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Coenzyme Q10 microemulsion ion-activated gel: a promising ophthalmic delivery system for enhanced corneal protection and sustained release

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Abstract

Purpose This study aimed to evaluate a novel microemulsion ion-activated gel system for the ophthalmic delivery of coenzyme Q10 (CoQ10).

Methods Various CoQ10 microemulsion ion-activated formulations were prepared and fully assessed for physical and chemical parameters, assay and related substances, in vitro release, rheological properties, in vitro cytotoxicity and ophthalmic retention. A preliminary pharmacokinetic study was also performed in rabbits.

Results The formulations met the specified criteria, showing a droplet size of 24.5 ± 2.0 nm for microemulsions, increasing slightly to 39.6 ± 3.5 nm for the microemulsion gels. They exhibited a 24-hour sustained in vitro release (80.0% ± 3.2%) and increased viscosity upon contact with artificial tears containing Ca^{2+} and K^{+} ions. The no-film dissolution method and in vitro models indicated first-order release kinetics ($r = 0.987$). The preparations demonstrated good tolerance and non-irritating properties, with a Draize score of 0–0.55 in rabbits, and provided a 2-hour extension in drug retention on the ocular surface compared with microemulsions alone. In ultraviolet B (UVB)-exposed rats, corneal epithelial damage was reduced and antioxidant marker levels (superoxide dismutase, malondialdehyde) were significantly improved.

Conclusion This novel system is a promising preparation for ophthalmic CoQ10 delivery, offering sustained release and protection against UVB-induced corneal damage.

Keywords Coenzyme Q10, Microemulsion gel, Ophthalmic drug delivery systems, Corneal damage, Ion activated

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Introduction

Coenzyme Q10 (CoQ10) is one of the participating substances in the electron transport chain and aerobic respiration in eukaryotic mitochondria. It can have an insulin-like function and antihypertensive effect in the treatment of diabetic neuropathy and can also be used to alleviate the side effects of radiation and chemotherapy in the treatment of patients with cancer [1, 2]. Reports have shown that CoQ10 is an effective antioxidant in cells, helping scavenge free radicals, and is the only naturally occurring, yet regenerative, fat-soluble antioxidant [3]. In addition, CoQ10 has preventive and therapeutic effects on certain ophthalmic diseases, such as glaucoma, retinal injury, dry eye disease, cataracts and corneal injury [4–9]. However, the protective effect of topically administered CoQ10 on corneal ultraviolet B (UVB) injury remains unclear.

Due to its chemical structure, CoQ10 has poor water solubility, resulting in slow absorption and affecting its therapeutic efficacy in diseases [10]. Delivering the drug to the eye is highly challenging for clinicians, as many barriers in the eye prevent the dose from reaching the site [11–13]. Microemulsions are a good choice in the delivery of compounds with poor water solubility, their unique properties enhancing the solubility of poorly water-soluble drugs, thereby improving their efficacy [14–16]. However, in terms of application in ophthalmic diseases, microemulsions have their limitations due to their inability to overcome drug stimulation completely through effective tear drainage and blinking effects [17]. This problem can be effectively resolved by adding in situ gel materials to the microemulsions [18]. There are currently three types of systems for achieving this: pH-triggered, temperature-dependent and ion-activated systems. Microemulsion-based gels have the combined advantages of microemulsions and gel agents, substantially improving the solubility of drugs with poor water solubility, improving drug loading and increasing the adherence of the preparation, resulting in a delayed-release effect. However, this involves composite preparation with poor stability. The ion-activated gel system utilises the properties of certain polysaccharides, such as gellan gum, to form a gel network in the presence of specific cations, such as Ca^{2+} and K^{+}. These cations cross-link the polymer chains by interacting with their carboxyl groups, thereby increasing the viscosity and forming a gel structure. This process is reversible and can be modulated by the concentration of the cations and the polymer. The gelation process plays a crucial role in the sustained release of the entrapped drug, as it controls the diffusion of the drug through the gel matrix. The biocompatibility of gellan gum and its responsiveness to physiological cation concentrations make it an attractive candidate for ocular drug delivery systems. Ion-activated systems have been evaluated by the use of gellan gum for the ocular delivery of many drugs [16]. These systems allow the drugs to be easily instilled into the eyes at room temperature by adjusting the pH and temperature [19]; furthermore, they have good biocompatibility, as reported in our previous studies [20, 21].

Our novel approach merges microemulsions with an ion-activated in situ gel matrix for CoQ10 delivery. This not only enhances CoQ10's solubility but also prolongs its retention on the ocular surface, potentially offering better corneal protection. In this study, we developed a microemulsion-based ion-activated in situ gel system for CoQ10 and tested its ability to sustain release while protecting rat corneas against UVB-induced damage. Such an approach may prove clinically relevant for preventing UVB-related corneal injury and managing various ocular surface disorders.

Materials and methods

Materials and animals

Materials

Coenzyme Q10 was obtained from Hangzhou Kangrun Pharmaceutical Co., Ltd. (Anji, China). Labrafac® lipophile wl1349 (medium chain triglycerides [MCTs]), Cremophor® EL (CrEL, surfactant), Capmul® MCM C8 EP (C8, cosurfactant) and gellan gum were kindly gifted by Gatteffese (Saint-Priest, France), BASF Corporation (Guangzhou, China), ABITEC Corporation (Columbus, OH, USA) and Zhejiang Zhongken Biological Technology (Tongxiang, China), respectively. The levels of superoxide dismutase (SOD) and malondialdehyde (MDA) were determined using enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Biotech, Nanjing, China). Haematoxylin and eosin (H&E) staining was conducted using cell-staining kits (Boster Biological Technology, Wuhan, China).

Consistent with previous reports [22], the simulated tear fluid (STF) comprised the following: NaCl (6.78 g), KCl (11.38 g), $NaHCO_3$ (2.18 g) and $CaCl_2$ (0.063 g), dissolved in 1 L of water to obtain simulated tears with an electrolyte level similar to that of tears.

Animals

Thirty-six New Zealand White rabbits (18 male and 18 female) weighing 2.0–2.5 kg were provided by the Laboratory Animal Center of Wuhan University (experimental animal certificate: 4210000440). Forty-eight healthy adult Wistar rats (24 male and 24 female) weighing 2.0–2.2 kg and with no eye disease were purchased from Hubei Experimental Animal Research Center (experimental animal certificate: 4200696091). This study was approved by the Institutional Animal Care and Use Committee of General Hospital of Central Theater Command, China (No. SCXK2023-0139).

Preparation of the coenzyme Q10 microemulsion and microemulsion gel

Preparation of the microemulsion

Before finalising the formulation, extensive preliminary studies were conducted to optimise the composition. Various ratios of MCT: CrEL: C8 (2:5:1, 3:5:1, 4:5:1 w/w/w) were evaluated for their ability to solubilise CoQ10. The 3:5:1 ratio was selected, as it provided optimal solubility (> 10 mg/mL CoQ10) while maintaining acceptable viscosity and clarity. For the gellan gum concentration, a range of 0.2–0.6% w/v was tested, with 0.4% w/v showing the best balance between initial flowability and subsequent gel strength when exposed to artificial tears.

As previously reported [23], the oil phase was prepared using a weight ratio of MCT: CrEL: C8 of 3:5:1 (w/w/w). Coenzyme Q10 (10% w/w relative to the oil phase) was dissolved in the oil phase under moderate stirring (400 rpm) for 30 min at 40 °C. Subsequently, the oil phase was slowly titrated with deionised water at a ratio of 1:10 (v/v), with continuous stirring (300 rpm) at room temperature until a clear and transparent microemulsion was obtained. The final concentration of CoQ10 in the microemulsion was 10 mg/mL.

Preparation of the microemulsion gel

To prepare the ion-activated gel base, gellan gum was added to deionised water at a concentration of 0.4% (w/v) and then placed in a 90 °C water bath for 15 min to ensure complete dissolution. The solution was cooled to 40 °C, passed through a 0.45-μm filter and maintained at 25 °C. Finally, the CoQ10 microemulsion (Sect. 2.2.1) was slowly mixed with the gellan gum solution in a ratio of 10:1 (v/v) under gentle magnetic stirring (200 rpm) for 10 min. This yielded the CoQ10 microemulsion ion-activated gel, wherein the gellan gum forms a gel matrix upon contact with Ca^{2+} and K^{+} ions from tears.

Note The ratio of gellan gum (0.4% w/v) was optimised based on preliminary tests, aiming for a suitable viscosity that would permit easy instillation yet form a stable gel in the ocular environment.

Physicochemical characterisation of microemulsion gel

Particle size and morphology analysis

A laser particle analyser (Zetasizer ZS90, Malvern Instruments Ltd., UK) and transmission electron microscope (Model JEM-100CXII, JEOL Ltd., Japan) were used to monitor the particle size and morphology of the microemulsion and microemulsion gel, respectively. The results are shown in Table 1; Fig. 1A–B.

Table 1 The results of characterization of microemulsion and microemulsion-gel

Preparation	Particle size (nm, $n=3$)	Viscosity (mPa*s, $n=3$)	pH ($n=3$)
Microemulsion	24.5 ± 2.0	6.9 ± 1.0	7.2 ± 0.4
Microemulsion-gel	39.6 ± 3.5	7.8 ± 2.0	7.3 ± 0.6

Viscosity measurements

Viscosity measurements were performed at 25℃ ± 0.1℃ using a rotary viscometer (Model NDJ-4, Shanghai Changji Instruments Co., Ltd., China). For the samples of microemulsion, microemulsion gel and microemulsion gel containing STF (in the ratio of 40:7), the deflection angles (α) on the dial at the rotation rate of 60 rpm were read, and the corresponding specific factor coefficients were recorded. The viscosity (η) of the system was calculated according to the Mark–Houwink equation:

$$\eta = K \cdot M^{\alpha},$$

where K and α are known as the Mark–Houwink parameters and are related to the type of polymer, type of solvent and temperature, M is the molecular weight and η is the characteristic viscosity. The results are shown in Table 1.

Measurement of pH

The pH was measured at 25℃ using a pH meter (Delta320, Mettler Ltd., UK). The results are shown in Table 1.

Assay and related substance method

High-performance liquid chromatography (HPLC) was used for quantifying CoQ10 and detecting related substances. The HPLC system (Agilent 1260, USA) comprised a quaternary pump, autosampler and ultraviolet (UV) detector. A C18 column (150 mm × 4.6 mm, 5 μm) was used with the mobile phase: methanol: isopropanol (70:30, v/v) at a flow rate of 1.0 mL/min. The detection wavelength was 275 nm, the column temperature was 30 °C and the injection volume was 20 μL. Related substances were evaluated by monitoring any additional peaks in the chromatogram with retention times differing from CoQ10.

In vitro drug release studies

A modified no-film dissolution method was employed, which uses a self-made diffusion cell (Chagnzhou Guohua Instruments Co., Ltd., China) and a thermostatic shaker. Briefly, the release medium (200 mL of STF) was kept at 34 °C ± 1 °C to mimic ocular surface temperature. The microemulsion gel (2.0 mL) containing 10 mg/mL of CoQ10 was placed into 5-mL vials, followed by adding STF at a ratio of 7:40, and stirred gently. Each vial was then placed in the 250-mL STF bath. The entire setup was shaken at 50 rpm [24]. At fixed intervals, samples (1.0 mL) of the dissolution medium were withdrawn and

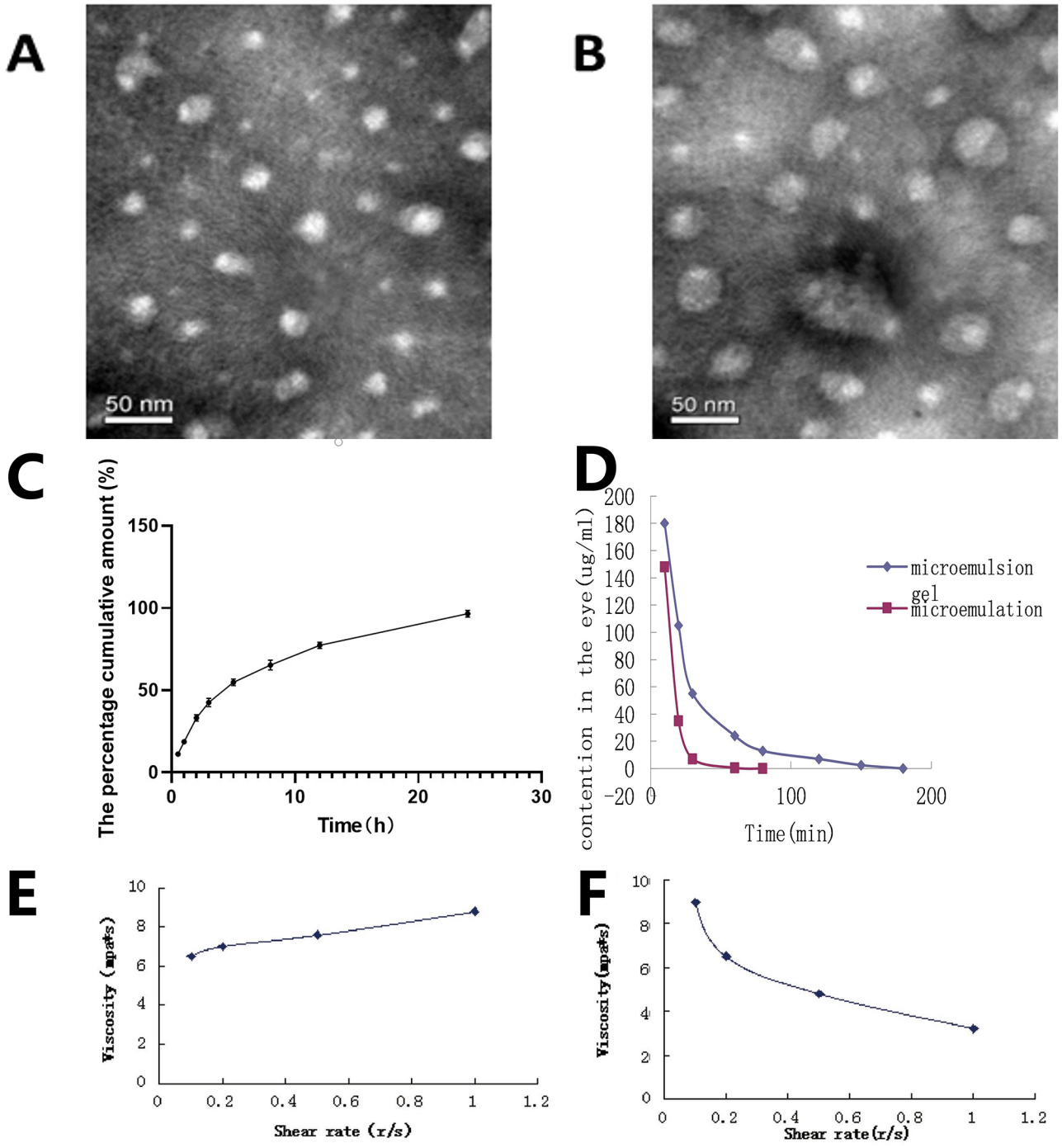

Fig. 1 Transmission electron microphotograph of CoQ10 microemulsion (**A**) and microemulsion-gel (**B**). Scale bar = 100 nm. Images are representative of $n = 3$ independent preparations. (**C**) Cumulative release profile of CoQ10 from microemulsion-gel versus time. Data points represent mean ± SD ($n = 3$). *$p < 0.05$ compared to microemulsion control. (**D**) Precorneal retention of CoQ10. Drug concentration was measured in rabbit conjunctival sac ($n = 4$ per group) over time. Data shown as mean ± SD. **$p < 0.01$ versus microemulsion group. Rheological behavior of CoQ10 ophthalmic formulations (**E**) Viscosity versus shear rate for microemulsion-gel before ion exposure ($n = 3$). (**F**) Viscosity versus shear rate after exposure to artificial tear fluid ($n = 3$). Statistical analysis: repeated measures ANOVA with Dunnett's post-hoc test, *$p < 0.05$

replaced with fresh STF. Samples were filtered through a 0.45-μm filter, and 20 μL was injected into the HPLC system for CoQ10 determination. The results are shown in Fig. 1C.

Rheological properties analysis

Rheological properties analysis was performed on microemulsion ion-activated gel formulations without cations at 25℃ ± 0.1℃ using the rotational viscometer (Model

NDJ-4). The viscosity of the microemulsion in situ gel containing CoQ10 (A) and the viscosity with the added STF (B) were determined. The viscosity (η) of A and B was calculated at the following shear rates (C): 0.1, 0.2, 0.5 and 1 s^{-1}; each sample was measured three times, and the mean of the results was recorded. The Mark–Houwink equation was used to calculate the apparent viscosity at each shear rate, and the results were plotted against the shear rate to determine pseudoplastic behaviour. The value of *K* was determined using an instrument constant calculation table and used in the Mark–Houwink equation to obtain the apparent change of viscosity corresponding to the different shear rates (D).

Draize rabbit eye irritation test

Thirty-six New Zealand White rabbits were used in the Draize eye irritation test. After inhalational anaesthesia with 5% isoflurane, 50 µL of the test formulation was instilled into the right eye, whereas the left eye received saline as a control, once daily for 7 days. Each day, the eyes were examined under a UV lamp to assess any corneal lesions. The degree of keratopathy was scored according to the Draize method [25].

Biosafety evaluation

Before the animal studies, the biosafety of the CoQ10 microemulsion gel was evaluated through the following tests:

In vitro cytotoxicity assay

Human corneal epithelial cells (HCECs) were seeded in 96-well plates at 5×10^3 cells/well and allowed to attach overnight. Various concentrations (0.1–1.0 mg/mL) of microemulsion gel were added. After 24 h, MTT reagent (0.5 mg/mL) was added, and the absorbance at 570 nm was measured. Cell viability (%) was calculated relative to the untreated control.

Haemolysis assay

Fresh rabbit blood was collected in heparinised tubes and diluted (1:1) with normal saline. Different concentrations of microemulsion gel (0.1–1.0 mg/mL) were incubated with 2% RBC suspension at 37 °C for 1 h. The mixture was then centrifuged at 1,000 g for 10 min, and the absorbance of the supernatant was recorded at 540 nm. Haemolysis (%) = [(A_sample– A_blank) / (A_total– A_blank)] × 100, where A_total is the absorbance for distilled water (100% lysis).

Acute ocular irritation test

A modified Draize eye test was performed on three rabbits, instilling 50 µL of the microemulsion gel in one eye, with the other eye serving as control (saline). Eyes were examined at 1, 24, 48 and 72 h for redness, swelling and discharge. The mean score was recorded according to Draize criteria.

Ophthalmic retention in rabbits' eyes

Four New Zealand White rabbits were used to evaluate the retention of CoQ10 in the conjunctival sac. After inhalational anaesthesia (5% isoflurane), 50 µL of microemulsion gel or microemulsion (both containing CoQ10 at 10 mg/mL) was administered into the right conjunctival sac. At predetermined intervals, 8-mm-diameter filter paper was placed onto the eye for 1 min, then removed and weighed [26]. The filter paper was extracted with 50 µL of absolute ethyl alcohol, vortexed for 5 min and centrifuged at 12,000 rpm for 10 min. Then, 20 µL of supernatant was injected into the HPLC (Sect. 2.3.4) for CoQ10 quantification.

Protective properties of topically administered coenzyme Q10 microemulsion gel

Animals

Forty-eight Wistar rats were divided into three groups: control, model (UVB-exposed only) and therapy (UVB + CoQ10). Four days before UVB exposure, saline was instilled into the left eyes, whereas microemulsion gel containing 10 mg/mL of CoQ10 was instilled into the right eyes. This daily regimen continued for 3 more days post-UVB.

Ultraviolet B exposure

The UVB source was a self-made lamp box with a wavelength of 295 nm and an intensity of 3.6 J/cm [27]. The UVB exposure was administered for 5 min/day to the test group and sustained for 3 days. A slit lamp microscope (Model SL120, Carl Zeiss, Germany) was used to observe epithelial layer damage (as fluorescein staining) in each eye of all the rats 8 h after each exposure to UVB. In this study, the standard Draize test protocol was followed to evaluate the irritancy of the formulation in the eyes of the New Zealand White rabbits [27]. All procedures were conducted in accordance with the guidelines and were approved by the Institutional Animal Care and Use Committee.

Morphology observation

Eight hours after the final UVB exposure, the rats were humanely euthanised via an intraperitoneal injection of 200 mg/kg of pentobarbital, and the corneas were isolated. Half of each cornea was fixed in 4% paraformaldehyde for 24 h, paraffin embedded and H&E stained. The remaining half was stored at – 20 °C for biochemical analyses (Sect. 2.8.4). Corneal sections were examined using an optical microscope (BX517-32P01, Olympus, Japan).

Superoxide dismutase and malondialdehyde level determination

The rats' corneas were minced, treated with liquid nitrogen for 30 min and subsequently diluted with 0.9% NaCl solution to obtain homogenates in the ratio of 1:9 (w/v) and centrifuged at 3,000 rpm/min for 15 min to obtain a supernatant concentration of 10% of the homogenate [28]. The supernatant of the homogenate was diluted in buffer solution (0.9% NaCl solution) in concentrations of 0.5%, 0.5% and 0.1%, and the SOD and MDA levels were subsequently determined.

Additional in vitro and pharmacokinetic studies

In vitro release studies

A Franz diffusion cell was used as an additional confirmation of CoQ10 release, with the receptor compartment filled with phosphate-buffered saline (pH 7.4) containing 0.5% Tween 80. Samples were withdrawn at 0, 2, 4, 8, 12 and 24 h and analysed by HPLC (Sect. 2.3.4).

Stability testing

The physical and chemical stability of the CoQ10 microemulsion gel was evaluated at 4 °C, 25 °C and 40 °C over 6 months. At 0, 1, 3 and 6 months, samples were evaluated for appearance, pH, viscosity, assay (CoQ10 content), related substances and in vitro release profiles. Deviations of CoQ10 content beyond ± 5% were considered significant changes.

Preliminary Pharmacokinetic study

A pilot pharmacokinetic study was conducted in six rabbits, receiving a single topical dose of the CoQ10 microemulsion gel. Aqueous humour samples were collected at 0.5, 1, 2, 4 and 8 h post-administration via paracentesis. Each sample (100 µL) was mixed with 100 µL of methanol, vortexed (5 min) and centrifuged (12,000 rpm, 10 min). Supernatant (20 µL) was injected into the HPLC system (Sect. 2.3.4). The concentration–time profile was used to estimate key pharmacokinetic parameters (C_{max}, T_{max}).

Statistical analysis

Statistical analysis was performed using the SPSS (v19.0, IBM, Armonk, NY, USA) software package. All data were presented as the mean ± standard error or standard deviation, as specified. Physicochemical measurements, including particle size, viscosity and pH, were analysed using one-way ANOVA followed by Dunnett's test. In vitro release profiles were evaluated using repeated measures ANOVA with the Bonferroni correction. For in vivo studies, paired comparisons were analysed using the two-tailed Student's *t*-test, and multiple group comparisons, such as SOD and MDA levels, were performed using one-way ANOVA with Dunnett's post hoc test. Non-parametric data including Draize scores were analysed using the Mann–Whitney U test. Statistical significance was set at $p < 0.05$.

Table 2 Physicochemical properties of CoQ10 formulations

Formulation	Droplet size (nm)	Viscosity (mPa·s)	pH	CoQ10 assay (mg/mL)
Microemulsion	24.5 ± 2.0	7.2 ± 0.5	7.2 ± 0.4	9.7 ± 0.3
Microemulsion gel	39.6 ± 3.5	9.8 ± 1.2	7.3 ± 0.6	9.8 ± 0.2
+STF (40:7)	—	120 ± 4.2	—	—

CoQ10: Coenzyme Q10; STF: Simulated Tear Fluid

Results

Physicochemical characterisation of microemulsions and microemulsion gels

Particle size analysis

The mean droplet size was 24.5 ± 2.0 nm for the prepared microemulsions and 39.6 ± 3.5 nm for the microemulsion gels (Table 1), which conforms to the typical microemulsion droplet size [29]. Transmission electron microscope images (Fig. 1A–B) confirmed spherical droplets of uniform size. Incorporating the ion-activated gel slightly increased the droplet size, suggesting that the microemulsions are well dispersed in the gel.

Viscosity measurements

The viscosity of ophthalmic solutions should be < 20.0 mPa·s [30, 31], and ophthalmic instillation should improve with higher viscosity [32]. The viscosity values of the microemulsions and microemulsion gels were < 10.0 mPa·s, which is within the required viscosity of eye drops. However, upon mixing with STF (ratio of 40:7), the viscosity increased up to 120 mPa·s ± 4.2, indicating that ions in the STF triggered gelation.

Measurement of pH

The pH is also important for ophthalmic preparations, with an ideal pH of 7.2 ± 0.2 when instilled into the eye [32]. Tears can adjust the pH of ophthalmic preparations to physiological levels [33], meaning if the pH of the ophthalmic preparation fluctuates across a small range, it can also be adjusted by tears. As shown in Table 1, the pH value of the microemulsions was 7.2 ± 0.4 and that of the microemulsion gels was 7.3 ± 0.6.

Assay and related substances

The CoQ10 content (assay) was 9.8 ± 0.2 mg/mL (98% of the theoretical 10 mg/mL), and related substances were < 1.0%, indicating satisfactory purity (Table 2).

In vitro drug release studies

The results (Fig. 1C) show that the microemulsion ion-activated gel containing CoQ10 exhibited sustained

release behaviour, reaching a cumulative release of 80.0% ± 3.2% at 24 h, similar to the results of existing experimental studies [34]. The results of the fitting of each release data are shown in Table 3, and according to the principle that the larger the *r* is, the better the fit, it can be seen that the first-order equation is better than the Higuchi equation, releasing the drug slowly and non-constantly as required. Monovalent or divalent cations in solutions interact with the carbonyl groups of the polymer chains and participate in the formation of interchain hydrogen bonds, which stabilise the double helices. Each of the two double helices aggregates in the reverse direction to form a three-dimensional gel network. The drug is released by diffusion through the orifice, which is in accordance with the first-order equation.

Comparison of the release kinetics across different formulations revealed that the microemulsion gel system exhibited the most sustained release profile (Table 3). The normal eye drops showed the fastest release rate ($k = -0.22$), followed by the simple microemulsion ($k = -0.17$), whereas the microemulsion gel system had the slowest release rate ($k = -0.13$). This demonstrates the superior controlled release properties of the microemulsion gel system.

Rheological properties analysis

The rheological properties of the microemulsion gels were studied, particularly upon exposure to lacrimal fluid. The results are shown in Fig. 1E–F. The systems generally exhibited low viscosity, and there was no marked change with the increase in angular velocity; however, their viscosity increased greatly when they were in contact with the ions in the tear fluid, which suggests that the system is a pseudoplastic fluid. Eye drops are preferred as a pseudoplastic fluid, given that the pseudoplastic effect on the anterior corneal membrane should be as small as possible [35].

In vivo residence studies

Rapid removal of eye drops in the eye also results in the decreased efficacy of the drops [36]. The drug concentration profile over time on the rabbit corneal surface is shown in Fig. 1D. After administering the CoQ10 microemulsion gel preparation, the drug concentration in the conjunctival sac of the rabbits was significantly higher than that when using the CoQ10 microemulsion, especially within 60 min. During the first 30 min, the microemulsion almost completely disappeared from the eye capsule. Compared with the microemulsion, the microemulsion gel wash-out was slower and the drug residence time of the latter increased by more than 2 h.

Irritation test

The safety of ophthalmic preparations is extremely important and was a major consideration in this study. The Draize rating scale of eye irritant experiments was used to assess the irritation in the rabbits' eyes.

The results of the ocular irritation studies are shown in Fig. 2A–B. Clinical investigations revealed that no ocular damage to the cornea, iris or conjunctivae was visible and that the structure of corneal epithelial cells was normal compared with those in the control group (NEs: 3, 5, 6, 10, 12 and 14), demonstrating that the microemulsion gels are non-irritants (average total score: 0–0.55). Rabbit eyes are more sensitive to irritants than human eyes [37]; therefore, this result indicates that the gels can be considered safe for human eyes.

Protective properties of topically administered coenzyme Q10 microemulsion gel

The results of the slit lamp observations are shown in Fig. 2C–E. Compared with the control group, there was apparent epithelial damage (evaluated by fluorescein staining) in UVB-treated eyes, whereas the eyes with locally administered CoQ10 had comparatively little epithelial damage; however, there was still some epithelial damage compared with the control group.

As shown in Fig. 2F–H, the corneal epithelium cells in the control group were 4–6-layer squamous epithelium cells, packed tightly and arranged in an orderly manner with normal morphology [7]. The cornea eyes following UVB irradiation indicated damage to the corneal epithelium surface cells; cell layers were chaotic and disordered, the cell shapes were non-normal and red blood cells emerged. The Bowman layer and substrate layer showed no significant changes. In the eyes that received locally administered CoQ10, the morphology of the cornea epithelial cells was similar to that of the control group.

As shown in Table 4, the SOD levels were decreased, whereas the MDA levels were increased ($p < 0.05$) in the corneas following UVB irradiation. When 10 mg/mL of CoQ10 was locally administered for 4 days before and 3 days after UVB treatment, the MDA content was no different compared with the control group and significantly

Table 3 In vitro release kinetics of CoQ10 formulations

Formulation	First order equation	R^2	Higuchi equation	R^2
Normal eye drops	$Ln(F_\infty\text{-}F) = -0.22t + 4.56$	0.9935	$F = 25.31t^{(1/2)} + 2.87$	0.9589
Simple microemulsion	$Ln(F_\infty\text{-}F) = -0.17t + 4.49$	0.9902	$F = 22.84t^{(1/2)} + 2.95$	0.9634
Microemulsion-gel	$Ln(F_\infty\text{-}F) = -0.13t + 4.43$	0.9871	$F = 20.57t^{(1/2)} + 3.01$	0.9677

CoQ10: Coenzyme Q10

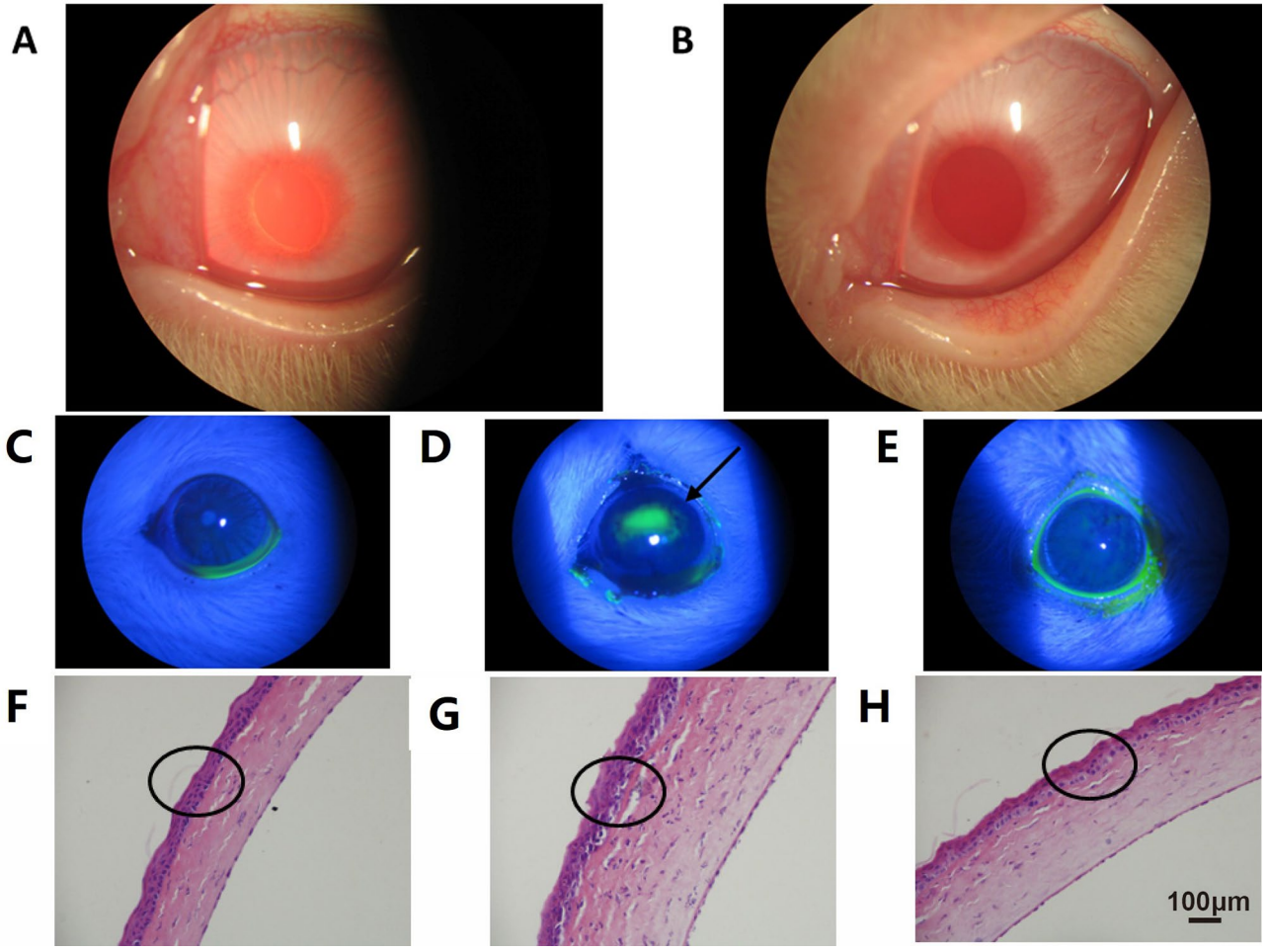

Fig. 2 The graph of lamp examination (**A**) Normal saline group; (**B**) CoQ10 microemulsion-gel. The results of slit lamp examination (**C**) Normal group; (**D**) Model group; (**E**) Therapy group. The results of morphological observations (40×) (**G**) Control groups; (**G**) Model group; (**H**) Therapy group

Table 4 The results of SOD, MDA determination($n=8$)

Groups	SOD(U/mgprot)	MDA(nmol/mgprot)
Control groups	30.4 ± 1.4	3.2 ± 0.5
Eyes got UVB irradiation	18.9 ± 0.9*	7.6 ± 0.3*
Eyes treated with CoQ10	25.8 ± 1.1**	4.9 ± 0.6**

Explain: *compare to control groups, $p<0.05$; **compare to UVB irradiation eyes, $p<0.05$

SOD, Superoxide Dismutase; MDA, Methane Dicarboxylic Aldehyde

CoQ10: Coenzyme Q10; UVB: ultraviolet B

reduced ($p<0.05$) compared with the UVB-treated eyes. Moreover, the SOD level was no different compared with the control group and was significantly increased ($p<0.05$) compared with the UVB-treated eyes.

Additional in vitro and pharmacokinetic findings

The Franz cell tests confirmed sustained release up to 24 h. Stability tests showed < 5% CoQ10 degradation at 4 °C and 25 °C over 6 months but approximately 8% at 40 °C by 3 months. Pharmacokinetic data indicated a C_{max} of 0.52 ± 0.11 μg/mL at 2 h, indicating adequate transcorneal penetration.

Biosafety evaluation results

The in vitro cytotoxicity results showed > 90% HCEC viability at up to 1 mg/mL. The haemolysis assay revealed < 5% haemolysis, indicating good compatibility. The acute ocular irritation test on rabbits confirmed no signs of irritation (scores within the 'non-irritating' range).

Discussion

The corneal epithelium shields the eye by absorbing UVB light, protecting internal structures such as the lens and retina. However, long-term exposure to UVB can damage the cornea, killing epithelial cells and causing oxidative stress due to reactive oxygen species [38, 39]. This oxidative damage can lead to corneal and conjunctival lesions [23, 29]. Coenzyme Q10, known for its antioxidant properties, can mitigate this damage and is beneficial for treating related diseases [40–42]. In this study, rats exposed to

UVB showed mild corneal damage when treated with a CoQ10 microemulsion gel, supporting previous findings that CoQ10, whether orally or topically administered, protects against UVB-induced corneal damage [7].

In this study, we designed and verified a novel topical administration of CoQ10 against UVB damage in corneas. To improve its efficacy, we selected various microemulsions to enhance the water solubility of CoQ10 [10]. In addition, we added an in situ gel material into the microemulsion to increase the pre-corneal residence time of the delivery system and enhance ocular bioavailability [18]. To reduce irritation to the ocular tissues, we selected gellan gum as the ion-activated gel material [16]. The formulated CoQ10 microemulsion gel eye drops have acceptable physicochemical and pseudoplastic properties, long-term precorneal residence and good tolerance by the eye. Moreover, the developed microemulsion gel can enhance the water solubility of CoQ10. In vitro, the solution is a yellow fluid, but it changes to the gel phase immediately when dropped in the eye. This decreases the number of applications necessary per day and leads to better patient compliance. Furthermore, our study shows that a single ocular irradiation with an effective dose of UVB induces cornea damage and affects its antioxidant defence systems. Due to its free radical scavenging and antioxidant properties, the administration of CoQ10 is capable of protecting eye tissue, thus reducing the harmful effects of UVB. Interestingly, a microemulsion ion-activated gel containing CoQ10 can effectively utilise the ions in tears to achieve sustained release delivery of insoluble drugs in the eye, which provides ideas for the development of ocular drug delivery systems.

The corneal epithelium protects internal ocular structures by absorbing UVB light. However, excessive UVB can cause oxidative stress and damage corneal epithelial cells [23, 29]. Coenzyme Q10's antioxidant properties help mitigate this damage [40–42]. Our results suggest that topical CoQ10 microemulsion gel offers improved residence, sustained release and effective antioxidant protection against UVB-induced corneal damage, aligning with prior reports on CoQ10's protective effects in ophthalmic tissues [7].

Compared with other in situ gelling systems, our ion-activated approach uses gellan gum triggered by lacrimal ions, reducing the need for external pH or temperature changes. The novelty lies in the specific ratio of MCT: CrEL: C8 (3:5:1), combined with 0.4% gellan gum, which balances solubility, stability and in situ gelation upon contact with tears. Additionally, we showed that the formulation remains stable for months at lower temperatures and can protect corneal tissue from UVB damage. Future studies will focus on optimising the system using a design of experiments (DoE) approach, testing it in larger animal models and eventually pursuing clinical trials. Although a formal DoE approach was not used in this study, our systematic preliminary screening effectively identified suitable formulation parameters. The decision to use conventional optimisation rather than DoE was based on our prior experience with similar systems and the need to first establish basic feasibility. Future research could apply the DoE methodology to further refine the formulation, particularly for scale-up production processes and manufacturing, while also addressing important regulatory considerations for widespread clinical use.

Conclusion

In this study, we introduced a novel microemulsion ion-activated in situ gelling system for the sustained ophthalmic delivery of CoQ10. The gel is triggered by naturally occurring ions in tears, increasing ocular retention. Our findings demonstrate that this formulation significantly reduces UVB-induced corneal epithelial injury and maintains robust antioxidant defences. Although the present results are promising, further optimisation and larger-scale testing are necessary to fully validate its clinical potential. This platform holds promise as a new approach to ophthalmic drug delivery, enhancing therapeutic outcomes for various ocular disorders.

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Author contributions

Dong SH, Gao Y, Li Y, Wu D, Chen Y, and Chen SH made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All procedures using animals in this study were performed according to a protocol by the Instiutional Animal Care and Use Committee of General Hospital of Central Theater Command and were approved by the General Hospital of Central Theater Command Experimental Animal Ethics Committee (No. SCXK2023-0139).

Consent for publication

All authors agree to submit for consideration for publication in the journal.

Competing interests
The authors declare no competing interests.

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