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Acrylic/cyclodextrin hydrogels with enhanced drug loading and sustained release capability

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Abstract

The influence of the proportion of acrylamidomethyl-γ-cyclodextrin (γ-CD-NMA) on loading and release of the hydrophobic triamcinolone acetonide (TA) and the hydrophilic propranolol (PR) by acrylic acid hydrogels was evaluated. γ-CD-NMA was synthesized by condensation of γ-cyclodextrin (γ-CD) with *N*-(hydroxymethyl) acrylamide. Hydrogels were prepared with γ-CD-NMA and sodium acrylate (3 M or 4 M), using *N*,*N*′-methylen(bisacrylamide) (BIS) as cross-linker, by free radical polymerization into glass moulds of 2 mm wide and were cut as discs (10 mm diameter). γ-CD-NMA did not modify the pH-dependent swelling of the hydrogels, but significantly increased the swelling degree in the 40:60 ethanol:water, medium in which TA can be dissolved. Hydrogels prepared with γ-CD-NMA above 5% (w/w of total monomers) showed a remarkably higher capacity to load TA, e.g., 33 mg/g dry hydrogel versus 0.6 mg/g dry hydrogel without γ-CD-NMA. This is explained by the formation of 1:1 inclusion complexes of TA with γ-CD mers that overcomes the lack of interactions with the acrylic groups of the network. The release of TA in water, 0.1 N HCl, or pH 6.8 phosphate buffer was sustained for at least 24 h, whatever the pH and the composition of the medium used. In contrast, loading of PR from the water solutions was greater for hydrogels prepared with 3 M acrylate than with 4 M acrylate, irrespective to their content in γ-CD-NMA, and in less than 2 h ca. 80% PR was released. The lower affinity of PR for the γ-CD cavities, compared to the strong intensity of the electrostatic interactions with the acrylic acid groups, explains why the incorporation of γ-CD-NMA did not increased the loading and control release capacity of the hydrogels of this hydrophilic drug. In summary, the copolymerisation of CD with acrylic monomers can provide highly hydrophilic pH-sensitive networks which load large amounts of hydrophobic drugs and release them in a sustained way.

Keywords: Acrylic hydrogels; γ-Cyclodextrin; Inclusion complexes; Triamcinolone acetonide; Propranolol; Controlled release

1. Introduction

The interest of hydrogels as platforms for drug delivery systems is raising continuously; their suitability for pharmaceutical applications being mainly determined by their mechanical properties, drug loading and controlled drug release capability. Drug-loaded hydrogels may act as reservoirs that release the drug, immediately or in a sustained way, by mechanisms such as diffusion or erosion. They can be also used as targeting agents for site-specific delivery, using two main approaches: (a) systems that modify their structure, swelling or eroding, in response to changes in the characteristics of the physiological medium (*smart* hydrogels) and (b) devices in which the drug is covalently bonded to the polymer via a labile covalent bond that can broken at specific biological conditions (Yuk and Bae, 1999; Peppas et al., 2000; Alvarez-Lorenzo and Concheiro, 2003). A recent approach consists in the development of high loading capacity hydrogels that can interact effectively with the drug through ionic or hydrophobic bonds under specific conditions (Sen and Yakar, 2001; Alvarez-Lorenzo and Concheiro, 2002; Jimenez-Kairuz et al., 2003; Rodríguez et al., 2003). The loading efficiency can even be increased by arranging, using molecular imprinting technology, the interacting groups in the bulk of the hydrogel to adapt them to the molecular structure of the drug. Using this approach, hydrogels with specificity for given drugs can be created (Alvarez-Lorenzo and Concheiro, 2004; Hilt and Byrne, 2004; Hiratani and Alvarez-Lorenzo, 2004).

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To modulate the release properties, additives able to modify the polarity and/or the viscosity of the microenvironment, altering the diffusion of the drug, can be incorporated to the hydrogels. These effects are more remarkable when the additive (e.g., surfactant, cyclodextrin) interacts with the drug or the polymer chains, altering the drug–hydrogel interactions (Blanco-Fuente et al., 2002; Alvarez-Lorenzo and Concheiro, 2003). The ability of cyclodextrins (CDs) to form inclusion complexes, by taking up a whole drug molecule or rather some non-polar part of it into the hydrophobic cavity, may considerably alter drug solubility and size of diffusive molecules and consequently, the release behaviour of polymeric systems (Loftsson and Brewster, 1996; Bibby et al., 2000; Quaglia et al., 2001; Uekama, 2004). The diffusivity of CD complexes is also conditioned by the potential interactions with the polymeric components of the formulation, which, in some cases, can even alter the swelling behaviour of the polymer network (Ikeda et al., 2000; Blanco-Fuente et al., 2002). If the drug–CD complex has a high association constant and its diffusion out of the matrix is impeded, a sustained drug release can be achieved (Pose-Vilarnovo et al., 2004).

Recently, highly cross-linked polymer networks having the CDs covalently bonded to the network have been prepared. With this aim in mind, several procedures to obtain CDs with groups able to copolymerise with acrylic or vinyl monomers have been developed (Lee et al., 2001; Liu and Fan, 2002; Hussain et al., 2005). Asanuma et al. (2000, 2001) have synthesized rigid networks by assembling CDs with a spatial distribution that allows each to fit a portion of a target molecule. Using a vinyl monomer of 6-*O*-glucosyl-CD and *N*,*N*′-methylen(bisacrylamide) (BIS) as cross-linker, polymer networks with high affinity for vancomycin and cefazolin were created. Piletsky et al. (1999) have combined bisacryloyl-β-CD and 2-acryloylamido-2,2′-dimethylpropane sulfonic acid to obtain a network with an enhanced affinity for amphiphilic molecules, such as phenylalanine. Propranolol (PR) release from microcapsules prepared with cross-linked CDs was delayed when the proportion in CD increased (Pariot et al., 2002). The incorporation of CDs with ionic groups can provide networks that also undergo phase transitions owing a change in the pH of the medium (Nozaki et al., 1997; Liu and Fan, 2002). CD-pullulan microspheres have been shown to be a useful stationary phase in chromatographic separations, able to retain compounds that strongly interact with the CD (Fundueanu et al., 2003).

In the pharmaceutical field, CDs may be especially useful to overcome some flakes of low cross-linked hydrogels, in particular the limited uptake of hydrophobic drugs and the slight control of the release of hydrophilic drugs (Rodríguez-Tenreiro et al., 2006). Recently, *N*-isopropylacrylamide/maleic-derivative of β-CD hydrogels have been proposed to uptake chlorambucil and to release it at pH-dependent rate (Liu et al., 2004). However, there is as yet little information on the applications of copolymerised CDs on this field.

The aim of this work is to prepare acrylic hydrogels with a high drug loading capacity by incorporating a γ-CD based monomer, i.e. acrylamidomethyl-γ-cyclodextrin (γ-CD-NMA), and to evaluate the influence of the proportion of γ-CD-NMA on the loading and the release of two drugs with recognized ability to form inclusion complexes: triamcinolone acetonide (TA) which is almost insoluble in water and did not interact with carboxylic acid groups (Klein et al., 2000; Shin et al., 2000), and propranolol a fairly soluble drug that strongly interacts with acrylic networks (Blanco-Fuente et al., 2002; Fundueanu et al., 2003). Taking into account that acrylic hydrogels present excellent mechanical properties, bioadhesivity and pH-responsive behaviour and are useful as basis for many drug delivery systems to be administered by different transmucosal routes (Shin et al., 2000; Alvarez-Lorenzo and Concheiro, 2003; Edsman and Hägerström, 2005), optimisation by copolymerisation with CDs, of their loading and release behaviour may be of enormous practical importance.

2. Materials and methods

2.1. Materials

TA and PR·HCl were supplied by Roig Farma (Spain). Sodium acrylate was from Aldrich Chemical Company (USA), *N*,*N*′-methylen(bisacrylamide) was from Fluka Chemie-AG (Switzerland). *N*-(hydroxymethyl)acrylamide (NMA), *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), and ammonium persulfate (APS) were supplied by Sigma–Aldrich (Spain). γ-CD was Cavamax W8 from Wacker (Germany), acetonitrile was from Merck (Germany). Distilled water was used for the synthesis and the washing of the hydrogels. Ultrapure water (MilliQ®, Millipore Spain, resistivity >18 MΩ cm) was used for HPLC experiments. All other reagents were of analytical quality.

2.2. Synthesis of acrylamidomethyl-γ-cyclodextrin (γ-CD-NMA)

γ-CD (17.12 g) and NMA (13.36 g) were added to 1% HCl solution (50 ml) in a three-neck reactor, equipped with a mechanical stirrer, a thermometer, and a condenser (Lee et al., 2001). The solution was heated at 80 °C. After 30 min, acetone (300 ml) was added to stop the reaction and precipitate the γ-CD-NMA. The reactor was kept at 4 °C for 12 h. After, the precipitate was separated by filtration (Albet® 145, Spain), repeatedly washed with acetone (200 ml) and filtered (four cycles), and finally dried under vacuum for 2 days at room temperature, and stored at 4 °C. About a 95% of the initial of γ-CD was incorporated to the monomer.

2.3. Characterization of γ-CD-NMA

2.3.1. Elemental analysis

The content in N, C, H, and O was determined using a Carlo Erba 1108 analyser (Italy).

2.3.2. Proton nuclear magnetic spectra

^{1}H NMR spectra of γ-CD and γ-CD-NMA were obtained on a Varian Inova 750-MHz FT-spectrophotometer (USA), using deuterated water as solvent. The peaks of γ-CD were assigned as follows: H-C(1) = 5.11 ppm; H-C(2) = 3.65 ppm;

H-C(3) = 3.93 ppm; H-C(4) = 3.59; H-C(5) = 3.86 ppm; H-C(6) = 3.87 ppm (Kamigauchi et al., 2004).

2.3.3. FTIR analysis

IR spectra of γ-CD-NMA monomer were recorded over the range 400–4000 cm^{-1}, in a Bruker IFS 66V FT-IR spectrometer (Germany) using the potassium bromide pellet technique.

2.3.4. Differential scanning calorimetry (DSC)

The DSC experiments were carried out, in duplicate, using a DSC Q100 (TA Instruments, New Castle DE, USA) with a refrigerated cooling accessory. Nitrogen was used as purge gas at a flow rate of 50 ml/min. The calorimeter was calibrated for cell constant and temperature using indium standard (melting point 156.61 °C, enthalpy of fusion 28.71 J/g) and for heat capacity using sapphire standards. All experiments were performed using non-hermetic aluminium pans, in which 5–10 mg samples were accurately weighed, and then just covered with the lid. The samples were loaded on an autosampler tray. Samples for DSC study were program-heated from 25 to 300 °C, or heated from 25 to 150 °C, cooled to 0 °C, and then heated again until 300 °C, always at the rate of 10 °C/min.

2.4. Synthesis of hydrogels

Two sodium acrylate solutions were prepared by dissolving in water the monomer to reach final concentrations of 3 or 4 M. After to portions of these solutions, BIS was added up to a final concentration of 13 or 39 mM. To 10 ml of each resulting solution, γ-CD-NMA (50, 100, 150, 200, 250 or 300 mg) was added under stirring. The 24 resulting solutions, plus 4 reference solutions without γ-CD-NMA, were kept at 4 °C for 30 min, and then TEMED (100 μl) and APS (100 μl of a 4 wt.% solution) were incorporated into each of them. The solutions were immediately injected into moulds constituted by two glass plates (10 cm × 10 cm) separated by a 2 mm wide teflon frame, which were previously cooled at −18 °C. Polymerization was carried out, at room temperature, for 24 h. The hydrogels were carefully removed from the moulds, covered with water, and discs of hydrogel (10 mm diameter × 2 mm thickness) were immediately cut using a cork-borer. The discs were repeatedly washed with water for 7 days. The removal of unreacted monomers was monitorized by measuring the absorbance in the 190–800 nm range of the washing solutions. Once the absorbance was below 0.001 in the whole range, the discs were dried at 37 °C until constant weight.

2.5. Structural and mechanical characterization of hydrogels

2.5.1. FTIR analysis and differential scanning calorimetry (DSC)

The experiments were carried out as described above.

2.5.2. Swelling degree

The degree of swelling of the hydrogels was evaluated immersing the discs in aqueous media of pH 1.2 (0.1 N HCl), 3.0, 5.5, 6.8 or 8.0 (phosphate buffers) or in 40:60 ethanol:water, using the following expression:

$$\%\, Q = \frac{W_s - W_d}{W_s} \times 100$$

where W_s and W_d represent the weight of the disc at the swollen and at the dry states, respectively. The experiments were carried out in triplicate.

2.5.3. Rheometry

The storage (elastic, G') and the loss (viscous, G'') moduli of each hydrogel, fully swollen in water, were evaluated in triplicate at 25 °C, applying 0.5% strain and angular frequencies of 0.05–50 rad/s in a Rheolyst AR1000N rheometer (TA Instruments, UK) equipped with an AR2500 data analyzer, an environmental test chamber and a solid torsion kit. The samples were fixed between two clamps separated 9.0 ± 0.5 mm. The total analysis time of each sample (13 min) was short enough to prevent drying.

2.6. Drug loading

Dried hydrogel discs were loaded, at 37 °C, by immersion in 15 ml of drug solutions for 48 h, inside capped containers. Each experiment was repeated six times. For loading with TA, the drug solution (1 mg/ml) was prepared in 40:60 ethanol: water. The absorbance of each solution was measured at 240 nm (HP Agilent, Germany) before and after immersion of the hydrogel. For loading with PR, each disc was immersed in an aqueous drug solution of 0.070 mg/ml (30 ml) or 0.135 mg/ml (15 ml). The absorbance at 290 nm of the solutions was used to calculate the absorbed amount. Finally, the loaded discs were dried at 37 °C.

2.7. Drug release

The release experiments were carried out, at 37 °C, in 30 ml of water, 0.1 N HCl, or pH 6.8 phosphate buffer. TA-loaded hydrogels made with 3 M sodium acrylate and 39 mM BIS, with and without 9.4% of γ-CD-NMA, were evaluated. Samples of 2.5 ml of the release medium were withdrawn at pre-established times and replaced with the same volume of fresh solution. TA concentration in the samples was determined by an HPLC procedure adapted from Tymes (1977). Briefly, a LiChroCART® RP-18 (5 μm) column kept at 40 °C, an UV–vis detector (240 nm, L-4500 Diodearray Detector, Merck-Hitachi), and acetonitrile:water 40:60 (1.3 ml/min flow) were used. TA retention time was observed at about 3.5 min. The PR release experiments from hydrogels made with 3 or 4 M sodium acrylate and 39 mM BIS, without or with γ-CD-NMA, were carried out in a similar way, in 25 ml of medium, but the absorbance of the samples was measured spectrophotometrically at 290 nm (HP Agilent, Germany) and immediately returned to the container. The experiments were carried out by triplicate. Before analysing the samples, both analytical methods were validated for sensitivity, recovery, linearity, accuracy and precision.

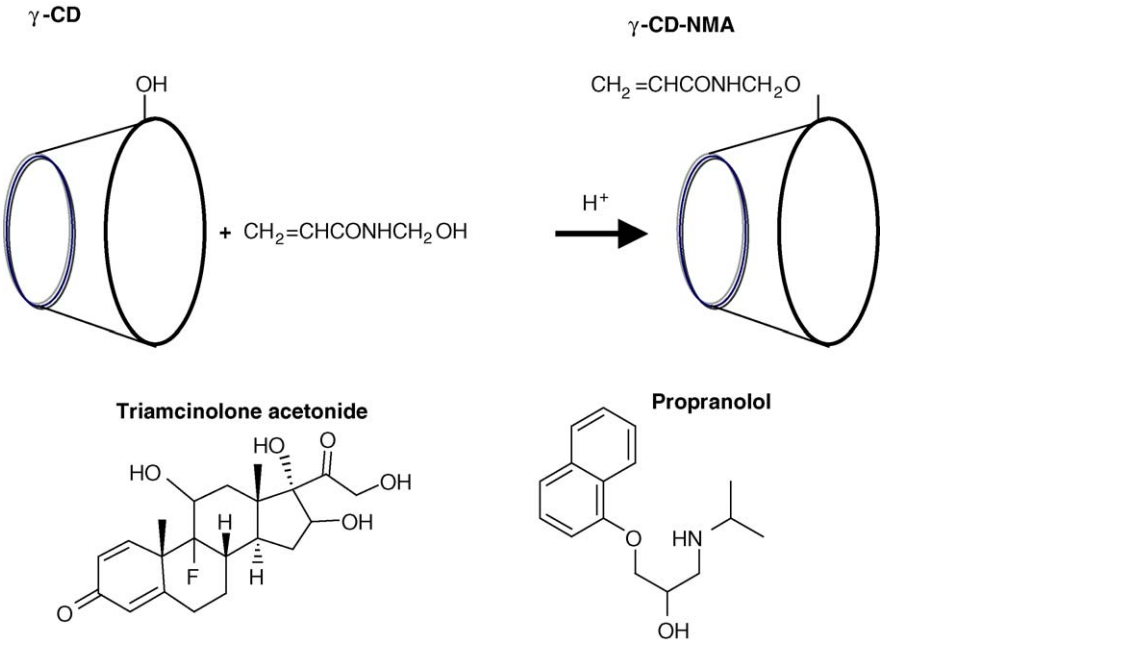

Fig. 1. The molecular structure and the reaction scheme of γ-CD with *N*-(hydroxymethyl)acrylamide (NMA), in acid medium, to obtain γ-CD-NMA, and drugs used for hydrogel loading and release studies.

2.8. Statistical analysis

To compare release profiles, the percentages of TA released at 0.5, 2 and 5 h and of PR released at 0.25 and 1 h were statistically analyzed using a one-way (hydrogel composition at each pH evaluated) analysis of variance and a pairwise Student–Newman–Keuls multiple comparison (Statgraphics Plus 5.1, Statistical Graphics Corp.).

3. Results and discussion

3.1. Synthesis and characterization of γ-CD-NMA

In order to synthesize the monomer of CD, γ-CD-NMA, the conditions were chosen to obtain 1 mmol of CH_2=CH– per gram of γ-CD (Fig. 1). Therefore, as reported by Lee et al. (2001) for the synthesis of β-CD-NMA, the initial NMA/CD mol ratio was 10. The reaction time (30 min) was enough to complete the process without a significant degradation of the γ-CD-NMA (Lee et al., 2001). This procedure is carried out in aqueous environment and uses acetone as the only organic solvent, avoiding toxic organic solvents which is very convenient to prepare materials intended for biomedical applications.

The formation of γ-CD-NMA was confirmed by elemental analysis, ^{1}H NMR and FTIR. The content in N (0.98%) and the proportion of vinylic protons (at 6.06 and 5.69 ppm; Hussain et al., 2005) indicated the presence of one acrylamidomethyl group per cyclodextrin molecule on average. Compared to the FTIR spectrum of γ-CD, the spectrum of γ-CD-NMA shows two clear additional bands at 1670 and 1570 cm^{-1}, which correspond to the amide I C=O stretching peak and to the amide II NH stretching peak (Fig. 2). A minor increase in a band with maximum at 1628 cm^{-1} related to the C=C vinyl stretching peak was also observed. These changes prove that γ-CD-NMA was indeed synthesized. γ-CD-NMA molecular weight is estimated as 1381 Da (from the molecular weight of one γ-CD and one NMA substituent).

The DSC scans of both the unmodified CD and γ-CD-NMA were similar, showing only an endothermic peak that is characteristic of solvent evaporation, around 100 °C. The temperature of decomposition of both products was above 250 °C (Rodríguez-Tenreiro et al., 2004).

3.2. Synthesis and structural characterization of hydrogels

The synthesis of the hydrogels was carried out in water by free radical polymerisation of sodium acrylate and γ-CD-NMA

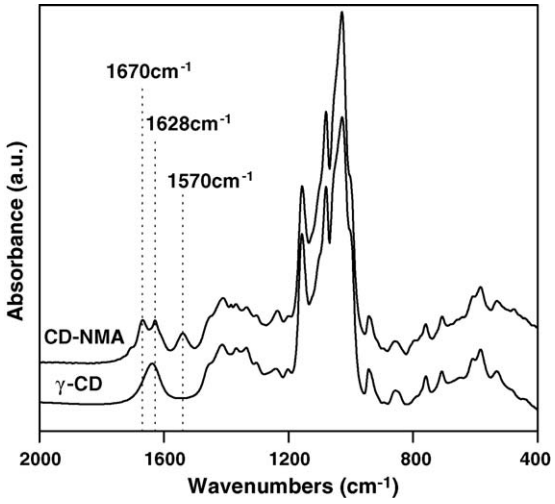

Fig. 2. FT-IR spectra of γ-CD and γ-CD-NMA.

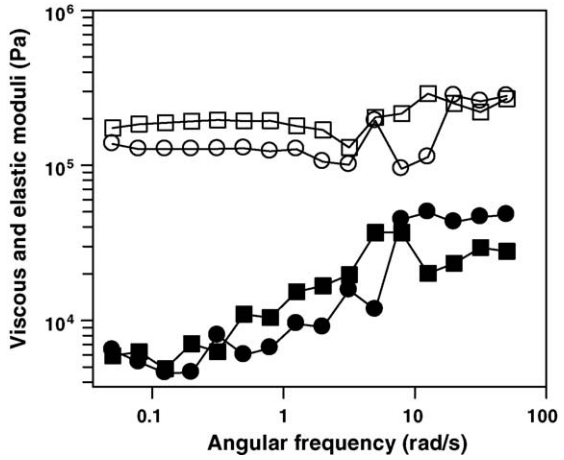

Fig. 3. Viscoelastic behaviour (G', open symbols; G'', solid symbols) of two representative 4 M sodium acrylate and 39 mM BIS hydrogels prepared with (squares) or without (circles) γ-CD-NMA.

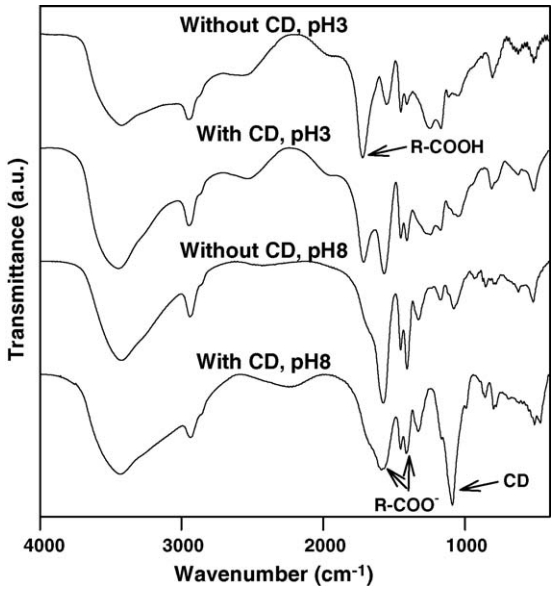

Fig. 4. FT-IR spectra of dried hydrogels synthesized with 4 M sodium acrylate and 39 mM BIS and different contents in γ-CD-NMA, as a function of the pH in which they were previously swollen.

in the presence of cross-linker BIS. γ-CD-NMA showed a high solubility in water and was easily dissolved (up to 300 mg) in the 10 ml monomer soup. After adding the initiators of reaction, gelation was observed to occur in the moulds in few minutes. However, 24 h were allowed to pass before removal from the moulds for a complete polymerisation. Hydrogels prepared with 3 M sodium acrylate and the lower BIS concentration (13 mM) were too fragile to be handled and, therefore, discarded. The other hydrogels, independently of their γ-CD-NMA proportion, were highly flexible and transparent after removal from the moulds and no visual differences were noticed among them. When subjected to an oscillatory stress, these all hydrogels showed G' and G'' values (Fig. 3) of around 10^5 and 10^4 Pa, respectively, and practically independent of angular frequency, which is characteristic of a well-structured polymer network. The presence of cyclodextrin in the network did not cause any relevant change in the viscoelastic properties of the hydrogels. The G' and G'' values were in the range considered adequate for hydrogels that combine a consistency enough to resist handling with a high flexibility (Hiratani and Alvarez-Lorenzo, 2004).

DSC scans showed that the glass transition temperature of the dried acrylate hydrogels was not modified when copolymerised with γ-CD-NMA, which was around 130 °C. This means that γ-CD-NMA does not act as plasticizer; being therefore effectively attached to the network. Liu and Fan (2002) found similar results when a maleic-derivative of β-CD was copolymerised with *N*-isopropylacrylamide.

The FTIR spectra of hydrogels that were dried after removal from acid medium, showed the characteristic bands of acrylic acid groups at 1720 cm^{-1} (Fig. 4). A pH 8 buffer solution neutralized the acrylic acid groups and, in consequence, after immersion of hydrogels in this medium, the FTIR spectra showed a decrease of the intensity of this band and an increase in the peaks at 1570 and 1407 cm^{-1} (Juang and Storey, 1998). The presence of cyclodextrins becomes evident by a broadening of the hydroxyl group band around 3400–3300 cm^{-1}. At an acidic pH, the characteristic peaks of saccharide ether groups around 1000–1200 cm^{-1} were overlapped with those of the acrylic groups, when the hydrogels were neutralized, the cyclodextrin bands were clearly evident (Fig. 4), confirming the copolymerisation of γ-CD-NMA.

The hydrogels prepared with γ-CD-NMA at a proportion around or above 5% (w/w total monomer content) swelled significantly more in ethanol:water (Fig. 5A) and in pH acid media (Fig. 5B) than hydrogels without γ-CD-NMA. In general, the degree of swelling of hydrogels with ionisable moieties mainly depends on four variables: content in ionizable groups and cross-linking density of the network, and pH and ionic strength of medium (Ricka and Tanaka, 1984; Alvarez-Lorenzo and Concheiro, 2002; Zhang et al., 2004). Sodium acrylate hydrogels without γ-CD-NMA showed the typical shrunken-to-swollen phase transition in water at pH above the pK_a of their carboxylic groups, i.e. 4.5 (Barreiro-Iglesias et al., 2004). It is interesting to note that the swelling degree of these hydrogels at pH 3 was greater when the hydrogels were immersed directly in this buffer from water, than when they were previously immersed in pH 1.2 buffer to have all acrylic groups in the acid form (Fig. 5B). In the first case, the ionisation of acrylic acid groups, which remain neutralized with sodium ions, prevented the collapse of the network structure at pH 3 (dotted lines). The incorporation of γ-CD-NMA to the hydrogels significantly contributes to increase the swelling degree in aqueous solutions of acidic pH owing to the hydrophilic character and the bulky structure of CD molecules, which disturb the hydrogen bonding interactions among the acid groups of the acrylic network (Rodríguez-Tenreiro et al., 2004). On the other hand, the presence of γ-CD-NMA in the hydrogel enhances its affinity for the ethanol:water 40:60 solutions, because of the greater solubility of γ-CD-NMA in this less polar medium, compared to sodium acrylate. Since the maximal degree of swelling in water and the G' and G'' values of all hydrogels were similar, independently

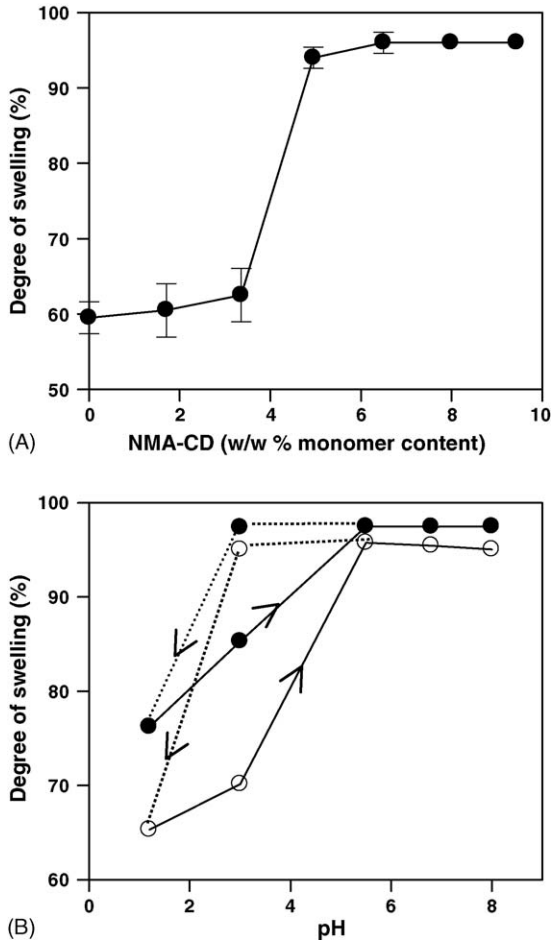

Fig. 5. Dependence of degree of swelling of hydrogels synthesized with 3 M sodium acrylate and 39 mM BIS on the content in γ-CD-NMA (A; ethanol: water 40:60 medium) and on pH (B; hydrogels prepared without (◯) or with 9.4% of γ-CD-NMA (●)). A hysteresis cycle was observed for the dependence of the degree of swelling on pH: the swelling at pH 3 was larger for hydrogels that were previously immersed in a neutral pH medium (dotted lines) than for hydrogels that were immersed in pH 1.2 solution (continuous lines). Standard deviation values are shown for each data point ($n = 3$) on the graph.

of their content in γ-CD-NMA, it can be concluded that copolymerisation with γ-CD-NMA did not caused an increase in the cross-linking density of the acrylic hydrogels (Alvarez-Lorenzo and Concheiro, 2002).

3.3. Triamcinolone loading and release

Hydrogels prepared without γ-CD-NMA were only able to load small amounts of TA (0.6 mg/g, with S.D. of 0.2), owing to the lack of the affinity of the drug for the hydrogel components and to the low degree of swelling in the ethanol/water medium (Fig. 5A) used for TA loading because solubility requirements (Klein et al., 2000; Ahn et al., 2002). When the amount of γ-CD-NMA used in the synthesis was 150 mg or above, the hydrogels could notably swell and sorb the drug. The greater degree of swelling of the discs may make the diffusion easier of drug molecules inside the network, to be efficiently hosted by the CD cavities and, in consequence, loaded into the hydrogel. Hydrogels prepared with a 9.4% γ-CD-NMA (i.e. 300 mg of γ-CD-NMA upon synthesis) were able to load 32 (S.D. 2) mg TA/g gel. TA can form inclusion complexes with free γ-CD with a 1:1 stoichiometric ratio and an affinity constant of 9920 M^{-1} (Fundueanu et al., 2003). Molecules with such a high binding constant to cyclodextrin are commonly used to determine the amount of cyclodextrin present in cross-linked networks, when the molecules are only taken up by the cyclodextrin cavities as in this case (Fundueanu et al., 2003, 2004). Assuming that all γ-CD-NMA monomers used to prepare the hydrogel were incorporated to the network and taking into account the stoichiometry of the inclusion complexes, a loading of 1.09 (S.D. 0.03) mol of TA per mol of γ-CD-NMA was obtained. This clearly indicates that, indeed, practically all γ-CD-NMA monomers were successfully copolymerised into the hydrogel.

To analyse the TA release behaviour from the hydrogels, those made from 3 M sodium acrylate and 39 mM BIS, without and with 9.4% of γ-CD-NMA, were immersed in water, 0.1 N HCl (pH 1.2) or pH 6.8 phosphate buffer (Fig. 6). Statistically significant differences ($\alpha < 0.05$) in released amounts were observed between hydrogels with or without 9.4% of γ-CD-NMA, at 0.5, 2 and 5 h. Hydrogels prepared with γ-CD-NMA can sustain the release for 24 h. It is interesting to note that despite the remarkably different degree of swelling of the hydrogels at these pHs, the release rate was similar in both media and also in water. This means that the high affinity for the CD cavity controls the release of this hydrophobic drug, independent to the volume phase transition of the hydrogels. This finding together with the well-known bioadhesive properties of the acrylic acid hydrogels in acidic medium (Edsman and Hägerström, 2005) make them potentially useful as platforms for controlled delivery systems to administer TA by transmucosal vias, e.g., buccal and vaginal (Ahn et al., 2002; Nicolazzo et al., 2005).

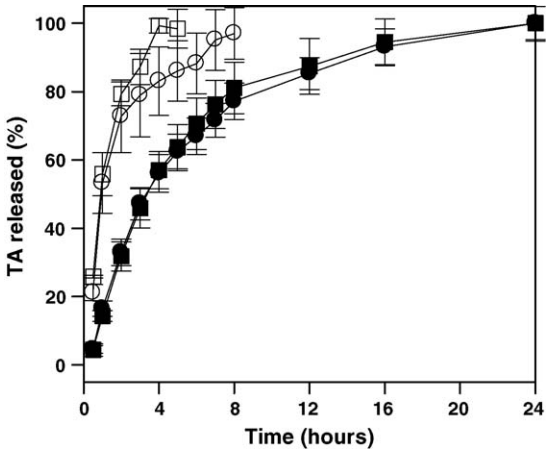

Fig. 6. Triamcinolone release profiles, in 0.1 N HCl (◯, ●) and in pH 6.8 phosphate buffer (□, ■) from hydrogels synthesized with 3 M sodium acrylate and 39 mM BIS without (◯, □) or with 9.4% of γ-CD-NMA (●, ■). Note that the content in TA of hydrogels prepared without γ-CD-NMA was significantly lower (0.6 mg/g-dried gel) than that of hydrogels prepared with 9.4% γ-CD-NMA (32 mg/g-dried gel). Standard deviation values are shown for each data point ($n = 3$) on the graph.

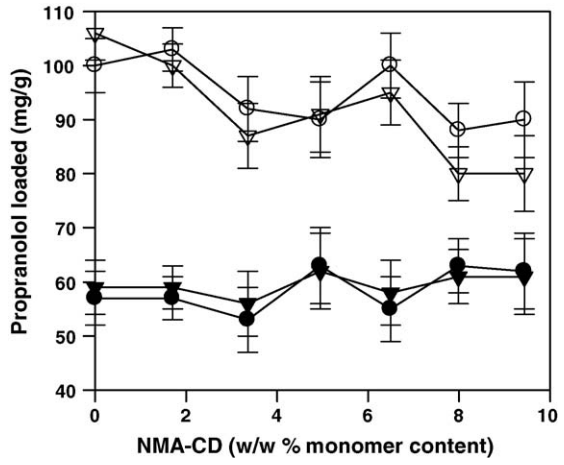

Fig. 7. Propranolol loaded by hydrogels made with 3 M (◯, ▽) or 4 M (●, ▼) sodium acrylate without or with different contents in γ-CD-NMA. Loading was carried out in 30 ml of 0.070 mg/ml (◯, ●) or 15 ml of 0.135 mg/ml (▽, ▼) of propranolol HCl aqueous solutions. Standard deviation values are shown for each data point ($n = 6$) on the graph.

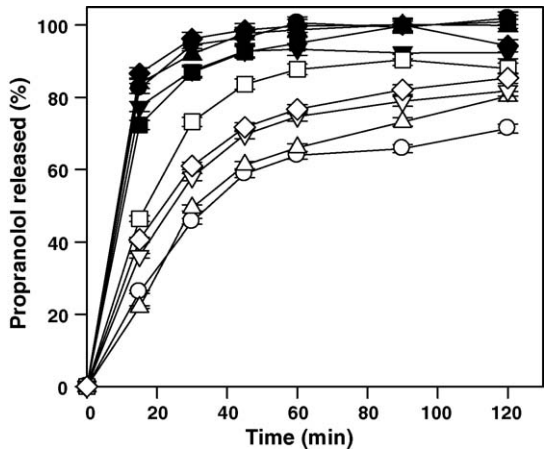

Fig. 8. Propranolol release profiles, in 0.1 N HCl (full symbols) and in phosphate buffer pH 6.8 (open symbols) from hydrogels synthesized with 3 M sodium acrylate, 39 mM BIS and different proportions of γ-CD-NMA (1.70% ◯, ●; 3.36% □, ■; 4.95%, △, ▲; 6.50% ▽, ▼; 7.99% ◇, ♦). Standard deviation values are shown for each data point ($n = 3$) on the graph.

3.4. Propranolol loading and release

All hydrogels showed a great affinity for PR and were able to sorb more than the 80% of the drug in solution. The amounts loaded were similar by immersion of the discs both in 30 ml of 0.070 mg/ml or 15 ml of 0.135 mg/ml of PR in water, i.e. only dependent on the PR quantity but independent of the PR concentration in the outer solution. The loading was significantly larger for gels prepared with 3 M acrylate compared with those synthesized with 4 M acrylate (Fig. 7). The highest value was observed for 3 M acrylate hydrogels without γ-CD-NMA; the incorporation of γ-CD-NMA resulting in a progressive decrease of the loading, which is related to a diminishing in the content in acrylic acid groups per mass of hydrogel. The lower amount of PR loaded by hydrogels synthesized with 4 M acrylate can be attributed to a steric hindrance that makes it difficult for drug molecules to interact with all acrylic acid groups in this network. Although the cross-linking degree (as the molar ratio of cross-linker to AA) of 4 M acrylate hydrogels is lower than that of 3 M acrylate hydrogels, the former ones contain a greater number of monomers by volume unit. This can create a steric impediment for the penetration of more drug molecules in the network. All 4 M acrylate hydrogels had a similar loading capability disregarding their content in γ-CD-NMA.

The main driving force for loading seems to be the electrostatic interaction between the protonized amino group of the drug and the ionised acrylic acid groups of acrylate moieties (Tarvainen et al., 1999; Blanco-Fuente et al., 2002). Strong ionic interactions of poly(acylic acid) Carbopol® gels with other cationic drugs such as procaine (Realdon et al., 1998) or metoclopramide (Jimenez-Kairuz et al., 2003) have been already reported. We have previously shown that the addition of β-CD (not bound) and the formation of the PR/β-CD inclusion complexes (affinity constant of 220 M^{-1}) hinder Carbopol®/PR interactions and, in consequence, modify the swelling, bioadhesion, and release properties of hydrogels obtained by a compression/heating procedure (Blanco-Fuente et al., 2002). Despite the affinity constant of propranolol for γ-CD is expected to be greater than for β-CD (Fundueanu et al., 2003), the results shown in Fig. 6 indicate that once the CDs are attached to the network, they do not prevent such an interaction of PR with the acrylic acid moieties. In this sense, an adequate distribution of the CD cavities and the ionic groups in the network may even create adequate receptors for the cationic drug, allowing simultaneous interaction with both components of the hydrogel. This effect is not shown in an enhanced loading as more CD is copolymerised, but it may increase the overall affinity of the drug for the binding sites inside the hydrogel (Piletsky et al., 1999).

The release of PR (Fig. 8) in 0.1 N HCl was faster (80% in 15 min) than in the buffer pH 6.8 (around or less than 80% in 120 min) despite the discs were almost collapsed at acid pH and completely swollen near neutral pH (see Fig. 5). Again, this is in contradiction with a drug release mechanism controlled by a simple volume phase transition. The greater release rate at acid pH (significant differences $\alpha < 0.05$ between the release at each pH from each hydrogel) is explained by the higher solubility of the drug at this pH, with a consequent decrease in its affinity for the CD cavity and, especially, by the competition of the hydrogen ions of the medium for the binding to the acrylic acid groups ($pK_a = 4.5$). These factors prompt a fast release of the drug at acid pH despite the shrinking of the network. In buffer pH 6.8, both the PR molecules and acrylic acid residues are ionized, but the high ionic strength of phosphate buffer weakens their electrostatic interactions and facilitates the release. Nevertheless, some ionic associations are still expected, which together with some affinity of the drug for the cyclodextrin cavities, provides at pH 6.8 a weak control of the release process through the swollen matrix. No clear relationship between the PR release rate and the proportion of γ-CD-NMA in the network could be found.

4. Conclusions

Copolymerization of an acrylic monomer of γ-CD with acrylate sodium allows hydrogels that combine good mechanical properties and pH-sensitive swelling behaviour with an improved loading and release capacity for hydrophobic drugs with high affinity for the CD cavities, such as triamcinolone acetonide, to be obtained. These properties make them potentially interesting in the design of controlled release systems, based on hydrogels, for administration of hydrophobic drugs by different routes.

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