

# Studies on Chitin. IX. Crosslinking of Water-Soluble Chitin and Evaluation of the Products as Adsorbents for Cupric Ion

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## Synopsis

Water-soluble chitin was successfully crosslinked to varying extents with glutaraldehyde in homogeneous aqueous solutions to improve the properties as an adsorbent for metal cations, and the effects of crosslinking were discussed. Complete insolubilization was achieved with the fivefold excess aldehyde, but, in terms of adsorptivity of  $\text{Cu}^{2+}$ , the chitin crosslinked at an aldehyde/amino group ratio of 1.0 was found to exhibit remarkable capacity and was much superior to others. The desorption of  $\text{Cu}^{2+}$  from the adsorption complex was also attained effectively at pH 2.0. These results indicated that the loose crosslinking was quite simple and efficient to produce high capacity adsorbents for practical use. Thermal behavior of the cross-linked chitin was examined by TMA and TGA; a softening phenomenon was observed at 145°C.

## INTRODUCTION

Chitin, the most abundant naturally occurring amino polysaccharide, is undoubtedly one of the most promising and attracting resources present in quantity. Utilization study of chitin has therefore been extensively carried out recently. Various possibilities have been examined so far, but further basic studies seem to be necessary to withdraw its full potential.

Among some interesting properties of chitin, chelating ability arising from its characteristic structure is especially noteworthy.<sup>1-3</sup> In a previous paper<sup>4</sup> we reported the adsorption behavior of chitin and its congeners with various degrees of deacetylation. In a series of partially deacetylated chitins with 15–95% deacetylation, a sample with 50% deacetylation prepared under homogeneous conditions (water-soluble chitin) showed maximum adsorption. This high adsorption capacity was ascribable primarily to its remarkable hydrophilicity in cooperation with the relatively high amino group content. Difficulties were, however, sometimes encountered in isolating the adsorption products owing to the water-solubility of the 50% deacetylated chitin. These results indicate the importance of hydrophilicity and suggest that, in order to develop adsorbents of high capacity, it is essential to make chitin derivatives highly hydrophilic and yet insoluble in water. Loose crosslinking of the water-soluble chitin is probably a promising way to achieve insolubilization without sacrificing the high hydrophilicity and most of amino groups. Crosslinking would also be effective to prevent dissolution in acidic media as Masri and coworkers reported for chitosan under heterogeneous conditions.<sup>5</sup>

In this paper, we report the crosslinking reaction of the water-soluble chitin with various amounts of glutaraldehyde under homogeneous conditions and also the induced effects on some properties including the binding of cupric ion.

## EXPERIMENTAL

### Materials

Water-soluble chitin with about 50% deacetylation was prepared under homogeneous conditions as reported previously.<sup>6</sup> The chemicals were reagent grade and used without further purification.

### Measurements

X-ray diffraction diagrams of polymers were obtained with the use of nickel-filtered  $\text{CuK}_\alpha$  radiation. TMA and TGA were carried out on films with a Rigaku 8100 at a heating rate of 5°C/min in air.

### Reaction of Water-Soluble Chitin with Glutaraldehyde

Water-soluble chitin (0.3 g) was dissolved in 30 mL of deionized water at 0°C,<sup>6</sup> and the solution was diluted with water to 0.8%. A given amount of glutaraldehyde in water (50%) was added to the solution with mechanical stirring at room temperature. After 1 h the gelatinous mixture was poured into acetone. The precipitated polymer was collected on a filter, washed thoroughly with acetone, and dried. The crosslinked polymer was finely pulverized (100 mesh), extracted with methanol in a Soxhlet apparatus for 14 h, and dried *in vacuo*.

### Solubility Test

Powdered crosslinked chitin samples were treated with a 100-fold amount of water at 0°C in the same way as in dissolving water-soluble chitin.<sup>6</sup> The insoluble material was filtered with a glass filter, washed with water, dried *in vacuo*, and weighed.

### Collection of $\text{Cu}^{2+}$

To 25 mL of an aqueous solution of  $\text{CuCl}_2$  ( $5.0 \times 10^{-4}$  mol/L) was added 50 mg of a crosslinked chitin sample. After stirring for 72 h at room temperature, the mixture was centrifuged and filtered with a glass filter. The  $\text{Cu}^{2+}$  concentration in the filtrate was determined by the diethyldithiocarbamic acid (DDTC) method as follows. A 1 mL aliquot of the filtrate was acidified with 1N HCl, and 10 mL of 20% citric acid solution and 10 mL of 5% EDTA solution were added. The solution was then basified with aqueous ammonia and left standing for 20 min. It was diluted with water to 50 mL, and 5 mL of 0.2% DDTC solution was added. After vigorous shaking, the DDTC/cupric ion complex was extracted with 10 mL of carbon tetrachloride. The absorbance of the extract was measured at 436 nm, and the  $\text{Cu}^{2+}$  concentration was determined using a calibration curve.

### Desorption of $\text{Cu}^{2+}$

An adsorption complex (6 mg) obtained from  $\text{Cu}^{2+}$  and a crosslinked chitin (aldehyde/amino group ratio, 1.0) was suspended in 5 mL of diluted hydrochloric acid of pH 2.0. After stirring the mixture for a given time, it was filtered with a glass filter. The  $\text{Cu}^{2+}$  content in the filtrate was determined by the DDTTC method described above.

### Preparation of Films

Films were cast from the reaction mixture of crosslinking on a polyethylene film.

## RESULTS AND DISCUSSION

### Crosslinking of Water-Soluble Chitin

The solubility of water-soluble chitin should be suppressed without harming the outstanding adsorption capacity so as to overcome the difficulty in isolating the adsorption products. Loose crosslinking with a dialdehyde was anticipated to be appropriate for this purpose.

The water-soluble chitin was subjected to the reaction with glutaraldehyde in a homogeneous aqueous solution. The amount of aldehyde groups used was varied from 0.3 to 5 mol for 1 mol of the amino group in the water-soluble chitin. The mixture became gelatinous in the course of the reaction. Gelation took place rapidly in 1–2 min when the aldehyde was used in excess. The products were precipitated in acetone and obtained as off-white solids. The results are summarized in Table I.

Water solubility of the resulting crosslinked chitins was determined as a measure of the extent of crosslinking. The derivatives were treated with water at 0°C in the same manner as in dissolving water-soluble chitin. After filtration, the percentage of the dissolved chitin derivatives was calculated, and the values are included in Table I. As shown in the table, the water-soluble part decreased with an increase in the ratio of the aldehyde in the reaction, and the complete insolubilization was attained by crosslinking using fivefold excess glutaraldehyde. For comparison, water-soluble chitin was treated with a monoaldehyde, acetaldehyde, at an aldehyde/amino group ratio of 0.3. The resulting derivative showed much better solubility as expected, and as much as 85% of the sample was still soluble in water, which was in contrast to a small value (9.2%) shown by glutaraldehyde under the same conditions. A slight reduction in solubility of the reaction product with acetaldehyde can simply be explained in terms of a decrease in hydrophilicity caused by the transformation of the hydrophilic amino groups into the less hydrophilic imino linkages, since there seems to be no chance of crosslinking by the monoaldehyde.

The IR spectra of the crosslinked water-soluble chitins were similar to that of the original water-soluble chitin. No distinct difference was observed for all the samples except the one prepared at an aldehyde/amino group ratio of 5.0, whose spectrum showed a small shoulder at  $1710\text{ cm}^{-1}$  ascribable to the presence of unreacted pendant aldehyde groups as illustrated in Figure 1.

TABLE I  
Crosslinking of Water-Soluble Chitin with Glutaraldehyde

Water-soluble chitin (g)	Glutaraldehyde (mol)	Reactant ratio --NH <sub>2</sub> :--CHO	Time for gelation (min)	Yield (g)	Solubility in water (%)
0.30	$1.24 \times 10^{-4}$	1:0.3	15	0.24	9.2
0.15	$1.44 \times 10^{-4}$	1:0.7	10	0.12	5.6
0.15	$2.06 \times 10^{-4}$	1:1	1-2	0.12	4.6
0.30	$6.20 \times 10^{-4}$	1:1.5	1-2	0.31	1.4
0.15	$1.00 \times 10^{-3}$	1:5	1	0.17	0

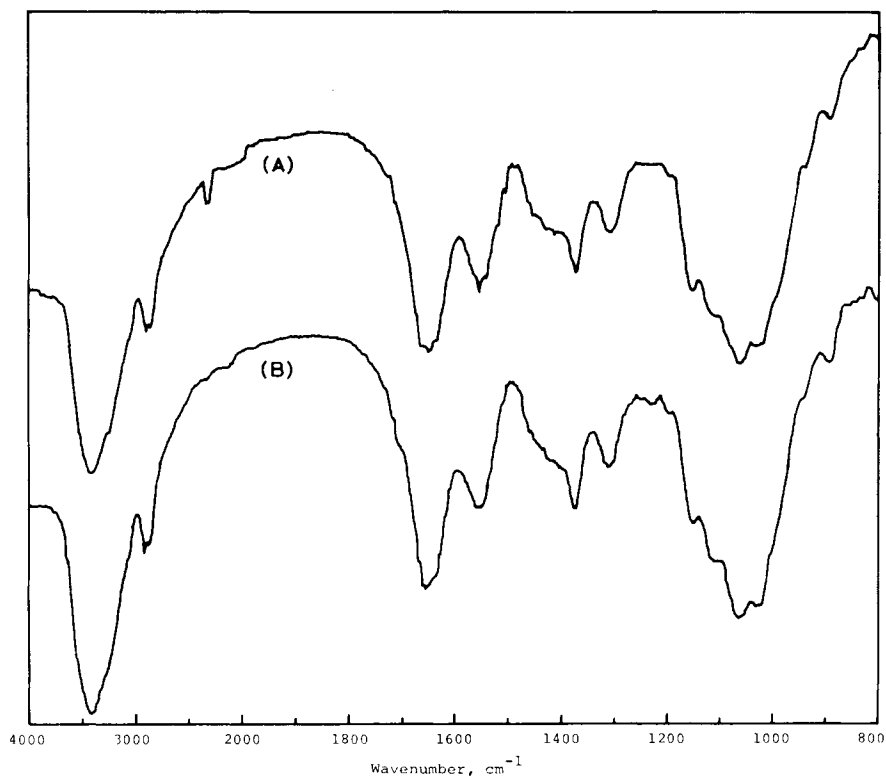


Fig. 1. IR spectra of water-soluble chitins crosslinked with glutaraldehyde at the aldehyde/amino group ratio of: (A) 1.0 (KBr); (B) 5.0 (KBr).

## Properties of Crosslinked Chitins

### *Adsorption and Desorption of Cu<sup>2+</sup>*

An aqueous CuCl<sub>2</sub> solution was treated with finely powdered (100 mesh) water-soluble chitins crosslinked to various extents to examine the effects of crosslinking. Figure 2 shows the relation between the aldehyde/amino group ratio in the crosslinking reaction and the percentage of Cu<sup>2+</sup> collected. The collection percentage gradually increased as the aldehyde proportion was increased from 0.3 and reached a maximum at 1.0. It then decreased markedly with an increase in the aldehyde proportion. The relatively low uptake of Cu<sup>2+</sup> by the lightly crosslinked chitins is probably due to the incomplete destruction of close arrangement of the rigid chitin molecules with strong intermolecular hydrogen bonding. The remarkable adsorption achieved by the sample at the ratio of 1.0 may be attributable to its high hydrophilicity and the easy accessibility of chelating groups, primarily amino groups, which were brought about by the moderate extent of crosslinking leading to loose arrangement of the polysaccharide molecules. The reduction in adsorption by highly crosslinked chitins appears to arise from decreases in the amino group content by the Schiff base formation and in the swelling property by the increased crosslinking and hydrophobicity. The swelling behavior strongly depended on the extent of crosslink-

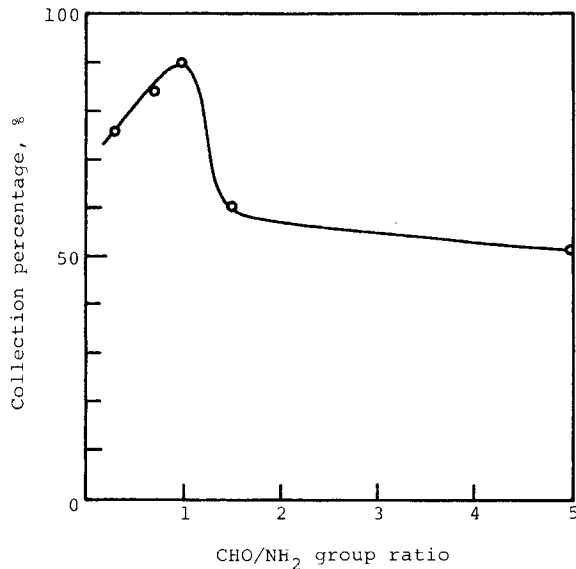


Fig. 2. Adsorption of  $\text{Cu}^{2+}$  by crosslinked water-soluble chitins.

ing, and the chitins prepared at the ratio above 1.5 showed rather poor swelling ability.

When the pH value of the cupric chloride solution was lowered, no adsorption was observed below 2. This suggests that desorption of the adsorbed  $\text{Cu}^{2+}$  may be effected at pH 2.0, regenerating the adsorbents. Prior to the desorption study, it was necessary to examine the stability of the crosslinked chitin under acidic conditions. The crosslinked chitin (aldehyde/amino group ratio, 1.0), on treatment with a solution of pH 2.0 for 24 h, showed no apparent change, and its almost insoluble nature in water was confirmed to be retained, indicating the considerable stability in acidic media. Thus desorption of  $\text{Cu}^{2+}$  from the adsorption complex from the crosslinked chitin (aldehyde/amino group ratio, 1.0) was examined at pH 2.0. As shown in Figure 3, the  $\text{Cu}^{2+}$  was found to be recovered rapidly and effectively. The recovery percentage was as much as 80% in 1 h and 100% in 24 h.

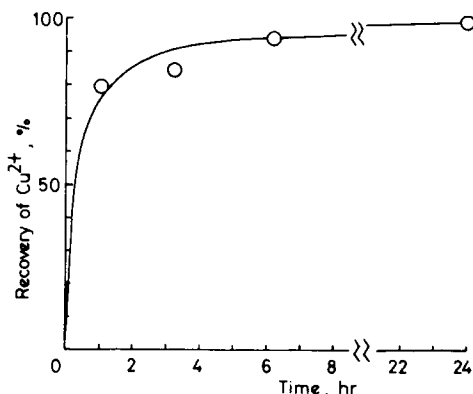


Fig. 3. Desorption of  $\text{Cu}^{2+}$  from the adsorption complex at pH 2.0.

Consequently, the results from these adsorption and desorption experiments revealed that the loose crosslinking of water-soluble chitin with glutaraldehyde is quite efficient to produce one of the most powerful adsorbents for practical use from the standpoints of high collection capacity, effective desorption, and durability in repeated use.

#### *Thermal Behavior*

The effects of crosslinking were then examined in terms of thermal behavior using the crosslinked chitin prepared at an aldehyde/amino group ratio of 1.0 as the most typical example. Films of the crosslinked chitin were subjected to thermomechanical analysis (TMA) by the tensile loading method along with those of the uncrosslinked, original water-soluble chitin. Both kinds of films showed shrinkage on heating until about 120°C as shown in Figure 4. According to thermogravimetric analysis (TGA), these sample showed a weight reduction in the same temperature range, indicating the shrinkage in TMA to be on account of desorption of water from the films. The crosslinked film, however, showed expansion in the subsequent temperature range, 145–220°C, as evident by the TMA curve. It should be noted that the partially crosslinked water-soluble chitin exhibited a softening phenomenon at 145°C, in spite of the fact that all of chitin, partially de-

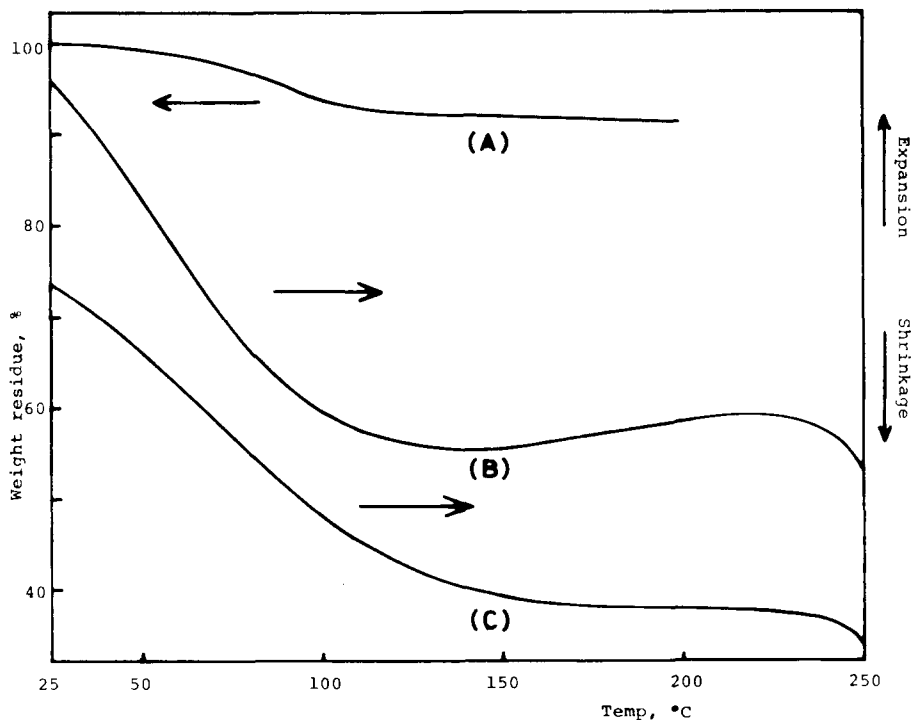


Fig. 4. Thermal analysis of water-soluble chitin and the crosslinked one (aldehyde/amino group ratio, 1.0) at a heating rate of 5°C/min in air: (A) TGA curve for the crosslinked water-soluble chitin; (B) TMA curve for the crosslinked water-soluble chitin; (C) TMA curve for water-soluble chitin.

acetylated chitin, and chitosan show no such a phenomenon. This peculiar thermal behavior is considered to be interpreted by the interference of arrangement and thus intermolecular hydrogen bonding of the polysaccharide molecules by loose crosslinking.

### *Crystallinity*

The crosslinked water-soluble chitins were amorphous similar to the original water-soluble chitin, judging from the X-ray diffraction diagrams where no crystalline peaks were observed even after annealing of the samples. This amorphous nature appears to ensure the high adsorption ability.

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