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Cross-linked hydroxypropyl-β-cyclodextrin and γ-cyclodextrin nanogels for drug delivery: Physicochemical and loading/release properties

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ABSTRACT

Due to their size and high surface-to-volume ratio, nanogels can give some unique drug delivery opportunities. A novel technique to prepare cyclodextrin (CD) nanogels, in which the cross-linking takes place simultaneously with an emulsification/solvent evaporation process, has been implemented. The aqueous phase consisted of γ-cyclodextrin (γCD) or hydroxypropyl-β-cyclodextrin (HPβCD) at a fix concentration of 20% (w/w) with or without hydroxypropyl methylcellulose (HPMC) or agar at various concentrations. The incorporation of the cross-linking agent, ethyleneglycol diglycidyl ether (EGDE), was essential for the nanogel formation. By contrast, nanogels could be formed in the absence of surfactant such as Span 80, which can be attributed to the emulsion stabilizing effect of CDs by forming inclusion complexes with the organic solvent at the interface. Gas chromatography–mass spectrometry (GC–MS) analysis of the nanogels confirmed that dichloromethane levels were below the safety limit and, therefore, that these conditions of the organic solvent evaporation (60 °C for 180 min) led to nanogels that satisfy residual solvent requirements. Infrared analysis (IR), transmission electron microscopy (TEM) and dynamic light scattering (DLS) provided information about the cross-linking degree, the size and the size distribution of the nanogels. The ability of the nanogels to host a molecule that can form inclusion complexes and to sustain its release was tested using 3-methylbenzoic acid (3-MBA) as a probe with a high affinity for both β-cyclodextrin (βCD) and γCD. Permeability tests confirmed that 3-MBA was indeed taken up by the nanogels and then slowly released.

1. Introduction

Hydrogels are three-dimensional hydrophilic polymer networks (Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009) that can take up several times their weight in water, which endows them with biocompatibility features. However, residual reagents or organic solvents used during their synthesis can lead to toxic reactions. Hydrogels can be prepared as macroscopic networks or they can be confined to smaller dimensions leading to microgels (>1 μm) or even nanogels (<1 μm) (Oh, Drumright, Siegwart, & Matyjaszewski, 2008). Nanogels combine the advantages of hydrogels and nanocarriers. The nanometric size and the high surface-to-volume ratio of nanoparticulate drug delivery systems bestow them with unique technological advantages regarding enhancement of drug bioavailability and drug targeting (Stefánsson & Loftsson, 2010). Compared to liposomes and micelles, nanogels are more physically stable and do not disassembly upon dilution in physiological fluids (Sanson & Rieger, 2010). Nano-, micro- and macro-hydrogels can be created according to either the "bottom-up" or the "top-down" approaches (Kumar & Khan, 2010). The "bottom-up" method starts form the building blocks (molecules, clusters) that are subsequently linked together by physic interactions or chemical bonds (Daoud-Mahammed, Couvreur, & Gref, 2007; Gref et al., 2006; Stupp et al., 1997). This approach enables a precise control of the structure and morphology of the networks. The "top-down" method generates nanoparticles by physical, chemical or mechanical grinding of large particles or clusters (Lyuksyutov, Paramonov, Dolog, & Ralich, 2003).

The advantages of nano-sized hydrogels as drug carriers include ease dispersion in aqueous media, suitability for both passive and active drug targeting, enhanced bioavailability of both low and high molecular weight drugs, and, when adequate chemical groups are present, stimuli-triggered drug release (Guerrero-Ramirez, Nuno-Donlucas, Cesteros, & Katime, 2008a, 2008b; Kabanov & Vinogradov, 2009; Samah, Williams, & Heard, 2010; Wang, Xu,

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Yang, & Yang, 2008a, 2008b, 2008c). Limitations regarding drug loading and ability to control drug release have prompted the search for building blocks that possess high affinity for specific drugs through electrostatic, van der Waals and/or hydrophobic interactions. However, enhanced drug loading can lead to the collapse of the nanogel matrix structure and phase separation and, consequently, to a strong entrapment of drug molecules. To avoid phase separation, the nanogel matrix should possess a hydrophilic surface (Kabanov & Vinogradov, 2009). Incorporation of cyclodextrins (CDs) to the matrix structure has a two-fold aim; namely, (1) to provide an affinity-based mechanism of drug loading and control of drug release through inclusion complex formation, and (2) to enhance the hydrophilicity of the polymer matrix. The benefits of CD incorporation to conventional macrohydrogels for sustained delivery have already been demonstrated (Alvarez-Lorenzo, Hiratani, & Concheiro, 2006; Moya-Ortega, Alvarez-Lorenzo, Sigurdsson, Concheiro, & Loftsson, 2010). As occurred when dissolved in water, CDs attached to polymer networks can form complexes with hydrophobic drugs of suitable size (Loftsson & Duchene, 2007; Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2006; Santos et al., 2009; Zhang, Xue, Gao, Huang, & Zhuo, 2008). Nevertheless, the environment provided by the network may interfere with the drug-CD interaction; the dissociation of the complexes being more difficult due to the high local concentration of CDs (Rodriguez-Tenreiro, Alvarez-Lorenzo, Rodriguez-Perez, Concheiro, & Torres-Labandeira, 2007; Jansook & Loftsson, 2009). Previously, β-cyclodextrin (βCD) microgels were synthesized by inverse-emulsion polymerization, using epichlorohydrin as cross-linker and paraffin as organic phase (Liu, Fan, Kang, & Sun, 2004). More recently, miniemulsion polymerization has been applied to prepare α-cyclodextrin (αCD) nanoparticles (185–1600 nm) cross-linked with isophorone diisocyanate in hexadecane (Baruch-Teblum, Mastai, & Landfester, 2010).

The aim of the present work is to develop a versatile simple emulsion-solvent evaporation technique for cross-linking of CDs to form nanogels. This technique allows for incorporation of other saccharides or saccharide derivatives, such as cellulose ethers or agar–agar, into the matrix structure. Here we report the synthesis, physicochemical characterization, and loading/release performance of γ-cyclodextrin (γCD) and 2-hydroxypropyl-β-cyclodextrin (HPβCD) nanogels. These CDs were chosen due to their ocular compatibility. The ultimate purpose of the project is to apply the nanogels developed to topical delivery to the eye of drugs that require a sustained delivery, such as antiglaucoma or antimicrobial agents or anti-inflammatory drugs (Saarinen-Savolainen, Järvinen, Araki-Sasaki, Watanabe, & Urtti, 1998).

2. Materials and methods

2.1. Materials

γ-Cyclodextrin (γCD, W8) and 2-hydroxypropyl-β-cyclodextrin (HPβCD, W7 HP, Mw 1309.24 Da) were purchased from Wacker (Barcelona, Spain), hydroxypropyl methylcellulose (HPMC, Methocel K4M Premium EP) from Colorcon Iberica S.L. (Barcelona, Spain), agar–agar from Guinama (Valencia, Spain), ethylene glycol diglycidyl ether (EGDE, 50% (w/w) solution in water) from Fluka (St. Louis, IL, USA), dichloromethane (CH_2Cl_2) reagent grade (stabilized) from Normasolv, Scharlau, S.A. (Barcelona, Spain), sorbitan monooleate (Span 80) from Fluka (Schnelldorf, Germany), 3-methyl benzoic acid (3-MBA) from Merck (Darmstadt, Germany). The water used was purified by reverse osmosis (MilliQ®, Millipore, Spain). Other reagents used were of analytical grade.

2.2. Synthesis of CD nanogels

An emulsion-solvent evaporation technique with simultaneous cross-linking was implemented as follows.

2.2.1. Preparation of the aqueous phase

Several aqueous phases of different composition were prepared. 4 ml of EGDE were added to 10 ml of a 20% (w/w) γCD or HPβCD solution in aqueous 0.2 M sodium hydroxide solution and the solution stirred for 5 min. Separately, HPMC was dispersed into aqueous 0.2 M sodium hydroxide solutions to obtain 1% (w/w) and 2% (w/w) HPMC solutions. γCD (2 g) was added to 10 ml-aliquots of the HPMC solutions. In the same way, agar–agar was dispersed into aqueous 0.2 M sodium hydroxide solution to obtain 1% (w/w) solution and 2 g of HPβCD were added to 10 ml of this solution. After homogenization, 4 ml of EGDE were added under stirring. EGDE was not added to one of the mixtures to evaluate its role on the formation of the nanogels. The solutions were heated at 60 °C for 25 min to initiate the cross-linking reaction.

2.2.2. Preparation of the organic phase

Span 80 was dissolved at various concentrations (0, 0.5, 1 or 2%, w/v) in dichloromethane.

2.2.3. Preparation of the W/O emulsions

The aqueous phase (14 ml) was added to the organic phase (20 ml) and the mixture was homogenized using an Ultra-Turrax T25 (Janke & Kunkel, INK-Labortechnik, Germany) at 8000 rev./min for 30 s. The emulsion was kept under magnetic stirring (100 rev./min) at 60 °C for 30 min.

2.2.4. Breaking of the emulsions

Immediately following heating of the W/O emulsions, the emulsions were poured into 100 ml of distilled water and stirred at 60 °C for 180 min for the complete evaporation of CH_2Cl_2.

2.2.5. Dialysis of the nanogels dispersions

Aliquots of each resulting colloidal system (50 ml) were transferred to dialysis bags (MWCO 12–14 kDa) and then placed into beakers containing water. The water was replaced every 12 h during 3 days in order to eliminate impurities. The colloidal dispersions were then frozen in liquid nitrogen, kept at −80 °C and lyophilized (VirTis Genesis freeze-dryer, USA).

The nanogel formulations were identified using the code *Cyclodextrin-polysaccharide*$_{x,y}$, being *cyclodextrin* γCD or HPβCD, *polysaccharide* HPMC or agar, *x* the concentration of HPMC or agar–agar (0, 1 or 2%) in the aqueous phase and *y* the concentration of Span 80 in the organic phase (0, 0.5, 1 or 2%) used during preparation of a given nanogel (Table 1).

2.3. Dichloromethane residual content

Gas chromatography (Finnigan Trace GC ultra Thermo, USA)-mass spectrometry (Finnigan Trace DSQ Thermo, USA) was used to determine residual CH_2Cl_2 in the freeze-dried nanogels applying the head space technique. The samples were analyzed in full-scan and single ion monitoring (SIM) modes, according the conditions indicated in the supporting information SI1. Dichloromethane quantification limit was 1 ppm.

2.4. Characterization of γCD nanogels

2.4.1. FTIR analysis

IR spectra of γCD nanogels were recorded over the range 400–4000 cm^{-1}, in a Bruker IFS 66 V FT-IR spectrophotometer

Table 1
Surfactant concentration, yield of the preparation process, and results of the DLS analysis of nanogels suspensions, reported as peak intensity area, hydrodynamic radio (r_h) and mass distribution (M). The nanogels are identified by the cyclodextrin and the polysaccharide (if any) with a numeric subindex, which indicates the concentration of the polysaccharide in the aqueous phase followed by the concentration of Span 80 in the organic phase.

Nanogel type	Span 80 (%)	Yield (%)	Peak	Area	r_h (nm)	M (%)
$\gamma CD_{0,0}$	0	34.4	1	6.14	4.15	100
$\gamma CD_{0,0.5}$	0.5	34.6	1	15.22	10.82	99.53
			2	46.39	93.68	0.47
$\gamma CD_{0,1}$	1	26.0	1	17.74	73.71	75.57
			2	883.35	395.06	24.43
$\gamma CD_{0,2}$	2	26.2	1	0.68	13.75	75.62
			2	293.79	151.36	24.38
$\gamma CD_{0,4}$	4	45.7	–	–	–	–
$\gamma CD\text{-}HPMC_{1,0.5}$	0.5	28.0	1	58.16	8.51	100
$\gamma CD\text{-}HPMC_{1,1}$	1	18.7	1	0.22	2.02	98.99
			2	0.52	13.75	0.73
			3	128.59	119.08	0.28
$\gamma CD\text{-}HPMC_{1,2}$	2	19.1	1	0.05	3.26	86.95
			2	0.73	22.22	3.78
			3	134.91	93.68	9.27
$\gamma CD\text{-}HPMC_{2,0}$	0	11.8	1	57.11	8.51	100
$\gamma CD\text{-}HPMC_{2,0.5}$	0.5	29.3	1	167.09	22.22	100
$\gamma CD\text{-}HPMC_{2,1}$	1	24.6	1	1.23	6.70	96.99
			2	104.10	93.68	3.01
$\gamma CD\text{-}HPMC_{2,2}$	2	28.6	1	0.19	10.82	59.82
			2	171.78	119.08	40.18
$HP\beta CD_{0,0.5}$	0.5	35.7	1	1.67	2.02	99.77
			2	90.76	57.99	0.23
$HP\beta CD_{0,1}$	1	30.4	1	0.07	2.57	89.39
			2	0.53	10.82	9.05
			3	5.90	55.62	1.34
			4	144.03	244.53	0.21
$HP\beta CD\text{-}Agar_{1,0.5}$	0.5	26.5	1	6.10	3.26	99.85
			2	124.55	73.71	0.15
$HP\beta CD\text{-}Agar_{1,1}$	1	11.7	1	1.81	3.26	99.63
			2	330.67	119.08	0.37

(Bruker, Ettlinger, Germany) using the potassium bromide pellet technique.

2.4.2. Transmission electron microscopy (TEM)

5 μl drops of the colloidal nanogel systems were placed on grids covered with carbon film. After 1 min, the excess of sample was carefully removed with a tip of filter paper. The samples were dried and observed using a Philips CM-12 TEM apparatus (FEI Company, The Netherlands) set up at 100 kV. The magnification to observe the nanogels was 66,000- or 110,000-fold and the diameter was measured using a calibrated scale. Samples were sufficiently dense to direct observation; that is, staining with solutions of heavy metal was not necessary.

2.4.3. Dynamic light scattering (DLS)

The DLS measurements were performed using an ALV-5000 F optical system equipped with CW diode-pump Nd:YAG solid-state laser (400 mW) operated at 532 nm (Coherent Inc., Santa Clara, CA, USA). The intensity scale was calibrated against scattering from toluene. The nanogels dispersions were filtered (Millipore® 0.45 μm, Ireland) into the quartz cell (previously washed with condensing acetone vapor) and maintained between 20 and 37 °C. The diffusion coefficient was deduced from the standard second-order cumulant analysis of the autocorrelation functions measured at 90° angle. The experiments were carried out in triplicate and the apparent hydrodynamic radius ($r_{h,app}$) of the nanogels was calculated from the apparent diffusion coefficient applying the Stokes-Einstein equation (González-Gaitano et al., 2002; Ukhatskaya et al., 2010):

$$d = \frac{kT}{3\pi\eta D^*} \quad (1)$$

where d is the hydrodynamic diameter (m), k the Boltzmann constant (J/K), T the temperature in Kelvin degrees, η the solvent viscosity (kg/(m s)) and D^* the diffusion coefficient (m^2/s). Size distribution graphs, which represent dependences of relative intensity of scattered light versus hydrodynamic radii of nanogels, were drawn. The method employed for characterization of cyclodextrin (González-Gaitano et al., 2002) and aminocalix (Ukhatskaya et al., 2010) aggregation was used in order to estimate the mass distribution of the nanogels between size populations existing in the colloidal system. The mass distribution was calculated using the equation:

$$M_i = \frac{A_i/R_i^a}{\sum A_i/R_i^a} \times 100 \quad (2)$$

where M_i is the mass distribution percentage, A_i is the intensity area, R_i is the hydrodynamic radius of the size population i and a is the shape parameter, which equals to 3 for spherical particles (González-Gaitano et al., 2002; Ukhatskaya et al., 2010).

2.4.4. Stability studies

Aqueous dispersions of freshly prepared nanogels were centrifuged at 5000 rpm for 10 min or 10,000 rpm for 30 min to test the tendency of nanogels to precipitate, simulating the aging process during storage.

2.5. In vitro permeation studies

The release of 3-MBA from aqueous dispersions of nanogels (2%, w/v) was tested using Franz diffusion cells (SES GmbH, Analysesysteme, Germany) consisting of donor and receptor compartments separated by a semi-permeable cellophane membrane (MWCO 3500), which was previously soaked overnight in water. The diffusion area was 0.785 cm^2. The donor phase (2 ml) consisted of aqueous 3-MBA solution (0.08 mg/ml). Freeze-dried nanogel powder, γCD or HPβCD (2%, w/v) was added to the solution and the system allowed to equilibrate for 60 h. For comparison permeation of 3-MBA was also determined from pure aqueous solutions containing no nanogel. The receptor phase (5.5 ml of water) consisted of pure water and was kept under constant stirring (magnetic bar, 300 rpm). Aliquots of the receptor phase (750 μl) were withdrawn at pre-established times, 3-MBA concentration was determined by UV spectrophotometry (λ = 281 nm; Agilent 8453, Germany), and the withdrawn aliquots returned to the corresponding cell. The release rate was characterized though the kinetic constant (K_H), i.e. the Higuchi constant, estimated as the slope of the linear section of the amount of drug in the receptor chamber versus the $t^{1/2}$ plots. The steady state flux was calculated as the slope of linear portion of the plot of the amount of drug released in the receptor chamber versus time (Jansook & Loftsson, 2009; Messner, Kurkov, Flavià-Piera, Brewster, & Loftsson, 2009).

3. Results and discussion

3.1. Synthesis of CD nanogels

The synthesis was based on our previous work on monolithic networks bearing CDs which were directly cross-linked with diglycidylethers under mild aqueous conditions (Alvarez-Lorenzo et al., 2006; Moya-Ortega et al., 2010; Rodriguez-Tenreiro et al., 2006; Rodriguez-Tenreiro, Alvarez-Lorenzo, et al., 2007; Rodriguez-Tenreiro, Diez-Bueno, Concheiro, Torres-Labandeira, & Alvarez-Lorenzo, 2007; Santos, Couceiro, Concheiro, Torres-Labandeira, & Alvarez-Lorenzo, 2008). Adaption of the bottom-up procedure of hydrogel synthesis from the macroscale to the nanoscale, while preserving the functional features of the bulk system, is a challenge (Sanson & Rieger, 2010). Furthermore, environmental concerns prompt search of a greenish synthesis approach that reduces the amount of organic solvents needed. Therefore, we implemented a technique in which the cross-linking takes place simultaneously with an emulsification/solvent evaporation process in order to render CD nanogels (Fig. 1).

The aqueous phase consisted of γCD or HPβCD at a fix concentration of 20% (w/w) with or without HPMC or agar. The incorporation of the cross-linking agent, EGDE, was necessary to obtain nanogels. In the absence of EGDE, nanogels were not formed and unbound γCD precipitated when the emulsion was broken. The EGDE:CD 1:1 (w/w) ratio was chosen in order to have sufficient cross-linking agent to react with 2 out of 3 hydroxyl groups present on each CD glucopyranose unit. This weight ratio had previously been identified as adequate to obtain well-structured conventional hydrogels (Rodriguez-Tenreiro et al., 2006). Once EGDE was added to the aqueous phase, the solution was heated for 25 min at 60 °C in order to trigger the reaction with the CDs before mixing with the organic phase. Less time led to the migration of EGDE to the organic

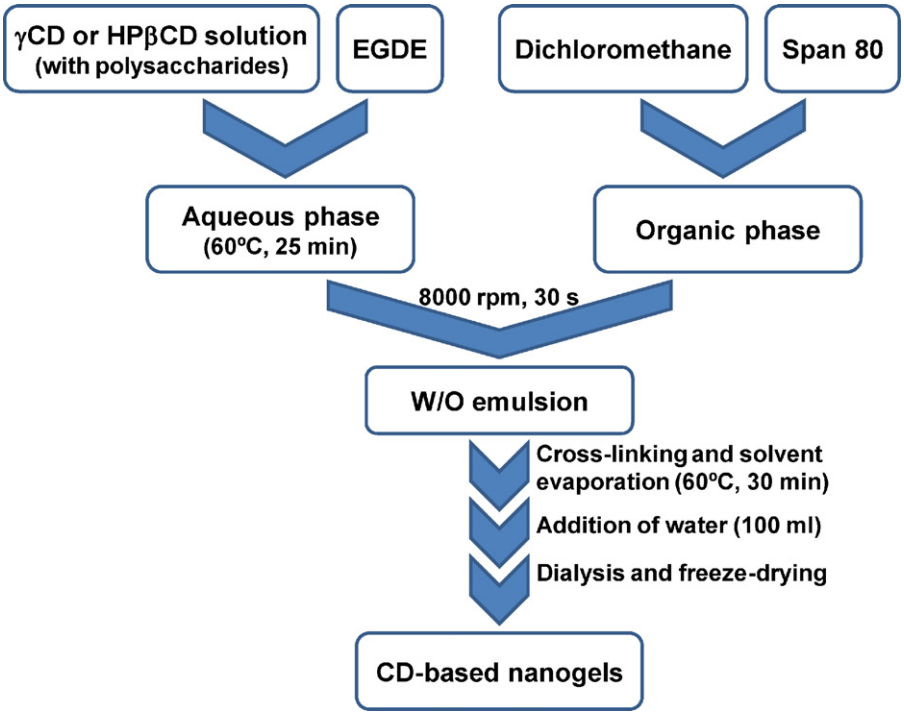

Fig. 1. Flow chart for the preparation of the nanogels.

solvent and a poor reaction yield, while heating for more than 30 min increased the risk of formation of a conventional hydrogel in the aqueous solution prior to the addition of the organic phase.

Various surfactant concentrations were tested (Table 1). However, nanogels could be obtained even in the absence of the surfactant (Span 80). CDs such as γCD and HPβCD can exert an emulsion stabilizing effect through complex formation between the organic solvent, dichloromethane, and CD at the water/dichloromethane interface (Inoue, Hashizaki, Taguchi, & Saito, 2009). This complex may have surfactant-like properties and, consequently, may exert an emulsion stabilizing effect. The size and distribution mass of the nanogels were affected by both the polysaccharide and the surfactant proportion, as discussed below.

3.2. Dichloromethane residual content

The acceptable amounts of residual dichloromethane in active substances, excipients and medicinal products after processing are regulated by guidelines of the International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) and the European Pharmacopoeia (Inoue et al., 2009). Dichloromethane is an EP Class 2 solvent, that is, a solvent associated with less severe toxicity which should be limited in order to protect patients from potential adverse effects. According to the EP the permitted daily exposure for dichloromethane is 6.0 mg/day and the concentration limit in a pharmaceutical product is 600 ppm, disregarding the dosage form and administration route. A GC–MS analysis of the nanogels revealed dichloromethane levels close to the quantification limit; namely 1 ppm. Therefore, the conditions used for the organic solvent evaporation (60 °C for 210 min) led to nanogels that satisfy residual solvent requirements.

3.3. Characterization of γCD nanogels

The formation of cross-links during the nanogel formation was evident by changes in the IR region of the ether bond signals of the lyophilized nanogels, as compared to those of pure γCD, HPβCD, HPMC and agar–agar (supporting information S2). The broadening of the bands in the 1100–1200 cm^{-1} region (due to stretching of C–O ether bonds) indicated that the CDs were indeed linked together through ether bonds, by the chemical reaction of EGDE epoxy groups with some CD hydroxyl groups. Particularly, there is a predominance of primary alcohols (1030–1050 cm^{-1}) in the raw CDs and polysaccharides compared to the signal of the ether bonds (1082–1117 cm^{-1}). By contrast, the nanogels displayed a strong increase in this latter signal. The increase in the absorbance ratio A_{1082}/A_{1033} is an index of the cross-linking density of the network. No signal appeared at 1250 cm^{-1} confirming that no unreacted EGDE remains in the hydrogels.

The morphology of CD nanogels, both freshly prepared and 2 days after rehydration of the freeze-dried powder, was observed using TEM (Fig. 2). No notable differences in the size and size distribution (10–400 nm) among the formulations before and after lyophilization were observed; that is, dried nanogels swelled in water to their original dimensions, i.e. the dimensions of freshly synthesized nanogels. A more precise analysis of the hydrodynamic radius and size distribution of the nanogels were carried out using DLS. The particle size mass-distribution (Table 1) was obtained as previously described (González-Gaitano et al., 2002; Ukhatskaya et al., 2010) assuming that the nanogels were spherical (Eq. (2)). Unimodal size populations were obtained for $\gamma CD_{0,0}$, γCD-$HPMC_{1,0.5}$, γCD-$HPMC_{2,0}$, and γCD-$HPMC_{2,0.5}$ nanogels (Fig. 3). Two size populations were observed in the case of $\gamma CD_{0,0.5}$, $\gamma CD_{0,1}$, $\gamma CD_{0,2}$, γCD-$HPMC_{2,1}$ and γCD-$HPMC_{2,2}$ nanogels. The smallest size population observed in each of the γCD-$HPMC_{1,1}$, γCD-$HPMC_{1,2}$, $HP\beta CD_{0,0.5}$, $HP\beta CD_{0,1}$, HPβCD-$Agar_{1,0.5}$ and HPβCD-$Agar_{1,1}$ nanogels corresponds to monomeric CD units that remain free in the nanogel dispersions. In general, the γCD nanogels were larger than the HPβCD nanogels and incorporation of HPMC or agar increased the size of the nanogels. The DLS results indicate that when no surfactant is present, or when just 0.5% Span 80 is added, CDs are the main species responsible for the droplets stability in the emulsion during the cross-linking. Under these conditions, the size distribution of the nanogels was narrower than when 1 or 2% Span 80 was present. The incorporation of Span 80 resulted in the formation of larger nanogels that coexisted with population of smaller nanogels.

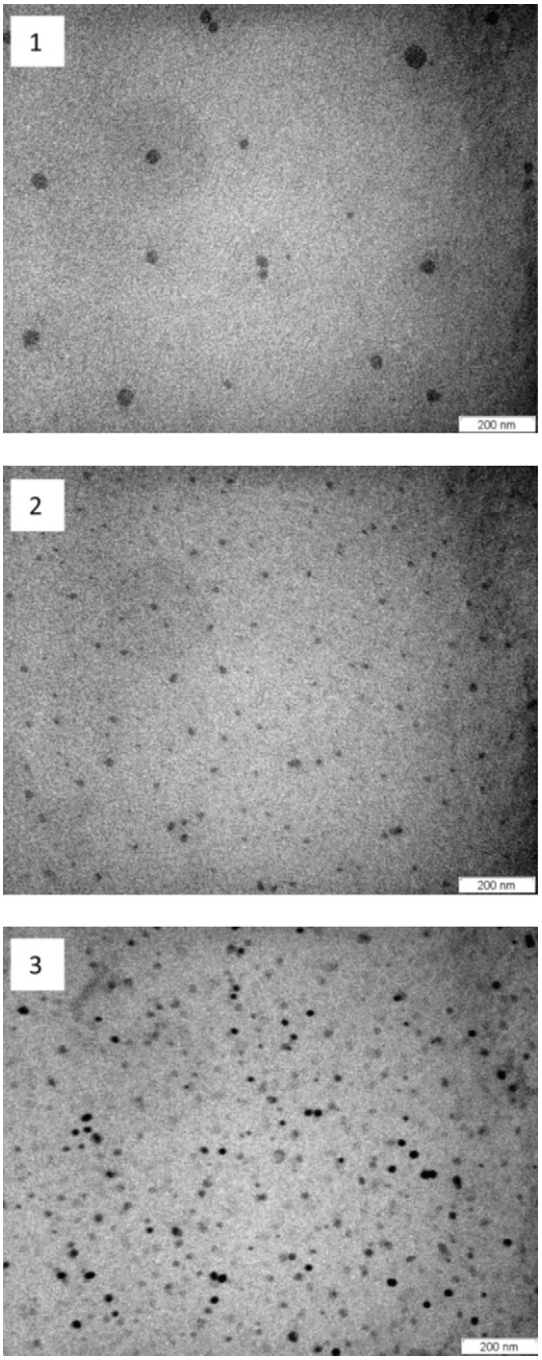

Fig. 2. TEM micrographs of $\gamma CD_{0,0}$ (1), γCD-$HPMC_{2,0.5}$ (2) and $HP\beta CD_{0,0.5}$ (3) after purification with a magnification of 66,000.

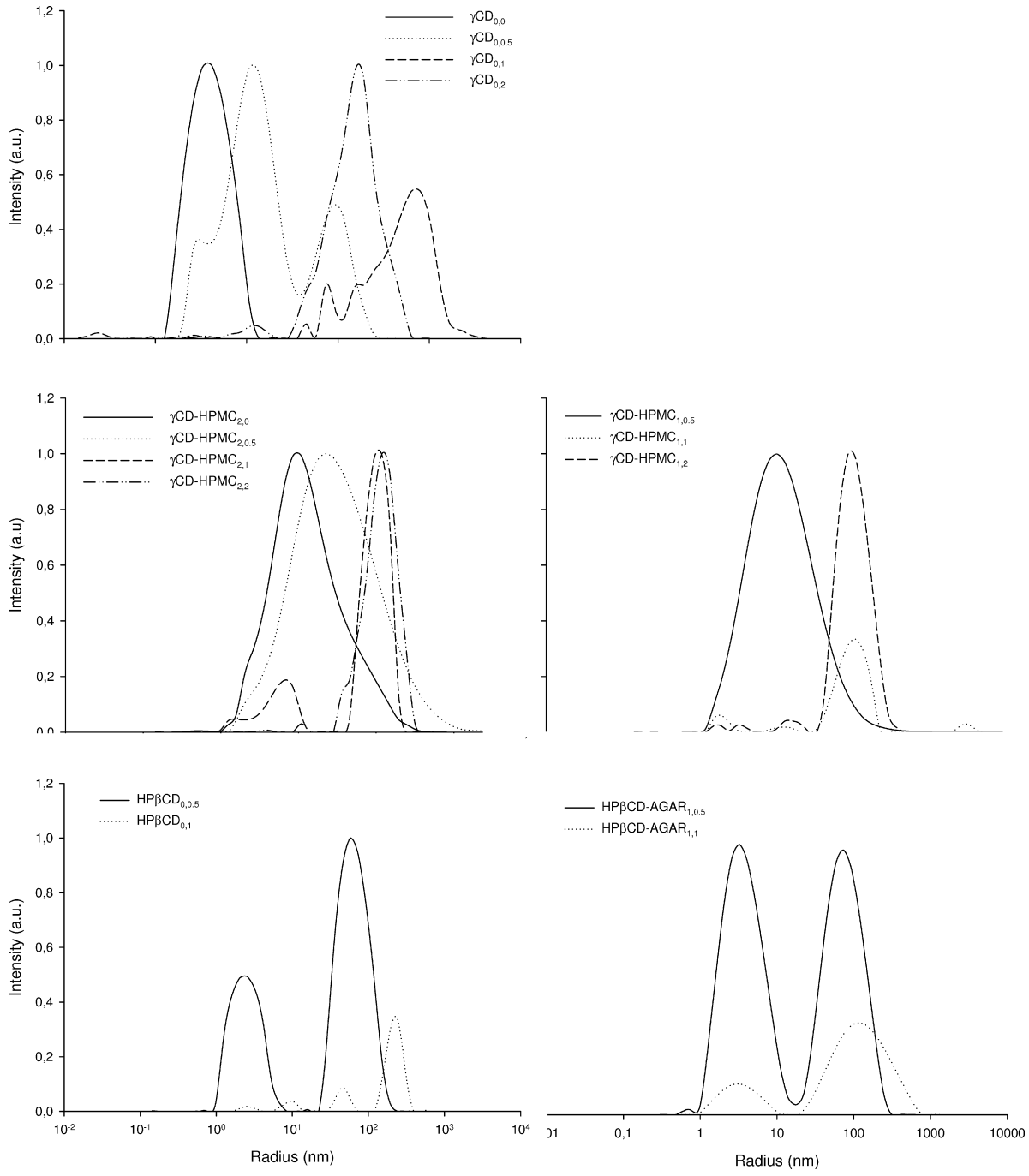

Fig. 3. Intensity fraction size distributions of the apparent hydrodynamic radius of nanogels, as measured by DLS.

3.3.1. Stability studies

The colloidal systems obtained after dispersion of the nanogels in water were subjected to centrifugation in order to test their tendency to precipitate. Centrifugation at 5000 rpm for 10 min did not cause any precipitation. After 30 min at 10,000 rpm a small amount of precipitate was formed and the precipitation was more intense in the case of γCD-HPMC than in the case of γCD nanogels. The supernatants were taken and filtered through a 0.22 μm filter, giving a still cloudy dispersion after filtration, which indicates that the non-precipitated nanogels had a diameter below the mesh size of the filter. The appearance of the systems is shown in Fig. 4. In all cases, the precipitate was easily redispersed by gentle shaking.

3.4. In vitro release studies

The ability of the nanogels to host a molecule and to sustain its release was tested using 3-MBA as a probe with high affinity for both βCD and γCD (Fundueanu et al., 2003; Santos et al., 2009, 2008). Lyophilized γCD nanogels or HPβCD nanogels (2%, w/v) were incubated in 3-MBA (0.08 mg/ml) solution for 60 h at room temperature and then diffusion of 3-MBA from the nanogels (2 ml aliquots) was evaluated using a dialysis membrane that did not allow passing of the gels but allowed permeation of the individual 3-MBA molecules. Experiments with 3-MBA solutions to which monomeric γCD or HPβCD had been added were simultaneously

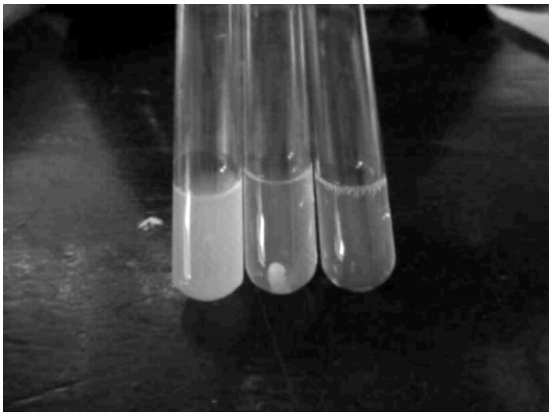

Fig. 4. Photograph of the systems after the centrifugation process. From left to right: Original colloidal system, γCD nanogel precipitated, and colloidal system after filtration (0.22 μm).

carried out. As a general trend, the nanogels prepared with 1 or 2% Span 80, namely $\gamma CD_{0,1}$, $\gamma CD_{0,2}$, $\gamma CD\text{-}HPMC_{1,1}$, $\gamma CD\text{-}HPMC_{2,1}$, $\gamma CD\text{-}HPMC_{2,2}$, $HP\beta CD_{0,1}$, $HP\beta CD\text{-}Agar_{1,1}$, showed sustained release of 3-MBA (Fig. 5). This finding suggests that 3-MBA was indeed taken up by the nanogels and, then, slowly released.

The flux of 3-MBA from every nanogel system, as well as from solutions with and without monomeric γCD or HPβCD, was calculated from the sustained part of the permeability profiles (Table 2). The nanogels led to smaller flux in comparison with the monomeric CDs and the free 3-MBA in absence of CD and gel. That is, nanogels enabled sustained drug delivery.

Table 2
Values of K_H and correlation coefficient (r^2) for 3-MBA release from the nanogels $\gamma CD_{0,1}$, $\gamma CD\text{-}HPMC_{1,1}$, $\gamma CD\text{-}HPMC_{1,2}$, $\gamma CD\text{-}HPMC_{2,0}$, $\gamma CD\text{-}HPMC_{2,1}$, $\gamma CD\text{-}HPMC_{2,2}$ and $HP\beta CD_{0,1}$ using the Higuchi model.

Nanogel	Flux (μg/h) [t = 3–10 h]	r^2	Higuchi model	
			K_H ($h^{-1/2}$)	r^2
3MBA	1.297	0.931	7.43	0.937
γCD	1.441	0.879	8.55	0.981
$\gamma CD_{0,0}$	0.562	0.854		
$\gamma CD_{0,0.5}$	0.517	0.835		
$\gamma CD_{0,1}$	0.639	0.992	5.02	0.974
$\gamma CD_{0,2}$	0.101	0.714		
$\gamma CD\text{-}HPMC_{1,0.5}$	0.444	0.991		
$\gamma CD\text{-}HPMC_{1,1}$	0.569	0.821	4.43	0.965
$\gamma CD\text{-}HPMC_{1,2}$	0.712	0.948	4.48	0.967
$\gamma CD\text{-}HPMC_{2,0}$	0.316	0.984	6.64	0.978
$\gamma CD\text{-}HPMC_{2,0.5}$	0.329	0.771		
$\gamma CD\text{-}HPMC_{2,1}$	1.042	0.993	6.64	0.979
$\gamma CD\text{-}HPMC_{2,2}$	0.551	0.924	6.75	0.969
HPβCD	0.407	0.944	4.79	0.907
$HP\beta CD_{0,0.5}$	0.499	0.935		
$HP\beta CD_{0,1}$	0.366	0.784	4.20	0.973
$HP\beta CD\text{-}Agar_{1,0.5}$	0.466	0.924	4.18	0.964
$HP\beta CD\text{-}Agar_{1,1}$	0.512	0.892		

The Higuchi model was applied in order to gain an insight into 3-MBA release from the nanogels. The equation states that the release of a compound (drug) is proportional to the square root of time:

$$Q = K_H t^{1/2} \quad (3)$$

where K_H is the Higuchi constant expressed as $(2ADC_S)^{1/2}$, where A is the diffusion area, D is the diffusion coefficient,and C_S is the

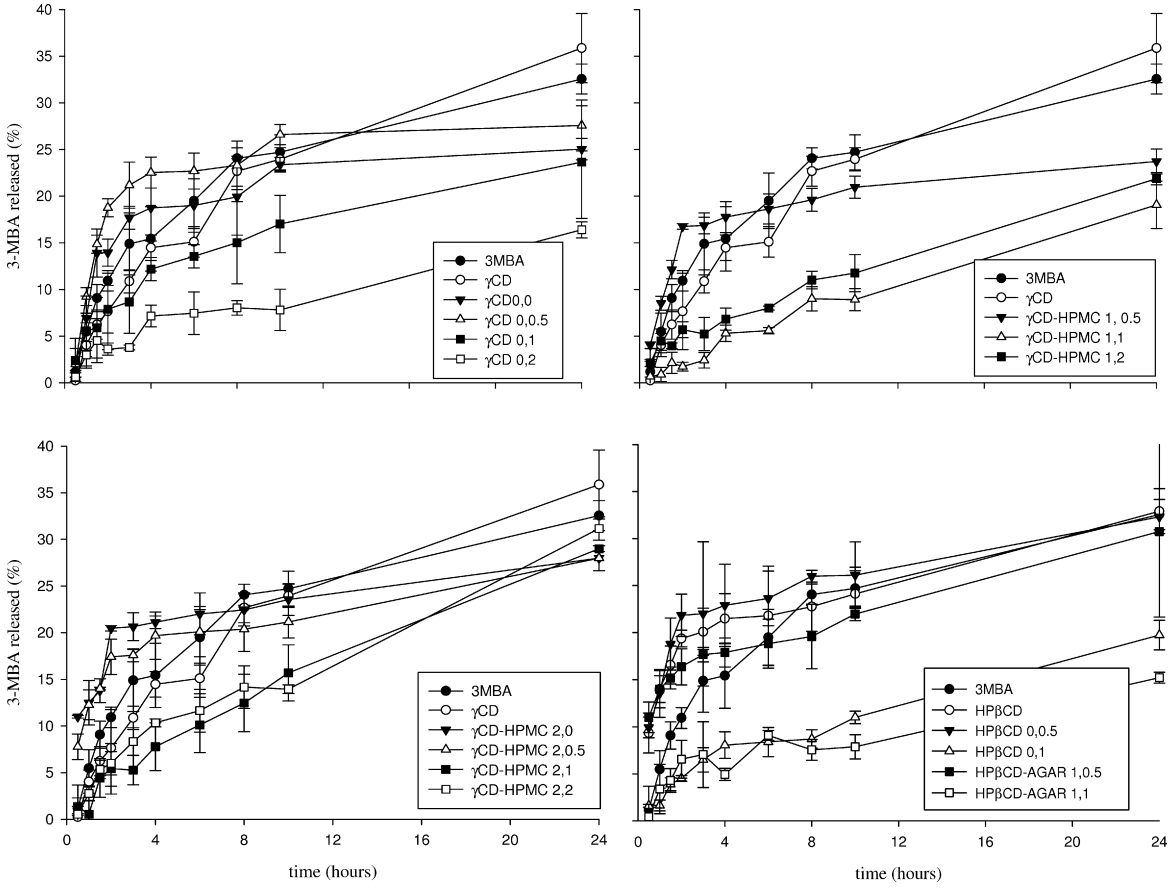

Fig. 5. Permeation profiles of 3-MBA in water alone or in presence of the raw γCD and HPβCD (code without subindex) or from the different synthesized nanogels through a semi-permeable membrane with MWCO of 3500 Da.

drug solubility in the system. This equation fitted some release profiles depicted in Fig. 5 ($r^2 > 0.97$ in Table 2), indicating that the release of 3-MBA from those nanogels is a diffusion controlled process.

4. Conclusions

Based in our previous synthesis experience we have implemented a technique in which the cross-linking takes place simultaneously with an emulsification/solvent evaporation process in order to render CD nanogels. γCD, HPβCD, HPMC and agar were used as components. The incorporation of the cross-linking agent EGDE was necessary to form nanogels, which however could be obtained in absence of surfactant such as Span 80. The size and mass distribution of the nanogels were affected by both the polysaccharide and the surfactant concentration. No notable differences were observed between the freshly prepared and rehydrated nanogels regarding particle size and the morphology; that is, after rehydration lyophilized nanogels that had been stored under dry conditions for 2 days were indistinguishable from freshly prepared nanogels. The nanogels prepared with 1 or 2% Span 80 were able to load 3-MBA and to sustain its release through a diffusion-controlled mechanism. All these features make the γCD and HPβCD nanogels promising carriers for both controlled and targeted drug delivery.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.11.005.

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