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Research Paper

New Cyclodextrin Hydrogels Cross-Linked with Diglycidylethers with a High Drug Loading and Controlled Release Ability*

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Purpose. The goal of the study is to develop new hydrogels based on cyclodextrins cross-linked with ethyleneglycol diglycidylether (EGDE) under mild conditions, to be used as carriers of amphiphilic drugs. Also, it aims to characterize the cross-linking and the drug loading and release processes.
Methods. The cross-linking of hydroxypropyl-β-cyclodextrin (HPβCD) with EGDE, in the absence or presence of hydroxypropylmethylcellulose (HPMC) Methocel® K4M, was optimized applying oscillatory rheometry and Fourier transform infrared. Hydrogels were characterized regarding swelling in water, ability to load diclofenac, and release after different drying treatments.
Results. Solutions of HPβCD (14.28%), without or with HPMC (0.2–1.0%), provided firm and transparent hydrogels after cross-linking with EGDE (14.28%), in which around two thirds of the OH groups were cross-linked. The incorporation of HPMC progressively reduced the gel time and the swelling degree of hydrogels. HPβCD hydrogels efficiently loaded diclofenac and sustained the release for several hours. The presence of HPMC slowed the release from swollen hydrogels, but promoted it from hydrogels dried before the loading and also before the release.
Conclusions. HPβCD hydrogels with good mechanical properties and tunable loading and release ability can be obtained by direct cross-linking with EGDE.

KEY WORDS: amphiphilic drugs; cross-linking; ethyleneglycol diglycidylether (EGDE); hydrogels; hydroxypropylmethylcellulose (HPMC); hydroxypropyl-β-cyclodextrin (HPβCD); rheology.

INTRODUCTION

Hydrogels are particularly suitable materials for developing efficient and safe new drug delivery systems for both conventional and new biotechnological drugs, mostly because of (1) their high content in water, which allows a fast diffusion of small molecules, (2) the easiness with which it is possible to combine enough mechanical strength to resist handling and physiological stress, with a flexibility that prevents mechanical damage to the tissues, and (3) the versatility of the techniques used for preparing them (1,2). Nevertheless, hydrogels have two important limitations: their exiguous capacity to load poorly water-soluble drugs, which have low affinity for hydrophilic networks, and their poor ability to sustain, once activated, the release of hydrophilic drugs, which diffuse through the hydrogel as freely as in pure water (3). Some of the most recent approaches to solve these drawbacks involve the inclusion of the drug in vesicles such as micelles or liposomes (4–6) or the application of molecular imprinting technology (7–9). In addition, the incorporation of cyclodextrins by different means to the polymer network also seems very promising. Cyclodextrins can be found in hydrated polymer-based matrices as free molecules that can delay drug release; the great hydrodynamic size of the drug–cyclodextrin complexes makes the diffusion through the network difficult (10,11). Additionally, if the mesh of the network is small and only the free drug molecules can diffuse out, the affinity of the drug for the cyclodextrin may regulate the release rate. The presence of a hydrophilic polymer can enhance this affinity, whereas the competition of the components of the environment can prompt dissociation and release (12,13). On the other hand, cyclodextrins can act themselves as cross-linking agents by complexation of hydrophobic chains of amphiphilic polymers, which leads to the formation of supramolecular hydrogels (14,15).

Cyclodextrins can also form hydrogels through covalent bonds among themselves and/or with other compounds. These chemically cross-linked materials make the formulation of hydrophobic drugs in hydrophilic media possible, combining the versatility of the hydrogels and the complexation capability of cyclodextrins (16–20). This type of hydrogel has been shown useful in developing drug delivery systems to administer the drug by different routes, e.g., oral, buccal, or transdermal, minimizing adverse side effects and improving the pharmacological effects (21). Compared to other approaches that require the use of organic solvents and

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sophisticated carriers, the preparation of cyclodextrin hydrogels involves simpler technological processes, which are foreseeable to be easy to scale-up, and more biocompatible and stable materials (20,21).

To chemically attach the cyclodextrins, vinyl or acrylic monomers of cyclodextrins able to copolymerize with other monomers can be used (18,21–24). In this way, hydrogels of *N*-isopropylacrylamide and a maleic derivative of β-cyclodextrin (βCD) with an enhanced loading of a hydrophobic drug, chlorambucil, have been prepared. These hydrogels showed a pH-dependent release rate, which is given by the maleic groups of the βCD monomer (17). Cyclodextrin hydrogels can be also obtained by using cross-linking agents able to react with their hydroxyl groups (20,24,25). Unmodified βCD and poly(vinyl alcohol) were cross-linked with epichlorohydrin to obtain hydrogels able to load and sustain the release of salicylic acid (25). Interpenetrated networks of poly-βCD (produced by reaction with epichlorohydrin) and cross-linked *N*-isopropylacrylamide have shown a more sustained release of ibuprofen than hydrogels that only contain *N*-isopropylacrylamide (24). The affinity of the cyclodextrin for the drugs is not expected to be decreased when incorporated to the hydrogel. In fact, linear cyclodextrin polymers obtained by polycondensation with epichlorohydrin do not only maintain but also notably increase the affinity of the cyclodextrin for the guest molecules, making it possible to significantly improve the solubilizing effect of cyclodextrins in cosmetic and pharmaceutical formulations (26).

This work is focused on the development of new biocompatible cyclodextrin-based hydrogels, made with cyclodextrins alone or combining them with cellulosic derivatives, to be used as the main components of drug delivery systems and biomedical devices. This paper reports on a new cyclodextrin cross-linking procedure, carried out in water, under mild conditions, and uses ethyleneglycol diglycidylether (EGDE) as cross-linker, which has two epoxy groups in its structure, both of similar reactivity and able to react simultaneously with the hydroxyl groups of cyclodextrins. EGDE has been shown to be an adequate cross-linker of polysaccharides and of DNA (27–29), and its low toxicity makes its use in the preparation of protective coatings inside food and drink cans common (30,31). This cross-linking method does not require any modification in the cyclodextrin structure, thereby avoiding the use of acrylic or vinyl monomers in the preparation in which the number and the position of the polymerizable groups are hardly foreseeable (32). Compared to the already developed hydrogels based on cyclodextrin monomers, this new approach allows a step to obtain the hydrogel in a fast and predictable way. The unreacted substances, if any, are nontoxic, and therefore, although washing is recommended before use, it may not be mandatory. The cross-linking can be rheologically monitored to optimize the conditions to prepare the hydrogels. Several hydrogels were prepared with hydroxypropyl-β-cyclodextrin (HPβCD) or with HPβCD and hydroxypropylmethylcellulose (HPMC), and the influence of HPMC proportion on the physical properties of the hydrogels and on their capacity to load diclofenac and to control its release was evaluated. Diclofenac is a suitable candidate for formulation in sustained delivery systems for different routes (33,34). It contains two strong hydrophobic rings and a hydrophilic carboxylic acid group (35; Fig. 1), which provides it with an amphiphilic character that makes it challenging to achieve a thermodynamic stabilization of its hydrophobic groups in water and, at the same time, to avoid a burst release once administered. The inclusion of the hydrophobic rings of diclofenac in the HPβCD is thermodynamically favorable and can be enhanced in the presence of HPMC (11). Therefore, it is expected that the hydrogels can load this drug significantly and sustain its release efficiently.

MATERIALS AND METHODS

Materials

Hydroxypropyl-β-cyclodextrin (D.S. 4.6) was a gift from Janssen Pharmaceutische (Geel, Belgium); HPMC Methocel® K4M (batch MM87050902K) was provided by Dow Stade GmbH (Stade, Germany); ethyleneglycol diglycidylether (EGDE) (50% w/w in water) was from Fluka Chemie GmbH (Deisenhofen, Germany); diclofenac sodium was from Analema (Vigo, Spain). Water purified by reverse osmosis (MilliQ®, Millipore, Madrid, Spain) with a resistivity above 18.2 MΩ cm^{-1} was used.

Rheological Characterization of the Cross-Linking Process

A 20% w/w HPβCD solution in 0.2 M NaOH (code #20-0) was prepared under stirring. Different amounts of HPMC were added to 5-ml portions of this solution to have final HPMC concentrations of 0.2, 0.4, 0.6, 0.8, and 1.0% w/w. The resulting systems thereafter were denoted by codes #20-

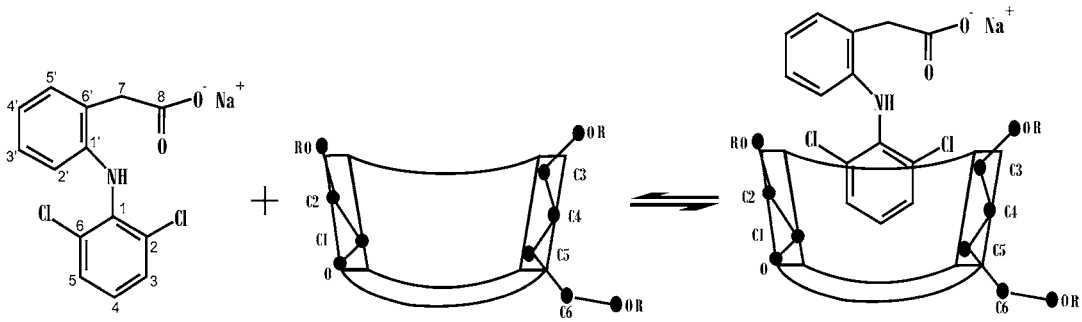

Fig. 1. Structure of diclofenac sodium, a cross-section of hydroxypropyl-β-cyclodextrin (HPβCD), and a schematic drawing of the 1:1 inclusion complex (R: –H; $–CH_2–CHOH–CH_3$).

0.2, #20-0.4, #20-0.6, #20-0.8, and #20-1, respectively. A control solution was prepared without HPβCD having a 1.0% w/w HPMC (code #0-1). Once homogenized, EGDE (2 ml of the 50% w/w in water solution) was added to each dispersion and stirred for 2 min at 20°C. Immediately, portions of the dispersions were transferred to the Peltier plate of a Rheolyst AR-1000N rheometer (TA Instruments, Newcastle, UK) equipped with an AR2500 data analyzer and a cone plate (2°, 6-cm diameter) measuring geometry. Liquid paraffin was put around the gap of the sample to prevent solvent evaporation. The rheological behavior of the dispersions was evaluated at 50°C in duplicate. The evolution in time of the storage and loss moduli (G' and G'', respectively) and of tan δ ($=G''/G'$) was recorded for an angular frequency of 0.1 rad/s by measuring these parameters for the first 120–600 min of the gelation process. The gelation time (t_{gel}) was estimated as the time at which G' and G'' crossover (tan δ = 1) (29).

Synthesis of HPβCD and HPβCD-*co*-HPMC Hydrogels

Solutions of HPβCD, HPβCD–HPMC, and HPMC prepared as for the rheological analysis were transferred to test tubes (8.6-mm internal diameter), hermetically closed, and kept at 50°C for 12 h to obtain the HPβCD, HPβCD-*co*-HPMC, and HPMC hydrogels, respectively. After cooling down, the hydrogels were carefully removed from the glass and were immersed in ultrapure water for 12 h to swell. The hydrogels were then transferred to 10 mM HCl solution for 12 h to neutralize the alkaline medium and were finally immersed in water for 1 week. Cylindrical pieces of each gel (4- to 5-mm thickness) were cut and maintained in water.

Characterization of Hydrogels

Infrared and Raman Spectroscopy

Samples of hydrogels were oven-dried at 40°C until they reached constant weight and were powdered in a mortar. Infrared (IR) spectra were recorded over the range 400–4000 cm^{-1} in a Bruker IFS 66V Fourier transform (FT)–IR spectrophotometer (Bruker, Ettlinger, Germany) using the potassium bromide pellet technique. Raman spectra were recorded at room temperature with a FT-Raman Bruker spectrometer (Bruker) equipped with a FRA 106 Raman module. Radiation of 1064 nm from a Nd:YAG laser was used for excitation. The acquired spectra were the result of 150 scans, and the spectral resolution was 1 cm^{-1}.

Swelling Studies

The amount of water absorbed by the hydrogels was estimated by the difference between the weight of the fully swollen hydrogel (W), after careful wiping of the surface with a soft tissue, and the weight of the hydrogel after being dried (W_0) at 40°C until equilibrium, and referred to the weight of the dried hydrogel:

$$Q = (W - W_0)/W_0 \quad (1)$$

To study the swelling process, dry samples of hydrogels (about 30 mg, 5-mm diameter and 1.5-mm thickness) were immersed in 10 ml of water and were weighed at preestablished time intervals. The kinetics of water uptake was characterized by fitting the data obtained up to 60% of the content in water to the following empirical equation (36):

$$W_t/W_\infty = K_w t^{0.5} \quad (2)$$

where W_t is the amount of water absorbed at time W_∞ is the amount of water absorbed at equilibrium, and K_w is a rate constant.

Diclofenac Loading by the Hydrogels

Cylindrical pieces of the hydrogels (8.6-mm diameter × 4- to 5-mm thickness) were placed, directly or after oven-drying at 40°C, in aqueous solutions of diclofenac sodium (10 ml of 0.1 or 0.5 wt.%) for 1 week at room temperature. Diclofenac remaining in the loading solution was determined spectrophotometrically (λ = 276.5 nm), and the amount loaded was estimated by subtracting it from the initial content.

Characterization of Diclofenac-Loaded Hydrogels

Diclofenac–Cyclodextrin Interactions

Raman spectra of dried loaded hydrogels were recorded as described above. Lorentzian deconvolution (Origin 7.0, OriginLab Corporation, Northampton, MA, USA) was used to separate the unresolved bands in the 1500- to 1650-cm^{-1} region into the three characteristic peaks of diclofenac (1579, 1582, and 1603 cm^{-1}). These peaks are related to its carboxylic group and the dichlorophenyl and phenyl acetate rings and can be altered by inclusion complex formation (37). Diclofenac–HPβCD and diclofenac–HPβCD–HPMC inclusion complexes were used as references. To prepare these inclusion complexes, equimolar amounts of drug and cyclodextrin were dissolved in water. To a portion of this solution, HPMC was added to obtain a final concentration of 0.4% (w/v). The systems were left to stand for 24 h at 4°C and were then freeze-dried in a Labconco Lyph-lock 6 apparatus (Kansas City, MO, USA) (−34°C, 48 h) after being frozen by immersion in liquid nitrogen. Loaded hydrogels were oven-dried at 40°C or were freeze-dried under the same conditions as the complexes before the Raman analysis. Spectra of the drug as supplied and after dissolution in water and oven-drying or freeze-drying were also recorded and used as controls.

Diclofenac Release

Loaded hydrogels (about 30-mg dried weight) were rinsed with water and immersed, directly or after a drying step in an oven at 40°C, in 25-ml water at room temperature, under sink conditions (drug solubility about 9 mg/ml) (35). The amount of diclofenac sodium released was measured spectrophotometrically (λ = 276.5 nm) in periodically taken samples and again was placed in the same vessel so that the liquid volume was kept constant. The experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Characterization of the Cross-Linking Process

The cross-linking of HPβCD with EGDE was carried out in NaOH 0.2 M. 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 (38). Heating is required for the reaction to proceed; whereas at room temperature, no cross-linking is observed, at 50°C, the reaction rate is fast enough to finish the cross-linking process in a few hours. Because at this temperature both the cyclodextrins and the cellulose ethers remain stable, these conditions were chosen to carry out the process (29).

Firstly, several HPβCD and EGDE concentrations were assayed to elucidate those at which hydrogels can be formed. Once EGDE was added to the HPβCD solutions, a sample of each resultant solution was immediately transferred to the Peltier plate of the rheometer and the rest of the solution to a test tube of 8.6-mm internal diameter. The tubes were kept at 50°C, and the rheological analyses were carried out at the same temperature. We observed, both visually and rheologically, that under the chosen reaction conditions, minimum concentrations of 10% w/w of HPβCD and of 14.28% w/w of EGDE are required in the final solution to obtain hydrogels. Therefore, as a basis to prepare the hydrogels, 5 ml of 20% HPβCD solutions was mixed with 2 ml of EGDE commercial solution (50% w/w in water) to have a final concentration of 14.28% of HPβCD and 14.28% of EGDE. This EGDE concentration is enough to react with 1.64 mol/l of hydroxyl groups. Taking into account that the concentration of HPβCD is 0.11 mol/l and that each molecule of this cyclodextrin possesses 21 hydroxyl groups, the EGDE added can react with 71% of the total hydroxyl groups of HPβCD. In the truncated cone structure of cyclodextrins, the OH-2 and OH-3 groups are located on the wider side of the torus, whereas the OH-6 is positioned on the narrower side and is directed away from the cavity (39). The reactivity of these OH groups is as follows: OH-6 > OH-2 > OH-3 (40). The OH-6 is more basic than the secondary OH groups, and therefore, it can react easily in strong alkaline solutions. By contrast, the reactivity of OH-2 is favored in weak alkaline solutions. The limited reactivity of the OH-3 groups is attributed to the steric hindrance and to hydrogen bonding between this hydroxyl and the ring oxygen of an adjacent glucopyranose (41). Therefore, in the HPβCD solution used to prepare the gels, there are 1.54 mol/l of the most reactive OH-6 and OH-2 groups. The mean degree of substitution of the HPβCD was of 4.6 hydroxypropyl groups per molecule of cyclodextrin. The OH groups of the hydroxypropyl substituents are as reactive as the OH-6 and can also participate in the cross-linking process. Consequently, a decrease in the number of reactive OH groups is not expected because of the substitution process. In summary, the amount of EGDE incorporated as a cross-linker is enough to react with at least two thirds of the OH groups of HPβCD and to obtain consistent hydrogels.

In a second step, hydrogels were prepared from solutions containing HPMC, HPβCD, and EGDE to evaluate the effects of the cellulose ether. HPMC, a linear polymer constituted by glycopyranose units similar to those of HPβCD, should participate in the cross-linking process to form HPβCD-*co*-HPMC hydrogels. HPMC, with a content in methoxyl groups of 22.4% and in hydroxypropoxyl groups of 8.3% (total degree of substitution of 1.65) (42) and a mean molecular weight of 84,200 Da, was used, and its influence on the kinetics of the cross-linking reaction was evaluated. As in the case of HPβCD, the reactivity of the OH groups of the HPMC ranks in the following order: OH-6 > OH-2 > OH-3.

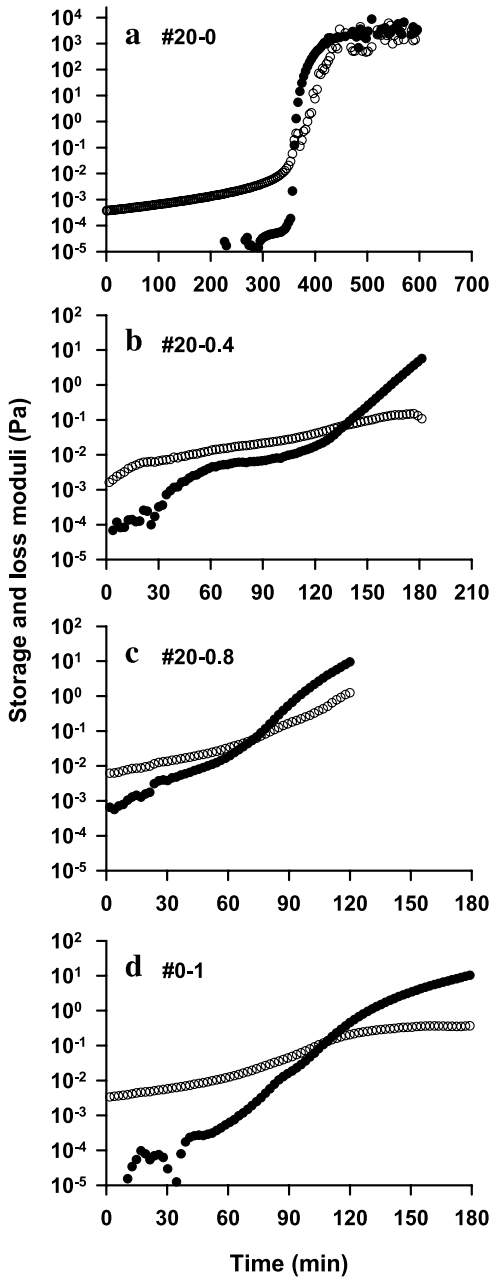

Fig. 2. Time dependence of the storage (G', •) and loss (G'', ○) moduli for systems prepared from 20% HPβCD solutions without hydroxypropylmethylcellulose (HPMC) (#20-0), from 20% HPβCD solutions containing 0.4% (#20-0.4) or 0.8% (#20-0.8) HPMC, and from 1.0% HPMC solutions without HPβCD (#0-1). In all cases, the final ethyleneglycol diglycidylether (EGDE) proportion in the solutions was 14.28%.

Then, in a 1.0% aqueous dispersion of this HPMC, around 0.1 mol/l of OH groups is available for cross-linking.

HPMC concentration range was chosen to be above the critical overlap concentration ($c^* = 1/[\eta]$) but below its entanglement concentration ($c_{ent} = 10/[\eta]$). In this way, intra- and intermolecular junctions of the cellulose ether themselves are minimized (29,43), and, consequently, the likelihood of having an even distribution of HPβCD and HPMC in the hydrogel is increased. The intrinsic viscosity of HPMC in water is 7.31 dl/g at 298 K, and its critical overlap concentration is 0.137% (44). The lowest HPMC concentration evaluated (0.2% in the HPβCD solution, equivalent to 0.143% in the final mixture with EGDE) was just above this value. The greatest HPMC concentration (1.0% in the HPβCD solution or 0.714% in the final mixture) was well below the entanglement concentration (1.37%). Even at this HPMC concentration, the low viscosity of the solutions makes them easy to handle. As can be seen in Fig. 2, at time zero (i.e., when the cross-linking has not occurred yet), the G'' values of HPβCD–HPMC solutions were only one order-of-magnitude greater (10^{-2} Pa) than those of HPβCD solutions without HPMC (10^{-3} Pa), which clearly indicates that the contribution of HPMC itself to the consistency of the non-cross-linked systems is of little relevance compared with that caused by the cross-linking process (five to six orders of magnitude). In fact, in the absence of EGDE, all HPβCD and HPβCD–HPMC solutions showed a rheological behavior typical of diluted polymer dispersions, with low G'' and negligible G' values.

The angular frequency (0.1 rad/s) at which the studies were carried out allowed the monitoring of the cross-linking process because all dispersions show before reaction with EGDE a mainly viscous behavior. Under this condition, a number of G' and G'' data points were recorded without distorting the forming network. The time-sweep experiments at this angular frequency showed that, when EGDE was absent, G' and G'' values remained practically constant in time. The addition of EGDE caused at 50°C a relatively fast increase in both moduli; G' was initially lower than G'', but it increased faster, being greater than G'' after a time, and finally reaching a plateau region (Fig. 2). The gel time, i.e., the time at which the crossover of G' and G'' occurs ($\tan \delta = 1$) (28), decreased as follows: #20-0 (360 min) > #20-0.2 (240 min) > #20-0.4 (138 min) > #0-1 (109 min) > #20-0.6 (80 min) > #20-0.8 (72 min) > #20-1 (15 min). Therefore, in the presence of HPMC, the gelation process was faster and the final values of G' and G'' were greater. This can be explained by the greater content in reactive hydroxyl groups and also because the long HPMC chains favor the cross-linking process in the growing network.

In summary, HPβCD and HPβCD-*co*-HPMC hydrogels can be prepared under mild conditions by this one-step procedure. The rheological results indicate that, in 6 h, consistent networks were formed. To insure the end of the reaction, the preparation of hydrogels went on for 12 h.

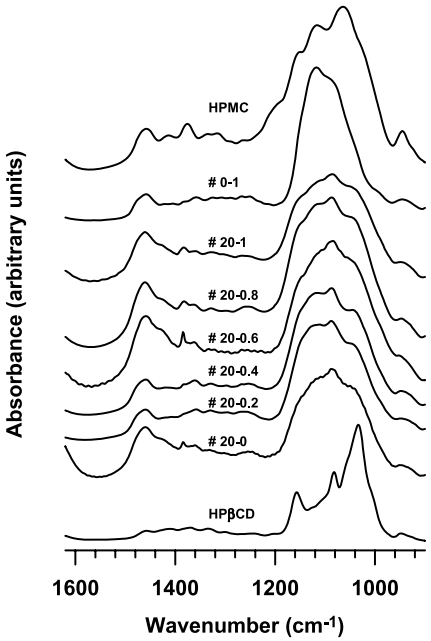

Fig. 3. Infrared spectra of HPβCD and HPMC powder and dry hydrogels in the region 1600–800 cm^{-1}. Hydrogels were prepared from 20% HPβCD solutions without HPMC (#20-0) or with 0.2% (#20-0.2), 0.4% (#20-0.4), 0.6% (#20-0.6), 0.8% (#20-0.8), or 1.0% (#20-1) HPMC, and from 1.0% HPMC solutions without HPβCD (#0-1).

Structural Characterization of the Hydrogels

Hydrogels, once removed from the tubes, were transparent and easy to handle (firm but flexible). After immersion in water, their diameter increased from 8.6 mm up to around 12 mm, showing a smooth and continuous surface. These observations are characteristic of a homogeneously cross-linked hydrogel that does not lose material by disentanglement of the polymer chains (43).

The degree of the cross-linking was determined by the changes observed in the region of the ether bond signals of the IR spectra of dried hydrogels using HPβCD and HPMC powders as references (Fig. 3). In the 1120- to 1050-cm^{-1} range, HPβCD has a lower absorbance at 1082 cm^{-1} than at 1033 cm^{-1} ($A_{1082}/A_{1033} = 0.73$), and HPMC shows less intense peaks at 1117 cm^{-1} than at 1066 cm^{-1} ($A_{1117}/A_{1066} = 0.95$), which is characteristic of the predominance of primary alcohols in the structure of these substances

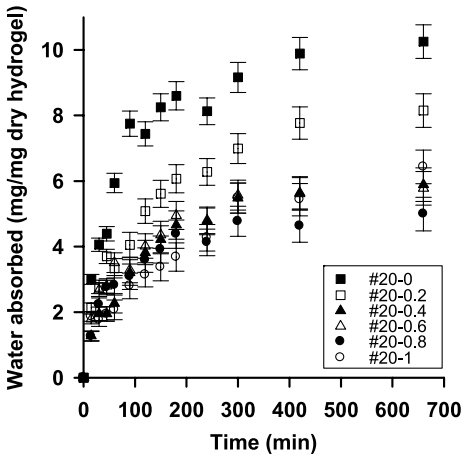

Fig. 4. Swelling profiles of dry hydrogels in water. The composition of the hydrogels is indicated by legend as in Fig. 3.

(29,45). By contrast, in the hydrogel, a remarkable increase in these ratios was observed: the A_{1082}/A_{1033} is in the range 1.03–1.13, and the A_{1117}/A_{1066} is in the range 1.01–1.13. This means an increase of about 60% of the A_{1082}/A_{1033} ratio in the HPβCD and of about 10% for the A_{1117}/A_{1066} ratio in the HPMC. These results indicate that around 60% of the primary OH of HPβCD have been transformed into secondary OH to form cross-linking junctions, which agrees with the expected two thirds of hydroxyl groups of HPβCD cross-linked by EGDE in the hydrogel. The 10% cross-linking of the OH groups of HPMC, observed both in the presence and in the absence of HPβCD, was similar to the one previously reported for other cellulose derivatives when the cross-linking process was finished (29). No signal appeared at 1250 cm^{-1}, confirming that no unreacted EGDE remains in the hydrogels.

The high affinity of the dried hydrogels for water was reflected in the profiles of swelling degree vs. time (Fig. 4). Although all swelled relatively fast, their final degree of swelling was notably dependent on the proportion of HPMC. The hydrogel prepared only with HPβCD (#20-0) was able to uptake ten times its dry weight in water, whereas the HPβCD-*co*-HPMC hydrogel prepared with the highest HPMC proportion (#20-1) increased only up to four times its weight. The dried hydrogel prepared only with HPMC (#0-1) looked like a film and was more difficult to handle. The degree to which it swelled after remaining in water for 12 h was 5.6 mg water/mg gel. The lower swelling degree of HPβCD-*co*-HPMC hydrogels is explained by the attainment of a higher cross-linker density; the long chains of this cellulose ether acted as bridge points among several cyclodextrins.

The amount of sorbed water depended linearly on $t^{1/2}$ ($r^2 > 0.99$) for all the hydrogels studied; that is, Fickian behavior was maintained in spite of the swelling of the network (36). All hydrogels showed a similar rate of swelling around 0.050–0.069 $min^{-0.5}$, except those prepared with HPβCD and the highest HPMC proportion (#20-1) that had a significantly lower slope (about 0.037 $min^{-0.5}$). The latter value is similar to those previously found for hydrogels prepared with other cellulose ethers in the absence of cyclodextrins (29). In any case, water sorption rates indicate that water molecules easily penetrate the structure of the networks.

Diclofenac Loading and Release

In general, loading and release from hydrogels mainly depend on (1) the easiness with which the drug molecules diffuse into the hydrogel, which is determined by the degree of cross-linking and the affinity of water for the network, and (2) the affinity of the drug for the components of the network (2,3,7,46). As mentioned above, the mesh size of all HPβCD and HPβCD-*co*-HPMC hydrogels prepared is enough to allow the exchange of the water and small size molecules. However, there are compositional and structural differences among the hydrogels that can affect their loading and release behavior. It was macroscopically observed that HPβCD-*co*-HPMC hydrogels with a slightly greater degree of cross-linking have a lower affinity for water (Fig. 4) than HPβCD hydrogel (#20-0). This can hinder the diffusion of drug molecules. The presence of the overlapped HPMC chains may also contribute to this effect. Therefore, when the hydrogel is loaded by immersion in a suitable drug solution, it is expected that the penetration of the drug inside HPβCD-*co*-HPMC hydrogels should occur in a much slower way, and, in the absence of other factors, the amount loaded may be less.

Table I shows the amounts of diclofenac loaded by each hydrogel. Loading was similar when disregarding the discs that were added to the drug solution in the swollen state or previously dried, which can be explained by the fast swelling of the dried hydrogels and by their ability to completely recover their initial form after the drying process.

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, can be estimated using the following equation proposed by Kim *et al.* (46):

$$Loading\,(aqueous\,phase) = \left(V_s/W_p\right) \times C_0 \qquad (3)$$

where V_s is the volume of water sorbed by the hydrogel, W_p is the dried hydrogel weight, and C_0 is the concentration of the drug in the loading solution. If we consider the data of swelling shown in Fig. 4, when a 0.1% diclofenac solution is used, the mean amounts (standard deviations) loaded for the hydrogels by this mechanism are as follows: #20-0, 9.9 (0.5) mg/g; #20-0.2, 7.8 (0.4) mg/g; #20-0.4, 5.6 (0.3) mg/g; #20-0.6,

Table I. Diclofenac Loaded (mg/g dry hydrogel) by Immersing Dry Hydrogels in 0.1 or 0.5% Diclofenac Solution, and Values of the Partition Coefficient Between the Polymer Network and Drug Loading Solution, Calculated According Eq. (4)

	0.1% Solution		0.5% Solution	
Hydrogel code	Diclofenac loaded (mg/g)	*K*	Diclofenac loaded (mg/g)	*K*
#20-0	27 (1)	17.1 (0.8)	106 (19)	11.3 (0.6)
#20-0.2	20 (3)	12.2 (0.6)	81 (21)	8.4 (0.4)
#20-0.4	17 (2)	11.4 (0.6)	68 (12)	8.0 (0.4)
#20-0.6	16 (2)	10.4 (0.5)	58 (10)	6.0 (0.3)
#20-0.8	15 (1)	10.0 (0.5)	58 (12)	6.6 (0.3)
#20-1	17 (3)	11.6 (0.6)	57 (9)	6.0 (0.3)
#0-1	25 (2)	19.4 (0.9)	97 (23)	13.8 (0.7)

Mean values and, in parentheses, standard deviations. Hydrogel codes as in Fig. 3.

5.6 (0.3) mg/g; #20-0.8, 5.0 (0.2) mg/g; #20-1, 5.4 (0.3) mg/g; #0-1, 5.6 (0.2) mg/g. When a 0.5% diclofenac solution is used, amounts five times greater are estimated. The actual amounts loaded, which are shown in Table I, were 2.1–4.5 times greater. These results clearly indicate that the affinity of diclofenac for the network also plays a role in the loading process.

Because the pK_a of diclofenac is around 4 (35), it is expected that the ionized form predominates in the loading (pH 6.7) and release (pH 6.0) media. Nevertheless, even under these conditions, the solubilized drug could establish hydrophobic interactions with the network and form inclusion complexes with cyclodextrin cavities through its aromatic rings (47). This may explain the difference between the actual loading results and the values predicted using Eq. (3) for HPβCD and HPβCD-*co*-HPMC hydrogels. To gain an insight into the role of the interaction of diclofenac with cyclodextrin units, the partition coefficient K between the polymer network and the drug loading solution was estimated from the following expression (46):

$$Loading\,(total) = \left[\left(V_s + KV_p\right)/W_p\right] \times C_0 \qquad (4)$$

where V_p is the volume of dried polymer and the other variables as defined in Eq. (3). The partition coefficient is an index of the affinity of the drug for the network. The values of K (Table I) clearly prove that, despite that diclofenac is ionized and its ability to interact with the cyclodextrin cavities may be reduced (48), the cyclodextrin units play an important role in the loading of the hydrogel.

The high loading of HPMC-alone hydrogel (#0-1) is also explained by the hydrophobic interactions between the anhydroglucose units of the HPMC chains and the aromatic rings of diclofenac. A similar sorption phenomenon has been previously described for the other structurally related nonsteroidal anti-inflammatory drug, ibuprofen, for which a noncooperative association with cellulose ethers was observed in solutions below its critical micellar concentration (49). More than half of the anhydroglucose units were bound to ibuprofen molecules (49). Because diclofenac is more hydrophobic than ibuprofen (50), this type of interaction may be enhanced and contribute strongly to its loading by the HPMC hydrogel. Taking into account the composition of this hydrogel (about 3.27×10^{-4} U g of repeating anhydroglucose per gram of dry hydrogel), the total amount of drug loaded (a mean value of 3.14×10^{-4} mol/g), and the amount of drug that the hydrogel can load by simple equilibrium of its aqueous phase with the 0.5% diclofenac solution (0.91×10^{-4} mol/g), the number of diclofenac molecules bound to each anhydroglucose repeating unit of polymeric network was estimated to be 0.68. In HPβCD hydrogels (which have 3.84×10^{-4} mol of cyclodextrin per gram), the number of diclofenac molecules bound by each anhydroglucose unit is estimated to be in the range 1:4 and 1:5. Because each HPβCD is formed by seven anhydroglucose units and the contribution of the HPMC to the total content in anhydroglucose units is quite low, a drug/cyclodextrin molar ratio close to 0.61 can be assumed. Therefore, in HPβCD hydrogels, the loading seems to be mainly driven by the interaction of diclofenac molecules with the cyclodextrin cavities. The ability of resins made of cross-linked cyclodextrin to load organic compounds (phenol, benzoic acid, aniline, chlorobenzoic acid isomers, and tyrosine) through inclusion complex formation has been previously reported (16). A Langmuir or Freundlich type of isotherm is suitable to relate the amount absorbed by the resins with the concentration of the drug in the loading medium (16). Our findings (Table I) are in agreement with these results and also indicate an important dependence of the total amount loaded on diclofenac concentration in the surrounding solution. Because diclofenac is an amphiphilic drug, some molecules could be also loaded by self-association with those bonded to the network. This phenomenon has been observed to occur in ibuprofen– and diflunisal–cyclodextrin complexes in solution, below their critical micellar concentrations (47).

To gain an insight into the state of the drug in the cyclodextrin hydrogel, the Raman spectra of hydrogel samples were compared with those of diclofenac–HPβCD freeze-dried inclusion complexes. The Raman spectrum of diclofenac presents different bands associated with the phenyl acetate and the dichlorophenyl rings. The interaction of the drug with HPβCD to form an inclusion complex can take place through both rings, but it seems to be stronger through the dichlorophenyl one (11). To study the formation of the complexes, the changes in the Raman spectra of the drug loaded into the hydrogel were analyzed (37). In the region between 1500 and 1650 cm^{-1}, diclofenac shows three bands, and HPβCD and HPMC do not show any specific band that can interfere (Fig. 5). The 1579-cm^{-1} band is assigned to the $O_1C_8O_2$ asymmetric stretching vibration

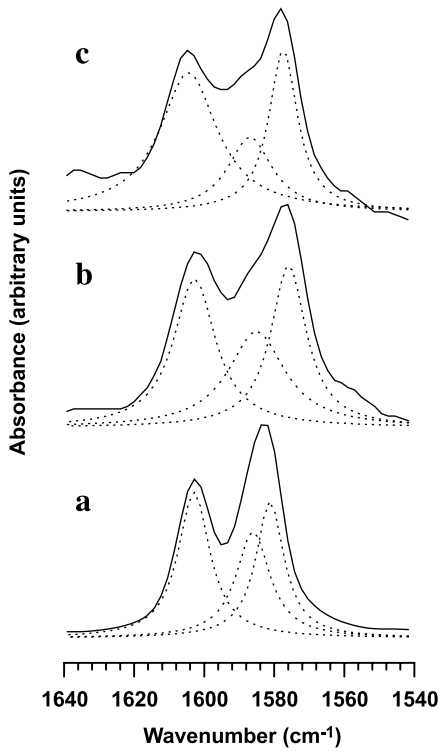

Fig. 5. Fourier transform–Raman spectra of diclofenac sodium (a), #20-0 hydrogel loaded with diclofenac sodium (b), and 1:1 diclofenac sodium HPβCD inclusion complex (c). Lorentzian deconvolution of Raman bands in the 1540- to 1640-cm^{-1} range is also shown as dotted lines.

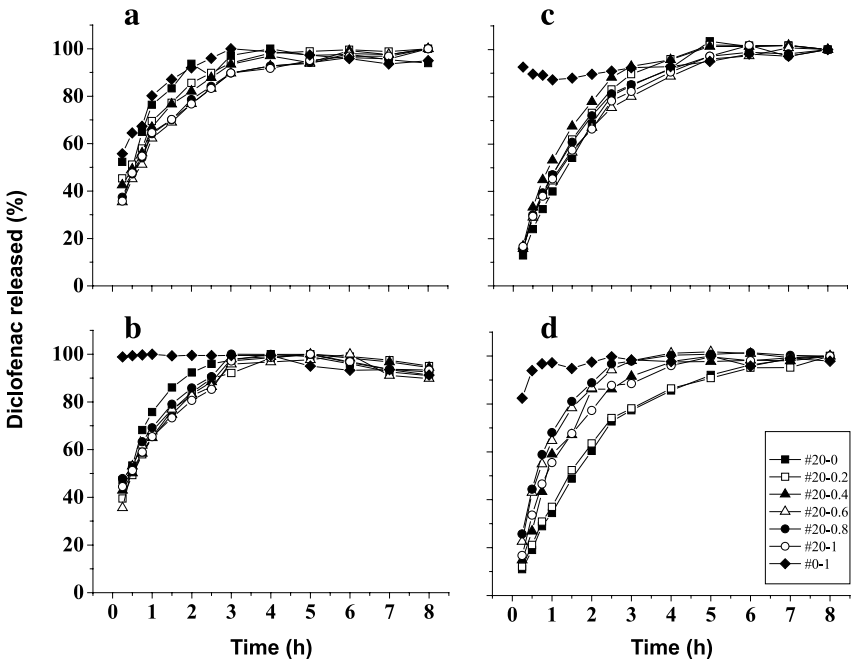

Fig. 6. Diclofenac sodium release profiles from hydrogels loaded in aqueous solutions of 0.5% w/w diclofenac sodium. Hydrogels not subjected to any drying step (a); hydrogels dried before loading and dried again before the release test (b); hydrogels only dried before the release (c); and hydrogels only dried before loading (d). Hydrogel legend as in Fig. 3.

(Fig. 1), whereas the bands at 1582 and 1603 cm^{-1} are related, respectively, with the dichlorophenyl and phenyl acetate ring stretching vibrations. Some of these bands became wider and shifted to greater wave numbers when inclusion complexes were formed by freeze-drying of drug–HPβCD solutions (see below). Similar changes were observed in the spectra of loaded cyclodextrin hydrogels (Fig. 5). Control samples of the drug, processed under the same conditions as the hydrogels and the complexes, did not show any change in their spectra. Therefore, the shifts observed in the complexes with HPβCD and in the loaded hydrogels cannot be attributed to changes on drug structure caused by the simple processing of the samples. Lorentzian deconvolution of this spectral region led to the resolution of the three characteristic peaks of the drug. The widths of diclofenac bands increased when the drug was forming inclusion complexes with HPβCD, and the maxima of the peaks were shifted except for the 1603-cm^{-1} band. This suggests that the interaction between the phenyl acetate ring of the drug with the cyclodextrin cavity is weak. In contrast, the peaks associated with the dichlorophenyl ring were significantly shifted from 1582 to 1588 cm^{-1}. These results, which were similar for all complexes disregarding the presence of HPMC in the preparation medium, indicate that diclofenac–HPβCD complexation is mainly driven by the inclusion of the dichlorophenyl ring in the cyclodextrin cavity. Raman spectra of the loaded HPβCD or HPβCD-*co*-HPMC hydrogels presented shifts similar to those observed for the complexes, with peaks at 1588 cm^{-1} (#20-0) and at 1589 cm^{-1} (#20-0.4). HPMC hydrogel (#0-1) also showed a shift to 1589 cm^{-1}. This confirms the existence of hydrophobic bonding, also in this hydrogel, between the drug and the anhydroglucose units. Because such an interaction with HPβCD is only possible when the drug enters into the cavity (the hydrophilic OH groups are orientated toward the exterior; Fig. 1) (21, 39), we can conclude that the interaction between the drug and the cyclodextrin in the hydrogel is similar to that which occurs in common host–guest complexes.

Figure 6 shows the diclofenac release profiles from hydrogels loaded in 0.5% diclofenac solution, which were very similar to those obtained for hydrogels loaded in 0.1% diclofenac. Some samples were directly assayed, i.e., not dried either before loading or before the release test (wet loaded–wet released). Hydrogels that were dried before loading and also again before the release test (dried loaded–dried released) and hydrogels with intermediate treatments (wet loaded–dried released; dried loaded–wet released) were also evaluated.

The release from the HPMC hydrogel, without incorporating HPβCD (#0-1), was practically immediate disregarding the thermal treatment. In contrast, the HPβCD and HPβCD-*co*-HPMC hydrogels were able to sustain the release for several hours, owing to the ability of cyclodextrins to provide a more hydrophobic environment than that of the linear chains of HPMC. The relatively fast release of a significant fraction of the dose at the beginning of the experiment can be related to drug molecules that were simply loaded by equilibrium of the aqueous phases, without effectively interacting with the network. These results clearly indicate that the cyclodextrin also plays an important role in the control of the release of diclofenac. It has been reported that although the stronger the binding constant, the slower the dissociation kinetics, the release of the drug from common cyclodextrin complexes is practically instantaneous when the complexes are diluted in aqueous fluids (13,48,51). Therefore, in general, cyclodextrin solutions do not provide a sustained release. However, in the case of formulations based on cyclodextrin hydrogels, the dilution phenomena are minimal because the

cyclodextrins are covalently attached to each other, and the volume of water that can enter the hydrogel is limited by its own network. This should provide a microenvironment rich in cavities available to interact with the surrounding drug molecules, and, as a consequence, cyclodextrin hydrogels could serve as platforms of new sustained release devices (17,24).

The effect of the proportion of HPMC was noticeable in the HPβCD-*co*-HPMC hydrogels that were not dried (wet loaded–wet released) or that suffered two drying steps (dried loaded–dried released), but the influence was the opposite. HPMC decreased the release rate in the former hydrogels but promoted the release in the latter ones. HPβCD hydrogels that were not dried (wet loaded–wet released) did not show significant changes in the degree of swelling during the release process, and the binding to the HPβCD plays the main role in the control of the release. The presence of HPMC leads to a greater cross-linking and a lower swelling degree (Fig. 4), which may explain the slightly slower release from HPβCD-*co*-HPMC hydrogels.

HPβCD-*co*-HPMC hydrogels that suffered two drying steps (dried loaded–dried released) showed a faster release as the HPMC proportion in the hydrogel increased. In these hydrogels, two concomitant effects can occur: (1) an enhancement of the drug–HPMC hydrogen bonds and hydrophobic interactions during the drying; and (2) a sweeping of the drug along by water during the swelling. Additionally, the entrance of water toward the hydrogel core and the diffusion of the drug can be facilitated by the salting out of the HPMC by diclofenac (52). These effects together with the affinity of the drug for the HPβCD determine the release rate. As can be seen in Fig. 5b and d, HPβCD hydrogel and HPβCD-*co*-HPMC hydrogel with the lowest proportion of HPMC released the drug at a significantly lower rate, despite their lower degree of cross-linking and greater degree of swelling.

We have previously found that cationic cellulose hydrogels cross-linked with EGDE can load diclofenac by electrostatic interactions and are able to sustain the release in water or pH 8 phosphate buffer for 4 h (29). The new HPβCD and HPβCD-*co*-HPMC hydrogels showed an even greater ability to load and to sustain the release of diclofenac for several hours. Therapeutic doses of diclofenac are in the range of 50–100 mg per day (53), and HPβCD and HPβCD-*co*-HPMC hydrogels can perfectly fulfill this requirement, being potentially useful as a basis of drug delivery systems for different routes (20,33,34). To improve the performance of these hydrogels to uptake and release strong hydrophobic drugs will be the aim of future work.

CONCLUSIONS

Cyclodextrin hydrogels that show good mechanical properties and high ability to load and sustain the release of an amphiphilic drug, diclofenac, can be obtained by direct cross-linking with diglycidylethers under mild conditions. Loading is mainly driven by the affinity of the drug for the cyclodextrin units, which also control the release process. This versatile procedure also allows the incorporation of other structurally related polymers, such as HPMC, to the network, which can contribute to the improvement of the physical properties and to the modulation of the release behavior.

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