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List of Abbreviations and Acronyms

DP	2, 6-diamino pyridine
ACT	2-acetyl-5-chloro thiophene
DPACT	N-[1-(5-chloro-thiophen-2-yl)-ethyl idene]-pyridine-2, 6-diamine
Zn-DPACT	zinc-ligand complex
ν	Stretching
L	Ligand
DMSO	Dimethylsulfoxide
AAS	Atomic Absorption Spectroscopy
FTIR	Fourier Transform Infrared
TLC	Thin Layer Chromatography

Abstract

The Schiff base ligand N-[1-(5-chloro-thiophen-2-yl)-ethylidene]-pyridine-2, 6-diamine, DPACT, was synthesized by condensation reaction of 2-acetyl-5-chlorothiophene and 2, 6-diaminopyridine in the presence of concentrated hydrochloric acid as a catalyst.Zn(II) complex with the synthesized ligand was prepared by reacting zinc (II) chloride and the ligand (1:1) in methanol solvent. The purity of the synthesized compounds wasmonitored usingTLC,and characterized by melting point, solubility, conductivity measurements,atomic absorption data, and infrared spectra. The analytical data showed, the stoichiometryZn(II) to DPACT of the complex to be 1:1. Infra-red spectral data showed that the ligand behaves as bidentate with (N, S) donor sequence towards Zn(II) metal ion. The Zn(II) complex was formulated as [Zn ($C_{11}H_{10}ClN_3S$) Cl_2 (H_2O) $_2$].Based on the analytical data obtained, an octahedral geometry and non-electrolyte behavior was proposed for the Zn((II)-DPACT complex.The ligand and its zinc complex were screened for their antibacterial activity against bacterial species. Activity data show that the antibacterial activity of the complex is higher than that of the parent ligand.

Key words: Synthesis, Schiff base, Metal complex, characterization, octahedralgeometry, antibacterial activity.

1. INTRODUCTION

Heterocyclic compounds are organic compounds that contain a ring structure and atoms in addition to carbon, such as sulfur, oxygen or nitrogen, as part of the ring. They may be either simple aromatic rings or non-aromatic. Heterocyclic aromatics are not actually pure hydrocarbon but ring systems that have at least one atom in the ring that is other than carbon. Heterocyclic compounds that follow the Huckel conditions are aromatic [1]. Forexample,pyridine and Thiophene are considered aromatic because they are cyclic planar molecule with 6 π electrons in the ring, satisfying the rule of (4n + 2) π electrons.

Heterocycles have constituted one of the largest areas of research in organic chemistry. They play an important role in biochemical processes assidegroups of the most typical and essential constituents of living cells, DNA and RNA. Sulfur and nitrogen,containing heterocyclic compounds have maintained the interest of researchers in organic synthesis. The grounds of this interest were their biological activities and unique structures that led to several applications in different areas of pharmaceutical and agrochemical research or, more recently, in material sciences [2].

Nitrogen and sulfur aromatic heterocycles are formally derived from aromatic carbon cycles with a heteroatom taking the place of a ring carbon atom or a complete CH=CH group. The presence of heteroatom results in significant changes in the cyclic molecular structure due to the availability of unshared pairs of electrons and the difference in electronegativity between heteroatom and carbon. Therefore, nitrogen and sulfur heterocyclic compounds display physicochemical characteristics and reactivity quite different from the parent aromatic hydrocarbons [3].

In the pharmaceutical industry, pyridine has been a bioisosteric replacement of benzene ring. Literature reports show that pyridine containing compounds possess antioxidant, antivirial, anticancer, antibacterial and antifungal activities [3].

Thiophene is considered to be aromatic and "π - excessive". This is because the five sp^2 – hybridized atoms may sustain a 6-π-electron system. Each carbon atom contributes one electron to the system and the lone pair provides the remaining two electrons.

Nevertheless, theoretical calculations suggest that the degree of aromaticity is less than that of benzene. The participation of the lone electron pairs on sulfur in the delocalized π-electron system is significant. Thiophenes are used as synthetic intermediates, taking advantage of the susceptibility of the carbon atoms adjacent to sulfur toward electrophilic reactions. [4].

Thiophene derivatives have been very well known for their therapeutic applications. Thiophene nucleus is one of the most important heterocycles exhibiting remarkable pharmacological activities. [5].

Many therapeutic agents having thiophene moiety such as Cefoxitin, cephalothin, cephaloridine, temocillinhave been developed possessing antimicrobial activity. In other words it can be stated that thiophene moiety serves as a royal warrior against almost all types of microbes [6].

The treatment of infectious diseases still remains an important and challenging problem because of a combination of factors including new kind of infectious diseases and the increasing number of medicinal drug resistant pathogens [7- 11]. In spite of a large number of antibiotics and chemotherapeutic drugs available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new classes of antimicrobial agents [12, 13]. Therefore there is a real perceived need for the discovery of new compounds endowed with antimicrobial activities.

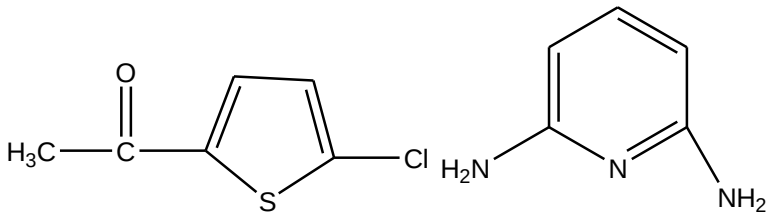
The chemistry of biological science has produced a number of compounds that are now employed as antibacterial agents. Such type of compounds revealed great promise in this area is the Schiff bases [14].

A Schiff base (azomethine), named after Hugo Schiff, is a functional group that contains carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group, but not to hydrogen. Schiff bases are of the general formula $R_1R_2C=N-R_3$, where R_3 is an aryl or alkyl group that make the Schiff base a stable imine [15]. Schiff bases can be synthesized from an aromatic heterocyclic amine and an aromatic heterocyclic carbonyl

compound by neucleophilic addition forming a hemiaminal, followed by a dehydration to generate an imine.

Schiff bases are animportantclass of ligands that coordinate to metal ions viaazomethinenitrogen and have beenstudiedextensively. Schiff base ligands form a stable complex with different transition metal ions. Schiff bases having multidentate coordination sites are known to form complexes with transition metal ions readily [16-17].

Schiffbase metal complexes show great diversity in their varied biological activities as anticonvulsant [18], antifungal [19–23], anti-HIV [24], antiviral and anticancer [25] antimicrobial [26–31] and antibacterial [32] agents. In the present investigation,attempt has been made to synthesize a ligand containing pyridine and thiophene derivatives, capable of bonding to a metal ion through different characteristic chelating sequences such as NS,(NS) from condensation reaction of 2,6-diaminopyridine (DP) and 2-acetyl-5-chlorothiophene (ACT) and its Zn(II) complex. The structures of DP and ACT are shown in Figure 1.



I. 2-acetyl-5-chlorothiophene (ACT) **II.** 2,6-diamino pyridine (DP)

Figure -1. Structure of compounds used to synthesize the ligand

1.1. Objectives

1.1.1. General Objective of the Study

The main objective of this work was to synthesize, characterize and study antibacterial activities of metal complex of ligand derived from Schiff bases.

1.1.2. SpecificObjectives

- To prepare Schiff base ligand (DPACT) from condensation reaction of 2, 6-diaminopyridine (DP) and 2-acetyl-5-chlorothiophene (ACT).
- To synthesizeZn(II) complex with DPACT ligandthrough direct method.
- To characterize the obtained ligand andits Zn(II) complex using melting point determination, solubility study, molar conductance measurement, AAS determination and Infrared spectral studies.
- To evaluate the antibacterial activity of the ligand and its Zn(II) complex on selected bacterial species:*Staphylococcus aureus* and *Escherichia coli* by disk-diffusion method.

1.2. Statement of the Problem

The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. Metal-based drugs represent a novel group of antibacterial and antifungal agents.In the last few years so many studies have been done on the structure and chemical behavior of Schiff base ligands and their metal complexes to find out an alternative.Schiff bases are very attractive class of ligands because of their ease of preparation and simple modification of both stearic and electronic properties.However, no literature report has been found on the synthesis of Schiff base ligand from 2,6-diamino pyridine (DP) and 2-acetyl-5-chloro thiophene (ACT) and its Zn (II) complex. Therefore, the present study attemptedto synthesize, characterize and studied theantibacterial activity of ligand derived from 2,6-diamino pyridine and 2-acetyl-5-chlorothiophene and its Zn(II) complex.

Thus, the present work attempted to investigate the way to:-

- Synthesize Schiff base ligand (DPACT) from 2, 6-diamino pyridine and 2-acetyl-5-chloro thiophene.
- Obtain the metal [Zn (II)] complex with the synthesized ligand DPACT.
- Study the structural characterizationandevaluate the antibacterial activity of Zn(II)-DPACT complex.

1.3. Significance of the Study

In spite of a large number of antibiotics and chemotherapeutic available for medical use, at the same time the emergence of old and new antibiotic resistance created in the last decades revealed a substantial medical need for new metal complexes of antimicrobial agents. Therefore, the newly prepared Zn(II)-DPACT complex may be less toxic and/or act through a distinct mechanism from those of well-known classes of antimicrobial agents to which many clinically relevant pathogens are now resistant. 2, 6-diaminopyridine and 2-acetyl-5-chlorothiophene are commercially availableand zinc is essential in many biological systems. In view of this, DPACT was synthesized and its Zn(II) complex was obtained for the first time. Thus, the results of this study may provide the following advantages:-

- The synthesized Zn(II) complex may be potential candidates for developing a drug in the future.
- The researcher developed a new learning process by using various experimental method and instruments employed in this research.
- This thesiscan be used as a resource for other researchers.

2. REVIEW OF RELATED LITERATURE

2.1. Synthesis of Schiff Base Ligands

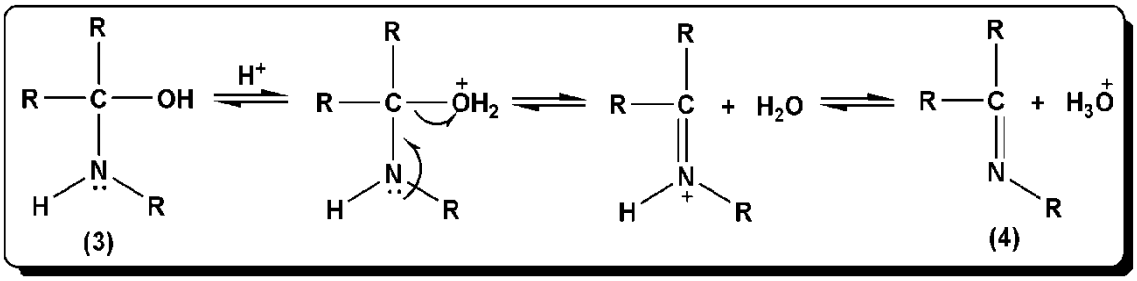
Schiff base ligands are most commonly synthesized by condensation of primary amines with aldehydes and/or ketones in alcoholic media at relatively low temperatures (below 300 °C) [15].

1234

Scheme 1: General synthesis of Schiff bases [16].

The preparation of Schiff bases involves a variety of conditions and is brought about by mixing carbonyl compounds (**1**) and amines (**2**) in various proportions and employing a range of solvents (Scheme 1). The formation of Schiff bases (**4)** is generally favored by making use of dehydrating agents. A great care should be taken for the purification of Schiff bases as they are degradable [15, 16].

The acid/base catalysis or heating is employed for the synthesis of Schiff bases as the reactions are mostly reversible. The Schiff bases are formed by the reaction of amines with carbonyl compounds andgive an unstable addition compound carbinolamine (**3)**. The compound thus obtained loses water molecule. The dehydration step during formation of Schiff base is the rate determining step and is catalyzed by acid (**Scheme 2**). The removal of product or separation of water from the reaction mixture assists the formation of the product.



Scheme2: Ratedetermining step in the synthesis of Schiff bases.

In the preparation of Schiff bases, high concentration of acid is not required due to basic character of amines. The formation of carbinolamine cannot occur and equilibrium will be shifted towards the left side of the reaction because protonated amine does not act as nucleophile. As a result mildly acidic pH is quite good for the formation of Schiff bases. Moreover, bases can also catalyze dehydration of carbinolamines. This reaction shows similar trends as E_2 elimination of alkyl halides nevertheless, it involves an ionic intermediate and completes in two steps [33]. The formation of Schiff bases is a combination of two type of reactions i.e., elimination after addition. Schiff bases can undergo hydrolysis on silica gel and due to this reason, purification of Schiff bases by chromatography is not recommended.

The nitrogen atom of azomethine group possesses a lone pair of electrons in sp^2 hybridized orbital and has got greater significance in chemistry of Schiff bases as well as their excellent chelating abilitywhich is enhanced when nitrogen atom is present in the vicinity of one or more donor groups. The azomethine group carrying ligands have achieved a considerable interest in coordination chemistry. [33].

2.2. Transition Metal Complexes of Schiff Base Ligands

A metal complex is a chemical species which contains a metal atom or ion bonded to a greater number of ions or molecules than would be expected from simple valence considerations. The ions or molecules that are bonded or co-ordinated with the metal are termed ligands. The co-ordination number of a metal in a complex is defined as the total number of ligand atoms bonded to the metal. Coordination number and geometries are determined by a number of factors notably the size of the metal. The numbers of potential

metal-bonding sites in a ligand is indicated by use of terms such as monodentate, bidentate, tridentate, etc [34].

The treatment of Schiff base ligands with metal salts gives metal complexes of the Schiff base under suitable experimental conditions. The most important parameters of solvothermal metal-Schiff base complex synthesis are temperature, the concentration of the metal salt and the ligand, solubility of the reactants, and the PH of the solution.

Many coworkers [35] reported the use of 2,6-diaminopyridine in synthesis of macro-cyclic ligand for chelation with metal ions such as Ni(II), Cu(II), Cr(III), La(III), Pb(II), Cd(II) and Zn(II) which has opened the fascinating area of research in coordination chemistry.Reviews which describe the synthesis and characterization of Schiff base ligands and their metal complexes have been previously published [36-37].

Many metal complexes containing pyridine moiety possess a wide spectrum of medicinal properties, including activity against tuberculosis, leprosy, and bacterial and viral infections. They have also been found to be active against influenza, protozoa, smallpox, malaria and certain kinds of tumors and have been suggested as possible pesticides and fungicides. Their activity has frequently been thought to be due to their ability to chelate trace metals [38].

2.3. Zinc(II) Complexes

Large numbers of Zn(II) complexes are known and coordination numbers of 4 and 6 are the most common. This metal ion is known in four (tetrahedral) and six coordinate (octahedral) stereochemistry. Square planar geometry is not common in Zn(II) complex. Zn(II) complexes are diamagnetic and they do not possess any d-d transition due to d^{10}electron configuration. The divalent zinc ion is exceptionally stable with respect to oxidation and reduction.. The d^{10}electron configuration of Zn^{2+} indicates that zinc complexes are not subject to ligand field stabilization effects and so coordination number and geometry is only dictated by ligand size and charge [39].

Complexes of zinc with sulfur ligands have been studied widely because of their biological importance and their use as accelerator in the vulcanization of rubber [40]. Zinc dithiocarboxylate complexes have close relationship with the metals Ni(II) and

Cu(II) in their formation of stable complexes with S-donor ligands. The stability and stereochemistry of a particular compound depends on the size and polarizing power of Zn(II) ion and the steric requirements of the ligands. In enzymes, zinc shows a strong preference for tetrahedral coordination, which enhances both the Lewis acidity of a zinc center and the Brønsted acidity of a coordinated water molecule. Therefore, zinc binds strongly to many proteins [40].

Zinc has a specific role in bioinorganic processes because of the unique properties of the coordination compounds of the Zn(II) ions: i) Zn(II) can easily be 4-, 5-, or 6-coordinated, without a marked preference for six coordination. In coordination compounds, where there is no ligand-field stabilization energy and the coordination number is determined by a balance between bonding energies and repulsion among the ligands. Tetrahedral complexes have shorter metal-donor distances than five- coordinate complexes, and the latter have shorter ones than six-coordinate complexes, whereas the ligand repulsion increases in the same order. Thus Zn(II) forms four coordinated tetrahedral complexes. ii) As a catalyst, zinc in enzymes is exposed to solvents, which is most often, water. A coordinated water molecule exchanges rapidly, because ligands in Zn(II) complexes are kinetically labile. This, again, can be accounted for by lack of preference for a given coordination number by the zinc ion [41].

2.4. Biological Importance of Metal Complexes

Metal complexes with labile ligands have long been known to undergo ligand substitution reactions with bio molecular targets. Metal ions can bind to nitrogen, sulfur or selenium atoms of the histidine, cysteine, or selenocysteine residues in proteins [42].

Metal complex provides better opportunities to use as therapeutic agents. The results found showed that the metal complexes were found to be more active than the ligand.The lipophilicity of the drug is increased through the formation of chelates and drug action is increased due to effective permeability of the drug into the site of action. Metal ions bound with ligands in some process, and to oxidize and reduce in biological systems [43].

Patel *et al* studied the drug based copper (II) complexes with levofloxacin in presence of 2, 2’-bipyridylamine (bpd). It shows antibacterial activity [44]. Insulin zinc complex with

different coordination structures have shown a blood glucose lowering effect to treat type 2 diabeties [45]. In diabetes intake of chromium metal complex shown considerable reduction in the glucose level. Nair *et al* reported that Co(II), Ni(II), Cu(II) and Zn(II) complexes of the Schiff base derived from indole-3-carboxaldehyde and *m*-amino benzoic acid. They were synthesized and characterized by elemental analysis, molar conductance, IR, UV–Vis, magnetic moment. The antibacterialtest of the synthesized ligand and the complexes were screened by disc diffusion method [46].

The other important biological aspect of Schiff base metal complexes is their enhanced anti-bacterial activity [47]. The microorganisms adsorb metal ions on their cell walls and as a result respiration processes of cells are disturbed and protein synthesis is blocked which is the requirement for further growth of organisms. The growth inhibition effects of metal ions are considerable. The only passage of lipid soluble material is favored by the lipid membrane that surrounds the cell in accordance with the overtone's concept of cell permeability, as the antifungal activity is controlled by lipophilicity factor. The overlap of ligand orbitals and the behavior of metal ions to share charge with the donor groups is reduced upon chelation. Besides this, the delocalization of π electrons over the whole ring is due to chelation and lipophilicity of complexes is enhanced. The proliferation of microorganisms is further restricted because the penetration of complexes in lipid membranes is facilitated by increased lipophilicity. The impermeability of microbial cells and differences in ribosomes of cells are the major reason for variations in the effectiveness of different compounds against a variety of organisms. In most of the cases, ligands are less effective antifungal agents than their metal complexes[48].

3. EXPERIMENTAL

3.1 Chemicals and reagents

All the starting chemicals and solvents used in this work were of analytical reagent grade (AR) and were used without further purification. The main starting chemicals, 2-acethyl-5-chlorothiophene and 2, 6-diamino pyridine were acquired from H. CHANDANMAL & Co., India. In addition zinc chloride, concentrated hydrochloric acid and sodium acetate,concentrated nitric acid, silver nitrate, $K_4Fe(CN)_6$solvent like ethanol, methanol, petroleum ether; DMSO, ethyl acetate, deuterated methanol, deuterated chloroform, acetone and distilled water were used.

Table1. Physical properties of the main starting materials used in this study.

Starting compounds	MolecularFormula (g/mol)	Molecular mass (g/mol)	M.P (^oC)
2-acethyl-5-chloro thiophene, 99%	C_6H_7ClOS	160.62	49-52 (Lit.)
2,6-diamino pyridine,98%	$C_5H_7N_3$	109.13	117-122 (Lit)

3.2 Instruments and Experimental Conditions

Infrared spectralmeasurements for the synthetic compounds were recorded by usingKBr pellets in FT-IR spectrophotometer in the rangefrom 400-4000 cm^{-1}. Estimation of zinc metal in the prepared complex was recorded by using atomic absorption spectroscopy. The solubility of the synthesized ligand and its metal complex in various solvent was checked at room temperature. All weighing were performed by using Electronic meter balance, Model AB 54. The melting point temperature for the synthesized compounds was determined using IA-9200 Digital melting point apparatus. Molar conductance of the prepared compounds in DMSO was recorded at room temperature using a Jenway model

4330 conductivity meter.Thin layer chromatography was carried out using TLC plate coated with silica gel. In addition Oven, water bath and whatman 40 filter papers were used. Analytical test and spectroscopic studies were made at Department of Chemistry, Addis Ababa University.

3.3. Synthesis

3.3.1. Synthesis of N-[1-(5-chloro-thiophen-2-yl)-ethylidene]-pyridine-2,6-diamine ligand[49]

The new N-[1-(5-chloro-thiophen-2-yl)-ethylidene]-pyridine-2,6-diamineligand, DPACT, was prepared by using equi-molar quantities of 2-acetyl-5-chlorothiophene and 2,6-diaminopyridine.A solution 2,6-diaminopyridine (0.67g,6.22x 10^{-3} mol) in hot methanol ((10mL) was slowly added to a solution of 2-acetyl-5-chlorothiophene (1.00g, 6.22x 10^{-3} mol) in hot methanol (15 mL) in 100mL flat bottomed flaskand concentrated hydrochloric acid was added (3 drops) to the mixture. The reaction mixture was then refluxed on water bath at 60^{o}C for 8 h. The progress of the reaction was followed by careful observation and TLC test. Reddish brown color was observed after 7hreflux but no single spot for the TLC test at this time. It was after 8hrsof refluxing that a single spot was observed and the resulting reaction mixtures was transferred into 100mL beaker at room temperature and left open for evaporation. The reddish brown oily solid obtained after 3days was washedwith cold methanol and petroleum ether to remove impurities, and finally dried in a desiccator over anhydrous $CaCl_2$. The yield of recrystallized ligand was 0.788g (47%). The melting point of the resulting colored solid product was found to be 140-142°C.

Scheme 3: Synthesis of the ligandN-[1-(5-chloro-thiophen-2-yl)-ethylidene]-pyridine-2, 6-diamine

3.3.2. Synthesis of DPACT-Zn(II)Complex [49]

Zinc(II) chloride (0.100 g, 0.0014 mol) mixed with sodium acetate (0.005g) and dissolved in hot methanol (20 mL) and the ligand DPACT (0.184 g, 0.0014 mol) were separately dissolved in hot methanol (20 mL). The methanolic solution of DPACT was added drop wise while stirring,with magnetic stirrer, to a hot methanolic solution of the zinc (II) chloride and sodium acetate in 100mL beaker. The mixture was refluxed for 1h. The dark brown precipitate obtained after cooling to room temperature was separated by filtration using what man filter paper, washed several times with petroleumether (20 mL) and followed by cold ethanol and then dried in a desiccator over anhydrous $CaCl_2$.The yield of the obtained powder was 0.198g (69.7%). The melting point was found to be 212-214^{o}C.

3.4. Antibacterialactivity

The samples of the synthesized ligand and its zinc complex were tested for antibacterial activities against bacterial stains and evaluated against Gram-positive bacteria *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*.

4. RESULTS AND DISCUSSION

The ligand, DPACT, was prepared by refluxing the appropriate amount of a methanolic solution of 2-acetyl-5-chloro thiophene (ACT) with 2, 6-diaminopyridine (DP) in 1:1 molar ratio in the presence of drops conc. hydrochloric acid. The progress of the reaction was monitored by careful observation and using TLC test. The formation of single spot after 8 h has beenconsidered asan indication of the formation of the Schiff base ligand. Melting point of the synthesized ligand was determined to be 140-142^{0}C.The red brown ligand (DPACT) obtained is stable at room temperature. It is soluble in DMSO, hot methanol and hot ethanol, but insoluble in chloroform and petroleum ether.

The metal complex of Zn (II) was synthesized by direct reaction of the ligand DPACT with zinc (II) chloride in 1: 1 mole ratio in the presence of sodium acetate which gave dark brown colored compound. The complexis soluble in ethanol, methanol and DMSO and insoluble in petroleum ether and chloroform..The resulting product was studied in terms of melting point, conductivity solubility, qualitative test and (AAS and IR).

4.1. Physical Characteristics

Table2: Colour, yield, meltingpoint of the ligand and its zinc complex

Compound	**F. w (g/mol)**	**Colour**	**Yield (%)**	**M. pt (^{o}C)**
DPACT	251.5	Red Brown	47.0	140-142
Zn-DPACT	423.9	Dark-Brown	69.7	212-214

4.2. Solubility of the ligand and its zinc complex in differentsolvents

Solubility test of the ligand and its zinc complex was done in order to findthe suitable solvents that could be utilized for analytical and spectroscopic measurements. The observedsolubility of the compounds synthesized istabulated(table3).

Table 3: Solubility of the synthesized compounds

Compound	DMSO	Ethanol	Methanol	Chloroform	Petroleum ether
DPACT	Soluble	Soluble	Soluble	Insoluble	insoluble
Zn-DPACT	Soluble	Soluble	Soluble	Insoluble	insoluble

4.3. Zinc Test[50]

The qualitative test of zinc in the Zn(II)-DPACT was performed by the addition of 3 drops of $K_4Fe(CN)_6$ solution to HNO_3solution of the complex which gave a white precipitate. The precipitate is due to the formation of $K_2Zn[Fe(CN)_6]$whichconfirms the presence of zinc in the complex. The reaction between the complex and $K_4Fe(CN)_6$ is shown as follows

$$Zn^{2+} + 2K^{+} + [Fe(CN)_6]^{-4} \longrightarrow K_2Zn[Fe(CN)_6]$$

4.4. Chloride Test in the Complex

20mg of the Zn(II) complex was dissolved in 5ml concentrated nitric acid and digested for complete oxidations of organic component which is diluted to 100 ml using distilled water after evaporation of HNO_3 and digested for 2 hours. When 0.5M solution of silvernitrate was added to the cooledacidic solution and left for overnight, whiteprecipitate,which is due to the formation of silver chloride, was observed. Thisconfirms the presence of chloride in the Zn(II) complex. The reaction between silver ion and chloride ion is shown below:

$$Ag^{+} + Cl^{-} \rightarrow AgCl(s)$$

4.5. Determination of Molar conductivity [51]

DPACT and Zn(II)-DPACT complex (0.006 g DPACT and 0.009 g Zn-DPACT) were separately dissolved in 25 ml DMSO. The synthesized compounds are insoluble in common organic solvents like cold methanol and cold ethanol but soluble in DMSO. Thus, DMSO was used as solvent for the samples to ensure complete dissolution of the

samples. Molar conductivities of 10^{-3} M of their solution were measuredat room temperature (27^{o}C).

Specific conductance (K) of the ligand and Zn(II) complex obtained were 3x 10^{-6} and 7 x $10^{-6}\Omega^{-1}cm^{2}mol^{-1}$, respectively. The molar conductance (Λ_M) of the ligand and complex were calculated using the relation Λ_M = 1000K/C (where C is the molar concentration of the metal complex solution, and K is specific conductance). The molar conductivities of the DPACT and Zn(II)-DPACT complex were found to be 3 and 7.5 $\Omega^{-1}cm^{2}mol^{-1}$,respectively. The results obtained were tabulated in Table 3.

Themolar conductivity values of both the ligand and the Zn(II) complex are too low to account for any dissociation; therefore, the compounds are considered to be non-electrolytes. Furthermore, the molar conductance value of the complex suggested that the chloride ions were inside the coordination sphere and bonded tothe metal ion. Therefore, the complex may be formulated as $[ZnLCl_2 (H_2O)_2]$, L=DPACT.

Table 4: Conductivity values of the synthesized compounds

Compound	Molar conductivity ($\Omega^{-1}cm^{2}mol^{-1}$)	Type of electrolyte
DPACT	3.0	Non-electrolyte
Zn-DPACT	7.5	Non-electrolyte

4.6. Estimation of zinc metal in the complex by AAS

Zinc contentof the complex was determined using atomic absorption spectroscopy (AAS).

Zn(II)-DPACT complex (20 mg) was placed in a clean and dry beaker, to which 10 mLportion of conc. HNO_3 was added and the content was heated gently in a hood until a few drops remained in the beaker. Then 10 mL of additional conc. HNO_3 was added in the beaker and heated slowly until a few drops remained. The latter procedure was

repeated for three times until all the organic componentof the complex was decomposed. Then the residue was dissolvedand diluted using distilled water in a 50 mLflask. The solution was subjected to AAS study after appropriate dilution.Based on the absorbance data, the amount of Zn(II) in the complex was calculated.

$$\%Zn = \frac{concentration(ppm)\ X\ Volume\ diluted\ to\ X\ 100}{mass\ of\ sample\ taken\ X\ 1000}$$

$$\%Zn = \frac{55.68ppmX\ 50\ ml\ X\ 100}{20\ mg\ X\ 1000} = 13.92$$

The metal percentage obtained from this calculation was used to assess the molecular mass of the complex and to arrive at the metal to ligand ratio in the complex by comparing with the theoretically calculated percentage composition of zinc in Zn(II) complex.

The percentage of zinc in the complex was determined to be 13.92%(experimental value) while the theoreticallycalculate percentage composition of Znin [$ZnC_{11}H_{14}Cl_3N_3O_2S$] complex is 15.42%.Based on the theoretically calculated and experimental value the molar mass of the complex was found to be 425.03 and469.83, respectively.The variation of calculated and found mass may be connected with the presence of impurities. Therefore, it is concluded that the metal to ligand ratio for the complex is 1: 1. Based on this data the chemical formula of the complex is proposed to be [$ZnLCl_2\ (H_2O)_2$].

4.7. IR spectra Analysis

The IR spectra of the free ligand and its metal complex were carried out using KBR pellets in the range of 4000 – 400 cm^{-1}. The important IR frequencies of the ligand and the metal complex and their assignments are given (Table 5).

The IR spectrum of DPACT (**Figure 2**) shows medium intensity bands in the range 3443-3194 cm^{-1}which are due to primary amine N-H stretching vibrations, νNH_2. This indicates that one $–NH_2$ group of 2,6-diaminopyridine is left unreacted and the

multiplicity of this bands is attributed to intermolecular hydrogen bonding. The frequency observed at 3443 $cm^{-1}$is due to the intermolecular hydrogen bonding as well as asymmetric O–H stretching vibration of the water or methanol alcohol presentin the compound as impurity. The absorption frequency at 3335 cm^{-1} is due to the symmetric N-Hstretching vibration mode. Thebandat3194cm^{-1}isduetoaromaticringstretching**.**The frequency at 2925 $cm^{-1}$is due to C–H symmetric stretching present in the aromatic nuclei and asymmetric C–Hstretching of CH_3 group [52].

The FTIR spectrum of the free ligand showed the absence of characteristic carbonyl [$\nu_{C=O}$] band in the range 1760-1690 cm^{-1} 2-acetyl-5-chlorothiophene. Instead, the appearance of a new strong band at 1631 cm^{-1} is assigned to the $\nu_{C=N}$ double bond vibration arising from the condensation reaction of the two reactants to form the proposed ligand**.** The spectrum also showed a strong absorption band at 1460 cm^{-1} corresponding to the C-S-Cstretching mode of vibration [53].

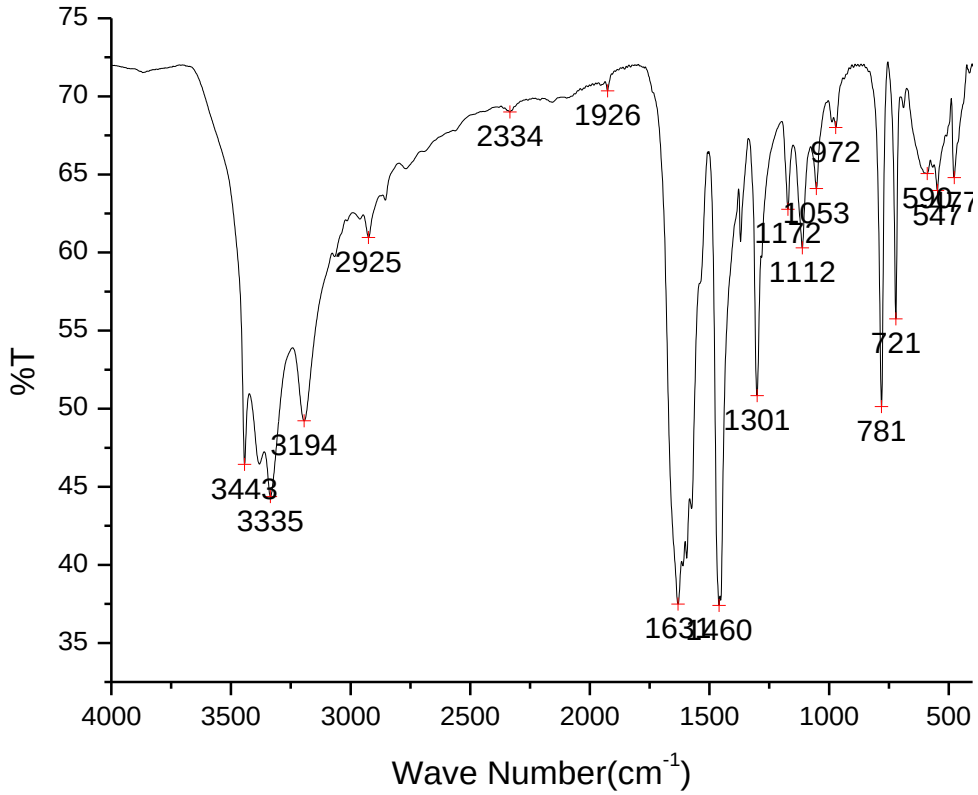


Figure 2**: IR** Spectrum ofDPACT

The IR spectrum of the complex was compared with that of the free ligand in order to determine the coordination sites that may be involved in chelation.There were some guide peaks in the spectra of the ligand, which were helpful in achieving this goal.The position and the intensities of these peaks are expected to change upon chelation. New peaks are also guide peaks, as is water, in chelation. In the IR spectra of Zn(II)-DPACT complex (**Figure 3**), the broad peak that appeared around 3505 cm^{-1} is due to coordinated water which commonly appears in the range of 3550-3200 cm^{-1}. Furthermore, coordinated water molecules exhibit rocking, twisting and waging mode in the lower frequency region 500-1000 cm^{-1} of the metal complex. It can be explained as overlapping result of primary amine (-NH_2 stretching) and coordinated water molecules.

Upon comparison, it was determined that the υC=N stretching vibration is found in the free DPACT ligand at 1631 cm^{-1}.This band was shifted to lower wave number (1575cm^{-1}) in the complex, indicating the participation of the azomethine nitrogen in coordination (Zn−N). The sharp IR ligand band at 1460 cm^{-1}, υC-S-C of thiophene moiety, shifted to 1418 cm^{-1} for the metal complex. This confirms the coordination of sulphur to Zn(II) ion.

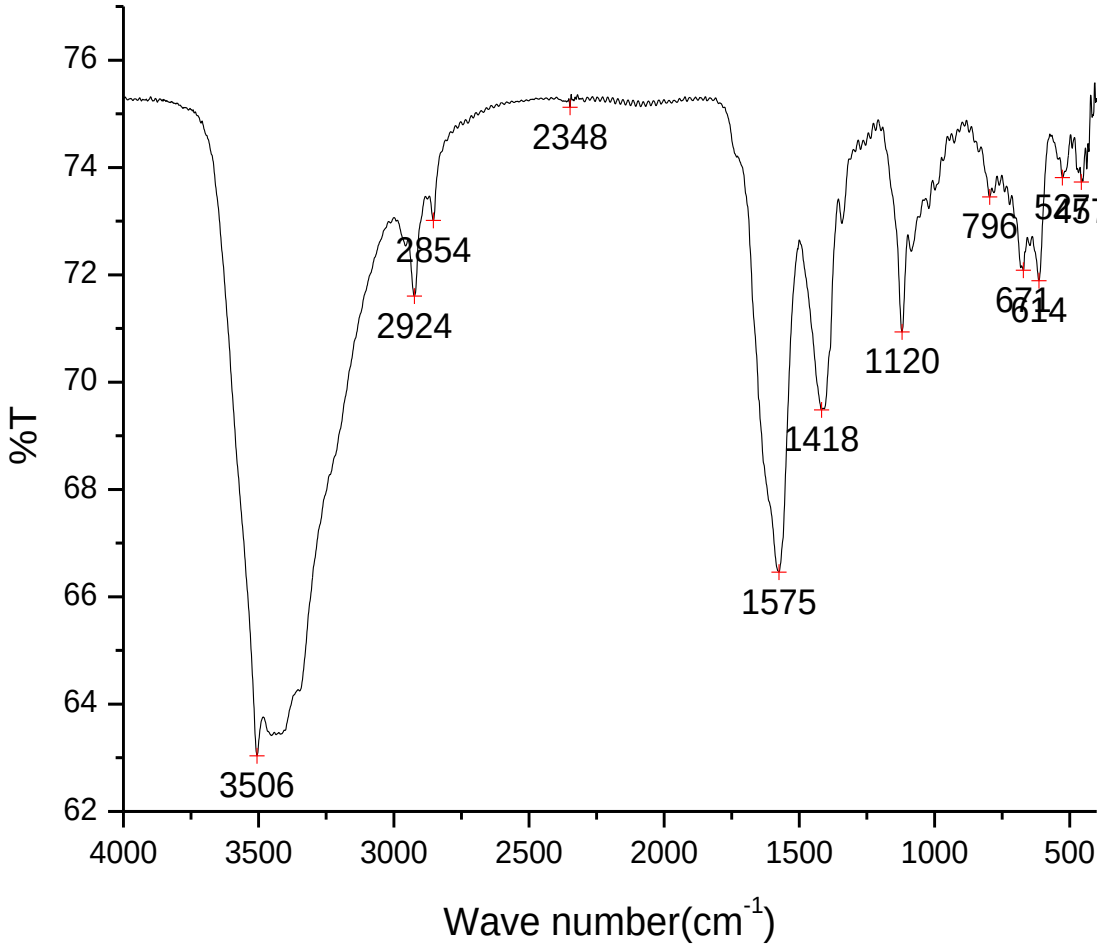


Figure -3**:** IR SpectrumZn(II)-DPACT Complex

The bands at 527 $cm^{-1}$and 451 cm^{-1}in the metal complex were assigned to υZn-N andυZn-Smodes, respectively. The new bands that appeared at 614-671cm^{-1} in the spectra of the complex indicates the coordination of water molecules to the metal. Therefore, from the IR spectra, it is concluded that the DPACT ligand behaves as a neutral bidentate ligand coordinated to the metal ion via azomethine N and thiophene S.

The main IR data of the spectra of Schiff base ligand (DPACT) and its complex are presented in **Table 5**.

Table 5: Characteristic IR bands (cm^{-1}) of DPACT and its Zn(II) complex.

Compound	**ν(NH$_2$)/ H$_2$O**	**ν(C=N)**	**ν (C-S-C)**	**ν(Zn-O)**	**ν (Zn-N)**	**ν (Zn-S)**
DPACT	3444, 3335, 3194	1631	1460	---	---	---
DPACT-Zn	3506	1575	1418	671,614	527	451

4.8. Anti-bacterialActivity Test

The anti-bacterial activity of the ligand and its Zn(II) complex was carried out against the *Escherischia Coli* and *Staphylococcus aureus* using disc diffusion method using DMSO as a solvent [54].Unfortunately, the antibacterial activity of zinc chloride was not checked and no standardized antibacterial agent that was compared with the activity of the synthesized compounds.

A comparative study of the growth inhibition zone values of Schiff base and its complex indicate that the metal complex exhibit higher anti-bacterial activity than the free ligand and the same is indicated from the results given in the **Table 6**. The result indicates the Zn(II) complex show higher activity (maximum inhibition zone) against both *E.Coli*and *S.aureus*at 1 mg/mLconcentration than the ligand.

Table 6: Growth inhibition zone of bacteria in mm

Compound	*E.coli*	*S.aureus*
DPACT	16	18
Zn(II) DPACT	19	23

This is probably due the greater lipophilic nature of the complex. Such increased activity of the metal chelate can be explained on the basis of Overtone's concept and Tweedy's chelation theory [54]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the anti-microbial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups. Further, it increases the delocalization of the π electrons over the whole chelate ring and enhances the lipophilicity of the complex [54].

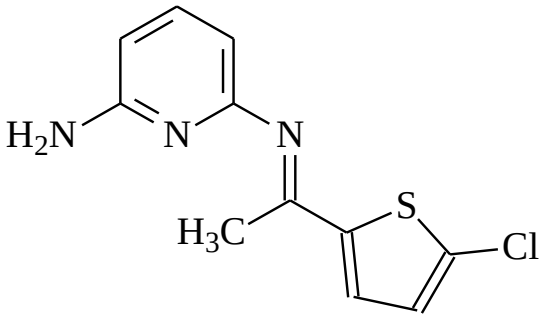
The same possible explanation [55] for this increase in the activity upon chelation is that, in the chelated complex, positive charge of the metal is partially shared with donor atoms present on ligand and there is an electron delocalization over the whole chelating ring. This, in turn, increases the lipid layers of the bacterial membranes.

5. CONCLUSION

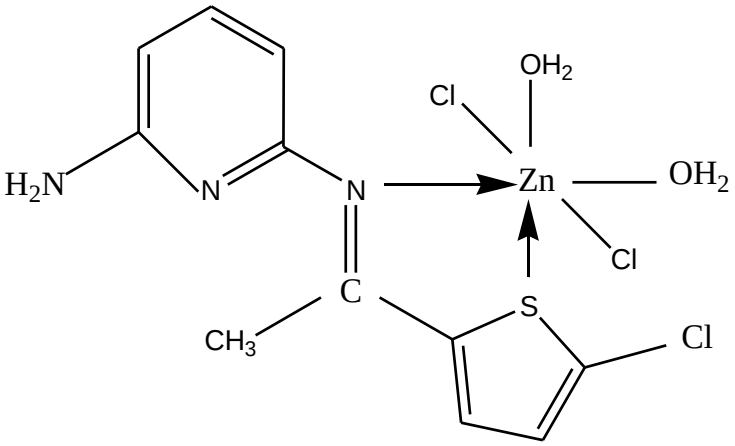
A Schiff base ligand, N-[1-(5-chloro-thiophen-2-yl)-ethylidene]-pyridine-2, 6-diamine (DPACT), was synthesized by condensation reaction of 2, 6-diaminopyridine and 2-acetyl-5-chlorothiophene in the presence concentrated hydrochloric acid as a catalyst. The new Zn(II) complex with the ligand was obtained by direct method. The synthesized compounds were characterized by melting point determination, solubility test, conductivity measurement and spectral studies (AAS and IR).The formation of Schiff base ligand from thecondensation reaction of is confirmed by the absence of C=O stretching vibration of the thiophene derivative.

The results of this study indicated that the ligand is coordinated to the metal as a neutral bidentate (NS) ligand and formulated as $[Zn(DPACT)Cl_2(H_2O)_2]$. The analytical and spectral studies are supportive evidences for the coordination of the C=N (of the imine) and C-S of the ligand with the Zn(II) ion. The low conductivity of the Zn(II) complex reveals the coordination of the chloride ion in the inner sphere and non-electrolyte nature of the metal complex. The metal complex has higher antibacterial activity than the ligand.Based on the spectral and conductivity measurements the following structures (**Schemesbelow**)are proposed for the DPACT ligand and its Zn(II) complex.

Present observations may serve as an initial step to further investigate and establish the structure of the Zn(II) complex and the use of the complex for the practical application as an antibacterial drug.



Scheme 4: Structure of the proposed Ligand



Scheme 5: Structure of the proposed Zn (II) Complex

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