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Complex formation equilibria of imipenem with some transition metal ions. Ternary complex formation reactions involving Cu(II) with imipenem and various bio-relevant ligands

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ABSTRACT

Imipenem is one of the β-lactam antibiotics (β-lactamase inhibitors), which are reported to be the most important class of drugs that are capable of inhibiting the bacterial enzyme to protect the β-lactam antibiotic from destruction. In view of the biological importance of imipenem as drug, the ligation behavior of imipenem is studied in order to get an idea about its potentiality towards some transition metals in *in-vitro* systems. The binary complex formation equilibria with the metal ions Cu(II), Ni(II), Co(II), Mn(II), and Zn(II) were investigated potentiometrically. The effects of dioxane as a solvent, on the protonation constant of imipenem and the formation constants of Cu(II)-imipenem complexes were discussed. The ternary copper(II) complexes involving imipenem and various biologically relevant ligands containing different functional groups, as amino acids, amides, dicarboxylic acids and DNA constituents were investigated. The stability constants of the complexes are determined. The mechanisms of complex formation are speculatively discussed based on the calculated stability constant values. The ternary complexes are formed by simultaneous reactions. The concentration distributions of various species formed in solution were also evaluated as a function of pH.

1. Introduction

β-Lactam antibiotics (β-lactamase inhibitors) have long been known to behave as relatively efficient chelating agents. Imipenem (Imip) is one of these antibiotics. They are the most important class of drugs that are capable of inhibiting the bacterial enzyme to protect the β-lactam antibiotic from destruction [1]. The belief that antibiotics action is related to the ability of these compounds to form complexes with metal ions has stimulated investigations of the complexing properties of antibiotics as ligands [2]. This is due to the fact that some metal complexes are known to exhibit remarkable anti-tumour, antifungal, antiviral and special biological activities and the efficacies of some therapeutic agents are known to increase upon co-ordination [3-6]. Many drugs possess modified pharmacological and toxicological properties when administered in the form of metallic complexes [7]. It was reported that some metal-based antibiotics such as bleomycin, streptonigrin, and bactracin have gained recognition and are more effective than pure drugs [3,8]. The development of metal-based drugs with promising pharmacological application has been reported as transition metals can interact with a number of negativelycharged molecules due to different oxidation states they possess. It seems therefore to be of considerable interest to conduct investigations of solution equilibria and biological activity of binary metal ion imipenem complexes.

Despite the fact that the action of copper in humans has been intensively studied, the clinical picture of copper status is not always so straightforward, and less is known about the role of copper complexes in medicine. Yet it is evident that such compounds could be very important in medicinal procedures, and their role has probably been underestimated [9]. Therefore, the interaction of Cu(II) ion with therapeutically administered drugs is a subject of considerable interest [10]. In addition ternary complexes formed between metal ions and two different bio-ligands, may be considered as models for substrate-metal ionenzyme interactions and other metal ion mediated biochemical interactions [11]. Literature survey on imipenem reveals that no work has been reported on the formation constants of binary metal complexes with imipenem or ternary complexes of Cu(II) with imipenem and bio-ligands containing different functional groups. Consequently we thought it is worthwhile to study the interaction of Cu(II) with imipenem in the presence of ligands of biological significance as amino acids, peptides, or DNA units, including the study of the mechanism of their formation, calculation of stability constant, and evaluation of the concentration distribution of various species in solution. This may be useful in elucidating the mechanism of actions of this class of drugs and understanding the driving forces leading to formation of such complexes in biological systems.

The present study is a continuation of our previous work directed to study complex formation equilibria with biologically active ligands [12-16]. The aim of the present work is to conduct several investigations to study the complexing properties of imipenem, as one of the new β-lactam antibiotics as ligand, and to obtain information on the protonation

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equilibria and the binary and ternary complex forming ability of imipenem by establishing the composition and stabilities of the species formed. The second point of importance is the investigation of complex formation equilibria in solvents of lower polarity that represent some biological microenvironments, or simulate to some degree the situation in active site cavities. In spite that *in vivo* reactions take place in aqueous media, it has been reported that water is not an ideal model for *in vivo* reactions. In enzymes membranes and other biologically important media, the pK_a values are far different from those in water, as these media tend to be lipophilic rather than hydrophilic. Therefore studies involving the calculation of the protonation constants in media other than water should provide some understanding of the chemistry of biorelevant ligands in biological systems. The effects of dioxane as a solvent on both the protonation of Imip and the formation constants of Cu(II) complexes with Imip are discussed.

2. Experimental

2.1. Materials and reagents

Imipenem was obtained from Sigma Chem. Co. Glycine, alanine, phenylalanine, proline, tyrosine, tryptophane, threonine, L-histamine dihydrochloride, L-histidine.HCl, ethanolamine.HCl together with the dicarboxylic acids as, oxalic, malonic, succinic, adipic and cyclobutane-1,1-dicarboxylate anion (CBDCA) were also provided by Sigma Chem. Co. The amides (Glycylglycine, glutamine, aspargine and glycineamide) and the DNA constituents (Uracil, uridine, thymine, thymidine, inosine, inosine 5'-monophosphate) were supplied by BDH-Biochemicals Ltd. For stability constant determination, solution of Imip (0.01 mol/dm^3) was prepared in two equivalents of HNO_3 acid (0.02 mol/dm^3), freshly prepared solutions of Imip were used for all the measurements. L-Histidine.HCl (0.01 mol/dm^3) was prepared in one equivalent of HNO_3 acid (0.01 mol/dm^3), $Cu(NO_3)_2.2H_2O$ was provided by DBH. The copper content of the solutions was determined by complexometric EDTA titrations [17]. Carbonate free NaOH (titrant) was prepared and standardized against potassium hydrogen phthalate solution. All solutions were prepared in deionized water.

2.2. Instrumentations

Potentiometric measurements were made using a Metrohm 751 Titrino. The titroprosessor was calibrated with standard buffer solutions potassium hydrogen phthalate (pH = 4.008) and a mixture of KH_2PO_4 and Na_2HPO_4 (pH = 6.865) prepared according to NBS specifications [18] at 25±0.1 °C and I = 0.1 mol/dm^3. The electrode was calibrated by the method proposed by Irving [19]. The pH meter readings were converted into hydrogen ion concentration by titrating a standard acid solution (0.01 M), the ionic strength of which was adjusted to (0.1 M) with $NaNO_3$, with standard base (0.10 mol/dm^3). The pH is plotted against p[H]. The relationship pH - p[H] = 0.05 was observed. [OH$^-$] value was calculated using a pK_w value of 13.997 [20]. All potentiometric titrations were carried out in a double-walled glass cell of 50 mL capacity. The temperature of all solutions was maintained at 25±0.05 °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 ($NaNO_3$).

2.3. Procedure and measuring technique

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. The stability constant of the complexes involving bivalent metal ions with imipenem was determined by titrating 40 cm^3 of a solution mixture of (1.25 ×10 $^{-3}$ mol/dm^3) of the metal ions, Imip ligand (2.5×10^{-3} mol/dm^3) and $NaNO_3$ (0.1 mol/dm^3).

The formation constant of the mixed ligand complexes formed with Cu(II) were determined by titrating solution mixtures containing equivalent amounts of Cu(II), Imip and other ligands (1.25 ×10^{-3} mol/dm^3) in concentration ratio 1:1:1 for amino acids, dicarboxylic acids, and peptides. All titrations were performed in a purified N_2 atmosphere using aqueous NaOH (0.05 mol/dm^3) as titrant.

In the present work the effect of dioxane on the protonation constant of Imip and the formation constants of Cu(II)-Imip complexes taken as an example of binary complexes were also performed in water/dioxane mixtures of different composition, containing 10-50% dioxane. The pH-meter readings (B) recorded in dioxane-water solutions were converted to hydrogen ion concentration [H$^+$] by using the widely used relation given by the Van Uitert and Hass equation, Equation 1 [21] as shown below,

$$\text{Log}\,[H^+] = B + \text{Log}\,U_H \quad (1)$$

where Log U_H is the correction factor for the solvent composition and ionic strength for which B is read. Values of pK_w in dioxane-water mixtures were determined as described previously [22,23]. For this purpose, various amounts of standard NaOH solution were added to a solution containing 0.1 mol/dm^3 $NaNO_3$. The [OH$^-$] was calculated from the amount of base added. The [H$^+$] was calculated from the pH value. The product of [OH$^-$] and [H$^+$] was taken. The mean values obtained in this way at 25 °C for Log [H$^+$][OH$^-$] are pK_w = 14.17, 14.60, 15.12 and 15.49 for 12.5, 25, 37.5 and 50% dioxane-water solutions respectively.

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

$$l(Cu) + p(Imip) + q(L) + r(H) \rightleftharpoons (Cu)_l(Imip)_p(L)_q(H)_r \quad (2)$$

$$\beta_{lpqr} = \frac{[(Cu)_l(Imip)_p(L)_q(H)_r]}{[Cu]^l[Imip]^p[L]^q[H]^r} \quad (3)$$

The calculations were obtained from *ca*. 100 data points in each titration using the nonlinear least-squares computer program MINIQUAD-75 [24]. 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 without giving any systematic bias in the magnitudes of various residuals [24]. The results obtained in Table 1-6 are at best reliable to two or three significant figures. The concentration distribution diagrams were obtained using the program SPECIES [25] under the experimental condition used.

2.4. Spectrophotometric measurements

Spectrophotometric investigations of Cu-Imip complexes were performed by scanning the visible spectra of solution mixtures (A-C). Under the experimental conditions and after neutralization of the hydrogen ions released during complex formation, it is supposed that the complexes have been completely formed.

(A). 1.0 cm^3 (0.01 mol/dm^3) Cu (II) ion
(B). 1.0 cm^3 (0.01 mol/dm^3) Cu(II) ion + 1.0 cm ^{3} (0.01 mol/dm^3) Imip + 2.0 cm^3 (0.01 mol/dm^3) NaOH
(C). 1.0 cm^3 (0.01 mol/dm^3) Cu(II) ion + 1 cm^3 (0.01 mol/dm^3) Imip + 4.0 cm^3 (0.01 mol/dm^3) NaOH

In each case the final volume was brought to 25 cm^3 by addition of deionized water, and the ionic strength is adjusted at *I* = 0.1 mol/dm^3 using $NaNO_3$.

Table 1. Formation constants of the binary complexes of imipenem at 25 °C and I = 0.1 M ionic strength.

System	P [a]	q [a]	r [a]	Log β [b]	S [c]
H^+-Imp	0	1	1	9.86 (0.04)	4.99×10^{-6}
	0	1	2	13.40 (0.06)	
Co-Imp	1	1	0	9.63 (0.02)	4.69×10^{-7}
	1	1	1	13.03 (0.04)	
	1	1	-1	3.70 (0.08)	
Cu-Imp	1	1	0	10.16 (0.01)	1.18×10^{-7}
	1	1	1	13.42 (0.04)	
	1	1	-1	4.43 (0.03)	
Mn-Imp	1	1	0	8.83 (0.04)	5.31×10^{-7}
	1	1	1	13.14 (0.04)	
	1	1	-1	2.46 (0.10)	
Ni-Imp	1	1	0	10.01 (0.02)	9.49×10^{-7}
	1	1	1	12.16 (0.55)	
	1	1	-1	4.57 (0.12)	
Zn-Imp	1	1	0	8.87 (0.03)	5.68×10^{-7}
	1	1	1	12.91 (0.04)	
	1	1	-1	1.62 (0.09)	

[a] p, q and r are the stoichiometric coefficient corresponding to Cu(II), Imip and H^+, respectively.
[b] Standard deviations are given in parentheses.
[c] Sum of squares of residuals.

Table 2. Solvent effect on the dissociation constant of imipenem and the formation constant of the Cu-Imip complex.

System	% Dioxane (*v:v*)	p [a]	q [a]	r [a]	Log β [b]	pK_1^H	S [c]
Cu-Imip	12.5	0	1	1	9.96 (0.03)		2.91×10^{-6}
		0	1	2	13.63 (0.02)		
		1	1	0	10.36 (0.01)	5.72	5.62×10^{-7}
		1	1	1	12.12 (0.02)		
		1	1	-1	4.64 (0.07)		
Cu-Imip	25.0	0	1	1	10.14 (0.07)		4.10×10^{-6}
		0	1	2	13.59 (0.05)		
		1	1	0	10.69 (0.03)	5.85	1.27×10^{-6}
		1	1	1	12.62 (0.03)		
		1	1	-1	4.84 (0.09)		
Cu-Imip	37.5	0	1	1	10. 64 (0.04)		4.48×10^{-6}
		0	1	2	13.89 (0.04)		
		1	1	0	10.95 (0.029	6.00	7.29×10^{-7}
		1	1	1	12.84 (0.095)		
		1	1	-1	4.95 (0.088)		
Cu-Imip	50.0	0	1	1	10. 94 (0.04)		4.87×10^{-6}
		0	1	2	14.31 (0.04)		
		1	1	0	11.22 (0.03)	6.21	6.25×10^{-7}
		1	1	1	13.62 (10.8)		
		1	1	-1	5.01 (0.09)		
Cu-Imip	62.5	0	1	1	11. 04 (0.04)		3.82×10^{-7}
		0	1	2	14.41 (0.04)		
		1	1	0	11.52 (0.03)	6.40	6.10×10^{-7}
		1	1	1	13.72 (10.8)		
		1	1	-1	5.12 (0.09)		

[a] p, q and r are the stoichiometric coefficient corresponding to Cu(II), Imip and H^+, respectively.
[b] Standard deviations are given in parentheses.
[c] Sum of squares of residuals.

3. Results and discussion

In the present work the stoichiometric protonation constants of the studied secondary ligands *viz*. (amino acids, amids, dicarboxlic acids and DNA units) were determined under the experimental conditions used to determine the stability constants of the binary and ternary Cu(II) complexes. The values obtained are consistent with data reported in the literature [26].

3.1. Binary complexes involving Imip with the metal ions Cu(II), Ni(II), Co(II), Zn(II) and Mn(II)

Imipenem contains several potential donor atoms that might be involved in coordination to the metal ions. The sulfur atom of imipenem is a typical thioether group. Thioethers are good nucleophilic centers that can fairly form chelates with metal ions. Therefore, coordination of imipenem to the metal ions is suggested most likely to occur through the sulfur atom and the amino group located next to the thioether center.

The potentiometric titration data of the binary metal complexes of some selected metal ions as Cu(II), Ni(II), Co(II), Zn(II) and Mn(II) with Imip together with the proton association constant of Imip are listed in Table 1. The potentiometric titration curve for each of the studied metal ions with Imip is significantly lower than Imip titration curve. This corresponds to the formation of a complex through release of proton. The potentiometric titration curve of Cu(II)-Imip, taken as a representative of the binary complexes is presented in Figure 1. The titration data were fitted to various models. The data were fitted satisfactorily with the model involving the formation of the species 110, 111 and 11-1. The good fit between the experimental and theoretical curves is an indication of the validity of the complex formation model. The concentration distribution diagram of various species as a function of pH for the binary Cu-Imip and Co-Imip complexes is shown in Figure 2.

3.1.1. Visible electronic spectra

The electronic absorption spectra of aqueous Cu(II) and solution mixture of Cu(II) and Imip are compared. The spectrum of the hydrated Cu(II) ion (Mixture A) consists of a broad, weak band with a maximum wavelength at 815 nm, attributed to the $^2E_g \rightarrow {}^2T_{2g}$ transition [27,28]. This band undergoes a blue shift to 605 nm in the spectrum of $[Cu\text{-}Imip]^{2+}$

complex (Mixture B) (Figure 3). Upon increase of pH, further shift in the absorption spectrum to λ_{max} = 585 nm is observed (Mixture C) corresponding to the formation of $[Cu(Imip,H_{-1})]^{+}$ complex. The shift toward shorter wavelength in the absorption spectrum with increasing pH may be taken as evidence, supporting the potentiometric measurements, for the induced ionization of one of the hydroxyl group of Imip upon complex formation, calculated as the specie with the stoichiometry 11-1.

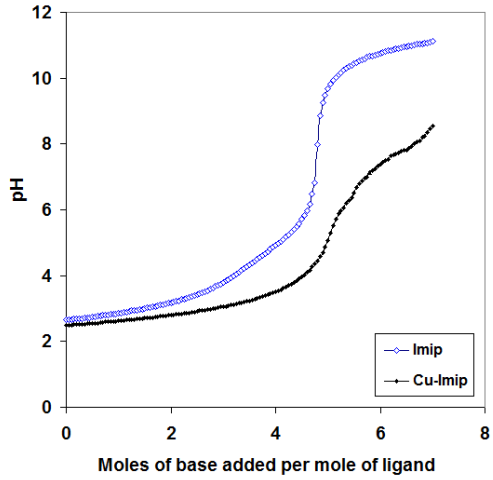


Figure 1. Potentiometric titration curves of the Cu-Imip system.

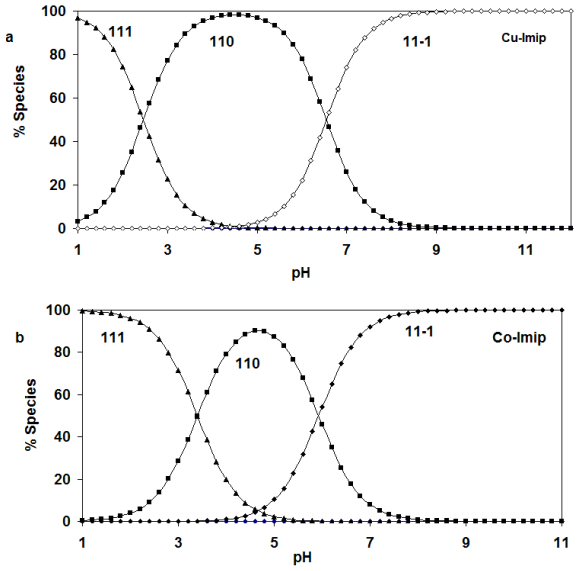


Figure 2. Concentration distribution of various species as a function of pH in the Cu-Imip (a) and Co-Imip (b) systems.

3.1.2. Effect of solvent

Study of the effect of solvent on the acid dissociation constant of a ligand and the formation constant of bioactive metal complexes has important biological implications, as it allows extrapolating the data into physiological conditions. It is well established that the "effective" or "equivalent solution" dielectric constants in protein [29,30] or active site cavities of enzymes [31] are small compared to that in bulk water. Hence by using aqueous solutions containing 10-50% dioxane, one may expect to simulate to some degree the situation in active site cavities [32]. In the present work the effect of dioxane on the protonation constant of Imip and the formation constants of Cu(II)-Imip complexes taken as an example of binary complexes was studied. Careful examination of media effects on the equilibrium constants (Table 2) reveals the following features:

(1) pK_a of Imip increases linearly with increasing percentage of organic solvent in the medium, Figure 4. This may be correlated with the ability of a solvent of relatively low dielectric constant to increase the electrostatic forces between the proton and the ligand and consequently the pK_a value increases.

(2) The stability constant (Log K_1) of the Cu^{2+}-Imip complex increases with increase of dioxane concentration, Figure 4. This can be interpreted in terms of an electrostatic model. In general, lowering of the dielectric constant of a medium (by increasing dioxane content) favors the interaction between the Cu(II) ion and Imip and consequently the stability constant of the complex increases. In general, the stability of compounds containing O-H link increases with increasing organic content of the solvent, due to the decrease in the dielectric constant of the bulk solvent. As the dielectric constant decreases the ion-ion interaction involving the proton and the anionic oxygen donor of the ligand increases to a greater extent than the ion dipole interaction between the proton and the solvent molecule. This finding is in agreement with literature data [33].

(3) The deprotonation constant of Cu(II)-Imip complexes (pK_1^H = Log β_{110} – Log β_{11-1}) increased with increasing dioxane proportion. This change may be related to the decrease of the dielectric constant of the medium and an increase of the electrostatic forces between the Cu (II) cation and the anionic oxygen donor of Imip.

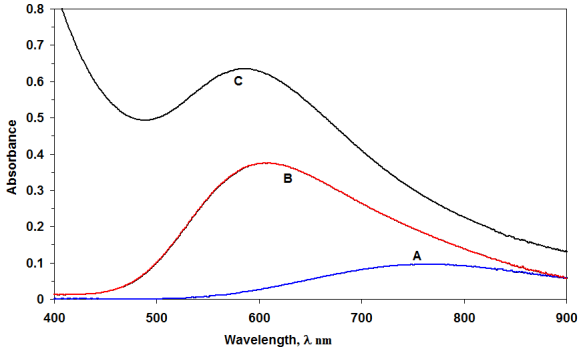


Figure 3. Visible spectra of (Cu-Imip) system, curves A: Cu^{2+}, B: $[Cu(Imip)]^{2+}$, C: $[Cu(Imip,H_{-1})]^{+}$.

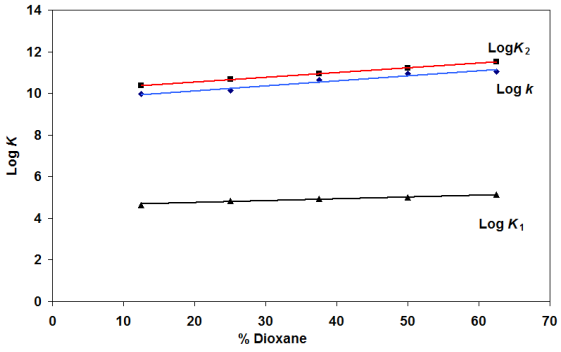


Figure 4. Effect of solvent on the protonation constant of Imip and the formation constant of Cu-Imip systems. Curves Log K corresponds to Imip, Log K_1 corresponds to 110, Log K_2 corresponds to 11-1.

Table 3. Stability constants of the ternary species in the Cu(II)-Imip-amino acid systems and proton-association constants and their binary stability constants at 25 °C and I = 0.1 M ionic strength.

System	l [a]	p [a]	q [a]	r [a]	Log β [b]	Log β^{Cu}_{CuL}	Log β^{Cu}_{CuL2}	S [c]
$[Cu(H_2O)_4]^{2+}$	1	0	0	-1	-6.44 (0.07)			9.6×10^{-7}
	1	0	0	-2	-12.99 (0.02)			
Imip	0	1	0	1	9.86 (0.04)			1.7×10^{-8}
	0	1	0	2	13.40 (0.06)			
Cu-Imip	1	1	0	0	10.16 (0.01)	10.16		3.5×10^{-7}
	1	1	0	1	13.42 (0.04)			3.1×10^{-9}
	1	1	0	-1	4.43 (0.03)			
Cu-Imip-Glycine	0	0	1	1	9.60 (0.01)	8.62	13.57	1.6×10^{-7}
	0	0	1	2	11.93 (0.02)			3.0×10^{-7}
	1	1	1	0	17.39 (0.01)			
	1	1	1	-1	16.70 (0.02)			
Cu-Imip-Alanine	0	0	1	1	9.69 (0.01)	7.99	14.62	3.5×10^{-7}
	0	0	1	2	11.89 (0.007			
	1	1	1	0	17.69 (0.02)			
	1	1	1	-1	18.70 (0.03)			
Cu-Imip-β-phenyl-alanine	0	0	1	1	9.12 (0.01)	7.53	13.73	2.0×10^{-8}
	0	0	1	2	11.01 (0.03)			
	1	1	1	0	23.41 (0.02)			4.3×10^{-8}
	1	1	1	-1	20.03 (0.01)			
Cu-Imip- Proline	0	0	1	1	10.52 (0.01)	8.60	15.09	4.4×10^{-8}
	0	0	1	2	12.03 (0.04)			
	1	1	1	0	23.58 (0.02)			1.6×10^{-8}
	1	1	1	-1	19.70 (0.03			
Cu-Imip- L-Threonine	0	0	1	1	9.11 (0.01)	8.66	14.13	7.0×10^{-8}
	0	0	1	2	11.32 (0.02)			
	1	1	1	0	23.25 (0.02)			2.2×10^{-7}
	1	1	1	-1	12.70 (0.06)			
Cu-Imip- Tryptophan	0	0	1	1	9.52 (0.01)	13.26	15.36	3.2×10^{-8}
	1	0	1	0	7.72 (0.02)			
	1	1	1	0	22.10 (0.03)			8.4×10^{-7}
	1	1	1	1	26.26 (0.01)			
	1	1	1	-1	20.76 (0.02)			
Cu-Imip- Tyrosine	0	0	1	1	10.18 (0.02)	10.17	15.81	1.1×10^{-8}
	0	0	1	2	19.42 (0.03)			
	0	0	1	3	22.28 (0.02)			
	1	1	1	0	4.22 (0.03)			9.2×10^{-7}
	1	1	1	1	29.70 (0.04)			
	1	1	1	-1	19.87 (0.04)			
Cu-Imip- Histidine	0	0	1	1	9.53 (0.01)	11.48	19.70	1.8×10^{-7}
	0	0	1	2	15.81 (0.02)			
	1	1	1	0	26.50 (0.05)			
	1	1	1	1	31.13 (0.05)			
Cu-Imip- Histamine	0	0	1	1	9.88 (0.01)	10.20	17.53	2.4×10^{-8}
	0	0	1	2	15.97 (0.01)			
	1	1	1	0	26.20 (0.02)			2.5×10^{-7}
	1	1	1	1	32.30 (0.02)			
Cu-Imip- Ethanolamine	0	0	1	1	7.94 (0.01)	4.91		3.5×10^{-7}
	1	1	1	0	16.64 (0.06)			2.6×10^{-7}
	1	1	1	-1	8.50 (0.08)			

[a] l, p, q and r are the stoichiometric coefficients corresponding to Cu(II), Imip acids and H^+, respectively; The coefficient -1 refers to a proton loss.
[b] Log β of Cu-Imip-amino acids.
[c] Standard deviation are given in parentheses.
[d] Sum of square of residuals.

3.2. Ternary complex formation equilibria

The formation constant of the binary complexes of amino acids, dicarboxylic acids and amides with Cu(II) presented in Tables 3-5 were taken from the literature [12,13]. The results show that the chelating potential of the 1:1 Cu(II) complexes with Imip and other ligands, are found to be in the same order. Consequently the ligation of Imip and amino acids, dicarboxylic acids or of the amides to Cu(II) will proceed simultaneously. The validity of this model was verified by comparing the experimental potentiometric data with the theoretically calculated (simulated) curve.

3.2.1. Amino acid complexes

The titration data of the ternary complexes with amino acids and Imip fit satisfactorily with formation of the species: Cu(Imip), Cu(L), $Cu(L)_2$, Cu(Imip)(L), Cu(Imip)(LH) and $Cu(Imip)(LH_{-1})$, where L is the studied amino acids. These results were further verified by comparing the experimental potentiometric data with the theoretically calculated (simulated) curve, to support the ternary complex formation model.

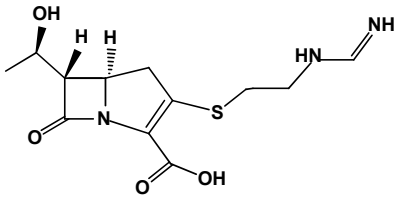
Phenylalanine forms a more stable complex than alanine, although the amino group of phenylalanine (pK_a =9.12) is less basic than that of alanine (pK_a = 9.96) [12,15] This may be due to some stacking interactions between the phenyl group of phenylalanine and the aromatic moiety of Imip as shown in Scheme 1. This will contribute to the stabilization of the formed complex.

The titration data of threonine fit satisfactory with the formation of the complex species $[Cu(Imip)LH_{-1})]^+$. This species is formed due to the induced ionization of the extra binding β-alcoholato-group of threonine as mentioned in the literature [34]. The pK_a value of the β-alcoholato-group incorporated in the Cu(II) complex (Log β_{1110} – Log β_{111-1}) is 10.55. This is in good agreement with that reported in literature for the Cu-threonine complex [12,13,35]

Table 4. Formation constants of the binary and ternary complexes in the Cu(II)-Imip-dibasic acid systems at 25°C and 0.1M ionic strength.

System	l [a]	p [a]	q [a]	r [a]	Log β [b]	pK_a [c]	S [d]
Oxalic acid	0	0	1	1	4.10 (0.00)	4.10	1.3×10^{-9}
	0	0	1	2	5.78 (0.01)	1.68	3.7×10^{-7}
	1	0	1	0	6.98 (0.05)		
	1	0	2	0	11.27 (0.09)		
	1	1	1	0	22.81 (0.04)		1.6×10^{-8}
	1	1	1	1	24.05 (0.04)	1.24	
Cyclobutane-1,1-dicarboxylic (CBDCA)	0	0	1	1	5.54 (0.01)	5.54	5.4×10^{-8}
	0	0	1	2	8.77 (0.02)	3.23	
	1	0	1	0	6.54 (0.03)		1.7×10^{-7}
	1	0	2	0	9.19 (0.09)		
	1	0	1	1	10.59 (0.04)		2.2×10^{-7}
	1	1	1	0	20.66 (0.03)		8.2×10^{-8}
	1	1	1	1	22.68 (0.03)	2.02	
Malonic acid	0	0	1	1	5.42 (0.00)	5.42	
	0	0	1	2	8.19 (0.01)	2.77	2.0×10^{-9}
	1	0	1	0	5.62 (0.05)		
	1	0	2	0	9.06 (0.08)		
	1	0	1	1	9.98 (0.09)		3.3×10^{-8}
	1	1	1	0	20.27 (0.03)		
	1	1	1	1	23.77 (0.03)	3.5	2.9×10^{-8}
Succinic Acid	0	0	1	1	5.35 (0.00)	5.35	5.3×10^{-10}
	0	0	1	2	9.41 (0.01)	4.06	
	1	0	1	0	4.50 (0.01)		1.8×10^{-9}
	1	0	2	0	7.42 (0.03)		
	1	0	1	1	9.76 (0.01)		
	1	1	1	0	19.71 (0.05)		5.2×10^{-6}
	1	1	1	1	20.56 (0.04)	0.86	
Adipic acid	0	0	1	1	5.28 (0.00)	5.28	3.5×10^{-9}
	0	0	1	2	9.61 (0.01)	4.33	
	1	0	1	0	4.18 (0.09)		2.9×10^{-8}
	1	0	2	0	7.93 (0.07)		
	1	0	1	1	8.99 (0.06)		
	1	1	1	0	18.86 (0.08)		1.4×10^{-6}
	1	1	1	1	19.10 (0.09)	0.24	

[a] l, p, q and r are the stoichiometric coefficients corresponding to Cu(II), Imip, dibasic acids and H^+, respectively.
[b] Log β of Cu-Imip-dibasic acids. Standard deviations are given in parentheses.
[c] The pK_a of the protonated species (Log β_{1111}–Log β_{1110}).
[d] Sum of square of residual.



Imipenem

Scheme 1

The pK_a value of the coordinated alcohol group in the ethanolamine complex (8.14) is considerably smaller than of the threonine complex. This could be explained according to the reaction scheme where the alcohol group in ethanolamine is coordinated to copper center, where the OH group in threonine remains attached prior to deprotonation. Due to the donation of the electron pair on oxygen to the metal center, the OH bond can be considerably weekend and the ionization of a proton occurs at fairly low pH. It is also noticed that the Log β_{1110} value in ethanolamine is smaller than those for the amino acids. This may be attributed to the weaker coordination tendency of the neutral alcohol group compared to the negatively charged carboxylate group.

Histidine is a ligand having amino, imidazole and carboxylate group as possible binding sites. Histamine has only two binding sites via, amino and carboxylate group. Hence, histidine coordinates in either glycine-like or histamine-like mode. The stability constant values of histidine and histamine are considerably higher than those of amino acids. This indicates that both histidine and histamine would preferably coordinate through amino and imidazole group. Histamine has been shown to form protonated complex species (1111). The acid dissociation constants of the protonated species are given by the following equation.

$$pK^{H}_{Cu(Imip)L} = \text{Log } K^{Cu(Imip)}_{Cu(Imip)(L)(H)} - \text{Log } K^{Cu(Imip)}_{Cu(Imip)(L)} \quad (4)$$

The pK_a value for the histamine complex is (6.10), being lower than that of the protonated amino group (-NH_3^+) in the histamine ligand (pK_a = 9.85) but closer to that of the protonated imidazole (pK_a = 6.12), considering the increase in acidity due to complex formation. This reveals that the proton in the protonated complex would be located mainly on the imidazole group.

Estimation of the concentration distribution of the various species in solution provides a useful picture of metal ion binding. The speciation diagram for complexes of threonine, taken as a representative amino acid, is given in Figure 5. The [Cu(Imip)(Threonine)] complex species predominates in the physiological pH range and attains a maximum concentration of 100 %, between pH = 5.5 and 8.0. Upon increase of pH the hydroxo species [Cu(Imip,H_{-1})(Threonine)] starts to form up pH = 8 and attains a maximum concentration of 100 % at pH = ~12 .

3.2.2. Dicarboxylic acid complexes

The titration data of the ternary complexes with dicarboxylic acids and Imip fit satisfactorily with formation of the species: Cu(Imip), Cu(L), Cu(Imip)(L) and Cu(Imip)(LH)., Where L is dicarboxylic acids. The results in Table 4 show that the formation constants of the 1:1:1 complexes involving formation of five-membered chelate ring as in oxalic, and six-membered chelate rings as in malonic and CBDCA are higher than those involving seven-membered, as in succinic, and eight-membered chelate ring as in adipic acid.

Table 5. Formation constants of the binary and ternary complexes in the Cu(II)-Imip- amides at 25 °C and 0.1 M ionic strength.

System	l [a]	p [a]	q [a]	r [a]	Log β [b]	pK_a [c]	S [d]
Glycinamide	0	0	1	1	7.88 (0.00)	7.88	4.6×10^{-8}
	1	0	1	0	4.75 (0.04)		6.4×10^{-7}
	1	0	1	-1	-1.58 (0.02)		
	1	1	1	0	22.42 (0.06)		2.2×10^{-6}
	1	1	1	-1	20.37 (0.05)	2.05	
Glycylglycine	0	0	1	1	7.97 (0.01)	7.97	2.5×10^{-8}
	0	0	1	2	11.01 (0.01)	3.04	
	1	0	1	0	5.50 (0.02)		1.7×10^{-8}
	1	0	1	-1	1.14 (0.01)		
	1	1	1	0	22.46 (0.06)		3.1×10^{-6}
	1	1	1	-1	18.50 (0.05)	3.96	
DL-Aspargine	0	0	1	1	8.55 (0.01)	8.55	5.9×10^{-8}
	0	0	1	2	10.79 (0.03)	2.24	1.0×10^{-8}
	1	0	1	0	6.21 (0.03)		
	1	0	1	-1	-2.40 (0.07)		4.1×10^{-7}
	1	1	1	0	21.51 (0.01)		
	1	1	1	-1	20.32 (0.02)	1.19	
L-Glutamine	0	0	1	1	9.06 (0.01)	9.06	1.1×10^{-8}
	0	0	1	2	11.19 (0.02)	2.19	6.4×10^{-7}
	1	0	1	0	7.69 (0.01)		
	1	0	1	-1	-1.59 (0.02)		3.8×10^{-8}
	1	1	1	0	22.27 (0.01)		
	1	1	1	-1	18.93 (0.01)	2.34	

[a] l, p, q and r are the stoichiometric coefficients corresponding to Cu(II), Imip, amides and H^+, respectively; the coefficient -1 refers to a proton loss.
[b] Log β of Cu-Imip-amides. Standard deviations are given in parentheses.
[c] The complex pK_a of the amides.
[d] Sum of square of residuals.

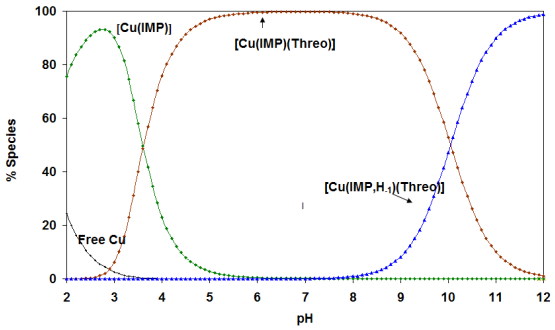


Figure 5. Concentration distribution of various species as a function of pH for the [Cu(IMP)-Threonine] system at a concentration of $1.25\text{x}10^{-3}$ mol/dm^3, for Cu, Imip and threonine. I = 0.1 and T= 25 °C.

This may be explained on the premise that the five and six-membered rings are more favored energetically than the seven and the eight-memberd rings.

It is interesting to note that CBDCA has a 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 than the latter dicarboxylic acid. The pK_a of the protonated species is 2.02, a value lower than that of the free H-CBDCA (5.68), which indicates acidification upon first chelation to Cu(II) through one carboxylate group by 3.64 pH units (5.68-2.02).

3.2.3. Amide complexes

The ionized amide residue of the peptide, [-CONH-], behaves as an important ligating group, and coordinates to metal ions as Cu(II), Pd(II), and Ni(II), through binding with the ionized amide group [36]. In the present investigation, ternary complex formation of amides proceeds also through simultaneous reaction. The species to be considered are Cu(Imip), Cu(L), Cu(LH$_{-1}$), Cu(Imip)(L) and Cu(Imip)(LH$_{-1}$), and their formation constants are given in Table 5. The amide may form the Cu(Imip)(L) complex by coordination through the amine and carbonyl groups. It was reported that on increasing the pH, the coordination sites should switch from carbonyl oxygen to amide nitrogen [37,38]. However, since the stability constant value of the 111-1 species is in fair agreement with those of amino acids, where there is no induce ionization. Therefore, it is assumed that the 111-1 species is formed through coordination of the hydroxyl group forming the hydroxo-complex. The speciation diagram of glycylglycine complex, as a representative amide, is given in Figure 6. The mixed ligand species [Cu(Imip)L] (1110) starts to form at pH = ~2 and with increasing pH, its concentration increases reaching a maximum of 80 % at pH = 3. Further increase of pH is accompanied by a decrease in 1110 complex concentration and an increase in [Cu(Imip)LH$_{-1}$] (111-1) complex concentration.

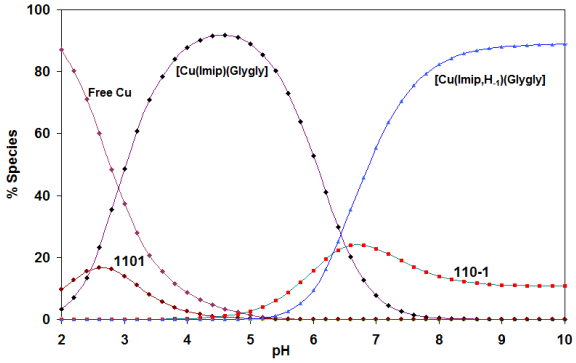


Figure 6. Concentration distribution of various species as a function of pH for the [Cu(Imip)(Glycylglycine] system at a concentration of 1.25 mmol/ dm^3, for Cu, Imip and Glycylglycine . I = 0.1, T =25 °C.

3.2.4. DNA-unit complexes

In this study, the ternary complex formation equilibria involving Cu(II) with Imip and some selected DNA units like uracil, uridine, thymine, inosine and inosine-5'-monophosphate were also studied, and the concentration distribution diagrams of the formed complexes species have been evaluated. On Comparing the stability constant value of Cu(II)-Imip with those of Cu(II)-DNA unit complexes it is clear that the value of the Cu(II)-Imip complex is considerably higher than those of Cu(II)-DNA unit complexes.

Table 6. Formation constants of [Cu-Imip]-DNA complexes, at 25 °C and 0.1 M ionic strength.

System	p	q	r [a]	Log β [b]	pK_a [c]	S [d]	Log K^{Cu}_{CuD}	Δ Log K
Cu(Imip)-OH	1	0	-1	-6.62 (0.01)		5.7×10^{-8}		
	1	0	-2	-16.85 (0.05)				
Uracil	0	1	1	9.28 (0.06)	9.28	2.4×10^{-8}		
	1	1	0	7.70 (0.04)		1.5×10^{-6}	5.49	2.21
	1	2	0	13.24 (0.01)				
Uridine	0	1	1	9.01 (0.01)	9.01	1.1×10^{-7}		
	1	1	0	7.94 (0.05)		5.4×10^{-7}	4.03	3.91
	1	2	0	13.40 (0.01)				
Thymine	0	1	1	9.58 (0.00)	9.58	8.7×10^{-8}		
	1	1	0	9.10 (0.03)		5.9×10^{-7}	5.77	3.33
	1	2	0	12.58 (0.06)				
Thymidine	0	1	1	9.55 (0.00)	9.55	8.1×10^{-8}		
	1	1	0	9.69 (0.02)		1.4×10^{-7}	4.70	4.99
	1	2	0	13.60 (0.08)				
Inosine	0	1	1	8.43 (0.01)	8.43	1.1×10^{-6}		
	1	1	0	7.80 (0.02)		2.2×10^{-7}	4.50	3.30
	1	1	1	13.57 (0.03)	5.70			
Inosine 5′-monophosphate	0	1	1	9.21 (0.01)	9.21	2.3×10^{-8}		
	0	1	2	15.21 (0.01)	6.00			
	1	1	0	8.40 (0.01)		5.8×10^{-8}	3.50	4.90
	1	1	1	15.30 (0.01)	6.90			
	1	1	2	21.50 (0.02)	6.20			

[a] p, q and r are the stoichiometric coefficients corresponding to[Cu-Imip], DNA units and H^+, respectively.
[b] Log β of [Cu-Imip]-DNA units. Standard deviations are given in parentheses.
[c] The pK_a of the protonated species (Log β_{111}–Log β_{110}).
[d] Sum of square of residuals.

This implies a stepwise mechanism for the formation of the ternary complexes of Cu(II)-Imip with DNA units, whereby Cu(II)-Imip complex is first formed due to its greater stability, followed by chelation of DNA constituent. The results are presented in Table 6. The stepwise reaction of the ternary complex formation could be described by the equilibria presented in Equation (5) and (6).

$$Cu + Imip \rightleftharpoons Cu(Imip) \quad (5)$$

$$Cu(Imip) + D \rightleftharpoons Cu(Imip)D \quad (6)$$

(Charges are omitted for simplicity)

Uracil, uridine, and thymine have only a basic nitrogen donor atom (N_3-C_4O group) in the measurable pH range. Fitting the potentiometric data of these pyrimidinic species was found to be consistent with the formation of the 1:1 and 1:2 complexes with $Cu(Imip)^{2+}$ ion. The inductive effect of the extra electron-donating methyl group of thymidine increases the basicity of its N3 group, consequently the thymidine complex is more stable than that of uridine.

As a result of the high pK_a values of the pyrimidines (pK_a > 9) and the fact that they are monodentates, their complexes are formed only above pH = 6, supporting the view that the negatively charged nitrogen donors of the pyrimidine bases are important binding sites in the neutral and slightly basic pH ranges. The calculated values show that the mixed ligand complexes of nucleosides are less stable than the corresponding bases. This is due to the steric hindrance caused by the suger residue in the nucleotides, which may reduce their complexation tendency with the metal ions and consequently reduces the overall basisty of the formed complexes considerably. Inosine and inosine 5′-mono-phosphate (IMP), as examples of purines, are reported to have two metal ion binding centers, N_1 and N_7 nitrogen's. Inosine forms the complexes 110 and 111, while inosine 5′-monophosphate forms 110, 111 and 112 complexes. The extra stabilization of inosine-5′-monophosphate complex with $Cu(Imip)^{2+}$ compared to that of inosine is due to the triply negatively charged 5′-imp^{3-} ion. The protonated species formed in case of inosine-5′-monophosphate corresponds to N_7 coordinated complex, where the N_1 nitrogen and the phosphate group are protonated. Figure 7 shows the speciation of inosine 5′-monophosphate complex, where the species distribution of the complexes is plotted against pH. From Figure 7 it is clear that the species 112 and 111 are formed in the acidic pH region. The pK_a values of the protonated species of the inosine-5′-monophosphate complex which corresponds to N_1H group is 6.5 (Log β_{112} - Log β_{111}). Whereas, the second protonated species which corresponds to the $-PO_2(OH)$ has a pK_a value of 6.2 (Log β_{111} - Log β_{110}). This means that acidification by 2.71 pK_a units (9.21 to 6.50) for the N_1H groups will occur upon complex formation, which is in consistent with that reported previously for inosine 5′-monophosphate and guanosine 5′-monophosphate [39]. The different coulombic forces operating between the ions resulting from the negatively charged phosphate group in inosine-5-monophosphate would result in extra stabilization of its complexes species compared to that of pyrimidines. By way of comparison of the relative stability of the ternary complexes formed with (Cu-IMP) and the DNA unites, through the stepwise mechanism, to the corresponding binary Cu-DNA unit complexes, the ΔLog K values are calculated using Equation (7).

$$\Delta\text{Log } K = \text{Log } K^{CuIMP}_{Cu(IMP)D} - \text{Log } K^{Cu}_{CuD} \quad (7)$$

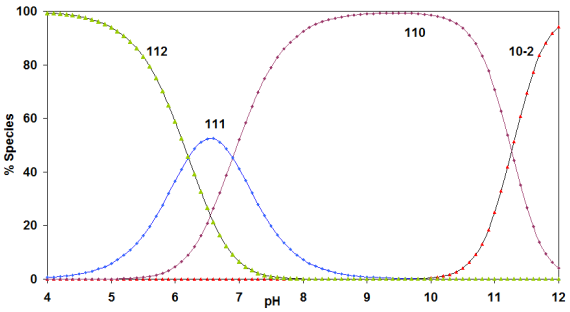


Figure 7. Concentration distribution of various species as a function of pH for the [Cu(Imip)]-inosine-5-monophosphate system at a concentration of 1.25 mmol/dm^3 for Cu(Imip) and 2.5 mmol/dm^3 for inosine .

ΔLog K has been widely accepted and used for many years [12,40] and the advantages in using ΔLog K in comparing

stabilities of ternary and binary complexes have been reviewed. It expresses the effect of the bounded primary ligand towards an incoming secondary ligand (L). One expects to obtain negative values for ΔLog *K* since more coordination positions are available for the bonding of ligand (L) in the binary than in the ternary complexes. This indicates that the secondary ligand (L) DNA form more stable complexes with copper (II) ion alone than with the Cu(II)-Imip. It is of interest to note that for all the systems listed in Table 6, the values of ΔLog *K* are invariably positive. This means that the DNA constituents form more stable complexes with Cu(Imip) than with the free Cu(II) ion.

4. Conclusion

The present investigation describes the complex formation equilibria of imipenem with some selected metal ions of biological significance, to give an idea about its potentiality towards metal ions in biological fluids. The protonation constant of Imip as well as the Cu-Imip complex formation were studied in dioxane-water mixture of different composition in order to check the complexation tendancy of imipenem in media of lower polarity. This allows extrapolating the investigation to some biological micro-environment of low polarity, as active sites of enzymes and side chain proteins. The results show that the formation of the Cu-Imip complex will be more favored in biological environments of low dielectric constant.

The investigation also describes the ternary complex formation equilibria of imipenem with Cu(II) and some selected biorelevant ligands containing different functional groups and occurring *in vivo,* as amino acids, peptides, dicarboxylic acids and DNA units. Amino acids and peptides form highly stable 1:1 complexes and the corresponding hydroxo species through a simultaneous reaction mechanism. Ternary complex formation with DNA units proceeds through a stepwise mechanism. DNA constituents form both 1:1 and 1:2 complexes. In particular such mixed-ligand coordination is likely to occur with bacterial nucleic acid. The earlier observation that copper-tetracycline complexes can bind DNA units where tetracycline itself cannot is in accordance with our results for imipenem, where Cu-Imip-DNA complex formation is favored as reflected from the positive values of ΔLog *K*.

The stability data presented in this work arise mainly from data fitting of potentiometric titrations. The ligand and complexes may have interesting biological activity. This would require specially designed research conducted by specialized biologists. It is hoped that the present investigation will be a significant contribution for understanding the antibiotic action in biological fluids where all the selected ligands are present simultaneously.

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References

[1]. Beth, B. M.; Brian, S. K. *Antimicrob. Agents Chemother.* **2002**, *46*, 3978-3980.
[2]. Shehata, M. R.; Shoukry, M. M.; Barakat, M. M. *J. Coord. Chem*. **2004**, *57*, 1369-1386.
[3]. Sekhon, B. S. *J. Pharm. Educ. Res.* **2010**, *1*, 21-25.
[4]. El-Ajaily, M. M.; Abdlseed, F. A.; Ben-gweirif, S. *E-J. Chem*. **2007**, *4*, 461-466.
[5]. Sultana, N.; Arayne, M. S. *Pak. J. Pharm Sci.* **2007**, *20*, 305-310.
[6]. Arayne, S.; Sultana, N.; Haroon, U.; Mesaik, A. M. *Bioinorg. Chem. Appl*. **2009**, 2009, 914105-914115.
[7]. Raman, N; Joseph, J.; Sakthivel, A.; Jeyamurugan, R. *J. Chil. Chem. Soc*. **2009**, *54(4)*, 354-357.
[8]. Ming, L. J. *Med. Res. Rev*. **2003**, *23*, 697-762.
[9]. Iakovidis, I; Delimaris, I.; Piperakis, S. M. *Mol. Bio. Intern.* 2011, 594529-594542.
[10]. Melnik, M.; Potocnak, I.; Macaskova, L.; Miklos, D.; Holloway, C. E. *Polyhedron* **1996**, *15*, 2159-2164.
[11]. Garcia-Raso, A.; Fiol, J. J.; Adrover, B.; Tauler, P.; Pons, A.; Mata, I.; Espinosa, E.; Molins, E. *Polyhedron* **2003**, *22*, 3255-3264.
[12]. Shoukry, A. A. *J. Sol. Chem*. **2011**, *40*, 1796-1818.
[13]. Shoukry, A. A. *Trans. Metal Chem*. **2005**, *30*, 814-827.
[14]. Shoukry, A. A.; Mohamed, M. M.; Shoukry, M. M. *J. Sol. Chem*. **2006**, *35*, 853-868.
[15]. Shoukry, M. M.; Shoukry, M. M.; Hafez, M. N. *J. Coord. Chem.* **2009**, *63(4)*, 652-664.
[16]. Shoukry, A. A.; Brindell, M.; Van Eldik, R. *Dalton Trans*. **2007**, 4169-4174.
[17]. Welcher, F. J. The Analytical Uses of Ethylenediamine Tetraacetic Acid. Van Nostand, Princeton 1965.
[18]. Vogel, A. E. Text Book of Quantitative Chemical Analysis, Longman, Harlow, 5th edn., Chap. 15, pp. 555, 1989.
[19]. Irving, M. H.; Miles, M. G.; Pettit, L. D. *Anal. Chim. Acta.* **1967**, *38*, 475-488.
[20]. Stark, J. G.; Wallace, H. G. Chemistry Data Book Murray, London p. 75. 1975
[21]. Van Uitert, G. L; Hass, C. G. *J. Am. Chem. Soc*. **1971**, *75*, 451-455.
[22]. Motekaitis, R. J.; Martell, A. E.; Nelson D. A. *Inorg. Chem*. **1984**, *23*, 275-283.
[23]. Shoukry, A. A.; Al-Mhayawi S. R. *Eur. J. Chem*. **2013**, *4(3)*, 260-267.
[24]. Gans, P.; Sabatini, A.; Vacca, A. *J. Inorg. Chim. Acta* **1976**, *18*, 237-239.
[25]. Pettit, L. D. SPECIES, available program supplied to the authors, Academic Software, Old Farm, Timbly, Otley, 1993 York, LS21 2PW, UK
[26]. Shehata, M. R.; Shoukry, M. M.; Nasr, F. M.; Van. Eldik R. *Dalton Trans.* **2008**, 779-786.
[27]. Cotton, F. A.; Wilkinson, G. Basic Inorganic Chemistry, Part 3. Wiley, New York, pp. 353, 1973
[28]. Shoukry, M. M.; Saeed, A.; Khairy, E. M. *Trans. Metal Chem.* **1989**, *14*, 347-350.
[29]. Kramer-Schnabel, U.; Linder, P. W. *Inorg. Chem*. **1991**, *30*, 1248-1254.
[30]. Rees, D. C. *J. Mol. Biol.* **1980**, *141*, 323-326.
[31]. Rogersa, N. K.; Mooreb, G. R.; Sternberga, M. J. E. *J. Mol. Biol.* **1985**, *182*, 613-616.
[32]. Akerlof, G.; Short, O. A. *J. Am. Chem. Soc*. **1953**, *75*, 6357-6362.
[33]. Dogan, A.; Koseoglu, F.; Kılıc, E. *Anal. Chem.* **2001**, *295*, 237-239.
[34]. Sigel, H.; Martin, R. B. *Chem. Rev.* **1982**, *82*, 385-426.
[35]. Grenouillet, P.; Martin, R. P.; Rossi, A.; Ptak, M. *Biochim. Biophys. Acta.* **1973**, *322*, 185-194.
[36]. Shoukry, M. M.; Khairy, E. M.; Khalil, R. G. *Trans. Met. Chem*. **1997**, *22*, 465-470.
[37]. Savago, I.; Kiss, A.; Farkas, E.; Sanna, D.; Marras, P.; Micerain, G. *J. Inorg. Biochem.* **1997**, 65, 103-108
[38]. Daniele, P. G.; Zerbinati, O.; Zelano, V.; Ostacoli, G. *Dalton Trans.* **1991**, 2711-2715.
[39]. Sigel, H.; Massoud, S. S.; Corfu, N. A. *J. Am. Chem. Soc.* **1994**, *116*, 2958-2971.
[40]. Sigel, H. *Angew. Chem. Int. Ed.,* **1975**, *14*, 394-402.