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Characterisation and antimicrobial activity studies of the mixed ligand complexes of Cu(II) with 8-hydroxyquinoline and salicylic acids†

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Abstract. Mixed ligand complexes of copper(II) with 8-hydroxyquinoline and various salicylic acids were isolated. Elemental analysis, conductometric and IR data of these complexes were recorded. The ESR and electronic spectral data of these complexes indicate distorted square planar coordination for central copper ion. The σ bond and inplane π bond coefficients α^2 and β^2 respectively, of these complexes indicate partial covalency in the metal ligand bonding. The antimicrobial activity studies show that the mixed ligand complexes act as more effective toxic agents than *bis* (8-hydroxyquinolinato) copper(II) against certain bacteria and fungi. The lipophilic tendency of these complexes is determined and its influence on their antimicrobial activity is critically examined. A probable mechanism for the antimicrobial activity of these complexes is discussed.

Keywords. 8-hydroxyquinoline; salicylic acid; mixed complexes; characterisation; antimicrobial activity.

1. Introduction

The copper chelate of 8-hydroxyquinoline is recognised as one of the most highly effective fungicide and is used in preservation of textiles, cordage, paints and various industrial products (Albert *et al* 1953). Though its utility as an agricultural fungicide was well established, its applicability has been limited by its prohibitive cost. Powell (1954) observed that its cost factor can be minimised by combining the copper chelate with low cost fungicides without losing its protective properties against certain plant diseases. This may be due to the formation of mixed complexes. The mixed ligand complexes play an important role in controlling the antimicrobial activity of the metal chelates as their formation can greatly bring changes in their structure, stability, liposolubility etc., By suitably selecting combinations of hetero chelating agents, the situation can be exploited to attain the desired effect on the metal chelates.

Saxena and Mishra (1978) have prepared number of mixed ligand complexes of Cu, Fe, Zn, Co and Mn with 8-hydroxyquinoline and thiocyanate and studied their antimicrobial activity against various bacteria and fungi. Saxena *et al* (1979) have reported the preparation of mixed ligand complexes of various metals like Cu, Ni, Co and Fe with salicylic acid and oxine, pyridine, nicotinic acid, β-picoline as secondary ligands and studied their antifungal activity. We have taken up a systematic study to understand the relation between physicochemical properties and the antimicrobial

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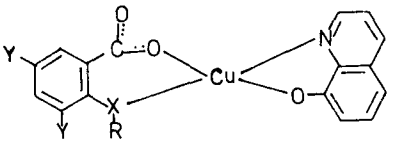


R = H or $COCH_3$, X = O or S, Y = Cl, Br, I, NO_2, H

activity of the mixed ligand complexes of Cu(II) with 8-hydroxyquinoline and various substituted salicylic acids as hetero ligands with the general formula (scheme I). The results are presented in this paper.

2. Experimental

All the chemicals used were of analytical grade (BDH) reagents.

2.1 *Physical measurements*

IR spectra were recorded on a Perkin Elmer model 577 spectrophotometer (4000–200 cm^{-1}) using the KBr disc technique. The electronic spectra were recorded as diffuse reflectance spectra on a Beckman DK-2A ratio recording spectrophotometer in the 400–2500 nm region. ESR solution data (DMF) at room (RT) and liquid nitrogen temperature (LNT) were recorded on a Varian instrument at X-band. The conductivity of the complexes was measured at 27°C by Systronics conductivity bridge 305.

2.2 *General method for the preparation of the complexes*

Equimolar solutions of salicylic acid or substituted salicylic acids (0·2 M), 8-hydroxyquinoline (0·2 M) and copper(II) acetate monohydrate (0·2 M) in 80% aqueous methanol were mixed and stirred for 30 min. The solid product was removed by filtration, washed with several volumes of water and boiled in acetone to remove any free ligand. After filtration, the complex was dried at 70°C for 12 hr.

2.3 *Composition of the complexes*

The percentage composition of metal and nitrogen in these complexes were determined by the following procedures.

2.3a. % *metal*: 100 mg of the sample was decomposed by digestion with 1 ml of sulphuric acid, adding three to six drops of 100 vol. hydrogen peroxide to clear the digest. Afterwards the contents of the Kjeldahl flask were rinsed out into a 100 ml conical flask with distilled water and the solution was boiled for several minutes to destroy the excess of peroxide. The copper present in the peroxide free solution was then determined by idometric titration with sodium thiosulphate (Ingram 1962).

2.3b. % *nitrogen*: 25 mg of the sample was pyrolysed in a stream of hydrogen and the gases were passed over a nickel/magnesia catalyst heated to 350°C. The ammonia formed was absorbed in boric acid and titrated with 0.01 N hydrochloric acid (Dixon 1968).

2.4 *Antimicrobial activity*

The antimicrobial activity of the dimethyl formamide solutions of these compounds were examined *in vitro* by serial dilution method (Schaub *et al* 1958) against various bacteria and by paper disc method (Jasper *et al* 1958) against fungi. All the stock cultures were supplied by the Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India. Peptone water and saline water were used for making the inoculum for bacteria (18 hr culture) and fungi respectively. Nutrient broth and Sabourand's dextrose agar (Hindustan Dehydrated Media, Bombay) were used as test media for bacteria and fungi respectively. The minimum inhibitionary concentration (MIC) (μg/ml) of the compounds against bacteria and the average zone of inhibition (mm) of the compounds at 1000 μg/ml against fungi are given in table 2. All the tests were carried out in duplicate.

3. Results and discussion

The elemental analysis and other physical measurements (table 1) show that the copper forms a 1:1:1 mixed ligand complex with 8-hydroxy-quinoline and substituted salicylic acids. The low conductance (2–4 mhos cm^2/g) of these complexes show that they are of non-electrolyte type.

3.1 *IR data*

The important peaks of the IR spectra of these complexes agree with the proposed structures. In all the mixed ligand complexes, the symmetric and asymmetric stretching vibrations of the (O–C–O) group (Goyal and Khosla 1980) of salicylic acids are observed at 1420 cm^{-1} and 1570 cm^{-1} respectively. Charles *et al* (1956) reported that

Table 1. Analytical data, physical measurements and distribution data of the complexes.

Compound	Colour	Decomp. temp. (°C)	% of N		% of Cu		Percentage extraction of Cu(II) as mixed complex into chloroform from 7·4 pH phosphate buffer
			cal	obs	cal	obs	
Cu(OX) (SA)*	Green	266	4·06	4·19	18·43	18·51	55
Cu(OX) (Cl-SA)	Yellowish green	270	3·69	3·68	16·76	16·70	72
Cu(OX) (2Br-SA)	Yellowish green	253	2·79	2·72	12·64	12·69	79
Cu(OX) (2I-SA)	Green	252	2·35	2·33	10·66	10·72	90
Cu(OX) ($2NO_2$-SA)	Yellowish green	290	9·66	9·62	14·61	14·70	68
Cu(OX) (Thio-SA)	Green	260	3·88	3·68	17·61	17·50	48
Cu(OX) (Ace-SA)	Bluish green	227	3·62	3·64	16·43	16·49	76

(OX) = 8-hydroxyquinoline; (SA) = salicylic acid; (Cl-SA) = 5, chloro salicylic acid; (2Br-SA) = 3,5-dibromo-salicylic acid; (2I-SA) = 3,5-diiodo salicylic acid; ($2NO_2$-SA) = 3,5-dinitro salicylic acid; (Thio-SA) = thio salicylic acid; (Ace-SA) = Acetyl salicylic acid.

* Reported earlier Saxena *et al* 1979.

Table 2. Antimicrobial activity of copper complexes with 8-hydroxyquinoline and substituted salicylic acids.

	Anti bacterial activity MIC (μg/ml)**								Antifungal activity (mm)			
	Gram-Positive		Gram-negative						Average zone of inhibition at 1000 ppm			
Compound	1	2	3	4	5	6	7	8	a	b	c	d
Oxine	12·5	12·5	100	100	100	100	100	100	8	8	7	8
$Cu(OX)_2$	6·2	12·5	25	25	50	100	50	50	11	18	12	12
Cu(OX) (SA)	10	25	50	50	50	12·5	25	25	8	12	14	10
Cu(OX) (Cl-SA)	10	25	50	25	50	50	50	50	9	12	14	10
Cu(OX) (2Br-SA)	10	25	50	6·2	100	1·5	12·5	6·2	9	12	14	10
Cu(OX) (2I-SA)	12	25	50	50	50	100	50	25	8	12	14	10
Cu(OX) ($2NO_2$-SA)	6·2	12·5	25	12·5	25	25	25	50	8	12	17	12
Cu(OX) (Thio-SA)	12·5	35	75	25	25	25	25	50	9	10	12	9
Cu(OX) (Ace-SA)	25	6·2	100	50	100	12·5	25	100	10	10	14	9

1. *Staphylococcus albus*; 2. *Staphylocous auresus*; 3. *Shigella schmitzi*; 4. *Shigella sonnei*; 5. *Proteus morganii*; 6. *Vibrio cholerae*; 7. *Escherichia coli*; 8. *Salmonella typhi.*

a. *Pencillium* spp; b. *Aspergillus niger*; c. *Trychophyton rubrum*; d. *Aspergillus fumigatus.*

incubation period: **24 hr at 37°C; *48 hr at 30°C.

in several 8-hydroxyquinoline complexes of divalent metals, the $\nu_{(C-O)}$ appeared at 1120 cm^{-1} region and the region position of the band slightly varies with the metal. The $\nu_{(C-O)}$, observed in the free oxine molecule at 1090 cm^{-1}, shifted to higher frequencies in all the mixed complexes giving a strong absorption band at 1110 cm^{-1}. This clearly indicates the coordination of 8-hydroxyquinoline in these complexes. The observed sharp peaks at 310–320 cm^{-1} and 260–280 cm^{-1} may be assigned to the M–O and M–N stretching frequencies respectively (Ohkaku and Nakamoto 1971).

3.2 *Ligand field and* ESR *spectra*

In all these complexes the crystal field symmetry of the copper ion may be taken as axial arising out of two oxygens of salicylic acids and nitrogen and oxygen atoms of 8-hydroxyquinoline, forming a distorted square planar coordination (distorted D_{4h} symmetry). Electronic and the ESR spectra of copper(II) compounds with square planar and octahedral coordination stoichiometry can be easily understood using figure 1 (Hathaway and Billing 1970; Reedijk 1981). Such a distortion will be smallest for strongly coordinating axial ligands and will increase when the coordinating power of the axial ligands decreases, resulting in the observation of only one overlapping band. Since only one broad maximum in between 15000 to 16000 cm^{-1} (ΔE) is observed in DMF of these compounds the nonexistence of axial coordination or at best weak axial coordination of the solvent may be taking place. Regular tetrahedral complexes of Cu(II) show no *d–d* absorption bands in the region 10000 to 20000 cm^{-1} (Gersman and Swalen 1962).

The spin Hamiltonian parameters ($g_{\|}$, $g_{\perp}$ and $A_{\|}$) derived from frozen solution (DMF) spectra at liquid nitrogen temperature are given in table 3. The molecular orbital parameters α^2 and β^2 which describe the fraction of the copper *d* orbitals used in σ bond and inplane π bond respectively are calculated by the standard procedure (Hathaway and Billing 1970; Reedijk 1981; Gersman and Swalen 1962; Nieman and Kivelson 1961) employing equations (a)–(c) and taking $K = 0{\cdot}43$ (Nieman and Kivelson 1961).

$$g_{\|} = g_e - 8\lambda\alpha^2\beta^2/\Delta E_{xy} \quad \text{(a)}$$

$$g_{\perp} = g_e - 2\lambda\alpha^2\beta^2/\Delta E_{xz} \quad \text{(b)}$$

$$A_{\|} = p[-\alpha^2(K + 4/7) + (g_{\|} - 2) + 3(g_{\perp} - 2)/7] \quad \text{(c)}$$

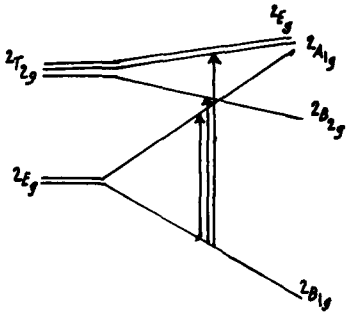


Figure 1. Ligand-field splittings for tetragonal copper(II) complexes.

Table 3. ESR and electronic spectral data.

Compound	Solution data at LNT			*d-d* transition energy (cm^{-1})	α^2	β^2
	$g_\parallel$	$g_\perp$	$A_\parallel cm^{-1}$			
$Cu(OX)_2$	2·244	2·025	168×10^{-4}	16000	0·759	0·768
Cu(OX) (SA)	2·299	2·029	149×10^{-4}	16340	0·762	0·960
Cu(OX) (Cl-SA)	2·297	2·035	153×10^{-4}	14815	0·774	0·852
Cu(OX) (2Br-SA)	2·299	2·041	149×10^{-4}	16000	0·767	0·934
Cu(OX) (2I-SA)	2·305	2·044	151×10^{-4}	15385	0·739	0·951
Cu(OX) ($2NO_2$-SA)	2·302	2·052	149×10^{-4}	16000	0·775	0·934
Cu(OX) (Thio-SA)	2·298	2·069	168×10^{-4}	15385	0·751	0·901
Cu(OX) (Ace-SA)	2·291	2·026	158×10^{-4}	16340	0·779	0·914

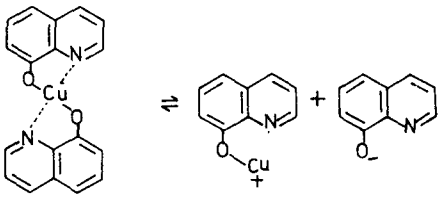
The accuracies in the evaluated values of $g_\parallel$, $g_\perp$ and $A_\parallel$ are $\pm 0{\cdot}004$, $\pm 0{\cdot}006$ and $\pm 5 \times 10^{-4}$ respectively.

The σ bond coefficient α^2 describes the covalency between the central paramagnetic ion and its ligands. The α^2 values from table 3 indicate a partial covalency in σ bond, ranging in between the complete ionic bond ($\alpha^2 = 1$) and the complete covalency ($\alpha^2 = 0{\cdot}5$) for all these complexes. The α^2 values are comparable to those found for other ternary complexes which have shown to possess axial symmetry (Kuska *et al* 1967). The inplane π bonding coefficient β^2 is sensitive to back donations from the filled $d_{xz} \cdot d_{yz}$ orbitals from copper. From examination of β^2 values given in table 3 it appear that significant back donation occurs in $Cu(OX)_2$ and Cu(OX) (Thio-SA) complexes.

3.3 *Antimicrobial activity*

In general all the mixed complexes possess comparable activity against the bacteria and fungi studied with that of binary copper oxinate though in some cases the mixed complexes are more effective toxic agents. Simple salicylic acids and their binary chelates have very low activity against these organisms which may be due to their higher water solubility. The antimicrobial activity of (8-hydroxyquinolinato) copper(II) complexes were explained by several workers (Albert *et al* 1953; Block 1955; Zentmyer *et al* 1960; Esposito and Fletcher 1961; Gershon *et al* 1966) assuming that this complex first penetrates the cell and at the site of action it undergoes dissociation to a 1 : 1 copper(II) 8-hydroxyquinoline complex which will then become the toxic entity by combining with and blocking metal binding sites on enzymes as shown below (scheme II).

Thus the 1 : 2 chelate due to its liposolubility is necessary to take the toxic moiety. *i.e.* the 1 : 1 charged complex which is unable to penetrate the cell membrane due to its charge. According to Overtone's concept (Overtone 1901) of cell permeability, the lipoid membrane that surrounds the cell favours the passage of only lipid soluble materials due to which lipo solubility has been considered as one of the important factors which control the antimicrobial activity of any toxic agent. The partition of the toxic agent between oleyl alcohol or chloroform and 7.4 pH phosphate buffer (pH of the biological medium) system has been considered as a good model to understand the lipophobic or lipophilic tendency (Dwyer and Mellor 1964). The distribution of all these mixed complexes in between chloroform and 7.4 pH phosphate buffer are determined to understand their lipophilic tendency and the results are included in



Dissociation of 1 : 2 copper oxine complex

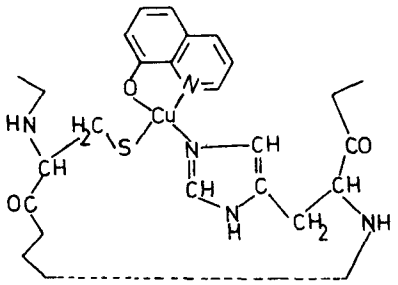


table 1. As expected the mixed complexes have lower partition coefficient in chloroform due to the presence to hydrophilic salicylate ion than compared to copper(II) 8-hydroxyquinoline complex which is quantitatively extractable under these conditions.

Thus the mixed complexes in many cases are better toxic agents though they have lower lipophilic character which indicates different mechanism may be working at the site of action. The probable mechanism at the site of action can be understood by considering the dissociation of the mixed complex into corresponding 1 : 1 binary complexes as shown below.

$$2\,Cu(OX)(SA) \longrightarrow Cu(OX)^+ + Cu(SA)^+ + OX^- + SA^-$$

Such a kind of dissociation of ternary complex to form binary complexes to an extent of 25% each can be reasonably expected to be in equilibrium due to statistical reasons (Sigel 1975). Thus in the mixed complexes in addition to 1 : 1 copper oxinate, 1 : 1 copper salicylate may be also acting as a toxic agent which increases their activity. The 1 : 1 copper salicylate is better transported to the site of action as a mixed complex than the *bis*(salicylato)copper(II) complex which is found to possess very low activity most likely due to its higher hydrophilic character.

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