



***In-Vitro* CT-DNA Cleavage of Newly Synthesized Zn(II) Complex with P-Mercaptoaniline and Benzoate Ion**

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ABSTRACT

Coordination complex of Zn(II) with 4-mercaptoaniline(4-MTP or 4-ATP) were synthesized under microwave irradiation. The synthesized complex was characterized by elemental analysis, metal estimation, UV-Visible, conductance measurement, IR, Far-IR & NMR (proton and carbon) spectral studies. The metal complex was found to mononuclear, neutral and colorless in nature which are confirmed by the elemental analysis and molar conductance studies. The geometry for 1:2:2 complex exhibits to be square planar were confirmed by its spectral absorption study. The ligand was coordinated to the metal ion through its 'N' or 'S' atom and diamagnetic nature of the complex to be confirmed by the IR, Far-IR and NMR spectral studies. The electrochemical properties of complex shows the well redox properties of them with Zn(II)/Zn(I) couple by predicting the values of anodic and cathodic current. *In-vitro* antibacterial and antifungal activities of microorganisms viz., *S. Aureus*, *bacillus* and *C. Albicans* were carried out by Agar disc diffusion method using Amikacin and ketoconazole as standard control and DMSO as solvent control. The metal complex shows enhanced biological activities than the 4-ATP. The CT-DNA cleavage study of 4-ATP and its Zn(II) complex were performed and calculated the IC50 values. The results show the antioxidant property of 4-ATP and its complex.

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INTRODUCTION

4-amino thiophenol or p-mercaptoaniline is an organic compound which are very much interested in nanotechnology, especially gold electrodes (Yilmaz *et al.*, 2020), fabricate DNA sensor (Chen

et al., 2017) and functionalized MWCNTs (Shafaatian *et al.*, 2016). N-donor character of 4-ATP shows the catalytic oxidation (Silva *et al.*, 2013) and biosensor (Yilmaz *et al.*, 2008). 4-amino thiophenol Schiff base metal complexes show enhanced bio-potential viz., anticancer (Li *et al.*, 2009), anti-inflammatory (Taha *et al.*, 2018), anticonvulsant, etc., (Zhang *et al.*, 2012). Microwave-assisted synthetic reactions are attractive because less time consuming to form product easily and efficiently with high purity (Lanjekar and Rathod, 2021) and eco-friendly than the normal heating method (Aziz *et al.*, 2017). In the literature, there is no systematic study of the 4-amino thiophenol itself as a ligand in complexes so the present study focused on the synthesis of Zn(II) complex with 4-amino thiophenol and benzoate ion under microwave irradiation and characterized by various physicochemical, spectral and biological studies.

MATERIALS AND METHODS

Zinc nitrate, 4-amino thiophenol, sodium benzoate, solvent DMSO, methanol, ethanol was used as such without further purification, which are AR grade.

Preparation of zinc complex

The mononuclear Zn(II) complex was prepared by adding 4-amino thiophenol 0.84 g (6.72 mmol) in 10 mL methanol, sodium benzoate 0.97 g (6.72 mmol) in 10 mL ethanol to the zinc nitrate 1g (3.36 mmol) in 10 mL methanol (Scheme 1). The entire mixture was irradiated on a microwave oven (CATA-R, model). The obtained complex was filtered, washed with ethanol: water, dried in desiccators and kept in a container. At 950-1200°C temperature, elemental analysis of the complex was carried out by Vario make EL-III model instrument. The metal ion estimated volumetrically using standard procedure. Molar conductance of 10^{-3} M complex predicted by Systronic Conductivity Bridge. The Cyclic voltammogram of the complex was recorded in DMSO solution at room temperature was measured by using Versa Stat (Princeton Applied Research-Make) electrochemical work station. The UV spectra of 4-ATP and complex were recorded on Varian make, CARY-5000 model, UV-VIS-NIR Spectrophotometer by solid-state DRS method. IR spectra of ligand and its Zn(II) complex predicted by Shimadzu, FT-IR, 8400 S Model IR spectrometer. Using Bruker, Germany makes 3000 Hyperion Microscope with Vertex 80 FTIR system, the low frequencies range of metal complex (Far-IR) was measured. ^1H and ^{13}C -NMR spectral data of the 4-ATP and Zn(II) complex were recorded in DMSO-d₆ using 500 MHz instruments.

DNA Cleavage

The DNA cleavage study of the metal complex was predicted using supercoiled pBR322 DNA by agarose gel electrophoresis method. The reaction mixture (20 μL) containing pBR322 DNA, 50 mM Tris-HCl at pH 7.4, 50mM NaCl and 10 mM H₂O₂ followed by the addition of Millipore water. Then the solutions were incubated at 37°C for 1 hr.

RESULTS AND DISCUSSION

The colorless Zn(II) complex is insoluble in water but soluble in DMSO, DMF and ethanol. The results of elemental analysis and metal estimation indicate the molecular formula of the complex is $[\text{Zn}(4\text{-ATP})(\text{Ben})_2]$ with 1:2:2 (M: L1:L2) stoichiometry ratio of the metal and ligands. The molar conductance of the synthesized complex in 10^{-3} M concentration show below $50 \text{ Ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$, indicating their non-electrolyte nature (1:0 type), which is

further confirming by no anions and cations present outside the coordination sphere of the complex (Tas *et al.*, 2010).

UV spectra of the complex

The UV-spectrum of the 4-ATP shows the value of λ_{max} at 271 nm corresponds to $\pi\text{-}\pi^*$ transition, but in Zn(II) complex, this is shifted to 242 nm due to the C-T band (charge transfer spectra). The d-d transition is not present in Zn(II) complex because of its filled 'd' subshell. This assignment is confirming the square planar geometry of the complex (He *et al.*, 2009).

Cyclic voltammetry

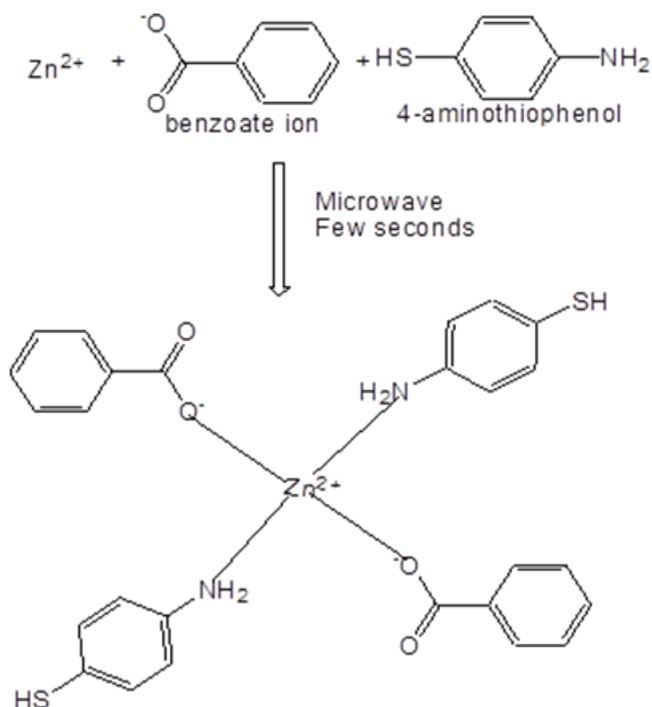
The cyclic voltammogram of Zn(II) complex shows the cathodic peak potential E_{pc} at -0.4656V, anodic peak potential E_{pa} at 0.4113 and ΔE_p is -0.8769 V. this assignment confirming by one-electron transfer quasi reversible reaction of Zn(II)/Zn(I) couple reaction which is further confirming the IPA/IPC at 0.8722 V (Figure 1) (El-Maali *et al.*, 2005).

FT-IR spectral analysis of complex

The IR spectral data of 4-ATP shows aromatic C-H at 3071 cm^{-1} , aromatic NH(asymmetric) at 3418 cm^{-1} and symmetric NH at 3089 cm^{-1} . The C-N & C-S stretching frequencies at 3027 cm^{-1} and 1307 cm^{-1} . In metal complex, these are shifted to higher / lower values. The C-N and C-S stretching frequency of complex gives higher shift of stretching frequency at 3055 cm^{-1} and 1352 cm^{-1} , respectively, indicating the 4-ATP coordinate (Figure 3) to the metal ion through 'N' or 'S' atoms. In complex, the additional stretching frequencies of aromatic C-H at 3100 cm^{-1} , aromatic C=C at 1494 cm^{-1} and conjugated C=O at 1605 cm^{-1} (asymmetric) and 1572 cm^{-1} (symmetric) attributed to the presence of additional ligand benzoate ion coordinated to complex through an oxygen atom (Figure 2). The coordination mode was further confirmed by Far-IR spectral data at 463 cm^{-1} for $\nu(\text{M-N})$, 312 cm^{-1} for $\nu(\text{M-S})$ and for 4-ATP 448 cm^{-1} for $\nu(\text{M-O})$ of benzoate ion respectively (Faniran *et al.*, 1976; Raman *et al.*, 2008).

NMR Spectra of 4-ATP and its complex

^1H -NMR spectrum of 4-aminothiophenol shows the chemical shift at 3.384 ppm (t, 2H), 2.499pp (s, 1H), 7.885-7.873 ppm (d, J=6 Hz, Ortho-H), 7.263-7.275 ppm (d, J=6Hz, meta-H). In Zn(II) complex, these chemical shift values are shifted to downfield or up-field at 4.650 ppm (t, 2H), 3.360ppm (s, 1H), 7.931-7.948 ppm (d, J=8.5Hz, Ortho-H), 7.394-7.409 ppm (d, J=7.5Hz, meta-H) after the complex formation of ligands to the metal ion, in addition to the above chemical shift values the other chemical shifts



Scheme 1: Preparation of Zn(II) complex

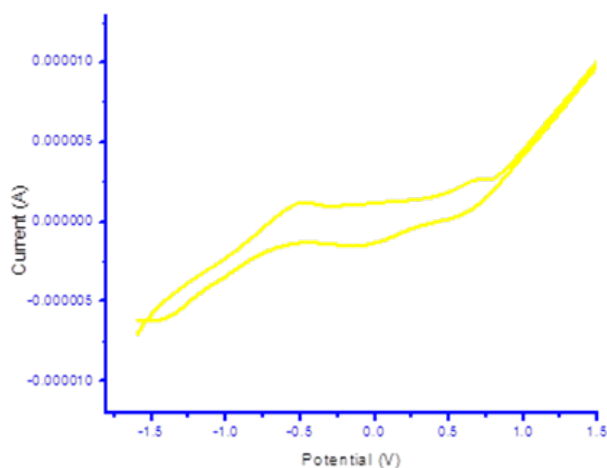


Figure 1: Cyclic voltammogram of 4-ATP complex of Zn(II)

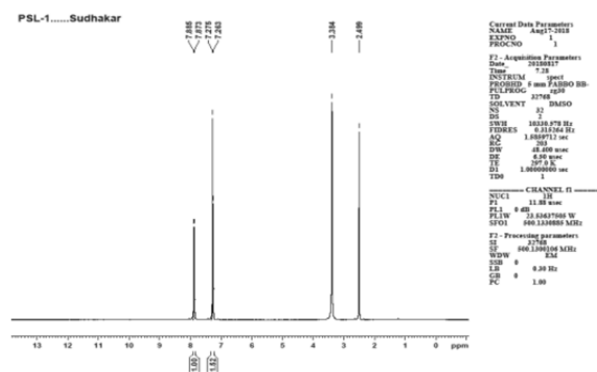


Figure 3: ¹H-NMR spectrum of 4-ATP

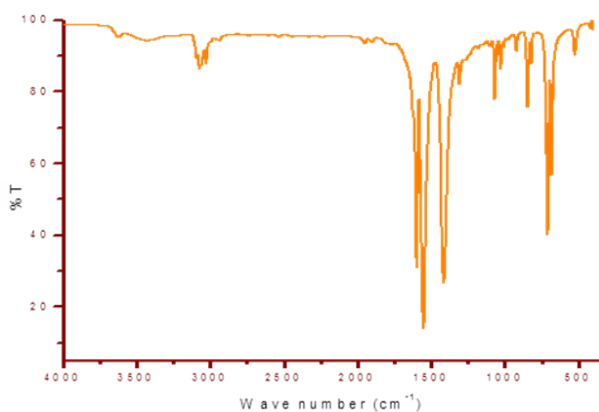


Figure 2: IR spectrum of 4-Aminothiophenol

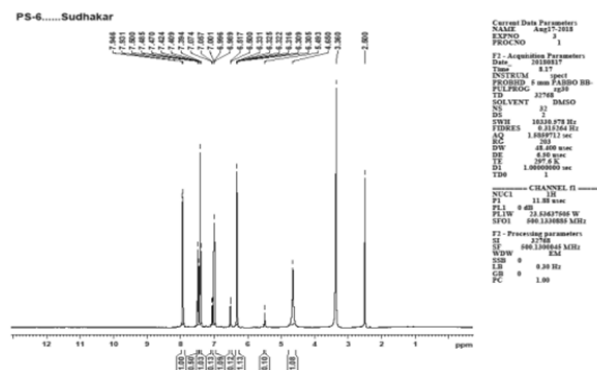


Figure 4: ¹H-NMR spectrum of Zn(II) complex

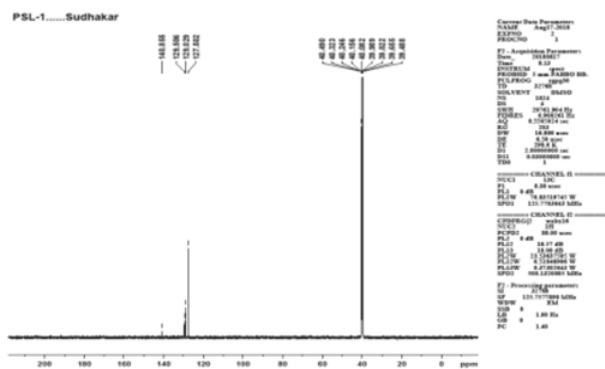


Figure 5: ¹³C-NMR spectrum of 4-ATP

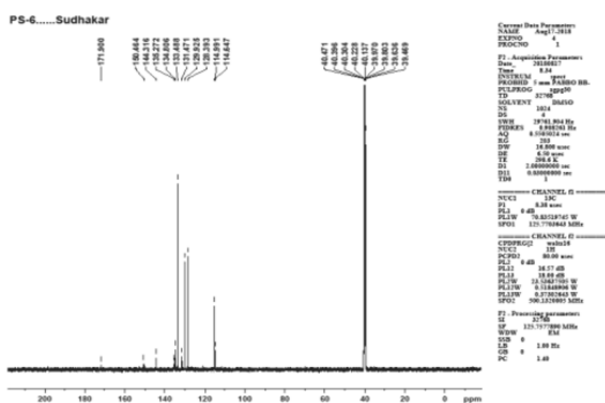


Figure 6: ¹³C-NMR spectrum of Zn(II) complex

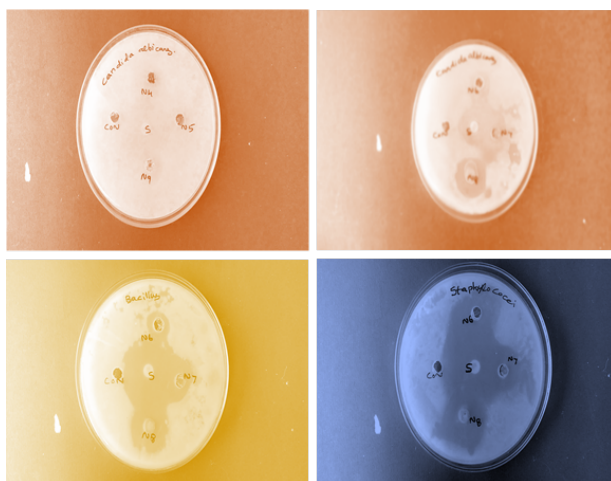
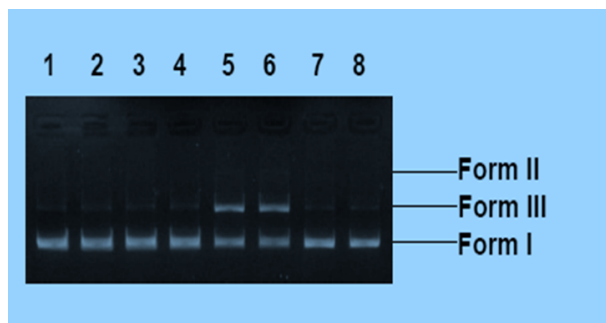


Figure 7: Bio-potential activities of 4-ATP and Zn(II) complex

are observed at 5.493ppm (s, para-H), 6.305-6.316 ppm (t, J=5.5Hz, meta-H) and 6.500-6.517 ppm (t, J=8.5Hz, ortho-H) respectively confirming the mixed ligand benzoate ion also present in the complex (Figure 3 and Figure 4) (Alaghaz *et al.*, 2015).

The ¹³C-NMR spectrum of 4-ATP shows the chemical shift values at 129.029 ppm (C1), 129.506ppm(C2), 127.502ppm (C3) and 140.955 ppm (C4). In metal complex, these are shifted to down/up-field at 131.471 ppm (C1), 133.488



Lane 1 –PUC19 DNA -Control
 Lane 2 –DNA + H2O2 (1mM)
 Lane 5–DNA + H2O2 (1mM) + PS6(40µM)
 Lane 6 –DNA + H2O2 (1mM) + PS6(50µM)
 Lane 7 –DNA + H2O2 (1mM) + PSL1(40µM)
 Lane 8 –DNA + H2O2 (1mM) + PSL1(50µM)

Figure 8: DNA Cleavage

ppm(C2), 114.991ppm (C3) and 150.464 ppm (C4). The mixed ligand benzoate ion also gives the chemical shift values of five different carbon atoms at 171.90 ppm (C1), 144.316 ppm(C2), 129.393 ppm (C3), 129.926 ppm (C4) and 134.806 ppm (C5) (Figure 5 and Figure 6) (Denizot and Lang, 1986).

Bio-potential activities

The bio-potential activities of the metal complex show greater activity than the 4-ATP, according to Tweedy Overtone’s chelation theory indicating the polarity of metal ion reduced and partial sharing of positive charge on the metal ion to the donor group of ligand (4-ATP). The greater the lipophilicity nature of the complex, the cell permeability of the lipid membrane that surrounds the cell favours the passage of lipid-soluble materials. The delocalization of π electrons on the chelate ring also increase lipophilicity; such increase lipophilicity facilitates the penetration of the complex into the lipid membrane and then blocks the metal-binding sites on enzymes of microorganisms (Figure 7). The other factors also favour biological activities such as non-electrolytic nature, size, the charge on the metal ion and geometry of the complex (Parekh *et al.*, 2005).

DNA Cleavage studies

The DNA cleavage of circular plasmid DNA is subjected to electrophoresis; relatively fast migration will be observed for the intact supercoil form (Form-I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower-moving open circular form (Form-II). If both strands were cleaved, a linear form (Form-III) that migrates between Form-I and Form-II will be generated. The cleavage effect upon irradiation of the plasmid pUC19 DNA in the presence of Zn(II) complex and 4-ATP in two different concentrations has been tested and is shown in Figure 8. It is clear that the Zn(II)

complex in the presence of H₂O₂ exhibit DNA cleavage activity in both concentrations; Form-III (linear form) increases and Form-I (supercoiled form) decreases. The control DNA alone does not show any apparent cleavage (Sitlani *et al.*, 1992; Kumar *et al.*, 2010).

CONCLUSION

The Zn(II) metal complex were successfully synthesized and characterized. The ligand coordinated to the metal ion through nitrogen or sulphur atom and the additional mixed ligand coordinated through oxygen atom resulting in the formation of four coordinated square planar complexes. This complex is non-electrolyte, stable diamagnetic was confirmed by spectral and physicochemical studies. Biologically active against tested microorganisms. It can also be deduced from this study that the antibacterial growth inhibition ability of the synthesized complex increased with increasing concentration.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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