Original Research Paper

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chemistry

PREPARATION AND CHARACTERIZATION OF CARBOXYMETHYLCHITOSAN

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ABSTRACT Chitosan was reacted with trichloroacetic acid at room temperature for different reaction times (10h) and employing two chitosan/trichloroacetic acid molar ratios (1:4.3). The carboxymethylation of chitosan was confirmed by 1H NMR and 13C NMR spectroscopy. The carboxymethylchitosans had average degrees of substitution ranging from 0.52 to 1.44 as determined by analysis.

KEYWORDS :

Introduction:- Chitin, a polysaccharide usually isolated from the carapaces of marines animals such as crabs and shrimps, is a homopolymer composed of 2-acetamide-2-deoxy-D-glucopyranose units linked by bonds. Chitosan is a copolymer of 2-amino-2-deoxy-D-glucopyranose and 2-acetamide-2-deoxy-D-glucopyranose also linked by bonds which is commercially available from the deacetylation of chitin.

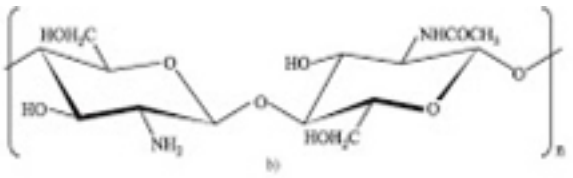

Figure 1. Schematic representation of the primary structure of chitin (a) and chitosan (b).

The industrial production of chitin is growing due to the great stimulus of the food processing industry for the utilization of its refuses, mainly shells of shrimps and crabs, and mainly to the properties of these polymers, such as biocompatibility, biodegradability and their ability to interact with different substances, such as heavy metals and pesticides. Thus, chitin, chitosan and their derivatives prepared by chemical modifications have been used in many applications in the food industry, in cosmetic formulations, for medical and pharmaceutical applications, in the agriculture and in the wastewater treatment for the removal of metallic ions and humic acids.The amino groups of chitosan are weak bases which are predominantly protonated when pH < 6.5, leading to the solubilization of the polymer only in acid dilute solutions. However, the poor solubility of chitosan when pH>6.5 is a serious drawback in many of its potential applications. Thus, the preparation of chitosan derivatives has been envisaged to overcome its limited solubility in aqueous media. Such an adequate chemical modification results, for instance, when the carboxymethylation of chitosan is carried out since carboxymethylchitosan is soluble in a wide range of pH.The antimicrobial activity of chitosan and carboxymethylchitosan may allow their application in agriculture for inhibiting the growth of fungi and bacteria during storage of fruits and vegetables. Such an application is specially interesting for the food industry since these polymers have low toxicity and because they are adequate to oral administration. The limited solubility of chitosan to acid media also limits its use as an antimicrobial agent in the food industry since the low pH may favor deleterious reactions on the food, altering its color and flavor. However, as carboxymethylchitosan is soluble in a wider range of pH its application in this field does not suffer from this drawback. The affinity of chitin, chitosan and derivatives to metal ions, such as Cu, Cd, Pb, Ni, Co, has also been reported, allowing their application for the treatment of industrial effluents. Also in this case the carboxymethylchitosan presents some advantages as compared to chitosan for complexing more efficiently Co+2 and presenting affinity for a larger number of ions. Other important applications of carboxymethylchitosan include the medical and pharmaceutical areas, mainly for the controlled release of drugs, orthopedic devices and tissue adhesion.

The properties and applications of carboxymethylchitosan are strongly dependent on its structural characteristics, mainly the average degree of substitution and the locus, amino or hydroxyl groups, of the carboxymethylation. Thus, the aim of this work is to evaluate the effect of the reaction conditions, essentially the molar ratios chitosan /trichloroacetic acid and the reaction time, on the characteri stics of carboxymethylchitosan.

Preparation of Carboxymethylchitosan:- The procedure described for the carboxymethylation of cellulose, purified chitosan (3g) was dispersed in 65mL of ethanol. After 30 minutes of magnetic stirring at room temperature, 40g of aqueous NaOH (40%) and 24g of trichloroacetic acid/ethanol solution were added to the suspension. The reaction proceeded to the desired time at room temperature and the solid product was then filtered, suspended in 250mL of methanol and neutralized with glacial acetic acid. The product was extensively washed with 60% isopropanol and dried at room temperature. Different carboxymethylchitosan samples were prepared by employing different reaction times (10h) and molar ratios of chitosan/monochloroacetic acid (1:4.3). By employing the molar ratio 1:4.3 and carrying out the carboxymethylation reaction for 10h resulted in the samples QC10 respectively. The carboxymet hylchitosan samples QC10E were obtained when the reactions were extended for 17h, respectively, employing the molar ratio 1:8.6.For the purification of these derivatives, 1.5g of the sample were dissolved in 1.5L of aqueous solution of 0.1M NaCl. The resulting solution was filtered and the carboxymethylchitosan was precipitated upon addition of absolute ethanol. Then, the carboxymethylchitosan was washed with water mixtures of increasing ethanol content (75%, 80% and 90%) and finally with absolute ethanol.

NMR Spectroscopy:-The 1H NMR and 13C NMR spectra of chitosan and carboxymethylchitosans were acquired at 80 °C by using a 200MHz spectrometer (Bruker AC200). For acquiring the 1H NMR spectra of chitosan and carboxymethylchitosans their solutions were prepared at concentrations 10mg/mL and 20mg/mL, respectively. Both polymers were dissolved in D2O/HCl (100/1 v/v) for 1H NMR but solutions of carboxymethylchitosan in D2O were used for 13C NMR.

Results :-The carboxymethylation of chitosan occurs selectively according to the conditions used in the reaction, a complex mixture of products is generally obtained when ordinary conditions are used. The carboxymethylation reaction of chitosan may introduce carboxym ethyl groups in the hydroxyl groups bonded to the carbon atoms 3- and 6- of the glucopyranose unit. The amino group is also a reactive site and two carboxymethyl groups can be introduced. As the reaction is not generally complete, some units of glucosamine as well as acetylglucosamine units coming from the partial deacetylation of chitin may also occur. It is also necessary to take into account the combinations that can occur involving the presence of carboxymethyl groups in the different structural units and then at least 12 different units should be considered to compose the chains of carboxym ethylchitosan. Thus, the complete characterization of this derivative of chitosan may present difficulties due to its structural complexity.The 1H NMR spectrum of chitosan the signal centered at 2.00 ppm corresponds to the hydrogens of the methyl belonging to the acetamido groups. The signal observed between 3.10 and 2.90 ppm corresponds to the hydrogen bonded to the C2 glucosamine ring, while the signals between 3.30 and 4.00 ppm correspond to hydrogens bonded to the carbon atoms C3, C4, C5 and C6 of the glucopyranose that are overlaped. The hydrogen bonded to the anomeric carbon (C1) gives rise to the the signals in the range 4.40 & 5.00.The 13C NMR of

chitosan shows signals at 177.9 ppm and at 25 ppm, which are assigned to the carbonyl carbon of -COCH3 and the methyl carbon (CH3), respectively. These signals are less intense than the other signals, even though the average degree of acetylation of the sample is not small . Indeed, the different carbons have very different relaxation times and the acquisition of spectrum was not optimized to allow a quantitative analysis. The signal at 101.3 ppm is assigned to the hydrogen bonded to carbon C1 of chitosan and the signals in 59.6 ppm, 73.1 ppm, 81.1 ppm, 78.6 ppm and 64 ppm are assigned to carbons C2, C3, C4, C5 and C6 of glypyranose respectively.

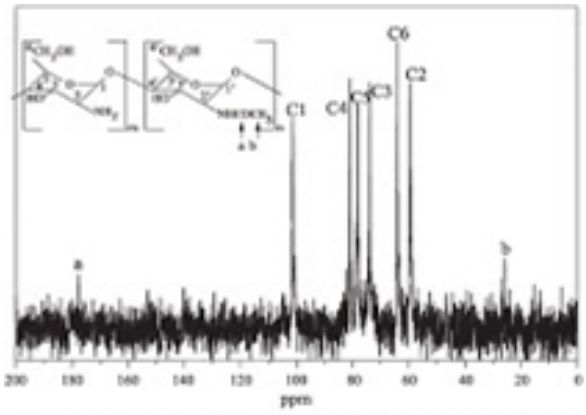

Figure 3. ^{13}C NMRspectrum of chitosan (sample Q) in D_2O/HCl (1:1 v/v) at 80 °C.

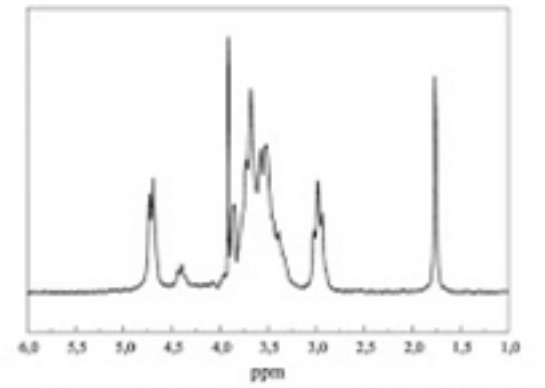

Figure 2. ^1H NMR spectrum of chitosan (sample Q) in D_2O/HCl (1:1 v/v) at 80 °C.

Conclusions:-The characterization of carboxymethylchitosan was difficult due to its structural complexity. Indeed, the 1H NMR and 13C NMR spectra and the titrations of the samples prepared in this work showed the occurrence of O- and N-carboxymethylation in all cases. The degrees of substitution were not proportionally increased when the reaction proceeded for longer times but the use of a higher excess of trichloroacetic acid resulted in more substituted samples.

The structural characteristics of carboxymethylchitosan are strongly affected by the stoichiometry of the reaction and that other variables such as the reaction temperature, should be studied reduce the occurrence of N-carboxymethylation.

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