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Short communication

Influence of some reactional parameters on the substitution degree of biopolymeric Schiff bases prepared from chitosan and salicylaldehyde

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Abstract

Some reactional parameters as mol ratio (salicylaldehyde:free amino groups), reaction time and temperature were investigated in order to improve the substitution degree (DS) in the preparation of biopolymeric Schiff bases from chitosan. In this case, the reaction of chitosan and salicylaldehyde was used as a probe system in order to produce the Schiff base. The use of 50% (mol/mol) salicylaldehyde excess, reaction time of 18 h and temperature of 55 °C permitted to obtain a DS of 60% without evidences of hydrolysis of the biopolymeric matrix or changes in its acetylation degree.

Keywords: Chitosan; Schiff bases; Degree of substitution

1. Introduction

Chitin and *chitosan* are linear polysaccharides containing 2-acetamido-2-deoxy-D-glucopyranose (*GlcNAc*) and 2-amino-2-deoxy-D-glucopyranose (*GlcN*) units joined by $\beta(1 \rightarrow 4)$ glycosidic bonds (Signini & Campana Filho, 1999; Lamarque, Viton, & Domard, 2004). The proportion of *GlcNAc* in relation to the *GlcN* units is defined as the degree of N-acetylation (DA) (Chatelet, Damour, & Domard, 2001; Tsaih & Chen, 2003) and differentiates chitin ($DA > 0.5$) from chitosan ($DA \leqslant 0.5$). However, this definition is just an approximation and the difference is also defined practically by solubility in aqueous acidic medium, in which chitosan is soluble while chitin is not (Dung, Milas, Rinaudo, & Desbrières, 1994; Kurita, Kamiya, & Nishimura, 1991; Lamarque et al., 2004; Signini & Campana Filho, 1999).

In chitosan structure predominates the GlcN residues whose free amino groups at C-2 position allows its solubility in acidic medium (Rinaudo, Pavlov, & Desbrières, 1999) and permits reactions with several substituents resulting in a wide series of modified biopolymers with a large spectrum of applications (Peter, 1995).

Among these substituted biopolymers there are the Schiff bases, obtained by the reactions of the free amino groups of chitosan with an active carbonyl compound such as aldehyde or ketone (Hirano et al., 1999; Kurita, Mori, Nishiyama, & Harata, 2002; Moore & Roberts, 1981; Sashiwa & Shigemasa, 1999).

These bases present the characteristic imine group (–RC=N–) is their structures (Cimerman, Galic, & Bosner, 1997) and offer several possibilities such as in reactions requiring the protection of chitosan C-2 amino groups (Baba, Kawano, & Hirakawa, 1996; Yang & Yuan, 2001) or in reactions with metal ions for enhancing the adsorption/complexation properties of the biopolymer yielding a material with potential analytical and environmental applications (Baba et al., 1996; Becker, Schlaak, & Strasdeit, 2000; Hall & Yalpani, 1980;

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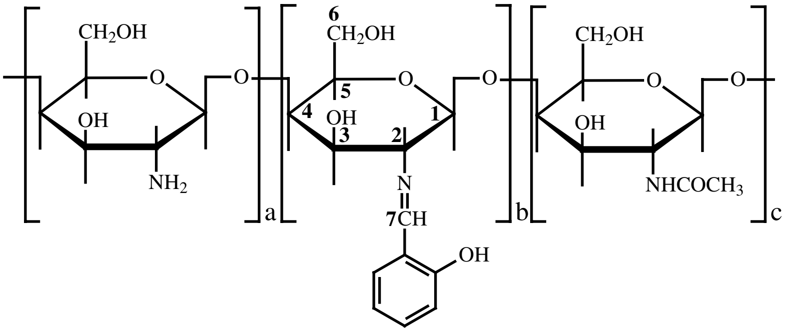

Fig. 1. Representative structure for biopolymeric Schiff bases prepared by the reaction of free amino groups from 2-amino-2-deoxy-D-glucopyranose (*GlcN*) units and salicylaldedyde. The ratio of Schiff base (b) in relation to the unreacted amino groups (a) is called degree of substitution (DS).

Hu, Shi, Tang, Fang, & Kennedy, 2001; Rodrigues, Laranjeira, de Fávere, & Stadler, 1998).

Some papers concerning the synthesis, characterization and thermal behavior of biopolymeric Schiff bases prepared from chitosan have already been described (Santos, Dockal, & Cavalheiro, 2005a; Soliman, 2001; Tirkistani, 1998). Of course, the extension of Schiff base formation, called degree of substitution (DS), is a very important parameter that plays an important role on the applications mentioned above and whose determination have also been issued in the literature (Kurita et al., 2002; Santos et al., 2005a; Santos, Dockal, & Cavalheiro, 2005b).

Although the successful preparation of the biopolymeric Schiff bases from chitosan described by these authors, any effort in order to maximize the extension of the substitution has been described and, in some cases, a very low DS was obtained.

In this sense, the main objective of the present work is to evaluate the effect of some experimental conditions in order to reach a highest DS in the preparation of biopolymeric Schiff bases. This evaluation has not been performed in previous work (Santos et al., 2005b). The reaction from chitosan and salicylaldehyde, Fig. 1, was used as a probe. Reactional parameters such as mol ratio of salicylaldehyde:free amino groups, reaction temperature and time were studied.

2. Experimental

2.1. Chitosan purification

The chitosan used in this work was a technical grade commercial product of medium molecular weight from crab shells (Aldrich/USA). The purification was attained by the dissolution of the crude commercial product (approximately 1 g) in 300 mL of dilute 0.5 mol L^{-1} acetic acid solution. The dissolution of the polysaccharide was assured by stirring the initial suspension during 12 h and precipitated in the hydrogel form by carefully adding concentrated NH_4OH. The chitosan hydrogel was washed with water until neutrality followed by ethanol. The final product was dried at 60 °C under reduced pressure. The purified sample was kept under reduced pressure in a desiccator over silica gel (Signini & Campana Filho, 1999). The DA of the purified sample was determined by ^1H NMR as 11.2%.

2.2. Preparation of biopolymeric Schiff bases

Salicylaldehyde (Aldrich, PA) was used without additional purification. The biopolymeric Schiff bases were synthesized by dissolving 400 mg of the previously purified chitosan with 25.0 mL of dilute 0.15 mol L^{-1} acetic acid solution in a homemade reactor vessel immersed in a thermostated bath at 25 °C, during 12 h, to assure its dissolution in a hydrogel form. Then, a desired amount of salicylaldehyde previously dissolved in 10.0 mL of ethanol was added to the chitosan solution.

This reaction mixture was let to react under the different experimental conditions as follows:

- mol ratio of salicylaldehyde: free amino groups of 1.00:1.00, 1.05:1.00, 1.10:1.00, 1.50:1.00, and 2.00:1.00. The mixtures were stirred during 12 h at 25 °C. The number of moles of free amino groups, 88.8%, was calculated on the basis of the DA, determined by ^1H NMR;
- reaction temperatures of 35, 45, and 55 °C. The ratio of salicylaldehyde:free amino groups was kept at 1.50:1.00 stirring the mixtures during 12 h;
- reaction time of 6, 18, and 24 h. The ratio of salicylaldehyde:free amino groups was kept at 1.50:1.00 stirring the mixtures at 55 °C.

Deep yellow gels revealed the formation of the Schiff base on the biopolymeric matrix. The resulting gels were collected by filtration, washed several times with ethanol to remove any unreacted aldehyde, dried at 60 °C under reduced pressure yielding a yellow powder that was kept in a desiccator over silica gel.

2.3. Infrared spectroscopy

The infrared spectra were recorded in an MB102 FT-IR spectrophotometer (Bomen-Michelson). The chitosan and its biopolymeric Schiff bases were dried overnight at 60 °C under reduced pressure and mechanically well-blended with 100 mg of KBr in an agate mortar. The pellets were desiccated for 8 h at 60 °C under reduced pressure and the IR spectra were recorded, against KBr disk as a reference, by accumulation of at least 64 scans with a resolution of 2 cm^{-1}.

2.4. ^1H *nuclear magnetic resonance*

The solution ^1H NMR spectra were measured in a Brucker AMX 400 spectrometer. For these measurements, a suspension of 10 mg of chitosan with 1 mL of 1% D_2O/HCl (v/v) solution were prepared and stirred for 15 h. All the measurements were performed at 70 °C, using 16 scans pulse accumulation and the LB parameter of 0.30 Hz. The spectral width and data points were 8000 Hz and 32K points, respectively. This procedure was repeated for the biopolymeric Schiff bases.

3. Results and discussion

The main bands observed in the IR spectra of the chitosan, Fig. 2a, were: the axial stretching of OH and C–H groups at 3417 and 2907 cm^{-1} respectively, which appears superimposed to the N–H stretching band; C=O stretching band (amide I) at 1654.8 cm^{-1}, N–H angular deformation at 1598.8 cm^{-1} (amide II); CH_3 symmetrical angular deformation at 1386.7 cm^{-1} and C–N amino groups axial deformation 1323.7 cm^{-1}, besides the two characteristic polysaccharide bands, 1076.2 and 1155.2 cm^{-1} due to the C–O stretching from $\beta(1 \rightarrow 4)$ glycosidic bonds (Brugnerotto et al., 2001; Santos et al., 2005a, 2005b; Shigemasa, Matsuura, Sashiwa, & Saimoto, 1996).

The IR spectra of the biopolymeric Schiff bases, Fig. 2b, presented a strong absorption band at 1631.6 cm^{-1} attributed to the C=N vibrations characteristic of imines (Colthup, Daly, & Wiberley, 1990) which is not observed in chitosan. On the other hand, there is no evidence of bands characteristic of the free aromatic aldehydes near to 1665 cm^{-1}. The bands at 1581.5, 1278.7, and 752.2 cm^{-1}, are attributed to the C=C, C–O, and C–H stretching in the aromatic ring of the aldehyde, respectively (Santos et al., 2005a, 2005b; Silverstein, Bassler, & Morrill, 1994; Singh, Srivasta, & Singh, 1967).

The purified chitosan and its biopolymeric Schiff base were characterized by ^1H NMR spectroscopy. For chitosan, Fig. 3a, DA is deduced from the exact ratio of 1/3 between the integrated resonances of the methyl proton signal of the 2-acetamido-2-deoxy-D-glucan (δ 2.04 ppm) and the proton at C-2 position in the 2-amino-2-deoxy-D-glucopyranose units (δ 3.21 ppm) (Hirai, Odani, & Nakajima, 1991; Lamarque et al., 2004; Signini & Campana Filho, 1999).

For biopolymeric Schiff bases, Fig. 3b, DS is deduced from the exact ratio of 1/1 between the integrated resonances of the proton at C-7 position in the imine groups (δ 10.5 ppm) and the proton at C-2 position in the 2-amino-2-deoxy-D-glucopyranose units (δ 3.77 ppm) (Santos et al., 2005a, 2005b). The complete proton assignments are presented in Fig. 1.

Some papers concerning the determination of the DS from elemental analysis data have already been presented (Kurita et al., 2002; Rodrigues et al., 1998). However, we believe this is not so exact, since the water present can severely interfere in the results.

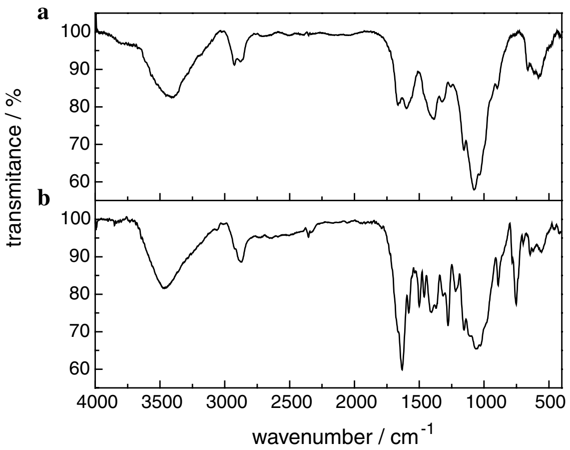

Fig. 2. Typical infra red spectra for (a) chitosan, DA = 11.2% and (b) biopolymeric Schiff base, DS = 21.9%.

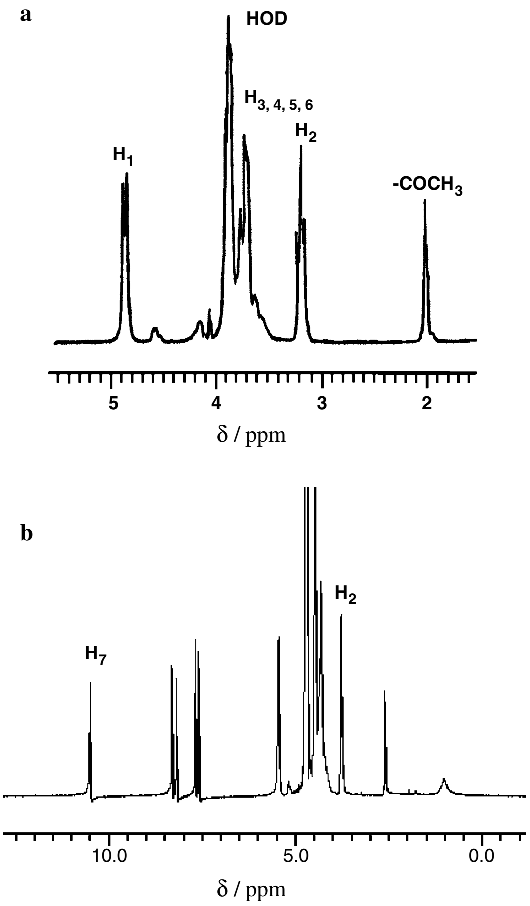

Fig. 3. Typical ^1H NMR spectrum for (a) chitosan, DA = 11.2% and (b) biopolymeric Schiff base, DS = 21.9%.

The DS obtained from ^{1}H NMR was used to evaluate the effect of the mol ratio, reaction time and temperature in the extension of the substitution by salicylaldehyde in the chitosan matrix.

3.1. Effect of mol ratio

The DS of the biopolymeric Schiff bases obtained at 25 °C after 12 h of reaction using different excess amount of salicylaldehyde are presented in Table 1.

It is possible to observe that the mol ratio lead to an increase on the DS value up to 1.50 salicylaldehyde:1.00 chitosan. The small increase observed for 2.00:1.00 is not enough to justify the waste of the aldehyde reagent.

Table 1
Effect of the mol ratio (salicylaldehyde:chitosan free amino groups) on the DS determined by ^{1}H NMR

Mol ratio[a]	DS (%)[b]
1.00:1.00	22
1.05:1.00	25
1.10:1.00	27
1.50:1.00	34
2.00:1.00	36

[a] Salicylaldehyde:free amino groups.
[b] At 25 °C, after 12 h of reaction.

3.2. Effect of reaction temperature

The effect of the temperature on the DS of biopolymeric Schiff bases obtained with 1.50:1.00 mol ratio during 12 h of reaction is presented in Table 2.

The temperature seems to be the most remarked effect in the DS of biopolymeric Schiff bases. However, temperatures much higher than 55 °C could cause hydrolysis of chitosan in the acidic media (Zhishen & Dongfeng, 2002) and this temperature was set as the higher to be used in the substitution reaction.

Table 2
Effect of the temperature on the DS determined by ^{1}H NMR

Temperature (°C)	DS (%)[a]
25	34
35	40
45	44
55	58

[a] Mol ratio 1.50:1.00, reaction time of 12 h.

3.3. Effect of reaction time

The effect of the reaction time on the DS of biopolymeric Schiff bases obtained with 1.50:1.00 mol ratio at 55 °C is presented in Table 3. The DS increases with the reaction time up to 18 h. No change in the acetylation degree was observed during the reaction.

Table 3
Effect of the reaction time on the DS determined by ^{1}H NMR

Reaction time (h)	DS (%)[a]
6	34
12	58
18	60
24	62

[a] Mol ratio 1.50:1.00, temperature 55 °C.

These studies revealed that the mol ratio (salicylaldehyde:free amino groups), reaction temperature and time influences the DS and should be optimized in order to improve the resulting amount of Schiff base sites on the biopolymeric chain, represented by the DS. However, care should be taken in order to prevent hydrolysis of the biopolymeric chain and deacetylation of the chitosan matrix.

The conditions here investigate permitted us to obtain a DS from 20% to 60% by increasing the salicylaldehyde excess, temperature and reaction time, without hydrolysis and/or deacetylation of the biopolymeric matrix. Earlier studies conducted at room temperature and without excess gave a DS of only 17% (Santos et al., 2005a, 2005b).

4. Conclusion

The present work suggested that the DS in the preparation of Schiff bases from chitosan and aldehydes could be increase since optimized conditions are used during the substitution reaction. Using chitosan and salicylaldehyde as a probe system the best conditions that improved the DS were: mol ratio 1.50:1.00 (salicylaldehyde:free amino groups), temperature of 55 °C and reaction time of 18 h. These conditions gave a relatively higher DS without waste of salicylaldehyde, easiness in removing any unreacted aldehyde and prevention of chitosan chain hydrolysis.

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