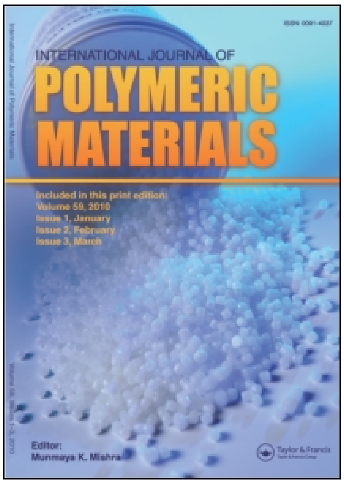

International Journal of Polymeric Materials
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713647664

Chitosan with phosphonic and carboxylic group: New multidentate ligands

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To cite this Article Ramos, Viviana M. , Rodríguez, María Susana , Agulló, Enrique , Rodríguez, Nora M. and Heras, Angeles(2002) 'Chitosan with phosphonic and carboxylic group: New multidentate ligands', International Journal of Polymeric Materials, 51: 8, 711 — 720

To link to this Article: DOI: 10.1080/714975833

URL: http://dx.doi.org/10.1080/714975833

International Journal of Polymeric Materials, 51:711–720, 2002
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0091-4037/02 $12.00 + .00
DOI: 10.1080/00914030290046264

CHITOSAN WITH PHOSPHONIC AND CARBOXYLIC GROUP: NEW MULTIDENTATE LIGANDS

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Chemical modifications of polysaccharides are increasingly studied and they have potential providing new applications. N-methylene phosphonic N-methylene carboxylic chitosan was obtained in water soluble form using N-methylene phosphonic chitosan and glyoxylic acid (via aldimine formation) under reduction conditions with sodium borohydride. The modified chitosan was characterized by ^{1}H NMR, ^{13}C NMR and FTIR spectroscopy. A practical simple and safe preparative procedure has been established to obtain this novel water-soluble and filmogenic chitosan. Taking advantage of the introduction of multidentate ligands into chitosan macromolecule this derivative opens interesting perspectives including the posibility to chelate Ca(II) and others bivalent metals with technological and biomedical potential applications.

Keywords: N-methylene phosphonic chitosan, phosphonomethylation, carboxymethylation, chitosan soluble derivative, ^{1}H NMR, ^{13}C NMR

Received 27 December 2000; in final form 31 December 2000.

This work was supported by CONICET (Argentina), CIC (Provincia de Buenos Aires), SECyT (Universidad Nacional del Sur, Bahía Blanca, Argentina) and MAT97-0702 and APC1997-0047 (España).

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INTRODUCTION

Chitin is the most abundant amino polysaccharide among the naturally ocurring polysaccharides. Chitosan represents partially deacetylated chitin and is considered to be a random-type copolymer of N-acetyl-D-glucosamine and D-glucosamine [1].

Recently much attention has been paid to chitosan as a potential polysaccharide resource owing to its specific structure and properties. An aspect of the current interest in chitosan is related to the biological and biotechnological characteristic properties of chitosan itself and its derivatives [2].

A number of potential applications of chitosan such as in hematology, immunology, rheology, selective interaction *etc.*, would require, however, the preliminary preparation of aqueous chitosan solutions. The cationic nature of chitosan limits the versatility of such solutions because certain acids in excess quantity are required in order to form water soluble chitosan salts. Nevertheless, several efforts have been reported to prepare functional derivatives of chitosan by chemical modifications [3 – 7].

Early works on the chemical derivatization of chitosan showed that water-soluble N-carboxymethylchitosan can be obtained from crustacean chitosan and glyoxylic acid under reducing conditions *via* aldimine formation [8]. On the other hand it is possible to link covalently new functionalities to chitosan without affecting the integrity of the polysaccharide, this approach has been followed in order to introduce methylene phosphonic group into chitosan [9, 10].

The aminoacids containing methylene phosphonate groups have well-know strong chelating properties owing to the donor effect of the amine group ($—NH_2$) into the molecule ($—NH_2—CH_2—PO_3^{2-}$) and the monodentate ligand $—PO_3^{2-}$. They have a tendency to form chelates in ring form with the possibility of different conformations owing to the metal ion nature [11 – 14].

In addition to solubility, reactivity is another important requisite to make possible versatile molecular design. For this purpose groups such as phosphonate and carboxylate are expected to be promising candidates in view of solubility and reactivity of the derivative. The resulting derivative would be useful as a soluble and reactive precursor for further modifications that can be carried out only in homogeneous solution.

Our original approach to the carboxymethylation of the N-methylene phosphonic chitosan (NMPC) consisted of reacting the free amino groups to yield a novel polifunctional amine derivative. This multidentate soluble derivative has potential chelating ability not only for transitions metals ions but also for calcium ions [3, 15 – 18].

The present work describes the preparation and characterization of a new chitosan multidentate ligand carrying carboxylic and phosphonic groups: N-methylene phosphonic and carboxylic chitosan (NMPCC).

MATERIALS

Preparation of Chitosan

Chitosan from shrimp shells was prepared directly by heterogeneous deacetylation at 136°C with 50% (w/w) NaOH for 1 hour.

Synthesis of N-methylene Phosphonic Chitosan

NMPC was prepared with phosphorous acid and formaldehyde according to a previous work [9].

Synthesis of N-methylene Phosphonic and Carboxylic Chitosan

NMPC (1 g) was suspended in distilled water (100 mL), glyoxylic acid was added (400 mg) after stirring for 20 minutes. The molar ratio of glyoxylic acid/amine was 2.6. The final pH was $2-3$. The polymer in solution is then reduced by a solution containing 200 mg of sodium borohydride (dissolved in a small quantity of water) that was delivered to hydrogenate the Schiff base double bond or to obtain a final pH of 4.8. The clear solution was dialysed against demineralized water for 48 hours or until pH of water was rised to 6.8, in dialysis tubing with a M.W. cut-off value of 12.400. Finally, the solution was frozen and freeze-dried.

METHODS

X-ray Diffraction Spectrometry

The material in a 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 K_α radiation ($\lambda = 0.154$ nm).

NMR Spectroscopy

^{13}C and ^{1}H NMR measurements were performed on a AMX500 Brukker NMR spectrometer under a static magnetic field of 125 MHZ and 500.13 MHZ respectily 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 situ*.

Film Casting

NMPCC powder (1.0 g) was dissolved in 100 mL of water. Films could be cast by evaporation from 10 mL of this solution in polystyrene Petri dishes at 37°C (overnight).

IR Spectroscopy

Fourier Transform Infrared (FTIR) spectra recorded on a thin film of NMPCC have been obtained between 4000–400 cm^{-1} on a Nicolet "Nexus" 470 FT-IR spectrometer (DTGS detector).

Scanning Electron Microscopy

A JEOL JSM-35 CF scanning electron microscope was used to characterize the surface of NMPCC particles. The samples were prepared by gold coating using a sputter coater, Pelco 91000. The thickness of the film was 300 Å.

Determination of Viscosity

The measure of viscosity of NMPCC (1%w/v) was made on a Broockfield viscometer at rotational velocity of the spindle of 50 rpm at 25°C.

RESULTS AND DISCUSSION

The chitosan used for the synthesis of NMPC was characterized and the results were: acetylation degree 16%, moisture 11%, ash 0.38%, viscosity 50 mPa.s and elemental analysis (%) C, 39.48; H, 6.93; N, 7.29.

The introduction of phosphonic acid function in the chitosan macromolecule with phosphorous acid and formaldehyde yielded a chitosan derivative (NMPC) with different properties from chitosan and was previously described [9].

The chemical identity of the NMPC was assessed by FT-IR, ^{1}H and ^{13}C NMR spectrometry. It was thus demonstrated to give two species I and II in a ratio around 28/72.

$$\text{Chitosan—NH—CH}_2\text{—PO}_3\text{H}_2 \quad \text{I}$$

$$\text{Chitosan—N—(CH}_2\text{—PO}_3\text{H}_2)_2 \quad \text{II}$$

The degree of acetylation was 0.16, the degree of substitution was 0.33 with N-monosubstitution of 0.09 and N,N-disubstitution of 0.24, the rest of the amino groups being in the free form (0.41). The results of physical and chemical properties of NMPC were: moisture 10%, ash 12%, viscosity 22.5 mPa.s and elemental analysis (%) C, 32.66; H, 6.79; N, 5.42; P, 5.3.

The introduction of a methylene carboxylic acid function in the methylene phosphonic chitosan macromolecule *via* Schiff reaction with glyoxylic acid and reduction with sodium borohydride yielded a novel polifunctional amine derivative (Fig. 1).

The FTIR spectrum in Figure 2 taken on a film of NMPCC modified chitosan shows the characteristics peaks at 2500–3500 cm^{-1} (P—OH), 1066 cm^{-1} (P—OH), 918 cm^{-1} (P=O) and revealed a strong band at

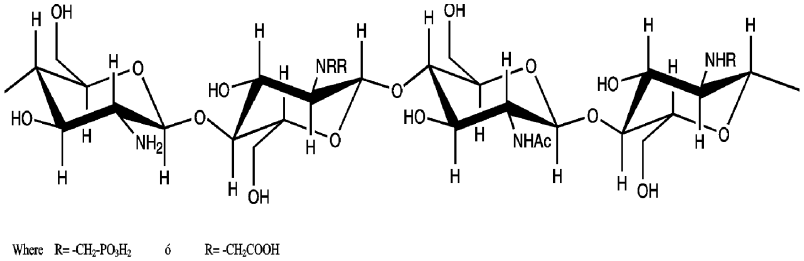

FIGURE 1 Chemical structure of N-methylene phosphonic N-methylene carboxylic chitosan.

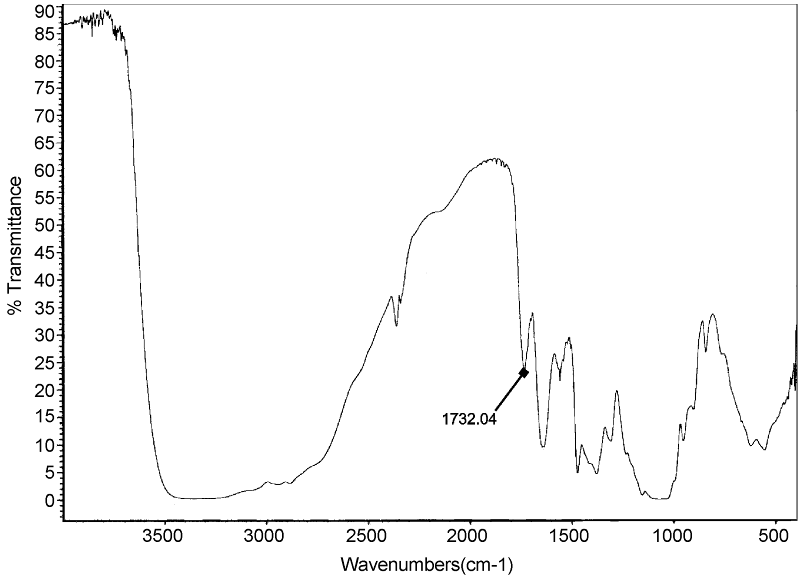

FIGURE 2 FTIR spectrum of a thin film of NMPCC treated with HCl. The band at 1732 cm^{-1} assigned to protonated carboxyl group is indicative of the substitution.

1732 cm^{-1} (C=O). The last band is indicative of a high degree of substitution, which is confirmed by the elemental analysis data: (%) C, 38.64; H, 7.56; N, 5.51; P, 2.60.

The chemical derivatization introduced carbon, oxygen and sodium into the derivative NMPC with a resulting N:C ratio for NMPCC (sodium form) of 0.1426, well below the N:C ratio for NMPC (0.1623).

In the case of NMPCC the ^{1}H NMR spectra shows the modification due to the introduction of NH—CH$_2$—COO$^-$ group replacing the free amino group.

The two forms monosubstituted and disubstituted are distinguishable on the ^{1}H NMR spectrum (Fig. 3) because of two different chemical shifts of H1 which appear at 4.97 ppm and 5.20 ppm with normalized integrals (Σ H-1 = 100%) in a ratio around 16 : 84. The normalized integrals are significantly affected by the carboxymethylation.

The ^{1}H NMR results were confirmed by ^{13}C NMR spectrum from the normalized integrals of C1 (Σ C-1 = 100%) with a signal at 98 ppm corresponding to the disubstituted form (Fig. 4).

The diffractograms obtained on the powders prepared as indicated above showed amorphous water-soluble derivative; most of the diffraction bands were depressed or absent (Fig. 5).

The spongy freeze-dried NMPCC shows flakes with porous surface when the material was examined by Scanning Electron Microscope (Fig. 6).

The derivative results in a high solubility compound in aqueous media with clear, limpid and colourless solutions.

The films obtained were translucent, brilliant and mechanically resistant and had homogeneous appearance.

The physico-chemical properties of NMPCC were: moisture 10%, ash 3.73%, viscosity 4.5 mPa.s.

The preparation of NMPCC is very readily effected, does not require warming or cooling, and requires only commercially available reagents.

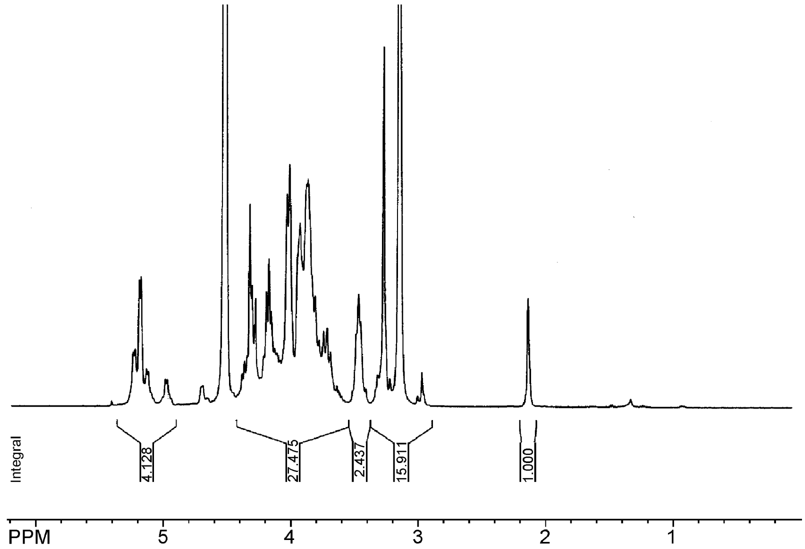

FIGURE 3 ^{1}H-NMR spectrum of NMPCC dissolved in 2% (w/w) DCl/D_2O. Polymer concentration 20 g/L.

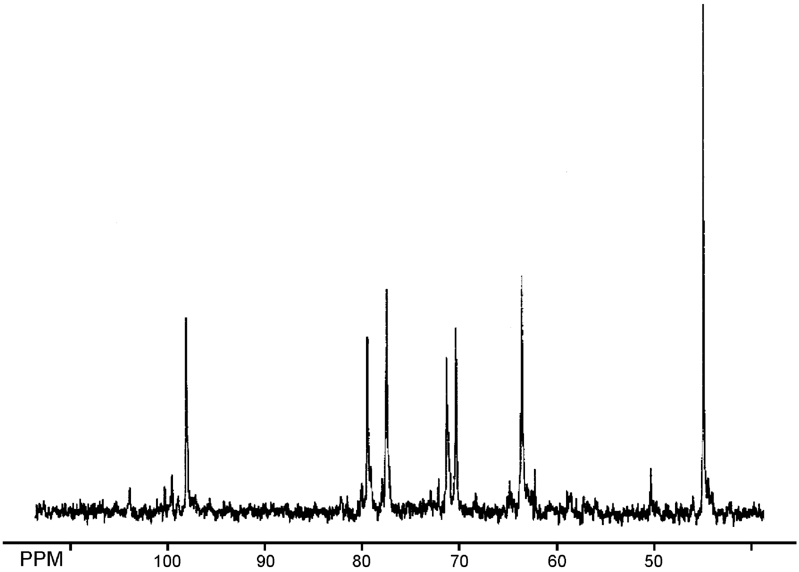

FIGURE 4 ^{13}C-NMR spectrum of NMPCC dissolved in 2% (w/w) DCl/D_2O. Polymer concentration 20 g/L.

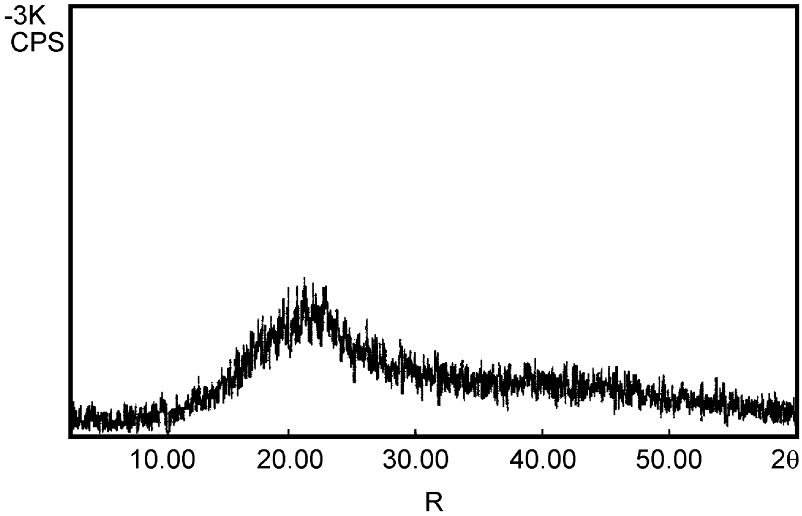

FIGURE 5 X-ray diffraction exhibiting complete absence of crystallinity.

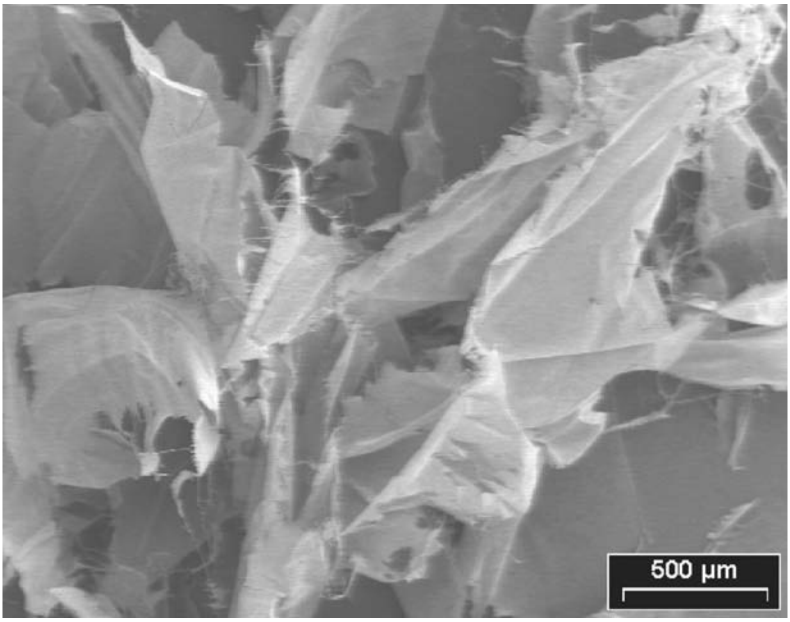

FIGURE 6 Electron micrograph for NMPCC freeze-dried sample (x 44).

The derivative produced is a variety of chitosan containing acetyl, phosphonomethyl, carboxymethyl, and free amino groups in proportions controlled through the choice of the starting chitosan and the amount of glyoxylic acid used. The selected conditions were those corresponding to delivery of sodium borohydride (5%, 8.0 mL) according to Muzzarelli, 1994 [8].

Excess glyoxylic acid does not seem to be necessary to reach high degree of substitution in view of the high reactivity of the aldehyde function.

The reactivity is so high that disubstituted N-carboxymethylation is unavoidably produced, even under mild conditions.

FTIR determinations in conjunction with NMR analysis permitted identification of the structure of the product which is partly N-monocarboxymethylated (0.02) N,N dicarboxymethylated (0.48) and N-acetylated depending on the level of deacetylation of the starting chitosan.

CONCLUSION

A practical simple and safe preparative procedure for N-methylene phosphonic carboxylic chitosan has been established for the first time. The identity of this new compound has been confirmed.

Taking advantage of the reactivity of the precursor we have prepared a novel multidentate derivative. It is biodegradable, film-forming and opens interesting perspectives with the posibility to chelate Ca(II) and others bivalent metals such us Cu(II), Cd(II), Zn(II), *etc*., and for additional technological and biomedical potential applications.

The complete solubility of NMPCC is another peculiar characteristic of this novel polyampholyte and should favor its applications in various fields.

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