Characterization techniques and experimental studies of macrocyclic compounds
2.1 CHARACTERIZATION TECHNIQUES AND EXPERIMENTAL STUDIES

In recent years, immense development in the field of coordination chemistry has taken place due to the availability of powerful analytical techniques. The spectral techniques provide substantial information regarding the structure of macrocyclic ligand and their complexes. The structural characterizations and morphology of the synthesized macrocyclic ligands and their complexes were performed by FT-IR, NMR, Mass, ESR, UV-vis spectroscopy, TGA/DTA, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). The procedure for evaluation with short account of the instruments used is discussed as follows:

2.1.1 Fourier Transform Infrared (FT-IR) spectroscopy

The infrared spectroscopy is a basic and potent tool in structural analysis of a compound which results from transition between vibrational and rotational energy levels because various functional groups absorb the characteristic frequencies of IR radiations. The IR region of the electromagnetic spectrum exhibit a wide spectrum of wavelength from 200-4000 cm$^{-1}$ or the region between 0.78 and 1000 µm. It is categorized into three regions near, mid and far IR, with wave numbers in the electromagnetic spectrum 14,000-4,000 cm$^{-1}$ (wavelength ranges from 0.78 to 2.5 µm), 4,000-200 cm$^{-1}$ (wavelength ranges from 2.5 to 50 µm) and 200-10 cm$^{-1}$ (wavelength ranges from 50 to 1,000 µm), respectively. The IR absorption bands are generally represented in the form of spectrum containing wavelengths (or wave numbers) on the x-axis and absorption intensity (or percent transmittance) along the y-axis.

Transmittance (T) is defined as the ratio of radiant power transmitted by the sample (I) to the radiant power incident on the sample ($I_0$).\(^2\) Absorbance (A) is the logarithm to the base 10 of the reciprocal of the transmittance (T).

$$A = \log_{10} \left( \frac{1}{T} \right) = -\log_{10} T = -\log_{10} \left( \frac{I}{I_0} \right)$$  \hspace{1cm} (2.1)

In infrared spectroscopy, wavelength is measured in terms of “wavenumbers” having unit as cm$^{-1}$.

$$Wave \ number = \frac{1}{wavelength \ (cm)}$$ \hspace{1cm} (2.2)

**Basic principle**

When infrared light is passed through a sample, some frequencies are transmitted through it while some of the frequencies are absorbed. The absorption of IR radiations is limited to the compounds having slight energy differences in the possible
vibrational and rotational states.

For the absorption of IR radiation, the vibrations or rotations within a molecule must lead to net change in the dipole moment. Thus, the fluctuations in the dipole moment of the molecule interact with alternating electrical field of the radiation. In the absorption of the radiation, only transitions for which change in the vibrational energy level ($\Delta \nu = 1$), can occur as most of the transition will occur from stable $\nu_0$ to $\nu_1$ and the frequency corresponding to its energy is known as the fundamental frequency. Figure 2.1 shows various vibrational modes (stretching and bending) exhibited by a molecule.

- Bending frequencies are lower than the corresponding stretching frequencies.
- Bonds to heavier atoms than hydrogen have lower stretching frequencies.
- Single bonds (except for bonds to hydrogen) have lower stretching frequencies compared to the corresponding double bonds which further have lower frequencies than triple bonds.
2.1.2 Nuclear magnetic resonance spectroscopy
In our work Bruker Avance II 400 NMR spectrometer has been used (Figure 2.2). This technique is one of the most frequently used techniques to interpret the structure of chemical species.

![Figure 2.2 NMR Spectrometer](image)

The NMR spectroscopy is based on the measurement of electromagnetic radiation in the radio-frequency region (~ 4 to 900 MHz). In case of NMR study, the nuclei of atoms are involved in the absorption process in contrast to infrared, ultraviolet and visible absorption studies.

It is possible to induce the transitions between oriented nuclei by irradiating the processing nuclei (protons) with radiowaves (electromagnetic radiations) of suitable frequency. Subsequently, the absorption occurs and the nuclei in lower energy spin state flip to the high energy state. The nuclei are said to be in resonance with the applied radiation when this spin transition occurs and thus this resonance is due to the magnetic behavior of the nuclei, it is called Nuclear Magnetic Resonance. In general, there are two methods to scan an NMR spectrum.

First, by keeping the magnetic field ($H_0$) constant and changing the frequency of oscillator i.e., Pulsed or Fourier Transform (FT-NMR) which can record the
spectrum within the time period of 5 seconds. However in the second method, the frequency of oscillator is kept constant while the magnetic field is varied i.e., Continuous-Wave (CW) which may takes 2-5 minutes to scan the spectrum.

In both the methods, the sample is placed in a powerful magnetic field. All the nuclei are charged particles, and this charge spin on the nuclear axis due to which a magnetic dipole is developed along the axis. Similarly, the protons and neutrons which constitute nuclei of atoms also spin about their axis and each of them possesses angular momentum. Like electron, each proton and neutron have a spin quantum number of 1/2 and therefore the nucleus will have a resultant nuclear spin, if the protons and neutrons are not paired. The numerical value of this nuclear spin quantum number, I, depends upon number of protons and neutrons with parallel and anti-parallel spins. The dependency of ‘I’ on mass number and atomic number of the nuclei is represented as:

<table>
<thead>
<tr>
<th>Atomic mass</th>
<th>Atomic number</th>
<th>Nuclear spin quantum number, I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Odd</td>
<td>Odd or even</td>
<td>1/2, 3/2, 5/2, --- etc.</td>
</tr>
<tr>
<td>Even</td>
<td>Odd</td>
<td>1, 2, 3, --- etc.</td>
</tr>
<tr>
<td>Even</td>
<td>Even</td>
<td>0</td>
</tr>
</tbody>
</table>

In agreement with the above general rules:

- The nuclei of $^{32}$S$_{16}$, $^{16}$O$_8$, $^{12}$C$_6$, and $^4$He$_2$ have I = 0, and therefore give no NMR signals.
- The nuclei such as $^1$H$_1$, $^3$H$_1$, $^{13}$C$_6$, $^{15}$N$_7$, $^{19}$F$_9$, $^{31}$P$_{15}$ have I = 1/2; $^{11}$B$_5$, $^{23}$Na$_{11}$, $^{35}$Cl$_{17}$, $^{79}$Br$_{35}$, $^{81}$Br$_{35}$ etc. have I = 3/2 and $^{17}$O$_8$ has I = 5/2, show NMR signals.
- The nuclei such as $^2$H$_1$ and $^{14}$N$_7$ have I = 1 while $^{10}$B$_5$ has I = 3, show NMR signals.

Since the atomic nuclei are associated with charge therefore, a spinning nucleus generates a small current and has a finite magnetic field associated with it. The magnetic dipole of the nucleus fluctuates with each element. When a spinning nucleus is placed in an external magnetic field, it will experience a torque (force perpendicular to the axis of the nuclear magnet) which will tend to align with the external field. For a nucleus with a spin of 1/2 there are two possible orientations, i.e., parallel to the field (low energy) and opposite to the field (high energy). The parallel orientation being lower in energy is slightly more populated than the anti-parallel
orientation. The $\Delta E$ is designated as the difference in energy between the two spin states.

The exact amount of electromagnetic radiation necessary for resonance depends on both the strength of the external magnetic field and on the characteristics of the nucleus being examined.

$$\Delta E = h\nu = \frac{\hbar}{2\pi} H_o$$  

----- (2.3)

where, $\Delta E$ denotes the energy difference between the two spin states. $H = \text{Planck’s constant}$ and $\gamma = \text{gyromagnetic ratio}$ having the value of $26,750$.

The nucleus of the proton placed at field strength of $14,100 \text{ G}$ or $1.41 \text{ T}$, undergoes resonance when irradiated with radiation in the $60 \text{ MHz}$ (Radio-wave region). Higher magnetic fields such as in case of superconducting magnets, require high energy radiation and correspondingly results in high resolution. Nowadays, FT (Fourier Transform) instruments are commonly used to observe the resonance of nuclei of several different elements in a single instrument (up to $800 \text{ MHz}$).

**$^{13}$C NMR spectroscopy**

$^{13}$C NMR spectroscopy can be used to complement the previously explained hydrogen NMR information. $^{13}$C NMR is a crucial tool in chemical structure elucidation in organic chemistry, analogous to $^1$H NMR. In case of $^{13}$C NMR, no multiplicity of the signals is observed due to the low natural isotopic abundance of $^{13}$C (only $1.1\%$) and therefore it is very challenging to find two $^{13}$C linked together inside the carbon backbone of the molecule which results in the absence of coupling.

The information about the identification of different type of carbon atoms present in the molecule of an organic compound can be obtained using $^{13}$C NMR spectrum. This technique also helps to identify any functional group present along with giving indications towards the solution of the structure.

The primary difference between $^{13}$C NMR and $^1$H NMR spectra are:

- Broad range of resonance (0-200 ppm) for common carbon atoms (typical range for protons 1-10 ppm).
- No integration of carbon spectra.

Another significant difference between $^1$H and $^{13}$C NMR spectroscopy is that diamagnetic effects are dominant in the shielding of hydrogen nucleus, whereas paramagnetic effects are the dominant contributions to the shielding of the $^{13}$C nucleus. $^{13}$C NMR spectrum comprises of discrete, sharp lines corresponding to each
non-equivalent carbon atom. These resonances are usually in the wide range 0-200 ppm with the TMS reference peak. The coupling constants for $^{13}$C-$^1$H are large (100-250 Hz) and thus interpretation of the $^{13}$C spectra can be problematic because of the overlapping $^{13}$C-$^1$H multiplets. $^{13}$C NMR spectra are recorded under double resonance conditions to simplify the spectrum, where the coupling of $^1$H to $^{13}$C is destroyed. Complete $^1$H coupling is accomplished by irradiating the $^1$H to resonance region with a broad band width radio frequency radiation, termed “noise”, sufficient to cover the entire $^1$H resonance region. The $^{13}$C NMR spectrum thus obtained contains only singlet resonances corresponding to its chemical shifts.

In NMR spectroscopy, the chemical shift of a nucleus, is the difference between the resonance frequency of the nucleus relative to a standard molecule generally taken as tetramethyilsilane (TMS, Si(CH$_3$)$_4$). Chemical shift ($\delta$) is measured in (ppm) and can be calculated using following equation.

$$\delta = (\nu - \nu_{ref}) \times 10^6/\nu_{ref}$$ -----(2.4)

where $\nu$ and $\nu_{ref}$ are the resonance frequencies of a sample nucleus and the nuclei of TMS.

2.1.3 Mass spectrometry (MS)

The mass spectra were obtained using WATERS Q-TOF premier mass spectrometer. MS is one of the most accurate analytical techniques applied to pure samples as well as complex mixtures. During MS, the effect of source of ionizing energy over the sample molecule is observed. This technique ionizes the chemical species (solid, liquid or gas) on bombardment with an electron beam having energy to sorts the ions based on their mass-to-charge ratio (m/z). Since the bulk of the ions which are produced in the mass spectrometer, have a unit positive charge, the value of m/z corresponds to the fragment molecular weight. The steps involved in its assessment are given in Figure 2.3.
Figure 2.3

The mass spectrometer displays the plot of relative intensity vs. the mass to charge ratio (m/z). The most intense peak in the spectrum is called base peak and assigned a value of 100%. The other peaks are reported as percentage of the base peak. The progression of fragmentation follows the simple chemical pathways and the ions that are formed, will reflect the most stable cations and radical cations, which that molecule can form. Many molecules with especially labile protons do not show molecular ions, e.g., alcohols. The identification of fragments can be done by their mass-to-charge ratio. The highest molecular weight peak observed in a spectrum will typically represent the parent molecule, minus an electron, and is termed the molecular ion peak (M\(^+\)). Generally, small peaks are also observed above the calculated molecular weight due to the natural isotopic abundance of \(^{13}\)C, \(^2\)H, \(^{15}\)N etc. Many molecules with especially labile protons do not display molecular ions, e.g. in alcohols, the highest molecular weight peak occurs at m/z one less than the molecular ion (M-1). Fragments can be identified by their mass-to-charge ratio, but it is often more informative to identify them by the mass which has been lost, i.e., loss of a methyl group will generate a peak at M-15; loss of an ethyl, M-29, etc.

Mass spectrometry finds various applications in organic chemistry, including:
- Determination molecular mass.
- Elucidation of the structure of an unknown substance.
- Verify the identity and purity of a known substance.
- Provide data on isotopic abundance.

2.1.4 Ultraviolet-visible (UV-vis) spectroscopy

**Figure 2.4 UV-vis Spectrophotometer**

In our work Pye-Unican 8800 spectrophotometer has been used (Figure 2.4). UV-vis spectroscopy has been used to analyze various metal complexes having d-d transition.\(^5\) When a molecule absorbs radiation, its energy increases which is equal to the energy of the incident photon expressed by the relation

\[
E = h\nu = \frac{hc}{\lambda}
\]

----- (2.5)

where \(h\) and \(\nu\) are Planck's constant and frequency, respectively. \(\lambda\) is the radiation’s wavelength and \(c\) is the light velocity.

Usually, the compounds absorb light in the spectral range of 200-900 nm (where the wavelength of 200-340 nm are referred as ultraviolet region and from 340-900 nm is the visible region), resulting in the excitation of electrons of the molecules from ground state to higher electronic states. In transition metals, all the five ‘d’ orbitals (\(d_{xy}, d_{yz}, d_{xz}, d_z^2\) and \(d_{x^2-y^2}\)) are degenerate, however, in coordination
compounds due to the presence of ligands, the degeneracy of d orbitals is destroyed and split into two i.e., $t_{2g}$ ($d_{xy}$, $d_{yz}$ and $d_{xz}$) and $e_{g}$ ($d_{z^2}$ and $d_{x^2-y^2}$) in case of an octahedral complex and $t$ and $e$ in a tetrahedral complex. During octahedral complexes, the set of $t_{2g}$ orbitals goes below the original level of degenerate orbitals while this case is reversed during tetrahedral complexes [Figure 2.5 (a and b)].

![Diagram](image)

**Figure 2.5** (a) Ligand field splitting of ‘d’ energy levels in an octahedral complex. (b) Ligand field splitting of a tetrahedral complex, ‘g’ subscript is omitted in $T_d$ symmetry.

One or more intense bands are commonly observed at energy higher than the ligand field absorption bands, which go off scale unless log $\varepsilon$ is plotted. These are referred to as charge transfer bands which correspond to electron transfer processes.
that might be either metal→ligand (M→L) or ligand→metal (L→M).

The two laws that govern the absorption of light by molecules are Beer’s Law that relates the absorption to the concentration of absorbing solute and the Lambert’s Law that relates the total absorption to the optical path length. Thus, the Beer-Lambert law is expressed by the following equation:

\[ \log \left( \frac{I_0}{I} \right) = \varepsilon c l \quad \text{or} \quad \varepsilon = \frac{A}{c l} \]  

----- (2.6)

where, \( I_0 \) is the incident light intensity (or the intensity passing through a reference cell), \( I \) is the light transmitted through the sample solution, \( \log (I_0/I) \) is the absorbance (A) of the solution (formerly called the optical density, OD), \( c \) is the concentration of solute (in mol L\(^{-1}\) or mol dm\(^3\)), \( l \) is the sample path length (in cm or m \( \times 10^2 \)) and \( \varepsilon \) is the molar absorptivity (formerly called the molecular extinction coefficient, in L mol\(^{-1}\) cm\(^{-1}\), m\(^2\) mol\(^{-1}\) \( \times 10^2 \)).

**Instrumentation detail**

The basic components of a UV-vis spectrophotometer are the light source, holder, diffraction grating in a monochromator or a prism to separate the different wavelengths of light, and a detector. The tungsten filament serve as a radiation source, a deuterium arc lamp, which is continuous over the ultraviolet region (190-400 nm), continuous Xenon arc lamp, light emitting diodes (LED) for the visible wavelengths. The detector is a photomultiplier tube, a photodiode, a photodiode array or a charge-couple device (CCD). Single photodiode detectors and photomultiplier tubes are embedded with scanning monochromator that filter the light so that only light of a single wavelength can reach the detector at a time. There are two types of spectrophotometer, a single beam or a double beam. In a single beam, \( I_0 \) is calculated by removing the sample, whereas in the double beam instrument, the light split into two beams before it reaches the sample where one beam is used as the reference while the other beam passes through the sample. The reference beam intensity is taken as 100% transmission (or zero absorbance), and the measurement displayed is in the ratio of the two beam intensities. Samples for UV-vis spectrophotometry was used as liquids placed in cuvette (made of fused silica or quartz glass), although the absorbance of gases and even of solids can also be done.

**2.1.5 Electron spin resonance (ESR) spectroscopy**

In the present thesis work, ESR spectra of complexes (solid) were quantified at room temperature on ES-DVT4 spectrometer (Figure 2.6).
ESR spectroscopy involves the absorption of microwave frequency by an unpaired electron when it is exposed to a strong magnetic field.\(^5\)

The plot between the absorbed energy and the magnetic field is called the electron paramagnetic resonance spectrum. This method is operated for the structural analysis of the molecular systems or ions with unpaired electrons such as NO\(_2\), NO etc., species such as methyl and diphenylpicryl hydrazyl free radicals etc., odd-electron molecules, rare earth ions, transition metal complexes etc. having spin-degenerate ground states in the absence of magnetic field.\(^6,7\) Hence, this method is employed to understand the symmetry of surroundings of the paramagnetic ion and the nature of its bonding to the nearest neighboring ligands. For an electron of spin \(S = 1/2\), the spin angular momentum quantum number will have values of \(m_s = \pm 1/2\). In absence of magnetic field, the two values of \(m_s\) i.e. +1/2 and -1/2 will give rise to a doubly degenerate spin energy state. However in presence of static magnetic field, this degeneracy is lifted due to the Zeeman splitting of the levels leading to the non-degenerate energy levels. Application of an oscillating magnetic field of appropriate frequency will induce transitions between the Zeemen levels and the energy is absorbed from the electromagnetic field. If the static magnetic field is slowly varied, the absorption displays a series of maxima. The low energy level will have the spin magnetic moment aligned with the field and correspond to the quantum number \(m_s = -1/2\).
1/2 whereas the high energy state will have the spin magnetic moment opposed to the field and correspond to the quantum number \( m_s = +1/2 \). The energy, \( E \) of the transition is given by the following equation:

\[
E = h\nu = \frac{2}{2.7} \beta B_0
\]

where \( h \) is Plank’s constant, \( \nu \) is the frequency of radiation, \( \beta \) is the Bohr magneton, \( B_0 \) is the magnetic field strength and \( g \) is the spectroscopic splitting factor (gyromagnetic ratio). The magnitude of \( g \) also depends on the orientation of the molecules having the unpaired electron with respect to the applied magnetic field. In case of perfect cubic symmetry, the \( g \) value does not depend on the orientation of the crystal and is said to be isotropic and if the value of \( g \) depends on the orientation of the crystal then it is said to be anisotropic.

The energy levels splitting in ESR occurs under the effect the internal crystalline field and applied magnetic field. While studying a paramagnetic ion in a diamagnetic crystal lattice, there are two types of interactions i.e., dipolar interaction (interactions between the paramagnetic ions) and crystal field interaction (interactions between the paramagnetic ion and the diamagnetic neighbors). The dipolar interaction will be negligible for small doping of paramagnetic ion in the diamagnetic host. The crystal field interaction of paramagnetic ion with diamagnetic ligands alters the magnetic properties of the paramagnetic ions. According to crystal field theory, the ligands influence the magnetic ion through the electric field which they produce and modify orbital motion. The electrostatic screening by the outer electronic shells affects the crystal field interaction. The influence of magnetic field of one paramagnetic ion on the dipole moments of the similar neighbouring ions gives rise to the dipole-dipole interaction. The arrangements of the neighbour and the direction of their dipole moments influence the local field at any given site. Therefore, the resultant magnetic field (which varies from site to site giving a random displacement of the resonance frequency of each ions and thus broadening the line widths) on the paramagnetic ion will be the vector sum of the external field and the local field.

Hyperfine interactions are mainly magnetic dipole interactions between the electronic magnetic moment and the nuclear magnetic moment of the paramagnetic ion. The quartet structure in the ESR of Cu(II) ion and octet in the ESR of vanadyl ion are the results of the hyperfine interactions. An additional hyperfine structure may also be observed due to the interaction between magnetic electrons and the surrounding nuclei which is defined as super hyperfine structure. This effect was first
observed in ammonium hexachloroiridate and subsequently for a number of transition metal ions in several hosts.  

2.1.6 Thermogravimetric analysis (TGA)/Differential thermal analysis (DTA)

In the thermogravimetric analysis, the thermal stability of the sample is evaluated as a function of time or temperature. The plot of mass or mass percent as a function of time is known as thermogram or thermal decomposition curve. In the present work, the thermal stability of the samples was evaluated by TGA/DTA studies (Shimadzu system-DTG-60H Japan) (Figure 2.7). TGA consist of a sample pan supported by a precision balance and placed in a furnace which is heated during the experiment. The various components of the sample are decomposed and the weight percentage of resulting mass change can be recorded as the temperature increases. So, during thermal dissociation, the weight of any volatile ligand expelled is measured and the empirical formula of the product may usually be deduced. The mass of the sample is observed during the experiment. To restrict undesired reactions, the atmosphere in the chamber is purged with a gas that flows over the sample and exits through an exhaust. DTA is a technique used for quantitative analysis and identification of the chemical composition of substances by observing their thermal
behavior upon heating. It measures the temperature difference between the complex and a reference material as a function of temperature. The endothermic and exothermic behavior of complexes at high temperature can be obtained using DTA.\textsuperscript{11} The differential temperature is then plotted against time or temperature is called DTA curve or thermogram.

2.1.7 *Scanning electron microscopy (SEM)*

![Figure 2.8 Scanning Electron Microscope](image)

In this work, SEM images were recorded using Scanning Electron Microscope (JEOL-JAPAN) at different magnifications, equipped with an energy dispersive X-Ray spectrocope (EDX) for elemental analysis and bulk composition with an operational voltage of 15-20 KV (*Figure 2.8*). Scanning Electron Microscopy is used for the surface morphology analysis and to evaluate average crystallite size of the samples.\textsuperscript{12} It furnishes information about various morphologies such as needle like, rod like or quasi-spherical, irregular broken ice rock, lamellar, inter-granular and the intra-granular pores along with the sub-structural defects within the grains, which may arise because of the absence of homogeneity and stoichiometry.

**Basic principle**

The atomic measurements made using SEM is based on the principle of irradiating the specimen with a finely focused electron beam. This focused energetic electron beam
scan the surface of the specimen and results in the transfer of energy to the spot focused. These injected bombarding primary electrons remove the electrons from the specimen itself which are collected by a positively biased grid or detector, where it is translated in form of signal. This is further converted into image after signal amplification and system analysis, and finally displayed on the screen.

### 2.1.8 Transmission Electron Microscopy (TEM)

![Transmission Electron Microscope](image)

**Figure 2.9 Transmission Electron Microscope**

TEM is one of the most widely used techniques where a beam of electrons is transmitted through a sample to form an image. This technique provides information about the morphology, crystal structure, size and shape of compounds on a very fine scale. In the present work, the morphology of the bacteria treated with synthesized compounds was evaluated by transmission electron microscopy, (TEM, Hitachi H-7500 Japan) set at an accelerating voltage of 120 KV (**Figure 2.9**). Transmission electron microscopes have advantage of imaging at a significantly high resolution than light microscopes because of having small de Broglie wavelength of electrons.\(^\text{13}\)

**Basic principle**

It works on the principle of the optical (light) microscope but uses electrons instead of light. Therefore, electromagnetic (EM) lenses is used that work by utilizing the Lorentz force; as magnetic field is altered, this will tune the focus on the lens and the
resulting image. Electrons are sent from an electron gun comprises of tungsten filament at different accelerating voltages. The transmission of electron beam is highly dependent on the properties (density, composition etc.) of material being examined. The electrons are passed through a condenser lens and then objective lens to change the beam before coming in contact with the sample. The sample is placed between two objective lenses and the first image is created and projected onto a fluorescent screen. The quality of the image is correlated to the electron wavelength which is further dependent on electron speed.

The sample used for TEM study must be of low density and should be re-sized before viewing under TEM that can be done using variety of methods such as mechanical thinning, electrochemical thinning, ion milling etc.

2.1.9 Magnetic susceptibility measurements

Over the years, there have been a number of techniques used to evaluate magnetic susceptibilities of transition metal complexes that provide extensive information in assigning their structures. These include the NMR method, the Gouy method, and the Faraday method. The magnetic moment resulting from the motion of electrons contributes to the bulk magnetic properties and magnetic susceptibility values help in the determination of the magnetic moments. Several kinds of magnetic phenomenon such as diamagnetism, paramagnetism and antiferromagnetism or ferromagnetism are observed in chemical substances. In general, the transition element compounds are paramagnetic while the diamagnetism is associated with substances having closed shell electrons in an applied magnetic field where the electron spin moment and orbital moment of the individual electrons balance one another so that there is no magnetic moment. The interaction between dipoles of neighboring atoms gives rise to ferromagnetism and antiferromagnetism.

If a substance is placed in a magnetic field, $H$, then the magnetic induction, $B$ with the substance is given by:

$$ B = H + 4\pi I $$ ---- (2.8)

where, $I$ is the magnetization intensity. The $B/H$ ratio is called magnetic permeability of the material and is given by:

$$ B/H = I + 4\pi (I/H) = I + 4\pi K $$ ---- (2.9)

where $K$ is called the magnetic susceptibility per unit volume or volume susceptibility. $B/H$ is the ratio of density of line of force within the substance to the
density of such lines in the same region in the absence of sample. Therefore, the volume susceptibility in vacuum is zero \([B/H = 1]\).

The effective magnetic moment \((\mu_{\text{eff}})\) for a particular substance can be calculated from the gram magnetic susceptibility using the following equation:

\[
\mu_{\text{eff}} = 2.48 \left(\chi_{M}^{\text{corr}}(T)\right)^{1/2} \text{B.M.}
\]

where, \(T\) is the absolute temperature at which the experiment is performed. The magnetic properties of any individual atom or ion will result from some combination of two properties that is the inherent spin moment of the electron and the orbital moment resulting from the motion of the electron around the nucleus. The magnetic moments are usually expressed in Bohr Magnetons (B.M.). The magnetic moment of a single electron is given by:

\[
\mu_s = g\sqrt{S(S + 1)} \text{B.M.}
\]

where \(S\) is the total spin quantum number arising from unpaired electrons and \(g\) is the gyromagnetic ratio. For Mn\(^{2+}\), Fe\(^{3+}\) and other ions with \(S\) as ground state, there is no orbital angular momentum. However, the transition metal ions in their ground state \(D\) or \(F\) being most common possess orbital angular momentum. For such ions, as Co\(^{2+}\) and Ni\(^{2+}\), the magnetic moment is given by:

\[
\mu_{(S+L)} = \sqrt{4S(S + 1) + L(L + 1)} \text{B.M.}
\]

In which \(L\) represents the total orbital angular momentum quantum number for the ions.

The spin magnetic moment is insensitive to the environment of metal ion but the orbital magnetic moment is not. For an electron, in order to have an orbital angular momentum and thereby an orbital magnetic moment with reference to a given axis, it must be possible to transform the orbital into a fully equivalent orbital by rotation about that axis. For octahedral complexes the orbital angular momentum is absent for \(A_{1g}\), \(A_{2g}\) and \(E_g\) term, but can be present for \(T_{1g}\) and \(T_{2g}\) terms. Magnetic moments of the complex ions with \(A_{2g}\) and \(E_g\) ground terms may depart from the spin-only value by a small amount. The magnetic moments of the complexes possessing \(T\) ground terms usually differ from the high spin value and vary with temperature. The magnetic moments of the complexes having \(6A_{1g}\) ground term are very close to the spin-only value and are independent of the temperature.

For octahedral and tetrahedral complexes in which spin-orbit coupling causes a split in the ground state, an orbital moment contributions is expected. Even no
splitting of the ground state appears in cases having no orbital moment contribution; an interaction with higher states can appear due to spin-orbit coupling giving an orbital moment contribution.

When magnetic susceptibility is considered on the weight basis, the term gram susceptibility ($\chi_g$) is used. The gram susceptibility is measured by the following formula:

$$\chi_g = \frac{\Delta W}{W} \cdot \frac{W_{\text{std}}}{\Delta W_{\text{std}}} \chi_{\text{std}} \quad \text{----- (2.13)}$$

where $\chi_g = \text{Gram Susceptibility}$, $\Delta W = \text{Change in weight of the unknown sample with magnet on and off}$, $W = \text{Weight of the unknown sample}$, $\Delta W_{\text{std}} = \text{Change in weight of standard sample with magnets on and off}$, $W_{\text{std}} = \text{Weight of standard sample}$ and $\chi_{\text{std}} = \text{Gram susceptibility of the standard sample}$. The effective magnetic moment ($\mu_{\text{eff}}$) can be calculated from the gram magnetic susceptibility for a particular substance using the following equation:

$$\mu_{\text{eff}} = 2.48 \left[ \chi_M^{\text{corr}}(T) \right]^{1/2} \quad \text{----- (2.14)}$$

where $T$ is the absolute temperature at which the experiment is performed.

### 2.1.10 Elemental analysis

In this process, the sample is analyzed for the stoichiometric composition of the ligand as well its metal complexes. Carbon, hydrogen and nitrogen (C, H and N) analyses were performed on a Perkin Elmer elemental analyzer. Chlorine was estimated using conventional method\textsuperscript{18} where a known amount of the sample was decomposed in a platinum crucible and dissolved in water in concentrated nitric acid. The solution was then treated with AgNO$_3$ solution. The precipitate so obtained was dried and weighed. During metal estimation,\textsuperscript{19} a known amount of complex was decomposed with mixture of HNO$_3$, HClO$_4$ and H$_2$SO$_4$ acids and dissolved in water and made upto known volume so as to titrate it with standard EDTA.

### 2.1.11 Conductivity measurements

The conductivity measurement is one of the simplest and commonly available techniques used to study the nature of the complexes because it provides direct information regarding whether a given compound is ionic or covalent.

The resistance of a sample of an electrolytic solution is defined by the following equation:

$$R = \rho [1/A] \quad \text{----- (2.15)}$$
where, \( l \) is the path length of a sample and \( A \) is the cross sectional area. The symbol, \( \rho \) is the proportionality constant known as resistivity or specific resistance. The reciprocal of resistivity is known as conductivity, ‘\( \kappa \)’

\[
\kappa = \frac{l}{\rho} = \frac{l}{RA}
\] 

----- (2.16)

Since, \( l \) is in cm, \( A \) is in \( \text{cm}^2 \) and \( R \) in ohms (\( \Omega \)), the unit of ‘\( \kappa \)’ is \( \Omega^{-1} \text{ cm}^{-1} \) or \( \text{S cm}^{-1} \).

### 2.1.12 Molar conductivity

Molar conductance was measured using a Systronic type 302 conductivity bridge equilibrated at 25 ± 0.01 °C. The molar conductance (\( \Lambda_m \)), was measured using the equation:

\[
\Lambda_m = \frac{\text{cell constant} \times \text{conductance}}{\text{concentration of solute (mol cm}^{-3}\text{)}}
\] 

----- (2.17)

If the conductivity ‘\( \kappa \)’ is in \( \Omega^{-1} \text{ cm}^{-1} \) and the concentration \( C \) is in mol \( \text{cm}^{-3} \), then the molar conductivity \( \Lambda \) is in \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) and is defined by

\[
\Lambda = \frac{\kappa}{C}
\] 

----- (2.18)

1:1 electrolyte may have value of 70-95 \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) in nitromethane, 50-75 \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) in dimethylformamide and 100-160 \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) in methyl cyanide. Similarly a solution of 2:1 electrolyte may have a value of 150-180 \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) in nitromethane, 130-170 \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) in dimethylformamide and 140-220 \( \Omega^{-1} \text{ cm}^{2} \text{ mol}^{-1} \) in methyl cyanide.

### 2.2 BIOLOGICAL STUDIES

#### 2.2.1 Antimicrobial assessments

The antibacterial activity was determined by agar well diffusion method against bacterial and fungal isolates. The microbial cultures were adjusted to 0.5 McFarland standards, which is comparable to a microbial suspension of approximately \( 1.5 \times 10^8 \) CFU/mL. 10 mL of agar media was poured into each petri plate and plates were swabbed with inoculums of the test microorganisms and kept for 15 min for adsorption. The wells were bored into the seeded agar plates by sterile cork (6 mm diameter), and loaded with a 50 \( \mu \)L volume having concentration of 1 mg/mL of each compound reconstituted in the dimethyl sulfoxide (DMSO). The plates were incubated at 37 °C for 24 h. Antibacterial activity was evaluated by measuring the zone of growth inhibition against the test microorganisms. The negative control was taken as the medium with dimethyl sulfoxide (DMSO) whereas media with standard antibiotics were used as positive controls.
The antimicrobial properties of the ligand and metal complexes against bacterial and fungal isolates were measured by micro dilution method using 96 micro well plates, according to NCCLS recommendations. The stock of compounds at a concentration of 10 mg/mL in DMSO was prepared and further converted to desired concentration of 1 mg/mL in methanol. 100 μL of media into all wells of pre-sterilized microtiter plate was allotted. Thereafter, two fold serial dilutions were performed from well 1-10. Liquid culture for test microorganisms was grown in suitable medium for the require time period at 37 °C. Then, optical density of liquid culture was measured at 600 nm and the dilution was performed to adjust the number of cells from 10^4-10^7 CFU/mL. Suitable control was also comprised in this study.

2.2.2 XTT assay: Antibiofilm analysis

Biofilm inhibition test is performed using the XTT assay, based on reduction of the soluble tetrazolium salt, 2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazolium-5-carboxanilide (XTT) by mitochondrial dehydrogenases to formazan product. This method quantifies bacterial/fungal growth by measuring bacterial/fungal metabolism in-vitro, using appropriate concentrations of XTT and an electron transfer agent, such as menadione. The intensity of the color change corresponds to the number of metabolically active (live) cells. The biofilms assay was performed using sterile, 96-well microtiter plates. Following the incubation (2 h) of respective bacterial and fungal cell suspension (at the density of 10^4-10^7 cells) in the wells at 37 °C, the plates were rinsed with PBS to remove loosely attached and un-adhered cells. Further, the plates were incubated with 100 μL per well of RPMI media for another 4 h at 37 °C to allow the biofilm development. After 4 h of incubation, when biofilms were still developing, the biofilms were given dark exposure of treated sample. Consequently, the plates were incubated at 37 °C for another 20 h. Finally, the sodium salt of XTT was dissolved in phosphate buffer to 1 mg/mL concentration, filter-sterilized and stored at -80 °C. Menadione was dissolved in acetone to 1 mM followed by immediate sterilization before each experiment. Using rotator incubator (100 rpm), 100 μL of XTT/menadione solution was then loaded to each well of 96-microtiter plate followed by 100 μL of the pure culture and 100 μL of tested samples then covered (in dark) and incubated at 37 °C for 5 h. After the incubation, the biofilm production inhibition was observed measuring the colorimetric change (at 450 nm) by water soluble formazan product using a microplate reader (BioRad, USA). The wells without biofilms were taken as a blank.
2.2.3 Cell viability assay (MTT)

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used for assessing the growth of cells that reduces the tetrazolium yellow dye, MTT into insoluble formazan by mitochondrial dehydrogenase of living cells. The cytotoxicity of compounds can be examined via dose response curves when the amount of purple formazan produced by untreated control cells. The cancer cell lines were sustained in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum along with antibiotic antimycotic solution. The growing cells were seeded at a density of $0.5 \times 10^3$ cells per well in a 96-well plate and cultured for 24 h in a humidified atmosphere in presence of 5% CO$_2$ at 37 °C. The stock solutions of the test compounds were prepared in DMSO. The cells were subsequently exposed to various concentrations of compounds. The plates were incubated for 48 h and cell proliferation was estimated by adding 20 µL of MTT dye (5 mg/mL in phosphate buffered saline, PBS) per well. The plates were further incubated (37 °C) for 4 h in a humidified chamber. The formazan crystals appeared due to the reduction of dye by viable cells in each well. At the end of incubation period, the culture media were discarded and cells were washed with 100 µL HBSS (Hank’s balanced salt solution) and then dissolved in 100 µL DMSO. The extent of MTT reduction was recorded spectrophotometrically at 540 nm. The cytotoxicity is expressed as the concentration of compound inhibiting cell growth by 50% (IC$_{50}$). The IC$_{50}$ values were determined using GraphPad Prism 3.0 computer program.

2.2.4 Radical scavenging potential: DPPH assay

Antioxidants play an important role as health protecting factor as they reduce the risk for chronic diseases including cancer and heart disease. Free radicals are an atom or molecule bearing an unpaired electron and shows highly reactive nature, capable of initiating rapid chain reactions that destabilize other molecules and thus, generating more free radicals. These free radicals are deactivated by antioxidants which act as an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. The hydroxyl, peroxyl and the superoxide anion are harmful free radicals which are constantly being produced as a result of metabolic reactions in living systems. There are several diseases which have been reported due to the presence of free radicals, such as atherosclerosis, cancer, liver cirrhosis, diabetes, etc.
The free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) which is widely used to test the ability of compounds to measure their antioxidant by acting as free radical scavengers or hydrogen donors. DPPH free radical method is an antioxidant assay based on the process of electron-transfer that produces a violet solution in methanol. DPPH free radical is stable at room temperature and gets reduced in the presence of an antioxidant molecule, giving rise to colorless methanol solution. The DPPH assay provides an easy and rapid way to determine antioxidants by spectrophotometry. It gives a maximum absorption at 517 nm (purple colour). The absorbance decreases when an antioxidant species react with DPPH, as it is reduced to the DPPH-H, resulting in decolorization (yellow colour) with respect to the number of electrons captured. The extent of decolorization is indicative of antioxidant behavior of a particular compound. Ascorbic acid was used as the reference compound. The scavenging activity was calculated by following formula:

\[
\% \text{ inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \tag{2.19}
\]

where \( A_{\text{control}} \) is the absorbance of the L-ascorbic acid (Standard). \( A_{\text{sample}} \) is the absorbance of different compounds.

### 2.2.5 Fluorescence spectral studies for DNA and HSA binding

#### 2.2.5.1 Sample preparation of stock solutions

For the preparation of 20 mM phosphate buffer (pH = 7.4), double distilled water was used. By dissolving the desired amount of HSA or CT-DNA in 20 mM phosphate buffer (pH = 7.4), a stock solution of CT-DNA or HSA was made. A stock solution of the compounds was made by dissolving the complex in an aqueous solution of DMSO as the co-solvent and dilution was made with the corresponding phosphate buffer (pH = 7.4) to obtain desired concentrations. The final DMSO concentration was kept below 5% v/v. The CT-DNA concentration was determined by the absorption intensity at 260 nm using the molar absorption coefficient value of 6600 M\(^{-1}\) cm\(^{-1}\). The purity of CT-DNA solution was confirmed by UV absorbance ratio (\( \frac{A_{260}}{A_{280}} = 1.9 \)), indicating that CT-DNA is free from protein impurity. While HSA concentration was evaluated (Perkin-Elmer \( \lambda \)-25 spectrometer) using the molar absorption coefficient using \( E_{1\%}^{1\text{cm}} \) of 5.3 at 280 nm. Drug solution of 2 mM concentration was made in 20 mM phosphate buffer (pH = 7.4) and diluted to desired concentrations in the same buffer.
2.2.5.2 Fluorescence quenching measurement of DNA and HSA binding

All the experiments involving interaction of the complexes with CT-DNA and HSA were conducted in phosphate buffer adjusted to pH 7.4. When molecules which have absorbed light are in a higher electronic state, they must lose their excess energy to return back to the ground state. Thus, if the excited molecule returns to the ground state by emitting light, it exhibits fluorescence and spectrum thus obtained is termed as emission spectrum.

In case of DNA binding study, fluorimetric titration measurements were performed using spectrofluorophotometer model RF-5301 (Shimadzu Japan) at 25±0.1 °C with a 1.00 cm optical path length cell, equipped with a 150 W Xenon lamp and both the excitation and emission slits were adjusted at 5 nm and 10 nm, respectively. The intrinsic fluorescence was recorded by exciting the CT-DNA solution at 478 nm and emission spectra were recorded in 500-700 nm range. For the authentication of binding parameters, 1:1 ratio of DNA-EB (50 µM) complex solution was taken in quartz cell and the fluorimetric titrations were done with increasing concentration of tested compounds (0-90 µM). Non-fluorescent or weakly fluorescent compounds can often be reacted with strong fluorophores and thus enabling them to be examined quantitatively. On this basis molecular fluorophore, EB was used which emits fluorescence in presence of CT-DNA due to its strong intercalation.

For HSA binding study, fluorescence measurements were done on F-2700 Fluorescence spectrophotometer (HITACHI). In fluorescence quenching experiments, HSA (5 µM) was titrated using varying concentration of compounds (0-45 µM) in 20 mM of phosphate buffer at pH 7.4. The fluorescence emission spectra were recorded in range of 300-500 nm with a quartz cuvette of 1.0 cm path length after setting slit width of both excitation and emission at 10 nm. To minimize the effect of tyrosine residues in the emission signal, all the samples were excited at 295 nm. For correction of background of fluorescence, appropriate blanks corresponding to the buffer were subtracted. The intensity at 340 nm (tryptophan) was used to determine the binding parameters.

Quenching of the fluorescence of EB bound to DNA or HSA were evaluated with increasing amount of metal complexes as a second molecule and Stern-Volmer quenching constant $K_{sv}$ was obtained from the following equation.\(^{31}\)

$$\frac{F_0}{F} = 1 + K_{sv}[Q]$$

\[ \text{----- (2.20)} \]
where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of complexes (quencher) respectively. $K_{sv}$ and $Q$ denote a well-known linear Stern-Volmer constant and quencher concentration, respectively. The value of $K_{sv}$ is obtained by the ratio of slope to intercept. The plot of $F_0/F$ versus $[Q]$ showed a good linear relationship. The linearity of Stern-Volmer plot suggests that only one type of quenching process occurs either static or dynamic. To further study the quenching process, the bimolecular quenching constant, $k_q$, can be calculated using the equation:

$$k_q = K_{sv} \tau_0$$  \hspace{1cm} (2.21)

where the fluorescence lifetime of biological macromolecule ($\tau$) is $\sim 10^{-8}$ s.

For static binding reaction, the binding constant ($K_b$) and binding stoichiometry ($n$) for complex and macromolecule system was evaluated from modified Stern-Volmer equation.

$$\log \left( \frac{F_0}{F-1} \right) = \log K_b + n \log [Q]$$  \hspace{1cm} (2.22)

### 2.2.6 Calculations of energy transfer

The absorption spectra of each complex (2 μM) and fluorescence spectra of HSA (2 μM) were scanned in a similar way as discussed in section 2.2.5.2 in range of 300-400 nm. If the HSA (donor) and complex (acceptor) spectra overlap each other then donor-acceptor pair will be considered within Forster distance so that energy transfer might be taken place.\(^\text{32}\) The efficiency of energy transfer ($E$) can be used to evaluate the distance between the metal complex (acceptor) and the fluorophores in the protein (donor). The efficiency of energy transfer ($E$) could be calculated by following equation:\(^\text{33}\)

$$E_{FRET} = \frac{R_0^6}{(R_0^6 + r_0^6)} = 1 - \frac{F}{F_0}$$  \hspace{1cm} (2.23)

where $F$ and $F_0$ are the fluorescence intensities of HSA in the presence and absence of metal complex, $r$ is the distance between acceptor (bound metal complex) and donor (Trp214 residue of HSA) and $R_0$ is the critical distance when the transfer efficiency is 50%. The value of $R_0$ was calculated using the following equation:

$$R_0^6 = 8.8 \times 10^{25} K^2 n^4 \Phi J$$  \hspace{1cm} (2.24)

In the above equation, $K^2$ is the spatial orientation factor describing the relative orientation in space of the transition dipoles of the donor and acceptor, $n$ is the refractive index of the medium, $\Phi$ is the fluorescence quantum yield of the donor and $J$ is the overlap integral of the fluorescence emission spectrum of the donor with the absorption spectrum of the acceptor which could be calculated by equation:
\[
J = \frac{\sum F(\lambda) \varepsilon(\lambda) \lambda^4 \Delta \lambda}{\sum F(\lambda) \Delta \lambda}
\] ----- (2.25)

where, the parameters \(F(\lambda)\) denotes the corrected fluorescence intensity of the donor at wavelength \(\lambda\) and \(\varepsilon(\lambda)\) is the UV molar absorption coefficient of the acceptor at the wavelength \(\lambda\). For HSA study, the value of \(K^2 = 2/3\), \(n = 1.336\) and \(\Phi = 0.188\).

### 2.2.7 Circular dichroism measurements

![CD Spectrometer](image)

*Figure 2.10 CD Spectrometer*

In the present work, circular dichroic spectra were obtained on JASCO J-815 CD spectrometer (Jasco Corporation; Tokyo, Japan) (*Figure 2.10*) at 25 °C equipped with a 150W Xenon lamp, 2.0 mm quartz cuvette and temperature control unit. Circular dichroism spectroscopy is an excellent technique with a high degree of analytical selectivity and thus helps in direct determination of optically active drugs. CD has following two major advantages:

(i) It is extremely sensitive to absolute configuration along with the conformational features, which are often completely obscured in ordinary absorption spectrum.

(ii) Wide range of solvents can be used to study with relatively much smaller sample amounts.

The degree of selectivity is sufficient to determine enantiomers in mixtures without any chromatographic separation. Circularly polarized light represents a wave
where the electrical component spirals around the direction of propagation of the ray, either clockwise or counterclockwise. Within the absorption band, the molar absorptivity for right and left handed circularly polarized light is different, i.e., \((\varepsilon_d - \varepsilon_l) \neq 0\). This effect converts linearly polarized light into elliptically polarized light and is known as circular dichroism.\(^\text{34}\) Circular dichroism graphs are plots of molecular ellipticity \([\Theta]\) against wavelength. The amplitude of d component will be greater than the 1 component. When 1 component of the substance absorbs left circularly polarized light, more strongly than d component which absorb the right circularly polarized light i.e., \(\varepsilon_l > \varepsilon_d\). Furthermore, if \(\varepsilon_d > \varepsilon_l\) then the d component will be retarded more than the 1 component (\textit{Figure 2.11}).

![Figure 2.11](image)

The ellipticity, that is the angle whose tangent is ratio of minor axis of the ellipse OB to the major axis OA, is denoted by \(\Theta\). The molecular ellipticity \([\Theta]\) can be shown by the relationship: \(^\text{35}\)

\[
\Theta = 3305(\varepsilon_l - \varepsilon_d) \quad \text{----- (2.26)}
\]

CD spectroscopy has following applications:
- UV CD is used for the study of secondary structure of proteins.
- UV/vis CD is employed to examine charge-transfer transitions.
- Near-infrared CD provides information about the geometric and electronic structure.
- Vibrational CD is used for structural studies of small organic molecules along with proteins and DNA.\(^\text{36}\)
All the observed CD spectra were baseline subtracted for buffer and the CD results were expressed in terms of MRE (mean residue ellipticity) in deg cm$^2$ d mol$^{-1}$ as equation.

$$MRE = \frac{\Theta_{obs}}{10 \pi c l}$$

----- (2.27)

where c is the molar concentration of the protein, n is the number of amino acid residues (585) and l is the path length (0.1 cm). The $\alpha$-helical contents of free and combined HSA were calculated from the MRE value at 208 nm using the equation as described by Basu et al.$^{37}$

$$\% \alpha-helix = \frac{MRE_{222} - 2340}{30300} \times 100$$

----- (2.28)

where MRE$_{222}$ is the observed MRE value at 222 nm.

2.2.8 Molecular docking studies

Molecular docking is a key tool in structural molecular biology of ligand-protein interaction and its computer-assisted drug design to predict the predominant binding mode of a ligand with a protein of known three-dimensional structure.$^{38}$ Docking utilizes an energy-based scoring function for the identification of the energetically most favorable ligand conformation when bound to the target. Successful docking methods discover high-dimensional spaces efficiently and use a scoring function that properly ranks candidate dockings. Docking found many applications such as it perform virtual screening on large libraries of compounds, rank the results, and propose structural hypothesis of how the ligands inhibit the target. In general, lower energy scores represent better protein-ligand bindings compared to higher energy values. Therefore, molecular docking can be formulated to optimize the preferable ligand-binding mode with the lowest energy.$^{39}$

In our experiment, the rigid molecular docking studies were performed using an interactive molecular graphics program, HEX 8.0.0 software which is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein, DNA molecule and enzymes.$^{40}$ The structure of compounds were sketched by CHEMSKETCH (http://www.acdlabs.com) and converted to pdb format from mol format by OPENBABEL (http://www.vcclab.org/lab/babel/). The three dimensional crystal structure of B-DNA dodecamer d(CGCGAATTGGCG)$_2$ and HSA dodecamer was converted in pdb format (PDB ID: IBNA) and (PDB ID: 1AO6), respectively, and utilized as a
template. All the water molecules surrounding of DNA and HSA were removed to avoid hindrance in docking process. All other docking parameters were kept as default. PyMol (http://pymol.sourceforge.net/) and CHIMERA (www.cgl.ucsf.edu/chimera) were used for visualization purpose. The docked conformation with the lowest energy was selected for analysis.
2.3 REFERENCES


