4.2.9. Cleavage of Plasmid pUC18 DNA

The ability of [Cu$_3$(L$^3$)Cl$_6$], [Ni$_3$(L$^3$)Cl$_6$], [Mn$_3$(L$^3$)Cl$_6$], [CuNi$_2$(L$^3$)Cl$_6$] and [NiCu$_2$(L$^3$)Cl$_6$] complexes to perform DNA cleavage in the presence of H$_2$O$_2$ has been studied by agarose gel electrophoresis using supercoiled pUC18DNA in a medium of 50 mM Tris–HCl/NaCl buffer. All the complexes show considerable DNA cleavage ability at high concentrations. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoil form (Form I). If scission occurs on one strand (nicking), the supercoil will relax to generate a slower moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) that migrates between Forms I and II will be generated [33].

Figure 4.22 shows the gel electrophoretic results of trinuclear complexes interaction with plasmid pUC18 DNA. The control experiments did not show any apparent cleavage of DNA (lane 1 & 2). The [Cu$_3$(L$^3$)Cl$_6$] trinuclear complex in the presence of H$_2$O$_2$ (lane 3) at higher concentration (50µM) shows more cleavage activity. The supercoiled plasmid DNA was completely degraded. This shows that a slight increase in the concentration over the optimal value led to extensive degradations, resulting in the disappearance of bands on agarose gel. The [Ni$_3$(L$^3$)Cl$_6$] trinuclear complex in the presence of H$_2$O$_2$ resulting the conversion of supercoiled form (Form I) into linear form (Form III) (lane 4). [Mn$_3$(L$^3$)Cl$_6$] homotrinuclear complex in the presence of H$_2$O$_2$ (lane 5) at higher concentration (50µM) shows cleavage activity in which supercoiled DNA (Form I) cleaved and supercoiled form converted to open circular form (Form II).
The heterotrinuclear [CuNi₂(L³)Cl₆] complex in the presence of H₂O₂ (lane 6) at higher concentration (50μM) shows cleavage activity in which supercoiled DNA (Form I) cleaved and supercoiled form converted to open circular form (Form II). [NiCu₂(L³)Cl₆] trinuclear complex in the presence of H₂O₂ resulting the conversion of supercoiled form (Form I) into linear form (Form III) (lane 7). These highly reactive hydroxyl radical species can cleave the DNA by abstraction of the hydrogen atom from the deoxyribose sugar. The cleavage ability of the homotrinuclear Cu(II) and heterotrinuclear complexes are higher than the homotrinuclear Ni(II) and Mn(II) complex.

Figure 4.22. Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H₂O₂ and metal complexes: Lane 1, DNA alone; Lane 2, DNA alone + H₂O₂; Lane 3, DNA + [Cu₅(L³)Cl₆] + H₂O₂; Lane 4, DNA + [Ni₃(L³)Cl₆] + H₂O₂; Lane 5, DNA + [Mn₃(L³)Cl₆]+ H₂O₂; Lane 6, DNA+ [CuNi₂(L³)Cl₆] + H₂O₂; Lane 7; DNA + [NiCu₂(L³)Cl₆]+ H₂O₂.
4.2.10. Antimicrobial assay

The main of the production and synthesis of any antimicrobial compound is due to inhibit the causal microbe without any side effects on the patients. In addition, it is worthy to stress here on the basic idea of applying any chemotherapeutic agent which depends essentially on the specific control of only one biological function and not multiple ones. The chemotherapeutic agent affecting only one function has a highly sounding application used in the present time, affect both cancerous diseased cells and healthy ones which in turns affect the general health of the patients, Therefore, there is a real need for having a chemotherapeutic agent which controls only one function [34].

In testing the antibacterial activity of these entire complexes more than one test organism was used to increase the chance of detecting antibiotic principles in tested materials. All of the tested compounds show a remarkable biological activity against different types of Gram positive and Gram negative bacteria. The data are listed in Table 4.7 and Figures 4.23–4.25 describes the difference between the antimicrobial activities of the trinuclear complexes. The biological activity of [Cu₃(L₃)Cl₆], [Ni₃(L₃)Cl₆], [Mn₃(L₃)Cl₆], [CuNi₂(L₃)Cl₆] and [NiCu₂(L₃)Cl₆] homo/heterotrinuclear complexes are higher than that of the standard streptomycin. In addition, the biological activity of the homotrinuclear Cu(II) and heterotrinuclear complexes are higher than the homotrinuclear Ni(II) and Mn(II) complex.

All the homo and heterotrinuclear complexes seem to inhibiting Gram positive and Gram negative bacterial strains. The importance of this unique
property of the investigated Schiff base complexes lies in the fact that, it can be applied safely in the treatment of infections caused by any of these particular strains. Since almost all scientists working in the field of search for new antitumor depends basically on the line of antibiotics affecting Gram negative bacteria. Moreover, there are certain organisms which have proved difficult to treat and most of them are Gram negative rods, it is therefore believed that all the complexes which are biologically active against both the Gram negative strains may have something to do with the barrier function of the envelop of these Gram negative strains activity.

Table 4.7. Antibacterial activity of the homo and heterotrinuclear Schiff base metal complexes

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Klebsiella pneumoniae (mm)</th>
<th>Escherichia coli (mm) Concentrations</th>
<th>Staphylococcus aureus (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(μg/ml)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25  50  75  100</td>
<td>25  50  75  100</td>
<td>25  50  75  100</td>
</tr>
<tr>
<td>[Cu3(L3)Cl6]</td>
<td>11  14  17  19</td>
<td>11  13  15  18</td>
<td>12  14  17  19</td>
</tr>
<tr>
<td>[Ni3(L3)Cl6]</td>
<td>10  12  14  18</td>
<td>10  12  14  15</td>
<td>11  13  15  16</td>
</tr>
<tr>
<td>[Mn3(L3)Cl6]</td>
<td>10  13  16  18</td>
<td>10  13  14  15</td>
<td>11  14  16  17</td>
</tr>
<tr>
<td>[CuNi2(L3)Cl6]</td>
<td>10  12  15  17</td>
<td>10  13  15  17</td>
<td>11  13  16  18</td>
</tr>
<tr>
<td>[NiCu2(L3)Cl6]</td>
<td>11  13  16  18</td>
<td>10  13  15  18</td>
<td>11  14  17  18</td>
</tr>
</tbody>
</table>
Figure 4.23. Difference between the antimicrobial activities of the trinuclear metal complexes against Klebsiella pneumoniae

Figure 4.24. Difference between the antimicrobial activities of the trinuclear metal complexes against Escherichia coli
Figure 4.25. Difference between the antimicrobial activities of the trinuclear metal complexes against Staphylococcus aureus

4.3. Conclusion

The design and synthesis of three homo/heterotrinuclear Cu(II), Ni(II) and Mn(II) Schiff base complexes have been demonstrated in this report. All the three complexes were synthesized from p-phenylenediamine, benzil and 2-aminobenzaldehyde containing N₄ donors set in different environments. They were characterized by spectral and analytical data. The molar conductance values indicate that the complexes were non electrolytic in nature and also indicate that the chloride ion present inside the coordination sphere. FT-IR spectra reveal that the azomethine stretching frequency observed for all the complexes suggesting the complex formation and far IR spectra indicate that the chloride ion bonded to the metal ion. The UV-Vis, magnetic susceptibility and EPR spectral data of the complexes suggest a distorted octahedral geometry around the central metal ion.
and terminal metal ions. The DNA cleavage studies reveals that the homo and heterotrinuclear copper(II) complexes cleaved DNA was more effectively as compared homotrinuclear nickel(II) and homotrinuclear manganese(II) complexes. The antibacterial activity of the homotrinuclear Cu(II) and heterotrinuclear complexes are higher than the homotrinuclear Ni(II) and Mn(II) complexes.
REFERENCES


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CHAPTER V

SUMMARY AND CONCLUSION

The work described in the thesis concerns with the reactions of some of the Cu(II), Ni(II), VO(II) and Mn(II) complexes with p-phenylenediamine, o-phthalaldehyde/2,3-pentanedione/benzil and 2-aminobenzaldehyde. The new homo and heterotrinuclear Schiff base complexes are formed with N₄ donor sets. The structures of these new synthesised complexes have been investigated by using various physico chemical methods. The complexes have been effectively used as DNA cleavage and antibacterial studies. Some of the complexes used as a DNA binding studies. These complexes have effectively bind to CT DNA. The antibacterial properties of the complexes have also been screened against some pathogenic bacteria.

Chapter I deals with the general introduction to Schiff base, biological importance of the copper, nickel, vanadium and manganese metal ions and brief discussion of their applications in various methods. The synthetic methodologies of homo and heterotrinuclear Schiff base metal complexes derived from p-phenylenediamine, o-phthalaldehyde (or) 2,3-pentanedione (or) benzil and 2-aminobenzaldehyde.

Chapter II deals with the details on various experimental and characterization techniques employed in the present study and also provides the information about the spectral characterization of two homo and three heterotrinuclear Schiff base metal complexes. These metal complexes were synthesized by using p-phenylenediamine, o-phthalaldehyde and 2-aminobenzaldehyde. Trinuclear Schiff base metal complexes were synthesized
by two step synthetic route. The mononuclear copper(II) and nickel(II) complexes of p-phenylenediamine as the ligand system in which the amine group could be modified to attach additional nitrogen donor sites through Schiff base condensation has been attempted. The monometallic complex, is allowed to react with 2-aminobenzaldehyde and metal salts, complexes of Cu(II), Ni(II) and VO(II) to afford homo and hetero trinuclear metal complexes of the types Cu₃, Ni₃, Cu₂Ni, Ni₂Cu and Cu₂VO. The composition and geometry of the trinuclear metal complexes were proposed based on elemental analysis, molar conductance, infrared, electronic, thermal, magnetic and EPR studies. Molar conductance values suggest that complexes are electrolytes in nature. The IR, electronic, EPR, magnetic studies of the metal complexes were used to determine the type of the coordination and geometries of the trinuclear complexes. The electronic and magnetic susceptibility studies reveal that the geometry of the central metal ion (Cu(II), Ni(II)) is distorted octahedral and that of 2-side terminal metal ions (Cu(II), Ni(II)) is square planar and VO(II) is square pyramidal. The redox behaviour of the trinuclear complexes was explained by cyclic voltammetry. Thermal gravimetric analyses for the complexes were obtained to give information concerning the thermal stability of the complexes. The results of thermal analyses showed good agreement with the theoretical formula as suggested from the elemental analyses. The interaction of the complexes with calf thymus DNA (CT-DNA) was studied using absorption spectra, cyclic voltammetry and viscosity measurements. They exhibit absorption hypochromicity, and the specific viscosity increased during the binding of the complexes to calf thymus DNA. The shifts in the oxidation-reduction potential and changes in peak current on addition of DNA
were shown by cyclic voltammetric measurements. The pUC18 DNA cleavage study was monitored by gel electrophoresis method. From this study, it was found that the complexes cleave pUC18 DNA in presence of the oxidant H₂O₂. Finally, the homotrinuclear and heterotrinuclear complexes were tested in vitro against some pathogenic bacteria to assess their antimicrobial properties. The MIC value against the growth of micro-organisms is much larger for heterotrinuclear complexes than the other homotrinuclear complexes.

In chapter III, the spectral characterization and biological properties of 2,3-pentanedione complexes have been discussed. Complexes of Cu(II), Ni(II) and Mn(II) containing a novel three homo and two heterotrinuclear Schiff base metal complexes were synthesized by using p-phenylenediamine, 2,3-pentanedione and 2-aminobenzaldehyde. The mononuclear complexes have been synthesised and are assembled in the step wise to form homo and hetero metallic trinuclear complexes. This methodology can be employed to build complexes with more metal centers. The characterization and nature of bonding of the trinuclear complexes have been deduced from elemental analysis, molar conductivity, FT-IR, UV-Visible, cyclic voltammetry, thermal, magnetic and EPR studies. Molar conductance values indicate that all the trinuclear complexes were non electrolytic in nature. FT-IR spectra reveal that the azomethine stretching frequency observed for all the complexes suggesting the complex formation. The distorted octahedral structures were assigned to the trinuclear complexes based on spectral data (electronic, EPR and magnetic studies). The subnormal magnetic moment values were observed for trinuclear complexes suggesting that one more metal ion present in the coordination sphere, trinuclear nature of the complexes. The EPR spectra of the
complexes were recorded on powder samples with room temperature. The resonance line at the higher magnetic field shows an increased broadness than the monomer precursor and this indicates that the presence of copper and manganese ions were found to have more than one environment. Redox behaviour of the complexes was explained by cyclic voltammetry. Thermal studies explain the content of a particular component in a complex changes with its composition and structure. The pUC18 DNA cleavage study was monitored by gel electrophoresis method. The results suggest that all the homo and hetero trinuclear Cu(II), Ni(II) and Mn(II) complexes cleaves pUC18 DNA in presence of the oxidant H2O2. The in vitro antimicrobial activity of the synthesized complexes have been tested against the Gram negative bacteria like Klebsiella pneumoniae, Escherichia coli and Gram positive bacteria Staphylococcus aureus. The trinuclear Schiff base complexes were found to be more active.

In chapter IV, spectral characterization, DNA cleavage and antimicrobial activities of benzil complexes are elaborately discussed. The template condensation of benzil, p-phenylenediamine and metal salts in 1:2:1 molar ratio yielded the mononuclear Schiff base complex with N4 donors. The mononuclear complex has been used as a ligand for further condensation with 2-aminobenzaldehyde and metal salts to obtain the new three homo and two hetero trinuclear Cu(II), Ni(II) and Mn(II) Schiff base complexes. The characterization and nature of bonding of the trinuclear complexes have been deduced from elemental analysis, molar conductivity, FT-IR, UV-visible, cyclic voltammetry, thermal, magnetic and EPR studies. The molar conductance values indicate that the complexes were non electrolytic in nature. The UV-Vis, magnetic
susceptibility and EPR spectral data of the complexes suggest a distorted octahedral geometry around the central metal ion and terminal metal ions. The redox behaviour of the Cu(II), Ni(II) and Mn(II) homo and heterotrinuclear complexes was studied by cyclic voltammetry. Thermal gravimetric analyses determine the thermal stability of the complexes and also showed good agreement with the theoretical formula. The pUC18 DNA gel electrophoresis experiment was conducted at room temperature using our synthesised trinuclear complexes in the presence of H₂O₂ as an oxident. As can be seen from the results at high concentration, all the trinuclear complexes exhibit nuclease activity. Control experiment using DNA alone does not show any significant cleavage of DNA. From the observed results, we have concluded that from the complexes, copper complex cleaves DNA was more effectively as compared nickel and manganese complexes. The in vitro biological screening effects of the investigated compounds were tested against the Gram negative and Gram positive bacteria by disc diffusion method. A comparative study of minimum inhibitory concentration values of the complexes indicates that the all trinuclear metal complexes exhibit higher antibacterial activity.
List of Publications

Publications connected to the thesis


Publications not connected to the thesis


11. P. Jayaseelan, S. Prasad, S. Vedanayaki and R. Rajavel “Synthesis, Spectral Characterization, anti-microbial activities, DNA binding and
cleavage studies of new binuclear Schiff base metal complexes derived from 3,3’ diaminobenzedine” accepted by Arabian Journal of Chemistry.

Papers presented in International / National Conferences


International / National Conferences/ Workshops Attended


3. Academics sponsored Two day Lecture Workshop on FRONTIERS IN CHEMISTRY held at Department of Chemistry, National College, Tiruchirappalli, 13 & 14, March 2009.

4. One day Workshop on Recent Developments in Nanomaterials Research, held at Department of Physics, Periyar University, Salem-11, 31st March 2008.