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N-methylene phosphonic chitosan. Effect of preparation methods on its properties

V.M. Ramos[a,*], N.M. Rodríguez[b], M.F. Díaz[a], 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]*Departamento de Química, Instituto de Investigaciones en Química Orgánica (INIQO), 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

N-methylene phosphonic chitosan is prepared from chitosan with different acetylation degrees and sources using three reaction times. The degree of substitution is not dependent on chitosan's source and degree of deacetylation but it depends on reaction time. Data are confirmed by ^{1}H NMR and FT-IR. A significant decrease of viscosity and molecular weight is observed due to derivatization. It is demonstrated that *N*-methylene phosphonate chitosan is a powerful chelating agent of transition metal and calcium ions.

Keywords: Phosphonomethylation; Chitosan soluble derivative; *N*-methylene phosphonic chitosan; ^{1}H NMR; Metal ions chelate

1. Introduction

Chitosan is a potential polysaccharide resource owing to its specific structure and properties (Kumar, 2000). The cationic nature of chitosan limits the versatility of aqueous solutions because addition of certain acids in excess quantity is required in order to form water-soluble chitosan salts. Many efforts have been reported to prepare functional derivatives of chitosan by chemical modifications, in order to increase the solubility in water (Dung, Milas, Rinaudo, & Desbrieres, 1994; Kubota, Tatsumoto, Sano, & Toya, 2000; Muzzarelli, 1988; Muzzarelli, Ilari, & Petrarulo, 1994; Muzzarelli, Ilari, & Tomasetti, 1993).

Removal of one or two hydrogen atoms of amino groups of chitosan and introduction of some hydrophilic substituent by chemical modification results in a solubility improvement in aqueous solvents.

A previous work has stated that the incorporation of methylene phosphonic groups into chitosan allowed solubility in water under neutral conditions without decreasing its filmogenic properties (Agulló, Ramos, Rodríguez, & Heras, 2000; Heras, Rodríguez, Ramos, & Agulló, 2001).

On the other hand in recent years, the ability of chitosan to chelate metal ions has been extensively studied and hypotheses about the binding sites were made. In the case of the hydrophilic chitosan derivatives, the binding ability of the polymer could be enhanced, thanks to increased solubility of the polysaccharide, presence of certain functions such as amino and carboxyl groups and depressed tendency to establish hydrogen bonds (Muzzarelli, 1988; Muzzarelli et al., 1998; Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982; Muzzarelli & Zattoni, 1986).

Based on the report by Schwarzenbach, Ackermann and Ruckstuhl (1949) phosphonic complexing agents have been considered as effective as or even more than those containing carboxylic groups. The aminoalkylphosphonic ligands ($-NH_2-CH_2-PO_3^{2-}$) have well-known strong chelating properties owing to the donor effect of the amine group ($-NH_2$) and the monodentate ligand $-PO_3^{2-}$ (Hendrickson, 1967). They have the tendency to form chelates in ring structure with the possibility of different conformations owing to the metal ion nature.

In our case, in the *N*-methylene phosphonic chitosan ($NH_2-CH_2-PO_3^{2-}$) the presence of an amine group from

* Corresponding author. Tel.: +54-2914-595100; fax: +54-2914-595110.

E-mail addresses: vramos@criba.edu.ar (V.M. Ramos).

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chitosan combines these two effects, increasing the metal-binding abilities (Westerback & Martell, 1956; Westerback, Rajan, & Martell, 1965).

In this paper to show a final characterization of this phosphonic derivative, the effects of different chitosan sources, degree of deacetylation and reaction-time on the physical–chemical behavior of *N*-methylene phosphonic chitosan (NMPC) are presented, together with preliminary data on chelating properties.

2. Experimental

Preparation of chitin and chitosan. Chitin was isolated from shells' waste from two different sources (*Pandalus borealis*, *Artemesia longinaris*). The material was homogenized and the product was rinsed in order to remove the organic material. It was then 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. The characteristics were: source *Pandalus borealis* acetylation degree 0.5%, particle size 1.5 mm, moisture 5.3%, ash 0.09%, viscosity 15 mPa s (1% w/v in 1% acetic acid at 25 °C); source *Artemesia longinaris* acetylation degree 0.8%, particle size 1.5 mm, moisture 4.6%, ash 0.47%, viscosity 30 mPa s (1% w/v in 1% acetic acid at 25 °C).

Synthesis of N-methylene phosphonic chitosan (*NMPC*) *and sodium N-methylene phosphonate chitosan* (*NMPC-Na*). Chitosan solution 2% (w/v) in glacial acetic acid 1% (v/v) was prepared. 1 part (by weight) of chitosan was used and 1 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 to 70 °C with reflux and 1 part of formaldehyde 36.5% (by weight) was added dropwise for 1 h. Heating was protracted at the same temperature for different period of time (7, 20 and 30 h). The 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. NMPC (acid form) was obtained in this fashion.

To obtain sodium NMPC-Na, the reaction mixture was then neutralized with sodium bicarbonate (final pH 7.0) and dialyzed against sodium hydroxide 0.1000 M and alternating with water, in dialysis tubing. Finally, the solution was frozen and freeze-dried.

Metal ion chelation and insolubilization. NMPC-Na (30 ml, 1.0% w/v) was dialyzed (cut-off value of 12.400 Da) against the metal salt solution (300 ml, 0.1000 M) during 24 h with constant stirring. The insoluble chelate was filtered on Whatman filter paper and analyzed by atomic absorption spectrometry.

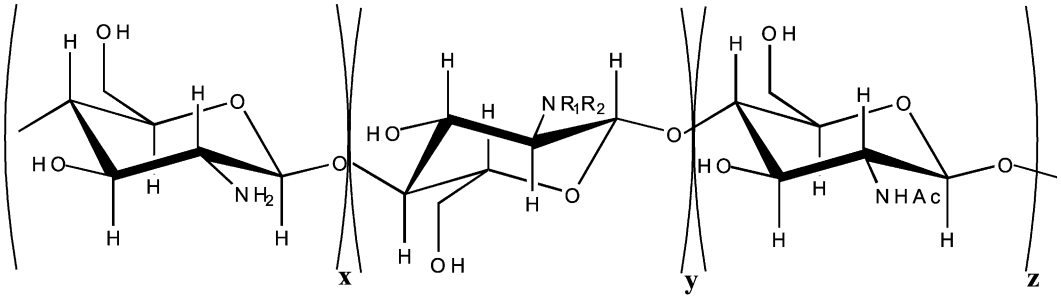
Atomic absorption spectrometry. Analyses were carried out with Sequential Plasma Shimadzu ICPS 1000 III spectrometer.

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. ^{1}H NMR measurement was performed on a AMX500 Brukker NMR spectrometer under a static magnetic field of 500.13 MHz 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 (Hirai, Odani, & Nakajima, 1991).

IR spectroscopy. The infrared spectra were recorded with a Nicolet FT-IR spectrometer, model Nexus 470-670-870. The micro-cup of the accessory was filled with the sample (1.0 mg) ground with anhydrous KBr (130 mg).

Differential scanning calorimetry. To study thermal properties DSC was performed using a DSC PYRIS. The baseline was obtained using empty aluminum pans. A sample was encapsulated in a pan. A heating rate of 10 °C/min and a temperature range of 30–340 °C were selected for scanning in the N_2 gas atmosphere (flow rate: 20–30 ml/min).



$R_1 = H$, $R_2 = CH_2PO_3H_2$
$R1 = R_2 = CH_2\text{-}PO_3H_2$

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

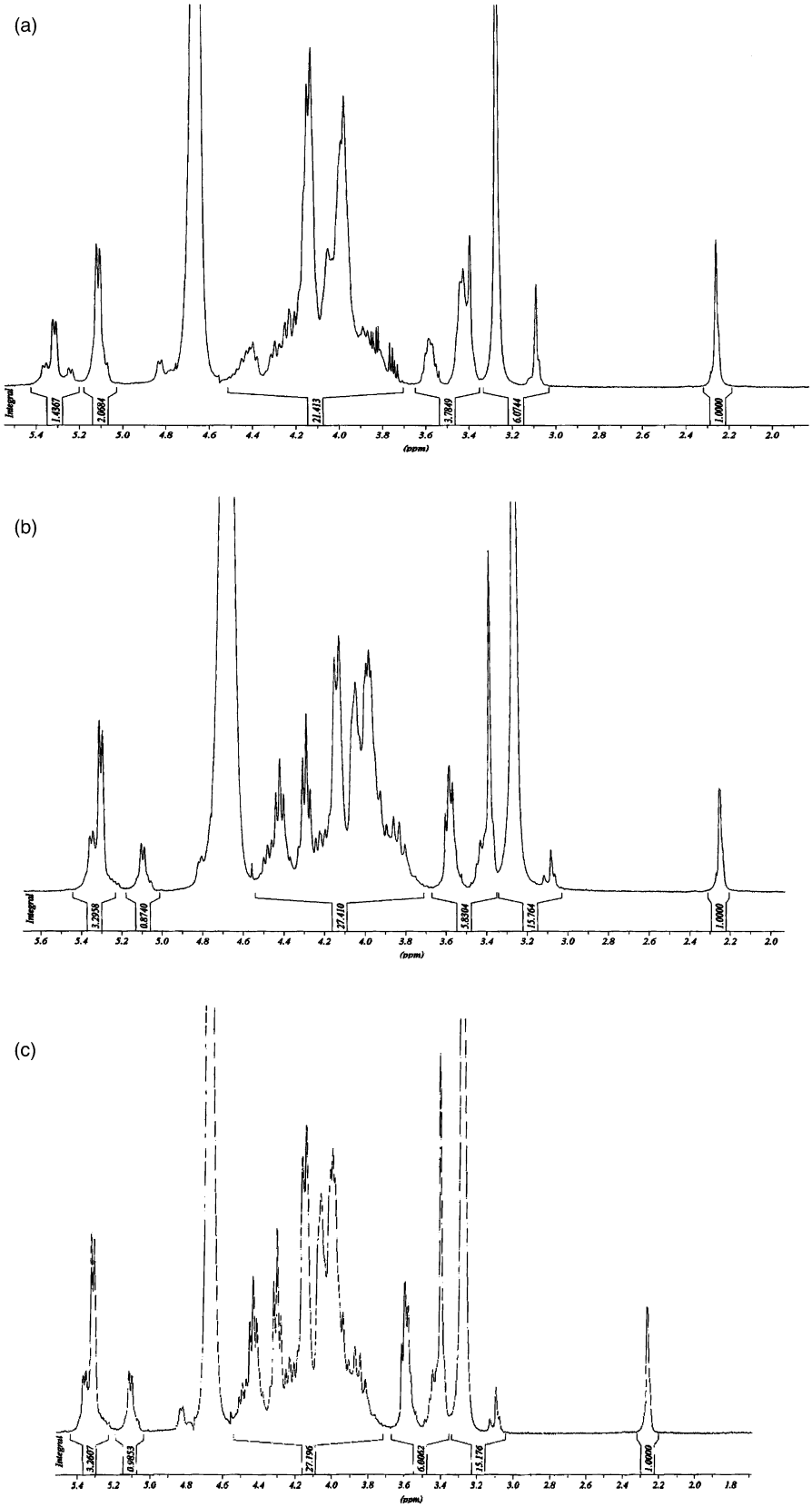


Fig. 2. ^{1}H-NMR spectrum of NMPC at 7 h reaction time (a), NMPC 20 h (b), and 30 h (c) dissolved in 2% (w/w) DCl/D_2O. Polymer concentration 20 g/l.

Determination of viscosity. The measure of viscosity was made on Broockfield (DV-II) viscometer at rotational velocity for spindle 21 of 50 rpm at 25 °C. The sample volume was 10 ml.

Determination of molecular weight (M_v). The solvent system used was 0.1 M AcOH–0.2 M NaCl. The values for the Mark–Houwink equation constants K and a were 1.81×10^{-3} and 0.93, respectively (Roberts, 1992).

3. Results and discussion

Chemical identity. In a previous report we showed for the first time the synthesis of chitosan derivative carrying phosphonic group on the amine function (Heras et al., 2001).

The introduction of methylene phosphonic group into chitosan macromolecule with phosphorous acid and formaldehyde yielded a derivative (Fig. 1) with different properties.

We stated the formation of the monophosphonic secondary amine (I) and that of the tertiary diphosphonic amine (II)

$$\text{Chitosan}-\text{NH}-\text{CH}_2-\text{PO}_3\text{H}_2 \qquad \text{(I)}$$

$$\text{Chitosan}-\text{N}(-\text{CH}_2-\text{PO}_3\text{H}_2)_2 \qquad \text{(II)}.$$

In the case of this derivative ^{1}H NMR spectrum shows the modification due to the introduction of $\text{NH}-\text{CH}_2-\text{PO}_3\text{H}_2$ group replacing the free amino group. Besides this modification, for all of them (7, 20 and 30 h time reaction), the spectrum is just the same as that obtained for chitosan. The spectra show the following chemical shift ^{1}H NMR $\delta = 5.10$ (I H_1), $\delta = 5.30$ (II H_1), $\delta = 3.40$ (I H_2), $\delta = 3.58$ (II H_2), $\delta = 3.78$–4.25 ($H_3H_4H_5H_6$), $\delta = 3.25$ (I $\text{N}-\text{CH}_2-$), $\delta = 3.08$ (II $\text{N}-\text{CH}_2-$) and $\delta = 2.25$ (NCOCH_3) (Pretsch, Clerc, Seibl, & Simon, 1983; Silverstein, Bassler, & Morrill, 1991). The two forms (I and II) are distinguishable on the spectrum because of two different chemical shifts of H-1 appear and were observed for the derivatives with different time of reaction (Fig. 2).

^{1}H NMR study was done to evaluate the degree of substitution (%). Especially the H1 integrals of species I and II were estimated for the different derivatives (7, 20, 30 h time of reaction). The summarized results (Table 1) confirmed an inversion of substitution for the NMPC with time of reaction of 20 h and without later modification. On the other hand the substitution results were the same for the derivatives obtained from different sources.

Table 1
1H NMR chemical shifts and normalized integrals of NMPC ($\sum$H-1 = 100%)

	NMPC *Pandalus borealis*					
	7 h		20 h		30 h	
Proton H1	I	II	I	II	I	II
δ (ppm)	5.10	5.30	5.08	5.35	5.01	5.32
Integral (%)	61.51	38.49	20.96	79.04	23.21	76.79

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

	C	N	C/N[a]	H	P	DS
Chitosan	39.48	7.29	6.31	6.93		
NMPC (7 h)	32.66	5.42	7.03	6.79	5.30	0.72
NMPC (20 h)	34.68	5.15	7.86	7.10	7.93	1.54
NMPC (30 h)	33.50	4.96	7.93	7.37	7.51	1.57

[a] Carbon/nitrogen molar ratio.

The degree of substitution of the NMPC derivatives 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 in the chitosan chain, since the monosaccharide include one nitrogen. The degree of phosphonomethylation for 7, 20 and 30 h time reaction were estimated to be 0.72, 1.54 and 1.57, respectively.

In addition to these results, normalized integrals (Table 1) and elemental analysis (Table 2) made on chitosan derivatives indicate that the degree of substitution increased from 0.37 to 0.48 for 20 h time reaction without an important increase with major time reaction. In all cases, degree of acetylation was constant and the rest of the amino were in the free form (Table 3). The degree of substitution is independent of chitosan's deacetylation degree.

IR spectroscopy. The infrared spectra of the NMPC derivatives shows the same position and intensity at 1654 cm^{-1} band due to amide carboxyl group. The δ NH 1534, 1541 and 1545 cm^{-1} for 7, 20 and 30 h derivatives were observed, respectively. While the ν P–OH shows a slight shift around 1070 cm^{-1}. The more significant evidence was observed for the intensity ratio: ν P–OH/δ NH: 2.42 (7 h); 4.27 (20 h) and 6.55 (30 h). These values suggest an increasing substitution as a function of the time reaction (Fig. 3).

Solubility test. The solubility of NMPC was evaluated in various solvents and aqueous media at a wide range of pH. The results are summarized in Table 4.

Table 3
Degree of substitution of chitosan derivatives

NMPC time reaction (h)	Degree of substitution		
	N-mono	*N*,*N*-di	Total
7	0.23	0.14	0.37
20	0.10	0.38	0.48
30	0.11	0.35	0.46

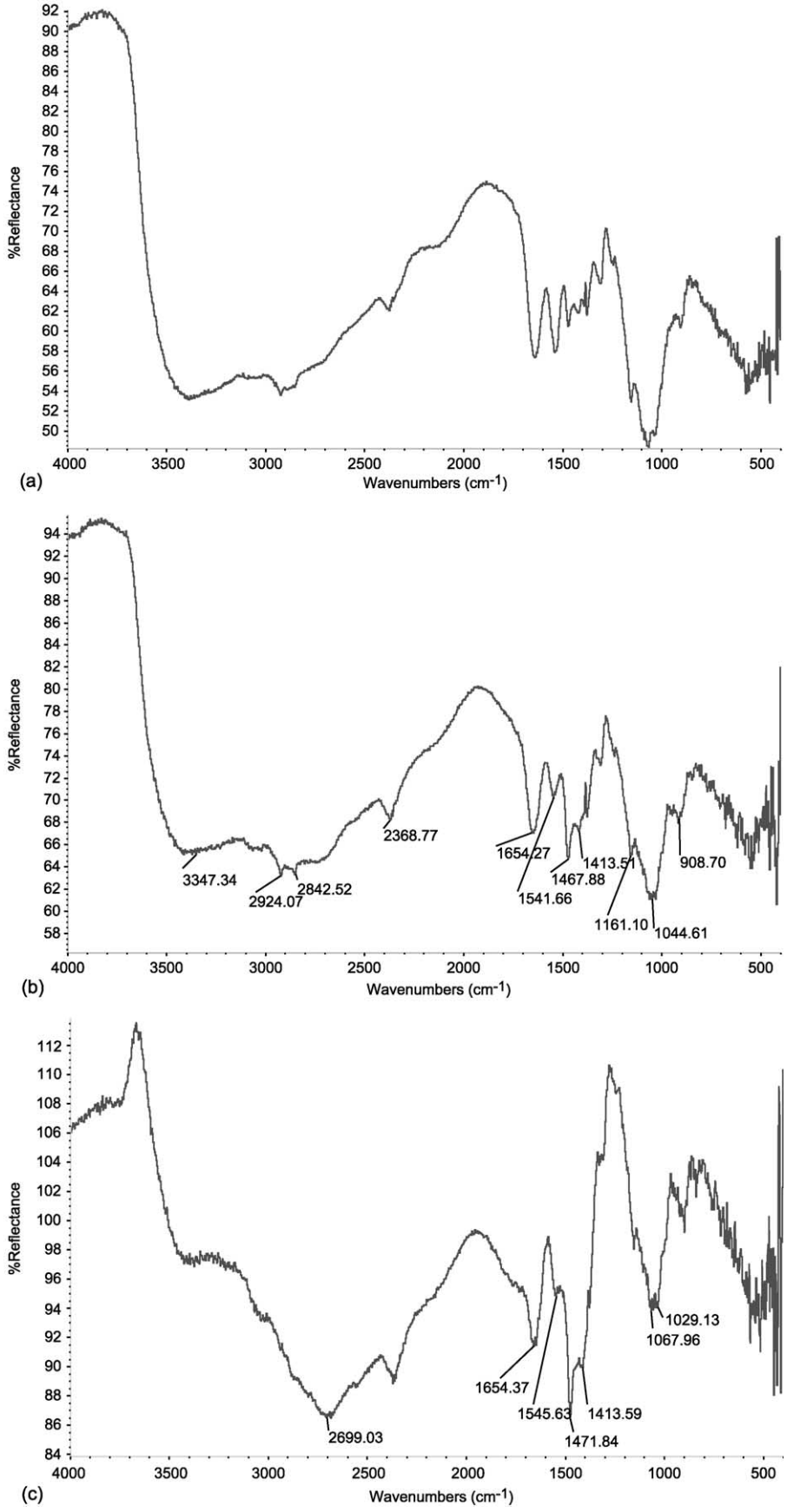


Fig. 3. FT-IR spectrum of NMPC 7 h reaction time (a), NMPC 20 h, (b) and 30 h (c).

Table 4
Solubility of chitosan and its derivatives

Solvent	Chitosan		NMPC					
	Pandalus borealis	*Artemesia longinaris*	*Pandalus borealis*			*Artemesia longinaris*		
			7 h	20 h	30 h	7 h	20 h	30 h
H_2O	I	I	S	S	S	S	S	S
Ethanol	Sw	Sw	Sw	Sw	Sw	Sw	Sw	Sw
Pyridine	I	I	Sw	Sw	Sw	Sw	Sw	Sw
DMSO	I	I	Sw	Sw	Sw	Sw	Sw	Sw
Acetone	I	I	Sw	Sw	Sw	Sw	Sw	Sw
Petroleum ether	I	I	Sw	Sw	Sw	Sw	Sw	Sw
HCI (0.1000 M)	S	S	S	S	S	S	S	S
Chloroform	I	I	Sw	Sw	Sw	Sw	Sw	Sw

Sw: swelling; I: insoluble; S: soluble. The sample (10 mg) and 5 ml of solvent.

Table 5
Effect of the concentration on the NMPC derivative's solubility

Concentration (%)	NMPC					
	Pandalus borealis			*Artemesia longinaris*		
	7 h	20 h	30 h	7 h	20 h	30 h
0.2	S	S	S	S	S	S
1	S	S	S	S	S	S
2	LVG	LVG	LVG	LVG	LVG	LVG
3	LVG	LVG	LVG	HVG	LVG	LVG
4	LVG	LVG	LVG	HVG	LVG	LVG
5	LVG	LVG	LVG	HVG	LVG	LVG

LVG: low viscosity gel; HVG: high viscosity gel; S: soluble.

All prepared chitosan derivatives were soluble in water and acidic media, swelled in DMSO and gelled in protic organic solvents.

From Table 4 we can assess that neither chitosan source nor synthesis reaction time affects the solubility behavior in spite of the progressive substitution.

On the other side, the increase in polymer concentration gives, in the same way, an increases gel consistency. Concentration between 0.2 and 1.0% w/v results in fully water-soluble solution and from 2.0 to 5.0% w/v give gels with different viscosity (Table 5).

Viscosity. Chitosans (from *Pandalus borealis* and *Artemesia longinaris*) viscosities were evaluated between 0.2 and 5.0% w/v in acetic acid solution 1%v/v (Fig. 4).

The viscosity studies from derivatives show for 20 and 30 h reaction time a significative decrease (98%) for both sources. On the other hand, for 7 h time of reaction

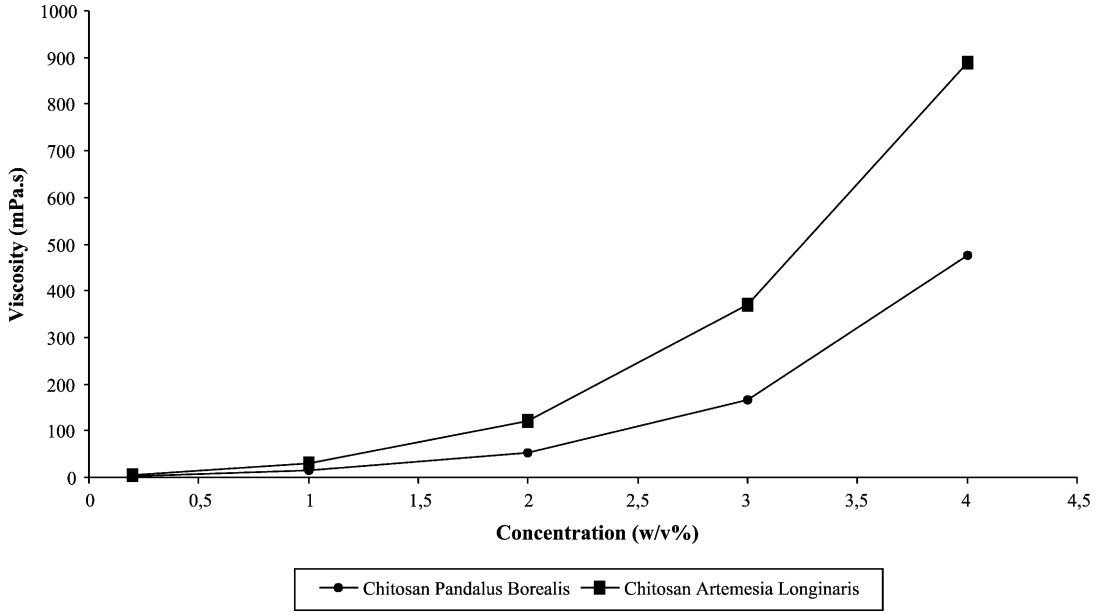


Fig. 4. Chitosan's viscosity as a function of concentration.

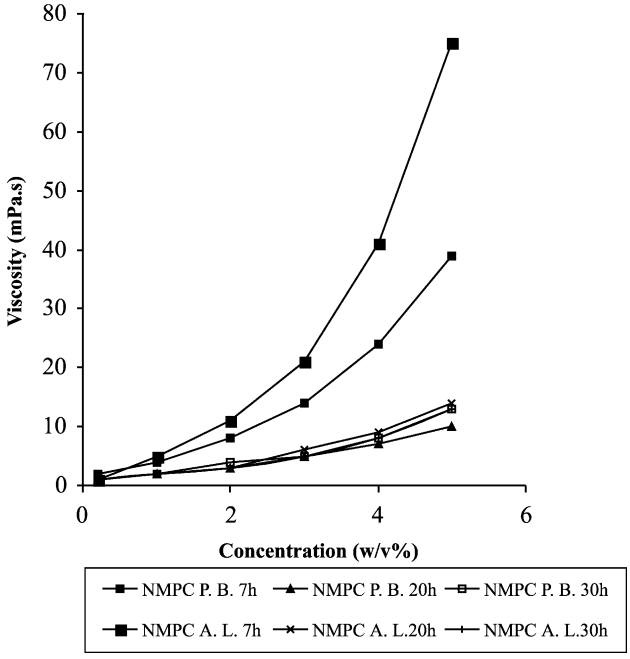


Fig. 5. Concentration dependence of NMPC derivatives viscosity.

the viscosity was related to the original chitosan's viscosity and decreased 95% (Fig. 5).

The relation viscosity–pH was studied (Fig. 6). From pH 6 to 11 viscosity increases, and after that it decreases. Slowly increase of pH leads to turbid alkaline viscous solutions. The alkali had to be added very rapidly to avoid the formation of precipitate (pH 12).

Molecular weight. The molecular weight of derivatives were in the range of 136.000–50.000 Da, lower than the original chitosan (Fig. 7). The derivatization decreases the molecular weight in 57.7% for 7 h time reaction. The decrease is more significative for 20 and 30 h time reaction owing to the chain cleavage, 81.3 and 85.4%, respectively.

Differential scanning calorimetry. DSC thermograms of powdered NMPC were evaluated and the value of decomposition temperature is 226 °C in all cases (Fig. 8). The endotherm centered at approximately 100 °C represents evaporation of water. Usual inspections of

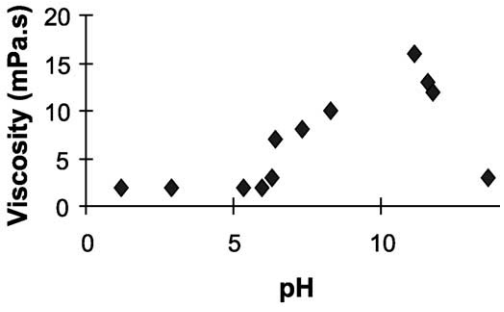


Fig. 6. Dependence of derivative viscosity with the pH.

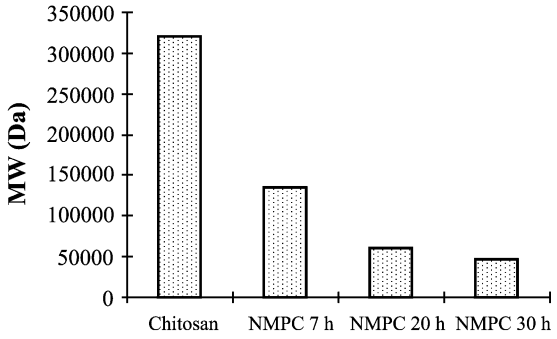


Fig. 7. Molecular weight of NMPC derivatives prepared with 7, 20 and 30 h reaction time.

the samples showed that they were dark brown after thermal treatment.

X-ray diffraction spectrometry. As a consequence of the phosphonomethylation the crystallinity decreases in comparison with the values obtained for the peaks 2θ 9.7 and 19.2 of the chitosan. As well as a shift to higher 2θ values for the second peak.

Film casting. For all derivatives film forming capacity was maintained as the same as the original chitosan.

Metal ion binding. Our original approach consisted of NMPC sodium salt preparation in order to get chelating capacity. One of the characteristic properties is its ability to chelate not only transition-metal ions, but also calcium ions (Table 6). Insoluble NMPC metal ion chelates yield readily settled as hydrated solids within minutes after mixing. The presence of NMPC polymer gives amorphous chelates studied by X-ray diffraction spectrometry.

NMPC-metal ion complex does not keep the filmogenic properties.

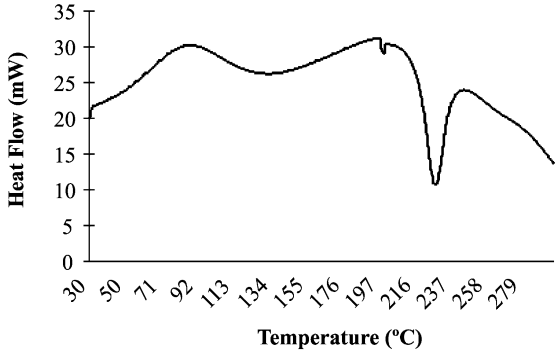


Fig. 8. DSC of NMPC derivative showing the position of the decomposition peak.

Table 6
NMPC metal ion chelates yielding

Ion	Ca^{2+}	Mg^{2+}	Cd^{2+}	Cu^{2+}	Fe^{2+}
mg ion/100 mg chelate	6.22	1.76	18.46	9.70	6.33
Molar ratio chelate/ion	2.5	5.5	1.8	2.3	3.14

4. Conclusions

The resulting product is a variety of NMPC derivatives containing acetyl methylene phosphonic acid and free amino groups in proportions easily controlled through the choice of the starting chitosan (degree of acetylation and molecular weight) and the time of reaction. Preparation of such polymers in 'one pot reaction' yield a simple and reproducible method.

The time of reaction enhances the degree of phosphonomethylation from 0.72 to 1.54 for 7 and 20 despite this, improvement is not achieved for 30 h. As well the degree of substitution is not dependent on chitosan's source and degree of deacetylation.

Furthermore viscosity and molecular weight show a significant decrease due to derivatization.

N-methylene phosphonate chitosan is a powerful chelating agent and opens interesting perspectives. The chemical and biochemical properties of these chelates will be studied, including analytical data, biological significance, functionality in food and cosmetic formulations.

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