# Metal-coordinating controlled oxidative degradation of chitosan and antioxidant activity of chitosan-metal complex

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**Dedicated to Professor Chengye Yuan**

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**Abstract**

A new method of metal-coordinating controlled oxidative degradation of chitosan leading to low molecular weight chitosan with uniform molecular weight distribution is reported in this paper. Chitosan is converted into chitosan-metal complex in which chitosan chain can be easily broken at weak points caused by coordinating bond. IR, UV, XRD, DSC and elemental analysis proved coordination between chitosan and the metal ion and the presence of weak points existing on the chitosan chain of complexes. Coordinating conditions (such as metal amount, speed of stirring, speed of metal salt addition) are controlled to obtain the complex with uniform metal distribution and uniform weak points distribution. $H_2O_2$ is added to break chitosan chain at those weak points to get low molecular weight chitosan. Degradative speed with different metal ion is different as follow: $Cu^{2+} > Co^{2+} > Mn^{2+} > Ni^{2+}$. With the increase of oxidant amount, temperature, and pH, and decrease of $O_2$ content, degradative velocity increase. HPLC/GPC spectra show that degradative velocity of complexes is faster than that of chitosan, and the molecular weight distribution is much more narrow than that of chitosan. Low molecular weight chitosan and its complexes with Cu(II) or Co(II) possess good $O_2^{\cdot -}$ scavenging activity.

**Keywords:** Chitosan-metal complexes, chitosan, oxidative degradation, low molecular weight chitosan, $O_2^{\cdot -}$, scavenging activity

## Introduction

Chitosan, copolymer of 2-amino-2-deoxy-β (1,4)-D-glucose and 2-acetamido-2-deoxy-β (1,4)-D-glucose units, is N-deacetylated product of chitin which is the second most abundant natural

polysaccharide. Chitosan and its derivatives have received most attention and are currently used in many fields. Low molecular weight chitosan (LCTS) is especially important in medicine, food, cosmetics and agriculture because of its special physiological activity such as antitumor, antifungal, enhancing immunity, lowering cholesterol levels, water retention, and so on.[1-7] But effectiveness of these chemicals has been found to be dependent on their molecular size. For example, from five samples with Mw 1500, 3000, 5000, 8000, 13000, the sample with Mw 1500 has best antifugal ability, and the samples with Mw 1500 and Mw 3000 have best water retention ability. Lowering blood fat ability of the Mw 8000-20000 chitosan is better than that of the oligomeric chitosan. So, it is very important to control the molecular weight of degraded chitosan to satisfy the requirement of its various utilization. At present, numerous methods have been proposed for degradation of chitosan. Enzymatic degradation (such as chitosanase, lipase, proteinase, polysaccharase), physical degradation (by X-ray, light, microwave), chemical methods, including acidic hydrolysis with some inorganic acid (such as HCl, HF, $H_2SO_4$, $H_3PO_4$, $HNO_2$) or some organic acid (such as HOAc, formic acid) and oxidative hydrolysis with oxidant (such as $H_2O_2$, $O_3$, $CH_3COOOH$), are the usual methods for chitosan degradation.[8-16] Except degradation with chitosanase, which can get chitosan oligomer with 6-8 glucose residues, other methods often produce the low molecular weight chitosan with large molecular weight distribution, and so the yield of low molecular weight chitosan with particular molecular weight is low. In addition, the degradation with chitosanase has some other restrictions, such as, the loss of chitosanase activity, difficult product separation, and so on. Therefore, an improved new method is needed to produce low molecular weight chitosan with narrow molecular weight distribution.

The aim of this work is to develop a new method of metal-coordinating controlled oxidative degradation for chitosan degradation leading to the low molecular weight chitosan with uniform molecular weight distribution, and so to increase the yield of chitosan with particular molecular weight. The main ideas are as follow: the –OH and -$NH_2$ groups on the skeleton of chitosan are good ligands to coordinate with transition metal ions and rare earth metal ions to get chitosan-metal complex. Coordinating bond may weaken a bond near the coordinating site and cause some weak points on the chitosan chain. Very dilute metal salt solution with different concentration is added dropwise to chitosan's dilute solution in acetic acid to obtain chitosan-metal complex whose metal-distribution on chitosan is uniform and the molecular size of glucose residues between each two weak points is uniform. A very small amount (50-100 micro liters (μL)) $H_2O_2$ is added to the complex to cut the chitosan chain at those weak points to produce degraded chitosan of uniform molecular size. Hydrogen peroxide was selected as the oxidant because its product of reduction is $H_2O$, which simplifies the process of separation. The solution of degraded chitosan complex is passed through a cation exchange resin column to eliminate metal ions on the chitosan. After separation and lyophilization, the dry low molecular weight chitosan is obtained (Figure 1).

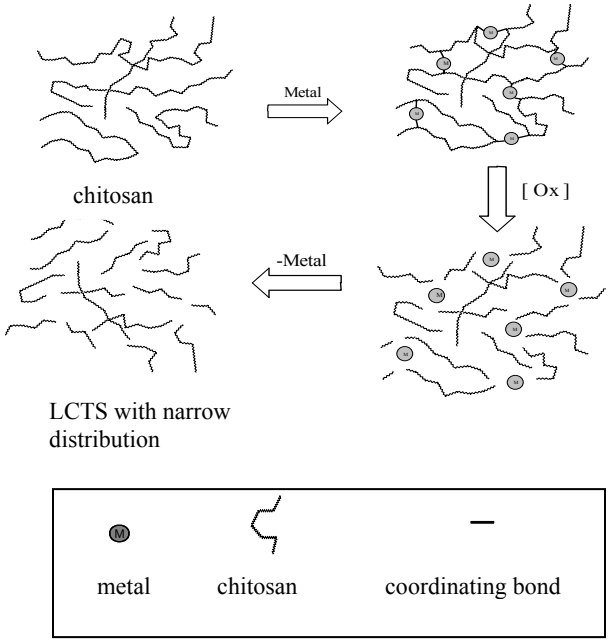



**Figure 1.** Scheme of metal-coordinating controlled oxidative degradation.

## Results and Discussion

### Effect of metal ion on chitosan degradation

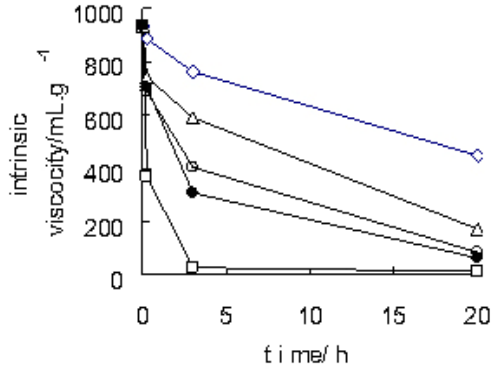



**Figure 2.** Effect of metal type on chitosan degradation. CTS(◇), CTS-Cu(□), CTS-Ni(△), CTS-Co(○), CTS-Mn(●)
(metal: chitosan 1: 8, coordinated 5 h, 50 μL $H_2O_2$, 25°C, air).

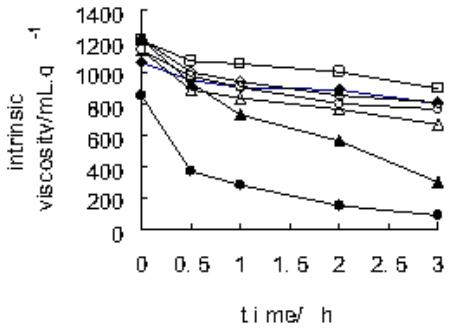



**Figure 3.** Effect of copper amount on chitosan degradation. Molar ratio of copper ion with glucose residues: ◆(0), ●(1:8), ▲($10^{-1}$: 8), △($10^{-2}$: 8), □($10^{-3}$: 8), ○($10^{-4}$: 8), ◇($10^{-5}$: 8)
(coordinated 5 h, 50 μL $H_2O_2$, 25 °C, air).

Four transition metal ions ($Cu^{2+}$, $Mn^{2+}$, $Co^{2+}$, $Ni^{2+}$) were selected to investigate the effect of metal on chitosan degradation. The molar ratio of metal salt with glucose residues was set to 1:8 in order to provide eight glucose residues between coordinating sites. It was expected that the chitosan chain will break at some weak points nearby the coordinating sites to form chitosan oligomer with DP8 which has a very good antitumor ability. The intrinsic viscosity is plotted versus time during degradation (Figure 2).

Fig. 2 shows that the natural intrinsic viscosity of chitosan (CTS) is 933 $mL.g^{-1}$. With the increase of time, intrinsic viscosity decrease. When degraded for 3 h, the intrinsic viscosity is 28, 405, 307, 590, and 763 $mL.g^{-1}$ for CTS-Cu, CTS-Co, CTS-Mn, CTS-Ni and CTS respectively. This result shows that the intrinsic viscosity of all complexes decreases faster than that of chitosan, and that viscosity decrease velocity is different with the type of metal ion as: $Cu^{2+} > Mn^{2+} > Co^{2+} > Ni^{2+}$.

The intrinsic-viscosity-reducing velocity of chitosan-copper complex is much higher compared to chitosan, and so, seven different copper ion amounts (molar ratio with glucose residues 0: 8, 1:8, $1x10^{-1}$: 8, $1x10^{-2}$: 8, $1x10^{-3}$: 8, $1x10^{-4}$: 8, and $1x10^{-5}$: 8) were selected to investigate effect of copper amount on chitosan degradation. The intrinsic viscosity of chitosan versus time is shown in Fig. 3. Intrinsic viscosity of the chitosan-Cu systems with 1:8, $1x10^{-1}$: 8 and $1x10^{-2}$: 8 diminishes much faster than that of chitosan, and the viscosity of the other chitosan-Cu systems decreases slowly but still faster than that of chitosan. The result shows a little Cu could enhance the velocity of chitosan degradation, which suggests that there must be some other reaction in the system of chitosan-copper complex, for example chitosan-copper complex would catalyze decomposition of $H_2O_2$.

**Effect of temperature on chitosan degradation**

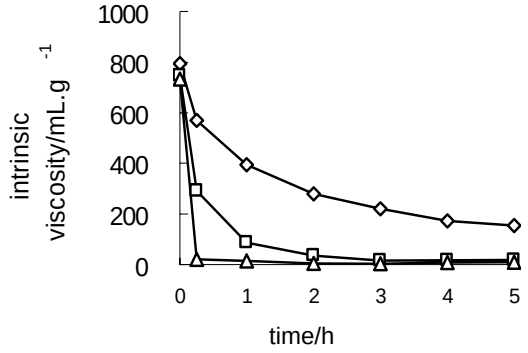



**Figure 4.** Effect of temperature on chitosan degradation. 25 °C (◇), 40 °C (□), 60 (△) ($Cu^{2+}$: chitosan 1: 8, coordinated 5 h, 50 μL $H_2O_2$, air).

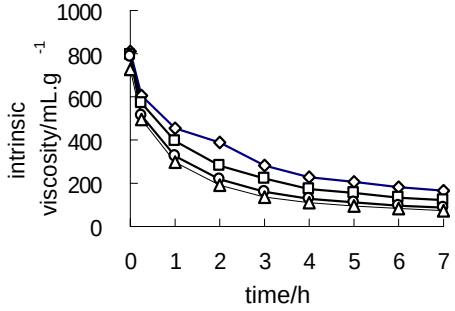



**Figure 5.** Effect of $H_2O_2$ amount on chitosan degradation. 50 μL(◇), 100 μL(□), 150 μL(○), 200 μL(△) ($Cu^{2+}$: chitosan 2: 8, coordinated 5 h, 50 μL $H_2O_2$, air).

Chitosan-copper complex was selected to investigate the influence of temperature on chitosan degradation. 0.1 mol/L HOAc-0.2 mol/L NaOAc solution was used as solvent, concentration of chitosan was 1%. After coordinating for 5 h at room temperature, the reactors were placed into water baths with different temperature (25, 40, and 60 $^{o}$C), and 50 μL $H_2O_2$ in 10 mL water was added into the reactor. Viscosity was determined at 30 $^{o}$C, and intrinsic viscosity was plotted versus time as shows in Figure 4.

With the increase of temperature, viscosity-reducing velocity increases. After degrading for 2 h, viscosity of chitosan at 60 $^{o}$C decreases from 733.8 to 20.4 mL.g$^{-1}$, but that of chitosan at 25 and 40 $^{o}$C decreases just from 794.3 to 570.4, and 746.6 to 292.2 mL.g$^{-1}$ respectively. These results suggest that chitosan degradative velocity could be obviously improved by increasing temperature. But too high temperature is not suitable for controlling chitosan degradation; therefore, 25 $^{o}$C is recommended for chitosan-metal complex degradation.

**Effect of $H_2O_2$ amount on chitosan degradation**

$H_2O_2$ is used as oxidant to degrade chitosan and chitosan-copper complex. 10 mL $H_2O_2$ solution with different $H_2O_2$ amount (50, 100, 150, and 200 μL) is dropped into chitosan-metal complex solution. Intrinsic viscosity of chitosan is plotted versus time in Figure 5. The plot shows that with the increase of $H_2O_2$ amount, intrinsic-viscosity-reducing velocity increases.

**Effect of pH on chitosan degradation**

Chitosan with higher molecular weight is soluble only in acidic solution, therefore, five pH values (1.04, 2.00, 3.05, 4.01, 5.00) were selected to investigate effect of pH on chitosan degradation. Because acidic hydrolysis is possible for chitosan, chitosan-Cu whose degradative velocity is the fastest was adopted in order to eliminate error brought by the acidic hydrolysis. For chitosan with different pH, the intrinsic viscosity is plotted versus time as Figure 6 shows. With the increase of pH, the intrinsic-viscosity-reducing velocity increases. Intrinsic viscosity of chitosan whose pH is 5.00 decreases fastest, and that of chitosan with pH 1.02 decreases most slowly. This suggests the higher the pH, the faster chitosan degradation. Hydroxy radical, $^{\bullet}$OH, plays an important role in the $H_2O_2$ oxidative chitosan degradation.[17] Protons will scavenge $^{\bullet}$OH in the solution, therefore, the higher acidity of the solution will slower chitosan degradation. It is possible to control chitosan degradation through controlling pH of chitosan solution therefore. The pH of mixture of 30 mL 1% HOAc and 60 mL water is about 4.5, so the solution used to dissolve chitosan is suitable for chitosan degradation.

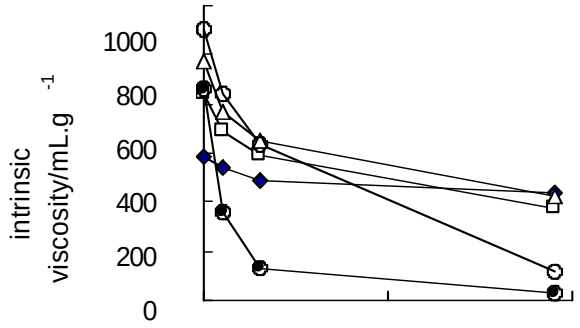



**Figure 6.** Effect of pH on chitosan degradation. pH value1.04 (◆), 2.00(□), 3.05(△), 4.01(○), 5.00(●)
($Cu^{2+}$: chitosan 1: 8, coordinated 5 h, 50 μL $H_2O_2$, 25 °C, air).

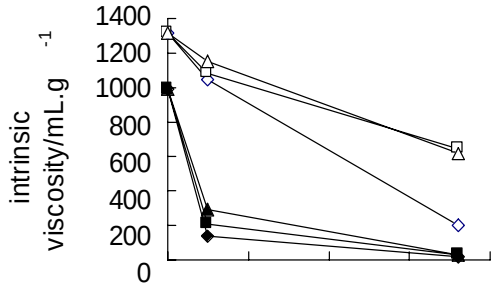



**Figure 7.** Effect of atmosphere on chitosan degradation CTS-$N_2$(◇), CTS -air(□), CTS -$O_2$(△), CTS -Cu-$N_2$(◆), CTS -Cu-air(■), CTS -Cu -$O_2$(▲)
($Cu^{2+}$: chitosan 1:8, coordinated 5 h, 50 μL $H_2O_2$, 25 °C).

**Effect of atmosphere on chitosan degradation**

When $H_2O_2$ is used as oxidant for chitosan degradation, there should be some oxygen released. So, oxygen-removing could increase chitosan degradation. Therefore the effect of atmosphere on chitosan degradation is investigated. Chitosan degradation is executed in three different atmosphere: pure $N_2$, air, and pure $O_2$. Results of chitosan degradation is plotted versus time in Figure 7. With the decrease of oxygen in the degradation system, the intrinsic-viscosity-reducing velocity increase. Viscosity-reducing velocity of both chitosan and chitosan-copper complex is as follow: $N_2$ > air > $O_2$.

**Effect of free radical scavengers on chitosan degradation**

In order to verify whether a radical reaction also happen in chitosan-complex degradation, two OH scavengers, dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF), were used to investigate the effect of scavenger on chitosan degradation. After reacting for 5 h, 5 mL scavenger or 5 mL H2O as comparison was added to chitosan solution with stirring, and 100 μL H2O2 was added into the reactor. Intrinsic viscosity is plotted in Figure 8.

After coordinating for 19.5 h, the viscosity of chitosan-Cu decreases from 822 to 8 $mL.g^{-1}$, but that of chitosan-Cu with DMSO and DMF decreases from 819 to 419 $mL.g^{-1}$, and 831 to 264 $mL.g^{-1}$ respectively. The degradation of chitosan is obviously slowered by $^{\bullet}OH$ scavenger DMSO and DMF, which suggests there should be $^{\bullet}OH$ produced in the degradative system. So, chitosan-Cu degradation should also be a radical reaction like chitosan degradation with $H_2O_2$, and the difference between $Cu^{2+}$ and other metal ion is probable due to catalysis of chitosan-Cu to $H_2O_2$ decompsition.

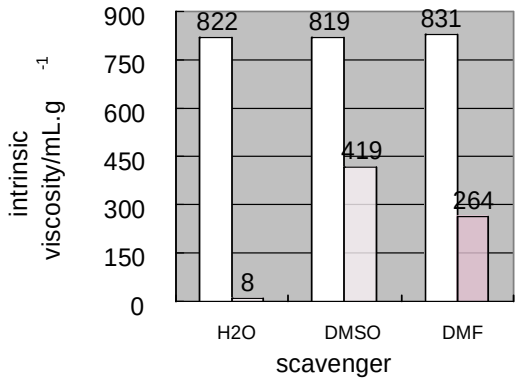



**Figure 8.** Effect of free radical scavenger on chitosan degradation with 19h(□ 0 h; ▯ 19 h) ($Cu^{2+}$: chitosan 1: 8, coordinated 5 h, 100 μL $H_2O_2$, 25°C, air).

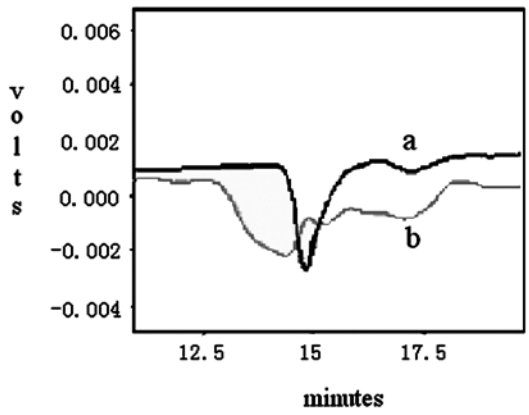



**Figure 9.** Chromatograms obtained with HPLC/GPC and differential refractometry with chitosan-Cu degraded for 2 h (a) and chitosan degraded for 40 h (b).

**Molecular weight distribution of degraded chitosan from GPC analysis**

Coordinated on chitosan metal was removed by passing chitosan solution through a cation exchange column. The absence of copper in the eluate was verified by atomic absorbance chromatography. HPLC/Gel permeation chromatography (GPC) was used to determine the molecular weight distribution of degraded chitosan. The molecular weight distribution of chitosan degraded for 2 h in the form of chitosan-copper complex (as 1) is much narrower than that of chitosan directly degraded for 40 h (as 2), and the molecular weight of the former is similar to that of the latter (Figure 9). These chromatograms suggest that chitosan chain in chitosan-metal complex is easier to be broken than chitosan itself, and chitosan-Cu complex can be degraded to low molecular weight chitosan with much narrower distribution in a shorter time than chitosan.

**$O_2^{\cdot-}$ scavenging ability of chitosan and its complex**

A sample dissolved in PBS buffer solution with $0.5\times10^{-2}$ g/mL mass concentration has been added to a mixture of methionine (Met), Vitamin $B_2$ ($V_{B2}$) and nitroblue tetrazolium chloride (NBT). Absorbance has been determined after reacting for about 30 min. Scavenging $O_2^{\cdot-}$ abilities of polymer chitosan (PCTS), degraded chitosan (DCTS, Mv 10,000), D-glucosamine (GA), and degraded chitosan metal complexes (DCTS-Cu, DCTS-Co) are listed in Table 1.

**Table 1.** $O_2^{\cdot-}$ scavenging property of chitosan and DCTS –metal complexes

| | control | GA | PCTS | DCTS | DCTS –Cu(II) | DCTS –Co(II) |
|---|---|---|---|---|---|---|
| Absorbance | 0.269 | 0.243 | 0.234 | 0.055 | 0.043 | 0.058 |
| $O_2^{\cdot-}$ scavenged/% | 0 | 9.5 | 13 | 80.3 | 84.1 | 78.4 |

The data in Table 1 illustrate PCTS and GA has a very low $O_2^{\cdot-}$ scavenging ability, just 13%, and 9.5% respectively, but DCTS and its complexes(DCTS-Cu, DCTS-Co) all have good $O_2^{\cdot-}$ scavenging ability. The $O_2^{\cdot-}$ percent is 80.3% for DCTS, 84.1% and 78.4% for DCTS-Cu and DCTS-Co respectively.

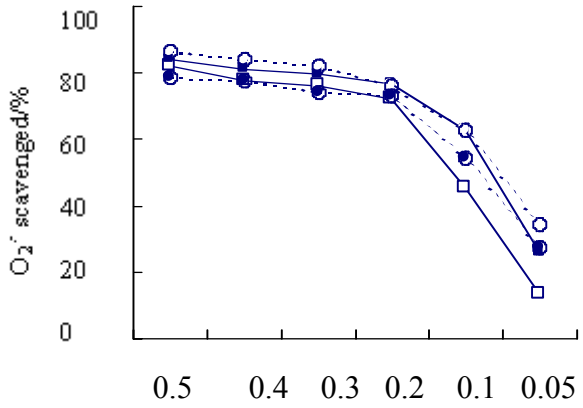



**Figure 10.** Plots of mass concentration of samples vs their $O_2^{\cdot-}$ scavenging activity(□: DCTS1, ■: DCTS1-Cu, ●: DCTS2, ○: DCTS2-Co).

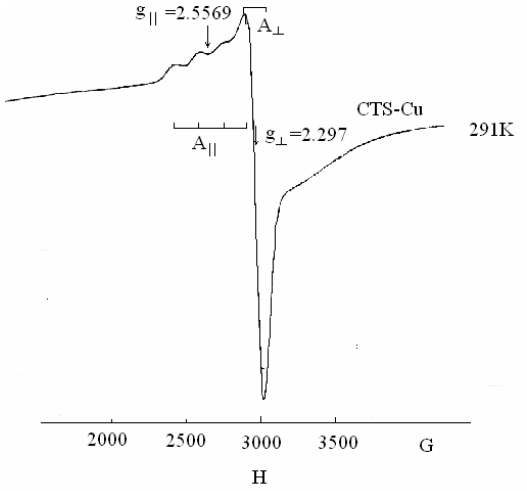



**Figure 11.** ESR spectra of chitosan-Cu.

Effect of mass concentration of samples on $O_2^{\cdot-}$ scavenging ability has been investigated with five concentrations of $0.5\times10^{-2}$, $0.4\times10^{-2}$, $0.3\times10^{-2}$, $0.2\times10^{-2}$, $0.1\times10^{-2}$, and $0.05\times10^{-2}$ g/mL. The $O_2^{\cdot-}$ scavenged percent is plotted versus mass concentration in Figure 10. DCTS1 and DCTS2 is uncoordinated chitosan corresponding to DCTS1-Cu(II).DCTS2-Co(II) respectively. With the decrease of mass concentration, $O_2^{\cdot-}$ scavenging ability of every sample decrease. The $O_2^{\cdot-}$ scavenged is 25.9%, 27.5% for DCTS1-Cu(II).DCTS2-Co(II) with concentration of $0.05\times10^{-2}$ g/mL respectively. $O_2^{\cdot-}$ scavenging ability of DCTS1 is lower than that of DCTS1-Cu(II) with same concentration, but $O_2^{\cdot-}$ scavenging ability of DCTS2 is higher than that of DCTS2-Co(II). ESR spectra ($g_{\|}$.=2.5569 > $g_{\perp}$.=2.297, Figure 11) suggested chitosan-Cu has a square-planar arrangement of four nitrogen ligands.[18] The structure is propitious to $O_2^{\cdot-}$ coordinating with Cu(II) from axis direction to form intermediate Cu(II)-complex-$O_2^{\cdot-}$. Electron transports from $O_2^{\cdot-}$ to

Cu(II) to produce Cu(I), and then $O_2^{\cdot -}$ is oxidized to $O_2$. The mechanism shown above is similar to that of superoxidase (SOD)[19], so $O_2^{\cdot -}$ scavenging ability of DCTS1-Cu complex is higher than DCTS1.

## Conclusions

We have developed a new method for chitosan degradation called metal-coordinating controlled oxidative chitosan degradation. In this method chitosan is converted into chitosan-metal complex before oxidative degradation because degradation of complex can be more easily controlled to form low molecular weight chitosan with uniform molecular weight distribution than chitosan itself. Degradation degree and points of fission of chitosan can be controlled by changing coordination and degradation conditions. Infrared absorption spectroscopy (IR), Ultraviolet absorption spectroscopy (UV), X-ray diffraction (XRD), differential Scanning calorimetry (DSC) and elemental analysis verify that there is coordination between chitosan and metal ions, and chitosan chain of complex can be broken easier than that of chitosan itself. Intrinsic viscosity and molecular weight distribution of degraded chitosan show that degradative velocity of complexes is faster than that of chitosan, and the molecular weight distribution of the former is much more narrow than that of the latter. With the increase of metal ion amount, $H_2O_2$ amount, pH, and temperature, intrinsic viscosity-reducing velocity of chitosan and chitosan-metal complex increase, and when $O_2$ content decrease, intrinsic viscosity-reducing velocity increase. Small amount of Cu can enhance the velocity of chitosan degradation, which suggests chitosan-copper complex can catalyze some reaction during chitosan degradation, for example decomposition of $H_2O_2$. Degraded chitosan and its metal complexes express good scavenging ability to $O_2^{\cdot -}$, and chitosan-copper complex shows better scavenging ability to $O_2^{\cdot -}$ than chitosan because its geometry (square-planar) favours reaction of $O_2^{\cdot -}$ reacting with $Cu^{2+}$.

## Experimental Section

**General Procedures. Preparation and characterization of chitosan-metal complex.** 0.5 g chitosan (90% degree of deacetylation) was dissolved in 30 mL 1% HOAc solution for 20 h at room temperature, then filtered to remove insoluble material. 50 mL distilled water was added to this chitosan solution. Then 10 mL of metal salt aqueous solution with different concentration was added dropwise with stirring. After stirring at room temperature for a period of time, 0.1% $Na_2CO_3$ aqueous solution was used to neutralize and precipitate chitosan-metal complex. Before filtering and final drying, rinsing with double-distilled water was done to eliminate free metal ion on the surface of complex precipitate. PE 1000 FTIR (KBr) spectrometry was utilized to determine the structure of chitosan and chitosan-metal complex. The morphology of chitosan and

its metal complexes was examined with D/MAX-RC X-ray diffractometer at the $CuK_\alpha$ ray($\lambda$=1.54 Å). Powder of the sample was deposited on the surface of a glass plate which was introduced in the diffractometer. Measurement was executed from 5-100° with irradiation conditions of 40 kV and 80 mA, and a scanning rate of 2° /min of diffraction angle 2θ. Thermal analysis has been done with Perkin Elemer DSC-7 differential Scanning calorimetry (DSC) at $N_2$ atmosphere with heating rate of 10 $^oC$ /min to analysis structure of the complex. UV analysis has been conducted with Shimadazu UV-2101PC spectroscopy at the range of 190nm-400nm. Elemental analysis has been done with Perkin Elemer 2400 elemental analysis instrument with CHNS/O mode. ESR spectra has been obtained on JEOL JES-FEIXG electron spinning resonance spectroscopy at modulation frequency 100kHz, modulation width $6.3x10^{-4}$ T, and 291 K.

**Degradation of chitosan.** 10 mL $H_2O_2$ aqueous solution with different concentration is added to chitosan-metal complex solution to oxidatively degrade chitosan at room temperature with stirring. 20 mL solution was taken out at different reaction time to determine viscosity with Ubbelohbe-type viscometer at 30 $^oC$. After degradation, the chitosan-metal complex solution was transported into a cation-exchange column to eliminate the metal ion, then chitosan-metal complex was reversed to chitosan. Metal content in the demetaled chitosan was measured with atomic absorbing spectroscopy, and it was verified that no metal was present in the demetaled chitosan solution. HPLC (with JASCO PU-1596 high pressure gradation pump, RI-1530 differential refractometer, Jordi Gel GBR 1000A GPC column) was used to determine the molecular weight distribution of degraded chitosan, degassed aqueous acetonitrile solution (acetonitrile: water 65: 35/v: v) was used as eluent at flow velocity of 1.0 mL/min. 0.1% $Na_2CO_3$ is utilized to neutralize chitosan solution. Alcohol was added to the solution to deposit degraded chitosan. The mixture was filtered, rinsed, and dried in vacuum at 50 $^oC$ to get dry low molecular weight chitosan. Chitosan was processed at the same condition and the result was compared with that of chitosan-metal complex.

**Determination of $O_2^{\cdot-}$ scavenging ability.** Colorimetry was used to determine scavenging $O_2^{\cdot-}$ ability of low molecular weight chitosan and its complexes. 0.5 mL Met, $V_{B2}$ and NBT with concentration $3\times10^{-3}$ mol/L, $18\times10^{-6}$ mol/L, and $225\times10^{-6}$ mol/L separately were added to colorimetric cuvette, and finally 0.5 mL aqueous chitosan or chitosan-metal complex was added to the cuvette. The cuvette was irradiated with daylight lamp for about 30 min until the color of control cuvette changed, and then the samples were placed into a 721 spectrometer to determine the absorbance. Undegraded chitosan and D-glucosamine was detected with the same method. Scavenging $O_2^{\cdot-}$ ability of the sample was calculated by the following eqution:

$$scavenging \quad ability = \frac{A_0 - A}{A_0} \times 100\%$$

where $A_0$ is absorbance of control A is absorbance of sample

## Compound characterization

**Table 2.** Elemental analysis of chitosan and its metal complexes(%)

| complexes | C | H | N | O | M |
|---|---|---|---|---|---|
| $(C_{6.19}H_{11.19}O_{4.09}N.0.45H_2O)n$ | 42.87(42.93) | 6.51(6.47) | 8.01(8.09) | 42.11(41.99) | |
| $(C_{6.19}H_{11.19}O_{4.09}N.0.77H_2O)_{8.8}.Cu(OAc)_2$ | 37.30(37.48) | 6.84(6.73) | 6.88(7.02) | 42.80(42.64) | 3.81(3.65) |
| $(C_{6.19}H_{11.19}O_{4.09}N.0.46H_2O)_{22.2}.Mn(OAc)_2$ | 41.01(41.14) | 7.01(6.84) | 7.59(7.74) | 42.01(41.82) | 1.29(1.37) |
| $(C_{6.19}H_{11.19}O_{4.09}N.0.73H_2O)_{11.8}.Co(OAc)_2$ | 38.49(38.65) | 7.00(6.82) | 7.02(7.25) | 42.88(42.76) | 2.87(2.59) |
| $(C_{6.19}H_{11.19}O_{4.09}N.0.26H_2O)_{14.2}.Zn(OAc)_2$ | 40.59(40.85) | 6.78(6.65) | 7.61(7.67) | 40.78(40.61) | 2.48(2.52) |

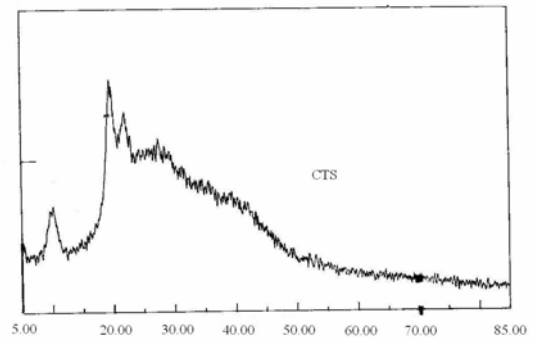
**Table 3.** Spectra and DTA of chitosan and its metal complexes

| samples | IR($cm^{-1}$) | | | | | | | | UV(nm) | DSC… |
|---|---|---|---|---|---|---|---|---|---|---|
| | $\nu_{N-H+O-H}$ | $\nu_{C=O}$ | $\delta_{as(NH_3^+)}$ | $\delta_{N-H}$ | $\nu_{C-N}$ | $\nu_{C-O-C}$ | $\nu_{as(C-OH)}$ | $\nu_{as(C-OH)}$ | | |
| CTS | 3429.4 | 1658.9 | 1630.2 | 1599 | 1409.9 | 1150.5 | 1091.5 | 1025.9 | 203 | 98.140.358 |
| CTS-Cu | 3437.1 | 1658.9 | 1622.6 | 1597.1 | 1408.5 | 1155.6 | 1100.6 | | 231.5 | 99.285.355 |
| CTS-Mn | 3434.7 | 1657 | 1627.8 | 1599.2 | | 1155 | 1087.9 | 1025.9 | 227 | 92.324.375 |
| CTS-Co | 3447.5 | 1658.9 | 1627.8 | 1599.2 | 1409.9 | 1155.6 | 1074.6 | 1025.9 | 233.5 | 91.311.375 |
| CTS-Zn | 3447.7 | 1661.0 | 1627.8 | 1590.8 | 1407.6 | 1155.6 | 1098.6 | 1025 | 236 | 121.315 |

Results of elemental analysis in Tab. 2 show chitosan coordination with metal ion because every complex contains some metal ion. Tab. 3 shows FTIR, UV and DSC spectra for chitosan and its complexes. In the FTIR spectra after coordination, peaks due to $\nu_{N-H+O-H}$ at 3429.4 $cm^{-1}$, $\delta_{as(NH3^+)}$ at 1630.2 $cm^{-1}$, and $\delta_{N-H}$ at 1599 $cm^{-1}$ moved at a range of 0-18.3 $cm^{-1}$; peak due to $\nu_{C-N}$ at 1409.9 $cm^{-1}$ moved at a range of 0~4.1 $cm^{-1}$; peak due to $\nu_{as(C-OH)}$ at 1091.5 $cm^{-1}$ also moved. This behavior could be attributed to the fact that $NH_2$ and OH are coordinated with metal ions. Glucosidic bond peak at 1150.5 $cm^{-1}$ due to $\nu_{C-O-C}$ moved about 5 $cm^{-1}$. UV spectra of each complex has absorption around 230 nm. Highest absorption of CTS-Zn, CTS-Co, CTS-Cu, CTS-Mn is at 236nm, 233.5nm, 231.5nm, 227nm respectively, which could be attributed to $n\rightarrow\pi^*$ of acetyl from metal salts. From thermal analysis data, dehydration endotherms at 98-140$^oC$ has moved. Thermal decomposition temperature of chitosan located at 358 $^oC$ moved to lower temperature in chitosan-metal complexes at the range of 34~73 $^oC$, which indicates that chitosan chain of complexes can be broken more easily. The third endotherm of complexes belongs to metal salt.

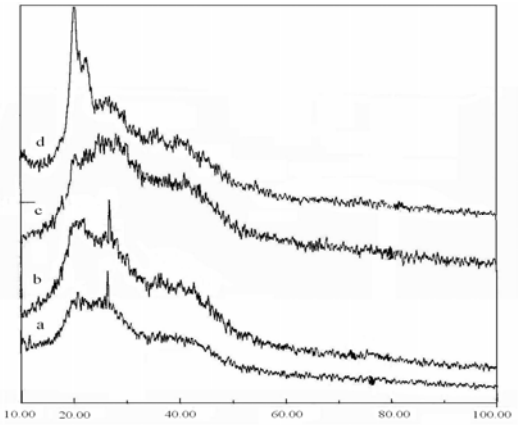
**$CuK_{\alpha}$ X-ray diffraction of chitosan and chitosan-metal complexes**

When chitosan is exposed to X-ray diffraction analysis, it gives a diffractogram with five peaks characteristic of crystalline regions (Fig. 1(a)) at 9.83, 10.58, 19.78, 22.45 and 27.71$^{o}$ But CTS-Cu gives a diffractogram without characteristic peak of crystalline region, CTS-Co gives a diffractogram with two peaks at 21, 26.61$^{o}$, CTS- Zn gives a diffractogram with one peak at 26.98$^{o}$, CTS-Mn also gives a diffractogram with two peaks. The x-ray diffractograms of chitosan and its metal complexes show chitosan has a polymorph of higher crystallinity than its complexes, so complexes have more amorphous domains which allows a better accessibility to reactants and thus a better reactivity.




2θ

(a) X-ray diffractogram of chitosan




2θ

(b) X-ray diffractograms of CTS-Co(a), CTS-Zn(b), CTS-Cu(c), CTS-Mn(d)

**Figure 12.** X-ray diffractograms of chitosan and its metal complexes.

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