JOURNAL OF PHYSICAL ORGANIC CHEMISTRY
J. Phys. Org. Chem. 2002; **15:** 852–857
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/poc.559

Synthesis and coordinating ability of chitosan dithiocarbamate and analogs towards Cu(II) ions†

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Received 18 April 2002; revised 10 July 2002; accepted 15 July 2002

ABSTRACT: Potassium chitosan dithiocarbamate (ChitDTC) was synthesized and characterized by elemental analysis and solid-state ^{13}C NMR spectroscopy. The degree of substitution (DS) (number of substituents per 100 2-amino-2-deoxyglucopyranoside units) and molalities of the groups were calculated for acetylamino, amino and dithiocarbamate substituents in chitin, chitosan and ChitDTC. A comparative study of the retention and exchange of Cu(II) was performed with chitin, chitosan and ChitDTC at 30 °C, pH 6.0 (0.1 M succinate). The stability constants, K_X, for the heterogenous equilibrium

$$\text{ChitX} + \text{Cu(II)} \overset{K_X}{\rightleftharpoons} \text{ChitXCu(II)}$$

were calculated assuming that complexing of Cu(II) ions was largely in a 1:1 ratio with respect to each group. The values of K_X for each group were NHAc 45.1, DTC 3.14×10^3, and NH_2 1.12×10^4. It is proposed that in the presence of succinate, the main species of aqua Cu(II) ion present in solution must be the uncharged Cu(II)–succinate complex that prevents the acetylamino, amino and DTC groups from forming complexes higher than 1:1. Also, this may explain the fact that the stability constant for DTC was lower than for the amino group. The method allows the characterization of the chelating ability of each group in ChitDTC with respect to metal ions. Copyright © 2002 John Wiley & Sons, Ltd.

KEYWORDS: chitosan dithiocarbamate; Cu(II) complex; stability constants; chitin; chitosan

INTRODUCTION

Chitosan is a linear polymer of β-(1 → 4)-linked 2-amino-2-deoxy-D-glucopyranose residues, which is prepared from chitin by *N*-deacetylation. Partially *N*-acetylated chitosan is commercially available. Chitin and chitin derivatives have been intensively studied and used as antimicrobials, food additives, agricultural and biomedical materials, etc.[1] Chitosan and partially acetylated chitosan are more reactive than chitin because the primary free amino groups are distributed regularly in its chain. Various applications depend on information about the distribution of the substituted amino groups and the pK_a value of the amino group.[2]

Chitin and chitosan show a high ability for chelation with transition metal cations, but they are indifferent to alkali and alkaline earth metal ions.[3] Functionalization of the primary amino group can increase the electron dative power. This is the case with dithiocarbamates that have been used extensively for the detection and quantitative determination of copper, and they form stable complexes with many metal ions that are sparingly soluble in water.[4a,5] Consequently, chitosan dithiocarbamate was expected to have a good chelating ability for transition metal ions.[6] However, establishing the percentages of retention of different metal ions by chitosan dithiocarbamate is insufficient for the characterization of the dithiocarbamate (DTC) group's capacity to form complexes with metal ions, because there is a distribution of substituents (acetylamino, NH_2, NH_3^+, DTC) present in the polysaccharide matrix.

In this work, we developed a method to synthesize chitosan dithiocarbamate (ChitDTC) while avoiding other secondary reactions, and we characterized the independent chelating abilities of the acetylamino, amino and dithiocarbamate groups with respect to Cu(II).

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†Presented in part at the Sixth Latin American Conference on Physical Organic Chemistry (CLAFQO-6), held at Isla Margarita, Venezuela, during December 2001.

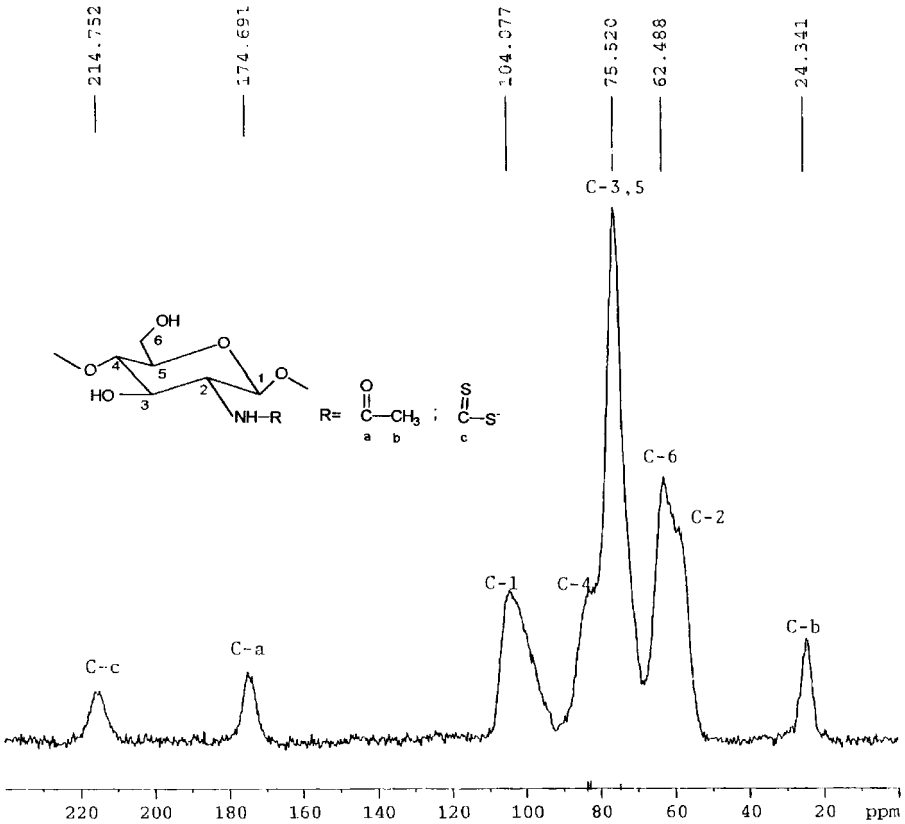


Figure 1. ^{13}C CP/MAS-TOSS NMR spectrum of chitosan dithiocarbamate

EXPERIMENTAL

All chemicals were of analytical grade and were used without further purification. Aqueous solutions were prepared using distilled, deoxygenated water. Chitin, supplied by Aldrich, was from crab shells, and high molecular weight chitosan was also obtained from Aldrich.

Sulfur content was determined using a LECO SC132 analyzer. Solid-state ^{13}C NMR spectra were obtained using cross-polarization (CP), magic angle spinning (MAS) and total sideband supression (TOSS) with a Bruker MSL-300 spectrometer. The metal ion retention–elution experiments were performed by measuring their atomic absorption spectra with a Hitachi Z 8230 spectrometer.

Potassium chitosan dithiocarbamate (ChitDTC)

Chitosan (1 g) was dispersed in 35 ml of 0.2 M $KHCO_3$–K_2CO_3 (pH 10) solution and stirred for 1 h, after which a solution of 0.74 ml of CS_2 in 10 ml of acetone was added and the dispersion was allowed to react for 9 h. The solid was treated with a solution buffered at pH 4 (0.1 M, acetate) and then washed with water, ethanol and diethyl ether. The product was dried under vacuum over P_2O_5.

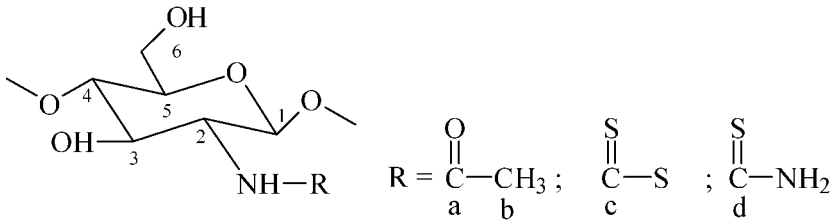
The solid-state ^{13}C NMR spectrum of ChitDTC is presented in Fig. 1 and a comparison of the spectra of chitin, chitosan, and ChitDTC is shown in Table 1.

Several attempts under different conditions were made to obtain ChitDTC using concentrated ammonia as the basic medium, as has been described for the synthesis of dithiocarbamates.[7] However, the ^{13}C NMR spectra showed the formation of the thiourea derivative (182.7 ppm) along with the formation of the dithiocarbamate (214.7 ppm). Muzzarelli *et al.*, using this method, followed the formation of ChiDTC by IR spectroscopy, and the band at 1480 cm^{-1} was assigned to the N—C═S moiety.[6a] The dithiocarbamate group absorbs at of 1481–1493 and 1449 cm^{-1} and the substituted thioureas at 1493–1449 cm^{-1}.[8] Therefore, it is not possible to distinguish between these two groups by IR spectroscopy and the product was probably contaminated by the thiourea derivative.

Degree of substitution (DS)

The DS was defined as the number of substituents per 100 2-amino-2-deoxyglucopyranoside units,[9] and it was calculated from the solid-state ^{13}C NMR spectra of chitosan and ChitDTC. For ChitDTC, the DS was also calculated from sulfur elemental analysis. Both methods gave very similar values.

Table 1. ^{13}C NMR spectral data (δ, ppm) for chitin, chitosan and chitosan dithiocarbamate[a]



	Chitin[b]	Chitosan	ChitDTCNH$_4$	ChitDTCK
C-1	104.3	105.2	103.3	104.1
C-2	55.7	57.8	57.0	57.0
C-3	75.5	76.1	74.9	75.5
C-4	83.7	86.0	~81.0	~81.0
C-5	74.0	75.8	74.9	75.5
C-6	61.4	60.4	61.8	62.5
C-a	174.3	173.9	174.9	174.7
C-b	23.4	24.1	23.8	24.3
C-c[c]			215.6	214.8
C-d[c]			182.7	

[a] Solid state, CP/MAS-TOSS.
[b] Ref. 18.
[c] Ref. 5i.

Metal ion retention and exchange experiments

Preliminary experiments were carried out to determine the time needed to reach the equilibrium concentration of the metal ion in solution in the presence of chitin, chitosan and ChitDTC.

For the metal ion retention experiments, 100 mg of the solid were stirred for 1–2 h at 30 °C with 50 ml of a 1 mM solution of the metal ion buffered at pH 6.0 with 0.1 M succinate. The suspension was centrifuged and samples of the supernatant were taken for atomic absorption determination of Cu(II).

Exchange experiments were performed by washing carefully with water the solid product of the retention run and dispersing it in 25 ml of 3 M KCl solution, buffered with 0.1 M succinate at pH 6.0, and stirring for 1 h at 30 °C. The suspension was centrifuged and the supernatant was analyzed by atomic absorption spectrometry for Cu(II).

RESULTS AND DISCUSSION

Degree of substitution and molalities of groups of chitin derivatives

The DS with respect to acetylamino, amino and dithiocarbamate groups were calculated from the solid-state ^{13}C NMR spectra. The areas of the signals of C═S, C═O and CH_3 at 215, 175 and 24 ppm, respectively, were compared, using as internal standard the area of the signal of C-1 at 104 ppm or, alternatively, the area of the signal that included C-2, -3, -4, -5 and -6 in the range 41–91 ppm divided by 5. The results were averaged and are shown in Table 2.

Chitosan presented a degree of acetylation of $DS_{NHAc} = 28.3$. For ChitDTC, the value $DS_{DTC} = 21.6$, calculated from the NMR spectrum, was very close to that calculated from the S content, $DS_{DTC} = 21.9$. The values of the DS for each group allowed the calculation of the molalities of each group in the samples of chitin, chitosan and ChitDTC.

Retention and exchange of Cu(II)

Preliminary experiments showed that aqueous solutions of Cu(II) ions become acidic, and the solid-state ^{13}C NMR spectra of the ChitDTC samples, after chelation, showed no sign of the DTC group at 215 ppm. Also, the chelation capacities of chitosan and ChitDTC under these conditions were very similar. It has been reported that the capacity of Cu(II) retention by ChitDTC is a maximum at pH 6.8 and decreases at pH 3.0 and 12.0.[6a] It is well known that dithiocarbamates decompose under acidic conditions.[4] For instance, the acid decomposition of 2-hydroxyethyldithiocarbamate, an analog of chitosan dithiocarbamate, occurs at pH 4 with a half-life of 39 min at 25 °C.[4b] For this reason, in order to avoid the decomposition of chitosan dithiocarbamate, the solutions of the metal ion were buffered at pH 6.0 with succinate. The concentration of Cu(II) was kept lower than the solubility product of $Cu(OH)_2$.

The retention and exchange abilities of Cu(II) of chitin,

Table 2. Degree of substitution and molalities of groups in chitin derivatives[a]

		Chitin (203.09)[b]		Chitosan (172.95)[b]		ChitDTCK (197.60)[b]	
Substituent	MW, ring	GS	m^0_{NHAc}	GS	m^0	GS	m^0
$NHCOCH_3$	203.09	100	4.92	28.3 ± 3.0	1.63	28.3 ± 3.0	1.43
NH_2	161.07			71.8 ± 0.9	4.15	50.2 ± 0.6 (49.8)	2.54 (2.52)
$NHCS_2K$	275.32					21.6 ± 0.3 (21.9)	1.09 (1.11)

[a] Calculated from solid state ^{13}C NMR spectrum. Values in parentheses were calculated from 7.09% S content.
[b] Average ring molecular weight of one ring.

Table 3. Retention of Cu^{2+} by chitin derivatives[a]

	Cu^{2+} in solution, $(m_{Cu})^{sln}$	Cu^{2+} in solid, $10^2(m_{Cu})^{sod}$
Chitin	6.88×10^{-4}[b]	14.8
Chitosan	5.70×10^{-5}[b]	46.3
ChitDTCK	8.81×10^{-6}[c]	9.47

[a] Solution of $CuSO_4$ at pH 6.0 (10 mM, succinate) in equilibrium with 100 mg of solid.
[b] 50 ml, initial concentration 9.83×10^{-4} m.
[c] 10 ml, initial concentration 9.56×10^{-4} m.

chitosan and chitosan dithiocarbamate was compared. The results are shown in Tables 3 and 4.

Stability constants

The stability constants assume that the metal ion forms complexes with one substituent of the glucosamine ring of the polyssacharide matrix (1:1 ratio), without a contribution of intermolecular complexation:

$$\text{ChitX} + \text{Cu(II)} \overset{K_X}{\rightleftharpoons} \text{ChitXCu(II)} \qquad (1)$$

The stability constant K_x of a group is given from equilibrium (1) by the following equation:

$$K_X = \frac{(m_{Cu})^{sld}}{[m^0_X - (m_{Cu})^{sld}](m_{Cu})^{sln}} \qquad (2)$$

where m^0_X is the initial molality of the group in the solid, and $(m_{Cu})^{sld}$ and $(m_{Cu})^{sln}$ are the molalities of Cu(II) ions in the solid and the solution, respectively, at equilibrium. When the mass w of the solid is suspended in a volume v of the Cu ion solution, at equilibrium, the number of gram-atoms of metal ion transferred to the solid is given by the equation

$$x = [(m_{Cu})^{sln}_0 - (m_{Cu})^{sln}]\frac{v}{1000} = (m_{Cu})^{sld}_X \frac{w}{1000} \qquad (3)$$

According to the NMR spectrum, we can assume that there are only acetylamino groups in chitin and, therefore, from Table 3, $K_X = K_{NHAc} = 45.1$.

The calculation of K_X for chitosan has to take into account the fact that in addition to the amino groups, there are also acetylamino groups present in the solid and, consequently, from the volume v of the solution there are x gram-atoms of Cu(II) chelated with the NHAc groups and y gram-atoms chelated with the amino groups. The value of x can be calculated from K_{NHAc} and then y is obtained from the following equation, allowing the calculation of K_{NH_2} from Eqn. (2):

$$y = [(m_{Cu})^{sln}_0 - (m_{Cu})^{sln}]\frac{v}{1000} - x \qquad (4)$$

In the case of ChitDTC, the metal ion can complex with three different groups, and correction for the complexation with NHAc and NH_2 is necessary. Calculation of x and y from the values of K_{NHAc} and K_{NH_2} allowed the calculation of z, the number of gram-atoms of Cu(II) chelated with the dithiocarbamate group:

$$z = [(m_{Cu})^{sln}_0 - (m_{Cu})^{sln}]\frac{v}{1000} - x - y \qquad (5)$$

The values of the stability constants of the acetylamino, amino, and dithiocarbamate groups shown in Table 5 were obtained from Tables 2 and 3. They follow the order NH_2 >DTC >NHAc. Accordingly, the exchange experiments showed that the concentration of Cu(II) released from the solids followed the inverse order (Table 4).

Despite the fact that copper is one of the most abundant transition elements in biological systems, the behavior of the aqueous form of the Cu(II) ion remains poorly understood.[10] The first solvation shell yields an octahe-

Table 4. Exchange of ChitXCu at 30 °C[a]

	Cu^{2+} in solution, $10^5(m_{Cu})^{sln}$
Chitin	15.3
Chitosan	1.26
ChitDTCK	2.36

[a] 25 ml of 3 M KCl at pH 6.0 (0.1 M succinate) in equilibrium with 100 mg of the solid obtained from the retention experiments.

Table 5. Stability constants for Cu(II) ions with some groups contained in chitin derivatives, at 30 °C[a]

X	K_X
NHAc	45.1
DTCK	3.14×10^3
NH_2	1.12×10^4

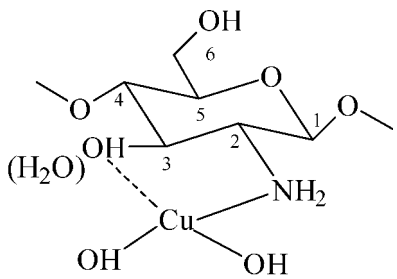
[a] At pH 6.0, 0.1 M succinate; free amino base corrected for $pK_a = 6.4$.

dral $[Cu(H_2O)_6]^{2+}$ complex[11] that exchanges with water at a high rate. Experimental and theoretical results[10] suggest that the solvated complex undergoes frequent transformations between square-pyramidal and trigonal pyramidal configurations where five-fold coordination is preferred.

Addition of a ligand such as NH_3 displaces successive water molecules:[12]

$$[Cu(H_2O)_6]^{2+} + NH_3 \overset{-H_2O}{\rightleftharpoons} [CuNH_3(H_2O)_5]^{2+} \overset{-H_2O}{\rightleftharpoons} [Cu(NH_3)_2(H_2O)_4]^{2+} \quad (6)$$

Electron-withdrawing substituents such as the acetyl group decrease the donor power of the amino group and consequently the stability constant of the acetylamino group is low. In the case of chitosan, the dependence of the Cu(II)–chitosan complex on the pH suggested that at pH 6.0 the composition was a 1:1 Cu(II):glucosamine unit.[13] This model is in agreement with that obtained in the solid state[14] and with potentiometric and circular dichroism measurements, which are consistent with the formation of only one uncharged complex with a single Cu per NH_2, $[CuNH_2(OH)_2]^0$,[2] where the fourth site could be occupied by a water molecule or a neighboring OH on C-3, as shown in I.



I

The reaction of Cu(II) with succinate:

$$[Cu(H_2O)_6]^{2+} + succ^{2-} \rightleftharpoons [Cu(succ)(H_2O)_4] \quad (7)$$

produces a neutral complex, $[Cu(succ)(H_2O)_4]^0$ ($-\Delta G° = 3.6$–3.7 kcal mol^{-1}) (1 kcal = 4.184 kJ),[15,16] the structure of which has been proposed to be square-pyramidal.[16] Therefore, at pH 6.0 (0.1 M succinate), the main species of Cu(II) present in solution must be the Cu(II)–succinate complex, which prevents the amino group of chitosan from forming complexes higher than 1:1. The stability constant for 1:1 Cu(II):glucosamine at 30 °C (in the absence of succinate) is 1.09×10^5 M^{-1},[15] i.e. about 10-fold higher than that for the Cu(II):glucosamine unit in chitosan.

A 1:1 ratio can also be expected for the bidentate dithiocarbamate group of ChitDTC. For Cu(II) aqua ion and dimethyldithiocarbamate, the existence of both 1:1 and 1:2 ratios was demonstrated by spectrophotometry.[17] There is very little interaction between the dithiocarbamate moiety and the Cu(II) aqua ion in the complexes.[5c,k] Therefore, the interaction with the uncharged Cu(II)–succinate complex is bound to increase the $\Delta G°$ of the reaction

$$[Cu(succ)(H_2O)_4] + ChitNHCS_2^- \rightleftharpoons [Cu(succ)(ChitNHCS_2)(H_2O)_2]^- \quad (8)$$

because of the decrease in electrostatic attraction compared with the charged aqua ion, promoting a 1:1 complex. This may also explain the fact that the stability constant for dithiocarbamate was lower than for the amino group.

CONCLUSIONS

Potassium chitosan dithiocarbamate (ChitDTC) was synthesized and the degree of substitution (DS) (number of substituents per 100 2-amino-2-deoxyglucopyranoside units) and molalities of the groups were calculated for acetylamino, amino and dithiocarbamate substituents in chitin, chitosan and ChitDTC.

The stability constants, K_X, at 30 °C, pH 6.0 (0.1 M succinate) for the reaction of acetylamino, amino and DTC groups with Cu(II) were NHAc 45.1, DTC 3.14×10^3 and NH_2 1.12×10^4, assuming that complexing of Cu(II) ions is largely in a 1:1 ratio with respect to each group.

It is proposed that in the presence of succinate, the uncharged Cu(II)–succinate complex prevents the acetylamino, amino and DTC groups from forming complexes higher than 1:1, and that this may also explain the fact that the stability constant for DTC was lower than that for the amino group.

Acknowledgements

This work was written in part during the stay of E.H. at the Institute of Fundamental Research in Organic Chemistry at Kyushu University, as Visiting Professor. E.H. thanks Dr Shinjiro Kobayashi for the invitation. We

thank University College London for the solid-state NMR spectra that were obtained through the ULIRS Solid State NMR Service.

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