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Spectroscopic, Analytical and Biological Properties of New Copper(II) Complexes of Antiviral Drug 2-Amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one (Acyclovir)

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Two new copper(II) complexes with the antiviral drug acyclovir (H_2L) have been synthesized: the mononuclear complex $[Cu(H_2L)Cl_2]\cdot 1.5H_2O$ and the binuclear one $[Cu_2(H_2L)_3(H_2O)_2Cl\cdot 5(H_2O)$. The complexes have been characterized using elemental analysis, conductivity, atomic absorption, electronic, IR and mass spectra, magnetochemical and thermogravimetric methods. In the mononuclear complex $[Cu(H_2L)Cl_2]\cdot 1.5H_2O$, copper(II) is coordinated bidentately with the N and O groups of a drug molecule forming a nearly square-planar structure of the type CuL, with a CuNO chromophore. In complex $[Cu_2(H_2L)_3(H_2O)_2Cl\cdot 5(H_2O)$ each copper atom is coordinated with one N atom from one ligand, one oxygen atom from one ligand molecule which serve as bridges in the formation of the binuclear complex $[Cu_2(H_2L)_3(H_2O)_2Cl\cdot 5(H_2O)$. In aqueous solutions, a binuclear violet complex $[Cu_2(H_2L)_3(H_2O)_2Cl\cdot 5(H_2O)$ is formed with an square pyramid structure. Measurable antiferromagnetic interactions between the two paramagnetic Cu(II) centers are responsible for some interesting magnetic properties. Using the Job method, the compositions of these complexes were determined. Protonation constants of the acyclovir and stability constants of its Cu^{2+} complexes were determined by potentiometric titration method in 50 % methanol-water mixtures at 25.00 ± 0.02 ºC under nitrogen atmosphere and ionic strength of 0.10 M sodium chloride. It has been observed that acyclovir has two protonation constants. The divalent metal ion Cu^{2+} forms CuL_2, $Cu_2H_2L_2$ and Cu_2L stable complexes with acyclovir. The voltammetric behaviour of the complexes were studied using cyclic voltammetric technique. Different parameters were tested to optimize the conditions for the characterization of the complexes. The dependence of current intensities and potentials on pH, concentration, scan rate, nature of the buffer were also investigated. The antimicrobial activity studies of the acyclovir and it's complexes have been studied against some gram positive species: (*Corynebacterium xerosis, Bacillus brevis, Bacillus megaterium, Bacillus cereus, Mycobacterium smegmatis, Staphylococcus aureus, Micrococcus luteus, Enterococcus faecalis*) and gram negative (*Pseudomonas aeruginosa, Klebsiella pneumoniae, Escherichia coli, Yersinia enterocolitica, Kluyveromyces fragilis, Saccharomyces cerevisiae, Candida albicans*) bacteria.

Key Words: Acyclovir, Metal complexes, Antimicrobial activity, Cyclic voltammetric, Potentiometric titration.

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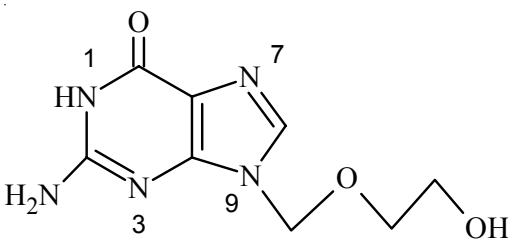
INTRODUCTION

Acyclovir is an antiviral drug which acts against the Herpes viruses, including herpes simplex 1 and 2 (cold sores and genital herpes), varicellazoster (shingles and chicken pox) and the Epstein-Barr virus (mononucleosis)[1]. Despite *in vitro* activity against herpes viruses and a favourable toxicity profile, many potential applications of acyclovir are limited by its poor absorption. Acyclovir is absorbed slowly and incompletely from the human gastrointestinal tract. The exact mechanism of absorption of acyclovir is not fully characterized. Oral bioavailability is reported[2] to be between 15 and 30 %. The poor absorption is considered to be a result of characteristics of the drug itself and not its delivery vehicle[3]. Several approaches initially were tried to improve after the oral bioavailability of acyclovir[4]. Experiments with esters of a number of amino acids were favourable, with the valine-esterified compounds demonstrating the best properties. Addition of the valine moiety to acyclovir results in a substrate for active transport mechanisms in the human intestine. The valine-esterified compound has similar polarity and ionization at physiological pH; thus, an improvement in passive diffusionrelated uptake would not be expected[5]. The prodrug, valacyclocir, synthesized by the addition of a naturally occurring amino acid, l-valine to acyclovir, results in the achievement of plasma acyclovir concentrations superior to those obtained with oral acyclovir, while requiring less frequent administration. Valacyclovir is at least as effective as oral acyclovir for a number of indications[6].

Acyclovir's chemical name is 2-amino-1,9-dihydro-9-[(2-hydroxyethoxy)methyl]-6*H*-purin-6-one (**Scheme-I**). There has been an interesting report in acyclovir-metal complex for example acyclovir-Pt(II)[7]. Turel *et al.* synthesized and characterized three different copper(II) complexes of acyclovir too. These formulas are $[Cu(acv)_2(H_2O)_2](NO_3)_2$[8], $[Cu(acv)_2(H_2O)_3](NO_3)_2 \cdot H_2O$[9] (acv: acyclovir), $[Cu(acvmp)_2(H_2O)_2] \cdot H_2O$ (acvmp = acyclovir monophosphate)[10]. As can see these structures, M:L ratio has been found like 1:2 in three articles.



Scheme-I. Chemical structure of acyclovir

The classical coordination complex, *cis*-DDP or cisplatin (*cis*-diamminedichloroplatinum)[11], has been the subject of much recent attention towards the metal-based chemotherapy because of its beneficial effects in the treatment of cancer[12]. The major disadvantage of *cis*-DDP is the occurrence of primary or acquired resistance[13]. To ameliorate this disadvantage, much effort has been put in the development of new antitumour metal coordination compounds having capability of overcoming resistance to *cis*-DDP[14]. Hitherto, the majority of antitumor metal complexes synthesized and characterized have been structural analogs of *cis*-DDP[15]. However, there has been a leveling off[16] or perhaps even a decrease in the number of new compounds of this type; possibly because it is beginning to transpire that substantial advances are unlikely to be made with these compounds. At the same time, there has been an emergence of new structural types of metallic complexes often with promising activity and able to circumvent *cis*-DDP resistance[17]. Thus, a lot of drugs have raised much interest and in some cases the highest activity is associated with a metal complex[18-21]. For instance, in the course of present studies, we have shown that antiviral drug valacyclovir and antihypertensive drug propranolol dithiocarbamate metal(II) complexes (metal: Cu(II), Co(II), Ni(II), Cd(II) and Zn(II)) have very effective antimicrobial activity[22,23]. These encouraging results led us to study the screening of drugs-metal(II) complexes as antibacterial agents.

EXPERIMENTAL

Acyclovir was kindly provided by Biofarma Pharm. Ind. (Istanbul, Turkey) and was used without further purification. All other chemicals were of analytical reagent grade. Elemental analyses (C, H, N) were performed by the TUBITAK Instrumental Analysis Laboratory (Besevler, Ankara, Turkey) using a Carlo Erber 1106 elemental analyser. Infrared spectra were obtained using KBr discs (4000-400 cm^{-1}) on a Shimadzu 8300 FT-IR spectrophotometer. The electronic spectra in the 200-900 nm range were obtained on a Shimadzu UV-160 A spectrophotometer. Magnetic measurements were carried out by the Gouy method using $Hg[Co(SCN)_4]$ as calibrant. Molar conductances of the ligand and its copper complexes were determined in DMSO (*ca.* 10^{-3} M) at room temperature using a Jenway Model 4070 conductivity meter. The mass spectral analyses were carried out on a VG ZabSpec spectrophotometer. The metal content of each complex was determined by an Ati Unicam 929 Model AA Spectrometer in solutions prepared by decomposing the compounds in aqua regia and then subsequently digesting in concentrated HCl. Electrochemical studies were carried out with a Iviumstat Electrochemical workstation equipped with a low current module (BAS PA-1) at a platinum disk electrode and a platinum microelectrode of 10 lm diameter (BAS). Cyclic voltammetric measurements were made at room

temperature in an undivided cell (BAS model C-3 cell stand) with a platinum counter electrode and an Ag/AgCl reference electrode (BAS). All potentials are reported with respect to Ag/AgCl. The solutions were deoxygenated by passing dry nitrogen through the solution for 0.5 h prior to the experiments and during the experiments the flow was maintained over the solution. Digital simulations were performed using DigiSim 3.0 for windows (BAS, Inc.). Experimental cyclic voltammograms used for the fitting process had the background subtracted and were corrected electronically for ohmic drop.

Stock solution of acyclovir was prepared in the purified methanol[24]. Double distilled conductivity (Millipore system) water was used as aqueous medium. All the other chemicals used were of AR grade and were used without further purification. Stock solutions of 0.03 M $CuCl_2$ were standardized using an appropriate indicator by EDTA titrations[25]. Sodium hydroxide solutions were prepared as 50 % methanol-water mixture and its concentration and absence of carbonate were frequently checked by means of Gran plots[26] using potassium hydrogen phthalate (Merck) as the acid. 0.10 M acid solutions prepared from Merck. Hydrochloric acid were titrated against standardized 0.10 M NaOH solution[27]. The ionic strength of each solution was adjusted to 0.10 M by the addition of NaCl as supporting electrolyte.

Potentiometric titrations: Potentiometric titrations were carried out in a thermostated 80 mL glass vessel. It was equipped with a combined pH electrode (Orion Inlab 412 combined glass electrode), nitrogen inlet and outlet tubes, a magnetic stirrer and titrant inlet. The electrode was modified by exchanging its aqueous NaCl solution consisting of 0.10 M NaCl saturated with AgCl. The e.m.f was measured using an Orion 960 automatic titrator. The pH measurement of proton-ligand and metal-ligand systems of acyclovir were made with containing carbonate-free NaOH at a known (*ca.* 0.10 M) concentration at 25.00 ± 0.02 °C with ionic strength 0.100 M NaCl. Temperature was maintained constant inside the cell at 25.00 ± 0.02 °C, by the circulating water by a Haake thermostatted bath (precision ± 0.02). The potentiometric cell was calibrated before each experiment to obtain -log[H^+] values (pH) for the titration medium[28]. The ion products (K_w = [H^+][OH^-]) were calculated at a constant ionic strength of 0.100 M with NaCl in 50 % aqueous methanol solutions based on measurements of [OH^-] and pH in several series of experiments. The standardization of the combined pH electrode was also checked in the alkaline range by addition of excess NaOH. By assuming the E^0 cell value determined in the acidic range to be reliable and the [OH^-] concentration of a base added in excess, the reproducible values of pK_w were calculated to examine 50 % aqueous methanol solution[29,30]. The pK_w value obtained is 14.90 in this medium.

Potentiometric titrations were carried out at constant temperature and in an inert atmosphere of nitrogen with CO_2-free standardized 0.10 M NaOH

in a 50 mL solution containing 0.10 M NaCl:(i) 2.00×10^{-3} M HCl (for cell calibration); (ii) 3.00×10^{-3} M HCl + 1.50×10^{-3} M acyclovir (for the protonation constants of acyclovir); (iii) 3.00×10^{-3} M HCl + 1.50×10^{-3} M acyclovir + 1.50×10^{-3} M $CuCl_2$ (for the stability constant of the 2:2 M:L complex); (iv) 6.00×10^{-3} M HCl + 3.00×10^{-3} M acyclovir + 1.50×10^{-3} M $CuCl_2$ (for the stability constant of the 1:2 M:L complex).

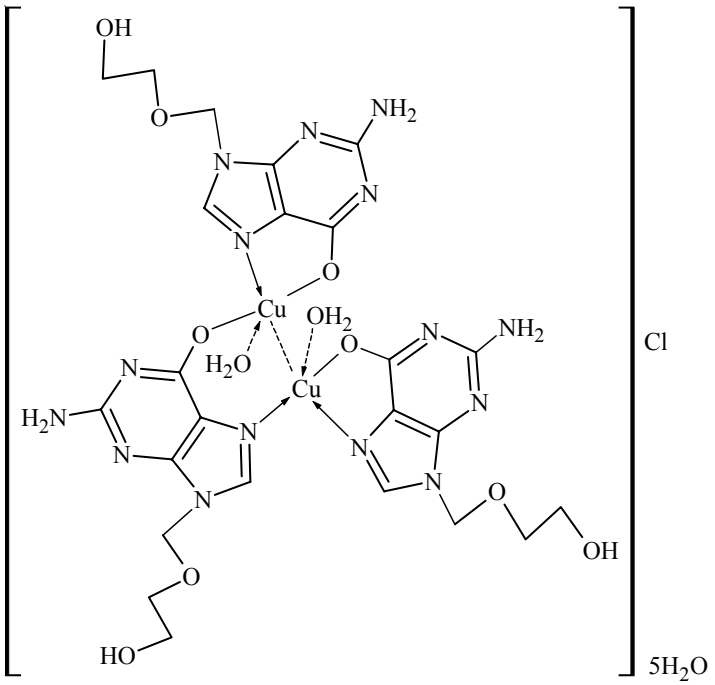
Data processing: The protonation and stability constants of acyclovir were evaluated by iterative non-linear least squares fit of potentiometric equilibrium curves through mass balance equations for all the components expressed in term of known and unknown equilibrium constants using a computer program BEST[31]. All the models converged at $r < 0.03$ pH units of the observed pH values, which is considered to be an acceptable fit. The equilibrium constants reported in this paper were obtained as averaged values of three titrations. Selection of the equilibrium models was based on critical evaluation of the least squares fitting results, namely analysis of the statistical parameters.

Determination of stoichiometry of metal complexes: Job's method[32] was used to determine, in water, the stoichiometric ratios for the reactions between the H_2L and the metal ions. The solution were prepared by mixing solutions of both components with equal molar concentrations (1.0×10^{-3} M) in ratios varying from 1:9 to 9:1. The complexes were prepared by mixing, in water, an adequate stoichiometric amount of the H_2L and the corresponding salt.

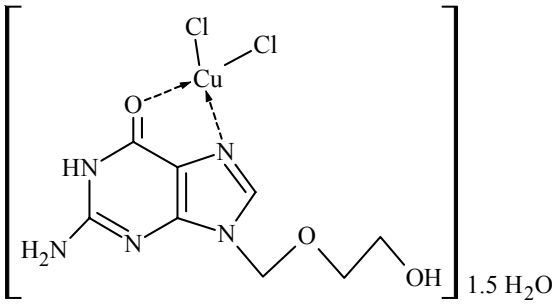
Preparation of the binuclear and mononuclear complexes: A solution of 1.05×10^{-4} mol $CuCl_2$ (0.014 g) in H_2O (15 mL) was added to an aqueous solution of 1.57×10^{-4} mol H_2L (0.0353 g in 15 mL H_2O), the M:L molar ratio being Cu:H_2L = 1:10. Dilute aqueous NaOH was used to raise the pH from 5.5 to 10 at room temperature. At the end of the reaction, the dark violet precipitate (**Scheme-II**) was filtered off, washed with EtOH and MeOH and dried *in vacuo*.

Fine green crystals (**Scheme-III**) of the mononuclear complex were obtained after mixing MeOH solutions of the reagents in a molar ratio Cu:H_2L:NaOH = 1:1:1 (0.0215 g $CuCl_2 \cdot 2H_2O$ (1.6×10^{-4} mol) in 15 mL MeOH; 0.0347 g (1.54×10^{-4} mol) H_2L in H_2O/MeOH (1/9, v/v); 0.0062 g (1.54×10^{-4} mol) NaOH in H_2O/MeOH (1/9, v/v). At the end of the reaction, the green precipitate was filtered off, washed with EtOH and MeOH and dried *in vacuo*.

Antimicrobial activity: *in vitro* antimicrobial activity of the compounds was tested by the tube dilution technique. Each of the test compounds and standards: ampicillin trihydrate, streptomycin and nystatin, was dissolved in water at concentrations of 60 µg/mL. Further dilutions of the complexes and standards in the test medium were prepared at the required quantities



Scheme-II. Chemical structure of the binuclear copper(II) complex $[Cu_2(HL)_3]Cl(H_2O)_5$ of H_2L



Scheme-III. Chemical structure of the mononuclear copper(II) complex $[Cu(H_2L)Cl_2]\cdot 1.5H_2O$ of H_2L

of 60 µg/mL concentration. The minimum inhibitory concentrations (MIC) were defined as the lowest concentrations of the compounds that prevented visible growth. It was determined that the solvent had no antimicrobial activity against any of the test microorganism. All the compounds were tested for their *in vitro* growth inhibitory activity against *Corynebacterium xerosis* UC 9165, *Bacillus brevis* NRS, *Bacillus megaterium* DSM 32, *Bacillus cereus* EU, *Mycobacterium smegmatis* CCM 2067, *Staphylococcus*

aureus Cowan 1, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* FMC 5, *Enterococcus faecalis* ATTC 15753, *Micrococcus luteus* LA 2971, *Yersinia enterocolitica* CMC 120 and *Escherichia coli* DM] and three yeasts [*Kluvyeromyces fragilis* A 230, *Saccharomyces cerevisiae* WET 136 and *Candida albicans* ATCC 1023].

Antibacterial activity assay: The cultures were obtained in Mueller-Hinton Broth (Difco) for all the bacteria after 18-36 h of incubation at 37 ± 1 ºC. Testing was carried out in Mueller-Hinton Broth at pH 6.4 and a two-fold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation, the last tube with no micro-organism growth was recorded.

Antifungal activity assay: The yeasts were maintained in Sabouraud Dextrose Broth (Difco) after incubation for 48 h at 25 ± 1 °C. Testing was performed in Sabouraud Dextrose Broth at pH 6.4 and the twofold dilution technique was applied. A set of tubes containing only inoculated broth was kept as controls. After incubation, the last tube with no growth of yeast was recorded[3].

RESULTS AND DISCUSSION

Two complexes were characterized by C, H and N analytical data and other spectroscopic methods. Analytical data are given in Table-1 and are consisted with the proposed structures. The binuclear complex is soluble in water while the mononuclear complex is insoluble. The gravimetrical analysis results indicate the presence of the chloride in an appropriate ratio in the complexes. The size of the crystals obtained does not permit the determination of X-ray diffraction on single crystals.

TABLE-1
ANALYTICAL DATA FOR COPPER(II) COMPLEXES WITH ACYCLOVIR

Compound (colour)	m.w.	Yield (%) / (m.p. ºC)	Found (Calcd.) %			
			C	H	N	Cu
$[C_{24}H_{39}Cu_2N_{15}O_{11}]Cl\cdot 5H_2O$ (Green)	961.24	45 (316)	29.99 (29.94)	4.61 (4.67)	21.86 (21.82)	13.22 (13.20)
$[C_8H_{11}Cl_2CuN_5O_3]\cdot 1.5H_2O$ (Violet)	386.66	73 (254)	24.80 (24.84)	3.69 (3.65)	18.15 (18.11)	16.40 (16.45)

The electronic spectra of H_2L and binuclear complex were recorded in water. In the spectra of the H_2L, the aromatic band in 268 nm is attributed to benzene π-π* transitions. The electronic spectrum of the mononuclear complex lies at 612 nm in DMSO, while the binuclear complex exhibits a band at 701 nm. These data are in accord with the intensity of the observed

bands which are characteristic of Cu(II) complexes with a square pyramide and square planar symmetry[33]. No absorbance was found in the 360-400 nm range, typical for polynuclear Cu(II) complexes with oxygen-containing bridging ligands[34].

Job's continuous variation method is applied in order to determine the suitable ratio between H_2L and copper ions. The results show that M:L= 1:1 for $Cu(H_2L)Cl_2 \cdot 1.5H_2O$ or 2:3 for $Cu_2(HL)_3Cl(H_2O)_5$ complexes were formed between the H_2L and copper ions as shown in Fig. 1.

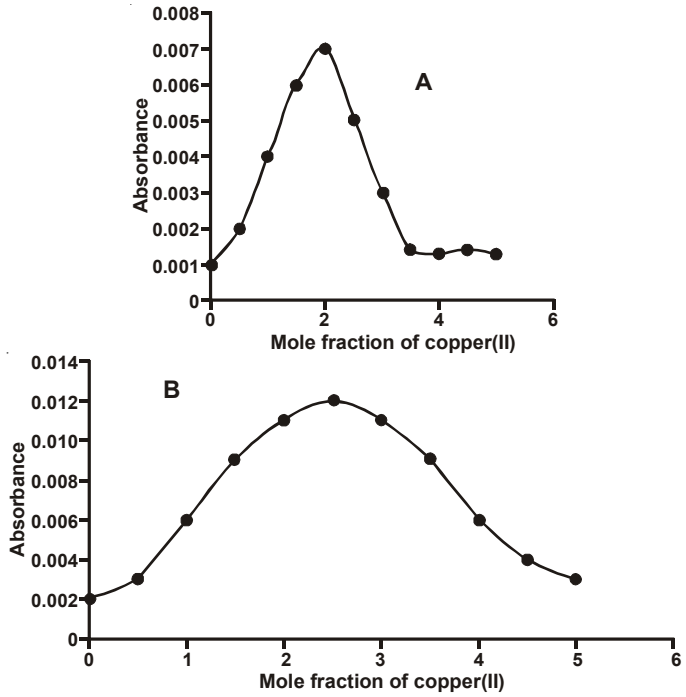


Fig. 1.

The key IR bands of free H_2L and its complexes are given in Table-2. H_2L exhibits bands at 3315, 1637 and 763 cm^{-1} which are due to the different modes of vibrations of the NH, ν, δ and ρ. On complexation, these bands are not affected, indicating that the amino group is not involved on complexation. The ligand gave strong split carbonyl bands at 1695 cm^{-1} corresponding to ν(C=O) of the amide. However, this band of the ligand are shifted towards lower wave number by *ca.* 20 and 40 cm^{-1}, respectively on complexation, indicating that the C=O group is either involved on the structural configuration of the complex or the phenomenon of tautomerism is assigned. The band corresponding to $\nu(C\text{-}N)_{amide}$ in the free ligand, at 1485 cm^{-1} is shifted on complexation, *i.e.* the C-N(7) of the five membered ring

TABLE-2
INFRARED SPECTRAL DATA (cm^{-1}) OF THE COPPER(II) COMPLEXES

Compound	ν(OH)	ν(NH_2)	ν(C=O)	ν(NH)	ν(M-N)	ν(M-O)
H_2L	3440.8	3186.2	1695.3	3315.4, 1637.5, 763.8	–	–
$[C_{24}H_{39}Cu_2N_{15}O_{11}]Cl\cdot 5H_2O$	3440.0	3209.3, 2929.7, 2732.9	1639.4	1550.7	420.5	704.0
$[C_8H_{11}Cl_2CuN_5O_3]\cdot 1.5H_2O$	3431.1	3315.0, 2875.7	1675.4	1504.4	422.4	717.5

in acyclovir is involved on the complexation. This indicates that N(7) is more donating than N(3), because of the resonance stabilization of N(7)[35,36]. In addition, the presence of coordinated water is observed in the infrared spectra of $Cu_2(HL)_3Cl(H_2O)_5$ as indicated by broad band at 3440 cm^{-1}.

The positive-ion FAB mass spectra of the mono- and binuclear complexes show peaks in the m/z 386.4 (20 %) and 962.75 (50 %), respectively. These peaks may be due to the molecular ion peaks ($[M]^+$). In spectra of the mononuclear complex, the peaks at m/z 385.3 (60 %) can be attributed to $[M+H]^+$ ion peak. In the spectra of the binuclear complexes, the highest intensity peaks at m/z 872.45 (100 %) may be assigned to the loss of the five moles hydrate groups[34-36].

The ratios of the metal present in two complexes were determined by atomic absorption spectroscopy. The complexes were decomposed in HNO_3/H_2O_2 (1/1) and then dissolved in 1.5 N HNO_3. The amounts of Cu was determined (Table-1). They support the structures given in the **Schemes II** and **III**[37,38].

Equivalent conductance of H_2L and both complexes were carried out in 1×10^{-3} M DMSO solutions and the values were found to be for $Cu_2(HL)_3Cl(H_2O)_5$ and $Cu(H_2L)Cl_2\cdot 1.5H_2O$, 6.3 and 3.9 Ω^{-1} cm^2 mol^{-1}, respectively indicating that both complexes are weak electrolytes. H_2L was found to be a non-electrolyte in DMSO[36-38].

Magnetic measurements[39,40]: Magnetic measurements were recorded at room temperature and the effective magnetic moment (μ_{eff}) values of the complexes are calculated and support the structures for the mononuclear and binuclear complexes. The magnetic susceptibilities of these complexes obey the Curie-Weiss law, indicating an antiferromagnetic interaction between the pair of copper ions. The magnetic moment of the binuclear Cu(II) complex has been found to be 1.85 BM. Due to steric interactions of the larger size of the ligand the lower coordination number five has been assigned to this complex. The binuclear Cu(II) complex may have the usual

square-pyramide structure. In this complex, three ligands act as bidentates (O, N) neutral ligand. The magnetic moment of the mononuclear Cu(II) complex (1.80 BM) lies as expected for the copper(II) chelates. This value suggests that the copper atom is in a square-planar environment.

Thermal decomposition studies[39,40]: The two complexes were placed into dried Schlenk tube against a dry N_2 flow at atmospheric pressure. The complexes were then heated to the desired temperature under static N_2.

Thermal analysis of $Cu(H_2L)Cl_2 \cdot 1.5H_2O$: The TG of the complex $Cu(H_2L)Cl_2 \cdot 1.5H_2O$, shows the formation of the unhydrous complex by 192 ºC, which indicates the loss of the water of hydration; between 244 and 391 ºC further decomposition leads to $Cu(H_2L)$ (observed mass loss: 25.63; theoretical 25.35 %) and finally a species of mass corresponding to CuO by 750 ºC (observed residual mass: 13.5; theoretical 13.8 %).

Thermal analysis of $Cu_2(HL)_3Cl(H_2O)_2$: In thermal study of $Cu_2(HL)_3Cl(H_2O)_5$ in N_2 atmosphere the mass loss started slowly from 36.9 ºC in TG curve and an inclined slope from 119 to 232 ºC with a mass loss of 9.41 % (calcd., 9.35 %) indicates the removal of all the five molecules of water. The mass loss continues in TG and showing another inclined slope from 232 to 461 ºC with 32.87 % mass loss, which apparently indicates the formation of a $Cu_2(HL)_2$ that may be an intimate copper complex of the HL. The calculated mass loss to get the compound is 32.51 %. In DTA curve the peak in the range of 200 to 700 ºC which consists of an endotherm with DTA_{min} of 200 ºC and an endotherm with DTA_{max} of 700 ºC corresponds to the dehydration and decomposition phenomena. The TG curve is almost stable up to 461 ºC and then a mass gain of 23.27 % up to 573 ºC is observed (obsd. mass loss, 23. 35 %) which is almost stable up to 674.3 ºC (obsd. mass loss, 21.84 %). The species generated at 674.3 ºC starts loosing mass and at the end of the scanning up to 1100 ºC with a mass loss of 86.80 % attributed the formation of metallic Cu.

Electrochemistry[41]: The electrochemical properties of the complexes $Cu(H_2L)Cl_2 \cdot 1.5H_2O$ and $Cu_2(HL)_3Cl(H_2O)_5$ have been investigated (Table-3). The cyclic voltammograms of the complexes in the different buffer media

TABLE-3
ELECTROCHEMICAL DATA OF THE COPPER(II) COMPLEXES

Complex	Media	E_{pa} (V)	E_{pc} (V)	$E_{1/2}$ (V)	E_p (mV)
$[C_{24}H_{39}Cu_2N_{15}O_{11}]Cl \cdot 5H_2O$	BRT (pH=2)	-0.340	1.320	0.490	-1.660
	BRT (pH=5)	-0.020	-0.420	-0.220	0.400
	H_2SO_4 (0.5 M)	0.040	0.130, 0530	0.085	-0.090
$[C_8H_{11}Cl_2CuN_5O_3] \cdot 1.5H_2O$	BRT (pH=2)	0.160, -0.780	0.110, 0.560	0.135	0.050
	BRT (pH=5)	-0.920, -0.050	-1.120, -0.480	-1.020	0.200
	H_2SO_4 (0.5 M)	0.250	0.150, 0.530	0.200	0.100
	Aqua	-0.080	-0.350, 0.580, 1.440	-0.215	0.270

are given in Fig. 2A-G. These buffer media are BRT (pH = 2), BRT (pH = 5) and H_2SO_4 (0.5 M). In the BRT (pH = 5), the complex $Cu(H_2L)Cl_2$-$1.5H_2O$ shows one anodic and cathodic peaks at -0.340 and 1.320 V. In like manner, in the pH = 5, there are the cathodic at -0.420 V and anodic peak at -0.020 V. Finally, in the 0.5 M H_2SO_4 solution, the complex has one anodic peak at 0.040 V and two anodic peaks at 0.130 V and 0.053 V. This process is reversible shown as follows:

$$Cu(II) + e^- \longleftrightarrow Cu(I)$$

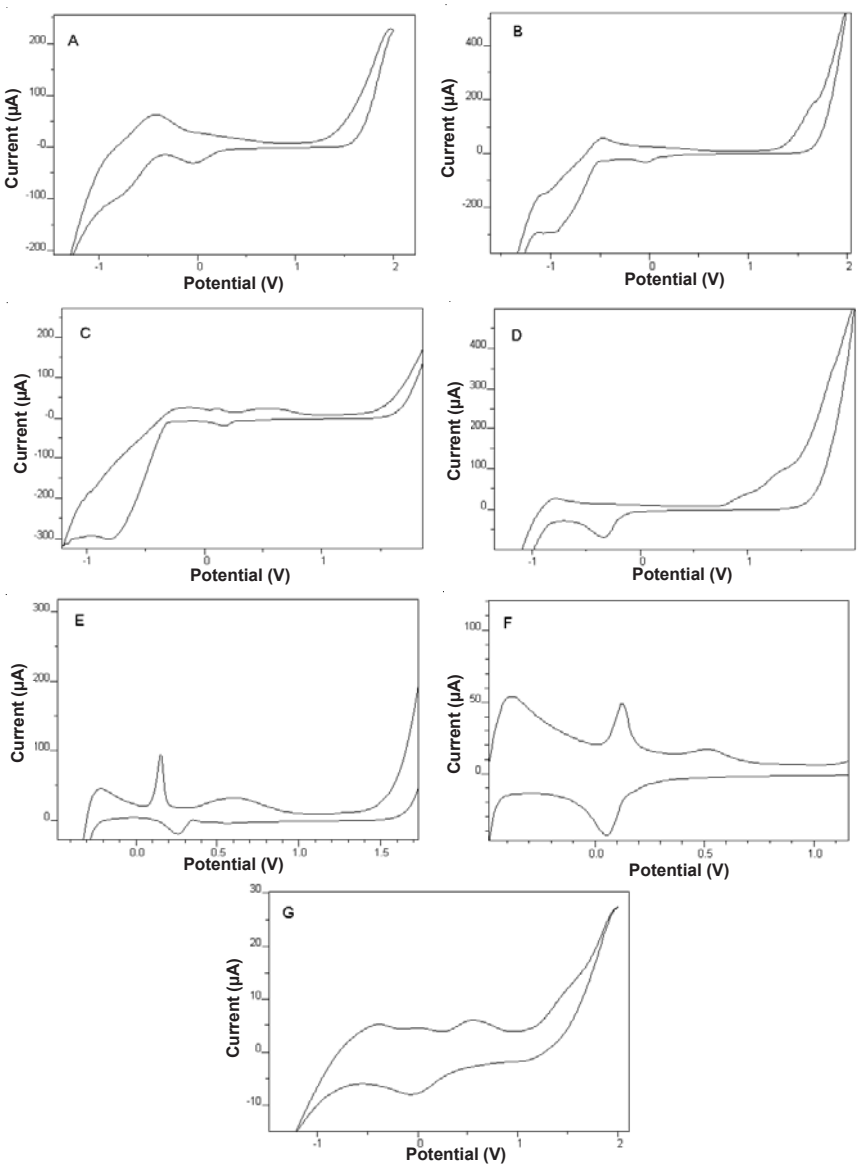


Fig. 2

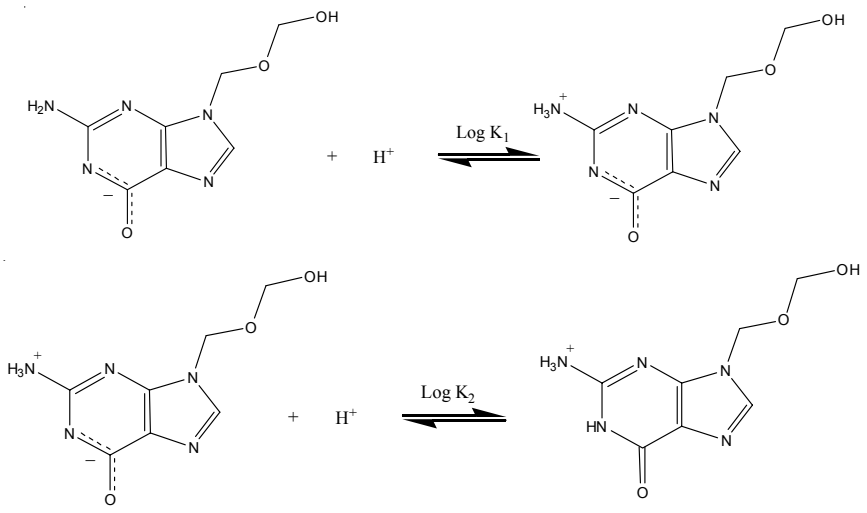
In the binuclear $Cu_2(HL)_3Cl(H_2O)_5$ complex, four different media have been used for electrochemical studies. As seen from CV curves and obtained data, the complex has two anodic and cathodic peaks. The peaks shift to more anodic or cathodic region according to mononuclear Cu(II) complex. In the 0.5 M H_2SO_4 solution of the mononuclear and binuclear Cu(II) complexes are observed as a weak and strong shoulders in the range from about -1.8 to -1.5 V, respectively. These reduction peaks are assigned to the one-electron process.

The electrochemical process is reversible for the binuclear Cu(II) complex. This process is:

$$Cu(II)\ Cu(II) + e^- \longleftrightarrow Cu(II)\ Cu(I) + e^- \longleftrightarrow Cu(I)\ Cu(I)$$

when the obtained data is studied, pH of the solution has been affected the oxidation-reduction phenomena.

Protonation constants of acyclovir: The mixture methanol-water 50:50 % was the chosen solvent for present study. In such a medium, the acyclovir and its metal complexes are soluble giving stable solutions. The stoichiometric protonation constants of the acyclovir were determined in 50 % methanol-water mixture at 25.00 ± 0.02 ºC and these constants, log K_1 and log K_2 are 10.05 ± 0.02 and 12.05 ± 0.03 respectively (**Scheme-IV**). It was seen that present protonation constants of acyclovir are similar to literature value. The value of protonation constant of acyclovir in aqua media as 11.87 and 9.35[26-32]. However in literature, no protonation constants of acyclovir is reported earlier in methanol-water mixture. Therefore, the protonation constant calculated in this study may significantly contribute to the literature.



Scheme-IV. log K_1 and log K_2 equilibrium reactions of acyclovir

As the titration curve of acyclovir in Fig. 3, it can be seen that there are two end-points at a=1 and a=2 for acyclovir. According to the results obtained from this titration curve it can be concluded that the acyclovir have two protonation constants. The log K_1 and log K_2 are related to the protonation of amino group and nitrogen in benzene ring for acyclovir, respectively.

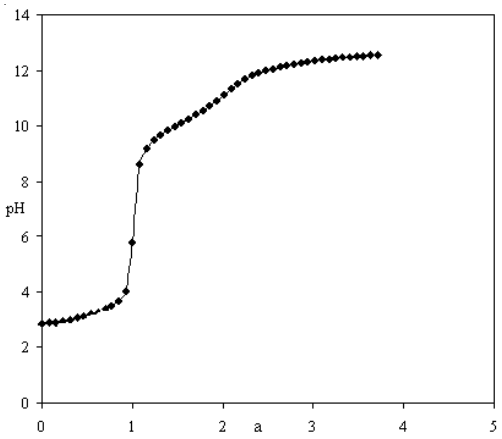


Fig. 3.

Stability constants of acyclovir-Cu complexes: The potentiometric titration curve of the acyclovir with equivalents of ligand to metal ion for Cu^{2+} is shown in Fig. 4. The metal ion Cu^{2+} depresses the titration curve of the free ligand by the release of protons according to the abilities of the metal ions to bind to the acyclovir. As the titration curve of the complexes formed by copper with the acyclovir is examined, two inflection points can be observed approximate at a = 2 and a = 4 for the acyclovir. This inflection point can be explained by the formation of complexes of CuL_2.

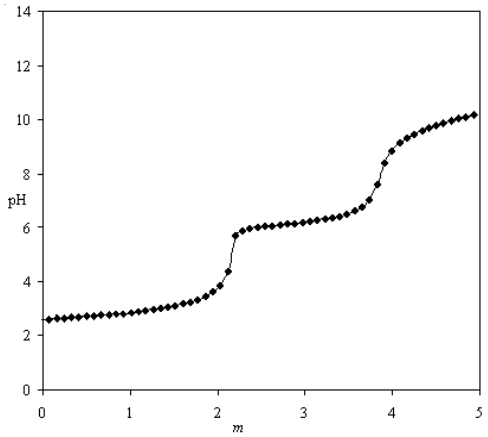
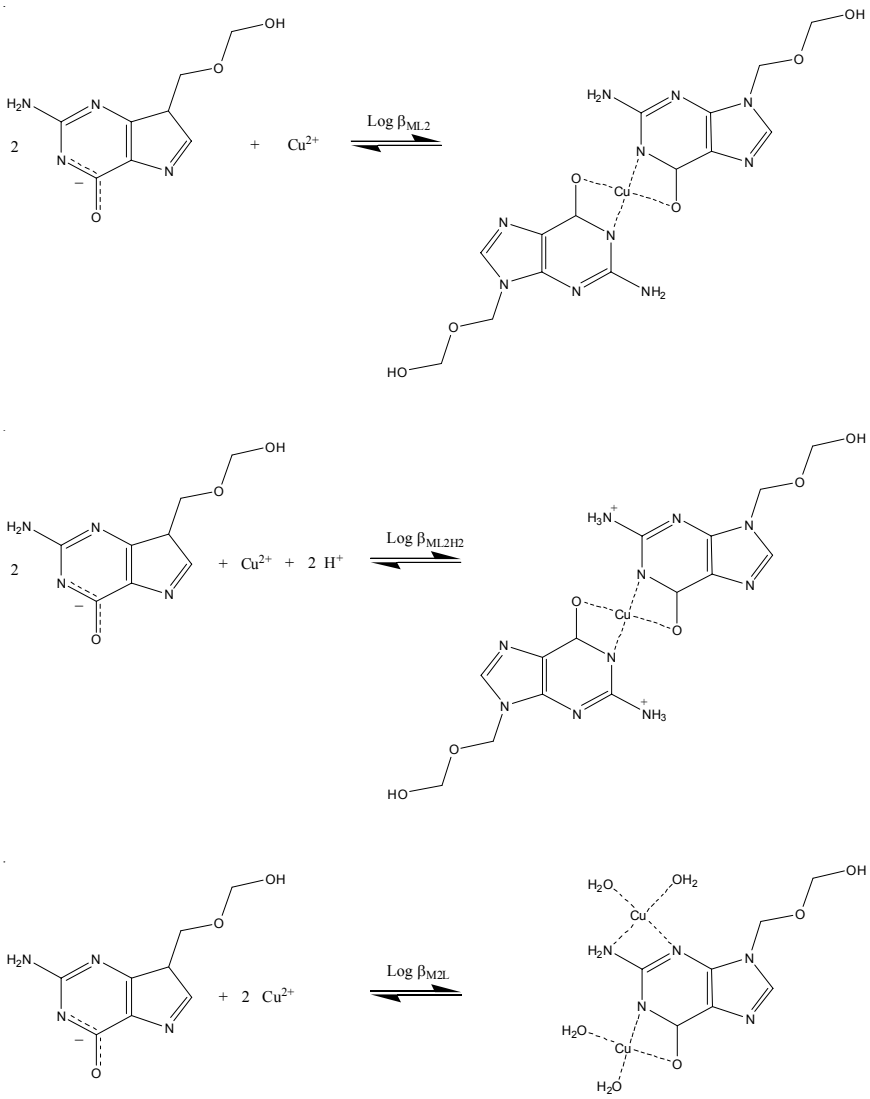


Fig. 4.

The stoichiometric stability constants of Cu^{2+} complexes of acyclovir were determined in 50 % methanol-water mixture at 25.00 ± 0.02 ºC and these constants are tabulated in Table-4 and equilibrium reactions of acyclovir-Cu^{2+} complexes are shown in **Scheme-V**. It is seen from Table-4 that the concentration CuL and CuHL complexes are less than the other species concentration's in 50 % methanol-water mixture. So, they aren't calculated in this study.



Scheme-V. Equilibrium reactions of acyclovir-Cu^{2+} complexes

TABLE-4
STABILITY CONSTANTS OF ACYCLOVIR-Cu^{2+} COMPLEXES IN 50 % METHANOL-WATER MIXTURE (μ = 0.100 M NaCl, t = 25.00 ± 0.02 ºC)

$L + Cu^{2+} \rightleftharpoons CuL$	Less than
$L + Cu^{2+} + H^{+} \rightleftharpoons CuHL$	Less than
$2L + Cu^{2+} \rightleftharpoons CuL_2$	$\log \beta_{ML_2} = 18.21 \pm 0.03$
$2L + Cu^{2+} + 2H^{+} \rightleftharpoons CuL_2H_2$	$\log \beta_{MH_2L_2} = 30.96 \pm 0.04$
$L + 2Cu^{2+} \rightleftharpoons Cu_2L$	$\log \beta_{M_2L} = 19.78 \pm 0.02$

In order to investigate change with pH in the concentration of the complexes, which acyclovir formed with Cu^{2+}, the stability constant values are evaluated using SPE computer program and the species distribution curves are drawn. In Fig. 5, if the distribution diagram for Cu-acyclovir system is examined, it would be seen that the complex form with CuL_2 is dominant in between pH = 6-12. This species forms approximately 99 % at pH = 10. CuH_2L_2 complex forms between pH 2-7. This CuH_2L_2 complex forms 85 % at pH = 2 and Cu_2L complexes form at pH= 2-8 range. It is also seen that protonated species of acyclovir is HL dominantly at pH = 2-8 range. Another protonated species H_2L is formed at pH = 2-4.

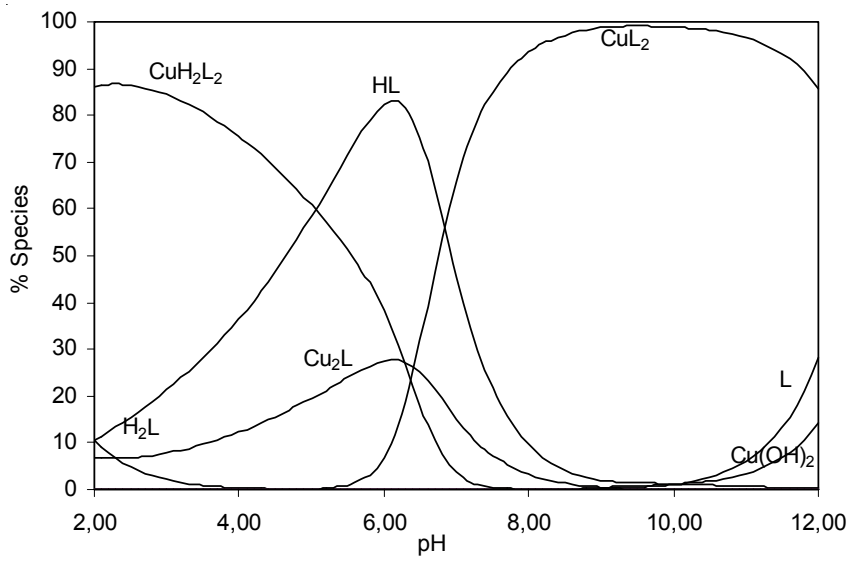


Fig. 5

Antimicrobial studies: Antibacterial activities of copper(II) complexes have been carried out against the 12 bacterias. The test solutions were prepared in DMSO. The results of the antibacterial activities are summarized in Table-5. While the ligand has not shown any biological activity, the complexes

TABLE-5
in vitro ANTIFUNGAL ACTIVITY DATA OF
MONO AND BINUCLEAR COPPER(II) COMPLEXES

Compounds	1	2	3	4	5	6	7	8	1'	2'	3'	4'	1"	2"	3"
H_2L	6	7	8	8	5	9	7	8	6	10	10	8	7	9	6
$[C_{24}H_{39}Cu_2N_{15}O_{11}]Cl\cdot 5H_2O$	17	12	17	17	16	23	19	16	11	19	31	25	11	16	13
$[C_8H_{11}Cl_2CuN_5O_3]\cdot 1.5H_2O$	24	18	22	20	29	20	20	24	15	22	35	30	16	20	14
$CuCl_2\cdot 2H_2O$	32	28	32	33	26	34	36	37	22	26	28	26	34	38	36
Ampicillin	9	16	14	13	20	12	25	33	17	8	10	17	15	30	10
Streptomycin	7	19	16	15	21	14	22	23	15	10	13	16	20	22	7
Nystatin	5	NT	NT	NT	NT	NT	NT	NT	NT	9	NT	NT	15	15	21

Gram positive bacteria: 1 = *Corynebacterium xerosis*, 2 = *Bacillus brevis*, 3 = *Bacillus megaterium*, 4 = *Bacillus cereus*, 5 = *Mycobacterium smegmatis*, 6 = *Staphylococcus aureus*, 7 = *Micrococcus luteus*, 8 = *Enterococcus faecalis*.
Gram negative bacteria: 1' = *Pseudomonas aeruginosa*, 2' = *Klebsiella pneumoniae*, 3' = *Escherichia coli*, 4' = *Yersinia enterocolitica*.
Yeasts: 1" = *Kluyveromyces fragilis*, 2" = *Saccharomyces cerevisiae*, 3" = *Candida albicans*. NT: Not tested, MIC, µg/mL.

have shown, well enough, high biological activity against the all bacteria and yeasts. The complexes were found to posses remarkable bacterial and fungicidal properties; it is however interesting to note that the biological activity becames enhanced on undergoing complexation with the metal ion. From the structure point of view of the prepared compounds with their effects on microbial testing, it is clear that formation of the chelate derivatives in the 1:1 molar ratio (M:L) sometimes increase the biological activity as appeared to be the case for the Cu(II) complex. Moreover, the enhanced activities of the metal complexes compared to the ligand may be due to the oxygen and nitrogen atoms around the central metal ion arising from chelation in a 2:3 molar ratio (M:L). Such an increased activity for the metal chelates as compared to the free ligand can be explained on the basis of chelation theory[42-45]. Chelation considerably reduces the polarity of the metal ion because of the partial sharing of its positive charge with the donor groups and possible *p*-electron delocalization over the chelate ring. Such chelation could increase the lipophilic character of the central metal atom, which subsequently favours the permation through the lipid layer of cell membrane. The mode of action of the complexes may involve the formation of the hydrogen bond through the free -NH_2 and ring N atom (in the purin fragment) groups with the active centers of the cell constituents resulting in the interference with a normal cell process[45]. Both complexes have high antimicrobial activity against the yeast cultures used in this study, as compared to the standard antifungal antibiotics ampicillin, streptomycin and nystatin. Notably, the mononuclear $Cu(H_2L)Cl_2$ complex shows a higher antiyeast activity

than the binuclear $Cu_2(HL)_3Cl(H_2O)_5$ complex. $Cu(H_2L)Cl_2$ complex has stronger antiyeast activity than those of standard antibiotics against some bacteria and yeasts.

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