

RESEARCH ARTICLE

Synthesis, Characterization and Biological Activity of Azo Dye Derived from 2-methoxyaniline and its Complexes

Hasan S. Mohammed*, Saif D. K. Alzamili

Department of Chemistry, Science College, Al-Qadisiyah University, Al Diwaniyah, Iraq

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ABSTRACT

The azo ligand of guanine namely 8-[2-methoxyphenylazo]-guanine (MPAG) was prepared and characterized by elemental analysis, mass spectroscopy, ¹H-NMR, FTIR and UV-vis spectroscopies. The Cu(II) and Zn(II) complexes of MPAG ligand were prepared under mole ratio equal to 2:1 as MPAG ligand to metal ions and characterized by ¹H-NMR, FTIR, UV-vis, XRD powder and mass spectroscopies and elemental analysis, magnetic susceptibility as well as molar conductance. The solvents did not have a significant effect on the NAPAG ligand, and the MPAG ligand exhibited potential changes under different pH values. The MPAG ligand may be utilized as a colorimetric sensor for the particles of Co²⁺, Ni²⁺, Cu²⁺ and Zn in watery solutions. The MPAG ligand is tridentate and the complexes are electrolyte and octahedral.

Keywords: Antifungal, Complexes, Guanine, Naked eye, pH sensor.

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INTRODUCTION

The hetero azo dyes are utilized in clothing manufacturing as well as in healthcare for phototherapy, anti (viral, fungal, and oxidant) effects.¹⁻³ Azo derivatives have been researched towards microorganisms that cause a variety of human diseases, *Escherichia coli* bacteria.^{4,5}

Orthomethoxyaniline (ortho-Anisidine) is one of three isomers of the aniline derivatives including ortho, meta and para methoxy aniline. It is important in the production of dyes, medicines, and perfumes. The orthomethoxy aniline has shown better dissolvable when compared to aniline as well as the fact monomeric excellent resistance to rust.^{6,7}

The metal ions may seep to groundwater and possibly seep to drinking water and leech into the soil, leading to accumulating these ions in plants and animals, and may cause serious health risks in humans. The naked eye method is an important, facile method, high selectivity and sensitivity for detecting metal ions such as iron, copper, zinc, and ions based on azo dyes and schiff base dyes.⁸⁻¹² The azo guanosine derivatives exhibited a remarkable photoisomerization process in liquid that play important role in biological process and high optic data storage.¹³ As a result, we are interested in the synthesis and characterization of new azo ligands for guanine and study their antifungal activity.

MATERIALS AND METHODS

The solvents used in our study were reagent grade. The ortho-methoxy aniline 99% purity, hydrochloric acid (concentrated), sodium nitrite, guanine 98%, sodium hydroxide, CuCl₂.2H₂O and ZnCl₂ were supplied from Sigma Aldrich.

The data of elemental analysis were done on Euro Ea 300 C.H.N element analysis. The molar conductance was measured for the complexes of MPAG ligand via Cond.720 (WTW). UV-vis spectra were recording using a (Shimadzu UV-1650 PC) UV-vis Spectrophotometer. IR- spectra were taken on a (Shimadzu, FT-IR- 8400S) fourier transform infrared spectrophotometer 4000–400 cm⁻¹ with samples prepared as KBr disk. ¹H-NMR spectra of MPAG ligand and zinc complex were done by Bruker 400 MHz spectrophotometer at 298 K in DMSO d₆.

Synthesis of MPAG Ligand

Ortho-methoxyaniline (0.5 g, 0.00246 mol) was dissolved in 10 mL of distilled water, followed by the addition of (2 mL) of hydrochloric acid. Sodium nitrite (0.27 g, 0.00246 mol) was dissolved in 7 mL of distilled water and then was put under cooling by ice bath. It was added to the cold solution of ortho-methoxyaniline with stirring to form diazonium salt. Guanine (0.6 gm, 0.00246 mol) was dissolved in 20 mL of distilled water, and then 0.7 g of sodium hydroxide was added.

*Author for Correspondence: hasan.sh.mohammed@qu.edu.iq

The solution of azo guanine was filtrated and washed by water with a little bit of cold ethanol.

Preparation of Metal Complexes

The 20 mL of MPAG ligand (0.6 g, 0.00168 mol) was dissolved in hot 20 mL DMSO solvent then was added to hot aqueous solution of 0.075 g, 0.00084 mol of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ or 0.09 g, 0.00084 mol of and ZnCl_2 . The reaction mixture was refluxed for 2 hour. The mixture was cooled until a dark color precipitate of complex was filtrated, washed by distillate water, and dried under vacuum.

RESULTS AND DISCUSSION

The 8-[2-methoxyphenylazo]-guanine ligand (MPAG) was prepared from the reaction of the diazonium salt of orthomethoxyaniline prepared in an acidic condition with the basic solution of the amino base (guanine). The MPAG ligand was precipitated in a neutral to slightly acidic environment from the reaction solution as shown in Scheme 1.

Solvent effect on MPAG Ligand

Methanol, ethanol, dimethyl sulfoxide, and tetrachloromethane solvents were among the solvents utilized to demonstrate the effect of solvents on the behavior of MPAG ligand. The MPAG ligand was not significantly affected by the different solvent types, with the exception of bathochromic behavior and high redshift for MPAG ligand in the dimethyl sulfoxide solvent (Figure 1).

Acidity Effect on the MPAG Ligand

Figure 2 shows that MPAG ligand spectrum in the basic medium exhibited high bathochromic behavior accompanied with dark red color for the aqueous solution. That is possible due to losing the proton, which leads to increasing the resonance effect on all molecule of MPAG. While in the acidic medium at the same concentration, a slight decrease in the absorption

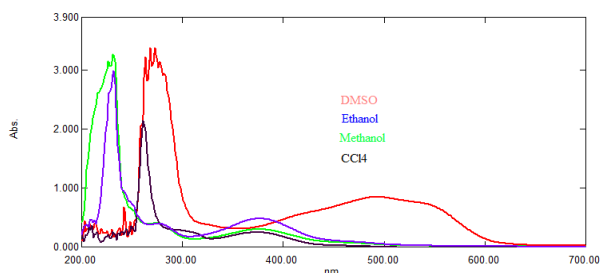
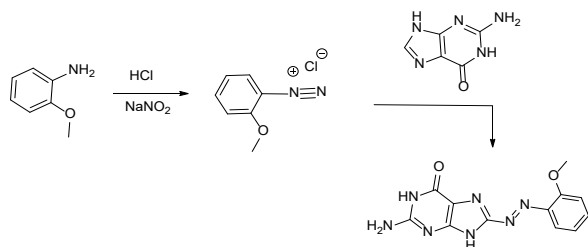


Figure 1: UV-vis spectra of MPAG ligand in different solvents.



Scheme 1: Preparing steps of 8-[2-methoxyphenylazo]-guanine ligand (MPAG).

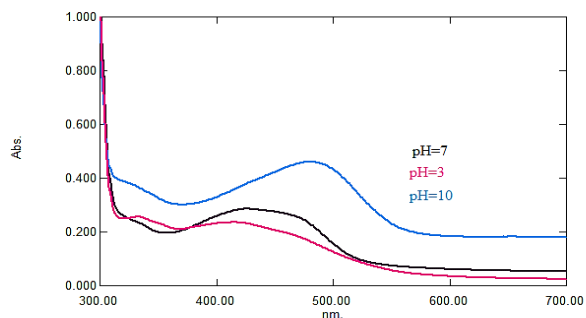


Figure 2: UV-vis spectra of MPAG ligand under different values of pH

was observed, accompanied by yellowish-orange color as shown in Figure 3. That may be attributed to the protonation process, which decreases the resonance effect. On the other hand, in the neutral state, the absorption was middle between acidic and basic conditions, and the color was reddish-orange.

Complexes of MPAG Ligand

Solid metal complexes of metal ions (copper (II) and zinc (II)) for the ligand of 8-[2-methoxyphenyl azo]-guanine were prepared by reacting an equivalent of the metal ion with two equivalents of ligand 8-[2-methoxyphenylazo]-guanine as shown in Scheme 2. This molar ratio of 2:1 [metal: ligand] was adopted depending on the results of molar ratio that indicated the reaction of ligand 8-[2-methoxyphenyl azo]-guanine with metal ions in this ratio as in Figure 4 for the complexes.

The MPAG ligand exhibited colorimetric selectivity for Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} using their chloride salts in water-ethanol at room temperature. When the MPAG ligand mixed with these metal ions separately, we see a remarkable color change as shown in Figure 5, which could easily be detectable by naked-eye these metal ions in solution aqueous.¹⁴

The azo ligand 8-[2-methoxyphenyl azo]-guanine and its solid metal complexes were identified by accurate quantitative

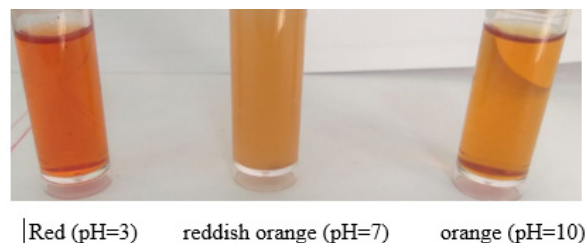
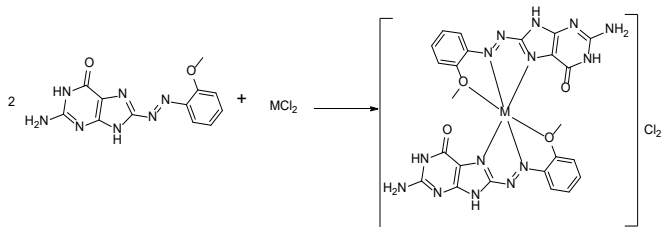


Figure 3: Colors of MPAG ligand under different values of pH in aqueous solutions.



Scheme 2: Preparation steps of (M= Cu(II) and Zn(II)) for MPAG ligand.

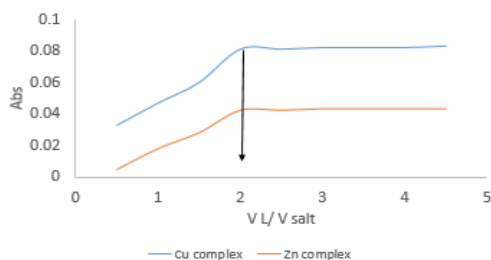


Figure 4: Mole ratio of Cu(II) and Zn(II) ions with MPAG ligand.

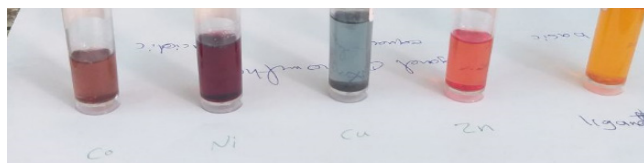


Figure 5: Chemosensor of Co^{2+} , Ni^{2+} , Cu^{2+} and Zn^{2+} with MPAG ligand in aqueous solution.

analysis of the elements (C.H.N). The results of these analyses were recorded in Table 1, and a great convergence was found when comparing the obtained values with the theoretically calculated values.

Mass spectrometry was used to determine the presence of the 8-[2-methoxyphenyl azo]-guanine ligand. A signal reflecting the MPAG ligand's mass exhibited at 285.3, along with signals for methoxybenzene at 108.2 and guanine at 151.3. That indicates to release azo group as nitrogen. The zinc complex also displayed a signal at 706.5, indicating to its molecular weight and formula, $\text{ZnC}_{24}\text{H}_{22}\text{N}_{14}\text{O}_4\text{Cl}_2$. Additionally, a signal corresponding to the molecular weight of the copper complex appeared at 704.4.

The molar conductivity of the copper and zinc(II) complexes was performed in dimethylformamide (DMF) solvent at room temperature. The conductivity values of the copper and zinc(II) complexes were 133 and $160 \Omega^{-1} \text{cm}^2 \text{mol}^{-1}$, respectively. Which indicates that the coordination compounds have an electrolytic nature, in a ratio of 1:2.

NMR Spectra

The MPAG ligand was characterized by nuclear magnetic resonance spectroscopy. The MPAG ligand showed a number of signals representing the number of ligand protons. A signal appeared at 14.72 ppm belonging to NH, and signals of phenyl ring appeared in the aromatic region in range of 6.94–7.66 ppm, including the protons of the NH_2 group. On the other hand, a singlet signal appeared at 3.84 ppm representing O- CH_3 . These chemical displacements in ppm of ligand are 14.72 (2H, NH,s),

7.66 (1H₄, m), 7.48 (1H₁, d), 7.29 (1H₃, t), 7.15 (1H₂, m), 6.94 (2H, NH₂, m), 3.84 (3H, O- CH_3 , s). The $^1\text{H-NMR}$ spectrum of the zinc complex of ligand as shown in Figure 6 was similar to the spectrum of the ligand except for the chemical shifts of protons' signal positions changed with double integration for number of protons. The chemical shifts in ppm of zinc complex of MPAG ligand are 12.20 (2H, NH,s), 10.37 (2H, NH, s), 7.42 (2H₄, d), 7.18 (2H₁, d), 6.95 (2H₃, 2H), 6.62 (2H₂, m), 6.17 (4H, NH₂,s), 3.85 (3H, O- CH_3 , s).

Infrared Spectra of 8-[2-methoxyphenyl azo]-guanine Ligand and its Metal Complexes

Infrared spectroscopy is an important technique for determining of active functions in compounds. Where 8-[2-methoxyphenyl azo]-guanine ligand showed many identity peaks listed in Table 2. The N-H group appeared at (3458 and 3383) cm^{-1} which did not suffer changing in the complexes. As well as other groups such as C-H aromatic at 3059 cm^{-1} and C-H aliphatic at 2928 cm^{-1} . The C=C function was at 1564, and C=O at 1695 cm^{-1} and NH_2 at 1660 cm^{-1} as well as bending vibration of CH aromatic at 746 and 702 cm^{-1} , which in turn did not suffer a change after the coordination of the ligands to metal ions in the prepared complexes. The ligand 8-[2-methoxyphenylazo]-guanine showed absorption of C=N at 1612 cm^{-1} , which suffered a change in location and intensity

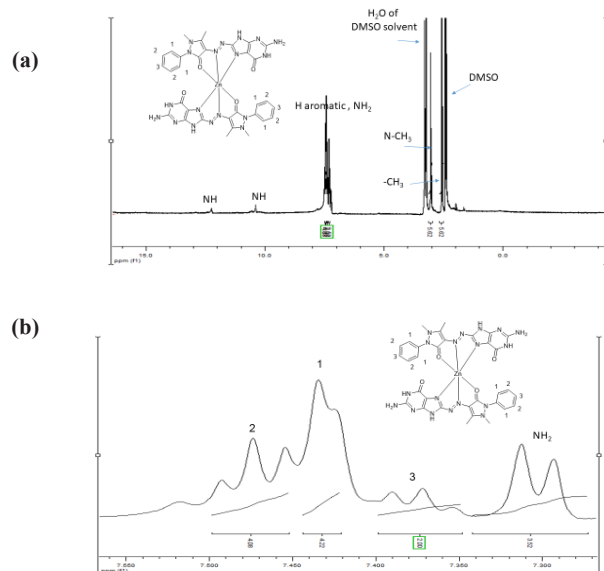


Figure 6: $^1\text{H-NMR}$ spectrum of zinc complex for MPAG ligand in DMSO (d₆). (a) $^1\text{H-NMR}$ spectrum of zinc complex for MPAG ligand. (b) Aromatic area of $^1\text{H-NMR}$ spectrum of zinc complex for MPAG ligand.

Table 1: Physical properties and results of elemental analysis of MPAG ligand and its complex

Compound	Color	Melting point ($^{\circ}\text{C}$)	Yield%	Elemental analysis		
				C_{exp} (C_{theo})	H_{exp} (H_{theo})	N_{exp} (N_{theo})
Ligand ($\text{C}_{12}\text{H}_{11}\text{N}_7\text{O}_2$)	orange	120	85	50.34 (50.53)	3.45 (3.89)	34.28 (34.37)
Copper complex $\text{C}_{24}\text{H}_{22}\text{N}_{14}\text{O}_4\text{Cl}_2\text{Cu}$	brown	282	60	25 (40.89)	2 (3.15)	2 (27.82)
Zinc complex ($\text{C}_{24}\text{H}_{22}\text{N}_{14}\text{O}_4\text{Cl}_2\text{Zn}$)	dark red	285	80	25 (40.78)	2 (3.14)	2 (27.74)

in spectra of complexes, which indicates its consistency with the metal ions in the complexes, as its frequency increased by 25–23 cm^{-1} in consistency.¹⁵ The ligand 8-[2-methoxyphenyl azo]-guanine showed absorption of N=N group at 1483 cm^{-1} , and suffered a change in location and intensity in spectra of complexes. This indicates its consistency with metal ions in complexes, as its frequency decreased by 6 to 8 cm^{-1} under coordinating. The ligand also showed absorption of the C-O-C group at 1286 cm^{-1} , which suffered a change in location and intensity in the complexes' spectra, indicating its consistency

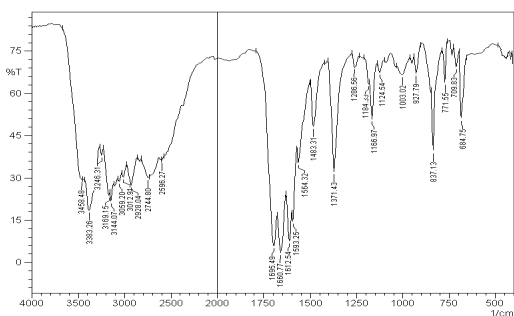


Figure 7: FTIR spectrum of MPAG ligand in KBr disk.

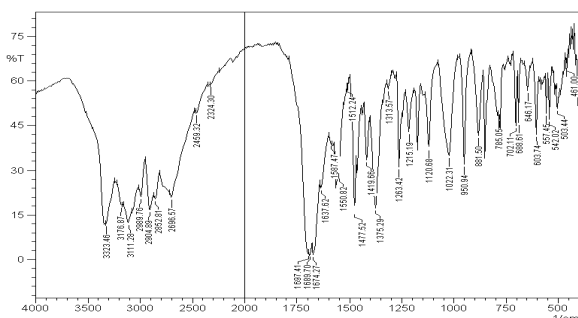


Figure 8: FTIR spectrum of zinc complex for MPAG ligand in KBr disk.

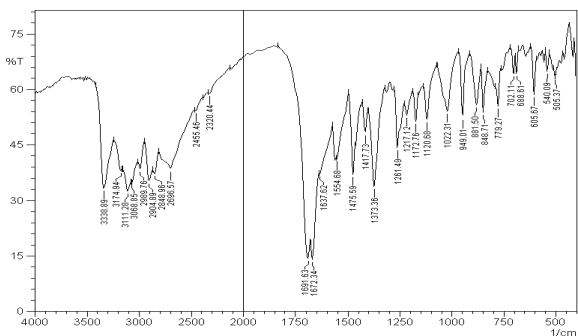


Figure 9: FTIR spectrum of copper complex for MPAG ligand in KBr disk.

Table 2: Important vibrations of active functions of MPAG ligand and its complexes

Compound	N-H	C-H Aromatic	C-H Aliphatic	C=O	NH ₂	C=N	C=C	N=N	C-O-O	M-N	M-O
MPAG ligand	3458, 3383	3059	2928	1695	1660	1612	1564	1483	1286	–	–
Zinc complex	3329, 3176, 3111	3055	2989, 2904	1697	1674	1637	1550	1477	1261	503	461
Copper complex	3329, 3113	3057	2989, 2904	1697	1672	1562	1552	1475, 1464	1261	503	447

with the metal ions in the complexes, as its frequency decreased by about 35 cm^{-1} under coordinating. New bands appeared in the spectra of complexes that were not present in the spectrum of MPAG ligand. The peaks appeared at 505–501 cm^{-1} due to M-N absorption and at 470–445 cm^{-1} due to M-O absorption (Figure 7-10).^{4,15}

UV-vis Spectra

Ultraviolet-visible spectroscopy is an important spectroscopic technique in organic chemistry, where it is preferable that the absorption be in the visible region or close to the red region. That leads to use in medicine and for photosensitizers. In inorganic chemistry, it is useful also to determine the electronic transitions, which help to predict the shape of coordination compounds.¹⁶ The spectrum of MPAG ligand as shown in Figure 10, showed an absorption band at 273 nm that could be attributed to the $\pi \rightarrow \pi^*$ transition, as well as band located at 510 nm that could be attributed to $n \rightarrow \pi^*$.¹⁷ The spectrum of the copper complex showed a band at 284 nm due to the ligand transition. The band at 469 nm is due to the ${}^2B_{1g} \rightarrow {}^2E_g$ ($dxz, dyz \rightarrow dx^2-y^2$) electron transition. The band at 626 nm is due to ${}^2B_{1g} \rightarrow {}^2B_{2g}$ ($dxy \rightarrow dx^2-y^2$) electron transition. These electronic transitions are consistent with octahedral complexes of copper with a magnetic susceptibility of 1.85 B.M.¹⁸ The spectrum of zinc complex of MPAG ligand showed a band at 264 nm due to the $\pi \rightarrow \pi^*$ transition of the ligand and a band at 528 nm due to the $n \rightarrow \pi^*$ transition, which suffered a redshift in the zinc complex relative to MPAG ligand that also explains the dark color of the zinc complex relative to the MPAG ligand.

The patterns of powder XRD for MPAG ligand, Cu(II) and Zn(II) complexes recorded in the range of $[\theta = 0-80]$ which were depicted in Figures 11-13. All patterns exhibited sharp crystalline peaks, indicating the prepared compounds' crystalline phase. The crystalline size of these compounds were in the range of nanoparticles that were calculated by using Scherer equation and Xpert software. The powder XRD

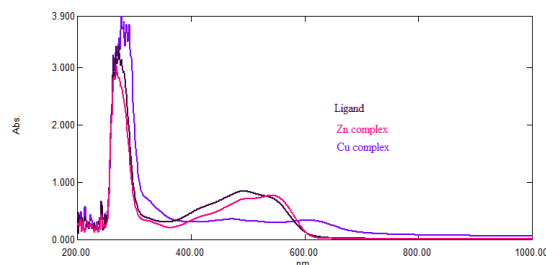


Figure 10: UV-vis spectra of MPAG ligand and its complexes in DMSO solvent at room temperature.

patterns of complexes are completely different compared to the MPAG ligand, demonstrating the formation of coordination compounds of Cu(II) and Zn(II) metal ions.¹⁹ Peak pos. [$^{\circ}$ 2Th] of MPAG ligand are 9.084, 12.249, 13.905, 18.125, 20.989, 22.646, 23.45, 25.308, 26.062, 27.167, 27.87, 30.532, 31.788, 33.596, 36.962, 39.473, 45.551, 51.578, 57.104. Peak pos. [$^{\circ}$ 2Th] of copper complex are 9.66, 11.449, 14.133, 17.781, 21.979, 25.282, 27.828, 31.958, 34.091, 45.584, 50.883. Peak pos. [$^{\circ}$ 2Th] of zinc complex are 9.729, 11.312, 13.927, 27.76, 31.751, 45.377, 56.596, 75.383.

The antifungal activities of coordination compounds has been widely described. The coordination compounds showed strong antifungal properties,^{20,21} therefore, requires to the development of new compounds such as complexes of azo guanine ligand. The *in-vitro* antifungal activities of the compounds were tested against *Aspergillus niger* using sabroid



Figure 14: Antifungal activity of copper complex of MPAG against *Aspergillus niger*.

agar by diffusion method. All the synthesized complexes of MPAG ligand showed stronger antifungal activities than MPAG-free ligands as shown in Figure 14 for copper complexes of MPAG ligand.

CONCLUSION

The MPAG ligand of guanine azo and its Cu(II) and Zn(II) complexes were prepared and characterized by different techniques. It can quickly be identified the formation of the complexes of Co(II), Ni(II), Cu(II) and Zn(II) ions by the naked eye under mixing of these metal ions with MPAG ligand. The MPAG ligand is sensor for various values of acidity. The MPAG ligand and its complex are crystalline materials and nanoparticles.

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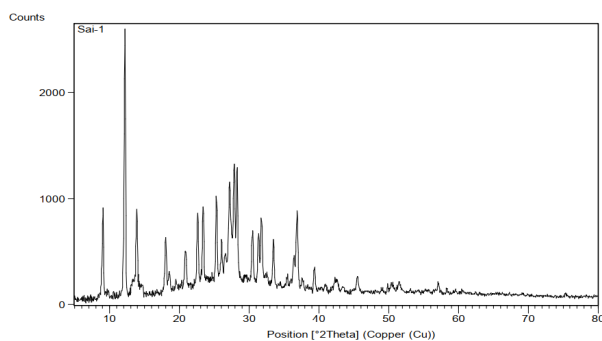


Figure 11: XRD spectrum of MPAG ligand.

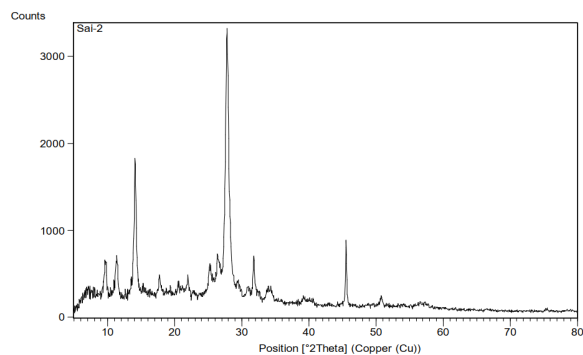


Figure 12: XRD spectrum of Cu complex.

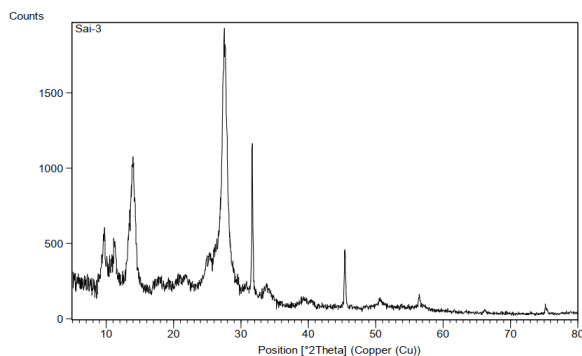


Figure 13: XRD spectrum of Zn complex.

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