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RESEARCH

Preclinical Safety Testing of Novel Silica Eye Drop as an Ophthalmic Carrier Matrix

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Abstract

Purpose Topical eye drops are common for treating eye diseases, often requiring multiple daily doses. Silica Matrix technology enables drug delivery systems that release drugs over time. This study aimed to develop and characterize a silica-based eye drop, as well as a 14-day tolerance and toxicity assessment to evaluate its potential as a drug carrier in ophthalmology.

Methods Silica Eye Drop is a silica-based composite containing silica microparticles, made via sol-gel chemistry and spray drying, along with silica hydrogel. The product was terminally sterilised by gamma irradiation and characterised by particle size, silica content, in vitro dissolution time, droplet size, applicability, endotoxin content, sterility, and short-term stability. Four New Zealand rabbits received one drop daily in one eye's conjunctival sac for 14 days, while the other eye served as a control. During the study, eye conditions, blood chemistry, and postmortem histology were monitored.

Results The final formulation contained 23% (w/w) silica microparticles (average diameter 3.21 μm) and 77% (w/w) silica hydrogel, with 18.2 wt% silica, dissolving in 24 h. The average droplet volume was 31.3 ± 4.4 μl, easily dispensed manually from a single-dose unit with acceptable size variability. A 2-month stability study showed no major changes in product properties. Finally, in the rabbit toxicity study, both the treated and untreated eyes showed similar tolerability levels.

Conclusions The Silica Eye Drop was successfully developed and manufactured. Easy to use with consistent quality, it demonstrated the safety and potential of silica platform technology for a carrier material in topical ophthalmic products.

~ 100 Word Summary In this study, a novel silica-based eye drop formulation was described and evaluated. Silica microparticles were synthesised and incorporated through a Fill and Finish process, followed by sterilization and characterization for particle size, silica content, dissolution, droplet size, microbiological purity, and short-term stability. In vivo tolerability was assessed in four New Zealand rabbits over 14 days with daily administration. The optimised formulation contained 23% (w/w) silica microparticles and 77% (w/w) silica hydrogel (18.2 wt% silica). Microparticles (D50 = 3.21 μm) fully dissolved within 24 h. No adverse ocular effects occurred, confirming excellent safety, stability, and therapeutic potential.

25–30 Word Teaser A novel Silica Eye Drop formulation was developed, showing full dissolution in 24 h and good tolerability in rabbits, highlighting its promise for future ocular therapies.

Keywords Silica hydrogel · GLP study · Eye drops · Silica matrix · Tolerability · Ophthalmology

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Abbreviations

API	Active Pharmaceutical Ingredient
DMSO	Dimethyl Sulfoxide
EMA	European Medicines Agency
GLP	Good Laboratory Practice
HCl	Hydrochloric Acid
HS GCFID	Headspace Gas Chromatography Flame Ionization Detector
ICH	International Council for Harmonisation
IOP	Intraocular Pressure
IPA	Isopropyl Alcohol
NZW	New Zealand White (rabbit)
NaOH	Sodium Hydroxide
OECD	Organisation for Economic Co operation and Development
Ph Eur	European Pharmacopoeia
PSD	Particle Size Distribution
RH	Relative Humidity
Rpm	Revolutions per Minute
SAL	Sterility Assurance Level
SDR	Spray Dried Residue
SDU	Single Dose Unit
Si	Silicon
SiA	Silicic Acid
SiO_2	Silicon Dioxide
TEOS	Tetraethyl Orthosilicate
TRIS	Tris(hydroxymethyl)aminomethane
VDmax	Maximum Validated Dose
w/w	Weight per Weight
wt%	Weight%

Introduction

Eye drops are non-invasive medications used to treat eye diseases. In ophthalmology, topical medications usually need to be administered multiple times daily, often several times to keep a consistent level in the eye tissues [1]. For instance, antibiotics for keratitis, steroids for ocular inflammations, or moisturizing drops for dry eye syndrome may be applied up to 6 times daily. This requirement results from the limited residence time of the drug on the ocular surface following application, which can challenge patient adherence to treatment regimens [2]. The conjunctival sac naturally holds a limited volume of about 30 µl (non-blinking state) [3]. This small capacity, coupled with the eye's protective mechanisms, decreases ocular bioavailability and increases the need for frequent dosing. Understanding this dynamic is crucial for optimizing treatment outcomes and ensuring that medications are effective. Maintaining drug presence at the site of action for an extended period is necessary, as this can contribute to reduced application frequency, lowered dosage, increased absorption, improved drug efficacy, and patient compliance. To enhance ocular bioavailability, innovative drug delivery systems, including inserts, contact lenses, mucoadhesives, collagen shields, and penetration enhancers, have been developed [4, 5]. Additionally, vesicular systems like liposomes, niosomes, and nanoparticles, along with in situ forming gels, significantly improve ocular therapy outcomes [6–9].

Silica (silicon dioxide, SiO_2) gel matrices have been investigated as carrier materials for controlled and sustained delivery of active substances [10–14]. The adjustable biodegradation rate of silica and its nanosized pore structure, combined with mild and aqueous processing conditions of silica gel, make it an ideal material for controlled drug delivery. Its potential applications in ophthalmology open new possibilities for enhancing patient care. Silica matrix is a synthetic inorganic biodegradable polymer made from amorphous silicon dioxide, produced through a chemical process known as the sol-gel process. Silica microparticles are produced by spray drying of the silica sol and then mixed with another dilute aqueous silica sol (i.e. silica hydrogel) to produce a viscous silica composite structure. An active substance can be encapsulated in the silica microparticles upon spray drying. In contact with body fluids, the silica composite material dissolves over time, and the embedded active substance is released concomitantly under the control of silica dissolution. The dissolution of the Silica Matrix takes place mainly through a surface erosion mechanism [15]. Eroded silica is freely dispersed in tissue and excreted via urine in the form of a very weak inorganic acid, silicic acid. The biodegradation process does not change the pH within the silica gel nor in the surrounding tissue. The manufacturing process can be adjusted to vary the number of hydroxyl groups and the specific surface area of silica matrix, thereby influencing the rate of silica biodegradation and the release rate of the active substance [16–18].

Previously, silicon-based topical ophthalmic formulations have been developed for ocular drug delivery. In principle, these formulations consisted either of mesoporous silica nanoparticles or silicon hybrid nanoparticles with various functional chemical groups or decorations [19, 20]. In both types of formulations, there are the major differences to the Silica Matrix technology described in the current study such as silica particle size (nanoparticles vs. microparticles), very different manufacturing chemistries and form giving processes for the particles, use of organic solvents (multiple organic solvents vs. none), industrial scalability of the manufacturing processes, and, finally, the actual formulation (nanoparticles in a solution vs. 3-dimensional silica-silica composite structure made of silica microparticles and highly aqueous silica hydrogel). Nevertheless, silicon-based drug delivery systems have demonstrated that various active

substances for treating ocular diseases can be incorporated into the topical carrier material, such as agents that lower eye pressure and inhibit neovascularization [20–22].

The aim of this study was to develop a Silica Eye Drop formulation and characterise it as a promising drug carrier platform for the treatment of eye diseases. A 14-day topical ocular tolerance and toxicity study was run with Silica Eye Drop in rabbits. Silica composite material is a new excipient for eye drops, and its ocular tolerance and safety have not been previously demonstrated.

Materials and methods

Reagents

Reagent-grade (98%) tetraethyl orthosilicate (TEOS) and L (+)-tartaric acid were purchased from Aldrich Sigma. 0.1 M HCl and 0.1 M NaOH solutions were obtained from Merck. Silica standard solution (1000 µg/ml Si in H_2O), isopropanol (IPA), and sodium peroxide pellets were sourced from VWR Chemicals. Nitric acid (67–70%) and Dimethyl sulfoxide (DMSO), bioreagent grade, were supplied by Fisher Chemicals. 4-amino-3-hydroxy-1-naphthalene sulfonic acid was from Alfa Aesar. Sodium bisulfite was from Acros Organics. Ethanol (≥99.5%, ETAX Aa) was from Anora Group Oyj. Deionised water (18.2 MΩ.cm) was prepared in-house using a Millipore Milli-Q system. All other chemicals and reagents used were of analytical grade and were used as received without further purification.

Materials

60-mL straight containers with screwed caps used in dissolution testing were purchased from Corning. The primary packaging materials for the eye drop were single-dose units (SDU) (Code/Ref: AA03898ABAAQ00C) produced by Lameplast Spa, Italy, while the secondary packaging was a LamiZip stand-up pouch (PET/LDPE/aluminum) purchased from DaklaPack.

Manufacture and Characterisation of Silica Eye Drop

Silica Eye Drop is a silica composite material that consists of silica microparticles embedded in silica hydrogel. Silica microparticles and hydrogel are used to control the dissolution rate and rheological properties, such as viscosity, of the composite. The silica microparticles can also act as a reservoir for an active pharmaceutical ingredient (API). However, in the current study, no active substance was incorporated into silica microparticles. The manufacturing of Silica Eye Drop is a three-step process, which includes the manufacturing of the silica microparticles, compounding silica microparticles with dilute silica sol, and packaging into SDUs. The product was terminally sterilised by gamma irradiation. A flow scheme of the manufacturing process is shown in Fig. 1.

The process for manufacturing silica microparticles began with the hydrolysis of the silica precursor, TEOS, in water. The reaction was catalysed by 0.1 M HCl at pH 2, where TEOS was hydrolysed completely. The hydrolysed species form a nano-scale silica cluster-water suspension, known as silica sol. The silica sol was cooled down to 5–10 °C and diluted with ethanol. The pH of the sol was adjusted with 0.1 M NaOH solution to 5.8–6.2 before spray drying. The sol was pumped into a spray dryer (Mobile Minor, PSD 1 from GEA, Denmark) via a tube reactor and spray dried to form silica microparticles. The following spray drying parameters were used: Inlet temperature 180 °C, process flow rate for nitrogen gas 80 kg/h (3 bar), atomization flow rate for nitrogen gas 12 kg/h (1.5 bar), and pump feed rate 35 g/min. Silica microparticles were collected in a collection vessel of the pilot-scale spray dryer. Silica microparticles were manufactured in a class C clean room (EU-GMP certified facility). After the manufacture, silica microparticles were packed in a polyethylene bag and an aluminum laminated bag as a secondary package. They were stored at 5 ± 3 °C prior to Fill and Finish manufacture.

The Fill and Finish process was performed in an ISO 8 classified clean room. In this process, a diluted silica sol was prepared and mixed with silica microparticles to produce a suspension. The suspension was allowed to become a gel by stabilising at ambient temperature for a day. The eye drop is a continuous silica-silica composite gel material whereby the hydrogel plays an important role in preserving the rheological property and/or preventing sedimentation or phase separation of the silica microparticles. The pH of the sol was adjusted with 0.1 M NaOH solution to 6.0–6.5 prior to mixing with the silica microparticles. The suspension was transferred into pre-sealed SDUs by pipetting. The target filling volume was 0.8 ml per SDU, which represents a large overfill, as only one drop (0.03 ml) is used from each SDU. Each SDU was closed tightly with a Lameplast SDU cap. The filled and tightly closed SDUs were placed in a tube rotator to prevent particle sedimentation while the suspension contents were allowed to gel and stabilize at ambient temperature for approximately 24 h. The filled SDUs were labelled and packed in labeled resealable aluminum pouches (secondary package). After the Fill and Finish manufacture, the SDUs in secondary packages were stored at 5 ± 3 °C °C before sterilisation by gamma irradiation at 25 kGy.

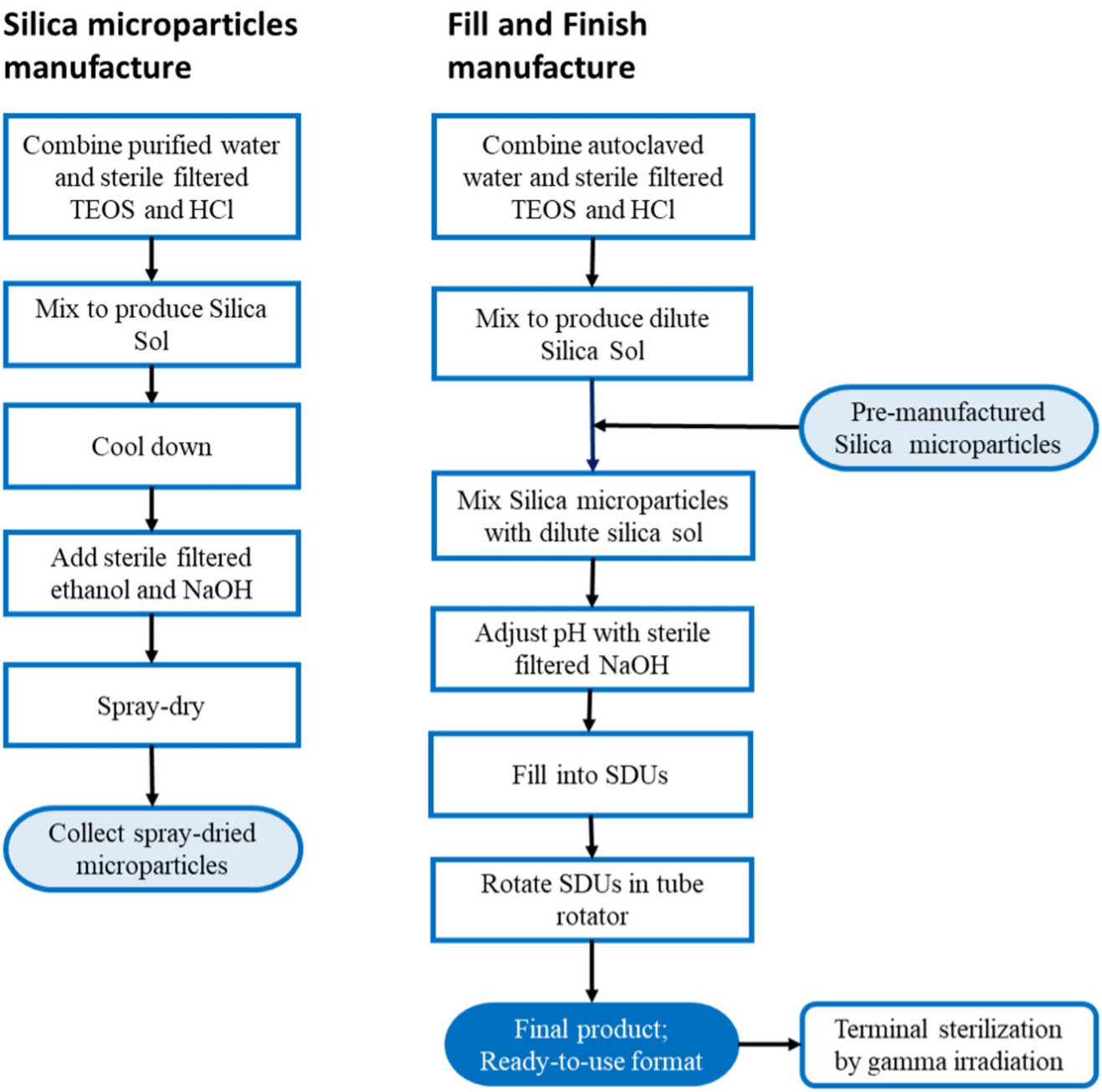

Fig. 1 Flow charts for the manufacturing of Silica Eye Drop. The flow chart on the left illustrates the manufacture of silica microparticles by spray drying, while the flow chart on the right depicts the Fill and Finish of the final eye drop product

Characterisation of Silica Eye Drop

Particle Size Analysis

Silica microparticles present in the eye drop were controlled for their particle size distribution (PSD), which is determined using a validated laser diffraction method (Laser Helo Sympatec with cuvette and R3 lens). 20 mg of silica microparticles were suspended in 20 ml of ethanol. The suspension was added dropwise, under stirring, to a cuvette containing about 50 ml of ethanol until an optical density of 5% to 25% was achieved. The measurement was performed in triplicate.

In Vitro Dissolution Test

To establish the in vitro release kinetics of silica, a dissolution testing method with silica under sink conditions was

used. Briefly, 25–35 mg of Silica Eye Drop were weighed into a dissolution vessel. 50 ml of dissolution buffer, 50 mM TRIS buffer (pH7.4 @37°C), was added, and the vessel was transferred to a shaking water bath at + 37 °C and 60 strokes per minute. At specified time points (1 h, 2 h, 3 h, 4 h, 5 h, 6 h, 9 h, and 24 h), the sample was centrifuged at 4500 rpm for 5 min. Silica content in the supernatant was analysed while the pellets were resuspended in fresh 50 ml dissolution buffer. The dissolution vessel was returned to the water bath shaker (+ 37 °C and 60 rpm). The cycle continued until the final dissolution time point. The silica concentration in the dissolution samples was measured with a spectrophotometer (Jenway 630, Cole-Parmer Ltd, UK). The method involves derivatisation, whereby a blue colour complex is formed in a reaction containing ammonium molybdate in an acidic environment [23]. The intensity of the blue color was measured spectrophotometrically at a wavelength of 820 nm and was directly proportional to the concentration of dissolved silica. Three parallel dissolution tests were carried out per batch.

Silica Content Analysis

The total content of silica in the eye drop was determined by spectrophotometry. The aim of this experiment was to understand the eye drop composition, i.e., how much silica is present in the formulation. In addition, silica assay was used to calculate the cumulative release of silica from the in vitro dissolution experiments. A sample of Silica Eye Drop (20–30 mg) was dissolved in 50 ml of 2% NaOH at 37 °C and 60 rpm for 3 days in a water shaking bath. After complete dissolution of the sample, the sample solution was diluted 100 times in water. The same spectrophotometric method used for analysing the dissolution samples was used for the determination of total silica content.

Ethanol Content Analysis

Silica microparticles contain some residual amount of ethanol. Ethanol was produced as a by-product in the sol-gel process and was also used for dilution of the sol prior to spray drying. Potentially, high ethanol content could cause irritation in the living eye. The determination of ethanol in Silica Eye Drop was carried out by headspace-gas chromatography using a flame ionisation detector (HS-GC-FID, Agilent G1888-6890 N). Sample solution preparation involved dissolving 30–40 mg Silica Eye Drop in a 20 ml glass vial with 50 ml of 2% NaOH at 37 °C and 60 rpm for 3 days in a water shaking bath. 20 µg/ml of isopropanol was used as an internal standard. The chromatographic separation was carried out on a DB-624, 30 m x 0.53 mm, 3 µm GC column (Agilent) with a gradient oven temperature ramp from 35 °C, 20 °C/min to 90 °C, 50 °C/min to 240 °C hold for 5 min and nitrogen constant gas flow at 1.5 ml/min. The inlet temperature was 220 °C, and the split ratio was 1:50 with a 4 mm deactivated glass liner. The headspace temperature conditions were set as follows: oven at 80 °C, loop at 210 °C, and transfer line at 220 °C.

Endotoxin

An endotoxin test was conducted on the EndoSafe Nex-Gen PTS device. This device performed endotoxin testing according to Ph. Eur. 2.4.16/USP<85>Method D chromogenic technique. The Detection limit of the method was <0.176 EU/mg.

Sterility

Sterility assurance level (SAL) of 10^{-6} was demonstrated by sterilization dose substantiation (gamma irradiation) using the VDmax 25 kGy method in accordance with the requirements in ISO 11137-2 [24–26]. The maximum allowable dose was determined to be 35 kGy. The VDmax 25 kGy method was used to establish 25 kGy as the sterilisation dose for products manufactured frequently or infrequently in large or small batches. For the validation of a single lot or infrequent production batches, 10 products were tested for bioburden, and then a dose audit was performed on 10 products irradiated at the verification dose. A SAL 10^{-6} was demonstrated by experimental evidence that the product bioburden was less resistant than a Standard Distribution of Resistances (SDR) for which a SAL of 10^{-6} was obtained at 25 kGy. The resistance to the sterilisation process was demonstrated by irradiation of the test items at a verification dose (VDmax) for which a SAL of 10^{-1} was obtained for the SDR. A SAL of 10^{-1} would result in 1 non-sterile sample per 10 items tested. Bioburden was determined using the validated most probable number method. The verification dose according to ISO 11137-2 was equal to 2.7 kGy. Sterility testing by a validated direct inoculation method for 10 test items irradiated at the verification dose was performed. A sterilisation dose of 25 kGy was established to obtain a SAL 10^{-6} since there was not more than one positive test of sterility out of the ten product items tested.

Stability Study

A stability study was conducted with Silica Eye Drop to test stability during storage and the preclinical in-use phase. The purpose of stability studies was to provide information on changes in the quality of the product over time because of environmental factors such as temperature, humidity, and light on the product. The following analyses were performed

in the study at time points 0, 1 month, and 2 months: Visual inspection, silica content, ethanol content, in vitro dissolution testing, and pH. The storage conditions were based on ICH Q1 guidelines for stability testing of drug substances and drug products. The study was conducted at 5 ± 3 °C and 25 ± 2 °C/60 ± 5% relative humidity (RH).

GLP Toxicity Study in Rabbits

The study was conducted according to the EMA guideline on non-clinical local tolerance testing (2015), OECD Test No. 405 (2021), Directive 2010/63/EU on animal protection, the Finnish Animal Protection Act (497/2013) and Decree (564/2013), OECD guidance on humane endpoints (2000), and OECD Principles of Good Laboratory Practice (1998). The investigation has been approved by the National Laboratory Animal Board of Finland (Care and Use Committee) with license number ESAVI/26,478/2019 and was performed with the 3Rs principle (replacement, reduction, refinement).

The biocompatibility, safety, and tolerability of Silica Eye Drop were investigated in four female New Zealand White (NZW) rabbits in compliance with Good Laboratory Practice (GLP). One drop of Silica Eye Drop was administered daily for 14 days to the conjunctival sac of one (left) eye of four animals. One drop (approximately 0,05 ml) of sterile 0.9% NaCl was administered in the conjunctival sac of the other eye (right) as a control. The eyes were checked daily before applying the test items by a veterinarian. The degree of eye irritation/corrosion was repeatedly evaluated by scoring lesions of the conjunctiva, cornea, and iris. The veterinarian used fluorescein (Kunze Indopharm B.V., Netherlands) to detect any possible damage to the cornea on study Days 2, 8, and 15. The scoring was performed according to OECD/OCDE Test No. 405: Acute Eye Irritation/Corrosion. Corneal opacity (0–4), iris involvement (0–2), conjunctival redness (0–3), and chemosis (0–4) were assessed using the standard OECD 405 grading scales, with higher scores signifying greater severity. Intraocular pressure was repeatedly measured in both eyes on study Day − 5 (pre-test) and on study Days 1 (prior to the first dose), 2, 8, and 15. Measurements were done by the veterinarian using the Icare Tonovet Plus (Icare, Finland) device. The animals were restrained with a towel, and after approximately two minutes, the measurements were performed. Three acceptable subsequent results from both eyes were collected from each animal, and an average of these three measurements was calculated. Other possible effects on the eye and adverse systemic effects were also recorded to provide a complete evaluation of the effects. Clinical signs were observed twice daily, and blood sampling for hematological analyses, coagulation tests, and clinical chemistry was taken at baseline and termination.

After 14 days of treatment (on study Day 15), the animals were sacrificed by exsanguination after inducing deep anesthesia using Medetomidine (Cepetor vet 1 mg/ml, CP-Pharma Handelsges. mbH, Germany) and Ketamine (Ketaminol vet 50 mg/ml, Intervet International B.V., Germany). Some animals were additionally given Euthasol 400 mg/ml (Produlab Pharma B.V., the Netherlands) intravenously to complete the euthanasia. Scheduled necropsy was performed in all animals, and all macroscopic changes were recorded. At necropsy, eyes with optic nerves, lids, conjunctivae, and nictitating membranes were collected from all animals into Davidson's solution (both sides for paired organs). The tissues were kept in Davidson's fixative for 24 to 27 h, after which they were transferred into 70% ethanol. The fixed tissues were sent to AnaPath GmbH, Department AnaPath Services, Switzerland. Trimming, processing, embedding in paraffin wax, cutting, and staining by eosin and hematoxylin were performed at AnaPath GmbH, Department AnaPath Services, Switzerland. Images were acquired using a Zeiss Axioplan microscope (Carl Zeiss, Oberkochen, Germany) equipped with a Zeiss AxioCam 105 color camera for transmitted-light imaging. A professional veterinary pathologist and an ophthalmologist evaluated the sections. Histological images were processed using Fiji (ImageJ2, version 2.16.0; National Institutes of Health, Bethesda, MD, USA; released in 2024) for calibration, scale-bar generation, and basic color normalization. Final adjustments, including uniform brightness and contrast refinement, were performed in Adobe Photoshop 2024 (Adobe Inc., San Jose, CA, USA). No structural modifications, alterations of tissue morphology, or manipulations affecting the scientific content of the images were made.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 10.4.1 (GraphPad Software, San Diego, CA, USA). For each time point, differences in intraocular pressure (IOP) between the control and treated groups were analysed using an unpaired t-test. P-values ≤ 0.05 were considered statistically significant.

In addition, the similarity factor (f_2) was calculated as described in USP<1090>to compare the dissolution profiles obtained under different storage conditions and time points. The f_2 value is a statistical parameter used to assess the similarity between two dissolution profiles, where values between 50 and 100 indicate that the profiles are considered similar.

Results and Discussion

Characterisation and Stability of Silica Eye Drop

The Silica Eye Drop formulation contained 23% (w/w) silica microparticles and 77% (w/w) silica hydrogel. By visual inspection, the eye drop was a white to nearly white homogeneous material. Figure 2 shows a photograph of the final eye drop product in its primary package. The silica microparticles in the eye drop exhibited a monomodal particle size distribution with D10 at 1.69 µm, D50 at 3.21 µm, and D90 at 5.78 µm. The D-values represent the fraction of the particles that are below the given values. For example, the D10 value is the diameter at which 10% of the sample's mass comprises particles with a diameter less than this value. The amount of silica in the final eye drop product was measured to be 18.2 ± 0.65 wt%, the ethanol content was 3.9 wt%, and the pH was 6.1. Silica Eye Drop was shown to dissolve completely in 24 h with an in-house in vitro in-sink dissolution testing method. Silica microparticles play a major role in controlling the dissolution rate of Silica Eye Drop. The dissolution rate of silica is influenced by several manufacturing parameters, including the precursor ratios (i.e., molar ratio of water to TEOS), aging of the silica sol, pH of the silica sol, nature of the diluent, and spray drying parameters. As mentioned above, silica microparticles are produced by the hydrolysis and condensation of TEOS via an acid-catalysed reaction. A lower water-to-TEOS molar ratio and ethanol as diluent in the manufacturing process resulted in silica microparticles with a fast dissolution rate. Unlike traditional mesoporous silica systems [27], which rely on the loading of drugs into pores, the silica matrix in this study is non-porous [28]. The dissolution of the matrix occurs through the surface erosion of the silica matrix in bodily fluids rather than through diffusion. Additionally, the rate of biodegradation can be finely tuned, enabling controlled release that can be tailored over a duration of days to months. The droplet size variation was determined by gravimetry from ten different SDUs. The average droplet weight was 33.8 ± 4.8 mg, corresponding to 31.3 ± 4.4 µl. The minimum and maximum weights were 29.1 mg and 39.1 mg, respectively. It was concluded that dispensing the droplet manually from the SDU results in droplets with acceptable variability in size. This was substantiated by the content uniformity test results, which conformed to the European pharmacopeia [Ph. Eur 2.9.40]. The application of a droplet was easy and required only a minimum amount of pressing force by fingers. The Silica Eye Drop formulation contained very low levels of endotoxin; the measured endotoxin content was below 0.2 EU/mg, which is the limit of quantitation of the method. Low endotoxin and bioburden (0.36 CFU/g) indicated a good control strategy for endotoxin and microbial contamination during the manufacturing process of the eye drop used in this study. The product was terminally sterilised by gamma irradiation at 25 kGy, and sterilisation dose substantiation confirmed that the irradiation dose of 25 kGy was adequate to reach SAL 10^{-6} level for the product. This study also demonstrated that the Silica Eye Drop formulation tolerates well gamma irradiation without any sign of loss of critical properties needed for an eye drop.

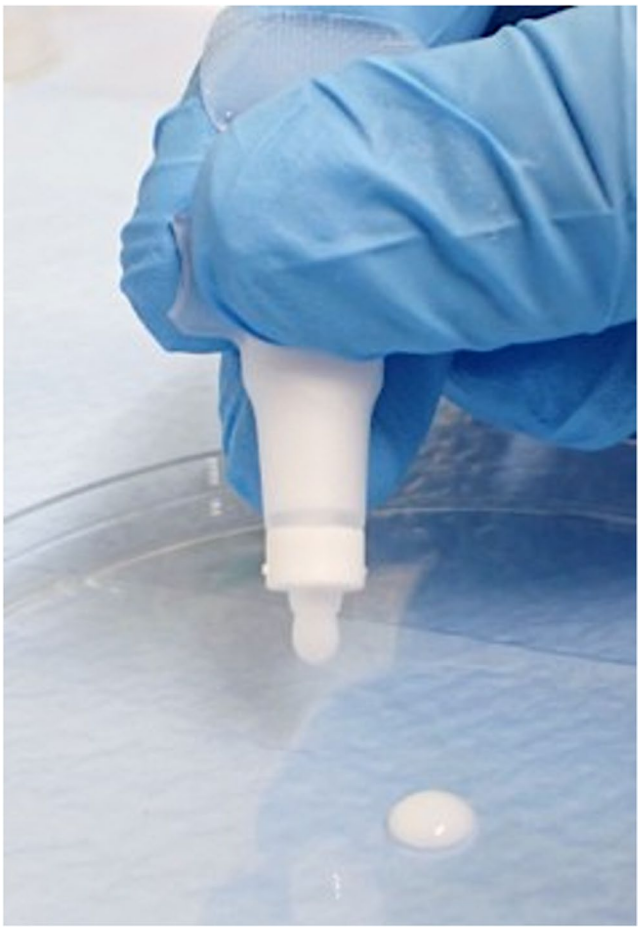

Fig. 2 A picture representation of the Silica Eye Drop product. Silica microparticles-silica hydrogel composite material inside single-dose units (SDU, primary package) and a drop of the product on a petri dish. The droplet weight is 33.8±4.8 mg, corresponding to 31.3±4.4 µl. Data are presented as mean±SD

A 2-month preliminary stability study was conducted with Silica Eye Drop to establish adequate stability of the product needed for the in vivo rabbit study. By visual inspection, the product looked the same, i.e., white to almost white homogenous suspension at all time points and both conditions. No changes were seen in silica or ethanol content over time. After 2 months, a slight decrease in pH from 6.1 to 5.9 and 5.7 was recorded at 5±3 °C and 25±2 °C/60±5%RH, respectively. Although the dissolution rate of silica was slower at 1 month and 2 months than

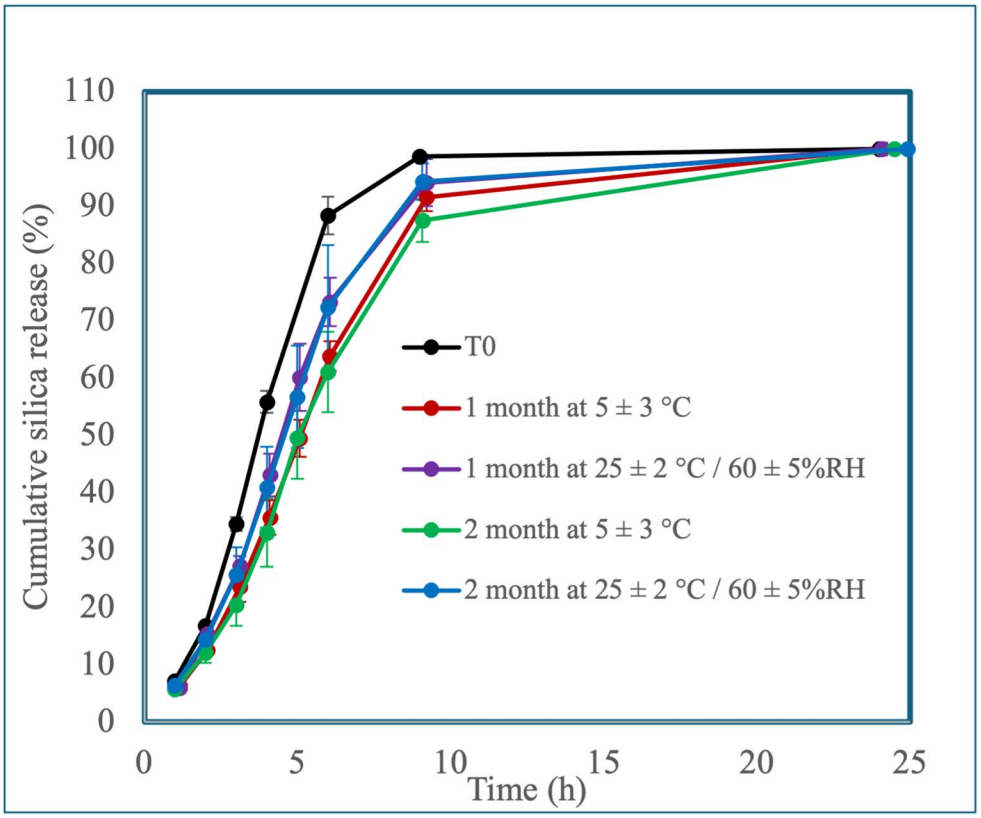

Fig. 3 Dissolution curves for Silica Eye Drop at 5±3 °C and 25 °C/60%RH storage conditions for 0, 1, and 2 months. The data represent the average of three replicate dissolution tests per sample, and standard deviations are represented by error bars

at the 0-month timepoint (Fig. 3), all the silica dissolved within 24 h at all conditions studied. In addition, the similarity factor (f2) calculated against T0 for samples stored at 25±2 °C/60±5%RH and 5±3 °C was 66 and 56, respectively, at the 2-month timepoint. Since f2 was ≥50, the dissolution profiles were considered equivalent. In conclusion, Silica Eye Drop was stable for at least 2 months and can be stored for this period of time either at 25±2 °C/60±5%RH or 5±3 °C.

GLP Toxicity Study

All animals gained weight normally during this study (data not shown). No unusual clinical signs were recorded in any of the animals. The clinical observations of the eyes recorded are summarised in Table 1. A total of 4 left eyes that were treated and 4 right eyes that served as controls. No severe irritation or corrosion was observed in any of the animals. Scores of grades 1 to 2 of test item residues were observed in the conjunctival sac of the test eyes in two out of four animals during eye examinations on Days 3, 7, 8, 10, and 15. Eyelid swelling (chemosis) above normal was observed in both control and treated eyes of the animals. The Silica Eye Drop group had 8 observations on the test (left) eye and 4 on the control (right) eye. These changes were not persistent. There were no other reactions in the eyes. The results of the haematological analyses, coagulation tests, and clinical chemistry analyses were comparable between pre- and post-treatment. Histopathological evaluation revealed no treatment-related findings in the examined organs, tissues, or structures (Fig. 4). Minimal (grade 1) mononuclear cell infiltrates in the nictitating membrane were observed in one eye treated with Silica Eye Drop. Focal mononuclear infiltrate, grade 2, was observed in the eyelid dermis of one control eye. The findings were considered incidental/background changes that sometimes occur in this type of study.

IOP (intraocular pressure) values for treated and control eyes are shown in Fig. 5. IOP values for all animals in this study remained within the normal reference range for laboratory animals despite showing a variation over 14 days (12–25 mmHg). This may be due to the fact that the IOP can be influenced by restraint techniques, animal stress, diurnal or circadian rhythm, eye position, sedation or anesthesia, corneal thickness, and several other variables [29]. Therefore, the observed fluctuations were considered to be within physiological limits and were not attributed to the treatment. Chemosis, which is the swelling of the conjunctiva, was observed in the test and control groups. In rabbits, these changes can be triggered by various factors, including environmental conditions often present in animal holding centers [30]. Additionally, stress resulting from the experiment may weaken a

Table 1 Summary of clinical observations of the eyes recorded in the rabbit GLP toxicity study (frequency of incidence)

Silica Eye Drop															
Study day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. RESIDUE OF TEST ITEM IN THE EYE															
Left eye	0/4	0/4	1/4	0/4	0/4	0/4	1/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4	1/4
Right eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
2. AMOUNT OF THE TEAR FLUID															
Left eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Right eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
3. CONJUNCTIVAE															
Left eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Right eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
4. CHEMOSIS															
Left eye	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4	2/4	0/4	3/4	0/4	0/4	1/4	0/4
Right eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4	0/4	1/4	0/4	0/4	1/4	0/4
5. IRIS															
Left eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Right eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
6. CORNEA															
Left eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Right eye	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

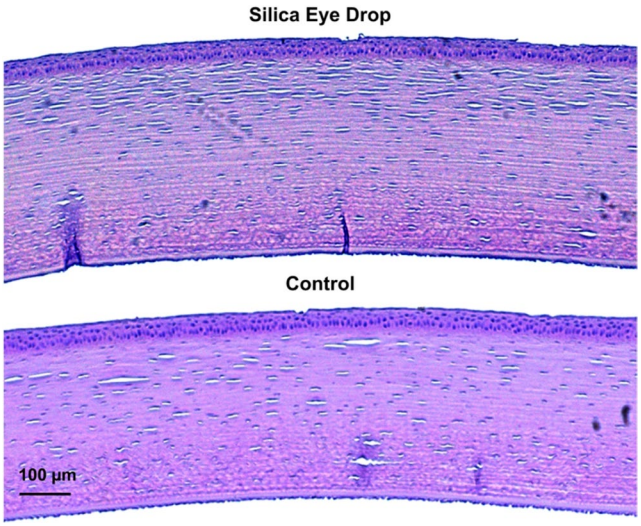

Fig. 4 Hematoxylin and eosin (H&E)–stained sections of the cornea in the rabbit GLP toxicity study. The image was acquired using bright-field microscopy at 5× objective magnification

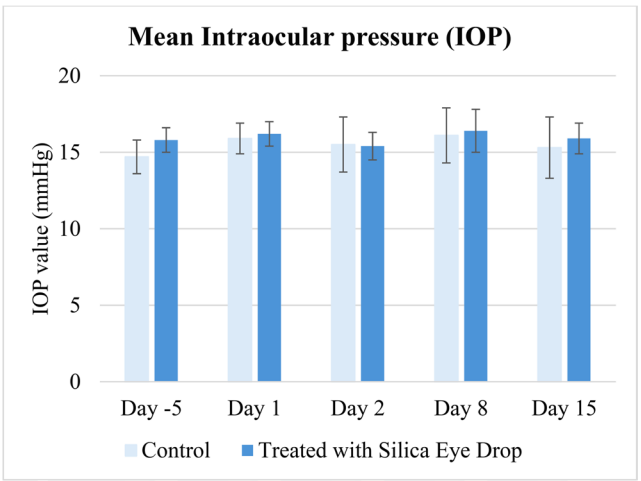

Fig. 5 Intraocular pressure values in control and treated eyes in the rabbit GLP toxicity study. Data are presented as mean±SD; sample sizes of 4 are reported for each group

rabbit's immune system, increasing its susceptibility to infections that can lead to conjunctival swelling. This explains the minor histological changes observed in the tissues of some animals in both study groups. After administering Silica Eye Drop, significant absorption of the main compounds of the medication into the eye structures or directly into the bloodstream is not expected due to their very low quantities and physicochemical properties. When the drop is in contact with tear fluid, the silica material begins to dissolve into silicic acid (SiA). This material may then be transported via the nasolacrimal duct. Additionally, some SiA could be absorbed directly into the bloodstream through the epithelium of the conjunctival sac and later excreted in the urine by the kidneys. Compared to other sources of silica and SiA, the amount contained in this material represents only trace levels of daily intake for humans [31–35]. It was concluded that 14-day repeated daily dosing of Silica Eye Drop was well-tolerated and considered to be safe and non-irritant. This is in line with a recent study in Sprague-Dawley rats, which received an ocular topical dose of nonporous silica nanoparticles four times a day for one month [36]. The authors of this study did not find any significant local or systemic safety concerns for the nanocarrier material after a 12-week follow-up period.

Conclusion

The Silica Eye Drop formulation was designed with the following target product properties: a dissolution time of approximately 24 h in the conjunctival sac and tear fluid, easy administration by the patient, a drop weight of around 33 mg (30 µl), and a sterile composition that is non-toxic and well-tolerated. The results in this study demonstrate that a silica composite eye drop formulation meeting these criteria was successfully developed. The eye drop formulation containing 23% (w/w) silica microparticles and 77% (w/w) silica hydrogel dissolved in 24 h according to the in vitro dissolution results. Moreover, in the rabbit in vivo GLP study with daily dosing for 14 days, no residual silica material was detected before the next dosing in 9/10 cases. Additionally, no unusual clinical signs were observed during the experiment. At necropsy, no abnormalities were found in any of the animals. No pathological changes were observed in this study; the control and test groups showed similar results throughout the entire period without any statistically significant differences. IOP values for all animals in this study remained within the normal reference range for laboratory animals. The data show that Silica Eye Drop is safe and well-tolerated for once-a-day use for 14 days in NZW rabbits without causing any toxic effects in tissues and organs, either locally or systemically. The Silica Eye Drop formulation was easy to administer, and the average droplet weight was 33.8±4.8 mg, corresponding to 31.3±4.4 µl, which is in line with the target value. In conclusion, this study demonstrates the potential of the silica platform technology as a drug carrier material in topical ophthalmology. Additional experiments are necessary, particularly those that involve combining the Silica Matrix with a drug, to evaluate clinical efficacy and pharmacokinetics of this technology.

Author Contributions L. L. – conceptualisation and study design, M. N. A-P., H. A. – supervision, M. N. A-P., P. S. – analytical development and stability studies (experiments), M. V., H. A. – formulation development and manufacturing (experiments), M. N. A-P., A. P., P. S., M. V., H. A. – data collection, analysis, and interpretation, M. N. A-P., A. P., P. S. – preparation of tables and figures, L. L., M. N. A-P., A. P. – manuscript writing. All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Data Availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Competing Interests M.N. A-P., P.S. M.V., and H.A. are employees of DelSiTech Ltd., where the formulation development and physicochemical properties were analysed. However, in vivo tolerability and safety studies were conducted independently by a third party not affiliated with DelSiTech Ltd. The authors affirm that this separation of roles ensured that the objectivity and integrity of the tolerability data remained unaffected by the company.

References

1. Ali M, Byrne ME. Challenges and solutions in topical ocular drug-delivery systems. Expert Rev Clin Pharmacol. 2008;1:145–61. https://doi.org/10.1586/17512433.1.1.145.
2. Gote V, Sikder S, Sicotte J, Pal D. Ocular drug delivery: present innovations and future challenges. J Pharmacol Exp Ther. 2019;370:602–24. https://doi.org/10.1124/jpet.119.256933.
3. Forrester JV, Dick AD, McMenamin PG, Roberts F, Pearlman E. General and ocular pharmacology. The eye. Elsevier; 2016. pp. 338–69. https://doi.org/10.1016/B978-0-7020-5554-6.00006-X. .e1.
4. Gaudana R, Ananthula HK, Parenky A, Mitra AK. Ocular drug delivery. AAPS J. 2010;12:348–60. https://doi.org/10.1208/s12248-010-9183-3.
5. Jumelle C, Gholizadeh S, Annabi N, Dana R. Advances and limitations of drug delivery systems formulated as eye drops. J Controlled Release. 2020;321:1–22. https://doi.org/10.1016/j.jconrel.2020.01.057.
6. Fedorchak MV, Conner IP, Schuman JS, Cugini A, Little SR. Long term glaucoma drug delivery using a topically retained gel/microsphere eye drop. Sci Rep. 2017;7:8639. https://doi.org/10.1038/s41598-017-09379-8.
7. Almeida H, Amaral MH, Lobão P, Lobo JMS. In situ gelling systems: a strategy to improve the bioavailability of ophthalmic pharmaceutical formulations. Drug Discov Today. 2014;19:400–12. https://doi.org/10.1016/j.drudis.2013.10.001.
8. Stefansson E, Loftsson T, Larsen M, Papp A, Kaarniranta K, Munk MR, et al. Topical treatment of diabetic macular edema using dexamethasone ophthalmic suspension: a randomized, double-masked, vehicle-controlled study. Acta Ophthalmol. 2023;101:22–33. https://doi.org/10.1111/aos.15215.
9. Wang Y, Wang C. Novel eye drop delivery systems: advance on formulation design strategies targeting anterior and posterior segments of the eye. Pharmaceutics. 2022. https://doi.org/10.3390/pharmaceutics14061150.
10. Forsback A-P, Noppari P, Viljanen J, Mikkola J, Jokinen M, Leino L, et al. Sustained in-vivo release of Triptorelin acetate from a biodegradable silica depot: comparison to Pamorelin® LA. Nanomaterials. 2021;11:1578. https://doi.org/10.3390/nano11061578.
11. McGowan A, Gennemark P, Akieh-Pirkanniemi M, Wirman L, Davies N, Elebring M, et al. Injectable biodegradable silica depot for controlled subcutaneous delivery of antisense oligonucleotides with beyond monthly administration. Mol Pharm. 2024;21:143–51. https://doi.org/10.1021/acs.molpharmaceut.3c00663.
12. Noppari P, Forsback A-P, Terjung C, Marttila A, Leino L, Unger F, et al. Development of injectable long-acting controlled release intravitreal depot of BAY224 using biodegradable silica microparticle-silica hydrogel composite. Invest Ophthalmol Vis Sci. 2020;61:1357.
13. Tyagi P, Koskinen M, Mikkola J, Leino L, Schwarz A. Silica microparticles for sustained zero-order release of an anti-CD40L antibody. Drug Deliv Transl Res. 2018;8:368–74. https://doi.org/10.1007/s13346-017-0408-1.
14. Tyagi P, Koskinen M, Mikkola J, Sarkhel S, Leino L, Seth A, et al. Injectable biodegradable silica depot: two months of sustained release of the blood glucose lowering peptide, Pramlintide. Pharmaceutics. 2022;14:553. https://doi.org/10.3390/pharmaceutics14030553.
15. Rosenholm JM, Sahlgren C, Lindén M. Towards multifunctional, targeted drug delivery systems using mesoporous silica nanoparticles – opportunities & challenges. Nanoscale. 2010;2:1870. https://doi.org/10.1039/c0nr00156b.
16. Jokinen M, Koskinen M, Areva S. Rationale of using conventional sol-gel derived SiO_2 for delivery of biologically active agents. Key Eng Mater. 2008;377:195–210. https://doi.org/10.4028/www.scientific.net/KEM.377.195.
17. Viitala R, Jokinen M, Tuusa S, Rosenholm JB, Jalonen H. Adjustably bioresorbable sol-gel derived SiO2 matrices for release of large biologically active molecules. J Sol-Gel Sci Technol. 2005;36:147–56. https://doi.org/10.1007/s10971-005-5286-1.
18. Viitala R, Jokinen M, Maunu SL, Jalonen H, Rosenholm JB. Chemical characterization of bioresorbable sol–gel derived SiO2 matrices prepared at protein-compatible pH. J Non-Cryst Solids. 2005;351:3225–34. https://doi.org/10.1016/j.jnoncrysol.2005.08.023.
19. Baran-Rachwalska P, Torabi-Pour N, Sutera FM, Ahmed M, Thomas K, Nesbit MA, et al. Topical siRNA delivery to the cornea and anterior eye by hybrid silicon-lipid nanoparticles. J Control Release. 2020;326:192–202. https://doi.org/10.1016/j.jconrel.2020.07.004.
20. Kim S-N, Ko SA, Park CG, Lee SH, Huh BK, Park YH, et al. Amino-functionalized mesoporous silica particles for ocular delivery of brimonidine. Mol Pharm. 2018;15:3143–52. https://doi.org/10.1021/acs.molpharmaceut.8b00215.
21. Hu C, Sun J, Zhang Y, Chen J, Lei Y, Sun X, et al. Local delivery and sustained-release of nitric oxide donor loaded in mesoporous silica particles for efficient treatment of primary open-angle glaucoma. Adv Healthc Mater. 2018. https://doi.org/10.1002/adhm.201801047.
22. Mohammadpour M, Jabbarvand M, Hashemi H, Delrish E. Prophylactic effect of topical silica nanoparticles as a novel antineovascularization agent for inhibiting corneal neovascularization following chemical burn. Adv Biomed Res. 2015;4:124. https://doi.org/10.4103/2277-9175.158039.
23. Yang H, Li C, Wei C, Li M, Li X, Deng Z, et al. Molybdenum blue photometry method for the determination of colloidal silica and soluble silica in leaching solution. Anal Methods. 2015;7:5462–7. https://doi.org/10.1039/C5AY01306B.
24. International Organization for Standardization. ISO 11737-2:2019 Sterilization of health care products — Microbiological

methods — Part 2: Tests of sterility performed in the validation of a sterilization process. 2019.
25. International Organization for Standardization. ISO 11737-1:2018 Sterilization of health care products — Microbiological methods — Part 1: Determination of the population of microorganisms on products. 2018.
26. International Organization for Standardization. BS EN ISO 11137-2:2015+A1:2023 sterilization of health care products — Radiation — Part 2: Establishing the sterilization dose. London. 2015. https://doi.org/10.3403/30326234.
27. Castillo RR, Lozano D, González B, Manzano M, Izquierdo-Barba I, Vallet-Regí M. Advances in mesoporous silica nanoparticles for targeted stimuli-responsive drug delivery: an update. Expert Opin Drug Deliv. 2019;16:415–39. https://doi.org/10.1080/17425247.2019.1598375.
28. Noppari P, Jokinen M, Dargelas F, Mikkola J, Leino L. CHAPTER 9. Parenteral Delivery of Therapeutic Proteins, Peptides and Small Molecules Using Biodegradable Silica. In: <book-title update="added">Implantable technologies: peptides and small molecules drug delivery. 2021. p. 188–215. https://doi.org/10.1039/9781839164958-00188.
29. Gilger BC, Cook CS, Brown MH, editors. Standards for ocular toxicology and inflammation. Cham: Springer International Publishing; 2018. https://doi.org/10.1007/978-3-319-78364-2.
30. Varga M. Rabbit Basic Science. In: Textbook of Rabbit Medicine. Elsevier; 2014. p. 3–108. https://doi.org/10.1016/B978-0-7020-4979-8.00001-7.
31. Jugdaohsingh R. Silicon and bone health. J Nutr Health Aging. 2007;11:99–110.
32. Jurkić LM, Cepanec I, Pavelić SK, Pavelić K. Biological and therapeutic effects of ortho-silicic acid and some ortho-silicic acid-releasing compounds: new perspectives for therapy. Nutr Metab (Lond). 2013;10:2. https://doi.org/10.1186/1743-7075-10-2.
33. Sripanyakorn S, Jugdaohsingh R, Elliott H, Walker C, Mehta P, Shoukru S, et al. The silicon content of beer and its bioavailability in healthy volunteers. Br J Nutr. 2004;91:403–9. https://doi.org/10.1079/BJN20031082.
34. Calcium silicate. Silicon dioxide/silicic acid gel added for nutritional purposes to food supplements. EFSA J. 2009;7. https://doi.org/10.2903/j.efsa.2009.1132.
35. Dekkers S, Krystek P, Peters RJB, Lankveld DPK, Bokkers BGH, van Hoeven-Arentzen PH, et al. Presence and risks of nanosilica in food products. Nanotoxicology. 2011;5:393–405. https://doi.org/10.3109/17435390.2010.519836.
36. Kim M, Park J-H, Jeong H, Hong J, Choi WS, Lee B-H, et al. An evaluation of the in vivo safety of nonporous silica nanoparticles: ocular topical administration versus oral administration. Sci Rep. 2017;7:8238. https://doi.org/10.1038/s41598-017-08843-9.