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Original Research Article

Spectroscopic Characterization, DNA Binding and Antibacterial Activity of Cu(II), Co(II) Complexes of Antipyrine Derivatives

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ABSTRACT

The DNA-binding properties of the Cu(II) and Co(II) complexes have been investigated by UV spectra, fluorescence spectroscopy , thermal analyses and SEM . The results confirmed that the intercalations of the complexes Cu(II) and Co(II) with DNA are stronger than ligand. The anti-bacterial activities have also been studied, copper complexes showed good anti-bacterial activity against gram positive and gram negative bacteria.

Keyword: Complexes; UV spectra; fluorescence spectroscopy; thermal analyses; DNA and anti-bacterial activity

INTRODUCTION

The transition metal complexes of 4-aminoantipyrine and its derivatives have been extensively examined due to their wide applications in various fields like biological, analytical and therapeutically [1–4]. Further, they have been investigated due to their diverse biological properties as antifungal, antibacterial, analgesic, sedative, antipyretic and anti-inflammatory agents [5-7].

Recently, new series of Co(II), Ni(II) and Cu of ligand 3,3'-thiodipropionic acid bis (4-amino-5-ethylimino-2,3-dimethyl-1-phenyl-pyrazoline prepared and characterized on the basis of elemental analysis, IR, Mass, ^{1}H-NMR and ^{13}C-NMR spectral studies. The screening of biological activities of ligand and its complexes against the fungi and the pathogenic bacteria indicated that the complexes show the enhanced activity in comparison to free ligand [8]. Copper (II) chelates have been found to interact with biological systems and to exhibit antineoplastic activity [9–11] and antibacterial,

antifungal [12, 13], and anticancer activity [14]. Some copper(II) N,S,O/N,N donor chelators are good anticancer agents due to strong binding ability with DNA base pair[15]. DNA binding metal complexes have been extensively investigated during the past several decades because they can be used as potential anticancer durgs, DNA structural probes, DNA-dependent electron transfer probes, DNA foot printing, sequence-specfic cleaving agants and so on [16-18].

A novel Schiff base ligand (hesperet-in-2-hydroxyl benzoyl hydrazine and Cu(II), Zinc(II), Ni(II) complexes have been synthesized and characterized. The ligand and Zn(II) complex can emit fluorescence in solid state and their emission spectra are highly solvent – dependent. In addition DNA binding properties of the ligand and their metal complexes have been investigated by electronic absorption spectroscopy, fluorescence spectra, ethidium bromide displacement experiment, potassium iodide quenching experiments, salt effect and viscosity measurements. Results exhibit that all metal complexes and ligand bind to DNA via an intercalation binding mode. Cu(II), Zn(II) and Ni(II) complexes found to possess potent antioxidant activity and be better than the free ligand alone and some standard antioxidants like vitamin C and mannital [19].

In the present study, the DNA-binding behaviour of $Cu(HL)(OH)ClO_4$ and $Co(HL)(H_2O)_2Cl_2$ complexes are explored by UV spectra, emission spectroscopy, thermal analysis of ligand and Cu(II), Co(II) complexes, SEM of Co(II) complex and antibacterial activity.

MATERIALS AND METHODS

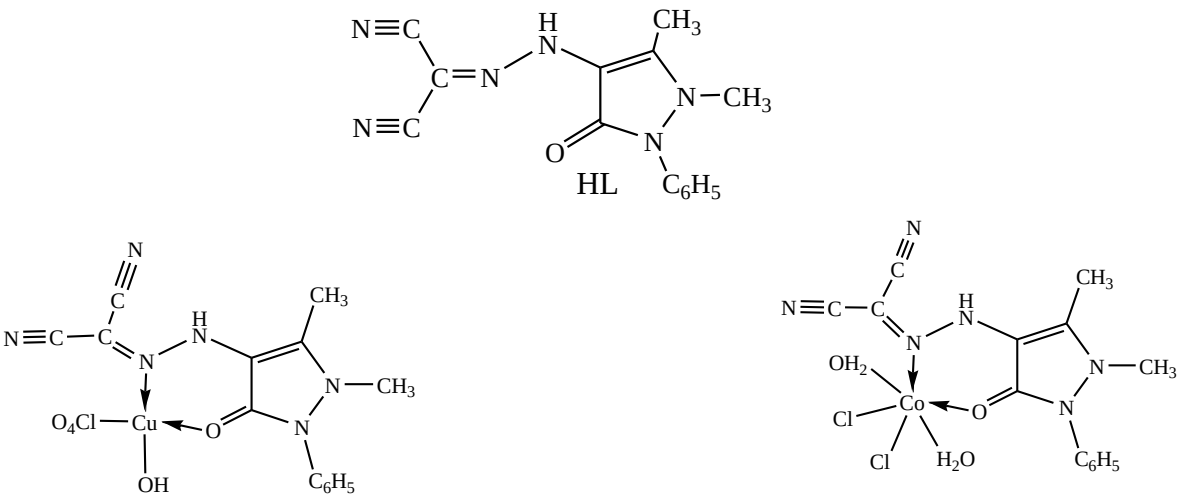
All the reagents and chemicals employed for the preparation of the ligands and their Cu(II), Co(II) complexes were of the best grade available (Merck) and used without further purification.

Physical measurements

The electronic spectra were carried out as solution (10^{-3}M) in DMSO using a Perkin- Elmer Lambada 4B Spectrophotometer (type CD6N). The fluorescence spectra were carried out using LS 45 PerkinElmer Fluorescence Spectrometer. Thermogravimetric analysis (TGA) was carried out in air using a Schimadzu (Japan) thermal analyzer at a heating rate of $10^{o}Cmin^{-1}$ in the temperature range 25-600^{o}C using platinum crucibles. Scanning electron microscopy (SEM) images were taken in Quanta FEG 250 equipment.

Preparation of the ligand and complexes

The ligand and copper (II), cobalt(II) complexes were prepared and characterized as described [20] and have the following structures (Scheme 1).



Scheme 1: Chemical structures of the ligand and their complexes

Biological test

Prepared Cu(II), Co(II) complexes was incubated with different concentrations of a purified DNA (heparine) sample. Concentrations started from 0.1 mM to 0.001 mM DNA. All Complex-DNA samples were subjected to the same conditions of measurements of complexes and ligands alone.

Antibacterial activity (in vitro) of Co(II) and Cu(II) complexes were studied against Gram positive (*Streptococcus pyogenes*) and Gram negative (*Escherichia coli)* bacteria at two concentrations (l mg/l and 5 mg/l) by using Broth Dilution Method [21] with some alterations, to investigate the inhibitory effect of the synthesized Co(II) and Cu(II) complexes on the sensitive organisms *Streptococcus pyogenes* as Gram positive bacteria and *Escherichia coli* as Gram negative bacteria. Broth medium was prepared by using Brain Heart Infusion (BHI) broth and distilled water. Compounds in measured quantities were dissolved in DMSO which has no inhibition activity to get two different concentrations (1mg/L and 5mg/L) of compounds. The bacteria were then cultured for 24 h at 37^{o}C in an incubator. One ml of the standard bacterial culture was used as inoculation in a nutrient broth. Growth was calculated at 650 nm using Spectrophotometer. The growth rate of different bacteria in absence as well as in presence of test compounds was performed for each concentration. Absorption measurements were accomplished by spectrophotometer after 24 and 48 h of incubation and used to calculate the % inhibition. Antibacterial activity studies were carried out at Genetic Engineering and Biotechnology Research Institute, Department of Environmental Biotechnology at Sadat City University, Egypt.

RESULTS AND DISCUSSION

Electronic absorption spectra, fluorescence spectra of ligand and metal complexes

The electronic spectral bands of ligand and copper (II), Co(II) complexes in solution DMSO are shown in Table (1). The ligand has an intensive band at λ_{max} equal 380nm and a less

intensive band at 365 nm after interaction of ligand with DNA. While the electronic absorption spectra of Cu (II), Co (II) complexes show maximum bands at 390nm, 410nm and 385nm, 400nm after interaction of complexes with DNA.

Complexes binding with DNA through intercalation usually results in hypochromism and bathochromism (red shift) in the absorption spectra and the extent of spectral changes are closely related to the DNA-binding affinities of $[Cu(HL)(OH)ClO_4]$and $[Co(HL)(H_2O)_2Cl_2]$ complexes. The spectral shifts for intercalation mode are usually greater than those in a groove-binding mode. In the presence of calf thymus DNA, the electronic absorption spectra for all these complexes exhibit hypochromism and bathochromism. The binding of the cobalt(II)and copper(II) complexes to the CT-DNA helix is examined by an increase of the absorption bands of complexes indicating that there are the involvement of strong interactions between complexes and the base pairs of DNA [22]. The absorption spectra of their complexes and ligand in the absence and presence of CT-DNA are shown in Figure (1). On the other hand, the interaction of the ligand and complexes **1** and **2** at zero time, after two days with DNA was studied using fluorescence spectroscopy.

Support for the above intercalative binding mode also comes from the emission spectroscopy of the ligand and Cu(II), Co(II) complexes. The emission property of the ligand (HL) and Cu(II), Co(II) complexes were recorded at room temperature (298K) in 1×10^{-6} (M) in DMSO solution given in Figure (1 and 2). In the absence of metal ions the fluorescence of the ligand at zero time and after two days are probably quenched by the occurrence of a photo induced electron transfer (PET) process due to the presence of lone pair of electrons in the ligand [23]. It is evident that the fluorescence emission intensity of the ligand decreases dramatically depending on the complex formation with the metal ions. While, the fluorescence of emission of Co(II),Cu(II) complexes with DNA are higher in intensity than their complexes alone. Binding of complexes **1** and **2** to DNA was found to increase the fluorescence intensity. The emission spectra of two complexes in the absence and presence of CT-DNA are shown in Figure (1 and 2). After binding to CT-DNA, the emission intensity of these complexes **1** and **2** increased sharply and reaches as high intensity at 425nm for Co(II) complex than Cu(II) complex at 420 nm and when all are excited at 300 nm in DMSO solution.

Table 1: Solution DMSO electronic spectra of ligand (HL) , Co(II) and Cu(II) complexes.

No.	Compound	I λ_{max}	II λ_{max}
	HL	380	365
1	$Cu(HL)(OH)ClO_4$	390	385
2	$Co(HL)Cl_2(H_2O)_2$	410	400

I= ligand and complexes at zero time

II = ligand and complexes with Heparin at zero time

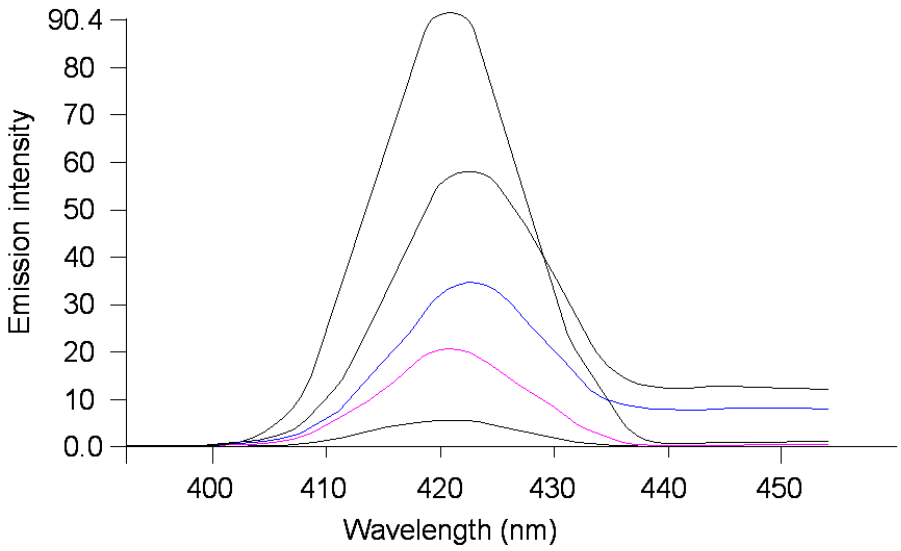


Fig.1: Fluoroscence intensity of copper complex with 5 μg/ml heparine (green); ligand after two days(red); 100 μ g/ml of ligand at zero time; copper complex after two days(purpule); 100 μg/ml of copper complex at zero time

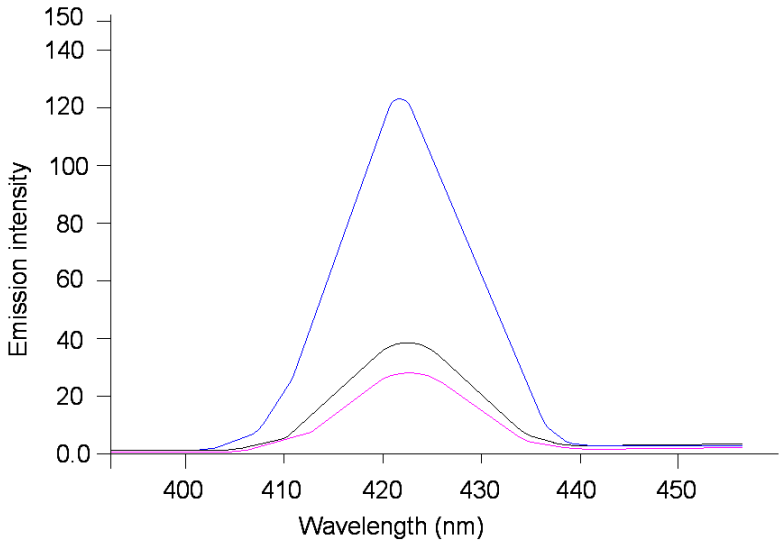


Fig.2: Fluoroscence intensity of cobalt complex with 5 μg/ml heparine (blue); cobalt complex after two days(green); 100 μg/ml of cobalt complex at zero time(pink)

Thermal analysis (DTG / TGA TGA)

Ligand

The TGA curve for ligand shows weight loss of 5.9% (theoretical 5.7%); in temperature range of 50-137◦C. The exothermic DTG peak recorded at 110, 130 reflect that loss of NH_2 of ligand. These results agree well with the composition of the ligand determined from elemental analysis and IR spectrum [20]. The TG curve indicates a thermal stability till 137◦C which coincides with the melting point of the ligand (130◦C). The TG curve also shows two

decomposition step in temperature range137-600◦C, with the total weight loss of 100.0% (found 98.8%), for the first and second steps.

Complexes

The corresponding data are summarized in Table 2. The thermal behavior of copper(II) and cobalt(II) complexes are different in behavior. The thermal properties of ligand, copper(II) and cobalt(II) complexes were investigated by TGA and DTG, under nitrogen atmosphere from 25 to 800◦C are listed in Table copper(II)complex shows weight loss of (Calc. 16.0%, 23.4%, 24.8% and Found 16.8%, 23.3%, 25.0%) in temperature range122- 257◦C, 257 -443◦C and 444-799◦C associated with three exothermic DTA peaks, two peak weaks at 218◦C, 333◦C and one strong peak at 517◦C. On other hand, the TG curve of cobalt(II) complex shows weight loss of (Calc. 11.6.0% and Found 11.4%) in temperature range 197- 423◦C participate with two weak exothermic DTA peaks at 195-243 and 397-438◦C. Thermogravimetric analysis reveals that Co(II) complex is thermally stable than Cu(II) complex.

Table 2: Thermogravimetric analysis for 4-azomalononitrile antipyrine ligand, copper (II) and cobalt (II) complexes

No.	Compound	DTG/°C	TG/°C	Mass loss% Cal. (F.)	Leaving species
	HL	110, 130 370, -540	50- 137 137 -600	5.7(5.9) 98.7(98.8)	NH_2 Decompostion
1	$Cu(HL)(OH)ClO_4$	 218 333 517	 122 -257 257 -443 444 -799	16.7(16.8) 23.4(23.3) 24.8 (25.0)	C_6H_5 0.25L, $C_2H2ClO4$
2	$Co(HL)(H_2O)_2\ Cl_2$	 195-243 397- 438	 197 - 423	 11.6(11.4)	 $2H_2O+ NH_2$

SEM of cobalt(II) complexes

SEM micrographs of the Co(II)) complex was shown in Fig. 3.

SEM picture of the Co(II) complex shows that the particles are agglomerated with controlled morphological structure and the presence of small grains in non-uniform size. The SEM images of Co(II) complex exhibits irregular shaped grains.

The average grain size (35 µm), for Co(II) complex.From SEM images, it is found that the complex is polycrystalline with micrometer sized grains.

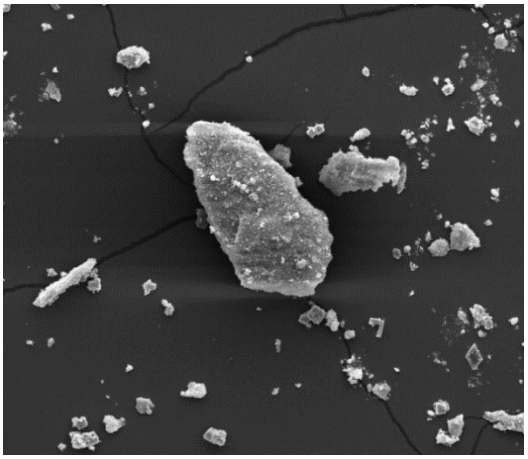


Fig. 3: SEM image of particle of cobalt(II) complex

Antibacterial activities

Complexes of Cu(II) and Co(II) were screened for anti-bacterial activity against S. pyogenes as Gram-positive bacteria and E. coli and as Gram-negative by Broth Dilution Method (Fig4 and 5). The results confirm that the copper complex is more activity than cobalt complex and ligand for S. pyogenes (5gm/L) and less activities for the other concentration (1mg/L) against same microorganisms under identical experimental conditions (Table 3).

Table 3: Inhibition (%) of ligand and Co(II) complexes aganist *S.pyogenes* and *E.Coli* bacteria.

Compound	Inhibition (%)			
	S.pyogenes		E.Coli	
	1mg/mL	5mg/mL	1mg/mL	5mg/mL
HL	35.0	77.0	41.0	79. 0
$Cu(HL)(OH)ClO_4$	93.0	98.0	45.40	94
$Co\ (HL)(H_2O)_2Cl_2$	91	95	47	92.0

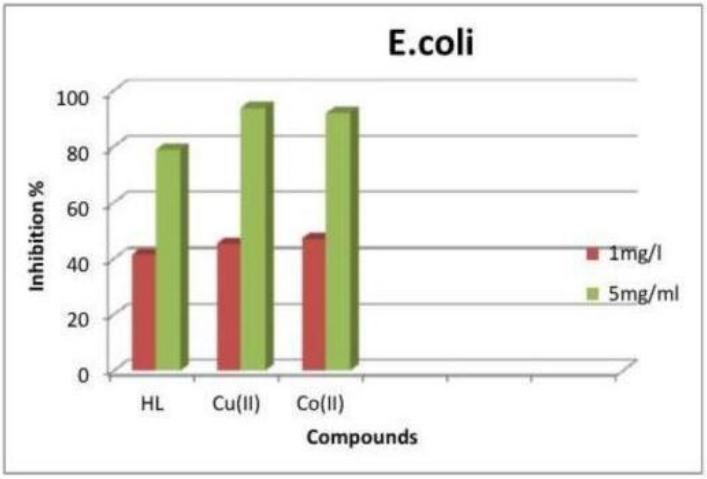


Fig. 4: In vitro antibacterial activities of ligand and their complexes against *E.Coli*

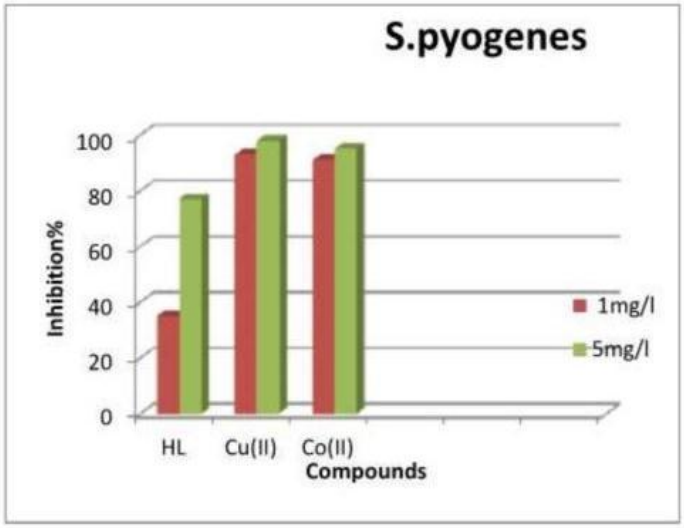


Fig. 5: In vitro antibacterial activities of ligand and their complexes against *Streptococcus pyogene*

CONCLUSION

In summary, DNA-binding properties was investigated, also Spectroscopic studies and thermal analysis supported that the complexes of Cu(II), Co(II) can intercalate into DNA base pairs via antipyrine ligand. Cobalt (II) complex is thermally stable than copper complex(II). Our results evident that the complexes **1** and **2** and ligand possess antibacterial activity and copper(II) complex is more active than cobalt(II) complex and ligand .

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