

SYNTHESIS, SPECTRAL CHARACTERIZATION, AND BIOLOGICAL ACTIVITIES OF COBALT(II) COMPLEXES OF SCHIFF BASES DERIVED FROM O-VANILLIN AND P-VANILLIN WITH 3-AMINOPYRIDINE

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Highlights

- Two 3-aminopyridine derived Schiff bases were synthesized.
- Six cobalt (II) complexes of the Schiff bases were synthesized.
- The Schiff bases and the cobalt (II) complexes were characterized.
- Antimicrobial and brine shrimps activities of the complexes and the Schiff bases were examined

ABSTRACT

Two biologically active Schiff base GL1 and GL2 were synthesized in equimolar reaction of 3-aminopyridine with *o*-vanillin and *p*-vanillin. The synthesized Schiff bases were reacted with cobalt acetate, cobalt chloride or cobalt chloride with trimethylamine by using a molar ratio of Schiff base: cobalt salt as 2:1. The characterization of Schiff bases and cobalt complexes was done by UV-visible in methanol, diffused reflectance, FTIR (mid and far), mass spectrometry studies while ¹H NMR and ¹³C NMR were used to further characterize the Schiff bases. The FTIR results confirmed the bidentate binding of the Schiff bases with cobalt center after the formation of the complexes. The UV-visible spectra and diffused reflectance spectra data obtained were used to further investigate the electronic structure as well as the properties of Schiff bases and their cobalt complexes. On the basis of the spectral studies, distorted tetrahedral geometry has been assigned for the Co(II) complexes. The antimicrobial activities of the two Schiff bases GL1, GL2 and their cobalt(II) complexes were tested against laboratory isolated *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and one fungus strain, *Aspergillus niger*. The antimicrobial evaluation demonstrates significant activity of the Schiff bases and the cobalt complexes against the four known human pathogens. The brine shrimps lethality study confirmed that the compounds are concentration and geometry dependent. The studies showed the complexes were higher in biological activity than the corresponding Schiff bases.

Keywords: 3-aminopyridine Schiff bases, cobalt(II), antimicrobial activity, brine shrimps test

INTRODUCTION

The widespread and continuous existence of antibiotic-resistant microorganism is a major worldwide problem. This has given rise to several innovative researches for antibiotic drug discovery. The quest to produce antibiotic drugs to combat drug resisting microbes has led to the considerable attention received by Schiff bases and their transition metal complexes. They are known to have simple synthetic methods and have effective biological importance as a result of their diverse pharmacological properties (Saxena, 1987; Williams, 1972; Furst, 1963). A number of *in vivo* studies surveyed, have indicated that biologically active compounds usually contain heteroatoms and become more bacteriostatic and carcinostatic upon chelation (Jain, Jain and Pitre, 2002; Hania, 2009).

The heteroatoms- nitrogen, oxygen and sulphur- are significant in the coordination of metals. They serve at the active sites of numerous metallo-biomolecules. Cobalt, copper and nickel are known to have great affinity for coordination to the heteroatoms because of small size and high nuclear charge. For instance, tetradentate Schiff bases with a N₂O₂ donor atom set are well-known to form complexes with various metal ions (Atkins *et al.*, 1985; Boucher & Coe, 1976; Yuan Ruo, Chai Ya-Qin, Liu Dong, Gao De, Li Jun-Zhong, 1993). Many complexes with N₂O₂ donor atom, have proven antitumor, antibacterial, antifungal, antiviral properties (Cui, Dong, Li, Li & Chen, 2012; Gurumoorthy, Mahendiran, Parbhu, Arulvasu & Rahiman, 2014; Bhattacharyya, Sen, Harms & Chattopadhyay, 2015). The synthesis of Schiff bases and their metal complexes has led to

increase in the development of the field of bioinorganic and coordination chemistry. It is long known that many of these complexes serve as models for biologically important species. Consequently, many studies have been carried out to examine their geometry and the biological properties of the Schiff bases and metal complexes. Coordination complexes of substituted salicylaldehyde Schiff bases are known to have shown diverse structures with a wide range of bonding interactions, electronic as well as biological properties (Soliman & Linert, 1999; Zolezzi, Decinti & Spodine, 1999; Elmali, Zeyrek, Elerman & Durlu, 2000; Hegazy and Gaafar, 2012).

In this paper, we report the synthesis of Schiff bases derived from the condensation reaction of 3-aminopyridine with *o*-vanillin or *p*-vanillin. The isolated Schiff bases were reacted with cobalt acetate, cobalt chloride or cobalt chloride with trimethylamine to form cobalt(II) complexes. The Schiff bases and their corresponding cobalt(II) complexes were investigated for *in vitro* antimicrobial activity against three well known bacteria and a fungus. Their cytotoxicity effects on brine shrimps were also examined. The results obtained from the characterization and the biological activities were used to confirm the effect of variation of the size and nature of the substituents on the coordination geometry around the metal ion, electronic and their biological properties.

Experimental

Materials and methods

The 3-aminopyridine, *o*-vanillin, *p*-vanillin were purchased from Sigma–Aldrich. Cobalt acetate and cobalt chloride purchased from E. Merck were used without further purification.

Physicochemical Measurements

Mid infrared spectra were recorded on a Perkin-Elmer spectrum 2000 FT-IR spectrometer. The spectra were determined using a KBr beam splitter and a DTG detector, in the region 4000 - 400 cm^{-1} with typically 16 scans at an average resolution of

4 cm^{-1} . Samples were run as mulls in Nujol on KBr windows. Far infrared spectra were recorded on a Perkin-Elmer spectrum 2000 FT-IR spectrometer. The spectra were recorded in the region 650 – 100 cm^{-1} with typically 64 scans and at an average resolution of 12 cm^{-1} . Samples were run as mulls in Nujol on polyethylene windows. Carbon, hydrogen and nitrogen combustion microanalyses were carried out using a Fisons Elemental Analyzer 1108 CHNS-O. Melting points were determined using a Gallenkamp melting point apparatus. The results were uncorrected. The ultraviolet-visible (UV-vis) spectra were recorded on a Varian Cary 500 spectrophotometer. The electronic properties were examined in methanol. The near-infrared-visible (NIR-VIS) spectra were carried out using a digital Cary 500 (Varian) spectrophotometer. All the measurements were made at room temperature. The NIR-VIS and UV-vis spectra data were employed for the analysis of the Schiff bases and the complexes. The diffuse reflectance spectra of the isolated Schiff bases and the complexes were measured by using a Cary 500 UV-vis/NIS spectrometer fitted with a Cary diffuse reflectance accessory. The spectra were collected as % reflectance at a spectra bandwidth of 2 nm and at a rate of 200 nm / min between 200 – 800 nm for UV-vis measurement and 700 – 2000 nm for visible-near infrared (Vis-NIR) region.. Polytetrafluoroethylene (PTFE) powder was used as background. The spectra have been smoothed and derivatized by using the Savitsky-Golay method employing 45 and 55 convolution points for UV-vis and vis – NIR respectively. The spectra were then converted to absorbance for comparison with solution spectra. The Schiff bases and their corresponding cobalt complexes were analysed for their purity by microanalysis and IR spectra. Cobalt analyses was done using inductively coupled plasma (ICP). ^1H NMR and ^{13}C NMR spectra of Schiff base GL1 and GL2 were recorded on an Avance Bruker AMX 400 MHz spectrometer using deuterated chloroform as solvent and tetramethylsilane (TMS) as the internal reference. All spectra were recorded at ambient temperature. Chemical shifts were measured in parts per million (ppm) downfield of

the reference signal.

BIOLOGICAL ACTIVITY

Preparation of microbial cultures (Antibacterial susceptibility screening, inoculation and incubation)

The antibacterial activities of Schiff bases GL1 and GL2 and their cobalt (II) complexes were tested against laboratory isolated *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and one fungus strain, *Aspergillus niger*, using the disc diffusion method (Madigan, Martinko & Parker, 1998). The microorganisms were supplied by the Microbiology Department, Rhodes University, South Africa. Nutrient agar and broth, malt extracts were supplied by Biolab. Antibiotic assay discs, 12.7 mm diameter and sterile 0.45 µm membrane filters were purchased directly from Sigma-Aldrich.

Nutrient agar for bacteria and malt extract agar for the fungus were separately inoculated with the isolated microorganisms (Ernst and Rogers, 2005). 4 mg/ml, 1 mg /ml and 0.5 mg/ml of the synthesized Schiff bases and their cobalt complexes were dissolved in methanol and absorbed on the 12.7 mm diameter antibiotic disc paper and were placed firmly on the agar media. The plates were incubated at 32 °C for bacteria 18–24 hours and at 37 °C for fungus for 10 – 14 hours. The soaked antibiotic assay discs were air dried under a lamina flow hood and placed on each of the inoculated malt extracts plates with the help of forceps flamed from time to time. All the procedures highlighted above were done aseptically under a lamina flow hood. After incubation, the resulting inhibition zones on the plates were measured. The diameters of inhibition zones around the discs were measured to the nearest mm, the average diameter was recorded and compared to that of the control. The control samples were absorbed in methanol only. The data reported in Table 5 are averages of three experiments.

Bioassay (Brine shrimp lethality test)

The assay method described by Anderson et al., with minor modifications was used for the

lethality test of the Schiff bases and their cobalt complexes (Anderson, Goetz & McLaughlin, 1991). Brine shrimp (*Artemia salina* leach) eggs were hatched in a shallow rectangular plastic dish (22×32 cm), filled with artificial seawater and used after 48 hours. A 100 µL of sea water containing 10-13 *Artemia* nauplii was added to micro-well containing 150 µL, giving a final volume of 250 µL per well with test solutions at concentrations 400, 200, 100 and 50 µL/mL. The micro well plates were covered and left in a dark cupboard for observation after 24 hours. Each well was examined under a Leica dissecting microscope and the numbers of dead (non-motile) as well as live nauplii were counted. The percent death at each dose was determined. 100 µL of methanol was added to live larvae and the total number of larvae per well counted. The LD₅₀ and 95 % confidence were determined using probit analysis method described by Finney (Finney, 1971). 'R' data analysis computer program was used to analyzed the numerical data generated from the brine shrimps assay and to address the problems of disparity (R Data, 2000).

Procedure for the synthesis of Schiff bases GL1

Equimolar quantities (0.76 g, 5 mmol) of *p*-vanillin in 5 ml ethanol was added to (0.471 g, 5 mmol) 3-aminopyridine in 5 ml ethanol and refluxed for 1 hour. After cooling, the crystalline product was filtered and recrystallized from ethanol, and then dried under reduced pressure over silica. The reaction for the synthesis of GL1 is shown in Scheme 1.

Procedure for the synthesis of Schiff bases GL2

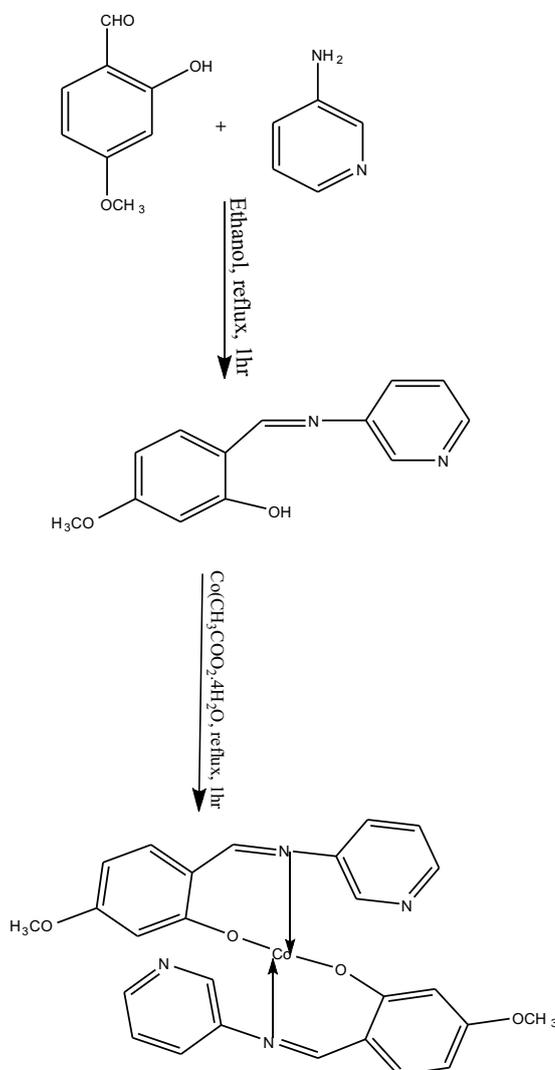
Equimolar quantities (0.76 g, 5 mmol) of *o*-vanillin in 5 ml ethanol was added to (0.471 g, 5 mmol) 3-aminopyridine in 5 ml ethanol and refluxed for 1 hour. After cooling, the crystalline product was filtered and recrystallized from ethanol, and then dried under reduced pressure over silica.

Syntheses of Cobalt(II) complexes of GL1B and GL2B

To an ethanolic solution (5 ml) of 3-aminopyridine (0.471 g, 5 mmol) was added 5 ml of ethanolic solution of *p*-vanillin or *o*-vanillin

(0.76g, 5 mmol). The mixture was refluxed in a round bottom flask. After refluxing for 1 hour, the solution was allowed to cool at room temperature. A methanolic solution (5 ml) of cobalt acetate (0.6226 g, 2.5 mmol) was added to

the ethanolic solution of the GL1 and GL2 respectively and the mixture was further refluxed for another 1 hour. The mixture was allowed to form precipitate. The precipitate was filtered, washed with diethyl ether, and cold methanol. The



Scheme 1: Reaction pathways for obtaining GL1 Schiff bases and one of its proposed complexes.

Syntheses of GL1A and GL2A complexes from cobalt(II) salts

A methanolic solution (10 ml) of cobalt chloride (CoCl₂·6H₂O), (0.5948 g, 2.5 mmol) was added to 5 mmol of each of the (10 ml) GL1 and GL2 solution. The mixture was refluxed in a 100 ml round bottom flask for 2 hours. The complex was allowed to precipitate. The precipitate was filtered, washed with diethyl ether, dried in the oven at 50 degree celcius and kept dry for analyses.

Syntheses of GL1C and GL2C complexes from cobalt(II) salts

To a solution of 3-aminopyridine (0.471 g, 5 mmol) in tetrahydrofuran (10 ml) was added *p*-vanillin (0.76g, 5 mmol) or *o*-vanillin (0.76g, 5 mmol) and refluxed for 30 minutes. The mixture cooled, and filtered. The mixture of trimethylamine (0.6969 ml, 5 mmol) and cobalt chloride (CoCl₂·6H₂O, 0. 5948 g, 2.5 mmol) in methanol was added to the filtered Schiff base

GL1 and GL2 solution respectively. The mixture was refluxed for another 1 hour. The solution was allowed to cool to room temperature. The precipitate obtained was filtered, washed with diethyl ether, cold methanol and dried over silica gel under reduced pressure.

RESULTS AND DISCUSSION

The physicochemical data for the Schiff bases GL1, GL2 and their corresponding cobalt complexes are listed in Tables 1 and 2 respectively. The two Schiff bases were obtained in good yield from the 1:1 *o*-vanillin or *p*-vanillin with 3-aminopyridine. The resulting solids are intensely coloured, and stable in air. They are soluble in most organic solvents, but poorly soluble in water.

Table 1: Microanalysis and analytical data for the 3-aminopyridine-based Schiff bases GL1 and GL2

No.	Molecular formula	% Found (Calculated)			Color	Yield (%)	M.p. (°C)	Molar mass	M+
		% C	% H	% N					
GL1	C ₁₃ H ₁₂ N ₂ O ₂	67.93 (68.41)	5.23 (5.30)	12.05 (12.27)	yellow	67	114-5	228	228
GL2	C ₁₃ H ₁₂ N ₂ O ₂	67.57 (68.41)	5.28 (5.30)	11.94 (12.27)	orange	75	119-20	228	228

Table 2: Microanalysis and analytical data for the cobalt complexes of GL1, GL2.

No.	Molecular formula	Molar mass	% Found (Calculated)				Color	Yield (%)	M.p. (°C)
			% C	% H	% N	% Co			
GL1A	C ₂₆ H ₂₆ ClCoN ₄ O ₅	568.9	55.08 (54.89)	4.06 (4.61)	9.95 (9.85)	10.29 (10.36)	Green	66	181-2
GL1B	C ₂₆ H ₂₂ CoN ₄ O ₄	522.4	59.26 (59.78)	4.16 (4.25)	10.53 (10.72)	11.36 (11.28)	greenish yellow	84	316-8
GL1C	C ₂₆ H ₃₀ CoN ₄ O ₇	569.5	54.72 (55.03)	4.47 (4.97)	9.19 (9.84)	10.47 (10.35)	greenish yellow	87	316-8
GL2A	C ₂₆ H ₂₆ ClCoN ₄ O ₆	584.9	53.72 (53.39)	4.87 (4.48)	9.51 (9.58)	10.37 (10.08)	leaf green	66	181-2
GL2B	C ₂₆ H ₂₃ CoN ₄ O ₄	522.4	59.52 (59.78)	4.11 (4.25)	10.74 (10.72)	11.48 (11.28)	greenish yellow	78	262-3
GL2C	C ₂₆ H ₂₃ CoN ₄ O ₄	522.4	59.81 (59.78)	4.76 (4.25)	10.35 (10.72)	11.66 (11.28)	greenish yellow	77	262-3

The elemental analysis data of the Schiff bases and the cobalt complexes are consistent with the proposed stoichiometry as shown in Table 2. The proposed structures of the Schiff bases and the cobalt complexes were identified using elemental analysis, mid- and far-infrared, UV-vis in methanol, diffused reflectance. ¹H NMR and ¹³C NMR were further used to characterize the Schiff bases. The GL1 and GL2 with their corresponding cobalt complexes gave a sharp melting point indicating the isolation of fairly pure Schiff bases and complexes. The microanalysis analysis for C, H, N and O as well as the molecular weight of the complexes obtained were in agreement with the predicted formula for the Schiff bases and complexes. An outline of the proposed structure for GL1 and GL2 are depicted in Schemes 1.

¹H NMR spectra and ¹³C NMR spectra

The diagnostic NMR spectra data for GL1 and GL2 are listed in Table 3. The observed spectra showed signals in the δ 7.2–8.6 (m) region for the aromatic protons. The formation of the GL1 and GL2 is confirmed by the presence of C=N azomethine proton signal appearance at δ 8.47 ppm and δ 8.65 ppm respectively (Bilge, Kiliç, Hayvali, Hökelek & Serap, 2009). Singlet signals appearance at δ 3.78 and 3.94 ppm are attributed to the *para* and *meta*-methoxy protons for GL1 and GL2 respectively. The chemical shifts at 13.13 ppm and 13.09 ppm implies the predominance of enol-imine tautomer (Nazir, et al., 2000). All the carbon peaks were observed in the ¹³C NMR at the expected positions. The structures of the Schiff bases were also confirmed by sharp signals of ¹³C NMR spectra.

Table 3: ^1H and ^{13}C chemical shift of GL1 and GL2

No.	Schiff base		$\underline{\text{H}}\text{C}=\text{N}$	$\text{C}-\underline{\text{O}}\text{H}$	$\text{O}-\underline{\text{C}}\text{H}_3$
1	GL1	^1H	8.47 (s, 1H)	13.13 (s, 1H)	3.78 (s, 3H)
		^{13}C	163.6	164.4	55.5
2	GL2	^1H	8.65(s, 1H)	13.09 (s, 1H)	3.94 (s, 3H)
		^{13}C	164.6	151.2	56.2

FTIR spectra

The important infra-red data of the Schiff bases, GL1, GL2 and the cobalt complexes are given in Table 4. The mid IR and far IR spectra of the Schiff bases and their cobalt (II) complexes provide information about the cobalt-Schiff base bonding. The FTIR spectra data of GL1, GL2 and their isolated cobalt complexes are listed in Table 4. The main stretching frequencies and their assignments are listed in Table 4. The formation of the Schiff base GL1 and GL2 is supported by the appearance of a strong band at 1610 and 1615 cm^{-1} due to $\nu(\text{C}=\text{N})_{(\text{imine})}$ in the IR spectra of these Schiff base (Yeap, Ha, Ishizawa & Suda, 2003). The Schiff bases exhibit a broad band centred at 3442 cm^{-1} . This suggests the involvement of the 3-OH or 4-OH group in the intramolecular hydrogen bonding with the lone pair of azomethine nitrogen (Maurya, Jaiswal & Verma, 1998). It also suggests that the Schiff bases GL1 and GL2 exist in enol form in the solid state (Maurya, Chourasia & Sharma, 2008).

In order to ascertain the bonding modes of the bidentate GL1 and GL2 to the cobalt metal, the vibrations of the GL1, GL2 were compared with their corresponding cobalt (II) complexes. The IR

spectra vibration observed at 1610 cm^{-1} and 1615 cm^{-1} for GL1 and GL2 assigned to $\nu(\text{C}=\text{N})_{(\text{imine})}$ shifted to higher wavenumber in GL1B, GL1C, but shifted to lower wavenumber in GL1A suggesting coordination through N atom of the azomethine (Sliverstein, Bassler & Morrill, 2005; Tyagi, Chandra and Tyagi, 2014). Similarly, the vibrations of GL2A, GL2B and GL2C appeared at lower wavenumber relative to GL2. Another diagnostic vibration is the C-O stretching frequencies which occur at 1297 cm^{-1} and 1259 cm^{-1} in GL1 and GL2 respectively. The $\nu(\text{C}=\text{O})_{(\text{imine})}$ stretching frequencies of the complexes appeared at lower wavenumber. A medium band observed in the region between 3442-3438 cm^{-1} due to O-H stretching modes are indicative of lattice water (Singh et al., 2002). In addition, two weaker bands in the region 755-795 and 702-718 cm^{-1} are attributed to Co(-OH) rocking and wagging mode of vibrations, respectively (Ejidike and Ajibade, 2015). The band which appeared at approximately 1578 cm^{-1} assigned to $\nu(\text{C}=\text{N})$ of the pyridine moiety shifted to higher frequencies in the spectra of the complexes (Abd-elzاهر, 2004). This shift suggests that the nitrogen of the pyridine participated in the formation of the complex.

Table 4: Mid and far Infrared frequencies (cm^{-1}) of the GL1, GL2 and their cobalt complexes

	$\nu\text{O}-\text{H}$	$\nu\text{C}=\text{N}_{(\text{imine})}$	$\nu\text{C}=\text{N}_{(\text{py})}$	$\nu\text{C}-\text{O}$	$\nu_a\text{M}-\text{O}$	$\nu_s\text{M}-\text{O}$	$\nu_a\text{M}-\text{N}$	$\nu_s\text{M}-\text{N}$	other	
GL1	3442	1610	1586	1297	-	-	-	-	-	-
GL1A	3441	1609	1568	1207	591, 572	545	497, 430	413, 393	340	366
GL1B	3440	1615	1574	1210	576	521	496	409	-	365, 318
GL1C	3438	1615	1575	1211	576	520	496	409	-	364, 318
GL2	3440	1615	1565	1259	-	-	-	-	-	-
GL2A	3439	1608	1541	1235	566	530	494	447	255	287
GL2B	3441	1614	1539	1234	541	520	470	347	271	-
GL2C	3442	1614	1563	1245	546	523	478	350	278	-

The coordination of cobalt to the GL1 and GL2 is also confirmed by the appearance of new bands in the spectra of the metal complexes in the far infrared region. The stretching of cobalt–oxygen and cobalt–nitrogen bands of the complexes appeared in the lower wavelength region in the range of 591–520 cm^{-1} and 498–347 cm^{-1} also signifying the complexation through oxygen and imine or pyridine nitrogen atoms from the ligand (Nakamoto, 1997). The appearance of medium bands at the range of 498–347 cm^{-1} is attributed to the ν_{asym} and ν_{sym} (M-N) bond (Abdallah & Zayed, 2010). The big difference between the asymmetry and the symmetry of the M-N vibration might be an indication of high covalent character. In addition, the stretching frequencies in the range of 591–520 cm^{-1} are attributed to the ν_{asym} and ν_{sym} cobalt-oxygen bond formation (Tyagi et al., 2014). In addition, a band at 305 is assigned to the ν (M-Cl). Thus the IR spectral data results provide strong evidences for the complexation of the bidentate GL1 and GL2 Schiff bases and also suggests that the cobalt(II) coordinated through azomethine or pyridine nitrogen and through oxygen of the aromatic ring.

Electronic Spectra

The important electronic spectra data of the Schiff bases GL1, GL2 and their complexes in

methanol are listed in Table 5. The various bands are grouped into range between 210–245 nm, 280–290 nm, 302–372 nm and 421 nm. The first and the second bands are attributed to benzene π - π^* , the third is attributed to imino π - π^* transitions while the band above 400 nm shown by these Schiff bases is attributed to imino n - π^* transition (Canpolat & Kaya, 2004). The appearance of the band above 400 nm implies significant quantity of keto-amine tautomer of the Schiff bases in methanol. The complexes showed similar electronic transitions as observed in the Schiff bases GL1 and GL2. However, the transition of the complexes shifted towards higher wavelengths or lower energy. The shift is position of the methoxy or type of cobalt salt used dependent. The cobalt chloride-based complexes of GL1 in methanol, shows maxima at about 360, 285, 240 and 205 while the cobalt acetate-based complexes show maxima at slightly higher wavelengths. Interestingly, the GL2 complexes appeared at much higher wavelengths than the GL1 complexes. All the observed maxima are attributed to charge transfer bands. The shift maybe attributed to the donation of the lone pairs of the nitrogen atom ($\text{N} \rightarrow \text{M}$) or the ($\text{O} \rightarrow \text{M}$) of the Schiff bases to the metal ion. The d-d bands were not seen or too low to be seen.

Table 5: Electronic spectra data (nm) in 3.0×10^{-3} mol dm^{-3} , diffuse reflectance and brine shrimps lethality data for GL1, GL2 and their cobalt(II) complexes

	Methanol 3.0×10^{-3} mol dm^{-3} (nm)						Diffuse reflectance (nm)	LD50	Standard error	Pseudo R
GL1	210	225	245	285				4.30	0.06	0.980
GL1A	205		240	285	-	360	366, 583, 625, 1400- 1200	4.25	0.11	0.900
GL1B				280	338	373		5.47	0.12	0.991
GL1C	207	-		275	-	377		5.21	0.12	0.988
GL2		225		275	-	416		4.49	0.04	0.930
GL2A	211		240	270	285	391-	389, 582, 636, 1400- 1200	4.46	0.14	0.906
GL2B	205		245	280	335	401		3.96	0.17	0.889
GL2C	205		241	279	-	400		5.62	0.08	0.913

However, in order to further characterize the complexes, the diffused reflectance technique was used to examine them in the UV-vis and the NIR-UV-vis regions. The spectra of the complexes are illustrated in Figures 1 and 2 respectively. On the basis of their diffused reflectance spectra, the cobalt complexes can be classified as tetrahedral because their spectra show absorption bands at 1400–1200, 600–500, and slightly below 400 nm (Ashmawy & Amer, 1986). The band at 1400–1200

nm can be assigned to low symmetry components of the second tetrahedral transition ${}^4\text{A}_2 \rightarrow {}^4\text{T}_1(\text{F})$. The band at 600–500 nm is assigned to the ${}^4\text{A}_2 \rightarrow {}^4\text{T}_1(\text{P})$ transition. However, while the bands for the cobalt acetate or cobalt chloride with triethylamine of the complexes are similar, they have lower intensity that are not well resolved like those of cobalt chloride GL1A, GL2A. However, we still suggest distorted tetrahedral coordination

for the GL1B, GL1C, GL2B, GL2C complexes. The result confirms that the four – coordinate tetrahedral and the six – coordinate octahedral are the idealized geometries for few, if any complexes

have been shown to attain symmetry high enough to be called tetrahedral or octahedral in the group theoretical sense (Carlin, 1965).

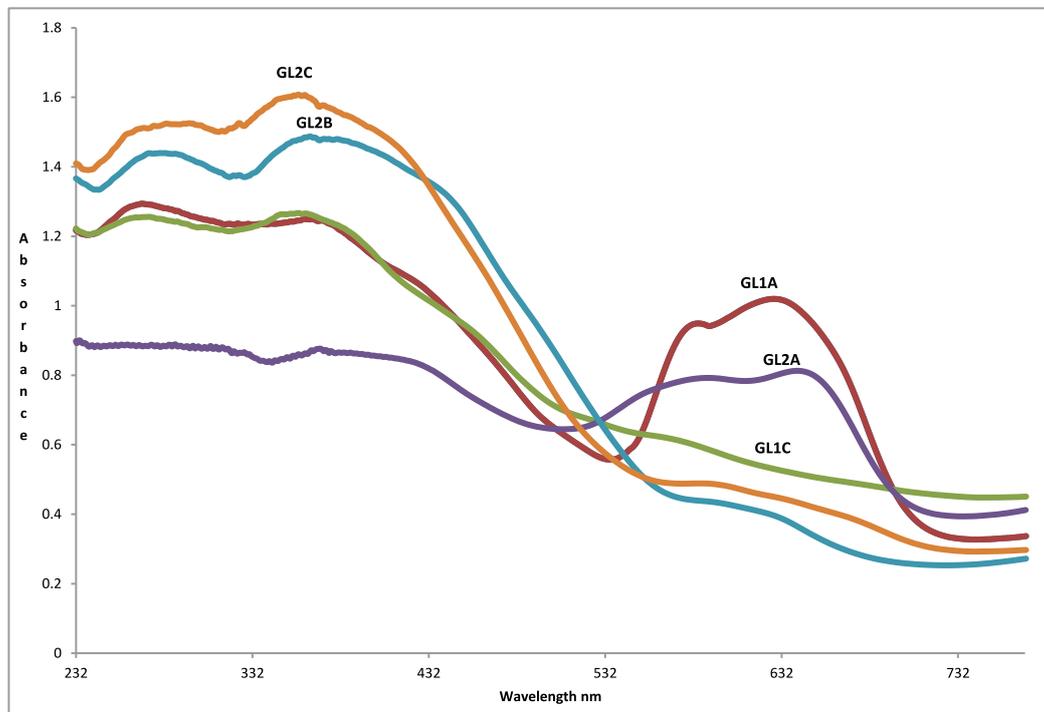


Figure 1: Diffused reflectance spectra of cobalt complexes in the UV-vis region

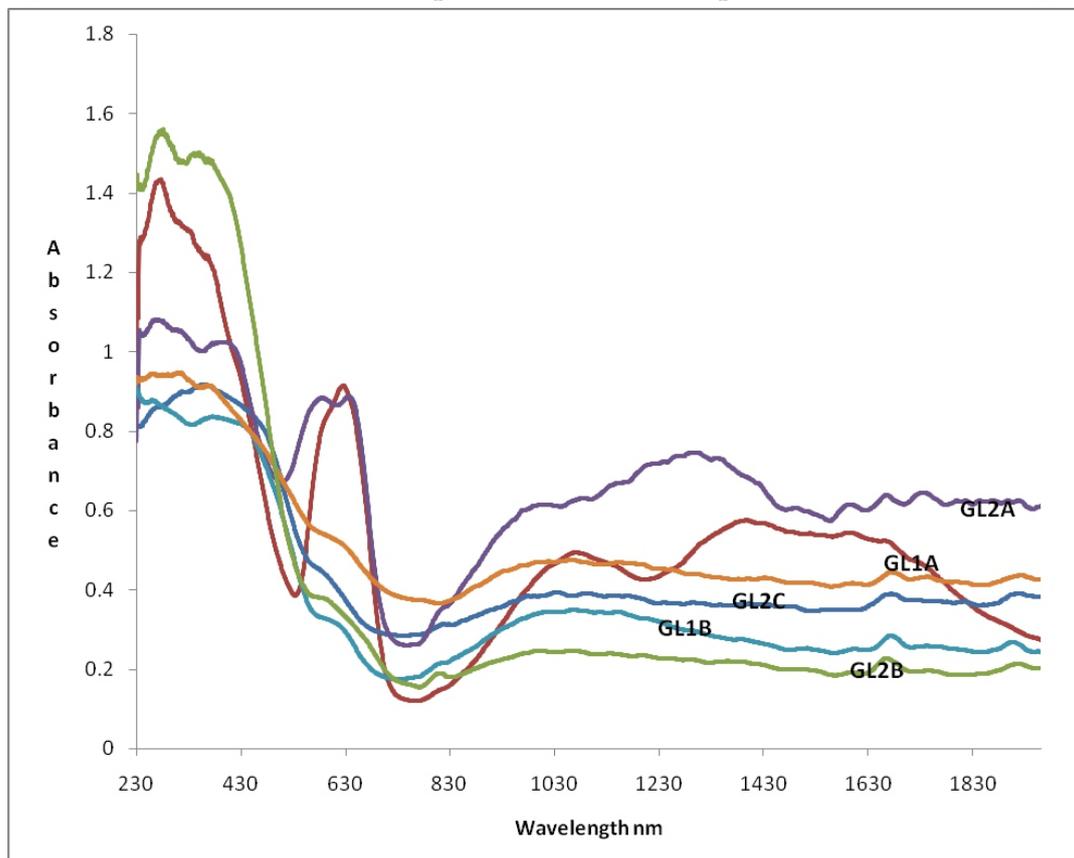


Figure 2: Diffused reflectance spectra of cobalt complexes in the NIR-Uv-vis region

Biological Activities

Antimicrobial Activity

The free Schiff bases GL1, GL2 and GL1A, GL1B, GL2A, GL2B complexes were tested for their *in vitro* antibacterial activity against the bacterial species *Staphylococcus Aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and fungus, *Aspergillus niger*. by zone of inhibition method using agar diffusion method (Frankle *et al.*, 1970; Yousif *et al.*, 2017). The comparison of the biological activities of the synthesized Schiff bases and their complexes shows the following results. Cobalt complex of GL1 showed broad range of antimicrobial activity against all the tested microorganism more than the other complexes. The cobalt chloride-based complex GL1A is more active than the GL1B and GL1C complexes. Generally, varying toxicity of the chelated compounds and the Schiff bases against the tested microorganism were observed and toxicity is concentration dependent. The strong activity of most of the complexes with the two Schiff bases against *E. coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria) and *Aspergillus niger* is well observed. This is attributed to their ability to combine with the lipophilic layer in order to enhance the membrane permeability of the Gram-negative bacteria. The increase in lipophilicity enhances the penetration of GL1, GL2 and their synthesized cobalt complexes into the lipid membranes and thus restricts further growth of the organism (Raman *et al.*, 2009; Maldonadol *et al.*, 1995; Tumer *et al.*, 1999; Imran *et al.*, 2007). Although there is sufficient increase in the antibacterial activity of the complexes as well as the Schiff bases, they could not fully reach the effectiveness of the standard drug, ampicillin, but more active than the antifungal drug used. The toxicity of GL1 and its complexes on *S. aureus* are more than those of GL2 and its complexes. This may imply that the presence of OCH₃ group in the *para* position imparted high interaction with the double membrane of the *S. aureus* than the *meta*-methoxy (Mohamed, Zayed, & Abdallah, 2010). The less activity of some of the compounds against microorganism can be ascribed to the creation of alternative pathways that have been modified by the microorganism to get a way

around so as to survive the effect of the drugs (Yeaman & Yount, 2003).

Brine Shrimps Lethality Test

The brine shrimp lethality test was employed in this study to determine the toxicity of the various Schiff bases and their cobalt(II) complexes, hoping to find a correlation between this assay and cytotoxicity (Meyer *et al.*, 1982). The LD₅₀ values of the synthesized compounds are listed in Table 5. The test samples showed different mortality rate at different concentration on the brine shrimps. The activities are concentration and geometry dependent. GL2B appeared to be the most toxic to the brine shrimps because it has the lowest LD₅₀ value.

CONCLUSION

In the present study, two novel Schiff bases and six cobalt complexes were synthesized and characterized by physico-chemical methods. The cobalt ions were complexed with nitrogen of the imine or pyridine and C-O producing distorted tetrahedral geometry complexes. The antibacterial and antifungal data given for the compounds presented in this study showed that the metal complexes generally have better activity than Schiff bases GL1, GL2 and less activity than antibacterial standard but better activity than the antifungal drugs used.

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