4.1 Molecular docking

Molecular docking can be defined as an optimization problem, which would describe the “best-fit” orientation of a ligand that binds to a particular protein of interest and is used to predict the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction because of its applications in medicines. Docking predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex [199]. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions.

The associations between biologically relevant molecules such as proteins, nucleic acids, carbohydrates and lipids play a central role in signal transduction. Furthermore, the relative orientation of the two interacting partners may affect the type of signal produced (e.g., agonism vs antagonism). Therefore docking is useful for predicting both the strength and type of signal produced.

Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets which in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs [200]. Based on biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.
Molecular docking can be thought of as a problem of “lock-and-key”, where one is interested in finding the correct relative orientation of the “key” which will open up the “lock” (where on the surface of the lock is the key hole, which direction to turn the key after it is inserted, etc.). Here, the protein can be thought of as the “lock” and the ligand can be thought of as a “key”. Since both the ligand and the protein are flexible, a “hand-in-glove” analogy is more appropriate than “lock-and-key” [201]. During the course of this process, the ligand and the protein adjust their conformation to achieve an overall “best-fit” and this kind of conformational adjustments resulting in the overall binding is referred to as “induced-fit” [202].

The focus of molecular docking is to computationally simulate the molecular recognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligand such that the free energy of the overall system is minimized.

4.2 Docking approaches

Two approaches are particularly popular within the molecular docking community. One approach uses a matching technique that describes the protein and the ligand as complementary surfaces [203-205]. The second approach simulates the actual docking process in which the ligand-protein pairwise interaction energies are calculated [206]. Both approaches have significant advantages as well as some limitations. It is clear that the simulation is computationally expensive, having to explore a large energy landscape. Grid-based techniques are outlined below.
4.3 **Shape complementarity**

Geometric matching/shape complementarity methods describe the protein and ligand as a set of features that make them dockable [207]. These features may include molecular surface/complementary surface descriptors. In this case, the receptor’s molecular surface is described in terms of its solvent-accessible surface area and the ligand’s molecular surface is described in terms of its matching surface description. The complementarity between the two surfaces amounts to the shape matching description that may help in finding the complementary pose of docking the target and the ligand molecules. Another approach is to describe the hydrophobic features of the protein using turns in the main-chain atoms. Yet another approach is to use a Fourier shape descriptor technique [208-210]. The shape complementarity based approaches are typically fast and robust, they cannot usually model the movements or dynamic changes in the ligand/protein conformations accurately, although recent developments allow these methods to investigate ligand flexibility. Shape complementarity methods can quickly scan through several thousand ligands in a matter of seconds and actually figure out whether they can bind at the protein’s active site, and are usually scalable to even protein-protein interactions. They are also much more amenable to pharmacophore based approaches, since they use geometric descriptions of the ligands to find optimal binding.

4.4 **Simulation**

In this approach, the protein and the ligand are separated by some physical distance, and the ligand finds its position into the protein’s active site after a certain number of “moves” in its conformational space. The moves incorporate rigid body transformations such as translations, rotations, as well as internal changes to the ligand’s structure including torsion angle rotations. Each of these moves in the
conformation space of the ligand induces a total energetic cost of the system. Hence, the system's total energy is calculated after every move. The obvious advantage of docking simulation is that ligand flexibility is easily incorporated, whereas shape complementarity techniques must use ingenious methods to incorporate flexibility in ligands. Also, it more accurately models reality, whereas shape complimentary techniques are more of an abstraction.

Simulation is computationally expensive, having to explore a large energy landscape. Grid-based techniques, optimization methods, and increased computer speed have made docking simulation more realistic.

Molecular docking is an important computational tool to predict the plausible interactions between the drug and nucleic acid in a non-covalent fashion, which plays an important role both for molecular recognition of nucleic acid as well as for the rational design of new chemotherapeutic drugs [211]. The small molecules bind with DNA have attracted attention in the medicinal design of anticancer and anti-AIDS drugs [212].

4.5 Interactions of Proteins

The normal functioning of a living cell is not only due to gene expression but also due to different proteins and their associations with each other. The activities of many proteins depend on their interactions with each other or with small intracellular signalling molecules. The interactions of protein in the biological systems are most important. There are two types of interactions in the field of structural biology as follows.

1. Protein–Protein interactions

2. Protein – Ligand interactions
4.5.1 **Protein –Protein interactions**

Protein-protein interaction determines the molecular structure of complexes formed by two or more proteins without the need for experimental measurement. The ions and molecules are in constant collision both inside and outside the cells. When two molecules come together, they are repelled because of the presence of weak non-covalent bonds. The different specific weak bonds or non-covalent interactions between complementary regions are able to bind two protein molecules. These interactions within a protein molecule cause it to fold into three dimensional conformations. Thus, many proteins in a cell interact with each other harmoniously so that their activities are regulated.

4.5.2 **Protein-ligand interactions**

The functions of almost all proteins depend on their ability to bind with other molecules namely ligand, a ligand may be an activator, inhibitor or substrate or all the three. This is because the ligand-binding sites on proteins and corresponding ligands are complementary with each other. The binding ability of a protein molecule depends on the strength of binding. Any alteration in the tertiary or quaternary structure of proteins induced by a ligand is called allosteria. For example, many ligands such as ATP, oxygen and cAMP cause allosteric change in target proteins. In addition, other allosteric ligands such as calcium ions and GTP also act on proteins and regulate cellular functions.

When a protein molecule binds with several molecule of a ligand, the binding of one ligand molecule affects the binding of subsequent ligands. This type forms graded binding and is called co-operativity in which the proteins respond efficiently even at small changes of ligand concentration. Thus protein-ligand
interaction enhances the binding and shows a positive co-operativity whereas inhibit the binding denotes negative co-operativity. For instance, haemoglobin is an example for positive co-operativity. It has four heme subunits in which the binding of oxygen to one of the subunits induces a conformational change. This spreads to other subunits to bind additional oxygen molecule.

4.6 Hydrogen bonding interaction

Hydrogen bond results from a dipole-dipole force with a hydrogen atom bonded to nitrogen, oxygen or fluorine (thus the name ‘hydrogen bond’, which must one be confuse with a covalent bond to hydrogen). The hydrogen bond is a very strong bond, but weaker than covalent or ionic bonds. However, it also has some features of covalent bonding nature: it is directional, strong and produces their inter atomic distances shorter than sum of van der Waals radii. The hydrogen bond is somewhere between a covalent bond and an electrostatic intermolecular interactions. This type of bond occurs in both inorganic molecules (such as water) and organic molecules (such as protein).

A hydrogen atom attached to relatively electronegative atom is hydrogen bond donor. This electronegative atom is usually fluorine, oxygen, or nitrogen and is called hydrogen bond acceptor, regardless of whether it is bonded to a hydrogen atom or not. The following figure shows the intermolecular hydrogen bonding interactions in the form of X-H....O.
The protein donor is a polar molecule that exhibits large dipole moment. Often, the positively charged hydrogen atom points towards an electron rich acceptor molecule. The fact that an electron rich region exists in the acceptor molecule implies already that the acceptor has relatively large dipole moment as well. Carbon can also participate in hydrogen bonding, that is C-H.....O interaction, which is now called as “non-classical hydrogen bonding”, especially when the carbon atom is bound to several electronegative atoms, as in the case of chloroform, CHCl₃ and N-H.....O, N-H.....N and O-H...O types are called “classical hydrogen bonding”.

The length of hydrogen bond depends on bond strength, temperature and pressure. The bond strength itself is depending on temperature, pressure, bond angle and environment (usually characterized by local dielectric constant). Hydrogen bonding results from interplay of electrostatic and quantum mechanical forces. If electrostatic interactions dominate, then the hydrogen bond is weak; in strong hydrogen bond, quantum mechanical interactions dominate. The strong hydrogen bonds do not occur in biological systems.
4.7 Molecular docking in Schiff base

Schiff bases are characterized by the \(-N=CH-(azomethine)\) group which is important in elucidating the mechanism of trans-amination and racemisation reaction in biological systems. The transition metal complexes of 4-aminoantipyrine and its derivatives have been extensively examined due to their applications in biological, analytical and therapeutical fields. Further, they have been investigated due to their diverse biological properties as antifungal, antibacterial, analgesic, sedative, antipyretic, anti-inflammatory and DNA binding agent.

4.8 Docking study in Schiff base metal complexes

In our present study, we have chosen the crystal structure of glucosamine-6-phosphate synthase (PDB ID Ijxa) was obtained from protein data bank. Crystallographic water molecules were removed from the protein. The molecular docking tool, Hex 6.0 interface on the windows 7 operating system was used for docking and scoring. The PDB file of the structure for ligand and complex was done by Chemoffice software. The structure was minimized by using the Gaussian-09 software. The optimized structure was used for molecular docking.
CHAPTER V
RESULTS AND DISCUSSION

5.1. Synthesis and characterization of FAAPBT and its Co(II), Ni(II),
Cu(II) and Zn(II) complexes

5.1.1 Synthesis and characterization of FAAPBT

Synthesis of FAAPBT

The Schiff base ligand Furfurylidene-4-aminopyrine-2-aminobenzothiazole
(FAAPBT) was prepared from furfuraldehyde, 4-aminoantipyrine and
2-aminobenzothiazole as described in Sec. 2.2.1.

Characterization

Solubility

FAAPBT is sparingly soluble in common organic solvents such as
methanol, ethanol, acetone and completely soluble in DMF and DMSO.

Elemental analysis

Elemental analysis of FAAPBT is given in Table 5.1.2.1. The
experimental results are in good agreement with theoretical values.

IR spectrum

The IR spectrum of the ligand (Fig. 5.1.1.1) shows characteristic-\(\text{CH}=\text{N}\)
bands in the region 1646 cm\(^{-1}\). (Table 5.1.2.3)
The IR spectrum of the furfuraldehyde shows a strong band at 1675 cm\(^{-1}\), which corresponds to the \(\nu_{(\text{CHO})}\) and the infrared spectrum of 4-aminoantipyrine shows strong bands at 3432 and 3328 cm\(^{-1}\) corresponding to the \(-\text{NH}_2\) stretching frequency. On condensation, these bands disappear and a new band appears at 1590 cm\(^{-1}\), which is assigned to \(\nu_{(\text{HC}=\text{N})}\). This demonstrates the condensation between the aldehyde group in furfuraldehyde and amino group in 4-AAP, resulting in the formation of furfurylidene-4-aminoantipyrine. The peak due to the \(>\text{C}=\text{O}\) of furfurylidene-4-aminoantipyrine is observed around 1630 cm\(^{-1}\). On condensation with 2-aminobenzothiazole, this peak disappears and a new peak appear at 1646 cm\(^{-1}\), which is assigned to the \(\nu_{(\text{C}=\text{N})}\).

Further, the spectrum of ligand shows the medium intensity band at 1242 cm\(^{-1}\), which can be assigned to \(\nu_{(\text{C-N})}\), and the strong band in the 1612 cm\(^{-1}\) region is assigned to aromatic ring \(-\text{C}=\text{C}-\) stretching vibration. The other series of weak and strong bands between 3100 and 2800 cm\(^{-1}\) are related to \((-\text{C-H})\) modes of vibrations. The \((-\text{C-S-})\) stretching frequency of the benzothiazole ring was observed at \(\sim 750\) cm\(^{-1}\) region.
Electronic spectrum

The electronic absorption spectra were recorded at 300 K in DMSO solution. The electronic spectra of the ligand (Fig. 5.1.1.2) shows broad band at 318 and 370 nm which can be assigned to n-\(\pi^*\) transitions of the azomethine (-CH=N) and imine (>C=N) chromophores. In addition, other intense absorption band at higher energy 220-265 nm is due to \(\pi-\pi^*\) transition of the benzene ring of the Schiff base ligand.

\(^1\)H NMR spectrum

\(^1\)H NMR spectrum of FAAPBT (Fig. 5.1.1.3) shows multiplet signal at 7.2-7.6 ppm, due to aromatic protons [213]. There is a singlet signal at 9.6 ppm due to azomethine proton. Signals at 2.5 and 3.3 ppm are due to aliphatic protons.
**DART mass spectrum**

The DART mass spectrum of FAAPBT is shown in Fig. 5.1.1.4. It shows a characteristic molecular ion peak at 414 m/z corresponding to the formula C\text{23}H\text{19}N\text{5}O\text{5}. Elemental analysis values are in good agreement with the values calculated from the molecular formula assigned to the ligand.

**Thermal analysis**

The thermogram of the ligand (Fig. 5.1.1.5) was recorded from room temperature to 600 °C at a heating rate of 10 °C. The ligand shows two stages of decomposition. The melting of ligand takes place at 145 °C. This is indicated by a endothermic peak in the DTA curve. The ligand is stable after 400 °C as seen as a horizontal line in the TG curve.

From the above results, the proposed structure of the ligand is given in Fig. 5.1.1.6.
**Cyclic voltammetry**

Cyclic voltammogram of FAAPBT (Fig. 5.1.1.7) was recorded at 300 K in acetonitrile solution in the potential range -1.6 to 1.6 V. The ligand shows one irreversible couple with $E_{pc} = -0.5$ V vs Ag/AgCl and the associated anode peak at $E_{pc} = -1.1$ V. The large separation $\Delta E = 0.6$ V indicates irreversible couple.

**Powder XRD**

The X-ray diffraction pattern of the ligand is shown in Fig. 5.1.1.8. The crystallite size of the ligand was calculated from Scherrer’s formula described in Sec. 2.4.9. The grain size of ligand obtained is 43 nm. This indicates that the ligand is in nanocrystalline phase.
SEM

The surface morphology of the ligand is shown in Fig. 5.1.1.9. The SEM micrograph was recorded with energy of 20 KV with magnification 200 X. The figure shows irregularly shaped particles.
5.1.2 Synthesis and characterization of Co(II), Ni(II), Cu(II) and Zn(II) complexes of FAAPBT

Synthesis of metal complexes

Co(II), Ni(II), Cu(II) and Zn(II) complexes using FAAPBT were synthesized as described in Sec. 2.2.2.

Characterization of metal complexes

Solubility

The metal complexes are stable at room temperature. They are sparingly soluble in common organic solvents, but soluble in DMF and DMSO. The analytical and physical data of the complexes are given in Table 5.1.2.1 Elemental analysis indicates that the found and calculated values are within acceptable limits (± 0.5).
Molar conductance

The molar conductance data of the metal(II) complexes measured in DMSO for 0.001 M solutions are given in Table 5.1.2.2. The molar conductance values fall in the range of 5-20 Ω^{-1} cm^{2} mol^{-1}, which is the expected range for the complexes to behave as non-electrolytes [165]. Thus, the present complexes have non-electrolytic nature as evidenced by the involvement of acetate ions in coordination. This result was further confirmed from the chemical analysis of CH₃COO⁻ ion, not precipitated by addition of FeCl₃.
IR spectra

IR spectra provide a lot of valuable information on coordination reaction. The IR spectra provide some important information regarding the skeleton of the complexes. In order to study the binding mode of the Schiff base to the metal in the complexes, the IR spectrum of the free ligand was compared with the spectra of the complexes (Table 5.1.2.3). There were some significant differences between the metal(II) complexes (Fig. 5.1.2.1.a-d) and the free ligand upon chelation as expected. The IR spectra of the ligand shows characteristic -C=N bands in the 1646 cm⁻¹ region which are shifted to lower frequencies, 1630-1604 cm⁻¹ in the spectra of complexes [214]. The ligand also shows HC=N- band in 1590 cm⁻¹ region and it is shifted up to 1582 cm⁻¹ in the complexes. It confirms the formation of -CH=N bond as well as the lack of carbonyl group in the original aldehyde. The presence of bands in the region 1573-1562 cm⁻¹ and 1333-1312 cm⁻¹ characteristic of asymmetric and symmetric COO⁻ stretching vibrations respectively with $\Delta \nu = \sim 250$ cm⁻¹ [215]. The complexes also display bands in the 442-421, 572-552 cm⁻¹ region due to the formation of M-N and M-O bonds [216]. Therefore, it was concluded that the Schiff base behaves as a bidentate ligand coordinated to the metal ions via the (-CH=N) and (>C=N) groups.
**Electronic spectra**

The electronic absorption spectra can often provide quick and reliable information about the ligand arrangement in transition metal complexes. It also serves as a useful tool to distinguish among the square-planar, octahedral or tetrahedral geometries of the complexes. The absorptions in the ultraviolet region are attributed to transitions within the ligand orbital and those in the visible region are due to allowed metal-to-ligand charge transfer transitions. The electronic absorption spectra were recorded at 300 K in DMSO solution. The absorption regions, assignments and the geometry of the complexes are given in Table 5.1.2.4.

The electronic spectrum of the ligand shows broad band at 318 and 370 nm which can be assigned to \( n-\pi^* \) transitions of the azomethine (-CH=N) and imine (>C=N) chromophores. On complexation this was shifted to lower wavelength (Fig. 5.1.2.2.a-d), suggesting the coordination of azomethine nitrogen. In addition, other intense absorption band at higher energy 220-265 nm is due to \( \pi-\pi^* \) transition of the benzene ring of the Schiff base ligand. The spectrum of Co(II) complex shows two bands in 600-700 nm region, which can be attributed to \( ^4T_{1g} (F) \rightarrow ^4T_{2g} (P) \) and \( ^4T_{1g} (F) \rightarrow ^4A_{2g} (F) \) [Sec. 2.4.5] transitions for octahedral geometry. The Ni(II) complex shows three bands at 983, 755 and 503 nm corresponding to the octahedral geometry with the following transitions \( ^3A_{2g} (F) \rightarrow ^3T_{2g} (F), \) \( ^3A_{2g} (F) \rightarrow ^3T_{1g} (F) \) and \( ^3A_{2g}(F) \rightarrow ^3T_{1g} (P) \) respectively. The Cu(II) complex shows three bands at 535, 619 and 770 nm due to \( ^2B_{1g} \rightarrow ^2B_{2g}, \) \( ^2B_{1g} \rightarrow ^2E_g \) and \( ^2B_{1g} \rightarrow ^2A_{2g} \) transitions [166, 116], respectively. The Zn(II) ions have d\(^{10}\) configuration and is diamagnetic in nature. According to the empirical formula, an octahedral geometry is proposed for this complex.
Magnetic measurements

Magnetic susceptibility measurements of transition metal complexes give an indication about geometry of the ligands around central metal ions. Magnetic susceptibility values of the complexes along with the geometry are given in Table 5.1.2.5. The observed magnetic moment value of Co(II) complex is 4.81 BM, which is the expected range for octahedral Co(II) complexes. For octahedral Ni(II) complexes, $\mu_{\text{eff}}$ is in the range 2.9-3.9 B.M. In the present case, the Ni(II) complex has the magnetic moment of 3.91 B.M. in accordance with octahedral geometry. The magnetic moment obtained for the present Cu(II) complex is 1.94 B.M. The magnetic moment obtained is in accordance with the proposed octahedral geometry. Zn(II) complex with d$^{10}$ electronic configuration is diamagnetic and have octahedral geometry. The magnetic moment values of the complexes are given in Table 3. The magnetic moment data indicate paramagnetic Co(II), Ni(II) and Cu(II) complexes [172].
The $^1H$ NMR spectrum of Zn(II) complex (Fig. 5.1.2.3) displays signals for aliphatic and aromatic protons with chemical shift values in accordance with the proposed structure. $^1H$ NMR spectrum of ligand shows multiplet at 7.2-7.6 ppm due to aromatic protons [213]. There is a singlet at 9.6 ppm due to azomethine proton. Signals at 2.5 and 3.3 ppm are due to aliphatic protons. In the $^1H$ NMR spectrum of the Zn(II) complex, the azomethine proton signal is shifted downfield compared to the free ligand due to the deshielding of the azomethine group due to coordination with Zn(II) ion [217]. The signals at 6.5 and 6.8 ppm are due to furfuryl protons.
The mass spectrum of ligand and its complexes were recorded and their stoichiometric compositions are compared. The molecular ion peak for ligand (FAAPBT), (C_{23}H_{19}N_{5}OS) is observed at 413 m/z. The DART mass spectrum of Cu(II) complex is given in Fig. 5.1.2.4. Similar fragmentation pattern is observed for Co(II), Ni(II) and Zn(II) complexes. The molecular ion peaks of Co(II), Ni(II), Cu(II) and Zn(II) complexes are observed at 627, 626, 631 and 632 m/z, which confirm the stoichiometry of the metal complexes to be [M(FAAPBT) (OAc)_{2}(H_{2}O)_{2}]. Elemental analysis values are in good agreement with the values calculated from the molecular formulae assigned to these complexes which are further supported by DART-mass studies [220]. The mass spectral fragmentation pattern of the metal complexes is given in Scheme 5.1.2.1.
ESR spectra

The ESR spectrum of the Cu(II) complex at 300 K shows one intense absorption band at high field (Fig. 5.1.2.5.a & b), which is isotropic due to the tumbling motion of the molecules. However, this complex in the frozen state (Table 5.1.2.6) shows four well resolved peaks with low intensities in the low field region and one intense peak in the high field region. The magnetic susceptibility value reveals that the copper complex has a magnetic moment 1.94 B.M. corresponding to one unpaired electron, indicating that the complex is mononuclear. This fact is also evident from the absence of half field signal, observed in the spectrum at 1600 due to the $m_s = \pm 2$ transitions, ruling out any Cu-Cu interaction [218]. The $g$ values are in the order $g_\parallel > g_\perp > 2.0023$ corresponding to the presence of an unpaired electron in the $d_{x^2-y^2}$ orbital.

For Cu(II) complex, $g_\parallel$ is a parameter sensitive enough to indicate covalency. The fact that $g_\parallel$ is less than 2.3 is an indication of significant covalent character to M-L bond [219]. From the observed values, it is clear that $A_\parallel = 160 > A_\perp = 40$; $g_\parallel = 2.36 > g_\perp = 2.08 > 2$ and the e.s.r. parameters of the complex coincide well with related systems which suggest that the complex has octahedral geometry and the system is axially symmetric. This is also supported by the fact that the unpaired electron lies predominantly in the $d_{x^2-y^2}$ orbital.
Thermal analysis

The thermograms of metal complexes were recorded in the temperature range from room temperature to 1000 °C at the heating rate of 10 °C per minute under nitrogen atmosphere. The initial decomposition temperature of the metal complexes indicate the relative thermal stability of these systems. The Co(II), Ni(II), Cu(II) and Zn(II) complexes show three stages of decomposition (Fig. 5.1.2.6.a-d). The first stage decomposition takes place in the temperature range 120-180 °C. The first stage of decomposition corresponds to the loss of coordinated water. The second stage corresponds to the loss of pyrazole and furfuraldehyde moieties. The third stage of decomposition corresponds to the loss of 2-aminobenzothiazole moiety. From the above observations, the metal(II) complexes are found to be thermally stable than the ligand [221].

Based on the above results, the proposed structure of the complexes is shown in Fig. 5.1.2.7.
Cyclic voltammetry

Cyclic voltammogram helps to evaluate the effect of ligand on the redox potential of the central metal ion in complexes. It has been shown to be a particularly useful technique in studies of unusual oxidation states of metal complexes [222]. The cyclic voltammograms of ligand and its metal complexes were recorded at 300 K in acetonitrile solution. Co(II) complex was recorded in the potential range -0.4 to 1.4 V, while Ni(II), Cu(II) and Zn(II) complexes were recorded in the potential range -0.6 to 1.4 V with scan rate 20 mV/S. The Co(II) complex shows two irreversible peaks one at Epc = 0.8 V corresponding to Co(II)/(I) couple. The other peak at Epc = -0.05V during the reverse scan corresponds to Co(II)/(III) couple. The peak to peak separation $\Delta E$ is 0.85 V indicating the process to be irreversible.

Ni(II) complex shows two irreversible peaks one at Epc = 0.6 V and the other at Epc = 1.0 V corresponding to Ni(II)/(I) couple. The Cu(II) complex (Fig. 5.1.2.8.a-d) shows quasi reversible cathodic peak at Epc = 0.65 V vs Ag/AgCl and the associated anode peak at Epa = 0.55 V corresponding to the formation of one electron oxidation Cu(II)/III couple. The peak to peak separation $\Delta E$ is 0.1 V confirming the process as quasi reversible. The Zn(II) complex shows quasireversible peak at Epc = 0.65 V vs Ag/AgCl and the associated anode peak at Epa = 0.55 V corresponding to Zn(0)/(II) couple. The peak to peak separation value $\Delta E = 0.1$ V corresponds to quasi reversible process [223].
Powder XRD

XRD pattern of the Co(II), Ni(II), Cu(II) and Zn(II) complexes were recorded in the 20 range 10-80°. The X-ray diffraction of the complexes are shown in Fig. 5.1.2.9.a-d. The crystallite size of the ligand and its complexes was calculated from Scherrer’s formula described in Sec. 2.4.9. The Cu(II), Ni(II) and Zn(II) complexes are nanocrystalline with grain sizes 22, 28 and 43 nm respectively. The Co(II) complex does not show any peak indicating that it is amorphous in nature [224].
SEM

The SEM micrographs of Co(II) and Ni(II) complexes were recorded at an energy of 20 KV with magnification 1000 X. The Cu(II) complex was recorded at an energy of 20 KV with magnification 1500 X. The Zn(II) complex was recorded at an energy of 20 KV with magnification 500 X. The SEM micrographs of the complexes are shown in Fig. 5.1.2.10.a-d. The SEM micrograph of ligand differs significantly from the complexes. The Co(II) complex shows bar-like structure. The Ni(II) complex shows faceted-microcrystals. Agglomerated morphology is present for the Cu(II) complex. For Zn(II) complex bar-with layered structure is present.
5.1.3 Biological studies

Antimicrobial analysis

A comparative study of the MIC values of the ligand and its metal complexes indicates that complexes exhibit higher antibacterial and antifungal activity compared to those of the free ligand (Table 5.1.3.1 and 5.1.3.2). Compounds containing -CH=N group have enhanced antimicrobial activity than >C=C< group. The growth of certain microorganisms containing >C=C< group, though capable of absorbing O₂ are not related with the growth of microorganisms. Such increased activity of the complexes can be explained on the basis of Overtone’s concept and Tweedy’s chelation theory [225].

According to Overtone’s concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only the lipid-soluble materials due to which liposolubility is an important factor which controls the antibacterial and antifungal activity. Chelation reduces the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups and possible π-electron delocalization over the ring. Such chelation could increase the lipophilic character of the central metal atom. The increased lipophilicity subsequently favours the permeation through the lipid layer of cell membrane. These complexes also disturb the synthesis of the proteins that restricts further growth of the organism. The variation in the effectiveness of different complexes against different organisms depend either on differences in the permeability of the cells of the microbes or on difference in ribosome of the microbial cells [226]. The synthesized compounds have electron releasing substituent and coordinated acetate increases the microbial activity.
**Effect of hetero atoms**

From the observations, the higher inhibition of microbial growth is due to uncoordinated hetero atoms. In the complexes, the ligand has some uncoordinated donor atoms which enhance the activity of the complexes by bonding with the trace elements present in microorganisms. This can combine with the uncoordinated site and inhibit the growth of microorganisms. The uncoordinated hetero atoms present are O, N and S.

**Mode of action**

The mode of action of antimicrobials may involve various targets in microorganisms.

(i) Interference with the cell wall synthesis, damage as a result of which cell permeability may be altered (or) they may disorganize the lipoprotein leading to the cell death.

(ii) Deactivate various cellular enzymes, which play a vital role in different metabolic pathways of these microorganisms.

(iii) Denaturation of one or more proteins of the cell, as a result of which the normal cellular processes are impaired.

Formation of a hydrogen bond through the azomethine group with the active centre of cell constituents, resulting in interference with the normal cell process.
DNA Binding Studies

DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA. A variety of small molecules interact reversibly with double stranded DNA, primarily through three modes; (i) electrostatic interactions with the negative charged nucleic sugar-phosphate structure, which are along the external DNA double helix and do not possess selectivity (ii) binding interactions with two grooves of DNA double helix and (iii) intercalation between the stacked base pairs of native DNA [227]. The absorption spectra are the most common means to examine the interaction between metal complex and DNA. Binding of complexes with DNA by intercalation usually results in hypochromism and red shift due to the intercalative mode involving a strong stacking interaction between the aromatic chromophore and the base pairs of DNA. The magnitude of the hypochromism and red shifts are commonly found to depend on the strength of the intercalative interaction [228].

The complexes exhibit intense absorption band in the UV region, which is attributed to intraligand (IL) \( \pi-\pi^* \) transition of the coordinating groups and addition of increasing amounts of CT DNA result in hypochromism and bathochromic shift in the UV spectrum of complexes. These spectral characteristics (Table 5.1.3.3) suggest that the complexes bind to DNA by intercalation. After intercalating the base pairs of DNA, the \( \pi^* \) orbital of the intercalated ligand can couple with the \( \pi \) orbital of the base pairs, thus decreasing the \( \pi-\pi^* \) transition energy and resulting in bathochromism. On the other hand, the coupling \( \pi \) orbital is partially filled by electrons, thus decreasing the transition probabilities and concomitantly resulting in hypochromism [229].
**DNA cleavage studies**

Gel electrophoresis via the nicking assay is an effective method for the determination of DNA damage to the double helix, allowing for the determination of strand breaks. Analytes are separated depending upon their relative charges and size. These properties depend on the analyte ability to move through the gel. A sample is injected into the gel. A potential is then applied across the gel and depend on their properties, the analytes are separated resulting in a series of bands across the gel. Gel electrophoresis experiment using pUC 18 DNA were performed with metal complexes in the presence and absence of H$_2$O$_2$ as oxidant. The naturally occurring super coiled form when nicked gives an open circular relaxed form up on further cleavage results in the linear form. The complexes cleave DNA more efficiently in the presence of oxidant, which may be due to the formation of hydroxyl free radicals.

The production of hydroxyl radical due to the reaction between the metal complex and oxidant may be explained as shown below.

$$H_2O_2 + M^{n+} \rightarrow M^{n+1} + \cdot OH + OH^-$$

The hydroxyl radicals participate in the oxidation of deoxy ribose moiety followed by hydrolytic cleavage of sugar phosphate back bone [230]. The cleavage efficiency is measured by determining the ability of the complex to convert the super coiled DNA into nicked open circular form or sheared form. Control experiments using DNA alone do not show any significant cleavage pUC18 DNA even on exposure to longer period. It is seen that there is considerable increase in the intensity of bands for open circular form in the case of Cu(II) complex and Ni(II) complex. This indicates that the above complexes have nicking activity. There is no appreciable level of increase in the intensity of bands of open circular form for Co(II) and Zn(II) complexes.
**SOD activity**

SOD mimetic activities of the ligand and its Co(II), Ni(II), Cu(II) and Zn(II) complexes have been measured. The SOD mimetic activities of the present complexes were examined by the NBT assay as described in Sec. 3.4. The observed IC$_{50}$ values of present complexes are compared with various reported complexes and are less active than native SOD [231]. The ligand, Co(II), Ni(II) and Zn(II) complexes have moderate activity and Cu(II) complex has higher activity (Fig. 5.1.3.1). A strong ligand may oppose the interaction of the Cu(II) with superoxide radicals, being unfavourable to the probable formation of intermediate copper superoxide adduct (Table 5.1.3.4) [232].

\[
\text{Cu}^{2+} + \text{O}_2^- + 2. \text{H}^+ \rightarrow \text{Cu}^{3+} \text{H}_2\text{O}_2
\]
5.1.4 Molecular docking

The docking results are summarized in Table 5.1.4.1. The residues that have been reported to be involved in the protein ligand interactions are also given in the table. Molecular docking of the complex formed between ligand and protein indicates that the ligand is well positioned in the gorge. Docking results in energy minimized docked structures. The lowest score is obtained by the Ligand. Among the complexes, the highest negative score is obtained for the Ni(II) complex. The lowest score is obtained by the Cu(II) complex. The docking score of the complexes are in the order Ni(II)> Co(II)> Zn(II)>Cu(II). The details about binding pattern of the complexes are obtained from the structural analysis of docked structures (Fig. 5.1.4.1.a-e). The number of hydrogen bonds present is maximum (7) in the Ni(II) complex. The number of hydrogen bonds is similar (3) in the Co(II), Cu(II), and Zn(II) complexes. The docking studies further indicate that the complexes are more active than the ligand.
5.2 Synthesis and characterization of mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) with FAAPBT and Phen

Solubility

The mixed ligand Co(II), Ni(II), Cu(II) and Zn(II) complexes synthesized are stable at room temperature. They are insoluble in common organic solvents and are soluble in DMF and DMSO. The analytical and physical data of the complexes are given in Table 5.2.1

Elemental analysis

Elemental analysis indicates that calculated and found values are within acceptable limits (± 0.5) (Table 5.2.1).

Molar conductance

The molar conductance data of the mixed ligand complexes measured in DMF for 0.001 M solutions are given in Table 5.2.2. The values fall in the range of 6.9-18.6 Ω^{-1}cm^{2} mol^{-1}, which is within the expected range for the complexes to behave as non-electrolytes [165]. Thus, the present complexes are non-electrolytic in nature.
**IR Spectra**

The IR spectra of the complexes are compared with those of the free ligand in order to determine the coordination sites involved in chelation (Fig. 5.2.1.a-d). There are some significant differences in IR spectra of the metal(II) complexes than the free ligand upon coordination. The infrared spectrum of the Schiff base shows strong bands at 1590 cm\(^{-1}\) and 1646 cm\(^{-1}\) attributed to the \((-\text{CH}=\text{N})\) and \( (>\text{C}=\text{N})\) stretching vibrations respectively [24, 233]. On complexation, these bands are shifted (Table 5.2.3), indicating that the nitrogen atoms of the azomethine and imino groups coordinated to the metal ion. The presence of bands in the region 1494-1479 cm\(^{-1}\) and 1305-1295 cm\(^{-1}\) are characteristic of asymmetric and symmetric COO\(^{-}\) stretching vibrations respectively [234].

All the complexes show bands in the regions 1050-1014 and 725-701 cm\(^{-1}\) which can be assigned to 1,10-phenanthroline ring –CH and \( >\text{C}=\text{N}\) stretching vibrations respectively [235]. The complexes also display bands in the 454-431, 507-493 cm\(^{-1}\) region due to the formation of M-N and M-O bonds respectively [236]. Therefore from the IR spectra, it is concluded that the Schiff base behaves as a bidentate ligand coordinated to the metal ions via \((-\text{CH}=\text{N})\) and \( (>\text{C}=\text{N})\) groups. The other coordination sites of metal(II) ions are occupied by two acetate groups and two positions are occupied by \( (>\text{C}=\text{N})\) groups of 1,10-phenanthroline ligand.
Electronic spectra

The electronic absorption spectra of the mixed ligand complexes were recorded at 300 K in DMF solution (Fig. 5.2.2.a-d). The absorption regions, assignments and the geometry of the complexes are given in Table 5.2.4. The electronic spectra of the ligand shows broad band at 318 and 370 nm which can be assigned to n-π* transitions of the azomethine (-CH=N) and imine (>C=N) chromophores. On complexation this is shifted to lower wavelength, suggesting the coordination of azomethine nitrogen. In addition, other intense absorption band at higher energy 220-265 nm is due to π-π* transition of the benzene ring of the Schiff base ligand. The electronic spectrum of Co(II) complex shows one band at 1035 nm region, which can be attributed to $^4T_{1g} (F) \rightarrow ^4T_{2g} (F)$ transition for octahedral geometry [Sec. 2.4.5]. The Ni(II) complex shows one band at 1083 nm corresponding to octahedral geometry with the following transition $^3A_{2g} (F) \rightarrow ^3T_{2g} (F)$. The Cu(II) complex shows one band at 735 nm due to $^2E_g \rightarrow ^2T_{2g}$ transition, characteristic for distorted octahedral geometry [166, 237]. The Zn(II) ions have d$^{10}$ configuration and is diamagnetic in nature. As indicated by the empirical formula, an octahedral geometry is proposed for this complex.
**Magnetic measurements**

The observed magnetic moment value of Co(II) complex is 5.1 BM, which is the expected range for octahedral Co(II) complexes. For octahedral Ni(II) complexes, $\mu_{\text{eff}}$ is in the range 2.9-3.9 B.M. In the present case, the Ni(II) complex has the magnetic moment of 3.2 B.M. in accordance with octahedral geometry. The magnetic moment obtained for the present Cu(II) complex is 1.93 B.M. The magnetic moment obtained is in accordance with the proposed distorted octahedral geometry [172, 168]. Zn(II) complex with d$^{10}$ electronic configuration is diamagnetic and have octahedral geometry. The magnetic moment values of the complexes are given in Table 5.2.5.
$^1$H NMR Spectrum

The $^1$H NMR spectrum of Zn(II) complex is given in Fig. 5.2.3. The signal for azomethine proton (-CH=N-) in the ligand appears as a singlet at 9.6 ppm. In the $^1$H NMR spectrum of the Zn(II) complex, the azomethine proton signal is shifted downfield compared to the free ligand due to the deshielding of the azomethine group on coordination with Zn(II) ion. The multiplet present in the 7.2-7.5 ppm range is due to the aromatic protons of the ligand. The signal for pyrazolone ring carbon attached methyl protons (-CH$_3$) appears as a singlet at 2.5 ppm, while pyrazolone ring nitrogen attached methyl protons (>N-CH$_3$) appears as a singlet at 3.3 ppm [159]. The signals at 6.5 and 6.8 ppm are due to furfuryl protons. In the Zn(II) complex, a new peak at 2.4 ppm indicates the coordination of acetate group.
Mass spectra

The mass spectrum of Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes were recorded. The molecular ion peaks of Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes are observed at 772, 771, 775 and 778 m/z which confirms the stoichiometry of the metal complexes to be \([\text{M(FAAPT)(OAc)}_2(\text{phen})]\). Elemental analysis values are in agreement with the values calculated from the molecular formulae assigned to these complexes which are further supported by DART-mass studies.
ESR spectra

ESR spectra of the Cu(II) complex was recorded at room temperature (300 K) and at liquid nitrogen (77 K) temperature (Fig. 5.2.4.a & b) in DMSO. The ESR spectral data are given in Table 5.2.6. The $g_{||}$ and $g_{\perp}$ values were calculated from the spectrum using tetracyanoethylene (TCNE) free radical as the “g” marker as described in Sec. 2.4.7. It was reported [238] that $g_{||}$ is less than 2.3 for covalent character and greater than 2.3 for ionic character of the metal-ligand bond in complexes. In the present Cu(II) complex, $g_{||}$ is less than 2.3 is an indication of significant covalent character in M-L bond. The $g_{||}$ and $g_{\perp}$ values are $>2.04$, consistent with an elongated tetragonally distorted octahedral stereochemistry with all the principal axes aligned parallel. The G factor calculated using eqn 18 [Sec. 2.4.7] is $>4.0$ suggests that the local tetragonal axes are only slightly misaligned and the exchange interactions between Cu(II) centers in the solid state are negligible [239]. This fact is also evident from the absence of half field signal, observed in the spectrum at 1600 due to the $m_s = \pm 2$ transitions, ruling out any Cu-Cu interaction. The $g$ values are in the order $g_{||} > g_{\perp} > 2.0023$ corresponding to the presence of an unpaired electron in the $d_{x^2}-d_{y^2}$ orbital.

From the observed values (Table 5.2.6), it is clear that $A_{||}=115 > A_{\perp}=80$; $g_{||}=2.25 > g_{\perp}=2.06 > 2$ and the e.s.r. parameters of the Cu(II) complex suggest that the complex have tetragonally distorted octahedral geometry. This is also supported by the fact that the unpaired electron lies predominantly in the $d_{x^2}-d_{y^2}$ orbital.
**Thermal analysis**

The thermograms of the metal complexes were recorded in the temperature range from room temperature to 700 °C. The Co(II) and Zn(II) complexes show four stages of decomposition. Ni(II) complex shows two stage of decomposition. The initial decomposition temperature of Co(II) complex is lower compared to the Ni(II), Cu(II) and Zn(II) complexes. The decomposition of Cu(II) complex takes place at higher temperature (290 °C), indicating that it is more thermally stable. Zn(II) complex shows the final decomposition at 472 °C, which is the higher final decomposition temperature compared to the Co(II), Ni(II), and Cu(II) complexes.

Based on the elemental analysis, IR, electronic, \(^1\)H NMR, mass, ESR spectral data, magnetic moment and thermal analysis, the proposed structure of the complexes is given in Fig. 5.2.5.
Cyclic voltammetry

Cyclic voltammetry offers a rapid location of redox potentials of the electro active species. The cyclic voltammograms of ligand and its metal complexes were recorded at 300 K in DMSO solution in the potential range -1.0 to 1.2 V with scan rate 0.1 V/s.

The cyclic voltammogram of the Co(II) complex shows an irreversible cathodic peak at 0.3 V. The present Ni(II) complex is electrochemically inert and does not show any peak in the cyclic voltammogram. The Cu(II) complex (Fig. 5.2.6.a-d) displays a cathodic peak at 0.5 V versus Ag/AgCl with the corresponding anodic peak at -0.04 V on the reverse scan. The peak separation value ($\Delta E_p = 0.54$ V) indicates quasi-reversible character for the one electron transfer of metal-based Cu(II)/Cu(I) couple. The Zn(II) complex shows a irreversible anodic peak at -0.25 V [223].
**Powder XRD**

The powder XRD patterns of the mixed ligand complexes were recorded in the range $2\theta = 0$-80 $\degree$ and are given in in Fig. 5.2.6.a-d. From the observed $d_{\text{XRD}}$ patterns, the crystalline size of the complexes are calculated from Scherrer’s formula described in Sec. 2.4.9. In the present study, Co(II), Ni(II), Cu(II) and Zn(II) complexes are nanocrystalline with grain size 19, 20, 14 and 12 nm respectively [227].
SEM

The SEM micrographs of Co(II) and Zn(II) complexes were recorded at an energy of 20 KV with magnification 100 X. The Ni(II) complex was recorded at an energy of 20 KV with magnification 50 X. The Cu(II) complex was recorded at an energy of 20 KV with magnification 40 X. The SEM micrographs of the Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes are shown in Fig. 5.2.7.a-d. SEM micrographs of the metal complexes show controlled morphological structure with the presence of small grains in non-uniform size. The SEM micrographs of Co(II) and Cu(II) complexes exhibit broken rock like structure. The Ni(II) and Zn(II) complexes show irregularly shaped particles.
5.2.1 Biological studies

Antimicrobial analysis

The in vitro biological screening of ligand and its mixed ligand metal complexes were tested against bacterial species S. aureus, E. coli and P. aeruginosa and fungal species A. niger, A. flavus and C. albicans by agar well diffusion method as described in Sec. 3.1.1 and 3.1.2. The results of the antibacterial and antifungal activities are summarized in Table 5.2.1.1 & 5.2.1.2. The standards used are Gentamycin for antibacterial and Amphotericin for antifungal activities.

In the present study, the antimicrobial screening of the mixed ligand metal complexes indicate that the complexes possess higher growth inhibition potential compared to those of the ligand. Among the metal(II) mixed ligand complexes, Cu(II) complex exhibits higher antimicrobial activity. The antimicrobial activity of the metal complexes increases with increase in concentration of the complexes. The reason for the high antimicrobial activity of Cu(II) complex can be explained in terms of the effect of copper metal ion on the normal cell process. The complexation reduces the polarity of the metal ion by the partial sharing of metal ion positive charge with donor groups and electron delocalization over the chelate ring. Thus, the lipophilic character of the central metal ion is enhanced, which results in a higher capability to penetrate the microorganisms through the lipid layer of the cell membrane [240].
DNA binding studies

The DNA binding of the complexes were carried out by the method described in Sec. 3.2. UV absorption spectroscopy is an effective method to examine the binding mode of DNA with compounds. After intercalating into the DNA base pairs, the π* orbital of the intercalated compounds can couple with the π orbital of the DNA base pairs, thus decreasing the π→π* transition energy and resulting in bathochromism. The coupling π orbital is partially filled by electrons, decreasing the transition probabilities along with hypochromism. Generally, the binding of an intercalative molecule to DNA is always accompanied by hypochromism and/or significant bathochromism in the absorption spectra due to the strong stacking interactions between the aromatic chromophore of the compounds and DNA base pairs [241].

The absorption band of the Co(II) complex at 377 nm exhibits hypochromism of 16 % and bathochromism of 8 nm. The absorption band of Ni(II) complex at 334 nm exhibits hypochromism of 22 % and bathochromism of 10 nm. The absorption band of Cu(II) complex at 356 nm shows hypochromism of 27 % and bathochromism of 9 nm. The Zn(II) complex at 372 nm shows hypochromism of 17 % and bathochromism of 3 nm. These results (Table 5.2.1.3) suggest that the mixed ligand complexes are interacting with DNA through intercalation. The intrinsic binding constant (K_b) values are also calculated (1.8 x 10^5, 2.2 x 10^5, 3.1 x 10^5 and 1.2 x 10^5 M^-1 for the mixed ligand Co(II), Ni(II), Cu(II) and Zn(II) complexes respectively).
DNA cleavage studies

The DNA cleavage of the metal complexes was done according to the procedure described in Sec. 3.3. Gel electrophoresis experiment using pUC18 DNA was performed with metal complexes in the presence of H$_2$O$_2$ as oxidant (Fig. 5.2.1.1). The naturally occurring super coiled form when nicked gives an open circular relaxed form up on further cleavage results in the linear form. The DNA cleavage of the mixed ligand complexes is given in Fig. 5.2.1.1. The complexes cleave DNA more efficiently in the presence of oxidant, which may be due to the formation of hydroxyl free radicals. The production of hydroxyl radical due to the reaction between the metal complex and oxidant is shown below.

\[
\text{H}_2\text{O}_2 + \text{M}^{n+} \rightarrow \text{M}^{n+1} + \cdot \text{OH} + \text{OH}^-
\]

The hydroxyl radicals participate in the oxidation of deoxy ribose moiety, followed by hydrolytic cleavage of sugar phosphate back bone [230]. The cleavage efficiency is measured by determining the ability of the complex to convert the super coiled DNA into nicked open circular form or linear form. Control experiments using DNA alone do not show any significant cleavage of pUC18 DNA. It is seen that there is considerable increase in the intensity of bands for open circular form in the case of Co(II), Ni(II) and Zn(II) complexes. Present Cu(II) complex completely cleaves the DNA in the presence of H$_2$O$_2$. The results indicate that the above complexes have DNA nicking activity. The DNA cleavage activity of the metal complexes are in the order, Cu(II) > Ni(II) > Co(II) > Zn(II).
**SOD activity**

The SOD mimetic activities of the present complexes were examined by the NBT assay as described in Sec. 3.4. The observed IC$_{50}$ values (Table 5.2.1.4) of present complexes are compared with various reported complexes. All the complexes have moderate activity and Cu(II) complex has higher activity (Fig. 5.2.1.2). A strong ligand may oppose the interaction of the Cu(II) with superoxide radicals, being unfavourable to the probable formation of intermediate copper superoxide adduct [232].

\[ \text{Cu}^{2+} + \text{O}^{2-} + 2\, \text{H}^+ \rightarrow \text{Cu}^{3+} \, \text{H}_2\text{O}_2 \]
Cytotoxicity assay

Cytotoxicity assay of the complexes were done as described in Sec. 3.5. The E.coli AB 1157, proficient to repair damage in the DNA is considered for cytotoxicity assay. Stannous chloride was taken as standard compound. The results (Table 5.2.1.5) show that the mixed ligand Co(II) and Zn(II) complexes are more toxic than the Cu(II) complex. The Cu(II) complex shows moderate cytotoxicity. In the present study the Ni(II) complex does not show any cytotoxicity.
Anticancer activity of newly synthesized mixed ligand metal complexes was investigated on human cervical carcinoma (HeLa) cells by MTT assay as given in Sec. 3.7.3. The results are expressed in terms of IC$_{50}$ values. The complexes are applied in the concentration range 0.1-100 µM. The data obtained by the MTT assay show that the Cu(II) and Zn(II) complexes have very good inhibitory effect on the growth of human cervical carcinoma (HeLa) cells (Fig. 5.2.1.3.a-c). Table 5.2.1.6 illustrates the IC$_{50}$ values of the compounds. The Cu(II) complex shows higher activity with IC$_{50}$ value 8.3 µM. The Zn(II) complex also exhibits good anticancer activity with IC$_{50}$ value 8.33 µM. The Co(II) complex gives IC$_{50}$ value at 72.35 µM. The ligand and Ni(II) complex has lowest growth inhibitory activity against HeLa cells (>100 µM).
Anti-tuberculosis activity

The antitubercular activity of the ligand and mixed ligand metal complexes were ascertained using MABA method explained in Sec. 3.6. The antitubercular results are summarized in Table 5.2.1.7, which clearly show the differential sensitivity of mycobacterial strain towards the test samples. Anti-tuberculosis activity of the complexes is due to their metal binding properties. The present study reveals an effective increase in potency of the ligand when chelated with the metal ions. The Cu(II) and Z(II) complexes are found to be effective growth inhibitors of mycobacterium tuberculosis strain, whereas the Co(II) and Ni(II) complexes exhibit moderate activity. The ligand also possesses moderate activity due to the presence of pyrazolone and benzothiazole moieties. The remarkable increase in the activity of the complexes is due to the presence of 1,10-phenanthroline moiety and the metal ions [242]. The presence of these moieties imparts the growth inhibition activity of mycobacterium tuberculosis. The results implies the importance of the Cu(II) complex and may be utilized for the design and development of novel therapeutic agent for tuberculosis.
5.2.2 Molecular docking

The docking results are summarized in Table 5.2.2.1. The residues that have been reported to be involved in the protein ligand interactions are given in the Table 5.2.2.1. The highest negative score is obtained for the Cu(II) complex. The docking score is similar in Co(II), Ni(II) and Zn(II) complexes. The antimicrobial screening obtained for the complexes are in accordance with the docking results. The details about binding pattern of the complexes are obtained from the structural analysis of docked structures (Fig. 5.2.2.1.a-d). The docking results also indicate that the Cu(II) complex can act as an anti cancer agent. This is in correlation with the results obtained for anti cancer activity of the Cu(II) complex. The number of hydrogen bonds present is similar in all the complexes. There is similarity between the theoretical and experimental values.
5.3 Synthesis and characterization of mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) with FAAPBT and AP

**Solubility**

The mixed ligand complexes synthesized are stable at room temperature. The complexes are soluble in DMF and DMSO and insoluble in common organic solvents.

**Elemental Analysis**

Elemental analysis results are given in Table 5.3.1. Elemental analysis indicates that the found and calculated values are within acceptable limits (± 0.5).

**Molar Conductance**

The molar conductance data of the mixed ligand complexes fall in the range of 3.3-12.5 $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$, which confirm that the present complexes are non-electrolytes (Table 5.3.2) [165].
IR spectra

A comparative study of IR spectra of the mixed ligand complexes with ligand (Fig. 5.3.1.a-d) reveals that several peaks are shifted, vanished or have newly appeared. The infrared spectrum of the Schiff base shows two strong bands, one at 1590 cm\(^{-1}\) and another at 1646 cm\(^{-1}\). These bands are assigned to the (-CH=N) and (>C=N) stretching vibrations respectively [55]. A shift in these frequencies (Table 5.3.3) is observed in all complexes due to the coordination of metal through nitrogen atoms of the -CH=N and >C=N groups. In all the complexes a broad band is present in the region 3435-3406 cm\(^{-1}\), indicates the presence of coordinated water molecules [243].

The band at 3354 cm\(^{-1}\) for \(\nu(\text{OH})\) in the free 2-aminophenol ligand disappears on complexation, indicating coordination of –OH group through deprotonation [244]. The absence of the bands due to NH\(_2\) group in the spectra of complexes indicate the involvement of amino group in coordination through deprotonation. The complexes also display bands in the 439-417, 590-497 cm\(^{-1}\) region due to the formation of M-N and M-O bonds respectively [245]. From the IR spectra, it is concluded that the Schiff base behaves as a bidentate ligand coordinate to the metal ions via (-CH=N) and (>C=N) groups. The other coordination sites of metal(II) ions are occupied by two water molecules and two positions are occupied by the –OH and NH\(_2\) groups of 2-aminophenol through deprotonation.
**Electronic spectra**

The electronic absorption spectra of mixed ligand complexes are recorded at room temperature in DMF solution (Fig. 5.3.2.a-d). The absorption assignments of the complexes along with the geometry are given in Table 5.3.4. The electronic spectra of the ligand shows broad band 318 and 370 nm which can be assigned to n-π* transitions of the azomethine (-CH=N) and imine (C=N) chromophores. On complexation this is shifted to lower wavelength, suggesting the coordination of azomethine nitrogen. In addition, other intense absorption band at higher energy 220-265 nm is due to π-π* transition of the benzene ring of the Schiff base ligand.

The electronic spectrum of Co(II) complex shows two absorption bands one at ~1075 nm and another one at 345 nm assignable to $^{4}T_{1g}$ (F)$\rightarrow^{4}T_{2g}$ (F) and $^{4}T_{1g}$ (F)$\rightarrow^{4}T_{1g}$ (P) transitions respectively, which is characteristic for octahedral Co(II) complex. The electronic spectrum of the Ni(II) complex shows one absorption band at 387 nm assignable to $^{3}A_{2g}$ (F)$\rightarrow^{3}T_{1g}$ (P) transition. This is characteristic of six coordinated octahedral Ni(II) complexes. The distorted octahedral Cu(II) complex, displays one absorption band at 464 nm, corresponding to $^{2}B_{1g}\rightarrow^{2}E_{1g}$transition. The Zn(II) complex exhibits two intra ligand transitions at 287 and 335 nm and is diamagnetic. According to the empirical formula, an octahedral geometry is proposed for the Zn(II) complex [246, 172, 168].
Magnetic measurements

Magnetic susceptibility values of the complexes along with the geometry are given in Table 5.3.5. The magnetic moment value of the Co(II) complex is 5.14 BM. The magnetic moment value is 3.20 BM for the Ni(II) complex which confirms six coordinate octahedral geometry. The $\mu_{\text{eff}}$ for the Cu(II) complex is 1.91 BM, which is characteristic for distorted octahedral geometry around Cu(II) ion. The Zn(II) complex is diamagnetic and has octahedral geometry.
The $^1H$ NMR spectrum of Zn(II) complex was recorded in DMSO d6 (Fig. 5.3.3). The signal for azomethine proton (-CH=N-) in the ligand appears as a singlet at 9.6 ppm [247]. In the $^1H$ NMR spectrum of the Zn(II) complex, the azomethine proton signal is shifted downfield compared to the free ligand due to the deshielding of the azomethine group on coordination with Zn(II) ion. The multiplet present in the 7.2-7.5 ppm range is due to the aromatic protons of the ligand as expected. The signal for pyrazolone ring carbon attached methyl protons (-CH$_3$) appear as a singlet at 2.5 ppm, while pyrazolone ring nitrogen attached methyl protons (>N-CH$_3$) appear as a singlet at $\delta$ 3.3 ppm [136]. The signals at 6.5 and 6.8 ppm are due to furfuryl protons.
Mass spectra

The mass spectrum of mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) with FAAPBT and AP were recorded. The molecular ion peak for ligand \((C_{23}H_{19}N_{5}O_{5})\) is observed at 413 \(m/z\). Whereas the molecular ion peaks of Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes are observed at 617, 616, 621 and 623 \(m/z\) which confirms the stoichiometry of the metal complexes to be \([M(FAAPBT)(AP)(H_2O)_2]\). The mass spectra of ligand and its complexes exhibit other peaks for several fragments. Elemental analysis values are in good agreement with the values calculated from the molecular formulae assigned to these complexes which are further supported by DART-mass studies.
ESR spectra

ESR spectra of the Cu(II) complex was recorded at room temperature (300K) and at liquid nitrogen temperature (77K) in DMSO (Fig. 5.3.4.a & b). The ESR spectral data are given in Table 5.3.6. The $g_{||}$ and $g_{\perp}$ values were calculated from the spectrum using tetracyanoethylene (TCNE) free radical as the “g” marker as described in Sec. 2.4.7. In the present Cu(II) complex, $g_{||}$ is less than 2.3 is an indication of significant covalent character to M-L bond. The $g_{||}$ and $g_{\perp}$ values are $>2.04$, consistent with an elongated tetragonally distorted octahedral stereochemistry. If the G factor calculated using eqn 18 [Sec. 2.4.7] $>4.0$ suggests that the exchange interactions between Cu(II) centers in the solid state are negligible [248].

The absence of half field signal at 1600 G due to the $\Delta m_s = \pm 2$ transitions, ruling out any Cu-Cu interaction. The g values are in the order $g_{||} > g_{\perp} > 2.0023$ corresponding to the presence of an unpaired electron in the $d_{x^2}-d_{y^2}$ orbital. From the observed values, it is clear that $A_{||}=110 > A_{\perp}=82; g_{||}=2.13 > g_{\perp}=2.03 > 2$ and the ESR parameters of the Cu(II) complex suggest that the complex has tetragonally distorted octahedral geometry and the unpaired electron lies predominantly in the $d_{x^2}-d_{y^2}$ orbital.
Thermal analysis

The thermal stabilities of ligand and its mixed ligand metal complexes were investigated using TG and DTA under nitrogen atmosphere with a heating rate of 10 °C per minute from 40 °C to 700 °C. The Co(II) and Zn(II) complexes show four stages of decomposition. The Ni(II) and Cu(II) complexes complex exhibits three stages of decomposition. Thermograms of Co(II), Ni(II), Cu(II) and Zn(II) complexes show weight loss around 151-223 °C indicates the presence of two coordinated water molecules in these complexes [249]. The other decomposition stages correspond to the loss of 2-aminophenol and decomposition of organic moieties of the Schiff base ligand. The initial decomposition temperature of Co(II) complex is higher compared to the Ni(II), Cu(II) and Zn(II) complexes, indicating it is more thermally stable. The Zn(II) complex shows the final decomposition at 457 °C, which is the higher final decomposition temperature compared to the Co(II), Ni(II) and Cu(II) complexes.

Based on the elemental analysis, IR, electronic, $^1$H NMR, mass, ESR spectral data, magnetic moment and thermal analysis, the proposed structure of the complexes is given in Fig. 5.3.5.
Cyclic voltammetry

The cyclic voltammograms of the mixed ligand complexes were recorded at room temperature in DMSO solution in the potential range -1.0 to 1.2 V with scan rate 0.1 V/s.

The cyclic voltammogram of the Co(II) complex shows an irreversible cathodic peak at 0.5 V. The present Ni(II) mixed ligand complex is electrochemically inert and does not show any peak in the cyclic voltammogram. The Cu(II) complex displays a cathodic peak at 0.25 V versus Ag/AgCl with the corresponding anodic peak at 0.45 V on the reverse scan. The peak separation value (ΔEp = 0.20 V) indicates quasi-reversible character for the one electron transfer reaction of metal-based Cu(II)/Cu(I) couple. The Zn(II) complex shows an irreversible [233] anodic peak at 0.65 V.
**Powder XRD**

The powder XRD patterns of the mixed ligand complexes were recorded in the $2\theta = 0-80$ A range and are shown in Fig. 5.3.7.a-d. From the observed $d_{XRD}$ patterns, the crystallite size of the complexes was calculated from Scherrer’s formula described in Sec. 2.4.9 [250]. In the present study, Co(II), Ni(II), Cu(II) and Zn(II) complexes do not show any peaks in the diffractogram, indicating that they are amorphous in nature.
The SEM micrographs of Co(II) complex was recorded at an energy of 20 KV with magnification 5000 X. The SEM micrographs of Ni(II), Cu(II) and Zn(II) complexes were recorded at an energy of 20 Kv with magnifications 1000 X, 2500 X and 2400 X respectively. The SEM micrographs of the Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes are shown in Fig. 5.3.8.a-d. The SEM micrograph of Co(II) complex exhibits spherical structured particles with small grains. Ni(II) complex exhibits irregularly shaped particles with small grains. The Cu(II) and Zn(II) complexes show cauli flower like morphology.
5.3.1 Biological studies

Antimicrobial analysis

The *in vitro* biological screening of ligand and its mixed ligand metal complexes were tested against bacterial species *S. aureus*, *E. coli* and *P. aeruginosa* and fungal species *A. niger*, *A. flavus* and *C. albicans* by agar well diffusion method as described in Sec. 3.1.1 and 3.1.2. The results of the antibacterial and antifungal activities are summarized in Table 5.3.1.1 & 5.3.1.2. The standards used are Gentamycin for antibacterial and Amphotericin for antifungal activities. The present study reveals that the complexes possess higher growth inhibition potential. Among the mixed ligand metal(II) complexes, Cu(II) complex exhibits higher antimicrobial activity. The antimicrobial activity of the metal complexes increases with increase in concentration of the complexes. Increased activity of the metal complexes can be explained based on the Overtone’s concept and Tweedy’s chelation theory [225].
**DNA binding studies**

The DNA binding of the complexes were carried out by the method described in Sec. 3.2. UV absorption spectroscopy is an effective method to examine the binding mode of DNA with compounds. Hyperchromic effect and hypochromic effect are the spectral features of DNA concerning its double helical structure. The spectral changes will give clue about changes in DNA in its conformation and structure after the drug bind to DNA [251]. Here the Co(II) complex exhibits hypochromism (17 %) in the absorption band at 380 nm and bathochromism of 16 nm. The absorption band of Ni(II) complex at 312 nm exhibits hypochromism of 15 % and bathochromism of 3 nm. The absorption band of Cu(II) complex at 315 nm exhibits hypochromism of 12 % and bathochromism of 7 nm. The Zn(II) complex at 284 nm exhibits hypochromism of 14 % and bathochromism of 2 nm. These results (Table 5.3.1.3) suggest that the mixed ligand complexes are interacting with DNA through intercalation. The intrinsic binding constant ($K_b$) values calculated are found to be as $1.1 \times 10^5$, $1.3 \times 10^5$, $2.2 \times 10^5$ and $1.6 \times 10^5$ M$^{-1}$ for the Co(II), Ni(II), Cu(II) and Zn(II) mixed ligand complexes respectively.
**DNA cleavage studies**

The DNA cleavage of the metal complexes was done according to the procedure described in Sec. 3.3. DNA cleavage is controlled by relaxation of supercoiled circular form of pUC18DNA into nicked circular form and linear form. When circular plasmid DNA has been subjected to electrophoresis, the fastest migration is observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between [67]. The cleavage efficiency (Fig. 5.3.1.1) of the mixed ligand complexes has been measured by determining the ability of the complex to convert the supercoiled DNA into nicked open circular form or linear form. Control experiments using DNA alone does not show any significant cleavage of pUC18 DNA. The Cu(II) complex completely cleaves the DNA. The Co(II), Ni(II) and Zn(II) complexes exhibit enhanced cleavage activity than ligand in the presence of H₂O₂. The order of activity of the mixed ligand complexes is given as Cu(II) > Co(II)>Zn(II) > Ni(II).
**SOD Activity**

The SOD mimetic activities of the mixed ligand Co(II), Ni(II), Cu(II) and Zn(II) complexes were examined by the NBT assay as described in Sec. 3.4. It has been established that the reaction of SOD and synthetic metal systems that have SOD-like activity involves a two-step reaction [252].

\[
M_{\text{ox}} + O_2^- \rightarrow M_{\text{red}} + O_2
\]

\[
M_{\text{red}} + O_2^- + 2H^+ \rightarrow M_{\text{ox}} + H_2O_2
\]

The observed IC\(_{50}\) values of present complexes are compared with various reported complexes. All the complexes have moderate (Table 5.3.1.4) activity and among them Cu(II) complex has higher activity. The complexes show (Fig. 5.3.1.2) the following order of superoxide radical scavenging activities; Cu(II) > Zn(II) > Ni(II) > Co(II).
Cytotoxicity assay

Cytotoxicity assay of the complexes are described in Sec. 3.5. The *E. coli* AB 1157, proficient to repair damage in the DNA is considered for cytotoxicity assay. Stannous chloride is taken as standard compound. The results (Table 5.3.1.5) show that the Co(II) and Ni(II) mixed ligand complexes are more toxic than the Cu(II) and Zn(II) complexes. In the present study the Cu(II) complex does not show any cytotoxicity against *E.coli* AB 1157, whereas Zn(II) complex exhibits moderate cytotoxicity.
In vitro anticancer studies

Anticancer activity of newly synthesized mixed ligand metal complexes was investigated on human cervical carcinoma (HeLa) cells by MTT assay as given in Sec. 3.7.3. The results were expressed in terms of IC_{50} values. The complexes were applied in the concentration range 0.1-100 µM. Table 5.3.1.6 illustrates the IC_{50} values for the complexes tested. The data obtained in the MTT assay show that the Cu(II) mixed ligand complex has very good inhibitory (0.8 µM) effect on the growth of human cervical carcinoma (HeLa) cells. The Zn(II) complex possesses moderate (68.81 µM) growth inhibition activity (Fig. 5.3.1.3.a-d). The Co(II) complex has the lowest growth inhibitory activity with IC_{50} value 462.8 µM. The Ni(II) complex also has lowest growth inhibitory activity against HeLa cells with IC_{50} value 264.2 µM.
5.3.2 Molecular docking

Cavities were detected and depending on this, particular constraint was created within which docking takes place. Docking was performed with default settings to obtain a population of possible confirmations and orientations for the ligands at the bonding sites. The structural analysis of docked structures gives significant details about the binding pattern of these complexes.

According to the docking studies, the best score is obtained for the Cu(II) complex. The results show that the Cu(II) complex has the ability to act as anticancer agent. It is predicted from docking studies that Cu(II) complex adopt an acceptable confirmation with in the active site of PDB ID1jxa and significant binding interactions have well been noticed from the Fig. 5.3.2.1.a-d. There is correlation between the theoretical and experimental values obtained. Molecular docking of the complex formed between the ligand and the protein indicates that the ligand is well positioned in the gorge. The docking results in energy minimized docked structures. The residues that have been reported to be involved in protein-ligand interactions are described in Table 5.3.2.1. The number of hydrogen bonds formed is found to be maximum (3) in the Cu(II) complex. For the Co(II), Ni(II) and Zn(II) complexes, equal number (2) of hydrogen bonds are present. The docking score is same for Co(II) and Zn(II) complexes. The lowest score is obtained in the case of Ni(II) complexes. The results of docking are in accordance with the antimicrobial results.