



Hydroxytriazene Derived from Sulphanilamide: Spectrophotometric and Biological Applications

LAXMI KUNWAR CHAUHAN¹, KSHIPRA NIMODIA¹, PRADHYUMAN SINGH RANAWAT¹,
AJAY KUMAR GOSWAMI¹ and PRABHAT KUMAR BAROLIYA^{1*}

¹Department of Chemistry, Mohanlal Sukhadia University, Udaipur-313001, Rajasthan, India.

*Corresponding author E-mail: prabhatkbaroliya@mlsu.ac.in

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ABSTRACT

In this investigation, we report synthesis, spectrophotometric application and antimicrobial activities of 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido)phenyltriazene(HCNT) and its Fe(III) complex $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$. The complex has been synthesized by traditional as well as mechanochemical routes. These compounds have been characterized and screened for antimicrobial activity against bacterial strains i.e. *E. coli*, *S. aureus*, *S. pyogenes*, *P. aeruginosa* and fungal strains i.e. *A. clavatus*, *A. niger*, *C. albicans* using broth microdilution method. The results indicate that the compounds may serve as better bactericides compared to fungicides and the molar composition of iron(III) complex was found 1:2 (Fe:HCNT) by spectrophotometric study.

Keywords: Sulphanilamide, Antimicrobial activity, Mechanochemical synthesis, Iron complex.

INTRODUCTION

Hydroxytriazenes have been synthesized and used as analytical reagents for spectrophotometric determination during last many years¹⁻². This is an organic compound which have linear -N(OH)-N=N- moiety and reported as a good chelating agent having nitrogen and oxygen donor atoms. This class of compounds and their metal complexes has been studied for many biological activities such as wound healing³, antimicrobial⁴⁻⁷, insecticidal⁸, anti-inflammatory^{7,9-11}, antioxidant¹², analgesic¹³⁻¹⁴, antidiabetic¹⁵⁻¹⁶ activities etc. Hydroxytriazene's derivatives have been also used for photo-induced

green synthesis of azo dyes¹⁷⁻¹⁸. Literature survey revealed that the molecules bearing sulphonamide moiety play promising role in modern drug discovery and medicinal chemistry. The sulfonamide group is considered as a pharmacophore because this group showed a broad spectrum of biological activities. Hydroxytriazenes have good chelating ability to form complex with Fe(III) ions. Only few reports were found on spectrophotometric determination of Fe(III) metal using sulpha drug based hydroxytriazene¹⁹. In view of this, sulphanilamide based hydroxytriazene and its $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ (Fe-HCNT) complex have been synthesized using traditional as well as mechanochemical route and screened for antimicrobial activity.



EXPERIMENTAL MATERIAL AND METHODS

All chemicals and reagents used were of AR grade. The purity of synthesized compounds has been checked by TLC (Thin Layer Chromatography). Melting point was determined by one side open capillary method. IR spectra were recorded with Bruker-Alpha FT-IR spectrometer using KBr pellet at Department of Chemistry, MLS University, Udaipur. ^1H and ^{13}C NMR spectra were recorded with Bruker Avance II 400 MHz NMR spectrometer at SAIF, Chandigarh. Mass spectra were obtained using XEVO-G2SQTOF-MS ES⁺ mass spectrometer at MNIT, Jaipur. TGA was carried out on DTA-TGA Perkin Elmer 6000 at Department of Physics, MLS University, Udaipur. Spectrophotometric determinations of Fe(III) were carried out on ELICO SL-210 double beam UV-Visible spectrophotometer at Department of Chemistry, MLS University, Udaipur. Antimicrobial activity has been performed on bacterial strains viz. *E. coli*, *S. aureus*, *S. pyogenes*, *P. aeruginosa* and fungal strains viz. *A. clavatus*, *A. niger*, *C. albican sat* Microcare laboratory, Surat (Gujarat). All used pathogenic strains were of microbial type culture collection (MTCC).

Synthesis of 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido)phenyltriazene (HCNT)

Hydroxytriazenes can be synthesized by three methods as reported in literature²⁰⁻²². We used diazocoupling reaction with hydroxylamine method for synthesis of HCNT which is described below.

Step-I: Reduction of 4-chloronitrobenzene

4-chloronitrobenzene (3.15 g) was dissolved in a solution of alcohol (14 mL) and distilled water (14 mL) and stirred. In this mixture, saturated solution of 1.06 g of ammonium chloride was added and warm up about 50°C. After then, zinc dust (4 g) was added in small lots with continuous stirring and temperature was maintained between 55°C to 60°C. When the addition of zinc dust was completed, the stirring was continued for some more time and then filtered. The obtained filtrate (4-chlorophenylhydroxylamine) was chilled in freeze and used in Step-III.

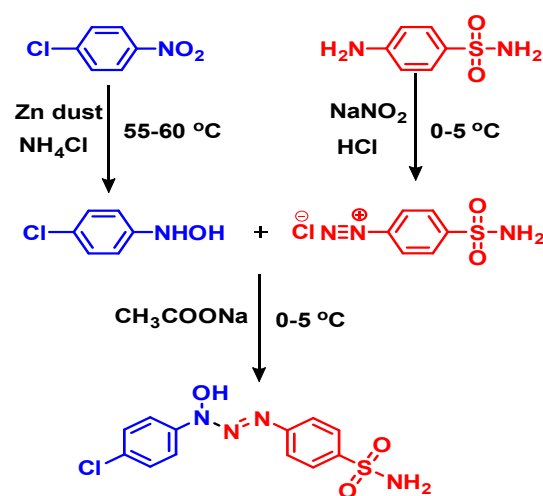
Step-II: Diazotization of Sulphanilamide

Sulphanilamide (1.72 g) was dissolved in a solution of con. HCl and distilled water. This solution

was placed on ice bath and saturated solution of sodium nitrite was added with continuous stirring and maintained temperature around 0-5°C. The diazotized product 4-sulfamoylbenzenediazonium chloride was obtained which was directly used for coupling with 4-chlorophenylhydroxylamine.

Step-III: Coupling of 4-sulfamoylbenzenediazonium chloride with 4-chlorophenylhydroxylamine

4-Chlorophenylhydroxylamine solution was kept on ice bath to maintain temperature around 0°C. After then, 4-sulfamoylbenzenediazonium chloride solution was added drop wise in hydroxylamine with continuous stirring. Saturated sodium acetate solution was used to maintain pH (5-6) of reaction mixture. After the completion of reaction, the crude product was precipitated out. The obtained crude product was recrystallized using ethyl alcohol. All the steps of synthesis are described in scheme 1.



Scheme 1. Synthesis of 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido)phenyltriazene

Synthesis of Fe(III) complexes

Iron complex of 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido)phenyltriazene with Fe(III) has been synthesized by using conventional as well as mechanochemical method.

Conventional method

In a 100 mL beaker, HCNT (0.65 g) was dissolved in methanol and in another beaker $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ salt (0.40 g) was dissolved in double distilled water. A small amount of tris buffer solution was added to methanolic solution of HCNT to adjust pH 6 and then Fe(III) ions solution was

added to HCNT solution dropwise with continuous stirring. Resultant instant black colour was appeared in the solution. When addition of Fe(III) ions was completed, the mixture was stirred for another 30 minutes. The obtained product (Fe-HCNT) was washed and filtered and then recrystallized using ethyl alcohol.

Mechanochemical method

Mechanochemistry deals with either liquid assisted grinding (LAG) or ball milling. The Fe(III) complex has been prepared by ball milling method using SPEX 8000M High Energy Ball Mill. The respective quantities of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (0.40 g) and HCNT (0.65 g) were taken to prepare solvent free complex. Mixture was grinded in the mortar pestle initially and transferred to stainless steel vial and grind for 30 min in ball mill. After grinding black coloured product (Fe-HCNT-M) was collected and washed with chilled distilled water and dried.

Spectrophotometric determination of Fe(III) with HCNT

All spectrophotometric measurements have been done at room temperature. Following solutions were prepared for spectrophotometric determination of Fe(III).

HCNT solution

A 100 mL of fresh stock solution of 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido) phenyltriazene (1×10^{-3} M) was made ready by dissolving requisite quantity of HCNT in methanol. Further, dilutions were done as and when required.

Fe(III) solution

A stock solution of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (1×10^{-2} M) was prepared in double distilled water containing 1-2 drops of conc. nitric acid. Standardization was done by complexometric titration using EDTA and sulphosalicylic acid as an indicator at pH 2.5. This stock solution was used to prepare solution of different concentration as required.

Buffer solution

- Tris buffer solution: 100 mL of 1% solution of tris buffer was prepared by dissolving 1 g of tris buffer in double distilled water.
- Perchloric acid solution: 100 mL of 1% HClO_4 solution was prepared by diluting 1 mL of HClO_4 in double distilled water.

Procedures

Determination of working wavelength:

To determine the suitable working wavelength, the solution of Fe(III) and HCNT were prepared in 1:10 molar ratio respectively, having 4×10^{-5} M Fe(III) and 4×10^{-4} M HCNT. Absorption spectrum of the $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ complex as well as HCNT were recorded in the wavelength region 300-500 nm against reagent blank and working wavelength was found 402 nm. The maximum absorbance of HCNT reagent was obtained at 354 nm Figure 1.

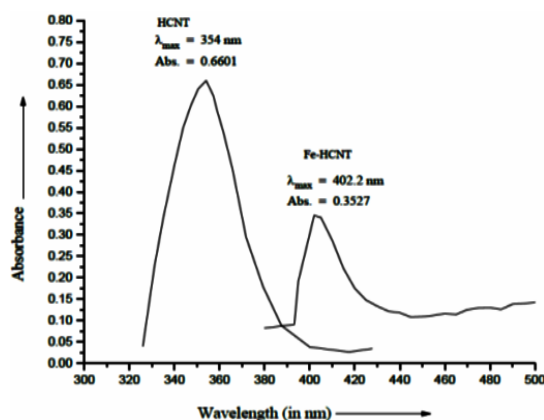


Fig. 1. Absorption spectrum of HCNT and Fe-HCNT complex

Effect of pH on absorbance

A series of a solutions were prepared in the molar ratio 1[Fe(III)]:10 [HCNT] having same concentration used in working wavelength determination with different pH range and absorbance was measured at 402 nm. Effect of pH on absorbance was studied in pH range 1 to 9. constant and maximum absorbance was found in the pH range 5 to 6.5 and the colour of developed complex was black Figure 2.

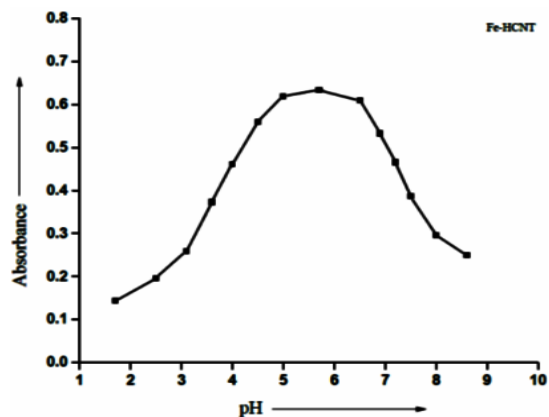


Figure 2: Effect of pH on absorbance

Effect of time on absorbance

The effect of time on absorbance of Fe-HCNT complex formation was studied under optimum conditions. The absorbance of Fe-HCNT complex was measured for 35 minute. This study suggests that formed Fe-HCNT complex was stable with time and did not decomposed Figure 3.

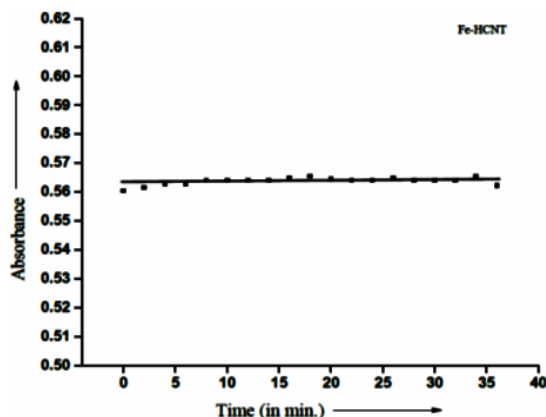


Fig. 3. Determination of time stability of complex

Validation of Lambert - Beer law

The validation of Lambert-Beer law has been studied by measuring the absorbance of solution by varying Fe(III) and HCNT concentration under optimum conditions. For this study, a set of solution containing Fe(III) and HCNT in molar ratio 1:10 have been prepared. This law was studied in 2×10^{-5} M to 4×10^{-4} M concentration range Figure 4.

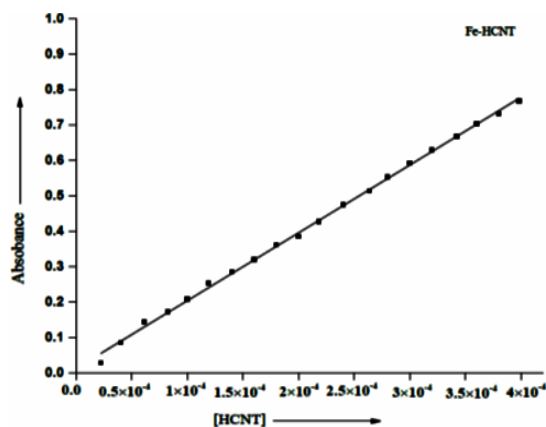


Fig. 4. Calibration curve (Beer's Law)

Sandell's sensitivity

The molar absorptivity of the Fe-HCNT complex was calculated from Lambert-Beer law's plot and it is found to be $\epsilon = 1915.82 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$. Using this value Sandell's sensitivity was calculated which is $29.14 \mu\text{g cm}^{-2}$. This value shows that the method

is sensitive and satisfactory for the determination of Fe(III) from solution.

Determination of molar composition of Fe-HCNT complex

In the present research work two methods were used for established molar composition of Fe-HCNT complex namely Job's and Mole ratio method.

Job's method

Job's method of continuous variation is based on change in absorbance with variation in concentration of both reagent and metal by keeping total volume constant. For this purpose, a set of solutions was made up in $1[\text{Fe}^{3+}]:10[\text{HCNT}]$ molar ratio with varying concentration range 3.8×10^{-4} M to 0.1×10^{-4} M for Fe(III) and 2×10^{-5} M to 3.9×10^{-4} M for HCNT. The absorbance has been measured at 402 nm under optimum conditions. The composition of complex was confirmed 1:2 [Fe:HCNT] by this method Figure 5.

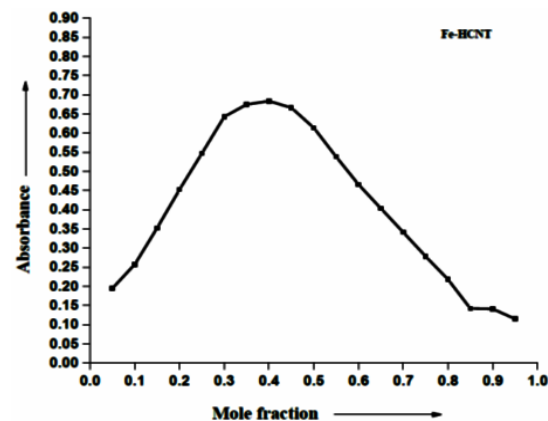


Fig. 5. complex composition by Job's method

Mole ratio method

Mole ratio method of Yoe and Jones is based on the constant metal concentration and varying reagent concentration. A series of a solution has been prepared having Fe(III):HCNT in molar ratio range 1:0.5 to 1:10 in which concentration of Fe(III) kept constant i.e. 4×10^{-5} M and HCNT concentration has been varied from 2×10^{-5} M to 4×10^{-4} M. The absorbance was measured at 402 nm under optimum conditions. By this method, the complex composition was again established as 1:2 [Fe(III):HCNT] that was agreed with Job's method. The stability constant of Fe-HCNT complex has also been calculated by this method. The calculated

stability constant (β) and free energy were found 4.0257×10^{10} and $-60481.99 \text{ J Mole}^{-1}$ respectively, for Fe-HCNT complex Figure 6.

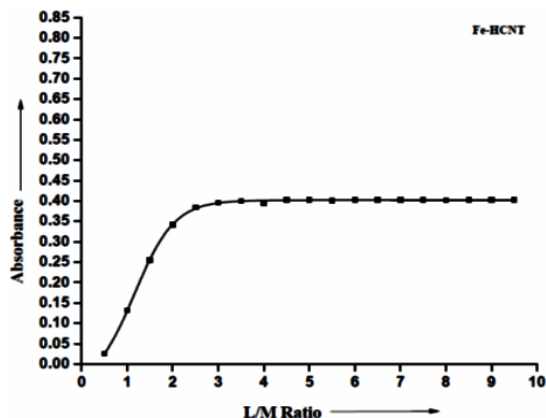


Fig. 6. Complex composition by Mole ratio method

Ion interference studies: The effect of foreign ions on Fe(III) determination has been studied. Ion interference studies in presence of 10 ppm, 50 ppm and 100 ppm of the following 10 cations and 12 anions were performed: K^+ , Ba^{2+} , Sr^{2+} , Na^+ , Pb^{2+} , Mg^{2+} , Hg^{2+} , Zn^{2+} , NH_4^+ , Ca^{2+} , F^- , Cl^- , Br^- , I^- , SO_4^{2-} , SO_3^{2-} , NO_3^- , NO_2^- , OH^- , CO_3^{2-} , $\text{C}_2\text{O}_4^{2-}$ and CH_3COO^- ions. For interference study a set of solution was prepared in methanol having $4 \times 10^{-5} \text{ M}$ Fe(III) and $4 \times 10^{-4} \text{ M}$ HCNT reagent and optimum pH condition was maintained. In each set of the solution 1 mL of foreign ion solution of 100 ppm, 50 ppm and 10 ppm were added and absorbance was measured at 402 nm after 10 min of addition. It was found that 10 ppm of Mg^{2+} , Br^- and 50 ppm of Mg^{2+} , Sr^{2+} and Br^- were interfered while Mg^{2+} , Sr^{2+} , Br^- , Ba^{2+} , Pb^{2+} , Zn^{2+} , Oxalate ions were tolerated upto 100 ppm level. Remaining 15 anions viz. K^+ , Na^+ , Mg^{2+} , NH_4^+ , Ca^{2+} , F^- , Cl^- , I^- , SO_4^{2-} , SO_3^{2-} , NO_3^- , NO_2^- , OH^- , CO_3^{2-} , acetate did not interfere upto 100 ppm level in the Fe(III) determination.

Biological activity

Antimicrobial Activity

Antimicrobial activities have been assessed by broth micro dilution method²³ and MIC values were determined against bacterial strains viz. *E. coli*, *S. aureus*, *S. pyogenes* and *P. aeruginosa* and fungal strains viz. *A. clavatus*, *A. niger* and *C. albicans*. MIC is a lowest concentration of antimicrobial drug that inhibits the visible growth of microorganisms after incubation. In this assay, Mueller-Hinton agar has been used as nutrient to grow the drug suspension²⁴. The stock solution has been prepared of 2000 $\mu\text{g/mL}$ and dilutions in DMSO were made. The concentration of samples has been taken 1000, 500 and 250 $\mu\text{g/mL}$ in primary screening and active samples were diluted to 200, 100 and 50 $\mu\text{g/mL}$ in secondary screening. The inoculum size of test microbes has been adjusted to 10^8 Cfu/mL . The tubes were incubated at 37°C for overnight and visible growth of tested microorganisms was observed. The reported antimicrobial activity was compared with standard drugs, viz. Ampicillin, Chloramphenicol and Greseofulvin. The obtained results are shown in Table 2.

RESULTS AND DISCUSSION

Characterization of (3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido)phenyl)triazene; HCNT : Light brown shining crystals (Yield 3.41 g, 80.23%), m.p. 155°C ; FT-IR (KBr, cm^{-1}): $\nu\text{O-H}$ (3362 cm^{-1}), $\nu\text{N-H}$ (3262 cm^{-1} , 3185 cm^{-1}), $\nu\text{N=N}$ (1443 cm^{-1}), $\nu\text{C-N}$ (1236 cm^{-1}), $\nu\text{N-O}$ (1321 cm^{-1}), $\nu\text{S=O}$ (1155 cm^{-1}). $^1\text{H NMR}$: δ (ppm) = 7.28 (s, 2H), 7.63 (m, 4H), 7.81 (d, 2H, $J=8.8 \text{ Hz}$), 8.13 (d, 2H, $J=9.0 \text{ Hz}$), 12.35 (s, 1H). $^{13}\text{CNMR}$: δ (ppm) = 114.7, 121.6, 127.2, 129.2, 134.5, 137.6, 141.5, 143.0. Mass (m/z): ($\text{C}_{12}\text{H}_{11}\text{ClN}_4\text{O}_3\text{S}$) cal. 326.75; found 326.55.

Table 1: Physical Characteristics of solid complex

Metal complex	Molecular formula	Colour and Crystal shape	Mol.Wt.	m.p.	% Yield
[Fe(HCNT) ₂ (H ₂ O) ₂] Conventional	$\text{C}_{26}\text{H}_{32}\text{Cl}_2\text{FeN}_8\text{O}_8\text{S}_2$	Black powder	775.46	210	63 %
[Fe(HCNT) ₂ (H ₂ O) ₂] Mechanochemical	$\text{C}_{26}\text{H}_{32}\text{Cl}_2\text{FeN}_8\text{O}_8\text{S}_2$	Black powder	775.46	207	71 %

Characterization of solid metal complexes

Solid complex Fe-HCNT (prepared by conventional method) and Fe-HCNT-M (prepared by mechanochemical method) have been characterized

by physical properties (Table 1) and spectral techniques such as FT-IR and TGA- DTA.

IR spectroscopic characterization

IR spectra have been obtained in range

between 4000-450 cm^{-1} . The bands at 3362, 3262 corresponding to $\nu_{\text{O-H}}$, $\nu_{\text{N-H}}$ in the spectrum of HCNT and are disappeared in the Fe-HCNT complex because H^+ ion removed during the complex formation. The $\nu_{\text{N=N}}$ and $\nu_{\text{N}\rightarrow\text{O}}$ bands for the tautomeric triazene-1-oxide [$\text{-NH-N=N}(\rightarrow\text{O})$] are appeared at 1423 and 1326 cm^{-1} , respectively. The band $\nu_{\text{C-N}}$ and $\nu_{\text{N-N}}$ were merged to one band which indicates delocalization of π electrons in chelate ring. New bands in the range of 415-624 cm^{-1} , which were not appeared in the free HCNT may be attributed to Fe-O and Fe-N vibrations, this appearance supports the involvement of N and O atom in complexation with iron metal.

Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis provides information about the presence and absence of H_2O molecules inside or outside of coordination sphere of complex. TGA curve showed 4.09% and 4.97% mass loss between 100 to 200 $^\circ\text{C}$ for Fe-HCNT and Fe-HCNT-M respectively, which reveals presence of two H_2O molecules in coordination sphere of metal complexes. Such a obtained molecular formula of complexes was $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ and octahedral geometry was established Figure 7.

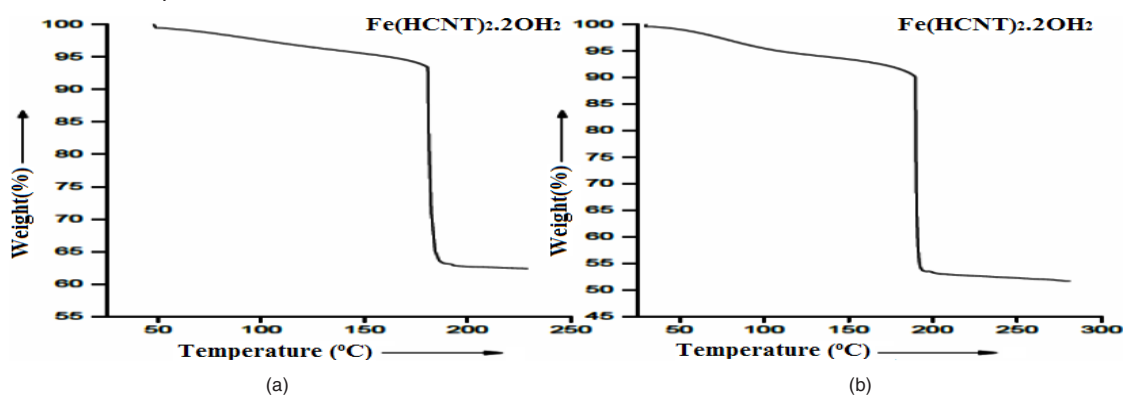


Fig. 7. (a) TGA curve for $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ conventional

(b) TGA curve for $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ mechanochemical

Differential Thermal Analysis (DTA)

This analysis measured the temperature difference between the sample and a thermally inert reference material. This analysis deals with the enthalpy

change. DTA curve is a plot of the differential temperature ($T_{\text{sample}} - T_{\text{reference}}$) against temperature. In DTA curve downward peak was observed which shows reaction is exothermic in nature Figure 8.

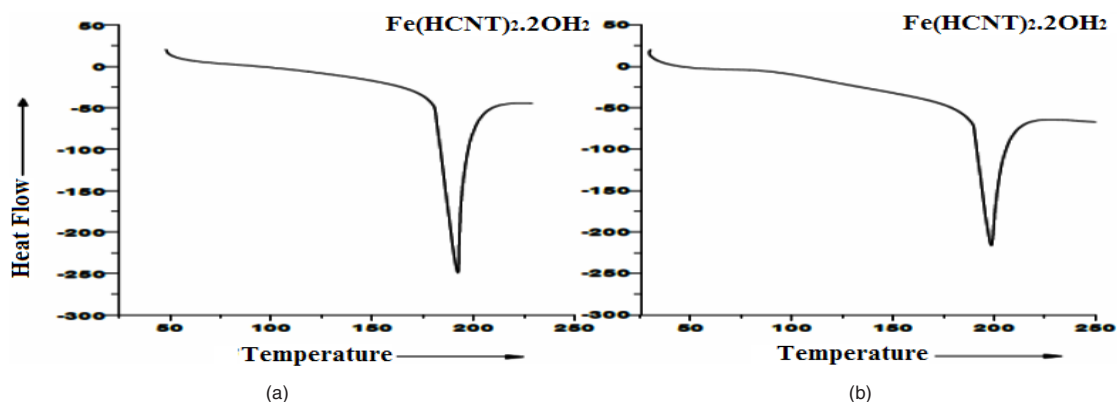


Fig. 8. (a) DTA curve for $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ conventional

(b) DTA curve for $[\text{Fe}(\text{HCNT})_2(\text{H}_2\text{O})_2]$ mechanochemical

Antimicrobial activity

The obtained result indicates that compounds are good antibacterial agent than antifungal. Compounds show good antibacterial result against *S. aureus* bacterial strain compared to standard drug. The metal complex, Fe-HCNT-M

which was synthesized by mechanochemical route was showing good activity against *E. colias* compared to Fe-HCNT because ball milling increases surface area of the metal complexes which responsible for increasing in biological activity.

Table 2:Antimicrobial screening of hydroxytriazene and Fe(III) complex

Compound	MIC($\mu\text{g/mL}$)						
	Bacterial strain				Fungal strain		
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. Pyogenes</i>	<i>C. albicans</i>	<i>A. niger</i>	<i>A. clavatus</i>
HCNT	250	125	62.5	250	500	500	1000
[Fe(HCNT) ₂ (H ₂ O) ₂] Conventional	500	250	100	500	500	500	>1000
[Fe(HCNT) ₂ (H ₂ O) ₂] Mechanochemical	250	250	100	500	500	500	1000
Ampicillin	100	-	250	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Greseofulvin	-	-	-	-	500	100	100

CONCLUSION

In summary, 3-hydroxy-3-(4-chlorophenyl)-1-(4-sulphonamido)phenyltriazene and its Fe(III) complex have been synthesized via conventional as well as mechanochemical route and characterized by various spectral techniques. The obtained result indicates that complex composition is [Fe(HCNT)₂(H₂O)₂] and octahedral geometry of the complex is proposed. The synthesized compounds were found moderate to good antibacterial agent than antifungal agent. The mechanochemical synthesis has been carried at low cost and increased product yield

compared to traditional method.

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Conflicts of Interest

The authors declare no conflict of interest.

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