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DNA cleavage studies of Zn(II) complex with 1-acetyl-4-phenyl-3-thiosemicarbazide

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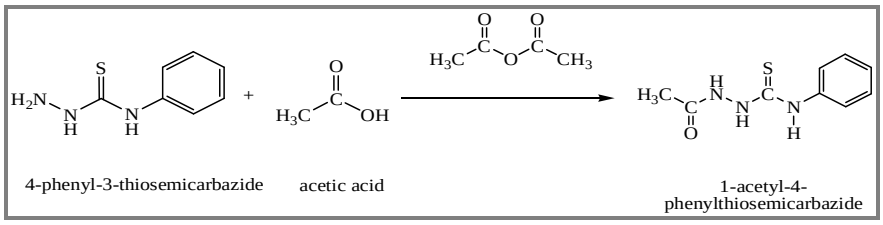
ABSTRACT

Zn(II) metal complex with 1-acetyl-4-phenyl-3-thiosemicarbazide (APTSC) was synthesized and characterized by LCMS, IR, ^{1}H-NMR, D_2O exchangeable ^{1}H-NMR, ^{13}C-NMR, UV-Visible spectrum, TGA and molar conductance measurements. The studies revealed the composition of complex as ML_2. Zn(II)-APTSC complex promote hydrolytic cleavage of plasmid pBR322.

Graphical Abstract



Synthesis of 1-acetyl-4-phenyl-3-thiosemicarbazide (APTSC)

Keywords: LCMS, IR, TGA, DNA cleavage studies.

INTRODUCTION

Transition metal complexes of thiosemicarbazide, plays significant role because of their bioinorganic applicability [**1, 2**]. By the dissociation of the hydrazinic proton, thiosemicarbazide bind to a metal ion as bidentate N, S-donor, forming five-membered chelate rings [**3**]. These complexes have been potentially helpful biologically. (viz, antibacterial, antimalarial, antiviral and antitumor) [**2-7**]. Thiosemicarbazide have become a great research interest due to their simple method of preparation, perfect complexation not only of transition but also non-transition p-block elements, their attractive structural characteristics, along with the probability of their analytical applications.

Due to binding and cleavage of nucleic acids remain at the centre of cellular transcription and translation, these substrates are marked targets for therapeutic intervention and the development of diagnostic investigation of nucleic acid structure. The characterization of DNA identification by small redox or photoactive transition metal complexes has been significantly helpful by studying the DNA cleavage activity [**8-11**].

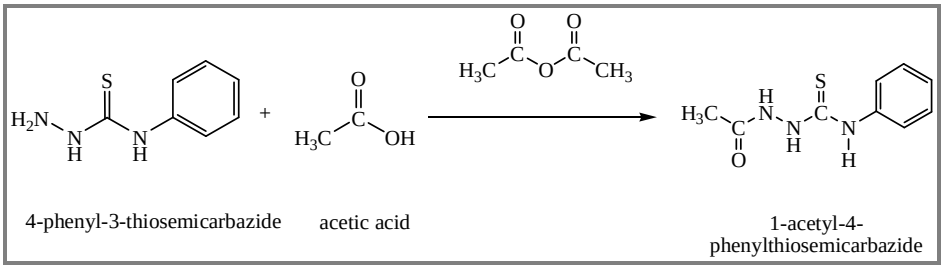
The present work explains the synthesis, characterization, of 1-acetyl-4-phenyl-3-thiosemicarbazide (APTSC) and DNA cleavage study of the chelate and its metal complex with Zn(II) ions.

MATERIALS AND METHODS

All the chemicals and solvents used were of Anala R grade. $ZnCl_2$ salt was used for the synthesis of complex.

Conductivity measurements of the metal complex was carried out in DMSO (1×10^{-3} M) using Digisun digital conductivity meter model D1 909. LCMS of all the compounds were recorded on LCMS 2010 A, Shimadzu spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were taken on Bruker 400 mHz NMR spectrophotometer. IR spectra were recorded in KBr phase ($4000cm^{-1}$ to $250cm^{-1}$) on Schimadzu IR prestige-21 FTIR spectrophotometer. UV spectrum was obtained from Schimadzu UV 2450 spectrophotometer within the range of 200-1000 nm. Thermo gravimetric analyses of the complex was recorded on TA model DTG 60 H SHIMADZU in temperature range of 0ºC-1100ºC with a ramp of 20ºC min^{-1}. DNA cleavage experiment was performed with the help of Biotech electrophoresis system supported by Genei power supply over a potential range of 50-500V, visualized and photographed by Biotech Transilluminator system.

Synthesis of 1-acetyl-4-phenyl-3-thiosemicarbazide (APTSC): To 3 gm of 4-phenyl-3-thiosemi carbazide (1.79 mmol), 1.03 mL of acetic acid (1.79 mmol) and 1.68 mL acetic anhydride (1.79 mmol) were added and refluxed for 40min on water bath (scheme 1) [**12**]. The progress of the reaction was monitored by TLC and then transferred the reaction mixture into a petridish. After 30 min white solid of APTSC separated out. It was recrystallized in ethanol and water mixture. It was soluble in methanol, ethanol and DMF. (M.P:170-172ºC).



Scheme 1. Synthesis of 1-acetyl-4-phenyl-3-thiosemicarbazide (APTSC).

Synthesis of metal complexes: Aqueous $ZnCl_2$ metal salt solution (1.2 mmol) was added to hot methanolic solution of APTSC (0.5 g, 2.3 mmol), in 1:2 (M:L) molar ratio and was refluxed for 10 h. The pH of the solution was regulated by the addition of small quantity of methanolic ammonium hydroxide solution. White coloured Zn(II)-APTSC complex was separated and filtered in hot condition, washed with hot methanol and double distilled water to remove unreacted ligand and metal salts respectively, then washed with petroleum ether and ultimately dried in vacuum.

RESULTS AND DISCUSSION

Characterization of 1-acetyl-4-phenyl-3-thiosemicarbazide (APTSC)

LC-MS: Liquid chromatograms of APTSC showed (Figure 1 inset) a single peak with retention time of 0.743 min indicating its purity. APCI-Positive1 mass spectrograph of the APTSC revealed molecular ion peak $[(C_9H_{11}N_3OS)+1]^+$ at m/z 210 (cal 209). Other fragmentation peaks at m/z 115 $[C_2HN_3OS]^+$ and m/z 101 $[C_2HN_2OS]^+$ were also observed**.**

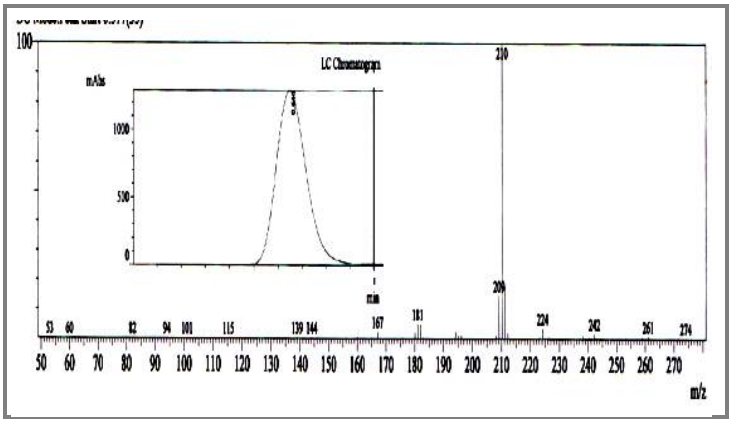


Figure1. LC-Mass spectrum of APTSC.

IR: IR spectrum of the APTSC showed (Figure 2) characteristic bands for the stretching vibrations at 3261cm^{-1}(νN$_4$-H), 3142 cm^{-1} (νNH amide), 3030 cm^{-1} (νN-H thioamide), 2872 cm^{-1}(νCH$_3$), 1693 cm^{-1} (νC=O), 1247 cm^{-1} (νC=S), 1049 cm^{-1} (νC-C) and 990 cm^{-1} (νN-N) [**13, 14**].

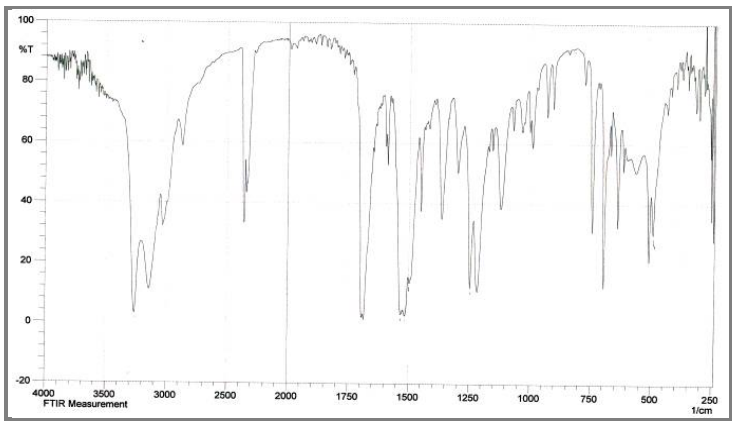


Figure 2. IR spectrum of APTSC.

^{1}H-NMR: ^{1}H-NMR spectrum of APTSC (Figure 3) showed peaks at δ9.94 ppm (s, 1H, OH), δ9.73 ppm (s, 1H, NHC$_6$H$_5$), δ8.92 ppm (s, 1H, NHCS), δ7.59 ppm (d, 2H, C$_6$H$_5$), δ7.56 ppm (t, 2H, C$_6$H$_5$) , δ7.51 ppm (t, 1H, C$_6$H$_5$), δ3.6 ppm (s, 1H, SH), δ2.09 ppm (s, 1H, NH) and δ1.88 ppm (s, 3H, CH$_3$). The D$_2$O exchangeable ^{1}H-NMR has been presented as inset.

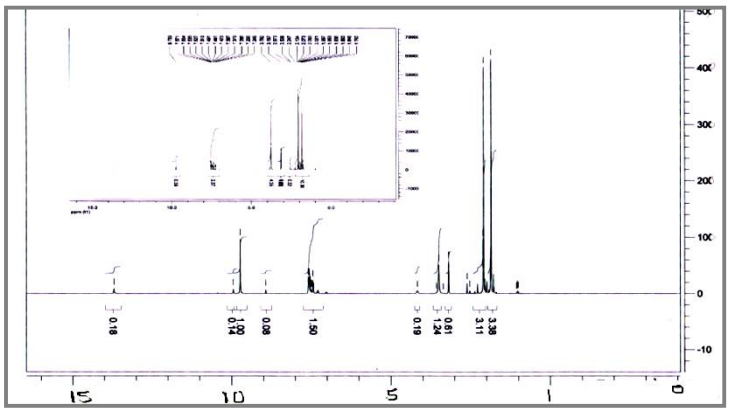
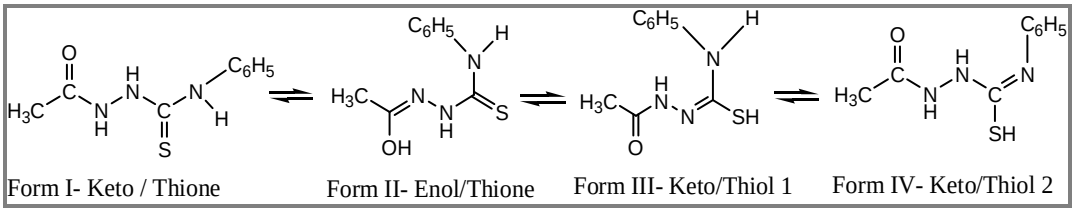


Figure 3. ^{1}HNMR and D$_2$O exchangeable NMR spectrum of APTSC.

More number of peaks observed, as against expected from number of hydrogens present may be due to tautomerism in the compound. At two positions APTSC can shows tautomerism. Chemical shift of NH proton was D_2O exchanged.



Keto-enol and thione-thiol forms of APTSC.

^{13}C-NMR: The chemical shift values of ^{13}C-NMR of the APTSC (Figure 4) have been observed at δ30 ppm (CH_3 carbon), δ127-149 ppm (C_6H_5 carbon), δ167-169 ppm (C=O, C-OH carbon), δ207 ppm (C=S carbon). The signals of C=O and C=S splits into two each indicating keto-enol and thione-thiol tautomerism.

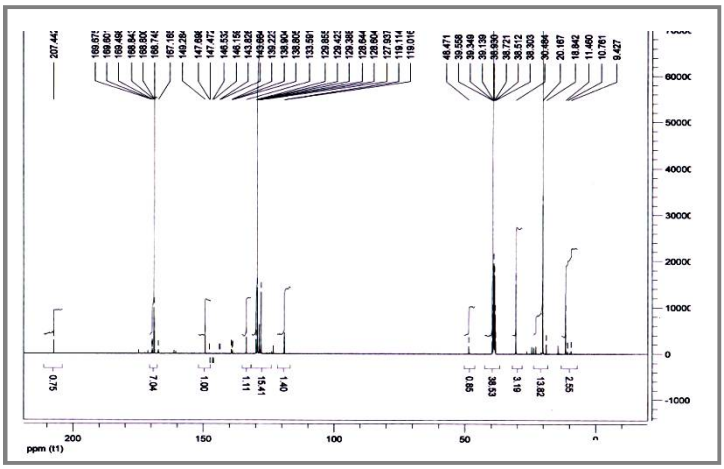


Figure 4. ^{13}C-NMR spectrum of APTSC.

UV-Visible: The electronic absorption spectrum of the APTSC showed (Figure 5) bands at 328 nm (30,487 cm^{-1}) corresponding to π→ π* (C_6H_5), 270 nm (37,037 cm^{-1}) for n→ π* (C=O) and 206 nm (48,543 cm^{-1}) due to n→ π* (C=S) transitions respectively.

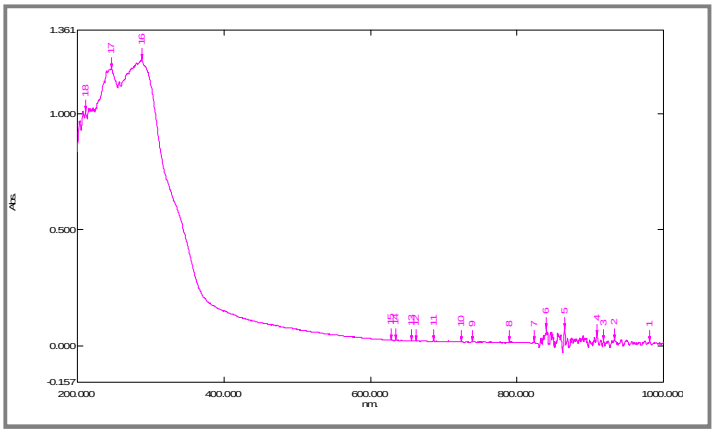


Figure 5. UV-Visible spectrum of APTSC.

Characterization of Zn(II)-APTSC complex: Zn(II) complex of APTSC was quite stable to air and moisture, amorphous and was dissolved in DMF and DMSO decomposed above 300ºC. Molar conductivity recorded in DMSO was 9 $ohm^{-1}cm^{-1}mol^{-1}$ indicating the non electrolytic nature of the complex. Volhard's [**15**] test revealed the absence of chloride ion.

Mass spectrum: The mass spectrum of Zn(II)-APTSC complex (Figure 6) showed $[M+1]^+$ peaks at m/z 518.38 $[ML_2.2H_2O]^+$, m/z 500.38 $[ML_2.H_2O]^+$, m/z 481.38$[ML_2]^+$, m/z 275 $[ML+1]^+$.

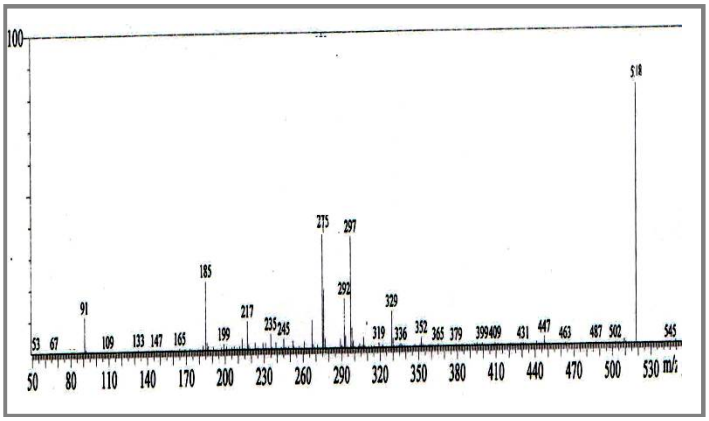


Figure 6. Mass spectrum of Zn(II)-APTSC.

Thermogravimetric analysis: Thermogram of Zn(II)-APTSC complex (Figure 7) showed decomposition of the complex in five steps. In the first step, the weight loss of 7% upto 200ºC indicated the loss of two coordinated water molecules. Thereafter the decomposition of the complex occurred in various steps, leaving the residue of 29% indicating partial decomposition of the complex.

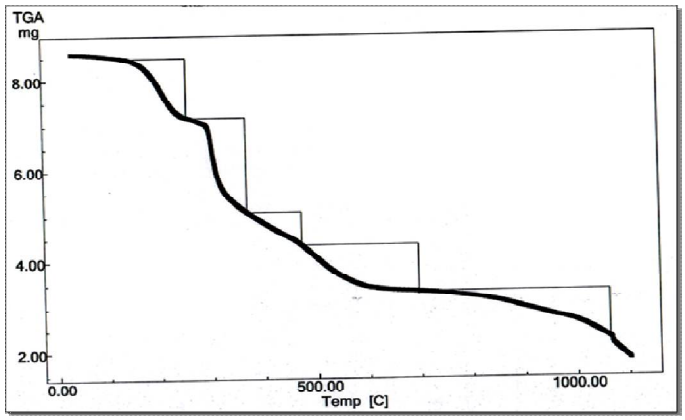


Figure 7. Thermogram graph of Zn(II)-APTSC.

IR: In the IR spectrum of APTSC the $\nu_{NH\ (N1)}$ peak which was observed at 3142 cm^{-1}, was absent in the Zn(II)-APTSC spectrum and an extra peak corresponding to $\nu_{C=N}$ was observed at 1598 cm^{-1}, indicating the dissociation of NH-'N'(1) during complexation (Figure 8). The $\nu_{C=O}$ peak observed at 1693 cm^{-1} in the IR spectrum of APTSC was absent in the complex and an extra peak corresponds to ν_{C-O} is observed at 1100 cm^{-1} indicating deprotonation of amide proton from oxygen in the enol form and participation of 'oxygen' in the bonding. The $\nu_{NH\ (N2)}$ peak observed in the ligand spectrum at 3030 cm^{-1} has been shifted to higher frequency region at 3100 cm^{-1} in the complex indicating the participation of 'N'(2) in the bonding without dissociation of hydrogen. The $\nu_{C=S}$ peak, observed at 1247 cm^{-1} in the ligand spectrum has been shifted to higher frequency region 1265 cm^{-1} in the IR

spectrum of the complex supporting 'N'(2) coordination. Therefore 'N'(2) and 'O' act as potential donor sites forming a five membered chelate with Zn(II) ion. From Far-IR region of the spectrum, there has been a clear evidence for the presence of extra peaks in the complexes corresponding to ν_{M-N} (437-487 cm^{-1}) [**16**], ν_{M-O} (405-435cm^{-1}), and ν_{M-OH2} (400-440cm^{-1}).

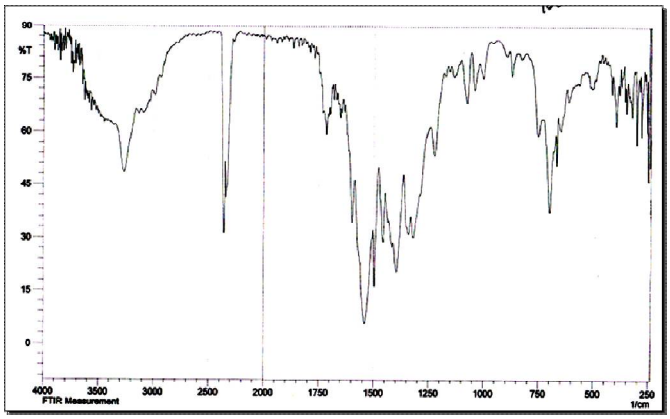


Figure 8. IR spectrum of Zn(II)-APTSC.

Based on the discussions of all the analytical, spectral techniques employed, the following tentative structure (Figure 9) has been proposed.

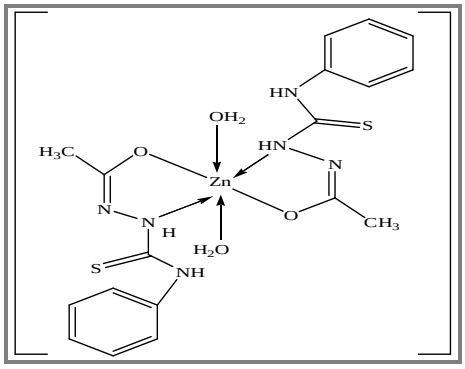


Figure 9. Tentative Structure of Zn(II)-APTSC.

DNA cleavage studies: Super coiled (SC) plasmid DNA, commonly seen in bacteria cells, will be cyclic super coiled double strand made up of several thousand base pairs. This has been main

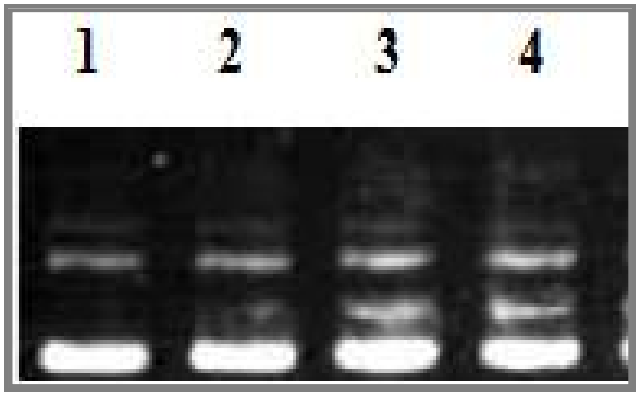


Figure 10. Agarose gel electrophoresis pattern for the cleavage of supercoiled pBR 322 DNA by complex. Lane 1, DNA control, Lane 2-4 DNA+ Zn(II) (20,40,60 μM resp.), of APTSC.

substrate for hydrolytic cleavage. Metal ions in the complexes help as Lewis acids to activate the phospho-diester links for nucleophilic attack and metal coordinated water species function as a nucleophile. DNA when subjected to electrophoresis, the intact SC form migrates faster. When scission occurs due to action of complex, SC form will relax to nicked (NC) form that migrates slowly. Cleavage of both types of strands leads to linear form which migrates between SC and NC forms, because shorter molecules migrate more easily through the pores of the gel [**17, 18**]. In the present investigation it is observed (Figure 10) that the complex promotes hydrolytic cleavage of plasmid pBR322 to certain extent due to scission in SC forms of DNA to NC forms.

APPLICATION

The formation of the thiosemicarbazide compounds can be used as the potential antibiotic agents against some known pathogenic organisms and can be used as drugs.

CONCLUSION

Spectral and analytical studies indicate that APTSC forms distorted octahedral complex in 1:2 (M:L) composition with Zinc(II) ion. DNA cleavage studies reveal that the complex can cleave SC form of plasmid DNA.

ACKNOWLEDGEMENTS

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