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A Novel Tricompartmental Macrocyclic Cu(Ii) Complexes: Synthesis, Characterization and Their Electrochemical and DNA Binding Studies

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Abstract: **A novel tricompartmental Cu(II) complexes were designed and synthesized. The binuclear Cu(II) complexes were prepared and then condensed with a series of mononuclear Cu(II) complexes to form trinuclear complexes as shown below. All the complexes were characterized by using FT-IR and UV-Visible spectral studies. 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}= - 1.24 V), the binuclear Cu(II) complex shows two quasireversible single electron reduction potentials at (E^1_{pc}= - 0.79 V and E^2_{pc} = -1.22 V) whereas, the trinuclear Cu(II) complex shows three quasireversible single electron reduction potentials at (E^1_{pc} = -0.65 V , E^2_{pc}= -0.94 V and E^3_{pc}= -1.18 V). The interaction of the complexes with calf thymus-DNA was studied using absorption and fluorescence spectral techniques.**

I. Introduction

Synthesis and characterization of new binuclear Cu(II) complexes have made an impact in the field of bioinorganic chemistry [1-3]. Copper (II) complexes have been found in the treatment of many diseases including cancer [4]. The versatile synthetic pathway of these complexes *via* template condensation of the appropriate dialdehyde and diamine precursor around the metal centers [5-8]. Several macromonocyclic complexes of ligands, containing ether oxygen and tertiary nitrogen and phenolic oxygen as coordinating atoms were reported in literature [9]. For Robson' s pioneering work on binuclear Cu(II) complexes with phenolate endogeneous bridge, numerous work has been carried out [10, 11]. Transition metal complexes have been widely exploited, 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 [12-14]. Hence, in recent years, the synthesis of unsymmetrical binuclear Cu(II) complexes has gained more attention. Metal complexes of the Schiff base ligands have variety of applications including clinical [15], analytical [16], industrial [17] and biological [18] fields. In addition to their important roles in catalysis and organic synthesis [19], this work focuses on the synthesis of trinuclear Cu(II) complexes with symmetrical and unsymmetrical macrocyclic ligands containing alkyl oxyimino group (salamo) [20-22].

II. Experimental

A. Materials and measurements

2,6-diformyl 4,methyl phenol was prepared according to the literature [23]. Tetra(n-butyl)ammonium perchlorate (TBAP), which is used as the supporting electrolyte in electrochemical measurement, was purchased from Fluka (Caution TBAP is potentially explosive; hence, care should be taken in handling the compound). Calf thymus DNA (CT-DNA) was purchased from Bangalore Genie (India). All the other chemicals and solvents were of analytical grade and were used without any further purification. Elemental analysis was carried out on a Carlo Erba model 1106 elemental analyzer. FT-IR spectrum was obtained on a Perkin-Elmer FTIR spectrometer with samples prepared as KBr pellets. UV-vis spectrum was recorded using a Perkin-Elmer Lambda 35 spectrophotometer operating from 200 to 1400 nm with quarts cells; are given in $M^{-1}cm^{-1}$. A CH11008 Electrochemical analyzer was used using a three-electrode cell in which a glassy carbon electrode is the working electrode, a saturated Ag/AgCl reference electrode and a platinum wire is used as an auxiliary electrode under oxygen free conditions. The concentration of the complexes are 10^{-3} M. TBAP (10^{-1} M) was used as the supporting electrolyte.

III. General methods

A. Synthesis

Synthesis of precursor ligands L^1

The acyclic ligand L^1 was prepared according to the literature (Welsh et al., 1977). Benzil (0.02 mol) was dissolved in ethanol and a slight excess of 1,3- diaminopropane (0.01 mol) in ethanol was added dropwise to the hot solution. The reaction mixture was refluxed for 3 hrs. The resulting white crystalline product was collected, washed with cold ethanol, and dried in vacuum. Yield: 3.2g (81%) M.P: 93- 98 ^oC

Synthesis of precursor ligand L^2

An ethanolic solution of ligand L^1 (0.01mol) was refluxed with excess of hydrazine hydrate and cooled to room temperature. The white solid product formed was then washed with ethanol. The product was dried under vacuum and recrystallized using ethanol. Yield: 2.2g (70%), M.P: 130- 133 ^oC

Synthesis of precursor ligand L^3

To the solution of precursor L^2 (0.01mol) is dissolved in chloroform was added the acetonitrile solution of 2,6-diformyl-4-methylphenol (0.02mol) at 0^oC 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%), Ele. Anal; $C_{49}H_{42}N_6O_4$; Cal, for C, 75.56; H, 5.44; N, 10.07; O, 8.22; Found ; C, 75.52; H, 5.40; N, 10.75; O, 8.20.

Synthesis of binuclear Cu (II) complex ML^4

The ML^3 (1.119 g, 1.8 mmol) is dissolved in acetonitrile was added to a acetronitrile solution of Cu(II) perchlorate hexahydrate (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 ^oC). After slow evaporation of the solvent at 25 ^oC, the brown solid compound obtained was washed with methanol and dried in vacuum.Yield:1.45g (70%).Ele. Anal; $C_{55}H_{58}Cu_2N_6O_4$; Cal, for C, 66.45; H, 5.88; Cu, 12.78; N, 8.45; O, 6.44; Found; C, 66.40; H, 5.83, Cu, 12.76, N, 8.43, O, 6.43

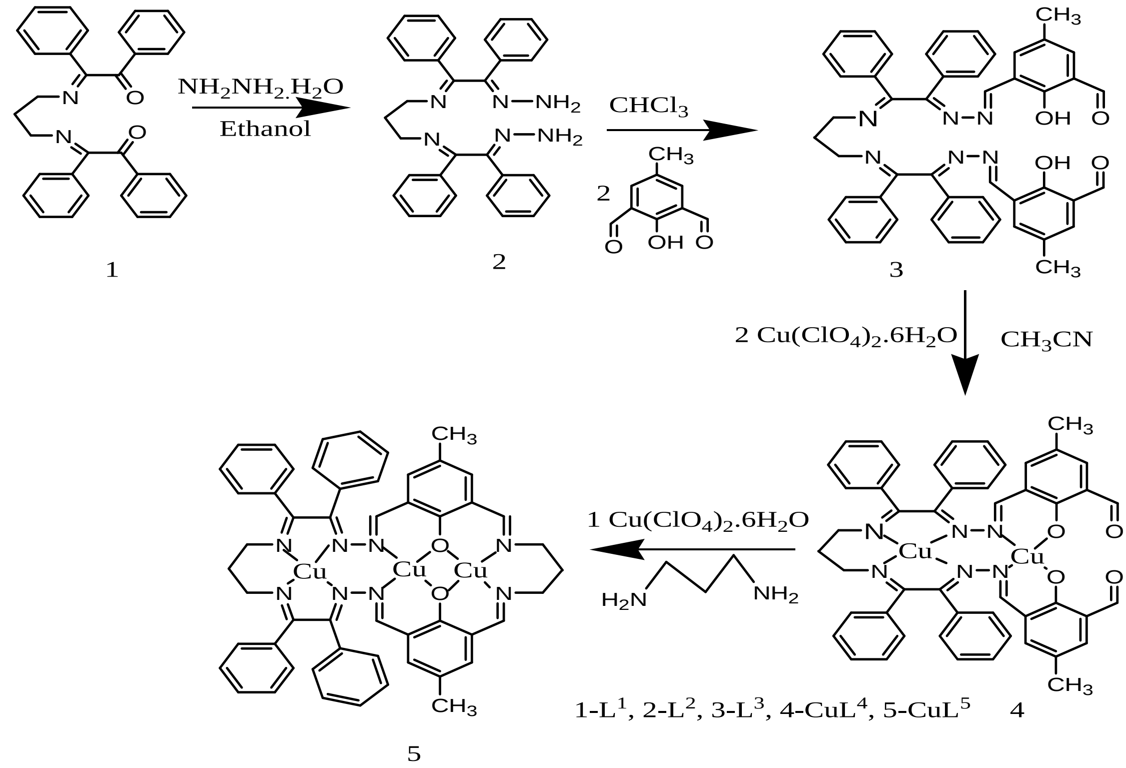
Synthesis of trinuclear Cu (II) complex ML^5

The ML^4 (1.085 g, 1.7 mmol) is 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 24hrs. 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 ^oC, the brown solid obtained was washed with methanol and dried in vacuum (**Scheme 1**).

Yield: 1.9 g, (80%).Ele. Anal; $C_{62}H_{76}Cu_3N_8O_2$; Cal, for C, 64.42; H, 6.63; Cu, 16.49; N, 9.69; O, 2.77; Found; C, 64.40; H, 6.60; Cu, 16.48; N, 9.67; O , 2.75.

B. DNA binding studies

The concentration of calf thymus DNA (CT-DNA) was determined from the absorption intensity at 260 nm with a ε value [24] of 6600 M^{-1} cm^{-1}. Absorption titration experiments were made using different concentrations of CT DNA, keeping the concentration of the complexes constant, with due correction for the absorbance of the CT DNA itself. The fluorescence spectral method using ethidium bromide (EB) as a reference was used to determine the relative DNA binding properties of the complexes. Fluorescence intensities of EB at 620 nm with an excitation wavelength of 510 nm were measured at different complex concentrations. The relative binding tendency of the complexes to DNA was determined from a comparison of the slopes of the lines in the fluorescence intensity versus complex concentration plot. The apparent binding constant ($K_{app)}$) was calculated using the equation $K_{EB}[EB]/K_{app}[complex]$, where the complex concentration is about a value at a 50% reduction of the fluorescence intensity of EB and $K_{EB} = 1.0x10^7\ M^{-1}$ ([EB]=1.3μM) [25]



Scheme 1

IV. Results and discussion

A. IR Spectral studies

IR spectrum for complex shows ν(C=N) peak [26] at 1620-1630 cm^{-1}. Moreover, the disappearance of ν(C=O) at 1678cm^{-1} in the complexes are indicative of effective Schiff base condensation. The binuclear complexes show two peaks near 1100cm^{-1} and one peak near 620 cm^{-1}. Of the two peaks, one peak is split [27] due to the presence of the coordinated perchlorate ion while the other peak due to the presence of the uncoordinated perchlorate ion does not show any splitting.

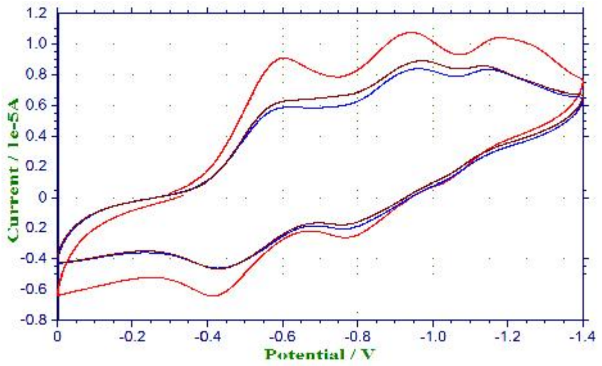
B. Electrochemical studies

The electrochemical behavior of symmetrical and unsymmetrical trinuclear Cu(II) complex has been studied by cyclic voltammetry (CV) in CH_3CN containing 0.1M TBAP. The electrochemical data are summarized and the CV of the Cu(II) complex are depicted **Figure 2**.

$$Cu^{II}Cu^{II}Cu^{II} \rightleftharpoons Cu^{I}Cu^{II}Cu^{II} \rightleftharpoons Cu^{I}Cu^{I}Cu^{II} \rightleftharpoons Cu^{I}Cu^{I}Cu^{I}$$

The cyclic voltammetric studies of the trinuclear complex were studied within a range of 0 to -1.4V. The unsymmetrical Cu(II) complexes are associated with three quasi reversible reduction waves. The first reduction potential ranges from -0.4 to -0.7V and the second reduction potential lies in the range from -0.8 to -1.03 V and the third reduction potential lies in the ranges from -1.09 to -1.39 V. 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:2 Cyclic voltammogram of complex in the absence and presence of CT-DNA.



C. Absorption spectral studies

The binding ability of the complex with CT-DNA was characterized by measuring their 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. Absorption titration experiment of Cu(II) complex in buffer solution was performed by using a fixed complex concentration to which increments of the DNA stock solution were added. The binding of Cu(II) complex to duplex DNA led to decreases in the absorption intensities with a small amount of red shift in the UV-vis absorption spectrum (**Figure:3**). To understand the affinity of the complex towards the CT-DNA, the binding constant was calculated and it is found to be 1.5x10^{4} M^{-1}. The better binding ability of the complex with CT-DNA may be due to the extension in conjugation between the phenyl moieties with imine groups. Complex due to the minor bathochromic shift and hypochromism is expected to have groove binding with CT-DNA.

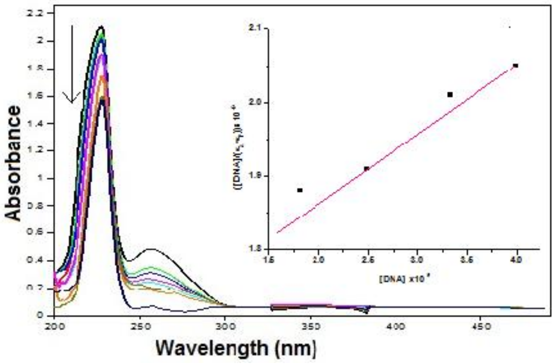


Figure 3: Absorption spectrum of complex in the absence and presence of CT-DNA (0-250 μM) at room temperature in 50 mM Tris-HCl buffer (pH 7.5). The arrow shows the absorbance change upon increasing addition of DNA. Inset shows the plot of (ε_a-ε_f)/(ε_b-ε_f) Vs [DNA].

D. Fluorescence spectral studies

The fluorescence spectroscopy technique is an effective method to study metal interaction with DNA. The addition of complex to DNA pretreated with EB, results in a decrease in fluorescence intensity which indicates that the complex competitively bind with DNA compared to the EB. The reduction in the intensity of the emission band is a measure for the DNA binding propensity of the complex. The EB bound DNA quenching curve is shown in **Figure 4**. In the linear fit plot of I_o/I Vs [complex]/[DNA], K_b is given by the ratio of slope /Intercept. The K_{sv} value for complex is 3.1.The apparent DNA binding constant of 5.2x10^{6} M^{-1} was derived for the complex.

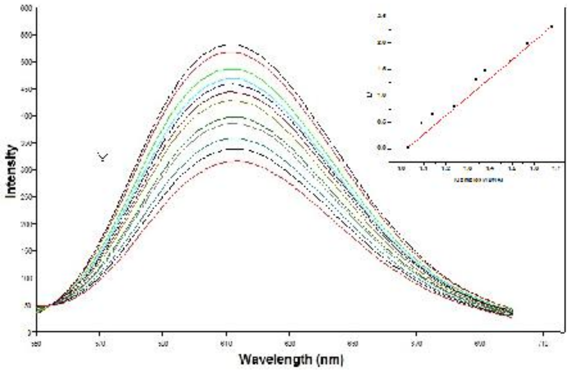


Figure 4: Fluorescence titration spectrum in the absence and presence of CT-DNA. The inset is the plot of Io/I Vs [complex] / [DNA].

Conclusion

The novel Schiff base ligands and their Cu(II) complex were designed, synthesized and characterized. Electrochemical studies of the macrocylic trinuclear copper (II) complexes display three electron transfer quasi-reversible wave in the cathodic region. The binding mechanism of the complexes with DNA was observed to be a groove binding.

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