



therapy is the first-line treatment while steroidal drugs are a second-choice for patients with chronic DME or who do not respond to anti-VEGF injections. Corticosteroids downregulate the synthesis of inflammatory mediators and VEGF through genomic mechanisms within the cytosol, binding to glucocorticoid receptors (Cunningham et al., 2008; Gaballa et al., 2021), and non-genomic mechanisms via phospholipase A2 inhibition, thereby reducing vasopermeability and leukocyte migration (Gaballa et al., 2021; Stewart, 2012). Current available intravitreal products comprise triamcinolone acetonide (TA) injectable suspension 1 to 4 mg (Kenalog-40, Bristol-Myers Squibb; Triescence®, Alcon; and Trivaris®, Allergan), dexamethasone implant 0.35 or 0.7 mg (Ozurdex, Allergan), and fluocinolone acetonide implant 0.19 mg (Retisert®, Bausch + Lomb; and Iluvien®, Alimera Science) (Cunningham et al., 2008; Gaballa et al., 2021; Schmidt-Erfurth et al., 2017). TA inhibits the inflammatory response, reducing leukocyte migration, fibroblast proliferation, capillary dilatation, and edema formation, thereby improving visual acuity (Sarao et al., 2012; Sorrentino et al., 2021). TA significantly suppresses the expression of pro-inflammatory proteins such as tumor necrosis factor α (TNF- α), interleukin 6 (IL-6), and interleukin 1-beta (IL-1 β) (Mizuno et al., 2007). The therapeutic effect of intravitreal TA may persist for 2–4 months, denoting a high residence time in the vitreous cavity (Thrimawithana et al., 2011). TA increases visual acuity and reduces retinal thickness within 1 h after intravitreal administration (Sonoda et al., 2011).

Although the intravitreal route is the most efficient one to deliver the drug to the posterior segment of the eye, it may cause changes in the vitreous body with a consequent increase of pressure over the retina and, in some cases, severe ocular complications such as retinal detachment (Cunningham et al., 2008; Thrimawithana et al., 2011). Moreover, because TA is administered in a heavy depot-formulated suspension, it may have direct toxic effects on the retina and optic nerve (Schmidt-Erfurth et al., 2017; Yeung et al., 2004). Safety concerns emerged due to the high levels of drug achieved intraocularly over extended periods that may increase intraocular pressure (Daruich et al., 2015; Sarao et al., 2012). Therefore, there is a need to develop products that can provide sustained release of TA while overcoming the adverse events of intravitreal injections.

Contact lenses (CLs) are non-invasive devices that may serve as advantageous platforms for the topical sustained release of ophthalmic drugs (Alvarez-Lorenzo et al., 2019; Pereira-da-Mota et al., 2022). Their prolonged contact with the eye facilitates drug penetration into the ocular tissues (Li & Chauhan, 2006). Although their potential for this purpose is recognized, the commercialization of the first drug-eluting CLs (Johnson & Johnson's ACUVUE Theravision CLs) – containing ketotifen for the treatment of allergic conjunctivitis – only started in 2021 (FDA News, 2021). The use of CLs for the vehiculation of lipophilic drugs, like TA, remains a challenge since their low aqueous solubility hampers their loading and the achievement of therapeutic concentrations in the eye when released. The design of CLs with specific affinity for such drugs could be achieved by incorporating molecules containing chemical groups able to simultaneously interact with the polymeric network of the hydrogel and the target drug (Alvarez-Lorenzo et al., 2019; Pereira-da-Mota et al., 2022; Toffoletto et al., 2021).

One strategy to increase the ability of hydrogels to host lipophilic drugs relies on the capability of cyclodextrins (CDs) to form inclusion complexes (Loftsson et al., 2010; Loftsson & Brewster, 2011; Lorenzo-Veiga et al., 2019). CDs are cyclic oligosaccharides formed by six (α -CD), seven (β -CD), or eight (γ -CD) α -(1,4)-linked glucose units that adopt a toroidal structure with a hydrophilic outer surface and a lipophilic inner cavity (Jacob & Nair, 2018). Random substitution of the hydroxyl groups with hydroxypropylated substituents to obtain e.g. HP- β -CD results in improved aqueous solubility (Jacob & Nair, 2018; Rekharsky & Inoue, 1998). Some commercial eye drop formulations already contain hydroxypropylated CDs as solubilizing excipients (Loftsson & Brewster, 2011; Lorenzo-Veiga et al., 2019). In the case of TA, it was found that HP- β -CD (10 % w/v) improves the aqueous solubility of the drug by 25-

fold (Miro et al., 2012).

The present work explored the possibility of incorporating pristine HP- β -CD in 2-hydroxyethyl methacrylate (HEMA) hydrogels to enhance TA loading and release. Two approaches were tested: (i) incorporation of CDs in the monomers solution before polymerization (HEMA/i-CD) and (ii) post-treatment of the hydrogel with CDs (HEMA/p-CD). In previous studies, CDs were incorporated in CLs by (a) modifying first the CDs with vinyl or acrylate monomers to copolymerize them with the hydrogel monomers (Dos Santos et al., 2008; Phan et al., 2014), or (b) introducing monomers with epoxide or glycidyl groups in the hydrogel for reaction with the hydroxyl groups of CDs (Dos Santos et al., 2010). The first approach involves many steps for obtaining CD monomers with the right double bond content. Since the acrylic derivatives of CDs are usually multifunctional, they increase the cross-linking density of the hydrogel, making the networks too rigid for ophthalmic purposes. The second approach entails using monomers that are not standard components of CLs and require quite drastic conditions for the reaction and then intense downstream washing. To overcome the disadvantages of both approaches, in the present study, pristine HP- β -CD ophthalmic excipient was used without previous modifications, and its incorporation was performed before (HEMA/i-CD) and after (HEMA/p-CD) the hydrogels polymerization. The use of non-modified HP- β -CD implies the challenge of incorporating enough CDs so that they do not leave the CL prematurely and can adjust the drug release rate to the therapeutic demand. To meet this challenge, we investigated to what extent HP- β -CD can be spontaneously grafted to the HEMA network during the free radical polymerization of the CL and also to elucidate whether the post-processing during product sterilization can reinforce the formation of drug-CD inclusion complexes without triggering leakage of HP- β -CD to the sterilization medium. Although there is a paucity of information on the grafting of acrylic chains to pristine CDs, it has been shown that a variety of polysaccharides can be integrated into acrylic networks in the presence of initiators (such as 2,2'-azobis(2-methylpropionitrile); AIBN) that simultaneously generate free radicals in both the acrylic monomers and the hydroxyl groups of the glucopyranose units (Ajaz et al., 2022; Malik et al., 2017; Thakur et al., 2013).

Sterilization is a mandatory step in producing insertable medical devices, including CLs. Drug-loaded hydrogels are often sensitive to conventional steam-heat and gamma-radiation sterilization, which may compromise the material properties, the drug release profiles, and the drug activity (Bento et al., 2023; Galante et al., 2018; Pereira-da-Mota et al., 2021; Topete et al., 2020; Van Cauwenbergh et al., 2022). In the present work, high hydrostatic pressure (HHP) was employed as an alternative sterilization method since it uses relatively low temperature and does not involve radiation exposition. HHP has been barely explored in the field of drug-CD formulations compared to steam-heat sterilization, but the available information suggests that HHP may aid the formation of drug-CD inclusion complexes in solution (Hu et al., 2012; Zong et al., 2010). Thus, the present work relies on the hypothesis that the incorporation of CDs within the CL hydrogel followed by high-pressure sterilization may assist the formation of TA complexes into the hydrogel and help regulate the delivery of this drug to the ocular surface through an affinity-driven mechanism.

The effects of the CDs and HHP sterilization on the material properties, namely in water content, transmittance, oxygen and ion permeability, roughness, and stiffness, were studied. The presence of HP- β -CD along the CL thickness was monitored using Raman microscopy. TA was loaded by soaking in a drug suspension. Afterward, *in vitro* drug release tests were performed under sink conditions. Adsorption of two of the main proteins of lacrimal fluid (albumin and lysozyme) onto the materials was studied, and biocompatibility was evaluated through cytotoxicity and HET-CAM irritability assays. The permeability of TA through the cornea and sclera was assessed *ex vivo*, and the TA ocular distribution *in vivo* after CL application was estimated using a mathematical model. Finally, the anti-inflammatory activity of the released drug was investigated.

2. Experimental section

2.1. Materials

Triamcinolone acetonide (TA) was purchased from Acofarma (Madrid, Spain). 2-Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (purity $\geq 98\%$, EGDMA), 2,2'-azobis(2-methylpropionitrile) (AIBN), dichlorodimethylsilane, phenolphthalein, lysozyme from egg white, and sodium chloride (NaCl) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Polyvinylpyrrolidone Kollidon® 30 (PVP) was from BASF (Heidelberg, Germany), and 2-hydroxypropyl- β -cyclodextrin, Cavasol® W7 HP PHARMA (molar substitution per anhydrous glucose unit = 0.59–0.73, HP- β -CD) was kindly provided by Ashland (Schaffhausen, Switzerland). Other reagents and solvents are described in the Supplementary Material file.

2.2. Hydrogel preparation

2.2.1. Hydrogels with incorporated HP- β -CD (HEMA/i-CD)

PVP (0.12 g) solution in HEMA (6 mL) and EGDMA (91 μ L) (Fig. S1) was bubbled with nitrogen for 15 min and degassed by ultrasounds for 5 min. To prepare HEMA/i-CD hydrogels, HP- β -CD (609 mg) dissolved in DMSO (1 mL) was added to the monomers solution; final HP- β -CD concentration of 8 % (w/w). Initiator AIBN (10 mg) was added, and the solution was injected into a mold comprised of two presilanized glass plates separated by a Teflon frame of 0.3 mm thickness. After polymerization (50 °C for 12 h and at 70 °C for an additional 24 h), the hydrogel was washed with DD water at room temperature (RT) three times a day for five days to remove unreacted molecules. Control pHEMA-based hydrogels were prepared similarly without HP- β -CD (HEMA control). The hydrogels were cut into disks of 10 mm diameter (6 mm for cell tests) or dumbbell-shaped specimens (2.5 mm width and 10 mm gauge length) for mechanical tests, dried in an oven at 60 °C for two days, and stored at RT in closed flasks until use.

2.2.2. Hydrogels post-treated with HP- β -CD (HEMA/p-CD)

HEMA/p-CD were prepared by immersing hydrated disks of HEMA control hydrogels in DMF:0.9 % (w/v) NaCl aqueous solution (50:50 v/v) containing 10 % (w/v) HP- β -CD and 3 % (w/v) NaOH, at a ratio of 6.4 mL/cm² of hydrogel surface at 80 °C for 24 h. Afterward, the hydrogels were washed by immersion in DD water at 80 °C for 5 min (five cycles), at 60 °C for 24 h (three cycles), in ethanol (96 %) for 24 h (three cycles), and in DD water at RT for 24 h (three cycles). Finally, the disks were dried at 60 °C for 48 h.

2.3. Quantification of HP- β -CD

CDs available for complexation within the hydrogels were estimated using the phenolphthalein method (Kettel et al., 2012). A 10 g/L phenolphthalein stock solution was prepared in 96 % (v/v) ethanol. Then, 5 mL of the phenolphthalein solution was added to 0.5 L of a 0.1 M NaOH solution to obtain a purple coloration. Dried hydrogel disks were immersed in 3 mL of the alkaline phenolphthalein solution and kept for 24 h in the dark. The concentration of phenolphthalein was determined spectrophotometrically at 553 nm (UV–Vis spectrophotometer, Thermo Scientific, Multiscan GO, Porto Salvo, Portugal). Phenolphthalein loaded by the hydrogels was calculated as the difference between the initial and final amounts in the solution.

2.4. Hydrogel characterization

Raman spectra¹ of the dry samples were recorded using a confocal Raman microscope.

Hydrogel water content was determined from the relative weight gain of the dried disks (W_d) after immersion in 0.9 % NaCl at 25 °C (W_h), according to Eq. (1):

$$\text{Water content (\%)} = 100 \times (W_h - W_d) / W_h \quad (1)$$

Water contact angles with the hydrogels were determined by the captive bubble method¹.

The transmittance of hydrated disks (swollen in 0.9 % NaCl) was recorded in a UV–Vis spectrophotometer ranging from 200 to 700 nm with 1 nm intervals.

Hydrogels' ion permeability and oxygen transmissibility were analyzed using a polarographic oxygen sensor and a conductivity meter, respectively¹.

Tensile tests¹ were carried out to evaluate the mechanical performance of the materials. Young's modulus, elongation to break, and tensile strength were taken from the stress-strain curves.

Surface roughness¹ was examined using an atomic force microscope.

2.5. TA loading and release

Dried disks were immersed independently in 3 mL of TA suspension (0.3 g/L) and kept for seven days at RT with orbital shaking (180 rpm). Afterward, the TA-loaded disks were removed, rinsed with water, carefully wiped with absorbing paper, and immediately transferred to 3 mL of 0.9 % NaCl solution.

In vitro release studies were conducted in 0.9 % NaCl medium (3 mL) at 32 °C and 180 rpm. At scheduled times, aliquots of the release medium (0.3 mL) were collected to measure TA concentration (spectrophotometrically at 240 nm) and replenished with equal volumes of fresh 0.9 % NaCl solution to maintain sink conditions.

Following the release studies, disks were rinsed and placed in vials with 3 mL of methanol to extract the drug remaining, and the absorbance was recorded at 249 nm. The procedure was repeated using fresh methanol until no drug could be detected. The amount of drug loaded was obtained from the sum of the extracted and released masses.

The network/water partition coefficient ($K_{N/W}$) was calculated based on the amount of drug loaded per unit mass of dry hydrogel (m_{Loaded}), the weight of the dry polymer (W_p), the concentration of the drug dissolved in the TA suspension (C), and the volumes of solvent sorbed (V_s) and dry polymer (V_p), through Eq. (2) (Kim et al., 1992):

$$K_{N/W} = [(m_{\text{Loaded}} \times W_p) / (C - V_s)] / V_p \quad (2)$$

The eventual release of CDs to the release medium was evaluated by high-performance liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), as described in the Supplementary Material.

2.6. Sterilization

Dried hydrogels were individually placed in polyamide/polyethylene bags containing 0.9 % NaCl aqueous solution or a TA suspension (0.3 g/L in 0.9 % NaCl solution). Undissolved drug (the solubility limit of TA in the NaCl solution at 25 °C is 0.0381 g/L) ensured the permanent saturation of the medium. A ratio of 3.8 mL of solution per cm² of hydrogel surface was maintained in all cases. The sealed packages were preheated at 70 °C for 5 min, placed inside an insulation basket with water at 70 °C, and pressurized in industrial scale high pressure equipment (Hiperbaric 55, Hiperbaric S.A., Burgos, Spain) for HHP sterilization at 70 °C and 600 MPa, for 10 min (Topete et al., 2020).

2.7. Protein adsorption

The adsorption of BSA and lysozyme onto the hydrogels was studied using a quartz crystal microbalance with dissipation (QCM-D, E4, Q-Sense, Gothenburg, Sweden). Gold-coated quartz crystals (5 MHz) were

¹ Details provided in Supplementary Material.

first treated with UV-ozone for 15 min and spin-coated with polystyrene (PS, 20 μ L, 2 % wt. in toluene) at 2000 rpm for 30 s. The monomer solutions used to prepare HEMA control and HEMA/i-CD hydrogels (vide Section 2.2.1) were deposited over the PS film by spin coating (5000 rpm, 30 s) and polymerized for 30 min at 50 °C, followed by 1 h at 70 °C (Vivero-Lopez et al., 2021). The crystals were then immersed in 0.9 % NaCl solution to remove unreacted molecules and left hydrated until the adsorption experiment.

Hydrogel-coated crystals were placed in the QCM-D cells, and the experimental baselines were acquired in 0.9 % NaCl solution at 32 °C. BSA (0.05 g/L) and lysozyme (1.90 g/L) solutions (prepared in 0.9 % NaCl solution) were added in independent experiments, followed by rinsing with 0.9 % NaCl solution to remove loosely bound protein molecules from the coated crystals. Normalized frequency and dissipation were monitored for the 3rd, 5th, 7th, 9th, and 11th harmonics throughout each experiment.

2.8. Drug permeability through ocular tissues

Fresh porcine eyes were obtained from a local slaughterhouse (SICASAL – Indústria e Comércio de Carnes, S.A., Gradil, Portugal) and transported immersed in PBS in an ice bath. Corneas and scleras were isolated and mounted in vertical Franz diffusion cells (epithelium and episclera, respectively, facing the donor chamber) and covered with 1 mL of 0.9 % NaCl solution. The receptor chambers were filled with 6.5 mL of 0.9 % NaCl solution and immersed in a bath at 36 °C. After 1 h, the solution in the donor chamber was removed, and a sterilized TA-loaded HEMA/i-CD HHP hydrogel was placed over the ocular tissues in 1 mL of fresh 0.9 % NaCl solution. Additional experiments were performed with 1 mL of a TA solution (0.060 g/L in ethanol:water 10:90 v/v) that mimicked the amount released by the hydrogels in 6 h. The donor chamber was covered with Parafilm® to prevent evaporation. At each time point (1, 2, 3, 4, 5, and 6 h), 1 mL aliquots were collected from the receptor chamber for analysis and replaced with fresh saline solution.

TA concentration was quantified using a 2695 Waters HPLC (Milford, MA, USA) coupled with a 2996 photodiode array detector equipped with a C18 column (150 $\times$ 4.6 mm², with 5 μ m pores) (ACE®, Aberdeen, UK) and a C18 20 $\times$ 3.9 mm Sentry™ guard column (Nova-Pak®, 4 μ m, Waters, Wexford, Ireland). Methanol:water 72:28 v/v was used as isocratic mobile phase at a flow rate of 0.8 mL/min and 25 °C. The injection volume was 50 μ L, and the detection wavelength was 249 nm (retention time of 4.6 min). A calibration curve of TA in 0.9 % NaCl solution was obtained between 0.0625 and 40 mg/L. The limit of detection (LOD) and limit of quantification (LOQ) values were 0.029 and 0.0625 mg/L, respectively.

After a six-hour experiment, aliquots from the solutions in the donor chambers were collected and quantified by HPLC. The corneas and scleras were removed from the diffusion cells and immersed in 1 mL of methanol:water (72:28 v/v), sonicated for two hours, and left overnight at RT. Then, the tissues were centrifuged (14,480 g, 10 min), and the supernatant was analyzed by HPLC at 254 nm.

2.9. Modeling of in vivo drug distribution

To predict the drug ocular distribution *in vivo* after the application of the developed TA-loaded hydrogels, a previously validated mathematical model (Toffoletto et al., 2023) was applied. Briefly, the drug concentration in the tears, aqueous humor, sclera and choroid, retina, and vitreous humor was predicted by modeling one mass balance equation for each tissue, obtained considering the anatomical and physiological parameters of the rabbit eye. The mass balance equations are ordinary differential equations because the spatial variations in tissue concentrations are neglected. Inputs to the model are drug- and CL-specific parameters, which include the *in vitro* drug release rate (*R*), the drug permeability values across the ocular barriers (*P*), the partition coefficients of the drug with the tissues (*K*), and the aqueous humor

bioavailability (*F* %). The values of *P*, *K*, and *F* were estimated based on reported data for similar drugs, in terms of molecular size and lipophilicity (Toffoletto et al., 2023) and are reported in Table S1 (Supplementary Material). The drug release from the contact lens over time, *f*, was modeled by fitting the *in vitro* experimental data to an exponential curve (Eq. (3)), where *M_{released}* and *T* are the amount of drug released and the time constant, both obtained by curve fitting. Then, the *in vitro* release rate (*R*) was calculated as in Eq. (4).

$$f = M_{\text{released}} \times (1 - e^{-t/T}) \quad (3)$$

$$R = df/dt = (M_{\text{released}}/T) \times e^{-t/T} \quad (4)$$

2.10. HET-CAM assay and cytocompatibility

The Hen's Egg Test on the Chorioallantoic Membrane (HET-CAM) test was performed to evaluate the irritability potential of the hydrogels according to the ICCVAM protocol (Gilleron et al., 1996; ICCVAM In Vitro Ocular Evaluation Report, 2020) as described in the Supplementary Material.

Human primary corneal epithelial cells (ATCC 700–010) were cultured in Corneal Epithelial Cell Basal Media supplemented with Corneal Epithelial Cell Growth kit components and 1 % antibiotics (penicillin-streptomycin solution) at 37 °C in a humidified atmosphere of 5 % CO₂. At 80 % confluency, cells were detached from the culturing flask using trypsin-EDTA and resuspended in culture media. Suspended cells were seeded in 24-well plates (1 $\times$ 10⁵ cells/well) and incubated under the same conditions for 24 h. The cytocompatibility of non-loaded and TA-loaded HEMA/i-CD hydrogels was assessed by indirect contact according to ISO 10993-5 guidelines (ISO, 2009). Cell culture inserts (Transwell®, Corning, Glendale, AZ, USA; 7 mm diameter) were inserted into the wells with cultured cells, and sterilized HEMA/i-CD hydrogels or the TA suspension were placed into the inserts. Then, 0.100 mL of fresh medium was added to cover the hydrogels completely. The viability of cells exposed to TA solutions of concentration 2.5 mg/L and 40 mg/L (diluted 10 $\times$ in culture medium from stock solutions in 0.9 % NaCl or ethanol:water 20:80 v/v, and sterilized by HHP), cells incubated in culture medium (negative control) and cells incubated in 10 % (v/v) DMSO in culture medium (positive control) was also evaluated.

After 24 h incubation, the inserts and culture media were removed and replaced with MTT solution (diluted 10 $\times$ in culture medium from a stock solution of 5 g/L) after washing the cell monolayer with PBS, followed by an incubation of 3 h at 37 °C and 5 % CO₂. Finally, MTT solvent (4 mM HCl and 0.1 % (v/v) IGEPAL in isopropanol) was added to each well, and the absorbance was read at 565 nm in a microplate reader (Infiniti 200 Pro, Tecan, Switzerland). The percentage of cell viability was obtained through normalization with the negative control, considered 100 % cell viability.

2.11. Anti-inflammatory activity

THP-1 human monocytes (ATCC TIB-202) were cultured in RPMI-1640 supplemented with 10 % FBS and 1 % antibiotics (penicillin-streptomycin solution) at 37 °C and 5 % CO₂. Suspended cells were placed into 24-well plates (9 $\times$ 10⁴ cells/well). Then, 200 nM PMA was added to promote the differentiation of THP-1 cells into macrophages, and the plates were incubated for 72 h. To induce a pro-inflammatory response, cells were exposed to culture medium with 100 μ g/L of LPS for 3 h. Then, inserts with non-loaded and TA-loaded HEMA/i-CD hydrogels (sterilized by HHP) were placed into the wells, following a period of incubation of 24 h at 37 °C and 5 % CO₂. A 2.5 mg/L TA solution (diluted as described in 2.9) was also tested in contact with the macrophages. Cells in culture medium were used as a negative control, while cells exposed to culture medium with LPS served as a positive control. Finally, cell culture supernatants were collected and stored at –80 °C until further assessment. The secretion of human IL-6 and TNF- α

cytokines was quantified by specific ELISA kits, following the manufacturer's instructions.

2.12. Statistical analysis

Quantitative data are expressed as mean $\pm$ standard deviation; $n \geq 3$ for all experiments. Statistical analysis was performed using GraphPad Prism (v.9.5.1) software (GRAPH PAD Software Inc., California, USA) as indicated in Supplementary Material. Statistical significance was set at $p \leq 0.05$ (*), $p \leq 0.01$ (**), and $p \leq 0.001$ (***)

3. Results and discussion

3.1. Synthesis of hydrogels containing HP- β -CD units

In this work, two strategies were explored to obtain pHEMA-based hydrogels containing CDs: 1) a post-treatment of the polymerized hydrogels with HP- β -CD (hydrogels HEMA/p-CD); and 2) incorporation of HP- β -CD in the monomers solution followed by polymerization (hydrogels HEMA/i-CD). No macroscopic modifications were observed compared with non-modified hydrogels (HEMA control).

HEMA/p-CD hydrogels were prepared by immersion of HEMA hydrogels in an alkaline medium containing HP- β -CD and DMF at 80 °C as previously reported (dos Santos et al., 2010). The hydrogels lacked glycidyl or epoxide groups that could covalently bind to CD molecules, but PVP could interact with HP- β -CD. PVP is poorly reactive and conjugation with CDs has been previously attempted via click chemistry previous modification of both PVP and CDs (Trellenkamp & Ritter, 2010). PVP's carbonyl groups have been shown to interact with peptides through hydrogen bonds when heated at 70 °C (D'Souza et al., 2003). Therefore, we attempted the reaction between PVP's carbonyl group and HP- β -CD by heating.

HEMA/i-CD hydrogels were prepared by dissolving HP- β -CD in the smallest possible volume of DMSO to solubilize HP- β -CD in HEMA monomers. A 8 % (w/w) concentration of HP- β -CD was selected to prepare both hydrogels. It has been previously shown that thermal initiators such as AIBN under the prolonged heating conditions used for CL polymerization (50 °C for 12 h and 70 °C for 24 h) can trigger radical reactions in carbohydrates (Binkley & Binkley, 2023). Therefore, we expected that part of HP- β -CD included in the monomers soup participates in the polymerization reaction and incorporates into the CL network.

3.2. Quantification of HP- β -CD units available

A phenolphthalein-based colorimetric method was used to estimate the content of available CDs in each hydrogel. It involves the complexation of phenolphthalein with CDs, determined by the uptake of phenolphthalein from the solution (Higuti et al., 2004; Kuwabara et al., 1998). CD units available to form inclusion complexes were calculated by subtracting the amount of phenolphthalein loaded by HEMA control hydrogel from the amount loaded by the modified hydrogels (Table 1) (Higuti et al., 2004).

HEMA hydrogels loaded a small amount of phenolphthalein, possibly in the aqueous phase. Post-treatment with CDs failed to endow HEMA/p-CD hydrogels with a higher phenolphthalein loading capacity,

suggesting that HP- β -CD were not successfully bonded. Contrarily, phenolphthalein uptake was remarkably enhanced by incorporating HP- β -CD into the monomers solution, i.e., in HEMA/i-CD ($p < 0.01$). Considering that one phenolphthalein molecule forms an inclusion complex with one HP- β -CD macromolecule (Higuti et al., 2004; Kuwabara et al., 1998), the amount of HP- β -CD per gram of HEMA/i-CD hydrogel was estimated to be 0.013 mmol/g dry hydrogel, corresponding to 18.43 mg/g dry hydrogel. The theoretical amount of HP- β -CD in the feed composition was 80 mg/g. This means that at least 23 % of the HP- β -CD were successfully grafted or chemically trapped into the HEMA network. The remaining fraction may have left the structure during washing in hot water. It is worth mentioning that HPLC/MS did not detect CDs in the subsequent washing steps.

Raman spectra analysis evidenced an increase in the bands typical of HP- β -CD in the HEMA/i-CD compared to the other two hydrogels (Fig. 1A). In agreement with the literature, the characteristic peak of the CD glucose ring vibrations appeared at 480 cm^{-1} (Deng et al., 2019). In the synthesized hydrogels, this band can only be attributed to HP- β -CD, and the monitoring of its intensity throughout the thickness of the hydrogels evidenced the presence of HP- β -CD from the surface to the bulk of HEMA/i-CD hydrogel (Fig. 1B). The peak at 1335 cm^{-1} due to scissoring mode vibrations of C—H bonds was also more intense in HP- β -CD spectrum (although not exclusive) compared to control HEMA hydrogel. In concordance, the HEMA/i-CD hydrogel bulk also showed an increase in the intensity of this peak (Fig. 1B).

3.3. Hydrogels characterization

CLs should combine comfort, adequate physical strength, and optical performance. Therefore, produced hydrogels were characterized concerning relevant physical and mechanical properties: water content, wettability, oxygen and ion permeabilities, tensile behavior, surface roughness, and transparency.

All dried hydrogels presented a fast swelling in 0.9 % NaCl solution, reaching equilibrium within 1 h. The liquid uptake was similar disregarding the presence of CDs (Table 2), and the values were within the range of those reported for pHEMA-based CLs (García-Fernández et al., 2013; Toffoletto et al., 2021; Vivero-Lopez et al., 2021). Previous studies on hydrogels involving copolymerized or grafted CDs reported some discrepancies in the effect of CDs on the equilibrium water content, which was in all cases of small magnitude (Dos Santos et al., 2010; Phan et al., 2014; Ribeiro et al., 2012). The contact angles (Table 2), obtained through the captive bubble method, were within the range for hydrophilic CLs (Lin & Svitova, 2010). The lower value corresponded to the hydrogel functionalized with CDs (HEMA/i-CD, $p < 0.05$), in good agreement with previous reports (Li et al., 2020). Regarding oxygen and ion permeabilities (Table S2 and Table 2, respectively), the results obtained for the designed hydrogels are of the same order of magnitude of commercial HEMA-based CLs with comparable equilibrium water contents (5–8.8 Dk and $10\text{--}29 \times 10^7 \text{ cm}^2/\text{s}$, respectively) (Efron et al., 2007; Guan et al., 2011; Pozuelo et al., 2014). Although according to some authors the diffusion characteristics are not expected to be altered by the presence of CDs (Ribeiro et al., 2012; Rosa dos Santos et al., 2009), here it was found that the oxygen transmissibility (which is independent of the materials' thickness and therefore only depends on its intrinsic properties) was generally higher for CD-functionalized hydrogels

Table 1

Phenolphthalein loaded by the hydrogels and available CD cavities for complexation in HEMA, HEMA/p-CD, and HEMA/i-CD hydrogels. Estimated CD content is also presented. ** denotes statistically significant comparisons ($p < 0.01$) between functionalized and control hydrogel (HEMA).

Hydrogel	Phenolphthalein loaded (mg/g dry hydrogel)	Available CD cavities (mmol/g dry hydrogel)	CD content (mg/g dry hydrogel)
HEMA	6.53 $\pm$ 0.32	—	—
HEMA/p-CD	5.93 $\pm$ 1.47	≈ 0	≈ 0
HEMA/i-CD	10.72 $\pm$ 0.16 (**)	0.013 $\pm$ 0.001	18.43 $\pm$ 1.75

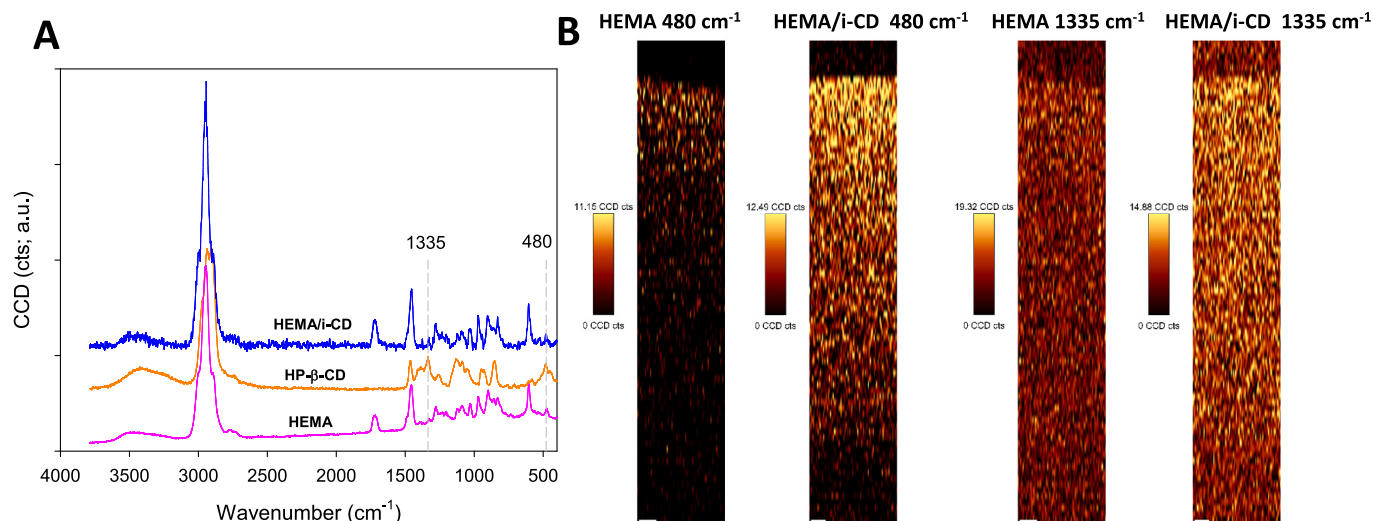


Fig. 1. (A) Raman spectra of control HEMA and HEMA/i-CD hydrogels and pristine HP-β-CD. The bands at 480 and 1335 cm^{-1} are typical of HP-β-CD; and (B) Raman microscope images of dried hydrogels (along the depth) recorded at 480 and 1335 cm^{-1} , showing the increase in HP-β-CD signals in a red to yellow scale. Scale bar 5 μm . (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2

Water content, water contact angle, Young's modulus, oxygen transmissibility, ion permeability, and surface roughness (S_a) of HEMA, HEMA/p-CD, and HEMA/i-CD hydrogels before and after sterilization by high hydrostatic pressure (HHP). + denotes statistically significant comparisons between non-sterilized and sterilized hydrogels and * between functionalized hydrogels and control (HEMA), as referred in Supplementary Material. ^a Units are 10^{-9} (cm/s) (mL $\text{O}_2/\text{mL} \times \text{mmHg}$).

Hydrogel	Water content (%)	Water contact angle ($^\circ$)	Young's modulus (MPa)	Oxygen transmissibility (Dk/L) ^a	Ion permeability $\times 10^7$ (cm^2/s)	Surface roughness (nm)
HEMA	35.2 ± 0.2	40.1 ± 3.1	1.13 ± 0.09	1.81 ± 0.02	27 ± 5	8.2 ± 2.1
HEMA/p-CD	34.2 ± 0.1 (*)	46.4 ± 5.2	0.94 ± 0.06 (**)	2.65 ± 0.04 (*)	28 ± 4	9.3 ± 1.0
HEMA/i-CD	36.9 ± 3.1	33.0 ± 0.4 (**)	0.69 ± 0.04 (***)	2.04 ± 0.10 (*)	33 ± 3	7.5 ± 2.8
HEMA HHP	34.2 ± 0.2	52.7 ± 2.3 (++)	0.78 ± 0.02 (+++)	1.78 ± 0.10	27 ± 5	8.3 ± 1.1
HEMA/p-CD HHP	34.1 ± 0.5	46.0 ± 8.3	0.88 ± 0.05	3.27 ± 0.02 (*,+)	28 ± 4	5.8 ± 2.0
HEMA/i-CD HHP	36.1 ± 1.7	39.8 ± 0.5 (*,++)	0.71 ± 0.03	1.91 ± 0.08	29 ± 6	5.3 ± 1.0

(Table 2). This increase may be due to oxygen transport through CD cavities, as suggested by López-de-Dicastillo et al. (López-de-Dicastillo et al., 2010).

As for the mechanical behavior, control hydrogels had Young's modulus of 1.13 ± 0.09 MPa (Table 2). The post-treatment with HP-β-CD (HEMA/p-CD) led to a minor decrease $\approx 17\%$ ($p < 0.001$), probably because of partial hydrolysis of some HEMA and PVP polymer chains in the alkaline solution (Kazantsev et al., 2017; Teella et al., 2014). The incorporation of HP-β-CD before polymerization (HEMA/i-CD) caused a decrease in the stiffness of $\approx 39\%$ ($p < 0.01$), which may be related to a plasticizing effect of the bulky HP-β-CD. In these HEMA/i-CD hydrogels, the small proportion of HP-β-CD incorporated is not expected to significantly increase the cross-linking density. Overall, the obtained Young's moduli values agreed with those reported for typical pHEMA CLs (0.2–2 MPa) (Kim et al., 2018). The tensile strength and elongation at break (Table S2) were also within the values reported for polymacon (Bausch & Lomb) and etafilcon A (1-Day Acuvue®, Johnson & Johnson) daily soft CLs (Food and Drug Administration, 2023). Concerning surface roughness (Table 2), the presence of CDs did not compromise the surface topography of the HEMA-based hydrogels ($p > 0.05$), in contrast with a study by Li et al. (Li et al., 2020) that reported an increase in roughness caused by uneven distribution of CD within the matrix. In all cases, the S_a values were lower than 10 nm and in the typical range of hydrophilic CLs (1.1–11.0 nm) (Giraldez et al., 2012; Ji et al., 2015; Lira et al., 2014). Finally, the light transmission of the hydrated hydrogels was, in all cases, superior to the 90 % threshold required for CLs in the visible range (Fig. S2), ensuring good transparency.

The effect of HHP sterilization (600 MPa at 70 $^\circ\text{C}$ for 10 min) on the hydrogel properties was also evaluated. HHP sterilization had no effect

on water content, light transmittance, ion permeability, and roughness, but slightly increased the oxygen transmissibility of HEMA/p-CD, increased the water contact angle of HEMA and HEMA/i-CD and decreased the elastic modulus of HEMA hydrogel by 31 %. Altogether, the properties analyzed remained within the typical values for hydrophilic CLs. These findings are in agreement with previous reports on intraocular lenses (Topete et al., 2020) and contact lenses (Pereira-da-Mota et al., 2021) that concluded that HHP sterilization did not compromise the liquid uptake, transmittance, surface morphology, and mechanical properties of the networks.

3.4. Triamcinolone acetonide loading

Control HEMA and HEMA/p-CD hydrogels loaded similar amounts of TA (approx. 4.4 mg/g dry hydrogel) (Table 3). The incorporation of HP-

Table 3

Amount of TA loaded by the hydrogels and network/water partition coefficient ($K_{N/W}$). + denotes statistically significant comparisons between non-sterilized and sterilized hydrogels and * between functionalized hydrogels and control (HEMA), as referred in Supplementary Material.

Hydrogel	TA loaded (mg/g dry hydrogel)	$K_{N/W}$
HEMA	4.41 ± 0.26	148.26 ± 0.52
HEMA/p-CD	4.39 ± 0.45 (*)	141.09 ± 3.43
HEMA/i-CD	6.14 ± 0.41 (*)	302.71 ± 20.85 (***)
HEMA HHP	3.78 ± 0.55	127.00 ± 0.44
HEMA/p-CD HHP	3.31 ± 0.38	106.08 ± 2.59
HEMA/i-CD HHP	9.72 ± 1.75 (+++)	479.77 ± 32.98 (***, +++)

β -CD before polymerization (HEMA/i-CD) significantly improved TA loading ($p < 0.05$) with a two-fold increase in partition coefficient (302.71 ± 20.85) compared to the non-modified hydrogel (148.26 ± 0.52). The high $K_{N/W}$ values indicate that the affinity of TA for the network and, especially for the HP- β -CD units inside, is much higher than for the aqueous phase in all hydrogels (dos Santos et al., 2008; Ribeiro et al., 2012; Rosa dos Santos et al., 2009).

It has been reported that pHEMA-based hydrogels can load and interact with steroids through hydrophobic interactions and hydrogen bonding (Bengani et al., 2020; Malaekhe-Nikouei et al., 2012; Schultz et al., 2011; Toffoletto et al., 2021). In HEMA/i-CD hydrogels the formation of TA:HP- β -CD inclusion complexes provides an additional loading mechanism (Jacob & Nair, 2018; Rekharsky & Inoue, 1998; Saokham et al., 2018). The TA aromatic ring can be included in the hydrophobic cavity of HP- β -CD in a ratio of 1:1 with a stability constant as high as 2780 M^{-1} (Miro et al., 2012).

Hydrogels immersed in TA suspension were submitted to HHP to explore whether such a sterilization method would further promote TA loading. In the case of HEMA and HEMA/p-CD hydrogels, the average loading capacity decreased slightly after sterilization, but the differences were not statistically significant. Differently, HEMA/i-CD hydrogels increased 1.6-fold TA loading after HHP sterilization (from 6.14 ± 0.41 to $9.72 \pm 1.75 \text{ mg/g}$ dry hydrogel; $p < 0.001$). Likewise, the partition coefficient of HEMA/i-CD after HHP was remarkably increased, evidencing the promoting effect of the sterilization treatment in the formation of inclusion complexes inside the hydrogel.

HHP has proven to facilitate the formation of inclusion complexes between β -CD and a variety of molecules such as vitamin C (Hu et al., 2012) and ursolic acid (Zong et al., 2010) with higher efficiency than other methods (e.g., coprecipitation, kneading, freeze-drying, and cogrinding). Cyclodextrin complexation is a thermodynamic process governed by enthalpic and entropic factors (Loftsson & Brewster, 2011; Rekharsky & Inoue, 1998). As such, from the Le Chatelier law, the increase in pressure during sterilization ($\approx 6000\times$) should shift the equilibrium where the reaction yields fewer molecules – that is, to aid the formation of the inclusion complex. Furthermore, the increase in temperature may favor the solubility of the hydrophobic molecules and the mobility of the polymer chains, facilitating drug diffusion into the network and, therefore, into CD cavities (Rekharsky & Inoue, 1998; Zong et al., 2010). Consequently, part of the drug solid particles in the loading medium are expected to dissolve during sterilization. This

temporary increase in solubility results in a higher concentration of TA in the aqueous phase, thereby increasing the possibility of complexation with CDs (Hu et al., 2012; Rekharsky & Inoue, 1998).

3.5. Triamcinolone acetonide release

All hydrogels sustained TA release for at least 24 h (Fig. 2). The amount of TA released after 24 h was 51 % higher for HEMA/i-CD hydrogel compared to HEMA hydrogel ($4.41 \pm 0.53 \text{ mg/g}$ vs. $2.93 \pm 0.22 \text{ mg/g}$; $p < 0.001$), in good agreement with the higher capacity of HEMA/i-CD hydrogel to load TA. However, the percentage released (relative to the total amount) in the first 24 h was similar, which evidenced the capability of the CDs to retain TA by forming complexes within the hydrogel.

TA release from HEMA control hydrogels is governed by the diffusion of the drug interacting with the polymeric network and the drug hosted in the aqueous phase (Alvarez-Lorenzo et al., 2019; dos Santos et al., 2008). In the case of HP- β -CD-containing hydrogels, the release is also regulated by the affinity of the drugs for the CDs and is triggered by dilution in the medium (Jacob & Nair, 2018; Loftsson & Brewster, 2011; Rekharsky & Inoue, 1998; Saokham et al., 2018). The high TA:HP- β -CD stability constant (2780 M^{-1} ; (Miro et al., 2012) explains the slower release rate.

The release profiles from HHP-sterilized hydrogels were remarkably distinct from those obtained with non-sterilized hydrogels (Fig. 3A). Sterilization increased 1.3-fold (from 2.93 ± 0.22 to $3.85 \pm 0.34 \text{ mg/g}$; $p < 0.01$) the amount of drug released at 24 h from HEMA hydrogels and 1.2-fold (from 4.41 ± 0.53 to $5.32 \pm 0.69 \text{ mg/g}$; $p < 0.01$) the release from HEMA/i-CD hydrogels. The amounts of TA loaded were similar for both non-sterilized and sterilized control HEMA hydrogels. A complete release was attained in 24 h for the sterilized hydrogel, while non-sterilized hydrogel still retained 46 % of the TA amount loaded. This finding suggests that HHP may alter the proportion of drug molecules interacting with the polymeric network and present in the aqueous phase (Topete et al., 2020). Differently, the HEMA/i-CD hydrogel still sustained TA release for at least 24 h. Although the total amount released was higher after sterilization (in good agreement with a significantly higher loading), the percentage of drug released from HEMA/i-CD was lower. These results confirmed that HHP favored TA:HP- β -CD complex formation, and since no CDs were detected in the release medium, a controlled decomplexation of TA inside the hydrogel

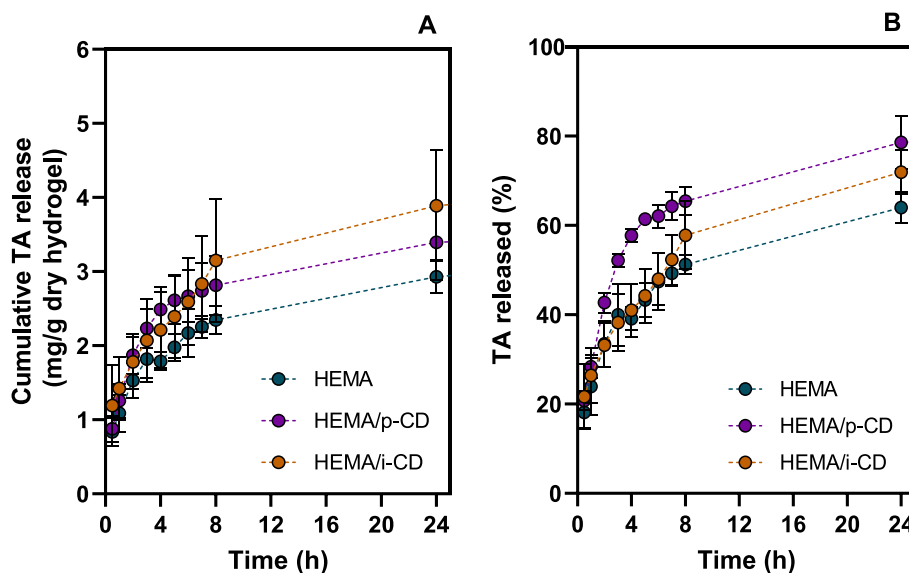


Fig. 2. (A) Cumulative release profiles from non-sterilized TA-loaded hydrogels and (B) % TA released relative to the total amount of drug contained in the hydrogels (B).

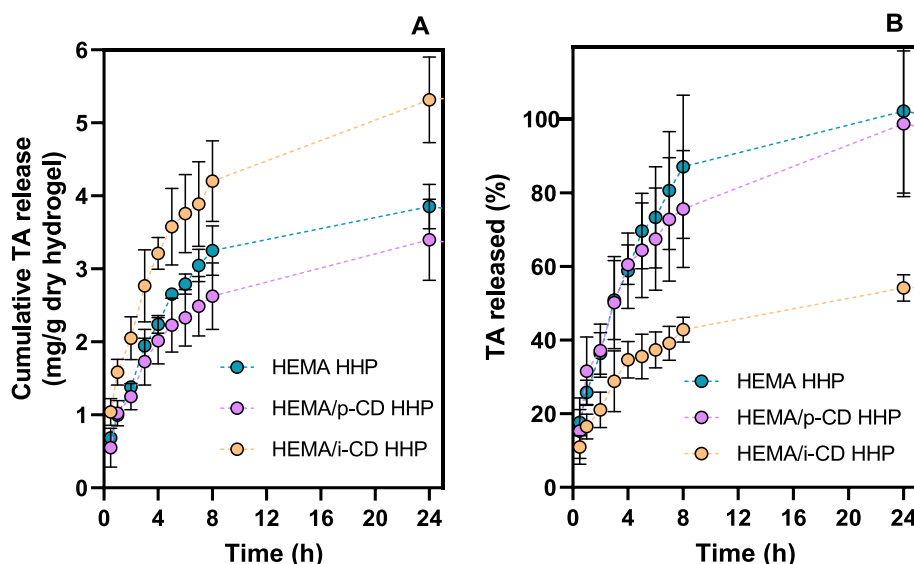


Fig. 3. (A) Cumulative release profiles from TA-loaded hydrogels submitted to HHP and (B) % TA released relative to the total amount of drug contained in the samples (B).

rendered sustained release. Thus, CDs play an essential role in increasing TA uptake and controlling the release when loaded hydrogels are submitted to HHP. Since the post-treatment of the hydrogels with HP- β -CD (hydrogel HEMA/p-CD) did not provide any advantage in TA loading and release, only HEMA/i-CD and HEMA hydrogels were considered for the subsequent experiments.

3.6. Protein adsorption

Deposition of proteins from tear fluid onto CLs should be minimized to prevent immunogenic and inflammatory responses on the ocular surface (Lord, Stenzel, Simmons, Milthorpe, 2006b). In the present study, the adsorption of BSA (0.05 g/L) and lysozyme (1.9 g/L) on HEMA and HEMA/i-CD hydrogels was evaluated through QCM-D. The time course of the normalized frequency and dissipation shifts upon the introduction of the protein solutions over the coated quartz crystals are presented in Figs. S3-S6. Normalized frequency ($\Delta f/n$) and dissipation (ΔD) variations for the 3rd harmonic are summarized in Table 4.

A decrease in frequency was verified after adding the protein solutions, revealing that the proteins adsorbed on the hydrogels. Additionally, the low dissipation values indicated the adsorbed layer's rigid nature (Vivero-Lopez et al., 2021). Thus, the Sauerbrey eq. (4) was used to predict the mass of the adsorbed layer per surface area of the crystals (Gispert et al., 2008),

$$\Delta m = C \times (\Delta f/n) \quad (4)$$

where C is the sensitivity constant for the 5-MHz quartz crystals (17.7 ng/cm²•Hz). The thickness of the adsorbed layer, t , was calculated through Eq. (5) (Jin et al., 2015), assuming the following values for the density of protein films (ρ_{protein}): 1.15 g/cm³ for BSA (Gispert et al., 2008) and 1.38 g/cm³ for lysozyme (Jin et al., 2015).

$$t = \Delta m / \rho_{\text{protein}} \quad (5)$$

The incorporation of HP- β -CD increased both the mass and thickness of the adsorbed BSA layer, but these values can still be considered small compared to other reported pHEMA-based hydrogels (150 ng/cm² (Mura-Galelli et al., 1991) and 170 ng/cm² (Castillo et al., 1984). Differences in mass adsorbed may relate to physicochemical properties of the hydrogels (e.g., water content, hydrophilicity, charge, or surface roughness) (Lord, Stenzel, Simmons, Milthorpe, 2006a; Luensmann & Jones, 2008) or differences in the experimental conditions (e.g., pH, temperature, protein solution concentration, flux). The small layer thickness (< 3 nm) suggests that BSA experienced conformational alterations upon interaction with the polymer surface (dimensions in solution of 8 × 3 × 3 nm) and/or that the protein adsorbed did not cover the surface completely.

Lysozyme's adsorbed mass was significantly higher than that observed for BSA in good agreement with the tested higher concentration of the former to resemble tear fluid conditions. In contrast to BSA, lysozyme layer mass and thickness decreased for HEMA/i-CD hydrogel compared to the control HEMA hydrogel. Values reported in the literature for lysozyme layer mass adsorbed in pHEMA-based hydrogels vary widely between 513 and 1330 ng/cm² (Teichroeb et al., 2008; Vivero-Lopez et al., 2021) yet are close to those found for the tested hydrogels. The thickness of the lysozyme layer adsorbed was in accordance with the lysozyme's dimensions (4.5 × 3 × 3 nm). In fact, lysozyme is known to form compact monolayers upon adsorption on pHEMA-based hydrogels (Lord, Stenzel, Simmons, Milthorpe, 2006a; Teichroeb et al., 2008).

Most studies report that protein adsorption decreases for more hydrophilic surfaces (Castillo et al., 1984; Luensmann & Jones, 2008; Teichroeb et al., 2008), as occurred for lysozyme onto HP- β -CD containing hydrogels. However, structural changes can play a major role in proteins with low conformational stability like BSA (Teichroeb et al.,

Table 4

Normalized frequency ($\Delta f/n$) and dissipation (ΔD) variations for the 3rd harmonic of resonance of QCM-D sensors coated with HEMA and HEMA/i-CD hydrogels when exposed to BSA and lysozyme solutions. Mass (Δm) and thickness (t) estimated for the proteins adsorbed layer are also presented.

Hydrogel	Protein	$\Delta f/n$ (Hz)	ΔD (x10 ⁻⁶)	Δm (ng/cm ²)	t (nm)
HEMA	BSA	-1.57 ± 0.42	-0.41 ± 0.32	28 ± 7	0.2 ± 0.1
HEMA/i-CD		-5.54 ± 3.57	0.51 ± 2.11	98 ± 63	0.9 ± 0.6
HEMA	Lysozyme	-27.51 ± 13.61	2.88 ± 2.95	487 ± 241	3.5 ± 1.7
HEMA/i-CD		-20.16 ± 2.60	1.76 ± 1.16	357 ± 46	2.6 ± 0.3

2008). Interactions between CDs and hydrophobic amino acids of BSA have previously been confirmed (Cooper et al., 1996) and may explain the higher protein layer mass for HEMA/i-CD hydrogel. Nevertheless, the incorporation of HP- β -CD had a small impact on protein adsorption compared to pHEMA-based hydrogels.

3.7. Ex vivo corneal and scleral permeability

The permeability of TA released from the most promising hydrogel (HEMA/i-CD HHP) was investigated *ex vivo* through porcine corneal and scleral tissues and compared to that of a free TA solution containing the amount of TA released from the hydrogel in 6 h in the *in vitro* release tests.

Accumulation of TA was higher in the sclera than in the cornea for both the hydrogel and the TA solution (Fig. 4A). Drug-eluting hydrogels led to lower amounts of TA accumulated in the cornea ($2.66 \pm 0.37 \mu\text{g}/\text{cm}^2$, $p < 0.01$) and sclera ($5.99 \pm 0.47 \mu\text{g}/\text{cm}^2$, $p < 0.001$) compared to the free TA solution. In the case of the TA solution, all free drug molecules are available to cross the tissues since the beginning of the experiment; differently, TA loaded in the hydrogel should diffuse out first. The efficient accumulation of TA in the cornea and sclera suggests that both tissues can constitute a depot from which TA can reach the posterior segment of the eye.

In the case of HEMA/i-CD hydrogels, TA permeated through the cornea and sclera was detected in the receptor after 2–3 h (Fig. 4B and C). Free drug solutions led to faster detection in the receptor medium (1 h) as the drug was more readily available. After 6 h, the amount of TA permeated from the hydrogels was $6.57 \pm 2.65 \mu\text{g}/\text{cm}^2$ for corneal tissues and $5.99 \pm 3.1 \mu\text{g}/\text{cm}^2$ for scleral tissues.

The flux (J), obtained from the slope of the linear regression of the mass of TA in the receptor chamber per diffusion area (0.567 cm^2) versus time, was used to calculate the apparent permeability coefficient (P) as follows:

$$P = J / [TA_{\text{donor chamber}}]_{t=6h} \quad (6)$$

TA concentration in the donor chamber after a 6 h test [$TA_{\text{donor chamber}}$] (Table 5) was similar for both the drug-eluting hydrogel and the TA solution in the case of cornea tests, but slightly lower in the sclera tests. P values for the TA solution were $18.07 \pm 0.88 \times 10^{-6} \text{ cm/s}$ and $19.17 \pm 0.63 \times 10^{-6} \text{ cm/s}$ for cornea and sclera, respectively (Table 5), and, as expected, were higher than for the loaded hydrogel. The trans-corneal and transcleral permeability and flux values here obtained are of the same order of magnitude as those previously reported for other ocular formulations, although significant variability in the data reported in the literature was found due to different experimental setups and tissue origin (Mora et al., 2005; Olsen et al., 1995; Prausnitz & Noonan, 1998; Tatke et al., 2019). The effect of 10 % ethanol on corneal and scleral permeability was considered negligible (Gautheron et al., 1992).

Drug permeation rate through the cornea depends on its molecular weight, concentration, and physicochemical properties, mainly the lipophilicity, given by the partition coefficient between *n*-octanol/buffer solution at pH = 7.4 ($\log D_{7.4}$) (Gaballa et al., 2021; Loftsson & Brewster, 2011). Compared to other corticosteroids evaluated for topical treatment of macular edema, such as dexamethasone or hydrocortisone (Cagini et al., 2021; Karasu et al., 2022; Y. Lin et al., 2022; Stefansson et al., 2023), TA presents a higher $\log D_{7.4}$ (2.58) – due to the acetamide group – resulting in higher corneal permeation (Bongiovi et al., 2017; Dave & Morris, 2016; Schoenwald & Ward, 1978). Contrarily, molecular size is the main limiting factor in scleral permeability since the drug should diffuse through a glycosaminoglycan matrix rich in collagen fibrils (Thrimawithana et al., 2011). TA presents a molecular weight of 434.5 g/mol, similar to hydrocortisone (362 g/mol) and dexamethasone (396 g/mol); therefore, a comparable transscleral permeability is expected. Previous reports on animal models with retinal diseases have confirmed the capability of topically applied TA to cross ocular barriers and reach the retina (Cheng et al., 2019). Overall, the results obtained in

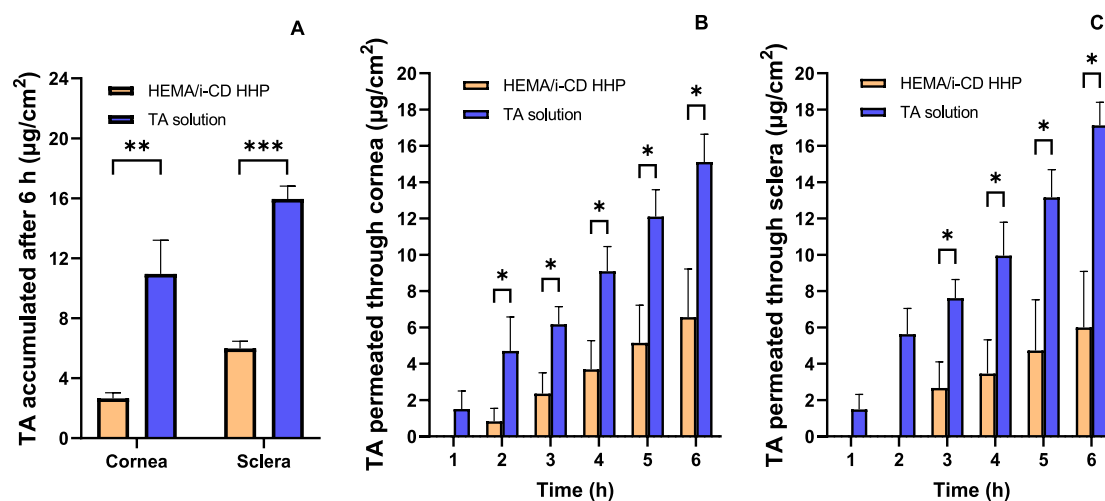


Fig. 4. (A) Amount of TA accumulated in corneal and scleral tissues after 6 h in contact with TA-loaded HEMA/i-CD HHP hydrogel or TA solution 60 mg/L. (B) Amounts of TA permeated through corneal and (C) scleral tissues from HEMA/i-CD HHP and TA solution (60 mg/L) as a function of time. * denotes statistically significant comparisons between HEMA/i-CD HHP hydrogel and TA solution, as referred in Supplementary Material.

Table 5

Flux (J) and apparent permeability coefficient (P) of TA through the cornea or sclera tissue obtained for TA-loaded hydrogel (HEMA/i-CD HHP) and a TA solution (60 mg/L). * denotes statistically significant comparisons between HEMA/i-CD HHP hydrogel and TA solution, as referred in Supplementary Material.

Formulation	Tissue	$[TA_{\text{donor chamber}}]_{t=6h}$ (mg/L)	J ($\mu\text{g}/\text{cm}^2 \cdot \text{h}$)	$P \times 10^6$ (cm/s)
TA solution	Cornea	40.9 ± 3.8	2.66 ± 0.13	18.07 ± 0.88
HEMA/i-CD HHP	Cornea	36.9 ± 2.4	1.42 ± 0.5 (*)	10.66 ± 3.79 (*)
TA solution	Sclera	42.7 ± 3.4	2.95 ± 0.10	19.17 ± 0.63
HEMA/i-CD HHP	Sclera	32.5 ± 2.2	1.32 ± 0.54 (*)	11.29 ± 4.61 (*)

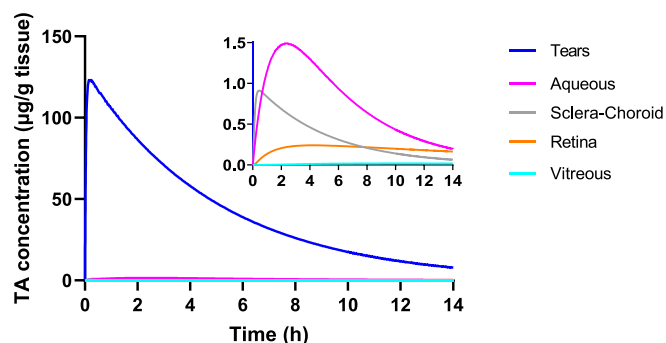


Fig. 5. TA concentration in the ocular tissues over time, normalized per tissue or fluid weight. A zoomed representation is shown in the figure insert.

our work confirmed the ability of TA released from CLs to cross the cornea and sclera from their outer face towards the internal structures of the eye. Therefore, the use of CLs as platforms for the sustained release of TA is expected to optimize the efficacy of the delivery in terms of minimizing unproductive loss and untoward systemic effects.

3.8. Prediction of *in vivo* efficacy

Regarding the clinical relevance of the proposed device, different doses varying between 1 and 4 mg of TA administered intravitreally were shown effective for treating DME for up to 3 months (Dubey, 2006; Jonas et al., 2004; Spandau, 2005). Such doses are considerably higher than the TA amount released by the designed CLs (i.e., ≈ 0.1 mg of TA on the ocular surface in a 14 h wearing time). Nonetheless, as pathologies affecting the posterior segment are generally chronic, repeated CL use could provide a cumulative therapeutic effect in the retina and a sustained drug delivery over time.

In vivo tests are required to assess the ability of the device to provide therapeutic drug doses in target tissues, as well as the safety of the proposed treatment. However, in the early stages of product development, mathematical simulations constitute a useful tool for the estimation of ocular drug distribution, with ethical and economic advantages over animal testing. Based on the *in vitro* release profile (which was fitted to an exponential curve, in Fig. S7), the drug concentration in the tears, aqueous humor, sclera-choroid, retina, and vitreous humor after a single CL use was predicted. TA is expected to distribute both to the anterior and posterior segments of the eye (Fig. 5). Although the minimum effective concentrations of TA needed in the vitreous cavity, choroid, or retina for a therapeutic outcome have not been reported in the literature, TA was

demonstrated to provide a neuroprotective effect and inhibit proinflammatory genes and cytokines (iNOS, TNF- α , and IL-1 β) at doses as low as 1 μ g/L and 9 μ g/L, respectively, in murine primary glial cells (Hong et al., 2012). According to the model prediction, the TA concentration in the retina is expected to reach a maximum value of 240 μ g/L after 4 h of CL wearing, and then be maintained at values above 150 ng/mL for at least 14 h, thus suggesting the therapeutic potential of the developed TA-eluting CLs for the treatment of the posterior segment of the eye.

3.9. Cytocompatibility and HET-CAM

HEMA/i-CD HHP hydrogels were highly compatible with primary human corneal epithelial cells, with viability values of 83.3 ± 6.6 % for non-loaded and 74.2 ± 4.7 % for TA-loaded hydrogels (Fig. 6A). To fully safeguard the non-toxicity of the hydrogels, the cell viability of two TA solutions covering the maximum solubility in an aqueous medium (40 mg/L) and the maximum concentration that the hydrogels can provide (2.5 mg/L) was also evaluated (Fig. 6B), with results of 83.6 ± 6.2 % and 89.7 ± 7.9 %, respectively. To check whether this reduction was due to dilution of the culture medium with the TA solvents (1:10), two additional controls, C TA 2.5 and C TA 40, were prepared in culture medium with 0.9 % NaCl or ethanol:water 20:80 v/v, respectively, and their impact was found negligible (cell viability ca. 95 %). Therefore, the TA eluted from CLs is not expected to produce any cytotoxic effect.

Furthermore, the HET-CAM test confirmed that non-loaded and loaded HEMA/i-CD HHP hydrogels did not trigger any irritation signs, namely hemorrhage, vascular lysis, or coagulation of CAM vessels (i.e., IS = 0) (Fig. S8). Consequently, the formulated hydrogels could be considered non-irritating devices for ocular tissues.

3.10. Anti-inflammatory activity

As a proof of concept of the therapeutic potential, the secretion of two inflammatory biomarkers involved in DME, namely IL-6 and TNF- α , by LPS-stimulated macrophages was evaluated after exposure to non-loaded and loaded HEMA/i-CD HHP hydrogels and TA solution (Fig. 7). Treatment with TA-loaded hydrogel significantly decreased the secretion of IL-6 compared to non-loaded hydrogel (≈ 68 % $p < 0.01$). Similar results were obtained for a TA solution containing the maximum amount of drug loaded by the hydrogel (2.5 mg/L). In the case of TNF- α , a decrease of 29 % and 27 % in the secretion of the cytokine was attained after exposure to loaded hydrogel and TA solution, respectively, compared to the non-loaded hydrogel. These results demonstrate the efficacy of TA-eluting HEMA/i-CD HHP hydrogels in dampening the secretion of pro-inflammatory cytokines.

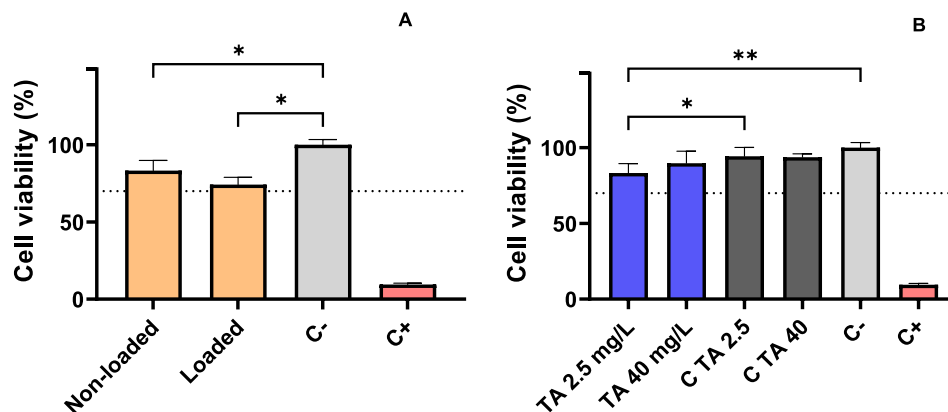


Fig. 6. (A) Cell viability of human corneal epithelial cells determined by MTT colorimetric assay after 24-h exposure to non-loaded and loaded HEMA/i-CD HHP hydrogels and (B) two TA solutions (2.5 mg/L and 40 mg/L). C TA 2.5 and C TA 40 refer to cells cultured in diluted culture medium with 0.9 % NaCl solution 1:10 or ethanol:water 20:80 v/v 1:10, respectively. Negative control (C-) and positive control (C+) represent cells cultured in culture medium without and with DMSO 10 %, respectively. The dashed lines correspond to 70 % cell viability. * denotes statistically significant comparisons, as referred in Supplementary Material.

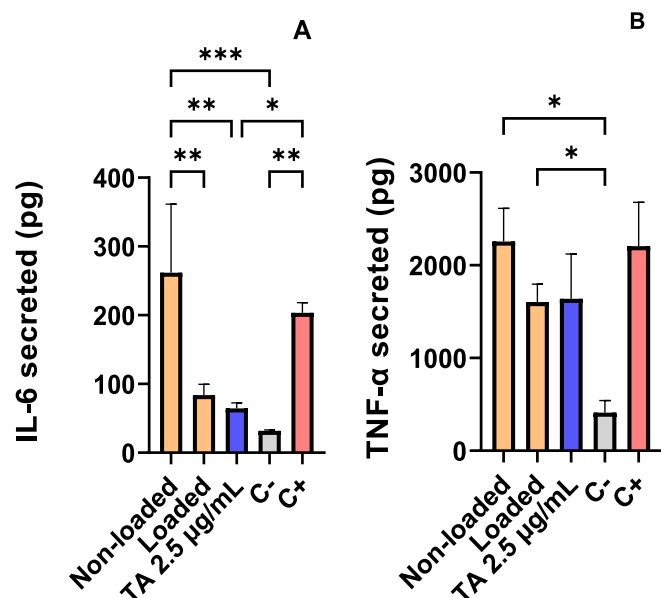


Fig. 7. Effect of the treatment with non-loaded and TA-loaded HEMA/i-CD HHP hydrogels and TA solution (2.5 mg/L) on the secretion of (A) IL-6 and (B) TNF- α from macrophages stimulated with LPS. Negative control (C-) refers to unstimulated macrophages without further treatment. Positive control (C+) refers to macrophages only stimulated with LPS without further treatment. *denotes statistically significant comparisons, as referred in Supplementary Material.

4. Conclusion

The purpose of this work was to design pHEMA-based hydrogels containing HP- β -CD and evaluate the impact of HHP sterilization on the loading and release of the poorly water-soluble drug TA. The findings indicate that the addition of HP- β -CD to the monomers mixture (HEMA/i-CD hydrogels) allowed effective incorporation of HP- β -CD into the acrylic network without compromising key properties for its use as therapeutic CL, such as water content, oxygen and ion permeability, stiffness, roughness, and light transmission. Moreover, HHP sterilization further improved the loading capacity of TA in HEMA/i-CD hydrogels by promoting host-guest interactions, which in turn resulted in an improved capability to sustain drug release during the 24-h CL wearing. Biocompatibility tests in human cell cultures and irritability tests confirmed the safety of the developed hydrogels. The *ex vivo* permeability tests and the reduction of the secretion of anti-inflammatory cytokines evidenced the suitability of the HP- β -CD functionalized material to deliver therapeutic amounts of TA on the ocular surface. A previously validated mathematical model demonstrated the potential of the designed device to deliver TA from the ocular surface to the posterior segment of the eye for at least 14 h, reaching therapeutic concentrations in the target tissues.

Overall, the obtained results confirm the starting hypothesis, that the application of HHP to CD-containing hydrogels promotes the formation of inclusion complexes with lipophilic drugs and is an easy-to-scale-up procedure to design TA-eluting daily CLs suitable for the treatment of diseases like diabetic macular edema. This study reports novel CLs-based hydrogels for the sustained release of TA on the ocular surface. Moreover, as far as the authors know, there was no study focusing on the development of HEMA-based hydrogels with incorporated pristine HP- β -CD and HHP to promote CD complexation within a hydrogel.

CRediT authorship contribution statement

Carolina Marto-Costa: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Nadia**

Toffoletto: Writing – original draft, Formal analysis. **Madalena Salema-Oom:** Writing – review & editing, Visualization, Validation, Resources, Methodology, Investigation. **Alexandra M.M. Antunes:** Resources, Methodology, Investigation. **Carlos A. Pinto:** Resources, Investigation. **Jorge A. Saraiva:** Resources, Investigation. **Ana S. Silva-Herdade:** Supervision. **Carmen Alvarez-Lorenzo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Ana Paula Serro:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

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.

Data availability

Data will be made available on request.

Acknowledgments

Work supported by Fundação para a Ciência e a Tecnologia (FCT) through [doi: 10.54499/UIDB/00100/2020] and [doi: 10.54499/UIDP/00100/2020] (CQE), [doi: 10.54499/UIDB/04585/2020] (CiiEM) and [doi:10.54499/PTDC/CTM-CTM/2353/2021] (SOL project), University of Aveiro and FCT/MCT through [UIDB/50006/2020 and UIDP/50006/2020] (LAQV-REQUIMTE) co-funded by the FEDER within PT2020 Partnership Agreement, and MCIN/AEI/10.13039/501100011033 Spain [PID 2020-113881RB-I00], Xunta de Galicia [ED431C 2020/17], and FEDER. The authors acknowledge SICASAL - Indústria e Comércio de Carnes, S.A. for the kind supply of porcine fresh eyes and to Dr. Nuno Taveira from the University of Lisbon for providing THP-1 human monocytes cell line. ASHLAND kindly provided Cavasol® W7 HP PHARMA (HP- β -CD). The Graphical Abstract was created by using pictures from Servier Medical Art., by Servier, licensed under a Creative Commons Attribution 3.0 Unported License (<https://creativecommons.org/licenses/by/3.0/>).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.carbpol.2024.121880>.

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