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# Heterogeneous degradation of chitosan with $H_2O_2$ catalysed by phosphotungstate

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## Abstract

Phosphotungstate (PTA), an insoluble catalyst synthesized with phosphotungstic acid (PWA) and chitosan in acidic reaction system by electrostatic attraction. The catalyst was characterized by DRS and FTIR spectra. Under the catalysis of PTA, the heterogeneous degradation of chitosan with $H_2O_2$ was achieved. The effect of volume of $H_2O_2$, dosage of catalyst, reaction temperature and time on the degradation was discussed by orthogonal tests. The experimental results show that chitosan can be effectively degraded with $H_2O_2$ under the catalysis of PTA. The catalyst, PTA, may have a promising application in oxidative degradation of polymer.

*Keywords:* Chitosan; Phosphotungstate; $H_2O_2$; Catalysis; Degradation

## 1. Introduction

Chitosan, a (1→4)-2-amino-2-deoxy-β-D-glucan derived from full or partial deacetylation of chitin, has been receiving great attention as novel functional material for its excellent properties such as biodegradation, immunological, antioxidant and antibacterial activities (Carmen & Roland, 1997; Cheng et al., 2003; Kofuji et al., 2005; Qi, Xu, Jiang, Hu, & Zou, 2004; Zhao, Wang, Zhao, & Pan, 2002). However, the utilization of chitosan has been scarcely developed because of its high molecular weight, water-insolubility and high viscosity of its solution. Compared with the ordinary chitosan, low molecular weight chitosan (LWCS) without altering its chemical structure has much improved water-solubility and some special biological, chemical and physical functions. LWCS has been prepared with different methods (Hsu, Don, & Chiu, 2002; Huang, Wang, Huang, Zhuo, & Guo, 2007; Tanioka et al., 1996; Terbojevich, Cosani, & Muzzarelli, 1996; Vårum, Ottøy, & Smidsrød, 2001; Wang, Huang, & Wang, 2005; Zhang & Neau, 2002), some of which show advantages, but also disadvantages.

$H_2O_2$ is a strong oxidant, producing free radicals in acidic, neutral and basic reaction systems, which can attack the β-D-(1–4) glycosidic bond and degrade chitosan. This technique is easy to handle, easily available and environmentally friendly (Chang, Tai, & Cheng, 2001; Qin, Du, & Xiao, 2002; Shao, Yang, & Zhong, 2003; Tian, Liu, Hu, & Zhao, 2003). However, the formation of radical groups is inefficient when $H_2O_2$ is used alone. Recently, to improve the efficiency, the combined degradation method using $H_2O_2$ and chemical or physical techniques such as catalysis of PWA (Huang et al., 2007), irradiation of ultraviolet light (Wang et al., 2005), radiation of microwave (Shao et al., 2003) and gamma radiation of $^{60}$Co source (Kang, Dai, Zhang, & Chen, 2007) have been studied. But to date there have been few reports about the degradation with $H_2O_2$ in heterogeneous phase under the catalysis of PTA.

PWA is a representative heteropoly acid with Keggin type, which is the most often studied due to their favorable acid and redox characteristics, greater stability and availability (Misono, 2001; Okuhara, Mizuno, & Misono, 1996; Pope, 1983). In a previous work, our group has

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shown that chitosan can be effectively degraded with $H_2O_2$ under the catalysis of PWA (Huang et al., 2007). However, PWA is often difficult to separate from the products due to its high solubility in water, methanol and acetone. Therefore, the catalysts supported PWA are widely applied. The supports including oxides such as $Al_2O_3$, $SiO_2$, $TiO_2$, diatomite, bentonite and active carbon, etc., apparently affect the structure and acidity, as well as the redox property of PWA (Wu et al., 1996). Moreover, chitosan is a cationic biopolymer, previous studies have shown that chitosan can interact with anionic matter to form either soluble or insoluble complexes which stabilized by electrostatic, ion-dipole and hydrophobic interactions (Vikhoreva, Babak, Galich, & Gal'braikh, 1997; Wei & Hudson, 1993). Thus, according to above theory, PTA was synthesized with protonated chitosan and PWA due to electrostatic attraction.

The aim of this work is to show the oxidative degradation of chitosan with $H_2O_2$ under the catalysis of PTA in heterogeneous phase. One advantage of the system is that PTA is easy to separate from LWCS, which improved the purity of LWCS. Other is that the oxidative degradation of chitosan occurs in heterogeneous phase, which avoided using acetic acid and made the preparation process of LWCS convenient.

## 2. Experimental

### 2.1. Materials

Original chitosan, obtained from Yuhuan Biology Engineering (Zhejiang, China), whose degrees of acetylation is 98.26%, its viscosity-average molecular weight (Mv) is about 210,000 determined based on viscosity measurements (Wang et al., 2005). Hydrogen peroxide, PWA , acetic acid, sodium acetate and other reagents, supplied by Nanyang Chemical Agent Corporation (Nanyang, China), are of analytical grade. The water used was distilled.

### 2.2. Preparation of catalyst PTA

Chitosan solutions were prepared by dissolving 3.0 g chitosan in 300 mL 1.5% (v/v) acetic acid solutions. Then 100 mL chitosan solutions were added into 3.0%, 5.0% and 7.0% (w/v) PWA solutions at a volume flow rate of 3.0 mL $min^{-1}$ (using a burette), respectively. Thus PTA (1), PTA (2) and PTA (3), which are hyaloid insoluble matter, were obtained, respectively. The products were washed thoroughly with distilled water and acetone, collected after drying in vacuum at 60 °C.

### 2.3. Oxidative degradation of chitosan under the catalysis of PTA

In order to reveal the contrast of catalysis of PTA, four experiments on the degradation of chitosan were designed. For each experiment, 1.5000 g chitosan was placed in a 50 mL conical flask and soaked thoroughly after adding 17 mL distilled water. Then PTA (1), PTA (2) and PTA (3) was added into the corresponding conical flask for experiment 2, 3 and 4, respectively, and the dosage for each catalyst was 0.03 g. But for experiment 1, catalyst was not used. After adding 3.5 mL 30% (wt%) $H_2O_2$ aqueous solutions for each experiment, the oxidation degradation of chitosan was carried out at 80 °C for 30 min. After reaction, the solutions were filtrated, the collected solid was washed with distilled water until the eluent reached pH 7, and then dried at 50 °C in vacuum. Furthermore, addition of ethanol into the resulting filtrate resulted in a precipitate, which is LWCS. The oligomers were washed thoroughly with acetone, collected after drying in vacuum at 50 °C.

### 2.4. Characterization techniques

The degradation ratio of chitosan was calculated according to the following equation:

$$R(\%) = \frac{M_0 + M_c - M_x}{M_0} \times 100 \tag{1}$$

where $R$ refers to degradation ratio, $M_0$, $M_c$ refers to the quantity of original chitosan and PTA, respectively. $M_x$ refers to the quantity of collected solid after degradation at different conditions.

The FTIR spectra of samples were obtained using a Nicolet 5700 FTIR spectrometer using KBr pellets, respectively.

The DRS spectra of samples were recorded on a Cary-500 Scan UV–vis-NIR spectrophotometer equipped with a HARRICK diffuse reflectance accessory.

## 3. Results and discussion

### 3.1. The choice of catalyst

For the degradation experiments, the using of different catalyst affected the degradation efficiency. The contrast of degradation ratio under different conditions is depicted in Fig. 1. As can be seen from Fig. 1, the degradation ratio was only 30% without catalyst, which indicated that the yield of free radicals formed by decomposing of $H_2O_2$ was rather slow when $H_2O_2$ was used alone. But in the presence of PTA (1), PTA (2) and PTA (3), the degradation ratio increased, which added up to 51%, 98% and 100%, respectively. It indicated that the catalysis of PTA improved as the ratio of the quantity of PWA to that of chitosan increased. Therefore, it can be predicated that the catalysis of PTA (3) is remarkable for oxidative degradation compared with other catalysts.

### 3.2. DRS and FTIR analyses of PTA (3)

Fig. 2 shows the DRS spectra of chitosan, PWA and PTA (3). For chitosan, a weak absorption band was evi-

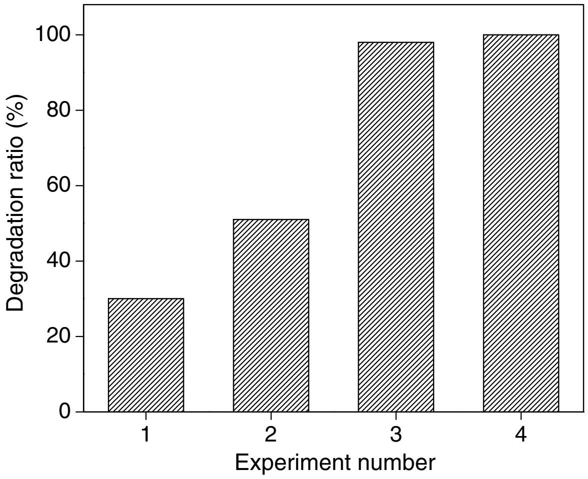

Fig. 1. The contrast of degradation ratio under different conditions.

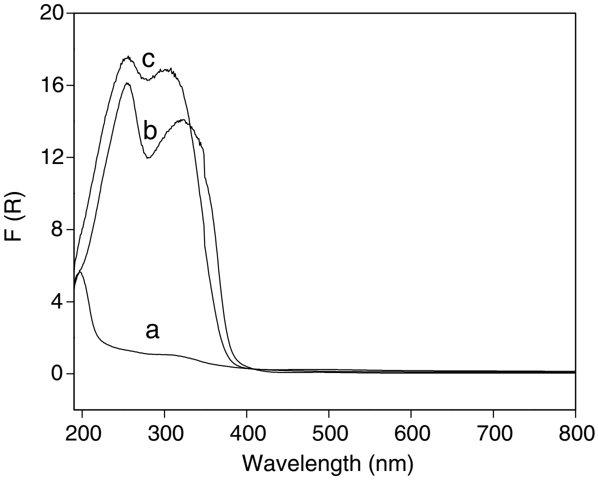

Fig. 2. DRS spectra of (a) chitosan, (b) PWA and (c) PTA (3).

dent around 203 nm, which was caused by the $n \rightarrow \sigma^*$ transition for amido groups of chitosan. There are two strong characteristic peaks at 256 and 325 nm in DRS spectra of PWA, which is assigned as $O_d \rightarrow W$ and $O_c$ $(O_b) \rightarrow W$ charge transfer transition, respectively.

But for PTA (3), the peak of chitosan at 203 nm was disappeared, the peak assigned as $O_d \rightarrow W$ charge transfer transition is still existed at 256 nm. However, the peak assigned as $O_c$ $(O_b) \rightarrow W$ charge transfer transition occurred at 312 nm, as shown in curve c. It indicates that PWA interact with chitosan, which results in the change of the electron density and bonds intensity.

Fig. 3 shows the FTIR spectra of chitosan, PWA and PTA (3). The main bands in the spectra of PWA are as follows (Huang, Wang, Yang, & Luo, 2006): the peak at 985 and 1081 cm$^{-1}$, correspond to the asymmetric stretching vibration of W=$O_d$ and P–$O_a$ bond, respectively. The asymmetric stretching vibration of W–$O_b$–W and W–$O_c$–W was observed at 895 and 805 cm$^{-1}$. In comparison with the characteristic absorption peaks of PWA, that of PTA (3) still existed, however, expect for the asymmetric stretching vibration of W–$O_b$–W, others were shifted as follows: the peaks of W=$O_d$, P–$O_a$ and W–$O_c$–W shifted to 978, 1087 and 817 cm$^{-1}$, respectively. It can be thought that these changes be due to the change of electron density of PWA. Moreover, the spectra of PTA (3) exhibited most of the characteristic absorption peaks of chitosan but with some differences.

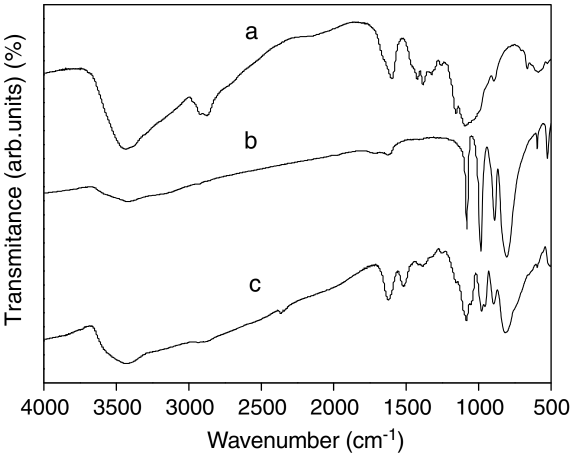

Fig. 3. FTIR spectra of (a) chitosan, (b) PWA and (c) PTA (3).

It is obvious that the spectra of PTA (3) exhibited the characteristic peaks of PWA and chitosan, which indicates that the heteropoly anions, $[PW_{12}O_{40}]^{3-}$, react with protonated chitosan by electrostatic attraction.

### 3.3. Orthogonal test

Owing to the degradation efficiency being the highest under optimum conditions, it was very important for oxidative degradation to look for these optimum parameters. However, study of the effect of changing single factor on degradation efficiency was not enough to judge what parameter was optimum because other factors were fixed under this condition. So the optimum parameters should be obtained by using orthogonal test design and mathematical analysis.

Reference to the design theory of orthogonal test, four controllable variables, volume of $H_2O_2$ ($A$), dosage of PTA (3) ($B$), temperature ($C$) and time ($D$), were selected for optimization. Three levels of each factor were investigated. The selected factors and levels were given in Table 1. The degradation ratio of chitosan was measured under the above-mentioned factors and levels, and a further

Table 1
The variables investigated and their levels

| Variables investigated | Levels of each variable | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| $A$: Volume of $H_2O_2$ (mL) | 1.5 | 2.5 | 3.5 |
| $B$: Dosage of PTA (3) (g) | 0 | 0.03 | 0.04 |
| $C$: Temperature (°C) | 60 | 70 | 80 |
| $D$: Time (min) | 10 | 20 | 30 |

Table 2
Experimental arrangement and test results

| Experiment No. | *A* | *B* | *C* | *D* | Degradation ratio (%) |
|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 1 | 3.7 |
| 2 | 1 | 2 | 2 | 2 | 64.7 |
| 3 | 1 | 3 | 3 | 3 | 92.4 |
| 4 | 2 | 1 | 2 | 3 | 22.2 |
| 5 | 2 | 2 | 3 | 1 | 99.1 |
| 6 | 2 | 3 | 1 | 2 | 68.6 |
| 7 | 3 | 1 | 3 | 2 | 46.8 |
| 8 | 3 | 2 | 1 | 3 | 91.2 |
| 9 | 3 | 3 | 2 | 1 | 96.6 |
| $K_1$ | 160.8 | 72.7 | 163.5 | 199.4 | |
| $K_2$ | 189.9 | 255 | 183.5 | 180.1 | |
| $K_3$ | 234.6 | 257.6 | 238.3 | 205.8 | |
| *R* | 73.8 | 184.9 | 74.8 | 25.7 | |

orthogonal analysis was carried out. Thus, the *K* and *R* values were calculated and presented in Table 2.

Obviously, the influential order of four factors on degradation ratio was $B > C \approx A > D$ by comparing *R* values in Table 2. Owing to the value of *R* for *B* factor being extremely high, it was shown that the contribution of *B* factor for degradation ratio was significant. Thus, dosage of PTA (3) has the greatest influence on degradation of chitosan. For each variance, level 3 is the best according to the value of *K*, so the optimum condition was $A_3B_3C_3D_3$. However, for dosage of PTA (3), there was no significant difference between the value of $K_2$ and that of $K_3$, that is to say, 0.03 g of PTA (3) is sufficient for the reaction system, therefore, level 2 is chosen for the sake of the saving of catalyst. Furthermore, according to the values of *R* for the other three factors, we can find that volume of $H_2O_2$, temperature and time have no significant influence on the degradation ratio compared with dosage of PTA (3). Consequently 3.5 mL of 30% (wt %) $H_2O_2$, 0.03 g of PTA (3), 80 °C of reaction temperature and 30 min of reaction time seem favorable for the oxidative degradation of chitosan with $H_2O_2$ under the catalysis of PTA (3). Thus, according to $A_3B_2C_3D_3$ with other fixed factors, chitosan was degraded in parallel for three times, each degradation ratio of chitosan reaches 100%. Therefore we rationally confirm the degradation condition to be the most optimum, namely, 30% (wt%) $H_2O_2$ 3.5 mL, dosage of PTA (3) 0.03 g, reaction temperature 80 °C and reaction time 30 min.

Moreover, LWCS produced under the optimum reaction conditions was precipitated by adding ethanol and dried at 50 °C in vacuum, its Mv is about 1500 analyzed by viscometry measurement (Wang et al., 2005).

### 3.4. DRS and FTIR spectral analyses of LWCS

Curve a, b in Fig. 4 show the DRS of original chitosan and LWCS produced under the optimum reaction conditions.

As can be seen, there is an absorption band at 200 nm in curve a, which was caused by the $n \rightarrow \sigma^*$ transition of amido groups in chitosan. For LWCS, two strong absorption bands were exhibited, as shown in curve b, one absorption band at 260 nm corresponds to the $n \rightarrow \sigma^*$ transition of amido groups, the other at 300 nm is assigned to the $n \rightarrow \pi^*$ transition of carbonyl or carboxyl groups (Wang et al., 2005). It is most likely that the new side group is formed during degradation.

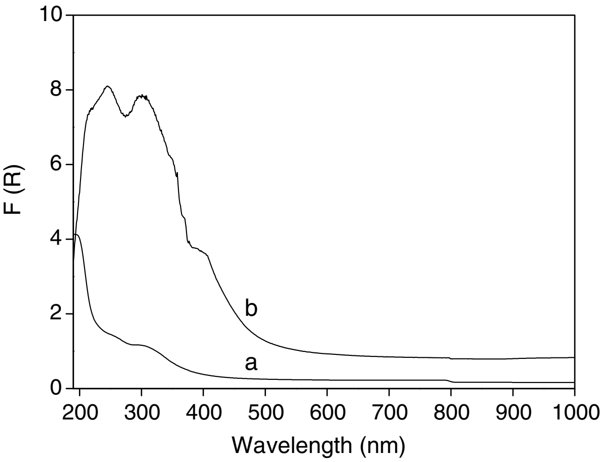

Fig. 4. DRS patterns of (a) original chitosan and (b) LWCS.

FTIR spectroscopy has been shown to be a powerful tool for the study of the physicochemical properties of polysaccharides. The FTIR spectra of initial chitosan and LWCS produced under the optimum reaction conditions is shown in Fig. 5.

The main bands in the spectrum of original chitosan are as follows: the intense band at around 3440 cm$^{-1}$ should be assigned to the stretching vibration of O–H and N–H, as well as to intermolecular hydrogen bonding within the polysaccharide molecules. The bands at 1602 and 599 cm$^{-1}$, correspond to the binding vibrations of the amido groups, the bands in the range 1158–895 cm$^{-1}$ are assigned to the characteristics of its polysaccharide structure (Peniche et al., 1999).

Compared with the spectrum of original chitosan, that of LWCS exhibited most of the characteristic absorption

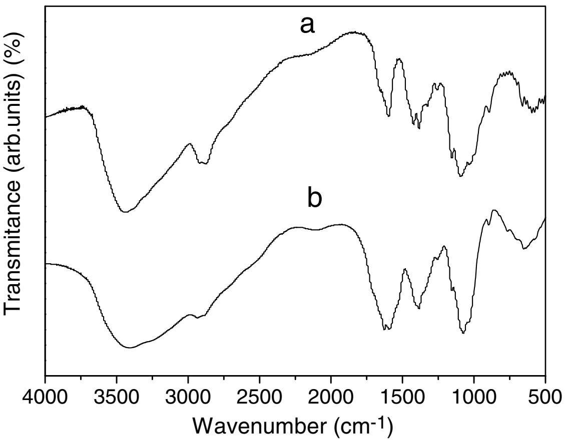

Fig. 5. FTIR spectra of (a) original chitosan and (b) LWCS.

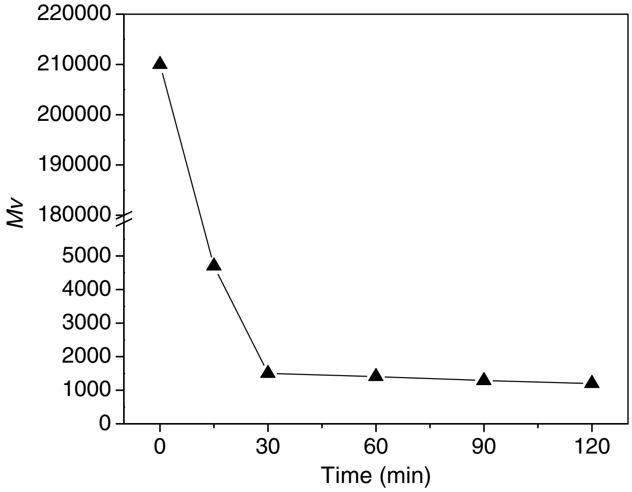

Fig. 6. Effect of reaction time on Mv of LWCS.

peaks of chitosan but with some differences. Moreover, a new absorption peak at 1634 $cm^{-1}$ was formed, which may be attributed to the absorbance of C=O, which indicates that the carboxyl or carbonyl groups do exist (Shao et al., 2003).

### 3.5. Effect of reaction time on Mv of LWCS

Chitosan was degraded under the optimum reaction conditions for 30, 60, 90, 120 and 150 min. After reaction, the resulting LWCS was obtained. Then the Mv of chitosan is plotted as a function of the reaction time. As shown in Fig. 6, the Mv of chitosan dropped sharply from 210,000 to 1500 within 30 min. It indicated that $H_2O_2$ was decomposed effectively and a great number of free radicals were produced within 30 min under the catalysis of PTA (3).

When chitosan was degraded continuously after 30 min, the Mv decreased slowly, finally reached 1200 after 120 min. It was obvious that the decreasing value of Mv was only 300 from 30 to 120 min, which indicated that the effect of PTA (3) on the decomposing of $H_2O_2$ was very slight after 30 min.

The new catalyst, PTA, was synthesized with heteropoly anions and protonated chitosan by electrostatic attraction, so the Keggin units, $[PW_{12}O_{40}]^{3-}$, still existed in the structure of PTA, as described in the DRS and FTIR spectral. Thus the Keggin units will react with $H_2O_2$ and lead to the rapid formation of hydroxyl radical (HO•) and hydroperoxide radical ($HO_2$•) (Huang et al., 2007), which are much more powerful oxidant, can quickly attack the β-(1,4) glycosidic bond of chitosan and served to decrease the average molecular weight of chitosan.

## 4. Conclusions

In heterogeneous phase, chitosan was effectively degraded with $H_2O_2$ under the catalysis of PTA (3). The optimum reaction conditions determined by orthogonal tests were as follows: 30% (wt %) $H_2O_2$ 3.5 mL, amount of PTA (3) 0.03 g, reaction temperature 80 °C and reaction time 30 min.

By FTIR and DRS analysis, it was presumed that the degradation be attributed to free radical formed by the reaction of PTA (3) and $H_2O_2$, which result in the rupture of glycosidic linkages and the formation of carbonyl groups.

One advantage of the degradation system is that PTA is easy to separate from LWCS, which improved the purity of LWCS. Other is that the oxidative degradation of chitosan occurs in heterogeneous phase, which avoided using acetic acid and made the preparation process of LWCS convenient.

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