



# Water-soluble *N*-carboxymethylchitosan derivatives: Preparation, characteristics and its application

Nguyen Tien An\*, Do Truong Thien, Nguyen Thi Dong, Pham Le Dung

Natural polymer laboratory, Institute of Chemistry, VAST, 18 Hoang Quoc Viet road, Hanoi, Viet Nam

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## ABSTRACT

In this work, only *N*-substituted chitosan derivatives (water-soluble *N*-carboxymethylchitosan derivatives: *N*-CMC) with different degrees of substitution were obtained by reaction of a fully deacetylated chitosan (derived from deacetylation of chitosan using decrystallized method) with monochloroacetic acid at pH 8 and temperature of 90 °C. The structure of *N*-carboxymethylchitosan and chitosan was characterized by IR, <sup>1</sup>H, <sup>13</sup>C and <sup>1</sup>H–<sup>13</sup>C NMR-HSQC spectra. In the IR spectrum of the *N*-carboxymethylchitosan, the appearance of peak at 1742 cm<sup>−1</sup> was assigned for CO group of –NH–CH<sub>2</sub>–COOH of substituted chitosan. In the <sup>1</sup>H NMR spectra, the peaks at about 3.81–4.06 ppm, assigned for –CH<sub>2</sub>– groups of –NH–CH<sub>2</sub>– and –N(CH<sub>2</sub>)<sub>2</sub>–, were the major feature, while in the <sup>1</sup>H–<sup>13</sup>C NMR-HSQC spectra, signals of –CH<sub>2</sub>– confirmed the presence of these two different substituted –CH<sub>2</sub>– groups. The degree of substitution (DS) of *N*-monosubstitution (DS<sub>N-mono</sub>) decreased from 0.47 to 0.03 meanwhile that of *N,N*-disubstitution (DS<sub>N,N-di</sub>) increased from 0.52 to 0.96 since the mass ratio of chitosan/monochloroacetic acid changing from 1/1 to 1/4. The *N*-carboxymethylchitosan derivatives have been used for adsorption Cu(II) ion from aqueous solution. The results shown that the optimum conditions for adsorption Cu(II) ion in nitrate solution were pH 6.5, temperature of 30 °C, for 60–90 min and the substituted chitosan derivative having DS<sub>N-mono</sub> of 0.16 and DS<sub>N,N-di</sub> of 0.81 had maximum adsorption capacity of 192 mg Cu(II) per gram of *N*-CMC.

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## 1. Introduction

Chitin is a naturally abundant biopolymer like cellulose and is distributed in the shell of crustacean such as crabs and shrimps, and the cuticle of insects and also in the cell wall of some fungi and microorganisms. Chitin consists of 2-acetamido-2-deoxy-(1-4)-β-D-glucopyranose residues (*N*-acetyl-D-glucosamine units) which has intra- and inter-molecular hydrogen bonds and is water-insoluble due to its rigid crystalline structure. Chitosan ideally consists of 2-amino-2-deoxy-(1-4)-β-D-glucopyranose residues (D-glucosamine units) and has no or a small amount of *N*-acetyl-D-glucosamine units, and is water-soluble as the salt with various acids on the amino group of D-glucosamine unit. Additionally, partially acetylated chitosan which has about 50% D-glucosamine unit is only able to dissolve in water (Sugimoto, Morimoto, Sashiwa, Saimoto, & Shigemasa, 1998).

Chitin and chitosan have been widely used in vast diversification, ranging from waste management to food processing, medicine and biotechnology. It becomes an interesting material in pharmaceutical applications due to its biodegradability and

biocompatibility, and low toxicity. Chitosan has found wide applications in conventional pharmaceutical devices as a potential formulation excipient. The use of chitosan in novel drug delivery as mucoadhesive, peptide and gene delivery as well as oral enhancer has been reported in the literature (Khan & Peh, 2002).

Although, there are also problems in using chitin and chitosan. One of them is that it is difficult to dissolve in water and neutral pH range. Otherwise, *N*-acetyl-D-glucosamine, is a monomer of chitin abundant in human body. It is a template for the glucosaminoglycans and glycoprotein, for instance, the hyaluronic acid, an important substance in human body. That is to say, the metabolism medium of both animals and vegetables is water. So, various studies were conducted to obtain the water-soluble derivatives of chitin and chitosan by chemical modification techniques. However, when chemical modifications change the fundamental skeleton of chitin and chitosan, the modified chitin and chitosan lost the original physicochemical and biochemical activities. On the other hand, the chemical modifications of chitin and chitosan may have an advantage, because the modification with a hydrophilic reagent would be expected to result in hydrophilic chitin or chitosan while keeping the fundamental skeleton intact. Some approaches for the graft reaction of hydrophilic reagent onto chitin and chitosan were reported as a technique to improve the affinity to water or organic

\* Corresponding author. Tel.: +84 4 7564308.

E-mail address: [nguyentienanh@gmail.com](mailto:nguyentienanh@gmail.com) (N.T. An).

solvents (Sugimoto et al., 1998). One of them was the carboxymethylation of chitosan through the direct alkylation using the monochloroacetic acid reagent.

The reactive sites for the carboxymethylation of chitosan are the amino and hydroxyl groups being present in its chain. The amino groups of chitosan are weak bases which are predominantly protonated when  $\text{pH} < 6.5$ , leading to the solubilization of the polymer only in acid dilute solutions. However, the poor solubility of chitosan when  $\text{pH} > 6.5$  is a serious drawback in many of its potential applications. Thus, the preparation of chitosan derivatives has been envisaged to overcome its limited solubility in aqueous media. Such an adequate chemical modification results, for instance, when the carboxymethylation of chitosan is carried out since *N*-carboxymethylchitosan is soluble in a wide range of pH.

According to the literature, some carboxymethylated chitosan derivatives and their applications have been studied. The choice of the appropriate reaction conditions and reagents allows the preparation of *N*-, *O*-, *N,O*- or *N,N*-dicarboxymethylchitosan. The antimicrobial activity of *N*-carboxymethylchitosan may allow its application in agriculture for inhibiting the growth of fungi and bacteria during storage of fruits and vegetables. Such an application was specially interesting for the food industry since these polymers have low toxicity and because they are adequate to oral administration. The limited solubility of chitosan to acid media also limits its use as an antimicrobial agent in the food industry since the low pH may favor deleterious reactions on the food, altering its color and flavor. However, as *N*-carboxymethylchitosan is soluble in a wider range of pH, its application in this field does not suffer from this drawback. The affinity of chitin, chitosan and its derivatives to metal ions, such as  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ca}^{2+}$ ..., has also been reported, allowing their application for treatment of industrial effluents. Also in this case, *N*-carboxymethylchitosan presents some advantages as compared to chitosan for complexing more efficiently  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$  and presenting affinity for a larger number of ions. Other important applications of *N*-carboxymethylchitosan include the medical and pharmaceutical areas, mainly for the controlled release of drugs, orthopedic devices and connective tissue (Chen & Park, 2003; de Abreu & Campana-Filho, 2005; Ge & Luo, 2005; Paulino, Minasse, Guilherme, Reis, Muniz, & Nozaki, 2006; Sun & Wang, 2006; Sun, Wang, & Wang, 2006).

The properties and applications of carboxymethylchitosan derivatives were strongly dependent on its structural characteristics, mainly the degree of substitution and the locus, amino or hydroxyl group. Although in previous paper, we have reported the carboxymethylation of chitosan through the reaction of chitosan with monochloroacetic acid but the conditions of reaction and the characteristics of *N*-carboxymethylchitosan derivatives have not been investigated. Thus, in this paper, the synthesis, characterization and evaluation ability of adsorption  $\text{Cu}(\text{II})$  ion in nitrate solution of water-soluble *N*-carboxymethylchitosan resulted from the direct alkylation of a fully deacetylated chitosan by monochloroacetic acid in presence of inorganic base were further discussed.

## 2. Experimental

### 2.1. Materials

The  $\beta$ -chitosan with a 60–65% degree of deacetylation was received from the deacetylation of chitosan using decrystallation method (Le Dung, Dong, & Mai, 2004). Monochloroacetic acid, sodium hydrogen carbonate were purchased from Merck (Germany). The heavy metal salt chosen  $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$  was analytical reagent grade. All other chemicals and reagents used were of analytical grade.

### 2.2. Preparation of fully deacetylated chitosan

The fully deacetylated chitosan was prepared according to the method of Le Dung et al. (2004). Initially, 1 g  $\beta$ -chitosan ( $\text{DA} = 60\text{--}65\%$ ) was dissolved in 1% hydrochloric acid solution to obtain a homogeneous solution, then concentrated sodium hydroxide (40%) was gradually added to reach concentration of sodium hydroxide of 5% at the end of procedure. The deacetylation of chitosan was carried out at  $80^\circ\text{C}$  for 3 h with stirring vigorously in air atmosphere. After that, chitosan was separated by 90% ethanol and washed many times by distilled water to remove any impurities. It was then dried at  $60^\circ\text{C}$  in an oven or lyophilisation. The finally product was investigated and used for synthesis of *N*-carboxymethylchitosan derivatives.

### 2.3. Synthesis of *N*-carboxymethylchitosan

The *N*-carboxymethylchitosan derivatives were synthesized according to our method described in An, Dung, Thien, Dong, & Nhi (2008). Briefly, fully deacetylated chitosan (1 g) was swollen in 100 ml of water for a day, then monochloroacetic acid (the weight was 1, 2, 3, 4 g, marked as *N*-CMC1, *N*-CMC2, *N*-CMC3 and *N*-CMC4, respectively) was added and the mixture was stirred strongly until all chitosan was dissolved in water to give a homogeneous solution; then the pH was adjusted to eight by slowly adding 5%  $\text{NaHCO}_3$  solution while continuing to stir vigorously. The reactant system became opaque due to precipitation of chitosan as the pH was raised, but reverted to a homogeneous solution on continued heating at  $90^\circ\text{C}$ . After heating at  $90^\circ\text{C}$  for different durations 1, 2, 3, 4, 5, 6 h, respectively, the solution was filtered and cooled to ambient temperature and the pH was adjusted to pH of 6 by using 1% HCl solution to precipitate out the product which was filtered off and washed to neutral with 90% ethanol. It was then dissolved up in dilute NaOH to give the neutral Na-salt of *N*-carboxymethylchitosan, which was isolated by being lyophilised (An et al., 2008).

### 2.4. Characteristics of fully deacetylated chitosan and *N*-carboxymethylchitosan derivatives

FTIR spectra of the fully deacetylated chitosan and the *N*-carboxymethylchitosan derivatives were recorded on the FTIR-Impact 410 spectrometer in the range between 4000 and  $400\text{ cm}^{-1}$ . All powder samples were compressed into KBr disks for the FTIR measurement.  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^1\text{H}$ - $^{13}\text{C}$  NMR-HSQC spectra of the fully deacetylated chitosan and the sodium salt of the *N*-carboxymethylchitosan derivatives were recorded on the 500 MHz Bruker Avance spectrometer, the sample concentration being about 10 g/l, in  $\text{D}_2\text{O}$  for  $^1\text{H}$  NMR and 70 g/l for  $^{13}\text{C}$  NMR spectra with *N*-CMC and 1%  $\text{CD}_3\text{COOD}/\text{D}_2\text{O}$  with chitosan, at 353 K.

Degree of deacetylation (DD) of the fully deacetylated chitosan was determined by using the data of  $^1\text{H}$  NMR spectroscopy. DD value was estimated from the equation:

$$\text{DD} = 100 - [1/I_{\text{CH}_3} \times 100]/I_{\text{H}_1}(\%)$$

Where  $I_{\text{CH}_3}$  was the integral of the hydrogen atom in  $\text{NHCOCH}_3$  group and  $I_{\text{H}_1}$  corresponded to the hydrogen atom of C1 in glucosamine units.

Degree of substitution at *N*-atom (DS) of *N*-carboxymethylchitosan derivatives was calculated using the following equation:

$$\text{DS}_{\text{N-mono}} = (I_{\text{H}_1' + \text{H}_1''} - I_{\text{COCH}_3}/3)/(I_{\text{H}_1} + I_{\text{H}_1' + \text{H}_1''})$$

$$\text{DS}_{\text{N,N-di}} = (I_{\text{H}_1})/(I_{\text{H}_1} + I_{\text{H}_1' + \text{H}_1''})$$

Where  $\text{DS}_{\text{N-mono}}$  and  $\text{DS}_{\text{N,N-di}}$  were degree of substitution at *N*-atom of monosubstituted and disubstituted derivatives, respectively.  $I_{\text{H}_1}$ ,

$I_{H1'}$  and  $I_{H1''}$  were the integrals to the hydrogen atom bonded to carbon-1 of disubstituted chitosan unit, carbon-1 of monosubstituted chitosan unit and carbon-1 of chitin unit.  $I_{CH3}$  was the integral relative of hydrogen atom in  $NHCOCH_3$  group.

### 2.5. Adsorption experiments

The *N*-CMC 0.1 g was dissolved in 50 ml of distilled water with stirring until a homogenous was obtained (the solution A). The solution of salt using for experimental (with various initial concentrations) was prepared by dissolving the  $Cu(NO_3)_2 \cdot 3H_2O$  in water (solution B). The pH of this solution was adjusted by using NaOH or HCl solutions. After that, the solution B was gradually added into the solution A while stirring vigorously. During the mixing of two solutions, precipitate was formed. After stirring at a defined time, the mixture was centrifuged and filtered. The metal ion concentration in the filtrate and initial concentration was determined by atomic adsorption spectrophotometry. The adsorption capacity was calculated as follows:  $Q = [V \times (C_0 - C)] / W$ ; where  $C_0$  and  $C$  were the concentration of  $Cu(II)$  ion before and after adsorption, respectively (mg/l),  $V$  was the total volume of the aqueous solution (l) and  $W$  was the dry weight of *N*-CMC (g).

## 3. Results and discussion

### 3.1. Preparation of fully deacetylated chitosan

Chitosan with a very low degree of acetylation (DA) approaching nearly total deacetylation, was rarely prepared due to the difficulty of deacetylation and risks of degradation. Therefore, in industry, many methods for deacetylation of chitosan have been reported such as the deacetylation of chitosan using highly concentrated sodium hydroxide (40–50%) and even with organic solvents (*N*-methylpyrrolidone or propan-2-ol) to increase the swelling capacity of chitosan, several successive treatments were required. For example, starting with a chitosan having a initial DA of 25%, to obtain chitosan with a final DA of 1%, the deacetylation had to be repeated three times. In comparison, decrystallised chitosan (suspension of chitosan in water due to gradually

precipitation of chitosan from aqueous solution) having an initial DA of 65% required only one deacetylation treatment in 5% aqueous NaOH to give a product with  $DD = 97 \div 99\%$ , no organic solvent being used. This was confirmed by recording FTIR and NMR of the chitosan before and after deacetylation (Le Dung et al., 2004).

The FTIR spectra of initial chitosan and the fully deacetylated chitosan resulted from the deacetylation of chitosan using decrystallised method were shown in Fig. 1.

In the spectrum of the initial chitosan, there are two absorption peaks at 1654 and 1598  $cm^{-1}$ , which correspond to the  $C=O$  stretch of the secondary amide and  $N-H$  bending of the primary amine, respectively. The spectrum of the fully deacetylated chitosan shown a reduction of the peak at 1664  $cm^{-1}$ , indicating that most of the secondary amide have been further changed to primary amine by the alkaline deacetylation. This would be approved by recording the  $^1H$  NMR spectrum (Fig. 2) and  $^{13}C$  and  $^1H$ - $^{13}C$ -HSQC NMR spectra (not shown) of the fully deacetylated chitosan:

The  $^1H$  NMR and  $^{13}C$  NMR chemical shifts of signals of chitosan could be assigned as follows:  **$^1H$  NMR:** H1 ( $\delta$  5.33 ppm), H2 (3.63), H3 (4.37), H4 (4.39), H5 (4.24), H6a (4.30), H6b (4.46),  $CH_3$  (of free  $OCOCH_3$  and chitosan units) (2.55–2.57).  **$^{13}C$  NMR:** C1 ( $\delta$  99.7 ppm), C2 (57.2), C3 (72.1), C4 (78.6), C5 (79.5), C6 (61.5),  $CH_3$  (no appearance),  $C=O$  (no appearance). The evidence of the deacetylation of chitosan could be seen in the  $^1H$  NMR spectrum (Fig. 2) with lower intensity in the *N*-acetyl peak at about 2.55–2.57 ppm than that of the initial chitosan ( $^1H$  NMR spectrum of initial chitosan and in the  $^{13}C$  NMR spectrum were not shown here) with the absence of peaks at 25 and 178 ppm corresponding to carbon of  $CH_3$  and  $C=O$  of  $NHCOCH_3$  group, respectively. The value of DD calculated was 98% indicating that the deacetylation was nearly completed.

### 3.2. Preparation of *N*-carboxymethylchitosan derivatives

Water-soluble carboxyalkylchitosan derivatives with the modified molecular chain containing the possible highest content of *N*-acetyl-D-glucosamine units, which was the repeat unit in chitin,

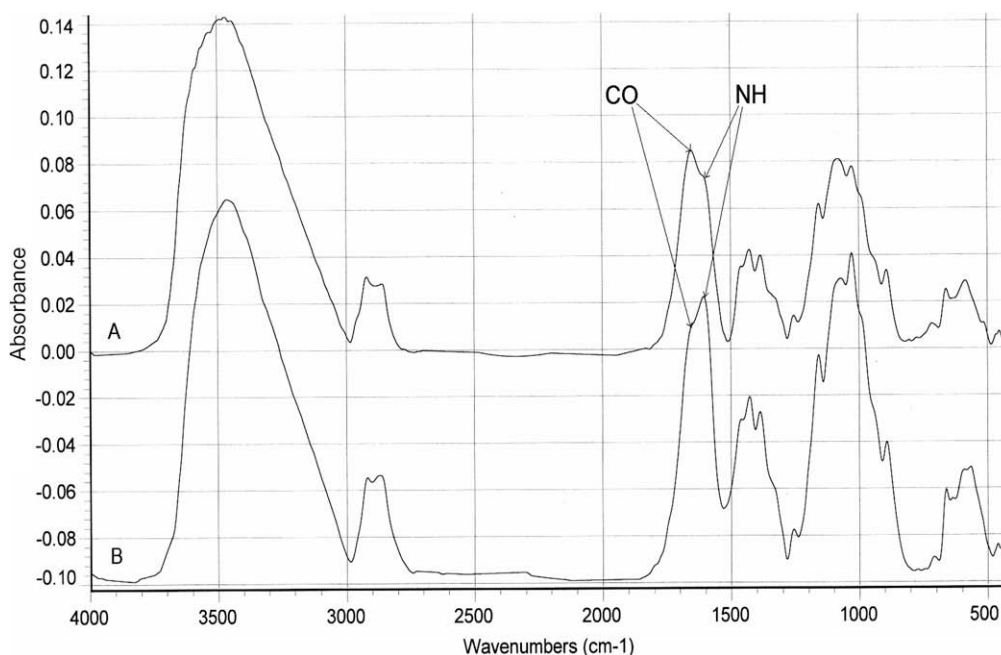


Fig. 1. FTIR spectra of initial chitosan (A) and fully deacetylated chitosan (B).

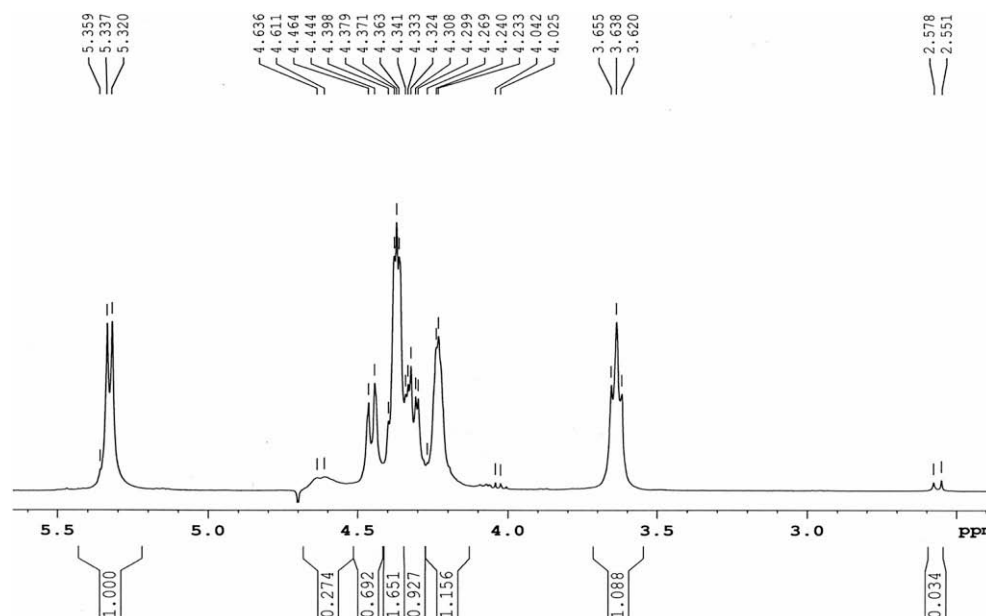
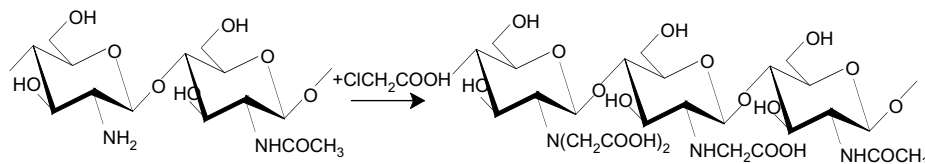


Fig. 2.  $^1\text{H}$  NMR spectrum of the fully deacetylated chitosan.

while retaining total water solubility, could be produced by three main methods such as: reductive alkylation, direct alkylation and Michael addition (An et al., 2008). The synthesis of *N*-CMC by the reductive alkylation, for example, the *N*-CMC was produced through the reaction of chitosan with appropriate carbonyl compound involved in two reaction steps: the first step was the formation of imine (or ketimine) ( $-\text{N}=\text{CHR}$ ;  $-\text{N}=\text{CR}_1\text{R}_2$ ) between the primary or secondary carbonyl group of the reagent with the  $-\text{NH}_2$  group of a chitosan unit. The second step was hydrogenation by  $\text{NaBH}_4$  reduction to convert the imine or ketimine linkages into saturated compounds, forming  $-\text{NH}-\text{CH}_2\text{R}$  or  $-\text{NH}-\text{CHR}_1\text{R}_2$  linkages. By the reductive alkylation, the reaction of chitosan with glyoxylic acid resulted in only the *N*-monocarboxymethylchitosan (*N*-monoCMC) when the molar ratio of glyoxylic acid: chitosan amine groups was  $\leq 2:1$ . If this ratio was greater than 3:1 both *N*-monoCMC and *N,N*-dicarboxymethylchitosan (*N,N*-diCMC) units were found in the product (An et al., 2008; Le Dung, Thien, Dong, Nhi, & An, 2005; Rinaudo, Le Dung, & Milas, 1992). If the procedure using a molar ratio  $>9:1$  and the reaction was repeated four times,

$0.7 > 0.47 > 0.2$ , respectively (Le Dung et al., 2004). In the mild alkaline medium (pH 8), however, only the amine group would be activated and so only *N*-substitution would take place. In our previous paper, the reaction of chitosan (DA = 5%) with monochloroacetic acid was carried out at pH 8 and temperature of  $90^\circ\text{C}$ . Although the chitosan was precipitated at this pH it would be gradually re-dissolved as the progressing time of reaction and at the end of the reaction all the chitosan molecules converted into solution as *N*-substituted chitosan derivatives, were *N,N*-diCMC with  $\text{DS}_{\text{di}} = 0.96$  and *N*-monoCMC with  $\text{DS}_{\text{mono}} = 0.03$ .

The synthesis and characterization of water-soluble *N*-carboxymethylchitosan resulted from the direct alkylation of a chitosan by monochloroacetic acid in presence of inorganic base were continuously discussed. Using the decrystallization of chitosan it was especially important for raising yield of reaction. The techniques for producing decrystallized chitosan have been defined clearly and presented in details previously (Le Dung et al., 2004). After carboxymethylation, the chitosan became a water soluble polyelectrolyte, following the reaction:



a compound of more than 90% was obtained (Le Dung et al., 2004). The *N*-CMC products were readily soluble in water at any such molar ratio, with no aggregation (gelation) being observed, provided that the pH was carefully controlled at  $4.5 \div 5$  by gradual addition of a diluted HCl solution throughout the hydrogenation reaction stage.

The synthesis of *N*-CMC derivatives by the direct alkylation of chitosan with monochloroacetic acid has been investigated in some previous works. It shown that at high alkali concentration (more than 25% of NaOH solution) the product would be mixture of *N*- and *O*-alkyl derivatives with substitution at the C(6) and C(3) OH groups and also some substitutions on the C(2)  $\text{NH}_2$  group were observed. The ease of substitution was in the order  $\text{C6} > \text{C3} > \text{C2}$  and the figures obtained for these reactions were

The FTIR spectrum of *N*-carboxymethylchitosan derivative was recorded (not shown). The characteristic absorption peaks of *N*-carboxymethylchitosan derivative were observed at  $3000 \div 4000\text{ cm}^{-1}$  ( $-\text{OH}$ ,  $-\text{NH}$ );  $1742\text{ cm}^{-1}$  (typical for  $\text{C}=\text{O}$  of  $-\text{COOH}$  group);  $2926\text{ cm}^{-1}$  ( $\text{vas}_{\text{CH}_2}$ );  $1635\text{ cm}^{-1}$  ( $\text{C}=\text{O}$  of  $-\text{COONa}$ );  $1396\text{ cm}^{-1}$  ( $\delta_{\text{CH}_2}$ );  $1158\text{ cm}^{-1}$  (twisting vibration of  $\text{CH}_2$ );  $1062$  and  $1024\text{ cm}^{-1}$  ( $\text{C}-\text{O}$  stretching vibration);  $896\text{ cm}^{-1}$  ( $\text{C}-\text{C}$  stretching). The characteristic absorption peak of amide II group observed at  $1603\text{ cm}^{-1}$  in the spectrum of chitosan and the appearance of peak at  $1742\text{ cm}^{-1}$  in the spectrum of *N*-carboxymethylchitosan indicating that the carboxymethylation could occur at the amino group of chitosan. This was approved by using the data of NMR spectra of the fully deacetylated chitosan and that of modified chitosans (Figs. 3–6).

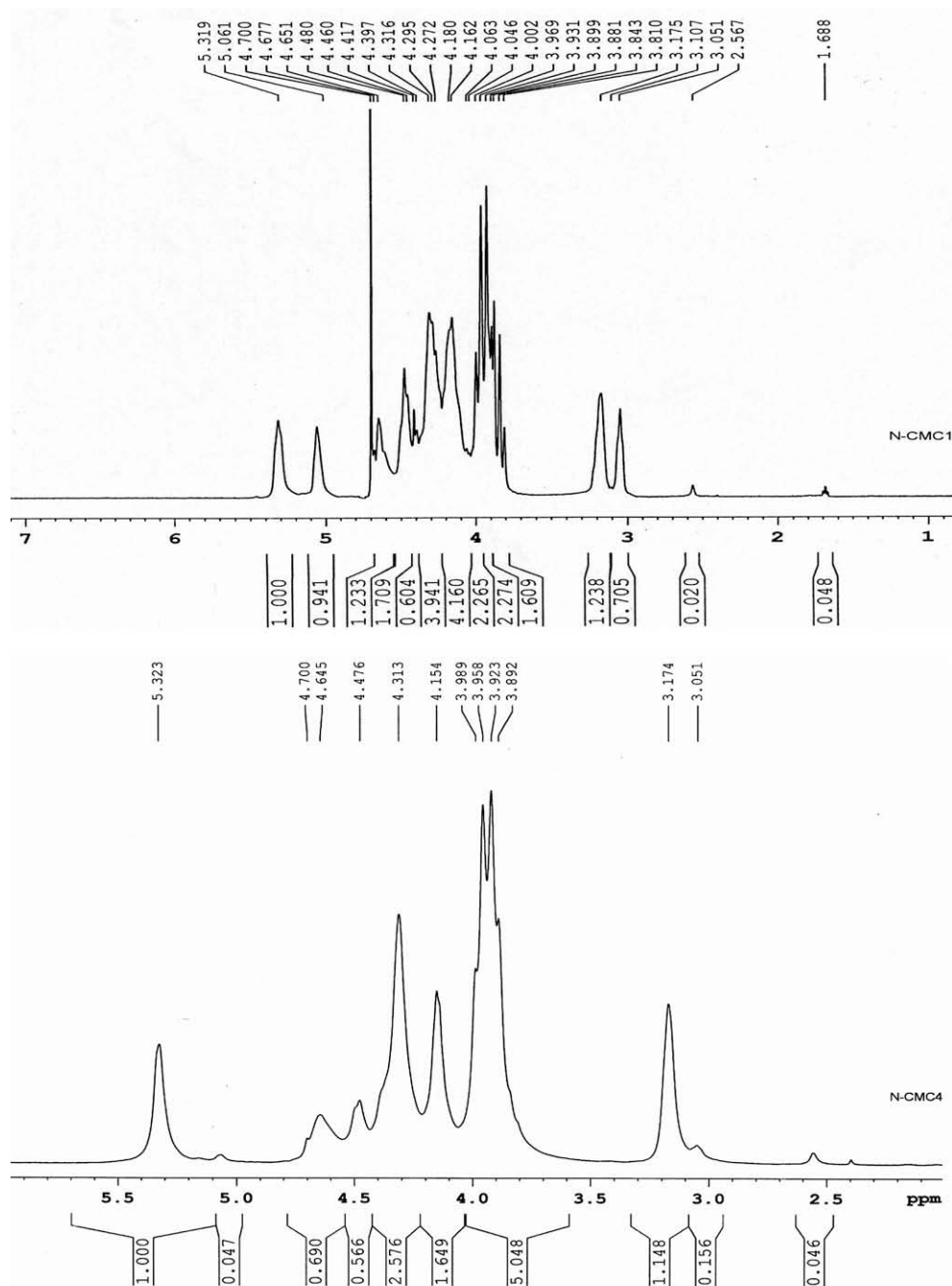
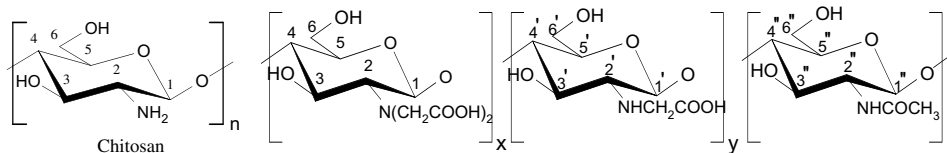


Fig. 3.  $^1\text{H}$  NMR spectra of *N*-CMC1 (CTS/MA = 1/1) and *N*-CMC4 (CTS/MA = 1/4).

Structures of chitosan and the *N*-carboxymethylchitosan derivative were shown as follows:



The  $^1\text{H}$  NMR chemical shifts of *N*-CMC's signals were assigned as follows: ***N*-CMC1**: H1 ( $\delta$  5.31 ppm), H1' + H1'' (5.06), H6a (4.48), H6b (4.31), H3 (4.39), H4 (4.29), H5 (4.17),  $\text{NHCH}_2\text{COOH}$  (3.89–3.81),  $\text{N}(\text{CH}_2\text{COOH})_2$  (4.06–3.93), H2 (3.17), H2' + H2'' (3.05–3.10), HOD (4.70),  $\text{CH}_3\text{CO}-$  (2.56). ***N*-CMC2**: H1 ( $\delta$  5.29 ppm),

H1' + H1'' (5.05), H6a (4.37), H6b (4.26), H3 (4.33), H4 (4.30), H5 (4.09),  $\text{NHCH}_2\text{COOH}$  (3.89–3.80),  $\text{N}(\text{CH}_2\text{COOH})_2$  (3.99–3.89), H2

(3.26), H2' + H2'' (3.09, HOD (4.70),  $\text{CH}_3\text{CO}-$  (2.46). ***N*-CMC3**: H1 ( $\delta$  5.28 ppm), H1' + H1'' (5.04), H6a (4.37), H6b (4.26), H3 (4.33), H4 (overlap), H5 (4.08),  $\text{NHCH}_2\text{COOH}$  (3.89–3.83),  $\text{N}(\text{CH}_2\text{COOH})_2$  (3.98–3.89), H2 (3.25), H2' + H2'' (3.08), HOD (4.77),  $\text{CH}_3\text{CO}-$  (2.46). ***N*-CMC4**: H1 ( $\delta$  5.32 ppm), H1' + H1'' (5.07), H6a (4.64),



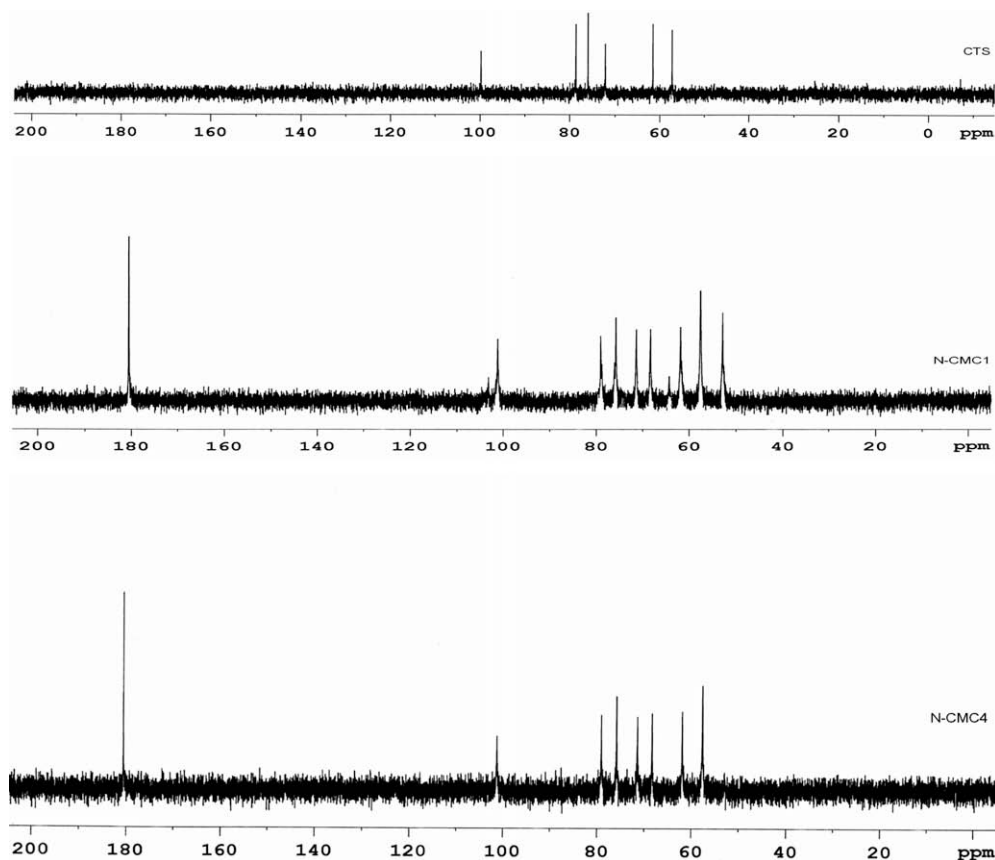


Fig. 4.  $^{13}\text{C}$  NMR spectra of the fully deacetylated chitosan (CTS); N-CMC1 (CTS/MA = 1/1) and N-CMC4 (CTS/MA = 1/4).

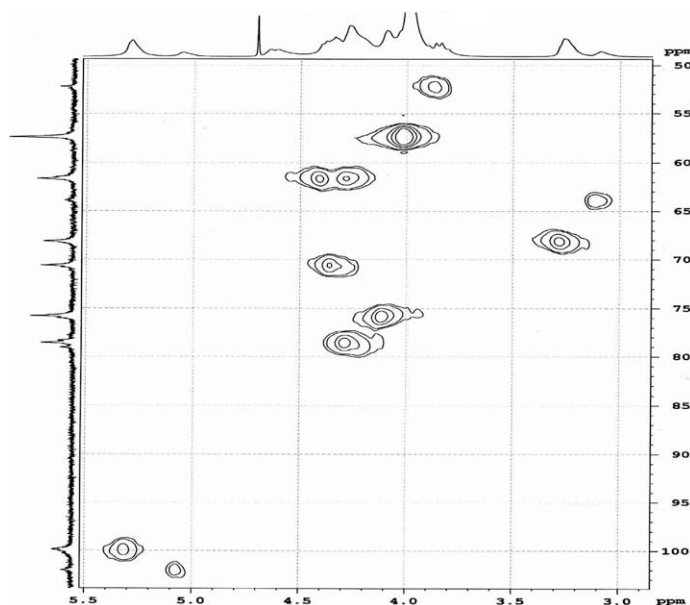


Fig. 5.  $^1\text{H}$ - $^{13}\text{C}$  NMR-HSQC spectrum of N-CMC2 (CTS/MA = 1/2).

H6b (4.47), H3 (4.31), H4 (overlap), H5 (4.15),  $\text{NHCH}_2\text{COOH}$  (no appearance),  $\text{N}(\text{CH}_2\text{COOH})_2$  (3.99–3.89), H2 (3.17),  $\text{H2}' + \text{H2}''$  (3.05), HOD (4.70),  $\text{CH}_3\text{CO}-$  (2.57). The structural modifications introduced by the carboxymethylation could be observed by comparing the  $^1\text{H}$  NMR spectrum of chitosan (Fig. 2) and to that of the N-CMC (Fig. 3). Indeed, this latter was considerably different and more complex than that of parent chitosan. This meant normally

in the chains of the N-carboxymethylchitosan there would be monomer units of: (assigned by  $^1\text{H}$  NMR, except the signals of H3 to H6): the chitin: ( $\overline{\text{DA}} \neq 0$ ) (assigned by 1 singlet of  $-\text{COCH}_3$  and 1 doublet of H1, 1 triplet of H2); the chitosan (in the case of their substitution degree was lower than one ( $\overline{\text{DS}} < 1$ )) by 1 doublet of H1, 1 triplet of H2); the N-monoCMC (by 1 doublet of H1, 1 triplet of H2 and 1 significant quadruplet of  $-\text{NHCH}_2-$ ) and the

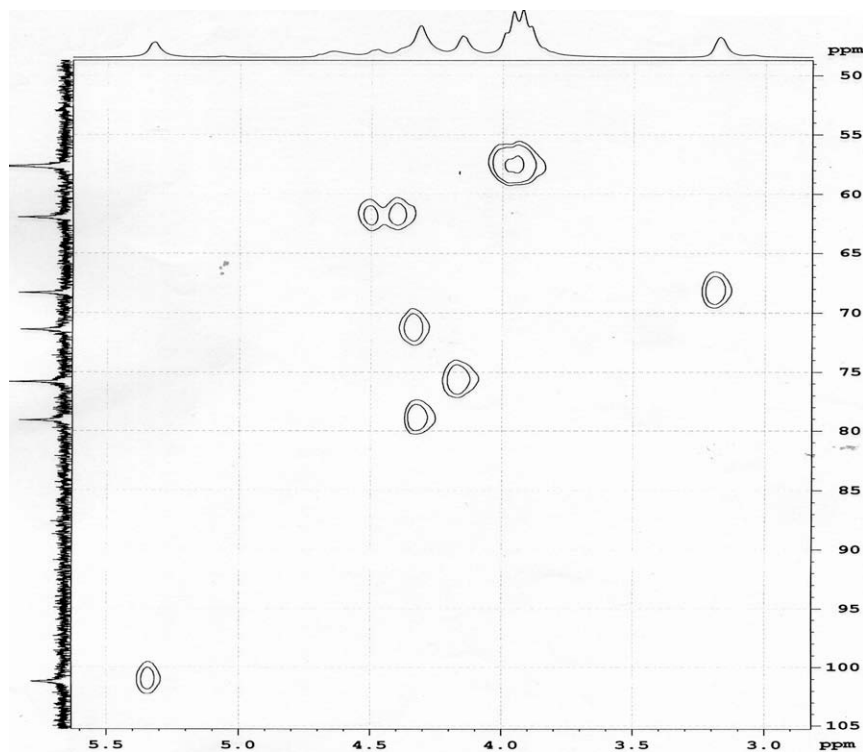


Fig. 6.  $^1\text{H}$ – $^{13}\text{C}$  NMR-HSQC spectrum of *N*-CMC4 (CTS/MA = 1/4).

*N,N*-diCMC (by 1 doublet of H1, 1 triplet of H2 and 1 significant quadruplet of  $-\text{N}(\text{CH}_2-)_2$ ) Le Dung et al., 2004. The occurrence of *N*-carboxymethylchitosan was denoted by the signals in the range 4.06–3.89 and 3.89–3.81 ppm, which were assigned to di- and mono-substitution of the amino groups, respectively. This was shown by the appearance of two quadruplet signals of mono- and disubstituted chitosan (Fig. 3).

The  $^{13}\text{C}$  NMR chemical shifts of *N*-CMC derivative signals were assigned as follows: ***N*-CMC1**:  $-\text{NH}-\text{CH}_2\text{COO}-$  ( $\delta$  53.1 ppm),  $-\text{N}(\text{CH}_2\text{COO}-)_2$  (57.7), C6 + C6' + C6'' (61.8), C2' + C2'' (64.3), C2 (68.1), C3 + C3' + C3'' (71.2), C5 + C5' + C5'' (75.7), C4 + C4' + C4'' (79.1), C1 + C1' + C1'' (101.2), CO of  $\text{COCH}_3$  and  $\text{COOH}$  (180.5). ***N*-CMC2**:  $-\text{NH}-\text{CH}_2\text{COO}-$  (52.1),  $-\text{N}(\text{CH}_2\text{COO}-)_2$  (57.3), C6 + C6' + C6'' (61.6), C2' + C2'' (63.9), C2 (68.1), C3 + C3' + C3'' (70.6), C5 + C5' + C5'' (75.7), C4 + C4' + C4'' (78.5–79.0), C1 (99.8), C1' + C1'' (102.0), CO of  $\text{COCH}_3$  and  $\text{COOH}$  (178.1). ***N*-CMC3**:  $-\text{NH}-\text{CH}_2\text{COO}-$  (52.9),  $-\text{N}(\text{CH}_2\text{COO}-)_2$  (57.6), C6 + C6' + C6'' (61.8), C2' + C2'' (64.2), C2 (68.3), C3 + C3' + C3'' (71.3), C5 + C5' + C5'' (75.8), C4 + C4' + C4'' (79.0), C1 + C1' + C1'' (101.1), CO of  $\text{COCH}_3$  and  $\text{COOH}$  (180.3). ***N*-CMC4**:  $-\text{NH}-\text{CH}_2\text{COO}-$  (no appearance),  $-\text{N}(\text{CH}_2\text{COO}-)_2$  (57.6), C6 + C6' + C6'' (61.9), C2' + C2'' (no appearance), C2 (68.4), C3 + C3' + C3'' (71.4), C5 + C5' + C5'' (75.9), C4 + C4' + C4'' (79.1), C1 + C1' + C1'' (101.2), CO of  $\text{COCH}_3$  and  $\text{COOH}$  (180.5). The  $^{13}\text{C}$  NMR spectra of *N*-carboxymethylchitosans (Fig. 3) shown various different units appeared in the structure of carboxymethylchitosan, the substitution was occurred mainly at  $\text{NH}_2$ -group of C2, so that the signal due to C2 was shifted from 57.2 to 68.3 ppm because of the electron-withdrawing effect of the carboxymethyl substituents. These features were taken as evidence that the carboxymethylation was taken place only at the amino group of C2 of chitosan (no carboxymethylation at hydroxyl groups of C3 and C6), resulting to both *N*-mono and *N,N*-disubstituted chitosan derivatives (when the mass ratio of chitosan/monochloroacetic acid was ranged from 1/1 to 1/3 and only *N,N*-disubstituted chitosan derivative if the mass ratio of 1/4).

### 3.2.1. Effect of ratio of chitosan to monochloroacetic acid

We examined the effect of the mass ratio of chitosan to monochloroacetic acid on the reaction by using four different ratios of the reactants (chitosan/monochloroacetic acid (CS/MA) = 1/1; 1/2; 1/3 and 1/4) while keeping the other conditions: pH 8, at 90 °C for 4 h. For each experiment the degree of substitution of substituted chitosan was investigated and the results were given in Table 1.

The degree of substitution (DS) of carboxymethyl group on the amino site could be also determined from  $^1\text{H}$  NMR spectra. According to the method described in the literature (Le Dung et al., 2005; Rinaudo et al., 1992),  $\text{DS}_{\text{N-mono}} \approx 0.32$  and  $\text{DS}_{\text{N,N-di}} \approx 0.82$  if all of H1, H1' and H1'' signals were used as reference. If (H2, H2' and H2'') signals were used as the internal reference the  $\text{DS}_{\text{N-mono}}$  and  $\text{DS}_{\text{N,N-di}}$  would be 0.28 and 0.76, respectively. However, as shown in the  $^1\text{H}$ – $^{13}\text{C}$ -HSQC NMR spectra (Figs. 5 and 6), the H signals of the  $-\text{NH}-\text{CH}_2\text{COO}-$  and  $\text{N}(\text{CH}_2\text{COO}-)_2$  groups were overlapped each other, so that the results were calculated from the method as mentioned above could be not so accurate. In this paper, the DS values of both *N*-mono and *N,N*-disubstitution could be calculated based on the integrals of H1 and H2 signals, as follow:  $\text{DS}_{\text{N-mono}} = (I_{\text{H2}} - I_{\text{CH3}/3}) / (I_{\text{H1}} + I_{\text{H1}'} + I_{\text{H1}'}) \approx 0.18$  and  $\text{DS}_{\text{N,N-di}} = I_{\text{H2}} / (I_{\text{H1}} + I_{\text{H1}'} + I_{\text{H1}'}) \approx 0.82$ . These results could be more accurate than that of above mentioned one because  $\text{DS} = \text{DS}_{\text{N-mono}} + \text{DS}_{\text{N,N-di}} \approx 1$  if  $\text{DA} = 0$ . However, as shown in the Figs. 5 and 6, the DS of *N*-monocMC and *N,N*-diCMC should be calculated by the reference of H1 signals.

**Table 1**  
DS values of *N*-carboxymethylation derivatives

Sample no.	$\text{DS}_{\text{N-mono}}$	$\text{DS}_{\text{N,N-di}}$	$\Sigma\text{DS}$
<i>N</i> -CMC1	0.47	0.52	0.99
<i>N</i> -CMC2	0.30	0.69	0.99
<i>N</i> -CMC3	0.16	0.81	0.97
<i>N</i> -CMC4	0.03	0.96	0.99

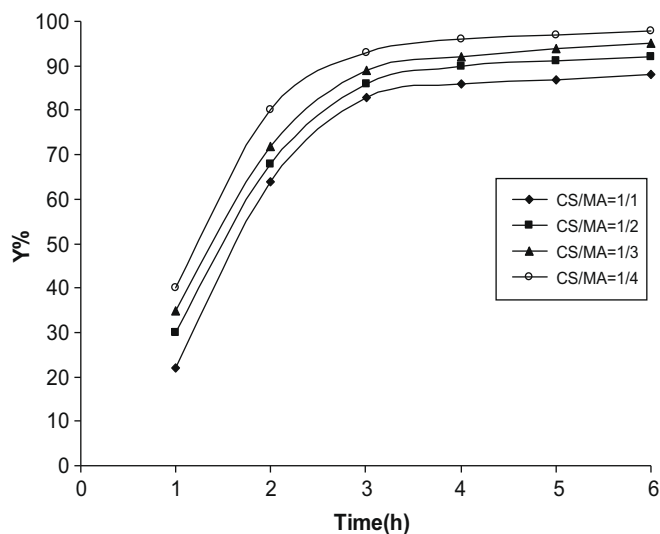


Fig. 7. Effect of reaction time on the yield of carboxymethylation.

### 3.2.2. Effect of reaction time

Effect of reaction time on the chitosan carboxymethylation was evaluated through the reaction yield as shown in Fig. 7 using four different ratios of the reactants (chitosan/monochloroacetic acid (CS/MA) = 1/1; 1/2; 1/3 and 1/4, respectively) while keeping the others conditions: pH 8, temperature of 90 °C. Fig. 7 shown that, the yield of the reaction increased as the reaction time was increased from 1 to 3 h but after that time there was no significant change of yield.

The molecular weight of *N*-CMC4 derivative was determined by MALLS (multiangle laser light scattering) as follows:  $M_n = 1.353 \times 10^5$ ;  $M_v = 1.685 \times 10^5$  and  $M_z = 1.62 \times 10^5$ . It shown that the initial chitosan ( $M_v = 1.95 \times 10^5$ ) has been depolymerized also in the carboxymethylation process.

## 3.3. Adsorption experiments

### 3.3.1. Effect of pH on metal ion adsorption

Indeed, the precipitation occurrence under different pH value for the different metals could lead to the misunderstanding and inaccurate interpretation of adsorption. Moreover, the comparison of adsorption performance would require an optimum pH value for

adsorption of the different metals before a well-founded conclusion could be reached. In the case of the sorption of metal ion, the chelation is very sensitive to pH value and usually adsorption does not occur at low pH value of the solution.

Fig. 8 shown the relationship between pH value of the original solution and adsorption capacity of *N*-CMCs for Cu(II). As seen in the Fig. 8, it was observed that the adsorption capacities of the Cu(II) increased with increasing the pH value of the solution until a maximum and then decreased with an increase in pH value. This could be explained as follows: At acidic pH, a decrease in the adsorption was attributed to the increase in ionic strength of solution and to the protonation of complexation sites. At alkaline pH, the Cu(II) ion in solution could form precipitate of copper hydroxide, which decreased the adsorption capacity. Whereas, with increase of pH value, the carboxymethyl and amino groups were free from the protonation, the adsorption mechanism may be partially replaced by a chelation mechanism, and so the adsorption capacity increased. The experimental result suggested that the optimum pH for Cu(II) ion adsorption of both *N*-CMC1 and *N*-CMC4 in the solution was around 6.5.

### 3.3.2. Effect of time

The experimental results of Cu(II) adsorption on *N*-CMCs versus time were shown in Fig. 9. The adsorption capacity of *N*-CMCs for Cu(II) ion increased sharply with the increase of adsorption time within 45 min and the equilibrium was reached at about 90 min for both *N*-CMC1 and *N*-CMC4. The fast adsorption rate of the *N*-CMC derivatives may probably be due to the state of *N*-CMCs at the beginning of adsorption procedure.

### 3.3.3. Effect of temperature

The influence of temperature on adsorption capacity of *N*-carboxymethylchitosan derivatives (*N*-CMC1 and *N*-CMC4) for Cu(II) ion was shown in Fig. 10. As seen from Fig. 10, the adsorption capacity of Cu(II) appeared to decrease as the temperature was raised from 30 to 50 °C and lightly changed as the temperature was raised from 50 to 60 °C. It found that the high temperature was to the disadvantage of adsorption and that the adsorption was an exothermic reaction. Furthermore, it should be noted that excessively high temperature could result in solvent evaporation or some desorptions, which affected the content of Cu(II) ion in solution.

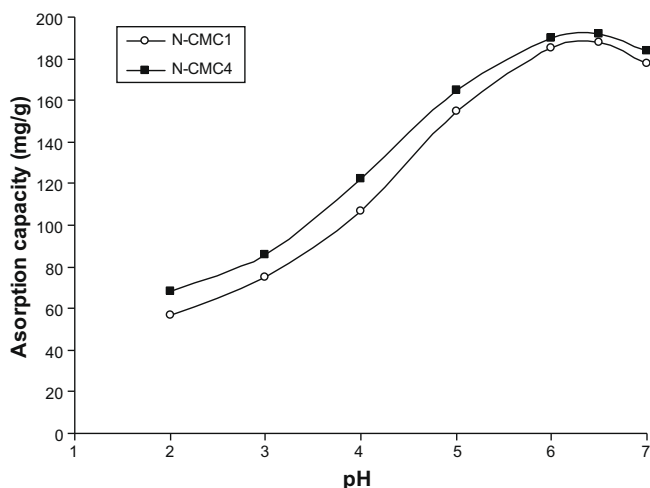


Fig. 8. Relationship between pH value of original solution and adsorption capacity of *N*-CMC for Cu(II) ion (the initial copper concentration of 0.02 mol/l, at 30 °C, shaking 24 h).

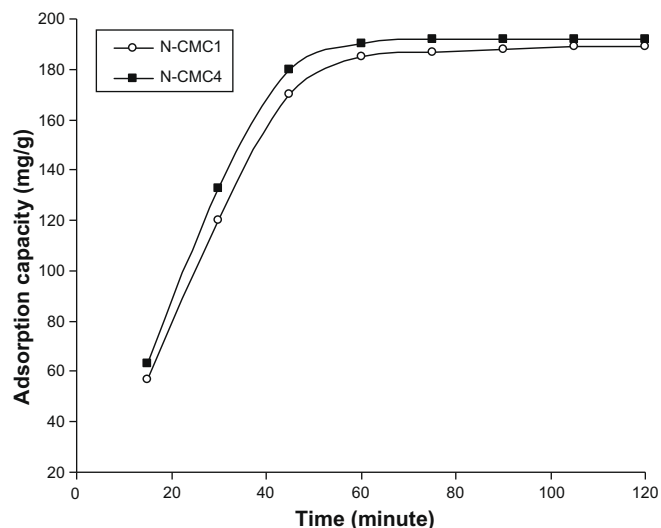


Fig. 9. Effect of adsorption time on the adsorption capacity of *N*-CMCs for Cu(II) ion (the initial copper concentration of 0.02 mol/l, 30 °C at pH 6.5).



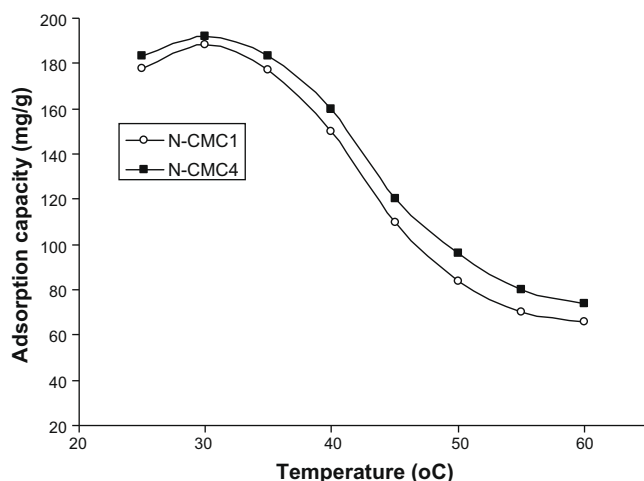


Fig. 10. Effect of adsorption temperature on adsorption capacity of *N*-CMC for Cu(II) ion (the initial copper concentration of 0.02 mol/l, shaking 24 h, pH 6.5).

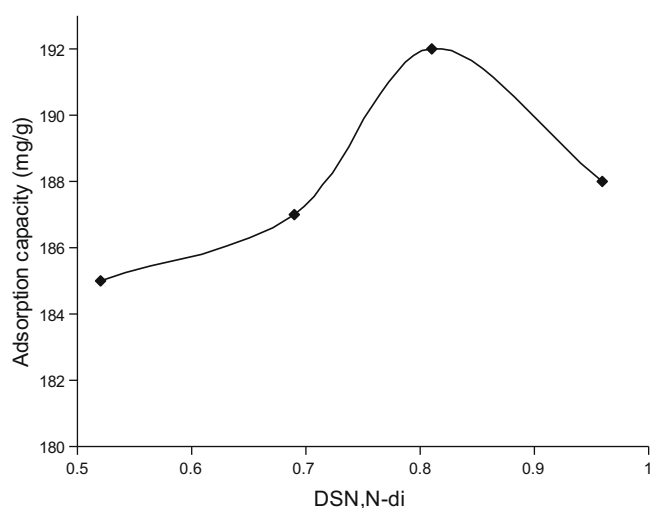


Fig. 11. Relationship between DS<sub>N,N-di</sub> and adsorption capacity of *N*-CMCs for Cu(II) ion in nitrate solution.

### 3.3.4. Effect of DS on metal ion adsorption

Fig. 11 shown the effect of degree of *N*-substitution (DS) on the adsorption capacity of *N*-CMCs for Cu(II) ion at the optimum adsorption time and pH value. It was observed that the adsorption capacity of Cu(II) ion in nitrate solution increased with increasing the DS<sub>N,N-di</sub> (or decreasing the DS<sub>N-mono</sub>) of *N*-CMCs. Considering the carboxymethyl substituting for hydrogen atom of the  $\text{—NH}_2$ , the amount of amino group decreased, whereas the adsorption capacity of Cu(II) in nitrate solution increased apparently with an increase of DS<sub>N,N-di</sub> between 0.52 and 0.81, which led to the conclusion that the carboxyl group participated in the Cu(II) adsorption process (this was confirmed by formation of precipitate

during the adsorption experiment). Otherwise, the adsorption capacity decreased evidently may be attributed to steric effect beyond a certain point. This phenomenon further suggested that the adsorption of *N*-CMCs for Cu(II) ion depended on not only carboxyl group but also on the other groups with different affinity, such as  $\text{—NH}_2$  and  $\text{—OH}$ .

## 4. Conclusions

Both of *N*-monocarboxymethylchitosan and *N,N*-dicarboxymethylchitosan derivatives were obtained by the direct alkylation of a low acetyl content chitosan with monochloroacetic acid at pH of 8 and temperature of 90 °C for 3 h. The DS of *N*-monosubstitution decreased from 0.47 to 0.03 while that of *N,N*-disubstitution increased from 0.52 to 0.96 since the mass ratio of chitosan/monochloroacetic acid changing from 1/1 to 1/4. The *N*-carboxymethylchitosan derivatives have been evaluated for adsorption Cu(II) ion from aqueous solution. The investigation of the adsorption, such as the pH, the temperature, the time, the DS of *N*-CMC shown that the optimum conditions for adsorption Cu(II) ion in nitrate solution were pH of 6.5, temperature of 30 °C for 60–90 min and the substituted chitosan derivative having DS<sub>N-mono</sub> of 0.16 and DS<sub>N,N-di</sub> of 0.81 had maximum adsorption capacity of 192 mg Cu(II) per gram of *N*-CMC.

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