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Dual-network hydrogels based on chemically and physically crosslinked chitosan/chondroitin sulfate

André R. Fajardo *, Silvia L. Fávaro, Adley F. Rubira, Edvani C. Muniz

Grupo de Materiais Poliméricos e Compósitos (GMPC), Chemistry Department, Maringá State University, Av. Colombo, 5790, 87020-900 Maringá, Paraná, Brazil

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ABSTRACT

The formation of a novel type of hydrogel that combines chemically and physically crosslinked networks in a dual-network approach is presented here. Chitosan (CHT) and chondroitin sulfate (CS) were chemically modified with glycidyl methacrylate (GMA) and then crosslinked. The chemical hydrogels (CHT- and CS-gel) were deposited in different vials filled with CS or CHT stock solutions to form the dual-network hydrogels. FTIR, TGA and XRD analyses were used to characterize the chemical and the dual-network hydrogels. The percentages of CS or CHT complexed to the CHT- and CS-gel networks were calculated from the HPLC data. SEM images and swelling assays indicated that the formation of a secondary network by polyelectrolyte complexation changes the morphologies and liquid uptake capacities of the chemical hydrogels. Hence, the data and discussion presented here enable the formation of dual-network hydrogels with very interesting properties, such as the ability to interact with charged specimens (i.e., drugs, proteins or metal ions), a desirable feature for a wide range of applications.

1. Introduction

Hydrogels are very attractive soft materials that have been applied in several fields due to their interesting and unique properties [1–3]. The three-dimensional (3D) hydrogel network is responsible for absorbing and retaining large amounts of water without dissolving or losing its structural integrity [4]. Chemical or physical crosslinking process can form this 3D network [5–8]. Chemically crosslinked networks (or chemical hydrogels) are usually formed by polymerization and parallel crosslinking of multifunctional monomers. This involves the synthesis of polymers with reactive groups and their subsequent crosslinking by reacting them with suitable crosslinking agents. On the other hand, in physically crosslinked networks (or physical hydrogels) the polymer chains are held together by electrostatic forces (polyelectrolytes), hydrogen bonds, hydrophobic interactions or chain entanglements. Over decades of research, chemical and physical hydrogels have been developed to provide different advantages/properties for applications in different fields (agriculture, hygienic industry, pharmaceutical and tissue engineering, for instance). Despite the constant enhancements in this area of research, both hydrogel types still show some disadvantages that restrict their application. In general, the crosslinking agents utilized to form chemical hydrogels show some level of toxicity, which restricts the use of the obtained hydrogel as a biomaterial. Furthermore, physical hydrogels may demonstrate poor mechanical properties in their swollen state and eventually disintegrate. Considering the different approaches used to overcome these disadvantages, a strategy focused on the formation of the hydrogel network may be a promising solution. Hence, we developed an original hydrogel formed by both chemical and physical crosslinked networks – a dual-network hydrogel. Firstly, a conventional chemical hydrogel based on methacrylated chitosan (CHT-*methacrylate*) is formed. In a second step, a physically crosslinked network is formed between the CHT-gel network and chondroitin sulfate (CS) due to the electrostatic interaction among the charged functional groups in the CHT and CS backbones [9]. CS, a sulfated glycosaminoglycan (GAG) component of the extracellular matrix (ECM), is a polyelectrolyte specimen with anionic charge density that can associate with polycationic moieties through electrostatic interactions [10–13]. This methodology was also tested in reverse: raw CS was chemically modified first and then chemically crosslinked (resulting in CS-gel). Then, a physically crosslinked network was formed between the CS-gel and CHT.

The complete characterization of these novel types of hydrogels was performed in this work and demonstrates that the formation of such a dual-network system combines, in the same material, the advantages of chemically and physically crosslinked networks. Additionally, the use of natural polymers such as CHT and CS broadens the range of applicability of the as-prepared hydrogels due to their low toxicity, biodegradability and biocompatibility. All of these features are desirable for applications in many fields (i.e., pharmacological, biological, environmental, etc.) [14].

* Corresponding author. Tel./fax: +55 44 3261 4215.
E-mail address: drefajardo@hotmail.com (A.R. Fajardo).

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2. Experimental

2.1. Materials

Chitosan (CHT, CAS 90-77-7), 85% deacetylated and with a viscometric mass (M_V) of 87,000 g/mol, was purchased from Golden-Shell Biochemical (China) [12]. Chondroitin sulfate (CS, CAS 9007-28-7) with M_w of 20,000 g/mol determined by GPC/SEC was kindly supplied by Solabia, Brazil. Note that the CS utilized in this work is a mixture of CS sulfated in the C_4 and C_6 positions. Glycidyl methacrylate (GMA, 97% CAS: 106-91-2) and *N*,*N*-methylenebisacrylamide (MBA, CAS 110-26-9) were purchased from Sigma–Aldrich (St. Louis, USA). All materials and reagents were used as received.

2.2. Characterization techniques

Gel permeation chromatography (GPC) analyses were performed at 25 °C in a Waters 150C system (Waters, Milford, USA) equipped with a multiangle laser light scattering detector (DAWN DSP-F, Wyatt, Hollister, USA) and two Shodex columns in series (OHPack 802 and 803) using water as a solvent. Infrared (IR) spectra were recorded with a Bomem (Model MB 100-C26) spectrometer, operating in the region from 4000 to 500 cm^{-1}, with a resolution of 4 cm^{-1}. ^1H nuclear magnetic resonance (NMR) and solid-state ^{13}C NMR spectra were recorded using a Varian spectrometer (Model Oxford 300, Illinois, USA). High performance liquid chromatography (HPLC) analyses were performed at 25 °C in a Surveyor Plus chromatographic workstation (Thermo Fisher Scientific, Illinois, USA) equipped with a PolySep-GFC-P 6000 chromatographic column (300 × 7.8 mm, Phenomenex) and a Surveyor Plus PDA Detector with a diode array, controlled by ChromChest™ software. An acetic acid solution (1.5 vol.%) was used as a mobile phase at a flow rate of 0.5 ml/min. The eluted analytes were monitored at λ = 212 nm. Thermogravimetry (TGA) was carried out in a Simultaneous Thermal Analyses System, Netzsch (Model STA 409 PG/4/G Luxx) at a scanning rate of 10 °C·min^{-1} under N_2 gas flowing at 20 ml/min in a range of temperatures from 22 to 800 °C. X-ray diffraction (XRD) measurements were performed using a DMAXB diffractometer (Rigaku, Japan) equipped with a Cu Kα radiation source in a scattering angle (2θ) from 5° to 70°, with a resolution of 0.02°, at a scanning speed of 2° min^{-1}. Scanning electron microscopy (SEM) images were recorded with a Shimadzu (Model SS550 Superscan, Kyoto, Japan) microscope coupled with an energy-dispersive X-ray (EDX) analyzer. Prior to the analyses, the samples were immersed in distilled water at room temperature up to the equilibrium swelling (~24 h). Then, the samples were frozen using liquid nitrogen. Thereafter, the frozen hydrogel samples were lyophilized on a freeze dryer (Christ, Alpha 1-2 LD Plus, Germany) at −55 °C for 24 h. The dried samples were carefully fractured and then gold-coated by sputtering before SEM visualization.

2.3. Synthesis of CHT- and CS-methacrylate

The strategy to prepare CHT- and CS-*methacrylate* was adapted from the literature [15,16]. Briefly, CHT (2 g; 23 μmol) was solubilized in an aqueous acid acetic solution (1.5 vol.%, 200 ml) at room temperature for 4 h. Afterwards, GMA (2.1 ml; 15.4 mmol) was added to the reaction system, which was then heated to 60 °C. The reaction was maintained under magnetic stirring for 12 h. The reaction system was cooled, and the material of interest was precipitated by the addition of ethanol (200 ml) and then recovered by filtration. The precipitate was washed several times with ethanol portions to remove all the unreacted chemicals. CHT-*methacrylate* was lyophilized at −55 °C for 24 h, yielding a white powder. **Yield**: 1.75 g (87.5%). FTIR (cm^{-1}): 3431 (O—H and N—H); 2928 and 2876 (C—H); 1728 (C=O_{ester}); 1657 (C=C); 1554 (N—H, bending); 1418 and 1384 (C—H, bending); 1316 (C—N); and 1162, 1054, and 1034 (C—O). ^1H NMR (300 MHz, D_2O, 298 K, δ, ppm): 6.02 (d, C=C**H**$_2$); 5.62 (d, C=C**H**$_2$); 4.79 (m, H-1^{GlcNAc} and H-1^{GlcN}); 4.52 (d, O—C**H**$_2$); 3.90–3.30 (m, C**H**, 5× C**H**$_2$, N**H** and O**H**); 3.77 (m, H-3,4,6^{GlcNAc} and H-3,4,6^{GlcN}); 3.57 (m, H-5,6^{GlcNAc} and H-5,6^{GlcN}); 3.40 (m, O—C**H**); 3.03 (m, H-2^{GlcN} and H-2^{GlcN}); 1.93 (s, C**H**$_3$); and 1.73 (s, C**H**$_3$) (Note: GlcN = *D*-glucosamine unit and GlcNAc = *N*-acetyl-*D*-glucosamine unit).

CS-*methacrylate* was synthesized using a similar methodology. CS (10 g; 500 μmol) was solubilized in water (100 ml) at room temperature for 4 h. The pH was adjusted to 3.5 by HCl (0.1 M) addition. GMA (2.1 ml; 15.4 mmol) was then added to the reaction system. The reaction system was heated to 60 °C and maintained under magnetic stirring for 12 h. The reaction system was cooled, and the CS-*methacrylate* was precipitated by the addition of ethanol (100 ml) and then recovered by filtration. The precipitate was washed several times with ethanol portions to remove all the unreacted chemicals. CS-*methacrylate* was lyophilized at −55 °C for 24 h to yield a white powder. **Yield:** 9.23 g (92.3%). FTIR (cm^{-1}): 3417 (O—H and N—H); 2972 and 2915 (C—H); 1719 (C=O_{ester}); 1633 (C=O_{amide} and C=C); 1574 (N—H, bending); 1421 and 1378 (C—H, bending); 1315 (C—N); 1250 (SO_2); and 1131 and 1035 (C—O). ^1H NMR (300 MHz, D_2O, 298 K, δ, ppm): 6.11 (d, C=C**H**$_2$); 5.70 (d, C=C**H**$_2$); 4.69 (d, O—C**H**$_2$); 4.48 (m, H-1^{GlcUa} and H-1^{GalNAc}); 4.30–3.20 (m, H-2,3,4,5,6^{GlcUA} and H-2,3,4,5,6^{GalNAc}, O—C**H,** C**H**, 5× C**H**$_2$, N**H**, and O**H**); 1.94 (s, C**H**$_3$); and 1.89 (s, C**H**$_3$) (Note: GlcUa = Glucuronic acid unit and GlcNAc = *N*-acetylgalactosamine unit).

2.4. Dual-network hydrogels formation

Initially, chemical hydrogels were formed. For this purpose, CHT-*methacrylate* (1.50 g) was solubilized in an acetic acid solution (1.5 vol.%, 50 ml) at room temperature for 4 h. MBA (45 mg; 29 μmol) and sodium persulfate ($Na_2S_2O_8$, 30 mg; 12.6 μmol) were added to the reaction system. The temperature was increased to 70 °C. The solution was maintained at 70 °C for 1 h to complete the crosslinking process. The as-formed CHT hydrogel (CHT-gel) was washed with distilled water for 24 h to achieve neutral conditions (ca. pH 7) and to remove the unreacted materials. The CHT-gel was cut into cubes (1 × 1 × 1 cm) and then oven-dried (50 °C) for 24 h. **Yield:** 1.323 g (dry weight).

Immersing the dried CHT-gel samples in a CS stock solution formed the physically crosslinked network. The CS stock solution was prepared by the addition of CS (2.5 g) in an acetic acid solution (1.5 vol.%, 50 ml). The CHT-gel samples were immersed in different vials filled with CS stock solution for different time intervals (6, 12, and 24 h). After each time interval, the CHT-gels physically crosslinked with CS (CHT-gel/CS) were removed and washed several times with distilled water and then oven-dried (50 °C) for 24 h. Aliquots of each vial were collected and stored after the formation of CHT-gel/CS to quantify the amount of CS complexed to the CHT-gel network.

Using a similar methodology, CS-gel/CHT hydrogels were formed. Initially, CS-*methacrylate* (7.50 g) was solubilized in distilled water (50 ml) at room temperature for 4 h. MBA (225 mg; 94 μmol) and $Na_2S_2O_8$ (225 mg; 145 μmol) were added, and the reaction system was maintained at 70 °C for 1 h to complete the hydrogel crosslinking. The as-formed CS hydrogel (CS-gel) was washed with distilled water for 24 h and then cut into cubes (1 × 1 × 1 cm) and oven-dried (50 °C) for 24 h. **Yield:** 5.890 g (dry weight).