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PREPARATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF NEW AZO LIGAND AND SOME OF IT'S METAL COMPLEXES

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ABSTRACT

The present investigation describes the synthesis, characterization and antibacterial activity of the new azo ligand and its metal complexes. The new azo compound 1-[(3-Trifluromethylphenyl)azo]-2-naphthol (TFMAN) was prepared from coupling reaction of trifluromethylphenyldiazonium chloride with 2-naphthol as coupling component in a basic medium. The prepared ligand was characterized by elemental and thermal analyses as well as FT-IR, UV-Vis and GC-mass spectroscopic technique. Metal chelate complexes of this azo ligand with metal ions Co(II), Ni(II), Cu(II) and Zn(II) were prepared by reacting (TFMAN) with the metal ions in a molar ratio of (1:2) (metal:ligand). All of the coordination compounds were identified by elemental analysis, flame atomic absorption, FT-IR, UV-Vis, magnetic susceptibility and conductivity measurements, in addition to thermal analyses and mass spectra for some of the chelate complexes. Based on the obtained results the geometry for the complexes was proposed. The invitro antibacterial activity of the synthesized compounds have been tested against gram positive and gram negative bacteria. The antibacterial activity assay results showed that metal complexes posses higher antibacterial activity compared to free ligand.

KEYWORDS: Azo-naphthol, chelate complexes, FT-IR, UV-Vis and GC, mass spectrum, antibacterial activity.

INTRODUCTION

Phenyl azo naphthol derivatives are famous homocyclic organic compounds, the usual preparation of this class involves diazotization of a primary aromatic amine or its derivatives, followed by coupling with naphthol molecule. [1] Aryl azo-2-naphthol form chelate complexes with variety of metal ions via coordination through nitrogen atom of the azo group and a strong electron donating oxygen atom in the ortho position next to the azo group. [2-6] Large number of azo dyes are used in analytical chemistry due to coloured complex formation with many metal ions in solution. [7-10] On the other hand some of metal-azo compounds are used as a drug such as cadmium complex with 4-(2-pyridyl azo)-resorcinol, which is used as an anti-tumour drug [11], also some of these organic dyes are used as antibacterial drug such as prontosil. [12] This study reports the preparation, characterization and antibacterial activity of new azo-naphthol ligand and its Co(II), Ni(II), Cu(II) and Zn(II) chelate complexes.

EXPERIMENTAL

MATERIALS AND METHODS

All the used reagents and solvents had at least analytical grade. Melting points was determined by open capillary tube method and are uncorrected by using a Stuart melting point SMP10. Mass spectra of the ligand and Cu(II) complex were obtained using MSD Direct Probe Spectrophotometer. Elemental analyses (C, H, and N) were carried out usinga C.H.N.O EA- 034mc elemental analyzer. Metal contents were determined by using Schimadzu AA-6300 atomic absorption Spectrophotometer. IR spectra were recorded on a Shimadzu 8000 FTIR spectrophotometer in the (4000-400) cm–1 range using KBr discs. The UV-Vis spectra were recorded in ethanol (1.0×10–3mol L–1) for the ligand and its metal chelate complexes with a Shimadzu 1800 Spectrophotometer using 1 cm quartz cuvettes from (1100-200) nm range. Magnetic susceptibilities were determined by faraday method at roomtemperature using Balance Magnetic (MSB-MKI) apparatus, and diamagnetic corrections for the ligand were calculated using Pascal'sconstant [13]. Molar conductance of chelate complexes were determined in N,N-dimethylformamide using conductivity meter Alpha-800 at 25 °C, the concentration of the solutions was (10–3 mol. L–1). Thermal analyses by TG and DTG were obtained by using Thermo Gravimetric Analyser (TGA), A Du-Pont Thermo balance Model 951, Tg 209 F1 Libra.

Preparation of 1-[(3-Trifluromethylphenyl) azo]-2-naphthol (TFMAN)

The azo-naphthol ligand (TFMAN) with the structure depicted in figure 1 was prepared as described in the earlier methods for azo dyes. In short details, 1.61 g of 3-trifluromethyl-aniline was dissolved in 30 mL of water and 2.5 mL of concentrated hydrochloric acid to prepare 10 mmol solution. This solution was diazotized below 5 °C with 13 mL of aqueous sodium nitrite (1.0 mol. L–1). A drop of the reaction mixture was tested from time to time with starch-iodide paper until nitrous acid persists in the solution for 10 min. The resulting diazonium chloride solution was added drop-wise with continuous stirring to 2-naphthol solution of 10 mmol concentration (1.44 g in 150 ml alkaline ethanol). The reaction mixture was stirred for 1 hr at 0-5 °C, and left in the refrigerator for overnight. The mixture was acidified with 0.1 N hydrochloric acid until the pH becomes 6. The soiled product was filtered off, washed with 500 mL cold water, air dried and recrystellized twice from hot ethanol and then dried in the oven at 80 °C for 6 hrs.

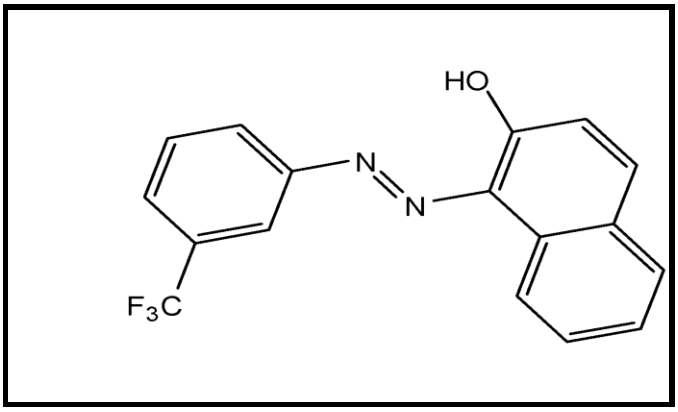

Figure1: Structure of the ligand (TFMAN)

Preparation of metal complexes

The metal complexes (1 mmol) were prepared by dissolving 0.632 g of the ligand in 50 ml hot ethanol and added drop by drop with stirring to a stoiciometric amounts of (1:2) (metal:ligand) molar ratio of Co(II), Ni(II), Cu(II), and Zn(II) chloride salts, dissolved in 10 mL hot distilled water. The resulting mixture solution was stirred under reflux for atleast 30 min. The colored precipitates formed at room temperature were filtered off, washed with 5 mL hot (1:1) ethanol-water to remove any traces of the unreacted starting materials and dried at 80 °C for overnight.

Analysis of ligand and complexes

Melting points was determined by open capillary tube method and are uncorrected by using a Stuart melting point SMP10. Mass spectra of the ligand and Cu(II) complex were obtained using MSD Direct Probe Spectrophotometer. Elemental analyses (C, H, and N) were carried out usinga C.H.N.O EA- 034mc elemental analyzer. Metal contents were determined by using Schimadzu AA-6300 atomic absorption Spectrophotometer. IR spectra were recorded on a Shimadzu 8000 FTIR spectrophotometer in the (4000-400) cm^{-1} range using KBr discs.

The UV-Vis spectra were recorded in ethanol (1.0×10^{-3}mol L^{-1}) for the ligand and its metal chelate complexes with a Shimadzu 1800 Spectrophotometer using 1 cm quartz cuvettes from (1100-200) nm range. Magnetic susceptibilities were determined by faraday method at roomtemperature using Balance Magnetic (MSB-MKI) apparatus, and diamagnetic corrections for the ligand were calculated using Pascal'sconstant. [13] Molar conductance chelate complexes were determined in N,N-dimethylformamide using conductivity meter Alpha-800 at 25 °C, the concentration of the solutions was (10^{-3} mol. L^{-1}). Thermal analyses by TG and DTG were obtained by using Thermo Gravimetric Analyser (TGA), A Du-Pont Thermo balance Model 951 ,Tg 209 F1 Libra.

Antibacterial activity

The *invitro* antibacterial effects of the synthesized ligand and all the complexes were investigated against several pathogenic bacteria by the well diffusion method [14] using the Mueller-Hinton Agar (MHA) as medium. The screened bacteria represented both Gram-positive bacteria (*Staphylococcus aureus, Streptococcus viridans,* and *Enterococcus fecalius*) and Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia* and *Pseudomonas flouresence*). All the bacteria used in this study were supplied from the microbiology laboratory, Department of Biology, Kufa University, Iraq. Twenty ml of sterilized (MHA) media was poured into each sterile petri dish. In the solidified medium suitably spaced wells of 6 mm in diameter were made using sterile cork borer. One ml of the spore suspension of each bacteria was spread all over the surface of cold solid media placed in the petri dish. The test compounds was dissolved in DMF to give (10^{-2} mol.L^{-1}) solutions. The wells were filled with 0.1 ml of the prepared compounds by using a micropipette. All the plates were incubated at 37 ^{0}C for overnight. The inhibition zones formed after 24 hrs by the compounds against the particular bacterial strain were measured and the antibacterial activity of the synthetic compounds were determined. The mean value obtained for three individual replicates were used to calculate the zone of growth inhibition for each compound.

RESULTS AND DISCUSSION

The reaction of aryl azo-naphthol ligand (TFMAN) with the metal ions Co(II), Ni(II), Cu(II), and Zn(II) gave different colored semi crystals, depending on the nature of metal ion. The metal complexes were air-stable, insoluble in water, but soluble in some common organic solvents. Decomposition occured with conc. nitric acid, and the resultant solution was used after suitable dilution for metal analysis. The elemental analysis data of the ligand and it's chelate complexes are given in table1. They were consistent with the calculated results from the empirical formula of each compound. It has been found that the theoretical values are in good agreement with the found values.

Table 1: The analytical and the physical data of the ligands and their complexes.

Comp. (colour)	*Molecular formula*	*M.PºC*	*Analysis Found. (Calc)*				*Ω-1cm2mol-1*	*μeff.(B.M.)*	**UV–visible (MeOH); λmax nm**	
			C%	*H%*	*N%*	*M% (Metal)*				
TFMAN (L) orange	**$[C_{17}H_{11}N_2OF_3]$**	193-195	64.32 (64.56)	3.41 (3.51)	8.63 8.86	-	**-**	**-**	**472 385**	**n→π***
									296 231	**π→π***
$[Co(L)_2(H_2O)_2]$ H_2O Dark brown	$[Co(C_{17}H_{10}N_2OF_3)_2(H_2O)_2]$ $.xH_2O$	209-215	54.63 (54.87)	3.29 (3.49)	7.43 (7.53)	7.82 (7.92)	15.74	3.78	465 392 253	C.T.
$[Ni(L)_2(H_2O)_2]$ H_2O Light brown	$[Ni(C_{17}H_{10}N_2OF_3)_2(H_2O)_2]$ $.xH_2O$	165-166	54.57 (54.89)	3.31 (3.49)	7.29 (7.53)	7.65 (7.88)	11.11	2.71	468 422	C.T.
$[Cu(L)_2(H_2O)_2]$ H_2O brown	$[Cu(C_{17}H_{10}N_2OF_3)_2(H_2O)_2]$ $.xH_2O$	157	54.38 (54.61)	3.32 (3.48)	7.22 (7.49)	8.17 (8.49)	11.26	1.69	475 417	C.T.
									300 258 226	
$[Zn(L)_2(H_2O)_2]_2$ H_2O Red orange	$[Zn\ C_{17}H_{10}N_2OF_3)_2(H_2O)_2]$ $.xH_2O$	159	52.88 (53.19)	3.52 (3.65)	7.15 (7.30)	8.34 (8.51)	11.90	Dia	471 410	C.T
									299 227	

The reaction of aryl azo-naphthol ligand (TFMAN) with the metal ions Co(II), Ni(II), Cu(II), and Zn(II) gave different colored semi crystals, depending on the nature of metal ion. The metal complexes were air-stable, insoluble in water, but soluble in some common organic solvents. Decomposition occured with conc. nitric acid, and the resultant solution was used after suitable dilution for metal analysis. The elemental analysis data of the ligand and it's chelate complexes are given in table1. They were consistent with the calculated results from the empirical formula of each compound. It has been found that the theoretical values are in good agreement with the found values**.**

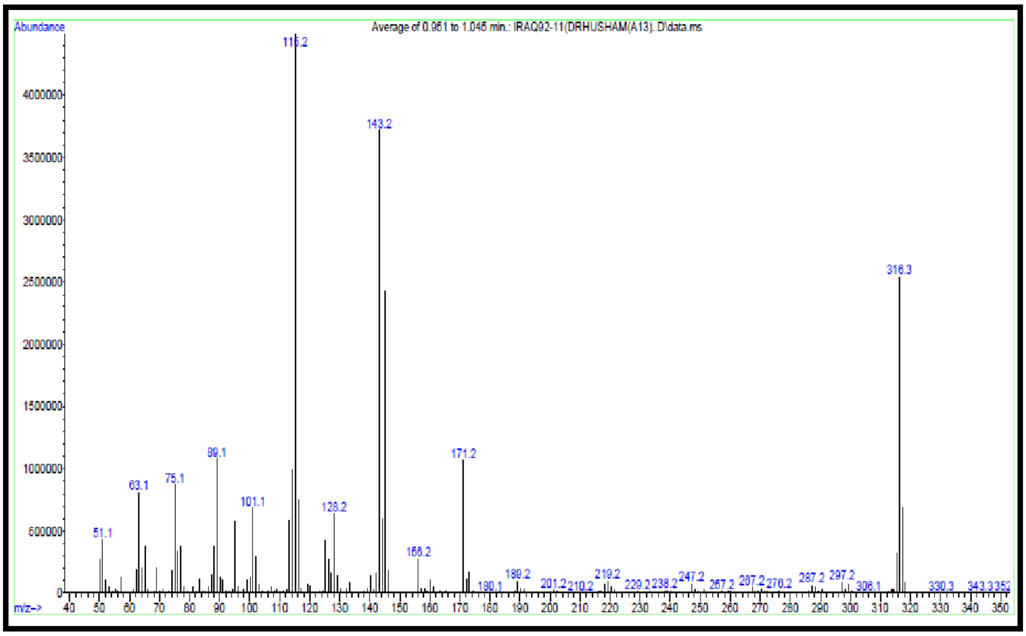

Figure 2: Mass spectra of (TFMAN) ligand

The mass spectrum was obtained for the ligand and its complexes. The mass spectrum of (TFMAN) ligand was presented in figure 2. The most important peaks were observed in the EI mass spectrum data of both ligand and its Cu(II) complex. The base peak of ligand at m/z = 316 is due to the $[C_{17}H_{11}N_2OF_3]^+$ ion. The different competitive fragmentation pathways of ligand give the peaks at different mass numbers at 77, 115, 127, 143, 145 and 171. The molecular ion peaks of Cu(II) complex was observed respectively at 316, 432 and 460 respectively. They were in good agreement with the molecular weight of the proposed structures and pathways of fragment for two compounds. [15] The coordination site of the ligand moieties was clearly evident from the IR spectral data as presented in figure 3 and 4.

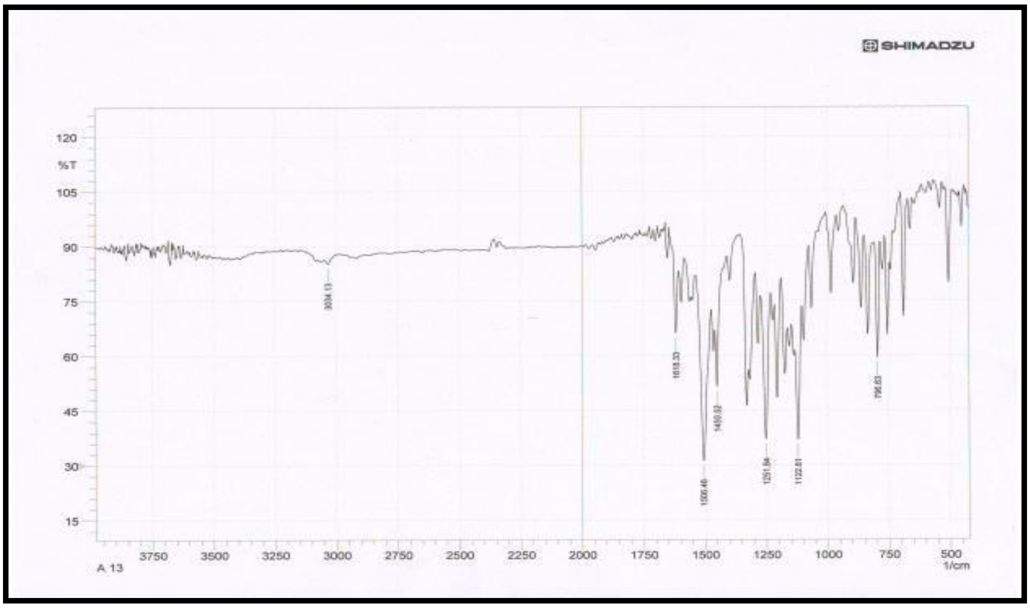

Figure 3: IR spectroum of (TFMAN) ligand

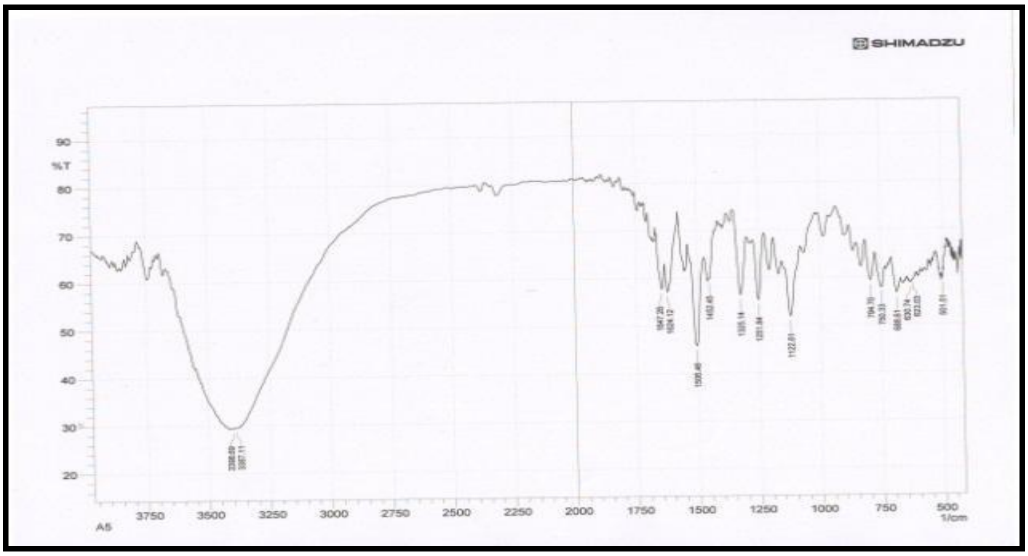

Figure 4: IR spectroum of the [Co(TFMAN)$_2$(H$_2$O)$_2$].xH$_2$O chelate complex

The most important IR absorption bands corresponding to the ligand and the complexes are presented in table 2. The observed band in the range 1450 cm^{-1} is due to ν (N=N) group of the ligand. [16] These bands are shifted to higher region during the complex formation indicating its participation in coordination with the metal ions. The sharp band in the range 748-760 cm-1 and 1506 cm-1 are due to ν (C–F) and ν (C=C) respectively. The medium intense band was observed in the range 1622 cm^{-1}. The appearance of these bands support the involvement of –OH, and N=N groups in the formation of new azo ligand complexes. Some bands in the range of 501-504 and 448-453 cm^{-1} which are not present in the free ligands assigned to ν (M–O) and ν (M–N) vibrations. [17,18] In all complexes the appearance of a broad band around 3566-3398 cm^{-1} in the spectra of the complexes suggests the presence of water molecules.

Table 2: IR frequencies (in cm–1) of the azo ligands and their complexes

Compound	ν(O-H) H2O hyd-	ν (N=N)	ν(M-O)	ν(M-N)	(C=C)	(C-F)
HL1(AZO)	3424	1450	---	---	1506	750
[Co(L1)2].2H2O	3398	1452	501	448	1506	750
[Ni(L1)2].2H2O	3385	1450	502	453	1510	748
[Cu(L1)2].4H2O	3387	1453	504	453	1506	751
[Zn(L1)2].2H2O	3566	1452	502	449	1506	760

The electronic spectra of the ligands and all the complexes were recorded in ethanol at room temperature. The UV–Vis spectral data of the ligands and their complexes are given in table 1. The aromatic peaks of (L_1) ligand at (385 and 472), (231 and 296) nm, which may be ascribed to, n→π* and π →π*, intra ligand charge transfer ransitions respectively, due to presence of conjugation in the ligand molecule. The electronic spectra of the azo ligand complexes show two intense transition peaks < 400 nm which are assigned to ligand centered transitions, along with intense peak at longer wavelength (226 – 468) nm that can be referred to the combination of MLCT [dπ(M2+)→π*(L)] transitions where π*(L). [19,20]

The molar conductance values of the coordination compound of mentioned metal ions under investigation were determined using (1.0×10^{-3} mol. L^{-1}) DMF solvent, as showed in table 1 are in the range of (11- 15) S. cm^{2}.mol^{-1}. These values suggest the presence of a non-electrolyte. [21] The magnetic moments of Co(II), Ni(II), Cu(II), and Zn(II) complexes were measured at room temperature and the obtained values are listed in table 1.

The Co(II) complex exhibited the magnetic moment value of 3.78 BM corresponding to three unpaired electrons which suggests an octahedral geometry. [22] The magnetic moment observed for Ni(II) complex lies in the range of 2.8-3.5 BM showing a value of 2.81 BM, which is consistent with the octahedral stereochemistry of the complex. [23] The Cu(II) complex exhibited a magnetic moment value of 1.69 BM, slightly lower than the spin-only value of 1.73 BM expected for one unpaired electron suggesting the possibility of an octahedral geometry. [24] In view of industrial application of azo dyes, it has been considered worthwhile to study thermal behavior of these compounds. We studied the thermal behaviour of ligands by thermogravimetry technique. TGA curves are represented in figure 5, which show loss of H_2O turn bact to fraction –OH of 2- naphthaol and binding with hydrogen atom to form water molecule. The observed temperature range was 50-198 ^{0}C. The weight loss was found to be 3.45. As shown, the calculated values for weight loss are in good agreement with observed TG values that confirm the structural integrity of the complexes. [25]

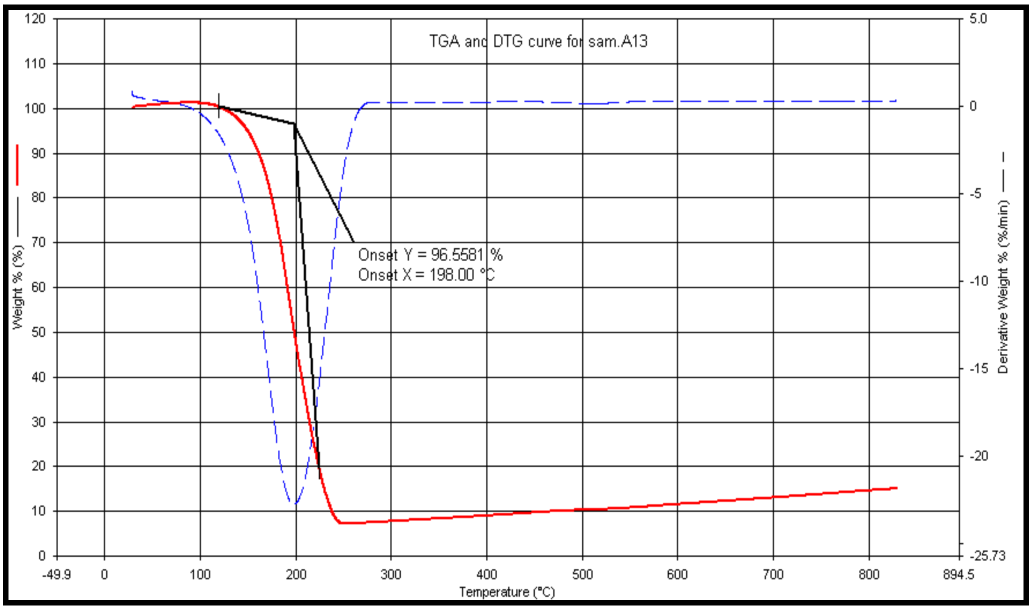

Figure 5: TGA of (TFMAN) ligand

The antibacterial effect of the ligand (TFMAN) and their complexes against both gram positive and gram negative bacteria was measured and tabulated in table 3. All of tested compounds exhibited remarkable antibacterial activity against tested bacteria. The statistical presentation is showed in figure 6. A comparative study of the antibacterial activity values of the ligand and their complexes indicate that the metal complexes exhibited higher antibacterial activity compared to the free ligand. This is probably due to the greater lipophilic nature of the metal complexes. The increased activity of the metal chelates can be explained on the basis of Overtone's concept of cell permeability. The lipid membrane that surrounds the cell favours the passage of lipid soluble materials due to which liposolubility is an important factor which controls the antibacterial activity. [26] On chelation, the orbital of each metal ion is made so as to overlap with the ligand orbital. Increased activity enhances the lipophilicity of complexes due to delocalization of π – electrons in the chelate ring. In some cases increased lipophilicity leads to breakdown of the permeability barrier of the cell. [27] The mechanism of action of antibacterial drug can be discussed under four headings, (1) inhibition of cell wall, (2) inhibition of cell membrane function(3), Inhibition of protein synthesis and (4) inhibition of nucleic acid thensis[28].

Table 3: Antibacterial activity data (zone of inhibition in mm) of ligand and metal complexes

Compound/ Bacteria	S. aureus	S. viridans	E. fecalius	E. coli	K. pneumonia	P. flouresence
TFMAN	+++	+++	++	+++	++	+++
$[Co(TFMAN)_2(H_2O)_2].xH_2O$	+++	+++	++	+++	+++	+++
$[Ni(TFMAN)_2(H_2O)_2].xH_2O$	+++	+++	+++	+++	+++	+++
$[Cu(TFMAN)_2(H_2O)_2].xH_2O$	+++	+++	+++	+++	+++	+++
$[Zn(TFMAN)_2(H_2O)_2].xH_2O$	+++	+++	+++	+++	+++	+++

Note: Highly active = +++ Inhibition zone => 12 mm. Moderately active = ++ Inhibition zone = 9-12 mm

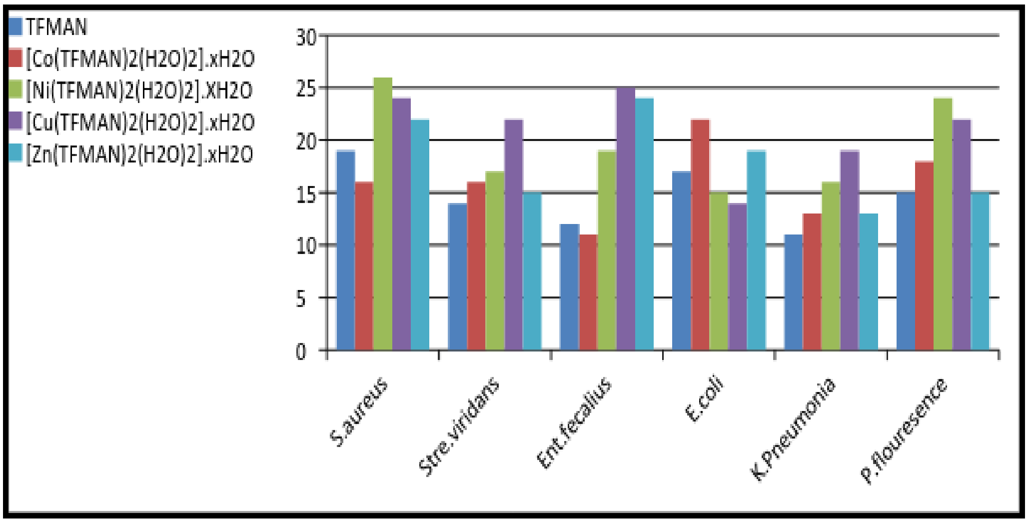

Figure 6: Statistical representation for antibacterial activity of ligand (TFMAN) and itsmetal complexes

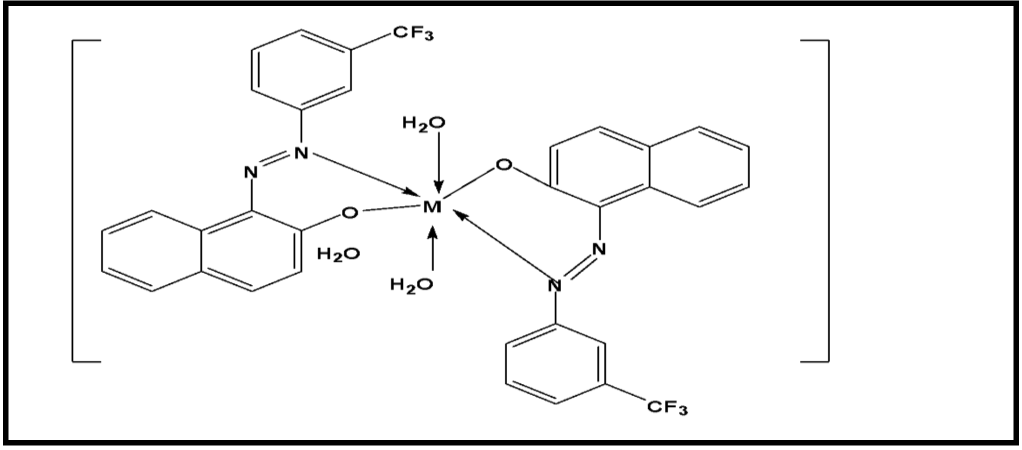

Figure 7: The suggested geometry of Co(II), Ni(II), Cu(II) and Zn(II), Chelate complexes M= Co, Ni, Cu, or Zn

CONCLUSION

The study describes the synthesis, characterization and antibacterial activity of the new azo ligand and its metal complexes. From the elemental analysis, molar conductance measurements, infrared data, electronic absorption and electron paramagnetic resonance we propose the chemical formulae and geometry for the metal(II)-azonaphthol complexes as presented in figure 7. A comparative study of the antibacterial activity of the ligand and their complexes indicate that the metal complexes exhibited higher antibacterial activity compared to the free ligand.

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