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An improved method for synthesizing *N*,*N*′-dicarboxymethylchitosan

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Abstract

Fully substituted *N*,*N*′-dicarboxymethylchitosan was obtained by the alkylation of chitosan at pH 8–8.5 and 90 °C using a monochloroacetic acid:chitosan weight ratio of 4:1. The water-soluble derivative is a chelating agent suitable for treatment of osteogenis or other applications involving chelation.

Keywords: Water-soluble chitosan; *N*-Monocarboxymethylchitosan; *N*,*N*′-Dicarboxymethylchitosan; Osteogenesis agent; Chelating agent

1. Introduction

There are three main methods disclosed in the literature for the preparation of *N*-carboxyalkylchitosan derivatives (Le Dung et al., 2005).

1.1. Reductive alkylation

The $-NH_2$ group of chitosan unit is reacted with the carbonyl group of, for example, glyoxylic acid, then hydrogenated by reaction with $NaBH_4$ or $NaCNBH_3$ to give the Chit–NH–CH_2–COOH and Chit-*N*(CH_2COOH)$_2$ structures, the ratio of mono-:di-substituted units depending on the ratio of reagent to chitosan used.

1.2. Direct alkylation

At mildly alkaline pH values, such as pH 8–8.5, alkyl halides such as monochloroacetic acid react preferentially with the amine groups of chitosan rather than with the hydroxyl groups, thus forming *N*-carboxymethylated chitosan derivatives.

1.3. Michael addition

A number of α,β-unsaturated carbonyl reagents, such as acrylic acid, react with the amine groups of chitosan by an addition mechanism. However this reaction gives *N*-carboxyethylated derivatives rather than the target *N*-carboxymethylated, so will not be considered further.

To date the products obtained by reductive alkylation or direct *N*-alkylation contain both *N*-carboxymethyl- and *N*,*N*′-dicarboxymethylchitosan units, the overall composition depending on the reaction conditions. Using the reductive alkylation process gave a product having approximately 70% *N*,*N*′-dicarboxymethylchitosan units (Rinaudo, Dung, & Milas, 1992), and this could be raised to 90% by repeating the reaction step a total of four times (Dung, Rinaudo, Milas, & Desbrieres, 1994). This product has been used for its chelating properties in both medical (Muzzarelli, Ramos, Stanic, Dubini, & Giardino, 1998) and non-medical applications (St Angelo & Vercellotti, 1992).

2. Experimental

2.1. Materials

The β-chitosan was from squid pens ($\overline{DA} \approx 10\%$) and the monochloroacetic acid was a commercially purchased reagent.

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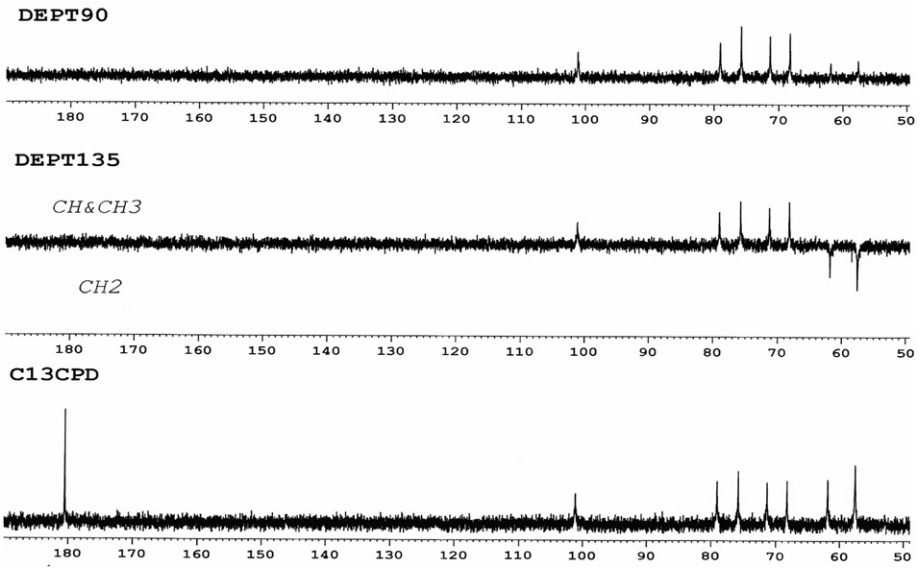

Fig. 1. ^{13}C-DEPT.NMR spectrum of *N*,*N*′-diCMC (500 MHz, 353 K, 70 g/l in D_2O).

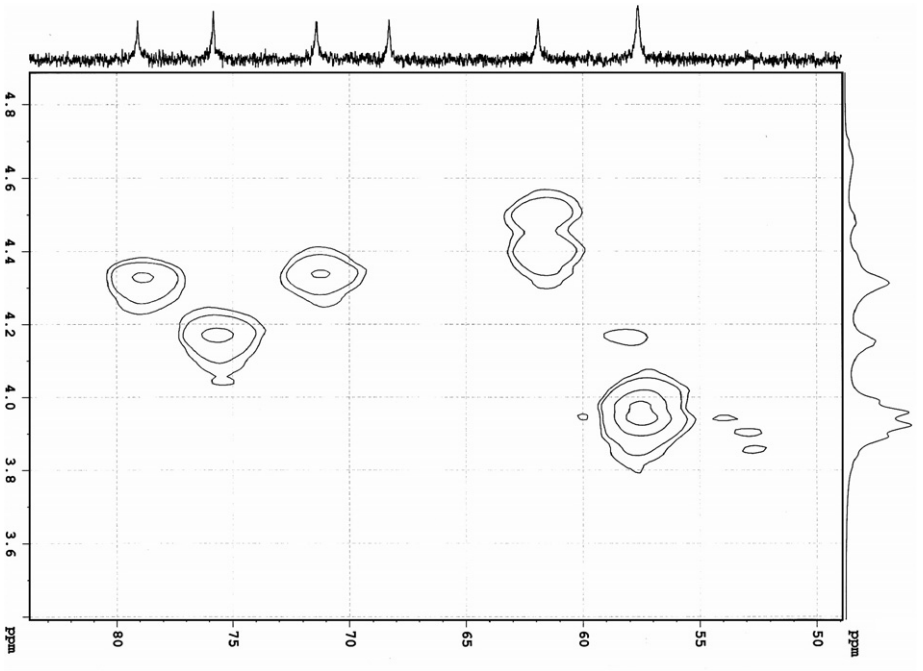

Fig. 2. ^{13}C–^{1}H NMR COSY spectrum of *N*,*N*′-diCMC (500 MHz, 353 K, 70 g/l in D_2O).

2.2. Preparation

β-Chitosan (1 g) was swollen in 100 ml of water for 24 h, then monochloroacetic acid (4 g) was added and the mixture stirred strongly until all β-chitosan was dissolved in water to give a homogeneous solution.; The pH was then adjusted to 8–8.5 by slowly adding 10% Na_2CO_3 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 °C. After heating at 90 °C the solution was cooled to ambient temperature and the pH lowered to pH 6 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*,*N*′-dicarboxymethylchitosan, which was isolated by drying at 60 °C or by being lyophilised.

2.3. Analysis

The ^{1}H and ^{13}C NMR spectra of the di-sodium salt of *N*,*N*′-diCMC were recorded on the 500 MHz Bruker

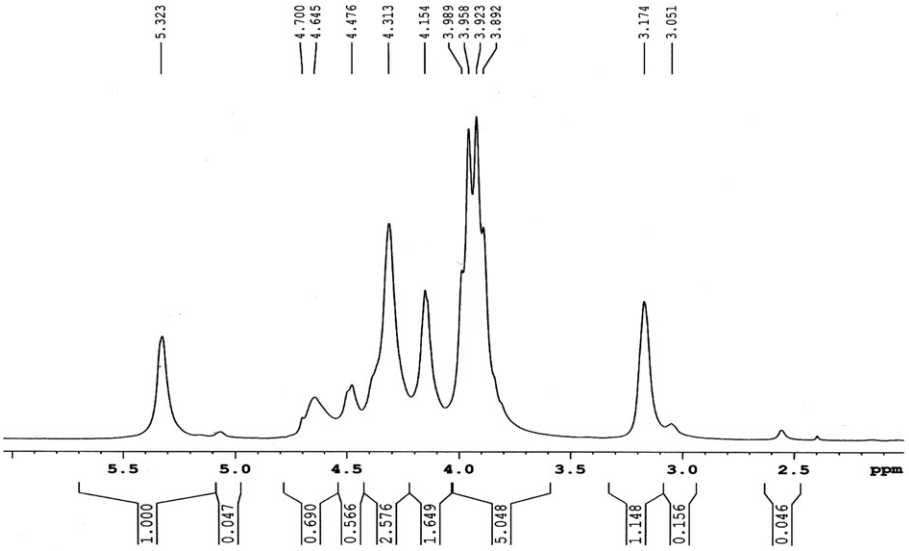

Fig. 3. ^{1}H NMR spectrum of *N,N′*-diCMC (500 MHz, 353 K, 10 g/l in D_2O).

Avance spectrometer, the sample concentrations being about 10 and 70 g/l, respectively, in D_2O at 353 K.

3. Results and discussion

The reductive alkylation reaction requires relatively expensive reagents and is not easy to apply on a large scale. In comparison the direct alkylation reaction at mildly alkaline pH values uses relative inexpensive reagents and is an easier process to scale up, so should be a route to cheaper products.

To date both techniques have been found to give both the *N*-carboxy- and *N,N′*-dicarboxymethylchitosan, depending on the reaction conditions. This means that in *N*-carboxymethylated chitosans prepared as previously described the polymer chains will contain the following structural units, as indicated by proton NMR (the signals of H3–H6 are omitted):

- Anhydro-*N*-acetyl-D-glucosamine, where the $\overline{DA} \neq 0$, shown by 1 singlet of $-COCH_3$ and 1 doublet of H1 and 1 triplet of H2.
- Anhydro-D-glucosamine, where the degree of substitution (DS) is less than 1, shown by 1 doublet of H1 and 1 triplet of H2.
- Anhydro-*N*-monocarboxymethyl-D-glucosamine, shown by 1 doublet of H1, 1 triplet of H2 and 1 significant quadruplet of $-NH(CH_2-)$.
- Anhydro-*N*-dicarboxymethyl-D-glucosaminethe shown by 1 doublet of H1, 1 triplet of H2 and 1 significant quadruplet of $-N(CH_2-)_2$.

Alkylation of chitosan with monochloroacetic acid at high alkali concentrations (more than 25% aqueous NaOH) gives mixed *N*- and *O*-alkyl derivatives with substitution at the C(6) and C(3) OH groups and also some substitution on the C(2) NH_2 groups. The ease of substitution is in the order C6 > C3 > C2 and the figures obtained for one reaction was 0.7 > 0.47 > 0.2 (Rinaudo et al., 1992). In the mildly alkaline medium of pH 8–8.5 however, only the amine groups will be activated and so only *N*-substitution will take place. Althought the chitosan was precipitated at this pH it will be gradually re-dissolved as the reaction proceeds and at the end of the reaction all the chitosan molecules will be in solution and fully di-*N*-substituted. It was considered that the ^{1}H NMR spectra of the *N*-carboxymethylchitosan ($\overline{DA} \neq 0$) consisting of many

Table 1
^{13}C NMR spectral data of *N,N′*-diCMC

Signals	ppm
N,N-diCMC	57.63
C6	61.91
C2	68.30
C3	71.42
C5	75.84
C4	79.10
C1	101.20
COOH	180.53

Table 2
^{1}H NMR spectral data of *N,N′*-diCMC

	ppm	Intergrals
H1 of *N,N′*-di-substituted chitosan	5.32	1.00
H1 of chitin unit (very weak)	5.07	0.47
H6a	4.70	0.69
H6b	4.40	–
H5	4.18	–
H4	4.35	–
H3	4.37	–
N,N′-diCMC ($-N(CH_2-)_2$)	3.99–3.89	5.05
H2	3.17	1.15
H2 in chitin unit	3.05	0.16
CH_3CO-	2.57	0.05

signals representing these 4 above-mentioned kinds of molecule, especially the two quadruplets of the *N*-mono-CMC and the *N*,*N*′-diCMC, will be complex; but the ^{1}H NMR spectra will be simpler in the case where all chitosan molecules are fully disubstituted, that means, there are not the chitosan signals and chitin one when its $\overline{DA} \approx 0$ (representing at H1 and H2) and even the quadruplet of *N*-mono-CMC. As a fully substituted *N*,*N*′-diCMC spectrum, there is only one signal of H1, one of H2 and one of significant *N*,*N*′-diCMC which was assigned to the [–N(CH$_2$)$_2$–] group, having 4 hydrogen atoms. One of these H1 or H2 signals could be used as the internal reference. The integral of the *N*,*N*′-diCMC signal of this derivative is $\overline{DS} \approx 1.25$, if the H1 signal is used as reference, ($\overline{DS} = 5.05/4/1 \approx 1.25$); but if the H2 signal is used as the internal reference $\overline{DS} = 5.05/4/1.15 \approx 1.08$, because there is the overlap of H5 signal, so it could be recognised that their degree of substitution of this reaction, that means the *N*,*N*′-dicarboxymethylation of chitosan, is $\overline{DS} = 1$, which is shown by Figs. 2 and 3.

Following alkylation at pH 8–8.5 and 90 °C, with the chit-NH$_2$ unit: ClCH$_2$COOH weight ratio of 1:4, it can be seen that there is almost one signal of the *N*,*N*′-dicarboxymethylchitosan only (Figs. 1–3) at 57.63 ppm (^{13}C.DEPT-NMR) and 3.99 to 3.89 ppm (^{1}H NMR) (See Table 1).

The ^{13}C.DEPT-NMR and its ^{1}H–^{13}C-COSY-NMR (Figs. 1 and 2) signals are shown at (ppm) (See Table 1):

The ^{1}H NMR signals are shown at (ppm) (See Table 2)

Because the residual level of *N*-acetylation is low the acetyl signal is very weak (1.7%). In all Figs. 1–3 there is no trace of the *N*-monosubstituted derivative. Based on the integrals of *N*,*N*′-disubstituted derivative or even the shapes of all their signals at H1, H2 regions, it could be considered that the derivative which was obtained by this way was fully substituted *N*,*N*′-dicarboxymethylchitosan. As the product yield is high it may also be assumed that nearly all the chitosan chains have been modified to the *N*,*N*′-disubstituted compound. The unused monochloroacetic acid could be recycled.

4. Conclusions

Fully substituted *N*,*N*′-dicarboxymethylchitosan was obtained by the alkylation of chitosan at pH 8–8.5, 90 °C for 3 h with monochloroacetic acid (1:4 weight ratio). Its neutral Na-salt is a water-soluble chelating agent particularly suitable for promotion of the osteogenis (Muzzarelli et al., 1998). The work presented here represents a new, simple and relatively inexpensive method for synthezising fully substituted *N*,*N*′-dicarboxymethyl-chitosan and its di-sodium salt.

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