

## Synthesis, characterization and biological activities of some transition metal complexes with 3-amino-5-bromo-benzo[b]furan-2-carboxamide Schiff base

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### ABSTRACT

This article represents the synthesis and characterization of metal complexes of transition metal ions namely Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Hg(II) using a Schiff base formed by the condensation of 3-amino-5-bromo-benzo[b]furan-2-carboxamide and citral. Structures of the synthesized compounds were elucidated based on analytical, elemental, UV-Vis, IR, Mass and ESR studies. All the complexes are soluble in DMF and stable at NTP. The low specific conductance values suggest the non-electrolytic nature of the complexes. Further the Schiff base and its metal complexes were screened in vitro against *Escherichia coli* and *Staphylococcus aureus* bacterial species and *Aspergillus niger* and *Aspergillus fumigatus* fungal species in order to evaluate their anti-bacterial and anti-fungal activities respectively. Further, all the metal complexes were subjected to DNA cleavage analysis.

**Keywords:** Benzo[b]furan Schiff base, metal complexes, spectral studies, Biological studies, DNA cleavage study.

### 1. INTRODUCTION

Schiff base are the compounds derived from an amine and any aldehydes or ketones, which on coordination with metal ions through the azomethine nitrogen, forms good chelating agents [1]. Benzo[b]furan derivatives are an active and important class of organic compounds known to be present in many natural products and possess physiological activity [2]. Benzo [b] furan compounds studied worldwide since, they occur in a variety of structural forms ranging from a simple molecule such as 5-methoxy-benzo [b] furan to highly complex molecules like morphine-A and B. The seed oil of a plant "Egonoki" (*Styrax Japonicus*) which is much common in Japan well known to contain a benzo[b]furan derivative called "Egonal". It is an effective synergist for rotenone pyrethrum against houseflies, mosquitoes, aphids and many other insects [3]. Baker's yeast contains a benzo[b]furan derivative, which acts as an antioxidant and prevents hemorrhagic liver neurosis in rats and haemolysis of red cells in vitamin deficient rats [4].

Over the years, Schiff base has drawn much attention of the chemist due to their vast biological and chemical properties. It is now well known that some drugs have increased their activity when administered as metal complexes [5].

A variety of Schiff bases containing various donor sites have been tried for the complexation and the structures were deduced with the help of spectral and magnetic data.

Citral or 3,7-dimethyl-2,6-octadienal is an acyclic monoterpene and an important constituent of lemongrass oil and the seed oil of several plants such as lemon myrtle, *Listea citrate*, *Litcea cubeba* and other *Rutaceae* plant family [6]. Citral has an appreciable antibacterial and antifungal activity [7]. There is less work has been done on the complexation behavior of citral with benzo [b] furan Schiff base, therefore, we considered, it's worthwhile to undertake the synthesis, structural, magnetic and spectral investigations with the biological activity of a legend and its complexes formed by the condensation of 3-amino-5-Bromo benzo [b] furan-2-carboxamide and citral.

### 2. MATERIALS AND METHODS

All the chemicals used in the present work are of AR grade and of the highest purity available. The metal and the chloride content were determined according to the Vogel's procedures [8]. The experimental techniques used were discussed in our earlier report [9]. Benzo[b]furan-carboxamide synthesized according to the literature procedure [10].

### 2.1. Synthesis of Schiff base 5-bromo-(3,7-dimethylocta-2,6-dien-1-ylidene)amino benzo[b]furan-2-carboxamide [L]

A mixture of 3-amino-5-bromo benzo[b]furan-2-carboxamide (0.1 mol) and 3,7-dimethylocta-2,6-dienal (Citral).(0.1 mol) in 30 mL ethanol was refluxed on water bath for about 8h with the addition of few drops of glacial acetic acid with occasional shaking the solution. The product, which was separated out as a crystalline solid on cooling, was collected and recrystallized from Ethanol.  $C_{19}H_{21}BrN_2O_2$ [L]: Mol.Wt = 389.29, m.p = 220°C, Yield= 68%.

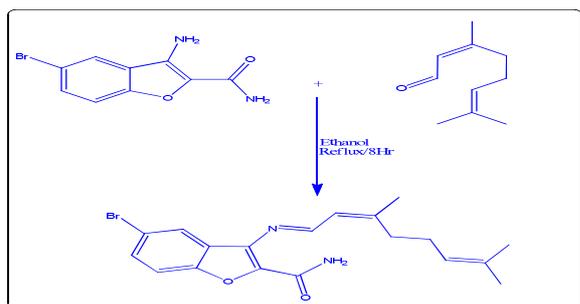


Figure - 1: Synthesis of ligand [L].

### 2.2. Synthesis of metal complexes

Metal chlorides (0.01 mol) were mixed with a Schiff base (0.01 mol) in an ethanolic medium 30 mL and the reaction mixture was refluxed for about 03 h. on water bath, then an aqueous solution of sodium acetate was added to the mixture to adjust the pH 6 to 7 and further refluxed for about an hour. Complexes were precipitated by adding distilled water. Further complexes separated were filtered off, washed with distilled water, then with alcohol, and finally dried in vacuum over fused calcium chloride.

### 2.3. Antibacterial and antifungal assay

The biological activities of synthesized Schiff bases and their Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Hg (II) complexes have been studied for their antibacterial and antifungal activities by agar diffusion methods respectively in DMF solvent against *Escherichia coli*, *Staphylococcus aureus* bacteria and *Aspergillus niger*, *Aspergillus fumigatus* fungi species [11,12]. The stock solution of the test compound was prepared by dissolving 10mg of the test compound in 10 mL of DMF solvent. The stock solution suitably diluted with sterilized distilled water to get 12.5 and 25µg/mL. Control for each solution was prepared by diluting 10mL of solvent instead of stock solution with sterilized distilled water.

The bacteria were subcultured in agar medium. The petridishesh were incubated for 24h at 37°C. Standard antibacterial drug, *Gentamicin* was also screened under similar conditions for

comparison. The fungi were subcultured in potato-dextrose agar medium. The petridishesh were incubated for 48h at 37 °C. Standard antifungal drug *Fluconazole* was also screened under similar conditions for comparison. Activity was determined by measuring the diameter of the zone showing complete inhibition.

### 2.3. DNA cleavage experiment

All the metal complexes are screened for their DNA cleavage activity. DMSO is used as the solvent and stock solution of the complexes prepared is 10 mM. The Preparation of culture media, Isolation of DNA and analysis of the Cleavage products by the agarose gel electrophoresis is performed according to the method used in our earlier report [9].

## 3. RESULTS AND DISCUSSION

The physical appearance and analytical results show that the complexes of Co(II), Zn(II), Cd(II) and Hg(II) possess 1:1 stoichiometry whereas Cu(II) and Ni(II) possesses 1:2 stoichiometry (Table 1). Lower molar conductance values show that the complexes are non-electrolytes indicating coordination of the two chlorides [13,14]. All the complexes are light colored, stable, and non-hygroscopic in nature having high melting points. The complexes are soluble in DMF and DMSO.

### 3.1. Spectroscopic and magnetic studies:

#### 3.1.1. IR spectral studies

A comparative interpretation of IR spectral data (Table 2) suggests that ligand (L) acts as a bidentate ligand in the investigated complexes, using carbonyl oxygen and azomethine nitrogen as donor atoms. The IR spectrum of the ligand (L) shows the strong band at 3327  $cm^{-1}$  attributed to  $\nu$  (N-H) stretching vibrations of the  $NH_2$  group [15], which is unchanged in the complexes indicating non-involvement of the nitrogen atom of  $NH_2$  group. The band at 1658  $cm^{-1}$  is due to the  $\nu$  (C=N) stretch and this frequency shifted to a lower value in the complex confirming the involvement of the (C=N) in the coordination with the metal ions [16]. The stretching vibrational band of the carbonyl group of a ligand lies at 1682  $cm^{-1}$  frequency [17]. This band shifts to the lower frequency side in all the complexes and become weaker in some cases. This suggests the coordination of the C=O group with metal ions. Further the bonding is supported by the appearance of new bands in the 426-482, 329-389 regions are assigned to  $\nu$ (M-N) and  $\nu$ (M-O) vibrations respectively [15]. This indicates that carbonyl oxygen and azomethine nitrogen atoms are involved in coordination.

Table - 1: Elemental analysis and physical characteristics of Ligand [L] and its metal complexes

Empirical formula	Mol. Wt.	M.P(°C)	Yield (%)	Elemental analysis (%) found(calculated)					$\mu_{\text{eff}}$ B.M.	$\chi_M$ ohm <sup>-1</sup> cm <sup>2</sup> mole <sup>-1</sup>
				C	H	N	M	Cl		
[C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ]	389.2	220	63	53.50(58.62)	03.09(5.44)	07.80(7.20)	--	--	--	--
[Cu(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	913.0	>300	65	43.44(49.99)	4.35(4.64)	5.11(6.14)	6.81(6.96)	7.21(7.77)	1.87	15.07
[Ni(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	908.1	>300	66	49.88(50.26)	4.39(4.66)	6.11(6.17)	6.26(6.46)	7.60(7.81)	2.96	17.80
[Co(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ] <sub>n</sub>	516.9	>300	72	43.22(43.96)	3.91(4.08)	5.00(5.40)	11.22(11.35)	13.56(13.66)	4.86	14.60
[Zn(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	521.9	>300	75	43.38(43.42)	3.94(4.03)	5.21(5.33)	12.19(12.44)	13.36(13.49)	—	15.19
[Cd(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	571.9	>300	68	39.78(39.85)	3.65(3.70)	4.82(4.89)	19.58(19.63)	12.20(12.38)	----	14.32
[Hg(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	659.9	>300	82	34.10(34.54)	3.11(3.20)	4.11(4.24)	30.12(30.36)	10.58(10.73)	----	13.10

Table - 2: The important IR frequencies (cm<sup>-1</sup>) of ligand (L) and its metal complexes

Compound	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{M-N})$	$\nu(\text{M-O})$
[L](C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> )	1682	1658	-----	----
[Co(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ] <sub>n</sub>	1679	1599	426	372
[Ni(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	1670	1599	450	389
[Cu(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	1671	1606	471	386
[Zn(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	1670	1580	436	379
[Cd(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	1672	1575	482	329
[Hg(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	1676	1578	453	377

Table - 3: Electronic spectral bands and ligand field parameters of the Co(II), Ni(II) and Cu(II) complexes in DMF (10<sup>-3</sup>M) solution

Complexes	Transitions in cm <sup>-1</sup>			Dq (cm <sup>-1</sup> )	B' (cm <sup>-1</sup> )	$\beta$	$\beta\%$	$\nu_2/\nu_1$	LFSC (K.cal)
	$\nu_1^*$	$\nu_2$	$\nu_3$						
[Co(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ] <sub>n</sub>	7300	16150	20441	863.79	936.98	0.965	3.21	2.21	14.81
[Ni(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	9920	15821	25581	992.34	775.43	0.746	25.44	1.59	34.02
[Cu(C <sub>19</sub> H <sub>21</sub> BrN <sub>2</sub> O <sub>2</sub> ) <sub>2</sub> Cl <sub>2</sub> ]	13600 - 16350			1497.5	----	----	----	----	25.67

$\nu_1^*$  calculated values

### 3.1.2. Mass spectral studies

In the present investigation, the LC mass spectrum of Ligand (L) shows the formation of the molecular ion (M+1) peak at m/z 389.2 and it is in good agreement with its suggested empirical formula as indicated from elemental analyses (Table 1) which supports the formation of the ligand (L).

### 3.1.3. Magnetic properties

Magnetic susceptibility measurements at the room temperature exhibits paramagnetism for Co(II), Ni(II) and Cu(II) complexes and they are listed in table 1. The cobalt complex exhibit magnetic moment value of 4.86 B.M. suggesting octahedral geometry [18,19]. The Ni (II) complex shows the magnetic moment value 2.96 B. M. slightly higher than spin only value (2.83 B.M), indicating an octahedral environment. The

observed magnetic moment value of the Cu (II) complex is 1.87 B.M. suggesting a distorted octahedral geometry [20].

### 3.1.4. Electronic spectral studies

The electronic spectra of the Co (II), Ni (II) and Cu (II) complexes were recorded for freshly prepared solution in DMF (10<sup>-3</sup> M) at room temperature Table 3. The electronic spectra of the Co (II) complex show two bands at 16150 and 20441cm<sup>-1</sup>. These two bands are assigned to <sup>4</sup>T<sub>1g</sub> (F) → <sup>4</sup>A<sub>2g</sub> (F) ( $\nu_2$ ) and <sup>4</sup>T<sub>1g</sub> (F) → <sup>4</sup>T<sub>2g</sub> (P) ( $\nu_3$ ) transitions, respectively in an octahedral environment [21]. The  $\nu_1$  band could not be observed, however, it can be calculated using an equation suggested by Underhill and Billing [22].

The Ni (II) complex exhibited two absorption bands, at 15821 and 25581 cm<sup>-1</sup> assignable to <sup>3</sup>A<sub>2g</sub> (F) → <sup>3</sup>T<sub>1g</sub> (F) ( $\nu_2$ ) and <sup>3</sup>A<sub>2g</sub> (F) → <sup>3</sup>T<sub>1g</sub> (P) ( $\nu_3$ ) transitions, respectively in an

octahedral environment. The  $\nu_1$  could not be observed due to limited range of the instrument, but was calculated by using a band fitting procedure [22]. The copper complex exhibit broad asymmetric band in the region 13600- 16350  $\text{cm}^{-1}$ . The broadness of the band indicates the three transitions  ${}^2B_{1g}(F) \rightarrow {}^2A_{1g}(\nu_1)$ ,  ${}^2B_{1g}(F) \rightarrow {}^2B_{2g}(\nu_2)$  and  ${}^2B_{1g}(F) \rightarrow {}^2E_g(\nu_3)$  which are similar in energy and gives rise to only one broad band. The broadness of the band may be due to Jahn-Teller distortion. All the data suggest the distorted octahedral geometry around Cu (II).

The octahedral geometry [23] is further supported by the values of ligand field parameters such as Racah inter-electronic repulsion parameter (B), ligand field splitting energy (10Dq), covalency factor ( $\beta$ ) and ligand field stabilization energy (LFSC) [24]. The 'B' values for the complex are lower than free ion values, which is an indication of orbital overlap and delocalization of d-orbitals. The  $\beta$  values obtained are less than unity suggesting a considerable amount of covalent character of the metal-ligand bonds. The  $\beta$  value of Ni (II) complex is less than Co (II) complex, indicating more covalency of M-L[25].

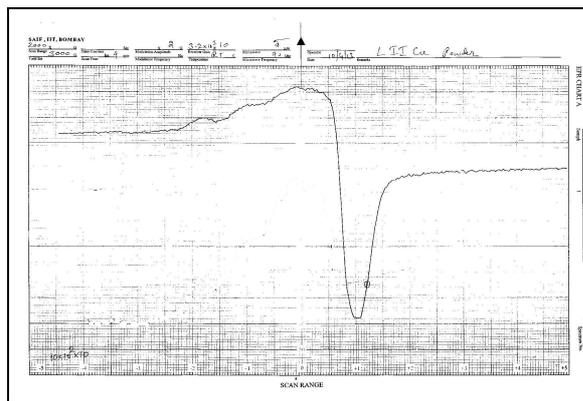


Figure - 2: ESR spectrum of Cu(II) complex of the ligand (L).

### 3.1.5. ESR spectral studies

The ESR spectrum of Cu(II) (Figure 2) complex at room temperature displayed a poorly resolved broad asymmetric signal in the Hamiltonian parameter range  $g_{||} = 2.3811$  and  $g_{\perp} = 2.0599$ . These values are in agreement with values for distorted octahedral structure with no hyperfine splitting in the parallel features. The exchange interaction parameter,  $G = (g_{||} - 2.00277) / (g_{\perp} - 2.00277) = 6.62$ , which is more than four, suggesting that there is negligible or no interaction between the copper centers [26]. Thus suggesting distorted octahedral geometry for the Cu(II) complex.

## 3.2. Biological activity screening

### 3.2.1. Antibacterial and Antifungal activity

All the metal complexes and the ligand (L) were screened against selected bacteria and fungi (Figure 3 and 4) to examine their anti-bacterial and anti-fungal activities. The susceptibility of the bacteria and fungi towards complex compounds were tested by measuring zone of inhibition (in mm) and compared with the parent ligand (L) with a concentration of 25  $\mu\text{g}/\text{mL}$  and 12.5  $\mu\text{g}/\text{mL}$ .

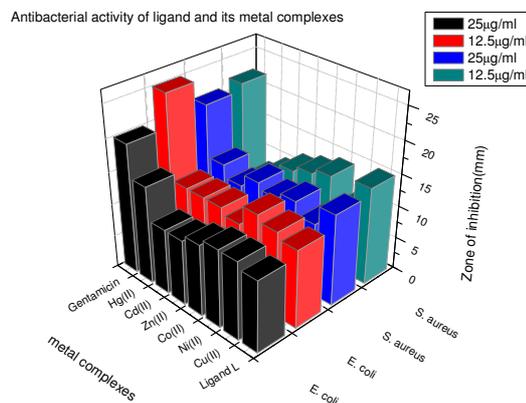


Figure - 3: Antibacterial activity of the Schiff base [L] and its metal complexes.

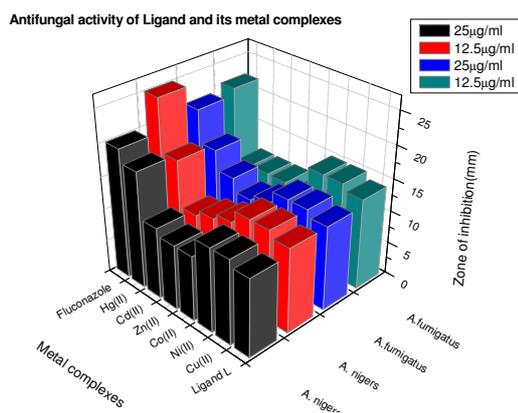


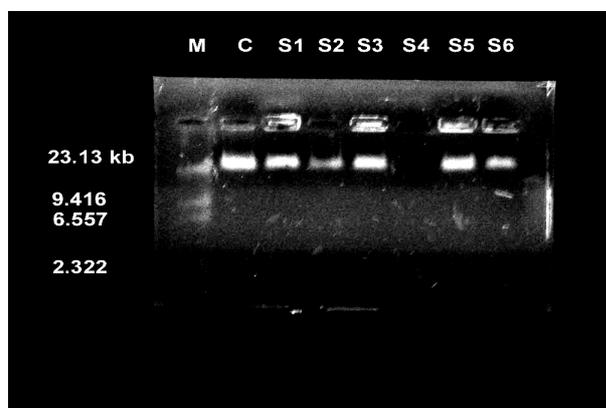
Figure - 4: Antifungal activity of the Schiff base [L] and its metal complexes.

All the complexes shows medium sensitivity against both bacteria and fungi species. The obtained results compared with the standard drugs *Gentamicin* and *Fluconazole* as antibacterial and anti-fungal drugs, respectively. In general, metal complexes are moderately more active than the legands because metal complexes may serve as a vehicle for activation of legands as the principle cytotoxic species [27-29].

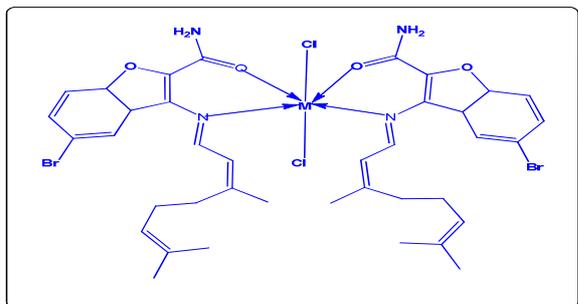
### 3.2.2. DNA Cleavage analysis

The Schiff base metal complexes are subjected to DNA cleavage analysis by agarose gel electrophoretic method and represented in the figure 5. The gel after the electrophoresis clearly revealed that the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The gel after electrophoresis

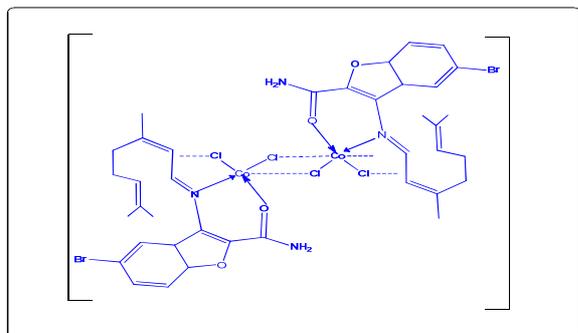
clearly revealed that, the intensity of all the treated DNA samples has diminished, possibly because of the cleavage of the DNA. The difference was observed in the bands of the complexes (lanes S1 – S6) compared to that of the control DNA of *S. aureus*. This shows that the control DNA alone does not show any apparent cleavage whereas complexes show. The complete cleavage of DNA is seen for Zn(II) complex (lane S4) and partial cleavage of DNA was observed for Ni(II) and Hg(II) complexes of the Schiff base [L] (lanes S2 and S6). The results indicate the important role of metal ions in these isolated DNA cleavage reactions. As the compound observed to cleave the DNA, it can be, concluded that the compound inhibits the growth of the pathogenic organism by cleaving the bacterial genome [29].



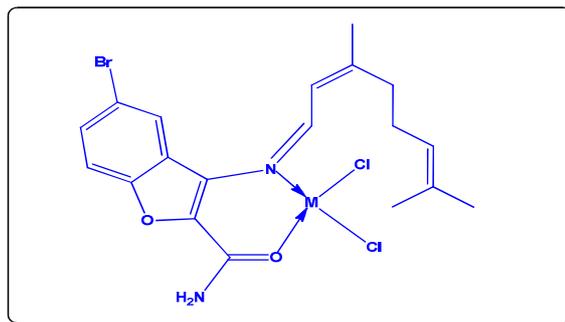
**Figure - 5:** DNA cleavage activity of Co(II), Ni(II), Cu(II), Zn(II), Cd(II) and Hg(II) Complexes of Schiff base [L] (S1 – S6).



**Figure - 6A:** M = Cu(II) or Ni(II).



**Figure - 6B:** Co(II) complex.



**Figure - 6C:** M= Zn(II) or Cd(II) or Hg(II) complex.

#### 4. CONCLUSION

On the basis of above results we conclude that the newly synthesized Schiff base acts as a neutral bidentate ligand coordinating through the oxygen of the amide group and nitrogen of the azomethine group. Analytical and spectral investigation reveals probable structures for the complexes as dimeric octahedral to Cu(II) and Ni(II) (Fig.6.A) complexes and polymeric octahedral to Co(II) (Fig.6.B) and tetrahedral to Co(II), Zn(II), Cd(II) and Hg(II) complexes (Fig.6C). DNA cleavage studies show that the complete cleavage of DNA is seen for Zn(II) complex and partial cleavage of DNA was observed for Ni(II) and Hg(II) complexes of the Schiff base [L].

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