Carbohydrate Polymers 83 (2011) 645–652

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and characterization of water-soluble O-carboxymethyl glucomannan derivatives

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

Natural Polymer Laboratory, Institute of Chemistry, VAST, 18-Hoang Quoc Viet Road, Hanoi, Viet Nam

ARTICLE INFO

Article history:
Received 24 March 2010
Received in revised form 4 August 2010
Accepted 13 August 2010
Available online 20 August 2010

Keywords:
Amorphophallus paeoniifolius
O-carboxymethyl glucomannan

ABSTRACT

Water-soluble O-carboxymethyl glucomannan derivatives (O-CMG) with different degrees of substitution were synthesized successfully by reaction of a konjac glucomannan (isolated from the tubers of *Amorphophallus paeoniifolius*, one of the most abundant *Amorphophallus* species in Viet Nam forest) directly with monochloroacetic acid (MCA) without methanol. The structure of O-carboxymethyl glucomannan derivatives was characterized by FTIR, ^{1}H, ^{13}C and ^{1}H–^{13}C NMR-HSQC spectroscopy. The conditions for synthesizing of O-CMG derivatives were also evaluated. The results shown that the optimal conditions for carboxymethylation of glucomannan were pH 10, temperature of 60 °C for 3 h. The degree of substitution ($\overline{DS}$) of O-substitution increased from 0.363 to 0.697 since the mass ratio (w/w) of glucomannan/monochloroacetic acid changed from 1/1 to 1/5.

1. Introduction

Amorphophallus sp. is grown in mountain or hilly areas in subtropical regions, mainly in the South East of Asia, including Viet Nam. It has been used as food and food additives in China and Japan for more than 1000 years. Glucomannan (GM) is a polysaccharide of the mannan family, very abundant in nature, specifically in softwoods (hemicellulose), roots, tubers and many plants bulbs. Despite the variety of sources, the most common used type of GM is named konjac glucomannan (KGM), which is extracted from tubers of *Amorphophallus* plants. Irrespective of its origin, GM is composed of β-1,4-linked D-mannose and D-glucose monomers. However, the mannose/glucose monomer ratio may vary depending on the original source of GM (Gao & Nishinari, 2004; Ishrud, Zahid, Viqar, & Pan, 2001; Koroskenyi & McCarthy, 2001; Xiao, Gao, & Zhang, 2000). Glucomannan and its derivatives have been investigated and used in many fields, such as food, film-forming, and biomedical (Vuksan et al., 1999, 2000). Although, the application of glucomannan is restricted for its viscosity and low solubility. So, various studies were conducted to improve the ability of dissolving of glucomannan in organic solvents, polar solvents and especially in water by chemical modification techniques, such as methylation, acetylation and carboxyalkylation. The preparation of dicarboxyglucomannan derivative has been proposed by Matsumura, Nishioka, and Yoshikawa (1991). This chemical modification has resulted in an important increase in glucomannan water solubility, and also in its ability to interact with positively charged polymers. These new features are very attractive for the design of new drug carriers. On the other hand, this chemical modification may also affect its biological activity. The methylation reaction of glucomannan has been done also for aiming at to increase the solubility of this polysaccharide in water (Kishida, 1979; Shatwell, Sutherland, Ross-Murphy, & Dea, 1991). Up to now, no pharmaceutical or medical application has been specifically reported for this derivative; however, it could be deduced that the increase of glucomannan water solubility could promote its use as a pharmaceutical excipient. The carboxymethyl glucomannan derivative was also synthesized (Kobayashi, Tsujihata, Hibi, & Tsukamoto, 2002). However, according to Kobayashi et al. KGM was etherified using MCA under the catalytic action of sodium hydroxide. The results shown that in the aqueous alkaline solution, KGM formed a gel and, consequently, carboxymethylation did not successfully proceed. Therefore, *methanol* was used as a disperse medium of KGM to facilitate the mechanical agitation for preventing a gel formation. The use of toxic and expensive chemicals as methanol could provoke some disadvantages of processing and applying this product both in food product and medicine. Otherwise, it seems to be some unexpected reactions have been taken place in this medium by means of the primary OH groups of methanol, that is to say its purification is more complicated.

Glucomannan isolated from *Amorphophallus paeoniifolius* is almost insoluble in water at ambient temperature. For enlarging the application of glucomannan and simplifying its preparation, in this work, the glucomannan was carboxymethylated by monochloroacetic acid only in aqueous medium without methanol for obtaining the water-soluble O-carboxymethyl glucomannan

* Corresponding author. Tel.: +84 4 37564308.
E-mail address: nguyentienanvhh@gmail.com (N.T. An).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.08.034

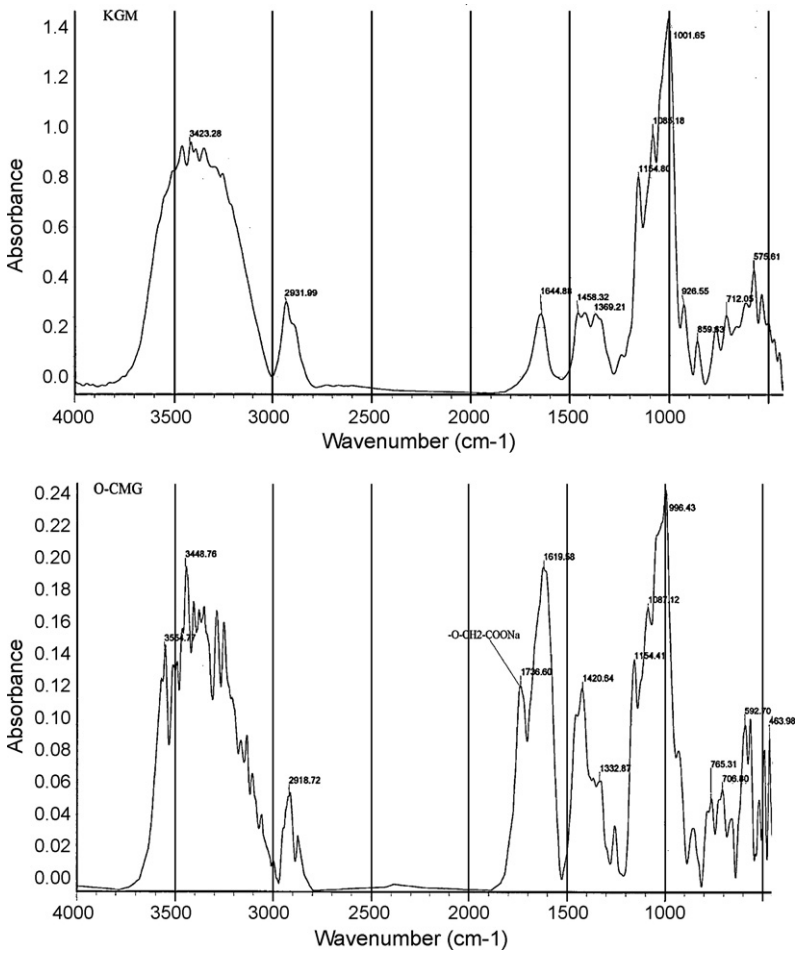

Fig. 1. FTIR spectra of KGM and O-CMG.

derivatives. The conditions for synthesis of O-CMGs such as ratio (w/w) of KGM/MCA, pH, time reaction were evaluated. The structure of carboxymethyl glucomannan derivatives was characterized carefully by mean of FTIR and NMR spectroscopy as evidences.

2. Experimental

2.1. Materials

Konjac glucomannan was isolated from tubers of *A. paeonifolius* (An, Dong, Thien, & Du, 2010). Monochloroacetic acid, sodium hydroxide, hydrochloric acid and ethanol were purchased from Merck Co. (Germany). All other reagents used were of analytical grade.

2.2. Synthesis of O-carboxymethyl glucomannan

The O-carboxymethyl glucomannan derivatives were synthesized as follows: konjac glucomannan (1 g) was dispersed in 100 ml of water by vigorously stirring, then monochloroacetic acid (the weight was 1, 2, 3 and 5 g, marked as O-CMG1, O-CMG2, O-CMG3 and O-CMG4, respectively) was added and the mixture was stirred strongly for 30 min; then the pH was adjusted to 8–10 by slowly adding 10% NaOH solution, while stirring continuously. The reactant system became quite transparent solution due to swelling of konjac glucomannan as the pH was raised, but reverted to a completely transparent solution when the temperature of system was up to 60 °C, then heating at 60 °C for different durations 1, 3 h, respectively. This solution was filtered and cooled to ambient tem-

Table 1
^{1}H NMR chemical shift data of O-CMGs (δ ppm) (10 g/l in D_2O at 353 K).

Signals	O-CMG1	O-CMG4
H1 of substituted units	6.085; 6.153	6.218
H1 of initial units	5.878	6.004
H2 of both un- and substituted units	4.292	4.283
H3 of both un- and substituted units	4.108	4.615; 4.54
H4 of unsubstituted units	4.126	4.077
H4 of substituted units	3.926	3.928
H5 of both un- and substituted units	4.108	4.436
H6a	4.345	4.436
H6b (overlapped with the signal of H2)	4.292	4.346
H signal of OCH_2COONa group	4.540	4.615; 4.54

Table 2
^{13}C NMR chemical shift data of O-CMGs (δ ppm) (70 g/l in D_2O at 353 K).

Signals	O-CMG1	O-CMG4
C1 of initial units	100.096	99.441
C1 of substituted units	97.08; 97.92	96.801
C2 of both un- and substituted units	72.035	71.820
C3 of both un- and substituted units	72.382	73.205
C4 of unsubstituted units	78.17; 77.78	–
C4 of substituted units	80.69; 80.89	80.671
C5 of substituted units	73.121	75.00
C5 of unsubstituted units	74.046	75.00
C6 of both un- and substituted units	61.39; 62.20	61.30
C signal of –**C**OONa group	178.241	178.309
C signal of methylene group (–O**C**H_2COONa)	70.59; 71.65	70.52; 71.30

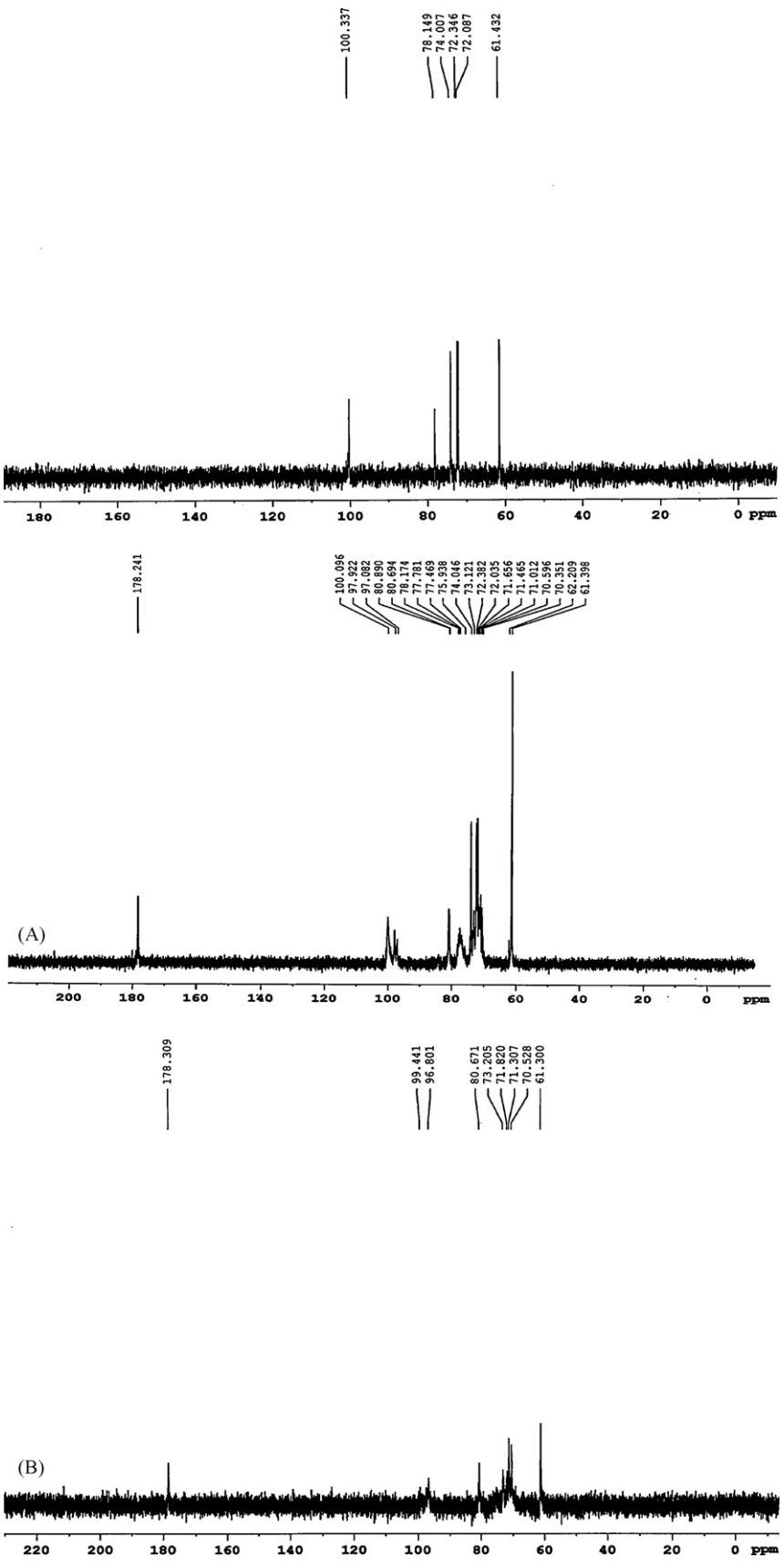

Fig. 2. ^{13}C NMR spectra of KGM, O-CMG1 (A) and O-CMG4 (B) (70 g/l, in D_2O at 353 K).

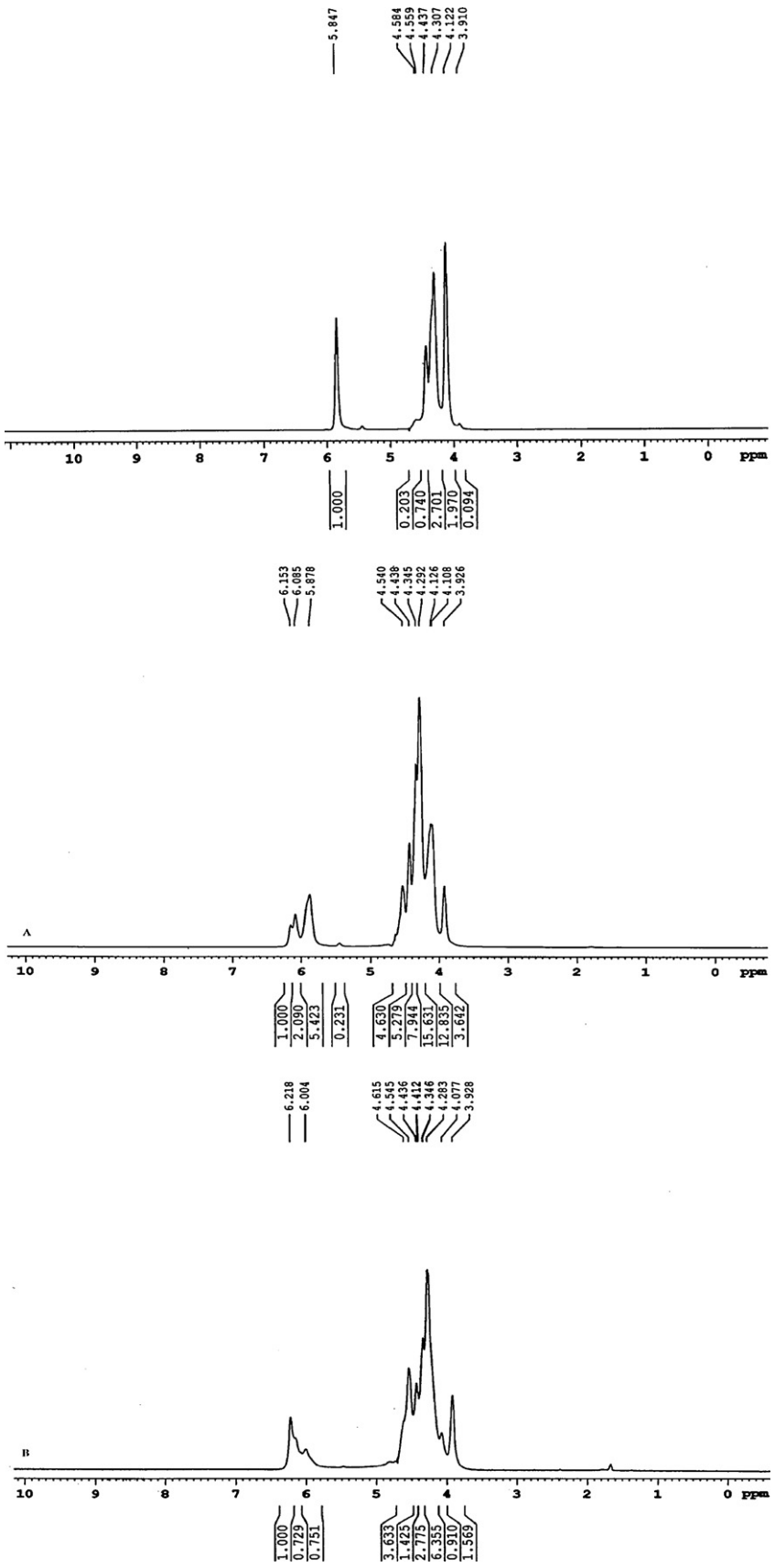

Fig. 3. ^{1}H NMR spectra of KGM, O-CMG1 (A) and O-CMG4 (B), (10 g/l in D_2O at 353 K).

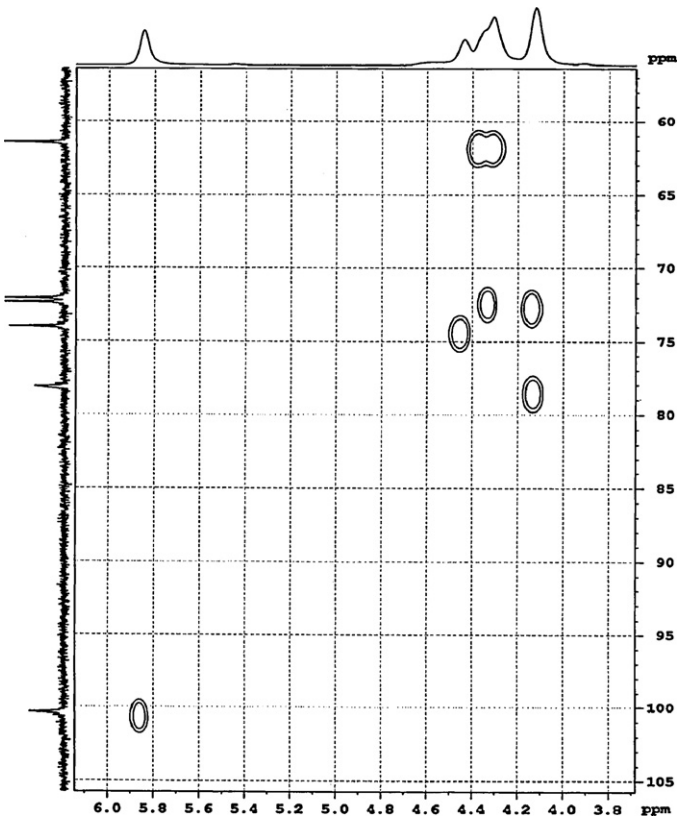

Fig. 4. ^{1}H–^{13}C NMR-HSQC spectrum of KGM (in D_2O, at 353 K).

perature then the pH was adjusted to pH 5 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 solution for obtaining the neutral Na-salt of O-carboxymethyl glucomannan, which was continuously washed to neutral by ethanol, then dried at 50 °C in oven for 3 h or lyophilized. The final product was used for further characterization.

FTIR spectra of the konjac glucomannan and the O-carboxymethyl glucomannan derivatives were recorded on the FTIR-Impact 410 spectrometer in the range of 4000–400 cm^{-1}. The powder samples were compressed into KBr pellets for the FTIR registration.

^{1}H, ^{13}C, ^{13}C Dept and ^{1}H–^{13}C HSQC-NMR spectra of the konjac glucomannan (initial material) and the sodium salt of the O-carboxymethyl glucomannan derivatives (product) were recorded on the 500 MHz Bruker Avance spectrometer, the sample concentrations being about 10 g/l for ^{1}H NMR and 70 g/l for ^{13}C NMR spectra in D_2O, at 353 K.

Degree of substitution at O-atom ($\overline{DS}$) of O-carboxymethyl glucomannan derivatives could be evaluated by their ^{1}H NMR integrals using the following formula:

$$\overline{DS} = (I_{H1'})/(I_{H1+H1'})$$

where $\overline{DS}$ is degree of substitution at O-atom of substituted derivatives. $I_{H1'}$ and $I_{H1+H1'}$ were the integrals of the hydrogen atom bonded at C1 of substituted glucomannan unit and C1 of all substituted and unsubstituted glucomannan unit, respectively.

3. Results and discussion

3.1. Characterization of O-carboxymethyl glucomannan

Kobayashi et al. said that the carboxymethylation could not performed successfully without using *methanol* as a dispersed agent. They had to use a large quantity of CH_3OH as medium of reaction. But the primary OH group of this one (a simple molecule as monomer) is also reactive as that of KGM (which are in the polymer chain). So the reaction of methanol with monochloroacetic acid could be occurred as unexpected reaction. According to our procedure, there was no need of methanol presence. Although both the glucomannan and monochloroacetic acid are not soluble in water so the carboxymethylation was occurred in the heterogeneous state. This reason might limit the ability of reaction and therefore the yield of carboxymethylation might be low. However, this problem could be overcome by doing as mentioned in Section 2, the monochloroacetic acid was added into the suspension of glucomannan. With the high speed of stirring and at raising temperature, the mixture of glucomannan and MCA was in the polydispersive state that means the contact ability of glucomannan with MCA was enhanced. After that, the addition of NaOH would result to the hydration of glucomannan, especially when the reaction temperature raised up to 60 °C. Although, this reason would not prevent monochloroacetic acid disperding into reaction medium, so the reaction occurred easily. The hydration of glucomannan would also lead the macromolecular chain of glucomannan to be more flexible, so that the hydroxyl groups became more active and readily reacted with the MCA by the nucleophilic substitution reaction for forming the O-CMG derivatives.

Table 3
Effect of the amount of monochloroacetic acid, pH and time (h) on the extent of carboxymethylation.

Samples	GM (g)	MCA (g)	pH	Time (h)	*R* (%)[a]	$\overline{DS}$
O-CMG1	1	1	8	1	1.8	–
	1	1	8	3	2.3	–
	1	1	10	1	128.3	0.363
	1	1	10	3	132.5	0.372
O-CMG2	1	2	8	1	4.1	–
	1	2	8	3	5.6	–
	1	2	10	1	141.8	0.416
	1	2	10	3	146.7	0.432
O-CMG3	1	3	8	1	4.6	–
	1	3	8	3	5.6	–
	1	3	10	1	149.6	0.498
	1	3	10	3	154.2	0.512
O-CMG4	1	5	8	1	5.1	–
	1	5	8	3	6.3	–
	1	5	10	1	158.6	0.652
	1	5	10	3	167.3	0.697

[a] *R* = water-soluble O-CMG product (g)/GM (g)×100%.

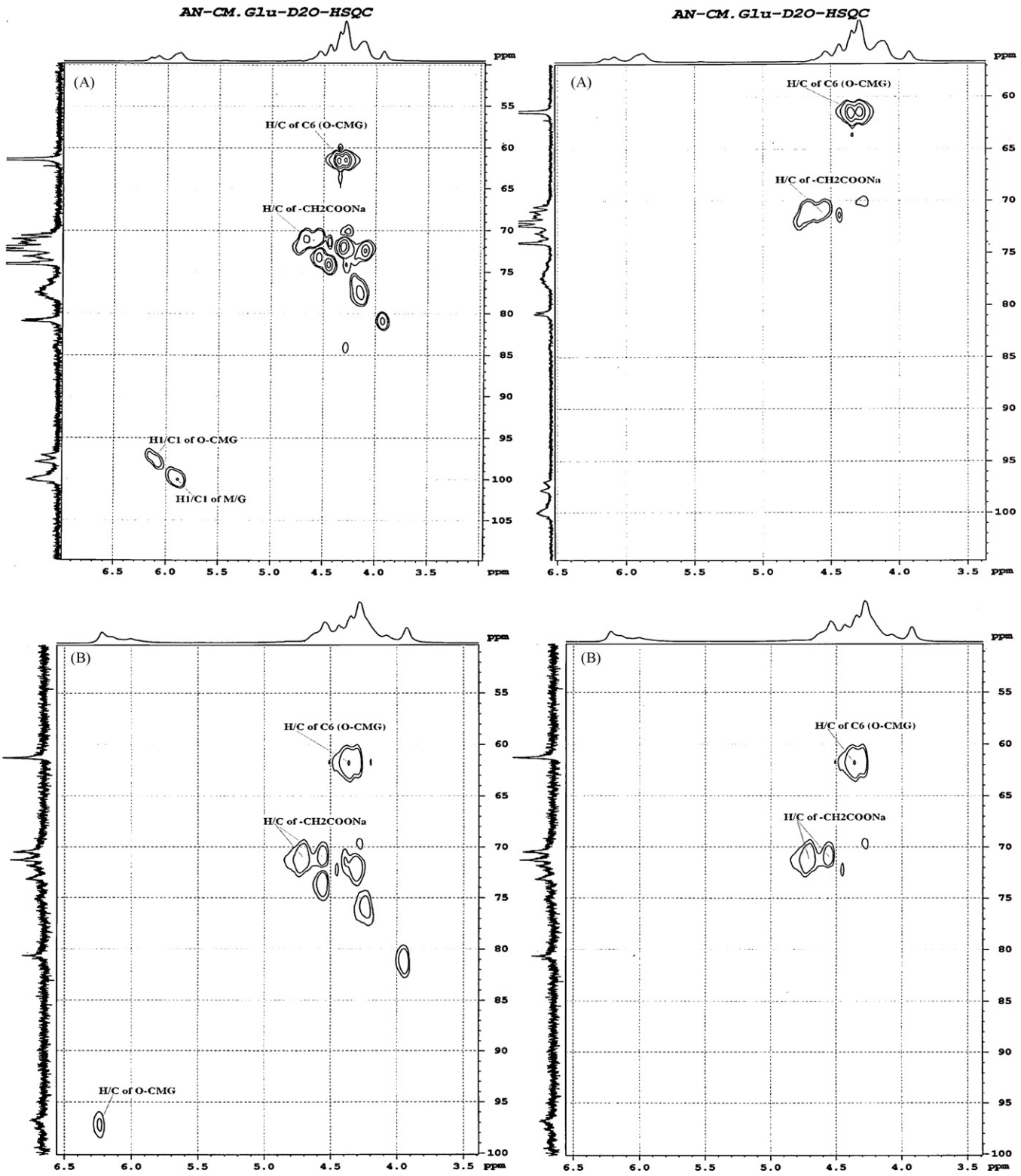

Fig. 5. $^{1}H-^{13}C$ NMR-HSQC spectra of O-CMG1 (A) and O-CMG4 (B).

3.1.1. FTIR analysis

The FTIR spectra of konjac glucomannan (KGM) and O-carboxymethyl glucomannan in the wavelength range of 4000–400 cm^{-1} were shown in Fig. 1.

In the spectrum of KGM, the wide band observed at 3000–3700 cm^{-1} could be attributed to the O–H stretching of the glucomannan. The bands at 2935 and 2887 cm^{-1} were attributed to the asymmetric stretching of C–H, while the band at 1646 cm^{-1} was described to adsorbed water and the bands at 1418 and at 1364 cm^{-1} to the angular deformation of C–H. The C–O ether bond showed stretching at 1154 cm^{-1} while the C–O alcohol bond showed stretching at 1085 cm^{-1}.

In the spectrum of O-CMG1, the characteristic absorption peaks of O-carboxymethyl glucomannan derivative were

observed at 3000–3700 cm^{-1} (–OH); 1736 cm^{-1} (C=O of –COOH); 2918 cm^{-1} (ν_{asCH_2}); 1619 cm^{-1} (C=O of –COONa); 1420, 1332 and 1158 cm^{-1} (δ_{CH_2}); 1087 and 996 cm^{-1} (C–O). The appearance of peak at 1736 cm^{-1} which was typical for –COOH group. It could be seen that the carboxymethylated reaction has taken place.

3.1.2. NMR analysis

The ^{13}C, ^{1}H, and ^{1}H–^{13}C HSQC-NMR spectra of KGM were shown in Figs. 2–4 and that of O-CMG derivatives were shown in Fig. 2A,B; Fig. 3A,B and Fig. 5, respectively.

The ^{1}H NMR and ^{13}C NMR spectra of glucomannan (Figs. 3 and 2, respectively) were registered directly in state of polymer chain, dissolved transparently in D_2O at 353 K (the hydration of glucomannan took place well at this temperature) (no NaOH be used as previously). The glucose and mannose units were overlapped, so that the spectrum was rather simple. The signals were assigned as follows:

In the ^{1}H NMR spectrum of KGM, the signal at 5.84 ppm was assigned to H1 proton of both mannose and glucose units (overlapped); the signals of H2–H6 proton were overlapped in region 3.91–4.84 ppm.

In the ^{13}C NMR spectrum of KGM, the signals were assigned as follow: C1 (δ 101.3 ppm); C2 (72.08); C3 (72.34); C4 (78.14); C5 (74.00) and C6 (61.43).

The NMR chemical shift data of O-CMG derivatives were summarized in the Tables 1 and 2.

The structural modifications introduced by the carboxymethylation could be observed by comparing the ^{13}C NMR and ^{1}H NMR spectrum of konjac glucomannan (initial material, Figs. 2 and 3) and that of the O-CMG derivatives (product, Fig. 2A,B and Fig. 3A,B). Indeed, this latter was considerably different and more complex than that of parent konjac glucomannan.

The ^{13}C NMR spectra of O-carboxymethyl glucomannan (Fig. 2A,B) showed various different degree of substitutions appeared in the structure of carboxymethyl glucomannan, the substitution was occurred at OH group of C6, so that the signal due to C2, C3 and C6 was shifted to the down-field because of the electron-withdrawing effect of the carboxymethyl substituents. The appearance of the signals at about 70.351–71.656 ppm in the ^{13}C NMR spectrum of O-CMG1 and the two signals at 70.528 ppm and 71.307 ppm in the ^{13}C NMR spectrum of O-CMG4, which they could be seen overall (as Tables 1 and 2) were assigned to C atom of methylene group of $-CH_2COO-$ substituted group. The signals assigned to the C atom of –COONa group were observed at 178.24 ppm (in the ^{13}C NMR of O-CMG1) and 178.309 ppm (in the ^{13}C NMR of O-CMG4). These features were taken as evidences that the carboxymethylation was taken place representing to the glucose ring –C(6)H_2–O–$\mathbf{CH_2}$COONa formation of glucomannan derivatives.

The occurrence of O-carboxymethyl glucomannan was also denoted by the signal at about 4.540 ppm (in the ^{1}H NMR spectrum of O-CMG1) and 4.615–4.545 ppm (in the ^{1}H NMR spectrum of O-CMG4), which were assigned to methylene proton of substitution of the OCH_2COONa groups.

The above discussions were affirmed by observation the ^{1}H–^{13}C NMR-HSQC spectra of KGM (Fig. 4) and that of O-CMG derivatives (Fig. 5). In the ^{1}H–^{13}C NMR-HSQC spectrum of O-CMG1 (Fig. 5A), the crosspeaks at δ 4.540/70.596 ppm and 4.540/71.012 ppm were due to H/C signals of the substituted methylene groups (the H signal of substituted group was overlapped at 4.540 ppm). The H/C signals were also observed in the ^{1}H–^{13}C NMR-HSQC of O-CMG4 derivative (Fig. 5B), the two dominant H/C crosspeaks of substituted methylene group could be identified at δ 4.545/70.528 ppm and 4.615/71.307 ppm.

3.2. Determination of the degree of substitution

The degree of substitution ($\overline{DS}$) for a O-carboxymethyl glucomannan derivative was defined as number of substitutions of hydroxyl groups per monomer unit of O-CMG. According to Kobayashi et al. the $\overline{DS}$ of carboxymethylated glucomannan samples could be determined by the titration (many errors in processing analysis), but in our method, the $\overline{DS}$ could be determined quite simply by the data of ^{1}H NMR spectra. However, it could be only determined using the integrals of H1 proton (as seen in the Fig. 5, the H/C crosspeak signals at about 6.1–6.3/96–98 ppm were assigned as H1 proton in substituted glucomannan, also by this one, it could be evaluated quantitatively the degree of substitution) because the signals of the other protons were overlapped as seen in the ^{1}H–^{13}C HSQC-NMR spectra. The $\overline{DS}$ was calculated using the formula presented in experimental part, the resulting $\overline{DS}$ value of O-CMG was approximately 0.363 and that of O-CMG4 was 0.697. These results are predominant than that of Kobayashi et al. (2002) by means of no unexpected reaction of methanol.

3.3. Various reaction conditions for synthesis of O-carboxymethyl glucomannan

The effect of reaction conditions on synthesis of O-carboxymethyl glucomannan was summarized in Table 3. Every carboxymethylation was carried out at 60 °C. The degree of carboxymethylation was investigated via the $\overline{DS}$ and R (R was the percent ratio of water-soluble O-CMG (g) per KGM). The results showed that the carboxymethylation of glucomannan was hardly occurred at pH 8. At the same amounts of MCA, the $\overline{DS}$ value and the ratio of water-soluble O-CMG per KGM increased with the increase of pH and reaction time. The $\overline{DS}$ value was also increased as the ratio (w/w) of MCA/KGM increased. The pH of reactive medium was an important factor of the preparation of O-CMG derivatives.

4. Conclusions

O-carboxymethyl glucomannan derivatives (O-CMGs) were synthesized successfully by the direct alkylation of a konjac glucomannan with monochloroacetic acid at pH 10 and temperature of 60 °C. The O-CMG derivatives were totally soluble in water. Their chemical structure was investigated quantitatively and qualitatively by IR and NMR spectroscopy. The appearance of the new signals as crosspeaks at about 4.5–4.8/70–73 ppm in the ^{1}H–^{13}C NMR-HSQC of O-CMG derivatives confirmed the presence of substituted methylene group (–$\mathbf{CH_2}$–COO–) on the carboxymethyl glucomannan macromolecule. Their degrees of substitution were calculated using the integrals of ^{1}H NMR signals and increased from 0.363 to 0.697 since the mass ratio of glucomannan/monochloroacetic acid changing from 1/1 to 1/5.

Without methanol, there are some advantages: no toxic, no need of product purification, higher yield by no concurrent reaction, lower cost of product, etc. These O-CMG derivatives with different $\overline{DS}$ could be used as film-forming for packaging or encapsulating, designing the new drug carriers, forming beads, micro and nanoparticles when interaction with positively charged polymers.

References

An, N. T., Dong, N. T., Dung, P. L., Thien, D. T., & Du, N. V. (2010). Characterization of glucomannan from some amorphophallus species in Vietnam. *Carbohydrate Polymers*, *80*, 308–311.

Gao, S., & Nishinari, K. (2004). Effect of deacetylation rate on gelation kinetics of glucomannan. *Colloids Surface B Biointerfaces*, *38*, 241–249.

Ishrud, O., Zahid, M., Viqar, U.-A., & Pan, Y.-J. (2001). Isolation and structure analysis of a glucomannan from the seeds of Libian dates. *Agricultural and Food Chemistry*, *8*, 3772–3774.

Kishida, N. (1979). Relationship between the quality of konjac flour and the molecular matter nature of konjac mannan. *Agricultural and Biological Chemistry*, *11*, 2391–2392.

Kobayashi, S., Tsujihata, S., Hibi, N., & Tsukamoto, Y. (2002). Preparation and rheological characterization of carboxymethyl konjac glucomannan. *Food Hydrocolloids*, *16*, 289–294.

Koroskenyi, B., & McCarthy, S.-P. (2001). Synthesis of acetylated konjac glucomannan and effect of degree of acetylation on water absorbency. *Biomacromolecules*, *2*, 824–826.

Matsumura, S., Nishioka, M., & Yoshikawa, S. (1991). Enzymically degradable poly(carboxylic acid) derived from polysaccharide. *Macromolecular Chemical Rapid Communications*, *12*, 89–94.

Shatwell, K. P., Sutherland, I. W., Ross-Murphy, S. B., & Dea, I. C. M. (1991). Influence of acetyl substituent on the interaction of xanthan with plant polysaccharides. III. Xanthan-konjac mannan systems. *Carbohydrate Polymers*, *14*, 131–147.

Vuksan, V., Jenkins, D. J. A., Spadafora, P., Stevenpiper, J. L., Owen, R., Vidgen, E., Brighenti, F., Josse, R., Leiter, L. A., & Bruce-Thompson, C. (1999). Konjacmannan (glucomannan) improves glycemia and other associated risk factors for coronary heart disease in type 2 diabetes. *Diabetes Care*, *22*, 913–919.

Vuksan, V., Stevenpiper, J. L., Owen, R., Swilley, J. A., Spadafora, P., Jenkins, D. J. A., Vidgen, E., Brighenti, F., Josse, R., Leiter, L. A., Zheng, X., & Novokmet, R. (2000). Beneficial effects of viscous dietary fiber from konjac-mannan in subjects with the insulin resistance syndrome. *Diabetes Care*, *23*, 9–14.

Xiao, Ch., Gao, S., & Zhang, L. (2000). Blend films from konjac glucomannan and sodium alginate solutions and their preservative effect. *Journal of Applied Polymer Science*, *3*, 617–626.