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Synthesis and characterization of new unsymmetrical macrocyclictrinuclearCu(II) complex and its electrochemical behaviour and DNA binding studies:

BappithaaKuppusamy[*], KokilaSelvarajan[**],KandaswamyMuthuswamy[**]

[*] Department of Inorganic Chemistry, University of Madras
[**]Department of Inorganic Chemistry, University of Madras

Abstract- TrinuclearCu(II) complex was designed and synthesized. The binuclear Cu(II) complex was prepared and then condensed with a mononuclear Cu(II) complex to form trinuclear complex as shown below. All the complexes were characterized by using FT-IR and UV-Visible spectral studies. ESR spectra and magnetic moments of the trinuclearCu(II) complex show the presence of antiferromagnetic coupling.The electrochemical properties of these complexes were studied in the range of 0 V to -1.4 V. The mononuclear Cu(II) complex shows single electron reduction potential at (E_{pc}= - 1.22V), the binuclear Cu(II) complex shows two quasireversible single electron reduction potentials at (E^1_{pc}= - 0.76 V and E^2_{pc} = -1.23 V) whereas, the trinuclear Cu(II) complex shows three quasireversible single electron reduction potentials at (E^1_{pc} = -0.67 V , E^2_{pc}= -0.935 V and E^3_{pc}= -1.17 V). The binding interaction of the complexes with calf thymus-DNA was studied using absorption and fluorescence spectral techniques.

INTRODUCTION

The interaction of coordination compounds with DNA has been of interest due to their possible application in cancer therapy [1-5] and molecular biology [6-7]. Transition metal ions are known to play very important roles in biological processes in the human body. Copper complexes have found possible medical uses in the treatment of many diseases including cancer [8-9]. Also copper complexes have been known to cleave DNA by different mechanisms like, hydrolytic [10] and oxidative pathways [11]. The chemistry of 2,6 diformyl-4-methylphenol and its derivatives is of a great interest in designing the compartmental ligands which can form polynuclear complex system having magnetic communication between the metal centers. The study of their stereochemical electronic, magnetic, catalytic spectroscopic and also biological properties have allowed the proposal of probes for many important applications [12,13]. A quantitative evaluation of the binding of these complexes with calf thymus DNA in solution and the damage to DNA in the presence of the complex compounds were studied. Transition metal complexes have been widely exploited for the purposes not only because of their unique spectral and electrochemical signatures but also due to the fact that by changing the ligand environment, one can tune the DNA binding and cleaving ability of a metal complex [14-16].The present work describes the synthesis and characterization of trinuclearCopper(II) and Nickel(II) complexes and derived from tricompartmental ligands. These ligands are capable of binding upto three metal centers in close proximity.

I. EXPERIMENTAL

2.1 Materials and instrumentation

Elemental analysis was carried out on a Carlo Erba Model 1106 elemental analyzer. IR spectra were recorded using Perkin Elmer FTIR model SPECTRUM 1, using simple dispersed in KBR pellet. Cyclic voltammograms were obtained on a CHI instruments electrochemical analyzer. The measurements were carried out under oxygen free condition using three electrode cell in which glassy carbon electrode serves as working electrode, platinum wire was used as auxiliary electrode and saturated Ag/AgCl electrode was the reference electrode. Tetra (n-butyl) ammonium perchlorate (TBAP) used as the supporting electrode was purchased from fluka and recrystallized from hot methanol in electrochemical measurements, (Warning -perchlorate salts are potentially explosive; hence care should be taken in handling TBAP). UV-Vis spectra were recorded using a Perkin Elmer Lambda 35 spectrophotometer operating in the range 200-1000 nm with quartz cell and ε is given in $M^{-1}cm^{-1}$. The emission spectra were recorded on a Perkin Elmer LS-45 fluorescence spectrometer. X-band ESR spectra were recorded at 25^oC on a Varian EPR-E 112 spectrometer using diphenylpicrylhydrazine (DPPH) as a reference.

II. SYNTHESIS

Synthesis of ligands L^1

Benzil (benzil)ethylenediamine was prepared from ethanolic solution (35 mL) of benzil (5.35 g, 25.4 mmol) and 25 ml solution of ethylenediamine (0.76 g, 12.7 mmol) in ethanol. The whole reaction mixture was refluxed for 3 hrs. After reducing the solvent, the

solution was cooled and the reddish yellow crystalline compound thus obtained was recrystallized from ethanol, collected and the product was dried in vacuum. Yield: 4.2g (81%), M.P: 86 - 89 ^{o}C

Synthesis of ligand L^2

An ethanolic solution of ligand L^1 (0.02 mol) was refluxed with excess of hydrazine hydrate and cooled to room temperature. The white solid product formed on evaporation was filtered off, collected and the product was dried under vacuum and recrystallized using ethanol. Yield: 2.5g (75%), M.P:150- 155 ^{o}C

Synthesis of ligand L^3

To the solution of precursor L^2 (0.01 mol) dissolved in chloroform was added the acetonitrile solution of 2,6-diformyl-4-methylphenol (0.02 mol) at 0 ^{o}C and it is allowed to stirred for overnight at room temperature. The resulting solid that separated out on evaporating the solution at room temperature was washed with ethanol and dried in vacuum. Yield: 1.2g (60%), Elemental Anal.; $C_{48}H_{40}N_6O_4$; Cal. for C, 75.37; H, 5.27; N, 10.99; O, 8.37; Found ; C, 75.32; H, 5.20; N, 10.80; O, 8.35.

Synthesis of binuclear Cu(II) complex ML^4

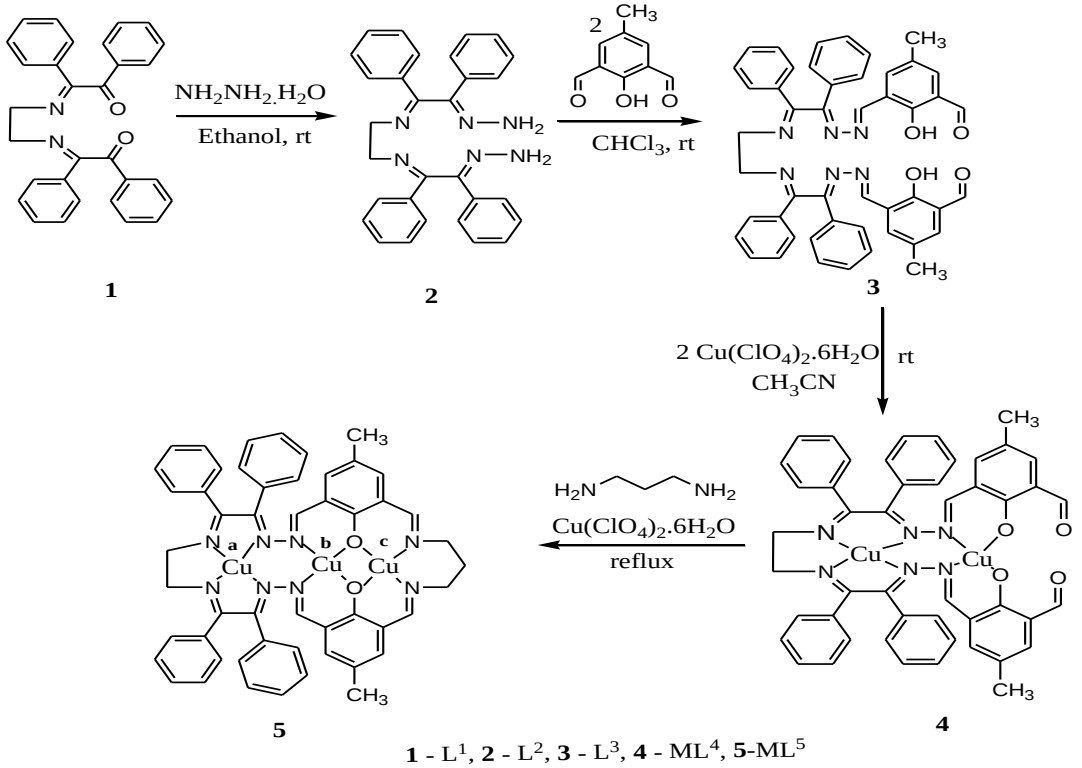
ML^3 (1.119 g, 1.8 mmol) dissolved in acetonitrile was added to the acetronitrile solution of Cu(II) perchloratehexahydrate (0.676 g, 1.8 mmol) and it is allowed to reflux for 24 hrs. After the reaction was completed, the reaction mixture was filtered and allowed to stand at room temperature (25 ^{o}C). After slow evaporation of the solvent at 25 ^{o}C, the brown solid compound obtained was washed with methanol and dried in vacuum.Yield:1.55g (70%).Elemental Anal; $C_{54}H_{56}Cu_2N_6O_4$; Cal. for C, 66.17; H, 5.76; Cu, 12.97; N, 8.57; O, 6.53; Found; C, 66.15; H, 5.75, Cu, 12.92, N, 8.53, O, 6.49.

Synthesis of trinuclearCu(II) complex ML^5

ML^4 (1.085 g, 1.7 mmol) dissolved in acetonitrile was added to an acetonitrile solution of Cu(II) perchlorate hexahydrate (0.632 g, 1.7 mmol) followed by the addition of 1,2-diaminopropane (0.102 g, 1.7 mmol) in ethanol. The solution was refluxed on water bath for 24 hrs. After the reaction was completed, the solution was filtered at hot condition and allowed to stand at room temperature. After slow evaporation of the solvent at 25^{o}C, the brown solid obtained was washed with methanol and dried in vacuum

Yield: 1.7 g, (80%), Elemantal Anal; $C_{61}H_{74}Cu_3N_8O_2$; Cal, for C, 64.16; H, 6.53; Cu, 16.69; N, 9.81; O, 2.80; Found; C, 64.13; H, 6.50; Cu, 16.66; N, 9.78; O, 2.75

(**Scheme 1**).



1 - L^1, **2** - L^2, **3** - L^3, **4** - ML^4, **5**-ML^5

III. DNA Binding experiments

Absorption spectral studies

Absorption spectral titrations were carried out in (50 mM Tris-HCI buffer, pH 7.5) buffer at room temperature to investigate the binding affinity between CT-DNA and complex. The concentration of CT- DNA was determined from the absorption intensity at 260 nm with a ε value [18] of 6600 $M^{-1}cm^{-1}$. Absorption titration experiments were carried out using various concentrations of CT- DNA, keeping the complex concentration constant, with due correction for the absorbance of the CT-DNA itself. The intrinsic binding constant, K_b for the complexes of [CuL] and [NiL] was determined from the spectral titration data using the following equation [19].

$[DNA]/(\varepsilon_\alpha-\varepsilon_f) = [DNA]/ (\varepsilon_a-\varepsilon_f) + 1/ K_b (\varepsilon_b- \varepsilon_f)$

whereε_a, ε_f and ε_b corresponds to A_{obsd}/ [complex], the absorbance for the free copper (II) complex, and the absorption for the copper (II) complex in the fully bound form, respectively.

Fluorescence spectral studies

The fluorescence spectral method using EB as a reference was used to determine the relative DNA binding properties of the complex to CT-DNA in 50 mM Tris-HCl / 1 mMNaCl buffer, pH 7.5. Flourescence intensities at 610 nm (excited at 510 nm) were measured after addition of complex. Stern- Volmer quenching constant K_{sv} of the complex [CuL], CT-DNA were determined from the equation $I_o/I = 1+K_{sv}$. The apparent binding constant (K_{app}) was calculated using the equation $K_{EB}[EB]/ K_{app}[compex]$, where the complex concentration was observed to be equal to the value at a 50% reduction of the fluorescence intensity of EB and $K_{EB} = 1x10^7$ M^{-1} ([EB] = 2 μM [20].

IV. Result and Discussion

IR Spectral studies

All the complexes were characterized by spectral studies. In IR spectra of the copper (II) complexes, (C=N) stretching frequency was observed [21] at 1620-1640 cm^{-1}. All the complexes showed two sharp peaks near 1,100 cm^{-1} and 629 cm^{-1} for perchlorate ions [22-24].

Electrochemical studies

The electrochemical behavior of the complexes was studied by cyclic voltammetry in DMF containing 10^{-1} M tetra (n-butyl) ammonium perchlorate over the range of (0 to -1.4 V). The copper (II) complex show three irreversible reduction waves in the cathodic potential region in the range (-0.43 to -0.70 V), and (-0.78 to -1.03 V) and (-1.10 to -1.33V). The copper (II) complex show three irreversible reduction waves in the anodic potential region in the range (-1.10 to -0.95 V), and (-0.82 to -0.62 V), and (-0.50 to -0.13V). Controlled potential electrolysis was also carried out at 100 mV more negative to the cathodic peak, and the results show that each wave corresponds to one electron transfer process, as follows.

The first and second reduction potential in the range of (E^1_{pc} -0.43 to -0.70 and E^2_{pc} -0.78 to -1.03 V) are attributed to the reduction of copper (II) placed in the left (a) and right corner (b) of the complex while the third reduction wave in the range of E^3_{pc} -1.10 to -1.03 V, is attributed to the reduction of copper (II) in central (c) compartment (Scheme 1). The cyclic voltammetric behaviour of the complex in presence of DNA in the same conditions showed slight positive shift in the redox potentials and the ip_c / ip_a value was found to decrease with the increase in DNA concentration.

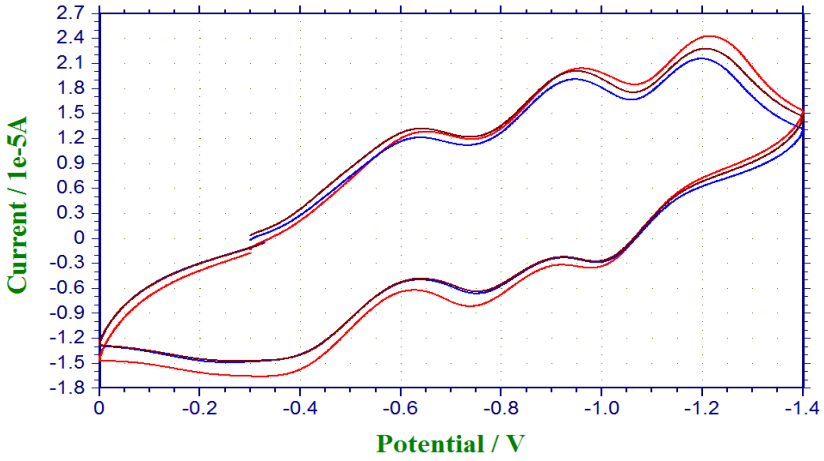


Figure:1**Cyclic voltammogram of complex in the absence and presence of CT-DNA**

Absorption spectral studies

The binding ability of the complex with CT-DNA was characterized by measuring the effects on electronic spectroscopy. In the present investigation, the interaction of macrocyclic trinuclear Cu(II) complex in DMF solution (10%) with CT-DNA has been investigated. Complex binding with DNA through intercalation usually results in hypochromism and bathochromism due to the intercalative mode involving a strong stacking interaction between an aromatic chromophore and the base pairs of DNA [25]. The binding of Cu(II) complex to duplex DNA led to decrease in the absorption intensities with a small amount of red shift in the UV-Visibe absorption spectrum (**Figure:2**). To compare quantitatively the affinity of the complex towards the CT-DNA, the binding constant was calculated and it is found to be $1.9x10^4$ M^{-1}. Complex due to the minor bathochromic shift and hypochromism is expected to have groove binding with CT-DNA.

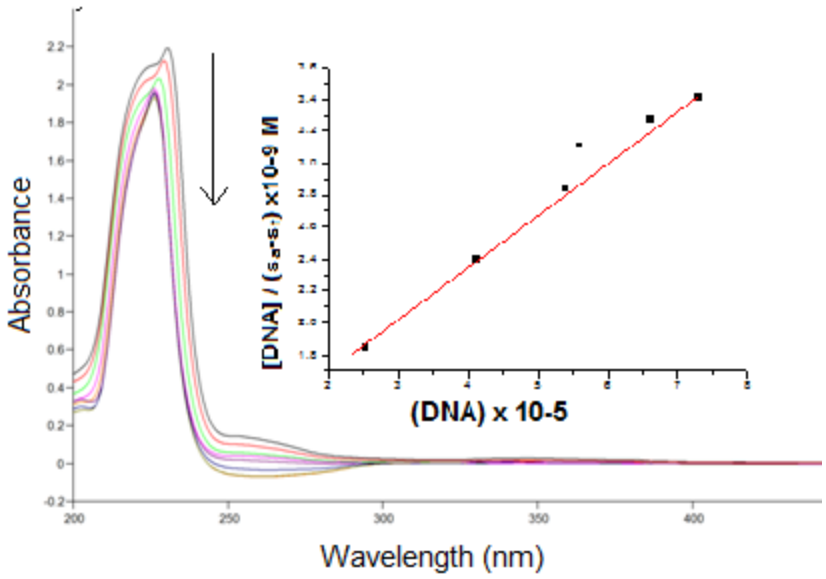


Figure 2:**Absorption spectrum of complex in the absence and presence of CT-DNA (0-250 μM) at room temperature in 50 mM Tris-HCl buffer (pH 7.5). The arrow shows the absorbance change upon increasing addition of DNA. Inset shows the plot of (ε_a-ε_f) / (ε_b-ε_f) Vs [DNA]**

Fluorescence spectral studies

The fluorescence spectroscopy technique is an effective method to study metal interaction with DNA. EB is one of the most sensitive fluorescence probes that can bind with DNA [26]. The fluorescence of EB increases after intercalating into DNA. If the metal intercalates into DNA, it leads to a decrease in the binding sites of DNA available for EB resulting in a decrease in the fluorescence intensity of the EB-DNA system [27]. The EB bound DNA quenching curve is shown in **Figure 3**. In the linear fit plot of I_o/I Vs [complex]/[DNA], K is given by the ratio of slope /Intercept. The stern-volmer quenching constant K value for complex is 2.1. The apparent DNA binding constant of 2.9x 10^4 M^{-1} was derived for the complex.

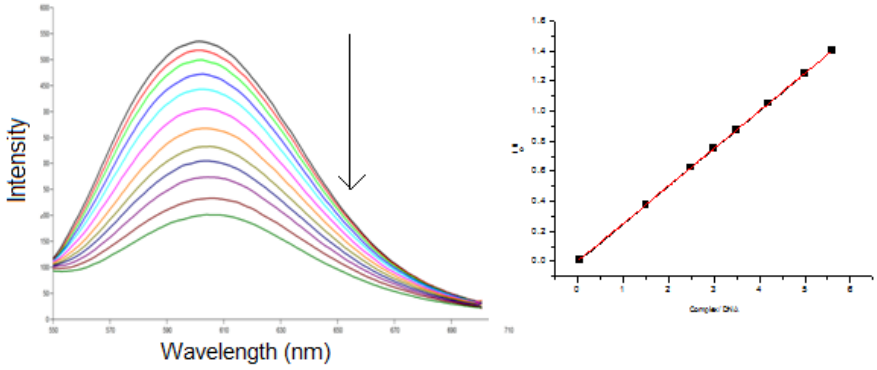
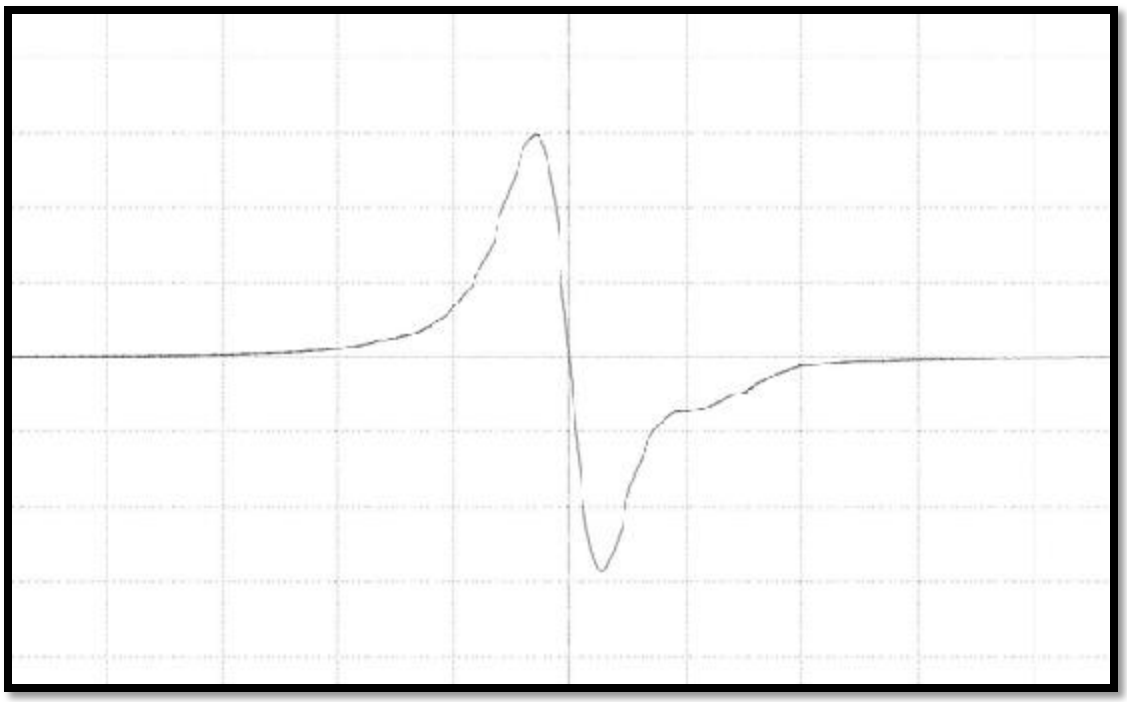


Figure 3**: Fluorescence titration spectrum in the absence and presence of CT-DNA. The inset is the plot of Io/I Vs [complex] / [DNA**

EPR Spectra

The EPR spectra of the complex at 298K were obtained in the X-band region. The EPR spectra of the copper complexes consists of a broad band centered at g = 2.06. The g values were calculated using the equation hν = gβH. The hyperfine splitting was not observed due to spin-spin coupling indicating the presence of anti-ferromagnetic interaction in the complex.



V. Conclusion:

The trinucleating ligand L and its Copper(II) trinuclear complex were synthesized and characterized by elemental analysis and spectroscopic techniques. The binding properties of the complex with CT-DNA were investigated by spectroscopic titrations like Absorption and Emission studies. The intrinsic binding constant K_b of the complex was found as $1.9x10^4$ M^{-1} through UV absorption spectroscopic titration studies.

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AUTHORS

First Author – Bappithaa Kuppusamy, Research Scholar, University of Madras, email address – bappithaa88@gmail.com
Second Author – Kokila Selvarajan,Research Scholar, University of Madras, email address – koki120780@gmail.com

Correspondence Author –Kandaswamy Muthuswamy,mkands@yahoo.com, 044-22202796.