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Quaternized Chitosan Quinoline Derivative with Potential Antibacterial Activity

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ABSTRACT

In this work, a quaternized chitosan derivative has been made by reacting chitosan with 2-hydroxy quinoline-3-carbaldehyde in presence of acetic acid to form a Schiff base intermediate, followed by reaction with sodium borohydride and methyl iodide to obtain the water soluble quaternized product. The heterocylclically modified quaternized compound was characterized by Fourier Transform Infra-Red (FTIR), X-rey Diffraction (XRD), Thermogravimetric Analysis (TGA) and UV-Visible spectroscopy. The antimicrobial activity of the quaternized product was investigated against Gram-positive and Gram-negative bacteria using agar well diffusion method. The results showed that the modified compound showed good inhibitory action attributed to the presence of hydrophobic quinoline moiety.

Keywords: Chitosan, 2-hydroxy quinoline-3-carbaldehyde, Schiff bases, Quaternized chitosan, Antimicrobial activity

INTRODUCTION

Chitosan, which is the result of the alkaline hydrolysis of the naturally occurring chitin biopolymer, is considered to be one of the highly versatile polymeric materials due to its biocompatibility, biodegradability and nontoxic property. Its structure is basically constituted of D-glucosamine units, with contents of N-acetyl-D-glucosamine in the range of 0-50% [1]. It has been applied in a variety of fields, such as medical applications, biotechnology, wastewater treatment and agriculture [2,3]. It has useful reactive groups such as a hydroxyl group and an amino group. However, the poor solubility greatly limits its applications. Much attention has been focused on modification of chitosan by using the reactive amino and hydroxyl group to make it water soluble [4]. The ammonium groups on chitosan, formed on protonation at physiological pH interact with the anionic groups on the microbial surface resulting in an impermeable coat on the bacterial surface preventing its growth [5]. The main objective of this work is to functionalize chitosan with heterocyclic moiety to enhance the biological activity and to improve its solubility in various solvents. Quinoline is a heterocyclic organic compound which has a wide range of industrial, pharmaceutical, and biological uses [6,7]. Quinoline and their derivatives are receiving increasing importance due to their potential antimalarial, antibacterial, antiviral, antifungal, antiasthmatic, antihypertensive, analgesic, anti-inflammatory, cytotoxic, antiplatelet and biosynthesis inhibitor activity [7-11].

Based on the above reports we have attempted to synthesize chitosan quinoline derivative and quaternized it. The quaternized derivative has been evaluated for biological activity against 2 strains of Gram-positive and Gram-negative bacteria and the data have been reported.

MATERIALS AND METHODS

Materials

Chitosan (Cs) (with specifications: 75-85% deacetylated, molecular weight: 310 kDa based on viscosity) was purchased from Sigma Aldrich (India). Aniline, acetic anhydride, acetic acid, methyl iodide (CH_3I), tetrahydrofuran (THF), dimethylformamide (DMF), sodium borohydride ($NaBH_4$), sodium iodide (NaI) were purchased from Spectrochim (India), N-methylpyrrolidone (NMP) and all other reagents were purchased from SRL Chemicals (India).

Synthesis of 2-hydroxy quinoline-3-carbaldehyde

2-hydroxy quinoline-3-carbaldehyde was synthesized according to method reported in literature [12]. To a mixture of THF was added aniline (1 eq) and acetic anhydride (1.2 eq) and the reaction was stirred at room temperature for about 8 h till the substrate was consumed as monitored by Thin Layer Chromatography (TLC). Later the mixture was precipitated in water, filtered and dried. To the mixture of phosphoryl chloride ($POCl_3$) in anhydrous DMF (1 eq) maintained at 0°C added acetanilide (1 eq) under stirring, refluxed over night to obtain a dark red solution. It was cooled and the completion of the reaction was confirmed by TLC. The mixture was precipitated in water to obtain a yellow precipitate

which was washed with water and dried. The obtained substituted 2-chloro quinoline-3-carbaldeyde was treated with 4 M HCl (1 eq) under microwave to obtain the 2-hydroxy quinoline-3-carbaldeyde (Scheme 1).

Synthesis of Schiff's bases of chitosan (BCS)

0.2 g of chitosan was dissolved in 10% acetic acid solution and 0.2 g 2-hydroxy quinoline-3-carbaldehyde dissolved in DMF was added slowly into the chitosan solution under continuous stirring for 6 h at room temperature. After 6 h the product was precipitated out in excess acetone and is filtered. The filtrate was washed with ethanol for 4 times to remove the unreacted aldehyde and later dried at 50°C for 24 h to obtain white solid product**.**

Synthesis of N-substituted chitosan (NSC)

The N-substituted Cs was synthesized according to the previous method (14). To 0.1 g of BCS in DMF solvent was added 2 ml of 10% aqueous $NaBH_4$ and the reaction mixture was allowed to stir at room temperature for 4 h later the product was precipitated in acetone, filtered and dried at 60°C for 24 h to obtain NSC.

Synthesis of quaternized quinoline chitosan (QTR-CS)

To prepare the quaternized chitosan derivative, NSC (0.2 g) was dispersed in NMP (10 ml) by stirring at room temperature for 12 h. To this mixture, 0.01 M NaOH (0.1 ml), NaI (0.3 g) and CH_3I (0.8 ml) were added and the reaction was carried out with stirring at 50°C for 20 h. The product was precipitated by the addition of excess acetone and filtered. The QTR-CS obtained was dried at 60°C for 24 h.

Characterization

Fourier transform infra-red (FTIR) spectroscopy

The infrared spectra of the Cs, BCS and QTR-CS were recorded on an IR Prestige- 21, FTIR spectrometer (Shimadzu, Japan) in the powder form.

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy

The ^{1}H-NMR spectra of QTR-CS was recorded on a 400 MHz Bruker (USA) spectrometer using D_2O as solvent.

Thermogravimetric analysis (TGA)

TGA of Cs and QTR-CS was carried out in a Shimadzu DTG-60 thermogravimetric analyzer. The samples were heated from 0-800°C at a heating rate of 10°C/min under flow of nitrogen during the analysis.

UV-visible spectroscopy

UV absorption spectra of QTR-CS were obtained using a Shimadzu UV-2550 spectrophotometer in the range 200-600 nm.

X-rey diffraction (XRD)

XRD of CS and QTR-CS were recorded employing X-ray diffractometer (Rigaku Miniflex 600-XRD Instrument, USA) using Cu K-radiation generated at 40 kV and 35 mA in the differential angle (2θ) range of 5-80°.

Scanning electron microscopy (SEM)

The surface micrographs of CS and QTR-CS were recorded using JOEL-JSM5800LV (Japan) SEM under a voltage of 10 kV with varying magnifications.

Antimicrobial activity assay

The antimicrobial activity of QTR-CS was determined using the agar well diffusion method. Nutrient agar medium was prepared, autoclaved at 120°C for 15 min. Then in the sterile condition the medium was poured into the plates and allowed to solidify. Bacterial suspension was prepared by taking a loopful of cultures of Gram-positive and Gram-negative bacteria dissolved in 1 ml of sterile distilled water separately for each culture. 0.1 ml of the bacterial suspension was placed on the nutrient agar medium and spread using a sterile swab. Wells of 6 mm diameter were made in the solidified media with the help of sterile borer followed by addition of 50 µl of the samples into each well. The plates were incubated at 37°C for 24 h and zone of inhibition was measured in millimeter (mm).

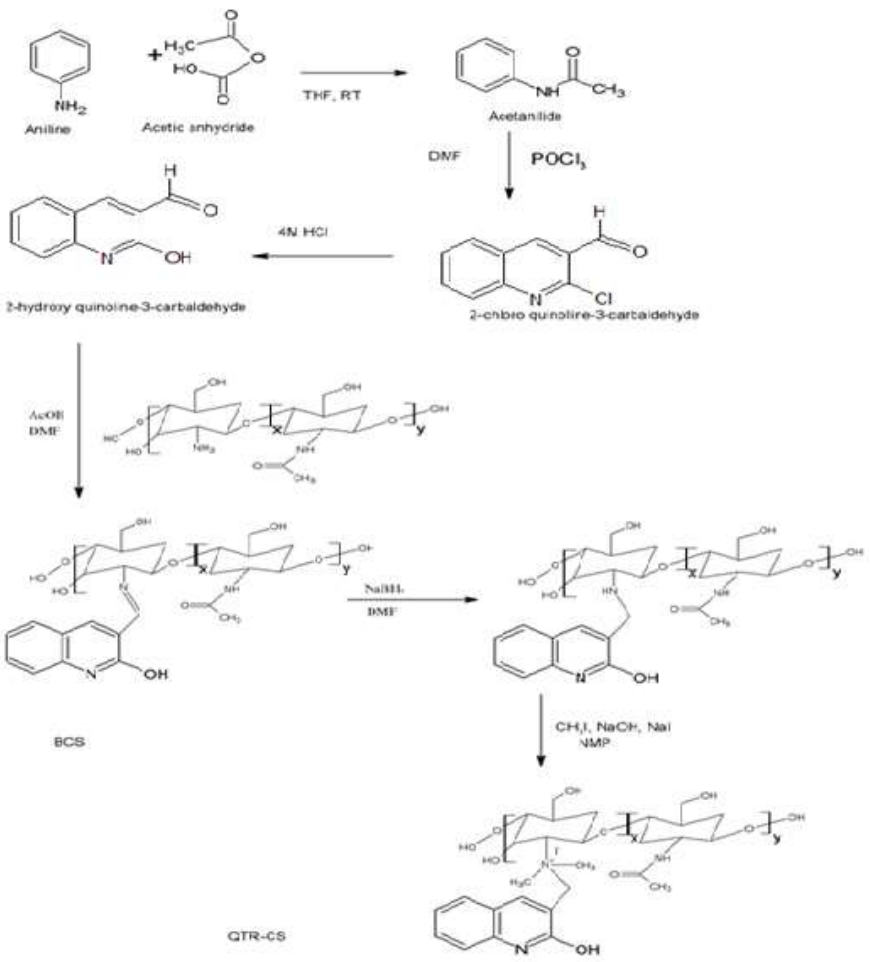
RESULTS AND DISCUSSION

Synthesis of quaternized chitosan

Cs has been reacted with 2-hydroxy quinoline-3-carbaldehyde to form the Schiff base, BCS which is further reduced to NSC and subsequently quaternized to obtain QTR-CS according to the reaction scheme presented in Scheme 1.

FTIR spectral analysis

In the FTIR spectra of pure Cs displayed in Figure 1, the broad band at around 3400 cm^{-1} is attributed to -NH and -OH stretching vibrations, a weak band at 2926 cm^{-1} is attributed to -CH stretching and the peaks at 1642 cm^{-1}, 1600 cm^{-1}, 1377 cm^{-1} and 1258 cm^{-1} are assigned to the C-O stretching, N–H bending and –NHCO stretching of the amide and C–N stretching respectively. The peak characteristic of the saccharide structure appeared at 895 cm^{-1} [13]. The symmetric stretching of bridge C-O-C is observed at 1160 cm^{-1} which is the characteristic structure of carbohydrate of Cs [14]. In the FTIR spectra of BCS shown in Figure 1b, a new peak appeared at 1648 cm^{-1} corresponding to the C=N characteristic vibration of imines, which is a confirmatory evidence for the formation of Schiff base [15] and peak at 1584 cm^{-1} is attributed to C-N stretching of Chitosan–quinoline respectively [16]. Upon quaternization new peaks appeared at 1656 cm^{-1}, which are assigned to quaternary ammonium groups. Peaks also appeared around 1410 cm^{-1}, attributed to the characteristic absorption of N-CH_3.



Scheme 1: Synthesis of quaternized chitosan 2-hydroxy quinoline-3-carbaldehyde

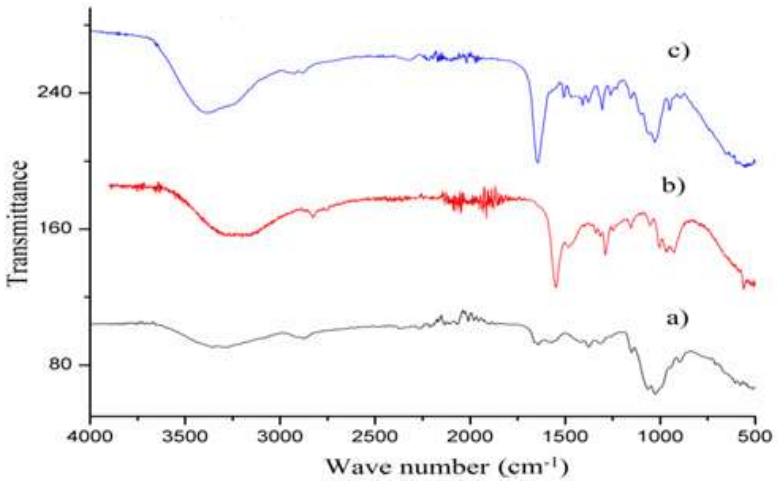


Figure 1: FTIR spectra of (a) Cs, (b) BCS and (c) QTR-CS

UV-visible spectra

The UV-visible spectra of Cs and QTR-CS are showed in the Figure 2. As seen in Figure 2a, Cs did not show any absorbance in the UV-visible region. The spectra of Figure 2b showed a broad peak at 333 nm indicating the presence of aromatic ring in the structure.

Solubility tests

The synthesized QTR-CS was dispersed in different polar and non-polar solvents at room temperature to check the solubility. The results showed in Table 1 indicates that substitution of an 2-hydroxy quinoline-3-carbaldehyde to the free amine group of Cs tentatively increases the

solubility of the derivative due to the loss of protons in the amine group results to increase its solubility in water due to quaternization. The QTR-CS sample gets completely soluble in water and acetic acid.

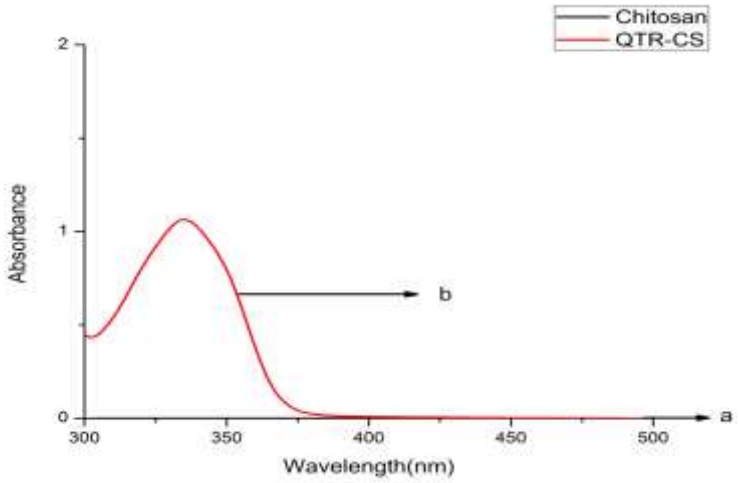


Figure 2: UV-visible spectra of (a) Cs and (b) QTR-CS

Table 1: Solubility of Cs and its derivatives in different solvents

Code	Solvent						
	NMP	Acetic acid	methanol	water	DMF	DMSO	Acetone
Cs	-	+	-	+/-	-	-	-
SBC	-	-	-	+/-	+/-	+/-	-
NCS	-	-	-	-	+/-	+/-	-
QTR	-	+	-	+	-	-	-

(+) readily soluble; (−) insoluble; (+/−) swells

XRD analysis

The X- ray diffractogram of Cs and QTR-CS derivatives are presented in Figure 3. The XRD analysis was used to determine the crystallinity of the sample. In Figure 3a the diffractogram of Cs with peaks at 2θ=10°, 21.09° and 30.0° Cs indicates the semi crystalline nature of the sample. The modification made in the sample results in the disappearance of the above two peaks and formation of a broad band located at low diffraction angle of 23°. Figure 3b, which is indicative of the loss in the crystallinity of the sample which makes the molecule ideal for biomedical applications.

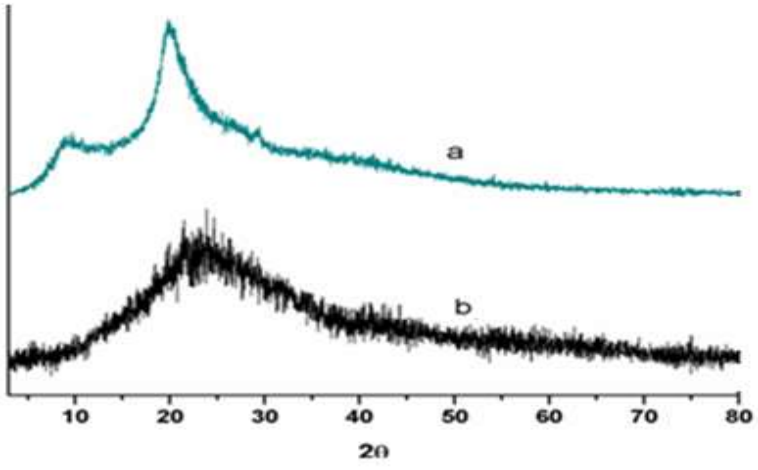


Figure 3: X-ray diffractogram of (a) Cs and (b) QTR-CS

TGA analysis

Figure 4 shows the TG thermograms of Cs and QTR-CS samples. Cs exhibits Figure 4a a two-step degradation process over the temperature range 25-600°C, resulting in complete weight loss at 580°C. The first stage of weight loss of 20% is in the range 25-100°C and is due to the presence of adsorbed water molecules. The second stage marks the degradation accounting to 40% weight loss occurring at 300°C is attributed to the decomposition of polysaccharide chain and the remaining 40% weight loss occurs gradually resulting in the oxidation of decomposed products to CO_2. The thermogram of QTR-CS Figure 4b reveals the low thermal stability of the sample compared to Cs. The degradation of the quaternized compounds starts at 190°C. 45% of the degradation takes place in the range 190-350°C. 30% of weight loss takes place in the range 350-600°C. Nearly 20% of the mass remains undecomposed at 600°C.

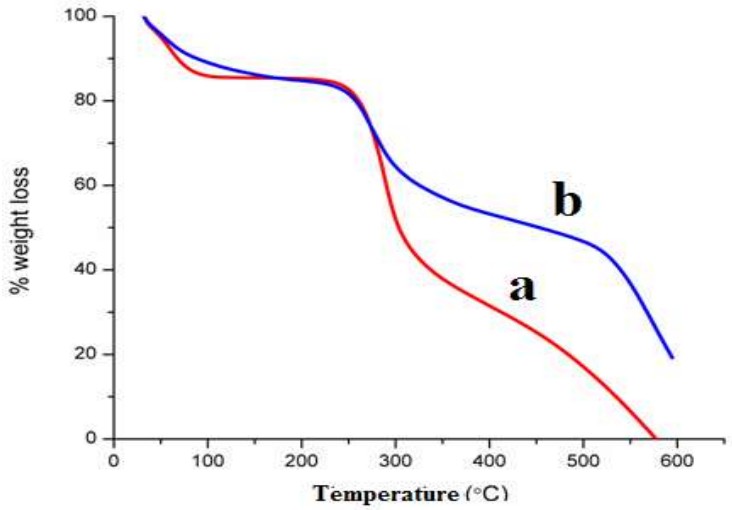


Figure 4: Thermogram of (a Cs and (b) QTR-CS

SEM analysis

The surface of Cs (Figure 5a) appears to be nonporous, smooth membranous consisting of crystallites, orifices and microfibrils. The surface completely changes on quaternization. The surface of QTR-CS appears smooth and homogeneous indicating the amorphous nature (Figure 5b).

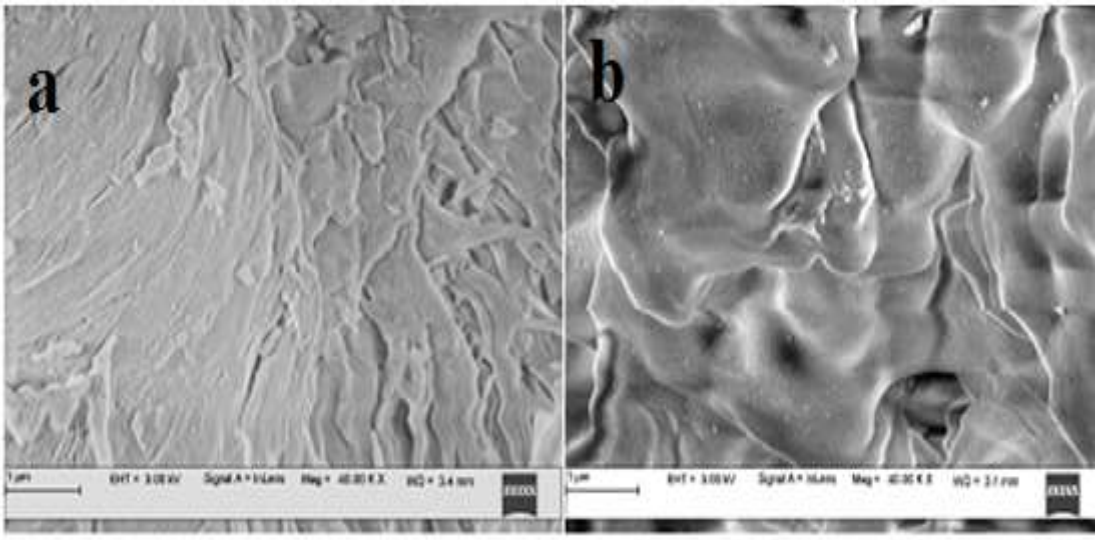


Figure 5: SEM images of (a) Cs and (b) QTR-CS

Antimicrobial activity

The synthesized QTR-CS was evaluated for their antibacterial activity against two Gram-positive and two Gram-negative bacteria namely, *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Escherichia coli* at concentrations of 0.2%, 0.1% and 0.05% considering water as control and tetracycline as the standard. The inhibitory effect was measured in terms of inhibitory zone of bacterial growth, the absence of clear zone indicating no inhibitory effect (Figure 6) and the data obtained is tabulated in Table 2.

Table 2: Antibacterial activity of QTR-CS against Gram-positive and Gram-negative bacteria

QTR-CS Concentration (%)	Inhibition zone (mm)			
	Gram-negative bacteria		Gram-positive bacteria	
	Pseudomonas aeruginosa	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcus pyogenes*
0.2	12	14	15	14
0.1	11	11	11	11
0.05	10	10	11	10
Tetracycline (10 µg/ml)	23	26	24	29

Several reports have shown the importance of polycationic nature of the compounds [17], in imparting biological activity to the target molecules. Quaternization of the quinoline modified Cs results in the formation of positively charged quaternary ammonium groups which increases its water solubility and enhances the antimicrobial activity [18,19]. The acceptable mechanism of the antibacterial activity of

quaternized CS is that the interaction between the quaternary ammonium groups and the anionic microbial cell surface results in the prevention of entry of nutrients [20]. Hydrophobicity of the quinoline moiety also plays an important role in inhibitory activity of quaternized CS derivatives. The negative charges on the cell surface of the Gram-negative bacteria are more in number compared to the Gram-positive bacteria, leading to higher extent of adsorption of the polycationic QTR-CS leading to better effect.

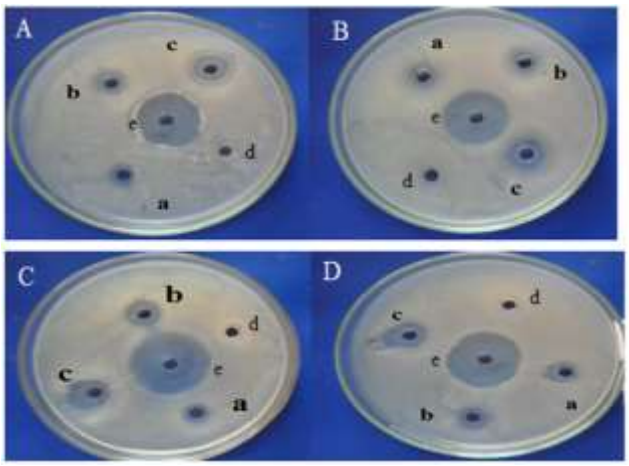


Figure 6: Inhibitory effect of (F) Cs and QTR-CS against (A) *Staphylococcus aureus*, (B) *Pseudomonas aeruginosa*, (C) *Streptococcus pyogenes* and (D) *Escherichia coli* at concentration (a) 0.05, (b) 0.1, (c) 0.2%, (d) Control (H_2O) and (e) Tetracyclic (10 µg/ml)

CONCLUSION

The quaternized chitosan-hydroxy quinoline derivative has been successfully made. The FTIR and UV-Visible spectroscopy confirms the modification made on chitosan. The compound appears to be amorphous in structure and exhibits smooth surface characteristics. The modification is reflected in the changes in thermal stability and degradation pattern of the modified compound. The modification is observed to impart water solubility and antibacterial activity to the compound. The presence of the heterocyclic moiety and the quaternary ammonium group is thought to be the key factors responsible for the observed antibacterial property of the modified chitosan derivative. In conclusion, the obtained results show that the modification of chitosan with heterocyclic substituent can be a promising material in enhancing the antibacterial property.

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