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Water-induced local ordering of chitosan polymer chains in thin layer films

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ABSTRACT

Carbon-13 NMR (CP-MAS and FSLG ^1H–^{13}C HETCOR) have been applied to chitosan salt films synthesized in acetic acid and exposed to different relative humidity environments (32% or 75%) at 20 °C for 1 month. It gives insight in the relationship between structure and functional properties according to the hydration level of this biomaterial as a film. The acetate ions trapped in the chitosan act as structuring agents between chitosan chains for the low hydration state. But, increasing the moisture content induces spontaneous removal of acetic acid and a subsequent modification in the film structure, with an increase in local ordering. HETCOR experiments also showed a multiplicity of signals for most of the observed carbon atoms and in particular those implied in the glycosidic linkage, which reveals different water-induced conformational states. Changing the water content allows to modify the polymer structure and therefore to modulate the properties such as controlled release of active compounds trapped in chitosan-based coatings, e.g., for medicated dressing or active packaging.

1. Introduction

Chitin is the second most abundant polysaccharide in nature after cellulose. Its estimated worldwide production is 10^{10}–10^{12} tons per year (Kumar, 2000). It generally comes from crustacean shells, insects, or mollusks. Chitin is a linear polymer of β-(1–4)-N-acetyl-D-glucosamine units. Chitosan is obtained from chitin by deacetylation in the presence of base agents, which gives a copolymer of glucosamine and N-acetylglucosamine units. This polymer contains three different polar functional groups, namely, hydroxyl (OH), primary amine (NH_2), and ether (C—O—C) groups, plus depending on the deacetylation level residual carbonyl (C═O) groups. The presence of these functional groups gives chitosan high water attracting capacity. Due to its positive charge on the amino group under acidic conditions, chitosan is often referred to as cationic polymer, which can easily bind to negatively charged molecules. This property, along with its biocompatibility, makes chitosan a very attractive polymer in various fields, such as in food and nutrition (edible film (Dutta, Tripathi, Mehrotra, & Dutta, 2009; Shahidi, Arachchi, & Jeon, 1999), antimicrobial film (Dutta et al., 2009)), in biotechnology (artificial skin, reconstituted tissue structure (Di Martino, Sittinger, & Risbud, 2005)), in material science, for drugs and pharmaceuticals (encapsulating material (Rinaudo, 2006)), in agriculture and environmental protection (chitosan membranes (Peter, 1995)), and recently in gene therapy (Kumar, 2000; Rinaudo, 2006).

The structure of chitosan has been investigated in aqueous solutions and solid state by various spectroscopic techniques (NMR (Domján, Bajdik, & Pintye-Hodi, 2009; Heux, Brugnerotto, Desbrieres, Versali, & Rinaudo, 2000; Webster, Osifo, Neomagus, & Grant, 2006), IR (Brugnerotto et al., 2001; Cárdenas, Cabrera, Taboada, & Miranda, 2004; Harish Prashanth, Kittur, & Tharanathan, 2002; Rinaudo, 2006), X-ray spectroscopy (Clark & Smith, 1937; Ogawa, Yui, & Okuyama, 2004; Okuyama, Noguchi, Miyazawa, Yui, & Ogawa, 1997; Saito, Tabeta, & Ogawa, 1987)) and by molecular modeling (MD simulation (Franca, Lins, Freitas, & Straatsma, 2008; Prathab & Aminabhavi, 2007), MM2 (Ferreira, Pedroni, Alimenti, Gschaider, & Schulz, 2008)). Main results obtained on crystals showed that fully deacetylated chitosan develops many types of structural complexes through several systems of intra and intermolecular hydrogen bonds. This polymorphism (Rinaudo, 2006)

Abbreviations: CS, chitosan (powder); CSF3, chitosan film equilibrated at 32% relative humidity (during 1 month); CSF7, chitosan film equilibrated at 75% relative humidity (during 1 month).

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can be classified into three types (Ogawa et al., 2004): (i) an anhydrous form in which the polymer backbone chains is an extended 2-fold helix (type I) (Yui et al., 1994); (ii) a hydrated crystal form with a different (relaxed-) 2-fold helix (type II) (Kumirska et al., 2010; Lertworasirikul, Tsue, Noguchi, Okuyama, & Ogawa, 2003; Okuyama et al., 2000); (iii) a chain pattern with an extended 5-fold helix with an asymmetric unit of one glucosamine (type III) (Cunha et al., 2012). Few structural data are available for an intermediate state, which can be referred to as low hydrated state. This last case covers a large range of applications of chitosan polymer in its uses as films, coatings, or encapsulation agent for controlled release of drug.

The good mechanical properties of chitosan films, comparable to many medium-strength commercial synthetic polymers, as well as the excellent oxygen-barrier properties in dry conditions, are closely related to its structure, and in particular to the hydrogen bonds network (Epure, Griffon, Pollet, & Avérous, 2011). The relationship between such properties and the polymer structure under various environmental conditions (water content, charge of the polymer, presence of other small solutes, pH) requires a better knowledge. The hydroxyl groups (a primary hydroxyl in C6 and a secondary hydroxyl in C3) and the highly reactive amino group in C2 concomitantly participate in intra- and intermolecular hydrogen bonds, resulting in the formation of different polymorphic forms whose properties may vary considerably.

The influence of water/acetate ion on the structure of chitosan is investigated in the present work on a molecular scale with solid state NMR. It aims at comparing a weakly hydrated state of chitosan film to a more hydrated state, focusing in particular on the hydrogen bond network. It is particularly interesting in order to get better insight in the relationship between structure and functional properties to target controlled release applications of active compounds from the chitosan layer (film or coating), for the pharmaceutical or the food industries. ^{1}H–^{13}C Cross Polarization Magic Angle Spinning (CP-MAS) is very sensitive to changes in the H-bonded structure of chitosan (Heux et al., 2000; Kameda, Miyazawa, Ono, & Yoshida, 2005) and two-dimensional (2D) spectroscopy gives further insights in the interactions between molecules. In particular, Frequency-Switched Lee–Goldberg (FSLG) ^{1}H–^{13}C Heteronuclear Correlations (HETCOR) NMR has been previously used to study the effect of plasticizers on chitosan film with 50% acetylation degree, dried 24 h at 50% Relative Humidity (RH), at a MAS frequency of 10 kHz, unraveling different mechanisms of plasticization though induced mobility and specific interaction with the polymer according to the type of plasticizer used (glycerol or polyethylene glycol) (Domján et al., 2009). The same technique, at a higher MAS frequency (18 kHz) is used in this study in order to better understand the influence of water/acetate ion on hydrogen bonding structure of chitosan-based film having an acetylation degree of 10%.

2. Materials and methods

2.1. Materials

Chitosan (CS) used for this study is a commercial grade supplied by France Chitine, powder 652, having a molar mass of 165 kg mol^{-1}. Its degree of deacetylation is approximately 90% (data given by the supplier). Table 1 gives the major ions present in the initial chitosan powder. A 2% (v/v) acetic acid (glacial 100%, Merck, Darmstadt, Germany) solution (pH 4.5) was used as dissolution medium for chitosan. Films were obtained by casting method followed by solvent evaporation in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) under controlled conditions of temperature (20 °C) and relative humidity (30%). Their thickness is around 60 μm.

Table 1
Anions and cations in the initial chitosan powder (CS).

Ion	Concentration (mg/g)	Method
Chloride	21.42	Ionic chromatography
Sulfate	0.53	
Sodium	102.80	Inductively coupled plasma atomic emission spectroscopy
Potassium	5.23×10^{-3}	
Copper	1.22×10^{-3}	
Iron	1.50	
Platinum	$<0.5 \times 10^{-3}$	High-resolution continuum source atomic absorption spectroscopy

2.2. Film equilibration

For studying the effect of relative humidity on chitosan-based films, the films were exposed to ambiances with temperature and relative humidity controlled conditions for 1 month to ensure equilibration. To that purpose films were stored in airtight cabinet containing saturated salt solutions of NaCl or $MgCl_2$ (Sigma–Aldrich) to fix the relative humidity at 75 ± 0.5% and 32 ± 0.5%, respectively. Cabinets were stored in a chamber (KBF 240 Binder, ODIL, France) which temperature is regulated at 20 ± 0.5 °C. These films stored at 75% or 32% RH were designed hereafter as CSF7 and CSF3, respectively.

As determined by Karl Fischer titrimetry, the water content (wt%, dry basis) was 6.45 (±0.12) for the chitosan powder, 19.66 (±1.09) and 5.28 (±0.82) for CSF7 and CSF3 films, respectively.

Films were chopped before being inserted into NMR rotors.

2.3. Elemental analysis

Elemental analysis was done using a Flash EA 1112 CHNS/O analyzer from Thermo Electron Corporation. It aims at determining the acetate ion acid content in chitosan film from the C/N ratio.

2.4. Solid state NMR spectroscopy

^{1}H–^{13}C CP-MAS spectra were recorded on a Bruker 300 spectrometer operating at frequencies of 300.1 and 75.5 MHz for ^{1}H and ^{13}C, respectively, at a temperature of 20 °C. The spectra were acquired with a 4-mm MAS probe at a spinning speed of 10 kHz. Hartmann–Hahn matching for the ^{1}H–^{13}C CP-MAS experiments was set on adamantane for ^{1}H and ^{13}C radio-frequency fields of ca. 60 kHz. Chemical shifts for ^{1}H and ^{13}C spectra were referenced to the signal of water (4.87 ppm) and to the methylene signal of adamantane (29.47 ppm), respectively. 360 transients were collected with a contact time of 1 ms and a recycling delay of 2 s, except for the sample CSF3 (5 s). No line broadening was applied during the processing.

Two dimensional FSLG ^{1}H–^{13}C HETCOR spectra were recorded on a Bruker 500 spectrometer with a 3.2-mm MAS probe at spinning speed of 18 kHz, at a temperature of 20 °C. 360 transients were collected with a contact time of 1 ms and a recycling delay of 2 s for each of the 128 increments recorded with an indirect window size of 16 kHz. SPINAL-64 proton decoupling with a strength of 70 kHz was used during the acquisition. Contour plots are constituted of 20 levels on a logarithm scale from 0.07 to 1. A 20 Hz Lorentzian line broadening in the direct dimension was applied during the processing. All spectra have been normalized independently to unity.