

# Synthesis and Property Studies of *N*-Carboxymethyl Chitosan

Qingping Song, Ze Zhang, Jiangan Gao, Chunmei Ding

Department of Biochemical Engineering, Anhui University of Technology and Science, Wuhu City, Anhui Province, 241000, People's Republic of China

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**ABSTRACT:** *N*-carboxymethyl chitosans (*N*-CMC) were synthesized from chitosan in water with chloroacetic acid instead of comparatively expensive glyoxylic acid. The optimal reaction conditions were 90°C and 4 h with a ratio of chloroacetic acid to chitosan 5 : 1(w/w). The degree of substitution of product exceeded 1.32. The *N*-carboxymethyl chitosans were characterized by XRD, FTIR, <sup>1</sup>H-NMR, and the water solubility and isoelectric

point of *N*-CMC with different degrees of substitution were determined. FTIR and <sup>1</sup>H-NMR data has confirmed that the substitution reaction occurred on the amino groups. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 119: 3282–3285, 2011

**Key words:** biopolymers; modification; synthesis; water-soluble polymers; FTIR

## INTRODUCTION

Chitin is the second most abundant natural biopolymer and exists largely in the shell of crabs, shrimps, the cuticle of insects, and the cell wall of some fungi and microorganisms. Chitosan (CTS), which can be readily obtained from chitin by deacetylation with alkali, was widely used in waster management, food processing, medicine, and biotechnology due to its low toxicity and biodegradability.<sup>1–4</sup> However, poor solubility in water has prevented it from enjoying more widespread utilization. To overcome this problem, many studies were inducted to obtain water-soluble chitosan derivatives by chemical modification.<sup>5–9</sup> Among the water-soluble derivatives, carboxymethyl chitosan (CMC) is the most important kind of chitosan derivatives due to its better solubility in water with wide pH value.<sup>10–12</sup> These developed CMC derivatives can be divided into *O*-carboxymethyl chitosan, *N,O*-carboxymethyl chitosan, and *N*-carboxymethyl chitosan according to the substitution position of carboxymethyl group.

In recent years, more and more attentions were paid to the synthesis of *N,O*- carboxymethyl chitosan and its application. But the methods for the synthesis of *N*- carboxymethyl chitosan have been scarcely reported. Muzzarelli<sup>13</sup> firstly reported that *N*-CMC can be synthesized by reducing the *in situ* generated

imine from the reaction of the amino group (–NH<sub>2</sub>) in chitosan with the aldehyde group (–CHO) in glyoxylic acid with NaBH<sub>4</sub> or NaBH<sub>3</sub>CN. But in this method, the starting material glyoxylic acid is relatively expensive, and two steps were involved.

In this article, *N*-CMC can be achieved from the reaction of chitosan with chloroacetic acid at neutral aqueous solution by using Na<sub>2</sub>CO<sub>3</sub> as binding acid agent. In neutral condition, the reaction activity of NH<sub>2</sub> is higher than that of OH, therefore, carboxymethylation can only occur in the NH<sub>2</sub> and *N*-CMC can be obtained.

## EXPERIMENTAL

### Chemicals

Chitosan, whose degree of deacetylation (DD) was calculated to be 93% by <sup>1</sup>H-NMR, was supplied by Jinhu biochemistry (Qingdao, Shandong Province, China). All other chemicals were of analytical reagent and directly used without further purification.

### Preparation of *N*-CMC

In a three-necked flask, chloroacetic acid (7.5 g) was dissolved in 30 mL distilled water and the resulted solution was adjusted to pH 7.0 with NaOH (3.17 g) in 70 mL distilled water. Then chitosan (1.5 g) was introduced into the above solution. The reaction mixture was heated to 90°C with vigorous stirring and maintained for 4 h. During the reaction, the pH value of the reactant system was controlled at pH 7.0 by dropping 20% Na<sub>2</sub>CO<sub>3</sub> to absorb HCl generated from the carboxymethylation process with an interval of 30

Correspondence to: C. Ding (dingchunmei@ahpu.edu.cn).

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**TABLE I**  
***N*-Carboxymethyl Chitosan Under Different Conditions**

Entry	Chloroacetic acid : Chitosan (mass ratio)	Temp (°C)	Time (h)	DS
1	4	85	3	0.68
2	4	90	3	1.01
3	4	95	3	1.14
4	3	90	3	0.72
5	3.5	90	3	0.82
6	4.5	90	3	1.11
7	5	90	3	1.19
8	5	90	2.5	0.98
9	5	90	3.5	1.29
10	5	90	4	1.32

minutes. After filtration for removal of the unreacted chitosan, the filtrate was diluted with 95% ethanol (v/v 1 : 3). The precipitate was filtered off, washed for two times again with absolute ethanol, and dried under vacuum to afford the final desired product *N*-CMC. The substitution degree of *N*-CMC was determined by potentiometric titration.<sup>14</sup>

#### Water solubility and isoelectric point

The solubilities of *N*-CMC with different DS were measured in water at room temperature. The sample was slowly added into 10 mL stirring water until the solution was saturated, with the sample usage referring to the corresponding solubility.

Isoelectric point of *N*-CMC was evaluated from the turbidity based on the method reported by Sashiwa.<sup>15</sup> The sample was dissolved in distilled water. The transmittance of the solution was recorded on a 721 spectrophotometer using a quartz cell (1 cm) at 600 nm. The pH dependence of the water solubility of CMC was also determined by measurement of transmittance of the solution (0.2 mg/mL). The *N*-CMC solutions with various pH values were prepared by adjusting with 0.5% aqueous HCl and NaOH.

#### Characterization

Fourier transform infrared (FTIR) spectra were recorded on a EQUINOX55 spectrometer (Bruker) with KBr pellets. XRD of chitosan and *N*-CMC chitosans were measured using an X-ray diffractometer (Model No. D8 Advance; DanDong, China). <sup>1</sup>H-NMR spectrum of *N*-CMC measured in D<sub>2</sub>O was obtained with a Bruker AV-300 spectrometer.

## RESULTS AND DISCUSSION

#### Carboxymethylation of chitosan

The *N*-CMC derivatives could hardly be obtained from the direct alkylation of chitosan with chloroacetic acid.

Currently, sodium hydroxide is the mostly used base to achieve this point. But in these cases, the strongly basic condition would lead to low reaction selectivity. Specifically, the reactivity of OH groups are higher than NH<sub>2</sub>, therefore, the product would be mixture of *N,O*-CMC derivatives with substitution on the OH groups of C<sub>6</sub> and C<sub>3</sub>, and also some substitutions on the NH<sub>2</sub> groups. Chen and Park<sup>10</sup> pointed out that the carboxymethyl groups were mostly introduced mainly onto the OH groups with a small amount onto the NH<sub>2</sub> groups. Here in this work, we have successfully developed an efficient method for selective alkylation of chitosan by strictly controlling the reaction system at pH 7.0 using Na<sub>2</sub>CO<sub>3</sub> at 90°C, the reactant activity of NH<sub>2</sub> group is higher than that of OH, therefore, *N*-CMC can be obtained.

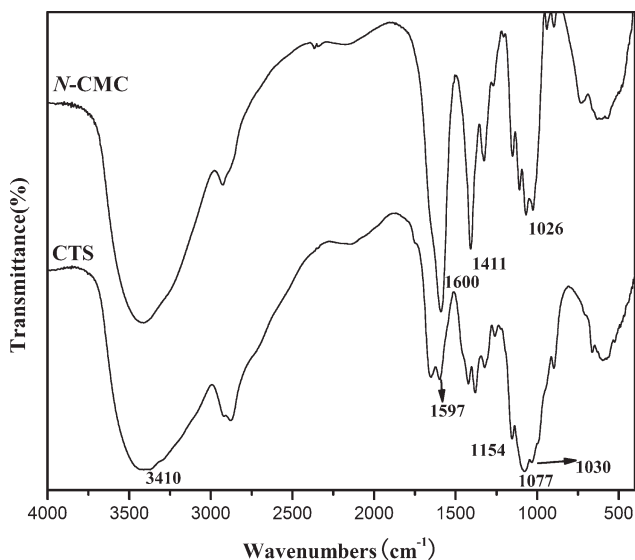
As shown in Table I, the DS of the product increased with the used amount of chloroacetic acid, reaction temperature, and reaction time. This reaction proceeded very slowly below 80°C, and little product was obtained. However, when the temperature reached 95°C, the product turned into yellow. Therefore, the temperature 90°C was the best. It was also observed that the amount of chloroacetic acid played an important role in synthesis of CMC. With the increase of the amount of chloroacetic acid, the DS increased accordingly and then changed little with further increase of the amount of chloroacetic acid more than 5 times (w/w) of CTS. At the same time, the increase of reaction time can lead to higher DS, but when the time exceeded 4 h, the DS increased slowly and product also turned into yellow. Therefore, the optimal reaction conditions were as follows: mass ratio of chloroacetic acid to chitosan 5 : 1; reaction temperature 90°C; reaction time 4 h. The DS value of product can be up to 1.32.

#### Water solubility and isoelectric point

The solubilities of *N*-CMC were measured in diluted water. The results are listed in Table II. It was found that all the products were well soluble in water. Solubilities of the products increased with the DS. When the DS is 1.32, the solubility of product can reach 0.83 g/10 mL. The isoelectric points (pI) with different DS were also shown in Table II. As the DS increased, the isoelectric point decreased close to pH 2.0. The increased DS results in a reduced number

**TABLE II**  
**Effect of *N*-Carboxymethyl Chitosan with Different DS on Solubility and pI**

DS	0.98	1.19	1.29	1.48
pI	2.10	2.00	1.95	1.80
Solubility	0.45	0.63	0.80	0.83

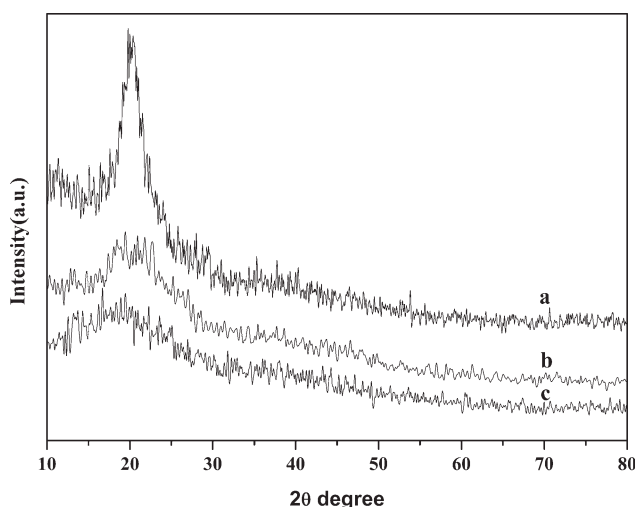


**Figure 1** FTIR spectra of chitosan and carboxymethyl chitosan; the spectra are shifted along the  $y$ -axis.

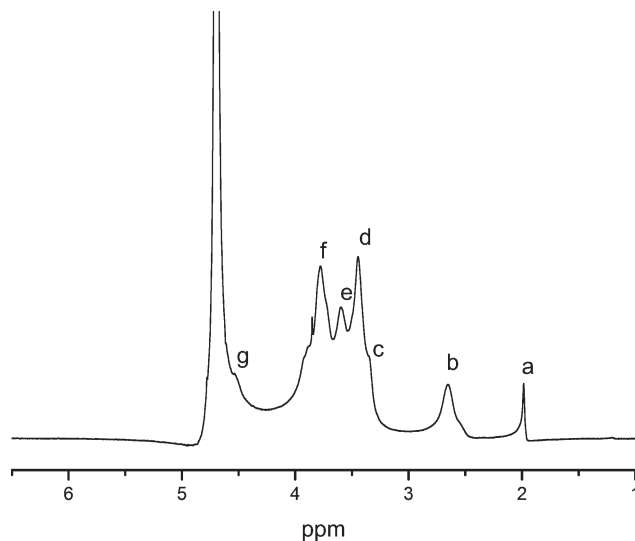
of amino groups and increased number of COOH groups on the chitosan. Therefore, isoelectric point decreased.

### FTIR spectra of CTS and CMC

The chitosan and *N*-CMC were identified by their FTIR spectra. As shown in Figure 1, the IR spectrum of chitosan shows peaks assigned to the saccharide structure at 1154, 1077, 1030  $\text{cm}^{-1}$ , and strong amino and OH characteristic band at around 3410  $\text{cm}^{-1}$ . The peak at 1597  $\text{cm}^{-1}$  is assigned to  $\text{NH}_2$ . In the IR spectrum of *N*-CMC the strong peaks at 1600 and 1411  $\text{cm}^{-1}$  could be assigned to the respective asymmetric and symmetric stretch vibration of  $\text{COONa}$ .<sup>16</sup> The peak of  $\text{NH}_2$  disappeared and was replaced by



**Figure 2** XRD of chitosan and carboxymethyl chitosan (b: DS = 0.82, c: DS = 1.32); the spectra are shifted along the  $y$ -axis.



**Figure 3**  $^1\text{H-NMR}$  of carboxymethyl chitosan.

that of 1600  $\text{cm}^{-1}$ , however the OH group only change from 1030 to 1026  $\text{cm}^{-1}$ . This indicated that carboxymethylation successfully occurred on the  $\text{NH}_2$  of chitosan nor on the OH.

### XRD analysis

The XRD of CTS (curve a) and CMC of low and high DS are shown in Figure 2. It can be seen that there were some differences of peak heights between them. CTS consisted of a major peak at  $2\theta = 20^\circ$ . Compared with CTS, CMC had a relatively broader peak at  $2\theta = 20^\circ$  and the peak significantly weaker. With the increase of DS from 0.82 (Curve b) to 1.32 (curve c), the peak weak accordingly. It is well known that the XRD peak is related to the size of crystallite, the weaker peak usually results from small crystallites.<sup>17</sup> Hence, in this reaction the chitosan was carboxymethylated, the crystalline structure was destroyed and the crystallinity disappeared.

### $^1\text{H-NMR}$ data analysis

The  $^1\text{H-NMR}$  chemical shifts of signals of *N*-carboxymethyl chitosan (DS:1.32) is shown in Figure 3. The basic assignment of the chitosan resonance is that: a is the resonance of  $\text{CH}_3$  of free  $\text{COCH}_3$  ( $\delta$  1.98 ppm), g is the H1 ( $\delta$  4.53 ppm), b is the H2 ( $\delta$  2.65 ppm), c is the H3 ( $\delta$  3.35 ppm), e is the H4 ( $\delta$  3.59 ppm), f are the H5-6 ( $\delta$  3.78 ppm). These resonances can be found in the  $^1\text{H-NMR}$  spectrum of chitosan described by Kubota et al.<sup>18</sup> The resonance signal of the protons from  $\text{NCH}_2\text{COOD}$  groups can be found at d ( $\delta$  3.44 ppm), which can be found at the  $^1\text{H-NMR}$  spectrum of *N*-carboxymethyl chitosan (Muzzarelli),<sup>19</sup> also described by Hjerde.<sup>20</sup>

The result showed that the amino groups were carboxymethylated.

### CONCLUSION

N-carboxymethyl chitosan was successfully prepared by reacting the chitosan with chloroacetic acid in aqueous solution. The optimal reaction conditions were as follows: mass ratio of chloroacetic acid to chitosan was 5 : 1, reaction temperature was 90°C, reaction time was 4 h. Using this method, the use of glyoxylic acid could be reduced. The degree of substitution of N-CMC exceeded 1.32 and thus this is a valuable preparative method.

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