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Poly(hydroxyethyl methacrylate-co-methacrylated-β-cyclodextrin) hydrogels: Synthesis, cytocompatibility, mechanical properties and drug loading/release properties

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Abstract

Copolymerization of hydroxyethyl methacrylate (HEMA) with a methacrylated-derivative of β-cyclodextrin (β-CD) was evaluated as a way to obtain hydrogels with tunable mechanical and drug loading and release properties, particularly for preparing medicated soft contact lenses. A fully methacrylated β-CD monomer was synthesized and added to the HEMA and cross-linker solution at concentrations ranging from 0.042 to 0.333 g ml^{-1} (i.e. 0.23–1.82 mol.%). Thermal polymerization led to transparent hydrogels with a degree of conversion above 74%, which showed a high cytocompatibility and did not induce macrophage response. The greater the content in methacrylated β-CD was, the higher the glass transition temperature, the lower the degree of swelling and free water proportion, and the greater the storage and loss moduli of the swollen disks. These findings are directly related to the increase in the degree of cross-linking caused by the methacrylated β-CD. Loading studies were carried out with hydrocortisone and acetazolamide, both able to form complexes with CDs in water and in lacrimal fluid. Hydrocortisone loading progressively decreased as the content in methacrylated β-CD rose due to a decrease in the volume of aqueous phase of the hydrogel. Acetazolamide loading showed a maximum for an intermediate content in β-CD (0.125–0.167 g ml^{-1}) owing to a balance between complexation with β-CD and hydrogel mesh size. The hydrogels sustained drug delivery for several days, the acetazolamide release rate being dependent on the β-CD content. An adequate selection of the content in β-CD enables pHEMA-co-β-CD hydrogels suitable for specific biomedical applications to be obtained.

Keywords: Cytotoxicity; Hydrocortisone; Acetazolamide; Cyclodextrin complexation; Soft contact lenses

1. Introduction

Poly(hydroxyethyl methacrylate) (pHEMA) hydrogels are widely used as components of biomedical devices and drug delivery systems owing to their high biocompatibility, thermal and chemical stability, and tuneable mechanical properties [1–6]. The main limitation of the highly hydrophilic pHEMA materials is their poor ability to effectively load drugs and to control the release in biological medium [7,8]. Different approaches have been assayed to enhance the potential of pHEMA hydrogels as drug carriers; mainly, the chemical bound of polymerizable drugs through biodegradable links [9] and the copolymerization with functional monomers containing ionic or hydrophobic groups able to interact with the drug molecules [10–13]. Copolymerization with cyclodextrin (CD) monomers that can form inclusion complexes with drugs would be a feasible alternative.

Materials with inner microenvironments rich in CD cavities available to interact with surrounding drug molecules offer considerable possibilities for achieving an efficient

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loading and sustained release [14–21]. The attachment of CDs to a three-dimensional network hinders the fast decomplexation of the drug that usually occurs when CD–drug solutions are diluted in physiological fluids [15]. High cross-linked polymer networks, made with acrylic or vinyl monomers of CDs are particularly promising materials for controlled delivery [16,17,22–24]. For example, acrylamidomethyl-γ-cyclodextrin significantly improved the triamcinolone uptake and the control of the release from acrylic acid hydrogels [25]. The combination of HEMA with CDs has received little attention to date. Microparticles for absorbing organic pollutants from waste water or for chromatographic separations have been prepared by attachment of β-CD to preformed pHEMA microspheres using glutaraldehyde [26] or by free radical polymerization/cross-linking of HEMA with 2-hydroxy-3-methacryloyloxy-propyl-β-CD [27]. Methacrylated-β-CD monomers have also been assayed as components of highly cross-linked photopolymerizable dental composites [28,29]. However, no references to application for drug delivery have been found.

The aim of the present work was to synthesize pHEMA-co-β-CD hydrogels with low proportions of cross-linker and a wide range of contents in β-CD, and to characterize them in terms of mechanical and viscoelastic properties, interaction with water (swelling and water states), cytotoxicity, and drug loading and release performance. β-CD is poorly soluble in HEMA and, therefore, a highly substituted methacrylic monomer has to be prepared first. The biocompatibility of the resultant hydrogels was evaluated by a recently proposed method based on macrophage response to methacrylate conversion [30]. Loading studies were carried out with hydrocortisone and acetazolamide, both of practical interest for the local treatment of ocular pathologies and able to form complexes with CDs in solution, thereby increasing their hydrosolubility [31,32]. It was previously observed that Sauflon PW soft contact lenses (made with *N*-vinyl pyrrolidone and methyl methacrylate) soaked in 5% acetazolamide solution can provide sufficient concentration to lower the intraocular pressure in a more efficient way than conventional local treatments, without significant systemic absorption [33]. Since pHEMA hydrogels are receiving increasing attention as medicated soft contact lenses for ocular sustained release [7,8,34], the incidence of copolymerization with methacrylated-β-CD on the optical properties of the hydrogels and on the ability to sustain drug release in lacrimal fluid was also evaluated.

2. Materials and methods

2.1. Materials

Ophthalmic grade 2-hydroxyethyl methacrylate (HEMA) was supplied by Merck (Germany). Methacrylic anhydride, 2,6-di-tert-butyl-4-methylphenol (BHT), pyridine, 2,2′-azo-bis(isobutyronitrile) (AIBN), ethyleneglycol dimethacrylate (EGDMA), 3-methylbenzoic acid (3-MBA); acetazolamide and hydrocortisone were from Sigma–Aldrich (Spain). β-Cyclodextrin (β-CD) was supplied by Roquette-Laisa (Spain). Ultrapure water (resistivity > 18.2 MΩ cm) was obtained by reverse osmosis (MilliQ®, Millipore Spain). All other reagents were of analytical grade.

2.2. Synthesis of (2,3-di-O-methacrylated-6-methacrylated)-β-CD

A procedure based on the template polymerization method developed by Saito and Yamaguchi [35] was used. β-CD (3.6 or 7.2 g) previously dried at 105 °C for 24 h and BHT (0.04 g) were dissolved in pyridine (36 ml). Then methacrylic anhydride (19.84 g) was added, and the system stirred for 2 h at room temperature. The solution was refluxed at 50 °C for 5 h and then poured into cold water (300 ml) and stored at 4 °C overnight for precipitation of the monomer. The precipitate was filtered (0.22 μm nylon membrane, Teknokroma, Spain) and purified by dissolution in methanol (20 ml) and reprecipitation in cold water (100 ml). The purification process was repeated three more times, then the precipitate was collected and dried under vacuum. ^{1}H NMR spectra of monomer dissolved in *d*-chloroform was recorded in a Bruker AMX500 apparatus (Germany) at 500 MHz: δ[ppm] = 5.18 (C(1)H of β-CD, 7H), 4.80 (C(2)H of β-CD, 7H), 4.60 (C(3)H of β-CD, 7H), 3.58 (C(4)H of β-CD, 7H), 3.95–4.37 (C(5)H of β-CD, 7H, and C(6)H of β-CD, 14H), 5.62–6.17 (C(7)H of CH_2=C in methacrylate) and 1.95 (CH_3 in methacrylate). The number of vinyl groups per β-CD unit was estimated as the ratio of the area of C(7)H peaks of the vinyl group and the total area of protons of C(1)H [35]. FT-IR spectra of the native β-CD and the resultant monomers were recorded over the range 400–4000 cm^{-1} in a Bruker IFS 66 V FT-IR spectrometer (Germany) using the potassium bromide pellet technique.

2.3. Drug–cyclodextrin complexation in solution

Acetazolamide or hydrocortisone were added in excess to β-CD solutions (0.2–1.2% w/v) in water or artificial lacrimal fluid (6.78 g l^{-1} NaCl, 2.18 g l^{-1} $NaHCO_3$, 1.38 g l^{-1} KCl, 0.084 g l^{-1} $CaCl_2 \cdot 2H_2O$, pH 8 [36]). The resultant suspensions were kept under oscillating movement (50 oscillations min^{-1}) at 25 °C for 7 days. Then samples were taken and filtered through 0.22 μm cellulose acetate membranes (Millipore®, Spain). The concentration of dissolved acetazolamide or hydrocortisone was determined by ultraviolet spectrophotometry (Agilent 8453, Germany) at 264 or 248 nm, respectively. The apparent affinity constant of the 1:1 drug:β-CD complexes was estimated from the A_L-type diagrams using Eq. (1) [37]:

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

where m is the slope of the plot and S_0 is drug solubility in absence of cyclodextrin.