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γ-Cyclodextrin hydrogels and semi-interpenetrating networks for sustained delivery of dexamethasone

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ABSTRACT

γ-Cyclodextrin (γCD) networks with tunable texture and complexation efficacy were prepared by direct cross-linking of γCD in aqueous medium containing hydroxypropyl methylcellulose or polyacrylic acid sodium salt (PAcNa) resulting in hydrogels or semi-interpenetrating networks (semi-IPN), respectively. The composition of the networks determined their hardness, compressibility and bioadhesion. γCD networks were loaded by immersion in autoclaved or sonicated dexamethasone suspensions with or without free γCD. Drug loading yield and release profile depended on the capability of the networks to form inclusion complexes. Free γCD in the loading medium decreased dexamethasone uptake (~50%) through competitive drug complexation and resulted in faster delivery. Such an effect was notable for γCD/PAcNa semi-IPNs due to their smaller mesh size limits the entrance of drug–γCD complexes. Hydrogels and semi-IPNs loaded in the absence of free γCD sustained dexamethasone release for several days. Relationships between the structure of the novel cytocompatible γCD networks and their properties, namely capability to host and to sustain the delivery of hydrophobic drugs, were elucidated.

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1. Introduction

The pharmaceutical suitability of hydrogels is mainly determined by the extent they can mimic mechanical behaviors of the body tissues, and can load drugs and control their release as a function of therapeutic demands (Kopecek, 2009). Hydrogels posses a highly hydrophilic character that may make the loading of hydrophobic drugs difficult and that limits their ability to control the release of the hydrophilic molecules (Alvarez-Lorenzo, Hiratani, & Concheiro, 2006; Kim, Bae, & Okano, 1992). Covalent binding of cyclodextrins (CDs) to the polymer networks has recently been seen as a promising way to overcome these drawbacks (Crini & Morcellet, 2002; dos Santos et al., 2009; Liu, Fan, Kang, & Sun, 2004; Mocanu, Mihai, LeCerf, Picton, & Moscovici, 2009; Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2006; Zhang, Xue, Gao, Huang, & Zhuo, 2008). The interest of CDs as integral components of hydrogels lies in that the capability of CDs to host drug molecules can be transmitted to the network, which enables the drug–CD affinity to play a relevant role in the loading and the release processes (Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2007a).

CD hydrogels are usually obtained by copolymerization of CD monomeric derivatives with acrylic or vinyl monomers (Demir, Kahraman, Bora, Apohan, & Ogan, 2008; dos Santos, Couceiro, Concheiro, Torres-Labandeira, & Alvarez-Lorenzo, 2008; Shan, Chen, Yang, Zhang, & Li, 2009; Siemoneit et al., 2006). In order to avoid the important drawbacks of the chemical modification of CDs (low reproducibility of the synthesis or residual toxic monomers), a direct procedure was developed to synthesize 2-hydroxypropyl-β-cyclodextrin (HPβCD) hydrogels using ethyleneglycol diglycidylether (EGDE) as cross-linking agent (Rodriguez-Tenreiro et al., 2006). These HPβCD-based hydrogels have been shown to be able to efficiently load and control the release of diclofenac, estradiol and sertaconazole (Lopez-Montero, dos Santos, Torres-Labandeira, Concheiro, & Alvarez-Lorenzo, 2009; Rodriguez-Tenreiro, Diez-Bueno, Concheiro, Torres-Labandeira, & Alvarez-Lorenzo, 2007b). Hydrogels prepared from natural γCD may be even more valuable since γCD forms complexes with a wide range of substances, possesses very favorable toxicological profile and is approved for almost any route of administration (Loftsson & Duchene, 2007; Munro, Newberne, Young, & Bär, 2004). Furthermore, the high aqueous solubility of γCD (18.5–39.0% at 20–35 °C; Szente, Szejtli, & Kis, 1998) may enable preparation of hydrogels with a high γCD content and, consequently, enhanced ability to complex lipophilic drug molecules.

The aim of this work was to prepare γCD hydrogels in water, under mild conditions and in one step, containing γCD solely or in combination with various hydrophilic polymers, such as hydroxypropyl methylcellulose (HPMC K4M) or polyacrylic acid

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sodium salt (PAcNa) of various molecular weights. HPMC is susceptible of simultaneous cross-linking rendering γCD-co-HPMC networks. On the other hand, PAcNa is not expected to react with EGDE but to lead to semi-interpenetrating networks, which could benefit from the bioadhesive properties of PAcNa (Rodriguez-Tenreiro et al., 2007b). Dexamethasone is a glucocorticoid commonly used as an anti-inflammatory drug. It is relatively lipophilic ($\log P = 1.8$) and permeates biological membranes quite easily. However, its low aqueous solubility (0.16 mg/ml) limits its clinical usefulness (Kristinsson et al., 1996). It has been recently reported that phase-solubility profiles of dexamethasone in aqueous solutions containing γCD are Bs-type, with an initial linear increase up to 5% w/w γCD and a slight synergic effect of 0.1% w/w HPMC (Jansook & Loftsson, 2008, 2009). Higher γCD or HPMC concentrations resulted in a brusque solubility reduction due to precipitation of the complexes. Cross-linking of γCD solutions may render hydrogels with enhanced capability to host dexamethasone avoiding insolubilization of the resultant complexes. The present work focuses on the incidence of hydrogels composition on their swelling, textural, and bioadhesion features. Cytocompatibility of the hydrogels and their capability to load dexamethasone and to control its release are also characterized in detail. We attempt to combine in a single material the loading capability of cross-linked CDs with the favorable properties of the hydrophilic polymers, which could results in an optimized vehicle for loading and delivering of hydrophobic drugs.

2. Materials and methods

2.1. Materials

γCD (W8) was purchased from Wacker Quimica Ibérica S.A. (Barcelona, Spain), polyacrylic acid sodium salt (PAcNa) 2100 and 5100 Da from Fluka (St. Louis, USA), hydroxypropyl methyl cellulose (HPMC) Methocel® K4M Premium EP from Colorcon Iberica S.L. (Barcelona, Spain), and ethyleneglycol diglycidylether (EGDE, 50% w/w in water) and dexamethasone from Sigma–Aldrich (St. Louis, USA). Water purified by reverse osmosis (MilliQ®, Millipore, Spain) with a resistivity above 18.2 $\text{M}\Omega\,\text{cm}^{-1}$ was used. All other chemicals used were of analytical reagent grade.

2.2. Synthesis of γCD hydrogels

2.2.1. Preparation of γCD solutions

γCD solutions (5, 10, 15 or 20 w/w) in 0.2 M NaOH were prepared under stirring. Two milliliters of 50% EGDE solution were added to 5 ml of each γCD solution and the systems were stirred for 2–5 min at room temperature.

2.2.2. Preparation of γCD–PAcNa and γCD–HPMC solutions

γCD was added under stirring to 10 ml of aqueous 0.2 M NaOH solution, containing either 2–4% w/w PAcNa (2100 or 5100 Da) or 2–3% w/w HPMC K4M solutions, until the γCD concentration had reached either 5 or 10% w/v. Once homogenized solutions were obtained, EGDE (4 ml of 50% w/w aqueous solution) was added to each solution and the systems were stirred for 2–5 min at room temperature. EGDE was also added to HPMC solutions containing no γCD.

2.2.3. Cross-linking procedure

Immediately after the addition of EGDE, the solutions were transferred to test tubes, which were hermetically closed and kept at 50 °C for 12 h. After cooling down, the hydrogels were removed from the tubes and immersed in water for 12 h to swell. The medium was replaced by 10 mM HCl for 12 h to neutralize the alkaline medium and, then, the hydrogels were immersed in water, which was replaced every 12 h for 1 week to thoroughly remove the unreacted substances.

2.3. Hardness and compressibility

Swollen hydrogel disks were characterized before and after autoclaving using a TA–TX Plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK) with a cylindrical aluminum probe (Ref. P/20). A disk of 5 mm thickness was placed on the platform and the probe was compressed into the sample at a rate of 1 mm/s and to a depth of 2 mm. Then, the probe was removed at 1 mm/s and the recovery of the sample was also monitored. The hardness was estimated as the maximum resistance to compression (i.e., the peak value in the force–distance plot) and the compressibility was quantified as the work carried out in the compression (i.e., the area under the force–distance plot) (Andrews & Jones, 2006). The modulus of deformability, ED, was estimated as the slope of the initial linear portion of the true stress–strain plot. The true stress was obtained from the force as follows:

$$\sigma_T = \frac{F(t)[h_o - \Delta h]}{A_o h_o} \tag{1}$$

and the Hencky's strain from the distance as follows:

$$\varepsilon_T = \ln\left(\frac{h_o}{h_o - \Delta h}\right) \tag{2}$$

In these equations h_o is the original height of sample, Δh is the change in height, $F(t)$ is the compressive force at time t, and A_o is the original cross-sectional area (Konstance, 1993).

2.4. In vitro bioadhesion assay

The bioadhesive properties of each hydrogel after swelling 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) (Stjernschantz & Astin, 1993) were evaluated in duplicate using a TA–TX Plus Texture Analyzer (Stable Micro Systems Ltd., Surrey, UK). The disk was glued (Loctite® SuperGlue3, Germany) on the lower face of the probe (Ref. P/20), whilst a piece of goat tanned leather was fixed on the lower platform (Blanco-Fuente, Vila-Dorrio, Anguiano-Igea, Otero-Espinar, & Blanco-Mendez, 1996). Then, the disk was allowed to contact the leather surface and a downward force (0.49 N) was applied for 10 min. The probe was then elevated at 5 mm/s, and the bioadhesion was determined as the detachment work.

2.5. Cytocompatibility studies

The tests for *in vitro* cytocompatibility were carried out according to the ISO 10993-5 protocol. Small disks of hydrogels were immersed in phosphate buffer pH 7.4 and autoclaved. Then, the disks were added to wells (24-well plates) containing Balb/3T3 clone A31 cells (200,000 cells per well, 2 ml) in DMEM F12 HAM (Sigma–Aldrich, USA) and kept in humidified incubator at 5% CO_2 and 37 °C. After 24 h, aliquots (100 μl) of the culture medium were taken and mixed with 100 μl of the reaction medium contained in the Cytoxicity Detection Kit$^{\text{PLUS}}$ (LDH, Roche). Blank (100 μl of medium), negative (50 μl of cells and 50 μl of medium) and positive (50 μl of cells and 50 μl of medium with 5 μl of lysis factor) controls were also prepared according to the kit protocol. The plates were incubated 10 min at 15–25 °C protected from light. Fifty microliters of stop solution was added to each well and the absorbance at 490 nm was immediately measured using an ELISA reader. The cytotoxicity was estimated as follows:

$$\text{Cytotoxicity } (\%) = \frac{\text{Abs}_{\text{exp}} - \text{Abs}_{\text{negative control}}}{\text{Abs}_{\text{positive control}} - \text{Abs}_{\text{negative control}}} \times 100 \quad (3)$$

2.6. Degree of swelling

The degree of swelling of the hydrogels was calculated as the difference between the weight of the fully swollen hydrogel (W) in water and the initial weight of the dried hydrogel disk (W_0):

$$Q\ (\%) = \frac{W - W_0}{W_0} \times 100 \quad (4)$$

2.7. Dexamethasone loading

Cylindrical pieces of the hydrogels were dried at 50 °C for 24 h and then immersed in aqueous suspension of dexamethasone (10 mg drug in 10 ml) for 1 week at room temperature. The suspensions were prepared either in water (systems denoted with an H) or in 50 mM γCD solution (systems denoted with a G). Some suspensions were autoclaved at 121 °C 20 min (key AH or AG) before the immersion of the hydrogels. Other suspensions were subjected to ultrasounds for 30 min at 25 °C (key SH or SG). The hydrogels were kept in the suspensions for 1 week at room temperature under oscillatory movement (25 osc/min). All experiments were carried out in triplicate.

The amount loaded by just a simple equilibrium between the aqueous phase of the network and the loading solution was estimated using the equation (Kim et al., 1992):

$$\text{Loading(aqueous phase)} = \frac{V_s}{W_p} C_0 \quad (5)$$

where V_s is the volume of water sorbed by the hydrogel, W_p the dried hydrogel weight, and C_0 the initial concentration of drug in the loading solution.

2.8. Dexamethasone release

Drug-loaded hydrogels were rinsed with water and immersed directly in 5 ml of water at room temperature. Samples of the release medium (1 ml) were taken periodically and replaced with the same volume of fresh water. The amount of dexamethasone released was measured using a Merck-Hitachi HPLC equipment that consisted of a L-6200 pump (operated at 1.5 ml/min), an automatic injector (AS-4000), a thermostat (L-5025) for the column (30 °C) and a UV detector (Diode-array, model L-4500) operated at 241 nm, connected to a computer by an interface (D-6000). A Symmetry® C18 5 μm column (3.9 × 150 mm; Waters, Ireland) was used. The mobile phase was acetonitrile (LiChrosolv®, Merck), tetrahydrofuran (LiChrosolv®, Merck) and water obtained by reverse osmosis (MilliQ®) (33:1:66). The volume injected was 20 μl and the retention time was 3.40 min.

3. Results and discussion

3.1. Synthesis of γCD hydrogels

Table 1 summarizes the composition of the γCD hydrogels and semi-IPNs. The synthesis was carried out in NaOH 0.2 M since the OH^- ions catalyze the ring opening of the oxacyclopropane of the EGDE to react with hydroxyl groups, such as those of the cyclodextrin glucopyranoses. Heating is required for the reaction to proceed; whereas at room temperature, no cross-linking was observed. At 50 °C the reaction rate was fast enough to complete the cross-linking process in a few hours (Rodriguez, Alvarez-Lorenzo, & Concheiro, 2003). Previous studies have shown that EGDE concentration has to be at least 14.28% w/w to obtain hydrogels from HPβCD or MβCD solutions (Rodriguez-Tenreiro et al., 2006). This EGDE concentration, which is sufficient to react with 1.64 mol/L of hydroxyl groups, was used for preparing the γCD hydrogels.

Twenty percentages of γCD solutions (~14.28% in the reaction mixture once EGDE was added) rendered too brittle hydrogels that became easily fragmented during handling. Taking into account that this γCD concentration corresponds to 0.110 mol/L and that each molecule of γCD possesses 24 hydroxyl groups, the EGDE added could react with 62% of the total number of hydroxyl groups on each γCD molecule. Contrary to what was observed previously in aqueous HPβCD or methylated βCD solutions, such a cross-linking density leads to hard but fragile hydrogels that break under stress. On the other hand, 5% γCD solutions (~3.6% in the reaction mixture) did not lead to hydrogel formation, which means that the distance between the hydroxyl groups of neighbor CD units is too great for the cross-linking agent to establish intermolecular bridges. Only 10% γCD (~7.1% in the reaction mixture) and 15% γCD (~10.7% in the reaction mixture) provided viscoelastic hydrogels with mechanical properties adequate to be handled without the risk of disintegration (Table 1). HPMC and PAcNa were added to these latter solutions in order to modulate the textural features of the γCD hydrogels. Additionally, 5% γCD solutions with 2% or 3% HPMC (1.43% and 2.86% in the reaction mixture) enabled formation of flexible hydrogels. The four types of networks synthesized (depicted in Fig. 1) were homogeneous and transparent, which means that γCD and the polymers did not undergo phase separation during the cross-linking.

3.2. Hardness and compressibility

Texture analysis showed that the force–distance curve registered for increasing forces was almost superimposable to that obtained during the removal (i.e., recovery) of the probe (Fig. 2). The

Table 1
Composition of γCD hydrogels upon cross-linking and their textural properties (ED: modulus of deformability). Mean ± standard deviation.

γCD (% w/w)	Polymer (% w/w)	EGDE (% w/w)	Hardness (N)	Compressibility (N mm)	ED (kPa)	Bioadhesion work (N mm)
14.28	–	14.28	7.9 ± 0.2	9.1 ± 0.1	0.064	n.d.
10.70	1.43% HPMC K4M	14.28	7.3 ± 0.1	7.8 ± 0.1	0.032	n.d.
7.14	–	14.28	3.2 ± 0.1	3.0 ± 0.2	0.015	n.d.
7.14	1.43% HPMC K4M	14.28	0.36 ± 0.02	0.32 ± 0.02	0.010	0.001
7.14	1.43% PAcNa 2100	14.28	4.2 ± 0.1	3.8 ± 0.3	0.014	0.030
7.14	2.86% PAcNa 2100	14.28	5.0 ± 0.1	5.5 ± 0.4	0.018	0.028
7.14	1.43% PAcNa 5100	14.28	7.2 ± 0.1	5.7 ± 0.1	0.042	0.001
7.14	2.86% PAcNa 5100	14.28	7.6 ± 0.4	6.0 ± 0.1	0.056	0.010
3.57	1.43% HPMC K4M	14.28	5.1 ± 0.2	4.2 ± 0.2	0.009	0.005
3.57	2.14% HPMC K4M	14.28	5.3 ± 0.2	4.5 ± 0.1	0.015	0.005
0	1.43% HPMC K4M	14.28	0.21 ± 0.01	0.29 ± 0.01	0.002	0.035
0	2.14% HPMC K4M	14.28	2.4 ± 0.1	1.97 ± 0.02	0.008	0.087

n.d., non-detectable.

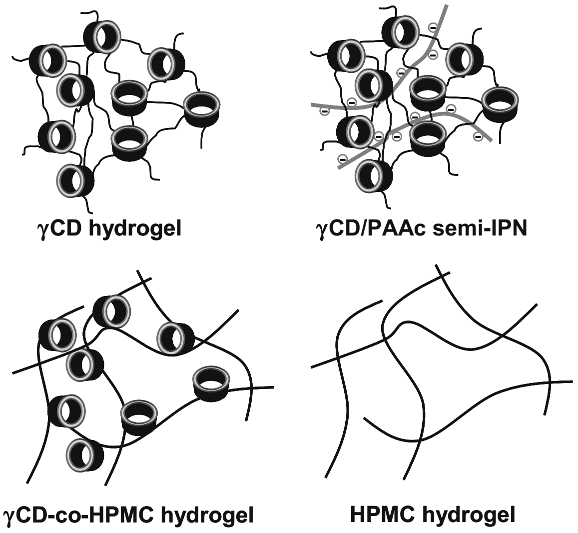

Fig. 1. Schematic view of the structure of γCD and γCD-co-hydroxypropyl methyl cellulose (HPMC K4M) hydrogels, γCD/polyacrylic acid semi-interpenetrating networks (semi-IPN), and HPMC solely hydrogels.

hardness and compressibility of the 14.28% γCD hydrogel were the highest, while those recorded for 7.14% γCD hydrogel were the smallest ones. Incorporation of HPMC or PAcNa enabled to modulate the textural behavior of the hydrogels being possible to cover a wide range of hardness and compressibility values (Table 1). In this sense, as expected from previous works (Rodriguez-Tenreiro et al., 2006), HPMC alone cross-linked by EDGE rendered quite soft and flexible hydrogels. These features were transmitted to the mixed HPMC-co-γCD networks, which could be manipulated without risk of breaking. On the other hand, PAcNa is not expected to be cross-linked by EGDE under alkaline conditions. Thus, a semi-interpenetrating network (semi-IPN) with a γCD mesh interpenetrated by the long PAcNa chains was formed. The greater the proportion of PAcNa was the greater the increase in hardness and compressibility of the semi-IPNs (Table 1). In this regard, the molecular weight of PAcNa played an important role. The 5100 Da variety led to remarkably greater hardness and compressibility. Such an effect is explained by the electrostatic repulsions among the interpenetrating chains, which force the mesh to be as expanded as much as possible to maximize the distance among the ionized groups of the PAcNa chains (Rodriguez-Tenreiro et al., 2007b).

Since the hydrogels are viscoelastic rather than purely elastic, the Young's modulus cannot be accurately calculated from the slope of the force–distance plot. The cross-sectional area and length of the hydrogel discs do change substantially when force is applied, and the equations developed for extensional rheometry assuming incompressibility are not valid since the engineering stress ceases to be an accurate measure (Lapasin & Pricl, 1995). Therefore, a modulus of deformability, ED, was estimated (Table 1) using the Hencky model, in which the true stress represents an adjustment of the engineering stress ($F(t)/A_0$) to account for cross-sectional area expansion of the deformed specimen (Konstance, 1993). ED is an index of the stiffness and has been widely used for characterizing hydrogels and soft materials of varied nature (Konstance, 1993).

Taking into account the results of textural analysis, the most adequate conditions for obtaining the hydrogels were as follows: 7.14% γCD alone or with 1.43% HPMC, 1.43% or 2.86% PAcNa, or 3.57% γCD with 1.43% or 2.14% HPMC. These hydrogels were chosen for further studies.

Artificial lacrimal fluid was used for the bioadhesion test in order to elucidate the behavior of the hydrogels under the less favorable physiological conditions, since in this fluid most acrylic acid groups of the semi-IPNs are ionized, diminishing the capability to establish hydrogel bonds (Andrews, Laverty, & Jones, 2009). γCD hydrogels are not bioadhesive (Table 1). Oppositely the presence of PAcNa or HPMC provided the hydrogels with certain bioadhesion capability.

3.3. Cytocompatibility studies

The cytocompatibility studies were carried out following the direct contact test as described in ISO 10993-5:1999, which enabled to evaluate the toxicity of both the hydrogel itself and of leached substances. Before the test, the hydrogel disks were immersed in phosphate buffer pH 7.4 and autoclaved. The hydrogels withstood this thermal treatment without breaking or changing their texture. As can be seen in Fig. 3, all hydrogels resulted to be highly cytocompatible.

3.4. Dexamethasone loading

In general, drug loading depends on the easiness of the drug molecules to diffuse into the hydrogel (determined by the degree of cross-linking and the affinity of water for the network), on the

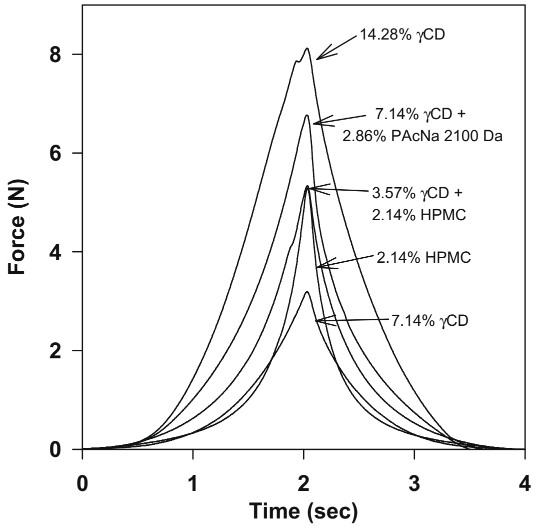

Fig. 2. Force–displacement curves for water-swollen γCD-based hydrogels.

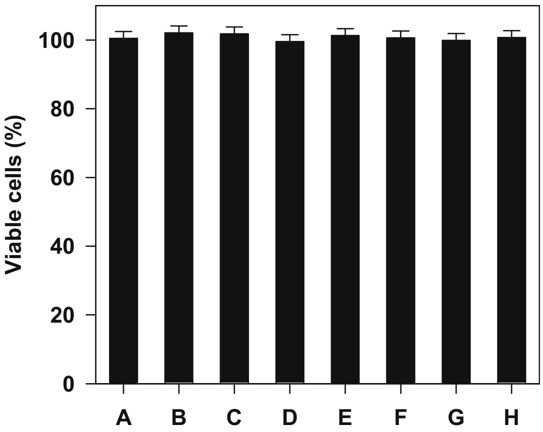

Fig. 3. Viability of Balb/3T3 clone A31 cells after being in contact with the hydrogels for 24 h. Codes: (A) 7.14% γCD hydrogel; (B) 7.14% γCD with 1.43% PAcNa 2100; (C) 7.14% γCD with 2.86% PAcNa 2100; (D) 7.14% γCD with 1.43% PAcNa 5100; (E) 7.14% γCD with 2.86% PAcNa 5100; (F) 7.14% γCD with 1.43% HPMC; (G) 3.57% γCD with 1.43% HPMC; and (H) 3.57% γCD with 2.14% HPMC.

Table 2
Degree of swelling (*Q*) and total amount released by the γCD hydrogels after being loaded in a dexamethasone suspension, containing 50 mM γCD (code G) or not (code H), that was autoclaved (code A) or subjected to ultrasounds (code S) before immersion of the hydrogels. Mean ± standard deviation.

γCD (% w/w)	Polymer (% w/w)	Loading conditions							
		Autoclaved-water (AH)		Autoclaved-cyclodextrin (AG)		Sonicated-water (SH)		Sonicated-cyclodextrin (SG)	
		Q (%)	mg/g	*Q* (%)	mg/g	*Q* (%)	mg/g	*Q* (%)	mg/g
7.14	–	763 ± 18	11.8 ± 1.1	780 ± 11	12.1 ± 2.2	663 ± 123	8.1 ± 0.3	783 ± 4	6.4 ± 1.1
7.14	1.43% PAcNa 2100	767 ± 41	13.1 ± 0.6	745 ± 42	6.6 ± 0.3	700 ± 37	10.3 ± 0.9	724 ± 54	5.5 ± 0.7
7.14	2.86% PAcNa 2100	649 ± 12	10.3 ± 0.3	657 ± 6	4.8 ± 0.4	627 ± 11	8.2 ± 0.1	650 ± 7	4.3 ± 0.2
7.14	1.43% PAcNa 5100	711 ± 5	12.7 ± 0.4	704 ± 18	5.9 ± 0.1	669 ± 33	8.5 ± 0.2	693 ± 6	4.2 ± 0.3
7.14	2.86% PAcNa 5100	534 ± 42	7.8 ± 0.3	569 ± 9	4.6 ± 0.2	543 ± 18	6.1 ± 0.1	562 ± 7	3.8 ± 0.9
7.14	1.43% HPMC	767 ± 1	10.4 ± 0.7	728 ± 15	11.6 ± 6.3	706 ± 66	7.3 ± 0.3	593 ± 140	5.0 ± 0.6
3.57	1.43% HPMC	1569 ± 63	13.4 ± 2.1	1619 ± 24	7.9 ± 0.2	1620 ± 33	12.2 ± 0.9	1543 ± 95	15.1 ± 4.8
3.57	2.14% HPMC	956 ± 122	8.6 ± 1.6	1032 ± 11	8.8 ± 1.2	977 ± 21	7.5 ± 1.5	1037 ± 37	9.5 ± 1.0
0	1.43% HPMC	4045 ± 244	8.9 ± 1.3	4206 ± 362	49.1 ± 14.1	3830 ± 310	12.8 ± 3.3	3779 ± 65	41.3 ± 2.6
0	2.14% HPMC	2485 ± 58	5.4 ± 1.2	2574 ± 40	42.0 ± 7.2	2430 ± 45	8.2 ± 2.0	2462 ± 45	21.1 ± 4.2

drug concentration in the surrounding loading solution, and on the affinity of the drug for the network structure (Rodriguez-Tenreiro et al., 2006). Regarding the first issue, all γCD hydrogels showed high affinity for water, swelling to a large extent when immersed in the loading solutions (Table 2). The presence of HPMC did not modify the degree of swelling of the 7.14% γCD hydrogels,

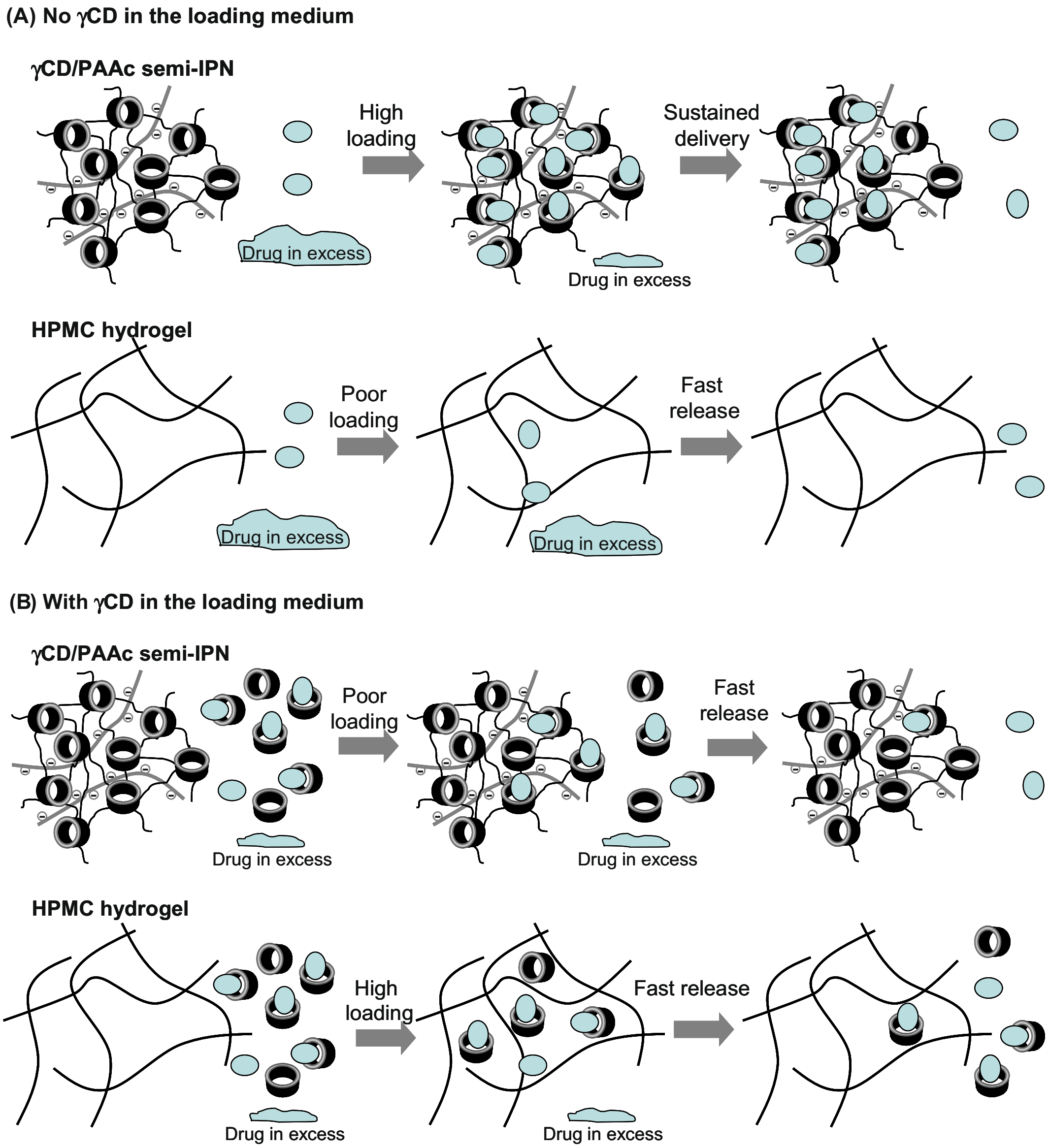

Fig. 4. Schematic view of the drug uptake and release by the hydrogels that behave more differently each other, when the loading was carried out in a dexamethasone suspension without (A) or with (B) free γCD and drug–γCD complexes.

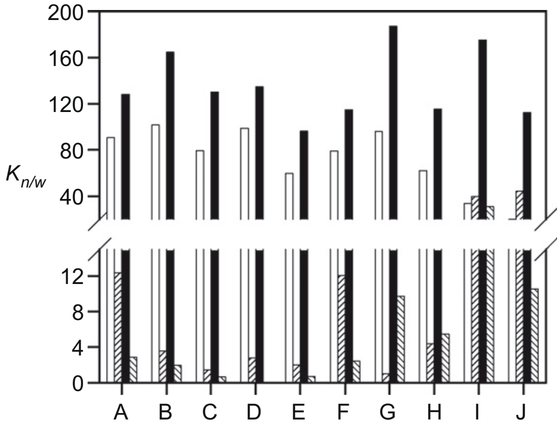

Fig. 5. Partition coefficient of the drug between the network and the loading medium, when the drug suspensions without or with γCD were autoclaved (AH white column and AG striped-up pattern column) or sonicated (SH black column and SG striped-down pattern column). Codes: (A) 7.14% γCD hydrogel; (B) 7.14% γCD with 1.43% PAcNa 2100; (C) 7.14% γCD with 2.86% PAcNa 2100; (D) 7.14% γCD with 1.43% PAcNa 5100; (E) 7.14% γCD with 2.86% PAcNa 5100; (F) 7.14% γCD with 1.43%HPMC; (G) 3.57% γCD with 1.43% HPMC; (H) 3.57% γCD with 2.14% HPMC, (I) 1.43% HPMC; and (J) 2.14% HPMC.

and PAcNa just caused a minor decrease. Remarkably, the 3.57% γCD hydrogels prepared with 1.43% or 2.14% HPMC behaved as superabsorbents.

In order to increase the concentration of dexamethasone in the outer solution, several approaches were tested. Drug suspensions (1 mg/ml) with or without γCD (50 mM) were autoclaved at 121 °C for 20 min or were sonicated for 30 min before immersion of the hydrogels. Dexamethasone concentrations achieved were 0.12 mg/ml in autoclaved aqueous suspension (AH), 0.06 mg/ml in sonicated aqueous suspensions (SH), and 0.60 mg/ml in autoclaved (AG) or sonicated (SG) suspensions prepared in 50 mM γCD solutions. It has been previously shown that the dexamethasone–γCD complex has a high affinity constant, which explains the observed solubilization enhancement (Jansook & Loftsson, 2008).

Table 2 summarizes the amount of dexamethasone loaded by the hydrogels after being immersed in the different suspensions. The amounts loaded by 7.14% γCD-based hydrogels were in the range of 3.8–13.1 mg/g. As expected from the drug solubility values, those hydrogels immersed in autoclaved suspensions loaded some more drug than those immersed in sonicated suspensions. However, the presence of free γCD as solubilizer agent did not facilitate the drug loading, despite the apparent enhanced drug solubility in the medium. In fact, the 7.14% γCD–PAcNa semi-IPNs loaded less drug when immersed in the suspensions containing drug–CD complexes. This finding can be attributed to that the interpenetration of PAcNa reduces the mesh size of the 7.14% γCD network, hindering the diffusion of the drug to the CD cavities of the network and of the drug–CD complexes towards to the network. It is interesting to note that this tendency reverts in the case of the 3.57% γCD-co-HPMC hydrogels, which have a higher degree of swelling and therefore a greater mesh size. For these hydrogels no relevant differences between the amount of drug loaded in the presence and in the absence of free γCD in the outer solution were observed. This means that the concentration of free drug in the outer solution determines the amount of drug that can be hosted by the γCD hydrogel. The superabsorbent HPMC alone hydrogels showed a remarkable increase in the amount of drug loaded when immersed in the suspensions containing drug–γCD complexes. HPMC hydrogels have a much higher mesh size than the γCD-based hydrogels and the drug–γCD complexes may easily diffuse inside the HPMC network. Fig. 4 schematically depicts the loading process by the hydrogels that behave more differently each other (i.e., the γCD/PAcNa semi-IPN and the HPMC network).

The amount loaded by just a simple equilibrium between the aqueous phase of the network and the loading solution, which leads the drug concentration within the hydrogel to be equal to that of the loading solution, was estimated from the degree of swelling of the hydrogels reported in Table 2 applying Eq. (5). 7.14% γCD-based hydrogels may uptake 0.4 or 0.9 mg/g when immersed in sonicated or autoclaved suspensions, respectively. 3.57% γCD-co-HPMC hydrogels can load in the aqueous phase 1–2 mg/g in the absence of γCD in the outer solution and 6–9 mg/g in the presence of γCD complexes. On the other hand, HPMC alone hydrogels load 1.5–4.5 mg/g in the absence of γCD in the outer solution and 15–25 mg/g in the presence of γCD complexes. These findings indicate that, when no γCD is present in the loading solution, the total uptake of dexamethasone by the hydrogels is at least one order of magnitude greater than the loading in their aqueous phase. Oppositely, when the loading is carried out in a medium containing γCD complexes, the total uptake is just 2–3 times greater than the amount of drug dissolved in the aqueous phase. As a whole, these results mean that the drug can establish hydrophobic interactions and form inclusion complexes with the network, but when free γCD exists in the loading medium, a competition with the CDs attached to the network is established, resulting in a less efficient loading.

To gain insight into the role of the complexation in the drug loading, the partition coefficient, K, between the polymer network and the drug loading solution was estimated from the following expression (Kim et al., 1992):

$$\text{Loading(total)} = \frac{V_s + KV_p}{W_p} C_0 \tag{6}$$

where V_p is the volume of dried polymer and the other symbols maintain the same meaning as Eq. (5). The values of K for γCD hydrogels ranked in the order SH (100–187) > AH (62–100) >> AG (1–12) ~ SG (0.1–9.7) (Fig. 5). It is clear that the presence of γCD in the loading solution remarkably decreases the dexamethasone network/water partition coefficient. This effect is less noticeable in the case of HPMC solely hydrogels due to the absence of competitive effects and to the greater mesh size of these hydrogels, compared to those also containing γCD, which enables the free movement of the dexamethasone–γCD complexes through the mesh of the network.

3.5. Dexamethasone release

Dexamethasone-loaded hydrogels were rinsed with water to remove deposits of insoluble drug particles and then immersed in the release medium. Drug release profiles are shown in Fig. 6. The γCD-based hydrogels loaded by immersion in γCD containing suspensions (AG and SG) showed a relatively rapid release; the process finishing in 12–24 h. By contrast, the γCD-based hydrogels loaded in media without γCD gave sustained drug release over a period of more than 3 days, particularly the semi-IPNs prepared with 1.43% PAcNa (2100 or 5100 Da) which controlled the release for almost 1 week. The sustained delivery displayed by the 7.14% γCD-based hydrogels and the 3.57% γCD-co-HPMC hydrogels is in agreement with the drug loading into the CD cavities of the polymer network. The time the gels sustained the delivery positively correlated with the drug affinity for the γCD-based hydrogels. The differences observed between the hydrogels loaded under distinct conditions suggest that the drug is not hosted so tightly when there is γCD in the outer solution. In media with drug–γCD complexes, the loading seems to have occurred just by

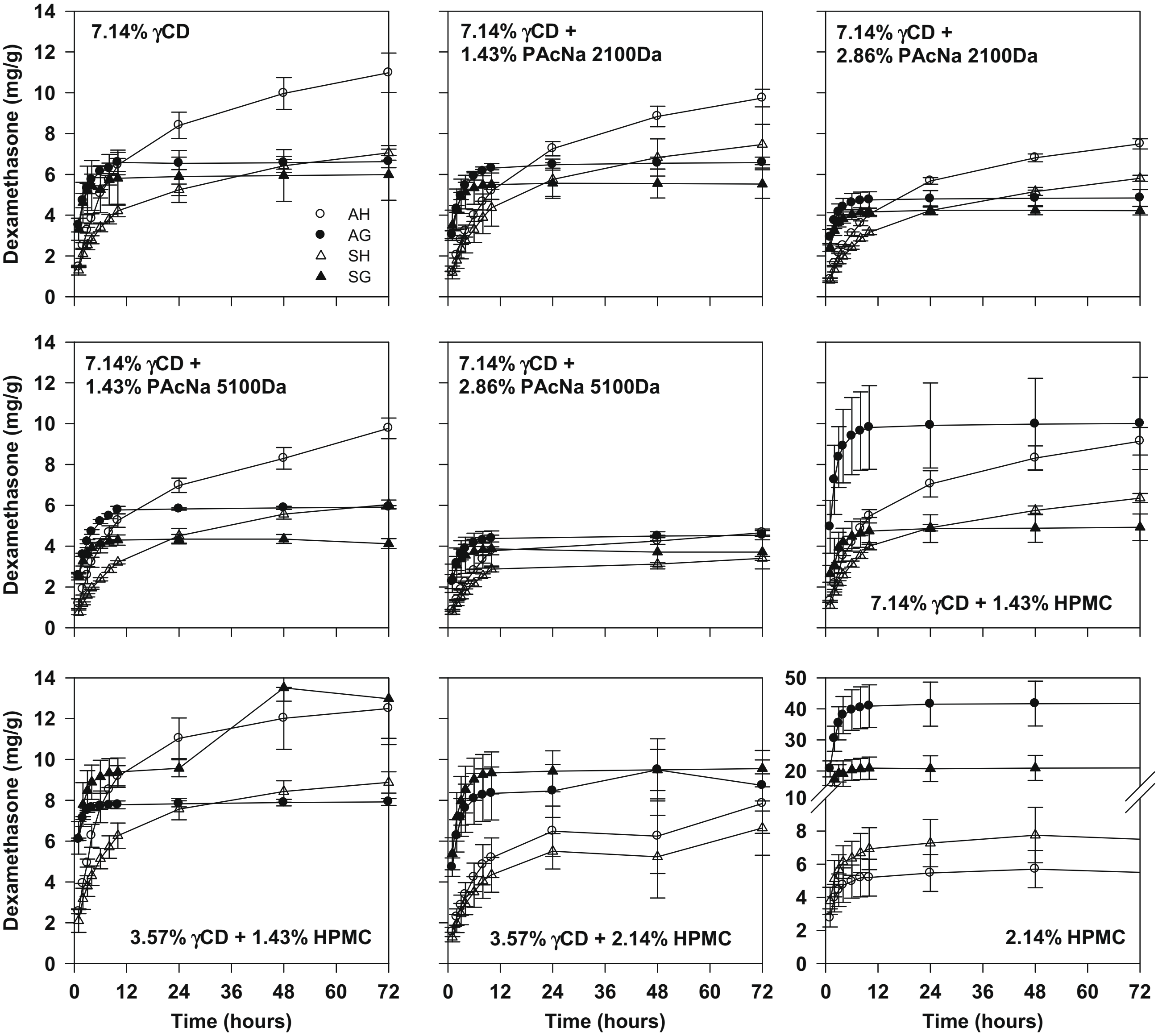

Fig. 6. Dexamethasone release from the γCD-based hydrogels and semi-IPNs that were loaded in different drug media.

diffusion of the complexes or by interaction of the drug with the more external CDs of the hydrogel. Consequently, the diffusion path of the drug molecules during release is shorter (as depicted in Fig. 4). The fact that the HPMC alone hydrogels showed a very rapid delivery confirms that the drug complexes that easily penetrated during loading also diffuse out the hydrogel without hindrance.

4. Conclusions

γCD-based networks can be obtained by direct cross-linking with diglycidylethers under mild conditions. It is possible to tune their textural properties and to enhance the bioadhesion by co-cross-linking with cellulose ethers or semi-interpenetration with poly(acrylic acid). The hydrogels showed ability to load and to sustain the release of dexamethasone owing to the affinity of the drug for the cyclodextrin units. Loading yield depends not only on the composition of the hydrogels but also on the characteristics of the loading medium. Particularly, complex formation in the hydrogel is competitively hindered by the presence of free γCD in the loading media. In sum, the procedure developed renders γCD-based hydrogels that combine a notable cytocompatibility with adequate texture and loading/release properties for being used as drug delivery vehicles.

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