2{\cdot}2H_2O$; Rozier et al., 1989) according to Vandamme and Brobeck (2005). The viscosity of sample solutions under different ionic environment was measured with angular velocity being at 15 rpm.

2.2.5. In vitro release studies

To determine the in vitro release of CsA, 10 ml of artificial tear fluid was placed in a vial and mounted vertically in a water bath at 34 ± 0.1 °C and 2 ml of formulation was added. The temperature and stir rate were remained at 34 °C and 50 rpm, respectively. Aliquots of 1 ml were withdrawn from the release medium and replaced by an equal volume at each sampling time. The amount of CsA was determined by HPLC.

2.2.6. Wettability evaluation

The wettability of the microemulsion in situ gelling system was determined by measuring the contact angle using the air-bubble capture technique reported by Shanker et al. (1995). The width (W) and height (H) of the bubble were measured visually using a microscope and the contact angle was calculated according to:

$$\theta = \cos^{-1}\left(\frac{2H}{W} - 1\right), \quad \theta < 90^{\circ}.$$

Male New Zealand albino rabbits used in these investigations were sacrificed. Then eyes were enucleated extra-orbitally with the connective tissue holding the conjunctival sac in place and by cutting the optic nerve so that the ocular globe retained its shape. Next, all accessory glands and connective tissue was removed from the eye. The enucleated eye was placed in oxygenated Ringer's solution (134 mM NaCl, 1.4 mM $MgCl_2{\cdot}6H_2O$, 1.5 mM $CaCl_2{\cdot}2H_2O$, 20 mM $NaHCO_3$, 5.0 mM Na_2CO_3, 5.0 mM KH_2PO_4 and 10 mM dextrose) maintained at 34 °C. Eyes were used for surface chemical characterization within 5 h of enucleation. All measurements were performed on eyes with a clear transparent cornea.

The kinetics of desorption from the ocular surface was determined for all formulations by measuring the contact angle at intervals of 5 min according to the following sequence. (a) The ocular surface was immersed in the test solution for 30 min. (b) The coated ocular surface was then submerged in the environmental chamber containing water. (c) The contact angle of water was measured on the submerged surface.

2.2.7. Ocular irritation testing

New Zealand albino rabbits weighing 2–3 kg were provided by the Animal Experimental Center of Shanghai Institute of Materia Medica. The animals were housed in standard cages in a light-controlled room at 19 ± 1 °C and 50 ± 5% RH and were fed a standard pellet diet and water ad libitum. All studies were approved by the Department of Laboratory Animal Research at Shanghai Institute of Materia Medica. Procedures involving animals were reviewed and approved by the Animal Ethics Committee at Shanghai Institute of Materia Medica.

Rabbits were divided into four groups (6 per group) and then treated twice a day (one drop in the right eye) with different formulations for 2 weeks. The left eyes served as controls and were treated with saline. The ocular condition was recorded every day and at 1 h after the last administration. According to the Draize test, ocular irritation scores for every rabbit were calculated by adding together the irritation scores for the cornea, iris and conjunctiva. The eye irritation score was obtained by dividing the total score for all rabbits by the number of rabbits. Irritation was classified according to four grades: practically non-irritating, score 0–3; slightly irritating, score 4–8; moderately irritating, score 9–12; and severely irritating (or corrosive), score 13–16.

2.2.8. In vivo evaluation

Studies were performed on fully awake male New Zealand albino rabbits. The following formulations were tested: F2 (CsA microemulsion Carbopol 980 gel system), F4 and F5 (CsA microemulsion in situ gelling systems) and the control CsA emulsion. Animals were divided into four groups (n = 4–6) and 100 μl of formulation containing 2 mg/ml CsA was placed in the cul-de-sac of both eyes of all animals, twice a day for 1 week. At different times after the last administration (1, 6, 24 and 32 h), rabbits were sacrificed. The eyes were proptosed and rinsed with normal saline. The cornea and conjunctiva were subsequently dissected in situ. Each tissue was rinsed with normal saline, blotted dry to remove any adhering drug and weighed. The excised tissue was minced with methanol to extract CsA and the methanol was then evaporated. Then another 500 μl of methanol was added, vortexed for 3 min, and centrifuged. A portion (50 μl) of supernatant was analyzed by HPLC. The average relative recovery of this analytical method was 96.52 ± 3.26%, and the absolute recovery was 71.18 ± 10.79%.

2.2.8.1. Preparation of CsA emulsion (control sample). The CsA emulsion was prepared according to US Patent 5,474,979 (Ding et al., 1995). In brief, 50 ml of water in a 100-ml glass beaker was heated to 70 °C. Then 1.0 g of polysorbate 80 and 2.2 g of glycerin were dissolved in it to make up the water phase. The oil phase was prepared by dissolving 200 mg of CsA in 2.5 g of castor oil at 60 °C. The oil phase was then added to the water phase and emulsified using a high-shear dispersing emulsifier (T25 basic, IKA Guangzhou, China) at 6500 rpm for 0.5 h. The coarse emulsion was then treated using a high-pressure emulsifier (Panda2K, GEA, Niro Soavi S.P.A, Italy) at an inlet pressure of 800 bar. Each batch was processed through the Panda2k system for eight discrete volume cycles and then collected in a glass beaker. After treatment, the emulsion was cooled in an ice-water bath.

Pemulen TR_2 (0.05 g) was dissolved in 50 ml of water with continuous stirring. The emulsion was then added to the gel solution with constant stirring until a uniform emulsion was obtained.

3. Results and discussion

3.1. Particle size distribution

High concentrations of surfactants may lead to ocular toxicity. Therefore, it may be better to decrease the quantity of surfactant and choose a non-spontaneous preparation process (Vandamme, 2002). In this case, microemulsions are not obtained spontaneously.

Table 2
Loading efficiency of the CsA microemulsion and the control sample

Formulation	C_{total} (mg/100 ml)	C_{free} (mg/100 ml)	Q_w (%)
Microemulsion	208.4 ± 6.9	12.1 ± 2.6	94.2
Control sample	49.8 ± 2.4	4.9 ± 1.1	95.1

A decrease in surfactant quantity required the use of a high-shear emulsifier, which provides a supply of energy.

The mean particle size was 54.5 ± 31.6 nm for the microemulsion and 208.8 ± 83.3 nm for the control emulsion.

3.2. Drug encapsulation efficiency

The encapsulation efficiency of CsA microemulsions was evaluated by determining the difference between the total amount in the microemulsion and the amount in the filtrate. The recovery obtained was 98.6%, indicating that this method for evaluating the loading efficiency was accurate. Table 2 shows that the CsA encapsulation efficiency was 94.2% for the microemulsion and 95.1% for the control emulsion, with no significant difference. This demonstrates that most of the drug was encapsulated within droplets for both the control emulsion and the microemulsion.

3.3. Transmission electron micrographic study

As observed from the TEM images (Fig. 2), there was no significant difference after addition of CsA microemulsion to the Kelcogel® gel solution and the CsA microemulsion retained its spherical microstructure in the in situ gelling system.

3.4. Rheology studies

Fig. 3 shows the rheology of different formulations. The CsA microemulsion system containing Carbopol 980 as the viscosity-enhancing agent exhibited higher viscosity (~650 cps) that remained almost stable with increasing angular velocity. The viscosity of the other formulations was much lower and they exhibited pseudoplastic rheology, as shown by shear thinning and a decrease in viscosity with increasing angular velocity. This might be beneficial for the production process, since too many bubbles in the gel solution would make accurate dosing very difficult. No significant difference was observed between 0.3% Kelcogel® solution and formulation F4, indicating that addition of CsA microemulsion did not affect the rheological behavior of the Kelcogel® solution.

The results in Fig. 4 demonstrate that dilution with artificial tear fluid dramatically increased the viscosity of F4 and F5, but not that of F2 or the control CsA emulsion. This phenomenon confirms the in situ gelling property of the microemulsion Kelcogel® system, with the viscosity ratio after/before dilution by tear fluid of approximately 10. No such significant change in viscosity was observed for F3, suggesting that a Kelcogel® concentration of 0.1% is too low for in situ gelling.

The ionic content of the preparations was varied, keeping the proportion of the ions Na^+/Ca^{2+} constant. The tear fluid ratio, TFR, is defined as:

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

Thus, in a solution with TFR = 0.6, all ions are at 0.6-fold the concentration found in tear fluid.

Fig. 5 shows the viscosity of F3, F4 and F5 under different ionic environments. The viscosity of all formulations increased greatly with increasing ionic content in a concentration-dependent manner up to TFR of 0.6, after which the viscosity decreased. Assuming

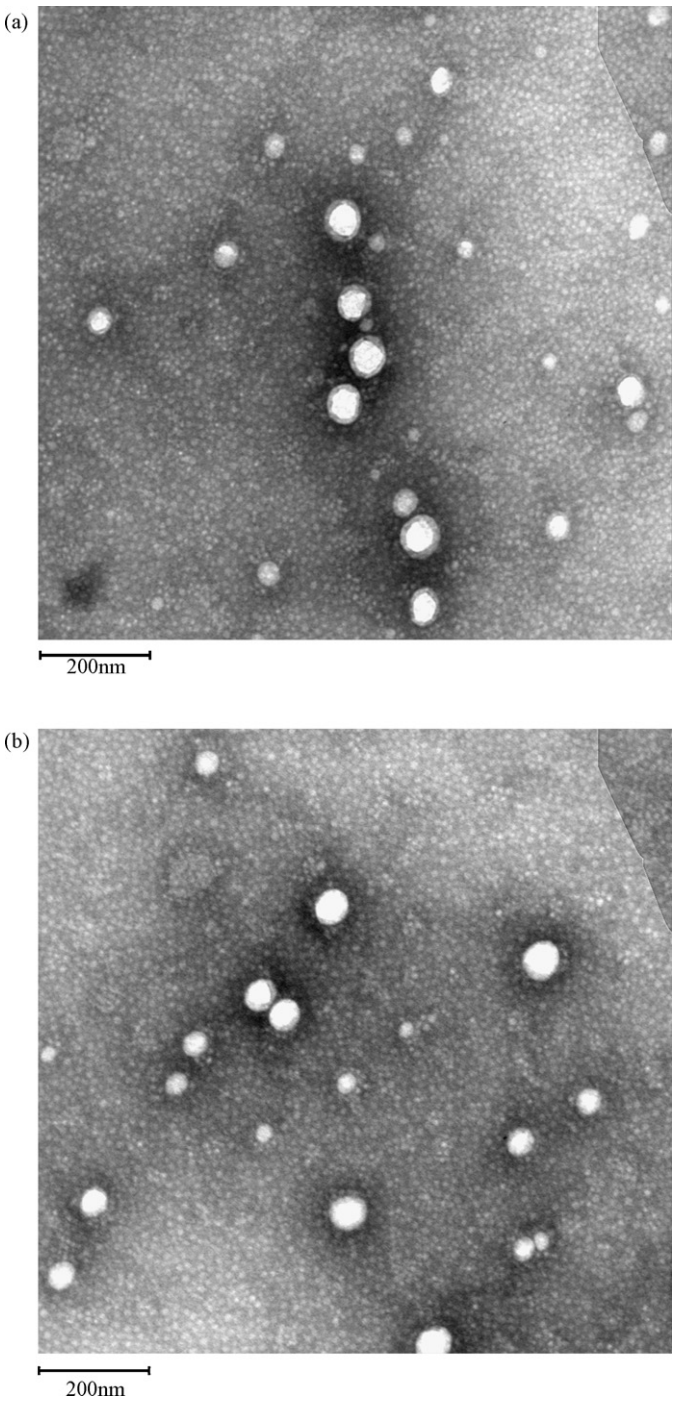

Fig. 2. TEM images of (a) CsA microemulsion and (b) CsA microemulsion in situ gelling system with 0.3% Kelcogel®.

that the volume delivered by a commercial eye dropper is approximately 20–50 μl and the tear volume in the eye is 7 μl, then after administration of a drop the ionic concentration would be 12–25% of the normal value (Paulsson et al., 1999). It can be observed in Fig. 5 that in this region the viscosity of microemulsion Kelcogel® formulations substantially increased, confirming that Kelcogel® can change into a gel when triggered by ions. At higher TFR (>0.6) the viscosity decreased, which has also been observed by other researchers. Polysaccharide solubility decreases with increasing salt content, and insoluble polysaccharide aggregates can act as heterogeneous nuclei, leading to the growth of microgels that

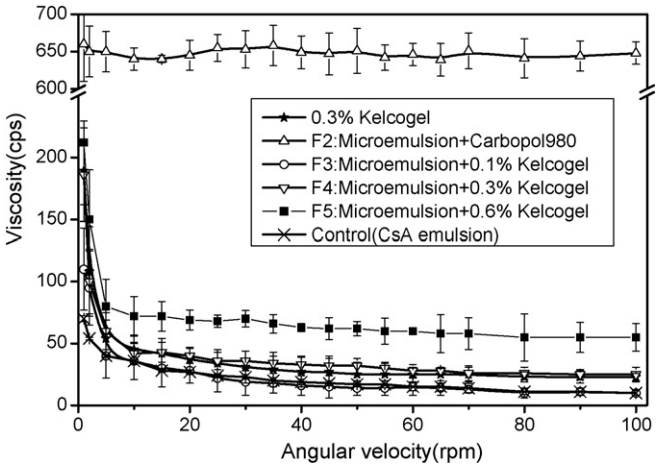

Fig. 3. Rheology profiles of different formulations.

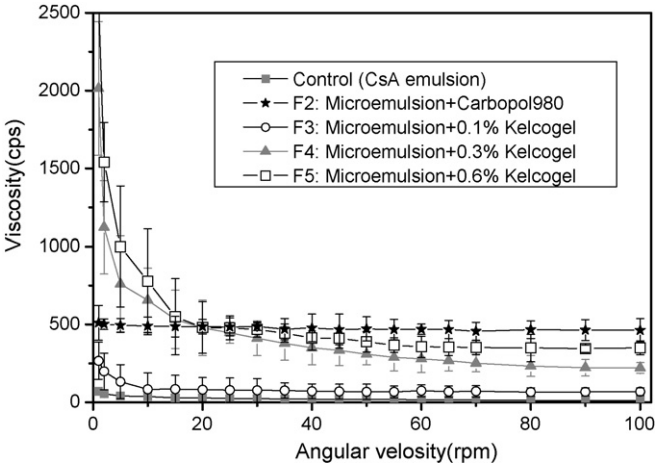

Fig. 4. Rheology profiles of formulations diluted with artificial tear fluid.

interconnect to form a weakened gel network (Paulsson et al., 1999).

On the other hand, administration of ophthalmic preparations should influence as little as possible the pseudoplastic character of the precorneal tear film. The ocular shear rate is very large, ranging from 0.03 s^{-1} during interblinking periods to 4250–28500 s^{-1} during blinking. Viscoelastic fluids with viscosity that is high at low shear rates and low at high shear are preferred (Srividya et al., 2001). As shown in Fig. 3, the in situ gelling system exhibited pseudoplastic rheology, as the viscosity decreased with increasing angular velocity.

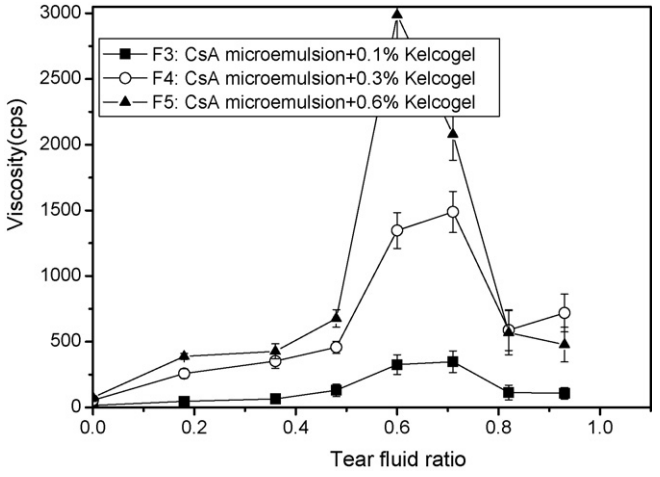

Fig. 5. Viscosity of formulations upon exposure to tear fluid as a function of the ionic concentration.

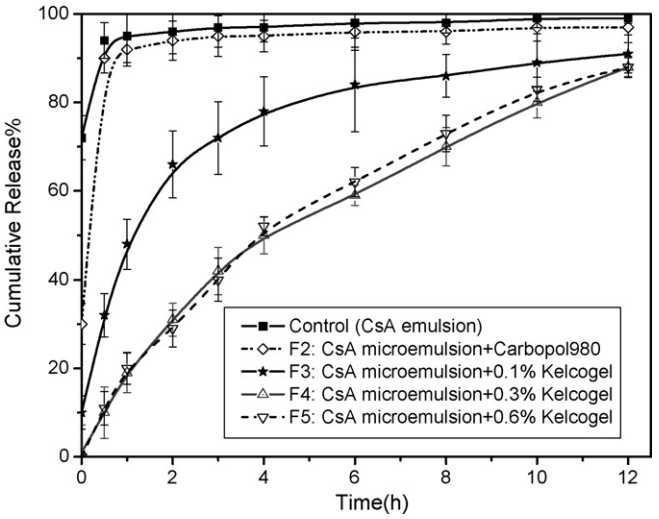

Fig. 6. In vitro drug release profiles of different formulations.

3.5. In vitro release

The CsA release profile versus time is illustrated in Fig. 6. For the CsA emulsion, approximately 70% of the drug was released immediately. For the Carbopol 980 formulation, 30% of the drug was released at the start, with 90% released by 1 h. For the 0.3% and 0.6% Kelcogel® formulations, 15% of the drug was released at 0.5 h and less than 80% was released by 10 h, with drug release continuing thereafter. No significant difference was observed between the 0.3% and 0.6% Kelcogel® formulations. The results demonstrate that Kelcogel® can retain CsA before release. The drug release rate of F3 (0.1% Kelcogel®) was much faster than that of the 0.3% and 0.6% formulations, and did not exhibit satisfactory sustained drug-release behavior.

3.6. Kinetics of desorption from the ocular surface

Fig. 7 shows the kinetics of desorption from the ocular surface for different formulations. The decrease in contact angle for Kelcogel® formulations was approximately 13°, whereas that for F2 (Carbopol 980) was approximately 6°. This indicates the better wettability of the in situ gelling system compared to the previously gelled system, which might contribute to its liquid property before gelling.

On the other hand, Kelcogel® formulations significantly prolonged the residence time on the ocular surface, with lag times in the order F5 > F4 > F2 > control emulsion. As mentioned for the rheology results, high viscosity would prolong the residence time of formulations on the ocular surface.

3.7. Ocular irritation

To exclude the effect of osmotic pressure, each formulation was measured (Osmomat 010, Gonotec, Germany) and was found to be equivalent to normal saline. Albino rabbits ($n = 24$) were treated with the formulations mentioned above to investigate ocular irri-

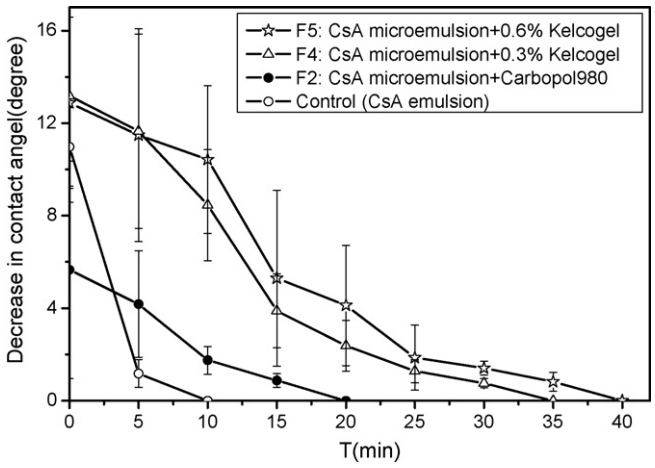

Fig. 7. Kinetics of desorption from the ocular surface for different formulations as measured by the captive bubble technique.

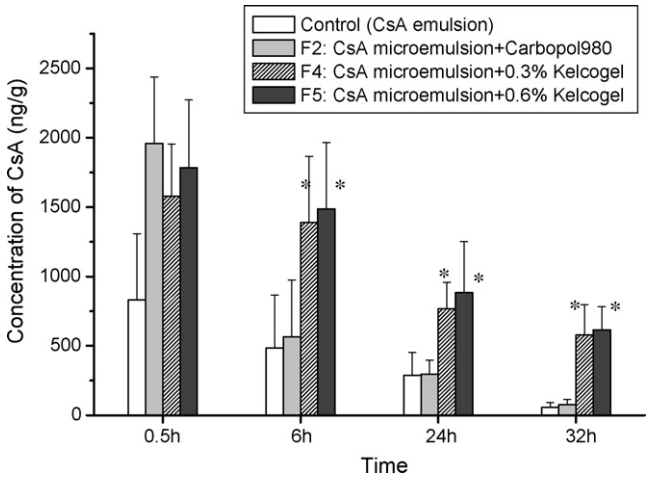

Fig. 9. Concentrations of CsA in the cornea after instillation of F2, F4, F5 and the control microemulsion. *$P < 0.05$.

tation. The results for multiple administrations are summarized in Fig. 8. No ocular damage or clinically abnormal signs were observed in the cornea, conjunctiva or iris. The irritation score for all groups was less than 1, indicating that all formulations exhibited excellent ocular tolerance.

3.8. In vivo evaluation

Concentration–time profiles of CsA in the cornea after topical instillation of the four formulations are shown in Fig. 9. Animals treated with both CsA microemulsion in situ gelling formulations (F4 and F5) had significantly higher corneal drug levels ($P < 0.05$) than those treated with CsA emulsion. More specifically, at 32 h post-instillation of CsA emulsion, the CsA concentration had decreased to subtherapeutic levels, whereas the microemulsion Kelcogel® formulations provided CsA levels high enough to adequately modulate the local immune response and suppress inflammatory processes (50–300 ng/g; Acheampong et al., 1999). Thus, clinically relevant ocular tissues might act as a reservoir for CsA when using the microemulsion in situ gelling system. Consequently, the microemulsion Kelcogel® formulations provided delayed pharmacological action compared to the other dosage forms. The high CsA level and prolonged residence time in the cornea suggest that the microemulsion in situ gelling system might provide an alternative for the treatment of corneal allograft rejection.

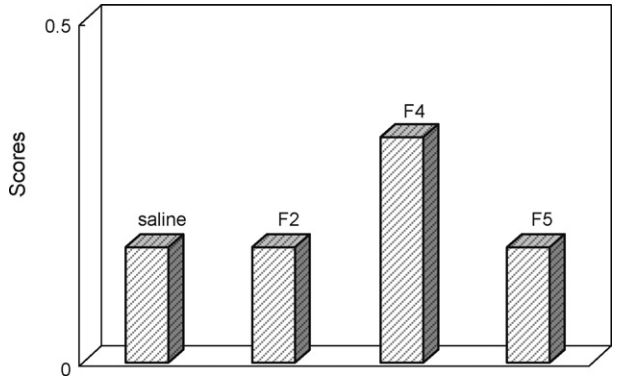

Fig. 8. Irritation scores of saline and different formulations. F2, CsA microemulsion + 0.4% Carbopol 980; F4, CsA microemulsion + 0.3% Kelcogel; F5, CsA microemulsion + 0.6% Kelcogel.

As observed in Fig. 9, the decrease in CsA concentration for the microemulsion Kelcogel® formulations was much slower than for CsA emulsion. This might be due to the sustained release of CsA from Kelcogel® gel. The $AUC_{0\rightarrow 32h}$ of CsA in the cornea for F5 was approximately three-fold greater than that for CsA emulsion. For F2, although the CsA concentration at 0.5 h after instillation was the highest, it sharply decreased to almost the same level as for the control group at 6 h. This is due to progressive dilution of Carbopol 980 gel during measurement (Carlfors et al., 1998).

No significant difference in CsA concentration was observed between in situ formulations with different Kelcogel® content, even though the CsA in situ formulation containing 0.6% Kelcogel® showed slightly higher corneal CsA concentrations than the 0.3% Kelcogel® formulation. It seems that CsA penetration into ocular surface tissues is affected by the release rate of CsA from its carrier in the dispersion medium (Kuwano et al., 2002). As observed from the in vitro release study, a higher concentration of Kelcogel® would lead to more sustained release of the drug.

Fig. 10 shows CsA concentrations in the conjunctiva after topical administration of the four CsA formulations. CsA levels in the conjunctiva decreased much faster than in the cornea. This could be attributed to the uptake of CsA microemulsion by antigen-presenting cells (Langerhans cells and macrophages; Baudouin et al., 1997) or to diffusion of the drug into the blood

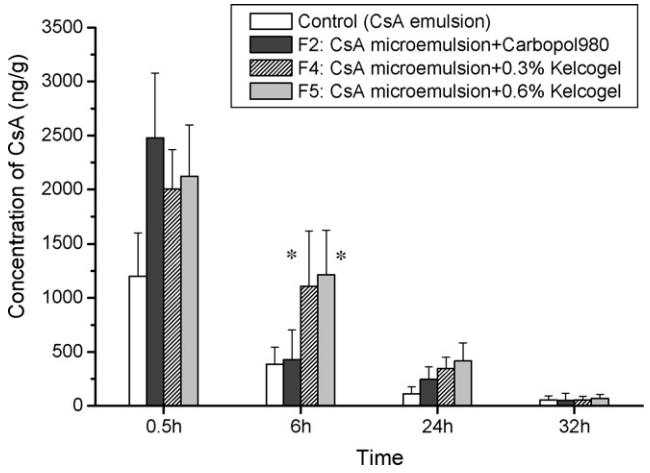

Fig. 10. Concentration–time profile of CsA in the conjunctiva after instillation of F2, F4, F5 and the control microemulsion. *$P < 0.05$.

and lymphatic vessels underlying the fine and leaky conjunctival epithelium.

4. Conclusions

In this study we investigated the potential of a microemulsion in situ electrolyte-triggered gelling system for specific delivery of CsA to external ocular tissue. Compared to two other formulations, the CsA in situ gelling system exhibited better wettability, higher CsA levels and prolonged residence in the cornea. At 32 h after administration, CsA concentrations delivered by this system remained at therapeutic levels in the cornea. Ocular irritation test revealed good compatibility of the system. Therefore, it is suggested that this CsA microemulsion in situ electrolyte-triggered gelling system might represent an alternative for preventing corneal allograft rejection.

Acknowledgement

This work was partly supported by The National High Technology Research and Development Program of China (863 Program) (2007AA021604), for which we are very grateful.

References

Acheampong, A.A., Shackleton, M., Tang-Liu, D.D., Ding, S., Stern, M.E., Decker, R., 1999. Distribution of cyclosporine A in ocular tissues after topical administration to albino rabbits and beagle dogs. Curr. Eye Res. 18, 91–103.

Baudouin, C., Brignole, F., Pisella, P.J., Becquet, F., Philip, P.J., 1997. Immunophenotyping of human dendriform cells from the conjunctival epithelium. Curr. Eye Res. 16, 475–481.

Bourlais, C.L., Acar, L., Zia, H., Sado, P.A., Needham, T., Leverge, R., 1998. Ophthalmic drug delivery systems-recent advances. Prog. Retin. Eye Res. 17, 33–55.

Carlfors, J., Edsman, K., Petersson, R., Jörnving, K., 1998. Rheological evaluation of Gelrite® in situ gels for ophthalmic use. Eur. J. Pharm. Sci. 6, 113–119.

Ding, S.L., Tien, W.L., Olejnik, O., Canyon, T., 1995. Nonirritating emulsions for sensitive tissue. US Patent 5,474,979A, 12 December.

Durrani, A.M., Farr, S.J., Kellaway, I.W., 1995. Influence of molecular weight and formulation pH on the precorneal clearance rate of hyaluronic acid in rabbit eye. Int. J. Pharm. 118, 243–250.

Kuwano, M., Ibuki, H., Morikawa, N., Ota, A., Kawashima, K., 2002. Cyclosporine A formulation affects its ocular distribution in rabbits. Pharm. Res. 19, 108–111.

Lallemand, F., Felt-Baeyens, O., Besseghir, K., Behar-Cohen, F., Gurny, R., 2003. Cyclosporine A delivery to the eye: A pharmaceutical challenge. Eur. J. Pharm. Biopharm. 56, 307–318.

Lang, J.C., 1995. Ocular drug delivery: conventional ocular formulations. Adv. Drug Deliv. Rev. 16, 39–43.

Meisner, D., Mezei, M., 1995. Liposome ocular delivery systems. Adv. Drug Deliv. Rev. 16, 75–93.

Paulsson, M., Hägerström, H., Edsman, K., 1999. Rheological studies of the gelation of deacetylated gellan gum (Gelrite®) in physiological conditions. Eur. J. Pharm. Sci. 9, 99–105.

Power, W.J., Mullaney, P., Farrell, M., Collum, L.M., 1993. Effect of topical cyclosporin A on conjunctival T cells in patients with secondary Sjögren's syndrome. Cornea 12, 507–511.

Price, M.O., Price, F.W., 2006. Efficacy of topical cyclosporine 0.05% for prevention of cornea transplant rejection episodes. Ophthalmology 113, 1785–1790.

Reidy, J.J., Gebhardt, B.M., Kaufman, H.E., 1990. The collagen shield: a new vehicle for delivery of cyclosporine to the eye. Cornea 9, 196–199.

Robinson, J.R., Mlynek, G.M., 1995. Bioadhesive and phase-change polymers for ocular drug delivery. Adv. Drug Deliv. Rev. 16, 45–50.

Rozier, A., Manuel, C., Groove, J., Plazonet, B., 1989. Gelrite: a novel ion activated in situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol. Int. J. Pharm. 57, 163–168.

Rozier, A., Mazuel, C., Grove, J., Plazonnet, B., 1997. Functionality testing of gellan gum, a polymeric excipient material for ophthalmic dosage forms. Int. J. Pharm. 153, 191–198.

Sanzgiri, Y.D., Maschi, S., Crescenzi, V., Callegaro, L., Topp, E.M., Stella, V.J., 1993. Gellan-based systems for ophthalmic sustained delivery of methylprednisolone. J. Control Release 26, 195–201.

Shanker, R.M., Ahmed, I., Bourassa, P.A., Carola, K.V., 1995. An in vitro technique for measuring contact angles on the corneal surface and its application to evaluate corneal wetting properties of water soluble polymers. Int. J. Pharm. 119, 149–163.

Srividya, B., Cardoza, R.M., Amin, P.D., 2001. Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. J. Control Release 73, 205–211.

Vandamme, Th.F., 2002. Microemulsion as ocular drug delivery systems: recent developments and future challenges. Prog. Retin. Eye Res. 21, 15–34.

Vandamme, Th.F., Brobeck, L., 2005. Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. J. Control Release 102, 23–38.

Zimmer, A., Kreuter, J., 1995. Microspheres and nanoparticles used in ocular delivery systems. Adv. Drug Deliv. Rev. 16, 61–73.