SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF IRON COMPLEX

MSc ANALYTICAL CHEMISTRY

CONTENTS

PAGE NO.

ABSTRACT

Coordination Chemistry has gone through a period of remarkable renaissance during the last few decades. The vital role it plays in life processes and industry is well known. The structure and bonding in Iron complex give excellent scope for theoretical studies. The present work is concerned with the synthesis of Fe(III) complex using a Schiff base prepared from 4-aminoantipyrine and salicylaldehyde;(SAAP). The ligand and the complex were characterized by elemental analysis, molar conductance, magnetic studies , UV-Visible and FT-IR spectroscopy. Using these data geometry of the complex is also determined . The antibacterial and antifungal activity of the ligand and complex is studied.

CHAPTER 1

INTRODUCTION

A long-established topic of chemistry, coordination chemistry has applications in practically all areas of research. There are hundreds of papers each year, which indicates that research in this area is moving quickly. Since the nineteenth century, when coordination compounds were first discovered, inorganic chemists have faced difficulties in understanding them. Over the past few decades, tens of thousands of compounds including mono, di, and multidentate ligands and metal ions, both transition and non-transition, have been described. To account for these molecules, standard bonding theories like Molecular orbital theory and Ligand field theory have been expanded.[1]

For more than a century, descriptive research predominated in the study of inorganic metal-containing substances. Since the discovery of electrons in 1897, which allowed for the later beginnings (1916) and development (1920s) of the electron theory of valence, real theoretical advancement towards understanding the structure and behaviour of this enormous class of inorganic compounds has only recently been possible[2]. Since then, theoretical inorganic chemistry has advanced incredibly quickly. The pioneering work of Lewis, Kossel, Langmuir, Sidgwick, Fajans, Pauling, Van Vleck, and many others who have expanded and amplified their concepts is primarily responsible for this.

Coordination chemistry is usually considered to have started with Tassert[3] discovery of hexamine cobalt (Ill) chloride $[Co(NH_3)Cl_3]$ in 1798. Later, several compounds were created and their properties were investigated. The structure of coordination compounds has been the subject of numerous theories.

By introducing his coordination theory in 1893, Alfred Werner, who is hailed as the "Father of Modern Chemistry," expanded on the topics. The first effective attempt to describe the bonding in coordination complexes was Werner's coordination theory. Later, Lewis[4] electronic theory of valency, which Sidgwick[5] extended to coordination compounds, brought

Werner's theories together. Complexes colour and stability could be predicted using Pauling's valence theory. However, the colour and variety of magnetic characteristics remain a mystery. Because of these factors, the Bethe and Van Vleck-proposed Crystal Field Theory (FT), whose ideas were notable for their clarity and capacity to adequately explain complex properties, has replaced it. The most recent advancement in the area is Molecular Orbital Theory (MOT), which is regarded as the most significant and precise method of studying chemical bonding. However, it hasn't replaced other theories.

In coordination chemistry, a structure's coordination number, or the number of ligands attached, is used to define it. However, even counting can occasionally be confusing.Normal coordination numbers range from two to nine, however the lanthanides and actinides can have several ligands. The size, charge, and electrical configuration of the metal ion and the ligands all affect how many bonds there are. There may be several coordination numbers for metal ions.

Numerous biological, industrial, and analytical fields use coordination chemicals. Researchers have recently come to understand the value of chelation therapy[6] and metal chemotherapy. They serve as both analytical reagents and catalysts in a wide range of metallurgical processes. Many transition metal complexes have recently been employed as non-linear optical materials.

Coordination complexes are crucial to the metabolic functions that keep life on earth going. Examples of coordination chemicals include the green plant pigment chlorophyll, the red blood pigment haemoglobin, and vitamin B_{12}. The blue colour of writing ink is one of many dyes and pigments that contain metal complexes. Coordination compounds are utilised as medicinal agents and are crucial for medical diagnosis. In positron emission tomography, the positron-emitting Cu^{2+} nuclei complexed with pyruvaldehydebis(4-methyl thiosemicarbazone) serve as a perfusion tracer[7]. The gadolinium $[Gd(DTPA)]^{2-}$ diethylenetriaminepentaacetic acid combination is a regularly utilised contrast agent in MRI method [8]. Metal complexes are being researched as possible novel medications (metallopharamceutics). Coordination compounds are a common component of many catalysts used in the chemical industry.

Coordination complexes' nutritional and pharmacological features have been examined.The field's scope has expanded to include a variety of disciplines, including molecular electronics, extended and microscopic materials, photonic materials, models for solid surfaces, and solid state chemistry.

1.1 SCHIFF BASE

Any primary amine will react with an aldehyde or ketone under particular circumstances to produce Schiff bases, which bear Hugo Schiff's name[9]. Schiff bases are structurally the nitrogen counterpart of an aldehyde or ketone (also known as imines or azomethine).

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

Numerous studies have shown the critical role of the lone pair of electrons on the trigonaly hybridised nitrogen atom of the azomethine group for both chemical and biological processes. By using a nucleophilic addition to create a hemiaminal and then a dehydration to create an imine, it is possible to create schiff bases from an aliphatic or aromatic amine and a carbonyl molecule.

Some of the most frequently utilised organic substances are schiff bases.They serve as dyes and pigments, catalysts, steps in the synthesis of chemical compounds, and stabilisers for polymers. Rhodopsin, a visual pigment, has an azomethine bond[10]. A wide spectrum of biological activities, such as antifungal, antibacterial, antimalarial, antiproliferative, anti-inflammatory, antiviral, and antipyretic characteristics, are also demonstrated by schiff bases. E-Coli and Staphylococcus are resistant to the antibacterial effects of isatin thiosemicarbazones. According to reports, rats treated with Schiff bases showed cytotoxic and antitumor effects[11]. Some isatin Schiff base complexes are efficient at treating conditions of the central nervous system. Rare earth compounds of furfural anthranilic acid Schiff bases have been shown to exhibit antitumor properties[12].

Complexes of Co(II),Cu(II),Ni(II), and Zn(II) were created using ligands like 1,1'-disubstituted ferrocene derived Schiff bases. These complexes were used for screening against pathogenic bacterial species like E-Coli, Staphylococcus aureus, and Klebsiella pneumonia and are a novel class of organometallic antibacterials[13]. Their biological functions depend on the imine group present.

According to reports, several of the Schiff bases are engaged in the fight against leukaemia[14]. There are numerous more Schiff bases that are said to have therapeutic effects and to have analgesic, antipyretic, anti-inflammatory, cardiotoxic, and diuretic action[15]. Another investigation claims that some Schiff bases prevent the roots of detached cabbage leaves from developing properly[16].In addition to biological activities, Schiff bases have a wide range of applications[17]. Reactions involving polymerization, oxidation, and breakdown are catalysed by aromatic Schiff bases[18]. Numerous metal chelate polymers have been produced by multidentate Schiff bases, and they have also been integrated into a number of other compounds to generate desired properties. Due to their colour, Schiff bases and their metal chelates have been used in the dye and pigment business. A few azomethines are added to fuel. The flexibility of Schiff bases during synthesis might make it possible to create multidentate ligands with a variety of structural types. A few aromatic Schiff bases have been employed as stabilisers for a variety of substances, such as lubricating oils, fuel oils, and jet fuels.In order to provide some polymers desirable properties like super conductivity, heat resistance, and resistance to light, oxidation, hardness, etc., Schiff bases have been added to a variety of polymers. Polymeric Schiff bases are a common choice today for electro photographic materials. Schiff bases also have a wide range of analytical uses in addition to these[19].Schiff bases on amino acids are being used in complexes[20].

Schiff bases made from aromatic acids and aromatic aldehydes are used in a wide range of analytical, inorganic, and biological chemical applications[21]. Many modern analytical equipment need organic reagents since they are crucial parts of the measuring system. They are utilised to enable the detection of increased selectivity and sensitivity in optical and electrochemical sensors as well as in a number of chromatographic techniques. Schiff bases are among the organic reagents that are actually employed; they have outstanding qualities, structural parallels to natural biological substances, comparatively simple synthesis methods, and the synthetic adaptability that allows design of appropriate structural properties.

Typically, multifunctional, hetero functional groups are present in heterocyclic compounds that comprise possible donor atoms N, O, and S[22].Fast-coloring dyes for leather, food packaging, wool, and other materials include Schiff base unsymmetrical complexes of 1:2 chromium[23] and chromium azomethine complexes. Fabrics made of cellulose and polyester are dyed using azo groups that include metal complexes. Polyfibres can be mass-dyed using certain metal complexes. A Schiff base's cobalt complex (salicylaldehyde with diamine) has high light resistance, great storage properties, and does not deteriorate even in gaseous acids (CO_2). New tetradentate Schiff bases are used as a chromogenic reagent to measure Ni in some samples of natural foods[24].

Polymeric Schiff bases have photoconductive qualities, are sensitive to light, and are stable when included in silver halide emulsions. Some hydroxyl azomethine compounds are used in complexometric titrations as indicators and as luminous reagents. Inorganic cations and anions are determined fluorometrically using them. They safeguard organic inhibitors in nitrocellulose coatings as well as act as inhibitors against acid corrosion of steel and aluminium.

Schiff bases are used in a variety of fields, including environmental chemistry[25], bioinorganic chemistry[26], metallic deactivators, catalysis[27], and separation processes.

Schiff base complexes have attracted considerable biological interest because they can be used to create metal-containing model systems that closely resemble biologically active systems. Schiff base complexes continue to draw numerous researchers due to their numerous industrial and academic applications.

1.2 CHEMISTRY OF IRON

The most prevalent oxidation states for iron are +2 and +3. Octahedral Fe(II) complexes predominate. Fe(II) electronic ground state is ^5D. It will be separated into $^5T_2$ and ^5E. There are only two possible quintet states, thus if one of these is the ground state, there can only be one d-d transition allowed[29]. There are also some known tetrahedral complexes that contain the $[FeCl]^{2-}$ ion in salts with big cations. Tetrahedral complexes have a high spin and $^5T_2$ and ^5E band at a frequency of 4000. In most cases, magnetic moments fall between 5.0 and 5.5 BM[29].

According to theoretical prediction, octahedral Fe(IlI) complexes have a larger propensity to produce charge transfer bands in the near UV region with significant low energy wings in the visible range, which mask the extremely spin prohibited d-d bands[30].

Fe(lIl) is isoelectronic with Mn(II), but less is known about the specifics of Fe(llI) spectra due to the trivalent ion's greater propensity to have charge transfer bands in the near UV region with strong low energy shoulders that obscure very weak, spin forbidden d-d bands. Fe(lIl) is nearly high spin in all its complexes except those with the strongest ligand,$[Fe(CN)_6]^{3-}$, $[Fe(bpy)]^{3+}$, $[Fe(phen)_3]^{3+}$and other tris complexes with amine, nitrogen atoms as Donors. With values of 2.3 BM, the low spin complexes with the $^5T_{2g}$ structure typically have a significant orbital contribution to their moments as they are near room temperature[31]. These seconds are temperature-dependent, falling to ≈1.9 BM at liquid nitrogen temperature. Depending on the ligands, five coordinated complexes can have high or low spin. High spin oxy-bridged nuclear complexes are present. There are numerous excellent complexes of spin crossover and low spin high spin equilibrium provided by Dithio carbonates and Schiff base complexes[32-33].

1.3 METALS IN BIOMEDICAL FIELD

The inorganic chemists and medical researchers were motivated to investigate new therapeutic areas based on diverse medication categories as a result of their understanding of the role of metals in biological systems. Understanding the structure, function, and significance of the metal ions in biological systems is a hard frontier of research today and forms the basis of bioinorganic chemistry. Copper, silver, and gold were utilised in Arabic, Hindu, and Chinese prescriptions over four millennia ago[34]. Living things include relatively high levels of sodium, potassium, and calcium, as well as trace or ultratrace amounts of many other metals like iron, copper, molybdenum, manganese, zinc, chromium, and vanadium. Divalent metal ions are functionally important for tertiary RNA stability, especially ribozyme stability[35]. The underpinnings of life depend on the role that metals play in metalloproteins and in activities including respiration, photosynthesis, biosynthesis, nitrogen fixation, and metabolism[37-39]. Many essential human functions require metal ions. Diseases can develop from a lack of certain metal ions; well-known examples are pernicious anaemia, which is brought on by an iron deficiency, and growth retardation[40] .

The attributes of the ligands utilised in complexation, which would endow the emerging complex with extraordinary qualities, are what determine the perfection of coordination compounds. Strongly basic ligands should have a high association constant with metal ions, according to expectations. The stability of metal complexes is increased by any substituent or structural modification that enhances the localization of negative charge on the donor atom without creating steric constraints[41]. Studies on the complexation of medicinal molecules with metal ions helped significantly to keep active interest in metal chelation of multidendate ligands. The usage of metal chelates is now extensively acknowledged in numerous departments of theoretical, practical, and related fields. In the event of metal poisoning, metal complexes and complex formers are employed for detoxification[42]. Numerous chemical chelating ligands play extremely specific therapeutic roles in how humans handle hazardous metal ions[43].

Chelation therapy, in which metal-specific chelating chemicals are supplied as medications to complex and facilitate elimination of undesirable excess metal, can help remove harmful metal. This feature relies on the soluble, quickly excretable chelates by either competing with chelating biological sites for the bound metal ions or sequestering metal ions in the circulation[44]. Numerous medical procedures use metal complexes with radioactive nuclei, including imaging of cancers, organs, and tissues[45].Because of the intricate interactions between metal ions and humic compounds found in water bodies, the biota is not poisonous[46]. The use of inorganic complexes that cleave DNA or RNA in a certain order has applications in biotechnology and the treatment of viral and cancerous disorders. Secondary tumour cells are treated with metallofullerenes, which are fullerene cage-enclosed carbon clusters containing lanthanides[47]. Metal complexes are necessary for the "chemical foot printing" method of finding protein binding sites on DNA. Moreover, the most frequently used anticancer drugs in the world, carboplatin and cisplatin, are biologically active metal complexes of platinum[48].

CHAPTER 2

REVIEW OF LITERATURE

Coordination chemistry is the field in which most scholarly, attractive and experimentally demanding frontiers in modern chemical science. The complexation reactions are used for the qualitative as well as quantitative analysis of metal ions. Extremely sensitive and selective organic reagents are used for the determination of metal ions. Co-ordination chemistry deals with metals and ligands were the metal coordination occurs when lone pair electrons from a ligand are donated to an empty orbital in a metal ion[49] . There are different classes of ligands such as classical, organo-metallic, cluster and bioinorganic. Classical ligand is known as Werner complex after coordination chemistry's founder Alfred Werner which is a ligand that binds through the lone pairs of the main group atom of the ligand[50]. The most of the metal-ligand interactions seen in nature are classical ligands. Metals have a definite choice for certain ligands and for certain geometries. Classical cases are the so-called Schiff-base couplings; in other cases rather unique ligands can be formed only when the metal is present.

A German chemist Hugo Schiff in 1864 developed a new class of organic compounds. The preparations of these compounds is simple that is they are prepared by condensing a carbonyl compound with an amine, generally refluxing in alcohol.[51]

Using 2-[4-(2-HO C_6H_4 CH=N)CMe=NC_6H_4COO], studies were conducted on the coordination chain polymers of the transition metal ions Ni(II), Mn (II) and Zn(II) by Patel et al., they were identified using elemental analysis, IR spectra, electronic spectra, magnetic measurements, and T.G. analyses.[52]

The mixed ligand complexes of Ni(II) or Fe(II) with 2,2'-bipyridylamine and the Schiff base 5-bromo salicylaldene-o-aminophenol have been created by Patel and colleagues and described using elemental analysis, magnetic and spectroscopic investigations, and T.G.A. The complexes' anti-microbial effects were examined when they were tested against bacteria, yeast, and fungi.[53]

Nutan Sharma and Tejpal Singh Chundwai studied the synthesis of some salicylaldehyde based Schiff bases in aqueous media. A new efficient procedure for the synthesis of a series of salicylaldehyde-based Schiff bases is described. The present work involves condensation of salicylaldehyde with various aromatic amines in water under microwave irradiation. This reaction condition allow the final product to be generated in excellent yields in a one-step procedure and microwave irradiation method gives advantages like reduction in reaction time, increase in conversion, reduced wastes, and good yields . The structures of the product are described by IR, 1HNMR and mass spectroscopic data[54]

A few complexes of Fe(III) ions with a pair of Schiff base ligand derived from 1,3-bis (aminomethyl) cyclohexane and salicylaldehyde or pyrogalaldehyde were synthesized and characterized by Sarojini and co-workers and are having tetrahedral geometries[55]

Hussaini.S.Y, A. Ahmad and S. Sani synthesised and characterised a new Schiff base prepared from the interaction of ethanolic solution of salicylaldehyde and aniline. They form complexes with divalent metal ions such as Ni(II) and Cu(II). Then their characteristics are studied using IR, H^1NMR, elemental analysis, potentiometry, molar conductance and melting point. The elemental data give that 1:2 ratio of metal ligand formation. The antibacterial activity is studied by using E-coli and Staphylococcus aureus.[56]

K.A.Maher and S.R.Mohammad condensed salicylaldehyde and their derivatives with amines in 1:1 ratio and 2:1ratio. It gives bi, tri and tetra dendate NO and N_2O_2 Schiff base ligands. It contains additional donor atoms like sulphur, oxygen, nitrogen etc... which is capable for making chelating ligands with metal complexes. This made a large possibility of synthesis of large numbers of bi, tri and tetradentate Schiff bases with different structures .[57]

P. Mosae et al. , synthesized Schiff base ligands of 4-aminoantipyrine and substituted 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 assosciation . 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 interaction is analyzed in detail.[58]

Mrinmony, Nayim Sepay, Corrado Rizzoli, Chandan Kumar Ghosh and Sandeep Sahaa's work report a distorted square pyramidal mononuclear copper(II) complex [Cu(L)(N3)] . This was synthesized from a tetradentate Schiff base (HL) and it is prepared by the condensation of salicylaldehyde and N-2-(aminoethyl)-1,3- propanediamine. These Complexes are characterized based on elemental analysis, IR spectroscopy, UV-Vis spectroscopy, ESI-MS, PXRD. The structure of the complex is confirmed using a single-crystal X-ray diffraction study.[59]

Studies on 4-aminoantipyrine based Schiff base transition metal complexes as potent anticonvulsant agents was made by Gurunath S Kuredkar et al.[60]

P.K. Parashar, R.C. Sharma, G. Moham's work derived Schiff bases from salicylaldehyde and 2-substituted aniline and their metal complexes of Cu(II), Ni(II), and Co(II) ions were synthesized and they are used for the study of anti-inflammatory and antiulcer activity. The compound salicylidene-anthranilic acid was found that it has efficient activities in the anti-inflammatory and antiulcer . The copper complexes showed more antiulcer activity than other metals.[61]

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 (synthesis, spectral, magnetic, structural characteristics,antimicrobial and DNA cleavage) of the metal complexes with heterocyclic Schiff bases of 4-aminoantipyrine with some aldehydes and oximes.[62]

Sheetal Jadhav and Kailas Kapadnis study Schiff bases and their metal complexes for very longer time to identify their unique properties. By condensing meta nitro aniline and benzaldehyde in ethanol in the presence of acetic acid they prepared isolated Schiff base and made complex derivatives with nickel, copper and cobalt. Then the NMR, IR and antimicrobial activities were studied .[63]

Dr Navneet Kumar, Pratima Sharma and Asha Pareek synthesised Schiff bases such as Salicyladehyde glycine, DL-2, 3-Diaminopropion-Salicyaldehyde, benzylidene glycine and 4- acetylamido benzylidene aniline in basic media using 2M NaOH. The compounds formed as yellow precipitates and then reacted with methanolic solution of copper per chlorate. Application of Schiff bases and their metal complexes as catalysts, in various biological systems, polymers and dyes are studied. They have important uses in birth control and food packages. These Schiff bases also show the antimicrobial activities, antifungal activities, antiviral activities.[64]

Transition metal complexes of Co(II) ,Ni (II) and Cu (II) metal ions 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 analyses,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 and orbital reduction factor (k) suggest the covalent nature of the complexes. [65]

S.N. Pradeepa, H.S. Bhojya, B.Vijay Kumar, K. Indira Priyadharshini, Atanu Bharik and T.V. Ravi Kumar Naik's work synthesised and characterised metal complexes of Schiff baseN'1,N'3-bis[(E)-(5-bromo-2-hydroxyphenyl)methylidene]benzene-1,3-dicarbohydrazide.DNA-binding studies were done using absorption spectroscopy, emission spectra, viscosity measurements and thermal denaturation studies and the experimental evidence indicated that, the Co(II), Ni(II) and Cu(II) complexes interact with calf thymus DNA through intercalation with an intrinsic binding constant Kb of 2.6×10^4 M^{-1}, 5.7×10^4 M^{-1} and 4.5×10^4 M^{-1}, respectively. They exhibited potent photodamage abilities on pUC19 DNA, through singlet oxygen generation with quantum yields of 0.32, 0.27 and 0.30 respectively. The cytotoxic activity of the complexes shows that they can act as potent-photosensitizers for photochemical reactions.[66]

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. These thiosemicarbazides 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.[67]

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.[68]

M. Rajashekhar, S. Sreedharan, R.Prabhu, V.Narayanan ,R.Jagathesh, N.Ramen and A. Kalilur Rahman work synthesised and studied Ni(II) and Cu(II) metal complexes of simple unsymmetrical Schiff-base ligands derived from salicylaldehyde/5-methylsalicylaldehyde and ethylenediamine or diaminomaleonitrile (DMN) . The ligands and their complexes were characterized by using elemental analysis, H^1NMR, FT IR, and mass spectroscopy. The electronic spectra of the complexes show d–d transitions in the region at 450–600 nm and the electrochemical studies of the complexes reveal that all mononuclear complexes show a one electron quasi-reversible reduction wave in the cathodic region. ESR spectra of the mononuclear copper(II) complexes show four lines is the characteristic of square-planar geometry having nuclear hyperfine spin 3/2. The studies show that copper (II) complexes have a normal room temperature magnetic moment value μ eff = 1.70–1.74 BM which is close to the spin only value of 1.73 BM. Kinetic studies on the oxidation of pyrocatechol to quinone using the copper(II) complexes as catalysts were also done. The in vitro antimicrobial activity of these compounds was tested against human pathogenic bacteria’s like Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas aeruginosa and Escherichia coli. The antifungal activity of these compounds was tested against Candida albicans. The studies show that the metal complexes have higher antimicrobial activity than the free ligands .[69]

Hassan Keypour et al. , synthesized two potentially heptadentate (N_4O_3) tripodal Schiff-base ligands : tris (3-(salicylidineimino)propyl)amine and tris(3-(4’-hydroxysalicylideneimino)-propyl)amine 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-hydroxysalicylaldehyde. The nickel (II) and copper (II) complexes of were obtained from its reactions of their salts in absolute methanol. These complexes were studied by IR and FAB-MS.[70]

The work of S.A. Abdul Latif, H.B. Hassib, V.M.Issa studied the formation constants of some transition metal ions like Cr(III), Mn(II), Fe(III), Ni(II) and Cu(II) binary complexes containing Schiff bases formed from condensation of salicylaldehyde with aniline (I), 2-aminopyridine (II), 4-aminopyridine (III) and 2-aminopyrimidine (IV). They were determined by pH metrically in ethanolic medium. For all binary complexes the formation constants were determined. They studied the infrared spectral bands corresponding to the active groups in the four ligands and the solid complexes. Using thermogravimetric analysis the solid complexes synthesised and studied and using Coats-Redfern equation the thermal dehydration and decomposition of complexes studied kinetically. The studies found that the thermal decomposition of the complexes follow second order kinetics and also the thermodynamic parameters of the decomposition are reported. Determining the pKa values spectrophotometrically the absorption spectra of ligand determined.[71]

H.N. Aliyu and H.Adamu studied the interaction of ethanolic solution of semicarbazide hydrochloride, benzaldehyde and morpholine yielded N-(I-Morpholinobenzyl) Semicarbazide Schiff base. The Schiff base complexes of manganese(II) and iron(III) were synthesized by refluxing the mixture of ethanolic solutions of the Schiff base and metal(II) chlorides respectively. These complexes were characterized by gravimetry, spectrophotometry, potentiometry , molar conductance and infrared analysis. [72]

Sheetal Jadhav and Kailas Kapadnis study Schiff bases and their metal complexes for very longer time to identify their unique properties. By condensing meta nitro aniline and benzaldehyde in ethanol in the presence of acetic acid they prepared isolated Schiff base and made complexes derivatives with nickel, copper and cobalt. Then the NMR, IR and antimicrobial activities were studied.[73]

The metal Schiff base complexes study is still going and it is a popular research topic now days. This compound is very useful as antimicrobial, antibacterial, antifungal, antiulcer, antitumour etc...

AIM AND SCOPE OF THE PRESENT INVESTIGATION

The present work include the synthesis and characterization of complexes of 4-aminoantipyrine with metal ions Fe(III). The complex was analyzed chemically. It was characterized by electrical conductivity, infrared and electronic spectral data. Using this information a tentative structure is also proposed.

CHAPTER 3

MATERIALS, METHODS AND INSTRUMENTS

This chapter outlines the details regarding the preparation of metal salt, ligand, complexes, purification methods, characterisation of the ligand,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 salt

In the present investigation ferric chloride was the transition metal salt used which are of AR quality

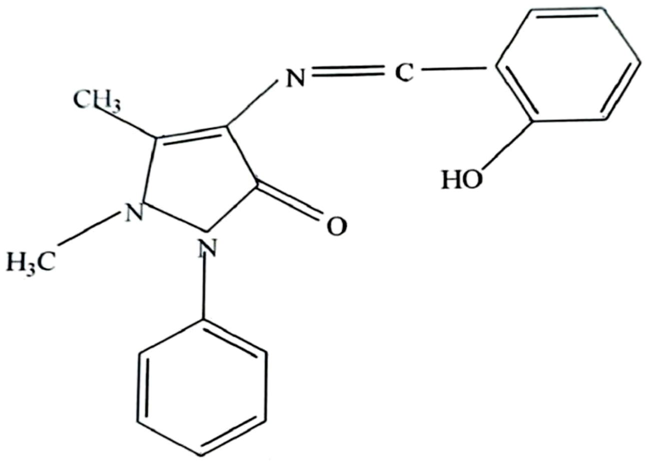
3.1.2 Solvent

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

3.2 PREPARATION OF LIGAND

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

3.2.1 Structure of ligand



3.3 PREPARATION OF COMPLEXES

The complexes are synthesized by a general method. Ethanolic or methanolic solutions of the metal salt (0.01 mol) and ligand (0.005 mol) 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 dessicator.

3.4 ANALYSIS OF COMPLEXES

3.4.1 ESTIMATION OF METAL

Estimation of Iron

Weighed out exactly 0.2 g of complex and taken in a previously weighed crucible. It was gently heated at first so that all the organic matter got burned off. Ignited strongly for about 2 hours so that it becomes red hot. Then it was cooled slowly and weighed. Iron was estimated as iron oxide.

Estimation of Chloride

The percentage of chlorine was determined by Volhard's method. The chloride complex was carefully decomposed with analar anhydrous sodium carbonate in kjeldahl's flask. The contents was extracted with water, acidified with nitric acid to decompose the carbonate and filtered free of any carbon particle[74].

3.4.2 SOLUBILITY OF COMPLEXES

Fe (III) complex was 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 complex 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 1.02 cm^{-1}.Approximately 10^{-3} M solutions were used.

3.5.2 Magnetic Susceptibility

Magnetic susceptibility of the complex was 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_0= 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 $\chi_M = \chi_g \times M$

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,

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

x_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 400-4000 cm^{-1} on Agilent Cary 630 FTIR Spectrometer at Kariavattom Campus.

3.5.4 CHNS Analysis

CHNS contents of the complex was determined by microanalytical method, using Heraeus – CHNS Rapid Analysis Instrument at STIC, Cochin.

3.5.5 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 was 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 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 complex was analyzed at Biogenics, Thiruvananthapuram.

CHAPTER 4

RESULTS AND DISCUSSION

In the present study the ligand used was a Schiff base. It was prepared from 4-aminoantipyrine and salicylaldehyde. Complex of Fe(III) was prepared using this Schiff base. Complex is an 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: $[Fe(SAAP)_2CI_2]Cl$.

Compound	Carbon %		Hydrogen %		Nitrogen %		Metal %	
	Cal	Obs	Cal	Obs	Cal	Obs	Cal	Obs
SAAP	70.56	70.64	5.26	5.18	13.27	13.65	-----	-----
$[Fe(SAAP)_2CI_2]Cl$	55.79	55.72	4.16	4.25	10.84	10.75	7.21	7.17

4.2 **Magnetic studies**

The magnetic moment value measured for Fe(III) complex is 5.3 BM . The magnetic moment value supports octahedral structure for iron complex.

4.3 **Molar conductance**

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

Complex	Molar conductance in methanol ($ohm^{-1}cm^{2}mol^{-1}$)	Assignment
$[Fe(SAAP)_2CI_2]Cl$	92	1:1 electrolyte

4.4 **UV-Vis spectra**

The ligand is characterized by two absorption bands in the UV region. A high intensity band 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 and the Complex

Compound	λ_{max}(nm)	Assignment
SAAP	398 321	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$
$[Fe(SAAP)_2CI_2]Cl$	423 354 523 552	$n \rightarrow \pi^*$ $\pi \rightarrow \pi^*$ ${}^{A}{}_{1g} \rightarrow T_{1g}$ ${}^{A}{}_{1g} \rightarrow T_{2g}$

4.5 FT-IR Spectra

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

The IR spectral data of the ligand SAAP and complex with Fe(III) is in agreement with an expected range. The band at 1490cm^{-1} in the ligand is attributed to azomethine group. This shifted to 1456 cm^{-1} in iron complex suggesting a coordination of metal ion to nitrogen of azomethine group. The band at 1650cm^{-1} in the ligand is attributed to carbonyl group. This is shifted to 1639cm^{-1} in iron complex suggesting a coordination of metal ion to oxygen of carbonyl group. The IR spectral data are as shown in table below:

Ligand ($\bar{\upsilon}$ cm^{-1})	$[Fe(SAAP)_2CI_2]Cl$ ($\bar{\upsilon}$ cm^{-1})	Assignment ($\bar{\upsilon}$ cm^{-1})
2939	2932	$\bar{\boldsymbol{\upsilon}}_{\text{C-H stch}}$
1490	1456	$\bar{\boldsymbol{\upsilon}}_{\text{C=N stch}}$
1650	1639	$\bar{\boldsymbol{\upsilon}}_{\text{C=O stch}}$
----	551	$\bar{\boldsymbol{\upsilon}}_{\text{M-O}}$
----	459	$\bar{\boldsymbol{\upsilon}}_{\text{M-N}}$

4.6 Structure of complex

On the basis of these observations and discussions it can be suggested that SAAP is acting as a bidentate ligand. The iron complex have coordination number six and assume octahedral geometry. The proposed structure for the complex is shown in Fig 4.1

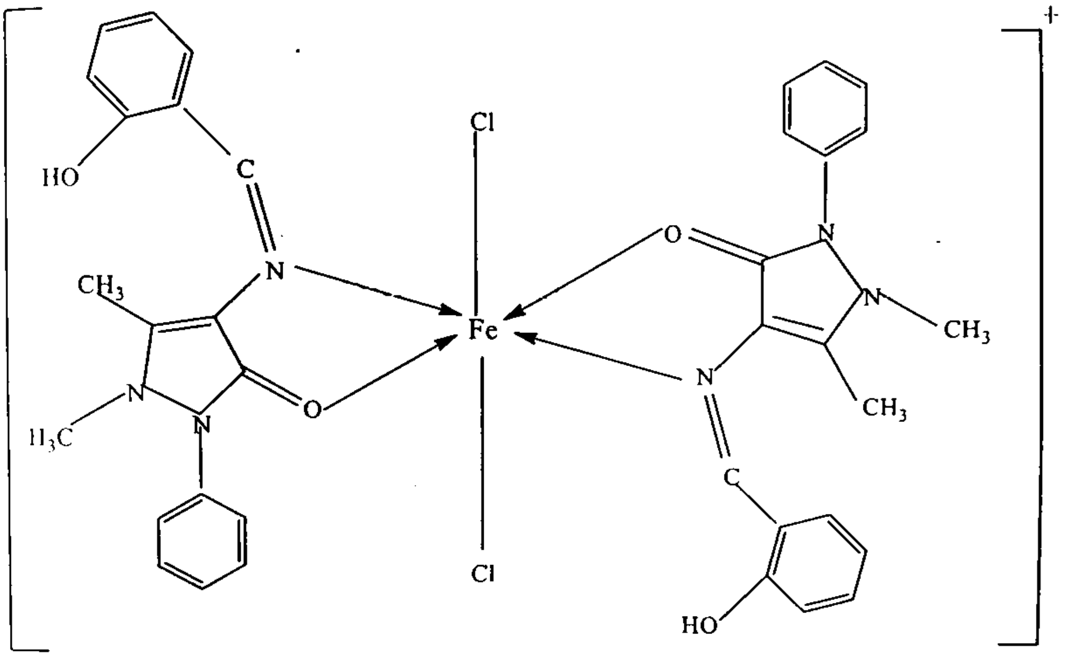


Fig 4.1 : Proposed structure for $[Fe(SAAP)_2CI_2]$

CHAPTER 5

BIOLOGICAL STUDIES

5.1 ANTIBACTERIAL ACTIVITY

The invitro biological screening effects of the investigated compound was tested against the bacteria Psuedomonas aeroginosa by using disc diffusion method by taking DMSO as solvent.

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 confluent lawn of growth. The diameter of zone of inhibition can be measured in centimeters.

REAGENTS

1.Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g 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 (25-30 ml/plate) while still molten.

2.Nutrient broth (1 L)

One litre of nutrient broth was prepared by dissolving 13g of commercially available nutrient medium (HiMedia) in 1000 ml 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:20 mg/ml)

PROCEDURE

Petriplates containing 20 ml Muller Hinton Medium were seeded with bacterial Pseudomonas aeroginosa (growth of culture according to McFards Standard) . Wells of approximately 10 mm was bored using a well cutter and 25 µl ,50 µl and 100 µl of sample was added to the well from a stock concentration of 0.1g/1ml . 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 around 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 complex is shown in the table below:

Compound	Bacterial inhibition zone(mm)
SAAP	2.0
$[Fe(SAAP)_2Cl_2]Cl$	1.2

The result indicates that the ligand and iron complex shows the antibacterial activity against Psuedomonas aeroginosa.

5.2 ANTIFUNGAL ACTIVITY

Inorder 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 antimycotic (Clotrimazole). Streptomycin was used as a positive control.

Compound	Fungal Inhibition Zone(mm)
SAAP	2.6
$[Fe(SAAP)_2Cl_2]Cl$	2.2

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

Fig 4.2: Antibacterial activity of SAAP complexes

Fig 4.3: Antibacterial activity of ligand (SAAP)

Fig 4.4 : Antifungal activity of SAAP complexes

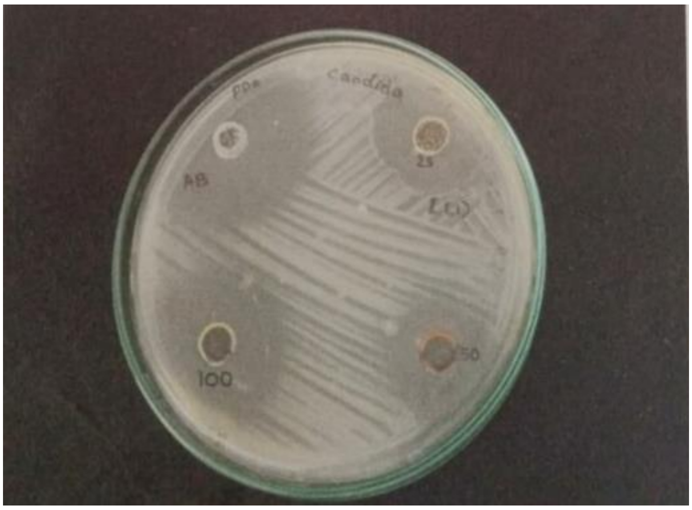


Fig 4.5: Antifungal activity of ligand (SAAP)

CONCLUSION

A Schiff base is prepared from 4-aminoantipyrine and salicylaldehyde; (SAAP). The complex of Fe(III) was prepared using SAAP. Characterization of the ligand and complex has been done on the basis of analytical and physicochemical methods. From the spectral and magnetic data it is concluded that the iron complex possess octahedral geometry having the formula $[Fe(SAAP)_2Cl_2]Cl$. The ligand and metal complex were screened for its biological activities against Pseudomonas aeroginosa and candida albicans. The ligand and the complex show better antimicrobial activity

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