Research Article

Microwave Assisted Synthesis, Characterization and Antimicrobial Evalution of Chitosan Triazine Serine Hydroxymate (CT-SH)

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ABSTRACT

Microwave assisted synthesis method is a green methodology in the synthesis of chitosan triazine serine hydroxamate (CT-SH). The product was characterized by fourier transform infrared (FT-IR), H^1 NMR, Mass spectrometry, SEM. The thermal stability of chitosan derivative was studied using thermo gravimetric analysis (TGA). Also the chitosan derivative possesses a high antibacterial activity against gram negative & positive bacteria and fungus.

Keywords: Microwave assisted synthesis, chitosan, serine, antimicrobial activity, TGA.

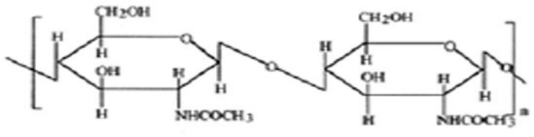
INTRODUCTION

Microwave assisted synthesis is an enabling technology for accelerating synthetic processes. Microwave organic synthesis opens up new opportunities to the synthetic chemist in the form of new reaction that are not possible by conventional heating.[1-2] Microwave enhanced heating based on "Microwave dielectric heating effect". Microwave dielectric heating lead to volumetric heating of the sample.[3]

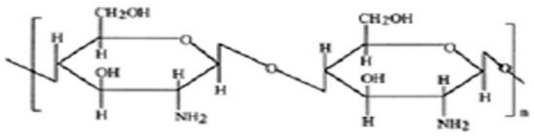
Microwave technology has major advantages includes decrease in reaction time, clean product formation, rapid volumetric heating, enhanced reaction selectivity and energy saving, compared with conventional heating method .[4-5]

Chitosan is a natural nontoxic, biocompatible, biodegradable, biopolymer.[6-8] Deacetylation of chitin yields chitosan, which is actually a copolymer Glc NAC and β-(1- 4)2-amino-2-deoxy-dglucopyranose with deacetylation and second most abundant natural polysaccharide next to cellulose.[9-11]

Serine [(2S)-2-amino-3-hydroxy propanoic acid] is a non-essential amino acid[12-13] and it natural form is L-isomer. It contain an α amino group which is in the protonated – NH^+. serine derived from glycine. [14-15] Fig 1



(a) Chitin



(b) Chitosan

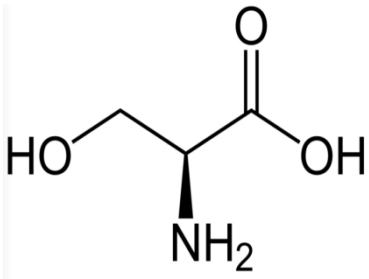


Figure 1: (a) Chitin (b) Chitosan (c) Serine

Serine encoded by the codon UCU, UCC, UCA, UCG, AGU, AND AGC is an α amino acid that is used in the biosynthesis of proteins.[16-17]

Chitosan possesses various biological properties like antibacterial, antifungal, antioxidant etc.[18-21] It has limited practical application due to its low solubilty at p^H value above 6.3.[22-23] The high number of amino group in chitosan molecules (increase in MW) lead to decrease in antimicrobial activity. [24-25]

MATERIALS

Chitosan was procured from local industry. Cyanuric chloride (sigma Chemicals Company). All other AR grade chemicals used were procured from sigma, Aldrich, loba chemicals, Ases chemical works. The antimicrobial strains (bacterial and fungal strain) were obtained from Dr. S.N. Medical College, Jodhpur (Rajasthan) India.

METHODS

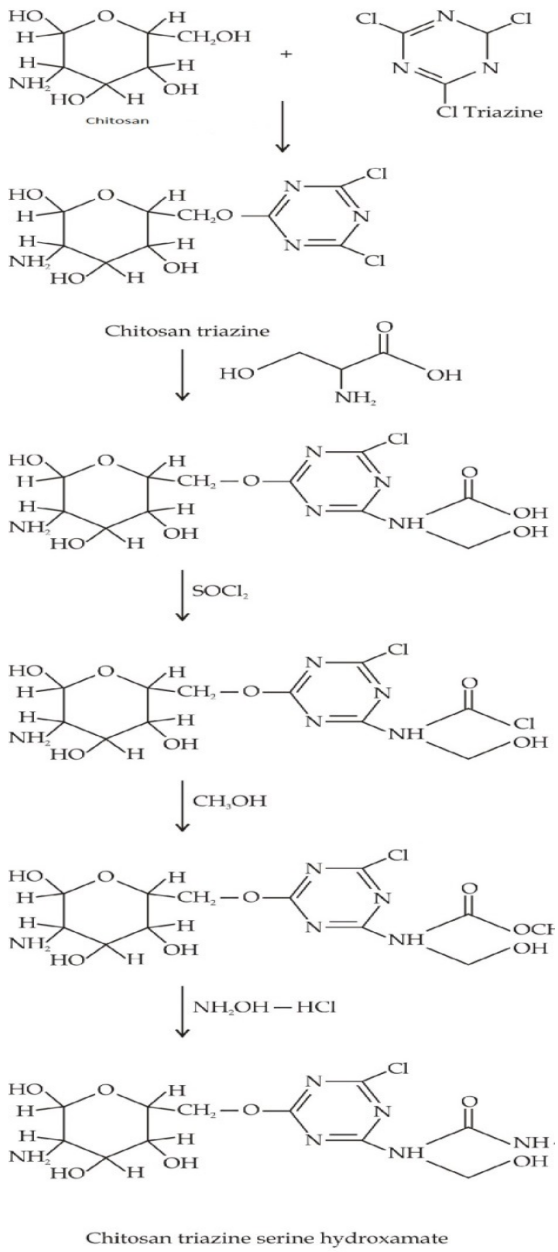
Synthesis of chitosan triazine

In round bottom flask 1 mole of chitosan slurried in maximum quantity of DMSO maintained at about 5°c by cooling in ice bath with content magnetic stirring. Further 1 mole of cyanuric chloride (Triazine) was added with continuous stirring and the p^H was adjusted to 9-10 then reaction mixture was subjected to microwave for 15 minutes.

The product was filtered on a vacuum pump and washing was done with 80% aqueous methanol containing nitric acid to remove impurities .the washed product was dried in an oven at 50° c.

Synthesis of chitosan triazine serine hydroxamate

Chitosan triazine was added to serine dissolved in DMSO and slurred for hour with constant magnetic stirring. Then the reaction mixture was subjected to microwave for 15 minutes. serine derivative of chitosan triazine formed was taken in round bottom flask excess methanol and thionyl chloride were added and temperature raised up to 40°C at rotavapour for two hour. Now methanolic solution of hydroxyl amine chloride was added to get the hydroxamate of the ester with pH was maintained at 9 to 9.5 by sodium bicarbonate solution. The chitosan derivative thus produced was filtered off and washed with double distilled water and finally dried. Fig 3



Synthesis of chitosan triazine serine hydroxamate (CT-SH)

Characterization

The newly chitosan derivative was characterized by FTIR spectroscopy, H^1 NMR Spectroscopy, Mass spectrometry, Scanning Electron Microscopy (SEM) and Elemental Analysis (EDX)

FT-IR Analysis

IR Spectra was recorded with BRUKER spectrophotometer.

H^1 NMR Analysis

NMR Spectra was determined by Bruker AV-II 300 MHz FT-NMR Spectrometer. The compound was dissolved in DMSO.

Mass spectral Analysis

DART-MS was recorded on a JEOL-AccuTOF JMS-T1OOLC Mass spectrometer having a DART (*Direct analysis in real time*) source. The compound was subjected as such in front of DART source. Dry Helium was used with 4 LPM flow rate for ionization at 350°C. The orifice 1 was set at 28 V.

Thermo gravimetric analysis

TGA was carried out using TGA Q 500 V 6.7 Build 203. The thermogram of the compound was analysed for their thermal stability up to 600°C under inert atmospheric conditions.

Elemental Analysis

The element determine by SEM EDX Method.

Antimicrobial Activity

An anti-microbial is a substance that inhibits the growth of micro-organisms such as bacteria, fungi. The minimum inhibitory concentration (MIC) of chitosan ranged from 0.005 to 0.1%depending on the species of bacteria, fungi and molecular weight of chitosan.[26]

The bacterial strain *Escherichia coli, klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Fungal strains like C*andida albicans, Candida tropicalis.* The pathogenic organisms were obtained from S.N. Medical College, Jodhpur. Ampicillin (antibacterial) and Voriconazole (anti-fungal) were used as standard drugs.

Antimicrobial sensitivity test using Agar well diffusion method.

Preparation of inoculum

The bacterium and fungi were inoculated in 1% peptone, 0.5% yeast extract and 1%NaCl. The inoculation was conducted at 37°C for 24h with shaking. The obtained suspension was diluted with the same peptone medium solution.

The plates were inoculated by dipping a sterile spread into inoculums. The sterile spread was streaked all over the surface of the medium. Finally the sterile spread was passed round the edge of the agar surface. The inoculum

was dried for a few minutes, at room temperature, with the lid closed.

Antimicrobial susceptibility test

Wells of approx 6 mm were dig on the sterile agar plate. Solution of compound was filled in well using micropipette. The plates were incubated in an upright position at 37°C for 24 h. Thediameter of inhibition zones formed was measured in mm with transparent ruler and the results were recorded.

The newly formed derivative was characterized by FTIR spectroscopy, H^1 NMR Spectroscopy, Mass spectrometry, Scanning Electron Microscopy (SEM), Elemental analysis.

FT-IR Analysis

FT-IR spectrum of the compound shows a peak at 3617.9cm^{-1} NH stretching in –NH-OH Indicates formation of hydroxamate, 2487.94 cm^{-1} due to NH structure in primary amine, 1692.34cm^{-1} due to –c=o stretching in CONH, 802.06cm^{-1} due to –CH deformation .Fig 2

RESULTS AND DISCUSSION

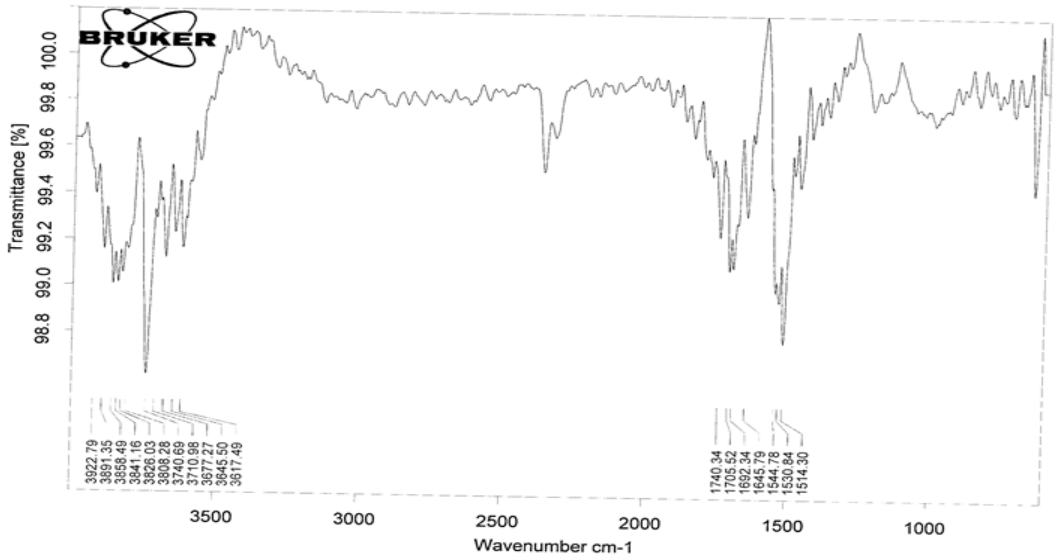


Figure 2: IR Spectra

H^1 NMR Analysis

The compound was dissolved in DMSO.^{1}H NMR peak interpretation shows peak at 7.26 δ due to aromatic proton (Ar-H), medium signal 2.0 δ to 3.5 δ may be due to methylene proton, the downfield signal arises in the range of 1.5 -3.2 δ may be due to amine (–C=O-N**H**-OH) moiety. Fig 3

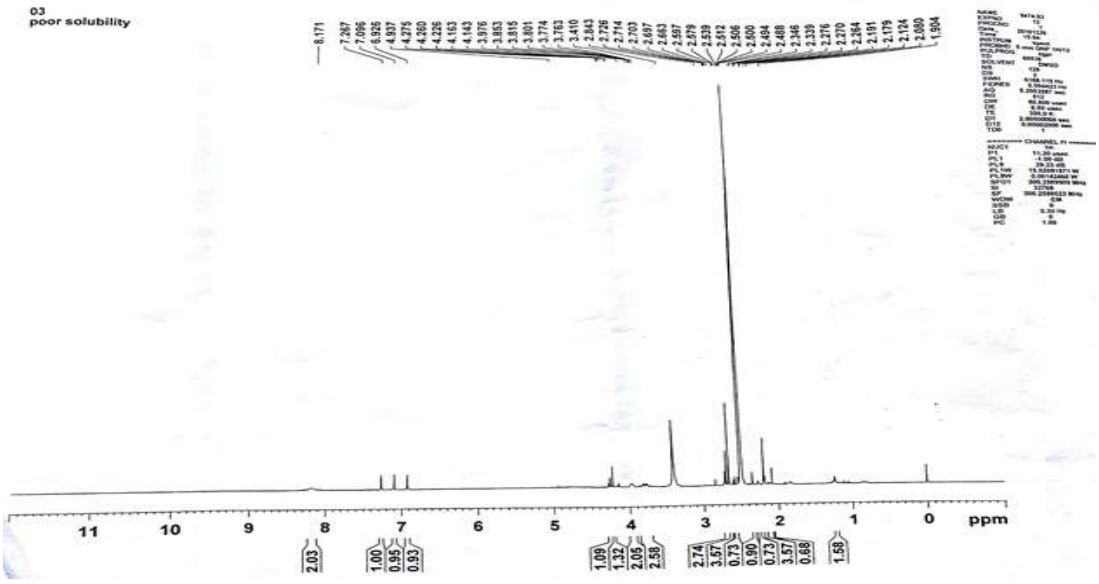


Figure 3: NMR SPECTRA

Mass spectral Analysis

The base peak of CE-AH obtained at 234.17. Fig 4

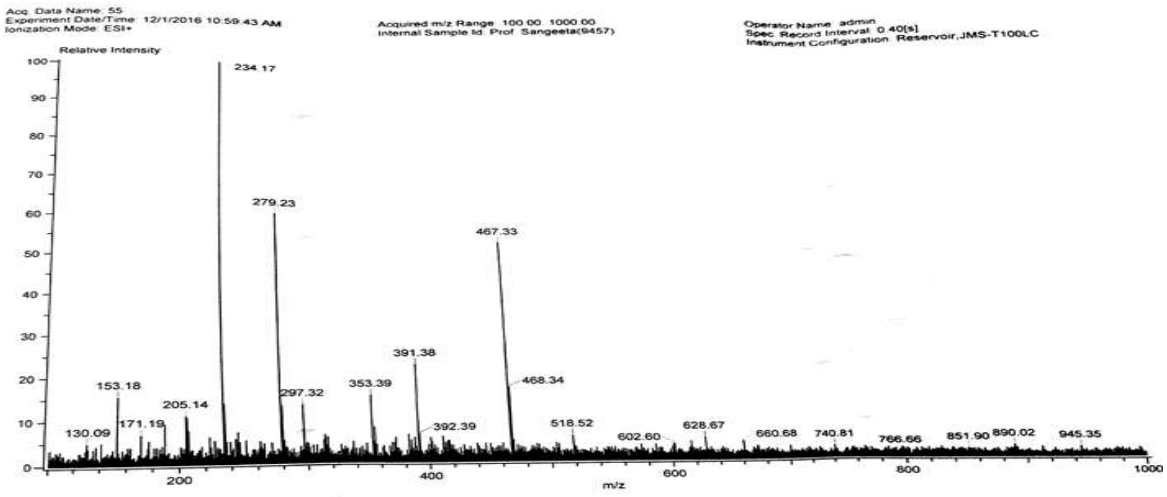


Figure 4: MASS SPECTRA

Thermo gravimetric analysis

TGA is a technique which is use for menstruate quantity and change of pace in the mass of the sample as a function of temperature. It breaks at three points. First at 85°C, here 5-6% weight loss occurs mainly due to desorption of water. Second breakdown at 250-300°C and 12-14% weight loss occur. Third breakdown at 425°C. here maximum 80% weight loss occur due to break down of weaker bonds and loss of organics. It is clear that formation of chitosan derivatives increases its thermal stability. Fig 5

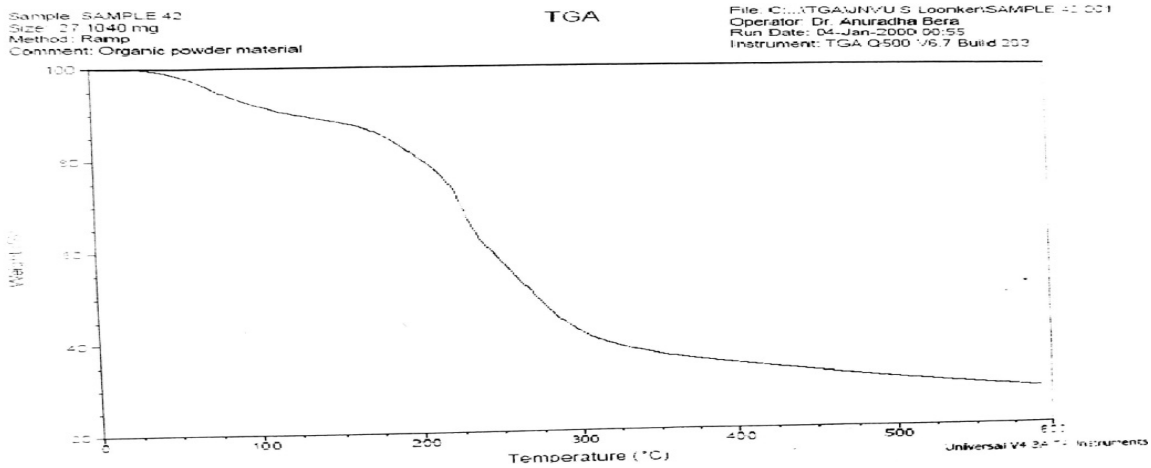


Figure 5: TGA of (CT-SH)

Scanning Electron Microscopy (SEM) and Elemental Analysis (EDX)

The SEM technique used to information about sample surface topography and composition. Newly synthesized derivative surface is craggy, honeycomb and unequal size of particals. Fig 6

An energy dispersive X-Ray analysis (EDX) is also used elemental identification and qualitative composition.[27] its elemental composition such as carbon, hydrogen, nitrogen and sulphur. The major elements of the composite are carbon and nitrogen. Composition of newly synthesized derivative is given Element carbon (weight%-38.82 & Atomic %-49.56) and Nitrogen (weight % -47.13 & Atomic % -40.95).

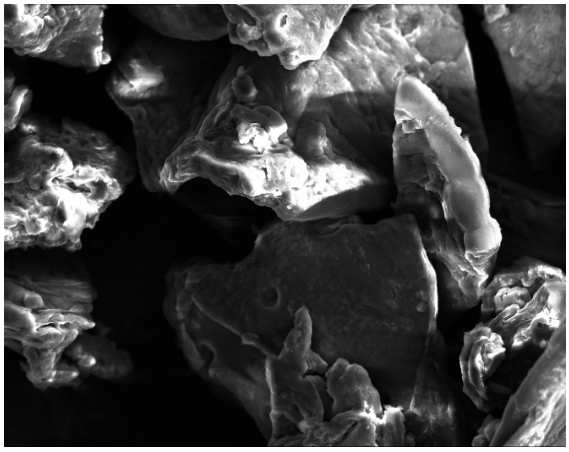
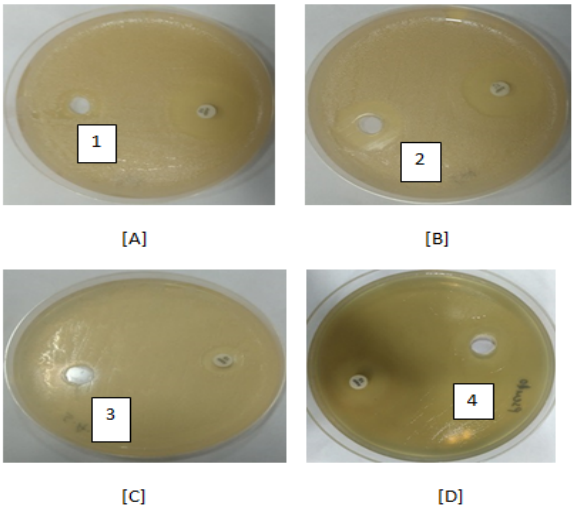


Figure 6: SEM image

Antimicrobial Activity

The antimicrobial activity of newly chitosan derivatives against bacterial **(**gram negative &gram positive) and fungal strain are given table 1. It shows inhibition zone against *Escherichia coli, klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa.* Fungal strains like C*andida albicans, Candida tropicalis* do not grow in presence of the compound. Fig 7



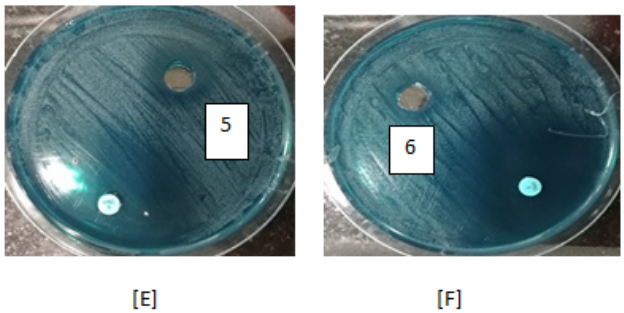


Figure 7: Photographs showing Antibacterial& Antifungal activity [A],[B],[C],[D],[E],[F]

Table 1: Anti bacterial & Anti fungal activity of CT-SH

S. No.	Bacterial/Fungal strains	Type	Zone of Inhibition 250µg/ml
1	***Escherichia coli***	Gram Negative	6mm
2	***klebsiella pneumonia***	Gram Negative	12mm
3	***Staphylococcus aureus***	Gram Positive	10mm
4	***Pseudomonas aeruginosa***	Gram Negative	15mm
5	***Candida albicans***	-	13mm
6	***Candida tropicalis***	-	9mm

CONCLUSION

In this study, synthesis of chitosan derivatives by an efficient management technique as compare to conventional method. ^{1}H NMR, IR, MASS, SEM, EDX, TGA analyses are described the polymer structure in much shorter time. The synthesized compound showed significant antimicrobial activity against gram positive and against gram negative bacteria and fungi.

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