

# PREPARATION AND ANTI-BACTERIAL ACTIVITY OF CHITOSAN- IODINE COMPLEX

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Chitosan-iodine (CI) complexes were prepared by freezing mixtures of chitosan and KI-I<sub>2</sub> solutions and then thawing them. The complexation was recognized by specific coloring and the CI complexes exhibited absorption spectra with a peak at around 500 nm. The available iodine concentration in the CI complexes increased remarkably. The values of  $A_{500}$  and I<sub>2</sub> concentration in the CI complexes were almost kept with storage at 4°C for 3 months, while the values decreased sharply at room temperature. The CI complex prepared with the concentration of 0.05% chitosan and 0.0025% iodine inhibited completely the growth of *Escherichia coli* for 24 hr, whereas 0.05% chitosan showed no antibacterial activity and 0.0025% iodine showed weak antibacterial activity. The synergistic effect of the CI complex was suggested.

## Introduction

Chitosan coagulates bacterial cells in aqueous solution. Positive-charged amino groups in chitosan are attached to negative-charged bacterial cell walls, therefore, chitosan and bacteria make coagulant flocks[1]. Chitosan has also healing activity[2]. On the other hand, Iodine, which is extracted from an underground salt water in Japan, is used as disinfectants. One famous disinfectant, poly-vinyl-pyrrolidone-iodine complex, has been used all over the world. However, poly-vinyl-pyrrolidone is utilized only as an iodine-holder. We have studied chitosan-iodine (CI) complex as a new anti-bacterial material.

## Materials and Methods

### Chitosan and other chemical reagents

Chitosan was offered by Dainichi Color Industry Co. Ltd., Tokyo, Japan. The molecular weight of the chitosan was about  $1.0 \times 10^5$ , and the degree of de-acetylation (DDA) is 99%. Iodine and other chemical reagents were purchased from Japanese reagent makers as the highest quality grade.

### Preparation of chitosan-Iodine(CI) complex

CI complex was prepared by Yajima method [3]. Various concentrations of chitosan and iodine (I<sub>2</sub>-KI, [I<sub>2</sub>]/[KI]=0.10) were dissolved in acetate buffer solution (0.33 M acetic acid + 0.10 M sodium acetate, pH 4.1). The solution was frozen at -20 °C, and then thawed at 4 °C. CI complex was colored with violet. The complex was preserved at 4 °C.

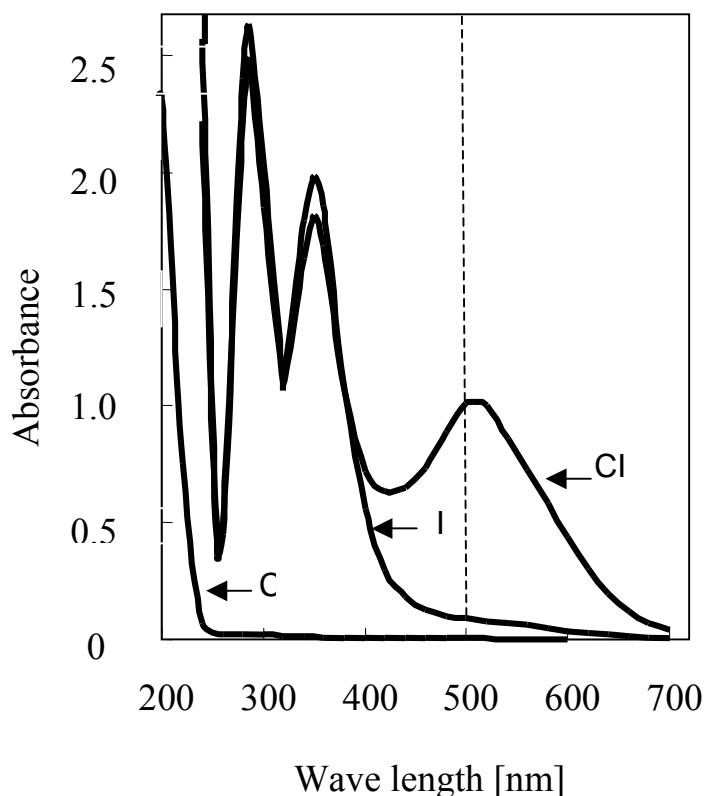
### Anti-bacterial activity

*Escherichia coli* IFO3301 was used as a standard bacterium. *E. coli* was inoculated to an ordinary bouillon medium (Eiken Chemicals, Co.Ltd, Tokyo, Japan) containing various concentrations of CI complex. The culture medium in an L-shaped test tube was incubated at 37 °C with Monod shaker. The growth of the bacterium was monitored by the absorbance (660 nm) of the culture suspension.

## Results and Discussion

### Characteristics of CI complex

UV and visible spectra of CI complex, chitosan, and iodine are shown in Figure.1. Absorbance at 500 nm increased by complex formation between chitosan and iodine. CI complex was colored with violet. Available iodine concentrations of I<sub>2</sub>-KI and CI complex at the same iodine quantity, were 78 mg/ml and 498 mg/ml, respectively. These data indicate that chitosan holds I<sub>2</sub> and I<sub>3</sub><sup>-</sup> in the polymers.



**Figure 1:** UV and visible spectra of CI complex, chitosan, and iodine

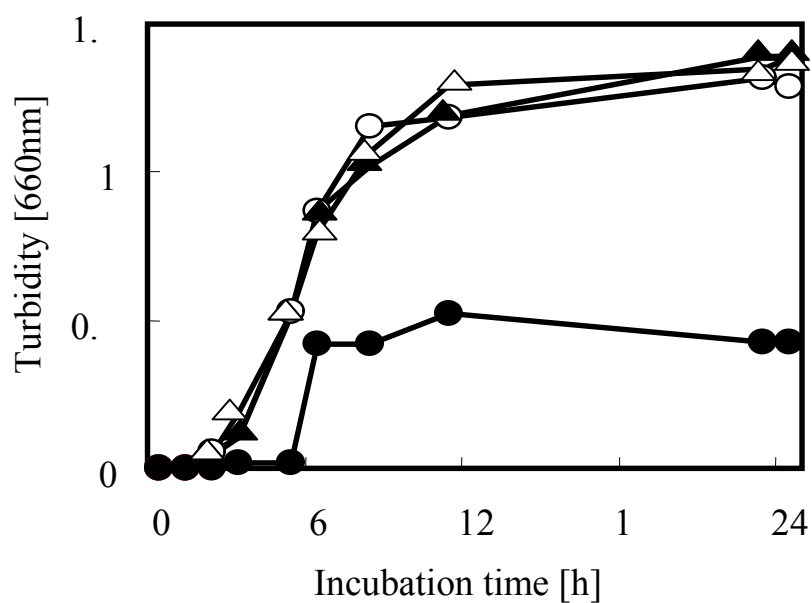
C: chitosan; I: iodine (I<sub>2</sub>-KI solution); CI: chitosan-iodine complex.

### Anti-bacterial activity of CI complex

Anti-bacterial activities of CI complex are shown in Figure 2 and Figure 3. Chitosan and iodine did not inhibit the growth of *E. coli* at the final concentrations at 0.025% and 0.0025%, respectively. However, The CI complex at the final concentrations of 0.025% chitosan and 0.0025% iodine, inhibited the bacterial growth for 6 hours. The CI complex at the final concentration of 0.025% chitosan and 0.005% iodine, also inhibited for over 24 hours the bacterial growth. The synergistic effect of the CI complex was suggested.

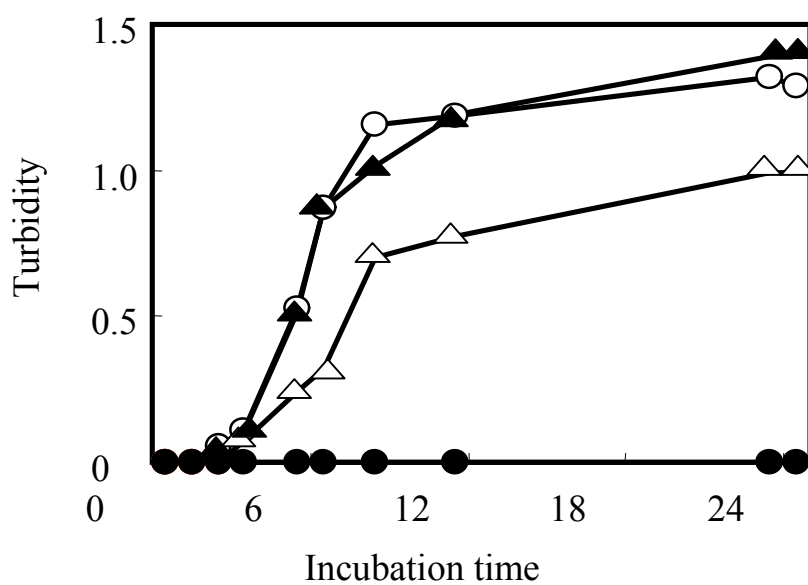
### Temperature stability of CI complex

Temperature stability are shown in Figure.4. Available iodine concentration of CI complex was kept at 4 C for 3 months, however, it decreased at room temperature within 1 week. We thought examination of temperature stability for CI complex was needed necessary.



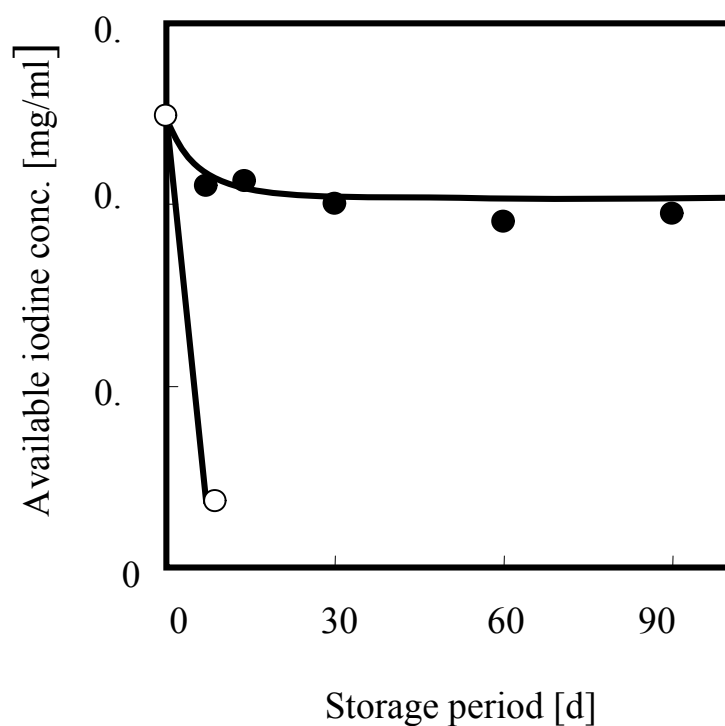
**Figure 2:** Growth curves of *Escherichia coli* with CI complex, chitosan, iodine (1),

○ :control; ● : 0.025% chitosan + 0.0025% iodine; △ : 0.0025% iodine  
 ▲ : 0.025% chitosan.



**Figure 3:** Growth curves of *Escherichia coli* with CI complex, chitosan, iodine (2)

○ :control; ● : 0.025%chitosan + 0.005% iodine; △ :0.005%iodine;  
 ▲ : 0.025% chitosan.



**Figure 4:** Temperature stability of CI complex  
 ● : at 4°C; ○ : at 20°C (room temperature).

#### References

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- [3] H. Yajima, M. Morita, M. Hashimoto, H. Sashiwa, T. Kikuchi, T. Ishii : *Int. J. Thermophys.*, **22**(4), 1265-1283 (2001).