

Synthesis and Characterization of a Novel Polysaccharide-based Self-healing Hydrogel

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ABSTRACT

Polysaccharide-based hydrogels have attracted great interest for potential industrial and biomedical applications. However, the structural and functional integrity of hydrogels are often compromised by external mechanical forces or chemical degradation. This paper reports the fabrication of a self-healing hydrogel composite consisting of an oxidized xanthan gum (OXG) and chitosan (CS). Fourier transform infrared (FT-IR) spectroscopy showed successful formation of Schiff base bonding between CS and OXG. The hydrogels successfully self-healed at room temperature and pressure without any external stimulus such as water, pH level, and increase in temperature. A weight ratio of 1:1 (CS:OXG) results in a good balance between the self-healing capability and the mechanical strength of the prepared hydrogel.

Keywords: *chitosan; oxidized xanthan gum; hydrogel; self-healing*

INTRODUCTION

Hydrogels are three-dimensional, polymeric networks that have the capability to absorb and retain large amounts of water. They have gained significant attention in industrial and biomedical applications such as drug delivery, tissue engineering and regenerative medicine. However, the hydrogels' integrity in terms of form, fit and function is at risk by either mechanical or chemical degradation in in-vivo environments (Li *et al.*, 2017). Thus, hydrogels with the intrinsic ability to heal itself whenever it is exposed to destructive factors have the potential to be great candidates to deal with this issue. Hydrogels that have the ability to self-heal or autonomously repair itself in micro- and macro- structure as well as restore its mechanical and rheological properties are defined as "self-healing hydrogels", which can either be extrinsic or intrinsic depending on their self-assembly mechanisms in response to damage. Extrinsic self-healing hydrogels are those that have embedded healing agents into their matrices and

oftentimes require heating. Intrinsic self-healing hydrogels on the other hand are impelled by the chemical bonding of the polymer materials that have the ability to restructure for infinite times (Hia *et al.*, 2016). One of the chemical bonds operating in intrinsic self-healing hydrogels is the imine bond, also known as Schiff base, which forms dynamic interactions between amine (NH₂) groups and aldehyde (CHO) groups of the polymers involved.

Polysaccharides are excellent candidates in fabricating such composite gels due to the fact that they are cheap, available, biocompatible, and biodegradable (Altay & Gunasekaran, 2013). Chitosan (CS) is one of the polysaccharides that form hydrogels. It is produced by *N*-deacetylation of chitin, which is found in fungi, cell walls of algae, or in crustaceans and is comprised of randomly distributed β -(1 \rightarrow 4)- α -glucosamine and *N*-acetyl- α -glucosamine units (Domard & Domard, 2001; Islam *et al.*, 2017). In the chemical structure of CS, there are abundant reactive NH₂ groups that make

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chitosan a good candidate material to form dynamic Schiff base. Xanthan gum (XG) on the other hand, is a polysaccharide produced from the bacterium *Xanthomonas campestris* having repeating units composed of D-glucose, D-mannose, D-glucuronic acid, acetal-linked pyruvic acid, and O-acetyl (Hassler & Doherty, 1990). It can be polymerized or crosslinked with other polysaccharides to generate gels or films with different mechanical and rheological properties. XG could form Schiff base interaction with CS through modification by periodate-mediated oxidation leading to C–C cleavage between (–CHOH)₂ groups to generate dialdehydes (Guo *et al.*, 2014). This oxidation process introduces a large number of aldehyde groups into the XG chains, which improve its reactivity and provides Schiff base bonding interaction with the NH₂ group of CS.

In this paper, we hereby report a novel hydrogel with a self-healing capability prepared from chitosan and oxidized xanthan gum.

EXPERIMENTAL

Materials. Chitosan in medium molecular weight (MMW), concentrated acetic acid, sulfuric acid (H₂SO₄), absolute ethanol (EtOH), hydrochloric acid (HCl), hydroxylamine hydrochloric acid (NH₂OH(HCl)), xanthan gum, chitosan, and potassium metaperiodate (NaIO₄) were obtained from Sigma-Aldrich and used as received. Infrared spectroscopy (IR) was obtained using Nicolet FT-IR Spectrophotometer by KBr pellet technique.

Oxidation of Xanthan Gum. The oxidation of xanthan gum (1.0 g) was done by dissolving in distilled water (25 mL), and adding 10 mL of 0.96 g/mL NaIO₄. The pH of the mixture was maintained at pH 5 using 1 M H₂SO₄ and stirred at 600 rpm at 40 °C for four (4) hours in the dark (Guo *et al.*, 2014). The oxidized xanthan gum (OXG) was precipitated into a beaker of absolute ethanol. The precipitates were filtered, washed with distilled water, and vacuum-dried overnight to afford a white powder.

The degree of oxidation of xanthan gum was determined by determining its aldehyde content via titration with hydroxylamine hydrochloride. The oxidized xanthan gum (100 mg) was dissolved in 10 mL distilled water and the pH was adjusted to 7.60 using 1 M NaOH. NH₂OH(HCl) (0.72 M; 4 mL) was added to the mixture and stirred for 4 hours at 40 °C. The resulting mixture was then titrated with 0.5 M NaOH with its volume recorded as V_c. The same concentration of xanthan gum was also titrated to serve as the blank and the titrant needed was recorded as V_b. Each set was done in triplicates

and the aldehyde content was calculated using the equation

$$\text{aldehyde content (\%)} = \frac{(V_c - V_b) \times C_{\text{NaOH}}}{8 \times \frac{m}{M}} \times 100$$

where C_{NaOH} is the concentration of NaOH in molarity, *m* is the dried mass in g of the sample, and *M* is the approximate molecular weight of the repeating units of xanthan gum, which is 934 g/mol.

Swelling Ratio Determination. The dried hydrogels were immersed in 20 mL distilled water for 24 hours and weighed afterwards. The swelling ratios of the hydrogels were determined using the formula

$$\text{swelling ratio} = \frac{(W_s - W_d)}{W_d} \times 100$$

where W_s is the mass of the swollen hydrogel and W_d is the mass of the overnight dried hydrogel.

Self-healing Assay. The self-healing assays were done by slicing the hydrogel into two parts using a glass cover slip. The two separated portions were then put side-by-side to come in contact. The time until the hydrogel fully self-healed was recorded for each of the treatments. The self-healed hydrogels were then lifted to show that the linkages were stable and reflective of self-healed materials. To further demonstrate the self-healing capability of hydrogels, weights were connected on one side of the gel using a thin wire and binder clips. For the CS-OXG of ratio 1:1, they were dipped into a dye to better demonstrate the self-healing of the gel.

RESULTS AND DISCUSSION

Oxidation of Xanthan Gum. The periodate oxidation of xanthan gum was confirmed by comparing the FT-IR spectra of the raw xanthan gum (XG) with the oxidized xanthan gum (OXG) (Figure 1). Three similar bands, at 1200-1000 cm⁻¹ (C–O and C–C stretching vibrations of the hexopyranosyl), 1415 cm⁻¹ (OH bending) and 3700-3000 cm⁻¹ (OH stretching vibration) were observed for both spectra. However, spectral differences can be seen at 2830 cm⁻¹ and 2720 cm⁻¹, where two peaks appeared in the OXG. These two peaks indicate the presence of the aldehyde groups. The appearance of another new peak at 1730-1720 cm⁻¹ in the OXG spectra was an indication of the successful periodate oxidation process, where this peak was attributed to the C=O stretching vibration in the aldehyde. This observation was supported by the study of Fan *et al.* (2001), where the aldehyde IR absorbance peak in periodate oxidation process is located in this region. It is not easy to detect the presence of aldehyde

groups because it readily interacts with water or neighboring groups to generate hydroxyl group absorbance in the IR spectrum. However, properly drying the sample prior to FT-IR subtraction, would make it easier for the aldehyde group to be seen and characterized in the region $1730\text{--}1720\text{ cm}^{-1}$.

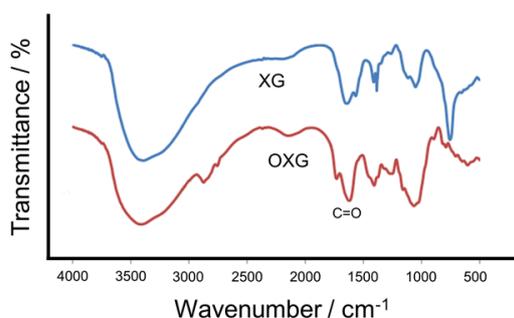


Figure 1. FT-IR Spectra of Xanthan Gum (XG; above) and Oxidized Xanthan Gum (OXG; below).

The presence of aldehyde groups in OXG was further confirmed by titration of the OXG samples by hydroxylamine hydrochloric acid. Essentially, hydroxylamine HCl reacts with the carbonyl groups of the OXG and forms Schiff bases liberating one HCl for every single aldehyde present on OXG—the liberated HCl, titrated against NaOH—enable the determination of the aldehyde content in an OXG sample. XG was also titrated to serve as the blank. The average volume of the NaOH titrant used for the blank and for the OXG was determined to be 7.89 mL and 15.60 mL, respectively. The average aldehyde content in OXG is found out to be 45.02%.

Formation of Hydrogels. The chitosan (CS) and the OXGs were then mixed in different weight ratios (CS:OXG = 1:0.5, 1:1 and 1:1.5) and immediately formed into hydrogels. The textures of the hydrogels were not very smooth as a result of the inherent coarseness of the MMW chitosan. The viscosities of the CS-OXG hydrogels were observed to decrease as the ratio of OXG increases (Figs 2B-D).

The FT-IR spectrum of each hydrogel confirmed the presence of the expected linkages (Figure 3). For the CS-XG hydrogel, it is consistent with the work of Rao *et al.* (2018) where peaks at around 1630 cm^{-1} and a carboxylic $\text{C}=\text{O}$ stretching peak at 1088 cm^{-1} indicate a successful formation of a complex with supplementary hydrogen bonding interactions between the negatively charged XG and positively charged CS. For the CS-OXG hydrogels, it is evident that there is the presence of imine bonds at 1640 and 1690 cm^{-1} . Moreover, broad peaks at about 3400 cm^{-1} indicate the presence of OH groups for all of the hydrogels affirming their water contents or ability to hold water.

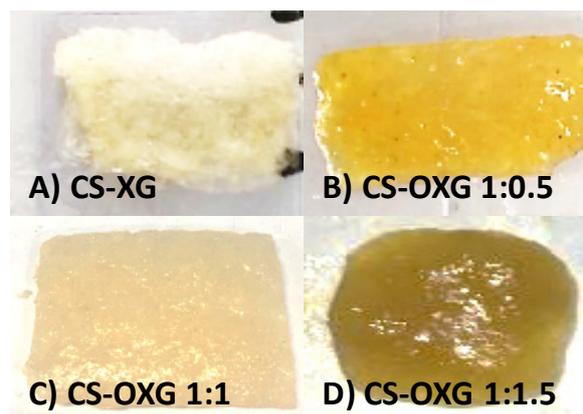


Figure 2. Prepared Hydrogels in Different Composition by Weight Ratios of Chitosan (CS):Oxidized Xanthan Gum (OXG).

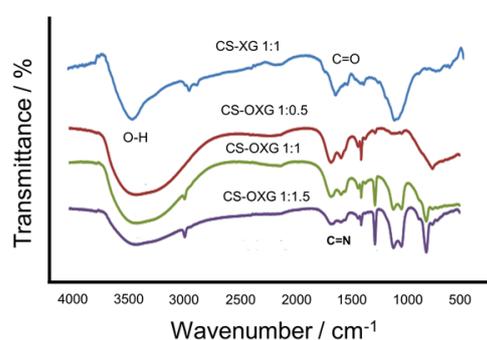


Figure 3. FT-IR Spectra of the Prepared Hydrogels in Different Composition by Weight Ratios of Chitosan (CS):Oxidized Xanthan Gum (OXG). Also, the FT-IR Spectrum of the Chitosan:Unoxidized Xanthan Gum (CS-XG) at 1:1 Weight Ratio is Shown.

The swelling ratio of the hydrogels was found to be inversely proportional to the amount of OXG (Table 1). This is attributed to the increase in aldehyde content in OXG, where more crosslinking interactions occur, which reduced the swelling ratio. This finding was supported by De Carvalho & Grosso (2014) that an increase in crosslinking results in a decrease in the water binding capacity, thereby leading to a decrease in the swelling ability of the prepared hydrogels. Moreover, the use of dye absorption on the CS-OXG hydrogels confirms its differences in the ability to absorb.

Table 1. Swelling Ratios of the Hydrogels

	W_{dried}	W_{swollen}	Swelling Ratio
CS-XG 1:1	0.27g	*	*
CS-OXG 1:0.5	0.28 g	1.06 g	2.79
CS-OXG 1:1	0.29 g	0.85 g	1.93
CS-OXG 1:1.5	0.28 g	0.54 g	0.93

*Got dissolved into the solution during the swelling experiment

Self-healing Assay. The hydrogels were cut into two parts and allowed to directly contact with each other to facilitate the self-healing process (Figure 4).



Figure 4. Illustration of the Self-healing Assay Used for the Hydrogels.

The CS-XG hydrogel did not self-heal (Figure 5). For the CS-OXG having a weight ratio 1:0.5, it was able to fully self-heal after 120 minutes. After its separated portions got reconnected, a counterweight was applied to check its self-healing characteristic. It was able to withstand a counter weight of 5.30 g attached to one side before the film got broken.

	Before cutting	After cutting	Self-healed	Healed test via counter-weights
CS-XG 1:1				
CS-OXG 1:0.5				
CS-OXG 1:1 Undyed				
CS-OXG 1:1 Dyed				
CS-OXG 1:1.5				

Figure 5. The Results of the Self-healing Assay Applied on Various Hydrogels Prepared in Different Composition by Weight Ratios of Chitosan (CS):Oxidized Xanthan Gum (OXG). The results from the chitosan: unoxidized xanthan gum (CS-XG) at 1:1 weight ratio is also shown.

For the CS-OXG of ratio 1:1, it was able to fully self-heal after 90 minutes of contact. At 1:1 weight ratio, it can carry a heavier counterweight of 6.50 g attached to one side of the gel before it got separated into two pieces again. The same procedure was also done with CS-OXG of ratio 1:1.5 where it was allowed to self-heal for about 210 minutes. However, when the weight was added, it broke in less than 3 seconds. All prepared CS:OXG hydrogels

successfully self-healed at room temperature and pressure without any external stimulus such as water, pH level and increase in temperature. Furthermore, the hydrogels still have the ability to self-heal even when they were cut into small pieces.

To sum up, the optimal weight per weight ratio for self-healing of CS-OXG hydrogels is 1:1. This can be explained by the aldehyde groups in OXG being optimally crosslinked with the amino groups of CS by the Schiff base reaction. In addition, the aldehyde content of 45.02% of OXG results in an enhanced self-healing character of the hydrogel. An increase in the oxidation degrees of OXG would result to an increase in the extent of crosslinking. These kinds of hydrogels can be further improved into physiological materials, which are usually beneficial for application in the biomedical field.

CONCLUSION

The oxidation of the xanthan gum (XG) by periodate oxidation afforded aldehyde groups (45%) that when combined with chitosan, the oxidized xanthan gum (OXG) results in the preparation of hydrogels with self-healing capabilities. These were demonstrated to interact with the NH_2 groups of the chitosan (CS), and as verified by FT-IR measurements, showed effective crosslinking between CS and OXG through dynamic Schiff base linkages. All prepared CS:OXG hydrogels (at different weight ratios of CS:OXG=1:0.5; 1:1 and 1:1.5) successfully self-healed at room temperature and pressure without any external stimulus such as water, pH level and increase in temperature. Furthermore, the hydrogels still have the ability to self-heal even when they were cut into small pieces. A similar hydrogel prepared from the mixture of chitosan and unoxidized xanthan gum did not exhibit similar self-healing property.

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