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Poly-(cyclo)dextrins as ethoxzolamide carriers in ophthalmic solutions and in contact lenses

M.J. García-Fernández[a], N. Tabary[b], B. Martel[b], F. Cazaux[b], A. Oliva[c], P. Taboada[d], A. Concheiro[a], C. Alvarez-Lorenzo[a,*]

[a] Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain
[b] Unité Matériaux et Transformation (UMET), Ingénierie des Systèmes Polymères, Université de Lille 1, F-59655 Villeneuve D'Ascq, France
[c] Departamento de Ingeniería Química y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de La Laguna, 38200 La Laguna, Tenerife, Spain
[d] Departamento de Física de la Materia Condensada, Facultad de Física, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain

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ABSTRACT

Efficient ophthalmic therapy requires the development of strategies that can provide sufficiently high drug levels in the ocular structures for a prolonged time. This work focuses on the suitability of poly-(cyclo)dextrins as carriers able to solubilize the carbonic anhydrase inhibitor (CAI) ethoxzolamide (ETOX), which is so far used for oral treatment of glaucoma. Topical ocular treatment should notably enhance the efficiency/safety profile of the drug. Natural α-, β- and γ-cyclodextrins and a maltodextrin were separately polymerized using citric acid as cross-linker agent under mild conditions. The resultant hydrophilic polymers exhibited larger capability to solubilize ETOX than the pristine (cyclo)dextrins. Moreover, they provided sustained drug diffusion in artificial lachrymal fluid. Interestingly the poly-(cyclo)dextrins solutions facilitate the loading of remarkably high doses of ETOX in poly(2-hydroxyethyl methacrylate)-based contact lenses. Exploiting ionic interactions between functional groups in the contact lenses and remnant free carboxylic acids in the citric acid linkers of poly-(cyclo)dextrins led to the retention of the drug-loaded poly-(cyclo)dextrins and, in turn, to sustained release for several weeks.

1. Introduction

The unique ability of natural α-, β-, and γ-CDs to form inclusion complexes with a wide variety of substances has been largely exploited to face up to relevant drug formulation problems related to deficient solubility, stability, permeability and organoleptic features (Brewster & Loftsson, 2007). As the knowledge in CDs progresses, novel applications are coming up, particularly for development of modified drug release formulations exhibiting from sustained to targeted delivery (Mellet, Garcia, & Benito, 2011; Moya-Ortega, Alvarez-Lorenzo, Concheiro, & Loftsson, 2012). In this sense, combination of the features of CDs with those of polymers is particularly attractive (Concheiro & Alvarez-Lorenzo, 2013; Morin-Crini & Crini, 2012). Far from having a detrimental effect on the ability to form complexes, CDs cooperatively work when they are close together, provoking a displacement in the complex formation equilibrium toward the complexed species; this in turn prevents rapid release once in contact with the physiological fluids (Danel et al., 2013; Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2007; Rosa dos Santos et al., 2010). Moreover, non-inclusion complexes become also possible with the polymer backbone participation. These findings make CDs to be outstanding building blocks for the development of polymeric drug delivery systems with a therapeutic potential still to be fully explored (Simoes et al., 2013; Van de Manakker, Vermonden, van Nostrum, & Hennink, 2009).

Several approaches can be followed to prepare linear and branched CD polymers: (i) grafting of CDs to preformed polymers (Auzély-Velty, 2011); (ii) polymerization of monomer derivatives of CDs bearing reactive double bonds (e.g. acrylic substituted CDs) suitable for reaction with other acrylic/vinyl monomers (Yamaguchi, Kobayashi, Takashima, Hashidzume, & Harada, 2011); or (iii) cross-linking of CDs with bi- or multi-functional reagents, such as epichlorohydrin, biepoxides and diisocyanates (Renard, Deratani, Volet, & Sebille, 1997; Simoes et al., 2013). Concerns on sustainability and human and environmental toxicity are prompting the application of green chemistry principles to polymer synthesis (Beach, Cui, & Anastas, 2009). For example, in the context of approach (iii), notable efforts are been made to choose innocuous cross-linkers and solvents. Citric acid has been shown suitable to form ester groups with the hydroxyl groups of CDs via dehydration,

* Corresponding author. Tel.: +34 981563100x15239; fax: +34 981547148.
E-mail address: carmen.alvarez.lorenzo@usc.es (C. Alvarez-Lorenzo).

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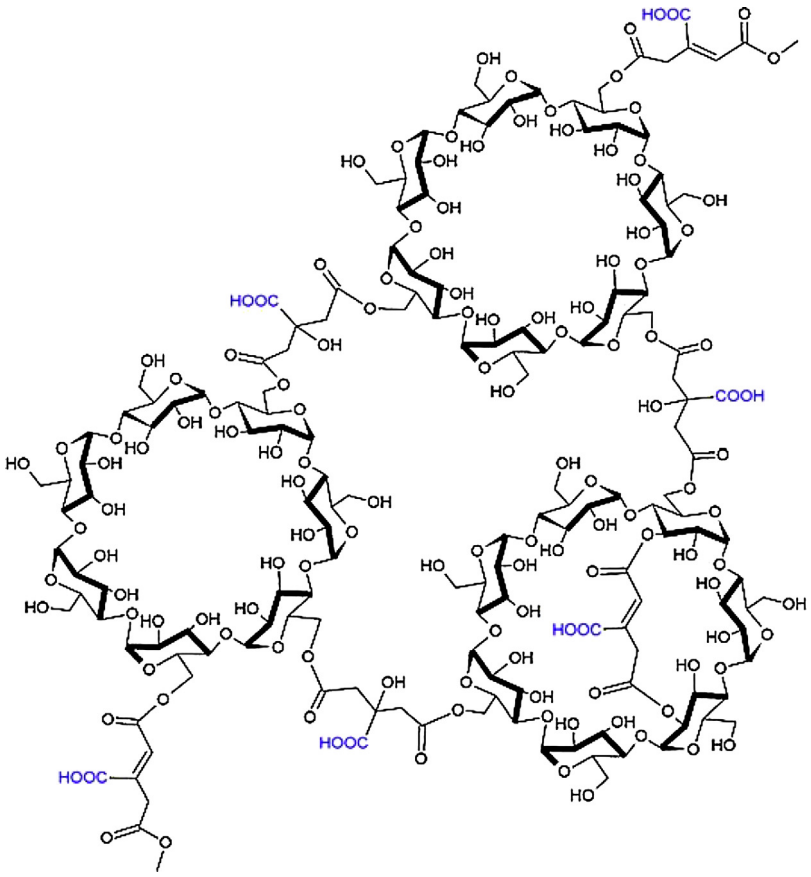

Fig. 1. Structure of the water-soluble polymer of CDs cross-linked with citric acid.

forming an anhydride intermediate (Martel, Weltrowski, Ruffin, & Morcellet, 2002). The method involves a minimum amount of water as solely solvent since the reaction takes place when the CD and citric acid solution, containing an adequate catalyzer, is dried and then cured at 140–170 °C under vacuum. In fact, cross-linking of CDs with polycarboxylic acids has been shown useful for the coating of medical devices, such as vascular prosthesis, catheters, or gauzes (Blanchemain et al., 2007, 2008; Concheiro & Alvarez-Lorenzo, 2013; Garcia-Fernandez et al., 2013), and more recently hip prostheses coated with hydroxyapatite (Taha et al., 2013). The layer of CDs endows the surface of the device with capability to host a variety of substances, including dyes and drugs, and regulate their release rate (Blanchemain et al., 2012; El Ghoul et al., 2010). In vivo studies carried out with vascular prosthesis have shown that the citric acid cross-linked CDs layers are biocompatible, and eventually disappear some months after implantation (Jean-Baptiste et al., 2012).

The aim of this work was to adapt the cross-linking of CDs with citric acid to the preparation of water soluble polymers (Fig. 1) that act as solubilizing agents of ophthalmic drugs. Particularly, we focused on ethoxzolamide (ETOX), a carbonic anhydrase inhibitor (CAI) that exhibits high ocular permeability but is poorly soluble in water, which prevents the attainment of therapeutic ocular levels. CAIs are in the first line of treatment of glaucoma and are mostly administered through the oral route at relatively high doses (Supuran, 2010). Since carbonic anhydrases are ubiquitously distributed in the body, systemic administration leads to side effects (Supuran, 2008). The topical administration of CAIs to the eye has been shown to notably decrease these side effects and be more patient compliant (Prausnitz & Noonan, 1998). ETOX solubilization by means of polymeric micelles (Ribeiro et al., 2012) or forming inclusion complexes with hydroxypropyl-β-CD (Loftsson et al., 1994) has been previously reported. To carry out the work, polymers of α-CD, β-CD, γ-CD, methyl-β-CD, hydroxypropyl-β-CD and maltodextrin were prepared having an approximate cross-linker:(cyclo)dextrin 50:50 weight ratio, and their ability to solubilize ETOX and to regulate its diffusion was compared to that of pristine components. Ocular compatibility was evaluated by means of the HET-CAM assay, an alternative to animal testing (NICEATM-ICCVAM, 2012). Since short permanence time of ophthalmic solutions on the ocular surface usually compromises drug bioavailability and therapeutic effects length, the second aim of this work was to elucidate the feasibility of incorporating ETOX-loaded poly-(cyclo)dextrins into soft contact lenses. Hydrogel-based contact lenses have attracted notable interest as ocular bandages able to combine the primary function of vision correction with that of sustained release drug depots (Alvarez-Lorenzo, Yañez, & Concheiro, 2010; Rosa dos Santos et al., 2009). Previous attempts to load ETOX into contact lenses involved bioinspired, imprinted design of the lens network in order to create high affinity domains able to directly interact with the drug (Ribeiro et al., 2011a, 2011b). Despite the success of this approach, the loading was still limited because it was carried out by soaking in an aqueous drug solution without solubilizing agents and thus a small drug concentration gradient was attained. It can be hypothesized that poly-(cyclo)dextrins enhance the loading of ETOX in contact lenses by increasing the concentration in their aqueous phase, which may also promote specific drug-network interactions. Moreover, if the contact lenses possess functional groups suitable for ionic interaction with free carboxylic acid groups in the poly-(cyclo)dextrins, more sustained drug release compared to free CDs should be attained.

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD), maltodextrin (MD, Glucidex® 19), hydroxypropyl-β-cyclodextrin (HPβCD, Kleptose® HPB, MS = 0.84), and methyl-β-cyclodextrin (MβCD, Crysmeb®, DS = 0.50) were provided by Roquette (Lestrem, France). α-Cyclodextrin (α-CD) and γ-cyclodextrin (γ-CD) were provided by Wacker (Bürghausen, Germany). The term (cyclo)dextrins or poly(cyclo)dextrins was used to name systems indifferently based on cyclodextrins and maltodextrins. Sodium chloride (NaCl), citric acid monohydrate (CTR) and sodium dihydrogen hypophosphite ($NaH_2PO_2{\cdot}H_2O$) were from Aldrich Chemicals (Saint Quentin Fallavier, France). Dialysis membranes (500–1000 and 6000–8000 MWCO) were from Spectrum laboratories (France), hydroxyethyl methacrylate (HEMA) from Merck (Spain), and N-(3-aminopropyl) methacrylamide hydrochloride (APMA) from Polysciences (Germany). Ethoxzolamide (ETOX), ethyleneglycol dimethylacrylate (EGDMA), azobisisobutyronitrile (AIBN) and dichloromethylsilane were from Sigma Aldrich (St. Louis, MO, USA). Purified water was obtained by reverse osmosis (MilliQ®, Millipore Iberica S.A., Madrid, Spain).

2.2. Synthesis of poly-(cyclo)dextrins

Water soluble polymers of MD, α-CD, β-CD, γ-CD, HPβCD and MβCD, respectively named polyCTR-MD, polyCTR-αCD, polyCTR-βCD, polyCTR-γCD, polyCTR-HPβCD and polyCTR-MβCD, were synthesized as previously reported (Martel et al., 2002; Martel, Ruffin, Weltrowski, Lekchiri, & Morcellet, 2005). Briefly, aqueous solutions (200 mL) of sodium dihydrogen hypophosphite (30 g/L), CTR (100 g/L) and the corresponding CD or MD (100 g/L) were prepared under stirring at room temperature. Water was totally removed from the solutions using a Rotavapor (Büchi, Switzerland). The resulting dried mixture was then heated at 140 °C in oil bath during 30 min also under vacuum (~60 mbar). The polymer was redispersed in water (200 ml) and then filtered on sintered glass funnel to remove the insoluble fraction. The filtrate was concentrated in rotavapor and dialyzed (6000–8000 MWCO) against water (2 L, exchanged every 12 h) for 3 days. Finally, the polymer was recovered as a white powder by freeze-drying. The yield of each polymer fraction (soluble and gel) was calculated as the ratio of the weight of the obtained product to the weight of CTR plus CD in the initial solution. The catalyst was neglected as only traces of phosphorus were detected by elemental analysis in the final product.

2.3. NMR spectroscopy

NMR spectra of the polymers (10 mg in 600 μL D_2O) were recorded using a spectrometer Bruker Avance™ 400 equipped with a Bruker Ultra-Shield 9.4T (proton Larmor frequency of 400.33 MHz) and BBI probe (^{1}H, X).

2.4. Molecular mass

The molecular mass of the poly-(cyclo)dextrins were determined from static light scattering (SLS) intensities of 1–50 g/l polymer solutions recorded at 25 °C using an ALV-5000F (ALV-GmbH, Germany) instrument with vertically polarized incident light of wavelength λ = 488 nm supplied by a CW diode-pumped Nd:YAG solid-state laser (Coherent Inc., Santa Clara, CA) operated at 2 W. The intensity scale was calibrated against scattering from toluene. Measurements were made at the scattering angle of 90° to the incident beam. Solutions were filtered through Millipore Millex filters (Triton free, 0.22 μm pore size) and equilibrated at 25 °C for 30 min before measurement. Experiment duration was in the range 5–15 min and each experiment was repeated two or more times. The basis for analysis of SLS intensities was the Debye equation

$$\frac{K^*c}{I - Is} = \frac{1}{Mw} + 2A_2c + \cdots$$

where I is the intensity of light scattering from solution relative to that from toluene, Is, c is the concentration (in g dm^{-3}), Mw is the mass-average molar mass of the solute, A_2 is the second virial coefficient (higher coefficients being neglected), and K^* is the appropriate optical constant ($K^* = 4\pi^2(dn/dc)^2 n_{ref}^2/(R_{ref}N_a\lambda^4)$). Values of the specific refractive index increment, dn/dc, were determined to be 0.123 (polyCTR-βCD), 0.121 (polyCTR-γCD) and 0.126 (polyCTR-MD) (±0.00001) m^3 kg^{-1} by analysing the concentration dependence of the polymer refractive index by using a refractometer RA-510M (Mettler-Toledo, Spain). Other quantities used were the Rayleigh ratio of toluene for vertically polarized light, $R_{ref} = 2.57 \times 10^{-5}\,[1 + 3.68 \times 10^{-3}(t\text{-}25)]$ cm^{-1} (t in °C) and the refractive index of toluene, $n_{ref} = 1.4969 \times [1 - 5.7 \times 10^{-4}(t\text{-}20)]$.

Molecular mass was also determined using gel permeation chromatography (GPC) in an equipment consisting in one pre-column (separation range: 100–1,800,000 g/mol) and 3 columns Shodex OH-pak 30 cm (802.5HQ, 804HQ, 806HQ), Waters 515 HPLC pump and a 717 plus Autosampler (Waters), Differential Refractometer Waters 2414, and LS multi-angles mini DAWN TREOS (Wyatt Technol.). Molecular mass estimation was performed using Astra 6.0.1 software (Wyatt Technol.). In this case dn/dc values were determined analyzing the concentration dependence of the polymers refractive index using a Differential Refractometer Waters 2414 with mobile phase as polymer solvent. The estimated values were 0.122 (polyCTR-βCD), 0.122 (polyCTR-γCD) and 0.121 (polyCTR-MD) m^3 kg^{-1}. The mobile phase was 0.1 M $NaNO_3$ with NaN_3 in Millipore quality water at flow of 0.5 ml/min at 25 °C. Polymer was dissolved in mobile phase (2.49–2.59 mg/mL) and then filtered (Chromafill XTra, Macherey-Nagel, 0.45 μm/25 mm) before injection (50 μL).

2.5. HET-CAM test

The Hen's Egg Test-Chorioallantoic Membrane (HET-CAM) assay was carried out using fertilized broiler chicken eggs (Avirojo, Pontevedra, Spain) incubated for 11 days (37 ± 0.3 °C and 60 ± 2% RH) following the ICCVAM-recommended test method protocol (NICEATM-ICCVAM, 2012). The eggs were rotated 5 times per day to prevent the adhesion of the embryo to one side of the egg. The top of the eggshell was removed with a rotary saw (Dremel 300; Breda, Netherlands). The inner membrane was wetted with 0.9% NaCl solution, and the eggs were placed again in the climatic chamber for 30 min. Then, the 0.9% NaCl solution was removed, and the inner membrane detached with a forceps. The polymer solution (50 g/L in phosphate buffer pH 7.4, 300 μL) was poured on the chorioallantoic membrane and the potential irritation was monitored during 5 min paying attention to the following processes: hemorrhage (bleeding from the vessels; H_{time}), vascular lysis (disintegration of blood vessels; L_{time}), and coagulation (protein denaturation, intra and extra vascular complications; C_{time}). The experiments were done in triplicate, using 0.9% NaCl solution as negative control and 0.1 N NaOH solution as positive control.

The irritation score was calculated as:

$$IS = \left[\left(\frac{301 - H_{time}}{300}\right)\cdot 5\right] + \left[\left(\frac{301 - L_{time}}{300}\right)\cdot 7\right] + \left[\left(\frac{301 - C_{time}}{300}\right)\cdot 9\right]$$

2.6. ETOX solubilization

CD, MD, and the corresponding polymer solutions were prepared in 0.9% NaCl covering a wide range of concentrations. ETOX was added in excess (1 mg/mL) to the solutions and then they were kept under magnetic stirring for 48 h at room temperature and protected from light. The systems were filtered (0.45 μm PTFE membrane; Fisher Scientific, Spain) and aliquots of the solutions were diluted with ethanol: 0.9% NaCl solution 7:3 for spectrophotometric quantification of ETOX (λ = 303 nm, Shimadzu UV 1800, France). The apparent stability constant of ETOX inclusion complexes and the binding efficiency (BE) of polyCTR-CDs were estimated from the slope of phase solubility diagrams in the initial linear range following the same procedure as for the stability constant of 1:1 complexes and the complexation efficiency (CE), respectively, of CD dispersions (Higuchi & Connors, 1965; Loftsson, Hreinsdóttir, & Másson, 2005). In the case of polyCTR-CDs, the CD molar concentration was estimated from the CD content of each polymer. The same procedure was applied to the solutions based on MD and polyCTR-MD.

2.7. ETOX diffusion

ETOX solutions in β-CD, γ-CD, MD (7.5 and 15 g/L) and their corresponding polymers (15 and 30 g/L) in NaCl 0.9% were prepared as described above. Aliquots of the solutions (3 mL) were placed in the donor compartment of Franz-Chien diffusion cells, separated from the receptor with cellulose dialysis membrane (MWCO 500–1000 Da, Spectrum Lab., Rancho Dominguez, CA, USA). The receptor medium (7 ml of 0.9% NaCl solution containing 0.3% SDS) was kept under stirring at 25 °C. At pre-established time intervals, samples of receptor medium (0.5 mL) were taken and the absorbance measured at 303 nm. The samples were immediately returned to the receptor compartment. The diffusion coefficients were estimated by fitting the Higuchi equation (Higuchi, 1962):

$$\frac{Q}{A} = 2^{\bullet} C_0^{\bullet} \left(\frac{D^{\bullet} t}{\pi}\right)^{1/2}$$

where Q is the amount of ETOX (mg) released at time t (s), A the diffusion area (cm^2), C_0 the initial concentration of ETOX in the formulation (mg/ml), and D the diffusion coefficient (cm^2/s).

2.8. Preparation of hydrogel-based contact lenses

EGDMA (476 μL) and AIBN (0.049 g) were dissolved in HEMA (30 ml). APMA (0.214 g) was added to half of this solution. Portions of monomers solutions were then carefully injected into molds constituted by two glass-made plates (pretreated with dichloromethylsilane) and a silicone frame (0.9 mm thickness). Polymerization was carried out at 50 °C for 12 h and then at 70 °C for 24 h. Poly-HEMA and poly-HEMA-co-APMA sheets were removed from the molds and immediately immersed in boiling water (500 mL) for 30 min to remove unreacted monomers and to facilitate the cutting of disks (10 mm diameter) with a cork borer. The disks were washed in 0.9% NaCl (500 mL, 24 h) and then in water (24 h). Finally, the disks were dried at 50 °C and weighed.

2.9. ETOX loading in hydrogel-based contact lenses

ETOX (1 mg/mL) was added to solutions of CD or MD (15 g/L) or their polymers (30 g/L) in water. After stirring during 48 h at room temperature protected from the light, the dispersions were filtered (0.45 μm PTFE membrane) and the concentration of ETOX was quantified as described above. Dry disks of poly-HEMA and poly-HEMA-co-APMA hydrogel (0.078 ± 0.009 g) were immersed into 2 mL of ETOX solutions and kept in the dark. At pre-established time intervals, the absorbance of the loading solution was spectrophotometrically monitored at 303 nm. The amount of drug loaded by each hydrogel disk was calculated from the difference between initial and final drug concentration. Degree of swelling in the loading medium was estimated from the weights of the disks at the dry state (W_i) and once swollen (W_f) as follows:

$$Q(\%) = \frac{W_f - W_i}{W_i} \times 100$$

2.10. ETOX release from hydrogel-based contact lenses

Drug-loaded hydrogel discs were removed from the loading solutions (their surfaces were carefully wiped with absorbent paper) and immediately immersed in artificial lachrymal fluid (6.78 g/L NaCl, 2.18 g/L $NaHCO_3$, 1.38 g/L KCl, 0.084 g/L $CaCl_2 \cdot 2H_2O$; pH 8.0) at 25 °C. The volume of medium, initially 2 mL was progressively increased up to 10 mL in the first 48 h to maintain sink conditions. The amount of released drug was spectrophotometrically monitored at 303 nm.

3. Results and discussion

3.1. Synthesis and characterization of poly-(cyclo)dextrins

Polymers were prepared with CDs differing in size and substituents as well as with MD in order to elucidate the possibilities of preparing materials having different ability to interact with active substances. The temperature and the time of polymerization were the same for all CDs and MD. ^{1}H NMR analysis was carried out to determine the ponderal composition of the different polymers. As an example, the spectrum in Fig. 2 corresponding to polyCTR-βCD displays the signal of the glucopyranose units of CDs, H1 at 5.01 ppm, H3, H5, H4 and H2 between 3.58 and 3.99 ppm, protons of ester groups between 4 and 4.60 ppm, methylene groups of the citrate crosslinks appearing between 2.76 and 2.96 ppm, and finally two singlets at 6.32 and 7.62 ppm due to cis and trans aconitic esters, respectively. The latter species visible in Fig. 1 were issued from a side reaction consisting of the dehydration of the citrate crosslinks (Wyrzykowski, Hebanowska, Nowak-Wiczk, Makowski, & Chmurzyński, 2011). Based on the integrations values of H1 (CD) and citrate methylene signals (CH_2-CTR), the ponderal composition of polyCTR-βCD was estimated to consist of 51 wt.% in β-CD moieties and 49 wt.% in citrate cross-links. The content in CD of polyCTR-αCD, polyCTR-γCD, polyCTR-MβCD and polyCTR-HPβCD was 59.1, 55.0, 57.7 and 66.2 wt.%, which is in close agreement with the feed ratio. Similarly, the content in MD of polyCTR-MD was 65.6 wt.%.

Static (SLS) and dynamic light scattering (DLS) measurements of the synthesized CD-based polymers were carried out to gain an insight into their structure. The intensity-averaged population distributions of the poly-CDs obtained from DLS data (see Fig. S1 in Supplementary Material) revealed the existence of several relatively well-defined species. In particular, there exists a common population for all samples with sizes ranging ca. 1–5 nm, whilst other populations are present at larger sizes (centered at ca. 40 nm for polyCTR-γCD, and at ca. 10 and 70 nm for polyCTR-MD). In fact, the cross-linked (cyclo)dextrin polymers can relate to nanosponges, as called by Trotta and collaborators (Trotta, Zanetti, & Cavalli, 2012). The latter populations observed by DLS might well correspond to the existence of large species from the synthetic process which, in any case, denotes a certain sample polydispersity of the as-synthesized polymers. At this respect, it is necessary to bear in mind that these polymers do not form micelles due to their high hydrophilicity. The Debye plot of polyCTR-βCD,

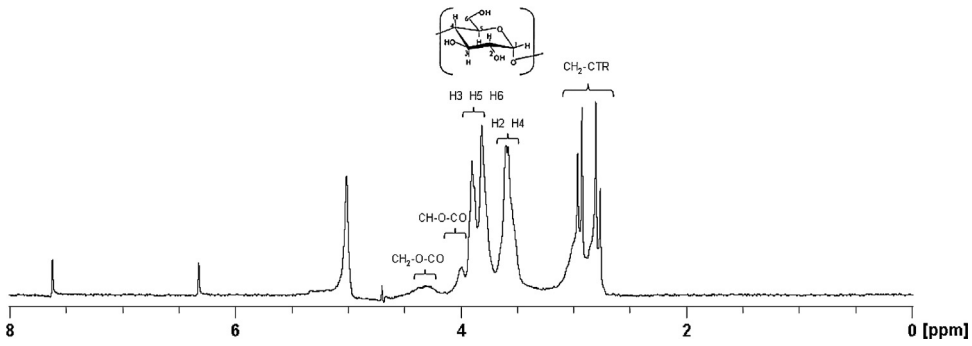

Fig. 2. ^{1}H NMR in D_2O of polyCTR-βCD.

polyCTR-γCD, and polyCTR-MD obtained considering the whole population (Fig. S2) rendered mass-weighted molecular mass of 33,490 ± 5520, 37,130 ± 6120 and 774,590 ± 84,700 g/mol for polyCTR-βCD, polyCTR-γCD and polyCTR-MD, respectively (determined from the intercepts of Fig. S2 in Supplementary Material). Ignoring other minor correction factors in the present case such as intra- and inter-particle interference scattering, as observed from the intensity dissymmetry ratios (I_{45}/I_{135}) below 1.03, we can simply assume that scattered intensity scales as molecular mass (and molecular mass as $r^3_{h,app}$). Then, to derive more precisely the "true" molecular mass of the present CD-based polymers, subtraction of the contributions to scattered intensity of the large-size populations present in polyCTR-γCD and polyCTR-MD polymers (estimated to be ca. 29 and 93%, respectively, from the areas of the intensity-averaged population distributions) was performed. In this way, from the intensity-corrected Debye plots (Fig. 3) molecular masses of 25,030 ± 3980 and 50,570 ± 6550 g/mol were obtained for polyCTR-γCD and polyCTR-MD, respectively. Differences in molecular mass can be attributed to the distinct size of the starting species. MD Glucidex®19 consists of small oligosaccharides along with polysaccharides of larger molecular mass (ca. 8000 g/mol; Avaltroni, Bouquerand, & Normand, 2004). Therefore, for a similar degree of polymerization, polyCTR-MD should be larger than polyCTR-βCD and polyCTR-γCD.

The GPC analysis corroborated molecular masses in the range of those estimated by SLS by fractioning the differences

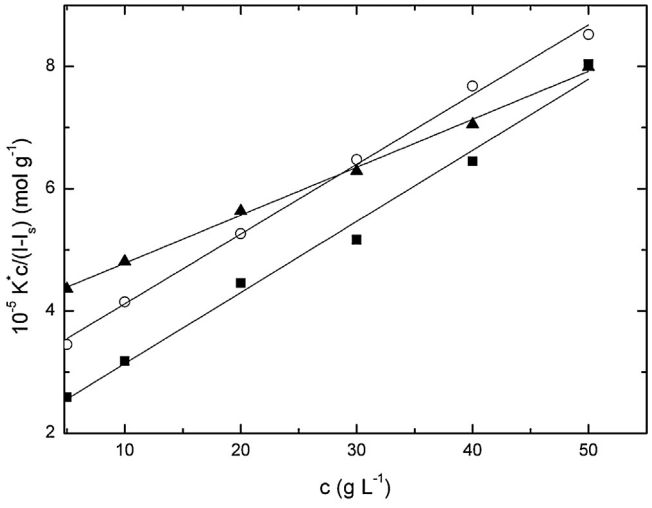

Fig. 3. Intensity-corrected Debye plots for aqueous solutions of polyCTR-βCD (○), polyCTR-γCD (▲) and polyCTR-MD (■) at 25 °C. The mass-weighted molecular mass determined from the intercepts resulted to be of 33,490 ± 5520, 25,030 ± 3980 and 50,570 ± 6550 g/mol for polyCTR-βCD, polyCTR-γCD and polyCTR-MD, respectively.

species observed in solution, and it also confirmed the greater molecular mass of polyCTR-MD (50,870 ± 6200 g/mol), compared to polyCTR-βCD (31,400 ± 4360 g/mol) and polyCTR-γCD (17,800 ± 1420 g/mol).

3.2. Cytocompatibility

The potential ocular irritancy of pristine CDs and MD and their polymers was evaluated according to the HET-CAM test following the NICEATM-ICCVAM protocol, which is as an alternative to the Draize eye rabbits test for the evaluation of ocular formulations (NICEATM-ICCVAM, 2012). The HET-CAM assay is a simple, fast and cheap test that provides measurable indices of the biocompatibility of a material (Cazedey, Carvalho, Fiorentino, Gremião, & Salgado, 2009; Valdes, Kreutzer, & Moussy, 2002). According to IS values, materials behave as non-irritating (0–0.9), weakly (1–4.9), moderately (5–8.9) or severe (9.21) irritating. The test is valid if the negative control does not induce irritation and the positive control causes severe irritation. As expected from the cytocompatibility of pristine CDs and MD, none of the polymers induced hemorrhage, lysis or coagulation; the IS being 0.0 (see Fig. S3 in Supplementary Material) despite the high concentration evaluated (50 g/L). This was also the case of the negative control. The positive control led to an IS of 19.7 ± 0.1. Therefore, the HET-CAM test indicates that the polymers may be well tolerated when used in ocular formulations. Recent studies with animals bearing for 6 months polyester vascular prostheses coated with polyCTR-HPβCD did not evidence any signs of acute or chronic, local or systemic toxicity (Jean-Baptiste et al., 2012).

3.3. ETOX solubility

CDs notably enhanced the solubility of ETOX in 0.9% NaCl (Fig. 4), although to a lower extent than that reported for block copolymer micellar systems (Ribeiro et al., 2012). The solubility ranked in the order: HPβCD > βCD > MD > γCD > αCD ≅ MβCD. It is interesting to note that MD also acted as hydrosolubilizing agent, probably through hydrophobic interactions of the drug aromatic ring with anhydroglucose units, as observed for other hydrophobic drugs and cellulose ethers (Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2006), but also forming helical inclusion complexes (like amylose) (Wangsakan, Chinachoti, & McClements, 2001). Cross-linking with CTR notably improved the ability of CDs to solubilize ETOX (Fig. 4 and Table 1). The effect was particularly intense in the case of polyCTR-γCD, which exhibited BE = 0.85; namely, polymerization increased the affinity of γCD 40-fold. In the case of the other CDs, the increase in affinity was between 8-fold (αCD and MβCD) and 3-fold (βCD), except for HPβCD and MD that did not alter their interaction capability after polymerization (Table 1). Increments of up to 5-fold

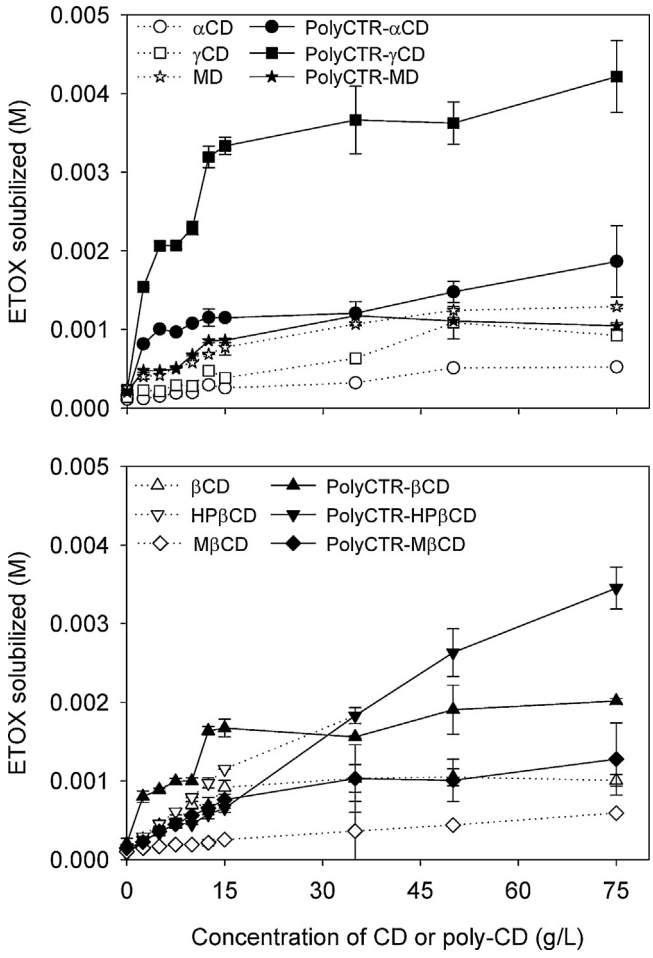

Fig. 4. Phase solubility diagrams of EtOX in NaCl 0.9% in the presence of various CDs, MD and their polymers.

have been previously observed for polyCTR-βCD, compared to βCD, when interacting with risperidone and verapamil (Danel et al., 2013). It should be noted that the pH did not alter ETOX solubility. The pH of the polyCTR-CD solutions was around 3, while that of pristine CDs was 7.6. The experiments with pristine CD solutions were repeated at pH 3 adjusted with citric acid, obtaining solubility values similar to those recorded at pH 7.6. Thus, the differences in $K_{1:1}$ and BE observed for CDs and their polymers are not caused by a change in pH neither to the presence of free citric acid in the medium.

The high complexation ability of the poly-CDs can be attributed to a synergy effect between hydrophobic interactions (inclusion in the CDs), and hydrophilic and electrostatic interactions with the polymer network (citric acid moieties) (Danel et al., 2013). The crosslinked macromolecular structure allows a cooperative action between neighboring CD cavities, or between the CDs and the polymeric network in the complexation of bulky and poorly water soluble drugs (Danel et al., 2013).

Table 1
Stability constant and binding efficiency of ETOX estimated from the linear region of the phase solubility diagrams in CD, MD and their polymer solutions.

Solubilizing agent	Diagram type	$K_{1:1}$ (M^{-1})	BE
αCD	A_N	85.4	0.012
PolyCTR-αCD	A_N	756.4	0.151
βCD	B_S	433.8	0.059
PolyCTR-βCD	B_S	1225.1	0.245
HPβCD	A_L	732.4	0.102
PolyCTR-HPβCD	A_L	576.6	0.081
MβCD	A_L	75.8	0.011
PolyCTR-MβCD	A_L	625.7	0.093
γCD	A_N	130.2	0.018
PolyCTR-γCD	B_S	5448.1	0.851
MD	B_S	274.4	0.038
PolyCTR-MD	B_S	261.3	0.078

3.4. ETOX diffusion

Since polyCTR-βCD and polyCTR-γCD rendered the highest solubility and BE values, further studies were carried out with these polymers and their corresponding CDs and using MD and its polymer as reference. ETOX release was evaluated using Franz-Chien diffusion cells having membranes of 500–1000 Da MWCO to ensure that only the drug passes to the receptor compartment. Therefore, the diffusion rate should be determined by the easiness of the complexes to be broken as well as by the contribution of the free and bound CDs or polymers to the microviscosity (Pose-Vilarnovo et al., 2004). Since the content in CD of each polymer is roughly 50%, solutions of CDs at 0.75 and 1.5% and of polymers at 1.5 and 3.0% were prepared, and ETOX was solubilized as explained above. A control ETOX solution in water (0.030 mg/ml) completed drug diffusion in 30 h. βCD, γCD and MD solutions led to ca. 30% released, while the proportion of drug diffused in 30 h from the polyCDs and polyMD was much lower (Fig. 5). Since ETOX concentration in each system was different (see Fig. 4), a direct comparison of the diffusion profiles is not possible. Thus, for comparative purposes, the diffusion coefficients D were estimated (Table 2).

The magnitude of D value obtained for ETOX in NaCl 0.9% is typical of small molecules diffusing in water (Table 2). Therefore, the dialysis membrane itself is not a limiting barrier for ETOX transport from the donor to the receptor compartments. βCD and γCD diminished 4- to 11-fold the D value. At 0.75%, γCD delayed more the

Table 2
Diffusion coefficients of EtOX and microviscosity of various CD, MD and their polymer solutions.

Solubilizing agent	Concentration (g/L)	D (cm^2/s) × 10^6	Microviscosity (mPa s)
None	–	92.50 (8.91)	0.707
βCD	7.5	22.58 (2.16)	2.897
βCD	15.0	7.86 (3.49)	8.323
PolyCTR-βCD	15.0	1.11 (0.47)	59.10
PolyCTR-βCD	30.0	0.278 (0.075)	235.51
γCD	7.5	16.58 (4.13)	3.945
γCD	15.0	12.58 (3.97)	5.200
PolyCTR-γCD	15.0	4.26 (0.64)	15.34
PolyCTR-γCD	30.0	1.34 (0.48)	48.89
MD	7.5	0.810 (0.570)	80.70
MD	15.0	0.077 (0.036)	848.62
PolyCTR-MD	15.0	0.036 (0.010)	1831.2
PolyCTR-MD	30.0	0.010 (0.001)	6435.2

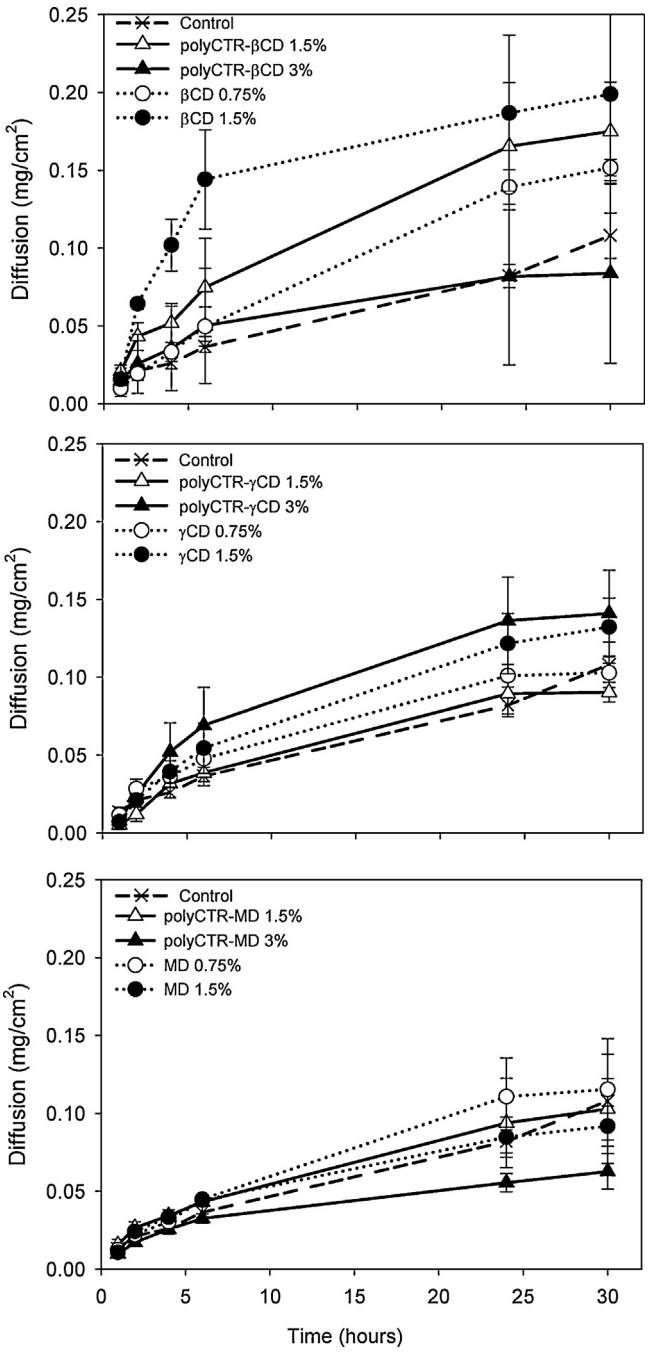

Fig. 5. Diffusion of ETOX from water (control), and βCD, polyCTR-βCD (top), γCD, polyCTR-γCD (mid plot), MD and polyCTR-MD (bottom) solutions.

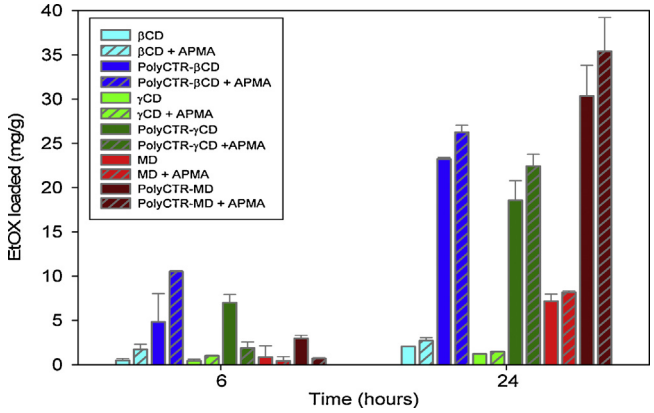

Fig. 6. Loading of ETOX by soaking of poly-HEMA (continuous bars) and poly-HEMA-co-APMA (stripped bars) hydrogels into drug solutions prepared with CD, MD or their polymers.

release than βCD, probably because the greater hydrodynamic size of the former. However, the higher decrease in D was caused by βCD at the highest concentration tested probably due to the stronger affinity constant and, in turn, greater complexation efficiency. The effect of MD on ETOX diffusion was more remarkable. As indicated above, MD Glucidex®19 contains small oligosaccharides along with polysaccharides of larger molecular mass (ca. 8000 g/mol; Avaltroni, Bouquerand, & Normand, 2004). The entanglement of the chains, together with the drug–MD hydrophobic interactions and/or helical inclusion complexes, explains the strong resistance against diffusion. Microviscosity, i.e. the effect of the polymer on drug movement at the microscopic scale, was estimated as follows (Alvarez-Lorenzo, Gomez-Amoza, Martinez-Pacheco, Souto, & Concheiro, 1999):

$$\eta = \eta_0 \bullet \frac{D_0}{D}$$

In this equation, η_0 is the viscosity of the solvent, D_0 drug diffusion coefficient in the solvent and D drug diffusion coefficient in the formulation (Table 2). MD at 1.5% provoked 3 orders of magnitude increase in the microviscosity compared to the solvent alone. The increments were just one order of magnitude in the case of βCD or γCD.

Polymerization of CDs notably diminished D, being 2- to 3-orders of magnitude lower than that recorded in the solvent alone (Table 2). Accordingly, the microviscosity was up to 335 times greater (for polyCTR-βCD at 3.0%). Polymerization of MDs led to the smallest D and the largest microviscosity, as the cross-linking with citric acid reinforces the entangled structure of the polymers (and notably increased the molecular mass) as well as the affinity of the drug.

3.5. Poly-(cyclo)dextrins as coadyuvants in the preparation of medicated contact lenses

Hydrogel-based contact lenses were prepared by copolymerization of HEMA with low proportion of cross-linker EGDMA, following a previous protocol (Ribeiro et al., 2011a). Some hydrogels were also synthesized adding APMA as functional monomer potentially useful to interact with free carboxylic acid groups remnant in the poly-(cyclo)dextrins (Andrade-Vivero, Fernandez-Gabriel, Alvarez-Lorenzo, & Concheiro, 2011). Former attempts to load ETOX in poly-HEMA hydrogels by soaking for 48 h in drug aqueous solution resulted in only 0.99 mg drug per gram of dried material; 40% drug was released in 48 h to NaCl 0.9% under sink conditions (Ribeiro et al., 2011a). Application of the molecular imprinting approach resulted in minor increase in drug loading because of the limited drug concentration that can be attained in the soaking medium. This limitation was overcome with the use of the poly-(cyclo)dextrins. Soaking in polyCTR-βCD or polyCTR-γCD resulted in notably greater amounts loaded just after 6 h (Fig. 6). This rapid loading can be attributed to the high solubilization ability of polyCTR-βCD or polyCTR-γCD, forming species that do not cause a too detrimental effect on drug diffusion (as discussed above). It is expected that both free and complexed ETOX enter into the aqueous phase of the network and can establish non-specific interactions with poly-HEMA chains. The loading equilibrium was reached at 24 h, attaining outstandingly high loadings with polyCTR-βCD and polyCTR-γCD, but also with polyCTR-MD. As discussed above, the

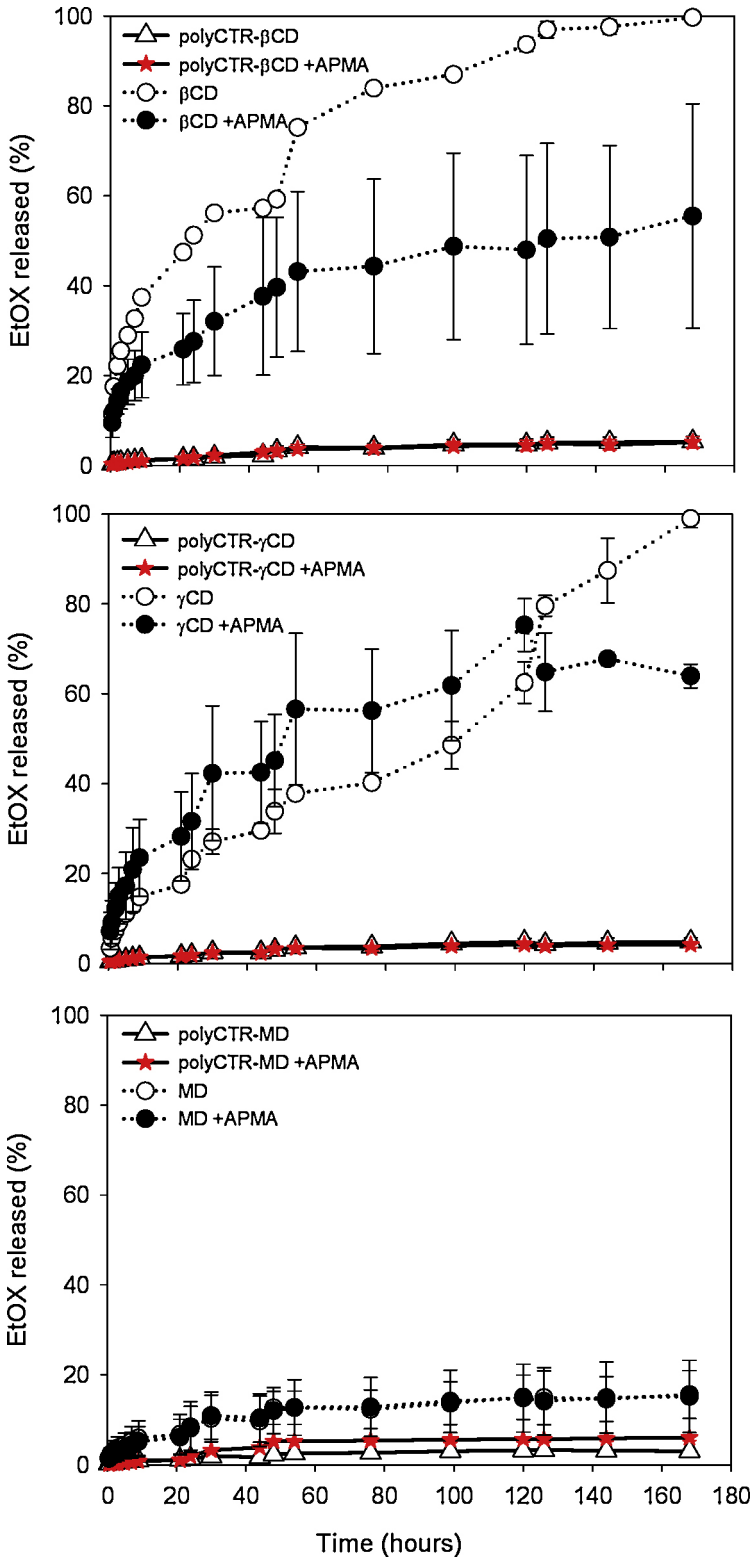

Fig. 7. Percentage of ETOX released in artificial lachrymal fluid from poly-HEMA (open symbols) and poly-HEMA-co-APMA (full symbols) previously loaded by soaking in βCD, polyCTR-βCD, γCD, polyCTR-γCD, MD and polyCTR-MD.

large polyCTR-MD provided greater apparent solubility values of ETOX, but took more time in the diffusion process. Copolymerization of HEMA with APMA slightly increased the uptake process, probably because of some ionic interactions with the polyCTR chains. The degree of swelling of poly-HEMA (52.8%, s.d. 1.9) and poly-HEMA-co-APMA (51.4%, s.d. 2.2) hydrogels was very similar and therefore this variable did not influence the loading process.

If the weight of a contact lens is about 50 mg, the soaking in the poly-(cyclo)dextrins may enable the preparation of lenses medicated with at least 1 mg drug per lens. Commercial eye-drops of related CAIs contain 1 to 10 mg/ml and are intended to be applied

twice a day, resulting in the delivery of 0.05 to 0.5 mg in each eye-drop. Since the ocular bioavailability is estimated to be 10-times lower, the ETOX-loaded poly-HEMA hydrogels contain drug sufficient for at least 10 days.

Although it is not easy to simulate drug release from a contact lens, we have carried out release tests in artificial lachrymal fluid under static conditions for comparison purposes (Fig. 7). Amounts of ETOX released are plotted in Fig. S4 (Supplementary material). According to Fig. 7, faster release was observed for hydrogels loaded by soaking in CD-ETOX or MD-ETOX solutions, compared to the hydrogels loaded in ETOX solutions with polyCTR-CD or polyCTR-MD. Poly-HEMA hydrogels loaded with CD-ETOX complexes sustained drug release for 6 days, attaining 100% drug release. On the other hand, poly-HEMA hydrogels loaded by soaking in ETOX solutions containing MD released the drug more slowly (< 20% in 6 days), which is in agreement with the lower drug diffusion coefficients in the presence of MD compared to CDs.

Hydrogels loaded with ETOX from polyCTR-CDs or poly-CTR-MD solutions only released 5–8% drug in one week (Fig. 7), as expected from the slower diffusion of the drug when forming complexes with the poly(CD)dextrins. Nevertheless, the total amount released was similar or somewhat greater than that provided by hydrogels loaded by soaking in CDs or MD solutions.

4. Conclusions

Poly-(cyclo)dextrins obtained by cross-linking of CDs with citric acid under environmental-friendly conditions can perform as multi-host agents of hydrophobic drugs, such as ETOX, notably enhancing the apparent aqueous solubility and enabling the regulation of drug diffusion. This effect was particularly significant in the case of polyCTR-γCD (complexation efficiency of 0.85; polymerization increased the affinity of γCD 40-fold) and to a lesser extent in the case of polyCTR-βCD. The HET-CAM test confirmed the ocular compatibility due to the non-irritating character of these polymers. These promising results were used to demonstrate the feasibility of incorporating ETOX-loaded poly-(cyclo)dextrins into poly(2-hydroxyethyl methacrylate)-based soft contact lenses as sustained release ocular bandage. Poly-(cyclo)dextrins facilitate the loading of high doses of ETOX in the contact lenses (at least 1 mg drug per lens). Finally, the release tests in artificial lachrymal fluid indicated the retention of the drug-loaded polymers and a more sustained release than that achieved when the hydrogel lenses were loaded by soaking in ETOX-pristine CD solutions.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.08.003.

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