

Extended release of ketotifen from silica shell nanoparticle-laden hydrogel contact lenses: in vitro and in vivo evaluation

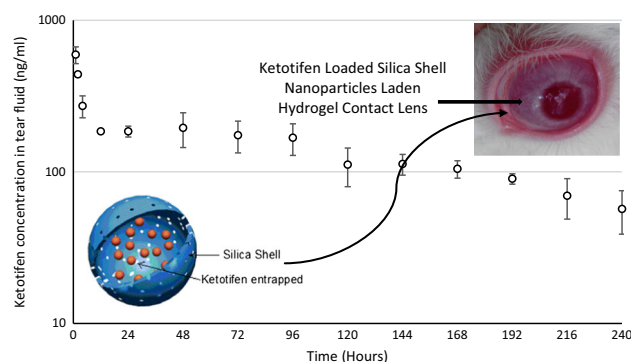
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Abstract Ketotifen an anti-allergic drug delivered via eye drops has major limitations, including poor ocular bioavailability and poor patient compliance. The objective of the research work was to fabricate ketotifen loaded microemulsion laden hydrogels and silica shell nanoparticle-laden (prepared from microemulsion using octyltrimethoxysilane) hydrogels to achieve extended ocular drug delivery. The porous silica shell membrane was synthesized at the liquid interface of microemulsion, which facilitates the prolongation of drug release duration from hydrogels. Drug encapsulated microemulsion and silica shell nanoparticles were dispersed separately in pre-monomer mixture, and fabricated to hydrogel. For comparison, hydrogel with direct drug entrapment was also fabricated. Significant loss in transmittance and physical properties was observed in hydrogels with direct drug entrapment. While, microemulsion and silica shell nanoparticle-laden hydrogels did not show significant effect on transmittance and physical properties. The in vitro drug release data showed extended release of

ketotifen from hydrogels in following order: direct loading < microemulsion < silica shell nanoparticles. The results of cytotoxicity and animal study confirmed the safety of hydrogels. The in vivo pharmacokinetic study showed extended ketotifen release for more than 10 days. The results demonstrated the translational potential of silica shell nanoparticles for extended drug delivery without compromising the critical lens properties.

Graphical Abstract



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1 Introduction

Currently, more than 90 % of ophthalmic drugs are delivered through eye drops. It has been well accepted by ophthalmic practitioners; however, it suffers from critical shortcomings which limit its therapeutic efficacy [1]. On instillation, eye drops are constantly diluted and washed away by reflex tearing, absorption through the conjunctiva and draining through the nasolacrimal system (prone to systemic absorption and related side effects). Thus, only 1–3 % of drug is available to ocular tissue for therapeutic

effect [2]. Frequent dosing is required with high drug loading to maintain the drug levels within the therapeutic window, which result in poor compliance in patients [3, 4].

Previous efforts in developing new ophthalmic drug delivery systems are focused on different formulations including in situ forming gels, nanoparticles, liposomes and niosomes, most of them have stability issues, ocular inserts have problem of burst release, collagen shields with poor patient compliance, ointments that cause blurred vision, etc. [5–10]. Most of them have achieved some success in terms of improving the fractional drug intake and reducing systemic absorption, but still they suffer from different issues, such as low bioavailability, blurring of vision, discomfort, toxicity to the cornea, etc. [11, 12]. Thus, there exists an urgent need for novel ophthalmic drug delivery systems to control the drug release over an extended period of time. These all findings and literature led us to explore contact lenses. Contact lenses as a drug delivery device have shown improvement in bioavailability (>50 %) [13, 14]. Therapeutic contact lenses release the drug in post and pre-lens tear film with residence time greater than 30 min, in comparison to 5 min with eye drop therapy [15, 16]. Higher bioavailability through contact lenses allows reduction in dose (and frequency) thereby reduces the systemic absorption and its related systemic side effects.

Therapeutic contact lenses are prepared by a conventional method of soaking, whereby contact lenses are soaked in concentrated drug solution, which in turn absorbs drug through its aqueous polymeric channels. Weak interactions between drug and contact lens material led to low drug loading and failure to extend the drug release duration [17, 18]. To overcome this drawback, different methodologies have been proposed like, molecular imprinting [19, 20], use of vitamin E [21], drug loaded colloidal structures [6, 22], etc. Although therapeutic contact lenses have been explored by many scientists to deliver ophthalmic drugs, they suffered from many limitations, such as low drug loading, loss of critical lens properties like transparency, swelling, oxygen permeability, ion permeability, storage modules, etc. [23].

The purpose of this study was to explore drug loaded microemulsion and silica shell nanoparticle-laden hydrogels, to achieve extended ocular drug delivery. Microemulsions are dispersions of oil in water or water in oil that are thermodynamically stable due to the significant lowering of the interfacial tension by the adsorption of amphiphiles on the surface. Microemulsion allow high drug loading and the tightly packed surfactant at the oil–water interface of microemulsions provide a barrier to drug transport, which can be used to control and extend the drug release duration [24]. In the present study porous silica shell membrane was synthesized at liquid interface of

microemulsion to extend the drug release duration from hydrogels [25]. Thus the objective of the research paper was to fabricate ketotifen-loaded microemulsion and silica shell nanoparticle-laden hydrogels for extended ocular drug delivery without affecting the critical lens properties.

2 Materials and methods

2.1 Materials

Hydroxyethylmethacrylate (HEMA), Methacrylic acid (MAA), Darocur[®] (trimethyl benzoyl-biphenyl-phosphine-oxide), Ethyleneglycoldimethacrylate (EGDMA), Trimethoxy(octadecyl)silane (OTMS), Pluronic F 127, Tween 80, and PEG 400 were acquired from Sigma Aldrich Chemicals (St. Louis, MO, USA). Ketotifen fumarate was purchased from Flaming Lab. LTD (A.P., India). Rabbit corneal epithelial cell lines ATCC[®] CCL60TM were obtained from NCCS (Pune, India). Ultrapure water (18.2 MΩ cm) was obtained from Synergy U.V. Millipore water purification system.

2.2 Synthesis of microemulsions and silica shell nanoparticles

Two batches of microemulsion coded MET and MEP were synthesized. Ketotifen fumarate was converted to its base form by raising the pH of the aqueous solution. A solution of ketotifen in isopropyl myristate (0.2 gm in 1.5 ml) and a solution of Pluronic F127 (1.12 gm) and Tween 80 (4.48 gm) in 1:4 ratio was prepared in deionized water (4.48 ml). The drug solution prepared in oil was added drop-wise to the surfactant solution with continuous stirring until it became clear and it was coded as MET microemulsion. Similarly MEP microemulsion was prepared using PEG 400 in place of Tween 80. These two batches of microemulsion MET and MEP were used to synthesize silica shell nanoparticles coded as SST and SSP. The silica shell nanoparticles were prepared by incorporating 25 mg of OTMS to 10 gm of the microemulsion. The resultant mixture was heated at 100 °C with continuous stirring at 100 RPM for 15 min. Molecules of OTMS being amphiphilic, orient themselves at the oil/water interface of microemulsion. To polymerize the OTMS on the surface, 0.5 ml of 1 N HCl solution was added for each gram of freshly prepared mixture. The addition of HCl resulted in hydrolysis of the OTMS molecules followed by condensation on the surface of the oil globules, thus forming silica shell nanoparticles. The hydrolysis and condensation reactions were performed in a water bath at 60 °C for 6 h [24].

2.3 Characterization of ketotifen-loaded microemulsion and silica shell nanoparticles

2.3.1 Dynamic light scattering

Globule size of microemulsion and particle size of silica shell was measured using photon correlation spectroscopy (Malvern Nano-ZS, UK).

2.3.2 Transmission electron microscopy (TEM)

The morphology of the drug-loaded microemulsion globules and silica shell nanoparticles was examined by TEM (Philips Tecnai 20 G2, FEI) with a 100 kV accelerating voltage capable of point-to-point resolution. The sample was stained with 0.5 % aqueous solution of phosphotungstic acid and directly positioned on the copper electron microscopy grids. Bright-field imaging at increasing magnification were used to reveal the form and size of the microemulsions and silica shell nanoparticles.

2.4 Synthesis of hydrogels

Formulation-laden hydrogel sheets were synthesized by free radical polymerization. Pre-monomer mixture composed of 48 %w/w HEMA, 1 %w/w MAA, 0.5 %w/w EGDMA, and 50.5 %w/w water was admixed with 10 %w/w formulations [26]. All four formulations (MET, MEP, SST and SSP) were used for study (Table 1). The hydrogels were cured by adding the formulation laden pre-

monomer solution (with 0.5 %w/w photo initiator Darocur®) between two moulds (glass plates) coated with Teflon to prevent adherence and were separated by 0.1 mm spacer. The mould was placed in UV transilluminator and the sheets were polymerized by UV photo polymerization at 365 nm for 45 min [14, 27]. Similarly, hydrogels for direct ketotifen loading (direct loading method) were also fabricated, by adding required quantity of ketotifen (0.25, 0.5, 0.75 and 1 %w/w) in pre-monomer mixture.

2.5 Characterization of hydrogels

2.5.1 Transmittance

Transmittance of contact lenses should not be altered by loading of ketotifen formulation in contact lenses. The transparency of hydrogels was measured by light transmittance test using UV–Vis spectrophotometer. A fully hydrated (soaked in STF for 24 h) hydrogel was fixed to the inner side of quartz cuvette. The cuvette was placed in a spectrophotometer and the transmittance was measured at 630 nm. All measurements were recorded in triplicate [14].

2.5.2 Swelling

The previously weighed (initial dry weight) hydrogel sheets were placed in STF (2 ml) at 25 °C for 24 h. After achieving hydration, the swollen hydrogels were removed and patted dry with blotting paper, and reweighed. The % swelling of hydrogels was calculated as follows [28]:

$$\% \text{ Swelling} = \frac{\text{Weight of fully swollen and hydrated hydrogel} - \text{Weight of dry hydrogel}}{\text{Weight of dry hydrogel}} \times 100 \quad (1)$$

Table 1 Formulations of direct loading, microemulsion and silica shell nanoparticle-laden hydrogels

Code	Direct drug laden hydrogels			
	DL-125	DL-250	DL-375	DL-500
Ketotifen in pre monomer mixture (%w/w)	0.25	0.50	0.75	1
Ketotifen loading in hydrogels (µg)	125	250	375	500
Code	Microemulsion laden hydrogels		Silica shell laden hydrogels	
	MET	MEP	SST	SSP
Ketotifen loaded in formulation (%)	2	2	1.24	1.24
Formulation in pre monomer mixture (%w/w)	10	10	10	10
Ketotifen loading in hydrogels (µg)	100	100	65	65

2.5.3 Dynamic surface contact angle

Dynamic surface contact angle was measured to assess the influence of formulation ingredients on the spreadability/wettability of hydrogels. Dynamic contact angle of Ultra-pure water on each hydrogel sheet blotted with soft blotting paper was measured at 25 °C using Theta Optical Goniometer (KSV Instrument, USA) applying the sessile drop method [18].

2.5.4 Na⁺ ion permeability

Extended wear hydrogel contact lenses must have Na⁺ ion permeability greater than 1.5×10^{-6} mm²/min to maintain homeostasis of ion concentration between contact lens and cornea (i.e. post lens tear film) and the lens motion should be free from friction and abrasion. The Na⁺ ion permeability of hydrogels was measured using the modified conductivity meter. This study was performed as per our previous published work [18]. The hydrogel sheet was attached to the bottom of the donor tube (10 mm inner diameter) filled with 0.1 M NaCl solution (5 ml) and placed in a receiving chamber containing 50 ml of ultra-pure de-ionized water. The receiving chamber was placed on the thermostat maintained at 34 °C temperature with continuous stirring (50 RPM). The dynamic conductivity of the receptor solution was monitored by conductivity meter and converted to salt concentration (NaCl) using the standard calibration plot. The apparent Na⁺ ion permeability was calculated based on concentration of Na⁺ ions released in receiving chamber with time, and fitted to diffusion model [29].

2.5.5 Drug release rate

Formulation-laden hydrogel sheets were immersed in 2 ml of STF as a release medium in a glass vial. The in vitro flux studies were carried out at 35 °C at 100 RPM using a mechanical shaker incubator. The volume of STF was not enough to maintain ideal sink conditions, thus at each sampling time interval, the whole release medium was replaced with fresh STF. The amount of ketotifen released was quantified by HPLC using column (*Grace Smart RP-18*, 250 × 4.6 mm, 5 µm, 100 Å, USA) equipped with a UV detector (298 nm) [30].

2.5.6 Mathematical model for diffusion coefficient and release kinetics

The diffusion coefficient of ketotifen from hydrogels was calculated as described earlier by Furqan et al. 2015 [18]. The ketotifen release profiles from the hydrogels below 65 % were fitted to Eq. 2.

$$\frac{M_t}{M_\infty} = 4 \left[\frac{Dt}{\pi L^2} \right]^{1/2} \quad (2)$$

The diffusion coefficient was calculated from the slope obtained from plot of fractional ketotifen release versus ($t^{0.5}/L$). M_t represents the cumulative amount of ketotifen released from hydrogels at time t , M_∞ represents release at infinite time, and L the thickness of the lens in mm in Eq. 2. To evaluate the fitness of the data with Fickian release profile, the empirical power law Eq. 3 was used.

$$\log \frac{M_t}{M_\infty} = k + n \log(t) \quad (3)$$

The kinetic constant (k) shows the structural and geometric characteristics of the formulation-laden hydrogels, and the diffusional exponent (n) reveals the information about the physical mechanism controlling the ketotifen release from hydrogels.

2.5.7 Estimation of therapeutic dose

The dose of ketotifen eye drops (0.025 %w/v solution) for the treatment of allergic conditions is one drop twice a day, i.e. amount of daily dose delivered (considering 2 drops ≈ 100 µL) is 25 µg. The ocular bioavailability through eye drop therapy is only about 5 %, which suggests that the therapeutic requirement is about 1.25 µg/day. Many scientific studies have proved that the bioavailability of drug to target tissue is more than 50 % through contact lenses. Assuming 50 % bioavailability from contact lenses, the release rate requirement for ketotifen would be 2.5 µg/day or 104 ng/h [31].

2.6 Cytotoxicity study

The cytotoxicity study was conducted prior to animal studies, to evaluate whether the leachable extract from the formulation laden hydrogel contact lenses shows any local toxicity or not [32, 33]. The cells were plated on 24-well flat-bottomed plates, with each well at a density of 1×10^4 cells, and incubated for 24 h at 37 °C in the CO₂ incubator. The cells were cultured in a complete medium consisting minimum essential medium with 10 % fetal calf serum and 1 % penicillin–streptomycin incubated at 5 % CO₂/37 °C/relative humidity (RH) > 90 % for 24 h. After 24 h of incubation (~1 doubling period), the medium was replaced with test extract and again incubated for 24 h (5 % CO₂, 37 °C, RH > 90 %). Commercial contact lenses of Bausch and Lomb (Methafilcon) were used in the study for comparison. The cytotoxicity was evaluated by 3-[4, 5-dimethylthiazol-2-yl]-3, 5 diphenyltetrazolium bromide (MTT) assay. The culture medium was aspirated and the cells were washed twice with phosphate buffer solution

after 24 h. The cells were incubated with 100 μ L of the MTT solution (0.5 mg/mL) in Modified Eagle Medium for 2 h at 37 °C. The formazan crystals were dissolved in dimethyl sulfoxide and the absorbance was measured at 540 nm. The results of the experiments were evaluated to determine cell viability (%).

$$\text{Cell viability (\%)} = T/C \times 100 \quad (4)$$

where T is the test treatment over control C values. If viability is reduced to <70 % of the control, it has a cytotoxic potential.

2.7 Animal study

The experiment was performed using healthy Swiss albino mice and white New Zealand rabbit of either sex weighing between 30–40 gm and 1.7–3.2 kg respectively, obtained from the central animal house, Maliba Pharmacy College, Bardoli. The animals were housed under standard laboratory conditions of temperature (25 ± 2 °C), maintained on a 12 h light/dark cycle and had free access to food and water. The animals were acclimatized to the laboratory environment 1 h before the experiments. The protocol of study was approved by the Institutional Animal Ethics Committee (IAEC) (Protocol No: MPC/IAEC/05/2014) and experiments were conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animals).

2.7.1 Systemic toxicity study

The SST ketotifen loaded silica shell nanoparticle-laden hydrogel batch was selected as it showed the most promising results. This SST batch was extracted to determine whether the leachable extract would cause any acute systemic toxicity following injection into mice or not. Based on the ratio of 4 gm hydrogel material in 20 ml of the vehicle, the hydrogel was extracted in 0.9 % sodium chloride solution (SC) and sesame oil (SO) [as extraction vehicles] at 37 °C for 72 h. Sodium chloride (0.9 %) solution and sesame oil were used to extract water-soluble and oil-soluble components from hydrogels respectively. The vials were agitated occasionally during extraction. The blank extraction vehicles (SC and SO) were used as a control. The mice were identified and weighed individually before dosing. Six mice (per extract) were injected with the test sample extract at a dose of 50 ml/kg. Six mice were similarly injected with the corresponding control. The SC was injected by the intravenous (IV) route, while the SO was injected by the intraperitoneal route. The mice were returned to their cages and observed for any adverse reactions immediately after dosing, and at the regular

interval of 4, 24, 48, and 72 h. The animals were also weighed after 3 days.

2.7.2 Ocular Irritation study

The ketotifen loaded silica shell nanoparticle-laden hydrogel SST batch was extracted to determine whether the leachable extract from the hydrogel produced any irritation to the ocular tissue of the rabbit or not. Based on the ratio of 120 cm² (hydrogel): 20 ml of the vehicle, the selected hydrogel was extracted in 0.9 % SC and SO at 37 °C for 72 h. The vials were agitated occasionally during extraction. The extraction vehicles without hydrogels served as control blanks. The rabbits were identified and weighed prior to treatment (before 24 h). The eyes of each rabbit were examined clinically with an auxiliary light source. A 0.2 ml dose of appropriate extract was instilled in the lower conjunctival sac of the right eye of each rabbit (six rabbits per extract). The lids were gently held closed for a second following instillation. The corresponding control blanks (vehicles) were similarly instilled into the left eye. Rabbits were returned to the cages after treatment and examined at 1, 24, 48, and 72 h after instillation for any ocular reactions using an auxiliary light source. Reactions were recorded and scored as per the USP and ISO system for grading ocular lesions.

2.7.3 In vivo pharmacokinetic study

The in vitro drug release profiles of novel drug delivery system like lenses are poorly correlated with in vivo drug release performance. The authors investigated the ability of the ketotifen loaded silica shell contact lenses (Batch SST, 65 μ g loading) to elute drug safely and effectively in New Zealand white rabbit's eyes. Sterilized contact lenses were carefully placed on cornea under the nictating membrane without anesthetization (n = 4 rabbits, left eye control, right eye contact lens). At regular time intervals, 5 μ L of tear fluid was collected via capillary action from the lower eyelid and conjunctival sac using 5 μ L disposable glass capillaries. During sampling, rabbits were transferred to the rabbit restrainer, and care was taken not to cause lacrimation during sampling. The capillaries were flushed several times (total 85 μ L volume) with distilled water. The collected fluid was treated with 10 μ L perchloric acid (0.5 M) to precipitate protein, and 500 μ L of methanol was added to it. After vortex oscillation and centrifugation (freeze centrifuge, 10,000 RPM for 15 min) the supernatant was collected and analyzed for ketotifen content. Ketotifen in tear samples was quantified by LC–MS (TSQ Quantum Access, Thermo Scientific, USA) [18].

3 Results

3.1 Characterization of ketotifen-loaded microemulsion and synthesized silica shell nanoparticles

The mean globule size was found to be 74.10 and 84.24 nm for microemulsion MET and MEP batches respectively, which is typical for any ideal microemulsion. Particle size was 126.54 and 104.2 nm for silica shell batches SST and SSP respectively. The TEM images of microemulsion batches showed spherical oil globules with diameter below 100 nm, which is in agreement with the globule size results obtained using Zetasizer. A clear image of silica shell with a thickness of 12 nm was observed in the TEM report (Fig. 1), of SSP batch, while in case of SST batch it could not be measured due to blurred image.

3.2 Characterization of hydrogels

3.2.1 Transmittance

Direct drug-laden hydrogels showed significant decrease ($P < 0.05$) in % transmittance with increase in ketotifen loading (Table 2). On the other hand transmittance was good ($>90\%$) in case of microemulsion and silica shell nanoparticle-laden hydrogels, due to nano size of globules/particles.

3.2.2 Swelling

The effect of formulation-inclusion on the swelling behaviour of hydrogels is shown in Table 2. In direct ketotifen loaded batches, the % swelling was significantly reduced with increase in ketotifen loading in hydrogels. While in case of ketotifen loaded microemulsion hydrogels, the % swelling was increased. Statistical results showed a significant increase ($P < 0.05$) in swelling behavior in microemulsion laden hydrogels when compared with hydrogels without formulations (blank). In silica shell nanoparticle-laden hydrogels, the % swelling decreased with no statistical significance ($P > 0.05$) when compared with blank hydrogels.

3.2.3 Dynamic surface contact angle measurement

Dynamic surface contact angle increased with direct ketotifen laden hydrogel batches with increase in ketotifen loading as compared to hydrogels without formulation (blank) (Fig. 2). The contact angle for microemulsion laden hydrogels (MET and MEP) was lowered by 7° – 10° in comparison to blank hydrogels, which was expected, due to the presence of free surfactant molecules in hydrogel matrix. However, with silica shell laden hydrogels (SST and SSP) significant change was not observed ($P > 0.05$) in dynamic contact angle in comparison to blank hydrogels.

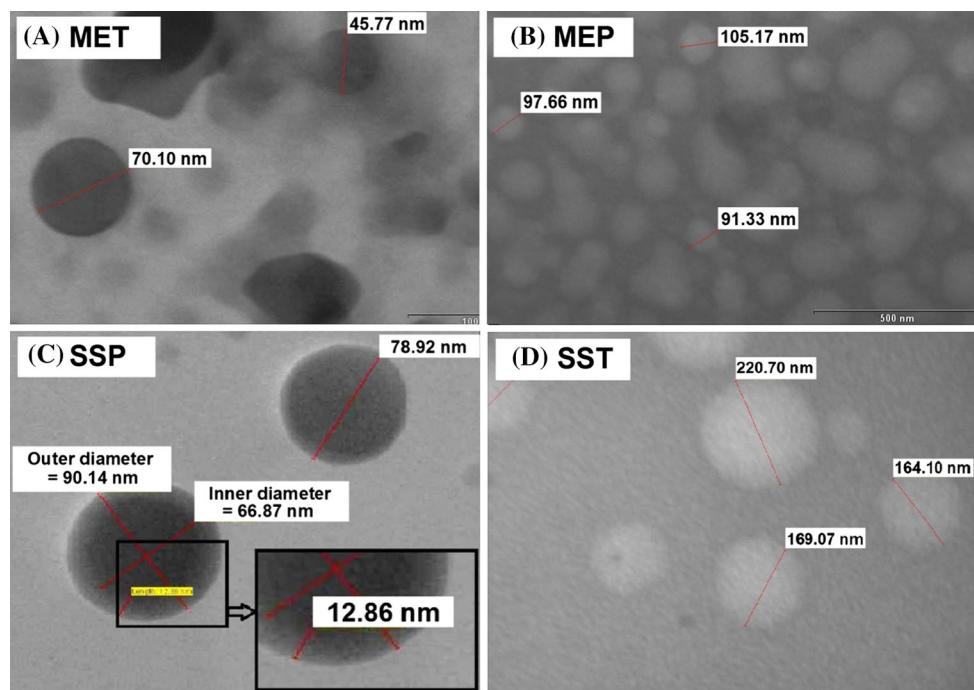
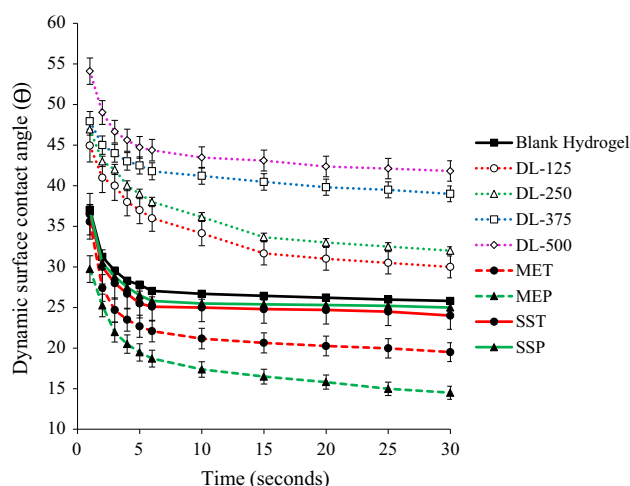


Fig. 1 TEM image of microemulsion and silica shell nanoparticles **a** MET, **b** MEP, **c** SSP, and **d** SST

Table 2 Physical properties and cell viability of hydrogel sheets

Hydrogel type	Swelling (%) (n = 3)	Transmittance (%) (n = 3)	Na ⁺ ion permeability [P _{ion} × 10 ⁻⁶ (mm ² /min)] (n = 3)	% Cell viability (mean ± SD, n = 4)
Blank sheet	78.50 ± 0.09	98.30 ± 0.05	6.38 ± 0.19	95.60 ± 3.42
DL-125	71.71 ± 0.40	93.10 ± 0.10	2.25 ± 0.25	NA
DL-250	67.29 ± 0.89	91.10 ± 0.05	1.84 ± 0.37	NA
DL-375	66.67 ± 2.77	89.90 ± 0.10	1.30 ± 0.26	NA
DL-500	61.20 ± 0.47	87.10 ± 0.15	1.06 ± 0.15	NA
MET	88.30 ± 0.84	96.90 ± 0.05	6.16 ± 0.11	85.60 ± 7.72
MEP	85.72 ± 0.78	94.80 ± 0.05	6.86 ± 0.45	87.60 ± 8.32
SST	80.82 ± 0.84	90.40 ± 0.10	6.06 ± 0.37	82.08 ± 5.37
SSP	79.39 ± 0.78	91.30 ± 0.01	6.56 ± 0.25	83.60 ± 6.42

**Fig. 2** Dynamic surface contact angle of Milli Q-water on blank and formulation laden dry hydrogels (n = 3)

3.2.4 Na⁺ ion permeability

The values of apparent Na⁺ ion permeability are shown in Table 2. The ion permeability in direct ketotifen-laden hydrogels reduced significantly ($P < 0.01$) as the amount of ketotifen in hydrogels was increased. Apparent ion permeability in microemulsion and silica shell laden hydrogels did not show significant differences ($P > 0.05$) when compared with the blank hydrogels.

3.2.5 Drug release rate

3.2.5.1 Release rate of drug from direct ketotifen laden hydrogels Four hydrogels coded DL-125, DL-250, DL-375 and DL-500 were analysed, containing 125, 250, 375, and 500 µg of drug respectively in each 50 µL volume of hydrogel sheet. The cumulative drug release (µg) and release rate (ng/h) profiles are shown in Fig. 3. All four batches showed an initial burst release (67–72 %), followed

by sustained delivery of drug. A significant improvement/enhancement ($P < 0.01$) in release rate duration was observed with increase in drug loading. Release rate of ketotifen within the therapeutic level (105 ng/h) for DL-125, DL-250, DL-375 and DL-500 batches was found to be 3, 5, 7 and 8 days respectively. It was not possible to calculate diffusion coefficients, release exponents n and k values due to initial high release, as Eqs. 2 and 3 are valid for only initial 65 % drug release profile.

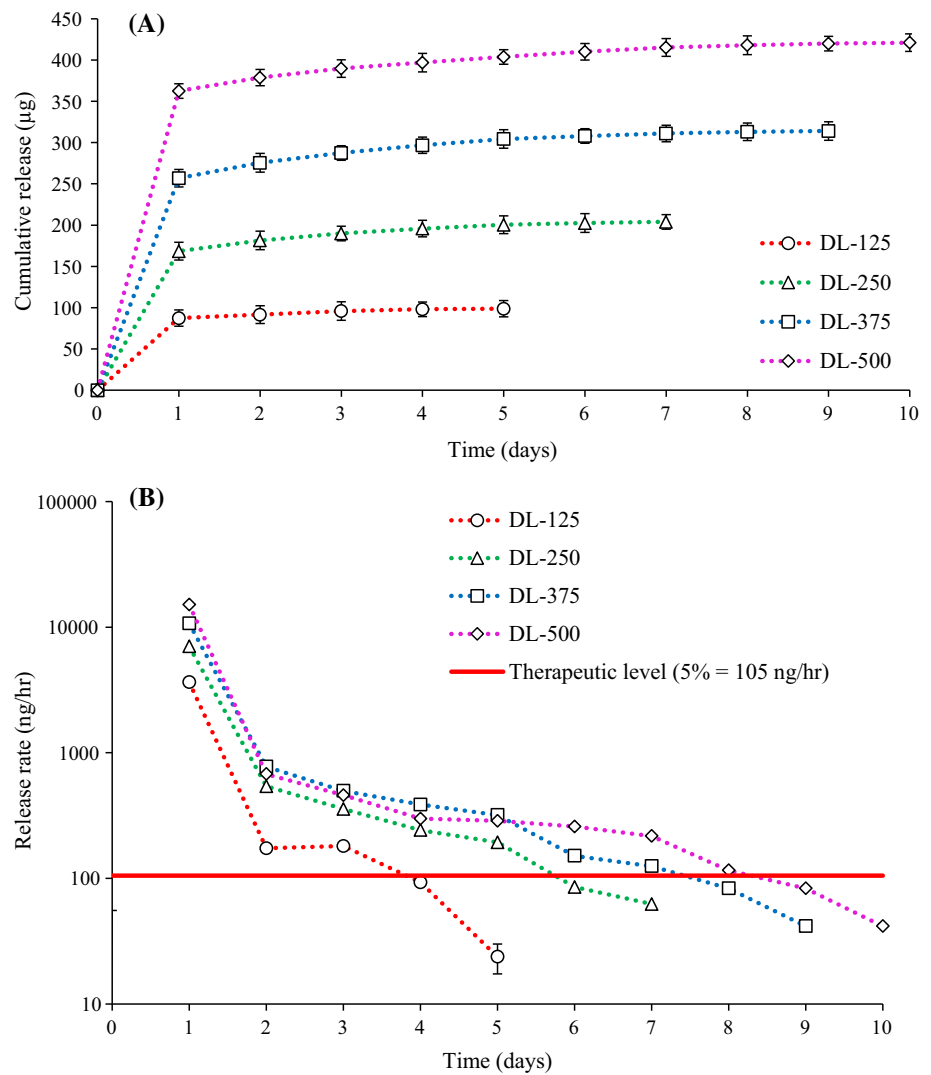
3.2.5.2 Release rate of drug from microemulsion and silica shell laden hydrogels Cumulative and release rate (ng/h) profiles of microemulsion and silica shell laden hydrogels are shown in Fig. 4. Microemulsion and silica shell laden hydrogels showed extended release with low ketotifen loading of 100 and 65 µg respectively, in comparison to the direct loading method. The MET hydrogel showed more extended release in comparison to MEP hydrogel. Results were also supported by the low values of the diffusion coefficient, i.e. 1.91×10^{-7} and 2.20×10^{-7} mm²/s for MET and MEP hydrogels respectively (Table 3).

Silica shell nanoparticle-laden hydrogels (SST and SSP) also showed extended drug release up to 9 days, with 65 µg loading (low drug) in comparison to 100 µg loaded microemulsion-laden hydrogels (Fig. 4). Hydrogel SST batch showed zero order release kinetics (Diffusional exponent $n = 0.93$). The SST hydrogel showed more extended drug release in comparison to SSP. The SST and SSP hydrogels showed diffusion coefficient values of 6.00×10^{-7} and 8.89×10^{-7} mm²/s respectively (Table 3). Both microemulsion and silica shell laden batches showed Fickian controlled diffusion mechanism for drug release from hydrogels.

3.3 Cytotoxicity test

Cell viability of microemulsion and silica shell nanoparticle-laden hydrogel extracts were significantly lower

Fig. 3 a Cumulative release of ketotifen from hydrogels prepared by direct loading method. **b** Release rate (ng/h) of ketotifen per day from hydrogels prepared by direct loading method. Values are shown as mean $\pm$ standard deviation ($n = 6$). Therapeutic release rate required for contact lenses was calculated according to 5 % absorption from eye drops



($P < 0.05$) in comparison to extract of commercial contact lens and blank hydrogels (Table 2).

3.4 Animal study

3.4.1 Systemic toxicity study

Silica shell nanoparticles (SST batch) showed the most promising results, so it was selected for further in vivo safety study. During the observation period of 3 days, no abnormal behavior such as convulsions or prostration, or no unscheduled death was observed. The test group injected with sesame oil extract as well as its control group appeared un-groomed or untidy at the 4 h of dosing. All other animals appeared normal during the study. A minor increase in body weight was recorded in the test group after 3 days, which was statistically not significant ($P > 0.05$) in comparison to control group.

3.4.2 Ocular irritation study

The conjunctival sac of the rabbit's right eye was treated with test extract and left eye received physiological saline or sesame oil as the control. An evaluation was performed by gross macroscopic examination using auxiliary light source. No symptoms of ocular irritation such as opacity of the cornea, inflammation or swelling of the iris, conjunctivae redness, chemosis, and discharge were observed after instillation of test extract at various time intervals. Redness and chemosis of conjunctivae of grade 1 was observed with the sesame oil test as well as control group, which reduced with time.

3.4.3 In vivo pharmacokinetic study

The tear fluid concentration profile for ketotifen loaded silica shell laden contact lenses (Batch SST, 65 μg loading)

Fig. 4 a Cumulative release of ketotifen from microemulsion and silica shell nanoparticles laden hydrogels. The total amount of ketotifen loaded in each hydrogel was 100, 100, 65 and 65 μg for MET, MEP, SST and SSP batches respectively. **b** Release rate (ng/h) of ketotifen per day from micro emulsion and silica shell nanoparticles laden hydrogels. Values are shown as mean $\pm$ standard deviation ($n = 6$). Therapeutic release rate required for contact lenses was calculated according to 5 % absorption from eye drops

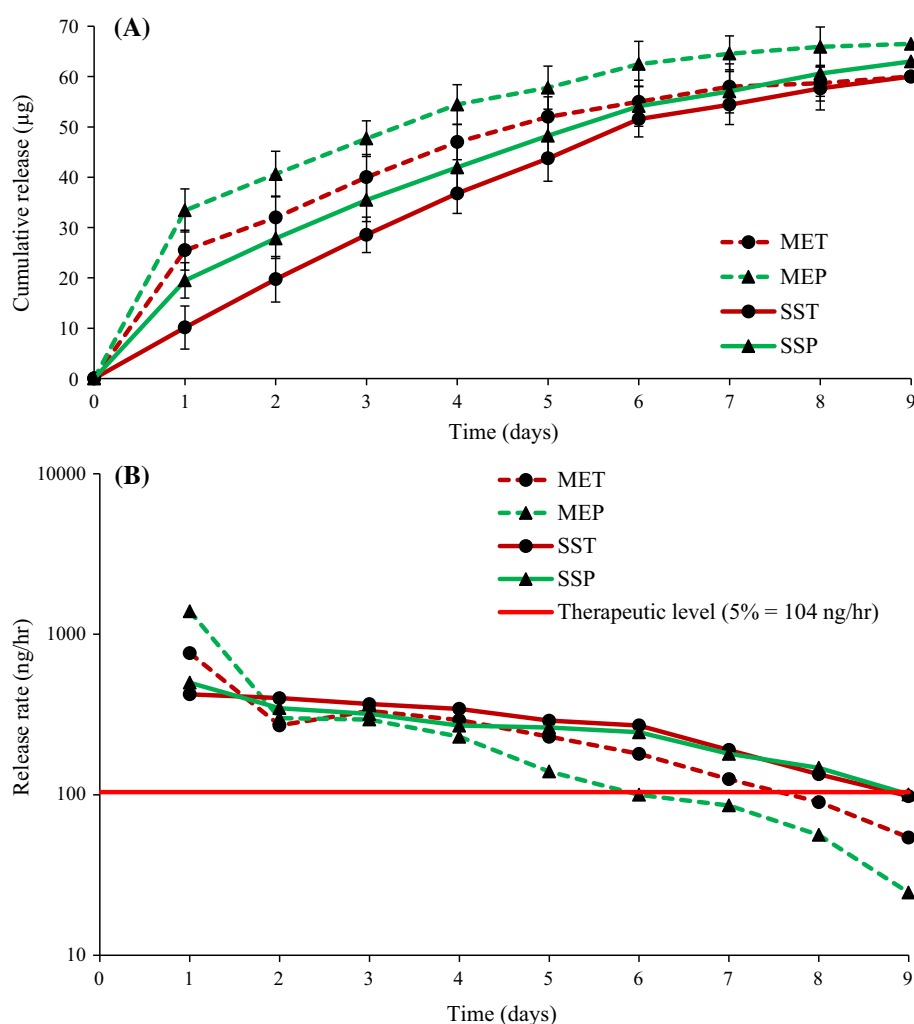


Table 3 Drug release kinetics of microemulsion and silica shell laden hydrogels

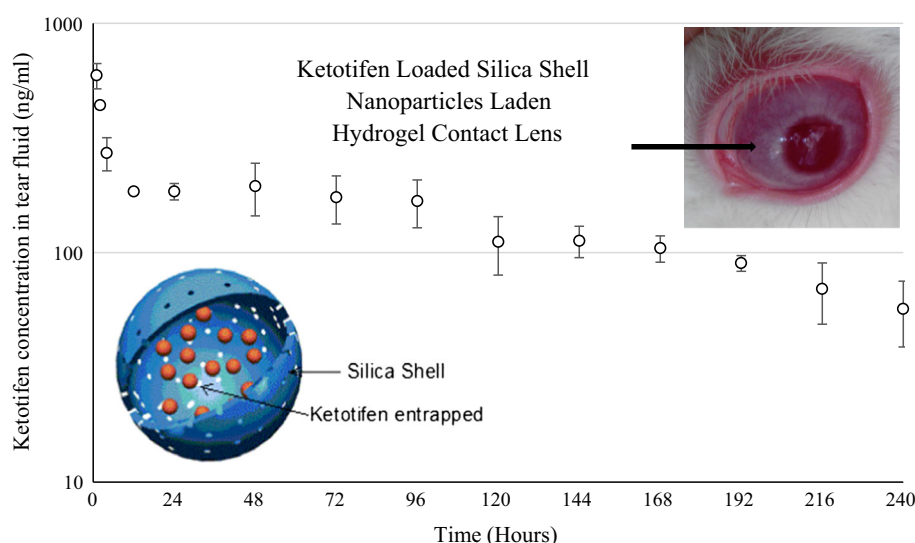
Hydrogels	Average drug loading (μg)	Release within therapeutic level (105 ng/h) (Days)	Diffusion coefficient ($D = \text{mm}^2/\text{s}$)	Regression coefficient (R^2)	Diffusional exponent (n)	Intercept k
MET	100	7	1.91×10^{-7}	0.97	0.40	1.06
MEP	100	6	2.20×10^{-7}	0.99	0.32	1.25
SST	65	9	6.00×10^{-7}	0.99	0.93	0.05
SSP	65	9	8.89×10^{-7}	0.99	0.55	0.72

is shown in Fig. 5. The contact lenses showed an initial burst release ($C_{\text{max}} = 592.6 \pm 74.46 \mu\text{g}/\text{ml}$), followed by a steady ketotifen release in tear fluid, clearly demonstrating an extended release of ketotifen for 10 days. The high values of $\text{AUC} = 33,092 \pm 1346 \text{ ng}\cdot\text{h}/\text{ml}$ and $\text{MRT} = 14.6 \pm 2.47 \text{ h}$ suggest the potential of ketotifen loaded silica shell laden contact lenses to extend the drug release duration.

4 Discussion

Zetasizer and TEM reports confirmed that, silica shell synthesis at the interface results in increase in oil globule size from its original microemulsion. The TEM report showed that, the MET and MEP batches had an increase in average diameter by 52.44 and 19.96 nm, after conversion to respective silica shell nanoparticles SST and SSP. This

Fig. 5 Ketotifen tear fluid concentration–time profile after application of ketotifen loaded silica shell laden contact lenses (SST). Each point represents the mean $\pm$ SD ($n = 4$)



value could be predicated as the thickness of silica shell synthesized at interface. The increase in diameter of oil globule (SSP) by an average of 8 nm could be due to destabilization of microemulsion during silica shell synthesis. The presence of silica shell instead of flexible liquid surfactant interface provides zero order release kinetics (controlled release). The pore size and thickness of silica shell may be optimized to achieve a desired controlled release rate according to therapeutic requirements for different drug molecules.

The transmittance of contact lenses should be greater than 90 % for clear vision. In case of direct ketotifen laden hydrogels, the transmittance decreases with increase in ketotifen loading, this could be due to brownish red colour of ketotifen base and its solubility in pre monomer mixture. No significant reduction in transmittance was observed in drug-loaded microemulsion and silica shell nanoparticle-laden hydrogels, due to low drug loading and encapsulation of drug in the oil phase. Pre-monomer mixture with these formulations remained clear, indicating the stability of microemulsion and silica shell nanoparticles in pre-monomer solution. Batches DL-375 and DL-500 failed to be used for contact lens application, since their % transmittance values were less than 90 %.

The formulation laden hydrogels were found to be hydrophilic and rapidly swelled when immersed in STF. They can be considered to belong to FDA Group 2; i.e., hydrophilic with high water content [34]. In the direct ketotifen loaded batches, the % swelling reduced with increase in ketotifen loading in hydrogels, possibly due to the hydrophobic nature of ketotifen. In case of ketotifen loaded microemulsion hydrogels, the % swelling increased, which could be due to the presence of free hydrophilic surfactant molecules in hydrogel sheets. In both the cases,

it was not acceptable, as changes in swelling behavior alter the dimension and other critical properties of biomaterial. The % swelling was predicted to fall in silica shell nanoparticle-laden hydrogels, due to reduction in water intake by hydrophobic silica nanoparticles. However, statistical results showed non-significant reduction ($P > 0.05$) in % swelling, which could be due to the presence of free surfactant molecules in hydrogels. The outcomes clearly indicate that, the presence of silica shell does not alter the swelling behavior of hydrogels.

The wettability of direct ketotifen laden hydrogels was significantly lower (increase in contact angle) due to the presence of ketotifen (hydrophobic nature). For microemulsion laden hydrogels, the contact angle decreased which was expected due to the presence of free surfactant molecules in hydrogel matrix. However, with silica shell laden hydrogels no significant change in dynamic contact angle was observed in comparison to blank hydrogels. Thus the wetting behavior was not affected due to the presence of hydrophobic silica particles, supporting the results of swelling study. The reason could be the presence of free surfactant molecules which were detached from the interface of microemulsion during silica shell synthesis. Thus silica shell laden hydrogels were the preferred formulations, as they did not cause any change in contact angle, thus avoiding any changes in swelling behavior and in dimensions of the contact lenses.

The ion permeability for direct ketotifen-laden hydrogels decreased as the amount of ketotifen in hydrogels was increased, which might be due to the hydrophobic nature of ketotifen. The ion permeability for batch DL-375 and DL-500 was found to be less than $1.5 \times 10^{-6} \text{ mm}^2/\text{min}$, which is not suitable for contact lens application. The ion permeability for microemulsion and silica shell laden

hydrogels did not show significant differences as compared to blank hydrogels, suggesting that the presence of microemulsion and silica shell nanoparticles in hydrogels do not interfere with Na^+ ion permeability. Therefore the contact lenses fabricated using these biomaterials/hydrogels would not cause abrasion and damage to the cornea/eye upon insertion.

In vitro drug release profile of direct ketotifen loaded batches (direct entrapment) showed an initial burst release, which might be due to the rapid diffusion of the drug from the aqueous matrix of hydrogel and low molecular weight of ketotifen. The cumulative drug release data indicate that the amount of ketotifen released was dependent on drug loaded in hydrogels. A significant improvement ($P < 0.01$) in release rate duration was observed with increase in drug loading. However, batch DL-375 and DL-500 cannot be used for contact lens application, due to insufficient swelling, transmittance and ion permeability characteristics. Thus, therapeutic release up to 5 days is possible with direct ketotifen loaded hydrogels, with constraint of high burst release and high drug loading.

Microemulsion laden hydrogels showed extended in vitro drug release, due to the presence of a tightly packed surfactant at the interface (oil–water), which offers a physical barrier to drug transport from microemulsion-hydrogels. Drug release profiles of microemulsion laden hydrogels (MET and MEP) suggest that the drug release was affected by the type of co-surfactant used for study. The MET hydrogel showed more extended drug release in comparison to MEP hydrogel, suggesting the higher stability of microemulsion prepared by Tween 80 in comparison to PEG-400. The higher stability was due to the long chain (C_{18}) of Tween 80 gripped in the oil phase, resulting in extended release up to 7 days in comparison to 6 days for MEP with same drug loading of 100 μg (Fig. 4).

Ketotifen-loaded silica shell hydrogels did not show burst release, due to the resistance offered by the solid porous silica shell deposited or coated on oil globules. Silica shell hydrogels showed extended release up to 9 days, clearly signifying the potential for controlled drug delivery. The SST hydrogel showed extended release in comparison to SSP, which might be due to greater predicted thickness of silica shell. The lower values of intercept k for SST and SSP hydrogels in comparison to microemulsion hydrogels clearly indicated a higher affinity of the drug to silica nanoparticles. Thus, using the novel approach of silica shell nanoparticles, zero order release was achieved, as the drug molecules that were entrapped inside the nanoparticles encountered additional resistance offered by the solid porous silica. Likewise by changing the thickness and porosity of the silica shell layer, the desired release rate of drugs can be tailored according to different therapeutic regimens.

Cell viability of microemulsion and silica shell nanoparticle-laden hydrogel extracts were significantly lower, which might be due to the presence of surfactant molecules. However, the extracts from formulation laden hydrogels passed the cytotoxicity test as the cell viability was greater than 70 %. In acute systemic toxicity study (in mice), no abnormal behavior was observed during the study period. The test group mice injected with sesame oil extract as well as its control group appeared un-groomed or untidy at the 4 h of dosing, which was considered an obvious effect due to the unctuous nature of sesame oil. In ocular irritation study, redness and chemosis of grade 1 was observed in sesame oil test as well as control groups which again could be due to the non-polar nature of sesame oil. The symptoms of ocular irritation were not observed after instillation of test extract of saline at various time intervals as compared to the control group. The in vivo results confirmed the clinical potential of the drug loaded silica shell nanoparticle-laden hydrogels.

The contact lenses showed good compatibility, with no irritation and protein deposition during the study period. They demonstrated extended release of ketotifen for 10 days (Fig. 5). The controlled and extended release of ketotifen from contact lenses maintained a stable drug concentration in the tear fluid, despite the tear drainage and tear-film turnover rate. The reported eye drop therapy results showed an exponential decrease in drug concentration in tear fluid, and disappear after 3 h of treatment [35]. The high values of AUC and MRT suggest the potential of ketotifen loaded silica shell laden contact lenses to extend the drug release duration.

5 Conclusion

The present work focused on developing therapeutic soft contact lenses using drug loaded microemulsion and silica shell nanoparticles for extended ophthalmic drug delivery without altering critical lens properties. The extended release of ketotifen from hydrogels was in following order: Direct loading < Microemulsion < Silica shell nanoparticles. The silica shell batch (SST) showed the highest potential for extended drug delivery, as it showed zero order release kinetics up to 9 days, even with low drug loading of 65 μg . Direct ketotifen loading (DL-375 and DL-500) method was not effective as it altered the optical and swelling properties, and the ion permeability of hydrogel contact lenses, while microemulsion and silica shell nanoparticle-laden hydrogels did not show significant effect on these properties. The cytotoxicity and animal studies demonstrated the safety of silica shell nanoparticle laden hydrogels. The silica shell laden contact lenses (SST) showed an extended release of ketotifen in the pre corneal area of rabbit eyes,

which led to a smoother drug concentration and a longer retention of drug in rabbit eyes. This work demonstrated that the drug loaded silica shell nanoparticle-laden soft contact lenses, could offer an alternative approach to the current eye drop therapies and may serve as an ocular drug delivery platform capable of alleviating many ocular diseases.

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Compliance with ethical standards

Conflict of interest There are no potential conflicts of interest to disclose this work.

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