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Potentiometric Binary Stability Constant Studies of the Complexes of Some Biologically Important Transition Metal Ions with Cysteine

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ABSTRACT

A lot of the traditional chelating agents have been reported to be toxic, non-biodegradable, and rigid towards the recovery of bonded metal ions. The drawbacks with these chelators necessitate a search for their alternatives such as amino acids. Binary complexes of biologically important transition metal ions: Cu(II), Ni(II), Co(II), and Mn(II) with cysteine have been studied potentiometrically at about 27 ℃ in aqueous medium using Irving-Rossotti titration technique. The data obtained from the Irving-Rossotti titration technique were used to evaluate the proton-ligand and metal-ligand stability constants. The proton-ligand stability constants were: log K_1H 8.4 (pKa for thiol group) and log K_2H 10.7 (pKa value for NH_2-group). The binary metal-ligand stability constant values for 1:1 (M: L) complexes, log K, as evaluated were in the order of Cu(II) > Ni(II) > Co(II) > Mn(II); which is in agreement with the Irving–Williams order of the divalent metals of 3d series metal ions. Though the redox-active nature of Cu(II)-cysteine atmosphere must have affected the Cu(II)-cysteine stability constant. Hence, this work agreed with previous reports that cysteine is fit for use as alternative traditional chelants for relocating metal ions and for detoxification of metal poisoning and allied applications.

Keywords: Metal ions, Ligand, Potentiometric titration, Binary formation constant

INTRODUCTION

Stability constant is an equilibrium constant that quantifies the strength of the interaction between the ion and the ligand. It can be used for the purposes of: assessment of metal-ligand interactions; validating theoretical models; determining biological relevance; chelation therapy/detoxification; metal transport; drug function and design; and anticancer/antimicrobial agents [1-3].

Cysteine is a versatile ligand that forms complexes with 3d transition metals. There are several reports of the preparation and characterization of 3d transition metals complexes with cysteine [4-6]. Many chelators are widely used as scavengers to relocate metals to or away from

target sites due to their ability to form strong bonds with different metal ions. However, a good number of these common chelating agents have been reported to be toxic, non-biodegradable, and rigid towards the recovery of bonded metal ions [7]. Therefore, there is need for alternative chelating agents with good credentials [8] which are nontoxic and flexible for recovering bound metal ions. Amino acids are regarded as essential integral foundation of living organisms. Amino acids unite to form proteins which are abundant in our body system. The reactions of proteins with metals in our bodies is then inevitable. More so, cysteine is a sulfur-containing amino acid crucial for biological systems, primarily due to its thiol group, which facilitates disulfide bonds for protein structure, acts as a redox switch for protein function, and binds to metals. Therefore, understanding the stability constants of biologically important metal ions with cysteine is essential for their potential applications [9] and gaining insight into the metal-protein activity in the body. Amino acids are metal ions coordinating agents via their amino (NH_2), carboxylic (COO^-) and thiol (-SH) groups etc [10, 11]. They can be used in the treatment of metal poisoning [9]. They are good alternatives of the traditional chelants [12].

The presence of metals in biological fluids has significant influence on the therapeutics [13]. Thus, the study of metal complexes of biologically active ligands is important because it gives the understanding of interaction between the protein and metals in the biological systems. In addition, these studies will help to identify the atoms or groups that are responsible for binding to metal ions, or are used in retention of toxic heavy metal ions [9]. The degree of metal-ligand complex formation is therapeutically useful as the pharmacological relevance of a drug is related to its status (either in free or complexed form) [14].

Copper is vital for good number of enzymes for catalyzing physiologically important reactions. Cu(II) has a strong ability to form complexes with proteins, peptides, and enzymes in the living organisms [9]. On the otherhand, cobalt is found in vitamins (B_{12} and B_9) that facilitate the natural production of red blood cells. Nickel can replace other metal ions in enzymes and proteins which then bind to cellular compounds containing O, S, and N atoms such as enzyme and nucleic acids. The deficiency of Ni can impair intestinal absorption of iron and thus causes anemia. In addition, 90% of glucose and glycogen levels in the liver and serum are reduced upon nickel deficit [9].

Although there are many methods for studying the stability of proton-ligand and metal-ligand complexes, pH-metry is most frequently used because of its accuracy and reliability [13,

15]. In the determination of formation constants of complexes some of these precautions are considered: Firstly, the concentration of the ligand should be higher than that of the metal so as to prevent hydrolysis of metal ions. Secondly, the ionic strength must be kept at ≤ 0.2 mol/dm^3 to hinder the formation of ion pair between the anionic species and the cationic species or strong electrolytes [14]. Furthermore, it has been revealed that the stability constants increase when metal ion with high covalent index interacts with a ligand that possesses high polarizability [8].

There is paucity of comparative metal ligand stability constants studies of 3d transition metal ions with cysteine. In this work the stability constants of some 3d transition metal ions with cysteine are compared. Therefore, this study reports the comparison of potentiometric binary stability constants of complexes of some biologically important transition metal ions with cysteine using Calvin-Bjerrum titration technique as applied by Irving and Rossotti [16], to investigate the stability constants of these metal ions with cysteine.

MATERIALS AND METHODS

Materials

All the potentiometric measurements were carried out on a pH meter (Hanna Pocket pH meter - HI - 96107). The meter was calibrated using standard buffer pH ≈ 4.00, pH ≈ 7.00, and pH ≈ 9.00 respectively. L-Cysteine (Sigma-Aldrich, 97%), NaOH (MolyChem, 96%), HCl (JHD, 37%), $CuCl_2$. $2H_2O$ (LOBA CHEMIE PVT. LTD, 96%), $NiCl_2$. $6H_2O$ (ACS, 97%), $CoCl_2$. $6H_2O$ (Kermel, 97% pure) and $MnCl_2.4H_2O$ (ACS, 98%) used were all of analytical grade. Distilled water was used for the preparation of the solutions.

Potentiometric Studies

The potentiometric studies were performed using Calvin-Bjerrum titration technique as applied by Irving and Rossotti [16]. Calvin-Bjerrum method, as modified by Irving and Rossotti, is widely used pH titration technique for determining the stability constants of metal-ligand complexes in solution. The method relies on the principle that the formation of metal-ligand complexes involves the displacement of protons from the ligand molecule. This reaction causes a change in the pH of the solution, which is monitored by pH meter. By comparing the pH titration curves of an acid, and acid plus ligand, and an acid plus ligand plus metal ion, one can determine: (a) Proton-ligand stability constant (pK_a) – related to the basicity of the ligand and (b) metal-ligand stability constant

(log K)- related to the strength of the metal-ligand bond [16]. Therefore, the experimental procedure for the potentiometric titration is described as follows.

The following solutions of acid; cysteine (ligand) and the metal solutions were prepared and titrated against CO_2-free 0.04 mol/dm^3 NaOH solution at about 27 ℃:

(a) 3 mL 0.04 mol/dm^3 HCl;

(b) Solution (a) + 4 mL 0.03 mol/dm^3 cysteine; and

(c) Solution (b) + 2 mL 0.04 mol/dm^3 metal (Cu(II), Ni(II), Co(II), and Mn(II)) chloride solutions, respectively.

In all the titrations, the total volume was maintained constant at 50 mL. The method of Calvin-Bjerrum as adopted by Irving and Rossotti has been employed to determine log K values [13, 14]

RESULTS AND DISCUSSION

Agrawal *et al*. [2] determined stability constants of binary complexes of Cu(II), Ni(II) and Mn(II) transition metal ions with cetrizine and benzoic acid by pH metrically method. Formation constants were also calculated by using Calvin Bjerrum method as modified by Irving and Rossoti. The stability constants were discussed in terms of order of stability and correlated with atomic number, ionization potential, electro negativity and reciprocal of ionic radii. Observed trend in the order of stability constant was Cu(II) > Ni(II) > Mn(II), which are in accordance with the William- Irving series [2]. Furthermore, metal-ligand complexes of Ni(II), Co(II), and Cu(II) containing cysteine had been synthesized previously [4]. The focus in this work was to perform comparative metal-ligand stability constants of some 3d transitions using cysteine. It has been shown in the literature that, studies of relative stability constants are possibly made for reported metal-ligand complexes without the necessity of their laboratory synthesis again [1, 2].

The Irving-Rossotti potentiometric titration

The proton dissociation constants of the cysteine and its complexes of Cu(II), Co(II), Mn(II) and Ni(II) have been determined in aqueous medium at 0.04 mol/dm^3 NaOH and 27 ℃. Therefore, the potentiometric titration curves of cysteine and its metal ions complexes are presented in Figure 1. During the titrations, no precipitate was formed because of the absence of hydroxo complexes [8].

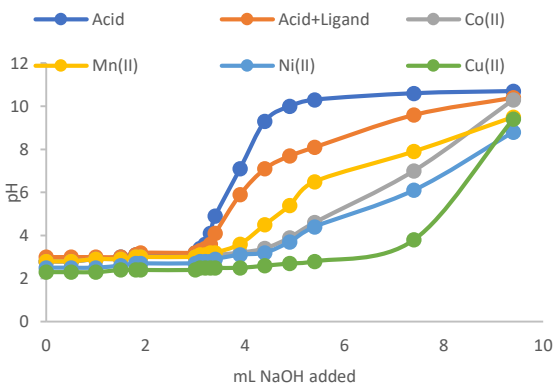


Figure 1: The titration curves of cysteine and its metal ions complexes

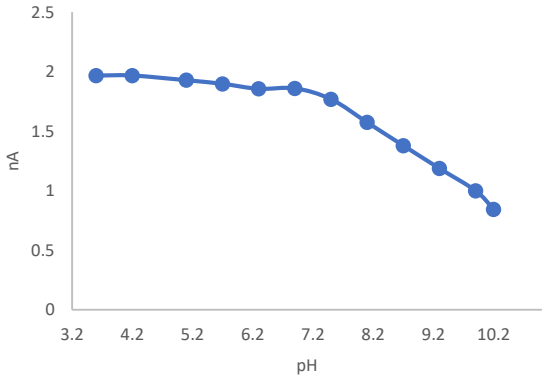


Figure 2: pH-nA curves for Cysteine

Proton-Ligand Stability Constant

From Figure 1 the titration curves indicate that the ligand and metal curves are shifted to the right of the acid titration curve. The shift is due to the release of proton from the ligand as previously observed [8, 9, 13, 14, 17]. This confirms the formation of proton-ligand and metal-ligand complexes [16] through displacement of proton (s) from the ligand [8]. The values of n_A (the degree of formation of the proton complex) were determined by employing the following Equation 1 as previously described by Gayakwad *et al*. [18]; where Y=number of replaceable hydrogen ion; V°=total volume, 50 mL; V_1 =volume of alkali required by the acid; V_2 =volume of alkali used by acid and ligand; N^o =concentration of alkali; E°=total strength of acid; and TcL°=total concentration of ligand.

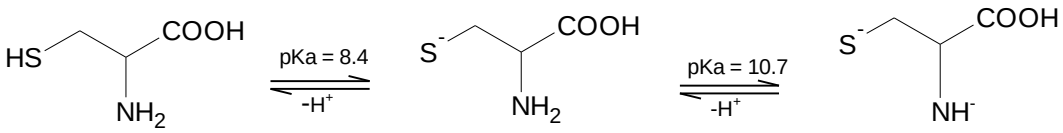
Equations 2 and 3 [19], were then used under integral method of stability constant determination; by putting the value of n_A = 0.5 in Equation 2, we obtain log K_2H = pH. Similarly, by using 1.5 for n_A in the Equation 3, we obtain log K_1H = pH. Therefore, a plot between n_A and pH will give log K_2H and log K_1H corresponding to values of pH at n_A equal to 0.5 and 1.5 [9, 13]. By this, the values of log K_1H and log K_2H were then determined from the curves (Figure 2) corresponding to n_A values of 0.5 and 1.5. It was noted that log K_1H and log K_2H represent the first and second proton formation constants of ligand, respectively. Therefore, log K_1H of 8.4 (lower pKa value) corresponds to the proton from thiol group; and log K_2H 10.7 (higher pKa value) is associated to the proton of the NH_2-group in the cysteine (Scheme 1). The condition of the experiment did not permit the determination of the pKa value for the COOH group. Hence, the

thiol group along carboxylate were implicated in the complex formation of these metal ions and the cysteine [13, 20]. Similarly, Al-Mohaimeed and Alothman observed that S-methylcysteine exhibits pKa value of 8.65 and has found that the sulfur atom contributes in the complex formation process [21].

$$n_A = Y - \frac{(V_2 - V_1)(N^o + E^o)}{(V^o + V_1)T_{CL^o}} \quad (1)$$

$$\log K_2 = pH + log\frac{n_A}{1 - n_A} \quad (2)$$

$$\log K_1 = pH + log\frac{n_A - 1}{2 - n_A} \quad (3)$$



Scheme 1: Proton dissociation from -SH and -NH_2 of cysteine

Metal-Ligand Stability Constant

The binary stability constant was determined using Irving and Rossotti Potentiometric technique [14]. The average number of ligands attached per complex ion **(**$\bar{n}$) can be calculated from the Equation 4 [14]. In the Equation 4, V_n = volume of alkali used for acid + ligand + metal ion titration; TcM^o = total concentration of the metal ion, the rest of term are as given in Equation 1. The free ligand exponent, pL was calculated using Equations 5 [9, 13, 14]. Integral method for stability constants determination were deployed on the Equations 6 and 7 for the evaluation of log K_1 and log K_2, respectively [19]. The approach involves taking the value of $\bar{n}$ = 0.5 in Equation 6 to form logK_1 = pL. More so, when $\bar{n}$ is assumed as 1.5 in the Equation 7, we obtain logK_2 = pL. It implies that a graph between $\bar{n}$ and pL with corresponding values of pL at $\bar{n}$ equal to 0.5 and 1.5 gives log K_1 and log K_2, respectively [9, 13, 14]. Subsequently, plots of pL vs $\bar{n}$ (Figures 3-6) were made as previously performed [13]. After the plots, the stability constants were obtained as presented in Table 1. The stability constant for $\bar{n}$ = 1.5 were not detected at the condition of these experiments. Usually, formation constant or the pL value at n= 0.5 indicates a 1:1 (M: L) composition; whereas, the pL value at n=1.5 shows M: L of 1: 2; a less stable complex [14]. The

values of the stability constants for 1: 1 (M: L) were 7.5, 12.2, 12.8, 33.2 for Mn(II), Co(II), Ni (II), and Cu(II), respectively.

The order of the stability constants is: Cu(II) > Ni(II) > Co(II) > Mn(II); which is in agreement with the Irving–Williams order of the divalent metals of 3d series metal ions. In addition, lack of formation of 1: 2 (M: L) in this work may be due to the experimental conditions such as the nature of metal ion, concentration of ligand, and ionic strength as previously supported in the past [13].

$$\bar{n} = \frac{(V_n - V_2)(N^o + E^o)}{(V_o + V_2) n._A TcM^o} \quad (4)$$

$$pL = \log[\frac{1 + \beta_1[H^+] + \beta_2[H^+].^2}{(TcL^o - \bar{n}TcM^o)} \times \frac{V_o + V_3}{V_o}] \quad (5)$$

$$\log K_1 = log\frac{\bar{n}}{1-\bar{n}} + pL \quad (6)$$

$$\log K_2 = pL + log\frac{(\bar{n}-1)K_1[L]}{(2-\bar{n})K_1[L]} \quad (7)$$

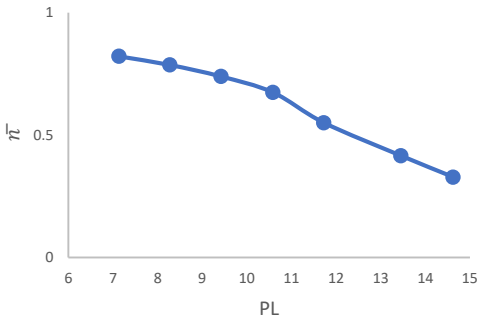


Figure 3: Plot of $\bar{n}$ against (pL) for Co (II)-Cys

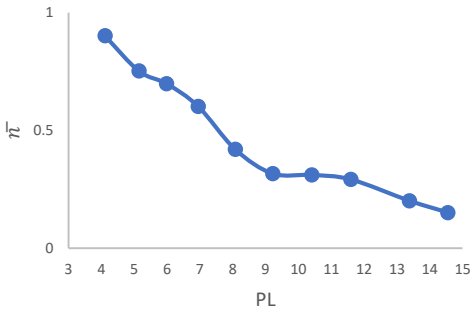


Figure 4: Plot of $\bar{n}$ against (pL) Mn (II)-Cys

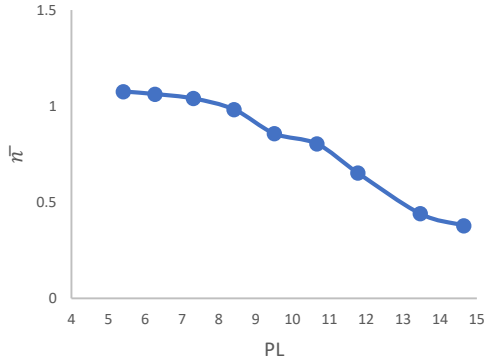


Figure 5: Plot of $\bar{n}$ against (pL) Ni (II)-Cys

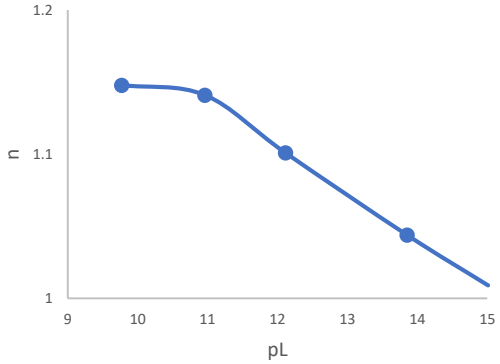


Figure 6: Plot of $\bar{n}$ against (pL) Cu (II) -Cys

Table 1: Characteristics properties of the metal ions and their binary stability constants of the metal-Cys complexes

ions	RI / pm	Z	IP kJ/mol	EN	Species	Log K_1 (M: L; 1:1)
Cu (II)	73	29	2704	1.75	Cu (II)-Cys	33.2
Ni (II)	72	28	2490	1.75	Ni (II) -Cys	12.8
Co (II)	79	27	2404	1.7	Co (II) - Cys	12.2
Mn (II)	80	25	2226	1.6	Mn (II) -Cys	07.5

Note: Cys = cysteine, RI = ionic radius, Z = atomic number, IP = ionization potential, EN = Allred-Rochow electronegativity

According to Adam *et al*. [16], the binary metal complexes of Cu(II) and Ni(II) with some amino acids showed formation constants (via Irving and Rossotti titration technique) of Cu(II)-leucine and Ni(II)-leucine as 8.15 and 5.87, respectively; and 8.12 and 5.78 for Cu(II)-isoleucine and Ni (II)-isoleucine, respectively [16]. In addition, Ishola *et al*. [8] observed that the binary formation constants of Cu(II)-L-tyrosine, Co(II)-L-tyrosine, and Pb(II)-L-tyrosine were 6.40, 4.20, and 6.98, respectively [8], as similarly found in this report. Furthermore, potentiometric determination of stability constant of coordination complex of pyrazinamide with Fe(III) was carried out by Kosasy *et al*. [14]. The pL at $\bar{n}$ = 0.5 (i.e. the stability constant for Fe(III)- pyrazinamide) was 2.75. On the otherhand, the pL value at $\bar{n}$=1.5 gave stability of 1.6 [14]. More so, the binary and ternary complexes of Fe(III), Pb(II), Co(II), Al(III), La(III), Sr(II), Cr(III), Ti(II), and Zr(II) with sulphathiazole and glycine have been studied potentiometrically at 25˚C ± 0.1˚C and I = 0.1 M $NaClO_4$ in 25% (v/v) pure ethanol-water medium [13]. They found that both Al(III) and Zr(IV)

ions form 1:1, 1:2, and 1:3; M: L complexes [13]. Whereas, some metal ions like; Zr(IV), Sr(II), Al(III), Fe(III), Th(IV), and Pb(II) form M: L complexes of 1:1 and 1:2. Furthermore, Co(II), Cr (III), Ti(II) and La(III) only gave complex of 1:1 as relatedly reported in our work [22]. Once again, this may be due to the nature of metal ion, concentration of ligand and ionic strength [13]. Again, the order of stability of the different binary complexes formed between the sulphathiazole and the metal ions as they investigated was in the Irving-Williams order [13]; Fe(III) > Co(II) > Ti(II) > Zr(IV) > Al(III) > La(III) > Cr(III) > Sr(II) > Pb(II) > Th(IV).

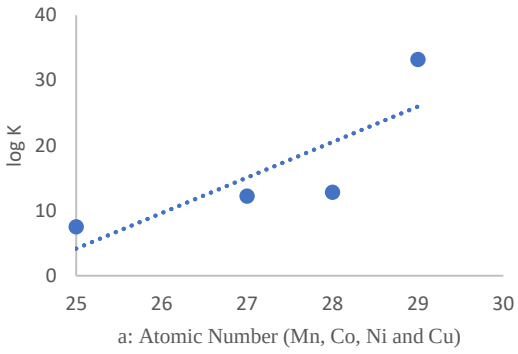
In another development, complex formation equilibria of 2-hydroxy-1-naphthoic acid with Cu (II), Ni (II), Co (II) and Mn (II) and the ternary complexes involving Cu (II) with 2-hydroxy-1-naphthoic acid and some amino acids were studied. The stability of the complexes followed the trend Cu (II) > Ni (II) > Co (II) > Mn (II) which is in agreement with the Irving–Williams order of the divalent metals of 3d series metal ions [23]. Meanwhile, proton-ligand stability constant and metal-ligand stability constant of chlorosubstituted pyrazoles and isoxazoles by Calvin Bjerrum titration as applied by Irving-Rossotti was also reported [15]. The metal-ligand stability constants as found were within the range of 5.343 - 3.644 [15]. Belkher et al. [9] also used Irving-Rossotti titration technique for potentiometric studies of stability constant of the complexes of some essential transition metal ions with *L-Valine* and found the order of stability as Cu (II) > Ni (II) > Co (II) [9], as similarly observed in this research work.

Therefore, assuming the interaction of the metal ion and the ligand is electrostatic and for metal ions of similar electronic configuration, the stability constants for complexes of the metal ions of the same charge should be inversely proportional to metal ion radius (RI) [23]. This has caused the differences of stability of the complexes as found here; Cu (II) > Ni (II) > Co (II) > Mn (II). The M: L stability constant again is directly proportional to the electronegativity, atomic number, and ionization potential of the metal as described in Table 1. In addition, the relationship of M: L stability constants with ionic radii can be shown further when log K values are plotted against the reciprocal of ionic radius [23] (Figure 7(d)).

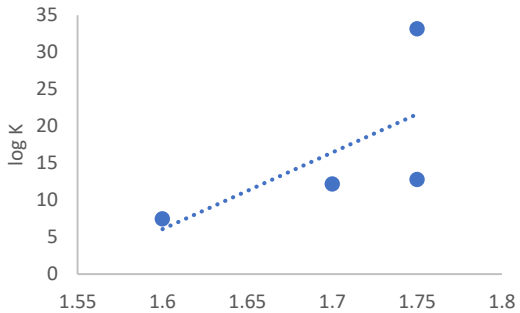
It is noteworthy that increasing electronegativity (EN) of the metal ions will decrease the electronegativity difference between the metal atom and the donor atom of the ligand. The metal–ligand bond would have more covalent character, resulting into greater stability of the metal complex. Plotting log K values against EN of metal atoms gives a straight line (Figure 7(b)). In addition to this, it has been found that ionization potential (IP) of the metal ions also has an effect

on the stability of the formed complex. A linear correlation has been observed between log K and second ionization potentials of divalent metal ions [23]. Plotting log K values against the ionization potential of the metal atoms gives more or less straight line (Figure 7(c)). Similarly, increase in atomic number just like EN and IP increases the stability constant, see (Figure 7(a) because of the favourable effect such enhanced M-L covalent character. However, log K value for the Cu (II) complex deviates significantly when log K values of metal chelates are plotted against properties of the metal ions. The ligand field will give Cu(II) some extra stabilization due to tetragonal distortion of the octahedral symmetry [23]. Ishola *et al*. also stated that the extra stability exhibited by Cu(II)-Thio complex could be linked to the unique electronic configuration of Cu(II) and the Jahn-Teller effect [8].

In general, Cu(II) -Cys complexes have been reported to be very unstable [24] and that Cu (II) has high tendency to oxidize cysteine [24]. The redox-active nature of Cu and Cys can result into formation of versatile and unusual coordination species [25]. These can also affect the stability constant of the Cu(II) – Cys as seen in this work.



a: Atomic Number (Mn, Co, Ni and Cu)



b: Allred-Rochow Electronegativty (Mn, Co, Ni and Cu)

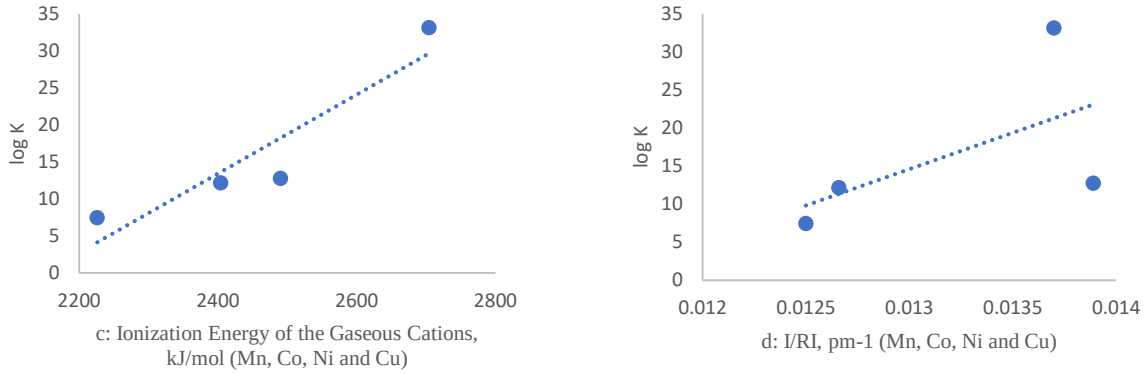


Figure 7: Effect of metal ion properties on the stability constants of the metal complexes

CONCLUSION

Binary complexes of biologically important transition metal ions; Cu(II), Ni(II), Co(II), and Mn (II) with cysteine have been studied potentiometrically at about 27 ℃ in aqueous medium using Irving-Rossotti titration technique. The data obtained of the Irving-Rossotti titration technique were used to evaluate the proton-ligand and metal-ligand stability constants. The proton-ligand stability constants were; log K_1H 8.4 (pKa for thiol group) and log K_2H 10.7 (pKa value for NH_2-group). The binary metal-ligand stability constant values for 1:1 (M: L) complexes, log K as evaluated were found in the order of Cu(II) > Ni(II) > C (II) > M(II); which is in agreement with the Irving–Williams order of the divalent metals of 3d series metal ions. Hence, this work agreed with previous claimed that cysteine is fit for use as alternatives to traditional chelants for relocating metal ions and for detoxification of metal poisoning and allied applications.

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