

## Carboxymethylchitosan의 특성화 및 금속 결합 성질

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(1986년 6월 24일 접수)

## Characterization and Metal Ion Binding Properties of Carboxymethylchitosan

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(Received June 24, 1986)

**Abstract:** Carboxymethylchitosan (CMChitosan) was prepared by reacting "alkali chitin" with monochloroacetate in 18 w/v% NaOH at 30°C for 5 hours. The resulting polymer was characterized by IR spectroscopy, acid-base titration and viscometry. The free amine content of the CMChitosan was 1.39 meq/g, and the pKa of the ammonium group in the polymer was 7.0. The amount of carboxylic group in the polymer was 1.42 meq/g. The CMChitosan was soluble in water regardless of pH, and various transition metal ions bind to the polymer even at low pH. In CMChitosan-Cu<sup>2+</sup> solution, Cu<sup>2+</sup>-COO<sup>-</sup> chelate formed in acidic solution, whereas Cu<sup>2+</sup>-NH<sub>2</sub> chelate was produced at high pH. The cooperativity of Cu<sup>2+</sup> binding observed in chitosan solution was not revealed in CMChitosan solutions.

### INTRODUCTION

Chitin and its N-deacetylated product (chitosan) have drawn considerable amount of interests because of their wide range of applicabilities.<sup>1</sup> Among these, the metal ion binding properties were most extensively studied due to the promises using them in inorganic chromatography and in heavy metal removal from polluted water.<sup>1-5</sup> The binding site of chitin and chitosan for metal ions is generally believed to be the free amine groups. However, the binding ability of the polymers for various metal ions is not *directly* proportional to the degree of the free amine content of the polymers.<sup>4</sup> Rather, the binding was observed to be closely related to the conformation of the polymer, and inter-and in-

tramolecular cooperative chelation depending on the pH, ionic strength and the metal ion to chitosan ratio was revealed for binding of Cu<sup>2+</sup> and Ni<sup>2+</sup>.<sup>5</sup>

To enhance and extend the applicabilities of chitin and chitosan, various chemical modifications of chitin were attempted. These include O-carboxymethylation of chitin and chitosan<sup>6,7</sup>, O-acylation,<sup>8</sup> N-acylation<sup>9,10</sup> and N-carboxymethylidation<sup>11</sup> of chitosan, N-sulfation of chitosan<sup>12</sup>, and cross-linking of chitin and chitosan.<sup>13</sup> Anionic carboxylate group is also a good ligand for many metal ions. The incorporation of the group onto chitosan is expected to result in large alternation of metal ion binding properties as well as polyelectrolytic behaviors of the polyion. In addition, the resulting polymer would behave as a polyampholyte, of which proper-

ties depend strongly on pH and ionic strength. The polyampholyte is of interest as a physicochemical bridge between synthetic polyelectrolyte and many ionic biopolymers such as proteins. Also, the polyampholyte can be utilized in studies for many polyelectrolyte catalyzed reactions of biomimetics.<sup>14</sup> For these purposes, the detailed characteristics of the polymer are required.

In this report, we present the characteristics and metal ion binding properties of O-carboxymethylchitosan (CMChitosan) prepared by reacting "alkali chitin" with monochloroacetate.

## EXPERIMENTAL

**Reagents** Chitin and chitosan were obtained from Tokyo Kasei. Chitosan was purified as described, and the degree of N-deacetylation of the sample was 0.64(4.0 meq/g).<sup>15</sup> Monochloroacetic acid was from Junsei, and other chemicals were commercially available reagent grade. Demineralized distilled water was used.

**Preparation of O-Carboxymethylchitosan** Carboxymethylation of chitin was performed by the method of Okimasu<sup>6</sup> with some modification of work-up procedures. Chitin powder soaked in 42w/v% NaOH was reacted with sodium monochloroacetate (obtained by neutralization of the acid with NaOH) at 30°C for 5 hours. The final concentration of NaOH in the reaction mixture was 18 w/v%. After filtering off the unreacted suspension of chitin through glass wool, the pH of the filtrate was adjusted to 7 with HCl. The solution was dialyzed against running water for three days and followed by precipitation by the addition of acetone. The precipitate was dialyzed exhaustively against distilled water and finally freeze-dried to obtain white-pulpy solid (overall yield was ca 50%).

**IR Spectra of Carboxymethylchitosan Films** 100 mg of CMChitosan was dissolved in 3 ml of 0.1 N HCl or 0.1 N NaOH solution of solvent composition of 20% ethanol-80% water. The CMChitosan films were prepared on glass plates by pouring and drying the pastes. IR spectra of the films were taken from a Perkin-Elmer 683 IR spectrophotometer.

### Determination of Carboxylate and Amine Groups

30 mg of CMChitosan was dissolved in 1.5 ml of 1 N NaOH and precipitated by addition of 37 ml ethanol. The precipitate was washed with 18 ml ethanol by suspension and centrifugation eight times till Na<sup>+</sup> is free in ethanol. The final CMChitosan pellet was redissolved in aqueous 0.05 N HCl and the concentration of sodium ion was determined from a Perkin-Elmer 2380 AA spectrophotometer. The concentration of carboxylate groups was assumed to be the same as that of sodium ion. The free amine content of CMChitosan was determined by potentiometric titration of acidic CMChitosan solution with NaOH solution under nitrogen atmosphere as described previously.<sup>15</sup>

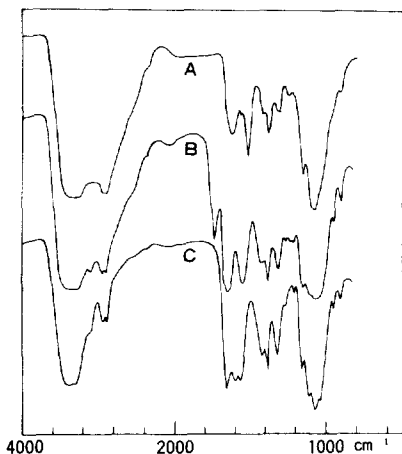
### Metal Ion Binding on CMChitosan by Equilibrium

**Dialysis** 30 mg of CMChitosan was dialyzed against 200 ml of  $5 \times 10^{-4}$  M metal ion solutions for 1 day at room temperature with continuous stirring. The pH's of solutions were adjusted with 0.01 M cacodylate buffer to the desired values and the ionic strength of the solutions was maintained at 0.1 M with NaCl. The concentrations of metal ions inside and outside the cellulose dialysis bag were determined by using a Perkin-Elmer 2380 AA spectrophotometer.

**Other Measurements** Difference UV spectra of CMChitosan-Cu<sup>2+</sup> solutions were taken from a Beckman 8B UV-Vis spectrophotometer using mixing tandem double cells of pathlength of 0.882 cm. Viscosity of solutions was measured by using an Ubbelohde viscometer at 25°C.

## RESULTS AND DISCUSSION

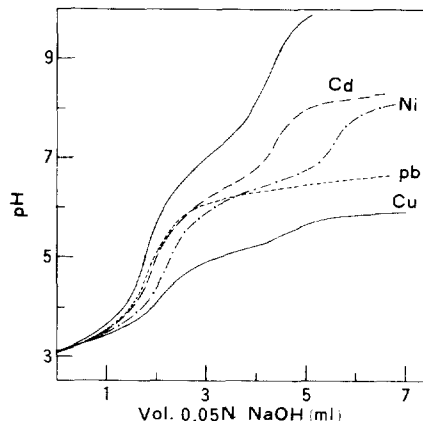
The carboxymethylation of chitin by treating "alkali chitin" with monochloroacetate accompanies N-deacetylation of acetamide groups.<sup>6</sup> Fig. 1 shows IR spectra of the CMChitosan films and they were compared to that of chitosan film. The band at 1,740 cm<sup>-1</sup> in the spectrum of CMChitosan film prepared from an acidic solution belongs to carboxylic acid group (COOH). This band is substituted by 1,600 cm<sup>-1</sup> band, which is assigned to the band of carboxylate anion (COO<sup>-</sup>), in the spectrum of



**Fig. 1.** IR spectra of chitosan film obtained from pH 2.1 solution (A), CMChitosan film from pH 2.1 (B) and CMChitosan film from pH 8.9 (C).

CMChitosan film obtained from a basic solution. These assignments match well with the absence of the bands in spectrum of chitosan. The absorption frequencies of COOH and COO<sup>-</sup> groups in CMChitosan (O-carboxymethylated) are 10-20 cm<sup>-1</sup> higher than the corresponding frequencies in the spectrum of N-(carboxymethylidene)chitosan.<sup>12</sup> The bands at 1,660 and 1,560 cm<sup>-1</sup> in the spectra of CMChitosan films do not change with pH, and are assigned to those of the remaining N-acetamido group (NHCOCH<sub>3</sub>). The amide bands, amide I(CO stretching, 1,660 cm<sup>-1</sup>) and amide II(N-H deformation, 1,560 cm<sup>-1</sup>), appear at the same frequencies in the spectrum of chitin.<sup>16</sup> In the spectrum of chitosan film, the 1,620 and 1,510 cm<sup>-1</sup> bands (-NH<sub>3</sub><sup>+</sup>) predominate over 1,660 and 1,560 cm<sup>-1</sup> bands (-NHCOCH<sub>3</sub>) reflecting the N-deacetylation in chitosan. These -NH<sub>3</sub><sup>+</sup> bands are less evident in the spectra of CMChitosan, despite of considerable extent on N-deacetylation in the polymer. However, this is in line with the absence of the -NH<sub>3</sub><sup>+</sup> absorption bands in the spectrum of carboxymethylcellulose-chitosan blend.<sup>17</sup>

The content of free amine group (by N-deacetylation) and pK<sub>a</sub> of the -NH<sub>3</sub><sup>+</sup> group were determined by potentiometric titration of acidic CMChitosan solution with NaOH. Fig. 2 shows the titration curves. The pK<sub>a</sub> of glycolic acid (HOCH<sub>2</sub>COOH) is



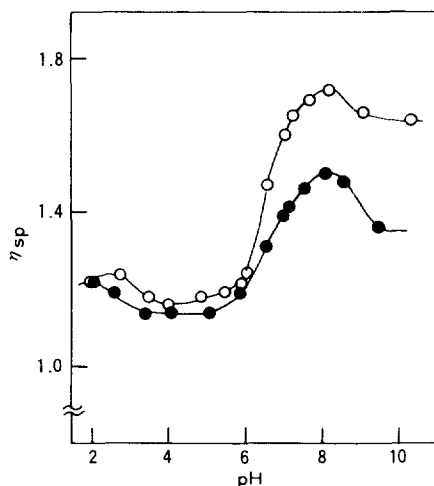
**Fig. 2.** Titration curves of 100 mg CMChitosan in 20 ml of 0.025 N HCl solutions. Top curve is for the solution without other added salt, and used for determination of content of amine group and pK<sub>a</sub> of the group in CMChitosan. The other curves were taken in the presence of 0.01 M divalent metal ions.

3.83, and it is expected that the pK<sub>a</sub> of COOH group in CMChitosan is much lower than this value due to intramolecular electrostatic attraction between -COO<sup>-</sup> and -NH<sub>3</sub><sup>+</sup> groups. Thus, the group titrated between two inflection points of the titration curve, pH 5.1 and 8.9, is soundly assumed to be -NH<sub>3</sub><sup>+</sup>. The amount titrated in this pH range, and thus the amine content in CMChitosan, was estimated to be 1.39 meq per gram of the dried polymer. The degree of neutralization,  $\alpha$ , were calculated at various pH's and pH vs log  $\alpha/(1-\alpha)$  plot gave a straight line (the plot is not shown in this article) as in case of chitosan.<sup>15</sup> This supports further that only -NH<sub>3</sub><sup>+</sup> group is titrated in the pH range of 5.1-8.9. The pK<sub>a</sub> of the group is the intercept of the plot and the value was 7.0. This value is in between the pK<sub>a</sub> values of the same group in chitosan (6.2) and in D-glucosamine (7.8).<sup>15</sup>

The sodium ion content of basic CMChitosan determined by AA spectrometry was 1.42 meq/g. This value can be assumed to be the content of COO<sup>-</sup> group, which is the counter ion of Na<sup>+</sup>, in the polymer. These analytical results suggest that the net charge of CMChitosan is negative and the charge per polyion increases as -NH<sub>3</sub><sup>+</sup> group is titrated, provided that the carboxylic groups are

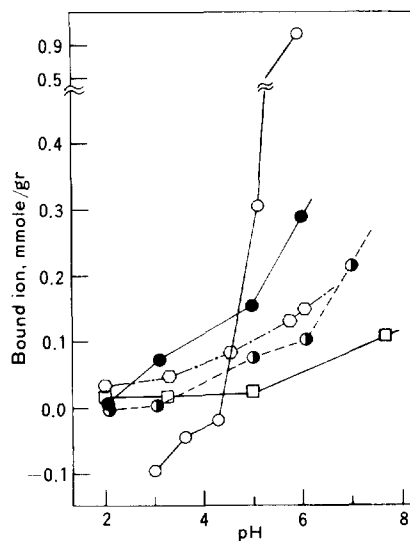
completely dissociated i.e., at  $\text{pH} > 4$ . The CM-Chitosan sample prepared in this investigation was soluble in water over wide pH range studied, pH 3-11. Fig. 3 shows the variation of specific viscosity ( $\eta_{sp} = \eta_{\text{soln}}/\eta_{\text{soln}}^{-1}$ ) of the solution with pH. The sharp increase in  $\eta_{sp}$  with pH above pH 6 reflects the expansion of the polymer chain due to the deprotonation of  $-\text{NH}_3^+$  group which results in increased negative charge density of the polymer. This pH dependence matches well with the results of titration shown in Fig. 2. Also, the virtual invariance of viscosity in pH 4-5 supports our assumption that neither  $\text{COO}^-$  nor  $\text{NH}_3^+$  group in the CMChitosan is titrated in the pH range. Addition of NaCl leads to large decrease in viscosity of the solution, especially above pH 6. This is a typical salt effect on the viscosity of polyelectrolyte solutions. And the effect is more pronounced as the charge density of the polymer increases.

The amounts of bound metal ions to one gram of CMChitosan were calculated from the analytical data of metal ion concentrations inside and outside cellulose bags after equilibrium dialysis at ionic strength of 0.1 M: bound ions per gram of



**Fig. 3.** Specific viscosity of 7 mg/ml CMChitosan solutions as function of pH. The desired solutions were prepared by mixing of the CMChitosan solutions in 0.025 N HCl and 0.025 N NaOH. (○) are data taken in the absence of added salt, (●) are data in the presence of 0.5 M NaCl.

$\text{CMChitosan} = (C_{\text{in}} - C_{\text{out}}) \times \text{Vol. of inside} / \text{weight of CMChitosan used}$ . The results are shown as functions of pH in Fig. 4. In all cases, the amounts of bound metal ions to CMChitosan steadily increase with pH, and the decreasing order of binding affinity was  $\text{Cu}^{2+} > \text{Pb}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+}$ . In contrast to binding to CMChitosan, binding of  $\text{Cu}^{2+}$  to chitosan increases sharply near pH 4.5. This result is in good agreement with our previous report that the metal ion binds to the polymer cooperatively in solution near pH 5.<sup>5</sup> Below pH 4.5, the concentration of  $\text{Cu}^{2+}$  inside the dialysis bag containing chitosan was rather less than the value outside the bag. This might be due to Donnan effect of the positively charged chitosan. Binding of metal ions to CMChitosan even in acidic solutions was also noticed in titration curves of polymer obtained in the presence of the metal ions (Fig. 2). The shift of the titration curves to lower pH values by the presence of metal ions is an indication of binding of the ions to CMChitosan which results in release of proton



**Fig. 4.** The variation of bound metal ions to CMChitosan vs pH. 30 mg of CMChitosan was dialyzed against 200 ml of  $5 \times 10^{-4}$  M metal ion solutions at ionic strength of 0.1 M. Volume inside the dialysis bag was ca. 10 ml: (●) for Cu, (○) for Pb, (◐) for Ni and (◻) for Cd. (○) are data for binding of Cu to Chitosan.

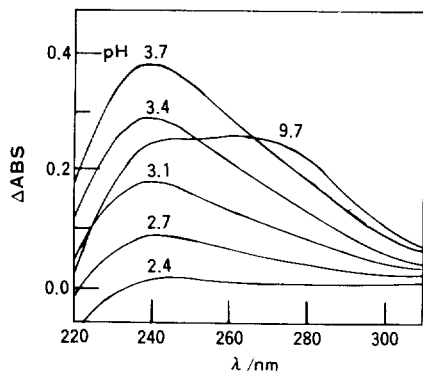


Fig. 5. Difference UV absorption spectra of 4 mg/ml CMChitosan- $5 \times 10^{-4}$  M  $\text{Cu}^{2+}$  solutions at pH values shown. Light pathlength of the cell is 0.882 cm.

due to competition of the metal ions with  $\text{H}^+$  for common binding sites,  $\text{NH}_2$ , or due to electrostatic interaction between the bound cations and  $-\text{NH}_3^+$  groups. The effect of metal ions to the titration curves is closely parallel with binding affinity of the ions to CMChitosan shown in equilibrium dialysis studies.

Fig. 5 shows UV difference spectra of  $\text{Cu}^{2+}$ -CMChitosan at various pH. In acidic pH, the spectra display an absorption peak at 238 nm. This is the typical carboxylate to copper charge transfer band<sup>18</sup> and indicates that  $\text{Cu}^{2+}$  ions bind mainly to  $\text{COO}^-$  and the binding increases with pH. At high pH, for example pH 9.7, the spectrum shows another band near 270 nm, and this can be attributed to the involvement of  $\text{NH}_2$  group to the copper complexes.<sup>5</sup> This spectral change with pH confirms that both  $\text{COO}^-$  and  $\text{NH}_2$  group in CMChitosan serve as binding sites to  $\text{Cu}^{2+}$ , depending on pH of the solution.

**Acknowledgement** The work was supported by a grant from Asan Foundation, 1984.

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