Spectroscopic and DNA Binding Studies on a Novel Water Soluble Binuclear Copper (II) Schiff base Complex

Wahengbam Bembee Devi[1], R. K. Bhubon Singh[2]

[1]Department of Chemistry, Manipur University, Imphal-795003, Manipur, India
wbembee@yahoo.com

[2]Department of Chemistry, Manipur University, Imphal-795003, Manipur, India
bhubonsingh@gmail.com

Abstract: ***A novel water soluble binuclear copper (II) Schiff base complex have been synthesized by template condensation reaction method using 1, 2-diamino-2-methylpropane, furfural and copper (II) nitrate trihydrate in methanol. The newly prepared complex was characterized by elemental analyses, FT-IR, electronic spectra (UV-Vis) and thermogravimetric analysis techniques and was found to be water soluble. EPR study in room temperature as well as in frozen (77 K) dmso solution clearly indicates the complex is binuclear in nature. A DNA binding study by spectroscopic, cyclic voltammetry and viscosity measurement studies suggests the said complex is an avid binder to Calf Thymus DNA.***

Keywords: water soluble, Schiff base cooper (II) complex, EPR, DNA binding.

1. Introduction

Schiff bases are organic compounds formed by the condensation of a primary amine with an aldehyde or ketone. They were characterized by presence of carbon-nitrogen double bond (C=N) functional group and are related to potential biological and pharmacological compounds having anticancer, anti-tubercular, anti-inflammatory and antimicrobial activities [1]-[3]. Their biological properties can be altered depending on the presence of different substituent groups present on the parent structural unit or by complexation with variable metal ions. The coordination chemistry of Schiff bases has undergone noticeable development due to ease of preparation, good chelating property etc. Water soluble Schiff base complexes are rare [4]. The objective of the present work is to synthesize and characterize novel water soluble copper (II) Schiff base complex in good yield using lesser amount of solvents.

2. Experimental

All the chemicals were of analytical reagent grade and used as received without further purifications. Elemental analyses were carried out in Perkin Elmer 2400 Series II CHN analyzer. Infrared spectra were recorded in Shimadzu FTIR-8400 spectrometer in solid using KBr discs. Electronic spectra were recorded in Perkin-Elmer Lamda 35 UV/VIS Spectrometer. Thermal analyses were carried out on a Perkin Elmer STA 6000 Thermal Analyzer at 10ºC min^{-1} within the temperature range 25-900ºC under nitrogen atmosphere. EPR spectra were recorded using JEOL, JES-FA200 ESR spectrometer.

2.1. Synthesis of the complex, $[Cu_2(NO_3)_2(Nfmmpd)_2]$ (1)

The reaction scheme for the synthesis of the complex is summarized in Figure 1. Experimental details were given below:

To 5 ml methanolic solution of 1, 2-diamino-2-methylpropane (0.2 ml, 2 mmol), furfural in 5 ml methanol (0.16 ml, 2 mmol) was added drop by drop and the content was stirred for about 1 hour, till colour of the solution changes to yellow. To this precursor solution 10 ml methanolic solution of $Cu(NO_3)_2$. $3H_2O$ (482 mg, 2mmol) was added slowly for about 1 hour and the reaction mixture was stirred magnetically till colour changes ceases. Blue coloured, microcrystalline complex was obtained on slow evaporation of the reaction mixture. Yield: 370 mg (≈ 85%). Anal.Calc. for $C_{18}H_{28}N_8O_{14}Cu_2$ (Mr. 706.55 g mol^{-1}): C, 30.56; H, 3.99; N, 15.84. Found: C, 30.50; H, 3.98; N, 15.77. IR bands (ν in cm^{-1}): 3257, 3134, 2979, 1747, 1641, 1604, 1566, 1465, 1377, 1332, 1261, 1145, 1120, 1093, 1026, 966, 941, 883, 827, 800, 861, 767, 684, 594, 507, 460, and 433.

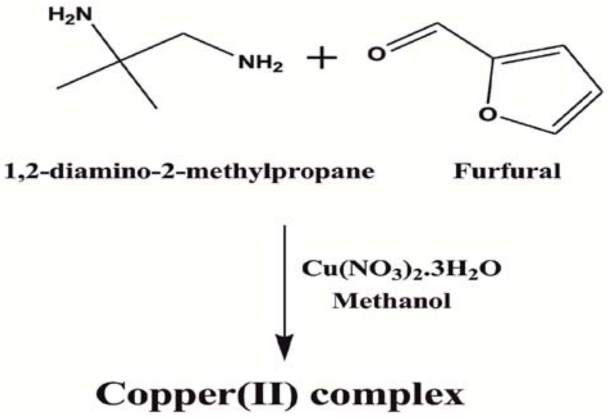

Figure 1: Reaction scheme for the synthesis of the complex

3. Results and Discussions

In the present work, a template synthesis method for the preparation of the binuclear copper (II) Schiff base complex is described in high yield using lesser amount of solvent. The prepared complexes were found to be soluble in water, methanol, ethanol, DMSO, DMF, acetonitrile etc. Analytical and spectroscopic analysis revealed that the complex was 1:1 (metal-ligand) stoichiometry.

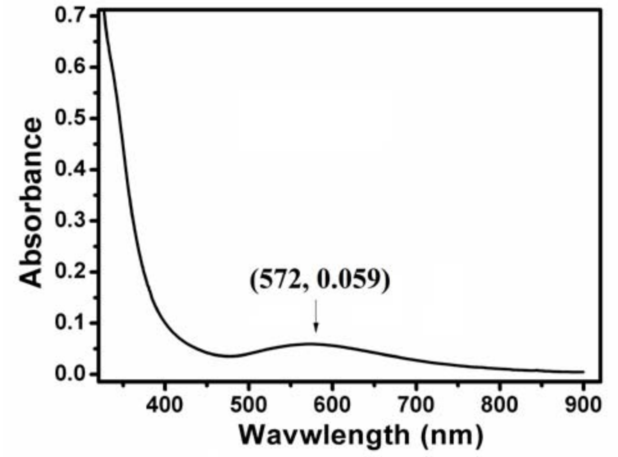

Figure 2: Electronic spectra of the complex in DMSO

The electronic spectra of the complex (Figure 2) were recorded in DMSO at a concentration of about 1x10^{-4} M, which shows a weak broad absorption band *ca*. 578 nm with a weak shoulder bands around 340 nm , assignable to the $_2T_{2g} \rightarrow _2E_g$ transition, suggesting an octahedral geometry around the Cu(II) ion [5]. In the FT-IR spectra of the complex, the azomethine ν(C=N) band was observed at 1641 cm^{-1}, which is an indication of Schiff base formation [6], [7]. The ν(Cu-N) and ν(Cu-O) vibration modes were assigned to bands at 507 and 460 cm^{-1}, respectively. According to Nakamoto et.al., [8], the bridging bidentate NO_3 stretching were observed at 1519, 1291 and 1008 cm^{-1} for Cu(II) complexes and the newly prepared copper(II) complex shows IR bands at ca. 1535, 1261 and 1026 cm^{-1} assignable to NO_3 bidentate bridging mode.

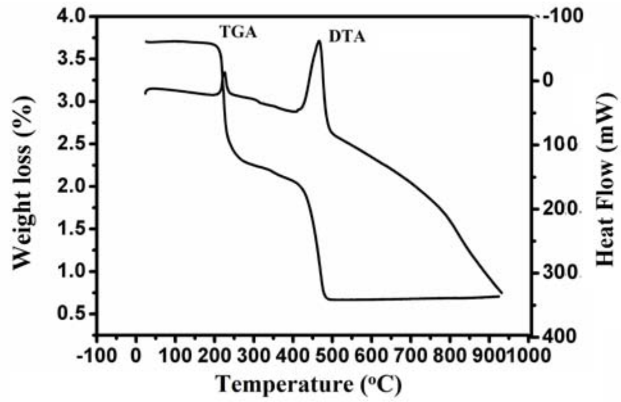

Figure 3: Thermogravimetric curves of the complex

In the thermal analysis data of the complex, loss of ligand molecules was started *ca.* 150°C and was completed at 510°C with respective endothermic peaks at 225 and 464°C, Figure 3. There is no indication of presence of lattice or coordinated water molecules and the formation of the complex was thermodynamically allowed.

EPR spectra of the complex at room temperature as well as in frozen (77 K) DMSO solution are shown in Figure 4. The room temperature epr spectrum of the complex (Figure 4 (a)) consists of a weak signal at g = 4.2561, with unresolved hyperfine coupling at the half field (H ≈ 1600 G). The weak signal corresponds to the forbidden transition (Ms = ± 2) among the S = 1 coupled states, whereas signal observed around g = 2.0000 region correspond to the allowed fine structure transitions (Ms = ± 1). The epr spectrum of the complex dissolved in DMSO at 77 K (frozen complex, Figure 4 (b)) showed anisotropic spectrum. The displayed spectra suggest the complex structure is dissociated in DMSO solution presumably due to coordination by the solvent molecules [9], [10]. The $A_{\parallel}$ (G), $g_{\parallel}$, $g_{\perp}$ and g_{av} values were found to be 190 G, 2.3466, 2.0580 and 1.4282 respectively and are comparable to those other reported binuclear copper(II) complexes [11]. The room temperature magnetic moment value of the complex was found to be 1.65 B.M which is lower than 1.73 (S= 1) value, indicating antiferromagnetic coupling between the two Cu(II) ions [12].

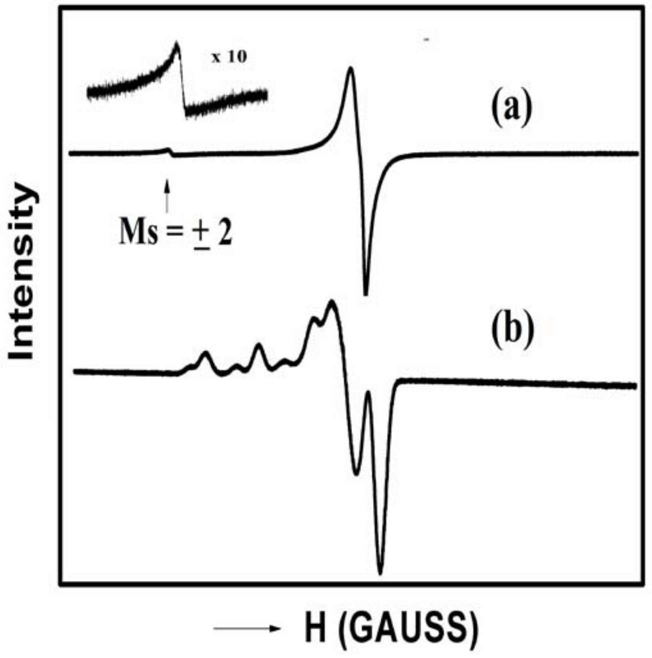

Figure 4: EPR spectra of the complex in (a) room temperature solied; (b) frozen (77 K) DMSO solution

A DNA binding study with the newly prepared copper(II) complex was carried out by monitoring electronic absorption titrations, a thermal behaviour study, cyclic voltametry and viscosity measurement methods. Electronic absorption titrations were carried out on a Perkin Elmer UV-vis Lambda 35 spectrophotometer. By maintaining a constant concentration of the complex (approximately 1x10^{-4} M) and varying the concentration of the CT-DNA (1.3 to 5.3x10^{-4} M) solution added, the absorbance for each addition of the DNA concentration was then recorded. The intrinsic binding constant was determined from the absorption spectral data [13] by application of the equation,

$$[DNA]/ (\varepsilon_a - \varepsilon_f) = [DNA]/ (\varepsilon_b - \varepsilon_f) + 1/ K_b (\varepsilon_b - \varepsilon_f) \quad (1)$$

Where, ε_a, ε_f and ε_b correspond to extinction coefficients of the apparent, free and bound metal complexes, respectively. [DNA] is the molar concentration of DNA and was measured by electronic absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 258 nm. The solution of CT-DNA was prepared by using a 5mM, tris-HCl buffer (pH = 7.1).

The ratio of UV-absorbance at 260 nm and 280 nm, A_{260}/A_{280}, was found to be *ca.*1.9, indicating that the DNA was satisfactorily free of protein [14]. A plot of $[DNA]/(\varepsilon_b-\varepsilon_f)$ *vs*. [DNA] was plotted with K_b calculated by a ratio of the slope to the y-intercept. The absorption spectrum of the complex in presence of increasing concentration of the CT-DNA (1.3, 2.0, 2.6, 3.3, 4.0, 4.6, 5.3x 10^{-4} M) showed a considerable amount of hyperchromism with a slight red shift (Figure 5). The intrinsic binding constant (K_b) of the complex was found to be $7.72x10^5$ M^{-1}.

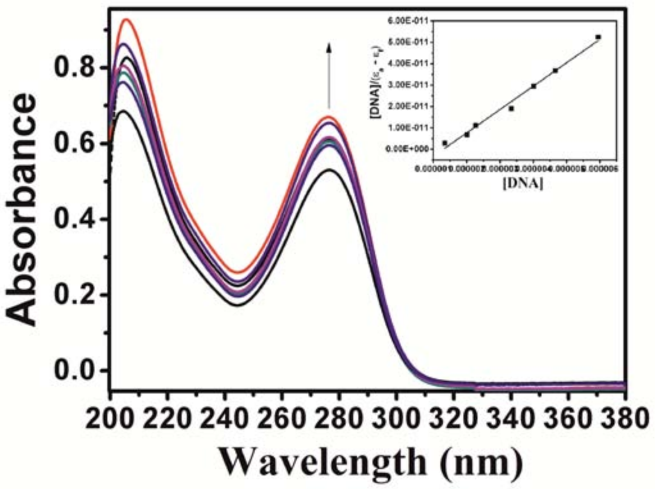

Figure 5: Absorption spectra of the complex in absence and presence of different concentrations of CT-DNA. The arrow indicates the effect of DNA on absorption curve of the respective compounds. Inset plot of $[DNA]/(\varepsilon_a-\varepsilon_f)$ vs. [DNA] and a linear fit of the titration curve.

A thermal behaviour study of DNA was performed by measuring the absorption intensity of CT-DNA at 258 nm at a temperature range 25-100^{0}C, both in the absence and presence of the complex. The thermal denaturation behaviour in the presence of the ligand and complexes provides insight into conformational changes when the temperature is raised. The strength of interaction between the complex and the DNA can also be monitored. As the temperature of the solution increases, the double stranded DNA tends to dissociate into a single strand thereby displaying a hyperchromic effect on the absorption spectra of DNA, which was observed. The DNA melting temperature, T_m, where half of the total base pairs becomes non-bonded, was determined experimentally and found to be 76^{0}C which increases to 78, 80 and 81^{0}C for the complex with increasing ratio of DNA to compound concentrations (0, 0.02, 0.05 and 0.10), respectively, which indicates the prepared complex might have intercalative mode of DNA binding with CT-DNA [15].

The cyclic voltammogram (CV) of the complex was recorded in absence and presence of the CT-DNA concentrations in the range -1.0 to 1.0 V. In presence of variable concentrations of the CT-DNA (20-80 µl), the cyclic voltammogram of the complexes shows a minor but significant anodic and cathodic shift with a change in the current peaks respectively (Table 1). The observed changes in the peak currents of the complex in presence of CT-DNA may be the result of slow diffusion of the complex to the DNA molecules, forming an equilibrium mixture of the free and DNA-bound complex to the electrode surface [16]. As a complement to the absorption spectral titrations, emission spectroscopy and a DNA melting temperature experiments, the CV data suggests that the respective complexes bind the DNA helix.

Table 1: Cyclic voltammetric data of complex in the absence and presence of CT-DNA

CT-DNA concentration (µl)	*Complex*					
	E_{pa}(V)	*E_{pc} (V)*	*ΔE (V)*	*I_{pc} (1x 10^{-5}A)*	*I_{pa} (1x 10^{-5}A)*	*I_{pc}/I_{pa}*
0	-0.069	-0.587	0.517	-0.723	1.058	0.683
20	-0.093	-0.636	0.543	-1.193	1.022	1.167
40	-0.136	-0.625	0.488	-1.072	1.031	1.039
60	-0.184	-0.610	0.426	-1.062	0.962	1.104

In order to further clarify the DNA interaction mode, viscosity measurements have been carried out (on an Ostwald's viscometer at 25±0.1^{0}C) with CT-DNA (37 µM) by using variable concentrations of the complex (1.26-6.25 µM). Flow time was measured with a digital stop watch and each sample was measured at least three times and an average flow time was calculated and the data were presented as $(\eta/\eta_o)^{1/3}$vs. [Q]/[DNA], where, η = viscosity of the DNA in presence of the ligand and complexes; η_o= viscosity of DNA alone; [Q] = concentrations of the free ligand and its complexes. Hydrodynamic measurements such as viscosity measurements which are sensitive to length increase are regarded as the least ambiguous and the most critical tests of binding in solution in the absence of crystallographic structural data [17]. In classical intercalation, the DNA helix lengthens as base pairs are separated to accommodate the bound adducts leading to increased DNA viscosity whereas a partial non-classical intercalation causes a bend in the DNA helix reducing its effective length and its viscosity. The effect of complex on the relative viscosity of CT-DNA was shown in Figure 6.

It has been observed that on addition of complex, the relative viscosity increases steadily, which suggests that the newly prepared complex was bound to CT-DNA by intercalative mode of binding [14]. The said results were consistent with our spectroscopic and cyclic voltametry measurements.

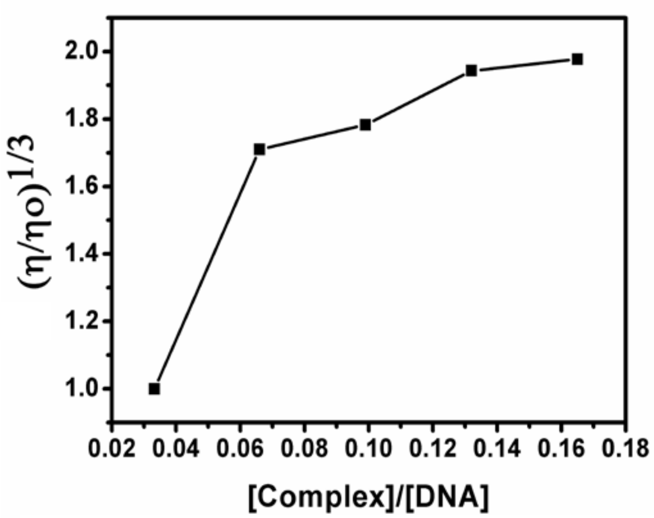

Figure 6: Effect of increasing amount of the complex on the relative viscosity of CT-DNA in a Tris- HCl buffer. [DNA] = 37 µM, [Complex] = 1.26-6.25 µM.

4. Conclusion

The present work focuses on synthesis, characterization and spectroscopic studies on a novel binuclear water soluble copper (II) Schiff base complex from 1,2-diamino-2-methylpropane and furfural in good yield. Water soluble binuclear Schiff base complexes are very rare. The preparation of such type of complexes should be encouraged to study the role of chemical compounds in chemistry, medicine and biology.

5. Acknowledgement

The financial assistance provided by CSIR, New Delhi is highly acknowledged.

References

[1] R. Villar, I. Encio, M. Migliaccio, M. G. Gil, V. Martinez-Merino, "Synthesis and cytotoxic activity of lipophilic sulphonamide derivatives of the benzo[b]thiophene 1,1-dioxide," Bioorg. Med. Chem.12, pp. 963-968, 2004.
[2] G.G. Mohamed, M.S. Carmen, "Metal complexes of Schiff base derived from sulphametrole and o-vanilin: Synthesis, spectral, thermal characterization and biological activity," Spectrochim. Acta A 66, pp. 949-958, 2007.
[3] C.V. Sastri, D. Eswaramoorthy, L. Giribabu, B. G. Maiya, "DNA interactions of new mixed-ligand complexes of cobalt(III) and nickel(II) that incorporate modified phenanthroline ligands," J. Inorg. Biochem. 94, pp. 38-145, 2003.
[4] B. K. Singh, D. Adhikari, "Complexation Behaviour Of Schiff Base Ligands With Transition MetalL Ions," International Journal of Basic and Applied Chemical Sciences 2, pp. 84-107, 2012.
[5] M. Khedr, H. M. Marwani, "Synthesis, Spectral, Thermal Analyses and Molecular Modeling of Bioactive Cu(II)-complexes with 1,3,4-thiadiazole Schiff Base Derivatives. Their Catalytic Effect on the Cathodic Reduction of Oxygen," Int. J. Electrochem. Sci., 7, pp. 10074 – 10093, 2012.
[6] W. Bembee Devi, R. K. Bhubon Singh, "Syntheses, Characterization and Spectroscopic Studies on Schiff base Complexes of Diaminomaleonitrile," International Journal of Science and Research. 2, pp. 312-318, 2013.
[7] R. Vafazadeh, M. Kashfi, "Kinetics of the Ligand Exchange Reaction Between Tetradentate Schiff Base N,N'-ethylen-bis (salicylaldimine)and Cu(N,N'-propylen-bis(salicylaldimine))," Acta Chim. Slov. 57, pp. 310–317, 2010.
[8] K. Nakamoto, "Infrared and Raman Spectra of Inorganic and Coordination Compounds," 3rd edn.John Wiley & Sons, U. S. A, 1977.
[9] C. P. Pradeep, P. S. Zacharias, S. K. Das, Synthesis and characterization of a chiral dimeric copper(II) complex: Crystal structure of [Cu2(m-Cl)2(HL)2]×H2O(H2L = S-(-)-2-[(2-hydroxy-1-phenyl-ethylimino)-methyl]-phenol), J. Chem. Sci., 117, pp. 133–137, 2005.
[10] O. I. Singh, M. Damayanti, N. Rajen Singh, R. K. Hemakumar Singh, M. Mohapatra, R. M. Kadam, "Synthesis, EPR and biological activities of bis(1-n-butylamidino-O-alkylurea)copper(II)chloride complexes: EPR evidence for binuclear complexes in frozen DMF solution,"Polyhedron 24, pp. 909-916, 2005.
[11] L. Jaideva Singh, N. Shantibala Devi, S. Pramodini Devi, W. Bembee Devi, R.K. Hemakumar Singh, B. Rajeswari, R.M. Kadam, "Spectroscopic studies on bis(1-amidino-O-alkylurea) copper(II) sulphate complexes where alkyl = methyl, ethyl, n-propyl or n-butyl: EPR evidence for binuclear complexes," Inorg. Chem. Comm. 13, pp. 365-368, 2010.
[12] R. L. Dutta, A. Syamal, Elements of Magnetochemistry, 2nd edn. East-West Press, New Delhi, 1993.
[13] A. Wolfe, G. H. Shimmer, T. Meehan, Polycyclic aromatic hydrocarbons physically intercalate into duplex regions of denatured DNA, Biochemistry 26, pp. 6392-6396, 1987.
[14] M.E. Reichman, S.A. Rice, C.A. Thomas, P. Doty, A Further Examination of the Molecular Weight and Size of Desoxypentose Nucleic Acid, J. Am. Chem. Soc. 76, pp. 3047-3053, 1954.
[15] W. Bembee Devi, R. K. Bhubon Singh, J. P.Jasinski, J.A. Golen, A new two dimensional copper(II) coordination complex with sulphonamide: Synthesis, Crystal Structure and DNA Binding Study, Inorg. Chem. Comm. 21, pp. 163-167, 2012.
[16] C. Moucheron, A. K.-D. Mesmaeker, S. Choua, Photo physics of Ru(phen)2(PHEHAT)2+: A Novel "Light Switch" for DNA and Photo-oxidant for Mononucleotides, Inorg. Chem. 36, pp. 584-592, 1997.
[17] S. Satyanarayana, J.C. Dabrowiak, J.B. Chaires, Neither delta□ nor lambdatris(phenanthroline)ruthenium(II) binds to DNA by classical intercalation, Biochemistry 31, pp. 9319–9324, 1992.

Author Profile

Wahengbam Bembee Devi received the B.Sc. and M.Sc. degrees in Chemistry from Manipur University in 2004 and 2008, respectively. She is now working as a Research Scholar at Department of Chemistry, Manipur University, Imphal, Manipur, India. Her research interests are in the fields of Inorganic synthesis, Coordination Chemistry, Spectroscopic Analysis and Bioinorganic Chemistry.

Dr. Bhubon Singh did his M. Sc, M. Phil and Ph. D. from Manipur University, Canchipur Imphal in the year 1986, 1989 and 1992 respectively. He joined Biramangol College Sawombung, Imphal as a lecturer in 1991, then worked as a Reader at the Department of Chemistry, Nagaland University from1999- 2007. He is currently working as an Associate Professor at Department of Chemistry, Manipur University. His research interests are in the field of Themochemistry, Supramolecular and Bioinorganic Chemistry, and magneto-structural study of coordination complexes

.