

Study on Partially Oxidized Sodium Alginate with Potassium Permanganate as the Oxidant

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ABSTRACT: In this article, partial oxidation of sodium alginate with potassium permanganate (KMnO_4) was researched successfully. The effects of KMnO_4 on the properties of partially oxidized sodium alginate were discussed. It was found that the degree of oxidation and degradation rate of sodium alginate could be controlled by oxidation conditions, as monitored by the reduction of viscosity and decrease of molecular weight. This work pro-

vides a new method to obtain partially oxidized alginate and to improve degradability of alginate for biomedical applications. © 2009 Wiley Periodicals, Inc. *J Appl Polym Sci* 113: 3585–3589, 2009

Key words: sodium alginate; potassium permanganate; partially oxidized; degradability; biomaterials; degradation; modification

INTRODUCTION

The injectable polymer scaffolds which are biocompatible and biodegradable are important biomaterials for tissue engineering and drug delivery. Hydrogels derived from natural proteins and polysaccharides are ideal scaffolds for tissue engineering as they resemble the extracellular matrices of the tissue comprised of various amino acids and sugar-based macromolecules. Although alginate, a naturally derived polysaccharide, is an attractive material and has been widely used in a variety of biomedical applications owing to its biocompatibility and ability to form hydrogel, its slow and uncontrollable degradation can be an undesirable feature. And with hydrogel degradation, high molecular weight strands are released, which may have difficulty being cleared from the body. Usually, degradability is a critical property of materials utilized in tissue engineering. To apply alginate to injectable systems for tissue engineering, drug delivery and other medical applications, scientists have done much good work to enhance its properties. Partially oxidized alginate with sodium periodate is a popular method, which creates acetal groups susceptible to hydrolysis.^{1–4} In this study, an alternative method to partially oxidized alginate is evaluated.

As a strong oxidizing agent, potassium permanganate (KMnO_4) shows potential to partially oxidize

alginate more effectively. And KMnO_4 is cheaper, safer and more accessible than sodium periodate. However permanganate is difficult to control the degree and speed of oxidation.⁵ The effects of KMnO_4 on the properties of partially oxidized alginate were discussed, including molecular structure, degree of oxidation, molecular weight, and degradability. The degradability and degree of oxidation of sodium alginate were monitored by the reduction of viscosity and decrease of molecular weight. The work provides a new method to obtain partially oxidized alginate and to improve the properties of alginate for biomedical applications.

MATERIALS AND METHODS

Materials

Sodium alginate (SA) with high guluronate content (79%) was purchased from Sinopharm Chemical Reagent (Beijing, China). KMnO_4 , sodium chloride (NaCl), calcium chloride (CaCl_2), sulfuric acid (H_2SO_4), hydroxylamine hydrochloride, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Guangzhou chemical reagent factory (Guangzhou, China). All chemical reagents were of analytical grade and used as received.

Methods

KMnO_4 oxidizing sodium alginate

SA were diluted to 0.5–2% w/v in deionized water. 0.025 mol/L KMnO_4 aqueous solution was prepared as follows. KMnO_4 (3.2 g) was dissolved in 1 L of

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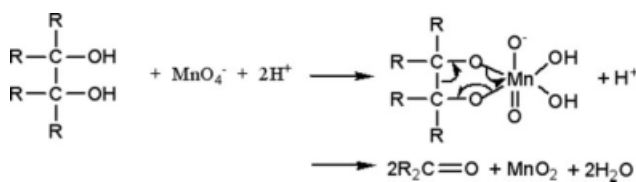


Figure 1 Mechanism of vicinal diols oxidized with KMnO_4 .

deionized water and boiled for about 1 h and left to stand overnight. MnO_2 by filter and the solution was transferred to an amber glass-stoppered bottle. The concentration of KMnO_4 solution was determined by titration.

Sodium alginate solutions (100 mL) were oxidized with KMnO_4 by stirring and in the dark at room temperature until the color of mixed solutions changed from violet to colorless. H_2SO_4 (1 mol/L) was added to the mixed solutions as a catalyst and the pH was maintained at 3–4 during the oxidation process. NaCl was added at the end of the reaction; the solution was subsequently dialyzed (cut off $M_w = 10,000$), filtered, and lyophilized.

Structure character

The microstructure of partially oxidized sodium alginate was investigated and compared with that of natural sodium alginate by FT-IR Paragon 1000 Fourier transform infrared spectrometer (Perkin Elmer, USA).

Circular dichroism (CD) spectra of samples were measured using J-810 circular dichroism spectrometer (JASCO Corporation, Japan) in the range of 185–400 nm, bandwidth 1 nm, time constant 2 s, scan speed 20 nm/min, path length 0.1 nm.

Degree of oxidation

Degree of oxidation was quantified by measuring the aldehyde content of these modified polymers using hydroxylamine hydrochloride titration via oxime derivatization to the following reaction and the procedure, as previously described.⁶

In brief, an oxidized polymer solution was added dropwise to a 2 mol/L hydroxylamine hydrochloride solution, whose pH was also adjusted to 4 with HCl. During the reaction, the pH of the mixture was maintained at 4 with adding NaOH solution. Aldehyde levels are then calculated based on the total consumption of NaOH using the following equation:

$$\text{Degree of oxidization} = \frac{N_{\text{NaOH}}}{W_{\text{SA}}/198} \times 0.5 \times 100\%$$

N_{NaOH} (mol): the total consumption of NaOH; W_{SA} (g): the weight of SA; 198: the weight of per SA cell.

Viscosity average molecular weight of samples

The intrinsic viscosity ($[\eta]$) measurements of 0.1% alginate solution were carried out in H_2O at 35°C with an Ubbelohde viscometer. The viscosity average molecular weight (M_η) was calculated from $[\eta]$:

$$[\eta](100\text{L/g}) = 2.0 \times 10^{-5} \times M_\eta$$

Degradation

To form hydrogels, 1% alginate solutions were mixed with 1% CaCl_2 deionized H_2O solution (ratio of 1 mL CaCl_2 to 1 mL alginate) with quick stir. The resulting mixture was cast between two glass plates separated with 1 mm spacers and allowed to gel for 30 min. Gel disks were punched out using an 11.1 mm puncher. The slices were weighed, incubated in 100 mL vials filled with simulated body fluid (SBF), and placed in a BS-2F water shaker bath (Honghua Instruments, Jiangsu Jintan, China) at 37°C . The surfaces of slices were dried with filter paper before they were weighed. The degradation process was followed by the daily recording of the mass retention of the slices. The SBF in the vials was replaced everyday too. The results are the averages of three separate specimens per polymer.

RESULTS AND DISCUSSION

Conceivable mechanism of KMnO_4 oxidizing sodium alginate

Just as sodium periodate, KMnO_4 is a strong oxidant and oxidizes vicinal diols to two aldehyde groups, as shown in Figure 1. If excess KMnO_4 is used, aldehyde groups can be further oxidized to carboxyl groups.

The chemical structure of alginate, which is a linear block copolymer of β -D-Mannuronic acid (M unit) and α -L-Guluronic acid (G unit), shown in Figure 2, was uronic acids and vicinal diols in its structure. Hence, it is possible to open saccharide rings between vicinal diols leaving two aldehyde groups via oxidized with KMnO_4 . The conceivable mechanism of KMnO_4 oxidizing sodium alginate is shown in Figure 3.

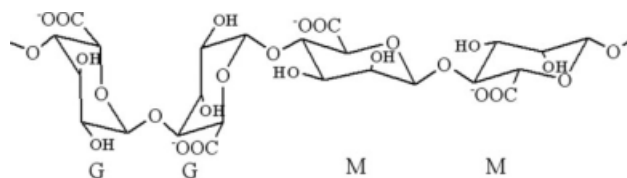


Figure 2 Molecular structure of sodium alginate.

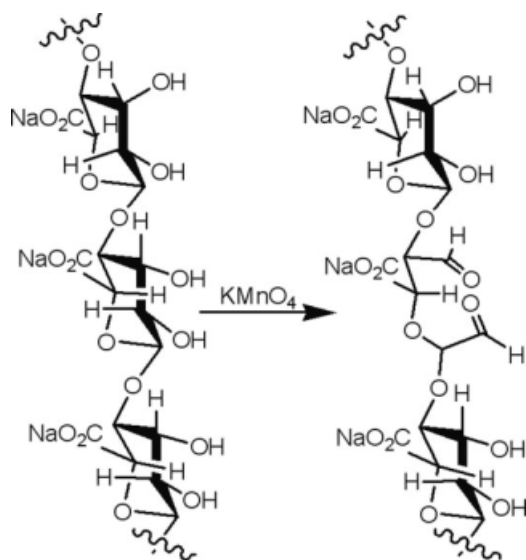


Figure 3 Possible structure of KMnO_4 -oxidized sodium alginate.

Structure character of partially oxidized sodium alginate

In natural SA, there are lots of strong polar hydroxyl groups which are easy to form hydrogen bond with another electron donating groups, such as $-\text{NH}_2$, $-\text{OH}$ and $\text{C}-\text{O}-\text{C}$. The FT-IR spectrum of SA shows a broad band to assigned to hydroxyl groups at 3438 cm^{-1} , and $\text{C}-\text{O}$ stretching vibration absorption peak at 1034 cm^{-1} [shown in Fig. 4(a)]. And because of existing in sodium salt, carboxyl groups in SA formed carboxylate anion, in which both $\text{C}=\text{O}$ bonds were similar without the difference between single and double bonds. So antisymmetric and symmetric stretching vibration absorption peaks appeared at 1626 cm^{-1} and 1384 cm^{-1} . Owing to the effect of ring tension of hexatomic ring in SA, $\text{C}-\text{H}$ stretching vibration absorption peak appeared at 2926 cm^{-1} .⁷ Compared with natural SA, partially oxidized SA presented a new characteristic peak at 1727 cm^{-1} [as shown in Fig. 4(b)], which was assigned to an aldehyde group $\text{C}=\text{O}$. Another characteristic peaks $\text{C}(\text{O})-\text{H}$ of aldehyde groups should be at 2820 cm^{-1} and 2720 cm^{-1} , which were covered by $\text{C}-\text{H}$ alkyl absorption peak. Therefore, SA was shown to be oxidized with KMnO_4 , and aldehyde groups were formed in the alginate structure. The result also further supports the above mechanism of KMnO_4 oxidizing SA.

In the SA molecular structure, O(4) of M unit is at tiled bond, while O(4) of G unit is at axial bond. Their conformations are just opposite. So there is optically active difference in their CD spectra. The change of M and G units in SA structure can be observed from CD spectra.⁷

There were an up peak at 200 nm and a down peak at 210 nm in the natural SA CD spectra (shown in Fig. 5), which indicated M and G units, respectively. But after oxidation with KMnO_4 under acidic condition, the characteristic peak assigned to M units was weak at around 200 nm in the CD spectra. KMnO_4 ($0.005 \times 10^{-2}\text{ mol/L}$) provided the almost same effect on oxidation of SA, as $0.02 \times 10^{-2}\text{ mol/L}$ KMnO_4 . In these CD spectra, there were only the characteristic peaks of G units at 210 nm, just like monosaccharide. While without acid, the change of CD spectra could not happen even if with high concentration $0.4 \times 10^{-2}\text{ mol/L}$ KMnO_4 . Addition of an acid catalyst was an important factor in the oxidation reaction.

During the process of oxidation, oxidant KMnO_4 cleaved glycosidic bonds of M units, which were weak relatively. On the other hand, almost all vicinal diols $\text{C}-\text{C}$ bonds of G units were cleaved into aldehyde groups with KMnO_4 . Except for a few integral G units which were distributed in isolation, there were hardly unoxidized G long blocks in the oxidized SA. Hence, the character of G monosaccharide was put up on the CD spectra of oxidized SA.

Effect of reaction conditions on the properties of partially oxidized SA

Degree of oxidation

The natural SA and uncatalyzed KMnO_4 -oxidized SA showed no signs of aldehyde groups. While under acidic condition, KMnO_4 exerted a strong

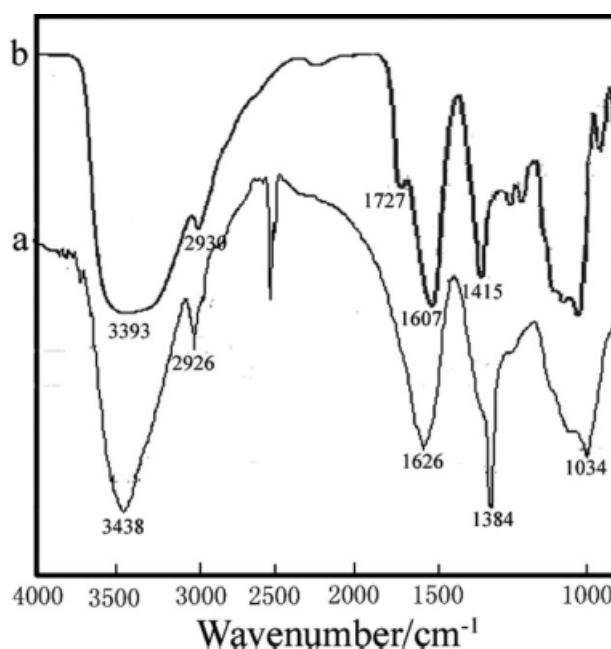


Figure 4 FT-IR spectra of natural SA (a) and partially oxidized SA with $0.005 \times 10^{-2}\text{ mol/L}$ KMnO_4 pH = 3 (b).

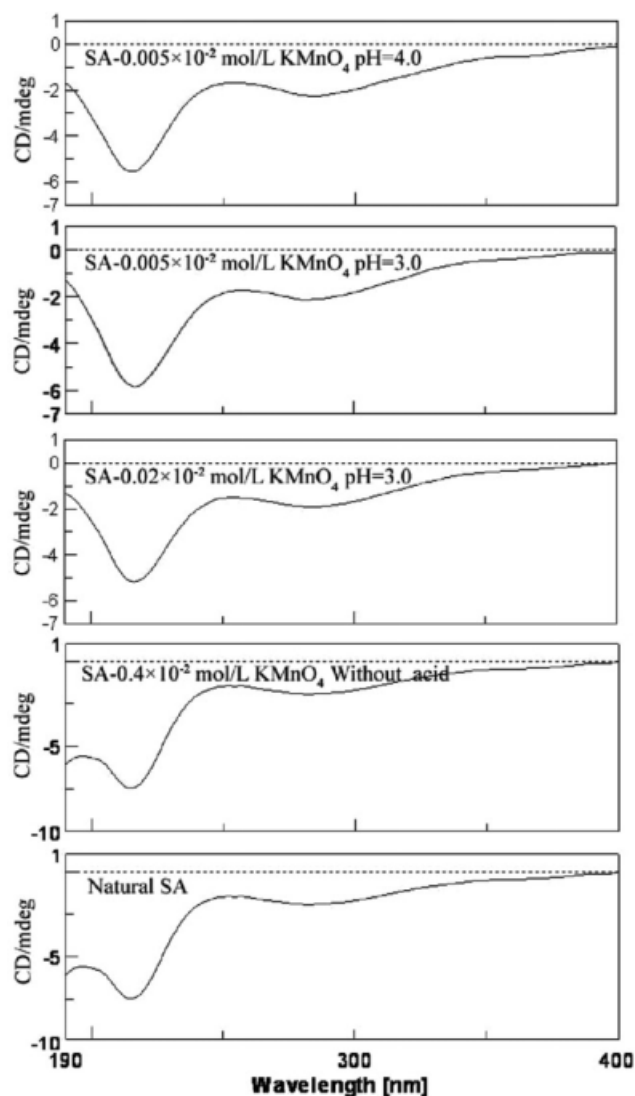


Figure 5 CD spectra of natural SA and partially oxidized SA.

oxidation ability on SA. The concentration of KMnO_4 and pH value of oxidation reaction had obvious effect on degree of oxidation of partially oxidized SA. As shown in Figure 6, the degree of oxidation of samples was increased markedly with an increase of KMnO_4 concentration. With a decrease in pH of the reaction solution, the degree of oxidation of SA increased.

When the KMnO_4 concentration went up to 0.05×10^{-2} mol/L, the degree of oxidation reached a peak. Considering that the excess KMnO_4 could further oxidize aldehyde groups into carboxyl groups, the presence of excess KMnO_4 made the degree of oxidation decrease, as shown in Figure 6.

Molecular weight

Oxidation had an important effect on the molecular weight of SA, as shown in Figure 7. It was shown that

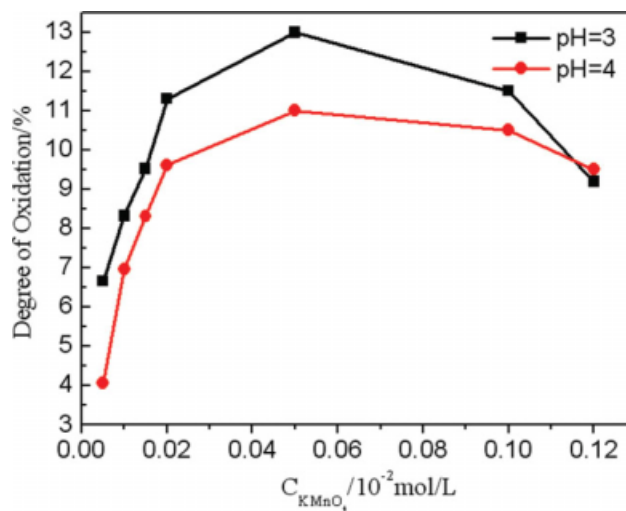


Figure 6 Effect of concentration of KMnO_4 on the degree of oxidation of partially oxidized SA. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

M_n of partially oxidized SA samples were lower than that of natural SA. With an increase of KMnO_4 concentration, M_n of partially oxidized SA decreased steadily. When the KMnO_4 concentration increased from 0 to 0.01×10^{-2} mol/L, pH = 4, M_n of partially oxidized SA decreased from 568,000 to 298,000. While at pH = 3, the M_n of partially oxidized SA decreased to 226,000. The pH value of solution had also an influence on the molecular weight, as shown in Figure 7. The M_n downtrend of SA in pH = 3 solution was more rapid than that in pH = 4. When the KMnO_4 concentration was 0.02×10^{-2} mol/L, the M_n of partially oxidized SA was 220,000 at pH = 4, while it was 156,000 with pH = 3. Hence, it was indicated that both

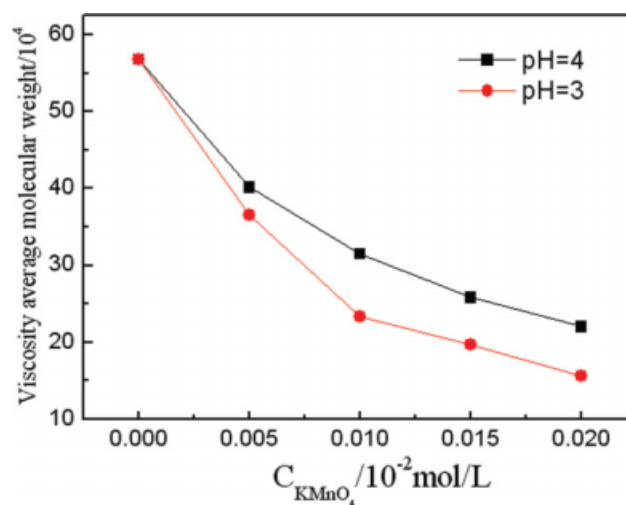


Figure 7 Viscosity average molecular weight (M_n) of partially oxidized SA samples. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

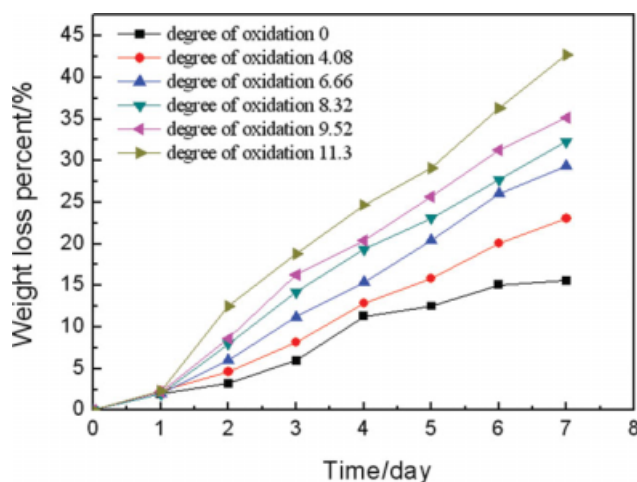


Figure 8 Degradation of SA samples with different degrees of oxidation at 37°C, pH = 7.4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

pH value and oxidant quantity had an important influence on M_n of partially oxidized SA samples. To obtain samples with definite molecular weight, pH value and oxidant quantity should be controlled.

Degradation

Degradation of SA samples with different degrees of oxidation at 37°C at pH = 7.4 is shown in Figure 8. Degradation rate of natural SA was slow and reached a steady level after 4 days at pH = 7.4, 37°C. And the most weight loss percent was only 23% after 7 days. Degradation of partially oxidized SA samples had a perceptible improvement. With the increase of degree of oxidation of partially oxidized SA from 0 to 11.3, the degradation rate sped sharply. The most weight loss percent of partially oxidized SA samples was about 43% with degree of oxidation 11.3. It was about twice as rapid as natural SA. But if degree of oxidation was exorbitant, the samples could not form stable hydrogel and dissolved in solution.

After oxidation, one vicinal diols C—C bond in SA structure were cleaved into two aldehyde groups, forming an open-ring structure. This change could

make the β 2 glycosidic bond freely rotating, and its molecular movement just liked the acetal structure, which was easy to hydrolyze.⁸ At the same time, as the foregoing statement, the molecular weight of SA decreased with oxidation. It also led to the increase of degradation rate of SA. Therefore oxidation with KMnO_4 was a practical way to improve the degradability of SA.

CONCLUSIONS

From the experimental study in detail, it was discovered that sodium alginate can be partially oxidized with KMnO_4 under acidic condition.

Oxidation of SA resulted in cleaving vicinal diols into two aldehyde groups. The degree of oxidation can be controlled through adjusting pH value and quantity of KMnO_4 . After oxidation, the molecular weight of SA reduced. The degradation rate of oxidized SA was dependant on the degree of oxidation of SA. Therefore, this work provides a new method for the chemical modification of SA, and overcomes the poor degradability of the natural SA.

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