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# Synthesis and characterization of Schiff bases from chitosan and salicylaldehyde derivatives

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## Abstract

The introduction of salicylaldehyde and derivatives at the C-2 nitrogen position of chitosan with low degree of acetylation has been investigated. Chitosan was reacted with salicylaldehyde and its 5-bromo, 5-chloro, 5-nitro, 5-methyl and 5-methoxy derivatives. The resulting biopolymeric Schiff bases were characterized by elemental analysis (C, H, N), IR spectroscopy, $^1$H NMR spectroscopy and conductimetric titration. The $^1$H NMR spectroscopy was used for the determination of degree of acetylation of chitosan. The degree of acetylation was determined from the ratio between the integrated area of the proton signal corresponding to the acetamido group (H-Ac) and that of the proton (H-2) of the GlucN unit. A procedure for the determination of the degree of substitution based on $^1$H NMR data is proposed. The substitution degree was calculated from the ratio between the integrated area of the proton signal of the imine group and of the proton (H-2) of the GlucN unit. Substitution degrees varying from 4.6 to 68.5% depending on the *R* group have been measured. No significant change on the acetylation degree during the reaction was observed.

*Keywords:* Chitosan; Schiff bases; Degree of substitution

## 1. Introduction

Chitosan is the *N*-deacetylated derivative of chitin, although this *N*-deacetylation is almost never complete (Matteus, 1997). Chitin is the most important natural polysaccharide after cellulose, and is found in crustacean shells or in fungi cell walls. The term chitosan is usually used when the polymers become soluble in a dilute acid solution. The solubility is also controlled by the distribution of the acetyl groups remaining along the chain (Matteus, 1997). This biopolymer has applications ranging from cosmetics, artificial skin, photography, food and nutrition, ophthalmology and wastewater treatment (Narang,1990; Ravi-Kumar, 2000).

The chitosan is insoluble in water, but it dissolves in aqueous solutions of organic acids as acetic, formic, citric, besides inorganic acids, as diluted hydrochloric acid resulting in viscous solutions. (Rinaudo; Pavlov, & Desbriéres, 1999). It is well known that some of the structural characteristics such as degree of acetylation (DA) and molecular weight controlled the solubility the in chitosan.

The determination of the DA of chitosan has been largely discussed in the literature. The main techniques suggested for this determination are conductimetric titration, infrared spectroscopy, $^1$H NMR, HPLC, thermal analysis, and others (Jiang, Chen, & Zhong, 2003; Raymond, Morin & Marchessault, 1993).

The presence of amminic groups in the polymeric chain leads to the possibility of a several chemical modifications, including the preparation of Schiff bases by reaction with aldehydes and ketones (Moore & Roberts, 1981; Muzzarelli, Jeunieux, & Gooday, 1985).

The reaction of chitosan with aromatic aldehydes in acetic acid to produce the corresponding Schiff bases has

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been described by Tirkistani, 1998. This author prepared, characterized and investigated the thermal properties of the derivatives.

Schiff base compounds containing an imine group (–RC=N–), are usually formed by the condensation of a primary amine with an active carbonyl. Its attractiveness as analytical reagents raises from the fact that they enable simple and inexpensive determinations of various organic and inorganic substances (Cimerman, Galic, & Bosner, 1997).

The insertion of functional groups in the chitosan matrix may improve its capacity of interaction with metallic ions by complexation. In this sense the modification of chitosan with aldehydes to produce Schiff bases may result in a potentially complexing material for metallic species with potential analytical and environmental applications (Hall & Yalpani, 1980; Rodrigues, Laranjeira, de-Favere, & Sadler, 1998).

According to Kurita, Mori, Nishiyama, and Harata (2002) the introduction of carbonyl groups in a chitosan results in Schiff bases whose degree of substitution is dependent on the stoichiometric amount of aldehyde used in the reaction. The degree of substitution (DS) of the Schiff bases was calculated from ratio C/N of elemental analysis.

The present work describes the preparation of biopolymeric Schiff bases by the reaction of low molecular weight chitosan with salicylaldehyde and five derivatives: 5-bromo, 5-cloro, 5-nitro, 5-methyl, 5-methoxy. The characterization of products has been performed by elemental analysis, $^1$H NMR and infrared spectroscopy.

## 2. Experimental

### 2.1. Materials

Chitosan with low molecular weight was supplied by Aldrich (cat. number 44,865-9) and purified according to Matteus (1997) and Signini and Campana-Filho (2001).

Salicylaldehyde and its 5-bromo, 5-chloro, 5-nitro, 5-methyl, 5-methoxy derivatives and deuterium oxide (Aldrich) were used without additional purification.

### 2.2. Methods

The $^1$H NMR spectra were measured in a Brucker AMX 400 spectrometer. For these measurements, a suspension of 10 mg of samples with 1 mL of 1% HCl/$D_2O$ (v/v) solution and was prepared and stirred for 15 h. All the measurements were performed at 343 K, using pulse accumulating of 16 scans and the LB parameter of 0.30 Hz. The spectral width and data points were 8000 Hz and 32 K points, respectively. 3-(trimethylsilyl)-1-propanesulfonic acid-d4 (TSPA from Aldrich) was used as an external standard.

The elemental analyses were performed in a FISONS-EA1108 analyzer.

For the IR spectra the samples (1.5 mg), which were dried overnight at 60 °C under reduced pressure, were mechanically mixed with 100 mg of KBr, previously dried. A KBr disc was prepared from the mixed power and dried for 15 h at 110 °C under reduced pressure. The IR spectra were recorded in an MB102 FT-IR spectrophotometer (Bomen-Michelson) using a disk of 100 mg KBr as a reference.

The molecular weight distribution and subsequent characteristics were obtained in an AVS-350 viscometer coupled to an automatic dilution device Schott Gerate ViscoDoser AVS 20. The molecular weight of the chitosan was estimated based on the parameters described by Mark–Houwink equation $[\eta]=K_m M^a$ where $K_m=0.076$ and $a=0.76$ (Rinaudo, Milas, & Dung, 1993). The average viscometric molecular weight $M_V=69{,}000$ was estimated from the intrinsic viscosity determined in a 0.3 mol L$^{-1}$ acetic acid–0.2 mol L$^{-1}$ sodium acetate buffer solution at 25±0.01 °C as the mean of five replicates.

### 2.3. Chitosan sample purification

The chitosan samples (CA) were purified by dissolution in 0.5 mol L$^{-1}$ $CH_3COOH$ during 15 h. The resulting solution was filtered through 5 and 0.8 μm cellulose nitrate membranes (Sartorius) successively and then neutralized with 15 mol L$^{-1}$ $NH_4OH$ up to pH 8. The precipitate was washed with water several times followed by methanol. The resulting gel was then dried at room temperature for 18 h (Matteus, 1997; Signini & Campana-Filho, 2001).

### 2.4. Synthesis of biopolymeric Schiff bases

Previously purified chitosan was dissolved in 0.1 mol L$^{-1}$ acetic acid and stirred at room temperature for 6 h. Then salicylaldehyde derivatives dissolved in methanol (2:1, v/v) were added to the resulting viscous solution. This mixture was stirred for 16 h. The formation of a deep yellow gel reveals the formation of the Schiff base on chitosan matrix. The resulting gel was concentrated in a rotatory evaporator under vacuum and washed with ethanol several times, filtered and dried at room temperature in a vacuum oven at 60 °C for 15 h.

The Schiff bases were prepared from salicylaldehyde (SB-1); and its 5-bromo (SB-2), 5-cloro (SB-3), 5-nitro (SB-4), 5-methyl (SB-5) and 5-methoxy (SB-6) derivatives.

## 3. Results and discussion

In the Schiff base preparation, chitosan acts as the primary amine reacting with aromatic aldehyde to produce the corresponding biopolymeric derivative. The

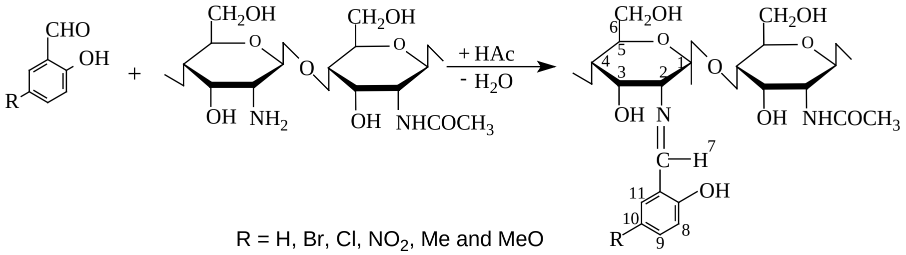

Fig. 1. Reaction scheme of the chitosan modified with substituted salicylaldehydes, where R=H(SB-1), Br(SB-2), Cl(SB-3), $NO_2$(SB-4), Me (SB-5), MeO(SB-6).

Table 1
Elemental analysis and characteristic IR bands of chitosan and biopolymeric Schiff bases

| Sample | Element (%) | | | | Infrared ($cm^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | H | N | C/N | $\upsilon_{C=O}$ (amide I) | $\delta_{NH}$ (amide II) | $\upsilon_{C=N}$ (imine) | $\delta_{CH}$ (aromatic ring) | $\upsilon_{antis}$ (bridge C-O-C) | $\upsilon_{C–O}$ (phenolic) | $\upsilon_{C=C}$ (aromatic ring) |
| CA | 43.5 | 6.49 | 7.92 | 5.49 | 1655.9 | 1602.8 | Absent | Absent | 1154.2 | Absent | Absent |
| SB-1 | 53.0 | 5.39 | 5.74 | 9.23 | nd | nd | 1631.5 | 754; 891 | 1154.0 | 1276.4 | 1580.9 |
| SB-2 | 44.4 | 3.65 | 4.05 | 11.0 | nd | nd | 1633.4 | 783; 817 | 1149.8 | 1273.2 | 1571.6 |
| SB-3 | 49.9 | 4.10 | 4.54 | 11.0 | nd | nd | 1636.3 | 784; 818 | 1151.0 | 1273.8 | 1577.7 |
| SB-4 | 47.9 | 4.11 | 8.52 | 5.62 | nd | nd | 1640.4 | 727; 835 | 1147.1 | 1233.4 | 1545.4 |
| SB-5 | 56.4 | 5.68 | 5.21 | 10.8 | nd | nd | 1636.6 | 782; 822 | 1153.8 | 1279.0 | 1590.8 |
| SB-6 | 55.1 | 5.48 | 4.46 | 12.3 | nd | nd | 1640.2 | 787; 817 | 1162.0 | 1271.7 | 1593.7 |

CA, Chitosan Aldrich; SB, Schiff base (1, 2, 3, 4, 5 and 6); nd, not detected; $\upsilon$, stretching; $\delta$, axial deformation.

representation of the Schiff base preparation reaction is presented in Fig. 1, which also presents the atom assignment used in this discussion.

When two equivalents of the aromatic aldehyde were used, the mixture becomes a gel in the course of the reaction. All the biopolymer Schiff bases formed are deep yellow, stable in air and insoluble in common organic solvents.

Table 1 presents the most significant bands found in the IR spectra of chitosan and the produced Schiff bases. The main bands observed in the IR spectra of the chitosan sample were: the axial stretching of OH group in 3368.9 $cm^{-1}$, which appears superimposed to the N–H stretching band; an amide band (amide I) at 1655.9 $cm^{-1}$; N–H angular deformation at 1602.8 $cm^{-1}$ (amide II); $CH_3$ symmetrical angular deformation at 1381.7 $cm^{-1}$, C–N axial deformation at 1422.1 $cm^{-1}$ and C–N amino groups axial deformation 1323.7 $cm^{-1}$, besides the three characteristic polysaccharide bands, 1154.2,1075.7 and 1030.6 $cm^{-1}$ (Brugnerotto, Lizardi, Goycoolea, Argüelles, & Desbrières, 2001).

The IR spectra of the biopolymeric Schiff bases present a strong absorption band between 1631.5 and 1640.4 $cm^{-1}$ attributed to the C=N vibrations characteristic of imines (Colthup, Daly, & Wiberley, 1990). On the other hand, there is no evidence of the characteristic band related to free aromatic aldehydes group near 1665 $cm^{-1}$. The IR spectroscopy confirms the imine formation represented by the absorption in the 1540–1590 $cm^{-1}$ region, which is not present in the original chitosan sample. This absorption is attributed to the C=C stretching in the aromatic ring of the aldehyde.

The elemental analysis results of the chitosan and the six biopolymeric Schiff base derivatives are also presented in Table 1. The results are quite different from those

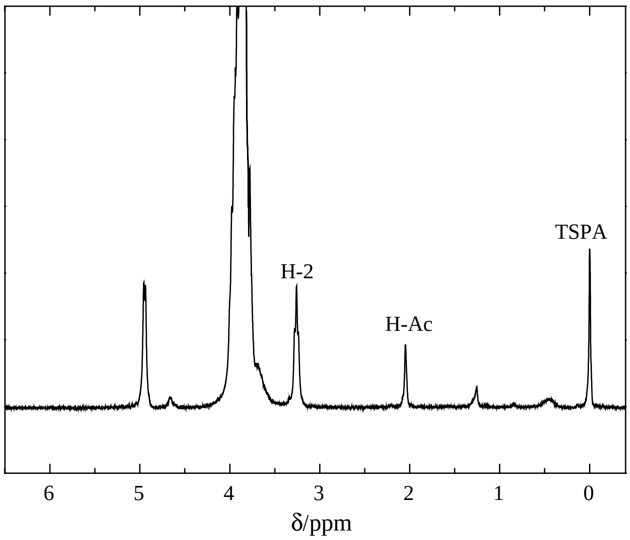

Fig. 2. $^1H$ NMR spectrum of chitosan (sample CA). Concentration: 10 mg/mL in $HCl/D_2O$ at 343 K. TSPA is the reference signal; H-Ac is the proton of the acetyl group and H-2 is defined in Fig. 1.

expected for a 100% degree of substitution, suggesting that the substitution is not complete. However the presence of salycilaldehyde and its derivatives caused an increase in the carbon and hydrogen content in the resulting materials compared with the starting chitosan. A decrease in nitrogen/carbon ratio suggests that the substitution occurred, but in different degrees depending on the modifier used (Skorik et al, 2003). The chitosan is strongly linked to water molecules and, in the solid state, the biopolymer can present distorted structures that are easily hydrated (Rinaudo, 2001).

The occurrence of the substitution is in total agreement with the results obtained by IR and $^1$H NMR described bellow.

Evidences of intramolecular hydrogen bonding between the *o*-phenolic −OH and the imine nitrogen of the Schiff bases are observed only for the SB-4 sample since a displacement of c.a. 40 cm$^{-1}$ is observed in the IV band of C–O phenolic bond (Table 1).

In addition the H-7 proton in this sample presented the smaller chemical shift in the $^1$H NMR spectra when compared with the other biopolymeric Schiff bases prepared in this work (Table 2).

The degree of acetylation ($\overline{\mathrm{DA}}$) is an important characteristic of chitosan and was determined from the ratio between and *N*-acetylated glucosamine (GlcNAc, 2.04 ppm) and the area of the proton 2 signal in the pyranose ring (GlcN, 3.2 ppm), in the $^1$H NMR spectra (Shigemasa et al., 1996; Rinaudo et al., 2001; Signini & Campana-Filho, 1999).

The $^1$H NMR spectrum of the unmodified chitosan obtained at 343 K is presented in Fig. 2. In this temperature the spectrum allows a better resolution of the signals. The calculated value using the Eq. (1) was 10% of acetylation.

$$\overline{\mathrm{DA}} = \left(\frac{A_{\mathrm{CH3}}}{3A_{\mathrm{H-2}}}\right)100 \qquad (1)$$

where $\overline{\mathrm{DA}}$, is the degree of acetylation, $A_{\mathrm{CH3}}$ is area of peak of the three protons of acetyl group and $A_{\mathrm{H\text{-}2}}$ is area of peak of proton H-2 (see Table 2).

Table 2
Assignments of the $^1$H NMR spectra signals and $\overline{\mathrm{DS}}$ for the biopolymeric Schiff bases

| Sample | Chemical shift (δ), ppm | | | | | %$\overline{\mathrm{DS}}$ |
|---|---|---|---|---|---|---|
| | H-1 | H-2 | H-Ac | H-7 | H-8,9,10,11 | |
| CA | 4.94 | 3.26 | 2.04 | Absent | Absent | – |
| SB-1 | 4.93 | 3.23 | 2.05 | 9.96 | 7–8 | 17.1 |
| SB-2 | 5.41 | 3.74 | 2.57 | 9.94 | 7–8 | 4.6 |
| SB-3 | 5.40 | 3.71 | 2.55 | 9.98 | 7–8 | 5.3 |
| SB-4 | 5.10 | 3.40 | 2.22 | 9.89 | 7–8 | 19.5 |
| SB-5 | 4.93 | 3.25 | 2.06 | 9.91 | 7–8 | 34.1 |
| SB-6 | 4.93 | 3.26 | 2.06 | 9.95 | 7–8 | 68.5 |

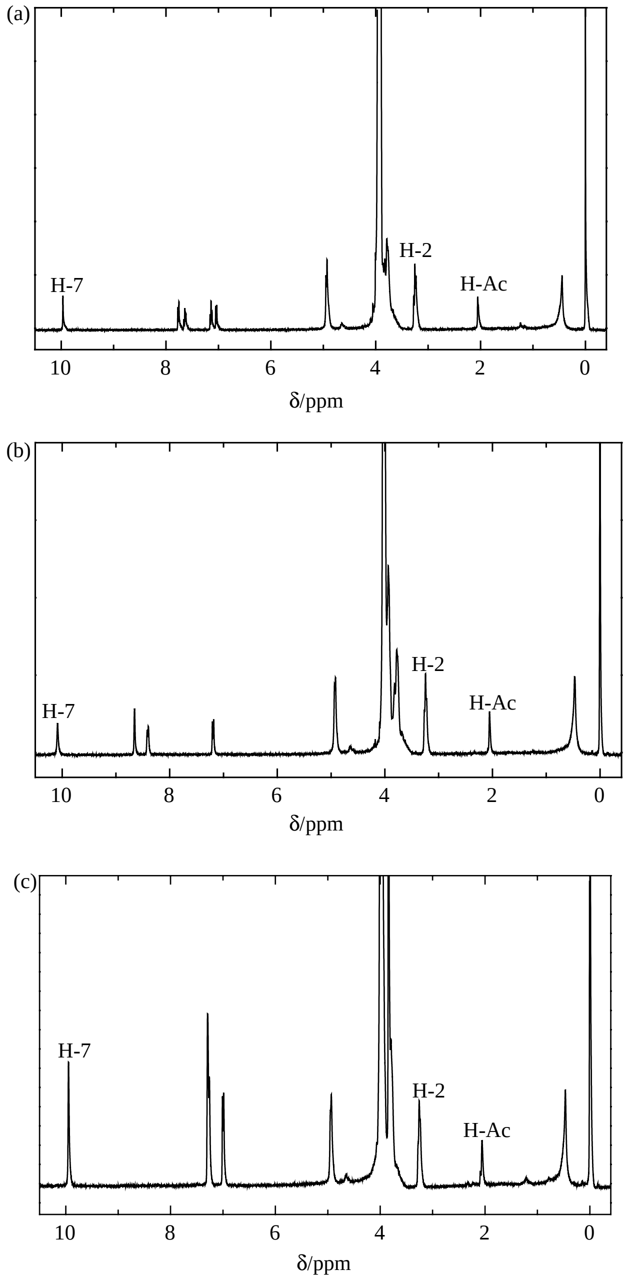

Fig. 3. $^1$H NMR spectrum of sample SB-1 (a), SB-4 (b) and SB-6 (c). Concentration: 10 mg/mL in HCl/$D_2O$ at 343 K. H-Ac is the proton of the acetyl group; H-7 e H-2 are defined in Fig. 1.

The assigments of the $^1$H NMR signals of the biopolymeric Schiff bases were performed in attempt to characterize them, according to the literature (Silverstein et al., 1994) and are described in the Table 2.

The $^1$H NMR spectra of the biopolymeric Schiff bases (Fig. 3) presented significant alterations in relation to the original chitosan. The substitution could also be confirmed by $^1$H NMR spectroscopy in the six samples.

These spectra also allowed us to propose a method to determine the degree of substitution. The determination is based on the ratio of the areas of the proton in the imine groups (H-7 in Fig. 1) and the proton of the pyranose ring (H-2 in Fig. 1), as represented in Eq. (3):

$$\overline{\mathrm{DS}} = \left(\frac{A_{\mathrm{imine}}}{A_{\mathrm{H-2}}}\right)100 \qquad (3)$$

where $\overline{\mathrm{DS}}$, is the degree of substitution. $A_{\mathrm{imine}}$, is the area of the pick of proton imine and $A_{\mathrm{H\text{-}2}}$ is the area of the pick of proton H-2.

The calculated values using the Eq. (3) and the areas ratio described above varied from 4.6 to 68.5%, as presented in Table 2.

Kurita et al. (2002); Rodrigues et al. (1998), and Skorik et al. (2003), suggested the determination of the $\overline{\mathrm{DS}}$ from elemental analysis data. However, we think this is not adequate, since the water present can severely interfere in the results. $^1$H NMR spectra of SB-1, SB-4 and SB-6 are presented in Fig. 3 examples.

The $^1$H NMR spectrum of SB-1 compound is presented in Fig. 3a. The area of the signal corresponding to imine group proton (between 9 and 10 ppm) and that of the H-2 proton of the ring pyranose determined the degree of substitution of the biopolymeric Schiff bases. Any modification in the acetylation degree during the Schiff base preparation has not been observed.

According to the $\overline{\mathrm{DS}}$ in Table 2, the order of substitution is:

$$5-\mathrm{MeO} > 5-\mathrm{Me} > 5-\mathrm{NO_2} \approx \mathrm{H} > \mathrm{Cl} \approx \mathrm{Br}$$

The differences can be related with the electronic effects of substituents, which are composed of two main parts: a field/inductive component and a resonance component, according to Hansch, Leo, and Taft (1991).

The highest $\overline{\mathrm{DS}}$ observed for the 5-MeO should be related to the inductive effect of this substituent which is considered a moderately activating agent in aromatic substitutions (Allinger, Cava, Johnson, Lebel, & Stevens 1976; Sollomons, 1992).

The weakly activating agent 5-Me presented a $\overline{\mathrm{DS}}$ value higher than the 5-$NO_2$ substituent, which can be explained by the strong deactivation effect of the nitro group.

However, the 5-Cl and 5-Br substituents presented the lowest $\overline{\mathrm{DS}}$, although they are considered weakly deactivating groups. This can be explained by the fact that their inductive effect is more important than the resonance effect, while in 5-$NO_2$ the inverse behavior is observed (Allinger et al., 1976).

## 4. Conclusions

Six biopolymeric Schiff bases were successfully prepared by the reaction of chitosan with salicylaldehyde and its 5-$NO_2$, 5-Br, 5-Cl, 5-MeO and 5-Me derivatives under mild acidic conditions. The degree of substitution was determined by a proposed $^1$H NMR spectrometric procedure. It appears that the inductive/field and resonance effects control the substitution degree.

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