PHYSICO-CHEMICAL STUDIES ON
NEW METAL CHELATES

ABSTRACT

Thesis submitted for the award of the degree of

Doctor of Philosophy
IN
CHEMISTRY

BY
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DEPARTMENT OF CHEMISTRY
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ABSTRACT

Macrocyclic metal complexes arose wide interest owing to their diverse biological applications viz. enzyme modelling; synthetic ribonuclease investigations; as nucleobase recognition agents, in the formation of luminescent sensors and new generation metal based drug (in chelation therapy). There is a lot of literature available on bio-medical diagnostic application of polyazamacrocyclic complexes viz. radiotherapy as positron emission tomography (PET), blood pool imaging as biofunctional agents, e.g. bound to monoclonal antibodies. Thus these complexes are entering fast in the area of therapeutic medicine as anticancer and anti-HIV agents. It has long been recognized that cyclic molecules offer potentially useful binding sites for guest molecules and ions that result in their use as models for carrier molecules in the studies of selective uptake and transport of oxygen in biological system. The enhancement of the thermodynamic stability of these macrocyclic complexes compared to similar noncyclic tetraamine ligands together with their potential kinetic inertness are important for applications in medicine.

The metallated macrocycles can adopt a variety of configurations and the relative energies of these configurations are dependent on the nature of substituents on the cyclam ring, size of the metal ion and interactions between the metal and NH groups of the cyclam ring, additional ligands as pendants (free ligands or complex moiety), including solvent and counterions. Transition metal complexes with nitrogen containing ligands have been extensively studied in order to mimic reactions in (i) the redox function of
various metalloenzymes in living systems (ii) the formation and reactivity of dioxygen in synthetic, industrial and biological process. In enzymes, metals have several roles (i) redox as in superoxide dismutase like activity (ii) structural and catalytic. Dioxygen binding and activation in dinuclear metal complexes is of current interest owing to the physiological metabolism of dioxygen at bimetallic biosites. Oxygenation of homodinuclear metal complexes like Cu$^+$Cu$^+$ and Fe$^{II}$Fe$^{II}$ have been studied with the aim of providing models of hemocyanin and hemerythrin. It is well known that dioxygen reduction in cytochrome c oxidase is promoted by CuFe pair in close proximity and this has stimulated interest in the oxygenation behavior of heterodinuclear complexes having different combinations of metal ions. To rationalize these findings, we have synthesized a series of metal complexes and these complexes were characterized by various spectroscopic tools i.r, e.p.r and n.m.r spectroscopy. Particularly important physical techniques, such as uv.vis spectroscopy, cyclic voltammetry, viscosity measurements were employed to study the interaction of the complexes with H$_2$O$_2$/ with pyrocatechol in presence of dioxygen / with DNA to detect the mode of binding that can provide valuable mechanistic information.

A new series of binuclear complex of the type [M-mac- M'(L)$_4$] Cl$_2$(ClO$_4$)$_2$ derived from 14-membered hexaazacyclotetradecane and second generation tetra-pyridyl complexes- tetra(3-acetyl pyridine) of Mn$^{II}$, Ni$^{II}$ and Cu$^{II}$ chloride have been synthesized. On the basis of spectral data, it is inferred that all the binuclear complexes are consistent with square pyramidal geometry and are ionic in nature. The catalytic
oxidation and mechanism of binding to the complexes by hydrogen peroxide have been studied kinetically. The progress of the reaction of the representative complex, \([\text{C}_{36}\text{H}_{50}\text{N}_{10}\text{O}_{4}\text{CuCo}]\text{Cl}_2(\text{ClO}_4)_2\), with \(\text{H}_2\text{O}_2\) in aqueous DMSO (95:5) at room temperature has been monitored by measuring absorbance changes at 344 nm (\(\lambda_{\text{max}}\) of binuclear \(\text{Cu}^{II}\)-\(\text{Co}^{II}\) complex with \(\text{H}_2\text{O}_2\)) under varying concentrations of \(\text{H}_2\text{O}_2\) (0.10-0.16 mol dm\(^{-3}\)). Pseudo-first order rate constants, \(k_{\text{obs}}\), have been calculated using linear least squares regression method. The redox behavior of the binuclear complex \([\text{C}_{36}\text{H}_{50}\text{N}_{10}\text{O}_{4}\text{CuCo}]\text{Cl}_2(\text{ClO}_4)_2\) has been studied in presence and in absence of \(\text{H}_2\text{O}_2\) at a scan rate of 0.1 V s\(^{-1}\) by cyclic voltammetry. The cyclic voltammogram of the \(\text{Cu}^{II}\)-\(\text{Co}^{II}\) complex reveals quasi-reversible redox waves for one electron transfer attributed to \(\text{Cu}^{II}/\text{Cu}^{I}\) and \(\text{Co}^{III}/\text{Co}^{I}\) redox couples. On interaction with \(\text{H}_2\text{O}_2\), \(E^0\) values shift considerably as the coordination environment changes from five coordinate to six coordinate geometry due to catalytic oxidation of the complex by \(\text{H}_2\text{O}_2\).

Metal oxo complexes are important intermediates in enzymatic and catalytic oxidations with molecular oxygen and hydrogen peroxide. Iron (III) peroxo and high valent synthetic iron oxo intermediates play a central role in many metabolically important reactions. Iron species play a dual role as both precursors in active oxo form and active oxidants. The pyrazole-based ligands incorporated into the macrocyclic framework provide a suitable environment to cover the structural and spectroscopic properties of two different metal ions in concord. New oxygen carriers have been synthesized by the interaction of the \(\text{Cu}^{II}/\text{Ni}^{III}\) derivative of the bis(5-nitroindazolyl)methane complex with

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M = Fe$^{III}$, Ni$^{II}$, Co$^{II}$, to yield binuclear complexes. These complexes have been
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Investigations on DNA binding studies have been focused owing to their relevance to the cellular molecular target and macrocyclic complexes bind to DNA through a number of interactions e.g. hydrogen bonding or $\pi$-stacking interaction or intercalation. New modulated pentacoordinate complexes $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{Cu}]\text{ClO}_4$, $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{Co}]\text{ClO}_4$ and $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_2\text{Ni}]\text{ClO}_4$ have been synthesized by the interaction of 1,8-dihydro 1,3,6,8,10,13 Cu(II), Co(II), Ni(II) hexaazamacrocycles and Hsaleza N-(2-hydroxy benzyl)-2-amino-1 ethanol ligand. All the complexes have been characterized by uv-vis
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The work embodied in this thesis entitled “Physico-chemical studies on new metal chelates” is the result of original research carried out by Miss. Shaiba Parveen under my supervision and is suitable for the award of Ph.D. degree.

(Sartaj Tabassum)
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Shaiba Parveen
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Sartaj Tabassum, Shaiba Parveen and Farukh Arjmand, Transition Metal Chemistry. (Accepted).

Sartaj Tabassum, Shaiba Parveen and Farukh Arjmand, Org. Biomol. Chem (Communicated).

4. New modulated metallic macrocycles, electrochemistry and their interaction with calf thymus DNA.
Sartaj Tabassum, Shaiba Parveen and Farukh Arjmand, Biochimica et Biophysica Acta (Communicated).
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CHAPTER I

INTRODUCTION
INTRODUCTION

Macrocyclic polyamines have received much attention because of their biological activities and numerous potential bioinorganic applications viz. magnetic resonance imaging (MRI) [1-4], biocatalysts, diagnostic and therapeutic radiopharmaceuticals [5,6], hydrolytic enzyme models, synthetic ribonuclease, nucleobase recognition agents, oxygenase promoters [7]. Recent developments in coordination chemistry have produced numerous macrocyclic complexes through appropriate coordination geometry. Among them a large number of synthetic biofunctional macrocyclic compounds were prepared and investigated.

Saturated macrocyclic polyamines containing six or more nitrogen donors exhibit specific new properties anticipated from their assemblies of amines or linear polyamines [8] due to pronounced ability to bind a large variety of metals to form stable metal complexes and in many cases, undergo marked conformational changes during binding. Hexaazamacrocycles are interesting and versatile receptor molecules capable to coordinate one or two metal ions and also encapsulate anionic guests via. electrostatic interaction and hydrogen bonding [9-11]. One pot metal template condensation involving amines and formaldehyde have been employed for the easy, high yielding and stereospecific synthesis of various saturated hexaazamacrocyclic metal complexes [12-15]. They can be used as models of metalloenzymes to understand the reactivity change caused by the proximity of two metals [16-20]. The strong affinity of hexaazamacrocycles and their selective binding of metals result in their use as models for carrier molecules in studies of the selective uptake and transport of metal ions [21,22].
as metal catalysts [23] and active site mimics of metalloenzymes eg superoxide dismutase [24], as agents which cleave phosphate esters including DNA and RNA [25] in medical techniques [26] and as anti-HIV agents [27]. De Clercq and coworkers have studied the effect of aromatic linkers on the HIV activity of bicyclams [28]. They found that the activity depends highly on the substitution of the aromatic linker connecting the cyclam rings. For example 2,6 and 3,5- pyridine linked bicyclams are potent inhibitors of HIV-1 and HIV- 2 replication, whereas the 2,5 and 2,4 substituted pyridine linked compounds exhibit substantially reduced activity (Figure 1).

**Figure 1.** Models of pyridine- linked bicyclams in which pyridine nitrogen participates in pendant complexation along with four nitrogen atoms of the adjacent cyclam ring.
If the binding of bicyclams to the molecular target is not transition metal mediated, then the pendant heteroatom of linker may hydrogen-bond to the proton shared between the nitrogen group of the macrocyclic ring thereby inducing an unfavorable conformation with respect to anti-HIV activity.

Metallo-radiopharmaceuticals containing a metallic radionuclide e.g. $^{67}$Cu are used for the diagnosis or therapy of various diseases, including cancer, thrombosis, kidney and liver abnormalities and cardiological and neurological disorders [29]. A target specific metallo-radiopharmaceutical can be coupled to targeting biomolecules, such as antibodies or peptides, through a bifunctional chelator (e.g. cyclam) which covalently links to the targeting molecule and coordinates to the metallic radionuclide (Figure 2).

**Figure 2.** Schematic structure of a radiopharmaceutical. Cyclam binds to a radionuclide and is linked to a biological targeting molecule.
Shorter lived copper radionuclides are currently used in lipophilic copper complexes for measuring blood flow and hypoxia. The longer lived copper radionuclides such as $^{64}$Cu and $^{67}$Cu are used in the development of copper-labelled biological molecules for tumor targeting using monoclonal antibodies and peptides.

Copper(II) complexes with macrocyclic ligands such as bifunctional chelators, 4-[(1,4,8,11-tetraazacyclotetradecane-1-yl) methyl] benzoic acid (CPTA) and 6-[p-(bromoacetamido) benzyl]- 1,4,8,11-tetraazacyclotetradecane –1, 4,8,11-tetraacetic acid (BAT) (Figure 3), are well studied and have been employed in the formulation of $^{64/67}$Cu(II)- labeled conjugates for human clinical trials [30,31].

Figure 3. (a) 4-[(1,4,8,11-tetraazacyclotetradecane-1-yl) methyl] benzoic acid (CPTA) (b) 6-[p-(bromoacetamido) benzyl]- 1,4,8,11-tetraazacyclotetradecane –1, 4,8,11-tetraacetic acid (BAT).
The oxygen activation mechanism by heme and non-heme iron catalysts have continued to fascinate bio-inorganic chemists [32,33]. Such mechanism provides new insight in biocatalysts and drug design as well as in the development of new approaches for industrial catalysts [34]. Nature has evolved biological mononuclear and binuclear non heme iron(III) centers which are capable of binding dioxygen/H$_2$O$_2$ to produce oxidants Fe peroxide or ferryl species [35,36]. These iron (III) peroxide species are also interesting as potential new catalysts for the oxidation of organic substrates. Such type of catalysts are implicated as intermediates in the mechanism of oxygen activation biomolecules with either heme e.g., cytochrome P-450 [37] mimics for oxidation, (electron removal) [38] heme oxygenase/ heme peroxidases [39], olefin epoxidation, alkane hydroxylation, olefin cis-dihydroxylation [40], cyclopropanylation [41], radical formation [42], or non heme iron centers e.g. methane monooxygenase [43-46], antitumor drug bleomycin [47] and Rieske dioxygenases [48] as well as superoxide reductase from anaerobic bacteria [49]. A particularly interesting non-heme iron requiring natural product e.g. iron bleomycin (Fe-BLM) is a metalloglycopeptide derived antibiotic with antitumor activity which is capable of bringing about oxidative DNA cleavage and oxidation of a number of organic substrates with dioxygen or H$_2$O$_2$ to generate a short lived Fe$^{III}$-OOH intermediate [50-53]. Fe-BLM consists of four rings anchored via branches to a central nitrogen atom (N4Py) creating pentadentate ligands PMA.
and PYML which provide a very similar ligand environment around the iron center like Fe$^{II}$BLM, Fe$^{II}$PMA and Fe$^{II}$PYML [54,55]. Mascharak and co-workers designed the PMAH ligand and Fe$^{III}$-OOH complexes derived from pentadentate N5 ligands such as N4Py, Py5, tpen, trispicMeen and bztpen (Figure 4) that closely matched the ligand environment around the iron center.

Figure 4. Pentadentate ligand such as N4Py, Py5, tpen, trispicMeen, and bztpen
N4Py=N,N-bis(2-pyridyl-methyl)-N-bis(2-pyridyl)methylamine; Py5=2,6-bis(methoxy)di(2-pyridyl)methylpyridine; tpen=N,N,N',N'-tetrakis(2-pyridylmethyl)-1,2-diaminoethane; trispicMeen = N-methyl-N,N,N'-tris(2-pyridylmethyl)-1,2-diaminoethane; bztpen=N-benzyl-N,N,N'-tris(2-pyridylmethyl)1,2-diaminoethane and PMAH=2-(2',5'-diazapentyl)-5-bromopyrimidine-6-carboxylic acid N-[2-(4'-imidazolyl)ethyl]amide.
These ligands react with O₂ to generate low spin iron (III) intermediate, which have EPR parameters nearly identical to those of activated BLM. Similarly, the treatment of the iron(II) complex [(N₄Py)Fe” (CH₃CN)] (ClO₄)₂ with excess H₂O₂ in acetone affords a transient purple species (λₘₐₓ = 530 nm, ε = 1100 M⁻¹ cm⁻¹) which exhibits EPR signals at g = 2.17, 2.12, 2.12 and 1.98 resembling those of the low spin Fe”” center of activated BLM. The purple intermediate of Fe””-OOH species was also established using electrospray ionization mass spectroscopy with prominent peak found at m/z 555 and 753 corresponding to the positive and negative molecular ions, {[N₄PyFeOOH](ClO₄)}⁺ and {[(N₄Py)FeOOH] (ClO₄)₃⁻}⁻ [56, 57].

Hydrogen peroxide is the powerful oxidant thermodynamically and environment friendly, as on decomposition it yields water and oxygen. Many reactions with H₂O₂ are limited by the kinetics of reaction, through the evolution of enzymes such as haloperoxidase (chloroperoxidases [58] and bromoperoxidases) [59] and the thiolperoxidases (glutathione peroxidase and other seleno enzymes) [60,61]. H₂O₂ poses the threat of oxidative damage to cellular structure protein and metabolites. Certain degenerative and disease states such as aging [62] and cancer [63] have been attributed to oxidative damage by H₂O₂. To combat this hazard, nature has evolved enzymes such as catalase and peroxidase which catalyzes the decomposition of H₂O₂ to useful and innocuous products water and dioxygen. The resting state of the catalase enzyme contains iron(III) heme.

\[ 2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2 \]
There are two main types of redox catalysts for hydrogen peroxide. Fenton type catalysts which donate a single electron to the peroxide and need a reduction step in practice, usually photochemical or electrochemical to complete the catalytic cycle. Oxygen atom transfer catalysts, however, do not require exogenous electron donors. This type includes the peroxidase enzymes that depend upon general base catalysis to remove a proton from hydrogen peroxide during the formation of the active intermediate at neutral pH [64].

Peroxidases are heme containing ubiquitous enzymes in nature that catalyze the oxidation of wide array of organic and inorganic compounds by hydrogen peroxide or organic peroxides [65]. For example, the reaction of hydrogen peroxide with ferric porphyrins is involved in several classes of enzymes like peroxidase, catalase, cytochrome P450 enzymes [66,67]. Horseradish peroxidase (HRP) is an extra cellular plant enzyme and it is found in high concentration in horseradish roots that takes part in regulation of cell growth and differentiation, polymerization of cell wall components, metabolism, essential for important pathogenic defense reactions [68]. It has been extensively characterized and catalyzes the oxidation of various molecules (organic aromatic substrates) by H₂O₂ (Figure 5). The active site of HRP contains a protoheme that is bound to the enzyme through axial coordination by the imidazole side chain of a proximal histidine residue. The catalytic cycle of HRP involves stepwise two electron oxidation of the ferric heme by H₂O₂ generating an oxoferryl proto porphyrin species, the second intermediate is formed by one electron reduction of HRP-I where the Fe(IV) =O center is maintained and the porphyrin π cation radical is reduced [69]. HRP has
been involved in a number of applications such as diagnostic arrays, biosensors, bioremediation, polymer synthesis [70] and other biotechnological processes [71].

Numerous model compounds of peroxidases namely pinch porphyrins such as [1,9-bis (2-pyridyl)- 2,5,8- trizanonane] (protoporphyrinato) iron (III), - (mesoporphyrinato) - iron-(III) and (deuteroporphyrinato) iron (III) were synthesized from the parent compound

Figure 5. The catalytic cycle of Horseradish peroxidase, catalyzes the oxidation of various molecules by $H_2O_2$. 
chloro (porphyrinato) iron (III), (mesoporphyrinato) iron (III) and (deutero porphyrinato) iron (III) and 1,9-bis(2-pyridyl) - 2,5,8 - triazanonane (picdien). The structural, electronic, magnetic properties and the peroxidase activity [72] of these complexes was determined in the presence of substrate (guaiacol) and oxidant H₂O₂.

The proposed mechanism of iron(III) porphyrin by the oxidation with H₂O₂ is represented in Figure 6 [73].

![Figure 6. The catalytic oxidation of the iron(III) porphyrin with hydrogen peroxide.](image)

The kinetics of the reaction of iron (II) complexes of the macrocyclic ligands 8-methyl-1, 4-bis(2-pyridylmethyl)-1,4,8-triazacycloundecane (L¹) and 1-methyl-5-9-bis(2-pyridylmethyl) -1,5,9 - triazacyclododecane (L²) with hydrogen peroxide was studied in phosphate buffer medium at pH 7.0. Before the reaction, the spectrum of L¹Fe(II)
exhibited two peaks at 250 and 395 nm. The spin allowed transitions $^{5}T_{2g} \rightarrow ^{5}E_{g}$ due to change transfer were found in the low energy region. But after reaction with $H_2O_2$, the spectrum was markedly changed and only one broad CT band was observed (Figure 7) between 250 and 400 nm with a shoulder at 340 nm attributed to $^{6}A_{1g} (^{6}s) \rightarrow ^{4}T_{2g} (^{4}P)$ transition for high spin iron (III).

![Absorption spectra](image)

**Figure 7.** Absorption spectra of $5.0 \times 10^{-4} \text{ M } L^1\text{Fe}^{II}$ before (solid line) and after reaction (broken line) with $5.55 \times 10^{-3} \text{ M } H_2O_2$ in 0.05 M phosphate buffer at pH 7.0. Inset: plot of absorbance change versus $[H_2O_2]: [L^1\text{Fe}^{II}]$ ratio measured at 320 nm using the same buffer solution.

The stoichiometry of these oxidation reaction is shown in equation (1)

$$2L^{1}\text{Fe}^{II} + H_2O_2 + 2H^+ \rightarrow 2L^{1}\text{Fe}^{III} + 2H_2O \quad \text{Equation (1)}$$
The reaction of an excess of \( \text{L}^\dagger \text{Fe}^{\text{II}} \) with \( \text{H}_2\text{O}_2 \) was observed at \( \lambda_{\text{max}} \) 320 nm at different concentration of \( \text{H}_2\text{O}_2 \). The absorbance increased linearly with increasing \( \text{H}_2\text{O}_2 \) concentration (15.3, 30.6, 45.9, 61.2, 76.5 and 91.8 \( \mu \text{M} \)) as shown by the traces A to F depicted in Figure 8. The reaction consisted of two irreversible pseudo-first order reactions. Hence the rate law [74] which holds good is.

\[
(+X) k_a \rightarrow A \xrightarrow{(+Y)/k_b} C\]

\[
A_t - A_e = a_1 \exp(-k_{\text{obs}} t) + a_2 \exp(-k_{\text{obs}} t) \quad \text{Equation (2)}
\]

Figure 8. Typical two-exponential reaction traces recorded in the presence of an excess of \( \text{L}^\dagger \text{Fe}^{\text{II}} \) (1.0 \( \times \) 10\(^{-3} \) m) over \( \text{H}_2\text{O}_2 \). \( [\text{H}_2\text{O}_2] = 15.3 \) (trace A), 30.6 (B), 45.9 (C), 61.2 (D), 76.5 (E) and 91.8 \( \mu \text{M} \) (F).
The enigmatic nature of the heme-copper binuclear moiety has provoked biomimetic efforts by inorganic chemists to generate synthetically derived heme-copper Fe(III)-Cu(II) $\text{H}_2\text{O}_2$/dioxygen reactions, leading to heme-Cu/superoxo, heme-Cu/peroxo, or O-O cleaved products [75]. These binuclear assemblies (Figure 9) have importance in

**Figure 9.** Synthetically derived heme-Cu assemblies possessing reduced iron(II) and copper(I) metal ions.
biological respiration, catalysis, magnetic exchange, electron transfer phenomena, modelling metalloprotein active site, synthetic considerations and in dioxygen activation chemistry [76-88].

Gunter and Murray [89] prepared a binuclear heme copper complex adapting the concept of the “picket-fence” porphyrin in their ligand design (Figure 9). In the presence or absence of axial ligand (Me₂SO or 1- methylimidazole) at ambient temperature, the binuclear complex [[(P) FeII CuI(N₄)]⁺ reacted with dioxygen to yield oxidized compound (Figure 10).

Another system that provides heme containing Fe(II)/Cu(I)/O₂ reactions is a group of binuclear complexes in which a Cu-tris (2-pyridyl methyl) amine moiety is tethered to a FeII-tetraphenyl porphyrinate compound (Figure 9). Upon oxygenation, all of them are low temperature stable high spin “heme-peroxo-copper” complexes possessing strong

![Figure 10. Reaction of the binuclear complex [(P)FeII CuI(N₄)]⁺ with dioxygen.](image-url)
magnetic coupling between iron and copper. A series of capped porphyrin complexes in which an imidazole or pyridine axial base is covalently bound to one side of a porphyrin whereas a triazacyclononane or N, N', N''-tribenzyl tris (amino ethyl) amine (TBTren) copper ligand is attached to the opposite side (Figure 11) [90,91].

Karlin and coworkers have extensively studied the heme/Cu/O_2 and Cu/O_2 reactivities where μ-η^2: η^2 side on peroxy (vs μ-1,2 end-on peroxy) ligation considerably reduces the ν(O-O) values (805-830) → 710-760 cm^{-1} [92,93] and they also observed that tetradentate containing systems possess either end-on Fe^{III} (μ-1,2-peroxo)-Cu^I or Fe^{III}-(μ-η^2-η'-peroxo)-Cu^{II} structures, while the tridentate Cu-ligand containing systems possess side-on Fe^{III}-(μ-η^2: η^2-peroxo)-Cu^{II} structure (Figure 12).
Figure 12. "Side-on" and "end-on" coordination of the Tri-dentate and Tetra-dentate Cu-ligand containing system.

Metal interaction with catechol 1,2-dihydroxybenzene and derivatives (Figure 13) are being found in an increasing number of biological system with functions ranging from metal ion internalization to biomaterial synthesis [94-98]

Figure 13. Catechol (1,2-dihydroxybenzene) and other ortho-dihydroxy moieties.
The unique metal binding and redox capabilities of catechol also provide interesting catalytic roles for these ligands such as enzymatic degradation of many aromatic compounds [99]. Catechol is known to be easily oxidized by molecular oxygen to o-quinone, but however, under particular conditions, i.e. when catechol is bound to a metal center, breaking of the aromatic ring with formation of muconic acid derivatives is observed. This reaction is commonly found in the metabolic pathway of aerobic microorganism, which uses aromatic substances as a carbon source and it is performed with the help of metalloenzymes referred as ring cleaving dioxygenases [100]. The active site of these metalloenzymes contain penta-coordinated Fe(III) or Fe(II) ions, which are essential for the catalytic activity, bound to protein ligands (mostly histidines and tyrosines) and water molecules. [101-104]. These dioxygenases can be divided into two classes on the basis of the site of cleavage of the aromatic ring and of the oxidation state of the metal ion (Figure 14).

![Figure 14. Modes of catechol cleavage catalyzed by catechol 1,2-dioxygenases (intradiol cleavage) and catechol 2,3-dioxygenases (extradiol cleavage).](image-url)
The extradiol dioxygenase catalyze the cleavage of the ring adjacent to the vicinal hydroxyl groups and contains iron(II) ion. [105,106]. The intradiol dioxygenases employ iron(III) and open the ring by breaking the C-C bond between the hydroxyl groups. In both classes, the substrate catechol has been proven to bind directly to the metal center [107,108]. Many iron(III) complexes have been synthesized and studied as model for extradiol cleavage of the catechols [109-114]. Oxygenation of a mixture of FeCl$_2$.4H$_2$O or FeCl$_3$, 3,5 di-tert-butyl catechol (DBCH$_2$) and pyridine derivatives in organic solvents affected intradiol and extradiol cleavage products together with 3,5 di-tert-butyl benzoquinone. Extradiol cleavage products were favoured by using FeCl$_2$.4H$_2$O and DBCH$_2$ in aqueous THF (Figure 15).

Figure 15. Proposed mechanism for the extradiol-cleaving catechol dioxygenases.
UV-vis spectroscopy is particularly useful method for identifying and characterizing a complete array of metal catecholate complexes owing to unique d-d charge transfer due to metal catecholate bands [115-122]. A wide variety of iron porphyrin derivatives were expected to mimic the oxygenase activity of cytochrome P-450 and its selectivity in oxygen atom transfer reactions like epoxidation and alkane and arene hydroxylation. The square planar bis(dimethylglyoximate) bis(N-methyl imidazole) iron(II) [Fe(Hdmg)₂(MeIm)₂] complex with 4N donor equatorial ligands structurally similar to the iron porphyrin species found in heme type monooxygenases also possess catecholase activity under dioxygen. The complex [Fe(Hdmg)₂(MeIm)₂] catalyzes the oxidative dehydrogenation of 3,5-di-tert-butyl catechol (H₂dbcat) to the corresponding 1,2 benzoquinone which is observed at 400 nm (Figure 16) [123].

Figure 16. Ferroxime-catalyzed oxidation of H₂dbcat by O₂ monitored every 2 min (l = 1mm). Formation of dtbq detectable at 400 nm. [Fe(Hdmg)₂(MeIm)₂]₀ = 0.44 x 10⁻³, [H₂dbcat]₀ = 9.08 x 10⁻³, [O₂] = 1.1 x 10⁻³ mol dm⁻³.
The electrochemical behavior of a series of iron(III) complexes of tetradeutate tripodal ligand have been investigated by cyclic voltammetry. These complexes show a cathodic as well as anodic wave for one electron reduction process. The $i_p$ vs $V^{1/2}$ plot is linear but the values of peak current ratio $i_{pa}/i_{pc}$ (1.0-0.6) and peak potential separation $\Delta E_p$ (68-132) mv suggest fairly reversible to irreversible processes. The coordination of H$_2$dbcat to the complex exhibit only the oxidation wave by lowering of Fe(III)/Fe(II) couple and depresses the $E_{1/2}$ by 100-500 mv. This is consistent with one of the suggested functions of the iron(III) center in the enzyme viz promoting the loss of both protons of the substrate. 

It is relevant observation that the presence of catechol lowers the potential of the iron(III) center in the enzyme [124, 125].

The interaction of transition metal complexes and DNA has been extensively studied [126] mainly owing to their applications in pharmaceutical industries [127-133] as chemotherapeutic agents for the treatment of cancer [134-137], lymphomas, [138] acquired immunodeficiency syndrome (AIDS), anti HIV agents, [139-141] bacterial infections as well as the treatments of many other ailments [142,143].

There are number of modes through which small molecules such as metal complexes can interact with DNA [144-154]. These binding modes are (i) external binding by electrostatic attraction (ii) intercalation of planar aromatic molecules between the base pairs of the DNA helix (iii) major groove binding and minor-groove binding (Figure 17). The intercalation binding mode has been extensively studied and is rather well understood [155-157].
Figure 17.  
(i) external binding,  
(ii) intercalation  
(iii) groove binding.

Binding studies of small molecules to DNA are very important in the development of new therapeutic reagents and DNA molecular probes [158,159]. A large number of octahedral complexes of first-row transition metal ions have been found to the minor or major groove of DNA [160].

The cationic metal ions compensate the negative charges of the sugar phosphate backbone, the nature of these cations plays an important role in the folding of RNA [161]. Metal complexes that bind covalently to DNA have been known to function as anti-tumor agents [162]. Macro cyclic compounds have found utility in a number of medical applications including imaging application and as potential HIV inhibitors. A polyamino dizinc(II) complex \([(\text{N-bisdien})\text{Zn}_2(\text{II})\text{Cl}_2](\text{ClO}_4)_2\) (Figure 18) has been synthesized as a nucleobase receptor molecule and in vitro anti-tumor investigation shows that this compound is a potent inhibitor of tumor cell growth with \(\text{IC}_{50}\) values below 10 micromolar [163]. The copper complexes of macrocycle/intercalator prepared from
cyclam (1,4,8,11-tetraazacyclotetradecane) and the 1- and 2- substituted anthraquinones (1-[(2-aminoethyl)amino]anthracen-9,10-dione(1C2mac), 1-[(3-aminopropyl)amino]anthracene-9,10-dione (1C3mac), 2-[(3-aminopropyl)amino]anthracene-9,10-dione (2C3mac) (Figure 19) were allowed to react with plasmid DNA to study the effect of these complexes as DNA intercalators.

Figure 18. Bis(Zn-cyclen) Zn-Cyclen LZn

Figure 19. Structure of metal complex of cyclam/anthraquinone adducts.
Addition of the macrocycle increased the extent and effect of DNA intercalation and addition of copper metal ion increased the effect still further [164].

It was observed that even though the position of the side chain was accommodated better in the major groove of DNA due to the larger space available, other interactions like electrostatic interactions between the cationic groups in the side chains of the intercalators and negative electrostatic potential of the minor groove and close van der Waals contacts within the minor groove could not be ruled out. The binding process of these macrocycles was monitored using UV-vis spectroscopy to ascertain the binding of complexes with salmon sperm DNA. The absorbance was measured at a maximum of 314 nm for 1C2mac and 316 nm for Cu-1C2mac over a range of DNA concentrations (Figure 20). A steady decrease in intensity of the absorbance with addition of each increment of salmon sperm DNA was observed and the binding constants $k_b (4.7 \times 10^{-3}$ and $6.2 \times 10^{-3}$) of 1C2mac and Cu-1C2mac were determined from the half reciprocal plots.

![Figure 20](a) UV spectrophotometric titration curves and (b) half reciprocal plot for 1C2mac.
A series of inert cationic cobalt(III)-sar (sar-sarcophagine = 3,6,10,13,16,19-hexaaza bicyclo [6.6.6] icosane) cage complexes (Figure 21) covalently linked to polycyclic rings such as anthracene or phenanthrene were known to act as DNA binding and cleaving agents [165]. The hydrophobic polycyclic aromatic groups bound to the hydrophilic cobalt(III)-sar cages are effectively solubilised in water and they have the prospect of intercalating with DNA as these cationic complexes associate with the negatively charged phosphodiester backbone of DNA.

Interaction of a chiral phorphyrin [(TPP)Co(Trp)] where TPP = tetraphenyl phorphyrin and Trp = L-tryptophan with calf thymus DNA was studied spectrophotometrically. The complex [(TPP)Co(Trp)] after interaction with calf thymus DNA, exhibited a shift in the absorption spectrum and a large hypochromicity, indicating an intercalating binding mode. This observation was further confirmed by the electrochemical behavior of
[(TPP)Co(Trp)] before and after interaction with calf thymus DNA. The complex experienced a negative shift in $E_{1/2}$ and a decrease in $E_p$. The ratio of cathodic to anodic peak currents $i_{pc}/i_{pa}$ was $\approx 1$ for [(TPP)Co(Trp)] (Figure 22) while for DNA bound complex $i_{pc}/i_{pa} < 1$ suggesting that calf thymus DNA moiety was bound strongly to the complex [(TPP)Co(Trp)] (Figure 23). Kinetic studies of the DNA-porphyrin complex revealed a pseudo-first order rate law as the plot of $k_{obs}$ versus calf thymus DNA was linear passing through the origin [166].

Figure 22. Cyclic voltammogram of the [(TPP)Co(Trp)] complex in $H_2O/MeOH$ (93:5) at scan rate of $0.1Vs^{-1}$. 
Figure 23. Cyclic voltammogram of the [(TPP)Co(Trp)] complex after addition of calf thymus DNA in H$_2$O/MeOH(95:5) at scan rate of 0.1Vs$^{-1}$. 

Potential / V

Current le-5A

Segment 1:
Ep = -0.308V
eh = -0.376V
Ip = -1.348e-4A
Ah = -1.011e-4C
Ep = 0.809V
eh = 0.551V
Ip = -1.681e-6A
Ah = -3.375e-7C
Present work

Non-heme biomimetic catalyst are the subject of extensive current research owing to the fact that these complexes are involved in dioxygen metabolism and perform a variety of physiological functions such as dioxygen carriers, oxidases, oxygenases and superoxide dismutase [167-169]. Interest has been mainly on mononuclear first row transition metal complexes with a variety of ligands viz., macrocyclic, capping multidenate (di-, tri- and tetra-dentate) or binucleating ligands containing pyridine, imidazole, pyrazole, quinoline or histidine groups [170]. Interaction of redox-active metallic salts with bis[di(2-pyridyl)methyl]amine leads to the first generation of pyridyl complexes [171,172]. Similarly, the tetra-pyridyl ligand, bis[1, 1-di(2-pyridyl)ethyl]amine and its complexes represent the second generation of this family of ligands [173]. New methodology has been charted to synthesize functional and highly effective biomimetic catalyst for coordination to the desired redox-active metal center. Novel binuclear macrocyclic complexes have been synthesized by tethering together 14-membered 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic Ni(II)/Co(II) complexes and the second generation tetra pyridyl-tetra(3-acetyl pyridine) Mn(II), Ni(II) and Cu(II) chloride complexes which has enormous scope due to the vacant sixth coordination site. These complexes have been characterized by various physico-chemical methods and the redox properties of binuclear complex \([C_{36}H_{50}N_{10}O_{4}CuCo]Cl_2(ClO_4)_2\) were studied by cyclic voltammetry. In order to explore as an oxygen carrier, kinetic studies of the binuclear
complex \([\text{C}_3\text{H}_5\text{O}_1\text{N}_1\text{O}_4\text{CuCo}]\text{Cl}_2(\text{ClO}_4)_2\) with \(\text{H}_2\text{O}_2\) were carried out spectrophotometrically. The progress of the reaction was monitored by measuring absorbance change at 344 nm (\(\lambda_{\text{max}}\) of binuclear Cu(n)-Co(n) complex with \(\text{H}_2\text{O}_2\)) and pseudo-first order rate constants \(k_{\text{obs}}\) were obtained from the slope of the straight line by linear least squares regression method.

Transition metal complexes of the bis[3-(2-pyridyl) pyrazol-1-yl] methane ligand have also attracted much attention in which two bi- or multidentate chelating pyridyl/pyrazolyl fragments are linked by a flexible \(-\text{CH}_2\) spacer [174]. Kitajima et al. prepared pyrazole-based copper complexes that have proved useful as functional models for hemocyanin and tyrosinase [175, 176]. Such ligands have the ability to coordinate donor atoms to a single metal ion or to act as a bridge between different metal ions to give polynuclear complexes with helical topologies [177]. In this context, an attempt has been made to synthesize bis(5-nitroindazolyl) methane complex which is also a bidentate ligand with a bridging CH\(_2\) spacer group. Tethering bis(5-nitroindazolyl) methane Cu(II), Ni(II) complexes with 14-membered hexaazamacrocycle [M-mac] where M = Fe(III)/Ni(II)/Co(II) yielded binuclear complexes which are capable of binding dioxygen. These complexes have been characterized by several spectroscopic methods i.r, e.p.r, uv-vis, \(^1\)H-n.m.r, \(^{13}\)C-n.m.r, 2D cosy n.m.r and cyclic voltammetry. Kinetic studies of the binuclear complex \([\text{C}_{38}\text{H}_{42}\text{N}_{18}\text{O}_{9}\text{FeCu}]\text{Cl(NO}_3)_2(\text{ClO}_4)_2\) with \(\text{H}_2\text{O}_2\) have been performed. The progress of the reaction was monitored by measuring the absorbance change at 367 nm (\(\lambda_{\text{max}}\) of the binuclear Fe(III)-Cu(II) complex with \(\text{H}_2\text{O}_2\)) with respect
to time under varying concentrations of H$_2$O$_2$. On the basis of kinetic studies, it has been inferred that the active site before interaction with H$_2$O$_2$ is square pyramidal, while after interaction with H$_2$O$_2$, the coordinated site becomes saturated and is octahedral.

Iron(III) porphyrin models [178-181] have been extremely useful in bioinorganic and biomimetic chemistry which catalyze a wide range of biological and chemical oxidations with dioxygen either as a mono-oxygen transfer or electron transfer agent [182-185]. Organotin compounds with various uni- and bidentate ligands has prompted the preparation of many new organotin compounds due to their various industrial and agricultural applications [186, 187]. The tin (IV) complexes of protoporphyrin inhibit the enzyme heme oxygenase which is responsible for hyperbilirubinemia in neonates [188]. These porphyrin complexes have also been used in photodynamic therapy [189-191] and have good selectivity towards tumors because of accumulation in cancer cells [192, 193]. A great deal of attention has been paid to iron (III) porphyrin containing pendant arms or Sn$^{IV}$ moiety as still there is a vacant room for selective mechanistic studies. In this series, new substituted porphyrin derivatives were synthesized by the interaction of new organotin complex [C$_{23}$H$_{19}$NOSn] with Fe(III), Cu(II), Ni(II) porphyrin. The catalytic activity of binuclear complex [C$_{67}$H$_{49}$N$_5$OFeSn]Cl in presence of pyrocatechol was studied spectrophotometrically and by cyclic voltammetry. The reaction can be easily monitored by increase in absorbance at $\lambda_{max}$- 400 nm to afford extradiol cleavage product and the $V_{max}$ and $K_m$ parameters were obtained from the Michaelis-Menten methods.
The increasing interest in using macrocycles and their coordination compounds for binding with DNA has motivated us to explore the application of macrocyclic transition metal complexes. Studies have shown that these macrocyclic complexes can react with DNA in different binding fashion and exhibit effective nuclease activities. Introducing oxygen attached pendant with free alcoholic group would perhaps modulate the hexaazamacrocycle to carry out specific biochemical process through which the cancer inhibition would take place [194]. Thus the new modulated macrocycles have been synthesized form the reaction of 14-membered 1,8 dihydro 1,3,6,8,10,13 Cu(II), Co(II), Ni(II) hexaazamacrocycle with Hsalea N-(2-hydroxybenzyl)-2-aminol-ethanol ligand. Interaction of the Cu(II) and Co(II) modulated macrocycle with calf thymus DNA were studied by electronic absorption titration, cyclic voltammetry and viscosity measurements. The experimental results suggest that complex \([\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Cu}]\text{ClO}_4\) binds to calf thymus DNA through partial intercalation of the aromatic ring into the base pair of DNA while complex \([\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Co}]\text{ClO}_4\) binds to calf thymus DNA by electrostatic mode.
CHAPTER II

EXPERIMENTAL
CHAPTER II

EXPERIMENTAL METHODS

The following techniques were employed to characterize the complexes

1. Infrared spectroscopy
2. Ultra-violet and visible spectroscopy
3. Nuclear magnetic resonance spectroscopy
4. Electron paramagnetic resonance spectroscopy
5. Molar conductance measurements
6. Cyclic voltammetry
7. Kinetic studies
8. Viscometry

Infrared spectroscopy

The infrared spectroscopy is a useful technique to characterize a compound. It results from transition between vibrational and rotational energy levels. I.r. region of the electromagnetic spectrum covers a wide range of wavelength from 200 to 4000 cm⁻¹. It has been found that in i.r. absorption, some of the vibrational frequencies are associated with specific groups of atoms and are the same irrespective of the molecules in which this group is present. These are called characteristic frequencies [195] and their constancy results from the constancy of bond force constants from molecule to
molecule. The important observation that the i.r. spectrum of a complex molecule consists of characteristic group frequencies which makes i.r. spectroscopy, an unique and powerful tool in structural analysis.

**Ultraviolet and visible spectroscopy**

When a molecule absorbs radiation, its energy is increased. This increased energy is equal to the energy of the photon expressed by the relation

\[ E = h\nu \]

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

where \( h \) is Planck's constant, \( \nu \) is the frequency, \( \lambda \) is the wavelength of the radiation and \( c \) is the velocity of light. Most of the compounds absorb light 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. \( d_{xy}, d_{yz}, d_{xz}, d_{x^2-y^2} \) and \( d_{z^2} \) are degenerate. However, in coordination compounds due to the presence of ligands, this degeneracy is destroyed and d orbital split into two groups \( t_{2g} \) (\( d_{xy}, d_{yz}, \) and \( d_{xz} \)) and \( e_{g} \) (\( d_{z^2} \) and \( d_{x^2-y^2} \)) in an octahedral complex and \( t \) and \( e \) in a tetrahedral complex. The set of \( t_{2g} \) orbitals goes below the original level of degenerate orbitals in octahedral complexes and the case is reversed in tetrahedral complexes (Figure 24). At energy higher than the ligand-field absorption bands,
Figure 24(a) *Splitting of the d energy levels in an octahedral complex*

Figure 24(b) *Ligand-field splitting of tetrahedral complex*

we commonly observe one or more very intense bands that go off scale unless log ε is plotted. These normally are **charge transfer bands**, corresponding to electron transfer processes that might be ligand → metal (L → M) or metal → ligand (M → L). M → L transitions occur for metal-ion complexes that have filled, or nearly filled, t_{2g} orbitals with ligands that have low-lying empty orbitals. These empty orbitals are ligand π* orbitals in complexes such as those of pyridine, bipyridine, 1,10-phenanthroline, CN⁻, CO and NO. Figure 25 shows overlap of a t_{2g} metal orbital and π* of CO.
Figure 25. Metal-carbon double bonding.

The L → M charge-transfer (C-T) spectra have been studied more thoroughly. The intense bands are for Laporte-allowed transitions common of the (ligand) \( p \rightarrow d \) (metal) type. The ionization energy for the ligand (or its ease of oxidation) as well as the oxidation state and electron configuration of the metal (or its ease of reduction) determine the energy of the transition. No net oxidation-reduction usually occurs, because of the short lifetime of the excited state [196].

Nuclear magnetic resonance spectroscopy

The nuclei of certain isotopes possess a mechanical spin or angular momentum. The n.m.r. spectroscopy is concerned with nuclei having spin quantum number \( I = 1/2 \), example of which include \(^1\text{H}, \, ^{13}\text{C}, \, ^{31}\text{P}\) and \(^{19}\text{F}\).

For a nucleus with \( I = 1/2 \), there are two values for the nuclear spin angular momentum quantum number \( m_I = \pm 1/2 \) which are degenerate in the absence of a magnetic field. However, in the presence of the magnetic field, this degeneracy is destroyed such that the
positive value of $m_1$ corresponds to the lower energy state and negative value to higher energy state separated by $\Delta E$.

In a n. m. r. experiment, one applies strong homogeneous magnetic field causing the nuclei to precess. Radiation of energy comparable to $\Delta E$ is then imposed with radio frequency transmitter is equal to precision or Larmor frequency and the two are said to be in resonance. The energy can be transferred from the source to the sample. The n. m. r. signal is obtained when a nucleus is excited from low energy to high energy state.

**Electron spin resonance spectroscopy**

E.p.r spectroscopy [197] is the branch of absorption spectroscopy in which radiation having frequency in the microwave region is absorbed by paramagnetic energy levels of electrons with unpaired spins. The magnetic energy splitting is done by applying a static magnetic field. For an electron of spin $S = 1/2$, the spin angular momentum quantum number will have values of $m_s = \pm 1/2$. In absence of magnetic field, the two values of $m_s$ i.e. $+1/2$ and $-1/2$ will give rise to a doubly degenerate spin energy state. If a magnetic field is applied, this degeneracy is lifted and leads to the non-degenerate energy levels. The low energy level will have the spin magnetic moment aligned with the field and correspond to the quantum number $m_s = -1/2$. On the other hand, the high energy state will have the spin magnetic moment opposed to the field and correspond to the quantum number $m_s = +1/2$. 

Conductance measurements

The conductivity measurements is one of the simplest and easily available techniques used to study the nature of complexes. It gives direct information regarding whether a given compound is ionic or covalent. For this purpose, the measurement of molar conductance ($\Lambda_m$), which is related to the conductance value in the following manner is made.

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

Conventionally, solutions of $10^{-3}$ M strength are used for the conductance measurements. Molar conductance values of different types of electrolytes in a few solvents are given as, 1:1 electrolyte has a value of 80-115 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in methanol, 65-90 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in dimethylformamide, 50-70 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in dimethylsulphoxide and 35-45 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in ethanol [198, 199]. Similarly a solution of 2:1 electrolyte has a value of 160-220 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in methanol, 130-170 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in dimethylformamide and 70-90 ohm$^{-1}$ cm$^2$ mol$^{-1}$ in ethanol.
Cyclic voltammetry

Cyclic voltammetry involves the measurement of current-voltage curves under diffusion controlled, mass transfer conditions at a stationary electrode, utilizing symmetrical triangular scan rates ranging from a few millivolts per second to hundred volts per second. The triangle returns at the same speed and permits the display of a complete polarogram with cathodic (reduction) and anodic (oxidation) waveforms one above the other. Two seconds or less is required to record a complete polarogram [200].

Consider the reaction

\[ \text{O} + \text{ne} \rightarrow \text{R} \quad (i) \]

Assuming semi-infinite linear diffusion and a solution containing initially only species O. With the electron held at a potential \( E_i \) where no electrode reaction occur. The potential is swept linearly at \( v \) v/sec so that the potential at any time is

\[ E(t) = E_i - vt \]

or \[ E_{\text{peak}} = E_{1/2} - 0.0285 \]

The rate of electron transfer is so rapid at the electrode surface that species O and R immediately adjust to the ratio according to the Nernst equation which is as follows,

\[ C_0(0,t) = C_0^* - [nFA(\pi D_0)^{1/2}]^t \int (\pi)(t-\tau)^{-1/2} \, d\tau \quad (ii) \]

\[ i = nFAC_0^*(\pi D_0\sigma)^{1/2} \times (\sigma t) \quad (iii) \]

Redox (electron-transfer) reactions of metal complexes can be investigated by cyclic voltammograms. An electrode is immersed in a solution of the complex and voltage is
swept while current flow is monitored. No current flows until oxidation or reduction occurs. After the voltage is swept over a set range in one direction, the direction is reversed and swept back to the original potential. The cycle may be repeated as often as desired. Figure 26 shows the cyclic voltammograms (CV) for a reversible one-electron redox reaction such as,

\[
\text{CpFe(CO)LMe} \longrightarrow \text{CpFe(CO)LMe}^+ + e^-
\]

Sweeping the potential in an increasing direction oxidizes the complex as the anodic current \(i_a\) flows; reversible reduction of \(\text{CpFe(CO)LMe}^+\) generates cathodic current \(i_c\) on the reverse sweep. The magnitude of the current is proportional to the concentration of the species being oxidized or reduced.

The measured parameters of interest on these cyclic voltammograms are \(i_{pa}/i_{pc}\) the ratio of peak currents and \(E_{pa} - E_{pc}\) the separation of peak potentials. For a Nernstian wave with stable product, the ratio \(i_{pa}/i_{pc} = 1\) regardless of scan rate, \(E_x\) and diffusion coefficient, when \(i_{pa}\) is measured from the decaying current as a base line. The difference between \(E_{pa}\) and \(E_{pc}\) (\(\Delta E_p\)) is a useful diagnostic test of a Nernstian reaction. Although \(\Delta E_p\) is slightly a function of \(E_x\), it is always close to \(2.3RT/\text{nF}\).
The technique yields information about reaction reversibilities and also offers a rapid means of analysis for suitable systems. The method is particularly valuable to study interaction of metal ions to H$_2$O$_2$, catechol and DNA as it provides a useful compliment to other methods of investigation, such as uv/vis spectroscopy. In cyclic voltammetric experiments, all solutions were deoxygenated via. purging with N$_2$ at room temperature.

**Kinetic studies**

In a closed constant volume system, the rate of a chemical reaction is defined as the rate of change with time of the concentration of any of the reactants and products. The concentration can be expressed in any units of quantity per unit volume e.g. moles per liter, moles per cubic centimeter. The rate will be defined as positive quantity regardless of the component whose concentration change is measured.

Consider the general chemical reaction

\[ \text{aA} + \text{bB} \rightarrow \text{cC} + \text{dD} \]

**Figure 26.** (a) Cyclic potential sweep  (b) Resulting cyclic voltammogram
The rate can be expressed as

\[ \frac{-dA}{dt}, \frac{-dB}{dt}, \frac{dC}{dt}, \text{ or } \frac{dD}{dt} \]

where A, B, C and D designate the concentration in arbitrary units.

The rate of a chemical reaction is not measured directly instead the concentration of one of the reactants or products is determined as a function of time. A common procedure for determining the reaction order is to compare the experimental results with integrated rate equations for reactions of different orders. For a first order rate equation, integrating by separate variables using integration limits such that at \( t = 0 \), \( c = c_0 \) and at \( t = t \), \( c = c \).

\[ \frac{-dc}{dt} = kc \]

or

\[ \ln (c_0/c) = kt \]

If the reaction is first order, a plot of \( \ln c \) or \( \log c \) versus time should give a straight line with a slope of \(-k\) or \(-k/2.303\) respectively (Figure 27). The dependent variable chosen is the decrease in concentration of reactant. If this variable is designated as \( x \) and \( c_0 \) is the initial concentration,

\[ \frac{dx}{dt} = k(c_0-x) \]

\[ \ln c_0 / (c_0-x) = kt \]

If however, the conditions for a given reaction are such that one or more of concentration factors are constant or nearly constant during a reaction, these factors are included in the
constant \( k \). In this case, the reaction is said to be of pseudo-\( n^{th} \) order or kinetically \( n^{th} \) order where \( n \) is the sum of the exponents of those concentration factors which alter the reaction [202]. This situation is true for catalytic reactions where the concentration of catalysts remains constant throughout the reaction, if one reactant is in large excess over another so that during the reaction there is only a small percentage change in the concentration of the former reactant.

**Viscosity Measurements**

Viscosity measurements were carried out using Ostwald’s viscometer at \( 29 \pm 0.01^\circ C \). Flow time was measured with a digital stop-watch. Each sample was measured three times and
an average flow time was calculated. Data were presented as $(\eta/\eta_0)$ versus binding ratio ([Cu]/[DNA]), where $\eta$ is a viscosity of DNA in the presence of complex and $\eta_0$ is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution ($t >100s$) corrected for the flow time of buffer alone ($t_0$), $\eta = t - t_0$. 
CHAPTER III

New binuclear oxygen carriers of the type \([M-\text{mac}-M'(L)_4]Cl_2(ClO_4)_2\) where \(M-\text{mac} = \text{Co(II)}\) and \(\text{Ni(II)}\) 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane, \(M' = \text{Mn(II)}, \text{Ni(II)}, \text{Cu(II)}\) and \(L = \text{tetra(3-acetyl pyridine)}\) and their kinetic studies with \(H_2O_2\).
CHAPTER III

Experimental

All chemicals were of reagent grade and used without further purification. N,N-dimethylformamide, 3-acetyl pyridine (Fluka), ethylenediamine, ammonia, perchloric acid (Merck), formaldehyde (Ranbaxy), hydrogen peroxide (30% w/v), MnCl₂.4H₂O, CoCl₂.6H₂O, NiCl₂.6H₂O and CuCl₂.2H₂O (BDH) were used as received. Microanalyses of the complexes were carried out on a Elementar Vario EL III Carlo Erba 1108 Elemental Analyzers. Molar conductance was measured at room temperature on a Digisun Electronic Conductivity Bridge. I.r. spectra (200-4000 cm⁻¹) were recorded on a Carl-Zeiss Specord M-80 Spectrophotometer in Nujol mull. The electronic spectra of all the complexes in N,N-dimethylformamide were recorded on a (ESP-300) Systronic 119 Spectrophotometer using 1 cm path length quartz cell. ¹H and ¹³C n.m.r spectra were performed on Bruker DRX-300 NMR spectrometers at 300 MHz at 298 K using DMSO-d₆ and D₂O as the solvent. Cyclic voltammetric measurements were carried out on a CH Instrument Electrochemical Analyzer. High purity, aqueous DMSO (95:5) solvent was used for the cyclic voltammetric (c.v) studies with 0.4 M KNO₃ as supporting electrolyte. The electrochemical cell employed a three electrode configuration with platinum disk working electrode, platinum wire counter electrode and Ag/AgCl as a reference electrode. Kinetic experiments were performed under pseudo-first order
conditions using Systronic 119 spectrophotometer. The progress of the reaction was monitored by measuring absorbance changes at 344 nm ($\lambda_{max}$ of binuclear Cu$^{II}$-Co$^{II}$ complex with H$_2$O$_2$) with respect to time under varying concentrations of H$_2$O$_2$. Pseudo-first order rate constants $k_{obs}$ were obtained from the slope of the straight line by linear least squares regression method.

**Synthesis of 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic complex** [C$_8$H$_{22}$N$_6$Ni](ClO$_4$)$_2$

The synthesis was carried out by the method reported earlier [203]. To a stirred methanolic solution (50 cm$^3$) of NiCl$_2$.6H$_2$O (0.1 mol), were added slowly ethylenediamine (99%, 0.2 mol), formaldehyde (36%, 0.4 mol) and ammonia (25%, 0.2 mol). The resulting mixture was refluxed for ca. 24 h until a dark orange solution resulted. The solution was cooled at room temperature and filtered under vaccum to remove nickel hydroxide. Excess perchloric acid in methanol was added to the filterate and the mixture was kept in the refrigerator. The yellow crystals which separated out, were washed thoroughly with methanol and dried in vacuo. The crystals were recrystallized from acetonitrile.

**Synthesis of 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic complex** [C$_8$H$_{22}$N$_6$Co](ClO$_4$)$_2$

To a stirred methanol (50 cm$^3$) suspension of CoCl$_2$.6H$_2$O (0.1 mol), were added ethylenediamine (99%, 0.2 mol), formaldehyde (36%, 0.4 mol) and ammonia (25%, 0.2
mol). The mixture was refluxed for ca. 24 h and then cooled at room temperature. An excess amount of perchloric acid in methanol was added to the filtrate and the mixture was kept in the refrigerator. The orange crystals which separated out, were washed thoroughly with methanol and dried in vacuo. The crystals were recrystallized from acetonitrile.

(Scheme 1)

**Synthesis of complex [C$_{28}$H$_{28}$N$_4$O$_4$Cu]Cl$_2$**

CuCl$_2$.2H$_2$O (1.70 g, 0.01 mol) was dissolved in cold EtOH (15 cm$^3$). 3-acetyl pyridine (4.36 cm$^3$, 0.04 mol) was added to this solution in 1:4 molar ratio. A sky blue product was formed immediately which was washed thoroughly with Et$_2$O and dried in vacuo.

**Synthesis of complex [C$_{28}$H$_{28}$N$_4$O$_4$Mn]Cl$_2$**

3-acetyl pyridine (4.36 cm$^3$, 0.04 mol) was added to the ethanolic solution (15 cm$^3$) of MnCl$_2$.4H$_2$O (1.97 g, 0.01 mol) in 4:1 molar ratio. Off white product was formed immediately at mixing which was washed thoroughly with Et$_2$O and dried in vacuo.

**Synthesis of complex [C$_{28}$H$_{28}$N$_4$O$_4$Ni]Cl$_2$**

In ethanolic solution (15 cm$^3$) of NiCl$_2$.6H$_2$O (2.37 g, 0.01 mol), 3-acetyl pyridine (4.36 cm$^3$, 0.04 mol) was added to this solution in 1:4 molar ratio. A light green product was appear immediately at mixing which was washed thoroughly with Et$_2$O and dried in vacuo.

(Scheme 2)
Synthesis of binuclear complex \([C_{36}H_{50}N_{10}O_{4}MnCo]Cl_2(ClO_4)_2\)

A mixture of 1,8-dihydro-1,3,6,8,10,13-hexaazaclotetradecane macrocyclic Co(II) complex \((0.459 \text{ g}, 1 \text{ mmol})\) in DMF \((15 \text{ cm}^3)\) and the monometallic Mn(II) complex of 3-acetyl pyridine \((0.610 \text{ g}, 1 \text{ mmol})\) in warm DMF \((15 \text{ cm}^3)\) in 1:1 molar ratio were brought to reflux. The resulting mixture was then kept overnight in refrigerator. A dark brown precipitate was obtained after filtration, washed with Et\(_2\)O and dried in vacuo.

Synthesis of binuclear complex \([C_{36}H_{50}N_{10}O_{4}MnNi]Cl_2(ClO_4)_2\)

To a cold solution of 1,8-dihydro-1,3,6,8,10,13-hexaazaclotetradecane macrocyclic Ni(II) complex \((0.459 \text{ g}, 1 \text{ mmol})\) in DMF \((15 \text{ cm}^3)\) was added monometallic Mn(II) complex of 3-acetyl pyridine \((0.610 \text{ g}, 1 \text{ mmol})\) in DMF \((15 \text{ cm}^3)\) with heating in 1:1 molar ratio. The resulting mixture was heated to reflux and then kept overnight in refrigerator. A dark brown precipitate was isolated after filtration, washed with Et\(_2\)O and dried in vacuo.

Synthesis of binuclear complex \([C_{36}H_{50}N_{10}O_{4}NiCo]Cl_2(ClO_4)_2\)

\((0.459 \text{ g}, 1 \text{ mmol})\) of the 1,8-dihydro-1,3,6,8,10,13-hexaazaclotetradecane macrocyclic Co(II) complex in DMF \((15 \text{ cm}^3)\) was added to the boiling solution of monometallic Ni(II) complex of 3-acetyl pyridine \((0.614 \text{ g}, 1 \text{ mmol})\) in DMF \((15 \text{ cm}^3)\) in 1:1 molar ratio. The resulting solution was refluxed, cooled and kept overnight in refrigerator. A black solid precipitated out after filtration which was washed with Et\(_2\)O and dried in vacuo.
Synthesis of binuclear complex \([C_{36}H_{50}N_{10}O_4NiNi]Cl_2(ClO_4)_2\)

(0.459 g, 1 mmol) of monometallic Ni(II) complex of 3-acetyl pyridine (0.614 g, 1 mmol) was dissolved in DMF (15 cm\(^3\)) by heating. To this solution, (0.459 g, 1 mmol) of 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic Ni(II) complex in DMF (15 cm\(^3\)) was added in 1:1 molar ratio. The resulting solution was heated to reflux. A dark brown precipitate was obtained after filtration, washed with Et\(_2\)O and dried in vacuo.

Synthesis of binuclear complex \([C_{36}H_{50}N_{10}O_4CuCo]Cl_2(ClO_4)_2\)

The 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic Co(II) complex (0.459 g, 1 mmol) was dissolved in DMF (15 cm\(^3\)) without heating. The monometallic Cu(II) complex of 3-acetyl pyridine (0.619 g, 1 mmol) was also dissolved in DMF (15 cm\(^3\)) by heating on water bath. Both solutions in 1:1 molar ratio were mixed and refluxed. The resulting mixture was kept overnight in a refrigerator. A dark green precipitate was obtained after filtration, washed with Et\(_2\)O and dried in vacuo.

Synthesis of binuclear complex \([C_{36}H_{50}N_{10}O_4CuNi]Cl_2(ClO_4)_2\)

(0.459 g, 1 mmol) of monometallic Cu(II) complex of 3-acetyl pyridine was dissolved in DMF (15 cm\(^3\)) by heating. This solution was added to a cold solution of (0.459 g, 1 mmol) of 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic Ni(II) complex in DMF (15 cm\(^3\)) in 1:1 molar ratio. The resulting solution was brought to reflux. A dirty green precipitate was isolated after filtration, washed with Et\(_2\)O and dried in vacuo. (Scheme 3)
Scheme 1

\[ M = \text{Co}^{II}, \text{Ni}^{II} \]

\[ [M\text{-mac}] \]

Scheme 2

\[ M' = \text{Mn}^{II}, \text{Ni}^{II} \text{ and Cu}^{II} \]

\[ [M'(L)_4]\text{Cl}_2 \]
Scheme 3

\[
[M\text{-mac}]\, + \, [M'(L)_4Cl_2] \rightarrow [M\text{-mac}\,M'(L)_4]\, \text{Cl}_2\cdot(\text{ClO}_4)_2
\]
Results and discussion

The physical and analytical data of monometallic and binuclear complexes are given in Table 1. The monometallic complexes of 3-acetyl pyridine are in powdered form and soluble in DMF/DMSO. All binuclear complexes are air stable, insoluble in MeOH/EtOH but highly soluble in DMF/DMSO. The molar conductivity (90 ohm⁻¹ cm²mol⁻¹) of binuclear complex [C₃₆H₅₀N₁₀O₄CuCo]Cl₂(ClO₄)₂ in DMSO (10⁻³M) shows that the complex is 1:4 electrolyte.

I.R. spectra

The 14-membered 1,8-dihydro-1,3,6,8,10,13-hexaaazacyclotetradecane macrocyclic complexes exhibit a single broad band at ca. 3320 cm⁻¹ which is attributed to ν(N-H) of the coordinated secondary amines. However, this band remains unaltered on coordination with tetra(3-acetyl pyridine) M'Cl₂ [where M' = Mn(II) Ni(II) and Cu(II) ]. The i.r. spectra of tetra(3-acetyl pyridine) monometallic complexes reveal bands at 1598, 1045 and 680 cm⁻¹ respectively, due to pyridine ring vibrations [204] and these bands remain unaltered in the binuclear complexes. A sharp and strong band at 1697 cm⁻¹, which is attributed to ν(C=O) of acetyl pyridine group, splits on coordination with the 14-membered 1,8-dihydro-1,3,6,8,10,13-hexaaazacyclotetradecane macrocyclic complexes. In the present complexes, the ν(C=O) shifts negatively and appears at 1637 cm⁻¹ after complexation. This strongly supports the contention that one of the pyridyl oxygen atoms is involved in coordination [205]. All the binuclear complexes exhibit strong antisymmetric stretching bands at ca. 1080-1095 cm⁻¹ and sharp antisymmetric bending
bands at ca. 624 - 630 cm⁻¹, an indication of uncoordinated perchlorate anions. In the far i.r region, ν(M-N) and ν(M-O) bands appear in the region 430-470 cm⁻¹ and 375-415 cm⁻¹ respectively [206], further confirming the coordination of metal ion of the macrocyclic complexes through carbonyl oxygen atom of monometallic 3-acetyl pyridine complex. The i.r bands of the complexes are given in Table 2.

Electronic spectra

The electronic spectra of monometallic tetra(3-acetyl pyridine) Cu(II) complex shows absorption band at ca. 22883 cm⁻¹ attributed to ²B₁g → ²E₁g transition, characteristic of square planar geometry [207]. A shoulder band observed at 11,248 cm⁻¹ is assigned to dₓz, dᵧz → dₓ²−ᵧ², dₓᵧ → dₓ²−ᵧ² transitions [208]. The electronic spectra of Co(II) macrocyclic complex displays two bands in the higher energy region at 21,276 and 26,652 cm⁻¹, assigned to ¹A₁g → ²E₁g and charge transfer L → M bands respectively, supporting square planar geometry. However, in the binuclear Cu(II)-Co(II) complex, two shoulder bands at 24,330 and 12,722 cm⁻¹ correspond to a LMCT transition and d-d transition respectively, suggesting square pyramidal geometry [209].

The electronic spectra of monometallic tetra(3-acetyl pyridine) Ni(II) complex exhibits absorption bands at 24,390 and 16,260 cm⁻¹ assignable to ¹A₁ → ¹E and ¹A₁ → ¹A₂ transitions respectively, which is characteristic of square planar geometry [210]. The Ni(II) macrocyclic complex exhibits a band at 22,573 cm⁻¹ indicating a square planar geometry around Ni(II). Two bands observed at ca. 27,100 and 21,505 cm⁻¹ in the binuclear Ni(II)-Ni(II) complex assigned to ³B₁(F) → ³E(P) and ³B₁ → ³A₂(P)
transitions are consistent with a square pyramidal geometry [211] around NiII. The electronic spectra of Mn(II)-Ni(II) show absorption band at 24,752 cm⁻¹ which also commensurates with pentacoordinate geometry [212] around Ni(II). Two medium intensity bands at ca. 20,883 and 17,513 cm⁻¹ observed in the binuclear Mn(II)-Co(II) are attributed to pentacoordinate environment [213]. The electronic spectra of Cu(II)-Ni(II) complex displays a band at ca. 26,881 cm⁻¹ which is characteristic of five coordinate square pyramidal geometry [214]. The Ni(II)-Co(II) complex also reveals Ni(II) atom in the square pyramidal environment [215].

**E.P.R spectra**

The e.p.r spectrum of tetra(3-acetyl pyridine) Cu(II) complex at room temperature shows 
\[ g_\\| = 2.23 \] and \[ g_\\perp = 2.05 \] values respectively, characteristic of square planar geometry. The higher value of \[ g_\\| \] as compared to \[ g_\\perp \] indicate the presence of unpaired electron in the \[ d_{t_1^2} \] orbital [216]. The e.p.r spectrum of binuclear Cu(II)-Co(II) displays \[ g_\\perp = 2.06 \] and \[ g_\\| = 2.22 \] values, which is consistent with square planar geometry around Cu(II).

**N.M.R spectra**

\(^1\)H-n.m.r spectrum of the macrocyclic complex \([C_8H_{22}N_8Ni]_2(ClO_4)_2\) exhibits characteristic CH₂ protons and NH protons at 2.4-2.8 ppm and 6.45 ppm respectively. The \(^1\)H n.m.r spectrum of binuclear complex \([C_{36}H_{50}N_{10}O_4NiNi]Cl_2(ClO_4)_2\) reveals no change in these signals suggesting nonparticipation of NH protons in complex formation. The \(^1\)H n.m.r spectrum of the binuclear complex reveals CH₃, CH₂ and pyridine protons
at 0.7-1.2, 2.4-2.7 and 7.44-8.19 ppm respectively. The $^{13}$C n.m.r spectra of the complex $[C_8H_{22}N_6Ni].(ClO_4)_2$ and the binuclear complex $[C_{36}H_{50}N_{10}O_4NiNi]Cl_2.(ClO_4)_2$, both reveal characteristic CH$_2$-CH$_2$, CH$_2$-NH, and pyridine ring carbons at 31.9 ppm, 39.36 ppm and 140 ppm respectively. However, there is an additional signal in the binuclear complex due to C=O carbon which appears in the range 150-160 ppm confirming the coordination through carbonyl oxygen to the metal of macrocycle.

**Electrochemical properties**

The cyclic voltammogram of the binuclear Cu(II)-Co(II) complex in aqueous DMSO (95:5) at a scan rate of 0.1 Vs$^{-1}$ is shown in Figure 28a. The figure reveals two quasi-reversible couples for one electron transfer reaction with $E^0$ values of +0.157, -0.501V and +0.116, -0.303V respectively. The peak current ratio $i_{pa}/i_{pc}$ is ~ 1 implying quasi-reversible electron transfer, but the $\Delta E_p$ values are greater than the, nernstian values ($\Delta E_p$= 59 mV) for a one electron redox system. This clearly indicates reorganization of the coordination sphere during electron transfer and this observation is authenticated by a number of reports for copper(II) complexes [217]. At different scan rates 0.1, 0.2, 0.3 Vs$^{-1}$, there is no change in $E^0$ values (Figure 28b) which supports quasi-reversible electron transfer. The first couple is attributed to Cu(II) $\rightarrow$ Cu(I) process and the second redox couple involves Co(II) $\rightarrow$ Co(I) system at second M(II) center. Reaction of the binuclear Cu(II)-Co(II) complex with H$_2$O$_2$ in aqueous DMSO (95:5) reveals a shift in $E^0$ values (0.013, 0.001V) at the same scan rate (0.1Vs$^{-1}$). The reaction proceeds through the coordination of H$_2$O$_2$ to the Co(II) center and there is change in
coordination environment from five coordinate to six coordinate geometry. The change is shown in cyclic voltammogram (Figure 29). The cyclic voltammogram after interaction with H$_2$O$_2$ exhibits two quasi-reversible couples corresponding to Cu(II) $\rightarrow$ Cu(I) and Co(III) $\rightarrow$ Co(II) redox reactions. The cyclic voltammogram indicates that there is a shift in redox peaks due to oxidation of second metal center by H$_2$O$_2$. The mechanistic pathway has been confirmed by kinetic studies.

**Figure 28.** (a) Cyclic voltammogram of the binuclear complex [C$_{36}$H$_{50}$N$_{10}$O$_4$CuCo]$\text{Cl}_2$. (ClO$_4$)$_2$ in aqueous DMSO (95: 5) at room temperature at a scan rate of 0.1 V$s^{-1}$.
Figure 28. (b) Cyclic voltammogram of the binuclear complex \([\text{C}_3\text{H}_5\text{N}_1\text{O}_4\text{CuCo}]\text{Cl}_2 (\text{ClO}_4)_2\) in aqueous DMSO (95: 5) at room temperature at a scan rate of 0.1, 0.2, 0.3 Vs\(^{-1}\).

Figure 29. Cyclic voltammogram of the binuclear complex \([\text{C}_3\text{H}_5\text{N}_1\text{O}_4\text{CuCo}]\text{Cl}_2 (\text{ClO}_4)_2\) with \(\text{H}_2\text{O}_2\) in aqueous DMSO (95: 5) at room temperature at a scan rate of 0.1 Vs\(^{-1}\).
Kinetic studies

The representative complex \([\text{C}_{36}\text{H}_{50}\text{N}_{10}\text{O}_{4}\text{CuCo}]\text{Cl}_2(\text{ClO}_4)_2\) was allowed to interact with H$_2$O$_2$ in aqueous DMSO (95:5) at 30°C temperature and the progress of the reaction was monitored by measuring absorbance changes at 344 nm with respect to time under varying concentration of H$_2$O$_2$ (c = 10x10^-2 - 16x10^-2 mol dm^-3). The plot of $-\log A$ versus time ($r > 0.996$) is linear (Figure 30) and shows that the reaction is significantly shorter under these conditions and yields a slope for the first order rate constant $k_{obs}$. The effect of [H$_2$O$_2$] was studied at constant concentration of complex (c = 1x10^-3 mol dm^-3). A linear plot was obtained when the pseudo-first order rate constants was plotted against [H$_2$O$_2$] (Figure 31) which indicates the reaction is first order on [H$_2$O$_2$].

Mechanistic studies show that the reaction involves oxo adduct formation and a change in coordination geometry. The complex \([\text{C}_{36}\text{H}_{50}\text{N}_{10}\text{O}_{4}\text{CuCo}]\text{Cl}_2(\text{ClO}_4)_2\) exhibits absorption bands at 411 and 786 nm characteristic of square pyramidal geometry, while in the oxo adduct, two bands were observed at 344 and 892 nm which are consistent with octahedral geometry around Co(II). The cleavage of oxygen bond is coupled with the oxidation of Co(II) $\rightarrow$ Co(III). The proposed mechanism is shown in Scheme 4. According to equation (3), the plot of $k_{obs}$ versus H$_2$O$_2$ should give a linear plot if the proposed mechanism is correct.
On the basis of proposed mechanism, the following rate law holds good [218].

\[ k_{\text{obs}} = k_1 k_2 \frac{[H_2O_2]}{k_{-1} + k_2} \text{ Equation (3)} \]

This rate law is consistent with the mechanism of binuclear complex as proposed in Scheme 4.
Figure 30. Plot of absorbance versus time of the binuclear complex \([C_{36}H_{50}N_{10}O_{4}CuCo]Cl_2(\text{ClO}_4)_2\) \((c = 1 \times 10^{-3} \text{ mol dm}^{-3})\) at varying concentration of \(H_2O_2\).

Figure 31. Plot of \(k_{obs}\) versus \(H_2O_2\) at varying concentration \((c = 10 - 16 \times 10^{-2} \text{ mol dm}^{-3})\).
Scheme 4
Table 1. Physical and analytical data of monometallic and binuclear complexes

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Colour</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
<th>C</th>
<th>Found (calcd) (%)</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C₁₀H₂₈N₄O₄Mn]Cl₂</td>
<td>Off white</td>
<td>300±3</td>
<td>75</td>
<td>54.69</td>
<td>(55.09)</td>
<td>4.54</td>
<td>9.09</td>
</tr>
<tr>
<td>[C₁₀H₂₈N₄O₄Ni]Cl₂</td>
<td>Light green</td>
<td>255±2</td>
<td>72</td>
<td>54.50</td>
<td>(54.75)</td>
<td>4.60</td>
<td>9.10</td>
</tr>
<tr>
<td>[C₁₀H₂₈N₄O₄Cu]Cl₂</td>
<td>Sky blue</td>
<td>225±4</td>
<td>80</td>
<td>54.60</td>
<td>(54.32)</td>
<td>4.51</td>
<td>9.00</td>
</tr>
<tr>
<td><a href="ClO%E2%82%84">C₈H₂₂N₆Co</a>₂</td>
<td>Orange</td>
<td>265±3</td>
<td>60</td>
<td>20.90</td>
<td>(20.87)</td>
<td>4.80</td>
<td>8.19</td>
</tr>
<tr>
<td><a href="ClO%E2%82%84">C₈H₂₂N₆Ni</a>₂</td>
<td>Yellow</td>
<td>252±3</td>
<td>65</td>
<td>20.92</td>
<td>(20.88)</td>
<td>4.83</td>
<td>18.13</td>
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Table 1. Contd.

<table>
<thead>
<tr>
<th>Complexes</th>
<th>Colour</th>
<th>M.p. (°C)</th>
<th>Yield (%)</th>
<th>C</th>
<th>Found (calcd) (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>([C_{36}H_{50}N_{10}O_{4}MnCo]Cl_2(ClO_4)_2)</td>
<td>Dark brown</td>
<td>230 ±4</td>
<td>50</td>
<td>40.40</td>
<td>4.70</td>
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<td>13.10</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(40.38)</td>
<td>(4.67)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([C_{36}H_{50}N_{10}O_{4}MnNi]Cl_2(ClO_4)_2)</td>
<td>Dark brown</td>
<td>285±3</td>
<td>55</td>
<td>40.30</td>
<td>4.70</td>
<td></td>
<td>13.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(40.39)</td>
<td>(4.67)</td>
<td></td>
<td></td>
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<tr>
<td>([C_{36}H_{50}N_{10}O_{4}NiCo]Cl_2(ClO_4)_2)</td>
<td>Black</td>
<td>235±3</td>
<td>55</td>
<td>40.40</td>
<td>4.63</td>
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<td>12.98</td>
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<td></td>
<td>(40.24)</td>
<td>(4.65)</td>
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<tr>
<td>([C_{36}H_{50}N_{10}O_{4}NiNi]Cl_2(ClO_4)_2)</td>
<td>Dark brown</td>
<td>195±3</td>
<td>53</td>
<td>40.30</td>
<td>4.80</td>
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<td>13.10</td>
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<td></td>
<td></td>
<td>(40.25)</td>
<td>(4.65)</td>
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<td></td>
</tr>
<tr>
<td>([C_{36}H_{50}N_{10}O_{4}CuCo]Cl_2(ClO_4)_2)</td>
<td>Dark green</td>
<td>290±2</td>
<td>56</td>
<td>39.97</td>
<td>4.61</td>
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<td>12.89</td>
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<td></td>
<td></td>
<td>(40.05)</td>
<td>(4.63)</td>
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<td></td>
</tr>
<tr>
<td>([C_{36}H_{50}N_{10}O_{4}CuNi]Cl_2(ClO_4)_2)</td>
<td>Dirty Green</td>
<td>310±2</td>
<td>48</td>
<td>39.99</td>
<td>4.61</td>
<td></td>
<td>12.88</td>
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<td></td>
<td>(40.06)</td>
<td>(4.63)</td>
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### Table 2. I.r. spectra of the complexes (cm\(^{-1}\))

<table>
<thead>
<tr>
<th>Complexes</th>
<th>(v(\text{N-H}))</th>
<th>(v(\text{C=O}))</th>
<th>(v(\text{C-N}))</th>
<th>(v(\text{C-H}))</th>
<th>(v(\text{py}))</th>
<th>(v(\text{ClO}_4))</th>
<th>(v(\text{M-N}))</th>
<th>(v(\text{M-O}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{C}<em>{28}\text{H}</em>{28}\text{N}_4\text{O}_4\text{Mn}]\text{Cl}_2)</td>
<td>--</td>
<td>1697</td>
<td>--</td>
<td>--</td>
<td>1595</td>
<td>1035</td>
<td>690</td>
<td>--</td>
</tr>
<tr>
<td>([\text{C}<em>{28}\text{H}</em>{28}\text{N}_4\text{O}_4\text{Cu}]\text{Cl}_2)</td>
<td>--</td>
<td>1695</td>
<td>--</td>
<td>--</td>
<td>1598</td>
<td>1045</td>
<td>680</td>
<td>--</td>
</tr>
<tr>
<td>([\text{C}<em>{36}\text{H}</em>{50}\text{N}_{10}\text{O}_4\text{MnCo}]\text{Cl}_2(\text{ClO}_4)_2)</td>
<td>3370</td>
<td>1697</td>
<td>1431</td>
<td>2390</td>
<td>1600</td>
<td>1085</td>
<td>470</td>
<td>375</td>
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<tr>
<td>([\text{C}<em>{36}\text{H}</em>{50}\text{N}_{10}\text{O}_4\text{MnNi}]\text{Cl}_2(\text{ClO}_4)_2)</td>
<td>3385</td>
<td>1697</td>
<td>1425</td>
<td>2375</td>
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<td>1034</td>
<td>687</td>
<td>465</td>
</tr>
<tr>
<td>([\text{C}<em>{36}\text{H}</em>{50}\text{N}_{10}\text{O}_4\text{NiCo}]\text{Cl}_2(\text{ClO}_4)_2)</td>
<td>3390</td>
<td>1695</td>
<td>1430</td>
<td>2365</td>
<td>1602</td>
<td>1030</td>
<td>686</td>
<td>450</td>
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<tr>
<td>([\text{C}<em>{36}\text{H}</em>{50}\text{N}_{10}\text{O}_4\text{NiNi}]\text{Cl}_2(\text{ClO}_4)_2)</td>
<td>3340</td>
<td>1697</td>
<td>1410</td>
<td>2350</td>
<td>1600</td>
<td>1042</td>
<td>684</td>
<td>455</td>
</tr>
<tr>
<td>([\text{C}<em>{36}\text{H}</em>{50}\text{N}_{10}\text{O}_4\text{CuCo}]\text{Cl}_2(\text{ClO}_4)_2)</td>
<td>3368</td>
<td>1697</td>
<td>1435</td>
<td>2371</td>
<td>1600</td>
<td>1042</td>
<td>685</td>
<td>445</td>
</tr>
<tr>
<td>([\text{C}<em>{36}\text{H}</em>{50}\text{N}_{10}\text{O}_4\text{CuNi}]\text{Cl}_2(\text{ClO}_4)_2)</td>
<td>3423</td>
<td>1697</td>
<td>1404</td>
<td>2371</td>
<td>1597</td>
<td>1044</td>
<td>682</td>
<td>430</td>
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</table>
Table 3. $^1$H- n.m.r spectra of the binuclear complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>-CH$_3$</th>
<th>-CH$_2$</th>
<th>-NH</th>
<th>Pyridine ring</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{36}$H$</em>{50}$N$_{10}$O$_4$Ni]Cl$_2$(ClO$_4$)$_2$</td>
<td>0.7-1.2</td>
<td>2.4-2.7</td>
<td>13.02</td>
<td>7.44-8.19</td>
</tr>
</tbody>
</table>

Table 4. $^{13}$C- n.m.r spectra of the binuclear complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>-CH$_3$</th>
<th>-C =O</th>
<th>CH$_2$-CH$_2$</th>
<th>CH$_2$-NH</th>
<th>Pyridine ring</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{36}$H$</em>{50}$N$_{10}$O$_4$Ni]Cl$_2$(ClO$_4$)$_2$</td>
<td>24.0</td>
<td>160</td>
<td>31.9</td>
<td>39.36</td>
<td>140</td>
</tr>
</tbody>
</table>
Synthesis and characterization of new synthetic oxygen carriers

A kinetic study of the reaction of the binuclear iron(III)-copper(II) complex with $\text{H}_2\text{O}_2$
CHAPTER IV

Experimental

N,N-dimethylformamide, 5-nitroindazole (Fluka), ethylenediamine, ammonia, perchloric acid (Merck), formaldehyde (Ranbaxy), hydrogen peroxide (30% w/v), FeCl₃ (anhydrous), CoCl₂·6H₂O, NiCl₂·6H₂O, CuCl₂·2H₂O, Cu(NO₃)₂·Ni(NO₃)₂ (hydrated) (BDH) were used as received. Microanalyses of the complexes were conducted on a Elementar Vario EL III Carlo Erba 1108 Elemental Analyzers. Infra-red spectra (400-4000 cm⁻¹) were recorded on a Shimadzu 8201 PC Spectrophotometer in Nujol mull. The electronic spectra of all the complexes were recorded on a (ESP-300) Systronic 119 Spectrophotometer using a 1cm path length quartz cell. ¹H n.m.r, ¹³C n.m.r and 2D cosy n.m.r spectra were recorded on an AMX-500 NMR Spectrometer using DMSO-d₆ as solvent. Cyclic voltammetric measurements were carried out on a CH Instrument Electrochemical Analyzer. High purity, aqueous DMF (95:5) was used as solvent for the cyclic voltammetric (c v) studies with 0.4 M KNO₃ as supporting electrolyte. The electrochemical cell employed a three electrode configuration with a Pt disk working electrode, Pt wire counter electrode and Ag/AgCl as a reference electrode. Kinetic experiments were performed under pseudo-first order conditions using a Systronic 119 Spectrophotometer. The progress of the reaction was monitored by measuring the absorbance change at 367 nm (λ_max of the binuclear Fe(III)-Cu(II) complex with H₂O₂) with respect to time under
varying concentrations of \( \text{H}_2\text{O}_2 \) Pseudo-first order rate constants \( k_{\text{obs}} \) were obtained from the slope of the straight line by the linear regression method.

**Synthesis of the ligand \([\text{C}_{15}\text{H}_{10}\text{N}_6\text{O}_4]\)**

5-nitroindazole (3.26 g, 20 mmol) was dissolved in absolute EtOH (100 cm\(^3\)). \( \text{H}_2\text{CO} \) solution (0.84 cm\(^3\), 10 mmol) was added to this solution in a 2:1 molar ratio. The reaction mixture was refluxed for ca. 30 h and allowed to stand overnight in a refrigerator. The cream compound which formed was washed thoroughly with Et\(_2\)O and purified by column chromatography, using silica gel as adsorbent.

**Synthesis of the bis(5-nitroindazolyI) methane copper complex**

\([\text{C}_{30}\text{H}_{20}\text{N}_{12}\text{O}_8\text{Cu}]\)(\(\text{NO}_3\))\(_2\)

The ligand \( \text{C}_{15}\text{H}_{10}\text{N}_6\text{O}_4 \) (0.676 g, 2 mmol) was dissolved in MeOH (100 cm\(^3\)). Cu(\(\text{NO}_3\))\(_2\) (hydrated) (0.241 g, 1 mmol) was added to this solution in a 2:1 molar ratio and the mixture was refluxed for ca. 48 h. The mixture was then allowed to stand for ca. 24 h in refrigerator. The dark green crystals which were obtained were filtered, washed with Et\(_2\)O and dried in vacuo.

**Synthesis of the bis(5-nitroindazolyI) methane nickel complex**

\([\text{C}_{30}\text{H}_{20}\text{N}_{12}\text{O}_8\text{Ni}]\)(\(\text{NO}_3\))\(_2\)

A solution of Ni(\(\text{NO}_3\))\(_2\) (hydrated) (0.290 g, 1 mmol) in MeOH (10 cm\(^3\)) was added to a methanol solution (100 cm\(^3\)) containing ligand \( \text{C}_{15}\text{H}_{10}\text{N}_6\text{O}_4 \) (0.676 g, 2 mmol) in 1:2 molar ratio. The resulting solution was refluxed for ca. 48 h and was then allowed to
stand for ca. 24 h in refrigerator. A light green precipitate in powder form was obtained after filtration, washed with Et<sub>2</sub>O, and dried in vacuo.

(Scheme 5).

**Synthesis of the 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane-iron (III) perchlorate [C<sub>8</sub>H<sub>22</sub>N<sub>6</sub>FeCl] (ClO<sub>4</sub>)<sub>2</sub>**

To a stirred methanol solution (50 cm<sup>3</sup>) of anhydrous FeCl<sub>3</sub> (0.1 mol) were slowly added ethylenediamine (0.2 mol), formaldehyde (0.4 mol) and ammonia (0.2 mol). The resulting mixture was refluxed for ca. 24 h until a dark orange solution appeared. This solution was cooled at room temperature and filtered under vacuum. Excess perchloric acid in methanol was added to the filtrate and the mixture was kept in refrigerator. The light yellow crystals were separated, washed thoroughly with methanol, dried in vacuo and recrystallized from acetonitrile.

A similar procedure was adopted for the synthesis of 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane Ni(II) and Co(II) complexes.

(Scheme 6)

**Synthesis of binuclear complex [C<sub>38</sub>H<sub>42</sub>N<sub>18</sub>O<sub>6</sub>FeCu]Cl(NO<sub>3</sub>)<sub>2</sub> (ClO<sub>4</sub>)<sub>2</sub>**

The bis(5-nitroindazolyl)methane copper(II) complex (0.5 g, 0.5 mmol) was dissolved in MeOH (15 cm<sup>3</sup>). The macrocyclic complex of iron(III) (0.285 g, 0.5 mmol) was also dissolved in MeOH (10 cm<sup>3</sup>). The solutions were mixed in a 1:1 molar ratio. The light green precipitate was obtained immediately, which was washed with Et<sub>2</sub>O and dried in vacuo.
Synthesis of binuclear complex \([C_{38}H_{42}N_{18}O_8CuCo](NO_3)_2(ClO_4)_2\)

To a methanol solution (15 cm\(^3\)) of bis(5-nitroindazolyl) methane copper(II) complex (0.432 g, 0.5 mmol) was added a solution of the 1,8-dihydro-1,3,6,8,10,13-hexaaazamacrocyclic cobalt(II) complex (0.229 g, 0.5 mmol) in DMF (10 cm\(^3\)) in 1:1 molar ratio. The solution was heated to refluxed for 8 h. The light brown product formed was filtered off, washed with Et\(_2\)O and dried in vacuo.

Synthesis of binuclear complex \([C_{38}H_{42}N_{18}O_8NiNi](NO_3)_2(ClO_4)_2\)

A mixture of bis(5-nitroindazolyl) methane nickel(II) complex (0.429 g, 0.5 mmol) in MeOH (15cm\(^3\)) and 1,8-dihydro-1,3,6,8,10,13-hexaaazamacrocyclic nickel(II) complex (0.229 g, 0.5 mmol) in DMF (10 cm\(^3\)) in 1:1 molar ratio was refluxed for 8 h. The yellowish green product was isolated after filtration which was washed with Et\(_2\)O and dried in vacuo (Scheme 7).

\[
\begin{align*}
\text{O}_2\text{N} \quad & \quad \text{N} + \text{CH}_2\text{O} \\
\text{EtOH} & \quad \text{H}_2\text{O} \\
\text{MeOH} & \quad \text{M(NO}_3)_2 \\
\text{MeOH} & \quad \text{M(NO}_3)_2 \\
\text{M} = \text{Cu}^{ll} \text{ and Ni}^{ll} \\
\text{Scheme 5}
\end{align*}
\]
Scheme 6

Bis(5-nitroindazolyl) methane metal complex + [M-mac]

\[
\text{M} = \text{Co}^{II}, \text{Ni}^{II} \quad \text{M} = \text{Fe}^{III}
\]

Scheme 7

\[
\text{M} = \text{Fe}^{III}
\]
Results and discussion

I.R spectra

The i.r. spectrum of the 1,8 dihydro-1,3,6,8,10,13-hexaazacyclotetradecane macrocyclic complex shows a stretching band at 3325 cm$^{-1}$ due to the v(N-H) band of the coordinated amine groups. However in the spectra of the binuclear complexes, this band remains unaltered on coordination with the bis(5-nitroindazolyl) methane complex. An intense band appears in the 1600-1640 cm$^{-1}$ and 1400-1410 cm$^{-1}$ range attributed to v(C=N) and v(C-N) stretching band of bis(5-nitroindazolyl)methane complex respectively, v(C=N) remains unaltered while v(C-N) is shifted to the 1440-1480 cm$^{-1}$ region in the binuclear complexes indicating that the nitrogen atom of 5-nitroindazole is coordinated to the metal of the hexaazamacroyclic complex. A band at 2840 cm$^{-1}$, assigned to the v(CH$_2$) vibration, remains unaltered. All binuclear complexes exhibit a strong band at 1348 cm$^{-1}$ due to v(NO$_2$) (sym stretch). Two characteristic strong antisymmetric stretching bands were also observed in the 1085-1095 cm$^{-1}$ region, and a sharp antisymmetric bending band at 630-645 cm$^{-1}$ region confirm the presence of ionic perchlorate which acts only as a counter ion [219]. The spectra display two bands at 480-495 cm$^{-1}$ and 500-520 cm$^{-1}$ region due to Fe-N and Cu-N respectively, strongly supporting coordination of the metal atom of the hexaazamacroyclic complex through the ring nitrogen of 5-nitroindazole, while the nitrogen of the azomethine group is coordinated in the monometallic bis(5-nitroindazolyl) methane complex [220].
**Electronic spectra**

The electronic spectrum of the iron(III) macrocyclic complex exhibits bands at 26,595 and 23,474 cm$^{-1}$ corresponding to $^2T_{2g} \rightarrow ^2T_{1g}$, $^2A_{1g}$ and $^2T_{2g} \rightarrow ^4T_{2g}$ transition, respectively, characteristic of the five-coordinate iron(III) [221]. The bis(5-nitroindazolyl) methane copper(II) complex displays a broad band in the 11,000-18700 cm$^{-1}$ range due to a combination of $^2B_{1g} \rightarrow ^2A_{2g}$, $^2B_{1g} \rightarrow ^2B_{2g}$ and $^2B_{1g} \rightarrow ^2E_g$ transitions which commensurate with the square planar geometry around Cu(II) metal ion [222], whereas in the binuclear Fe(III)-Cu(II) complex, two bands were observed at 24,330 cm$^{-1}$ and 14,880 cm$^{-1}$ due to the overlapping of the LMCT transition and d-d transition respectively, indicating square-pyramidal geometry around the Fe(III) metal ion [223, 224]. The electronic spectrum of the cobalt (II) macrocyclic complex exhibits two bands in the higher energy region at 21,276 and 26,652 cm$^{-1}$, attributed to $^1A_{1g} \rightarrow ^2E_{1g}$ and charge transfer $L \rightarrow M$ bands respectively, suggesting square planar geometry around Co$^{II}$ ion [225]. Two medium intensity bands occur at 22,624 and 14,848 cm$^{-1}$ in the binuclear Cu(II)-Co(II) complex which possesses a five coordinate square pyramidal geometry around cobalt (II) [226]. The electronic spectrum of the nickel(II) macrocyclic complex shows a broad band at 22,573 cm$^{-1}$, characteristic of square-planar geometry. The band at 15,625 cm$^{-1}$ has been assigned to square planar geometry for the bis(5-nitroindazolyl) methane nickel(II) complex [227]. However, the pentacoordinate environment around nickel (II) is observed in the binuclear
Ni(II)-Ni(II) complex at 26,385 cm⁻¹ and 23,474 cm⁻¹ region which has been assigned to ³B₁(F) → ³E(P) and ³B₁ → ³A₂(P) transitions, respectively [228].

E.P.R spectra

The e.p.r spectrum of the bis(5-nitroindazolyl)methane copper(II) complex was recorded at room temperature in the solid state, which show two signals corresponding to g_L=2.03 and g_H = 2.2 values respectively, characteristic of square planar geometry [229]. The e.p.r spectrum of the binuclear Fe(III)-Cu(II) complex exhibits g_L = 2.05 and g_H = 2.2 values which is also compatible with square planar geometry around copper(II) with an approximate {dxy}¹ or {dx²-y²} ground state.

N.M.R studies

¹H-n.m.r and ¹³C-n.m.r of the binuclear complex [C₃₈H₄₂N₁₈O₈NiNi(NO₃)₂(ClO₄)₂ are given in Tables 7 and 8 respectively. On the basis of ¹H-n.m.r, complete assignments have been made for the binuclear complex [C₃₈H₄₂N₁₈O₈NiNi(NO₃)₂(ClO₄)₂ using the 2D n.m.r technique (Figure 32 and Table 9).

The ¹H-n.m.r spectrum of the bis(5-nitroindazolyl) methane nickel complex exhibits a phenyl proton signal (C₆H₃) at 7.0-7.2 ppm. The signal arising from the CH=N proton of the complex appears at 13.7 ppm. A singlet, observed at 3.3 ppm, was assigned to the CH₂ protons of formaldehyde. The ¹³C-n.m.r spectra of the complex shows multiplets in the 112-125 ppm range which correspond to the phenyl carbons. A singlet at ca. 39-40 ppm is ascribed to the CH₂ carbons due to the formaldehyde group. The ¹³C-n.m.r spectra
exhibited a multiplet in the 138-141 ppm range, attributed to C=N carbons associated with the pyrazole nitrogen.

$^1$H-n.m.r and $^{13}$C-n.m.r spectra of the binuclear complex $[C_{38}H_{42}N_{18}O_8NiNi](NO_3)_2(ClO_4)_2$ show a down field shift in the CH=N signal observed in the 8.4-9.2 ppm and 131-134 ppm ranges respectively confirming that the coordination occurs through pyrazole nitrogen to the metal [230]. Additional peaks were observed in the 2.0-2.9 ppm region which are attributed to NH-CH$_2$-CH$_2$-NH protons in the $^1$H-n.m.r and 25.1 ppm for NH-CH$_2$-CH$_2$-NH carbons in the $^{13}$C-n.m.r spectra. The $^1$H-n.m.r spectra of the binuclear complex also show signals at 6.6-6.7 ppm due to NH protons of the macrocycle.

![Figure 32. 2D cosy-n.m.r spectrum of the binuclear complex $[C_{38}H_{42}N_{18}O_8NiNi](NO_3)_2(ClO_4)_2$ at 300K](image)
Electrochemical studies

The redox behavior of the binuclear Fe(III)-Cu(II) complex was investigated by cyclic voltammetry in aqueous DMF (95:5) at a scan rate of 0.1 Vs\(^{-1}\). The cyclic voltammogram of the binuclear Fe(III)-Cu(II) complex reveals two electrochemical responses with \(E^0\) values \(-0.093\, \text{V}, -0.739\, \text{V}\) and \(-0.628\, \text{V}\) respectively (Figure 33).

The first electrochemical response shows a quasi-reversible wave for the one electron transfer corresponding to the Cu(II)-Cu(I) redox couple for \(E^0\) values \(-0.739\, \text{V}\) and \(-0.628\, \text{V}\) respectively (\(\Delta E_p = 111\, \text{V}\)). The \(\Delta E_p\) values for the Cu(II)-Cu(I) redox couple are consistent with many earlier reports [231]. For a reversible wave, \(E_p\) is independent of scan rate and \(i_p\) (as well as the current at any point of the wave) is proportional to \(V^{1/2}\). The second electrochemical response shows an irreversible wave at an \(E_p\) value of \(-0.093\, \text{V}\) which is attributed to the reduction of the Fe(III) \(\rightarrow\) Fe(II) couple. The cathodic process leading to the Cu(I)/Fe(II) species seems to be associated with the reorganization of the coordination sphere around the metal, since the second electron transfer centered on the iron atom did not appear to be electrochemically reversible [224].

The cyclic voltammogram of the binuclear Fe(III)-Cu(II) complex, after interaction with \(\text{H}_2\text{O}_2\) in aqueous DMF (95:5), shows a shift in \(E_p\) (84 mv, 81 mv) as well as in \(E_{1/2}\) (98 mv, 78 mv) values respectively, at the same scan rate (Figure 34). Coordination of oxygen is favoured in the binuclear Fe(III)-Cu(II) complex, as it stabilizes the iron complex by the generation of six coordinate iron(IV) species. There is supporting evidence for iron(IV) species also in horseradish peroxidase [232, 233]. The complex is
oxidized by H$_2$O$_2$ in the same manner to the extent of two equivalents. The mechanistic pathway can be understood by a well-laid out Redox Scheme 8, as shown below.

Scheme 8

There are two possible mechanisms for peroxidase oxidation; one where H$_2$O is eliminated or another where OH and H$^+$ are formed in heterolysis/homolysis. However, the irreversibility of the cyclic voltammogram responses (shift in E$^0$ and E$_{1/2}$ values) for iron leads to the formation of a six-coordinate Fe(IV) = O species and formation of H$_2$O molecules. This observation is also consistent with the kinetic data.
Figure 33. Cyclic voltammogram of the binuclear complex \([C_{38}H_{42}N_{18}O_{8}FeCu]\ Cl(NO_3)_2\ (ClO_4)_2\) in aqueous DMF (95: 5) at room temperature at a scan rate of 0.1 \(Vs^{-1}\).

Figure 34. Cyclic voltammogram of the binuclear complex \([C_{38}H_{42}N_{18}O_{8}FeCu]\ Cl(NO_3)_2\ (ClO_4)_2\) with \(H_2O_2\) in aqueous DMF (95: 5) at room temperature at a scan rate of 0.1 \(Vs^{-1}\).
Kinetic studies

The kinetic studies on the binuclear complex \([C_{38}H_{42}N_{18}O_8FeCu]Cl(NO_3)_2(CIO_4)_2\) were performed at 367 nm \(\lambda_{\text{max}}\) of the binuclear complex \([C_{38}H_{42}N_{18}O_8FeCu]Cl(NO_3)_2(CIO_4)_2\) with \(H_2O_2\) with a freshly prepared solution of \(H_2O_2\) at 30°C. Kinetic experiments were carried out under pseudo-first order conditions with varying concentrations of \(H_2O_2\) (10-16) \(10^{-3}\) mol dm\(^{-3}\) and a fixed concentration of metal complex \((c = 1 \times 10^{-3}\) mol dm\(^{-3}\)).

The absorption time traces of the binuclear Fe(III)-Cu(II) complex reveal bands at 400 nm and 804 nm (Figure 35). Upon interaction with \(H_2O_2\), there is a shift in the 400 nm wavelength (ca. 33 nm) corresponding to the decrease in intensity of absorption with respect to time (Figure 36). The rate constants \(k_{\text{obs}}\) were calculated by the linear regression method. The slope of such a linear plot gives the rate of absorbance change (Figure 37).

As suggested in the electrochemical mechanistic studies, hydrogen peroxide reacts irreversibly with the iron(III) complex to form an iron(IV) peroxo species. Absorbance time traces fitted reasonably well to a single exponential function, and a plot of the pseudo-first order rate constants \(k_{\text{obs}}\) versus \([H_2O_2]\) shows a linear dependence (Figure 38) confirming that Fe(IV)=O oxygenated species formation are practically irreversible [74, 234]. The following mechanistic pathway was proposed [73, 235].

\[
\begin{align*}
(Mac)\text{-}Fe^{\text{III}} & + H_2O_2 \xrightarrow{k_1} (Mac)\text{-}Fe^{\text{III}}HOOH \\
(Mac)\text{-}Fe^{\text{III}}HOOH & \xrightarrow{k_2} (Mac)\text{-}Fe^{\text{IV}=O} + H_2O \\
\text{(Oxo-species)}
\end{align*}
\]
According to above proposed mechanism (Scheme 9), the corresponding rate law equation was derived.

\[ k_{\text{obs}} = \frac{k_1 k_2 [\text{H}_2\text{O}_2]}{k + k_2} \quad \text{Equation (4)} \]

Equation (4) holds good as a straight line plot of \( k_{\text{obs}} \) versus \([\text{H}_2\text{O}_2]\).
Figure 36. Electronic spectra of the representative complex \([C_{38}H_{72}N_{18}O_{8}FeCu]Cl\)
\((NO_3)_2(ClO_4)_2 (c = 1 \times 10^{-2} \text{ mol dm}^{-3})\) after interaction with \(H_2O_2\) with respect to time.
Scheme 9

\[
\begin{align*}
N_02^- + H_2O_2 & \rightarrow k_1 \cdot Cl(ClO_4)_2(NO_3)_2 \\
& + H_2O
\end{align*}
\]
Figure 37. Plot of absorbance versus time of the binuclear complex $[C_{38}H_{42}N_{18}O_{6}FeCu]$ $\text{Cl(NO}_3\text{)}_2(\text{ClO}_4\text{)}_2$ ($c = 1 \times 10^{-3}$ mol dm$^{-3}$) at varying concentration of $H_2O_2$.

Figure 38. Plot of $k_{obs}$ versus $H_2O_2$ at varying concentration $c = (10-16) \ 12.5 \times 10^{-3}$ mol dm$^{-3}$.)
<table>
<thead>
<tr>
<th>Complex</th>
<th>Colour</th>
<th>M.P (°C)</th>
<th>Yield (%)</th>
<th>Found (calcd) (%)</th>
<th>C</th>
<th>H</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C\textsubscript{38}H\textsubscript{42}N\textsubscript{18}O\textsubscript{8}FeCu]Cl(NO\textsubscript{3})\textsubscript{2}(ClO\textsubscript{4})\textsubscript{2}</td>
<td>Light green</td>
<td>230</td>
<td>68</td>
<td>33.60 (33.66)</td>
<td>3.09 (3.10)</td>
<td>20.63 (20.67)</td>
<td></td>
</tr>
<tr>
<td>[C\textsubscript{38}H\textsubscript{42}N\textsubscript{18}O\textsubscript{8}CuCo]NO\textsubscript{3}\textsubscript{2}(ClO\textsubscript{4})\textsubscript{2}</td>
<td>Dark brown</td>
<td>240</td>
<td>60</td>
<td>34.50 (34.49)</td>
<td>3.20 (3.17)</td>
<td>21.09 (21.18)</td>
<td></td>
</tr>
<tr>
<td>[C\textsubscript{38}H\textsubscript{42}N\textsubscript{18}O\textsubscript{8}NiNi]NO\textsubscript{3}\textsubscript{2}(ClO\textsubscript{4})\textsubscript{2}</td>
<td>Yellowish green</td>
<td>250</td>
<td>65</td>
<td>34.59 (34.62)</td>
<td>3.20 (3.18)</td>
<td>21.19 (21.26)</td>
<td></td>
</tr>
<tr>
<td>Complex</td>
<td>v(N-H)</td>
<td>v(C-N)</td>
<td>v(CH\textsubscript{2})</td>
<td>v(NO\textsubscript{2})</td>
<td>v(C≡N)</td>
<td>v(ClO\textsubscript{4})</td>
<td>v(Fe-N)</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>-------------------------</td>
<td>------------------------</td>
<td>--------</td>
<td>------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>[C\textsubscript{38}H\textsubscript{42}N\textsubscript{18}O\textsubscript{8}FeCu]Cl(NO\textsubscript{3})\textsubscript{2}(ClO\textsubscript{4})\textsubscript{2}</td>
<td>3325</td>
<td>1460</td>
<td>2840</td>
<td>1342</td>
<td>1627</td>
<td>1085\textsubscript{630}</td>
<td>495</td>
</tr>
<tr>
<td><a href="NO%5Ctextsubscript%7B3%7D">C\textsubscript{38}H\textsubscript{42}N\textsubscript{18}O\textsubscript{8}CuCo</a>\textsubscript{2}(ClO\textsubscript{4})\textsubscript{2}</td>
<td>3320</td>
<td>1458</td>
<td>2820</td>
<td>1336</td>
<td>1622</td>
<td>1089\textsubscript{640}</td>
<td>490</td>
</tr>
<tr>
<td><a href="NO%5Ctextsubscript%7B3%7D">C\textsubscript{38}H\textsubscript{42}N\textsubscript{18}O\textsubscript{8}NiNi</a>\textsubscript{2}(ClO\textsubscript{4})\textsubscript{2}</td>
<td>3322</td>
<td>1480</td>
<td>2835</td>
<td>1340</td>
<td>1640</td>
<td>1095\textsubscript{645}</td>
<td>480</td>
</tr>
</tbody>
</table>
Table 7. $^1$H-n.m.r data of the binuclear complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>C$_6$H$_3$</th>
<th>NH-CH$_2$-CH$_2$-NH</th>
<th>-CH$_2$</th>
<th>-NH</th>
<th>-CH=N</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{38}$H$</em>{42}$N$_{18}$O$_8$NiNi(NO$_3$)$_2$(ClO$_4$)$_2$]</td>
<td>7.0-7.2</td>
<td>2.0-2.9</td>
<td>3.3</td>
<td>6.6-6.7</td>
<td>8.4-9.2</td>
</tr>
</tbody>
</table>

Table 8. $^{13}$C-n.m.r data of the binuclear complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>C$_6$H$_3$</th>
<th>NH-CH$_2$-CH$_2$-NH</th>
<th>-CH=N</th>
<th>CH$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{38}$H$</em>{42}$N$_{18}$O$_8$NiNi(NO$_3$)$_2$(ClO$_4$)$_2$]</td>
<td>112-125</td>
<td>25.1</td>
<td>131-134</td>
<td>39.0-40.0</td>
</tr>
</tbody>
</table>
Table 9. $^1$H-n.m.r. assignments of the binuclear complex $[C_{38}H_{42}N_{18}O_8NiNi](NO_3)_2ClO_4)_2$ and correlation with 2D cosy- n.m.r. spectra (ppm).

<table>
<thead>
<tr>
<th>Protons</th>
<th>$^1$H-n.m.r.</th>
<th>COSY correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_6H_3$</td>
<td>7.0-7.2</td>
<td>7.0-7.5</td>
</tr>
<tr>
<td>NH-CH$_2$-CH$_2$-NH</td>
<td>2.0-2.9</td>
<td>2.0-3.0</td>
</tr>
<tr>
<td>-CH$_2$</td>
<td>3.3</td>
<td>3.5</td>
</tr>
<tr>
<td>-NH</td>
<td>6.6-6.7</td>
<td>6.5-6.7</td>
</tr>
</tbody>
</table>
CHAPTER V

Systematic studies on Sn$IV$ protected Fe$III$ porphyrins: oxidation of pyrocatechol by new binuclear model compound.
CHAPTER V

Experimental

Materials and Methods

Pyrrole (Merck), benzaldehyde (CDH), BF3 etherate (Acros), 2,3-dicholoro 5,6 dicyano-
p-benzoquinone (Merk-schuchardt), triphenyltin chloride (Merck), 2-hydroxypyridine
(Fluka), FeCl3 (anhydrous), NiCl2.6H2O, CuCl2.2H2O (BDH), dimethyl sulfoxide, tris
(hydroxy methyl) amine ethane (Merck), pyrocatechol (S.d fine chemical), tetra n-butyl
ammonium bromide (Lancaster) were used as received. Stock solution (2.5 M) of BF3
etherate was prepared in CHCl3. Column chromatographic separations used Merck silica
gel. Removal of solvent was carried out under vacuum using a rotary evaporator.

Microanalyses (C, H ,N) of the complexes were performed with a Elementar Vario EL
III Carlo Erba 1108 Elemental Analyzers. Molar conductance measurements of the
complexes [C67H49N50FeSn]Cl and [C23H19NOSn] were measured with an Elico model
CM 180 Conductivity Bridge, using a freshly prepared solution of 10^{-3} M complex in
DMSO and MeOH at room temperature. Infrared spectra (200-4000 cm^{-1}) were recorded
on Carl-Zeiss Specord M-80 Spectrometer in Nujol mull . The electronic absorption
spectra of the complexes were recorded on a Systronic 119 Spectrophotometer (ESP-300)
using 1 cm path length quartz cell. ^1H and ^13C n.m.r spectra were performed on Bruker
DRX-300 NMR spectrometers at 300 MHz at 298 K using DMSO-d_6 and D_2O as the
solvent. E.p.r measurements were obtained on powdered Cu(II) complexes at liquid nitrogen temperature using Varian E 112 X-band Spectrometer with modulation frequency of 100 KHz, modulation amplitude of 1G, microwave radiation power of 5 mw, field setting of 3000G and scan range of 2000G. Cyclic voltammetric experiments were performed using CH Instruments Electrochemical Analyzer. The redox potentials of the binuclear complex [C₆₇H₄₉N₅₀FeSn]Cl in absence and in presence of pyrocatechol (substrate) were determined by cyclic voltammetry in non aqueous DMSO containing 0.4 M tetra n-butyl ammonium perchlorate (TBAP) as a supporting electrolyte at room temperature. A conventional three-electrode system was employed with a platinum microcylinder as working electrode, platinum wire as auxiliary electrode and Ag/AgCl as a reference electrode respectively.

**Catecholase activity**

The kinetics of binuclear complex [C₆₇H₄₉N₅₀FeSn]Cl was carried out with pyrocatechol at different concentration (3 x 10⁻³, 4 x 10⁻³, 5 x 10⁻³, 6 x 10⁻³ M) in presence of O₂ spectrophotometrically. The progress of the oxidation reaction was monitored by measuring increase of the absorption band at about 400 nm with respect to time in DMSO saturated with O₂/buffer pH-8 solution at room temperature. The kinetic parameter Vₘₐₓ and Kₘ were evaluated from Michaelis -Menten method.

**Synthesis of porphyrin [C₄₄H₃₀N₄]**

The reaction was performed in three-necked round bottom flask fitted with a reflux condensor and a inlet port. The inlet port immersed in the solution with nitrogen flow
rates maintained at about 2 ml per min. Equimolar solution of benzaldehyde (0.25 ml, 2.5 mmol) and pyrrole (0.17 ml, 2.5 mmol) in chloroform was magnetically stirred at room temperature. After purging the solution for 5-10 min, the appropriate amount of BF₃ etherate (100μl of 2.5 M solution in CHCl₃) was added through a syringe and the reaction vessel was shielded from ambient lighting. Porphyrin yields were determined by removing 500μl aliquots from the reaction mixture and injected into oxidizing 3000μl of 10⁻² M solution of 2,3-dichloro-5, 6-dicyano-1, 4 benzoquinone in refluxing toluene. With an oxidizing solution of DDQ, the oxidation of porphyrinogen to porphyrin and pyrrylmethanes to pyrrylmethenes occurs almost instantaneously. The resulting black product was purified by column chromatography using silica gel as adsorbent. The column was washed with CHCl₃ containing 10-20% ethyl acetate to elute the porphyrin. The first two-porphyrin fractions contained 10% impurities while the third and final fractions appeared pure. These three porphyrin fractions were combined and concentrated to obtain a crude product which upon recrystallization gave tetraphenylporphyrin. The purity of porphyrin was determined from the intensity of soret band at 420 nm by uv-vis spectroscopy and also by thin layer chromatography in chloroform: petroleum ether.

**Synthesis of metalloporphyrin [C₄₄H₃₅N₄Fe]Cl**

A mixture of [H₂TPP] (0.614 g, 1mmol) and FeCl₃ (anhydrous) (0.162 g, 1mmol) in MeOH (25 cm³) was heated to reflux in 1:1 molar ratio for 12 h. The resulting dark brown solution was concentrated to ca. 10 cm³ by rotary evaporator and allowed to cool
overnight in refrigeration. A black product was isolated and washed thoroughly with hexane and dried in vacuo.

**Synthesis of metalloporphyrin [C₄₄H₃₀N₄Ni]**

This complex was prepared using nickel chloride (0.237 g, 1 mmol) and [H₂TPP] (0.614 g, 1 mmol) by a procedure similar to that described for iron(III) porphyrin.

**Synthesis of metalloporphyrin [C₄₄H₃₀N₄Cu]**

This complex was prepared using copper chloride (0.170 g, 1 mmol) and [H₂TPP] (0.614 g, 1 mmol) by a procedure similar to that described for iron(III) porphyrin.

(Scheme 10)

**Synthesis of organotin complex [C₂₃H₁₉NOSn]**

Methanolic solution of triphenyltin (0.385 g, 1 mmol) and 2-hyroxypyridine (0.95 g, 1 mmol) was heated to reflux for about 12 h and this resulting mixture was concentrated by rotary evaporator up to one-fourth which is washed with hexane and dried in vacuo.

(Scheme 11)

**Synthesis of organotin metalloporphyrinate [C₆₇H₄₉N₅OFeSn]Cl**

A solution of iron(III) porphyrin (0.705 g, 1 mmol) in MeOH (20 cm³) was added to a solution of organotin complex [C₂₃H₁₉NOSn] (0.443 g, 1 mmol) in methanol (15 cm³). The resulting solution was brought to reflux for about 12 h. A brown precipitate was collected by filtration, washed thoroughly with hexane and dried in vacuo.

**Synthesis of organotin metalloporphyrinate [C₆₇H₄₉N₅ONiSn]**

The same procedure was employed to prepare the binuclear nickel(II) porphyrin complex using nickel(II) porphyrin (0.672 g, 1 mmol) and organotin complex [C₂₃H₁₉NOSn] (0.443 g, 1 mmol).
Synthesis of organotin metalloporphyrinate [C$_{67}$H$_{49}$N$_{50}$OCuSn]

The same procedure was used to prepare the binuclear copper(II) porphyrin complex using copper(II) porphyrin (0.677g, 1mmol) and organotin complex [C$_{23}$H$_{19}$NOSn] (0.443 g, 1mmol).

(Scheme 12)

RCHO + Tetrapyromethane $\rightarrow$ Porphyrinogen

oxidation

M$^{III}$, Ni$^{II}$, Cu$^{II}$

Metalloporphyrin

Porphyрин

Scheme 10
Scheme 11

Metaoporphyrin + (Triphenyltin chloride + 2-Hydroxypyridine) → metalloporphyrin + (Triphenyltin chloride + 2-Hydroxypyridine)

Scheme 12
Results and discussion

Tetrphenylporphyrin was synthesized by Lindsey’s method [236] in two steps from commercially available starting material in which pyrrole and benzaldehyde undergo acid-catalyzed condensation to form porphyrinogen. Porphyrinogen is converted to the porphyrin in a second step upon addition of an oxidant 2,3-dichloro-5,6 dicyano-1,4 benzoquinone. Purification of porphyrin was accomplished by column chromatography. Metallation of the tetrphenylporphyrin ligand with Fe(III), Ni(II), Cu(II) in methanol in 1:1 molar ratio resulted in metalloporphyrins (Scheme 10) and the reaction of triphenyltin and 2-hydroxypyridine yielded the organotin complex \([\text{C}_{23}\text{H}_{19}\text{NOSn}]\) (Scheme 11). The desired new substituted binuclear organotin scaffold was obtained by the reaction between metalloporphyrins and organotin complex \([\text{C}_{23}\text{H}_{19}\text{NOSn}]\) (Scheme 12). The presence of Sn(IV) in the complex protects Fe(III) due to its Lewis acid nature. The molar conductance value of the 10^{-3} DMSO solution of the complex \([\text{C}_{67}\text{H}_{49}\text{N}_{5}\text{OFeSn}]\)Cl is 55 ohm^{-1} cm^2 mol^{-1} indicating that the complex is 1:1 electrolyte type. The organotin complex \([\text{C}_{23}\text{H}_{19}\text{NOSn}]\) in 10^{-3} M methanol solution having molar conductance value 10 ohm^{-1} cm^2 mol^{-1} show the non electrolytic nature of the complex.
**I.R spectra**

The infrared spectrum of the new organotin complex \([C_{23}H_{19}NOSn]\) derived from triphenyltin and 2-hydroxypyridine exhibits the characteristics absorption bands at 1540, 1069 and 691 cm\(^{-1}\) due to vibrational modes of pyridine ring. Disappearance of the bands corresponding to \(\nu(\text{Sn-Cl})\) stretching mode vibrations at 340-360 cm\(^{-1}\) region and appearance of new characteristics \(\nu(\text{Sn-O})\) [237] vibration at 443 cm\(^{-1}\) confirming the coordination of tin atom through oxygen of 2-hydroxypyridine. On complexation with metalloloporphyrin, the i.r spectra of the all the binuclear complexes show low shift in frequencies of the pyridine ring vibration which occurs at 1533, 1036 and 673 cm\(^{-1}\) clearly suggesting the involvement of nitrogen atom of hydroxypyridine. The \(\nu(\text{M-N})\) symmetric stretches are assignable in the region 470-487 cm\(^{-1}\) which further imply the direct coordination of nitrogen atom of hydroxypyridine to the metal atom. Another prominent band has been observed in the 460-464 cm\(^{-1}\) region corresponding to \(\nu(\text{Sn-O})\) vibration.

**Electronic spectra**

The electronic spectrum of iron(III) porphyrin in methanol solution exhibited the characteristic soret band at 24752 cm\(^{-1}\) which is consistent with a four coordinate environment around iron(III) [238] whereas the electronic spectrum of binuclear complex \([C_{67}H_{49}N_2OFeSn]\text{Cl}\) in DMSO is dominated by an intense broad peak at 23,923 cm\(^{-1}\) (Figure 39 ) typical of five coordinate geometry around iron(III) [239].
The electronic spectrum of the nickel(II) porphyrin display a single band at 20,876 cm\(^{-1}\) due to the \(A_{1g} \rightarrow 1B_{1g}\) transitions, consistent with the adoption of a square planar geometry [240] while the binuclear complex \([C_{67}H_{49}N_{50}NiSn]\) shows a higher energy band at 25,062 cm\(^{-1}\) region which could be assigned to \(3B_{1}(F) \rightarrow 3A_{2},3E(P)\) transition, characteristic of a square pyramidal coordination around nickel(II) [241].

A single band centered at 24,096 cm\(^{-1}\) in the electronic spectrum of the Cu(II) porphyrin attributable to the \(2B_{1g} \rightarrow 2E_{g}\) charge transfer transition and its supports square planar geometry [242]. However in the binuclear complex \([C_{67}H_{49}N_{50}OCuSn]\), one peak at 24,096 cm\(^{-1}\) remain the same and other peak at 23,094 cm\(^{-1}\) appears which correspond to d-d (\(d_{xy} \rightarrow d_{x^2-y^2}\)) transition, close to square pyramidal geometry [243].
E.P.R spectra

The solid state X-band e.p.r spectra recorded at liquid nitrogen temperature (77K) on the powdered samples of mononuclear \([\text{C}_{44}\text{H}_{30}\text{N}_{4}\text{Cu}]\) and binuclear complexes \([\text{C}_{67}\text{H}_{49}\text{N}_{50}\text{CuSn}]\) are depicted in Figure 40(a) and (b) respectively. The e.p.r spectrum of mononuclear complex \([\text{C}_{44}\text{H}_{30}\text{N}_{4}\text{Cu}]\) display well resolved broad signals of easily assignable parallel and perpendicular components \(g_{\perp}=2.16\) and \(g_{||}=2.03\) respectively [244] with \(g_{\text{av}}=1.39\) values which were calculated by the formula \(g_{\text{av}}^2=2g_{\perp}^2+g_{||}^2/3\) indicating the considerable covalent bonding in the mononuclear complex. The e.p.r parameters (g-values) \(g_{||}>g_{\perp}>g_{\epsilon}(2.0023)\) are typical of axially symmetric \(d^9\) copper(II) complex in a ground state doublet with an unpaired electron in the \(d_{x^2-y^2}\) orbital indicating square planar geometry. While the e.p.r spectrum of binuclear complex \([\text{C}_{67}\text{H}_{49}\text{N}_{50}\text{CuSn}]\) at liquid nitrogen temperature exhibit a broad and almost symmetrical features centered at \(g=2.13\), bear close resemblance to the five coordinate geometry around Cu(II) [243].

![Figure 40](image)

**Figure 40** (a) X-band e.p.r spectrum of mononuclear complex \([\text{C}_{44}\text{H}_{30}\text{N}_{4}\text{Cu}]\) at liquid nitrogen temperature (77K).
Figure 40 (b) X-band e.p.r spectrum of binuclear complex [C₆₇H₄₉N₅OCuSn] at liquid nitrogen temperature (77K). Both spectra were recorded at 100 KHz modulation, microwave frequency 9.1 GHz and a field set of 3000 G.

N.M.R spectra

$^1$H and $^{13}$C n.m.r spectra of nickel(II) substituted organotin porphyrin are shown in Figure 41(a) and (b). The $^1$H and $^{13}$C n.m.r signals and information from the synthesis were used to assign a structure to a newly synthesized porphyrin.

$^1$H n.m.r spectra of nickel(II) porphyrin show the presence of multiplets CH protons in the range 3.7-4.1 ppm [245] and a signal observed at 7.46 ppm due to doublet pyrrole protons [246]. This complex displays another doublet at 7.75-7.86 ppm which correspond to the phenyl protons [247]. On the other hand, $^1$H n.m.r spectra of nickel(II) substituted organotin complex consists of multiplets CH protons at 3.19-3.59 ppm, pyrrole protons at 7.4 ppm and phenyl protons occur at 7.6 ppm, respectively. Furthermore, the presence of new signals of pyridine protons are manifested as narrowly split multiplets at $\delta$ 6.3 (d,1H), $\delta$ 6.46 (t,1H) and $\delta$ 6.49 (m,2H) indicating the coordination of nitrogen of the pyridine ring to the metal ion [248].
Similarly, $^{13}$C n.m.r spectra of the nickel(II) porphyrin and nickel(II) substituted organotin porphyrin exhibits a singlet at 39.0-39.6 ppm which can be assigned to CH carbon. A multiplet appearing at 110-125 ppm may be ascribed to the phenyl carbons [249]. Two signals at 130 ppm and 136 ppm are due to the presence of pyrrole $\alpha$C carbons and pyrrole $\beta$C carbons respectively [250, 251]. However in the $^{13}$C n.m.r spectra of the binuclear complex $[C_{67}H_{49}N_{5}ONiSn]$, additional signal appeared at 145 ppm, corresponding to pyridine ring carbon, further corroborated the proposed structure [252].

![Figure 41](image)

**Figure 41** (a) $^1$H-n m r spectrum of the binuclear complex $[C_{67}H_{49}N_{5}ONiSn]$ obtained in $D_2O$ at 298 K
Electrochemical studies

The electrochemical feature of the representative binuclear organotin complex [C₆₇H₄₉N₅OFeSn]Cl (c = 1 x 10⁻³ mol dm⁻³) was studied using cyclic voltammetry at room temperature over a potential range from 0.4V to -1.2V in DMSO containing 0.4 M tetra-n-butyl ammonium perchlorate (TBAP) as a supporting electrolyte. The cyclic voltammogram for the binuclear complex [C₆₇H₄₉N₅OFeSn]Cl (Figure 42) depicts a well formed cathodic wave [E_{pc} (cathodic peak potential) = -0.835 V] as well as an anodic wave [E_{pa} (anodic peak potential) = -0.717 V] with E_{1/2} value at -0.776 V at a scan rate of
0.1Vs⁻¹. This complex undergoes a quasi-reversible redox wave corresponding to one
Fe(III)/Fe(II) reduction couple and also an additional irreversible cathodic peak which
occurred at \( E_{pc} = -0.652 \) V. The separation between the oxidation and reduction
potentials for the quasi-reversible couple is 118 mV [253] and the ratio of anodic peak
current to cathodic peak current \( (I_{pa}/I_{pc}) \) is equal to unity suggesting a fairly quasi-
reversible redox process.

In presence of molecular oxygen, the cyclic voltammogram differs considerably from the
free complex (in absence of O₂) as the cathodic peak appears at \( E_{pc} = -0.897 \) V at a scan
rate of 0.1Vs⁻¹. There is shift of (62 mV) in \( E_{pc} \) of the complex after oxygenation but no
anodic peak potential corresponding to the reoxidation of Fe(II) to Fe(III) was detected in
the presence of dioxygen [254] (Figure 43).

The cyclic voltammetry for the catechol cleaving dioxygenase activity of the complex
\([C_{67}H_{49}N_{50}FeSn]\)Cl towards pyrocatechol was examined in presence of molecular
oxygen at a scan rate of 0.1Vs⁻¹. On addition of pyrocatechol to the complex
\([C_{67}H_{49}N_{50}FeSn]\)Cl in presence of O₂, the cyclic voltammogram displays an irreversible
wave of cathodic peak \( E_{pc} \) at -0.878 V with no anodic peak coupled to it (Figure 44 a).
These features can be associated with the reduction of catechol protons to produce
quinone species [255, 256]. The CV scan shows an irreversible wave of two cathodic
response at 0.898 V and 0.246V (former response is shifted by 20 mV) after sometime
(10 min) (Figure 44 b) which exhibits the complete conversion of pyrocatechol to
extradiol cleavage product at the Fe(III) center.
Figure 42. Cyclic voltammogram of free binuclear complex \([C_{67}H_{49}N_3OFeSn]\)Cl in non-aqueous (DMSO + 0.4 M TBAP) at room temperature at a scan rate of 0.1 Vs\(^{-1}\).

Figure 43. Cyclic voltammogram of binuclear complex \([C_{67}H_{49}N_3OFeSn]\)Cl in non-aqueous (DMSO + 0.4 M TBAP) after passing O\(_2\) at room temperature at a scan of 0.1 Vs\(^{-1}\).
Figure 44 (a) Cyclic voltammogram of binuclear complex $[C_{67}H_{49}N_5OFeSn]Cl$ in non-aqueous (DMSO + 0.4 M TBAP) saturated with $O_2$ in presence of added pyrocatechol at the initial stage at room temperature at a scan rate of 0.1 $Vs^{-1}$.

Figure 44 (b) Cyclic voltammogram of binuclear complex $[C_{67}H_{49}N_5OFeSn]Cl$ in non-aqueous (DMSO + 0.4 M TBAP) saturated with $O_2$ after completion of catalytic reaction with pyrocatechol at room temperature at a scan rate of 0.1 $Vs^{-1}$.
Kinetic studies for catecholate activity

The kinetic experiment of the binuclear complex \([C_{67}H_{49}N_{50}FeSn]\)Cl has been studied with pyrocatechol for catecholase activity at room temperature in aerobic conditions. The catalytic oxidation of pyrocatechol via extradiol cleavage product was monitored by spectrophotometry after injecting a complex solution prepared in DMSO into the solution of pyrocatechol in DMSO. A typical experiment was carried out as follows. 100 \(\mu\)l of a complex solution \((c = 1 \times 10^{-3} \text{ mol dm}^{-3})\) was added to a 1 cm path length cell containing 3 ml of DMSO saturated with \(\text{O}_2\) and 100 \(\mu\)l of aqueous buffer TRIS pH - 8.0. The reaction kinetics was followed by the addition of different concentration of 100 \(\mu\)l of catechol solution \([\text{pyrocatechol}] = 3.0 \times 10^{-3} \text{ to } 6.0 \times 10^{-3} \text{ M}\). The course of the reaction was monitored for almost 20 min at regular time intervals by scanning the spectra every 4 min. The absorption spectrum of the binuclear complex \([C_{67}H_{49}N_{50}FeSn]\)Cl revealed band at 418 nm but upon addition of pyrocatechol to the reaction solution in presence of \(\text{O}_2\), the spectral changes occurred and brown solution changed into dark grey solution and a wide band centered at 700 nm appeared immediately due to the catecholate to iron(III) charge transfer as a result of catechol binding. The band at 418 nm disappeared and a new peak generates at 400 nm indicating the solution is absorbing dioxygen and pyrocatechol is being converted into 5 formyl-2 furanone by monitoring the increasing absorbance at 400 nm (Figure 45).

A kinetic treatment on the basis of Michaelis-Menten approach was applied. The catalytic reaction also shows a dependence on the concentration of both the complex and
pyrocatechol and the second order reaction rate was deduced from the slope of trace at 400 nm by the method of initial rate (Figure 46). The Lineweaver Burk plots gave $V_{\text{max}} = (5.5 \times 10^{-3} \text{ M}^{-1} \text{ S}^{-1})$ and $K_M = (7.0 \times 10^{-4} \text{ M})$ (Figure 47). This kinetic experiment demonstrated that the iron(III) complex was found to be active toward oxidation of catechol by O$_2$ to afford extradiol cleavage product (Scheme 13) which was characterized by its uv-vis absorption spectrum ($\lambda_{\text{max}} = 400 \text{ nm}$) [257].

In the reaction route of extradiol oxygenation, the substrate (catechol) loses both of its proton upon coordination to the iron center followed by partial oxidation of the catecholate due to ligand to metal charge transfer, resulting in a substrate with semiquinone species (formation of the seven coordinate intermediate species which is feasible) [258, 259]. The next step involves the binding of O$_2$ to Fe (III) center. The electron transfer from metal to O$_2$ in the Fe(III)-O$_2$ species results in a superoxide like moiety. The bound O$_2$ attacks the carbon adjacent to the enediol unit in a Michael type addition to form peroxy intermediate that decomposes by a Criegee type rearrangement to the desired extradiol cleavage product [260, 261].
Figure 45. **Progress of the reaction of binuclear complex** \([C_{67}H_{49}N_5OFeSn]Cl\) **saturated with** \(O_2\) **after adding Tris-HCl buffer (pH 8.0) and** pyrocatechol **showing increase in absorbance as monitored by visible spectroscopy at 4.0, 8.0, 12.0, 16.0 min.**

![Graph showing absorbance vs. wavelength](image)

**Wavelength (nm)**

**Absorbance**

\[\lambda_{\text{max}} = 400 \text{ nm}\]

**Figure 46.** **The plot of enzyme kinetics of the binuclear complex** \([C_{67}H_{49}N_5OFeSn]Cl\) **with pyrocatechol.** The reaction were performed in DMSO saturated with \(O_2\), Tris-HCl buffer (pH 8.0) and pyrocatechol of different concentration (a) \(3 \times 10^{-3} \text{ M}\) (b) \(4 \times 10^{-3} \text{ M}\) (c) \(5 \times 10^{-3} \text{ M}\) (d) \(6 \times 10^{-3} \text{ M}\).
Figure 47. Dependence of the reaction rates on the concentration of pyrocatechol (substrate) for the oxidation reaction catalyzed by binuclear complex \([C_{67}H_{49}N_5OFeSn]Cl\).

Scheme 13
<table>
<thead>
<tr>
<th>Complex</th>
<th>Colour</th>
<th>M.P (°C)</th>
<th>Yield (%)</th>
<th>Found (calcd) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C_{23}H_{19}NOSn]</td>
<td>Off white</td>
<td>70</td>
<td>40</td>
<td>C: 62.23 (62.30), H: 4.26 (4.28), N: 3.15 (3.16)</td>
</tr>
<tr>
<td>[C_{67}H_{49}N_5OFeSn]Cl</td>
<td>Dark brown</td>
<td>240 (d)</td>
<td>35</td>
<td>C: 70.10 (70.00), H: 4.28 (4.26), N: 6.10 (6.09)</td>
</tr>
<tr>
<td>[C_{67}H_{49}N_5ONiSn]</td>
<td>Black</td>
<td>120</td>
<td>32</td>
<td>C: 72.06 (72.10), H: 4.40 (4.39), N: 6.21 (6.28)</td>
</tr>
<tr>
<td>[C_{67}H_{49}N_5OCuSn]</td>
<td>Brown</td>
<td>140</td>
<td>30</td>
<td>C: 71.69 (71.75), H: 4.35 (4.37), N: 6.20 (6.24)</td>
</tr>
</tbody>
</table>
Table 11. I. r spectra of the complexes (cm⁻¹)

<table>
<thead>
<tr>
<th>Complex</th>
<th>v(C-N)</th>
<th>v(M-N)</th>
<th>v(Sn-O)</th>
<th>v(Py)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C₅₇H₄₉N₅OFeSn]Cl</td>
<td>1404</td>
<td>487</td>
<td>460</td>
<td>1540, 1069, 691</td>
</tr>
<tr>
<td>[C₅₇H₄₉N₂ONiSn]</td>
<td>1397</td>
<td>474</td>
<td>464</td>
<td>1515, 1075, 691</td>
</tr>
<tr>
<td>[C₅₇H₄₉N₅OCuSn]</td>
<td>1405</td>
<td>470</td>
<td>455</td>
<td>1601, 988, 602</td>
</tr>
</tbody>
</table>
Table 12. $^1$H-n.m.r data of the complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>CH</th>
<th>Pyrrole protons</th>
<th>C$_6$H$_5$</th>
<th>Pyridine ring</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{C}<em>{44}\text{H}</em>{30}\text{N}_4\text{Ni}]$</td>
<td>3.7-4.1</td>
<td>7.46</td>
<td>7.75-7.86</td>
<td>–</td>
</tr>
<tr>
<td>$[\text{C}<em>{67}\text{H}</em>{49}\text{N}_5\text{ONiSn}]$</td>
<td>3.19-3.59</td>
<td>7.4</td>
<td>7.6</td>
<td>6.43-6.49</td>
</tr>
</tbody>
</table>

Table 13. $^{13}$C-n.m.r data of the complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>CH</th>
<th>Phenyl carbon</th>
<th>Pyrrole $\alpha$C</th>
<th>Pyrrole $\beta$C</th>
<th>Pyridine ring</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{C}<em>{44}\text{H}</em>{30}\text{N}_4\text{Ni}]$</td>
<td>39.6</td>
<td>125</td>
<td>128</td>
<td>135</td>
<td>–</td>
</tr>
<tr>
<td>$[\text{C}<em>{67}\text{H}</em>{49}\text{N}_5\text{ONiSn}]$</td>
<td>39.6-40.7</td>
<td>110-120</td>
<td>130</td>
<td>136</td>
<td>145</td>
</tr>
</tbody>
</table>
New modulated metallic macrocycles, electrochemistry and their interaction with calf thymus DNA.
CHAPTER VI

Experimental

Chemicals
NiCl₂.6H₂O, CuCl₂.2H₂O, CoCl₂.6H₂O (BDH), salicylaldehyde, KBH₄ (Lancaster), ethanolamine, ethylenediamine, formaldehyde, perchloric acid, ammonia, tris(hydroxy methyl) amine methane (Merck), HCl are of analytical reagent grade and were used as received. Preparation of tetrabutylammonium perchlorate (TBAP) for electrochemical work were performed as reported in literature [262]. Calf thymus DNA was obtained from sigma. Solution of calf thymus DNA in Tris HCl buffer gave a ratio of UV absorbance at 260 and 280 nm of ca. 1.9:1 indicating that DNA was sufficiently free of protein [263].

Physical measurements
Elemental analysis of C, H and N of the complexes was conducted on Elementar Vario EL III Carlo Erba 1108 Elemental Analyzers. The electrical conductivity measurements were performed on an Elico Model CM 180 Conductivity Bridge, using a freshly prepared solution of the 10⁻³ M complexes in DMSO at room temperature. Infrared spectra were recorded in the wavenumber range of 4000-400 cm⁻¹ with Shimadzu 8201 PC Spectrophotometer in Nujol mull. Electronic spectral studies of the complexes were determined on a Systronic 119 Spectrophotometer (ESP-300) using 1 cm path length quartz cell in wavelength region 300-950 nm reported as λ_max/ cm⁻¹. ¹H and ¹³C n.m.r and 2D cosy n.m.r spectra were recorded at 300MHz on Bruker DRX-300 NMR.
Spectrometer using D₂O, DMSO-d₆ as solvent. Solid state EPR investigations of complex [C₁₇H₃₄N₇O₂Cu].ClO₄ at liquid nitrogen temperature (77K) were performed on a Varian E 112 X-band Spectrometer. The instrumental parameters were: field setting of 3000 G, scan range of 2000 G, field modulation amplitude of 1G, microwave radiation power of 5 mV using tetracyanoethylene (TCNE) (g = 2.011) as field marker. Cyclic voltammetric experiments were performed using CH Instruments Electrochemical Analyzer. The redox potentials of the complexes [C₁₇H₃₄N₇O₂Cu].ClO₄ and [C₁₇H₃₄N₇O₂Co].ClO₄ in absence and in presence of calf thymus DNA were determined by cyclic voltammetry in non aqueous DMSO containing 0.4 M tetra n-butyl ammonium perchlorate (TBAP) as a supporting electrolyte at room temperature. A conventional three electrode system was employed with a platinum microcylinder as working electrode, platinum wire as auxiliary electrode and Ag/AgCl as a reference electrode respectively. All formal potentials were taken as the average of the anodic and cathodic peak potentials E₁/₂. All the kinetic experiments involving interaction of the complex with CT DNA were performed in aerated buffer (0.01M, pH 7.5) on USB-2000 Ocean Optics Spectrophotometer. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600 dm³ mol⁻¹ cm⁻¹ at 260 nm [264]. Viscosity measurements were carried out using Ostwald’s viscometer at 25°C. Flow time was measured with a digital stop-watch. Each sample was measured three times and an average flow is calculated. Data were presented as η/η₀ versus binding ratio ([M]/[DNA]) [265] where η is a viscosity of DNA in the presence of complex and η₀
is the viscosity of DNA alone. Viscosity values were calculated from the observed flow time of DNA containing solution \( t > 100 \text{s} \) correct for the flow time of buffer alone \( t_0 \),

\[ \eta = t - t_0 \] [266].

**Synthesis of Hsalea N-(2-hydroxy benzyl)-2-amino-1 ethanol**

For the preparation of Hsalea N-(2-hydroxy benzyl)-2-amino-1 ethanol ligand, the reaction was carried out according to the previous literature method [267] which is given below.

2-amino-1 ethanol (3.6ml, 60 mmol) and salicylaldehyde (6.3 ml, 60 mmol) was dissolved in 100 ml ethanol. The resulting mixture was allowed to stir for half h, then 3.2 g solid KBH\(_4\) was carefully added portion wise with stirring and was refluxed for 2h, meanwhile the reaction solution changed slowly from yellow to colorless. The solvent was removed under reduced pressure by rotary evaporator. The resulting viscous residue was dissolved in water (40 ml) and the solution was extracted with chloroform (3 x 40 ml). The chloroform extracts were combined and dried over anhydrous sodium sulphate, and dried solution was filtered and half of CHCl\(_3\) was again evaporated on a rotary evaporator. A white crude product was obtained by drop wise addition of concentration HCl, washed with ether and dried.

(Scheme 15)

**Synthesis of the 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane-Cu(II) perchlorate \([C_8H_{22}N_6Cu\) (ClO\(_4\)]\)\(_2\)**
To a stirred methanol solution (50 cm$^3$) of CuCl$_2.2$H$_2$O (0.1 mol) were slowly added ethylenediamine (0.2 mol), formaldehyde (0.4 mol) and ammonia (0.2 mol). The resulting mixture was refluxed for ca. 24 h until a dark blue solution appeared. This solution was cooled at room temperature and filtered under vacuum. Excess perchloric acid in methanol was added to the filtrate and the mixture was kept in refrigerator. The purple-red crystals were separated, washed thoroughly with methanol, dried in vacuo and recrystallized from acetonitrile.

A similar procedure was adopted for the synthesis of 1,8-dihydro-1,3,6,8,10,13-hexaazacyclotetradecane Co(II) and Ni(II) complexes.

(Scheme 16)

**Synthesis of new modulated macrocycle [C$_{17}$H$_{34}$N$_7$O$_2$Cu] ClO$_4$**

A solution of (0.740 g, 0.4 mmol) of 1,8-dihydro 1,3,6,8,10,13 hexaazamacroyclic copper (II) complex in warm DMF (15 cm$^3$) was slowly mixed to a cold solution of Hsalea ligand (0.356 g, 0.4 mmol) in MeOH (10 cm$^3$) in equimolar quantities. Aqueous NaOH (0.1 ml, 4 M, 0.4 mmol) was added to this solution. The resulting mixture was heated to reflux. A black product was obtained after concentrating the refluxing material, washed with n-hexane and dried in vacuo.

**Synthesis of new modulated macrocycle [C$_{17}$H$_{34}$N$_7$O$_2$Co] ClO$_4$**

This complex was prepared with 1,8-dihydro 1,3,6,8,10,13 hexaazamacroyclic cobalt(II) complex (0.736 g, 0.4 mmol) and Hsalea ligand (0.356 g, 0.4 mmol) by the same method as used for the first preparation of copper analogue.
Synthesis of new modulated macrocycle $[C_{17}H_{34}N_7O_2Ni]ClO_4$

This complex was prepared with 1,8-dihydro 1,3,6,8,10,13 hexaazamacroyclic nickel (II) complex (0.736 g, 0.4 mmol) and Hsalea ligand (0.356 g, 0.4 mmol) by the same method as described for Cu (II) complex.

(Scheme 17)

\[
\begin{align*}
\text{Salicylaldehyde} & \quad + \quad \begin{array}{c} \text{Aminoalcohol} \\ \text{1} \end{array} \quad \xrightarrow{\text{KBH}_4, \text{H}_2\text{O}} \quad \begin{array}{c} \text{Hsalea ligand} \\ \text{N-(2 hydroxybenzyl) -2 amino 1-ethanol} \\ \text{1} \end{array}
\end{align*}
\]

Scheme 15

\[
\begin{align*}
\text{M = Cu}^{II}, \text{Co}^{II}, \text{Ni}^{II}
\end{align*}
\]

Scheme 16
Scheme 17

\[ \text{Hexaazamacrocycle} + \text{Hsalea ligand} + (\text{ClO}_4)_2 \cdot \text{NaOH} \rightarrow \text{product} \cdot \text{ClO}_4 \]
Results and discussion

The Hsalea ligand was prepared by direct condensation of 2-amino-1 ethanol with salicylaldehyde in ethanol followed by reduction with KBH₄ in situ (scheme 15). Further new modulated macrocycles were synthesized by reaction of 1,8-dihydro1,3,6,8,10,13 hexaazamacrocyclic complex [M = Cu(II), Co(II), Ni(II)] with Hsalea ligand. The isolated complexes are sketched in scheme 17. The coordination geometry of the central metal ion is square pyramidal in the complexes which is proposed on the basis of spectroscopic studies. The molar conductance of the complexes in 10⁻³ M solution in DMSO are observed in the range 55-65 ohm⁻¹ cm² mol⁻¹ corresponding to 1:1 electrolyte indicating that one ClO₄⁻ is present which is further authenticated by the elemental analysis and i.r spectral bands. The complexes are soluble in DMF and DMSO.

I.R Spectra

The infrared spectra of all the 1,8 dihydro 1,3,6,8,10,13 hexaazamacrocyclic complexes present a single broad stretching vibration at 3320 cm⁻¹ belongs to the v(N-H) of the coordinated amines, while in the spectra of new modulated macrocycles, this stretching vibration remain essentially unchanged. Bands due to v(C-O) vibration at 3090-3098, 2932-2970, 2850-2862, 1550-1560, 1440-1462, 1360-1396, 1250-1277 cm⁻¹ region can be observed in the spectra of new modulated macrocycles remain unaffected by metal complexation are consistent with metal coordination of oxygen atom of Hsalea ligand. A band of medium intensity at 760-778 cm⁻¹ signature of aromatic ring vibration is also occurred. The spectra of these complexes show a very strong band ~1090 cm⁻¹ and a
medium band at $\sim 629 \text{ cm}^{-1}$ due to uncoordinated ClO$_4^-$. All the complexes exhibit a broad medium intensity band at 3380-3399 cm$^{-1}$ can be ascribed to $\nu$(OH), suggesting the presence of ligand in the structure. This has been further corroborated by the appearance of medium $\nu$(M-O) bands at 468-474 cm$^{-1}$ [268] and $\nu$(M-N) bands at 515-575 [269] cm$^{-1}$ prove the coordination of oxygen atom of salicylaldehyde to the metal of macrocyclic complexes.

**Electronic spectra**

The main absorption of the solution electronic spectra of freshly prepared 1,8 dihydro 1,3,6,8,10,13 hexaazamacroyclic complexes and its new modulated macrocycles in DMF, DMSO examined in the 33333-11111 cm$^{-1}$ region. The UV-vis spectrum of complex $[C_{17}H_{34}N_{7}O_{2}Cu]$ ClO$_4$ reveals the high energy absorption band at 25,509 and 23,923 cm$^{-1}$ attributed to strong LMCT bands assigned to $\pi \rightarrow \pi^*$ and d $\rightarrow \pi^*$ due to the coordination of deprotonated phenoxy group to Cu(II) of hexaazamacrocycle, consistent with square pyramidal geometry [270], in contrast 1,8 dihydro 1,3,6,8,10,13 hexaazamacroyclic copper(II) complex exhibits a low energy intense broad band at 16,835 cm$^{-1}$ assigned to square planar geometry [271].

The electronic absorption spectrum of the complex $[C_{17}H_{34}N_{7}O_{2}Co]$ ClO$_4$ possess intense band at 24,330 cm$^{-1}$ attributed to MLCT transition, close to square pyramidal geometry around Co$^{II}$ ion, are compared to the absorption spectrum of the 1,8 dihydro 1,3,6,8,10,13 hexaazamacroyclic cobalt(II) complex which lies at 21,186 cm$^{-1}$ assigned to square planar environment. The absorption band are blue shifted compared to its new modulated
macrocycles. Similarly, 1,8 dihydro-1,3,6,8,10,13 hexaazamacrocyclic nickel(II) complex reveals only one broad band at 22,522 cm\(^{-1}\) due to square planar geometry of Ni(II) metal ion. On the other hand, the absorption spectrum of new modulated macrocyclic complex \([C_{17}H_{34}N_7O_2Ni] ClO_4\) exhibited a single band at 23,923 cm\(^{-1}\) followed by a shoulder peak in the 20000-16666 cm\(^{-1}\) region attributed to \(^3\)B\(_1\) (F) \(\rightarrow\) \(^3\)A\(_2\), \(^3\)E(P) and \(^3\)B\(_1\) (F) \(\rightarrow\) \(^3\)E(F) transition respectively [211]. The presence of distinguishable absorption maxima indicates that a pentacoordinate geometry around Ni(II) ion.

**E P R Spectra**

The solid state X-band e.p.r spectrum of complex \([C_{17}H_{34}N_7O_2Cu] ClO_4\) was measured at liquid nitrogen temperature (77K) with remarkable differences being observed between the e.p.r spectra of 1,8 dihydro 1,3,6,8,10,13 hexaazamacrocyclic copper(II) complex and its new modulated macrocycle \([C_{17}H_{34}N_7O_2Cu] ClO_4\).

The e.p.r spectrum of 1,8 dihydro 1,3,6,8,10,13 hexaazamacrocyclic copper(II) complex consists of a very broad axial symmetrical line shape with \(g_{11} = 2.18\), \(g_\perp = 2.07\) values and \(g_{av} = 2.10\). The \(g_{av}\) was calculated from the formula \(g_{av}^2 = 2g_\perp^2 + g_{11}^2/3\). The sequence \(g_{11} > g_\perp > 2.0\) are typical of axially symmetric \(d^9\) copper (II) complex in a ground state doublet with the unpaired electron in the \(d_{x^2-y^2}\) orbital. Moreover the relevant \(g_{11}\), \(g_\perp\) and \(g_{av}\) parameter are in good agreement with the coordination geometry around the metal ion. The present complex exhibit an essentially square planar coordination geometry [244]. The liquid nitrogen temperature e.p.r spectrum of complex
[C_{17}H_{34}N_{7}O_{2}Cu] ClO_4 with broad feature is accompanied by the appearance of only a single isotropic signal at g = 2.09 (depicted in Figure 48) possess mononuclear five coordinate square pyramidal environment around copper(II) ion with dz^2 ground state [272].

Figure 48. X-band e.p.r spectrum of complex [C_{17}H_{34}N_{7}O_{2}Cu] ClO_4 at liquid nitrogen temperature (77K.), spectrum were recorded at 100KHz modulation, microwave frequency 9.1 GHz and a field set of 3000G.
N.M.R spectra

The $^1$H and $^{13}$C n.m.r spectra of the complex $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Ni}]\text{ClO}_4$ were compared to the Hsalea N-(2-hydroxybenzyl)-2-amino-1-ethanol ligand in order to elucidate the structure of the complex. $^1$H, $^{13}$C-n.m.r and 2D cosy n.m.r spectra of the complex $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Ni}]\text{ClO}_4$ are shown in Figure 49(a), (b) and (c) respectively. In $^1$H n.m.r, the 1,8 dihydro 1,3,6,8,10,13 hexaaazamacroyclic nickel(II) complex exhibit the characteristics resonances at 6.0 ppm as a sharp singlet due to NH proton and at 2.42-2.72 ppm for the CH$_2$ protons of the ethylenediamine framework [273]. The $^1$H n.m.r of the Hsalea ligand has a large number of sharp non overlapping signals and have good resolution. In $^1$H n.m.r, the aromatic protons are clustered at 7.35-7.41 ppm and appear as a four proton multiplet. The signal in the high field region are generating a doublet at 3.20-3.23 ppm corresponding to NHCH$_2$ functionality. In addition, two signals are easily detected in the range 4.29-4.41 ppm and 3.85-3.89 ppm for the two protons as a doublet which are attributed to ArCH$_2$ and CH$_2$OH group respectively [267]. While the $^1$H n.m.r spectra of the complex $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Ni}]\text{ClO}_4$ in comparison to free Hsalea ligand revealed downfield shift in aromatic protons due to the formation of M-O bonds. The rest of the spectrum involves characteristics signals which are present in Hsalea ligand. $^1$H n.m.r also contain resonance at 6.5 ppm and in the range 2.49-2.87 ppm due to NH protons and CH$_2$ protons of the macrocycle. The assignment of the proton signals were also obtained from 2D cosy n.m.r which exhibits the relatively same resonances in $^1$H n.m.r
at 2.5, 3.2, 3.3, 3.85-3.9, 7.3, 4.2-4.4, 6.3 ppm due to CH$_2$, NHCH$_2$, CH$_2$OH, aromatic protons, ArCH$_2$ and NH protons respectively.

$^{13}$C n.m.r spectrum of Hsalea ligand was characterized by various resonances due to CH$_2$-NH carbons in the range 49.2-50.7 ppm and phenyl ring carbons at 122.9 ppm [274, 275].

A multiplet and singlet which appears in the range 157 ppm and 58.8 ppm may be assigned to C-OH carbons and CH$_2$OH carbons respectively [276, 277]. On the other hand $^{13}$C n.m.r of the complex [C$_{17}$H$_{34}$N$_7$O$_2$Ni] ClO$_4$ in comparison to Hsalea ligand, also revealed downfield shift only in the C-OH carbons in the range 161-163 ppm indicating the formation of complexes. An additional signal at δ 38.7-43.7 ppm, δ 55.4 ppm and δ 69.0 ppm are observed due to N-C-C, N-C-C-N and N-C-N carbons of the macrocycle, authenticates that oxygen atom of Hsalea ligand is attached to the metal of macrocyclic complex [203]. These n.m.r data are compatible with the structure of new modulated macrocycles.
Figure 49. (a) $^1$H-n.m.r (b) $^{13}$C-n.m.r spectra of the complex $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Ni}]\text{ClO}_4$ obtained in DMSO at 298 K.
Figure 49. (c) 2D cosy n.m.r spectrum of the complex [C_{17}H_{34}N_{7}O_{2}Ni] ClO_{4} obtained in DMSO at 298 K.
Cyclic voltammetry

The electrochemical properties of complex \([C_{17}H_{34}N_7O_2Cu]\) ClO\(_4\) in absence and in presence of DNA were studied in non aqueous DMSO containing 0.4 M TBAP by means of cyclic voltammetry at room temperature within sweep range from + 0.4 V to -1.2 V. The cyclic voltammogram of the complex \([C_{17}H_{34}N_7O_2Cu]\) ClO\(_4\) undergoes well shaped quasi-reversible redox wave for one electron oxidation and reduction attributed to the Cu(II/I) couple with \(\Delta E_p\) value of 124 mV [278] at a scan rate of 0.1Vs\(^{-1}\) (Figure 50a). For this couple, the half wave potential \(E_{1/2}\), taken as the average of the cathodic peak potential \(E_{pc}\) and the anodic peak potential \(E_{pa}\) being -0.684V. The \(\Delta E_p\) value is larger than the nemstian value (59 mv) and the ratio of anodic to cathodic peak currents \(i_{pa}/i_{pc} \sim 1\), characteristics of one electron transfer process. On addition of calf thymus DNA to the complex solution \([C_{17}H_{34}N_7O_2Cu]\) ClO\(_4\), the voltammetric pattern alters significantly as the complex reveals large shift in \(\Delta E_p\) value = +.386 V as well as small shift in \(E_{1/2}\) values = 0.651 V at a scan rate of 0.1 Vs\(^{-1}\) (Figure 51) The ratio of \(i_{pa}/i_{pc} = 1.6\) greater than unity, suggests that the strong binding of calf thymus DNA. The negative shift in \(E_{1/2}\) values and positive shift in \(E_p\) values indicate that the complex favorably binds with DNA. The cyclic voltammogram of complex \([C_{17}H_{34}N_7O_2Co]\) ClO\(_4\) in absence of DNA features a quasi-reversible redox wave with \(\Delta E_p = 168 \text{ mV} [279]\) at a scan rate of 0.1 Vs\(^{-1}\) shown in Figure 52a. The measured half wave potential \(E_{1/2}\) for the Co(II)/(I) couple is -0.644 V. The ratio of anodic to cathodic peak current is 0.6 closely resemble to the
criteria of quasi-reversible. In the cyclic voltammogram of the complex \([C_{17}H_{34}N_{7}O_{2}Co]\) ClO\(_4\) upon addition of DNA, cathodic peak potential experienced a positive shift while the anodic peak potential shifted to more negative potential at the same scan rate (Figure 53). In this case \(E_{1/2} = -0.635\) V and \(\Delta E_p = +0.328\) V. The complex also shows decrease in \(i_{pa}/i_{pc}\) values = 0.3. Therefore, the complexes \([C_{17}H_{34}N_{7}O_{2}Cu]ClO_4\) \([C_{17}H_{34}N_{7}O_{2}Co]ClO_4\) have different mode of binding with calf thymus DNA. At different scan rate 0.2, 0.3 Vs\(^{-1}\), in both complex, the anodic to cathodic peak to peak separation \(\Delta E_p\) increases slightly (Figure 50b and 52b). The ratio of equilibrium constant \(k_{2+}/k_{1+}\) for the binding of the M(II) and M(I) form of complexes (Scheme 18) can be rationalized from the net shift in \(E_{1/2}\) by using the equation (5).

\[
E_b^0 - E_f^0 = 0.059 \log \left( k_{1+}/k_{2+} \right) \quad \text{Equation 5}
\]

Where \(E_f^0\) and \(E_b^0\) are the formal potential of the 2+/1+ couple in the free and bound forms respectively. \(k_{2+}\) and \(k_{1+}\) couple are the corresponding binding constants for the 2+/1+ species to DNA respectively. For complex \([C_{17}H_{34}N_{7}O_{2}Co]ClO_4\) , the ratio of binding constants of Cu(II) and Cu(I) species is nearly about 1 suggest that the both species interact with DNA to the same extent, while the ratio of binding constants of Co(II) and Co(I) species is less than 1 for complex \([C_{17}H_{34}N_{7}O_{2}Co]ClO_4\) , which indicate the stabilization of Co(II) species occur [280].

\[
\begin{align*}
M^{II} + e^- & \rightleftharpoons M^{I} \\
K_{2+} & \\
M^{II} + CTDNA + e^- & \rightleftharpoons M^{I} - CTDNA \\
K_{1+}
\end{align*}
\]

Scheme 18
Figure 50. Cyclic voltammogram of complex \([C_{17}H_{34}N_{7}O_{2}Cu]ClO_4\) in non aqueous (DMSO + 0.4 M TBAP) at room temperature (a) at a scan rate of 0.1 \(Vs^{-1}\) (b) at scan rate of 0.1, 0.2 and 0.3 \(Vs^{-1}\).
Figure 51. Cyclic voltammogram of the complex \([\text{C}_17\text{H}_{34}\text{N}_7\text{O}_2\text{Cu}]\text{ClO}_4\) after addition of CT DNA in non aqueous (DMSO + 0.4 M TBAP) at room temperature at a scan rate of 0.1, 0.2 and 0.3 \(Vs^{-1}\).

Figure 52 (a) Cyclic voltammogram of complex \([\text{C}_17\text{H}_{34}\text{N}_7\text{O}_2\text{Co}]\text{ClO}_4\) in non aqueous (DMSO + 0.4 M TBAP) at room temperature at a scan rate of 0.1 \(Vs^{-1}\).
Figure 52 (b) Cyclic voltammogram of the complex \([\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Co}]\text{ClO}_4\) in non aqueous (DMSO + 0.4 M TBAP) at room temperature at a scan rate of 0.1, 0.2 and 0.3 Vs\(^{-1}\).

Figure 53. Cyclic voltammogram of the complex \([\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Co}]\text{ClO}_4\) after addition of CT DNA in non aqueous (DMSO + 0.4 M TBAP) at room temperature at a scan rate of 0.1, 0.2 and 0.3 Vs\(^{-1}\).
Binding studies with calf thymus DNA

In order to determine the DNA binding characteristics of complexes $[C_{17}H_{34}N_7O_2Cu]ClO_4$ and $[C_{17}H_{34}N_7O_2Co]ClO_4$, the interaction of the complexes with calf thymus DNA was monitored using absorption spectroscopic titration of complex $[C_{17}H_{34}N_7O_2Cu]ClO_4$ and $[C_{17}H_{34}N_7O_2Co]ClO_4$ with DNA at room temperature at fixed concentration ($8.3 \times 10^{-5}$ M) of the complex and increasing concentration of DNA ($1.7 \times 10^{-5}$, $2.5 \times 10^{-5}$, $3.3 \times 10^{-5}$, $4.1 \times 10^{-5}$ M). The electronic absorption spectra of complexes $[C_{17}H_{34}N_7O_2Cu]ClO_4$ and $[C_{17}H_{34}N_7O_2Co]ClO_4$ were characterized by an intense ligand centered transition in UV region at 235 and 247 nm respectively. Within each series of titration experiments by the addition of increasing concentration of CT DNA to solution of $[C_{17}H_{34}N_7O_2Cu]ClO_4$, the electronic spectra showed no shift in absorbance maxima but characteristically progressive decrease in absorbance (hypochromicity) is observed (Figure 54 and scheme 19) due to the intercalative mode which involve a strong stacking interaction between an aromatic chromophore. The extent of hypochromism is commonly consistent with the strength of intercalative interaction. In contrast to complex $[C_{17}H_{34}N_7O_2Cu]ClO_4$, the electronic spectra of complex $[C_{17}H_{34}N_7O_2Co]ClO_4$ upon titration with calf thymus DNA exhibits large hyperchromism with an appreciable bathochromic shift which is shown in (Figure 55). The large hyperchromism observed in complex $[C_{17}H_{34}N_7O_2Co]ClO_4$ supports electrostatic binding of calf thymus DNA. For comparison, the binding strength of complexes $[C_{17}H_{34}N_7O_2Cu]ClO_4$ and $[C_{17}H_{34}N_7O_2Co]ClO_4$, their intrinsic binding constant with calf thymus DNA were
obtained by monitoring the change in absorbance at 235 and 250 nm respectively with increasing concentration of DNA according to equation [281]

\[
\frac{[\text{DNA}]}{(\varepsilon_a - \varepsilon_f)} = \frac{[\text{DNA}]}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{k_b (\varepsilon_b - \varepsilon_f)}
\]

where $[\text{DNA}]$ is the concentration of DNA. $\varepsilon_a$ is the apparent absorption coefficient which was obtained by calculating $A_{\text{obs}}/[M]$. $\varepsilon_f$ and $\varepsilon_b$ are the extinction coefficient for the free complex and the complex in the fully bound form respectively. A plot of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$ was made to yield the ratio of the slope to the $y$ intercept. The intrinsic binding constant for the complex $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Cu}]\text{ClO}_4$ and $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Co}]\text{ClO}_4$ was determined to be $6.8 \times 10^4 \text{ M}^{-1}$ and $1.8 \times 10^4 \text{ M}^{-1}$ respectively. The $k_b$ value for $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Co}]\text{ClO}_4$ is higher than those for $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Cu}]\text{ClO}_4$.

![Absorbance](image)

**Figure 54.** Absorption spectral traces of complex $[\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Cu}]\text{ClO}_4$ in Tris-$\text{HCl}$ buffer upon addition of CT DNA. Inset: Plots of $[\text{DNA}]/(\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$ for the titration of DNA with complex, experimental data points; full lines, linear fitting of the Data.
Figure 55. Absorption spectral traces of complex $[C_{17}H_{34}N_7O_2Co]ClO_4$ in Tris-HCl buffer upon addition of CT DNA. Inset: Plots of $[DNA]/\varepsilon_\alpha$ versus $[DNA]$ for the titration of DNA with complex, experimental data points; full lines, linear fitting of the Data.
Scheme 19
Viscosity measurements

The effect of complexes $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Cu}]\text{ClO}_4$ and $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Co}]\text{ClO}_4$ on the relative specific viscosity of DNA was examined with calf thymus DNA. In order to assess the binding mode and strength of these complexes with DNA, hydrodynamic measurements were carried out which are sensitive to length change (i.e., viscosity and sedimentation), least ambiguous and most critical test of binding in solution [282]. A classical intercalation model is known to cause a significant increase in the viscosity of a DNA solution due to an increase in lengthening in the DNA helix. On the other hand, a partial or nonclassical intercalation of the ligand would reduce the DNA viscosity [283]. Representative plots of $\eta/\eta_0$ versus $[M]^{2+}/[\text{DNA}]$ are presented in Figure 56. For the viscosity of DNA bound to complex $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Cu}]\text{ClO}_4$ and $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Co}]\text{ClO}_4$, there is a negative change of viscosity with increasing concentration of complex. All these observations suggest that the complex $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Cu}]\text{ClO}_4$ binds to DNA by nonclassical semi intercalation model which produced bends or kink in the DNA and hence reduced its viscosity while complex $[\text{C}_{17}\text{H}_{34}\text{N}_{7}\text{O}_{2}\text{Co}]\text{ClO}_4$ is a binder of calf thymus DNA due to electrostatic interaction.
Figure 56. Effects of increasing amount of complex $[C_{17}H_{34}N_7O_2Cu]ClO_4$ (▲) $[C_{17}H_{34}N_7O_2Cu]ClO_4$ (■) on the relative viscosity of CT DNA at 29° + 0.1°C. $[\text{DNA}] = 6 \times 10^{-4} \text{ M}$. 

Relative specific Viscosity ($\eta/\eta_0$)
Table 14. Physical and analytical data of the complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Colour</th>
<th>M.P  (°C)</th>
<th>Yield (%)</th>
<th>Found (calcd) (%)</th>
<th>C   (%)</th>
<th>H   (%)</th>
<th>N   (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C\textsubscript{17}H\textsubscript{34}N\textsubscript{7}O\textsubscript{2}Cu].ClO\textsubscript{4}</td>
<td>Black</td>
<td>230</td>
<td>65</td>
<td></td>
<td>38.60</td>
<td>6.39</td>
<td>18.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(38.41)</td>
<td>(6.40)</td>
<td>(18.45)</td>
</tr>
<tr>
<td>[C\textsubscript{17}H\textsubscript{34}N\textsubscript{7}O\textsubscript{2}Co].ClO\textsubscript{4}</td>
<td>Dark brown</td>
<td>240</td>
<td>60</td>
<td></td>
<td>38.82</td>
<td>6.44</td>
<td>18.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(38.75)</td>
<td>(6.45)</td>
<td>(18.61)</td>
</tr>
<tr>
<td>[C\textsubscript{17}H\textsubscript{34}N\textsubscript{7}O\textsubscript{2}Ni].ClO\textsubscript{4}</td>
<td>Dark brown</td>
<td>340</td>
<td>60</td>
<td></td>
<td>38.75</td>
<td>6.45</td>
<td>18.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(38.82)</td>
<td>(6.47)</td>
<td>(18.64)</td>
</tr>
</tbody>
</table>
### Table 15. IR spectra of the complexes (cm$^{-1}$)

<table>
<thead>
<tr>
<th>Complex</th>
<th>ν(C-O)</th>
<th>ν(OH)</th>
<th>ν(CH$_2$)</th>
<th>ν(C-N)</th>
<th>ν(Ar)</th>
<th>ν(ClO$_4$)</th>
<th>ν(M-N)</th>
<th>ν(M-O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>{17}$H$</em>{34}$N$_7$O$_2$Cu].ClO$_4$</td>
<td>3090, 2955&lt;br&gt;2860, 1550&lt;br&gt;1440, 1396&lt;br&gt;1264</td>
<td>3398</td>
<td>2370</td>
<td>1350</td>
<td>760</td>
<td>1077&lt;br&gt;627</td>
<td>515</td>
<td>470</td>
</tr>
<tr>
<td>[C$<em>{17}$H$</em>{34}$N$_7$O$_2$Co].ClO$_4$</td>
<td>3098, 2970&lt;br&gt;2850, 1559&lt;br&gt;1462, 1364&lt;br&gt;1277</td>
<td>3466</td>
<td>2371</td>
<td>1324</td>
<td>778</td>
<td>1094&lt;br&gt;629</td>
<td>575</td>
<td>474</td>
</tr>
<tr>
<td>[C$<em>{17}$H$</em>{34}$N$_7$O$_2$Ni].ClO$_4$</td>
<td>3095, 2932&lt;br&gt;2862, 1560&lt;br&gt;1451, 1360&lt;br&gt;1250</td>
<td>3499</td>
<td>2373</td>
<td>1306</td>
<td>760</td>
<td>1081&lt;br&gt;626</td>
<td>530</td>
<td>468</td>
</tr>
</tbody>
</table>
Table 16. $^1$H-n.m.r data of the ligand and complex (ppm)

<table>
<thead>
<tr>
<th>Complex</th>
<th>Ar-H</th>
<th>Ar-CH$_2$</th>
<th>CH$_2$OH</th>
<th>NHCH$_2$</th>
<th>NH</th>
<th>CH$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C$<em>9$H$</em>{13}$NO$_2$.HCl.1.5H$_2$O]</td>
<td>7.35-7.41</td>
<td>4.29-4.41</td>
<td>3.85-3.89</td>
<td>3.20-3.23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[C$<em>{17}$H$</em>{34}$N$_2$O$_2$Ni] (ClO$_4$)</td>
<td>7.89-7.99</td>
<td>4.213</td>
<td>3.49</td>
<td>3.14</td>
<td>6.5</td>
<td>2.49-2.87</td>
</tr>
<tr>
<td>Complex</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;NH</td>
<td>CH&lt;sub&gt;2&lt;/sub&gt;OH</td>
<td>CH</td>
<td>C=C</td>
<td>Phenyl ring</td>
<td>Aromatic carbons</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>-----</td>
<td>------</td>
<td>-------------</td>
<td>------------------</td>
</tr>
<tr>
<td>[C&lt;sub&gt;4&lt;/sub&gt;H&lt;sub&gt;10&lt;/sub&gt;NO₂·HCl1.5H₂O]</td>
<td>49.2-50.7</td>
<td>58.8</td>
<td>117.9</td>
<td>119.7</td>
<td>122.9</td>
<td>133.8-134.6</td>
</tr>
<tr>
<td>[C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;24&lt;/sub&gt;N&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;Ni]ClO₄</td>
<td>46.8</td>
<td>58.1-59.7</td>
<td>114.8-118.9</td>
<td>120.1-120.4</td>
<td>128.4-129.0</td>
<td>132.9-133.7</td>
</tr>
</tbody>
</table>
Table 18. \(^1\)H- n.m.r assignments of the complex \([\text{C}_{17}\text{H}_{34}\text{N}_7\text{O}_2\text{Ni}] \text{ClO}_4\) and correlation with 2D COSY n.m.r spectra (ppm)

<table>
<thead>
<tr>
<th>Protons</th>
<th>(^1)H-n.m.r.</th>
<th>COSY correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>ArH</td>
<td>7.89-7.99</td>
<td>7.3</td>
</tr>
<tr>
<td>ArCH(_2)</td>
<td>4.213</td>
<td>4.2-4.4</td>
</tr>
<tr>
<td>CH(_2)OH</td>
<td>3.49</td>
<td>3.85-3.9</td>
</tr>
<tr>
<td>NHCH(_2)</td>
<td>3.14</td>
<td>3.2-3.3</td>
</tr>
<tr>
<td>NH</td>
<td>6.5</td>
<td>6.3</td>
</tr>
<tr>
<td>CH(_2)</td>
<td>2.49-2.87</td>
<td>2.5</td>
</tr>
</tbody>
</table>
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