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2022-03

Balla , A , Ruponen , M , Valtari , A , Toropainen , E , Tuomainen , M , Alvarez-Lorenzo , C , del Amo , E M , Urtti , A & Vellonen , K-S 2022 , ' Understanding dexamethasone kinetics in the rabbit tear fluid : Drug release and clearance from solution, suspension and hydrogel formulations ' , European Journal of Pharmaceutics and Biopharmaceutics , vol. 172 , pp. 53-60 . https://doi.org/10.1016/j.ejpb.2022.01.005

http://hdl.handle.net/10138/347475
https://doi.org/10.1016/j.ejpb.2022.01.005

European Journal of Pharmaceutics and Biopharmaceutics 172 (2022) 53–60

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Understanding dexamethasone kinetics in the rabbit tear fluid: Drug release and clearance from solution, suspension and hydrogel formulations

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ARTICLE INFO

Keywords
Hydrogel
Suspension
Dexamethasone
Solution
Pharmacokinetics
Precorneal residence
Tear fluid

ABSTRACT

Rapid precorneal loss of topically applied eye drops limits ocular drug absorption. Controlling release and precorneal residence properties of topical formulations may improve ocular drug bioavailability and duration of action. In this study, we evaluated *in vivo* ocular pharmacokinetics of dexamethasone in rabbits after application of a drug solution (0.01%), suspension (Maxidex® 0.1%), and hydrogels of 2-hydroxyethyl methacrylate (HEMA) and acrylic acid (AAc) copolymers. The rabbits received a single eyedrop (solution or suspension) or dexamethasone-loaded hydrogel topically. Dexamethasone in tear fluid was sampled with glass capillaries and quantitated by LC-MS/MS. Higher dexamethasone exposure (AUC) in the tear fluid was observed with the suspension (≈3.6-fold) and hydrogel (12.8-fold) as compared to the solution. During initial 15 min post-application, the highest AUC of dissolved dexamethasone was seen after hydrogel application (368 min*μg/mL) followed by suspension (109.9 min*μg/mL) and solution (28.7 min*μg/mL. Based on kinetic simulations, dexamethasone release from hydrogels *in vivo* and *in vitro* is comparable. Our data indicate that prolonged exposure of absorbable dexamethasone in tear fluid is reached with hydrogels and suspensions. Pharmacokinetic understanding of formulation behavior in the lacrimal fluid helps in the design of dexamethasone delivery systems with improved ocular absorption and prolonged duration of action.

1. Introduction

Eye drops are the most widely used dosage form in the treatment of anterior segment diseases, such as dry eye disease, glaucoma, and ocular infections and inflammations[1,2] . Topically applied eye drop solutions constitute approximately 90% of the currently marketed ophthalmic formulations [3,4]. They have a short duration of action (a few hours up to one day) because the instilled drug is rapidly eliminated from the ocular surface in a few minutes, mainly due to reflex lacrimation, nasolacrimal solution drainage, and systemic absorption through conjunctiva [5].

Ophthalmic suspensions are widely used useful in the topical administration of poorly water-soluble drugs. However, comparison of fluorometholone penetration to the rabbit aqueous humor reveals that dose normalized drug concentrations after 0.1% suspension instillation were smaller than the levels after administration of low dose (15 μg/ml) in solution [6]. Furthermore, aqueous humor bioavailability (0.1%) for ophthalmic brinzolamide after topical suspension administration has been reported to be at the low end of bioavailability range of ophthalmic eye drop solutions of various drugs (0.07–4.31%) [7]. Not all suspended particles dissolve in the tear fluid and, therefore, the ocular bioavailability is affected by the suspension residence time and dissolution rate

Abbreviations: $AUC_{soluble}$, Dissolved dexamethasone fraction in the suspension; CL_{conj}, Conjunctival clearance; CL_{cornea}, Corneal clearance; CL_{total}, Total clearance; CL_{TT}, Clearance by tear turnover; $F_{soluble}$, Fraction dissolved; NCA, Non-compartmental analysis; P_{conj}, Permeability in the conjunctiva; P_{cornea}, Permeability in the cornea; S_{conj}, Surface area of conjunctiva; S_{cornea}, Surface area of cornea.

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https://doi.org/10.1016/j.ejpb.2022.01.005
Received 6 October 2021; Received in revised form 13 January 2022; Accepted 18 January 2022
Available online 2 February 2022

in the lacrimal fluid as was recently shown for indomethacin [8].

Even though eye drops are a non-invasive drug administration method, difficulties in eye drop instillation have led to poor patient compliance. For example, only 28% of glaucoma patients are correctly using their eye drop medication [9]. Proper instillation of the eye drops is particularly problematic for elderly patients or handicapped persons.

One way to improve patient compliance and reduce dosing frequency is to develop topical drug formulations with prolonged ocular retention and sustained drug release. Various formulation approaches have been investigated to prolong the duration of topically applied drugs (e.g. viscous and mucoadhesive formulations, ointments, nano- and microparticles, inserts, punctal plugs, and hydrogels) [10–18]. Hydrogels, are widely used in soft contact lenses and they are well tolerated in the eye [19,20]. They can control drug release and prolong dosing intervals [21]. Although *in vitro* and *in vivo* studies with topical ophthalmic hydrogels have been carried out, *in vitro/in vivo* correlation and functionality of drug containing hydrogels in the tear fluid are still poorly understood.

Ocular pharmacokinetic top-down models and bottom-up simulations are important tools for prediction and understanding of dosage form functionality *in vivo*. The simulations can reveal relationships between the administered dose, release rate and observed concentrations in ocular tissues, tear fluid, and plasma thereby facilitating pharmacokinetic understanding [22]. The models have been applied in few cases also to pharmacokinetics in the tear fluid [8,23–26]. The models and simulations are tools that can reduce the use of laboratory animals, speed up drug development and reduce the costs of the development processes.

Our main objective was to investigate kinetics in the tear fluid of dexamethasone after being administered as eye drops (solution and suspension) or hydrogels. Hydrogels based on 2-hydroxyethyl methacrylate were prepared and characterized for their physicochemical properties, dexamethasone loading, and release. Then, dexamethasone levels were evaluated in rabbit tear fluid after topical delivery of the formulations. Pharmacokinetic models and simulations were used to improve understanding of dexamethasone and formulation kinetics in the tear fluid. Excellent *in vitro – in vivo* correlation was achieved with the hydrogels.

2. Materials and methods

2.1. Materials

HEMA and AAc were from Merck (Darmstadt, Germany). Ethylene glycol dimethacrylate (EGDMA), trifluoroacetic acid (TFA), 2,2′-azobis (2-methylisopropionitrile) (AIBN), dichlorodimethylsilane, and dimethyl sulfoxide (DMSO) were from Sigma-Aldrich (St. Louis Missouri, USA). Acetonitrile (ACN) was from Honeywell International Inc. (Morris plains, New Jersey, USA). Phosphate buffer saline (PBS) was from Life Technologies Limited (Paisley, UK). Dexamethasone was purchased from Sigma-Aldrich (Steinheim am Albuch, Germany) and dexamethasone-d5 from Toronto Research Chemicals Inc (Ontario, Canada). The dexamethasone solution (0.01%) was prepared in PBS, pH 7.4 by diluting it from 10 mg/mL stock solution in DMSO. The commercial dexamethasone suspension Maxidex® 0.1% (Alcon Laboratories, Inc.; South Freeway Fort Worth, Texas, USA) was used.

2.2. Polymerization and hydrogel manufacture

Three sets of monomer mixtures were prepared (Table 1). HEMA, EGDMA, and AAc were poured into vials to which AIBN was added and then they were mixed at room temperature under magnetic stirring at 400 rpm. After complete dissolution the mixtures were injected into molds of two glass plates (14 cm × 12 cm) that had been pretreated with dichloromethylsilane and separated by a silicone frame (thickness 0.3 mm). The molds were transferred to oven where polymerization was carried out at +50 °C for 12 h and then at +70 °C for 24 h.

Table 1
Composition of the monomer mixtures in hydrogels.

Material	Hydrogel 1	Hydrogel 2	Hydrogel 3
HEMA (M)	8	8	8
EGDMA (mM)	8	8	8
AIBN (mM)	10	10	10
AAc (mM)	–	100	200

HEMA 2-hydroxyethyl methacrylate, EGDMA = ethylene glycol dimethacrylate, AIBN = azobisisobutyronitrile , AAc = Acrylic acid.

After polymerization, the hydrogel sheets were cleaned in boiling distilled water for 15 min to remove unreacted monomers. Then, they were cut into circular discs with 10 mm diameters. The hydrogels were placed in Milli-Q water under magnetic stirring (200 rpm) at room temperature for 3 days. The water was replaced 3 times a day to remove unreacted monomers and the presence of remnants was monitored by measuring absorbance of water medium (UV–Vis spectrophotometer Agilent 8453, Germany) in the 190 – 800 nm range. Finally, the hydrogels were dried at 40 °C for 24 h.

2.3. Swelling, transmittance and mechanical properties

Swelling of the hydrogels was studied in water and in 0.9% sodium chloride (4 mL) at room temperature for 24 h. After incubation, the hydrogels were removed and patted dry with tissue and weighed at predetermined time points. Their initial dry weights were recorded before placing them into solution. The percentage of swelling of each hydrogel was calculated as follows (Eq. (1)):

$$Swelling\% = \frac{Ws - Wd}{Wd} * 100 \quad (1)$$

where, W_d and W_s represent the weight of initial dried and swollen hydrogels, respectively.

Transmittance of swollen hydrogels, after fitting to the wall of a quartz cell filled with water, was recorded in the 190–800 nm range (UV–Vis Spectrophotometer Agilent 8453, Germany).

Stress–strain plots of water-swollen discs were recorded in duplicate using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Ltd., Surrey, UK) fitted with a 5 Kg load cell. The discs were fixed to the upper and lower clamps (4 mm gap) and subjected to a crosshead speed of 0.1 $mm{\cdot}s^{-1}$. The Young's modulus was calculated from the slope of the linear portion of the tensile stress versus tensile strain curves.

2.4. HET-CAM test

Ocular irritation potential of hydrogels was evaluated with Hen's Egg Test on Chorio-Allantoic Membrane (HET-CAM). Fertilized hen's eggs (50–60 g; Coren, Spain) were incubated at 37 °C and 60% relative humidity for 8 days, as previously described [27]. After incubation (9th day), the eggs were cut open on the air space using a rotary saw (Dremel 3000, Konijnenberg, Breda, Netherlands) to expose the inner membrane, which was hydrated with 0.9 % sodium chloride solution for 30 min. Then, the solution was carefully pipetted out and the hydrated inner membrane was removed to permit feasible access to the CAM. Then, hydrogels were laid on top of the CAM for 5 min. In parallel, 0.9% sodium chloride and 0.1 N sodium hydroxide solutions (300 μL) were tested as negative and positive controls, respectively. During 5 min, the vessels of the CAM were observed for hemorrhage, vascular lysis, or coagulation.

2.5. In vitro release of dexamethasone from hydrogels

2.5.1. Dexamethasone loading and release

Loading. The dried hydrogels were weighed and immersed in PBS

solution (4 mL) containing 0.1 mg/mL of dexamethasone. The hydrogels were incubated at room temperature without shaking and samples (100 μL) were collected from drug loading solution at pre-determined time points for up to 24 h. The drug concentration was determined with HPLC, and the amount of dexamethasone loaded to the hydrogels was calculated from the difference between the initial and final amount of drug in the loading solution.

Release. Dexamethasone-loaded hydrogels were gently wiped with paper and immersed in 2 mL of release solution (0.9% sodium chloride solution) at +37 °C under stirring and maintained sink condition with replacement of the release solution (2 mL) at every sampling point till 48 h with an equal volume of fresh release solution to retain the needed driving force for drug release. The collected samples were stored at −20 °C until HPLC analysis.

2.5.2. HPLC analysis

The standards and quality controls. The calibration standards were prepared in duplicate at the concentration range of 5–100 μg/mL. The quality control samples (40 and 80 μg/mL) were independently prepared in triplicate.

HPLC conditions. The HPLC analysis was performed with Shimadzu Technologies HPLC control system, CBM-10 AV equipped with an autoinjector (SIL −20AC), a pump (LC −20AD), column oven (CTO-10AV), and a UV/Visible detector (SPD −10AV). Zorbax Eclipse XCB-C18 column (4.6 × 50 mm, 1.8 μm; Agilent) column maintained at 40 °C was used for analysis. The isocratic mobile phase contained 0.1% trifluoroacetic acid (Sigma-Aldrich) in acetonitrile (LC-MS Chromasolv, Honeywell, Riedel-de Haen) and 0.1% trifluoroacetic acid in Milli-Q-H_2O (30:70 v/v). Flow rate was 1 mL/min, injection volume was 5 μL and the detection wavelength was 240 nm. The retention time of dexamethasone was 3.1 min. The mobile phase was degassed before use.

2.5.3. Statistical analysis

Statistical analysis for comparison of characterization, *in vitro* loading and release properties of hydrogels was performed using one-way ANOVA test where level of significance was $P < 0.05$ in Excel. Then, individual *t*-test for two groups assuming equal variance was performed followed by Bonferroni correction (with two tail P value < 0.0167).

2.6. Pharmacokinetics in rabbit tear fluid

2.6.1. In vivo experiment

New Zealand male albino rabbits (2.5–4 kg, Envigo Laboratories, UK) were used in the study. The animals were housed individually under standard laboratory conditions of 12-h dark-light cycles and were provided with a normal pellet diet with water ad libitum. Animal studies demand set by EU directive 2010/63/EU and all animal experiments were approved by the National Project Authorization Board. During the experiment, the rabbits were restrained in holders, but they could move their heads freely.

The hydrogel (#3, Table 1), dexamethasone solution (0.01% in PBS) and commercial dexamethasone suspension Maxidex® 0.1% (Alcon) were tested *in vivo* (n = 6–8). The hydrogels were autoclaved (121 °C, 30 min) and loaded with dexamethasone under aseptic condition as described above. After drug loading, the hydrogels were cut into smaller pieces (diameter of 8 mm) with a sterilized cutting tool. The loaded hydrogels were gently wiped with paper and carefully placed on the rabbit cornea under the nictitating membrane without local anesthesia. The dexamethasone solution and suspension were administered onto the upper cornea-scleral limbus of the rabbit eye (25 μL/eye) and the eye was kept open for 1 min after installation. The experiments were repeated with the same set of rabbits after three weeks.

Tear fluid samples (1 μL) were collected at the edge of the lower eyelid at pre-determined time points using 1 μL disposable capillary (Drummond Scientific Company, USA). The collected tear fluid samples were stored at −80 °C until LC-MS/MS analysis.

2.6.2. LCMS/MS analysis

Samples. The tear fluid samples were thawed on ice for 10 min and then spun down for 1 min at 13,000 rpm, +4 °C. The samples were diluted (1:20–1:2000 depending on time points) with the internal standard solution (40 ng/mL dexamethasone-d5 in Milli-Q water with 1% formic acid and 50% ACN). Then, the samples were vortexed for 1 min, centrifuged at 14,000 rpm for 10 min at +4 °C and the supernatants were collected for LC-MS/MS analyses.

The standards and quality controls. The calibration standards (0.5–500 ng/ml) were prepared in duplicate in internal standard solution. The quality control samples were independently prepared at three concentrations (2.5, 25, 250 ng/mL). The internal standard solution described above was used as the diluent. The standards and quality controls were processed in the same way as the samples.

LC-MS/MS. The LC-MS/MS system consisted of an Agilent 1290 series liquid chromatograph and an Agilent 6495 triple-quadruple mass spectrometer (Agilent Technologies, Inc., USA) with electrospray ionization. Poroshell 120 SB-C18 column (2.1 mm × 50 mm, 2.7 μm, Agilent) was maintained at 50 °C. A binary mobile phase with a gradient elution was used. Solvent A was 0.1 % formic acid in milli-Q water and solvent B was methanol. For solution and hydrogel samples the gradient was: 0.0 to 2.0 min: 30%→90% B, 2.0 to 2.5 min: 90% B, 2.5 to 2.6 min: 90%→30% B, 2.6 to 4.0 min 30% B. The flow rate was 0.5 mL/min. The gradient for suspension samples was as follows: 0.0 to 5.0 min: 40%→100% B, 5.0 to 5.5 min: 100% B, 5.5 to 5.6 min: 100%→40% B, 5.6 to 7.0 min 40% B The flow rate was 0.3 mL/min. The injection volume was 2 μL. Nitrogen was used as a drying, nebulizer, and collision gas. The following ion source conditions were employed: positive ion mode, drying gas temperature 200 °C, drying gas flow 16 L/min, nebulizer pressure 25 psi. The data was analyzed with Agilent Mass Hunter Quantitative Analyzed software (vB.09.00, build 9.0.647.0, Agilent Technologies, CA, USA). The MS/MS parameters are presented in supplementary information, Table S1.

2.6.3. Pharmacokinetic analysis

Non-compartmental analysis (NCA) of mean dexamethasone concentrations in tear fluid after application of solution, suspension and hydrogel were conducted with Phoenix WinNonlin (build 8.3, Certara L. P.). Moreover, compartmental naïve-pooled analysis was also conducted for the solution and suspension administrations using one- and two-compartment models using uniform, 1/predicted concentration (1/Yhat) and 1/predicted concentration^2 (1/Yhat^2) weighting methods in curve fitting. Based on visual inspection of the plot and coefficient of variation (CV%) of estimated parameters one or two compartmental model was selected.

Areas under the curve ($AUC_{0-\infty}$, $AUC_{0\text{-}15\ min}$, $AUC_{0\text{-}120\ min}$) were estimated. We further estimated a theoretical AUC value for the dissolved dexamethasone fraction in the suspension ($AUC_{soluble}$). Based on dexamethasone solubility of 0.11 μg/μL [28], the fraction dissolved ($F_{soluble}$) was calculated:

$$F_{soluble} = \frac{Solubility}{ConcentrationofMaxidex} \quad (2)$$

And used in following equation:

$$AUC_{soluble} = AUC_{suspension} * (F_{soluble}) \quad (3)$$

2.7. Pharmacokinetic simulations

STELLA® software (v8.1.1, isee systems) with fourth order Runge-Kutta integration algorithm was used in the simulation. The pharmacokinetic model for rabbit eye was built modifying our earlier models ([29]; Fig. 1). Since the release of dexamethasone from the hydrogel during first two hours followed better zero order kinetics than first order

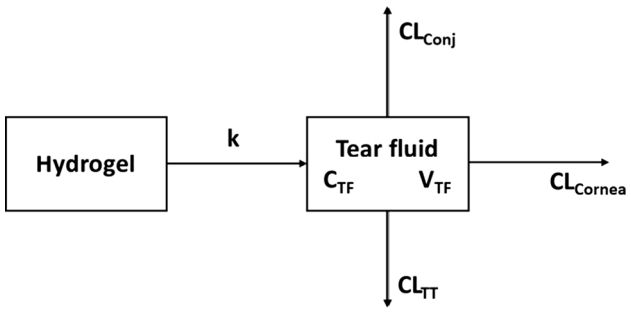

Fig. 1. A model for dexamethasone kinetics in the tear fluid after hydrogel application. Meaning of the symbols are as follows. k, release rate of dexamethasone from the hydrogel; CL_{Conj}, clearance from tear fluid by conjunctival absorption; V_{TF}, tear fluid volume; CL_{Cornea}, clearance from tear fluid by corneal absorption; C_{TF} concentration of dexamethasone in tear fluid; CL_{TT}, clearance from lacrimal fluid by tear turnover.

kinetic, the *in vitro* release rate (k = 18.2 μg/h) was used in the model as drug input for the simulation of two hours. The simulation was run using two conjunctival clearance (CL_{conj}) values in rabbit eyes: experimental value for timolol (10.4 μL/min) [30], and the estimated value for dexamethasone (31.95 μL/min). The estimated CL_{conj} was calculated based on permeability in the conjunctiva (P_{conj})(7.5×10^{-5} cm/s, [31]) and half of total conjunctival surface area (S_{conj}) of 14.2 cm^2 [32]. Half of the S_{conj} was used assuming that the administered drug would be absorbed across conjunctiva mainly from the lower fornix of conjunctiva The parameter values are given in the supplementary information (Table S2).

Clearance of dexamethasone (CL_{total}) from tear fluid was estimated as follows.

$$CL_{total} = CL_{conj} + CL_{TT} + CL_{cornea}. \quad (4)$$

Then, the predicted concentration of dexamethasone in tear fluid should be dependent on drug release rate from hydrogel (J) and clearance of released drug from the tear fluid (CL_{total}) (Eq. (5)) The *in vivo* release rate was assumed to be equal to the *in vitro* release rate.

$$C_{SS} = \frac{J}{CL_{total}} \quad (5)$$

The simulated dexamethasone concentrations in tear fluid were then compared to the experimental values from *in vivo* study.

3. Results

3.1. Characterization of the hydrogels

Dried hydrogels were transparent and swelled rapidly upon immersion in water and 0.9% sodium chloride solution (Fig. 2). Liquid uptake was dependent on the amount of AAc in the hydrogels. All swollen hydrogels were optically transparent (>80%) and had similar Youngs moduli, with mean values of 0.41, 0.47, and 0.44 MPa for hydrogels 1, 2, and 3, respectively (no statistical differences). The stress–strain plots of water-swollen hydrogels are shown in Supplementary information (Fig. S1).

3.2. Biocompatibility assessment with HET-CAM test

In HET-CAM the hydrogels did not induce lysis, hemorrhage, or coagulation (Fig. 3).

3.3. Dexamethasone loading and release in vitro

A rapid increase in dexamethasone loading took place, mostly in 2 h for all hydrogels (Fig. S2). AAc slightly increased the loading levels of dexamethasone in the hydrogels (Table 2).

A rapid release of dexamethasone (average rate 18.2 μg/h) was observed from the hydrogels in the first two hours (Fig. 4). Thereafter, gradually declining release rate was seen until 12 h and then the release remained relatively constant. Dexamethasone release was fastest from hydrogel-3 (with highest AAc concentration) and slowest from hydrogel-1 (Fig. 4). However, not all dexamethasone was released in 48 h. The released percentage of the loaded dexamethasone amounts at 48 h were 61.2 ± 11.2, 68.1 ± 7.3, and 75.9 ± 6.9 % for hydrogel-1, hydrogel-2 and hydrogel-3, respectively.

3.4. In vivo pharmacokinetics in rabbit tear fluid

Hydrogel-3 was selected to the *in vivo* studies, and it was well tolerated, causing no visible symptoms (redness, swelling). Since different doses were used (solution 2.5 μg, suspension 25 μg and hydrogel 56.88 μg), we dose normalized the tear fluid concentrations to dexamethasone dose in the suspension (25 μg) assuming linear kinetics. The dose normalized (25 μg) concentrations of dexamethasone in tear fluid are shown in Fig. 5, while actual dexamethasone concentrations in the tear fluid without dose-normalization are in Fig. S3.

The dexamethasone concentrations in the tear fluid after topical hydrogel administration remained rather constant for 120 min (mean concentrations were 18 ± 1.8 μg/mL). After instillation of a suspension, dexamethasone concentrations in tear fluid dropped ≈ 5-fold within 5

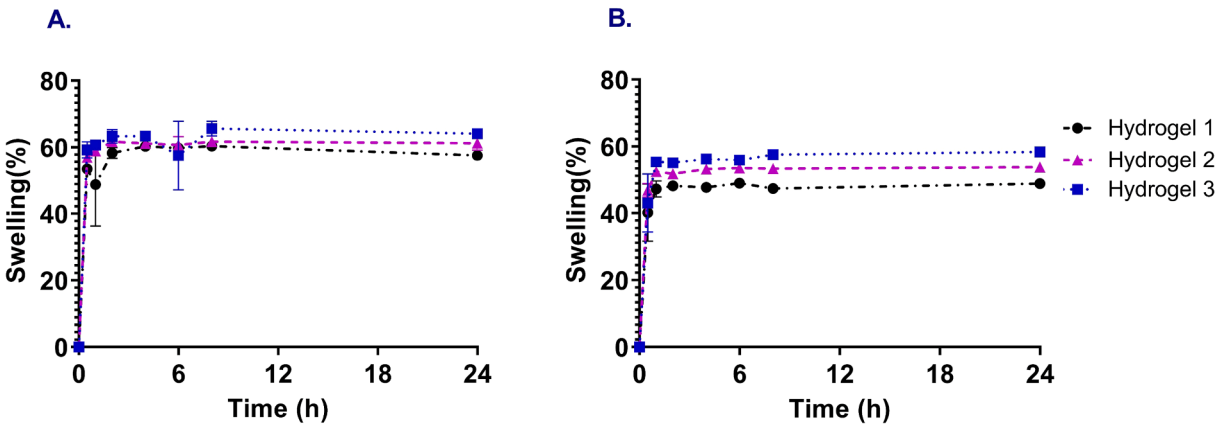

Fig. 2. Swelling (%) of hydrogels in A) water, and B) 0.9% sodium chloride solution during 24 h. The results are expressed as a mean ± standard deviation (SD), n = 3.

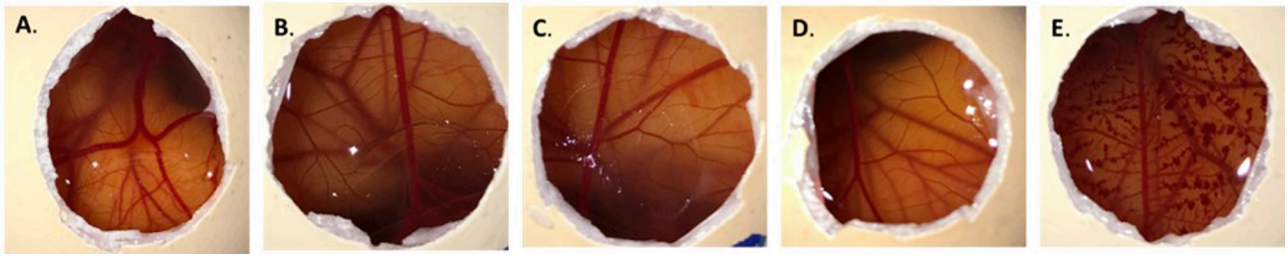

Fig. 3. Chorioallantoic membranes after 5 min exposure to A) hydrogel-1, B) hydrogel-2, C) hydrogel-3, D) 0.9% sodium chloride (negative control) and E) 0.1 N sodium hydroxide (positive control).

Table 2
Amounts of dexamethasone loading into hydrogels during 24 h incubation and dexamethasone release from hydrogels during 48 h (mean ± standard deviation (SD), n = 3).

Hydrogel	AAc (mM)	Dexamethasone loading (μg/mg dry hydrogel)	Dexamethasone release rate (μg/mg dried hydrogel)
Hydrogel-1	–	4.5 ± 0.2	2.8 ± 0.6
Hydrogel-2	100	4.8 ± 0.3	3.3 ± 0.1
Hydrogel-3	200	5.1 ± 0.3	3.8 ± 0.1

min (from 274.6 ± 60.2 to 58.4 ± 25.8 μg/mL) and further ≈100-fold decrease was seen in 20 min post-application (to 2.8 ± 0.4 μg/mL). Thereafter, a slower decrease in the dexamethasone concentration was observed until 120 min (0.04 ± 0.01 μg/mL).

After instillation of dexamethasone solution, the drug concentration in the tear fluid decreased ≈13 times, from 1.9 ± 0.4 μg/mL at 5 min to 0.15 ± 0.01 μg/mL at 15 min after application (Fig. S3). After that time point the dexamethasone concentrations were below the limit of quantification.

3.5. Pharmacokinetic analysis and parameters

One compartmental model was fit to dexamethasone tear fluid concentrations after solution administration, while 2- compartmental model was fitted for the suspension concentration data. The $1/yhat^2$ weighing method was selected for solution and 1/yhat weighing method was used for suspension, based on visual inspection and low CV% compared to other methods as done in previous study. Supporting information, Fig. S4.

The AUCs of dexamethasone in tear fluid are reported in Table 3. The rank order of AUC values was hydrogel > suspension > solution.

Because tear fluid samples after suspension administration contain both soluble and particulate dexamethasone, $F_{soluble}$ value of 0.11 was

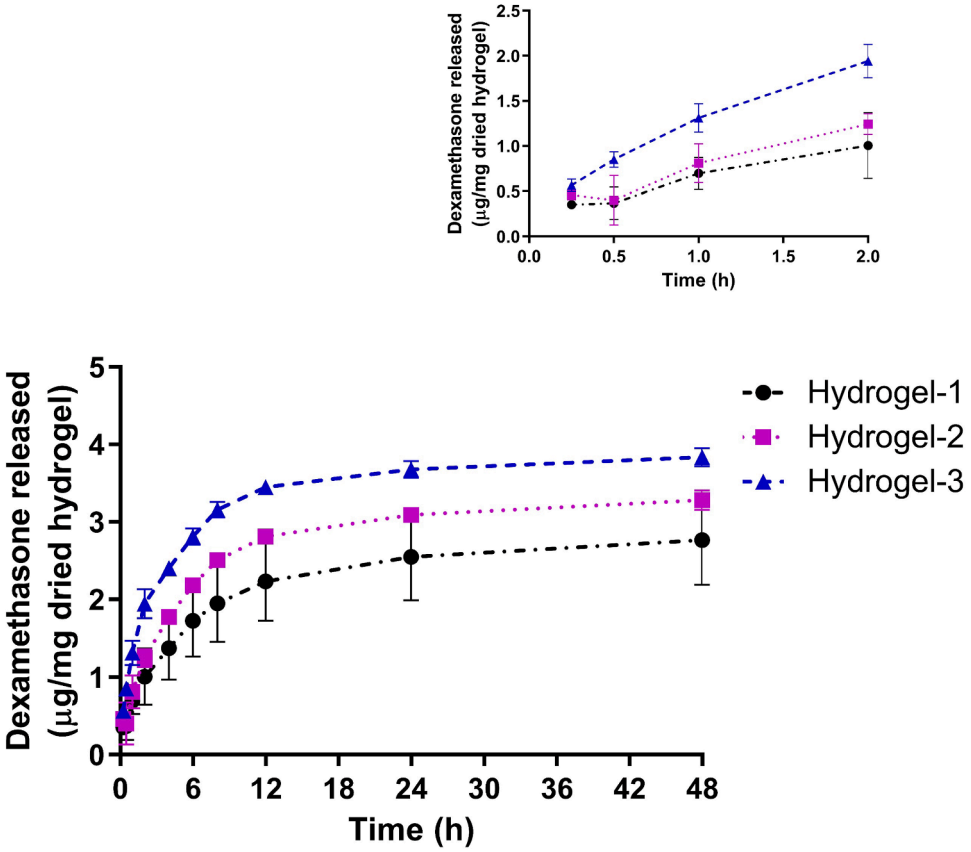

Fig. 4. Cumulative dexamethasone release in 0.9% sodium chloride from hydrogel-1(circle), hydrogel-2 (square), and hydrogel-3 (triangle) as a function of time. The results are expressed as a mean ± standard deviation (n = 3).

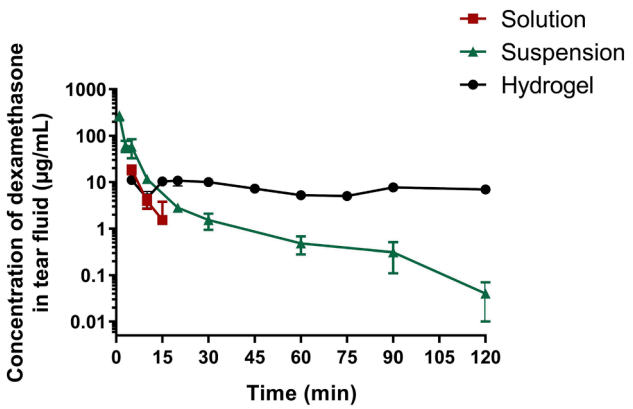

Fig. 5. Dexamethasone concentrations in the tear fluid after topical application of dexamethasone suspension (Maxidex®, 25 µg dose, triangles), dexamethasone solution (normalized to 25 µg dose, squares) and dexamethasone-loaded hydrogel (normalized to 25 µg dose, circles). Means ± SEM are shown (n = 5–8).

Table 3
Area under curve (AUC) values of dexamethasone in tear fluid after topical administration of a solution, suspension and hydrogel. CA = compartmental analysis. NCA = non-compartmental analysis.

Formulation (Dose, µg)	CA $AUC_{0\ to\ \infty}$ (min*µg/mL)	NCA $AUC_{(0\text{-}15\ min)}$ (min*µg/mL)	NCA $AUC_{(0\text{-}120\ min)}$ (min*µg/mL)
Solution (2.5 µg)	29.7	28.7	29.3
Suspension (25 µg)	889*	999	1083
	97.8*◇	109.9◇	119.2◇
Hydrogel (56.9 µg)	–	368	2117

*: $AUC_{0\text{-}120\ min}$ corresponding to the initial phase of the two-compartmental model fitted to the concentration–time profile of dexamethasone suspension in tear fluid (productive phase when the drug ocular absorption occurs)
$AUC_{0\ to\ \infty}$: Area under curve from 0 to infinity.
$AUC_{(0\text{-}15\ min)}$: Area under curve from 0 to 15 min.
$AUC_{(0\text{-}120\ min)}$: Area under curve from 0 to 120 min.
◇: estimated contribution of soluble dexamethasone fraction (0.11) (Eq. (3)).

used to determine AUC for the absorbable dissolved dexamethasone ($AUC_{soluble}$) in the suspension. $AUC_{soluble}$ during 0–15 min was 109.9 min*µg/mL, that is 3.8-fold higher than $AUC_{0\text{-}15min}$ of the solution (28.7 min*µg/mL) and 3.3-fold lower than the $AUC_{(0\text{-}15\ min)}$ of the hydrogel (368 min*µg/mL).

3.6. Simulation of dexamethasone concentrations in the tear fluid

The simulated and measured dexamethasone concentrations in tear fluid after hydrogel administration are shown in Fig. 6. We used *in vitro* release rate (18.2 µg/h) during the first two hours in the simulations. Two CL_{conj} values were used (10.4 µL/min, 31.95 µL/min) were used in the simulations to estimate dexamethasone concentration in the tear fluid. The experimentally determined dexamethasone concentrations were in the same range with the simulated values (Fig. 6) suggesting that the hydrogels are releasing dexamethasone *in vivo* approximately at the same rate as in *in vitro* conditions.

4. Discussion

In this study, we present ocular pharmacokinetic analysis of topical hydrogel, placed on the surface of eye as contact lens, solution and suspension using dexamethasone as the drug. HEMA was chosen as the main component of the hydrogels because it is widely used in commercial soft contact lenses [33–35]. The swelling of synthesized hydrogels in water was found to be in a similar range as previously reported [27,36]. An initial burst of uptake and release of dexamethasone was observed in all three hydrogel formulations. This is not surprising as these hydrogels have relatively high-water content (≈60 %) and dexamethasone is a small molecule (392.5 g/mol). Previously, similar rapid release kinetics from hydrogels were reported for other small molecule drugs (e.g. cromolyn sodium, ketorolac tromethamine, ciprofloxacin) [37,38]. Maximum uptake (5.07 ± 0.30 µg/mg dried hydrogel) of dexamethasone was observed in hydrogel containing highest AAc concentration. Carboxylic acid moiety of AAc contributes to a higher affinity for dexamethasone through weak interactions with the hydroxyl and carbonyl groups of dexamethasone. In another study [39], the *in vitro* uptake of dexamethasone sodium phosphate (0.845 mg/ml) ranged from 58 to 88 ug/lens in commercial hydrogels with HEMA polymer but the study lacked the release data. In our *in vitro* study, dexamethasone release ranged from 61 to 76% with rapid release within initial two hours. In previous study [40], the *in vitro* release percentage of dexamethasone 21 phosphate disodium salt from commercial hydrogels with HEMA polymer ranged from 44 to 84 % with maximal release within the initial hour, which is in line with our release data.

In our *in vivo* study, dexamethasone concentration in the tear fluid was 15.8 ± 2.8 µg/mL at 120 min after hydrogel administration. $AUC_{0\text{-}120min}$ value was about 72 times higher for hydrogel (2116.8 min µg/mL) as compared to the solution (29.3 min µg/mL). The higher precorneal

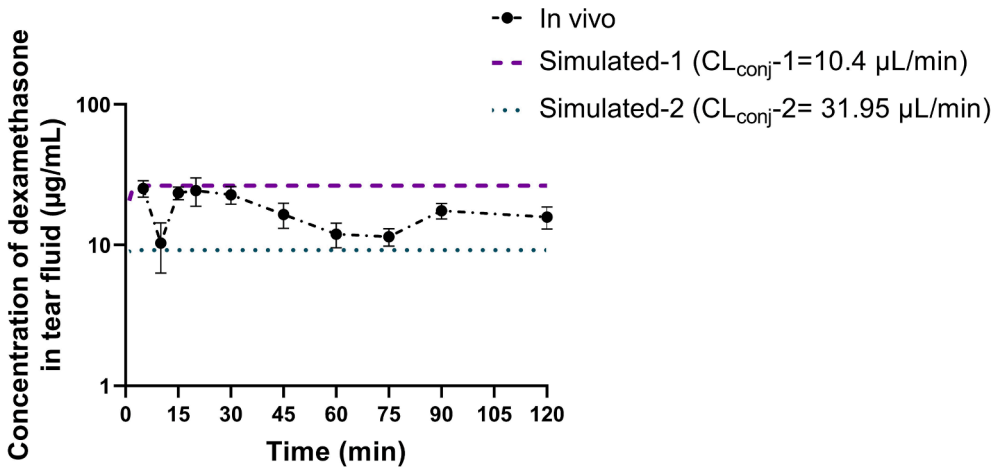

Fig. 6. Simulated and observed *in vivo* dexamethasone tear fluid concentrations after application of hydrogel. The observed *in vivo* results are expressed as mean ± standard error of the mean (SEM) (n = 5).

retention and exposure in the tear fluid indicates better efficacy of dexamethasone hydrogel. To our knowledge, no prior *in vivo* study comparing tear fluid concentrations of dexamethasone exist in the literature. While, previous *in vivo* studies carried out in rabbit have demonstrated 1.5-fold and 7-fold longer retention of timolol and ketotifen fumarate respectively in tear fluid following hydrogel application compared to solutions [43,44]. In another *in vivo* pharmacokinetic study in rabbit, a dramatic increase in mean residence time (>100 fold) and AUC (>1000 fold) of hyaluronic acid in tear fluid was observed with hydrogel administration [45].

A single dose of nearly saturated dexamethasone solution (0.01%) or dexamethasone suspension (0.1%) was topically applied to rabbits *in vivo*. The dexamethasone tear fluid concentrations after solution administration were one order of magnitude lower than after suspension delivery (Fig. S2). As dexamethasone exists as both solid and dissolved forms in the suspension samples, we further compared AUC for absorbable dissolved dexamethasone to the solution. In addition to higher exposure of absorbable dexamethasone in tear fluid (Table 3), the suspension retained longer in tear fluid as compared to solution (Fig. 5). The AUC values for absorbable dexamethasone for initial 15 min from three formulations showed that hydrogel (368 min μg/mL) provided the highest drug exposure in the tear fluid while the solution provided the lowest values (28.7 min μg/mL).

In vivo drug release rate in the lacrimal fluid may deviate from *in vitro* release rate in sink conditions. The reasons for the possible deviations include: (1) different compositions and concentrations of the buffers in the media; (2) different volumes of the release media *in vivo* and *in vitro*; (3) reflex eye blinking; (4) drug solubility; (5) loss of sink conditions *in vivo* for many reasons. *In vivo* release in the tear fluid is not well understood, and *in vitro – in vivo* correlation would be desirable for drug development. Previously release of relatively water-soluble timolol from silicone inserts was estimated to be equal *in vitro* and *in vivo* in the tear fluid of rabbits [46] and humans [26]. Deviations between *in vitro* and *in vivo* release rates are more likely with poorly water-soluble drugs since *in vitro* release studies are performed in sink conditions to avoid any solubility limitations in drug release. Even though tear fluid has very small volume (7 μL), *in vivo* release of dexamethasone was estimated to be similar with *in vitro* release based on our simulations (Fig. 6). This is due to the effective clearance of the released dexamethasone from the tear fluid. Indeed, the average dexamethasone tear fluid concentrations after hydrogel administration (18 ± 1.8 μg/mL) is less than 20% of the solubility of dexamethasone in water (110 μg/mL) [28], suggesting that the sink conditions are maintained *in vivo* in the tear fluid.

5. Conclusion

Pharmacokinetics of dexamethasone in lacrimal fluid was evaluated after delivery in solution, suspension, and hydrogel. The hydrogel delivery resulted in highest soluble dexamethasone exposure and prolonged concentrations in the tear fluid. Also, instillation of suspension resulted in increased exposure to soluble dexamethasone in lacrimal fluid as compared to the solution. Results from pharmacokinetic simulations and experimental data suggest that the hydrogel released dexamethasone similarly *in vitro* and *in vivo* suggesting that sink conditions were maintained for dexamethasone in the lacrimal fluid. Successful *in vitro in vivo* extrapolation will be useful in further development of gels and other topical ocular formulations. The presented gel formulations have potential as long-acting drug delivery system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work is supported by IT-DED3 from European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No GA 765608 and by the Government of Russian Federation Mega-grant W03.031.0025. The School of Pharmacy mass spectrometry laboratory is supported by Biocenter Kuopio and Biocenter Finland.

Mrs. Lea Pirskanen is acknowledged for her technical support and Dr. Soledad Anguiano Igea and Angela Varela for their guidance on hydrogel preparation.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2022.01.005.

References

[1] A. Patel, Ocular drug delivery systems: an overview, World J. Pharmacol. 2 (2) (2013) 47, https://doi.org/10.5497/wjp.v2.i2.47.
[2] K. Cholkar, S.P. Patel, A.D. Vadlapudi, A.K. Mitra, Novel strategies for anterior segment ocular drug delivery, J. Ocul. Pharmacol. Ther. 29 (2) (2013) 106–123, https://doi.org/10.1089/jop.2012.0200.
[3] C. Le Bourlais, L. Acar, H. Zia, P.A. Sado, T. Needham, R. Leverge, Ophthalmic drug delivery systems – recent advances, Prog. Retin. Eye Res. 17 (1) (1998) 33–58, https://doi.org/10.1016/S1350-9462(97)00002-5.
[4] D. Gulsen, A. Chauhan, Ophthalmic drug delivery through contact lenses, Investig. Ophthalmol. Vis. Sci. 45 (2004) 2342–2347, https://doi.org/10.1167/iovs.03-0959.
[5] T. Sørensen, F.T. Jensen, Tear flow in normal human eyes. Determination by means of radioisotope and gamma camera, Acta Ophthalmol. 57 (4) (1979) 564–581, https://doi.org/10.1111/j.1755-3768.1979.tb00504.x.
[6] J.W. Sieg, J.R. Robinson, Vehicle effects on ocular drug bioavailability I: Evaluation of fluorometholone, J. Pharm. Sci. 64 (6) (1975) 931–936, https://doi.org/10.1002/jps.2600640606.
[7] A. Fayyaz, V.-P. Ranta, E. Toropainen, K.-S. Vellonen, A. Valtari, J. Puranen, M. Ruponen, I. Gardner, A. Urtti, M. Jamei, E.M. del Amo, Topical ocular pharmacokinetics and bioavailability for a cocktail of timolol, betaxolol and atenolol in rabbits, Eur. J. Pharm. Sci. 155 (2020) 105553, https://doi.org/10.1016/j.ejps.2020.105553.
[8] E. Toropainen, S.J. Fraser-Miller, D. Novakovic, E.M. Del Amo, K.-S. Vellonen, M. Ruponen, T. Viitala, O. Korhonen, S. Auriola, L. Hellinen, M. Reinisalo, U. Tengvall, S. Choi, M. Absar, C. Strachan, A. Urtti, Biopharmaceutics of topical ophthalmic suspensions: Importance of viscosity and particle size in ocular absorption of indomethacin, Pharmaceutics 13 (4) (2021) 452, https://doi.org/10.3390/pharmaceutics13040452.
[9] B.F. Gomes, A.F. Paredes, N. Madeira, H.V. Moraes, M.R. Santhiago, Assessment of eye drop instillation technique in glaucoma patients, Arq. Bras. Oftalmol. 80 (2017) 238–241, https://doi.org/10.5935/0004-2749.20170058.
[10] A. Ludwig, The use of mucoadhesive polymers in ocular drug delivery, Adv. Drug Deliv. Rev. 57 (11) (2005) 1595–1639, https://doi.org/10.1016/j.addr.2005.07.005.
[11] A.S. Mundada, J.G. Avari, In situ gelling polymers in ocular drug delivery systems: a review, Crit. Rev. Ther. Drug Carrier Syst. 26 (1) (2009) 85–118, https://doi.org/10.1615/CritRevTherDrugCarrierSyst.v26.i1.30.
[12] P.K. Suresh, G. Barsa, A.K. Sah, S.J. Daharwal, Ocular implants as drug delivery device in opthalmic therapeutics: an overview, Res. J. Pharm. Technol. 7 (2014) 665–676.
[13] C. González-Chomón, A. Concheiro, C. Alvarez-Lorenzo, Soft contact lenses for controlled ocular delivery: 50 years in the making, Ther. Deliv. 4 (9) (2013) 1141–1161, https://doi.org/10.4155/tde.13.81.
[14] P.-L. Destruel, N.i. Zeng, M. Maury, N. Mignet, V. Boudy, In vitro and in vivo evaluation of in situ gelling systems for sustained topical ophthalmic delivery: state of the art and beyond, Drug Discov. Today. 22 (4) (2017) 638–651, https://doi.org/10.1016/j.drudis.2016.12.008.
[15] T.J. Nagelhout, D.A. Gamache, L. Roberts, M.T. Brady, J.M. Yanni, Preservation of tear film integrity and inhibition of corneal injury by dexamethasone in a rabbit model of lacrimal gland inflammation – induced dry eye, J. Ocul. Pharmacol. Ther. 21 (2) (2005) 139–148, https://doi.org/10.1089/jop.2005.21.139.
[16] R.C. Nagarwal, S. Kant, P.N. Singh, P. Maiti, J.K. Pandit, Polymeric nanoparticulate system: a potential approach for ocular drug delivery, J. Control. Release 136 (1) (2009) 2–13, https://doi.org/10.1016/j.jconrel.2008.12.018.
[17] Y.H. Yung, I. Toda, C. Sakai, A. Yoshida, K. Tsubota, Punctal plugs for treatment of post-LASIK dry eye, Jpn. J. Ophthalmol. 56 (3) (2012) 208–213, https://doi.org/10.1007/s10384-012-0125-8.
[18] A.-M. Lobo, L. Sobrin, G.N. Papaliodis, Drug delivery options for the treatment of ocular inflammation, Semin. Ophthalmol. 25 (5-6) (2010) 283–288, https://doi.org/10.3109/08820538.2010.518522.

[19] P.C. Nicolson, J. Vogt, Soft contact lens polymers: an evolution, Biomaterials 22 (24) (2001) 3273–3283, https://doi.org/10.1016/S0142-9612(01)00165-X.
[20] J. Kopecek, Hydrogels: from soft contact lenses and implants to self-assembled nanomaterials, J. Polym. Sci., Part A: Polym. Chem. 47 (22) (2009) 5929–5946, https://doi.org/10.1002/pola.23607.
[21] S. Gause, K.H. Hsu, C. Shafor, P. Dixon, K.C. Powell, A. Chauhan, Mechanistic modeling of ophthalmic drug delivery to the anterior chamber by eye drops and contact lenses, Adv. Colloid Interface Sci. 233 (2016) 139–154, https://doi.org/10.1016/j.cis.2015.08.002.
[22] R. Robinson, Nimit Worakul, Ocular pharmacokinetics/pharmacodynamics, Eur. J. Pharmac. Biopharmac. 44 (1997) 71–83, https://doi.org/10.1016/S0939-6411(97)00064-7.
[23] K.J. Himmelstein, I. Guvenir, T.F. Patton, Preliminary pharmacokinetic model of pilocarpine uptake and distribution in the eye, J. Pharm. Sci. 67 (5) (1978) 603–606, https://doi.org/10.1002/jps.2600670507.
[24] M.C. Makoid, J.W. Sieg, J.R. Robinson, Corneal drug absorption: an illustration of parallel first-order absorption and rapid loss of drug from absorption depot, J. Pharm. Sci. 65 (1) (1976) 150–152, https://doi.org/10.1002/jps.2600650141.
[25] M.C. Makoid, J.R. Robinson, Pharmacokinetics of topically applied pilocarpine in the albino rabbit eye, J. Pharmac. Sci. 68 (4) (1979) 435–443, https://doi.org/10.1002/jps.2600680411.
[26] A. Urtti, H. Rouhiainen, T. Kaila, V. Saano, Controlled ocular timolol delivery: systemic absorption and intraocular pressure effects in humans, Pharm. Res. Off. J. Am. Assoc. Pharm. Sci. 11 (1994) 1278–1282, https://doi.org/10.1023/A:1018938310628.
[27] F. Alvarez-Rivera, A.P. Serro, D. Silva, A. Concheiro, C. Alvarez-Lorenzo, Hydrogels for diabetic eyes: naltrexone loading, release profiles and cornea penetration, Mater. Sci. Eng., C 105 (2019) 1–9, https://doi.org/10.1016/j.msec.2019.110092.
[28] T. Loftsson, H. Friðriksdóttir, S. Thórisdóttir, E. Stefánsson, The effect of hydroxypropyl methylcellulose on the release of dexamethasone from aqueous 2-hydroxypropyl-β-cyclodextrin formulations, Int. J. Pharm. 104 (2) (1994) 181–184, https://doi.org/10.1016/0378-5173(94)90194-5.
[29] V.P. Ranta, M. Laavola, E. Toropainen, K.S. Vellonen, A. Talvitie, A. Urtti, Ocular pharmacokinetic modeling using corneal absorption and desorption rates from in vitro permeation experiments with cultured corneal epithelial cells, Pharm. Res. 20 (2003) 1409–1416, https://doi.org/10.1023/A:1025754026449.
[30] I. Ahmed, R.D. Gokhale, M.V. Shah, T.F. Patton, Physicochemical determinants of drug diffusion across the conjunctiva, sclera, and cornea, J. Pharm. Sci. 76 (8) (1987) 583–586, https://doi.org/10.1002/jps.2600760802.
[31] C. Loch, S. Zakelj, A. Kristl, S. Nagel, R. Guthoff, W. Weitschies, A. Seidlitz, Determination of permeability coefficients of ophthalmic drugs through different layers of porcine, rabbit and bovine eyes, Eur. J. Pharm. Sci. 47 (1) (2012) 131–138, https://doi.org/10.1016/j.ejps.2012.05.007.
[32] M.A. Watsky, M.M. Jablonski, H.F. Edelhauser, Comparison of conjunctival and corneal surface areas in rabbit and human, Curr. Eye Res. 7 (5) (1988) 483–486, https://doi.org/10.3109/02713688809031801.
[33] A. Tieppo, C.J. White, A.C. Paine, M.L. Voyles, M.K. McBride, M.E. Byrne, Sustained in vivo release from imprinted therapeutic contact lenses, J. Control. Release 157 (3) (2012) 391–397, https://doi.org/10.1016/j.jconrel.2011.09.087.
[34] X. Tian, M. Iwatsu, A. Kanai, Disposable 1-day Acuvue contact lenses for the delivery of lomefloxacin to rabbits' eyes, CLAO J. 27 (2001) 212–215. https://pubmed.ncbi.nlm.nih.gov/11725984/.
[35] L.i. Zhang, G.-J. Zheng, Y.-T. Guo, L. Zhou, J. Du, H. He, Preparation of novel biodegradable pHEMA hydrogel for a tissue engineering scaffold by microwave-assisted polymerization, Asian Pac. J Trop. Med. 7 (2) (2014) 136–140, https://doi.org/10.1016/S1995-7645(14)60009-2.
[36] M. Vivero-lopez, A. Muras, D. Silva, A.P. Serro, A. Otero, A. Concheiro, C. Alvarez-lorenzo, Resveratrol-loaded hydrogel contact lenses with antioxidant and antibiofilm performance, Pharmaceutics 13 (2021) 532, https://doi.org/10.3390/pharmaceutics13040532.
[37] G.A. Lesher, G.G. Gunderson, Continuous drug delivery through the use of disposable contact lenses, Optom. Vis. Sci. 70 (12) (1993) 1012–1018, https://doi.org/10.1097/00006324-199312000-00004.
[38] C.C.S. Karlgard, L.W. Jones, C. Moresoli, Ciprofloxacin interaction with silicon-based and conventional hydrogel contact lenses, Eye Contact Lens. 29 (2) (2003) 83–89, https://doi.org/10.1097/01.ICL.0000061756.66151.1C.
[39] C.C.S. Karlgard, N.S. Wong, L.W. Jones, C. Moresoli, In vitro uptake and release studies of ocular pharmaceutical agents by silicon-containing and p-HEMA hydrogel contact lens materials, Int. J. Pharm. 257 (1-2) (2003) 141–151, https://doi.org/10.1016/S0378-5173(03)00124-8.
[40] A. Boone, A. Hui, L. Jones, Uptake and release of dexamethasone phosphate from silicone hydrogel and group I, II, and IV hydrogel contact lenses, Eye Contact Lens. 35 (2009) 260–267, https://doi.org/10.1097/ICL.0b013e3181b26c49.
[43] H. Hiratani, A. Fujiwara, Y. Tamiya, Y. Mizutani, C. Alvarez-Lorenzo, Ocular release of timolol from molecularly imprinted soft contact lenses, Biomaterials 26 (11) (2005) 1293–1298, https://doi.org/10.1016/j.biomaterials.2004.04.030.
[44] J. Xu, X. Li, F. Sun, In vitro and in vivo evaluation of ketotifen fumarate-loaded silicone hydrogel contact lenses for ocular drug delivery, Drug Delivery 18 (2) (2011) 150–158, https://doi.org/10.3109/10717544.2010.522612.
[45] F.A. Maulvi, T.G. Soni, D.O. Shah, Extended release of hyaluronic acid from hydrogel contact lenses for dry eye syndrome, J. Biomater. Sci. Polym. Ed. 26 (15) (2015) 1035–1050, https://doi.org/10.1080/09205063.2015.1072902.
[46] A. Urtti, J. Pipkin, G. Rork, T. Sendo, U. Finne, A. Repta, Controlled drug delivery devices for experimental ocular studies with timolol 2. Ocular and systemic absorption in rabbits, Int. J. Pharm. 61 (3) (1990) 241–249, https://doi.org/10.1016/0378-5173(90)90215-P.