

Synthesis of (2-pyridyl)-Acetyl Chitosan and Its Antioxidant Activity

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ABSTRACT. In this paper, chloroacetyl chitosan (CACTS) was prepared at first. In the molecules of CACTS, there are active chlorine groups, which can take part in other reactions. Thus, number of chitosan derivatives will be obtained after chlorine is substituted. Choosing pyridine as the active group, a novel water-soluble chitosan derivative, (2-pyridyl)-acetyl chitosan (PACTS) was obtained and its antioxidant activity against hydroxyl radicals and superoxide radicals was assessed. The results indicated that PACTS had better antioxidant activity than that of chitosan, carboxymethyl chitosan (CMCTS), hydroxypropyl chitosan (HPCTS), and Vitamin C. And the IC_{50} values against hydroxyl radicals and superoxide radicals were 0.31 mg/mL and 0.21 mg/mL, respectively.

Key words: synthesis, (2-pyridyl)-acetyl chitosan, antioxidant activity

INTRODUCTION

As a non-toxic, biocompatible and biodegradable polymer, chitosan has attracted much attention for its unique biological activities.¹⁻⁷ However, the application of chitosan is limited because of its poor solubility in water or high pH region. So it is important to improve the soluble property of chitosan. In order to increase the solubility, many derivatives have been synthesized such as hydroxypropyl chitosan (HPCTS), carboxymethyl chitosan (CMCTS) and so on.⁸ In this paper, a novel water-soluble chitosan derivative, (2-pyridyl)-acetyl chitosan (PACTS) was synthesized.

It has been found that chitosan has antioxidant activity, and that, many chitosan derivatives were synthesized and their antioxidant activity was assessed accordingly.⁸⁻¹⁰ Among various reactive oxygen species, both of hydroxyl radical and superoxide radical have strong chemical activity, which can damage a wide range of essential biomolecules such as amino acids, proteins, and DNA.¹¹ In this paper, the antioxidant activity against hydroxyl radicals and superoxide radicals of PACTS were estimated.

EXPERIMENTAL

Chitosan was purchased from Qingdao Baicheng biochemical Corp. (China). The degree of deacetylation was 97% and the viscosity average-molecular weight was 7.0×10^4 . The other reagents were all analytical grade and used without further purification.

The derivatives were synthesized based on the earlier

method (Fig. 1):¹²

CACTS, 1.61 g chitosan was dispersed in 100 mL N-methyl-2-pyrrolidone (NMP) and stirred for 12 h at room temperature (r t). Then 0.02 mol chloroacetyl chloride was added. After stirring for 24 h at r t, the solution was precipitated in ether and the precipitate was washed with methanol and ether by turns and lyophilized and CACTS was obtained.

PACTS, 0.2 g CACTS was dissolved into 10 mL of dimethyl sulfoxide (DMSO) at 60 °C and 0.5 mL pyridine was added with stirring for 20 h. The solution was collected by precipitation with excess acetone. After drying at 60 °C for 24 h, PACTS was obtained in 87% yield (The yield is based on the ratio of PACTS and added chitosan).

CMCTS and HPCTS were synthesized according to the method of Xie.¹³

The antioxidant activity against hydroxyl radicals was carried out according to Guo.¹⁴ The reaction mixture, total volume 4.5 mL, containing the samples of chitosan derivatives, was incubated with EDTA- Fe^{2+} (220 μ M), safranine O (0.23 μ M), H_2O_2 (60 μ M) in potassium phosphate buffer (150 mM, pH 7.4) for 30 min at 37 °C. The absorbance of the mixture was measured at 520 nm. Hydroxyl radicals

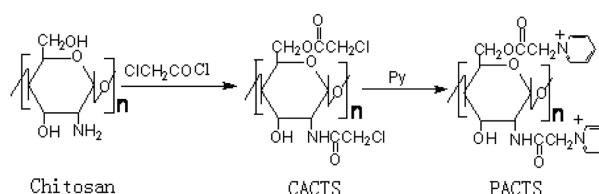


Figure 1. Synthetic pathway of PACTS.

bleached the safranin O, so decreased absorbance of the reaction mixture indicated decreased hydroxyl radicals scavenging ability (equation 1).

Scavenging effect (%)

$$\frac{[(A_{\text{sample } 520\text{nm}} - A_{\text{blank } 520\text{nm}}) / (A_{\text{control } 520\text{nm}} - A_{\text{blank } 520\text{nm}})] \times 100}{(1)} \quad (1)$$

Where $A_{\text{blank } 520\text{nm}}$ is the absorbance of the blank (distilled water, instead of the samples) and $A_{\text{control } 520\text{nm}}$ is the absorbance of the control (distilled water, instead of H_2O_2).

The scavenging of superoxide radicals was carried out according to an earlier method.¹⁵ Different concentrations of each derivatives of chitosan were mixed separately with PMS (30 μM), NADH (338 μM), and NBT (72 μM) in Tris-HCl (16 mM, pH 8.0) and then incubated at room temperature for 5 min. The absorbance was measured and the capability of scavenging superoxide radicals was calculated using the following equation (2):

Scavenging effect (%)

$$(1 - A_{\text{sample } 520\text{nm}} / A_{\text{control } 520\text{nm}}) \times 100 \quad (2)$$

Where $A_{\text{control } 560\text{nm}}$ is the absorbance of the control (Tris-HCl buffer, instead of NADH).

Results and Discussion

The IR spectra data of chitosan, CACTS and PACTS are shown in Figure 2. The IR spectra data of chitosan shows peaks assigned to the saccharine structure at 895 and 1155 cm^{-1} and amino characteristic peaks at around 1600 cm^{-1} .

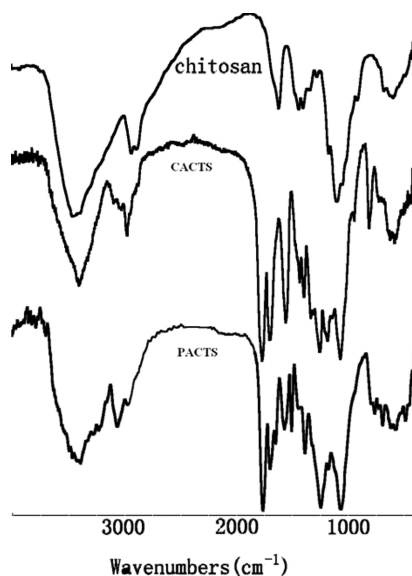


Figure 2. IR spectra data of chitosan, CACTS and PACTS.

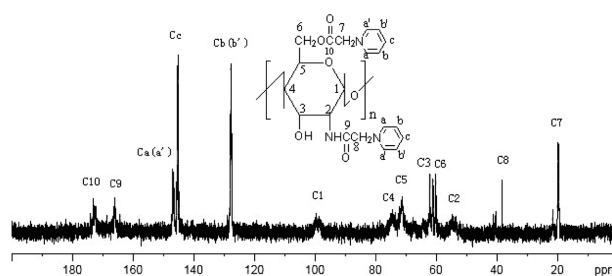


Figure 3. ^{13}C NMR spectra of PACTS.

For CACTS, there are strong peaks at 1749, 1682 and 790 cm^{-1} , attributed to groups of carbonyl and C-Cl,¹⁶ which indicates that CACTS was synthesized. For PACTS, there are peaks at 1492, 1640 and 3056 cm^{-1} , attributed to the characteristic absorbance of pyridine.¹⁷ The characteristic absorb of C-Cl groups at 790 cm^{-1} disappeared after pyridine was grafted.

Figure 3 presented the ^{13}C NMR of PACTS. In the ^{13}C NMR spectra of PACTS, the chitosan backbone signals can be seen at 99.7 ppm (C_1), 74.5 ppm (C_4), 71.3 ppm (C_5), 62.2 ppm (C_3), 60.2 ppm (C_6), 55.8 ppm (C_2).¹⁸ Also the graft evidences are clear. The peaks at 173.1 ppm and 166.9 ppm are corresponded to carbonyl groups.¹⁷ The strong peaks at 146.8 ppm, 145.5 ppm, 127.8 ppm are corresponded to pyridine ring carbons.¹⁹ The peaks at 20.0 ppm and 39.5 ppm can be assigned for methylene carbons.¹⁸ Above ^{13}C NMR results further confirmed that PACTS was obtained.

The hydroxyl radicals, generated by the Fenton reaction in this system, was scavenged by chitosan, chitosan derivatives and Vitamin C. Figure 4 shows that the scavenging effect of all the samples raised with the increases

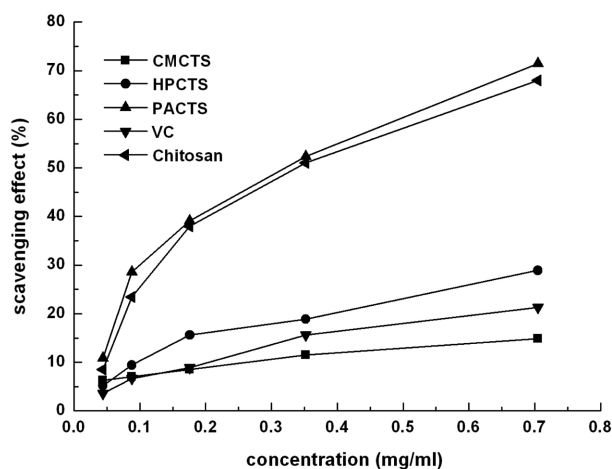


Figure 4. Scavenging effect of chitosan, chitosan derivatives and Vitamin C on hydroxyl radicals.

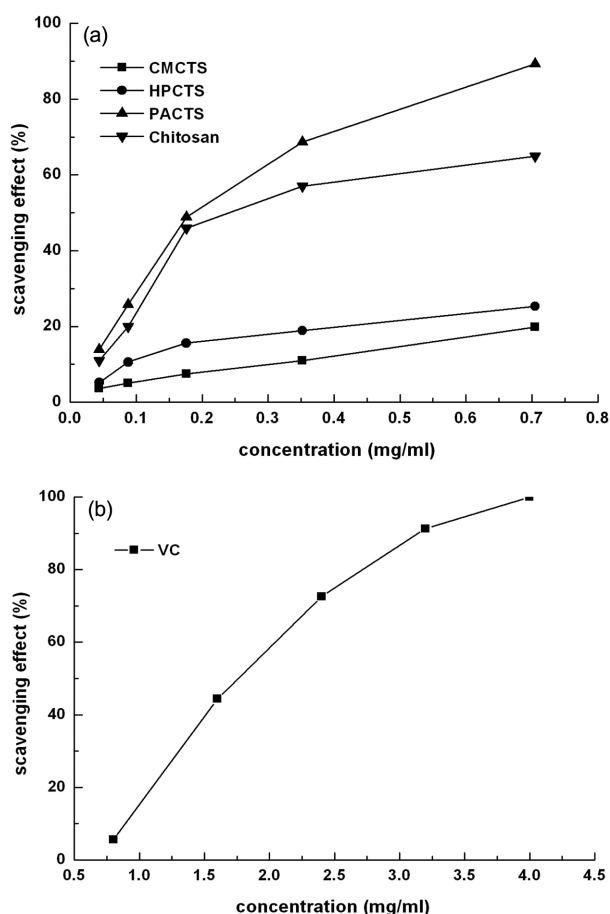


Figure 5. (a) Scavenging effect of chitosan, chitosan derivatives on superoxide radicals. Values are mean \pm SD (n=3). (b) Scavenging effect of VC on superoxide radicals. Values are mean \pm SD (n=3).

of concentration. Among all the chitosan derivatives and Vitamin C, PACTS has the best scavenging effect against hydroxyl and the % scavenging effect is 71.5 at 0.71 mg/mL. Much attention has been put on CMCTS and HPCTS because of their good water-solubility. But, the antioxidant activity of these two compounds against hydroxyl radicals is very soft, and the scavenging effect is 14.9% and 28.9%, respectively, at 0.71 mg/mL. Compared with Vitamin C, CMCTS and HPCTS have similar scavenging effect and they all have poorer activity than chitosan. However, PACTS has better activity at tested concentration and the IC_{50} was determined to be 0.31 mg/mL.

Figure 5(a) shows the inhibitory effect of chitosan, CMCTS, HPCTS, and PACTS against superoxide radicals. All derivatives have inhibitory ability against superoxide radicals, and the activity is affected by the concentration. It is obvious that PACTS has better scavenging effect than that of chitosan, CMCTS and HPCTS at all the tested con-

centrations, and the IC_{50} of PACTS was determined to be 0.21 mg/mL. However, IC_{50} of CMCTS and HPCTS can not be read at the tested concentration. Figure 5(b) shows the superoxide radicals scavenging ability of Vitamin C, and the IC_{50} is 1.7 mg/mL.

Conclusion

As shown in Scheme I, CACTS has similar structure with chitin and it can not dissolved in water at any pH. In the molecules of CACTS, there are active chlorine groups, which can take part in other reactions. Thus, number of chitosan derivatives will be obtained after chlorine is substituted. Choose pyridine as the active groups and one novel water-soluble chitosan derivative, PACTS is obtained. Compared with the other two water-soluble derivatives of chitosan - CMCTS and HPCTS, PACTS has better antioxidant activity against hydroxyl radicals and superoxide radicals. Further study about this kind of chitosan derivatives and similar compounds is necessary.

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