

Synthesis and Biological Evaluation of Schiff Base Metal Complexes of Cu(II), Co(II) and Ni(II) using (DFMPM) and L-Alanine

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Abstract

Schiff base nano complexes of Cu(II), Co(II) and Ni(II) of general composition ML have been synthesized using DFMPM and L-alanine. The complexes were prepared by standard methods. The melting point, elemental analysis, molar conductance, magnetic moment studies were conducted and the complexes were characterized by IR, UV-Vis, ¹H NMR, SEM, and XRD. Based on the above studies the ligand was bidentate and the complexes were hexa coordinated proposing octahedral geometry to the complexes and are nano crystalline in size. Biological studies such as antibacterial, antifungal and DNA cleavage studies of the complexes indicated that the complex have better antibacterial, antifungal and DNA cleavage properties. The XRD and SEM studies revealed the nano crystalline nature of the complexes. The conductance measurements indicated the non-electrolytic nature of the complexes and are used for the removal of these metals from water.

Keywords: DFMPM, L-alanine, Schiff base, nano crystalline, DNA cleavage and anticancer activity

1. Introduction

Transition metal complexes with Schiff base ligands have been extensively investigated of as physical and chemical properties and their antimicrobial and anticancer agents. Schiff base complexes derived from aminoacids are important due to their ability to possess unusual configurations and biological importance [1-4].

DFMPM is prepared from cardanol which formed Schiff base ligand (L) with L-alanine. The present investigation involved the synthesis and characterization of the Schiff base Ligands (L) and the complexes of Cu(II), Co(II), Ni(II) ions. The above complexes were characterized by elemental analysis, conductance measurements, magnetic susceptibility, uv-visible, IR, powder XRD, SEM, antimicrobial activity, DNA cleavage and anticancer activity.

2. Materials and Physical Measurements

Cardanol was obtained from M/S Satya Cashew, Chennai, India. Formaldehyde (37% solution) hydrochloric acid, epichlorohydrin, sodium hydroxide and other chemicals used were of AR grade quality obtained from Merk Chemicals. All the solvent used was purified by standard methods[9]. The micro analytical data (C, H, N) were collected using Perkin Elmer 2400 instrument. The metal ion intake were estimated by standard methods.

IR spectra were obtained using PE IR spectrum instrument model : system 2000. ¹HNMR spectra were obtained using AMX-300 MHz, FT NMR Spectrometer. Conductance measurements were obtained using systronics-305 conductivity meter. Electronic spectra of the ligands and its complexes was obtained using Perkins Elmer Lambda-25 UV-visible spectrometer in the range of 200-1100 nm. Surface morphological studies was obtained using JSM-5610 scanning electron microscope.

2.1 Preparation of Schiff base Ligand (L) with DFMPM and L-alanine.

The Schiff base Ligand (L) were prepared by mixing equimolar ethanolic solution of DFMPM and L-alanine in ethanol and refluxed for 2 hour and then cooled the mixture and the Schiff base was separated and recrystallized from ethanol and washed thoroughly with diethyl ether. (fig 1)[5]

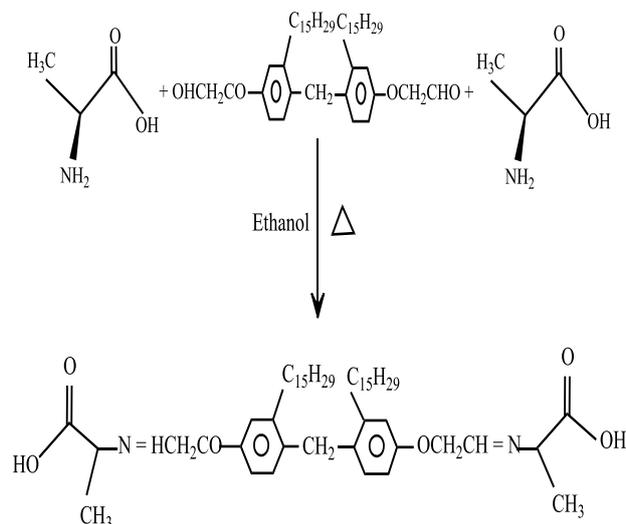


Fig.1 Schiff base Ligand (L)

2.2 Preparation of the metal complexes:

Schiff base complexes of Ligand(L) were prepared by the addition of hot ethanolic solution (60°C) of Ligand(L) and aqueous solution nitrates of Cu(II), Co(II), Ni(II) ions in drop by drop in 2:1 molar ratio. The mixture was stirred for 12 hours at 65°C. The precipitated solids were washed thoroughly by ethanol and then by diethyl ether. The solid complexes were dried in vacuum desiccator.

3. Results and Discussion

All the metal complexes prepared above are coloured complex are stable towards air and have high melting point. The complexes are insoluble in water and common organic solvents but are soluble in DFM and DMSO.

3.1 Elemental analysis

The analytical data suggested that all complexes are mono nuclear with ligand coordinated to the central metal atom. The metal to ligand ratio in all the complexes was 1:2 The details are given in Table. 1 and shows the Fig.2.

Table.1 Physical characteristics and analytical data of the complexes

Ligand/Complexes	Yield %	colour	Molecular formula	Molecular wt	Melting point	Elemental analysis		
						C	H	N
Ligand (L) C ₄₇ H ₈₄ O ₂ N ₂	62	Brown	C ₄₇ H ₈₄ O ₂ N ₂	826	228	76.99 (76.86)	11.86 (11.12)	3.35 (3.34)
[Cu(L)(NO ₃) ₂]	58	Light Green	CuC ₁₀₆ H ₁₉₆ O ₁₀ N ₆	1775.54	>250	71.64 (70.98)	11.03 (10.98)	4.73 (4.53)
[Co(L)(NO ₃) ₂]	57	Brown	CoC ₁₀₆ H ₁₉₆ O ₁₀ N ₆	1770.9	>250	71.82 (71.78)	11.06 (11.02)	4.74 (4.53)
[Ni(L)(NO ₃) ₂]	59	Brown	NiC ₁₀₆ H ₁₉₆ O ₁₀ N ₆	1770.69	>250	71.83 (71.78)	11.06 (10.98)	4.74 (4.58)

3.2 Molar Conductivity:

The molar conductivity values are given in table.2 and shows the Fig.3. The molar conductivity value were in the range of 13-15ohm⁻¹ cm² mol⁻¹ it showed that all the complexes are non-electrolytes because their conductivity value were low.

Table 2: Molar conductance data of ligand and its complexes

Ligand/complexes	Molar conductance Ohm ⁻¹ cm ² mol ⁻¹	Nature
Ligand(L) C ₄₇ H ₈₄ N ₂ O ₂	-	non-electrolyte
[Cu(L)(NO ₃) ₂]	13	non-electrolyte
[Co(L)(NO ₃) ₂]	15	non-electrolyte
[Ni(L)(NO ₃) ₂]	14	non-electrolyte

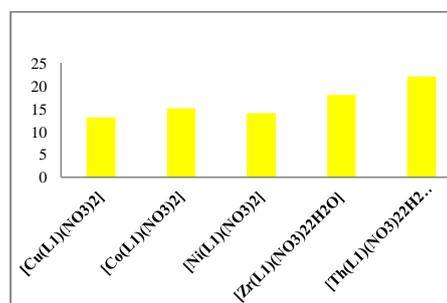


Fig. 3 Molar conductance data of the complexes

3.3 IR Spectra studies of ligand and its complexes:

Selected IR Spectra data for the ligand and its complexes are given in Table 3. The IR Spectrum of the free ligand (fig 4) is characterized mainly the strong bands of 2924cm^{-1} , 2854.65cm^{-1} , 1604.77cm^{-1} and 1381cm^{-1} which are attributed to the stretching frequencies of $\nu_{\text{C-H}}$, $\nu_{\text{C-O}}$, $\nu_{\text{C=N}}$ $\nu_{\text{-CH}_3}$ respectively.

The FT-IR Spectrum of the ligand is compared with the spectra of the complexes. The Characterization of absorption bands 3448cm^{-1} – 337.57cm^{-1} range were attributed to -OH group of the lattice water or the coordinated water. In the IR spectrum of the complexes (Fig 4-9) the absorption bands in the range 2924.09cm^{-1} –

2399.45cm^{-1} were assigned to $\nu_{\text{C-H}}$ stretching frequencies. The absorption bands in the range 2854.65cm^{-1} – 2059.98cm^{-1} were assigned to $\nu_{\text{C-O}}$ stretching frequencies[5,6]. The $\nu_{\text{C=N}}$ bands for the free ligand in the spectra of the complexes 1604.77cm^{-1} – 1627.92cm^{-1} suggesting coordination of both the nitrogen atoms of metal . The absorption bands at 1327cm^{-1} - 1311.59cm^{-1} is assigned to free CH_3 group. It shows that the terminal CH_3 is not involved in bonding. The imine peak in the metal complexed showed change in Schiffs compared to the ligand indicating coordination of the imine nitrogen atom to the metal ion due to coordination. The assigned to the coordinate nitro group with the central metal atom 779cm^{-1} - 748cm^{-1} is assigned to $\nu_{\text{M-N}}$ bond and 509cm^{-1} - 563cm^{-1} is assigned to $\nu_{\text{M-O}}$ band [7].

Table 3 : Selected FTIR frequencies (cm^{-1}) of the ligand and complexes

Ligand/Complexes	ν O-H	ν C-H	ν C-O	ν C=N	ν CH ₃	ν M-N	ν M-O
Ligand (L) $\text{C}_{53}\text{H}_{98}\text{O}_4\text{N}_2$	–	2924.09	2854.65	1604.77	1381	–	–
[Cu(L)(NO ₃) ₂]	3448.72	2924.09	2854.65	1604.77	1327	779	509.21
[Co(L)(NO ₃) ₂]	3402.43	2947.23	2391.73	1620.21	1381.03	732.95	578.64
[Ni(L)(NO ₃) ₂]	3433.29	2931.8	2376.3	1643.35	1381.03	779.24	617.23

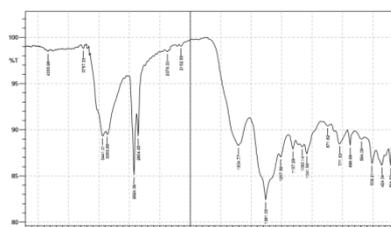


Fig. 4 FTIR spectrum of Schiff base ligand (L)

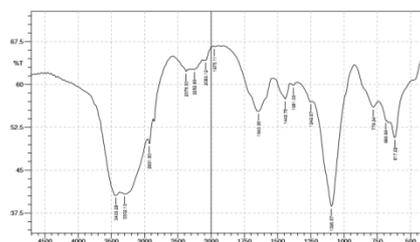


Fig.7 FTIR spectrum of Ni(II) complex of ligand(L)

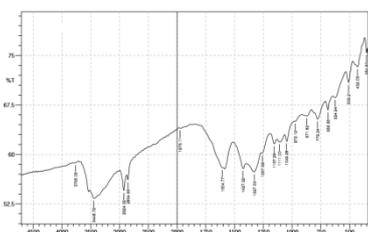


Fig. 5 FTIR spectrum of Cu(II) complex of ligand(L)

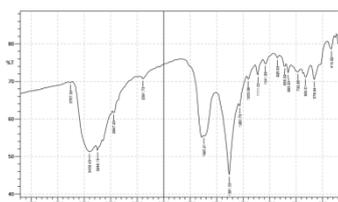


Fig. 6 FTIR spectrum of Co(II) complex of ligand(L)

3.4 Electronic Spectra:

The electronic spectral data are given Table 4 and the respective spectra. The nature of the ligand field around the metal ions has been derived from uv-visible spectra. The uv-visible spectra of ligand(L) shows the peak around at 274nm which are assigned to $\pi\text{-}\pi^*$ transition of the C-N chromophore respectively. The uv-visible spectra of the Cu(II) complex showed absorption band of 275nm , 266nm and 251nm respectively, corresponding to the ${}^2\text{B}_{1g} \text{--} {}^2\text{E}_{1g}(\text{p})$ transition and indicative of a distorted octahedral geometry of the complexes[8,9]

The uv-visible spectra of the Co(II) complexes showed absorption band at 264nm , 255nm and 247nm respectively corresponding to the ${}^4\text{T}_{1g}(\text{F}) \text{--} {}^4\text{T}_{1g}(\text{F})$ exhibited a d-d transition of the

complexes. Which is characteristic of octahedral geometry.

The uv-visible spectra of the Ni(II) complexes show exhibited absorption band at 278nm, 272nm and 229nm which can be tentatively assigned to the ${}^3A_{2g}(F) \rightarrow {}^3T_{2g}(P)$ transition which is the characteristic of octahedral geometry.

Table.4 : uv-visible spectral data of the ligand (L) and its complexes

Ligand/Complex	$\lambda_{max}(nm)$
Ligand(L) $C_{47}H_{84}N_2O_2$	281, 274, 270
[Cu(L)(NO ₃) ₂]	275, 266, 251
[Co(L)(NO ₃) ₂]	264, 255, 247
[Ni(L)(NO ₃) ₂]	278, 272, 229

3.5 Magnetic susceptibility measurements (BM)

The magnetic susceptibility values of the complexes of given in Table 4.5 Cu(II) complex showed magnetic moment of 2.0 BM indicating distorted octahedral nature of the complexes. The Co(II) complex showed magnetic moment of 5.2 Bm for indicating the formation of high spin octahedral geometry of the complex. The Ni(II) complex showed the magnetic moment of 2.7 BM indicating octahedral nature of the complexes.

Table 5: magnetic susceptibility values of the complexes

Complex	Magnetic susceptibility (BM)
[Cu(L)(NO ₃) ₂]	2.0
[Co(L)(NO ₃) ₂]	5.2
[Ni(L)(NO ₃) ₂]	2.7

¹HNMR Spectra:

The ¹HNMR spectrum of schiff base ligand (L) (Fig 4.10) exhibit a multiplet signal at ($\delta=7.031ppm - 7.021ppm$) is due to substituted aromatic ring protons. The multiplet at ($\delta=6.63ppm-6.608ppm$) and ($\delta=5.70ppm-5.425ppm$) were due to olefinic protons of the side chain and $-O-CH_2$ group of the ligand respectively [10,11]

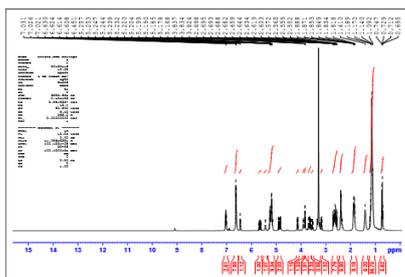


Fig. 8 : The ¹HNMR spectrum of schiff base Ligand(L)

The ¹HNMR spectrum of the Cu(II) complex of Ligand(L) show the (Fig.11) exhibit a signal at ($\delta=7.148ppm$) is due to substituted aromatic ring protons. Olefinic protons of the side chain ($\delta=6.709ppm - 6.978ppm$). The signal at ($\delta=8.2ppm$) is due to the $-CH=N$ protons. The siglet signal at ($\delta= 0.753ppm$) were due to the presence of $-CH_3$ group. The signal in the range ($\delta=0.795ppm - 0.909 ppm$) were due to the presence of saturated $-CH_2 -CH_2-$ group of the complex.

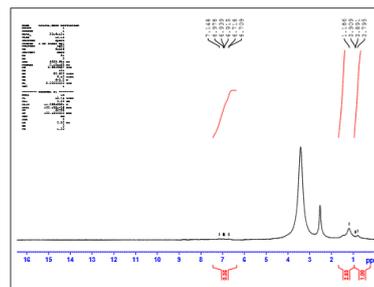


Fig. 9 : The ¹HNMR spectrum of Cu(II) complex of Ligand (L)

Based on the observations in elemental analysis, IR spectra, uv-visible spectra and ¹HNMR spectral studies, the proposed structure of metal Schiff base Ligand and complexes. [M(L)(NO₃)₂] are given in Fig 10-11

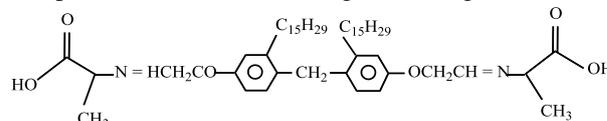


Fig.10 : Structure of Schiff base Ligand(L)

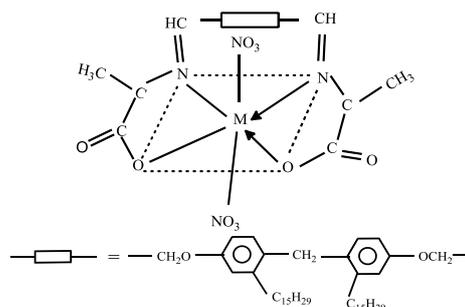


Fig. 11 : General structure of Schiff base complexes of Cu(II), Co(II) and Ni(II) M = Cu(II), Co(II) and Ni(II)

3.6 XRD Analysis

The powder XRD for the Ni(II) complexes were performed the XRD data. The diffractogram for the complexes are given in (Fig12). The grain size of the complexes was calculate using scherrer's formula. The strong broad peaks confirm the complexes formation and appearance of large feeble peaks indicates the complex to be microcrystalline. The calculated grain size 3.43nm this values suggested the crystals of the Ni(II) complexes is nano size [12].

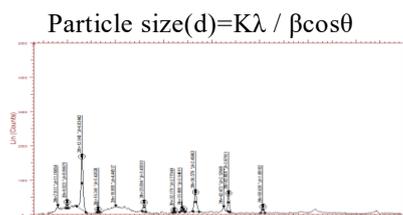


Fig. 12 XRD spectrum of Ni(II) complex of (L)

3.7 SEM Analysis

The surface morphology of the complexes was studied using JSM 5610 scanning electron microscope. The SEM images of Ni(II) complex is given fig (13). The SEM image showed that the complexes is nanocrystalline in nature. Careful examination of the single crystal clearly indicated the nano scale size of the single crystal of the complexes. The SEM images showed that all the complexes are nanocrystalline in nature showed rough, granular and pitted surface .

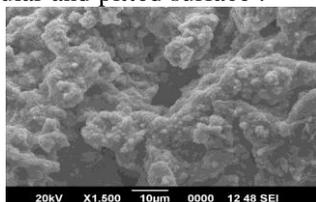


Fig. 13 SEM image of Ni(II) Complex of Ligand(L)

4.1 Antimicrobial activity.

The results of the antibacterial and antifungal screening of the Schiff base and the metal complexes with *Staphylococcus aureus*, *E.Coli*, *Klebsiella pneumonia* *Streptococcus mutans* and *Aspergillus niger*, *Aspergillus flavus* SP by disc diffusion method are given in Table.4. The antimicrobial activity was estimated based on the size of inhibition zone in the discs. Test drug solution of (100 µg) of each compound was prepared by dissolving 1mg of each compound separately in 1ml of DMSO. Standard antibiotic Amikacin 5mg /disc was used as standard for bacteria. Standard antibiotic Flucanazole 5µg/disc was used as standard for fungi[13,14].

The results of antimicrobial activity substantiate the findings of earlier researchers that biologically active compounds become active and less biologically active compounds become more active upon coordination. Such enhancement in biological activity of metal complexes can be explained on the basis of Overtone's concept and chelation theory. The present investigation suggest that all the metal complexes of the ligand bearing metal ion, pyridine ring, -N = CH- group, have comparatively more biological activity. This antibacterial activity serves as a basis for the chemical modification directed towards the development of new class of antimicrobial agents.

4. Biological Screening

Table 6: Antimicrobial activity data of ligand and its complexes

Ligand / complexes	Antibacterial activity (mm)				Antifungal activity (mm)	
	Staphylococcus aureus	Streptococcus mutans	E.coli	Klebsiella pneumonia	Aspergillus niger	Aspergillus flavus
Ligand $C_{69}H_{110}N_6O_4$	7	7	7	7	7	7
[Cu(L)(NO ₃) ₂]	8	11	10	9	8	7
[Co(L)(NO ₃) ₂]	11	10	9	8	7	12
[Ni(L)(NO ₃) ₂]	10	9	10	8		
PC	20	16	20	19	18	17

From the results it was found that the Cu(II) complex exhibit maximum antibacterial activity against *Streptococcus mutans* But Co(II) and Ni(II) shows the moderate activity towards the *Streptococcus aureus* and *E.coli*. It showed that the maximum activity against Cu(II) complex when compared to other complexes . It showed that Co(II) Complex the maximum antifungal activity against *Aspergillus flavus* When compared to other complexes.

4.2 DNA cleavage studies

The DNA cleavage ability of the complexes is monitored by gel electrophoresis. All the metal complexes are able to convert super coiled DNA in to open circular DNA. The results of DNA

cleavage are given in Fig.14[15,16].The result revealed the damage of DNA in Cu(II), Co(II), Ni(II) complexes could be attributed to the cleavage of DNA. The DNA cleavage activity of complexes is in the order Cu(II) > Co(II) > Ni(II) > with lambda-DNA. The DNA cleavage activity occurred as evidenced by the total appearance of DNA. On the basis of these results it is concluded that prominent DNA cleavage activity was observed in the presence of an oxidizing agent H₂O₂.



Fig.14 Gel electrophoresis diagram of the complexes of Ligand(L)

Lane 1 : DNA + Cu(L) + H₂O₂

Lane 2 : DNA + Co(L) + H₂O₂

Lane 3 : DNA + Ni(L) + H₂O₂

Lane 4 : Control DNA

Lane 5 : Marker

Lane 6 : DNA + L + H₂O₂

5. Conclusion

Schiff base metal complexes of Cu(II), Co(II), Ni(II), were synthesized from DFMPM using L-alanine were clearly described and characterized on the basis of analytical and spectral data. The XRD, SEM analysis indicate that the crystals of Schiff base metal complexes of nanocrystalline in nature. . Antibacterial study showed that the complexes of Cu(II), Co(II), Ni(II),.The more active than the complex of Cu(II) complexes. Antifungal activity showed that the complexes of Cu(II), Co(II), Ni(II) The more active than the complex of Co(II) when compared to other complexes. The DNA cleavage activity of complexes is in the order Cu(II) > Co(II) > Ni(II) > with lamda- DNA.

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