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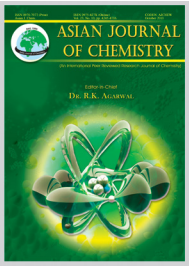

Preparation and Antibacterial Activity of Ag/Chitosan Complex†

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While retaining the original activity of chitosan (CTS), water-soluble carboxymethylated chitosan (CM-CTS) was first synthesized *via* the carboxymethylation of chitosan with chloroacetic acid and then complexed with silver ions to form stronger antimicrobial agent (Ag/CM-CTS). FTIR was employed to characterize and confirm the structures of each product. Antimicrobial tests show that Ag/CM-CTS has much higher antimicrobial activity than both CTS and CM-CTS against ether *E. coli* or *B. subtilis*. The minimum inhibitory concentrations of Ag/CM-CTS against *E. coli* and *B. subtilis* are 0.1 and 0.2 mg/mL, respectively.

Key Words: Antibacterial agent, Chitosan, Silver ion, Carboxymethylation.

INTRODUCTION

With the improvement of living standards, the demand for ecological and functional textiles is rising. In the past decades, antimicrobial textiles have attracted so much attention that antimicrobial agents with excellent overall properties were largely needed.

Chitosan, the second most abundant natural polymer, is non-toxic, biocompatible and biodegradable. And more importantly, chitosan and its derivatives were found to have biological activities, like antitumor[1], antiulcer[2], anticoagulation[3], as well as antimicrobial[4,5] and their antimicrobial activity is effective to a wide range of microorganisms including fungi, algae and bacteria[6]. Different from most other antimicrobial agents, chitosan has high reactivity and processability owing to its special molecular structure and polycationic nature[7]. However, chitosan only dissolves in acidic solution and its antimicrobial ability is not high enough for finishing textiles. In present work, we first synthesized a water-soluble carboxymethylated chitosan (CM-CTS) by carboxymethylation of original chitosan with chloroacetic acid and then CM-CTS was complexed with silver ions to form stronger antimicrobial agent-Ag/CM-CTS. The antimicrobial activities of CTS, CM-CTS and Ag/CM-CTS were evaluated and compared.

EXPERIMENTAL

Chitosan (CTS) with 95 % degree of deacetylation was obtained from Zhejiang Jinqiao Biochemical Co. Agar, peptone and beef extract were purchased from Shanghai Chemical Reagent Co. All other chemicals involved were of analytical grade and used without further purification. The microorganisms tested (*E. coli* and *B. subtilis*) were provided by the Key Lab of Biotechnology of Hefei University.

Preparation of carboxymethyl chitosan (CM-CTS): Chitosan powder was first dissolved in 1.0 % acetic acid and then diluted in isopropanol. NaOH was added in a ratio of 1:4 (CTS: NaOH, w/w). After 12 h of basification at room temperature, chloroacetic acid was added in a ratio of 1:4 (CTS: $ClCH_2COOH$, w/w) and reacted with chitosan at 60 ºC for 10 h. After cooling down, glacial acetic acid was added to neutralize the solution to pH 7.0. CM-CTS powder was filtered out, rinsed repeatedly with 75 % ethanol till no chloride ions was detected and dried under vacuum below 50 ºC.

Preparation of Ag/chitosan complex (Ag/CM-CTS): $AgNO_3$ and ethylenediamine tetraacetic acid (EDTA) in a molar ratio of 1:1 were dissolved in water on the basis of 1 g/L $AgNO_3$. Whereafter, CM-CTS was dissolved in the above solution in a ratio of 1:0.05 (CM-CTS:$AgNO_3$, w/w). The mixture was agitated for 1 h at room temperature and, then acetone was added in to give white powder, which was subsequently rinsed repeatedly with 75 % ethanol till no silver ions could be detected and dried under vacuum below 50 ºC.

Characterization: FTIR spectra were measured with a spectral resolution of 1 cm^{-1} on a Nicolet NEXUS 870 FTIR spectrophotometer using KBr powder at room temperature.

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Antibacterial assessment: The antibacterial activities of CTS, CM-CTS and Ag/CM-CTS complexes were evaluated using the agar plate method. Typically, a loopful of each culture was spread to give single colonies on nutrient agar (agar 15 g, peptone 10 g, beef extract 3 g, NaCl 3 g in 1 L water; pH 7.0) and incubated at 37 ºC for 24 h. A representative colony was picked out with a wire loop and place in nutrient broth (peptone 10 g, beef extract 3 g, NaCl 3 g in 1 L water; pH 7.0), which was then incubated at 37 ºC overnight. By appropriated diluting with sterilized distilled water, each culture containing *ca*. 10^5-10^6 CFU/mL was prepared which was used for the antibacterial test. Samples were dissolved in distilled water at various concentrations from 0.01-1.0 mg/mL and then were autoclaved at 121 ºC for 20 min. A loop of each suspension was inoculated on each nutrient medium with sample or control added. Then, the inoculated plates were incubated at 37 ºC for 48 h and the colonies were counted and the minimal inhibitory concentrations (MIC) values were obtained. Each test was repeated three times at least.

RESULTS AND DISCUSSION

FTIR characterization of Ag/CM-CTS complexes: Fig. 1 shows the FTIR spectra of CTS, CM-CTS and Ag/CM-CTS. CM-CTS have a similar FTIR spectrum to CTS, both showing O-C stretching vibration band at 1067 cm^{-1}, -NH_2 bending vibration at 1606 cm^{-1} and -OH and -NH_2 overlapped stretching vibration band at *ca*. 3437 cm^{-1}. The only exception is that an absorption band appears at 1413 cm^{-1}, which is assigned to asymmetric stretching vibration band of -COO- group, indicating that CM-CTS has been successfully synthesized. The FTIR spectrum of Ag/CM-CTS complex is similar to that of CM-CTS, but the frequency of some vibration band changed a little. For example, the -OH and -NH_2 vibration band shifted from 3437-3430 cm^{-1}, the asymmetric stretching vibration of -COO^- transferred from 1413-1397 cm^{-1} and -NH_2 bending vibration varied from 1606-1631 cm^{-1}, all suggesting that -NH_2, -OH and -COO^- groups have participated in the complexation between Ag^+ and CM-CTS.

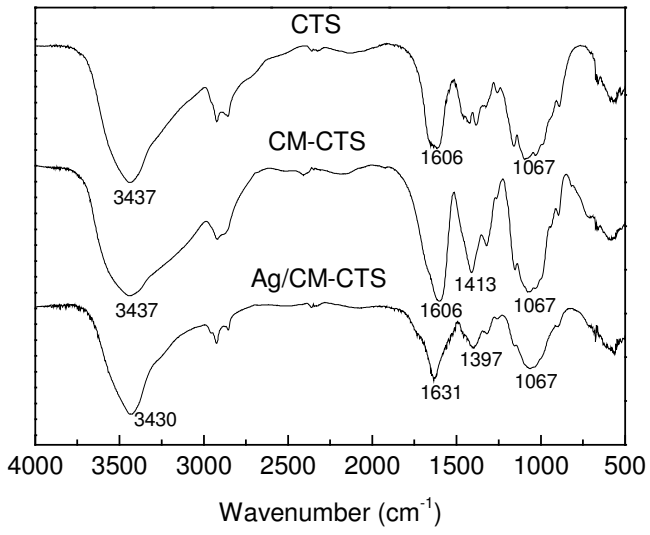


Fig. 1. FTIR spectra of CTS, CM-CTS and Ag/CM-CTS

Comparison of antibacterial performance between CTS, CM-CTS and Ag/CM-CTS: CTS, CM-CTS and Ag/CM-CTS solutions with same concentration (0.2 mg/mL) were employed to test antibacterial performance against the common gram-negative bacterium (*E. coli*) and gram-positive bacteria (*B. subtilis*). Their inhibitory rates were calculated by the colony counting method and the results are displayed in Table-1.

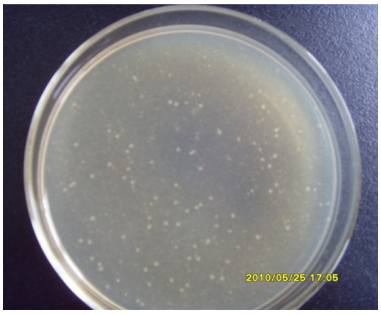
TABLE-1
COMPARISON OF ANTIBACTERIAL RATES BETWEEN CTS, CM-CTS AND AG/CM-CTS

Microorganisms		Sample			
		Control	CTS	CM-CTS	Ag/CM-CTS
E. coli	Colony No.	170	103	98	0
	Inhibitory rate (%)	–	39	42	100
B. subtilis	Colony No.	187	125	116	0
	Inhibitory rate (%)	–	33	38	100

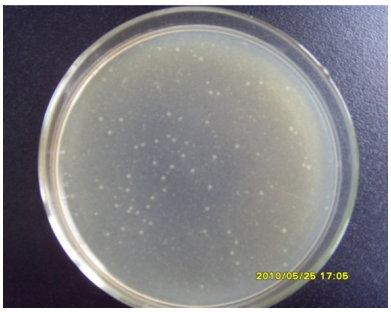
As is seen in Table-1, both CTS and CM-CTS have certain antibacterial activities against ether *E. coli* or *B. subtilis*, which is accordant with literature reports. As expected, Ag/CM-CTS shows much higher antibacterial performance than ether CTS or CM-CTS. Generally, chitosan displays antibacterial activities only in acid solutions, in which chitosan has so many positively charged -NH_3^+ groups that interact strongly with negatively charged surfaces of bacteria, obstructing the normal substance exchanges *via* the cell walls and thus inhibiting the growth of bacteria. After complexation between Ag^+ and CM-CTS, according to the Lewis acid-base theory, Ag^+ ions show stronger electron-accepting ability than H^+, leading to the increase of positive charge density on chitosan, which reinforcing the adsorption of chitosan onto the cell surfaces, finally resulting in the higher antibacterial activity of Ag/CM-CTS complex than ether CTS or CM-CTS.

Antibacterial assessment of Ag/CM-CTS complexes: Figs. 2 and 3 display the culture media in which the tested microorganisms, *E. coli* and *B. subtilis*, have been inoculated and incubated. As is seen, Ag/CM-CTS shows remarkable antibacterial effects on both *E. coli* and *B. subtilis*. Comparing with the control group (Fig. 2a), Ag/CM-CTS with 0.03 mg/mL of concentration (Fig. 2b) exhibits 59 % inhibitory rate and, its antibacterial effect improves apparently with the increase of concentration. 0.1 mg/mL Ag/CM-CTS has completely inhibited the growth of *E. coli*. As shown in Fig. 3, it seems that Ag/CM-CTS complexes show a somewhat weaker antibacterial activity against gram-positive bacteria. Ag/CM-CTS with 0.03 mg/mL concentration (Fig. 3b) exhibits 56 % inhibitory rate against *B. subtilis* and, no growth is observed till the concentration was increased to 0.2 mg/mL (Fig. 3c).

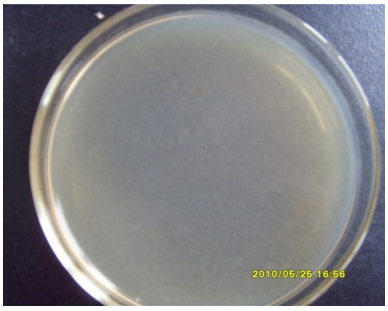
Table-2 shows the detailed data describing the antibacterial activities of Ag/CM-CTS complexes against *E. coli* and *B. subtilis*, from which the minimum inhibitory concentration (MIC) can be obtained. Herein, MIC is defined as the lowest concentration of compounds at which the microorganisms tested do not show visible growth. Therefore, MICs for *E. coli* and *B. subtilis* are 0.1 and 0.2 mg/mL, respectively, implying that Ag/CM-CTS has better antibacterial activity against *E. coli* than *B. subtilis*.



(a) Control

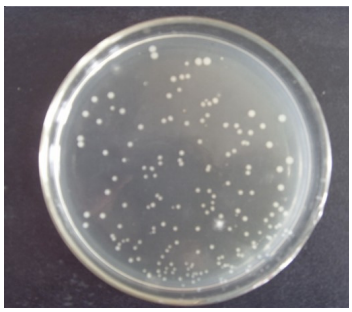


(b) 0.03 mg/mL Ag/CM-CTS

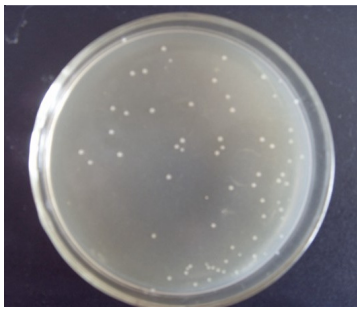


(c) 0.1 mg/mL Ag/CM-CTS

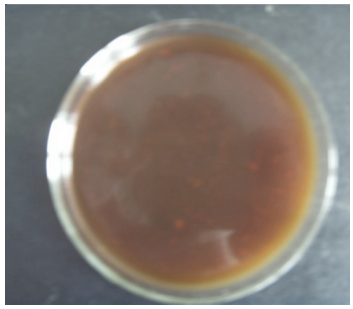
Fig. 2. Antibacterial effect of Ag/CM-CTS in different concentrations on *E. coli*



(a) Control



(b) 0.03 mg/mL Ag/CM-CTS



(c) 0.2 mg/mL Ag/CM-CTS

Fig. 3. Antibacterial effect of Ag/CM-CTS in different concentrations on *B. subtilis*

TABLE-2
ANTIBACTERIAL ASSESSMENT OF
Ag/CM-CTS IN DIFFERENT CONCENTRATIONS

Microorganisms		Concentration (mg/mL)						
		0.01	0.03	0.06	0.1	0.2	0.5	1.0
E. coli	No. (s)[a]	96	69	20	0	0	0	0
	No. (c)[b]				170			
	R (%)[c]	44	59	88	100	100	100	100
B. subtilis	No. (s)	108	82	56	29	0	0	0
	No. (c)				187			
	R (%)	42	56	70	85	100	100	100

[a]No. (s): The colony number in sample nutrient medium. [b]No. (c): The colony number in control nutrient medium. [c]R: Inhibitory rate, equaling to No./s divided by No./c.

Conclusion

Water-soluble chitosan was synthesized by carboxymethylation of chitosan with chloroacetic acid in mild conditions. Ag/CM-CTS complexes were prepared *via* the complexation reaction between Ag^+ ions and CM-CTS. FTIR analyses confirm that $-NH_2$, -OH and $-COO^-$ groups on CM-CTS have participated in the complexation. Ag/CM-CTS displays much higher antibacterial activity than both CTS and CM-CTS against ether *E. coli* or *B. subtilis*. And it seems that Ag/CM-CTS has better antibacterial activity against gram-negative bacteria than gram-positive bacteria.

ACKNOWLEDGEMENTS

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