



Microwave-Assisted Synthesis, Spectral and Biopotential Characterization of Cr(III), Cu(II) and Zn(II) Complexes with 4-Aminothiophenol and Benzoate Ion

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Bioactive metal complexes of Cr(III), Cu(II), and Zn(II) were synthesized with 4-aminothiophenol and benzoate ion under microwave irradiation. The synthesized metal chelates were characterized by physical, spectral, biological studies. The geometry and magnetism of all the complexes were monitored by electronic spectra and magnetic studies. The metal complexes possess the metal-ligand ratios in the order of 1:3:3 metal:ligand1:ligand2 for Cr(III), 1:2:2 metal: ligand1:ligand2 for Cu(II) and Zn(II) complexes were predicted based on the elemental analysis and metal estimation. The synthesized metal chelates are monomeric and neutral in nature and they show well redox properties based on the results of conductivity and electrochemical studies. Octahedral geometry of Cr(III), tetragonal geometry of Cu(II) and square planar geometry of Zn(II) complexes confirmed by IR, far-IR, NMR and EPR spectral studies. The crystalline nature and lattice parameters of Zn(II) complex were predicted by using the X-ray diffraction powder method. Biopotential activities of 4-aminothiophenol compared with metal chelates and standard drug namely chloramphenicol for bacterial and fluconazole for fungal strain by Agar disc diffusion method using *Streptococci*, *Shigella* and *C. albicans*. The results of DNA cleavage of pBR322 DNA and binding nature of ct-DNA to Cr(III) complex possess the binding and cleavage nature of them by predicting the binding constant of the metal complex.

Keywords: 4-Aminothiophenol, Benzoate, Metal chelates, Agar disc diffusion, Gel electrophoresis.

INTRODUCTION

4-Aminothiophenol or *p*-mercaptoaniline is an organic building block compound used in sensors on gold electrodes [1], self-assembled layer on gold electrode [2], gold nanoparticles, electrochemical immunosensor and electroactive support for sensing. Nitrogen sulfur donor's ligand shows stereochemical, electrochemical and electronic properties, they are a very good precursor for the synthesis of Schiff base which are having applications in the field of pharmaceutical and material chemistry [3]. 4-Aminothiophenol was used to functionalized MWCNTs [4] and also enhanced the catalytic oxidation, electrochemical reduction, asymmetric catalysis, surface modified electrode, complexing ability to some toxic metals, and DNA electrochemical biosensor [5,6]. 4-Aminothiophenol Schiff base metal complexes showed enhanced biological activities like, anticancer, anti-inflammatory, anticonvulsant, etc. [7,8].

The microwave-assisted reactions nowadays are an attractive area screwcap because the reactions are completed within a few minutes instead of classical prolonged to reflux [9,10]. In the literature, there is no systematic study of 4-aminothiophenol itself as a ligand in complexes, so present study focused on the synthesis of metal complexes of Cr(III), Cu(II) and Zn(II) with 4-aminothiophenol and benzoate ion under microwave irradiation and characterized by various physico-chemical, spectral and biological studies.

EXPERIMENTAL

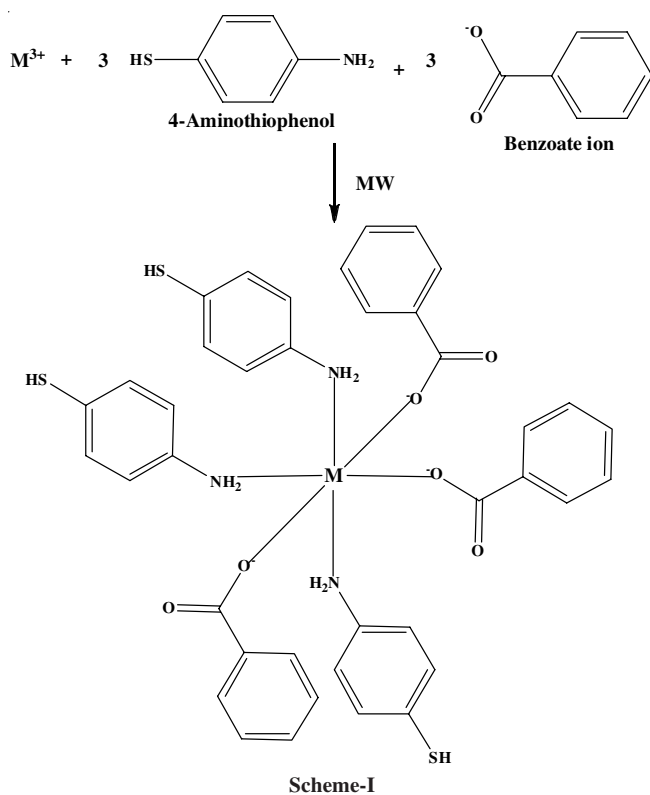
The chemicals, *viz.* chromium nitrate, copper nitrate, zinc nitrate, 4-amino-thiophenol, sodium benzoate, solvent DMSO, methanol, ethanol were purchased from a chemical company and were of AR grade.

Synthesis of metal complexes: The mononuclear Cr(III) complex was synthesized by mixing 4-aminothiophenol (0.938 g, 7.42mmol) in methanol, sodium benzoate (1.080 g, 7.49 mmol)

in ethanol to chromium nitrate (1.000 g, 2.349 mmol) in methanol. The mixture was mixed and microwave irradiated on a microwave oven (CATA-R, model). The precipitated greenish-blue colour complex was filtered, washed with ethanol, dried in desiccators, and kept in an air-tight glass container.

Cu(II) complex was prepared by the addition of 4-aminothiophenol (1.03 g, 8.27 mmol) in methanol, sodium benzoate (1.190 g, 8.27 mmol) in ethanol to copper nitrate (1.000 g, 4.13 mmol) in methanol solution. The above mixture was irradiated with a microwave oven (CATA-R, model). The precipitated blue colour complex was filtered, washed with ethanol, dried in desiccators and kept in an air-tight glass container.

The diamagnetic Zn(II) complex was synthesized by the addition of 4-aminothiophenol (0.840 g, 6.72 mmol) in methanol, sodium benzoate (0.970 g, 6.72 mmol) in ethanol to zinc nitrate (1.000 g, 3.36 mmol) in methanol solution. The mixture was irradiated on a microwave oven (CATA-R, model). The precipitated colorless complex was filtered, washed with ethanol, dried in desiccators and kept in an air-tight glass container (**Scheme-I**).



Characterization: Elemental analysis and the metal estimation of the synthesized complexes were carried out using Vario make EL-III model elemental analyzer and standard volumetric and colorimetric analysis, respectively. For conductance studies, 10^{-3} M metal complexes were mixed with CH_3CN and the conductance was measured at room temperature using Systronic Conductivity Bridge. The electrochemical property of metal complexes was measured using Versa Stat (Princeton Applied Research-Make) electrochemical analyzer in DMSO

solvent. The electronic spectra of 4-aminothiophenol and its metal complexes in the solid-state were measured using a UV-VIS-NIR spectrophotometer (Varian make, CARY-5000 model). Using Shimadzu, FT-IR, 8400 S Model IR spectrometer, IR spectra of ligand and its metal complexes were recorded. The far IR spectra of the complex were recorded in a Bruker; Germany makes 3000 Hyperion Microscope with Vertex 80 FTIR system model instruments.

Biopotential activity: The biological activities of 4-aminothiophenol and its metal complexes were carried out by Agar disc diffusion method at three different concentrations viz., 50, 100 and 150 μL using *Streptococci*, *Shigella*, *Bacillus* and *C. albicans*. The experiments were performed in triplicate. The antimicrobial potential of test compounds was determined based on the mean diameter of the zone of inhibition around the disc in millimeters. The zones of inhibition (MIC) of the tested microorganisms by the samples were measured using a Millimeter scale.

DNA-cleavage: The DNA cleavage of supercoiled pBR322 DNA to the metal complex was carried out by using a reaction mixture containing pBR322 DNA, 50 mM Tris-HCl, NaCl, 10 mM H_2O_2 mixed with 20 μL Millipore water for the final volume. Then the mixed solutions were incubated at 37 $^\circ\text{C}$ for 1 h. They checked by the agarose electrophoresis method. Prepare sufficient electrophoresis buffer (usually $1\times\text{TAE}$ or $0.5\times\text{TBE}$) to fill the electrophoresis tank.

DNA Binding: The binding of *ct*-DNA with the complex was studied using the UV absorption spectral method. The stock solution of calf thymus was prepared using mM Tris-HCl/20 mM, NaCl buffer and stored at 4 $^\circ\text{C}$. The concentration of DNA was determined from its absorption intensity at 260 nm with a molar extinction coefficient of $6600\text{ M}^{-1}\text{ cm}^{-1}$. Absorption titration experiments were made using different concentrations of DNA (1-10 μM) used 200-1100 nm, while keeping the complex concentration constant (25 μM).

RESULTS AND DISCUSSION

All the synthesized metal complexes are stable under normal conditions, the molecular formula of the complexes were derived based on the elemental analysis and metal estimation (Table-1). The conductivity of the 10^{-3} M complex solution indicates the non-electrolyte nature of the complexes because of their non-polar nature. The conductance values at 1.6, 2.5 and $1.8\ \Omega^{-1}\text{ cm}^2\text{ mol}^{-1}$, respectively for Cr(III), Cu(II) and Zn(II) complexes further confirmed by 1:0 type electrolyte with the absence of cations and anions in the outside of the complexes [11].

Electrochemical property: The redox properties of Cr(III) complex gives the well-defined redox process which gives E_{p_c} at -1.1082V and E_{p_a} at -0.5777 V. The peak to peak separation ΔE_p is at -0.5303 V and i_{p_a}/i_{p_c} is at 0.5398 A indicating the quasi-reversible one-electron transfer redox process with Cr(III)/Cr(II) (Fig. 1). The standard electrode potential E° is at -1.138 V also confirmed by the quasi-reversibility [12].

UV-visible studies: The UV-visible spectral study of the 4-ATP shows only one peak at 271 nm, which corresponds to the $\pi\text{-}\pi^*$ transition while in Cr(III) complex three transition

TABLE-1
ANALYTICAL DATA OF THE COMPLEX

Complex	Colour	Yield (%)	Conductivity (ohm ⁻¹ cm ² mol ⁻¹)	Elemental analysis (%): Calcd. (found)					
				C	H	O	N	S	M
[Cr(4-ATP) ₃ (Benz) ₃]	Greenish blue	76	32.00	54.42 (54.28)	04.18 (04.90)	11.16 (11.88)	04.88 (04.50)	11.16 (12.02)	06.05 (06.60)
[Cu(4-ATP) ₂ (Benz) ₂]	Dark green	90	50.00	51.81 (51.90)	02.33 (02.54)	10.63 (10.89)	09.30 (09.60)	10.63 (10.98)	10.55 (10.60)
[Zn(4-ATP) ₂ (Benz) ₂]	Colorless	78	36.00	51.65 (51.87)	02.31 (02.90)	10.59 (10.89)	09.27 (09.87)	10.56 (10.90)	10.82 (11.02)

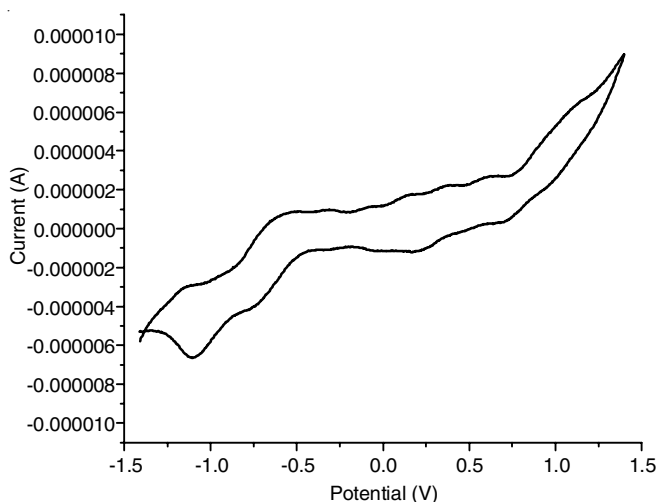


Fig. 1. Cyclic voltammogram of Cr(III) complex

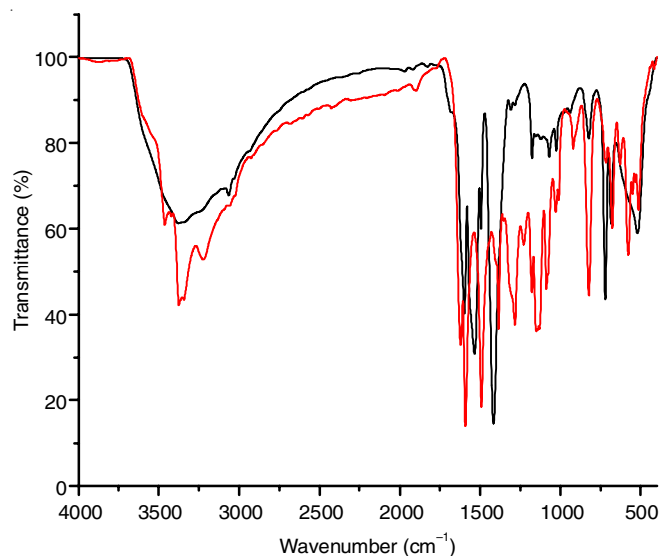


Fig. 2. IR spectra of Cr(III) complex and 4-ATP

possible at 575 nm, 356 nm and 267 nm with the Rache parameter B value at 746 corresponding to the ${}^4T_{2g} \rightarrow {}^4A_{2g} (v_1)$ $10Dq$, ${}^4T_{1g}(F) \rightarrow {}^4A_{2g} (v_2)$ $18Dq$, ${}^4T_{2g} \rightarrow {}^4A_{2g} (v_3)$ ($12Dq+15B$), respectively indicates the octahedral geometry around Cr(III) complex. Due to the presence of three unpaired electrons in the complex, the magnetic moment is at 3.89 BM and the hybridization is d^2sp^3 is further confirmed by the octahedral geometry [13].

The Cu(II) complex is a ' d^9 ' system according to Jahn Teller distortion of two geometries was possible. The UV-visible spectrum of Cu(II) complex shows three absorption bands at 620 nm, 350 nm and 265 nm, respectively indicating the three possible transitions ${}^2A_{1g} \rightarrow {}^2B_{1g}$, ${}^2B_{2g} \rightarrow {}^2B_{1g}$ and ${}^2E_g \rightarrow {}^2B_{1g}$ transitions confirmed their tetragonally distorted octahedral geometry, which is further confirmed by magnetic moment at 1.85 BM and ν_2/ν_1 value of 1.771 cm^{-1} [14]. The UV spectra of Zn(II) complex shows no significant absorption in the visible region due to its completely filled d -subshell. These complex give charge transfer spectra at 287 nm, in agreement with the filled d -orbital (d^{10}) electronic configuration of the divalent metal ions. These transitions suggest the square-planar geometry for Zn(II) complex [15].

FT-IR studies: The IR spectra of 4-ATP (Fig. 2) show a strong peak for the asymmetric N-H group of the aromatic ring at 3418 cm^{-1} and the weak peak at 3089 cm^{-1} for the symmetric N-H group of aromatic ring. In Cr(III), Cu(II) and Zn(II) complexes the asymmetric stretching frequencies are shifted to higher wave numbers at 3377 cm^{-1} , 3362 cm^{-1} , and 3366 cm^{-1} whereas the symmetric stretching frequencies are

shifted to lower wave number at 3010 cm^{-1} in Cr(III) complex but at higher wave number at 3222 cm^{-1} and 3185 cm^{-1} in Cu(II) and Zn(II) complexes, respectively indicating the coordination of 4-aminothiophenol through its amine nitrogen atom *via* monodentate mode, which is further confirmed by the shifting of aromatic C-N stretching frequency of 4-aminothiophenol at 3027 cm^{-1} to higher wave number in all the complexes at 3030 cm^{-1} and 3055 cm^{-1} . The mixed anionic ligand benzoate ion exhibits the vibrational stretching frequencies of symmetric and asymmetric C=O, aromatic C-H, and aromatic C=C in the ranges of $1600-1550 \text{ cm}^{-1}$, $1550-1450 \text{ cm}^{-1}$, $3250-3000 \text{ cm}^{-1}$, and $1500-1400 \text{ cm}^{-1}$, respectively after the formation of complexes through an oxygen atom of carboxylic groups [16]. These are move towards higher or lower vibrational frequencies in the corresponding region in all the complexes confirmed by its coordination through an oxygen atom. The metal-chelate atom linkage and the coordinating group were confirmed by the far-IR spectral data at lower frequencies of $\nu(M-N)$ and $\nu(M-O)$ coordination mode [17].

NMR studies of 4-aminothiophenol and zinc complex:

The ${}^1\text{H}$ NMR spectrum (Fig. 3) of 4-aminothiophenol in DMSO- d_6 provides the following signals. Thiol (S-H) as a singlet at δ 3.384 ppm, aromatic (*ortho*) C-H at δ 7.873-7.885 ppm (2H, $J = 8.86$ Hz) as a triplet, aromatic (*meta*) C-H at δ 7.263-7.275 ppm (2H, $J = 6.002$ Hz) as a triplet and NH_2 triplet at δ 7.100-7.120 ppm (2H, $J = 10.00$ Hz). In zinc complex spectrum (Fig. 4), these are moved to low or high field regions

90°. The unit cell volume is at 360.87 g/m³. The facts $a = b = c$; $\alpha = \gamma = 90^\circ$, $\beta = 90^\circ$ confirming both 4-aminothiophenol (4-ATP) and zinc complex are monoclinic crystal system with nanocrystalline phase [20,21].

EPR studies: EPR spectrum of copper complex in LNT shows the peaks corresponding to the g-tensor values such as g_{\parallel} at 2.4266 and g_{\perp} at 2.1393 from the available literature the copper complex exhibits the trend $g_{\parallel} > g_{\perp} > g_e$ indicating the unpaired electrons are present in the $d_{x^2-y^2}$ orbital of copper ion. From the g values, the tetragonal geometry is confirmed by the interaction parameters G at 3.0970, which is less than 4.0, which means the exchange coupling is possible over the tetragonal geometry and the metal-ligand covalent character also confirmed by its $\alpha^2(\text{Cu})$ value is found at 0.4138 is also equal to 0.5 indicating the covalent character of M-L bond of copper complex [22].

Biological activity: The biopotential activity carried out by Agar disc diffusion method using amikacin and ketoconazole as standards and DMSO as solvent control and the results of Cr(III) complex compared with 4-aminothiophenol. The results indicated that the complex shows enhanced biological activity than the ligand due to the chelate nature of the complex. The synthesized metal complexes show polar and non-polar properties together and this shows the permeation to the cell membrane and lipophilicity is the other factor that enhances the activity [23].

DNA cleavage studies: The characterization of DNA recognition by transition metal complex has been aided by the DNA cleavage chemistry associated with redox or photo-activated metal complexes. When 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 [24]. The cleavage effect upon irradiation of the plasmid pUC19 DNA in the presence of complex Cr(III) and ligand 4-aminothiophenol in two different concentrations has been tested and is shown in Fig. 8. It is clear that the complex DNA + H₂O₂ (lane 2), DNA + H₂O₂ + PS1 (lanes 3 & 4), DNA + H₂O₂ + PSL1 (lanes 7&8) does not show any significant cleavage of plasmid DNA when compared to the control. The complex is observed to cleave the DNA; it can be concluded that the Cr(III) complex may inhibit the growth of the pathogenic organism by cleaving the DNA of *E. coli*.

DNA binding studies; DNA binding constant of ct-DNA predicted from absorption of charge transfer spectral peaks at 248 nm for 4-aminothiophenol, 265 nm for Cr(III) and 274 nm for Zn(II) complexes, respectively. These spectral data show the hypochromic red shift detected in complexes, which are assigned by stacking interactions between the aromatic chromophore of 4-aminothiophenol in the complexes with DNA base pair the binding of the metal complexes either by electrostatic or groove binding, which leads to the partial opening of the double helix at phosphate moiety. From the spectrophotometric titration curve, the binding constant of 4-aminothiophenol and its metal complexes was predicted by using the equation:

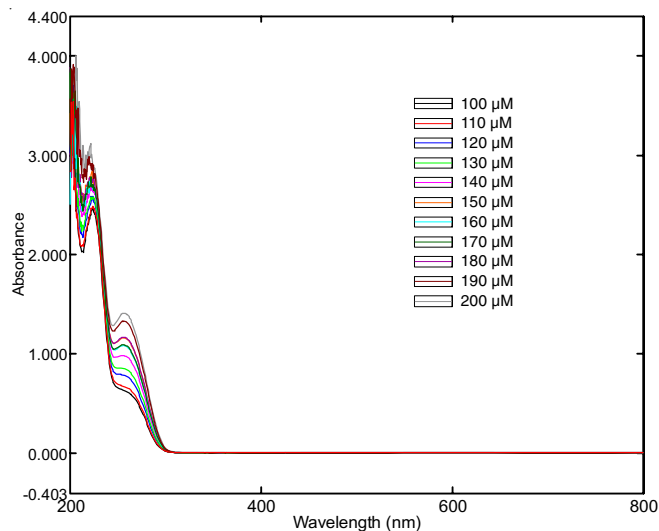


Fig. 8. DNA overlay absorption spectrum of 4-aminothiophenol

$$\frac{[\text{DNA}]}{(\epsilon_a - \epsilon_f)} = \frac{[\text{DNA}]}{(\epsilon_a - \epsilon_f)} + \frac{1}{K_b(\epsilon_b - \epsilon_f)}$$

where [DNA] is the concentration of the DNA in base pairs, ϵ_a , ϵ_b and ϵ_c corresponding to $A_{\text{obsd}}/[\text{complex}]$, the molar extinction coefficient for the free complexes and the molar extinction coefficient for the complex in fully bound form, respectively (Fig. 9).

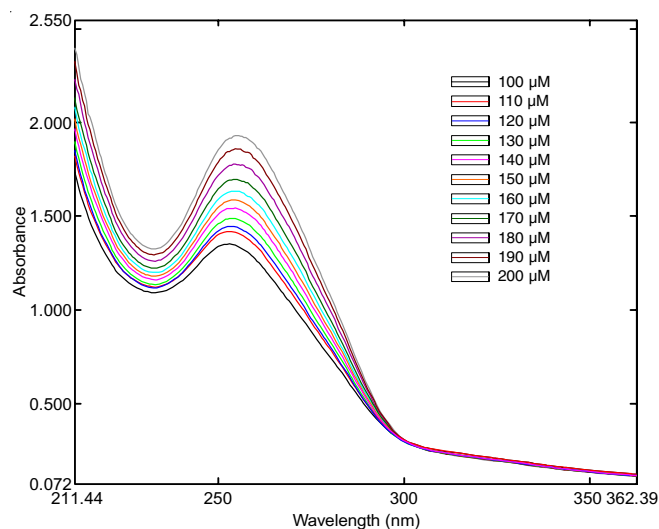


Fig. 9. DNA overlay absorption spectrum of Cr(III) complex

The binding constant K_b of 4-aminothiophenol at $4.518 \times 10^3 \text{ M}^{-1}$, for Cr(III) complex at $4.827 \times 10^3 \text{ M}^{-1}$ and for Zn(II) complex at $4.991 \times 10^3 \text{ M}^{-1}$, respectively confirm the binding nature of 4-aminothiophenol and its metal complexes to the DNA in the order of 4-aminothiophenol > Cr(III) complex and 4-aminothiophenol > Zn(II) complex [25].

Conclusion

The mononuclear Cr(III), Cu(II) and Zn(II) metal complexes were successfully synthesized and characterized based on the physico-chemical, spectral and biological activity.

All the metal complexes are neutral and non-electrolyte. Ligand 4-aminothiophenol coordinated to the metal ion through nitrogen atom of 4-aminothiophenol and oxygen atom benzoate ion resulting in the formation of six-coordinated octahedral and four coordinated square planar complexes. The complexes are biologically active against all the tested microorganisms. It can also be deduced from this study that the antibacterial growth inhibition ability of the synthesized complexes increased with increasing concentration. The DNA binding and cleavage studies also show the binding nature of the complex to DNA through intercalation.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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