



Anti-Microbial Studies of Ag(I) and Cu(II) via Acylation of Thiourea Ligands with Benzoyl Chloride

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Abstract

Amide complexes of Cu²⁺ and Ag⁺ metal ions derived from the acylation of thiourea ligands with benzoyl chloride were synthesised in acetone and recrystallised in ethanol/dichloromethane mixture of 1:1. The complexes all gave a good yield and were characterised on the basis of solubility, melting point, FTIR, UV-visible spectrometry and XRD analysis to determine the mode of binding of the ligands to each of the metal ion and the lattice crystal. The complexes were soluble in non-polar solvents like ethanol and have moderate melting points. The bidentate nature of the metal complexes is indicated by the shifting of $\nu(\text{C}=\text{O})$ stretching vibrations from the range 1643-1690cm⁻¹ to lower range of 1420-1595cm⁻¹ which is attributed to the absorption of the C-O stretching participating in the coordination. The shifting of the $\nu(\text{C}=\text{S})$ stretching vibration from 723.1cm⁻¹ to 1397.8cm⁻¹ for Cu(II) and 1267.3cm⁻¹ for Ag(I) suggesting a coordination of the metal through the C-S. The sharp diffraction peaks obtained at different angles of incidence indicates the crystalline nature of the metal complexes, the Cu(II) complex is triclinic, while the Ag(I) complex showed a cubic lattice. Inhibition properties of the complexes against three bacteria (*Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi*) and a fungus (*Candida albicans*) showed that the complexes have higher antimicrobial activities than the ligands, this could be attributed to chelation. The antimicrobial activities, however, were lower than the standard antibacterial drugs Imipenem and anti-fungi, Amphotericin at the studied conditions.

Keywords: Anti-microbial studies, Ag(I) and Cu(II), acylation of thiourea ligands with benzoyl chloride

1. Introduction

The emergence of microbial species with lower sensitivity to antibiotics and the quantity of multidrug tolerant bacteria are both constantly rising. This rise is attributed to the inappropriate use of immunosuppressive drugs, antibiotics with wide spectrum, organ transplantation, and the ongoing HIV infection epidemic in developing nations. Synthetic drugs, while potentially expensive, are insufficient for treating diseases; consequently, new infection-fighting strategies are needed to control microbial infection. This research will attempt to explore the possibility of using the proposed synthesized metal complexes as antimicrobial agents against some commonly occurring bacteria and fungi. This work aimed at assessing the antimicrobial capabilities of N-substituted complexes of Ag(I) and Cu(II).



A combination compound can be formed by joining stoichiometric compounds that are stable. Essentially, there are two types of reactions that occur in solution: those that, in solution, regain their identity (complexes) and those that lose it (double salts). The $[\text{Cu}(\text{H}_2\text{O})_2(\text{NH}_2)_4]^{2+}$ (cuprammonium ion) and the $[\text{Fe}(\text{CN})_6]^{4-}$ (ferrocyanide ion), for example, are found in solid and solution as distinct entities. Square brackets are used to denote complex ions, and coordination compounds are those that contain such ions. It is primarily the behavior of metals or ions in solutions that determines the characteristics and behavior of complexes in such solutions. Ions of the transition metals specifically, have the ability to form numerous complexes that are stable. Ligands such as aqua or water can coordinate to free metal ions while in solution (Guha and Lee 2022; Malinowski et al., 2020). Antibiotic-resistant microbes cause diseases that kill millions of people each year. Widely accessible antibiotics generally used for treatments can be made inactive by the development of strains that are resistant; this diminishes their clinical usefulness, thus resulting in future use restrictions. The efficacy of future antimicrobial therapy may be diminished by considering broad-spectrum and low-toxicity antibiotics necessitating further introduction of medications with higher selective toxicity. Furthermore, therapeutic failure of antimicrobials brought by incessant use of restricted antibiotics might raise rates of morbidity and mortality as well as treatment expenses. In synthesizing antibiotics with new activation targets, the activity of compounds with known antimicrobial potential should be considered; such compounds will be synergistic in the production of drugs useful against illnesses related to multi-resistant microbes. A novel approach to drug production suggests studying metal ions-antibiotics interactions. This considers three areas: one of which targets microbial resistance; the other places emphasis on the action mechanisms that are foreign to the pathogens; and last but not least, considers the toxicity of the complex ions. (Santosa et al., 2014). Prior to the introduction of organic antibiotics in the middle of the 20th century, complexes of metals or compounds were used as antibacterial agents for thousands of years. In battling communicable diseases, metal-oriented antibiotics hold out hope for sustainability. Efficaciousness in small dosages with no resistance is the ultimate goal of antimicrobials. In the hope that resistance will decrease, metals are becoming more and more popular as biocidal and antibacterial agents. Metal ions are known to target more than one cellular activity, which causes pleiotropic effects on microbial cells (Raymond et al., 2017). It is an acceptable fact that a variety of metals considered for antimicrobial agents are of the d-block, which is typical of the transition metals; however, some metals or metalloids also possess antimicrobial properties. Additionally, some effectiveness of metals has been demonstrated in persister cells, which are typical cells that are dormant and resistant to antibiotics (Raymond et al., 2017). Some metal ions are essential for various activities; certain metal ion deficiencies can cause diseases such as anemia and stunted growth, which can be associated to lack of iron and dietary zinc. Copper deficiency causes infantile cardiac disorders. The alarming pace of the rise of antibiotic resistance has led to a daily decline in the effectiveness of medicines against bacteria (gram-positive and gram-negative). Thus, the necessity to create new compounds with novel modes of action and broad objectives is paramount (Mjos et al., 2014). The synthesis of new metal-oriented medications has been greatly aided by inorganic molecules, especially metal complexes. There is currently a marked increase in interest in the field of scientific study known as medical inorganic chemistry for the evolution of metal complexes intended for use as drugs and medical devices for diagnosis. A major area of study within the discipline of chemistry concerning bioinorganic compounds focuses on the introduction of metals or their ions coupled to biological systems, which enhances the management of disorders (Ravinderpal et al., 2019). One typical property of metals is their propensity to readily shed electrons, generating cations that often dissolve in biological medium. The role that metallic elements play in biology is in this cationic state. In contrast to important mediums like proteins and DNA, which are rich in electron, metal ions are, however, electron deficient. There is a widespread tendency for cations to attach and bond with the biological components; hence, the attractive force originates from the opposing charges (Mjos et al., 2014, Natarajan et al., 2012).

The ability of cations to bind to numerous electronegative ions, such as oxygen essential for life, is based on such concepts. Metals, including Cu, Zn, Fe, and Mn, are integrated into metalloenzymes, or proteins catalysis, which help with several spontaneous reactions essential to life. Clinical trials on novel Metall medicines, like antibiotics induced by metals, antibacterials, antivirals, and several others are encouraged by the existence of metal complexes (Saddam et al., 2017). It is known that ligands like urea and modified thioureas can coordinate with metal ions of the



transition elements to form stable compounds that could be neutral or charged; most of these compounds have even undergone structural characterization. The possible applications of urea and thiourea derivatives as insecticides, antibacterial, antiviral, and antifungal agents, as well as methods to separate metal ions from other substances, have drawn interest in their chemistry (Saddam et al., 2018). Thiourea ligands are interesting due to their three possible coordination sites: through 'N' atom or 'S' of the NH or CS groups respectively. This is in addition to their diverse applications. A leading cause of mortality, particularly in underdeveloped nations, is infectious disease (Jackson et al., 2018). One of the greatest medical achievements of the 20th century has long been cited as the preparation of antibiotic medications, especially antibiotics drugs. Resistance to antimicrobial agents is currently a global public health concern. In the search for innovative therapies, recent studies have shown that connecting organic medications to metal-containing fragments can increase their activity (Orie et al., 2021).

In our endeavour to prepare metal-based medicinal compounds, we provide the synthesis, characterization, and antimicrobial analyses of N-amide complexes of copper (II) and silver (I), or the amine-acyl chloride reaction.

2. Results and Discussion

Results

XRD Data

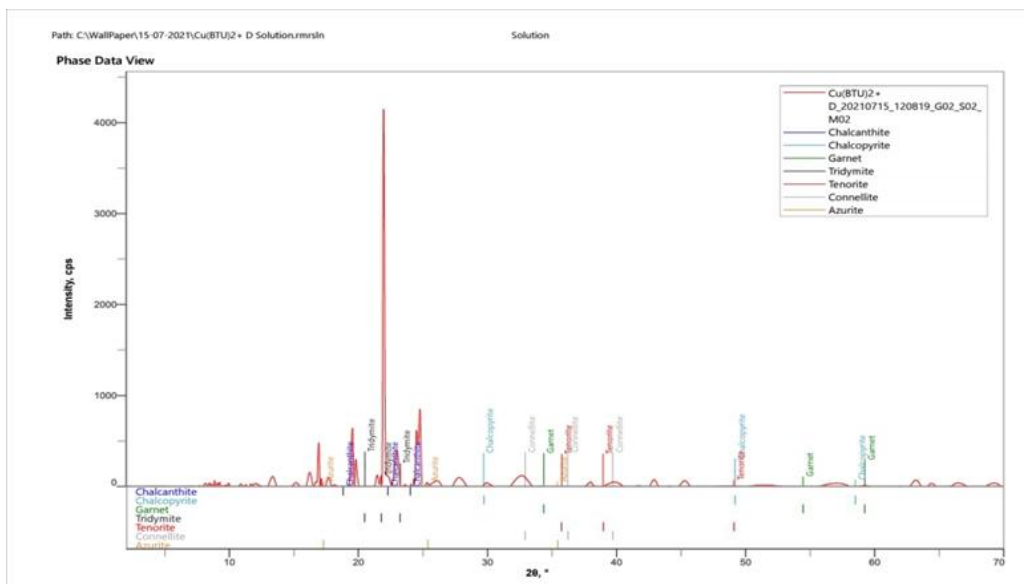
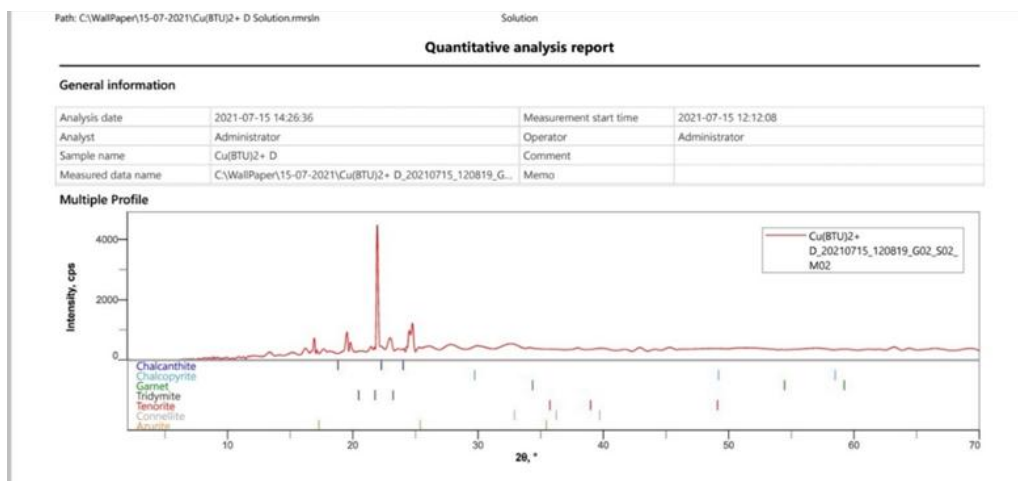


Figure 1: Phase data View of Cu(II) N-Benzoylthioureato complex with ICDD



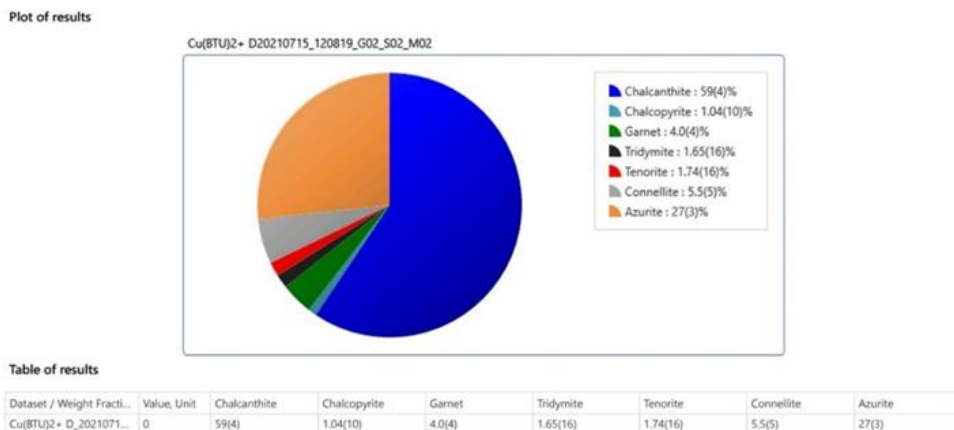


Figure 2: Peak of Cu(II) N-benzoylthioureato complex at FWHM

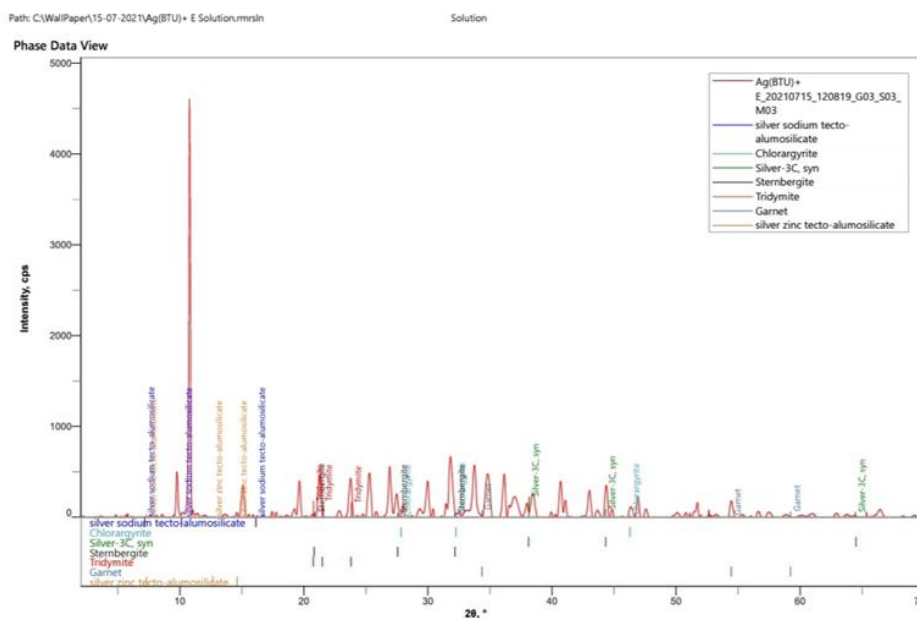
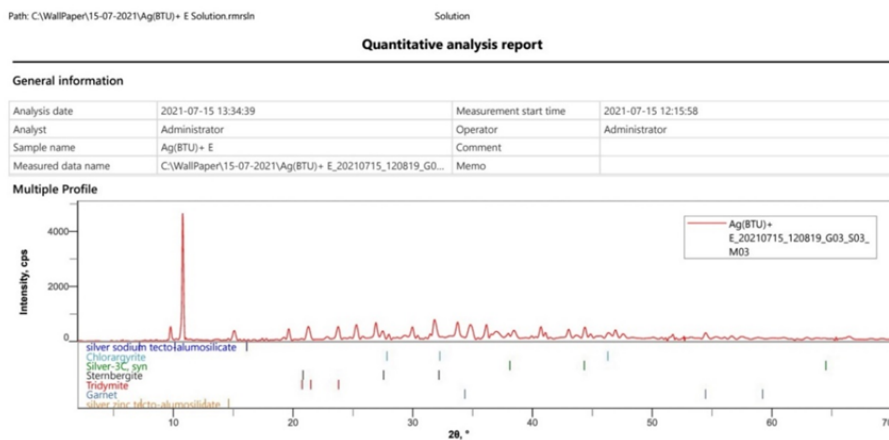


Figure 3: Peaks and phase matching of silver (I) N-benzoylthioureato complex with ICDD



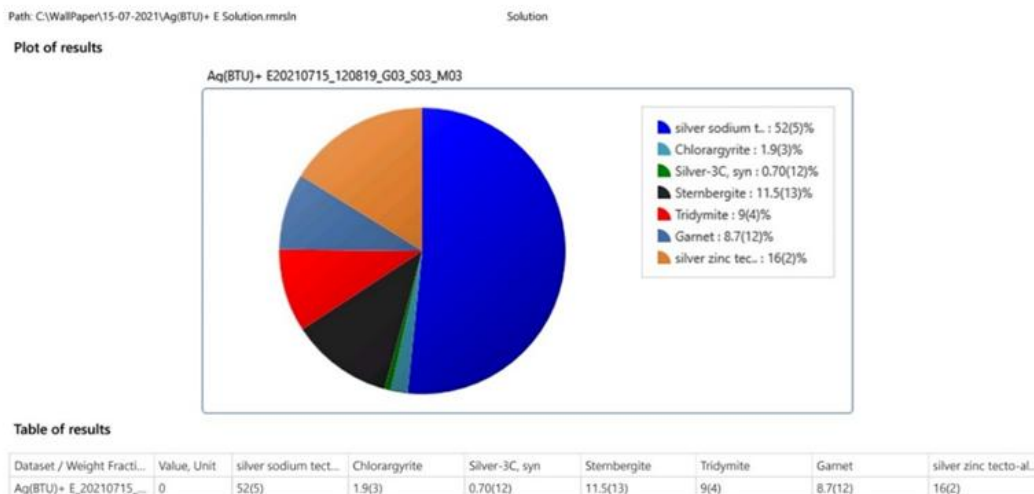


Figure 4: Peak of Silver (I) N-benzoylthioureato complex at FWHM

Physical properties and solubility test result



Figure 5: Zones of inhibitions of ligands and metal complexes and tube dilution of the test samples.

Table 1: Physical characteristics of the ligands and metal complexes

S/N	Ligand/Complex	Colour	M.Pt (°C)	Yield %	Physical appearance
1	L ²	White	110.5	88	Crystalline
2	[Cu(II)L ²]	Sky-blue	150.2	82	Crystalline
3	[Ag(I)L ²]	Dark-blue	188.7	78	Crystalline

Table 2: solubility of the ligands/complexes in different solvents.

S/N	Ligand/Complex	Distilled Water	Ethanol	DMSO	DCM	Acetone	Chloroform	CCl ₄	DCM/ ETOH
1	L ²	SS	SS, +Δ, S	SS	NS	NS	NS	NS +Δ, SS	SS, +Δ S
2	[Cu(L ²)]	SS	NS, +Δ, SS	NS	NS	NS	NS	S, +Δ, SS	NS, +Δ, SS
3	[Ag(L ²)]	NS	NS, +Δ, SS	NS	NS	NS	NS	SS	NS

KEY S= Soluble, SS= slightly soluble, NS= Not soluble, +Δ = plus heat

Table 3: Infrared spectral data for the ligand and metal complexes

S/N	Ligand/Complexes	λ_{\max} (nm)
1	N-benzoylthiourea (L^2)	903.4
2	[Cu(L^2)]	808.5
3	[Ag(L^2)]	400

Table 4: IR-spectral data of the ligands and complexes.

Peaks	L^2	[Cu(L^2)]	[Ag(L^2)]
ν (N-H)	3239.1	3239.1	3242.8
ν (C=O)	1692.2	1476.0(C-O)	1595.3(C-O)
ν (C-N)	1088.8	1606.5	1703.4
ν (C=S)	723.1	1397.8	1267.3

Table 5: crystal data of the complexes

Parameters	L^2	Cu(L^2)	Ag(L^2)
Crystal formula	$C_8H_8N_2SO$	C_8H_8CuSO	$C_8H_8N_2AgSO$
Formula weight(g/mol)	180.9	243.77	288.09
Crystal size (nm)		61.3	60.5
Crystal system		Triclinic	Cubic
Space group		p-1(2)	Pm-3m (221)
a (Å)		5.057	4.684
b (Å)		5.245	4.684
c (Å)		7.817	4.684
α , (°)		108.98	90
β , (°)		108.003	90
γ , (°)		90.422	90
Final magnetic moment (μ_B)		0.334	0.03
Formation energy/atom eV		-1.557	-1.09

Antimicrobial test results

Table 6: Zones of inhibition of the ligand along with their metal complexes (mm)

S/N	Compound	E.C	S.T	S.A	C.A
1.	L^2	15	16	25	22
2.	Cu(II) L^2	17	15	19	18
3.	Ag(I) L^2	21	19	26	27
4.	Imipenem	28	24	26	-
5.	Amphotericin	-	-	-	27

N.A = No activity, E.C = *Escherichia coli*, S.T = *Salmonella typhi*, S.A = *Staphylococcus aureus*, C.A = *Candida albicans*

Table 7: Minimum inhibitory concentration of ligand along with its complexes (mg/mL)

S/N	Compound	E.C	S.T	S.A	C.A
2.	L^2	3.13	12.5	12.5	6.25
4.	Cu(II) L^2	3.13	3.13	1.56	12.56
5.	Ag(I) L^2	1.56	12.5	6.25	12.5



Table 8: Minimum bactericidal/fungicidal concentration of the ligand along with its complexes (mg/mL)

S/N	Compound	E.C	S.T	S.A	C.A
2.	L ²	6.25	25	12.5	12.5
4.	Cu(II)L ²	3.13	12.5	1.56	12.5
5	Ag(I)L ²	1.56	25	12.5	25

3. Discussion

Physical Characteristics of the Prepared Complexes

The details of the noticeable characterization of the ligand and metal complexes are shown in Table 1; both the ligand and the complexes are crystalline solids. As typical of transition metal, the complexes are characterized by different colours. Cu(II)L² showed sky blue, Ag(I)L² showed dark blue. The table also shows the melting points of the ligand and the prepared complexes and their physical appearance. The L² ligand has a melting point of 110.5 °C which is lower than its Cu(II) and Ag(I) complexes. The melting point is also low if compared to the values 130-132 °C of N,N-dimethyl-N'-4-fluoro benzoyl thiourea reported by Gun et al. (2013). The [Cu(II)L²] has its melting point increased to 150.2 °C while that of [Ag(I)L²] is 188.7 °C. The change in the melting points of the complexes in contrast to ligands also suggests possible complexation. Nur et al. (2012), in the synthesis, characterization, and antibacterial studies of Cu(II) complexes of thiourea, the ligand 1,2-bis (N'-2-methoxybenzoylthioureido)-4-chlorobenzene has a melting point of 192.7 °C, after complexation, the melting point of the Cu(II) complex formed was 169.0°C, hence there was a decrease in melting point after complexation. The behavior of the ligand with the metal complexes agreed with the literature of Nur et al. (2012), Gun et al. (2013) and according to Iorungwa et al. (2019), complexation has a greater influence on melting points which could indicate the stability of complexes.

Solubility

The results of solubility tests of the complexes and the ligand in some solvents are shown in Table 2. The table shows that the ligand with the complexes are slightly soluble in non-polar solvents like ethanol and tetrachloromethane but completely soluble on application of heat. The ligand N-benzoylthiourea is barely soluble in ethanol but entirely soluble in a dichloromethane/ethanol mixture of 1:1. The solubility of the complexes in non-polar solvents explains the molecular nature of the complexes, which suggests that they are non-polar compounds (Yiase et al., 2014). The forces of attraction that act between the particles of a nonpolar compound are weak dispersion forces, which prevent nonpolar compounds from dissolving in water. Nonetheless, the non-polar molecules exhibit a stronger attraction towards one another in comparison to the polar water molecules.

Electronic Spectral

The UV-visible spectral data of the ligand with the complexes were recorded in ethanol between 400 and 950 nm at ambient temperature. The electronic spectra data of the ligand N-benzoylthiourea had λ_{\max} at 903.4 nm, as shown in Table 3. The complexes showed sharp absorption bands at 808.5 nm for Cu(II) complex and 400 nm for the Ag(I) complex. The shifting in the λ_{\max} of these bands to lower wavelengths suggests possible electronic transition and as such coordination of the ligand to the metal ions thereby resulting in the formation of the complexes. Bands in the visible region are attributable to transitions or metal charge transfer, d-d transitions, and transitions centered on the ligand (Yiase et al., 2014 and Gajendra et al., 2010). According to Iorungwa et al. (2019), these transitions could be from $n \rightarrow \sigma^*$, $n \rightarrow \pi^*$, $\pi \rightarrow \pi^*$.

IR Spectra

The IR spectra of the acylated ligand with the metal complexes were compared to examine the chelation behavior. FT-IR was taken with KBr pellets between 4000 cm⁻¹ and 600 cm⁻¹. FT-IR spectra of the synthesized N-benzoyl thiourea, and its Cu(II) and Ag(I) complexes showed sharp and strong absorption bands at (3261.4, 3239.1, 3339.7, 3239.1, 3339.7, 3242.8) cm⁻¹; which are attributed to the N-H group's stretching vibration. $\nu(\text{C}=\text{O})$ stretching vibrations were also observed in the range 1643-1690 cm⁻¹, which is consistent with findings from the literature. The $\nu(\text{C}=\text{O})$ bands were shifted to lower frequencies following complexation, which signifies the coordination of the



metal. This is evident by the strong peak recorded at 1420–1595 cm^{-1} region due to the absorption of the C–O stretching vibration that took part in the coordination (Nur et al. 2012).

The $\nu(\text{C}=\text{S})$ stretching vibration was observed at 723.1 cm^{-1} , which was expected. The metal complexes of the benzoyl thiourea after complexation showed $\nu(\text{C}=\text{S})$ stretching vibrations at higher frequencies of 1397.8 cm^{-1} for Cu(II) and 1267.3 cm^{-1} for Ag(I), suggesting a coordination of the metal through the C-S; this shows that the complexes are bidentate (Gun et al., 2013). Another intense absorption band is observed at 1600-1703.4 cm^{-1} which indicates C-N fragments. These conclusions conform with other literature reviewed (Siti et al., 2011; Gun et al., 2006).

X-Ray Diffraction Analysis

X-ray diffraction (XRD) was used to assess the crystal complexity of the metal complexes. A crystal is a solid made up of atoms arranged in a three-dimensional periodic pattern.

As a result, the atomic configurations in gases and liquids lack the necessary need for periodicity, which sets crystals apart from these substances fundamentally. However, not all solids are crystalline; some, like glass, are amorphous and lack a regular atomic structure within. In actuality, an amorphous solid and a liquid are identical, even though the former is frequently referred to as an "undercooled liquid."

The XRD data analysis reviewed that the sharp diffraction peaks obtained at different angles of incidence indicate the crystalline features of the metal complexes. The peaks were automatically matched with the data of the Joint Committee on Powder Diffraction Standard (JCPDS) now the International Centre for Diffraction Data (ICDD). The phase matching was restricted to mineral identification, in which emphasis was placed more on the possible phases containing the transition metal chelates. From the quantitative data obtained, different phases of the metals were obtained, which gave a possible idea that the metals were coordinated to the ligands. The possible ICDD data are shown in figures 1-4.

Since composite materials may consist of more than one crystalline material, this is indicated by variations in the positions of the peaks. The XRD data indicates the space grouping of all the phases identified; the space group can be used to determine the lattice symmetry of these complexes from standard data. Lattices serve as a kind of skeleton or structure around which the real crystal is constructed. They consist of unit cells, on which the three vectors x , y , and z describe the unit cells' sizes and forms. These vectors, also referred to as the cell's crystallographic axes, define the cell. They can also be explained by their lengths (a , b , c) and the angles (α , β , γ) that separate them. The lattice constants and lattice parameters of the unit cell are represented by these lengths and angles. Not only does the unit cell determine the vectors x , y , and z , but they also define the entire point lattice that allows for the definition or classification of the crystal (Cullity, 1956).

Table 5 and Fig 1-4 showed the crystal data of the complexes. The $[\text{Cu}(\text{L}^2)]$ was recorded at $2\theta \sim 21.9243^\circ$ with FWHM of 0.1378° and d -spacing of 4.05414\AA . The complex has a crystallite size of 61.3 nm, a crystal system (lattice symmetry) as triclinic with space grouping of P-1 and space group number 2. That of $[\text{Ag}(\text{L}^2)]$ was recorded at $2\theta \sim 10.7976^\circ$ with FWHM = 0.13782° and inter-planar spacing of 8.1938\AA . The complex has an average crystallite size of 60.5 nm with space grouping of Pm-3m and space group number 221, which is Cubic, in which lattice parameters $a = b = c$. The Copper II complex was recorded at a matching standard data DB card N0 00-001-0301 while the N-benzoyl thiourea to Silver (I) at DB card N0 01-004-078. The lattice symmetries of the complexes were influenced by their crystallite size (Jain et al., 2013)

Scherer's equation was used to calculate the crystallite sizes of the complexes.

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where D is the average crystallite size, K is a dimensionless shape factor with value 0.9, λ equals the X-ray wavelength, Cu $K\alpha$ is 1.5406\AA , β equals the line broadening at FWHM in radians, θ equals the Bragg's angle in degree.

Antimicrobial studies

The MIC (MBC/MFC) and inhibition zones, which are provided in Tables 6-8, were used to quantitatively examine the antimicrobial sensitivity of the ligands and metal complexes as well as their potency. The primary goal of the



antimicrobial screening process was to ascertain the pathogenic bacteria' susceptibility to the metal complexes and the doses at which their apparent development could be totally stopped or eliminated, making them potentially useful therapeutic agents. An antimicrobial effect of benzylamides and their complexes against three strains of bacteria was investigated (gram-positive *Staphylococcus aureus* and gram-negative *Escherichia coli*, *Salmonella typhi*) as well as fungi, *Candida albicans*

The minimum concentration of inhibition and minimum bactericidal or fungicidal concentration tests were conducted using the agar-dilution method to determine the minimum concentration at which the organism did not die and expanded beyond the spot of inoculation and the concentration at which the organism died. Table 6 presents the findings of the measurements of the susceptibility zones in diameter (mm). The clear areas surrounding the well that killed the germs were known as the susceptibility zones. Figure 5. In varying degrees, the amide ligands and the complexes showed inhibitory effects on the growth of the various species of microorganisms that were tested. Compared to positive controls, imipenem and amphotericin, their zones of inhibition were appreciable. As was seen, the antibacterial activity of the amide ligands increased when they were chelated to the metal ions (complexes), but the free ligand was only somewhat active against the microbe species.

The Cu(II) complexes showed much activity since they were active on all the organisms tested. The biological activities of the complexes varied with the test organisms. In general, both ligand and their metal complexes demonstrated efficacy on *S. aureus* and *C. albicans* with MIC ranging from 1.56 mg/mL - 12.5 mg/mL and inhibition zones of 8 mm -27 mm. This is contrasted with the control, Imipenem, a popular antibiotic that exhibited high inhibition zones measuring 24–28 mm.

The ligand with the complexes showed high efficacy on fungus (*Candida albicans*), which was comparable to a high extent to the control, Amphotericin which showed an inhibition zone of 27 mm at 0.05 mg/mL, this result is in close agreement with Chenielle *et al.* (2009), in their studies on crude extracts from *Callistemon* leaves, in which amphotericin was used as the positive control against *C. albicans*; the zone of inhibition obtained was 25 mm. Certain prevalent infections caused by *E. coli*, *S. typhi*, *S. aureus*, and *C. albicans* could plausibly be treated using the complexes made with N-benzoylthiourea as therapeutic agents.

The enhanced biochemical potential of bioactive species may be attributed to the higher activities of complexes (metal chelates) as compared to free ligands. Chelation decreases the polarity of the metal ion by partially sharing the positive charge with ligand donor atoms, which involves orbital overlaps and partial sharing of the metal ion's positive charge with donor groups, potentially leading to π -electron delocalization throughout the entire molecule. This makes the complex more lipophilic, which favours permeability through the lipid layers of the microbe membrane and may improve the complexes' penetration into the lipid membranes, allowing them to obstruct the metal binding sites in the microorganisms' enzymes (Iorungwa *et al.*, 2019). These complexes also interfere with the cell's respiratory function, blocking the production of proteins and preventing the organisms from growing further (Gajendra *et al.*, 2010).

Table 7 provides the minimum inhibitory concentration (MIC) values for each microbial organism. From the table, it is easy to see that the microorganism determines how efficacious the ligands and complexes are. For example, the sole gram-positive bacteria *Staphylococcus aureus* needed a low concentration of the ligand and the complexes (MIC of 1.56–12.56 mg/mL) to stop visible growth, and the gram-negative bacteria *Salmonella typhi* and *Escherichia coli* also needed a lower concentration of the ligand and the complexes (MIC range 1.56–12.5 mg/mL) to stop visible growth. To stop the fungus *Candida albicans* from growing visibly, the same MIC range applies. Table 8 lists the minimal bactericidal/fungicidal concentrations (MBC/MFC) for every organism.

Cu(II) and Ag(I) complexes have higher MBC/MFC values than their corresponding ligands. The L2 (N-benzoylthiourea) ligand showed reasonable efficacy against the tested organisms; however, their complexes showed MBC values of 1.56-25 mg/mL with zones of inhibitions (8-27 mm) indicating good antimicrobial potency.

4. Conclusion

The study revealed that amid complexes of Cu(II) and Ag(I), were synthesized from acylation of thiourea with benzoyl chloride. The complexes obtained exhibited good antimicrobial efficacy against the test organisms.



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