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Synthesis and Characterization of a Modified Chitosan

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Summary: Chitosan has been modified by incorporating a cationic moiety N- (3-chloro 2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) onto its primary amine group in aqueous alkali medium. A series of modified chitosan copolymers (Chito-cat-1 to Chito-cat-5) was synthesized by varying the CHPTAC to chitosan ratio. The modified chitosan copolymers have been characterized by viscometry, elemental analysis, infrared spectroscopy (IR), thermal analysis and X-ray diffractometry. From the above investigations, it has been confirmed that the cationic moiety was successfully incorporated onto chitosan.

Keywords: biopolymer; cationic; chitosan; CHPTAC; copolymer

Introduction

Chitosan is a partially deacetylated derivative of chitin. Chitin is one of the most abundant biopolymer in nature and commercially extracted from exoskeleton of crustacean, e.g. crab, shrimp etc.[1–3] Chitosan displays unique properties such as biodegradability, biocompatibility and nontoxicity.[4–7] It is used in food, personal care, biomedical, wastewater treatment and other specific applications.[4,8–12] But such a versatile material is only soluble in dilute organic and inorganic acids,[1] which limits its applications. Many researchers have reported that incorporation of other materials may widen its applications.[13–19] There are two types of reactive groups in chitosan on which incorporation of other moiety has been done, the first is the amino group and the second is the hydroxyl group. The incorporation of monomer imparts some unique properties, such as, solubility in neutral water, anti bacterial properties, anti oxidant properties and flocculation properties.[20] We are very much interested in incorporating a cationic moiety onto chitosan. There are few reports available where cationic moiety has been successfully incorporated onto various polysaccharides.[21–24] The main objective of this paper is to synthesize a modified chitosan by incorporating a cationic moiety N-(3-chloro 2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) onto chitosan. A series of modified chitosan (Chito-cat-1 to Chito-cat-5) has been synthesized. The modified products have been characterized by viscometry, elemental analysis, infrared spectroscopy (IR), thermal analysis, and X-ray diffractometry (XRD).

Experimental Part

Materials

Chitosan (degree of deacetylation: 86%, molecular weight: 1.24×10^4 dalton) was a gift from Central Institute of Fisheries Technology, Cochin, India. N-(3-Chloro 2-hydroxypropyl) trimethyl ammonium chloride (CHPTAC) was procured from Lancaster Synthesis Company, England. Analytical grade sodium hydroxide and hydrochloric acid were purchased from E. Merck Limited, Mumbai, India. Isopropanol was supplied by S. D. Chemicals, Mumbai, India.

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Synthesis

Chitosan (0.7 g) was taken in 250 ml of distilled water. The mixture was heated to 33 °C under controlled stirring for 30 minutes. 15 ml of 1(N) NaOH was added. After 15 minutes, an aqueous solution of CHPTAC was added to the reaction mixture. The reaction was then allowed to continue for next 18 hours. Dilute hydrochloric acid was added to the mixture to lower the reaction pH below 7 (Scheme 1). This stops the reaction.[25] The modified chitosan was then precipitated in excess of isopropanol. The precipitate was washed several times to remove the unreacted CHPTAC. It was then dried under vacuum at 60 °C for 2 hours. A series of modified chitosan (Chito-cat-1 to Chito-cat-5) was thus prepared by modifying the amount of CHPTAC added (Table 1).

Characterization

Intrinsic Viscosity Measurement

Intrinsic viscosity measurement was carried out using a Ubbelohde Viscometer (Cannon Ubbelohde, State College, PA) at 30 °C. The viscosity were measured in dilute aqueous solution. The pH of the polymer solution was acidic in nature. The time of flow of the solution was measured at four different concentrations. From the time of flow of the polymer solutions (t) and that of the solvent (t_0, for distilled water), the relative viscosity ($\eta_{rel} = t/t_0$) was obtained. Specific viscosity (η_{sp}) was calculated from the relation $\eta_{sp} = \eta_{rel} - 1$. The reduced viscosity ($\eta_{red} = \eta_{sp}/C$) and the inherent viscosity ($\eta_{inh} = \ln\eta_{rel}/C$) of the polymer samples were plotted at different concentration(C, dL/g). The curves were extrapolated. The extrapolated curves meet at zero concentration. The point of intersaction at zero concentration is the measure of the intrinsic viscosity of the polymer sample.[26] The intrinsic viscosity values of the modified products are reported in Table 1.

Elemental Analysis

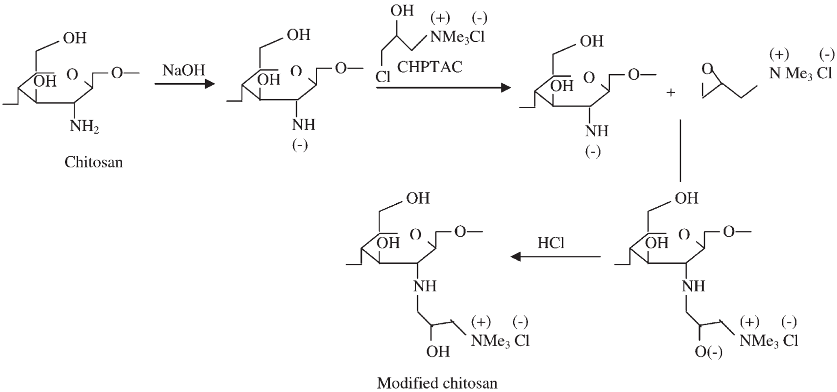
The elemental analysis was performed using Carlo-Erbo 1108 Elemental Analyzer. The percentage of carbon, nitrogen and hydrogen was estimated.

Infrared Spectroscopy

Infrared spectra were recorded on a Perkin Elmer-630, Cetus apparatus using KBr disks under nitrogen atmosphere. Approximately 6 mg of the dried sample was taken with 200 mg of IR grade KBr and 40 mg of the mixture was used to prepare a pallet.

Thermal Analysis

Thermogravimetric analysis (TG) and differential thermogravimetric analysis (DTG) waere performed using Staton Red croft (STA-625) thermal analyzer. The analysis was performed under continuous flow of



Scheme 1.
Modification of chitosan with CHPTAC.

Table 1.
Synthesis details of CHPTAC modified chitosan[a].

Modified chitosan	Molar ratio of CHPTAC to MSU of chitosan[b]	Degree of cationization (%)[c]	Intrinsic Viscosity (dl/g)
Chito-cat-1	0.3977	1.77	0.32
Chito-cat-2	0.6818	7.08	1.51
Chito-cat-3	0.9681	9.40	2.443
Chito-cat-4	1.2272	14.99	2.736
Chito-cat-5	1.3863	15.94	3.245

[a] The reactions were carried out at 33 °C for 18 hours.
[b] MSU is the monosaccharide unit.
[c] Degree of cationization (%) = (Nitrogen content in modified chitosan − Nitrogen content in virgin chitosan) × 100/Nitrogen content in CHPTAC.

dry nitrogen gas at a heating rate of 10 °C min^{-1}.

X-ray Diffractometry

X-ray diffraction patterns of the samples were obtained by the PW-1840 diffractometer and a PW-1716 X-ray generator with CuK_α radiation. The relative intensity was recorded in the scattering range (2θ) of 10 degree to 50 degree.

Results and Discussion

The incorporation of CHPTAC moiety onto chitosan was undertaken in alkaline condition. A series of modified chitosan (Chito-cat-1 to Chito-cat-5) was prepared by varying the CHPTAC to chitosan ratio (Table 1).

Intrinsic Viscosity Measurement

The intrinsic viscosity of a polymer is the measure of its hydrodynamic volume in solution, which in turn depends upon its molecular weight, structure and nature of the solvent and the temperature of the medium. Table 1 shows that the intrinsic viscosity value of the modified chitosan increases with the increase in the CHPTAC to chitosan ratio. As the other parameters remain same, this is a direct indication of the increase in molecular weight. It is believed that with the increase in the content of the CHPTAC moiety in the reaction mixture, large number of the CHPTAC moiety is attached to the chitosan backbone and increase the molecular weight.

Elemental Analysis

The success of the reaction can be confirmed by elemental analysis. Table 2 shows that the percentage of nitrogen content increases with increase in the CHPTAC to chitosan ratio. It means that larger number of CHPTAC moiety was attached to the chitosan with the increase in the CHPTAC to chitosan ratio. It is observed from Table 1 that the degree of cationicity of the modified product increases with the increase in the nitrogen content.

Infrared Spectroscopy

Infrared (IR) spectroscopy is one of the most common characterization tools for the investigation of chemical modification. Figure 1 shows the IR spectra of chitosan, Chito-cat-2 and CHPTAC moiety. The IR spectrum of chitosan shows a broad O-H stretching band at 3427 cm^{-1}. A peak at 1377 cm^{-1} represents the C–O stretching of primary alcohol group. Peak at 1660 cm^{-1} represents the acetylated amine group of chitin, which indicates that the sample is not fully deacetylated. A strong bending vibration for $-\text{NH}_2$ is found at 1591 cm^{-1}.

Table 2.
Elemental analysis.

Sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)
Chitosan	40.4	6.68	7.65
CHPTAC	39.9	8.40	7.34
Chito-cat-1	40.0	8.29	7.78
Chito-cat-2	40.4	8.45	8.17
Chito-cat-3	40.2	8.47	8.34
Chito-cat-4	41.0	8.85	8.75
Chito-cat-5	40.5	8.97	8.82

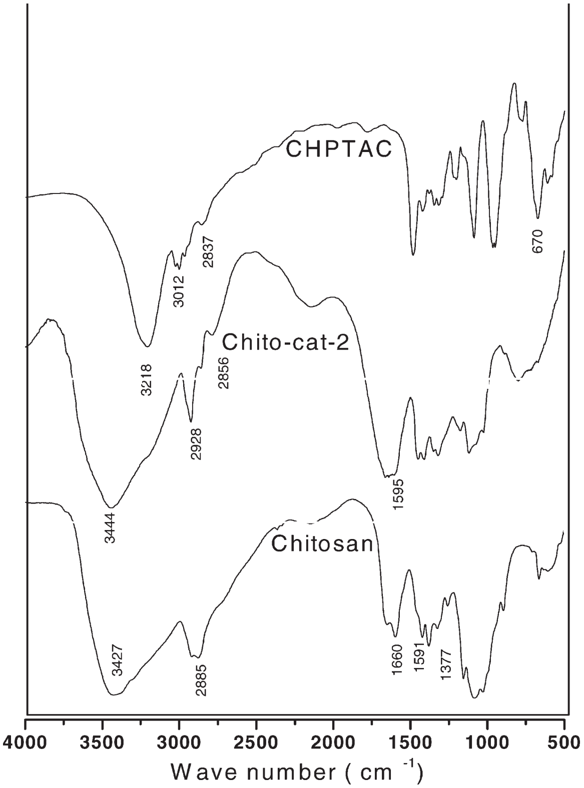


Figure 1.
IR spectra of chitosan, chito-cat-2 and CHPTAC.

The IR spectrum of CHPTAC shows methyl and methylene symmetric stretching vibration at 3012 cm^{-1} and 2837 cm^{-1} respectively. A strong band at 670 cm^{-1} corresponds to the C-Cl stretching.

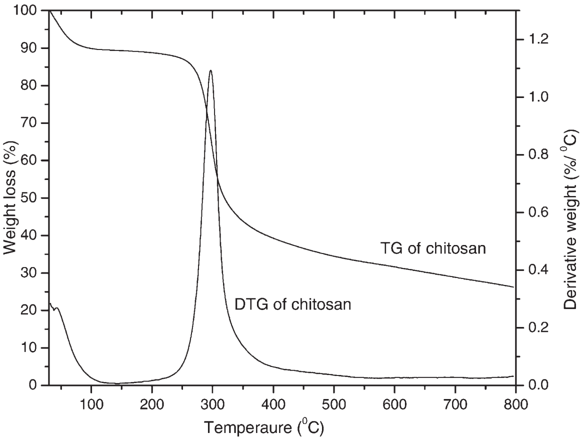


Figure 2.
Thermal analysis of chitosan.

Chito-cat-2 shows decrease in the band intensity at 1595 cm^{-1}, associated with $-NH_2$ bending vibration. This is a direct indication of the incorporation of CHPTAC moiety onto the primary amine group of

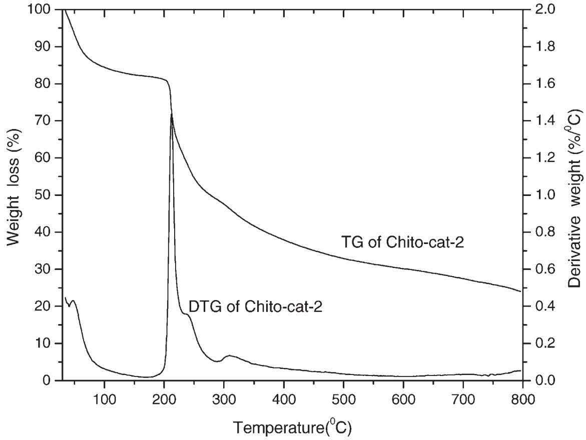


Figure 3.
Thermal analysis of Chito-cat-2.

chitosan. The appearance of intense peaks at 2928–2856 cm^{-1} is contributed to the increase in the number of the methyl group by the incorporation of the CHPTAC moiety.

Thermal Analysis

The thermogravimetric analysis (TG) and differential thermogravimetric analysis (DTG) of chitosan and Chito-cat-2 are represented in Figure 2 and 3 respectively. Figure 2 shows two stages of weight loss. The first stage of weight loss, which starts at 34 °C is due to the loss of adsorbed and bound water.[27] The second stage of weight loss starts nearly at 250 °C. This is due to the degradation of chitosan chain. Figure 3 also shows two distinct zones of weight loss. The

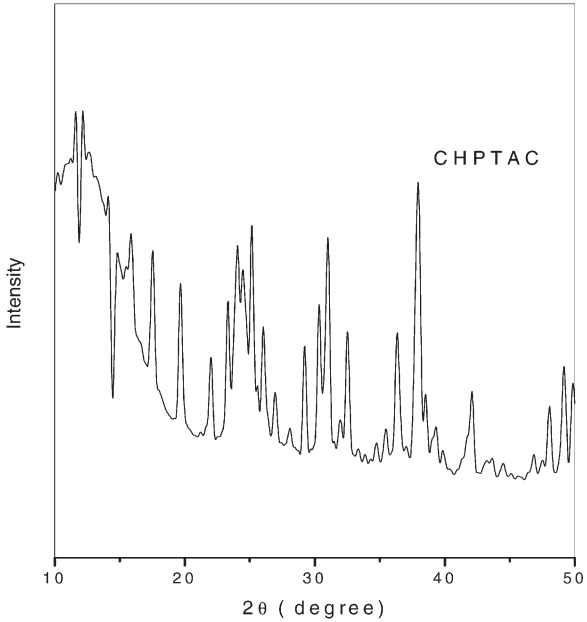


Figure 4.
X-ray diffractogram of CHPTAC.

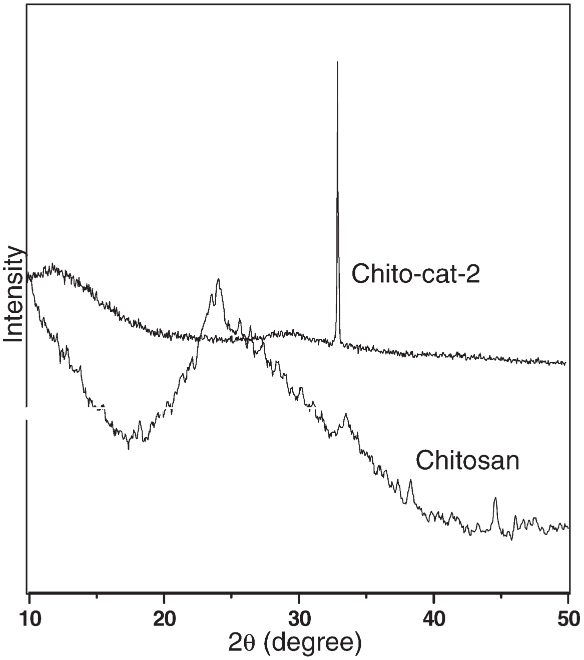


Figure 5.
X-ray diffractogram of chitosan and Chito-cat-2.

first zone of weight loss is associated with the release of adsorbed water from the polymer. The second zone of weight loss, registered between 206–243 °C, is due to the decomposition of the CHPTAC moiety attached to the chitosan.

X-ray diffractometry

Figure 4 shows the highly crystalline nature of the CHPTAC. The crystalline nature of CHPTAC is absent in the Chito-cat-2 (Figure 5). The X-ray diffractogram of chitosan (Figure 5) shows a strong reflective at 10 degree and two other reflectives at 18.2 and 23.9 degree. This is an indication of the hydrated crystalline nature of the chitosan. It also shows a distinct reflective at 33.4 degree. The reflective at 33.4 degree is due to the presence of the hydroxyapatite salt in the chitosan starting material.[1,2] The modified chitosan looses its characteristics reflectives at 10, 18.2 and 23.9 degree. This may be due to the disruption of the ordered structure of the chitosan by the incorporated CHPTAC moiety. The abnormally strong peak at 33.4 degree in the modified chitosan is believed to be due to the increase in the crystallinity of the hydroxyapatite salt by the treatment of alkali and heat during the modification.[1,2] This is also reflected from the increase in the crystallinity index value (calculated from the IR data). The crystallinity index of the virgin chitosan has increased from the 1.053 to 8.8 after modification.

Conclusion

The above study concluded the successful synthesis of CHPTAC modified chitosan. The increase in the CHPTAC to chitosan ratio increases the molecular weight as well as the nitrogen content in the modified chitosan. The IR spectroscopic analysis provides strong evidence of incorporation of CHPTAC moiety onto chitosan. The thermal analysis shows that modified chitosan has lower thermal stability than the unmodified chitosan. The X-ray diffraction study indicates substantial change in crystallinity after modification.

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