Carbohydrate Polymers 74 (2008) 704–706

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Spectroscopic investigation of the VO^{2+}/chitosan interaction

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ARTICLE INFO

Article history:
Received 7 February 2008
Received in revised form 21 April 2008
Accepted 23 April 2008
Available online 29 April 2008

Keywords:
Chitosan–VO^{2+}
Complexes
IR spectra
Structural model

ABSTRACT

Different oxovanadium (IV) complexes were prepared by interaction of aqueous $VOSO_4{\cdot}5H_2O$ solutions with chitosan solutions in diluted acetic acid, or by direct interaction of the vanadyl salt with chitosan suspensions in water. A detailed analysis of the IR spectra of pure chitosan and its VO^{2+} complex is presented. On the basis of this analysis, it is suggested that VO^{2+}/chitosan interaction involves only N-donors of the biopolymer.

1. Introduction

Chitin, a high molecular weight linear polymer of 2-acetamido-2-deoxy-D-glucopyranose units linked together by 1,4-glycosidic bonds, is the most widespread amino polysaccharide in nature and is estimated annually to be produced almost as much as cellulose (Merzendorfer & Zimoch, 2003). It is the source material of chitosan, which is the *N*-deacetylated product of chitin. Although a sharp nomenclature border does not exist between chitin and chitosan, the term "chitosan" usually refers to copolymers of 2-amino-2-deoxy-D-glucopyranose where the degree of deacetylation is generally more than 60% (Fig. 1).

As chitosan contains multiple amino, hydroxyl, and acetamido groups it can easily generate complexes with numerous metal cations (Lima & Airoldi, 2004; Ogawa, Oka, & Yui, 1993; Taboada, Cabrera, & Cárdenas, 2003; Varma, Desphande, & Kennedy, 2004; Vold, Vårum, Guibal, & Smidsrød, 2003; Wang, Du, & Liu, 2004). On the other hand, it is non-toxic, biocompatible, and biodegradable. For this reason, chitosan and some of its chemically modified forms had attained increasing interest from the pharmaceutical point of view, as a useful and extremely versatile drug delivery agent (Ravi Kumar, Muzzarelli, Muzzarelli, Sashiva, & Domb, 2004).

Numerous recent studies have shown that a number of simple or complex vanadium compounds present an important insulinomimetic activity, which can be exploited for the search of new ways for the treatment of diabetes mellitus (Baran, 1997, 2004; Thompson, 1999; Thompson & Orvig, 2000; Rehder, 2003).

In this context, chitosan has been proposed as an effective vehicle for the controlled vanadium release (Kofuji, Qian, Murata, & Kawashima, 2005). Therefore, it was interesting to investigate the characteristics of the oxovanadium(IV)/chitosan interaction in order to attain an insight into the structural characteristics of the generated complex. Kinetic and equilibrium studies on the adsorption of VO^{2+} on chitosan were performed some years ago (Jannson-Charrier, Guibal, Rousssy, Delanghe, & Le Cloirec, 1996), but structural aspects of the interaction were not considered.

2. Experimental section

2.1. Materials

Chitosan (⩾75% deacetylated) was purchased from Sigma. All the other employed reagents and solvents were from Merck, analytical grade.

A standard solution of chitosan was obtained dissolving 4.0 g of chitosan in 400 mL of 1% acetic acid solution, and stirred during about 2 h at room temperature. After three days, the non-dissolved particles were separated by filtration from the solution, using a G2 fritted glass funnel.

2.2. Preparation of the complexes

Fifty milliliters of the standard solution of chitosan was diluted with 25 mL of distilled water. To this solution a small portion of $VOSO_4.5H_2O$ (100, 200, 300, 400 or 500 mg), dissolved in the minimum amount of water, was added. The mixture was heated at 70–80 °C, under continuous stirring, during 2 h. After cooling, the ob-

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doi:10.1016/j.carbpol.2008.04.031

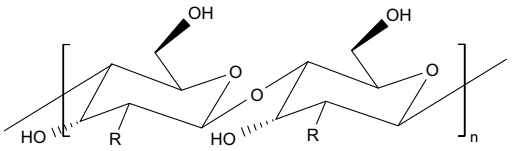

Fig. 1. Schematic structure of chitosan (in chitosan most R = $-NH_2$ and some of them = $-NHOC-CH_3$; in chitin all R = $-NHOC-CH_3$).

tained dark green solution was filtered off. The complex was precipitated from this solution by addition of a double volume of absolute ethanol. After a few hours, it was filtered off, washed repeatedly with absolute ethanol, and finally dried in an oven at 60 °C during 2 days.

A second series of experiments was performed interacting directly vanadyl, sulfate with solid chitosan. In this case, 620 mg of finely particulated chitosan (obtained by milling the commercial chitosan sample during about 2 h in a vibrating mill (ball mill Retsch, model MM-200), using agate sample holders and balls) was suspended in 50 mL of distilled water and then 620 mg of $VOSO_4{\cdot}5H_2O$ was dissolved in this solution. The mixture was left in contact during 1 week and was stirred during 6 h each day. The final pH value of the solution was ca. 2.5. On the 7th day, the solid material was filtered off using a G3 fritted glass funnel, washed repeatedly with cold water, and finally dried at 60 °C during 2 days.

2.3. Vanadium content of the complexes

The vanadium content of the obtained complexes was determined by direct ignition of the samples in a muffle furnace in air, with slow heating up to 600 °C, and weighted as V_2O_5 (Furman, 1939). The determined values, for each of the starting VOSO4.5H_2O quantities (mg/VO), were as follows: 100 mg/VO = 3.70%V; 200 mg/VO = 6.85%V; 300 mg/VO = 8.85%V; 400 mg/VO = 11.90%; 500 mg/VO = 12.35%V. As can be seen there is a definite tendency to reach a plateau as the concentration of VO^{2+} increases, in a similar way as observed in the direct interaction of other divalent metal cations (for example, Cu(II) and Co(II)) with chitosan suspensions (Lima & Airoldi, 2004).

On the other hand, in the direct interaction of oxovanadium (IV) solutions with suspended chitosan the amount of retained vanadium was much lower. Values determined in three independent preparations ranged between 5.95 and 6.65%V.

2.4. Spectroscopic studies

The infrared spectra in the spectral range between 4000 and 400 cm^{-1} were recorded as KBr pellets with a Bruker IFS 66 FTIR instrument. A total of 60 scans were accumulated. Spectral resolution was ±4 cm^{-1}. Raman spectra were obtained with the FRA 106 Raman accessory of the mentioned spectrophotometer, using the 1064 nm line of a solid state Nd:YAG laser for excitation. Spectral resolution was ±4 cm^{-1}.

3. Results and discussion

3.1. Infrared spectra

The FTIR spectra of free chitosan and of one sample of its oxovanadium (IV) complex, obtained in solution (prepared with 400 mg of vanadyl sulfate, vanadium content 11.90%), are shown in Fig. 2. The spectra of the other complexes, prepared with different amounts of the vanadyl cation, are entirely similar. The proposed assignments are presented in Table 1 and briefly commented, as follows:

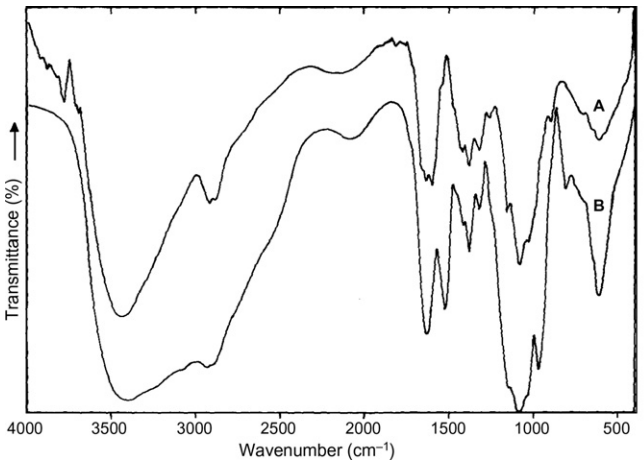

Fig. 2. FTIR spectra of commercial chitosan (**A**) and of one of the prepared VO^{2+}/chitosan complexes (**B**).

Table 1
Assignment of the infrared spectra of free chitosan and of the VO^{2+} complex of chitosan (band positions in cm^{-1})

Chitosan	VO^{2+}/chitosan complex	Assignment
3427 vs, br		ν(O–H) + ν(N–H)
	3410 vs, br	ν(O–H)
	3045 sh	ν(N–H)
2920 m, 2870 sh	2927 m, 2845 sh	$\nu(CH_2)$ + $\nu(CH_3)$ + ν(CH)
1630 sh	1631 vs	amide I
1597 s	1529 s	amide II + $\delta(NH_2)$
1417 w	1419 sh	$\delta(CH_2)$ + $\delta(CH_3)$
1379 m	1383 m	δ(CH)
1317 w	1325 w	amide III
1251 vw		
1153 w	1150 sh	ν_{as}(C–O–C) bridge
1080 vs, 1030 sh	1090 vs	ν(C–O–C) pyranose ring
	968 m	ν(V = O)
899 vw	901 sh	ring
	806 m	
710 sh, 672 sh		
606 s	613 s	γ(OH) out of plane

vs, very strong; s, strong; m, medium; w, weak; vw, very weak; sh, shoulder; br, broad.

- In free chitosan, only one very strong and broad band, assignable to the O–H and N–H stretchings, can be identified. Its position suggests the presence of relatively important hydrogen bonds (Smith, 1999). In the complex, a weak splitting of this broad band can be observed, and the separation of the two contributions has been suggested in our assignment.
- Some of the characteristic C–H vibrations (Smith, 1999) could be assigned to both free chitosan and the complex. The C–H stretching bands identified as a doublet (2920/2870 cm^{-1} in chitosan; 2927/2845 cm^{-1} in the complex) are surely of complex nature, because several symmetric and antisymmetric stretching modes appear in this region: ν(CH), $\nu(CH_2)$ from the CH_2OH groups and $\nu(CH_3)$ from the *N*-acetyl groups.
- The behavior in the medium IR region is specially interesting. The so-called amide I band, which is essentially the C=O stretching vibration of the amide group (Parker, 1971; Smith, 1999), is only seen as a weak shoulder in free chitosan, partially superimposed with the strong IR band located at 1597 cm^{-1}. This band is originated by the superposition of the so-called amide II band of the secondary amide groups and the deformational scissoring mode, $\delta(NH_2)$, of the primary amine groups (Parker, 1971; Smith, 1999). The amide II vibration is mainly a mixed vibration involving the N–H in-plane bending and the

C–N stretching vibration (Parker, 1971). In the complex these two bands are clearly split in the form of two relatively intense bands.

- The amide III band, which is only found in secondary amides and is also originated in a similar vibrational mixing as the amide II band (Parker, 1971), presents similar intensities in the free chitosan and in the complex, but is slightly displaced to higher energies in the last case. Besides, this band and the previous one (1379 cm^{-1} in free chitosan) may also be involved in the in-plane bending of the hydroxyl groups (Smith, 1999).
- The weak IR band found at 1153 cm^{-1} in free chitosan and as a shoulder at 1150 cm^{-1} in the complex was assigned to the antisymmetric C–O–C bridge stretching vibration by comparison with data reported for the FTIR spectrum of cellulose acetate (Ilharco & Brito de Barros, 2000). Also the position of the C–O–C stretching of the pyranose ring is supported by comparison with those data and with that of previous investigations of chondroitin sulfate A (Etcheverry, Williams, & Baran, 1994). This C–O–C stretching of the pyranose ring is probably partially superimposed with the ν(C–N) motion of the primary amine groups (Smith, 1999).
- The out-of-plane bending of the OH-groups (Smith, 1999; Ilharco & Brito de Barros, 2000) is seen with similar intensity in both cases, but slightly displaced to higher energies in the case of the complex.
- The characteristic V⎓O stretching of the oxocation is seen as a medium intensity IR band at 968 cm^{-1}, lying somewhat higher than that usually found in VO^{2+} complexes of simple sugars, in which coordination takes place by two pairs of deprotonated OH-groups (Baran, 2001).
- The presence or absence of water molecules in the complex cannot be established spectroscopically because the O–H stretching modes may be overlapped by the very strong 3410 cm^{-1} absorption and the corresponding deformational mode, usually of medium intensity, by the very strong 1631 cm^{-1} band.

Unfortunately, the corresponding Raman spectra gave no additional support to this vibrational-spectroscopic analysis. In the case of free chitosan only a limited number of well-defined bands (2883, 1413, 1379, 1263, 1208, 1098 and 892 cm^{-1}) could be seen whereas the complexes did not give Raman signals at all, presenting only one very strong and broad band centered at about 3250 cm^{-1}.

Concerning the FTIR spectra of the samples obtained by direct interaction of vanadyl sulfate with chitosan suspensions, they are also very similar to that shown in Fig. 2B, although most of the bands are usually somewhat broader and not so well defined.

3.2. Structural considerations

On the basis of the above-performed spectroscopic analysis it is possible to propose a model for the VO^{2+}/chitosan interaction. The displacement to lower frequencies of the band assigned to the amide II/$\delta(NH_2)$ mode in the complex together with the slight energy increase of the C–O–C pyranose ring stretching, which also involves a ν(C–N) motion of the amine group, as well as the apparent frequency diminution of the stretching mode of this group, clearly support their participation in bonding. On the other hand, the hydroxyl groups seem not to be involved in bonding, as both the ν(OH) and the γ(OH) vibrations are only slightly displaced, on going from free chitosan to the complex.

These spectroscopic results point to the exclusive participation of amino and *N*-acetyl-amino groups of chitosan in bonding, with the additional participation of water molecules in coordination, generating complex moieties of the type $[VO(N_{ch\text{-}it})_2(H_2O)_2]^{2+}$. The N-donors may be provided by the same chitosan chain, as in the case of the Fe(III) complex (Nieto, Peniche-Covas, & Del Bosque, 1992) or by two parallel opposing chains, generating a *trans*-diamine arrangement, similar to that recently proposed for the Pd(II) complex (Johannesen, Petersen, Duus, & Skrydstrup, 2007).

Acknowledgements

This work has been supported by the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET) and the Universidad Nacional de La Plata (UNLP). The author is a member of the Research Career from CONICET.

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