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Synthesis, characterization and evaluation of reactional parameters on substitution degree of *N*-hexyl-*N*-methylene phosphonic chitosan

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ABSTRACT

N-methylene phosphonic chitosan (NMPC) is treated with hexyl aldehyde to give an imine, which is easily converted into *N*-hexyl-*N*-methylene phosphonic chitosan (HNMPC) under mild conditions. The structure of this new chitosan derivative is characterized by FT- IR, ^{1}H, ^{13}C, ^{31}P, $^{1}H^{13}C$–HSQC NMR, SEM and XRD. The influence of reactional parameters on the substitution degree (DS), evidenced that a mol ratio 1.50:1.00 (hexyl aldehyde: free amino groups); a reaction time of 1 h. and 45 °C of temperature afford the best DS. HNMPC molecular weight is 12,768.62 Da. It shows good emulsifying properties giving o/w emulsions with high stability in time. Microscopic observation as well as particle size distribution show an unimodal droplet size distribution with low droplet diameters. Preliminary tests lead us to believe that this new polymer has good film forming properties.

1. Introduction

The sugar backbone of chitosan consists of β-1,4-linked D-Glucosamine with a variable degree of N-acetylation, a structure very similar to that of cellulose, except that the hydroxyl group on the C-2 position is replaced by an acetyl amino group.

Chitosan possesses −OH and -NH_2 groups that can give rise to hydrogen bonding or can be exploited for production of derivatized chitosan (Dung, Milas, Rinaudo, & Desbriéres, 1994; Rinaudo, 2006, Ma, 2008; An, Dung, Thien, Dong, & Nhi, 2008; Alves & Mano, 2008; Mourya & Inadmar, 2008; Badawy & Rabea, 2012; 2013). From a chemical standpoint, the incorporation of new chemical groups or moieties provides versatile materials with specific functionalities and modified physical and biological properties. These derivatives are non-toxic, have a wide range of applications and the raw material sources are unlimited.

Chitosan has been processed in different ways to get modified physicochemical properties. Some of them, with the aim of improving its solubility, chemical and even biological properties (Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Kurita, 2001; Mourya & Inadmar, 2008; Kumar, Dutta, & Dutta, 2009; Badawy, Rabea, & Taktak, 2014; Hu et al., 2016; Zang et al., 2017). It is largely known for its activity against a wide range of microorganisms, in which the most acceptable antimicrobial mechanism is found to include the presence of charged groups in the polymer backbone and their ionic interactions with bacteria wall constituents (Goy, Morais, & Assis, 2016).

Degree of substitution (DS) is a parameter that plays a very important role on the applications of chitosan derivatives and its determination has been well documented in the literature (Kurita, Mori, Nishiyama, & Harata, 2002; Pestov et al., 2016; Santos, Dockal, & Cavalheiro, 2005; Santos, Dockal, & Cavalheiro, 2005).

The successful preparation of *N*-methylene phosphonic chitosan (NMPC) was opportunely reported, as well as, the effect of preparation methods on its properties (Heras, Rodríguez, Ramos, & Agulló, 2001; Ramos, Rodríguez, Díaz et al., 2003, Ramos, Rodríguez, Rodríguez et al., 2003). NMPC proved to have film forming properties like chitosan, but with the improvement of an increased solubility over an extended pH range. This derivative was also modified and grafted with alkyl chains to obtain amphiphilic properties that have potential applications in cosmetics (Ramos, Rodríguez, Díaz et al., 2003, Ramos, Rodríguez, Rodríguez et al., 2003). Hydrophobicity is adjusted according to the length of the alkyl chain or the substitution degree on amine function (Aranaz, Harris, & Heras, 2010; Desbrières, Martínez, & Rinaudo, 1996; Desbrières, 2004; Keisuke, Satoko, Yasuhiro, & Manabu, 2002; Philippova & Korchagina, 2012; Sashiwa & Shigemasa, 1999; Uragami, Kato, & Miyata, 1997; Viegas de Souza et al., 2013). Reductive amination of carbonyl compounds was carried out to obtain *N*-lauryl- (LNMPC) and *N*-propyl-*N*-methylene phosphonic chitosan (PNMPC) (Ramos, Rodríguez, Díaz et al., 2003, Ramos, Rodríguez, Rodríguez et al., 2003; Zuñiga, Debbaudt, Albertengo, & Rodríguez, 2010). Although successful preparation and characterization of these novel biopolymeric derivatives was informed, any effort in order to

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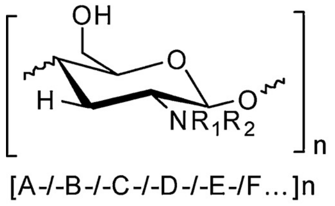

	R_1	R_2
A	-H	$-COCH_3$
B	-H	-H
C	-H	$-CH_2-PO_3H_2$
D	$-CH_2-PO_3H_2$	$-CH_2-PO_3H_2$
E	-H	$-CH_2-(CH_2)_4-CH_3$
F	$-CH_2-(CH_2)_4-CH_3$	$-CH_2-(CH_2)_4-CH_3$

Fig. 1. Chemical structure of *N*-hexyl-*N*-methylene phosphonic chitosan (HNMPC).

maximize the extension of substitution degree has yet to be described.

The main object of the present work is to inform the synthesis and characterization of *N*-hexyl-*N*-methylene phosphonic chitosan (HNMPC) (Fig. 1), as well as the influence of some reactional parameters (mol ratio of aldehyde: free amino groups, reaction temperature and time) on DS in the preparation of the biopolymeric derivative. Solubility, film forming properties and potential emulsifying capacity of the new derivative are analyzed.

2. Materials

Chitin was isolated from shrimp shells waste from (*Pleoticus muelleri*) Ingeniero White Port, Bahía Blanca, Argentina. The material was homogenized and the product was rinsed to remove the organic material. Afterwards, it was treated with 9% (w/w) NaOH at 65 °C for 90 min to remove proteins and finally, demineralized with 10% (v/v) HCl at 20 °C for 15 min. The product in the aqueous solution was then washed and dried.

Chitosan was prepared directly by heterogeneous deacetylation of chitin at 136 °C with 50% (w/w) NaOH for one hour. Its characteristics were: molecular weight 52,980.96 Da; acetylation degree 11.8%; moisture 12%; ash 0.39%; viscosity 63mPas (1% w/w in 1% acetic acid at 25 °C) measured with a Brookfield model DV-IV + viscometer at 50 RPM with spindle 21 (Brookfield, Stoughton, Ma.).

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

Phosphorous acid dissolved in water (1:1 w/w) at room temperature for one hour was added dropwise with stirring to a 2% chitosan solution (w/v) in glacial acetic acid 1% (v/v).After that, the temperature of the reaction vessel was raised up to 70 °C and one part of formaldehyde 36.5% (by weight) was added over 1 h with reflux and left overnight at the same temperature. The obtained solution was dialyzed against 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, the solution was freeze-dried (Heras et al., 2001). NMPC characteristics were: moisture 10.9%; ash 4.0%; viscosity 80 mPa.s (1% w/v at 25 °C). NMPC substitution degree (DS) was determined by elemental analysis as 1.54.

2.2. Syntesis of N-hexyl-N-methylene phosphonic chitosan (HNMPC)

NMPC (1 g) was suspended in distilled water-methanol in a ratio 1:1 (45 mL). This reaction mixture was let to react under different experimental conditions:

- Mol ratio of hexyl aldehyde and free amino groups of 1.00:1.00, 1.10:1.00, 1.50:1.00 and 2.00:1.00.The mixtures were stirred during 30 min at 25 °C (free amino groups of NMPC were determined by ^{1}H NMR).
- Reaction time of 1, 2 and 3 h. The ratio of 1.50:1.00 hexanal: free amino groups was kept and stirred at room temperature.
- Reaction temperatures of 25°, 35°, 45° and 55 °C. The ratio of 1.50:1.00 hexanal: free amino groups was maintained stirring for 1 h.

Then, reduction was carried out with an excess of sodium borohydride which was added dropwise and then left stirring overnight at room temperature. The reaction mixture was dialyzed against demineralized water until it reached a pH of 6.8 (dialysis tubing with a MW cut off value of 12,400). The solution was freeze-dried and HNMPC was obtained in a good yield (88%).

3. Methods

3.1. X-ray diffraction spectrometry

X-Ray Diffraction data was collected using a Rigaku D-Máx. III C Diffractometer (Cu $K\alpha$, $\lambda = 1.5406$ Å) irradiated at 35 kv-15 ma. DATASCAN was the acquisition data program used. Data was analyzed using JADE 8 and the PDF4 database of the International Centre for Diffraction Data (ICDD).

3.2. NMR spectroscopy

^{13}C, ^{1}H, ^{31}P and ^{1}H^{13}C–HSQC NMR spectra were recorded on a Varian VNMRS-400 instrument spectrometer at 70 °C. HNMPC (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. VNMRJ 3.0 was used for data acquisition and MNova software for data processing.

3.3. FTIR spectroscopy

Fourier Transform Infrared (FTIR) spectrum was performed ranging from 4000 cm^{-1} to 400 cm^{-1} using a Nicolet FT-IR instrument. KBr discs were prepared by blending anhydrous KBr with HNMPC (1%). The spectrum was collected using 32 scans with a resolution of 4 cm^{-1}. Data analysis was performed using the spectral analysis software OMNIC 8.0 (Thermo Fisher Scientific, USA).

3.4. Solubility tests

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