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In vitro release of metformin hydrochloride from sodium alginate/polyvinyl alcohol hydrogels

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ABSTRACT

Hydrogels, based on polysaccharides have found a number of applications as drug delivery carriers. In this work, hydrogels of full characterized sodium alginate (Mn 87,400 g/mol) and commercial poly(vinyl alcohol) (PVA) sensitive to pH and temperature stimuli were obtained using a simple, controlled, green, low cost method based on freeze-thaw cycles. Stable hydrogels of sodium alginate/PVA with 0.5:1.5 and 1.0:1.0 w/v concentrations showed very good swelling ratio values in distilled water (14 and 20 g/g, respectively). Encapsulation and release of metformin hydrochloride in hydrogels of 1.0:1.0 w/v sodium alginate/PVA was followed by UV spectroscopy. The hydrogel released a very low amount of metformin hydrochloride at pH 1.2; the highest release value (55%) was obtained after 6 h at pH 8.0. Also, the release of metformin hydrochloride was studied by ^{1}H NMR spectroscopy, the temporal evolution of methyl group signals of metformin showed 30% of drug release after 3 h.

1. Introduction

Alginic acid is a structural component of marine brown algae (Phaeophyceae) comprising up to 40% of dry matter; it is an unbranched copolymer of 1 → 4 linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues arranged in homopolymannuronic (MM), homopolyguluronic (GG), and heteropolymeric (MG) blocks (Haug, Larsen, & Smidsrød, 1974; Painter, 1983).The composition of alginic acids expressed as M/G ratio, and the distribution of the monomers in blocks depend on the species (Craigie, Morris, Rees, & Thom, 1984; Panikkar & Brasch, 1997; Larsen, Salem, Sallam, Mishrikey, & Beltagy, 2003; Leal, Matsuhiro, Rossi, & Caruso, 2008; García-Ríos, Ríos-Leal, Robledo, & Freile-Pelegrin, 2012).Within a particular species, M/G ratio and block composition depend on tissue type, season of harvest, habitat, etc. (Venegas, Matsuhiro, & Edding, 1993; Matsuhiro, Martínez-Gómez, & Mansilla, 2015). Gelation of alginate is mainly achieved by the exchange of sodium ions with divalent cations such as Ca^{2+}, Zn^{2+}, and Mn^{2+}.

Hydrogels are three-dimensional polymer networks made up of hydrophilic polymers, crosslinked to form a water-insoluble material that may absorb from 20% up to thousands of times their dry weight in water (Hoare & Kohane, 2008; Deligkaris, Shiferaw, Olthuis, & van den Berg, 2010; Hamidi, Azadi, & Rafiei, 2008).They are generally considered biocompatible due to their structural similarity with the macromolecular-based components in the body (Hoffman, 2012; Langer & Peppas, 2003). Hydrogels may exhibit swelling behavior dependent on the external environment; those which exhibit pH-dependent swelling behavior contain either acidic or basic pendant groups. This kind of polymeric systems is relevant in drug delivery applications (Brannon-Peppas & Peppas, 1991; Li, Ng, Yew, & Lam, 2005). Additionally, hydrogels may provide desirable protection to drugs from the harsh environment in the vicinity of the release site. Drug-loaded hydrogels may act as reservoirs that release the drug by mechanisms such as diffusion or erosion (Amsden, 1998). Sodium alginate is a biodegradable, biocompatible, and non-toxic biopolymer that has been extensively used in the preparation of hydrogels; however, ionotropically crosslinked alginate hydrogels show low stability and lose their initial mechanical strength as time passes (Funami et al., 2009). Poly(vinyl alcohol) (PVA) is a biocompatible semi-crystalline hydrophilic polymer which form hydrogels by chemical or physical crosslinking. Freeze-thaw process is the most effective and green method to produce physically crosslinked PVA hydrogels without the presence of crosslinking agent (Bolto, Tran, Hoang, & Xie, 2009; Gupta, Kumar, Upadhyay, Surekha, & Roy, 2009; Ricciardi, Auriemma, Gaillet, de Rosa, & Lauprêtre, 2004). PVA hydrogels have interconnected macropores allowing unhin-

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dered diffusion of solutes of practically any size. They have good mechanical properties, high water content and are stable at room temperature (Kulkani, Sreedhar, Mutalik, Setty, & Sa, 2010; Hua, Ma, Li, Yang, & Wang, 2010).

Metformin hydrochloride is a drug widely used in the management of non-insulin dependent diabetes mellitus, which improves glucose tolerance lowering both basal and postprandial plasma glucose (Oh et al., 2003; Nayak & Pal, 2013a,b). Metformin HCl has a short half-life of 1.5-1.6 h and an oral bioavailability of 40–60%.

In this work, we report on the application of a green and simple method, that is freeze/thaw cycle process for the preparation of sodium alginate/PVA hydrogels with very good water absorbency and pH sensitivity. Additionally, the in vitro release of metformin, as model drug from these hydrogels, at pH similar to gastrointestinal environment was evaluated.

2. Experimental

2.1. Materials and methods

Sodium alginate with a M/G ratio of 0.7 was obtained by alkaline extraction of the brown seaweed *Desmarestia menziesii* (Phaeophyceae). The extraction, purification and characterization were described elsewhere (Martínez-Gómez, Mansilla, Matsuhiro, Matulewickz, & Troncoso-Valenzuela, 2016). Poly(vinyl alcohol) with an average molecular weight of 98,000 g/mol and a degree of hydrolysis of 98–99%, and 99.9% deuterium oxide were purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA. FT-IR spectra of polymers in KBr were recorded in the 4000–400 cm^{-1} region using a Bruker IFS 66υ instrument; 32 scans were taken with a resolution of 4 cm^{-1} (Leal et al., 2008). NMR spectra were recorded on a Bruker Avance 400 spectrometer (Bellerica, MA, USA) operating at 400.13 MHz (^{1}H) and 100.62 MHz (^{13}C) at 25 °C, after isotopic exchange with D_2O (3 × 0.75 mL) using D_2O as solvent.

2.2. Thermal analysis

Thermal properties of the PVA, sodium alginate and hydrogels were measured using a differential scanning calorimeter (DSC, TA Instrument, model 2920, New Castle, DE, USA). The polymer samples were previously dried under vacuum at 56 °C. The dried samples (about 10 mg) were deposited on DSC sample holders and submitted to a heating/cooling cycle to erase the sample thermal history. Subsequently, samples were heated at 10 °C/min up to 230 °C–250 °C and then cooled to room temperature. Samples of hydrogels were freeze with liquid nitrogen and milled; the samples were dried under vacuum prior to analysis.

2.3. AFM imaging

Extended multimode AFM Nanoscope IIIa with an E scanner (Digital Instruments, Santa Barbara, CA, USA) was used to image the molecular structures of the sodium alginate and PVA. Imaging was carried out in air at room temperature using commercial AC200TS three-sided silicon probes (Olympus). The surfaces were scanned in the intermittent contact mode (tapping mode) with a scan rate of 0.4 Hz. Samples of sodium alginate and PVA were dissolved in distilled water (0.1 mg/mL), filtered through 0.45 Millipore filters and deposited onto freshly cleaved surfaces.

2.4. Hydrogels preparations and swelling ratio determination

Hydrogels from aqueous solutions of 2% (w/v) PVA were prepared according to Ricciardi et al. (2004) with some modifications. The aqueous solutions were poured on 12-well microplates (Evergreen Scientific, Los Angeles, CA, USA) and submitted to one to twelve freeze/thaw cycles consisting of a freezing (20 h at −40 °C) and thawing steps (4 h at room temperature). The stable hydrogels samples were dried at 40 °C for 24 h in an oven.

Blends of aqueous solution of sodium alginate/PVA with 1.5:0.5, 1.0:1.0, and 0.5:1.5% w/v ratio were prepared by dissolving PVA in distilled water as above, and aqueous solution of sodium alginate was slowly added, cooled to room temperature and kept at this temperature for 3 h to eliminate air bubbles. The aqueous polymers solutions were subjected to several repeated freeze/thaw cycles. The resulting hydrogels were dried in an oven at 40 °C for 24 h. The dried hydrogels were immersed in distilled water at room temperature, and the weight of the swollen sample at different times was measured; the excess of water on the sample surface was removed with 5 Whatmann filter (Whatmann International, Ltd, Maidstone, England). The swelling ratio was calculated according to the following equation (Hua et al., 2010):

$$Swellingratio = \frac{w_s - w_d}{w_d}$$

where w_s and w_d are the weights of swollen and dry hydrogels, respectively.

2.5. Effects of pH and temperature in swelling ratio

The pH and temperature effects were investigated by determining swelling ratios of hydrogels in different media. The dried samples were first immersed in 100 mL HCl solution (pH 1.2) for two hours. At specific time intervals, samples were taken out, dried with filter paper, and weighed; then, they were transferred into 100 mL sodium carbonate buffer (pH 6.5) for 2 h and weighed. The process was repeated in carbonate buffer (pH 8.0) for 2.5 h and at pH 6.5 in carbonate buffer for 65.5 h (Nair, Kritchevsky, & Setchell, 1971).

2.6. Preparation and characterization of hydrogels loaded with metformin hydrochloride

Metformin hydrochloride (50 mg) was added to solutions of sodium alginate:PVA (0.5:1.5 and 1.0:1.0 p/v), and dispersed by mechanical stirring. The resulting solutions were poured on 12-well micro plates and submitted to freeze/thaw cycles as in *2.4*. Samples were characterized by ATR FT-IR spectroscopy using a Spectrum Two equipment (Perkin Elmer, Waltham, MA, USA).

2.6.1. Scanning electron microscopy analyses (SEM)

Gold coated hydrogels were analyzed in a NanoSEM NPE 67 equipment (FEI, OR, USA) with an accelerating voltage of 5 kV.

2.6.2. In vitro release studies of metformin hydrochloride

The in vitro drug release assays were performed in 100 mL glass flasks containing HCl solution (pH 1.2), followed by sodium carbonate buffers (pH 6.5 and pH 8.0). The amount of drug release was determined by withdrawing 1 mL of solution at specific time intervals for 72 h. The collected aliquots were measured for drug content using a UV–vis spectrophotometer (Spectronic-Genesys 5, Thermo Fisher Scientific, Waltham, MA, USA) at 233 nm, using pure metformin hydrochloride as blank. Equal volume of fresh HCl or carbonate buffer solution was replaced into the release medium to maintain constant volume. The UV standard absorbance curve for metformin hydrochloride was established in the concentration range 2–20 μg/mL.

2.7. Release study by ^{1}H NMR spectroscopy

The release of metformin was investigated with Bruker Avance 400 spectrometer operating at 400.13 MHz at 27 °C. Hydrogel