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Characterization of a Dialdehyde Chitosan Generated by Periodate Oxidation

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Abstract

This article reports the oxidation of Chitosan by using periodate and the characterization of the dialdehyde chitosan (DAC) generated by the reaction. Oxidised chitosan were further characterised by FTIR analysis, ¹H NMR and TGA. The morphological analyses of oxidized chitosan by SEM confirmed the non change of elongated and fibrous network of chitosan. FTIR and TGA show no significant change on chemical structure and thermal properties successively. ¹H NMR study confirmed the presence of imines generated by the reaction between dialdehyde chitosan and amino groups of chitosan (spontaneous assembling).

Keywords: Periodate; Selective oxidation; Chitosan; Dialdehydes

1. Introduction

Currently, much interest has been raised in the development of environmentally friendly materials based on natural polysaccharides [1].

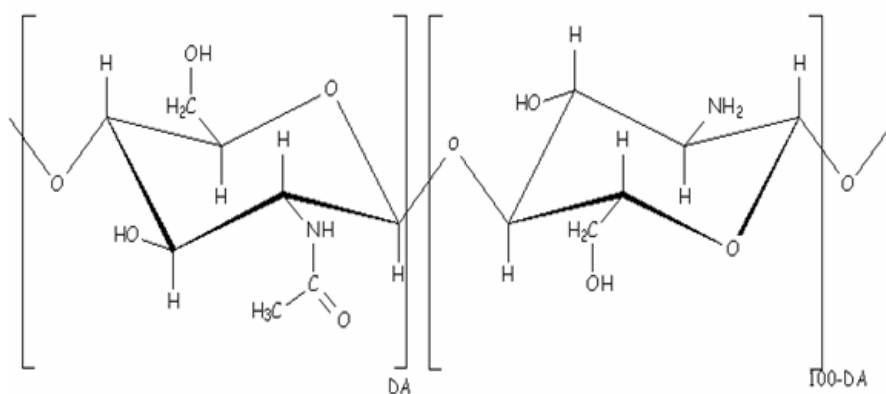
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Periodate oxidation has been widely used as a routine method for elucidation of structures in complex carbohydrates, and its earliest applications helped to interpret fundamental structures and novel functionalities in many polysaccharides such as cellulose, starch [2], glycerol related compounds [3] and xyloglucan [4]. The periodate ion, IO_4^- , attacks vicinal diols to cleave the carbon-carbon bond by an oxidation reaction, leading to the formation of a dialdehyde [5,6]. In addition to the vicinal diols, other 1,2-dioxygenated groups and 1,2-amino alcohols are also oxidatively cleaved by the periodate. N-acetylation of the amino group, however, prevents cleavage [6]. Opening of the pyranosidic rings may also result in increased chain flexibilities, and thereby reduced chain extensions, as demonstrated for alginates [7].

Periodate oxidation of polysaccharides generally seems to give the expected structures as long as the degree of oxidation is relatively low.

Chitin and chitosan are biopolymers having immense structural possibilities for chemical and mechanical modifications to generate novel properties, functions and applications [8,9], as biomedicine [10,11], pharmaceuticals [12-14], metal chelation [15,16], food additives [17], and in the fabrication of sensors or biosensors [16-18].



Scheme 1. Chemical structure of chitosan

Chitosan is a deacetylated derivative of chitin which is isolated from naturally occurring crustacean shells [19-22]. Chitosan has been proposed to have a unique set of properties, including little or no toxicity [23], biocompatibility [24], biodegradability [25], immunogenic activity [26] and antibacterial effects [27]. Chitosan is a linear β -(1-4)-linked polysaccharide composed of repeating units of glucosamine and a small amount of N-acetylglucosamine residues (Scheme 1). The charged state of the primary amine at the C-2 position of the glucosamine residues can be easily altered by pH. At lower pH (<6.5), the amino group is protonated in acidic solutions, and chitosan can act as a water-soluble cationic polyelectrolyte. At higher pH (>6.5), the polymer loses its charge and becomes insoluble.

The authors in [6,8] oxidized chitosans with different chemical compositions (FA = 0.05–0.65) (FA: fraction of acetylated units) and different molecular weights ($M_w = 36.000\text{--}460.000$) partially and with excess periodate. Partial periodate oxidation of chitosans led to a pronounced increase in the chain flexibility as shown by a

gradual decrease in persistence length. Also in another study by Yan Feng and al., periodate oxidation of chitosan was performed and led to a dialdehyde chitosan (DAC) used in a biosensor elaboration. [16]

Here, we present the preparation of a dialdehyde chitosan (DAC) and characterization by ¹H NMR and FTIR studies, TGA and SEM.

2. Materials and methods

2.1. Materials

Chitosan (C) with DDA ~ 80% - 90% in powder form was purchased from Marinard Biotech, Quebec, Canada. One lot of chitosan was used in this study: PR-7-4-61. The degree of deacetylation (DDA %) was determined by ¹H NMR spectroscopy [28].

Analytical grade sodium periodate was purchased from Sigma-Aldrich. All the other chemicals used are of analytical grade and used as received.

2.2. Methods

2.2.1. Oxidation of chitosan

Mix 1g chitosan ([GlcN]= 5.34 mM) in suspension with 50 ml HCl (10⁻³M) (pH ranging from 4-5) with magnetic stirring. Mixed with 1ml aqueous solution of sodium periodate 0.534 mM, P₀ = 0.1 (P₀ = moles of NaIO₄/moles of GlcN). The reaction was carried out at 4°C in the dark for 30 minutes. After reaction, to eliminate the unreacted periodate, add 1 ml ethylene glycol. The oxidized chitosan was washed by distilled water for 4 hand freeze dried.

2.2.2. FTIR analysis

A Nicolet 6700 FTIR -ATR crystal spectrometer, Canada, connected to software of the OMNIC operating system (version 8.2 Thermo Nicolet) was used to obtain FTIR spectra of chitosan. Powder of chitosan samples were placed in contact with attenuated total reflectance (ATR) on the small sampling area of ZnSe crystal at controlled ambient temperature (25°C). FTIR spectra were collected in the frequency range of 4000-450cm⁻¹ by co-adding 64 scans and at a resolution of 4 cm⁻¹. All spectra were rationed against a background of an air spectrum. After every scan, a new reference air background spectrum was taken. The sampling area was carefully cleaned by wiping with acetone and dried with soft tissue before filling in with the next sample. The spectra were recorded as transmittance values at each data point in triplicate.

2.2.3. NMR analysis

Deuterium oxide, deuterium chloride 35% wt. / vol. Deuterium oxide and glacial acetic acid were purchased from Aldrich Chemical.

For all tests, the solutions of oxidized chitosan were prepared by stirring at room temperature 10 mg of oxidized chitosan in a solution composed of 1.96 ml of D₂O and 0.04 ml of DCl and 600 µl of DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) 1M and waiting about half an hour to ensure complete dissolution of the polymer. In these solutions, DCl is in excess compared with amino groups of chitosan so that the polymer is easily dissolved.

¹H NMR spectra were acquired on a Varian Mercury 500 MHz spectrometer. The experiments were run at 70°C, temperature at which the solvent (HOD) peak does not interfere with any of chitosan's peaks. After dissolution, approximately 1 ml of the oxidized chitosan solution was transferred to a 5 mm NMR tube. The sample tube was inserted in the magnet and allowed to reach thermal equilibrium by waiting 10 min before performing the experiment. The ¹H NMR experiment was a single pulse sequence with presaturation of the solvent. A 90° pulse corresponding to a pulse width of 11 ms was used. The delay before the application of the pulse was 6 s and the acquisition time was 2 s for a total relaxation time (recycle time) of 8 s between each transient.

2.2.4. Thermal analysis

The thermal gravimetric analysis was carried out on a TGA 2950 Hi-Res Thermogravimetric Analyzer Canada, at a heating rate of 10°C/min under nitrogen atmosphere. The mass of the samples was generally in the range of 2-3 mg. The sample pan was placed in the balance system equipment and the temperature was raised from 0 to 600°C.

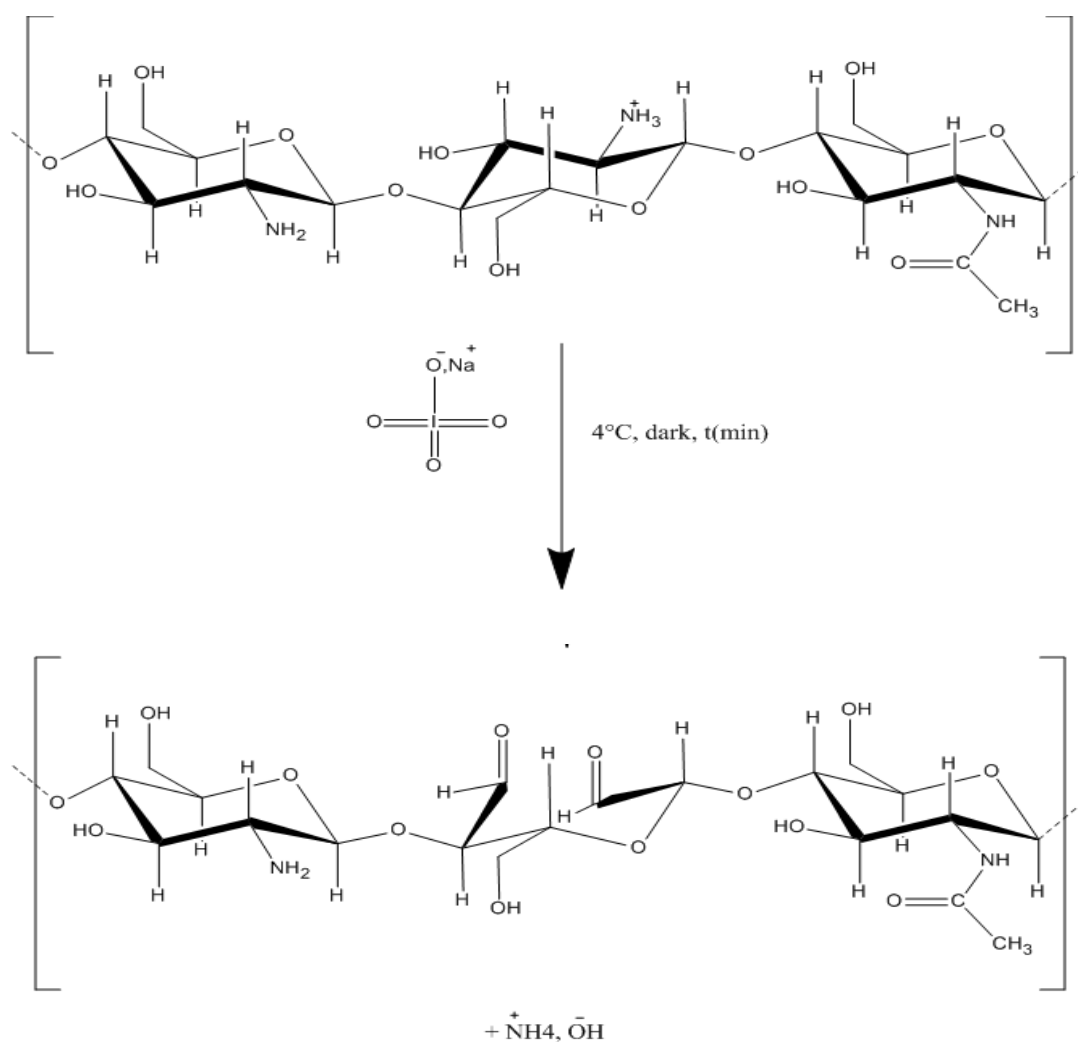
2.2.5. Morphological analysis

Conventional high vacuum scanning electron microscopy (SEM) images were also taken to visualize the structure of oxidized chitosan.

Chitosan and oxidised chitosan were freeze and dried for 24 h and were sprayed on silicon wafer substrate then sputter-coated with gold (Agar Manual Sputter Coater; Marivac Inc, Montreal, QC, Canada) and imaged using a Quanta 200 FEG Environmental Scanning Electron Microscope (FEI Inc, Hillsboro, OR). Observations were performed at 20 kV using the high-vacuum mode.

3. Results and discussions

The oxidation of chitosan using NaIO₄ was analyzed by FTIR (Fig. 1). The oxidation of chitosan (i.e. conversion of secondary hydroxyl groups into aldehydic functionality) must be confirmed by the appearance of characteristic –CO band at 1742 cm⁻¹, but it is known that The dialdehydes tend to react with hydroxyls in adjacent residues to form intramolecular hemiacetals (not shown here) [29]. The characterization and the content of dialdehydes (including their hemiacetals) are not easily determined. Most of the hemiacetal linkages in oxidised polysaccharides can be cleaved by a reduction treatment with NaBH₄ (not used here). Using NaBH₄ may give better results.



Scheme 2. Oxidation reaction of chitosan by periodate

In this work we partially oxidized chitosan with a very few amount of periodate. It is clearly seen from Fig.1 that the absorption patterns of the spectrum of chitosan is similar to that of the literature and suggesting good quality of chitosan biopolymer used [30]. Detailed examination and comparison of chitosan and dialdehyde chitosan spectra reveals that there is no major changes on characteristic bands of chitosan. The slight change is shown at the spectra were observed to have a band located at 3428.32 cm^{-1} indicates the OH stretching, the C-H stretching bands within $2870\text{--}2880\text{ cm}^{-1}$, the skeletal vibrations involving the C-O-C stretching band at 1030 or 1070 cm^{-1} , the $-\text{CH}_2$ bending centered at 1420 cm^{-1} , the anti-symmetric stretching of the C-O-C bridge around 1160 cm^{-1} , $1315\text{--}1320\text{ cm}^{-1}$ (amide III band), $1620\text{--}1630\text{ cm}^{-1}$ ($-\text{NH}$ bending of NH_2) and $890\text{--}900\text{ cm}^{-1}$ (C-O-C bridge as well as glucosidic linkage) [30].

NMR spectroscopy is extensively used to investigate chemical structure in polysaccharides, and has also been used in studies of periodate oxidised polysaccharides. In this work, NMR-spectroscopy has been used to investigate the structure of periodate oxidised chitosan.

Periodate oxidation of chitosan have been relatively little explored, with only a few studies on the periodate oxidation reaction and products formed. However, [28] studied the periodate oxidation and the physical characterisation of oxidised chitosans more in detail. The periodate oxidation of chitosan obviously leads to changes in the chemical structure. It is desirable to characterise the chemical structure of the oxidised chitosan in order to get a better understanding of the periodate oxidation of chitosans. The cleavage of C2-C3 in GlcN units leads to the formation of a dialdehyde. These dialdehydes are capable of existing in a variety of forms, and in solution, equilibrium occurs between the various forms. In water, they may exist as a hydrated, acyclic aldehyde, hemiacetals or hemialdals or as combinations of these. In polysaccharides these links may be intermolecular as well as intramolecular. The structures of chitosan and dialdehyde chitosan DAC are presented in Scheme 2. Fig. 2 presents the 500 MHz ¹H NMR spectrum of chitosan at 70°C. Table 1 shows the chemical shifts of chitosan protons in D₂O/DCl at 70°C.

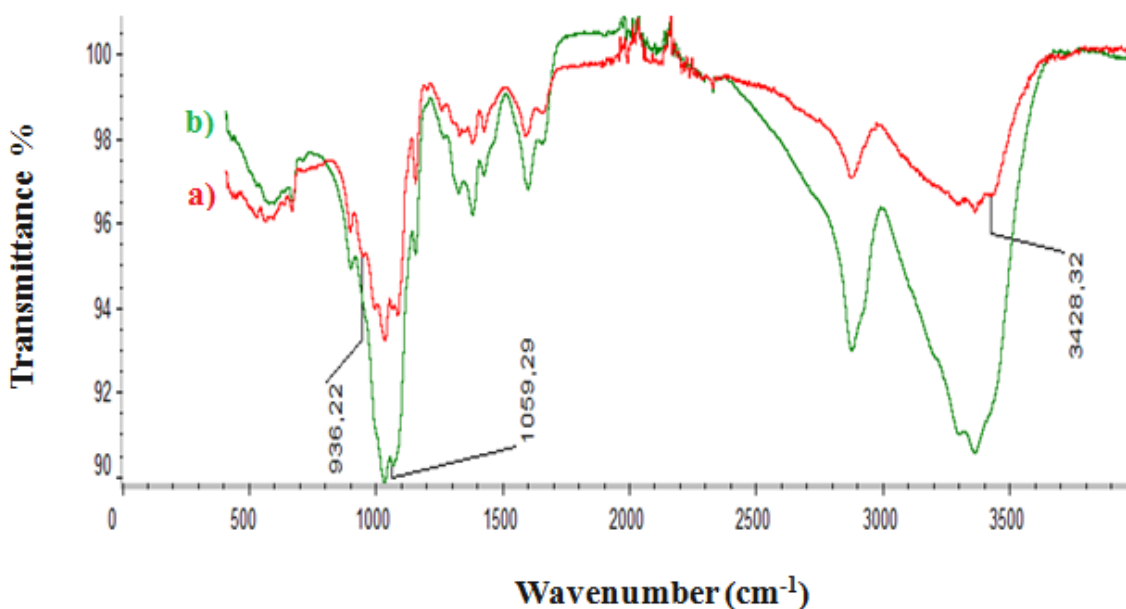


Fig. 1. FTIR spectra of a) chitosan, b) oxidized chitosan

Table 1: Chemical shifts of protons of chitosan and DAC at 70 °C in D₂O/DCl

		Protons					Acetone	DSS	Imine
		H1-D	H1-A	H-2/6	H-2-D	H-Ac			
Chemical shifts (ppm)	Chitosan	4.76	4.46	3.76	3.06	1.89	2.05	-	
	Dialdehyde chitosan (DAC)	5.1	4.8	4.1	3.4	2.2	-	0.00	8.24

¹H NMR spectroscopy of oxidized chitosan did show insignificant deviation from the spectra of unoxidised chitosan. ¹H NMR spectrum of the DAC is shown in Fig. 3. Oxidation causes the rupture of carbon–carbon bond and forms two aldehyde groups in each oxidized monomeric unit (Scheme 2). Hydroxyl groups on the carbons 2 and 3 of the repetitive unit were oxidized by sodium periodate. Therefore, new reactive groups having larger rotational freedom along the polymer backbone were obtained which can be used in our further chemical modifications.

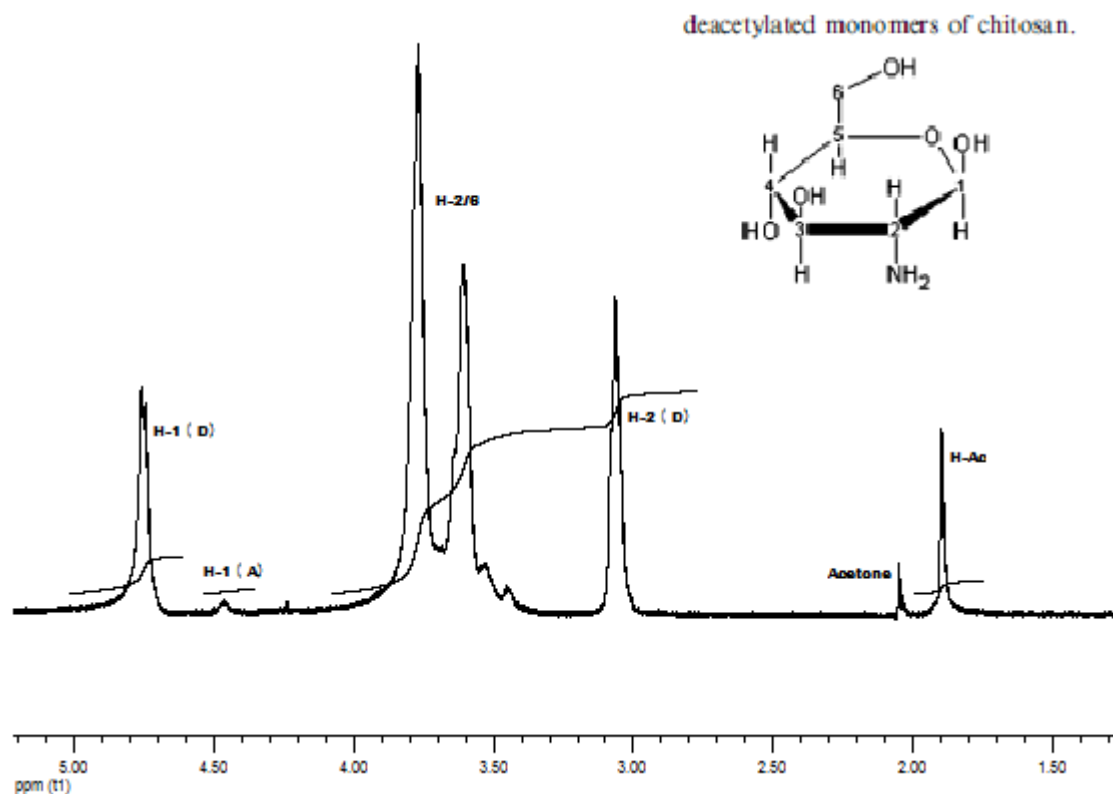


Fig. 2. ¹H NMR spectrum at 70°C of chitosan.

No peaks were observed at chemical shifts characteristic for aldehydes (around 10 ppm). This suggests that all the aldehydes present are hydrated or exist as hemiacetals or hemialdals, as observed previously by various techniques in other periodate oxidized polysaccharides [28].

DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid) is a chemical compound used in proton- and carbon-related NMR spectroscopy as a calibration standard, similar to tetramethylsilane (TMS), but with much higher water solubility. Whereas TMS is the most common NMR standard used in organic solvents such as chloroform or benzene, DSS or its sodium salt is more often used for protein experiments in water. The low electro negativity of the silicon shields the nine identical methyl protons. The result is a high intensity proton signal further up field (at lower chemical) than almost all peaks found in naturally occurring organic molecules. The resulting standard peak is easily identified as such and set to chemical shift 0 ppm.

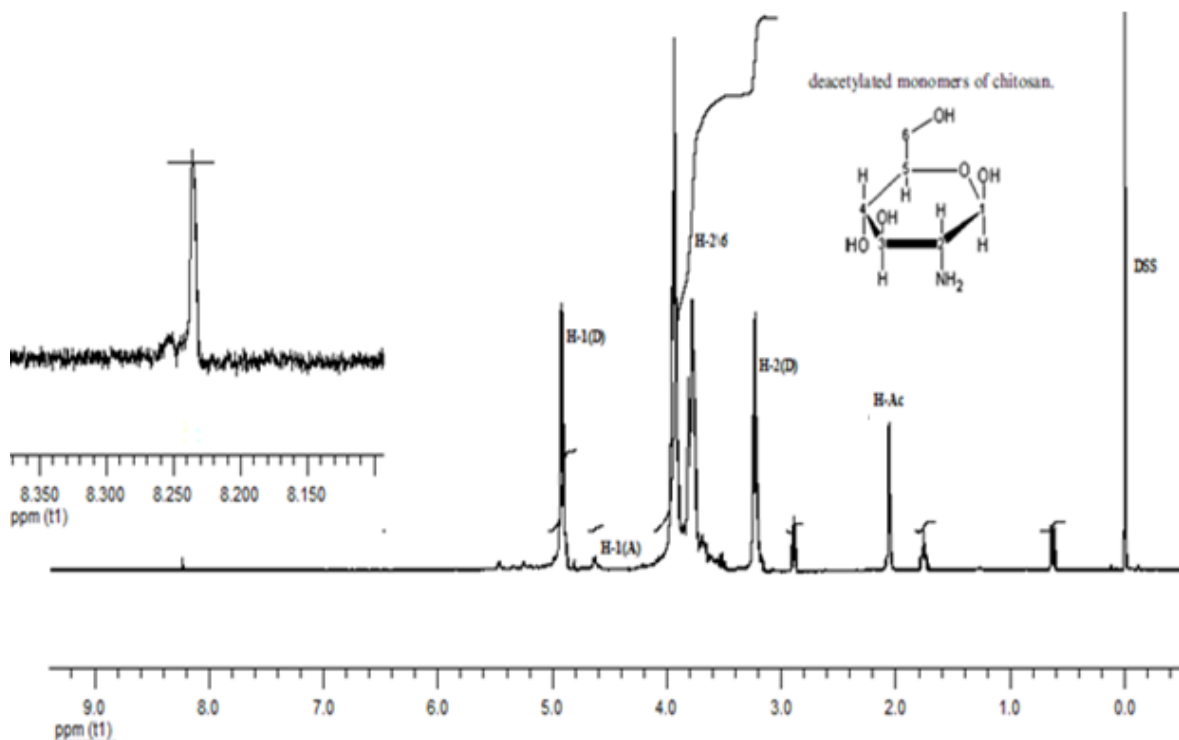


Fig. 3. ¹H NMR spectrum at 70°C of oxidized chitosan.

The proton spectrum of DSS also exhibits minor peaks at 2.9 ppm (triplet), 1.8 ppm (pentet), and 0.7 ppm (triplet) at an intensity of 22% of the reference peak at 0 ppm. However, these peaks appear much smaller than 22% of the height of the reference singlet because of their width (i.e. multiplicity). If these peaks pose a problem, a deuterated version of DSS is available at much higher cost. We were able to attribute the imine proton to the singlet peak at 8.2 ppm [31]. Periodate oxidised chitosan (aldehydes) may react with amines chitosan through Schiff base formation.

The SEM images of chitosan and oxidized chitosan at high vacuum and at different magnifications are shown in Fig.4, showing that there is no change of elongated and fibrous network of chitosan, but on the surface of oxidized chitosan we can see a slight degradation of some leaves.

TGA of chitosan showed a weight loss in two stages. The first stage ranges between 23 and 88°C and shows about 4% loss in weight corresponds to the loss of adsorbed and bound water. The second stage of weight loss started at 242°C and continued up to 316°C during this time there was 42% weight loss due to the degradation of chitosan.

The pattern of degradation was same for oxidized chitosan. The first stage ranges between 23 and 100°C and shows about 5% loss in weight. The second stage decomposition was observed from 200°C and continued up to 315°C during this time there was 42% weight loss due to the degradation of chitosan. The weight loss increased steeply with temperature. The results are shown in Fig. 5.

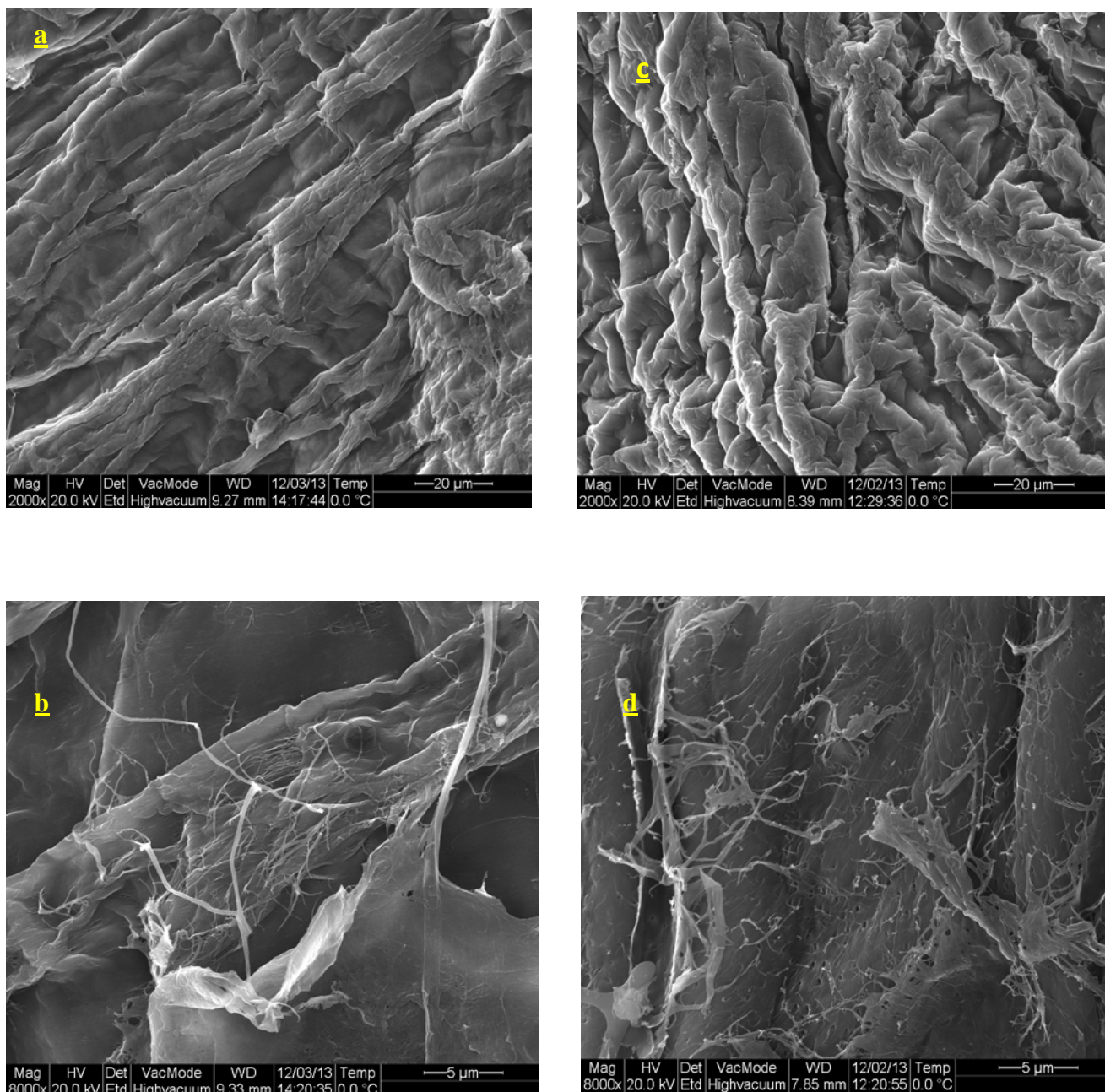


Fig.4. SEM images of (a&b) chitosan and (c&d) oxidized chitosan (DAC) at different magnifications

4. Conclusion

We have accomplished the synthesis and characterization of an oxidized chitosan generating a dialdehyde chitosan. Chitosan and oxidized chitosan are characterized by FTIR and TGA, these two analyzes show no significant change on chemical structure and thermal properties successively. Oxidized chitosan has fibrous network structure such as chitosan. These results are significant for our work because chitosan was partially oxidized with a slight amount of periodate to generate just few opening rings (dialdehyde).

¹H NMR study confirmed the presence of imines (peak at 8.2 ppm) generated by the reaction between dialdehyde chitosan and amino groups of chitosan.

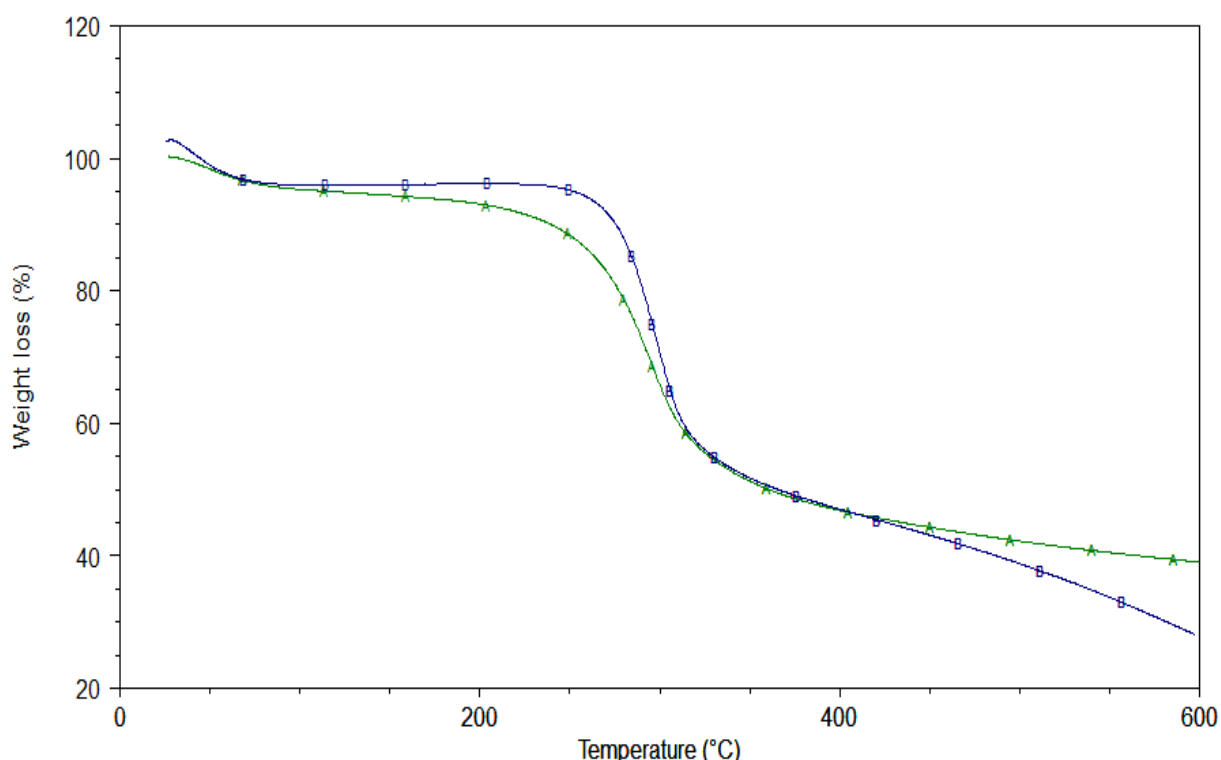


Fig. 5. Thermal properties of A) oxidized chitosan, B) chitosan.

GlcN residues of chitosan bearing free amino groups at C-2 position allow chemical substitution producing several chitosan derivatives with a large spectrum of applications. Among these derivatives, Schiff bases which obtained by reacting these free amino groups of chitosan with active carbonyl compounds such as aldehyde or ketone. The characteristic imine groups (-RC = N-) of these Schiff bases offer several potential analytical and environmental applications by enhancing the adsorption / complexation properties. Moreover, some Schiff bases of chitosan are reported to have antimicrobial activity. This approach can be used to produce various potential therapeutic hydrogels.

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