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Research Article

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Complex Formation Equilibria of Ternary Complexes of Cu(II) Involving Pyridine-2-Carboxylic Acid and Various Biologically Relevant Ligands

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Abstract

Binary and ternary complexes of Cu^{2+} involving pyridine-2-carboxylic acid (picolinic acid; PA) and amino acids, peptides or DNA constituents(HL) have been investigated. Ternary complexes of amino acids or peptides are formed by simultaneous reactions. Amino acids form the [Cu(PA)L] complex, whereas peptides form [Cu(PA)L] and [Cu(PA)(LH^{-1})]. The ternary complexes of Cu^{2+} with picolinic acid and DNA are formed in a stepwise process, whereby binding of Cu^{2+} to picolinic acid is followed by ligation of the DNA constituents. The stability of the ternary complexes is compared with the stabilities of the corresponding binary complexes. DNA constituents form both 1:1 and 1:2 complexes with$[Cu(PA)]^+$. The concentration distribution of the complexes in solution was evaluated. Probable mode of chelation with picolinic acid and various biologically relevant ligands was discussed. Absorption spectra of the binary and ternary complexes of Cu^{2+} were also investigated. The isolated metal chelates, [Cu(PA)(Cl_2)(H_2O)$_2$] and [Cu(PA)$_2$(Cl_2)], are characterized by elemental analysis and infrared measurements.

Keywords: Cu(II), pyridine 2-carboxylic acid, amino acids, peptides, DNA constituents, stability constant.

1. Introduction

The study of ternary complexes of transition metal ions with amino acids, peptides or DNA units has been the subject of increasing research efforts [1–6], which have revealed the role of metal ions at the molecular level. Cu^{2+} among other transition metal ions provides an active centre in many enzymes. It seems therefore to be of considerable interest to conduct several investigations covering binary and ternary complexes of Cu^{2+} involving the pyridine-2-carboxylic acid (picolinic acid; PA), which contains a basic nitrogen (pyridine) and possesses π-acceptor properties, which are believed to be involved in π – π stacking effects with purine and pyrimidine bases.

In this respect, it is very interesting to refer to the work of Bensichem and Forrell [7] who discovered a remarkable increase in the cytotoxicity of trans-[Pt(py)$_2$$Cl_2$] complexes as compared to the inactive trans-[Pt(NH_3)$_2$$Cl_2$],formed by introducing aromatic nitrogen ligands. In continuation of our previous work on binary and ternary complexes of pyridine 2-carboxylic acid, [8], amino acids, peptides and DNA constituents [9-14], we describe here the equilibrium associated with the formation of binary and ternary complexes involving Cu^{2+}, pyridine 2-carboxylic acid and bioactive ligands.

2. Experimental

2.1. Materials and reagents

Picolinic acid (PA) was purchased from Aldrich. Amino acids (glycine, alanine, β-phenylalanine, valine, proline, threonine, serine, lysine, l-histamine· 2HCl, l-histidine·HCl, l-ornithine, methionine and S-methylcysteine) together with mercaptoethanol, methylamine, and imidazole were provided by Sigma. The peptides (glycylglycine, glutamine, aspargine and glycinamide) were also purchased by Sigma. The DNA constituents; uracil, uridine, inosine, inosine 5-monophosphat (IMP), adenine, guanosine, guanosine 5-monophosphate (GMP) and $Cu(NO_3)_2{\cdot}2H_2O$ were supplied from BDH. The copper content of the solutions was determined by complexometric titrations [15]. Carbonate free NaOH (titrant) was prepared and standardized against a potassium hydrogen phthalate solution. All solutions were prepared using de-ionized water.

2.2. Synthesis of the solid complexes

The two complexes; $[Cu(PA)Cl_2(H_2O)_2]$ and $[Cu(PA)_2Cl_2]$ were prepared by direct mixing and dissolving the ligand PA ;(0.123 g, 1 mM) or (0.246 g, 2 mM) and $CuCl_2 . 2H_2O$ (0.170 g, 1 mM) in 20 mL distilled water .Each mixture was stirred for 2h. The resulting precipitates were filtered and washed thoroughly with water, ethanol and diethyl ether and dried overnight under vacuum. (yield: 80–85%). The dried complexes were subjected to elemental analysis. Anal. Calcd. for $[Cu(PA)Cl_2(H_2O)_2]$; $C_6H_9NO_4CuCl_2$ (%) (MW 293.61): C, 24.50; H, 3.07; N, 4.77. Found:C, 24.39; H, 3.18; N, 4.70. Anal. Calcd. for $[Cu(PA)_2Cl_2]$; $C_{12}H_{10}N_2O_4CuCl_2$ (%) (MW 380.72): C, 37.82; H, 2.63; N, 7.35. Found: C, 37.79; H, 2.74; N, 7.90.

2.3. Apparatus and measuring techniques

Potentiometric measurements were made using a Metrohm 751 Titrino. The titroprosessor and electrode were calibrated with standard buffer solutions prepared according to NBS specifications [16]. All potentiometric titrations were carried out at 25 ± 0.05°C in a double-walled glass cell of 50 ml capacity. The temperature of all the solutions was maintained at 25 ± 0.05°C by circulation of thermostatted water through the outer jacket of the cell. The solutions were stirred with a magnetic stirrer and all titrations were performed in triplicate at an ionic strength of 0.1 $mol{\cdot}L^{-1}$ ($NaNO_3$). Electronic spectra were recorded on a Shimadzu UV-160A spectrophotometer.IR spectra were measured on a 8001-PC FTIR Shimadzu spectrophotometer using KBr pellets.

2.4. Equilibrium measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 cm3) solution (1.25×10^{-3} $mol{\cdot}L^{-1}$) of constant ionic strength 0.1 $mol{\cdot}\ L^{-1}$, adjusted with $NaNO_3$. The stability constant of the $[Cu(PA)]^+$ complex was determined by titrating 40 cm^3 of a solution mixture of Cu^{2+}($1.25 \times 10^{-3}mol{\cdot}L^{-1}$), PA ($1.25\times10^{-3}mol{\cdot}\ L^{-1}$) and $NaNO_3$($0.1mol{\cdot}L^{-1}$). The formation constants of the mixed complexes were determined by titrating solution mixtures containing equivalent amounts of Cu^{2+}($1.25 \times 10^{-3}mol{\cdot}\ L^{-1}$), (PA) and other ligands in the concentration ratio 1:1:1 for amino acids and peptides, and 1:1:2 for the DNA constituents. All titrations were performed in a purified N_2 atmosphere using aqueous 0.05 $mol{\cdot}L^{-1}$NaOH as titrant.

The general four components equilibrium can be written as follows (charges are omitted for simplicity):

$$l(Cu)+p(PA) + q(L)+ r(H) \rightleftharpoons [(Cu)_l(PA)_p(L)_q(H)_r] \quad (1)$$

$$\beta_{lpqr} = \frac{[Cu_l PA_p L_q H_r]}{[Cu]^l [PA]^p [L]^q [H]^r} \quad (2)$$

The calculations were obtained from ca. 100 data points in each titration using the computer program MINIQUAD-75 [17]. The stoichiometry and stability constants of the complexes formed were determined by trying various possible composition models. The model selected gave the best statistical fit and was chemically consistent with the titration data. The results obtained are shown in Tables 1 and 2. The concentration distribution diagrams were obtained using the program SPECIES [18].

2.5. Spectrophotometric measurements

Spectrophotometric investigation of the binary and ternary complexes was performed by scanning the visible spectra of solution mixtures (A-D). Under the prevailing experimental conditions and after neutralization of the hydrogen ions released associated with complex formation, it is assumed that the complexes have been completely formed. In each mixture (A-D), the volume was adjusted to 10 mL by addition of distilled water and the ionic strength was kept constant by using $NaNO_3$ (0.1 $mol{\cdot}L^{-1}$).

A. 1.5 mL Cu^{2+}(0.01 mol.L^{-1}) + 1mL $NaNO_3$ (1 mol.L^{-1}).
B. 1.5 mLCu^{2+}(0.01 mol. L^{-1}) + 1.5 mL PA (0.01 mol. L^{-1}) + 1.5 mL NaOH (0.01mol·L^{-1}) + 1mL $NaNO_3$ (1 mol. L^{-1}).
C. 1.5 mL Cu^{2+}(0.01 mol. L^{-1}) + 1.5 mL PA (0.01 mol. L^{-1}) + 1.5 mL glycylglycine (0.01 Mol. L^{-1}) + 3.0 mL NaOH (0.01 mol. L^{-1}) + 1m L $NaNO_3$ (1mol.L^{-1}).
D. 1.5 mL Cu^{2+}(0.01 mol.L^{-1}) + 1.5 mL PA (0.01 mol. L^{-1}) + 1.5 mL glycylglycine (0.01 Mol. L^{-1}) + 4.5 mL NaOH (0.01 mol. L^{-1}) + 1 mL $NaNO_3$ (1 mol. L^{-1})

2.6. Antimicrobial Activity

The antibacterial activities of the synthesized complexes were evaluated at Biotechnology Laboratory of EPRI using the diffusion disc method against *Bacillus subtilis* and *Staphylococcus aureus* as Gram (+ve), *Escherichia coli* and *Pseudomonas aeruginosa* as Gram (-ve) bacteria, while the fungicidal activity was evaluated against *Aspergillus fumigatus* and *Geotricum candidum*.

Table 1: Stability constants of binary systems Cu(II)-PA, Cu(II) -L and proton-association constants at 25 °C and I =0.1 M $NaNO_3$.

System	L	P	Q	r[a]	log β[b]
Picolinic acid (PA)	0	1	0	1	5.22 (0.008)
	0	1	0	2	6.74 (0.04)
	1	1	0	0	6.07 (0.12)
Glycine	0	0	1	1	9.64 (0.01)
	0	0	1	2	12.17 (0.02)
	1	0	1	0	8.17 (0.02)
	1	0	2	0	14.93 (0.04)
Alanine	0	0	1	1	9.80 (0.01)
	0	0	1	2	12.62 (0.03)
	1	0	1	0	8.03 (0.03)
	1	0	2	0	14.77 (0.05)
Valine	0	0	1	1	9.68 (0.00)
	0	0	1	2	12.18 (0.01)
	1	0	1	0	8.11 (0.02)
	1	0	2	0	14.73 (0.03)
Proline	0	0	1	1	10.65 (0.009)
	0	0	1	2	13.18 (0.01)
	1	0	1	0	8.60 (0.03)
	1	0	2	0	15.97 (0.05)
β-phenylalanine	0	0	1	1	9.20 (0.01)
	0	0	1	2	11.81(0.03)
	1	0	1	0	7.30 (0.02)
	1	0	2	0	14.25 (0.03)
Methionine	0	0	1	1	9.23 (0.02)
	0	0	1	2	12.04 (0.04)
	1	0	1	0	7.72 (0.03)
	1	0	2	0	14.16 (0.05)
S-methylcysteine	0	0	1	1	8.65 (0.02)
	1	0	1	0	8.03 (0.01)
	1	0	2	0	14.81 (0.02)
Threonine	0	0	1	1	9.06 (0.009)
	0	0	1	2	11.07 (0.03)
	1	0	1	0	8.34 (0.02)

	1	0	2	0	14.80 (0.04)
	1	0	1	-1	1.06 (0.01)
Serine	0	0	1	1	9.17 (0.01)
	0	0	1	2	11.54 (0.03)
	1	0	1	0	8.04 (0.02)
	1	0	2	0	14.54 (0.04)
	1	0	1	-1	1.08 (0.03)
Ornithine	0	0	1	1	10.58 (0.03)
	0	0	1	2	19.43 (0.04)
	0	0	1	3	20.98 (0.05)
	1	0	1	0	11.85 (0.04)
	1	0	2	0	15.95(0.07)
	1	0	1	1	19.69 (0.03)
Lysine	0	0	1	1	10.44 (0.01)
	0	0	1	2	19.66 (0.02)
	1	0	1	0	11.83 (0.02)
	1	0	1	2	15.12 (0.03)
	1	0	1	1	19.44 (0.02)
Histidine	0	0	1	1	9.48 (0.01)
	0	0	1	2	15.76 (0.01)
	0	0	1	3	17.92 (0.04)
	1	0	1	0	10.65 (0.01)
	1	0	2	0	18.68 (0.03)
	1	0	1	1	18.39 (0.02)
Histamine	0	0	1	1	9.88 (0.03)
	0	0	1	2	15.94 (0.05)
	1	0	1	0	9.39 (0.02)
	1	0	2	0	15.12 (0.05)
	1	0	1	1	17.34 (0.02)
Penicillamine	0	0	1	1	10.41 (0.02)
	0	0	1	2	18.29 (0.03)
	0	0	1	3	19.55 (0.09)
	1	0	1	0	15.71 (0.04)
	1	0	2	0	29.02 (0.06)
Imidazole	0	0	1	1	7.06 (0.01)
	1	0	1	0	4.23 (0.01)
	1	0	2	0	7.57 (0.02)
Mercaptoethanol	0	0	1	1	9.52 (0.01)
	0	0	1	2	12.16 (0.02)
	1	0	1	0	11.61 (0.01)
Glycylglycine	0	0	1	1	8.26 (0.009)
	0	0	1	2	11.44 (0.02)
	1	0	1	0	6.46 (0.01)
	1	0	1	-1	1.73 (0.02)
Glycinamide	0	0	1	1	8.06 (0.01)
	1	0	1	0	4.58 (0.01)
	1	0	1	-1	0.64 (0.03)
Glutamine	0	0	1	1	8.99 (0.03)
	1	0	1	0	7.53 (0.04)
	1	0	1	-1	1.71 (0.01)

Aspargine	0	0	1	1	8.56 (0.03)
	1	0	1	0	7.38 (0.04)
	1	0	1	-1	1.41 (0.04)
Inosine	0	0	1	1	8.80 (0.01)
	1	0	1	0	4.50 (0.02)
Inosine-5'-monophosphate	0	0	1	1	9.02 (0.01)
	0	0	1	2	15.24 (0.01)
	1	0	1	0	4.59 (0.03)
Uracil	0	0	1	1	9.18 (0.03)
	1	0	1	0	5.29 (0.04)
Uridine	0	0	1	1	9.01 (0.03)
	1	0	1	0	4.85 (0.04)
Adenine	0	0	1	1	9.65 (0.03)
	0	0	1	2	13.90 (0.04)
	1	0	1	0	5.86 (0.01)
Guanosine	0	0	1	1	9.13 (0.01)
	0	0	1	2	11.11 (0.01)
	1	0	1	0	4.42 (0.01)
Guanosine-5'-monophosphate	0	0	1	1	9.48 (0.01)
	0	0	1	2	15.81 (0.02)
	1	0	1	0	4.47 (0.01)

[a] l, p,q and r are the stoichiometric coefficient corresponding to Cu^{2+}, PA, (bioactive ligands) and H^{+}, respectively.
[b] Standard deviations are given in parentheses.

Table 2: Stability constants of the ternary species in the Cu^{2+}-PA-L systems at 25 °C and I = 0.1 M $NaNO_3$.

System	l	P	q	r[a]	log β[b]	Δlog K
Glycine	1	1	1	0	13.15 (0.03)	-0.87
Alanine	1	1	1	0	13.09 (0.03)	-0.84
Valine	1	1	1	0	13.21 (0.02)	-0.88
Proline	1	1	1	0	14.49 (0.02)	-0.18
β-phenyl alanine	1	1	1	0	13.34 (0.04)	-0.03
Methionine	1	1	1	0	13.60 (0.01)	-0.19
S-methylcysteine	1	1	1	1	13.81 (0.02)	-0.29
Threonine	1	1	1	0	14.17 (0.03)	-0.24
	1	1	1	-1	7.02 (0.01)	-0.11
Serine	1	1	1	0	14.10 (0.02)	-0.01
	1	1	1	-1	7.10 (0.02)	-0.05
Ornithine	1	1	1	0	17.83 (0.01)	-1.52
	1	1	1	1	25.45 (0.01)	-0.31
Lysine	1	1	1	0	17.70 (0.04)	-1.40
	1	1	1	1	25.28 (0.01)	-0.23
Histidine	1	1	1	0	16.52 (0.04)	-0.20
	1	1	1	1	24.15 (0.03)	-0.31
Histamine	1	1	1	0	15.38 (0.01)	-0.08
	1	1	1	1	23.22 (0.02)	-0.19
Penicillamine	1	1	1	0	21.45 (0.02)	-0.33
Imidazole	1	1	1	0	10.23 (0.007)	-3.4
Mercaptoethanol	1	1	1	0	17.88 (0.03)	0.2
Methylamine	1	1	1	0	11.58 (0.007)	-1.29
Glycylglycine	1	1	1	0	12.12 (0.03)	-0.41
	1	1	1	-1	6.89 (0.02)	-0.91
	1	1	1	-2	-2.63 (0.03)	

	1	0	2	0	14.16 (0.05)	
Glycinamide	1	1	1	0	10.55 (0.02)	-0.10
	1	1	1	-1	5.75 (0.01)	-0.91
	1	1	1	-2	-4.52 (0.02)	
Glutamine	1	1	1	0	13.37 (0.03)	-0.23
	1	1	1	-1	6.67 (0.05)	-1.11
	1	1	1	-2	-3.36 (0.07)	
Aspargine	1	1	1	0	13.13 (0.03)	-0.32
	1	1	1	-1	6.56 (0.02)	-0.92
	1	1	1	-2	-3.21 (0.04)	
Inosine	1	1	1	0	11.20 (0.03)	0.63
Inosine-5'-monophosphate	1	1	1	0	11.55 (0.03)	0.89
Uracil	1	1	1	0	11.65 (0.03)	0.29
Uridine	1	1	1	0	11.25 (0.03)	0.33
Adenine	1	1	1	0	12.92 (0.03)	0.99
Guanosine	1	1	1	0	10.95 (0.03)	0.46
Guanosine-5'-monophosphate	1	1	1	0	11.15 (0.03)	0.61

[a] l, p,q and r are the stoichiometric coefficient corresponding to Cu^{2+}, PA, (bioactive ligands) and H^+, respectively. [b] Standard deviations are given in parentheses.

Results and Discussion

The acid dissociation constants of the ligands and the formation constants of their binary complexes were determined under the same experimental conditions of ionic strength and temperature used to study the ternary complexes. The results obtained listed in Table 1 are in good agreement with the literature data [19].

3.1. Ternary Complex Formation Equilibria Involving Amino Acids and Peptides

Ternary complex formation may precede either through a stepwise or simultaneous mechanism depending on the chelating potential of picolinic acid and the other ligands. The formation constants of the 1:1 Cu^{2+} complexes with picolinic acid, amino acids or peptides are of the same order (Table 1). Consequently the ligation of picolinic acid (PA) and amino acids, or peptides(HL), will proceed simultaneously. The titration data of the ternary complexes with amino acids and picolinic acid fit satisfactorily with formation of the species: [Cu(PA)], [Cu(L)] and [Cu(PA)(L)]. Serine forms, in addition to the previously mentioned complexes the [Cu(PA)(LH^{-1})] species. This complex is formed through induced ionization of the β-alcohol group, as mentioned in the literature [20]. Ornithine and lysine are α-amino acids having an extra amino group, which may be protonated. Consequently, the protonated ternary complex was detected. Ornithine and lysine may bind to Cu^{2+} ion as α-amino acid (N,O-donor set) or by α- and ω-amino groups (N,N-donor set). The stability constants of the ternary complexes (Table 2) are higher than those of α-amino acids, indicating that lysine and ornithine are ligating by the two amino groups. The species distribution of ornithine, taken as a representative amino acid, is given in Fig. 1. The protonated 1111 complex species predominates with a formation degree amounting to 94.3% at pH 5.8, whereas the deprotonated species 1110 attains a maximum concentration of 97% at pH 10.6. Therefore, the species 1111 predominates in the physiological pH range. For clarity the species with concentrations less than 5% were neglected in the concentration distribution plot.

Histidine is a tridentate ligand having amino, imidazole and carboxylate groups as binding sites. For $[Cu(PA)]^+$, only two of the three binding sites are involved in complex formation, but histamine has two binding sites via the imidazole and amino groups. Hence, histidine coordinates in either a glycine-like or histamine-like mode. The stability constant values of histidine and histamine are of the same magnitude and are considerably higher than those of the amino acids, indicating that both histidine and histamine would coordinate preferably through the amino and imidazole groups.

The potentiometric data of the [Cu(PA)]-peptide system were fitted by various models. The most acceptable model was found to be consistent with the formation of the complexes with stoichiometric coefficients 1110 and 111-1. In the 1110 case, the peptide is bound through the amino and carbonyl oxygen groups. On increasing the pH, the coordination sites should switch from the carbonyl oxygen to the amide nitrogen. Such a change in coordination centers is now well documented [21, 22]. The amide groups undergo deprotonation and the [Cu(PA)(LH^{-1})] complexes are formed. The pK^H values are calculated by the following equation:

$$pKH = \log \beta_{1110} - \log \beta_{111\text{-}1} \quad (3)$$

It is noteworthy that the pK^H for the glycinamide complex is lower than the pK^H values of other peptides. This signifies that the more bulky substituent group on the peptide may serve to hinder the structural change in going from the protonated to deprotonated complexes. On the other hand, the pK^H of the glutamine complex is relatively much higher than the others. This is due to the formation of a seven-membered chelate ring, which would be more strained and less favoured. Therefore, under physiological conditions (pH≈7.4) glutamine would coordinate in its protonated form, whereas glycinamide would preferably coordinate in the deprotonated form. The distribution diagram of the glycylglycine complex is given in Fig. 2. The mixed ligand species [Cu(PA)L] (1110) starts to form at pH~3.4 and, with increasing pH, its concentration increases reaching a maximum of 33% at pH = 5. A further increase of pH is accompanied by a decrease in the 1110 complex concentration and an increase in [Cu(PA)LH^{-1}] (111-1) complex formation.

Although the constant β_{1110} expresses the overall stability of the ternary complex [Cu(PA)L] as expressed by Eq. (1), it does not represent directly the binding strength between the amino acids and the copper(II) ion. The tendency towards ternary complex formation can be evaluated in various ways. Δlog K has been widely accepted and used for many years [23] and the advantages in using Δ log K in comparing the stabilities of ternary and binary complexes have been reviewed. The Δlog K values for protonated and deprotonated ternary complexes are given by Eqs. (4) and (5) whereas those of the induced deprotonated peptide complex can be calculated using Eq. (6).

$$\Delta \log K = \log \beta_{1111} - \log \beta_{1100} - \log \beta_{1011} \quad (4)$$
$$\Delta \log K = \log \beta_{1110} - \log \beta_{1100} - \log \beta_{1010} \quad (5)$$
$$\Delta \log K = \log \beta_{111-1} - \log \beta_{1100} - \log \beta_{101-1} \quad (6)$$

The values of Δlog K for the ternary complexes studied in this paper are listed in Table 2. The theoretical Δ log K value for a distorted-octahedral Cu^{2+} complex is -0.9 [24]. The tendency to form ternary complexes was compared with this value, so that if Δ logK is greater than -0.9, this should be taken as an indication that the ternary complex is favored. The Δ logK values of α-amino acids are less negative than the theoretical value(-0.9). This may be considered as evidence for the occurrence of enhanced stabilities involving π back-donation from the negatively charged amino acid to the π -system of the picolinic acid. The Δ logK for the ornithine mixed ligand complex (1110) is more negative than-0.9. This may be described on the premise that ornithine is a tridentate ligand and that two coordination sites are available in the $[Cu(PA)]^+$ complex. The ΔlogK values for ternary complexes of phenylalanine are much less negative than-0.9. This may be explained on the premise that the non-coordinating aromatic side groups of these amino acids can approach the pyridine ring of picolinic acid and exert a stacking interaction.

The Δ logK values for the induced deprotonated peptide ternary complexes, [Cu(PA)(LH^{-1})] are also considerably more negative than -0.9. This may be taken as an indication that formation of the ternary peptide complexes is less favoured than of binary ones. This may be explained on the premise that the deprotonated peptide is coordinated with the free Cu^{2+} ion as a tridentate ligand, whereas in the ternary complex two coordination sites are available in the $[Cu(PA)]^+$ complex.

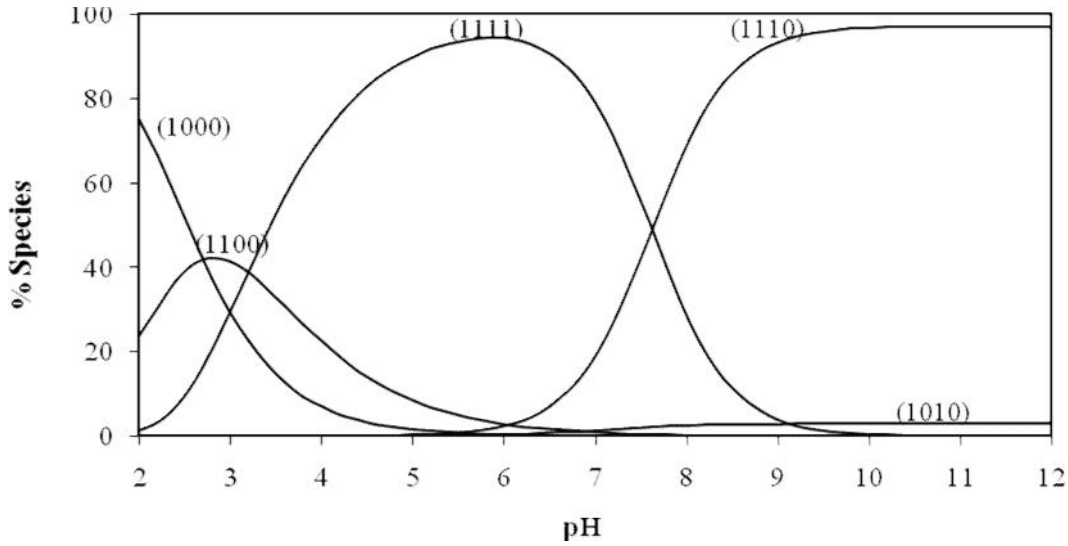


Fig. 1. Concentration distribution of various species as a function of pH in the Cu-PA-ornithine system.

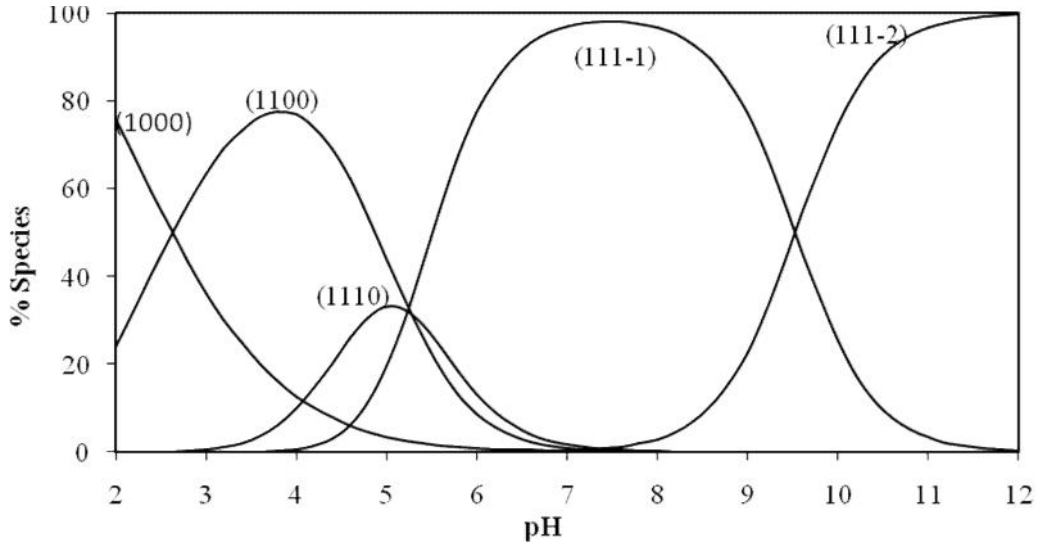


Fig. 2. Concentration distribution of various species as a function of pH in the Cu-PA-Glycylglycine system.

3.2. Complex formation equilibria involving DNA constituents

In the ternary complexes of DNA constituents, (D), the potentiometeric titration curves of the mixed ligand system Cu(II)-PA-uracil (as a representative of DNA species)are shown in figure 3. In this respect, the formation of a ternary complex was ascertained by comparison of the mixed-ligand titration curve with the composite curve obtained by graphical addition of uracil titration data to that of the Cu(II)-PA titration curve. The mixed ligand system was found to deviate considerably from the resulted composite curve, indicating the formation of a ternary complex. Thus, the following equilibria can be written to describe the formation of the ternary complex (charges are omitted for simplicity).

$$Cu + PA \rightleftharpoons [Cu(PA)] \quad (7)$$

$$[Cu(PA)] + D \rightleftharpoons [Cu(PA)D] \quad (8)$$

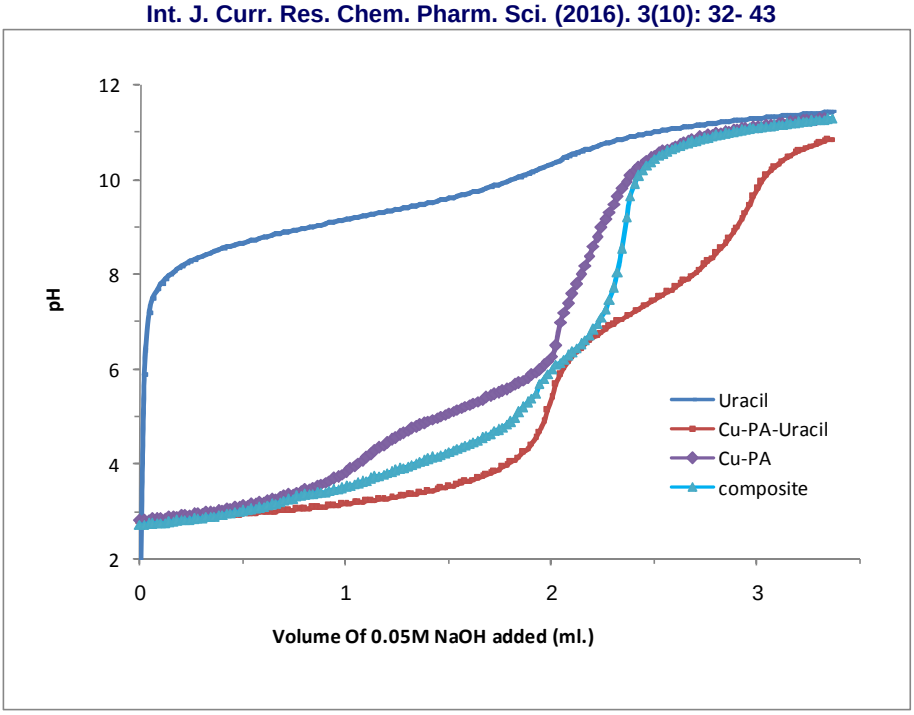


Fig.3. Potentiometric titration curve of Cu-PA- uracil system.

The pyrimidines, uracil and uridine have only basic nitrogen donor atoms (N_3-C_4O group) in the measurable pH range and as a consequence they form 1:1 and 1:2 complexes with $[Cu(PA)]^+$ ion. As a result of the high pK_a values of pyrimidines (pK_a> 9) and the fact that they are monodentate, the complexes are formed only above pH 6, supporting the view that the negatively charged nitrogen donors of pyrimidine bases are important binding sites in the neutral and slightly basic pH ranges. The purines inosine,inosine5-monophosphate,guanosine and guanosine 5-monophosphate have two metal ion binding centres at N_1 and N_7. The pH dependent binding of these N-donors was already reported.

The relative stability of the ternary complexes formed through a stepwise mechanism, as compared to those of the corresponding binary complex, is expressed in terms of Δlog K as defined by Eq. (9).

$$\Delta \log K = \log k^{Cu(PA)}_{Cu(PA)D} - \log k^{Cu}_{CuD} \quad (9)$$

The Δ log K values (Table 2) are invariably positive. This means that DNA constituents form more stable complexes with $[Cu(PA)]^+$ than with the free Cu^{2+} ion. This fact may be taken as evidence for a stacking interaction between the picolinic acid ring and the DNA constituent.

3.3.Spectrophotometric measurements

The spectrum of the aquated Cu^{2+} ion (mixture A) consists of a broad, weak band with a maximum λ_{max} at 810 nm being attributed to the $^2T_{2g} \leftarrow {}^2E_g$ transition [25, 26]. The spectral bands of the binary and ternary Cu^{2+} complexes are quite different from that of the aquated Cu^{2+} ion both as regards the position of the λ_{max} and their average molar absorptivity as shown in Fig. 4. The spectrum of the $[Cu(PA)]^+$ complex (mixture B) shows an absorption maximum at 717 nm. On the other hand, the spectra obtained from the ternary complex of the Cu^{2+} with PA and glycylglycine as a function of the number of equivalents, a, of base per ligand (mixtures C and D) exhibit a band at 645 nm (mixture C). The shift toward shorter wavelengths in the spectrum is an indication of the presence of an additional nitrogen center coordinated to Cu^{2+} in the complex. As the value of an increases, (mixture D) there is a progressive shift of the absorption maximum towards shorter wavelengths appearing at 633 nm. This may be taken as evidence supporting the potentiometric measurements for the induced ionization of peptide hydrogen upon complex formation.

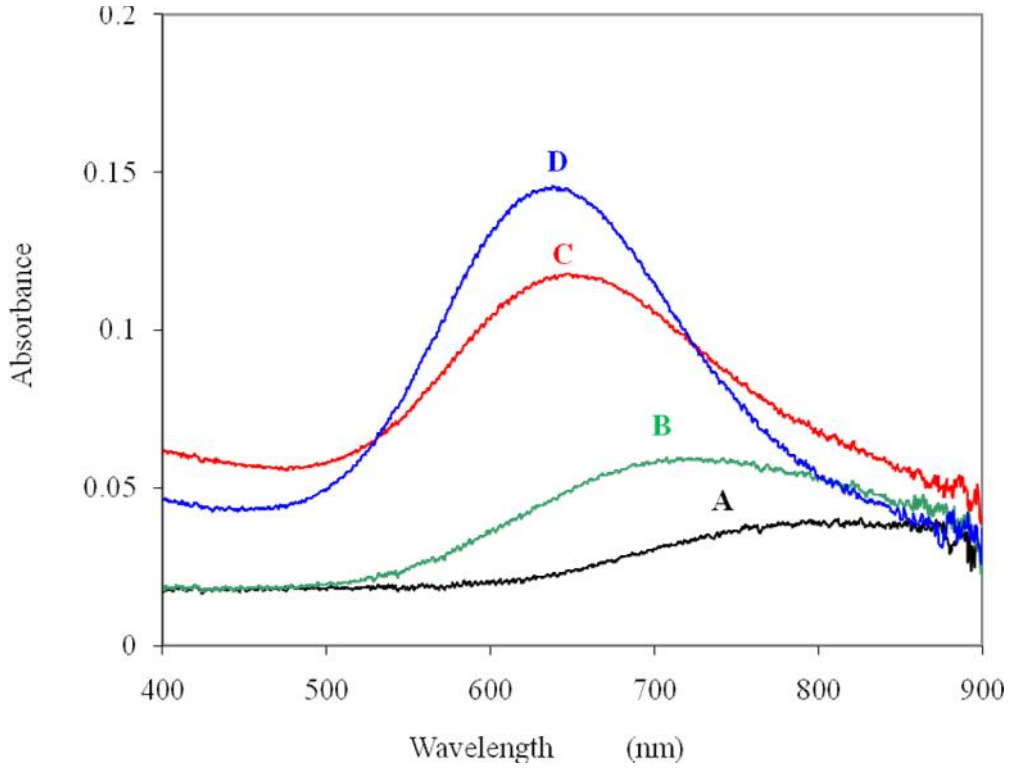
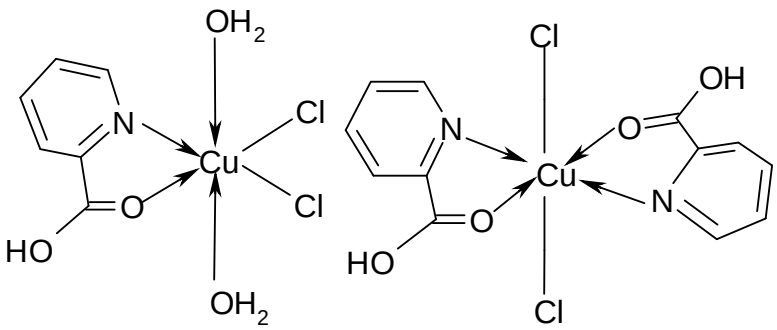


Fig.4.Visiblespectraofthe[Cu-PA-glycylglycine]system.(MixturesA-Daredescribedintheexperimentalpart).

3.4. IR spectra of the solid complexes

The IR spectra of the two isolated solid Cu^{2+} complexes, derived from picolinic (PA) acid, show that the ligand (PA) coordinates in a bidentate manner through the carbonyl (CO) and the (C=N) groups forming a stable five-member ringaround theCu^{2+} ion. The observation of the OH group at ≈ 3500cm^{-1} indicates that this group is not participating in bonding. On the other hand the carbonyl at 1714 and the C=N at 1650 cm^{-1} bands in the IR spectrum of PA are shifted to lower wave numbers in case of the spectra of the two Cu^{2+} complexes (1:1 and 1:2). The carbonyl band is observed at 1675 and the azomethine (C=N) at 1475 cm^{-1}, respectively. The observation of these bands confirms that PA coordinates without displacement of a proton from the carboxylic group. Also, the existence of the chloride ions is taken as additional evidence that the ligand (PA) coordinates to the Cu^{2+} ion without losing the proton from the carboxylic group. The insolubility of the complexes in H_2O suggests that the complexes are neutral as shown in Fig. 5. Finally, the weak bands at 460 and 420cm^{-1}, which are obscured in the IR spectrum of the free ligand (PA), are assigned respectively, to ν(M-N) and ν(M-Cl) vibrations. All these foundations suggest that the two isolated complexes in the solid state, $[Cu(PA)(Cl_2)(H_2O)_2]$ and $[Cu(PA)_2(Cl_2)]$, are neutral and have a distorted-octahedral structure.



$[Cu(PA)Cl_2(H_2O)_2]$ complex$[Cu(PA)_2Cl_2]$ complex

Fig. 5.Chemical structure of isolated complexes.

3.5. Antimicrobial activity

The main target of the synthesis and production of any antimicrobial compound is to inhibit the causal microbe without any side effects on the patients. Transition metal complexes are well known to have an enhanced antimicrobial activity [27, 28], therefore it seems interesting to screen the biological potential of the metal complexes against different species of bacteria and fungi. The in vitro antimicrobial activity of the compound was tested against *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram-negative bacteria) also for their antifungal activity against *Aspergillus fumigatus* and *Geotricum candidum* and then compared with standard antibacterial and antifungal drug. The results are displayed in Table 3. Based on comparing the biological activity of the compound with the standards ampicillin for G (+ve) bacteria, Gentamicin for G (-ve) bacteria (antibacterial agent) and amphotericin B (antifungal agent), Cu-Picolinate complex has inhibitory action against *Bacillus subtilis* and *Staphylococcus aureus* (Gram-positive bacteria), and this action is higher than that of ampicilline standard, but has slight antibacterial activity against Gram-negative (*Escherichia coli*) and no antibacterial activity against *Pseudomonas aeruginosa* when compared with standard Gentamicin. The examined compound also showed antifungal activity against *Aspergillus fumigatus* and *Geotricum candidum*.

These results can be rationalized on the basis of chelation theory [29]. On chelation, the polarity of the metal ion will be reduced to a greater extent mainly because of the partial sharing of its positive charge with the donor groups within the chelate ring system formed during the coordination and also possibly due to π-electron delocalization on the whole chelate ring system. This process of chelation, in turn, increases the lipophilic nature of the central metal atom, which subsequently favors its permeation more efficiently through the lipid layer of the microorganism resulting in the interference with normal cell process, and hence destroying them more aggressively [30]. The variations in the effectiveness of the different compounds against different microorganisms depend on the impermeability of the cells of microbes or difference in ribosome of the microbial cells [31]

.

Table 3.Antimicrobial activity of $[Cu(PA)]^+$ complex (inhibition zone diameter (mm/mg sample)).

Compounds	Microbial species					
	Bacteria				Fungi	
	P. aeruginosa	***E.coli***	***B.subtilis***	***S.aureus***	***A. fumigatus***	***G. candidum***
$[Cu(PA)Cl_2(H_2O)_2]$ Complex	-	9.2	21.7	20.6	16.8	19.1
Control (DMSO)	0.0	0.0	0.0	0.0	0.0	0.0
Standard						
Ampicilline	-	-	20	18	-	-
Gentamicin	17	22	-	-	-	-
Amphotericin B	-	-	-	-	17	19

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