SYNTHETIC, SPECTROSCOPIC AND TOXICOLOGICAL ASPECTS OF NEWELY DESIGNED TETRAAZAMACROCYCLIC COMPLEXES OF COPPER (II)

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ABSRACT

Brine shrimp leathility of a new series of tetraazamacrocyclic complexes of Copper (II) have been prepared by the template condensation reaction of dicarboxylic acids (malonic, succinic, adipic, glutaric) with 1,10-phenenthroline in 1:2:2 molar ratios. Structures and bonding of the macrocyclic complexes have been proposed based on elemental analyses, IR, Electronic and X-r-Ray spectral studies. An octahedral geometry for these complexes has been proposed as the binding sites are the Nitrogen atoms of the macrocycles The formation of the complexes as $[Cu(L^n)Cl_2]$ has been established on the basis of the chemical composition. The complexes have also been screened against Macrophomina phaseolina, Fusarium oxysporum, Aspergillus niger Pseudomonas cepacicola, Staphylococcus aureus and Xanthomonas campestris and the positive findings have been discussed.

Keywords: *Copper(II), Tetraazamacrocycles, toxicological aspects, brine shrump leathility.*

INTRODUCTION

Recent years have witnessed a great deal of interest in the area of synthesis and characterization of transition metal complexes with macrocyclic ligands. There is a great interest in the study of this branch of chemistry because of its importance in supramolecular chemistry, materials chemistry, and biochemistry[1-4]. There has been a spectacular growth in the interest in metal complexes with tetraazamacrocyclic ligands, followed by the extensive work on metal control template synthesis of macrocyclic species[5]. It has been reported that the macrocyclic complexes with tetraazamacrocyclic ligands, such as Cyclan, Cyclam or bycyclam exhibit antitumor or anti HIV activity, which stimulates researchers to do more exploitation on their derivatives[6].

The template method has been widely described and exploited for the preparation of a number of different types of metal encapsulated macrocyclic compounds. However, the importance of the non-template procedure for isolating metal ion free polyazamacrocycles have also been emphasized by some authors. Numerous reports continuously appear in literature regarding the design of novel macrocyclic polyaza as well as mixed polyazamacrocyclic ligands owing to their use as a model for study of the biologically important systems including determination of binding sties of metalloproteins as well as the study of the host-quest interactions etc.[7] Macrocyclic complexes of transition metals having both oxo and aza groups in a ligand are well known for the ligands having dioxotetraza, tetraooctaaza and tetraoxotetraaza moieties. Tehse complexes of nitrogen donor ligands have been studied in detail, on account of their interesting stereochemistry and wide practical utility[8,9].

In this communication, we have focused on the toxicological properties of macrocyclic copper (II) complexes with 1,10-phenanthroline chelators.

EXPERIMENTAL

The chemicals including malonic acid, succinic acid, glutaric acid, adipic acid and pthallic acid (Fluka), 1,10-phenanthroline (E.Merck), $CuCl_2.2H_2O$(BDH) were used as obtained.

Synthesis of Complexes : The reaction was carried out in 1:2:2 molar ratios. Metal chloride (5m mol) was dissolved in methanol (25mL) and cooled in an ice bath. To this was added 1,10-phenanthroline (corresponding to metal chloride) in methanol (25 mL) and put in a magnetically stirred 100 mL round bottom. The reaction was followed by the addition of dicarboxylic acid (corresponding to the metal chloride) in methanol (25 mL). The resulting mixture was stirred for 24-25 hrs. The solid product was isolated by filteration, repealedly washed with the same solvent and dried in vaccum (yield 57%). The compound was recrystallized in benzene and dried again in vaccuo.

Analytical methods and Physical measurements : Nitrogen and chlorine were estimated by Kjeldahl's and Volhard's method respectively. Conductivity measurements were made with a systronic (Model 305) conductivity bridge in dry dimethyl formamide. Molecular weights were detramined by the Rast Camphor Method. The IR spectra of the solid samples were recorded as KBr discs on a Nicolet Magna FTIR-550 spectrophotometer. Electronic spectra in dimethyl sulphoxide were recorded, in the range 200-600nm using method as the solvent on a UV-160A Shimadzu spectrophotometer. X-ray powder diffraction spectra of the compound was obtained on a Philips (Model P.W. 1840) automatic diffractometer using Fe(Kα) target with Mg filter. The wavelength used was 1.9373 Å and the reflections from 5-65^oC were recorded.

RESULTS AND DISCUSSION

All the complexes are coloured solids and soluble in most of the organic solvents like methanol, benzene, dichloromethane, tetrahydrofuran, dimethylformamide and dimethylsulphoxide. The molar conductivity of the macrocyclic copper complex in dimethylsulphoxide is 18-29 $ohm^{-1}cm^2mol^{-1}$, suggesting non-electrolyte. Their molecular weight determinations showed them to be monomeric in nature. Physical properties and analytical data of the complex are given in Table 1.

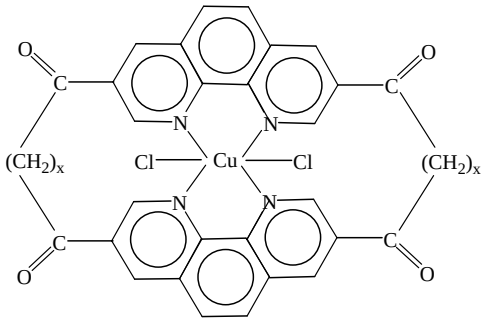
The IR spectra of the starting matericals and their copper complexes wera recorded and their comparative studies confirmed the formation of macrocyclic complexes with proposed coordination pattern[10]. The significant band in the compounds observed at 1600-1610 cm^{-1} is assigned to the (C=N) vibration. This suggests bidentate (NN) coordination of phenanthroline in the compounds. The four amide bnds are present in the regions 1600-1710, 1418-1500, 1220-1250 and 570-655 cm^{-1} assignable to amide I, amide II, amide III and amide IV, respectively[11,12].

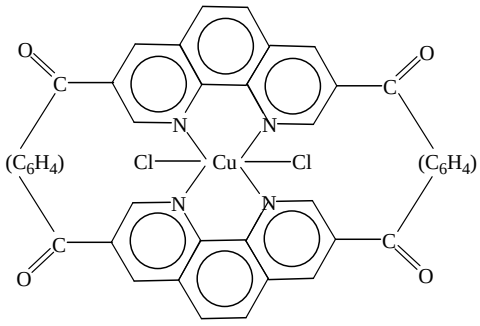
The electronic spectra of the copper complex in the powder from has been recorded at room temperature and exhibits an oxial type of signal with g values. The diamide macrocyclic complex gave g_{11} values. Value at 2.24 and g_1 value at 2.12 for the copper(II) complex which indicates an essentially d_x^2-y^2 ground state for the copper(II) ion. The oxial spectrum with g_{11}>g, > 2.04 is consistent with distorted octahedral[12] structure around cu^{2+}; ion. It has been reported that the g_{11} value of a copper(II) complex can be used as a measure of the covalent character of the metal-ligand[13] bond. If this value ismore than 2.3, the

environment is essentially ionic and values less than this limit are indicative of a covalent environment. The g_{11} values shows considerable covalent character in the present complex.

In order to ascertain the lattice dynamics of these compounds X-ray diffraction of the compound $[Cu(L^4)Cl_2]$ has been recorded. The observed interplanar spacing values ('d' in A°) have been measured from the diffractogram of the compound and the miller indices h, k and l have been assigned to each d value and 2θ angles are reported in Table 2. The results show that the compound, belongs to 'orthorhombic' crystal system having unit cell parameters as a = 32.872, b = 13.001 c = 19.850.
$\alpha = \beta = \gamma = 90°$, respectively, max. dev. of $2\theta = 0.9$.

The following structure can be suggested for Cu (II) complexes on the basis of spectral evidences and their monomeric nature.





Where, X = 1, 2, 3, 4

Antifungal Activity: The antifungal activity of the compounds have been evaluated against *Macrophomina phaseolina, Fusarium oxysporum* and *Aspergillus niger* by the Radial Growth Method[14] using czapek's agar medium. The compounds were directly mixed with the medium in 50, 100 and 200 ppm concentrations. Controls were also run and three replicates were used in each case. The linear growth of the fungus was obtained by measuring the diameter of the fungal colony after four days. The amount of growth inhibition in all the replicates was calculated by the equation, Percent inhibition = C–T/C×100, where, C is the fungal colony in the control plate and T is the diameter of the fungal colony in the test plate.

Antibacterial Activity: The antibacterial activity of the compounds was evaluated by the Inhibition Zone Technique[14]. The compounds were tested against *Pseudomonas cepacicola, Staphylococcus aureus* and *Xanthomonas campestris.*

Mode of Action

The chelation theory[14] accounts for the increased activity of the metal complexes. The chelation reduces the polarity of the metal atom mainly because of partial sharing of its positive charge with the donor groups and posible π-electron delocalisation within the whole chelating ring. The chelation increases the lipophilic nature of the central atom which subsequently favours its permeation through the lipid layer of the cell membrane.

The degradative enzymes produced by the microorganism are important in host infection. For food deterioration and break down of organic matter. The enzyme production is here intended to mean both synthesis of the enzyme by the microorganisms and activity of the enzyme in the medium after it is produced. Since the metal complexes inhibit the growth

of microorganism it is assumed that the production of enzyme is being affected and hence the microorganism is unable to utilize the food for itself or the intake of nutrients in suitable forms decreases and consequently the growth of microorganism is arrested, while higher concentration proves fatal. The higher concentration destroys the enzyme mechanism by blocking any of the metabolism path way and due to the lack of availability of proper food, the organism dies.

The results of biological activity have been compared with the conventional fungicide, Bavistin and the conventional bactericide streptomycin used as standards. The results achieved out of these studies have been enlisted in Tables 3 and 4 in which the antifungal activity indicated that the metal chelates are more active than their parent amines and dicarboxylic acids. Similar trends were observed, in case of antibacterial activity.

Brine Shrimp Lethality

The eggs of Brine Shrimp, Artemia Salina (Leech) were hatched in a small tank divided by a net containing brine water. One part of the tank contained the eggs and on the other part, a light source was placed in order to attract the nauplii. Two days were allowed to hatch all the eggs and the nauplii were sufficiently matured for experiment[15].

The test compounds dissolved in DMSO were applied at five concentrations 5, 10, 20, 40 and 80 μ gm/ml. However not more than 50 μl of DMSO was added to nauplii in each vial. For each concentration, one vial containing the same value of DMSO plus brine water was used as a control group

After 24 hours of incubation the vials were observed with the help of a magnifying glass and the number of survivors in each vial were counted and noted. From this data the mean percentage of mortality of the nauplii was calculated for each concentration.

CONCLUSION

The Brine Shrimp Lethality of the compounds $[Cu(L^1)Cl_2]$, $[Cu(L^2)Cl_2]$, $[Cu(L^7)Cl_2]$ and $[Cu(L^8)Cl_2]$, were performed. The LC_{50} values for all compounds were calculated and are given in Table 5.The rate of mortality of the nauplii was increased with the increase in concentration of each sample. A plot of Log of sample's concentration versus percentage of mortality showed a linear correlation. From the graph, the LC_{50} values of the samples were calculated and they were found 29.51, 14.51, 9.12 and 5.75 μ gm/ml, respectively.

Drug activity depends on the size, shape and degree of ionization of the drug molecule normally. It is found that the specific type of biological activity of a molecule is dependent upon more than just one functional group. Consequently, the addition of a single functional group to an inert organic substance doses not ordinarily imbue a molecule with a specific biological activity since more than one functional group normally is required for potent activity.

From this observation we conclude that compound $[Cu(L^1)Cl_2]$ and $[Cu(L^2)Cl_2]$ have got little cytotoxic activity than that of compounds $[Cu(L^3)Cl_2]$ and $[Cu(L^4)Cl_2]$. Among the compound $[Cu(L^1)Cl_2]$ and $[Cu(L^2)Cl_2]$, $[Cu(L^1)Cl_2]$, showed less activity than $[Cu(L^2)Cl_2]$. Similarly a comparison of compounds $[Cu(L^3)Cl_2]$ and $[Cu(L^4)Cl_2]$ indicated that $[Cu(L^4)Cl_2]$ is more active than compound $[Cu(L^3)Cl_2]$. From these observations we may conclude that combination of adepic acid make the compound $[Cu(L^4)Cl_2]$ highly toxic for brine shrimp nauplii.

The compound $[Cu(L^2)Cl_2]$ got significant antimicrobial activity but showed less cytotoxicity, therefore, the compound has potentiality to be a safe and effective antibiotic. But further extensive investigations on higher animal model is necessary to study it's other toxic effects.

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TABLE-1: PHYSICAL PROPERTIES AND ANALYTICAL DATA OF THE MACROCYCLIC COMPLEXES OF COPPER (II)

Compound	Colour & M.p.(^{0}C)	Yield(%)	Elemental analyses(%) Found (Calcd.)			Mol. Wt. Found (Calcd.)
			N	Cl	Cu	
$[Cu(L^1)Cl_2]$	Dark brown 177	54	7.64 (8.54)	9.40 (10.80)	8.28 (9.68)	574 (593)
$[Cu(L^2)Cl_2]$	Brown 152	46	8.53 (8.64)	10.51 (10.94)	9.37 (9.80)	602 (621)
$[Cu(L^3)Cl_2]$	Brown 168	52	8.30 (8.45)	10.13 (10.69)	9.02 (9.58)	630 (649)
$[Cu(L^4)Cl_2]$	Brown	49	7.52	9.99	8.90	641

	159		(8.27)	(10.47)	(9.38)	(677)
$[Cu(L^5)Cl_2]$	Dark Brown 162	51	7.82 (8.04)	9.82 (10.18)	8.77 (9.13)	685 (696

TABLE -2 : X-RAY DIFFRACTION DATA OF THE COMPOUND $[Cu(L^4)Cl_2]$

Peak No.	2θ obs	2θ calcd	d-spacing obs	h	k	l
1.	17.90	17.86	6.227	5	0	1
2.	17.90	18.04	6.227	0	2	1
3.	19.10	19.01	5.839	5	1	0
4.	19.10	19.29	5.839	2	2	1
5.	21.60	21.50	5.170	3	1	3
6.	22.40	22.51	4.987	0	0	4
7.	26.10	25.97	4.290	7	1	1
8.	26.90	26.73	4.165	7	1	1
9.	28.60	28.43	3.922	0	2	4
10.	32.20	32.22	3.493	8	0	3
11.	34.70	34.53	3.248	0	3	4
12.	34.70	34.76	3.248	10	0	1
13.	34.70	34.70	3.248	1	3	4
14.	42.50	42.34	2.673	1	2	2

Refined Values, a = 32.872, b = 13.001 and c = 19.850, $\alpha = \beta = \gamma = 90°$ respectively.
max dev. of 2θ = 0.9

TABLE-3 : FUNGICIDAL SCREENING DATA OF THE CU(II) MACROCYCLIC COMPLEXES (PERCENT GROWTH INHIBITION AFTER 4 DAYS AT 25±2°C, CONE IN PPM)

Compound	*Macrophomina phaseolina*			*Fusarium oxysporum*			*Aspergillus niger*		
	50	**100**	**200**	**50**	**100**	**200**	**50**	**100**	**200**
$[Cu(L^1)Cl_2]$	69	87	89	81	89	92	78	86	88
$[Cu(L^2)Cl_2]$	72	80	90	70	79	91	76	82	90
$[Cu(L^3)Cl_2]$	74	83	-	-	-	-	76	84	92
$[Cu(L^4)Cl_2]$	79	89	94	84	90	96	83	90	98
$[Cu(L^5)Cl_2]$	51	72	88	50	71	92	47	74	90
Standard (Bavistin)	82	100	100	85	100	100	86	100	100

TABLE-4 : BACTERIAL SCREENING DATA OF CU (II) MACROCYCLIC COMPLEXES (PERCENT GROWTH INHIBITION AFTER 24 HOURS AT 28±2°C)

Compound	*Pseudomonas cepacicola*		*Staphylocuccus aureus*		*Xanthomonas campestris*	
	500	**1000**	**500**	**1000**	**500**	**1000**
$[Cu(L^1)Cl_2]$	4	13	6	8	8	13
$[Cu(L^2)Cl_2]$	7	6	3	5	5	2
$[Cu(L^3)Cl_2]$	5	8	4	7	6	9
$[Cu(L^4)Cl_2]$	6	9	6	9	7	11
$[Cu(L^5)Cl_2]$	7	10	9	11	9	12
Standard (Streptomycin)	2	3	5	7	15	7

TABLE -5 : BRINE SHRIMP LETHALITY OF MACROCYCLIC COMPLEXES OF CU(II)

Conc. Sample (C) μgm/ml	log C	Number of Applied Shrimps in I, II and III Vial	Number of Survivors in I, II and III Vial	% of Mortality in I, II and III Vial	Mean Value of Mortal-ity	LC_{50} μgm/ml
Compound $[Cu(L^1)Cl_2]$						
5	0.7	11,10,11	6,6,6	36.4,40.0,36.4	37.6	
10	1.0	10,10,10	6,6,5	40.0,40.0,40.0	43.3	
20	1.3	11,12,11	6,6,6	45.0,50.0,45.0	46.7	29.51
40	1.6	11,10,10	5,5,5	54.0,50.0,54.0	51.3	
80	1.9	10,10,11	4,4,5	60.0,60.0,54.5	58.2	
Control	0.0	10,11,10	10,11,10	00.0,00.0,00.0	00.0	
Compound $[Cu(L^2)Cl_2]$						
5	0.7	10,10,10	6,6,7	45.5,45.5,50.0	36.7	
10	1.0	11,11,10	6,6,5	54.5,60.0,54.5	47.0	
20	1.3	11,12,11	5,6,5	60.0,60.0,63.6	56.3	14.15
40	1.6	10,10,11	4,4,4	40.0,40.0,30.0	61.3	
80	1.9	10,10,10	3,4,3	70.0,70.0,70.0	70.0	
Control	0.0	11,12,10	11,12,10	00.0,00.0,00.0	00.0	
Compound $[Cu(L^3)Cl_2]$						
5	0.7	11,11,10	5,5,4	54.5,54.5,60.0	56.3	
10	1.0	10,11,10	4,4,4	60.0,63.6,60.0	61.2	
20	1.3	10,10,11	3,3,4	70.0,70.6,63.6	67.9	9.12
40	1.6	11,11,11	3,3,3	72.7,72.7,72.7	72.7	
80	1.9	11,11,11	2,2,2	81.8,81.8,81.8	81.8	
Control	0.0	13,10,11	13,10,11	00.0,00.0,00.0	00.0	
Compound $[Cu(L^4)Cl_2]$						
5	0.7	10,10,11	5,5,6	50.0,50.0,45.6	48.5	
10	1.0	10,10,10	4,4,4	60.0,60.0,60.0	56.7	
20	1.3	11,11,10	3,3,3	63.6,63.6,60.0	62.4	5.75
40	1.6	10,10,10	2,2,3	70.0,70.0,70.0	70.0	
80	1.9	10,10,10	4,4,5	80.0,80.0,70.0	76.7	
Control	0.0	10,10,11	10,10,11	00.0, 00.0, 00.0	00.0	

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