



Faculty of Science

Department of Chemical and Forensic Sciences

**SYNTHESIS, CHARACTERISATION, AND ANTI-BACTERIAL
STUDIES OF Cu(II), Co(II), Ni(II), AND Fe(II) COMPLEXES WITH
SCHIFF BASES**

by

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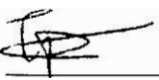
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DEDICATION

My thesis pays tribute to my mum, who has meant a lot to me and still does. Even though she is no longer with us, her memories still have an impact on my life.

ABSTRACT

According to studies, illnesses caused by drug-resistant bacteria cause approximately seven hundred deaths annually. Although several drugs are currently being developed in response to this matter, it is reported that the majority of these drugs are simply derivatives of the current class of antibacterial drugs, and as a result, they will only provide a temporary solution because bacterial cells will eventually develop resistance to them as well. In this regard a completely new class of compounds that are somewhat unconventional and could also provide new pathways and modes of action that are not observed by the current class could be introduced. Researchers have demonstrated that metal complexes have a positive degree of anti-bacterial potency.

The goal of this research work was to synthesize, analyse, and test antibacterial activities of Schiff base ligands denoted as 3N and 4N, based on the position of nitro group in the ligand, and their corresponding metal complexes. Schiff base ligands were synthesised through a condensation reaction between 4-nitrobenzaldehyde / 3-nitrobenzaldehyde and 2-amino-5-chlorobenzophenone to form 4N and 3N respectively. They were then complexed with Cu(II), Co(II), Fe(II), and Ni(II) metals and characterized by FTIR, ^1H NMR, and ESI-MS. Metal complexes were denoted as Cu4N, Cu3N, Co4N, Co3N, Fe4N, Fe3N, Ni4N, and Ni3N. All metal complexes, except for Cu3N and Cu4N, could not be comprehensively characterized by ^1H NMR due to their paramagnetic nature. According to FT-IR and NMR analysis, the "O" and "N" donor atoms of the Schiff base ligand took part in interaction with the metal (II) ions. The expected molecular formula of ligands and metal complexes were confirmed by relating their expected molecular weights with m/z values from ESI-MS data.

Gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* were used as test organisms to determine the antibacterial activity of the ligands and complexes. The test was done through a broth micro dilution test to find the minimum inhibitory concentration. When compared to their respective ligands, all metal complexes except Ni4N, displayed significant activity against both strains. Furthermore, the complexes were more active against gram-positive bacteria. This may suggest that these compounds' mode of action could be selective towards gram-positive bacteria.

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LIST OF ABBREVIATIONS

DNA : Deoxyribonucleic acid

CDC: Centre for Disease Control

AR: Antibiotic Resistance

MRSA: Methicillin-Resistant Staphylococcus aureus

AMR: Antimicrobial Resistance

FTIR: Fourier Transmission Infrared Spectroscopy

UV: Ultra-Violet

^1H NMR: Proton Nuclear Magnetic Resonance spectroscopy

ESI-MS: Electro-Spray Ionisation Mass Spectrometry

SC-XRD: Single-crystal X-ray Diffraction

MIC: Minimum Inhibitory Concentration

ROS: Reactive Oxygen Species

TLC: Thin-Layer Chromatography

TMS: Tetramethylsilane

ppm: Parts per million

1.0 INTRODUCTION

1.1 BACTERIA

1.1.1 Structure

Bacteria, Archaea, and Eukarya are the three fundamental domains into which all living things are typically classified based on their structural and biochemical properties, and DNA sequencing [1]. All other living things, including animals, plants, fungi, are classified as eukaryotes. Prokaryotes are bacteria and archaea which are single celled microorganisms with no nuclei and other membrane enclosed organelles [2].

The bacterial morphology is less complex when compared to eukaryotic cells. This is owing to the lack of nucleus and membrane enclosed organelles like mitochondria and endoplasmic reticulum. The morphological structures that bacteria have enable them to carry out some physiological processes. For example, flagella are used for locomotion, while fimbriae and pilli are used to collect food from a cell's surroundings and aid in adherence to other cells and surfaces [3]. Bacterial cells are enclosed by some layers called the cell envelope. The cell envelope is made up of the cytoplasmic membrane, the peptidoglycan cell wall, and the external membrane. Some bacteria have a capsule as depicted in Figure 1 which acts as a protection to the bacteria from ingestion and destruction by white blood cells [4].

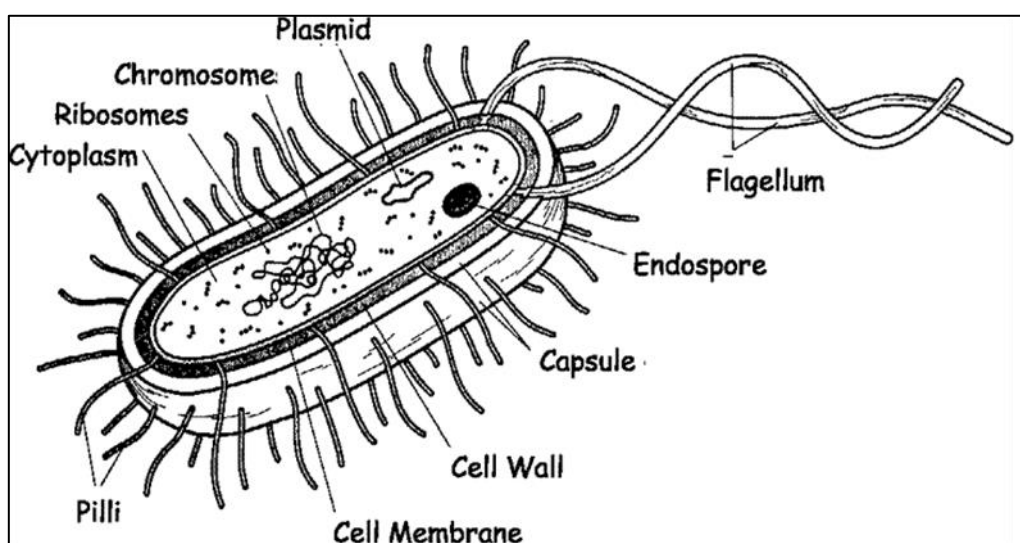


Figure 1: The schematic diagram of bacterial cell structure [4].

The cell wall, which is made up of peptidoglycan, is situated on the surface of the cell membrane. It aids in determining if a bacteria is gram positive or gram negative. Gram-positive bacteria have a dense peptidoglycan layer on top of the plasma membrane in contrast to gram-negative bacteria, which have a thin peptidoglycan between 2 membranes (surface and plasma). This difference is depicted more clearly in Figure 2.

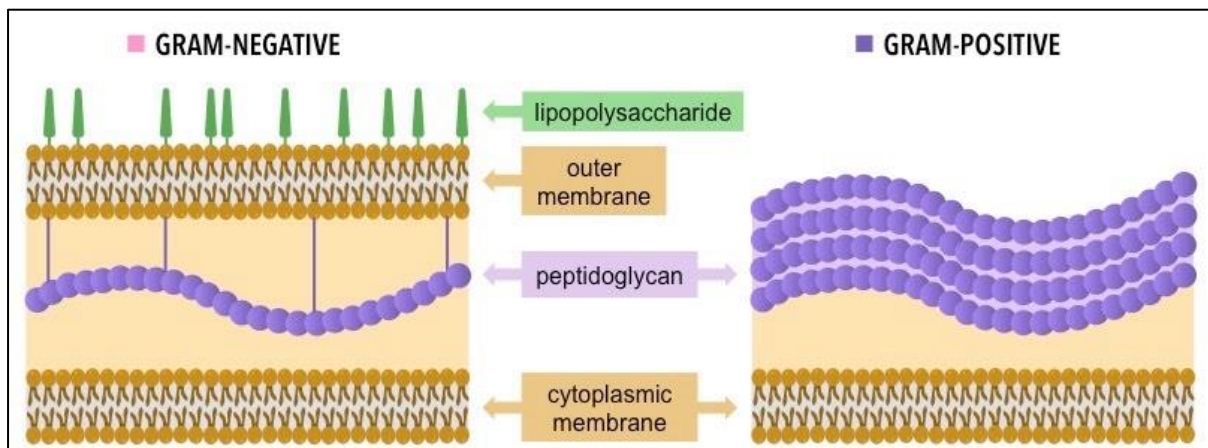


Figure 2: The cell composition of gram-positive vs gram negative bacteria [5].

Beneath the cell wall lies the cell membrane which is the most vital layer as it encompasses the cytoplasm, and it is mainly composed of water, enzymes, organic molecules, and other important cell components. The plasma membrane is also made up of a phospholipid bilayer, it is a permeability barrier for the majority of molecules and it is the site where molecules are transported into cell [6]. It also helps keep the interior of the bacteria in a highly organized state by selectively allowing only a certain ions and molecules access into the interior as well as to the exterior. Plasma membrane also plays significant roles in synthesis of lipids and cell wall components, cell respiration, and process of photosynthesis [7].

The membrane is made of the phospholipid bilayer which makes a strong barrier between two aqueous compartments. The phospholipid molecule is made of phosphate group and a lipid. This molecule is asymmetric with hydrophilic polar heads and hydrophobic non-polar tails. A number of models have been suggested before to describe the arrangement and morphology of the plasma membrane, but the “fluid mosaic model” has been the widely accepted theory. According to this model

phospholipid layers are arranged opposite to each other facing opposite direction. They are arranged in such a way that the hydrophilic polar ends of the phospholipid are directly exposed to the external environment and the internal or cytosol. These two environments are usually aqueous, and in case of an exposure to a nonpolar environment/solvent the membrane shifts the arrangement and the non-polar ends of the phospholipid face the external environment and this ends up damaging the membrane.

The phospholipid bilayer is integrated with molecules like proteins distributed throughout the layer. Peripheral proteins which are loosely attached on the external part of the membrane can move freely as they are loosely bound to the layer. Integrated proteins are totally merged into the bilayer and are strongly attached such that they cannot freely move.

1.1.2 Pathogenicity

Bacteria like other living organisms evolve over time. They gain or lose some genomic traits that allow them to adapt. This process of adaptation may afford them the ability to invade a particular environment better, surviving longer or avoid being detected by the immune response. Some of these adaptations does bring havoc to the human body. Bacteria may develop heightened virulence factors which will allow them to effectively cause diseases.

They have an ability through their fimbriae or pilli to adhere to surfaces within the body and establish a microbial presence and multiply. Other bacteria have adhesins on the tips of this pilli that gives them the ability to bind firmly to certain cells in the body [8]. *Neisseria gonorrhoeae* for example, have a pilli which allows it to bind specifically to oligosaccharide receptors on the epithelial cells [9].

Formation of biofilms (collection of one or more types of microorganisms) is another bacterial adaptation that promotes invasion or colonization of a human body by the bacteria [10]. This occurs when bacteria form polysaccharide-rich sticky webs that bind bacterial cells into a protected community, providing safety from antibiotics or natural body defense [10]. Also, bacteria can produce harmful toxins that are meant to either attack other bacteria or counter act the body's natural defense mechanisms [11]. Toxins normally cause lysis of cells or trigger destructive Immune reactions. Some diseases have symptoms that are entirely attributable to toxin production, with damage

happening at the site of infection, whereas others may travel through the bloodstream and cause symptoms elsewhere in the body [11].

The compounds that make up the bacterial cell wall can set off prevailing set of chemical reactions within an immune system. For example, the peptidoglycan in gram-positive bacteria and its side products, can stimulate a fever or inflammation [12]. Liposaccharide which is found on the surface of cell membrane of a gram-negative bacteria is endotoxin. Endotoxins can induce extremely high fevers, shock, or skin lesions, all of which can be fatal. Exotoxins can be produced by either type of bacteria. These include proteins that cause cytolysis which occurs when a cell bursts due to an osmotic imbalance, or receptor-binding proteins that can cause cell death [13].

Bacteria can evade our immune systems through a variety of mechanisms.

- They may modify surface proteins to avoid detection.
- Hide within body cells
- Inactivate anti-bacterial defenses

Some bacteria's most pathogenic characteristics is capsules. Capsules can imitate the surface of the host cell, protecting bacteria from immune responses. [11].

Basically, bacteria have a varied range of ways they can evade the immune system and cause illnesses. Some might express one virulence mechanism while others might express several different mechanisms. Altogether these mechanisms are what triggers symptoms, diseases and even death. Mycobacterium tuberculosis is a strain of bacteria which caused one of the deadliest infectious diseases (tuberculosis) in the early 21st century [14]. This airborne disease is among the deadliest diseases in the world, affecting nearly 2 billion people worldwide. World Health Organisation (WHO) estimated that 1.4 million people died from TB in 2019 worldwide. Other serious bacterial diseases include cholera, gonorrhoea, syphilis, diphtheria, and others [15].

1.2 ANTIBIOTICS

1.2.1 Brief history

Antibiotics are medications that destroy or slow down the growth of bacteria. The discovery and successive large-scale production of antibiotics was one of the most crucial medical developments in history [16]. Many infectious diseases were no longer

a threat and life quality and expectancy were greatly improved as a result of these drugs [17].

The foundation of the modern “antibiotic era” is normally associated with Paul Ehrlich and Alexander Fleming [18]. German scientist, Paul Ehrlich noticed that some dyes only colored certain types of bacterial cells. He concluded that it must be possible to develop chemical compounds that can kill specific bacteria while not harming other cells [19]. In 1904 he started an extensive screening program where he discovered that arsphenamine worked well to treat syphilis. This became the first modern antibiotic. Paul Ehrlich’s systematic screening method became the foundation of drug discovery efforts in the pharmaceutical sector, resulting in the discovery of hundreds of medications, including a variety of antibacterial drugs. In 1928, Scottish bacteriologist, Alexander Fleming discovered penicillin when he saw that *Staphylococcus aureus* could not grow in a culture that had a green mold [20]. The mold, known as *Penicillium notatum*, produced an active agent known as penicillin. However, Fleming encountered difficulties with its purity and provided the *Penicillium* to anyone who requested it [18]. In 1940, a paper outlining the purification of penicillin in clinically useful amounts was published by an Oxford team led by Howard Florey and Ernest Chain [20]. They shared the 1945 Nobel Prize in Medicine with Alexander Fleming for their contributions to the development of the first mass-produced antibiotic. [18].

1.2.2 Mode of Action

There are so many different classes of antibiotics that have been discovered ever since the first anti-bacterial drug [21]. All of them exert their action against the bacteria differently. Some classes of antibiotics act against one type of bacteria and fail against another. This is based entirely on their modes of action.

Aminoglycosides are a class of antibiotics that kill off gram-positive bacteria specifically by inhibiting the protein synthesis [22]. Bacteria use proteins for many purposes like structure formation, as enzymes, or for transport. A cell needs ribosomes to convert DNA into proteins. Ribosomes are part of a cell where protein synthesis takes place. The ribosome contains the rRNA (ribosomal RNA) molecules which direct the stitching together of amino acids to make a protein molecule, a process called translation. Ribosomal subunits form a sandwich on the strand of

mRNA (messenger RNA) as shown in Figure 3, attracting tRNA (transfer RNA) molecules bound to amino acids. The ribosome subunits decode the mRNA sequence into a long chain of amino acids forming a polypeptide, or a new protein. Inhibiting these ribosome subunits from performing their roles will automatically stop process of protein synthesis. Eukaryotic and prokaryotic ribosomes are different from each other. Human cells have 60s and 40s subunits while bacterial cells have 50s and 30s subunits [23]. These differences are exploited by aminoglycosides for selectivity in killing off the bacteria and not affecting the host.

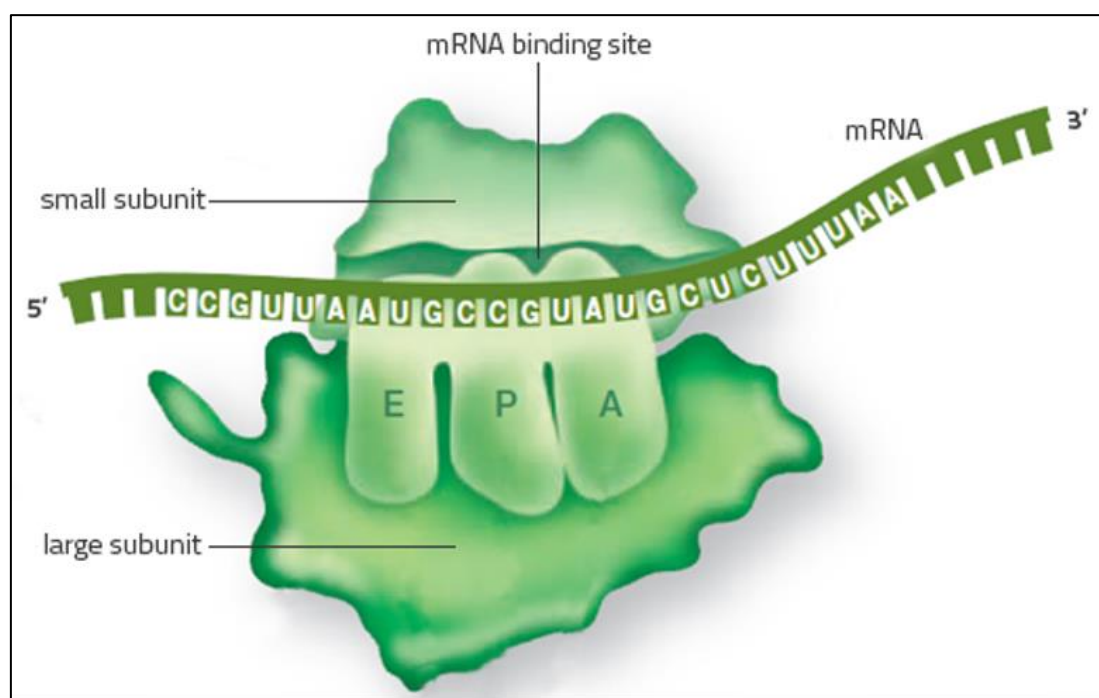


Figure 3: Ribosome structure [24]

Cephalosporins are class of antibiotics that kill off both gram positive and gram-negative bacteria by preventing cell wall production [25]. Cell walls are made of peptidoglycan, which is arranged in crystal lattice composed of sugars that are connected by amino acids/peptide links. Cephalosporins break these bonds between proteins that hold the sugar molecules together. This leads to the breaking apart of the cell wall. Without the cell wall, the water will rush into the cell due to its hyper osmotic environment, resulting in cell burst. Examples of antibiotics in this class of antibiotics is Cefazolin and Cefadroxil. Beta-lactam is another class of antibiotic that kills the bacteria through this mode of action [26]. Penicillin G, an antibiotic belonging to this class has four-membered β -lactam ring that can bind to DD-transpeptidase to irreversibly inactive it [27]. DD-transpeptidase is one of bacterial enzymes that are

involved in synthesis of bacterial cell wall [28]. As a result, the bacteria are unable to construct their cell wall. Weakening of the cell wall ultimately leads to cell burst due to osmotic pressure.

Quinolones/Fluoroquinolones- This class of antibiotics kills off both gram-negative and gram positive bacteria by inhibiting DNA from replicating [29]. In order for bacteria to stay alive, the DNA needs to replicate and create more copies of itself. The double stranded bacteria DNA is usually wrapped around itself and to make copies it first needs to unwind. Topoisomerases are enzymes that participate in the overwinding or underwinding of DNA. Quinolones and fluoroquinolones stop the topoisomerase from unwinding the DNA and therefore stop DNA from replicating consequently preventing bacteria growth. Other class of antibiotics include Macrolides which kills gram positive bacteria by inhibiting protein synthesis, sulfonamides inhibit folate synthesis, and glycopeptides who inhibit cell wall synthesis.

1.3 ANTIBACTERIAL RESISTANCE

Antibacterial resistance occurs when an antibiotic fails to damage the bacterial population sufficiently to treat the disease. Resistance frequently starts with a large bacterial population where a small percentage is naturally resistant to the antibiotic, through random genetic alterations or by gaining resistant genes from other microbes. [15].

Bacteria adapt to antibiotic attacks using two main genetic tactics. Alteration in genes that are linked to the compound's mechanism of action, as well as gaining of external DNA coding from resistant strains through horizontal gene transfer (HGT). According to a report by Centre for Diseases Control and prevention (CDC) [30], some other forms of antibiotic resistance mechanisms include,

- Restricting an antibiotic an access to the internal morphology of a bacterial cell, e.g. Gram-negative bacteria have an outer membrane that shields them from their surroundings. This membrane can be used to prevent antibiotics from penetrating the interior.
- Eliminating antibiotics either by discarding them out though their cell wall pumps or using enzymes to break down the drug.

- Coming up with new cellular processes that evades antibiotics mode of action or change the morphology of the antibiotic's target

Antibiotics kill most vulnerable portion of the microbial population and leave few strains that are resistant. Continued use of this particular antibiotic will eventually lead to development of major resistant strains in the population.

According to a 2018 World Health Organization report, resistance to penicillin ranged from zero to 51 percent [31]. The report further states that between 8% and 66% of *E. coli* causing urinary tract infection was resistant to ciprofloxacin, a common antibiotic used for the treatment of this condition. This report confirms the global severity of the antibiotic resistance crisis.

For as long as antibiotics have been around, microorganisms have been developing defenses against them. Antibiotic resistance was identified in the early 1940s, following the first large-scale clinical applications of penicillin[19]. In 1940s penicillin was introduced, around 1960s, over 80% of *S. aureus* strains were found to be resistant to penicillin [32]. Tetracycline was introduced in 1948, and resistance was eroding its effectiveness by the end of the 1950s [33]. Erythromycin was discovered in 1952, and resistance to it appeared in 1955. [34]. A lab-synthesised drug called methicillin was developed in 1960 to specifically combat penicillin resistance, however within a year, *S. Aureus* developed resistance against it as well, and was later called MRSA, methicillin-resistant *Staphylococcus aureus*. [35]. Bacteria adapted each time pharmaceutical chemistry created a new class of antibiotics with a new molecular shape and mode of action.

The CDC first released the AR Threats Report in 2013 [30]. According to the report, at least 2 million people get infected by antibiotic-resistant strain in the United States. It further states that, about 250,000 people are hospitalized for *Clostridium difficile* (*C. difficile*) infections, and at least 23,000 died from bacterial infections.

According to a 2019 CDC (Center for Disease Control) report on AR THREATS, over 2.8 million infections caused by antibiotic resistant strains occur in the United States each year, with over 35,000 people dying from the infections [30]. Furthermore, in 2017, there were 223,900 issues of *Clostridioides difficile*, and about 12,800 people died from bacterial infections. Some of the minimum morbidity and mortality estimates from antibiotic resistant infections that were recorded were multidrug resistant

Pseudomonas aeruginosa, with around 6700 annual estimated cases and 440 estimated annual number of deaths [30].

1.4 METAL COMPLEXES

Humans depend on metal ions for a several functions. Some of the diseases that can result from lack of metal ions include, pernicious anaemia due to lack of iron, heart disease in babies caused by copper deficit, and growth retardation due to lack of dietary zinc [36]. The use of metals for health and healing is an old practice that has been carried out for thousands of years [37]. According to Chang *et al*, the Chinese, as far back as 2500 BC, practised what is known as chrysotherapy, where they used elemental gold to treat various illnesses [38].

Since the discovery of cisplatin and its success in cancer treatment, more medicinal applications of various metal complexes have been investigated [39]. A lot of different coordination compounds have been discussed regarding the development of new antitumor agents [40]. This massive study of coordination compounds gave rise to the discipline of bioinorganic chemistry, which examines metal complexes and their roles in biological systems. Bioinorganic chemistry opened new prospects for research in coordination compounds thus leading to the discovery of some anti-microbial and anti-inflammatory activities of most metal complexes [41]. Metal complexes consists of metal ions which are part of a chelating resin on which complexing agents, such as iminodiacetic or nitrilotriacetic acid, are chemically grafted [42].

Some metals are known to prevent cytotoxicity and in-vivo antagonize Cd- induced carcinogenesis [43]. Nowadays, metal and metalloid elements like platinum, copper, arsenic, silver, titanium, vanadium, iron, ruthenium, and technetium are included into many therapeutic drugs [44]. As an example, silver sulphadiazine is used in treatment of open wounds [45]. It works by stopping the growth of bacteria that may infect an open wound. Chromium complexes and Copper histidine complexes are used as antidiabetic supplement and for Menkes disease treatment respectively [44].

1.5-PROBLEM STATEMENT

Currently there is a serious global surge in emergence of multi-drug resistant bacterial infections and diseases that conventional antibiotics are not able to alleviate [46]. In 2016 O'Neil J estimated that around 700,000 people perish annually because of drug resistant bacterial infections [47]. Although there are several drugs being developed

currently to tackle this eminent problem of AMR (Anti-microbial Resistance), it is reported that most of these drugs are just derivatives of the current class of antibacterial drugs, as such, they will only provide a short-term solution as bacterial cells will eventually develop resistance to them too. In that regard a completely new class of compounds could be introduced that are somewhat unorthodox and could also provide new pathways and modes of actions that are not observed by the current class. Metal complexes have been demonstrated by previous researchers to have a positive degree of anti-bacterial potency [48],[49],[50]. The knowledge and research of antibacterial studies in this class of compounds is limited hence this research was undertaken.

1.6-AIM AND OBJECTIVES

The aim of this research is to synthesise, characterize, and to test antibacterial activity of Cu(II), Co(II), Fe(II), and Ni(II) complexes

Objectives

1. To synthesize Schiff base ligands.
2. To synthesis Cu(II), Co(II), Fe(II), and Ni(II) complexes with Schiff base ligands.
3. To use different characterization tools (FTIR, H-NMR, Elemental analysis, single crystal XRD, ESI-MS) to elucidate the structure of the compounds.
4. To run anti-bacterial screening of ligands and complexes.

2.0 LITERATURE REVIEW

2.1 METAL COMPLEXES AS ANTIMICROBIAL AGENTS

Coordination compounds, also known as metal complexes, are formed when a metal ion is attached to one or more molecules. Such complexes are made of a metal centre that are bounded by molecules called ligands. Ligands can be divided into monodentate or polydentate as shown in Figure 4. Monodentate ligands are those that bind to the metal through only one atom, and polydentate ligands have more than one donor atom and form chelates.

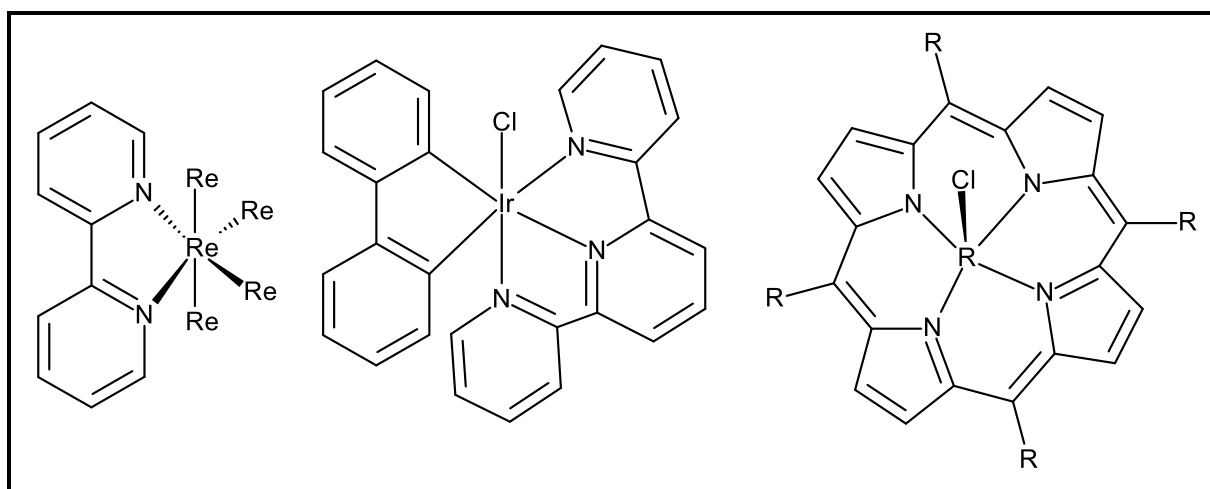


Figure 4: Examples of metal complexes adapted from [51]

Throughout the twentieth century, metal-containing compounds played a small but significant part in medicinal chemistry [52].

According to recent research, different metals cause diverse forms of microbial cell injury by causing protein dysfunction, oxidative stress, and membrane damage [53]. Metal compounds with antimicrobial properties, such as metallic coatings are being employed on medical devices and nanomaterials, which provide a wide range of uses in agriculture, and healthcare [54].

The role of coordination of metal complexes and ligands towards antibacterial activity is explained using Overtone's concept and chelation theory [55]. According to chelation theory, metal ion's polarity is highly reduced due to overlap of the ligand's orbital electrons and sharing of the metal ion's positive charge with the ligand's donor groups. The greater delocalization of electrons over the entire complex lowers the polarity of the complex, enhancing its lipophilicity. Overtone's concept state that the phospholipid membrane surrounding the cell allows only lipophilic molecules to pass

through. Many reports have also shown that metal complex's antimicrobial properties are sometimes greater than those of their typical organic medicines, due to the fact that metal complexation can result in variable degrees of synergistic action of both metal and ligand [50]. According to some reports, metal-based antimicrobial macromolecules are emerging as a viable alternative to conventional drugs due to their multiple mechanisms of action, which are a result of their unique properties, such as their capability to produce a variety of 3D geometries and metal's ability to exist in various oxidation states [53]. The geometry of a molecule is one of the most important aspects in determining its biological fate and activity and it has been reported that 3 dimensionality of molecules is associated to a greater clinical success rate [56]. Some reports demonstrated that various metal complexes had diverse modes of action towards bacterial cells, for example attacking of the citric acid cycle by a silver based complex, Inhibition of the TRX systems which leads to insufficient reducing power for DNA replication, and attacking of the bacterial cell membrane by a ruthenium complex [14], [57], [58]. Coordination of metal atom with protein thiol groups found in the cell wall, disrupt the cell wall synthesis of the bacterial cell during cell multiplication phase [59]. Because of these diverse biological activities, metal complexes have become increasingly popular in the field of research and medicine.

Chandrasekhar *et al* prepared three novel complexes of copper, cobalt and zinc metals with novel Schiff base ligand HL, [2((4-(dimethylamino)phenylimino)methyl)-4,6-di-*t*-butylphenol]. Their antimicrobial activity was studied against *Bacillus subtilis*, *S. aureus*, *Escherichia coli*, *Pseudo-monas aeruginosa* and fungal species of *Candida albicans*, *Pencillium chrysogenum* and *Aspergillus niger*. The results as shown in figure 5 (a) showed that metal (II) complexes had high antimicrobial potency in all pathogens compared to the Schiff base ligand as well as the standard drug, Amikacin. According to Chandrasekhar, antibacterial activity of the ligand and metal (II) complexes, is due to the generation of reactive oxygen species (ROS) [60]. The metal (II) ions and reactive oxygen species (ROS) attack the negatively charged bacterial cell wall, challenging the integrity of the cell wall leading to cell wall leakage and microbial death [60].

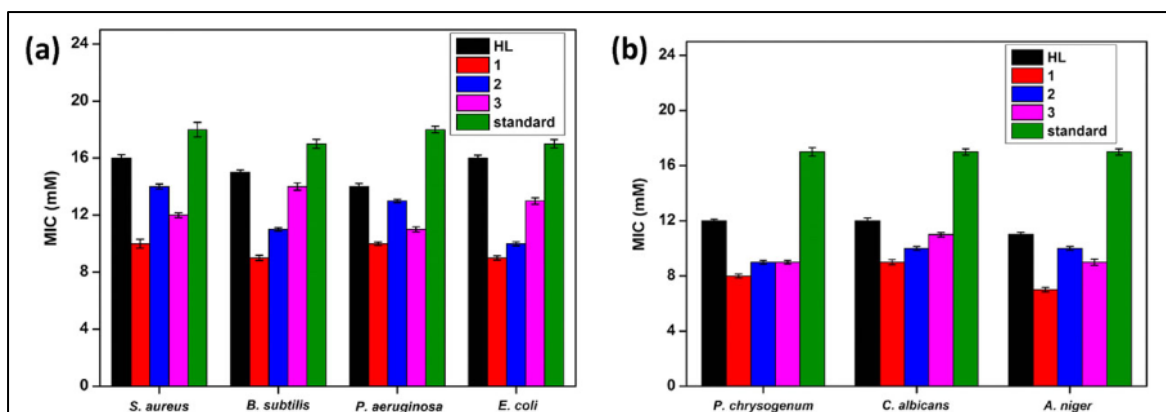


Figure 5: (a) Antibacterial and (b) antifungal results of Schiff base ligand HL and its metal (II) complexes 1–3 [60]

Chandrasekhar *et al*, further studied the mode of interaction of these complexes by the docking technique in order to understand the drug molecule-biomolecule interaction. Schiff base ligand exhibited high interaction towards the DNA duplex by binding to the minor groove region of the DNA. Metal complexes had better affinity than the Schiff base. Schiff base ligand had an atomic contact energy of -337.97 kcal/mol at the minor groove, but its equivalent metal (II) complexes had a significant increase in affinity, with ACE (atomic contact energy) values of -503.36, -381.67, and -388.21 kcal/mol, respectively. Both ligand and metal (II) complexes had a strong affinity for the minor DNA groove. The results indicated that the Schiff base ligand and metal (II) complexes interact with DNA preferably in the minor groove. Furthermore, the Cu(II) complex had the lowest ACE value of -503.36 kcal/mol, implying that it has a better DNA binding affinity than the Cobalt, Zinc complexes and ligand. This could be because Cu(II) complexes with aliphatic (t-butyl) groups have been shown to enhance hydrophobic interactions with DNA base pairs. The same compounds were further analysed for cell cytotoxicity against MCF-7 cancer cells. They showed remarkable results as they exhibited greater cytotoxic compared to the standard drug cyclophosphamide on MCF-7 cell.

2.1.1 Copper complexes

Cu(II) coordination plays an important role in the pharmacological profile of biological agents [61]. They show vast range of biological activity, including antiviral, anti-inflammatory, antitumor agents, enzyme inhibitor, anti-proliferative and biocidal [62]. Due to these various biological functions, copper has been coordinated with various organic ligands in an attempt to source and utilize its chemical properties in medicine

[63], [64]. According to Sovari *et al*, numerous scientific articles in the past few years have reported antibacterial properties of copper complexes [65].

Joseph *et al*, coordinated Schiff base ligands synthesized from furfurylidene-4-aminoantipyrine and aniline derivatives to copper to make Cu(II) complexes and tested them for antimicrobial activity [66]. The well diffusion experiment was used to test the effects of complexes against *S. aureus*, *E. coli*, *K. pneumoniae*, *P. vulgaris*, and *P. aeruginosa*, as well as the fungal species *A. niger*, *R. stolonifer*, *A. flavus*, *R. bataicola*, and *C. albicans*. Across all strains, all Cu(II) complexes showed excellent antimicrobial activity. MIC values for CuL1 and its ligand L1 ranged from 28-42 ug/ml and 60-72 ug/ml, respectively. The authors discussed the enhanced activity of metal complexes using Overtone's concept and Tweedy's chelation theory. These complexes also disrupt the cell's respiration process, interrupting the synthesis of proteins which limit the organism's ability to grow [66].

Joseph *et al*, studied the interaction of copper complexes with microorganisms, by mixing $[\text{CuL1}(\text{OAc})_2]$ with different microorganisms in a culture media. They recorded the UV-Vis spectra of the mixtures at different intervals of incubation. They stated that the 410 nm band of $[\text{CuL1}(\text{OAc})_2]$ complex vanished at first interval after interaction with the microorganisms. It was further noted that the $[\text{CuL1}(\text{OAc})_2]$ complex had a band at 332 nm, signifying that the complex underwent a first transition right away (shift to 410 nm), which was followed by a second transformation after 24 h. The loss of the Cu(I) complex's distinctive band at 332 nm suggests that a redox process occurred [66]. Some researchers suggest that this redox process may be responsible for antimicrobial activity and cell toxicity [67]. According to Raman *et al*, the redox potential of copper can be dramatically modified by modifying the ligand around the central copper ion. By potentially oxidizing essential cell components like lipoic acid, the O_2 and H_2O_2 created by such redox reactions causes cell toxicity. Furthermore, by inhibiting mitochondrial respiration and destroying cell viability, the reduced Cu(I) complex might disturb the process of DNA synthesis, energy production or ATP production [68].

Patel *et al*, synthesized and characterized $[\text{Cu(II)(PFL)(bpa)Cl}]\cdot 5\text{H}_2\text{O}$ (1) and $[\text{Cu(II)(LFL)(bpa)Cl}]\cdot 5\text{H}_2\text{O}$ (2) (BPA=bipyridyl amine, PFL=pefloxacin. LFL=levofloxacin) complexes as shown in figure 6 [69]. Compounds were screened

for antimicrobial activity on two Gram-positive and three Gram-negative bacteria. These compounds had diverse biological activity. Pefloxacin's antibacterial activity against *S. aureus*, *P. aeruginosa*, and *E. coli* was enhanced by coordination while its activity against *B. subtilis* and *S. merscences* was slightly low. Levofloxacin became more efficient against *B. subtilis* when coordinated, but less active against other microbes. According to Patel *et al*, this diversified biological activity could be related to Overtone's notion, chelation theory, or the impact of metal ions on regular cell processes [69]. Understanding and improving the action of metal complexes required determining their cellular uptake and even subcellular distributions [70]. Patel *et al*, used electronic absorption spectroscopy to assess DNA binding affinity of metal complexes. The intra ligand transitions of the complex were considerably disrupted in when the quantities of calf thymus DNA was increased, demonstrating interaction of the complex with DNA.

The binding constants (K_b) of the complexes were obtained by observing changes in absorbance at 274 nm when concentrations of CT DNA were increased [69]. The K_b values of both complexes were determined to be comparable to other DNA intercalative polypyridyl Ru(II) complexes although they were substantially lower than the K_b value of traditional intercalator ethidium bromide. These findings suggested that the complexes were attached to DNA via traditional intercalative mode or via partial intercalative mode [69].

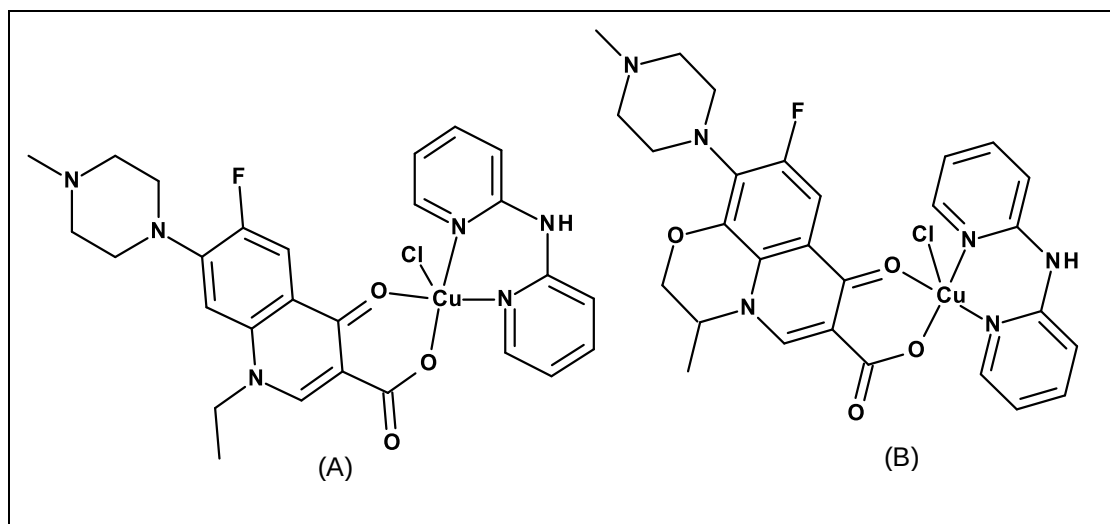


Figure 6: Structure of the complexes (A) $[Cu(II)(PFL)(bpa)Cl]$ and (B) $[Cu(II)(LFL)(bpa)Cl]$ [69].

Liu *et al*, synthesized crystal structure of Cu(II) complexes coordinated to thioureido ligands and studied their antimicrobial activities on *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Shewanella* [71]. They used agar paper diffusion method to determine the diameter of Zone of inhibition for each sample. The antimicrobial activities were calculated by the following equation.

$$\Delta r = \frac{d_1 - d_2}{2}$$

Where d_1 was the diameter of inhibition of zones and d_2 was the diameter of the wafer which was immersed in test samples. Based on their zones of inhibition the Cu(II) complexes, $[\text{Cu}(\text{L1})_2]$, $[\text{Cu}(\text{L2})_2]$ and $[\text{Cu}_2(\text{L3})_4(\text{DMF})_2]$ showed stronger inhibition than their corresponding ligands and CuCl_2 . The antibacterial activity of $[\text{Cu}(\text{L1})_2]$ was found to be equal to that of $[\text{Cu}(\text{L2})_2]$ [71]. This indicated that the position of fluorine did not have any impact on antibacterial activity since the difference between the two complexes was just the positioning of fluorine which was in para position in $[\text{Cu}(\text{L1})_2]$ and ortho position in $[\text{Cu}(\text{L2})_2]$ as depicted in Figure 7.

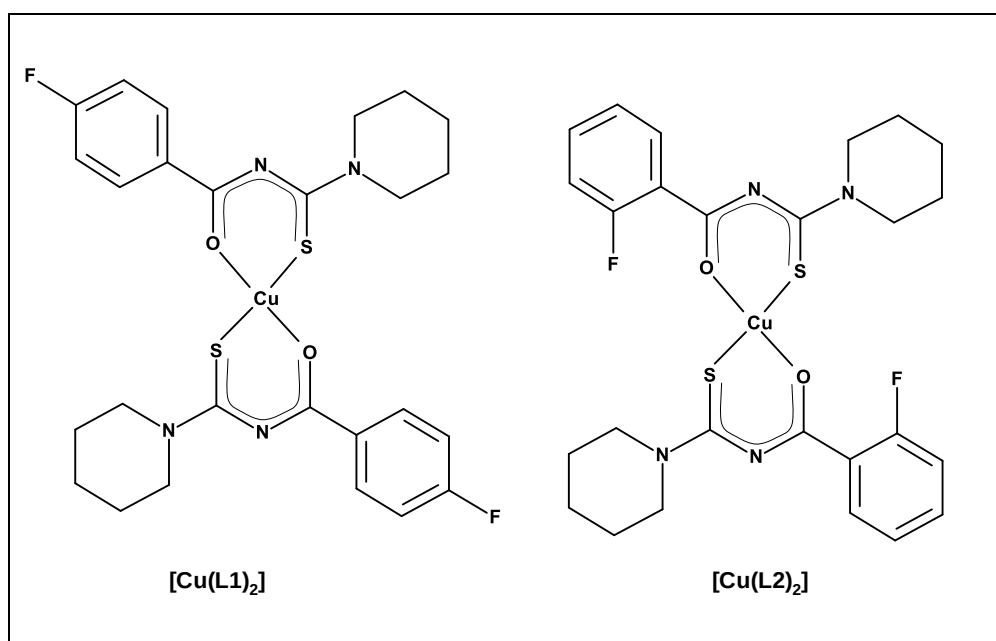


Figure 7: Proposed structures of $[\text{Cu}(\text{L1})_2]$ and $[\text{Cu}(\text{L2})_2]$ [71].

Oladipo *et al*, tested copper(II) N,N'-diarylformamidine dithiocarbamate complexes, for bacterial potency against Gram-negative, *S. typhimurium*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* and Gram-positive, *S. aureus* bacteria, including MRSA [72]. The

compounds showed excellent activities with the MIC values in the range of 6.25 ng/mL and 0.8 $\mu\text{g/mL}$. Krishnegowda and co-workers also prepared copper(II) complexes with 1-phenyl-1,3-butanedione which demonstrated strong activity against *B. cereus*, *Bacillus subtilis*, methicillin-resistant *S. aureus*, *E. coli*, *P. aerogenes* and *K. pneumonia* [73]. They had an impressive MICs ranging from 12.8–16.5 $\mu\text{g/mL}$, which was comparable to a standard drug ampicillin [73]. They found that the synthesis of these complexes was affected by both the counter ion and the solvents used in the reaction. Despite the fact that the reactions were conducted in the same manner, except changing solvent. Complex 1, synthesized under ethanol had a distorted square-pyramidal geometry as shown in Figure 8. Complex 2 which was synthesized in the solvent mixture of water: acetic acid had four coordination number (distorted square-planar geometry) as shown in Figure 8. Moreover, the stereochemistry of the complexes showed cis isomer for (complex 1) and trans for (complex 2) as confirmed by single crystal X-ray diffraction. In comparison, it was stated that complex 1 exhibited better inhibition compared to complex 2 due to effects of the solvent [73].

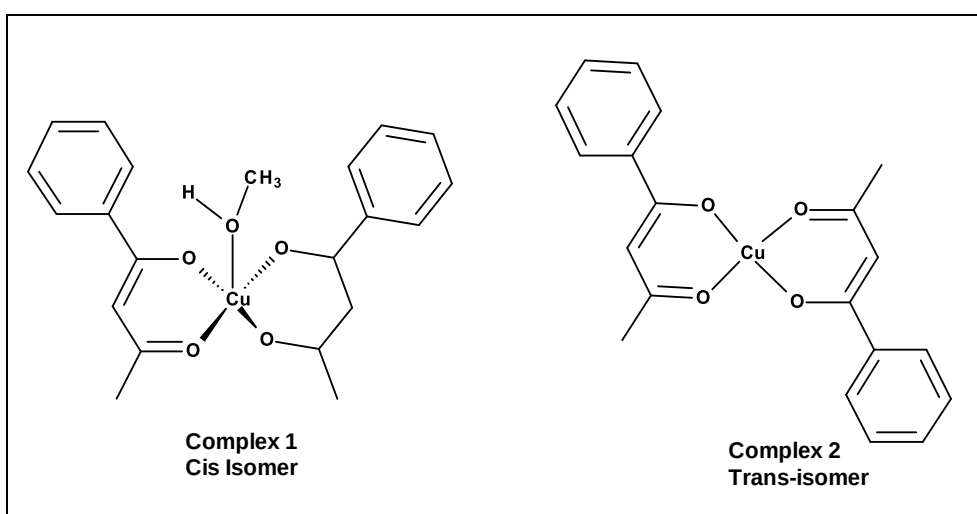


Figure 8: Depicting geometries of Complex 1 and complex 2 [73].

2.1.2 Cobalt complexes

Cobalt is a part of vitamin B₁₂ complex that aids in the prevention of anaemia and the production of erythrocytes [74], [75]. Because of the promising therapeutic and biological activities demonstrated by cobalt complexes, more research in cobalt complexes is undeniably necessary [76].

Gaëlle *et al*, reported antimicrobial studies of cobalt(II) and cobalt(III) complexes made from 1,10-phenanthroline with nitrate and azide co-ligands [77]. The cobalt(II)

[Co(phen)₃(NO₃)₂].2H₂O and Co(III) [Co(phen)₂(N₃)₂]NO₃ complexes were screened for antimicrobial activities against four pathogenic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*), and four fungi species *Candida albicans* ATCC P37039, *C. albicans* 194B, *Candida glabrata* 44B, *Cryptococcus neoformans* [77]. The coordination of the ligand to metal ion affected antibacterial efficacy against all tested strains with inhibitory zone sizes ranging from 13 to 38 mm. According to their results, cobalt (II) complex had better activity against bacteria, while cobalt(III) complex performed better against fungi. In general, the complexes remained effective against bacterial and fungal strains as compared to the reference drugs gentamycin and nystatin.

Irgi *et al*, synthesized cobalt(II) complexes by interacting Co(II) ion with oxolinic acid (a quinolone antimicrobial agent) and Lewis bases 2,20 -bipyridine, 2,20 -bipyridylamine, 1,10-phenanthroline, pyridine or 4-benzylpyridine shown in Figure 9 [78]. The compounds was tested against *Escherichia coli*, *Xanthomonas campestris*, *Staphylococcus aureus* and *Bacillus subtilis*, and were found to be active against most of the strains. They determined both IC₅₀ and MIC of the compounds. Based on the results, the compounds had equal activities against gram (-) bacterial strains by recording an MIC value of 2 mg/ml, while they showed less activity against the gram (+) strain, *S. aureus* recording an MIC value at 64 mg/ml or more [78].

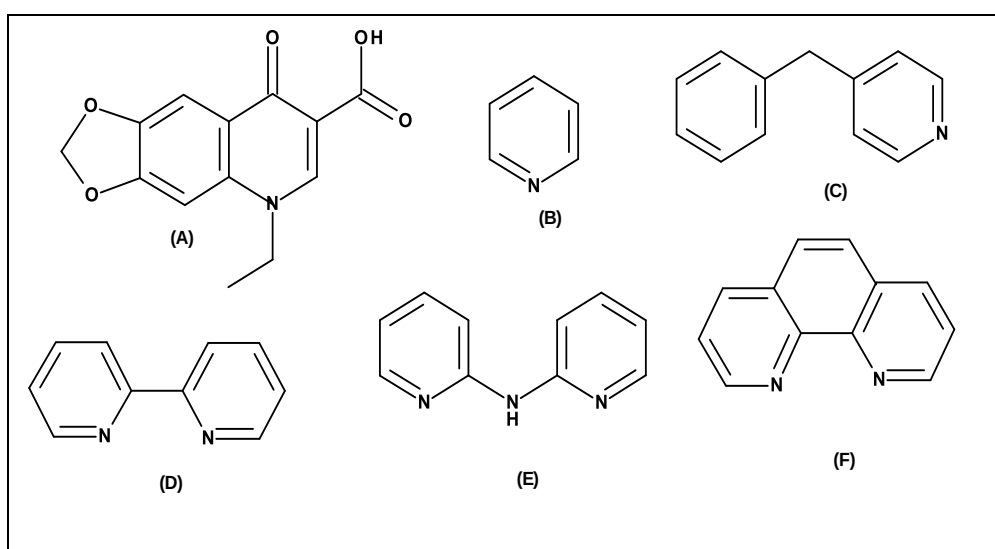


Figure 9: The structure of (A) oxolinic acid, (B) pyridine, (C)4-benzyl-pyridine, (D) 2,2-bipyridine, (E) 2,2-bipyridylamine, (F) 1,10-phenanthroline [78].

There are so many bio-active compounds with interesting biological properties that can be exploited [79]. However due to their low solubility, poor absorption and fast metabolism, their target delivery and bioavailability becomes poor which render them ineffective. These limitations can be overcome by enhancing their solubility through attaching metal ions, which improves their bioavailability and slow down the metabolism. Antimicrobial activities of curcumin have been previously reported by some studies [80], [81], [82]. Girish *et al*, coordinated cobalt (II) to curcumin, a plant extract which have biological properties such as antiviral, antibacterial, antifungal, and anti-cancer activities [83]. They complexed curcumin with cobalt to obtain Co(II)-complex as shown in Figure 10 and studied its antimicrobial activity.

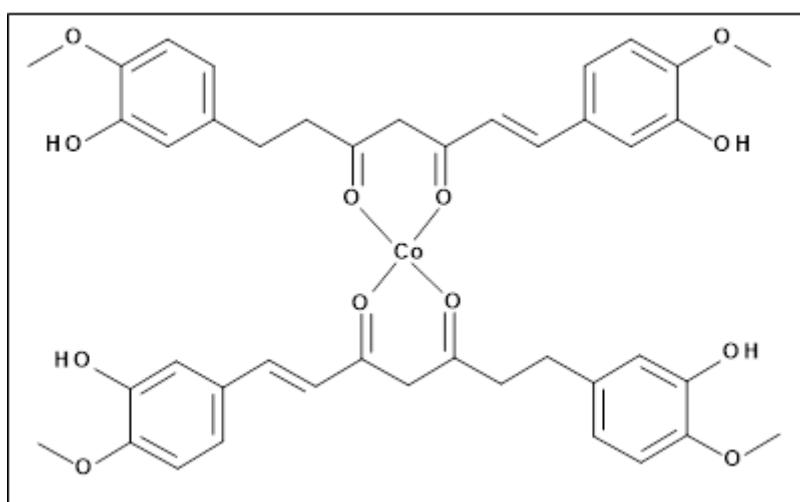


Figure 10: Proposed structure of Co(II) complex of curcumin [83].

Although both Co(II) complex and uncoordinated curcumin exhibited good antibacterial activity across most species, Co(II) complex had bigger zone of inhibition than the curcumin and standard antibiotic Azithromycin as shown in Table 1. This means that the introduction of Cobalt(II) ion to the curcumin enhanced its solubility which boosted bacterial uptake, which in turn raised antimicrobial activity[83].

Table 1: Antibacterial activity of Co(II)-Curcumin complex against bacteria [83].

Bacteria	Zone of inhibition (mm)		
	Curcumin	Co(II)-complex	Azithromycin
	6 mg/ml	6 mg/ml	(15 mg/disc)
<i>Bacillus subtilis</i>	15.1±0.3	21.1±0.3	20.4±0.3

<i>Escherichia coli</i>	11.1±0.3	13.8±0.2	14.3±0.3
<i>Klebsiella pneumoniae</i>	12.8±0.3	15.0±0.4	15.9±0.2
<i>Proteus vulgaris</i>	12.9±0.2	15.5±0.3	14.8±0.2
<i>Salmonella typhi</i>	13.8±0.2	16.5±0.3	17.2±0.3
<i>Shigella flexneri</i>	13.4±0.6	16.0±0.4	16.5±0.4
<i>Staphylococcus aureus</i>	14.4±0.2	19.5±0.4	18.8±0.2

2.1.3 Nickel complexes

Nickel is a d-block chemical element with the atomic number 28. It is crucial in the biological system because it's a critical element in the formation of secondary plant metabolites, which influences disease resistance [84]. It interacts with the iron in hemoglobin to aid oxygen transport, increase food absorption, and is considered a critical metal in various plant and animal enzyme systems [85].

There are several reports on nickel compounds and their biological activities like antimicrobial activities [86],[87], anticancer [88], and anti-viral activity [89]. In 2004, Shivankar and Taka used 8-hydroxyquinoline and deprotonated chiral saccharides such as (+)-glucose and (-)-fructose, as ligands to synthesize Co(II) and Ni(II) complexes [90]. They tested these metal complexes against bacteria such as *C. diphtheriae*, *E. coli*, *S. typhi*, *S. dysenteriae*, *S. aureus* and *V. cholerae* and compared their antibacterial activities. As per their findings, Co(III) complex had a superior antimicrobial activity compared to Ni(II) complex against several strains. Ni(II) complex however exhibited impressive activity on gram negative *E. coli* and gram-positive *S. aureus* with minimum inhibitory concentration of 100 ug/ml. Further studies were done on acute cytotoxicity of these complexes to determine their level of toxicity. Acute oral toxicity studies were done on albino rats. Respiratory disturbances, excitation, convulsions, tremors, and death were observed in animals following administration of the various doses. These observations suggested that the compounds were toxic.

Sheikhshoaie *et al*, synthesized Ni(II) and Zn(II) complexes [Ni(L1)], [Ni(L2)], and [Zn(L3)(DMSO)] as shown in Figure 11 and 12. They investigated their antibacterial efficacy on fungus species and eight bacteria species [91]. They synthesized nickel(II) and zinc(II) complexes with N₂O₂-Schiff base ligands made from diaminomaleonitrile and different aldehydes. The complexes were tested against Gram-positive bacteria

(*L. monocytogenes*, *E. faecalis*, *S. aureus*, *M. luteus* and *B. cereus*), some Gram-negative bacteria (*E. coli*, *K. pneumoniae*, *P. aeruginosa*) and a fungal species (*C. albicans*). All three complexes had descent antibacterial activities. The complex [Ni(L1)] demonstrated activity against three Gram positive bacteria but not against any Gram-negative bacteria. [Ni(L2)] showed no preference for Gram positive or Gram-negative bacteria as it was active across all but one *K. pneumonia* strain. $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ and complex [Ni(L2)] had antifungal activity against *Candida albicans*. *E. faecalis* and *L. monocytogenes* were resistant to ciprofloxacin and fluconazole, but susceptible to [Ni(L1)], [Ni(L2)], $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, and $\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$, respectively.

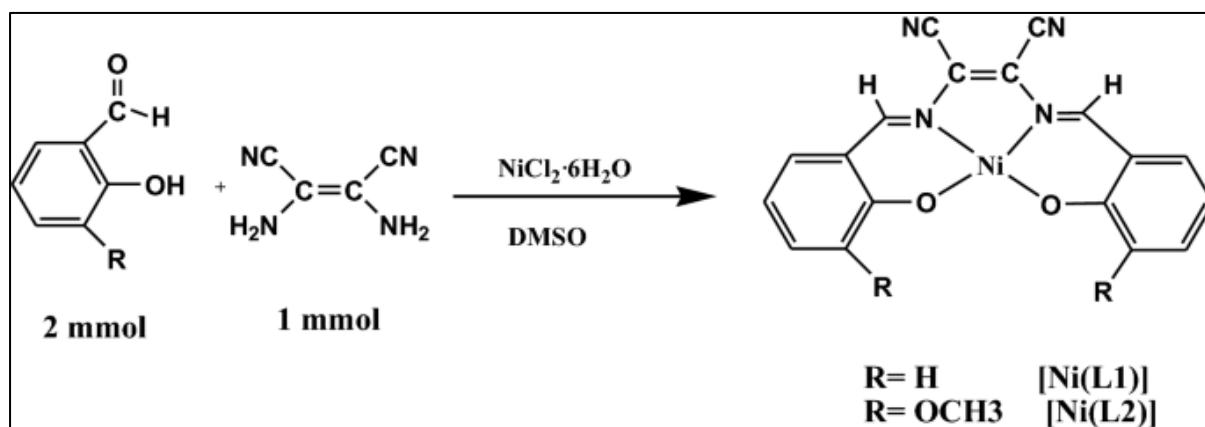


Figure 11: Schematic illustration of the synthesis of [Ni(L1)] and [Ni(L2)] complexes.
Adapted from [91]

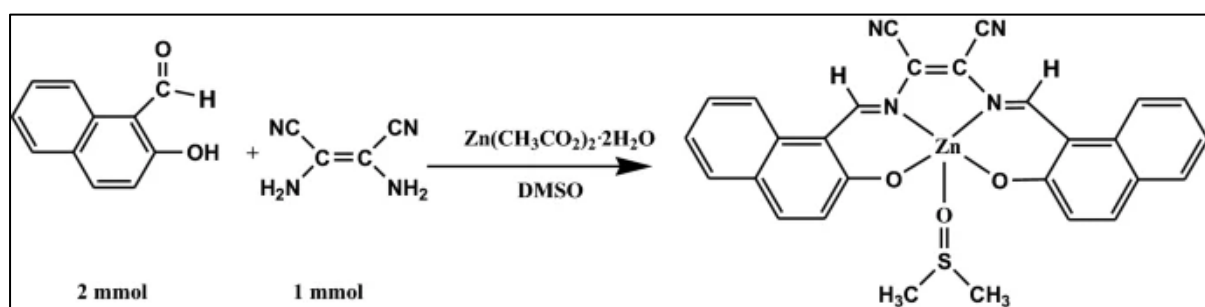


Figure 12: Schematic illustration of synthesis of [Zn(L3)(DMSO)] complex [91]

Sheikhshoaie used a microdilution experiment to find the minimum bactericidal concentration (MBC) of the complexes and their bactericidal properties [91]. MBC refers to the smallest amount of compound required to kill bacteria [91]. [Ni(L1)] and [Ni(L2)] had significantly lower MBC values than those for $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ while

[Zn(L3)(DMSO)] was also lower than that of $\text{Zn}(\text{CH}_3\text{CO}_2)_2 \cdot 2\text{H}_2\text{O}$. Therefore complexes did not only stop bacterial growth but also induced cell death.

2.1.4 Iron complexes

Iron is vital for nearly all living things as it plays a role in a variety of metabolic activities such as DNA synthesis, oxygen transport, and electron transport [92]. It is one of the most abundant transition metals on Earth and just like other d-block metals, Iron has been shown to exhibit some interesting biological properties [93]. Wani *et al*, did a review on iron complexes as anticancer drugs [94]. He reported that from 2000 to 2015, the amount of publications on iron complexes as anticancer drugs revealed a steady increase of interest in this field of research [94]. Iron complexes have also been the centre of interest for other biological studies [95][96][97].

Vinagreiro *et al*, synthesised Iron(II)–N-Heterocyclic carbene complexes as shown in Figure 13 (a-e), and tested their antibacterial activities against bacterial strains: *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 [98]. The minimum inhibitory concentration (MIC, $\mu\text{g}/\text{mL}$) values were used to measure antibacterial activity. The authors reported a substantial effect from the Fe(II)–NHC sidechain on antibacterial potency against Gram-negative and Gram-positive strain. The authors indicated that, amphiphilic hydroxyl groups contributed to excellent result in terms of MICs against *S. aureus*, with MIC values of 8 $\mu\text{g}/\text{mL}$ recorded with compounds (a) and (b) as shown in Figure 13. They also stated that, the presence of a hydrophobic methyl group (c) or dimethylacetamide groups (e) greatly reduced the activity. Further studies were done to evaluate the MICs of the most promising compounds against Gram-negative bacteria (*E.Coli*). The most lipophilic complex (d) was shown to be the most active of the compounds tested with MIC values 8 $\mu\text{g}/\text{ml}$.

Vinagreiro and his associates further investigated the potential of iron complexes to produce OH radicals under biological conditions. Significant UV-vis spectral changes were observed when H_2O_2 was added to a solution of Fe(II) complex (d). They reported that these changes were due to iron(II) oxidation. The results indicated that indeed metal complexes go through oxidation and there is potential ROS formation in biological conditions.

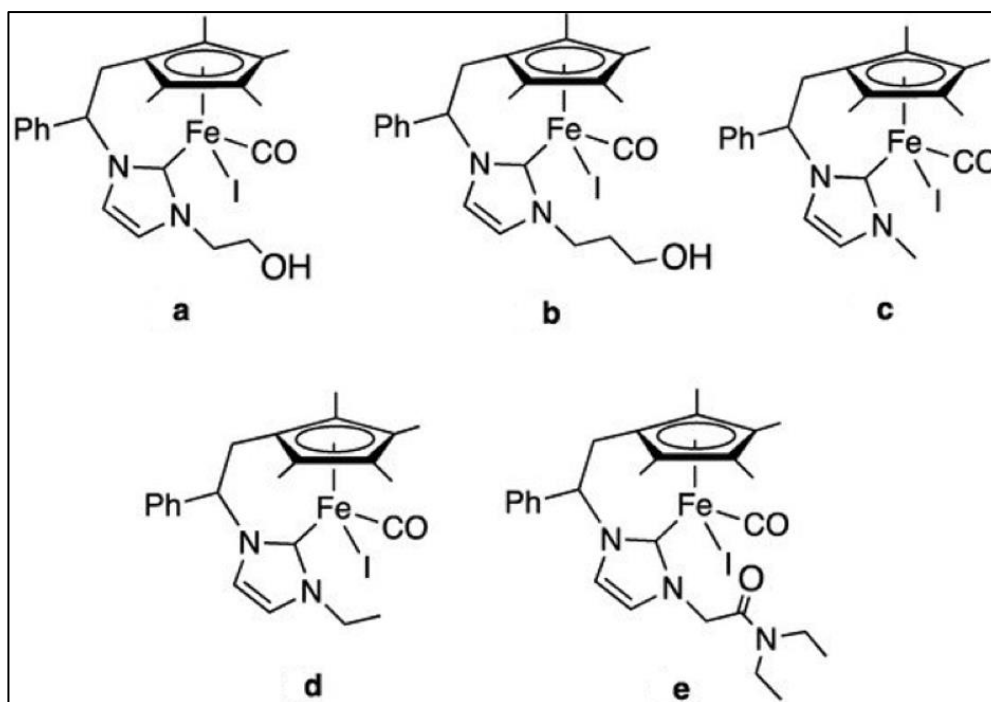


Figure 13: Iron(II)–NHC complexes evaluated against bacteria [98].

2.2 SCHIFF BASES

A Schiff base is a product of the condensation process between an aldehyde and a primary amine [99]. They have an azomethine ($-\text{C}=\text{N}-$) functional group which can be useful in the development of bioactive compounds. Schiff bases are a class compounds that has a variety of uses and applications, including analytical, biological, and inorganic chemistry [100]. Their wide range of biological activities like anti-inflammatory, antibacterial, anticancer, and antioxidant, grew Schiff bases popularity in the pharmaceutical industries [101], [102], [103], [104].

According to Raczuk *et al*, the biological applications of Schiff bases prompted the development of interest in the use of Schiff bases coordinated to metal ions in medicine in the second half of the nineteenth century [105]. Since then, this class of compounds has attracted researchers' interest in the development of the next high-end biopharmaceutical. Most metals have been coordinated with Schiff bases to generate stable metal complexes with a wide range of medicinal applications [106].

Patel and Patel, synthesised Schiff base derivatives through a condensation reaction of compound A shown in Figure 14 with substituted aromatic aldehydes and tested their antimicrobial and antifungal activities [107]. Schiff base derivatives showed decent activities at around 50% against gram-positive bacteria *S. aureus* and 60%

against *B. subtilis*. Schiff bases also showed decent activities (58.24–61.54%) against *P. aeruginosa* while compounds 4g and 5g had decent activities (56.66% and 64.29%, respectively) against *E. coli*.

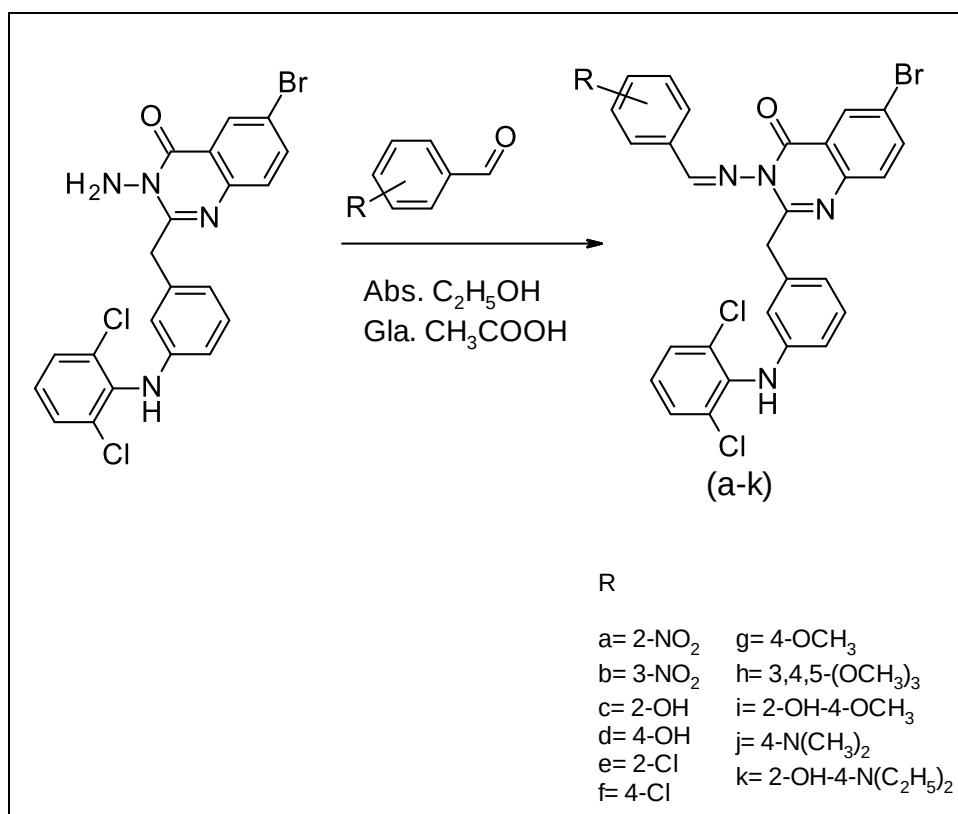


Figure 14: Schematic representation of the synthesis of 6-bromo-2-[2-(2,6-dichlorophenyl)amino]benzyl-3-substituted benzylideneamino-quinazolin-4(3H)-ones [107].

Matar *et al*, tested antimicrobial potency of metal complexes of Schiff bases made from benzaldehydes and 3,3'-diaminodipropylamine against gram positive and gram negative bacterial strains [108]. Their results showed that, the compounds had an antimicrobial activity against multi-drug resistant *S. Aureus* (MRSA) with MIC ranging between 200 and 2100 µg/mL. Compounds also showed an enhanced activity against *S.epidermidis Tu* and Meticillin-sensitive *Staphylococcus aureus* (MSSA). Compound (3e) as shown in figure 10, recorded the highest activity with MIC of 24 µg/mL against *S.epidermidis Tu* and (MSSA).

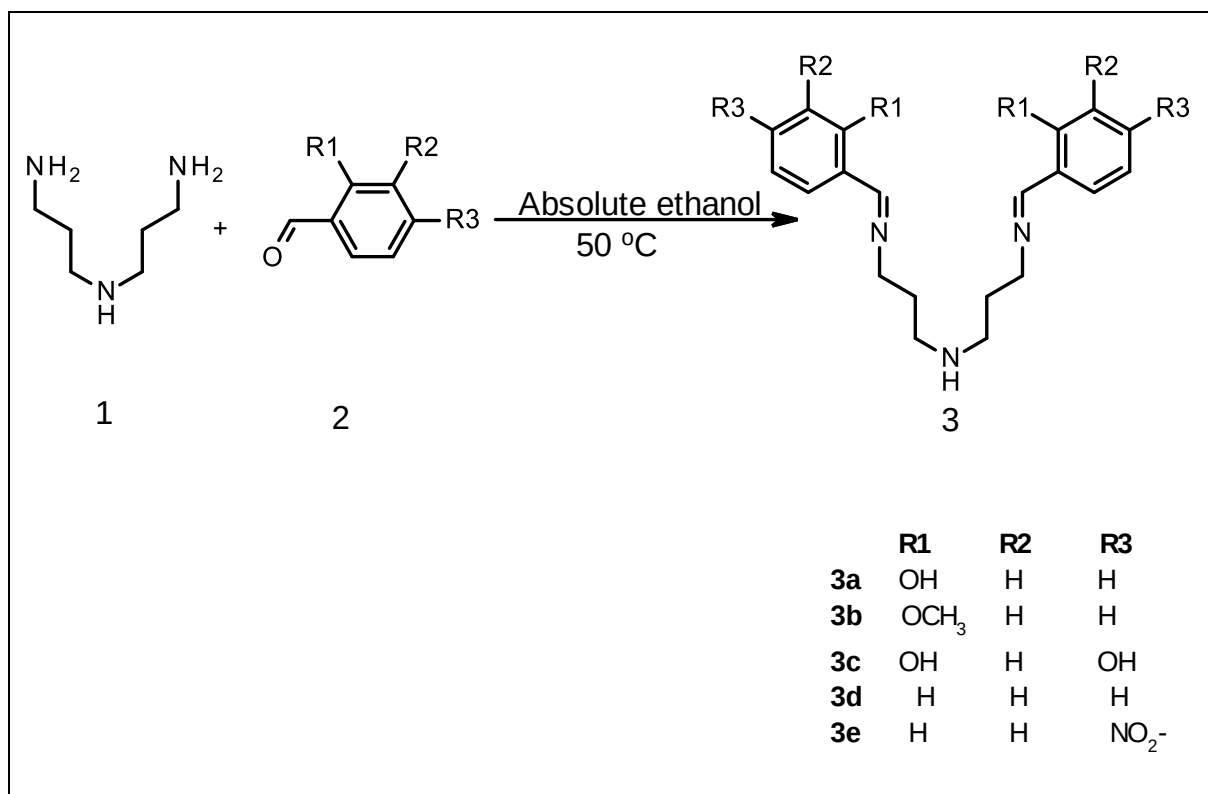


Figure 15: Synthesis of Schiff base compounds 3a-e

Some studies suggests that, by creating a hydrogen connection via the azomethine group with the active cellular components, Schiff bases can disrupt normal cellular processes and exert their antibacterial potency against bacterial strains [109], [110]. Additionally, other studies suggest that Schiff bases with diverse functional groups have a higher tendency to interact with nucleoside bases that is in the biosystem even when coordinated to metal ions [111].

3.0 MATERIALS AND METHODS

3.1 CHEMICALS

Chemicals and organic solvents were procured from Sigma-Aldrich, Alpha-Chemika, or Rochelle chemicals and were used without further purification. The reaction progress was monitored by thin-layer chromatography (TLC) on silica gel precoated aluminium foils (Sigma-Aldrich, Germany), and the spots were detected by exposure to UV lamp.⁴

Table 2: Chemicals and reagents

Chemical name	Supplier
Nickel chloride hexahydrate	Alpha Chemika
98% Iron(II) Chloride tetrahydrate	Sigma Aldrich
98% Cobalt(II) Chloride hexahydrate	Sigma Aldrich
98% Cu(II) Chloride dihydrate	Sigma Aldrich
99% 3-nitrobenzaldehyde	Sigma Aldrich
99% 4-nitrobenzaldehyde	Sigma Aldrich
98% 2-amino-5-chlorobenzophenone	Sigma Aldrich
99.99% Ethanol	Alpha Chemika
Methanol (HPLC grade)	Alpha Chemika
Acetic Acid glacial (Analytical reagent)	Rochelle Chemicals

3.2 INSTRUMENTS

Table 3: The main instruments used for structure elucidation of ligands and metal complexes

Instrument	Source	Use
Single crystal XRD Model: Bruker D8- Quest CCD Data Acquisition The crystal was at room temperature during data collection. The structures	Bruker (USA)	SC-XRD was used to provide thorough information with regard to the internal lattice of the crystalline compounds, including unit cell dimensions, space group, bond-lengths and angles as well as the

were solved by SHELXL structure solution program using Intrinsic Phasing and refined with the SHELXL refinement package using least squares minimization. The systems were used according to literature [112], [113]. The geometry properties, intermolecular energy calculations and calculation visualizations of the compound were performed using the Spartan 14 package [114]		positions of atoms within the lattice
Fourier Transmission Infrared Spectroscopy (FTIR) Model: Vertex 70v Accessory: A225/Q Platinum Attenuated Total Reflection (ATR), diamond crystal Software: Opus 7.8 Data Acquisition Result spectrum: Transmittance Resolution: 4 cm⁻¹ Sample scan time: 64 scans	Bruker Optik GmbH, Germany	FTIR was used to find and classify the functional groups expected in the compounds. Since the formation of schiff base involve the condensation reaction between an aldehyde and amine group, we expect to see the absence of an aldehyde and amine absorbance peaks and appearance of a characteristic peak of schiff base functional group. The FTIR was also used to determine whether the complexation took place by observing the position of schiff base peak in the metal complex.

Proton Nuclear Magnetic Resonance spectroscopy (^1H NMR) Model: Bruker AC 400 MHz Spectrophotometer Data Acquisition Internal standard: TMS Solvent: CDCl_3 chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS	Bruker	^1H NMR was used to elucidate the chemical structures of schiff base ligands and to determine whether complexation took place by observing the chemical shift of the azomethine proton in complexes.
ESI-MS Model: ABSciex 6500+Qtrap Data Acquisition Ionisation mode: Electron spray (positive mode and negative mode) Scan mode: Q1 Curtain gas: Nitrogen Curtain Gas(CURI) : 40 ion spray voltage: 5000 Temperature: 100 Ion source gas 1: 50 Ion source gas 2: 50 Declustering potential (DP): 100 Entrance Potential (EP): 10 Scan rate: 200 Da/s	ABSciex, Germany	ESI-MS was used to verify the structure of ligands and complexes using their mass to charge ratio. This instrument works by ionising the sample solution and then turning the solution into charged gaseous state. The gaseous state which contains some molecular ions and charged fragments of the molecule will then be transferred to mass analyser region where they are sorted and separated based on their mass to charge ratio (m/z value).The ions are then sent into detector devices, which measure their concentration and display the results as mass spectrum.

3.3 SYNTHESIS OF LIGANDS

The procedure for synthesis of Schiff base was adapted from Aslam *et al*, with minor modifications [115]. The synthetic scheme for the synthesis of 4N ligand is shown in Figure 16.

0.3022 g of 3-nitrobenzaldehyde / 4-nitrobenzaldehyde (2mmol) was dissolved in 5 ml ethanol. 2-amino-5-chlorobenzophenone (0.4634 g / 2 mmol) was dissolved in 5 ml ethanol. It was stirred at room temperature and slowly added to the 3-nitrobenzaldehyde / 4-nitrobenzaldehyde solution. 3 drops of acetic acid were added into the mixture. The contents were then refluxed for 18 h at 70 °C. After completion of the reaction as monitored by TLC (3:7 ethyl acetate: n-hexane), the solution was concentrated to one third of its initial volume through evaporation.

3.3.1 [5-chloro-2-[(3-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (3N)

A milky white solution was obtained and was allowed to stand overnight at ambient temperature. A white solid precipitate obtained was filtered, washed with cold ether, and dried under anhydrous CaCl₂ in a desiccator.

3.3.2 [5-chloro-2-[(4-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (4N)

A yellow solution was obtained and was allowed to stand for several minutes at room temperature. A yellow precipitate obtained was filtered, washed with cold ether, and dried under anhydrous CaCl₂ in a desiccator.

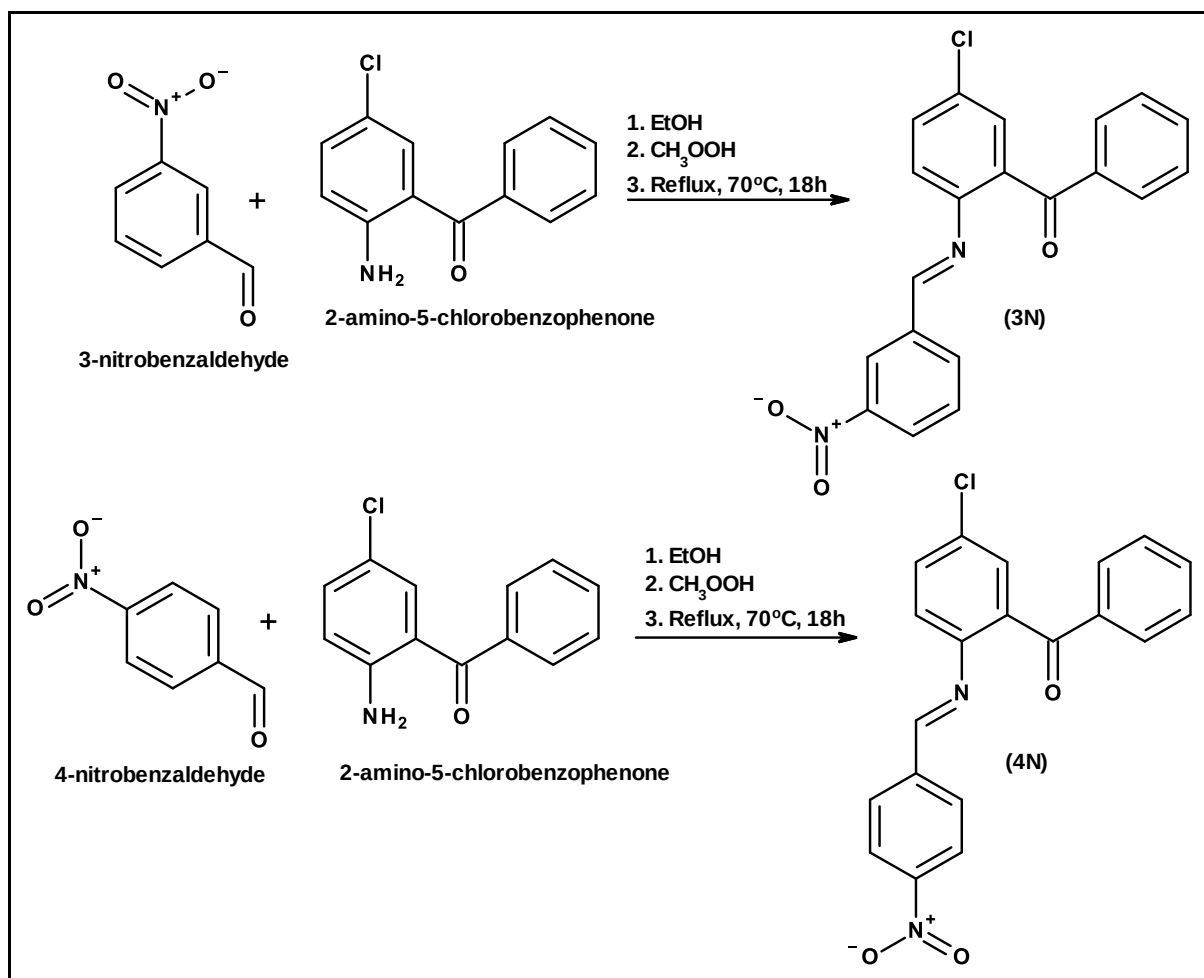


Figure 16: Synthetic scheme for synthesis of [5-chloro-2-[(3-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (3N) and [5-chloro-2-[(4-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (4N).

3.4 SYNTHESIS OF METAL COMPLEXES

Metal salts [$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, and $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$] were each dissolved in 10 ml methanol. Schiff base ligands (4N / 3N) were dissolved in 10 ml methanol. Methanolic solutions of respective metal salts were slowly added to a hot stirring methanolic solutions of ligands in 1:2 (metal: ligand) molar ratio. The mixture was refluxed for 4 h, and coloured solutions were obtained. The mixtures were left to stand overnight. Precipitate was recovered, it was filtered out, washed with methanol then diethyl-ether. The precipitate was dried in a desiccator over anhydrous CaCl_2 .

Table 4. Colour of precipitate obtained after filtration for each metal complex.

	Cu4N	Cu3N	Co4N	Co3N	Ni4N	Ni3N	Fe4N	Fe3N
Colour of the precipitate	yellow	Cream white	brown	brown	Yellowish green	Yellowish green	brown	Dark brown

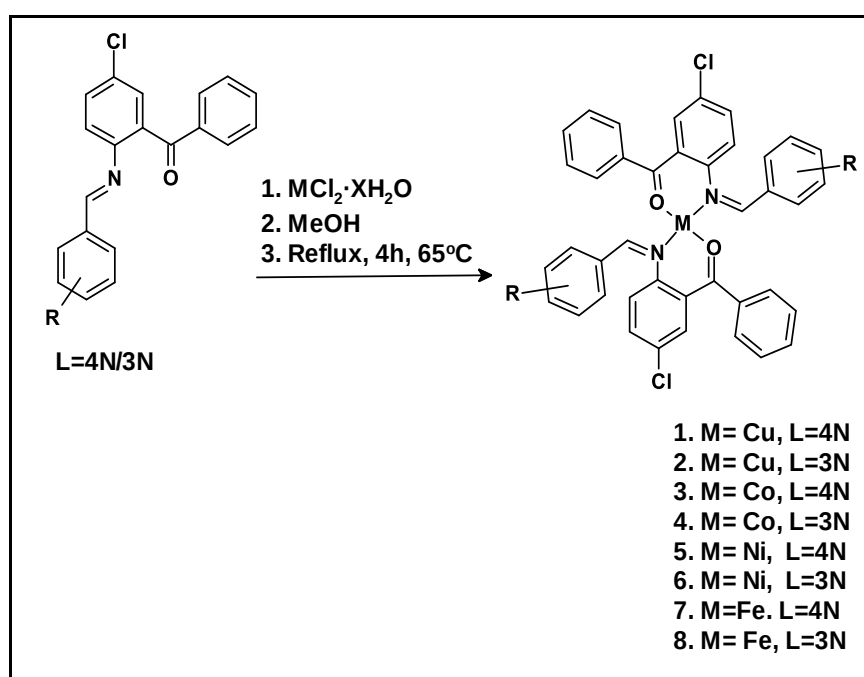


Figure 17: Synthetic scheme for metal complexes of 3N and 4N ligands with Cu, Co, Ni, and Fe metals.

3.5 SOLUBILITY PROFILING

Solubility profiling was done on ligands and the metal complexes. A total of 9 solvents were used to dissolve each sample. Test tubes were labelled accordingly, and a small portion of a test sample was then placed in each test tube. The solvents were added to each test tube containing the test samples using a plastic pipette. Observations were made to see which solvent would dissolve. Those that were partially dissolved or not dissolved at room temperature were placed in water bath, then heated. The temperature at which the test sample dissolved was recorded in table 5.

Table 5: Solubility of complexes and ligands in different solvents

	Acetone	DMSO	CCl₄	EtOH	MeOH	H₂O	DMF	Ethyl acetate	Hexane
4N	Soluble	soluble	Soluble	Not soluble	Partially soluble	Not soluble	Soluble	dissolves at 57 °C	Not soluble
Cu4N	Soluble	dissolves at 50 °C	Soluble	Not soluble	Not soluble	Not soluble	Soluble	Soluble	Not soluble
Co4N	Soluble	dissolves at 50 °C	Soluble	Not soluble	Not soluble	Not soluble	Soluble	Soluble	Not soluble
Fe4N	Soluble	dissolves at 60 °C	Soluble	Not soluble	Not soluble	Not soluble	dissolves at 55 °C	dissolves at 60 °C	Not soluble
Ni4N	Soluble	Soluble	Soluble	Partially soluble	Partially soluble	Not soluble	dissolves at 50 °C	dissolves at 50°C	Not soluble
3N	Soluble	Soluble	Soluble	Not soluble	Not soluble	Not soluble	Soluble	soluble	Not soluble
Co3N	Soluble	Soluble	Soluble	Soluble	Partially soluble	Not soluble	Soluble	soluble	Not soluble
Fe3N	Soluble	Soluble	Soluble	Partially soluble	Partially soluble	Not soluble	Soluble	Dissolve at 60 °C	Not soluble

Cu₃N	Soluble	Soluble	Soluble	Not soluble	Not soluble	Not soluble	Soluble	soluble	Not soluble
Ni₃N	Soluble	Soluble	Soluble	Partially soluble	Partially soluble	Not soluble	Soluble	dissolves at 50°C	Not soluble

3.6 ANTIBACTERIAL SCREENING

Schiff base ligands and metal complexes were tested for antimicrobial activities against Gram negative *Escherichia coli* and gram-positive *Staphylococcus aureus*.

The ligands and their metal complexes were prepared at a concentration of 4000 $\mu\text{g mL}^{-1}$ (using 100 % DMSO solution) to make a sample stock solution. Two-fold dilutions of compounds were prepared in 96-well microplates obtaining final concentrations ranging from 1.953125 $\mu\text{g mL}^{-1}$ to 2000 $\mu\text{g mL}^{-1}$. A control test was included which had a DMSO as the test medium using the same procedures as in the experiments.

To prepare dilutions, 100 μL of Mueller Hinton broth media was deposited into each microplate well. Then, in the first column of the microplate, 100 μL of sample stock solution (4000 $\mu\text{g mL}^{-1}$), was deposited. The final dilution of the first column was 2000 $\mu\text{g/mL}$. By using a multichannel pipette, 100 μL was transferred from the first column to the second column, homogenized with the 100 μL of broth already present in the well, and so on. As a result, each subsequent well had half the concentration of the preceding well.

The density of each bacterial suspension was standardized to a 0.4 optical density. 40 μL of the bacterial suspension was inoculated into each well. Two columns were used as positive control (without test sample and with the bacterium) and a negative control (without both antibiotic and bacterium). The lowest concentration that prevented bacterial growth, after 20 h incubation was regarded as the MIC. Each MIC assay was performed three times.

4.0 RESULTS AND DISCUSSION

4.1 SINGLE CRYSTAL XRD

Attempts were made to produce suitable crystals of all synthesised compounds for characterization using Single Crystal X-ray Diffraction (SC-XRD). Unfortunately only 4N ligand was successfully recrystallized, as such only SC-XRD data for 4N ligand is discussed.

4N ligand

The ligand crystalized in a monoclinic crystal system and it transformed under the $P2_1/n$ space group. The ORTEP structure as shown in Figure 18 (A) indicate that the nitro group is located in para position of the ring as expected. The structure lost its planarity due to the carbonyl oxygen being out of plane with aromatic protons. Table 6 shows the selected bond lengths of the different bonds within the structure. N2 and C6 have shorter bond length at 1.249 (3) Å while N2 and C7 had a longer bond length at 1.411(3) Å. This is expected as N2 and C6 have double bond while N2 and C7 have single bond and bond order is inversely proportional to bond length [116]. Similarly bond length of O3 and C9 is small as expected due to a strong double bond between the two atoms. The single crystal XRD data is in agreement with the expected structure of the ligand as represented in Figure 18 (B).

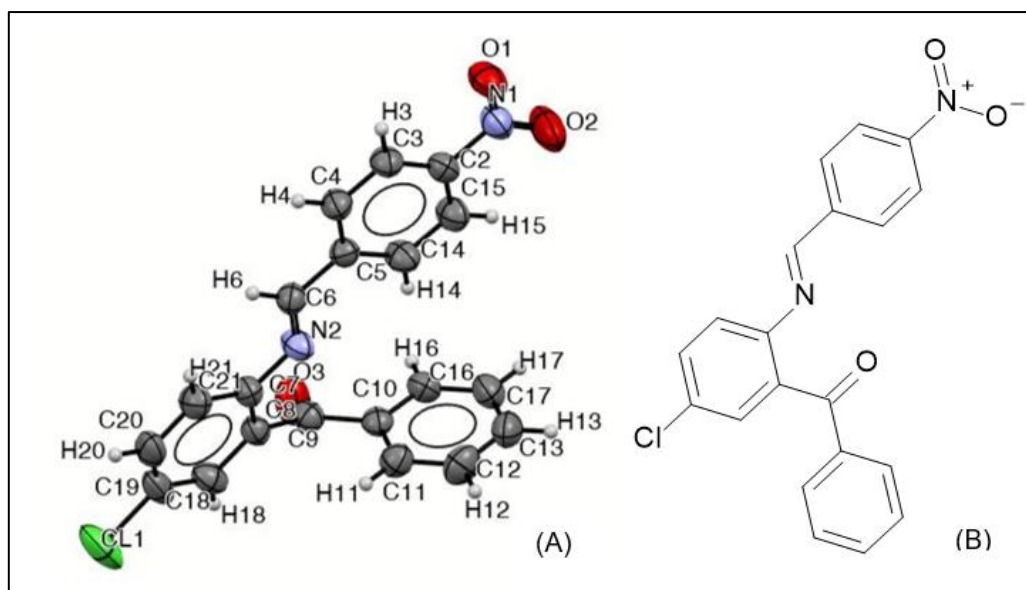


Figure 18: (A) Single Crystal XRD ORTEP structure at 50% probability, (B) Proposed structure.

Table 6: Bond Lengths for 4N Ligand.

Atom	Atom	Length/Å
O1	N1	1.218(3)
N1	O2	1.213(3)
N1	C2	1.477(3)
N2	C7	1.411(3)
N2	C6	1.249(3)
O3	C9	1.213(3)

4.2 FT-IR ANALYSIS

The existence of organic functional groups in a sample can be determined using Fourier Transform Infra-red Spectroscopy (FTIR). The functional groups available in the compound can be deduced by interpreting the spectrum data based on the infrared absorption frequency [117].

There are three main processes by which a molecule can absorb radiation. Either through absorption of light which causes a rise in rotational energy level, absorption of quantized energy which causes vibrational transition, and absorption of light leading to a rise in electron energy. The principle for IR absorption depends on the change in

dipole moment between atoms in molecule as it vibrates or rotates. The size of the charge difference between two atoms in a molecule and the distance between them determines the dipole moment. As the molecule vibrates, its dipole moment fluctuates, creating a field that matches with the radiation's electric field. When the vibration of the molecule matches the field of the radiation, the amplitude of the molecular vibration changes [118].

The infrared spectrum has two main regions: functional group region and fingerprint region. Frequencies less than 1500 cm^{-1} are considered to be in the fingerprint region, while the functional group region is commonly thought to range from 4000 cm^{-1} to around 1500 cm^{-1} . These regions are very useful when determining the identity of a compound. The functional group area includes vibrations that are more localized and characteristic of specific functional groups in organic compounds.

4.2.1 FT-IR analysis of [5-chloro-2-[(3-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (3N) and its metal complexes.

Synthesised Schiff base ligands contains carbonyl and azomethine functional groups which are used as a reference point to conclude as to whether the ligands formed or not. Since the ligands were made from condensation of 4-nitrobenzaldehyde/3-nitrobenzaldehyde and 2-amino-5-chlorobenzophenone, we expect that the band due to amino group and carbonyl carbon of an aldehyde would not appear in the IR spectrum of 4N and 3N ligands. The IR spectrum of 3N ligand in Figure 19 shows strong bands at 1647 and 1629 cm^{-1} which are assigned to (-C=O) and (C=N) , respectively. The benzene derivative vibrations are assigned to a strong peak at 709 cm^{-1} . A weak (-C-H) bending of aromatic compound appears at 1614 cm^{-1} . A strong peak at 1529 cm^{-1} is assigned to nitro (N-O^-) stretching. The medium peak at 837 cm^{-1} is assigned to (-C-Cl) stretching.

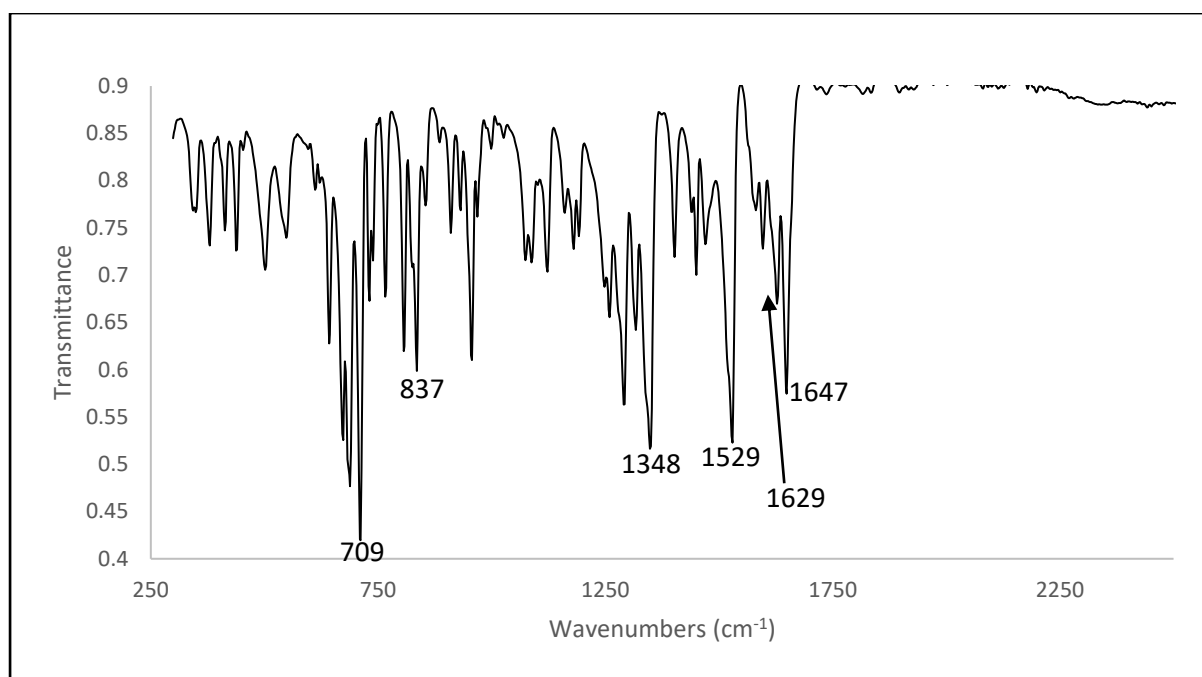


Figure 19: FT-IR spectrum of 3N ligand.

Results in Table 7 show that carbonyl carbon, (-C=O) bands have shifted towards higher frequencies in all metal complexes. This shifting indicates that the ligand has coordinated through the oxygen of the carbonyl carbon. This is because metal ions being cationic have pulled electron density from oxygen atom, affecting the polarity and dipole moment of carbonyl bond. According to Bako *et al*, dipole moment of polar molecule can be influenced by polarization from the cation's electrostatic charge [119]. Therefore, alteration in dipole moment of a polar molecule will have an effect on the natural vibration of the molecule and thus its infra-red absorption. The Schiff base peak did not move significantly in these metal complexes which is surprising as they are expected to be involved in coordination. It is suspected that there may be a weak Ligand-Metal interaction between the nitrogen and metal ions.

Table 7: The selected IR [cm^{-1}] peaks for the 3N ligand and its metal complexes.

	IR bands (cm^{-1})				
	3N	Co3N	Cu3N	Fe3N	Ni3N
(-C=O) ketone	1647	1658	1658	1658	1658
(-C=N-) schiff base	1629	1627	1629	1629	1627

4.2.2 FT-IR analysis of [5-chloro-2-[(4-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (4N) and its metal complexes

The FT-IR spectrum for 4N ligand is expected to be similar to that of 3N on the basis that it contains the same functional groups and the only difference is the position of a nitro group which cannot be differentiated by the FT-IR analysis. The FT-IR data for 4N ligand and its metal complexes is recorded in Table 7. The spectrum for 4N ligand as shown in Figure 20 shows sharp peaks at 1629 cm^{-1} and 1658 cm^{-1} which are assigned to the azomethine group (-C=N) and carbonyl group (-C=O) respectively. A strong peak at 1512 cm^{-1} is assigned to nitro (N-O-) stretching. A sharp, strong peaks at 686 and 713 cm^{-1} are assigned to benzene derivatives vibrations. A sharp medium peak at 852 cm^{-1} is assigned to (-C-Cl) stretching vibration. A sharp medium peak at 1594 cm^{-1} is assigned to (-C-H) bending vibration of an aromatic compound. Furthermore, the absence of a peak at $3300\text{-}3400\text{ cm}^{-1}$ and 1700 cm^{-1} which are characteristic peak for amino group and carbonyl carbon of an aldehyde indicates that the starting materials have been used up.

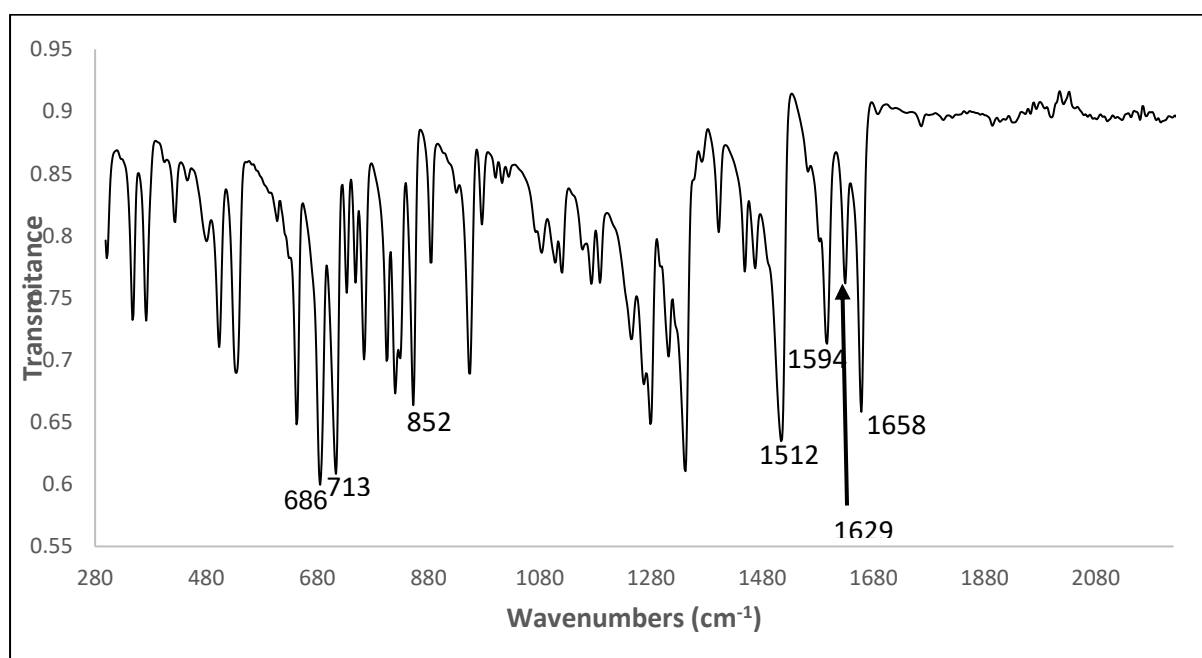


Figure 20: FT-IR spectrum of 4N ligand.

As expected, coordination of metals to 4N ligand had a slight impact on the FT-IR spectrum of the ligand. As shown in Table 8, the bands due to azomethine group, $\nu(\text{-C=N})$ shifted from 1631 cm^{-1} to lower frequency of 1600 cm^{-1} in metal complexes. This shifting indicates that the ligand has attached to the metal through azomethine

nitrogen. Surprisingly there was no significant shift for a carbonyl peak in metal complexes. It is suspected that there may be a weak metal-ligand interaction between the carbonyl oxygen and metal ions. FT-IR spectra of all compounds are shown in APPENDIX A.

Table 8: IR (cm^{-1}) data for the 4N ligand and its metal complexes.

	IR bands (cm^{-1})				
	4N	Co4N	Cu4N	Fe4N	Ni4N
(-C=O) ketone	1658	1654	1654	1654	1658
(-C=N-) schiff base	1631	1600	1600	1600	1600

4.3 NMR STUDIES

A nuclear magnetic resonance spectrometer (NMR) is an instrument that is normally used to determine a chemical compound's structure and conformation. NMR occurs when nuclear spin flip from low to high energy spin states due to the absorbance of radio frequency radiation. However not all nuclei are NMR active. The common NMR active nuclei are ^1H and ^{13}C . For ^1H NMR or proton NMR, there are 4 levels of information to be gained in relation to different parts of the spectrum. The first information to observe is the number of peaks that appear in the spectra. This data is important as it tells how many types of hydrogen are present. The next set of data to observe is the integration of each peak which gives a ratio for the number of hydrogens that give rise to each peak. This allows one to calculate the total number of hydrogens present in the sample [120].

The next set of information to observe is the chemical shift for each peak as well as the coupling patterns which provides information on the connectivity of chemical bonds. The structural electronical environment of the nucleus producing the signal determines chemical shift of that nucleus. Chemical shift (δ) refers to the position of a signal on the x-axis of an NMR spectrum. Protons in non-equivalent chemical surroundings produce signals at different chemical shifts. Upfield refers to the right-hand side of an NMR spectrum with smaller chemical shift values, while downfield refers to the left-hand side. The position at which the signal appears is mostly

determined by the electron distribution around the nuclei of interest [121]. If for example one introduce a more electronegative element into the sample. It would pull most of the electron density that surrounds the nuclei of interest, exposing the nuclei to the applied magnetic field. This will increase the nuclei resonating frequency and cause its signal to move downfield. Movement of a signal to a downfield region is called de-shield.

As stated above, the chemical environment surrounding the nuclei of interest will affect the position at which the signal of that nuclei appears. Consequently, all proton nuclei with similar chemical environment will experience the same applied magnetic field and hence resonate the same frequency. This means that they will give the same signal in the ^1H NMR spectrum. As an example in Figure 21, the total number of protons in the structure is six. However, the three H_1 protons are all in the same chemical environment and will show only one signal in ^1H NMR spectrum. The other three H_2 protons will also show only one signal. Therefore, the compound will only show two signals in the ^1H NMR spectrum.

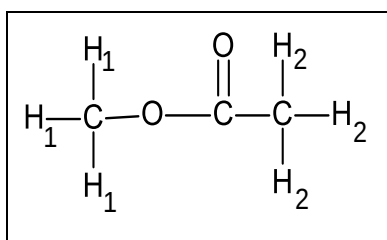


Figure 21: An illustration showing chemical equivalency of protons.

After synthesis, both ligands and metal complexes were taken to South Africa, University of Limpopo for ^1H NMR analysis.

4.3.1 [5-chloro-2-[(4-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (4N)

The ^1H NMR spectrum of 4N ligand, shown in Figure 20, shows the presence of azomethine, ($-\text{CH}=\text{N}$) proton singlet at 8.40 ppm. The absence of signal around $\delta = 1-2$ ppm and $\delta = 9-10$ ppm in the regions for amine group ($\text{R}-\text{NH}_2$) and aldehyde carbonyl group ($\text{R}-\text{CH}=\text{O}$) respectively, indicates a new compound has been formed. A chemical shift observed at 8.20 ppm which is a doublet was assigned to protons at position 19 and 23 as shown in Figure 22. These two positions are in the same chemical environment and as such, they absorb the same amount of external

magnetic field. Protons in position 20 and 22 are assigned the same chemical shift of 7.83 ppm which is a doublet due to chemical splitting by protons in position 19 and 23 respectively. Based on the same reasoning above, these two protons are assigned the same chemical shift.

Proton in position 14 is assigned a doublet at 7.18 ppm. This proton is being split by proton at position 12. The doublet for proton at position 12 and singlet for proton at position 11 do not appear in the spectrum. These protons are believed to be responsible for the multiple at 7.47 ppm. This is also backed by the integration value of 2 found in this peak. Two protons in position 2 and 6 are assigned the same chemical shift of a doublet observed at 7.69 ppm. Similarly, these two protons are chemically equivalent, hence they share the same chemical shift. A triplet and doublet of doublet is expected due to protons at position 1 and 5, however the spectrum shows a multiplet at 7.56 ppm with integration value of 3. This multiplet is assigned protons at position 1 and 5. Finally the total number of protons in the proposed structure is equal to the total sum of integration values, implying that all protons are accounted for and there are no impurities.

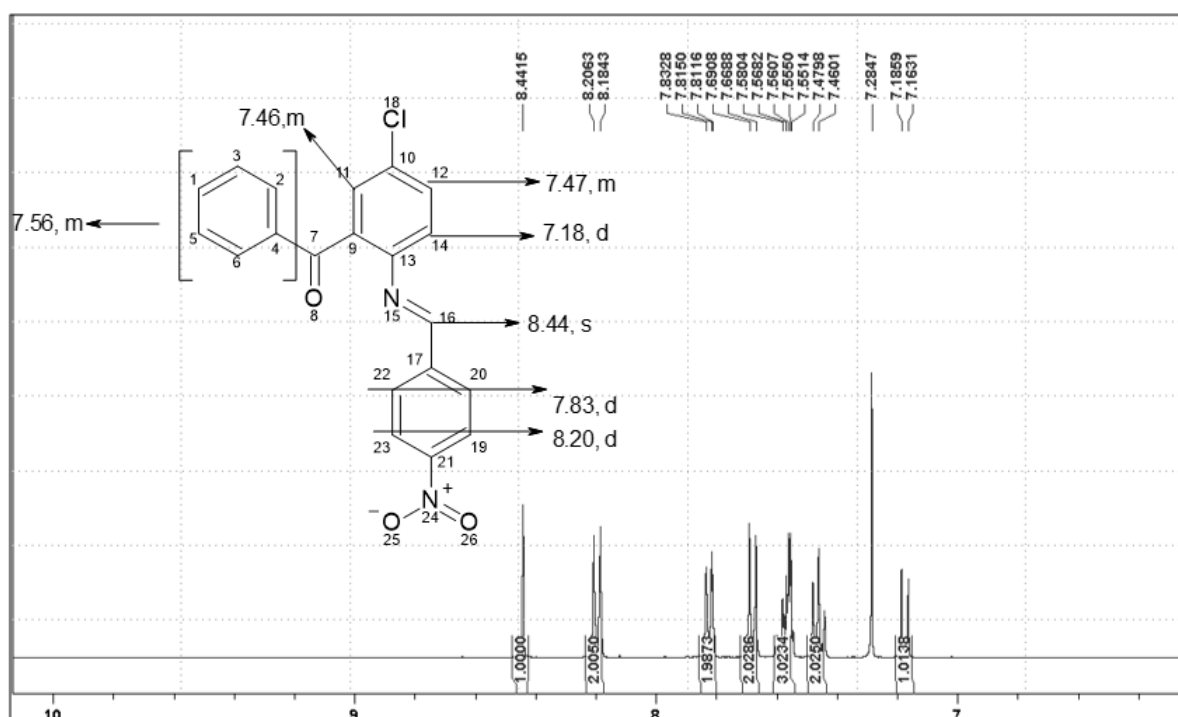


Figure 22: ¹H NMR spectrum of 4N ligand.

4.3.2 [5-chloro-2-[(3-nitrophenyl)methyleneamino]phenyl]-phenyl-methanone (3N)

The ^1H NMR spectrum of 3N ligand is presented in Figure 23. The spectrum shows a signal at 8.74 ppm indicating the characteristic peak of a Schiff base proton ($\text{R}-\text{CH}=\text{N}-$). The absence of a signal around 1-2 ppm and 9-10 ppm, the regions for amine group ($\text{R}-\text{NH}_2$) and aldehyde carbonyl group ($\text{R}-\text{CH}=\text{O}$) respectively, shows that the reaction was completed. A signal observed at 8.52 ppm which is a doublet is assigned to a proton at position 14 which is coupled by a proton at position 13. Two protons at position 12 and 14 each split proton attached to carbon 13 to doublets of doublets as they are not in the same chemical environment. This particular signal observed at $\delta = 7.83$ ppm is assigned to proton in position 13. Protons at position 18 and 6 are assigned to doublets appearing at 7.16 ppm and 7.28 ppm respectively. These two protons are coupling each other.

The proton at position 5 does not have any neighbouring proton, as such no chemical splitting will occur. It is worth noting that this peak is not very clear in the spectra as it forms part of the multiplet at position 7.47 ppm. In some cases involving aromatic compounds, signals from certain protons will overlap with other signals from protons that are not in the same chemical environment. This is because of the anisotropic induced magnetic effects due to the π system [122]. Both electronegative groups and π electrons affect the chemical shift of a signal independently and in some cases, the effect is such that the signals of two or more protons overlap one another even though they are not in the same structural electronical environment. In other words, the resolution of the peaks become poor [122]. This can also be observed with signals from protons in positions 23, 22, and 24. These protons are expected to show doublet, doublet of doublet, and triplet respectively. However, the ^1H NMR spectra have two signals at 7.64 and 7.47 ppm, they are treated as a large multiplet with poor resolution. The integration value of this multiplet signal shows that the number of protons giving this signals is 5.

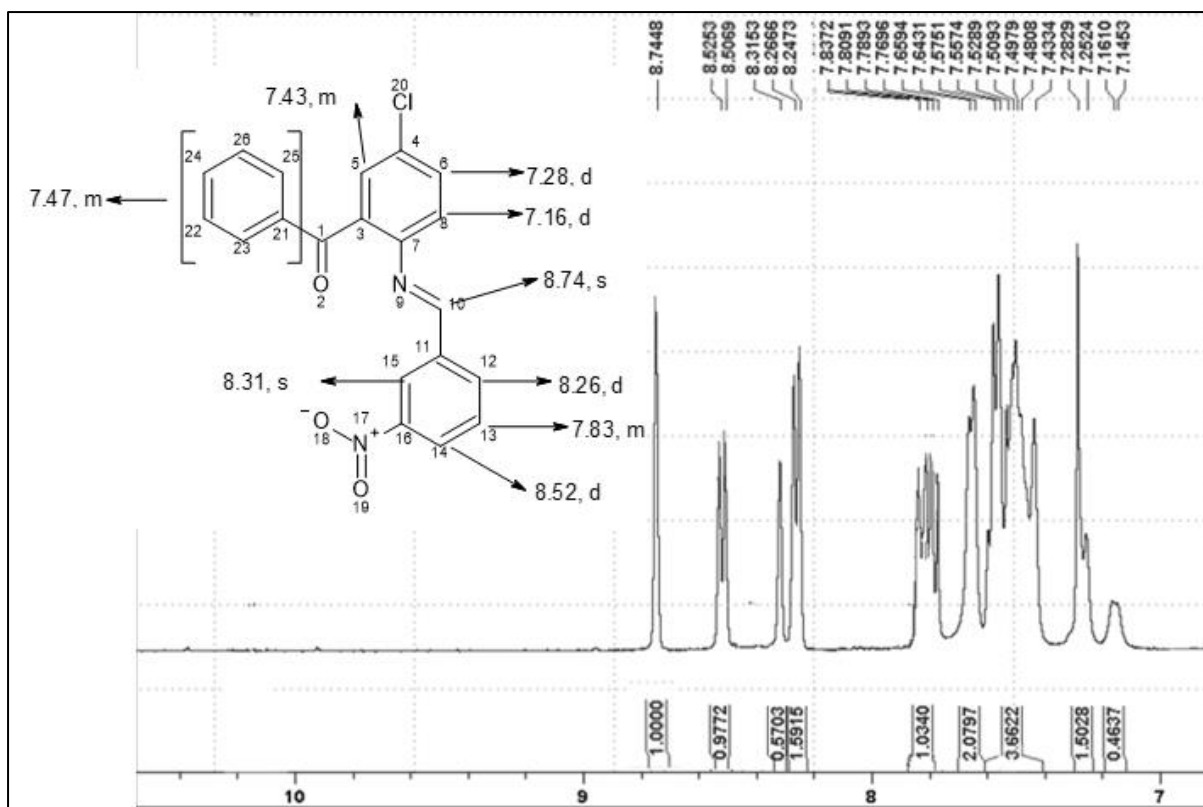


Figure 23: ^1H NMR spectrum for 3N ligand.

4.3.3 Metal complexes of 3N ligand

The ^1H NMR spectrum of Cu3N complex shows a downfield movement of the Schiff base ($-\text{CH}=\text{N}$) proton singlet from 8.74 ppm on a free ligand to 10.15 ppm. The discharging of electronic cloud from the nitrogen towards the Cu(II) ion, exerting a deshielding action on the proton, is attributed to the observed downfield shift of azomethine proton in the Cu3N complex. The spectrum displays a multiplet at 7.52 ppm which is attributed to protons in the phenyl ring of the ligand. It is difficult to assign accurate multiplicity to this signal as it is not well resolved. A doublet observed at 8.53 ppm is due to proton at position 14. A triplet at 7.81 ppm is assigned to a proton at position 15 which is coupled to protons in position 16 and 14. It could be expected that this proton would give a multiplicity of doublets of doublet since it is split by two inequivalent protons. However, appearance of triplet, could be due to anisotropic magnetic effects induced by the π system which affects the chemical shifts of signals. Two doublets at 7.67 ppm and 7.58 ppm are assigned to protons in position 10 and 11 respectively. These two signals shifted downfield in Cu3N complex as they were found in $\delta = 7.28$ and 7.16 ppm in the free ligand respectively. This is expected because during coordination the electron cloud that surrounds the carbonyl carbon of

the ligand is pulled towards the metal through nitrogen. This exposes the nucleus of these protons to a higher magnetic field. These findings were sufficient to conclude that metal coordination took place. Figure 24 shows assignment of ^1H NMR chemical shifts in Cu3N complex.

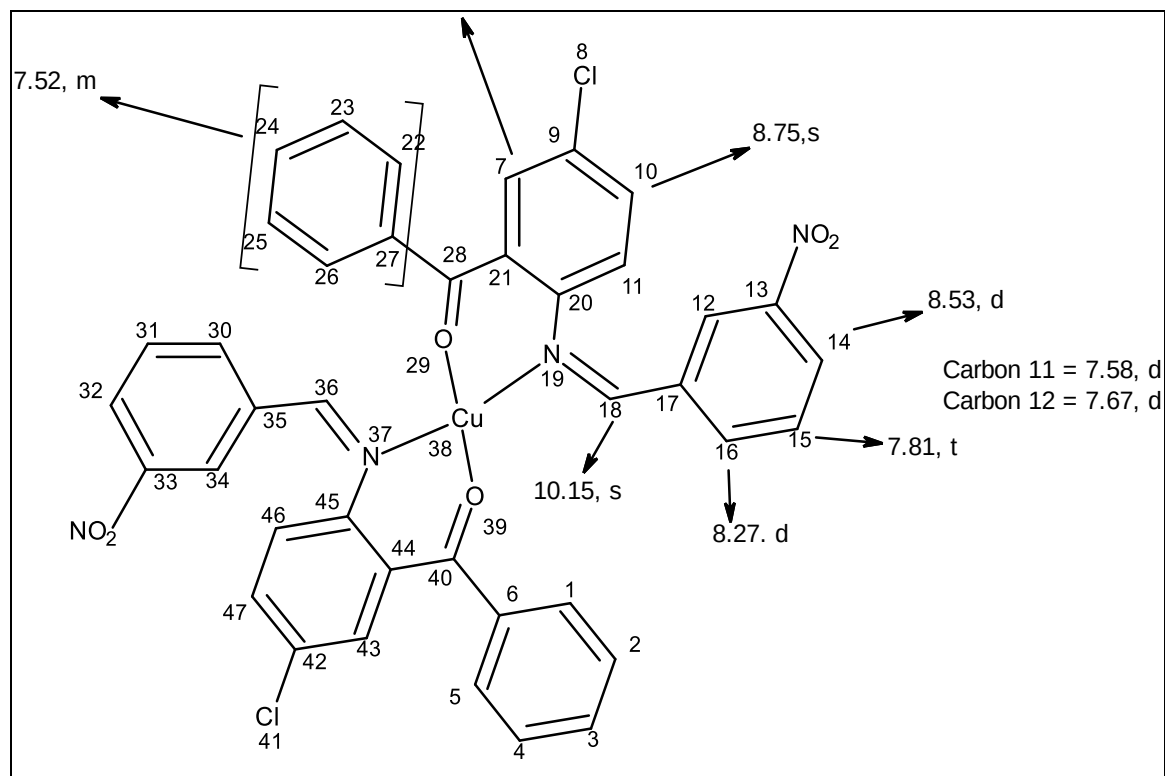


Figure 24: Assignment of ^1H NMR chemical shift in Cu3N.

4.3.4 Metal complexes of 4N ligand

The ^1H NMR spectrum of Cu4N complex shows a downfield movement of the Schiff base ($-\text{CH}=\text{N}$) proton singlet from 8.40 ppm on a free ligand to 10.18 ppm. The discharging of electronic cloud from the nitrogen towards the Cu(II) ion, exerting a deshielding action on the proton, is attributed to the observed downfield shift of azomethine proton in the Cu4N complex. The spectrum shows a multiplet at 7.66 ppm which is attributed to protons in the phenyl ring of the ligand as shown in spectrum 13 (Appendix B). It is difficult to assign accurate multiplicity to this signal as it is not well resolved. Doublets at 7.82 ppm and 8.44 ppm are assigned to protons at position 18 and 40 respectively. These two signals shifted downfield in Cu4N complex as they were found in 7.69 ppm and 8.20 ppm in the free ligand respectively. This is not surprising because during coordination the electron cloud that surrounds the carbonyl carbon of the ligand is pulled towards the metal through nitrogen. This exposes the

nucleus of these protons to a higher magnetic field. The spectrum also shows two doublets at 8.19 ppm and 8.10 ppm which are assigned to protons in position 12 and 14 respectively as shown in Figure 25. These signals also shifted downfield from 7.47 and 7.18 ppm in the free ligand. These findings were sufficient to conclude that metal coordination took place. It is worth noting that the analysis of spectrum of Cu₄N and Cu₃N complexes is done in reference to only one part of the complex even though the proposed structure has two ligands attached to the metal. This is because the two ligands are in the same chemical environment and the same protons in each ligand resonate the same magnetic field. As an example, in Figure 25, proton in position 16 resonate the same magnetic field as the proton in position 48 as they are in the same chemical environment.

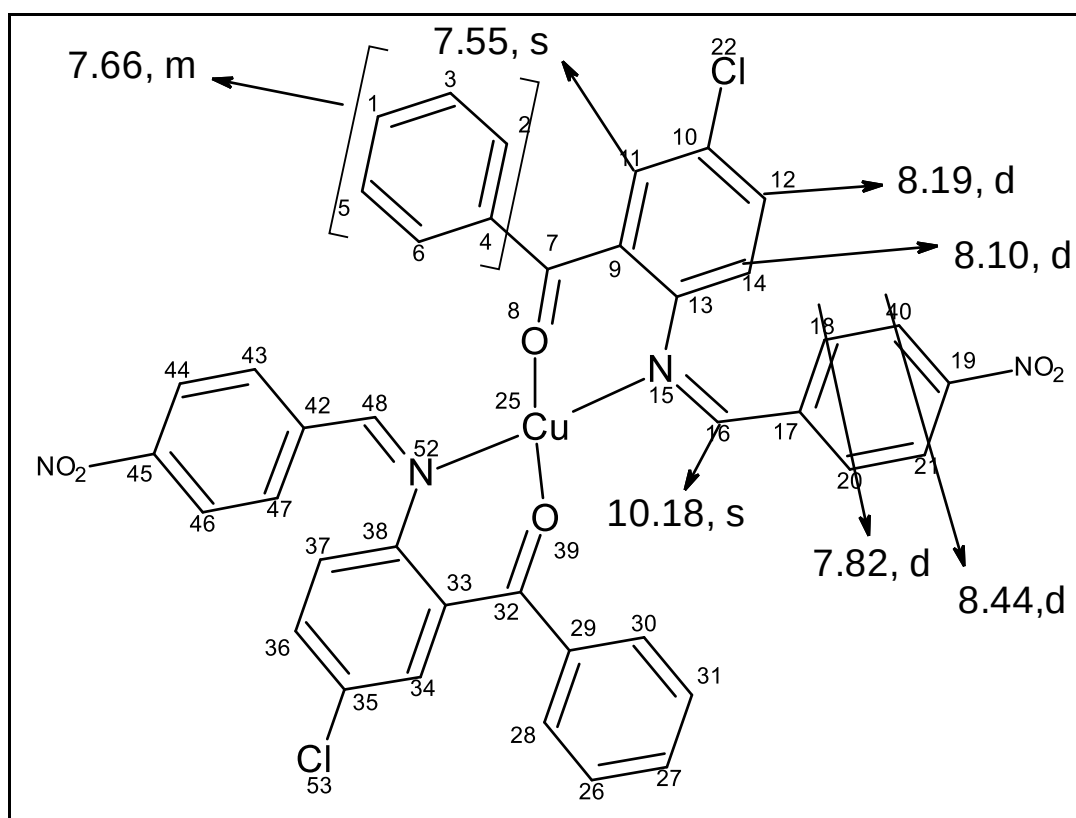


Figure 25: Assignment of ¹H NMR chemical shift in Cu₄N.

Structure elucidation is impossible in other metal complexes because of their paramagnetism. Coordinating them to the ligands causes a peak broadening on the ¹H NMR spectra making it impossible to extract more data regarding their overall structural make up. According to Satcher *et al*, ¹H NMR spectroscopy isn't often considered a suitable characterisation approach for paramagnetic compounds,

because of the slow electronic relaxation that leads to broad resonance [123]. However, following the movement of azomethine proton signal in these metal complexes could provide insight into whether or not complexation happened. The expectation is that, the azomethine protons in these ligands should move to a higher chemical shift upon complexation. Table 9 shows the chemical shifts of these metal complexes and clearly, the azomethine signal shifted downfield. The ^1H -NMR spectra of these compounds are displayed on Appendix B.

Table 9: Selected Chemical shift of azomethine proton in ligands and metal complexes

	3N	Co3N	Cu3N	Fe3N	Ni3N
Chemical shift of a schiff base proton (ppm)	8.74	10.09	10.15	10.25	10.04
	4N	Co4N	Cu4N	Fe4N	Ni4N
Chemical shift of a schiff base proton (ppm)	8.44	10.17	10.17	10.28	10.08

4.4 ESI-MS ANALYSIS

Mass spectrometry is an analytical tool that is normally used to determine the structure through molecular mass data of analyte molecules after their conversion to ions. The molecule is first ionised to obtain positive or negative charges in the mass spectrometer's ionisation source. The ions then pass through the mass analyser, arriving at the detector depending on their mass to charge ratio. The computer system then records useful signals according to mass per charge ratio as ions make contact with the detector. The signals are graphically displayed as a mass spectrum, which shows their relative abundance based on their m/z ratio.

4.4.1 3N ligand

The formation of Schiff base ligand (3N) was studied with ESI-MS spectra. The ligand was run in positive ion mode. The expected molecular formula of this ligand is established by comparing its molecular formula weight with m/z values. The ESI mass spectrum of the 3N ligand as shown in Figure 26 shows the parental ion peak at $[\text{MH}^+]$ 365 m/z consistent with $[\text{C}_{20}\text{H}_{15}\text{ClN}_2\text{O}_3]$ which has a molecular mass of 364.06 amu. The other peaks in the spectrum are assigned to different fragments of the ligand such

as $[MH^+]$ 335.8 m/z which is assigned to $[C_{20}H_{17}ClN_2O]$ after a loss of two O^{2-} ions, and $[MH^+]$ 319 m/z assigned to $[C_{20}H_{14}ClNO]$ after a loss of NO_2^- fragment. Scheme 1 shows a schematic diagram on mass spectral fragmentation patterns of 3N ligand. The fragmentation patterns are found to be consistent with its structures.

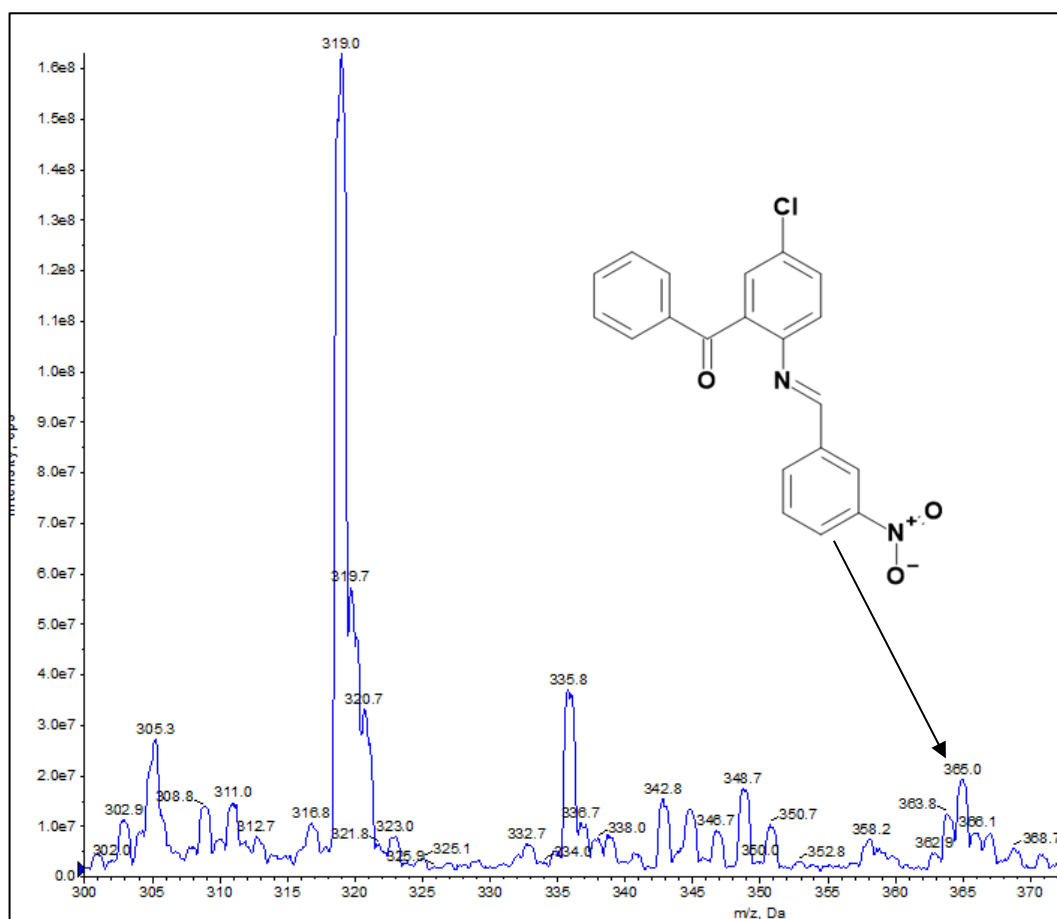
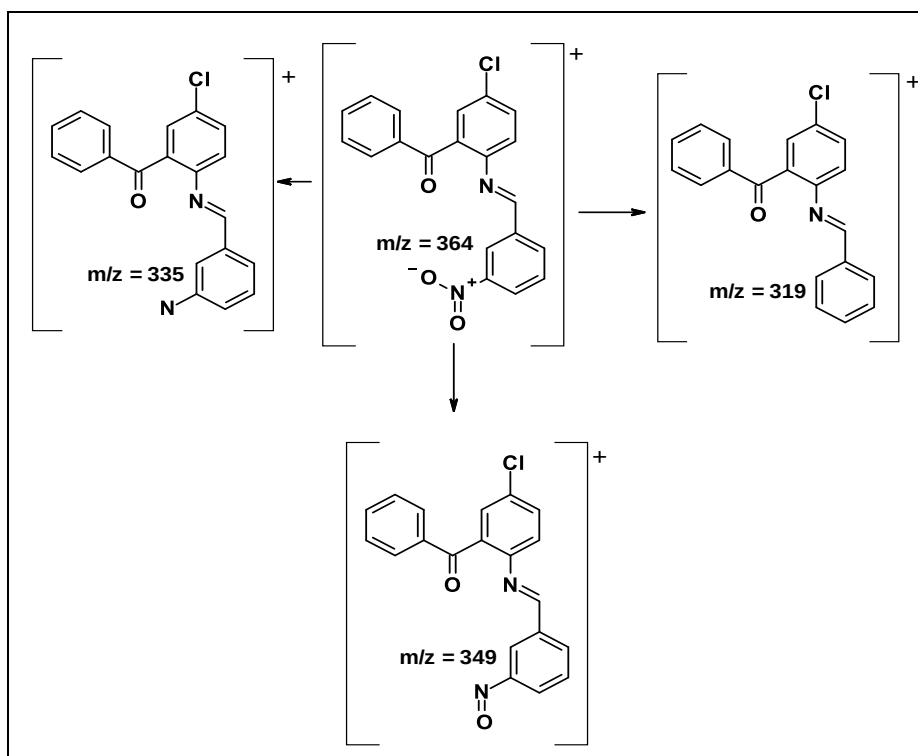


Figure 26: Mass spectrum of 3N ligand.



Scheme 1: Mass fragmentation patterns of 3N ligand [$C_{20}H_{14}ClN_2O_3$].

4.4.2 4N ligand

The ligand was run in positive ion mode. The ESI mass spectrum of the 4N ligand as shown in Figure 27 shows an expected parental ion peak at $[MH^+]$ 365 m/z agreeing with the molecular mass after protonation [$C_{20}H_{14}ClN_2O_3$] which has a molecular mass of 364.06 amu. The spectra show other peaks which are attributed to fragmentation of the parent molecular ion as shown in scheme 2. First, a loss of O^- radical give a fragment ion peak at $[MH^+]$ 347 m/z . Expulsion of O^{2-} ion followed by protonation at N site which gives an fragmented ion peak at $[MH^+]$ 335 m/z . The molecular fragment undergoes further fragmentation through two routes as shown in scheme 2. First, on loss of NH_3 radical giving an ion peak recorded at $[MH^+]$ 319 m/z , followed by detachment of C_4H_6 molecule which gives a fragment ion peak at $[MH^+]$ 269 m/z . The second route involve the simultaneous loss of NH_3 radical, O^{2-} ion and C_5H_5 molecule to give an ion peak recorded at $[MH^+]$ 241 m/z .

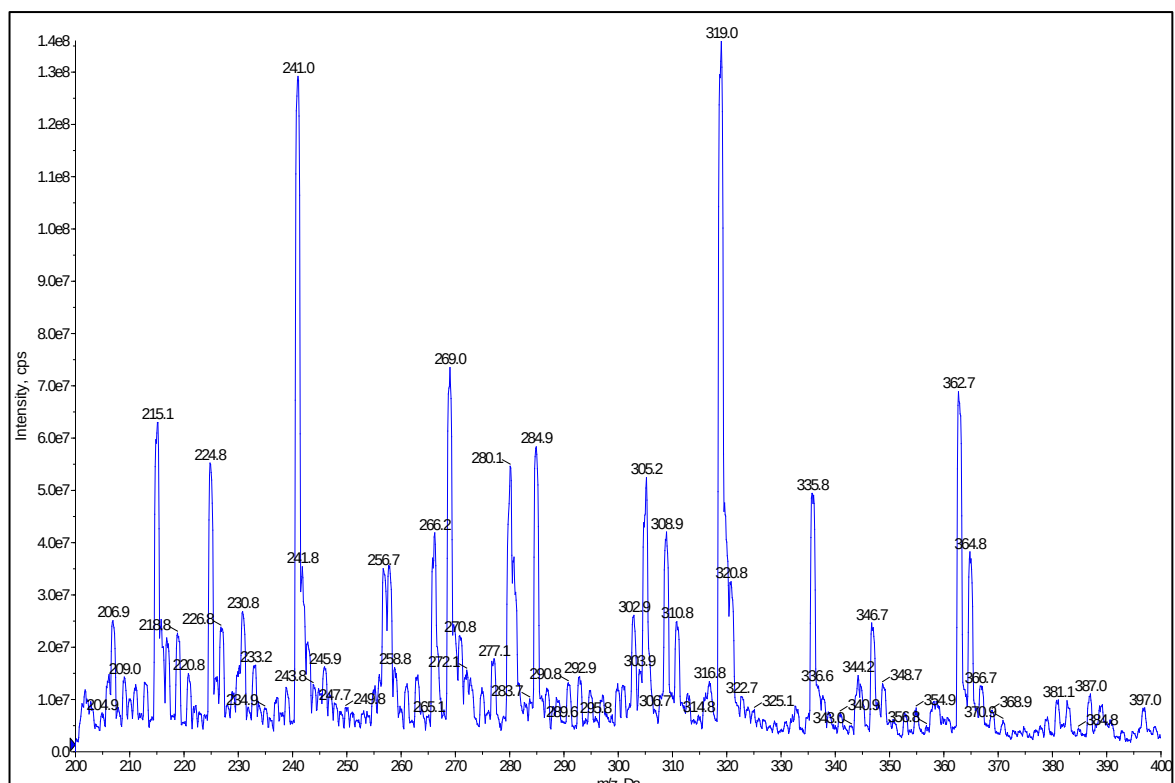
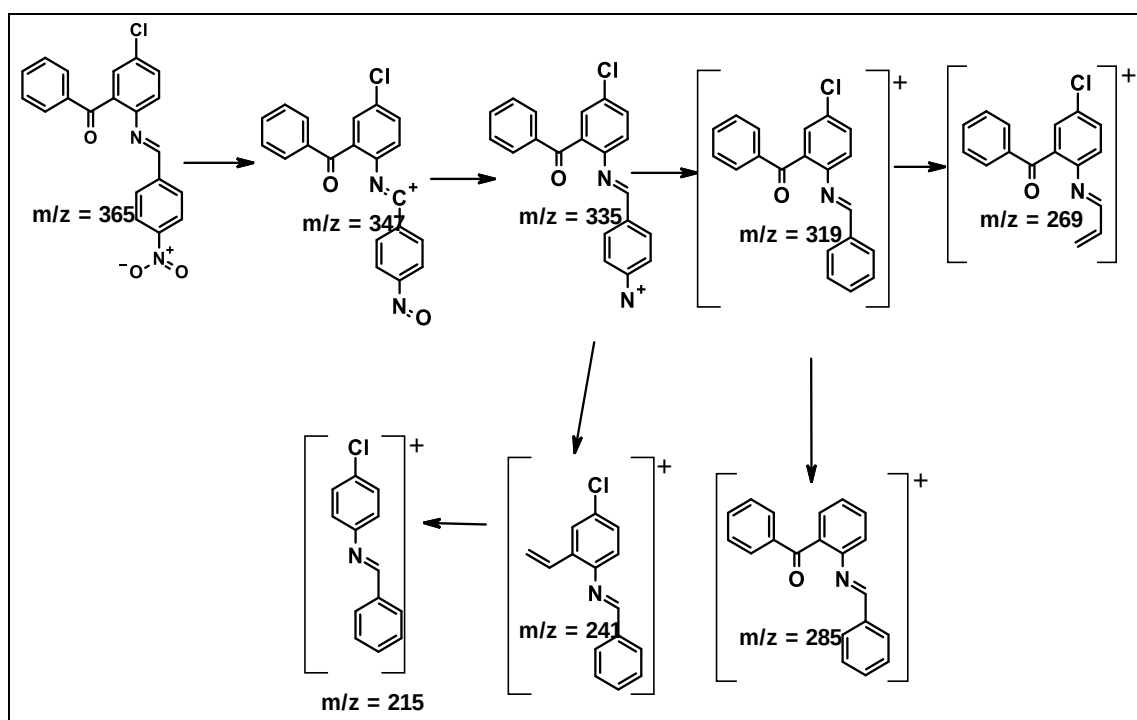


Figure 27: Mass spectrum of 4N ligand.



Scheme 2: Mass fragmentation pattern of 4N ligand $[C_{20}H_{14}ClN_2O_3]$.

4.4.3 Metal complexes

Cu3N complex

This complex was run in negative ion mode. The mass spectrum of Cu3N complex shows a peak at $[MH^-]$ 828 m/z and 830 m/z, which is assigned to a complex with 2 water molecules, $[Cu(C_{20}H_{14}ClN_2O_3)_2 \cdot 2H_2O]$ as shown in Figure 28. Further analysis of the spectrum shows several peaks that are due to adduct formation and also fragmentation of the molecule. Peaks at $[MH^-]$ 808 m/z and 790 m/z were attributed to Cu3N complex after loss of 1 water molecule, $[Cu(C_{20}H_{14}ClN_2O_3)_2 \cdot H_2O]$ and loss of 2 water molecules respectively $[Cu(C_{20}H_{14}ClN_2O_3)_2]$. Another peak appeared at $[MH^-]$ 844 m/z which is assigned to the Cu3N with 3 molecules of water $[Cu(C_{20}H_{14}ClN_2O_3)_2 \cdot 3H_2O]$. The spectrum also shows peaks which are considered to be due to the isotopic nature of chlorine and copper.

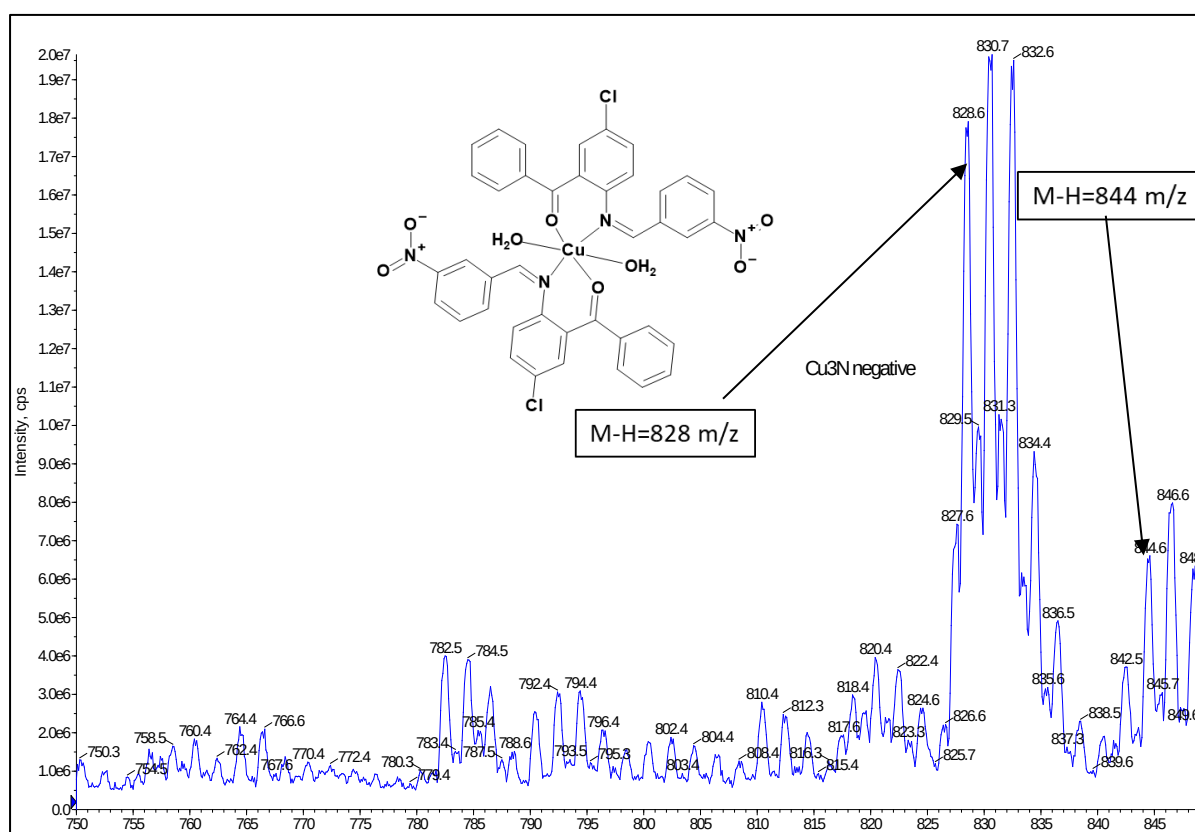


Figure 28: Mass Spectrum of Cu3N complex.

Cu4N complex

The complex was run in negative ion mode. The mass spectrum of Cu4N complex $[Cu(C_{20}H_{14}ClN_2O_3)_2 \cdot 2(H_2O)]$ have the parental ion peak at $[MH^-]$ 828.6 m/z agreeing

with the $[\text{Cu}(\text{C}_{20}\text{H}_{14}\text{ClN}_2\text{O}_3)_2 \cdot 2(\text{H}_2\text{O})]$ molecular formula. Some peaks are really close to the parental ion peak with a difference of $m/z = 2$, that is $[\text{MH}^-]$ 830.7 and 832.6 m/z . These peaks are believed to be due to isotopic effects of Cl ion [124]. The spectrum shows other peaks which are attributed to fragmentation of the parent molecular ion as well as addition of water molecules [125]. Some fragments with different m/z values appear at $[\text{MH}^-]$ 792.4 m/z corresponding to the complex after loss of 2 water molecules $[\text{C}_{40}\text{H}_{26}({}^{37}\text{Cl})({}^{35}\text{Cl})\text{CuN}_4\text{O}_6]$ and $[\text{MH}^-]$ 844 m/z which was attributed to a Cu(II) complex with 3 water molecules $[\text{Cu}(\text{C}_{20}\text{H}_{14}\text{ClN}_2\text{O}_3)_2 \cdot 3(\text{H}_2\text{O})]$. The peak at $[\text{MH}^-]$ 846 m/z is believed to be due to isotopic effects of chlorine ion and possibly isotopes of copper as well.

Co3N complex

The complex was run in positive ion mode. Molecular mass of Co3N as per the molecular formula $[\text{C}_{40}\text{H}_{26}\text{Cl}_2\text{CoN}_4\text{O}_6]$ is 787.07 g/mol and considering the isotopic effects of chlorine atoms in the molecule, the expected m/z peaks are $[\text{MH}^+]$ 788, 790, and 792 m/z . The observed peaks in the spectrum are 790.7 and 792.7 m/z confirming the mass of the product.

Co4N complex

This complex was run in positive ion mode. The mass spectrum of Co4N complex shows a molecular ion peak at $[\text{MH}^+]$ 824 m/z which corresponds to the molecular mass of the complex with two molecules of water $[\text{C}_{40}\text{H}_{26}\text{Cl}_2\text{CoN}_4\text{O}_6 \cdot 2\text{H}_2\text{O}]$ which have a molecular mass of 823.08 g/mol. The spectrum also shows a peak at $[\text{MH}^+]$ 806 m/z which correspond to the complex after it loses one molecule of water $[\text{C}_{40}\text{H}_{26}\text{Cl}_2\text{CoN}_4\text{O}_6 \cdot \text{H}_2\text{O}]$. Other peaks observed are attributed to fragmentation of the parent molecular ion [126]. Such peaks are $[\text{M}^+]$ 773.0 m/z owing to loss of O^- radical from one of the nitro groups in the ligand giving $[\text{C}_{40}\text{H}_{26}({}^{37}\text{Cl})\text{ClCoN}_4\text{O}_5]$, $[\text{M}]^+$ 729.0 m/z which is attributed to subsequent loss of O^- radical anion and O^{2-} ion which gave $[\text{C}_{40}\text{H}_{30}({}^{37}\text{Cl})\text{ClCoN}_4\text{O}_2]$. Another peak observed at $[\text{MH}^+]$ 713 m/z is believed to be of $[\text{C}_{40}\text{H}_{29}\text{Cl}_2\text{CoN}_3\text{O}_2]$ resulting from the loss of amino group.

Ni3N complex

Positive ion mode was used for analysing this complex. Mass spectrum of Ni3N shows a diminished peak of $[\text{M}^+]$ 806.1 m/z , corresponding to $[\text{C}_{40}\text{H}_{26}({}^{35}\text{Cl})({}^{37}\text{Cl})\text{N}_4\text{NiO}_6 \cdot \text{H}_2\text{O}]$ with a molecular mass of 806.06 g/mol. The spectrum also shows a medium peak of

$[M]^+$ 778.1 m/z which is due to loss of C_3H_3 radical anion after disintegration of the phenyl moiety. Other peaks observed at $[M]^+$ 750.1 and 722.1 m/z are believed to be due to loss of CH_3 radical and subsequent losses of O^- radical anion and O^{2-} ion.

Ni4N complex

Ni4N has a molecular mass of 786.06 g/mol, according to its chemical formula $[C_{40}H_{26}Cl_2N_4NiO_6]$. The ESI-MS was run in positive ion mode for this complex, and the anticipated m/z peaks are 787, 789, 791, and 793 m/z due to the isotopic masses of chlorine and Ni atoms in the molecule. The spectrum shows peaks at $[MH]^+$ 790.6 and 792.6 m/z which corresponded to a molecular mass per charge ratio of the complex after protonation.

Fe4N complex

The complex was run in positive ion mode. The Fe4N mass spectrum contains a moderate peak at $[MH^+]$ 824.9 m/z which is assigned to Fe4N complex with 2 molecules of water $[C_{40}H_{26}(Cl)_2FeN_4O_6 \cdot 2H_2O]$ which has a molecular mass of 824.07 g/mol. Another peak was observed at $[MH^+]$ 838.5 m/z which is attributed to $[C_{40}H_{26}Cl_2FeN_4O_6 \cdot 3H_2O]$ which has a molecular mass of 838.09 g/mol and the isotopic patterns of chlorine are observed at $[MH^+]$ 840.5 and 842.5 m/z. Further analysis of the spectrum shows a peak at $[MH^+]$ 729.7 m/z. This peak is due to a fragment of the parent molecule after the loss of CNO_2 molecule resulting in $[C_{39}H_{29}Cl_2FeN_3O_4]$ which has a molecular mass of 729.09 g/mol. Additionally the spectrum shows a peak at $[M^+]$ 702.7 m/z due to disintegration of the phenyl group leading to the loss of $C_3H_3^-$ radical to give $[C_{37}H_{26}Cl_2FeN_3O_4]$. Mass spectra of all metal complexes are shown in Appendix C.

4.5 ANTIMICROBIAL STUDIES

All compounds were tested for antimicrobial activity against gram negative *Escherichia coli* and gram-positive *Staphylococcus aureus*. The minimum inhibitory concentration (MIC) was used to estimate the antimicrobial activity for all compounds. This value defines the lowest concentration of a compound needed to inhibit or stop growth of bacteria. This value was achieved by employing broth micro dilution assay. The (MIC) values for all compounds against *E. coli* and *S. aureus* are recorded in Figure 29 and 30 respectively. The MIC values indicated that all complexes except Ni3N had enhanced activity compared to their respective ligands against both strains. Tweedy's

chelation theory and Overton's concept explain why the complexes have better antimicrobial activity than the ligands [127]. Tweedy's chelation theory states that chelation reduces the polarity of the metal ion due to ligand orbital overlap and partial sharing of the metal ion's positive charge with donor groups. This boosts the lipophilicity of the metal complexes [55]. Overton's concept of cell permeability states that lipid membrane surrounding the cell allows only lipid-soluble materials to pass through [126]. Metal complexes with increased lipophilicity can penetrate lipid membranes more easily, giving metal complexes more access to the bacteria's internal organelles for better antimicrobial action [55].

Several compounds exhibit good activity against both strains. Cu3N had the lowest MIC at 129 μ M and 118 μ M against *E.coli* and *S.aureus* respectively. This means that Cu3N demonstrated better activity compared to other complexes. It is also worth noting that while Ni3N complex exhibited decent activity against *S. Aureus* at 135 μ M, it had very low antimicrobial activity compared to its 3N ligand against *E. coli* which was recorded at 729 μ M. Although the mechanism of action of these metal complexes is not yet known, it is possible that Ni3N's mode of action could be dependent on the type of the bacterial cell. This is because it clearly had a decent activity against *S.aureus*. Additionally, it could be due to other aspects which influence the antibacterial activity. Nature of the ligand, nature of the metal ion, and coordination sites are some of the factors that influences the antibacterial potency of a metal complex [128].

Further analysis on the MIC data supports the suggestion that these compound's mode of action could be dependent on the type of the bacterial cell. Gram-positive strain (*S. aureus*) is generally more susceptible to the compounds as compared to gram-negative strain (*E. coli*). This is observed from their general trend as shown in Figures 29 and 30. The data in these figures shows higher MIC values for *E. coli* and relatively low MIC values for *S.aureus*. Some articles suggests that this observation could be because gram-negative bacterial cell membranes differ from those of gram-positive bacteria [129]. The gram-negative strains have an outer cell membrane which act as a shield and only allow passage of certain objects into the cell. The gram-positive strain lacks this layer of protection which makes it more susceptible to the complexes.

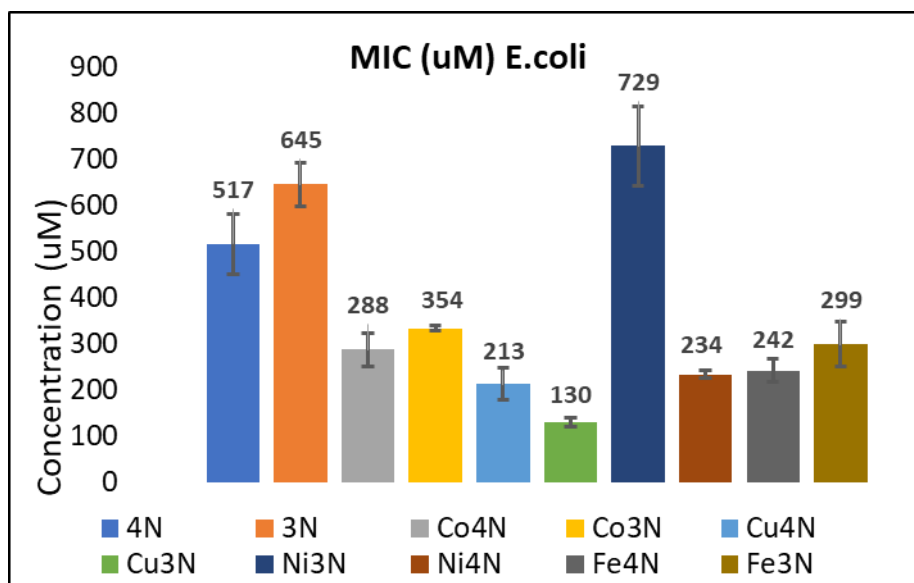


Figure 29: Average value of MIC (μM) of Metal complexes and their ligands in *E.coli* (N=3 independent determinations)

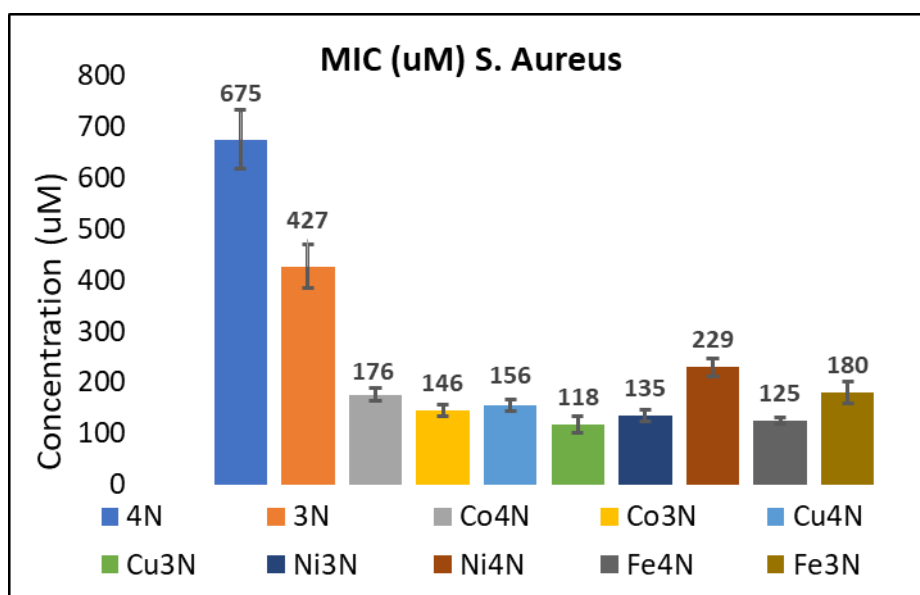


Figure 30: Average value of MIC (μM) of Metal complexes and their ligands in *S.aureus* (N=3 independent determinations)

5.0 CONCLUSION

These Schiff base ligands were first successfully synthesised from condensation reaction between 4-nitrobenzaldehyde/ 3-nitrobenzaldehyde and 2-amino-5-chlorobenzophenone. They were further coordinated to copper(II), cobalt(II), iron(II), and nickel(II) metal ions. The ligands and the metal complexes were characterised by FTIR, ^1H NMR and ESI-MS, all these techniques confirmed the successful synthesis of these ligands and the successful coordination of metal ions to the Schiff base ligands. However, the structural make up of metal complexes except Cu3N and Cu4N, could not be thoroughly elucidated by ^1H NMR as they are paramagnetic. Formation of crystals for most compounds was unsuccessful as such only 4N ligand was analysed using SC-XRD. The ligands and complexes were tested for antibacterial activity on gram positive *S.aureus* and gram negative *E.coli*. The complexes except Ni4N had impressive activity against both strains when compared to their respective ligands. Moreover, the complexes generally had higher activity against gram positive strain. It was concluded that this trend could mean that the mechanism of action of these complexes is selective towards gram positive bacteria. Conclusively, the results of this research work support the idea that metal complexes have great potential as antibacterial agents, as such more studies on the compounds of this nature are considerably necessary.

More biological studies such as cytotoxicity studies, anticancer studies, antimicrobial studies on multidrug resistant strains, and DNA binding affinity studies should be done on these metal complexes.

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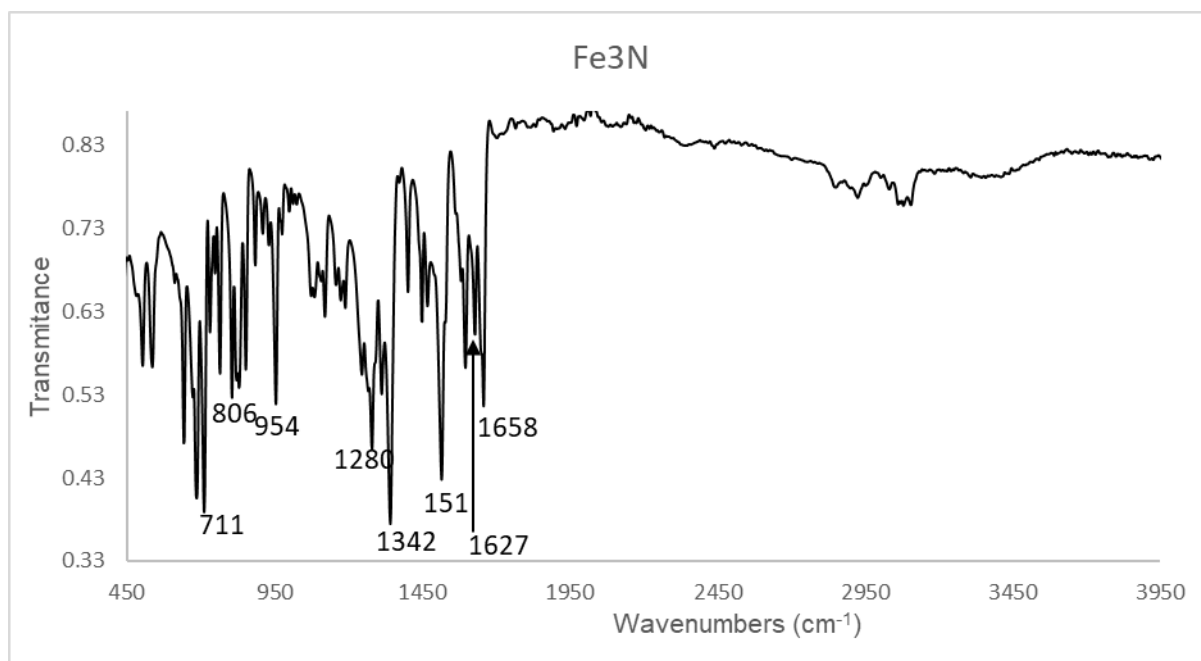
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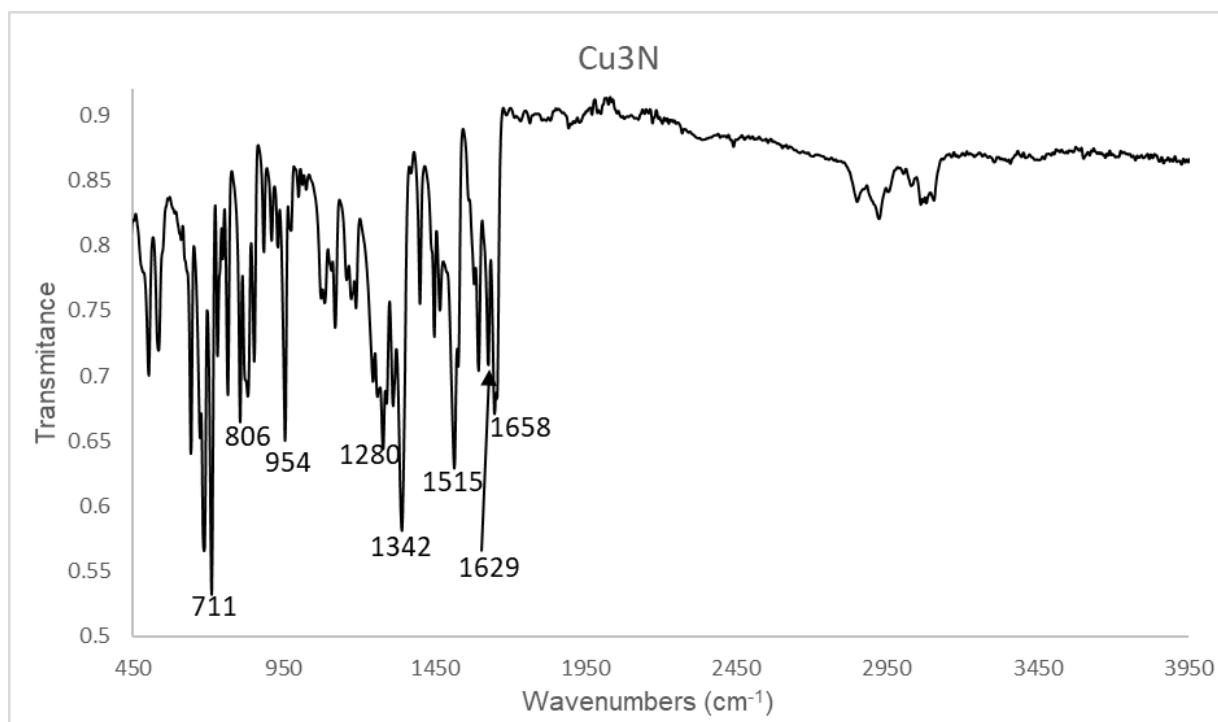
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7.0 APPENDICES

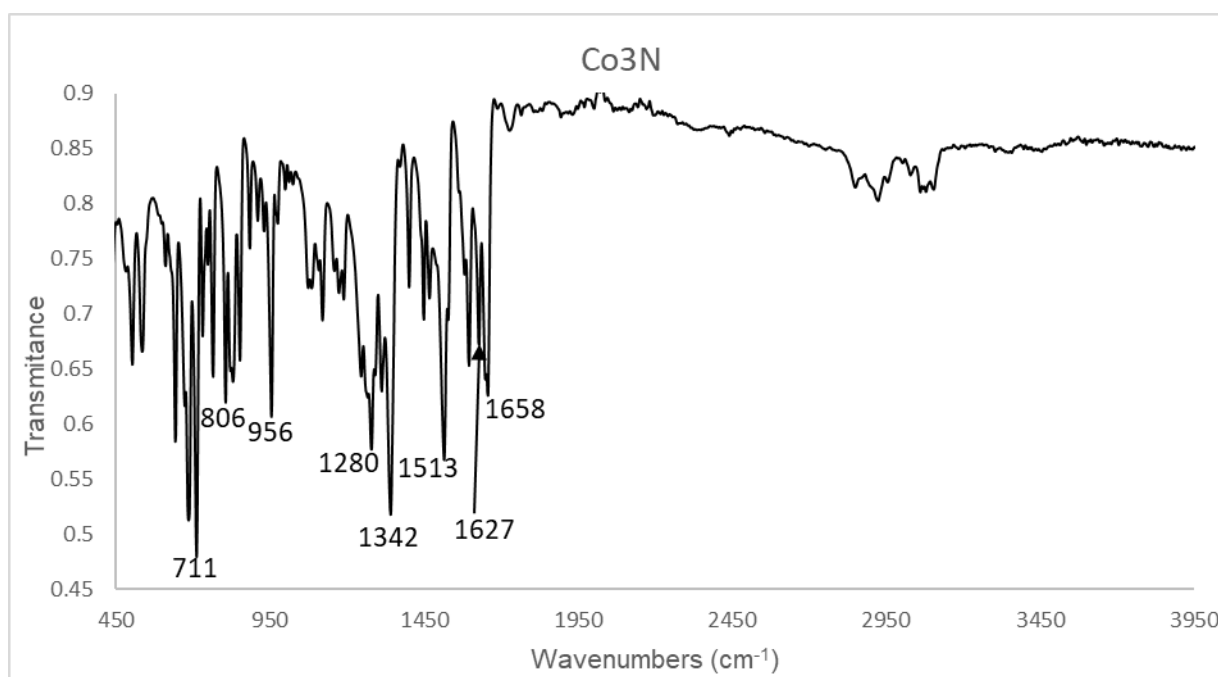
7.1 APPENDIX A; FTIR SPECTRA



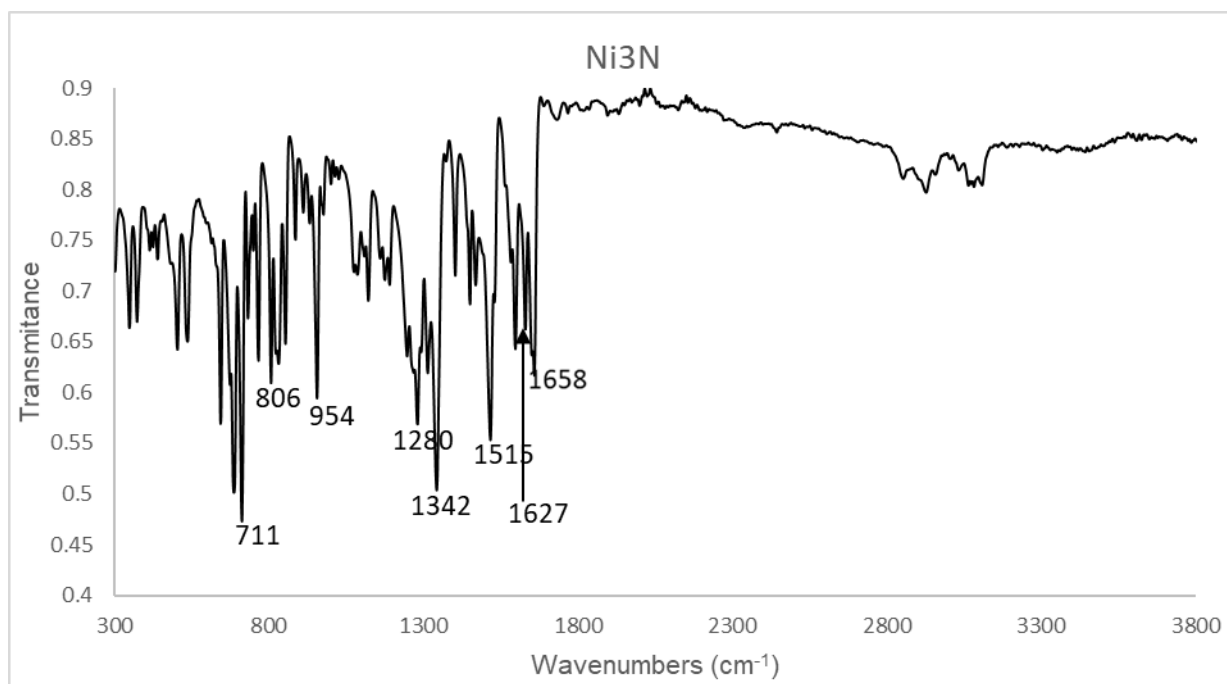
Spectrum 1. FT-IR Spectrum of Fe₃N complex



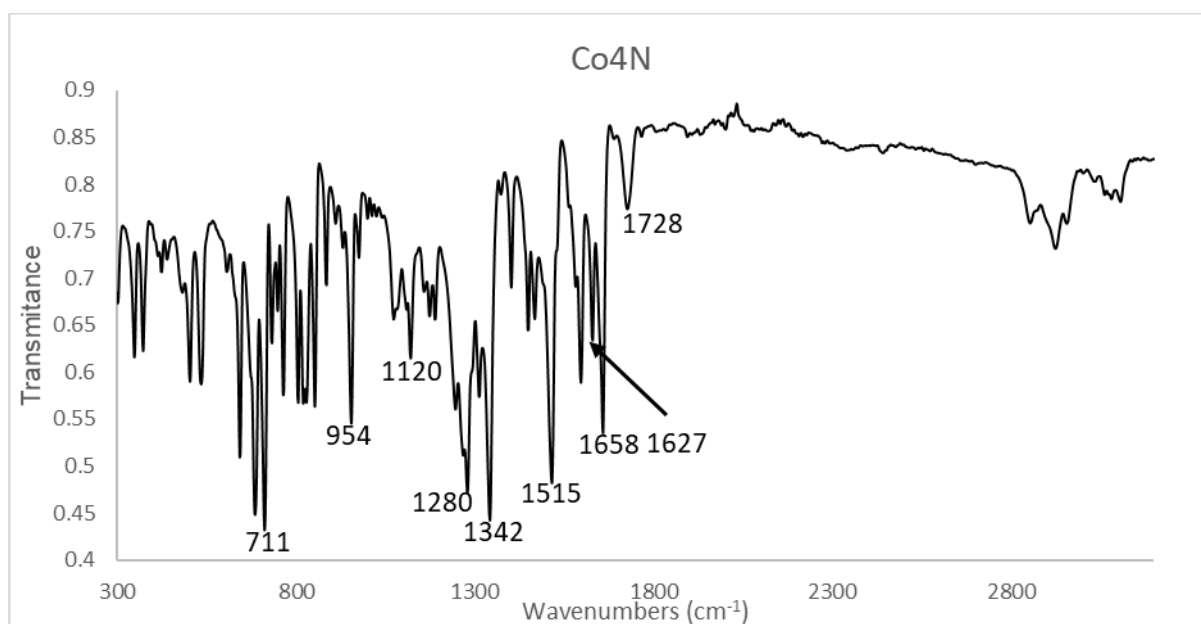
Spectrum 2. FT-IR Spectrum of Cu₃N complex



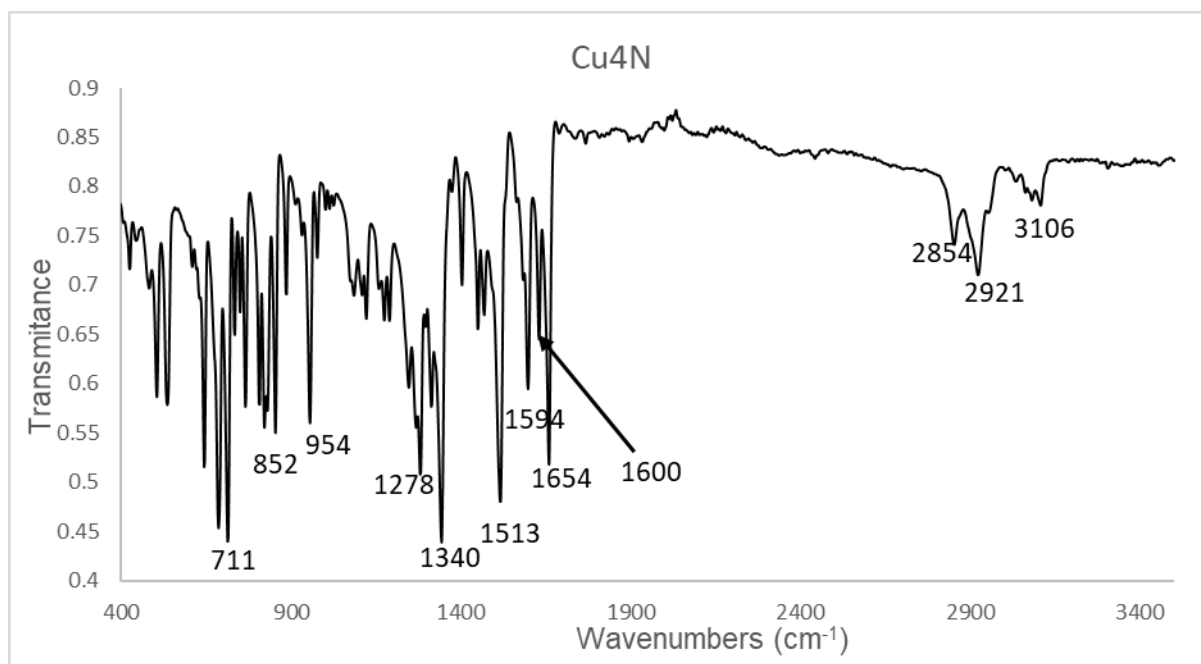
Spectrum 3. FT-IR Spectrum of Co₃N complex



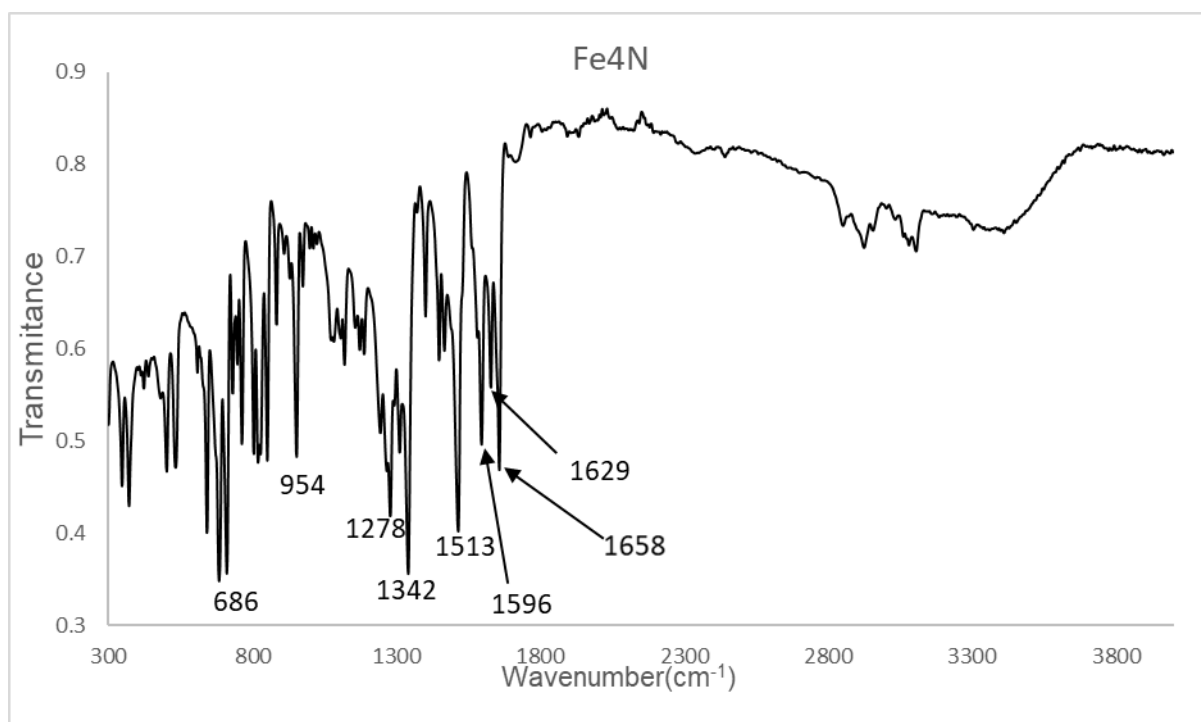
Spectrum 4. FT-IR Spectrum of Ni₃N complex



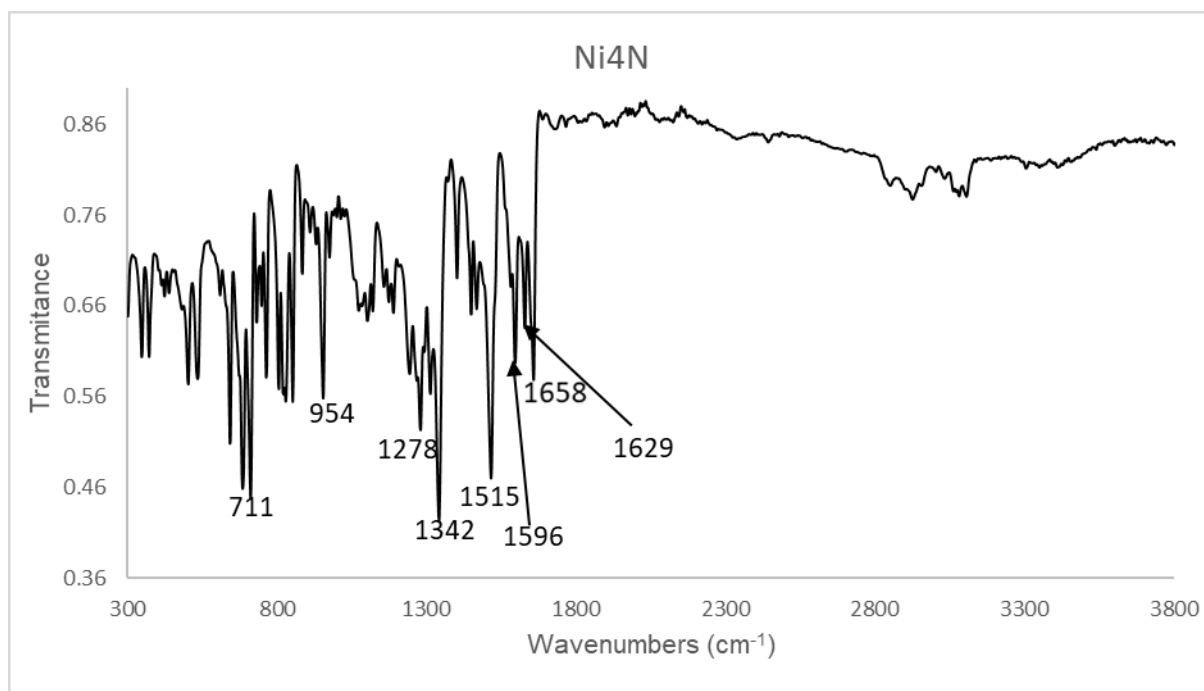
Spectrum 5. FT-IR Spectrum of Co₄N complex



Spectrum 6. FT-IR Spectrum of Cu₄N complex

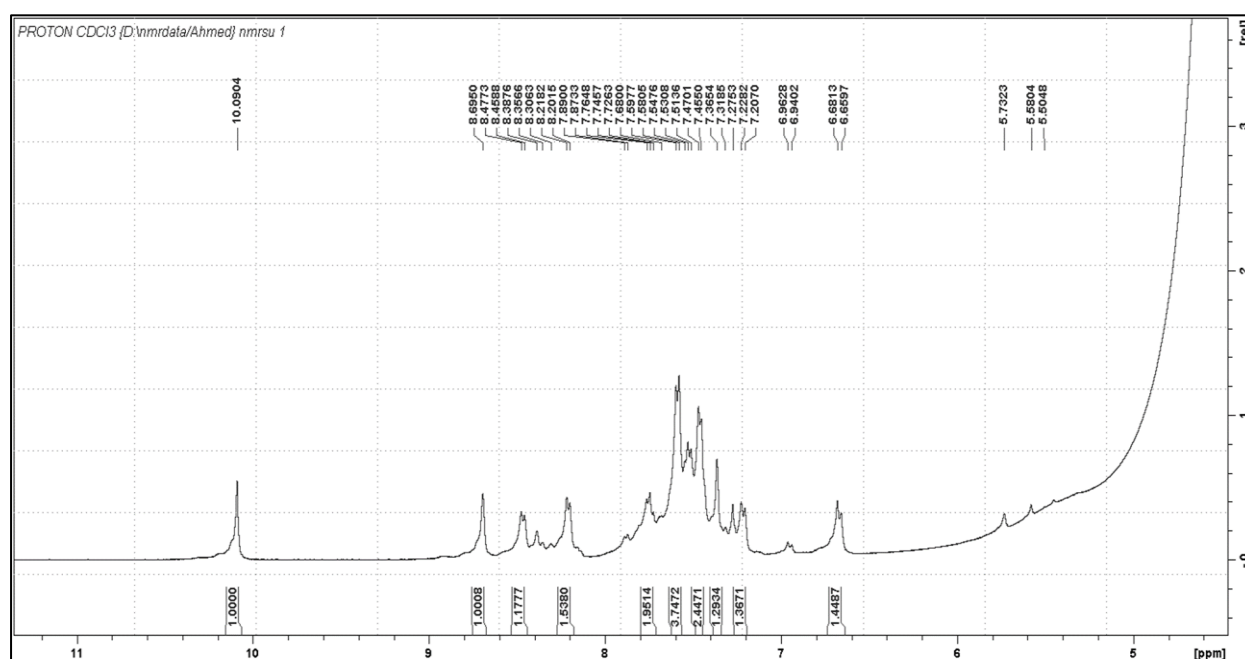


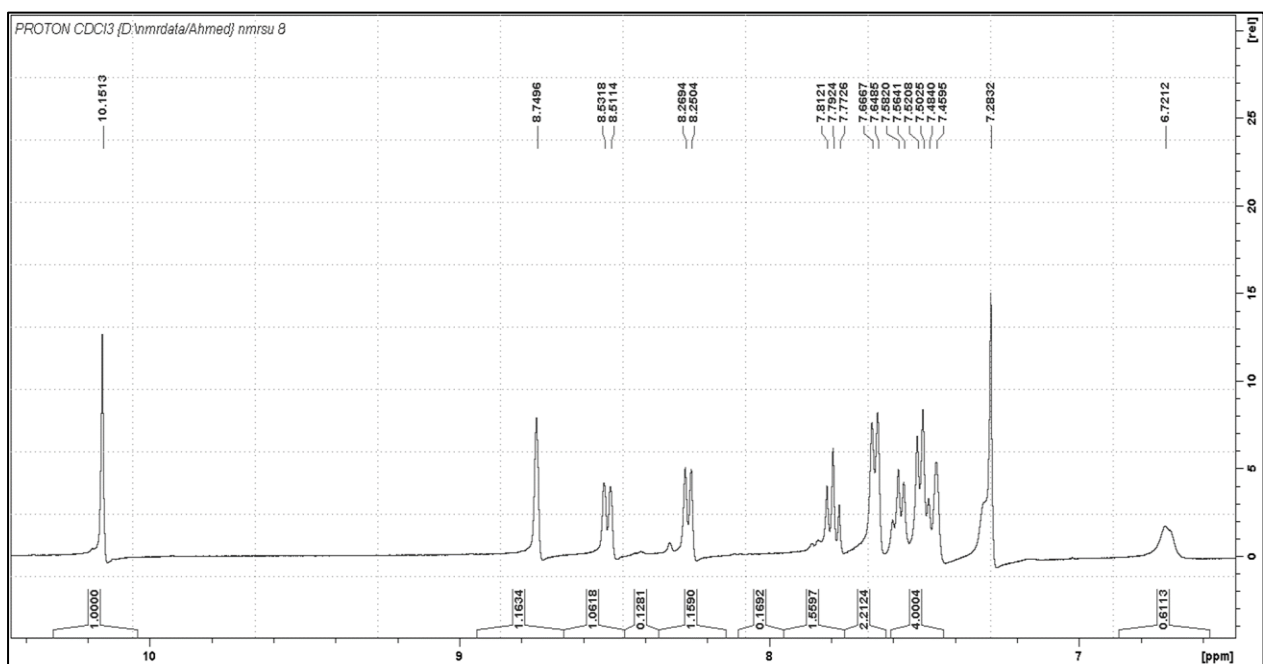
Spectrum 7. FT-IR Spectrum of Fe₄N complex



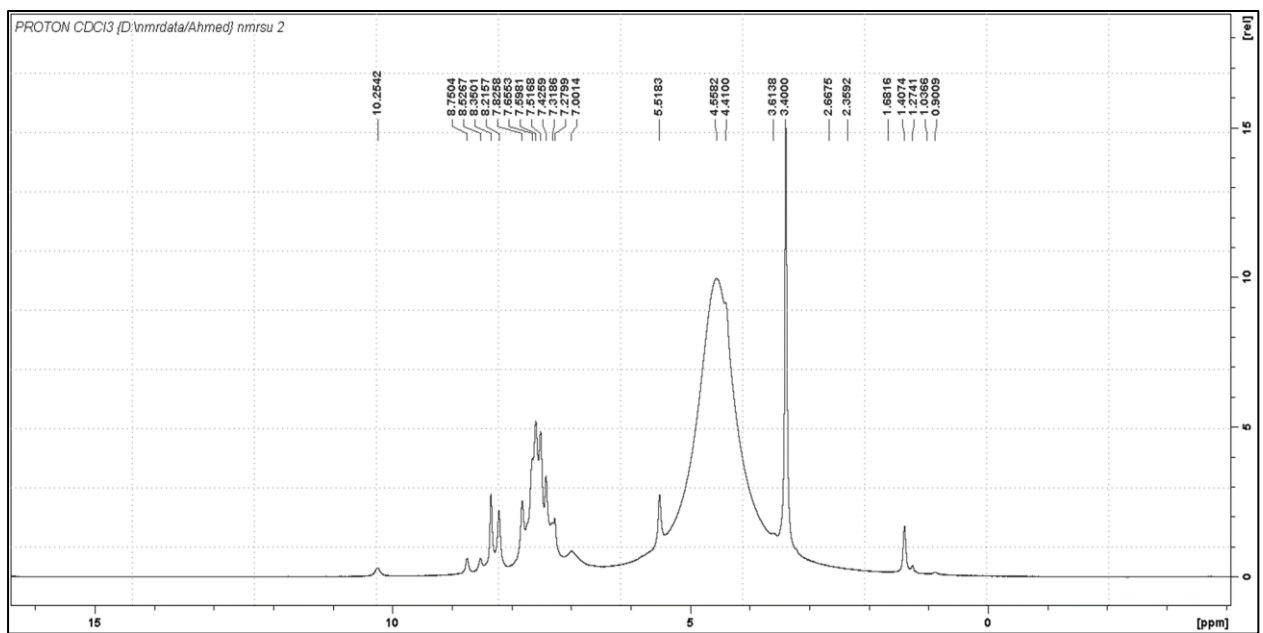
Spectrum 8. FT-IR Spectrum of Ni₄N complex

7.2 APPENDIX B; ¹H NMR SPECTRA

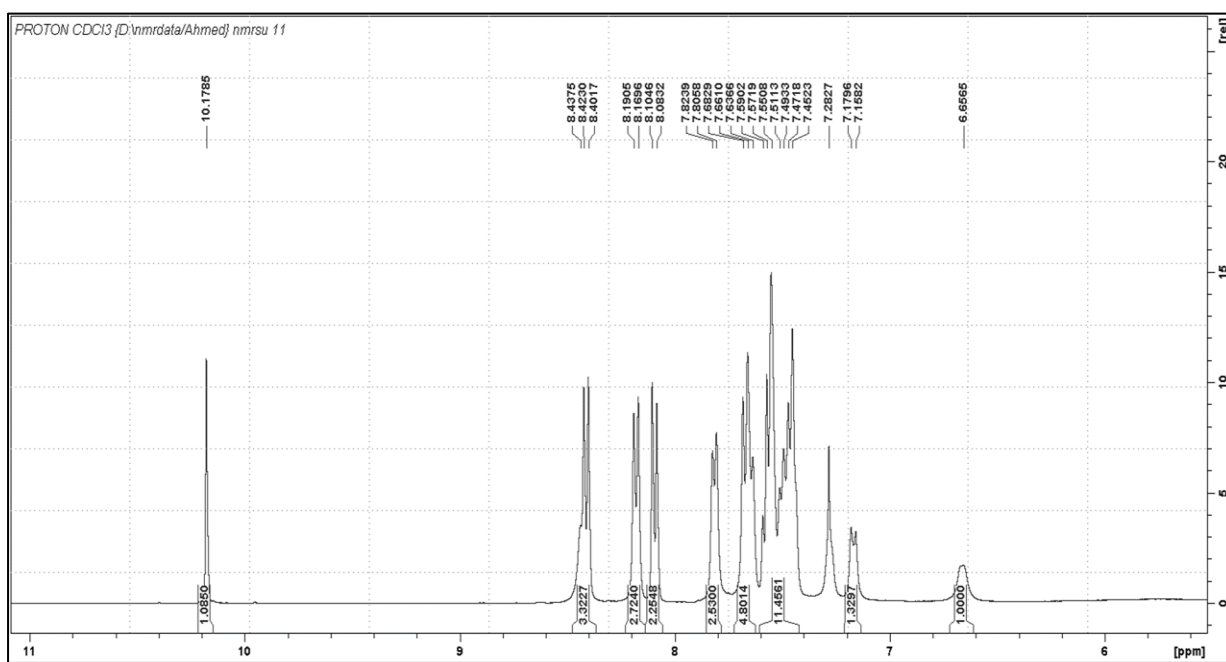
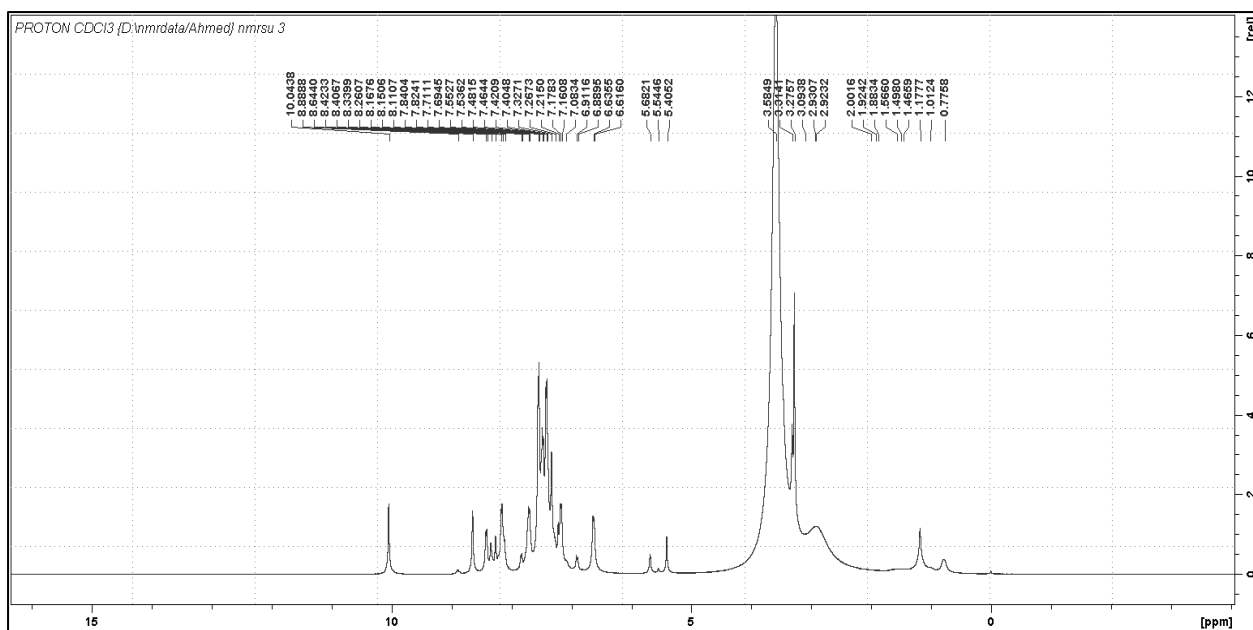


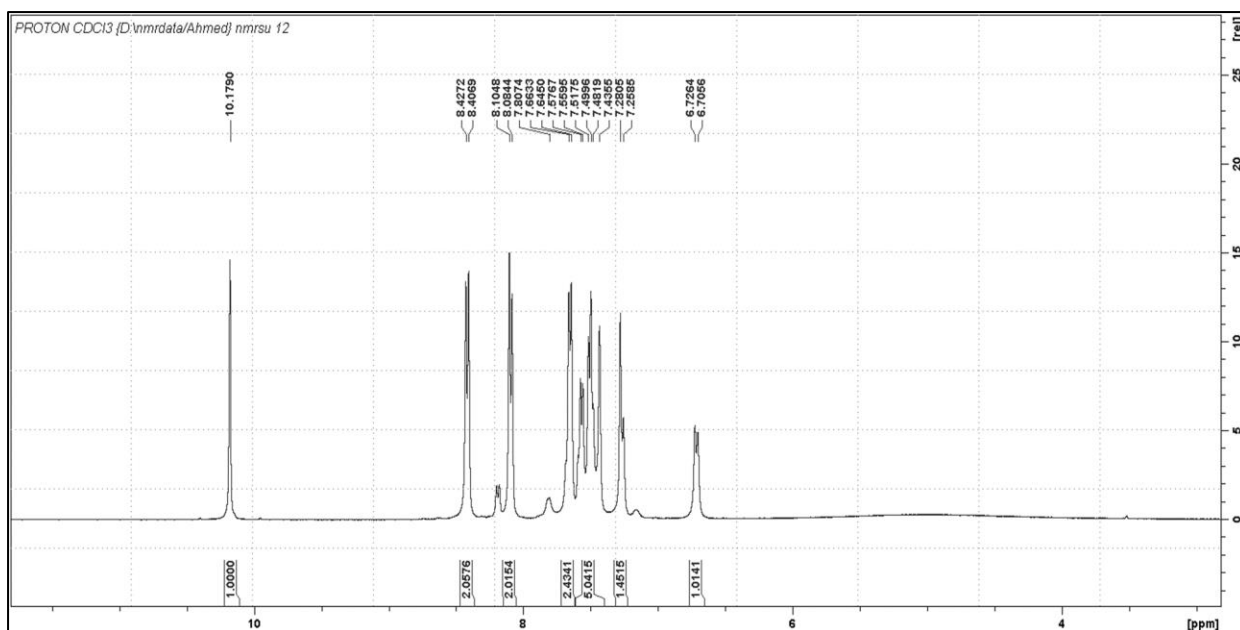


Spectrum 10. ^1H -NMR spectrum of Cu_3N complex

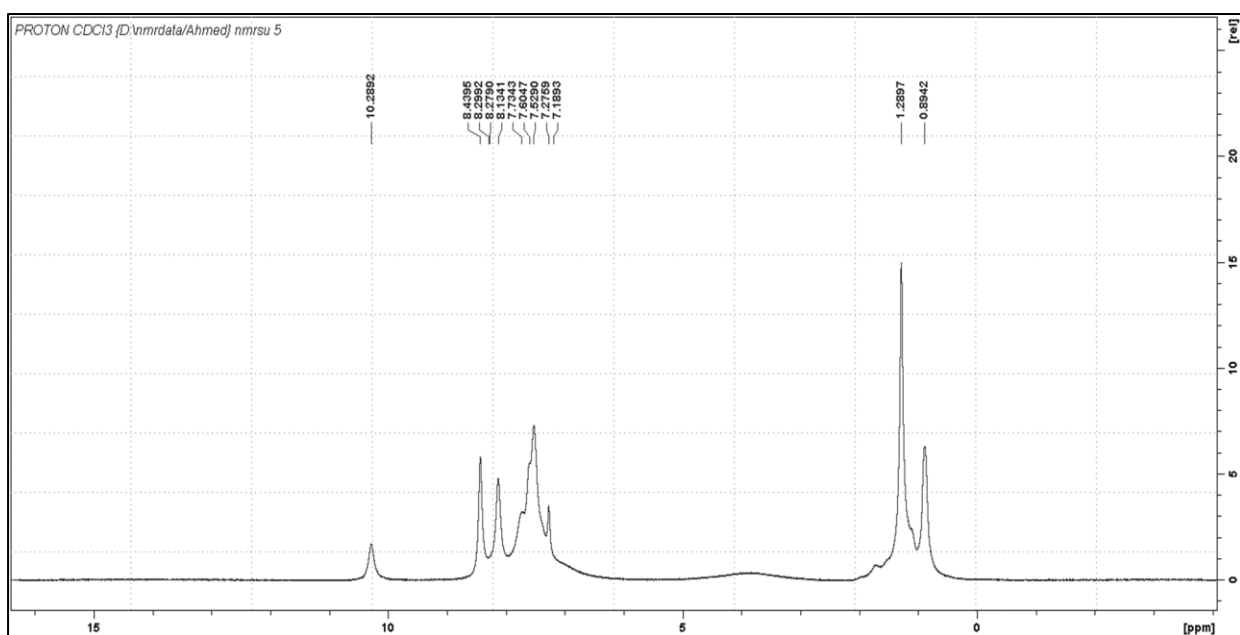


Spectrum 11. ^1H NMR spectrum for Fe₃N

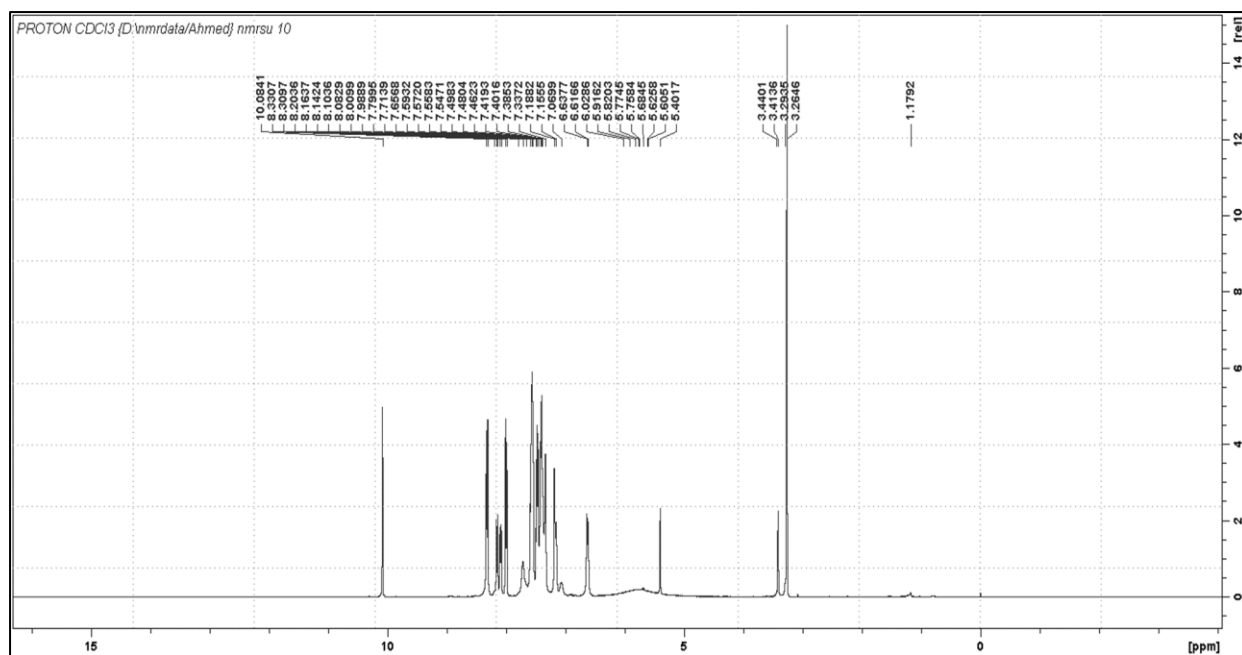




Spectrum 14. ^1H NMR spectrum of Co₄N

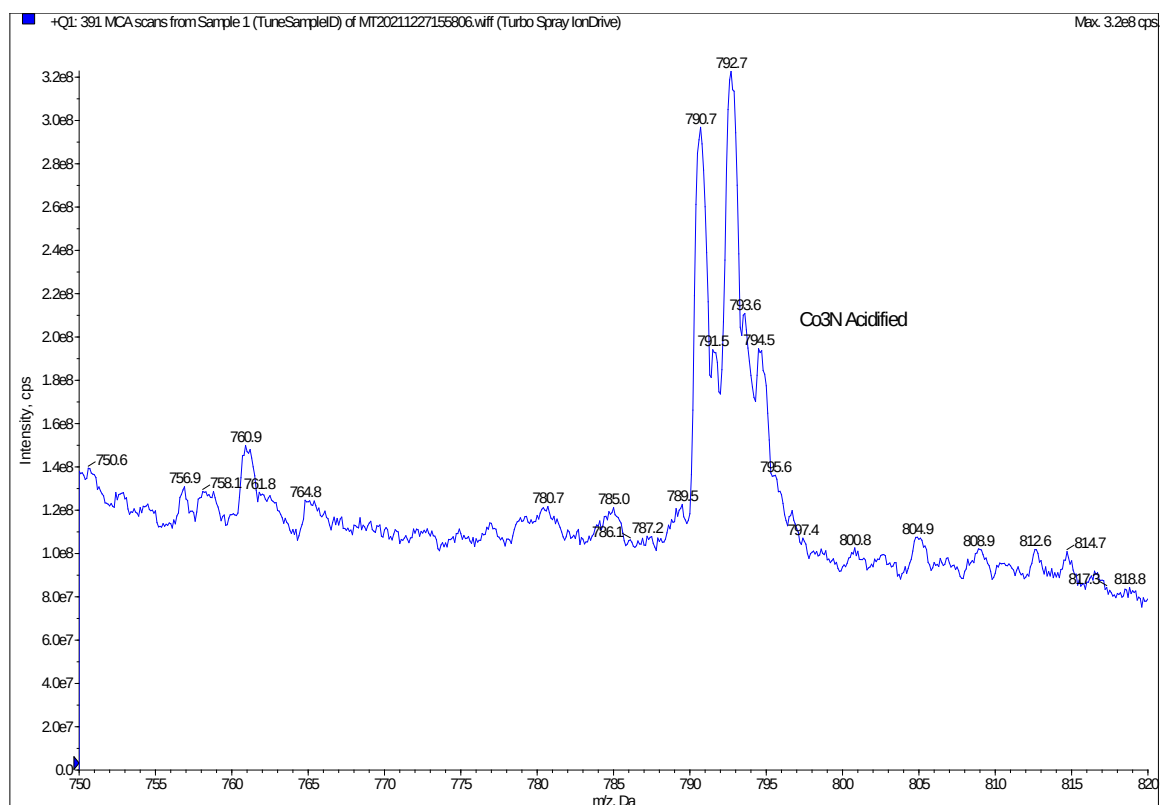


Spectrum 15. ^1H NMR spectrum of Fe₄N

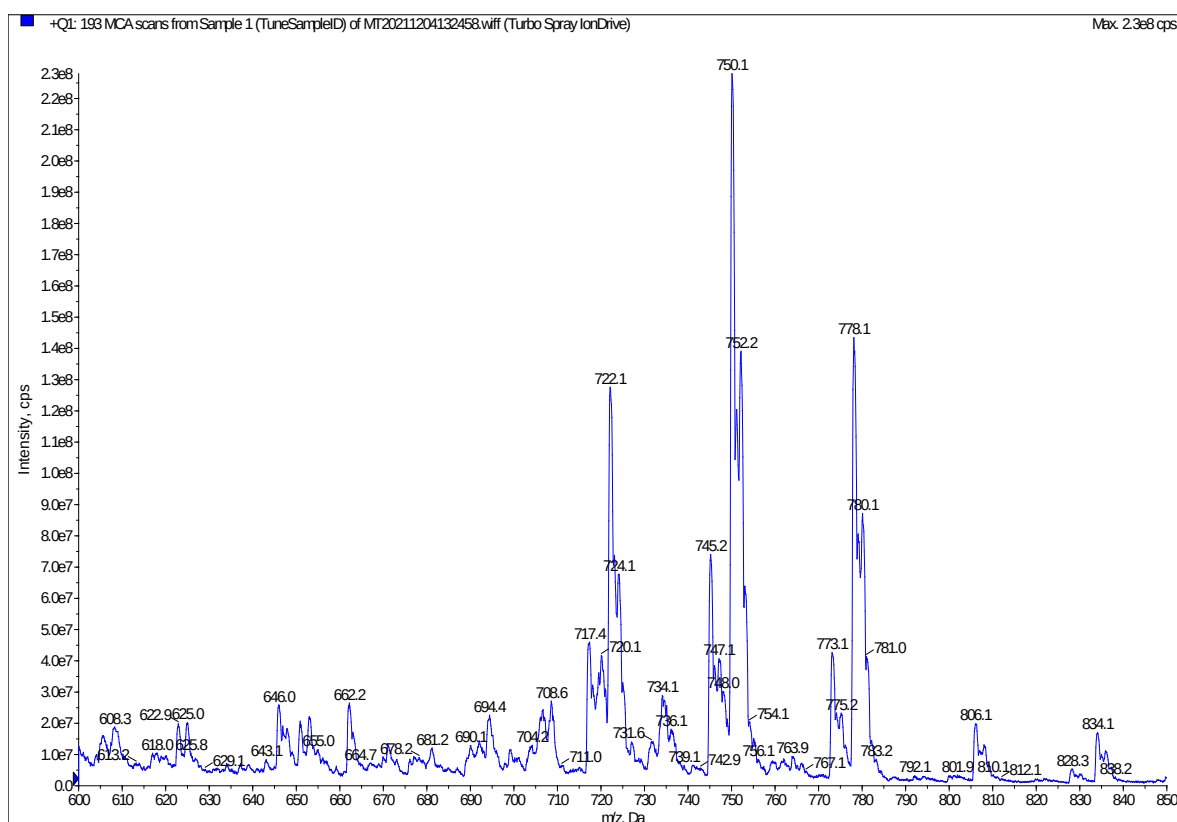


Spectrum 16. ¹H NMR spectrum of Ni₄N

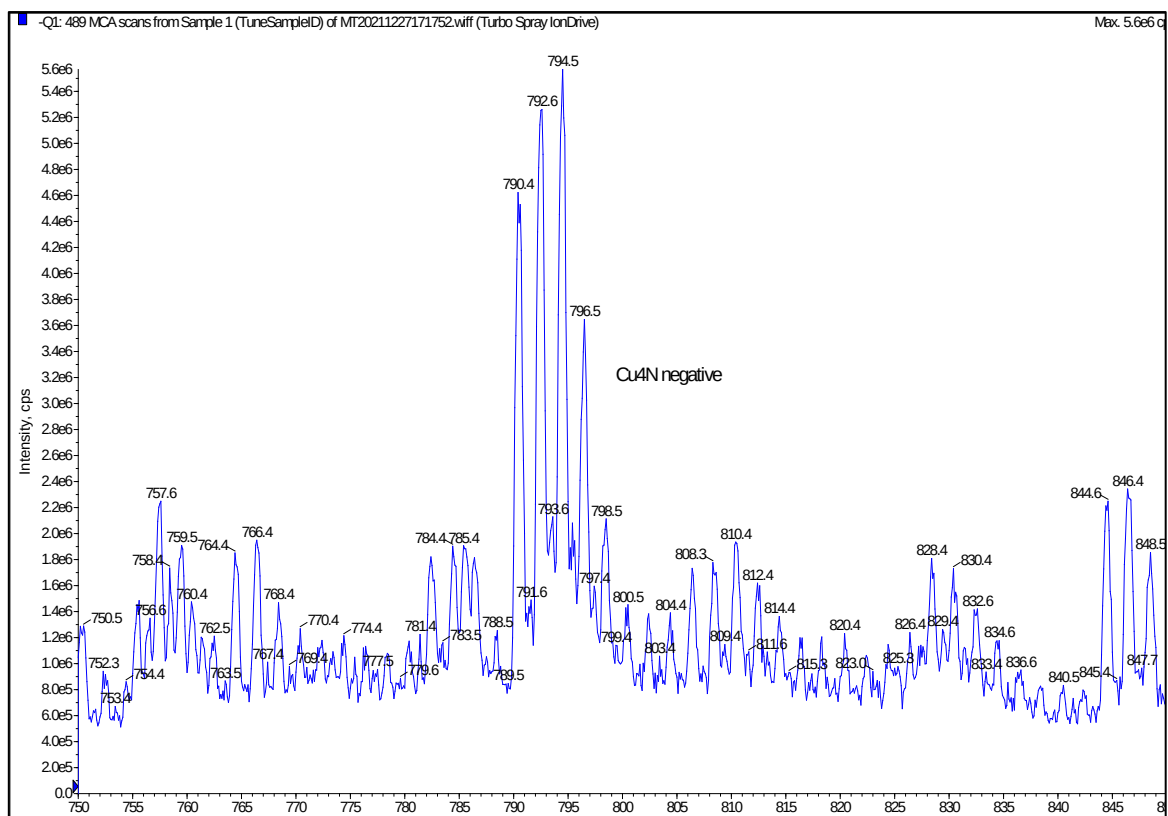
7.3 APPENDIX C; ESI-MS SPECTRA



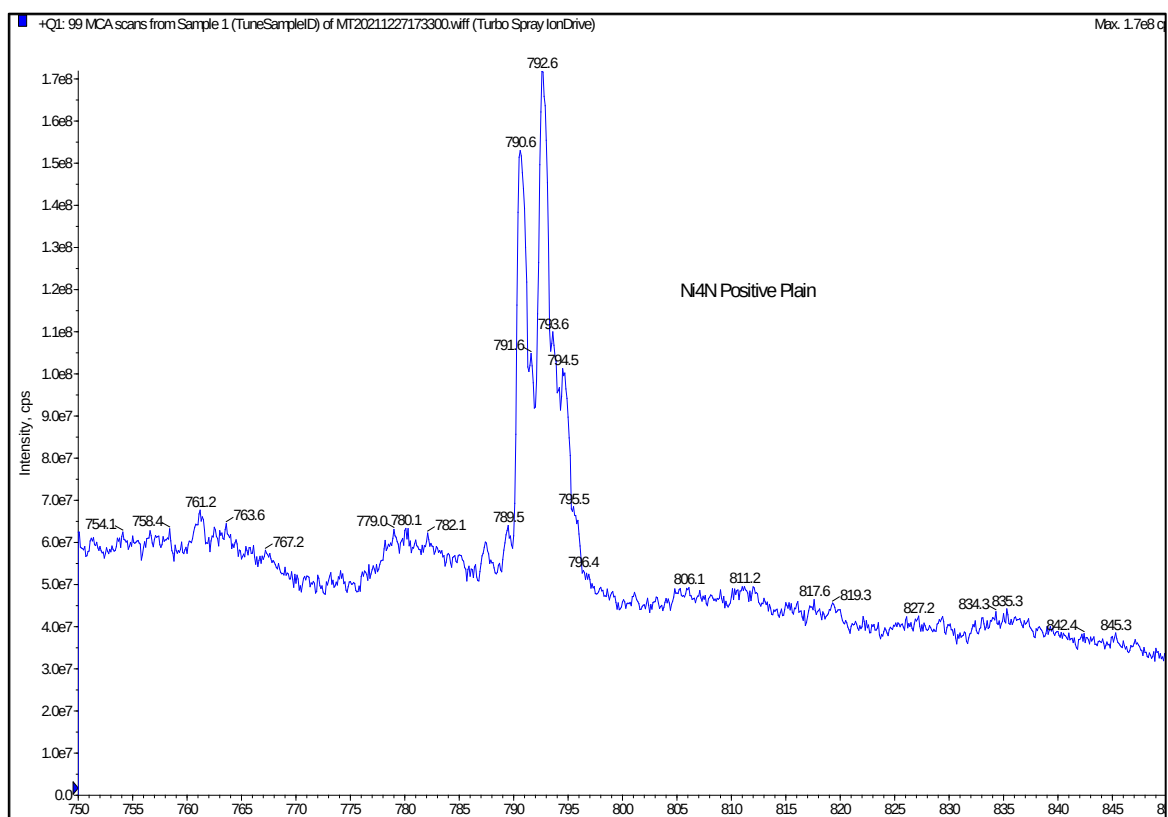
Spectrum 17. Mass spectrum of Co₃N complex



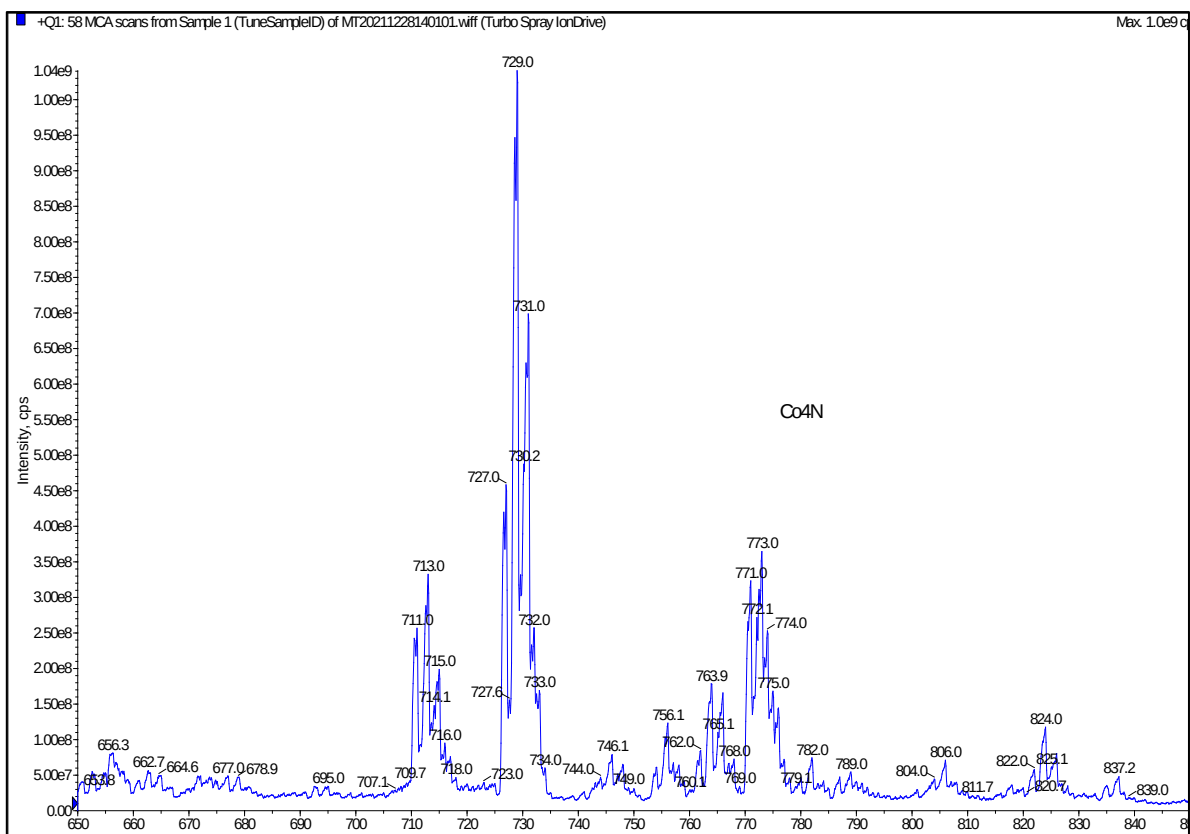
Spectrum 18. Mass spectrum of Ni₃N complex



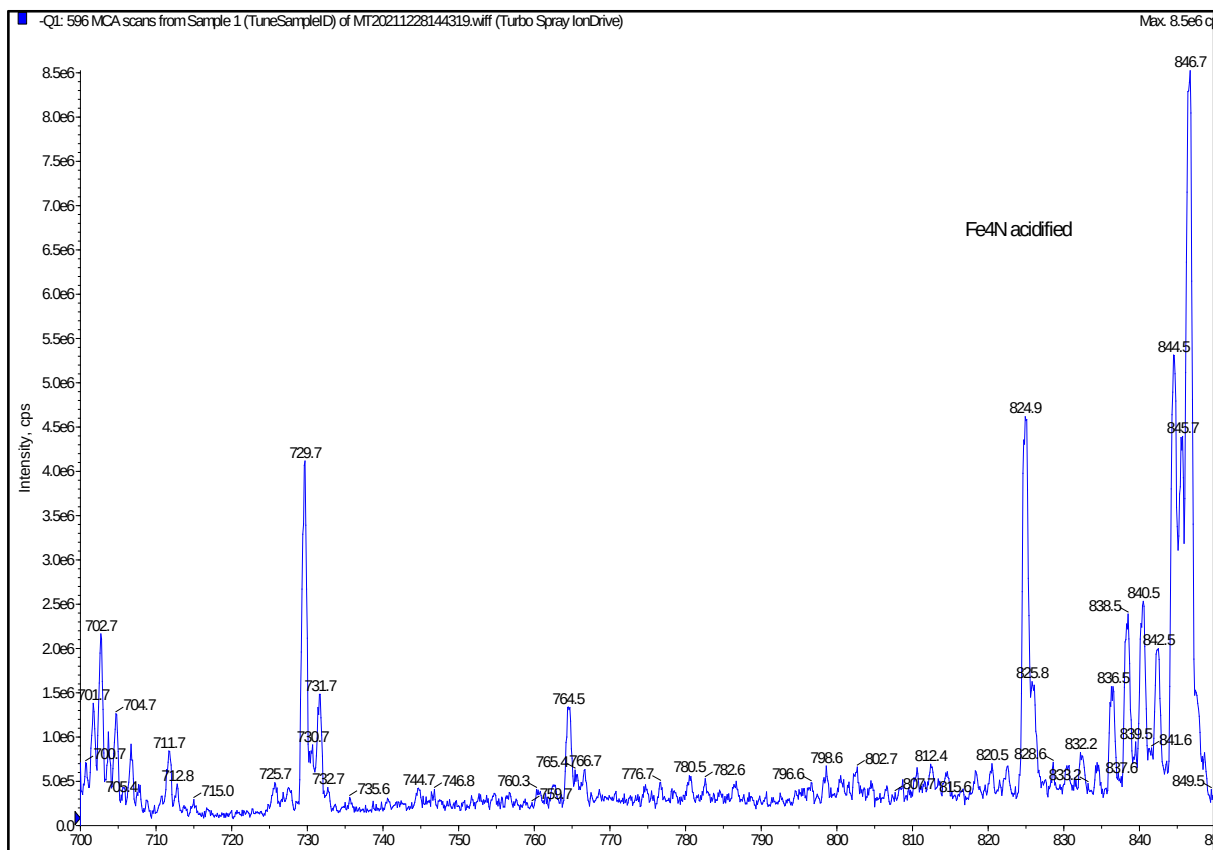
Spectrum 19. Mass spectrum of Cu₄N complex



Spectrum 20. Mass spectrum of Ni₄N complex



Spectrum 21. Mass spectrum of Co₄N complex



Spectrum 22. Mass spectrum of Fe₄N complex

7.4 APPENDIX D; CRYSTAL DATA AND STRUCTRE REFINEMENT FOR 4N

LIGAND_0m

Identification code	CoLtLAMELO_0m
Empirical formula	C ₂₀ H ₁₃ ClN ₂ O ₃
Formula weight	364.77
Temperature/K	294.00
Crystal system	monoclinic
Space group	P2 ₁ /n
a/Å	7.2296(7)
b/Å	20.257(2)
c/Å	11.9461(12)
α/°	90
β/°	98.095(4)
γ/°	90
Volume/Å ³	1732.1(3)
Z	4
ρ _{calc} /g/cm ³	1.399
μ/mm ⁻¹	0.243
F(000)	752.0
Crystal size/mm ³	0.6 × 0.5 × 0.3
Radiation	MoKα (λ = 0.71073)
2θ range for data collection/°	6.036 to 67.518
Index ranges	-10 ≤ h ≤ 11, -31 ≤ k ≤ 31, -18 ≤ l ≤ 18

Reflections collected	36820
Independent reflections	6849 [$R_{\text{int}} = 0.1059$, $R_{\text{sigma}} = 0.1017$]
Data/restraints/parameters	6849/0/235
Goodness-of-fit on F^2	1.026
Final R indexes [$ I \geq 2\sigma(I)$]	$R_1 = 0.0920$, $wR_2 = 0.1696$
Final R indexes [all data]	$R_1 = 0.1971$, $wR_2 = 0.2084$
Largest diff. peak/hole / $e \text{ \AA}^{-3}$	0.21/-0.28