Chitosan Modification by New Chromophore 4-(3-(4-nitrophenyl) thioureido) benzoic acid: Synthesis, Characterization and Evaluation

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ABSTRACT

In this investigation we have shown the preparation of carboxylic acid containing new chromophore : 4-(3-(4-nitrophenyl) thioureido) benzoic acid and its use.The chemical modification of chitosan, e.g., chitosan- acid salt complexes is done under mild conditions. The synthesis of chromophore was carried out by conventional methods and the novel polymer complex was soluble in most of the organic solvents. The modified chitosan was analyzed by various techniques like optical properties by UV and SHG spectroscopy which showed red shift. The antibacterial activity showed obvious effect against food pathogenic bacteria.

KEYWORDS: *Chitosan, New chromophore, SHG, Antimicrobial property*

INTRODUCTION

Chitin, the naturally occurring polymer and its derivatives from marine source have been extensively utilized for various applications due to their inherent characteristics of non-toxicity, biodegradability and stability. Chitosan has been utilized in biomedical areas, such as wound dressing, drug delivery, food packing and antibacterial agents as well as pharmaceutical.[1-5] Gel formation of chitosan is achieved by its interaction with small counter ions like sulphates, polyphosphates and glutaraldehyde crosslinking. These modification are well utilized in seed coating, gel entrapment of microbial organisms, embryo and whole cells.[6]

A new field has emerged for the usage of chitosan for the microencapsulation of drugs using differential techniques to optimize entrapment efficiency and kinetics of the drugs

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release.[7-10] Interestingly, chitosan has been acting as conduit for microencapsulated nerve growth factor for sustained release in a study conducted for facial nerves in rabbits and found to give efficient nerve repair effect with bio imaging.[11-12] Chitosan consists of single primary amino (-NH_2) group and two secondary alcoholic (-OH) groups for each unit of anhydro glycoside. It has tendency to react with negatively charged surfaces polymers and also undergoes complexation/chelation with metal ions as it possesses.[13]

Chitosan is insoluble in water and organic solvents as it is a relatively weak base and, however, it seems to solubilize effectively in dilute acidic(aqueous) medium (pH 6.5), that change the glucosamine units into a soluble form R-NH_3^+.[14-17] At lower pH levels chitosan exhibit to form gel as it is precipitated in alkaline medium or with poly anions. It shows remarkable biocompatibility due to its property of being well known cationic polymer and strong ability to interact with negatively charged species.[18,19]

Chitosan is soluble in acidic (aqueous) solutions which offer several possibilities/opportunities for various chemical modification of chitosan.[20-23] These chemical modifications allow obtaining feasibly processible products or for the purpose of synthesis of new chromophores that are used for bio-engineering, bioimaging and biomedical applications.[24-28]

As such chitosan is non-chromophoric nature, so an attempt has been made to make it chromophoric for optical applications. The authors' laboratory have made several in chromophore-chitosan based complex materials for biomedical applications.[29-32] It is possible to prepare covalently linked chromophore. In this paper an efficient method for preparation of chromophoric chitosan acid salt complex by using 4-(3-(4-nitrophenyl) thioureido) benzoic acid in aqueous alcoholic media is presented. The characterization is done by means of UV-Visible, FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectrometry techniques for its possible application in optical and biomedical area.

EXPERIMENTAL

General Chemicals

All reagents and chemicals for synthesis were purchased commercially and used without further purification and stored in a desiccator under vacuum having self-indicating silica. Dimethyl sulphoxide (DMSO) was dried over CaH_2 and then distilled under reduced pressure.

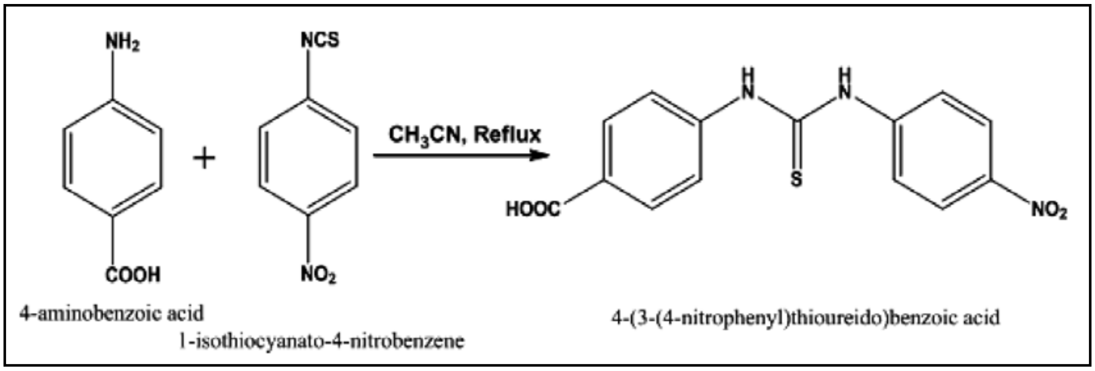
Measurements

Fourier Transform Infrared (FT-IR) spectra were recorded on Perkin Elmer RX1 FTIR spectrophotometer. UV-visible spectra were measured on a Systronics double beam spectrophotometer 2203 using 1.0 cm quartz cell at room temperature. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz Varian Mercury FT NMR and Bruker 300 MHz spectrometer respectively. Mass spectral analysis had been carried out with SYNAPT G2-S: 1TOF MS ES+: 2.05 eV.

Synthesis of 4-(3-(4-nitrophenyl) thioureido) benzoic acid

A mixture of 4-nitrophenyl thioisocyanate (10 mmol) and 4-aminobenzoic acid (10mmol) were charged to the round bottom flask having ACN(25 ml). The mixture was reflux for 5hrs. The solid was obtained which is filtered and purified by recrystallization from DMF – EtOH.

Yield: 2.69g (85%). FTIR (KBr Pellet, cm^{-1}): 3245, 1690, 1155, 1450



Scheme 1. Schematic representation of synthesis of new chromophore

^{1}H-NMR (400 MHz, DMSO-d_6,TMS) δ ppm: 12.6(s, 1H), 9.54 (d, 1J, J= 5.48). 9.20 (d, 1H, J = 4.88) 8.2-8.18(d, 2H, J=8.72), 8.18(m, 2H), 7.90(d, 2H, J=8.56) 7.71(d, 2H, J=9.16), 7.60-7.58(m, 2H)

^{13}C- NMR (100.62 MHz, TMS,$CDCl_3$,) δ ppm:166.9, 151.7, 146.0, 143.2, 141.2, 130.5, 125.1, 124.5.

UPLC/QTof-MS (*m/z*): 340[M + Na$^+$]

RESULTS AND DISCUSSION

4-hydrazino benzoic acid react with 4-nitrophenyl thioisocyanate in ACN under reflux condition (Scheme 1). Chromophore is well characterised by FTIR,^{1}H-NMR, ^{13}C-NMR spectroscopy and mass spectrometry. The characteristic signal of COOH protons at δ =12.6, pyrazole. The thiourea NH signals of chromophore shows at δ 9.54 and 9.20 in ^{1}H-NMR spectrum. The peak at 340 in mass spectra shows the [M + Na$^+$]

UV-Visible spectrum

In Figure 1, UV-visible spectrum of chromophore is given, that corresponds to the chromophore formed.

FTIR spectrum

The infrared spectrum of chromophore appears at 3245 cm^{-1}(OH groups of

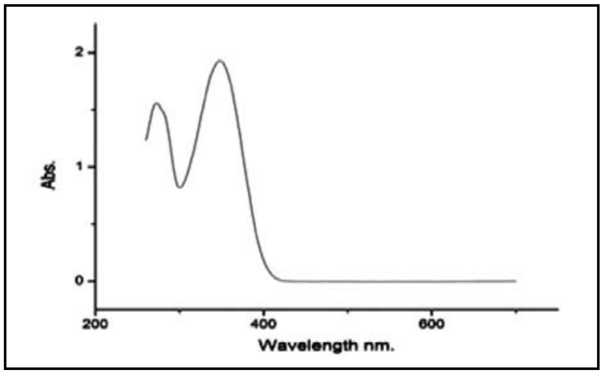


Fig. 1. UV-visible spectrum of chromophore

chromophore), 1690 cm^{-1}(-C=O stretching vibration), 1155 cm^{-1} (-C=S stretching), 1450 cm^{-1} (for -NO$_2$ group).

NMR spectrum

The ^{1}H-NMR spectrum of chromophore shown in Figure 2, δ = 12.6 ppm (s,1H) is due to COOH proton, δ =9.54 ppm (d,1H, J=8.40),δ = 9.20 ppm (d,1H,J=4.28), δ = 8.2-8.10 ppm (m, 2H,), δ =7.90 ppm (d,2H, J=8.56). δ =7.71ppm (d, 2 H, J=9.16), δ =7.60-7.88 ppm (m,2H) is due to aromatic protons of the chromophore.

The ^{13}C-NMR (100MHz) spectrum of chromophore (Figure 3) δ =166.95 ppm for HCOH, δ=151.7ppm, δ=146.01ppm, δ= 143, 141, 130, 125, 124, 117 that confirm the carbon skeleton of the chromophore formed.

Mass spectrum

Mass spectrum of the chromophore is shown in supplementary (S1). The prime peak *m/z* lies in 340, 324, 302, 176, 138 which correspond to various fragment ions –NH-CS-NH-, and different functional groups of diphenyl components. The result indicates that the formation of the chromophoric groups in the chromophore compound.

Modification of chitosan

Modified chitosan film was prepared by a method of solution casting.[27] In brief, a beaker

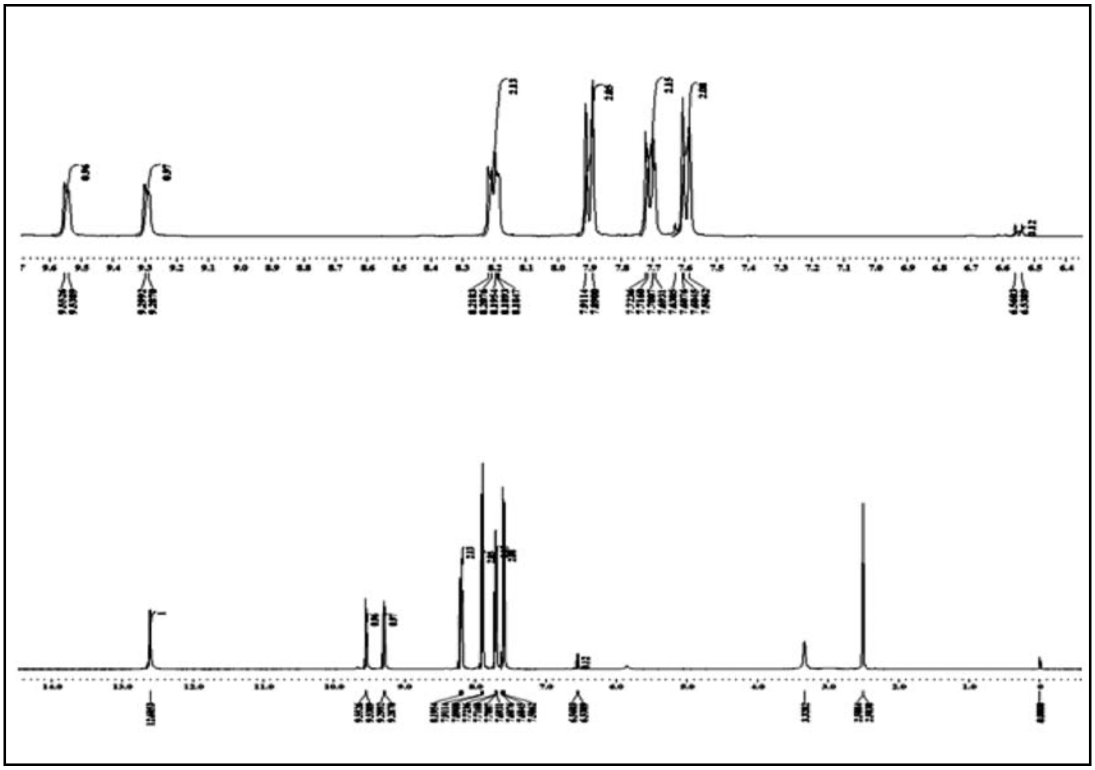


Fig. 2. ^{1}H-NMR spectrum of Chromophore

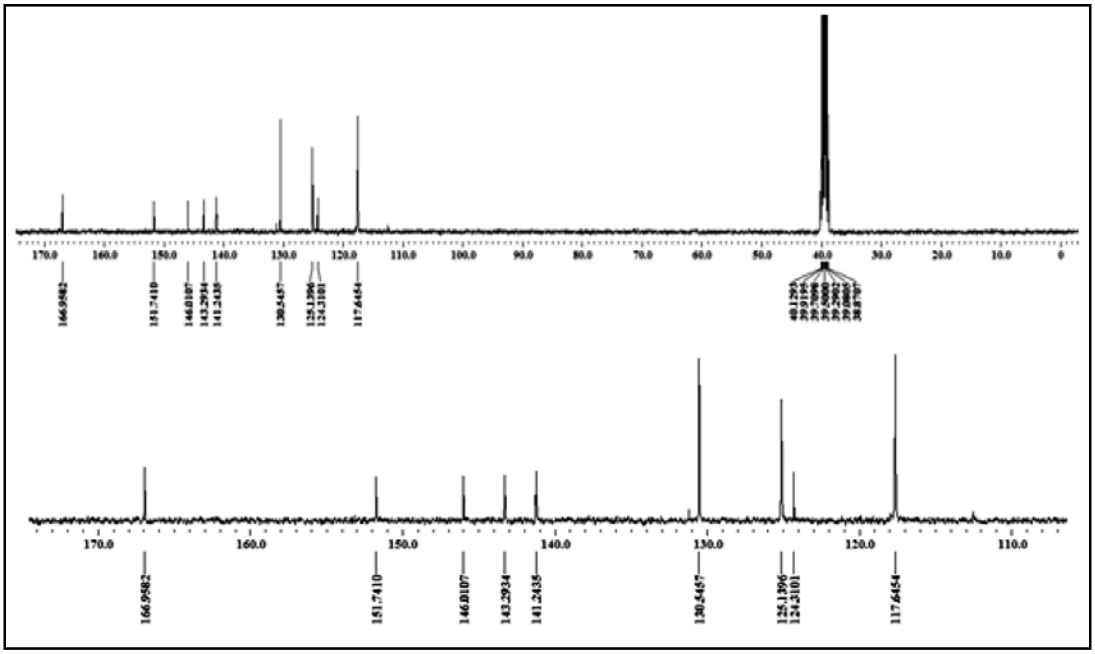
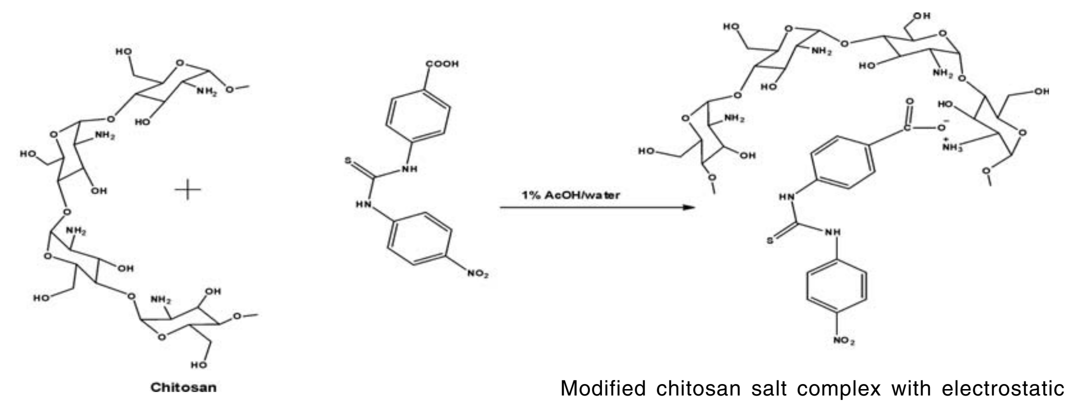


Fig. 3. ^{13}C-NMR Spectrum of Chromophore

of 100 ml, 500 mg of chitosan in 25 mL of 1% (v/v) aqueous acetic acid was prepared, stirred, and filtered to remove the undissolved matter. Then, above said prepared chromophore solution is added drop wise to the chitosan solution with vigorous stirring. A bubble-free



Modified chitosan salt complex with electrostatic interaction between -NH$_3^+$ group of chitosan and -COO$^-$ of acid. (Modified chitosan)

Fig. 4. Schematic representation of the formation of modified chitosan film

solution was spread over a clean and sterilized glass plate to a desired thickness and dried under atmospheric conditions at room temperature up to the dryness of the film. Finally, the resulting modified chitosan film (Figure 4) was prepared, carefully detached from the glass plate.

UV-Visible spectra

UV-Visible spectra of chromophore, pure chitosan and modified chitosan shown in Figure 5. Chitosan itself is transparent in the UV and visible region. UV-Visible spectra of modified chitosan film shows a broad band between the 280-380 nm due to the presence of aromatic ring and that particular peak is absent in chitosan.

FTIR spectra

The infrared spectrum at 3404 cm^{-1}(-OH), 3400-3000cm^{-1}(broad)(intermolecular hydrogen bonded), 2897 cm^{-1} (N-H due to NH$_3^+$ion

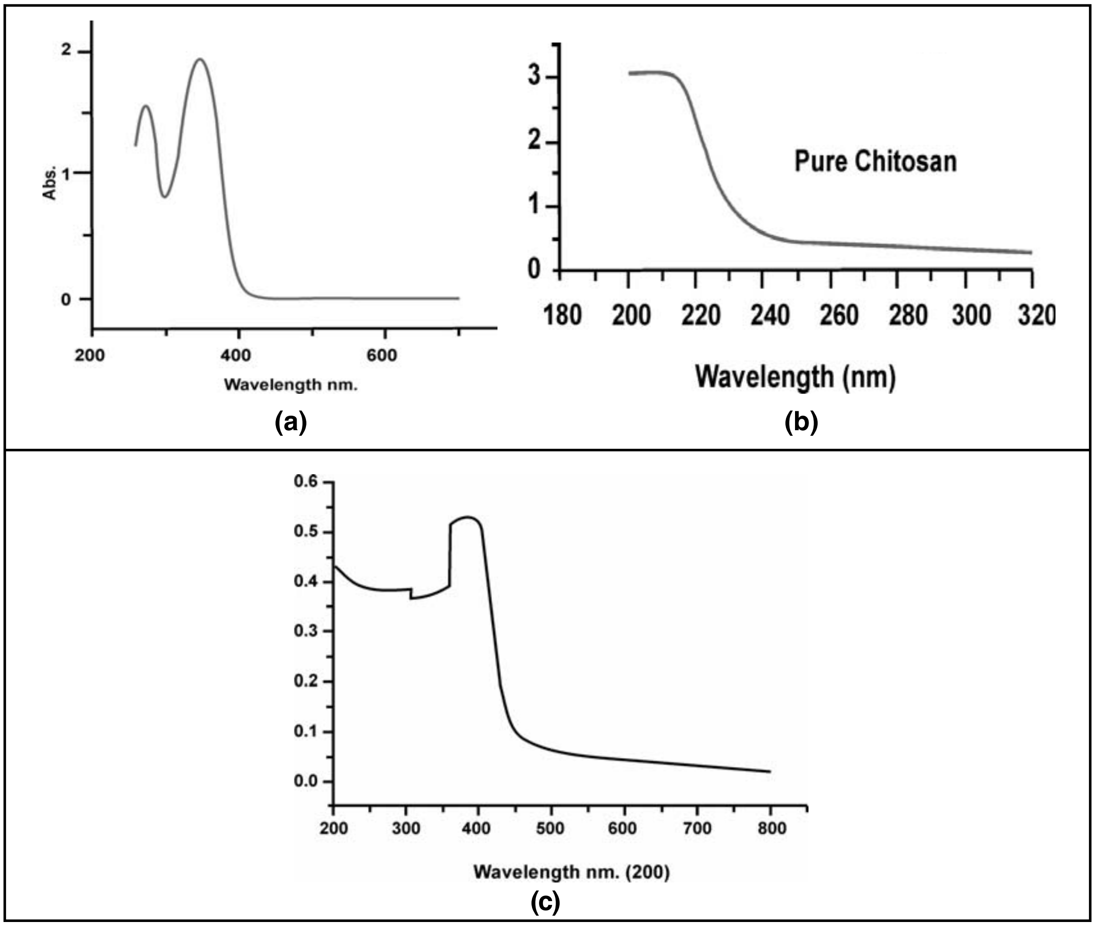


Fig. 5. UV-Vis spectra of (a) chromophore 4 (b) pure chitosan (c) CS4 in DMSO.

stretch), 2349 (aromatic C-H), 2155 cm^{-1} for carboxylate (-COO^{-}) ion, 1572 cm^{-1}(N-H bending stretching of amine -NH$_3^+$salt), 1397 (for phenolic OH),1079 cm^{-1}(ether linkage, C-O-C band stretching), 605 cm^{-1}(–C-H Cycloalk) which confirm the formation of modified chitosan (CS4) complex (Figure 6).

The FTIR spectrum of modified chitosan complex shows purely electrostatic nature of the interaction between chitosan (-NH$_3^+$ of chitosan and the acid, -COO^{-} of the chromophore) and chromophore. The new signal at 1628 cm^{-1}, probably due to the absorption band characteristic of symmetrical bending stretching of amine salt(due to interaction).[28] This result suggests that the -NH$_2$ group on chitosan chains were protonated by the H$^+$ supplied by acids.[33-34] The intensity of these bands depends on the amount and bulkiness of the acid. Degree of deacetylation (DD) also affects the intensity band, OH stretching which becomes broader and moves to a lower frequency.

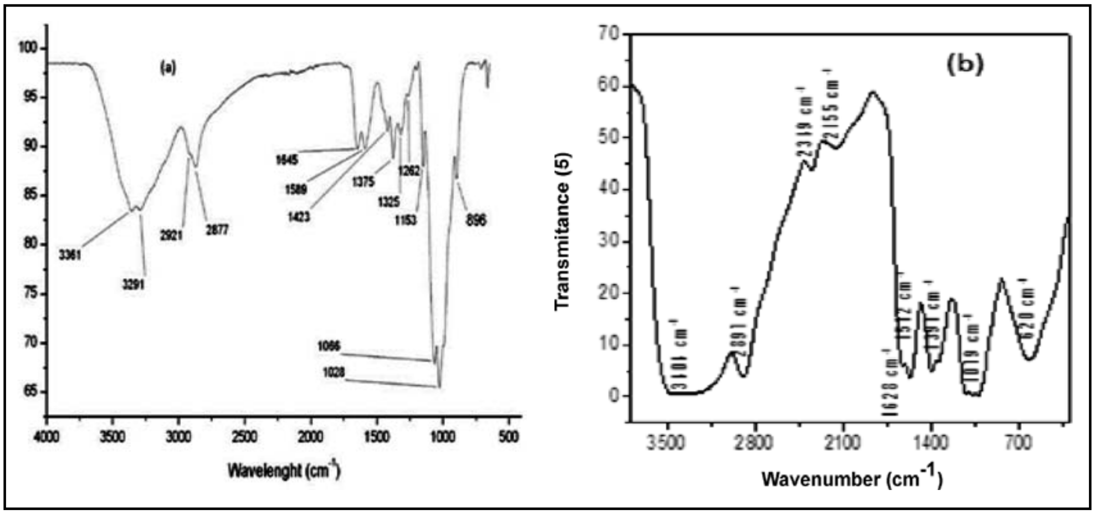


Fig. 6. FTIR spectra of (a) chitosan and (b) modified chitosan

Thermal Study

Thermogravimetric analysis (TGA)

Figure 7 shows the thermogravimetric analysis (TGA) of (a) pure chitosan and (b) modified chitosan discloses the fact that decomposition of the (a) and (b) carried out in three consecutive steps.

In case of modified chitosan decomposition of first step 12% mass loss is in the temperature range of 90-120^0 C. Due to the groups present in the side chain groups in derivative, second step 29.6 % mass loss in the temperature range of 250-320^0 C. Third step 33.2% mass loss is in the temperature range 410–450^0 C due to the loss of C=N, C=C and other moieties.

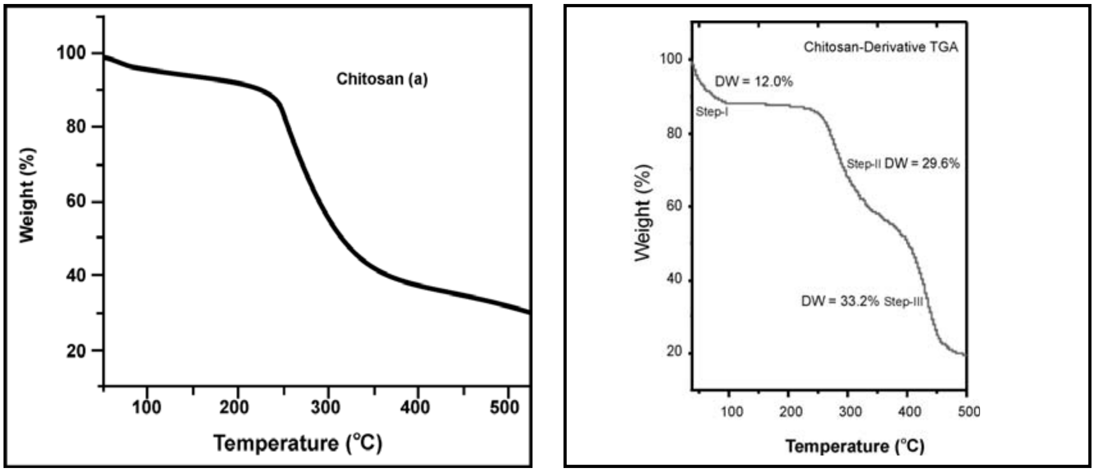
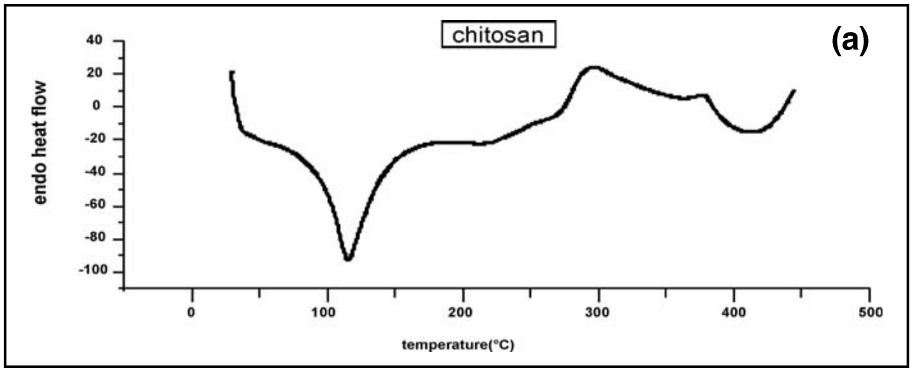


Fig. 7. TGA of (a) natural chitosan and (b) modified chitosan (CS4)

So the modified chitosan is comparatively more stable than natural chitosan, which is also supported by DSC.The electrostatic interaction of the present complex in water or other solvent is desirable. The stability of the film of modified chitosan is also well characterized. Further the NMR study also shows no deviation from the formation of the complex.

Differential scanning calorimetry (DSC) analysis

DSC curve of the modified chitosan (mCS) recorded in atmosphere of nitrogen from 10^{0}C temperature to 450^{0}C as exhibited in Figure 8. The temperatures for various thermal effects are shown for the mCS, the endothermic peaks at 100^{0}C probably due to the loss of humidity/ water loss from the sample. Endothermic peak at 210^{0}C is due to phase change and exothermic peak at 350^{0}C is probably due to excitation of thio urea coupling whereas exothermic peak at 425^{0}C represents the breaking of β-1,4-linkage of chitosan molecule and in most of the nitro group (–NO_2) containing chromophores such decomposition trends is observed.[35]



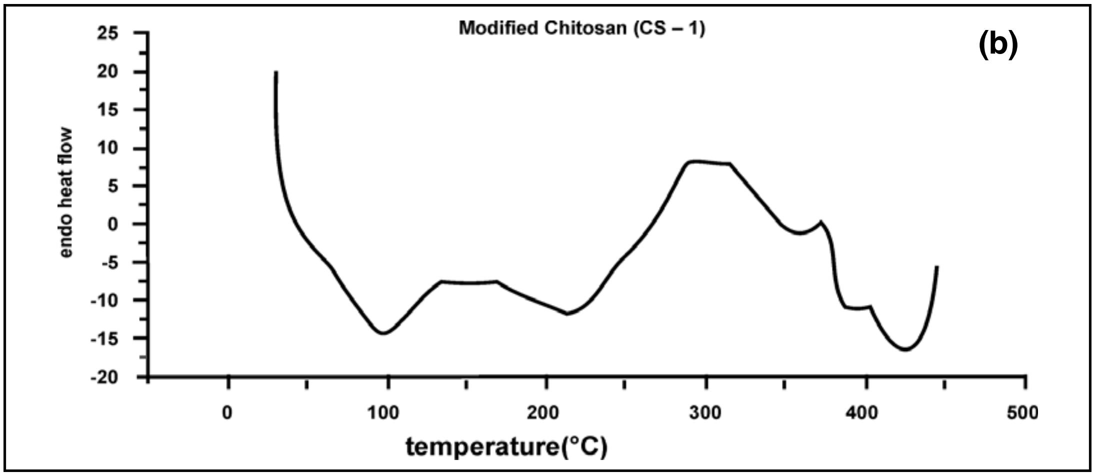


Fig. 8. DSC thermogram of (a) natural chitosan and (b) modified chitosan (CS4).

Second harmonic generation (SHG)

Second harmonic generation (SHG) materials, or commonly known as materials of frequency doubling, are important nonlinear optical (NLO) materials that are capable of converting a specific wavelengths (λ) of light into half of its original value.There is increasing interest in NLO materials. Based on the comprehensive list and our search of the literature, it appears that synthesized material is good examples of noncentro symmetric. Non-centro symmetric space group allows SHG activity. Powder SHG measurement of material exhibit that it is SHG active with particles ranging size from 50 to 90 µm when standardized with an efficiency of ~80x α-SiO_2 (Figure 9). The particle size of the ion associate complex was controlled by ultra-sonication method to get SGH activity.

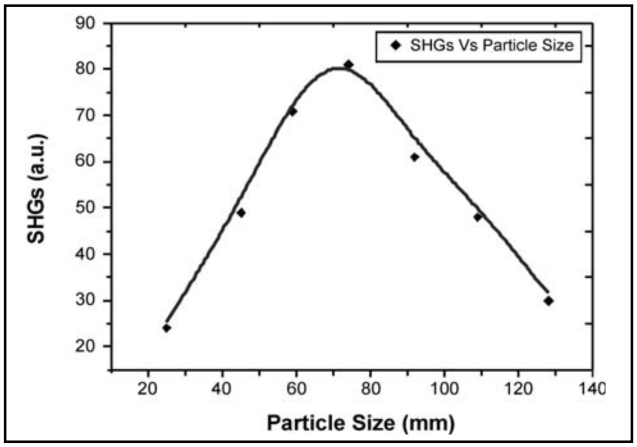


Fig. 9. SHG intensity curve as function of particle size of chitosan acid complex

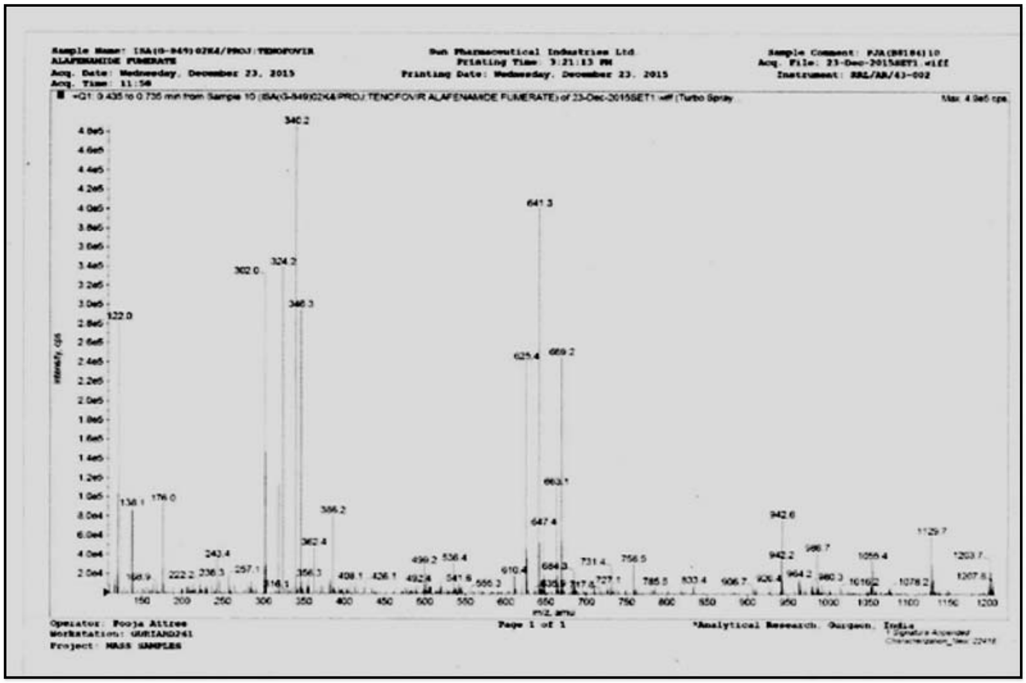
Antimicrobial effectiveness of film-forming solution

The antimicrobial activity of natural chitosan and modified chitosan were carried out using agar plate diffusion method.[36] In this method, the solution (1mg/mL) of the was absorbed in sterilized discs (approximately 60μL of solution) and the antimicrobial activity was evaluated against three different test cultures viz. gram negative bacteria *E.coli*, gram negative bacteria *P. aeruginosa*, gram positive bacteria *S.aureus*. The sterilized discs were placed on nutrient agar plates making grounds of the above test cultures. The plates were then incubated at 37°C for 24 h. The diameters of the inhibitory zone surrounding discs were then compared.

TABLE 1. Antibacterial study-Inhibition zones of film forming solution against different microbes

Sample code	Zone of inhibition in mm for the bacteria		
	E. coli	*P. aeruginosa*	*S. aureus*
Chitosan	18.2	15	16.1
Chromophore	20	17.1	18
Chitosan+chromophore	21	18	20.5

Supplementary Figure, S1



Sample Name: ISA(0-849)02K4/PROJ:TENOFOVIR ALAFENAMIDE FUMERATE
Acq. Date: Wednesday, December 23, 2015
Acq. Time: 11:50

Sun Pharmaceutical Industries Ltd
Printing Time: 3:21:13 PM
Printing Date: Wednesday, December 23, 2015

Sample Comment: PJA(B0184)10
Acq. File: 23-Dec-2015SET1.wiff
Instrument: RAL/AR/43-002

+Q1: 0.435 to 0.736 min from Sample 10 (ISA(0-849)02K4/PROJ:TENOFOVIR ALAFENAMIDE FUMERATE) of 23-Dec-2015SET1.wiff (Turbo Spray

Operator: Pooja Attree
Workstation: GURIAND241
Project: MASS SAMPLES

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*Analytical Research, Gurgaon, India

S1. Mass spectrum of chromophore

CONCLUSION

A new chromophore 4-(3-(4-nitrophenyl) thioureido) benzoic acid was synthesized and characterized by UV-Visible, FTIR, ^{1}H-NMR, ^{13}C-NMR spectroscopy and by mass spectrometry. This chromophore used for derivatization of chitosan and modified chitosan (mCS) was prepared and characterized their thermal, optical and anti-microbial properties. The modified chitosan is thermally more stable than natural chitosan. The thio urea containing modified chitosan shows optical activity and wide spectrum antimicrobial activity against microbes namely *E.coli, P. aeruginosa* and *S. aureus,* which is significant higher in comparison to the free chitosan. UV-visible spectroscopy showed a red shift. The present study indicate that the material may have a potential to be used for biomedical applications.

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