



Research Paper

Preparation and characterization of maleoylagarose/PNIPAAm graft copolymers and formation of polyelectrolyte complexes with chitosan

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ABSTRACT

A water soluble derivative in 98% yield with 23.1% incorporation of maleoyl groups was obtained by esterification of agarose with maleic anhydride. Graft copolymers were synthesized through vinyl groups of maleoylagarose with *N*-isopropylacrylamide using ceric ammonium nitrate or ammonium persulfate as initiator, by conventional method or microwave irradiation. High nitrogen content (4.6%) was obtained in the grafting process using ceric ammonium nitrate as initiator without microwave irradiation. Copolymers were characterized by FT-IR and NMR spectroscopies, TGA, DSC and morphological analysis by AFM and SEM microscopy, confirming the grafting of PNIPAAm onto polysaccharide backbone. Hydrogel films were obtained by ionic complexation between opposite charged groups of maleoylagarose-*g*-poly(*N*-isopropylacrylamide) and chitosan. The swelling of 1:1 w/v maleoylagarose-*g*-PNIPAAm:chitosan film was higher than 2:1 w/v film at 25 and 37 °C. 53% release *in vitro* of diclofenac sodium from 1:1 w/v maleoylagarose-*g*-PNIPAAm:chitosan was obtained at 37 °C and pH 6.0 with < 0.5 diffusional constant values.

1. Introduction

Agarose (→3-β-D-galactopyranosyl-1→4-3,6-anhydro-α-L-galactopyranosyl-1→) is the neutral fraction of agarans produced by red seaweeds of the Gracilariaceae and Gelidiaceae families. The amount of this polysaccharide depends on the species (Percival & McDowell, 1967; Matsuhira & Urzúa, 1990a, 1990b). The red alga *Ahnfeltia plicata* from southern Chile synthesizes agarose devoid of sulfate group with definite structure (Matsuhira, Barahona, Encinas, Mansilla, & Ortiz, 2014). This agarose constitutes an interesting biomaterial for the preparation of glycoconjugates with potential biotechnological applications, but is slightly soluble in water. Maleoylation of polysaccharides has proved to be a facile method for the preparation of soluble derivatives, which are useful as intermediates in graft copolymerization reactions (Hamcerencu, Desbrieres, Popa, Khoukh, & Riess, 2007; Hamcerencu, Desbrieres, Khoukh, Popa, & Riess, 2008; Kim, Won, & Chu, 1999; Mu & Fang, 2008; Tay, Pang, & Chin, 2012).

Hydrogels are three-dimensional polymer networks formed by cross linking of hydrophilic polymers, including polysaccharides, which are insoluble in water and may absorb up to thousands of times their dry weight in water. Hydrogels responsive to external stimuli such as pH, temperature, and ionic interactions have received much attention due to biomedical applications such as drug delivery, wound dressing and

tissue engineering matrices (Cirillo et al., 2015; Caló & Khutoryanskiy, 2015; Hamcerencu, Desbrieres, Popa, & Riess, 2009; Hoare & Kohane, 2008; Hoffman, 2002; Huang et al., 2006; Peppas, Bures, Leobandung, & Ichikawa, 2000; Prabakaran & Mano, 2006).

The grafting of *N*-isopropylacrylamide (NIPAAm) with some polysaccharides has been investigated. It has been found that free radical graft copolymerization in the polysaccharide backbone enhances hydrogels properties such as swelling, encapsulation and release of bioactive compounds (Dumitriu, Mitchell, & Vasile, 2010; Zhang, Wang, Guo, & Ma, 2014). In the last years, graft copolymerization of vinyl monomers into polysaccharides backbone has received much attention. Thakur, Singha, and Thakur (2012a, 2012b) grafted ethyl acrylate onto cellulose and lignocellulosic fibers using potassium persulfate as free radical initiator for the preparation of graft copolymer with better chemical and temperature resistance materials than cellulose.

According to Bharti, Mishra, and Sen (2013) copolymerization reaction *via* free radical mechanism which is based on chemical free radical initiator that generates free radical sites on polysaccharide backbone for the grafting of vinyl monomers can be considered as a conventional graft copolymerization method; by this method, the authors conducted the grafting of acrylamide onto oatmeal (1,3 and 1,6 linked β-D-glucan) backbone using cerium ammonium nitrate as free radical initiator. An efficient adsorbent for removing heavy metals was

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prepared by conventional graft polymerization of acrylonitrile onto crosslinked chitosan (Shankar, Gomathi, Vijayalakshmi, & Sudha, 2014). Yilmaz, Yalinca, Yahya, and Sirotna (2016) grafted (2-diethylamino)-ethyl methacrylate onto chitosan using potassium persulfate as free radical initiator; by crosslinking with glutaraldehyde they obtained a pH sensitive hydrogel. Also, the redox initiator ferrous ammonium sulfate/potassium persulfate system was employed for the graft copolymerization of ethyl acrylate and of butyl acrylate onto lignocellulosic material (Thakur, Thakur, & Gupta, 2013a, 2013b). Recently, Elella, Mohamed, ElHafeez, and Sabaa (2017) showed how new antibacterial copolymer can be obtained from non-biological active polysaccharide by grafting *N*-vinyl imidazole onto xanthan gum backbone by conventional free radical graft polymerization using potassium persulfate as initiator. Rani, Mishra, Sen, and Jaha (2012) grafted polyacrylamide onto agar using ceric ammonium nitrate as free radical initiator by conventional and microwave assisted methods; they found that the copolymer grafted by microwave assisted method showed better flocculation efficacy and waste water remediation than the copolymer prepared by conventional method. It has been described that microwave assisted graft copolymerization method presents several advantages such as much lower reaction times, inert atmosphere or toxic solvents are not required, and lead to higher graft incorporation onto polysaccharide in comparison with conventional free radical copolymerization (Kaur & Gupta, 2017; Singh, Kumar, & Sanghi, 2012). Thakur and Singha (2011) and Thakur et al. (2013c, 2013d, 2013e) accomplished the optimization of different reaction conditions for the preparation of graft copolymers of cellulose from different sources by microwave assisted copolymerization of vinyl monomers using potassium persulfate or ferrous ammonium sulfate/potassium persulfate system as free radical initiators. A detailed study on the preparation of grafted polyacrylamide onto agar by conventional and microwave assisted methods using ceric ammonium nitrate as initiator, and by microwave initiated free radical polymerization was published by Mishra, Sen, and Usha (2015). The first two methods afforded graft copolymers with potential applications in biomedicine and in bioremediation. In the past few years graft copolymers obtained by free radical copolymerization of vinyl monomers assisted by microwave irradiation onto natural polysaccharides such as sodium alginate, gellan gum, plantago psyllium, locust bean gum and carrageenan have been published (Arias et al., 2016; Giri, Verma, & Tripathi, 2014; Jatav & Agarwal, 2015; Rani & Singh, 2016; Rani, Mishra, & Sen, 2013). On the other hand, Rusu, Popa, Ibanescu, Danu, and Verestiuc (2016), synthesized pH sensitive hydrogels by free radical polymerization of *N*-maleoylchitosan with acrylic acid and ethyl acrylate with ammonium persulfate/*N,N,N',N'*-tetramethylethylenediamine system as initiator and they indicated that vinyl group of *N*-maleoylchitosan conducted to polymerization between this biopolymer and vinyl monomers. Previously, similar results were obtained by Hamcerencu et al. (2009) in the copolymerization of maleoylchitosan with NIPAAm and, by Shin, Kang, Park, and Yang (2002) in the copolymerization of maleoylated xanthan gum with NIPAAm.

Chitosan is a biocompatible and biodegradable polysaccharide, a basic polymer soluble in acidic medium which easily form hydrogels, microspheres, nanoparticles and films by polyelectrolyte complexation with anionic polymers (Coimbra et al., 2011; Luo & Wang, 2014; Costalat, David, & Delair, 2014; Kayitmazer, Koksall, & Kilic Iyilid, 2015). In this work, maleoylation of agarose was pursued in order to have an anionic, soluble derivative with terminal vinyl group for the copolymerization with *N*-isopropylacrylamide. Hydrogels were prepared by ionic interaction of the graft copolymer with chitosan and swelling properties of the obtained films and also, those loaded with a model drug (diclofenac sodium) were studied.

2. Experimental

2.1. Materials and methods

Agarose (AGA) was obtained by aqueous extraction of the red seaweed *Ahnfeltia plicata* (Ahnfeltiales, Rhodophyta) collected in southern Chile. The extraction, purification and characterization were previously described (Matsuhiro et al., 2014). Reagent grade chemicals and chitosan from shrimp shells, 75% deacetylated were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Diclofenac sodium was purchased from AK Scientific (Union City, CA, USA). FT-IR spectra of polymers in KBr were recorded in the 4000–400 cm⁻¹ region using a Bruker IFS 660 instrument and 32 scans were taken with a resolution of 4 cm⁻¹. NMR spectra in D₂O were recorded on a Bruker Avance 400 spectrometer (Bellerica, MA, USA) operating at 400.13 MHz (¹H) and 100.62 MHz (¹³C) at 25 °C, after isotopic exchange with D₂O (3 × 0.75 mL) using the sodium salt of 3-(trimethylsilyl)-1-propionic-2,2,3,3-d₄ acid as internal reference.

2.2. Esterification of agarose with maleic anhydride

Esterification was conducted by an adaptation of the method of Kim et al. (1999). Agarose: maleic anhydride in the molar ratio of 1:2 and 1:5. AGA (200 mg) was dissolved in 20 mL of *N,N*-dimethyl formamide, and 20 mg of lithium chloride was added with stirring at 90 °C under nitrogen atmosphere until dissolution. The temperature of the resulting solution was lowered to 60 °C, and 2.0 mL of triethylamine were added with stirring. After 15 min, the corresponding amount of maleic anhydride was added, and the mixture was stirred at 60 °C during 6 h under N₂. The reaction product was dialyzed against distilled water using Spectra/Por membranes (MWCO 3500) (Spectrum Laboratories, Rancho Dominguez, CA, USA) during 72 h, changing distilled water every two hours during the day, concentrated in vacuo and freeze dried. The degree of substitution was calculated by integration of ¹H NMR signals, according to Hamcerencu et al. (2008). Maleoyl groups were determined by the back-titration method (Tay et al., 2012). Maleoyl agarose (MAGA) (5.0 mg) was dissolved in 10 mL of 0.4 M NaOH with stirring at room temperature for 4 h. The excess of NaOH was titrated with 0.4 M HCl 0.4 M using phenolphthalein as indicator. Determinations were conducted in triplicate. Degree of substitution was determined according to Eq. (1).

$$DS = \frac{306 \times nCOOH}{m - 99 \times nCOOH} \quad (1)$$

where 306 g/mol is the molar mass of an anhydrous agarobiose unit, 99 g/mol is the net increase due to maleoyl group incorporation, *m* is the mass of the sample and *nCOOH* is the amount of COOH calculated from titration according to Eq. (2). MAGA (5.0 mg) was dissolved in 10 mL of 0.4 M NaOH with stirring at 25 °C for 4 h and titrated with 0.4 M HCl using phenolphthalein as indicator.

$$nCOOH = V_{NaOH} \times C_{NaOH} - V_{HCl} \times C_{HCl} \quad (2)$$

2.3. Copolymerization of maleoyl agarose with *N*-isopropylacrylamide

2.3.1. Ceric-initiated copolymerization

The copolymerization of MAGA with *N*-isopropylacrylamide using cerium ammonium nitrate as the free radical initiator was conducted according to Hosseinzadeh (2009) with some modifications. Briefly, MAGA (25 mg) was dissolved in 20 mL of distilled water at 25 °C and nitrogen gas was bubbled to remove oxygen. *N*-isopropylacrylamide (25–50 mg) and ceric ammonium nitrate (1 mL of 0.02 M solution)

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