SYNTHESIS SPECTRAL AND BIOLOGICAL STUDIES OF Zn (ll) COMPLEX WITH POLY DENTATE LIGAND

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ABSTRACT

Coordination chemistry is a far running branch of chemistry with applications in almost all branches of science. Research in this field is progressing rapidly with hundreds of publications every year.Coordination compounds have been a challenge to the inorganic chemists since the day they were identified, to the nineteeth century.

A Schiff base is prepared from 4–aminoantipyrine and salicylaldehyde; (SAAP). The Zn(II) complex is prepared using SAAP. The complex is prepared by the rection of methanolic solutions of the metal salt (0.01mol) and ligand (0.005mol) are mixed in the ratio 1:2.

Charecterization of ligand and complexes has been done on the basis of analytical and physic chemical methords. The resultant zinc complex $[Zn(SAAP)_2]So_4$ is studied on the basis of conductance mesurements , IR and NMR spectrul studies. From these studies the structure of complex will proposed ,which is in tetrahedral geometry. Antifungel and anti bacterial activity also have been studied.

CHAPTER– I

GENERAL INTRODUCTION

Coordination chemistry is an important and fascinating section of chemistry. During the last three decades, the industrial applications of metal complexes have gained significant importance, especially in the area of catalysis. The scope for further development of such applications is extensive. Several biological processes in the living cells involve metal complexes. Coordination chemistry is a subject which uniquely involves the applications of quantum spectroscopy, kinetics, catalysis, biology and industrial chemistry in its delineation. Thus, a clear understanding of coordination chemistry has become a necessity for chemistry students[1] .

Transition metals form a large number of complex compounds in which the metal atom are bound to a number of anions or neutral molecules. In modern terminology, such compounds are called coordination compounds. It has been a challenge to the inorganic chemist since the coordination compounds were identified in the nineteenth century. Today they comprise a large body of current inorganic research.

One class of coordination compounds, those involving metal-carbon bonds, is the focus of an entire sub discipline known as organometalic chemistry and the field of inorganic chemistry is centered on coordination compounds present in the biological systems[2].

The discovery of hexamine cobalt(II) chloride [$Co(NH_3)_6Cl_3$)] by Tassert[3] in 1798 is generally regarded as the beginning of coordination chemistry. Later many compounds were prepared and their properties studied. Many theories were proposed to explain the structure of coordination compounds.

In 1893, Werner proposed theory on the compound, which for the first time led to a clear understanding of the bonding in such compounds. The development of the quantum mechanical understanding of chemical bond subsequently resulted in clearer knowledge about the structure and bonding in the coordination compounds.

Later Werner's ideas , were reconciled by G.N.Lewis[4] in his electronic theory of valency, which was applied to coordination compounds by Sidgwick[5]. Pauling's valence theory was able to predict the colour and stability of complexes. But the colour and variation of magnetic properties cannot be explained. For this reason the crystal field theory(CFT) developed in 1930 by the physicists, Van Vleck and Bethe helped understand clearly the magnetic and spectral properties and also the stability of complexes.

Whatever deficiency existed in the understanding of coordination compounds was mostly removed by the application of the Molecular Orbital Theory(MOT) and Pauling's Valence Bond Theory(VBT) and this is the latest theory in co-oridination complexes.

Coordination compounds are those addition compounds which retain their identities in the crystalline state as well as in solution and their properties are completely different from those of the constituents[6]. A coordination compound usually involves coordinate bonds between Lewis acids and bases. Coordination complexes can be either neutral or charged. When the complex is charged, it is stabilized by the surrounding counter ions[7].

The study of organometallic chemistry assumed great significance after the preparation of ferrocene in 1957 by Kealy and Paulson and establishment of its structure by Wilkinson and Fischer,then coordination chemistry assumed a vital significance with the devolepment of bio inorganic chemistry, which is mainly the chemistry of coordination compounds.

The effectiveness of comp-lexes as catalysts has dramatically improved the efficiency of several industrials processes. Several coordination compounds are now routinely used in industries to benefit mankind.

The extensive kinetic study on reactions of metal complexes and the applications of several spectroscopic methods to establish their structure have further fine-tuned the understanding of this class of compounds[1].

Coordination compounds have a wide variety of applications in the area of analytical chemistry, metallurgy, biological systems, industry and medicine. Coordination compounds are present in the nature as well as artificially in industries. Some of the naturally occurring complex substances include Vitamin B_{12}, haemoglobin, chlorophyll, some dyes and pigments. They have major role in the homogeneous catalysis. Several organic substances can be produced using homogeneous catalysis[8].

Coordination compounds play a vital role in many living organisms as enzymes. Carbopeptidase is an example for a hydrolytic enzyme, which is involved in the digestion process. In this enzyme, Zinc ion is surrounded by many amino acid residues. Catalyse is a complex ion enzyme that contains iron-porphyrin complexes with a relative catalytic activity of ten billion times than a conventional catalyst. Similarly, magnesium- porphyrin complex is present in chlorophyll and cobalt corrin complex is present in Vitamin B_{12}[9].

1.1 SCHIFF BASE

Schiff bases, named after Hugo Schiff [10], are formed when any primary amine reacts with an aldehyde or ketone under specific conditions Structurally, a Schiff base (also known as imine razomethine) is a nitrogen analogie of an allehyde or ketone.

$$R\text{-}CH{=}O + H_2N\text{-}R' \longrightarrow R\text{-}CH{=}N\text{-}R' + H_2O$$

Various studies reveal that the presence of lone pair of electrons on trigonaly hybridized nitrogen atom of azomethine group is of paramount chemical and biological importance.

Schiff bases can be synthesized from an aliphatic or aromatic amine and a carbonyl compound by nucleophilic addition forming a hemiaminal, followed by a dehydration to generate an imine.

Schiff bases are some of the most widely used organic compounds. They are used as pigments and dyes, catalysis, intermediates in organic synthesis and as polymer stabilizers. The visual pigment rhodopsin contains an azomethine linkage[11]. Schiff bases also exhibit a broad range of biological activities including antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral and antipyretic properties Isatin thiosemicarbazones have antibacterial activity against E-Coli and Staphylococcus. Schiff hases are reported to exhibit antitumor and cytotoxic activities in rats[12]. Some isatin Schiff base complexes are effective in treating central nervous system disorders. Antitumor activity of rare earth complexes of furfural anthranilic acid Schiff bases have been reported [13].

Complexes of Co(II),Cu(II), Ni(II),Zn(II)were prepared with ligands such as a 1.1 disubstituted ferrocene derived Schiff bases and these were used for screening against pathogenic bacterial species like E-Coli, Staphylococcus aureus, Klebsiella pneumonia and are novel class of organometallic antibacterials[14] or azomethine groups are present in various natural, natural derived and non-natural compounds. The imine group present in critical to their biological activities.

Some of the Schiff bases have been reported to be active against leukemia[15] There are several other Schiff bases which are reported to be therapeutically active and possess antiinflammatory, antipyretic, analgesic, cardiotoxic and diuretic action[16] . According to another report some Schiff bases case retardation in out development in detached cabbage leaves[17] .

Apart from the biological activity. Schiff bases have found multitude of uses[18]. Aromatic Schiff bases exert catalytic influence on polymerization, oxidation and decomposition reactions[19].· Multidentate Schiff bases have yielded several metal chelate polymers and have been incorporated in several others to produce desired characteristics Schiff bases and their metal chelates have been used in dye and pigment industry, due to their colour. Some azomethines are used as fuel additives. Synthetic flexibility of Schiff bases may permit the synthesis of multidentate ligands of diverse structural type. Some aromatic Schiff bases have been used as stabilizers for variety of compounds which include jet fuels, fuel oils, lubricating oils etc. Schiff bases have been incorporated in several polymers to produce some desirable characteristics in the polymer such as super conductivity, heat resistance and resistance towards light, oxidation hardness etc. Nowadays,s polymeric Schiff bases are largely used as electro photographic materials. In addition to all these, Schiff bases find a variety of analytical applications[20]. Complexes with amino acid Schiff bases are receiving attention[21].

Schiff bases derived from aromatic acids and aromatic aldehydes have wide variety of applications in biological, inorganic and analytical chemistry[22]. Application of many new analytical devices requires the presence of organic reagents as essential components of the measuring system. They are used in optical and electrochemical sensors, as well as in various chromatographic methods, to enable detection of enhanced selectivity and sensitivity. Among the organic reagents actually used, Schiff bases possess excellent characteristics, structural similarities with natural biological substance, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties.

Metal chelates of certain multidentate Schiff bases have been found volatile enough for their separation using vapour phase chromatography, nuclear, medicinal applications of technetium(V). In general, heterocyclic compounds containing potential donor atoms N, O and S are due to presence of multifunctional, hetero functional groups[23]. Chromium azomethine complexes, Schiff base unsymmetrical complex 1:2 chromium[24] dyes give fast colours to leathers, food packages, wools etc. Azo groups containing metal complexes are used for dying cellulose polyester textiles. Some metal complexes are used to mass dye polyfibres. Cobalt complex of a Schiff base (salicylaldehyde with diamine) has excellent light resistant and storage ability and does not degrade even in acidic gases (CO2). Novel tetradentate Schiff bases act as a chromogenic reagent for determination of Ni in some natural food samples[25].

Polymeric Schiff base exhibit photoconductive properties, sensitivity and stability are associated with silver halide emulsion containing Schiff bases. Some hydroxyl azomethine compounds find application as luminescent reagents and as indicators in complexometric titrations. They are used for fluorometric determination of inorganic cations and anions. They act as inhibitors against acid corrosion of aluminium and steel and also provide protection of organic inhibitors in nitrocellulose coatings.

Schiff bases are applied in different areas such as electronic chemistry[26], bioinorganic chemistry, metallic deactivators, catalysis[27], separation process and environmental chemistry[28]. Major biological interest in Schiff base complexes stems from their suitability in designing metal containing model systems, which mimic biologically active systems[29]. Schiff base complexes continue to attract many researchers because of its wide applications in various industries and analytical fields.

1.2 CHEMISTRY OF ZINC

Since zinc forms no compounds in which the d orbital is partially filled, it is regarded as a non-transition element. However there is some resemblance to the d group elements in their ability to form complexes, particularly with ammonia, amines, halides and cyanides. In Zn(II) ions the probability of bonding between the metal ion and the ligand is very much lowered, since there is no ligand field stabilization effect in Zn(II) ions because of the completed d orbital. In most of complexes of Zn(II), the common coordination number is 6 (octahedral) or 4 (tetrahedral). Some 5 coordinated Zn(11) complexes have also been reported. Stereochemistry of the complexes is determined by the size of the ligand, electrostatic and covalent forces. Some square planar and distorted tetrahedral complexes are also reported.

Zn^{2+} tends to form stronger bonds to fluorine and oxygen. Zinc complexes of dithiocarbamate and other sulphur compounds are important accelerators in vulcanization of rubber Zinc β-diketonates also form 5-coordinated adducts with water, alcohol and nitrogen base. Five coordinated is often found for Zn, a recently reported example being hydrazine carboxylate complex, $Zn(NH_2NHCOO)_2$ [30]. Zinc appears to be o ne of the most biologically important metals.

In the past 15-20 years, more than 25 zinc containing proteins have been identified, most of them are enzymes. Some zinc complexes show fluorescent emission property both in solid state and in solution. The Zn^{2+} ion is contained in several dehydrogenases, aldolases, peptide, phosphates, an isomerase of yeast, a transphosphorylase, and a phospholipase attesting to its importance in carbohydrates, lipid and protein metabolism in virtually all organism[31].

1.3 METALS IN BIOMEDICAL FIELD

Awareness of the role of metals in biological systems directed the inorganic chemists and medical researchers to explore new therapeutic areas based on various categories of drugs. The use of metals and their compounds in the treatment of diseases goes back over four millennia as copper, silver and gold were used Arabic, Hindu and Chinese prescriptions[32]. Understanding the structure, function and relevance of the metal ions in biological systems is challenging frontier of research today and forms the core of bioinorganic chemistry. Sodium, Potassium and Calcium are present in living organisms in relatively large quantities and many other metals such as iron , copper , molybdenum , manganese , zinc , chromium and vanadium occur in trace or ultratrace amounts. Divalent metal ions play a functional role in stability of tertiary RNAs including ribozymes[33]. The function of metals in metalloproteins, their participation in respiratory. photosynthesis, biosynthetic, nitrogen fixation and metabolic processes are essential to the foundations of life[34-36] .Metal ions are required for many critical functions in humans. Scarcity of some metal ions can lead to diseases, well known examples are pernicious anemia resulting from iron deficiency and grown retardation[37].

The excellence of coordination compounds relies on the characteristics of the ligands used in complexation, as it would impart amazing qualities to the resulting complex. Strongly basic ligands would be expected to have large association constant with metal ions. Any substituent or structural effect that increases localization of negative charge on the donor atom without introducing steric factors increases the stability of metal complexes.

The use of metal chelates is in various branches of theoretical and applied and allied fields is now generally recognized and studies on complexation of drug molecules with metal ions helped immensely to sustain live interest in metal chelation of multidendide ligands. Metal complexes and complex formers are used for detoxification in the case of metal poisoning[38].

Many organic chelating ligands have very special clinical role in handling the toxic metal jons in humans. Chelation therapy can contribute to the removal of toxic metal in which metal specific chelating agents are administered as drugs to complex and facilitate excretion of unwanted excess metal. This property depends on the soluble, easily excretable chelates by sequestering metal ion in the circulation or competing with chelating biological sites for the bound metal ions[38].

Metal complexes having radioactive nuclei find many applications in medicine, such as in tumors, organ and tissue imaging[39]. Metal ions and humic substances present in water bodies complex themselves rendering the biota non toxic[40] .Inorganic complexes that cleave DNA or RNA in a sequence are of potential value in the treatment of cancer and viral diseases and have application in biotechnology[41,42] . Metallofullerenes made of carbon clusters that contain lanthanides trapped in fullerene cage are used for the treatment of secondary tumor cells[43]. The technique of locating protein binding site on DNA known as 'chemical foot printing' demands the requirement of metal complexes[44] .Moreover cisplatin and carboplatin are biologically active metal complexes of platinum, the most widely used anticancer agents in the world[45,46].

CHAPTER –II

REVIEW OF LITERATURE

The investigations on the coordination chain polymers of the transition metal ion Zn(II) with 2-[4-(2-HO C_6H_4 CH=N)CMe=NC_6H_4COO] L were done They were characterized by elemental analysis. IR spectra, electronic spectra, magnetic measurements and TG. analysis by Patel et al[47] .

Neutral complexes of Co(II), Ni(II), Cu(ll), Mn(II), VO(IV) and Zn(II) have been synthesized from the Schiff bass derived from salicylidene-4-aminoantipyrine and 2 aminophenol or 2-aminothiophenol. The structural features have been arrived at from salicylidene-4-aminoantipyrine and 2- aminophenol or 2-aminothiophenol. The structural features have been arrived at from their micro analytical, IR, UV-Vis. 'H NMR and ESR spectral data. The nonelectrolytic and monomeric nature of the complexes is evidenced by their magnetic susceptibility and low conductance data[48].

P.Mosac et al. synthesized Schiff base ligands of 4- aminoantipyrine and salicylaldehydes, and characterized using various spectroscopic techniques such as elemental analysis, UV-Vis, IR, and NMR. The single crystal X–ray structures were analyzed for their various weak H-bonding and dimeric association. Steric strain imposed by the methyl substitution on the 4-aminoantipyrine moiety of the Schiff base ligand, causing this small change of the Cu(II) geometry, along with various weak interactions is analyzed in detail.

Gurunath. S. Kuredkar et al., made studies on 4- aminoantipyrine based Schiff base transition metal complexes as potent anticonvulsant agents[49].

Ikechukwa P. Ejidike and Peter A. Ajibade synthesized and characterized Co(ll), Ni(II) , Zn(ll) and Co(ll) complexes of (3E)-3-[(2-{(E)-[1(2,4dihydroxyphenyl)ethylidene] amino} ethyl) imino]-1-phenylbutan-1-one derived from Co(II), Ni(l), Zn(11) and Cu(I) complexes of (3E)-3- [2-{(E)-[1-(2,44dihydroxyphenyl)ethylidene]amino}ethyl)imino]-1-phenylbutan-1-one derived from ethylene diamine, 2’4’-dihydroxyacetophenone and 1-phenylbutane 1,3-dione through FTIR.UV-Vis spectroscopy, and screened to establish their potential as antibacterial agents antioxidants and DPPH radical scavengers. The FTIR spectra showed that the ligand behaves as a dibasic tetradentate ligand with the dioxygen-dinitrogen donor atom system oriented towards the central metal ion. The analytical and spectroscopic data suggest a square planar geometry for Cu(ll) and Ni(II) complexes and an octahedral geometry for the Co(ll) complex. The ligand and their metal complexes were screened for antibacterial activity against Gram positive and Gram negative bacteria by the agar well diffusion method. In addition, the antioxidant activities of the complexes were also investigated through their scavenging effect on DPPH and ABTS radicals[50].

N. Raman et al. investigated the structural properties and biological studies of transition metal complexes derived from 4-aminoantipyrine. The most important results were made on the extensive studies (syntheses, spectral, magnetic, redox, structural characteristics, antimicrobial and DNA cleavage) of the metal complexes with heterocyclic Schiff bases of 4-aminoantipyrine with some aldehydes and oximes[51].

New binuclear Co(II), and Co(II) complexes of ONO tridentate heterocyclic Schiff base derived from 4-aminoantipyrine with salicylaldehyde have been synthesized and characterized on the bases of elemental analysis, UV-Vis. FTIR, and also by aid of molarconductivity measurements, magnetic measurements, and melting points by Omar Hamad Shihab Al-Obaidi. It was found that the Schiff bases with Cu(II) or Co(II) ion forming binuclear complexes on (1:1) "metal : ligand” stoichiometry. The molar conductance measurements of the complexes in DMSO correspond to be non-electrolytic nature for all prepared complexes. Distorted octahedral environment is suggested for metal complexes. A theoretical treatment of the formation of complexes in the gas phase was studied, and this was done by using the HyperChem-6 program for the molecular mechanics and semi-empirical calculations. The free ligand and its complexes have been tested for their antibacterial activities against two types of human pathogenic bacteria: the first type (Staphylococcus aureus) is Gram positive and the second type (Escherichia coli) is Gram by using agar well diffusion method). Finally, it was found that compounds show different activity of inhibition on growth of the bacteria[52].

Transition metal complexes of Co(ll), Ni(II) and Cu(II) metal ions[53] and ligand 3,3’-thiodipropionic acid bis(4-amino-5-ethylimino-2,3- dimethyl-1-phenyl-3-pyrazoline, have been synthesized and structurally characterized by Sulekh Chandra et al, through elemental analysis, molar conductance measurements, magnetic susceptibility measurements and spectral techniques like IR, UV and EPR. The nickel(II) complexes were found to have octahedral geometry. whereas cobalt(II) and copper(II) complexes were of tetragonal geometry. The covalency factor (β) and orbital reduction factor (K) suggest the covalent nature of the complexes. The ligand and its complexes have been screened for their antifungal and antibacterial activities against three fungi, ie, Altemaria brassicae, Aspergillus niger and Fusarium oxysporum and two bacteria; ie, Xanthomonas compestris and Pseudomonas aeruginosa.

Ram K. Agarwal et al, have synthesized a novel series of Schiff bases by condensation of 4-aminoantipyrine and various aromatic aldehydes followed by reaction with thiosemicarbazide[54]. These thiosemicarbazones are potential ligands toward transition metal ions. These complexes were characterized through elemental analysis, molecular weight, electrical conductance, infrared, electronic spectra, and magnetic susceptibilities at room temperature. Copper(II) complexes were screened for antibacterial and antifungal properties and have exhibited potential activity. Thermal stabilities of two representative complexes were also investigated.

Five Schiff bases derived from 4-aminoantipyrine and benzaldehyde derivatives (1) are prepared and their UV-Vis, IR. ^1H NMR and fluorescence spectra are investigated and discussed by Raafat M. Issa et al[55] .The electronic absorption spectra of the hydroxy-4-aminoantipyrine Schiff bases as well as the fluorescence spectra are studied in the organic solvents of different polarity. The UV-Vis absorption spectra of 4-aminoantipyrine Schiff hases are investigated in aqueous buffer solutions of varying P^H utilized for the determination of pK_a and ΔG of the ionization process. The reactions of the hydroxy compounds with Ni(II) and Cu(II) ions are also studied.

Shalin Kumar et al, studied the applications of Schiff bases and the metal complexes as catalysts in various biological systems, polymers and dyes and their use in birth control, food packages and as an oxygen detector.

Hassan Keypour et al. synthesized two potentially heptadentate (N_40_3) tripodal Schiff base ligands: tris(3-(salicylideneimino)propylamine and tris(3-(4'-hydroxysalicylideneimino) propylamine and have been prepared and characterized by various spectroscopic methods (IR FAB-MS, NMR). They are derived from the condensation reactions of tris(3-aminopropyl)amine (tpt) with 3 equivalents of either salicylaldehyde or the ring substituted salicylaldehyde 4–hydroxy salicylaldehyde. The nickel(II) and copper(II) complexes of were obtained from the its reactions Ni(ll) and Cu(ll) salts in absolute methanol. These complexes were studied by IR and FAB-MS[56].

A novel Schiff base, ethyl 4-[(E)-(2-hydroxy-4- methoxyphenyl)methylencamino] benzoate, was prepared and structurally characterized by Elena Pahontu et al., on the basis of elemental analyses, ^1H NMR,^{13}C NMR, UV-Vis and IR spectral data. Six new copper(II) complexes have been synthesized[57]. The characterization of the newly formed compounds was done by IR,UV– Vis, EPR, FAB-MS, elemental and thermal analysis, magnetic susceptibility measurements and malar electric conductivity. The crystal structures of Schiff base and the complex have been determined by single crystal X-ray diffraction studies. Both copper atoms display a distorted octahedral coordination type. This coordination is ensured by three phenol oxygen, two of which being related to the μ-oxo-bridge, the nitrogen atoms of the azomethine group and the sulphur atoms that come from the polydentate ligand. The invitro antimicrobial activity against Escherichia coli, Salmonella enteritidis. Staphylococcus aureus, Enterococcus and Candida albicans strains was studied and compared with that of free ligand. The complexes showed a better antimicrobial activity than the Schiff base against the tested microorganisms.

AIM AND SCOPE OF THE PRESENT INVESTIGATION

The present work include the synthesis and characterization of complexes of

4–aminoantipyrine with and Zn(II) metal ion. The complexe is analyzed chemically. They were characterized by electrical conductivity, infrared and electronic spectral data. Using this information a tentative structure is also proposed.

CHAPTER–III

MATERIALS, METHODS AND INSTRUMENTS

This chapter outlines the details regarding the preparation of metal salts, ligands, complexes, purification methods, characterisation of the ligands, details of various reagents used and methods used for the analysis of complexes. Physico-chemical measurements and various instrumentation techniques employed are also discussed .

3.1 REAGENTS

3.1.1 Metal salts

In the present investigation zinc sulphate was the transition metal salts used which is of AR quality.

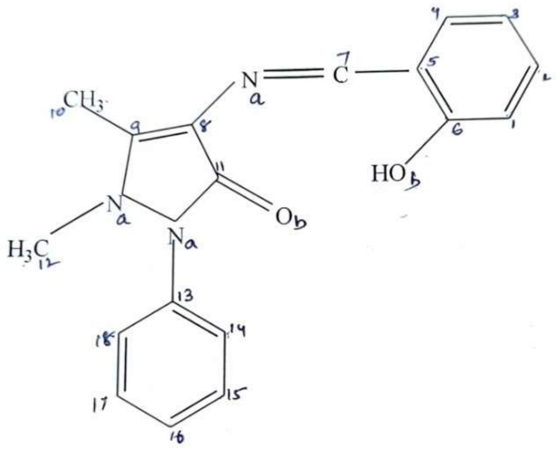
3.1.2 Solvent

Ethanol and methanol were used as solvents. The commercial samples of these solvents were purified by standard methods.

3.2 PREPARATION OF LIGAND

4-aminoantipyrine (2.033g, 10mM) in 40 ml of ethanol was stirred with salicylaldehyde (1ml, 10mM) refluxed for 3 hours. The resultant solution was cooled to room temperature. The yellow solid formed was filtered, repeatedly washed with ethanol and recrystallized from ethanol.

3.2.1 Structure of ligand



3.3 PREPARATION OF COMPLEXE

The complexes are synthesized by a general method . Methanolic solution of the metal salt (0.01mol) and ligand (0.005mol) are mixed in the ratio 1:2. The ligand solution is added gradually in small portions with good stirring to the metal salt solution when sudden colour change was occurred indicating the complex formation. Then it was kept under reflux for 2-3 hours, on a water bath for completion of reaction. Afterwards, the solid complexes formed were filtered, washed with ethanol to remove excess ligands. It was then dried in vacuum desicator.

3.4 ANALYSIS OF COMPLEX

3.4.1 ESTIMATION OF METAL

Estimation of zinc

About 0.2g of zinc complex was digested with conc. sulphuric acid and nitric acid in a Kjeldahl's flask. The resulting clear solution was evaporated to dryness and the residue obtained was extracted with water. The aqueous solution thus obtained was diluted to 50ml in a beaker and the excess acid was neutralized by adding ammonium hydroxide solution. The zinc content in the resulting solution was estimated by titrating with standard EDTA solution using Eriochrome black-T as indicator.

.3.4.2 SOLUBILITY OF COMPLEX

Zn(ll) complexe is insoluble in water, chloroform and ethanol and was found to be partially soluble in acetone and methanol and was completely soluble in dimethyl sulphoxide (DMSO).

3.5 PHYSICO CHEMICAL METHODS

3.5.1 Electrical Conductance

Molar conductance of the complexes in methanol is determined at room temperature (28°C) using direct reading type systronics conductivity Meter Model No.306. The cell constant of the conductivity meter was 1.02cm^{-1}. Approximately 10^{-3} M solutions were used.

3.5.2 Magnetic Susceptibility

Magnetic susceptibility of the complexes were measured at room temperature (28+2°C) on a Sherwood Scientific Magnetic Susceptibility Meter. The gram susceptibility was determined from the equation ,

$$\chi_g = \frac{L_x(R - R_0)}{m \times 10^9}$$

R_o= reading with empty tube.

R = reading with sample,

L=length of sample column,

W= weight of sample.

The molar susceptibility was calculated using the formula $\chi_M = \chi_g * M$

where M is the molecular mass of the substance. Then the molar susceptibilities were corrected for diamagnetism using Pascal's constants.

The effective magnetic moment was calculated using the formula,

$$\mu_{eff} = 2.84\sqrt{\chi_m^{corr} \times T}$$

χ_m^{corr}= molar susceptibility corrected for diamagnetism ,

T = temperature in Kelvin scale.

3.5.3 Infrared Spectroscopy

The importance of infrared spectroscopy lies in the fact that the characteristic infrared absorption band of a group occur at about the same frequency irrespective of the molecule in which the group is present. This makes IR spectroscopy a finger print for the identification and a powerful tool for studying the molecular structure .

The IR spectra of complexes were recorded by KBr pellet method in the range 400–4000cm^{-1} on Agilent Cary 630 FTIR spectrometer at Kariavatton Campuss.

3.5.4 CHNS Analysis

CHNS contents of the complexes were determined by micro analytical method, using Heraeus-CHNS Rapid Analysis Instrument at STIC, Cochin.

3.5.5 NMR Spectroscopy

At a particular combination of fields (one stationary and the other varying at some radio. frequency), energy is absorbed by the sample and the absorption can be observed as a change in the signal developed by a radio frequency detector and amplifier. This is related to the magnetic dipolar nature of spinning nuclei and this technique is known as the NMR spectroscopy.

The proton NMR spectrum of the ligand and zinc complex was recorded in Brucker-NMR spectrometer at 500 MHz at IISER, Thiruvananthapuram.

3.5.6 Biological studies

The biological activities or the therapeutical ability of any compound depends upon the minimum amount by which the chemical or substance is required to inhibit the growth or to kill the microorganism that causes the disease. The synthesized chemical ligand and complex were tested for their antimicrobial activity. Antimicrobial activity is the ability of a compound to inhibit the growth of a given microorganism. The disc diffusion method was used for the screening of the antimicrobial property of the test samples. The antimicrobial activity of the compound depends on the ability to form a compound-organism complex in the following order:

Uncomplexed metal > metal chelate> free ligand.

This order clearly indicates that the uncomplexed metal salts have greater ability to form the compound-organism complex. Higher activity of the metal chelates is due to the increased lipophilicity from inclusion of metal ion with the Schiff base.The biological activity of the ligand and the complexes were analyzed at Biogenics. Thiruvananthapuram.

CHAPTER IV

RESULTS AND DISCUSSION

In the present study, the ligand used was a Schiff base. It was prepared from 4–aminoantipyrine and salicylaldehyde. Zn(II) Complex is prepared using this Schiff base. Complex is amorphous solid and is soluble in acetone and DMSO.

4.1 Elemental Analysis

The microanalytical data are shown in the table given below. The experimental values are in good agreement with the theoretical value. From the analytical data obtained we suggest the following empirical formulae for the complex: [Zn (SAAP)$_2$]SO$_4$.

	Carbon %		**Hydrogen %**		**Nitrogen %**		**Metal %**	
compound	Cal	Obs	Cal	Obs	Cal	Obs	Cal	Obs
SAAP	70.56	70.64	5.26	5.18	13.72	13.65	…….	…….
[Zn(SAAP)$_2$]SO$_4$	55.85	55.79	4.16	4.23	10.85	10.70	8.44	8.38

4.2 Molar conductance

The molar conductance of the complex (10 $^{-3}$molar concentration) is carried out in methanol indicated that zinc complex is 1:1 electrolytes. The values are presented in the table below:

Complex	Molar conductance in methanol (ohm^{-1} cm^2 mol $^{-1}$)	Assignment
(Zn(SAAP)$_2$)SO$_4$	87	1:1 electrolyte

4.3 UV-Vis spectra

The ligand is characterized by two absorption bands in the UV region. A high intensity hand at 321cm^{-1} is attributed to $\pi \rightarrow \pi^*$ transition and a lower intensity band at 398cm^{-1} is attributed to $n \rightarrow \pi^*$ transition of azomethine group. The absorption bands in the complex is shifted to longer wavelengths compared to that of the ligand. Also new bands are appeared due to d-d transition.

Electronic spectral data of SAAP ,

COMPOUND	λ_{MAX}	ASSIGNMENT
SAAP	398 321	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$

4.4 FT-IR Spectra

Azomethine group (C=N) has a characteristic stretching frequency. In general, upon coordination to metal ions this band is shifted to lower frequencies with respect to the free ligand.

The IR spectral data of the ligand SAAP and the Zn(II) comple is in agreement with an expected range. The band at 1490cm^{-1} in the ligand is attributed to azomethine group. This is shifted to 1458cm^{-1} in the zinc complex suggesting a coordination of metal ions to nitrogen of azomethine group. The band at 1650cm^{-1} in the ligand is attributed to carbonyl group. This is shifted to 1676cm^{-1} in zinc complex suggesting a coordination of metal ions in oxygen of carbonyl group.

The IR spectral data are as shown in table below:

Ligand ($\bar{\nu}$ cm^{-1})	**$[Zn(SAAP)_2]SO_4$** ($\bar{\nu}$ cm^{-1})	**Assignment** ($\bar{\nu}$ cm^{-1})
2939	2927	$\bar{\nu}_{\text{C-H stretch}}$

1490	1458	$\bar{\nu}_{\text{C=N stretch}}$
1650	1676	$\bar{\nu}_{\text{C=O stretch}}$
------------	543	$\bar{\nu}_{\text{M-O}}$
------------	464	$\bar{\nu}_{\text{M-N}}$

4.5 NMR Spectra

Proton NMR spectra of the ligand SAAP shows the following signals: C_6H_6 multiplet at 6.9–7.6 ppm, azomethine proton at 9.7 ppm and –OH proton at 12.95 ppm. NMR spectrum is shown in Fig 4.1

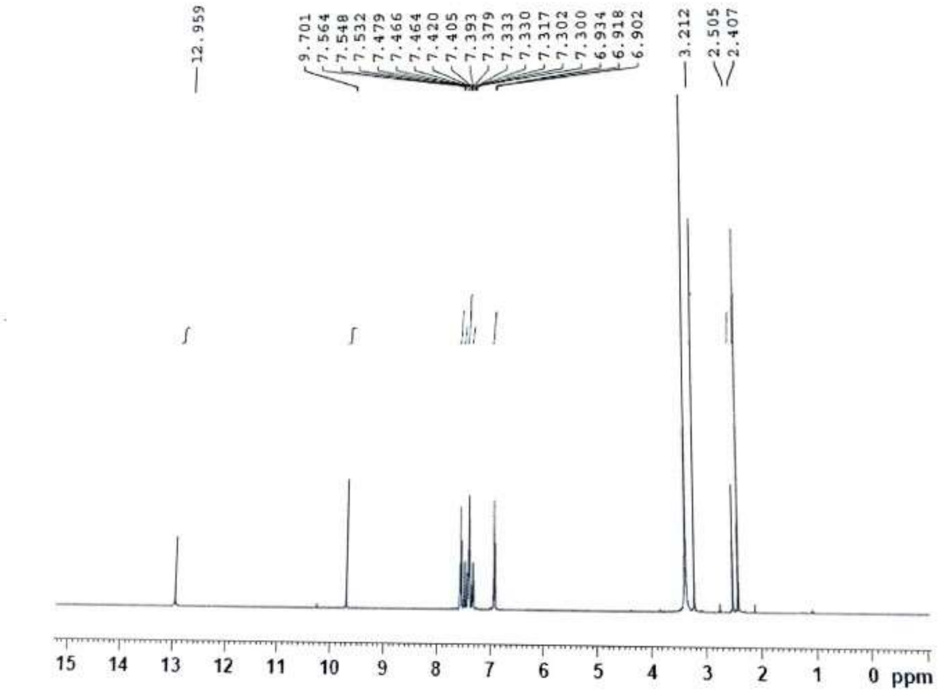


Fig 4.1:NMR spectrum of ligand (SAAP)

Proton NMR spectrum of the zinc complex shows the following signals: C_6H_6 multiplet at 6.8-7.6 ppm, azomethine proton at 9.91 ppm and –OH proton at 12.96 ppm, NMR spectrum is shown in Fig 4.2.

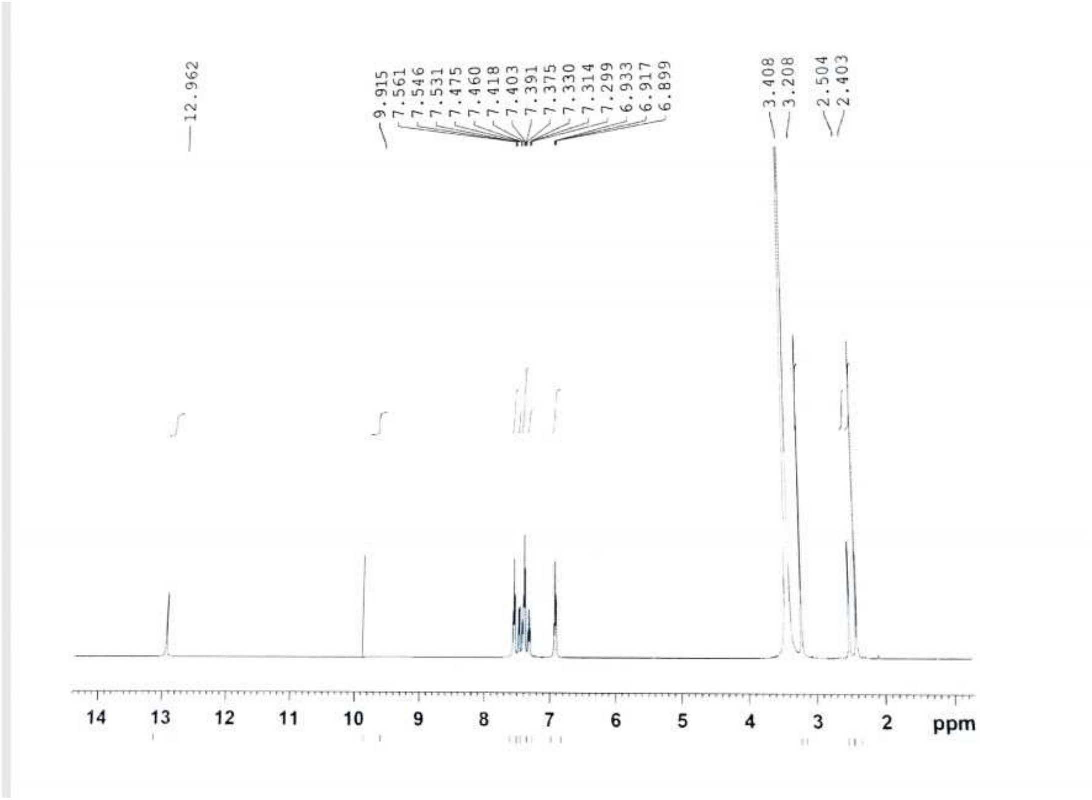


Fig 4.2: NMR spectrum of zinc complex: [Zn(SAAP)$_2$]SO_4

From the spectra of the ligand and the complex we can confirm that –C=N group is coordinated to the metal. The -OH group remains unchanged in its position indicates it is not coordinated to the metal.

4.6 Structure of complexes

On the basis of these observations and discussion it can be suggested that SAAP is acting as a bidentate ligand. The zinc complex has coordination number four and assumes tetrahedral geometry. The proposed structures for the complexes are shown in Fig 4.3:

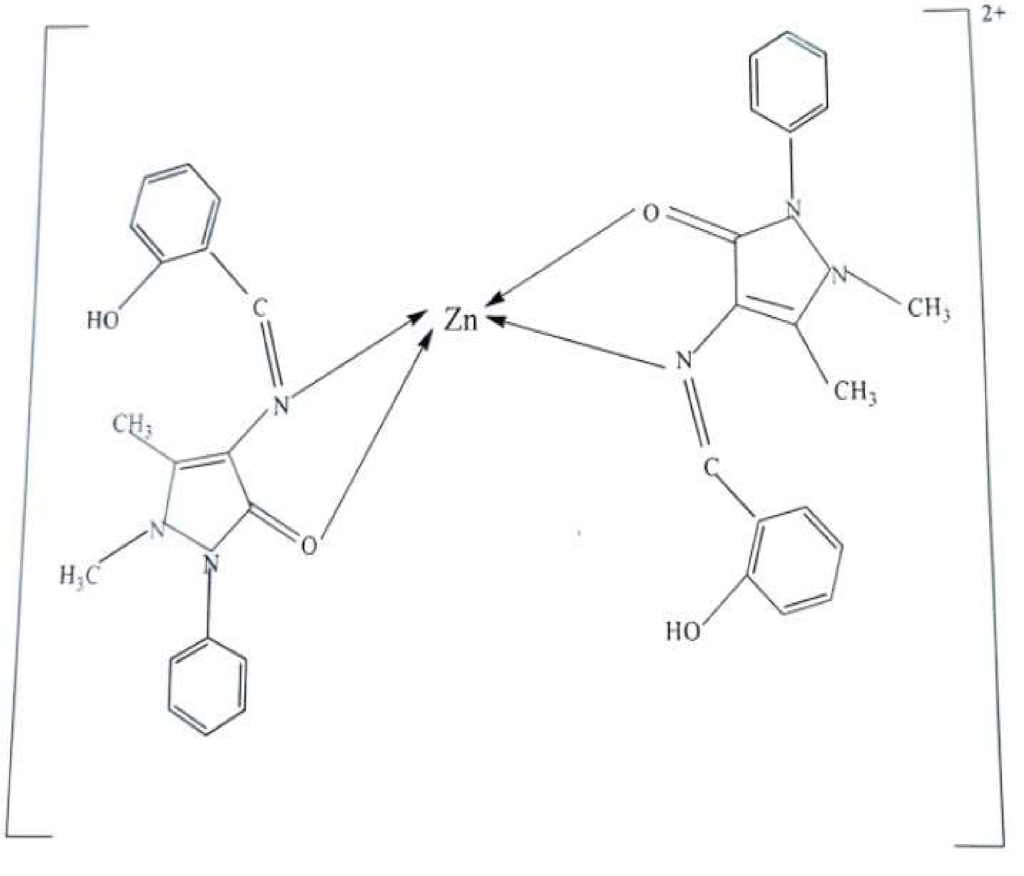


Fig 4.3: Proposed structure for $[Zn(SAAP)_2]SO_4$

CHAPTER V

BIOLOGICAL STUDIES

5.1 ANTIBACTERIAL ACTIVITY

The in vitro biological screening effects of the investigated compounds were tested against the bacteria Pseudomonas aeruginosa by using disc diffusion method by taking DMSO as solvent.

5.1.1 PRINCIPLE

The antimicrobials present in the plant extract are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in centimeters .

5.1.2 REAGENTS

1.Muller Hinton Agar Medium (IL)

The medium was prepared by dissolving 33.9g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured on to 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (IL)

One litre of nutrient broth was prepared by dissolving 13g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration :20mg/ml)

5.1.3 PROCEDURE

Petriplates containing 20ml Muller Hinton medium were seeded with bacterial Pseudomonas aeroginosa (growth of culture according to McFarts Standard). Wells of approximately 10 mm was bored using a well cutter and 25 µl, 50 µl and 100 µI of sample was added to the well from a stock concentration of 0.1g/I ml the plates were then incubated at 37°C for 24 hrs. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed and the well (NCCLS, 1993). Streptomycin was used as a positive control.

The study of the growth inhibition zone of the Schiff base indicated that the lipid membrane that surround the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the antimicrobial activity. The inhibition zone of antibacterial activity of SAAP and its complexes are shown in the table below:

compound	**bacterial inhibition zone (mm)**
SAAP	2.0
$[Zn(SAAP)_2]SO_4$	1.9

The result indicates that the ligand and Zinc complexes shows the antibacterial activity against pseudomonas aeroginosa.

5.2 ANTIFUNGAL ACTIVITY

In order to access the biological significance and ability of the sample, the minimum inhibitory activity was determined by Agar well diffusion method. Potato Dextrose agar plates were prepared and overnight grown different species of fungus as Candida albicans were swabbed Wells of approximately 10mm was bored using a well cutter and samples of different concentration was added, the zone of inhibition was measured after overnight incubation and compared with that of standard antimycistic (Clotrimazole) Streptomycin was used as a positive control.

compound	**Fungal inhibition zone (mm)**
SAAP	2.6
$[Zn(SAAP)_2]SO_4$	2.8

The result indicates that the ligand and zinc complex shows the highest antifungal activity against Candida albicans.

Fig 4.6 : Antibacterial activity of SAAP complexes

Fig 4.7 : Antibacterial activity of ligand (SAAP).

Fig 4.8 : Antifungal activity of SAAP complexes

Fig 4.9 : Antifungal activity of ligand (SAAP)

SUMMARY

A Schiff base is prfepared from 4-amino antipyrine and salicylaldehyde; (SAAP).The Zn(II) complex were prepared using SAAP. Charecterization of ligand and complex has been done on the basis of analytical and physic chemical methords. The tetrahedral geometrty is assigned for the zinc complex $[Zn(SAAP)_2]So_4$ on the basis of conductance mesurements , IR and NMR spectrul studies. The ligand and metal complex were screened for their biological activities against Pseudomonas aeroginosa and candida albicans. The ligand and the complex show better antimicrobial activity.

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