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Synthesis, Characterization and Antimicrobial Studies Metal Complexes of Co (II) and Cu (II) with Schiff Base Derived from 2,6-Diaminopyridine and 2-Hydroxybenzaldehyde

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Abstract

Complexes of Co (II), Cu (II) with Schiff base derived from condensation of 2,6-diaminopyridine and 2-hydroxybenzaldehyde were successfully synthesized and characterized on the basis of FT-IR, Molar conductance, Magnetic susceptibility, Melting point/Decomposition temperature and Uv-Visible spectroscopy. The Schiff base and the metal complexes are found to be insoluble in water and some solvents, but very much soluble in DMSO and DMF as revealed by their solubility test. The Schiff base melts at 220 °C while the complexes decomposed within range of 260 - 270 °C. Magnetic moment values of the complexes (2.04 – 3.97 BM) indicates that all the complexes are paramagnetic. The low molar conductance values ranges 40 - 46.1 $ohm^{-1}cm^2mol^{-1}$ indicate the non-electrolytic nature of the complexes. A peak at 1607 cm^{-1} from FT-IR analysis is assigned to azomethine nitrogen of the Schiff base which shifted to 1603 and 1611 cm^{-1} in Co (II) and Cu (II) Complexes respectively. Uv-Visible spectroscopy also revealed the coordination of Schiff base to the metal ions due to the appearance of new bands in the complexes that are absent in the ligand. Metal to ligand ratio were found to be 1:1 as suggested by Job's method of continuous variation. Furthermore, the antimicrobial activity of the Schiff base as well as its metal complexes were studied, the results suggest that the ligand and the metal complexes are active against both fungal and bacterial isolates at high and low concentration. However, the ligand shows a higher potent activity than the metal complexes.

Key Words: 2,6-diaminopyridine, 2-Hydroxybenzaldehyde, Characterization, Ligand, Metal Complex and Schiff base.

Introduction

Report of the rising emergence of drug resisting microbes and bacterial strains has become a global concern. The growing resistance of microbes to antibacterial and antifungal agents necessitates the development of new compounds to effectively target pathogenic microorganisms. It's found that bactericidal and fungicidal properties of drugs increase immensely by the incorporation of metal based systems into antibacterial molecules [1].

Schiff Bases and their metal complexes have become a subject of intense interest subsequent to their structural diversity and possession of potential bacterial activities such as anti-cancer, antitumor,

anti-bacterial, antifungal, antioxidant, anti-inflammatory and corrosion inhibition [2]. The compound (Schiff base) is named after a famous scientist called Hugo Schiff in the year 1864 and it's synthesized from the condensation reaction of primary amines and active carbonyl compounds. The compound has a general formula of RR'C=NR" (Where R, R' and R" is an organic side chain and can be alkyl, cyclohexyl, hydroxyalkyl, hydroxyaryl and others) [3]. A Schiff base can be referred to as nitrogen equivalent of a ketone or aldehyde, were the carbonyl group (C=O) is replaced by an Imine group (C=N). Therefore, an Imine contains (C=N) bond instead of a (C=O) bond. Imines demonstrate a very powerful and strong electron-donating capability, which makes it suitable in forming chemically stable metal complexes with transition metal ions [4].

The effective conjugation of aromatic aldehydes or ketones makes Schiff Base form with them more stable compared to Schiff Base form with aliphatic aldehydes or ketones, which tends to be easily polymerized [5]. Schiff base formation is a reversible reaction, therefore; acid or base catalysis and heating are commonly employed to drive the reaction to completion [6]. Schiff bases are versatile ligands capable of accommodating various metal ions through different coordination modes, enabling the synthesis of both homo and hetero metallic complexes with diverse stereochemistry. This flexibility is utilized in modeling active sites within biological systems. Numerous studies have explored Schiff bases and their complexes, revealing intriguing properties such as reversible oxygen binding, catalytic activity in olefin hydrogenation, photochromism, and complexation with toxic metals. Several biologically relevant Schiff bases exhibit antimicrobial, antibacterial, antifungal, anti-inflammatory, anticonvulsant, antitumor, and anti-HIV activities [7].

Schiff bases derived from Salicylaldehydes are found to be active against various pathogenic organisms [8]. Also, a transition metal complexes of Cu (II), Ni(II), Co(II), Fe(II) and Zn(II) with Schiff base ligand derived from derivatives of pyridine exhibit broad range of biological activities [9]. In these studies, the synthesis, characterization and antimicrobial studies of Schiff base derived from condensation of 2,6-diaminopyridine and 2-hydroxybenzaldehyde as well as its cobalt (II) and Cupper (II) metal complexes is reported.

Materials and Methods

Apparatus

All the glass wares used were washed with detergent, rinsed with distilled water and dried in an oven at 110 °C before used. Magnetic susceptibility measurement was conducted using Sherwood scientific mark 1 CB1 8HD magnetic susceptibility balance. Weighing of the reagents was done using an electric balance. Melting and decomposition temperature determination of the Schiff base and metal complexes were done using Stuart melting point apparatus (SMP10). Agilent

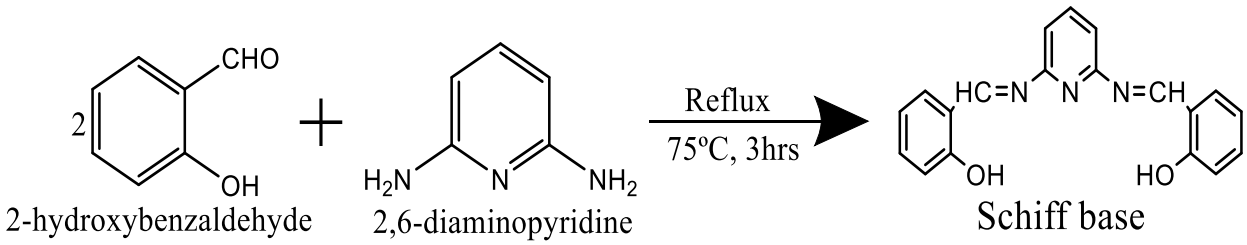
technologist FT-IR Carry 630 spectrophotometer, manufactured in Australia, was used to carry out all the infrared spectral analysis at the range of 4000 - 400 cm^{-1}. Molar conductivity measurements of all the complexes were recorded using Hana Instrument conductivity meter HI 9813-6, Romania. Metal to ligand Ratio Determination and Uv/Visible spectroscopic measurements (at the range of 200 - 700 nm) was conducted using Parking Elmer Uv/Visible spectrophotometer, manufactured in U.S.A, heating and refluxing was done using hot plate/stirring machine. Antimicrobial studies were carried out at Department of Microbiology, Bayero University Kano.

Reagents

All the reagents and solvents used in this work were analytically grade and were used without further purification. 2,6-diaminopyridine were obtained from sigma Aldrich while 2-Hydroxybenzaldehyde, metal (II) Chloride salts of Cobalt and Cupper as well as the solvents used: methanol, ethanol, pet-ether, diethyl ether, DMSO, CCl_4, DMF, distilled water, acetone, chloroform, n-hexane were all obtained from CDH (Central Drug House), Honeywell and Loba Chemie P.V.T Limited.

Synthesis of Schiff base Ligand

The Schiff base ligand was synthesized from the condensation of ethanolic solution of 2-hydoxybenzaldehyde (0.03 mol, 3.66g) with 2,6-diaminopyridine (0.015 mol, 1.6368g) in 20ml of ethanol (Scheme 1.0) in 1:2 molar ratios. The mixture was then refluxed for 3hrs at 75 ºC. The reaction mixture was concentrated and cooled at room temperature to obtain the product which was further washed with pet-ether and dried in a desiccator containing $CaCl_2$ [10].

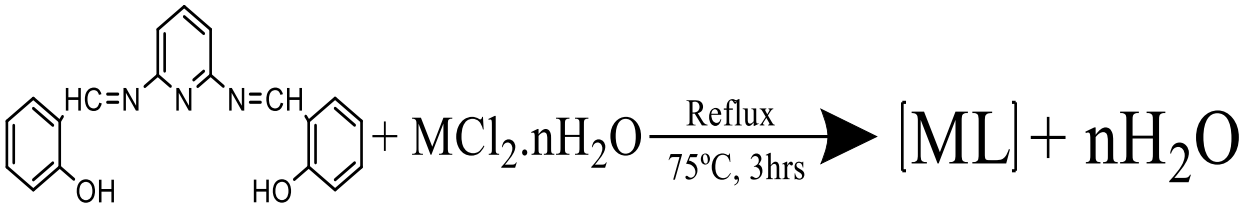


Scheme 1.0: Synthesis of Schiff base

Synthesis of Metal (II) complexes

The complexes were synthesized in a 1:1 Metal-Ligand molar ratio (Scheme 1.1). A solution of Schiff base (0.003 mol, 0.952g) in 25ml of hot ethanol/water mixture was added to a stirred solution of 0.003 mol of the metal chlorides (0.511g of $CuCl_2$, and 0.7137g of $CoCl_2.6H_20$) in 25ml of hot ethanol. The mixture was refluxed for 3hrs at 75

ºC. The reaction mixture was then concentrated & cooled at room temperature, washed with pet-ether and dried in a desiccator containing [10].



Scheme 1.1: Synthesis of Metal Complexes

Determination of Metal to Ligand Ratio

The coordination number of the ligand to the metal ion was determined using Job's method of continuous variation by the modification of procedure from Ibrahim *et al*, (2017). In this method, 3mmol aqueous solutions of both the ligand and the metal salt were prepared. Various ratios of ligand to metal salt (in milliliters) were mixed to maintain a total volume of 16ml in each mixture. The specific ratios used were 1:15, 3:13, 5:11, 7:9, 9:7, 11:5, 13:3, and 15:1. For each mixture, the mole fraction of the ligand was calculated. The solutions of the metal salts were scanned as blanks to determine the wavelength of maximum absorption (λmax) for the metal ion. The absorption measurements were then taken at these specific λmax values [11]. By plotting the absorbance values against the mole fraction of the ligand, the coordination number, which is the number of ligand molecules coordinating to the metal ion, was determined using expression below;

$$\acute{\mathbf{n}} = \frac{Xi}{1-Xi}$$

Where; ń = number of coordinated ligands

X_i = Ligand mole fraction at maximum absorbance.

Antimicrobial Test

The Antimicrobial activity of the Schiff base and metal(II) complex were assayed with bacterial (*Staph. aureus, Escherichia coli* and *Strept. pyogens*) and fungal (*Aspegilus niger* and *Aspergilus flavus*) isolates using disc diffusion method. 1mg of Schiff base and each metal complexes were weighed and dissolved completely in 1ml of dimethyl sulfoxide (DMSO) to prepare a 1000 µg/ml stock solution. Serial dilutions were performed to obtain working concentrations of 500 µg/ml and 250 µg/ml.

The sensitivity testing medium was prepared by weighing and dissolving each 48.5 g of Muller Hinton Agar (for antibacterial test) and 39 g of Potato Dextrose Agar (for antifungal test)

completely in 1000 ml of distilled water, which were sterilized by autoclaving at 121 ºC for 15 minutes, and then poured into sterile Petri dishes and allowed to solidify at 45 ºC. The test organisms were standardized by adjusting their turbidity to match the 0.5 McFarland standard using normal saline. The standardized organisms were spread on the prepared agar plates, and different concentrations (1000 µg/ml, 500 µg/ml, 250 µg/ml) of the Schiff base and metal complexes were applied. The plates were incubated for 24 hours at 37 °C for bacteria and 48 hrs at 28 - 30 °C for fungi. The diameter of zones of inhibition of the Schiff base and the corresponding metal complexes were recorded to evaluate the antimicrobial activity [12].

Results and Discussion

The Schiff base was prepared from the condensation of 2,6-diaminopyridine and 2-hydroxybenzaldehyde (Scheme 1). The Schiff base was found to be Orange in color with percentage yield 86.97 % as shown in Table 1. The high percentage yield indicates the feasibility of the reaction; the Schiff base melting point is found to be 220 ºC (Table 1). The high melting point value of the Schiff base indicates its thermal stability and purity [13].

The metal complexes are formed from the reaction between metal (II) salt and the Schiff base (Scheme 1.1), and they are found to be of black and light green color, the color impacted by the complexes is largely due to intra d-d electronic transition. The complexes have an appreciable percentage yield in the range of 81.7 - 86 % indicating the feasibility of the process of their synthesis and with a decomposition temperature within the range of 260 - 270 ºC (Table 1). The increased values of decomposition temperature indicate that the complexes are more air and thermally stable than the Schiff base due to chelation. The result corresponds with findings obtained by [14].

Table 1: Physical properties of Schiff base and Metal (II) Complexes

Compound	Color	Melting Point (ºC)	Decomposition Temperature (ºC)	Percentage Yield (%)
L	Orange	220	-	86.97
$[CoL(H_2O)_2]$	Black	-	270	86.00
$[CuL(H_2O)_2].H_2O$	Light green	-	260	81.70

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

Solubility Test carried out on the metal complexes and the Schiff base shown in Table 2 indicate that all the compounds are insoluble in water, non-polar solvents and slightly soluble in Ethanol and

methanol but readily soluble in polar aprotic solvent that have high dielectric constant (DMF, DMSO and Acetone). This indicates that the Schiff base and metal complexes are moderately polar compounds. The result is in agreement with result obtained by [15].

The results of the molar conductivity measurements taken in DMSO are shown in Table 3. The low molar conductivity values (40 - 46.1 × 10^{-6} ohm^{-1}cm^{2}mol^{-1}) suggest that all the metal (II) complexes are non-electrolytic in nature since a value expected for 1:1 electrolyte is suggested to be in the range of 50 – 70 ohm^{-1}cm^{2}mol^{-1} [16].

Table 2: Solubility Test Result of Schiff Base and Metal (II) Complexes

Compound	Dist. Water	EtOH	MeOH	DMF	DMSO	Acetone	CCl_4	n-Hex	D.E.E
L	IS	SS	SS	S	S	S	IS	IS	IS
[CoL(H$_2$O)$_2$]	IS	SS	SS	S	S	S	IS	IS	IS
[CuL(H$_2$O)$_2$].H$_2$O	IS	SS	SS	S	S	S	IS	IS	IS

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

S = Soluble, **SS** = Slightly Soluble **IS** = Insoluble **DMF** = Dimethyl Formamide **DMSO** = Dimethyl Sulfoxide, **D.E.E** = Diethyl ether, **n-Hex** = n-Hexane

Table 3: Molar Conductivity Measurement of the complexes in DMSO

Complex	Concentration (Moldm^{-3})	Specific Conductance (ohm^{-1}cm^{-1}) × 10^6	Molar Conductance (ohm^{-1}cm^{2}mol^{-1})
[CoL(H$_2$O)$_2$]	1 × 10^{-3}	46.1	46.1
[CuL(H$_2$O)$_2$].H$_2$O	1 × 10^{-3}	40.0	40.0

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

Table 4 shows the magnetic susceptibility measurement value for magnetic moment $\mu_{eff,}$ in Bohr Magneton (BM), the analysis is carried out at room temperature using magnetic susceptibility balance. Co (II) complex has a magnetic moment of 3.97 BM which is found to be closer to 3.87 BM reported by [17] which are consistent with octahedral geometry. The Cu (II) complexes has a magnetic moment of 2.04, the result falls within a range of (1.68 - 2.11) BM as reported by [18] for distorted octahedral geometry.

Table 4: Magnetic Susceptibility Values of the Metal (II) Complexes

Complex	Xg(g/mol)	Xm(g/mol)	μ_{eff} (BM)	Magnetic Property	Number of Unpaired Electrons
$[CoL(H_2O)_2]$	1.54×10^{-5}	6.615×10^{-3}	3.97	Paramagnetic	3
$[CuL(H_2O)_2].H_2O$	4.16×10^{-6}	1.740×10^{-3}	2.04	Paramagnetic	1

L = Ligand (Schiff base) = $C_{19}H_{15}N_3$

The selected vibrational frequencies for the Schiff base and its metal complexes are presented in Table 5. The IR spectral data of the ligand observed at 1607 cm^{-1} (Fig. 1) is a characteristic of azomethine nitrogen present in the ligand [19]. A band at 3365 cm^{-1} (Fig. 1) is a characteristics of intramolecular hydrogen bond v(-OH) stretching vibration, the value is closer to 3400 cm^{-1} reported by [20], for phenolic hydroxyl group v(-OH). On complexation the azomethine peak were shifted to 1603 and 1611 cm^{-1} (Fig. 3a and 4a) region in Co (II) and Cu (II) complexes indicating the coordination of the azomethine nitrogen of the ligand to the metal ions [21]. The formation of metal to ligand bonds was indicated from the appearances of new peaks in the spectra of the complexes that are absent in the ligand spectra, therefore, a peak observed at 666 cm^{-1} (Fig. 3b and 4b) in the IR spectra of Co (II) and Cu (II) is assigned to v(M-N) while the peak displayed at 589 and 592 cm^{-1} (Fig. 3b and 4b) is assigned to v(M-O) cm^{-1} for Co(II) and Cu(II) complex [22]. Furthermore, the complexes exhibit an IR bands at (3309 - 3327) cm^{-1} (Fig. 3a and 4a) which suggest the presence of water of crystallization in the metal (II) complexes, the values are closer to 3310 – 3447 cm^{-1} reported by [23].

Table 5: FT-IR Spectral Data of Schiff Base and Metal (II) Complexes

Compound	v(C=N) cm^{-1}	v(OH) cm^{-1}	v(M-N) cm^{-1}	v(M-O) cm^{-1}	v(H_2O) cm^{-1}
L	1607	3365	-	-	-
$[CoL(H_2O)_2]$	1603	3327	666	592	752
$[CuL(H_2O)_2].H_2O$	1611	3309	666	589	755

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

The results obtained in Table 6 shows the percentage of water of crystallization in the complexes. The appearance of a peak at around 3309 - 3327 cm^{-1} (Fig. 3a and 4a) in the IR spectra of the complexes confirm the presence of water of crystallization in all the complexes. However, in the lower region of the IR spectrum of complexes a broad band obtained in the range of 752 – 755 cm^{-1} (Fig. 3b and 4b) is assigned to v(-OH) of coordinated water molecules in the

complexes. The values were closer to 733 – 742 cm^{-1} reported by [24]. From the analysis, Cu (II) complex is found to be dihydrated while Co (II) complex is trihydrated, therefore, two of the water molecule are coordinated to the metal ions while others are water of crystallization, similar assertions are made by [25].

Table 6: Determination of Percentage of Water of Crystallization in the Complexes

Compound	Initial weight (g)	Weight loss (g)	% of Water of crystallization
$[CoL(H_2O)_2]$	0.105	0.0143	14.23
$[CuL(H_2O)_2].H_2O$	0.104	0.0090	8.650

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

The Uv-Visible specral data of the Schiff base and metal complexes shown in Table 7 were taken at the region between 200 – 700 nm using DMSO as solvent. The appearance of two absorption bands in the Schiff base ligand at the region of (258 and 302) nm is a characteristics of electronic transition assigned to π-π* and n-π* transition for phenyl ring and azomethine chromophore -CH=N, the values contrast with 276 and 313 nm assigned to π-π* and n-π* [19]. Subsequently these two electronic transition were also present in all the metal (II) complexes but they're either blue or red shifted to various intensities π-π* (244 and 262) nm and n-π* (308 and 310) nm for Co (II) and Cu (II) complexes respectively, these shifting of electronic transition in the spectra of the metal complexes confirm the coordination of the ligand to the metal ion.

Table 7: Uv-Visible Spectral Data of Metal (II) Complexes and Schiff Base

	Wavelength (λ) of Electronic Transition (nm)	
Compounds	**π - π***	**n - π***
L	258	302
$[CoL(H_2O)_2]$	244	308
$[CuL(H_2O)_2].H_2O$	262	310

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

The estimation of ligand to metal reaction ratio was determined using Job method of continuous variation. From the job's plot presented in Figures 5 and 6 and the working displayed in Table 8, the number of ligand coordinated to metal (**ń**) was determined to be approximately 1, indicating 1:1 metal to ligand ratio in all the metal complexes.

Table 8: Metal-Ligand Ratio Determination Using Job's Method

Complex	**X_i**	**1 - X_i**	**ń = $\frac{Xi}{1-Xi}$**
$[CoL(H_2O)_2]$	0.461	0.520	0.920
$[CuL(H_2O)_2].H_2O$	0.498	0.501	0.996

L = Ligand = $C_{19}H_{15}N_3O_2$

The metal complexes and the Schiff base were tasted for antibacterial activity against three bacterial isolates; *Staph. aureus, Escherichia coli* and *Strept. pyogens* microorganisms using ampiclox (100µg/ml) as control and for antifungal activity against two Fungicidal isolates; *Aspegilus niger* and *Aspergilus flavus* microorganisms using Nystatin (100µg/ml) as Control. The Antibacterial and Antifungal activities obtained for the Schiff base and its Metal (II) complexes are listed in Table 9a and 9b. All the compounds investigated exhibit a significant activity at low and high concentration against both the bacterial and fungal Isolates. The results obtained indicate that the Schiff base demonstrate a better activity than the Metal (II) complexes against (*Staph. aureus, E. coli and Aspergilus niger)* while the Metal (II) complex demonstrate better activity against (*Aspergilus flavus and Strept. pyogens).* The results obtained correspond to the findings obtained by [26].

Table 9a: Antibacterial activity of Schiff base and Metal (II) Complex

Compound	Antibacterial Inhibition Zone (mm)								
	Staph. Aureus			*E.Coli*			*Strept. Pyogens*		
Conc.(µg/ml)	**1000**	**500**	**250**	**1000**	**500**	**250**	**1000**	**500**	**250**
L	14	12	10	18	15	13	14	12	09
$[CoL(H_2O)_2]$	14	10	08	16	14	12	15	13	11
$[CuL(H_2O)_2].H_2O$	13	11	08	14	12	10	16	14	12
Ampiclox (100µg/ml)	31			33			28		

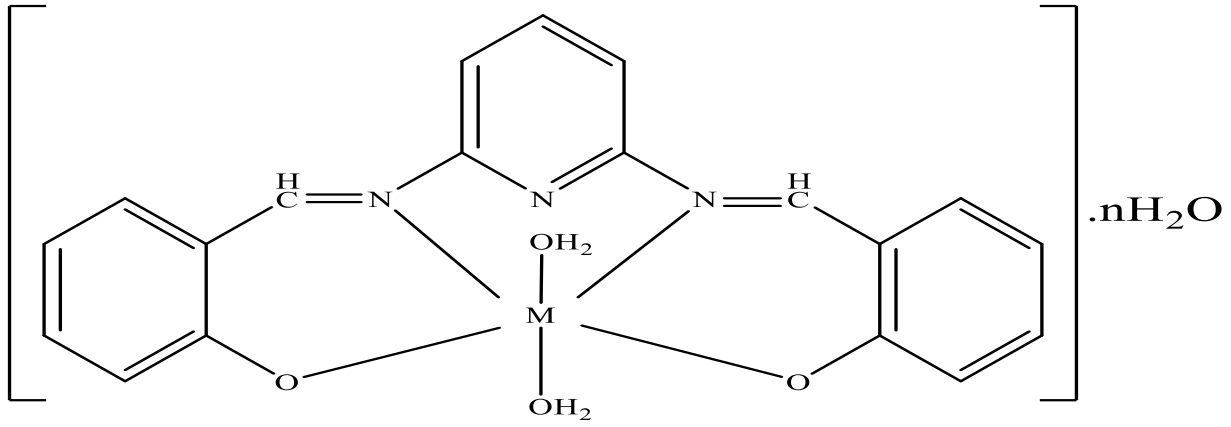
L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$

Conc. = Concentration

Table 9b: Antifungal activity of Schiff base and Metal (II) Complex

Compound	Antifungal Inhibition Zone (mm)					
	Aspergilus Niger			*Aspergilus Flavus*		
	1000µg/ml	**500µg/ml**	**250µg/ml**	**1000µg/ml**	**500µg/ml**	**250µg/ml**
L	15	13	11	14	12	10
$[CoL(H_2O)_2]$	13	11	09	17	15	12
$[CuL(H_2O)_2].H_2O$	13	10	09	16	14	11
Nystatin (100µg/ml)		25			30	

L = Ligand (Schiff base) = $C_{19}H_{15}N_3O_2$



where:
M = Co and Cu
n = 0, 1, 2, 3, 4.....

Figure 1: Proposed structure of the complexes

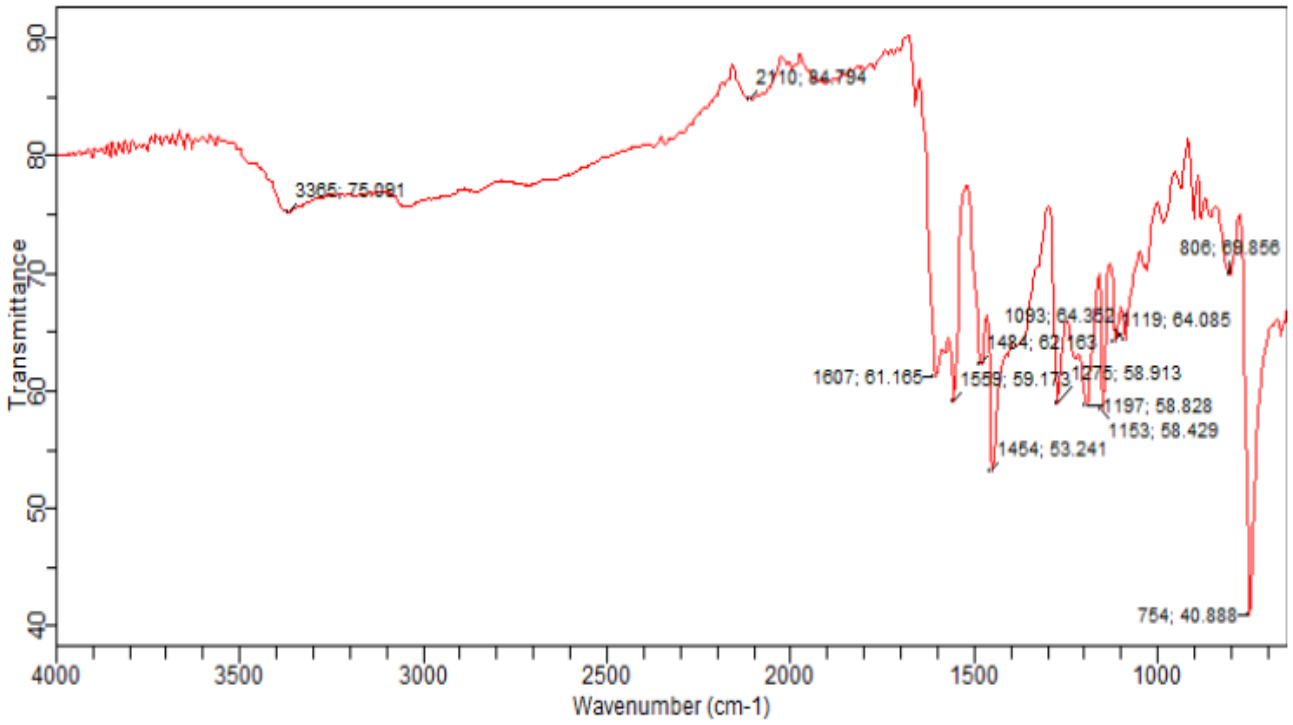


Figure 2: FT-IR Spectra of Schiff base

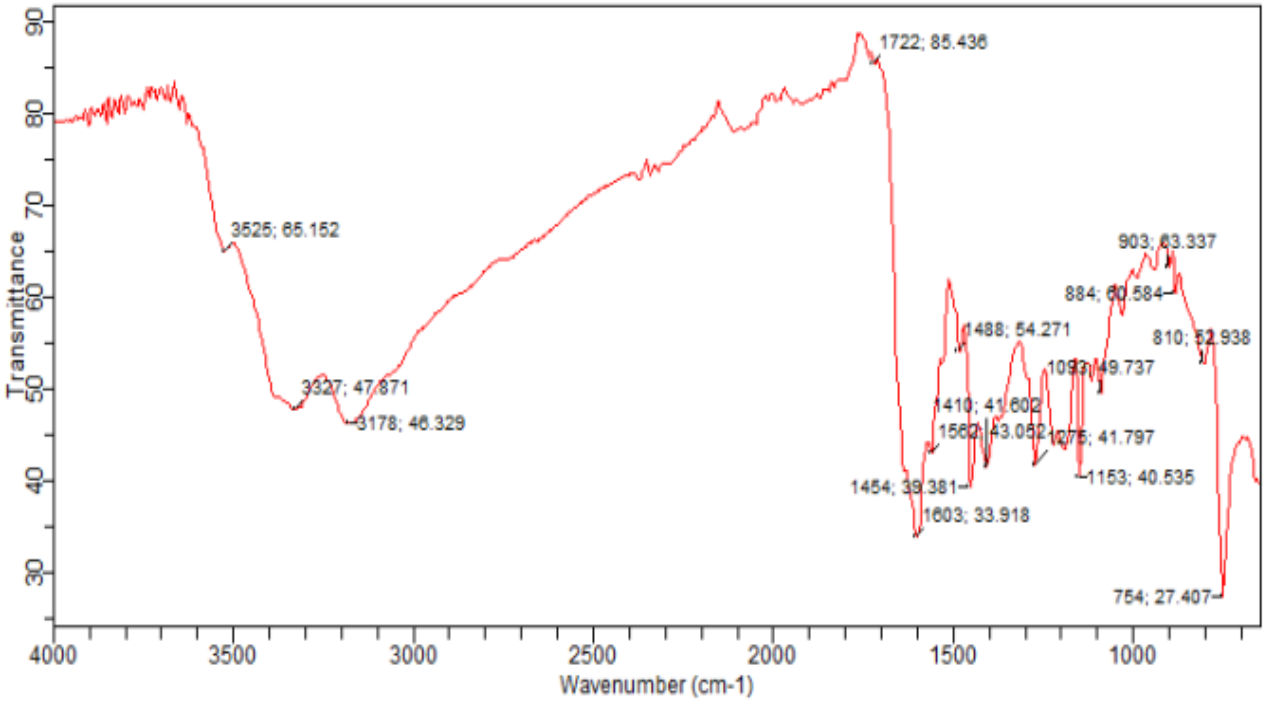


Figure 3a: FT-IR Spectra of Synthesis Co(II) Complex

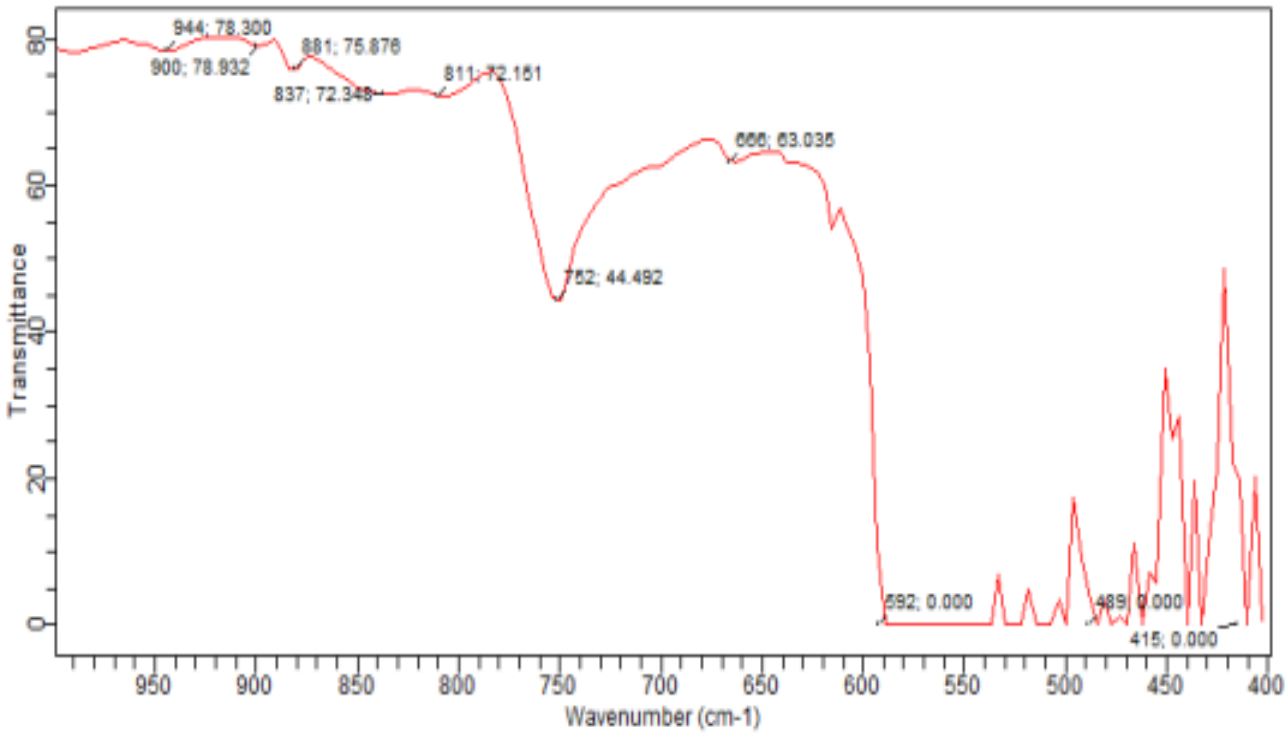


Figure 3b: FT-IR Spectra of Synthesis Co(II) Complex (lower region)

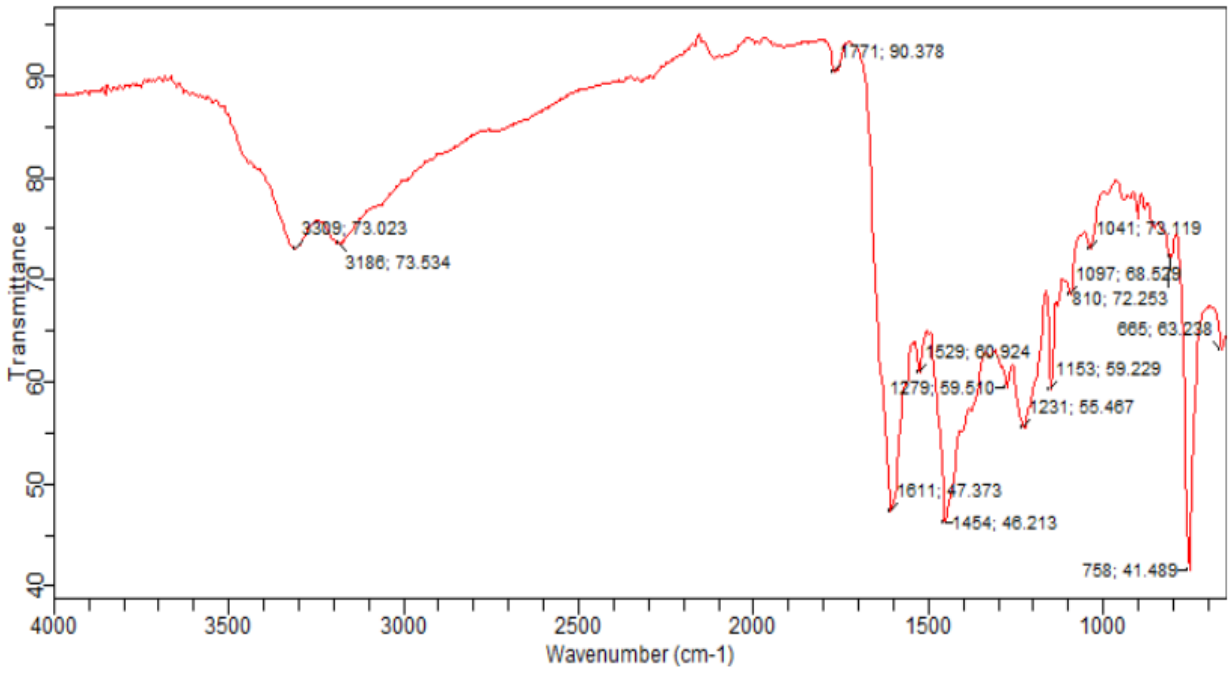


Figure 4a: FT-IR Spectra of Cu(II) Complex

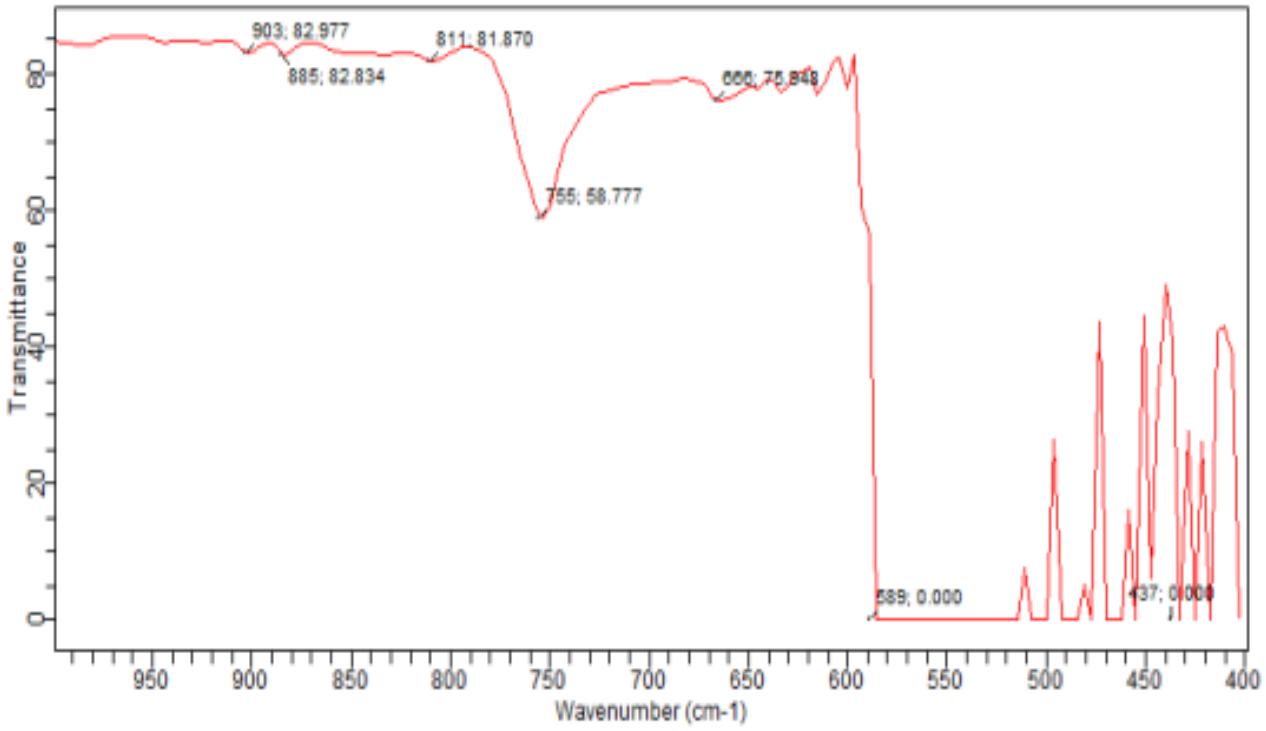


Figure 4b: FT-IR Spectra of Cu(II) Complex (lower region)

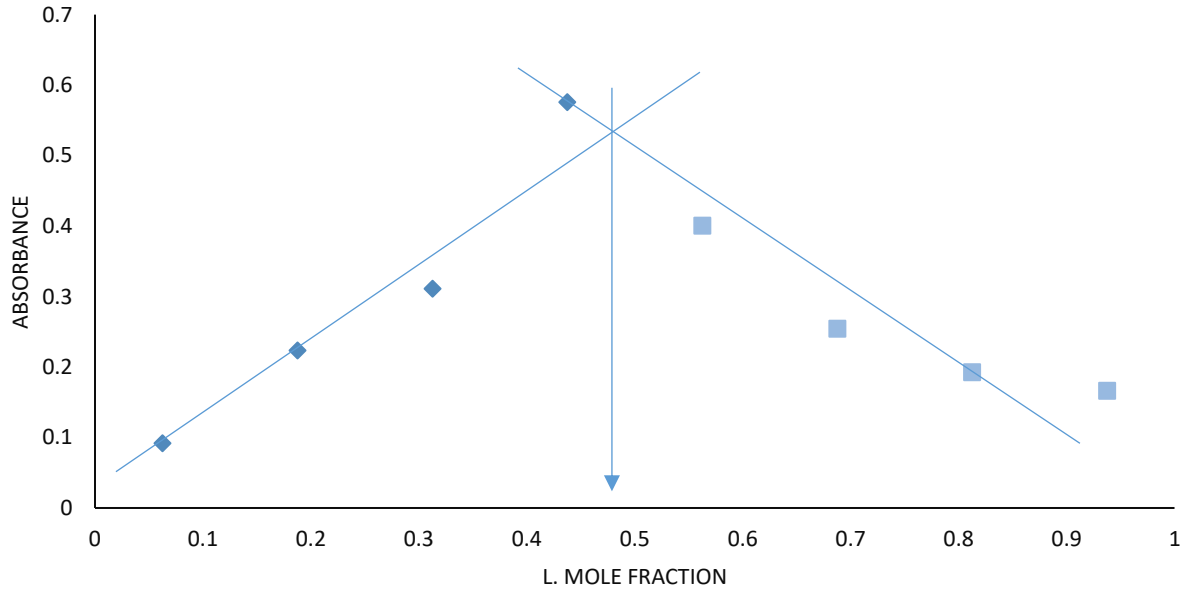


Figure 5: Plot of absorbance against ligand mole fraction at 674nm for Co(II) complex

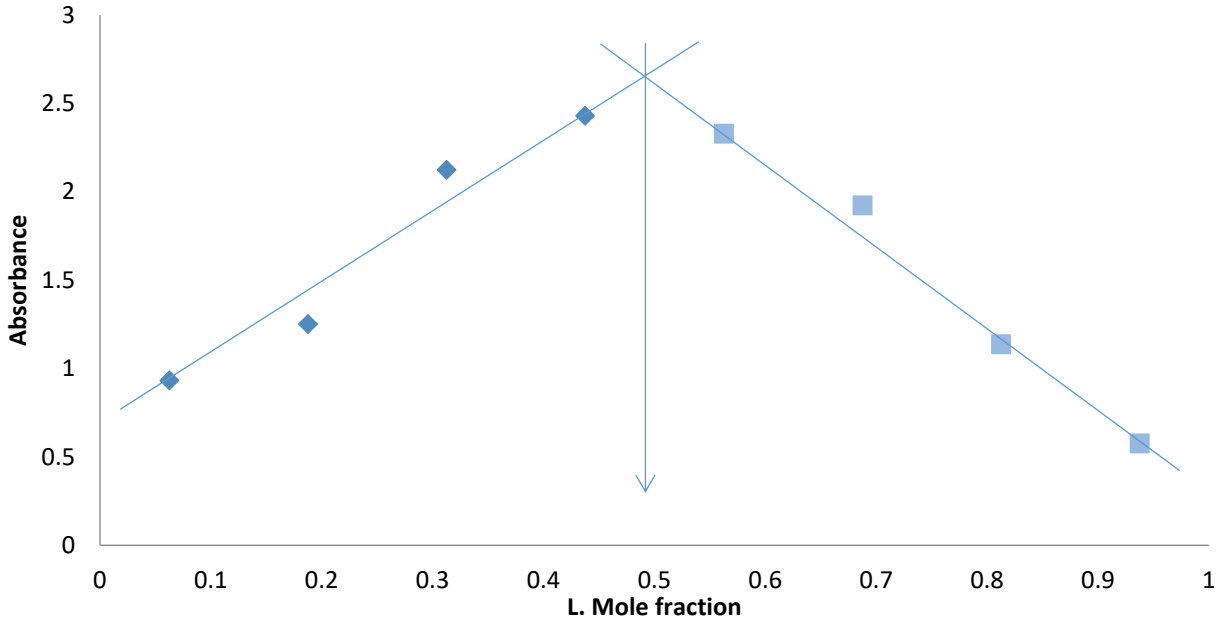


Figure 6: Plot of absorbance against ligand mole fraction at 437nm for Cu(II) complex

Conclusion

Metal (II) complexes of Co(II) and Cu(II) with Schiff base ligand derived from condensation of 2,6-diaminopyridine and 2-hydroxybenzaldehyde was successfully synthesized. The Schiff base and metal complexes was found to be colored, air stable and moderately polar compounds. The Schiff base behave as a weak field tetradentate ligand and the spectral studies indicate that the ligand coordinate to the metal ions through its azomethine nitrogen and oxygen of the phenyl group forming [ML] complexes. The metal complexes formed are neutral, high spin octahedral and paramagnetic complexes. Also, the Schiff base ligand and the metal complexes exhibit some level of Antibacterial and Antifungal activity at low and high concentrations after testing against some bacterial and fungal isolates.

Acknowledgements

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