CHAPTER 2
Experimental Techniques
EXPERIMENTAL TECHNIQUES

There are several physico-chemical methods available for the study of coordination compounds and a brief discussion of the techniques used in the investigation of the newly synthesized Schiff bases and their complexes described in the present work are given below:

1. Infrared Spectroscopy
2. Nuclear Magnetic Resonance Spectroscopy
3. Electron Paramagnetic Resonance Spectroscopy
4. Ultraviolet and Visible (Ligand Field) Spectroscopy
5. Magnetic Susceptibility Measurements
6. Mass Spectrometry
7. Molar Conductance Measurements
8. Elemental Analysis
9. Fluorescence Spectroscopy
10. X-Ray Crystallography
11. Antibacterial Activity
12. Analgesic Activity
INFRARED SPECTROSCOPY

When Infrared light is passed through a sample, some of the frequencies are absorbed while other frequencies are transmitted through the sample without being absorbed. The plot of percent absorbance or percent transmittance against frequency results is an infrared spectrum.

The term “infrared” covers the range of the electromagnetic spectrum between 0.78 and 1000 µm. In the infrared spectroscopy, wavelength is measured in “wavenumbers” which has unit as cm⁻¹.

\[ \text{Wave number} = \frac{1}{\text{wavelength in centimetres}} \]

\[ \nu = \frac{1}{\lambda} \]

It is useful to divide the infrared region into three regions; near, mid and far infrared.

<table>
<thead>
<tr>
<th>Region</th>
<th>Wavelength range (µm)</th>
<th>Wavenumber range (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near</td>
<td>0.78-2.5</td>
<td>12800-4000</td>
</tr>
<tr>
<td>Middle</td>
<td>2.5-50</td>
<td>4000-200</td>
</tr>
<tr>
<td>Far</td>
<td>50-1000</td>
<td>200-10</td>
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</tbody>
</table>

Theory of infrared absorption

The IR radiation does not have enough energy to induce electronic transitions but has enough energy to induce vibrational and rotational transitions having small energy difference between vibrational and rotational states in the molecule. For a molecule to absorb IR radiations, the vibrations or rotations within a molecule must cause a net change in the dipole moment of the molecule. The alternating electrical field of the
radiation (electromagnetic radiation consists of an oscillating electrical field and an oscillating magnetic field, perpendicular to each other) interacts with the fluctuations in the dipole moment of the molecule. If the frequency of the radiation matches the vibrational frequency of the molecule then the radiation will be absorbed, causing the change in the amplitude of molecular vibration. In the absorption of the radiation, only transition for which change in the vibrational energy is $\Delta V = 1$ can occur, since most of the transition will occur from stable $V_0$ to $V_1$, the frequency corresponding to its energy is called the fundamental frequency.

The group frequencies of certain groups characterize the group irrespective of the molecule in which these groups are attached. The absence of any band in the predicted region for the group indicates the absence of that particular group in the molecule.

**Molecular rotation**

Rotational levels are quantized, and absorption of the IR by gases yields line spectra. However, in solids, these lines broaden into a continuum due to molecular collisions and other interactions.

**Molecular vibrations**

The position of atoms in a molecule are not fixed; they are subjected to number of different vibrations. Vibrations fall into the two main categories of *stretching* and *bending*.
**Stretching:** Change in inter-atomic distance along bond axis

- Symmetric

- Asymmetric

**Bending:** Change in angle between two bonds. There are four types of bend:

- Rocking : In-plane Rocking
- Scissoring : In-plane Scissoring
- Wagging : Out-of-plane wagging
- Twisting : Out-of-plane twisting

**Vibrational coupling**

In addition to the vibrations mentioned above, interaction between vibrations can occur (coupling) if the vibrating bonds are joined to a single, central atom. Vibrational coupling is influenced by a number of factors *viz.* strong coupling of stretching vibrations occurs when there is common atom between the two vibrating bonds, coupling of bending vibrations occurs when there is common bond between vibrating groups, coupling between a stretching vibration and bending vibration occurs if the stretching bond is one side of an angle varied by bending vibration, coupling is greatest when the coupled groups have approximately equal energies, no coupling is seen between groups separated by two or more bonds.
Important Group Frequencies in the IR Spectra Pertinent to the Discussion of the Newly Synthesized Compounds.

1. Amines

*N-H Stretching Vibrations*

The N-H stretching vibrations occur in the region 3300-3500 cm\(^{-1}\) in the dilute solution\(^1\). The N-H stretching band shifts to lower value in the solid state due to extensive hydrogen bonding. Primary amines in the dilute solutions, in non-polar solvents give two absorptions i.e. symmetric stretch found near 3400 cm\(^{-1}\) and asymmetric stretch mode found near 3500 cm\(^{-1}\). Secondary amines show only a single N-H stretching band in dilute solutions. The intensity and frequency of N-H stretching vibrations of secondary amines are very sensitive to structural changes. The band is found in the range 3310-3350 cm\(^{-1}\) (low intensity) in aliphatic, secondary amines and near 3490 cm\(^{-1}\) (much higher intensity) in heterocyclic secondary amines such as pyrazole and imidazole.

2. C=N Stretching Frequency

Schiff bases (RCH=NR, imines), oximes, thiazoles, iminocarbonates etc., show the C=N stretching frequency in the 1471-1689 cm\(^{-1}\) region [1, 2]. The intensity of the C=N stretch is usually more than C=C stretch. The C=N undergoes positive or negative shift upon coordination [2].
3. **(C-O-C) Stretching Frequency of Furan**

Many workers [3,4] have reported medium intensity bands for $\nu$(C-O-C) of furan ring vibrations in the region 1020-1250 cm$^{-1}$.

4. **(C-S-C) Stretching Frequency of Thiophene**

(C-S-C) stretching frequency is observed at 850 cm$^{-1}$.

5. **M-N Stretching Frequency**

The M-N stretching frequency is of particular interest since it provides direct information regarding metal- nitrogen coordinate bond. Different amines complexes exhibited the metal-nitrogen frequencies in the 428-530 cm$^{-1}$ region [2].

6. **M-O Stretching Frequency**

Metal-oxygen stretching frequency has been reported to appear in different regions for different metal complexes [5]. The M-O stretching frequency lies in the range 550-595 cm$^{-1}$.

7. **M-S Stretching Frequency**

Metal-sulfur stretching frequency lies in the range 419-466 cm$^{-1}$.

8. **C=O Stretching vibrations**

Ketones, aldehydes, carboxylic acids, carboxylic esters, lactones, acid halides, anhydrides, amides and lactams show a strong stretching absorption band in the region of 1870-1540 cm$^{-1}$ [6]. Its relatively constant position and high intensity and relative freedom from interfering bands make it as one of the easiest band to recognize in infrared spectra.
9. Nitrate group frequencies

Free nitrate group exhibits strong band around 1383 cm\(^{-1}\) [7].

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

\( ^1 \text{H NMR Spectroscopy} \)

Nuclear magnetic resonance (NMR) spectroscopy is based on the measurement of electromagnetic radiation in the radio-frequency region of roughly 4 to 900 MHz. In contrast to ultraviolet, visible and infrared absorption, nuclei of atoms rather than outer electrons are involved in the absorption process.

Nuclear magnetic resonance spectroscopy is one of the most powerful tools available to the chemists and biochemists for elucidating the structure of chemical species.

There are two types of NMR spectrometers:

a) Continuous – Wave (CW)

b) Pulsed or Fourier transform (FT-NMR)

All early studies were carried out with continuous-wave instruments. Fourier transform spectrometers were available commercially around 1970. In both types of instruments, the sample is positioned in a powerful magnetic field that has strength of several teslas.

All nuclei carry a charge. In some nuclei this charge spin on the nuclear axis and this circulation of charge generates a magnetic dipole along the axis. Similarly protons and neutrons which constitute nuclei of atoms have the property to spin on their own axis and each of them possesses angular momentum. The net resultant of the angular momentum of all nuclear particles is called nuclear spin. For a nucleus having nuclear spin quantum number \( I \), there are \((2I + 1)\) spin states. It is known that the spin quantum number \( I \) is associated with mass number and atomic number of the nuclei as follows:
<table>
<thead>
<tr>
<th>Mass number</th>
<th>Atomic number</th>
<th>Spin quantum number</th>
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<tbody>
<tr>
<td>Odd</td>
<td>odd or even</td>
<td>1/2, 3/2, 5/2------</td>
</tr>
<tr>
<td>Even</td>
<td>even</td>
<td>0</td>
</tr>
<tr>
<td>Even</td>
<td>odd</td>
<td>1, 2, 3, ---------</td>
</tr>
</tbody>
</table>

The nuclei with $I = 0$, do not possess spin angular momentum and give no NMR signal. $^{12}$C, $^{16}$O and $^4$He fall into this category. The nuclei in which $I$ is half integral $(1/2, 3/2, 5/2 \text{ etc})$ e.g., $I = \frac{1}{2}$ include $^1$H, $^{19}$F, $^{31}$P and $^{15}$N and in the case of $^{11}$B, $I = 3/2$, $I$ is integral, the spin of $^2$H and $^{14}$N is 1 while that of $^{10}$B is 3.

Since atomic nuclei are associated with charge, a spinning nucleus generates a small current and has a finite magnetic field associated with it. The magnetic dipole of the nucleus varies with each element. When a spinning nucleus is placed in a magnetic field, the nuclear magnetic experiences a torque which tends to align with the external field. For a nucleus with a spin of $\frac{1}{2}$ there are two allowed orientations of the nucleus, parallel to the field (low energy) and against the field (high energy). Since the parallel orientation is lower in energy, this state is slightly more populated than the anti-parallel which has high energy state.

If the oriented nuclei are now irradiated with electromagnetic radiation of the proper frequency, the lower energy of the state will absorb a quantum of the energy and spin flip to the high energy state. When this spin transition occurs the nuclei are said to be in resonance with the applied radiation, hence the name Nuclear Magnetic Resonance. The amount of electromagnetic radiation required necessary for resonance depends on both the strength of the external magnetic field and on the characteristics of the nucleus being examined. The nucleus of the proton placed in 14,092 gauss field, undergoes resonance when irradiated with radiation in the 60 MHz (Radio-wave
region), higher magnetic fields such as those common in superconducting magnets, require high energy radiation and give a correspondingly high resolution.

\textbf{\textsuperscript{13}C NMR Spectroscopy}

\textsuperscript{13}C has a nuclear spin (I = \(\frac{1}{2}\)) and makes up 1.1\% of naturally occurring carbon to make carbon nuclear magnetic resonance spectroscopy (\textsuperscript{13}C NMR) a useful technique. Since carbon is the element central to organic chemistry, \textsuperscript{13}C NMR plays an important role in determining the structure of unknown organic molecules and the study of organic reactions. In particular, the \textsuperscript{13}C NMR spectrum of an organic compound provides information concerning:

- The number of different type of carbon atoms present in the molecule
- The electronic environment of different type of carbons
- The number of “neighbours” a carbon has (splitting)

The major difference between \textsuperscript{13}C NMR and \textsuperscript{1}H NMR spectra are:

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

\textsuperscript{13}C chemical shifts span slightly over 200 ppm in contrast to the typical 8 to 9 ppm range in the \textsuperscript{1}H NMR; thus considerably more structural information is generally available from \textsuperscript{13}C NMR chemical shift data. Another very important difference between \textsuperscript{1}H and \textsuperscript{13}C NMR spectroscopy is that diamagnetic effects are dominant in the shielding of the hydrogen nucleus, whereas paramagnetic effects are the dominant contributions to the shielding of the \textsuperscript{13}C nucleus. Long range shielding effects that
were important in the $^1$H-NMR are less important in $^{13}$C NMR. As a result, $^{13}$C chemical shifts generally do not parallel $^1$H chemical shifts. Since the spin number for $^{13}$C is the same for $^1$H, the same rules apply for predicting the multiplicity of the absorption.

A $^{13}$C NMR spectrum consists of discrete, sharp lines corresponding to each non-equivalent carbon atom. These resonance are typically in the range 0 to 220 ppm with the TMS reference peak at 0 ppm. The main feature of $^{13}$C NMR is its ability to give information concerning the chemical environment of carbon atoms. This helps to identify any functional group present as well giving clues towards the solution of the structure. The coupling constants for $^{13}$C-$^1$H are large (100-250 Hz) and thus interpretation of the $^{13}$C spectra can be difficult because of the overlapping $^{13}$C-$^1$H multiplets. To simplify the spectrum, $^{13}$C-NMR spectra are generally recorded under double resonance conditions in which 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.

**ELECTRON SPIN RESONANCE SPECTROSCOPY**

Electron spin resonance is a branch of absorption spectroscopy in which radiation of microwave frequency is absorbed by molecules possessing electrons with unpaired spins.

Gorter demonstrated [8,9] that paramagnetic salt when placed in a high frequency alternating magnetic field absorbs energy, which is influenced by the application of a static magnetic field either parallel or perpendicular to the alternating magnetic field.
The degeneracy of a paramagnetic ion is lifted in a strong static magnetic field and the energy levels undergo Zeeman splitting. Application of an oscillating magnetic field of appropriate frequency will induce transitions between the Zeeman levels and the energy is absorbed from electromagnetic field. If the magnetic field is slowly varied, the absorption shows a series of maxima. The plot between the absorbed energy and the magnetic field is called the electron paramagnetic resonance spectrum. A system exhibit paramagnetism wherever it has a resultant angular momentum. Such paramagnetic system includes, elements containing 3d, 4d, 4f, 5d, 5f, 6d etc. electrons, atom having an odd number of electrons like hydrogen, molecules containing odd number of electrons such as NO₂, NO etc. and free radicals which possess an unpaired electron like methyl free radical, diphenylpicryl hydrazyl free radical etc. are among the suitable reagents for EPR investigations. Splitting of energy levels in EPR occur under the effect of two types of fields, namely the internal crystalline field and applied magnetic field. While studying a paramagnetic ion in a diamagnetic crystal lattice, two types of interactions are observed, interactions between paramagnetic ions called dipolar interaction and the interaction between paramagnetic ion and diamagnetic neighbour called crystal field interaction. For small doping amount of paramagnetic ion in the diamagnetic host, the dipolar interaction will be negligibly small. The later interaction of paramagnetic ion with diamagnetic ligand modifies the magnetic properties of the paramagnetic ions. According to crystal field theory, the ligand influences the magnetic ion through the electric field, which they produce at its site and their orbital motion gets modified. The crystal field interaction is affected by the outer electronic shells.

The dipole-dipole interaction arises from the influence of magnetic field of one paramagnetic ion on the dipole moments of the similar neighbouring ions. The local
field at any given site will depend on the arrangements of the neighbours and the direction of their dipole moments. Thus resultant field on the paramagnetic ions will be the vector sum of the external field and local field. The resultant field varies from site to site giving a random displacement of the resonance frequency of each ions and thus broadening the line widths.

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 EPR of vanadyl ion is the results of hyperfine interactions. The origin of this can be understood simply by assuming that the nuclear moment produces a magnetic field \( B_N \) at the magnetic electrons and the modified resonance condition will be

\[
\Delta E = h\nu = g\beta B_N
\]

Where \( g \) is gyromagnetic ratio

\( \beta \) is Bohr Magneton

\( B_N \) is magnetic field

There may be an additional hyperfine structure also due to interaction between magnetic electrons and the surrounding nuclei called superhyperfine structure. The effect was first observed by Owens and Stevens in ammonium hexachloroiridate [10] and subsequently for a number of transition metal ions in various hosts [11,12].
ULTRA-VIOLET AND VISIBLE (LIGAND FIELD) SPECTROSCOPY

Most of the compounds absorb light somewhere in the spectral region between 200 and 1000 nm. These transitions correspond to the excitation of electrons of the molecules from ground state to higher electronic states. In a transition metal all the five d-orbitals viz. \(d_{xy}, d_{yz}, d_{xz}, d_{z^2}\) and \(d_{x^2-y^2}\) are degenerate. However, in coordination compounds due to presence of ligands this degeneracy is lifted and d-orbitals split into two groups called \(t_{2g}\) \((d_{xy}, d_{yz}, d_{xz})\) and \(e_g\) \((d_{z^2} \text{ and } d_{x^2-y^2})\) in an octahedral complex and \(t\) and \(e\) in tetrahedral complex. The set of \(t_{2g}\) orbitals goes below and the set of \(e_g\) orbitals goes above the original level of the degenerate orbitals in an octahedral complex. In case of the tetrahedral complexes the position of the two sets of the orbitals are reversed, the \(e\) going below and \(t\) going above the original degenerate level. When a molecule absorbs radiation, its energy equal in magnitude to \(h\nu\) and can be expressed by the relation:

\[
E = h\nu
\]

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

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

In order to interpret the spectra of transition metal complexes, the device of energy level diagram based upon ‘Russell - Saunders Scheme’ must be introduced. This has the effect of splitting the highly degenerate configurations into groups of levels having lower degeneracies known as ‘Term Symbols’.

The orbital angular momentum of electrons in a filled shell vectorially adds up to zero. The total orbital angular momentum of an incomplete d shell electron is
observed by adding \( l \) value of the individual electrons, which are treated as a vector with a component, \( m_l \) in the direction of applied field. Thus

\[
L = \sum_i m_l = 0, 1, 2, 3, 4, 5, 6
\]

\[S, P, D, F, G, H, I\]

The total spin angular momentum \( S = \sum_i s_i \) where \( s_i \) is the value of spin angular momentum of individual electrons. \( S \) has the degeneracy \( \tau \) equal to \( 2S + 1 \), which is also known as ‘Spin Multiplicity’. Thus a term finally denoted as ‘\( \tau L \)’. For example, if \( S = 1 \) and \( L = 1 \), the term will be \(^3P\) and similarly if \( S = 1\frac{1}{2} \) and \( L = 3 \), the term will be \(^4F\).

In general the terms arising from a \( d^n \) configuration are as follows:

\[
\begin{align*}
d^1d^9 & : \quad 2D \\
d^2d^8 & : \quad 3F, 3P, 1G, 1D, 1S \\
d^3d^7 & : \quad 4F, 4P, 2H, 2G, 2F, 2D, 2P \\
d^4d^6 & : \quad 5D, 3H, 3G, 3F, 3D, 2I, 1G, 1F, 1D, 1S \\
d^5 & : \quad 4S, 4G, 4D, 4P, 2I, 2H, 2G, 2F, 2D, 2P, 2S
\end{align*}
\]

Coupling of \( L \) and \( S \) also occurs, because both \( L \) and \( S \) if non-zero, generate magnetic fields and thus tend to orient their moments with respect to each other in the direction where their interaction energy is least. This coupling is known as ‘\( LS \) coupling’ and give rise to resultant angular momentum denoted by quantum number \( J \) which may have quantized positive values from \( |L + S| \) up to \( |L - S| \) e.g., in the case of \(^3P\) (\( L=1, S = 1 \)), \(^4F\) (\( L = 3, S = 1\frac{1}{2} \)) possible values of \( J \) representing state, arising from term splitting are 2, 1, 0 and 4\(\frac{1}{2}, 3\frac{1}{2}, 2\frac{1}{2} \) and \( 1\frac{1}{2} \). Each state specified by \( J \) is \( 2J+1 \) fold degenerate. The total number of states obtained from a term is called multiplet and
each value of $J$ associated with given value of $L$ is called component. Spectral transitions due to spin–orbit coupling in an atom or ion occurs between the components of two different multiplets while LS coupling scheme is used for the elements having atomic number less than 30, in that case spin-orbital interactions are large and electrons repulsions parameters decrease. The spin angular momentum of an individual electron couples with its orbital momentum to give an individual $j$ for that electron. The individual $j$s’ couple to produce a resultant $J$ for the atom. The electronic transitions taking place in an atom or ion are governed by certain ‘Selection Rules’ which are as follows:

1. Transitions between states of different multiplicity are forbidden.
2. Transitions involving the excitation of more than one electron are forbidden.
3. In a molecule, which has a centre of symmetry, transitions between two gerade or two ungerade states are forbidden.

It is possible to examine the effects of crystal field on a polyelectron configuration. The ligand field splitting due to cubic field can be obtained by consideration of group theory. It has been shown that an S state remains unchanged. P state does not split, and D state splits into two and F state into three and G state into four states as tabulated below: (Applicable for an octahedral ‘Oh’ as well as tetrahedral ‘Td’ symmetry).

\[
\begin{align*}
S & \rightarrow A_1 \\
P & \rightarrow T_1 \\
D & \rightarrow E + T_2 \\
F & \rightarrow A_2 + T_1 + T_2 \\
G & \rightarrow A_2 + E + T_1 + T_2
\end{align*}
\]
Transitions from the ground state to the excited state occur according to the selection rules described earlier. The energy level order of the state arising from the splitting of a term state for a particular ion in an octahedral field is reverse for the ion in the tetrahedral field. However, due to transfer of charge from ligand to metal or metal to ligand sometimes bands appear in the ultraviolet region of the spectrum. These spectra are known as ‘Charge Transfer Spectra’ or ‘Redox Spectra’. In metal complexes there are often possibilities that charge transfer spectra extend into the visible region to obscure d-d transition. However, these should be clearly discerned from the ligand bands, which might also occur in same region.

MAGNETIC SUSCEPTIBILITY MEASUREMENTS

The determination of magnetic moments of transition metal complexes have been found to provide ample information in assigning their structure. The main contribution to bulk magnetic properties arises from magnetic moment resulting from the motion of electrons. It is possible to calculate the magnetic moments of known compounds from the measured values of magnetic susceptibility.

There are several kinds of magnetism in substances viz. diamagnetism, paramagnetism and ferromagnetism or antiferromagnetism. Mostly compounds of the transition elements are paramagnetic. Diamagnetism is attributable to the closed shell electrons with an applied magnetic field. In the closed shell, the electron spin moment and orbital moment of the individual electrons balance one another so that there is no magnetic moment. Ferromagnetism and antiferromagnetism arise as a result of interaction between dipoles of neighbouring atoms.
If a substance is placed in a magnetic field of strength $H$, the magnetic induction $B$ within the substance is given by

$$B = H + 4\pi I$$

Where $I$ is the intensity magnetization or magnetic moment per unit volume and term $4\pi I$ is the contribution to $B$ by substance itself. The ratio $B/H$ is called magnetic permeability of the material and is given by

$$B/H = 1 + 4\pi(I/H) = 1 + 4\pi \chi_v$$

Where $\chi_v$ is called the magnetic susceptibility per unit volume or volume susceptibility. $B/H$ is the ratio of the density of lines of force within the substance to the density of such lines in the same region in the absence of sample. Thus the volume susceptibility of a vacuum is by definition zero since in vacuum $B/H = 1$. The volume susceptibility of a diamagnetic substance is negative while paramagnetic substances have positive susceptibilities.

Many studies are done using $\chi_g$, magnetic susceptibility per gram, which is $\chi_v$ divided by the density. Another useful form is $\chi_M$, molar magnetic susceptibility, which is $\chi_g$ times molecular weight.

Another measure of magnetic interaction that is often used is an effective magnetic moment ($\mu_{\text{eff}}$) where

$$\mu_{\text{eff}} = 2.828 \sqrt{\chi_M^{\text{corr}} \cdot T} \text{ BM}$$

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 these two properties i.e., 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 (BM). The magnetic moment of a single electron is given by

$$\mu_s = g \sqrt{S(S+1)} \text{ BM}$$

Where $S$ is the spin quantum number and $g$ is the gyromagnetic ratio. For Mn$^{2+}$, Fe$^{3+}$ and other ions whose ground states are S states there is no orbital angular momentum. In general, the transition metal ions in their ground state D or F being most common do possess orbital angular momentum. For ions such as Co$^{2+}$ and Ni$^{2+}$, the magnetic moment is given by

$$\mu_{(S+L)} = \sqrt{4S(S+1) + L(L+1)}$$

In which L represents the orbital angular momentum quantum number for the ion. The spin magnetic moment is insensitive to the environment of the metal ion but the orbital magnetic moment is not. In order for an electron 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 $E_g$ terms, but can be present for $T_{1g}$ and $T_{2g}$ terms. Magnetic moments of 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 complexes possessing T ground terms usually differ from the high spin value and vary with temperature. The magnetic moments of the
complexes having a $^6A_{1g}$ 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 contribution is expected. Even no splitting in 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 moments contribution.

Practically the magnetic moment value of an unknown complex is obtained on Gouy Magnetic Balance, Faraday method can also be applied for the magnetic susceptibility measurements of small quantity of solid samples.

The gram susceptibility is measured by the following

$$\chi_g = \frac{\Delta W}{W} \frac{W_{std}}{\Delta W_{std}} \chi_{std}$$

Where

$\chi_g$ = Gram Susceptibility

$\Delta W$ = Change in weight of the unknown sample with magnet on and off.

$W$ = Weight of the known sample

$\Delta W_{std}$ = Change in weight of standard sample with magnet on and off.

$W_{std}$ = Weight of standard sample.

$\chi_{std}$ = Gram Susceptibility of the standard sample.
MASS SPECTROMETRY

In mass spectrometry, a substance is bombarded with an electron beam having sufficient energy to fragment the molecule. The positive fragments which are produced (cations and radical) are accelerated in a vacuum through a magnetic field and are sorted based on the mass-to-charge ratio. Since the bulk of the ions produced in the mass spectrometer carry a unit positive charge, the value m/e is equivalent to the molecular weight of the fragment. The analysis of mass spectrometry information involves the re-assembling of fragments, working backwards to generate the original molecule. A very low concentration of sample molecule is allowed to leak into the ionization chamber (which is under a very high vacuum) where they are bombarded by a high-energy electron beam. The molecules fragment and the positive ions produced are accelerated through a charged array into an analyzing tube. The path of the charged molecules is bent by an applied magnetic field. Ions having low mass (low momentum) will be deflected most by this field and will collide with walls of the analyzer. Likewise, high momentum ions will not be deflected enough and will also collide with the analyzer wall. Ions having the proper mass-to-charge ratio, however, will follow the path of the analyzer, exit through the slit and collide with the collector. This generates electric current, which is then amplified and detected. By varying the strength of the magnetic field, the mass-to-charge ratio which is analyzed can be continuously varied.

The output of the mass spectrometer shows a plot of relative intensity vs the mass-to-charge ratio (m/e). The most intense peak in the spectrum is termed the base peak and all others are reported relative to its intensity. The peaks themselves are typically very sharp, and are often simply represented as vertical lines.
The process of fragmentation follows simple and predictable chemical pathways and the ions, which are formed, will reflect the most stable cations and radical cations, which that molecule can form. 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 \(^{13}\text{C},^{2}\text{H}\), etc. Many molecules with especially labile protons do not display molecular ions; an example of this is alcohol, where the highest molecular weight peak occurs at \(m/e\) 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. That is, loss of a methyl group will generate a peak at \(M-15\); loss of an ethyl at \(M-29\), etc.

**CONDUCTIVITY**

The resistance of a sample of an electrolyte solution is defined by

\[
R = \rho \frac{l}{A}
\]

Where \(l\) is the length of a sample of electrolyte and \(A\) is the cross sectional area. The symbol \(\rho\) is the proportional constant and is a property of a solution. This property is called resistivity or specific resistance. The reciprocal of resistivity is called conductivity, \(\kappa\)

\[
\kappa = \frac{l}{\rho} \frac{1}{l/RA}
\]

Since \(l\) is in cm, \(A\) is in \(\text{cm}^2\) and \(R\) in ohms \((\Omega)\), the units of \(\kappa\) are \(\Omega^{-1}\text{cm}^{-1}\) or \(\text{S cm}^{-1}\) (Siemens per cm).
MOLAR CONDUCTIVITY

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

$$\Lambda = \kappa/C$$

Where $C$ is the concentration of solute in mol cm$^{-3}$

Conventionally solutions of $10^{-3}$ M concentration are used for the conductance measurements. Molar conductance values of different types of electrolytes in a few solvents are given below:

A 1:1 electrolyte may have a 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 dimethyl formamide 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 dimethyl formamide, 140-220 $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ in methyl cyanide and 98-120 $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$ in dimethyl sulfoxide[13-15].

ELEMENTAL ANALYSIS

The chemical analysis is quite helpful in fixing the stoichiometric composition of the ligand as well as its metal complexes. Carbon, hydrogen and nitrogen analyses were carried out on a Perkin Elmer-2400 CHN elemental analyzer. Chlorine was analyzed by conventional method [16] for chlorine estimation, a known amount of sample was decomposed in a platinum crucible and dissolved in water in a little concentrated nitric acid. The solution was then treated with silver nitrate solution. The precipitate was then dried and weighed. For metal estimation [17], a known amount of complex was decomposed with a mixture of nitric, perchloric and sulphuric acids in a beaker. It
was then dissolved in water and made up to known volume so as to titrate it with standard EDTA.

**FLUORESCENCE SPECTROSCOPY**

With some molecules, the absorption of a photon is followed by the emission of light of a longer wavelength (i.e. lower energy). This emission is called fluorescence (or phosphorescence, if the emission is long lived). There are many environmental factors that effect the fluorescence spectrum; furthermore, fluorescence efficiency is also environmentally dependent. Because these parameters of fluorescence are more sensitive to the environment than are those of absorbance and because smaller amounts of material are required, fluorescence spectroscopy is frequently of greater value than absorbance measurement. With macromolecules, fluorescence measurements can give information about conformation, binding sites, solvent interactions, degree of flexibility, intermolecular distances and rotational diffusion coefficient of macromolecules. Furthermore, with living cells, fluorescence can be used to localize otherwise undetectable substances.

As with other physical methods, the theory of fluorescence is not yet adequate to permit a positive correlation between fluorescent spectrum and the properties of the immediate environment of the emitter; hence the utility of the procedure is based on establishing empirical principles from studies with model compounds.

The excited molecule does not always fluoresce. The probability of fluorescence is described by the quantum yield, $Q$ that is the ratio of the number of emitted to absorbed photons. Several factors determine $Q$, some of these are properties of the molecule itself (internal factors) and some are environmental.
The internal factors are not generally of the interest to the biochemist concerned with the properties of the macromolecules, environmental factors are more important. The effect of the environment is primarily to provide radiationless processes that compete with fluorescence and thereby reduce $Q$, this reduction in $Q$ is called quenching. In biological systems, quenching is usually a result of either collisional processes (either a chemical reaction or simply collision with the exchange of energy) or a long range radiative processes called resonance energy transfer. These factors are usually expressed in an experimental situation involving solutions as an effect of the solvent or dissolved compounds (called quenchers), temperature, pH, neighbouring chemical groups, or the concentration of the fluor.

It is important to know the distinction between a corrected spectrum and an uncorrected. It is common to plot a spectrum as the photomultiplier output versus wavelength, this is an uncorrected spectrum. Plotting fluorescence intensity or quantum yield produces a corrected spectrum.

To measure $Q$ requires the counting of photons because

$$Q = \text{photons emitted}/\text{photons absorbed}$$

$Q$ is dimensionless quantity

Because the energy, $E$, of the one photon is related to the frequency $\nu$ of the light by the relation $E = h\nu$, a measurement of the number of photons requires measuring the energy of the radiation and correcting for frequency. This usual method for determining $Q$ requires a comparison with a fluor of known $Q$, two solutions are prepared –one of the sample and one of the standard fluor- and with the same exciting source the integrated fluorescence (i.e. the area of the spectrum) of each is measured.
The quantum yield, \( Q_x \), of a sample X is

\[
Q_x = \frac{I_x Q_s A_x}{I_s A_x}
\]

Where \( Q_s \) is the quantum yield of the standard, \( I_x \) and \( I_s \) are the integrated fluorescence intensities of sample and the standard, respectively and \( A_x \) and \( A_s \) are the percentage of absorption of each solution at the exciting wavelength. Usually solutions are adjusted so that \( A_x = A_s \).

Two types of fluors are used in fluorescence analysis of macromolecules intrinsic fluors (contained in the macromolecules themselves) and extrinsic fluors (added to the systems, usually binding to one of the components).

For proteins, there are three intrinsic fluors - tryptophan, tyrosine and phenylalanine. The fluorescence of each can be distinguished by exciting with and observing at the appropriate wavelength. In practice, tryptophan fluorescence is most commonly studied, because phenylalanine has a very low Q and tyrosine fluorescence is frequently very weak due to quenching. The fluorescence of tyrosine is almost totally quenched if it is ionized, or near an amino group, a carboxyl group, or a tryptophan.

In special situations, however, it can be detected by excitation at 280 nm. The principle reason for studying the intrinsic fluorescence of proteins is to obtain information about conformation. This is possible because the fluorescence of both tryptophan and tyrosine depends significantly on their environment (i.e. solvent, pH, and presence of quencher, a small molecule, or a neighbouring group in the protein).
X-RAY CRYSTALLOGRAPHY

Undoubtedly the most important and useful technique, X-ray diffraction, has been in use since the early part of this century for the fingerprint characterization of crystalline materials and for the determination of their crystal structures.

X-rays and their generation

X-rays are electromagnetic radiations of wavelength ~ 1Å0 \( (10^{-10} \text{ m}) \) occurring between γ-rays and the ultraviolet region of electromagnetic spectrum. The X-rays used in almost all diffraction experiments are produced by a process that leads to monochromatic X-rays.

Commonly, two approaches have been used to treat diffraction by crystals as mentioned below:

1. The Laue equations
2. Bragg’s Law

The Laue equations provide a rigorous and mathematically correct way to describe diffraction by crystals. The drawback is that they are cumbersome to use. The alternative theory of diffraction based on Bragg’s law is much simpler and is used almost universally in solid state chemistry.

Structure factor

The X-ray scattering power of an atom is directly proportional to the number of electrons composing it and can be expressed by a scattering factor \( f \). In order to determine the combined scattering power of all the atoms in a unit cell, it is necessary to relate the differences between the pathlengths of X-rays scattered by each atom. This is done most conveniently by a geometric factor which is the function of the
position of each atom among the equipoints. When the amplitudes of the wavelets scattered by each atom in the unit cell are added, one obtains the so-called structure factor or structure amplitude.

\[ F_{hkl} = f_1 e^{2\pi i (hx_1 + hy_1 + lz_1)} + \ldots + f_N e^{2\pi i (hx_N + ky_N + lz_N)} \]

\[ = \sum_{n=1}^{N} f_n e^{2\pi i (hx_n + hy_n + lz_n)} \]

Where N is the total number of atoms contained in a unit cell. The exponential term expresses the relative phase of the radiation scattered by each atom, n as a function of its position in the unit cell \( x_n, y_n, z_n \).

In an actual X-ray diffraction experiment one measures the intensities rather than the amplitudes of the reflected beam. The intensity is directly proportional to the square of the amplitude

\[ I_{hkl} \sim F_{hkl}^2 \]

so that it is possible to determine the positions of the atoms in a unit cell, that is, the crystal structure, directly from the observed intensity values.

**Factors that effect intensities**

Intensities depend on several factors other than structure factor. The main factors are:


2. *Structure factor* – dependence on the position of atoms in the unit cell and their scattering power.
3. *Lorenz factor* – a geometric factor that depends on the particular type of instrument used and varies with \( \theta \). Usually lumped with polarization factor to give \( L_\rho \) factor.

4. *Temperature factor* – thermal vibrations of atoms cause a decrease in the intensities of diffracted beams and an increase in background scatter.

5. *Absorption factor* – absorption of X-rays by the sample depends on the form of the sample and geometry of the instrument. Ideally, for single crystal work, crystal should be spherical so as to have the same absorption factor in all directions.

**R- Factor and structure determination**

The measure of agreement between the individual, scaled \( F_{\text{obs}} \) and \( F_{\text{calc}} \) values is given by the residual factor or R-factor defined as follows

\[
R = \frac{\sum |F_{\text{obs}}| - |F_{\text{calc}}|}{\sum |F_{\text{obs}}|}
\]

The values of R guides one to solve the unknown crystal structures among other parameters. The lower the value of R, more likely the structure to be correct.

It is not possible to give hard and fast rules about the relation between the magnitude of R and the likely correctness of the structure, but, usually, when R is less than 0.1 to 0.2, the proposed structure is essentially correct. A structure which has been solved fully using good quality intensity data has R typically in the range 0.02 to 0.06.

**Space lattice and Unit Cell**

Crystals have definite orderly arrangements of their constituents (atoms, molecules or ions) in three dimensions. The positions of atoms, molecules or ions in a crystal relative to one another in space, are designated usually by points. Such a
representation is called space lattice (i.e. an array of points showing how molecules, atoms or ions are arranged at different sites in a three dimensional space).

A unit cell is the smallest repeating unit in the space lattice which when repeated over and over again results in a crystal of the given substance.

The three transactions selected as the edges of the unit cell are called the crystallographic axes a, b, c and the angles between them are called the interfacial angles, α, β, γ where

α is the angle between b and c (opposite the a axis)

β is the angle between c and a (opposite the b axis)

γ is the angle between a and b (opposite the c axis)

**Determination of unit cell contents**

The unit cell, by definition must contain at least one formula unit, whether it is be an atom, ion pair, molecule, etc. In centred cells and sometimes in primitive cells, the unit cell contains more than one formula unit. A simple primitive relation may be derived between the cell volume, the number of formula units in the cell, the formula weights and the bulk crystal density. The density is given by

\[ D = \frac{\text{mass/volume}}{\text{formula weight/molar volume}} = \frac{\text{FW/volume of formula unit}}{N} \]

Where N is Avogadro’s number. If the unit cell of volume V contains Z formula unit then

\[ V = \text{volume of one formula unit} \times Z \]

Therefore,

\[ D = \frac{\text{FW}}{V \times N} \]
V is usually expressed as cm$^3$ and density in grams per cubic centimetres.

**Symmetry in crystals**

Symmetry is the most characteristic property of the crystals. Symmetry in the crystals may be understood in terms of symmetry operation and symmetry elements.

There are two nomenclatures for labelling symmetry element.

a. The Hermann-Mauguin system used in crystallography

b. And the Schoflies system used in spectroscopy

Both the systems are well established, crystallographer require elements of space symmetry that spectroscopist do not, and spectroscopist use a more extensive range of point symmetry than crystallographer.

**The choice of unit cell and crystal system**

Theoretically, there can be 32 different combinations of elements of symmetry of a crystal. These are called 32 point groups or 32 systems. Some of the systems however have been grouped together so that we have only seven basic systems viz. Cubic, Orthorhombic, Tetragonal, Monoclinic, Triclinic, Hexagonal and Rhombohedral.

**ANTIBACTERIAL ACTIVITY**

The antibacterial activity was evaluated by agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standards, which is visually comparable to a microbial suspension of approximately $1.5 \times 10^8$ cfu/ml. 20 ml of agar media was poured into each Petri plate and plates were swabbed with a colony from the inoculums of the test microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 6 mm diameter, wells were bored into the seeded agar
plates and these were loaded with a 50µl volume with concentration of 10 mg/ml of each compound reconstituted in the dimethyl sulfoxide (DMSO). All 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 with Antibiotic Zone Scale. The medium with dimethyl sulfoxide (DMSO) as solvent was used as a negative control whereas media with Ciprofloxacin (standard antibiotic for gram positive) and Gentamicin (standard antibiotic for gram negative) were used as positive control. The experiments were performed in triplicates.

The MIC was tested against bacterial strains through a broth dilution method. In this method, the test concentrations of compounds were made from 2.5 to 0.01 mg/ml in the sterile wells of the micro-titre plates.

In a sterile microtitre plates (96-u-shaped wells) 50 µl of the sterile nutrient broth was poured in each well in three rows, then from a fresh inoculum so formed (10⁸ cfu/ml diluted with 100µl Nutrient broth to have 10⁶ cfu/ml) 50 µl of the suspension was poured in each well in the first and third row, second row was again filled with 50 µl of Nutrient broth, finally the drug sample 50µl was added in the first row diluting uniformly from 2.5 to 0.01 mg/ml till the 8th well. All the microtitre plates were wrapped properly with a sterilized foil and incubated at 37°C for 24 hours.

**ANALGESIC ACTIVITY**

The analgesic activity was studied by the Rat Tail-Flick test. The test was carried out by the method of Davies (1946). Albino rats of either sex weighing 125-150g were used. The rat was restrained in a Perspex restrainer and the reaction time was tested by placing the tail over the nichrome wire of the analgesiometer. The variac was adjusted at a point where the reaction time was found to be 3-6 seconds and the corresponding variac reading
was noted. The variac was set at the same point for subsequent testing of a particular animal. The reaction time of each animal was recorded before administering the test complexes and at intervals of 15 minutes for 60 minutes, after the administration of the complexes. The reaction time at each post treatment interval within a group was statistically compared with the initial reaction time by 1-way ANOVA test followed by LSD for pair-wise comparison.
REFERENCES


