SYNTHESIS, STRUCTURAL, ANTITUMOUR AND ANTIBACTERIAL STUDIES ON SOME TRANSITION METAL COMPLEXES OF SCHIFF BASES

Thesis submitted to the University of Calicut,
in partial fulfilment of the requirements
for the award of the degree of
DOCTOR OF PHILOSOPHY
In
CHEMISTRY

By

SREE KUMAR P.K. **M.Sc., M.Phil.**

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF CALICUT
KERALA – 673 635
INDIA

NOVEMBER 2007

Dr. GEETHA PARAMESWARAN
Professor (Rtd.)
Department of Chemistry
University of Calicut

Calicut University P.O.
Kerala – 673 635

CERTIFICATE

This is to certify that the thesis entitled "**SYNTHESIS, STRUCTURAL, ANTITUMOUR AND ANTIBACTERIAL STUDIES ON SOME TRANSITION METAL COMPLEXES OF SCHIFF BASES**" is an authentic record of the research work carried out by **Mr. SREE KUMAR P.K.**, under my supervision in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry of the University of Calicut, and further that no part thereof has been presented before for any other degree.

C.U. Campus
1st November 2007

Dr. Geetha Parameswaran
(Supervising Teacher)

DECLARATION

I hereby declare that the thesis entitled "**SYNTHESIS, STRUCTURAL, ANTITUMOUR AND ANTIBACTERIAL STUDIES ON SOME TRANSITION METAL COMPLEXES OF SCHIFF BASES**" submitted to the University of Calicut in partial fulfilment of the requirements for the Doctoral Degree in Chemistry is a bonafide research work done by me under the supervision and guidance of **Dr. Geetha Parameswaran**, Professor (Rtd.), Department of Chemistry, University of Calicut.

I further declare that this thesis has not previously formed the basis of any degree, diploma or other similar title.

C.U. Campus **SREE KUMAR P.K.**
1st November 2007

ACKNOWLEDGEMENT

I have no words to express my sincere thanks and gratitude to
Dr. Geetha Parameswaran, Professor (Rtd.), Department of Chemistry, University of Calicut, who as my teacher and guide, gave constant encouragement and whole-hearted support at all stages of this research work. I gratefully acknowledge the inspiring guidance, pertinent criticism, pregmatic suggestions and excellent hospitality extended to me during this tenure.

I express my profound gratitude to Dr. K.K. Krishnan Kutty, Professor and Head of the Department of Chemistry and former heads Dr. M.P. Kannan and Dr. K.K. Aravindakshan for having provided me with all the facilities to carry out this research work.

I sincerely appreciate the wholehearted co-operation and valuable help rendered by the teaching and non-teaching staff of Department of Chemistry, University of Calicut.

I am gratefully indebted to Dr. Prabha Balaram, Professor and Head, Division of Cancer research, RCC, Thiruvananthapuram, Suraj K. George and K.M. Sathyan (Senior Research Fellows, RCC, Tvm) for providing me adequate facilities and valuable suggestions to conduct antitumour studies.

I wish to extend my thanks to Dr. V. Thankamani, Professor and Head, Department of Biotechnology,

University of Kerala, for providing me, facilities to conduct anti bacterial studies on some selected complexes.

It is my pleasure to thank the authorities of SAIF Madras, SAIF Kochi, IIT Bombay and IICT Hyderabad, RCC Tvm, NIT Calicut and DDRC Tvm for providing necessary instrumental facilities and micro analysis of samples.

I am extremely thankful to a host of friends and fellow Research Scholars at Calicut University for their sincere co-operation and providing a friendly atmosphere during the course of this work.

With pleasure I acknowledge the assistance rendered by Dr. G. Madhu Kumar (Principal, NSS College Vazhoor) Dr. G. Unnikrishnan (NIT Calicut) and Arun V. (Research Scholar, CUSAT).

With heartfelt gratitude, I record my obligation to the NSS Management for granting permission to do my research work. I am also thankful to the Principal and staff of NSS College Pandalam and NSS College Manjeri for their help at different levels and words of encouragement.

My special thanks are due to University Grants Commission of Award of Teacher Fellowship under Faculty Improvement Programme.

I owe my debt of gratitude to Mr. Balu of Bina Photostat, Chenakkal and Mr. Shiju Jacob, Major Computers, Kottarakkara for their timely help in the neat

execution of data and printing of this thesis in a beautiful way.

Acknowledgements seems to be incomplete with out a word of thanks and appreciation to my family and to my Guru Acharya Yogananda Nath whose co-operation, patience and prayers helped me to materialise my dream of a thesis in to reality.

Above all, I thank God Almighty, who enabled me with philosophy, perception and motivation to present this work, after so many hurdles and obstacles. May THOU help all at times of need and make us obligated.

SREE KUMAR P.K.

PREFACE

In recent years the field of Coordination chemistry has experienced an impressive renaissance. Academic and industrial research in coordination chemistry is flourishing and the output of research papers and reviews is growing exponentially. In addition to the various applications in the applied science 'Complex Chemistry' plays vital roles in the chemistry of living matter.

Schiff bases have attracted considerable attention in terms with their chelating abilities and analytical applications. Their complexes coordinate through nitrogen atom of the azomethine group (>C=N) to the metal ion. These metal complexes could attain extra stability by keeping functional groups with replaceable hydrogen atoms such as –OH, -SH, etc, near enough to the azomethine group.

In the present investigation, bivalent tridentate and tetradentate Schiff base ligands and their transition metal complexes are prepared and characterized on the basis of elemental analysis, magnetic and conductance measurements, UV-Visible, IR, NMR and Thermogravimetric data. These results are summarized and presented in Part I. The present study describes mainly on the metal complexes of Schiff bases derived from a hydroxy ketone and a diketone. In this work some new ligands

namely

2-Hydroxy acetophenone 2-Aminothiophenol (HAPATP) or H_2L^1, 2-Hydroxyacetophenone 2-Aminophenol (HAPAP) or H_2L^2, Benzil 2-Aminothiophenol (BATP) or H_2L^3, Benzil 2-Aminophenol (BAP) or H_2L^4 and their transition metal complexes has been synthesized and characterized. Co(II), Ni(II), Cu(II) and Zn(II) are the metal ions used for the complexation.

Thermoanalytical studies of eight selected Schiff base complexes are carried out using TG technique. Valuable informations such as, temperature regions of stability, temperature of the completion of decomposition are evaluated from the TG curves. The results are interpreted in Part II. Thermal data further confirms the structure of the above complexes.

Part III and Part IV consist of biological studies of some selected metal complexes. In Part III antitumor studies of the new ligands and their metal complexes are described. The studies include *invitro* cytotoxicity, *invivo* cytotoxicity and tumor reduction experiments in BALB/c mice. The materials, methods and instruments used for the antitumor study are described in this part.

Part IV consists of antibacterial studies of some selected ligands and their metal complexes against some clinically important bacterial stains namely, *Escherichia coli*, *Staphylococcus*

aureus, *Pseudomonas aeruginosa* and *Proteus vulgaris*. The materials, methods and instruments used for the antibacterial screening study are described in this part. Part IV ends with a summary.

For the sake of brevity, symbols and formulae instead of names have been used in this thesis, which is given in the Abbreviations at the starting of thesis. A detailed list of references arranged in serial order is given at the end of each part.

The research work presented in this thesis has partly been published/ under publication as indicated below.

1. **Preliminary Antitumour studies on transition metal complexes of Schiff bases**:- Sree Kumar P.K., Suraj K. George, Sindhu Ramachandran C.V., Geetha Parameswaran and Prabha Balaram- Proceedings of the *26th Annual Convention of Indian Association of Cancer Research and International Symposium on Translational Research in Cancer* – Bhubaneswar, January 2007.

2. **Antitumour screening of Cu(II) and Zn(II) complexes of Schiff bases, on Tumour cells lines**:- Sree Kumar P.K., Suraj K.George, Sindhu R., G. Parameswaran and P. Balaram- Proceedings of the *International Conference on Materials for the Millennium* (Matcon, 2007) – CUSAT, Kochi, March 2007.

3. Synthesis, Biological, Spectral and Thermal studies of Co(II) and Zn(II) complexes of some new Benzil Schiff bases (communicated to *Asian Journal of Chemistry*.

4. Synthesis and Characterization of Cu(II) and Zn (II) metal complexes of a bivalent tridentate Schiff bases derived from 2-Hydroxy Acetophenone2-Aminothiphenol (communicated to *Journal of Indian Chemical Society*) .

5. Role of Zinc and Copper chelation in apoptosis mediated by 2-Hydroxyacetophenone 2-Aminothiphenol (communicated to *Biochem. Pharmacol*.).

ABBREVIATIONS

For the sake of easiness in description, the following abbreviations are used in this thesis.

ADI	-	Average daily intake
BAP	-	Benzil 2-Aminophenol
BATP	-	Benzil 2-Aminothiophenol
BM	-	Bohr Magneton
DLA Cells	-	Dalton’s lymphoma ascites cells
DMEM	-	Dulbecco’s Modified Eagle’s Medium
FCS	-	Fetal Calf Serum
HAPAP	-	2-Hydroxy acetophenone 2-Aminophenol
HAPATP	-	2-Hydroxy acetophenone 2-Aminothiophenol
HeLa Cells	-	Henrietta Lacks Cells
hrs	-	Hours
IC_{50}	-	Inhibitory concentration at 50%
ILS	-	Increase in life span
Ip	-	Intraperitonial
L	-	Deprotonated ligand molecule
LD_{50}	-	Lethal dose at 50%
M	-	Central metal atom
MTS	-	3-(4,5-dimethyl thiazol-2-yl)-5-(3-carboxy methoxy phenyl) – 2-(4-sulphophenyl)-2H-tetrazolium inner salt

MTT	-	3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NK Cells	-	Natural Killer Cells
PBS	-	Phosphate buffered saline
PMS	-	Phenazine metho sulfate
ppm	-	parts per million
TC	-	Total Count
TMC	-	Transition metal complexes.
TRIS	-	Tris hydroxy methyl amino methane
μg	-	Micrograms (10^{-6} g)

CONTENTS

PART -I

SYNTHESIS AND CHARACTERISATION

PART -II

THERMO GRAVIMETRIC STUDIES

PART -III

ANTI TUMOUR STUDIES

PART -IV

ANTI BACTERIAL STUDIES

CHAPTER I

INTRODUCTION

The field of coordination chemistry is one of the most intellectual, attractive and experimentally demanding frontiers in modern chemical sciences. It has grown in a half century from a readily defined and limited area into the most active research field of inorganic chemistry. Coordination compounds brought about a synthetic revolution in inorganic chemistry which leads to novel products of equally novel applications in wide range of areas such as analytical chemistry, fungicides, paints, pigments, polymers, pharmaceuticals, catalysis, and photoconductors. Complexation reactions are used in qualitative as well as in quantitative analysis. There are some extremely sensitive and selective organic reagents for the determination of metal ions. The role of coordination compounds in colorimetric, spectrophotometric and polarographic analysis is also significant.

Transition metal complexes acts as catalyst in many industrial processes like Wacker process, Oxoprocess, Monsanto processes etc. Many enzymes contain a small prosthetic group which is usually a complexed metal ion. Haemoglobin, Myoglobin, Chlorophyll and Cytochrome are some of the most important complex compounds in living systems.

Another exciting application of metal complexes is the photolytic spitting of water producing hydrogen. This process has immense potential for generating non polluting fuel which may be a solution for the fuel crisis. Another important development is the recognition of the vital role of metal complexes in biological systems and in the field of therapy. Many of the complexes and complex formers are known to be used as drugs in certain types of diseases and also for metal detoxification in the case of metal poisoning

SCHIFF BASES

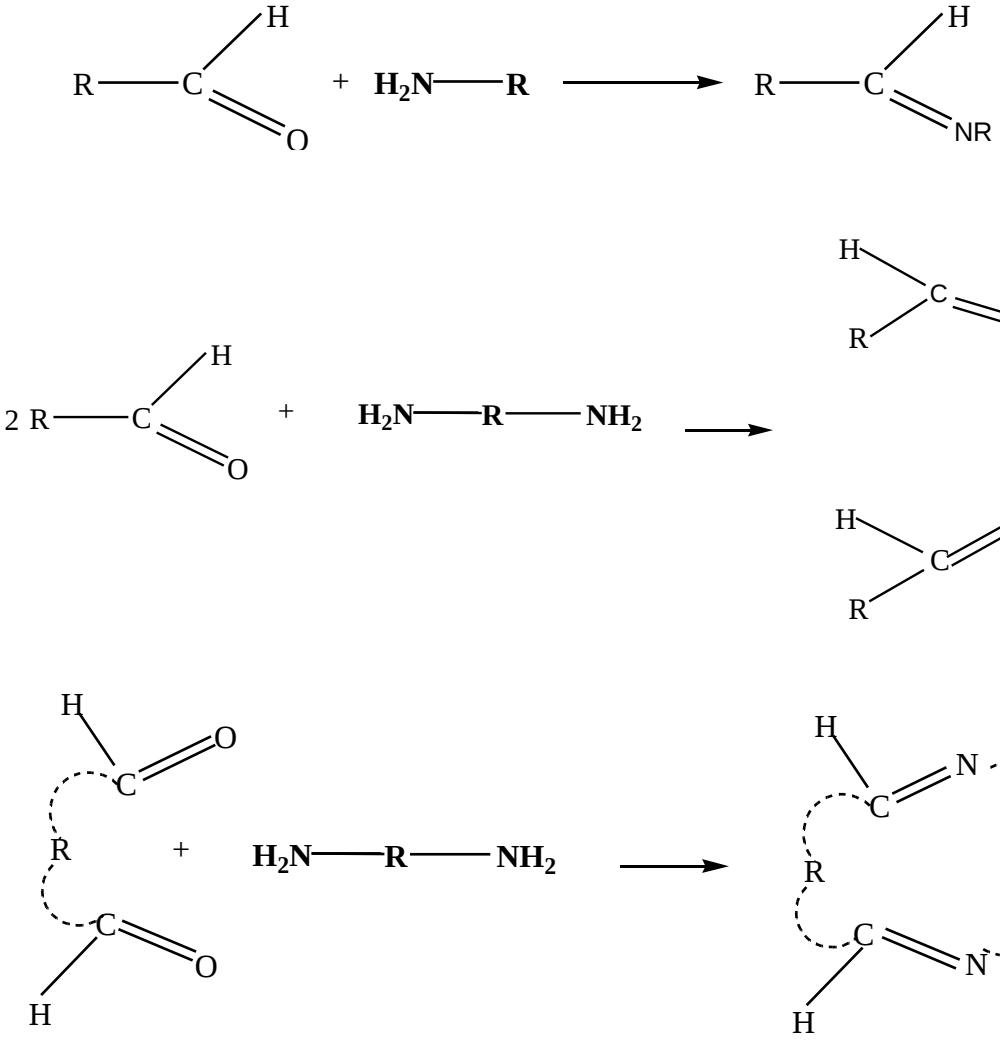
Schiff base complex compounds have taken a wide place in coordination chemistry and have important role in development of inorganic chemistry, biochemistry and environment chemistry. Schiff bases have a chelating structure and are in demand because they are straight forward to prepare and are moderate electron donors with easily tunable electronic and steric effects thus being versatile. These types of complexes with transition and heavy metals are used in organic synthesis, analytical reagent, metal ion catalyst and medicine (**Nelson SM, 1980; Fenton DE, 1986; Mukerjee S, 2006**). Schiff bases have been extensively studied because of their high potential permutations. Magnetic susceptibility, absorption spectra, elemental analysis, molecular weight determination, conductivity and thermal analysis of many

Schiff bases and their complexes have been reported (**Thomas R, 1982; Khalil MM, 1999; Mukhargee AK, 2004**). Several workers have reported that the rapidly developing field of bioinorganic chemistry is centered in the presence of coordination compounds in the living systems (**Ashash S, 2006; Jeong T, 2005**).

The general preparation of Schiff bases and reports of metal complexes of these types of ligands were first published in the 1860s (**Schiff H, 1864; Ettling C, 1840; Hobday MD, 1972**). Schiff bases or imines have the general formula RN=CR' where the R and R' are alkyl, aryl, cycloalkyl or heterocyclic groups. They are formed by condensation reaction that occurs when aldehydes and some ketones react with primary amines.

Imines play an important role in many biochemical reactions because some of the enzymes use an amine group of an amino acid to react with an aldehyde or ketone to form an imine linkage (**Solomons TWG, 1986**). It follows then, that when aldehyde or ketones are reacted with diamines in a stoichiometry of 2:1, a diimine compound is produced. N, N'-bis(salicylidine)-ethylenediamine (salenH_2) is a good example of a diimine Schiff base obtained from this type of reaction. Moreover, if dialdehydes or diketones are reacted with a diamine in a stoichiometry of 1:1, a

cyclic compound may be obtained. This type of reaction is used to prepare the mixed donor macro cycles.



Transition metal organic and organometallic chemistry lie at the interface between organic and inorganic chemistry because they look at the interaction between metal ions and organic molecules. The bonding ability of ligands depends on the nature of atoms which act as coordination sites, their electronegative and steric factors. The possibility of having a lone pair of electrons in

either π or sp^2 hybridised orbital or trigonally hybridised nitrogen in the C=N group is of the fundamental chemical and biological importance.

Transition metal ion acts as Lewis acids and can bind to lewis bases known as ligands (L) to give a coordination compound, or complex ML_n. The ligands bind in the first coordination sphere of the metal, while the second coordination sphere may accommodate such compounds as uncoordinated solvent molecules which act to stabilize the complex. The most common type of coordination complex is ML_6, which adopts an octahedral geometry (**Sargeson AM, 1984**). The ligands occupy the six vertices of the octahedron which allows them to minimize their M-L bonding distances, while maximizing their L-L non bonding distances. Obviously the geometry is named after the number of faces rather than the number of vertices present in the complex (**Crabtree RH, 1994**).

Complex ions of d-block elements exhibit certain special features when compared with the complexes of other block metals due to the presence of incompletely filled d-orbitals. The special features exhibited are

1. Great variety of colours observed in these complexes

2. Magnetic properties exhibited due to the presence of unpaired electrons

3. Important feature is the large number of possible oxidation states; even zero or negative oxidation states are possible

4. d-block metals bond with certain types of ligands such as CO, which very seldom bond to p-block metals.

All these special characters are exhibited by the d-block elements of the 4^{th} period, that is complexes of eight metals, Ti through Cu.

With a broad range of metal ions and ligands available to form coordination compounds in limitless numbers, the question arises- what makes certain ligands coordinate to certain metal ions. This can be explained by the hard and soft acid base (HSAB) principle whereby hard (Lewis) acids tend to combine with hard (lewis) bases, while soft acids prefer soft bases (**Shriver DF, 1994**).

Most metal ions in high oxidation states tends to bind saturated ligands where examples of these include NH_3 , H_2O, F^- which are known as hard ligands (**Sargeson AM, 1984**). The hard metal ions such as $Cr^{3+}$and Al^{3+} are low in electron density and require good σ donor ligands. Low oxidation state metals such as

Ag^{+}, Hg^{2+}, which are soft metals, tend to form strong complexes with unsaturated or polarisable ligands, including PPh_3 or C_2H_4 which are all soft ligands. The soft metal binds soft ligands because these metals have excess electron density by virtue of their reduced state. They avoid strong donor ligands and prefer ligands with which they can form covalent bonds, and that have available empty orbitals into which they can donate, also known as back bonding, some of their excess electron density (**Sargeson AM, 1984**). Ligands are generally nucleophilic because they have available electron lone pairs and the metal ion is electrophilic because it has available d-orbitals to accept those lone pairs.

The chemical character of many ligands can be profoundly altered on binding to the metal. For example in the case of molecular nitrogen metal complex, the nitrogen directly bonded to the metal becomes positively charged and the terminal nitrogen becomes negative, thus activating nitrogen towards chemical reactions (**Sargeson AM, 1984**).

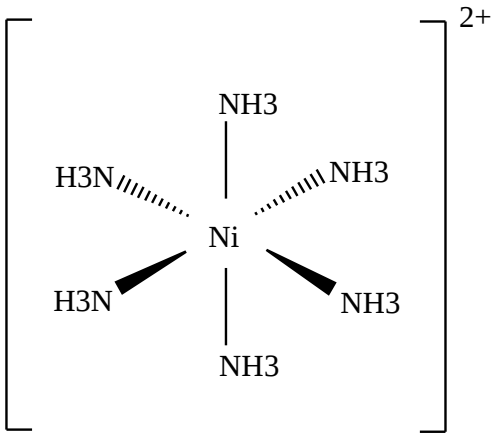
The properties of the metal ion can also be altered on complex formation. For example Co (III) is very strongly oxidising in a simple compound such as acetate which will even oxidize hydrocarbons. All the oxidizing power can be quenched by binding six ammonia ligands to give $[Co(NH_3)_6]^{3+}$ ion which lacks the severe electron deficiency of the acetate because of the presence of six

strong σ-donor ligands (**Sargeson AM, 1984**). These cobalt complexes represent examples of coordination compounds incorporating monodentate ligands. Monodentate ligands have only one donor atom attached to the metal, based on their denticity. Bidentate chelating ligands will be attached by two donor atoms, tridentate by three and so on.

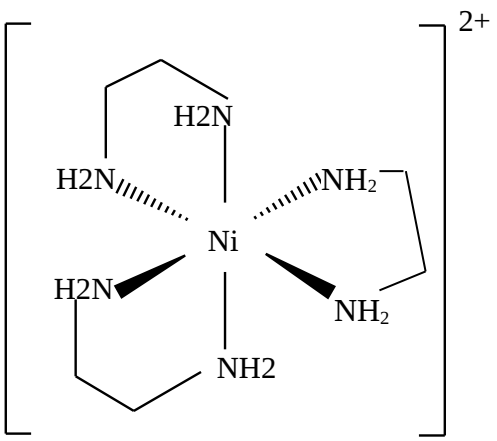
CHELATE EFFECT

If the coordinating ligands bear a functional group usually –OH,-SH, or –COOH sufficiently near to the site of condensation, then a very stable five or six membered chelate ring can be formed Chelated ligands have always played important roles in the field of medicine, catalysis, chemical analysis and geology. These ligands will donate lone pairs of electrons from the donor atoms to the same metal to give a ring compound, referred to as a chelate-from Greek word for 'claw'. The properties of chelating ligands arise from the ability of the ligand to sequester various metals dictated by the soft or hard nature of the two or more donor atoms. As a general rule, complex containing a five or six membered chelate ring has a higher formation constant than a complex that is otherwise similar, but lacks some or all of the connecting ring structure. For example if we compare the complexes tris(ethylene-diamine) Nickel(II) $([Ni(en)_3])^{2+}$ and Hexaammine Nickel(II), $([Ni(NH_3)_6])^{2+}$ both are Octahedral Nickel(II) species that contain six

nitrogen donors. The formation constants show that $([Ni\ (en)_3])^{2+}$ is about 10^{10} times more thermodynamically stable than $([Ni(NH_3)_6])^{2+}$ (**Cotton FA, 1995)**.



log K=8.6



log k=18.3

The term 'chelate effect' refers to the generalisation that chelate ligands form more stable complexes than analogous monodentate ligands (**Wulsberg G, 1987**). Relating this to ring structure, it is the enhanced stability of a complex system containing chelate rings as compared to the stability of a system that is similar but contains none or fewer rings (**Cotton FA, 1999**). In this case, the enthalpy and entropy components of a reaction favour the formation of the chelate complex. In some reactions, the enthalpy component can be unfavourable towards the chelate complex; however this contribution is generally small. Hence it is important to note that the chelate effect is essentially entropy driven and the

thermodynamically favourable formation of a chelate complex is due to the overall increase in the entropy of the system. The entropy of the system is increased because as the chelate complex is formed there will be a displacement of a greater number of molecules into the system increasing the disorder (**Cotton FA, 1995, 1999**).

Review shows that transition metal complexes of Schiff bases have emerged as highly effective catalyst for various important reactions. Catalysis plays an important role in synthesising a diverse group of molecules for the mass production of drugs and other chemical compounds. In drug developed, often only one of the two mirror images of a compound generally has the desired biological effect, while the other is ineffective or perhaps even harmful.

In order to ensure the safety of the chemical compound, it must be enantiomerically pure. So asymmetric catalysis used for designing a catalyst that is able to selectively control the formation of a desired stereo isomer, where new Schiff bases are playing a central role. Such a pentadentate sulphonamide ligand is reported by Karno et.al. (**Karno NG, 2001**). It was envisaged that a pentadentate ligand could coordinate to metals and leave an open coordination site that could bind and activate a substrate in a Lewis acid catalyzed reaction.

Another report by Genet et.al. (**Genet JP, 1986**) accounts for the use of palladium chiral complexes of Schiff bases in the stereo selective synthesis of α-amino acid. Tridentate Schiff base complexes of ruthenium (III) are used in the oxo transfer from tert ButOOH, to C-H by insertion (**Chatterjee D, 2004**). Catalysis of hydrocarbon, oxidation of cyclohexene, cyclohexane, cyclohexanol, toluene, tetrahydrofuran has been studied using various O-transfer agents. A mechanism involving intermediary of a high valent Ru(IV) –oxo species is proposed for the catalytic oxidation processes.

Application of Schiff base complexes in the field of catalysis is not only limited to the field of chemical catalysis but it extent even to the field of biological catalysis. In this area, report by Erskine et.al. is very significant (**Erskine PT, 1999**). They studied about Schiff base complexes formed by the yeast 5-aminolaevulinic acid dehydrates with the inhibitor laevulinic acid which has specific role during catalysis.

The next goal of this review is to demonstrate significant progress in the field of Schiff base coordination chemistry as it applies to medicinal field like drug designing, diagnostic tool etc. This aspect has gained momentum in the development of molecule which have active role in treatment of diseases like cancer, neurological diseases and heart diseases (**Cad VT, 2002; Verma**

M, 2004; Toshihiko Takeuchi, 1998). Report of Cad on synthesis and biological activities of new racemic gossypol and gossypolone and of (+) and (-) enantiomers and their gold complexes is an interesting example. They are stable at room temperature and it was suggested that gossypol and gossypolone dithianes and dithiolanes can be used as prodrug that target tumour cells.

Toshihiko Takeuchi et.al. (**Toshihiko 1998**) reported the selective inhibition of human α-thrombin by cobalt (III) Schiff base complexes. Human α-thrombin associated with the blood coagulation cascade, converts fibrinogen into fibrin, which ultimately forms blood clots. Cobalt (III) Schiff base complexes of class acacen bind histidine residues in active sites and on enzymes surfaces in a random fashion. Spectroscopic evidence indicates that the binding of these complexes is controlled by axial ligand substitution. They showed that the active site directed peptide linked to cobalt chelate leads to selective irreversible inhibition of thrombin.

Other developing areas of drug research include the study of antimicrobial activity of Schiff bases and enhancement in activity due to complexation. Dashora et.al. reported the synthesis (**Dashora R, 1986**) of organo silicon and organo lead complexes of Schiff bases from sulpha drugs. Complexes of the type $(CH_3)_2Si$

(ONN) $(C_6H_5)_2Si$ (ONN), $(C_6H_5)_2Pb$ (ONN) have been prepared. New (N-indoledene-DL-glycine, N-indolidiene-DL alanine, and N-indolidene-DL-valine were prepared and characterised by Nursen.et.al. (**Nursen S, 2003**) and their antimicrobial activities tested against different micro-organisms like *B.subtilis*, *S.aureus*, *E.coli* and *C.albicans*. The results of antibacterial screening of the Schiff bases ind-gly, ind-ala, and ind-val, at a concentration of 5000µg/cm^3 against all bacteria have been found and results indicate that amino acid Schiff bases shows more activity against *Staphylococcus aureus*, *E-coli* and *Bacillus polymyxa* than *Candida albicans*. Ind-gly was found to be active against both strains of *S.aureus*. *Escherichia coli* and *Bacillus polymyxa* were inhibited by ind-gly. Ind-val was found to be most active of them all. *E.coli* was the most sensitive. The activity of these substances may be due to the presence of electron donating effect.

Acylhydrazine derived furanyl and thienyl Schiff bases and their Cu(II) complexes were prepared and characterised by Zahid Chohan et.al. (**Zahid CH, 2000**). The Schiff bases and their complexes with different anions were tested for their antibacterial activity against bacterial species such as *E.coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonae*.

Raman et.al. (**Raman N, 2003**) screened Cu(II), Ni(II), Co(II)and Zn(II) compexes of Schiff bases formed by condensation

of acetyl acetone and p-anisidine by the diffusion technique using DMSO as solvent. The minimum inhibitory concentration MIC values were calculated at 37^0C for a period of 24h. It was found that all the complexes are anti microbial active and show higher activity than free ligand.

Modern researchers used to imitate the biosynthetic pathways by designing bio mimetic reactions that approximate natural reaction pathways. For example the relationship between redox properties and super oxide dismutase mimetic activity of thiohydrazone Cu (II) complexes was studied by Zdena et.al. (**Zdena DM, 1999**). The macro cyclic complexes showed more positive reduction potential and more activity than the open derivatives. From their results it follows that the structure and conformation of ligand has influence on the redox potential of central atom in the coordination compound.

In coherence with advances in analytical chemistry Schiff bases have emerged as cutting edge tools in sophisticated chemical analysis such as application of polyvinyl chloride membrane (**Susan S, 2004**). Electrodes based on two complexes of Schiff base 2,2'[4,4'-diphenylmethane bis (nitromethylidyne)] bis phenol, with copper(II) and iron(III) ions, and used for determination of triiodide ions, with lower limits of detection $4X10^{-6}$ and $6X10^{-6}$ mol/dm^3, respectively. The proposed electrode has

fast response, independent of pH and good selectivity for tri iodide ion over a variety of anions. The electrode is used in the determination of ascorbic acid in vitamin-C tablet.

Copper in alloys can be safely estimated by using the Schiff base method without interference from many, other metals in alloys. Elif Kormal used (**Elif K, 2002**) N, N'-disalicylidine-1, 3-diaminopropane as selective chelating titrant for copper(II). The stoichiometry of titration reaction and interference effects of some metals ions on titration of copper was studied. There was a good agreement between the results obtained by the proposed titration method and EDTA titration method.

In another report Papi et.al (**Papi S, 1994**) studied the synthesis, characterisation and application of metal complex of Nickel(II) with Schiff bases, 2-(2-pyridylmethyleneamino) phenol (PMAP) and 2-(2-quinolyl-methyleneamino) phenol (QMAP). The solution properties of Ni-QMAP were investigated at different pH. The chromophoric properties of the complex were enhanced with increase in pH, while stability decreased with time. The study of QMAP as a spectrophotometric reagent for the determination of small amounts of nickel was investigated. Dyeing properties of both complexes were investigated on polyamide 66 and the influence of the addition of another phenyl ring to the ligand

molecule, the dyeing properties of the complex is also investigated.

Guidelines for the molecular design of non-doping red emissive materials for OLED applications are presented in the investigation of Jia-An Gan et.al. (**Jia-An Gan, 2004**). They have reported the synthesis of Schiff base derivatives of 1, 8-naphthalimides for non doping OLEDs (Organic Light Emitting Diode) showing tunable emission colour from blue, green to red.

Several Schiff bases and their complexes were synthesised from raceacetophenone (**Nair R, 2006**) and their antibacterial activity was studied against *B.magaterium*, *E.coli*, *B.subtilis* and antifungal activity against *A.awamori*. On comparison of activity of ligand and complexes, presence of metal causes more inhibition i.e more activity.

The synthetic, spectroscopic, and biological studies of sixteen ring-substituted 4-phenylthiosemicarbazones and 4-nitrophenylthiosemi-carbazones of anisaldehyde, 4-chlorobenzaldehyde, 4-fluoro-benzaldehyde, and vanillin with ruthenium(III) and rhodium(III) chlorides are reported by Vinod K. et al. (**Vinod KS, 2007**). Their structures were determined on the basis of the elemental analyses, spectroscopic data (IR, UV, ^{1}H and ^{13}C NMR) along with magnetic susceptibility measurements, molar

conductivity and thermogravimetric analyses. On the basis of the above studies, three ligands were suggested to be coordinated to each metal atom by thione sulphur and azomethine nitrogen to form low-spin octahedral complexes with ruthenium (III) while forming diamagnetic complexes with rhodium (III). Both ligands and their complexes have been screened for their bactericidal activities and the results indicate that they exhibit a significant activity.

A series of 2-benzylideneaminonaphthothiazoles were designed and synthesized incorporating the lipophilic naphthalene ring to render them more capable of penetrating various biomembranes. Schiff bases were synthesized by the reaction of naphtha[1,2-*d*]thiazol-2-amine with various substituted aromatic aldehydes. 2-(2′-Hydroxy)-benzy-lideneamino-naphthothiazole was converted to its Co(II), Ni(II) and Cu(II) metal complexes upon treatment with metal salts in ethanol. All the compounds were evaluated for their antibacterial activities by paper disc diffusion method with Gram positive *Staphylococcus aureus* and *Staphylococcus epidermidis* and Gram negative *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. All the compounds moderately inhibited the growth of Gram positive and Gram negative bacteria. The results obtained validate the hypothesis that Schiff bases having substitution with halogens, hydroxyl group

and nitro group at phenyl ring are required for the antibacterial activity while methoxy group at different positions in the aromatic ring has minimal role in the inhibitory activity. The results also indicated that the metal complexes are better antibacterial agents as compared to the Schiff bases (**Faizul A, 2007**).

Reactions of diphenyllead(IV) chloride with benzil bis(thiosemicarbazone) (L^1H_6) and benzil bis(4-methyl-3-thiosemicarbazone) ($L^1Me_2H_4$) afforded the first complexes containing the diphenyllead(IV) moiety with bis(thiosemicarbazone) ligands. The new complexes show diverse structural characteristics depending on the ligand and the working conditions. The X-ray structure shows a distorted octahedral geometry around the lead atom, with the ligand molecules acting as NS chelates, but the nitrogen bonded to the metal is different; one of the triazines shows a novel behavior, since the nitrogen atom of the new imine group formed is the one that is bonded to the lead center, being a good example of linkage isomerism (**David G, 2007**).

SCHIFF BASES OF 2-AMINOPHENOL AND 2-AMINOTHIOPHENOL

Schiff base complexes of 2-Aminophenol and 2-Aminothiophenol are of wide interest because of their higher tendency for chelation stability, diversity of structure and speciality in applications. Tez Can has prepared and characterised the complexes of transition metals, rare earth metals and main group metals with Schiff base Salicylidene 2-Aminophenol and salicylidene-2-hydroxy-1-naphthyl amine (**Tez Can, 1984**). The synthesis of several new complexes of Cu(II), Ni(II), Co(II), Sn(II), Hg(II) etc with Schiff bases derived from 7-formyl-8-hydroxy quinoline(oxine) and 2-aminothiophenol have been reported by Sonabati and Bindary (**Sonabati AZ, 2000**). Ligands and complexes were characterised and the Schiff bases behave as mono basic and tridentate ligands coordinating through oxygen atom of the deprotonated phenolic group, the nitrogen atom of the azomethine group and pyridine.

New complexes of the Vanadium (IV) and oxovanadium (IV) with Schiff base ligands derived from the β diketone and 2-aminophenol with distorted octahedral geometry were characterised (**Abdel SD, 1998**). The spectroscopic results were used to compute the important ligand field parameters. Vanadium (IV) complexs exhibit promising catalytic activity towards the

aerobic oxidation of phenylene diamine to the corresponding semi oxide form.

Copper (II) complexes of Schiff bases of 5-nitro salicylaldehyde with 2-aminophenol and 4-aminophenol were prepared by Murty et.al. (**Jaya Murty J, 2000**). The complex showed mild to moderate activity against common pathogenic organisms. Maya devi et. al. (**Mayadevi S, 1997**) have synthesised and characterised some transition metal complexes of Schiff base -quinoxaline-2-carboxaline-2-aminophenol. A tetrahedral structure was assigned for Mn(II), Co(II), Ni(II), Cu(II) complexes, but for Fe(III) complex an octahedral dimeric structure was suggested. Pt complexes of 2-aminophenol with salicylaldehyde and 2-hydroxy-1-naphthaldyhyde were prepared. The dibasic tridentate nature of the ligands was established on the basis of IR studies. The complexes were found to be non electrolytes, diamagnetic and square planar.

Sanchez et.al. (**Sanchez G, 2002**) have synthesised new palladium (II) complexes with a tridentate PNO Schiff base ligand of amino phenol. Deprotonation of the Schiff base formed by the condensation of 2-(diphenylphosphino)benzaldehyde with 2-aminophenol in the presence of the appropriate Pd precursor ([$Pd(AcO)_2$] or

$[PdCl_2(PhCN)_2]$ form the corresponding neutral complexes in good yield.

Minu et.al. (**Minu GB, 2004**) have synthesised several Ru (II) Schiff base complexes derived from bis(pyrrole-2-carboxaldehyde)-3,4-toluenediimine, bis (pyrrole-2-carboxaldehyde)-1,2-cyclohexanediimine and bis (pyrrole-2-carboxaldehyde) ethylenediimine. All the complexes were characterised by analytical and spectroscopic methods and were found to be effective catalysts for the oxidation of primary alcohols in the presence of N-methyl morpholine-N-oxide as oxidant.

Besides the traditional applications, Schiff bases have emerged as analytical tool for precise determination of traces in even physiological systems as the application of Al(III) complex (**Sanchez G, 2002**) with salicylidene-o-aminophenol to the fluorometric determination of nucleic acids is a very good development in this field. In buffer medium of hexamethylene tetramine-HCl at pH 5.9 the Al (III) complex with salicylidene –o-aminophenol has a fluorescence peak at 508nm with excitation at 410nm. When nucleic acid coexists, it reacts with the complex within 8minutes at room temperature to produce a non fluorescent product, resulting in decrease of fluorescence intensity of the aluminium complex. On basis of this a new flourimetric method for nucleic acids determination is proposed. Compared with some

established fluorometric methods, this procedure is sensitive, selective, reliable and reproducible.

Investigation on new transition metal chelates of 3-methoxysalicyledene-2- aminothiophenol Schiff base have carried out by Soliman et.al. (**Soliman AA, 1999**) Co (II), Cu(II), and Zn(II) complexes of the Schiff base was prepared and characterised by elemental analysis, IR, NMR, thermo gravimetric analysis, conductometric and magnetic measurements. The Schiff base act as bivalent anion with tridentate ONS donors derived from the phenolic oxygen, azomethine nitrogen and thiophenolic sulphur. The formulae were found to be [ML.H_2O] and ML_2] for the 1:1 and 1:2 non electrolyte complexes, respectively. The thermal decomposition of the complex follows first order and complexes show ligand field transitions at 815 and 760nm at room temperature and are consistent with a pseudo tetrahedral kinetics and thermodynamic parameters of the decomposition were calculated.

Subrata Mandal. et.al have reported (**Subatra M**, **1995**) synthesis and characterisation of CuN_2S_2 complexes for modelling the blue protein active sites. Two new tetradentate ligands have been synthesised by Schiff base condensation of di isobutyraldehyde disulphide with mercaptoethylamine L^1 and 2-aminothiophenol L^2 respectively and then reducing the imine

linkage with sodium borohydride in refluxing methanol. In the free ligand the thiolate sulphur is protected with t-butyl groups, which are cleaved in the presence of Cu (II) salts to give neutral CuN_2S_2 complexes.

The condensation of o-aminothiophenol with 2-thiophene-dicarboxaldehyde yields 2-thiazolin derivative, rather than the expected Schiff base. However, upon reaction with metal ions, the thiazoline rearranges to the expected thiolate Schiff base. Complexes of Schiff base with Ni(II), Cu(II), Zn(II), Cd(II), Pb(II), Ag(I), and Pd(II) were isolated and characterised (**Tayim HA, 1983**). Schiff base derived from the reaction of 2,5-thiophene-dicarboxaldehyde and o-amino-benzenethiol gave 2,5-bis(benzothiazolidin-2-yl)thiophene. Schiff base reacted as neutral ligand with Pb(II) and as dianionic species with Cu(II), Cd(II), Zn(II) (**Salameh AS, 1983**).

Complexes of Ni(II), Co(II), Cu(II), Zn(II), Pd(II), Pb(II), with Schiff base derived from isatin and 2-aminothiophenol were synthesised and characterised by elemental analysis, molar conductance, magnetic moment and spectroscopic methods (**Khalifa MA, 1996**). Schiff bases derived from 5-nitrosalicylaldehyde and the amines, o-and p-aminophenols o-aminothiophenol and sulfanilic acid were prepared and characterised. These complexes were tested for antibacterial

activity against common pathogens and showed mild to moderate activity (**Murty JJ, 1998**).

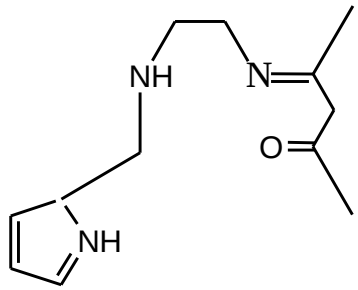
Synthesis, spectroscopic characterisation, redox and biological screening studies of some Schiff bases transition metal complexes derived from salicylidine -4-aminophenoland aminophenol and 2-aminothiophenol were studied by Raman et.al. (**Raman N, 2001**). Ruthenium(II)complexes with tetradentate Schiff base ligands derived by condensing o-aminophenol or o-aminothiophenol with glyoxal has been carried out. (**Viswanathan P, 2006**). An octahedral geometry was proposed for the complexes and it was effective catalyst for the oxidation of benzyl alcohol to benzaldehyde using N-methyl morpholine-N-oxide as co-oxidant.

The Schiff base has been prepared by condensing 4-[N.N-dimethylamino] benzaldehyde with o-aminophenol in ethanolic media, form new metal complexes with Ni(II), Fe(II) and Cr (III) ions. The elemental analysis data exhibit the formation of 1:1 [M:L] ratio. IR spectral data display that the complexation occurs through oxygen atom or the hydroxyl group in the o-aminophenol and the nitrogen atom of azo-methine group (**Maihub AA, 2007**).

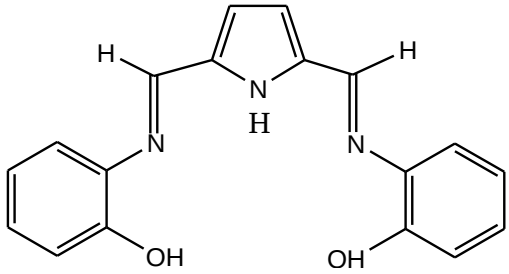
SCHIFF BASES OF DIKETONES AND HYDROXY KETONES

Tetradentate Schiff base ligands can conveniently be synthesised by condensing α-diketones, β-diketones, aldehydes with various diamines. Tetradentate Schiff bases with N_2O_2, N_3O, N_4 and N_2S_2 donor sets have been widely studied for their ability to coordinate with metal ions. Classical examples that have been most widely examined with N_2O_2 donor set are the derivatives of acetylacetone with various diamines.

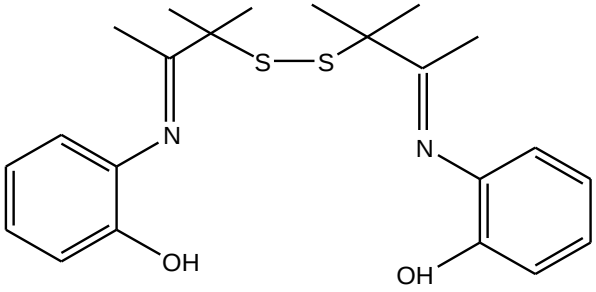
Recently large number of biologically active quadridentate ligands and its complexes has been reported. Gillian F. Morgan and coworkers (**Gillian F, 1990**) characterised the structural features of the new brain imaging agent ^{99}Tc [TcO(L)], a,novel technetium complex with N_3O donor set, currently undergoing clinical trial evaluation for cereberal perfusion.



A.T. Ramdan synthesised metal chelates of 2, 5-pyrrolediyl-bis (N-o-hydroxyphenylaldimine) (**Ramdan AT, 1992**). The Schiff base behave as a tetradentate ligand.



Rameswar Shukla and Bharadwaj have synthesised a new tetradentate Schiff base ligand by the condensation of diisobutyraldehyde disulphide with 2-aminophenol (**Rameswar S, 1993**). The ligand forms mononuclear manganese (II) complex with imine and phenolate ligation, which is relatively rare.

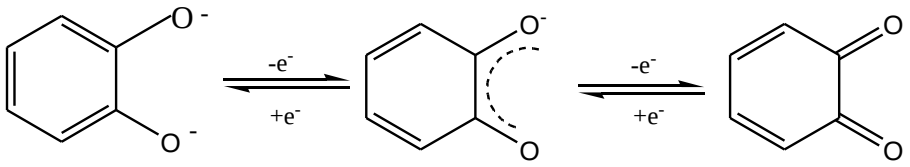


Tropolone, o-quinone, 9, 10-phenanthrenequinone etc. are formally

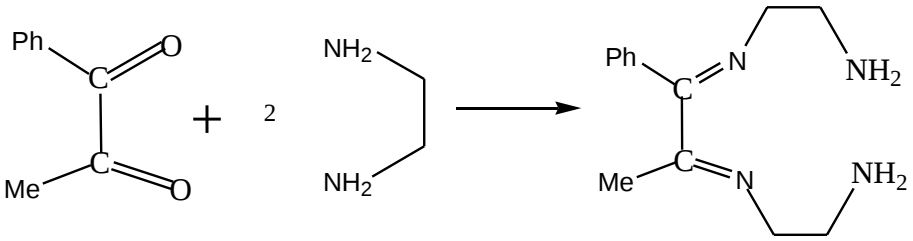
1,2-diketones. In many respects their coordination chemistry resembles that of the more familiar congener acetylacetone. An important feature is its ability to provide stable paramagnetic ligands upon reduction. Its relevance to bacterial photosynthesis attracts special attention.

The electronic structure of these congeners is such that it is capable of undergoing successive one electron reduction and the

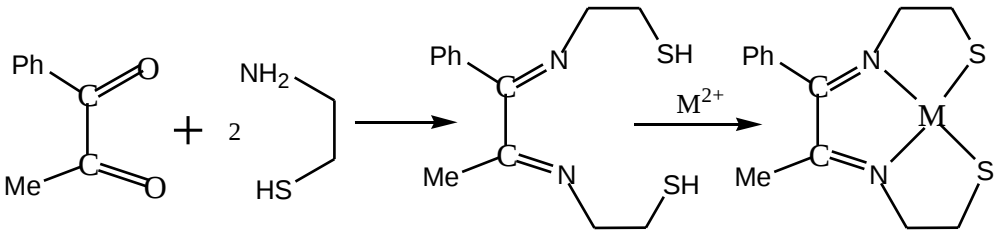
close similarity between the complexes of their compounds can be appreciated by considering the redox equation given below.



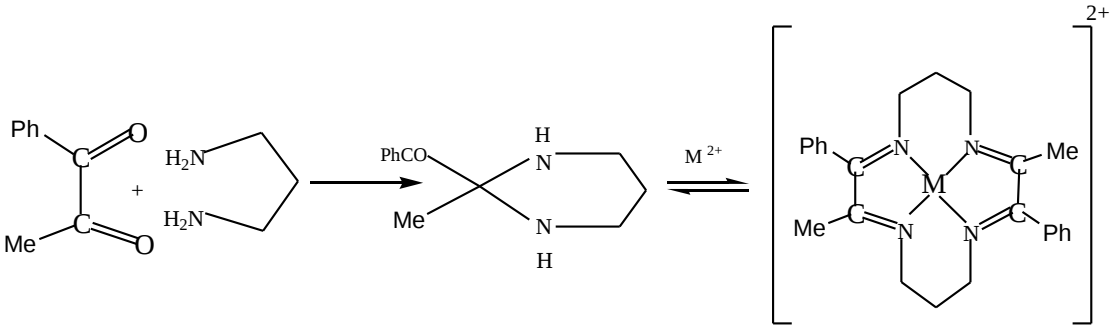
It was noted earlier that the planar, rigid nature and small bit angle of 1, 2-diketones favours twist rearrangement pathways in metal complexes. The synthesis of quadridentate diimine chelates usually require the combination of one equivalent of a dicarbonyl compound and 2 equivalents of a primary amine, or one equivalent of a diamine and two equivalent of a carbonyl compound.



Simple α-diimines are hydrolytically unstable, but can be stabilised as metal complexes by the virtue of formation of chelated 5-membered rings (**Krumhotz P, 1954**). The α-diketones and gloxal undergo metal template reactions with amines to yield complexes of multidentate ligands. The same reactions occur with amino alcohols and amino thiols (**Thomson MC, 1965; Elder MS, 1968**).



Template reactions between α-diketones and diamines have been used for the synthesis of complexes of macro cyclic ligands (**Jackel SC, 1972, Welsh WA, 1977**).



A variety of primary amines undergo reaction with substituted aldehydes or ketones to yield the related imines, which can be used as the ligands in the metal complexes. The imine carbon is more susceptible to nucleophilic attack, and hence to the hydrolytic cleavage; so the imine formation is reversible. Therefore, it is often more desirable to prepare such metal complexes directly from the amine and the aldehyde or ketone, in the presence of the metal ion (**Jackel SC, 1980; Lindoy LF, 1971**).

The prior coordination of the carbonyl group to the metal ion results in the activation of the carbonyl carbon to amine

nucleophilic attack. But it would be impossible for a precoordinated amine to act as a nucleophile and hence no kinetic template effect is involved. Numerous macrocylic chelated compounds have been prepared by this method.

It is noteworthy that dicarbonyl compounds undergo reactions with diamines to yield heterocyclic compounds rather than α-diimines. However, in the presence of suitable metal ions the tautomeric α-diimines structure is reoriented and stabilised through the formation of five membered chelate rings (**Stoufer RC, 1960**). Dihydrazones of α-diketones can form low spin tris complexes with iron (II) ion. Thus the complex with biacetyl dihydrazone is well characterised and related tris complexes derived from benzil and glyoxal were also low-spin (**Busch DH, 1956**). While bis complexes of the type $[FeX_2 (NN)_2]$ are paramagnetic with magnetic moment of 3 B.M.

Considerably much work has been carried on the low spin bis and tris complexes of bidentate and tridentate Schiff base of diketones. The facile template synthesis can be used to prepare free ligand by subsequent demetallation with EDTA.

Schiff bases, especially multidentate and macrocyclic ones are strong ligands for Zn. Derivatives of diketones form tetradentate Zn complexes in which the ligand controls the

stereochemistry of the complexes and provides numerous examples of unusual geometry about the metal atom, thus illustrating the coordination flexibility of these ions. The diimine formed by the condensation of benzil with ethylenediamine or 1,2-diamino benzene has been shown to have tetrahedral structure.

Neutral complexes of Cu(II), Ni(II), Co(II), and Zn(II) have been synthesised (**Raman N, 2002**) from benzil and 2-aminobenzyl alcohol and their antimicrobial activity was carried out . Most complexes have higher activity compared to the free base.

A series of antibacterial and antifungal compounds derived from various amino acids and diketone and their metal complexes were synthesised and characterised (**Chohan ZH, 2006**). The ligands and the complexes were screened for their antibacterial and antifungal activity. The results show metal (II) complexes are more active than the free ligands.

Schiff base derived from the reaction of the aldehydes,3-hydroxybenzaldehyde and 5-nitrosalicylaldehyde with amines, aniline, and o-aminothiophenol and their complexes with Co(II), Ni(II) were reported. The chemical shifts of the different protons in the NMR spectra of the prepared Schiff bases were also reported (**Kolwalker SD, 1996; Issa YM, 1998**).

Thiosemicarbazones of aromatic o-hydroxy aldehydes and ketones have attracted considerable interest because of their potential biological properties (**West DX, 1990**) and catalytic activities (**Barber DE, 1992**). These aromatic thiosemicarbazones most often coordinate as the dianion on loss of the phenoxy hydrogen and thiosemicarbazones to form mononuclear [M(ONS)X] (ONS represents the dianionic thiosemicarbazone) ligand coordinated via the phenoxy oxygen, azomethine nitrogen and thiolato sulphur and X represents a neutral molecule such as ammonia or pyridine (**Sorianco M, 1985**).

Palladium complexes of 2-hydroxyacetophenone N-ethyl thiocarbzone have been studied with regard to their structural and biological properties. The complexes were found to be triangular, trinuclear complex with bridging thiosemicarbazonato sulphur atoms (**Dimitra KD, 1997)**.

Vanadium (IV) complexes have an ability to catalyse the generation of ROS, so diverse vanadium complexes with different ligands linked to metal by carbon, nitrogen or oxygen atoms were synthesised. Among the various complexes, Bromo Hydroxyl acetophenone complex was found to have a potent sperm immobilizing activity and hence may be used as a contraceptive agent (**Osmond JD, 1999**).

Mixed ligand complexes of alkaline earth metals with 5-chlorosalicilaldehyde and o-hydroxyacetophenone have been prepared and characterised (**Prasad RN, 2002**). In recent years complexation studies of these metal ions have been undertaken following the recognition of various important roles of these metal ions in biological system.

Inorder to investigate the electronic, steric and geometric effect of a methyl group on imine carbon three optically active Schiff-base ligands of 2-hydroxyacetophenone with (1R,2R)-(-)1,2-diaminocyclohexane, (1S,2S)-(-)1,2-diphenylethylenediamine or R-(+)-2,2'-diamino-1,1'-binaphthalene were prepared and characterised (**Wen TG, 2002**).

Novel Pd^{II} and Pt^{II} complexes of substituted *o*-hydroxyacetophenone-glycine have been synthesized, and characterized by conductivity measurements, IR, UV and NMR spectra. The spectral data indicate that the ligands are monobasic bidentate, coordinating through imino nitrogen and the carboxylate group. A four coordinate square planar configuration has been proposed for all the complexes. The ligands, as well as their Pd^{II} and Pt^{II} complexes, exhibit potent cytotoxic activity against *Ehrlich ascites* tumour cells *in vitro*, but appear to be more active *in vivo* (**Offiong E, 2004**).

Several hexacoordinated ruthenium (III) complexes with appropriate Schiff bases having the donor groups (O,N) viz., o-hydroxy acetophenone ethylenediimine, o-hydroxy acetophenone propylenediimine, o-hydroxy acetophenone tetramethylenediimine and o-hydroxy acetophenone orthophenylenediimine in 1:1 molar ratio. All the complexes have been characterised on the basis of elemental analyses and spectral (IR, electronic and EPR), electrochemical and magnetic moment data. The antibacterial activities was tested for these complexes and found to be active (**Thangadurai T, 2002**).

A new complex of Cu with 2'-hydroxy-5'-methyl-acetophenone (HMAP) and triphenylphosphine (PPh_3) was prepared by the electrochemical oxidation of Cu in acetonitrile solutions. The final product, (MAP)Cu(PPh_3)$_2$, was crystallized from bulk solution and characterized by microanalysis, IR, Raman, ^{1}H NMR together with x-ray crystallographic determinations. The results showed that the complex was a chelate structure in which Cu(I) ion was coordinated with two oxygen atoms of the deprotonated ligand of MAP anion to form a six member ring, and two PPh_3 molecules participated in the coordination (**Yaxian Yuan, 2005**).

The synthesis of Cu(II) complexes derived from Schiff base ligands obtained by the condensation of 2-hydroxybenzaldehyde or terephtalic aldehyde with 4-amino-antipyrine is reported. The

newly prepared compounds were characterized by 1H-NMR, UV-VIS, IR and ESR spectroscopy. The determination of the antimicrobial activity of the ligands and of the complexes was carried out on samples of *Escherichia coli, Klebsiella pneumoniae, Acinetobacter boumanii, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida sp.* The qualitative and quantitative antimicrobial activity test results proved that all the prepared complexes are very active, especially against samples of *Ps. aeruginosa, A. Boumanii, E. coli* and *S. aureus* (**Tudor Rosu, 2006**).

The metal complexes of UO[II] and Th[IV] with Schiff bases derived from 2-aminopyridine and acetophenone/o-hydroxy acetophenone/o-amino acetophenone have been synthesized and characterized on the basis of elemental analyses, conductivity, IR, electronic and [1]H NMR spectral studies. The evidences show that the complexes with acetophenone and o-hydroxy acetophenone Schiff bases exhibit coordination number eight while coordination number ten with o-amino acetophenone Schiff bases. A comparative study of testicular atrophy in albino rats by the chelating agents and the corresponding UO[II] and Th[IV] complexes was carried out. A considerable reduction in the testes weight and weight of some accessory sex organs was observed.

The effect is more with the complexes than with the ligands (**Gudasi KB, 2006**).

Scope of the Present Investigation

Generally an extensive study of co-ordination compounds has centered on the behaviour of metal ions rather than the attached Schiff base ligands. But the properties of such ligands can be modified by the co-ordination to the metal ions. An overview of Schiff base metal chelates reveals that only few works have been carried out in the area of hydroxy ketones as well as 1,2-diketones. Such class of ketones together with aminophenols and aminothiols, act as the building blocks of many poly and macrocylic ligand systems of Schiff bases and their metal chelates. The present investigation has been designed to provide some modern and advanced results in this field.

In the present work, some new ligands namely 2-Hydroxy-acetophenone 2-Aminothiophenol (HAPATP), 2-Hydroxyacetophenone
2-Aminophenol (HAPAP), Benzil 2-Aminothiophenol (BATP), Benzil Aminophenol (BAP) have been synthesised and characterised. Their complexes with traditional transition metal ions such as Co(II), Ni(II), Cu(II) and Zn(II) were also isolated and characterised by various physico-chemical methods like UV, IR, NMR Spectra,

Molar conductance and Magnetic data. The thermal decomposition character of some representative complexes in static air atmosphere have been studied by TGA technique. Analysis of the TG curves provides some probable structural assignments for each stages of decomposition. These results also support the proposed geometry of the complexes.

CHAPTER II

MATERIALS, METHODS AND INSTRUMENTS

In this chapter a concise report of the general reagents employed for the present study are given. It also gives the theory and techniques of the analytical and physical methods used for the characterisation of the ligands and complexes synthesised. Materials methods and instruments for antitumor and antibacterial studies were given as a separate chapter in Part III and Part IV.

Materials

Analar grade samples of 2-Hydroxyacetophenone, Benzil, 2-Aminothiophenol and 2-Aminophenol supplied by E-Merck, BDH (India), Glaxo, Sigma Company, USA were used for the preparation of ligands, the metal source used for the synthesis of the complexes are acetates of Co(II), Ni(II), Cu(II) and Zn(II). Solvents like chloroform, carbon tetrachloride, dimethylformamide and dimethylsulphoxide were used as such, but the LR grade ethanol, methanol and acetone were purified by standard procedures (**Weissberger A, 1956; Vogel AI, 1962**). Specroscopic grade samples of the solvents were employed for the spectral measurements.

Methods

The following procedures were employed to test the purity of ligands and characterisation of their metal complexes.

CHN Analysis

Carbon, Hydrogen and Nitrogen content of the ligands and their metal complexes were determined by microanalysis on a Heraous-CHN-O-rapid analyser.

Estimation of Metals

Standard methods (**Vogel AI, 1978**) like volumetric, gravimetric, pyrolitic techniques were adopted for the estimation of metal content. The atomic absorption spectroscopy is also used for the confirmation of the metal percentage in selected samples.

For the volumetric and gravimetric estimations a common methods was used for decomposing the metal complexes. About 0.2gm of the complex was digested with concentrated nitric acid-perchloric acid mixture followed by Conc.HCl. The resultant solution was then quantitatively made up to 100 ml by using a definite volume of the solution, the metal content of the complex was estimated.

Amount of copper was determined iodometrically by the addition of KI and subsequent titration of liberated iodine by

standard sodium thiosulphate. The amount of Cobalt was estimated volumetrically by complexometric titration using standard EDTA solution and Xylenol Orange indicator. Gravimetrically Nickel was estimated by precipitating as dimethyl glyoximate. By complexometric titration using standard EDTA and Eriochrome Black-T indicator, the amount of zinc metal was estimated.

Almost all of these metals were estimated by pyrolysis method. About 0.2 gm of each complex was weighed out in a silica crucible and heated strongly. During the heating all the organic particles in the chelate was burnt of and the metallic oxide left behind was weighed. From the weight of the oxide metal percentage was calculated.

Estimation of Sulphur

Sulphur content in the complexes was estimated after oxidising it with nitric acid to sulphate. The sulphate was then determined gravimetrically (Furman NH, 1962) as $BaSO_4$.

Molar Conductance

Molar conductance measurements of the complexes were carried out in methanol, ethanol and double distilled water at 28 ± 2°C using a solution of 10^{-3} M concentration. The conductance measured can be used to find the electrolytic or non electrolytic nature of complexes.

Magnetic Moment

Magnetic susceptibilities of the complex were determined at room temperature by using a Gauy Balance (**Lewis J, 1963**). Diamagnetic Corrections were applied using Pascal's Constants taking into consideration of the magnetic contribution of various atoms and structural units (**Earnshaw N, 1968**). The effective magnetic moment (μeff) was calculated using the equation

$$\mu eff = 2.84 \sqrt{\chi' M \times T}$$

χ'M = molar susceptibility corrected for diamagnetism and T = absolute temperature.

The theoretical magnetic moments were calculated using the formula

$$\mu eff = g \sqrt{S \times (S+1)}$$

Electronic Spectra

The UV - Visible spectra of the ligands and complexes were recorded on a Shimadzu recording spectrophotometer using methanol, ethanol or distilled water as the solvent. Electronic spectral studies were carried out to supplement the information obtained from the magnetic measurements.

The ligands which are mainly organic compounds have absorption in the UV region and hence do have bands in the region of 200 to 350nm of the electromagnetic spectrum. In some cases these bands extends over to a higher wavelength region due to conjugation. But upon complexation with transition metal ions, due to interaction with the metal ion, there will be interesting change in the electronic properties of the system. For each complex the peak was assigned to a particular d-d transition and charge transfer spectra from metal to ligand (M→L) or ligand to metal (L→M) can be observed. These information can be processed to obtain the structure and geometry of the compounds (**Lever ABP, 1984**).

Infrared Spectra

The IR Spectroscopy is commonly used as a characterisation technique for metal complexes. The IR Spectra of the ligands and metal complexes were recorded in the range of 4000-400 cm^{-1} on a Schimadzu-IR -470 infrared spectrometer by KBr disc technique.

The importance of IR spectroscopy lies in the fact that the characteristic infrared absorption bands of a group occur at about the frequency irrespctive of the molecule in which the group is present.

Thermogravimetric Analysis

The thermogram of complexes were recorded non isothermally using a sample weight of 5mg in static air atmospehere at a rate of heating 10° or 15°C min^{-1}. Each mass loss consideration from the TG plot can be assigned to the decomposition of volatilisation of a particular group. The careful examination of such steps during the heating of each complex can be found to be in good agreement with the proposed structure.

NMR Spectra

NMR Spectra of some selected ligands and complexes (Zn Complexes of HAPATP and BAP) were carried out on BruckerDPX 300 MHz Machine using DMSO as solvent. In each case the spectra were analysed by considering the standard chemical shift values.

Instruments

The instruments used for the present investigations are given bellow.

1. Heraous – CHN – O – rapid analyser
2. Toshniwal conductivity bridge.
3. Gouy type magnetic balance.
4. Shimadzu UV-1602 Spectrophotometer
5. Shimadzu IR 470 Infrared Spectrophotometer
6. Hitachi R – 600 Spectrometer
7. Varian E4 Band Spectrometer
8. Perkin Elmer – 7 Series Thermobalance.
9. Brucker DPX – 300 MHz Machine

CHAPTER III

TRANSITION METAL COMPLEXES OF 2-HYDROXY ACETOPHENONE 2-AMINO THIOPHENOL (H_2L^1)

New vistas have opened up in the field of co-ordination chemistry with the designing of structure of complexes by the influence of metal ions (**Busch DN, 1963**). A perusal of literature showed major developments have been achieved in the research of co-ordination compounds with special emphasis on metal complexes of Schiff bases containing nitrogen, oxygen and sulphur atoms as donors (**Djebbar SS, 1997**). This may be due to their stability, pharmacological activity and potential applications in many fields.

The Schiff bases derived from 2-Hydroxy acetophenone are very interesting due to their ability to form various types of metal complexes. Biological activity of complexes derived from amino thiophenol has been extensively studied with respect to their antiviral, antitumor and antibacterial activities (**Thanga Durai TD, 2002**). In view of the importance of this class of complexes we have attempted to synthesize a novel ligand 2-Hydroxy acetophenone 2-Aminothiophenol (HAPATP), and to explore its structure and possibilities as an active biological agent.

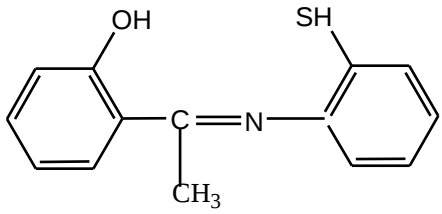
In this chapter we have described our results of the brief study of the transition metal complexes of the Schiff base derived from 2-Hydroxy acetophenone and 2-Aminothiophenol.

Synthesis of the Ligand

The ligand 2-Hydroxyacetophenone2-Aminothiophenol was prepared in ethonolicmedium from 2-Hydroxyacetophenone and 2-Aminothiophenol. An ethanolic solution of 2-Hydroxy acetophenone (0.01 mol) was mixed with and ethanolic solution of 2-Aminothiophenol (0.01 mol) and was refluxed for two hours on a water bath. The pale yellow precipitate formed was filtered, washed and dried over anhydrous $CaCl_2$. The melting point was found to be 105^0C.

Characterisation of the Ligand

The ligand 2-Hydroxy acetophenone 2-Aminothiophenol (HAPATP) was characterized on the basis of elemental analysis and spectral data as given in the Table I.3.1 and I.3.2. The UV, IR and NMR studies of the ligands showed the characteristic bands. Based on the above results the structure of the ligand was confirmed as



2-Hydroxy acetophenone 2-Aminothiophenol

STUDIES ON Co (II), Ni(II), Cu(II) AND Zn(II) COMPLEXES OF 2-HYDROXY ACETOPHENONE 2-AMINO THIOPHENOL- HAPATP (H_2L^1)

Preparation and characterization Co (II), Ni(II), Cu(II) and Zn(II) complexes of 2-Hydroxyacetophenone 2-Aminothiophenol-HAPATP (H_2L^1) are described in this part.

Synthesis of the Complexes

The complexes were prepared by slowly adding a hot aqueous solution of the metal acetate to a refluxing ethanolic solution of the ligand containing sodium acetate (0.5 gm), until the metal ligand ratio reached 1:1. The reaction mixture was refluxed for 1 hour and the complexes were precipitated. The precipitated complexes filtered, washed with water and alcohol and dried over anhydrous calcium chloride.

Co(II), Ni(II), Cu(II) and Zn(II) complexes were prepared by this method.

Characterisation of the Complexes

The complexes were characterized on the basis of elemental analysis, UV, IR and NMR spectral data, magnetic studies, conductance measurements and thermal studies as given in the Tables I.3.1 and I.3.2.

Results and Discussion

All the complexes are coloured, photostable and non hygroscopic. They are soluble in DMSO and in other common organic solvents. On the basis of elemental analysis Co(II), Ni(II), Cu(II) and Zn(II) complexes can be represented by the general formula $ML(H_2O)_3$ where M is the metal and L is the ligand moiety.

The analytical data of these complexes are presented in the Table I.3.1. The room temperature, magnetic moments and molar conductivities of the complexes are also given in the same table. Important IR spectral bands of the ligands and complexes are also given in the Table I.3.2.

Elemental Analysis

The complexes were analysed for metal and sulphur by standard methods (**Vogel AI, 1978; Furman NH, 1962**). The percentages of carbon, hydrogen and nitrogen were estimated by micro analytical methods. The results are given in the Table I.3.1.

Molar Conductance

Conductance measurements of these complexes in methanol at a concentration of 10^{-4} M at room temperature are in the range of 2-10 $ohm^{-1}cm^2mol^{-1}$. The very low values indicate that these

complexes behave as non-electrolytes and are neutral in nature (**Geory WJ, 1971**).

Magnetic Measurements

Magnetic susceptibility of the complexes was determined by Gouy balance. The measurements were made at room temperature and instrument was standardized using Hg $[Co(NCS)_4]$ as calibrant (**Figgis BN, 1958**). Table I.3.1 shows effective magnetic moment values calculated from the corrected magnetic susceptibility. Some indications of the structures and geometries of the complexes can be obtained from these magnetic moment values (**Dutta RL, 1992**).

Octahedral and tetrahedral Co(II) complexes differ in their magnetic properties. In high spin octahedral complexes of Co(II), the ground term is $^4T_{1g}$ which results in considerable orbital contributions. It is reported that octahedral high spin geometry can be assigned to Co(II) complexes if the measured value of the magnetic moment is in the range of 4.7-5.2 BM (**Lewis J,1963; King EA, 1968**). The expected magnetic moment is the spin only value for the three unpaired electrons ie 3.87BM. Here the Co(II) complex shows a magnetic moment of 4.82BM rather above the spin only value indicates octahedral geometry.

Ni(II) complex has a magnetic moment value of 3.5 BM, which is near to the normal range observed for octahedral Ni(II) complexes. This shows the presence of two unpaired electrons with the electronic configuration of $t_{2g}^6 eg^2 [^3A_2]$. Therefore an octahedral geometry can be assigned to the Ni(II) complex (**Lewis J, 1963; Masoud MS, 1991**).

The Cu(II) complexes usually have a distorted octahedral stereochemistry. A few are known with squareplanar or approximately tetrahedral geometry. In regular octahedral Cu(II) complexes ground term is ^2Eg and hence no orbital contribution is expected. The spin only magnetic moment values corresponding to one unpaired electron is 1.73BM but the observed values fall in the range 1.80-2.10BM. This is due to spin orbit coupling. In regular tetrahedral Cu(II) complexes, the ground term being a triplet state, orbital contribution is expected. So theoretically predicted magnetic moment value is 2.2BM (**Figgis BN, 1958**). But the reported values are in the range of 1.95-2BM (**Kumar NRS, 1999**). In our study the Cu(II) Complex gave a magnetic moment value of 1.96BM which indicates an octahedral geometry for the complex. The Zn(II) Complex was found to be diamagnetic.

Electronic Spectra

The electronic spectra of all the complexes of HAPATP are recorded in DMSO. Octahedral geometries are commonly found in Co(II) Complexes and such complexes are pale pink in colour. In the octahedral Co(II) complex, $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$ transitions occur in the region 1250-1000nm and 700-500nm respectively (**Bhara B, 1971**). In the present case the electronic spectrum of Co(II) Complex shows bands at 1066nm and 665nm which can be assigned to the d-d transitions of octahedral geometry. Low intensity of bands and the pink colour of the complex support octahedral geometry.

Studies by Jorgensen (**Jorgenson CK, 1956**) revealed that in the case of Ni(II) octahedral complexes, the d-d transitions are observed in the ranges of 1100-900nm [$3A_{2g}(F) \rightarrow 3T_{2g}(F)$], 650-500nm [$3A_{2g}(F) \rightarrow 3T_{1g}(F)$] and 400-350nm [$3A_{2g}(F) \rightarrow 3T_{1g}(P)$]. Most of the tetrahedral complexes of Ni(II) has intense blue colour due to the presence of an absorption band in the red part of the visible region (**Solimann AA, 1999**). In the present investigation Ni(II) complexes of HAPATP shows three absorption band at 444nm, 580nm and 900nm which can be attributed to an octahedral geometry (**Angela K, 2007**).

For octahedral Cu(II) complexes only a single band due to the transition $^2E_g \rightarrow {}^2T_{2g}$ (D) would result but the observed band is very broad and clearly contain several components which is a result of tetragonal distortion due to Jhan –Teller Effect (**Sutton, 1968**). In the case of Cu(II) complex of HAPATP, an absorption band at 694nm is assigned to a slightly distorted octahedral geometry for the complex. In the case of Zn(II) complex d-d transitions are not possible and the bands observed ~ 400nm are due to charge transfer.

Infrared Spectra

Infrared spectra of the ligand and complexes were examined in detail. The significant vibrational bands and their assignments are given in the Table I.3.2. IR Spectra of the ligand (HAPATP) show the absence of bands corresponding to ν NH_2 and νC=O. Instead of this a new prominent band at 1550-1690cm^{-1} due to azomethine linkage appeared in the ligand indicating the condensation of ketonic group with amino group (**Nakomoto K, 1970**). A peak ~3400cm^{-1} and ~1300cm^{-1} corresponds to ν(OH) stretching and bending vibrations. A weak band ~2550cm^{-1} corresponds to the ν(SH) vibration. Bands at ~760cm^{-1} probably indicate the C-S symmetric vibrations and this band is shifted to lower frequencies in all the complexes. The band at 1612cm^{-1} is the characteristic of the azomethine group (>C=N) present in the free ligand. A

downfeild shifting in this frequency region (1570-1595cm^{-1}) can be observed in all the complexes indicating that the ligand is coordinated to the metal through azomethine nitrogen. This also indicates the reduction of electron density in the azomethine ligand. Broad and strong band around 3300-3400cm^{-1} indicates coordinated water molecules present in the complexes. In the chelates of Co(II), Ni(II) and Cu(II), coordinated nature of water molecules is further supported by the appearance of new bands of medium intensity between 750-850cm^{-1} (**Clothup NB, 1975**). The weak ν (SH) vibration band at 2552cm^{-1} in the ligand disappears in the complexes showing the participation of SH group in chelation. The C-O band which appear ~1246 cm^{-1} in the ligand is shifted to lower frequency in the complexes indicating the co-ordination of the metal through phenolic oxygen. The new band found in complexes in the range 540-560cm^{-1} are assigned to ν(M-N) stretching mode. The ν(M-S) bands of the complexes appear in the range 450-500cm^{-1} and the new bands in the region 420-440cm^{-1} are correspond to ν(M-O) stretching vibrations (**Ferrare R, 1971**). The above data indicate that the ligand is dianionic tridentate ligand (ONS) coordinating to the metal through the deprotonated thiophenol, deprotonated phenol and azomethine nitrogen.

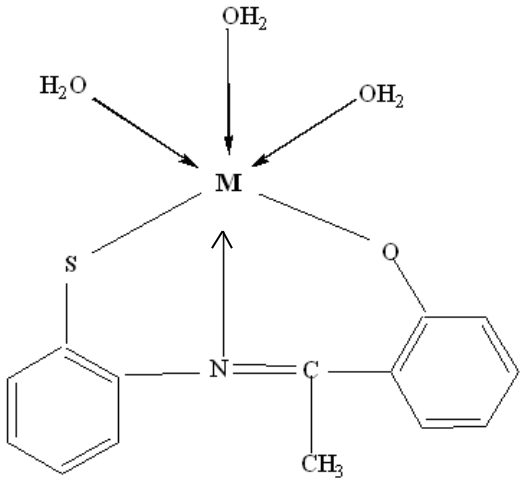
NMR Spectra

Proton NMR spectra has been useful in establishing the nature and structure of Schiff bases and complexes in solution. In the case of HAPATP complexes the ^{1}H NMR Spectra of diamagnetic Zn Complex, $[ZnL^{1}(H2O)_{3}]$ was compared with free ligand. The ligand gave a singlet at 12.5δ corresponding to OH protons, another singlet at 5.1δ corresponding to SH, and a singlet at δ 2.5 for the methyl protons. Aromatic multiplet was observed at the range 6.9-7.4 δ corresponding to 8 aromatic protons. In NMR spectra of the diamagnetic Zn complex it was found that the singlets due to SH and OH protons disappeared indicating the removal of these protons by chelation with Zinc (**Soliman AA, 1999**).

TG Studies

Cu(II) and Zn(II) complexes of HAPATP were subjected to thermal studies by non isothermal method. A three stage decomposition was observed for Cu(II) Complex whereas Zn(II) Complex undergoes a two stage decomposition. The observed mass loss in TG studies, agrees fairly well with the values calculated from pyrolitic experiments. Probable assignments of each decompositions are discussed in Part II.

The complexes can be represented by the following structure based on the above results.



M = Co(II), Ni(II), Cu(II) and Zn(II)

Table I.3.1

Microanalytical, magnetic and conductance data of transition metal chelates of 2-Hydroxyacetophenone 2-Aminothiophenol (H_2L^1)

Compound	C %	H %	N%	S %	M%	μ_{eff} B.M	$ohm^{-1} cm^2 mol^{-1}$.
H_2L^1	68.40 (69.10)	5.2 (5.34)	5.2 (5.76)	12.5 (13.16)			
$CoL^1(H_2O)_3$	47.9 (47.46)	4.7 (4.80)	4.02 (3.95)	9.1 (9.049)	16.25 (16.65)	4.82	3.7
$NiL^1(H_2O)_3$	47.23 (47.49)	4.56 (4.80)	4.01 (3.95)	9.4 (9.04)	16.12 (16.59)	3.5	5.5
$CuL^1(H_2O)_3$	47.02 (46.85)	4.15 (4.74)	3.92 (3.90)	8.9 (8.92)	17.02 (17.72)	1.96	2.53
$ZnL^1(H_2O)_3$	47.2 (46.61)	4.65 (4.71)	3.93 (3.88)	8.79 (8.87)	17.82 (18.13)	D	2.4

Calculated values are given in the parenthesis; D-diamagnetic, M – metal,
L-ligand

Table I.3.2

Characteristic Infrared absorption frequencies (cm^{-1}) of transition metal chelates of 2-Hydroxyacetophenone 2-Aminothiophenol (H_2L^1)

Compound	νH_2O cm^{-1}	$\nu C=N$ cm^{-1}	$\nu C-O$ cm^{-1}	ν C-S cm^{-1}	$\nu M-N$ cm^{-1}	$\nu M-O$ cm^{-1}
H_2L^1	3379br	1612 s	1246 m	763m		
$Co(H_2L^1)(H_2O)_3$	3308br	1585 s	1230 m	747m	540m	420w
$Ni(H_2L^1)(H_2O)_3$	3316br	1587	1230	750m	542m	428w

		s	m			
$Cu(H_2L^1)(H_2O)_3$	3329br	1580 s	1226 m	749m	520w	422w
$Zn(H_2L^1)(H_2O)_3$	3304br	1590 s	1227 m	742m	560m	438m

br – broad, s-strong, m-medium, w-weak

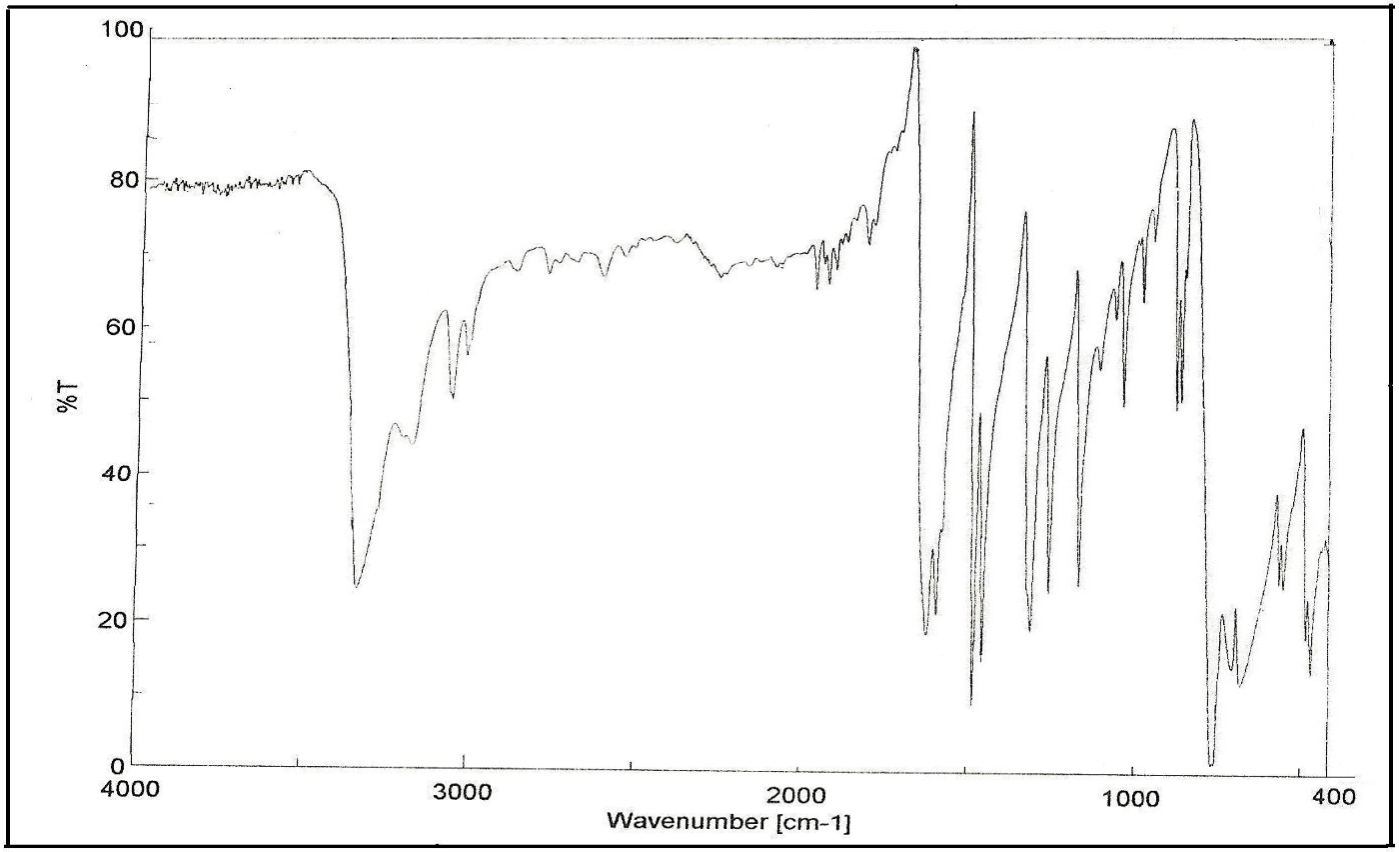


Figure I.3.1: Infra red spectra of HAPATP (H_2L^1)

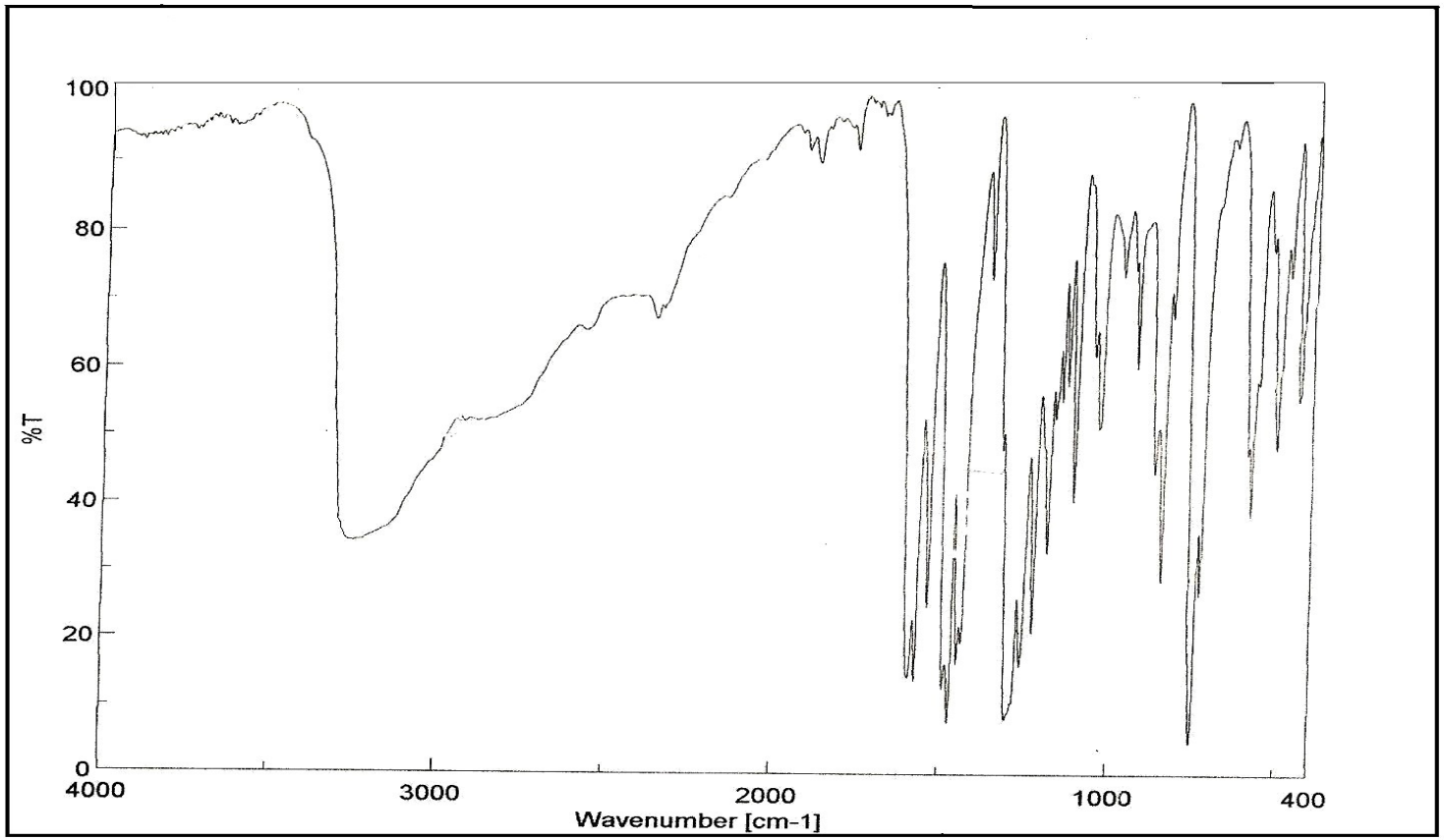


Figure I.3.2: Infra red spectra of $[ZnL^1(H_2O)_3]$

CHAPTER IV

TRANSITION METAL COMPLEXES OF 2-HYDROXY ACETOPHENONE 2-AMINOPHENOL (H_2L^2)

A survey of literature showed that there have been numerous studies on the Schiff bases and metal complexes derived from 2-Aminophenol. However little information is available on metal complexes of Schiff bases derived from 2-Hydroxyacetophenone and 2-Aminophenol. Hence it is considered to be worthwhile and interesting to investigate the properties, structures and geometries of the ligand 2-Hydroxyacetophenone 2-Aminophenol and its complexes with metal ions such as Co(II), Ni(II), Cu(II) and Zn(II).

The present study described the synthesis of a new tridentate Schiff base ligand derived from 2-Hydroxyacetophenone and 2-Aminophenol.

Synthesis of the ligand

For the preparation of the ligand H_2L^2, an ethanolic solution of

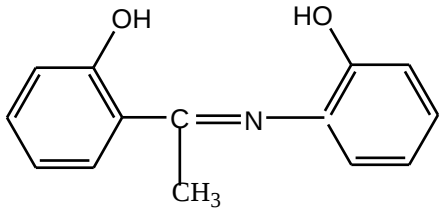
2-hydroxyacetophenone (0.01 mol) was reflexed with an ethanolic solution of 2-aminophenol (0.01 mol) for about 3 hours on a water bath. The product formed was filtered and washed with very dilute

alcohol. It was dried over anhydrous $CaCl_2$. The melting point of the ligand H_2L^2 was found to be 121°C.

Characterisation of the ligand

CHN analysis, IR and UV spetral data and thermal data were used for the characterisation of the ligand HAPAP. The calculated percentages of carbon, hydrogen and nitrogen were found to be in good agreement with observed values. Results are given in the Tables I.4.1 and I.4.2.

Bases on the above results the proposed structure of the ligand is given below.



2-Hydroxyacetophenone 2-Aminophenol

STUDIES On Co(II), Ni(II), Cu(II) AND Zn(II) COMPLEXES OF 2-HYDROXYACETOPHENONE 2-AMINOPHENOL - HAPAP (H_2L^2)

Synthesis of the Complexes

The complexes are prepared by adding a hot aqueous solution of the metal acetate slowly to a refluxing ethanolic solution of the ligand. The resulting solution was refluxed for two hours and then cooled. The separate complex was filtered washed with alcohol and dried.

Characterisation of the Complexes

The complexes were characterised on the basis of elemental analysis, UV and IR spectral data, magnetic studies, conductance measurements and thermal data.

Results and Discussion

All the complexes are coloured, stable and non hygroscopic powdered solids. They are insoluble in water but soluble in organic solvents like methanol, ethanol, acetone, DMSO, etc. The properties of the complexes were analysed on the basis of information obtained from analytical, physiochemical and spectral investigations

Elemental Analysis

The complexes were analysed for metal by standard methods. Carbon, hydrogen and nitrogen were estimated by microanalytical methods. Results are summarized in tables.

Molar Conductance

The conductance measurements in methanol were carried out at a concentration of 10^{-4} M at room temperature and the data are included in the Table I.4.1. All the chelates exhibited very low values of molar conductance (<10 ohm^{-1} cm^2 mol^{-1}) which indicate their non electrolytic nature.

Magnetic Measurements

The details of the methods for the theoretical prediction by magnetic behaviour of the complexes are already explained in the Chapter III. The observed magnetic moment values are summarised in Table I.4.1.

The magnetic moments for the spin free octahedral Co(II) complex ($4T_{1g}$) is higher than the spin only values and it may be due to orbital contributions of the ground state and first excited state. It is reported that the octahedral high spin geometry can be assigned to Co(II) complexes if the observed magnetic moment values are in the range of 4.7 – 5.2BM (**Wilkins RG, 1969**). In the

case of Co(II) complex of HAPAP shows a magnetic moment of 4.94BM prefers an octahedral geometry. The complex of Ni(II) have a magnetic moment value of 3.4BM which is very close to the value of octahedral geometry (**Wilkins RG, 1969**). The magnetic moment observed for Cu(II) complex of HAPAP was 1.9BM as expected for octahedral geometry. The Zn(II) complex was found to be diamagnetic.

Electronic Spectra

The electronic spectral data were found to be in good agreement with the conclusions arrived from magnetic susceptibility measurements.

In the present study Co(II) complex of HAPAP shows the absorption bands of 476nm and 562nm which can be assigned to the transitions [$^4T_{1g}(F) \rightarrow {}^4T_{2g}(P)$, $^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$] respectively. This suggests an octahedral geometry for the complex.

For Ni(II) complex two bands correspond to 564nm and 972nm were obtained. These data supports an octahedral geometry for the complex. In the case of Cu(II) complex absorption band was found to be at 678nm and can be assigned to the d-d transitions in an octahedral environment (**Waters JM, 1964**). For the Zn(II) complex of HAPAP d-d transitions are not possible and the band observed at 399nm is due to charge transfer.

Infra Red Spectra

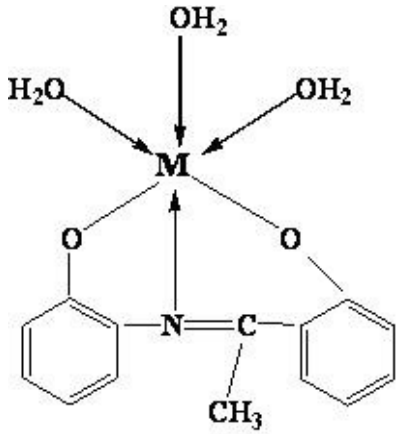
The infrared spectra of the ligand gave the azomethine stretching band at 1603 cm^{-1} and the broad band due to O-H stretching frequency ~3405cm^{-1}. The bands at 1368cm^{-1} and 1254cm^{-1} corresponds to the bending vibrations of O-H and C-O respectively. The IR spectra of the complexes were compared with that of the free ligand to resolve the changes that might have taken place during complexation. The ligand exhibit broad medium intensity bands in the 2700-2450cm^{-1} range, which are assigned to the intra molecular hydrogen bonding, O-H…N, vibrations. This situation is common for aromatic azo methine compounds containing ortho -OH groups and in complexes this band disappeared completely (**Khedry AM, 2005; Ben Saber SM, 2005**). The band at 1603cm^{-1} is the characteristic of the azomethine group present in the free ligand. The lowering of this frequency region to 1585cm^{-1} observed in Cu(II) and Zn(II) complexes of the ligand indicates the involvement of azomethine nitrogen in co-ordination (**Mahapatra BI, 1987**). The same phenomenon can also be observed in cobalt and nickel complexes of the ligand (Table I.4.2). Presence of a broad band around 3378cm^{-1} in Co(II), Ni(II), Cu(II) and Zn(II) complexes corresponds to the OH stretching of co-ordinated water which is further supported by new bands at 871-894cm^{-1}. New bands in the range 420-432cm^{-}

[1] and 520-540 cm^{-1} also appeared in all the complexes. These bands are due to the formation of M-O and M-N bonds (**Ferrare R, 1971**).

TG Studies

Complexes of Cu(II) and Zn(II) were subjected to thermal studies by non-isothermal method. The observed mass loss values from the TG curves were in good agreement with the values obtained from pyrolytic experiments. Probable assignments of each decompositions are discussed in Part II.

Bases on the above results an Hexa co-ordinated structure is assigned to all the metal complexes of HAPAP under study. The general structure of the complexes can be represented as follows.



M = Co(II), Ni(II), Cu(II) and Zn(II)

Table I.4.1

Microanalytical, magnetic and conductance data of transition metal chelates of 2-Hydroxyacetophenone 2-Aminophenol (H_2L^2)

Compound	C %	H %	N%	M%	μ_{eff} B.M	ohm^{-1} cm^2 mol^{-1}.
H_2L^2	73.61 (74.00)	5.24 (5.7)	6.06 (6.16)			
$CoL^2(H_2O)_3$	48.42 (49.71)	4.78 (5.03)	4.23 (4.14)	17.01 (17.43)	4.94	4.6
$NiL^2(H_2O)_3$	48.5 (49.74)	4.95 (5.03)	4.3 (4.14)	16.82 (17.38)	3.4	3.73
$CuL^2(H_2O)_3$	48.20 (49.04)	4.46 (4.96)	3.9 (4.08)	17.92 (18.54)	1.9	3.4
$ZnL^2(H_2O)_3$	47.56 (48.78)	4.4 (4.93)	4.11 (4.06)	17.9 (18.98)	D	5.1

Calculated values are given in the parenthesis; D-diamagnetic, M – metal,

L-ligand

Table I.4.2

Characteristic Infrared absorption frequencies (cm^{-1})of transition metal chelates of 2-Hydroxyacetophenone 2-Aminophenol (H_2L^2)

Compound	νH_2O cm^{-1}	$\nu C=N$ cm^{-1}	$\nu C-O$ cm^{-1}	$\nu M-N$ cm^{-1}	$\nu M-O$ cm^{-1}
H_2L^2	3405 br	1603 s	1254 m		
$CoL^2(H_2O)_3$	3378 br	1579 m	1225 m	520 m	420 w
$NiL^2(H_2O)_3$	3380 br	1586 s	1217 m	524 m	424 w
$CuL^2(H_2O)_3$	3378 br	1585 s	1216	540 m	421 w

			m		
$ZnL^2(H_2O)_3$	3385 br	1585 s	1226 m	525 m	432 w

br-broad, s-strong, m-medium, w-weak

CHAPTER V

TRANSITION METAL COMPLEXES OF BENZIL 2-AMINOTHIOPHENOL (H_2L^3)

The interest in the clinical use of sulphur containing chelating agents as vehicles for delivery of metals to various sites in biological systems as led to the study of Schiff bases derived from Aminothiophenol and Diketones. The properties and reactivity of Schiff base complexes derived from 1,2 Diketones and Aminothiophenols attracts many chemist due to their biological activity as antitumor, antifungal and antiviral agents. So it was thought worthwhile to study the interaction of the ligand Benzil 2-Aminothiophenol with some transition metals.

In this chapter we have described the synthesis of a new tetradentate Schiff base ligand obtained from Benzil and 2-Aminothiophenol. This ligand produces various metal complexes with different transition metal ions such as Co(II), Ni(II), Cu(II) and Zn(II).

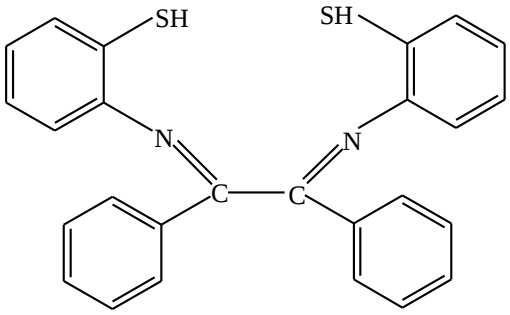
Synthesis of the Ligand

For the preparation of the ligand Benzil 2-Aminothiophenol (H_2L^3) an ethanolic solution of Benzil (0.01 mol) was refluxed with an ethanolic solution of 2-Aminothiophenol (0.02 mol) for two hours on a waterbath. When the light yellow crystals of the Schiff base began to separate the solution was cooled in ice, filtered washed and recrystallised from ethanol. The melting point of the compound was found to be 118°C.

Characterisation of the Ligand

Free ligand has been studies to obtain an insight into their structure and for comparison with their metal complexes. Elemental and spectral studies were carried out to characterise the compound these details are given in the Table I.5.1 and I.5.2. The calculated percentages of carbon, hydrogen and nitrogen were found to be in good agreement with the observed values.

Bases on the above results the structure of the ligand BATP can be given as follows.



Benzil 2-Aminothiophenol (BATP)

STUDIES ON Co(II), Ni(II), Cu(II) AND Zn(II) COMPLEXES OF BENZIL AMINOTHIOPHENOL -BATP (H_2L^3)

Synthesis of the Complexes

The metal complexes were prepared by adding an aqueous solution of the metal acetate to a refluxing ethanolic solution of benzil (0.01 mol) and 2-aminothiophenol (0.02 mol) continued the process for further two hours. The separated complexes were filtered, washed with very dilute alcohol and dried over anhydrous $CaCl_2$.

Co(II), Ni(II), Cu(II) and Zn(II) complexes of the ligand H_2L^3 were prepared by the above method.

Characterisation of the Complexes

The complexes were characterised on the basis of elemental analysis, UV and IR Spectral data, magnetic studies, conductance measurements and thermal data.

Results and Discussion

The nature of carbonyl and amine compounds favours the formation of Schiff base at equilibrium. When these compounds are aromatic the equilibrium favours the formation of the Schiff base since this brings two aromatic rings in conjugation. The metal

complexes of Benzil 2-Aminothiophenol are coloured, stable and non hygroscopic solids. They are insoluble in water but soluble in all organic solvents. The properties, structure and bonding of these complexes have been explained. On the basis of the information obtained from analytical, physicochemical and spectral investigations.

Elemental Analysis

The complexes were analysed for metal and sulphur by standard methods. Carbon hydrogen and nitrogen were estimated by microanalytical methods. The results of this analysis are summarised in Table I.5.1.

Molar Conductance

The conductance measurements in methanol were carried out at a concentration of 10^{-4} M at room temperature and the data are included in the Table I.5.1. All these chelates exhibit a very low value of molar conductance which indicates the non-electrolytic nature of all the complexes.

Magnetic Measurements

The observed magnetic momemnt values of the prepared complexes are summarised in Table I.5.1. Co(II) ion in octahedral ground state ($^4T_{1g}$) possesses considerable orbital contribution with

a magnetic moment in between 4.7-5.2BM. For tetrahedral Co(II) complex the ground term $4A_{2g}$ have no orbital contribution. But due to spin orbit coupling the magnetic moment values vary in the range 4.4-4.7BM. In the present case the magnetic moment of 5.1BM indicates that Co(II) ion is in an octahedral environment (**Lewis J, 1963**). The magnetic moment values of 3.12BM for Ni(II) complex is in accordance with octahedral complexes. Cu(II) complex of BATP possesses normal magnetic moment value of 1.92BM as expected for octahedral Cu(II) complexes. The Zn(II) complex is purely diamagnetic.

Electronic Spectra

The electronic spectra and the magnetic moments support the stereochemistry of the complexes. In the spectra of Co(II) complex the bands at 990nm and 520nm are associated to the transitions $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ and $^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$ respectively. These transitions are specified to the Co(II) ion in the field of octahedral symmetry.

For the Ni(II) complex two d-d transitions correspond to 634nm and 1010nm were observed, hence an octahedral stereochemistry can be assigned for the complex (**Jorgenson, 1956**). The octahedral geometry of Cu(II) complex is clear from the absorption band at 638nm. For the Zn(II) complex of BATP d-d

transitions are not possible and the bands obtained ~390nm are due to charge transfer (**Lever ABP, 1984**).

Infra Red Spectra

The ligand BATP shows bands at 1669cm^{-1} and 2589cm^{-1}, which are due to C=N stretching and S-H stretching respectively. The band at 742cm^{-1} is probably due to C-S stretching (**Koji N, 1962**). Absence of bands in the region 1705-1725cm^{-1} characteristic of C=O vibrations of 1,2diketones also indicate the involvement of carbonyl group in Schiff base formation. The IR spectra of the complexes were compared with that of the ligand to determine the changes that might have taken place during complexation. The band at 1669cm^{-1} is characteristic of the azomethine group present in the free ligand. Lowering of this frequency, to the region (1622-1630cm^{-1}), observed in all the complexes indicates the involvement of the C=N group in co-ordination. The stretching vibrations of S-H have no valuable help since they exhibit weak bands both in ligand and complexes but the participation of S-H group in chelation is confirmed from the shift of C-S stretching frequency of the ligand from 742cm^{-1} to lower frequencies in the complexes. The bands ranging from 515-543cm^{-1} and 460-470cm^{-1} are due to the presence of M-N and M-S bonds respectively. Presence of a broad band around 3400cm^{-1} in the case of metal complexes may be due to O-H stretching of co-ordinated water. The characteristic frequency of free acetate ions

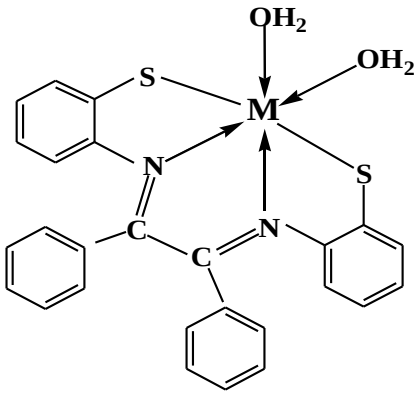
at 1550 and 1455 are absent in all the complexes. The non conducting nature and stochiometry of complexes indicate the absence of acetate ion. New bands corresponding to co-ordinated water also appears in all the complexes.

Therefore from the IR spectrum it is concluded that the ligand BATP behaves as a dianionic tetradentate ligand co-ordinates to the metal ion via deprotonated thiophenol and also azomethine group.

TG Studies

Cu (II) and Zn(II) complexes of BATP were subjected to thermogravimetric analysis. The mass loss data obtained from TG curves were compared with the data obtained from pyrolytic experiments. Both values well agree with in the limits of experimental error.

All the above results suggest octahedral structure for the Co(II), Ni(II), Cu(II) and Zn(II) complexes of BATP and is given as follows.



M= Cu, Zn, Co, Ni

M = Co(II), Ni(II), Cu(II) and Zn(II)

Table I.5.1

Microanalytical, magnetic and conductance data of transition metal chelates of Benzil 2-Aminothiophenol (H_2L^3)

Compound	C %	H %	N%	S%	M%	μ_{eff} B.M	$ohm^{-1} cm^2 mol^{-1}$.
H_2L^3	73.52 (73.58)	5.17 (4.71)	5.98 (6.6)	15.25 (15.09)			
$CoL^3(H_2O)_2$	60.02 (60.35)	4.10 (4.25)	5.32 (5.41)	12.59 (12.38)	10.98 (11.39)	5.1	2.7
$NiL^3(H_2O)_2$	59.83 (60.38)	4.02 (4.25)	5.61 (5.41)	12.25 (12.38)	11.1 (11.36)	3.12	5.64
$CuL^3(H_2O)_2$	58.20 (59.82.)	3.9 (4.21)	5.22 (5.36)	12.94 (13.03)	12.95 (12.81)	1.92	5.7
$ZnL^3(H_2O)_2$	58.93 (59.61)	4.12 (4.20)	5.25 (5.34)	12.59 (12.22)	12.07 (12.49)	D	6.84

Calculated values are given in the parenthesis; D-diamagnetic, M – metal,

L-ligand

Table I.5.2

Characteristic Infrared absorption frequencies (cm^{-1})of transition metal chelates of Benzil 2-Aminothiophenol (H_2L^3)

Compound	νH_2O cm^{-1}	$\nu C=N$ cm^{-1}	ν C-S cm^{-1}	$\nu M-S$ cm^{-1}	$\nu M-N$ cm^{-1}
H_2L^3	3450 br	1669 s	742 m		
$CoL^3(H_2O)_2$	3370 br	1630 s	727 m	467 w	525 w
$NiL^3(H_2O)_2$	3375 br	1629 s	716 m	468 w	525 w
$CuL^3(H_2O)_2$	3373 br	1622 s	716 m	468 m	515 m

$ZnL^3(H_2O)_2$	3382 br	1625 s	720 m	470 m	543 m

br-broad, s-strong, m-medium, w-weak

CHAPTER VI

TRANSITION METAL COMPLEXES OF BENZIL 2-AMINOPHENOL (H_2L^4)

An overview of co-ordination chemistry reveals that only few works have been carried out in the area of Schiff bases involving 1,2 diketones and aminophenols. In the present work tetradentate Schiff base ligand, Benzil 2-Aminophenol containing ONNO donor groups have been synthesised for the first time. This ligand produces various metal ions such as Co(II), Ni(II), Cu(II) and Zn(II).

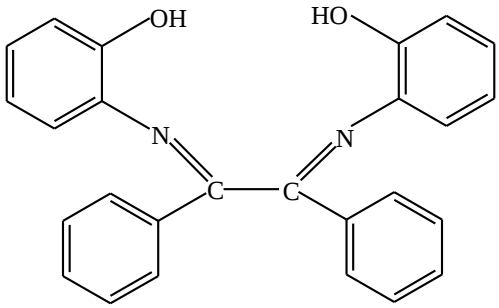
Detailed investigations on synthesis structural and biological aspects of the ligand Benzil aminophenol and its metal complexes are described in this chapter.

Synthesis of the Ligand

A hot ethanolic solution of Benzil (0.01 mol) was added drop wise to a stirred solution of 2-Aminophenol (0.02 mol) dissolved in ethanol. The mixture was refluxed for three hours and then cooled. The precipitate found was filtered, washed with alcohol and then dried over anhydrous $CaCl_2$. Melting point of H_2L^4 was found to be 131^{o}C.

Characterisation of the Ligand

The ligand BAP was characterised on the basis of elemental analysis and spectral data. The UV, IR and NMR spectra of the ligand show characteristic bands. Based on the above results the structure of the ligand was confirmed as given below.



Benzil 2-Aminophenol

STUDIES ON Co(II), Ni(II), Cu(II) AND Zn(II) COMPLEXES OF BENZIL 2-AMINOPHENOL – BAP (H_2L^4)

Synthesis of the Complexes

The complexes were prepared by refluxing an aqueous solution of the metal acetate with an ethanolic solution of the ligand. Sodium acetate (1gm) was added and the resulting solution was again refluxed for 3 hours on a waterbath. The separated complex was filtered, washed with very dilute alcohol and dried well. The metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) were prepared by this method.

Characterisation of the Complexes

The complexes were characterised on the basis of elemental analysis, UV, IR and NMR spectral data, magnetic studies, conductance measurements and thermal data. These results are given in the Tables I.6.1 and I.6.2.

Results and Discussion

The metal complexes of Benzil 2-Aminophenol are coloured, stable and non hygroscopic solids. They are insoluble in water but soluble in organic solvents.

Elemental Analysis

The complexes were analysed for metal percentage by standard methods. Percentages of carbon, hydrogen and nitrogen were estimated by microanalytical methods. The analytical data obtained is summarized in Table I.6.1.

Molar Conductance

Conductance measurements of metal complexes of BAP in methanol at a concentration of 10^{-4} M at room temperature are in the range of 2-10 $ohm^{-1}cm^{2}mol^{-1}$. The very low values indicate that these complexes behave as non-electrolytes and are neutral in nature.

Magnetic Measurements

The values of magnetic moments of the complexes of BAP are tabulated in Table I.6.1. The room temperature, magnetic moments of Co(II) complexes was found to be 4.98BM. The observed magnetic moment for spin free octahedral Co(II) have excess of spin only value and it may be due to orbital contributions of both ground state ($^{5}t_{2g}^{2}eg$) and the first excited state ($^{4}t_{2g}^{3}eg$). It is reported that an octahedral high spin geometry can be assigned to Co(II) complexes it the measured μ_{eff} value is in the range of 4.7-5.2BM. Ni(II) complex exhibited a magnetic moment value of

3.28BM which is very close to the spin only value of octahedral complexes. Hence an octahedral geometry can be assigned to the Ni(II) complex of BAP.

Cu(II) complex of BAP showed a magnetic moment value of 2.02BM. This value corresponds to one unpaired electron of the d^9 electronic configuration which supports the expected octahedral geometry around the metal ion. This value also shows the absence of any antiferromagnetic interaction. As expected, the remaining Zn(II) complex was found to be diamagnetic.

Electronic Spectra

The electronic absorption spectra of the ligand BAP (H_2L^4) exhibits two bands lying around 438nm and 312nm. These bands are due to n→ π* and π→π* transitions. These bands were shifted to higher energy in the complexes which indicates the involvement of the Schiff base in co-ordination.

The electronic spectrum of Co(II) complex gives only one characteristic band at 574nm due to $^4T_{1g}(F) \rightarrow {}^4T_{2g}(F)$ transitions. Ni(II) complex shows two d-d transitions at 877nm and 598nm, which can be assignable to $^3A_{2g}(F) \rightarrow {}^3T_{2g}(F)$ and $^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$ transitions. Cu(II) complex showed an absorption maxima at about 650nm which supports a slightly distorted octahedral geometry.

Zn(II) complex of BAP do not show any characteristic d-d transition bands.

Infra Red Spectra

The IR spectral data of the Schiff base ligand BAP (H_2L^4) and its metal complexes are presented in Table I.6.2. The spectra of the complexes were compared with that of the free ligand to determine the co-ordination sites which involved in chelation. There were some guide peaks in the spectra of the ligand, which were helpful in achieving this goal. In the case of BAP the band found at 1672cm^{-1} is due to the presence of azomethine linkage. The broad bands at ~3400cm^{-1} and ~1305cm^{-1} were respectively due to O-H stretching and bending vibrations. The shifting of C=N stretching band to lower frequency in the range of 1612-1630cm^{-1} indicating its participation in metal co-ordination. All the complexes showed peaks at 3300-3350cm^{-1} corresponding to the O-H stretching frequency of the co-ordinated water molecules. From IR spectra of the ligand and, complexes it is very clear that the bands were absent in the region of 1705-1725cm^{-1} indicates the absence of carbonyl stretching vibrations. The ligand exhibit a broad less intense band in the region 2650-2500cm^{-1} indicates the possibility of intra moledular hydrogen bonding between O-H and azo methine nitrogen. This band disappeared completely in complexes implies the involvement of O-H and >C=N in chelation. New bands

are found in the spectrum of the complexes in the region 540-568cm^{-1} and 424-433m^{-1} indicate the presence of M-N and M-O bonds respectively. The C-O bond in the free ligand at 1250cm^{-1} is shifted to lower frequency indicating the participation of phenolic group in the complex formation.

IR data showed that the ligand is tetradentate dianionic ligand coordinating to the metal ion through two azomethine nitrogen and two deprotonated phenolic oxygen atoms.

NMR Spectra

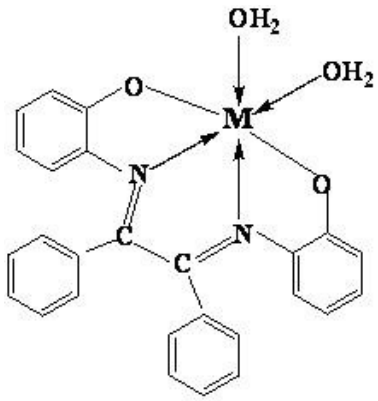
The NMR spectra of the Schiff base ligand BAP was recorded in DMSO solution using TMS as internal standard. The spectra of the diamagnetic Zn(II) complex was examined in comparison with that of the free ligand. The singlet at 10.2ppm corresponding to O-H protons in the free ligand disappears in Zn(II) complex indicates the co-ordination. A multiplet at 6.5-7.5ppm corresponds to aromatic protons. An additional peak at 3.36ppm showed the presence of two co-ordinated water, whose intensity corresponds to four hydrogen.

TG Studies

Cu(II) and Zn(II) complexes of BAP were subjected to thermal studies by non isothermal method. The thermogram of complexes were recorded using a sample weight of 5mg in static air

atmosphere. A three step decomposition was observed for Cu(II) Complex whereas Zn(II) Complex decomposes in two steps above 120°C. The probable assignments for each decomposition are described in Part II.

On the basis of the above results these complexes can be represented by the following structure.



M = Co(II), Ni(II), Cu(II) and Zn(II)

Table I.6.1

Microanalytical, magnetic and conductance data of transition metal chelates of Benzil 2-Aminophenol (H_2L^4)

Compound	C %	H %	N%	M%	μ_{eff} B.M	$ohm^{-1} cm^2 mol^{-1}$.
H_2L^4	79.17 (79.5)	5.4 (5.10)	6.84 (7.14)			
$CoL^4(H_2O)_2$	63.9 (64.33)	4.12 (4.53)	5.5 (5.77)	12.02 (12.15)	4.98	3.80
$NiL^4(H_2O)_2$	63.79 (64.36)	4.06 (4.53)	5.84 (5.77)	11.97 (12.11)	3.28	6.8
$CuL^4(H_2O)_2$	62.5 (63.73)	4.22 (4.49)	5.5 (5.71)	12.56 (12.97)	2.02	3.92
$ZnL^4(H_2O)_2$	63.0 (63.49)	4.28 (4.47)	5.54 (5.69)	12.78 (13.30)	D	6.2

Calculated values are given in the parenthesis; D-diamagnetic, M – metal,

L-ligand

Table I.6.2

Characteristic Infrared absorption frequencies (cm^{-1}) of transition metal chelates of Benzil 2-Aminophenol (H_2L^4)

Compound	νH_2O cm^{-1}	$\nu C=N$ cm^{-1}	$\nu C-O$ cm^{-1}	ν M-O cm^{-1}	ν M-N cm^{-1}
H_2L^4	3400 br	1672s	1250 m		
$CoL^4(H_2O)_2$	3341 br	1630s	1228 m	424 w	540 w
$NiL^4(H_2O)_2$	3356 br	1625s	1224 m	433 m	563 m
$CuL^4(H_2O)_2$	3350 br	1625s	1227 m	433 m	564 w
$ZnL^4(H_2O)_2$	3344 br	1612s	1227 m	430 m	568 w

br-broad, s-strong, m-medium, w-weak.

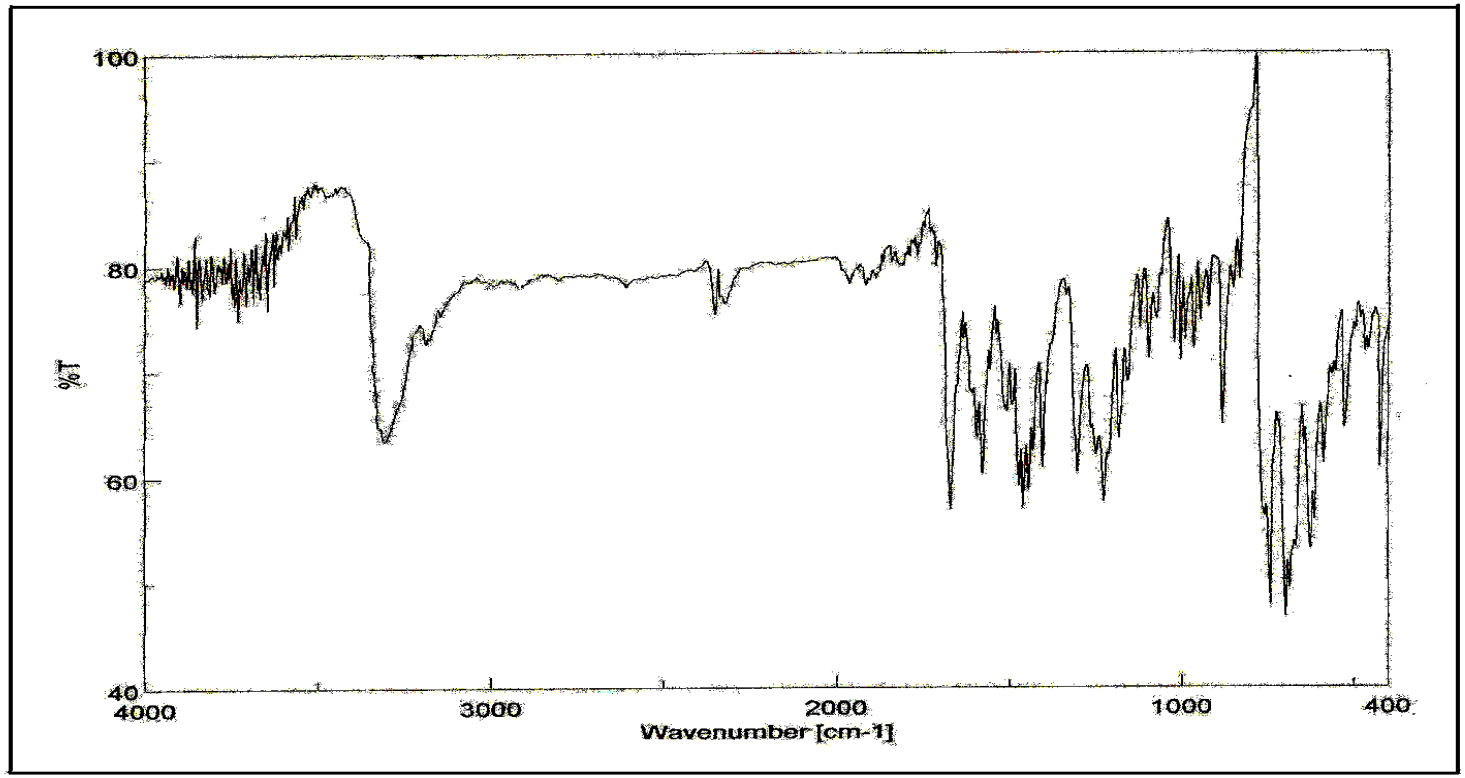

100
80
60
40
%T
4000
3000
2000
1000
400
Wavenumber [cm-1]

Figure I.6.1: Infra red spectra of BAP [H_2L^4]

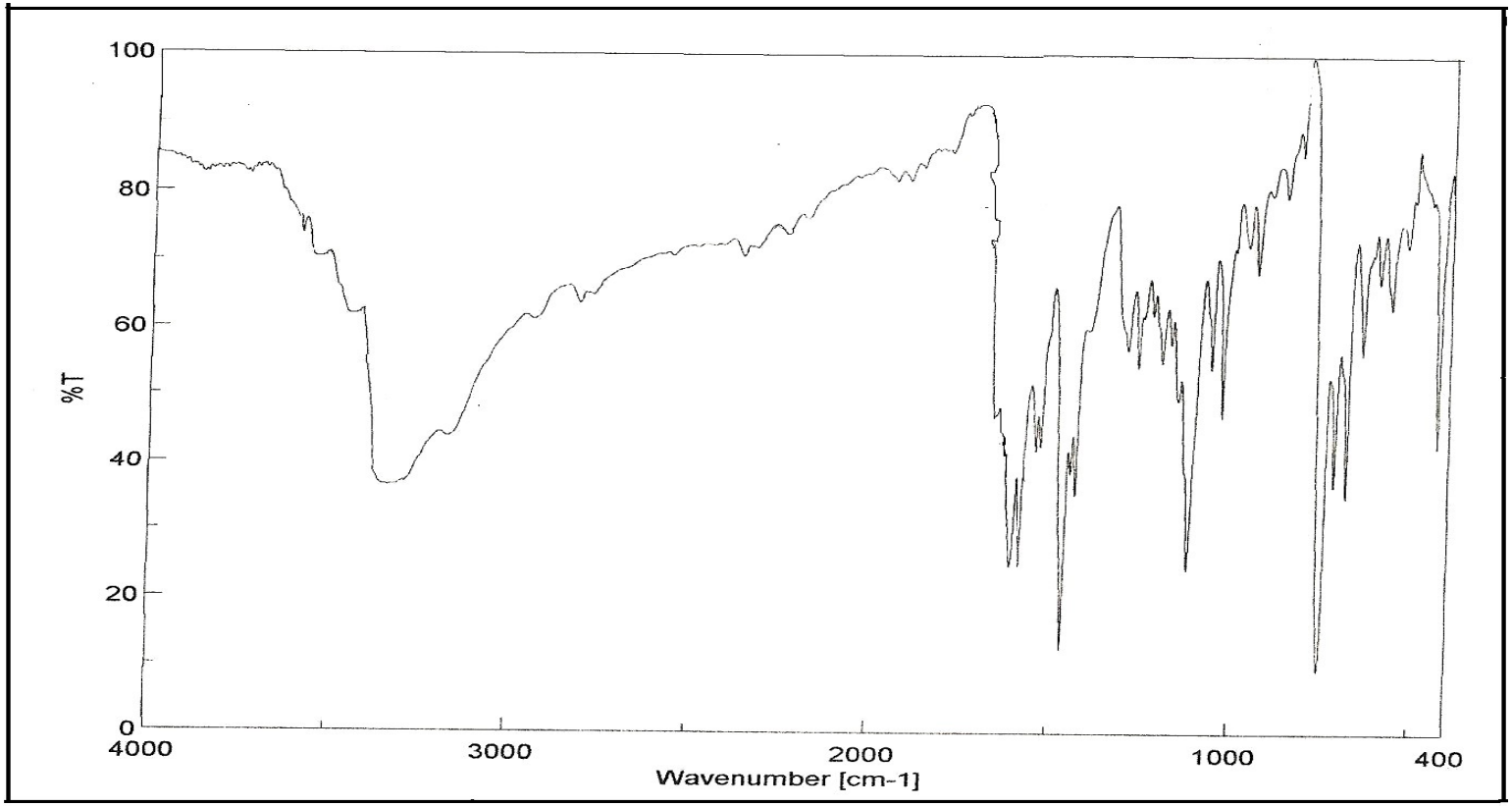


Figure I.6.2: Infra red spectra of $[CuL^4 (H_2O)_2]$

References

1. Abdel SD, Mohammed IA. *Transition Metal Chemistry*, 23:391-396, 1998.
2. Angela K, Mitu L. *Asian Journal of Chemistry*, 19: 7, 2007.
3. Ashash S, Shabana B. *Appln. Surf. Scien.,* 252: 4063, 2006.
4. Atef AT, Ramdan. *Thermo Chemica Acta*., 206: 327, 1992.
5. Baluja S, Solanki A, Kachhadi N. *Journal of Iranian Chemical Society*, 34: 312-313, 2006.
6. Barber DE, Richardson T, Crabtree RH. *Inorg. Chem.*, 31: 4709, 1992.
7. Ben Saber Sm, Maihub AA. *Micro Chem. J.* 81: 191, 2005.
8. Bhava B. *Indian Journal of Chemistry*, 9: 590, 1971.
9. Busch DH, Bailor JC. *J. Am. Chem. Soc*, 78: 1137*,* 1956.
10. Busch DN. *American Chemical Society*, 37: 1963.
11. Cad VT. Gossypol and its new derivatives, Synthesis and study of biological Activities. *Ph.D, Thesis,* Institute of Chemistry of Natural Substances, University Paris, 2002.
12. Chatterjee D, Mitra M, Shepherd R*. Inorganica Chimica Acta*, 357(4): 980-990*,* 2004.
13. Chinnaswamy J, Karuppannan N. *Transition Metal Chemistry*, 27:
    75-79*,* 2002.
14. Chohan ZH, Arif M, Muhammed AA, Claudiu TS. *Bioinorganic Chemistry and Application*., Article ID 83131, 2006.
15. Chohan ZH, Farooq MA, Claudiu TS. *Metal Based Drugs.*, 7: 171-177, 2000.

16. Clothup NB, Daly LH. Introduction to IR and Ramanspectroscopy Academic Press, 2nd Edn., 1975.

17. Cotton FA, Wilkinson G, Gaus PL. *Basic Inorganic Chemistry*, 3rd Ed., J. Wiley and Sons, New York, 1995.

18. Cotton FA, Willkinson G, Murillo CA, Bochmann M. *Advanced Inorganic Chemistry*, New York; John Wiley & sons, Inc, , 1999.

19. Crabtree RH. *The organometallic Chemistry of the Transition Metals*, John Wiley & Sons, Inc; New York, 1994.

20. Dashora R, Singh RV, Tandon JP. *Indian J. Chem, Sect.A*, 25(2): 188-190, 1986.

21. David GC, Elena L. *Inorg. Chem., Web Release Date*: October 16, 2007.

22. Dimitra K, Nikolaos K, Douglas X, Martinz JV. *Eur.J.Inorg. Chem.*,
861-63, 1998.

23. Djebbar SS, Benali BO. Polyhedron, 16: 2175-2182, 1997.

24. Dutta RL, Syamal A. *Elements of Magneto Chemistry*, 2nd Edn. Elsevier, 1992.

25. Earnshaw A, King EA. *Journal of Chemical Society*, Sect. A, 1968.

26. Eggleston, Jackel SC. *Inorg. Chem.*, 19: 1593, 1980.

27. Elder MS, Prinz GM, Thornton P. *Inorg. Chem.*, 7: 2426, 1968.

28. Elif K, Elsma K. *Talanta*, 58(4): 793-802, 2002.

29. Erskine PT, Newbold R, Roper J, Coker A, Warren MJ, Wood PM, Cooper SP. *Protein Science*, 8(6): 1250-1256, 1999.

30. Ettling C. *Ann.Chem. Pharm.*, 35: 241.

31. Faizul A, Satendra S. *J Zhejiang, Univ Sci. B*, 8(6): 446–452, 2007.

32. Fenton DE*. Pure Appl. Chem*., 58: 1437, 1986.

33. Ferrare R. Low frequency vibrations of Inorganic and co-ordination compounds, Plenum Press, New York, 1971.

34. Figgis BN, Nyholm RS. *Journal of Am. Chem. Soci*.: 4190, 1958.

35. Furman NH. Standard Methods of Chemical Analysis, D. Van Nostrand Company, 1962.

36. Genet JP, Ferroud D, Juge S, Montes JR. *Tetrahedron Lett.* 38: 4557-4576, 1986.

37. Geory WJ, *Co-ord. Chem. Rev.* 7: 81, 1971.

38. Gillian FM, Urich A, John RT. *J. Chem. Soc. Chem commun.*, 1772, 1990.

39. Gudasi KB, Nadagouda GS, Goudar TR. *Journal of the Indian Chemical Society*, 83: 376-378*,* 2006.

40. Hobday MD, Smith TD. *Coord.Chem.Rev*., 9: 311, 1972.

41. Issa YM, Omar HM, Soliman AA. *J.Indian, Chem.Soc*.: 73*,* 1998.

42. Jackels SC, Farmerry K, Barefield. *Inorg. Chem,* 11: 2893, 1972.

43. Jaya Murty J, Meha BH. *Orient. J.Chem*, 14: 129-131, 2001.

44. Jeong T, Lee HK, Jeong DC, Jeon S. *Talanta*, 65: 543*,* 2005.

45. Jia AG, Song QL. *Journal of Photochemistry and Photobiology,* 162:
399-406, 2004.

46. Jorgenson CK. *Acta Chem.:* 10, 1956.

47. Karno NG, Ratnaswamy S, Patric JW. *Tetrahedron,* 12: 1719-1722, 2001.

48. Khalifa MA, Hassan AM. *J. Chem.Soc. Pak.*, 18: 115-118,1996.

49. Khalil MM, Attia AE. *J.Chem. Eng. Data*, 44: 180, 1999.

50. Khedry AM, Gaber M. *Dyes and Pigments,* 67: 117, 2005.

51. Koji N. Infra red absorption spectroscopy, Holden day, Inc. 1962.

52. Kolwalker SD, Mehta BH. *Asian.J.Chem*, 8: 406-410*,* 1996.

53. Krumhotz P. *J. Am. Chem. Soc.*, 75: 2163,1954.

54. Kumar NRS, Nethji M, Patil KC. *Polyhedron*, 10: 365-371, 1991.

55. Lever ABP. Inorganic Electronic Spectroscopy, 2nd Edn. Elsevier, 1984.

56. Lewis J, Wilkins RG, Modern Co-ordination Chemistry, Interscience, New York, 1963.

57. Lindoy LF. *J. Am. Chem.Soc.*, 25: 379*,* 1971.

58. Mahapatra B, Das DK. *Indian Journal of Chemistry*, A26: 173, 1987.

59. Maryam L, Davar MB. *Synthesis and Reactiviy in Inorganic, Metal Orrganic and Nano metal Chemistry*, 31(8): 1519-1529*,* 2001.

60. Masoud MS, Hindawy AM. *Trans. Met. Chem.* 16: 372-376, 1991.

61. Mayadevi S, Yussuff KK. *Synth.React. Inorg. Met. Org. Chem*, 9:
624-629, 1997.

62. Minu GB, Li kam WH, Dosieah A, Ridana M, Lacour D. *Synthesis and Reactivity in Inorganic and Metal-organic Chemistry*, 34: 1-16, 2004.

63. Mukhargee AK, Saha AK, Rudra I, Rameesh S. *Inorg.Chim. Acta.:* 2004.

64. Mukherjee S, Samanta S, Roy BC. *Appl. Catal.A.*, 301: 79, 2006.

65. Murty JJ, Mehta BH. *Orient.J.Chem.*, 14: 129-131, 1998.

66. Nahar CT, Mukhedhar AJ. *J. Indian Chem. Soc*., 58: 343-346, 1981.

67. Nair R, Shah A, Baluja S, Chanda S. *J. Serb.Chem.Soc.*, 71(7): 733- 744., 2006.

68. Nakomoto K. IR Spectra of Inorganic and Co-ordination Compounds, 2nd Edn. Willey Interscience, 1970.

69. Nelson SM. *Pure Apple. Chem.*, 52: 2461, 1980.

70. Nursen S. *Gazi University Journal of Science*, 16(2): 283-88, 2003.

71. Offiong EO, Emmanuel N. *Transtion Metal Chemistry*, 25: 4, 2004.

72. Osmond JD, Yanhong Dong, Faith MU. *Biology of reproduction*, 60: 435-444, 1999.

73. Papi S, Koprivanac N, Grabari Z. *Dyes and Pigments*, 25(3): 229-240,1994.

74. Prasad RN, Mithlesh A, Madhulikaa S. *J.Serb.Chem.Soc.,* 67: 229-234, 2002.

75. Raman N, Kulandaiswamy A. *Polish.J. of Chem*., 76: 1085-1094, 2002.

76. Raman N, Kulandaisamy A. *Synth. React.Inorg,Met-Org.Chem.*, 31: 1270, 2001.

77. Raman N, Muthuraj V, Ravichandran S. *Proc. Indian Acad.Sci*, 115(3): 161-167, 2003.

78. Remeswar Shukla and Parimal KB. *Indian.J.Chem*., 32A: 767, 1993.

79. Sakyan I, Neela G, Truget G. *Web article*, Ankara University Science Faculty.

80. Salameh AS, Tayim HA. *Polyhedron*, 2: 829-34, 1983.

81. Sanchez G, Momblona F, Serrano JL, Garcia L. *Journal of Coordination Chemistry*, 55(8): 917-923, 2002.

82. Sargeson AM. *Pure Appl. Chem.*, 56: 1603, 1984.

83. Schiff H. *Ann.Chem. Pharm., Suppl*., 3: 343, 1864.

84. Shriver DF, Atkins PW, Langford CH. *Inorganic chemistry* 2nd Ed., Oxford University Press; Oxford , 1994

85. Soliman AA, Linert W. *Thermochimica Acta*, 338: 1-2, 67-75, 1999.

86. Solomons TWG. *Fundamentals of Organic Chemistry*,2nd ED., John Wiley and Sons, New York, 1986.

87. Sonabati AZ, Bindary AA. *Pol. J. Chem*., 4(5): 621-630, 2000.

88. Sorianco GM, Toscano RA, Valdes JM. *Acta Cryst.*, c41: 498, 1985.

89. Stoufer RC, Busch DH. *J.Am.Chem. Soc.*, 682: 3491, 1960.

90. Subatra M, Rameshwar S, Parimal KB. *Polyhedron*, 4: 15-16, 2063-2070,1995

91. Susan S, Abolfazal G, Mohammed AN, Hashem S. *Sensors and Acutators B*, *Chemical*, 98(15): 109-116, 2004.

92. Sutton, Electronic Spectra of Metal Complexes McGraw-Hill London, 1968.

93. Tayim HA, Salameh AS. *Polyhedron*, 2:1091-94, 1983.

94. Tez Can. *Chim.Acta. Turc*, 12(2): 376*,* 1984.

95. Thanga Durai TD, Natarajan K. *Tran. Met. Chemistry*, 27: 485-487, 2002.

96. Thangadurai TD, Natarajan K. *Indian journal of chemistry. Sect. A:* 41: 741-745, 2002.

97. Thomas R, Thomas J, Parameswaran G*. Ind. Journal of Chemistry,* A21: 83*,* 1982.

98. Thomson MC, Busch DH. *J. Am. Chem.Soc.*, 86: 3651*,* 1965.

99. Toshihiko T, Arnd B, Cindy MQ, Melvin IS, Thomas JM, Harry BG.
J. Am. Chem. Soc., 120: 8555-855*,* 1998.

100. Tudor R, Simona P, Veronica L, Carmen CRC. *Molecules*, 11: 904-914, 2006.

101. Verma M, Pandeya SN, Singh KN. *J. Acta Pharm.* 54(1): 49-56, 2004.

102. Vinod KS, Shipra S, Ankita S. *Bioinorg Chem Appl.* 2007.

103. Viswanathan P, Natarajan K. *Synthesis and Reactivity in Inorganic, Metal- organic and Nanometal Chemistry*, 36(5): 415-418, 2006.

104. Vogel AI. A textbook of Quantitative Inorganic Analysis, ELBS, 1978.

105. Vogel AI. Quantitative Inorganic Analysis Including Elemental Instrumental Analysis, 2nd Edn. Longmans, London, 1962.

106. Waters JM, Waters TN. *Journal of Chemical Society*, 2489, 1964.

107. Weissberger A, Proskauer DS. *Organic solvents*, Interscience, New York, 2, 1956.

108. Welsh WA, Reynolds GJ, Hanry PM. *Inorg. Chem.*, 16: 2558, 1977.

109. Wen TG, Zhuo Zheng. *Molecules*, 7:511-516, 2002.

110. West DX, Pandye SB, Sonawane RC. *Asian J. Chem. Rev.*,1: 125, 1990.

111. Wilkins RG, Lewis G. The magneto Chemistry or Complex compounds in co-ordination Chemistry, Interscience, New York, 1969.

112. Wulsberg G. Principles of Descriptive Inorganic Chemistry, Brookes/Cole Publishing; California, 1987.

113. Yaxian Y, Jianlin Y. *Synthesis and Reactivity in Inorganic, Metal Organic and Nano metal Chemistry*, 35(5): 385-390, 2005.

114. Zdena DM, Antonia MM, Teresa SW, Alander Valent. *Bioelectrochemistry*, 48(1): 109-116, 1999.

CHAPTER I

INTRODUCTION

Thermal decomposition studies of Inorganic metal complexes has been received considerable attention recently because it can be used in the structural and kinetic investigation of coordination compounds. This analytical method is composed of different techniques such as Thermogravimetry (TG), Differential Thermal Analysis (DTA), Derivative Thermogravimetry (DTG) and Differential Scanning Calorimetry (DSC). Thermogravimetric curves give valuable information on the temperature regions of stability and also on the temperature of inception of maximum rate and of completion of decomposition. DTA curves deals about the enthalpy changes occurring during heating and they give valuable information complementary to that derived from TG curves (**Duval C, 1963; Smoothers, 1966; Mackenzie RC, 1970**).

Thermogravimetry is one of the oldest thermoanalytical technique in which the weight loss of the sample is monitored in a chosen atmosphere (usually nitrogen or air) as a function of temperature. This method has been extensively used in different fields of science (**Christian S, 2004**). It also has wide application in the structural studies of Co-ordination compounds (**Yilmaz I,**

2004; Osmann AH, 2004). The resulting mass change versus temperature curve gives information about the thermal stability and composition of the intial sample. The instrument used is a thermobalance with a furnace programmed for a linear rise of temperature with time.

When the kinetic study is based on observation of the weight change, two approaches are possible namely isothermal (static) and non isothermal (dynamic) heating methods. The non isothermal method is the determination of the degree of transformation as a function of time during a linear increase of temperature compared with static method. Advantage of non isothermal method over isothermal method is that it requires smaller number of experimental data and the kinetic parameters may be determined from a single thermogravimetric curve for the whole temperature range (**Young DA, 1966**).

The derivative thermogravimetric curve may be obtained either by manual differentiation of the TG curve or by suitable instrumentation. It gives the relationship between the rate of weight change and the temperature. DTG curves have a number of peaks instead of steps. These curves have certain similarities to the DTA curves which allows to a certain extent, comparison to be made (**Paulik F, 1968; Wilburn FW, 1963**). TG is inherently quantitative and therefore it is an extremely important thermal

technique. The ability to analyse the volatile products during a weight loss is of great value in structural studies of metal chelates. The use of TG to generate a fundamental quantitative data from almost any class of compounds has led to its wide use in each and every field of science and technology *(***Wendlandt WW, 1964**).

Several factors could influence the nature of TG curve. The prime factors are, heating rate and sample size. Increase in either of which tends to increase the temperature of decomposition and to decrease the resolution between successive mass losses. Careful attention to consistency in experimental details normally results in good reproducibility.

The important fields in which the thermal analysis technique is applied can be summarised as follows.

Thermal Stability:– Related materials can be compared at elevated temperatures under a required atmosphere. The TG curve obtained can help to elucidate the decomposition mechanism.

Kinetic Studies:- Various methods exist for analysing the kinetic features of all types of weight loss or gain to understand the mechanisms in chemistry (**Galway AK, 1979**).

Material Characterisation

TG and DTA curves can be used to characterise materials for identification. It has obvious uses in the determination of moisture content of materials, water of hydration and of Carbon Monoxide and Carbon Dioxide evolution from carbonates etc (**Vatsala S, 1986**).

In Inorganic chemistry this method has been widely used to study the kinetic and thermal behaviour of Co-ordination compounds. The valuable information given by thermogravimetric analysis of Co-ordination compounds includes,

a) Temperature regions of stability

b) Temperature of inception of maximum rate.

c) Temperature of the completion of decomposition.

On the other hand the DTA curves give the information like enthalpy changes during the decomposition (**Brown ME, 2002**).

Scope of the Present Investigation

Much studies have not been reported on thermal decomposition reaction, kinetics and stability of metal chelates of Schiff bases derived from 1,2diketones and hydroxy ketones (**Scency CG, 1976**).

In this part the results of the studies, on the thermal decomposition of Cu(II) and Zn(II) complexes of 2-

Hydroxyacetophenone2-Aminothiophenol (HAPATP), 2-Hydroxyacetophenone2-Aminophenol (HAPAP), Benzil 2-Aminothiophenol (BATP) and Benzil 2-Aminophenol (BAP) are presented.

From the TG Curves the temperature regions of decomposition and corresponding mass loss have been noted. It also helps to define the stoichiometry, kinetics and mechanism of decomposition of metal complexes under consideration. The thermogravimetric results can be used to (i) decide whether the water molecules (if present) are inside or outside of the co-ordination sphere of the central metal ion and (ii) suggest probable assignments for the various stages of decomposition, which can be useful in structural studies.

CHAPTER II

MATERIALS, METHODS AND INSTRUMENTS

Materials

Analar grade chemicals supplied by Sigma, BDH, E-Merck, Glaxo were used for synthetic purpose. Commercial solvents were distilled by standard method. Detailed discussion regarding the reagents and their purity are given in part I.

Methods

The ligands 2-Hydroxyacetophenone 2-Aminothiophenol HAPATP (H_2L^1), 2-Hydroxyacetophenone 2-Aminophenol HAPAP (H_2L^2), Benzil 2-Aminothiophenol BATP (H_2L^3) and Benzil 2-Aminophenol BAP (H_2L^4) were synthesised by the procedures discussed in Part I. The preparative methods of the metal complexes and their characterization were also discussed in that part.

Instruments

Various instruments used for the thermogravimetric studies are

1. Perkin – Elmer 7 Series Thermal Analyser System.
2. Horizon III Mini Computer.

CHAPTER III

THERMOGRAVIMETRIC ANALYSIS OF Cu(II) AND Zn(II) COMPLEXES OF 2-HYDROXYACETOPHENONE 2-AMINOTHIOPHENOL (HAPATP) AND 2-HYDROXYACETOPHENONE 2-AMINOPHENOL (HAPAP)

Thermoanalytical studies play and important role in assigning the bonding and structure of Schiff base complexes (**Rao NS, 1990**). It also helps to define the stoichiometry, kinetics and mechanism of decomposition of metal complexes under consideration. Wendlandt and co-workers (**Wendlandt, WW, 1970; Chang FC, 1971; Perry DL, 1974**) studied the thermal properties of metal chelates with different types of complexing ligands. The use of thermoanalytical technique to follow the reaction of the metal ions during the course of thermal decomposition has been reported by Wendlandt.

The results of the studies on thermal decomposition of Cu(II) and Zn(II) complexes of 2-Hydroxyacetophenone Aminothiophenol $[H_2L^1]$ and 2-Hydroxyacetophenone Aminophenol $[H_2L^2]$ are discussed in this chapter.

Experimental

The metal complexes concerned were prepared and characterised in accordance with the procedure described in Part I. In the present investigation, heating rates were suitably controlled at 10°C min^{-1} under static air atmosphere and sample weight of 3-10mg were used for the whole study. The weight loss for each metal chelates was calculated with in the corresponding temperature ranges.

Treatment of Data

The instrumental TG and DTG traces were used as such. They are represented in Figures II.3.1 - II.3.4. The TG curves of the above metal chelates were studied in detail. The thermal data obtained for each metal chelates are given in Tables II.3.1 and II.3.2. Data from independent pyrolytic experiments are also included in these tables.

Results and Discussion

Structural analysis using various analytical techniques established that complexes of 2Hydroxyacetophenone 2-Aminothiophenol (HAPATP) and 2Hydroxyacetophenone 2-Aminophenol (HAPAP) have the general formula $[ML(H_2O)_3]$ were M=Cu(II) and Zn(II) and L = ligand moiety. The TG curves of this chelates also show decomposition pattern with different stages.

This data supports the above proposed formula for the metal chelates.

i. Cu(II) and Zn(II) complexes of HAPATP

Metal percentage from independent pyrolytic experiments and from thermal studies was found to agreeable with the calculated values, in the case of Cu(II) and Zn(II) complexes of HAPATP. In these complexes the decomposition started only above 120°C and the absence of decomposition at temperature ranges 60-90°C indicates that the water molecules present in the metal chelate are not in the lattice but co-ordinate to the metal ion (**Nikolaev AV, 1969**).

A three stage thermogram was obtained for $CuL^1 (H_2O)_3$. The first stage of the decomposition from 120-180°C stands for the removal of three co-ordinated water molecules with a mass loss of 13.34% (Calcd.15.06%). The second step involves the decomposition of aminothiophenol part of the organic ligand at temperature range of 180-230°C with a mass loss of 34.9% (Calcd.34.02%). The third decomposition in the temperature range 230-400°C with a mass loss of 30.66% (Calcd.28.72%) corresponds to the removal of 2-hydroxyacetophenone residue. Above 400°C, the metal oxide is formed and overall loss of mass as read from the TG curve is

comparable to the theoretical loss for the conversion of $CuL^1(H_2O)_3$ to CuO.

In the case of Zn(II) chelate the decomposition follows a two stage pathway. The first decomposition corresponds to the loss of three co-ordinated water molecules with a mass loss of 15.6% (Calcd.14.98%) at the temperature range of 110-180°C. The second step involves the complete decomposition of the ligand part corresponding to a mass loss of 64.10% (Calcd. 62.43%), which is the main decomposition stage.

ii. Cu(II) and Zn(II) complexes of HAPAP

The Cu(II) complex of the ligand HAPAP, shows a two stage decomposition pattern. The first stage is in the temperature range of 110-160°C, which may account for the loss of two co-ordinated water molecules, with a mass loss of 9.2% (Calcd. 10.52%). The second stage stands for the loss of one water molecule followed by the ligand part. Temperature range for this decomposition is 160-420°C and the corresponding weight loss is 68.30% (Calcd. 66.26%).

For $ZnL^2(H_2O)_3$ chelate also, a two stage decomposition pattern was observed. The first stage of decomposition at the temperature range of 100-200°C represents the loss of three water molecules and the hydroxyacetophenone part of the ligand. Here

the mass loss obtained is 48.14% (Calcd.50.23%). The second stage represents the removal of remaining part of the ligand with a mass loss of 30.32% (Calcd. 26.15%) in the temperature range of 200-450°C. Above 450°C the metal oxide is formed and overall weight loss is 78.46% (Calcd. 76.38%).

The temperature range of decomposition and the details of decomposition pattern of the Cu(II) and Zn(II) complexes of 2-Hydroxyacetophenone Aminothiophenol (HAPATP) and 2-Hydroxyacetophenone Aminophenol (HAPAP) are given in Tables II.3.1 and II.3.2. The thermal data obtained also support the assigned geometries of the ligands and complexes as explained in Part I.

Table II.3.1

Thermal Decomposition Data of Cu(II) and Zn(II) complexes of 2-Hydroxyacetophenone 2-Aminothiophenol (HAPATP)

Complex	Stage	Temperature range in TG °C	Loss of Mass Percentage			Probable Assignments
			From TG	Theoretical	From Pyrolysis	
$CuL^1(H_2O)_3$	I	120-180	13.34	15.06		Loss of 3 co-ordinated H_2O molecules
	II	180-230	34.90	34.02		Loss of aminothiophenol part
	III	230-470	30.66	28.72		Loss of hydroxy acetophenone part
			78.90	77.80	78.02	$CuL^1(H_2O)_3 \rightarrow CuO$
$ZnL^1(H_2O)_3$	I	110-180	15.60	14.98		Loss of 3 co-ordinated H_2O molecules
	II	180-500	64.10	62.43		Loss of ligand part
			79.70	77.41	78.50	$ZnL^1(H_2O)_3 \rightarrow ZnO$

Table II.3.2

Thermal Decomposition Data of Cu(II) and Zn(II) complexes of 2-Hydroxyacetophenone 2-Aminophenol (HAPAP)

Complex	Stage	Temperature range in TG °C	Loss of Mass Percentage			Probable Assignments
			From TG	Theoretical	From Pyrolysis	
$CuL^2(H_2O)_3$	I	110-160	9.20	10.50		Loss of 2 co-ordinated H_2O molecules
	II	160-420	68.30	66.26		Loss of one co-ordinated water + ligand part
			77.50	76.76	77.20	$CuL^2(H_2O)_3$ → CuO
$ZnL^2(H_2O)_3$	I	100-200	48.14	50.23		Loss of 3 co-ordinated H_2O molecules + hydroxy acetophenone part
	II	200-450	30.32	26.15		Loss of aminophenol part of the ligand
			78.46	76.38	78.10	$ZnL^2(H_2O)_3$ → ZnO

TGA

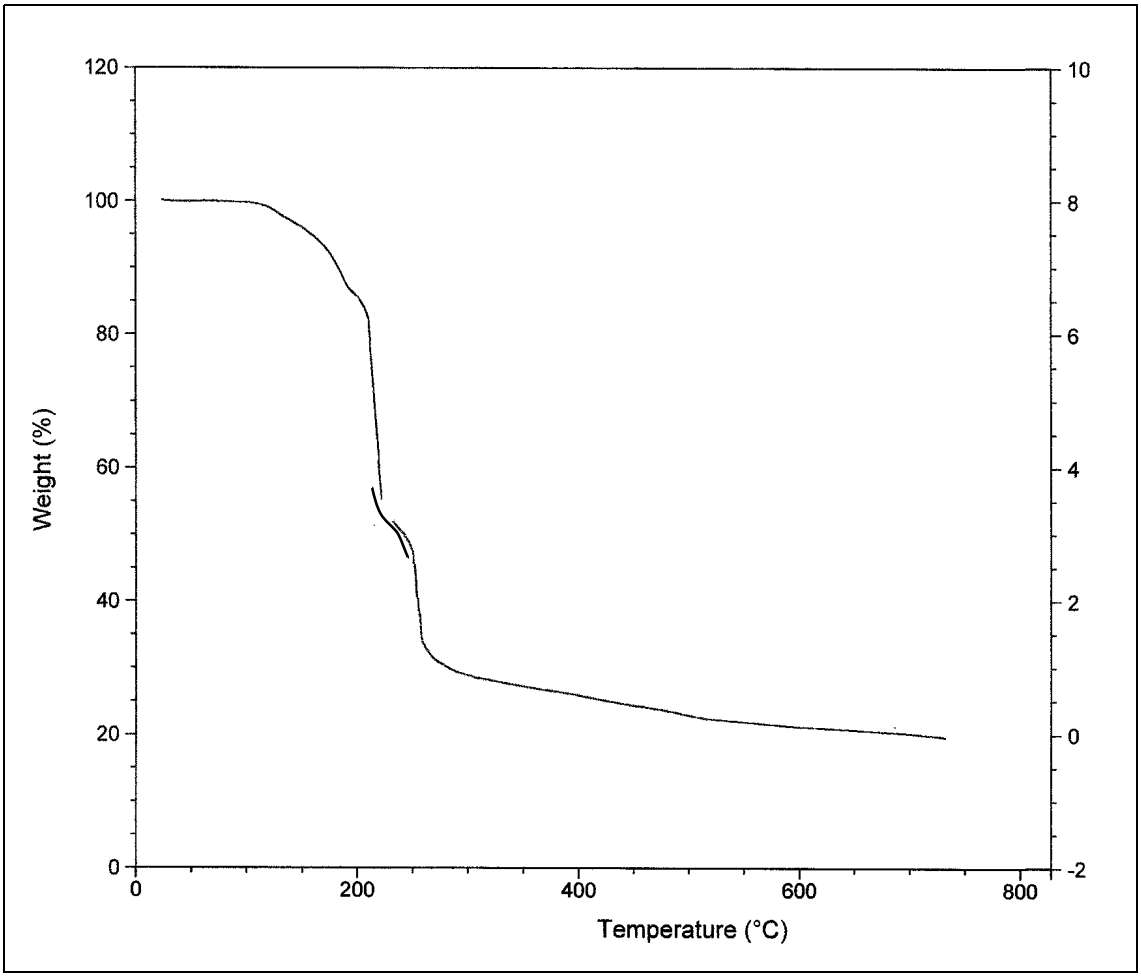


Figure II.3.1: TG Traces of $[CuL^1(H_2O)_3]$

TGA

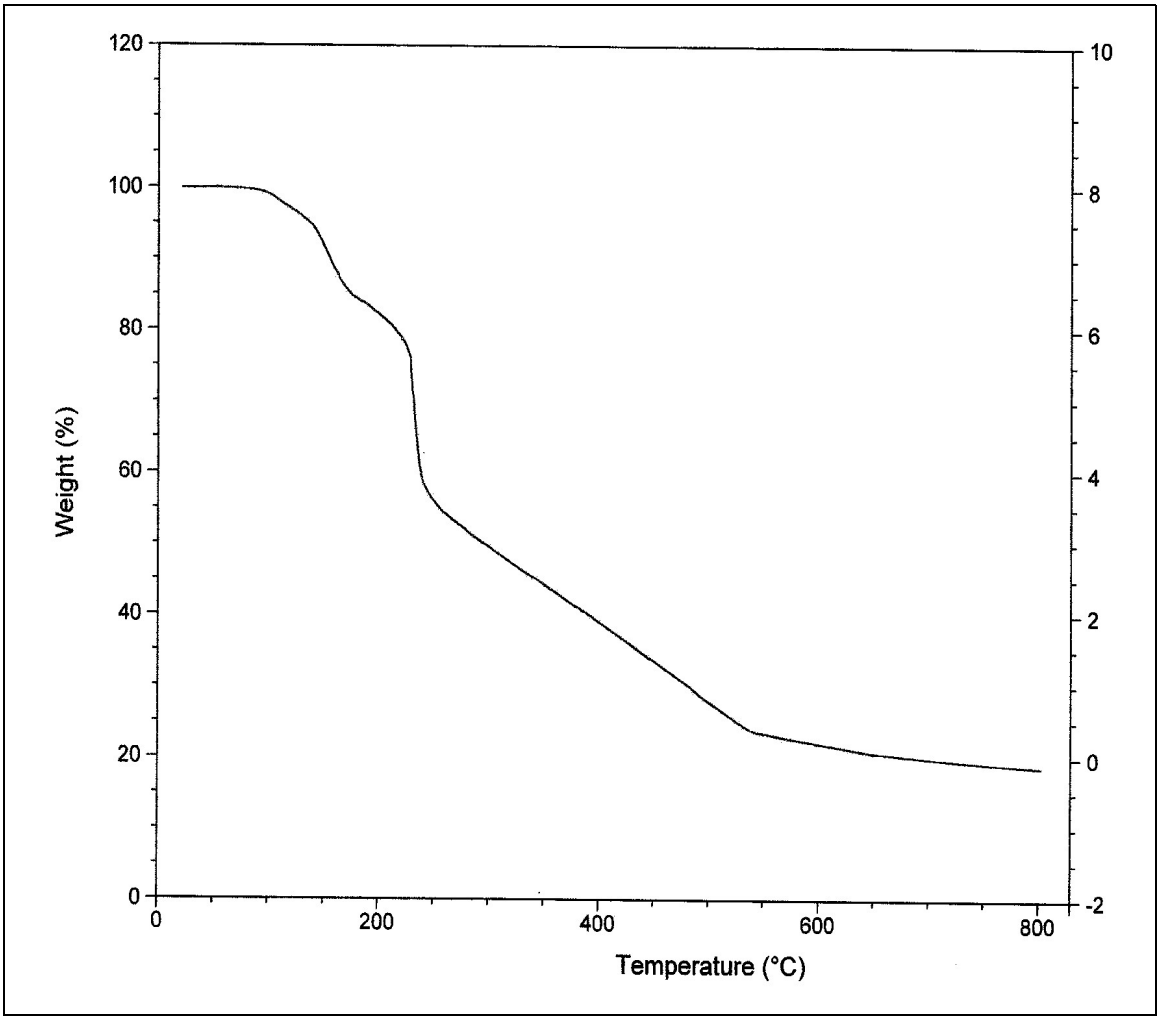


Figure II.3.2: TG Traces of $[ZnL^{1}(H_2O)_3]$

TGA

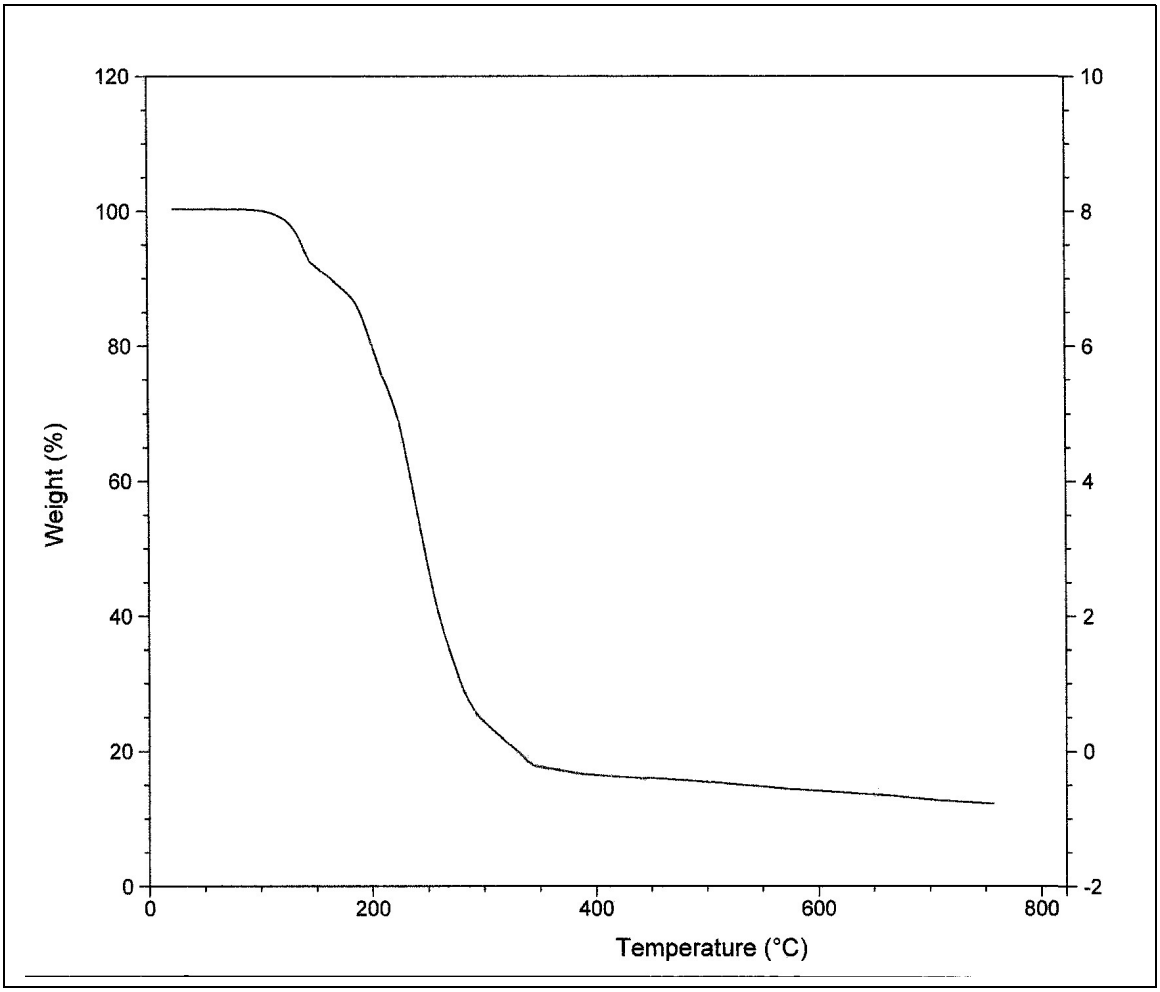


Figure II.3.3: TG Traces of $[CuL^2(H_2O)_3]$

TGA

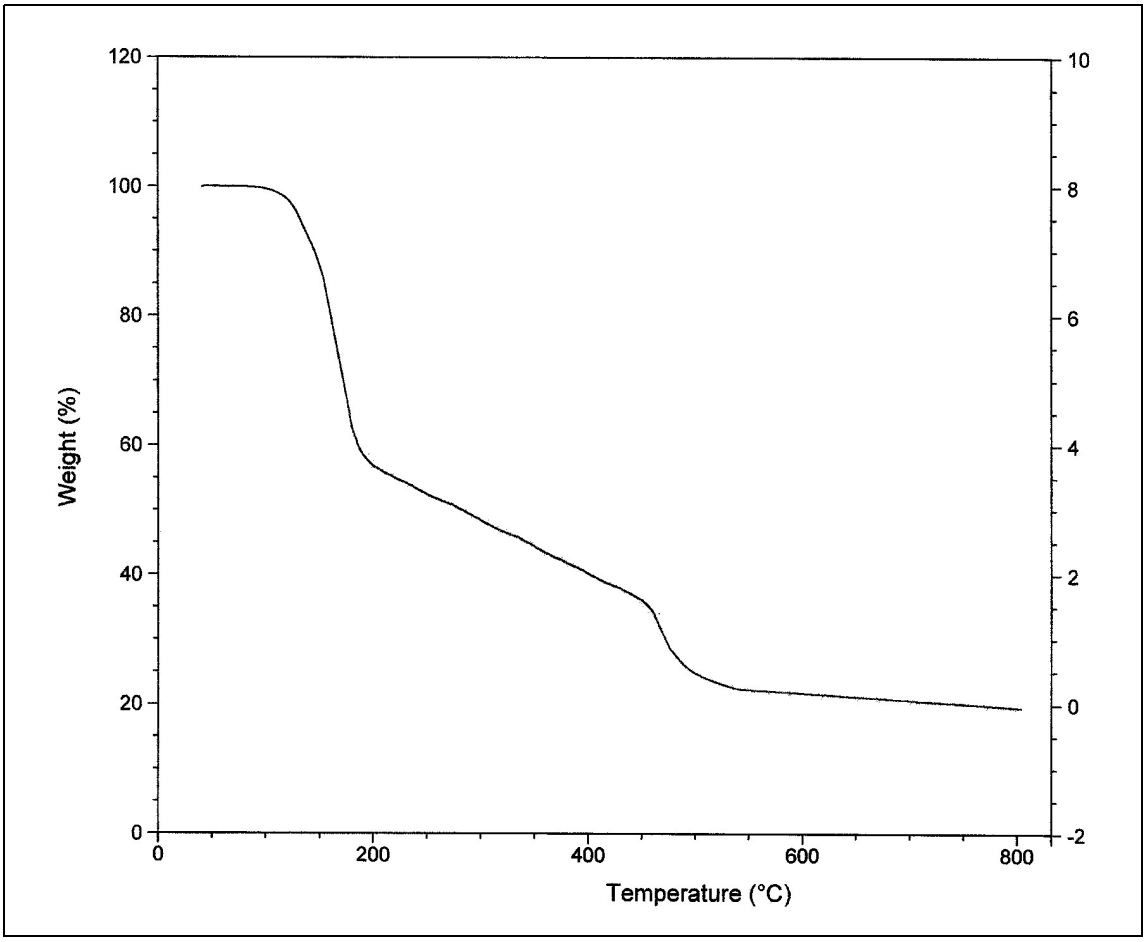


Figure II.3.4: TG Trace of $[ZnL^2(H_2O)_3]$

CHAPTER IV

THERMOGRAVIMETRIC ANALYSIS OF Cu(II) AND Zn(II) COMPLEXES OF BENZIL 2-AMINOTHIOPHENOL (BATP) AND BENZIL 2-AMINOPHENOL (BAP)

In this chapter the results of the thermal decomposition studies on Cu(II) and Zn(II) complexes of Benzil 2-Aminothiophenol (H_2L^3) and Benzil 2-Aminophenol (H_2L^4) are presented. The thermogravimetric results can be used to (i) decide whether the water molecules (if present) are inside or outside of the co-ordination sphere of the central metal ion and (ii) to suggest probable assignments for the various stages of decomposition (**Mohamed GG, 2006**).

Experimental

The preparation methods and characterisation of the Schiff base ligands and their complexes [Cu(II) and Zn(II)] are described in Part I. Thermal studies were carried out at a heating rate of 10^oC min^{-1} in static air atmosphere. The temperature ranges, the total mass loss and the probable assignments were given in Tables II.4.1 and II.4.2.

Treatments of Data

The typical TG curves in which mass is plotted against temperature are shown in the Figures II.4.1 – II.4.4. The temperature ranges in which the decomposition of the complexes takes place were also given in Tables II.4.1 and II.4.2. Data from independent pyrolytic experiments are included in this tables. These tables also summarise probable assignments for the metal complex during the various stages of decomposition.

Results and Discussion

Structural analysis using various analytical techniques established that complexes of Benzil 2-Aminothiophenol and Benzil 2-Aminophenol have the general formula $[ML(H_2O)_2]$ were M=Cu(II) and Zn(II) and L = ligand moiety. The TG curves of this chelates also show decomposition pattern with different stages. This data supports the above proposed formula for the metal chelates.

i. Cu(II) and Zn(II) Complexes of BATP

The TG curve of $[CuL^3(H_2O)_2]$ chelate shows a four stage decomposition pattern. The first stage at 95-160°C corresponds to the loss of two co-ordinated water molecules, with an estimated mass loss of 8.3% (Calcd.6.9%). The subsequent steps from 160-460°C corresponds to the removal of various organic parts of the ligand moiety, leaving metal oxide as residue. Probable

assignments and calculated weight loss corresponding to each decomposition stage is given in the Table II.4.1. The overall weight is 85.12% (Calcd 84.74%).

Zn(II) complex of BATP decomposed in three stages of which first stage is attributed to the loss of two co-ordinated water molecules. The temperature range for this decomposition is 130-170^{o} C with a mass loss of 7.69% (Calcd.6.87%). The second stage of the decomposition occurs within the temperature range 170-290^{o}C which corresponds to the removal of both aminothiophenol units. Here the mass loss was found to be 44.61% (Calcd.46.62%). The final stage accounts for the loss of benzil part of the ligand leaving ZnO as the residue.

ii. Cu(II) and Zn(II) Complexes of BAP

The TG curve of Cu(II) complex of BAP shows a three step decomposition pattern. The first stage at 150-200^{o}C corresponds to the removal of two co-ordinated water molecule and one aminophenol part of the ligand. Here the weight loss corresponds to 27.67% (Calcd. 29.00%). The second stage stands for the removal of second aminophenol part with a mass loss of 22.6% (Calcd. 21.65%). The final stage of decomposition occurs from 300-550^{o}C and it corresponds to the removal of Benzil part of the ligand with a mass loss of 32.30% (Calcd. 33.09%).

The Zn(II) complex decomposes in three steps above 100°C. In the first stage from 100-130°C, two co-ordinated water molecules were removed with a mass loss of 8.33% (Calcd. 7.35%). The second stage stands for the removal of benzil and one aminophenol part of the ligand. This decomposition takes place with in a temperature range of 130-360°C with a weight loss of 55.88% (Calcd. 57.80%). The third stage represents the loss of second aminophenol part of the ligand. The overall weight loss as read from the TG curve is comparable to the theoretical loss in mass for the conversion of $[ZnL^4(H_2O)_2]$ chelate to ZnO.

Table II.4.1

Thermal Decomposition Data of Cu(II) and Zn(II) complexes of Benzil 2-Aminothiophenol (BATP)

Complex	Stage	Temperature range in TG °C	Loss of Mass Percentage			Probable Assignments
			From TG	Theoretical	From Pyrolysis	
$CuL^3(H_2O)_2$	I	95-160	8.30	6.90		Loss of 2-co-ordinated H_2O molecules
	II	160-190	21.60	23.39		Loss of first aminothiophenol part
	III	190-230	24.92	23.39		Loss of second aminothiophenol part
	IV	260-460	30.30	31.06		Loss of benzil part of the ligand
			85.12	84.74	84.10	$CuL^3(H_2O)_2 \rightarrow$ CuO
$ZnL^3(H_2O)_2$	I	130-170	7.69	6.87		Loss of 2 co-ordinated H_2O molecules
	II	170-290	44.61	46.62		Loss of 2 aminothiophenol molecules
	III	290-510	30.31	30.95		Loss of benzil part of the ligand
			82.71	84.44	83.20	$ZnL^3(H_2O)_2 \rightarrow$ ZnO

Table II.4.2

Thermal Decomposition Data of
Cu(II) and Zn(II) complexes of Benzil 2-Aminophenol (BAP)

Complex	Stage	Temperature range in TG °C	Loss of Mass Percentage			Probable Assignments
			From TG	Theoretical	From Pyrolysis	
$CuL^4(H_2O)_2$	I	150-200	27.67	29.00		Loss of 2 co-ordinated H_2O molecules + first aminophenol
	II	200-300	22.60	21.65		Loss of second aminophenol part
	III	300-550	32.30	33.09		Loss of benzil part of the ligand
			82.57	83.74	82.80	$CuL^4(H_2O)_2$ → CuO
$ZnL^4(H_2O)_2$	I	100-130	8.33	7.35		Loss of 2 co-ordinated H_2O molecules
	II	130-360	55.88	57.80		Loss of first aminophenol + benzil
	III	360-520	20.01	18.32		Loss of second aminophenol part of the ligand
			84.23	83.47	82.50	$ZnL^4(H_2O)_2$ → ZnO

TGA

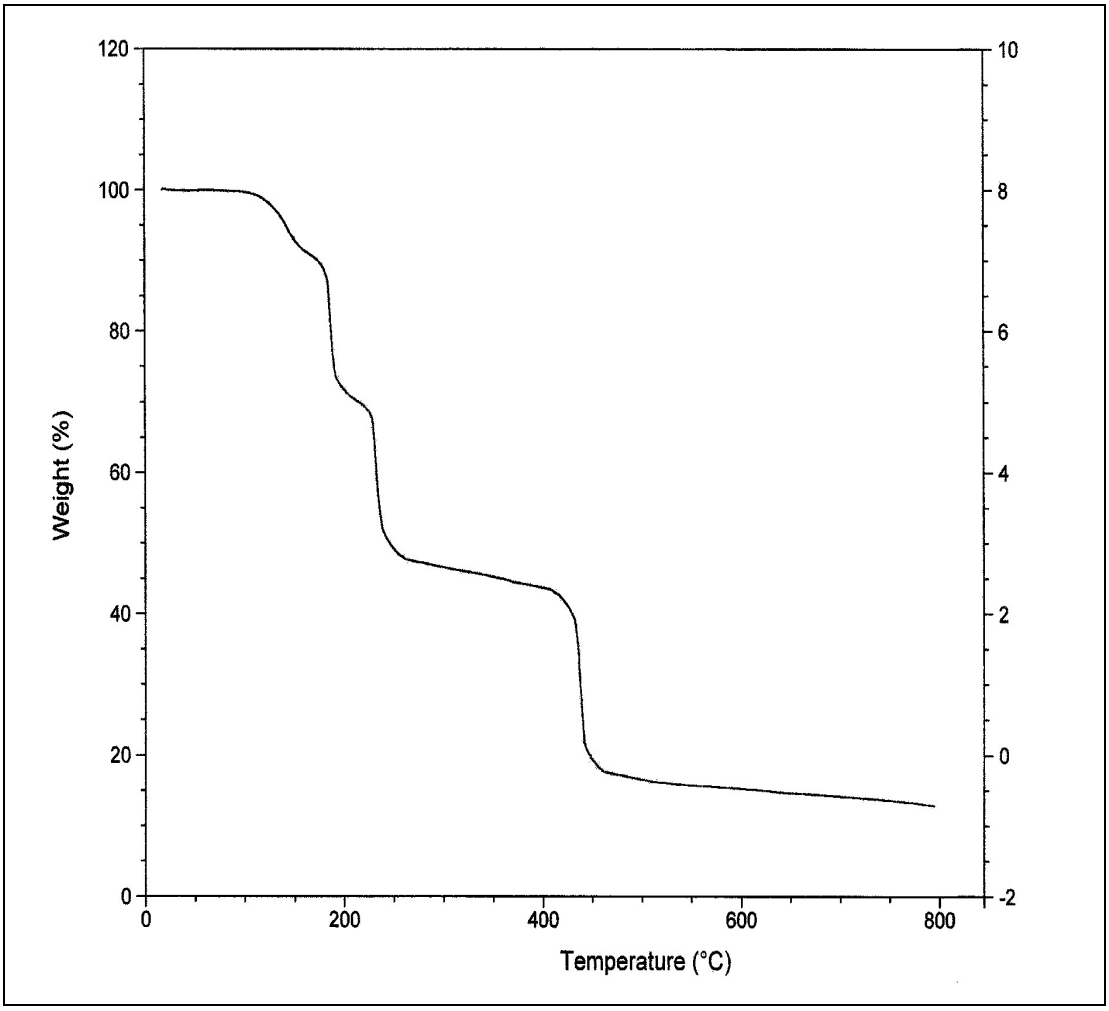


Figure II.4.1: TG Trace of $[CuL^3(H_2O)_2]$

TGA

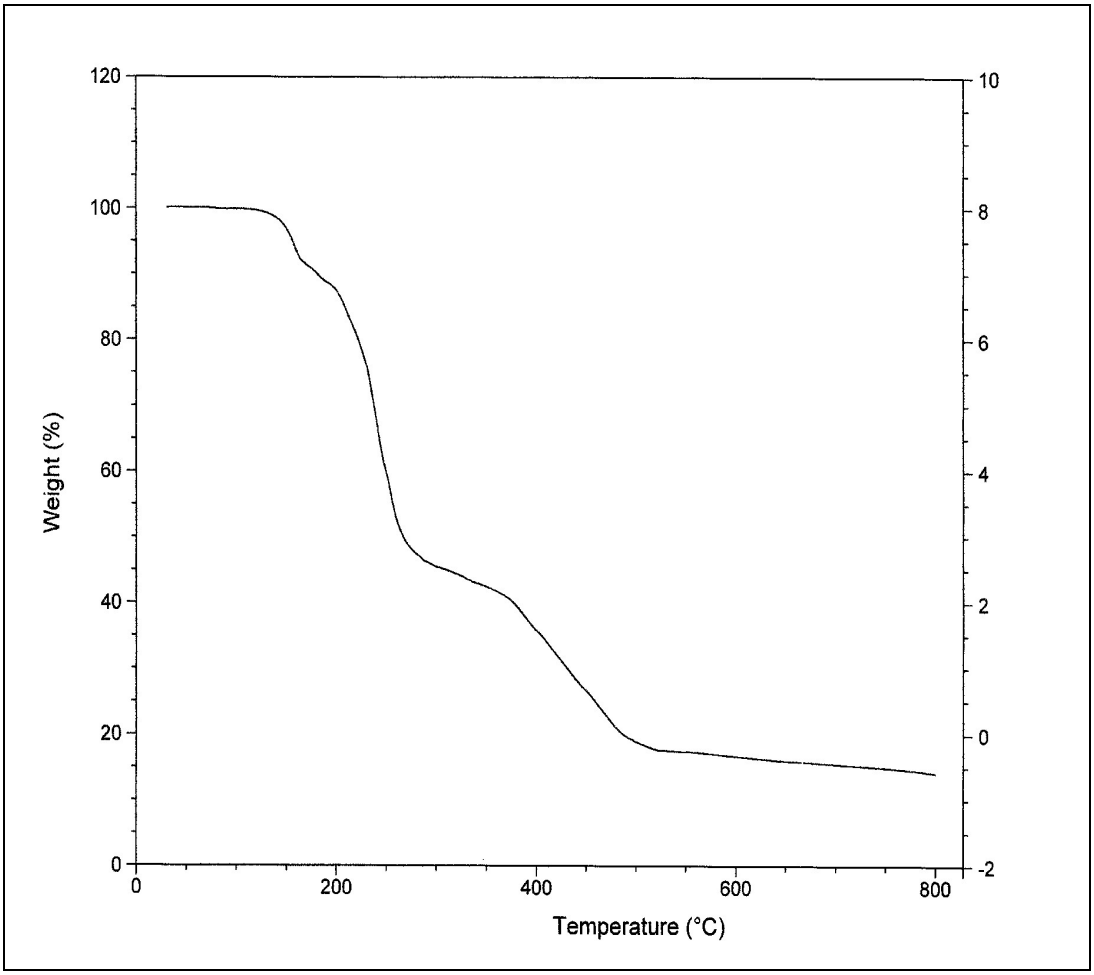


Figure II.4.2: TG Trace of $[ZnL^3(H_2O)_2]$

TGA

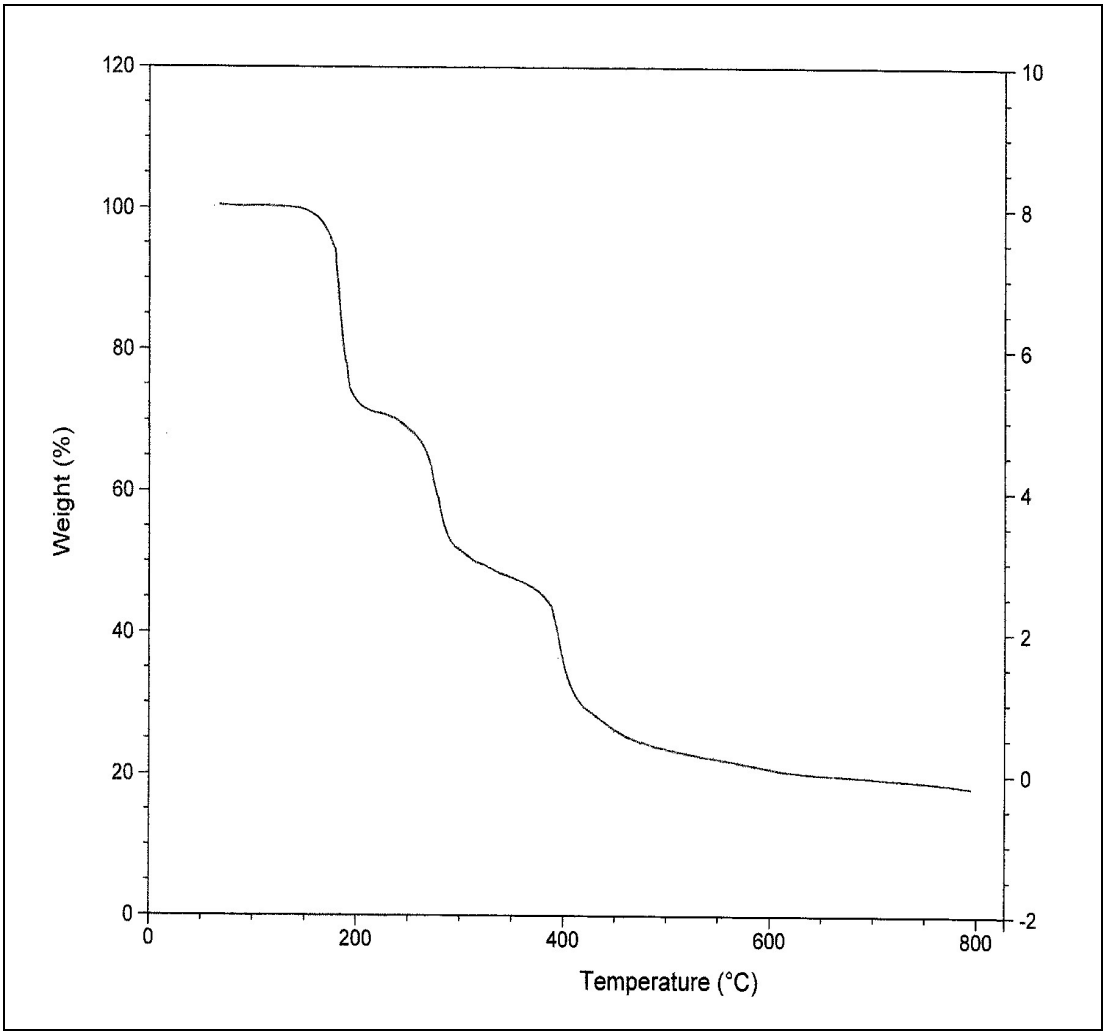


Figure II.4.3: TG Trace of $[CuL^4(H_2O)_2]$

TGA

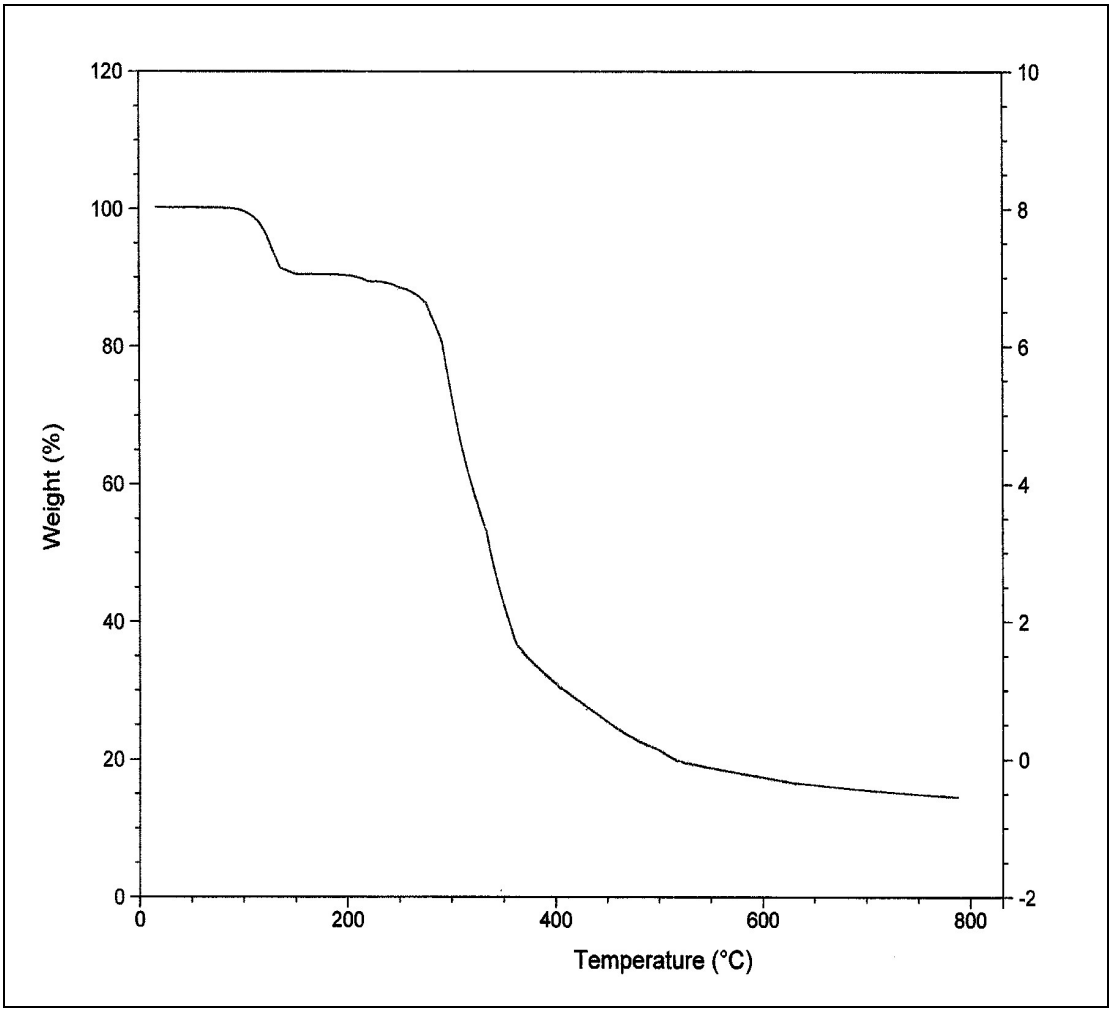


Figure II.4.4: TG Trace of $[ZnL^4(H_2O)_2]$

References

1. Brown ME. Introduction to thermal analysis, Techniques and applications, Topics in Thermal analysis and calorimetry, 1: 280, 2002.
2. Chang FC, Wendlandt WW. *Thermo Chem. Acta.* 2: 293, 1971.
3. Christian S. *Soil Sci. Sco. Am. J*, 68: 1656-1661, 2004.
4. Duval C. Inorganic Thermogravimetric Analysis, Elsevier, New York, 1963.
5. Galway AK, Brown ME. *Thermo Chemica. Acta.* 29: 129-146, 1979.
6. Mackenzie RC. The differential thermal analysis, Vol. I, London Academic Press, 1970.
7. Mohamed GG, Omar MM, Hindy AM. *Turkish Journal of Chemistry*, 30: 361-382, 2006.
8. Nikolaev AV, Logvinento VA. Thermal analysis, Academic Press, 779, 1969.
9. Osman AH, Bull AH, Gonda G. *Korean Chem. Soc.*, 25(1): 2004.
10. Paulik F, Erdey LZ. *Anal. Chem.* 160: 241, 1968.
11. Perry DL, Vaz C, Wendlandt WW. *Journal of thermal analysis*, 9: 76, 1974.
12. Rao NS, Reddy MG. *Biol. Met.* 3(1): 19-23, 1990.
13. Scency CG, Smith JF, Hill JO. *Journal of thermal analysis*, 9: 415, 1976.

14. Smoothers WJ, Yaochiang MS. Handbook of Differential thermal analysis, Chemical Publishing Company, New York, 1966.

15. Vatsala S, Parameswaran G. *Journal of thermal analysis*, 31: 883, 1986.

16. Wendlandt WW. Thermal methods of analysis, Interscience, New York, 1964.

17. Wilburn FW, Herford JR. *Sci. Instr.* 40: 91, 1963.

18. Yilmaz I, Cukurovali A. *Polish Journal of Chemistry*, 98: 663-672, 2004.

19. Young DA. Decomposition or solids, Pergamon Press, Oxford, 1966.

CHAPTER I

INTRODUCTION

Metals and Biology

The involvement of metal ions in biological processes and the role of metal complexes in biological systems have culminated in the emergence of a new branch viz., Bio-inorganic chemistry. Metal ions influence biological phenomena by interacting with organic functional groups on biomolecules, forming metal complexes. From this perspective, bioinorganic chemistry may be considered as coordination chemistry applied to biological questions. In general, bioinorganic chemists tackle such problems by first focusing on the elucidation of the structure of the metal complex of interest and then correlating structure with function. The attainment of solutions usually requires a combination of physical, chemical, and biological approaches. The chemical and physical properties of metals enable organism to undertake critical processes such as respiration, metabolism, nitrogen fixation, photosynthesis, development, nerve transmission, muscle contraction, signal transduction and protection against toxic and mutagenic agents (**Lippard SJ, 1994**). The alkali and alkaline earth metal cations such as Na^{2+}, K^{+}, Ca^{2+}, Mg^{2+} have importance

as structural elements, components of ion pumps in maintaining osmotic balance within cells.

Metalloenzymes many of which incorporate the transition metal cations Fe^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, $Mn^{2+}$and Zn^{2+} constitute about 30% of all the known enzymes (**Shriver DF, 1995**). Transition metal cations participate in a diverse range of biological catalytic, transport and redox functions (**Mills AL, 2002; Roat-Malone RM, 2002**). The utilization of metals for particular function is based on availability, size, stereochemistry, hard-soft acid-base character or reduction potential. In particular, transition metals are exploited for their ability to form stable cations and various oxidation states and geometries, and accept electrons from donor to form coordinate covalent bonds.

Metals and their compounds play a major role in medicine, inorganic chemistry and nutrition. The precious metals, gold and copper, have been used in medicines for thousands of years by the Egyptians, Arabs, Chinese, Indians etc. (**Orvig C, 1999**). The importance of zinc and iron in the diet and for the promotion of wound healing has long been known. On the other hand the toxic effects of metals such as arsenic, mercury and lead have also been long recognised (**Baldwin DR, 1999**). It is only within the last century that rational development of inorganic drugs commenced.

The modern discipline of inorganic medicinal chemistry is said to have begun in 1960's with the accidental discovery of the antitumour properties of cisplatin, [Pt $(NH_3)_2Cl_2$] (**Wong E, 1999; Blower PJ, 1999**). From this fortuitous find emerged the study of platinum anticancer agents, currently one of the most published areas of inorganic drug development. (**Blower PJ, 2004; Desoize B, 2002; Desoize B, 2004; Barnes KR, 2004; Haiduc I, 1990**) Before the discovery of cisplatin's anticancer qualities, the toxic and potentially carcinogenic properties of certain metal-containing substances may have been a factor delaying the investigation of metals for their biological activity (**Shaw CFI, 1999**).

The gold complex auranofin has been used for many years as an anti rheumatic. Although they share the anti-inflammatory qualities posessed by organic drugs aspirin and ibuprofen, gold drugs are additionally able to retard the arthritis and cause remission of the disease state (**Eisler R, 2003**).

Introduction to Cancer

Cancer embraces a multitude of diseases with different etiologies, presentations and degrees of severity. It poses an ever increasing threat to populations and health care systems on all fronts, local, national and international. It has no boundaries, affecting people across genders, ages, ethnicities and geography.

It comes under a class of diseases or disorders characterized by uncontrolled division of cells and the ability of these cells to spread, either by direct growth into adjacent tissue through invasion, or by implantation into distant sites by metastasis (where cancer cells are transported through the bloodstream or lymphatic system). There are many types of cancer. Severity of symptoms depends on the site and character of the malignancy and whether there is metastasis. The unregulated growth that characterizes cancer is caused by damage to DNA, resulting in mutations to genes that encode for proteins controlling cell division. Many mutation events may be required to transform a normal cell into a malignant cell. These mutations can be caused by radiation, chemicals or physical agents that cause cancer, which are called carcinogens, or by certain viruses that can insert their DNA into the human genome. Mutations occur spontaneously, and may be passed down from one cell generation to the next as a result of mutations within germ lines. However, some carcinogens also appear to work through non-mutagenic pathways that affect the level of transcription of certain genes without causing genetic mutation. Many forms of cancer are associated with exposure to environmental factors such as tobacco smoke, radiation, alcohol, and certain viruses. Some risk factors can be avoided or reduced.

Carcinogenesis, which means the initiation or generation of cancer, is the process of derangement of the rate of cell division due to damage to DNA. Cancer is, ultimately, a disease of genes. In order for cells to start dividing uncontrollably, genes which regulate cell growth must be damaged. Proto-oncogenes are genes which promote cell growth and mitosis, a process of cell division, and tumor suppressor genes discourage cell growth, or temporarily halt cell division in order to carry out DNA repair. Typically, a series of several mutations to these genes are required before a normal cell transforms into a cancer cell. Cancer pathology is ultimately due to the accumulation of DNA mutations that negatively effect expression of tumour suppressor proteins or positively effect the expression of proteins that drive the cell cycle. Substances that cause these mutations are known as mutagens, and mutagens that cause cancers are known as carcinogens (**Van Waes C, 2007**). Particular substances have been linked to specific types of cancer. Tobacco smoking is associated with lung cancer. Prolonged exposure to radiation, particularly ultraviolet radiation from the sun, leads to melanoma and other skin malignancies. Breathing asbestos fibers is associated with mesothelioma. In more general terms, chemicals called mutagens and free radicals are known to cause mutations. Other types of mutations can be caused by chronic inflammation, as neutrophil granulocytes secrete free

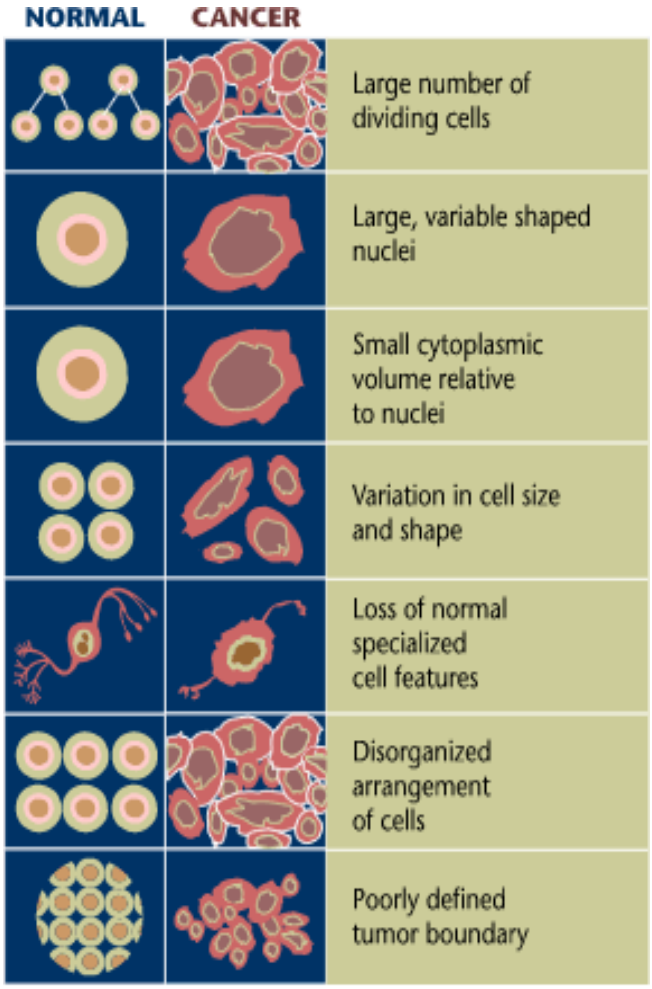
radicals that damage DNA. Chromosomal translocations, such as the Philadelphia chromosome, are a special type of mutation that involves exchanges between different chromosomes. Many mutagens are also carcinogens, but some carcinogens are not mutagens. Examples of carcinogens that are not mutagens include alcohol and estrogen. These are thought to promote cancers through their stimulating effect on the rate of cell mitosis. Faster rates of mitosis increasingly leave fewer opportunities for repair enzymes to repair damaged DNA during DNA replication, increasing the likelihood of a genetic mistake. A mistake made during mitosis can lead to the daughter cells receiving the wrong number of chromosomes, which leads to aneuploidy and may lead to cancer.

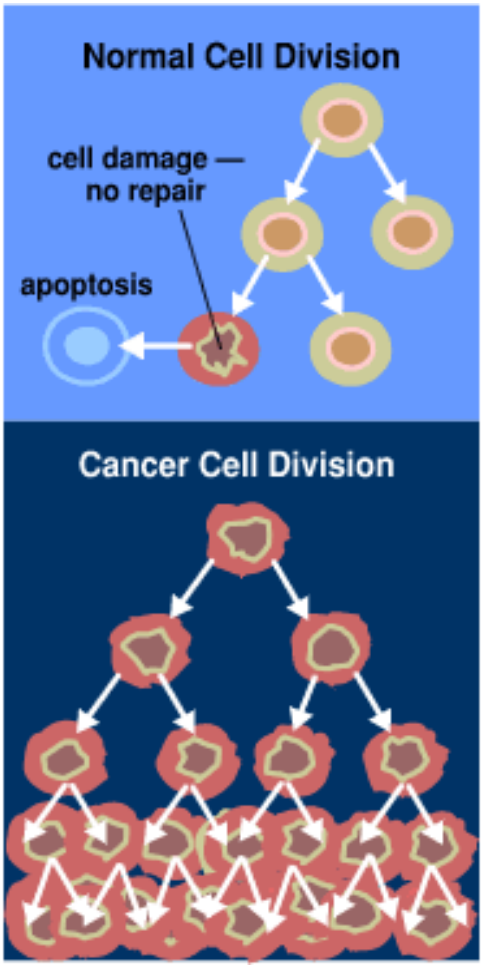
Furthermore, many cancers originate from a viral infection; this is especially true in animals such as birds, but also in humans, as viruses are responsible for 15% of human cancers worldwide. The main viruses associated with human cancers are human papilloma virus, hepatitis B virus, Epstein-Barr virus, and human T-lymphotropic virus (**Vile RG, 2006, 2007**). It is impossible to tell the initial cause for any specific cancer. However, with the help of molecular biological techniques, it is possible to characterize the mutations or chromosomal aberrations within a tumor, and rapid

progress is being made in the field of predicting prognosis based on the spectrum of mutations in some cases.

Malignant tumor cells have distinct properties:

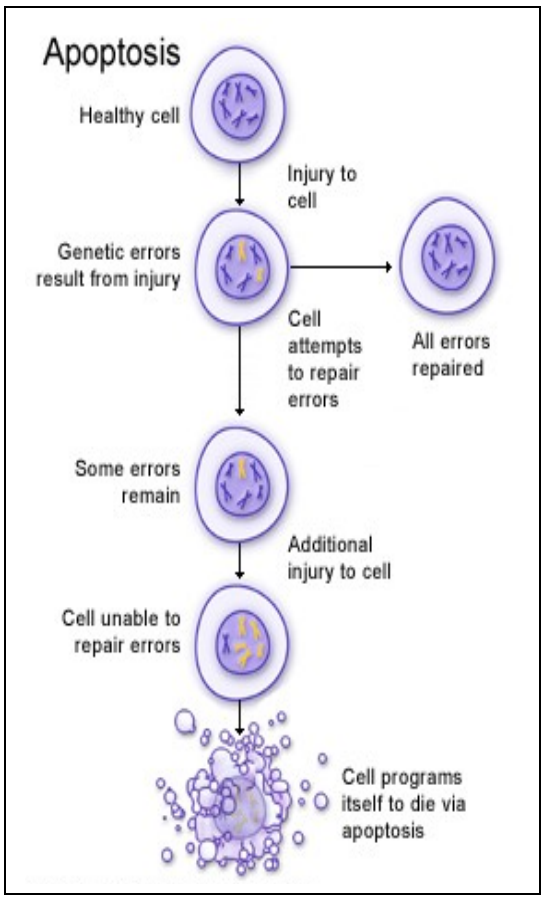
- evading apoptosis
- immortalitization due to overabundance of telomerase
- self-sufficiency of growth factors
- insensitivity to anti-growth factors
- increased cell division rate
- altered ability to differentiate
- no ability for contact inhibition
- ability to invade neighbouring tissues
- ability to build metastases at distant sites
- ability to promote blood vessel growth (angiogenesis)



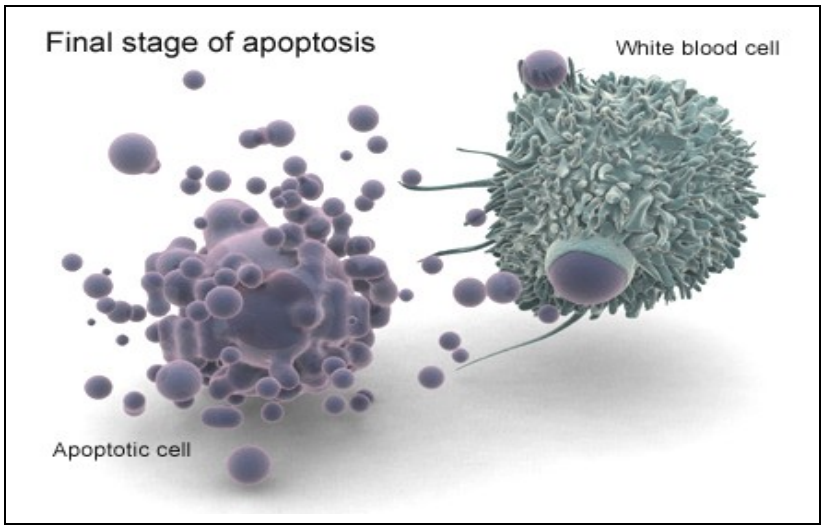


Characteristics of normal and cancerous cells

Cancer develops from cells that are capable of dividing. All tissues in the body contain some cells that can divide and renew themselves. A subset of the cell population in any tissue can differentiate into the functional cells of that tissue. The normal process of cellular differentiation ultimately leads to an adult, fully differentiated, "dead-end" cell that cannot, under ordinary circumstances, divide again. These fully differentiated cells are the workhorse cells in most tissues in the body. Under circumstances that are not clearly understood, cells that have the potential to divide can be changed by interaction with carcinogenic agents into a cell type that is capable of continued proliferation and thereby is prevented from achieving the normal state of complete differentiation. The carcinogen-altered cell is said to have undergone malignant transformation. Somehow, the genes controlling cell proliferation are locked in the "on" position when they should be in the "off" position, and the genes controlling differentiation are either not expressed or are expressed only imperfectly (**Nowell PC, 1986**). Cancer is one of the three leading causes of death in industrialized nations. Cancers are caused by the progressive growth of the progeny of a single transformed cell. Only by understanding these mechanisms, the manner in which cells are altered during malignant transformation be ascertained. Therefore, curing cancer requires that all the malignant cells be removed or destroyed without killing the patient (**Pardoll DM and Jaffee EM, 2000**).



A. Programmed cell death: Apoptosis



B. Final stages of Apoptosis

An attractive way to achieve this would be to induce an immune response against the tumor that would discriminate between the cells of the tumor and their normal cell counterparts. Immunological approaches to the treatment of cancer have been attempted for over a century, with tantalizing but unsustainable results. Experiments in animals have, however, provided evidence for immune responses to tumors and have shown that T-cells are a critical mediator of tumor immunity. More recently, advances in our understanding of antigen presentation and the molecules involved in T-cell activation have provided new immunotherapeutic strategies based on a better molecular understanding of the immune response (**Bevan MJ, 1995**). These are showing some success in animal models and are now being tested in human patients.

Treatment of Cancer

The diagnosis of cancer in the earlier stages is very difficult since there are many factors that different from patient to patients. The important once are patients' immunologic functions, performance status of the patients, the presence of complicating non-neoplastic diseases and the site of dominant metastases in patients with more advanced tumors.

Tumor is a form of tissue growth which was conventionally been divided into Benign and Malignant. Benign tumors grow slowly and do not harm the surroundings whereas the malignant tumors grow rapidly and invade surrounding tissues which lead to metastases. Antigens from such tumors react with embryonic tissue and may lost in the process of normal cell differentiation and those which escape or persist may finally lead to the development of cancer in later life.

At present there are number of methods for the treatment of cancer. They are surgery, radiation, chemotherapy and immunotherapy. The aim of these treatments is to increase the quality and duration of patients' life. In some cases integrated cancer treatment i.e. a combination of two or more of the above methods helps to attain a better recovery with in a short period.

Metallodrugs and Cancer

Cancer is a malignant disease in which rapid and uncontrolled proliferation of cells occurs. Defective cellular apoptosis (programmed cell death) is known to be significant to the development of cancer (**Kasibhatla S, 2003**). Therefore proper strategy when treating cancer is to attempt to induce apoptosis. Damage to DNA, the prime target of anticancer chemotherapy, triggers processes which lead to the prevention of

cellular reproduction, and death of the cell (**Kahlem P, 2004**). This cell death may occur as a result of apoptosis, or premature senescence due to the cell cycle being permanatly halted. A drug can interact with DNA by a number of modes in order to exert a negative effect, including alkylation, intercalation, groove binding and coordination to bases. (**Frausto da Silva JJR, 1994**). The drugs exerting an effect on mitochondria play an important role in energy production, molecular metabolism and regulation of apoptosis (**Pretson TJ, 2001**). Delocalised lipophilic cations are ideal drug candidates for targeting the mitochondria. They selectively accumulate within the mitochondria due to the high negative membrane potential and they are able to pass through the hydrophobic lipid layer due to the positive charge being delocalised over a large surface area (**Modica-Napolitano JS, 2001**). The difference in membrane potential between tumour cells and control epithelial cells has been found to be sufficient for ten-fold greater accumulation of lipophilic cations in cancerous mitochondria (**Modica-Napolitano JS, 2001**).

Traditionally, drugs used for cancer chemotherapy are organic compounds, either synthetic or natural products and include alkylating agents, antibiotics, alkaloids enzymes and hormones. There are now a large number of metal containing

compounds with antitumour properties in clinical use and are at varying stages of evaluation.

Transition Metal Complexes in Cancer

The essential role of transition metal ions in biological systems is well known. When designing metal complexes for therapeutic use the following events need to be considered: hydrolysis-protein binding-membrane transport-molecular target. Hydrolysis of metal complexes is important because of the aqueous milieu of biological systems, but the hydrophobic nature of cell membranes, vesicles and enzyme active sites requires consideration of lipophilic ligands in the design of complexes. Therefore, design of *metallo-drugs* requires bringing together organometallic chemistry with traditional aqueous coordination chemistry, a merger that is in its infancy. Whether or not the ultimate target of a *metallo-drug* is a protein, protein binding is always a factor in the medicinal use of such compounds. The greatest hurdle, however, is transport of metal complexes through cell membranes, which determines if metals enter cells with their ligands intact.

Cisplatin is the most widely used antitumor drug, especially for the treatment of testicular and ovarian cancers. However, cisplatin has some major drawbacks: severe toxic side effects, a

limited applicability to a relatively small range of tumors, and often occurring resistance alongwith kidney and gastrointestinal problems, including nausea, which may be attributed to the inhibition of enzymes through coordination of the heavy metal platinum to sulfhydryl groups in proteins. Accordingly a treatment with these metal chelates may counteract these symptoms. The second generation platinum compound, carboplatin, is used clinically worldwide, and two more cisplatin analogues, nedaplatin and oxaliplatin are also used clinically. (**Barnes KR, 2004**). Like cisplatin, all of these compounds are platinum (II) square planar complexes in which the ligands are arranged in a cis configuration, hence the similarities observed in their chemical properties and the mechanism of action (**Desoize B, 2004**). In search for other antitumor active metal complexes, several ruthenium complexes have also been reported to be promising as anticancer drugs.

Coordination Compounds as Anti-Tumour Agents

Coordination compounds have been found have application in the field of medicine as antiviral, antibacterial and antituberculosis drugs. The importance of metal complexes in cancer treatment started when Rosenberg noted the activity of *cis* – dichlorodiamine Platinum (II) against cancer. The approval of the same drug for human treatment in 1979 aroused the interest of coordination chemist towards cancer.

Currently one of the most widely used cancer drugs; cisplatin is effective for the treatment of testicular, breast, ovarian, colorectal, lung, cervical, bladder, head and neck cancers. (**Haiduc I, 1990; Guo Z, 1999; Reedijk, 1996**). Cisplatin, $Pt(NH_3)_2Cl_2$, is a square planar platinum(II) complex containing two amines, which are inert to substitution, and two chloride ligands, which serve as good leaving groups. While Pt $(NH_3)_2Cl_2$ can exist in both *cis*- and *trans*- forms, only the *cis*- configuration is effective. The *trans* isomer has no significant bioactivity. Over the past thirty years, major efforts have been directed towards investigating the way cisplatin exerts its effect on cells, in order to understand the reasons for its pharmacological and toxic properties, and to provide direction for the development of new drugs. The cytotoxicity of cisplatin can be attributed to the ability of the drug to modify the structure of DNA so that it is able to avoid excision repair by enzymes (**Berners-Price SJ, 1996**). The three major limitations to the use of platinum (II) cisplatin analogues as anticancer treatment are;

1. Severe adverse effects in patients, often resulting from lack of selectivity between tumour cells and healthy tissues.

2. The development of resistance to the drugs by tumours.

3. The restricted range of cancer that are responsive (**Wong E, 1999**).

Resistance to cisplatin is a growing problem which is still not properly understood. The mechanisms are extremely complex, but factors that mediate resistance may include reduced uptake and increased efflux of the drug, drug interaction with molecules other than DNA and increased DNA repair (**Desoize B, 2004**). Oxaliplatin demonstrates no cross resistance with cisplatin, and in addition shows good efficacy against tumours that are resistant to cisplatin. However, the side effects of oxaliplatin include significant and unpredictable sensory neurotherapy (**Barnes KR, 2004**). Trinuclear Pt (II) antitumour agents, BBR3464 is currently in phase II clinical trials. In preclinical trials, BBR3464 showed a lack of cross-resistance with cisplatin against various tumour lines, and was found to be 40 times more active (**Perego P, 1999; Ratesi G, 1999; Orlandi L, 2001**). The phase I and phase II clinical trials have demonstrated responses towards melanoma, ovarian, gastric, lung, colon and pancreatic cancers (**Perego P, 1999; Pratesi G, 1999; Jodrell DI, 2004; Kasparkova J, 2004**).

Antiarthritic gold (I) thiolates and phosphine gold (I) thiolates such as auranofin were tested for their anticancer activity in vitro and invivo in 1986 (**Merabelli CK, 1986**). The compounds exhibited activity against P388leuaemia in mice, with gold thiolates

found to be less active than the phosphine gold (I) thiolates, indicating that the phosphine ligand is important for activity. Budotitane and other diacidobis (β-ketonato) metal (IV) complexes containing Ti, Zr, Hf have shown antitumour activity against animal tumours (**Keppler BK, 1991; Clarke MJ, 1999**). Up to 200 variations of budotitane have been tested (**Keppler BK, 1993**). Variations involved the leaving groups, metal and β-ketonato ligand and it was found that asymmetric ligands are needed for high activity, with planar groups enhancing this activity. Ruthenium (II) and (III) antitumour compounds exhibit a wide variety of structural characteristics. They include octahedral complexes with amine and imine ligands, heteroaromatic ligands, DMSO Ligands. The relatively new complexes containing polyaminocarboxalate Ligands (such as PDTA = 1, 2-propylene diamine tetraacetate) and Ruthenium (II) or (III) complexes of more than one of the above ligands combined (**Clarke MJ, 2003; Alessio E, 2004**).

The "activation by reduction" hypothesis, put forward by Clarke *et al* suggests that Ru (III) drugs can be thought of as prodrugs, which are reduced to the more active R (II) species in vivo (**Clarke MJ, 1999; Clarke MJ, 1993**). Ruthenium complexes containing nitrogen donors which show anticancer activity include [RuCl $(NH_3)_5$], [cis-$RuCl_2$ $(NH_3)_4$] Cl, and (HIm) [*trans*-$RuIm_2Cl_4$]. The

probable reduction product of prototypical Ru(III) complexes such as $\{RuCl(NH_3)_5]^{2+}$is $[Ru(H_2O)(NH3)_5]^{2+}$.A mechanism by which this species induces its effect on nucleic acid function has been described by Clarke (**Clarke MJ, 1986**). RAP, H [cis-$RuCl_2$ (PDTA)] has been shown to dissociate from its chlorides in solution, while maintaining its +3 oxidation state (**Gonzalez Vilchez F, 1998**). The ruthenium (III) complex Him [trans-$RuCl_4$(DMSO-S)Im] (NAMI-A) is active against solid tumour metastases, which are usually insensitive to cisplatin (**Alessio E, 2004**). It is the only ruthenium complex to successfully complete a phase-I clinical trial (**Bacac M, 2004**).

Copper, Iron and Zinc as Antitumour Agents

Copper and iron although present in small amounts in the body, are vital for the normal functioning of several enzymes and proteins involved in energy metabolism, respiration and DNA synthesis. Their ionic forms are prone to participate in one-electron transfer reactions and can thereby catalyse redox reactions. So they generate free radicals which can induce apoptosis thereby initiating anti tumor activity. The uptake of zinc - *bis* (thiosemicarbazone) complexes in human cancer cells has been studied by fluorescence microscopy and the cellular distribution established, including the degree of uptake in the nucleus (**Andrew R, 2005**). Thiosemicarbazones and their metal

complexes have been known for many years to show a broad spectrum of therapeutic properties against a range of diseases with antibacterial, antimalarial, antiviral and antitumour activities. Despite a sustained level of interest in the pharmacological properties of such complexes, details of the cellular distribution of these complexes are scarce, particularly in living cells. Certain zinc thiosemicarbazone complexes have been shown to be active as anti-tumour agents, are as cytotoxic as cisplatin and are also effective against cisplatin resistant cell lines. (**Bernado H, 2004**). *N*-(2-hydroxyacetophenone) glycinate (CuNG) has been synthesized, which was initially found to be a potential resistance modifying agent and later found to be an immunomodulator in mice model in different doses. Oxidative stress is linked to carcinogenesis as well as to sensitivity or resistance of cancer cells to anticancer drugs (**Valko M, 2006**). The involvement of reactive oxygen species (ROS) in induction of apoptosis of various cancer cells, especially drug resistant cancers is well known. Often, the ability of a therapeutic agent to induce apoptosis of cancer cells depends upon the ability of cancer cells to generate ROS.

Moreover, low levels of ROS favor the expression of ABC transporters like P-gp. drug resistant cancers often show very low levels of ROS (**Park MT, 2005**). This is usually due to high intracellular reduced

glutathione (GSH) levels and enhanced activities of antioxidant enzymes like glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) (**Morales MC, 2005**). On the other hand, GSH is also required for phase II detoxification reactions, for example phase II enzymes like glutathione S-transferase (GST) isozymes require GSH for the conjugation of electrophilic drugs and xenobiotics. Therefore, high levels of GSH and GST have been implicated in drug resistant tumors.

Majumder *et al* (**Majumdar S, 2003**) reported the synthesis, toxicity and resistance reversal activity of the complex, viz., copper *N*-(2-hydroxyacetophenone) glycinate (CuNG) . The CuNG treatment alone at a lower dose (single administration) was found to completely heal EAC/Dox bearing animals from their tumor through immunomodulation in vivo. (**Mookerjee A, 2006**) Since copper has been reported by others to induce apoptosis by generation of ROS, and since CuNG is a copper (II) chelate, it warranted a study on its effect on ROS generation, which includes beside GSH, a number of antioxidant enzymes, like GPx, SOD and CAT.

Zhong,X (**Zhong X, 2006**) prepared mononuclear complexes of Zn(II), Cu(II), Mn(II), Co(II), Ni(II) with a new Schiff base ligand derived from 2,3-butanedione and thiosemicarbazide. Among these complexes, Cu (II) complex has highest antitumour

activity. Giuseppe Filomini *et al* Giuseppe Filomini, 2007), characterized the pro-apoptotic activity of two new synthesized isatin-Schiff base copper (II) complexes, obtained from isatin and 1, 3-diaminopropane or 2-(2-aminoethyl) pyridine: (Cu(isapn)) and ($Cu(isaepy)_2$), respectively. It was demonstrated that these compounds trigger apoptosis via the mitochondrial pathway. The extent of apoptosis mirrors the kinetics of intracellular copper uptake. Particularly, Cu ($isaepy)_2$ enters the cells more efficiently and specifically damages nuclei and mitochondria, as evidenced by atomic absorption analysis of copper content and by the extent of nuclear and mitochondrial integrity.

Mohammad Hashemi *et al* (**Heshemi, 2007**) reported the cytotoxic effects of intra and extra cellular zinc chelation on human breast cancer cells. Zinc serves many essential functions in mammalian cells and its important micronutrient in structural and regulatory functions. Zinc interacts with zinc bounding motifs, so called ‘zinc finger’ domains and thereby act as a cofactor for several hundred enzymes. Zinc also specifically bind to various membrane receptors, transporters and channels thereby modulating their activity (**Huang, 1997**). Co (II), Ni (II), Pd (II) and Pt (II) complexes of ortho-naphthaquinone (1, 2-dione) thio semicarbazone were synthesized and characterized by spectroscopic studies (**Znhra A, 2004**). *In vitro* anticancer studies

on cancer cells reveals that adding a thiocompound to a parent dione carbonyl, considerably enhances its antiproliferative activity. Carol Deegan et.al (**Deegan C, 2006**) reported the anticancer chemotheraptic potential of 1, 10-Phenanthroline 5, 6-dione (Phenadione) and its Co (II) and Ag (I) complexes in human cell lines. The results of their study suggest that Phenadione and its reported complexes may be capable of acting as highly effective anticancer therapies, which with careful administration could provide very potent and effective alternatives to cisplatin. Transition metal cations such as Cu (II) and Fe (II) bind to negatively charged DNA and have been shown to play and important role in the local formation of OH radicals (**Samuni A, 1981; Wijker CA, 1999**).

As discussed, the behaviour of metal complexes in biological systems is dependent on the following factors (1) the identity of the metal, (2) the oxidation state of the metal (3) the degree and type of derivatisation of the aromatic ligands. Regardless of the mode of action of these various drug candidates, it is established that activity can be greatly influenced by changes to the structure of the metal complex. The structure activity relationship that is observed may then provide more clues concerning the biological targets and the way cellular death is carried out. This in turn may

provide a rational strategy for much needed design and synthesis of new anticancer metallodrugs.

Scope of the Present Investigation

Many metallic elements play a crucial role in living systems. A characteristic of metals is that they easily lose electrons from the familiar elemental or metallic state to form positively charged ions which tend to be soluble in biological fluids. It is in this cationic form that metals play their role in biology. Metal ions are electron deficient, most biological molecules such as proteins and DNA are electron rich. The attraction of these opposing charges leads to a general tendency for metal ions to bind to and interact with biological molecules. Inorganic compounds have had an enormous impact on medicine, in particularly in the treatment of cancer. Recently there have been a number of reports highlighting the use of transition metal complexes as anticancer agents.

Co-ordination of a metal ion to a ligand can show large conformational changes, with concomitant bond elongations or compressions which can have drastic effects on its properties, which are most obvious in the spectroscopic and biological properties. But there are only few reports on the antitumour activities of Schiff bases derived from aminophenol, aminothiophenols, 1, 2-diketones and hydroxyketones. Our present study involves the synthesis of transition metal complexes of Schiff bases such as 2-Hydroxyacetophenone 2-Aminothiophenol (HAPATP),

2-Hydroxyacetophenone 2-Aminophenol (HAPAP), Benzil 2-Amino-thiophenol (BATP), Benzil 2-Aminophenol (BAP) and testing their cytotoxic potential in HeLa and DLA tumor cell lines.

Their ligands and complexes have characterized by analytical and spectroscopic methods which are discussed in Part I. The transition metal ions used for the complex formation are Co (II), Ni (II), Cu (II) and Zn (II). The specific objectives of the present study were; 1) Determination of *in vitro* antitumor activity. 2) Determination of *in vivo* antitumor activity 3) Acute toxicity testing for the most potential complex in BALB/c mice.

CHAPTER II

MATERIALS, METHODS AND INSTRUMENTS

Materials Required:

Cell line studies: HeLa and DLA cell lines were obtained from NCCS Pune, India and maintained at Laboratory of Tumor Immunology and Functional Genomics, Regional Cancer Centre, Thiruvananthapuram, India.

DMEM Media preparation:

DMEM	13.5g
NaHCO3	3.75g
HEPES	1.95g

Dissolved in 1000 ml of sterile distilled water. Added 500µL each of anti-mycotic and antibiotic solution. Filter sterilized, sealed and kept at 4oc.

FCS-DMEM Media

5-20% Fetal Calf Serum was added to the sterile DMEM media prior to use.

Phosphate Buffered Saline (PBS)

NaCl	8g
KCl	0.2g
Na2HPO4	1.44g
KH2PO4	0.02g
Distilled Water	1000 ml

Sterilized by autoclaving at 121°c for 15-20 minutes.

PBS-EDTA

EDTA	20g
PBS	100ml

Trypsin Solution

Trypsin	200mg
PBS-EDTA	100ml

Sterile distilled water and the whole solution was sterilized by filtration.

DMSO – Dimethyl Sulfoxide

MTS-PMS solution

Stock PMS (phenazine methosulfate) was dissolved in PBS at a concentration of 0.92 mg/ml DPBS (Dulbecco's Phosphate Buffered Saline). The solutions were then stored in light-protected tubes at -20°C. MTS and PMS detection reagents were mixed, using a ratio of 20:1 (MTS: PMS), immediately prior to addition to the cell culture at a ratio of 1:5 (detection reagents: cell culture medium).

Ethidium Bromide /Acridine Orange Dual Stain (100X Stock)

Ethidium Bromide	5 mg
Acridine Orange	1.5 mg

Dissolved in 0.1ml 95% ethanol and made up to 5 ml using distilled water. Thaw a 100 uL aliquot of the 100X Stock Solution and dilute 1/100 in phosphate buffered saline. Mix well. Store in an amber bottle at 4°C for up to one month. Add equal 25 µL volumes of cell suspension and ethidium bromide/acridine orange solution to a tube. Mix gently.

100mM TRIS

12.11g in 60ml distilled water. Adjusted pH to 8 and made upto 100ml and autoclaved.

100mM EDTA

3.722g in 100ml distilled water, autoclaved and stored at 4°C.

Lysis Buffer

100mM Tris	0.5ml.
100mM EDTA	0.2ml
Triton X 100	0.01ml
Distilled water	0.29ml

TBE Buffer (10X)

Tris	108g
Boric Acid	55g
0.5M EDTA	40ml

pH adjusted to 8 and made up to 1L with distilled water.

Loading dye

0.25 % Xylene cyanol ; 0.25% Bromo phenol blue. Dissolved in 30% glycerol.

Agarose : 2% ; 1.4 g for 70 ml TBE

Ethidium bromide: 3.5 µL of 10mg/ml stock for 70 ml Agarose-TBE mixture

RNase : 2µL of 10mg/ml for 500µL sample

Proteinase : 3µL of 20mg/ml for 500µL sample

Molecular weight marker (100bp).

Chemicals

The chemicals used for the preparation of complexes were of Analar grade quality- Merck. Hydroxyacetophenone, Aminothiophenol, Aminophenol, Benzil etc were obtained from Sigma, USA. Complexes were synthesized in our laboratory and characterized by elemental analysis, magnetic susceptibility and spectral studies as described in Part I. Solvents were purified by standard methods.

Zn-HAPATP : Zn-2Hydroxyacetophenone 2-Aminothiophenol

Cu-BAP : Cu-Benzil 2-Aminophenol

Cu-HAPAP : Cu-2Hydroxyacetophenone 2-Aminophenol

Cu-HAPATP : Cu-2Hydroxyacetophenone 2-Aminothiophenol

Metals	Ligands
M^1 - Cobalt M^2 - Nickel M^3 - Copper M^4 - Zinc	L^1-2 -Hydroxyacetophenone 2-aminothiophenol L^2- 2-Hydroxy acetophenone 2-amino phenol L^3 - Benzil 2-aminothiophenol L^4 - Benzil 2-amino phenol

Antitumour Animal studies and Toxicity testing:

Chemicals	Source
Fetal calf serum (FCS), MTT kit	Sigma chemicals
Dulbecco's Modified Eagle's Medium	Sigma chemicals
Ficoll-Paque plus	Amersham Biosciences
Phytohemagglutinin, MTS assay kit	Promega, USA.
N-2-hydroxyethyl-piperadineN'-2-ethane acid (HEPES)	Sigma, USA
Streptomycin	Gibco BRL
Amphotericin	Gibco BRL
Pencillin	Gibco BRL
Cyclophosphamide (CYP) - Ledoxan	Dabur India Ltd.

MTS -Non-radioactive cell proliferation assay kit

MTS - 2ml; PMS - 100µl

RBC lysing solution

NH_4Cl	8.29g
$KHCO_3$	1g
Na_2EDTA	37 mg
Dist. Water	1 L

pH 7.2

Leishman's Stain

Leishman’s stain	150 mg
Methanol	100 ml

Stain was constantly stirred overnight, filtered and used.

Turk’s fluid

Glacial acetic acid	1.5 ml
Crystal violet	1 drop
Distilled water	98.5 ml

Above preparation was stirred overnight, filtered and stored in a da rk bottle until use.

Hematoxylin

Hematoxylin	6.4 g
Ammonium alum (NH4)2SO4.Al2 (SO4)3.24(H2O)	60 g
Ethanol	200 ml
Glycerol	160 ml
Distilled water	640 ml

Mix for quite a while (about 2-4 hrs). Allow the stain to ripen in the dark for 6-8 weeks before using. Although it will work immediately after mixing the resulting stain is somewhat dull.

Eosin stain

Eosin Y	1 g
70% Ethanol	1 L
Glacial acetic acid	5 ml

Working solution**:** Dilute Eosin stock solution 1:1 with 70% ethanol, then add 2-3 drops of glacial acetic acid.

Acid Alcohol

Conc. HCl	4 ml
95% Ethanol	396 ml

Blueing Agent

Sodium bicarbonate	1 g
Distilled water	1 L

Methodology

Establishing Cell Cultures from Frozen Cells

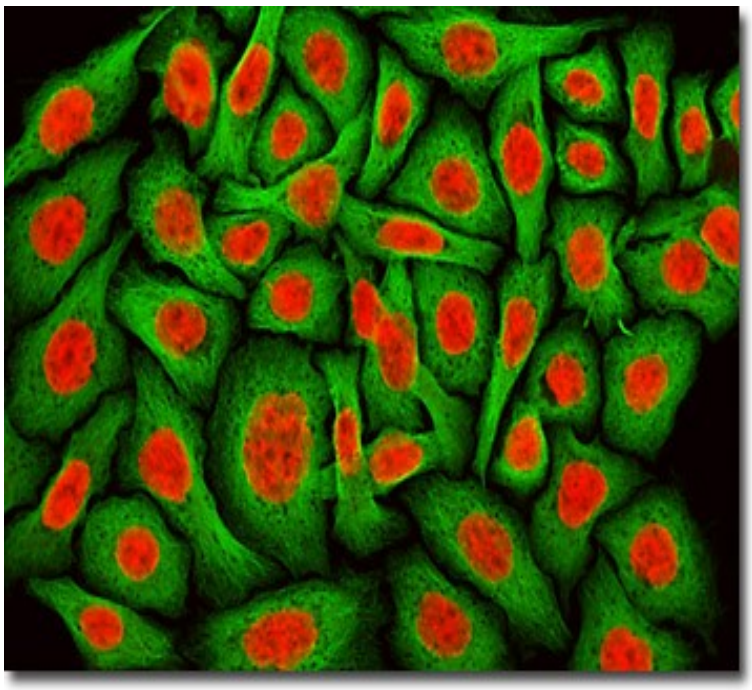
- Placed 10 ml of growth medium in a 15-ml conical tube.
- Thawed the frozen cryovial of cells within 40–60 seconds by gentle agitation in a 37°C water bath.
- Removed the cryovial from the water bath and decontaminated the cryovial by immersing it in 70% (v/v) ethanol (at room temperature).
- Transfered the thawed cell suspension to the conical tube containing 10 ml of growth medium.
- Collected the cells by centrifugation at 200 × g for 5 minutes at room temperature.
- Removed the growth medium by aspiration.
- Resuspended the cells in the conical tube in 5 ml of fresh growth medium.
- Added 10 ml of growth medium to a 75-cm2 tissue culture flask and transferred the 5 ml of cell suspension to the same tissue culture flask. Placed the cells in a 37°C incubator at 5% CO_2.
- Monitored cell density daily. Cells were passaged when the culture was at 50% confluency.

Preparation of Cell Liquid Nitrogen Stock

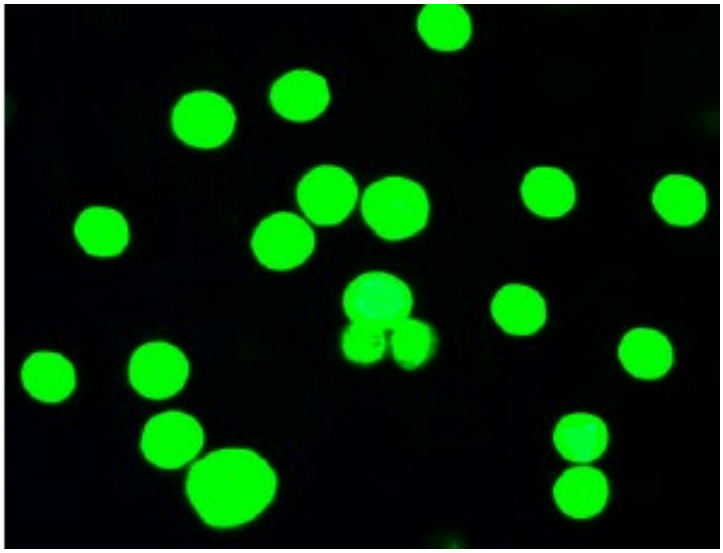
- When growing cells for the production of liquid nitrogen stock, cultures were maintained at 50% confluence.
- Collected cells from a healthy, log-phase culture. Removed the culture medium by aspiration. Trypsinized cells for 1–3 minutes in 1.5-ml of Trypsin-EDTA solution.
- Diluted the cells with 8.5 ml of growth medium. The serum in the medium inactivates the trypsin. Transfered the suspension to a 15-ml conical tube, then collected the cells by centrifugation at 600 × *g* for 5 minutes at room temperature.
- Removed the medium by aspiration. Resuspended the cell pellet in a minimal volume of growth medium (containing 10% fetal bovine serum). Counted the cells present in an aliquot of the resuspension using a hemocytometer.
- Diluted the cell suspension to 1×10^6 cells/ml in freezing medium, then dispensed 1-ml aliquots of the suspension into 2-ml cryovials.
- Frozen the cell aliquots gradually by placing the vials in a shell freezer and then placed in a –80°C freezer overnight.
- Transfered the vials of frozen cells to liquid nitrogen for long-term storage.

Passaging of Cells

- Removed the growth medium by aspiration. Washed cells once with 10 ml of phosphate-buffered saline.
- Trypsinized cells for 1–3 minutes in 1.5-ml of Trypsin-EDTA solution.
- Diluted the cells with 8.5 ml of growth medium to inactivate the trypsin.
- Transferred 1 ml of the cell suspension to a fresh 75-cm2 tissue culture flask and added 9 ml fresh growth medium. Placed the cells in a 37°C incubator at 5% CO_2.
- Monitored cell-density daily.



A. Fluorescent microscopic picture of HeLa cells



B. Fluorescent microscopic picture of DLA cells

Preparatory Phase for Cytotoxicity Assay

a. HeLa Cells

The cultured HeLa cells in T-75 flask were trypsinized, detached and centrifuged. The pellet was dissolved in FCS-DMEM media. 10 µl of the suspension was taken in hemocytometer and counted for average number of cells per ml. The required number of cells per well for cytotoxicity assay was 2500-5000 cells/well and calculated accordingly the required volume for 96 microtitre wells.

b. DLA Cells

The DLA cells were aspirated from the peritoneal cavity of Balb/C tumour mice, collected in PBS and cold-centrifuged. The pellet was dissolved in FCS-DMEM media. 10 µl of the suspension was taken in hemocytometer and counted the average number of cells per ml. The required number of cells per well for cytotoxicity assay was 5000 cells and hence calculated the required volume for 96 microtitre wells.

Calculation:

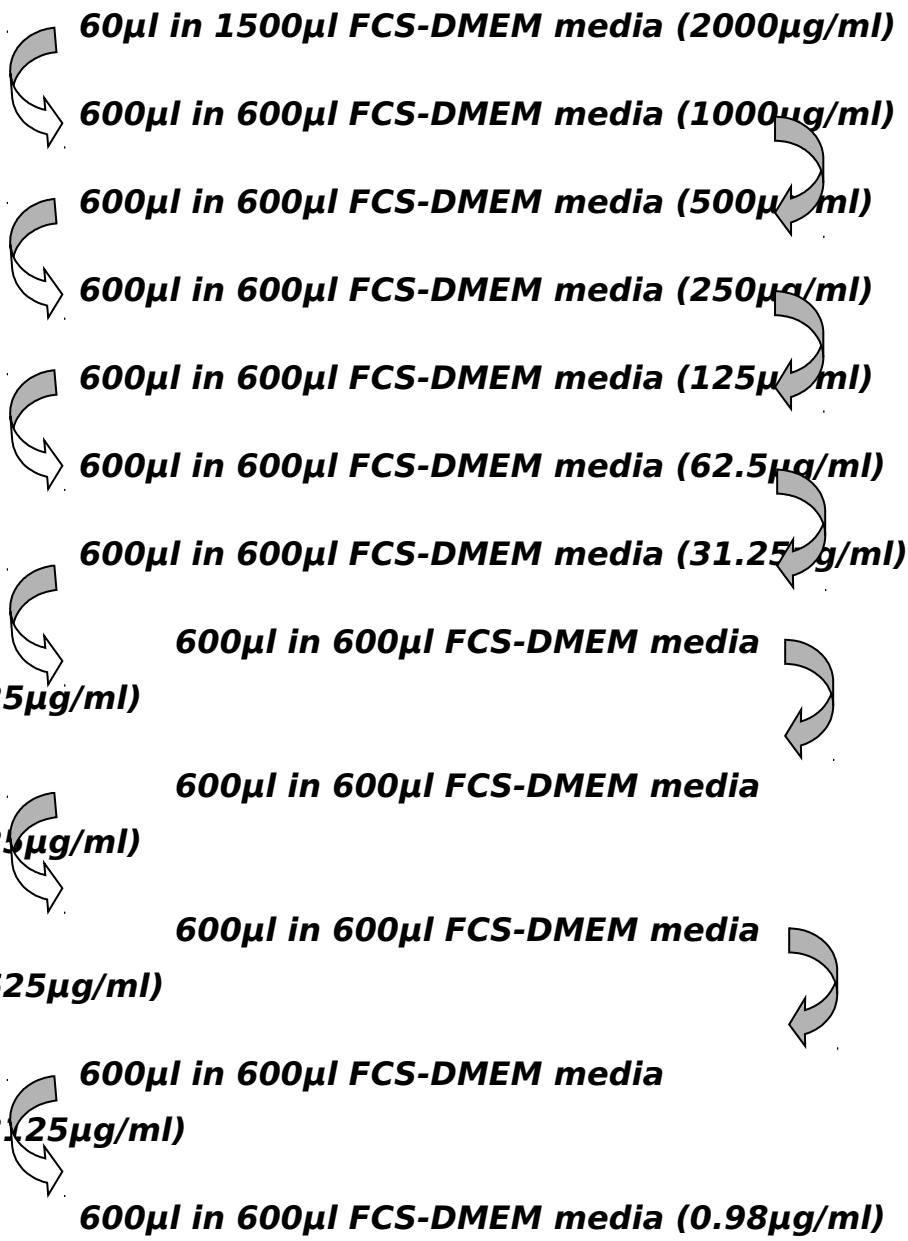
Average number of cells counted = 208 x 10^4 cells/ml or 208x10^3 cells/100µl

Required 5000 cells per well in 100 µl;

For 5000 cells/ well, the volume required is = (100 x 5000) / (208 x 10^3) µl

Therefore for 96 wells = (100 x 5000 x 96) / (208 x 10^3) µl

STOCK (50000µg/ml)



Serial dilution of Transition Metal Complexes - 2000µg/ml to 0.98µg/ml

Assessment of Cytotoxicity

Transition metal complexes (TMC's) were dissolved in DMSO and serially diluted using micropipettes. Cytotoxicity was then assessed using the Trypan Blue Dye exclusion staining and MTS non-radioactive cell proliferation assay.

Short-Term Cytotoxicity by TMC's using Trypan Blue

This dye exclusion test is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude certain dyes, such as Trypan blue, Eosin, or propidium, whereas dead cells do not. For Trypan Blue staining, HeLa/DLA cell lines were incubated with varying concentrations of the TMC's starting from 2000 µg/mL to 0.98µg/mL for 3 hours and 1% Trypan Blue was added for a minute and counted. Viable cells exclude dye while non-viable cells are blue colored. Cytotoxicity is determined by calculating percentage cell deaths. In the case of HeLa cells, the cells are detached by trypsinization prior to evaluating.

Determination of Cytotoxicty by TMC's in HeLa cells using MTS assay:

MTS assay uses the soluble tetrazolium salt, MTS, and it is versatile and offers several advantages over MTT and other cytotoxicity assays due to the solubility of the MTS formazan

product in culture medium. MTS is chemically reduced by cells into formazan, which is soluble in culture medium. The measurement of the absorbance of the formazan can be carried out using 96 well microplates at 490nm. The assay measures dehydrogenase enzyme activity found in metabolically active cells. Since the production of formazan is proportional to the number of living cells; the intensity of the produced color is a good indication of the viability of the cells.

HeLa cells (5000 cells / well), control 1 (medium only) and control 2 (medium + cells) were seeded in triplicates in 96 well microtitre plates and incubated for attachment at 37°C in 5%CO_2 incubator for 12 hours. After 12 hours, 100 µl of different concentrations of TMC's (concentration ranging from 2000 µg/ml to 0.98 µg/ml) were added to each well excluding the control wells. The plates were then incubated at 37°C in 5% CO_2 incubator for 48 hours. 20 µL of MTS-PMS solution was added and incubated in dark for another 4 hours and absorbances were recorded at 490 nm using ELISA plate reader. Graph was plotted with percentage cell cytotoxicity on Y-axis and concentration of TMC's (µg/ml) on X-axis.

Percentage cell viability is calculated using the formula (**James Kumi-Diaka *et al.*, 2004**).

$$\textbf{\% Cell viability} = A_T - A_B / A_C - A_B \times 100$$

Where A_T is the absorbance of test; A_C is the absorbance of control; A_B is the absorbance of the blank.

% Cell cytotoxicity = 100 – % cell viability

IC_{50} determination

IC_{50} value (inhibitory concentration at 50%; the concentration of the test substance required to reduce the light absorbance capacity of exposed cell cultures by 50%) was calculated using the formula: $AbsIC_{50} = (AbsIC_{100} + AbsIC_0) /2$. The mean absorbance generated by the "medium-only" control is denoted as IC_0. The mean absorbance generated by the "medium with cell" control is denoted as IC_{100}.

Determination of Cytotoxicity by TMC's in DLA cells

DLA cells (5000 cells / well), control 1 (medium only) and control 2 (medium + cells) were seeded in triplicates in 96 well microtitre plates and incubated at 37°C in 5%CO_2 for 6 hours. After 6 hours, 100 µl of different concentrations of TMC's (concentration ranging from 2000µg/ml to 0.98µg/ml) were added to each well excluding the control wells. The plates were then incubated at

37°C in 5% CO_2 incubator for 48 hours. 20 µL of MTS-PMS solution was added and incubated in dark for another 4 hours and absorbances were recorded at 490 nm using ELISA plate reader. Graph was plotted with percentage cell cytotoxicity on Y-axis and concentration of TMC's (µg/ml) on X- axis. Percentage cytotoxicity and IC_{50} was determined as mentioned before.

Deducing cell morphology by Ethidium Bromide/Acridine Orange Dual Stain

Acridine orange/Ethidium bromide staining is used to visualize nuclear changes and apoptotic body formation that are characteristic of apoptosis. Cells are viewed under a fluorescence microscope and counted to quantify apoptosis. Acridine orange (AO) is a nucleic acid selective fluorescent cationic dye useful for cell cycle determination. It is cell-permeable, and interacts with DNA and RNA by intercalation or electrostatic attractions. When bound to DNA, it is very similar spectrally to fluorescein. Ethidium bromide intercalates and stains DNA, providing a fluorescent red-orange stain. Although it will not stain healthy cells, it can be used to identify cells that are in the final stages of apoptosis - such cells have much more permeable membranes. Consequently, ethidium bromide is often used as a marker for apoptosis in cells populations and to locate bands of DNA in gel electrophoresis. The stain may also be used in conjunction with acridine orange (AO) in viable cell

counting. This EB/AO combined stain causes live cells to fluoresce green whilst apoptotic cells retain the distinctive red-orange fluorescence.

Drug induced apoptosis and necrosis were determined morphologically after labelling with acridine orange and ethidium bromide (**Duke & Cohen, 1992**). DLA cells (10^6 cells in 100µl) were cultured with 100 µl of different concentrations of Transition Metal Complexes at 37°C in 5% CO_2 incubator for 24 hours. Cells were pelleted and processed for apoptosis assay. Thawed 100 uL aliquot of the 100X A.O/Et.Br stock and diluted 1/100 in phosphate buffered saline. Added equal 25 µL volumes of treated/non-treated DLA cell suspension and ethidium bromide/acridine orange solution to a tube and observed under fluorescent microscope. Live cells were determined by the uptake of acridine orange (green fluorescence) and exclusion of ethidium bromide (red fluorescence) stain. Live and dead apoptotic cells were identified by perinuclear condensation of chromatin stained by acridine orange or ethidium bromide, respectively, and by the formation of apoptotic bodies. Necrotic cells were identified by uniform labelling of the cells with ethidium bromide

Determination of DNA Fragmentation using DNA Ladder Assay

Apoptosis has been characterized biochemically by the activation of a nuclear endonuclease that cleaves the DNA into multimers of 180-200 basepairs and can be visualized as an 'oligosomal ladder' by standard agarose gel electrophoresis known as DNA laddering. In DNA laddering assay, small fragments of oligonucleosomal DNA is extracted selectively from the cells whereas the higher molecular weight DNA stays associated with the nuclei. The isolated DNA is separated by electrophoresis and visualized using ethidium bromide.

Isolation of DNA

DLA cells (10^6 cells in 100µl) were cultured with 100 µl of different concentrations of Transition Metal Complexes at 37°C in 5% CO_2 incubator for 48 hours. Cells were pelleted and processed as follows.

- Pellet was dissolved in 1 ml lysis buffer.
- Added 0.02 ml of 10% SDS.
- Then added 2µl RNase (10mg/ml).
- Incubated at 56°C for 2 hours in dry bath.
- Added 3 µl proteinase K (20mg/ml).
- Incubated at 37°C for 2 hours (or overnight) in dry bath.

- Equal volume of isopropanol was added and centrifuged @ 10,000rpm at 4°C.
- Dried and dissolved in TE buffer (10-50µl).
- Stored at -20°C for further use.

DNA Laddering Assay

- 1.4g of Agarose was weighed and boiled to dissolve it in 70ml 1X TBE buffer (Tris-Borate-EDTA) to prepare 2% gel.
- Allowed to lower the temperature to about 50°C and 3.5 µl of 10mg/ml stock ethidium bromide was added and mixed well.
- Agarose-TBE was then poured onto a clean gel plate with comb inserted.
- After 5-10 minutes, when the gel was set, more 1x TBE buffer (~300ml) was poured and the comb was removed to load the mixture [5µl sample + 2µl of 6X loading dye (Bromophenol blue)].
- Power pack was connected and the voltage was set to 100V.
- After the initial movement of the sample + dye, a steady voltage of 80V was maintained for 2-3 hours.
- Analysed for DNA fragmentation in Gel Documentation system and ladder obtained is recorded.

Determination of Anti tumour activity in experimentally tumour

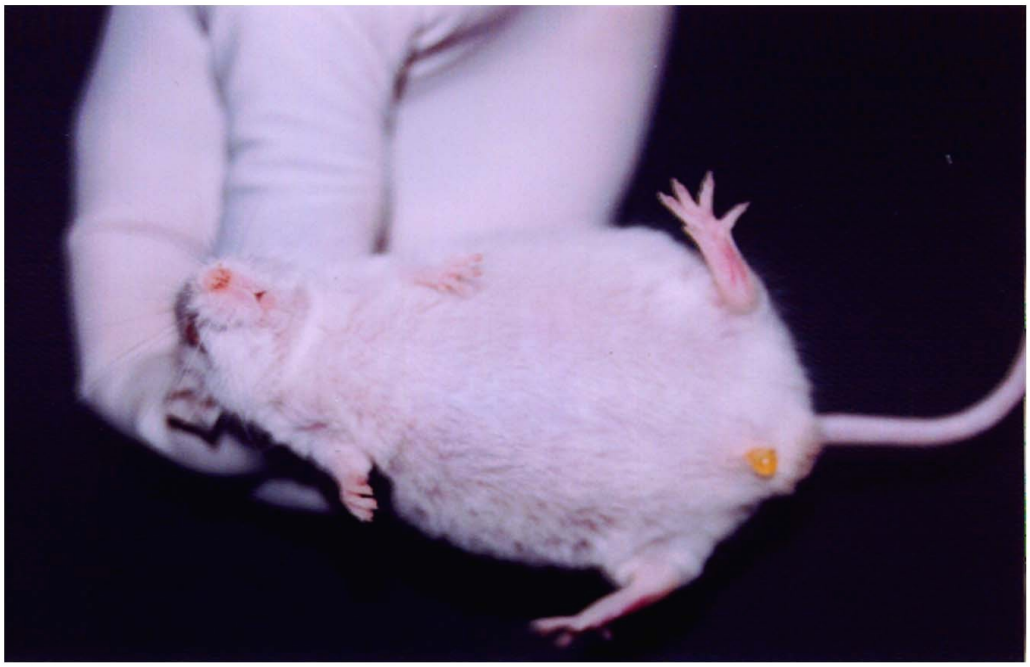
induced mice

Experimental animals

Studies were carried out using inbred strains of 6-8 weeks old BALB/c mice weighing 25±4 g housed in polypropylene cages with dark/light cycle (14/10 h). The animals were housed under controlled temperature and hygienic conditions. All animals were fed with standard pellet diet and water *ad libitum.* All procedures described were reviewed and approved by the **Institutional Animal Ethical Committee**.

Development of Lymphoma

Dalton's lymphoma cell lines were maintained in the inbred BALB/c mice. The lymphoma cells were collected from the peritoneum in cold PBS in asceptic conditions were pelleted after centrifugation. The cells were counted in a hemocytometer. Each experimental mouse received $1x10^6$ cells, intraperitoneally. The lymphoma was allowed to grow *invivo* for about 14 days. At the end of the experimental period the animals were sacrificed.



Normal mice (above) & DLA mice (below) after 17 days of tumour induction

Experimental Design

Transition metal complexes (TMC's) were dissolved immediately before intraperitoneal administration in 0.9 % saline. Inbred BALB/c mice weighing 25 ± 4g were divided into 6 groups, each consisting of 10 animals. Group I- Control (PBS), Group II- TMC (e.g Zn-HAPATP at a standardized dose of 5 mg/kg b.wt *i.p.* for 14 days), Group III served as DLA control (10^6 cells injected /animal), Group IV- DLA + TMC, Group V- DLA+CIS (2mg/kg b.wt. per animal *i.p.* post tumour inoculation) and Group VI- DLA+CIS+TMC. 24 hours after the last dose i.e.; after 14^{th} day, animals were sacrificed by cervical dislocation and the parameters were assessed.

Determination of Effect of TMC on Hemoglobin Concentration

Principle: Ferricyanide forms methemoglobin with hemoglobin, which is converted to cyanomethemoglobin by cyanide, which has an absorption at 540 nm.

Procedure: Blood (20µl) obtained from tail vein was mixed with 5ml of Drabkins reagent allowed to stand for 5 min at room temperature. Optical density (OD) was measured against regent blank. Haemoglobin content was calculated using the formula,

$$\text{g \% of Hb} = \frac{\text{O.D. of the Test}}{\text{O.D. of the Std.}} \times \frac{251}{1000} \times \text{Conc. of Std.}$$

Determination of Effect of TMC on Total Leukocyte Count

Principle: Blood was diluted in Turk's fluid, which contains a weak acid (acetic acid) to lyse RBC and a stain (crystal violet) for staining leucocytes. The number of cells in the large four corner squares of hemocytometer was counted.

Procedure: Blood was collected from tail vein and diluted with 3% acetic acid in saline solution so as to lyse all the erythrocytes. Dilutions of 1:100 or 1:20 were prepared; the Unopette ® micro collection system (**Becton Dickinson and Co.**) was used to prepare the dilutions. All samples were mixed using a Vortex prior to evaluating. Leucocytes were loaded on to the Neubauer hemocytometer and four large squares counted under a microscope using 10x objective. The Total White Blood Cells count was determined using the formula.

$$\text{Total count} = \frac{\text{Number of cells counted (N)} \times \text{Dilution factor} \times \text{Depth factor}}{\text{Area counted}}$$

Determination of Effect of TMC on Differential Count (D.C.) of leucocytes

Differential staining helps to study the morphology of leucocytes. There are five types of white blood cells.

- Neutrophils 40-75 %
- Eosinophils 5 %
- Basophils 0.5%
- Lymphocytes 20-50%
- Monocytes 1-5%

Normal mice differential staining show lymphocytes as the major population comprising 65-80% followed by neutrophils 10-20%, monocytes 2-6%, eosinophils 1-2% and basophils 0-2%.

Procedure

- A thin film of blood was made by smearing the drop of blood evenly across the glass slide using a glass spreader.
- Air dried and flooded the smear with 3 drops of Leishman's stain.
- After 3 minutes, flooded the smear with distilled water.
- Kept for 10 minutes, washed with tap water
- Observed under oil immersion 100 x objective.

The cells observed were;

Neutrophils: These granulocytes have segmented nuclei, with 2-5 lobes connected together by thin strands of chromatin and has a C shaped nucleus.

Eosinophils: They are the granulocytes having bilobed nuclei.

Basophils: Characterized by large cytoplasmic granules with obscure nuclei.

Monocytes: Agranulocytes having U shaped with reticular appearing chromatin.

Lymphocytes: Agranulocytes having a deeply stained nucleus which may be eccentric in location having small amount of cytoplasm.

Determination of Effect of TMC on Bone Marrow Cellularity

Bone marrow cells were obtained by flusing the femur bone with DMEM media containing 10% FBS and made a single cell suspension. Bone marrow cells were stained and checked for viability using Trypan blue in a hemocytometer and expressed as total live cells/ femur.

Determination of Effect of TMC on Lymphocyte Proliferation by MTS Assay

Principle: In 1968, Boyum described methods for isolation of mononuclear cells from circulating blood and bone marrow. In general, these procedures employed mixtures of polysaccharide and a radio opaque contrast medium. The solution contains sodium diatrizoate, adjusted to a density of 1.077± 0.001. This medium facilitates rapid recovery of viable lymphocytes from small volumes of blood, on centrifugation. It is usually employed as the initial isolation step prior to enumeration of T, B and null lymphocytes.

Lymphocyte proliferation assay (LPA) measures the ability of lymphocytes placed in short term tissue culture to undergo a clonal proliferation when stimulated *in vitro* by a foreign molecule, antigen or mitogen. CD4+ lymphocytes proliferate in response to antigenic peptides in association with class II major histocompatibility complex (MHC) molecules on antigen-presenting cells (APCs). This proliferative response of lymphocytes to antigen *in vitro* occurs only if the patient has been immunized to that antigen, either by having recovered from an infection with the microorganism containing that antigen, or by having been vaccinated. Therefore, some normal individuals may not respond to a given antigen, but most people will respond to at least one of several common microbial antigens. Antigen-specific T-cell proliferation is a major technique for assessing the functional capacity of CD4+ lymphocytes to respond to various stimuli. Almost everyone's lymphocytes can be stimulated to proliferate nonspecifically by stimulating them *in vitro* with the mitogen phytohemagglutinin (PHA) or pokeweed mitogen (PWM), or the antibody anti-CD3 (**Phillip Wong and Pamer EG, 2001**). However, these substances provide strong stimuli that are not antigen specific, and usually do not discriminate as well as antigens in reflecting different levels of immunodeficiency.

Isolation of Lymphocytes and Culturing

Splenic lymphocytes were obtained by gentle disruption of the spleen by passing through a wire mesh with DMEM medium with 10% FCS at 37°C under 5% CO2 in air. In the case of non-availability of spleen, peripheral blood cells diluted with DMEM was layered on the Ficoll Hypaque plus solution and centrifuged for a short period of time. Differential migration during centrifugation results in the formation of layers containing different cell types. Because of the lower density of erythrocytes and granulocytes, the lymphocytes are found at the interface between the plasma and the Ficoll Hypaque plus with other slowly sedimenting particle (Platelets and monocytes). Lymphocytes were then recovered from the interface and subjected to a short washing step with a balanced salt solution to remove any platelets, Ficoll Hypaque plus and plasma.

Principle: MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, first described by Mosmann in 1983, is based on the ability of a mitochondrial succinic dehydrogenase enzyme from viable cells to cleave the tetrazolium rings of the pale yellow MTT and form a purple formazan crystals which is largely impermeable to cell membranes, thus resulting in its accumulation within healthy cells. Solubilisation of the cells by the addition of a detergent results in the liberation of the crystals which are solubilized. The number of surviving cells is directly proportional to the level of the purple formazan product created. The color can then be quantified using a simple colorimetric assay. The results can be read on a multi well scanning spectrophotometer (ELISA reader). The CellTiter 96 ® AQ_{ueous} Non-Radioactive Cell Proliferation Assay Reagent (**Promega, USA**) is prepared by combining two solutions, MTS: 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (novel compound different from MTT without the need of adding DMSO to solubilise) and an electron coupling reagent, phenazine methosulfate (PMS). The mixed reagent is then added to cells. During the assay, MTS is converted to a soluble formazan product. Samples are read after a1–4 hour incubation at 490nm.

Procedure: Splenocytes were prepared by disrupting the spleen with DMEM medium and layered with the diluted blood over ficoll (1 ficoll: 3 blood). After 20 minutes of centrifugation at 2000 rpm to separate cells from the debris, upper layer is drawn off using a clean pasteur pipette, leaving the lymphocytes layer undisturbed at the interface. Using a clean pasteur pipette the lymphocyte layer was transferred to a clean centrifuge tube and washed it with DMEM media and cells were counted using a haemocytometer (**Cheesbrough and Mac, 1976**).

The isolated lymphocytes were suspended in DMEM medium to adjust the cell count ($1x10^6$cells/ml). After adding sufficient amount of cell suspension (≈200μl) per well in 96 well plates, 10μl of PHA is added in triplicates (without mitogen serves as control) and the microtitre plates were incubated for 48-72 hours at 37°C. 20μl of the combined MTS/PMS solution is pipetted into each of the 96 well assay plate containing 210 μl volume of lymphoctes and PHA. The amount of purple formazan was determined by measuring the absorbance at 490 nm after 4 hours of incubation in dark and cell proliferation was calculated by the formula T/C x 100, where T-Test O.D., C-Control O.D. The result obtained is the proliferation index or expressed as percentage relative cell viability.

Tumour Volume, Median Survival Time (MST) and Percentage

Life Span Analysis

Dalton's Lymphoma Ascites were injected in mice to develop tumour and the tumour volume were analysed on 7^{th}, 14^{th} and 21^{st} days after *i.p.* treatment of TMC at a dose of 5mg/kg body weight once daily starting from day 1 till the day of sacrifice. The volume in tumour induced as well as TMC administered animals were measured using a syringe and puncturing the peritoneal cavity so that no tumour cells are spilled out. The volume measured in ml. is noted on the respective days. The MST and % ILS was calculated by the formula (**Angela Garofalo et al., 2003**).

Median Survival Time; MST = (Date of 1st death + Date of last death) / 2

Percentage increment of life span; %ILS = (MST of test / MST of control) -1X100

Acute Toxicity Testing of TMC

Mice were acclimatized to laboratory conditions for 7 days prior to initiation of dosing. They were randomly assigned to cages and the individual animal was fur marked with picric acid. The females were nulliparous and non-pregnant. Mice were assigned to treatment groups of 3 males and 3 females. The mice were

deprived of feed for 16 hours before and 3 hours after administration of the test substance. The test substance, TMC at doses of 10mg/kg, 20mg/kg and 40mg/kg body weight were administered *i.p.* to mice of both sexes once at the starting day of experiment.

Observations of pharmacotoxic signs were made at 10, 30, 60, and 120 minutes and at 4 and 6 hours after dosing during the first day and daily thereafter for 7 days. The time of onset, intensity, and duration of these symptoms, if any, was recorded. All animals were observed twice daily for mortality during the 7-day period of study and LD-50 was also recorded. The weight of each mouse was recorded on days 0, 3 and 7. The group mean body weights were calculated. Animals' dead were immediately dissected to extract the serum for liver and renal function tests. Tissues and organs were preserved in 10% neutral buffered formalin for histopathological analyses. Histopathological and alteration in serum enzymes were done at DDRC, Thiruvananthapuram, India.

Histopathology of liver and kidney in mice

Haematoxylin and eosin staining protocol is used frequently in histology to examine thin sections of tissue. Haematoxylin stains cell nuclei blue, while eosin stains cytoplasm, connective tissue

and other extracellular substances pink or red. Eosin is strongly absorbed by red blood cells, colouring them bright red. Histopathological observations of liver and kidney were carried out in control and animals treated at the highest dose level of 40mg/kg.

Protocol for H & E Staining:

- Paraffin embedded slides are deparaffinized and rehydrated, frozens or vibratome sections are best mounted on slides and rehydrated;
- Slightly overstain the sections with hematoxylin, usually 3-5 minutes, depending upon section thickness and fixative (up to 20 min. if solution is not fully ripened);
- Remove excess stain in tap water, 2 min.
- Differentiate and destain a few seconds in acidic alcohol until sections look red, usually 4-5 dips.
- Rinse briefly in tap water to remove the acid;
- Blue in bicarbonate until nuclei stand out sharply blue, about 2 min.;
- Rinse in running tap water, 8 min.; Dehydrate and clear, or stain with eosin.

Eosin staining:

- Take hematoxylin stained slides from the last tap water rinse and place in 70% ethanol for 3 min.;
- Place slides in eosin for 2 min.;
- Take slides through 3 changes of 95% ethanol, 5 min. each;
- Then transfer to the first absolute ethanol of the clearing series.

Testing hematoxylin stain solution to see if it is still usable: Add several drops of stain to tap water (not distilled or deionized). If they turn bluish-purple immediately, it is satisfactory. However, if they change slowly, stays reddish or brownish, then the stain should be discarded.

Statistical Analysis

Statistical analysis was done using Analysis of Variance (ANOVA) followed by Tukey Kramer multiple comparisons test.

CHAPTER III

ANTI TUMOUR STUDIES ON SOME TRANSITION COMPLEXES OF SCHIFF BASES

The ability of medicinal formulations containing metal ions and related materials to cure a variety of diseases were well known from ancient times. In this contest it is logical to consider the extension of the use of co-ordination chemistry for medicinal purpose incorporating metal ions. Literature survey revealed that Schiff base metal complexes were well known for their antitumour activity. In continuation of the work carried out in our laboratory we tried to screen and study the antitumour activity of some selected metal complexes.

16 metal complexes of Co(II), Ni(II), Cu(II) and Zn(II) and their ligands namely 2-Hydroxyacetophenone 2-Aminothiophenol (HAPATP), 2-Hydroxyacetophenone2-Aminophenol (HAPAP), Benzil 2-Amino-thiophenol (BATP) and Benzil 2-Aminophenol (BAP) were tested for their short and long term *in vitro* cytotoxic action using DLA and HeLa tumour cell lines. Based on the studies four complexes were selected for the toxicity and apotosis studies. Two metal complexes were found to be more active. Among the two metal chelate, Zn-HAPATP (M_4L^1) was selected to study the effect on solid tumour reduction in BALB/c mice.

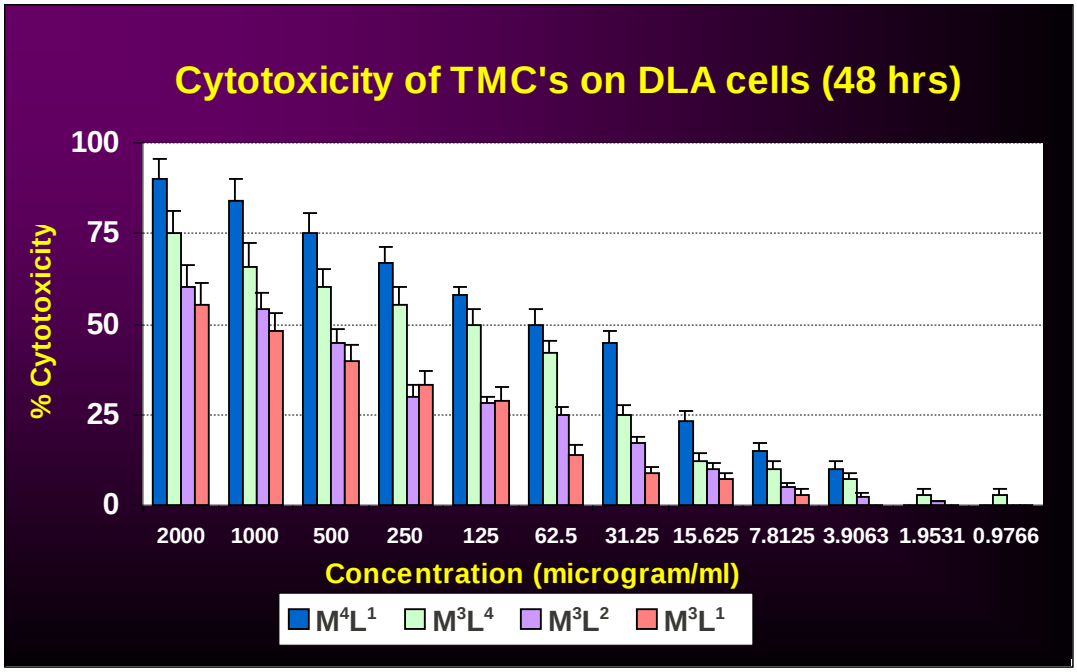
RESULTS

1. Effect of Transition Metal Complexes on Short Term *Invitro* Cytotoxicity

Results of short term cytotoxicity as obtained by Trypan Blue staining indicated 4 out of 16 complexes in about 12 concentrations screened showed promising cytotoxic activity against DLA (Table III.3.1) as well as HeLa (Table III.3.2) cell lines in about 3 hours. The cytotoxicity was more severe in DLA cells which brought about 90% of the cell death by M^4L^1 complex followed by M^3L^4 (75%), M^3L^2 (60%) and M^3L^1 (55%). In the case of HeLa cells, the cytotoxicity was observed in the order M^4L^1 (60%) > M^3L^1 (57%) > M^3L^2 (56%) > M^3L^4 (52%).

2. Effect of Transition Metal Complexes on Long Term *Invitro* Cytotoxicity

Results of long term cytotoxicity of Transition Metal Complexes by MTS assay confirmed cytotoxicity of these 4 complexes in the order Zn-HAPATP (M^4L^1) >Cu-BAP (M^3L^4) >Cu-HAPAP (M^3L^2) >Cu-HAPATP (M^3L^1) in both the cell lines (Figure. III.3.1). M^4L^1 showed the maximum cytotoxic ability in DLA as well as HeLa cell lines in all the doses tested. However, these 4 complexes were non-toxic in normal lymphocytes. The IC_{50} values obtained against DLA cells for M^4L^1 (62.5µg/mL) was the lowest and M^3L^1 (1350 µg/mL) was the highest (Table III.3.3) whereas IC_{50} values against HeLa for M^4L^1 (225 µg/mL) was the lowest and M^3L^1 (1450 µg/mL) was the highest (Table III.3.3).



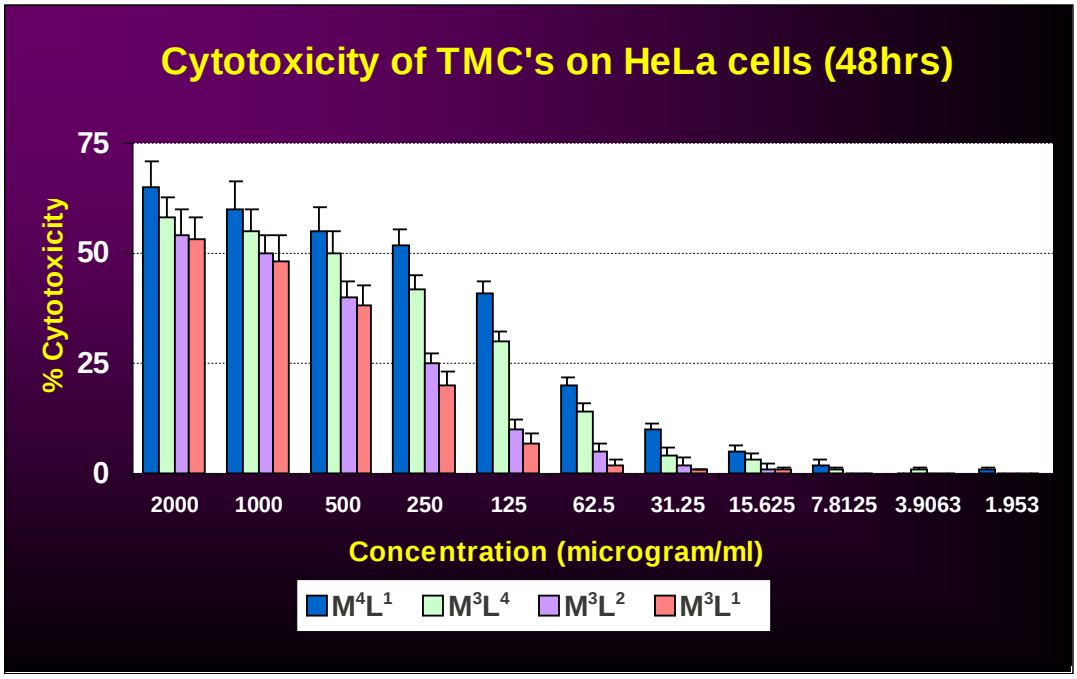


Figure III.3.1: Determination of Cytotoxicity by TMC's in DLA/HeLa cells

DLA/HeLa cells (5000/well) were cultured in the presence of TMC's at 37°C in 5% CO2 incubator for 48 hours. 20 µL of MTS-PMS added, incubated in dark for another 4 hours and absorbances were read at 490nm. Graph was plotted with percentage cell cytotoxicity on Y-axis and concentration of TMC's (µg/ml) on X- axis. Percentage cytotoxicity

was calculated according to James-Kumi Diaka et al, 2004. IC50 was calculated from the obtained graph.

3. Effect of M^4L^1 and M^3L^4 on Morphological Assessment of Apoptosis

DLA cells untreated after 12, 18 and 24 hours showed the green fluorescence representing 100% viability (Figure III.3.2A). DLA cells treated with 62.5µg/mL of M^4L^1 showed varying degrees of evident apoptosis in a time dependent manner. Nuclear and cytoplasmic condensation with blebbing of the plasma membrane, and formation of apoptotic bodies (Orange-red fluorescence) were prominent at the end of 18^{th} and 24^{th} hour; the latter being severe (Figure III.3.2C and III.3.2D). However, some cells showed necrosis (red fluorescence) as the membrane integrity was lost due to cell rupture releasing noxious cellular contents (Figure III.3.2C). M^3L^4 at an effective concentration of 125µg/mL showed similar but less intense effects at the end of 12^{th}, 18^{th} and 24^{th} hours (Figure III.3.3B, III.3.3C and III.3.3D).

4. Effect of Transition Metal Complexes on Tumor cell DNA fragmentation

Electrophoresis of DNA samples showed that M^4L^1 and M^3L^4 treated cells exhibited extensive double stranded DNA breaks, as evident by a ladder appearance, while the DNA of other TMC's treated DLA cells exhibited no laddering (Figure III.3.4). The positive control used for the study was a 100bp molecular weight marker. DLA cells treated with 62.5µg/mL M^4L^1, and 125µg/ml M^3L^4 for 48 hours showed significant DNA fragmentation as evident from characteristic DNA laddering (Figure III.3.4).

5. Effect of M^4L^1 on Blood picture and Bone marrow cellularity in mice

Intraperitoneal administration of M^4L^1 for a period of 14 days instigated a significant ($P<0.001$) increase of hemoglobin in DLA induced mice, whereas normal and Cisplatin treated DLA mice did not benefit on M^4L^1 treatment (Table III.3.4). Total count and bone marrow cellularity were significantly improved ($P< 0.001$)) on M^4L^1 treatment in tumour as well as Cisplatin treated tumour mice (Table III.3.4). But, these values were non-significant even after M^4L^1 treatment in normal mice. The differential staining revealed that majority of the cells are lymphocytes unlike neutrophils in the human blood. M^4L^1 administration showed a responsive increase in the counts of lymphocytes and neutrophils in the DLA mice ($P<0.001$) and not in other groups (Table III.3.4).

6. Effect of M^4L^1 on mice lymphocytic response to PHA

In control mice administered with M^4L^1; culturing splenocytes with PHA showed a proliferation index of 1.41 ($P<0.01$) while DLA mice showed greater significance ($P<0.001$) on M^4L^1 treatment in the rates of lymphocyte proliferation. However, Cisplatin also showed a proliferation index of 1.38 which unfortunately showed lesser effects on M^4L^1 treatment (Figure III.3.5).

7. Effect of M^4L^1 on Tumor volume, MST and percentage increment in life span

DLA induced mice on M^4L^1 treatment showed less promising regression on 7th and 14th day, but until 21st day ($P<0.001$) where it significantly reduced tumor volume (Figure III.3.6). However, the situation is just reverse in the Cisplatin treated tumor mice, where it decreased the tumor burden on all days, when compared to other groups. The standard anticancer drug itself is being effective against established tumours. But, the combination treatment of M^4L^1 and Cisplatin could effectively bring down the tumour volume slightly, although not upto that of Cisplatin alone. The MST in days (21) with respect to tumour control (17.8) was significant ($P<0.05$) which also could enhance the lifespan upto 18 % with M^4L^1 treatment alone (Table III.3.5). Here also, the anticancer drug – Cisplatin could effectively increase the lifespan upto 34.83 %, but only slightly more than the M^4L^1 and Cisplatin combination treatment (33.14%).

8. Acute toxicity testing of M^4L^1 in mice

Intraperitoneal administration of M^4L^1 at 20 mg/kg b.wt. in mice did not show any toxic effects and all of them were alive even after 7 days while at 40mg/kg body weight, 50% of the mice died within first 3-12 hours; being the LD50 of the drug M^4L^1 and rest 50 % of animals recovered the following day. However at 20 mg/kg, mild hypoactivity was observed for 4 hours post-administration and all animals appeared normal by the following day and throughout the 7-day observation period. Upto 20 mg/kg dose injected, the complex M^4L^1 appeared relatively safe. The serum enzyme levels showed a 3 fold increase in the 40 mg/kg b.wt. group which reciprocated toxic effects to liver and kidney, thus causing the death of some animals. At the same time, AST/SGOT (Aspartate amino transferase/ Serum Glutamate Oxaloacetate Transaminase), ALT/SGPT (Alanine amino transferase/ Serum Glutamate Pyruvate Transaminase) and ALKP (Alkaline phosphatase) were within safer limits upto doses of 20mg/kg b.wt (Figure III.3.7). Histopathology of liver showed intact portal triad with normal hepatocytes (Figure III.3.8A) which on intraperitoneal administration of 40mg/kg M^4L^1 ruptured liver chords with congestion of hepatocytes and hyperchromatic pyknotic nuclei (Figure III.3.8C). Kidney also showed normal equally spaced glomerulus (Figure III.3.9A) which underwent tubular degeneration and bleeding in the Bowman’s capsule (Figure III.3.9C) at a dose of 40mg/kg.

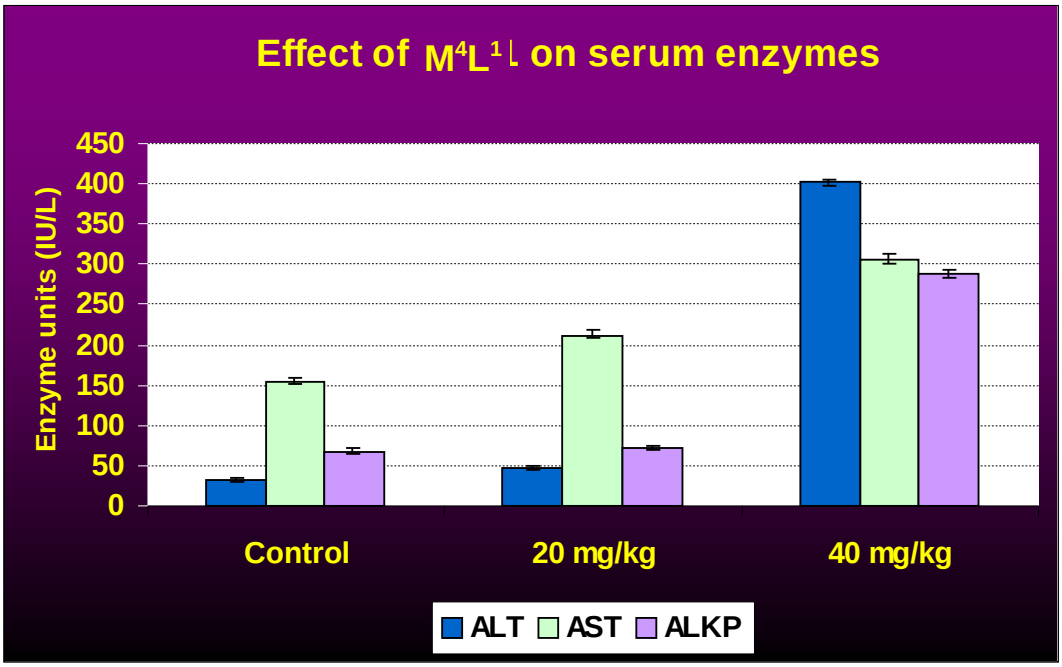


Figure III.3.7: Effect of M^4L^1 on Serum Enzymes

$M^4L^1$was administered intraperitoneally at doses of 20mg/kg and 40mg/kg/animal (n=6) once and observed for 7 days. In dead mice, blood was immediately drawn and liver function tests were performed. Data were expressed as mean ±SEM. Statistically significant differences at ***P<0.001, *P< 0.05, ns–non-significant, as compared with control group.

DISCUSSION

Metal ions influence biological phenomena by interacting with organic functional groups on biomolecules, forming metal complexes. ligand-a compound in which two or more atoms of the same molecule can coordinate with a metal to form a metal chelate. Usually, the metal in the metal chelate is tetra or hexacoordinated, but may be octacoordinated or more highly coordinated depending on the metal ions. The metal chelate will be uncharged, thus the number of acidic groups provided by its ligands will equal the oxidation state of the metal ion (**Petering DG, 1980**). Usually, the metal ligands will be relatively hydrophobic so as to impart solubility of the metal chelate in non-polar solvents.

Out of the 16 complexes screened, 4 of the transition metal complexes found to be cytotoxic against HeLa as well as DLA cell lines as obtained by Trypan Blue staining (Table III.3.1 and III.3.2). It follows the order Zn-HAPATP (M^4L^1) > Cu-BAP (M^3L^4), Cu-HAPAP (M^3L^2) >Cu-HAPATP (M^3L^1). So, M4L1 (Zn-hydroxy acetophenone aminothiophenol) was confirmed to be most potent of the complexes screened with a least IC50 value of 62.5 µg/mL for DLA cells and 225 µg/mL for HeLa cells (Table III.3.3). Zinc and Copper complexes examined in our experiments differ from each other by the Ligands. Each

component ligand as well as metal ion influences the physico-chemical and biological properties of the complexes in different way, which could explain the differences in their cytotoxic effects.

Cell death can occur by either of two distinct mechanisms, necrosis or apoptosis. Necrosis is a pathological process which occurs when cells are exposed to a serious physical or chemical insult. Apoptosis is a physiological and controlled process by which unwanted or useless cells are eliminated during development and other normal biological processes (**Wyllie AH, 1980**). Apoptotic cells were identified on the basis of morphological features that included contracted cell bodies, condensed, uniformly circumscribed and densely stained chromatin, or membrane-bound apoptotic bodies containing one or more nuclear fragments (**Shanker A, 2000**). The cell death due to apoptosis was further confirmed as assessed by Acridine Orange/Ethidium bromide dual staining and DNA laddering. Since M^4L^1 was the most potent which was effective against DLA than HeLa as evidenced by cytotoxicity assays, all the essential experiments were done using M^4L^1 on DLA itself. Apoptosis in DLA cells were obtained on M^4L^1 and M^3L^4 treatment at 12, 18 and 24 hours. DNA fragmentation as evidenced by 'ladder' was obtained for M^4L^1 and M^3L^4. The low molecular weight and lipid solubility of the Zinc and Copper complexes facilitate penetration of cell membranes. Depending

upon the specific type of complexes used, treatment might have resulted enhanced immune response to tumours, decreased tumour growth and increased survival of the mice as evidence by its extended life span.

Intraperitoneal administration of M^4L^1 at a dose of 5 mg/kg body weight/ animal/day for a period of 14 days was found to be optimal as calculated from the LD_{50} (40mg/kg - dose at which 50% of the animals died) and hence was followed for this evaluation. A dose of 20 mg/kg body weight was seen to have no adverse effects on the animal as per the biochemical examination of various parameters and histological examination of the liver and kidney. Initially, M^4L^1 caused a magenta discoloration of the tail, feet, eyes, nose and internal pinna of the ear almost immediately following injection which was very well stabilized the following day. The intensity of the color appeared to be dose-related. Transient discoloration of highly vascularized external appendages and mild hypoactivity were observed during the first 24 hours post-injection. The discoloration was transient with no apparent signs by 24 hours post-injection. All animals exhibited weight gains during the observation period. Gross examination of internal organs at necropsy was unremarkable. Histopathology of liver showed congestion of hepatocytes and hyperchromatic pyknotic nuclei (Figure III.3.8). Kidney also showed tubular degeneration and

bleeding in the Bowman's capsule at a dose of 40mg/kg (Figire III.3.9). This could be the reason for the death of the animal. In both the tissues, normal histopathology was observed until 20mg/kg dose. The serum enzymes were elevated only at doses of 40mg/kg indicating that the higher dose was unbearable for the mice which might have resulted in the death of the animal (Figure III.3.7). Mice inoculated with the DLA tumour was evaluated on 7th, 14th, & 21st days of M^4L^1 administration for the effect on body and organ weights, tumour volume, median survival time and percentage increment in life span. Body weights (+1.5 g) and organ weights were marginally increased (except liver and kidney) in the M4L1 treated animals while Cisplatin treated mice showed reduced body weights (- 2.0 g) with no changes in the organ weights. Reduction in tumour volume was observed in M^4L^1 treated DLA bearing mice at the end of 21st day and initially at the end of 7th day when co-administered with cisplatin (Figure III.3.6). The median survival time and percentage increment in life span also showed slight significant increase on M^4L^1 treatment in the DLA mice (Table III.3.5). 33.14 % increment in the life span was obtained in the combination treatment of Cisplatin with M^4L^1 when compared to DLA control mice, suggesting its promising role in tumour cell inhibition. By NCI criteria, a T/C exceeding 125% and an ILS

exceeding 25% indicate that the drug has significant anti tumour activity (**Plowman J, 1995**).

In the present study, M^4L^1 (5mg/kg) significantly increased the leukocyte count as well as the bone marrow cellularity in the tumour as well as Cisplatin treated tumour bearing mice, though not effective in the normal mice (Table III.3.4). Bone marrow is the site of hematopoiesis- the prime organ involved in the blood cell production. Many of the hematopoietic growth factors are also produced by the stromal cells, which reside in the close proximity to the precursors which enables to exhibit its functional redundancy and perform biological functions (**Paul W, 1999**). High amount of hematopoietic cells, particularly lymphocytes are localized in the bone marrow which are able to kill tumor cells or virus infected cells and play a vital role in immune response (**Pelczar MJ, 1990**). Thus, more amount of lymphocytes which upon presentation of antigenic determinants by the macrophages will initiate a cascade of immune responses involving cytokines, lymphokines etc. imparting anti-tumour potential in the DLA mice. The RBC's that supply oxygen to the body and WBC's which fight against infections also originate in the bone marrow. Increase in the WBC and BMC count signifies the ability of the M^4L^1 to mobilize the immune response against the various diseases including infections and cancer. The statistically significant elevation in the

hemoglobin count of M^4L^1 administered DLA bearing mice points out that M^4L^1 alone can contribute to overall immunity in tumour inoculated mice by producing more hematopoetic cells with profound Hemoglobin to circulation. The lymphocyte proliferation was considerably increased in normal as well as tumour bearing mice at a dose of 5mg/kg indicating possible immunostimulant effect (Figure III.3.5). Experimental evidence indicates that M^4L^1 achieved a sizeable peripheral pool of PHA-sensitive, naive T lymphocytes which ensures an improved immune response (**Pérez CS, 2001**). This shows that the M^4L^1 is able to induce lymphocyte clonal proliferation in normal as well as tumor bearing mice thus making them more reactive to neoplasmic challenges. Taken together, the results indicate the anti-tumour immunity imparted by M^4L^1 in the tumour inhibition.

The majority of anticancer drugs act as cytotoxic drugs. While anti-cancer drugs have proven useful in the treatment of cancer, they are not without harmful effects because of their potential to kill both cancer and normal cells. The serious deleterious effects prompt their discontinuous therapeutic applications (**Devasagayam TP, 2002**). The major drawbacks of the platinum based anticancer drug, cisplatin, are the serious side effects and acquired drug-resistance, which inevitably increases the drug dosage to patients. Accordingly, a new anticancer drug

that circumvents these clinical inconveniences is strongly desired to improve patient's quality of life. It is generally believed that cisplatin's anticancer action is triggered by formation of 1,2-intrastrand crosslinks on DNA, resulting in a severe DNA distortion. Most of the structurally related cisplatin-analogues show cross-resistance to cisplatin, probably due to their similar biological consequences. In this study, cisplatin - 2 mg/kg b.wt. suppressed leucocytes and bone marrow cells in tumour bearing mice (Table III.3.4). When M^4L^1 was administered in combination with cisplatin in DLA bearing mice, WBC counts and BMC were restored to near or above normal levels suggesting the ability of M^4L^1 to counteract myelosuppression. But the combination treatment did not initiate a boosted response to PHA by lymphocytes as well as in bringing down the tumour burden. The clear cut synergistic effect was noticeable only in improving the myelosuppression caused by the Cisplatin. Thus co-administration of these type of protective agents can reduce the undesired toxicity of the antineoplastic agent on healthy cells; thus, it enhances the treatment response. Furthermore, the metal complexes at the concentration checked did not inhibit the proliferation of normal splenocyte and bone marrow cells (data not shown), indicating that the cytostatic effect of the metal complexes was restricted to the tumor phenotype alone. Selective killing of tumor cells could be attributed to the fact

that tumor cells are unable to counter the load of mutations owing to their defective DNA repair mechanism (**Rosenberg B, 1985**).

Chelation in addition to its selectivty may increase the anti-tumour activity (**Furst A, 1963**); but this investigation is a primary one and farther tests are required to explore its actual mechanism of action and its probable effects on higher animal model and more cancer cell lines. Developing ligands which coordinate to a specific metal ion can build stable complexes with high selectivity. To achieve metal ion selectivity, the design of a ligand with preorganization and complementarity is needed which satisfies the requirements of the metal ion, allowing the formation of the ideal coordination geometry which might have enhanced biological activity (**Sigel H, 1998**). Apart from this ligands with nitrogen, oxygen and sulphur donor systems inhibit enzyme activity since the enzyme which require these groups for their activity appear to be especially more susceptible to deactivation by the metal ions through coordination.

Therefore, the areas of deficiency, toxicity, and optimum physiological response can be dramatically varied by considering a combination of these variables, as well as design features of the potential ligand which may be altered to tune the delivery of that metal ion into the biological system. This refinement of the biological properties of metal complexes by ligand modification,

along with the design of ligands to alter the homeostasis of endogenous metal ions, will provide many new therapeutic and diagnostic agents over the coming years and will direct medicinal inorganic chemistry into a discipline of central importance in medicine and science.

References

1. Alessio E, Mestroni G, Bergamo A, Sava G. *Metal ions in Biological System,* 42: 323-351, 2004.
2. Andrew R, Cawley JD, Jonathan R, Dilworth P. *Chem. Commun*:
   845-847, 2005.
3. Angela G, Elitza N, Luigi M, Carmen G. The Combination of the Tyrosine Kinase Receptor Inhibitor SU6668 with Paclitaxel Affects Ascites Formation and Tumor Spread in Ovarian Carcinoma Xenografts Growing Orthotopically. *Clin Cancer Res,* 9: 3476, 2003.
4. Bacac M, Hotze ACG, Vander S. *Journal of Inorganic Biochemistry*, 40: 1383-1396, 2004.
5. Baldwin DR, Marshall WJ. *Annuals of Clinical Biochemistry*, 36: 267-300, 1999.
6. Barnes KR, Lippard SJ. *Metal ions in Biological systems*, 42: 143-177, 2004.
7. Bernado H, Gambino D, Mini-Rev. *Med. Chem.,* 4: 31, 2004.
8. Berners-Price SJ, Barnham KJ, Frey U, Sadler PJ. *Chemistry-A European Journal*, 2:1283-1290, 1996.
9. Bevan MJ, Hogquist KA, Jameson SC. *Rev.Immun.,* 13: 93-126, 1995.
10. Blower PJ. Annual reports on the progress of Chemistry, *Inorganic Chemistry Section A,* 95: 631-655, 1999.
11. Blower PJ. Annual reports on the progress of Chemistry, *Inorganic Chemistry Section A*, 100: 633-658, 2004.
12. Carol D, Michael D, Malachy M. *Bioinorg Chem Appl.* 10: 1155, 2006.

13. Cheesbrough M, Mcarthur J, A Lab Manual for Rural Tropical Hospitals, Churchill Livingstone, London, 1976.

14. Clarke MJ, Jansen B, Marx KA, Kruger R. *Inorganica Chimica Acta,* 124: 13-24, 1986.

15. Clarke MJ, Zhu F, Frasa DR. *Chemical Reviews*, 99: 2511-2533, 1999.

16. Clarke MJ*. Coordination Chemistry Reviews,* 236: 209-233, 2003.

17. Clarke MJ. *Metal Complexes in Cancer Chemotherapy*: 129-156, 1993.

18. Desoize B, Madoulet C*. Critical reviews on Oncology/haematology,* 42: 317-325, 2002.

19. Desoize B. *Anticancer Research*, 24: 1529-1544, 2004.

20. Devasagayam TPA, Sainis KB. Immune system and antioxidants, especially those derived from Indian medicinal plants. *Ind. J. Exp. Biol.* 40: 639–655, 2002.

21. Duke RC, Cohen JJ. Morphological and biochemical assays of apoptosis. John Wiley & Sons. Inc., New York, 1: 11–13, 1992.

22. Eisler R, *Inflammation Research*, 52: 487-501, 2003.

23. Frausto da Silva JJR, Williams RJP*.* The Biological Chemistry of elements: The Inorganic Chemistry of Life; Oxford University Press: New York, 1994.

24. Furst A. Chemistry of Chelation in Cancer; *Springfield*, IL, 1963.

25. Giuseppe F, Giselle C. *J. Biol. Chem.,* 282(16): 12010-12021, 2007.

26. Gonzalez VF, Vilapana RB. *Journal of Inorganic Biochemistry*, 71: 45-51, 1998.

27. Guo Z, Sadler PJ. *Angewandte Chemie*. International edition in English, 38: 1512-153, 1999.

28. Haiduc I, Silvestru C. *Coordination Chemistry Reviews,* 99: 253-296, 1990.

29. Howayda S, Abd EA. *Infect Agent Cancer*. 2: 12, 2007.

30. Huang S, Mayeda A, Krainer AR, Spector DL. *Mol Biol Cell.* 8,6: 1143–1157, 1997.

31. James Kumi-Diaka, Potential mechanism of phytochemical-induced apoptosis in human prostate adenocarcinoma cells: Therapeutic synergy in genistein and -lapachone combination treatment ; *Cancer Cell Int,* 4: 5, 2004.

32. Jodrell DI, Evans TRJ. *European Journal of Cancer*, 40: 1872-1877, 2004.

33. Kahlem P, Doerken B, Schmitt CA*. Journal of Clinical Investigation*, 113: 169-174, 2004.

34. Kasibhatla S, Tseng B. *Molecular Cancer Theraupeutics*, 2: 573-580, 2003.

35. Kasparkova J, Fojta M, Farrell N, Brabec V. *Nucleic Acids Research,* 32: 5546-5552, 2004.

36. Keppler BK, Friesen C, Moritz HG, Vongerichten H, Vogel E. *Structure and bonding*, 78: 97-127, 1991.

37. Keppler BK, Friesen C, Vongerichten H, Vogel E. *Metal complexes in Cancer Chemotherapy:* 297-323, 1993.

38. Lippard SJ. *Principles of bioinorganic chemistry*; University science Books; California, 1994.

39. Majumdar S, Dutta P, Mookerjee A, Choudhari SK, *Chem. Biol. Interact,* 159: 90-103, 2006.

40. Majumdar S, Panda GS, Choudhari SK. *European J. Med. Chem.,* 38: 893-98, 2003.

41. Merabelli CK, Johnson RK. *Journal of Medicinal Chemistry*, 29: 218-223, 1986.

42. Mills AL. *Mannual of Environmental Microbiology* (2nd Edition); Hurst CJ. Ed 2002.

43. Modica-Napolitano JS, Aprille JR. *Advanced Drug Delivery Reviews*, 49: 63-70, 2001.

44. Mohammed H, Saied G. *European Journal of Pharmacology,* 557: 9-19, 2007.

45. Mookerjee A, Mookerjee BJ, Dutta P. *Clinical Cancer Res.,* 12: 4339-4349, 2006.

46. Morales MC, Perez-Yarza G. *Anticancer Research,* 25: 1945-51, 2005.

47. Nowell PC, Croce CM. *Am J Pathol*. 125: 1, 7–15, 1986.

48. Orlandi L, Colella G. *European Journal of Cancer*, 37: 649-657, 2001.

49. Orvig C, Abrams MJ. *Chemical Reviews* 99: 2201-2203, 1999.

50. Pardoll DM, Jaffee EM. *Journal of immuno therapy.,* 23: 4, 438-448, 2000.

51. Park MT, Kim MJ, Kang S. *Blood,* 105: 1724-33, 2005.

52. Paul W, Fundamental Immunology, 4th ed. Lippincott Raven, Philadelphia, 1999.

53. Pelczar MJ, Chan ECS, Krieg NR. *Microbiology*, 5th ed. Tata Mcgraw-Hill, New Delhi, 703–715, 1990.

54. Perego P, Caserini C, Gatti L. *Molecular Pharmacology*, 55: 528-534, 1999.

55. Pérez-Cuadrado S, and Rodriguez-Ramos JH. *Derivatives of pyroglutamic acid, preparation process and applications*. Munich, Germany, 2001.

56. Petering DH, *Metal Ions in Biological Systems*, Marcel, Dekker, New York, 197–229, 1980.

57. Phillip W and Eric GP. Antigen-Independent CD8 T cell Proliferation. *J Immunol,* 166: 5864-5868, 2001.

58. Plowman J, Dykes DJ. Human tumor xenograft models in NCI drug development. Humana, 101, 1995.

59. Pratesi G, Perego P. *British Journal of Cancer*, 80: 1912-1919, 1999.

60. Pretson TJ, Abadi A, Wilson L, Singh G, *Advanced Drug Delivery reviews*, 49: 45-61, 2001.

61. Reedijk. *J.Chemical Communication*: 801-806, 1996.

62. Roat-Malone RM. *Bioinorganic Chemistry*: A short Course; John Wiley & Sons; New Jersey, 2002.

63. Rosenberg B, *Cancer*, 55: 2303, 1985.

64. Samuni A, Chevion M, Czapski G. *J. Biol. Chem:* 256, 1981.

65. Shanker A, Singh SM. *Neoplasma* 47: 2, 2000.

66. Shaw CFI. *Chemical reviews* 99: 2589-2600, 1999.

67. Shriver DF, Atkins PW, Langford CH, *Inorganic Chemistry*; Oxford University press: Oxford 1995.

68. Sigel H. *Metal Ions in Biological Systems*, 33: Marcell Dekker, 1998.

69. Valko M, Rodes CJ, Moncol J, Izakovic M, Mazur M. *Chem. Biol. Interact,* 160: 1-140, 2006.

70. Van W, Bin Y. *Genome Biol.* 8(5): R78, 2007.

71. Van W, Ming Y. *Cancer Res*. 66,13: 6722–6731, 2006.

72. **Vile RG, Rosa MD.** *Cancer Res.*67(6): 2840-8, 2007.

73. **Vile RG, Sanchez-Perez.** *Gene Ther*. 2007.

74. **Vile RG.** *Gene Ther.* 15:1127-30, 2006.

75. Wijker CA, Lafleur MV, *Mutat Res.* 429(1): 27-35, 1999.

76. Wong E, Giandomenico CM, *Chemical reviews,* 99: 2451-2466, 1999.

77. Wong E, Giandomenico CM. *Chemical Reviews*, 99: 2201-2203, 1999.

78. Wyllie AH, Kerr JF, Currie AR. Cell death: the significance of apoptosis. *Int Rev Cytol,* 68: 251–306, 1980.

79. Zahra A, Ekkehard S. ***Inorganica Chimica Acta*** **357**: 271-278, 2004.

80. Zhong X, Yi J, Sun J. *Eur. J. Med. Chem.,* 41(9): 1090-92, 2006,.

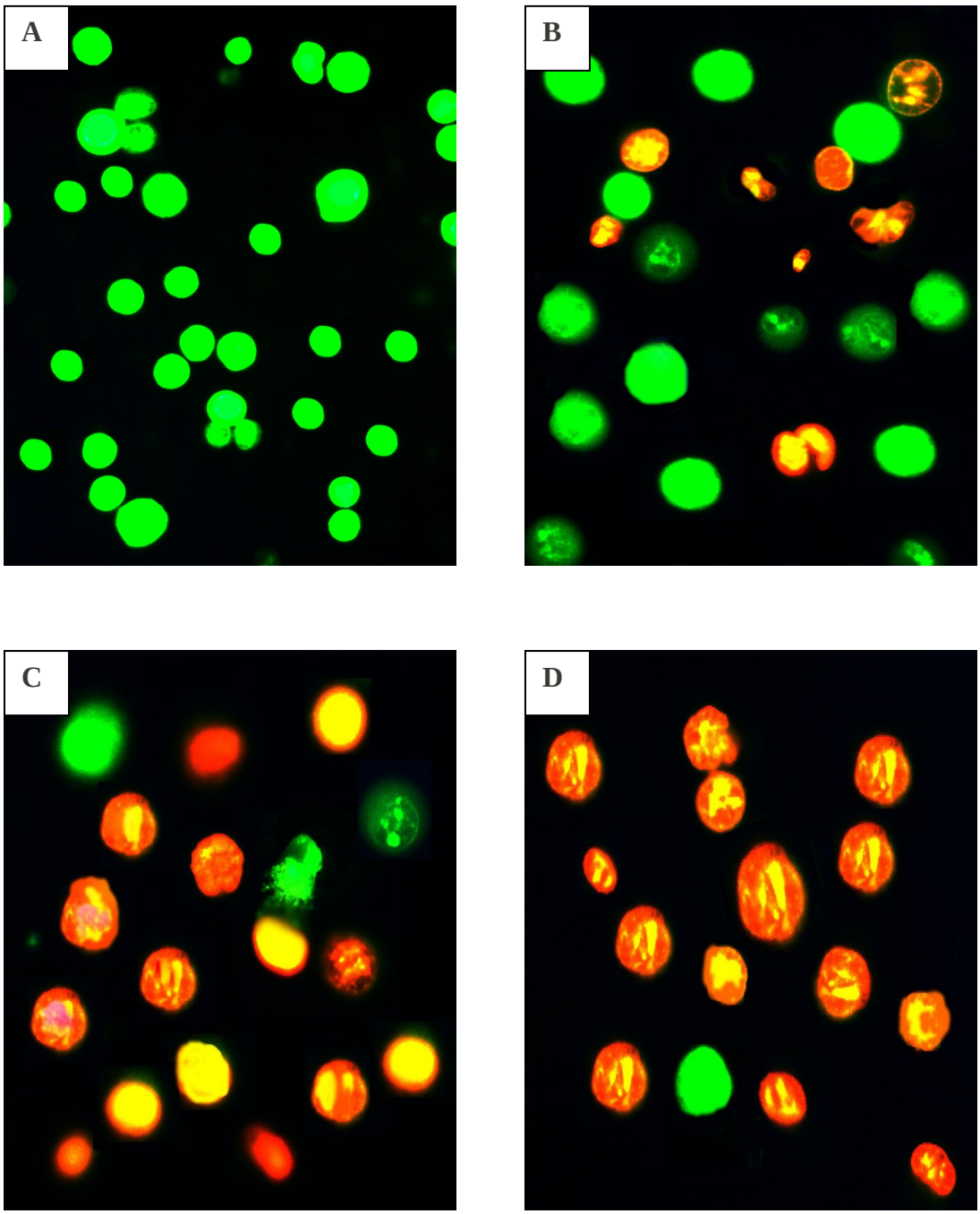


Figure III.3. 2: Morphological assessment of apoptotic DLA cells (M^4L^1)

DLA cells (10^6 cells) were cultured with 62.5µg/mL (IC_{50}) of M^4L^1 at 37°C in 5% CO_2 incubator for 24 hours. Equal volumes of treated/non-treated DLA cell suspension and ethidium bromide/acridine orange dye were mixed in a tube and observed under fluorescent microscope. Live cells fluoresce green and dead cells orange-red. Bright red cells denote necrosis. (A - Control, B- 12^{th} hour, C- 18^{th} hour, D-24^{th} hour).

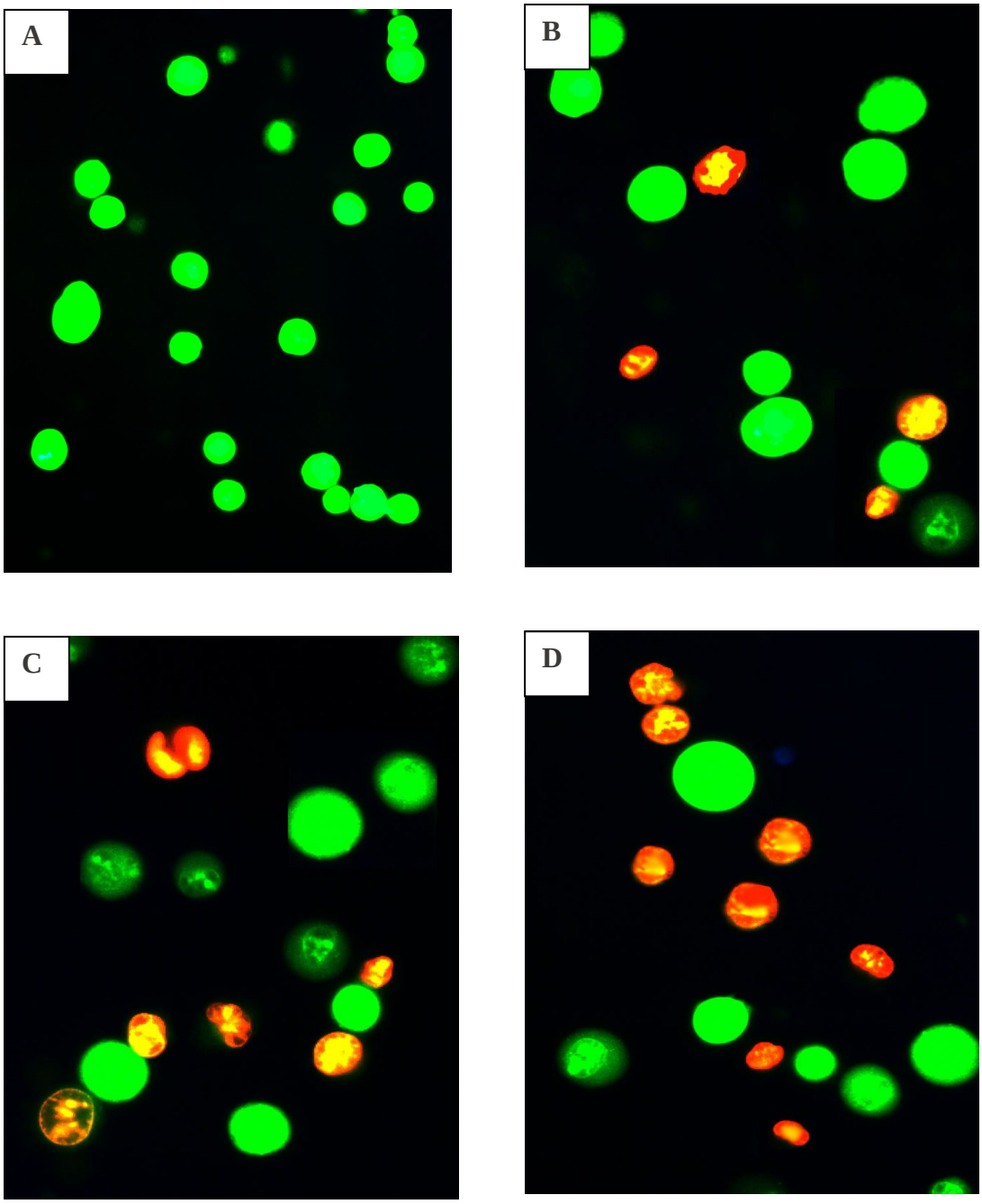


Figure III.3.3: Morphological assessment of apoptotic DLA cells (M^3L^4)

DLA cells (10^6 cells) were cultured with 125µg/mL (IC_{50}) of M^3L^4 at 37°C in 5% CO_2 incubator for 24 hours. Equal volumes of treated/non-treated DLA cell suspension and ethidium bromide/acridine orange dye were mixed in a tube and observed under fluorescent microscope. Live cells fluoresce green and dead cells orange-red. Bright red cells denote necrosis. (A - Control, B- 12^{th} hour, C- 18^{th} hour, D-24^{th} hour).

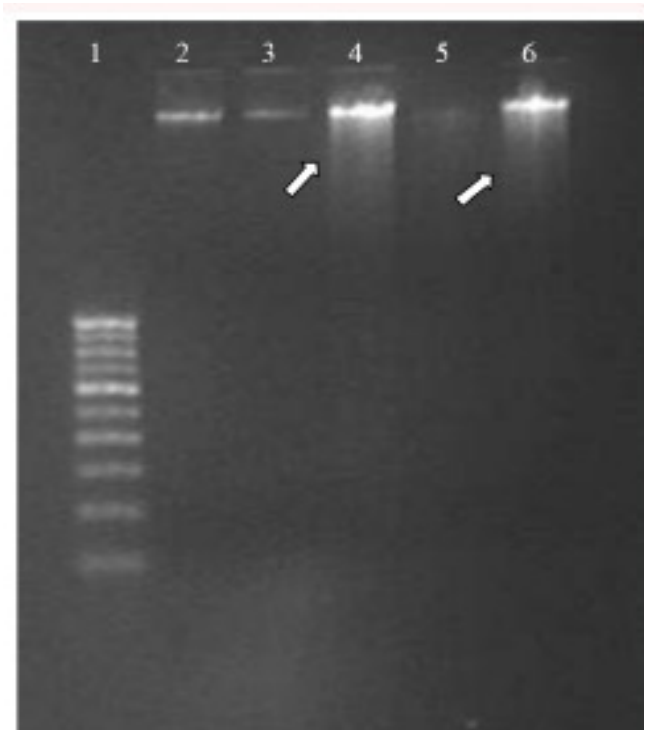


Figure III.3.4: DNA fragmentation by TMC's after 48 hours.

DLA cells (10^6 cells) were cultured with Transition Metal Complexes at 37°C in 5% CO_2 incubator for 48 hours. Cells were pelleted, DNA isolated and run in a 2% Agarose gel electrophoresis to obtain the characteristic DNA ladder. Lane 1: Molecular weight marker; Lane 2: M^3L^1; Lane 3: M^3L^2, Lane 4: M^4L^1; Lane 5: M^4L^3; Lane 6: M^3L^4. Arrows showing characteristic ladder appearance.

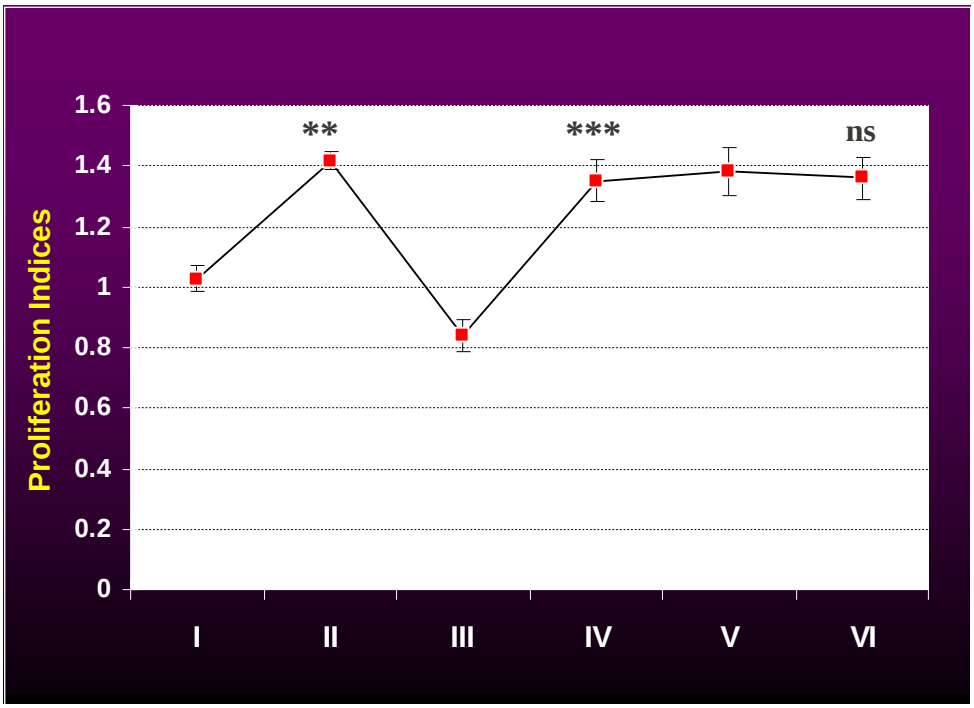


Figure III.3.5: Effect of M^4L^1 on PHA-induced lymphocyte proliferation

Mice were administered M^4L^1 (5mg/kg) daily for 14 days and Cisplatin (2mg/kg) once in DLA induced animals. Lymphocytes were isolated and cultured in the presence of PHA (mitogen) to induce proliferation. Proliferation indices were calculated by the formula; Test OD/Control OD. Group I – Control, II- M^4L^1, III-DLA, IV-DLA+M^4L^1, V-DLA+CIS, and VI-DLA+CIS+M^4L^1. Data were expressed as mean ± SD (n=6). Statistically significant differences at *P<0.05, **P<0.01, ***P<0.001, *ns* – non-significant, as compared with previous group.

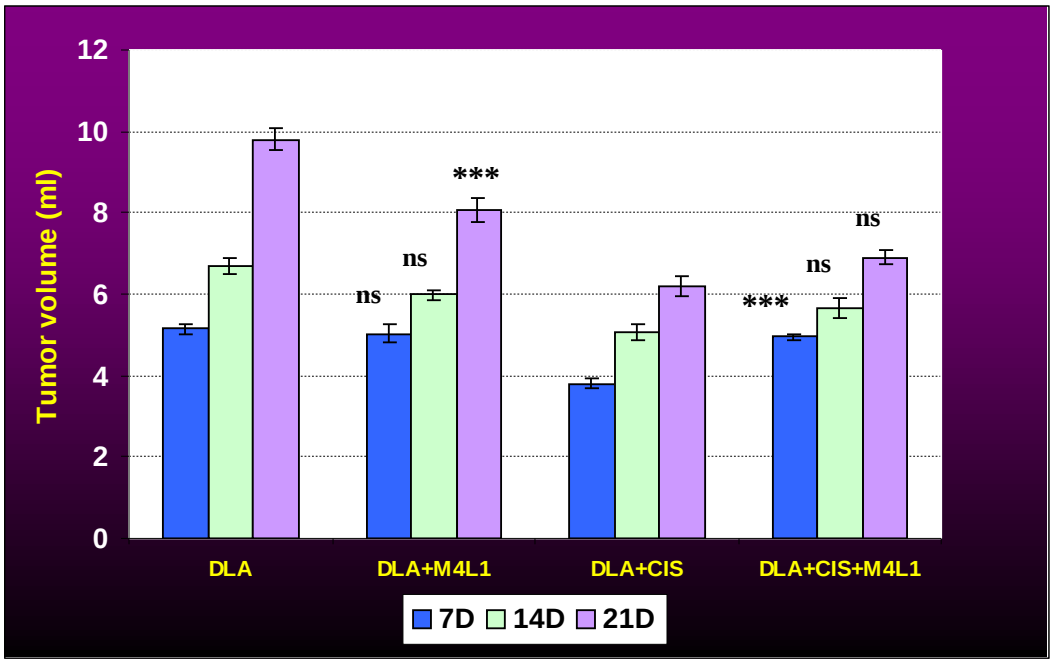


Figure III.3.6: Effect of M^4L^1 on Tumour Volume (7th, 14th and 21st days)

$M^4L^1$was administered at a dose of 5mg/kg/animal (n=6) for 14 days; 24h after DLA tumour induction (1million cells/animal). Cisplatin was administered 2mg/kg body weight/animal once. Data were expressed as mean ±SEM. Statistically significant differences at ***P<0.001, ns–non-significant, as compared with previous group.

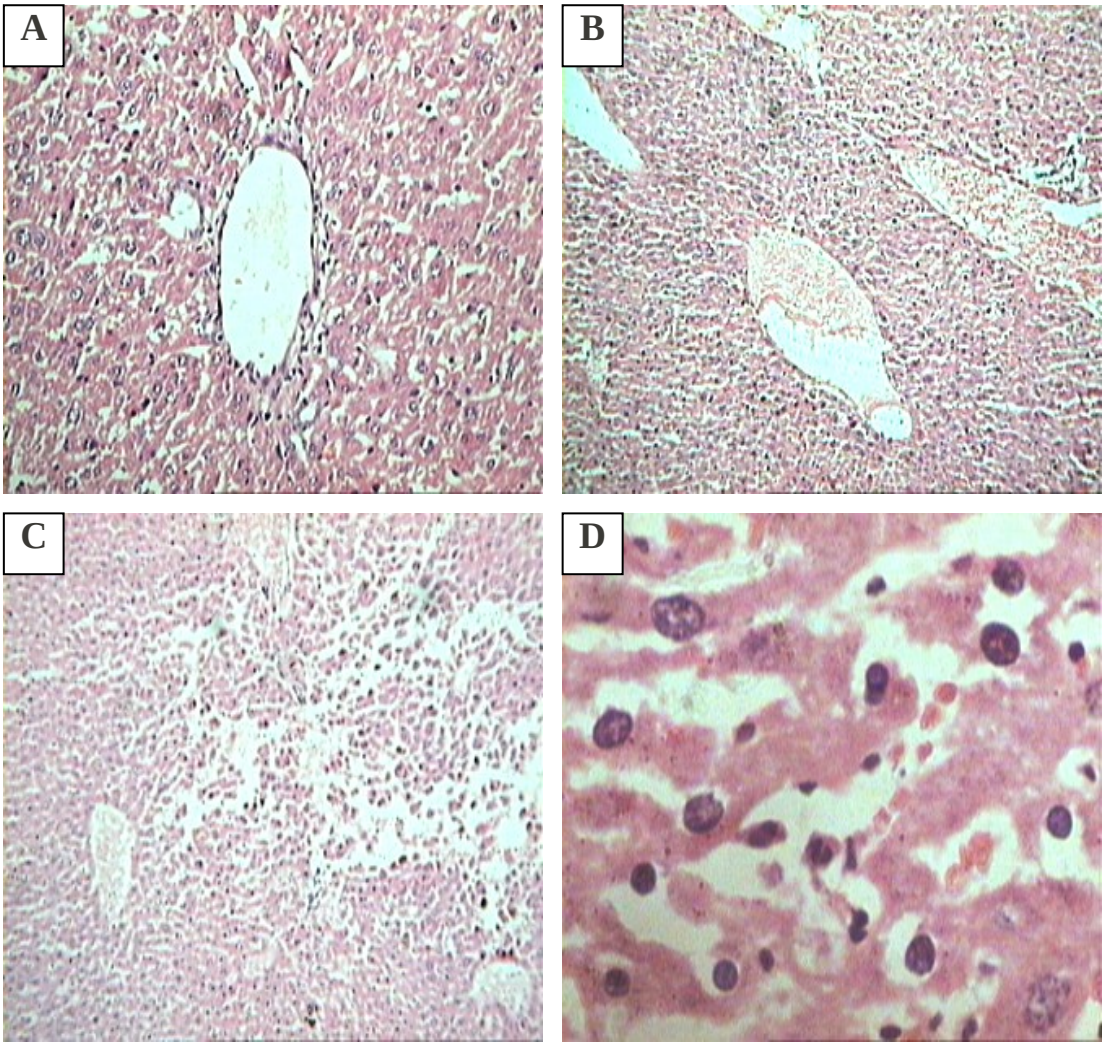
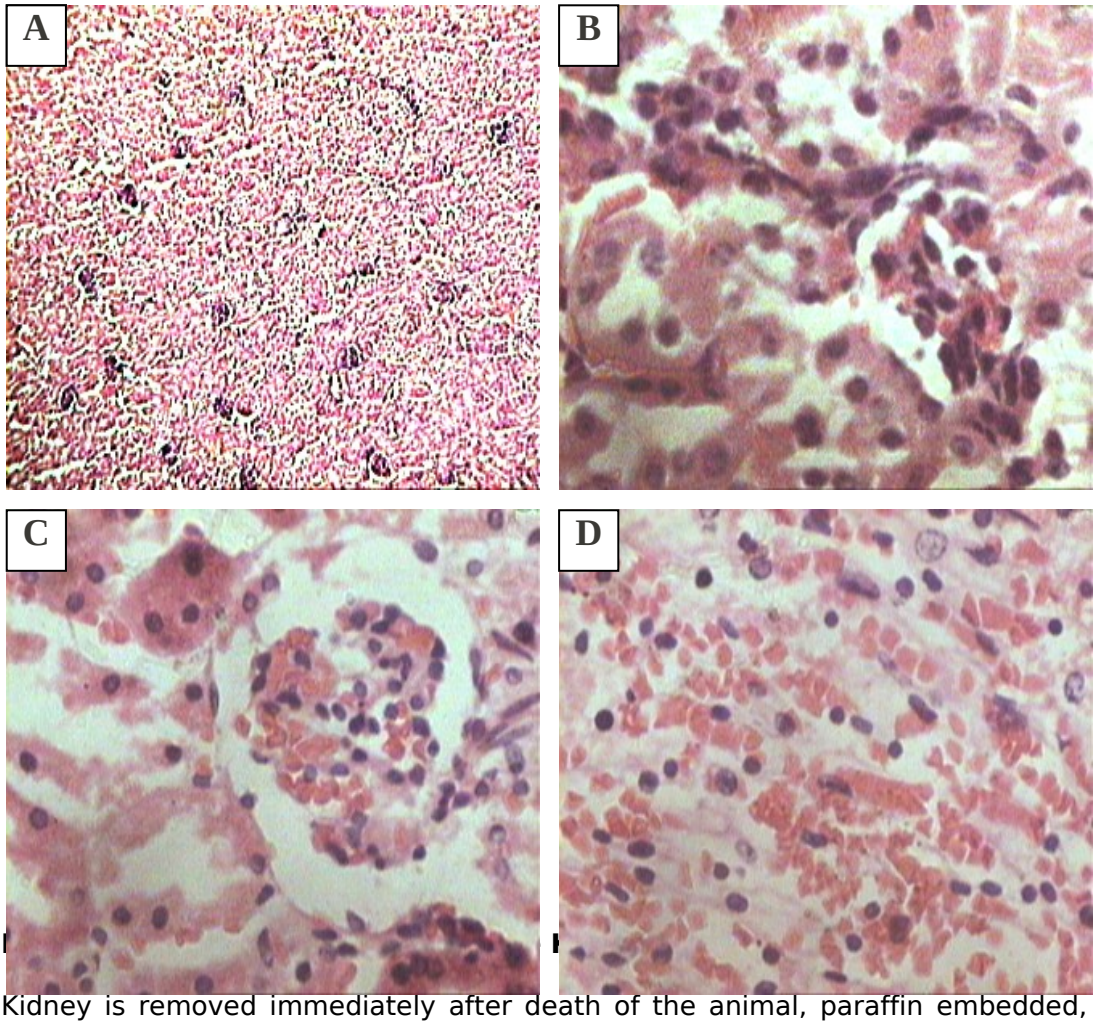


Figure III.3.8: Histopathology of the Liver

Liver is removed immediately after death of the animal, paraffin embedded, cut into thin sections and processed by H&E staining. A) Control liver – Portal triad showing intact hepatocytes B) Test liver (40mg/kg) - Congestion of hepatocytes C) Test liver (40mg/kg of M^4L^1) - Disruption of the liver chords, variation in size of nuclei; some are hyperchromatic pyknotic. Dialated sinusoids with frayed edges of the hepatocytes are also seen. D) Test Liver (40mg/kg of M^4L^1) - magnified 400X.



Kidney is removed immediately after death of the animal, paraffin embedded, cut into thin sections and processed by H&E staining. A) Control Kidney – Normal equally spaced glomerulus with intact tubules B) Control Kidney – magnified 400X C) Test Kidney (40mg/kg of M^4L^1) –Congested glomerulus with blood in the bowman's capsule associated with tubular degeneration; magnified 400X D) Test Kidney (40mg/kg of M^4L^1) – Severe medullary haemorrhage; magnified 400X.

Transition Metal Complexes	Percentage Cell Death at Concentration (µg/mL)											
	2000	**1000**	**500**	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**	**0.98**
M^1L^1	32	20	10	5	4	0	0	0	0	0	0	1
M^2L^1	20	14	7	5	4	3	2	0	0	0	0	0
M^3L^1	**55**	**48**	**40**	**33**	**29**	**14**	**9**	**7**	**3**	**0**	**0**	**0**
M^4L^1	**90**	**84**	**75**	**67**	**58**	**55**	**45**	**23**	**15**	**10**	**0**	**0**
M^1L^2	15	6	4	2	0	0	0	0	0	0	0	0
M^2L^2	30	27	25	18	12	0	0	0	0	0	0	0
M^3L^2	**60**	**54**	**45**	**30**	**28**	**25**	**17**	**10**	**5**	**2**	**1**	**0**
M^4L^2	40	23	10	7	5	2	0	0	0	0	0	0
M^1L^3	10	5	2	0	0	0	0	0	0	0	0	0
M^2L^3	12	7	0	0	0	0	0	0	0	0	0	0
M^3L^3	23	12	10	7	4	0	0	0	0	0	0	0
M^4L^3	45	41	40	32	25	22	10	5	4	0	0	1
M^1L^4	24	12	4	2	0	0	0	0	0	0	0	0
M^2L^4	20	10	7	4	5	2	0	0	0	0	0	0
M^3L^4	**75**	**66**	**60**	**59**	**57**	**42**	**25**	**12**	**10**	**7**	**3**	**3**
M^4L^4	37	30	30	28	27	21	17	14	12	11	5	2

Table III.3.1: Trypan Blue Dye Exclusion Staining for *Invitro* Cytotoxicity

DLA cells (5000 per well) were cultured with Transition Metal Complexes, at concentrations from 2000 µg/mL to 0.98µg/mL for 3 hours at 5%CO_2 and 37°C. 1% Trypan Blue was added to this suspension and counted in a microscope for percentage cell death.

Transition Metal Complexes	Percentage Cell Death at Concentration (µg/mL)											
	2000	**1000**	**500**	**250**	**125**	**62.5**	**31.25**	**15.63**	**7.81**	**3.91**	**1.95**	**0.98**
M^1L^1	44	30	25	18	12	6	4	4	3	2	1	1
M^2L^1	24	11	7	7	6	2	1	0	1	1	0	0
M^3L^1	**57**	**43**	**30**	**24**	**22**	**14**	**7**	**4**	**3**	**1**	**1**	**0**
M^4L^1	**60**	**57**	**54**	**52**	**45**	**21**	**14**	**12**	**15**	**10**	**5**	**2**
M^1L^2	14	7	6	3	2	1	1	1	0	0	0	0
M^2L^2	28	32	27	24	21	18	14	12	10	8	3	2
M^3L^2	**56**	**48**	**40**	**30**	**27**	**19**	**10**	**8**	**7**	**4**	**3**	**2**
M^4L^2	39	27	27	24	19	8	4	2	1	1	1	1
M^1L^3	29	12	7	6	4	2	1	1	0	1	0	0
M^2L^3	47	45	40	14	12	11	6	4	1	1	0	0
M^3L^3	18	12	14	4	3	2	1	1	1	1	1	0
M^4L^3	41	37	24	21	17	14	12	6	6	6	4	2
M^1L^4	12	7	8	5	6	3	2	1	1	0	0	0
M^2L^4	50	41	25	14	12	10	7	8	6	4	1	1
M^3L^4	**52**	**50**	**49**	**41**	**37**	**28**	**21**	**17**	**12**	**8**	**4**	**3**
M^4L^4	33	28	24	22	20	17	12	11	10	8	4	3

Table III.3.2: Trypan Blue Dye Exclusion Staining for *Invitro* Cytotoxicity

HeLa cells (5000 per well) were cultured with Transition Metal Complexes, at concentrations from 2000 µg/mL to 0.98µg/mL for 3 hours at 5%CO_2 and 37°C. Cells were detached after trypsinization and 1% Trypan Blue was added to this suspension and counted in a microscope for percentage cell death.

Table III.3.3: IC_{50} values of the Transition Metal Complexes

IC_{50} values as per MTS assay		
TMC's	**DLA**	**HeLa**
M^4L^1	62.5 µg/mL	225 µg/mL
M^3L^4	125 µg/mL	500 µg/mL
M^3L^2	750 µg/mL	1000 µg/mL
M^3L^1	1350 µg/mL	1450 µg/mL

Parameters / Group	Hb (g/dl)	Total Count (cells/mm³)	Differential Count (%)		Bonemarrow cellularity (cells/femur)
			Lymphocyte	Neutrophil	
Control	12.76 ± 1.26	7933 ± 559	59.16 ± 3.48	34.16 ± 1.72	13.98 ± 0.95
M^4L^1	12.73 ± 0.73 ^{ns}	7950 ± **145** ^{ns}	64 ± 3.41 ^{ns}	38.5 ± 2.43 ^{ns}	14.75 ± 0.78 ^{ns}
DLA	9.43 ± 0.81	10583 ± 441	37.33 ± 3.88	58.5 ± 6.15	11.4 ± 0.78
DLA+M^4L^1	13.86 ± 0.76 ***	8658 ± 495***	66.33 ± 4.45***	35.66 ± 3.26***	14.73 ± 1.3***
DLA+CIS	13.7 ± 0.92	6250 ± 504	58.83 ± 3.06	42.66 ± 4.50	5.98 ± 0.59
DLA+CIS+M^4L^1	13.01 ± 0.71^{ns}	8058 ± 354***	57.16 ± 4.66 ^{ns}	41.83 ± 3.54 ^{ns}	14.89 ± 0.83***

Table III.3.4: Effect of M^4L^1 on hematological parameters and bone marrow cells

M^4L^1 was administered at a dose of 5mg/kg for 14 days *i.p.* Cisplatin (2mg/kg) was administered *i.p.* once 24h post-tumour inoculation. Mice were sacrificed on the 15th day and the parameters were evaluated. Data were expressed as mean ± SD (n=6) on day 15 of the experiment. Statistically significant differences at *P<0.05, **P<0.01, ***P<0.001, *ns* – non-significant, as compared with previous group.

Group	MST (Days)	MST (T/C %)	% ILS
DLA	17.8 ± 1.17	--	--
DLA+M^{4}L^1	21 ± 1.78*	117.97	17.97
DLA+CIS	24 ± 1.41	134.83	34.83
DLA+CIS+M^{4}L^1	23.7 ± 1.75^{ns}	133.14	33.14

Table III.3.5: Median Survival Time (MST) and Percentage increment in life span (ILS)

MST = (x+y)/2; x - the earliest day when the number of dead animals is ≥ N/2; y - the earliest day when the number of dead animals is ≥ (N/2) +1; and N - the number of animals in the group. %ILS= (T-C)/C x 100 where T and C are MSTs of treated and control. Data were expressed as mean ± SD (n=6). Statistically significant differences at *P< 0.05, *ns*–non-significant as compared with previous group.

CHAPTER I

INTRODUCTION

The true masters of life on earth are not humans but microbes. Animals could not live in the absence of micro-organisms. Germ free animals are usually more susceptible to pathogens. We humans have lots of microbial friends that with in our intestine.

The normal microbiota use space, resources, nutrients and may produce chemicals that repel invading pathogens and possible diseases through bacterial interference. The relationship between the body and its normal microflora is an example of symbiosis (**Dubey and Maheswari, 1999**). The microbial symbiotic associations are essential to the livelihood of both the microorganism and its partner. Without microbial symbiotants most animals and plants could not survive in natural communities. In fact human kind enjoys a peaceful co-existence with a majority of micro organisms. (**Harwood and Greenberg, 1999)**

Humanbeings harbour a wide variety of micro organisms both on and in their bodies. The normal microflora are more or less constant and are broadly divided into residents and transients. The residents constitute a constant population which cannot be

completely removed while transients vary from time to time. The residents prevent permanent colonisation of the body by other organisms **(Alcomo E, 2001)**.

The normal and constant microflora of human body have adapted themselves to life in certain parts of the body. Besides these some microbes reside as temporary microflora. The pathogens among the microflora may cause disease, when the body's defence mechanism fails. Their abnormal multiplication can cause disease such as enteritis and entotoxic shock. Normal microflora is mainly found of *E.coli, Aerobactor aerugenes, Clostridiun sp., Staphylococcus sp., Streptococcus sp.,* Fungi, Diphtheroids and certain pathogenic and non pathogenic bacteria. They survive by receiving nutrition from the body (**Carpenter PL, 1977**).

The immune system provides protection against potential pathogens. Both commensalistic and mutualistic relationships are found between man and microbes. The frequency of microbial infection in humans has increased dramatically because of multidrug resistant microbial isolates like fungi and bacteria. The increasing clinical significance of drug resistant bacterial pathogens has lend additional urgency to microbiological and antibacterial research.

Many natural antimicrobial compounds can be used in food preservation systems but only a few have been exploited. Microbial control in foods could be assured by suppressing one or more essential factors for microbial survival (**Horace DG, 1982**). It could be possible by adding suitable substances (weak organic acids, hydrogen peroxide, chelators, organic biomolecules) and applying physical (temperature, packa-ging) and/or chemical procedures (pH, oxide-reduction potential, osmotic pressure) (**Ray, 1996; Brull S, 1999**). These procedures could kill or make some unviable microorganisms. There has been increasing concern of the consumers about foods free or with lower level of chemical preservatives because these could be toxic for humans

Lactic acid bacteria based cultures have been traditionally used to preserve fermented food in the tropics. In USA and other developed countries such fermented foods as yoghurt, buttermilk and fermented sausages are increasing in popularity as they are considered natural and healthy. Works were carried out to study the effectiveness of different antimicrobial packaging systems on the microbial quality decay kinetics during storage of Mozzarella cheese was evaluated. Lemon extract, at 3 different concentrations, was used as active agent, in combination with brine and with a gel solution made of sodium alginate. Results show an increase in the shelf life of all active

packaged Mozzarella cheeses, confirming that the investigated substance may exert an inhibitory effect on the microorganisms responsible for spoilage phenomena without affecting the functional microbiota of the product (**Conte A, 2007**).

Bacterial Strains

Bacteria have been one of the most studied test systems. They will thrive under a wide range of temperature. Some of them may grow throughout the range of 6-50°C. Usually these micro organisms are divided into two groups. One is Gram positive and the other is Gram negative. The Gram reaction is a procedure which was developed in 1884 by a Danish physician, Christian Gram, who detected the disease producing bacteria in animal tissues. The Gram stain reaction is determined by microscopic examinations of cells that have been successfully stained with Crystal Violet dye, treated with iodine solution and rinsed with acetone or alcohol. The Gram positive cells retain the violet stain while the Gram negative ones decolourise the solvent (**Glem LJ, 1966**). The bacterial strains studied during this research work are both Gram positive and Gram negative.

The major clinical pathogens among the normal microflora associated with the human body are *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* (**Boman HG, 2002**). Among these *Escherichia coli, Proteus*

vulgaris, Pseudomonas aeruginosa are Gram negative organisms while *staphylococcus aureus* belongs to gram positive category.

Escherichia coli (E.coli)

E.coli is an enteric organism and a part of normal flora which is a large heterogeneous group of Gram negative rods, whose natural habitat is the intestinal tract of human and animals (**Donnenberg, 2002**). In the intestine they generally do not cause disease and may even contribute to normal functions and nutrition. The bacteria become pathogenic only when they reach tissues outside the intestinal tract, particularly the urinary and billary tract, lungs causing inflammation at these sites. *E. coli* can generally cause intestinal and extra intestinal infections such as urinary tract infections, meningitis, peritonitis, septicemia and Gram negative pneumonia (**Feng P, 2007; Pearson H, 2007**).

Proteus vulgaris

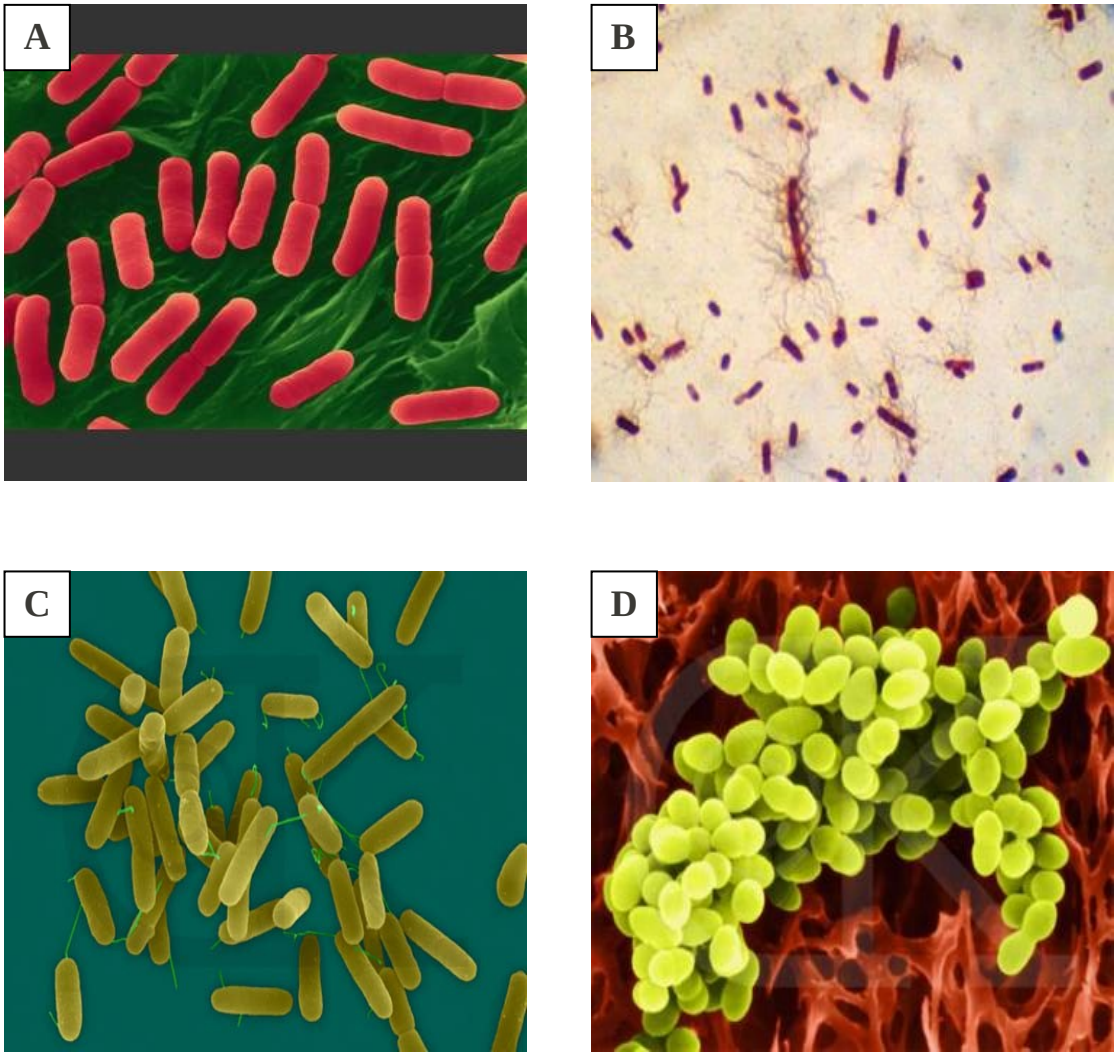
P. vulgaris is a rod shaped Gram negative bacterium that inhabits the intestinal tracts of animals and can be pathogenic. It can produce acid with the fermentation of glucose and sucrose. Optimal growing temperature is 23^{o}C in a facultative environment. In humans it can cause urinary tract infections and wound infections.

Pseudomonas aeruginosa

P. aeruginosa is a Gram negative, aerobic, rod shaped bacterium with unipolar motility. It secretes a variety of pigments including pyocyanin, fluorescein and pyorubin (**Iglewski BH, 1996**). Definitive clinical identification of *P. aeruginosa* often includes identifying the production of both pyocyanin and fluorescein as well as its ability to grow at 42°C (**King EO, 1954**). It is also capable of growth in diesel and jet fuel, where it is known as hydrocarbon utilising micro organism ("Hum bug") causing microbial corrosion. In humans it can cause septisemia, wound infections, burn infections and organ invasions (**Hachem RY, 2007**).

Staphylococcus aureus

S. aureus is the most common cause of *staph infections* is a spherical bacterium frequently live on the skin or in the nose of a person that can cause a range of illnesses from minor skin infections such as pimples, inpetigo and boils to life threatening diseases such as pneumonia, meningitis, Toxic Shock Syndrome (TSS) and septisemia (**Ryan KJ, 2004**).
S. aureus is a Gram positive coccus which appears as grape like clusters when viewed through a microscope and has large round yellow colonies when grown on blood agar plates (**Pyatkin, 1987**).



Bacterial strains

A) *Escherichia coli* B) *Proteus vulgaris* C) *Pseudomonas aeruginosa* D) *Staphylococcus aureus*

Growth of Bacteria

Bacteria can be found in all natural environments, often in extremely large numbers. As a group, they display exceedingly diverse metabolic capabilities and use almost any organic compound, and even some inorganic salts, as a food source. Some bacteria cause disease in humans, animals, or plants, but most are harmless or beneficial ecological agents whose metabolic activities sustain higher life-forms. Without bacteria, soil would not be fertile, and dead organic material would decay much more slowly. Some bacteria are widely used in the preparation of foods, chemicals, and antibiotics (**Gorbach SL, 1990**).

Bacterial Nutrition

All bacterial life has the same basic nutritional requirements which include:

1. **Source of Energy**. This may be light (the sun or lamps) or inorganic substances like sulfur, carbon monoxide or ammonia, or preformed organic matter like sugar, protein, fats etc. Without energy life can not exist and quickly dies or becomes inactive.

2. **Source of Nitrogen**. This may be nitrogen gas, ammonia, nitrate/nitrite, or a nitrogenous organic compound like protein or nucleic acid.

3. **Source Of Carbon**. This can be carbon dioxide or monoxide, methane, carbon monoxide, or complex organic material

4. **Source Of Oxygen**. All cells use oxygen in a bound form and many require gaseous oxygen (air), but oxygen is lethal to many microbes.

5. **Source Of Water**. All life requires liquid water in order to grow and reproduce

6. **Source of Minerals Like Iron, Zinc, Cobalt etc**. These are called Trace metals that are required by some enzymes to function.

The most common environmental conditions that a microbiologist considers are temperature, pH, oxygen, light, salt/sugar concentration and special nutrients. Each bacterium has an optimum range of these conditions within which it grows at a maximum rate. In some cases this may be a fairly broad range such as bacteria that can grow maximally over a 5 to 10 degree temperature span. The hydrogen ion concentration of the growth medium plays a very important role in bacterial growth. Most of the bacteria prefer to grow in neutral or near neutral pH (6.5-7.5). Highly acidic and alkaline pH is not suitable for bacterial growth (**Janocha R, 1995**).

The gaseous environment of the growth medium will affect bacterial growth. The important gases which influence bacterial growth are oxygen and carbon dioxide. Based on their requirements for oxygen, bacteria are generally classified into three groups.

1. Aerobic bacteria; growing in presence of free oxygen or air
2. Anaerobic bacteria; growing in absence of free oxygen or air
3. Facultative anaerobic bacteria; they are really aerobic but also have the capacity to grow in the absence of air or oxygen.

Anti Microbial Therapy

The control of infectious micro organisms is critical for the prevention and treatment of disease. Modern medicine is dependant on chemotherapeutic agents and most of them are antibiotic. The drug may be administered orally, intravenously or intramuscularly. All these affect, the invading pathogens but high doses may affect the host seriously (**Kell, 1997**).

Exposure time to an antimicrobial agent is the most important and frequently overlooked factor in the control of micro organism. Killing a microbial population is a gradual process except

in incineration. Young, actively growing cells are unusually susceptible to antimicrobial agents whereas mature or dormant cells are very resistant. The nature of medium in or upon which the organisms occur, influences the efficiency of the antimicrobial drugs (**Kadri MS, 2004**). Micro organisms are usually more resistant when suspended in a media of a reaction with a satisfactory pH for growth and killing by chemicals occurs rapidly as the pH changes from this value. A dry chemical placed in contact with bacteria is usually ineffective because moisture is essential for the disinfection process.

The first stage of antimicrobial therapy began in 1980 when Van Behring discovered that administration of immune serum could protect an immunologically naive animal against tetanus and diphtheria. This stage came to an end by the introduction of effective chemotherapy like sulphonamide. The introduction of antibiotics into clinical medicine was unquestionably one of the triumps of 20th century and physicians were able to treat most of the bacterial diseases. Unfortunately drug resistance inevitably developed (**Katherine M, 2004**).

Antibiotics are chemical substances excreted by some micro organisms which inhibit the growth and development of other microbes (**Levy S, 1998**). The study of antibiotics began in 1929 by experiment of Alexander Fleming. Some of these drugs were

obtained naturally. This naturally occurring drugs were chemically modified to enhance beneficial effects while minimising their toxic effects and the products thus obtained are called semisynthetic antibiotics. e.g. ampicillin, carbenicillin, methcilline,etc. Some drugs are synthesised by chemists artificially and are called synthetic drugs. e.g. sulphanomide, chloramphenicol, etc.

One of the major effect that an antibiotic can bring is the development of resistant varieties of microbes. The resistance developed by a microbe against an antibiotic may be due to many factors. This may occur due to inherent resistance, natural resistance, acquired resistance and vertical and horizontal evolution. Bacteria are able to exchange genes in nature by three processes - conjugation, transduction, and transformation. Since bacteria developed genes for drug resistance on plasmids, they are able to spread drug resistance to other strains and species during genetic exchange processes. The fast growth rate, high concentration of cells, genetic process of mutation and selection are responsible for the resistance and evolution of the bacteria (**Kfrisell W, 1982**).

The use of live bacteria to induce an immune response to itself or to a carried vaccine component is an attractive vaccine strategy. Advantages of live bacterial vaccines include their mimicry of a natural infection, intrinsic adjuvant properties and

their possibility to be administered orally. Derivatives of pathogenic and non-pathogenic food related bacteria are currently being evaluated as live vaccines. However, administration of live bacterial vaccines poses some risks. In addition, vaccination using recombinant bacteria results in the release of live recombinant organisms into nature. This places these vaccines in the debate on application of genetically modified organisms. (**Ann Detmer, 2006**).

Antibacterial drugs exert their action by interfering with either the structure or the metabolic pathways of bacteria. Important methods of antibiotic action include interfering with metabolic pathways, binding to the cytoplasmic membrane, inhibiting protein synthesis, inhibiting protein biosynthesis, inhibiting nucleic acid biosynthesis and disrupting cell wall biosynthesis.

Metals and Microbes

It is logical to observe that evolution has selected elements for tasks that fully related with chemical experience (**Bernsteen, 1977; Monod J, 1988; Hanzlik 1976**). For example Fe and Cu with stable oxidation states for electrons transfer, binding and activation of O_2, oxidation-reduction of substrates, Mo with three stable oxidation states for oxygen atom transfer; Zinc with flexible

stereochemistry for non redox catalysis; Ni and Co for catalysis involving formation and rupture of metal carbon bonds **(Fiabane AM, 1977**). Thus it can be stated that nature has made extensive use of metal ions in biological systems and their functions can be conveniently explained on the basis of various principles of co-ordination chemistry (**Melson GA, 1989; Hughes MN, 1981**).

The inorganic pharmacology considered to be an important field with more that 25 inorganic compounds being used in therapy as anti bacterial, antifungal and anticancer agents (**Louie AY, 1999; Walsh C, 2001; Bertini I, 1994**). Most of the heavy metals either along or in certain compounds exert a determinal effect upon micro organisms. The most effective are mercury, copper and silver. This ability of extremely small amounts of certain metals particularly silver, to exert a lethal effect upon bacteria is called oligo dynamic action (**Cotton FA, 1966**). This phenomenon is designed to demonstrate the zone of inhibition surrounding the metal after incubation. The effectiveness of these small amounts of metallic ions is believed to be due to high affinity of certain cellular proteins for the ion. Large amounts are accumulated in the cell from a dilute solution. Oligo dynamically active metals have been used in variety of applications such as treatments of water supplies, preparation of antiseptic articles like

bandages, ointments and in the impregnation of various fabrics (**David WM, 1972**).

The vital role of transition metal complexes of Schiff base ligands in anti microbial therapy has been witness for many years by the large number of publications and reviews. The reason for this sustained interest in this compounds are many but major among them must be their general easiness of preparation, diverse properties and uses as biological models. (**Mayor TJ, 2004; Cotton SA, 2005**).

The metal ions coordinate to the biological ligands through nitrogen, oxygen and sulphur atoms. The active sites of a large number of proteins and enzymes contains one or more metal ions. Their structural properties are often modulated by the co-ordination environment of metal ions (**Dhar SK, 1973; Hoare RJ, 1980; Schroedar A, 1975**). It has been variously estimated that approximately one third of all proteins and enzymes require metal ions as cofactors for biological functions.

For metal complexes showing antibacterial activity the following five principal factors have been considered: (i) **The chelate effec**t: Ligand like bipyridine, phenanthroline, *o*-phenyldiamine bound to metal ions in a bidentate fashion show higher antimicrobial efficiency than complexes with unidentate N-

donor ligands *e.g.* pyridine.) (ii) **The total charge of the complex**: Generally the antimicrobial efficiency decreases in the order cationic,_neutral ,anionic complex. This behavior may be related to the redox potential which is decreased in the same order. (iii) **The nature of the ion**. (iv) **The nature of the N-donor ligands**, and (v) **The nuclearity of the metal center in the complex**: Dinuclear complexes are more active than uninuclear ones.

Antibacterial Activity of Transition Metal Complexes

Dwyer and Meller reported that some metal complexes are very effective against certain microbial infections. It was found that metal chelates are more potent than the metals and chelating agents themselves. The early results on the antibacterial activity of transition metal ions and their complexes have been summarized by Dwyer (**Dwyer FP, 1964**). They found that Iron(II) and Ruthenium (II) Phenanthroline complexes have some bacteriostatic activity against Gram-positive and Gram-negative bacteria.

A series of copper complexes of some amino acid displayed antimicrobial activity against gram positive bacteria. Co(II), Cu(II), Ni(II) and Zn(II) complexes of amino acid derived compounds were evaluated for their antibacterial activity against bacterial species *E.coli*, *S.pneumomiae* and *S. typh*. The screening studies showed

that the metal complexes are more antibacterial than the simple uncomplexed ligand (**Chohan ZH, 1993**).

Co(II), Ni(II), Cu(II) and Zn(II) complexes of acylhydrazine derived pyrrolyl compounds were synthesised and evaluated for their antibacterial activity against *E. coli, P. aeruginosa* and *S. typh.* Here also the complexes are found to be more potent than their ligands (**Perrvez H, 2002**).

Some new transition metal complexes of ciprofloxacin-imines derived from ciprofloxacin and p-substituted anilines were synthesized and characterized. These ligands as well as their metal complexes were also evaluated for their antibacterial activity against several bacterial strains, such as *Staphylococcus aureus*, *Bacillus subtilus*, *Salmonella typhae*, and *E. coli*. It was found that metal complexes are more antibacterial as compared to uncomplexed ligands (**Muhammad I, 2007**).

Mixed ligand transition metal complexes of Cu(II), Ni(II) and Co(II) ions with Schiff base ligands derived from the condensation of O-hydroxy benzaldehyde with Amino phenols and nitrogen donor amine bases, e.g. Ethylenediamine, 2-Aminopyridine, O-Phenylenediamine or Thiocyanate have been synthesized. These complexes showed antibacterial and antifungal activity (**Saidul Islam M, 2001**).

Mixed-ligand transition metal complexes of Co (II) ions were synthesized, where, Maleicacid as a primary ligand and heterocyclic amine bases as secondary ligands have been used, respectively. Their anti-bacterial and anti-fungal activity has been evaluated. Disc diffusion methods were employed for anti-microbial assays against 14 pathogenic bacteria (5 Gram positive and 9 Gram negative) and 14 fungi. The complexes containing 8-Hyroxy-Quinoline as secondary ligand were having much more microbial activity than the other complexes (**Saidul Islam M, 2003**).

New complex of Cu(II) ion with Schiff base derived from the condensation of m-Aminophenol with o-Hydroxybenzaldehyde has been synthesized. The complex has the formulae [Cu(L)(NN)]. Antineoplastic activity of this complex has been carried out on Swiss Albino male mice (**Saidul Islam M, 2002**).

A study was done to investigate the biological activity of seven new chromium based coordination complexes against Gram-positive and Gram-negative bacteria, fungi and brine shrimp nauplii. The complexes showed good antibacterial activity at the concentration of 200μg $disc^{-1}$ and gave MIC values between 16-64μg ml^{-1} against the tested bacteria. The complexes gave comparatively better antibacterial activity against the Gram-negatives (**Chanmyia Sheikh, 2004**).

Preparation, characterisation and bioactivity of peroxo complexes of Mo (VI) containing organic acid and amine bases were carried out. The antimicrobial properties of the peroxo complexes of Mo (VI) indicated that both the complexes were stronger antibacterial and antifungal agents. However, the highest antifungal activity was shown by Mo-Alanine complex (**Nasrin J, 2007**).

Co(II), Ni(II), Cu(II) and Zn(II) complexes with three potentially tridentate ligands formed by coupling of diazotised anthranilic acid with 1,3-diketones have been synthesised and characterised. The Cu(II) complexes have been screened for their antibacterial properties. It has been observed that the metal complexes are more potent bactericides than the ligands (**Cheriayan M, 2007; Chandrappa GT, 1985**).

A new Schiff base ligand has been synthesised by the condensation of 2,4 dihydroxy benzaldehyde with p-benzyl oxy aniline in ethanol solution. The ligand and its Co(II), Ni(II) and Co(II) complexes were screened for their antibacterial and antifungal properties by agar well diffusion method. Accordingly to the UV and IR Spectra, the ligand is co-ordinated to the metal through the phenolic oxygen and the imino nitrogen (**Ispir E, Kurtoglu M, 2007**).

Scope of the Present Investigation

A review of the literature showed that transition metal complexes can be considered as an effective tool in antibacterial studies. It was seen that biological active compounds become more bacteriostatic by chelation process with metal ions. A plethora of literature exist on the antibacterial effect of phenolic derivatives on all kind of bacteria. It is worthy to mention that recently there are reports about the importance of hydrophobic effects in chemical-biological interaction which were brought about by QSAR analysis. (Quantitative Structure – Activity Relationships) (**Thakur A, 2006; Hansch C, 2001**). Recent developments of transition metal complexes as antibacterial agents look for a special attention in molecular and genetic level also.

In the present work, complexes of Co(II), Ni(II) Cu(II) and Zn(II) with two new Schiff bases i.e. 2-Hydroxyacetophenone2-Aminothiophenol (HAPATP) and Benzil 2-Aminophenol (BAP) have been synthesised and characterised as given in Part I. Further, their antibacterial activity towards some clinically important bacteria such as *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* was evaluated.

CHAPTER II

MATERIALS, METHODS AND INSTRUMENTS

All the complexes used in the study were synthesised and characterised as discussed in Part I. Analar grade chemicals and commercially available media were purchased from BDH, Glaxo and E.Merck. The micro organisms used were supplied from the stock collections of Department of Biotechnology, University of Kerala, Trivandrum.

All the glasswares used were of Borosil. They were washed thoroughly and rinsed with double distilled water. All the tubes and petridishes were sterilised at 121°C before preparing the samples.

Metals and ligands

Metals	***Ligands***
M^1 – Cobalt	L^1–2-Hydroxyacetophenone
M^2 – Nickel	2-Aminothiophenol (HAPATP)
M^3 – Copper	L^4 – Benzil-2-Aminophenol (BAP)
M^4 – Zinc	

General Methods of Determination of Antibacterial Activity

(1) Preparation of the Media

Nutrient Agar (**NA**) was used as the media for plate preparation and Nutrient Broth (**NB**) was used for culturing the bacterial strains.

Compositions of the Medium

a) Nutrient Agar (NA)

Peptone	10g
Beef Extract	10g
NaCl	5g
Agar	15g
Distilled Water	1000ml
pH (25^{o}C)	7.4±0.02

Dissolved ingredients in sufficient quantity of distilled water. Then it is sealed with a sterilised cotton plug and autoclaved at 121^{o}C for 15 minutes.

b) Nutrient Broth

Peptone	10g
Beef Extract	10g
NaCl	5g
Distilled Water	1000ml
pH (25°C)	7.4±0.02

Dissolve the ingredients by heating in distilled water. Distributed in 225ml quantities in 500ml conical flask, plugged with not absorption cottons. Autoclaved at 121°C for 15 minutes.

(2) Preparation of Agar Plates

1000 ml of pH adjusted NA is prepared and autoclaved. After autoclaving it is allowed to cool for some time at 42-45°C. Then it is poured into sterilised petri plates inside the Laminar flowhood chamber. The plates were allowed for solidification and dried and they kept for sterility checkup for 24 hours.

(3) Preparation of Sample Discs

Stock solutions of the synthesised ligands and complexes were prepared in DMSO. The compounds were suitably dissolved and diluted to obtain the concentrations ranging from 50$\mu g disc^{-1}$ to 500$\mu g disc^{-1}$. These samples were applied to paper disc having

5mm diameter (Whatman No:1) with the help of a micropipett. The disc were kept in an incubator for 24 hours at 37°C.

(4) Antibiotic Standard

Commercially available standard Gentamycin discs were used as a standard antibiotic, against all the bacterial strains studied.

(5) Antibacterial Screening

Antibacterial activity of the transition metal complexes and ligands was determined by the microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS) (**Villanova PA, 1997; Isenberg DH, 1998; Zgoda JR, 2001**) and paper disc diffusion technique (**Wayne PA, 2001**). 5-6 drops of sterilised water was taken in a test tube and with the help of inoculation loop, test organisms were taken off from the sland culture and diluted in the test tube. Using a sterile swab stick, the activated bacterial strains were spread over the entire surface of the Nutrient Agar plates in a uniform manner. Swab was used to obtain a continuous layer of microbe on the culture medium. This was then allowed to dry for 15-30 minutes. The paper discs which contains the samples were placed on Agar plates using sterile forceps. Each disc was placed with sufficient distance from each other. The plates were incubated at 37°C for 24 hours. The zone of

inhibition (diameter in millimeter) was then measured around the disc (**Tkaczynski T, 1995; Pasternak K, 2006**). A solvent control disc was also kept along with the test sample discs.

Apparatus and Equipments used

1. Petridishes
2. Sample dishes
3. Pipette-1ml and 10 ml capacity
4. Test tubes - 10ml, 25ml, 50ml capacity
5. Durham's tubes
6. Flasks-150ml, 250ml, 500ml, & 1litre
7. Microscopic slides
8. Microscope (Olympus Model K.H) Olympus India Pvt Limited
9. Incubator 37^{o}C
10. Electronic balance
11. Serological water bath
12. Air oven
13. Autoclave
14. Platinum loop.

CHAPTER III

ANTIBACTERIAL STUDIES OF Co(II), Ni(II), Cu(II) and Zn(II) COMPLEXES OF 2-HYDROXYACETOPHENONE 2-AMINOTHIOPHENOL (HAPATP) AND BENZIL 2-AMINOPHENOL (BAP)

Metal ions play a vital role in a wide variety of biological processes, through co-enzymatic systems. The interaction of these ions with biologically active ligands is matter of great interest. The use of organometallic drugs as antibacterial agents cannot be overlooked, as compared to antibiotics which now a days have became more prone to bacterial resistance. It is also well known that the elements of first transition series form biologically active transition metal complexes. Among these complexes of Cu(II) and Zn(II) found many applications in antimicrobial therapy. So the study of the antibacterial activity of complexes of Co(II), Ni(II), Cu(II) and Zn(II) ions are highly relevant.

In the present investigation the title Schiff bases and their metal complexes were evaluated for their antibacterial activity against some clinically important Gram negative and Gram positive bacterial strains. These include *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Synthesis and Characterisation of the Ligands and Complexes

The details regarding the synthesis and characterisation of Schiff bases HAPATP and BAP and their metal complexes [Co(II), Ni(II), Cu(II) and Zn(II)] were explained in Part I.

Determination of Antibacterial Activity

The analysis of the antibacterial activity of the newly synthesised ligands and their metal complexes were done by paper disc diffusion method as explained in Chapter II.

The prepared preset antibiotic agar plates were dried at 56°C for 45 minutes and cooled to room temperature. The sterile filter paper discs were dipped in various concentrations of the ligand and complex solutions ranging from 50μgdisc^{-1} to 500μgdisc^{-1}. These discs were placed at respective quarter on the surface of the agar plate. These plates were incubated for 24 hours at 37^{0}C and examined for clear zones of inhibition around the discs using a hard lens.

After 24 hours of incubation at 37°C, the zone of inhibition found around each disc were measured as diameter in mm and the results of the inhibition growth were recorded and interpreted. Both the ligands and their eight metal complexes along with the solvent control were screened by this method. The bacterial strains used for the study are, three-Gram negative organisms (*Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa)* and one gram positive organism *(Staphylococcus aureus)*.

Results and Discussion

The Schiff bases and their metal chelates were evaluated for their antibacterial activity against different bacterial strains at

concentrations ranging from 50μg $disc^{-1}$ to 500μg $disc^{-1}$. In testing the antibacterial activity of these compound we used more than one test organism to increase the chance of detecting the antibiotic potential of the tested materials. The antibacterial screening data are presented in the Tables IV.3.1 – IV.3.4. During the evaluation it was found that no inhibition zones were obtained with test samples of concentrations ranging from 50μg $disc^{-1}$ to 100μg $disc^{-1}$. But when the concentration was increased to 250μg$disc^{-1}$ to 500μg$disc^{-1}$ the metal complexes and the ligands exhibit well defined inhibition zones. But it is important to note that the complexes exhibit enhanced activity in contrast to free ligands in all the screening tests. This shows that the metal chelates are more antibacterial than the uncomplexed ligands.

In order to clarify any participating role of the solvent, DMSO in the bacterial screening, separate studies were carried out with DMSO as solvent control and it showed no meaningful activity against the bacterial strains under study. The activity of metal chelates was also compared with a known antibiotic, gentamycin (10μg $disc^{-1}$). The activities of the tested complexes were found to be less than that of the standard antibacterial agent used. For the standard drug, the exhibited inhibition zone diameter was in the

range of 15 – 25mm against all the bacterial strains used in this study.

The inhibition zone obtained for Co(II), Ni(II), Cu(II) and Zn(II) chelates of HAPATP and BAP against *E.coli* was given in Table IV.3.1. In both cases, the Cu(II) complexes showed considerable antibacterial activity than other metal complexes in the given concentration of 250μg $disc^{-1}$ and 500μg $disc^{-1}$. Zn(II) and Ni(II) complexes with 500μg$disc^{-1}$ also showed some moderate activity than their corresponding ligands. Hence it can be concluded that the metal ions in the complexes influence the antibacterial activity. Chelation can considerably reduce the polarity of the metal ion which in turn increases the lipophilic character of the chelate.

The antibacterial action of the title complexes and ligands against another Gram negative organism *P. vulgaris* is given in the Table IV.3.2. Here also the metal chelate showed better activity than their parent ligands. Among the metal complexes, the Cu(II) complexes of BAP showed better activity followed by Zn-HAPATP and Ni-BAP.

The maximum zone inhibition values were obtained against the Gram negative bacteria, *P.aerugenosa*. The enhanced activity was shown by Ni(II) and Cu(II) complexes of HAPATP and BAP. Here also the ligand showed least inhibition zone. The Zn(II) complex of

HAPATP shows some appreciable inhibition value than its counterpart, Zn-BAP. The results are given in the Table IV.3.3.

Table IV.3.4 shows the antibacterial effect of the metal complexes and their ligands on the Gram positive bacteria, *S.aureus.* A different effect of the metal complex was envisaged against this bacterial strain. In this case, only Cu(II) complexes of HAPATP and BAP showed some appreciable zone of inhibition. Other complexes and ligands showed poor antibacterial effect against this organism in comparison with other gram negative bacterial strains studied so far. Ni(II) and Zn(II) complexes of BAP were also found to be active towards *S.aureus* in the concentrations of 500$\mu g disc^{-1}$

From the above studies it can be concluded that metal chelates have higher antibacterial activity than the corresponding free ligands. Among the metal chelates studied, Cu-BAP was considered best and found active against all the four types of bacterial strains tested. This is because of an increase in cell permeability. Increased activity of metal chelate can be also explained on the basis of the overtone concept and chelation theory. According to the overtone concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only

lipid-soluble materials, in which lipo-solubility is an important factor that controls the antimicrobial activity.

On chelation, the polarity of the metal ion will be reduced to a greater extent due to overlap of ligand orbital and partial sharing of the positive charge of the metal ion with donor group. Further it increases the delocalization of π-electrons over the whole chelate ring and enhances the lipophilicity of complexes. This increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the metal binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism.

Antibacterial screening also showed that Ni(II), Cu(II) and Zn(II) chelates are more sensitive towards Gram negative bacteria such as *Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa.* Therefore it is claimed here that such chelates might have a possible antitumour effects since Gram negative bacteria are considered a quantitative microbiological method for testing beneficial and important drugs in both clinical and experimental tumour chemotherapy.

Antibacterial activity is also dependent on the molecular structure of the compound, the selected solvents and the bacterial

strains under consideration. The variation in the activity of different complexes against different microorganisms depends either on the impermiability of the cells of the microbes or difference in ribozomes in microbial cells. The screening studies of various Schiff base metal complexes and identifying their potentiality is essential because the successful prediction of a competent molecule and its drug like properties at the onset of drug design will pay off later in the field of drug development.

Table IV.3.1

Antibacterial Screening Data of L^1, L^4 and its metal complexes on *E.coli*

Bacteria	Conc. µg disc^{-1}	Diameter of Inhibition Zone (mm) for various samples									
		L_1	M^1L_1	M^2L^1	M^3L^1	M^4L^1	L_4	M^1L^4	M^2L^4	M^3L^4	M^4L^4
E.coli	250	5	6	8	13	11	5	9	12	16	14
	500	6	8	12	16	15	6	12	18	20	19

Table IV.3.2

Antibacterial Screening Data of L^1, L^4 and its metal complexes on *P. vulgaris*

Bacteria	Conc. µgdisc^{-1}	Diameter of Inhibition Zone (mm) for various samples									
		L^1	M^1L^1	M^2L^1	M^3L^1	M^4L^1	L^4	M^1L^4	M^2L^4	M^3L^4	M^4L^4
P. vulgaris	250	5	5	6	9	16	5	5	10	21	6
	500	5	6	6	12	20	5	6	16	28	8

M^1 – Cobalt

M^2 – Nickel

M^3 – Copper

M^4 – Zinc

L^1 – 2-Hydroxyacetophenone

2-Aminothiophenol (HAPATP)

L^4 – Benzil-2-Aminophenol (BAP)

Table IV.3.3

Antibacterial Screening Data of L^1, L^4 and its metal complexes on *P. aerugenosa*

Bacteria	Conc. μg disc^{-1}	Diameter of Inhibition Zone (mm) for various samples									
		L^1	M^1L^1	M^2L^1	M^3L^1	M^4L^1	L^4	M^1L^4	M^2L^4	M^3L^4	M^4L^4
P. aeruginosa	250	5	6	22	26	17	7	5	22	25	5
	500	5	7	28	29	20	8	6	28	30	7

Table IV.3.4

Antibacterial Screening Data of L^1, L^4 and its metal complexes on *S. aureus*

Bacteria	Conc. μg disc^{-1}	Diameter of Inhibition Zone (mm) for various samples									
		L^1	M^1L^1	M^2L^1	M^3L^1	M^4L^1	L^4	M^1L^4	M^2L^4	M^3L^4	M^4L^4
S.aureus	250	5	6	8	19	5	5	6	10	20	12
	500	5	7	10	23	7	6	6	14	24	15

M^1 – Cobalt

M^2 – Nickel

M^3 – Copper

M^4 – Zinc

L^1 – 2-Hydroxyacetophenone 2-Aminothiophenol (HAPATP)

L^4 – Benzil-2-Aminophenol (BAP)

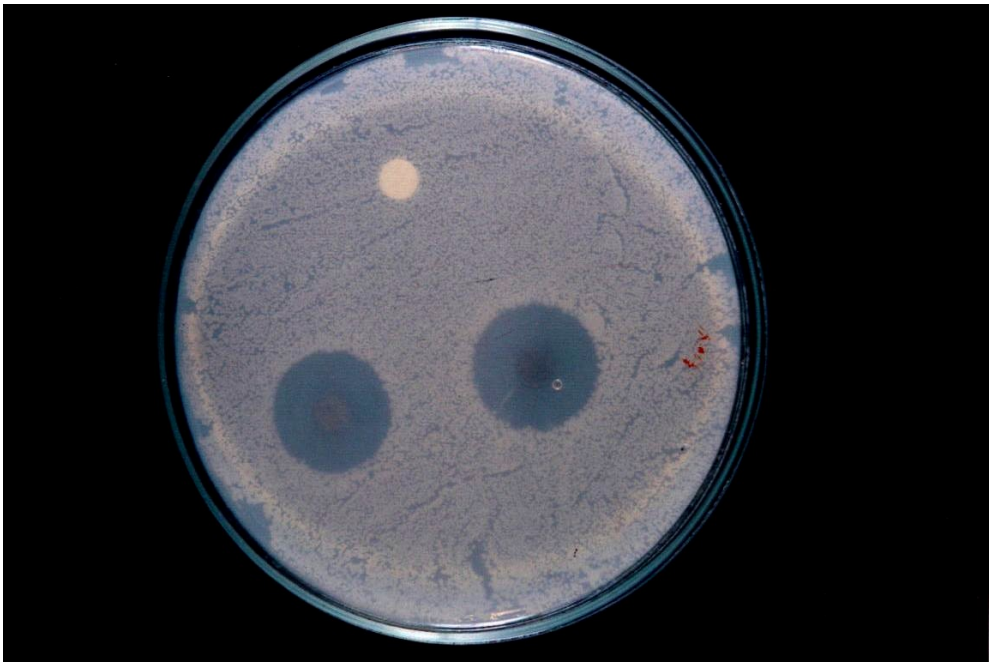


Figure IV.3.1
Zone of Inhibition of Ni(II) and Zn(II) complexes of BAP on *E.Coli*
(C-Solvent Control)

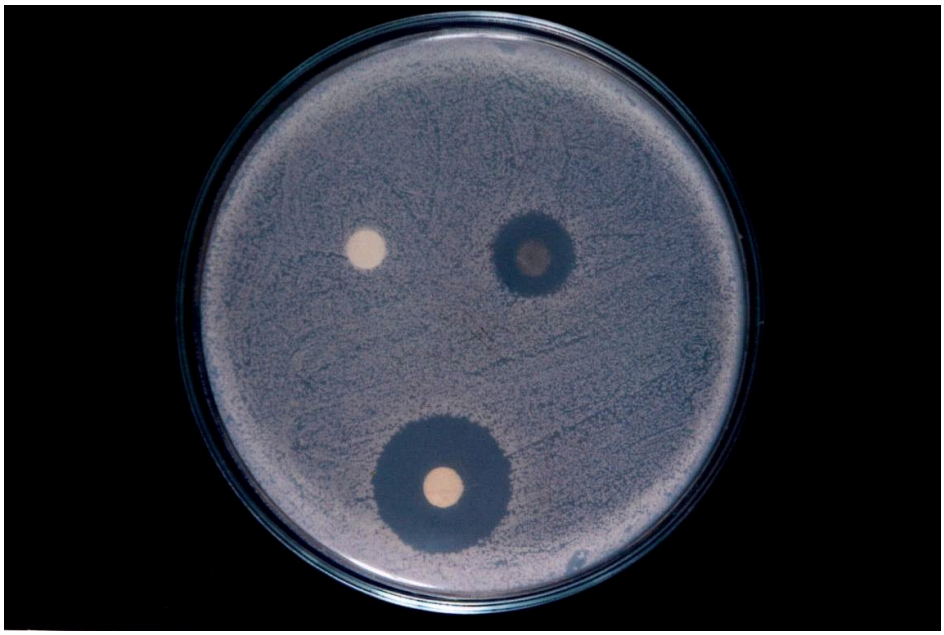


Figure IV.3.2

Zone of Inhibition of Cu(II) and Co(II) complexes of BAP on *E.Coli*
(C-Solvent Control)

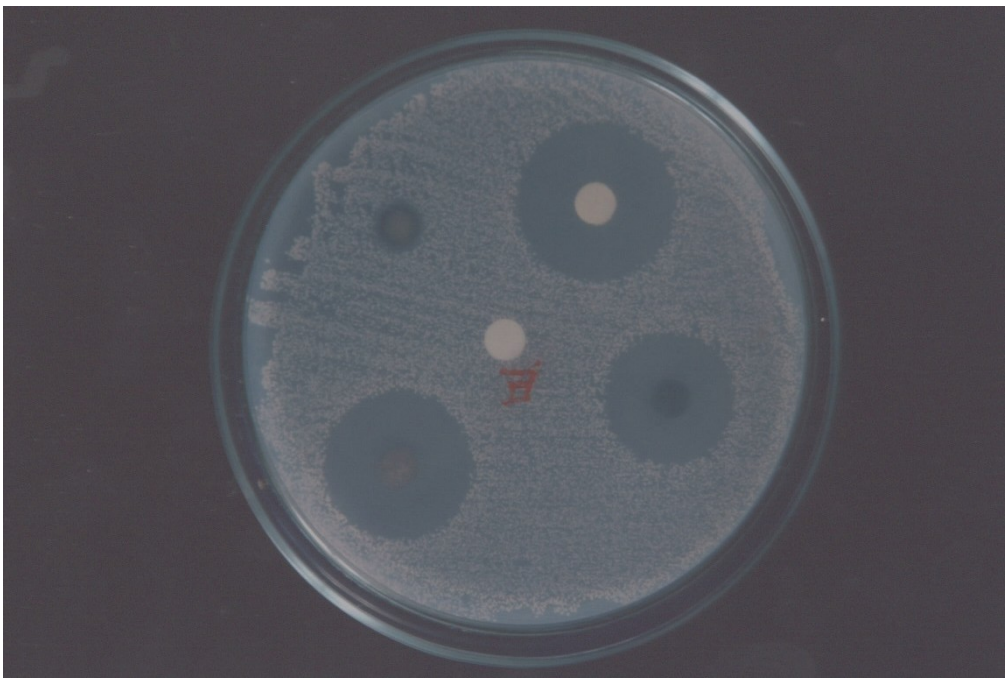


Figure IV.3.3
Zone of Inhibition of Co(II), Ni(II), Cu(II) and Zn(II) complexes of
BAP on *S. aureus*
(C-Solvent Control)

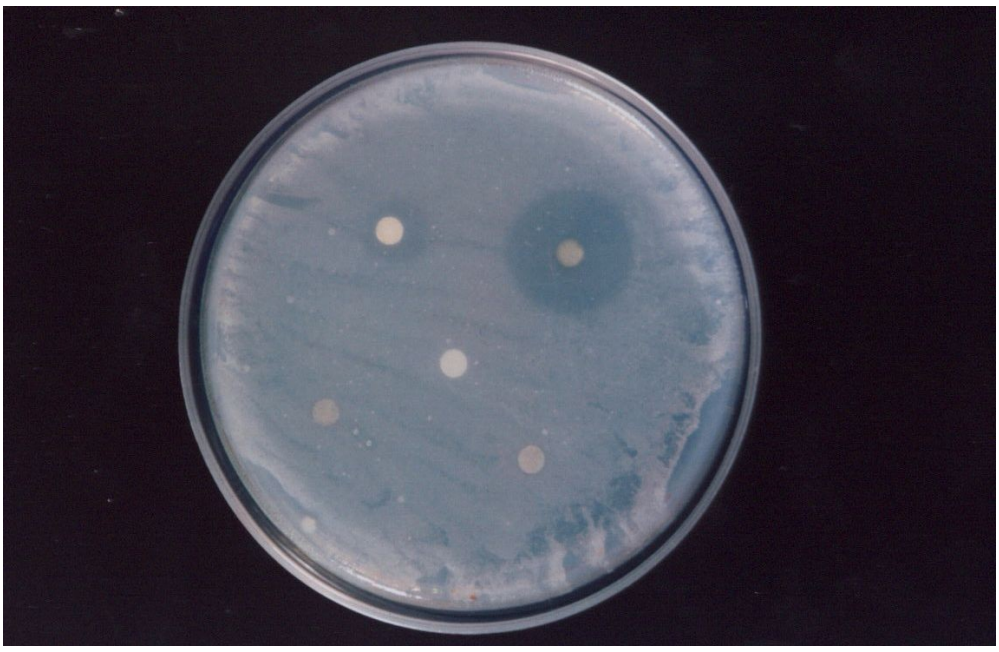


Figure IV.3.4

Zone of Inhibition of Co(II), Ni(II), Cu(II) and Zn(II) complexes of
HAPATP on *P.vulgaris*

(C-Solvent Control)

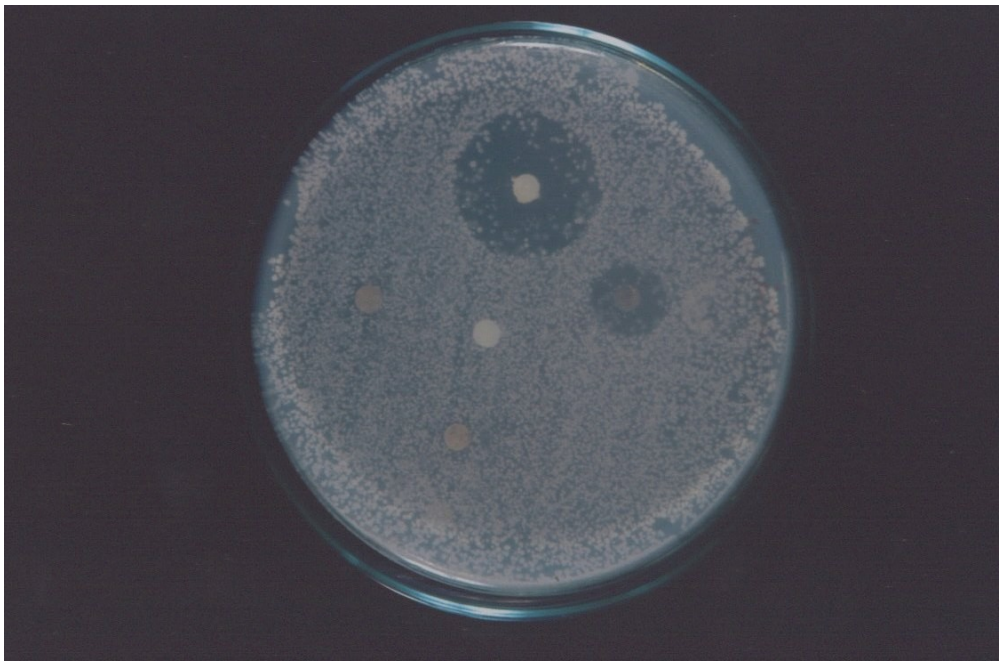


Figure IV.3.5

Zone of Inhibition of Co(II), Ni(II), Cu(II) and Zn(II) complexes of HAPATP on *S.aureus*
(C-Solvent Control)

References

1. Alcomo E. *Fundamentals of Microbiology* 6th Ed: 745, 2001.
2. Ann D, Jacob G. *Microbial Cell Factories*, 23: 5, 2006.
3. Bernsteen T. *Journal of Molecular Biology,* 112: 535, 1977.
4. Bertini I, Gray HB. Bioinorganic chemsitry, *Universal Science Books*, Mill Valley, 1994.
5. Boman HG, *Immunological Reviews* 173: 5-16, 2002.
6. Brull S, Coote P. *International Journal of Food Microbiology*, 50: 1-17. 1999.
7. Carpenter PL. *Microbiology*, Human Normal Microbiota: 329-330, 1977.
8. Chandrappa GT, Thimmaiah KN. *Inorganic Chim Acta,* 106: 81, 1985.
9. Chanmyia S, Shamim HM. *Pakistan Journal of Biological Sciences,* 7(3): 335-339, 2004.
10. Cheriyan M, Mohanan K. *Asian Journal of Chemistry*, 19(4): 283-288, 2007.
11. Chohan ZH and Humayun P. *J Res (Sci),* 4: 49, 1992.
12. Cotton SA, Chamic CR. 8: 129, 2005.
13. David WM, Martin PA, Victor WR, Daryl KG. *Harpers Review of Biochemistry,* 20th Ed., Language Medical Publication, 1972.
14. Dhar SK. Metal Ions in Biological Systems, Plenum New York, 1973.
15. Donnenberg M. *E.coli;* Virulance mechanism of a versatile pathogen, *Science Encyclopaedia,* Vol II, 2002.

16. Dubey RC, Maheswari DK. *A Textbook of Microbiology,* 551-552, 1999.

17. Dwyer FP. *Chelating Agents and Metal Chelates*, Academic Press, New York, 1964.

18. Feng P, Weagant S. Enumeration of *E.coli* and the coliform bacteria, *Bacteriological analytical manual*, 8th Ed., FDA/Center for food safety and Applied nutrition, 2007.

19. Fibane AM, Williams DR. Principles of Bio inorganic chemistry, *The Chemical Society*, London, 1977.

20. Glem LJ. *Chemistry of Organic Medicinal Products,* 4th Ed., John Willi & Sons, New York, 1966

21. Gutkoshi SB, Wiest JM. 13: 26-29, 1996.

22. Hanzlik RP. Inorganic aspects of biological and organic chemistry. Academic Press, New York, 1976.

23. Harwood CS, Greenberg EP. Megaroles of Microorganisms, *Science,* 1096, 1999.

24. Hoare RJ, Harisson PM. Metals in Biochemistry, Chapmen and Hall, New York, 1980.

25. Horace DG. The safety foods, *Connecticut Avi.* Pub. Comp, 1982.

26. Hughes MN. The inorganic chemistry of biological processes, 2nd Ed., Willey Chichester, UK, 1981.

27. Iglewski BH. Pseudomonas in Baron's Medical Microbiology, 4th Ed., Univ. of Texas, Medical Branch, 1996.

28. Isenberg DH, Essential Procedure for Clinical Microbiology. *American Society for Microbiology,* Washington, 1998.

29. Kadrim MS, Gash B. Antibiotic sensitivity and resistance profile of microorganism responsible for urinary tract infection observed in Kashmir, India, *Indian Journal of the Practising Doctors,* Vol. I, No.1, 2004.

30. Katherine M Shea. *Pediatrics*., 114: 3, 2004.

31. Kell DB. Target Practice – Novel approaches to antimicrobial chemotherapy TIBTECH 15: 334-336, 1997.

32. King EO, Ward MK. Two simple media for the demonstration of Pyocyajmin and fluoresin, *Laboratory Journal of Clinical Medicine*, 44(2): 301-307, 1954.

33. Kurtoglu M, Ispir E. *Asian Journal of Chemistry,* 19(2): 1239-1245, 2007.

34. Levy S. The antibiotic paradox; How miracle drugs are destroying the miracle, Plenum Publishers: 1-11, 1998.

35. Louie AY, Meate TJ. Metal complexes as enzyme inhibitors, *Chemical Rev,* 99: 2711 -2734, 1999.

36. Mayer TJ, McCleverty JA. Comprehensive co-ordination chemistry, Vol III. Pergamon Press, 2004.

37. Melson GA. Co-ordination chemistry of macro cyclic compounds, Plenum Publishers, New York, 1989.

38. Monod J, Wyman J, Changeux P. *Journal of Molecular Biology,* 12: 965, 1988.

39. Muhammad I, Javed I, Shahid I, Nazia IT. *Journal Biology,* 31: 67-72, 2007.

40. Nasrin J, Saidul IM. *Journal of Applied Sciences* 7(4): 597-603, 2007.

41. Pasternak K, Sztanke K. *Bioinorganic and Medicinal Chemistry*, ELSEVIER 14: 3635-3642, 2006.

42. Pearson H. The dark side of *E.coli*, *Nature,* 445-7123, 2007.

43. Pyatkin KD. *Microbiology with vibrology and immunology*, 121-122, 1987.

44. Rosenberg B, Van Camp L, Grimley EB, and Thomson AJ. *J. Biol. Chem.*, 242: 1347, 1967.

45. Ryan KJ, Ray CG. *Sherris Medical Microbiology*, 4th Ed., McGraw Hill, 2004.

46. Saidul IM, Akhter FM. *Journal of Biological Sciences,* 1(8): 711-713, 2001.

47. Saidul IM, Akhter FM. *Pakistan Journal of Biological Sciences,* 5(3): 335-337, 2002.

48. Saidul IM, Belayet HM. *Journal of Medical Sciences,* 3(4): 289-293, 2003.

49. Schroedar A. Elements in Living Systems, Plenum Publishers, New York, 1975.

50. Sinigaglia M, Delnobile MA. *Journal of Diary Science*., 2007.

51. Tkaczynski T, Janocha R. *Acta Pol. Pharm. Drug Res.* 52: 39, 1995.

52. Villanova PA. National Committee for clinical and Laboratory Standards, Methods for dilution antimicrobial susceptibility tests for bacteria, NCCLS Document M7-A4, 1997.

53. Walsh C. Enabling the science of life, *Nature,* 409: 226-231, 2001.

54. Wayne PA.NCCLS, DISC-Diffusion Eleventh International Supplement, NCCLS Document, M100-S11, 2001.

55. Zgoda JR, Porter JR. *Pharm. Biol.* 39: 221, 2001.

SUMMARY

Schiff base metal complexes have been considered as the 'Current Work Horse' in the development of Co-ordination chemistry. Flexibility of metal ions in an environment of a nucleophilic molecule or anions gave birth to large number and varieties of complexes. Recent explosion in the field of metal complex chemistry was not only due to the economic inspiration but also of the intrinsic curiosity and intellectual challenges confronted in Quantitative Structure – Activity Relationship (QSAR Analysis) of many of their compounds. This investigation is expected to provide valuable insight into the nature of metal ligand bonds and their thermal stability. High degree of biochemical interest in Schiff base Chemistry stems from their suitability in designing metal centred model systems, their mimic biologically active system. Many transition metal complexes play a crucial role in living systems. Hence it is proposed to study the biological mechanisms and also to find out some significant applications of Schiff bases and their metal chelates in biology.

In view of the versatile importance of Schiff base metal chelates we herein describe the study of the metal complexes of Schiff bases derived from 2-Hydroxyacetophenone 2-Aminothiophenol (HAPATP), 2-Hydroxy- acetophenone 2-

Aminophenol (HAPAP), Benzil 2-Aminothiophenol (BATP) and Benzil 2-Aminophenol (BAP) and their transition metal chelates. They have been synthesised and characterised with the help of analytical as well as physico-chemical methods. Co(II), Ni(II), Cu(II) and Zn(II) are the metal ions used for the complexation.

The thesis is divided into 4 parts. Part I deals with the synthesis and characterisation of the metal complexes derived from some new Schiff base ligands. There are 6 Chapters in Part I. The first chapter consists of an introduction and a critical review of the published work on metal complexes of Schiff bases derived from hydroxyketones, 1,2-diketones, aminophenols and aminothiophenols. Materials, methods and instruments used for the various studies during this work are given in Chapter II.

Synthesis and characterisation of Co(II), Ni(II), Cu(II) and Zn(II) complexes of HAPATP are described in Chapter III. Structural elucidation of these complexes has been made on the basis of microanalytical, magnetic and spectral data. These data suggest that HAPATP behave as a neutral bivalent tridentate ligand during complexation. All these complexes possess 1:1 metal ligand stoichiometry and are non electrolytes. Based on the above physico-chemical studies an octahedral geometry is suggested for all the complexes. Co(II), Ni(II) and Cu(II) complexes showed para magnetism while Zn(II) chelate is purely diamagnetic in nature.

Chapter IV deals with the preparation and characterisation of the Co(II), NI(II), Cu(II) and Zn(II) metal chelates of the ligand HAPAP. Microanalytical data reveals that there exists 1:1 stoichometry between metal and the ligand. Conductance data explains the non electrolytic nature of all these complexes. During the magnetic studies, it is observed that complexes of Co(II), Ni(II) and Cu(II) are paramagnetic while Zn(II) complex is diamagnetic. The spectral data suggest an octahedral structure for all the metal chelates. All the above results confirm that the ligand acts as a bivalent tridentate in all the metal complexes.

Chapter V describes the preparative as well as physico-chemical investigations of the metal complexes of the Schiff base, BATP. All the metals were found to form 1:1 complexes with the ligand. Conductance data explain the non electrolytic behaviour of the metal complexes.

Chapter V explains the preparation and characterisation of the metal complexes of BAP. Micro analytical data reveals that there exist an 1:1 stoichometry between the metal and the ligand in all these complexes. These complexes were found to be non-electrolytes in methanol. Except Zn(II), all other complexes were found to be paramagnetic in nature.

In both the above cases the ligands act as dianionic tetradentate type ligand. Part I ends with references.

Part II deals with the thermal studies of 8 metal complexes of the above Schiff bases. There are 4 chapters in this part. The first chapter gives an introduction about thermogravimetric analysis. Chapter II deals about the materials, methods and instruments used for the thermogravimetric studies.

Thermal decomposition studies of Cu(II) and Zn(II) complexes of HAPATP and HAPAP are discussed in Chapter III. A three stage decomposition pattern was observed for $CuL^1(H_2O)_3$, whereas the Zn(II) complex of the same ligand showed a two stage decomposition. The temperature regions and the probable assignments are presented in Table II.3.1. In the case of HAPAP a two stage decomposition pattern was observed for both Cu(II) and Zn(II) complexes. Results of this studies are summarised in Tables II.3.2.

Chapter IV explains the thermal decomposition studies of Cu(II) and Zn(II) chelates of BATP and BAP. Table II.4.1 and II.4.2 gives a detailed information regarding the temperature ranges and probable assignments for each decomposition stages of the metal chelates. Part II concludes with references.

Part III and IV consists of biological studies of some complexes.

In Part III antitumour activity of the metal chelates was discussed in detail. The studies include *in vitro* and *in vivo* antitumour study and acute toxicity studies in BALB/c Mice. Chapter I and II give and introduction to cancer and materials and methods employed in the present study. Chapter III explains the results of the above mentioned screening studies in detailed manner. Tables III.3.1-III.3.5 and Figures III.3.1-III.3.9, given the information about the antitumour screening of the transition metal complexes.

The screening studies confirm that Zn-HAPATP and Cu-BAP are the most active metal chelates with lowest IC_{50} value for DLA and HeLa cells. The low molecular weight and lipid solubility of the Zn and Cu complexes facilitate their penetration of cell membrance. The cell death due to apoptosis was further confirmed as assessed by Acridine orange/Ethidium bromide dual staining and DNA laddering. Depending upon the specific type of complexes used treatment has resulted enhanced immune response to tumours, decreased tumour growth and increased survival of the mice as evidenced by its extended life span. Experimental evidence indicates that the complex Zn-HAPATP achieved a sizable peripheral pool of PHA-sensitive naive T-lymphocytes which ensure

an improved immune response. This shows that the Zn(II) complex is able to induce lymphocytes clonal proliferation in normal as well as tumour bearing mice, thus making them more reactive to neoplasmic challenges. The abnormality observed with other complexes can be attributed to the steric hindrance of the complexes and also due to their membrane impermeable character.

Part IV deals with antibacterial studies of some selected complexes towards 4 clinically important bacterial strains. This part is divided into 3 chapters. Chapter I gives an introduction about antibacterial study. In Chapter II materials, methods and instruments used in the present study were discussed. In Chapter III results of antibacterial study was explained. It is important to note that metal complexes exhibit enhanced antibacterial activity than their free ligands (ie., 2-Hydroxyphenone2-Aminothiophenol and Benzil 2-Aminophenol). Among the metal complexes studied, Cu-BAP showed better antibacterial activity against all the bacterial strains studied especially towards the Gram negative organism, *P.aerugenosa.* This is because of an increase in the cell permeability of the metal complex. The lipid membrane which surrounds the cell favours the passage of only lipid soluble material and it is known that liposolubility is an important factor that controls antimicrobial activity. The variation in the activity of

different metal complexes against different bacteria depends either on the impermeability of the cells of the microbes or difference in ribozomes in microbial cells.

Part III and IV conclude with references.