2.1 Reagents used

2.1.1 Metal salts

The metal salts used in the synthesis of complexes were

1) \( \text{Ln(NO}_3\text{)}_3\cdot 6\text{H}_2\text{O} \) where \( \text{Ln} = \text{La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy and Er} \)

2) \( \text{LnCl}_3\cdot 6\text{H}_2\text{O} \) where \( \text{Ln} = \text{La, Pr, Nd, Sm, Eu, Gd, Tb, Dy and Er} \)

3) \( \text{Ln(ClO}_4\text{)}_3\cdot 6\text{H}_2\text{O} \) where \( \text{Ln} = \text{La, Pr, Nd, Sm, Eu, Gd, Tb, Dy and Er} \)
All metal salts were synthesized from the respective metal oxide. The metal oxides of lanthanum, praseodymium, neodymium, samarium, erbium and terbium were obtained (99.5% pure) from Indian Rare Earth Ltd, Udyogamandal Division, Kochi, Kerala. The oxides of cerium, europium, gadolinium and dysprosium of purity 99.8% were purchased from Aldrich Chemicals. For the present work, nitrate, chloride and perchlorate salts of lanthanum, cerium, praseodymium, neodymium, samarium, europium, gadolinium, terbium, dysprosium and erbium metals were prepared by reported methods [197]. The oxides were dissolved in the respective acid (50%) and the resulting solution was evaporated over a steam bath to get crystalline solids. All the salts were found to be highly hygroscopic and hence, kept in vacuum desiccator over P4O10 [197].

2.1.2 Solvents

The solvents used were methanol, absolute alcohol, acetone, acetonitrile, benzene, chloroform, dichloromethane, DMF, DMSO, ethyl acetate and nitrobenzene. All solvents were from E.Merck India or BDH with GR grade and used as such.

2.1.3 Other reagents

Nutrient agar, MH agar and potato dextrose agar (Himedia), 4-aminoantipyrine (BDH, New Delhi), pyridoxal hydrochloride (Sigma Chemicals), 2-aminophenol and 3-aminophenol (E.Merck) were 99.5% pure and used without further purification. The acids used for the preparation of salts were GR grade HCl, HNO3 and HClO4 supplied by Merck Chemicals.
2.2 Synthesis of 2,3-dimethyl-4-(iminopyridoxyl)-1-phenyl-3-pyrazoline-5-one (DIPP)

The Schiff base (DIPP) was synthesized by mixing ethanolic solution of 4-aminoantipyrine (1mmol, 30mL) and pyridoxal hydrochloride (1mmol, 30mL) in a 1:1 molar ratio and stirred well on a magnetic stirrer for half an hour and was allowed to stand for 30 minutes at room temperature and filtered. The yellow crystalline solid obtained was washed with ether followed by acetone and finally recrystallized from absolute alcohol [198]. Melting point was determined and purity was checked by TLC method. Yield: 85%, M.P: 278°C, Elemental data for C_{19}H_{21}N_{4}O_{3}Cl(388) found (calculated) %C=58.57(58.76), %H=5.2(5.6), %N=14.38(14.44).

![Scheme 1. Synthesis of 2,3-dimethyl-4-(iminopyridoxyl)-1-phenyl-3-pyrazoline-5-one (DIPP).](image1)

![Fig. 2.1 Crystal structure of DIPP](image2)
2.2.1 Infrared spectra

The IR spectra (KBr pellet technique) show very intense and strong band at 1603 cm\(^{-1}\) which is attributed to \(\text{C}=\text{N}\) stretching vibration of the azomethine group. A very intense band at 1649 cm\(^{-1}\) is assigned to \(\nu\text{C}=\text{O}\) stretching vibrations. A strong and intense frequency band observed at 1384 cm\(^{-1}\) is attributed to the stretching vibrations of the C-O (phenolic) \cite{199}.

2.2.2 \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra

The \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra of DIPP were recorded in DMSO-\(d_6\) solvent. The protons of aromatic ring appeared as multiplets in the range \(\delta= 7.39\text{-}7.5\) ppm. Signals at \(\delta= 2.5, 2.6\) and \(4.7\) ppm are attributed to nine methyl protons. Singlet signals at \(\delta= 3.5\) and \(\delta= 10.1\) ppm are attributed to protons attached to alcoholic OH and phenolic OH.

The \(^{13}\text{C}\) spectra of DIPP show the presence of carbon atoms corresponding to keto (206 ppm), methyl (38 ppm, 39 ppm, 40 ppm), C-OH...
(157ppm) and aromatic (126-129ppm) carbon atoms. These results confirm the proposed structure [200].

2.2.3 UV-Visible spectra

The spectra of complexes were recorded in DMF in the region 200-900nm. DIPP exhibits bands in the region 275nm and 385nm which are assignable to \( \pi \rightarrow \pi^* \) and \( n \rightarrow \pi^* \) transitions respectively [201].

2.3 Synthesis of 5-(hydroxymethyl)-4-\{(1Z)-[2-N(2-hydroxylphenyl)ethanimidoyl)-2-methyl]pyridine-3-ol-hydrochloride (HMHPMP\(^a\))

Ethanolic solution of pyridoxal hydrochloride (1mmol, 30mL) was added to the boiling solution of 2-aminophenol (1mmol, 30mL), refluxed for 5 hours and allowed to cool, filtered. The product was dried in vacuum desiccators. Fine dark brown crystalline solids were separated. This was further purified by washing with acetonitrile, followed by acetone, recrystallized from absolute alcohol [198]. Melting point and yield were determined and purity was checked by TLC method. CHN analysis was carried out. M.P:178\(^o\)C, Yield: 65\%, Elemental data for C\(_{14}\)H\(_{15}\)N\(_2\)O\(_4\)Cl(294) found (calculated) % C=57.51(57.14), %H=4.41(4.67), %N=10.31(9.98).

![Scheme 2. Synthesis of 5-(hydroxymethyl)-4-\{(1Z)-[2-N(2-hydroxylphenyl)ethanimidoyl)-2-methyl]pyridine-3-ol-hydrochloride (HMHPMP\(^a\)).](image)
2.3.1 Infrared spectra

The important features in the IR spectrum of ligand are strong and sharp vibrational frequency band at 1623 cm\(^{-1}\) ascribed to \(\nu\text{C}=\text{N}\), intense broad band at 3342-3353 cm\(^{-1}\) characteristic of \(\nu\text{(OH)}\) and a medium band observed at 1323 cm\(^{-1}\) attributed to C-O (pyridyl ring). Two strong bands, one at 1201 cm\(^{-1}\) attributed to \(\nu\text{(C-O phenyl ring)}\) and the other at 1110 cm\(^{-1}\) attributed to \(\nu\text{(C-O)}\) of the primary alcoholic group [199].

2.3.2 \(^1\text{H}\) and \(^{13}\text{C}\) NMR spectra

Broad signals observed at \(\delta = 2.4\) ppm is attributable to C-CH\(_3\) and \(\delta = 3.36\) ppm is attributed to OH (alc) protons. Signals observed at \(\delta = 8.1\) ppm is attributed to azomethine proton. Signals appeared as multiplet at \(\delta = 6.9-7.1\) ppm is attributed to aromatic protons. Singlet at \(\delta = 6.6\) ppm with respect to TMS is assignable for proton attached to phenolic –OH.
The $^{13}$C spectra of HMHPMP$^a$ show the presence of carbon atoms corresponding to methyl (31ppm, 39ppm, 40ppm), C-OH (158ppm, 156ppm) and aromatic (118ppm -142ppm) carbon atoms. These results confirm the proposed structure [200].

2.3.3 UV-Visible spectra

The electronic spectra of the ligand is characterized by three main absorption bands in the region 200-600nm. The high intensity bands observed in the region 240-255nm are attributed to the $\pi \rightarrow \pi^*$ transition of the benzene ring. A band at 285nm corresponds to $\pi \rightarrow \pi^*$ transition of the azomethine C=N while bands at 365nm attributed to n$\rightarrow$$\pi^*$ transition [201].

2.4 Synthesis of 5-(hydroxymethyl)-4-\{(1Z)-[2-N(3-hydroxyl phenyl)ethanimidoyl]-2-methyl\}pyridine-3-ol-hydrochloride (HMHPMP$^b$)

Methanolic solutions of 3-aminophenol (1mmol, 40mL) and pyridoxal hydrochloride (1mmol, 30mL) were mixed and refluxed for 5 hours, allowed to cool in air. A tarry mass was formed, which when kept in a desiccator over P$_4$O$_{10}$ for 2-3 days, fine dark brown crystalline solid was formed, separated and purified by recrystallization [198]. Yield: 56%, M.P:158°C, Elemental data of C$_{14}$H$_{15}$N$_2$O$_4$Cl(294) found (calculated) %C=57.65(57.05), %H=4.85(4.41), %N=10.1(9.5).

Scheme 3. Synthesis of 5-(Hydroxymethyl)-4-\{(1Z)-[2-N(3-hydroxylphenyl)ethanimidoyl]-2-methyl\}pyridine-3-ol-hydrochloride (HMHPMP$^b$).
2.4.1 Infrared spectra

The IR spectra of the ligand show a very intense and strong absorption band characteristic of azomethine (C=N) group at 1602 cm\(^{-1}\). Another prominent frequency band observed at 1317 cm\(^{-1}\) was attributed to the stretching vibrations of phenolic C-O(pyridyl ring). A medium intensity vibrational frequency band was displayed at 1245 cm\(^{-1}\) and 1190 cm\(^{-1}\) attributed to –C-O(phenolic) and C-O(alcoholic) [199].

2.4.2 \(^1\)H and \(^{13}\)C NMR spectra

The \(^1\)H NMR spectrum of the ligand displayed a singlet at \(\delta = 6.6\) ppm with respect to TMS assignable for proton attached to phenolic -OH. A complex multiplet was observed at \(\delta = 7-7.4\) ppm is attributed to aromatic protons. A sharp signal was observed at \(\delta = 3.44\) ppm is attributed to- CH\(_2\) protons. Signal at \(\delta = 6.3\) ppm (singlet) account for the N-H proton of the pyridoxal ring. A sharp singlet at \(\delta = 8.2\) ppm is assigned for azomethine proton [200].
The $^{13}$C spectra of HMHPMP$^b$ show the presence of carbon atoms corresponding to methyl (34ppm, 39ppm, 40ppm), C-OH (157ppm, 159ppm) and aromatic (138-142ppm) carbon atoms. These results confirm the proposed structure.

2.4.3 UV-Visible spectra

The spectra of HMHPMP$^b$ exhibit transitions around 268nm, which may be attributed to the $\pi \rightarrow \pi^*$ transitions and another at 340nm attributed to n→$\pi^*$ transitions [201].

2.5 Analyses of complexes- Physicochemical methods

2.5.1 TLC method

The purity of the synthesized compounds was checked by TLC technique using a mixture of chloroform and acetone (2:2 V/V) and various ratios of methyl acetate: acetone solvent mixture as eluents. The developed plate was kept in iodine chamber for spot location [202].

2.5.2 Melting point determination

The melting point of the synthesized ligands and complexes was determined on a Toshniwal melting point apparatus (Toshniwal Pvt. Ltd, India).

2.5.3 Elemental analysis

The chemical compositions of the Schiff bases and complexes were estimated by analyzing for the percentage of C, H and N on a Heracus CHN Rapid Analyzer (1104 28) at SAIF, CUSAT, Kochi. This method gives information regarding the metal-ligand ratio and quantification of simple molecules, solvent, water etc.
2.5.4 Estimation of metal content

The metal content in the complexes were analyzed by the ‘pyrolysis’ method [197]. About 1.5g of the complex was weighed accurately into a clean porcelain crucible. About five times its weight of finely powdered resublimed ammonium chloride was added and mixed well by shaking the crucible. The crucible was covered with a lid and heated gently till fuming was completed. The contents in the crucible were ignited to the metal oxide. The percentage of metal was estimated from the weight of the metal oxide obtained [197].

2.5.5 Estimation of chloride

0.1g of the complex was accurately weighed into a conical flask and 50mL methanol was added followed by 20mL 0.1N standard silver nitrate solution. About 10mL of 6N HNO₃ was added to acidify the solution. The contents were heated, cooled and titrated against 0.1N KSCN solution using ferric alum indicator [197].

2.5.6 Estimation of perchlorate

The amount of perchlorate in the complexes was estimated by titrating the chloride obtained by converting the perchlorate by reduction with NaNO₂. About 0.2g of finely powdered sodium nitrite was taken in a nickel crucible such that the bottom of the crucible was covered with a part of this sodium nitrite. Powdered complex was placed in it. Care should be taken to ensure that the complex does not touch any part of the crucible. The crucible was covered and transferred into a muffle furnace at 500ºC and removed after half an hour and cooled, dissolved in distilled water, transferred into a 250mL beaker, acidified with 6N nitric acid and titrated against KMnO₄ solution. 20mL 0.1N silver nitrate solution and 3mL nitrobenzene were added followed
by 1mL ferric alum indicator. The excess silver nitrate was back titrated with 0.1N potassium thiocyanate solution, until a faint brown color persisted [197].

2.5.7 Electrical conductance measurements

Electrical conductance measurement was used for the structural study of coordination compounds. From the value of the electrical conductance, nature of the coordination, the electrolytic nature of the complex and coordination number can be estimated. It helps to determine the number of ions present inside or outside the coordination sphere. Electrolytes obey Ohm's law just as metallic conductors do. Thus, the current $I$ passing through a given solution is proportional to the applied potential difference $V$. The resistance $R$ of the solution in ohms is given by

$$R = \frac{V}{I}$$

where the potential difference, $V$ is expressed in volts and the current, $I$ in amperes. The conductance, defined as the reciprocal of the resistance, of a homogeneous body of uniform cross section is proportional to the cross-sectional area $A$ and inversely proportional to the length $l$

$$\frac{1}{R} = \kappa \frac{A}{l}$$

where, $\kappa$ is the specific conductance (By international agreement, the reciprocal ohm is now called a Siemen). Molar conductance of a solution is obtained from the measured value of specific conductance. Specific conductance ($\kappa$) is related to the resistance ($R$) by the expression

$$\kappa = \frac{\text{Cell constant}}{R}$$
The cell constant is determined by measuring the resistance of a cell filled with a solution of known specific conductance. Molar conductance \( \Lambda \) of a solution is determined from the measured value of specific conductance from the equation

\[
\Lambda = \frac{(1000 \, K)}{c}
\]

\( \Lambda \) is the conductance \((cm^2mol^{-1}ohm^{-1})\), \( c \) is the concentration in \( molL^{-1} \) and \( K \) is the cell constant.

Once the cell constant has been determined, we can calculate molar conductance of any solution from experimental resistances by using above equation. The conductivities of all the complexes measured at room temperature using Toshniwal Conductivity Bridge with a dip type conductance cell with platinum electrode having cell constant 0.9658cm\(^{-1}\). The solvents used in the present study are methanol, ethanol, acetonitrile, DMF and nitrobenzene. The reference value for the molar conductivities of various common solvents [204] are tabulated in Table 2.1.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Electrolyte type</th>
<th>1:1</th>
<th>2:1</th>
<th>3:1</th>
<th>4:1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrobenzene</td>
<td>1:1</td>
<td>20-30</td>
<td>50-60</td>
<td>70-82</td>
<td>90-100</td>
</tr>
<tr>
<td>Methanol</td>
<td>1:1</td>
<td>80-115</td>
<td>160-220</td>
<td>290-350</td>
<td>450</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>1:1</td>
<td>120-160</td>
<td>220-300</td>
<td>340-420</td>
<td>500</td>
</tr>
<tr>
<td>DMF</td>
<td>1:1</td>
<td>65-90</td>
<td>130-170</td>
<td>200-240</td>
<td>300</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1:1</td>
<td>35-45</td>
<td>70-90</td>
<td>120</td>
<td>160</td>
</tr>
<tr>
<td>Acetone</td>
<td>1:1</td>
<td>100-140</td>
<td>160-200</td>
<td>270</td>
<td>-</td>
</tr>
</tbody>
</table>

2.5.8 Magnetic moment studies

Electrons have a magnetic moment that can be aligned with or in opposition to an applied magnetic field, depending on whether the spin
magnetic quantum number, $m_s$, is $\pm \frac{1}{2}$ or $\mp \frac{1}{2}$. For an atom or ion with only paired electrons, the individual electron contributions to the overall spin magnetic quantum number, $m_s$, giving a net value of zero i.e. $S = 0$. Such species are diamagnetic. If a diamagnetic material is placed between the poles of a strong magnet, it will experience repulsion for the applied field. The repulsion arises from circulation of the electrons caused by the applied field, resulting in an induced magnetic field in opposite direction. When the sample is suspended between the poles of the magnet and connected to the pan of an analytical balance, because of the induced diamagnetic repulsion, the sample will appear to weigh less in the magnetic field, compared to its true weight outside the field. When removed from the applied field, the sample has no residual magnetic moment, and its apparent weight will be its true weight. If the sample contains unpaired electrons, the overall spin quantum number will be greater than zero; i.e. $S > 0$, such species are paramagnetic. If a paramagnetic species is placed between the poles of a strong magnet, it will experience an attraction for the field, due to the alignment of the permanent paramagnetic moment with the applied field. If the sample is weighed with a Gouy balance, it will appear to be heavier in the magnetic field, compared to its true weight outside the field [205].

Magnetic susceptibility is the ratio of the intensity of magnetization induced in a substance to the magnetizing force or intensity of free field value. The magnetic moment $\mu$, results from both the spin and orbital contributions of these unpaired electrons. The presence of coordinated ligands around the metal ion quenches the orbital contribution to greater or lesser degree, making the spin contribution most important. The expected magnetic moment for an ion with a certain number of unpaired electrons can be estimated from the spin-only magnetic moment, $\mu_s$, which disregards orbital contributions
\[ \mu_s = g \sqrt{S(S+1)} \]  

Where \( g \) is the gyromagnetic ratio (\( g = 2.00023 \)) and \( S = n(\frac{1}{2}) \), where \( n \) is the number of unpaired electrons in the configuration. Substituting \( g = 2 \) and \( S = n(\frac{1}{2}) \) into equation (1), we can calculate the spin-only moment in terms of the number of unpaired electron.

\[ \mu_s = g \sqrt{n(n+2)} \]  

Magnetic moment is calculated from the measured magnetic susceptibility \( \chi \). The sample's magnetic susceptibility per gram called the gram magnetic susceptibility, \( \chi_g \).

\[ \chi_g = \frac{L}{m} [C(R-R_0) + \chi_v' A] \]

Where \( \chi_g \) = gram magnetic susceptibility, \( L \) = sample length in centimeters, \( m \) = sample mass in grams, \( C \) = balance calibration constant (different for each balance, calculated in this experiment using a calibration standard). In this case, it is 1, \( \chi_v' \) = volume susceptibility of air (0.029 x10\(^{-6}\)ergG\(^{-2}\)cm\(^{-3}\)), \( A \) = cross-sectional area of the sample, \( R \) = reading from the digital display when the sample (in the sample tube) is in place. The volume susceptibility of air usually ignored with solid samples, so equation becomes

\[ \chi_g = \frac{CL[R - R_o]}{m10^9} \]

The molar magnetic susceptibility, \( \chi_m \) is obtained from the gram magnetic susceptibility by multiplying with the molecular weight of the sample in units of gmol\(^{-1}\); i.e.

\[ \chi_m = \chi_g M \]
This experimentally obtained value of $\chi_m$ includes both paramagnetic and diamagnetic contributions ($\chi_A$ and $\chi_\alpha$ respectively). All sources of paired electrons (e.g., ligands, counter ions, core electrons on the paramagnetic species) contribute to the diamagnetic portion of the overall susceptibility. We are interested in the paramagnetic molar susceptibility, which is obtained by removing the diamagnetic contributions from $\chi_m$. Therefore,

$$\chi_A = \chi_m - \sum \chi_a$$

Thus

$$\mu_{\text{eff}} = 2.828 \sqrt{T \chi_A}$$

Lanthanide systems are materials with fascinating magnetic properties. The magnetic moments of the heavy lanthanide metals are mainly responsible due to their 4f electrons and their involvement in bonding. Being deeply buried in the atom, the f-electrons barely participate in coordination [206-210].

2.6 Spectroscopic methods
2.6.1 Infrared spectra

FTIR spectra of pure compounds are unique and regarded as molecular "fingerprint". Molecular bonds vibrate at various frequencies depending on the elements and type of bonds. For any given bond, it vibrates at several specific frequencies. The infrared spectrum of a molecule results due to the transition between two different vibrational energy levels when certain radiations of characteristic frequency fall on that molecule. For any given transition between two states, the ground state ($v_0$) to the first excited state ($v_1$) gives rise to a band called fundamental band. The energy of the ground state ($E_0$) and first excited state ($E_1$) given by
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\[ E_1 - E_0 = h\nu \] where \( \nu \) is the frequency of light

The fundamental vibrational frequency of a molecular ensemble can be expressed as

\[
\nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}}
\]

where, \( \nu \) is the fundamental vibration frequency, \( k \) force constant, and \( \mu \), the reduced mass. The reduced mass, \( \mu = \frac{m_1 m_2}{m_1 + m_2} \) where \( m_1 \) and \( m_2 \) are the masses of atoms involved in the chemical bond under consideration. This simple equation provides a link between the strength of the covalent bond between two atoms, the mass of the connecting atoms and the frequency of vibration [199].

If a ligand coordinates to a metal ion, some of the ligand bands are shifted to lower or higher frequency with prominent changes in position of the band. The vibrations are also sensitive to the nature of the anion. When the ligand have more than one donor atom, it is possible to determine the coordination sites from the observed band shifts. The observed frequency of the ligand is compared with that of the complexes. The ligand stretching band frequency can be increased or decreased. The lowering of the frequency is mainly due to the lowering of bond order upon complexation and the shift of bands to higher frequency is when the situation is reversed [211,212].

Thus the main conclusions that emerge from IR spectra are

1) The nature of anion coordination
2) The coordination sites of the ligand
3) The relative strengths of metal-ligand bonds
4) Coupled vibrations and skeletal vibrations of the whole molecule

When the anion is coordinated, it is possible to identify whether unidentatively, bidentatively or in some other manner by analyzing the IR spectral bands in a specified region. Thus in the case of nitrate ion and perchlorate ion, the mode of coordination could be predicted from the frequencies [213].

**Nitrate ion**

According to selection rules, the free nitrate ion exhibit one stretching and one *in plane* bending band in the infrared spectrum. According to resonance theory, free nitrate ion in the state is an average of the three structures (Scheme 4), the so called resonance hybrid, belonging to the molecular point group $D_{3h}$.

![Scheme 4](image)

When the nitrate ion is present as an anion in complex, it can be coordinated to the metal ion as unidentate, symmetric and asymmetric chelating bidentate and bridging bidentate ligand of various structures as given in Scheme 5.

![Scheme 5](image)
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In both unidentate and bidentate coordination, each of these bands split into two. The totally symmetric Raman active stretching mode becomes infrared active. These two can be identified from their stretching frequencies. The unidentate nitrate complex exhibit two N-O stretching bands at \( \sim 1420\text{cm}^{-1} \) and \( \sim 1305\text{cm}^{-1} \) whereas bidentate nitrate complex exhibit bands at \( \sim 1480\text{cm}^{-1} \) and \( \sim 1300\text{cm}^{-1} \). The difference \( \nu_4-\nu_1 \) is taken as an approximate measure of the covalency of the metal–nitrate bonding. For unidentate coordination, the separation is \( \sim 110\text{cm}^{-1} \) and for bidentate coordination, it is \( \sim 170\text{cm}^{-1} \) [213, 214]. The combination bands could also be used for structural elucidation. The number and relative energies of the combination frequencies \( (\nu_2+\nu_3) \) and \( (\nu_2+\nu_5) \) can be used as a tool to distinguish the various coordination modes of the nitrate group. A value \( \geq 50\text{cm}^{-1} \) in the separation of combination bands support bidentate coordination and \( \sim 8-25\text{cm}^{-1} \) suggests monodentate coordination. This combination frequency observed in the region of the IR spectrum \( 1800-1700\text{cm}^{-1} \) corresponds to monodentate coordination.

Perchlorate ion

A free perchlorate ion with a \( T_d \) symmetry has four fundamental vibrational bands in the region \( \sim 920-928\text{cm}^{-1} \), \( \sim 459\text{cm}^{-1} \), \( \sim 1100-1120\text{cm}^{-1} \) and \( \sim 615-625\text{cm}^{-1} \). Of the four, two of them \( \nu_3 \sim 1100\text{cm}^{-1} \) which was attributed to the asymmetrical stretching and \( \nu_4 \sim 624\text{cm}^{-1} \) attributed to the asymmetrical bending are IR active. However, all of them are Raman active. Both the IR bands are triply degenerate. The other two bands, \( \nu_1 \) band observed around the region \( \sim 930\text{cm}^{-1} \) assignable to the symmetrical stretching was non degenerate. Moreover, the symmetrical bending \( \nu_2 \) band observed at around \( 460\text{cm}^{-1} \) which was doubly degenerate were only Raman active.
Table 2.2 Vibrations of the perchlorate ion as a function of symmetry

<table>
<thead>
<tr>
<th>State of CrO₄</th>
<th>Symmetry</th>
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<tr>
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<td>T₄</td>
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</table>

Materials and Physical Methods
In the case of perchlorate complexes, the tetrahedral perchlorate ion changes its symmetry to $C_{3v}$ upon coordination. In unidentate coordination, $\nu_3$ and $\nu_4$ split into two bands and $\nu_1$ and $\nu_2$ vibrations which are Raman active in the free state become infrared active. Thus, three stretching and three bending frequencies are observed in the infrared spectrum. In bidentate coordination, the $T_d$ symmetry is lowered to $C_{2v}$, all the degenerate vibrations split to triplet, and we expect four stretching bands and four bending bands in the IR spectrum [215,216].

The metal–nitrogen and metal–oxygen bonds are most common in coordination compounds. Studies of these bond stretching and bending modes are highly important in elucidating the structure of the complex and the nature of bonding. When a ligand is coordinated to a metal ion, the electron density on the donor atom decreases and this drainage of free electron pair from the ligand increases the metal-ligand bond strength. Thus, these bond stretching vibrations are assigned by using either one or a combination of the following methods [217].

1) A comparison of infrared spectra between a free ligand and its metal complex.

2) The metal–ligand vibrations should be metal sensitive.

3) The metal–ligand vibrations show an isotopic shift if the ligand is isotopically substituted.

When a ligand is coordinated to the metal atom or ion, the symmetry properties of the ligand are not totally changed. Hence, interpretation of the bands in the vibrational spectrum of the complex is easy. On coordination, the electron density of donor atom decreases. Consequently, the bond order increases thereby the stretching frequency
also get modified. Thus, shifts in characteristic vibrational frequencies yield valuable informations regarding the mode of coordination. When functional group like OH, C=O, C=N are present, shift in frequency band is a strong evidence for coordination. The present investigation was carried out using Shimadzu IR 470 spectrophotometer in KBr pellets in the range 400-4000cm⁻¹. Far IR spectral analyses were done in the range 190-400cm⁻¹.

2.6.2 NMR spectra

Nuclear magnetic resonance spectroscopy (NMR) has become a very useful technique for determining the structure of organic compounds. A spinning charge generates a magnetic field and the resulting spin-magnet has a magnetic moment (µ) proportional to the spin in the presence of an external magnetic field (B₀), and two spin states exist, +½ or –½. The magnetic moment of the lower energy +½ spin state is aligned with the external field, that of the higher energy –½ spin state is opposed to the external field. The difference in energy between the two spin states is dependent on the external magnetic field strength, and is always very small. For NMR purposes, this small energy difference (∆E) usually given as a frequency in units of MHz (10⁶Hz), ranging from 20 to 900MHz, depending on the magnetic field strength and the specific nucleus being studied. The technique consisting of exposing the protons in a molecule to a field exactly corresponds to the spin state separation of a specific set of nuclei will cause excitation of those nuclei in the +½ state to the higher –½ spin state. NMR spectroscopy is therefore a probe used to examine the structure of molecules [200].

In ¹H NMR, number of signals in the NMR spectrum tells the number of different set of equivalent protons in a molecule. Each signal
corresponds to a set of equivalent protons. The position of signals in the spectrum is also important. It gives an idea about the nature of protons, whether aromatic, aliphatic, alcoholic, acetylenic, vinylic, etc. Multiplicity of the signal in a proton NMR spectra is given by \((n+1)\) where \(n\) is the number of coupling protons on neighbor atoms. The interpretation of NMR spectra of paramagnetic complexes is complicated due to signal broadening and large isotopic shift of resonance signals. The proton NMR spectra can conveniently be used to study the coordination sites of ligands and the way in which the ligand gets coordinated to the metal ion. The change in \(\text{N}=\text{CH}\) proton signal can be taken as an evidence for the coordination of the azomethine nitrogen [218-220]. The NMR studies of the ligand and diamagnetic lanthanum complex were done using Bruker DRX 500 NMR instrument up to 900Hz using the solvent DMSO-d6.

\(^{13}\)C NMR methods were employed for the characterization of the ligand. By inspecting the \(^{13}\)C spectrum, it is possible to recognize the nuclei that do not bear protons. It is also possible to recognize carbonyl group, alkene and alkyne carbon atoms. A \(^{13}\)C NMR spectrum consists of discrete sharp lines corresponding to each nonequivalent carbon atoms. This spectrum gives idea about the chemical environment of each carbon atom and the carbon framework of the complex molecule. The \(^{13}\)C NMR spectra can conveniently be used to study the coordination sites of ligands containing carbonyl group and the way in which it get coordinated to the metal ion. The change in \(\text{C}=\text{O}\) signal can be taken as an evidence for the coordination of carbonyl oxygen. The coordination through the ring nitrogen such as pyridine and pyrazolones can be established taking the changes in chemical shift of the carbons in the ring. The proton attached to the carboxylate ion and the hydrogen atom attached to the amino group is
unstable and are affected by the electronic configuration of the molecule caused by coordination [221].

In the present investigation, $^{13}$C NMR spectra was recorded on a Bruker DRX -500 spectrophotometer using DMSO–d$_6$ solvent.

2.6.3 UV-Visible spectra

Absorption spectra are useful tool to study the optically induced transitions, energy band and structure of compounds. This provides quick and reliable information about the arrangement of ligands around the metal atom. The collision of a photon of suitable energy with the appropriate molecule results in absorption of light. Beer–Lambert law relates the absorption of molecular species to the concentration (c), the path length (l) and the molar absorptivity ($\varepsilon$).

$$A = \varepsilon cl,$$

$\varepsilon$ is known as the extinction coefficient. The Beer–Lambert law holds for the majority of compounds over a wide range of experimental conditions. In any spectroscopic transition, both the ground level and excited level are equally important in determining the spectral features [201]. Electronic transitions occur when electrons within the molecule or ion move from one energy level to another and the vibrational and rotational motion of the molecule changes. Thus electronic spectrum consists of several absorption lines.

1) Ligand to metal charge transitions
2) Metal to ligand charge transitions
3) Intraligand transition involving one ligand orbital to another.
Generally, $n \to \pi^*$ transitions appear at longer wavelength than $\pi \to \pi^*$ transitions.

A comparison of the solution spectra of lanthanide complexes of various ligands with those of aqua complexes reveals that

1) Small displacement towards longer wavelength is commonly observed.

2) Development of fine structures.

3) Abnormal changes in the intensities of some of the bands.

The spectral intensities of trivalent lanthanide ions are tuned by appropriate choice of the medium. Lanthanide ions have the unique property of sharp spectral lines due to $f-f$ transitions. Since the $f$ orbitals are buried within the atom, they interact only very weakly with ligand orbitals. Consequently, the $f-f$ transitions, which occur in the visible region of the spectrum, give rise to very narrow bands and the lifetimes of their excited states are quite long lived.

These changes are attributed to the alterations in the inter electron repulsion parameter $\beta$ due to nephelauxetic effects which is a measure of the metal-ligand covalent bonding [222-225].

\[
\beta = \frac{V_{\text{complex}}}{V_{\text{aquo}}}
\]

The bonding parameter is expressed as

\[
b^{1/2} = \frac{1}{2}((1-\beta)^{1/2}]
\]

The Sinha parameter for covalency is expressed as

\[
\delta\% = \frac{(1-\beta)/\beta}{\times 100}
\]
The electronic spectral studies of the Schiff bases and their complexes were recorded at 300K in HPLC grade DMSO and DMF solutions using a Shimadzu UV 160A spectrophotometer in the range 190-900nm at SAIF, CUSAT, Kochi.

2.7 Thermal Analysis

Thermal analysis is a technique, which enable to measure physical properties of a substance and its reaction products as a function of temperature while the substance is subjected to a controlled temperature programme in specific atmosphere. The most common thermal analysis techniques are thermogravimetry (TG) and differential thermal analysis (DTA).

For the present study, the TG/DTA curves were recorded under a dynamic nitrogen atmosphere at a flow rate of 50mL/min and at a heating rate of 10K/min on a Perkin Elmer Thermal Analyzer, DT100201A, using Al$_2$O$_3$ crucibles. For the TG/DTA experiments the sample mass was between 10 -15mg.

In TG, a plot of mass (or % mass) as a function of time is called thermogram. Any weight change associated with chemical change can be studied from the data. The changes in mass may be due to the rupture of molecule or due to elimination of simple molecules, which are characteristic for a given compound. Decomposition, loss of water or gaseous products, etc. can cause mass loss. In DTA, relatively weak endothermic phenomenon was observed corresponding to the elimination of small gaseous molecules. In addition, any phase changes and corresponding energy changes are
Chapter 2

recorded in DTA. In DSC, usually melting point, enthalpies of fusion, phase transition etc. can be traced [226].

TG and DTA studies have much more importance in the case of coordination compounds. This may be because on varying temperature, the complexes may be changing to another product with the partial or complete removal of ligands, anions and coordinated or uncoordinated water molecules.

Non-isothermal studies are mainly focused on the phenomenological aspects. From this aspect, it is possible to find out the thermal stability of the substance. The kinetic analysis of decomposition can be examined with reference to different models of heterogeneous solid-state reactions. Under isothermal conditions, the rate constant k is independent on reaction time, hence,

\[ g(\alpha) = kt \]

Here two methods were followed for the kinetic study.

1) The Ozawa method and
2) The Coats–Redfern Method

The kinetic function \( g(\alpha) \) and linear heating rate \( \beta \) were made use in the modified Coats – Redfern equation.

\[
\log \left[ -\frac{\log(1-\alpha)^{1-n}}{T^2 \left(1-n\right)} \right] = \log \frac{AR}{BE} \left[ 1 - \frac{2RT}{E} \right] - \frac{E}{2.303RT} \quad \text{for } n \neq 1
\]

where \( g(\alpha) \) is the kinetic model function calculated for the fraction of compound reacted \( \alpha \) at temperature T and heating rate \( \beta \).
\[
\left(\frac{-\log(1-\alpha)}{T^2}\right) = \log \frac{AE}{B} \left[1 - \frac{2RT}{E}\right] - \frac{E}{2.303RT} \quad \text{for } n=1
\]

where, A is the frequency factor, E is the energy of activation [227].

A plot of \(\log \left[\frac{g(\alpha)}{T^2}\right]\) versus \(\log \left(\frac{1}{T}\right)\) is a straight line which is in agreement with a non-isothermal process. The slope \((E/R)\) of the straight line gives the energy of activation \(E_a\) and the y-intercept gives the frequency factor A.

### 2.8 Biological study

Bacteria are highly diverse. A fraction of these is pathogenic, with serious consequences for the health of humans and animals. They cause a vast spectrum of disease from food poisoning, toxic shock syndrome etc. Defence mechanisms are essential to prevent invasion and colonization by pathogens. The recognition of microbes is mainly due to binding of pathogen-associated molecular patterns. Efficiency in killing or inhibiting these microorganisms follow the sensing by bactericidal substances. The efficiency of killing or inhibiting the growth of microbes can be recognized by the screening tests. The methods adopted in the present investigations are described below. The effect of eight bacterial strains of clinical interest was used in the present investigation [228].

#### 2.8.1 Selected organisms

Isolates of bacteria were collected from Department of Microbiology and Biotechnology, CUSAT, Kochi, Kerala. \((Escherichia\ coli\ MTCC\ 443,\ Bacillus\ subtilis\ MTCC\ 441,\ Staphylococcus\ aureus\ MTCC\ 96,\ Pseudomonas\ aeruginosa\ MTCC\ 424,\ Bacillus\ cereus\ MTCC\ 430,\ Salmonella\ typhimurium\ MTCC\ 98,\ Klebsiella\ pneumonia\ MTCC\ 109)\).
This was mixed with saline (85%) and turbidity was adjusted to the standard inoculation of the Mc. Farland scale (10^6 CFU per millimeter) [229, 230].

2.8.2 Disc-plate method for (DMSO and DMF as solvent for metal complexes) sample preparation and sterilization:

A solution (1mg/mL in DMF /DMSO) of the test compound was filtered through a sterile membrane into a previously sterilized sample container. Cprofloxacin, 1mg/mL in distilled water was used as positive control [230].

2.8.3 Preparation of nutrient broth

A solution of the nutrient powder was prepared by dissolving 13g nutrient agar in 1 litre water, heated with frequent agitation and boiled for 1 minute. Dispense into sterilized flask. The opening of the flask was then covered with aluminium foil and the solution was autoclaved for 15 minutes at 121°C. Broth were poured into sterile tubes (fitted with lids) and incubated for 24 hours at 37°C [230].

2.8.4 Nutrient agar plates

A solution of the nutrient powder was prepared in an autoclave-safe Erlenmeyer flask by dissolving 28g agar in 1 litre water. The opening of the flask was then covered with aluminium foil and the solution was autoclaved for 15 minutes at 121°C. The solution was left to cool to bearable warmth. 15-20mL of this hot solution aseptically poured into each of the required number of sterilized petri dishes and each was covered with its lid. When the agar was set, the plates were inverted, placed in plastic bags and incubated at 37°C for 24 hours. Plates were then visually inspected for possible growth of micro-organisms. Different plates are used for different organism [230].
2.8.5 Obtaining a disc-plate

For each bacterial strain, 0.1mL of a pure culture suspended in nutrient broth was evenly spread over the surface of the nutrient agar using a sterile glass spreader and allowed for adsorption or diffusion of the microbial inoculums into the agar. For each test sample, a sterile paper disc (Whatman, 13mm diameter) was carefully placed on a lawn of microbial growth (Gülgür et al, 2003). 20µL test solution was dropped on the disc and labeled. Triplicate plates of each test sample for antibacterial and antifungal testing were prepared. The plates were inverted and sealed in plastic bags. This was incubated at the respective temperatures. After 24 hours these were removed and the surface of the nutrient agar examined. A clear zone, which is called inhibition zone or radial diffusion zone around a test-solution-dipped disc would indicate bactericidal activity against the bacterial species grown on the particular nutrient agar plate [230].

2.8.6 Minimum Inhibitory Concentration (MIC)

In clinical laboratories, MIC was used to establish the susceptibility of organisms. Further, MIC tests were used to determine the activity of new compositions and to confirm resistance of antibacterial agents. In dilution tests, microorganisms are tested for their ability to produce visible growth on a series of agar plates (agar dilution) or in micro plate wells of broth containing different dilutions of the antimicrobial agent. The lowest concentration of antimicrobial agent that will inhibit the visible growth of a microorganism is known as the MIC. The ranges of concentrations tested will depend on the organisms and antimicrobial agents being tested [231].
Chapter 2

A stock solution of desired concentration was prepared. Here we use 0.5 molar solution for each sample and desired dilutions are prepared. Twenty milliliter volumes of agar are used in 9cm Petri dishes for agar dilution MICs. A known concentration of bacteria is inoculated with different concentrations of the desired antimicrobial agent. Visible clouding indicates that the antibacterial agent has not worked and there is a visible growth of bacteria. Such clouding will not take place if the antimicrobial agents are successful in inhibiting or killing the bacteria. MIC value is determined by the lowest concentrations of the antimicrobial (mg/L) where no bacterial growth is visible.

2.8.7 Antifungal studies

Antifungal activity was assessed by disc diffusion method. The isolates of fungi were transferred from stocks to Potato dextrose agar and then sub cultured to enhance sporulation. Seven day-old cultures were covered with 1mL distilled water and the colonies were probed with the tip of a sterile Pasteur pipette to obtain the fungi. The suspensions were transferred to sterile tubes and allowed to sediment for 30minutes. The procedure for antibacterial screening was adopted for the disc applications.

2.8.8 Disc diffusion assay

All the tests were performed according to Esteban et.al [232]. The inoculums were evenly spread on the surface of petri dishes containing dextrose agar medium and it was exposed to air. The antifungal discs were applied to the plates, after which the plates were incubated at 25°C for 5-10days. After the colonies grew, the zone of inhibition around the discs were measured and recorded [233].
2.8.9 Algal studies

Most algae are aquatic, living in salt or fresh water. They produce oxygen during photosynthesis. In many ways, algae are useful and play significant role in human and animal life. Since algae require light for growth, they are seen at the top surface of water. Algae can be problematic when its reproduction becomes rapid. This makes water colored and mate of algae known as algae blooms, are produced. Algae blooms can increase the pH level of water as high as 9.5. Increase in pH destroys many natural processes in water. Prevention of algae blooms is the only method to maintain life in water.

2.8.10 Instrumentation

Growth rate of microalgae was estimated by measuring the absorbance of the culture at 750nm in Systronics Visiscan 167-model spectrophotometer. The absorbances of the cultures were converted into cell number.

\[
\text{Growth rate } r = \frac{(\ln N_t - \ln N_o)}{t}
\]

Where
- \(r\) = exponential growth rate
- \(N_t\) = population size at the end of time interval
- \(N_o\) = population size at the beginning of time
- \(t\) = length of time

Sterile algae nutrient medium (Table 2.3-2.5, Solution A-C.) were inoculated with algal cells. These were incubated for 3 days in an orbital incubator under continuous illumination at normal temperature (24-26°C). A portion of this culture was transferred into fresh sterile algal nutrient medium to prepare a secondary liquid culture. The culture was incubated for three days and subjected to analysis. The absorbance was measured for each culture before the addition of test samples. To 5mL of each culture, 1mL test solution was added and absorbance was again recorded. This is
the zero\textsuperscript{th} day reading. The test solution was kept under illumination for fifteen days and readings were recorded after every five days (Fig 2.1). The growth rate was calculated [234].

![Fig. 2.5 Algal culture medium](image)

### 2.8.11 Media composition

Conway Walne’s medium was prepared. The compositions are given below. For preparing media, 1mL of solution A (Table 2.3), 0.5mL of solution B (Table 2.4) and 0.1mL of solution C (Table 2.5) were added to 1000mL autoclaved seawater.

<table>
<thead>
<tr>
<th>Table 2.3 Solution A for Walne’s medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO\textsubscript{3}</td>
</tr>
<tr>
<td>Na\textsubscript{2}HPO\textsubscript{4}</td>
</tr>
<tr>
<td>Na\textsubscript{2}EDTA.2H\textsubscript{2}O</td>
</tr>
<tr>
<td>Citric acid</td>
</tr>
<tr>
<td>FeCl\textsubscript{3}.6H\textsubscript{2}O</td>
</tr>
<tr>
<td>MnCl\textsubscript{2}.4H\textsubscript{2}O</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
</tbody>
</table>
Table 2.4 Solution B for Walne’s medium

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>ZnCl₂</td>
<td>0.42g</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>0.4g</td>
</tr>
<tr>
<td>CoCl₂.6H₂O</td>
<td>0.4g</td>
</tr>
<tr>
<td>Am. Molybdate</td>
<td>0.18g</td>
</tr>
<tr>
<td>Distilled Water</td>
<td>100mL</td>
</tr>
</tbody>
</table>

Table 2.5 Solution C for Walne’s medium

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Thiamine HCl</td>
<td>200mg</td>
</tr>
<tr>
<td>Vitamin B₁₂</td>
<td>10mg</td>
</tr>
</tbody>
</table>

The cultures of *Dunaliella salina*, *Isochrysis*, *Marine chlorella* and *Nannochloropsis* were obtained from the department of Marine Biology, Microbiology and Biochemistry, School of Marine science, CUSAT, Kochi. Stock cultures of algae were raised in 1000mL Erlenmeyer flasks containing 500mL Walne’s medium. Illumination was provided by white fluorescent light of 2000 tux for a period of 72 hours.