Oxidative Degradation of Chitosan to the Low Molecular Water-Soluble Chitosan over Peroxotungstate as Chemical Scissors

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Abstract
Low molecular water-soluble chitosan was prepared by the depolymerization of chitosan in the presence of a series of catalysts with active WO3 sites. Both the peroxy species [W2O3(O2)2]3− and [PO4(WO3)2]4− showed high efficiency in the degradation of chitosan, indicating that the degradation mechanism did not follow the radical mechanism. That means δOH is not the active species, which has been proven by the fluorescence spectra. H2O2 acted as an oxidant to regenerate the active WO3 sites in the depolymerization of chitosan. The developed catalyst (TBA)2[PO4(WO3)2]4 is recoverable.

Introduction
Chitosan is a biopolymer obtained by partial deacetylation of chitin, which is the second most abundant polysaccharide in nature after cellulose[1,2]. However, chitosan has a high molecular weight and low solubility in most solvents, which limits its applications in agriculture[3,4], food and beverages[5], wastewater treatment[6-8], pharmaceuticals[9,10] and biomaterials[11]. By the degradation process chitosan can be converted to the low-molecular-weight chitosan which exhibits good water solubility. To date, acid hydrolysis, basic hydrolysis and oxidation-alkaline treatment[12–15], have been studied[12–15]. Recently, Huang et al. reported that a Keggin heteropoly acid, H3PW12O40, can efficiently catalyze oxidative degradation of chitosan with H2O2 to the LMWSC[16–18]. The authors presumed that 1) the peroxy species [PO4(WO3)2]4− formed by phosphotungstic acid in the presence of H2O2 is the real catalyst, 2) the peroxy species [PO4(WO3)2]4− accelerates the formation of free radicals by the disassociation of hydrogen peroxide, 3) the free radicals can attack the β-(1–4) glucosidic bond of chitosan to obtain the LMWSC. To the best of our knowledge, however, little experimental data is available to support this conjecture. Besides, some researchers[19,20] have reported that heteropolycylics with the Keggin structure [H3PW12O40] are degraded in the presence of H2O2 to form two peroxy species [PO4(WO3)2]4− and [W2O3(O2)2]3− (Figure 1). These results motivated us to reconsider why the real catalyst is not [W2O3(O2)2]3− but the peroxy species [PO4(WO3)2]4−, also it is not clear why the peroxy species [W2O3(O2)2]3− formed from H3PW12O40 showed no catalytic activity.

In this report, the isolated peroxy species (TBA)2[PO4(WO3)2]4 and K2[W2O3(O2)2]4 were directly employed as the catalyst to degrade chitosan. We demonstrated that not only the peroxy species [PO4(WO3)2]4−, but also [W2O3(O2)2]3− are highly efficient catalysts. The peroxy species itself can efficiently degrade chitosan without hydrogen peroxide. Moreover, the (TBA)2[PO4(WO3)2]4 catalyst can be recycled.

Experiment Section
Materials
H3PW12O40, Na3PW12O40, K2WO4, (NH4)2WO4, Na2WO4, EDTA, KSCN, SnCl2 and TiCl3 (AR) were purchased from Aladdin Industrial Corporation. H2O2 (Hydrogen peroxide, 30%) and Chitosan (DD≥90%) were purchased from Sinopharm Chemical Reagent Co., Ltd. The water used was distilled.

Synthesis of (TBA)2[PO4(WO3)2]4
The (TBA)2[PO4(WO3)2]4 was prepared according to the literature[19,21]. Hydrogen peroxide (30%) (10 mL, 100 mmol) was added to a solution of H3PW12O40 (1.65 g in 1 mL water). After 30 min, an aqueous solution of tetrabutylammonium hydroxide (1 M) was added until the pH reached 10. The precipitate was filtered out, washed several times with water, and then air dried. IR(KBr): ν[PO4]:1084, 1053 cm−1, ν(W–O): 964 cm−1, ν(O–O): 845 cm−1, νasym[WO3]: 593 cm−1, νsym

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\[ [\text{W(O)}_2]^- : 523 \text{ cm}^{-1}, \text{ Raman (in CH}_3\text{CN): } v(\text{PO}_4): 1050, 1024 \text{ cm}^{-1}, v(\text{W} = \text{O}): 957 \text{ cm}^{-1}, v(\text{O} = \text{O}): 840 \text{ cm}^{-1}, v_{\text{asym}}[\text{W(O)}_2]^- : 591 \text{ cm}^{-1}, v_{\text{sym}}[\text{W(O)}_2]^- : 520 \text{ cm}^{-1}. \]

**Synthesis of K\(_2\)[W\(_2\)O\(_3\)(O\(_2\))\(_4\)]**

K\(_2\)[W\(_2\)O\(_3\)(O\(_2\))\(_4\)] was prepared according to the literature\([19–21]\). A solution of K\(_2\)WO\(_4\) (5.0 g in 10 mL water) was placed in an ice-water bath. 30% H\(_2\)O\(_2\) (10 mL) was slowly added, and the solution turned light yellow. Dilute hydrochloric acid was added into the solution until the color just disappeared at pH = 2–3. Then the ethanol was added. The resulting white precipitate was filtered out, washed several times with ethanol and dried in air. IR (KBr): \( v(\text{W} = \text{O}): 966 \text{ cm}^{-1}, v(\text{O} = \text{O}): 854 \text{ cm}^{-1}, v_{\text{asym}}[\text{W(O)}_2]^- : 550 \text{ cm}^{-1}, v_{\text{sym}}[\text{W(O)}_2]^- : 615 \text{ cm}^{-1}, v_{\text{asym}}[\text{W}_2\text{O}_2]^- : 764 \text{ cm}^{-1}. \) Raman (solid): \( v(\text{W} = \text{O}): 958 \text{ cm}^{-1}, v(\text{O} = \text{O}): 861 \text{ cm}^{-1}, v_{\text{asym}}[\text{W(O)}_2]^- : 553 \text{ cm}^{-1}, v_{\text{sym}}[\text{W(O)}_2]^- : 537 \text{ cm}^{-1}, v_{\text{asym}}[\text{W}_2\text{O}_2]^- : 763 \text{ cm}^{-1}, v_{\text{sym}}[\text{W}_2\text{O}_2]^- : 450 \text{ cm}^{-1}. \)

**The typical procedure of the degradation of chitosan**

In a typical reaction, a mixture of 0.200 g of chitosan, 4.2 \( \mu \)mol tungsten (W) in the catalyst, 5 mL of the distilled water, 1 mL of 30% H\(_2\)O\(_2\), were stirred in the 50 mL round-bottomed flask for 20 min at different temperature. After the reaction, NaOH was added to the filtrate until pH = 7. The products were extracted with ethanol. The obtained white precipitate was filtered out, dried in vacuum and then analyzed.

**Determination of tungsten**

The filtrate was evaporated and calcinated at 400°C. Then the samples were digested by microwave with the digestion solvent of 40 mL NaOH solution (0.025 g/mL). After being digested for 30 min by medium-high level microwaves, the sample was cooled to room temperature, and 1 g EDTA and 30 mL deionized water were added into the beaker. Then the solution was transferred into 100 mL volumetric flask (I).

2 mL KSCN (0.5 g/mL), 4.5 mL SnCl\(_2\) (0.008 g/L) and 1.5 mL TiCl\(_3\) (0.15 g/mL) were added into 10 mL (I) solution, transferred 25 mL volumetric flask. Then the solution was measured on the Spectrophotometer 721 at \( \lambda = 406 \) nm.

**Analytical method**

The degradation ratio of chitosan was defined as follows:

\[
R\% = \frac{M_0 + M_{\text{cat}} - M_x}{M_0} \times 100
\]

where \( R \) refers to the degradation ratio of chitosan, \( M_0 \) refers to the quantity of the original chitosan, \( M_{\text{cat}} \) refers to the quantity of the catalyst, \( M_x \) refers to the quantity of the collected solid after degradation under different conditions.

**Figure 1. The chemical structure of peroxo species.**

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**Figure 2. The characterization of chitosan and the LMWSC, FTIR spectra (A) and DRS patterns (B).**

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Figure 3. The characters of chitosan and the LMWSC, XRD patterns (A), and the effect of the reaction time on the Mv of LMWSC (B).
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Figure 4. The oxidative degradation of chitosan, (A) H₃PW₁₂O₄₀ (0.35 μmol), Na₃PW₁₂O₄₀ (0.35 μmol) and H₂O₂ without any additives.
(C) (TBA)₃[PO₄(WO₄)₂]₄ (1.05 μmol), K₂[W₂O₇(O₂)₄] (2.1 μmol) and H₂O₂ without any additives. (D) Na₂WO₄ (4.2 μmol), (NH₄)₂WO₄ (4.2 μmol) and K₂[W₂O₇(O₂)₄] (2.1 μmol). Under the same reaction conditions: H₂O₂ (1 mL), H₂O(5 mL), 20 min. (B) Recycling of the filtrate at 65°C, 1 mL H₂O₂.
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The stockname viscosimeter was used to determine the intrinsic viscosities at 303 K. Chitosan was dissolved in 0.1mol/L CH₃COONa–0.2 mol/L CH₃COOH solution. The viscosity average molecular weight (Mᵥ) was calculated according to the following equation:[16–18].

\[
\eta = \eta_g + \frac{3\eta_g\eta_r}{4C}
\]

\[
\eta = 1.81 \times 10^{-3} M_v^{0.93}
\]

Here, \(\eta_g\) and \(\eta_r\) refer to the incremental viscosity and the relative viscosity respectively, \(C\) is the concentration of the LMWSC (g/mL), \(M_v\) is the viscosity average molecular weight.

The FT-IR spectra of samples were recorded on a NEXUS 670 FT-IR spectrometer with KBr pellets prepared by manual grinding. Raman spectra of samples were recorded on Renishaw RM1000 (\(λ = 514.5\) nm). Fluorescence spectra were acquired with an FLSP920 spectrofluorometer (Edinburgh Instruments Ltd, UK) at 20±1°C, equipped with a temperature-controlled circulator (Julabo, Germany). X-ray diffraction (XRD) patterns of samples were carried out on a PW 3040/60 X-ray diffractometer (Philips, Netherlands) with Cu Kα target at 40 kV and 40 mA. The samples were scanned from 5° to 70° of 2\(θ\). The UV-vis absorption spectra of the samples were measured in the range of 100–800 nm on a UV-vis spectrometer (Nilcolet Evolution 500, Thermo). The metal content of the product solution was measured on the Spectrophotometer 721 (Shanghai Analysis Instrument Company).

**Results and Discussion**

The characterization of chitosan

The chitosan and the LMWSC were characterized by FTIR in Figure 2A. The main bands of chitosan were as follows. The band at around 3422 cm\(^{-1}\) could be ascribed to the stretching vibration of O-H and N-H. The absorption peak at 1599 cm\(^{-1}\) corresponds to the binding vibration of the amido groups. An apparent carboxyl (–C = O) band at 1654 cm\(^{-1}\) is attributed to the residual acetyl. The band in the range 1157 cm\(^{-1}\) to 896 cm\(^{-1}\) belongs to the special absorb peaks of \(\beta\)-1,4 glycoside bond in chitosan[22]. The similar characteristics in the FTIR spectrum of the LMWSC can also be observed. Besides, a new absorption peak at 1720 cm\(^{-1}\) is observed in the spectrum of the LMWSC, which is assigned to the absorption of the carboxylic group (–COOH). It indicates the existence of the carboxylic group in the LMWSC. To further identify the carboxylic group of the LMWSC, DRS analysis are given in Figure 2B. There is an absorption band at 324 nm in the original chitosan, which is caused by the stretching vibration of O-H and N-H. The absorption peak at 1599 cm\(^{-1}\) corresponds to the stretching vibration of the amido groups. An apparent carboxyl (–C = O) band at 1654 cm\(^{-1}\) is attributed to the residual acetyl. The band in the range 1157 cm\(^{-1}\) to 896 cm\(^{-1}\) belongs to the special absorb peaks of \(\beta\)-1,4 glycoside bond in chitosan[22]. The similar characteristics in the FTIR spectrum of the LMWSC can also be observed. Besides, a new absorption peak at 1720 cm\(^{-1}\) is observed in the spectrum of the LMWSC, which is assigned to the absorption of the carboxylic group (–COOH). It indicates the existence of the carboxylic group in the LMWSC.

To further identify the carboxylic group of the LMWSC, DRS analysis are given in Figure 2B. There is an absorption band at 324 nm in the original chitosan, which is caused by the \(n\rightarrow\pi^*\) transition of residual acetyl (DD >90%). Compared with that of chitosan, the DRS spectrum of the LMWSC has a new absorption band at 357 nm caused by the \(n\rightarrow\pi^*\) transition of a carboxylic group in the LMWSC. Consequently, the DRS patterns reflected that the carboxylic group was formed, which coincided with the analysis of the FTIR spectra.

The X-ray powder patterns of chitosan and the LMWSC are shown in Figure 3A. The pattern of chitosan shows two characteristic peaks at \(2θ = 10.4^0\) and \(20.0^0\) which correspond to (1 0 0) and (0 2 0) reflections of the L-2 polymorph of chitosan[23]. For the LMWSC, the peak at \(20 = 10.4^0\) disappeared, and the strength of the peak at \(20 = 20.0^0\) decreased. It indicated the crystalline structure of the LMWSC was destroyed and the crystallinity decreased. LMWSC became more amorphous than chitosan[24]. It manifests that the
degradation of chitosan occurred preferentially from the amorphous region to the water-soluble molecular, which dissolved in the water. With the further degradation, the crystalline structure was destroyed thoroughly. Figure 3B shows the viscosity average molecular weight (Mv) of the LMWSC versus time on the (TBA)3{PO4[WO(O2)2]4} catalyst. After 20 min the Mv of the LMWSC can reach 5892.

The catalytic activities of the catalysts

We first reinvestigated the oxidative degradation of chitosan with H3PW12O40 and H2O2. Compared with H2O2, the degradation efficiency increased significantly (Figure 4A). This could be expected considering that both the acid hydrolysis of chitosan and the oxidative degradation of chitosan might occur in the presence of H3PW12O40. Thus, we switched to the salt Na3PW12O40. Interestingly, the degradation ratio of chitosan is not significantly changed when three Na+ substituted the position of protons in H3PW12O40, which allowed us to exclude the contribution of Brønsted acid catalysis for the reaction. In an attempt to interpret the results, we added an aqueous solution of tetrabutylammonium chloride to the solution of H3PW12O40 and 30% H2O2. The obtained white precipitate (TBA)3{PO4[WO(O2)2]4} was filtered out. It is worthwhile to note that the filtrate is still active for the degradation of chitosan although the catalytic activity is low. And most interestingly, the filtrate can be reused six times without loss of reactivity (Figure 4B). The results can be explained if (TBA)3{PO4[WO(O2)2]4} is not completely filtered out or the peroxo species [W2O3(O2)4]2− dissolved in the filtrate is also an active species. Therefore, we turned our attention to the two peroxo species {PO4[WO(O2)2]4}3− and [W2O3(O2)4]2−, which are always formed in the presence of H2O2.

The catalytic activity of the peroxo species

Next, we examined the oxidative degradation of chitosan by (TBA)3{PO4[WO(O2)2]4} and K2[W2O3(O2)4] with H2O2, separately. The results are shown in Figure 4C. We can see that the degradation ratio of chitosan is still high for the peroxo species, while only a very small amount of chitosan is degraded in the absence of peroxo species. Most interestingly, no significant change in the degradation ratio of chitosan was observed when (TBA)3{PO4[WO(O2)2]4} (the content of W atom: 4.2 μmol) and K2[W2O3(O2)4] with H2O2, separately. The results also motivated us to revisit the structure of the two peroxo species (Figure 1). Both of the peroxo species possess the same unit of W(O2). It indicates that the unit of W(O2) might be the active sites for oxidative degradation of chitosan.
chitosan. In order to prove the above suggestion, Na2WO4 or (NH4)2WO4 with H2O2 which could also form the active W(O2)3 sites[21,25] were used as the catalysts to degrade chitosan. As shown in Figure 4D, the two experiments exhibit the degradation ratio of chitosan as high as that of K2[W2O3(O2)4], which is consistent with our suggestion of the W(O2)3 active sites.

Except where noted, we are interested to know whether the K2[W2O3(O2)4] containing the active W(O2)3 sites can behave as the efficient oxidant without H2O2 in the preparation of LMWSC. Therefore, some comparative experiments were carried out and the results are summarized in Figure 5A. It is clear that the degradation ratio of chitosan was high with K2[W2O3(O2)4] in the absence of H2O2, while that was low with only H2O2. Moreover, the results further supported the hypothesis that the structure of W(O2)3 is the real active site in the degradation of chitosan because both of them contain an equal quantity of the peroxo group[10 - 11 mmol]. To the best of our knowledge, this is the first example of highly efficient utilization of the peroxo group to obtain the LMWSC. The peroxo species K2[W2O3(O2)4] itself was more efficient for the degradation of chitosan than hydrogen peroxide. This intriguing finding shows that we need to know more about the oxidative degradation of chitosan by K2[W2O3(O2)4] with H2O2.

The mechanism of the degradation of chitosan

It has been well documented in previous work that the unit of W(O2)3 is the active sites for oxidative different substrate such as epoxidation of olefins[26,27], deasulfituration of fuels[28], and oxidation of alcohols[29]. Our work also lead to the conclusion that W(O2)3 structures are the active sites. There are efficient for the degradation of chitosan, even in the absence of H2O2. However, in the previous work,[13,15] decomposition of H2O2 to free radicals is considered as a key factor in the degradation of chitosan. To further investigate the role of H2O2 in the oxidative degradation of chitosan by K2[W2O3(O2)4] with H2O2, the decomposition of H2O2 was detected according to the literature[30,31]. The results were summarized in Figure 5B. The fluorescence signal at 426 nm can be assigned to 2-hydroxynaphthoatuspic acid, which results from the capture of ·OH by terephthalic acid. Compared with the fluorescence of the system with only H2O2, that of the system with an extra Na2WO4 or K2[W2O3(O2)4] was much weaker under the same conditions(Figure 5B a,c), which indicated a low content of ·OH was in the system and the peroxo species exhibited its inherent poor activity for the decomposition of hydrogen peroxide[32–35]. Combining the above facts, H2O2 was suggested to act as an oxidant to re-form the structure of W(O2)3, on which chitosan was degraded. Hence, a plausible reaction mechanism might contain the active W(O2)3 sites cutting the β-1,4 glycoside bond in chitosan to obtain the LMWSC and the regeneration of the active sites (Figure 6).

Recycling of the catalyst (TBA)3{PO4[W(O2)2]4}L

The catalyst K2[W2O3(O2)4] can be dissolved in water, which is not beneficial for the recycle of the catalyst. Hence, we turned our attention to the water insoluble catalyst (TBA)3{PO4[W(O2)2]4}. When the reaction had proceeded for 15 min, the vessel was cooled to room temperature. Then the catalyst was filtered and the filtrate was used for the next run. The results were shown in Figure 7A. After removal of the catalyst, the degradation ratio of chitosan was obviously decreased, and only 0.6 μg/mL W was determined in the product solution. The degradation ratio of chitosan was slightly increased after 15 min probably due to the minute amount of W remained in the filtrate. Then, the recycling experiment of the catalyst was investigated. The results in Figure 7B show that the degradation ratio could still reach a high level of 96.2% after recycling 7 times. For comparison, the acid hydrolysis method was also used in the degradation of chitosan and hydrochloric acid of different concentration acid was employed (Figure 8). With the increase in the concentration of HCl, the degradation ratio of chitosan increased. Although the degradation ratio could reach 74.8% in 3mol/L HCl, the degradation ratio decreased to 62.9% in 4mol/L HCl. It could be ascribed that the low concentration of hydrochloric acid favors the chitosan dissolving in solution, while high concentration of hydrochloric acid restrains the chitosan dissolution[36]. What’s more, large amounts of inorganic acid are not friendly for the environment. It’s clear that the novel catalyst developed herein is promising for application in preparation of LMWSC.

Conclusions

In conclusion, this study showed that the peroxo species K2[W2O3(O2)4] itself was more efficient for the degradation of chitosan than hydrogen peroxide. Both the peroxo species [W2O3(O2)4]2– and {PO4[W(O2)2]4}L are effective catalysts for the oxidative degradation of chitosan to obtain LMWSC in water with high efficiency of hydrogen peroxide utilization. The structure of W(O2)3 was considered to be the active site. In addition, the new catalyst (TBA)3{PO4[W(O2)2]4}L was efficient for the degradation of chitosan, and the recovered catalyst could achieve a high degradation ratio of 96.2% after 7 times.

Author Contributions

Conceived and designed the experiments: ZM YW YH WW. Performed the experiments: ZM WW. Analyzed the data: ZM YW YH WW. Contributed reagents/materials/analysis tools: YW TW. Wrote the paper: ZM YW WW.

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