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# NMR study of the phosphonomethylation reaction on chitosan

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**Abstract**

*N*-Phosphonomethylation of chitosan reaction was studied and optimized using different reaction conditions. NMR spectroscopy was an important tool in this work to study this reaction and the α-aminomethylphosphonic acid function introduced onto chitosan was unequivocally characterized by $^{31}P$ and $^{13}C$ NMR analyses. But surprisingly, whatever the reaction conditions used, *N*-phosphonomethylation reaction of chitosan cannot be dissociated from a side reaction: the *N*-methylation reaction of chitosan. A mechanism was proposed to explain the formation of this side product.


*Keywords:* Chitosan; Phosphonomethylation; NMR

## 1. Introduction

Chitosan is a cationic natural biopolymer produced by alkaline *N*-deacetylation of chitin, the most abundant natural polymer after cellulose. It ideally consists of 2-amino-2-deoxy-(1-4)-β-D-glucopyranose residues (D-glucosamine units) and has no or small amount of *N*-acetyl-D-glucosamine units. Chitosan and derivatives are used in various fields [1,2]: treating water [3–5], biomedical [6–12], cosmetic [13], agricultural [14], food industrial [15,16]. It shows some biological activities such as immunological [17], antibacterial [18], and wound healing activity [19]. Moreover, chitosan is non-toxic and biodegradable [20,21].

Chemical modification of chitosan to generate new biofunctional materials is of prime interest because the modification would not change the fundamental skeleton of chitosan, would keep the original physicochemical and biochemical properties and finally would bring new properties depending on the nature of the group introduced.

Several techniques to obtain phosphate derivatives of chitosan have been proposed due to the interesting biological and chemical properties of such compounds. Among other, it could exhibit bactericidal [18] and metal chelating properties [22]. Introduction of groups such as phosphonic acid or phosphonate onto chitosan by reaction of phosphorylating agent onto the amino groups are known to increase the chelating properties [23–25] of chitosan and could modify its solubility. Phosphorylation of hydroxyle functions of chitosan to give phosphonate has been studied according two main ways (Scheme 1).

In one hand, the reaction is carried out between chitosan hydroxyle functions and phosphorus peroxyde in the presence of methanesulfonic acid [26–29]. On the other hand, the chitosan hydroxyle functions are reacted with phosphoric acid in the presence of urea [30]. The use of such derivatives concerns mainly biomedical [28,30] or metal chelating fields [26,27,29]. Phosphate derivatives of chitosan may also be obtained by interpolymer linkage of chitosan with tripolyphosphate or polyphosphate [31,32].

Few work deals with the introduction of α-aminomethylphosphonic acid functions onto chitosan using the Kabachnik-Fields reactions [33,34] in spite of the interest of such groups [23–25].

We describe in this work, our contribution to the synthesis of *N*-methylene phosphonic acid chitosan. A large part of this work consists on one hand, in variing the experimental conditions to optimize the synthesis, and on

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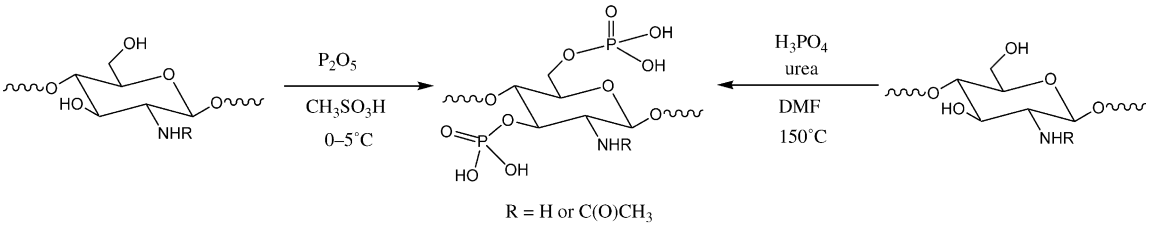



Scheme 1.

the other hand, in a complete NMR study of the reaction products. The formation of *N*-methylene phosphonic acid chitosan was unequivocally shown up by $^{31}P$ and $^{13}C$ NMR. Surprisingly a side product is revealed in the same time and we proposed a mechanism to explain its formation.

## 2. Experimental section

Chitosan obtained from Fluka is purified by dissolution in aqueous hydrochloric acid (0.2%) to get a solution with a polymer concentration of 1% (w/v) and is precipitated in aqueous NaOH solution (pH > 7). The residue is washed several times with deionized water to attain the water conductivity and finally freeze-dried. The viscosity-average molar mass ($\bar{M}_v$) is 330,000 g mol$^{-1}$ (determined by viscosimetry) [35] and the degree of deacetylation determined by $^{1}H$ NMR is 80% [36].

Phosphorus acid and formaldehyde (aquaous solution, 37%) were Acros products and used as received. Acetic acid and acetone were used without further purification.

$^{31}P$ and $^{13}C$ NMR spectra were recorded in $D_2O$ using a Bruker DRX400 Infrared spectra of chitosan and modified chitosan were obtained as KBr pellets using a Perkin–Elmer 16 PC spectrometer.

### 2.1. Synthesis of N-methylene phosphonic acid chitosan

The procedure of synthesis is described in Scheme 2. Typically, chitosan (0.5 g, 3.735 mmol corresponds to 2.988 mmol of $NH_2$, 1 $NH_2$ eq.) is dissolved in 50 mL of acetic acid 0.2 N. Phosphorous acid (4.9 g, 59.7 mmol, 20 eq.) and formaldehyde (4.7 mL, 59.7 mmol, 20 eq.) are added simultaneously to the former solution. The mixture is heated until 70 °C under nitrogen for 24 h. The solution is poured into acetone to precipitate polymer and this latter is washed with acetone in a soxhlet for 48 h to remove unreacted phosphorous acid and dried under reduced pressure.

## 3. Results and discussion

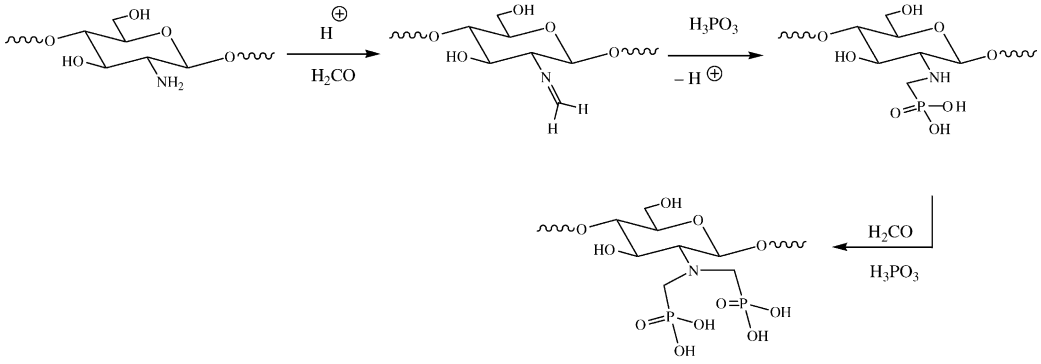
The reaction is conducted with a large excess of both phosphorous acid and formaldehyde at 70 °C (Scheme 2).

Nevertheless, the modified chitosan is ninhydrin positive, showing that the reaction of *N*-phosphonomethylation is not complete. The obtained white solid is soluble in water and acidic solution.

This product has been characterized by IR and NMR spectroscopy.

As it could be expected, IR spectrum (Fig. 1) shows the characteristic bands at 2500–3500 cm$^{-1}$ (υP–OH), at 1226 cm$^{-1}$ (δP=O) and at 924 cm$^{-1}$ (δP–O in P–OH). But the IR analysis cannot confirm unequivocally that the reaction occurred.

A $^{31}P$ NMR spectrum (Fig. 2) of the product shows the following signals: a peak at 0 ppm corresponds to the phosphoric acid $H_3PO_4$, a peak at +3.3 ppm is attributed to




Scheme 2.

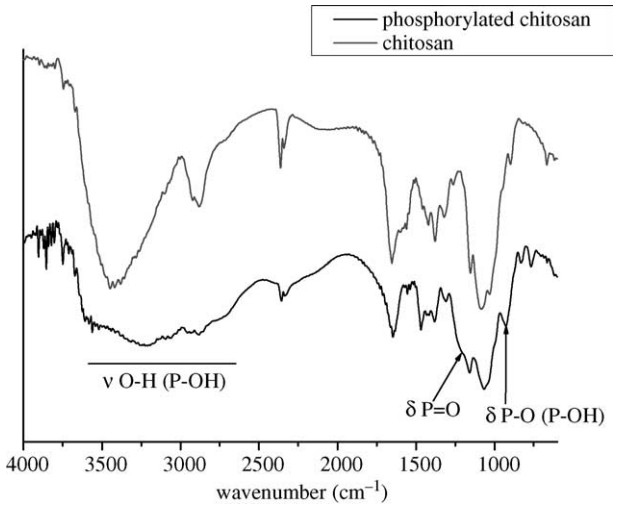



Fig. 1.

phosphorous acid $H_3PO_3$, a peak at +7 ppm corresponds to the α-aminomethylphosphonic acid function [37].

The presence of residual phosphorous acid shows that strong interactions occur between chitosan and $H_3PO_3$ that cannot be totally eliminated in spite of the soxhlet treatment. The presence of phosphoric acid could result from the oxidation of $H_3PO_3$ or from a side reaction. Above all, $^{31}P$ NMR analysis shows that the phosphonomethylation reaction occurs with the presence of the signal at +7 ppm [37].

Different experiments to evaluate the synthesis conditions were carried out and gave the following results:

- if the reaction is conducted with a stoichiometric ratio of $H_3PO_3$ and formaldehyde, or if $H_3PO_3$ and formaldehyde are not added simultaneously, then the phosphonomethylation reaction does not occur.
- if the reaction is conducted at a temperature inferior to 70 °C, no reaction occurs.
- if the reaction is conducted during 6 h, only a small quantity of the *N*-methylene phosphonic chitosan is detected and a best yield is obtained with a time reaction of 24 h.

In $^{13}C$ NMR spectrum of the reaction products, main peaks are located between 40 and 100 ppm (Fig. 3).

Out of this area, can be found peaks corresponding to the carbonyl and methyl groups of the *N*-acetyl-D-glucosamine units of chitosan at 171.7 and 21.1 ppm, respectively. The analysis of the spectrum (Fig. 3) shows that peaks at 56.8, 61.5, 69.3, 75.5 and 77.8 ppm are attributed to $C_2$, $C_6$, $C_3$, $C_5$ and $C_4$, respectively of the pyranose cycle [38] and the peaks located at 95.9, 97.3 and 98.2 ppm are attributed to the anomeric carbons. Other signals can be observed as two singlets at 42.5 and 43.0 ppm and one doublet at 54.2 ppm with a large scalar coupling (143 Hz). A DEPT NMR experiment allows the attribution of these signals; the doublet at 54.2 ppm corresponds to a secondary carbon and can be unequivocally assigned to the methylene group of an α-aminomethylphosphonic acid function. Indeed, the methylenic carbon is coupled with the phosphorus atoms to give a doublet with a large scalar coupling ($^1J_{CP}$= 143 Hz) [37]. The presence of one peak at +7 ppm in $^{31}P$ NMR and of one doublet at 54.2 ppm in $^{13}C$ NMR lets suppose that only occurs the monophosphonomethylation of the amino groups.

Peaks at 42.5 and 43 ppm correspond to primary or tertiary carbons and cannot be attributed to the *N*-methylene phosphonic-D-glucosamine units but to a side product. The $^{13}C$ NMR analysis confirms the *N*-phosphonomethylation reaction and reveals the formation of a side product.

The $^1H$ NMR spectrum (Fig. 4) shows the peaks attributed to chitosan units, the peaks in the anomeric area from 4.4 to 4.8 ppm and two additional peaks at 2.7 and 2.8 ppm.

To analyse this spectrum, a 2D $^1H$ $^{13}C$ NMR experiment gives the following informations (Fig. 5).

Anomeric carbons between 95.9 and 98.2 ppm are

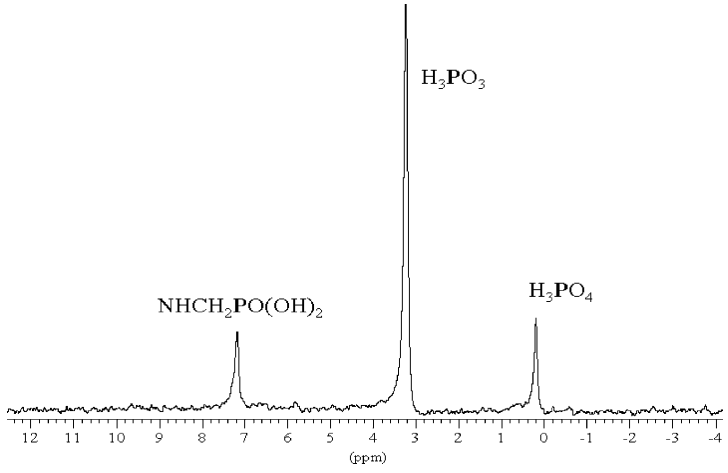



Fig. 2.

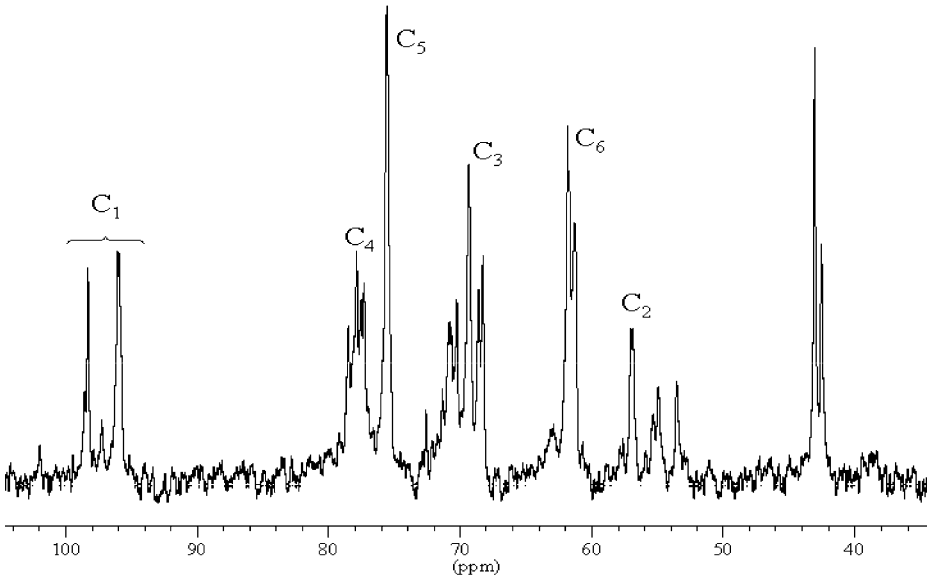



Fig. 3.

correlated to the anomeric protons between 4.4 and 4.8 ppm. The anomeric proton and carbon of the *N*-acetyl-D-glucosamine unit are located at 4.4 and 97.3 ppm, respectively. Anomeric proton and carbon of the D-glucosamine unit are located at 4.5 and 98.2 ppm, respectively. Finally, anomeric proton and carbon of the phosphorylated unit shift at 4.8 and 95.9 ppm, respectively.

The signal relative to the methylene group of the α-aminomethylphosphonic acid function at 54.2 ppm in $^{13}C$ NMR spectrum is correlated to the large signal between 2.9 and 3.8 ppm in $^{1}H$ NMR spectrum, that mainly corresponds to the protons of pyranose cycle indicating that protons attributed to this function cannot be clearly distinguished. Finally peaks at 2.7 and 2.8 ppm in $^{1}H$ NMR spectrum are correlated with the two singlets at 42.5 and 43 ppm in $^{13}C$ NMR spectrum and correspond also to a side product.

The $^{1}H$ and $^{13}C$ NMR analyses totally agree that a side product is formed during the phosphonomethylation reaction of chitosan.

We propose the mechanism outlined in Schemes 3 and 4 to explain the formation of this side product. The mechanism is based on the Leuckart–Wallach reaction

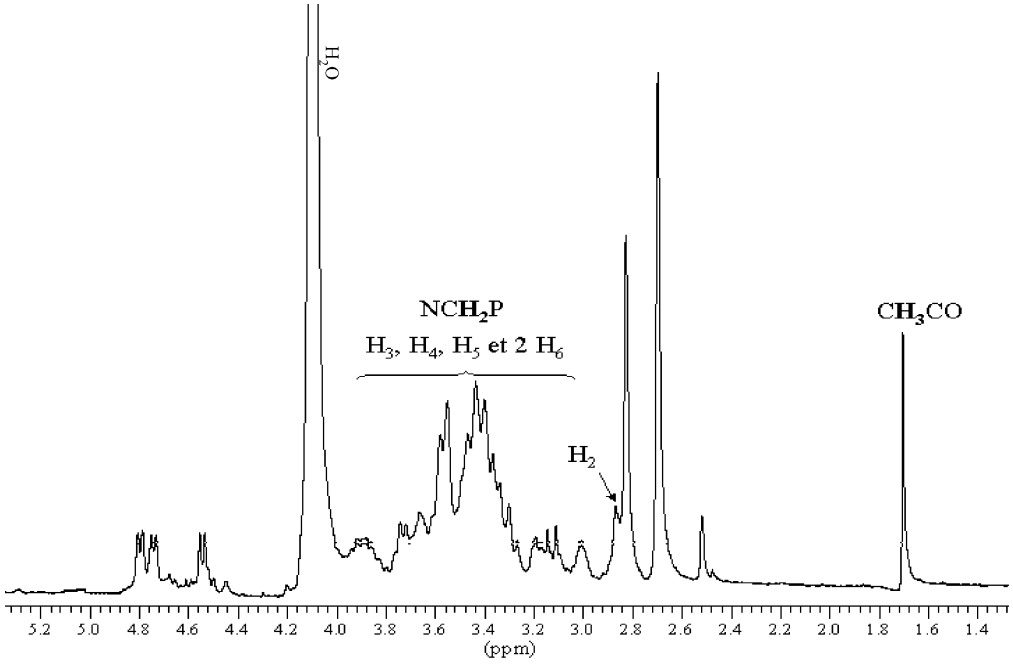



Fig. 4.

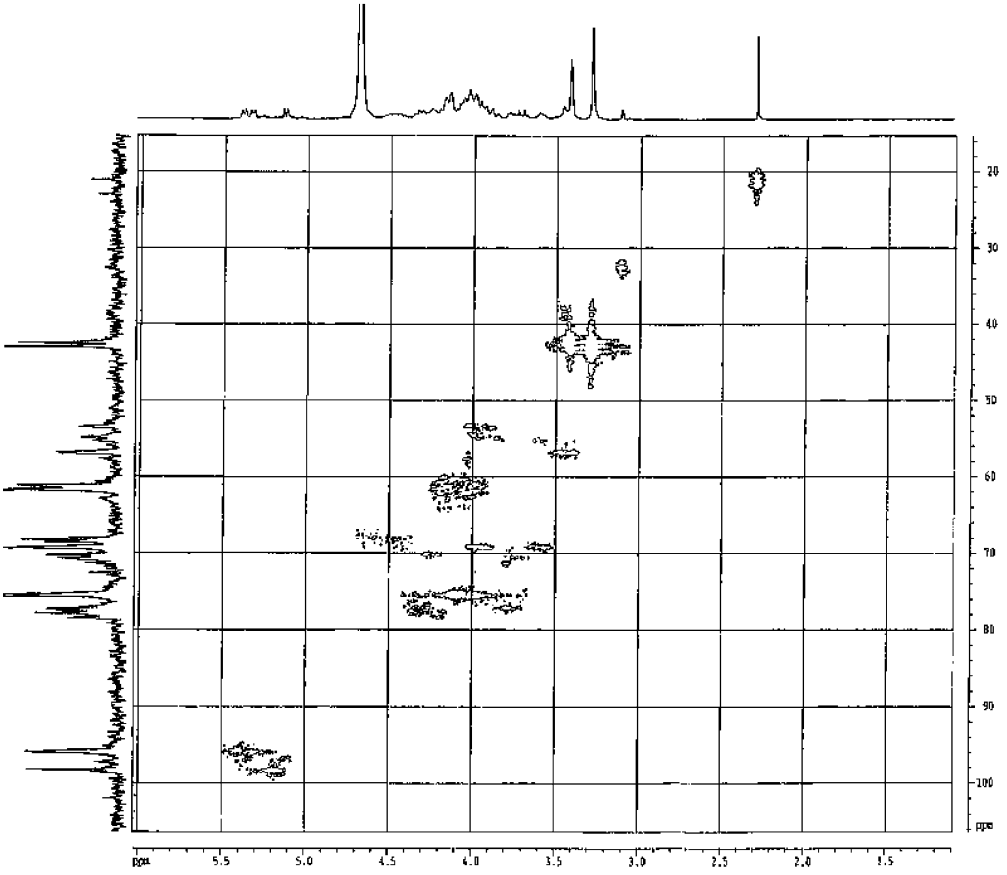


Fig. 5.

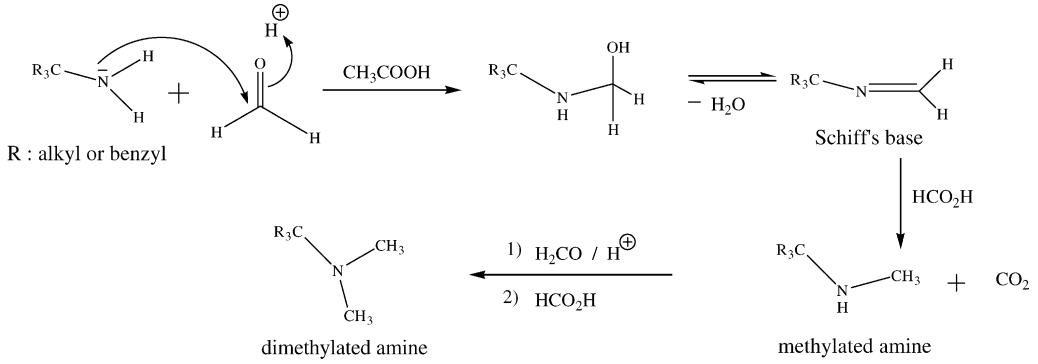
that consists in the reduction of the Schiff's base in the presence of formic acid [39,40] as described in Scheme 3.

Based on this reaction, we suppose that in the case of the reaction of chitosan with formaldehyde and phosphorous acid, $H_3PO_3$ has a similar behaviour than formic acid in the Leuckart–Wallach reaction to reduce the Schiff's base (Scheme 4) leading to *N*-methyl and *N,N*-dimethylchitosan.

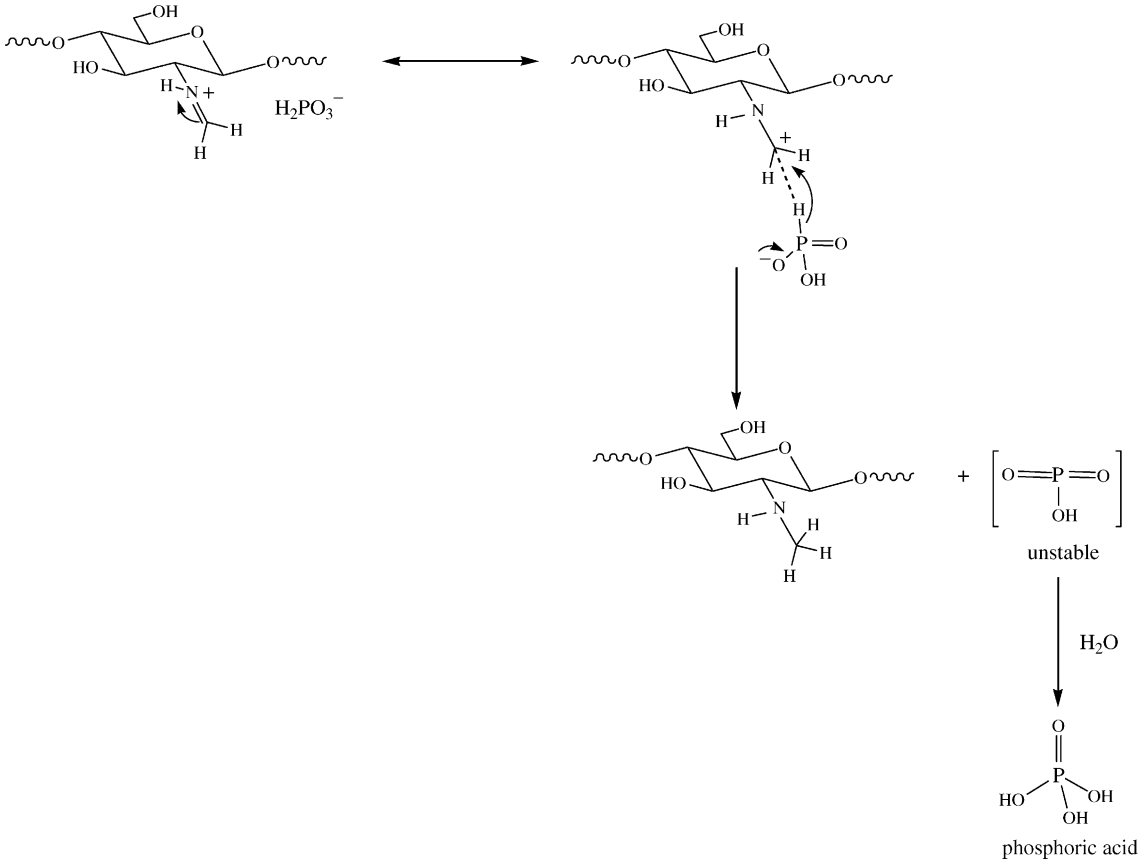
The new peaks observed in $^{1}H$ and $^{13}C$ NMR spectra are in accordance with the structure of the side products proposed. Peaks at 2.7 and 2.8 ppm in $^{1}H$ NMR spectrum and peaks at 42.5 and 43 ppm which correspond to tertiary carbons in $^{13}C$ NMR spectrum, are attributed to *N*-methyl and *N,N*-dimethyl-D-glucosamine units. Moreover, the mechanism shows the formation of $H_3PO_4$ that explain the presence of a peak at 0 ppm in $^{31}P$ NMR spectrum (Fig. 2).

If the phosphonomethylation reaction is carried out at a temperature inferior to 70 °C, or with a stoichiometric ratio of $H_3PO_3$ and formaldehyde or with a time reaction inferior to 6 h, only the *N*-methyl and *N,N*-dimethyl-chitosan are formed.

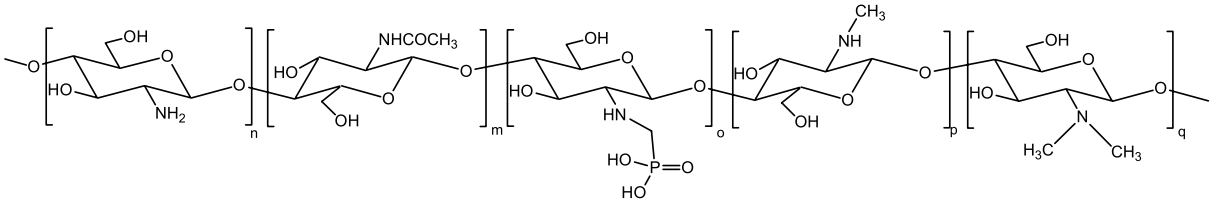
All these spectroscopic analyses allow us to conclude that the structure of the modified chitosan corresponds in fact to the following structure.




Scheme 3.




Scheme 4.



## 4. Conclusion

The experimental conditions for the phosphonomethylation of chitosan amino functions have been evaluated to conclude that this reaction is possible if it is carried out with a large excess of phosphorous acid and formaldehyde added simultaneously and heated for at least 6 h (better yield is obtained with 24 h) at 70 °C. The introduction of the α-aminomethylphosphonic acid function onto chitosan has been unequivocally confirmed by the peak at +7 ppm in $^{31}P$ NMR spectrum and the doublet at 54.20 ppm associated with a large scalar coupling in $^{13}C$ NMR. But $^{13}C$ and $^{1}H$ NMR analyses revealed the presence of a side reaction, which occurs according to a mechanism based on the Leuckart–Wallart reaction, leading to the *N*-methyl and *N,N*-dimethyl chitosan.

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