**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-mercaptopaniline(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. Albicance* 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-mercaptopaniline 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 (Shafat *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 IR spectral analysis of complex, the additional stretching frequencies of aromatic C-H at 3100 cm⁻¹, aromatic C=C at 1494 cm⁻¹ and symmetric NH at 3089 cm⁻¹. The C-N & C-S stretching frequencies at 3027 cm⁻¹ and 1572 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 ν(M-N), 312 cm⁻¹ for ν(M-S) and for 4-ATP 448 cm⁻¹ for ν(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 further confirming by no anions and cations present outside the coordination sphere of the complex (Tas et al., 2010).

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 [Zn(4-ATP)(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 Ohm⁻¹ cm² mol⁻¹, indicating their non-electrolyte nature (1:0 type), which is
Scheme 1: Preparation of Zn(II) complex

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

Figure 2: IR spectrum of 4-Aminothiophenol

Figure 3: $^1$H-NMR spectrum of 4-ATP

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

Figure 5: $^{13}$C-NMR spectrum of 4-ATP

Figure 6: $^{13}$C-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 $^{13}$C-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 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 $\pi$ 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 H$_2$O$_2$ 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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