The metal complexes of substituted pyrimidines, amino acids and imidazoles found are biologically more effective than their free state. They play a vibrant role in a great number of biological and enzymatic activities. Moreover, they are involved in a number of intracellular metabolic functions like energy production, hormone synthesis and transport of nutrients / oxygen in our human body. The effect of amino acids/imidazoles on the stability of substituted pyrimidine transition metal(II) complexes with substituted pyrimidine is of much attention in recent years. Complexation of metal(II) ions with 5-fluorouracil(5-FU: A) and amino acids(B)/imidazoles(B) offers simple models in enzymatic process. The binding and dissociation interactions of these mixed ligand complexes with DNA have significant role in nature. The oxidative cleavage interaction of DNA in photochemical method has been widely applied in the clinical and medicinal fields like photodynamic therapy of cancer. The chemistry of these mixed ligand complexes has a key role in biological systems as the mixed chelations in biological fluids generate definite structures which are confined in the storage and transport of dynamic substances through membranes.

The present investigation comprises the synthesis, structural characterization and pharmacological, nuclease and modeling studies on 24 mixed ligand complexes of 5-fluorouracil (5-FU:A) with Ni(II)/Cu(II)/Zn(II) ions in the presence of amino acids/imidazole enzyme constituents(B). The synthesized 24 mixed ligand complexes were structurally characterized by various spectral and analytical techniques. Mixed ligand Ni(II) and Cu(II) complexes show significant pharmacological and DNA activities. From the available knowledge of literature, it is found that this is the first
report on the *in vitro* pharmacological and nucleus activities of the above mixed ligand complexes under the same experimental conditions. The present work is also centered on the multiple equilibrium studies of 24 mixed ligand complex systems of 5-fluorouracil (5-FU: A) with Ni(II)/Cu(II)/Zn(II) ions in the presence of amino acids/imidazole enzyme constituents(B). This has been studied using pH-metric measurement with a combined pH electrode in aqueous medium at an ionic strength of $I = 0.15$ M. The pH-metric titration data have been analyzed with the aid of the computer program SCOGS.

This thesis is divided into six chapters. The essence of each chapter is delineated hereunder.

**CHAPTER 1**

**INTRODUCTION**

In the introductory chapter of the thesis, the biological importance of metal complexes and the ligands were described. Various experimental methods for the investigation of these complexes were discussed briefly. A brief review related to the present study is outlined in this chapter. The scope of the present investigation mainly includes the solid state and stability constant studies of the following mixed ligand complex systems:

(i) 5-Fluorouracil(5-FU) with Ni(II)/Cu(II)/Zn(II) ions in the presence of amino acids *viz.*, glycine(gly), L-alanine(ala), L-valine(val) and L-phenyl alanine(phe).

(ii) 5-Fluorouracil(5-FU) with Ni(II)/Cu(II)/Zn(II) ions in the presence of imidazole moieties *viz.*, imidazole(him), benzimidazole(bim), histamine(hist) and L-histidine(his).
CHAPTER 2
MATERIALS, METHODS AND EXPERIMENTAL TECHNIQUES

The second chapter is concerned with the materials employed, general procedure for the synthesis of MAB species, experimental procedure adopted and calculations used. Melting point of all the complexes was determined on Gallenkamp apparatus in open glass capillaries and is uncorrected. Metal content of the mixed ligand complexes was estimated gravimetrically by the standard procedures. Molar conductance of the complexes at room temperature was measured using an Elico CM 180 conductivity bridge by using 0.01 M KCl solution as calibrant. Micro analytical data were performed on Elementar Vario EL III analyzer. Fast atom bombardment mass spectra (FAB-MS) were recorded using a VGZAB-HS spectrometer in a 3-nitrobenzylalcohol matrix. Magnetic susceptibility measurements on powder samples were carried out on a Gouy balance. Electronic absorption spectra were recorded in DMSO (Dimethyl sulphoxide) solution with a Hitachi U-2000 double beam spectrophotometer (cell length, 1 cm) in 200–1100 nm range.

Vibrational spectra were recorded using KBr pellets on a JASCO FT/IR-410 spectrophotometer, in the 4000–400 cm\(^{-1}\) range. \(^1\)H and \(^{13}\)C NMR spectra of diamagnetic Zn(II) complexes were carried out in DMSO–\(d_6\) at room temperature using TMS (Tetramethylsilane) as internal standard on a Perkin Elmer R-32 spectrometer. Thermograms were recorded in dynamic nitrogen atmosphere with a heating rate of 10 °C/min using a Perkin Elmer (TGS-2 model) thermal analyzer. X-band EPR spectra of Cu(II) complexes at room temperature and liquid nitrogen conditions in DMSO medium were recorded on a Varian E-112 spectrophotometer using DPPH (2,2-diphenyl-1-picrylhydrazyl) as internal standard. Powder X-ray
diffraction (XRD) patterns were recorded with a Bruker AXS D8 advance powder X-ray diffractometer. Scanning Electron Micrography with Energy Dispersive Spectrometry association (SEM/EDS) using JSM-5610 scanning electron microscope was used for morphological evaluation. A detailed procedure for *in vitro* antimicrobial and antioxidant, DNA cleavage and binding activities were also described in this chapter. In view of many inherent advantages, pH-metric titrations have been chosen for the determination of stability constant at 310 ± 0.1 K. All the calculations have been done with the aid of SCOGS (Stability Constants Of Generalized Species) computer program and the metal speciation is determined with the help of HySS (Hyperquad Simulation and Speciation) program.

**CHAPTER 3**

**STRUCTURAL INVESTIGATION ON BIORELEVANT PYRIMIDINE-AMINOACID BASED MIXED LIGAND COMPLEXES**

In this chapter, synthesis and characterization of twelve mixed ligand complexes of 5-fluorouracil(A) and biologically active amino acids(B) namely glycine, L-alanine, L-valine and L-phenylalanine with three metal((II) ions *viz.*, Ni(II), Cu(II) and Zn(II) ions were carried out. All the M(II)–5-FU–amino acid mixed ligand complexes are air stable and non-hygroscopic in nature. They are insoluble in water and common organic solvents like benzene and petroleum ether but soluble in DMF, DMSO, acetonitrile and ethanol. The FAB mass spectra of complexes exhibit molecular ion ($M^+$) peak with $m/z$ ratio shows [M+1] pattern which supports the theoretical molecular weight calculated from the analytical data. The micro analytical data show that the metal to ligands ratio is 1:1:1. The observed low molar conductance value (< 26 Ω⁻¹ cm² mol⁻¹) in DMSO is consistent with their non-electrolytic nature.
From IR spectral data it is concluded that the 5-FU acts as bidentate ligand and forms metal chelates through the –N$_3$ and –C$_4$ carbonyl–O atoms. The amino acid ligands bind with M(II) ions in bidentate manner through amino–N and deprotonated carboxylato–O atoms. The presence of coordinated water molecule in M(II)–5-FU(A)–gly/ala/val/phe(B) complexes is indicated by a broad band in the region 3340–3240 cm$^{-1}$ and a weak band in the region 845–819 cm$^{-1}$ correspond to $\nu$(OH) rocking and wagging mode of vibrations of coordinated water molecule. The electronic absorption spectra of Ni(II) mixed ligand complexes show distorted octahedral geometry with $^3A_2g$ as ground state. The electronic spectra of Cu(II)–5-FU(A)–gly/ala/val/phe(B) complexes exhibit two bands, one band corresponds to LMCT transition and another broad band corresponds to $^2E_g(D) \rightarrow ^2T_2g(D)$ transition, results in distorted octahedral geometry. Zn(II) complexes show a band in the UV region due to M $\leftarrow$ L charge transfer for a distorted octahedral geometry. The decoupled NMR spectra confirm the disappearance of carboxylic proton peak, shift in the amino proton peak of amino acids and the presence of coordinated water molecule. The signal of C$_4$ carbonyl carbon of 5-FU at 158.97 ppm undergoes upfield shift in the complex (136.49–137.16 ppm) which confirms the binding mode of ligands A and B.

Thermogravimetric (TGA) and Derivative Thermogravimetric (DTA) analyses of the mixed ligand complexes were carried out at the temperature range of ambient to 900 °C under air stable condition. All the complexes show similar four stage decomposition. The calculated values of $C_s$ (kinetic order of the decomposition) indicate that the decomposition follows the first order kinetics. The observed order of $A_i$ values is phe $<$ val $<$ ala $<$ gly which is probably an indication of the distortion in
the environment and these values are comparable to the other reported distorted octahedral Cu(II) complexes. The powder X-ray diffraction pattern shows that the complexes have uniform phase crystalline structure. Morphology and particle size of all the complexes were investigated by scanning electron micrograph analysis from 500 to 10000 x magnification with the accelerating voltage of 15–30 kV. The SEM analysis of the complexes shows uniform matrix with homogeneous phase separation having particle size of 1 μm–100 μm. Based on the results, the following distorted octahedral structure is assigned to the mixed ligand complexes.

![Octahedral Structure](image)

where $R = H : \text{gly} ; \quad -\text{CH}_3 : \text{ala} ; \quad -\text{CH} (\text{CH}_3)_2 : \text{val} ; \quad -\text{CH}_2 \text{C}_6\text{H}_5 : \text{phe} ; \quad M = \text{Ni(II)}/\text{Cu(II)}/\text{Zn(II)}$.

All the 12 complexes are proved to be more bacteriostatic for three gram-positive bacteriae: *Bacillus subtilis*, *Staphylococcus saphyphiticus* & *Staphylococcus aureus* and two gram-negative bacteriae: *Escherichia coli* & *Pseudomonas aeruginosa* than fungistatic towards *Aspergillus niger*, *Enterobacter species* & *Candida albicans*. The higher scavenging activity of Cu(II)–5-FU(A)–phe(B) complex is attributed to the presence of extensive conjugation in the ligand moiety and its coplanarity. The considerable activity of DNA cleavage found with Ni(II) and Cu(II) mixed ligand complexes may also be partially due to the ability of these complexes to convert super coiled DNA into its open circular form. The order of binding ability of
metal complex with DNA is Cu(II) > Ni(II) > Zn(II) ions. The hexa-coordination of Cu(II)–5-FU(A)–phe(B) complex involving linkage of bidentate C4 carbonyl oxygen and N3 of 5-fluorouracil and monoprotic bidentate of L-phenylalanine ligands were studied by 3D molecular modeling.

CHAPTER 4

STRUCTURE AND DISTRIBUTION OF SPECIES FORMED BETWEEN NUCLEOBASE, 5-FLUOROURACIL AND Ni(II), Cu(II) and Zn(II) IONS IN AQUEOUS MEDIUM

In this chapter, the solution equilibria and structure of twelve Ni(II)/Cu(II)/Zn(II) mixed ligand complex systems with 5-fluorouracil [5-FU; (A)] and amino acids(B) viz., glycine(gly), L-alanine(ala), L-valine(val) and L-phenylalanine(phe) were undertaken pH-metrically in aqueous medium at 300, 310, 320 and 330 ± 0.1 K. The ionic strength is maintained at I = 0.15 M using NaClO4 as the supporting electrolyte. All the pH measurements were restricted to under pH 9.0 because above this level, all the systems undergo hydroxylation forming [M(OH)2] precipitate. All the mixed ligand species have distorted octahedral geometry and it has been found that the $\lambda_{max}$ values of Cu(II)–5-FU(A)–amino acid(B) systems in solution state remain unaltered with the change in pH of the medium (2.5–8.5).

Though, 5-FU shows four coordination sites viz., N1–H, C2=O, N3–H and C4=O, under the experimental conditions only one pK_a value (7.76) is observed which corresponds to the deprotonation at N3 site and this is comparable with the reported values. The highest stability for Ni(II)–gly(B) may be due to the absence of any bulky substituents in glycine ligand and the least stability for Ni (II)–phe(B) is due to the presence of bulky electron withdrawing phenyl groups. In addition to various binary species HA, MA, MA2, HB, H2B, MB and MB2, the formation of
mixed ligand species of stoichiometry MAB and MAB$_2$ are also detected in the pH-metric method. The formations of MAB species are found to be favoured in the pH range of 4.5–8.5. The obtained $\log K_{MAB}^M$ values of M(II)–5-FU(A)–amino acid(B) mixed ligand systems are comparable with $\log K_{MA}^M$ and $\log K_{MB}^M$ values of M(II)–5-FU(A) and M(II)–gly/ala/val/phe(B) binary systems demonstrating similar type of binding in MA, MB and MAB systems. The calculated $\Delta \log K_{MAB}^M$ values are found to be more positive for all the mixed ligand complexes. It demonstrates higher stability of mixed ligand complexes than their binary analogues. The observed $\log \chi'$ values are greater than 0.3, suggesting that MA and MB bonds in mixed ligand complex systems are stronger than in MA and MB binary complexes. It has been found that MAB$_2$ species are formed preferably above pH 5 under the experimental conditions. On the basis of the above findings, the following structures are proposed for MAB and MAB$_2$ type systems.
In MAB$_2$ system, the fifth and sixth positions are occupied by a second molecule of gly/ala(B) with the replacement of water molecules in MAB species. The calculated $\Delta \log K_{MAB_2}$ values of M(II)--5-FU(A)--gly/ala(B) complexes are more positive as compared to the statistically expected values indicating enhanced stabilities for MAB$_2$ systems. The Ni(II)--5-FU(A)--gly, ala, val & phe(B) species attain maximum stability at 54, 49, 83 & 50.66 % at pH 6.3, 6.7, 8.8 & 8.3, Cu(II)--5-FU(A)--gly, ala, val & phe(B) species attain maximum stability at of 62, 58, 91 & 90 % at pH 6.3, 6.0, 8.0 & 8.0 and Zn(II)--5-FU(A)--gly, ala, val & phe(B) species attain maximum stability at 61, 64, 63 & 62 % at pH 6.3, 6.5, 6.9 & 6.4 respectively. The negative values of $\Delta^\circ G$ and $\Delta^\circ H$ for binary and mixed ligand systems suggest that the complexation process is spontaneous and exothermic and are favored at low temperature. The observed high positive $\Delta^\circ S$ values for all species predict that the complexation of 5-FU(A) and amino acids(B) with M(II) ions is thermodynamically favorable under the experimental conditions.

Among the various mixed ligand species in solution state, Cu(II)--5-FU(A)--phe(B) system shows the highest biological activities and the observed order is Control > Cu(II)--5-FU(A)--phe(B) > Cu(II)--5-FU(A)--val(B) > Cu(II)--5-FU(A)--
ala(B) > Cu(II)–5-FU(A)–gly(B) > 5-Fluorouracil(A). The free radical scavenging activity of the mixed ligand complexes follow the order: Control >> Cu(II) > Ni(II) > Zn(II) > 5-FU(A). The solution state complex systems possess enhanced ability to cleave CT-DNA when compared to solid state complexes. This is attributed to the formation of several hydrogen bonds between the hetero ligands and CT-DNA.

CHAPTER 5

MORPHOLOGICAL AND PHARMACOLOGICAL INVESTIGATIONS ON SOME BIOPOTENT MATERIALS DERIVED FROM 5-FLUOROURACIL AND SUBSTITUTED IMIDAZOLES

This chapter deals with the synthesis and characterization of novel N₂O, N₃O and N₃O₂ type mixed ligand complex systems of Ni(II)/Cu(II)/Zn(II)–5-FU(A)–imidazole(him)/benzimidazole(bim)/histidine(his)/histamine(hist)(B) by means of analytical and spectral techniques. The synthesized mixed ligand complexes are highly air-stable and non-hygroscopic in nature. They are insoluble in water and common organic solvents like benzene, acetone, petroleum ether etc., but soluble in DMSO, DMF and dioxane. The observed low molar conductance value of the metal complexes in DMSO medium is in tune with the non-electrolytic nature. Fast atom bombardment mass spectrum shows the molecular ion (m/z) peaks corresponding to the stoichiometry of M(II)–5-FU(A)–him/bim/hist/his(B)(OAc)(OH₂)x.

From the IR spectra it is found that 5-FU acts as bidentate ligand which forms metal chelates through the deprotonated N₃ atom and oxygen atom of C₄ carbonyl group. The him/bim ligands bind with the M(II) ion in unidentate manner via imidazole ring –N₃ atom. The hist binds with M(II) ion in bidentate manner via imidazole ring –N and –N atom of side chain amino group. The ligand his forms stable chelates with M(II) ion through imidazole ring –N, amino –N and deprotonated
carboxylato –O atoms. The presence of coordinated water molecule in the complexes Ni(II)–5-FU(A)–him/bim/hist/his(B), Cu(II)–5-FU(A)–hist/his(B) and Zn(II)–5-FU(A)–hist/his(B) was confirmed from a broad peak in the region 3450–3380 cm\(^{-1}\) and the existence of two peaks in 846–816 cm\(^{-1}\) and 748–716 cm\(^{-1}\).

The electronic absorption spectra of Ni(II)–5-FU(A)–him/bim/hist/his(B) complexes display four spin allowed bands with distorted octahedral geometry of \(^3A_{2g}\) as ground state. The Cu(II)/Zn(II)–5-FU(A)–him/bim(B) complexes exhibit bands at 13441 & 26455 cm\(^{-1}\), 13680 & 25707 cm\(^{-1}\), 26738 cm\(^{-1}\) and 27624 cm\(^{-1}\) due to d–d electronic and M ← L charge transfer transitions respectively for distorted tetrahedral environment. Cu(II)/Zn(II)–5FU(A)–hist/his(B) display bands at 14690, 15337, 26576 and 26667 cm\(^{-1}\) respectively that are assigned to \(^2\)E\(_g\)(D) → \(^2\)T\(_{2g}\)(D) and M ← L charge transfer transitions with distorted octahedral environment. Based on the results, the following structures are assigned to the mixed ligand complexes.
In the $^{1}$H NMR spectra of diamagnetic Zn(II)–5-FU(A)–him/bim/hist/his(B) complexes, there are new peaks found at $\delta = 1.81–1.99$ ppm which indicate acetate (OAc) coordination with the Zn(II) ion. In addition, Zn(II)–5-FU(A)–hist/his(B) show a characteristic peak at 4.72 ppm which confirms the presence of coordinated water molecules. The decoupled $^{13}$C NMR signals confirm the presence of carbonyl and carboxylate functions which are in accordance with the assigned structure. EPR spectra of Cu(II)–5-FU(A)–him/bim/hist/his(B) complexes show $g$-tensor values of Hamiltonian parameters which follow the order: $g_{\parallel} (2.15) > g_{\perp} (2.04) > g_{e} (2.0023)$; $g_{\parallel} (2.11) > g_{\perp} (2.03) > g_{e} (2.0023)$; $g_{\parallel} (2.21) > g_{\perp} (2.05) > g_{e} (2.0021)$ and $g_{\parallel} (2.21) > g_{\perp} (2.06) > g_{e} (2.0023)$ respectively. Thermogravimetric analyses in the temperature range of ambient to 1100 °C under air stable condition for Ni(II)–5-FU(A)–him/bim(B), Ni(II)/Cu(II)/Zn(II)–5-FU(A)–hist(B), Cu(II)/Zn(II)–5-FU(A)–him/bim(B) and Ni(II)/Cu(II)/Zn(II)–5-FU(A)–his(B) complexes show four, four, three and three stages of decomposition respectively. The X-ray diffractogram of all the mixed ligand complexes were recorded in the 0–80° range. The observed crystalline sizes are in the range of 10–18 µm.

All the complexes show more significant antibacterial and less pronounced antifungal activities which follow the order: Control $>$ MAB $>$ MA $>$ Primary ligand(A). The moderate antioxidant activities shown by the mixed ligand complexes could be utilized as chemotherapeutic agents in preventing the progress of ageing and treatment of pathological diseases arising from oxidative stress. Histidine complexes are more active. The intrinsic binding constant ($K_b$) of Cu(II) complexes are high when compared to Ni(II) and Zn(II) complexes. All the complex systems have the ability to cleave the CT-DNA in the presence of $H_2O_2$. The activity of M(II) mixed
ligand complexes is due to the presence of imidazole moiety provides which additional Hydrogen bonding donor/acceptor atoms thereby enabling the molecule to stay in the minor groove of DNA with the help of van der Waal’s forces and H-bonds. The hexa-coordination of Ni(II)/Cu(II)/Zn(II)–5-FU(A)–his(B) mixed ligand complexes are confirmed by molecular modeling studies. The actual bond lengths and angles are very close to the optimal values which support the proposed geometry.

CHAPTER 6
MULTIPLE EQUILIBRIA AND SPECTROPHOTOMETRIC STUDIES ON SOME TRANSITION METAL COMPLEXES

The sixth chapter includes the equilibrium studies of mixed ligand complexes formed between Ni(II), Cu(II) and Zn(II) ions with primary ligand 5-fluorouracil (5-FU:A) and imidazole(him), benzimidazole(bim), L-histamine(hist) & L-histidine(his) as co-ligands(B) in aqueous medium containing NaClO₄ as supporting electrolyte at 310 ± 0.1 K. Dissociation constant of the primary ligand, 5- FU (0.015 M) and stability constants of binary (1:5) and mixed ligand systems (1:1:1) of Ni(II)/Cu(II)/Zn(II) ions in aqueous medium were determined. The ligand 5-FU shows four coordination sites viz., N₁–H, C₂=O, N₃–H and C₄=O. But under the experimental conditions, only one pKₐ value (7.76) is observed for 5-FU, corresponding to the deprotonation at N₃ site. Also the pKₐ value of N₃ site is lower than N₁ due to greater charge delocalization on N₃ atom. It is found that the stability constants of species formed between Cu(II) and different ligands are higher than other systems and this follows the Irving and Williams order as: Ni(II) < Cu(II) > Zn(II).

In the present experimental conditions, the different stoichiometric species formed in the mixed ligand systems of Ni(II)/Cu(II)/Zn(II)–5-FU(A)–
him/bim/hist/his(B) are HA, MA, MA₂, HB, MBH, MB, MB₂H, MB₂, MABH, MAB and MAB₂. Formation of MAB complexes increases steadily with rise in pH value and the favoured range is pH 2.5–8.0. This is shown by an increase in the absorbance with rise in pH at their λ_max values whereas λ_max remains unaffected. More positive log K and less negative Δlog K values indicate remarkable stability of these mixed ligand complexes. There is evidence for the formation of MAB₂ type complexes only with him ligand (< 10 %: above the pH 4.8) and not with other ligands. Formation of MABH species is found for M(II)–5-FU(A)–hist/his(B) systems in the pH range 4.5–5.5. All the systems show positive entropy (ΔS) values which indicate that the complexation is entropically favorable under the experimental condition. The negative value of change in free energy (ΔG) and enthalpy (ΔH) indicate that the chelation process is spontaneous and exothermic in nature and is favored at low temperatures. Square planar geometry is assigned for M(II)–5-FU(A)–him/bim(B) systems whereas distorted octahedral geometry is assigned for M(II)–5-FU(A)–hist/his(B) complexes. All the complexes show more significant antibacterial and less pronounced antifungal activities which follow the order: Control > MAB > MA > Primary ligand(A). It is found that M(II)–5-FU(A)–hist/his(B) complexes possess significant antioxidant activities due to the formation of monocationic conjugate species (NH₃⁺) and the presence of side chain amino and carboxyl groups in hist/his ligands. The Cu(II) mixed ligand complexes exhibit prominent DNA cleavage activity in the presence of H₂O₂ which was studied by gel electrophoresis method. Probably this may be due to the behavior of the Cu(II) ion to form redox couple in the presence of an oxidant. The proposed solution state structures for the possible mixed ligand species, MAB, MAB₂ and MABH are given as follows.
$M = \text{Ni}^{II}/\text{Cu}^{II}/\text{Zn}^{II}; X = \text{ClO}_4^-$