

# Water soluble derivatives obtained by controlled chemical modifications of chitosan

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Chemical modifications of polysaccharides are increasingly studied as they have the potential of providing new applications for such abundant polymers. Water soluble chitosan derivatives (quaternized and *N*-carboxylated chitosans) are prepared, using techniques which allow homogeneous modifications without any sharp decrease in molecular weight, and characterized using NMR spectroscopy and viscometry. In addition a new method for nearly complete deacetylation of chitin is proposed and the role of the acid used for solubilizing chitosan derivatives is mentioned.

## INTRODUCTION

Chitin is one of the most abundant natural polymers produced and therefore it is of interest to find new applications for this material (Roberts, 1992). It is a copolymer of  $\beta 1 \rightarrow 4$ -D-glucosamine and  $\beta 1 \rightarrow 4$  *N*-acetyl D-glucosamine with varying composition. The physical properties depend on the distribution of *N*-acetyl groups along the chain and on the average degree of *N*-acetylation (DA) (Rinaudo & Domard, 1989). The presence of the amino group at the 2-position allows specific reactions to be performed resulting in a more regular modified polymer than cellulose derivatives. When its DA is lower than 50%, the chitin becomes soluble in aqueous acidic solutions and is named chitosan. Up to the present time only a few cationic polymers exist and chitosan may be used as a flocculation and chelating polymer (Muzzarelli, 1973).

In this paper, some water soluble derivatives are prepared under conditions which give homogeneous modified chitins with low degree of degradation. A characterization using mainly viscosity, GPC and NMR is presented

## EXPERIMENTAL

The initial samples of chitosan (DA  $\simeq$  12.5%, 11%) were from Protan and Fluka, respectively.

A sample with DA = 56% is obtained from ABER

(France). A sample with DA  $\simeq$  2% was prepared from DA = 12.5% in our laboratory following the method described in this paper.

The viscosity measurements were performed with an Ubbelohde capillary (0.58 mm as diameter) at 25 °C in the range of the dilute regime.

Size exclusion chromatography was performed on our equipment (Tinland *et al.*, 1988) using on-line light scattering and concentration detectors allowing the molecular weight distribution and the average values  $\overline{M}_w$  and  $\overline{M}_n$  to be determined directly without calibration. The analysis of chitosans was previously described (Rinaudo *et al.*, 1993a,b) the solvent being a 0.3 M  $\text{CH}_3\text{COOH}$ –0.2 M  $\text{CH}_3\text{COONa}$  solution.

The degree of acetylation was determined using  $^1\text{H}$  NMR after dissolution of the polymer in  $\text{D}_2\text{O}$  at 80 °C using a 300 MHz spectrometer from Bruker;  $\text{CD}_3\text{CO}_2\text{D}$ ,  $\text{CH}_3\text{CO}_2\text{H}$  or HCl were added to solubilize the chitosan (Rinaudo *et al.*, 1992a,b). IR can be used (Roberts *et al.*, 1992) but this method is limited to samples with high DA values.

## RESULTS AND DISCUSSION

### Extensively deacetylated chitin

Chitosans with very low DA are rarely prepared due to the difficulty of deacetylation and risks of degradation. Industrial deacetylation is performed using highly concentrated hydroxides (40–50%) (Roberts, 1992).

We have previously obtained this polymer after three

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successive treatments in the presence of organic solvents (Domard & Rinaudo, 1983). Very recently, we have patented a new method for deacetylation using aqueous sodium hydroxide (5 or 10%) (Rinaudo *et al.*, 1993*b*). Under these conditions there is less degradation.

The method used is the following: the commercial raw material was first purified by solubilization in acetic acid (pH 4.5), filtered then reneutralized with NH<sub>4</sub>OH; the flocculated polymer is recovered by filtration, washed and dried at room temperature.

The purified chitosan was then dissolved in acetic acid, heated to a given temperature and poured into the solution of NaOH containing the reagents which had been shown to prevent polymer degradation.

Initially, we consider the different parameters playing a role on deacetylation at constant temperature (100°C): the polymer concentration expressed as chitin (% w/w), the NaOH concentration, the time of reaction and the presence of additives used to reduce polymer degradation (thiophenol (1 ml/g chitosan) or NaBH<sub>4</sub> (0.1 g/g chitosan)). The results are given in Table 1. The chitosans obtained were characterized by their degree of acetylation and their intrinsic viscosity. As demonstrated, the NaOH concentration plays an essential role; one shows that a 5% NaOH solution allows deacetylation. The NaBH<sub>4</sub> is recognized as an antioxidant and protected the polymer in addition to the thiophenol.

A reduction in reaction time also allows a reduction in degradation. We can conclude that condition “6” in Table 1 is to be recommended. In all the cases, the polymers were completely soluble at pH 4.5 as judged by filtration through a thin pore membrane ( $\phi < 0.2 \mu\text{m}$ ).

To investigate the kinetics, 1 g of the purified material (DA = 56%) was dissolved in 60 ml of water containing 0.4 ml of pure acetic acid. this solution was poured into an aqueous solution containing 4 g NaOH and 0.1 g NaBH<sub>4</sub> in 16 ml of water (NaOH 5%). This suspension

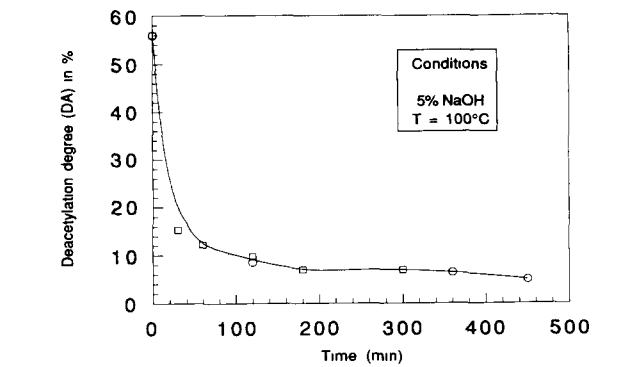


Fig. 1. Kinetics of deacetylation of chitosan by 5% NaOH in the presence of NaBH<sub>4</sub> (DA determined by <sup>1</sup>H NMR). The symbols □ and ○ correspond to two series of experiments.

was well stirred at 100°C under a nitrogen atmosphere. The kinetics of deacetylation was established by taking off a small aliquot of that suspension at fixed times, which is dispersed in water and washed to neutrality. The sample was washed twice with absolute ethanol and dried under vacuum at 25°C. The degree of residual acetylation was determined by NMR. The results are given in Fig. 1 for two series of measurements.

The minimum value obtained for DA in that case was 5% which seems to indicate that the characteristics of the initial sample determines the ability to deacetylate. From a sample DA ~ 12.5% one gets a final product in one run of 5 h with DA ~ 2%. Nevertheless the method proposed allows very effective deacetylation of the tested chitosans. It is clear that initial dissolution in an acetic acid medium is required to ensure good accessibility to the reagents.

In addition, it has also been established that the acid used for solubilization affects the value of DA determined first because the pH is not controlled and this can modify the solubility of the chitosan; in addition the chemical shifts are also modified (Fig. 2, Table 2).

Table 1. Conditions for deacetylation of chitosan and characteristics of the derivatives obtained

Conditions of reaction				DA (%)		[η] ml/g <sup>†</sup>
Reagents	Chitosan % w/w	Reaction time (hours)	IR	<sup>1</sup> H NMR <sup>†</sup>		
1	5% aq. NaOH	2.5	5	0	4.8	600
2	10% aq. NaOH	2.5	5	0	2	470
3	5% aq. NaOH + thiophenol	2.5	5	0	3 ± 1	710
4	5% aq. NaOH + NaBH <sub>4</sub>	1.25	3	0	3 ± 1	820
5	5% aq. NaOH thiophenol + NaBH <sub>4</sub>	1.25	5	0	3 ± 1	940
6	5% aq. NaOH + NaBH <sub>4</sub>	1.25	3	0	3 ± 1	975

<sup>†</sup>DA is determined in D<sub>2</sub>O/CD<sub>3</sub>COOD by <sup>1</sup>H NMR.  
<sup>‡</sup>Intrinsic viscosities are determined in AcOH 0.1N/NaCl 0.2N at 25°C (Roberts *et al.*, 1982). The properties of the initial chitosan were DA = 11%; [η] = 1020 ml/g.

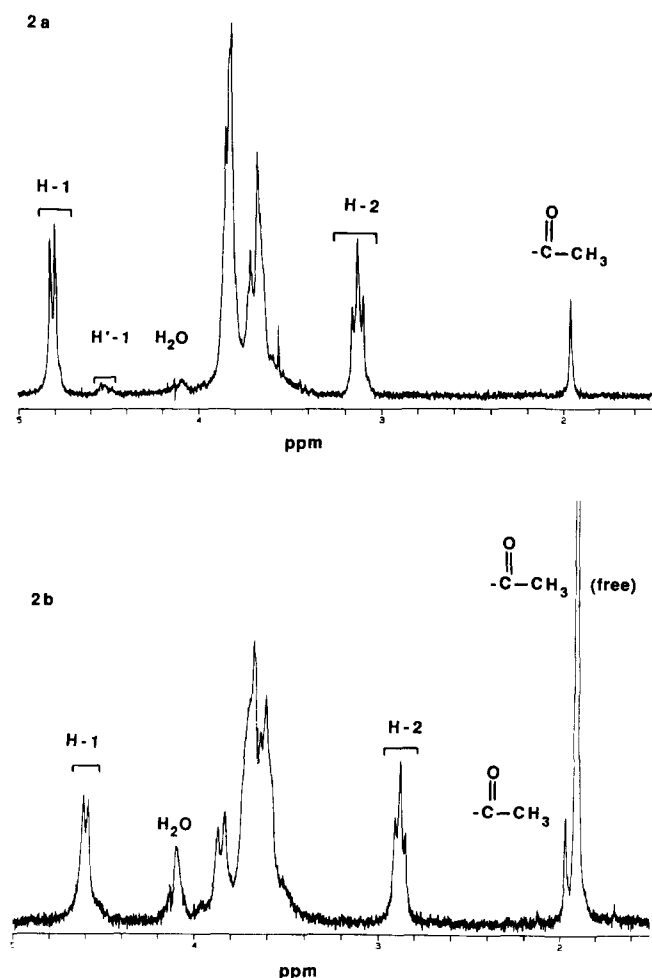


Fig. 2.  $^1\text{H}$  NMR spectrum for chitosan solubilized in HCl (a) or Ac-OH (b).

The main differences consist of the shifts of H-2 from 2.87 to 3.13 from  $\text{CH}_3\text{COOH}$  to HCl and the differentiation of two H-1 signals in HCl corresponding to the acetylated and the deacetylated forms of the chitosan in agreement with Hirai *et al.* (1991). The H'-1 signal is attributed to the acetylglucosamine units and is not modified by the nature of the acid or the pD of the solution. The H1 signal is attributed to the deacetylated units and its position depends upon the protonation. The degree of acetylation is determined from the integral of the  $\text{CH}_3$  peak of the acetyl group and is found to be 9.5%.

When  $\text{CH}_3\text{CO}_2\text{H}$  is used to stabilize chitosan the signal from the acetyl groups is not well separated from that of the  $\text{CH}_3$ - of the acid. This problem could be avoided if  $\text{CD}_3\text{CO}_2\text{D}$  was used. The degree of acetylation determined from the  $\text{CH}_3$  signal of the acetyl substituent is usually found to be lower when acetic acid is used compared with HCl (7.4%) due to the lowest resolution of the peaks assigned to acetyl groups.

The method of deacetylation proposed in this work has the advantage of preserving the molecular weight of the polymer (reflected by  $[\eta]$ ) and also using less chemi-

Table 2. Chemical shift of some protons of chitosan in different acids

	H1	H'1	H2	$-\text{CH}_3$
HCl	4.80	4.54	3.13	1.96
$\text{CH}_3\text{COOH}$	4.60	—	2.87	1.97

icals. This procedure is completely different from that described before (Domard & Rinaudo, 1983) and reduces the consumption of reagents. One also confirms that NMR is the most sensitive method of determining the DA.

### Quaternized chitosan

Chitosan is a water soluble cationic derivative only when the  $\text{pH} \leq 6$ . It is of interest to prepare a cationic derivative which is soluble over the total pH range. One previously proposed method is based on the dispersion of 5 g chitosan in 250 ml *N*-methyl-2 pyrrolidone reacting with  $\text{CH}_3\text{I}$  and NaOH (in molar ratio 1/15/2) for 3 h at 36 C (Domard *et al.*, 1986). After three steps, the degree of quaternization ( $\tau$ ) of 0.64 was determined by potentiometry and NMR (Domard *et al.*, 1987). The depolymerization of the polysaccharide was very large giving  $[\eta] = 64 \text{ ml/g}$  in NaCl 0.2 M (when the initial value was 800 ml/g).

A modification of the experimental conditions can be employed to reduce polymer degradation and control the different parameters affecting quaternization. The results are given in Table 3. The experimental condition adopted for series III, for example, involve dispersing purified chitosan (1 g, DA = 12.5%) in 40 ml of *N*-methyl-2-pyrrolidone and 13 g  $\text{CH}_3\text{I}$ , 2.4 g NaI, 5.5 ml of a 15% aqueous solution of NaOH. This dispersion was stirred at 60 C for a fixed time (from 30 min to 3 h). After 30 min, the modified polymers recovered were perfectly soluble in water. The presence of water is necessary to solubilize the NaOH added; after 15 min of reaction, the medium becomes completely homogeneous.

The counterion I is then exchanged to Cl by dissolving the quaternized polymer in a small quantity of water followed by the addition of HCl in ethanol.

In Table 3, the roles of the volume fraction of *N*-methylpyrrolidone, the time of reaction, sodium hydroxide ratio and temperature are examined. The polymers obtained were characterized by their intrinsic viscosity in 0.1 M NaCl and the degree of quaternization from NMR.

The  $^1\text{H}$  NMR spectrum is shown in Fig. 3. Following our previous work (Domard *et al.*, 1987), the signals at 2.95 ppm are attributed to the  $-\text{CH}_3$  groups of the quaternized site. The signal at 1.95 ppm corresponds to the *N*-acetyl group and we conclude that there is no variation of the DA under the conditions we have

Table 3. Conditions for quaternization and characteristics of the chitosan derivatives

	Conditions for reaction			Degree of quaternization ( $\tau$ )	$[\eta]$ ml/g NaCl 0.1N	Characteristics of quaternized chitosans by GPC		
	H <sub>2</sub> O (%) (Pyrrolidone/H <sub>2</sub> O)	Time (min)	Temperature (°C)			$\overline{M}_n$	$\overline{M}_w$	$[\eta]^{\dagger}$ (ml/g)
I	29.1	90	60	0.37	228	140.540	157.500	247
*(1/14.7/2.4)	29.1	180	60	0.42	200	97.880	115.300	185
II	16.6	30	60	0.40	157			
*(1/15/3.3)	16.6	60	60	0.47	146			
III	12	30	60	0.43	144			
*(1/15/3.45)	12	60	60	0.53	140			

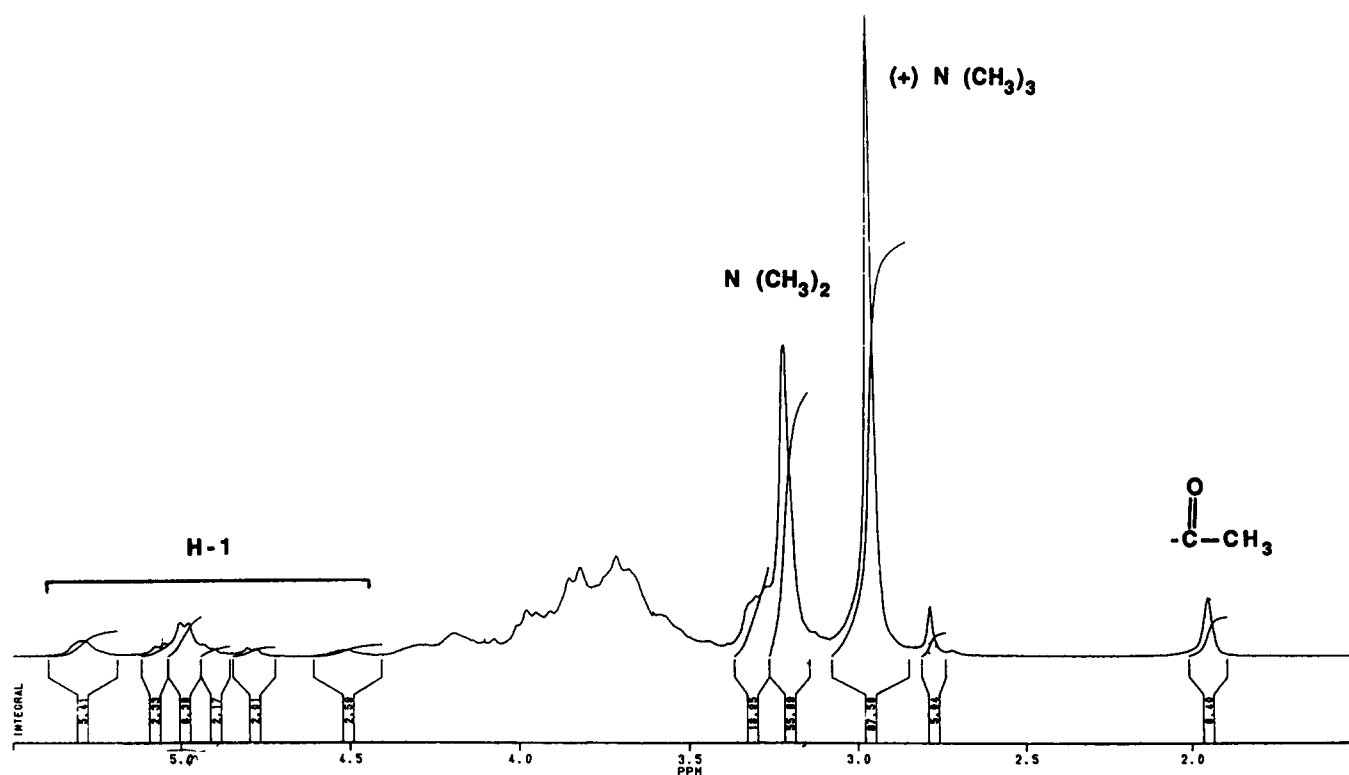
\*Molar ratio chitosan/CH<sub>3</sub>I/NaOH<sup>†</sup>Initial chitosan DA = 12.5%  $[\eta]$  = 735 ml/g<sup>‡</sup>Solvent: 0.2 M AcONa/0.3 M AcOH

adopted (condition I, Table 3). The better resolution of our <sup>1</sup>H NMR spectrum compared with the previous one allows us to identify at least four different anomeric protons depending on the substitution of the C-2 position; the integral of the H-1 signals is used as internal standard allowing the degree of quaternization  $\tau$  to be determined. A large peak appears at 3.2 ppm attributed to dimethylation of the nitrogen and a smaller one at ~2.8 ppm. When the water content decreases, a peak which nearly superimposes with that of the *N*-acetyl group appears. Moreover the degree of quaternization increases but molecular weight decreases due to the increase in the sodium hydroxide concentration. From

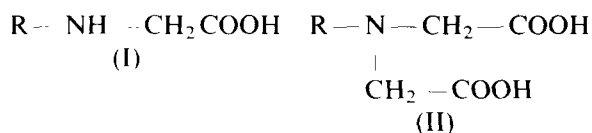
the intrinsic viscosity as well as the one-step  $\tau$  value obtained, it appears that the conditions adopted are more favourable than previously suggested by Domard *et al.* (1986). The degree of quaternization obtained in one step is larger and the degradation of the polymer lower. No evidence of *O*-methylation is obtained mainly due to lower reaction times.

#### *N*-Dicarboxymethylation

The introduction of carboxylic groups on chitin has been performed many times to produce an anionic water soluble derivative of chitin. The *O*- and

Fig. 3. <sup>1</sup>H NMR spectrum for a quaternized chitosan in D<sub>2</sub>O.

*N*-carboxymethylation was also previously described (Rinaudo *et al.*, 1992a,b) in which we described the distribution of substituents on each position C-6, C-3, and N by NMR measurements. *N*-Carboxymethylation was obtained by reaction of glyoxylic acid (March, 1985) on the  $-NH_2$  positions following a two step reaction in which forms I and II are obtained in different ratios:



These two forms were characterized by  $^1H$  and  $^{13}C$  NMR and previously discussed (Rinaudo *et al.*, 1992a,b).

The reaction was performed on chitosan (1g; DA  $\approx$  12.5%) which is first dissolved in 100 ml of 0.15 N acetic acid in water, filtered through 0.8  $\mu m$  membrane then treated with 4 g of glyoxylic acid at 25°C for 1 h in the molar ratio glyoxylic acid/chitosan  $\sim$ 9. The polymer in solution is then reduced by a solution containing 3.6 g of  $NaBH_4$  previously dissolved in a small quantity of water.

The *N*-carboxylated samples were precipitated with ethanol and recovered by centrifugation. The derivative is perfectly water soluble as judged from filtration tests previously mentioned. When necessary, the sample is redissolved in 100 ml of water to allow a second carboxymethylation to be performed as described above.

The degree of *N*-carboxymethylation was obtained by NMR and by conductimetric titration. The  $^1H$  NMR spectrum of the sample obtained from four successive reactions is given in Fig. 4. Compared to the spectrum previously published, the degree of substitution of N in the I and II forms can be determined. The sample appears to be nearly completely disubstituted (more than 90%) from the signal between 3 and 3.4 ppm. The peaks at 2.6 and 4.8 ppm are attributed to H-1 and H-2 of the acetylated units. The disubstitution is confirmed by  $^{13}C$  NMR spectroscopy (Fig. 5), the peak at 53.9 ppm being characteristic of disubstituted units; this spectrum is characteristic of the homogeneity of the derivative with the six signals of the sugar unit and that of the substituents at 180 ppm ( $-COOH$ ) and 53.9 ppm ( $N(CH_2)_2$ ), the monosubstitution being less than 10%.

The degrees of substitution (dicarboxylation) of N positions ( $x$ ) are determined from conductimetric titration of the acidic form by sodium hydroxide;  $x$  is obtained from the capacity of the polymer  $Q$  (expressed in equiv/g of solid material)

$$x = \frac{161 Q}{2 - 160 Q}$$

We obtained  $x = 0.95$ .

From the  $-CH_3$  signal in Fig. 4, one also demonstrates that there is no deacetylation during the carboxymethylation reaction.

Whatever the experimental conditions, the derivatives prepared were always totally soluble in aqueous solu-

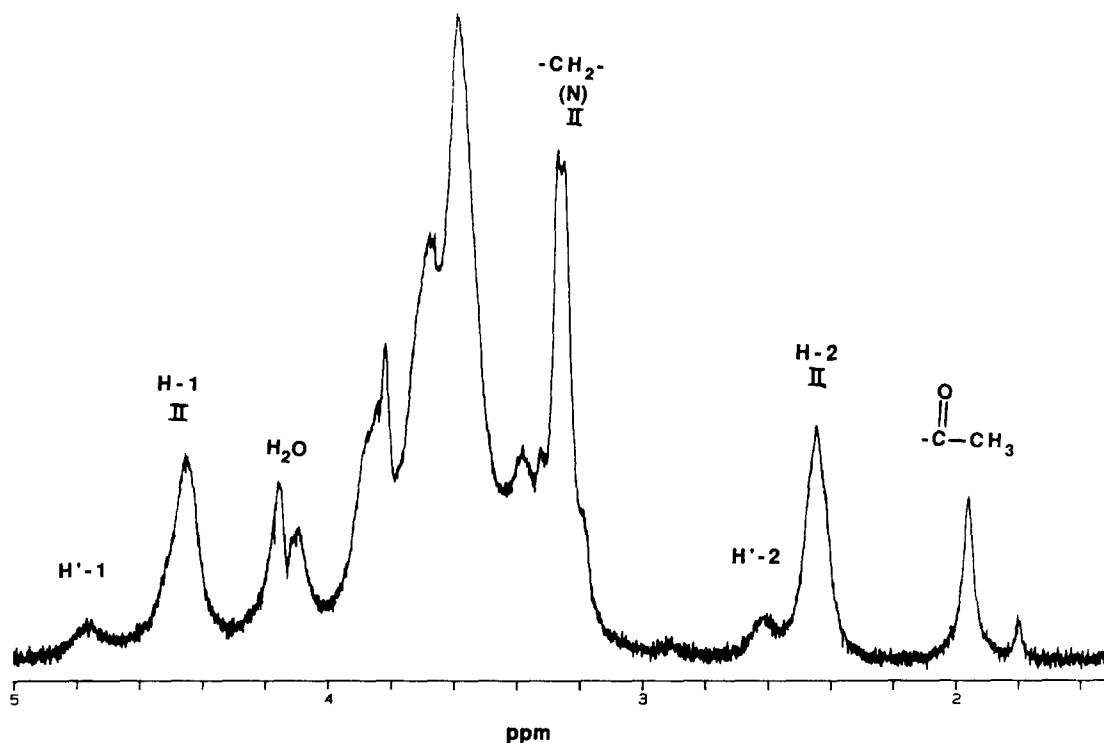


Fig. 4.  $^1H$  NMR spectrum for a *N*-carboxymethylated chitosan in  $D_2O$ .

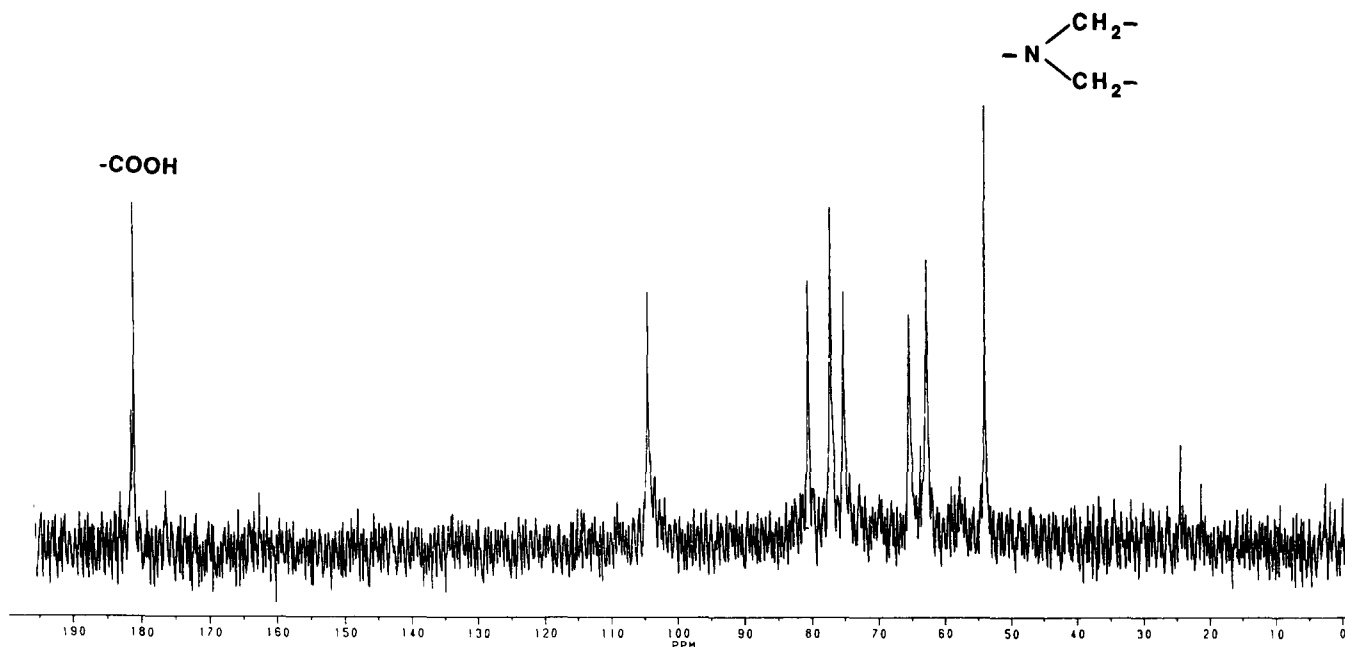


Fig. 5.  $^{13}\text{H}$  NMR spectrum for the *N*-carboxymethylated chitosan in  $\text{D}_2\text{O}$ .

tion over the whole pH range. Compared with literature data from Muzzarelli (Muzzarelli *et al.*, 1982) and Vercellotti (St Angelo *et al.*, 1992), it appears that they usually observed the formation of a gel with only 15–20% water soluble product; the insoluble material may originate from a low degree of substitution or secondary reactions such as crosslinking.

## CONCLUSION

Water soluble chitosan derivatives are prepared using techniques which allow homogeneous chemical modifications. Characterization of such compounds is presented particularly using NMR spectroscopy and viscosity measurements.

A new method for deacetylation of chitin is proposed allowing a nearly fully deacetylated derivative completely soluble in the presence of acetic acid to be obtained. The role of added acid for chitosan solubilization is mentioned and H-1 and H-2 NMR signals are characterized depending upon the acidic conditions adopted.

Quaternized chitosans are also prepared under conditions where polysaccharide degradation is minimized. A high degree of quaternization ( $\tau \sim 0.4$ ) is obtained in a single step reaction and the corresponding polymer is perfectly water soluble.

Moreover it is possible to propose nearly completely *N*-dicarboxylated chitosans, as demonstrated by  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy, which are also completely water soluble.

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