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Antibacterial Activity of a Chitosan Derivative Obtained in the Absence of a Solvent

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Abstract:A novel chitosan derivative was prepared through direct reaction of pure chitosan with acetylacetone in the absence of a solvent, and it was characterized by elemental analysis, Fourier transform infrared spectrometry (FTIR), and ^{13}C Nuclear Magnetic Ressonance (NMR) spectroscopy. Moreover, the antibacterial properties of the new biomaterial were tested by the direct contact method against multi-drug resistant strains of Staphylococcus aureus and Escherichia coli. The results from the characterization were consistent with the modification of the chemical structure made. The new derivative showed a better antibacterial activity than raw chitosan against E. coli strains, indicating that incorporation of imine link (Schiff base) enhanced its antibacterial activity against Gram-negative bacterium. On the contrary, this chemical change did not decrease its antibacterial activity against Gram-positive bacterium.

Introduction

The high prevalence of infectious diseases worldwide has motivated the search for new strategies to control the spread of multi-drug resistant (MDR) bacteria [1]. The search for new prophylactic and therapeutic resources that are more effective against MDR has mobilized workers of different areas in recent years. From this perspective, a growing interest has been given to the use of polymeric biomaterials with antimicrobial properties for prophylaxis and the treatment of infectious diseases [2].

Chitosan is a biopolymer obtained from chitin deacetylation under different conditions and an alkaline medium. The biological activities of chitosan, such as antimicrobial, antitumor, and anticoagulant activities, as well as, healing, have been studied in several works, which have shown different technological uses in medical and pharmaceutical areas [3, 4]. Structural changes of chitosan have been made in order to improve its properties, such as solubility, chemical stability, biocompatibility, and hydrophilicity [5], which can affect its biological activities, namely its antimicrobial activity [6].

Different modifier agents including glutaraldehyde [7], amino acids [8], aldehydes complex [9], ethylene sulfide [10, 11], ketone [12], epichlorohydrin [13], succinic anhydride [4, 14] and flavonoids [15] have been used for this purpose. In this work, a chemical modification of chitosan with acetyl acetone and without a solvent was made, and the antibacterial activity of the new derivative was tested against gram-positive and gram-negative bacteria.

Materials and Methods

Materials

Chitosan extracted from shell crab with an average degree of deacetylation (78%) was purchased from Primex. Acetylacetone (Riedel-de-Haen), sodium hydroxide (Dinâmica), acetic acid (VETEC), Brain Heart Infusion medium (HIMEDIA), Muller-Hinton Agar (HIMEDIA), and sodium chloride (IMPEX) were used without further purification.

Preparation of chitosan derivative

The reaction between chitosan (6.0 g) and acetylacetone (25 mL) was conducted without a solvent, under reflux and magnetic shake, at 140 ºC for 4 h (Fig. 1). The solid material was filtered and washed with water and acetone and further dried at 100 ºC.

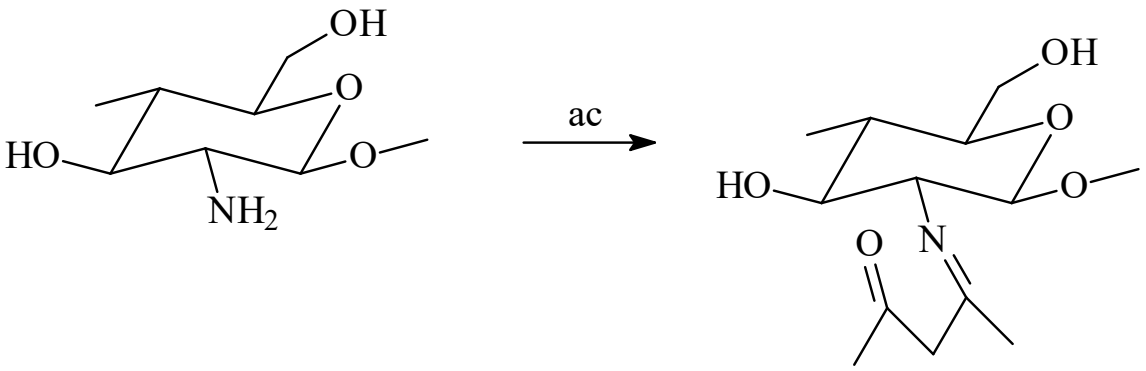

Fig. 1 - Synthesis route of CAC.

Characterization

The structure of the chitosan modified with acetylacetone (CAC) was characterized by: elemental analysis (nitrogen and sulfur percentages were determined through an elemental analyzer Perkin-Elmer, model PE-2400);^{13}C nuclear magnetic resonance (^{13}C NMR) (performed through a Bruker AC 300 at room temperature and the spectra were taken for ^{13}C and ^{29}Si using the cross-polarization technique with magic angle spinning with 4 kHz as the frequency of rotation. The pulse repetitions of 1 and 3 sand contact times were 3 s and 50 ms, respectively. The measurements were obtained at frequencies of 75.47 and 59.61 MHz for carbon);Fourier transform infrared spectrometry (FTIR) (performed by a FTIR Bomen, MB series spectrometer, by preparing KBr pellets with 1% of sample concentration, 32 scans, between 400 and 4000 cm^{-1}, and with 4 cm^{-1} of resolution.)

Antibacterial activity assay

The *in-vitro* antibacterial activity of the chitosan and CAC derivative against *Escherichia coli* (clinical isolate EC1)and *Staphylococcus aureus* (clinical isolate SA1)was investigated by direct contact test in accordance with Zheng and Zhu [16]. Stock solutions of chitosan and CAC derivative were prepared at 30,000 μg/mL in acetic acid 1% (m/v), pH 5.0. The inhibitory effect of the biomaterials was tested by adding 20 μL of the bacterial suspension (~10^5 CFU/mL, in BHI medium) and 20 μL of the biomaterials at 5000 μg/mL to petri dishes within Muller-Hinton agar. The plates were seeded by spread-plate method and incubated at 37 °C for 24h. The inhibitory effect was determined by counting the unit forming colony growing in the plates with and without the biomaterials tested.

Results and Discussion

A novel chitosan derivative CAC was synthesized without a solvent through a direct reaction with acetyl acetone. The elemental analysis data are shown in the Table 1. The effectiveness of the chemical reaction is evidenced by the decrease in the nitrogen content after the incorporation of the acetylacetone moiety (from 5.49 to 3.75 mmol/g), which led to an increase in the carbon/nitrogen ratio (from 6.13 to 10.44).

Table 1 – Percentage, molar amounts and ratio of Carbon (C) and nitrogen (N) for raw chitosan and chitosan modified with acetylacetone.

	%		mmol/g		
	C	N	C	N	C/N
Chitosan	40.43	7.69	33.69	5.49	6.13
CAC[a]	46.98	5.25	39.15	3.75	10.44

[a] CAC: Chitosan modified with acetylacetone.

The FTIR spectra of chitosan and CAC are presented in Fig 2. The FTIR spectra of chitosan (Fig. 2a) exhibited four characteristic peaks at 3400 cm^{-1}(corresponding to the OH and N-H groups), between 2880 and 2900 cm^{-1}(related to the C-H groups), at 1161 cm^{-1} (which can be attributed to the β-1,4-glycoside bond) and at 1051 cm^{-1}(which can be attributed to the C-O-C stretch of the glycopyranoside ring)[17]. The CAC FTIR spectrum, (Fig. 2b) presented an increase in the intensity at 1715 cm^{-1}, which can be attributed to the ketone carbonyl group. It also presented a new absorption band at 1650 cm^{-1}, which can be attributed to the formation of an imine bond (C=N). The results demonstrated that chitosan structural change occurred successfully.

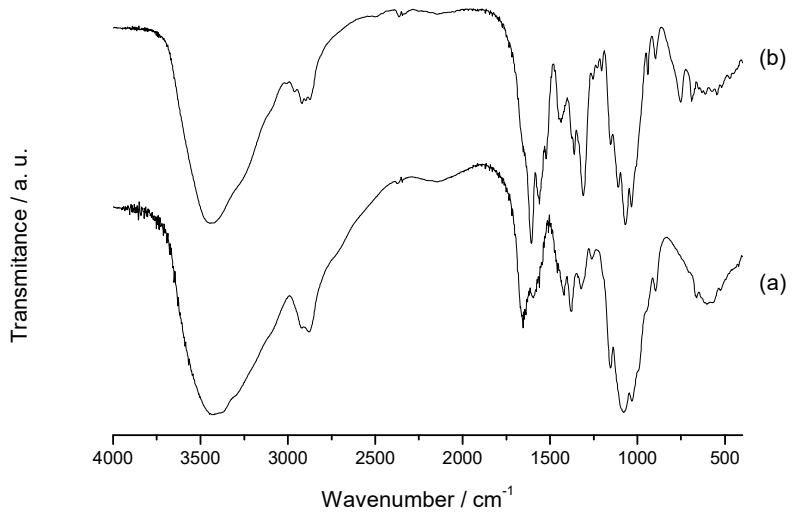

Fig. 2 – FTIR spectra of chitosan (a) and CAC (b).

The ^{13}C NMR spectrum of chitosan and CAC is displayed in Fig. 3. The raw chitosan showed characteristic peaks corresponding to its methyl groups (23 ppm) and the peaks related with the carbons 1 and 4 (105 and 84 ppm, respectively). The new peaks in the CAC spectrum (Fig. 3b), which emerged at 198 ppm and 168 ppm, correspond to the formation of imine bonds (C=N, Schiff base) and to the incorporation of carbonyl group (C=O) from acetyl acetone, respectively. It is also possible to verify the emergence of the peaks at 20 and 35 ppm, which can be attributed to the methyl groups from acetyl acetone and from chitosan molecule. The chemical shifts detected by ^{13}C NMR are in agreement with the structural changes verified by FTIR.

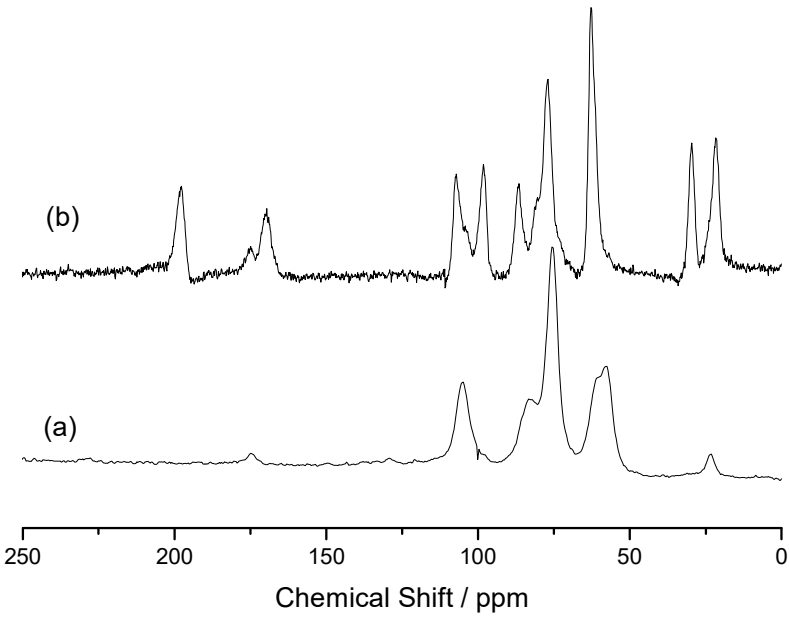

Fig. 3 – ^{13}C NMR spectra of chitosan (a) and CAC (b).

The antibacterial activities of the biomaterials studied against *E. coli*and *S. aureus* were tested by direct contact method (Table 2).

Table 2 – Inhibitory effect on the bacterial growth by the biomaterials tested.

	E. coli EC1	*S. aureus* SA1
	(%)	(%)
Saline	0.0	0.0
Acetic acid	0.3	0.0
Chitosan	16.8	32.1
CAC[a]	33.8	29.8

[a] CAC: Chitosan modified with acetylacetone.

For *E.* coli, the inhibitory effect shown by CAC was 2-fold higher than the inhibitory effect of the raw chitosan, indicating that the introduction of the acetyl acetone group with lipophilic nature enhanced the antimicrobial activity of the new biomaterial against the Gram-negative bacterium. On the other hand, the lipophilic radical did not affect the activity against *S. aureus* significantly. These results indicate that CAC is an antimicrobial material, which could be associated with other antiseptic agents in the control of both Gram-negative and Gram-positive bacteria.

Conclusion

In this study, we described a novel chitosan derivative after reaction with acetyl acetone without any solvent. The success of the reaction was proven through elemental analysis, FTIR, and^{13}C NMR. Moreover, the structural change improved the antibacterial activity of the new material against *E. coli*, which may be due to an increase in the affinity of the CAC for the outer membrane present in the surface of this Gram-negative bacterium. This result showed that CAC could be used in the prevention or in the treatment of bacterial infections.

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