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INVESTIGATION ON DNA CLEAVAGE ABILITY OF VANILLIN BASED SCHIFF BASE METAL COMPLEXES: SYNTHESIS AND CHARACTERIZATION

[1] M.Muthumariammal, [2] R.Asokarajan, [3] R. Pazhani and [1] R.Paulpandiyan

[1]Research Department of Chemistry, VHN Senthikumara Nadar College, Virudhunagar-626 001
[2] Principal, SreeVee College of Arts and Science, Dindigul-624 709
[3]Department of Basic Engineering, Government Polytechnic College, Nagercoil-629 004

Corresponding author: paulpandiansrs@gmail.com

ABSTRACT

A novel vanillin-based Schiff base ligand (L) has been synthesized from condensation between vanillin and benzene-1,2-diamine. Its metal complexes has been prepared and characterized by physical, spectral and analytical data. The complex have general formula [ML]Cl_2; where M= Co(II) and Cu(II) ion. The structural features have been derived from their elemental analysis, molar conductance, FT-IR. UV-*vis*., and EPR spectral studies. The metal complexes show a higher conductance value, supporting its electrolytic nature. Spectroscopic and other analytical data of the complex suggest square planar geometry. The nucleolytic cleavage activities of the complex were dissected on pUC18 plasmid DNA using agarose gel electrophoresis technique in the presence of H_2O_2 and the titled complexes show promising nuclease activity.

Keywords: vanillin based Schiff base ligand; electrolytic nature; square planar geometry; gel electrophoresis; nuclease activity

INTRODUCTION

Schiff bases are one of the most widespread and important class of ligands due to their simple preparation, selectivity and coordination towards the central metal ions. They are considered as most ‘privileged ligands’ containing an azomethine group (-CH=N) [1, 2]. These ligands are able to coordinate with various metal ions to form Schiff base metal complexes which increase their application in different fields such as catalysis and pharmacology. The efficiency of the Schiff bases as therapeutic agents has often been enhanced upon coordination to a metal [3, 4]. Over the years there has been a continuous curiosity of the biological activity in metal complexes. p-vanillin (4-hydroxy-3-methoxy benzaldehyde) which is a precursor of biologically active curcumin compound, derived Schiff base complexes have been found a variety of biological applications including antitumor, antimicrobial and anti-inflammatory activities [5, 6].

Bearing the above introduction in mind, herein it is interested in examining the DNA cleavage activity of Co(II) Schiff base metal complex. It has been obtained by the condensation of p-vanillin with o-phenylenediamine and copper chloride. They have been characterized by elemental analysis, molar conductance measurements, UV-*vis*., FT IR spectral studies. These complexes show lower conductance values, supporting their electrolytic nature. Spectroscopic and other analytical data of the complexes suggest square planar geometry. This Co(II) metal complex has been found to promote cleavage of pUC18 DNA from the super coiled form I to the open circular form II in presence of hydrogen peroxide by gel electrophoresis technique.

EXPERIMENTAL PROCEDURE

Synthesis

Synthesis of the Schiff base Ligand (L)

The Schiff base ligand was synthesized by benzene-1,2-diamine (2.1 g, 20 mmol) was mixed with vanillin (6.08 g, 40 mmol) and ethanol (50 mL). The reaction mixture was stirred thoroughly for a period of 3 h reflux. Brown colour crystalline solid was obtained after kept in refrigerator for two days, which was filtered and washed with ethanol followed by an excess of petroleum ether to remove any unreacted reagents. Washing was repeated for three times and the compound was recrystallized from ethanol. Schematic route for the synthesis of (L) is given in Scheme 1.

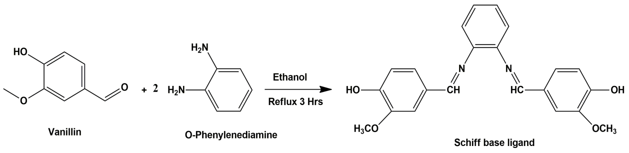

Fig:1. Schematic route for the synthesis of Schiff base ligand (L)

Synthesis of metal complexes

The metal complexes are prepared by mixing the appropriate molar quantity of ligand and metal chloride salt using the following procedure. A solution of metal chloride in ethanol (1 mmol) was stirred with an ethanolic solution of Schiff base ligand (L) (2 mmol), for 3 h on a magnetic stirrer at ambient temperature.

To the above stirring solution was refluxed for *ca* 2 h. Then the reaction mixture removed for ethanol at room temperature. The solid product formed was filtered and then recrystallized from ethanol and dried in *vacuo*.

Physical and chemical methods of characterization

The carbon, hydrogen and nitrogen contents of the Schiff base ligand and its copper complex are achieved at Sophisticated Analysis and Instrument Facility (SAIF), Central Drug Research Institute, Lucknow.

Estimation of metal

The metals, in general were estimated gravimetrically as their oxides by fusion with AnalaR ammonium oxalate [7]. In a typical experiment, about 0.3g of the dried complex was perfectly weighed in a formerly weighed silica crucible. AnalaR ammonium oxalate, nearly 3 parts by weight of the complex was added and the mixture was reduced to ashes slowly at first and then strongly using a Bunsen burner for 3 h. It was then cooled in a desiccator and weighed. The procedure was repeated till the final oxide weight was constant. From this, the percentage of metal in the complex was calculated.

FT- IR spectra

It is a powerful tool, popularly used as a characterization technique in which a sample is placed in the path of an IR radiation source and its absorption of different IR frequencies is measured. After absorbing electromagnetic radiation, the frequency of vibration of a bond increases, leading to a transition between the ground state and several excited states. These absorption frequencies represent excitations of vibrations of the chemical bonds and thus are specific to the type of bond and the group of atoms involved in the vibration. Therefore, IR spectroscopy may be employed to identify the type of bond between two or more atoms and identify functional groups. The Infra-red spectra of the ligands and their complexes were obtained as KBr discs in the range 400 – 4000 cm^{-1} recorded on an IR Affinity-1 FT-IR Shimadzu spectrophotometer. The percentage of transmission was recorded against wave number.

Electronic absorption spectra

This technique deals with the study of electronic transitions between orbital or bands of atoms, ions or molecules in the gaseous, liquid and solid state. The electronic absorption spectra of the Schiff bases and their metal complexes in the range 200-1100 nm in suitable solvents were recorded on a Shimadzu UV – 1601 spectrophotometer. Absorbance values were plotted against the corresponding wavelengths. The spectra of the complexes were recorded using DMSO or DMF (spectroscopic grade) as solvent. The electronic spectral measurements were used for assigning the stereochemistry of metal ions in the complexes based on the position and number of d-d transition peaks.

Electrical conductance studies

Conductance measurements of the complexes in solution were made mainly to verify the ionic formulation of the complexes, *i.e.* to see whether the anions of the metal salts remain inside or outside the coordination sphere of the central metal atom. The molar conductivity of the complex was measured on a Systronic model-304 digital conductivity meter using appropriate solvent. All measurements were corrected for the conductance of pure solvent by subtracting the conductance of pure solvent from that of the solution. Molar conductance in the solvent depends on the number of ions present in the solution, degree of dissociation, mobility of ions and temperature. The molar conductivity of the complexes was determined using the formula:

$$\Lambda m = (1000 \times \text{cell constant} \times \text{conductance}) / \text{concentration}$$

The values were compared with the standard values from the literature to find out the nature of the complexes whether they are electrolytes or non-electrolytes [8].

DNA cleavage efficacy

Interaction between the ligands and the complex with pUC18 plasmid DNA was studied by agarose gel electrophoresis. The samples were incubated for 3 h at 37°C, then 0.25% bromophenol blue, 0.25% xylene cyanol and 30% glycerol (3 μL) solutions were added. The DNA cleavage mixture was subjected to electrophoresis on 1.0 % agarose gel containing 0.3 μM ethidium bromide (EB). The gels were run at 50V for 1 h in Tris–acetic acid–ethylenediaminetetraacetic acid (TAE) buffer and the bands were photographed [9].

Results and discussion

The Cobalt complex has been synthesized from ligand having Schiff base (L). They are found to be stable in air. The ligand (L) is soluble in common organic solvents, but the complexes are soluble only in DMSO. The complexes have been characterized by the micro analytical data, IR, UV-Vis. and EPR spectra. Physical characterization, micro analytical and molar conductance data of the complexes are given in Table.3.1. The analytical data of the complexes accord well with the formula $[ML_2]Cl_2$; where M=Co(II). The high conductance of the chelates supports their electrolytic nature.

FT-IR spectroscopy

The functionalities of free ligand and their coordinating capability to form metal complex were investigated by FT-IR spectroscopy. The IR spectra for the ligand and metal complex have been recorded in the region 400-4000 cm^{-1} and shown in Fig.4.1. The spectrum of L shows absorption band in the region 1675 cm^{-1}, a characteristic feature of the $\upsilon(C=N)$ stretching mode.

Table 3.1 Physical characterization, analytical and molar conductance data of the synthesized compounds

Compound	**Yield (%)**	**Color**	**Calc. (Found) %**				**Formula Weight**	Λ_m (ohm^{-1} cm^2 mol^{-1})
			M	C	H	N		
L $[C_{22}H_{20}O_4N_2]$	75	Brown	-----	70.20 (69.91)	5.36 (5.12)	7.44 (7.63)	376	-----
$[CoL_2]Cl_2$ $[C_{44}H_{40}Co\ N_4O_8]Cl_2$	66	Pale green	7.26 (7.18)	65.10 (65.26)	4.97 (5.11)	6.90 (6.79)	812	124
$[CuL_2]Cl_2$ $[C_{44}H_{40}CuN_4O_8]Cl_2$	70	Black	7.78 (7.11)	64.74 (64.26)	4.94 (4.08)	6.86 (6.17)	816	110

This band is shifted towards lower frequencies in the spectra of cobalt complex 1635 cm^{-1} and copper complex 1640 cm^{-1} indicating the role of the azomethine group in coordination with metal ion [50]. A strong broad band is noticed in the range of 3425 - 3400 cm^{-1} for both the free ligand (L) and its metal complexes, which is due to the phenolic -OH group of vanillin moiety that implies the unbound nature in coordination [10]. The coordination of the azomethine group was further supported by the appearance of new band around 480 cm^{-1} which are due to $\upsilon_{(M–N)}$. This band was not identified in the spectrum of ligand.

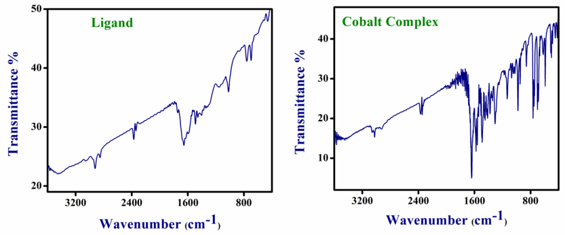

Fig.2 .IR spectra of Schiff base ligand and its cobalt complex

Electronic Spectroscopy

The geometry of these metal complexes has been deduced from electronic spectral data of the complex. Electronic spectra of Schiff base ligand **(L)** and its metal complexes were recorded at room temperature in DMSO medium in the range of 200 – 1100 nm. The absorption spectra of L and its respective metal complexes are given in Fig 4.2. The absorption spectrum of the L exhibits two characteristic bands at 37,313 and 28,653 cm^{-1} which correspond to the intra ligand charge transfer transitions of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ respectively. These two transitions are shifted due to the complex formation, either bathochromic or hypsochromic shift. In case of cobalt(II) complex, a strong absorption d-d band appeared at 19,047 cm^{-1} is assigned to $^1A_{1g} \rightarrow {}^1B_{1g}$ transition. Moreover in copper(II) complex, a strong absorption d-d band appeared at 17,543 cm^{-1} is assigned to $^2B_{1g} \rightarrow {}^2A_{1g}$ transition which is characteristic of square planar geometry [11].

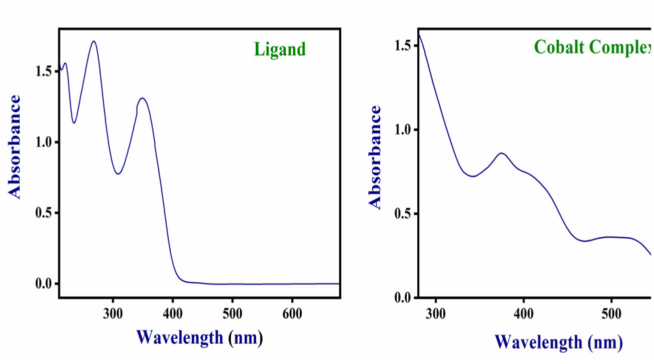

Fig. 3. UV-*vis*. spectra of Ligand and Cobalt complex in DMSO

EPR spectra

The X band EPR spectra of Cu(II) complex has been recorded in DMSO at liquid nitrogen temperature (LNT) and at room temperature (RT). The spectrum of the Cu(II) complex at RT shows one major intense absorption band in the high field and exist as isotropic due to the tumbling motion of the molecules. However, it shows four well-resolved peaks at LNT with low intensities around low field region and one intense peak in the high field region (Fig.4.3).

The spin Hamiltonian parameters of Cu(II) complex have been calculated and summarized in Table 4.1. From the spectral data, it is found that $A_{||}$ (113) > $A_{\perp}$(20); $g_{||}$ (2.38) > $g_{\perp}$(2.07)> 2.0025, which support the $d_{x^2-y^2}$ as the ground state, characteristic of square-planar geometry and axially symmetric. Further, in an axial symmetry, the g-values are related by the expression, $G = (g_{||} - 2.0025) / (g_{\perp} - 2.0025)$

This measures the exchange interaction between the copper centers in polycrystalline solid. As it is reported earlier, if the value of $G > 4.0$, the local tetragonal axes are aligned in parallel or only slightly distorted. While the value of $G < 4.0$, there is significant coupling exchange and the distortion is high. The observed value for the exchange interaction parameter for the Cu(II) complex is found to be 4.7 G, which suggests the local tetragonal axes are aligned parallel or slightly distorted and the unpaired electron occupies in the $d_{x^2-y^2}$ orbital. This infers the absence of exchange coupling between Cu(II) centers in the solid state [12].

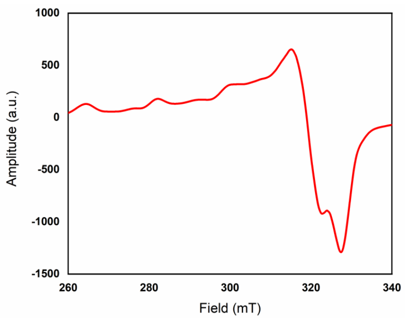

Fig.4. EPR spectrum of Cu(II) complex at LNT in DMSO

The degree of geometrical distortion is ascertained by the parameter $g_{||}/A_{||}$ ($A_{||}$ in cm^{-1}) with the values less than 142 cm^{-1} associated with the square-planar structures, whereas the higher values indicate the distortion towards tetrahedron [13]. With the above copper complex, the $g_{||}/A_{||}$ values are noticed at 210 cm^{-1}, exist in good agreement with significant deviation from planarity, which is further supported by the bonding parameter (α^2) value remaining less than unity.

Complex	g-tensor			A × 10^{-4} (cm^{-1})				
	$g_{||}$	$g_{\perp}$	g_{iso}	$A_{||}$	$A_{\perp}$	A_{iso}	**$g_{||}/A_{||}$**	**G**
Copper complex	2.39	2.08	2.35	114	19	54	209	4.8

The covalency parameters α^2 (covalent in-plane σ-bonding) and β^2 (covalent in-plane π-bonding) were calculated using the following equations. As we know, if $\alpha^2 = 1.0$, it indicates the complete ionic character, whereas if $\alpha^2 = 0.5$ signifies the 100% covalent bonding, based on the assumption of negligible small values of the overlap integral.

$$\alpha^2 = (A_{||}/0.036) + (g_{||} - 2.0025) + 3/7\,(g_{\perp} - 2.0025) + 0.04$$

$$\beta^2 = (g_{||} - 2.0025)\,(E / -8\lambda\alpha^2)$$

where $\lambda = -828\ cm^{-1}$ for the free copper ion and *E* is the electronic transition energy. From Table 4.1, the α^2 and β^2 values indicate that there is a substantial interaction occurs in the in-plane σ-bonding whereas the in-plane π-bonding is almost ionic. The lower value of α^2 (0.78) is compared with β^2 (1.19) which indicates that the in-plane σ-bonding is more covalent than in-plane π-bonding. These values are occurred to be in accordance with the report from other group [14]. Based on these interpretations, a square-planar geometry is proposed for this complex. The EPR spectral data of the copper(II) complex has provided the supportive evidence to the conclusion derived on the basis of electronic and magnetic moment values.

Table 3.1. The spin Hamiltonian parameters of Cu(II) complex in DMSO solution at LNT

Based on the above spectral data, the proposed structures of the metal complexes are given in Fig. .5

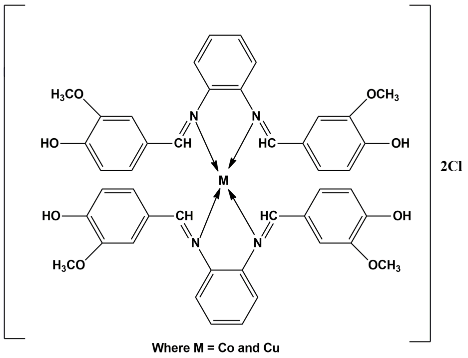

Fig 5 The proposed structure of the metal complexes

DNA Cleavage studies

Agarose gel electrorophoresis method

Gel electrophoresis experiments using pUC18 circular plasmid DNA was performed with the increase the concentration of cobalt complex in the presence of H_2O_2 as an oxidant. At micromolar concentrations for a 2 h incubation period, the complex exhibited moderate cleavage activity.

The agarose gel pattern of plasmid DNA comprises of two distinct conformations, one of it is accountable for the supercoiled DNA (Form I), and another for the nicked circular DNA (Form II). Altogether, the relaxed form of plasmid DNA is formed due to the cleavage of the DNA strands (nicking of DNA), known as the nicked circular form which migrates very slowly in the agarose gel. The linear form DNA (Form III) is generated to migrate between supercoiled and nicked circular forms, if both strands are cleaved [15]. Agarose gel electrophoresis can differentiate all the three different plasmid DNA conformations during analysis.

It is deduced that the cobalt complex has shown binding tendency with CT DNA exclusively. The cleavage activity is examined using supercoiled pUC18 DNA. It is incubated with different concentrations (Control 40, 60, 80 and 100 µM, respectively) of complex in TBE buffer at pH 7.2 for 3 h. Each complex tends to exhibit a pronounced cleavage activity in the presence of H_2O_2. It may be responsible for the formation of hydroxyl free radicals. Upon increasing the concentration of the complex, the ratio of Form I reduce gradually and there is partial transformation to Form II with concurrent increase in the intensity of the latter form, whereas the production of Form III also increases at 100 µM (Fig.5; lane 6). Thus, in the presence of activators, the concentration dependent cleavage of pUC18 DNA is observed in the case of complex as shown in Fig.4.4.

Fig.6 Gel electrophoresis pattern showing cleavage of pUC18 DNA (30 µM) treated with L and its cobalt complex at 35 °C after 2 h of incubation. Lane 1: DNA control; Lane 2: DNA + L+ H_2O_2; Lane 3, 40 µM of complex + DNA; Lane 4, 60 µM of complex + DNA; Lane 5, 80 µM of complex + DNA; Lane 6, 100 µM of complex + DNA

SUMMARY

A Schiff base ligand has been prepared by the condensation of p-vanillin and o-phenylenediamine. Its metal complexes has been synthesized and characterized by micro analytical data, IR, UV-Vis. and EPR spectra. The data show that they have composition of the type $[ML_2]Cl_2$ where M= Co(II) and Cu(II). The spectral data of the metal complexes suggest square planar geometry around the central metal ion. It shows good electrical conductance which reveals that this chelate was electrolytic in nature. Gel electrophoresis experiment infers that DNA cleavage is enhanced to significant extent in the presence of hydrogen peroxide. Active oxygen intermediate like hydroxyl radicals generated from hydrogen peroxide upon cleavage mechanism could play an important role. It has also been signified that concentration plays a vital role in increasing the cleavage activity.

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