SYNTHESIS, CHARACTERISATION AND BIOLOGICAL STUDIES OF MANGANESE COMPLEX

MSc. ANALYTICAL CHEMISTRY

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ABSTRACT

Schiff bases are the condensation products of primary amines with carbonyl compounds and they were first reported by Schiff in 1864. A large number of Schiff base complexes are characterized by an excellent catalytic activity in a variety of reactions at high temperature (>100ºC) and in presence of moisture. In recent years, there have been numerous reports of their use in homogeneous and heterogeneous catalysis. Transition metals are known to form Schiff base complexes and Schiff base have often been used as chelating ligands in the field of coordination chemistry. Their metal complexes have been of great interest for many years. Schiff base metal complexes have been widely studied because they have industrial, antifungal, antibacterial, anticancer, antiviral and herbicidal applications.

Schiff base is prepared from salicylaldehyde and 4-aminoantipyrine (SAAP). The complexes of Mn(II) were prepared using SAAP. The characterisation of ligand and complex is done on the basis of physic chemical methods and analytical methods. The structure of manganese complex is characterized by UV-Vis, FT-IR and conductivity test. This reports describes that the manganese complex posses octahedral geometry and having the formula $[Mn(SAAP)_2Cl_2]Cl$. The ligand and metal complexes were screened for their biological activities against Pseudonomas aeruginosa and candida albicans. Schiff base transition metal complexes have attracted wide attention due to their important role in analytical chemistry, organic synthesis, metallurgy, refining of metals, electroplating and photography.

CHAPTER 1

GENERAL INTRODUCTION

Coordination chemistry is one of the important branch in chemistry with application in almost all branches of science. The chemistry of coordination compound is an important and challenging area of modern inorganic chemistry. Research is the field of organic compound is progressing rapidly with hundreds of publications in every year.

The theoretical progress towards the structure and behaviour of organic compound could made after the discovery of electrons in 1897 which leads to the development of valence electron theory.

As early as 1798 Tassert carried out a reaction between cobalt chloride hexa hydrate $CoCl_26H_2O$ and NH_3. When he left the reaction mixture overnight in air, some orange coloured crystals were obtained. On analysis, these crystals were found to have the composition $CoCl_3.6H_2O$ [hexaammine cobalt(III)chloride].The discovery of the complex regarded as the beginning of coordination chemistry.

Alfred Werner (1866-1919), a Swiss chemist was the first to formulate his idea about the structure of coordination compounds. He prepared and characterised a large number of compounds and studied their physical and chemical behaviour by simple experimental technique. Later different theories have been developed to explain the bonding and properties of coordination compounds. The earliest of these is the valence bond theory developed by Pauling. It considers the bond formation by overlapping of hybridised atomic orbitals making use of resonant structures. The crystal field theory developed by Bethe and Van Vleck, explains the bonding in complexes. The latest development in the field is Molecular Orbital Theory considers the accurate approach to chemical bonding.

Coordination compounds are those addition compounds which retain their identities in the crystalline states as well as in solution and their properties are completely different from those of constituents. Coordination compounds find many application in electroplating, textile dyeing, and medicinal chemistry. Variety of metullargical processes, industrial calalysts and analytical reagents involve the use of coordination compounds.

Coordination compounds are of great importance in biological systems. The pigment responsible for photosynthesis, chlorophyll is a coordination compound of magnesium. Haemoglobin the red pigment of blood which act as oxygen carriers is a coordination compound of iron. Vitamin B12 is a cyanocobalamine the anti pernicious anaemia factor is a coordination compound of cobalt. So many of the catalysts are used in chemical industry make use of coordination compounds. And also coordination compounds are used in the field of medical diagnosis and are used as therapeutic agents. In recent years a large number of transition metal complexes are used as non linear optical material.

Coodination chemistry is an emerging field of chemistry in which it has high scope in the field of solid state chemistry, photonic material and extended microscopic material.

1.1 SCHIFF BASE

A Schiff base (Named after Hugo Schiff) is a compound with a general formula $R_1R_2C=NR_3$. They can be considered as a sub class of imines, being either secondary ketimines or secondary aldimines depending on their structure. The term is often synonymous with azomethine which refers specifically to secondary aldimines.

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

Schiff base are aldehyde or ketone compounds where the carbonyl group is replaced by an azomethine or imine group.

Schiff bases are formed when the primary amine is react with a ketone or an aldehyde under specific conditions. That means Schiff base is a nitrogen analogue of ketone or aldehyde in which the carbonyl group is replaced by an imine group. The synthesis of Schiff base occurs when the carbonyl group undergo condensation with the help on an imine under the distillation of azeotropic and to remove water formed in the system, molecular sieves are used.

Schiff bases have wide applications in the field of food industry, analytical chemistry, dye industry, fungicidal agrochemicals, catalysis, pharmaceutical field and biological activities. Not only have they played a seminal role in development of coordination chemistry but also they have an important role in the field of inorganic biochemistry, optical materials etc.

The important application of Schiff base in the field of catalysis include Fe(III), Co(III) and Ru(III) complexes 0f Schiff base derived from hydroxy benzaldehyde are used in oxidation of cyclohexanone in presence of hydrogen peroxide. The cobalt complexes are known to bind dioxygen more or less reversibly and therefore used to study as model compounds for natural oxygen carrier and for their use in organic synthesis due to their catalytic properties under mild condition.

The biological applications of Schiff base include antimalarial activity, antibacterial activity and antifungal activity etc. Schiff bases with 2,4-dichloro-5-fluorophenyl moiety have been proved to resist the growth of fungi like pencillium maeneffei, trichophyton mentagrophytes and aspergillus flavus. Schiff base derived from indoline-2,3-dione and 2- aminobenzoic acid and its tin complex showed antibacterial activity against Staphylococcus aureus. Schiff base also have high antiviral activities it include silver complexes in oxidation state 1 showed inhibition against cucumber mosaic virus. Schiff bases have emerged as promising antimalarial agents.

Schiff bases and their metal chelates have been used in dye and pigment industry, due to their colour. The aromatic Schiff bases have been used as stabilisers for variety of compounds which include fuel oils, lubricating oils, jet fuels etc. Schiff base are used to synthesise several polymers to produce some desirable characteristics include superconductivity, heat resistance and resistance towards oxidation, light etc. Organocobalt complexes with tridentate Schiff base act as an initiator of emulsion polymerisation and copolymerisation of dienyl and vinyl monomers. The hydroxyl azomethine compounds used as luminescent reagent and as indicators in complexometric titrations.

Schiff bases are applied in different areas such as electronic chemistry, bioinorganic chemistry, metallic deactivators, catalysis, separation process and environmental chemistry. Schiff base complex continue to attract many researchers because of its wide applications in various industries and analytical fields.

1.2 CHEMISTRY OF MANGANESE

Manganese is a **pinkinsh-gray, chemically active element**. It is a hard metal and is very brittle. It is hard to melt, but easily oxidized. Manganese is reactive when pure, and as a powder it will burn in oxygen, it reacts with water (it rusts like iron) and dissolves in dilute acids.

Manganese is a chemical element with symbol Mn and atomic number 25. Electronic configuration of Mn is $[Ar]3d^5 4s^2$. In its compounds Mn exhibits oxidation state from +2 to +7. The common oxidation states are +2, +4 and +7. Mn(II) is the most stable state. In acidic or neutral solutions it exist as $[Mn(H_2O)_6]^{2+}$, a pink hexa aqua ion, which is resistant to oxidation and it exists in basic medium as $Mn(OH)_2$ and is very easily oxidised even by air.

Mn is essential to iron and steel production. It is a key component of low cost stainless steel formulations and certain widely used aluminium alloys and it is used to decolourise glass and make violet coloured glass.

Mn(II) COMPLEXES

Although the normal coordination number of Mn(II) is 6S and it is not split in the ligand field but transforms in $^6A_{1g}$. This is the only sextuplet state possible. Excited states are quartet and doublet states. Therefore all transitions to higher states are spin and partially forbidden in octahedral fields. But due to weak spin orbit coupling these transitions do occur with weak intensity. This accounts for the pale pink colour of Mn complexes. In the tetrahedral environment transitions are still spin forbidden but no longer parity forbidden.

The range of polar absorbance of tetrahedral complex is 1 to 4 and octahedral complex is 0.01 to 0.04. The reason is due to mixing of p and d orbitals in the tetrahedral environment which is participated by the overlap of metal d orbital in the tetrahedral complexes.

Mn(II) have a t_{2g}^5 configuration when $\Delta_0$is high. This give rise to doublet ground state. For Mn(II) pairing energy is very high and only few low spin complexes such as $[Mn(CN)_6]^{4-}$, $[Mn(CN)_6(NO)]^{3-}$ etc are known.

The complex anions of Mn(II) includes MnX_3^- (octahedral with pyrovskite structure), MnX^{2-}(tetrahedral or polymeric octahedral with halide bridges) and $MnCl_6^{4-}$(octahedral). Acetonyl acetates and and alkoxides of Mn(II) polymerise to increase the coordination number. $Mn(acac)_3$ is trimeric. The structure of thiolates of Mn(II) have also been studied in detail. Recently several Schiff base complexes are also reported. Among the cyclopentadienyl of Mn(II) the most interesting is $[MnC_5H_4]_2Mn$, since it exhibits low spin-high spin crossover behaviour.

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. 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. The function of metals in metalloproteins, their participation in respiratory, photosynthesis, biosynthetic, nitrogen fixation and metabolic processes are essential to the foundation of life. Metal ions are required for many critical functions in humans.

Scarcity of some metal ions can lead to diseases, well known examples are pernicious anaemia resulting from iron deficiency and growth retardation.

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 0r structural effect that increases localisation of negative charge on the donar 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 multidentate ligands. Metal complexes and complex formers are used for detoxification in the case of metal poisoning.

Many organic chelating ligands have very special clinical role in handling the toxic metal ions in human. 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 metal. This property depends on soluble, easily excretable chelates by sequestering metal ion in the circulation or competing with chelating biological sites for the bound metal ions.

Metal complexes having radioactive nuclei find many applications in medicine, such as in tumors, organ and tissue imaging. Metal ions and humic substances present in water bodies complex themselves rendering the biota nontoxic. 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. Metallofullerenes made of carbon clusters that contain lanthanides trapped in fullerene cage are used for the treatment of secondary tumor cells. The technique of locating protein binding site on DNA known as ‘chemical foot printing’ demands the requirement of metal complexes. More over cisplatin and carboplatin are biologically active metal complexes of platinum, the most widely used anticancer agents in the world.

CHAPTER II

REVIEW OF LITERATURE

After a brief historical introduction, this manuscript is divided in three main sections. In the two first parts, the synthesis, reactivity, functions, and properties of tridentate Schiff base precursors and of quadridentate Schiff base metal complexes, respectively, are discussed through a literature survey including examples Co, Ni, Cu, Zn, Ru, Pd, Pt) and uranyl complexes. Emphasis is given to our research work based on ferrocenyl-containing tri- and tetradentate unsymmetrically-substituted Schiff base complexes of Ni(II) and Cu(II) starting from variously substituted ferrocenyl-*β*-diketones. The unsymmetrically-substituted Schiff base complexes present a wide range of remarkable al, biocidal, magnetic, and second-order nonlinear optical properties. The third section is devoted to the catalytic activity of Schiff base metal complexes that is discussed through thirteen major organic reactions, including copper-catalyzed azide–alkyne cycloaddition (CuAAC), Henry and nitro-Mannich reactions, hydrosilylation of ketones, aldol, cyclopropanation and epoxidation reactions, among others.

Neutral complexes of Mn(II), Cu(II) and Co(II) have been synthesised from the Schiff base 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, NMR and ESR spectral data. The nonelectrolytic and monomeric nature of the complex is evidenced by their magnetic susceptibility and low conductance data.

Schiff bases derived from thiophene carboxaldehyde and aminobenzoic acid , furaldehyde, benzimidazole , thiazole , furfuraldiamine , pyridine and benzyldithiocarbazate , pyrazolone , glucosamine , hydrazide , p-fluoro benzaldehyde , p-anisidiene , thiosemicabazones , and imidazolinones show antibacterial activity.

Desai et al. have been reported some oxime, semi carbazone, and thiosemicarbazones by using 2,4-dihydroxyacetophenone, 2,4-dihydroxy-5-nitroacetophenone, and 2,4-dihydroxy-5-bromo-acetophenone and were evaluated for their inhibitory effect against *S. aureus* and *E. coli* by agar diffusion technique. All the compounds exhibited great activity against Gram -ve bacteria, *i.e. E. coli* and poor activity against Gram +ve bacteria, *i.e. S. aureus*.

Mohmed et al. have reported some Fe (III), Cu (II), Zn (II), and UO2 (II) complexes of Schiff base obtained from 2-thiophene carboxaldehyde and 2-aminobenzoic acid and were tested for their antibacterial activity against bacterial species, *E. coli*, *P. aeruginosa*, *Streptococcus* pyogenes, and fungi (Candida). It is found that metal complexes are more effective against some bacterial species (Gram-positive and Gram-negative bacteria) as compared with the parent Schiff base ligand. It is also reported that Fe (III), Cu (II), Zn (II), and UO2 (II) complexes inhibited the growth of *E. coli* and could be employed in the treatment of common diseases such as septicemia, gastroenteritis, and urinary tract infections caused by *E. coli*. A series of Cu (II), Zn (II), and Ni (II) complexes of Schiff base ligands DAPY-{SalH} 42, DAPY-{SalH}2 43, DAPY-{4-OHBenz} 44 and DAPY-{4-NO2Benz} 45 were synthesized from 2,3-diaminopyridine (DAPY) and different aldehydes; salicylaldehyde (SalH), 4-hydroxy-benzaldehyde (4-OHBenz) and 4-nitro-benzaldehyde(4-NO2Benz) have been reported by Jeewoth et al. .

2,3-diamino-pyridine is significantly does not show activity against *P. aeruginosa*, *S. typhi*, *E. coli,* and *S. aureus,* but its Schiff base ligands have been found more effectual against these bacteria. The Cu (II) and Zn (II) complexes of the Schiff base DAPY-{4-NO_2Benz} show antibacterial activities against *P. aeruginosa* and *S. typhi*.

N.Raman 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, redox, structural characteristics, antimicrobial and DNA cleavage) of the metal complexes with heterocyclic Schiff bases of 4-aminoantipyrine with some aldehydes and oximes.

Hassan Keypour synthesized two potentially heptadentate (N4O3) tripodal Schiff base ligands: tris(3-(salicyldeneimino)propyl)amine and tris(3-(4-hydroxysalicylideneimino)-propyl)amine and have been prepared and characterised by various spectroscopic methods.

A novel Schiff base ligand of type HL was prepared by the condensation of amoxicillin trihydrate and nicotinaldehyde. The metal complexes of Co^{+2}, Ni^{+2}, Cu^{+2}, and Zn^{+2} were characterized and investigated by physical and spectral techniques, namely, elemental analysis, melting point, conductivity, ^{1}H NMR, IR, UV-Vis spectra, ESR, SEM, and mass spectrometry measurements. They were further analyzed by thermal technique (TGA/DTA) to gain better insight about the thermal stability and kinetic properties of the complexes. Thermal data revealed high thermal stability and nonspontaneous nature of the decomposition steps. The Coats-Redfern method was applied to extract thermodynamic parameters to explain the kinetic behavior. The molar conductance values were relatively low, showing their nonelectrolytic nature. The powder XRD pattern revealed amorphous nature except copper complex (1c) that crystallized in the triclinic crystal system. The EPR study strongly recommends the tetrahedral geometry of 1c. The structure optimization by MM force field calculation through ArgusLab 4.0.1 software program supports the concerned geometry of the complexes. The in vitro antibacterial activity of all the compounds, at their two different concentrations, was screened against four bacterial pathogens, namely, *E. coli, P. vulgaris, K. pneumoniae, and S. aureus,* and showed better activity compared to parent drug and control drug.

Over the last few years, many Schiff base complexes of metal ions have exhibited substantial catalytic activity in various reactions such as oxidation, Henry reaction, hydroxylation, aldol condensation, and epoxidation. The literature survey shows that, over the past few years, there have been few reports on the catalytic aldol and Henry reaction where Schiff base-based metal complexes act as homogeneous or heterogeneous catalysis. This review reorganizes the various transition metal Schiff base complexes as well as chiral Schiff base metal complexes, which have potential application in catalytic activity on aldol and Henry reaction. Schiff bases are an important class of chelating ligands due to its coordination tendency and their complexes exhibit a wide range of applications in various fields like anti-bacterial, anti-fungal, antiviral, anti-cancer, antioxidant, anti-inflammatory, magnetism, luminescence, conductivity, sensing, catalytic, and so on. This review mainly focuses on the catalytic activities of Schiff base ligands and their transition metal complexes to the aldol and Henry reaction.

The interaction of ethanolic solution of semi carbazide hydrochloride, benzaldehyde and morpholine yielded N-(1-Morpholinobenzyl) Semi carbazide Schiff base was studied by H.N. Aliyu and H. Adamu. The Schiff base complexes of Mn (II) and Fe (III) were synthesised by refluxing the mixture of ethanolic solutions of the Schiff base and metal (II)chlorides, respectively. These complexes were characterised by gravimetry, spectrophotometry, potentiometry, molar conductance and infrared analysis. The Schiff base and the complex compounds are not soluble in methanol and ethanol. The molar conductance of the Mn (II) and Fe (III) Schiff base complexes are measured are indicating their non electrolytic nature. The ratio of metal ion to Schiff base is determined potentiometrically and spectrophotometrically.

AIM AND SCOPE OF THE PRESENT INVESTIGATIONS

The present work include the synthesis and characterization of complexes of 4-aminoantipyrine with metal ion Mn (II). The complexes were analysed 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 salt, ligand, complex, purification methods, characterisation of the ligand, details of various reagents used and methods used for the analysis of complex. Physico-chemical measurements and various instrumentation techniques employed are also discussed.

3.1 REAGENTS

3.1.1 Metal salts

In the present investigation manganese acetate is the transition metal used which is of AR quality.

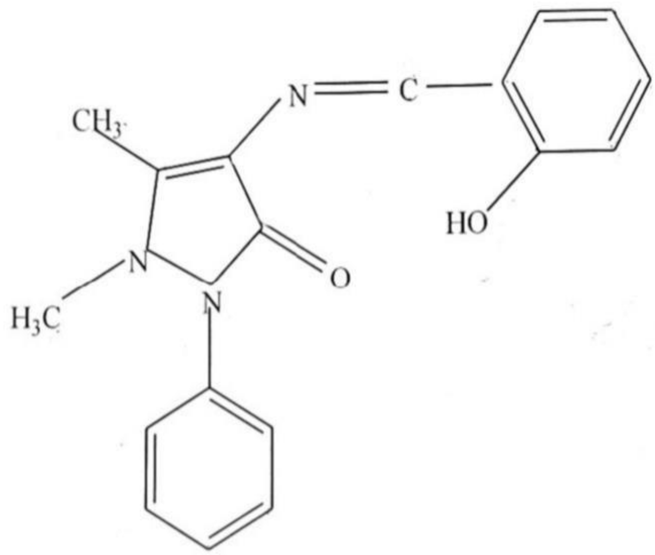
3.1.2 Solvents

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) for 3 hours. The resultant solution was cooled to room temperature. The yellow solid formed was filtered, repeatedly washed with ethanol and recrystallised from ethanol.

3.2.1 Structure of ligand



3.3 PREPARATION OF COMPLEX

The complex is synthesised by a general method. Ethanolic solutions of the metal salt (0.01 mol) and ligand (0.005mol) are mixed in the ratio 1:2. The ligand solution is added gradually in small portions with 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 complex formed was filtered, washed with ethanol to remove excess ligand. It was then dried in vacuum desicator.

3.4 ANALYSIS OF COMPLEX

3.4.1 ESTIMATION OF METAL

Estimation of manganese

About 0.2 g of manganese complex was digested with conc. sulphuric acid and nitric acid in a Kjeldahl's flask. The resulting clear solution was evapourated to dryness and the residue obtained was extracted with water. The aqueous solution thus obtained was diluted to 50 ml in a beaker and excess acid was neutralized by adding ammonium hydroxide solution. To this add 20g of ammonium chloride and excess of $(NH_4)_2HPO_4$ (2g solid). If a precipitate forms at this point dissolve it by addition of few drops of 1:3 HCl. Heat the solution to boiling and add 1:3 NH_3 solution dropwise and with constant stirring until a precipitate $Mn_3(PO_4)_2$ begins to form. Continue heating and stirring until precipitate becomes crystalline $MnNH_4PO_4.H_2O$. Then add ammonia solution and stir until no precipitate formed and its silky appearance remain unchanged, excess ammonia avoided. Allow solution to stand at room temperature for 2 hours. Filter through filter paper and washed with cold ice and NH_4NO_3 till free from chlorine. Then ignited in crucible and weighed.

3.4.2 SOLUBILITY OF COMPLEXES

Mn(II) complexes are 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 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 were measured at room temperature on a Sherwood Scientific Meter. The gram susceptibility was determined from the equation

$$\chi_g = L_x(R-R_0)/m \times 10^9$$

R- reading with sample R_0-reading with empty tube

L-length of sample column w-weight of sample

The molar susceptibility, $\chi_m = \chi_g \times M$

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

The effective magnetic moment was calculated using the formula

$$\mu_{eff} = 2.84(\chi_m^{corr} \times T)^{1/2}$$

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 fingerprint for the identification and a powerful tool for studying the molecular structure.

The IR spectra of complex was 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 micro analytical 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 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 complex> free ligand

This order clearly indicates that the uncomplexed metal salt have greater ability to form the compound-organism complex. Higher activity of the metal chelates is due to 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 IV

RESULTS AND DISCUSSION

In the present study the ligand used was a Schiff base. It was prepared from 4-aminoantipyrine and salicylaldehyde. Complexes of Mn (II) were prepared using Schiff base. Complex was amorphous solid and was 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 formula for the complex: $[Mn(SAAP)_2(AC)_2]$.

Compound	Carbon%		Hydrogen%		Nitrogen%		Metal%	
	Cal	Obs	Cal	Obs	Cal	Obs	Cal	Obs
SAAP	70.56	70.64	5.26	5.18	13.72	13.65	-------	-------
$[Mn(SAAP)_2(Ac)_2]$	61.14	61.07	4.87	4.93	10.69	10.62	6.99	8.38

4.2 Magnetic studies

The magnetic moment value measured for Mn(II) complex is 5.9 BM. The magnetic moment value support the octahedral structure of manganese complex.

4.3 Molar conductance

The molar conductance of the complex (10^{-3} molar concentration) was carried out in methanol indicated that manganese complex is a non electrolyte. The value is presented in the table below:

Complex	Molar conductance in methanol($ohm^{-1}cm^{2}mol^{-1}$)	Assignment
$[Mn(SAAP)_2(Ac)_2]$	34	Non electrolyte

4.4 UV-Vis spectra

The ligand is characterised by two absorption bands in the UV region. A high intensity band at 321 cm^{-1} is attributed to $\pi\rightarrow\pi^*$ transition and a lower intensity band at 398 cm^{-1} is attributed to $n\rightarrow\pi^*$ transition of azomethine group. The absorption band in the complex is shifted to longer wavelengths compared to that of 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	n→π*
	321	π→π*
$[Mn(SAAP)(Ac)_2]$	413	n→π*
	316	π→π*
	550	d-dtransition(weak shoulder)

4.5 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.

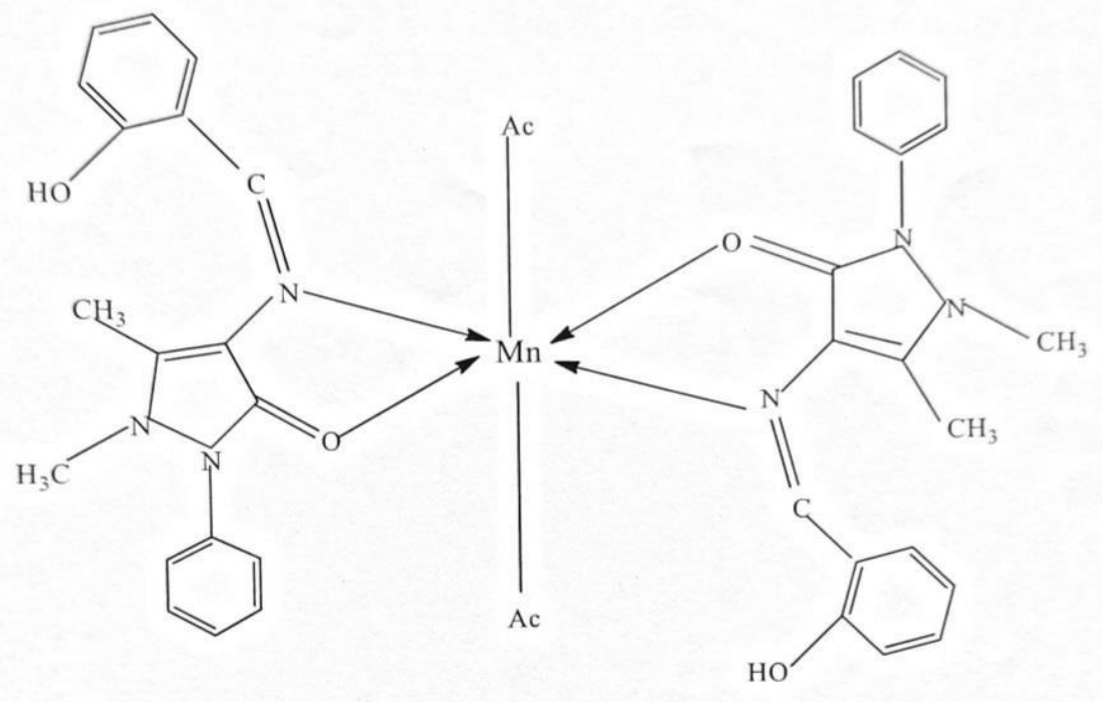
The IR spectral data of ligand SAAP and complex with Mn(II) are in agreement with an expected range. The band at 1490 cm^{-1} in the ligand is attributed to azomethine group. This is shifted to 1468 cm^{-1} in the manganese complex suggesting a coordination of metal ions to nitrogen of azomethine group. The band at 1650 cm^{-1} in the ligand is attributed to carbonyl group. This is shifted to 1674 cm^{-1} in manganese complex suggesting a coordination of metal ions to oxygen of carbonyl group.

The IR spectral data are as shown in table below:

Ligand (υ cm^{-1})	$[Mn(SAAP)_2(Ac)_2]$ (υ cm^{-1})	Assignment (υ cm^{-1})
2939	Ligand	$\bar{\upsilon}_{C\text{-}H\ stch}$
1490	1468	$\bar{\upsilon}_{C=N\ stch}$
1650	1674	$\bar{\upsilon}_{C=O\ stch}$
-----	534	$\bar{\upsilon}_{M\text{-}O}$
-----	467	$\bar{\upsilon}_{M\text{-}N}$

4.6 Structure of complex

On the basis of these observations and discussion it can be suggested that SAAP is acting as a bidentate ligand. The manganese complex have coordination number 6 and assume octahedral geometry. The proposed structure for the complex are shown below:



Proposed structure of $[Mn(SAAP)_2(Ac)_2]$

CHAPTER V

BIOLOGICAL STUDIES

5.1 ANTIBACTERIAL ACTIVITY

The invitro biological screening effects of the investigated compounds were tested against the bacteria Pseudomonas 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 a confluent lawn of growth. The diameter of zone of inhibition can be measured in centimeters.

REAGENTS

1. Muller Hinton Agar Medium (1L)

The medium was prepared by dissolving 33.3g of the commercially available Muller Hinton Medium (HiMedia) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121ºC for 15 minutes. The autoclaved medium was mixed well and poured on to 100 mm pertiplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

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 (121ºC) for 15 minutes.

3. Streptomycin (standard antibacterial agent)

PROCEDURE

Petriplates containing 20 ml Muller Hinton medium were seeded with bacterial pseudomonas aeruginosa (growth of culture according to McFards Standard). Wells of approximately 10mm was bored using a well cutter and 25µl, 50µl and 100µl of sample was added to the well from a stock concentration of 0.1g/ml. The plates were then incubated at 37ºC for 24 hours. The bacterial 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 favours the passage of only lipid soluble materials due to which lipo solubility is considered to be an important factor that controls the antimicrobial activity. The inhibition zone of antibacterial activity of SAAP and its complex are shown in the table below:

Compound	Bacterial inhibition (mm)
SAAP	2.0
$[Mn(SAAP)_2(Ac)_2]$	1.3

This result indicates that the ligand shows the highest antibacterial activity against Pseudomonas aeruginosa and manganese complex shows satisfactory antibacterial activity.

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 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 combined with that of standard antimycotic (Clotrimazole). Streptomycin was used as a positive control.

Compound	Fungal inhibition zone (mm)
SAAP	2.6
$[Mn(SAAP)_2(Ac)_2]$	2.0

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

Fig 4.6 Antibacterial activity of SAAP complexes

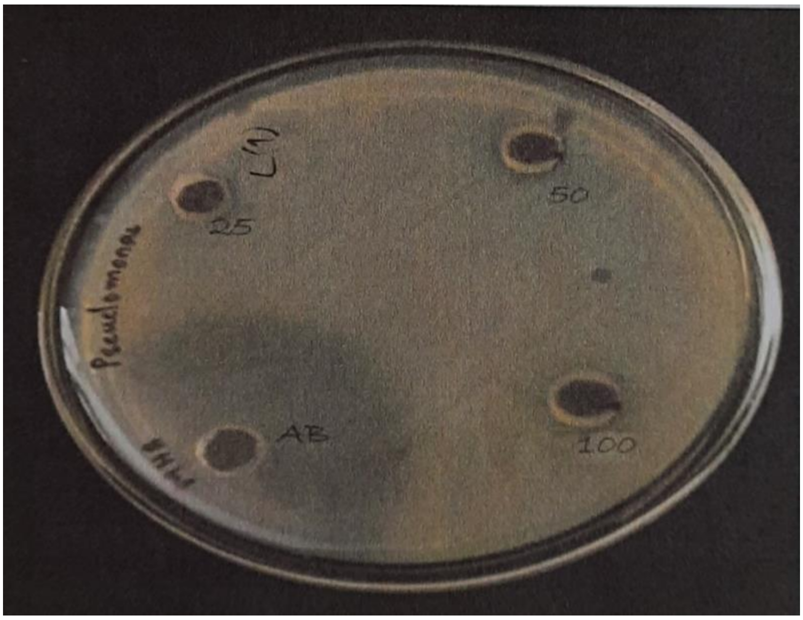


Fig 4.7 Antibacterial activity of ligand (SAAP)

Fig 4.8 Antifungal activity of SAAP complexes

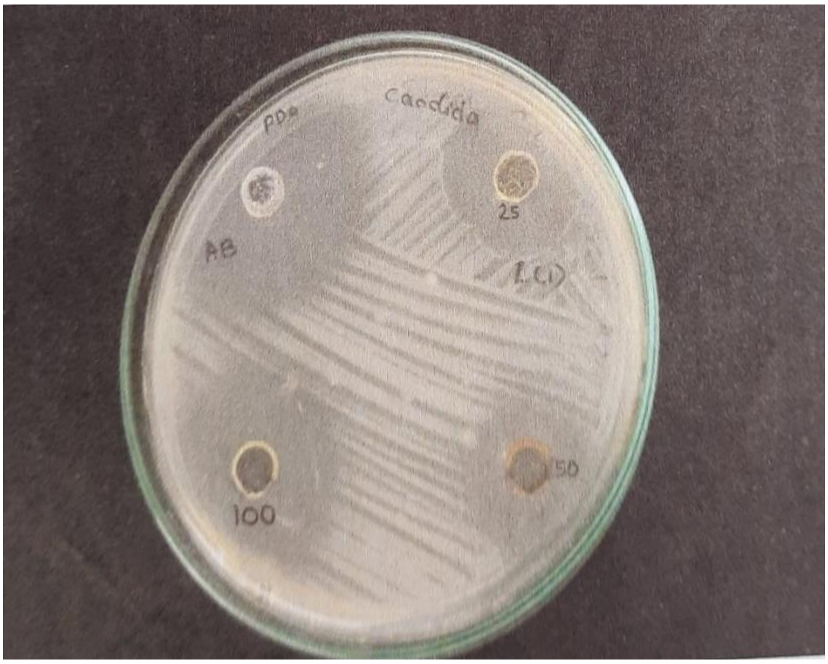


Fig 4.9 Antifungal activity of ligand (SAAP)

SUMMARY

A Schiff base is prepared from 4-aminoantipyrine and salicylaldehyde;(SAAP). The complex of Mn(II) were prepared using SAAP. Characterization of the ligand and complex have been done on the basis of analytical and physic chemical methods. From their spectral and magnetic data it is concluded that the manganese complex possess octahedral geometry having the formula $[Mn(SAAP)_2(Ac)_2]$. The ligand and metal complex was screened for their biological activities against Pseudomonas aeroginosa and candida albicans. The ligand and the complex shows better antimicrobial activity.

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