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Hydrophilic acrylic hydrogels with built-in or pendant cyclodextrins for delivery of anti-glaucoma drugs

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ABSTRACT

Carbonic anhydrase inhibitors (CAIs) are gaining interest for local treatment of glaucoma and other ocular disorders. The aim of this work was to elucidate the role of cyclodextrins (CDs) in the loading and the release rate of acetazolamide (ACT) and ethoxzolamide (ETOX) from N,N-dimethylacrylamide-co-N-vinylpyrrolidone (DMA-co-NVP) hydrogels, suitable as high water-content soft contact lenses. Two different approaches to incorporate the CDs were evaluated: (i) synthesis of CD monomers and copolymerization with DMA and NVP; and (ii) grafting of natural CDs to preformed hydrogels. Natural β-CD and γ-CD were tested in each approach. The effects of the preparation method on relevant functional features of the hydrogels as well as on cytocompatibility and drug delivery performance were studied in detail. The role played by the CDs strongly depends on the physicochemical properties of the drug and its ability to form complexes; being particularly relevant for slightly soluble molecules that have high affinity for the CDs. Otherwise, unspecific interactions with the network may screen the contribution of the CDs.

1. Introduction

Glaucoma is the generic name of a group of progressive optical neuropathies characterized by degeneration of retinal ganglion cells and their axons, with resultant visual field defects and loss of vision (Thylefors & Negrel, 1994). Recent data indicate that the number of people with open angle and angle closure glaucoma will rise up to 79.6 million in 2020; glaucoma being the second leading cause of blindness worldwide (Quigley & Broman, 2006). Carbonic anhydrase inhibitors (CAIs), such as acetazolamide (ACT) and ethoxzolamide (ETOX), are particularly useful for systemic (oral) antiglaucoma management, reducing the elevated intraocular pressure (IOP) characteristic of this disease (Kaur, Smitha, Aggarwal & Kapil, 2002). Their action mechanism consists in the inhibition of carbonic anhydrases at the eye and, thus the reversible conversion of carbon dioxide to bicarbonate and the secretion of aqueous humor. However, carbonic anhydrases are ubiquitously distributed in the body and systemic CAIs administration may lead to relevant collateral effects (Supuran, 2008). Topical formulations of the first generations of CAIs were initially unsuccessful due to low ocular bioavailability, related to their poor penetration coefficient and aqueous solubility. These limitations could be at least partially overcome by preparing inclusion complexes with cyclodextrins (CDs) (Granero, Maitre, Garnero & Longhi, 2008; Loftsson & Jarvinen, 1999; Loftsson, Stefansson & Kristinsson, 1996). Nevertheless, the search for topical formulations able to sustain the release and to provide better patient compliance is still on going.

The development of strategies to overcome the barriers for topical ocular delivery of drugs is a major challenge for pharmaceutical scientists (Hornof, Toropainen & Urtti, 2005; Koevary, 2003). In this sense, drug-eluting contact lenses can offer novel chances for the management of eye pathologies (Ribeiro et al., 2011a,b). Soft contact lenses (SCLs) can be loaded with drugs by soaking in drug solutions and, once applied onto the eye, they may sustain the release in the postlens lachrymal fluid (Alvarez-Lorenzo, Hiratani & Concheiro, 2006). SCLs increase significantly the residence time of the drug in the precorneal area, compared to the short time (2–5 min) achieved with common eye drops. The longer drug residence time on the cornea surface promoted by the SCL may result in higher drug flux through the anterior segment structures and, consequently, greater ocular bioavailability and lower

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side effects (Gulsen & Chauhan, 2004). Nevertheless, there are still a number of limitations associated with the use of SCLs as drug delivery devices. Usually the amount of drug incorporated in the lens matrix by presoaking is low due to a poor drug solubility in the aqueous phase of the SCL and/or to a low affinity of the drug for the polymeric network (Xu, Li & Sun, 2010). Several methods have been assayed to improve drug loading and the control of the release, such as the use of functional monomers and molecular imprinting (Ali, Horikawa, Venkatesh, Saha, Hong & Byrne, 2007; Alvarez-Lorenzo, Yañez, Barreiro-Iglesias, Concheiro, 2006; Alvarez-Lorenzo, Yañez & Concheiro, 2010; Hiratani, Fujiwara, Tamiya, Mizutani & Alvarez-Lorenzo, 2005; Venkatesh, Sizemore & Byrne, 2007), the drug impregnation applying supercritical fluids (Yanez et al., 2011), or the incorporation of the drug into colloidal structures, nanoparticles or microparticles to be dispersed in the polymeric network (Gulsen & Chauhan, 2004, 2005; Kapoor, Thomas, Tan, John & Chauhan, 2009). We have previously observed that biomimetic SCLs, with domains that resemble the composition and conformation of the active site of carbonic anhydrase, exhibit a remarkably longer affinity for ACT and ETOX than common SCLs (Ribeiro et al., 2011a,b). Recently, grafting of CDs to the SCL structure has been also shown to endow the networks with the ability to host drugs by forming dynamic inclusion complexes, which can regulate drug uptake and release through an affinity-driven mechanism, as previously reported for other CD hydrogels obtained by direct cross-linking (Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro & Torres-Labandeira, 2006), polymerization of CD monomers (e.g. acrylamidomethyl-γ-cyclodextrin or methacrylate-β-cyclodextrin) with hydroxyethyl methacrylate or sodium acrylate (Santos, Couceiro, Concheiro, Torres-Labandeira & Alvarez-Lorenzo, 2008; Siemoneit et al., 2006), or grafting of pristine CDs to preformed poly(hydroxyethyl methacrylate) networks (Santos et al., 2009, 2010).

The aim of this work was to explore the possibilities of using two natural CDs (β-CD and γ-CD) for modulating the loading and the release rate of ACT and ETOX (two CAIs of markedly different physicochemical properties; Supuran, 2008) from N,N-dimethylacrylamide-co-N-vinylpyrrolidone (DMA-co-NVP) hydrogels. DMA and NVP are common components of high water-content SCLs, thus their oxygen permeability and comfort on the eye are greater than those exhibited by other lenses (Wang, Tan, Zhang & Guang, 2008), but such high hydrophilicity may be an inconvenient for the uptake of the hydrophobic drugs. Two different approaches were evaluated to insert the CDs in the SCL structure: (i) synthesis of acrylamidomethyl-β-CD and acrylamidomethyl-γ-CD monomers and copolymerization with DMA and NVP; and (ii) grafting of β-CD and γ-CD to preformed hydrogels. The effects of the preparation method and CD-drug stability constant on relevant functional features of the hydrogels as well as on cytocompatibility and drug delivery performance were studied in detail.

2. Experimental

2.1. Materials

N,N-dimethylacrylamide (DMA), N-vinylpyrrolidone (NVP), ethylene glycol dimethacrylate (EGDMA), glycidyl methacrylate (GMA), N-(hydroxymethyl) acrylamide (NMA), acetazolamide (ACT) and ethoxzolamide (ETOX) were from Sigma–Aldrich Chemicals (St. Louis MO, USA). Azobisisobutyronitrile (AIBN) was from Acros Organic Co. (Geel, Belgium), γ-cyclodextrin (γ-CD) from Wacker Chemie AG (Munchen, Germany) and β-cyclodextrin (β-CD) from Roquette (Lestrem, France). Purified water was obtained by reverse osmosis (MilliQ®, Millipore Spain). All other reagents were analytical grade.

2.2. Phase solubility diagrams

Solutions of β-CD (0–0.0132 mol/L) or γ-CD (0–0.154 mol/L) were prepared in 0.9% NaCl and then 5-mL aliquots were added to glass vials containing ACT (60 mg) or ETOX (5 mg) in excess. Each system was prepared in sextuplicate; three replicates being immediately autoclaved (121 °C for 20 min). Then, the six replicates were kept at 37 °C under shaking (50 osc/min) for 96 h. The resultant suspensions were filtered through a 0.45 μm membrane (Sartorius®, Spain), the filtrates suitably diluted with ethanol and the absorbance measured at 264 nm (ACT) or 303 nm (ETOX) using a UV–visible spectrophotometer (Agilent 8453, Germany). The stability constants of the complexes were estimated as follows (Higuchi & Connors, 1965):

$$K_{1:1} = \frac{b}{S_0(1-b)} \quad (1)$$

In this equation, b represents the slope of the plot of the drug solubilized vs. CD concentration, and S_0 the equilibrium solubility of the drug in 0.9% NaCl. The complexation efficiency (CE) was calculated as follows (Brewster & Loftsson, 2007):

$$CE = \frac{[D \cdot CD]}{[CD]} = K_{1:1} \cdot S_0 = \frac{b}{(1-b)} \quad (2)$$

2.3. Synthesis of acrylamidomethyl-CD monomers

β-CD (15.0 g) or γ-CD (17.12 g) and NMA (13.36 g) were added to 1% HCl aqueous solution (50 mL) in a reactor and kept under stirring at 80 °C. After 30 min, acetone (300 mL) was added to stop the reaction and to precipitate β-CD-NMA and γ-CD-NMA monomers. The reactor was kept at 4 °C for 12 h. Then, the precipitate was separated by filtration (Sartorius®, Madrid, Spain) and repeatedly washed with acetone (200 mL) and filtered (four cycles). The monomers were finally dried under vacuum for 2 days at room temperature and stored at 4 °C (Siemoneit et al., 2006).

2.4. Synthesis of hydrogels with built-in CDs

NVP/DMA 20/80 molar ratio mixtures were prepared just by mixing the adequate volumes of the monomers (Table 1). β-CD-NMA or γ-CD-NMA were added (in the amounts indicated in Table 1) to 8-mL aliquots of NVP/DMA solution and kept under stirring until complete dissolution. The preparation of networks with high contents in γ-CD-NMA (300–800 mg; i.e., codes Cγ300 to Cγ800 in Table 1) required the previous dissolution of the monomer in 1 mL of DMSO. Then, EGDMA (80 mM) and AIBN (10 mM) were incorporated. The monomers solutions were injected into moulds constituted by two glass plates pretreated with dimethyldichlorosilane and separated by a silicone frame of 0.9 mm thickness (Alvarez-Lorenzo et al., 2002). The moulds were heated at 50 °C for 12 h and then at 70 °C for 24 h more. The hydrogels were removed from the moulds and immersed in boiling water for 15 min to remove any residual non-reacted component. Discs (10 mm in diameter) were cut from the wet films and immersed in water for 24 h, in 0.9% NaCl solution for 24 h, and again in water for some days, replacing the medium every 12 h until no absorbance of the medium in the UV–vis range was observed. The hydrogel discs were stored at the dried state.

Table 1
Monomeric composition of the hydrogels and their degree of swelling in water at 25 °C.

Formulation	NVP (mL)	DMA (mL)	EGDMA (mL)	AIBN (g)	GMA (mL)	DMSO (mL)	β-CD-NMA (mg)	γ-CD-NMA (mg)	Swelling (%)
C0	1.65	6.35	0.12	0.0135	–	–	–	–	77.9
Cβ100	1.65	6.35	0.12	0.0135	–	–	100	–	81.2
Cγ50	1.65	6.35	0.12	0.0135	–	–	–	50	80.4
Cγ100	1.65	6.35	0.12	0.0135	–	–	–	100	77.0
Cγ150	1.65	6.35	0.12	0.0135	–	–	–	150	78.2
Cγ200	1.65	6.35	0.12	0.0135	–	–	–	200	80.1
Cγ300	1.65	6.35	0.12	0.0135	–	1.0	–	300	79.6
Cγ400	1.65	6.35	0.12	0.0135	–	1.0	–	400	79.9
Cγ500	1.65	6.35	0.12	0.0135	–	1.0	–	500	78.4
Cγ600	1.65	6.35	0.12	0.0135	–	1.0	–	600	77.4
Cγ700	1.65	6.35	0.12	0.0135	–	1.0	–	700	79.5
Cγ800	1.65	6.35	0.12	0.0135	–	1.0	–	800	78.1
G1A	0.00	8.00	0.12	0.0135	0.22	–	–	–	80.5
G2A	1.65	6.35	0.12	0.0135	0.22	–	–	–	78.3
G3A	3.27	4.73	0.12	0.0135	0.22	–	–	–	79.6
G1B	0.00	8.00	0.12	0.0135	0.44	–	–	–	80.5
G2B	1.65	6.35	0.12	0.0135	0.44	–	–	–	79.9
G3B	3.27	4.73	0.12	0.0135	0.44	–	–	–	78.3

2.5. Hydrogels with pendant CDs

Different amounts of GMA were added to NVP/DMA mixtures (Table 1, codes starting with G) and, once mixed, EGDMA (80 mM) and AIBN (10 mM) were added. The monomer solutions were injected into moulds, polymerized and then washed as described above. 25 wet discs (10 mm in diameter) were immersed in 150 mL of dimethylformamide: 0.5 M NaCl aqueous solution 50:50 v/v mixture containing 80 mM β-CD or 80 mM γ-CD and 4.5 g NaOH, and kept at 80 °C for 24 h. Then the hydrogels were washed by immersion in water at 80 °C for 5 min (five cycles), in water at 70 °C for 24 h (three times), in ethanol (96%) for 24 h (three cycles) and in water at room temperature 24 h (three cycles). Then, the discs were dried at room temperature for 48 h.

2.6. Fourier transform infrared spectroscopy (FTIR)

FTIR-ATR (attenuated total reflection) spectra of pristine cyclodextrins, β-CD-NMA and γ-CD-NMA monomer, and dried hydrogels were recorded over the range 400–4000 cm^{-1} in a Varian-670 FTIR spectrometer equipped with a GladiATR™ (Madison Instruments, Madison WI, USA) fitted with diamond crystal.

2.7. Degree of swelling

Dried hydrogel discs were weighed (W_0) and immersed in water at room temperature. At pre-established time intervals, the discs were removed from the aqueous medium, their surface was carefully wiped and the weight recorded (W_t). The experiments were carried out in duplicate. The swelling ratio was estimated as follows:

$$Q\ (\%) = \frac{W_t - W_0}{W_0} \cdot 100 \quad (3)$$

2.8. Optical transparency

Fully swollen hydrogels were mounted on the side of the inside surface of a quartz cuvette and the transmittance was recorded, in duplicate, at 600 nm (UV–vis spectrophotometer, Agilent 8453, Germany).

2.9. Content in functional CDs

Dried hydrogel discs were immersed in 10 mL of 3-methylbenzoic acid (3-MBA) aqueous solution (0.12 mg/mL) and kept for 48 h in the dark. The concentration of 3-MBA was spectrophotometrically monitored at 281 nm (Agilent 8453, Germany). The total amount of 3-MBA taken up by discs was calculated as the difference between the initial and the final amounts in the solution. The experiments were carried out in triplicate.

2.10. Cytocompatibility

Dried hydrogel discs were immersed in phosphate buffer pH 7.4 and autoclaved (121 °C, 20 min). Then, the discs were placed in wells (24-wells plate) containing Balb/3T3 clone A31 cells (200,000 cells per well) in Dulbecco's Modified Eagle Medium DMEM F12 HAM (2 mL) (Sigma–Aldrich Chemicals, Madrid, Spain). The plates were kept in a humidified incubator at 5% CO_2 and 37 °C for 24 h. Then aliquots (100 μl) of medium were taken and transferred to 96-wells microplates, and mixed with the reaction mixture solution (100 μl) contained in the Cytoxicity Detection Kit^{PLUS} LDH, (Roche, Barcelona, Spain). Blank (100 μl of culture medium) and negative (50 μl of cells plus 50 μl of medium) and positive (50 μl of cells plus 50 μl of medium with 5 μl of lysis factor) controls were also prepared. The plates were incubated 30 min at 15–25 °C protected from light. A stop solution (50 μl) was added to the wells and the absorbance immediately measured at 490 nm (BIORAD Model 680 Microplate reader, USA). The experiments were carried out in triplicate.

2.11. ACT loading and release

Dried hydrogels discs were immersed in 5 mL of ACT aqueous solution (0.20 mg/mL) and kept for two days at room temperature protected from the light. The amount of ACT loaded by each hydrogel was calculated as the difference between the initial amount of drug in the solution and the amount remaining after loading, as determined by UV spectrophotometry at 264 nm (Agilent 8453, Germany). Drug-loaded discs were rinsed with water, their surface was carefully wiped and the discs were immediately immersed in 7.5 mL of 0.9% NaCl solution at room temperature. The amount of ACT released was measured spectrophotometrically at 264 nm, in samples periodically taken and again placed in the same vessel, so that the volume of liquid was kept constant. The experiments were carried out in sextuplicate per hydrogel composition.

2.12. ETOX loading and release

Discs of dried hydrogels were immersed in 5 mL of ETOX suspension (0.23 mg/mL) and kept for two days at room temperature. The ETOX-loaded discs were rinsed with water; their surface was carefully wiped and immediately transferred to 5 mL of 0.9% NaCl at room temperature. The amount of drug released was measured spectrophotometrically at 303 nm in samples periodically taken up and placed again into the same vessel. After 360 h in the release medium, the discs were rinsed with water and placed in vials with 5 mL of ethanol:water (70:30) mixture. The amount of drug extracted to the hydroalcoholic medium after 24 h was spectrophotometrically quantified at 303 nm. The experiments were carried out in sextuplicate per hydrogel composition.

3. Results and discussion

The suitability of CD-based hydrogels for performing as drug delivery systems is strongly dependent on the ability of the fixed CDs to host the drug of interest. Thus, the CDs should have affinity for the drug and that affinity should be maintained when copolymerized or grafted to the network. The information available about inclusion complexes of ACT and ETOX is limited to hydroxypropyl-β-cyclodextrin in water (Loftsson et al., 1994). Since the cyclodextrin chemical structure and the nature of the solvent determine the affinity of the drugs for the CDs, the first step of the work was to obtain the phase solubility diagrams of ACT and ETOX in 0.9% NaCl solutions of β-CD and γ-CD. This saline medium was chosen in order to mimic the physicochemical conditions of the lachrymal fluid. The next step was to prepare CD-containing hydrogels of two different structures: (i) networks in which the CDs were copolymerized with other monomers and thus integrated in the main backbone of the polymeric chains (hydrogels with built-in CDs); and ii) networks with hanging CDs that were obtained by grafting of pristine β-CD and γ-CD to preformed hydrogels (hydrogels with pendant CDs).

3.1. Phase solubility diagrams

The stoichiometry and stability constant of the inclusion complexes of ACT and ETOX were estimated from the phase solubility diagrams (Fig. 1). ACT and ETOX solubility in water at 25 °C is 0.70 mg/mL (Bock, Meier, Nyul, Hornegger & Michelson, 2010; Kaur et al., 2002) and 0.04 mg/mL respectively (Loftsson et al., 1994). The apparent solubility of ETOX linearly increased with the concentration of β-CD and γ-CD due to the formation of inclusion complexes. Solubility of ETOX increased 10-fold in 0.013 M β-CD and 21-fold in 0.154 M γ-CD solutions. The effect of the CDs on the solubility of ACT was smaller although still relevant; the increments being 3.8-fold in 0.013 M β-CD and 1.5-fold in 0.154 M γ-CD medium. It should be noticed that for both drugs, autoclaving helps the inclusion complex to be formed probably due to a temporal increase in drug solubility at high temperatures, which makes more drug molecules to be available to be hosted in the CD cavities (Cappello, Carmignani, Iervolino, La Rotonda & Saettone, 2001; Loftsson & Jarvinen, 1999). The phase solubility plots were A_L-type, which indicates that the complex is first order with respect to the complexing agent and also regarding to the drug (Higuchi & Connors, 1965). The stability constants (*Ks*) of 1:1 complexes for the ETOX and ACT with β-CD were greater than those found with γ-CD (Table 2). The *Ks* values calculated for the complexation of ETOX with β-CD and γ-CD were larger for non-autoclaved systems than for the autoclaved ones; the effect of thermal treatment on ACT complexes being less relevant.

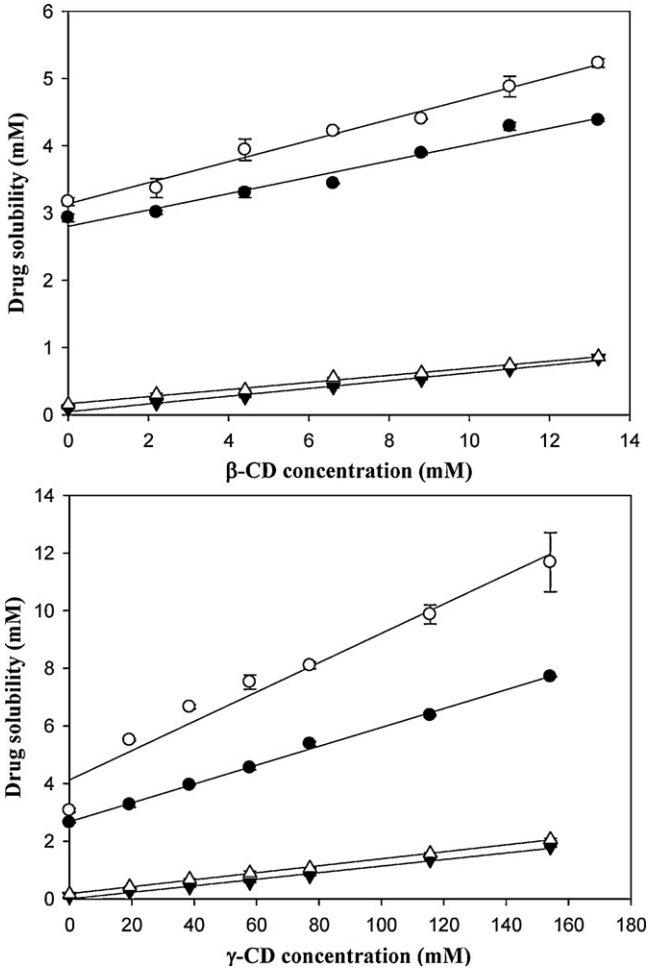

Fig. 1. Phase solubility diagrams for ACT and ETOX with β-CD and γ-CD at 37 °C in 0.9% NaCl: (●) ACT no autoclaved, (○) ACT autoclaved, (▼) ETOX no autoclaved, (△) ETOX autoclaved. The error bars represent the standard deviations ($n = 3$).

3.2. Synthesis of hydrogels with built-in CDs

Once we have confirmed that the drugs form inclusion complexes with β-CD and γ-CD, the next step was to synthesize monomers of these cyclodextrins suitable for polymerization with NVP and DMA, which are monomers commonly used as components of SCLs. The synthesis route for preparing β-CD-NMA and γ-CD-NMA has been previously described (Lee, Yoon & Ko, 2001; Siemoneit et al., 2006). Compared to the FTIR spectra of β-CD and γ-CD, the spectra of β-CD-NMA and γ-CD-NMA showed two additional bands at 1708 and 1544 cm^{-1}, which correspond to the amide I and II (C=O and NH) stretching peaks (Fig. 2). Vinyl (C=C) stretching peak was observed at 1628 cm^{-1}. The degree of substitution of the CDs with the NMA groups was estimated by comparison of the

Table 2
Complexation efficiency (CE) and stability constant ($Ks_{1:1}$) of ACT and ETOX with β-CD and γ-CD in 0.9% NaCl at 37 °C, before and after autoclaving.

Inclusion complex	Pre-treatment	CE	$Ks_{1:1}$ (M^{-1})	R^2
ACT:β-CD	None	0.152	39.1	0.957
ACT:γ-CD	None	0.033	11.9	0.999
ETOX:β-CD	None	0.060	644.9	0.997
ETOX:γ-CD	None	0.011	129.2	0.995
ACT:β-CD	Autoclaved	0.165	38.4	0.960
ACT:γ-CD	Autoclaved	0.066	19.1	0.828
ETOX:β-CD	Autoclaved	0.062	354.3	0.961
ETOX:γ-CD	Autoclaved	0.012	72.7	0.997

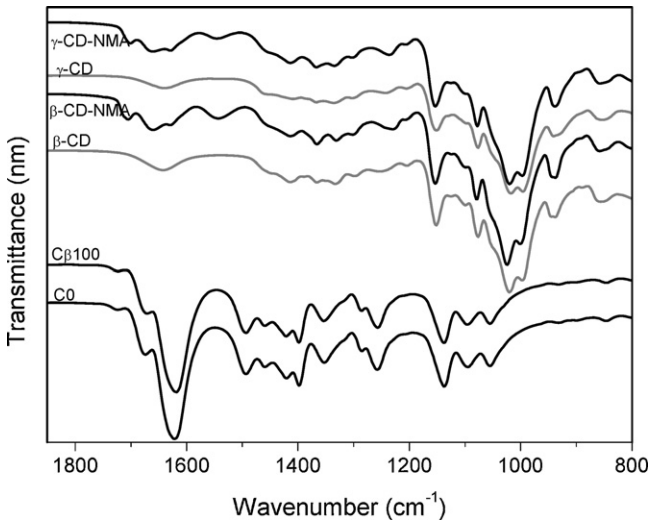

Fig. 2. FTIR spectra of pristine β-CD and γ-CD, β-CD-NMA and γ-CD-NMA monomers, and hydrogels C0 and Cβ100 (codes as in Table 1).

FTIR absorbance of secondary to the primary hydroxyl groups ratio of the CDs before and after reaction with NMA (Rodriguez-Tenreiro et al., 2006). FTIR spectra of β-CD and γ-CD raw materials showed 1000/1020 cm^{-1} absorbance ratios of 0.79 and 0.78, respectively, while for the monomers the ratio raised up to 0.98 and 0.89 respectively. These increments suggest that 1 hydroxyl group of each glucopyranose unit of the CD has reacted with a NMA monomer.

The synthesis of the hydrogels with built-in CDs was carried out by free radical polymerization of a NVP/DMA mixture (Table 1) with various proportions of β-CD-NMA (100 mg) and γ-CD-NMA (50–800 mg). FTIR spectra of NVP/DMA hydrogels without CD-NMA monomers showed peaks at 1720 cm^{-1} and 1394 cm^{-1} due to the carbonyl groups and the C–N stretching vibration of tertiary amine. The lactam group of the NVP and amide group of the DMA appeared at 1670 cm^{-1} (Fig. 2).

All hydrogels swelled rapidly when immersed in water and reached the equilibrium in less than 2 h. The degree of swelling was about 80% (Table 1), confirming the high affinity of the hydrogels for water.

3.3. Synthesis of hydrogels with pendant CDs

NVP/DMA networks were prepared using glycidyl methacrylate (GMA) as bi-functional monomer bearing both acrylic and epoxy groups. The attractiveness of GMA is related to the versatility of its epoxy group, which can react with the hydroxyl groups, such as those of CDs (Hornof et al., 2005; Santos et al., 2009). Copolymerization of DMA, NVP and GMA (Table 1) occurs via carbonic double bond cleavage and results in hydrogels that maintain the original reactivity of the epoxy ring. Thus, GMA mers act as grafting points for the binding of CDs to the preformed networks. β-CD and γ-CD grafting occurred in alkaline medium using DMF as cosolvent. This polar (hydrophilic) aprotic solvent is commonly used for SN_2 reactions involving epoxide groups and nucleophilic anions (as the hydroxyl groups of CDs) under alkaline conditions (Solomons & Fryhle, 2004, chap. 11).

FTIR spectra (not shown) of the hydrogels before and after CD grafting were similar, owing to the low proportion in weight of the CDs compared to the other components. Grafting did not alter the swelling degree of the hydrogels, which were similar to those recorded for DMA/NVP hydrogels without GMA (ca. 80%, Table 1). The hydrophilic monomers NVP and DMA are responsible for the high affinity of the dried hydrogels for water. The swelling was relatively fast and occurred in less than 1 h.

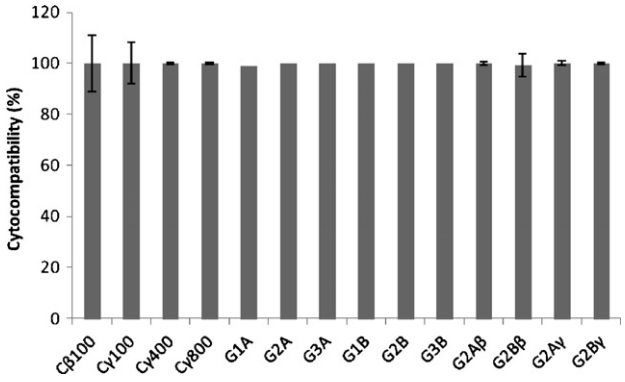

Fig. 3. Viability of Balb/3T3 cells after 24 h in direct contact with the hydrogels.

3.4. Light transmission, oxygen permeability and cytocompatibility

All swollen hydrogels were quite transparent (light transmission at 600 nm above 80%) and their oxygen permeability was modified neither by the copolymerization with the CD monomers nor the grafting of pristine CDs, and resulted to be in the 65–87 barrers range. The values of light transmission and oxygen permeability are in the range adequate for contact lenses (Gonzalez-Meijome, Compan-Moreno & Riande, 2008). Oxygen permeability is a relevant parameter for the viability of corneal cells. In fact, excellent biocompatibility with living tissues has been reported for NVP and DMA-based polymers and networks (Vijayasekaran et al., 1996). Nevertheless, the changes in composition due to the presence of CDs prompted us to evaluate the cytocompatibility of all hydrogels against the Balb/3T3 fibroblast cell line. Hydrogels made with β-CD-NMA or γ-CD-NMA monomers and grafted β-CD or γ-CD showed excellent cell compatibility (Fig. 3).

3.5. Availability of CD units for complex formation in the hydrogels

The content in CDs was determined by means of the typical organic compound (TOC) approach using 3-MBA as a probe with high affinity for β-CD ($1.3 \cdot 10^7$ M^{-1}) (Fundueanu et al., 2003; Nava-Ortiz, Alvarez-Lorenzo, Bucio, Concheiro & Burillo, 2009; Santos et al., 2008). All hydrogels with built-in CDs and those with pendant CDs were immersed in 3-MBA solutions. Hydrogels with built-in CDs took less 3-MBA than hydrogels with pendant CDs (Fig. 4). Hydrogels made with β-CD-NMA exhibited an affinity for 3-MBA larger than that of hydrogels prepared with γ-CD-NMA. From the amount of 3-MBA loaded by Cβ100 minus that loaded by C0 (control) hydrogels (Fig. 4), we estimated the number of 3-MBA molecules that are interacting with the β-CD mers, which resulted to be 0.007 mmol per gram of hydrogel. Taking into account that 1 molecule of 3-MBA can form complex with 1 β-CD mer, there should be 7.9 mg of β-CD mers per gram of hydrogel available for hosting 3-MBA. Since the total amount of β-CD mers should be 12.5 mg/g, roughly 63% of the β-CD-NMA added upon synthesis is effectively participating in the hosting process.

The ability of NVP/DMA/GMA hydrogels to load 3-MBA was remarkably increased after the grafting of β-CD or γ-CD (Fig. 4). The greater the proportion of GMA, the more β-CD or γ-CD could be grafted and, consequently, the higher the affinity for 3-MBA was (Fig. 4). Applying similar reasoning to that made above, we

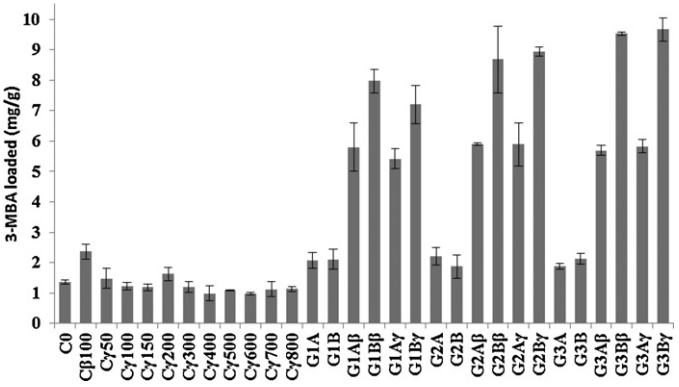

Fig. 4. 3-MBA loading by the hydrogels with built-in or pendant CDs.

estimated that the amount of β-CD or γ-CD grafted to the networks was ca. 15% of GMA proportion, for both two levels of GMA evaluated. This means that each CD unit is linked to the network through 6–7 of their hydroxyl groups or that the linking is less and some GMA mers are not involved in the grafting. We have previously observed that pHEMA-co-GMA networks can graft β-CD units through 2–3 hydroxyl groups (Santos et al., 2009). That could be also the case for the NVP/DMA/GMA hydrogels if half of GMA molecules would not have reacted with the CD units. This point is not easy to clarify since the non-reacted GMA mers open the epoxy group during the final washing step rendering hydroxyl groups that are hardly distinguishable from those of the CDs. Generally speaking, copolymerization with GMA followed by grafting of CDs results in hydrogels with more functional CDs available to interact with guest molecules and to form inclusion complexes.

3.6. Acetazolamide loading and release

When a hydrogel is immersed in an aqueous drug solution, the amount loaded mainly depends on both the drug concentration in the soaking solution and the affinity of the drug for the network. The amounts of ACT loaded by each hydrogel and the partition coefficient ($K_{N/W}$) values are shown in Fig. 5 and Table 3. The $K_{N/W}$ values were estimated from the equation (Kim, Bae & Okano, 1992):

$$\text{Loading (total)} = \left[\frac{\left(V_s + K_{N/W}V_p\right)}{W_p}\right] \cdot C_0 \quad (4)$$

where V_s is the volume of water sorbed by the hydrogel, W_p is the dried hydrogel weight, C_0 is the concentration of the drug in the

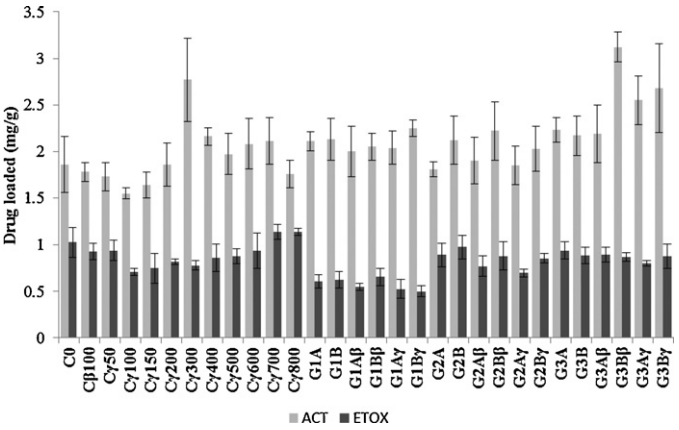

Fig. 5. Amounts of acetazolamide (ACT) and ethoxzolamide (ETOX) loaded by the hydrogels with built-in CDs or pendant CDs.

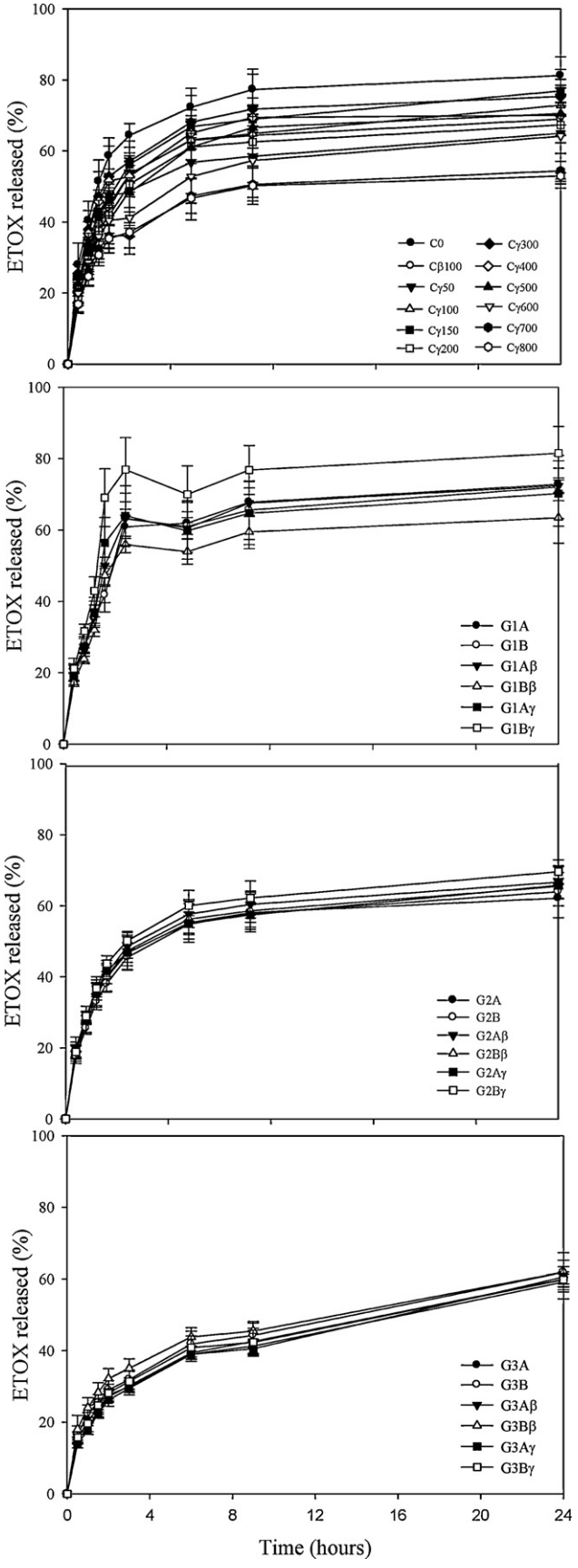

Fig. 6. ETOX release profiles from SCLs hydrogels formulations ($n = 6$).

Table 3
Amounts of acetalozamide (ACT) and ethoxzolamide (ETOX) loaded and network/water partition coefficients in hydrogels prepared with CD monomers or with grafted raw CDs.

Formulations	ACT (mg/g)	$K_{N/W}$	ETOX (mg/g)	$K_{N/W}$
C0	1.86 (0.30)	4.2 (1.5)	1.03 (0.16)	46 (6)
Cβ100	1.78 (0.10)	3.8 (0.4)	0.93 (0.09)	38 (4)
Cγ50	1.73 (0.15)	3.2 (0.7)	0.94 (0.11)	39 (5)
Cγ100	1.55 (0.06)	2.4 (0.4)	0.71(0.04)	28 (2)
Cγ150	1.64 (0.14)	2.9 (0.6)	0.75 (0.16)	28 (1)
Cγ200	1.86 (0.23)	4.0 (1.1)	0.82 (0.03)	33 (2)
Cγ300	1.90 (0.10)	4.6 (2.2)	0.78 (0.05)	33 (2)
Cγ400	2.16 (0.09)	5.8 (0.4)	0.86 (0.15)	36 (7)
Cγ500	1.97 (0.22)	4.7 (1.1)	0.88 (0.08)	37 (3)
Cγ600	2.08 (0.27)	5.7 (1.3)	0.94 (0.19)	40 (9)
Cγ700	2.11 (0.25)	5.7 (0.9)	1.14 (0.08)	49 (4)
Cγ800	1.76 (0.15)	4.2 (0.7)	1.14 (0.04)	50 (2)
G1A	2.11 (0.10)	4.8 (0.5)	0.61 (0.07)	25 (1)
G1B	2.13 (0.22)	5.1 (1.0)	0.62 (0.09)	23 (2)
G1Aβ	2.00 (0.27)	3.8 (1.2)	0.55 (0.04)	20 (2)
G1Bβ	2.05 (0.14)	4.1 (0.6)	0.65 (0.09)	26 (3)
G1Aγ	2.04 (0.18)	3.9 (0.8)	0.53 (0.10)	18 (3)
G1Bγ	2.25 (0.09)	5.7 (0.4)	0.50 (0.06)	18 (3)
G2A	1.81 (0.08)	3.7 (0.4)	0.89 (0.13)	35 (5)
G2B	2.12 (0.26)	4.7 (0.4)	0.98 (0.13)	37 (1)
G2Aβ	1.90 (0.25)	4.3 (1.2)	0.77 (0.11)	29 (2)
G2Bβ	2.22 (0.31)	4.3 (1.4)	0.88 (0.15)	34 (4)
G2Aγ	1.85 (0.21)	3.2 (0.9)	0.70 (0.04)	27 (2)
G2Bγ	2.03 (0.24)	4.3 (1.1)	0.86 (0.05)	35 (2)
G3A	2.23 (0.13)	5.5 (0.7)	0.94 (0.09)	39 (4)
G3B	2.17 (0.21)	5.7 (1.0)	0.89 (0.09)	37 (4)
G3Aβ	2.19 (0.31)	4.9 (1.4)	0.90 (0.08)	37 (4)
G3Bβ	3.12 (0.16)	9.7 (0.7)	0.87 (0.05)	36 (2)
G3Aγ	2.55 (0.26)	6.6 (1.2)	0.80 (0.03)	32 (1)
G3Bγ	2.68 (0.48)	7.7 (2.2)	0.88 (0.13)	34 (3)

loading solution and V_p is the volume of dried polymer. The $K_{N/W}$ values which are an index of the affinity of the drug for the network (Rodriguez-Tenreiro et al., 2006) clearly indicate that amount of ACT loaded was not affected by the incorporation of the β-CD-NMA and γ-CD-NMA monomers into the hydrogel network. Only larger $K_{N/W}$ values were found for G3Bβ and G3Bγ hydrogels; namely, those prepared with NVP/DMA 40/60 ratio and the highest proportion of GMA that, consequently, have the greatest content in grafted β-CD or γ-CD. Hydrogels loaded with ACT sustained the release for 3–6 h (Fig. S1); the release rate being slightly lower for networks synthesized with the highest proportion of γ-CD-NMA monomers but no remarkable differences were observed.

3.7. Ethoxzolamide loading and release

The poor aqueous solubility of ETOX forced that the loading was carried out by immersion in a drug suspension. All hydrogels showed a similar capability to host ETOX (Fig. 5), probably due to the strength of unspecific hydrophobic interactions with the polymer networks with and without CDs, as revealed by the high $K_{N/W}$ values (Table 3). It has been reported that DMA-co-NVP polymers can establish π–σ and π–π interactions through their lactam and amide groups with aromatic compounds such as ETOX, leading to charge-transfer complexes or electron donor-acceptor interactions (Queiroz, Franca, Abraham & San Roman, 2004; Ribeiro et al., 2011b). Only those copolymerized with the highest proportions of γ-CD-NMA monomers showed a slightly greater affinity for ETOX. The hydrogels prepared without NVP and copolymerized with GMA (codes G1A, G1B and derived from these) were the ones with the lowest uptake ability. This finding suggests that ETOX is more prone to interact with NVP than with DMA. The differences in affinity were more clearly seen when the release was evaluated (Fig. 6). In the case of hydrogels with built-in CDs, the higher the proportion of γ-CD-NMA, the slower the release was. On the other hand, the

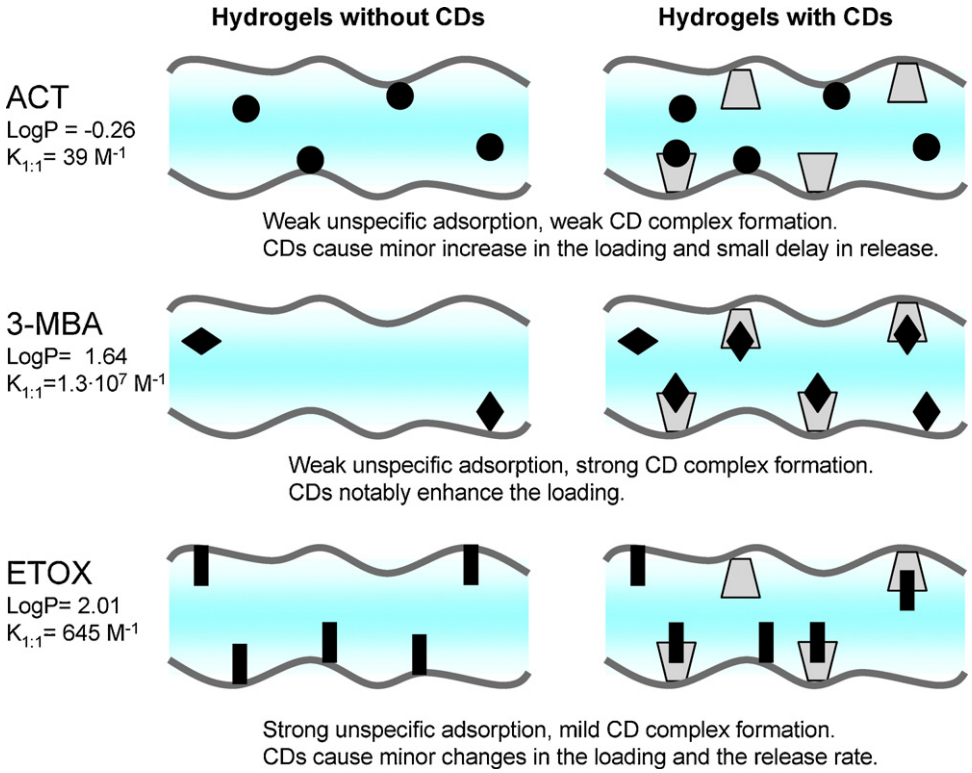

Fig. 7. Schematic view of the influence of the physicochemical properties of the drug (Supuran, 2008; and CS Chem Draw Ultra, Cambridge MA) and its affinity for the CDs (experimental data for ACT and ETOX, and data of 3-MBA from Fundueanu et al., 2003) on the loading/release in the NVP-co-DMA hydrogels with built-in or pendant CDs.

hydrogels with pendant CDs that sustained more the release were those synthesized with the greater proportion of NVP. Both types of hydrogels sustained the release for almost one week.

An overall analysis of the loading of 3-MBA and the loading/release of ACT and ETOX, enables us to identify three scenarios depending on the hydrophobicity and the cyclodextrin affinity of the drug (Fig. 7):

(i) hydrophilic molecules with low affinity for CDs (namely ACT) are mainly uptake in the aqueous phase of the hydrogels. The unspecific interactions with the backbone monomers are weak, and the complex formation with CDs plays a minor role in the loading and the control of release,
(ii) medium hydrophylic molecules with high affinity for CDs (e.g. 3-MBA) are mainly hosted in the hydrogels by means of complex formation. Thus the content in CDs determines the yield of loading,
(iii) hydrophobic, poorly-water soluble molecules with medium affinity for CDs (e.g. ETOX) are bound to the network through strong unspecific interactions, which overcome the effect of the complex formation with CDs. In this case, the composition of the backbone monomers determines the affinity of the drug for the hydrogel and thus the loading and the release processes.

4. Conclusions

Natural β-CD and γ-CD can form inclusion complexes with ACT or ETOX in aqueous solution; the affinity constant being one-order of magnitude larger for ETOX. When these CDs were copolymerized or grafted to NVP-co-DMA networks, no detrimental effects on the swelling, optical transparency or cytocompatibility were observed. In those networks, the drug can be hosted in three different states: free in the aqueous phase, nonspecifically interacting with the backbone monomers, or forming inclusion complexes with CDs. The balance between these three mechanisms, which depends on the physicochemical properties of the drug and its ability to form complexes, determines the role played by the CDs in the loading and release. Incorporation of CDs into the network is particularly beneficial for those molecules that are slightly soluble in water and exhibit high affinity for the CD cavity. The approach followed to prepare hydrogels with pendant CDs enables to incorporate them in a greater proportion, compared to the copolymerization of CD monomers, and makes the role of CDs in the loading/release of the CAIs more evident.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2012.01.053.

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