



Polarographic Determination and Antimicrobial Activity of Cu(II) Complex with 4-Chlorobenzylidene-4-aminoacetanilide

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Abstract: The electrochemical behaviour of the complex of Cu(II) with 4-chlorobenzylidene-4-aminoacetanilide (CAA) was studied. It was observed that CAA forms 1:1 complex with Cu(II) in between pH 6.5 to 7.1. It was found that the reduction process of Cu(II)-CAA complex is two electron reversible reduction process at D.M.E. The logarithm value of stability constant of Cu(II)-4-Chlorobenzylidene-4-aminoacetanilide (CAA) complex has been found to be 4.85. The redox properties of the complex was extensively investigated by electrochemical method using cyclic voltammetry (CV). The Cu(II) complexes exhibited quasi-reversible single electron transfer process. The Schiff base and its complex has been screened for their *in-vitro* antibacterial (*Escherichiacoli*, *Staphylococcus aureus*, *Vibrio cholerae* and *Bacillus subtilis*) and antifungal (*Aspergillus niger* and *Penicillium lilacinum*) activities by minimum inhibitory concentration (MIC) method.

Keywords: 4-Chlorobenzaldehyde, Polarography, Cu(II)-CAA complex, MIC.

Introduction

The coordination chemistry of the metal complexes has been widely studied because of their interesting pharmacological properties specifically the coordination chemistry of metal complexes of Schiff bases have been studied extensively¹⁻⁵ owing to their adequate flexibility and enough selectivity towards the central metal atom. In recent years the electrochemical techniques have led to the advancement in the field of analysis because of their sensitivity, low cost and relatively short analysis time when compared with other techniques. Additional application of electroanalytical techniques includes the determination of reaction mechanisms. Redox properties of a drug can give insights into its metabolic fate or pharmaceutical activity⁶⁻⁸. Thus, the aim of present work is to synthesize and characterize Cu(II) metal complex with newly synthesized Schiff base derived from 4-Chlorobenzaldehyde and 4-aminoacetanilide (CAA), possessing donor sites of carbonyl oxygen and azomethine nitrogen. The complex formation of Cu(II) with 4-Chlorobenzylidene-4-

aminoacetanilide (CAA) has been studied polarographically at D.M.E in aqueous/alcoholic medium. Overall stability constant of Cu(II)-CAA complex was determined. The electron transfer mechanism of the metal complex was investigated by the aid of cyclic voltammetry. The Schiff base and its metal complexes were screened for their antimicrobial activity. The study has been extended to compare action of ligand *v/s* its Cu(II) complex and thus an overall mechanism of drug action has been attempted.

Experimental

Carbon, hydrogen and nitrogen were estimated by using Elemental Analyzer Carlo Erba EA1108 analyzer. FTIR spectral analysis was carried out on a Shimadzu Corporation FTIR-spectrometer, model-8400S in KBr disc and electronic spectra (in MeOH) were recorded on Perkin-Elmer Spectrophotometer at Department of Chemistry at Sagar University, Madhya Pradesh, India. Molar conductivity measurements were recorded at room temperature by ELICO-CM-82 T Conductivity Bridge with a cell having cell constant 0.51 and magnetic moment was carried out by Gouy's method. Electrochemical behavior of the metal complexes was investigated by Ω Metrohm 797 VA Computrace (ion analyzer, Switzerland.) through electrochemical software version 3.1. A three-electrode cell was employed incorporating a dropping mercury electrode as working electrode, an Ag/AgCl (saturated KCl) reference electrode and a platinum wire counter electrode. Mass transport was achieved with a teflon-coated bar at approximately 400 rpm using a magnetic stirrer (KIKA Labortechnik, Germany). A systronics digital μ pH meter model-361 was used for pH measurements. All experiments were performed at room temperature and dissolved oxygen was removed by passing pure nitrogen through the solutions. Metal solution (0.01M) was prepared using $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ and ligand solution was prepared by dissolving CAA (0.01 M) in ethanol containing citric acid and Na_2HPO_4 solution as buffer to maintain pH. Ionic strength was kept constant by using KCl as supporting electrolyte and gelatin (0.002%) was used as maximum suppressor. The Polarographic study of Cu(II)-CAA has been done at D.M.E in aqueous/ethanolic medium. ESR spectra were recorded at RSIC, I.I.T, Mumbai on an X-band at a frequency of 9.07 GHz at a magnetic field strength of 2000 ± 4000 Gauss at a temperature of 300 K.

Materials

All the chemicals used were of reagent grade.

Synthesis of 4-Chlorobenzylidene-4-aminoacetanilide (CAA)

The ligand 4-Chlorobenzylidene-4-aminoacetanilide (CAA) has been synthesized by refluxing together the 4-chlorobenzaldehyde and 4-aminoacetanilide in methanol in equimolar ratio. The reaction mixture was refluxed on a waterbath for about 5-7 h. The coloured condensation product was re-crystallized with methanol. Purity was monitored by TLC using silicagel.

Synthesis of Cu(II)-4-Chlorobenzylidene-4-aminoacetanilide (CAA) complex

The metal complex has been synthesized by refluxing the methanolic solution of the copper salt CuCl_2 (0.01 mole) with the methanolic solution of the Schiff base (CAA) in 1:1 ratio on a water bath for 3-5 h. The refluxate was kept overnight. The coloured precipitate that appeared on cooling the solution, was washed with methanol and petroleum ether, successfully. The resulting metal complex was re-crystallized and then dried under reduced pressure over anhydrous CaCl_2 in a desiccator. The complex is air stable and slightly soluble in ethanol, methanol and acetone.

In vitro antibacterial and antifungal assay

The biological activities of synthesized Schiff base and its Cu(II) metal complex have been studied for their antibacterial and antifungal activities by agar and potato dextrose agar diffusion method respectively. The antibacterial and antifungal activities were done at 100, 200 and 500 µg/mL concentrations in DMF solvent by using four bacteria (*E. coli*, *S. aureus*, *V. cholerae* and *Bacillus subtilis*) and two fungi (*A. niger* and *P.liliacinum*) by the minimum inhibitory concentration (MIC) method⁹. These bacterial strains were incubated for 24 h at 37 °C and fungi strains were incubated for 48 h at 37 °C. Standard antibacterial (Streptomycin) and antifungal drug (*Nyastatin*) was used for comparison under similar conditions. Activity was determined by measuring the diameter of the zone showing complete inhibition (mm).

Results and Discussion

The formation of complex of CuCl₂.2H₂O with Schiff base in MeOH is presented in the following reaction.



where in L acts as a bidentate ligand. The metal complex is stable and hygroscopic in nature. The complex is insoluble in common organic solvents but soluble in DMF and DMSO. The elemental analyses shows that, the Cu(II) complex has 1:1 stoichiometry of the type ML₂Cl₂. The molar conductance values are too low to account for any dissociation of the complex in DMF, indicating the non-electrolytic nature of the complex in DMF (Table 1).

Table 1. Analytical and physical data of ligand and metal comple.

Mol.& Empirical Formula of compound	Colour	Mol.Weight/ M.P, °C	Yield %	Ω, ohm ¹ cm ³ mol ⁻¹	μ _m (B.M)
CAAC ₁₁ H ₁₁ N ₃ ClO	Brown	272.7(166)	79	-	-
[Cu(CAA)Cl ₂ (H ₂ O)]	Black	886.4(328)	77	57.3	2.02

Spectral studies

A sharp band at 1625 cm⁻¹ in the IR spectrum of the Schiff base ligand (>C=N) shifts downward by about 10-15 cm⁻¹ in the complex indicating coordination through azomethine nitrogen¹⁰. The band in Schiff base ligand at 1650 cm⁻¹ due to C=O vibration, appears at 1635±5 cm⁻¹ in the complex. This shows chelation of C=O group with metal ion. The spectra of complex exhibited new bands around 3445±20 cm⁻¹ and 755±10 cm⁻¹, which are assignable to stretching and rocking mode of water molecules¹¹. The new bands in the spectra of complex at 520±5 cm⁻¹ and 460±10 cm⁻¹ may be due to ν M-O and ν M-N vibrations, respectively¹².

Electronic, ESR spectral and magnetic studies

The electronic spectra of Cu^{II} Complex gives a band at 12295 cm⁻¹. The nature of the band corresponds to transition ²E_v-²T_{2v}. The values of ligand field parameters 10Dq, λ and LFSE comes to be 12295 cm⁻¹, (-) 731 and 146.83kJmol⁻¹, respectively. This favours an octahedral geometry of the complex^{13,14}. The values of ESR parameters viz. g_{II}, g, g_{av}, G and Δg are 2.1934, 2.1353, 2.1546, 1.4358 and 0.0581 for [Cu(CAA)Cl₂(H₂O)] complex. The magnetic

moment of Cu(II) complex is expected for one unpaired electron, which offers possibility of an octahedral geometry¹⁵.

Electrochemical Studies

Determination of half wave potential of Cu(II) with CAA

A 1×10^{-2} M Cu(II) solution in M/10 KCl has been used to obtain polarograms of Cu(II). This showed an $E_{1/2}$ at 0.225 Vs. Ag/AgCl. Polarographic study was done on Cu(II) with various concentration of CAA. The polarograms showed that half wave potential values shifted towards more negative value with increasing concentration of ligand indicating complex formation, further diffusion current was found to decrease regularly with increase of CAA concentration. Slope of the linear plots of $\log(i/i_d - i)$ vs. E_{de} was found to be in the range of 30-32 mV, thereby showing the reversible nature of reduction process involving two electrons. The plot of half wave potential $E_{1/2}$ vs. $\log C_x$ (where C_x = concentration of complex in m mole l^{-1}) have been found to be a straight line showing the formation of most stable complex. The coordination no. (j) of the metal complex is obtained from the slope of this plot, as may be expressed by:

$$d(E_{1/2})/d \log C_x = -j.0591/n,$$

where n = no. of electrons involved (here $n = 2$). The value of j was found to be 2. This shows that composition of the complex is 1:1 (metal: ligand). The tentative structure of 1:1 complex of Cu(II)-CAA has been given in Figure 1.

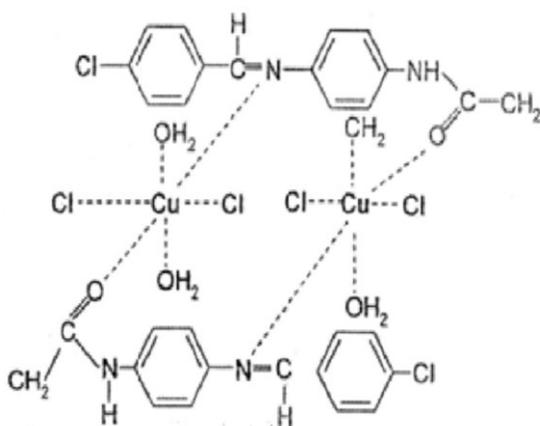


Figure 1. The tentative structure of 1:1 complex of Cu (II)-CAA.

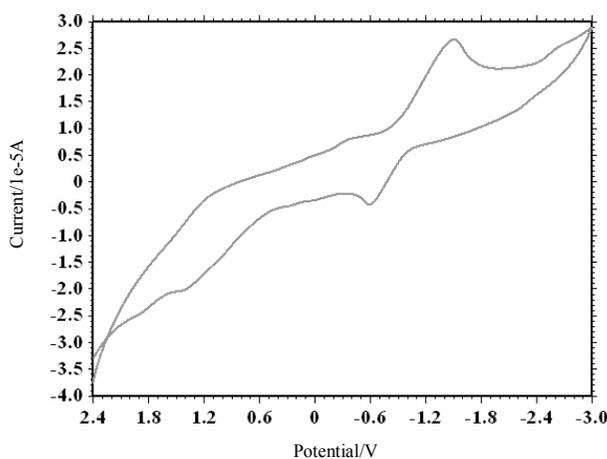
Determination of stability constant

The stability constant of the Cu(II)-CAA complex has been determined by classical method of Lingane, as the method is applicable for maximum coordination number and for the stability constant of highest complex formed. The $E_{1/2}$ has a linear correlation with ligand concentration; which shows that there is only complex formed. The following equation has been used to calculate the stability constant of the complex studied. $\Delta(E_{1/2}) = 0.0591/n \log \beta + j 0.0591/n \log C_x$. Here, $\Delta(E_{1/2})$ = Difference of half wave potentials of simple metal ion and complexed ion, n = number of transferred electron, $\log \beta$ = Stability constant of complex formed, j = Coordination number, C_x = concentration of ligand. Thus the average value of $\log \beta$ has been found to be 4.84. Polarographic data of Cu(II)-CAA are given in Table 2.

Table 2. Polarographic characteristics of Cu (II)-CAA.

S.No.	Cx	LogCx	-E _{1/2}	Logβ
1	0.00	0.00	0.24	-
2	0.01	-2.00	0.280	5.35
3	0.015	-1.8239	0.300	5.67
4	0.020	-1.6987	0.295	3.55
5	0.025	-1.6020	0.305	3.80
6	0.030	-1.5228	0.315	4.06
7	0.035	-1.4559	0.330	5.95
8	0.04	-1.3979	0.350	5.12
9	0.045	-1.3467	0.355	5.23

A cyclic voltammogram of Cu(II) complex is presented in Figure 2. Voltammogram displays a reduction peak at $E_{pc} = -1.4V$ with an associated oxidation peak at $E_{pa} = -0.6V$ at a scan rate of 50 mV/s. The peak separation of this couple (ΔE_p) is 0.8V and increases with scan rate. The ΔE_p is 1.1 and 1.4 at scan rates 100 mV/s and 200 mV/s respectively. Thus, the analyses of cyclic voltammetric responses at different scan rate give the evidence for quasi-reversible one electron reduction. The most significant feature of the Cu(II) complex is the Cu(II)/Cu(I) couple. The ratio of cathodic to anodic peak height was less than one. However, the peak current increases with the increase of the square root of the scan rates. This establishes the electrode process as diffusion controlled¹⁶.

**Figure 2.** Cyclic voltammogram of Cu(II) complex.

In-vitro antimicrobial assay

The antimicrobial results are systematized in Table 3. From the antibacterial studies it is inferred that, the Schiff base was found to be potentially active against *S. aureus* and *B. subtilis* at higher concentrations. Some of the complexes showed high antibacterial activity against *S. aureus* and *B. subtilis* than the parent ligands. In case of antifungal activity, the Schiff base was found to be inactive at low concentrations and their complexes were found to be active at higher concentrations. It is evident from the results that, the biological activity of all the metal complexes is higher than the ligands. This enhancement in the activity of the

metal complexes can be explained on the basis of chelation theory¹⁷. It is, however, known that the chelating tends to make the Schiff base act as more powerful and potent bacteriostatic agents, thus inhibiting the growth of bacteria and fungi more than the parent Schiff base¹⁸⁻²⁰.

Table 3. Antimicrobial results of Schiff base and its metal complexes.

Compound	Conc. µg/mL	Growth Inhibition against Bacteria in mm				Growth Inhibition against Fungi in mm	
		<i>S.aureus</i>	<i>B.subtilis</i>	<i>V.cholerae</i>	<i>E. coli</i>	<i>A.Niger</i>	<i>P. liliacinum</i>
C ₁₁ H ₁₁ N ₃ ClO	100	11	10	11	12	11	11
	200	13	12	12	15	13	14
	500	18	19	15	17	20	18
[Cu(CAA) Cl ₂ (H ₂ O)]	100	12	11	11	14	12	13
	200	15	13	13	17	16	15
	500	22	23	17	19	22	22
<i>Streptomycin</i>	500	28	30	29	27	25	24
<i>Nyastatin</i>	500	27	29	27	24	25	25
DMF	1 mL	-	-	-	-	-	-

Note: Less than 12mm - inactive; 12–16mm - moderately active; above 16mm - highly active.(-) means no activity.

Conclusion

In this study, complex of Cu(II), with Schiff base derived from 4-Chlorobenzaldehyde and 4-aminoacetanilide was synthesized and characterized. The Schiff base act as versatile bidentate ligand. The work has also opened up possibility of studying Cu(II)-CAA complex by D.C polarographic method. The value of log β has been found to be 11.72. This proves the validity of polarographic techniques for studies of 4-Chlorobenzylidene-4-aminoacetanilide (CAA) metal complex. The electrochemical properties of the metal complexes revealed the quasi-reversible one electron/two electron transfer redox process. The Schiff base and some of the metal complexes were found to be active against some of the representative bacterial and fungal strains.

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