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# Formation, Properties and Antimicrobial Activities of Cotton Xanthate-Cu(II)-Homosulfamine Complex

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To develop a cotton derivatives with prolonged antimicrobial activities, homosulfamine (Hs) was coupled to cotton xanthate (CX) via chelate bond in the presence of Cu(II) ion by one- and two-bath processes. In one-bath process, CX was treated with Cu(II)-Hs solution. In two-bath process, CX was treated with Cu(II) ion solution to produce CX-Cu(II) complex, which was isolated and treated in turn with Hs solution. Effects of concentration, Cu(II)/Hs ratio, and pH on the binding of Hs were investigated at 10°C. In one-bath process, binding of Hs took place readily with optimum pH around 5~6. The amount of binding increased to give a maximum within 5 min and decreased slowly to establish an equilibrium within an hour. In two-bath process, binding of Hs was much lower than that of one-bath process. Release of Hs from CX-Cu(II)-Hs was investigated by batch and flow method. Antimicrobial activities of CX-Cu(II)-Hs were tested against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* and it showed prolonged activity compared to that of free Hs.

**Key words :** Cotton xanthate-Cu(II)-homosulfamine complex, Cotton derivatives, Antimicrobial activity

## INTRODUCTION

During the past two decades, a great deal of papers have been published concerning with the development of polymeric drugs (Domb, 1994; Ottenbrite and Chiellini, 1992; Seymour *et al.*, 1991; Dunn and Ottenbrite, 1990; Jansen *et al.*, 1987). In the cases where the drugs were coupled to the preformed polymer by chemical means, the rate of delivery of the bioactive agent would, presumably, be determined by the nature of the bonding between the polymer matrix and the drug molecule. The type of these bondings ranges from the van der Waals forces, the ionic interactions, coordinated or chelate bonds or, hydrolyzable covalent bonds which differ in their binding energies. Since coordinate covalent bonds of chelate type vary in binding stability over orders of magnitude, the rate of drug release can be controlled to vary depending on the atomic characteristics of the metal and the ligands.

Neogi and Allen reported on the binding of a herbicide, picrolam, to a commercially available synthetic polymer, Dowex A-1, *via* chelate bond utilizing Fe(III), and Al(III) (Neogi and Allen, 1974). Kennedy *et al.* have reported on the coupling of antibiotics to cellulose, presumably, via chelate bond (Kennedy *et al.*, 1974). Morris *et al.* reported that a number of organic antimicrobial agents were durably bound to cotton fabrics in the form of metal complexes (Morris and Welch, 1983; Morris *et al.*, 1981). Kim *et al.* reported that various antimicrobial agents were coupled to cotton xanthate, which obtained by treating cotton with carbon disulfide in alkaline media, *via* chelate bond (Kim *et al.*, 1989; Lee *et al.*, 1996). These results demonstrate that controlled release systems *via* chelate bond are feasible.

In the present study, homosulfamine was linked to cotton xanthate *via* chelate bond with Cu(II) ion to produce cotton derivatives with prolonged antimicrobial activity. Optimum reaction conditions for the preparation of CX-Cu(II)-Hs, and the release of Hs from CX-Cu(II)-Hs were investigated. Antimicrobial activities of CX-Cu(II)-Hs were investigated against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Escherichia coli* employing ditch plate method, and the results were compared with that of free Hs.

## MATERIALS AND METHODS

Homosulfamine hydrochloride was purchased from Sigma Chemical Co., U.S.A. and used without further

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purification. Salts of metals were obtained from Wako Chemical Co., Japan. Adsorbent gause for medical use was employed as representing cotton matrices for this reaction. The material was cut down about 1×1 cm in size. The ingradients of medium used for antimicrobial test were received from DIFCO Laboratories, U.S.A.. The sources of bacteria employed were as follows; *Bacillus subtilis (NA-1), Staphylococcus aureus (ATCC 12228), Pseudomonas aeruginosa (ATCC-9027)* and *Escherichia Coli (TG-1).* IR and UV spectra were taken on a Bomem MB-100 and Shimadzu UV 2101-PC spectrophotometer, respectively, and Corning 150 pH meter was used for the pH measurements.

### Preparation of CX-Cu(II)-Hs complex

**Preparation of CX:** Three grams of cotton was reacted with 200 ml of 1N NaOH and 10 ml of carbon disulfide for 24 hrs at 15°C. As the reaction proceeded, the reaction mixture turned to orange color. The product was washed thoroughly with distilled water, acetone and dried.

**One-bath process:** One gram of CX was placed in a 200 ml of Hs-Cu(II) complex solution at 10°C. As the reaction proceeded, the pale-yellow matrix was turned into olive green. The reaction was proceeded until the amount of Hs bound to the matrix reached to the maximum, the material was washed three times with 100 ml of distilled water, 20 ml of acetone and dried.

**Two-bath process:** CX-Cu(II) complex was prepared by placing 1 g of cotton xanthate in 200 ml of Cu(II) solution at 10°C. After reacting for 2 hrs, the material was washed thoroughly with distilled water, and dried. CX-Cu(II) complex, thus prepared, was reacted with 200 ml of Hs solution at 10°C until the amount of Hs bound to the matrix reached to the maximum. The material was washed three times with 100 ml of distilled water, 20 ml of acetone and dried.

### Determination of Hs bound to CX-Cu(II)-Hs complex

The amount of Hs bound to the matrix was deduced by subtracting the amount in the bulk solution from the amount originally used for the preparation of CX-Cu(II)-Hs complex by measuring the absorbance at 230 nm where the presence of Cu(II) ion did not interfere with the analysis.

### Release of Hs bound to CX-Cu(II)-Hs complex

**Batch method:** Half grams of CX-Cu(II)-Hs complex 0.5 g, was placed in a flask containing 250 ml of distilled water (pH 5.5) and stirred at 10°C. Supernatant, 1.0 ml, was removed at varied time intervals and the concentration of Hs was determined by measuring the absorbance at 230 nm.

**Flow method:** Half grams of CX-Cu(II)-Hs complex was packed in a column (I.D.; 6 mm, height; 150 mm) and eluted with distilled water of pH 5.5 at 10°C using a peristaltic pump maintaining the flow rate at 1 ml/min. The amount of Hs eluted for each fraction was determined by measuring the absorbance at 230 nm.

### Antimicrobial activity of CX-Cu(II)-Hs complex

Antimicrobial activity tested against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus,* or *Pseudomonas aeruginosa* was carried out by ditch plate method reported previously (Kim *et al.*, 1989). To a culture plate (diameter: 90 mm, height: 15 mm) placed 30 ml of inoculum. Ditch plates were prepared by placing two stainless steel cylinders (I.D.: 9 mm, O.D.: 10 mm) before solidifying and allowed it to harden. After solidifying, the two steel cyclinders and the contents were removed to give two cylindrical ditches, to each of which sample (equivalent to 1.0 mg free Hs) was planted, and wetted with a few drops of agar solution. The plate was incubated at 37°C for 24 hours and the diameter of inhibition zone was determined. To investigate the duration of the antimicrobial activity of each sample, contents of the cylindrical ditch after 24 hours of incubation was removed and transfered to a ditch of freshly prepared plate as described above and incubated at 37°C for 24 hours. This procedure was continuously repeated until the inhibition of growth was no longer noticed. The same procedures were followed using 1.0 mg of free Hs to compare the antimicrobial activities of free and matrix-bound drugs. For antimicrobial activity test, one plate (two ditches) per sample to be tested was prepared.

## RESULTS AND DISCUSSION

### Preparation of CX-Cu(II)-Hs complex

The ligand group capable of forming chelate bond with metal ions in CX is xanthate or hydroxyl group, and that in Hs is amino group. In the present experimental conditions, Cu(II) ions can bind either with the ligand of drug molecule or with those of matrix.

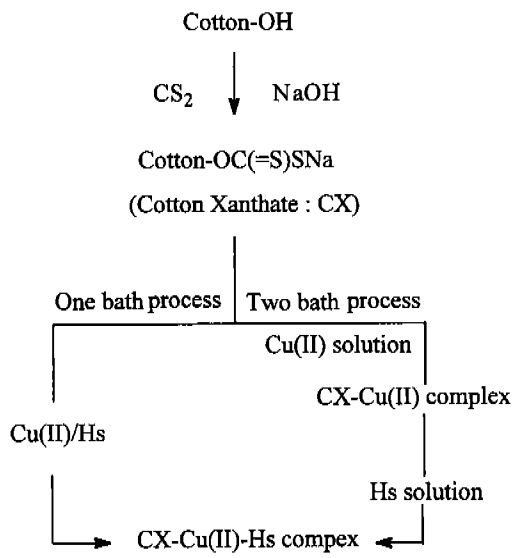
There are two factors which would determine the extent of formation and the type of complex in the presence of two different kinds of ligands. One is the avidity of the metal for the ligand. If the affinity of the two ligands, A and B, is substantially different, a complex with ligands with greater affinity should be formed. If Cu(II) ion possesses much greater affinity for the ligand in Hs to form Hs-Cu(II)-Hs, coupling of drug molecules to the matrix to form CX-Cu(II)-Hs will not be favored. This phenomena might be exhibited

especially in one-bath process, where matrix was introduced into a bath containing an excess of metal-drug complex solution. By the same token, if Cu(II) ion possesses much greater affinity for the ligand in CX to form CX-Cu(II)-CX, fomation of CX-Cu(II)-Hs will also be hindered greatly. This phenomena will predominate especially in two-bath process, where matrix is treated with a solution containing excess metal ion in the first step. Therefore binding of drug molecules will vary depending on the coupling method. If the two ligands are equally avid for a metal M, formation of mixed complex AMB is statistically favored by a factor of 2 over simple complex such as AMA or BMB. Therefore, if the affinity of the ligand in CX or Hs for Cu(II) ion is about the same magnitude, formation of CX-Cu(II)-Hs will be favored. The second factor, which acts as an important driving force in the present experiment, is the insolubility of cotton xanthate-metal-drug complex, which displaces the equilibria in favor of their formation. Even if the binding of Hs to CX *via* Cu(II) ion will take place mainly *via* the coordinate covalent bond, various type of bondings with different binding energies might be involved at the same time.

In the beginning, association of drug molecules to the insoluble matrix will take place readily to form CX-Cu(II)-Hs complex. At some point, the amount of binding will decrease because some of the weakly bound molecules dissociates until the reaction establishes an equilibrium. The amount of binding at equilibrium will depend on the stability of binding between Cu(II)-Hs and Cu(II)-CX.

General procedures for the preparation of CX-Cu(II)-Hs complex are shown in Scheme 1.

Binding of Hs to CX *via* Cu(II) took place readily, which was carried out by one- and two-bath processes.




**Scheme 1.** Preparation of CX-Cu(II)-Hs complex.

The results are shown in Fig. 1 and Fig. 2.

The amount of Hs bound to the matrix differed greatly depending on the coupling method, concentration and Cu(II)/Hs ratio. In one-bath process, when 200 ml of Cu(II)/Hs (4 mM/8 mM) solution was treated with 1 g of CX, the amount of Hs bound increased to give a maximum of 58 mg in 3 min and decreased until it reached to an equilibrium amount of 23 mg in 40 min. In cases where 200 ml of Cu(II)/Hs (8 mM/8

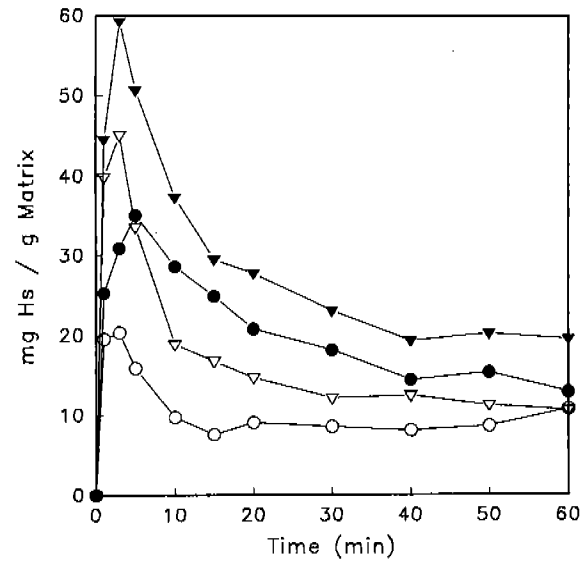



**Fig. 1.** Formation of CX-Cu(II)-Hs as a function of time in one-bath process at 10°C, pH 4.9. CX, 1 g, was treated with 200 ml of Cu(II)-Hs solution (symbol, Cu(II)/Hs mM ratio; ●, 4.0/4.0; ○, 8.0/4.0; ▼, 4.0/8.0; ▽, 8.0/8.0.).

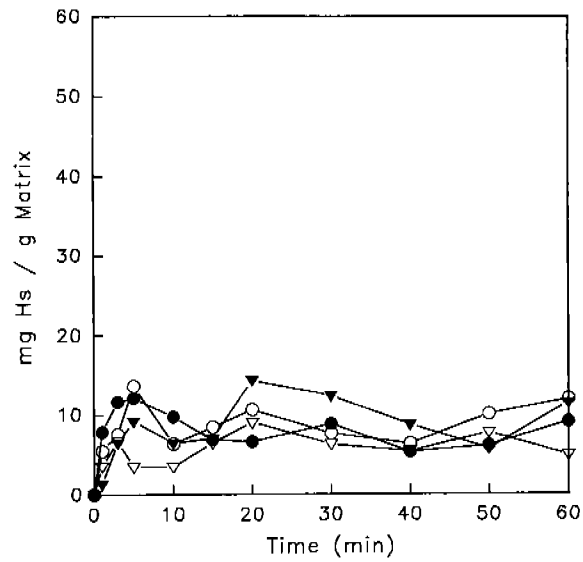



**Fig. 2.** Formation of CX-Cu(II)-Hs as a function of time in two-bath process at 10°C, pH 5.5. Samples were prepared by treating 200 ml of Hs solution (4 mM; circle, 8 mM; triangle) with 1g of CX-Cu(II), which was obtained from 1 g of CX and 200 ml of Cu(II) solution (4 mM; closed mark, 8 mM; open mark).

mM) solution was used, maximum amount of Hs bound per g matrix decreased to 45 mg, and decreased until it reached to an equilibrium amount of 13 mg in 30 min. When 200 ml of Cu(II)/Hs (4 mM/4 mM) solution was used, maximum amount of Hs bound reached to 35 mg in 5 min, and decreased until it reached to an equilibrium amount of 15 mg in 40 min. When 200 ml of Cu(II)/Hs (8 mM/4 mM) solution was used, maximum amount of Hs bound to the matrix reached to 20 mg in 3 min, and decreased until it reached to an equilibrium amount of 10 mg in 10 min. By increasing the concentration of Cu(II) ion from 4 to 8 mM, the amount of binding decreased at the same drug concentration level (closed vs open symbols in Fig. 1). By increasing the concentration of Hs from 4 to 8 mM, the maximum amount of binding increased, but the degree of dissociation also increased (trianglular vs circular symbols in Fig. 1). These results might suggest that much weaker secondary interaction forces might have been involved in this situation, which incresed the amount of binding in the beginning and decreased it by rapid dissociation. In two-bath process, Cu(II) in CX-Cu(II) is supposedly to bind either the ligand in the matrix to form CX-Cu(II)-CX or the ligand in the Hs to form CX-Cu(II)-Hs. As shown in Fig. 2, the amount of Hs bound per 1 g of matrix from two-bath process was around 8~10 mg, which was much lower than those from one-bath process, which suggested the preferred formation of CX-Cu(II)-CX instead of CX-Cu(II)-Hs. The same reasoning might be applicable to the one-bath process where high Cu(II) concentration or high Cu(II)/Hs ratio was applied, when the excess Cu(II) makes the reaction condition similar to that of two-bath process, resulting in the amount of Hs bound to the matrix to be low.

### Release of Hs from CX-Cu(II)-Hs complex

Release of Hs from CX-Cu(II)-Hs prepared by one-bath method was investigated by batch method by placing 0.5 g of sample in 250 ml of distilled water at 10°C, pH 5.5, and the results are shown in Fig. 3. In cases where the samples were prepared at Cu(II) concentration of 4 mM (closed symbols), the rate of release was relatively slow, and the extent of release was around 25~26% in 20 hrs. With the samples prepared at Cu(II) concentration of 8 mM (open symbols), the rate of release was fast, reaching to almost constant value in 1 hr, when the extent of release was around 41~66% depending on the ratio of Cu(II)/Hs. Release of Hs from CX-Cu(II)-Hs prepared by one-bath method was investigated by flow method using 0.5 g of sample and eluting with distilled water of pH 5.5 at 10°C, and the extent of release was around 13~43% (Fig. 4).

The degree of dissociation was higher with the samples prepared at higher concentration of Hs (trianglular symbols). These results are in accordance with what have been observed in binding experiments,

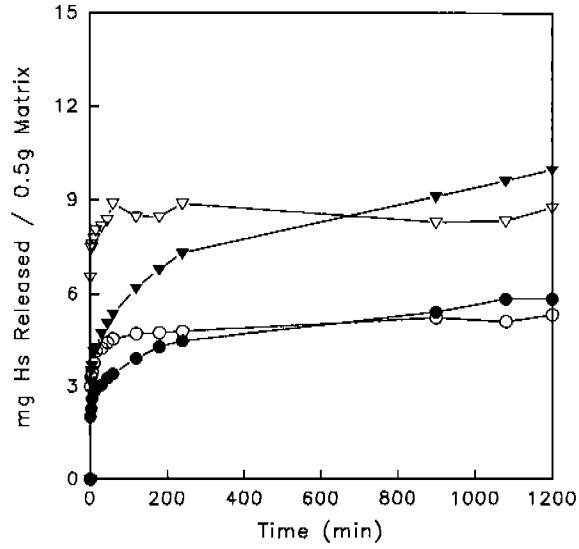



**Fig. 3.** Release of Hs from CX-Cu(II)-Hs prepared by one-bath process at different Cu(II)/Hs mM ratio by batch method (CX-Cu(II)-Hs, 0.5 g, was allowed to contact with 250 ml of bath solution at initial pH 5.5 at 10°C). The individual runs are (symbol, Cu(II)/Hs mM ratio, incorporated amount(Hs mg/0.5 g matrix)); ●, 4.0/4.0, 17.6; ○, 8.0/4.0, 13.1; ▼, 4.0/8.0, 27.4; ▽, 8.0/8.0, 13.5.

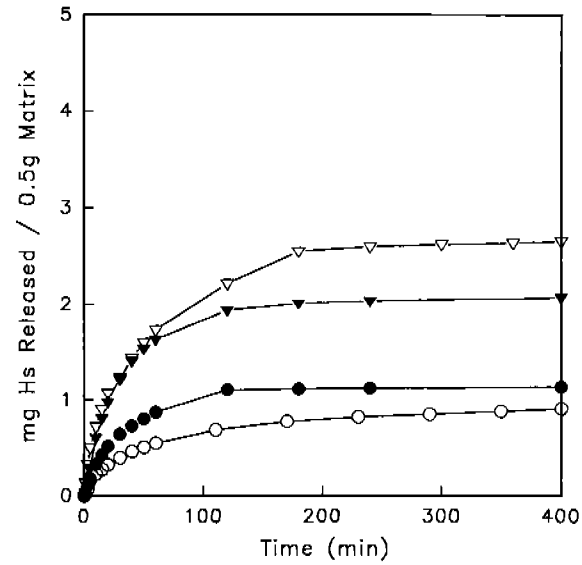
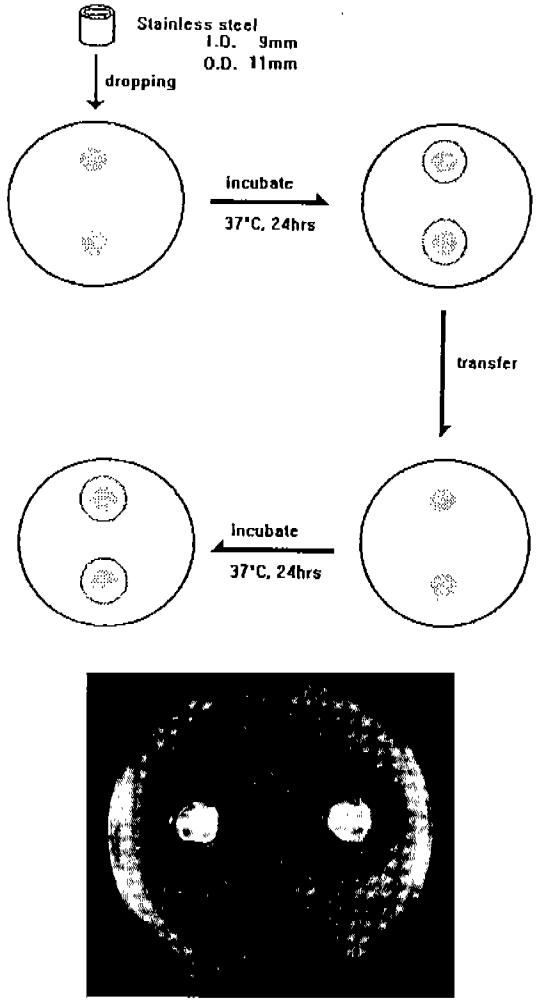



**Fig. 4.** Release of Hs from CX-Cu(II)-Hs prepared by one-bath process at different Cu(II)/Hs mM ratio by flow method (CX-Cu(II)-Hs 0.5 g; flow rate 1 ml/min; pH 5.5; 10°C). The individual runs are (symbol, Cu(II)/Hs mM ratio, incorporated amount (Hs mg/0.5 g matrix)); ●, 4.0/4.0, 6.84; ○, 8.0/4.0, 6.5; ▼, 4.0/8.0, 9.3; ▽, 8.0/8.0, 6.1.

where the amount of binding increased in the beginning, but decreased by rapid dissociation at conditions with high Hs concentration (trianglular vs circular symbols in Fig. 1), which suggested the involvement of low energy bonding in such a situation.




**Scheme 2.** Antimicrobial activity test by ditch plate method.

### Antimicrobial activities of CX-Cu(II)-Hs complex

Antimicrobial activities of CX-Cu(II)-Hs prepared by one-bath process was evaluated employing ditch plate method as shown in Scheme 2. The results are summarized in Table I. As long as the concentration of Hs released from CX-Cu(II)-Hs exceeds to that of minimum inhibitory concentration (MIC), zone of inhibition would be noticed. As expected, diameter of inhibition zone decreased on each successive test period for both free Hs, CX-Hs and CX-Cu(II)-Hs. CX-Cu(II)-Hs exhibited longer duration of activity compared with free Hs or CX-Hs, which suggested the role of Cu(II) in binding of Hs to CX. A series of control experiments were carried out by treating cotton itself with the solution of Cu(II) and Hs by following the same procedure as we employed in the preparation of CX-Cu(II)-Hs in the present experiment. It did not show antimicrobial activity, which proved indirectly that simply adsorbed free drug was almost completely removed by the washing procedures in the present experiments. Even though these data did not represent results on the quantitative basis, general tendency and durability of the antimicrobial activities of the material could be deduced.

## CONCLUSION

Cotton was derivatized to cotton xanthate by treating with carbon disulfide in alkaline media. Homosulfamine was coupled to cotton xanthate *via* Cu(II) ion producing olive-green colored product, CX-Cu(II)-Hs. The overall appearance and the physical strength were maintained during the reaction processes. Binding of homosulfamine was much favored in one-bath process, where cotton xanthate was treated with Cu(II)-Hs solu-

**Table I.** Antimicrobial activity of CX-Cu(II)-Hs complex

| Strains | Sample | Inhibition Zone in Days: Diameter (mm) | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| *B. subtilis* | Free (1.0 mg) | 23 | – | 21 | | | | | | | | | | | | | | | | | | | | | |
| | CX-Hs | 24 | – | 21 | | | | | | | | | | | | | | | | | | | | | |
| | CS-Cu(II)-Hs | 34 | 30 | 26 | 28 | 27 | 26 | 24 | 23 | 19 | 20 | 20 | 20 | 21 | 21 | 20 | 20 | 20 | 19 | 19 | 19 | 19 | 20 | 19 | 20 |
| *S. aureus* | Free (1.0 mg) | 24 | | | | | | | | | | | | | | | | | | | | | | | |
| | CX-Hs | 23 | | | | | | | | | | | | | | | | | | | | | | | |
| | CX-Cu(II)-Hs | 30 | – | 27 | – | 28 | – | 19 | – | 21 | – | 24 | | | | | | | | | | | | | |
| *P. aeruginosa* | Free (1.0 mg) | 28 | | | | | | | | | | | | | | | | | | | | | | | |
| | CX-Hs | 25 | | | | | | | | | | | | | | | | | | | | | | | |
| | CX-Cu(II)-Hs | 27 | – | 24 | – | 19 | – | 18 | – | 15 | – | 21 | – | 23 | – | 21 | – | 20 | | | | | | | |
| *E. coli* | Free (1.0 mg) | 21 | | | | | | | | | | | | | | | | | | | | | | | |
| | CX-Hs | 22 | | | | | | | | | | | | | | | | | | | | | | | |
| | CX-Cu(II)-Hs | 23 | 21 | 17 | | | | | | | | | | | | | | | | | | | | | |

The amount of sample used for the test was equivalent to 1.0 mg of free Hs.

tion in one step. CX-Cu(II)-Hs exhibited prolonged antimicrobial activity compared with free homosulfamine.

## ACKNOWLEDGEMENT

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