
Temitope E. Olalekan, Ph.D. and Temitope E. Bakare, M.Sc.

1Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan 200285, Oyo State, Nigeria.
2Department of Chemistry, Caleb University, PMB 21238, Ikeja, Lagos State, Nigeria.

E-mail: topeolalekan11@yahoo.com

ABSTRACT

Schiff base (SB) was synthesized by the condensation reaction of 2-aminobenzoic acid and p-hydroxybenzaldehyde. The reaction of the Schiff base with metal ions in an alkaline aqueous medium yielded the metal(II) complexes of the hydrolysed Schiff base product, identified as metal(II) complexes of 2-aminobenzoic acid. These compounds were characterized by physical and spectroscopic techniques.

The elemental analysis indicated the ligand to metal ratio as 2:1 in the complexes with general molecular formula M(L)_2 (L = 2-aminobenzoic acid; M = Mn, Co, Ni, Cu and Cd). IR data showed the ligand coordinated to the metal ion through the carboxylate oxygen and the amine nitrogen. Room temperature magnetic susceptibility and solid reflectance data suggested the complexes have four-coordinate geometry. The conductance measurements showed the complexes are non-electrolytes and are covalent compounds in DMSO. The compounds were screened for in-vitro antimicrobial activity against chosen strains of bacteria and fungi.

(Keywords: Schiff base, 2-aminobenzoic acid, metal(II) complexes, hydrolysis products, antimicrobial studies)

INTRODUCTION

2-aminobenzoic acid is very useful in synthesis of heterocyclic systems and other molecules. It serves as an excellent biochemical precursor to aromatic amino acids and it also forms an important part of several alkaloids (Dileep et al., 2011). The acid and its derivatives are useful in various applications such as sunscreen production (Beeby and Jones, 2001), perfumery (Yadav and Krishnan, 1998) and monitoring of glycosylation of proteins (Susindran et al., 2012). Anti-convulsant (Mendu et al., 2011) and anti-inflammatory activity (Dileep et al., 2011) of 2-aminobenzoic acid and its derivatives have been reported. Some transition metal anthranilates have demonstrated ability for hydrogenation (Susindran et al., 2011).

Hugo Schiff first reported the synthesis of Schiff bases (Schiff, 1864) containing imine group formed by a condensation reaction between primary amines and aldehydes. Schiff bases are more stable and non-polymerizing when an aryl group (minimum requirement) is attached to the nitrogen or to the carbon of the imine group (Wilkinson et al., 1987). The reaction can proceed in alkaline, acidic or neutral medium. This reaction is reversible under suitable conditions and is termed hydrolysis.

This research work reports the preparation of metal complexes by heating to reflux the mixture of a Schiff base with respective metal ions in an aqueous alkaline medium. Hydrolysis of the Schiff base was facilitated under these conditions. The resulting complexes were characterized and their antimicrobial potential was investigated.

MATERIALS AND METHODS

The reagents and solvents used in this research were of analytical grade and used as obtained from commercial suppliers (Sigma Aldrich and Qualichem). CHN analysis of the metal(II) complexes was carried out using Elementar Analysensysteme varioMICRO V1.6.2 GmbH. The infrared and electronic spectra of the ligand and metal(II) complexes were determined on Perkin Elmer Spectrum 11 FT-IR spectrophotometer in the region 4000–400 cm⁻¹ and Labomed double beam UV–Visible spectrophotometer in the range 190–900 nm.
respectively. Room temperature magnetic susceptibilities at 299K were measured using Sherwood magnetic susceptibility balance and diamagnetic corrections were calculated using Pascal’s constants. Molar conductance measurements of the complexes in DMSO were taken on 4150 Jenway conductivity meter. The melting points (uncorrected) of the synthesized compounds were determined using Gallenkamp melting point apparatus.

ANTIMICROBIAL ASSAY

**Diameter of Zone of Inhibition**

The ligand and metal(II) complexes were screened for in-vitro antibacterial and antifungal activity against the following pathogens using agar-well diffusion method: gram-positive Bacillus subtilis ATCC 6633, Staphylococcus aureus ATCC 6538, Methicillin-resistant Staphylococcus aureus (MRSA), gram-negative Escherichia coli ATCC 25922, Escherichia coli ATCC 23218, Salmonella typhi ATCC 33458, Klebsiella pneumoniae ATCC 13883, Pseudomonas aeruginosa ATCC 29853 and the fungi Candida albicans ATCC 10231 and Trichophyton rubrum ATCC 28189.

The procedure used for this assay followed that described by Chohan et al. (2006) in which, twenty four hour old bacterial inocula containing approximately 10⁵ colony forming units (CFU)/ml were used in the screening. The culture media were introduced by pour plate method into respective plates. After cooling, wells were dug in the nutrient agar in the case of bacteria (potato dextrose agar medium for fungi) with the help of a sterile metallic cork borer of 12 mm diameter. Recommended volume (100 µL of each test compound (5 mg/mL in DMSO) was introduced into the wells; hence each well contains 500 µg of test compound. Other wells supplemented with reference antibacterial drug- imipenem (10 µg/disc) and DMSO served as positive and negative controls for the antimicrobial studies respectively. The plates were incubated immediately at 37°C for 24 and 72 h for bacteria and fungi respectively. Activity was determined by measuring the diameter of zone (mm) showing complete inhibition.

**Minimum Inhibitory Concentration**

Serial dilution (two-fold) technique was used in the determination of minimum inhibitory concentration (MIC) of metal complexes. Bacterial and fungal inocula containing approximately 10⁵ colony forming units (CFU)/mL were used. MIC tests were run with the third subculture of microbes and samples were taken during the exponential phase of the microorganisms’ growth. Bacteria were inoculated on nutrient agar slants and incubated for 24 hour at 37°C while fungi were inoculated on potato dextrose agar slants and incubated for 72 h at 37°C.

**SYNTHESIS OF THE SCHIFF BASE AND METAL(II) COMPLEXES**

2-(4-hydroxybenzylidenamino)benzoic acid (SB): 2-aminobenzoic acid (1.00 g, 7.29 mmol) was dissolved in 5 mL hot absolute ethanol and p-hydroxybenzaldehyde (0.89 g, 7.29 mmol) in 5 mL hot absolute ethanol was added dropwise with stirring. The resulting solution was refluxed for 2 h. An orange precipitate formed upon cooling, which was filtered and washed with cold ethanol and allowed to dry in air. The product was recrystallised from hot ethanol and dried on silica gel. The mass of the Schiff base product was weighed as 0.71 g. The reaction procedure is presented in Scheme 1.

**Metal(II) Complexes:** Schiff base, SB (0.60 g, 2.49 mmol) and KOH (0.14 g, 2.49 mmol) were dissolved in 10 mL hot distilled water under stirring. Mass equivalent of 1.00 mmol of each metal salt (MnCl₂·4H₂O, CoCl₂·6H₂O, NiCl₂·6H₂O, CuCl₂·2H₂O, CdCl₂·2½H₂O) was dissolved in 5 mL warm distilled water and added in drops respectively. The mixture was heating under reflux for one hour during which colored precipitates formed. The mixture was filtered and the precipitate was washed with hot ethanol. The solid metal complex was dried in the oven at 70°C. The procedure for the synthesis of the metal complexes is given in Scheme 2 and their analytical data are recorded in Table 1.

RESULT AND DISCUSSIONS

The analytical data are presented in Table 1. The Schiff base and 2-aminobenzoic acid, L, have
melting points at 231-232 and 146-147°C respectively, which are lower than those of the metal complexes (>360°C). The compounds were derived in good yields of 63–93%. The experimental and calculated CHN values of the metal complexes were in close agreement with the proposed formula of ligand to metal ratio 2:1, ML₂, for each compound.

**Solubility and Molar Conductance**

The compounds were soluble in hot DMSO, slightly soluble in absolute ethanol and methanol and insoluble in water. The molar conductance of each metal(II) complex was measured in DMSO and the values obtained in the range 2.42–7.73 Ω⁻¹ cm² mol⁻¹ showed the compounds are non-electrolytes and neutral (Geary, 1971).

**Infrared Spectroscopy**

The assignment of the relevant IR bands of the Schiff base, 2-aminobenzoic acid and metal(II) complexes is recorded in Table 2. The Schiff base showed an intense absorption band at 1604 cm⁻¹ characteristic of the ν(CH=N) of imine group (Atmaram and Mangaonkar, 2011 and Kumar et al., 2013). The disappearance of ν(CH=N) in the metal(II) complexes indicated that hydrolysis of the ligand took place during complexation reaction.

Asymmetrical and symmetrical v(N–H) stretches of the free 2-aminobenzoic acid were found at 3501 and 3390 cm⁻¹. In the metal complexes, these bands were shifted to lower frequencies by 84-100 cm⁻¹ and 84–112 cm⁻¹ respectively. This suggested the Schiff base hydrolysis product was 2-aminobenzoic acid, and was bound to the metal(II) ions through the amine nitrogen (Al-Noor et al., 2013). A broad band at 3075 and 3241 cm⁻¹ due to carboxylic v(O–H) was observed in the spectra of the Schiff base and 2-aminobenzoic acid respectively. This band was absent in the spectra of metal(II) complexes, confirming deprotonation of the carboxylic acid (Raman and Raja, 2007). The sharp carboxylic v(C–O) of the free 2-aminobenzoic acid occurring at 1138 cm⁻¹ was shifted to lower frequencies in the range 1002–1069 cm⁻¹ in the metal(II) complexes, suggesting that ligand coordination to the transition metal ions was also through the carboxylate oxygen. Appearance of bands ranging between 516–521 cm⁻¹ and 408-425 cm⁻¹ in the complexes are characteristic of M–O and M–N bands respectively (Al-Noor et al., 2010).

**Electronic Spectra and Magnetic Moments**

Solid reflectance spectra of the compound were measured and recorded in Table 2. Absorption bands for the Schiff base at 29,760 cm⁻¹ (336 nm) and 34,602 cm⁻¹ (289 nm) were assigned to n→π* and π→π* transitions, respectively.

**Scheme 1**: Reaction Scheme for the Synthesis of Schiff Base.

**Scheme 2**: Reaction Scheme for the Synthesis of Metal(II) Complexes (M = Mn, x= 4; M= Co and Ni, x = 6; M=Cu, x= 2; M= Cd, x= 2.5).
A band of high intensity due to $n \rightarrow \pi^*$ transition was seen in the spectrum of 2-aminobenzoic acid at 29,940 cm$^{-1}$ (334 nm). Mn(II) complex showed two weak absorptions at 16,260 cm$^{-1}$ (615 nm) and 18,083 cm$^{-1}$ (553 nm), usually ascribed to tetrahedral geometry and were assigned to spin forbidden transitions $^6A_g \rightarrow ^4E_1$ ($\nu_1$) and $^6A_g \rightarrow ^4A_1$ ($\nu_2$), respectively. The magnetic moment of approximately 5.8 BM is normally observed for $d^5$ system due to lack of orbital contribution from the A ground term. The 4.8 BM magnetic moment value obtained for the Mn(II) complex suggested it could be antiferromagnetic at room temperature (Durot et al., 2003). Further temperature-dependent studies could be carried out to ascertain this observation.

The Co(II) complex showed absorption bands at 13,680 cm$^{-1}$ (730 nm) and 17,513 cm$^{-1}$ (570 nm) typical of tetrahedral geometry and these are assigned to $^4A_2 \rightarrow ^4T_1(P)$ and $^4A_2 \rightarrow ^4T_1(F)$ transitions. The magnetic moment value of 4.9 BM obtained for the complex suggested its tetrahedral geometry as well as metal-metal interaction in the complex (Rao and Reddy, 1990 and Earnshaw, 1980).

Absorption bands of 13,680 cm$^{-1}$ (730 nm) and 17,825 cm$^{-1}$ (560 nm) were observed for the Ni(II) complex. These are within the range for tetrahedral geometry and have been assigned to $^3T_1(F) \rightarrow ^3A_2$ ($\nu_2$) and $^3T_1(F) \rightarrow ^3T_1(P)$ ($\nu_3$) transitions, respectively (Satyaranayana and Nagasundara, 2004). The magnetic susceptibility value for the Ni(II) complex is 3.3 BM, which indicates a metal-metal interaction between neighbouring molecules of the complex (Osowole and Balogun, 2012).

Cu(II) complexes with tetrahedral and square planar geometry show absorptions below 10,000 cm$^{-1}$ (single and broad) and within the range 10,000–20,000 cm$^{-1}$ respectively (Tuncel and Selahattin, 2005 and Derebe et al., 2002). The Cu(II) complex synthesized has absorption bands at 14,925 cm$^{-1}$ (670 nm) and 17,857 cm$^{-1}$ (560 nm) assigned to $^2B_1g \rightarrow ^2A_1g$ and $^2B_1g \rightarrow ^2E_1g$ transitions, respectively. This suggests a square planar geometry for the compound. Its measured magnetic susceptibility value was 2.0 BM.

Cd(II) has a $d^{10}$ configuration, hence Cd(II) complexes are not expected to show d-d transitions (Housecroft and Sharp, 2008). The absorption at 24,271 cm$^{-1}$ (412 nm) in the spectrum of Cd(II) complex is due to charge transfer. The magnetic susceptibility measurement of the complex is 0.4 BM, which

### Table 1: Analytical Data of Ligands and the Metal(II) Complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Mass (g/mol)</th>
<th>Color</th>
<th>Melting Point (°C)</th>
<th>% Found (Calculated)</th>
<th>Molar Conductance ($\Omega^{-1}$ cm$^2$ mol$^{-1}$)</th>
<th>Magnetic Moment $\mu_{eff}$ (BM)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>241.24</td>
<td>Orange</td>
<td>231-232</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>82</td>
</tr>
<tr>
<td>L</td>
<td>137.14</td>
<td>Yellow</td>
<td>146-147</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mn(L)$_2$</td>
<td>327.21</td>
<td>Cream</td>
<td>&gt;360</td>
<td>51.47 (51.39)</td>
<td>3.96 (3.70) 8.33 (8.56)</td>
<td>7.7</td>
<td>4.8</td>
</tr>
<tr>
<td>Co(L)$_2$</td>
<td>331.21</td>
<td>Pink</td>
<td>&gt;360</td>
<td>50.49 (50.77)</td>
<td>3.82 (3.65) 8.27 (8.46)</td>
<td>2.6</td>
<td>4.9</td>
</tr>
<tr>
<td>Ni(L)$_2$</td>
<td>330.97</td>
<td>Green</td>
<td>&gt;360</td>
<td>50.83 (50.81)</td>
<td>3.65 (3.65) 8.10 (8.46)</td>
<td>2.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Cu(L)$_2$</td>
<td>335.82</td>
<td>Green</td>
<td>&gt;360</td>
<td>49.95 (50.07)</td>
<td>3.62 (3.60) 8.11 (8.34)</td>
<td>2.8</td>
<td>2.0</td>
</tr>
<tr>
<td>Cd(L)$_2$</td>
<td>386.68</td>
<td>White</td>
<td>&gt;360</td>
<td>43.82 (43.49)</td>
<td>3.26 (3.65) 6.91 (7.24)</td>
<td>4.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>

### Table 2: Infrared and Electronic Spectra of the Compounds (cm$^{-1}$).

<table>
<thead>
<tr>
<th>Compound</th>
<th>O-H (COOH)</th>
<th>N-H (amine)</th>
<th>CH= N</th>
<th>C=O</th>
<th>C-O</th>
<th>M-O</th>
<th>M-N</th>
<th>Wavelength $\lambda$ ($\text{cm}^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>3075</td>
<td>-</td>
<td>1604</td>
<td>1630</td>
<td>1166</td>
<td>-</td>
<td>-</td>
<td>29760, 34602</td>
</tr>
<tr>
<td>L</td>
<td>3241</td>
<td>3501, 3390</td>
<td>-</td>
<td>1677</td>
<td>1138</td>
<td>-</td>
<td>-</td>
<td>29940</td>
</tr>
<tr>
<td>Mn(L)$_2$</td>
<td>-</td>
<td>3415, 3305</td>
<td>-</td>
<td>1614</td>
<td>1066</td>
<td>520</td>
<td>424</td>
<td>16260, 18083</td>
</tr>
<tr>
<td>Co(L)$_2$</td>
<td>-</td>
<td>3401, 3141</td>
<td>-</td>
<td>1638</td>
<td>1037</td>
<td>518</td>
<td>418</td>
<td>13680, 17513</td>
</tr>
<tr>
<td>Ni(L)$_2$</td>
<td>-</td>
<td>3317, 3306</td>
<td>-</td>
<td>1639</td>
<td>1069</td>
<td>521</td>
<td>425</td>
<td>13680, 17825</td>
</tr>
<tr>
<td>Cu(L)$_2$</td>
<td>-</td>
<td>3417, 3278</td>
<td>-</td>
<td>1660</td>
<td>1039</td>
<td>519</td>
<td>425</td>
<td>14925, 17857</td>
</tr>
<tr>
<td>Cd(L)$_2$</td>
<td>-</td>
<td>3413, 3290</td>
<td>-</td>
<td>1637</td>
<td>1002</td>
<td>516</td>
<td>408</td>
<td>24271</td>
</tr>
</tbody>
</table>
showed it has a diamagnetic behavior (Golcu et al., 2005).

ANTIMICROBIAL POTENCY ASSAY

The diameters of inhibition zones of microbial growth by the test compounds are recorded in Table 3. 2-Aminobenzoic, L, Ni(II) complex and the solvent medium, DMSO, did not inhibit the growth of microbial strains at the concentration tested. The Schiff base inhibited the growth of methicillin-resistant Staphylococcus aureus (MRSA) by 16 mm diameter zone, Klebsiella pneumonia by 17 mm and Salmonella typhii by 20 mm.

Mn(II) complex was active against Escherichia coli, Klebsiella pneumonia and Candida albicans with inhibition zones 27, 20 and 15 mm, respectively. All the microbes except Escherichia coli were inhibited by Co(II) complex with zone diameters between 16 and 36 mm and the fungus Candida albicans was most susceptible. The Cu(II) complex was active against gram-positive MRSA (16 mm) and gram-negative Escherichia coli* (20 mm). The Cd(II) complex inhibited the growth of all the microorganisms except Trichophyton rubrum. The diameters of inhibition zones are in the range 19-32 mm.

The MIC of metal complexes, which showed diameter of zone of inhibition of at least 16 mm, was determined by serial dilution technique. The results of the complexes that showed activity against at least one of the microorganisms are plotted in Figure 1. Cd(II) complex showed high MIC activity, the growth of the microorganisms (except Trichophyton rubrum) was inhibited at concentrations as low as 0.49 µg/mL. The Co(II) complex showed the lowest degree of MIC activity with value of 250 µg/mL on all the organisms. All other complexes have activity within this range of values.

CONCLUSION

The anthranilic acid derived from in-situ hydrolysis of the Schiff base, (z)-2-(4-hydroxybenzylideneamino)benzoic acid, was coordinated to the metal(II) ions (Mn, Co, Ni, Cu and Cd) via the carboxylate oxygen and the amine nitrogen. The compounds all assumed four-coordinate geometry, majorly tetrahedral except Cu(II) complex, which exhibited square planar geometry. As metal-metal interactions were implied from the magnetic moments for Mn(II), Co(II) and Ni(II) complexes, their temperature-dependent behavior could be investigated. The proposed structures for the metal(II) complexes are presented in Figure 2.

Studies of the antimicrobial activity showed that the activity of the metal(II) complexes was generally lower than that of the standard drug, imipenem for the antibacterial studies. The MIC studies revealed the Cd(II) complexes with higher inhibitory potential than the other complexes and Co(II) complex has lowest inhibitory capacity. Mn(II) did not inhibit the growth of any gram-positive bacteria.

Table 3: Zone of Inhibition of Microorganisms (in mm) by the Compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>S. aureus</th>
<th>MRSA</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>E. coli*</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
<th>S. typhii</th>
<th>C. albicans</th>
<th>T. rubrum</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>12</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>L</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mn(L)₂</td>
<td>12</td>
<td>12</td>
<td>27</td>
<td>12</td>
<td>20</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Co(L)₂</td>
<td>21</td>
<td>22</td>
<td>16</td>
<td>12</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>36</td>
</tr>
<tr>
<td>Ni(L)₂</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cu(L)₂</td>
<td>12</td>
<td>16</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cd(L)₂</td>
<td>29</td>
<td>23</td>
<td>19</td>
<td>19</td>
<td>20</td>
<td>20</td>
<td>23</td>
<td>20</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>DMSO</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Imipenem</td>
<td>18</td>
<td>17</td>
<td>38</td>
<td>24</td>
<td>20</td>
<td>24</td>
<td>30</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

E.coli – Escherichia coli ATCC 25922, E.coli* – Escherichia coli ATCC 23218
The authors acknowledge Dr. B.C. Adebayo-Tayo of the Department of Microbiology, University of Ibadan, Nigeria, who carried out the antimicrobial assay procedure.

COMPETING INTERESTS

The authors declare that there is no conflict of interests.

REFERENCES


ABOUT THE AUTHORS

Dr. Temitope E. Olalekan, is a Lecturer at the University of Ibadan, Nigeria. She holds a Ph.D. degree in Chemistry from Rhodes University, Grahamstown, South Africa. Her research interests are in the areas of Coordination and Medicinal Chemistry.

Temitope E. Bakare, is a Lecturer at Caleb University, Lagos. He holds a Master’s degree in Inorganic Chemistry from University of Ibadan, Nigeria. His research interests are Bioinorganic and Physical Chemistry.

SUGGESTED CITATION