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Complex Formation Reactions of Promethazine Copper(II) and Various Biologically Relevant Ligands. Synthesis, Equilibrium Constants, Spectroscopic Characterization and Biological Activity

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Abstract Binary and ternary complexes of copper(II) involving promethazine, N,N-dimethyl-3-(phenothiazin-10-yl)propylamine (Prom) and various biologically relevant ligands containing different functional groups, were investigated. The ligands (L) are dicarboxylic acids, amino acids, amides and DNA constituents. The ternary complexes of amino acids, dicarboxylic acids or amides are formed by simultaneous reactions. The results showed the formation of Cu(Prom)(L) complexes with amino acids and dicarboxylic acids. The effect of chelate ring size of the dicarboxylic acid complexes on their stability constants was examined. Amides form both Cu(Prom)(L) complexes and the corresponding deprotonated species Cu(Prom)(LH_{-1}). The ternary complexes of copper(II) with (Prom) and DNA are formed in a stepwise process, whereby binding of copper(II) to (Prom) is followed by ligation of the DNA components. DNA constituents form both 1:1 and 1:2 complexes with $Cu(Prom)^{2+}$. The stability of these ternary complexes was quantitatively compared with their corresponding binary complexes in terms of the parameters $\Delta \log_{10} K$. The values of $\Delta \log_{10} K$ indicate that the ternary complexes containing aromatic amino acids were significantly more stable than the complexes containing alkyl- and hydroxyalkyl-substituted amino acids. The concentration distribution of various complex species formed in solution was also evaluated as a function of pH. The solid complexes [Cu(Prom)L)] where L = 1,1-cyclobutanedicarboxylic acid (CBDCA), oxalic and malonic acid were isolated and characterized by elemental analysis, infrared, TGA, and magnetic susceptibility measurements. Spectroscopic studies of the complexes revealed that the complexes exhibits square planar coordination with copper(II). The isolated solid complexes have been screened for their antimicrobial activities using the disc diffusion method against some selected bacteria and fungi. The activity data show that the metal complexes are found to have antibacterial and antifungal activity.

Keywords Copper(II) · Promethazine · Amino acids · Amides · DNA constituents · Stability constant · Spectroscopic characterization · Biological activity

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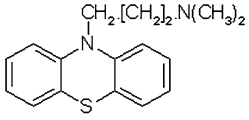
1 Introduction

The study of the formation of mixed ligand complexes is of growing importance, particularly due to their remarkable pharmacological activity and their role in different biological processes [1]. Many drugs possess modified pharmacological and toxicological properties when administered in the form of metallic complexes, as platinum anticancer drugs and Cu^{2+} complexes of bioactive compounds, which are the most widely studied complexes in this respect and have been proven efficient against diseases such as gastric ulcers, rheumatoid arthritis and cancer [2]. Ternary complexes involving an aromatic amine as the primary ligand and various amino acids, amides or DNA units as secondary ligands can serve as useful models for gaining a better understanding of enzyme-metal ion-substrate complexes, which play an important role in metalloenzyme-catalyzed biochemical reactions and other metal ion mediated biochemical interactions [3]. Among these compounds copper(II) complexes of heterocyclic amines are of great interest since they exhibit numerous biological activities such as antitumor [4], anticandida [5], antimycobacterial [6], antimicrobial activity [7], etc. In a number of biochemical processes Cu(II) is involved in mixed-ligand complex formation and ligand catalyzed complex formation reactions.

N-substituted phenothiazines are a pharmaceutically important class of tricyclic compounds. These compounds modulate a variety of biomedical processes such as the uptake of norepinephrine, dopamine, and acetylcholine neurotransmission, and the biological effect of histamine [8]. One of the recent studies on the pharmaceutical activities of phenothiazines reports on their ability to affect the antibiotic resistance of bacteria and tumor cells [9]. As a result of new pharmacological investigations, they are applied as antiarrhythmic drugs, lipid peroxidation inhibitors, and photodynamic virus inactivators [10]. Their low oxidation potential and the formation of stable radical cation are important reasons for their physiological activities [11, 12]. It has been suggested that the biological activity of phenothiazines is related to the ability of the central ring of the heterocycle to form a molecular complex at the receptor site [13]. In view of the above facts and the interest in studying the ligation behavior of such compounds, and in continuing our studies in complex formation equilibria of bio-relevant ligands such as amino acids [14–17], peptides [18, 19] and DNA [20–23] constituents, it seems to be of considerable interest to conduct several investigations covering binary and ternary complexes of copper(II) involving Prom and bio-relevant ligands. In this investigation, we report a quantitative study of the formation equilibria of binary and ternary complexes of copper(II) with Prom and some bio-relevant ligands. The solid complexes Cu(Prom)L where L = CBDCA, oxalic and malonic acid are synthesized and characterized using different physico-chemical methods like elemental analysis, I.R., magnetic moment determination, electronic spectral measurements and thermal analysis. The study also includes biological activity of the synthesized metal complexes since the synthesis and characterization of new metal complexes with antibacterial agents are of great importance for understanding the drug-metal ion interaction and for their potential pharmacological use.



Promethazine

2 Experimental

2.1 Chemicals

N,N-dimethyl-3-(phenothiazin-10-yl)propylamine (Prom) were obtained from Sigma Chem. Co. Glycine, phenylalanine, proline, tyrosine, tryptophane, threonine, L-histamine·2HCl, L-histidine·HCl, L-ornithine, ethanolamine·HCl together with the dicarboxylic acids, oxalic acid, malonic acid, succinic acid, adipic acid and (CBDCA) were provided by the Sigma Chem. Co. The amides used were glutamine, aspargine and glycineamide as well as glycylglycine as a model of peptides, which were also provided by Sigma Chem. Co. The DNA constituents uracil, uridine, thymine, thymidine, inosine, inosine 5′-monophosphate were supplied by BDH-Biochemicals Ltd. For stability constant determination, solutions of (Prom) were prepared in two equivalents of HNO_3 acid, freshly prepared solutions of (Prom) were used for all the measurements. L-histidine·HCl was prepared in one equivalent of HNO_3 acid, $Cu(NO_3)_2{\cdot}2H_2O$ was provided by BDH. The copper content of the solutions was determined by complexometric EDTA titrations [24]. Carbonate free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All chemicals were of the highest purity commercially available and were used without further purification. All solutions were prepared in deionized water by dissolving the chemicals shortly before use.

2.2 Synthesis of the Ternary Metal Complexes

The ternary complexes were prepared according to the method reported in the literature [14, 15] as follows: copper(II) chloride (265 mg, 1.56 mmol) dissolved in a minimum quantity of water was added slowly to (500 mg, 1.56 mmol) of the Prom with stirring. The dibasic acids (163 mg, 1.56 mmol for malonic), (224 mg, 1.56 mmol for CBDCA) and (197 mg, 1.56 mmol for oxalic) were then added to the above mixture with continuous stirring, followed by the addition of (166 mg, 1.56 mmol) of Na_2CO_3 to neutralize the released protons. The mixture was stirred with heating for further 2 h and stored overnight. The precipitated complex was isolated by vacuum filtration and washed three times with water, ethanol and diethyl ether. Elemental analyses as well as magnetic measurement data of the prepared metal complexes are presented in Table 5.

2.3 Antibacterial Activity

The metal complexes were evaluated for their antibacterial activity against Staphylococcus aureus (Gram-positive bacteria), Escherichia coli (Gram-negative bacteria) and also for their antifungal activity against Candida albicans and Aspergillus flavus using the disc diffusion technique as described in British Pharmacopoeia (2000). Nutrient agar was melted at 45.00 °C, and inoculated with the cell suspension (1:100) bacteria or yeast. The flask was shaken well and poured into a Petri-dish (15 cm in diameter). Filter paper discs (6 mm) Whatman No. 2 were thoroughly moistened with antibiotics (50 g) the treated discs were aseptically transferred and placed on the surface of the inoculated plates and kept in a refrigerator for 1 h to permit diffusion of antimicrobial substances. The plates were incubated at 37.00 °C, for 24 h in the case of bacteria and at 28.00 °C, for 48 h in the case of fungi. The zones of inhibition were measured in mm. The mean values of inhibition were calculated from triple reading in each test [25, 26].

The following media were used in studying the antimicrobial properties of drug complexes. The weights are given in gram per one-liter medium.

1. Nutrient agar medium (pH = 7.4): It consists of beef extract (1 g), yeast extract (2 g), peptone (5 g), sodium chloride (5 g), agar agar (15 g) and distilled water (100 cm^3).
2. Sabouroud's dextrose agar medium (pH = 5.6): It consists of peptone (10 g), dextrose (20 g), agar agar (15 g) and distilled water (100 cm^3). Moreover, some commercialized antibiotics were evaluated for their antibacterial activity and a comparison with the synthesized complexes present in this investigation was drawn.

2.4 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 [27] at $25.00 \pm 0.1\,^{\circ}C$, and $I = 0.1$ mol·dm^{-3}, potassium hydrogen phthalate (pH = 4.008) and a mixture of KH_2PO_4 and Na_2HPO_4 (pH = 6.865). The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.01 mol·dm^{-3}), the ionic strength of which was adjusted to 0.1 mol·dm^{-3} with $NaNO_3$, with standard base (0.10 mol·dm^{-3}) at 25 °C. The pH is plotted against p[H]. The relationship pH − p[H] = 0.05 was observed. The concentration of OH^- was calculated using a pK_w value of 13.997 [28]. All potentiometric titrations were carried out at $25.00 \pm 0.05\,^{\circ}C$, in a double-walled glass cell of 50 cm^3 capacity. The temperature of all solutions was maintained at $25.00 \pm 0.05\,^{\circ}C$ by circulation of thermostated 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·dm^{-3} ($NaNO_3$). The microchemical analysis of the separated solid complexes for C, H and N was performed in the Microanalytical Center, Cairo University. The analyses were performed twice to check the accuracy of the analytical data. IR spectra were measured on a 80486-pc FTIR Shimadzu spectrophotometer using KBr pellets. UV-visible spectra of freshly prepared solutions of the complexes in ethanol were measured at room temperature using a Shimadzu UV-160A recording spectrophotometer. A Shimadzu TGA-50H thermal analyzer was used to record simultaneously TGA and the differential curves. The measurements were carried out in N_2 (20 cm^3·min^{-1}) with a heating rate of $10\,^{\circ}C$·min^{-1} from 20.00 to 800.00 °C using platinum crucibles. The magnetic susceptibility measurements for the complexes were determined by the Gouy balance using $Hg[Co(NCS)_4]$ as a calibrant at room temperature [29].

2.5 Equilibrium Measurements

The acid dissociation constants of the ligands were determined potentiometrically by titrating the ligand (40 cm^3) solution (1.25×10^{-3} mol·dm^{-3}) of constant ionic strength 0.1 mol·dm^{-3} (adjusted with $NaNO_3$). The stability constant of the Cu(Prom) complex was determined by titrating 40 cm^3 of a solution mixture of Cu^{II} (1.25×10^{-3} mol·dm^{-3}), Prom ligand (2.5×10^{-3} mol·dm^{-3}) and $NaNO_3$ (0.1 mol·dm^{-3}). The formation constant of the mixed ligand complexes were determined by titrating solution mixtures containing equivalent amounts of Cu^{II} (1.25×10^{-3} cm^3), Prom and other ligands in the concentration ratio 1:1:1 for amino acids, dicarboxylic acids, and amides and, 1:1:2 for the DNA constituents. All titrations were performed in a purified N_2 atmosphere using aqueous 0.05 mol·dm^{-3} NaOH as titrant.

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

$$l(\mathrm{Cu}) + p(\mathrm{Prom}) + q(\mathrm{L}) + r(\mathrm{H}) \rightleftharpoons (\mathrm{Cu})_l(\mathrm{Prom})_p(\mathrm{L})_q(\mathrm{H})_r \quad (1)$$

$$\beta_{lpqr} = \frac{[(\mathrm{Cu})_l(\mathrm{Prom})_p(\mathrm{L})_q(\mathrm{H})_r]}{[\mathrm{Cu}]^l[\mathrm{Prom}]^p[\mathrm{L}]^q[\mathrm{H}]^r} \quad (2)$$

The calculations were obtained from approxmately 100 data points in each titration using the computer program [30] MINIQUAD-75. The stoichiometries 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 without giving any systematic drifts in the magnitudes of various residuals [30]. The results obtained are shown in Tables 1, 2, 3 and 4. The concentration distribution diagrams were obtained using the program SPECIES [31] under the experimental condition used.

2.6 Spectrophotometric Measurements

Spectrophotometric investigation of the binary and ternary complexes was performed by scanning the visible spectra of solution mixtures A–D. Under the experimental conditions and after neutralization of the hydrogen ions released, it is assumed that the complexes have been completely formed. Mixtures A–D were prepared to be used for spectrophotometric measurements. In each case the volume is made up to 10 cm^3 by water, and the ionic strength is kept constant by using $NaNO_3$ (0.1 $mol \cdot dm^{-3}$)

(A) 1.0 cm^3 Cu(II) (0.01 $mol \cdot dm^{-3}$) + 1.25 cm^3 $NaNO_3$ (0.8 $mol \cdot dm^{-3}$)
(B) 1.0 cm^3 Cu(II) (0.01 $mol \cdot dm^{-3}$) + 2.0 cm^3 Prom (0.01 $mol \cdot dm^{-3}$) + 2 cm^3 NaOH (0.01 $mol \cdot dm^{-3}$) + 1.25 cm^3 $NaNO_3$ (0.8 $mol \cdot dm^{-3}$)
(C) 1.0 cm^3 Cu(II) (0.01 $mol \cdot dm^{-3}$) + 1.0 cm^3 Prom (0.01 $mol \cdot dm^{-3}$) + 1.0 cm^3 glycylglycine (0.01 $mol \cdot dm^{-3}$) + 3.0 cm^3 NaOH (0.01 $mol \cdot dm^{-3}$) + 1.25 cm^3 $NaNO_3$ (0.8 $mol \cdot dm^{-3}$)
(D) 1.0 cm^3 Cu(II) (0.01 $mol \cdot dm^{-3}$) + 1.0 cm^3 Prom (0.01 $mol \cdot dm^{-3}$) + 1.0 cm^3 glycylglycine (0.01 $mol \cdot dm^{-3}$) + 4.0 cm^3 NaOH (0.01 $mol \cdot dm^{-3}$) + 1.25 cm^3 $NaNO_3$ (0.8 $mol \cdot dm^{-3}$)

3 Results and Discussion

The acid dissociation constants of the ligands and the formation constants of their binary complexes presented in Tables 1, 2 and 3 were determined under the same experimental conditions of ionic strength and temperature used to study the Cu(II)–Prom and the corresponding ternary complexes. The results obtained are in good agreement with the literature data [14, 15, 32]

3.1 Ternary Complex Formation

Ternary complex formation may proceed either through a stepwise or a simultaneous mechanism depending on the chelating potential of promethazine and other ligands. The formation constants of the 1:1 copper(II) complexes with Prom and those of amino acids, dibasic acids or amides, taken from the literature and cited in Tables 1, 2 and 3, are nearly of the same order. Consequently the ligation of Prom and amino acids, dicarboxylic acids, or of the amides will proceed simultaneously. The validity of this model was verified by comparing the experimental potentiometric data with the theoretically calculated (simulated) curve. Figure 1 presents such a comparison for the Cu–Prom–glycine system, taken as a representative one. Figure 2 presents the potentiometric titration curve for the Cu–Prom–Proline system according to the simultaneous mechanism.

Table 1 Formation constants of the binary and ternary complexes in the Cu^{II}–Prom–amino acids at 25 °C and 0.1 mol·dm^{-3} ionic strength

System	l	p	q	r^a	$\log_{10}\beta^b$	pK_a^c	$S^d/10^{-8}$	$\Delta\log_{10}K$
$[Cu(H_2O)_4]^{2+}$	1	0	0	−1	−6.44(0.07)			
	1	0	0	−2	12.99(0.02)		96	
Prom	0	1	0	1	8.49(0.01)	8.49	1.7	
	0	1	0	2	11.21(0.01)	2.72		
	1	1	0	0	8.32(0.01)		35	
							0.31	
Glycine	0	0	1	1	9.60(0.01)	9.6	16	
	0	0	1	2	11.93(0.02)	2.3		
	1	0	1	0	8.26(0.07)		30	
	1	0	2	0	13.57(0.09)			
	1	0	1	−1	0.34(0.08)			
	1	0	1	−2	10.07(0.06)			
	1	1	1	0	17.76(0.01)		35	1.18
	1	1	1	1	22.76(0.02)	5.0		
β-phenyl-alanine	0	0	1	1	9.12(0.01)	9.12	2.0	
	0	0	1	2	11.01(0.03)	1.89		
	1	0	1	0	7.53(0.04)		4.3	
	1	0	2	0	13.73(0.03)			
	1	0	1	−1	0.001(0.05)			
	1	0	1	−2	−10.08(03)			
	1	1	1	0	18.68(0.02)		14	2.83
	1	1	1	1	23.52(0.01)	4.84		
Proline	0	0	1	1	10.52(0.01)	15.51	4.4	
	0	0	1	2	12.03(0.04)	1.51		
	1	0	1	0	8.60(0.03)		1.6	
	1	0	2	0	15.09(0.07)			
	1	0	1	−1	1.29(0.04)			
	1	0	1	−2	−8.58(0.03)		5.5	
	1	1	1	0	18.45(0.02)		17	
	1	1	1	1	23.95(0.02)	5.5		1.53
L-threonine	0	0	1	1	9.11(0.01)	9.11	7.0	
	0	0	1	2	11.32(0.02)	2.21		
	1	0	1	0	8.66(0.03)		3.1	
	1	0	2	0	14.13(0.04)			
	1	0	1	−1	0.90(0.05)			
	1	0	1	−2	−8.39(0.02)			
	1	1	1	0	16.90(0.02)		22	−0.28
	1	1	1	1	21.84(0.03)	4.94		
	1	1	1	−1	8.30(0.06)	8.6		−0.36

Table 1 (*Continued*)

System	l	p	q	r^{a}	$\log_{10}\beta^{b}$	pK_a^{c}	$S^{d}/10^{-8}$	$\Delta\log_{10}K$
Tryptophan	0	0	1	1	9.52(0.01)	9.52	3.2	
	1	0	1	0	7.72(0.02)			
	1	0	1	1	13.26(0.01)			
	1	0	2	0	15.36(0.01)		0.83	
	1	1	1	0	18.57(0.03)			2.53
	1	1	1	1	24.64(0.01)	6.07	84	3.06
Tyrosine	0	0	1	1	10.18(0.002)	10.18	1.1	
	0	0	1	2	19.42(0.003)	9.24		
	0	0	1	3	22.28(0.02)			
	1	0	1	0	10.17(0.03)		1.3	
	1	0	1	1	17.83(0.02)			
	1	0	2	0	15.81(0.02)			
	1	0	2	1	25.2(0.04)			
	1	0	2	2	34.68(0.02)			
	1	1	1	0	19.22(0.03)		92	0.73
	1	1	1	1	29.70(0.04)	10.48		3.55
Histidine	0	0	1	1	9.53(0.01)	9.53	18	
	0	0	1	2	15.81(0.02)	6.28		
	1	0	1	0	11.48(0.01)		0.55	
	1	0	2	0	19.70(0.06)			
	1	0	1	1	15.75(0.04)			
	1	0	1	−1	6.82(0.03)			
	1	0	1	−2	−3.77(0.04)			
	1	1	1	0	20.07(0.05)		45	0.27
	1	1	1	1	24.93(0.05)	4.86		0.86
Histamine	0	0	1	1	9.88(0.01)	9.88	2.4	
	0	0	1	2	15.97(0.01)	6.09		
	1	0	1	0	10.20(0.01)		2.8	
	1	0	2	0	17.53(0.03)			
	1	0	1	1	14.89(0.01)			
	1	0	1	−1	3.48(0.04)			
	1	0	1	−2	−5.86(0.02)		25	
	1	1	1	0	19.50(0.02)			0.98
	1	1	1	1	25.70(0.02)	6.20		2.49
Ornithine	0	0	1	1	10.74(0.00)	10.58	1.0	
	0	0	1	2	19.62(0.01)	8.86		
	0	0	1	3	21.31(0.02)		16	
	1	0	1	0	11.71(0.03)			
	1	0	2	0	16.30(0.02)			
	1	0	1	1	18.42(0.06)			
	1	0	1	−1	1.86(0.07)		48	
	1	0	1	−2	−8.14(0.03)			

Table 1 (*Continued*)

System	l	p	q	r^{a}	$\log_{10}\beta^{b}$	pK_a^{c}	$S^{d}/10^{-8}$	$\Delta\log_{10}K$
	1	1	1	0	17.94(0.02)	8.52		−2.09
	1	1	1	1	26.60(0.04)			−0.14
Ethanolamine	0	0	1	1	7.94(0.01)	7.94	5.5	
	1	0	1	0	4.91(0.09)		78	
	1	0	1	−1	−3.09(0.09)			
	1	1	1	0	14.27(0.06)		180	1.04
	1	1	1	−1	6.50(0.08)	7.77		1.27

[a] l, p, q and r are the stoichiometric coefficients corresponding to Cu^{II}, Prom, amino acids and H^+ respectively; the coefficient −1 refers to a proton loss
[b] $\log_{10}\beta$ of Cu–Prom–amino acids. Standard deviation are given in parentheses
[c] The pK_a of the ligands and the protonated species
[d] Sum of squares of residuals

3.1.1 Complexes Involving Amino Acids

The titration data of the ternary complexes with amino acids and Prom fit satisfactorily with formation of the species: Cu(Prom), Cu(L), $Cu(L)_2$, Cu(Prom) (L) and Cu(Prom)(LH).

Ornithine (H_2L) is an α-amino acid having an extra amino group which may be protonated. Consequently the protonated ternary complex is detected. The coordination can be explained on the premise that ornithine is bound to $Cu(Prom)^{2+}$ by the amino and carboxylic groups, leaving the other amino group susceptible to protonation. The species distribution of ornithine, taken as a representative amino acid, is given in Fig. 3. The protonated 1111 complex predominates, amounting to 91.5% in the physiological pH range while the deprotonated species 1110 attains a maximum concentration of 93.5% at pH ≈ 10.0. Species with concentrations less than 5% were neglected in the concentration distribution plot for clarity.

Threonine has an extra binding center on the β-alcohol-group. This group was reported to participate in complex formation [33]. The potentiometric data fit much better assuming the formation of the Cu(Prom)(LH_{-1}) species. This complex is formed through induced ionization of the β-alcohol group, as mentioned in the literature [33]. The pK_a value of the β-alcoholato-group incorporated in the Cu(II) complex ($\log_{10}\beta_{1110} - \log_{10}\beta_{111-1}$) is 8.6. This is in good agreement with that reported in literature for the Cu–threonine complex [34]. Also ethanolamine forms the complex species 1110 and 111−1. The $\log_{10}\beta_{1110}$ value is smaller than those for the amino acids. This may be attributed to the weaker coordination tendency of an alcohol group compared to a carboxylate group. Charge effects will also be important since the alcohol is neutral, whereas the carboxylate group is negatively charged. The pK_a value of the coordinated alcohol group in the ethanolamine complex (7.77) is considerably smaller than that of the threonine complex. This is consistent with the reaction scheme where the alcohol group in ethanolamine is coordinated to the copper center, where the OH group in threonine is competing with carboxylate group in binding to $Cu(Prom)^{2+}$. Due to the donation of the electron pair on oxygen to the metal center, the OH bond can be considerably weakened and the ionization of a proton occurs at a lower pH.

Histidine is a tridentate ligand having, amino, imidazole and carboxylate groups as binding sites. With $Cu(Prom)^{2+}$, only two of the three binding sites are involved in complex formation. Hence, Histidine coordinates in either a glycine-like or histamine-like mode.

Table 2 Formation constants of the binary and ternary complexes in the Cu^{II}–Prom–dibasic acid systems at 25 °C and 0.1 mol·dm^{-3} ionic strength

System	l	p	q	r[a]	$\log_{10}\beta$[b]	pK_a^c	$S^d/10^{-8}$	$\Delta\log_{10}K$
Oxalic acid	0	0	1	1	4.10(0.00)	4.10	0.13	
	0	0	1	2	5.78(0.01)	1.68		
	1	0	1	0	6.98(0.05)		37	
	1	0	2	0	11.27(0.09)			
	1	1	1	0	21.81(0.04)		1.6	
	1	1	1	1	24.05(0.04)	2.24		
Cyclobutane-1,1-dicarboxylic	0	0	1	1	5.54(0.01)	5.54	5.4	
(CBDCA)	0	0	1	2	8.77(0.02)	3.23		
	1	0	1	0	6.54(0.03)		17	
	1	0	2	0	9.19(0.09)			
	1	0	1	1	10.59(0.04)		22	
	1	1	1	0	16.02(0.03)			1.16
	1	1	1	1	19.90(0.03)	3.88		0.99
Malonic acid	0	0	1	1	5.42(0.00)	5.42		
	0	0	1	2	8.19(0.01)	2.77	0.20	
	1	0	1	0	5.62(0.05)			
	1	0	2	0	9.06(0.08)			
	1	0	1	1	9.98(0.09)		3.3	
	1	1	1	0	15.87(0.03)			1.93
	1	1	1	1	19.20(0.03)	3.33	2.9	0.90
Succinic acid	0	0	1	1	5.35(0.00)	5.35	0.053	
	0	0	1	2	9.41(0.01)	4.06		
	1	0	1	0	4.50(0.01)		0.18	
	1	0	2	0	7.42(0.03)			
	1	0	1	1	9.76(0.01)			1.59
	1	1	1	0	14.41(0.05)		520	0.52
	1	1	1	1	18.60(0.04)	4.19		
Adipic acid	0	0	1	1	5.28(0.00)	5.28	0.35	
	0	0	1	2	9.61(0.01)	4.33		
	1	0	1	0	4.18(0.09)		2.9	
	1	0	2	0	7.93(0.07)			
	1	0	1	1	8.99(0.06)			
	1	1	1	0	13.86(0.08)		1.40	1.36
	1	1	1	1	18.01(0.09)	4.15		0.70

[a] l, p, q and r are the stoichiometric coefficients corresponding to Cu^{II}, Prom, dibasic acids and H^+, respectively

[b] $\log_{10}\beta$ of Cu–Prom–dibasic acids. Standard deviation are given in parentheses

[c] The pK_a of the protonated species ($\log_{10}\beta_{111} - \log_{10}\beta_{110}$)

[d] Sum of squares of residuals

Table 3 Formation constants of the binary and ternary complexes in the Cu^{II}–Prom-peptides at 25 °C and 0.1 mol·dm^{-3} ionic strength

System	l	p	q	r[a]	$\log_{10}\beta$[b]	pK_a^c	S[d] / 10^{-8}	$\Delta\log_{10} K$
Glycinamide	0	0	1	1	7.88(0.00)	7.88	4.6	
	1	0	1	0	4.75(0.04)		64	
	1	0	1	−1	−1.58(0.02)			
	1	1	1	0	12.51(0.06)		220	−0.56
	1	1	1	−1	4.81(0.09)	7.70		−1.93
Glycylglycine	0	0	1	1	7.97(0.01)	7.97	2.5	
	0	0	1	2	11.01(0.01)	3.04		
	1	0	1	0	5.50(0.02)		1.7	
	1	0	1	−1	1.14(0.01)			
	1	1	1	0	12.95(0.06)		310	−0.87
	1	1	1	−1	3.76(0.05)	9.19		−5.70
DL-aspargine	0	0	1	1	8.55(0.01)	8.55	5.9	
	0	0	1	2	10.79(0.03)	2.24	1.0	
	1	0	1	0	6.21(0.03)			
	1	0	1	−1	−2.40(0.07)		41	−0.77
	1	1	1	0	13.76(0.09)			−2.3
	1	1	1	−1	3.62(0.09)	10.14		
L-glutamine	0	0	1	1	9.06(0.01)	9.00	1.1	
	0	0	1	2	11.19(0.02)	2.19	64	
	1	0	1	0	7.69(0.01)			
	1	0	1	−1	−1.59(0.02)		88	−2.04
	1	1	1	0	13.97(0.01)			−3.2
	1	1	1	−1	3.53(0.07)	10.44		

[a] l, p, q and r are the stoichiometric coefficients corresponding to Cu^{II}, Prom, peptides and H^+ respectively; the coefficient −1 refers to a proton loss
[b] $\log_{10}\beta$ of Cu–Prom–peptides. Standard deviation are given in parentheses
[c] the complex pK_a of the peptides or of the peptide NH ionization
[d] Sum of squares of residuals

The stability constant values of histidine and histamine are of the same order and considerably higher than those of amino acids, which indicates that both histidine and histamine will preferably coordinate through the amino and imidazole groups.

3.1.2 Complexes Involving Dicarboxylic Acids

In the Cu(Prom)–dicarboxylic acid system the computer analysis of the pH titration data showed the presence of the 1:1:1 species and its protonated form. The results in Table 2 show that the formation constant of the 1:1:1 complex involving formation of five and six membered chelate rings as in oxalic acid, CBDCA and malonic acid, which are more favored energetically, are higher than those involving seven membered rings, as in succinic acid, and nine membered chelate ring as in adipic acid. It is interesting to note that CBDCA has a

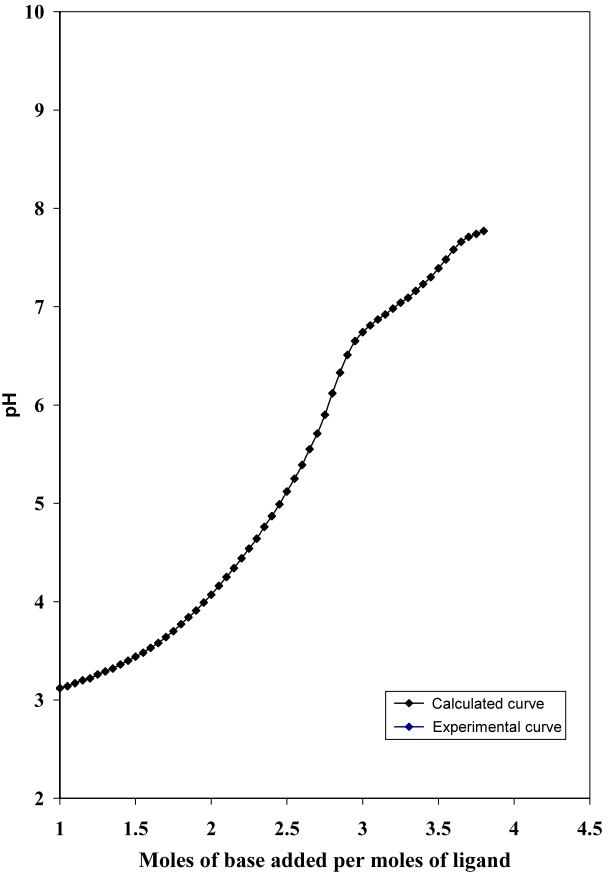


Fig. 1 Potentiometric titration curve for [Cu–Prom–glycine] system

higher stability constant than malonic acid, although they both form six-membered chelate rings. This may be due to the higher pK_a values of the former dicarboxylic acid. The pK_a of the protonated species is 3.88, a value lower than that of the free H-CBDCA, which indicates acidification upon first coordination to Cu(II) through one carboxylate group by 1.6 pH units (5.54–3.88). This lowering is due to coordination of one of the two carboxylic groups. The concentration distribution diagram of CBDCA complex, Fig. 4, shows that the main species in the physiological pH range is the closed ring form, 1110, which reaches a maximum concentration of ≈ 99% starting from pH ≈ 6.

3.1.3 Complexes Involving Amides

The analysis of the potentiometric data for the Cu(Prom)-amide system were fitted to 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, the amide is bound through the amino and carbonyl oxygen groups. On increasing the pH, the coordination sites should switch from carbonyl oxygen to amide nitrogen, with the release of the amide hydrogen, forming the species [Cu(Prom)(LH_{-1})]. Such changes in coordination centers is now well documented [35, 36]. The pK^H values of the amide groups incorporated in the

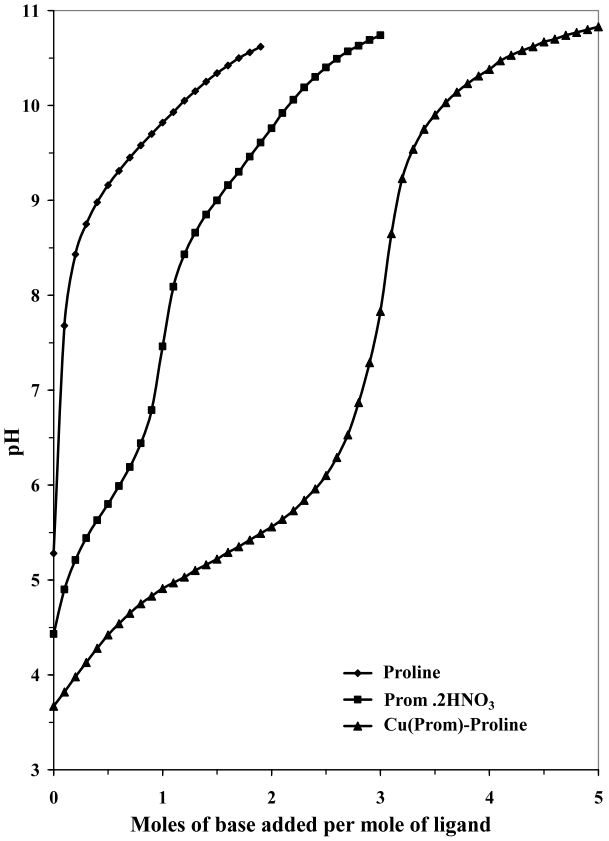


Fig. 2 Potentiometric titration curve of the [Cu(Prom)–proline] system

complexes are calculated by the following equation:

$$pK^{H} = \log_{10}\beta_{1110} - \log_{10}\beta_{111-1} \tag{3}$$

The relative magnitude of the pK^{H} values of the amides reflects the mode of coordination of the different amides to $Cu(Prom)^{2+}$ under normal physiological conditions. It is noteworthy that the pK^{H} for the glycineamide complex is lower than the pK^{H}s of other amides. This signifies that the more bulky substituent group on the amide may serve to hinder the structural change in going from protonated to deprotonated complexes. This implies that glycineamide will be present entirely in the deprotonated form. On the other hand, the pK^{H} of the glutamine complex is higher than the others. This is due to the formation of a seven-membered chelate ring, which would be more strained and less favored. Therefore, under physiological conditions (pH $\sim$ 7.4) glutamine will coordinate in its protonated form. The distribution diagram of the glutamine complex is given in Fig. 5. The mixed ligand species [Cu(Prom)L] (1110) starts to form at pH $\sim$ 4.5 and, with increasing pH, its concentration increases reaching a maximum of $\approx$ 87% at pH = 8. Further increase of pH is accompanied by a decrease in 1110 complex concentration and an increase in [Cu(Prom)LH_{-1}] (111–1) complex formation.

Before discussing the results of spectrophotometric measurements, it should be pointed out that the spectrum of aquated copper(II) ion (mixture A) consists of a broad, weak

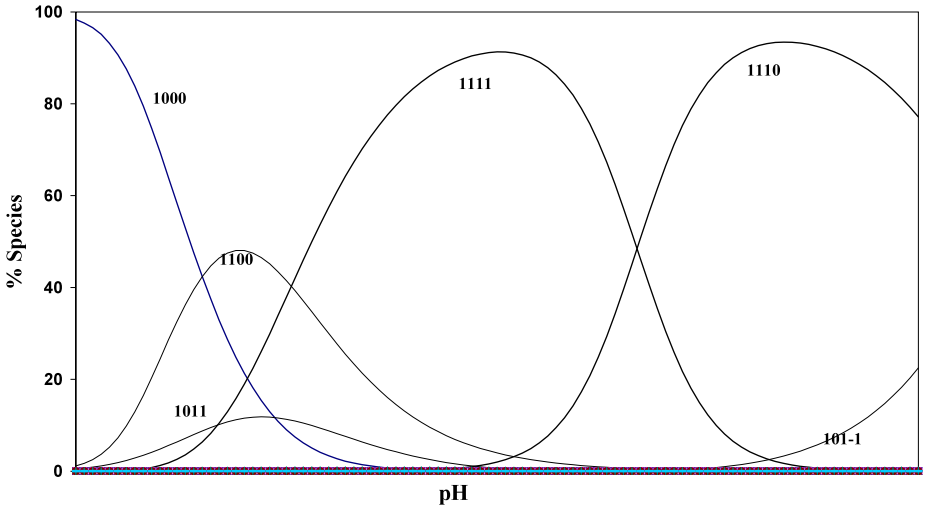


Fig. 3 Concentration distribution of various species as a function of pH in the [Cu–Prom–ornithine] system (each at a concentration of 1.25 mmol·L^{-1})

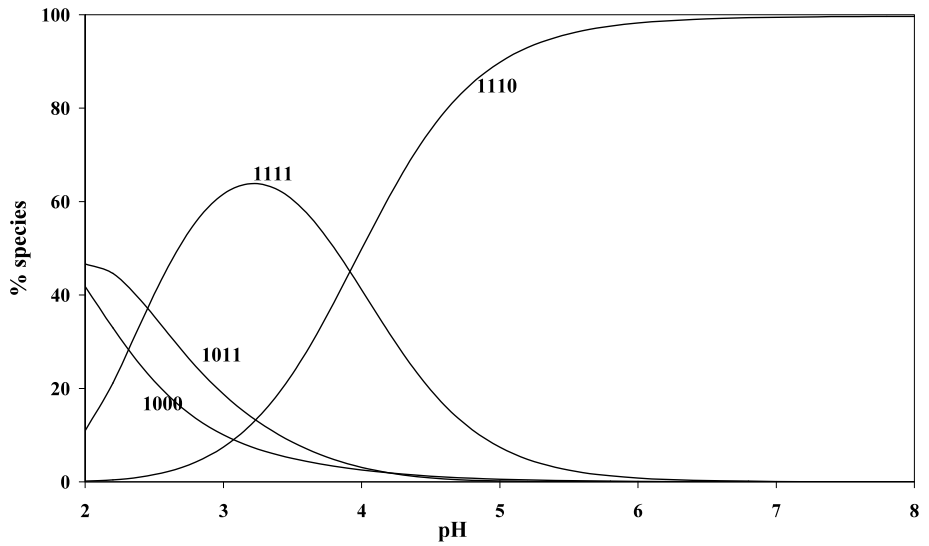


Fig. 4 Concentration distribution of various species as a function of pH in the [Cu–Prom–CBDCA] system (each at a concentration of 1.25 mmol·L^{-1})

band ($\varepsilon = 62$) with a maximum wave length at 817 nm, attributed to the $^2T_{2g} \leftarrow {}^2E_g$ transition [37, 38]. The spectral bands of the binary and ternary copper(II) complexes, shown in Fig. 6, are quite different from that of the aquated copper(II) ion, both as regards the position of the maximum wavelength and their average molar absorptivities. The spectrum of the $Cu(Prom)^{2+}$ complex (curve 1) shows an absorption maximum at 540 nm ($\varepsilon = 259$). On the other hand the spectra obtained from the ternary complex of copper(II) with Prom and glycylglycine as a function of equivalents, a, of base per

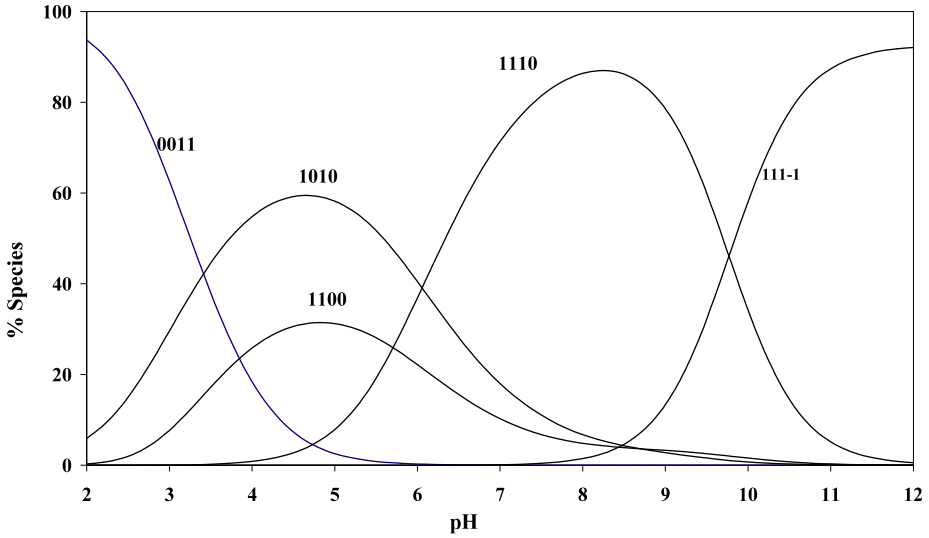


Fig. 5 Concetration distribution of various species as a function of pH in the [Cu–Prom–glutamine] system (each at a concentration of 1.25 mmol·L^{-1})

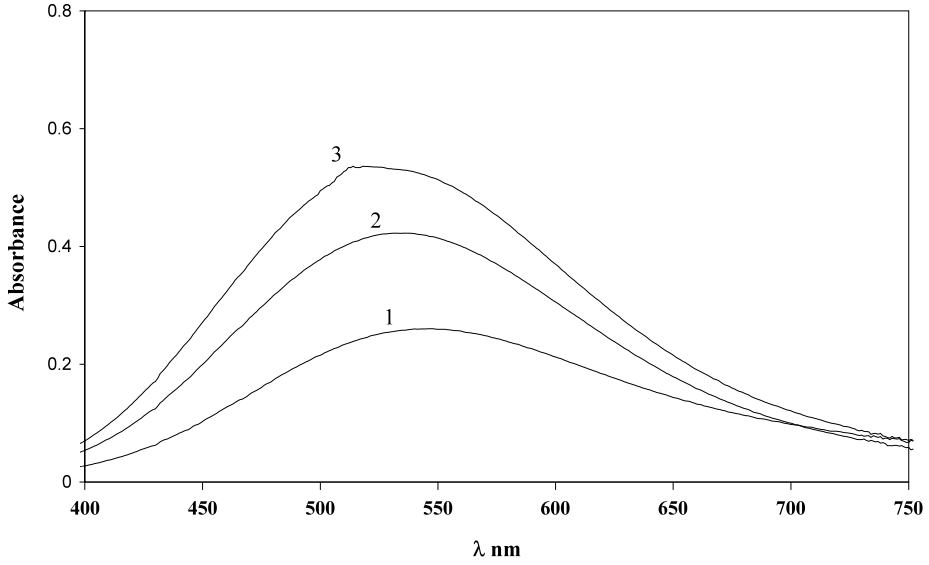


Fig. 6 Visible spectra of [Cu(Prom)–glycylglycinate]: Curve 1: $[Cu(Prom)]^{2+}$ at pH = 5.5 and I = 0.1 mol·dm^{-3}, Curve 2: [Cu(Prom)–glycylglycinate] at pH = 7.5 and $I = 0.1$ mol·dm^{-3}, Curve 3: [Cu(Prom)–glycylglycinate, H_{-1} at pH = 8.1 and $I = 0.1$ mol·dm^{-3} ($NaNO_3$)

ligand (curve 2), exhibits a band at 530 nm ($\varepsilon = 447$). The shift toward shorter wavelength in the absorption spectrum is an indication of the presence of an additional nitrogen center coordinated to copper(II) in the complex. As the value of a increases (curve 3) there is a progressive shift of the absorption maximum towards shorter wavelength ap-

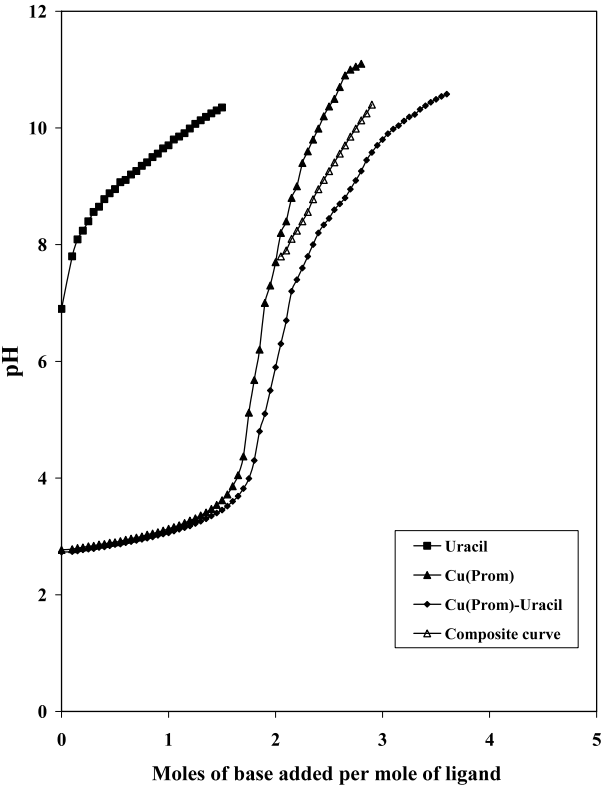


Fig. 7 Potentiometric titration curve in the [Cu–Prom]–uracil system

pearing at 520 nm ($\varepsilon = 537$). This may be taken as evidence, supporting the potentiometric measurement, for induced ionization of the amide hydrogen upon complex formation.

3.1.4 Complexes Involving DNA-Unit Constituents

In the ternary complexes of DNA constituents, D, the potentiometric titration curves of the mixed ligand system coincides with the 1:1 Cu^{II}–Prom curve in the region $0 < a < 2$ (a = number of moles of base added per mole of ligand, Fig. 7). In this respect, The Cu^{II}–Prom complex is first formed due to its greater stability compared to the Cu^{II}–DNA complex (Table 4). Beyond a = 2, the formation of the ternary complex was ascertained by comparison of the mixed ligand titration curve with the composite curve obtained by graphical addition of DNA titration data to that of the Cu^{II}–Prom titration curve. The mixed ligand system was found to deviate considerably from the resulting 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) (Eqs. 4 and 5)

$$\mathrm{Cu} + \mathrm{Prom} \rightleftharpoons \mathrm{Cu(Prom)} \tag{4}$$

$$\mathrm{Cu(Prom)} + \mathrm{D} \rightleftharpoons \mathrm{Cu(Prom)D} \tag{5}$$

The pyrimidinic species (uracil, uridine thymine and thymidine) have only basic nitrogen donor atoms (N_3–C_4O group) in the measurable pH range and as a consequence they form

Table 4 Formation constants of [Cu–Prom]–DNA complexes, at 25 °C and 0.1 mol·dm^{-3} ionic strength

System	p	q	r[a]	$\log_{10}\beta$[b]	pK_a[c]	S[d]$/10^{-8}$	$\log_{10} K^{Cu}_{CuD}$	$\Delta\log_{10} K$
Cu(Prom)–OH	1	0	−1	−6.62(0.01)		6.8		
	1	0	−2	−16.85(0.05)				
Uracil	0	1	1	9.28(0.006)	9.28	2.4		
	1	1	0	5.65(0.04)		1.5	5.49	0.16
	1	2	0	10.96(0.01)				
Uridine	0	1	1	9.01(0.01)	9.01	11		
	1	1	0	5.30(0.05)		120	4.03	1.27
	1	2	0	10.04(0.01)				
Thymine	0	1	1	9.58(0.00)	9.58	8.7		
	1	1	0	5.99(0.03)		19	5.77	0.22
	1	2	0	11.58(0.06)				
Thymidine	0	1	1	9.55(0.00)	9.55	8.1		
	1	1	0	5.52(0.02)		14	4.7	0.82
	1	2	0	12.60(0.08)				
Inosine	0	1	1	8.43(0.01)	8.43	3.1		
	1	1	0	5.52(0.03)		6.7	4.5	1.02
	1	1	1	12.55(0.04)	7.03			
Inosine 5′-monophosphate	0	1	1	9.21(0.01)	8.95	4.3		
	0	1	2	15.21(0.01)	6.32			
	1	1	0	6.25(0.01)		6.8	3.5	2.75
	1	1	1	13.59(0.02)	7.34			
	1	1	2	20.88(0.01)	7.29			

[a] p, q and r are the stoichiometric coefficients corresponding to [Cu–Prom], DNA units and H^+, respectively

[b] $\log_{10}\beta$ of [Cu–Prom]–DNA units. Standard deviation are given in parentheses

[c] The pK_a of the protonated species ($\log_{10}\beta_{111} - \log_{10}\beta_{110}$)

[d] Sum of squares of residuals

1:1 and 1:2 complexes with the $Cu(Prom)^{2+}$ ion. The thymidine complex is more stable than that with uridine, most probably owing to the higher basisity of the N_3 of thymidine, resulting from the inductive effect of the extra electron-donating methyl group. 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. Mixed ligand complexes of nucleosides are less stable than the corresponding bases as is evident from the stability constants given in Table 4. The presence of sugar residue imposes steric hindrance in nucleosides for their complexation with metal ions and reduces the overall basisity of metal complexes of nucleosides considerably. The purines inosine and inosine 5′-monophosphate (IMP) have two metal ion binding centers: the N_1 and N_7 nitrogens. The pH dependent binding of these N-donors was already reported. The results showed that inosine forms the complexes 110 and 111, while inosine 5′-monophosphate forms 110, 111 and 112 complexes. Inosine-5′-monophosphate (5′-IMP)

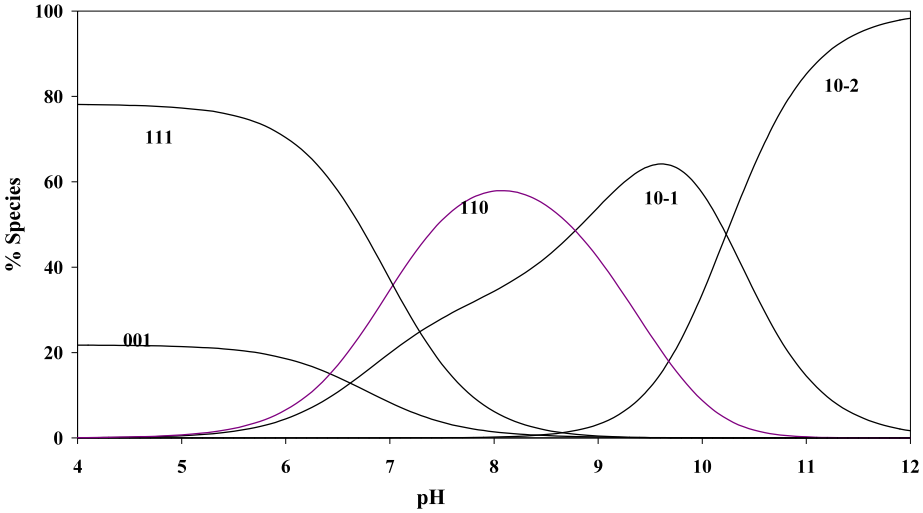


Fig. 8 Concentration distribution of various species as a function of pH in the [Cu–Prom]–inosine system (each at a concentration of 1.25 and 2.5 mmol·L^{-1})

forms a more stable complex with $[Cu(Prom)]^{2+}$ than that with inosine. The extra stabilization can be attributed to the triply negatively charged 5′-IMP^{3-} ion. The speciation of inosine complexes is presented in Fig. 8, where the species distribution of the complexes is plotted as a function of pH. The species 111 is formed in the acidic pH region and it corresponds to the N_7 coordinated complex, while N_1 nitrogen is in the protonated form. The pK_a of the protonated form ($\log_{10}\beta_{111} - \log_{10}\beta_{110}$) amounts to 7.03. In the case of IMP, the protonated species formed correspond to a N_7 coordinated complex, where the N_1 nitrogen and the phosphate group are protonated. The pK_a values of the protonated species of the IMP complex are 7.29 ($\log_{10}\beta_{112} - \log_{10}\beta_{111}$) and 7.34 ($\log_{10}\beta_{111} - \log_{10}\beta_{110}$). The former p$K_a$ value for corresponds to N_1H group and the second pK_a value to the-PO_2(OH). The N_1H groups were acidified upon complex formation by 1.92 (9.21 to 7.29) pK_a units. Acidification of the N_1H group upon complex formation is consistent with previous reports for IMP and GMP complexes [39]. The complexes formed with IMP are more stable than those of pyrimidines. The extra stabilization can be explained on the basis of different coulombic forces operating between the ions resulting from the negatively charged phosphate group.

3.2 Comparison of the Stability Constant of the Ternary Complexes with Those of the Binary Complexes

Although the constant β_{1110} expresses the overall stability of the ternary complex [Cu(Prom)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. $\Delta \log_{10} K$ has been widely accepted and used for many years [14, 15, 40] and the advantages of using $\Delta \log_{10} K$ in comparing stabilities of ternary and binary complexes have been reviewed. The $\Delta \log_{10} K$ value for protonated and deprotonated ternary complexes are given by Eqs. 6 and 7, while those of the induced deprotonated amide complex can be calculated using Eq. 8

$$\Delta \log_{10} K = \log_{10}\beta_{1111} - \log_{10}\beta_{1100} - \log_{10}\beta_{1011} \quad (6)$$

$$\Delta \log_{10} K = \log_{10} \beta_{1110} - \log_{10} \beta_{1100} - \log_{10} \beta_{1010} \quad (7)$$

$$\Delta \log_{10} K = \log_{10} \beta_{111-1} - \log_{10} \beta_{1100} - \log_{10} \beta_{101-1} \quad (8)$$

The values of $\Delta \log_{10} K$ for the ternary complexes studied in this paper are listed in Table 1. The theoretical $\Delta \log_{10} K$ value, for a square planar copper(II) complex is -0.9 [14, 15, 40]. The tendency to form ternary complexes was compared with this value, so that if $\Delta \log_{10} K$ is greater than -0.9, this this should be taken to indicate that the ternary complex is favored. The calculated $\Delta \log_{10} K$ values of α-amino acids are less negative than the theoretical value (-0.90). 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 N,N-dimethyl-3-(phenothiazin-10-yl)propylamine. The $\Delta \log_{10} K$ for ornithine mixed ligand complex (1110) is more negative than -0.90. This may be explained on the premise that ornithine is a tridentate ligand and that two coordination sites are available in the $Cu(Prom)^{2+}$ system.

The $\Delta \log_{10} K$ values for the ternary complexes of phenylalanine, tyrosine and tryptophan are much less negative than -0.9, or positive. This may be explained on the premise that the presence of an aromatic ring above the Cu(II) coordination plane is probably essential for preferential formation of ternary complexes.

The $\Delta \log_{10} K$ values for the induced deprotonated amide ternary complexes, $Cu(Prom)(LH_{-1})$ are also more negative than -0.90. This fact may be taken as an indication that the formation of the ternary amide complexes is favored over binary ones. This may be explained on the premise that the deprotonated amide is coordinated with the free Cu^{II} ion as a tridentate ligand, whereas in the ternary complex two coordination sites are available in $Cu(Prom)^{2+}$.

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 $\Delta \log_{10} K$ as defined by Eq. 9

$$\Delta \log_{10} K = \log_{10} K^{\mathrm{CuProm}}_{\mathrm{Cu(Prom)D}} - \log_{10} K^{\mathrm{Cu}}_{\mathrm{CuD}} \quad (9)$$

The $\Delta \log_{10} K$ values, Table 4, are invariably positive. This means that the DNA constituents form more stable complexes with $Cu(Prom)^{2+}$ than with the free copper(II) ion.

3.3 Characterization of Solid Complexes

All studied complexes are stable in air, allowing physical measurements. IR spectroscopy has proven to be a suitable technique to give information to elucidate the mode of bonding of the ligands to the metal ions. The IR spectra of the complexes [Cu(Prom)(CBDCA)] [Cu(Prom)(malonate)] and [Cu(Prom)(oxalate)] reveal the presence of coordinated water molecules, as indicated by a broad absorption bands at 3470, 3458 and 3435 cm^{-1}, respectively, two weaker bands at approximately 969 and 655 cm^{-1} for the CBDCA complex, at 970 and 620 cm^{-1} for the malonate complex, and at 924 and 614 cm^{-1} for the oxalate complex. These bands are assigned to OH stretching, rocking and wagging vibrations, respectively [41]. The presence of water in complexes was confirmed by TGA experiments in N_2. Bands in the 1654–1610 cm^{-1} region in the complexes are typical of coordinated carboxylate stretching [42]. This assignment is based on the fact that the unionized and uncoordinated COO^- stretching band occurs at 1750–1700 cm^{-1} whereas the ionized and coordinated COO^- stretching band appears at 1650–1590 cm^{-1} [43]. This indicates the participation of carboxylate group in the complex formation. The carboxylate stretching bands

Table 5 Microanalytical and physical data of the synthesized ternary complexes

Complexes empirical formula	M.Wt.	Color yield (%)	M.P. (°C)	Found C	(Calcd.) H	% N	μ_{eff} B.M.	λ_{max}/nm
[Cu(Prom)(CBDCA)]·(H_2O) $C_{23}H_{28}N_2O_5SCu$	507.5	Blue 90	250	52.8 (54.3)	5.7 (5.5)	5.4 (5.5)	2.0	659
[Cu(Prom)(malonate)]·(H_2O)$_2$ $C_{20}H_{26}N_2O_6SCu$	485.5	Blue green 85	265	49.7 (49.4)	5.6 (5.3)	5.9 (5.7)	2.2	744
[Cu(Prom)(oxalate)]·(H_2O) $C_{19}H_{22}N_2O_5Scu$	453.5	Light green 80	280	50.4 (50.2)	4.9 (4.8)	6.4 (6.1)	2.3	680

ν_{as} appear at 1651.5 cm^{-1} for the CBDCA complex, at 1653.6 cm^{-1} for the malonate complex, and at 1638.2 cm^{-1} for the oxalate complex, respectively. ν_s appears as a strong band at 1347.1 for the CBDCA complex, at 1391.3 for the malonate complex and at 1362.5 cm^{-1} for the oxalate complex, respectively. This also an indicates an unidentate coordination mode. The bands appearing in the 2958–2600 cm^{-1} region (medium to weak) can be assigned to C–H stretching vibrations in both the primary and secondary ligands. Low frequency bands corresponding to the vibration of the Cu–O and Cu–N bands have also been assigned for the complexes. The Cu·O bands appear at 449.3, 459.4, and 415 cm^{-1} and the Cu·N bands appear at 551.5, 509.1 and 504.3 cm^{-1} for the CBDCA, malonate and oxalate complexes, respectively, which further confirms formation of the complexes.

Copper(II) complexes have magnetic moments ranging from 1.7–2.2 B.M. [44]. Moments in the higher region between 1.9–2.2 B.M. are however, characteristic of four-coordinate complexes containing ionic or weak covalent bonds [45, 46]. The room temperature magnetic moments calculated for the three complexes [Cu(Prom)(CBDCA)], [Cu(Prom)(malonate)] and [Cu(Prom(oxalate)], presented in Table 5, are in this range, thus suggesting square planar geometry and a dsp^2 hybrid orbital being involved [47]. The square planar configuration has been further confirmed by scanning the electronic spectrum of the complexes in ethanol as a solvent. The spectrum showed broad d–d bands located around 13300–15500 cm^{-1} (Table 5) due to the $^2B_{1g} \rightarrow {}^2A_{1g}$ transition, which is consistence with that reported for square planar Cu(II) complexes [48].

3.4 Thermal Stability

The thermogravimetric analyses of the prepared complexes were carried out to confirm the suggested molecular formula. The analyses were performed in the temperature range 20–800 °C. The TGA analysis of the complexes exhibits mass loss in the temperature range 50–190 °C corresponding to the loss of water from the complexes. Generally, at higher temperature (>300 °C) all ternary complexes display rapid and successive weight loss steps revealing that their decomposition yielding metal oxide. The results were consistent with the analytical data.

3.5 Antimicrobial Activity

One of the recent studies on the pharmaceutical activities of phenothiazines reports on their efficiency to affect the antibiotic resistance to bacteria and tumor cells [9]. Also, copper

Table 6 The antibacterial and antifungal activity of the synthesized metal complexes

Compound	Inhibition zone diameter (mm/mg sample)			
	Gram-negative	Gram-Positive	Fungus	
	Escherichia coli (G−)	Staphylococcus aureus (G+)	Aspergillus flavus	Candida albicans
[Cu(Prom)–CBDCA]·H_2O	25	26(R)	31	12
[Cu(Prom)–malonate]·$(H_2O)_2$	17(R)	18(R)	16(R)	14
[Cu(Prom)–oxalate]·$(H_2O)_2$	14	15	20	11
Tetracycline	32	31		
Antibacterial agent				
Amphotericine B			18	20
Antifungunal agent				

R: Repellent (not complete inhibition)

chelates are well known to have an enhanced antimicrobial activity; therefore it seems interesting to screen the biological potential of the synthesized complexes, in vivo, against different species of bacteria and fungi. In testing the antimicrobial activity of these compounds, we used more than one test organism to increase the chance of detecting antibiotic principles in tested materials. All of the tested compounds show a remarkable biological activity against Gram-positive (G+) and Gram-negative (G−) bacteria and also fungi. The data are listed in Table 6. The biological activity of the metal complexes is governed by the following factors [49]: (i) the chelate effect of the ligands, (ii) the nature of the donor atoms, (iii) the total charge on the complex ion, (iv) the nature of the metal ion, (v) the nature of the counter ions that neutralize the complex, and (vi) the geometrical structure of the complex [50]. Furthermore, the increase in biological activity of the metal chelates may be due to the effect of the metal ion on the normal cell process. A possible mode of toxicity may be considered in the light of Tweedy's chelation theory [51]. Chelation considerably reduces the polarity of the metal ion because of partial sharing of its positive charge with the donor group and possible π-electron delocalization within the whole chelate ring system that is formed during coordination. Such chelation could enhance the lipophilic character of the central metal atom and hence increase the hydrophobic character and liposolubility of the complex favoring its permeation through the lipid layers of the cell membrane. This enhances the rate of uptake/entrance and thus the antimicrobial activity of the tested compounds. Accordingly, the antimicrobial activity of the complexes present in this investigation can be referred to the increase of their lipophilic character, which in turn deactivates enzymes responsible for respiration processes and probably other cellular enzymes, which play vital roles in various metabolic pathways of the tested micro-organisms. On comparing the biological activity of the synthesized complexes with the standards tetracycline (antibacterial agent) and amphotricine B (antifungal agent)), the following results are obtained:

(a) Using Escherichia coli (G−) and Staphylococcus aureus (G+): the biological activity of the CBDCA complex is higher than that of the malonate and oxalate complexes and slightly lower than that of the tetracycline standard.
(b) Using Aspergillus flavus fungus: the antifungal activity of the CBDCA and the oxalate complexes are higher than that of the standard antifungal agent Amphotericin B but the antifungal activity of the malonate complex is slightly lower than that of the standard.

(c) Using Candida albicans fungus: the biological activity of the malonate complex is found to be higher than that of CBDCA and the oxalate complexes, and all have lower values than that of the standard Candida albicans.

The importance of this lies in the fact that these complexes could be applied fairly in the treatment of some common diseases caused by E. coli, e.g. septicaemia, gastroenteritis, urinary tract infections and hospital acquired infections [52] or any other infections caused by any of these particular strains.

4 Conclusion

The present investigation may have important biological implications. The formation equilibria of Cu(II) complexes involving Prom and some ligands of biological significance were investigated. In combination with stability constants data of such $[Cu(Prom)]^{2+}$ complexes with dicarboxylic acids, amino acids, amides and DNA constituents, it will be possible to calculate the equilibrium distribution of the metal species in biological fluids where all types of ligands are present simultaneously. This would form a clear basis for understanding the mode of action of such metal species under physiological conditions. The amino acids and amide complexes are more stable than those of the DNA constituents. The present study shows clearly that deprotonation of the peptide bond was promoted under complex formation. Also, the slight difference in the side chain of the amides has an effect on the induced ionization of the amide proton. The β-alcoholate group in the side chain of the amino acid threonine have been found to play an essential role in the function of a number of proteolytic enzymes, for example chymotrypsin and subtilisin. $Cu(Prom)^{2+}$ promotes the ionization of the alcohol group of threonine with pK_a value of 8.6. This indicates that the participation of the OH group in complex formation is not contributing significantly in the physiological pH range.

The ternary complexes of CBDCA, oxalic and malonic acids were synthesized and characterized. The spectral studies revealed square planar geometry of the complexes. The results of antimicrobial activity show that the complexes exhibit antimicrobial properties. Also, the results indicated that the tested complexes were more active against Gram-negative than Gram-positive bacteria. The importance of this lies in the fact that such compounds may have a possible antitumor effect since Gram-negative bacteria are considered a quantitative microbiological method for testing beneficial and important drugs in both clinical and experimental tumor chemotherapy [53].

References

1. Zayed, M.A., Nour El-Dien, F.A., Mohamed, G.G., El-Gamel, N.E.A.: Synthesis and thermal characterization of new ternary chelates of piroxicam and tenoxicam with glycine and DL-phenylalanine and some transition metals. Spectrochim. Acta **64**, 216–232 (2006)
2. Raman, N., Joseph, J., Sakthivel, A., Jeyamurugan, R.: Synthesis, structural characterization and antimicrobial studies of novel Schiff base copper(II) complexes. Chil. Chem. Soc. **54**, 354–357 (2009)
3. García-Raso, A., Fiol, J.J., Adrover, B., Tauler, P., Pons, A., Mata, I., Espinosa, E., Molins, E.: Reactivity of copper(II) peptide complexes with bioligands (benzimidazole and creatinine). Polyhedron **22**, 3255–3264 (2003)
4. Ranford, J.D., Sadler, P.J.: Cytotoxicity and antiviral activity of transition-metal salicylato complexes and crystal structure of Bis(diisopropylsalicylato) (1,10-phenanthroline)copper(II). Dalton Trans. 3393–3399 (1993)

5. Majella, G., Vivienne, S., Malachy, M., Michael, D., Vickie, M.: Synthesis and anti-Candida activity of copper(II) and manganese(II) carboxylate complexes: X-ray crystal structures of $[Cu(sal)(bipy)]\cdot C_2H_5OH\cdot H_2O$ and $[Cu(norb)(phen)2]\cdot 6.5H_2O$ (salH2 = salicylic acid; norbH2 = cis-5-norbornene-endo-2,3-dicarboxylic acid; bipy = 2,2′-bipyridine; phen = 1,10-phenanthroline). Polyhedron **18**, 2931–2939 (1999)
6. Chen, C.-Y., Chen, Q.-Z., Wang, X.-F., Liu, M.-S., Chen, Y.F.: Synthesis, characterization, DNA binding properties, and biological activities of a mixed ligand copper(II) complex of ofloxacin. Transition Metal Chem. **34**(7), 757–763 (2009)
7. Zoroddu, M.A., Zanetti, S., Pogni, R., Basosi, R.: An electron spin resonance study and antimicrobial activity of copper(II)-phenanthroline complexes. J. Inorg. Biochem. **63**, 291–300 (1996)
8. Valzelli, L., Garattini, S.: Pharmocology of Phenothiazine. In: Clark, W.G. (ed.) Principles of Psychopharmacology, p. 255. Academic Press, New York (1970)
9. Fairhurst, R.M., Valles-Ayoub, Y., Neshat, M., Braun, J., Kidd, S.E., Hambley, T.W., Hever, A., Nelson, M.J., Molnar, J.: The antiplasmid action of some palladium(ii) complexes of phenothiazine based pharmaceuticals and the crystal structure of protonated trichloro[10-(3′-dimethylaminopropyl) phenothiazine-S]-palladium(II). J. Inorg. Biochem. **62**(3), 171–181 (1996)
10. Molnar, J., Tarodi, B., Galfi, M., Matkovics, B., Motohashi, N.: In vitro antiproliferative effects of tricyclic psychopharmaceutical agents and synergism with some resistance modifiers. Anticancer Res. **12**, 273–280 (1992)
11. Iida, Y.: The cation radical salts of phenothiazine and related compounds. Bull. Chem. Soc. Jpn. **44**, 663–667 (1971)
12. Morifumi, F., Akito, I., Tetsuro, M., Setsuo, T.: Lifetimes of radical anions of dicyanoanthracene, phenazine, and anthraquinone in the excited state from the selective electron-transfer quenching. J. Phys. Chem. **100**, 5382–5387 (1996)
13. Domelsmith, L.N., Munchausen, L.L., Houk, K.N.: Photoelectron spectra of psychotropic drugs. 1. Phenethylamines, tryptamines, and LSD. J. Am. Chem. Soc. **99**, 4311–4321 (1977)
14. Shoukry, A.A.: Complex formation reactions of (2,2′-dipyridylamine) copper(II) with various biologically relevant ligands. The kinetics of hydrolysis of amino acid esters. Transition Metal Chem. **30**, 814–827 (2005)
15. Shoukry, A.A., Mohamed, M.M., Shoukry, M.M.: Binary and ternary complexes of copper(ii) involving N,N,N,N-tetramethylethylenediamine (Me_4en) and various biologically relevant ligands. J. Solution Chem. **35**, 853–868 (2006)
16. Shoukry, A.A., Shoukry, M.M., Hafez, M.N.: Kinetics of base hydrolysis of α-amino acid esters catalyzed by palladium(II) piperazine complex. Cent. Eur. J. Chem. **8**, 797–805 (2010)
17. Shoukry, A.A., Shoukry, M.M.: Coordination properties of hydralazine Schiff base. Synthesis and equilibrium studies of some metal ion complexes. Spect. Chem. Acta A 686–691 (2007)
18. Shoukry, A.A., Shoukry, M.M.: Potentiometric studies of binary and ternary complexes involving cadmium(II) and nitrilo-tris(methyl phosphonic acid) with amino acids, peptides and DNA constituents. J. Ann. Chim. **97**, 733–744 (2007)
19. Shoukry, M.M., Shoukry, A.A., Hafez, M.N.: Complex formation reactions between $[Pd(piperazine)(H_2O)_2]^{2+}$ and biorelevant ligands: synthesis and equilibrium constants. J. Coord. Chem. **63**(4), 652–664 (2010)
20. Shoukry, M.M., Shoukry, A.A., Khalaf Allah, P.A., Hassan, S.S.: Equilibrium and kinetic investigation of the interaction of model palladium(II) complex with biorelevant ligands. Int. J. Chem. Kinet. **42**, 608–618 (2010)
21. Shoukry, A.A., Brindell, M., van Eldik, R.: Kinetics and mechanism of the substitution behaviour of Pd(II) piperazine complexes with different biologically relevant nucleophiles. Dalton Trans. 4169–4174 (2007)
22. Bugarcic, Z.D., Jancic, D.M., Shoukry, A.A., Shoukry, M.M.: Rate and equilibrium data for substitution reactions of [pd(dien)cl]+ with l-cystein and glutathione in aqueous solution. Monatsh. Chem. **135**, 151–161 (2004)
23. Shoukry, A.A., Rau, T., Shoukry, M.M., van Eldik, R.: Kinetics and mechanisms of the ligand substitution reactions of bis(amine) (cyclobutane-1,1-dicarboxylato)palladium (II). J. Chem. Soc. Dalton Trans. 3105–3112 (1998)
24. Welcher, F.J.: The Analytical Uses of Ethylenediamine Tetraacetic Acid. Van Nostand, Princeton (1965)
25. Grayer, R.J., Harbone, J.B.: A survey of antifungal compounds from higher plants, 1982–1993. Phytochemistry **37**, 19–42 (1994)
26. Mohamed, G.G., Soliman, M.H.: Synthesis, spectroscopic and thermal characterization of sulpiride complexes of iron, manganese, copper, cobalt, nickel, and zinc salts. Antibacterial and antifungal activity. Spectochim. Acta A 341–347 (2010)

27. Vogel, A.E.: Text Book of Quantitative Chemical Analysis, 5th edn., p. 555. Longman, Harlow (1989). Chap. 15
28. Stark, J.G., Wallace, H.G. (eds.): Chemistry Data Book, p. 75. Murray, London (1975)
29. Angelici, R.J.: Synthesis and Techniques in Inorganic Chemistry, 2nd edn., pp. 198–205. Saunders, Philadelphia (1977)
30. Gans, P., Sabatini, A., Vacca, A.: An improved computer program for the computation of formation constants from potentiometric data. J. Inorg. Chim. Acta **18**, 237–239 (1976)
31. Pettit, L.: Personal Communication. University of Leeds, Leeds (1993)
32. Shehata, M.R., Shoukry, M.M., Nasr, F.M., Van Eldik, R.: Complex-formation reactions of dicholoro(S-methyl-L-cysteine)palladium(II) with bio-relevant ligands. Labilization induced by S-donor chelates. Dalton Trans. 779–786 (2008)
33. Sigel, H., Martin, R.B.: Coordination properties of the amide bond stability and structure of metal ion complexes of peptides and related ligands. Chem. Rev. **82**, 385–426 (1982)
34. Grenouillet, P., Martin, R.P., Rossi, A., Ptak, M.: Interactions between copper(II) ions L-threoinine, L-allo-threonine and L-serine in aqueous solutions. Biochim. Biophys. Acta **322**, 185–194 (1973)
35. Savago, I., Kiss, A., Farkas, E., Sanna, D., Marras, P., Micerain, G.: Potentiometric and spectroscopic studies on the ternary complexes of copper(II) with dieptides and nucleobases. J. Inorg. Biochem. **65**, 103–108 (1997)
36. Daniele, P.G., Zerbinati, O., Zelano, V., Ostacoli, G.: Thermodynamic and spectroscopic study of copper(II)-glylcyl-L-histidylglycine complexes in aqueous solution. Dalton Trans. 2711–2715 (1991)
37. Cotton, F.A., Wilkinson, G.: Basic Inorganic Chemistry, pp. 353–379. Wiley, New York (1973). Part 3
38. Shoukry, M.M., Saeed, A., Khairy, E.M.: Equilibrium and hydrolysis of α-amino acid esters in ternary complexes of copper(II) involving glycyl-L-tyrosine. Transit. Metal Chem. **14**, 347–350 (1989)
39. Siegel, H., Massoud, S.S., Corfu, N.A.: Comparison of the extent of base back binding in complexes of divalent metal ions with guanine (GMP^{2-}), inosine (IMP^{2-}) and adenosine 5-monophosphate (AMP^{2-}). The crucial role of N-7 basicity in metal ion- nucleic base recognition. J. Am. Chem. Soc. **116**, 2958–2971 (1994)
40. Sigel, H.: Ternary Cu^{2+} complexes stability, structure, and reactivity. Angew. Chem., Int. Ed. **14**, 394–402 (1975)
41. Snell, F.D., Hilton, C.L.: Encyclopedia of Industrial Chemical Analysis, vol. 4, p. 74. Interscience, New York (1967)
42. Nakamoto, N.: Infrared and Raman Spectra of Inorganic and Coordination Compounds, 4th edn., pp. 228, 229, 237–239. Wiley, New York (1986),
43. Cotton, F.A., Wilkinson, G.: Basic Inorganic Chemistry, pp. 353–379. Wiley, New York (1973). Part 3
44. Cotton, F.A., Wilkinson, G.: Advanced Inorganic Chemistry. A Comprehensive Text, 3rd edn. (1972)
45. Kato, M., Jonassen, H.B., Fanning, J.C.: Copper(II) complexes with subnormal magnetic moments. Chem. Rev. **64**, 99–128 (1964)
46. Graddon, D.P., Heng, K.B.: Copper(II) chelates with 3-arylacetylacetones. Aust. J. Chem. **24**, 1059–1063 (1971)
47. Iskander, M.F., Khalil, T.E., Werner, R., Haase, W., Svoboda, I., Fuess, H.: Synthesis, reactivity and magnetochemical studies on copper(II) complexes derived from *N*-salicylidenearoylhydrazines. X-ray structure of [mononitratoOO(−1)(N-salicylidenatobenzoylhydrazine)ONO(−1)]copper(II) Monohydrate. Polyhedron **19**, 949–958 (2000)
48. Planiadavar, M., Natavajan, C.: Cobalt(II), nickel(II) and copper(II) complexes of some 2′-hydroxychalcones. Aust. J. Chem. **33**, 737–745 (1980)
49. Chohan, Z.H.: Antibacterial and antifungal ferrocene incorporated dithiothione and dithioketone compounds. Appl. Organomet. Chem. **20**, 112–116 (2005)
50. Russell, A.D.: Densification, Sterilization and Preservation, 4th edn. Lee and Febinger, Philadelphia (1991)
51. Tweedy, B.G.: Possible mechanism for reduction of elemental sulfur by Monilinia fructicola. Phytopathology **55**, 910–914 (1964)
52. Jawetz, E., Melnick, J.I., Adelberg, E.A.: Review of Medical Microbiology, 16th edn. LangMedical, Los Angeles (1979)
53. Inoue, T., Yamashita, Y., Nishihara, M., Sugiyama, S., Sonoda, Y., Kumabe, T., Yokoyama, M., Tominaga, T.: Therapeutic efficacy of a polymeric micellar doxorubicin infused by convection-enhanced delivery against intracranial 9L brain tumor models. Neuro-oncology **11**, 151–157 (2009)