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The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan

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Abstract—Five kinds of Schiff bases of chitosan and carboxymethyl chitosan (CMCTS) have been prepared according to a previous method and the antioxidant activity was studied using an established system, such as superoxide and hydroxyl radical scavenging. Obvious differences between the Schiff bases of chitosan and CMCTS were observed, which might be related to contents of the active hydroxyl and amino groups in the molecular chains.

1. Introduction

As one of the most abundant natural resources, chitosan has been attracting people's attention for its unique physiochemical characteristics and bioactivities.[1–4] With the development of the study on the sciences of chitosan, chitosan and its derivatives are being increasingly used in more and more fields, especially in biomedicine. It has been found that chitosan has antioxidant activity, and that, many chitosan derivatives were synthesized and their antioxidant activity was assessed accordingly.[5–8] In this paper, five Schiff bases of chitosan and CMCTS were prepared, according to Qu's method[9] and their antioxidant activity was measured.

2. Chemical

Chitosan was purchased from Qingdao Baicheng Biochemical Corp. (China). The degree of deacetylation was 97% and the viscosity average-molecular weight was 2.0×10^5. CMCTS was prepared according to a previous method,[10] and the synthesis of 2-hydroxy-5-chlorobenzaldehyde and 2-hydroxy-5-nitrobenzaldehyde was carried out according to Liu[11] and Zhang.[12] The Schiff bases of chitosan and CMCTS were synthesized as follows (Scheme 1). Three grams of chitosan (or CMCTS) was dispersed into 95% EtOH (100 ml), and various aldehydes were added with stirring. The mixture was refluxed for 8 h and then filter. Unreacted aldehydes and other inorganic products were Soxhlet-extracted with EtOH and ether for 2 days. The Schiff bases were obtained by drying under vacuum for 24 h. The elemental analysis results and the IR spectrum data of the derivatives are shown in Table 1 and Figure 1. There were strong peaks at about 1630–1660 cm^{-1} assigned to the characteristic absorbance of C=N, which showed the Schiff bases that were obtained.

3. The antioxidant activity of the derivatives of chitosan

3.1. Scavenging ability of hydroxyl radical

According to the reference,[13] the reaction mixture, total volume 4.5 ml, containing the Schiff bases samples, was incubated with EDTA-Fe^{2+} (220 μM), safranine O (0.23 μM), and H_2O_2 (60 μM) 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 radical bleached the safranine O, so decreased absorbance of the reaction mixture indicated decreased hydroxyl radical scavenging ability.

Keywords: Schiff bases; Chitosan; Carboxymethyl chitosan; Antioxidant activity.
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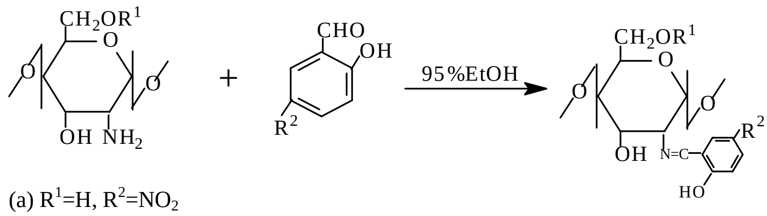

(a) R^1=H, R^2=NO_2
(b) R^1=CH_2COOH, R^2=NO_2
(c) R^1=H, R^2=Cl
(d) R^1=CH_2COOH, R^2=Cl
(e) R^1=CH_2COOH, R^2=H

Scheme 1. Synthetic pathway of the Schiff bases of chitosan and CMCTS.

Table 1. The elemental analysis results and the IR spectrum data of the Schiff bases of chitosan and CMCTS

Compound	Elemental analysis (%)			IR (KBr) (cm^{-1})
	C	N	H	
(a)	48.08	8.93	4.75	3441.31, 2876.05, 1640.47(C=N), 1530.75, 1069.18, 750.07
(b)	47.66	7.30	4.82	3444.76, 2923.16, 1660.13(C=N), 1542.44, 1508.40, 1437.25, 753.34
(c)	51.48	5.15	4.89	3446.26, 2892.36, 1631.90(C=N), 1577.81, 1482.93, 1072.81, 819.98
(d)	48.49	4.59	5.36	3445.45, 2878.52, 1642.09(C=N), 1557.97, 1408.82, 1138.40, 758.46
(e)	52.22	5.00	5.53	3438.89, 2891.21, 1632.57(C=N), 1573.69, 1503.96, 1457.45, 1067.92, 758.46

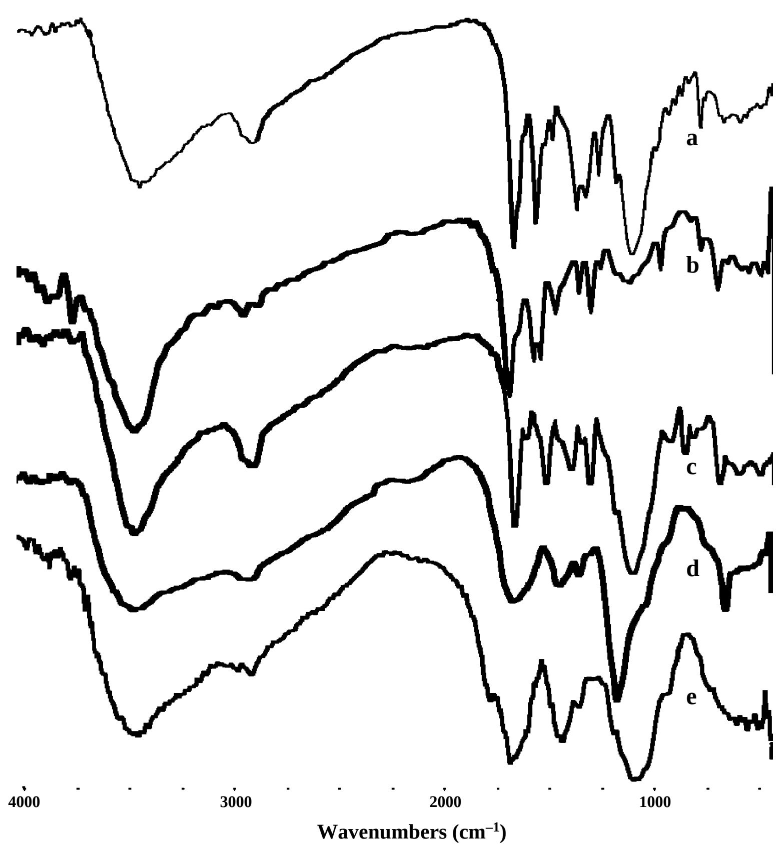

Figure 1. The IR spectra of the Schiff bases of chitosan and CMCTS.

$$\text{Scavenging effect } (\%) = [(A_{\text{sample 520 nm}} - A_{\text{blank 520 nm}})/(A_{\text{control 520 nm}} - A_{\text{blank 520 nm}})] \times 100,$$

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

3.2. Scavenging of superoxide radical

According to an earlier method,[14] different concentrations of each Schiff base of chitosan were mixed separately with PMS (30 μM), NADH (338 μM), and NBT (72 μM) in phosphate buffer (0.1 M, pH 7.4) and then incubated at room temperature for 5 min. The absorbance was measured and the capability of scavenging superoxide radical was calculated using the following equation:

$$\text{Scavenging effect } (\%) = (1 - A_{\text{sample 560 nm}}/A_{\text{control 560 nm}}) \times 100,$$

where $A_{\text{control 560 nm}}$ is the absorbance of the control (phosphate buffer, instead of NADH).

3.3. Statistical analysis

All data are expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test. The results were processed by computer programs: Excel and Statistical software (1999).

4. Results and discussion

4.1. Scavenging ability of a hydroxyl radical by all kinds of Schiff bases of chitosan and CMCTS

The hydroxyl radical, generated by the Fenton reaction in this system, was scavenged by the Schiff bases of chitosan and CMCTS. Figure 1 shows the percentage scavenging effect, and the scavenging effect increases with increases in the concentration of the Schiff bases. At a concentration of 1–5 mg/ml, the percentage scavenging effect was 15.6–45.1% for (a), and 14.4–51.7% for (c), but at the same time, for (b), (d), and (e), at a concentration of 1–5 mg/ml, the percentage scavenging effect ranged from about 6.2% to not more than 20.0%. As shown in Figure 1, there was a very interesting phenomenon in that the scavenging ability of (a) and (c) was better than that of others, and the scavenging rate was 45.1% and 51.7%, respectively, at maximum concentration of about 5 mg/ml. Polysaccharides with a scavenging effect on the hydroxyl radical have the same structural feature in that all of them have one or more alcohol or phenolic hydroxyl groups. And the scavenging ability was related to the number of active hydroxyl groups in the molecule. In the molecule of (a) and (c), there are the active hydroxyl groups in C_3 and C_6, but part of the hydroxyl groups are substituted by carboxymethyl in the molecule of (b), (d), and (e), so the scavenging ability has obvious differences. For all Schiff bases, phenolic hydroxyl was grafted to the chitosan's chain, but its antioxidant ability had not improved accordingly. The reason may be due to hydrogen bonds formed between the phenolic hydroxyl and the unreacted chitosan's hydroxyl.

4.2. Scavenging ability on superoxide radical by all kinds of Schiff bases of chitosan and CMCTS

The scavenging ability of the Schiff bases of chitosan and CMCTS is shown in Figure 2. The inhibitory effect of all kinds of Schiff bases on superoxide radical was concentration related, and at a concentration from 1 to 5 mg/ml, the percentage scavenging effect valued from 5.1% to 20.2% for (a), and from 8.8% to 16.1% for (c). Compared with CMCTS,[6] the scavenging ability of the Schiff bases of chitosan and CMCTS studied in our experiments especially (b), (d), and (e) on superoxide radicals was softer. And it proves that the Schiff bases are not of benefit to antioxidant activity. That is to say that the active amido may have important effects on the activity of antioxidant. And like the scavenging ability of hydroxyl radical, the scavenging ability on superoxide radical of (a) and (c) was better than that of others too (Fig. 3). It may be for the same reason that differences in activity were caused on account of the active hydroxyl. To CMCTS, the degree of substitution of C_3 and C_6 was about 0.79 in all, and to the Schiff bases, the group of NH_2 was changed to C=N in the degree of about 0.6. The antioxidant activity of the Schiff bases was reduced when the amido and hydroxyl were substituted, and it shows the importance of the active amido and hydroxyl on the antioxidant ability.

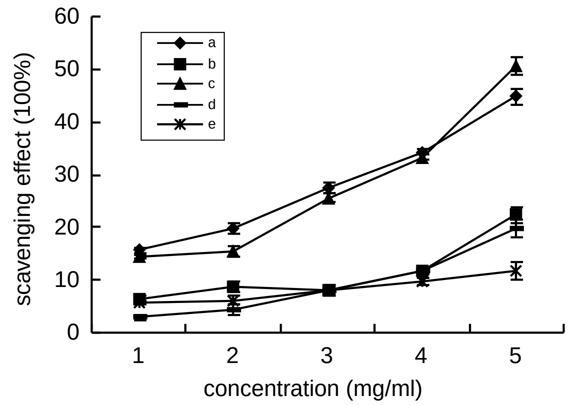

Figure 2. Scavenging effect of the Schiff bases of chitosan and CMCTS on hydroxyl radicals.

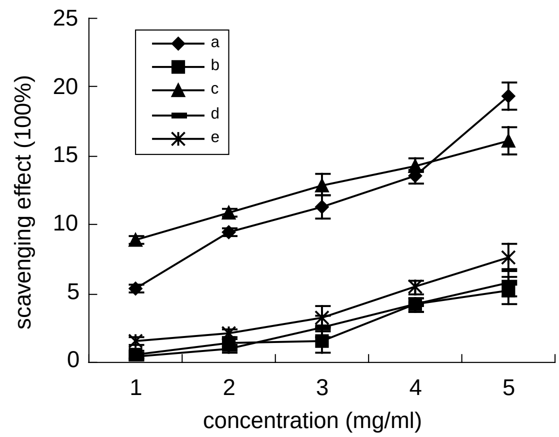

Figure 3. Scavenging effect of the Schiff bases of chitosan and CMCTS on superoxide radicals.

5. Conclusion

As it is well known, the bioactivity of chitosan is mainly attributes to the active hydroxyl and amino groups, and so does the antioxidant ability. A number of active hydroxyl and amino groups of chitosan were affected by intramolecular hydrogen bonds. The Schiff bases of chitosan destroyed part of the hydrogen bonds, but at the same time, new hydrogen bonds were formed,[9] and the NH_2 group was changed to C=N, so the activities of antioxidant did not show so much improvement. The relation between the molecular and bioactivity requires further research.

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