Synthesis and Characterization of New Chromophore 4-2-(2-hydroxy-5-(4 nitrophenyl) diazenyl) benzylidene) hydrazinyl) Benzoic Acid and its use in Chitosan Modification

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ABSTRACT

The present investigation deals with the preparation of carboxylic acid containing new chromophore : 4-2-(2-hydroxy-5-(4-nitrophenyl) diazenyl) benzylidene) hydrazinyl) benzoic acid and its use for chemical modification of chitosan, e.g., chitosan-acid salt complexes under mild conditions. Optical properties of chitosan acid complexes were evaluated by UV and SHG spectroscopy which showed red shift. Antibacterial activity of prepared chitosan acid complexes showed obvious effect against food pathogenic bacteria. The novel polymer complex was soluble in most of the organic solvents.

KEYWORDS: *Chitosan acid complexes, Chromophore, Optical property, Antimicrobial property.*

1. INTRODUCTION

Chitosan, a natural polymer has found its several utility due its advantages namely being biocompatible and biodegradable. It is a natural cationic polymer and it's characteristic to interact with opposite charges that exerts its ability to be bioadhesive and bacteriostatic. The structural and degree of deacetylation has vastly contributed for its anti-oxidant property as well as chelating agent. Its antimicrobial and anti-inflammatory characteristics have emphasized its use in biomedical application as haemostatic agent. It inhibits bleeding through procoagulant activity. Chitosan is proved to be advantageous in medical, pharmaceutical, agricultural, cosmetics and food processing industries. The pharmaceutical applications of chitosan have been utilized in

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drug and gene delivery, wound healing gauzes, tissue repair methodologies and tissue engineering[1-6].

Chitosan, a β-(1-4)-2-amino-2-deoxy-β-D-glucopyranose is a partially deacetylated chitin through alkaline hydrolysis and has been a favourite material for of research[7]. Chitosan has possess and one amino group and two hydroxyl groups in its monomeric form and soluble in acidic (aqueous) solutions. These characteristics offer wide range of possibilities/ opportunities for modifying it[8-11], so as to easily obtain more processible products or for the purpose of synthesis of new compounds which have optimal application for bio- engineering and biomedical application[12]. As such chitosan is non-chromophoric nature, so an attempt has been made to make it chromophoric for optical applications. In this paper an efficient method for preparation of chromophoric chitosan acid salt complex by using 4-2-(2 hydroxy-5-(4-nitrophenyl) diazenyl) benzylidene) hydrazinyl) 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 has been evaluated.

EXPERIMENTAL

General chemicals

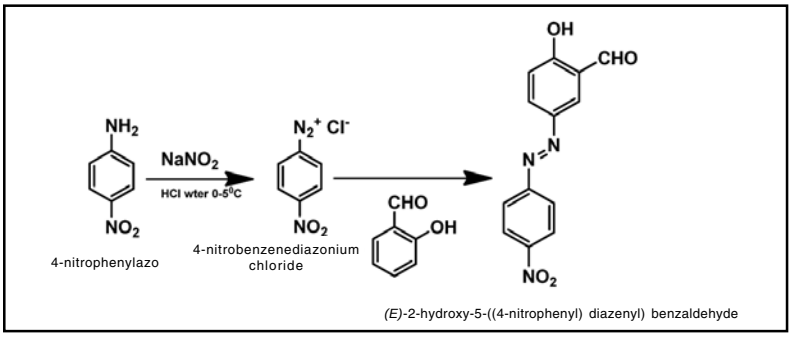
All reagents and chemicals for synthesis were purchased commercially and used without further purification and stored in a desiccator under vacuum having self-indicating silica. Dimethyl sulphoxide (DMSO) was dried over CaH_2 and then distilled under reduced pressure.

Measurements

Fourier Transform Infrared (FT-IR) spectra were recorded on Perkin Elmer RX1 FTIR spectro-photometer. 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 2-Hydroxy-5-(4-nitrophenylazo) benzaldehyde

This compound was prepared by an earlier reported procedure [27]. 4-Nitroaniline (3 g, 21.7 mmol) was added to a mixture of concentrated HCl of 5N and water. The mixture was heated at 85°C to obtain a clear solution and then, more HCl was added and cooled at 0°C. Aqueous $NaNO_2$ (1.49 g, 21.7 mmol) solution was added dropwise at 0°C and stirred at room temperature for 1 hr, affording a bright yellow solution. Salicylaldehyde (2.65 g, 21.7 mmol) in aqueous Na_2CO_3 solution was added drop wise over 1 hr to the bright yellow solution



Scheme 1: Schematic representation of synthesis of 2-Hydroxy-5-(4-nitrophenylazo)benzaldehyde

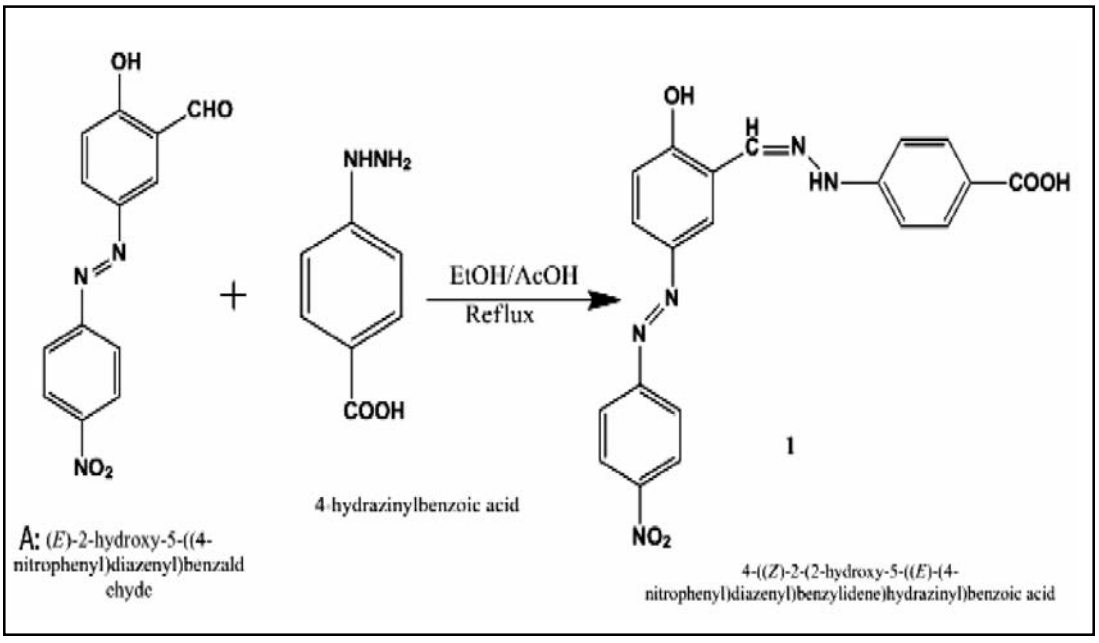
at 0°C and stirred at room temperature overnight. The reaction was neutralized with HCl and a brown crude solid was obtained which was filtered, dried and recrystallized with EtOH to afford the desired product as brown solid. Yield: 4.35 g (75%).

Synthesis of 4-(-2-(2-hydroxy-5-(4-nitrophenyl) diazenyl) benzylidene) hydrazinyl) benzoicacid(1) (chromophore)

To a solution of 4-hydrazino benzoic acid (173.2 mg, 1.14 mmol) in EtOH at 50°C, 2-Hydroxy-5-(4-nitrophenylazo) benzaldehyde (309 mg, 1.14 mmol) was added. Then 3-4 drops of acetic acid was added to the mixture. The reaction mixture was heated to reflux for 3-4 hrs. After completion of the reaction (monitored by TLC, R_f values are 0.54, 0.79, 0.90), the reaction mixture was allowed to cool at room temperature. The separated solid was filtered, washed with EtOH, dried and recrystallized from EtOH-DMF to obtain **1** as brown solid powder. Yield: 360 mg (78%).

RESULTS AND DISCUSSION

The chromophore **1** is prepared (Scheme 2) by refluxing of compound A with 4-hydrazinobenzoic acid in ethanol in the presence of catalytic amount of acetic acid in 84% yield. The Compound **A** is produced by diazotization of 4-nitroaniline followed by the reaction of the diazonium salt with salicyldehyde in aqueous Na_2CO_3 solution (Scheme 1). The formation of chromophore is confirmed by FTIR and ^1H NMR spectroscopy. The NH and COOH protons for carboxylic acid appeared at 11.57 ppm and 9.76 ppm, respectively. In FTIR the broad peak at 3720 cm^{-1} correspond to hydroxyl group and peak at 1720 pertaining to the C=O group.



Scheme 2: Schematic representation of Synthesis of 4-(-2-(2-hydroxy-5-(4-nitrophenyl) diazenyl) benzylidene) hydrazinyl) benzoic acid chromophore, 1

Elemental Analysis : Elemental analysis of $C_{20}H_{15}O_5N_5$ (%) : Calc. C 53.93,H 3.37, N 15.73; Found C 53.90,H 3.41, N 15.72

UV-Visible spectrum

UV-Visible spectrum (Figure 1) of acid chromophore shows a band between the 270-290 nm due to the presence conjugation in the chromophore formed..

FTIR spectrum

The infrared spectrum of chromophore 1 has been shown in Figure 2. The spectral band appears at 3720 cm^{-1} (OH groups of compound acid), 3410 cm^{-1}(N-H stretching), 1980 cm^{-1} (N=N- group), 1720 cm^{-1} (C=O group), 1610 cm^{-1} (CH=N) of chromophore.

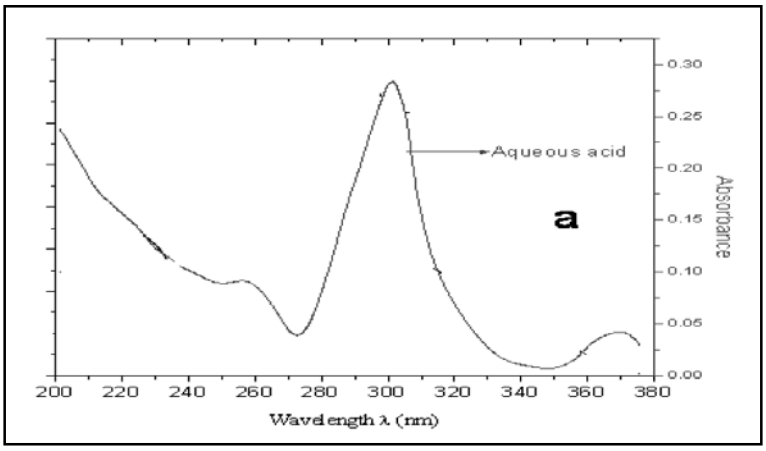


Fig.1. Electronic spectrum of synthesized chromophore, 1

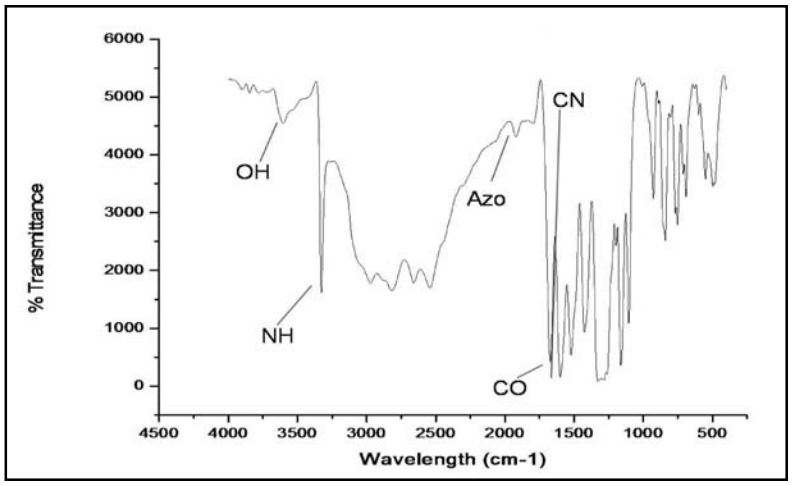


Fig. 2. FTIR spectrum of chromophore, 1

NMR spectrum

The ^{1}H-NMR spectrum has produced peak at chromophore (Figure 3) δ= 12.3 ppm (s,1H) is due to COOH proton, δ=11.31 ppm (s,1H) due to NH, and δ = 11.08 ppm (s,1H) is due to OH, δ = 8.39 ppm (d, 2H, J=8.7ppm), δ =8.3 ppm (d,2H,J=5.5ppm). δ = 8.02ppm (d,2H, J=9.1ppm), δ =7.8ppm(s,1H) is due to N=N, δ =7.81-7.84ppm (m,2H) is due to aromatic protons, δ=7.14ppm(d,2H,J=8.72 ppm), δ =7.09ppm(d,1H,J=8.7 ppm) of the chromophore.

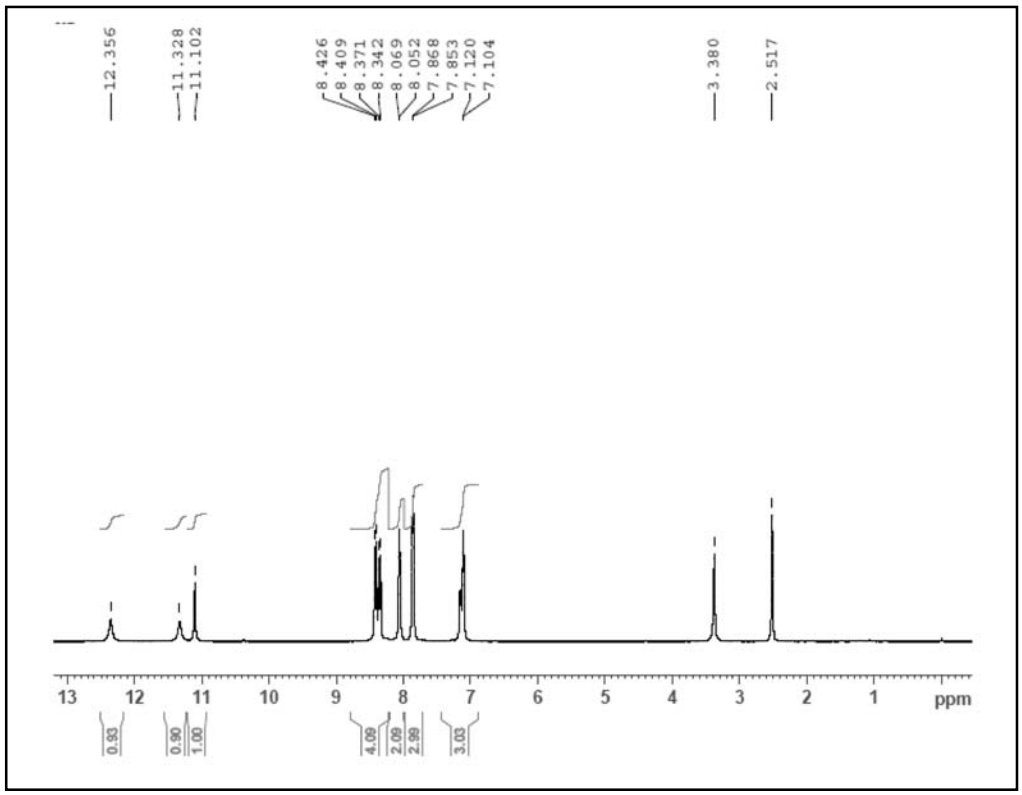
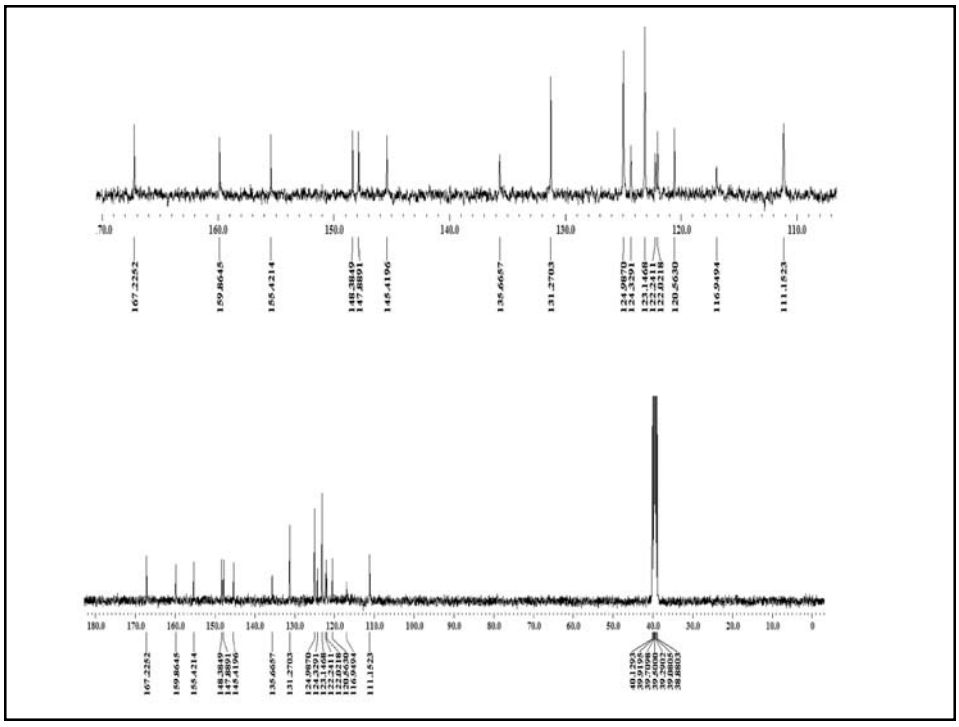


Fig.3. ^{1}H NMR Spectrum of chromophore

The^{13}C-NMR(100MHz) spectrum of chromophore (Figure. 4) δ = 167.2ppm for HCOH, δ =159.8ppm, δ = 188ppm, δ = 148, 147, 145, 135.6, 131.2, 124.9, 124.3 123.1, 122.0, 120.5, 116.9, 111.1, that confirms the carbon skeleton of the chromophore formed.

Mass spectrum of chromophore

Mass spectrum of the chromophore is shown in Figure 5. The prime peak *m/z* lies between 406-407. The corresponding fragment ions -CH=N-NH- and –N=N- of chromophores are linked of different functional groups based



Fig, 4. ^{13}C NMR Spectrum of chromophore

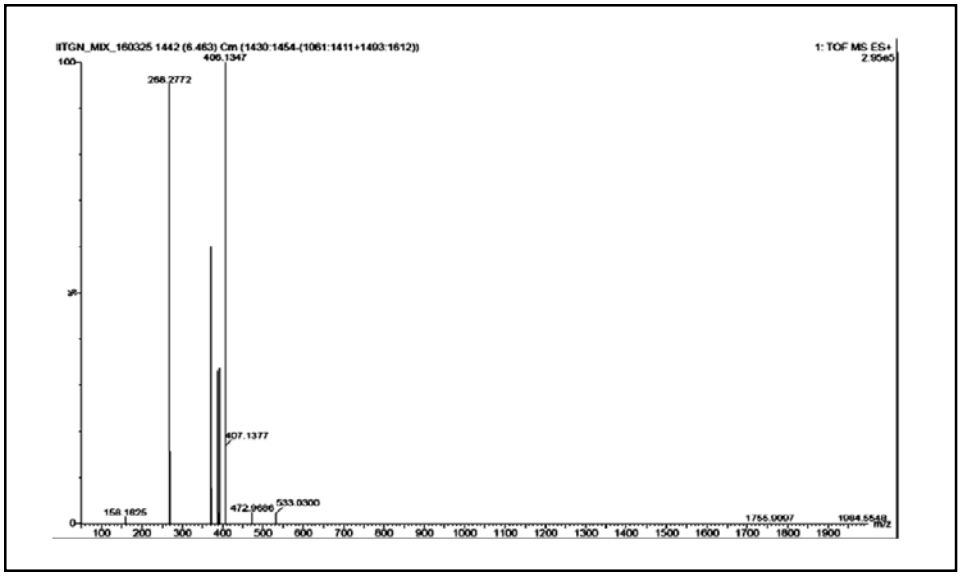


Fig. 5. Mass spectrum of chromophore

diphenyl components are at *m/z* 268 and 158 respectivly. The result indicates that the formation of the chromophoric groups in the chromophore.

Modification of chitosan

Modified chitosan film was prepared by a method of solution casting[9]. In brief, 500 mg of chitosan in 25 mL of 1% (v/v) aqueous acetic acid was taken in a 100 mL beaker, stirred and filtered to remove the undissolved matter. The solution of synthesized 4-(-2-(2-hydroxy -5-(4-nitrophenyl) diazenyl) benzylidene) hydrazinyl) benzoic acid chromophore, 1 (100 mg) was added dropwise to the chitosan solution with continuous stirring. The solution free from bubble was spread over a clean glass plate to a desired thickness and dried under normal conditions at room temperature up to the dryness of the film. Finally, the resulting modified chitosan film was prepared (Figure 6) and carefully detached from the glass plate.

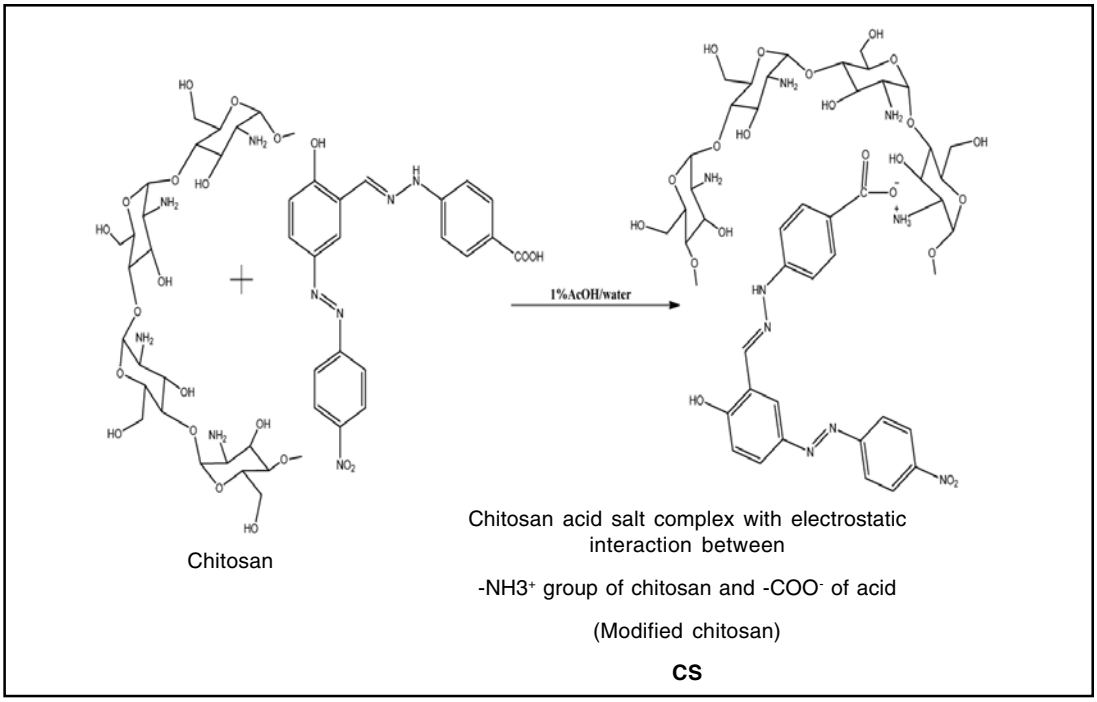


Fig. 6. Schematic representation of the formation of modified chitosan (CS) film

UV-Visible spectra

UV-Visible spectra of modified chitosan are shown in Figure 7. Chitosan itself is transparent in the UV and visible region. However, by incorporating synthesized chromophores to chitosan moiety, we have prepared chromophoric chitosan (modified chitosan). Electronic absorbance spectra (UV) of chitosan- acid film shows a broad band between

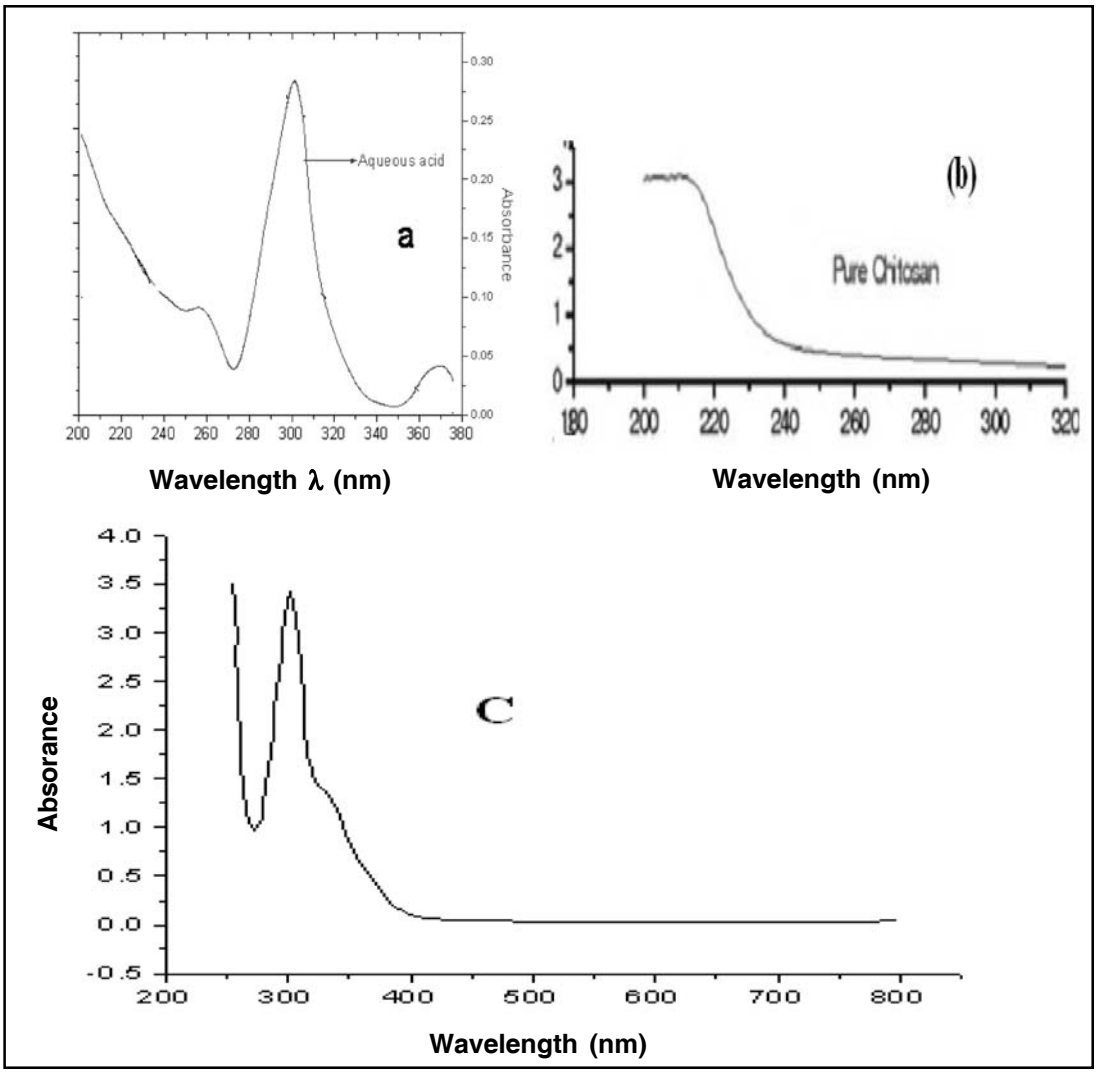


Fig. 7. UV-Vis. spectra of (a) chromophore (b) chitosan (c) modified chitosan (CS) .

the 280-330 nm due to the presence of aromatic ring and that particular peak is absent in chitosan. CS-chromophore (CS)shows λ_{max} at 330 nm due to the formation of the complex. Here Red Shift is obtained due to the modification of chitosan.

FTIR spectra

Figure 8 shows the FTIR spectra of chitosan (a) and modified chitosan(b). The absorption band of modified chitosan is compared with pristine chitosan. The spectrum of modified chitosan at 3487 cm^{-1}(-OH), 3300-3000

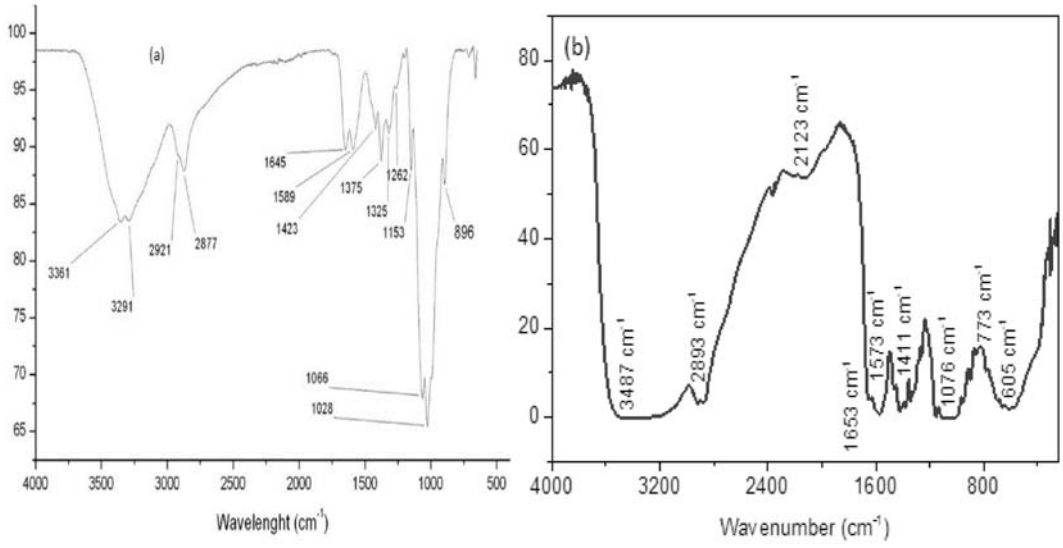


Fig. 8. FTIR spectra of (a) chitosan and (b) modified chitosan

(broad)(intermolecular hydrogen bonded), 2893cm^{-1}(N-H due to -NH_3^+ ion stretch), 2123 cm^{-1} (for N=N),1653 cm^{-1} for carboxylate ion, 1573cm^{-1} (symmetrical bending stretching of amine salt-NH_3^+), 1411 cm^{-1}(for phenolic OH),1076cm^{-1} (ether linkage, C-O-C band stretching), 773cm^{-1} (for Aromatic -C-H (monosubs.), 605cm^{-1}(-CH, cycloalk.), which confirm the formation of chitosan-chromophore (CS) complex.

The FTIR spectrum of chitosan acid salt complex showed purely electrostatic nature of the interaction between chitosan (-NH_3^+) and the acid (-COO^-), new signal at 1653 cm^{-1}, should be assigned to the absorption band characteristic of symmetrical bending stretching of amine salt[12]. This result suggested that the NH_2 group on chitosan chains were protonated by the H^+ supplied by acids [9-10]. 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.

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 chromophore and modified chitosan (CS) are the good examples of non-centro symmetric. Non-centro symmetric space group allows SHG activity. Powder SHG measurements on compounds indicate that they are SHG active with particles ranging size from 45 to 63 mm when standardized with

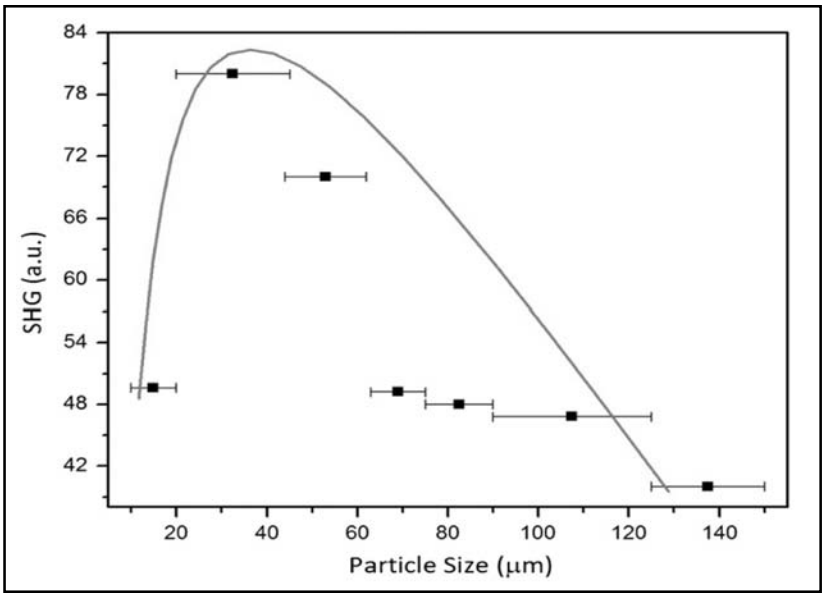


Fig. 9. SHG intensity curve as function of particle size of modified chitosan (CS).

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.

Antimicrobial effectiveness of film-forming solution

The antimicrobial activity of pure chitosan and modified chitosan were carried out using agar plate diffusion method[11]. In this method, the solution (1mg/mL) 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).

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	19.8	19.4	17.1
Chitosan+chromophore(CS)	21.9	18	20.5

CONCLUSIONS

A new chromophore 4-2-(2-hydroxy-5-(4-nitrophenyl) diazenyl) benzylidene) hydrazinyl) benzoic acid was synthesized by conventional method and characterized by UV-Visible, FTIR,^{1}H-NMR,^{13}C-NMR spectroscopy and by mass spectrometry. Thereafter, the above synthesized chromophore used as cross-linker of chitosan, and modified chitosan (CS) was prepared and characterized spectroscopically and their thermal, optical and antimicrobial properties are studied and it has been found that modified chitosan (CS) is thermally more stable compared to natural chitosan. The Schiff base containing modified chitosan has optical activity and also possess wide spectrum antimicrobial activity against different microbes *E.coli, P.aeruginosa* and *S.aureus* that is significantly higher in comparison to free chitosan. Our study has strongly pointed out the materials can be used as biomedical applications and bio-optical devices.

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