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Modified chitosan carrying phosphonic and alkyl groups

V.M. Ramos[a], N.M. Rodríguez[b], M.S. Rodríguez[a], A. Heras[c], E. Agulló[a,*]

[a]*Laboratorio de Investigaciones Básicas y Aplicadas en Quitina (LIBAQ), Departamento de Química, Universidad Nacional del Sur, Avenida Alem, 1253-8000 Bahía Blanca, Argentina*

[b]*Instituto de Investigaciones en Química Orgánica (INIQO), Departamento de Química e Ingenieria Química, Universidad Nacional del Sur, Avenida Alem, 1253-8000 Bahía Blanca, Argentina*

[c]*Unidad de RMN, Departamento de Química Física, Facultad de Farmacia, Universidad Complutense, Paseo Juan XXIII, 1-28040 Madrid, Spain*

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Abstract

The introduction of an alkyl chain onto a water soluble modified chitosan (*N*-methylene phosphonic chitosan) offer the presence of hydrophobic and hydrophilic branched for controlling solubility properties. A simple methodology for the preparation of a new chitosan derivative surfactant, *N*-lauryl-*N*-methylene phosphonic chitosan, has been developed. Its chemical identity was determined by FTIR and confirmed by ^{1}H and ^{13}C NMR. The degree of lauryl substitution was estimated to be 0.33. As a result of the amphiphilic properties, like surface activity typical for surfactants, this derivative opens new perspectives in pharmaceutical and cosmetic field.

Keywords: Chitosan amphiphilic derivative; *N*-methylene phosphonic chitosan; *N*-lauryl-*N*-methylene phosphonic chitosan; ^{1}H NMR; ^{13}C NMR

1. Introduction

Chitosan is a polysaccharide formed primarily from repeating residues of D-glucosamine, having primary amino groups, obtained by *N*-deacetylation of the natural polymer chitin. It is a remarkable biomaterial because of its numerous biological and immunological activities (Kumar, 2000). Moreover, chitosan itself is a nontoxic and biodegradable biopolymer, and therefore, a wide variety of applications of chitosan for biomedical materials have been reported over the last decades (Muzzarelli, 1997).

Its highly reactive free amino group offer great potential for further derivatization (Kurita, Kojima, Nishiyama, & Shimojoh, 2000; Sashiwa, Shigemasa, & Roy, 2000). Chitin and chitosan become water soluble through a chemical modification (Kubota, Tatsumoto, San, & Toya, 2000; Muzzarelli, Ilari, & Petrarulo, 1994). A previous work, has stated, that the incorporation of methylene phosphonic groups into chitosan allowed solubility in water under neutral conditions like NMPC (Agulló, Ramos, Rodríguez, & Heras, 2000; Heras, Rodríguez, Ramos, & Agulló, 2001).

Hydrophobically modified water soluble polymers or so-called associated polymers are important in a number of areas including enhanced oil recovery, and for formulation in coatings and personal care items (Nyström, Kjoniksen, & Iversen, 1999).

The introduction of an alkyl chain also offers several possibilities in molecular design. The utility of modifying chitosan with hydrophobic branches for controlling solubility properties has also been demonstrated (Holme & Hall, 1991; Nishimura et al., 1993). Recently, it was reported that the chitosan derivatives with both hydrophobic groups (long acyl groups) and hydrophilic groups (sulfate groups) could form micelles and solubilize hydrophobic compounds (Yoshioka, Nonaka, Fukuda, & Kazama, 1995). Moreover, it has been reported that polymer–micelle is better than other carriers for use as passive targeting carrier of anticancer drugs. *N*-lauryl carboxymethyl chitosan with both hydrophobic and hydrophilic groups was studied by Miwa et al. (1998) in connection with delivery of taxol to cancerous tissues. Other examples are the polymeric vesicles to encapsulation hydrophobic compounds like bleomycin (Sludden, Uchegbu, & Schatzlein, 2000).

The possibility to modify the water soluble NMPC allows to obtain an amphiphilic system in which the hydrophobic moiety counterbalances the electrostatic

* Corresponding author. Tel.: +54-291-4595-100; fax: +54-291-4595-110.

E-mail address: eagullo@criba.edu.ar (E. Agulló).

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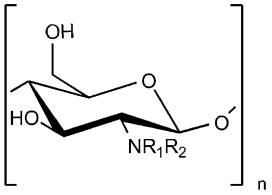
[-A-/-B-/-C-/-D-/-E-/...]$_n$



	R_1	R_2
A	- H	- CO-CH_3
B	- H	- H
C	- H	- CH_2-PO_3H_2
D	-CH_2-PO_3H_2	- CH_2-PO_3H_2
E	- H	- $(CH_2)_{11}$-CH_3

Fig. 1. Chemical structure of *N*-lauryl-*N*-methylene phosphonic chitosan.

repulsion but it also gets more tensioactive properties (Holme & Hall, 1991).

At the same time, chitosan has been employed as tissue repair preparations because it possess numerous biofunctional features: stimulates skin cicatrization, influences collagen deposition, being easily biodegradable by the lysosyme and thus easily resorbable, and it has bacteriostatic and bactericidal activities (Tucci, Belmonte Mattioli, Ricotti, & Biagini, 1999).

In many cases, emulsion stabilization is done by specially designed polymers which have hydrophilic and hydrophobic segments. They have been suggested as emulsifiers because they are absorbed at the interfacial surface stabilizing the emulsion, for this reason, these systems are promising in cosmetics and pharmaceuticals applications (Desbrieres, Rinaudo, Babak, & Vikhoreva, 1997; Özer, Balogen, Ertan, Muguet, & Yazan, 2000). In a previous work (Schulz, Rodríguez, Del Blanco, Pistonesi, & Agulló, 1998) the emulsifier properties of chitosan giving a multiple w/o/w emulsions was demonstrated.

In this paper, we reported the successful preparation of an *N*-alkyl derivative of the water soluble NMPC using a reductive *N*-alkylation with lauryl aldehyde to obtain a new amphiphilic hybrid material of synthetic and natural polymers.

2. Experimental

Preparation of chitin and chitosan. Chitin was isolated from shrimp shells waste from (*Pleoticus müller*i). The material was homogenized and the product was rinsed in order to remove the organic material. After this, it was treated with 9% (w/w) NaOH at 65 °C for 90 min to remove proteins, demineralized by treatment with 10% (v/v) HCl at 20 °C for 15 min, washed, and then dried. Chitosan was prepared directly by heterogeneous deacetylation of chitin at 136 °C with 50% (w/w) NaOH for 1 h. Its characteristics were: acetylation degree 6.0%, moisture 5.3%, ash 0.39%, viscosity 30 mPa s (1% w/v in 1% acetic acid at 25 °C).

Synthesis of N-methylene phosphonic chitosan. Chitosan solution 2% (w/v) in glacial acetic acid 1% (v/v) was prepared. By using one part (by weight) of chitosan and one part of phosphorous acid (by weight) dissolved in water was added dropwise with continuous stirring for an hour. Then the temperature of the reaction vessel was raised up to 70 °C and one part of formaldehyde 36.5% (by weight) was added dropwise over 1 h with reflux. Heating was protracted at the same temperature for 30 h. The clear pale yellow solution was dialyzed against demineralized water for 48 h or until pH of water was raised to 6.8, in dialysis tubing with a cut-off value of 12,400 Da. Finally, the solution was frozen and freeze-dried (Agulló et al., 2000; Heras et al., 2001). Its characteristics were: moisture 5.9%, ash 6.6%, viscosity 6.0 mPa s (1% w/v at 25 °C).

Synthesis of LMPC. NMPC (1 g) was suspended in distilled water–methanol in a ratio 1:1 (100 ml), lauryl aldehyde (1.5 g) was added and stirred for 30 min. Reduction was carried out with a sodium borohydride solution (0.5 g dissolved in 10 ml of water) in small portions for 2 h with mechanical stirring. The mixture was stirred at room temperature overnight. The reaction mixture was then neutralized with HCl 5 M solution and the LMPC was precipitated with methanol. The precipitate was filtered and washed with 90% methanol/water, methanol, hexane and acetone. LMPC (0.9 g) was obtained in this fashion with a moisture content 4.7% and ash 3.0%.

Another possibility is to obtain the LMPC sodium salt by dialyzing against demineralized water for 48 h or until pH of water raised to 6.8 (dialysis tubing with a MW cut-off value of 12,400) direct after the reduction with sodium borohydride solution. After that the compound was extracted with organic solvents to remove excess of aldehyde. Finally, the solution was frozen and freeze-dried (moisture 6.0% and ash 4.4%).

X-ray diffraction spectrometry. The material in the powder form was submitted to X-ray diffraction spectrometry by using a vertical powder diffractometer; the source was a rotating anode generator Rigaku Denki RU-300 and Ni filtered Cu Kα radiation ($\lambda = 0.154$ nm).

NMR spectroscopy. ^{13}C and ^{1}H NMR measurements were performed on a AMX500 Bruker NMR spectrometer under a static magnetic field of 125 and 500.13 MHz, respectively, at 70 °C. For those measurements, 10 mg of sample was introduced into a 5 mm ϕ NMR test tube, to which 0.5 ml of 2% (w/w) DCl/D_2O solution was added, and finally the tube was kept at 70 °C to dissolve the polymer in solution.

IR spectroscopy. Spectra were recorded with a Perkin Elmer Paragon 1000 (FTIR) spectrometer on translucent disk obtained by pressing the ground material with KBr.

Scanning electron microscopy. A JEOL JSM-35 CF scanning electron microscope was used to characterize the

Table 1
Solubility of LMPC acid form and sodium salt

Solvent	NMPC	LMPC acid form	LMPC sodium salt
H_2O	++	−	+
HCl (0.1 M)	±	++	+
NaOH (0.1 M)	++	±	+
EtOH	±	±	++
Acetone	±	±	++
Pyridine	−	++	+
DMSO	−	+	++
DMF	−	−	+

The sample (10 mg) and 5 ml of solvent (60 °C); ++, soluble; +, partially soluble; ±, swelled; −, insoluble.

surface of LMPC particles. The samples were prepared by gold coating using a sputter coater, Pelco 91000.

3. Results and discussion

The introduction of a hydrophobic alkyl chain onto NMPC's free amino groups by a reductive amine reaction leads to a new chitosan derivative surfactant. In this case, the introduction of C_{12} chain gives rise to *N*-lauryl-*N*-methylene phosphonic chitosan (LMPC). LMPC incorporated *N*-methylene phosphonic groups as hydrophilic moieties and lauryl groups as hydrophobic one. Fig. 1 shows the chemical structure of LMPC.

NMPC with a degree of substitution of 35% and the rest of the amino groups in the free form was obtained using the improved method (Heras et al., 2001) and then was *N*-alkylated with lauryl aldehyde (Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1983). The aldehyde was added to an aqueous suspension of NMPC resulting a viscous clear solution. The resulting pH was 4.9 and when sodium borohydride was added to stirred solution it became milky. The reaction can be manipulated with mild conditions of pH and temperature.

In front of NMPC, the alkyl substitution shows a decreased solubility in water, with evidence of micelles formation, and an increased one in organic solvents. In addition, by evaporation of LMPC concentrate solution the appearance of it becomes a brilliant white cream like a soft soup. The results of solubility test for LMPC (sodium salt) and LMPC are summarized in Table 1.

The alkyl branched chitosan shows a slight solubility in organic solvent at room temperature and is completely soluble at higher temperature solvents.

The infrared spectrum shows a 2923 and 2854 cm^{-1} bands, due to the aliphatic chain together with a contribution at 1454 and 720 cm^{-1}, with these exceptions, the spectrum of LMPC is coincident with that of NMPC.

In order to follow the modification due to the *N*-alkylation, the ^1H NMR and ^{13}C NMR spectra of chitosan, NMPC and LMPC were performed (Figs. 2 and 3).

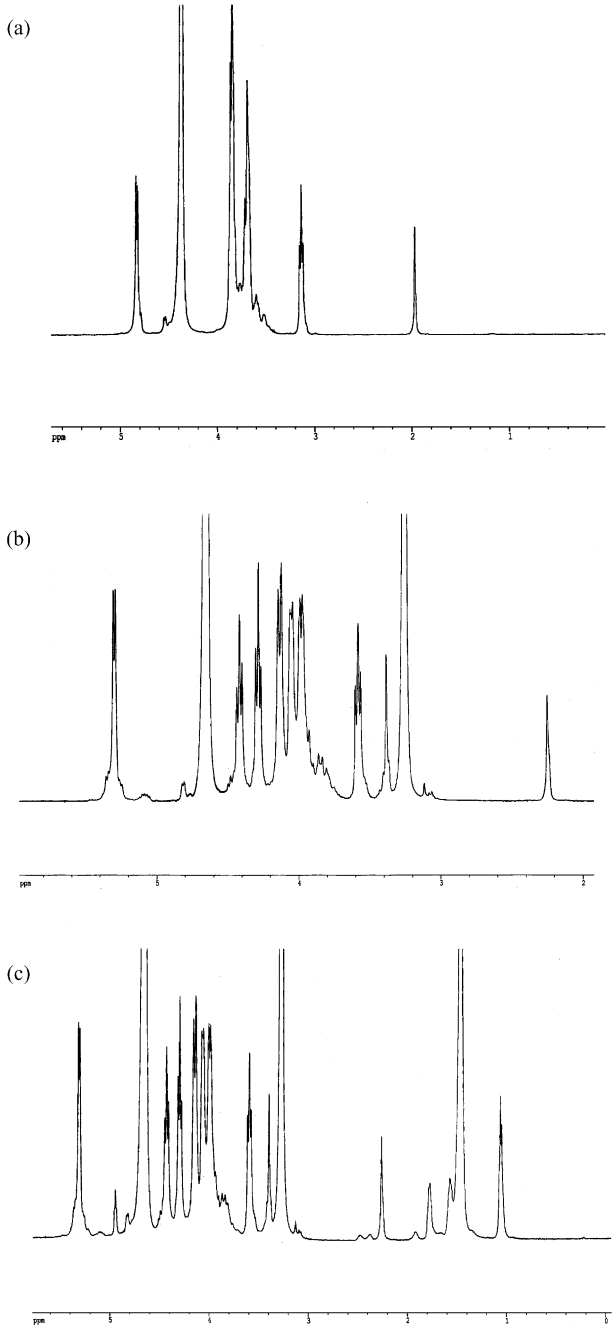


Fig. 2. ^1H NMR spectrum of chitosan (a), NMPC (b), and LMPC (c) dissolved in 2% (w/w) DCl/D_2O. Polymer concentration 20 g/l.

The assignments and chemical shifts of chitosan are: ^1H NMR $\delta = 4.85$ (H_1), $\delta = 3.18$ (H_2), $\delta = 3.45$–3.90 (H_3 H_4 H_5 H_6) and $\delta = 1.98$ ($NCOCH_3$). ^{13}C NMR $\delta = 101.0$ (C_1), $\delta = 58.0$ (C_2), $\delta = 73.5$ (C_3), $\delta = 79.5$ (C_4), $\delta = 77.5$ (C_5), $\delta = 64.0$ (C_6).

The preparation of NMPC reported in a previous work was with 7 h (Heras et al., 2001) but here we used 30 h reaction. In this case, we obtained only one substitution form (H1 and C1) as shows the ^1H NMR and ^{13}C spectra. NMPC: ^1H NMR $\delta = 5.3$ (H_1), $\delta = 3.58$ (H_2), $\delta = 3.78$–4.25 (H_3 H_4 H_5 H_6), $\delta = 3.25$ ($N–CH_2–$) and $\delta = 2.28$

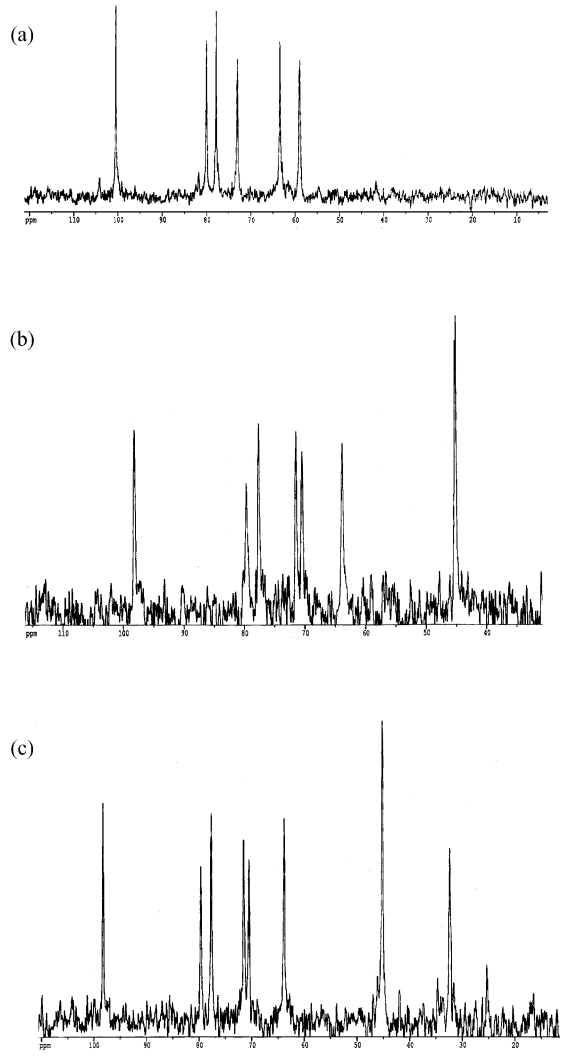


Fig. 3. ^{13}C NMR spectrum of chitosan (a), NMPC (b), and LMPC (c) dissolved in 2% (w/w) DCl/D_2O. Polymer concentration 20 g/l.

($NCOCH_3$). NMPC: ^{13}C NMR $\delta = 98.0$ (C_1), $\delta = 70.4$ (C_2), $\delta = 72.5$ (C_3), $\delta = 80.0$ (C_4), $\delta = 76.5$ (C_5), $\delta = 64.0$ (C_6) and $\delta = 45.0$ ($-CH_2-P$).

LMPC's ^1H NMR spectrum presents the signals at 1.0, 1.5 and 1.8 ppm attributed to the $-CH_3$, $-(CH_2)_{10}$ and $-N-CH_2-$ groups of the alkyl chain, respectively. The signal at 4.9 ppm is attributed to the CH–N=C– specie (Silverstein, Bassler, & Morrill, 1991). Besides this modification, the LMPC's ^1H NMR spectrum is just the same with that of the one NMPC. Furthermore the chemical shifts assignments

Table 2
Elemental analyses and degree of substitution (DS) of chitosan and its derivatives

	C	N	C/N[a]	H	P	DS
Chitosan	40.87	7.61	6.30	6.68		
NMPC	34.68	5.15	7.86	7.10	7.93	1.56
LMPC	50.17	4.97	11.76	8.26	1.12	0.33

[a] The molar ratio of carbon to nitrogen.

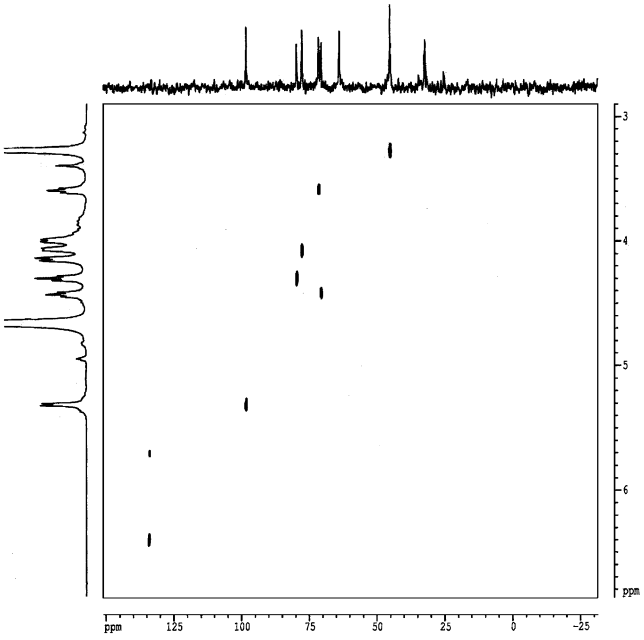


Fig. 4. $^1H-^{13}C$ NMR of LMPC.

for ^{13}C NMR were: $\delta = 97.5$ (C_1), $\delta = 70.4$ (C_2), $\delta = 71.5$ (C_3), $\delta = 80.0$ (C_4), $\delta = 76.5$ (C_5), $\delta = 64.0$ (C_6), $\delta = 45.0$ ($-CH_2-P$), $\delta = 42.4$ ($-N-CH_2$), $\delta = 32.5$ $(-CH_2-)_9$, $\delta = 22.6$ ($-CH_2-$) and $\delta = 13.9$ ($-CH_3$) (Pretsch, Clerc, Seibl, & Simon, 1983).

Assignments of ^1H and ^{13}C NMR spectra of LMPC were confirmed by two-dimensional heteronuclear chemical shift correlation spectroscopy (Fig. 4).

The degree of substitution of the LMPC was calculated by comparing the C and N molar ratio obtained from the elemental analysis in each derivative (Table 2). The increase in the molar ratio indicates the increasing carbon into the chitosan chain since the monosaccharide includes one nitrogen. The degree of phosphonomethylation and laurylation were stipulated to be 1.56 and 0.33, respectively.

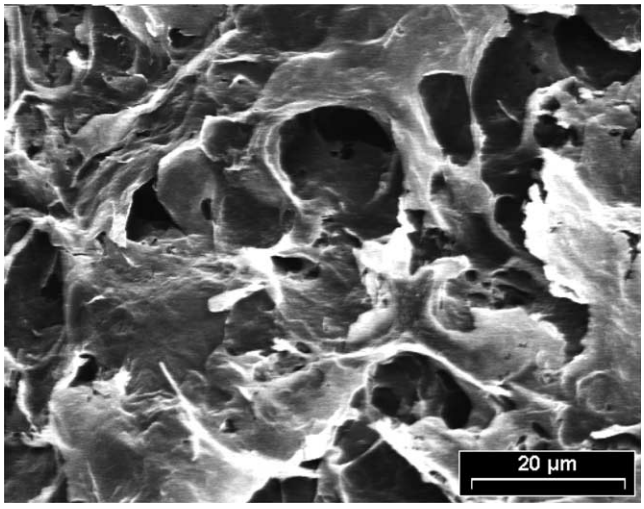


Fig. 5. LMPC electron micrograph (× 1500).

LMPC white powder has certain degree of crystallinity, its X-ray diffraction spectrum differ in terms of the 2θ value of the first peak as well as their intensities. The 2θ values of the chitosan from which it was obtained were $10°71'$ and $20°98'$; as a consequence of the *N*-phosphonomethylation, the first peak is shifted to lower 2θ values ($10°19'$) while the second peak appears at slightly higher 2θ value ($21°41'$). LMPC X-ray diffraction spectrum differ in terms of the 2θ value ($10°71'$ and $19°57'$) as well as with an increase in their intensities.

As shown in SEM photograph, the LMPC surface presents a slightly smooth structure (Fig. 5).

4. Conclusions

A methodology was developed for the preparation of a novel chitosan derivative surfactant carrying alkyl and phosphonic groups. Both *N*-phosphonomethylation and *N*-alkylation can be easily done under mild conditions.

On the water soluble NMPC, the presence of alkyl groups seems to weaken the hydrogen bond and provides good solubility in organic solvents. The chemical identity of LMPC was confirmed by FTIR and ^{13}C and ^{1}H NMR spectrometry.

Stabilization of emulsions may be done by specially surfactants like LMPC. Furthermore, it has positive influence to solubilize active hydrophobic compounds with essential roles in pharmaceutical and cosmetic field.

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