

Determination of Binding and Selective Efficacy of Two Chelating Agents towards Two Essential Metal Ions in Aqueous Solution

ISHOLA K. T¹, ODEDOKUN O. A², BOLARINWA O.T³, AJAYI, T.A⁴, ADEYEMI M.I.⁵

^{1, 2, 3, 4} Department of Chemistry, Federal College of Education (Special), Oyo, Oyo State, Nigeria.

⁵ Department of Integrated Science, Federal College of Education (Special), Oyo, Oyo State, Nigeria.

Abstract- Many transition metals such as cobalt and copper are involved in a large number of essential biological reactions and without them life could not exist. Lack of these metals as well as their presence at higher concentration can cause serious disruption of homeostasis in organism. Regulation of these metals could be achieved medically through chelating agents. Therefore, this study investigated chelating abilities and selectivity of thiobarbituric acid and L-methionine towards Cu (II) and Co (II) and their distribution in aqueous medium at 35 ° C as a function of pH using potentiometric titration method and Hyperquad Simulation and Speciation program (HySS). The ionic strength of solutions was maintained at 0.02 M using NaNO₃. The stability of ternary complexes with respect to binary complexes was determined by $\Delta \log K$. pH-metric analysis revealed chelating ability of the chelators towards the metal ions in the order of Cu(II) > Co(II) with L-methionine forming more stable binary complexes than thiobarbituric acid complexes. Ternary complexes were found to be more stable than the corresponding binary complexes. The chelators formed stable binary complexes with the metal ions within pH 4-8 and their mixture formed more stable ternary complexes within pH of 7.0-10.0. The ligands demonstrated high metal binding capability and different selectivity towards the investigated metal ions. Therefore, each ligand and their mixed-ligand complexes could be useful in regulating amount of the metal ions present in organisms.

Indexed Terms- Binary complexes, Chelating agent, Methionine, Speciation, Ternary Complexes, Thiobarbituric acid

I. INTRODUCTION

Transition metal ions inevitably exist as metal complexes in biological systems by their interaction with the numerous biomolecular chelating agents capable of forming chelates. The essential metals such as copper and cobalt have been found to interact amino acids, carboxylic acids, and protein in the body system to form binary and ternary complexes. The ability of metal ions to interact with some biomolecular ligands aids their transportation in organism thereby leading to existence of life [1]. The study of dynamic equilibria in the formation of chelates which occur in solution through transition metal ions and biomolecular ligands has significantly increased in field of coordination chemistry. Chelating agents have been previously applied for different purposes such as transportation of metals into body system, detoxification and remediation of metal pollutants [2, 3]. The formation and stability of many complexes have been extensively investigated using different methods to determine binding strength and selectivity of chelating agents towards different metal ions. The knowledge of effects of experimental conditions on the formation of complex, the nature of metal-ligand bonds and thermodynamic stability have been considered as essential factors for precise interpretation of behaviour of metal complexes in aqueous and non-aqueous media [4, 5].

Metal are essential for many metabolic reactions taking place in organism and lack of some of these metals as well as their presence at higher concentrations can cause serious disruption of homeostasis in organism. Chelation therapy has been applied to regulate amount of metal ion present in organism. Several chelating agents having different affinities for different metals have been applied in

chemical analysis, as water softeners, medicine, the transporter of metal, heavy metal detoxification and remediation [6, 7]. Most of the chelating agents being applied in the past have been reported to exhibit some shortcomings which demand a search for their replacement.

Therefore, there is an increase in the study of the interactions of different bimolecular ligands with different metal ions in the area of solution chemistry to obtain ideal chelating agents. The increase in the study has led to the provision of data on the binding properties and reactivity of the ligands, metal ions and their complexes which could be applied for metal extraction and remediation of metals [8].

The interaction of Mg(II), Ca(II) and Zn(II) with L-Aspartic acid and ethylenediamine in forming ternary complexes in various concentrations (0–60%, v/v) of Dimethylformamide-water mixtures with an ionic strength of 0.16 M using potentiometric technique was examined by [9]. The linear increase in the stability of the ternary complexes was observed which was attributed to decrease in the dielectric constants due to the dominance of the electrostatic forces. Stoichiometries and formation constants of binary and ternary complexes of Cu (II) with duloxetine and some selected amino acids at 25 °C and ionic strength 0.10 M were examined by [10] using potentiometric technique. Ternary complexes were reported to be more stable than the binary complexes. The stability constants of binary and ternary complexes of Cu (II) and Zn(II) transition metal ions with ethambutol hydrochloride antitubercular drug (L) and serine(R₁) valine(R₂) and amino acids (R) were investigated at 30 °C temperature and 0.1 M ionic strength (NaClO₄) in aqueous solution using potentiometric method by Magare and Ubale [11]. The more stability of the ternary complexes than the binary complexes observed in the study was attributed to the interactions outside the coordinated sphere such as formation of hydrogen bonding between coordinated ligands, charge neutralization, chelate effect and electrostatic interactions between non coordinated charge group of ligands.

Investigation of stability constant of ternary complexes of Fe(II), Co (II), Ni (II), Cu (II) and

Zn(II) with Nicotinamide and Alanine at 25±0.1 °C in 70%(v/v) ethanol - water medium at 0.1 M ionic strength was carried out by Jahangir et al. [12]. The increase in stabilities of the complexes observed was attributed to ionic radius of the metal ions. Stability constants of mixed ligand complexes of Ni (II) and Cu (II) metal ions with Diethylenetriamine and Ethylenediamine, Glycine, alpha-Alanine, Phenylalanine, Oxalic acid (ox) and Tyrosine at 25 °C in aqueous medium at ionic strength of 0.2 M has been evaluated [13]. Binary complexes were reported to exhibit higher stability than the corresponding ternary complexes.

In order to search for perfect potential chelating agents (non-toxic to biotic system or human health) that are selective towards different metal ions a lot of research have been conducted. Many researchers have investigated complexation reaction between different biomolecular ligands with metal ions in different solutions at different temperature. However, there is paucity of information on binding strength and selectivity of methionine and thiobarbituric acid towards essential metal ions which could be exploited for the ligands potential application in regulating the metal ions concentrations.. Therefore, this research investigated complexation reaction of L-methionine and thiobarbituric acid with copper and cobalt ions in aqueous medium using potentiometric titration method. This was carried out to provide data on their chelating ability and selectivity which could be considered for various applications in controlling the metal ions.

II. MATERIALS AND METHODS

• Materials

The chemical used include cobalt (II) nitrate hexahydrate, copper (II) nitrate, sodium nitrate, nitric acid, sodium hydroxide, double distilled water, ethanol, oxalic acid, methyl orange indicator, EDTA, L-methionine (Met) and thiobarbituric acid (Thio). They were of analytical grade and used without further purification.

The solutions of 0.002 M of the metal salts were prepared and the concentrations were determined by complexometric titration. Carbonate-free NaOH solution was prepared from double distilled water and

its concentration was standardized by oxalic acid. The ionic strength of each solution was adjusted to 0.02 mol/dm³ by addition of appropriate amount of standardized NaNO₃.

• Apparatus

The instruments used include Mettler Toledo pH-meter (FiveEasy™), Origin 8 program and Hyss program (Version 4.0.31), magnetic stirrer hot-plate, magnetic bar, volumetric flasks, Beakers, Thermometer, Burrete, Pipete, water bath and measuring cylinder. The pH meter electrode was calibrated before each titration using buffer solutions of 4,7 and 9. All the titrations were carried out within the slope of 99-105%.The temperature was maintained constant at 35 °C throughout the titrations and all the titrations were carried out in multiples. The errors that might occur from the liquid junction, the alkaline and acidic errors of the glass electrode were corrected by using Van Uitert and Hass equation [14].

• Experimental Methods

The following sets of titration mixture were prepared. The total volume in each set was kept at 40.00 ml in 100 ml glass beaker and allowed to attain the equilibrium. The ionic strength of the solutions was maintained at 0.02 M by addition of NaNO₃ salt and the solutions were magnetically stirred in the beaker in a water bath maintained at temperature of 35 °C. The solutions were titrated against standard alkali solution and the change in the pH of the solution was measured after each addition of 0.05ml of 0.13 M NaOH.

1. (5ml) Mineral Acid
2. (5ml) Mineral Acid + (5ml) Methionine,
3. (5ml) Mineral Acid + (5ml) Methionine + (5ml) metal ions (Cu(II), Co(II))
4. (5ml) Mineral Acid +(5ml) Thiobarbituric acid
5. (5ml) Mineral Acid + (5ml) Thiobarbituric acid + (5ml) metal ions (Cu(II), Co(II))
6. (5ml) Mineral Acid +(5ml) Thiobarbituric acid +(5ml) Methionine + (5ml) metal ion

The pH values obtained from pH meter readings were plotted against the volume of alkali added to each solution for above sets of titration and six titration curves were obtained [15, 16].

Determination of ligand, Metal-ligand and Metal-mixed-ligand Stability Constants

The proton–ligand and metal-ligand equilibrium constants for the ligands and the metal ions under different experimental conditions were determined by Calvin Bjerrum pH -titration as modified by Irving and Rossotti [17, 18]. The proton –ligand stability constant (\overline{nA}) and formation function (\overline{n}) of a metal-ligand (M-L) system were evaluated using:

$$\overline{nA} = y - \frac{(V_2 - V_1)(N^o + E^o)}{(V^o + V_1)T_{cl}^o}$$

$$\overline{n} = \frac{\text{total number of ligand L bound to metal M}}{\text{total number of metal present in system}}$$

$$\overline{n} = \frac{(V_3 - V_2)(N^o + E^o)}{(V^o + V_2)\overline{nA}T_{cm}^o}$$

$$pL = \log \left\{ \frac{1 + K_1^H [H^+] + K_1^H K_2^H [H^+]^2 + \dots}{T_{cl}^o - \overline{n}T_{cm}^o} \times \frac{V^o + V_2}{V^o} \right\}$$

Where,

y is number of dissociable protons

V₁, V₂ and V₃ are volumes of alkali employed to bring the solution 1, 2 and 3 to same pH values during acid, acid-ligand and acid-ligand-metal titrations respectively.

V^o is total volume of each solution

T_{cl}^o is total concentration of the ligand

T_{cm}^o is total concentration of metal ion

N^o is normality of NaOH

E^o is total concentration of mineral acid.

The number of complexes formed in the reaction was determined from the formation curve and the values of stability constants were evaluated.

The proton-ligand stability constants, K₁ and K₂ values of the ligands were obtained by employing the following expressions [6, 19].

$$\text{Log}k_3 = \text{pH} + \frac{\overline{nA}}{(1 - \overline{nA})} \quad (\overline{nA} = 0.2: 0.8)$$

$$\text{Log}k_2 = \text{pH} + \frac{\overline{nA} - 1}{(2 - \overline{nA})} \quad (\overline{nA} = 1.2: 1.8)$$

Stability constant of metal-ligand (logK₁ and logK₂) values were calculated using the following expressions [6].

$$\log k_2 = \log \frac{\bar{n}-1}{(2-\bar{n})} + pL \quad (\bar{n} = 1.2:1.8)$$

$$\log k_1 = \log \frac{\bar{n}}{(1-\bar{n})} + pL \quad (\bar{n} = 0.2:0.8)$$

The method adopted by [6] was employed in calculating stability constants of the ternary complexes due to simultaneous coordination of the ligands to a single metal ion. The ternary complexes were identified and evaluated from the shift in their titration curves as compared with those of the binary complexes. The following method was used to evaluate stability constants of the formed ternary complexes.

$$K_{MLPLS} = \frac{C_M - \left(\frac{1}{2}\right)[A] \cdot X}{\left(\frac{1}{2}\right)^3 [A]^3 \cdot X}$$

Where

$$[A] = \frac{2C_M + P - C_{OH} - [H]}{\frac{2[H^+]}{K_1^H + K_1^{H'}}$$

$$[X] = 1 + \frac{2[H^+]}{K_1^H + K_1^{H'}}$$

K_1^H = dissociation constant of first monobasic acid, $K_1^{H'}$ = dissociation constant of second monobasic acid, C_M = Total metal concentration, P = strength of acid, C_{OH} = strength of alkali, L_P = Primary ligand and L_S = secondary ligand.

III. RESULTS AND DISCUSSION

The change in pH of the solution is measured after each addition of a known amount of alkali solution to the reaction cell which contains the reaction mixture in aqueous medium. The titration curves (Figure 1) were drawn by plotting the measured pH values against the corresponding volume of alkali added.

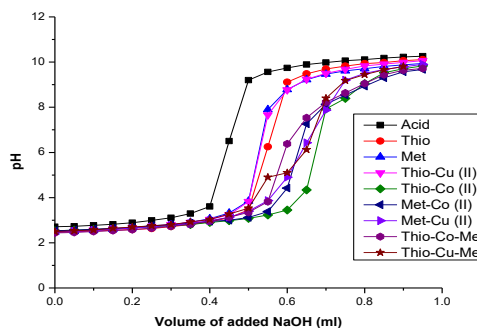


Figure 1: Potentiometric titration curves of Co (II) and Cu (II) binary and ternary complexes of methionine (Met) and thiobarbituric acid (Thio)

The methionine and Thiobarbituric acid curves as shown in Figure 1 were observed to lie below the acid curve for the same volume of added alkali. The position below the acid curve is attributed to deprotonation of the ligands [20]. The addition of Co (II) and Cu (II) ions to each of the ligand solutions lead to shifting of the pH of the ligands to lower pH values as observed on the curves. The shifts to lower pH are attributed to occurrence of complexation reaction and the formation of complexes through displacement of protons by the metal ions from the ligands [21, 22]. It was also observed from the figure that the ternary complexes of ThioCo(II)Met and ThioCu(II) Met curves coincide with the Co(II)Met and Cu(II)Thio complexes curves up to the pH of 4.2 and then deviated to lower pH. The deviations indicate the formation of the ternary complexes and non superimposable nature of the ternary complexes curves with either of the simple binary curves suggests simultaneous complexation of the two ligands to the metal ions [6]. The proton-ligand constants, metal-ligand constants and metal-mixedligand stability constants obtained from the above curves are shown in Tables 1 and 2 respectively.

Table 1 Proton-ligand and Binary complexes stability constants in water (I = 0.02 M NaNO₃) at 35°C

Ligand	LogK ₁ ^l	LogK ₂ ^H	LogK _{Ci} ^{Ci}	LogK _{Ci} ^{Ci}
Methionine ^[14]	-	10.5	877	7.25
			5.29	26

			50	
Thiobarbituric acid ^[14]	-	10.8	5.19	6.31
		750	21	22

Table 2: Stability constants of ternary complexes in water ($I = 0.02 \text{ M NaNO}_3$, $T = 35 \text{ }^\circ\text{C}$, $M = \text{Co (II) \& Cu (II)}$, $A = \text{Met \& B = Thio}$)

Complex	$\text{Log}K_{MAB}^M$	$\text{Lo}K_{MA}^M$	K_{MAB}^{MA}	$\text{Log}K_{MB}^M$	$\Delta\text{log}K$
[CoThioMet]	13.0203	5	7.8282	5.1921	+2.5332
		.2950			
[CuThioMet]	14.2467	7	7.9345	6.	+0.6819
		.2526		3122	

The $\text{Log}K_2^H$ value of 10.5877 evaluated from acid and Met curves as shown in Table 1 is found to close to that of the protonated amino group ($\text{p}K_a = 9.27$), but greater than that of the protonated COOH ($\text{p}K_a = 3.67$). The value was ascribed to the deprotonation at amino group (NH_2) of the ligand [23, 24]. The $\text{Log}K_2^H$ value of 10.8750 obtained for Thio was attributed to the deprotonation of protons at N_5H groups present in the ligand [3, 22]. Met and Thio coordinated to Cu (II) and Co (II) ions to form binary complexes with their stability constants in the order of $\text{Log}K_{\text{CoMe}}^{\text{Co}} > \text{Log}K_{\text{CoThio}}^{\text{Co}}$ and $\text{Log}K_{\text{CuMe}}^{\text{Cu}} > \text{Log}K_{\text{CuThio}}^{\text{Cu}}$ as shown in Table 1. Met was found to form more stable complex with copper ion than cobalt ion hence methionine is selective towards copper ion.

$\Delta\text{log} K$ was found to be positive for the complexes (Table 2). The positive $\Delta\text{log} K$ value suggested more stability of the ternary complexes than the corresponding binary complexes. The stability could be attributed to intramolecular hydrophobic stacking [25, 26]. The stability in order of $\text{Cu(II)} > \text{Co(II)}$ was observed for the complexes. The extra stability exhibited by Cu (II) complexes could be attributed to the unique electronic configuration on Cu (II) ion and distorted geometry [27].

• Distribution Curves as a Function of pH

Complexation behaviour of the metal ions and the ligands at different pH was analyzed through distribution curves as a function of pH. The distribution curves of the metal ions, the binary and ternary complexes show the relative abundance

of the metal ions and its complexes (chemical speciation) at different pH values. In picturing the nature of the equilibria existing in solution, species were distributed with change in pH of the solution. Speciation curves for the metal ions, their binary and ternary complexes are shown in Figure 2.

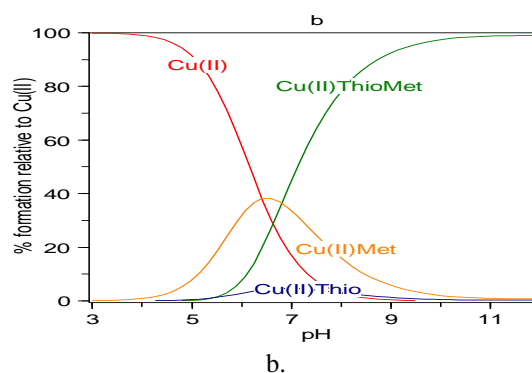
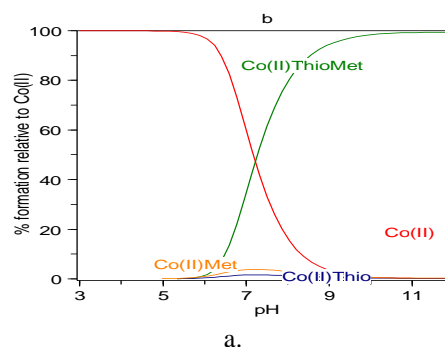


Figure 2: Distribution of species of Metal complexes with pH at 35 °C

(a) Co (II) complexes of Methionine and Thiobarbituric acid (b). Cu (II) complexes of Methionine and Thiobarbituric acid with pH at 35 °C

The percentage concentrations of Co (II) and Cu (II) ions were shown to decrease while the concentrations of binary complexes of CoThio, CoMet, CuThio and CuMet increased with an increase in pH as shown in Figure 2a and b, respectively. The decrease in the concentrations of the metal ions and increase in the concentration of the binary complexes were ascribed to the involvement of the metal ions in the formation of the complexes [28, 29]. The concentrations of free metal ions were found to be high in acidic pH values and decreased with increase in pH. Hence, in the acidic region, the metals were shown to be more bioavailable than in alkaline region. The complexes of CoMet and CoThio binary complexes started forming at the pH of 5.60 and 5.40, respectively. The complexes continued to form and reached maximum concentrations at the pH of 7.00 and 7.30 with concentrations of 7% and 4% respectively as shown in Figure 2a. A continuous decrease in the concentrations of the binary complexes at the pH values was observed with the formation of CoThioMet ternary complex. The formation of the complex occurred at pH of 5.60 and reached maximum level at pH of 9.16 with 97% concentration.

The binary complexes of Cu Met and CuThio (b) came to existence and reached maximum levels at the pH of 6.70 and 6.80 with concentrations of 45 and 2% , respectively. The complexes began to dissociate with increase in pH and disappeared completely at pH of 10.50 and 10.60, respectively. The formation of CuThioMet ternary complex began at pH of 4.16 and reached a maximum level (98%) at pH of 8.66. The continuous increase in the concentration of the ternary complex observed in the curves established the formation of the complexes. Also, the formation of the ternary complexes before any of binary complexes reached maximum level and suggests simultaneous coordination of the two ligands to the metal ion [29].

CONCLUSION

Amino acids and pyrimidine derivative are building blocks for metal chelation systems because they contain binding functionalities. Methionine and thiobarbituric acid are environmentally harmless and biodegradable chelating agents. The ligands are

found in this work to interact strongly with the metal ions in forming more stable ternary complexes than the binary complexes. The mixed-ligand demonstrates a strong ability to clutch copper ion thereby they are potential chelating agent capable of remediating the metal ion in aqueous solution. The ligand and its mixed-ligand could be applied for remediation of the metal ion at pH of 4.0 to 8.0 and 7.0-10, respectively.

REFERENCES

- [1] Schubert, J. (1981). Chelating Agents in Biological Systems. *Environmental Health Perspectives*, 40, 227-232
- [2] Janrao, D. M. P., Kayande, D. D. and Mulla, J. J. (2014). An over view of potentiometric determination of stability constants of metal complexes. *Scientific Reviews and Chemical Communications*, 4, 11-24.
- [3] Dharmendra, K. S. and Vijay, S. (2016). Formation constants of transition metal mixed ligand ternary complexes with biologically significant ligands. *Chemical Science Transactions*, 5(1): 272-278.
- [4] Berthon, G. (1995). *Handbook of Metal-Ligand Interaction in Biological fluids: Bioinorganic Medicine*. New York: Marcel Dekker.
- [5] Martell, A. E. and Hancock, R. D. (1996). *Metal complexes in aqueous solutions*. New York: Plenum Press.
- [6] Abdulbaset, A. Z., Mazahar, F.B. and Janrao, D.M. (2012). Study of stability constant of biological active molecules (drug) using potentiometric titration technique. *Journal of Chemical, Biological and Physical Sciences*, 2(1):67-80.
- [7] Tarek, K. (2014). *Organic Acids Chelating Agents*. Retrieved from http://tarek.kakhia.org/books_eng/Organic_Acids_Chelating_Agents.Tarek_Kakhia.pdf-accessed August, 2018.
- [8] Rajalakshmanan, E., Meti, M. M. and Hadgu, H. B. (2019). Chemical speciation of ternary complex of L-aspartic acid and ethylenediamine with essential metal Mg (II), Ca (II) and Zn (II)

- Ions in low dielectric media. *IOSR Journal of Applied Chemistry*, 12 (7): 52-59.
- [9] Amal, M. A. and Asma, A.A. (2019). Characterization by potentiometric procedures of the stability constants of the binary and ternary complexes. *Journal of Chemistry*, 2019, 1- 13.
- [10] Magare, B. K. and Ubale, M. B. (2013). Stability constants of transition metal complexes with piperacilin drug and alanine, glycine amino acids. *Asian journal of biochemical and pharmaceutical research*, 3,(3):91-95.
- [11] Kayande, D.D., Shivaji, J., Mazahar, F. and Megha, R. (2019). Stability constant study of transition metal complexes with pharmacologically active nicotinamide and alanine by pH Metric technique. *J. Biol. Chem. Chron.*, 5(1): 105-107.
- [12] Jahangir, M. A., Ullah, M.R. and Mousume, A(2018). Determination of stability constant of mixed ligand complexes of Ni(II) and Cu(II) metal ions with Diethylenetriamine (DET) and Ethylenediamine(en), Glycine (gly), alpha-Alanine (alpha-ala), Phenylalanine (ph-ala). Oxalic acid (ox), Tyrosine (tyr). *Journal of Ultra Scientist of Physical sciences*, 30(2):12-19.
- [13] Ishola, K. T., Oladipo, M. A., Odedokun, O. A., Olanipekun, O. T. (2020). Potentiometric Studies of Stability Constants and Speciation of Binary and Ternary Complexes of Metal (II) Ions with Amino Acids and Thiobarbituric Acid. *American Journal of Applied Chemistry*. 8 (1), 23-30.
- [14] Mohamed, M. k. and Abeer, A.((2000). Potentiometric studies on the formation equilibria of ternary complexes of some metals ions with dipicolinic acid and amino acids. *Journal of Chemical and Engineering data*, 45, 70-77.
- [15] Johaaghani, M. and Sotodehnejad, M. (2003). Model for therapeutic agent for Wilson's disease. *Iranian Journal of Chemistry and Chemical Engineering*, 22 (2):17-22.
- [16] Gabriela, V. F., Nora, B. P., Nora, B. D., and Paulina, M. M.(2008). Potentiometric and spectrophotometric study of 3-hydroxyflavone-La(III) complexes. *Journal of Chemical and Engineering Data*, 53 (6):1241-1245.
- [17] Senthilnithy, R., De costa, M.D.P and Gunawardhana, H.D.(2008). Fluorescence quenching and bonding properties of some hydroxamic acid derivatives by iron (III) and manganese (II). *Journal of the National Science Foundation of Sri Lanka*, 36 (3):191-196.
- [18] Promila, D.T. and Lonibala, R. (2010). Potentiometric Studies on the Complexation Reactions of N-(2-2-[1 (3-aminophenyl)ethylidene] hydrazino-2 oxoethyl)benzamide with Ni²⁺, Cu²⁺, and Cd²⁺ ions in aqueous-dioxane and Micellar media. *Journal of Chemical and Engineering data*, 55, 1166-1172.
- [19] Jeyaprakash, D., Paramasivam, S., Thirugnanasamy, E., Sutha, S. and Saravanan, R. (2014). Complexation equilibria and coordination aspect of Zn(II) complexes contain 2-aminobenzamide and some bioactive amino acid mixed ligands: pH-metric, spectroscopic and thermodynamic Studies. *Acta Chimica Slovica*, 61, 803-812.
- [20] Ayse, E., Tuba, S. and Suheylyla, P. (2005). Thermodynamics of binary and ternary complexes of 3-amino-1,2,4-triazole and amino acids with Ni(II) and Co(II). metal ion. *Journal of Serbian Chemical Society*, 70(8):1057-1066.
- [21] Naciye, T. and Aksoy, S. M. (2014). Complex formation of nickel(II) and copper(II) with barbituric acid. *International Journal of Analytical chemistry*, 2014, 1-5.
- [22] Liangnian, J.I. and Xueyi, L.E. (2002). Stacking interaction in metal complexes with compositions of DNA and heteroaromatic N-bases. *Chin. Sci. Bull.* 47, 1-9.
- [23] Ammar, R.A., Al-mutiri, R.A. and Abdalla, M.A. (2010). Equilibrium study of the Mixed-ligand complexes of copper(II) with adenine and amino acids in aqueous solution. *Journal of solution chemistry*, 39, 727-737.
- [24] Bhimrao, C. K., Pragat, M. D. and Balasaheb, R. A.(2010). Mixed-ligand complex formation of copper (II) with some aminoacids and drug

dapsone. *International journal of chemtech research*,2(2):1036-1041

- [25] Usama, E., Kenawy, I.M. and Abu, E.Y.G.(2007). Synthesis, thermal and spectral studies of first-row transition metal complexes with Girard-T reagent-based ligand. *Journal of Molecular Structure*, 871(3): 14-23.
- [26] Shriver, D. F. & Atkins, P. W. (1999) *Inorganic chemistry* (3rd ed.). Oxford: University press.
- [27] Serap, K., Dilek, B. A., and Aysen, K. C.(2014).The determination of the stability constants of mixed ligand complexes of creatinine-L-cysteine and creatinine-L-cysteine hydrochloride wit Co(Ii), Cd(Ii), Zn(Ii), Mn(Ii):Using potentiometric method. *Turkey Journal of Pharmaceutical Science*, 11(1):33-44.
- [28] Verma, S., Singh, D., Kumar, R., Shukla, B. k. and Krishna, V. (2015). Equilibrium study and Stability constants of mixed Ligand complexes of Biomolecules and Amino acids with Metal ions by Potentiometric method. *Research Journal of Chemical Sciences*, 5(3): 42-48.
- [29] Naciye, T. (2015). Stability constants of mixed ligand complexes of Nickel(II) with m adenine and some amino acids. *Bioinorganic Chemistry and Applications*, 2015, 1-9.