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# SYNTHESIS OF THIOUREA CHITOSAN AND THIOUREA CHITOSAN METAL COMPLEXES AND ANTIBACTARIAL ACTIVITY

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ABSTRACT

*Chitosan and its derivatives are becoming increasingly important natural polymers because of their unique combination of properties like biodegradability, biocompatibility and bioactivity, in addition to attractive physical and mechanical properties. Here we wish to report the synthesis and characterization of thiourea chitosan and its metal-complex. Thiourea chitosan was prepared by the reaction of chitosan with ammonium thiocyanate in ethanol. Its metal complex was prepared by dissolving thiourea chitosan in 1% $CH_3COOH$ solution follow by adding metal salt solution. Antimicrobial activities of the complexs were evaluated against three species of bacteria. These complexs had an effect on study bacteria, which minimum inhibition concentrate (MIC) values against bacteria were 2 times lower than those of Thiourea-chitosan; the complex had higher antibacterial activity on Staphylococcus aureus than E. coli and Pseudomonas aeruginosa.*

**Keywords**: *Chitosan, Thiourea chitosan, metal complex.*

## I - INTRODUCTION

Chitin, the source material for chitosan, is a high molecular weight linear polymer of 2-acetamido-2-deoxy-D-glucopyranose units linked together by 1,4-glycosidic bonds. Chitosan is a natural nontoxic biopolymer derived by the deacetylation of chitin. Unlike chitin, chitosan being soluble in dilute organic acids such as acetic acid, formic acid, lactic acid, is a chemically stable, white to pale yellow powder or flake. Chitosan with a positive charge, which is the basis of its use as a "sticking" agent. The positively charged molecules adhere to negatively charged pesticides and plant surfaces (figure 1) [1, 2].

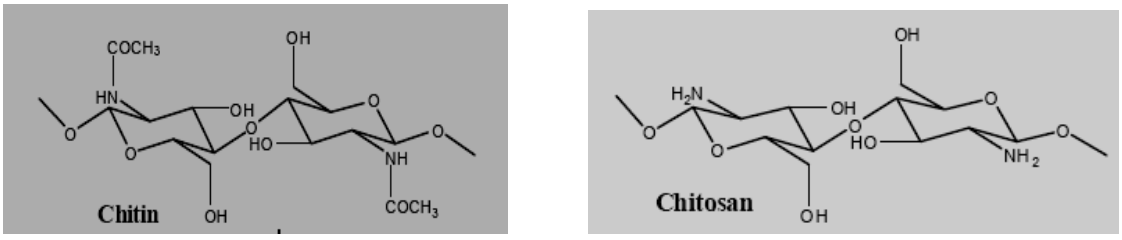


*Figure 1*: Chitin and chitosan structure

Both chitin and chitosan are becoming increasingly important natural polymers because of their unique combination of properties like biodegradability, biocompatibility and bioactivity, in addition to attractive physical and mechanical properties. Chitosan and its derivatives have attracted considerable interest due to their antimicrobial and antifungal activities [3]. The antimicrobial activities of chitosan depend on several factors such as the kind of chitosan (deacetylation degree, molecular weight), the pH of the medium and the temperature etc.

Consequently, Chitosan is used as a human dietary supplement for weight loss and cholesterol reduction. Chitosan is also used as a flocculating (i.e., settling) agent in wastewater treatment systems, a hydrating agent in cosmetics, a pharmaceutical agent in biomedicine, and an antimicrobial food wrap [4 - 7]. The chitosan bearing S-linkage in the structure was well known as a high antibacterial activity, but the preparation of this chitosan derivatives faced insoluble compounds. Here we wish to report preparation of thiourea chitosan in mild condition with high solubility in organic acid solution. However to improvement of thiourea chitosan antibacterial activity, the metal complex of thiourea chitosan was also prepared. The antimicrobial activity of thiourea chitosan and its metal complexes was evaluated on *S. aureus*, *E. coli* and *P. aeruginosa* (Fig. 2).

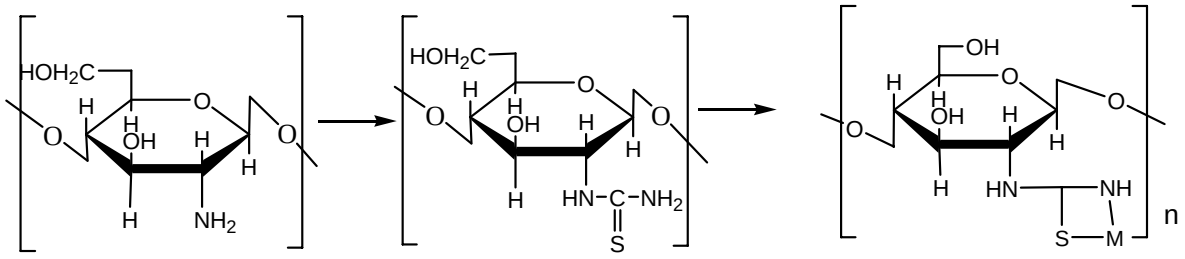


*Figure 2:* Synthesis of thiourea chitosan and metal-complexes

## II - MATERIALS AND METHODS

### 1. Materials

Chitosan with deacetylation degree of 80-90% was purchased from Nha Trang. The other reagents were of chemical grade and used without further purification.

IR spectra were recorded with KBr discs in the range of 4000 - 500 $cm^{-1}$ on FT-IR spectrophotometer; elemental analysis (C, H, N, O) were performed on elemental analyzer at laboratory of the Kaiserslautern University (Germany). Beef extract, glucose and peptone were purchased from Merck (Germany). The microorganisms were provided by Pasteur Institute (HCM City, Vietnam).

### 2. Methods

*a) Preparation of thiourea chitosan*

A mixture of 16.1 g (0.1 mol) chitosan powder, 15.2 g (0.2 mol) ammonium thiocyanate and 150 ml ethanol in a one-neck flask with magnetic stirring was refluxed for 12 h. After cooling down to room temperature, the precipitate was collected by filtration and washed with ethanol, and then was dissolved in 1000 ml of 1% (v/v) acetic acid solution. By adding 10% (w/v) NaOH or 5% ammonium solution into the solution and filtrating, the precipitate was collected and successively washed with water and finally dried at 40ºC to delivery 16.5 g of thiourea chitosan.

*b) Preparation of thiourea chitosan-Cu complex*

0.3 g of thiourea chitosan was dissolved in 30 ml of 1% (v/v) acetic acid, followed by addition of 25 ml of $Cu(NO_3)_2$ solution (0.01 g $Cu(NO_3)_2$). The resulting mixture was stirred for 3 h at room temperature, the precipitate obtained by adding 200 ml acetone into the mixture and collected by filtration, successively washed with

95% ethanol, and dried to give thiourea chitosan-Cu complex.

*c) Evaluation of antimicrobial activity*

The spectrophotometric determination of bacteria numbers method (Harley-Prescott 2002) was used to determine the minimum inhibition concentration (MIC) of chitosan, thiourea chitosan, thiourea chitosan-Cu (TC-Cu), and thiourea chitosan-Zn (TC-Zn) complex. The 1% (w/v) solutions of thiourea chitosan, thiourea chitosan- Cu and thiourea chitosan-Zn complex were prepared in 1% (v/v) acetic acid. Duplicate two-fold serial dilutions of each sample were added to nutrient broth (beef extract 5 g, peptone 10 g to 1000 ml distilled water, pH 7.0) for the last concentration 10 ppm to100 ppm. The blank sample of nutrient broth was used as a control. They were autoclaved at 121°C for 25 minutes. The culture of each bacterium was diluted by sterile distilled water to $10^5$ - $10^6$ CFU/ml. 0,2ml of each suspension, it was inoculated on nutrient medium with the samples and the control samples added (fix number of bacteria approximately $10^4$ CFU/ml). After injection, the tubes were incubated at 35°C and the samples were measuring by photometer 2 hours/each time throughout 20 houses. The MIC values were obtained based on the grown of bacteria. The MIC was considered to be the lowest concentration that completely inhibits against bacteria comparing with the control. The data was analyzed by Excels software.

## III - RESULTS AND DISCUSSION

### 1. Synthesis of TC and TC-metal complexes

In this paper, thiourea chitosan (TC) was prepared by the reaction of chitosan with ammonium thiocyanate in ethanol for 12 h at temperature from 70 to 80°C, which was easy to be dissolved in acetic acid solution. The metal ion-complexes were also prepared by adding metal salt solution to the thiourea chitosan solution. The presence of metal ion and S back bone, which remarkably enhanced the antimicrobial activity of chitosan [8, 9] showed a general method for preparing of chitosan through the grafting of sulfur compounds (thiourea, rubeanic acid) on chitosan backbone using glutaraldehyde as a linker. The obtained product is an insoluble solid and not used for bacteriostasis. Here we used different approach to introduce thiourea group into chitosan. It is well known that when temperature is above 80°C, a part of ammonium thiocyanate converts into its isomeric compound, thiourea. The strategy is that ammonium thiocyanate and chitosan are together heated to form chitosan thiocyanate and ammonia; similarly, chitosan thiocyanate is heated to be converted into thiourea chitosan. The preparation of thiourea chitosan is shown in figure 2. The structures of thiourea chitosan and thiourea chitosan—metal complexes were confirmed by FT-IR (figure 3).

The elemental analysis results of TC, TC-Zn and TC-Cu are shown in table 1. It could be seen that the contents of nitrogen in TC-Zn and TC-Cu were a little bit lower than that in TC. It was thought that the decrease was due to the presence of metal produced in the reactions of TC-$NH_2$ with metal salt.

*Table 1*: Elemental analysis results of TC and TC- metal ions sample

| Sample | Element analysis, % | | | |
|---|---|---|---|---|
| | C | H | N | S: Ratio |
| TC | 39.78 | 7.06 | 7.30 | 1:2 |
| TC-Zn | 37.90 | 6.64 | 6.47 | 1:2 |
| TC-Cu | 38.68 | 6.79 | 6.87 | 1:2 |

### 2. FT-IR spectra of TC and TC-metal complexes

The FT-IR spectrum of TC—metal complexes exhibits many alterations from that of TC (figure 3). The major differences are: (1) The wide peak at 3500 $cm^{-1}$, is corresponded to the stretching vibration of —$NH_2$ and —OH groups, shifted to lower frequency (3449 $cm^{-1}$ of Zn complex and 3433 $cm^{-1}$ of Cu — complex). (2) The absorb band at 1655 $cm^{-1}$ of TC assigned to the bending vibration of —$NH_2$ group shifted to lower frequency (1634 $cm^{-1}$ of Zn-complex and 1639 $Cm^{-1}$ of Cu-complex) indicated that the —$NH_2$ group was involved in

complexation. (3) The band at 1087 $cm^{-1}$ assigned to the second —OH group showed a significant shift to higher wave number (1119 $cm^{-1}$ for the both Zn-and Cu-complex) which was enhanced with increasing metal content. It suggested that the second —OH group got involved in complexation (Wang, Du, Lui, 2004) [10, 11]. Especially, the FT-IR spectra of thiourea chitosan-metal complexes have shown a new peak at 628 $cm^{-1}$ which peak confirmed the new bond between metal ion and S atom.

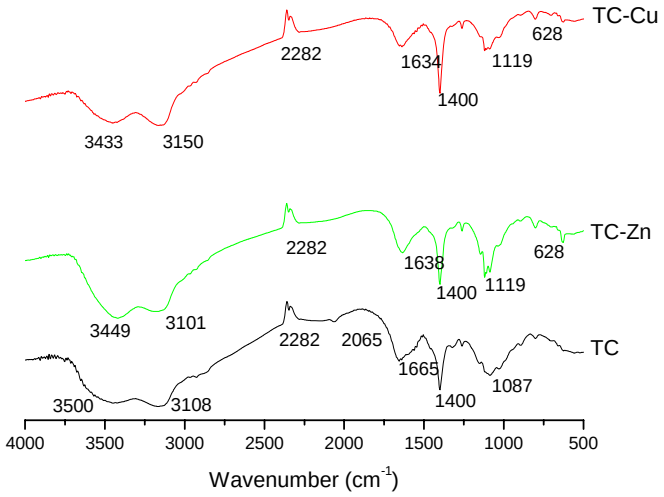


*Figure 3*: IR spectra of thiourea chitosan and its metal complexes

### 3. Antibacterial activity of TC-Zn complex on species *E. coli* and *Pseudomonas aeruginosa*

Based on research methods, after 20 hours incubation the optics density (OD) data of culture media with bacteria and substrate (TC-Zn complex) were obtained and presented in table 2 and figure 4. The data showed that the OD of samples had a relation between grown bacteria density and TC-Zn concentration in medium. The optic density of medium depended on concentration of grown bacteria in medium. The data showed that the TC-Zn complex had a wide effect on bacteria. The MIC of TC-Zn complex on species *E. coli* and *Pseudomonas aeruginosa* were approximately 50 - 60 ppm, while on species *Staphylococcus aureus* was 30 ppm.

The result of the antibacterial activities of TC-Cu on three species of bacteria showed that the TC-Cu complex had effect on bacteria. The MIC of the TC-Cu complex on species *E. coli* and *Pseudomonas aeruginosa* was approximately 60 ppm, while on species *Staphylococcus aureus* was 20 ppm.

Base on ours study, the results were showed that the antibacterial activity of TC-Zn complex was higher than chitosan. The MIC of TC-Zn complex on species *S. aureus* was smaller than chitosan about 20 times, and the MIC of it on *E. coli* was less than 10 times. The TC-Cu and TC-Zn complex showed higher effective on *S. aureus* species than that of *E. coli* and *P. aeruginosa* species.

## IV - CONCLUSION

We have successfully prepared thiourea chitosan in mild condition and its metal ion complexes.

In vitro antimicrobial activities of the complex were also evaluated against three species of bacteria included two kinds of Gram negative bacteria (*E. coli; Pseudomonas aeruginosa*) and one kind of Gram positive bacteria (*S. aureus*). These complexes showed

an effective antimicrobial activity. Their MIC values were much lower than those of chitosan and thiourea- chitosan. The complexes have better antibacterial activity on Gram positive bacteria (*S. aureus*) than that on Gram negative bacteria (*E. coli; Pseudomonas aeruginosa*).

*Table 2*: OD of study medium with species *E. coli*, *P. aeruginosa* and *S. aureus* after 20 hours TC- Zn complex incubation

| TC-Zn solution(ppm) | OD *E. coli* | OD *P. aeruginosa* | OD *S. aureus* |
|---|---|---|---|
| Blank sample | 0.220 | 0.230 | 0.230 |
| 10 | 0.217 | 0.238 | 0.207 |
| 20 | 0.147 | 0.205 | 0.147 |
| 30 | 0.144 | 0.159 | 0.130 |
| 40 | 0.141 | 0.138 | 0 |
| 50 | 0.104 | 0.112 | 0 |
| 60 | 0 | 0.107 | 0 |
| 70 | 0 | 0 | 0 |
| 80 | 0 | 0 | 0 |
| 90 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 |

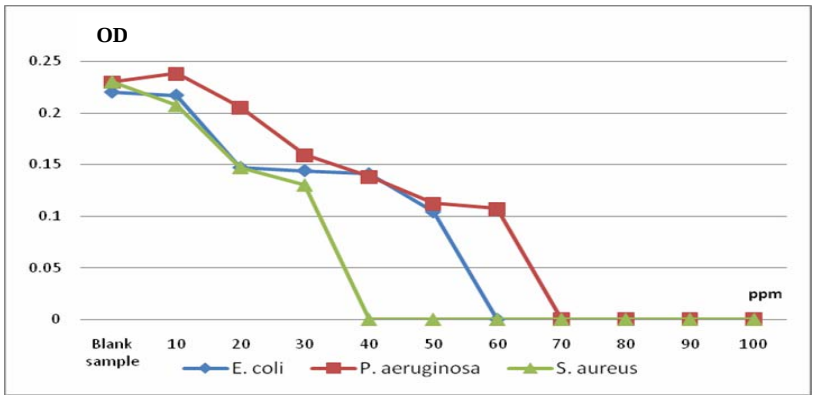


*Figure 4*: OD of *E. coli*, *P. aeruginosa* and *S. aureus* after 20 hours TC-Zn complex incubation

*Table 3*: Optical density (OD) of study medium with species *E. coli*, *P. aeruginosa* and *S. aureus* after 20 hours TC-Cu complex incubation

| TC- Cu solution, ppm | OD *E. coli* | OD *P. aeruginosa* | OD *S. aureus* |
|---|---|---|---|
| Blank sample | 0.230 | 0.230 | 0.230 |
| 10 | 0.201 | 0.237 | 0.221 |
| 20 | 0.199 | 0.214 | 0.202 |
| 30 | 0.187 | 0.169 | 0 |

| TC- Cu solution, ppm | OD *E. coli* | OD *P. aeruginosa* | OD *S. aureus* |
|---|---|---|---|
| 40 | 0.167 | 0.157 | 0 |
| 50 | 0.133 | 0.125 | 0 |
| 60 | 0.110 | 0.101 | 0 |
| 70 | 0 | 0 | 0 |
| 80 | 0 | 0 | 0 |
| 90 | 0 | 0 | 0 |
| 100 | 0 | 0 | 0 |

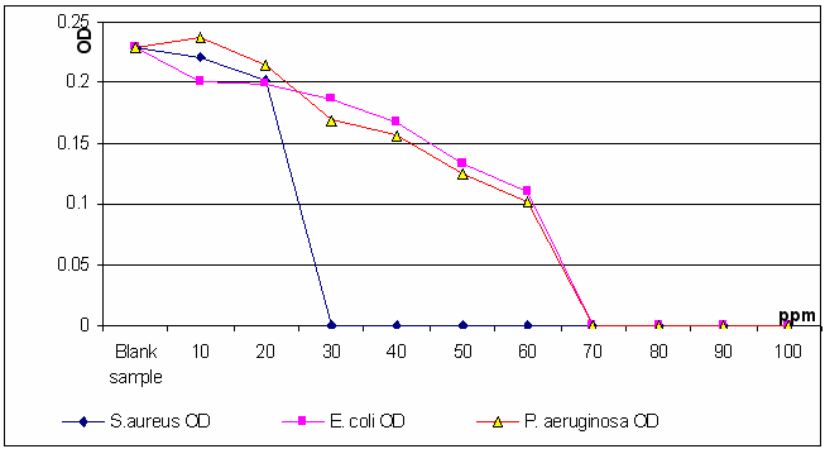


*Figure 5*: OD of *E. coli, P. aeruginosa* and *S. aureus* after 20 hours TC-Cu complex incubation

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