Carbohydrate Polymers 79 (2010) 475–480

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Synthesis and characterization of *N*-propyl-*N*-methylene phosphonic chitosan derivative

Adriana Zuñiga *, Adriana Debbaudt, Liliana Albertengo, María Susana Rodríguez

Instituto de Química (INQUISUR), Universidad Nacional del Sur-CONICET, Av. Alem 1253, (8000) Bahía Blanca, Argentina

ARTICLE INFO

Article history:
Received 15 December 2008
Received in revised form 30 July 2009
Accepted 6 August 2009
Available online 12 August 2009

Keywords:
Chitosan
Derivative
Solubility
Amphiphilic properties
N-Methylene phosphonic chitosan

ABSTRACT

A simple methodology for the preparation of a new chitosan derivative called *N*-propyl-*N*-methylene phosphonic chitosan (PNMPC) is proposed. Introduction of a propyl chain onto a modified chitosan (*N*-methylene phosphonic chitosan) offers the presence of hydrophobic and hydrophilic branches for controlling solubility properties of the new derivative. Its chemical identity was determined by FT-IR, ^{1}H, ^{13}C and ^{31}P NMR spectroscopy. The degree of propyl substitution estimated by elemental analysis was 0.64. Furthermore derivative molecular weight is about 60×10^3, X-ray diffraction and SEM showed certain degree of crystallinity and homogeneous surface with a rather packed structure. This derivative opens new perspectives in food, pharmaceutical and cosmetic fields.

1. Introduction

Chitosan, the deacetylated derivative of chitin, is a natural biopolymer consisting of β-1-4 linked *N*-acetyl glucosamine (GlcNAc) and glucosamine (GlcN) repeating units. It has a high molecular weight resulting in its low solubility in most solvents and shows bioactivity only in acidic medium, these reasons limit its applications especially in medicine and food industry.

To increase aqueous solubility and to improve biological, chemical and physical properties, many derivatives of chitosan have been synthesized (Alves & Mano, 2008; An, Dung, Thien, Dong, & Nhi, 2008; Dung, Milas, Rinaudo, & Desbriéres, 1994; Ma et al., 2008; Mourya & Inamdar, 2008; Rinaudo, 2006; Sui, Wang, Dong, & Chen, 2008).

In a previous work we described the synthetic strategy of a novel soluble chitosan derivative: the *N*-methylene phosphonic chitosan (NMPC) by the transformation through an additional functional group in a homogeneous, one step, reaction system for the purpose of creating a chitosan derivative that allowed solubility in water under neutral conditions (Heras, Rodríguez, Ramos, & Agulló, 2001). Lately a methodology was developed for the preparation of a derivative carrying alkyl and phosphonic groups (LMPC). On the LMPC the addition of alkyl groups seems to weaken the hydrogen bond and provides good solubility in organic solvents (Ramos, Rodríguez, Rodríguez, Heras, & Agulló, 2003). Moreover, the new derivative proves to be an amphiphilic system in which the hydrophobic moiety counterbalances the electrostatic repulsion but it also gets more tensioactive properties.

In this paper, we report the successful preparation of an *N*-alkyl derivative of the water soluble NMPC using a reductive *N*-alkylation with propyl aldehyde to obtain a new amphiphilic hybrid material of synthetic and natural polymer so-called *N*-propyl-*N*-methylene phosphonic chitosan (PNMPC).

2. Materials

2.1. Preparation of chitin and chitosan

Chitin was isolated from shrimp shells waste (*Pleoticus mülleri*). It was homogenized and rinsed with water to remove the organic material, then treated with 9% (w/w) NaOH at 65 °C for 90 min, to remove proteins and demineralized with 10% (v/v) HCl at 20 °C for 15 min, washed until neutral pH and dried.

Chitosan was prepared by heterogeneous deacetylation of chitin at 136 °C with 50% (w/w) NaOH for 1 h.

2.2. Synthesis of N-methylene phosphonic chitosan (NMPC)

A solution of phosphorous acid/water (1:1 w/w) was added dropwise with stirring, at room temperature for 1 h to chitosan 2% (w/v) in glacial acetic acid 1% (v/v). The temperature of the reaction vessel was raised to 70 °C and one part of formaldehyde 36.5% (by weight) was added dropwise over 1 h with reflux and left overnight at the same temperature. Solution was dialyzed against

* Corresponding author. Tel.: +54 291 4595100; fax: +54 291 4595187.
E-mail address: azuniga@criba.edu.ar (A. Zuñiga).

0144-8617/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2009.08.011

demineralized water for 48 h or until pH was raised to 6.8 in dialysis tubing with a cut off value of 12,400 Da. Finally solution was freeze-dried (Heras et al., 2001).

2.3. Synthesis of N-propyl-N-methylene phosphonate chitosan (PNMPC)

NMPC (1 g) was suspended in 100 mL distilled water–methanol (1:1 v/v), propyl aldehyde (1.5 g) was added and stirred for 30 min. Reduction was carried out with an excess of sodium borhydride which was added for 2 h and then left stirring overnight at room temperature. PNMPC sodium salt was obtained by dialyzing the reaction mixture against demineralized water for 48 h or until a water pH of 6.8 (dialysis tubing with a M_W cut off value of 12,400). The solution was freeze-dried.

3. Methods

3.1. Characterization of PNMPC

3.1.1. X-ray diffraction spectrometry

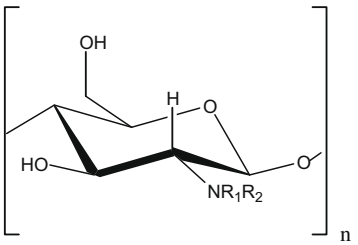
X-ray diffraction spectrometry data were collected using a Rigaku D-Máx. III C diffractometer (Cu Kα) irradiated at 35 kv–15 ma.

3.1.2. NMR spectroscopy

^{13}C, ^{1}H and ^{31}P NMR spectra were recorded on a Varian VNMRS-400 instrument spectrometer at 70 °C. PNMPC (23 mg) was dissolved in 0.5 ml of 5% (w/w) DCl/D_2O at 70 °C. Chemical shift values were recorded downfield from trimethylsilyl propionate sodium salt (TSP) as standard and PO_4H_3 (85%) for the ^{31}P NMR spectrum. The heterocorrelation was done with a Bruker DMX-500 instrument.

3.1.3. IR spectroscopy

The spectrum was recorded on a Nicolet FT-IR instrument. The KBr discs were prepared by blending anhydrous KBr with PNMPC (1%).



[A-/-B-/-C-/-D-/-E-/F…]n

	R_1	R_2
A	-H	$-COCH_3$
B	-H	-H
C	-H	$-CH_2-PO_3H_2$
D	$-CH_2-PO_3H_2$	$-CH_2-PO_3H_2$
E	-H	$-CH_2-CH_2-CH_3$
F	$-CH_2-CH_2-CH_3$	$-CH_2-CH_2-CH_3$

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

3.1.4. Solubility test

Solubility of PNMPC in different solvents was evaluated. Solutions of 10 mg of the polymer in 5 mL of each solvent were prepared.

3.1.5. Molecular weight determination

Weight-average molecular weight (M_w), number-average molecular weight (M_n) and molecular weight dispersion (M_w/M_n) were determined by a Waters-Breeze gel permeation chromatograph (Model 1525), connected to a Waters 2414 (Mod.410) Differential refractometer and a Dawn DSP Light scattering detector. A set of five columns connected in series Ultrahydrogel, Waters 7.8 × 300 mm of 120, 250, 500, 1000 and 2000 Å size pore were used. The temperature was maintained at 30 °C. The eluent was CH_3COOH/CH_3COONa pH 4.8, standards were Pullulans (Shodex Standard P-82. No. 30901-Showa Denko).

3.1.6. Elemental analysis

Elemental analysis were carried out with a Carlo Erba 1108 instrument. Gas separation was done by a gas chromatograph with a variable length Porapak column and a TCD detector.

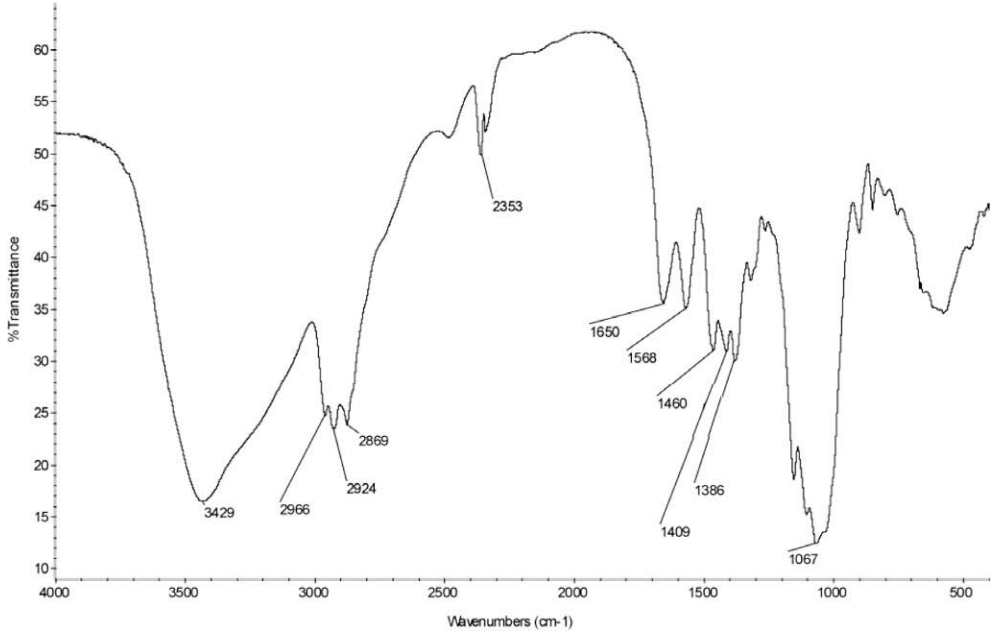


Fig. 2. FT-IR spectrum of PNMPC.