International Journal of Biological Macromolecules 95 (2017) 168–176

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Synthesis, characterization and biological activity of Cu(II), Ni(II) and Zn(II) complexes of biopolymeric Schiff bases of salicylaldehydes and chitosan

Eliene Leandro de Araújo[a], Hellen Franciane Gonçalves Barbosa[a], Edward Ralph Dockal[b], Éder Tadeu Gomes Cavalheiro[a,*]

[a] *Instituto de Química de São Carlos, Universidade de São Paulo, Av. Trabalhador São-Carlense 400, 13566-590, São Carlos, SP, Brazil*
[b] *Universidade Federal de São Carlos, Via Washington Luis, Km 235, 13560-900, São Carlos, SP, Brazil*

ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form 21 October 2016
Accepted 30 October 2016
Available online 13 November 2016

Keywords:
Schiff bases
Chitosan
Biological activity

ABSTRACT

Schiff bases have been prepared from biopolymer chitosan and salicylaldehyde, 5-methoxysalicylaldehyde, and 5-nitrosalicylaldehyde. Ligands were synthesized in a 1:1.5 mol ratio, and their Cu(II), Ni(II) and Zn(II) complexes in a 1:1 mol ratio (ligand:metal). Ligands were characterized by ^{1}H NMR and FTIR, resulting in degrees of substitution from 43.7 to 78.7%. Complexes were characterized using FTIR, electronic spectra, XPRD. The compounds were confirmed by the presence of an imine bond stretching in the 1630–1640 cm^{-1} and νMetal-N and νMetal-O at <600 cm^{-1}. Electronic spectra revealed that both Cu(II) and Ni(II) complexes present a square plane geometry. The crystallinity values were investigated by X-ray powder diffraction. Thermal behavior of all compounds was evaluated by TGA/DTG and DTA curves with mass losses related to dehydration and decomposition, with characteristic events for ligand and complexes. Schiff base complexes presented lower thermal stability and crystallinity than the starting chitosan. Residues were the metallic oxides as confirmed by XPRD, whose amounts were used in the calculation of the percentage of complexed metal ions. Surface morphologies were analyzed with SEM-EDAX. Preliminary cytotoxicity tests were performed using MTT assay with HeLa cells. Despite the differences in solubility, the free bases presented relatively low toxicity.

1. Introduction

Chitosan (Cs) [β-(1 → 4)-2-amino-deoxy-D-glucopyranose] is a natural biopolymer derived from the N-deacetylation of chitin [β-(1 → 4)-2-acetamido-deoxy-D-glucopyranose]. The Cs potential application in several fields of biomedicine, agriculture, environmental protection, among others has been studied [1]. Cs is a biocompatible, biodegradable, nontoxic, but it has limitations regarding its utility. The polymer presents low solubility in water in neutral and basic conditions, low surface porosity [2]. How Cs contains reactive free amine groups at the carbon-2 (C-2) position in deacetylated units, and hydroxyl units at the carbon-3 (C-3) and carbon-6 (C-6) positions in both acetylated (GLcNAc) and deacetylated units (GLcN) that allow many possibilities for chemical substitutions, as extensively reported in the literature [3]. Such reactions result in modified biopolymeric materials that represent alternatives to the natural raw material, with improved properties and a wide range of applications. Reaction of free amino groups on chitosan chain with a carbonyl group from an aldehyde or a ketone, produce a Schiff base (SB) (Fig. 1) [4–7]. Cs modified with Schiff bases have been widely investigated and the biological activity of such biopolymeric compounds include cell inhibition of carcinomas and antimicrobial action, such as, antibacterial activity antifungal activity that is improved when compared to the unmodified Cs and is strongly dependent on the nature of the substituent [8–10].

Schiff bases represent an important class of ligands in coordination chemistry, producing very stable complexes with several transition metals generally in (II) oxidation states [11–16]. Thus they have been extensively investigated regarding their electronic properties, catalytic activity in several chemical [17–21] and photochemical reactions as electrode modifiers in electroanalysis [22,23], as antitumor [24] antifungal, antiviral, antimalarial, and antibacterial agents [25], as mimetic models for the transport of oxygen in metalloenzyme complexes, as well as, in other types of applications.

---

* Corresponding author.
E-mail address: cavalheiro@iqsc.usp.br (É.T.G. Cavalheiro).

http://dx.doi.org/10.1016/j.ijbiomac.2016.10.109
0141-8130/

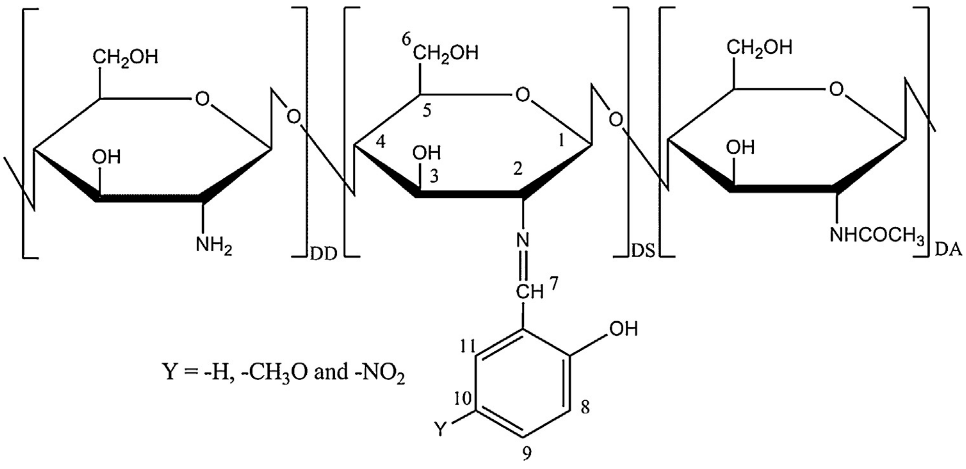

Fig. 1. Structural representation of the ligands biopolymeric Schiff base, where $\left(\overline{DD}\right)$ = degree of deacetylation, $\left(\overline{DS}\right)$ = degree of substitution and $\left(\overline{DA}\right)$ = degree of acetylation.

However, few studies have been performed regarding Cu^{2+}, Ni^{2+} and Zn^{2+} complexes with Schiff bases derived from salycilaldehyde and its derivatives anchored on Cs. Recently metallic complexes of Schiff bases derived from 3,5-Di-*t*-butylsalycilaldehyde on Cs matrix with zinc(II) have been prepared and characterized by FTIR spectroscopy and thermal analysis [26]. Mixed Zn^{2+} complexes of glycine and a Schiff base derived from salycilaldehyde and Cs were also prepared, characterized and investigated regarding its effect on the growth of liver cancer cells with improved action compared with complex without glycine [27], as well as, in the elimination of superoxide and peroxide anion, similar studies were developed with copper(II) complexes.

Anan et al. [28] synthesized and characterized a series of di and trivalent metals with a Schiff base derived from 4-hydroxysalicylaldehyde anchored on a Cs matrix, using FTIR, XRD, SEM images, ESR, RMN and determined the dissociation and stability constants of these complexes. Structures involved square planar or octahedral geometries depending on the metal, being the Cu(II), Ni(II) and Zn(II) of the second type and included an acetate anion in the coordination sphere. Nickel(II) complex of a tridentate Schiff base from a salicylaldehyde derivative on Cs matrix was also prepared and structurally characterized regarding its supramolecular structure on the basis of XRD data revealing a distorted octahedral character for the complex [29].

Concerning the possible biological activity and other uses of such compounds this paper describes the synthesis and characterization of biopolymeric Schiff bases obtained from the reaction of chitosan with salicylaldehyde, 5-methoxysalicylaldehyde, and

5-nitrosalicylaldehyde and their copper(II), nickel(II) and zinc(II) complexes (Fig. 2). The characterization of the ligands and complexes using FT-IR and thermal analysis (TGA/DTG-DTA) is also reported. To our knowledge, this is the first paper report the preparation of complexes Cu(II), Ni(II) and Zn(II) newly shynthesized Schiff bases derived from these salycilaldehydes substituted at position 5 on a Cs matrix.

2. Experimental

2.1. Materials

Salicylaldehyde, 5-methoxysalicylaldehyde and 5-nitrosalicylaldehyde (98% purity, Sigma-Aldrich), copper and nickel acetate (Mallinckrodt) and zinc acetate (Merck) were directly used in the synthesis, without further purification. Potassium bromide,

3-(4,5-dimethyltriazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) and deuterium oxide were purchased from Sigma-Aldrich. The culture media, Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), 0.25% trypsin-EDTA solution,

penicillin-streptomycin (PS) mixture were purchased from Invitrogen. All other chemicals were of analytical grade. The water used in all solutions was purified by Barnstead™ Easy pure® RoDi system from Thermo Scientific and presented conductivity of $18\,\Omega\,cm^{-1}$ at 25 °C.

Commercial chitosan (technical grade, Sigma-Aldrich, Cc) was purified according to a previously described procedure [30]. Briefly, the chitosan was suspended in 0.15 mol L^{-1} acetic acid, with stirring for 12 h until dissolution of the biopolymer, followed by filtration. Concentrated NH_4OH was then added to precipitate of the Cs, and the solid was filtered and washed with water until neutrality. The samples were then dried in a vacuum oven at 40 °C for 72 h. Finally, the degree of deacetylation ($\bar{D}D$) and degree of substitution ($\bar{D}S$) of salicylaldehydes was determined by 1H NMR. The data on average molar mass ($\bar{M}_W$) for the Cc and Cs were 414×10^3 and 418×10^3 respectively, and polydispersity (DP) were 3.7 and 4.6. Such data were calculated according to Brugnerotto et al. [31] and Kasai [32].

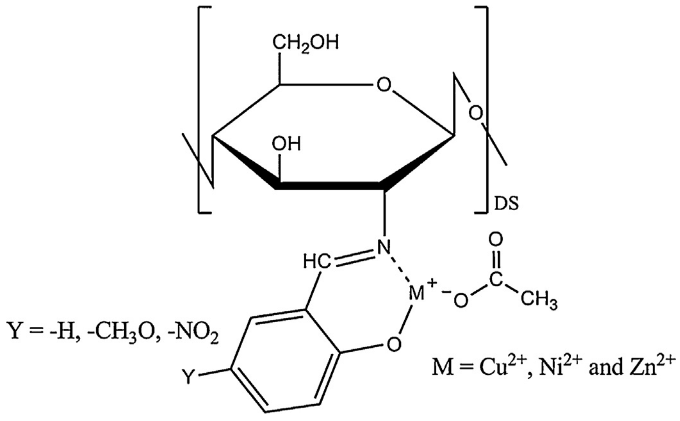

Fig. 2. Structural representation of the complexes.