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Synthesis, Spectral Characterization, Biological Activity and Dna Cleavage Studies of Cu(II), Ni(II) AND Zn(II) SCHIFF Base Complexes Derived from 2,4-Dihydroxy Benzaldehyde and P-Chloroaniline

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Abstract: Metal complexes of Schiff base derived from condensation of 2,4-dihydroxy benzaldehyde and p-chloroaniline were synthesised and characterized by elemental analysis, IR, ^1H NMR spectra, magnetic moment, molar conductance, UV– Visible and thermal analysis (TGA). From the elemental analysis data, the complexes were proposed to have the general formula [M (HL)] where M = Cu(II), Ni(II) and Zn(II). The molar conductance data revealed that all the metal chelates were non-electrolytes. The IR spectra showed that, HL is coordinated to the metal ions in a tetradentate manner with donor sites of the azomethine-N, phenolic-OH. The synthesized ligand, in comparison to its metal complexes were screened for their antibacterial activity against bacterial species, *Escherichia coli*, *Pseudomonas*, *Bacillus subtillus, Staphylococcus aureus*. The activity data showed that the metal complexes to be more potent / antimicrobial than the parent Shciff base ligand against one or more microbial species. From the magnetic moment and electronic spectra data, it is found that the geometrical structures of these complexes are square planar. The thermal behaviour of these chelates showed that decomposition of the anions and ligand molecules in subsequent steps.

Key Words: Schiff base, p-chloroaniline, 2, 4-dihydroxybenzaldehyde and antibacterial activity.

1. Introduction

Schiff bases are considered as a very important class of organic compounds which have wide applications in many biological aspects [1]. These wide applications of Schiff bases have generated a great deal of interest in metal complexes. Schiff base–transition metal complexes are one of the most adaptable and thoroughly studied systems [2,3].

These complexes have also applications in clinical [4] and analytical fields [5]. Some of Schiff base complexes are used as model molecules for biological oxygen carrier systems [6]. Tetradentate Schiff base complexes are well known to form stable complexes, where the coordination takes place through the N_2O_2 donor set [7–9].

The Zinc(II), Nickel(II) and Copper(II) complexes of Schiff bases derived from 2,4-dihydroxy benzaldehyde and amino acids have been shown to have stronger anticancer activity against Ehrlich ascites carcinoma (EAC). The promising class of bis(salicylaldiminato) metal Schiff base complexes have been shown to exhibit quadratic non-linear optical (NLO) properties, which are currently attracting considerable interest. In addition, there is a continuing interest in investigating the relationship between the redox potentials and other electrochemical parameters and spectral/geometrical parameters of Schiff base metal complexes, which could be the result of steric and electronic effects. The transition metal complexes having oxygen and nitrogen donor Schiff bases possess unusual configuration and structural liability and are sensitive to molecular environment. Four-coordinated Copper(II) complexes usually form a square planar coordination geometry that may be distorted to pseudo-tetrahedral geometry depending on the ligand environment.

The transition metals Zinc and Copper are some of the most frequently occurring elements integrated into essential biochemical pathways. There are a number of biologically important molecules showing the catalytic activity [10,11] or molecules involved in transfer processes, like oxygen transfer, and incorporating transition metals into their active sites. In contrast to Zinc(II), the Copper(II) ions are redox active and play a crucial role in catalytic sites of oxidoreductases. The cyclic redox process enables these ions to act as pro- or anti-oxidants. The published opinions on the structure and pro- or anti-oxidant activity relationships are, however, quite inconsistent [12–14]. It can be assumed from several scientific works that the quality of pro- or anti-oxidant action depends on the type of ligands forming the bioactive complexes [15,16].

This study presents the synthesis and characterization, anti-bacterial activities of new Cu(II), Ni(II), and Zn(II) complexes of Schiff base derived from 2,4-dihydroxy benzaldehyde and p-chloroaniline. The synthesised complexes have been characterized by elemental analysis, infrared, electronic spectra, thermal, and magnetic studies.

2. Experimental

Materials and Physical measurements

2,4-dihydroxy benzaldehyde was purchased from Aldrich and used without any further purification. The acetate salts of Cu(II), Ni(II), Zn(II), p-chloroaniline and solvents (AnalR /GR grade) were purchased from Merck, BDH, Loba chemicals and used without further purification. Double distilled water was used for the preparation of metal salt solutions.

The elemental analysis was carried out in Carlo-Erba elemental analyser 1108. The FTIR spectra were recorded in Perkin–Elmer spectrophotometer model 1800 in the ra nge of 4000–200 cm−1 in the KBr phase. The ^{1}H NMR spectra were recorded in DMSOd^6, 300 MHz FT 1H NMR (Bruker DRX 300) Spectrometer.

Synthesis of ligand

Synthesis of Ligand from p-Chloroaniline

The solution of 2,4-dihydroxybenzaldehyde was mixed with hot solution of p-chloroaniline in 20 ml ethanol. The resulting mixture was left under reflux for 2 hr and the solid product formed was separated by filtration, purified by crystallization from ethanol, washed with diethyl ether and dried in a vacuum over anhydrous calcium chloride. The yield of yellow colour product was 88% (Table 1).

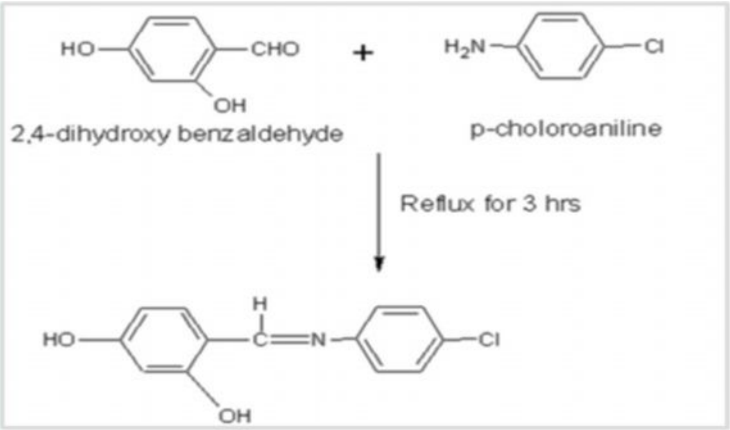


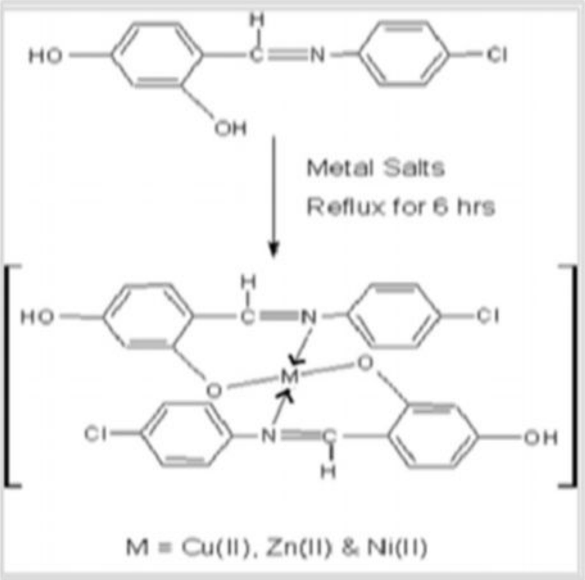
Fig 1: Synthesis of Ligand (HL).

Table-1: Analytical and Physical data for the ligand and its complexes.

Compound	Colour	M.pt 0(C)	Elemental analysis				Ëm (Ohm^{-1} cm^{2} mol^{-1})
			%M		%N		
			Exp	Cal	Exp	Cal	
L	Yellow	242	-	-	5.66	5.62	-
Cu(L)	Brown	276	11.51	11.55	5.03	5.04	13.54
Zn(L)	Pale Brown	295	11.66	11.67	5.02	5.04	14.32
Ni(L)	Dark Brown	252	10.54	10.56	5.09	5.12	14.89

Synthesis of Metal Complexes

All new metal complexes of the Schiff base HL were prepared by the same general procedure with stoichiometric amount of ligand and metal salts in a 2:1 mole ratio by the addition of hot solution of the appropriate metal acetate in ethanol to the hot solution of Schiff base in the same solvent. The resulting mixture was stirred under reflux for three hours where upon the complexes precipitated. They were collected by filtration, washed with a 1:1 ethanol: water mixture, diethyl ether and dried.



M = Cu(II), Ni(II) & Zn(II)

Fig 2: Synthesis of metal complexes.

Biological activity

0.5ml spore suspension (10−6 to 10−7 spore/ml) of e ach of the organisms to be investigated was added to a sterile agar medium just before solidification, then poured into sterile petri dishes (9 cm in diameter) and left to solidify. Using sterile cork borer (6mm in diameter), three holes (wells) were made in each dish, then

0.1ml of the compounds to be tested dissolved in DMF (100 (g/ml) were poured into these holes. Finally, the dishes were incubated at 37 °C for 48 h where clear or inhibition zones were detected around each hole. About 0.1ml DMF alone was used as a control under the same condition for each organism and by subtracting the diameter of inhibition zone resulting with DMF from that obtained in each case, both antimicrobial activities can be calculated as a mean of three replicates [17, 18].

Gel electrophoresis

The cleavage of pUC18 DNA was determined by agarose gel electrophoresis [19]. The gel electrophoresis experiments were performed by incubation of the samples containing 40 µM pUC18 DNA, 50 µM metal complexes and 50 µM H_2O_2 in Tris-HCl buffer (pH 7.2) at 37°C for 2 hours. After incubation, the samples were electrophoresed for 2 hours at 50 V on 1% agarose gel using Tris–acetic acid–EDTA buffer (pH 7.2). The gel was then stained using 1 µg/cm^3 ethidium bromide and photographed under ultraviolet light at 360 nm. All the experiments were performed at room temperature.

Cyclic Voltammetry

All voltammetric experiments were performed with a CHI 760 electrochemical analyzer, in single compartmental cells using tetrabutylammonium perchlorate as a supporting electrolyte. The redox behaviour of the complexes has been examined at a scan rate of 0.1 Vs^{-1} in the potential range +2.0 to –2.0 V. A three-electrode configuration was used, comprising a glassy carbon electrode as the working electrode, a Pt-wire as the auxiliary electrode, and an Ag/AgCl electrode as the reference electrode. The electrochemical data such as cathodic peak potential (Epc) and anodic peak potential (Epa) were measured.

Result and Discussion

The Schiff base, HL, is subjected to elemental analyses. The results of elemental analyses (C, H, N) with molecular formula and the melting point are presented in Table-1. The results obtained are in good agreement with those calculated for the suggested formula and the melting point is sharp indicating the purity of the prepared Schiff base. The structure of the Schiff base under study is given in Fig.1. The structure of this Schiff base is also confirmed by IR and ^1H NMR spectra, which will be discussed with metal complexes.

Composition and Structures of Schiff base complexes

The isolated solid complexes of Cu(II), Ni(II) and Zn(II) ions with the Schiff base HL ligand were subjected to elemental analysis (C, H, N, S and metal content), IR, ^1H NMR, magnetic studies, molar conductance and thermal analysis (TGA and DTA), to identify their tentative formula in a trial to elucidate their molecular structures. The results of elemental analysis, Table-1 are in good agreement with those required by the proposed formula.

Molar conductivity measurements

The complexes were dissolved in DMF and the molar conductivities of 10^{-3} M of their solutions at 25±2°C were measured. Table-1 shows the molar conductance values of the complexes. It is concluded from the results that the complexes are found to have molar conductance values of 13.54–14.89 Ω^{-1} mol^{-1} cm^2 indicating that these complexes are non-electrolytes. It also, indicates the non-bonding of the acetate anion to the metal ions.

IR Spectra

The IR data of the spectra of Schiff base and its complexes are listed in Table 2. The IR spectra of the complexes are compared with those of the free ligand in order to determine the coordination sites that may involve in chelation. There are some guide peaks, in the spectra of the ligand, which are of good help for achieving this goal. The position and/or the intensities of these peaks are expected to be changed upon chelation. New peaks are also guide peaks as well as water in chelation. These guide peaks are listed in Table-2. Upon comparison it was found that the azomethine υ(C=N)

stretching vibration is found in the free ligand at 1623 cm^{-1} . This band is shifted to lower wave numbers

(1590-1596 cm^{-1}) in the complexes indicating the participation of the azomethine nitrogen in coordination (M -N) [20].

Table-2: Infrared spectral data for Schiff base metal complexes.

Compounds	**(C=N)** **(cm^{-1})**	**C=C** **(cm^{-1})**	**(M–N)** **(cm^{-1})**	**(M–O)** **(cm^{-1})**
($C_{26}H_{20}N_2O_4Cl$)	1623	1525	--	--
[Cu($C_{26}H_{18}N_2O_4Cl$)]	1592	1478	456	515
[Ni($C_{26}H_{18}N_2O_4Cl$)]	1596	1511	478	519
[Zn($C_{26}H_{18}N_2O_4Cl$)]	1590	1495	459	512

^{1}H NMR spectra

A survey of literature revealed that the ^{1}H NMR spectroscopy has been proved useful in establishing the nature and Structure of many Schiff bases as well as their complexes in solutions. The ^{1}H NMR spectrum of Schiff base was recorded in dimethylsulfoxide (DMSO-d^6) solution using tetramethylsilane (TMS) as internal standard. The ^{1}H NMR spectra of the Schiff base ligand, its diamagnetic Zn(II) complexes and the chemical shifts of the different types of protons are analysed. The spectra of the complexes are examined in comparison with those of the parent Schiff base. Upon examinations it was found that the phenolic-OH signal, appeared in the spectrum of ligand at 12.82 ppm is completely disappeared in the spectra of its Zn(II) complexes indicating that the OH proton is removed by the chelation with Zn(II).

Table-3: Electronic spectroscopic data of the ligand and its metal complexes.

Electronic spectroscopy

	Absorption (nm)			
Compound	**ë → ë***	**n → ë***	**L→ M (CT)**	**d – d**
L	256	311	---	---
Cu(L)	261	305	427	510
Zn(L)	274	316	414	---
Ni(L)	259	347	436	515, 684

Magnetic susceptibility and electronic spectra Measurements

The UV–Visible spectra of the ligand and the complexes were recorded in DMF solution in the wavelength range from 200 to 800 nm. The spectra showed a sharp and intense band at 256 nm which is attributed to π-π^* transition within the benzene ring of the ligand and complexes molecule. The bands observed at 305 and 347 nm in the free ligand and complexes are reasonably accounted for n– π^* transitions for the phenolic-OH and azomethine moieties, respectively. The spectra of the complexes further display a band in the range 414-436 nm, which might be assigned to charge transfer transition from the ligand to metal ions (L→M) [21]. The bands in the regions respect to the ligand depend on the type of metal ions coordinated to the ligand and indicated coordination of phenolic-OH and azomethine moieties to the metal ions.

The Ni(II) complex reported herein has a room temperature magnetic moment value of 2.98 B.M. which is in the normal range observed for square planer Ni(II) complexes (μeff = 2.9–3.3 B.M.) [22]. This indicates that, the complexes of Ni(II) are four coordinate and probably square planer [23]. The square planer Cu(II) ion (d9) obtained at 625 was assign to the transition 2B1g→2B2g indicates square planer geometry [24]. The magnetic moment of 1.68 B.M. also indicates that planer geometry around Cu(II) complexes [25]. The complexes of Zn(II) is diamagnetic. In analogy with those described for Zn(II) complexes containing N–O donor Schiff bases [26, 27] and according to the empirical formulae of these complexes, we proposed an square planer geometry.

Thermal analysis (TGA and DTA)

In the present investigation, heating rates were suitably controlled at 10◦C under nitrogen atmosphere and the weight loss was measured from the ambient temperature up to 1000 ◦C. The weight losses for each chelate were calculated within the corresponding temperature ranges.

The thermogram of Cu(II) complex shows only one decomposition steps within the temperature range 100–6 00 ◦C. correspond to the loss of water molecules and loss of ligand groups with a mass loss of 39.16% (calcd. 44.42%). The TG curves of the Ni(II) chelates show only one stages of decomposition within the temperature range of 120–650 corresponds to the loss of water molecules and ligand groups with a mass loss of 39.23% (calcd. 44.28%). Similarly, the thermogram of Zn(II) complex shows only one decomposition steps within the temperature range 140–6 70 ◦C. correspond to the loss of water molecules and loss of ligand groups with a mass loss of 39.30% (calcd. 44.34%).

Cleavage of plasmid pUC18 DNA

DNA cleavage is controlled by relaxation of supercoiled circular form of pUC18 DNA into nicked circular form and linear form. When circular plasmid DNA is conducted by electrophoresis, the fastest migration will be observed for the supercoiled form (Form I). If one strand is cleaved, the supercoils will relax to produce a slower-moving open circular form (Form II). If both strands are cleaved, a linear form (Form III) will be generated that migrates in between.Figure-3 illustrates the gel electrophoresis experiments showing the cleavage of plasmid pUC18 DNA induced by the three complexes. The control experiments did not show any apparent cleavage of DNA (lanes 3 and 4). Complexes Ni(II) and Zn(II) in the presence of H_2O_2 (lanes 1 and 2) at higher concentration (50 µM) show cleavage activity inwhich supercoiled DNA (Form-I) cleaved and supercoiled form converted to open form (Form-II).Cu(II) complexes in the presence of H_2O_2 (lane 2) at higher concentration (50µM) shows more cleavage activity compared to complexes Ni(II) and Zn(II). The supercoiled plasmid DNA was completely degraded (100% cleavage efficiency was observed). This shows that a slight increase in the concentration over the optimal value led to extensive degradations, resulting in the disappearance of bands on agarose gel [28].

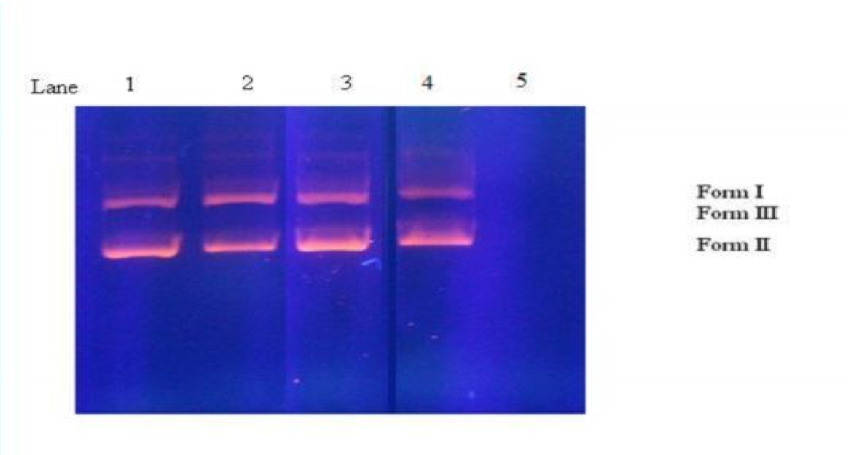


Figure-3: Changes in the agarose gel electrophoretic pattern of pUC18DNA induced by H_2O_2 and metal complexes:

Lane 1, DNA alone; Lane 2, DNA + Cu(II) complex + H_2O_2; Lane 3, DNA + Ni(II) complex + H_2O_2; Lane 4, DNA + Zn(II) complex + H_2O_2.

Biological activity

In testing the antibacterial activity of these compounds more than one test organism were used to increase the chance of detecting antibiotic principles in tested materials. The sensitivity of a microorganism to antibiotics and other antimicrobial agents was determined by the assay plates which incubated at 28 ◦C for 2 days for yeasts and at 37 ◦C for 1 day for bacteria. The data showed that in some cases the ligand has a higher or similar antbacterial activity than the selected standards (chloramphenicol and grisofluvine). Also, the presence of certain metal ions enhanced the antimicrobial activity of the ligand and in some cases a higher or similar activity than the selected standards were also observed (Table-4).

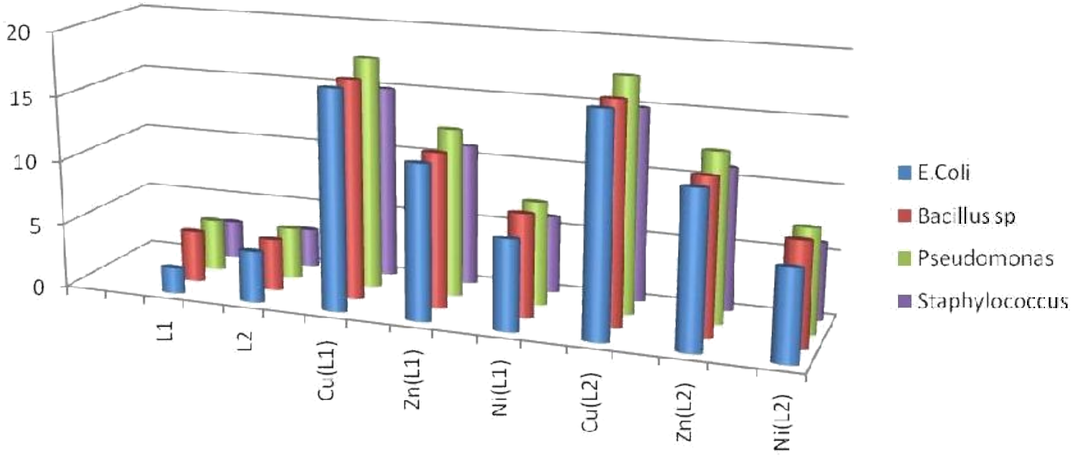


Figure-4: Difference between the antibacterial activities of the mononuclear metal complexes.

Table-4: Biological activity of the ligand, complexes.

Compound	E.Coli (mm)	Bacillus (mm)	Pseudomonas (mm)	Staphylococcus (mm)
Ligand	2	4	4	3
Cu(L)	17	17	18	15
Zn(L)	12	12	13	11
Ni(L)	7	8	8	6

Conclusion

The structures of the complexes of Schiff base with Cu(II), Ni(II), Zn(II) ions were confirmed by the elemental analyses, IR, NMR, molar conductance, magnetic, solid UV–VIS, and thermal analysis data. Therefore, from the IR spectra, it is concluded that HL behaves as a uninegatively tetradentate ligand coordinated to the metal ions via the azomethine-N, deprotonated phenolic-OH. It is found from the obtained the molar conductance data, that the complexes are non-electrolytes. The ^1H NMR spectra of the free ligand and its diamagnetic Zn(II) complex showed that the OH signal, appeared in the spectrum of HL ligand at 3.34 ppm is completely disappeared in the spectrum of its Zn(II) complex indicating that the OH proton is removed by the chelation with Zn(II) ion. On the basis of the above observations and from the magnetic and electronic measurements, square planer geometries are suggested for the investigated complexes. As a general conclusion, the investigated Schiff base behaves as a tetradentate ligand and its metal complexes structure can be given as shown in Fig.2. The antibacterial activity and DNA cleavage efficiency of the complexes have greater than that of ligand.

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