Sertaconazole-Loaded Cyclodextrin–Polysaccharide Hydrogels as Antifungal Devices[§]

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Abstract: The aim of the present work was to develop novel hydrogels for delivering sertaconazole based on cyclodextrins and various biocompatible polysaccharides. Sertaconazole is an antifungal agent very effective for treatment of *Candida albicans* infections. However its poor aqueous solubility is still a challenging issue for developing suitable formulations. Complexation with cyclodextrins is a very attractive route to overcome this limitation, simultaneously enhancing its antifungal effectiveness. Hydroxypropyl-β-cyclodextrin (HPβCD) hydrogels prepared by direct cross-linking in presence of methylcellulose (MC), hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), carboxymethyl cellulose (CMCNa), or dextran were transparent and swelled in water without dissolving, which enables the formation of microenvironments very rich in cyclodextrin cavities responsible for hosting the drug and control its release rate. HPβCD hydrogels showed a high capability to load sertaconazole (with partition coefficients from 22 to 470) while still combining high water affinity (superabsorbency), versatile biomechanical properties (hardness and compressibility) and sustained release behavior (up to 4 days). Importantly, sertaconazol-loaded hydrogels showed effectiveness against *Candida albicans* in culture medium. HPβCD-polysaccharide hydrogels could be useful as sertaconazole delivery systems for the treatment of mucosal infections.

Keywords: Sertaconazole nitrate, cross-linked cyclodextrins, cellulose ether hydrogel, antifungal activity, *Candida sp*.

INTRODUCTION

Opportunistic fungal infections, particularly those caused by *Candida albicans*, are an important factor of mortality and morbidity in infants, children, and patients with compromised immune system [1-4]. Amphotericin B and azoles are currently the most used drugs to manage fungal infections [5]. Sertaconazole is an effective fungicidal and fungistatic agent and has a broad-spectrum activity against dermatophytes, opportunistic filamentous fungi, and also Gram-positive bacteria [6-8]. When used for the treatment of dermatologic and gynaecological infections, it presents a good profile of security, high cutaneous permanence and low systemic absorption [9]. Despite these valuable features, the extremely low aqueous solubility of sertaconazole (<0.01% w/v) strongly limits its practical use [10] and the search for an adequate delivery system is still a challenging issue. Complexation with β-cyclodextrin (βCD) and with hydroxypropyl-β-cyclodextrin (HPβCD) has been shown as an effective approach to enhance solubility and dissolution rate in aqueous medium [11-13]. Nevertheless, the relatively high stability constant of the sertaconazole complexes entails a high risk of drug precipitation after administration due to dilution of the complexes in the biological fluids, displacement of the equilibrium towards decomplexation, and release of the drug in the poor solvent medium [14]. Such a risk could be minimized by using cross-linked cyclodextrin hydrogels, which can swell in the biological medium without significant dilution [15, 16]. Hydrogels are outstanding patient-friendly delivery systems that enable a precise release of drugs at the desired site for a finite time. This enhances the bioavailability of the drug at the affected site of the organism with minimal systemic exposure and collateral effects [17, 18]. Current non-cyclodextrin hydrogels have been shown useful for the local treatment of dermal and mucosal infections, including *Candida albicans* [19-21]. However, their hydrophilic nature prevents an effective loading of hydrophobic drugs and usually leads to a rapid release of polar drugs [22]. In the particular case of antifungals with protonizable groups, loading has been promoted using hydrogels with oppositely charged groups, although the release rate resulted to be very dependent on drug solubility at the pH of the medium [23]. Recently, fluconazole-loaded acrylic hydrogels that showed swelling-controlled release behavior have been prepared [21]. An adequate combination of ionizable and non-ionizable monomers enabled to achieve different degrees and rates of swelling at vaginal pH and, consequently, different release rates. Cyclodextrin networks can offer novel features since they make use of an unique mechanism to control drug loading and delivery: the affinity of the drug for the cyclodextrin cavities [15, 16, 24-28]. Cyclodextrin hydrogels combine the ability of the cyclodextrins to host hydrophobic drugs with the viscoelastic behav-

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ior and high water content of hydrophilic networks intended for topical or mucosal applications [15, 29].

In last years, we have developed a new approach to prepare in one step cyclodextrin hydrogels, with or without hydroxypropyl methylcellulose (HPMC), through condensation with ethyleneglycol diglycidylether (EGDE) [15, 30]. EGDE has two epoxy groups in its structure, both of similar reactivity and able to react with the hydroxyl groups of cyclodextrins and certain polysaccharides [31, 32]. This method does not require any modification in the cyclodextrin structure and takes places in aqueous medium under mild conditions, which are two important advantages towards an environmental-friendly ("green") chemistry. In previous papers, the contents in cyclodextrin and cross-linker and the kinetics of the cross-linking process were optimized, and the ability of the hydrogels to load and sustain the release of diclofenac and estradiol was demonstrated [15, 29, 33]. The aim of the present study was to develop and characterize novel hydrogels for delivering sertaconazole based on cyclodextrins and various polysaccharides (several non-ionic cellulose ethers, one ionic cellulose derivative, and dextran) of proved biocompatibility and used for first time for this application. The incidence of the nature and proportion of the polysaccharide on the swelling and mechanical properties of the hydrogels and on sertaconazole loading and release was studied in detail. Finally, the antifungal efficiency of drug-loaded hydrogels was tested in *Candida albicans* cultures.

MATERIALS AND METHODS

Materials

Sertaconazole nitrate (nitrate salt of 7-chloro-3-[-1-(2, 4-dichlorophenyl)-2-(1H-imidazol-1-yl)-ethoxy-methyl]benzo[b]thiophene; Mw 500.78 Da) was from Ferrer Internacional (Spain). Hydroxypropyl-β-cyclodextrin (HPβCD; D.S. 4.6, Mw 1310 Da) was supplied by Jansen Pharmaceutische (Belgium). Methylcellulose (MC, Methocel® A15C Premium EP, Mw 63, 000 Da) was from Colorcon Ltd. (UK); hydroxypropylcellulose (Nisso® HPC-M, Mw 570, 000 Da) from Nippon Soda Co. (Japan); hydroxypropyl methylcellulose (HPMC, Methocel® K4M, Mw 84, 200 Da) from Dow Stade GmbH (Germany). Sodium carboxymethylcellulose sodium (CMCNa, 400-800 cPs, Mw 125, 000 Da), sodium dodecylsulfate (SDS) and dextran from *Leuconostoc mesenteroides* (Mw 100, 000-200, 000 Da) were supplied by Sigma Aldrich (USA). Ethylenglycol diglycidylether (EGDE) was from Fluka Chemie GmbH (Germany). Water purified by reverse osmosis (MilliQ®, Millipore, Spain) with a resistivity above 18.2 MΩcm^{-1} was used. All other reagents were of analytical grade.

Phase Solubility Diagrams

Dissolutions of increasing concentration in HPβCD were prepared in water or in 0.25% w/v MC, HPC, HPMC, CMCNa or dextran aqueous solutions. Aliquots of these solutions (5 ml) were placed in ampoules containing sertaconazole in excess (20-25 mg). Some of these suspensions (two replicates) were autoclaved (Raypa AES-1219, Spain) at 121ºC for 20 min. The autoclaved and non-autoclaved suspensions were shaken at 25ºC and 50 rpm until equilibrium was reached (5 days), then filtered through 0.22 μm Millipore® cellulose acetate membrane filters (Teknokroma, Spain). The concentration of the dissolved drug was measured by UV spectrophotometry (Agilent 8453, Germany) at 302 nm. The apparent stability constant of the drug–cyclodextrin complexes was calculated from the slope (*m*) of the plot drug solubility (mM) versus HPβCD concentration (mM), and from the drug solubility in absence of cyclodextrins (S_o) [34].

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

Synthesis of HPβCD Hydrogels

Different amounts of MC, HPC, HPMC, CMCNa or dextran were added to 10 ml of HPβCD solution (20% w/w) in freshly prepared 0.2 M NaOH, up to a final concentration in polysaccharide of 0.4 or 0.8%. After homogenization, EGDE (4 ml) was added to each dispersion (10 ml) and stirred for two minutes at 20ºC. The systems were immediately transferred to test tubes (10 mm internal diameter), which were hermetically closed and kept at 50ºC for 24 h. After cooling down, the hydrogels were carefully removed from the moulds and immersed in water for 12 h to swell. Then, they were placed in a 10 mM HCl solution for 12 h to neutralize the alkaline medium and immersed in water once again. Finally, cylindrical pieces of each gel (4-5 mm thickness) were cut and maintained in water.

Characterization of HPβCD Hydrogels

Swelling

Dry samples of each hydrogel were immersed in 10 ml of water and weighed at pre-established time intervals. The kinetics of medium uptake was characterized by fitting the data obtained, up to 60% of the final content in water, to the following equation:

$$\frac{W_t - W_0}{W_\infty - W_0} = K_W \times t^{0.5} \quad (2)$$

where W_0 is the weight of the dried hydrogel, W_t the weight of the hydrogel at time t after immersion in the swelling medium, W_∞ the weight of the fully swollen hydrogel, and K_w is a rate constant. The equilibrium degree of swelling was estimated as follows:

$$Q = (W_\infty - W_0)/W_0 \quad (3)$$

Biomechanical Properties

Hardness and compressibility were determined using a TA-TX Plus Texture Analyzer (Stable MicroSystems Ltd., UK) fitted with a cylindrical aluminum probe (20 mm in diameter). A hydrogel disk of 8 mm thickness was placed on the platform and the probe was compressed into the sample at a defined rate of 1 mm/s and to a defined depth of 3 mm. Then, the probe was removed at 2 mm/s and the recovery of the sample was also monitored. Three replicate analysis of each sample were performed at room temperature. 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) [35]. The modulus of deformability, ED, was estimated from the initial linear portion of the force-distance plot, converting the force to a true stress using the expression:

$$\sigma_T = \frac{F(t)[h_0 - \Delta h]}{A_0 h_0} \quad (4)$$

and the distance to Hencky´s strain as follows:

$$\varepsilon_T = \ln\left(\frac{h_0}{h_0 - \Delta h}\right) \quad (5)$$

where h_0 is the original height of sample, Δh is the change in height, $F(t)$ the compressive force at time t, and A_0 the original cross-sectional area [36].

Sertaconazole Loading

Cylindrical pieces of each hydrogel (4-5 mm thickness) were placed in vials containing aqueous suspensions of sertaconazole (50 mg in 10 ml), which were put in a bath at 25ºC and subjected to 50 oscillations per minute for one week; some being firstly autoclaved for 20 min at 121 ºC. To determine the amount loaded, some hydrogels (three replicates) were immersed in 15 ml of 0.3% w/v SDS solution that were replaced every second day, for approximately one week, and the drug concentration in the washing medium was determined spectrophotometrically at 302 nm. The amount of drug loaded was estimated as the total amount of drug released to the washing medium. The amount loaded just by a simple equilibrium between the aqueous phase of the network and the loading solution was estimated using the following equation [37]:

$$Loading\ (aqueous\ phase) = (Vs/Wp)xC_0 \quad (6)$$

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

The affinity of the drug for the network was estimated as the partition coefficient, $K_{N/W}$, between the polymeric networks and the drug loading solution, as follows [37]:

$$Loading\ (total) = [(Vs + K_{N/W} Vp)/Wp]xC_0 \quad (7)$$

where Vp is the volume of dried polymer and the other symbols maintain the meaning of Eq. 6. The density of the dried hydrogels was assumed to be 1 g/ml.

Sertaconazole Release

Drug-loaded hydrogels were rinsed with water and immersed in 0.3% w/v SDS solution (30 ml, to ensure *sink* conditions) at room temperature. The drug concentration was measured spectrophotometrically in periodically taken samples and again placed in the same vessel, so that the liquid volume was kept constant. The experiments were carried out in triplicate. Once the test was finalized, the disks were weighed and they dried up to 50˚C (Heraeus stove, Spain) until constant weight.

Antifungal Activity

The capability of sertaconazole to inhibit the growth of *Candida albicans* was analyzed in liquid YPD medium (peptone, yeast extract, and dextrose at 2% each). 0.75 ml of a fungal preinoculate in stationary phase of growth were added to YPD medium and the growth of *Candida albicans* was followed through the changes in absorbance at 600 nm. Sertaconazole-loaded hydrogels were placed in 15 ml of the *Candida albicans* culture in exponential phase of growth and the systems were maintained under stirring (180 rpm) at 30ºC. The absorbance at 600 nm was periodically measured. The experiments were carried out in quadruplicate. Hydrogel disks without sertaconazole were used as controls. The percentage of growth was considered as the quotient of the absorbance registered for the medium to which sertaconazole-loaded hydrogels was added and for the medium containing *Candida albicans* without hydrogel.

RESULTS AND DISCUSSION

Dextran and four cellulose ethers with different substituents (MC, HPC, HPMC, and CMCNa) were chosen as components of HPβCD hydrogels due to their known hydrophilic character and biocompatibility [38, 39]. In addition, these polysaccharides alone have been shown to provide hydrogels with tuneable mechanical properties and potential as platforms for drug delivery [32, 40-42]. Complexation ability and, consequently, drug solubilizing efficiency are usually enhanced in the presence of hydrophilic polymers and by heating processes [34, 43]. Consequently, the effect of the chosen polysaccharides and of autoclaving on the complexation constant of sertaconazole with HPβCD was evaluated before preparing the hydrogels. The information obtained in this first step is relevant for understanding the loading/release behavior of the hydrogels [33].

Phase Solubility Diagrams

Sertaconazole solubility was notably enhanced in the presence of HPβCD, reaching up to 3 mM in 40 mM HPβCD (Fig. **1**). All diagrams were A_L type, which indicates the formation of 1:1 molar ratio complexes [34]. Table **1** shows the solubility values in solutions containing 1% (7.6 mM) HPβCD and 0.25% polysaccharide; 8-fold increase in sertaconazole solubility was observed in the HPβCD solution. The addition of CMCNa or MC slightly decreased the solubilization ability and the affinity constant, particularly when the HPβCD was above 10 mM and the systems were autoclaved. It is known that cellulose ethers can undergo precipitation or a sol to gel transitions when temperature rises; the polymer dehydrates and hydrophobic interactions among the cellulose backbones are promoted [39]. Such an increase in hydrophobicity can lead to a competition of the polymer with the drug for the cyclodextrin cavity, resulting in fewer cavities available for solubilizing sertaconazole.

Synthesis of HPβCD-Based Hydrogels

EGDE can act as a non-toxic cross-linker of cyclodextrins and polysaccharides forming ether bonds with hydroxyl groups; the reaction being catalyzed by OH^- ions and temperature [31-33]. We have observed that 0.2 M NaOH and 50 ºC are suitable conditions for obtaining hydrogels without compromising the stability of the cyclodextrins and HPMC [15]. Thus, these conditions were maintained to prepare the novel HPβCD-polysaccharide hydrogels. Most glycidylether groups of EGDE are consumed in the reaction and, if any still remains in the hydrogel, the washing with 0.01M HCl aq. medium opens the rings to give hydroxyl groups [44].

The reactivity of the hydroxyl groups of HPβCD as well as those of cellulose ethers is greater for those at C2 and C6. In the case of dextran (α-D-1,6-glucose-linked glucan with

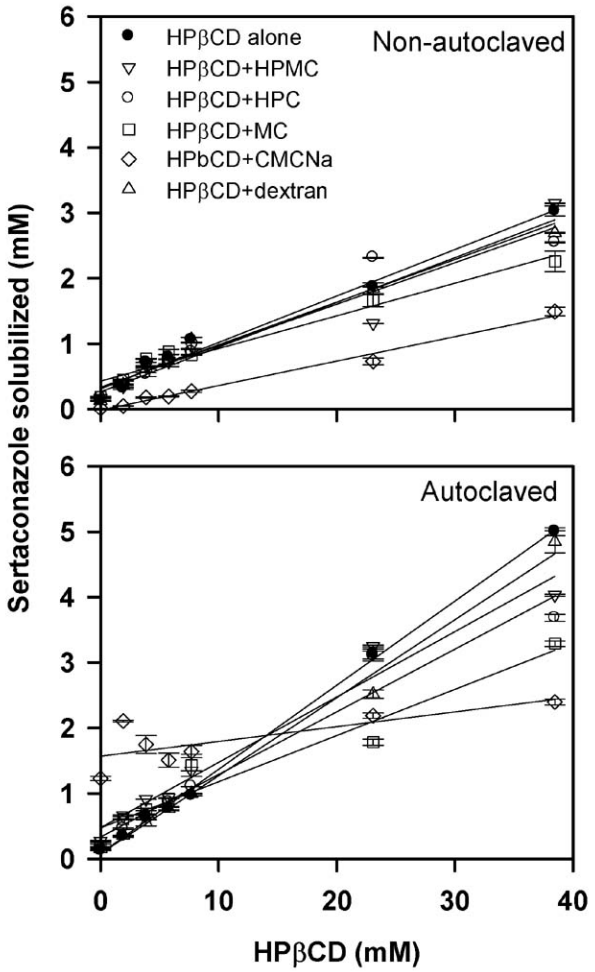

Fig. (1). Phase solubility diagrams of sertaconazole in aqueous solutions of HPβCD, in the presence or absence of 0.25% HPMC, HPC, MC, CMCNa or dextran.

Table 1. Sertaconazole Solubility in Media Prepared with 1% HPβCD and 0.25% of Various Polysaccharides, and Affinity Constants of Sertaconazole: HPβCD Complexes Before and After Being Autoclaved

System	Non-Autoclaved		Autoclaved	
	Solubility (mM)	K_{11} (M^{-1})	Solubility (mM)	K_{11} (M^{-1})
HP-βCD	1.06	610	0.96	1174
HP-βCD + 0.25% HPMC	0.97	587	1.35	887
HP-βCD + 0.25% MC	0.83	419	1.43	606
HP-βCD + 0.25% CMCNa	0.27	311	1.64	184
HP-βCD + 0.25% HPC	0.88	557	1.10	841
HP-βCD + 0.25% dextran	1.06	542	0.99	1082

side-chains 1-3 linked to the backbone units) the hydroxyls at C6 are occupied forming ether bonds among the glucose units to form the backbone, whereas some hydroxyls at C3 (around 5%) are substituted with branches of 1-2 glucose units long. The hydroxyl at C2 of the backbone as well as the hydroxyl at C2 and C6 of the branches can react with EGDE. The amount of EGDE added was sufficient to react with at least two thirds of all hydroxyls groups (of HPβCD and polysaccharide) present in the reaction medium. The polysaccharides were added to 20% HPβCD solutions up to 0.4 or 0.8%; the final concentration of polysaccharides in the pregel solution (after adding EGDE) being 0.29% or 0.57%, respectively. These concentrations were chosen to be below the critical entanglement concentration (i.e., ten-times the reciprocal value of the intrinsic viscosity reported in [45]) in order to obtain low viscosity solutions (before cross-linking) of individualized polysaccharide chains, avoiding inter- and intra-chain cross-linking and increasing the likelihood of an even distribution of both the polysaccharide and the HPβCD in the hydrogels. In fact, all hydrogels were viscoelastic and transparent and showed smooth and continuous surfaces.

Hydrogel Swelling

All hydrogels exhibited a fast swelling in the first 2 hours of the test, after which, the process slowed down until the equilibrium was reached approximately in 7 hours. Fig. (**2**) shows the swelling process of the HPβCD, HPβCD/MC and HPβCD/dextran hydrogels; the behavior of the other hydrogels being similar. Although the presence of the polysaccharide reduced the degree of swelling, all hydrogels took up high amounts of water and can be considered as superabsorbents (Table **2**). Hydrogels containing MC, CMCNa or HPC were the ones with the lowest degree of swelling, which can be attributed to the concomitance of two effects: i) a less hydrophilic character compared to HPβCD and ii) a higher degree of cross-linking due to an easier reaction of EGDE with the unsubstituted hydroxyl groups of cellulose.

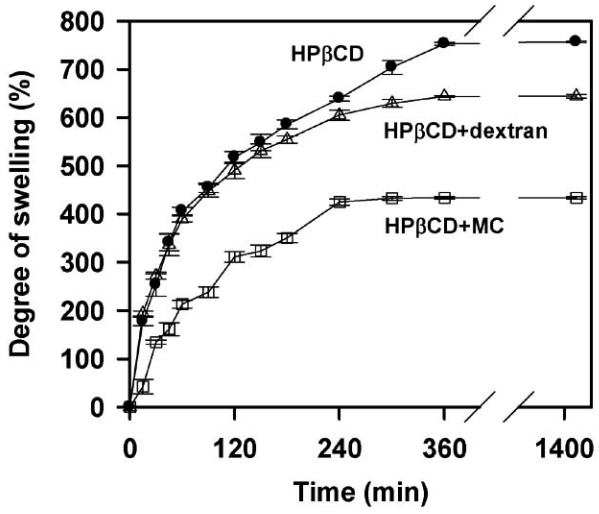

Fig. (2). Degree of swelling HPβCD hydrogels prepared without polysaccharides or with a 0.4% methylcellulose (MC) or dextran.

The swelling profiles fitted well to the square root kinetics, which implies that the water mainly penetrates in hydrogel by Fickian diffusion. The sorption rate constants (Table **2**) indicate that the water molecules can easily diffuse through the hydrogels.

Table 2. Degree of Swelling, Swelling Rate (r^2 >0.95), and Biomechanical Properties of HPβCD-Polysaccharide Hydrogels; Mean Values (Standard Deviations)

Hydrogel	Degree of Swelling (%)	Kw ($min^{-1/2}$)	Hardness (N)	Compressibility (N·mm)	Modulus of Deformability (ED; kPa)
HPβCD	765 (5)	0.048	4.22 (0.09)	3.1 (0.4)	35.5 (0.1)
HPβCD + HPMC 0.4%	710 (13)	0.048	4.29 (0.16)	3.9 (0.3)	43.8 (2.1)
HPβCD + HPMC 0.8%	664 (10)	0.050	5.87 (0.17)	5.0 (0.4)	57.0 (1.9)
HPβCD + MC 0.4%	433 (6)	0.061	9.33 (0.33)	7.8 (0.6)	115.0 (1.5)
HPβCD + MC 0.8%	514 (13)	0.062	3.31 (0.26)	2.5 (0.1)	49.5 (4.8)
HPβCD + CMCNa 0.4%	458 (13)	0.065	6.52 (0.36)	6.5 (0.8)	86.8 (1.9)
HPβCD + CMCNa 0.8%	456 (30)	0.071	9.38 (0.34)	8.2 (0.8)	110.0 (10.3)
HPβCD + HPC 0.4%	460 (6)	0.078	9.24 (0.78)	8.7 (0.5)	110.1 (10.3)
HPβCD + HPC 0.8%	417 (29)	0.071	5.82 (0.57)	4.2 (0.3)	89.1 (7.5)
HPβCD + Dextran 0.4%	644 (7)	0.049	3.97 (0.13)	4.3 (0.3)	45.5 (3.2)
HPβCD + Dextran 0.8%	568 (6)	0.048	4.7 (0.28)	3.7 (0.2)	59.3 (4.3)

Biomechanical Properties

Typical compression plots obtained for the water-swollen HPβCD hydrogels are shown in Fig. (**3**). In all systems, the force-distance curve registered during application of the force was almost superimposable to that obtained during the removal of the probe (i.e. recovery). The hardness and compressibility of hydrogels prepared with HPMC or dextran were similar to those of the HPβCD sole hydrogel (Table **2**) while the addition of other polysaccharides caused in general an increase in these parameters, which confirms the hypothesis of a greater effective cross-linking density in HPβCD/MC, HPβCD/CMCNa and HPβCD/HPC hydrogels. The HPβCD-based hydrogels are viscoelastic rather than purely elastic and, consequently, the Young's modulus cannot be strictly calculated from the slope of the force-distance plot [35, 36]. The cross-sectional area and length of the HPβCD-based hydrogel disks do change substantially while loads are applied, and the equations developed for extensional rheometry assuming incompressibility are not valid since the engineering stress ceases to be an accurate measure [46]. Therefore, the modulus of deformability, ED, was estimated 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 [47]. ED is an index of the specimen stiffness and has been widely used for characterizing hydrogels and soft materials of varied nature [36]. It is interesting to note that hydrogels prepared with 0.4% MC, 0.4% HPC or 0.8% CMCNa were particularly stiff. In the case of these two non-ionic cellulose ethers, 0.4% caused an increase in the consistency of the hydrogels but 0.8% decreased again the consistency. This is due to that the presence of a moderate proportion of long cross-linkable chains increases the yield of the cross-linking between the cyclodextrins and cellulose chains and, consequently, a more rigid network is obtained. By contrast, greater proportions of long cellulose chains make the network able to deform to a great extent. This is because cyclodextrins become diluted among the cellulose chains and the likelihood of that EGDE reacts only with cellulose chains increases. Conformational changes of cross-linked cellulose chains are easier than in the case of the rigid HPβCD toroids, and thus relatively high proportions of MC or HPC enhance the capability of the hydrogels to deform under stress. Oppositely, the greater the content in CMCNa, the greater the stiffness of the hydrogels was. This finding should be related to electrostatic repulsions and osmotic effects caused by the ionic cellulose chains, which decrease the freedom of movement of the network. Thus, the incorporation of polysaccharide, even at low proportions, strongly determines the mechanical behavior of HPβCD-based hydrogels; the effect being very dependent on the structure and ionic nature of the polysaccharide chains. Nevertheless, all hydrogels evaluated showed ED values in the range of data previously found for other hydrogels and had the consistency required to be easily handled without risk of disintegration, but were also deformable enough to be mechanically biocompatible [29, 48].

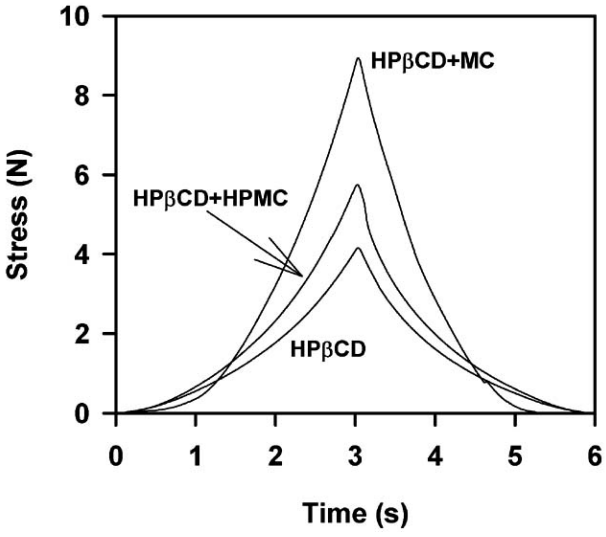

Fig. (3). Force-displacement curves for swollen HPβCD hydrogels prepared with 0.8% HPMC or 0.4% MC.

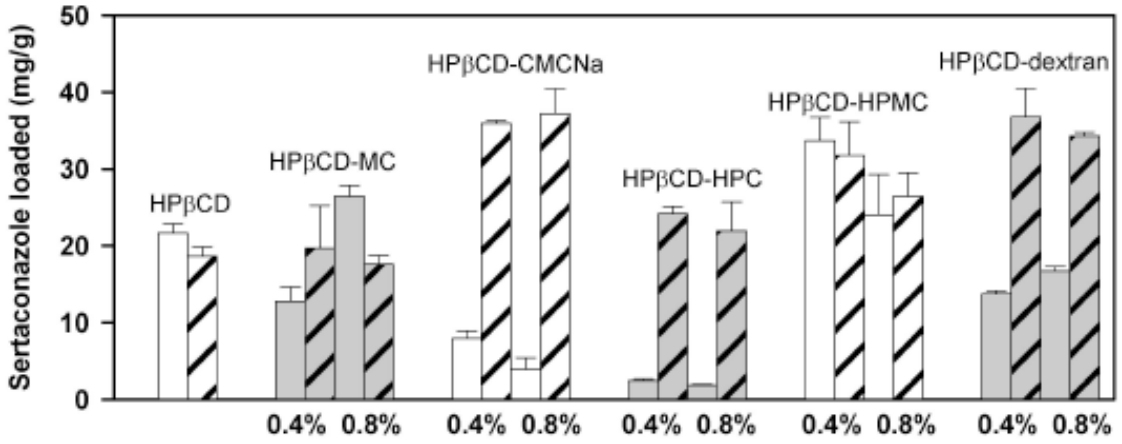

Fig. (4). Amounts of sertaconazole loaded by HPβCD hydrogels prepared without polysaccharides or with MC, CMCNa, HPC, HPMC or dextran at 0.4 or 0.8%. Columns with oblique-stripe fills identify hydrogels autoclaved during loading.

Loading and Release of Sertaconazole

Disks of each hydrogel were immersed in sertaconazole suspensions and some replicates were autoclaved to evaluate the effect of this thermal treatment on the loading capability. The hydrogels withstood this sterilization treatment without damage. Fig. (**4**) shows the amounts of sertaconazole loaded by each hydrogel, with or without applying autoclaving. HPβCD hydrogels loaded 21.7 mg/g and, when autoclaved, 18.7 mg/g. Hydrogels containing MC or HPMC loaded similar amounts or even greater. By contrast, non-autoclaved hydrogels made with HPC, CMCNa or dextran showed a significantly lower loading capability. Once autoclaved, HPC hydrogels reached similar values to those obtained for HPβCD sole hydrogels. In the case of CMCNa or dextran hydrogels, autoclaving enhanced so much the loading that these hydrogels become the ones with the greatest loading capability.

Sertaconazole can be loaded by diffusion into the inner aqueous phase of the hydrogel and by complexation with the cyclodextrin cavities. When the equilibrium is reached, the drug concentration should be the same in the aqueous phase of hydrogel as in the surrounding solution. Therefore, in the absence of other loading mechanisms, the greater the swelling of the hydrogels, the highest the loading in the aqueous phase [37]. Taking into account the aqueous solubility of sertaconazole (0.079 mg/ml), the maximum loading in the aqueous phase is 0.06-0.07 mg/g of hydrogel. These values are remarkably lower than any amount shown in Fig. (**4**). This means that most drug loaded by the network is interacting with its structural components, mainly HPβCD. In the case of HPβCD sole hydrogels, there are ca. 7 cyclodextrin units available per molecule of drug loaded, which means that the data shown in Fig. (**4**) are still far from saturation levels.

The affinity of the drug for the network was estimated as the partition coefficient, $K_{N/W}$, between the polymeric networks and the drug loading solution (Eq. 7) and resulted to be 273 and 235 for the HPβCD sole hydrogel before and after autoclaving. The HPβCD-polysaccharide hydrogels had $K_{N/W}$ values ranging from 22-30 for non-autoclaved HPβCD-HPC hydrogels to 454-470 for autoclave HPβCD-CMCNa hydrogels. This means that the hydrogels have a very remarkable affinity for the drug.

HPβCD-polysaccharide hydrogels have a 4% lower content in cyclodextrin than HPβCD sole hydrogels. This different content did not explain the notable differences observed in the amount of sertaconazole loaded by the different polysaccharide hydrogels. Hydrophobic sorption of drugs to MC and HPMC has been previously reported [15, 49]. Thus the hydrogels prepared with these polysaccharides loaded similar amounts to the HPβCD ones and showed similar $K_{N/W}$ values. On the other hand, CMCNa, HPC and dextran are more hydrophilic and this may create a barrier for the unspecific hydrophobic sorption of sertaconazole. Such a barrier can be overcome when autoclaving is applied, because of a temporal increase of the solubility of the drug in the aqueous medium that facilitates the complexation with the HPβCD units, which is the main driving force for the loading.

Sertaconazole release profiles from different hydrogels showed the influence of the polysaccharide used in their preparation as well as of autoclaving during the loading (Fig. **5**). All hydrogels showed a relatively fast delivery of drug in the first 24 hours, followed by a more sustained release step up to 4 days. It is important to note that the release studies were carried out under *sink* conditions in SDS micellar medium (as recommended for hydrophobic drugs) and, therefore, drug solubility is not a limiting step in the release profiles recorded. Sertaconazole powder dissolves in few minutes in SDS medium. Thus the sustained delivery is related to the capability of cyclodextrin cavities to retain the drug in the network, as previously reported for cyclodextrin-based hydrogels loaded with the hydrophobic hormone estradiol [33]. Decomplexation of a sertaconazole molecule from one cavity makes it available to complexate with a neighbour empty cavity; the likelihood of recomplexation being also dependent on the drug/cyclodextrin affinity. Therefore, drug movement through a hydrogel network dotted with many dimples can be envisioned as escaping a dimple to fall dawn in another one, which should be abandoned, and so on, up to reach hydrogel surface. The movement of a drug molecule may be faster when most dimples are occupied and the likelihood of recomplexation is less. Oppositely, as the hydrogel delivers the drug, the number of empty cyclodextrin cavities that are available for hosting the just-passing-through drug

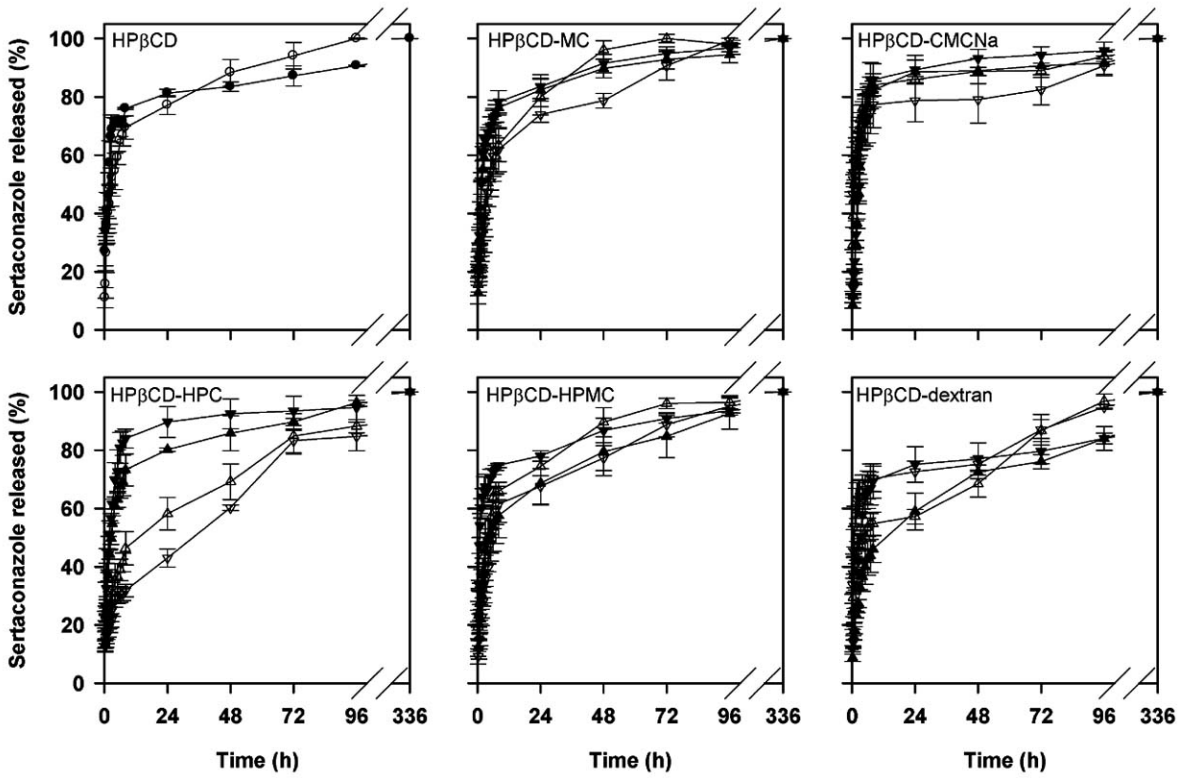

Fig. (5). Sertaconazole release profiles from HPβCD hydrogels prepared without polysaccharides or with MC, CMCNa, HPC, HPMC or dextran at 0.4% (up triangles) or 0.8% (down triangles). Full symbols correspond to hydrogels that were autoclaved during loading.

molecules increases. Furthermore, some previously released drug molecules could be attracted again towards the network. In the case of hydrogels containing polysaccharides, an increase in the cross-linking density (compared to HPβCD sole hydrogels) leads to an increase of tortuosity and to a minor mesh size which can also contribute to make drug release difficult. All these factors clearly explain that the non-autoclaved HPβCD/HPC hydrogels, which loaded the lower dose of drug, were those with the slowest release rate.

The amount of drug loaded by the HPβCD hydrogels, ca. 2%, is close to the content in drug of commercially available pharmaceutical creams, powders and solutions. As shown in Fig. (**5**), such content in sertaconazole is notably enhanced in HPβCD/HPMC hydrogels and in autoclaved HPβCD/CMCNa and HPβCD/dextran hydrogels. Taking into account the weight of the hydrogel disks prepared (~ 70 mg) and the antifungal activity of sertaconazole against *Candida albicans* (MIC_{50} = 0.07 mg/l; MIC = 0.63 mg/l), each HPβCD hydrogel disk contains sertaconazole enough to decrease the population of fungi to the half if immersed in 20 litres of medium, and to kill all fungi in a volume of 2 litres. Thus, sertaconazole-loaded disks when enter in contact with the small physiological volumes at the common sites of *Candida sp.* infections (e.g. vagina or mouth) should be adequate to efficiently treat this type of infections. The versatility of the hydrogels is increased by the fact that the size of the disks can be fixed at will to adjust the dose to specific requirements.

Antifungal Activity

The antifungal effectiveness of the sertaconazole-loaded hydrogels was verified using *Candida albicans* cultures in exponential phase of growth. Sertaconazole inhibits the ergosterol synthesis and, consequently, alters the cellular membrane formation causing the killing of the fungi [7]. Hydrogels without sertaconazole were used as controls in order to evaluate the direct effect of the cross-linked networks on the growth of *Candida albicans*. As can be observed in Fig. (**6**, dark grey columns), the networks themselves only caused a minor decrease in the growth rate. As a positive control, the same amount of drug (40 mg) as the maximum dose loaded by the hydrogel disks was dispersed in the culture medium (white column with horizontal lines, in Fig. (**6**)). Such a suspension diminished the *Candida* population up to a 50% in 24h. Sertaconazole-loaded hydrogels also significantly decreased the growth rate of *Candida albicans*. The inhibitory effect was particularly relevant at 24 h for hydrogels prepared with HPβCD sole or combined with MC or CMCNa, which killed the fungi as efficiently as the drug suspension despite of being loaded with lower amount of sertaconazole. Despite the culture medium is not as good solvent as the SDS micellar solutions used for the release experiments, hydrogels prepared with HPβCD sole or combined with MC or CMCNa are expected to deliver the drug faster than the others. Those hydrogels that sustained more the release, mainly prepared with HPC or HPMC, required more time to begin to evidence the antifungal effect.

CONCLUSIONS

Versatile cyclodextrin-polysaccharide hydrogels with tuneable biomechanical properties and capability to load sertaconazole and to regulate its release rate were obtained in a single-step using EGDE as cross-linker. The nature and proportion of the polysaccharide components play an impor-

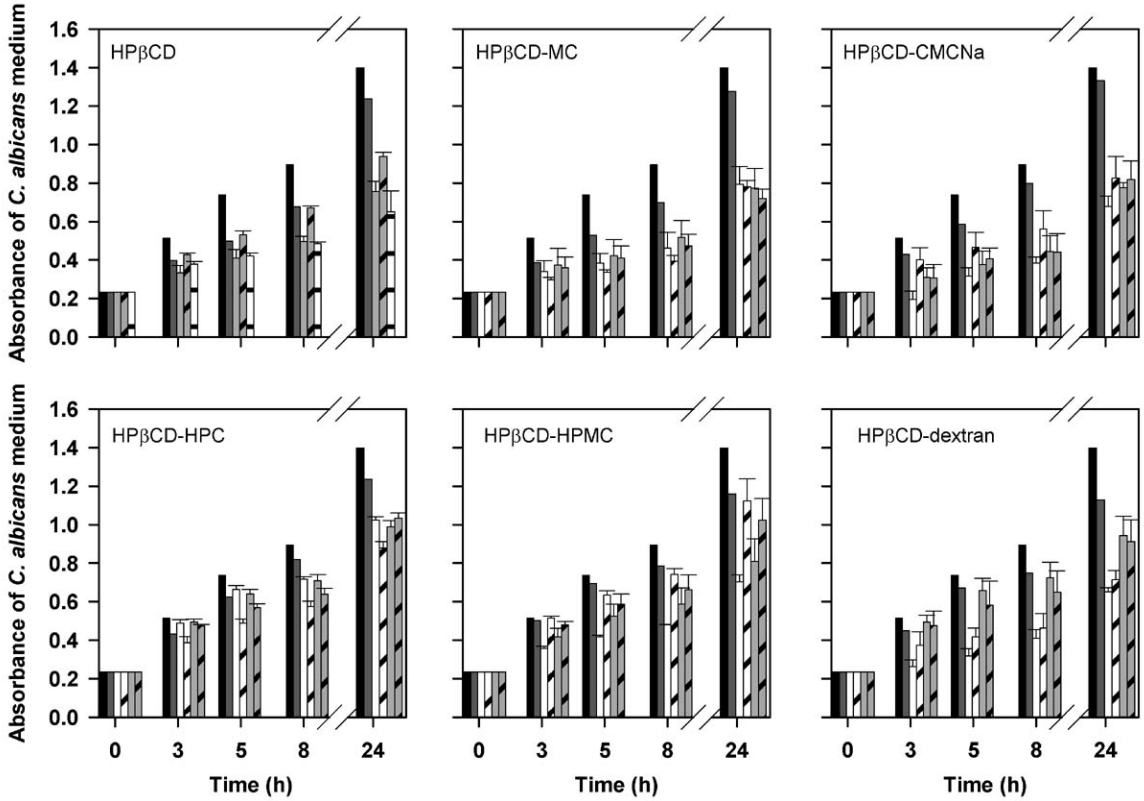

Fig. (6). *Candida albicans* growth in control medium (black column), in the presence of non-loaded hydrogel (dark grey column), or in the presence of sertaconazole loaded hydrogels. Upper left plot shows the effect of sertaconazole-loaded HPβCD hydrogels prepared without polysaccharides (grey columns) and of a drug suspension prepared with the maximum dose loaded by the hydrogels (white column with horizontal lines). The rest of the plots show the effect of sertaconazole-loaded HPβCD hydrogels prepared with different proportions of polysaccharides (0.4% white columns or 0.8% grey columns). Columns with oblique-stripe fills identify hydrogels autoclaved during loading.

tant role in the performance of the hydrogels. Sertaconazole loading is mainly driven by the drug affinity for cyclodextrin units. Autoclaving is also revealed as a key factor to facilitates the complexation and thus to regulate the delivery of the drug release. These hydrogels are capable of loading therapeutic doses of sertaconazole, to control its release for more than 24 hours and to provide proper antifungal activity against *Candida albicans*. The beginning of action was faster for those hydrogels that loaded more drug and released it faster. Therefore, the new cyclodextrin-polysaccharide hydrogels have a great potential as efficient carriers of antifungal drugs to be applied topically or on mucosa.

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REFERENCES

[1] Pfaller, M.A. Epidemiology of candidiasis. *J. Hosp. Infect.*, **1995**, *30*, 329-338.

[2] Dixon, D.M.; McNeil, M.M.; Cohen, M.L.; Gelling, B.G.; La Montane, J.R. Fungal infections: a growing threat. *Public. Health Rep.*, **1996**, *11*, 726-735.

[3] Hollenbach, E. Invasive candidiasis in the ICU: evidence based and on the edge of evidence. *Mycoses*, **2008**, *51*(Suppl. 2), 25-45.

[4] Marodi, L.; Johnston, R.B. Invasive Candida species disease in infants and children: occurrence, risk factors, management, and innate host defense mechanisms. *Curr. Opin. Pediatr.*, **2007**, *19*, 693-697.

[5] Zaoutis, T.; Walsch, T.J. Antifungal therapy for neonatal candidiasis. *Curr. Opin. Infect. Dis.*, **2007**, *20*, 592-597.

[6] Agut, J.; Palacín, C.; Sacristán A.; Ortiz, J.A. Inhibition of ergosterol synthesis by Sertaconazole in *Candida albicans*. *Arzneimittel Forsch*, **1992**, *42*, 718-720.

[7] Agut, J.; Palacín, C.; Salgado, J.; Casas, E.; Sacristán, A.; Ortiz, J.A. Direct membrane-damaging effect of Sertaconazole on *Candida albicans* as a mechanism of its fungicidal activity. *Arzneimittel Forsch* **1992**, *42*, 721-724.

[8] Carrillo-Muñoz, A.J.; Quindós, G.; Del Valle, O.; Santos, P.; Giusiano, G.; Guardia, C.; Eraso, E.; Ezkurra, P.A.; Tur-Tur, C.; Hernández-Molina, J.M. *In vitro* antifungal activity of sertaconazole nitrate against recent isolates of onychomycosis causative agents. *J. Chemother.*, **2008**, *20*, 521-523.

[9] Palacín, C.; Tarragó, C.; Ortíz, J.A. Sertaconazole: pharmacology of a gynecological antifungal agent. *Int. J. Gynaecol. Obstet.*, **2000**, *71*, 37-76.

[10] Albert, C.; Fernández, J.M.; Sacristán, A.; Ortiz, J.A. Physicochemical properties, analytical determinations and stability of sertaconazole nitrate. *Arzneimittel. Forsch*, **1992**, *42*, 695-698.

[11] Perdomo-López, I.; Rodríguez-Pérez, A.I.; Yzquierdo-Peiró, J.M.; White, A.; Estrada, E.G.; Villa, T.G.; Torres-Labandeira, J.J. Effect of cyclodextrins on the solubility and antimycotic activity of sertaconazole: Experimental and computational studies. *J. Pharm. Sci.*, **2002**, *91*, 2408-2415.

[12] Rodriguez-Perez, A.I.; Rodriguez-Tenreiro, C.; Alvarez-Lorenzo, C.; Taboada, P.; Concheiro, A.; Torres-Labandeira, J.J. Sertacona-

zole/hydroxypropyl-beta-cyclodextrin complexation: isothermal titration calorimetry and solubility approaches. *J. Pharm. Sci.*, **2006**, *95*, 1751-1762.

[13] Rodriguez-Perez, A.I.; Rodriguez-Tenreiro, C.; Alvarez-Lorenzo, C.; Concheiro, A.; Torres-Labandeira, J.J. Drug solubilization and delivery from cyclodextrin-pluronic aggregates. *J. Nanosci. Nanotechnol.*, **2006**, *6*, 3179-3186.

[14] Stella, V.J.; Rao, V.M.; Zannou, E.A.; Zia, V. Mechanisms of drug release from cyclodextrin complexes. *Adv. Drug Deliv. Rev.*, **1999**, *36*, 3-16.

[15] Rodriguez-Tenreiro, C.; Alvarez-Lorenzo, C.; Rodriguez-Perez, A.; Concheiro, A.; Torres- Labandeira, J.J. New cyclodextrin hydrogels cross-linked with diglycidylethers with a high drug loading and controlled release ability. *Pharm. Res.*, **2006**, *23*, 121-130.

[16] Siemoneit, U.; Schmitt, C.; Alvarez-Lorenzo, C.; Luzardo, A.; Otero-Espinar, F.; Concheiro, A.; Blanco-Méndez, J. Acrylic/cyclodextrin hydrogels with enhanced drug loading and sustained release capability. *Int. J. Pharm.*, **2006**, *312*, 66-74.

[17] Peppas, N.A.; Bures, P.; Leobandung, W.; Ichikawa, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.*, **2000**, *50*, 27-46.

[18] Hoare, T.R.; Kohane, D.S. Hydrogels in drug delivery: Progress and challenges. *Polymer*, **2008**, *49*, 1993-2007.

[19] Woolfson, A.D.; Malcolm, R.K.; Gallagher, R. Drug delivery by the intravaginal route. *Crit. Rev. Ther. Drug Carrier Syst.*, **2000**, *17*, 509-555.

[20] Zumbuehl, A.; Ferreira, L.; Kuhn, D.; Astashkina, A.; Long, L.; Yeo, Y.; Iaconis, T.; Ghannoum, M.; Fink, G.R.; Langer, R.; Kohane, D.S. Antifungal hydrogels. *Proc. Natl. Acad. Sci. USA*, **2007**, *104*, 12994-12998.

[21] Pulat, M.; Eksi, H.; Abbasoglu, U. Fluconazole release from hydrogels inclusing acrylamide-acrylic acid-itaconic acid, and their microbiological interactions. *J. Biomater. Sci. Polym. Edn.*, **2008**, *19*, 193-205.

[22] Alvarez-Lorenzo, C.; Hiratani, H.; Concheiro, A. Contact lenses for drug delivery achieving sustained release with novel systems. *Am. J. Drug Deliv.*, **2006**, *4*, 131-151.

[23] Sen, M.; Yakar, A. Controlled release of antifungal drug terbinafine hydrochloride from poly(N-vinyl 2-pyrrolidone/itaconic acid) hydrogels. *Int. J. Pharm.*, **2001**, *228*, 33-41.

[24] Asanuma, H.; Hishiya, T.; Komiyama, M. Efficient separation of hydrophobic molecules by molecularly imprinted cyclodextrin polymers. *J. Ind. Phenom.*, **2004**, *50*, 51-55.

[25] Crini, G.; Morcellet, M. Synthesis and applications of adsorbents containing cyclodextrins. *J. Sep. Sci.*, **2002**, *25*, 789-813.

[26] Liu, Y.Y.; Fan, X.D. Synthesis, properties and controlled release behaviors of hydrogel networks using cyclodextrin as pendant groups. *Biomaterials*, **2005**, *26*, 6367-6374.

[27] Davis, M.E.; Brewster, M.E. Cyclodextrin-based pharmaceutics: past, present and future. *Nat. Rev. Drug Discov.*, **2004**, *3*, 1023-1035.

[28] Rosa dos Santos, J.F.; Couceiro, R.; Concheiro, A.; Torres-Labandeira, J.J.; Alvarez-Lorenzo, C. Poly(hydroxyethylmethacrylate-co-methacrylate-β-cyclodextrin) hydrogels: synthesis, cytocompatibility, mechanical properties and drug loading/release properties. *Acta Biomater.*, **2008**, *4*, 745-755.

[29] Rodriguez-Tenreiro, C.; Diez-Bueno, L.; Concheiro, A.; Torres-Labandeira, J.J.; Alvarez-Lorenzo, C. Cyclodextrin/carbopol micro-scale interpenetrating networks (ms-IPNs) for drug delivery. *J. Control Release*, **2007**, *123*, 56-66.

[30] Alvarez-Lorenzo, C.; Rodriguez-Tenreiro, C.; Torres- Labandeira, J.J.; Concheiro, A. Method of obtaining hydrogels of cyclodextrins with glycidyl ethers, compositions thus obtained and applications thereof. PCT/ES2006/000096, WO2006-089993 A2, March **2006**.

[31] Yui, N.; Okano, T.; Sakurai, Y. Inflammation responsive degradation of crosslinked hyaluronic acid gels. *J. Control Release*, **1992**, *22*, 105-116.

[32] Rodriguez, R.; Alvarez-Lorenzo, C.; Concheiro, A. Cationic cellulose hydrogels: kinetics of the cross-linking process and characterization as pH-ion-sensitive drug delivery systems. *J. Control Release*, **2003**, *86*, 253-265.

[33] Rodriguez-Tenreiro, C.; Alvarez-Lorenzo, C.; Rodriguez-Perez, A.; Concheiro, A.; Torres-Labandeira, J.J. Estradiol sustained release from high affinity cyclodextrin hydrogels. *Eur. J. Pharm. Biopharm.*, **2007**, *66*, 55-62.

[34] Brewster, M.E.; Loftsson, T. Cyclodextrins as pharmaceutical solubilizers. *Adv. Drug Deliv. Rev.*, **2007**, *59*, 645-666.

[35] Andrews, G.P.; Jones, D.S. Rheological characterization of bioadhesive binary polymeric systems designed as platforms for drug delivery implants. *Biomacromolecules*, **2006**, *7*, 899-906.

[36] Konstance, R.P. Axial-compression properties of calcium caseinate gels. *J. Dairy Sci.*, **1993**, *76*, 3317-3326.

[37] Kim, S.W.; Bae, Y.H.; Okano, T. Hydrogels-swelling, drug loading and release. *Pharm. Res.*, **1992**, *9*, 283-290.

[38] Coviello, T.; Matricardi, P.; Marianecci, C.; Alhaique, F. Polysaccharide hydrogels for modified release formulations. *J. Control Release*, **2007**, *119*, 5-24.

[39] Doelker, E. Cellulose derivatives. *Adv. Polym. Sci.*, **1993**, *107*, 200-265.

[40] Anbergen, U.; Oppermann, W. Elasticity and swelling behavior of chemically cellulose ethers in aqueous systems. *Polymer*, **1990**, *31*, 1854-1889.

[41] Brondsted, H.; Andersen, C.; Hovgaard, L. Crosslinked dextran- a new capsule material for colon targeting of drugs. *J. Control Release*, **1998**, *53*, 7-13.

[42] Favre, E.; Girard, S. Release kinetics of low molecular weight solutes from mixed cellulose ethers hydrogels: a critical experimental study. *Eur. Polym. J.*, **2001**, *37*, 1527-1532.

[43] Nakai, Y.; Yamamoto, K.; Terada, K.; Watanabe, D. New methods for preparing cyclodextrin inclusion compounds. I. Heating in a sealed container. *Chem. Pharm. Bull.*, **1987**, *35*, 4609-4615.

[44] Huang, L.L.H.; Lee, P.C.; Chen, L.W.; Hsieh, K.H. Comparison of epoxides on grafting collagen to polyurethane and their effects on cellular growth. *J. Biomed. Mater. Res.*, **1998**, *39*, 630-636.

[45] Alvarez-Lorenzo, C.; Duro, R.; Gomez-Amoza, J.L.; Martinez-Pacheco, R.; Souto, C.; Concheiro, A. Influence of polymer structure on the rheological behavior of hydroxypropyl methylcellulose-sodium carboxymethyl cellulose dispersions. *Colloid Polym. Sci.*, **2001**, *279*, 1045-1057.

[46] Lapasin, R.; Pricl, S. *Rheology of industrial polysaccharides: theory and applications*, Blackie Academic and Professional: London, **1995**, pp. 560-562.

[47] Mohsenin, N.N.; Mittal, J.P. Use of rheological terms and correlation of compatible measurements in food texture research. *J. Texture Stud.*, **1977**, *8*, 395-408.

[48] Lee, K.Y.; Rowley, J.A.; Eiselt, P.; Moy, E.M.; Bouhadir, K.H.; Mooney, D.J. Controlling mechanical and swelling properties of alginate hydrogels independently by cross-linker type and cross-linking density. *Macromolecules*, **2000**, *33*, 4291-4294.

[49] Rodriguez, R.; Alvarez-Lorenzo, C.; Concheiro, A. Interactions of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels. Rheological and diffusional implications. *Eur. J. Pharm. Sci.*, **2003**, *20*, 429-438.

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