

Stability constants of ternary complexes β -lactam antibiotic drugs with bivalent transition metal(II) ions in the presence of aliphatic amino acids

Serap KARADERI^{1*}, Dilek BILGIC ALKAYA¹, Gulbin ERDOGAN¹, Aysen KURT CUCU¹

¹ Department of Analytical Chemistry, Faculty of Pharmacy, Marmara University, Basibuyuk 34854 Istanbul, Turkey.

* Corresponding Author. E-mail: skaraderi@marmara.edu.tr

Received: 28 March 2024 / Reviewed: 21 May 2024 / Accepted: 23 May 2024

ABSTRACT: Metal complex structures are important in many biological processes where coordination can occur between various metal ions and a wide variety of ligands. Here we compared bivalent transition metal(II) ions- ternary complexes with penicillin class amoxicillin and cephalosporin class cefaclor actives in the presence of aliphatic amino acids. The complex formation conditions and compositions of the complexes formed by the classical, economical and reproducible Irving-Rossotti method were investigated. Therefore, the formation conditions and compositions of binary and ternary complexes were investigated by the Irving Rossotti procedure. Ternary complexes are prepared by mixing metal(II) with active ingredients and the appropriate amount of primary ligand or L-asparagine (ASP) or L-glutamic acid (Glut). In the ternary systems, ASP and Glut are primary ligands (L) whereas amoxicillin (Amox) and cefaclor (Cefa) behave as a secondary ligand (Y). From the calculated formation constants of the complexes formed by the selected β -lactam antibiotics and amino acids with Cu(II) and Zn(II) metals, it was determined that the metal-ligand mole ratios were 1:1:1 (M:L:Y). At room temperature, the formation constants of the complexes were found by adjusting the ionic strength to 0.11 mol dm⁻³ with NaClO₄: $\log\beta_{(Cu(II)-ASP-Amox)}=15.83\pm 0.01$; $\log\beta_{(Cu(II)-Glut-Amox)}=20.76\pm 0.01$; $\log\beta_{(Cu(II)-ASP-Cefa)}=16.59\pm 0.01$; $\log\beta_{(Cu(II)-Glut-Cefa)}=5.72\pm 0.01$; $\log\beta_{(Zn(II)-ASP-Amox)}=5.32\pm 0.01$; $\log\beta_{(Zn(II)-Glut-Amox)}=4.62\pm 0.01$; $\log\beta_{(Zn(II)-ASP-Cefa)}=5.46\pm 0.01$; $\log\beta_{(Zn(II)-Glut-Cefa)}=4.52\pm 0.01$ respectively. The relative stabilities of the ternary complexes ($\Delta\log K$) were interpreted by comparison with the stabilities of the corresponding binary complexes, with negative $\Delta\log K$ values observed for all complexes.

KEYWORDS Amoxicillin; Cefaclor; L-asparagine; L-glutamic acid; Ternary complex

1. INTRODUCTION

Since 1967, there has been a growing interest in the use of metal complexes as pharmaceuticals due to their therapeutic applications. Several examples have been reported for essential metal ions, which exhibited activity both in vitro and in vivo [1-3]. In metal coordination chemistry, a ligand refers to a molecule or ion that binds to a metal ion through coordination bonds. Ligands typically have one or more electron-rich atoms, such as nitrogen, oxygen, or sulfur, which can donate electron pairs to the metal ion. The coordination of ligands to the metal ion forms in the formation of coordinate covalent bonds, where the ligand donates a pair of electrons to the metal ion. In the case of metal-ligand ternary complexes, two different ligands coordinate to a central metal ion. This coordination can occur in various geometries, including octahedral, square planar, or tetrahedral. The ligands can be the same or different chemical species, and their coordination with the metal ion can occur through different coordination sites or bonding modes.

Amoxicillin (Amox) and Cefaclor (Cefa) are β -lactam antibiotics commonly used for the treatment of lower and upper respiratory tract infections, genitourinary and gastrointestinal tract, soft tissue and skin infections, bacterial meningitis, and bacterial endocarditis as well as Lyme disease, as an antimicrobial agent [4-6]. Resistance of both gram-positive bacteria, and gram-negative bacteria to β -lactam antibiotics is mainly correlated with the forming of β -lactamases and sometimes, decreased carrying across the bacterial

Karaderi S, Alkaya D, Erdoğan G, Cucu A. Stability Constants of Ternary Complexes β -Lactam Antibiotic Drugs with Bivalent Transition Metal(II) Ions in the Presence of Aliphatic Amino Acids. J Res Pharm 2024; 28(6): 2215-2222.

cell wall [7-11]. According to the literature metal ions coordinate to the carboxyl, amide, and amino groups, exhibiting similar acid-base and ligand properties to dipeptides. In chelate formation, the polarity of the metal ion decreases due to interaction with donor groups and p-electron delocalization within the ring system. Thus, it increases the lipophilic nature of the central metal atom, which facilitates its penetration through the lipid layer of the membrane. Bioavailability of the drug as the metal complex crosses the membrane and its activity increases [12].

Copper (II) and Zinc (II) are an essential transition metals, and can form various complexes with coordination numbers ranging from 4 to 6, which are crucial for many enzymes [13, 14]. In the studies on the Cu (II)-Amox complex in the literature, it was reported that the copper complex of Amox significantly increased the antibacterial activity [15-26].

The most intense protein loss in the body after injury or infection periods occurs in skeletal muscle percent, respectively, due to the increase in water outside the skeletal water of the cell [4]. Amino acids have curical role importance in our life because their interaction with metal ions is of considerable interest as models in a variety of biological systems [11]. Likewise, ternary complexes between bioligand and amino acids with metals are receiving more attention [4-7]. Although the formation of metal(II)-amino acid binary complexes has been studied before, it was repeated in the experimental study to use equal conditions for ternary and binary systems [27, 28]. In the presented study, we explained the stability of divalent metal ions of mixed ligand ternary complexes and the stability order of ternary complexes according to the ligands used. For this reason, the stability constants active ingredients with Cu(II) and Zn(II), in the presence of two amino acids. ASP and Glut. Eight mixed ligand complexes, Cu(II)-Glut-Amox, Cu(II)-ASP-Amox, Cu(II)-Glut-Cefa, Cu(II)-ASP-Cefa, Zn(II)-Glut-Amox, Zn(II)-ASP-Amox, Zn(II)-Glut-Cefa, and Zn(II)-ASP-Cefa were determined via potentiometric titrations in aqueous solution (ionic strength $I = 0.11$) NaClO₄ at room temperature [29, 30]. It is aimed to understand the formation of ternary complexes between active ingredients, metal ions and aliphatic amino acids and to provide insight into the coordination chemistry [31, 32].

Here, the synthesis of ternary complex formation between antibiotic active ingredients, metal (II) ions and aliphatic amino acids through potentiometric titrations is reported. It is thought that the determination and characterization of the stability constants of these complexes may contribute to their behavior and potential applications in pharmaceutical and medicinal chemistry.

2. DISCUSSION

Protonated binary and ternary complexes were formed from potentiometric titrations in an aqueous solution at room temperature and $I = 0.11$ NaClO₄ was used in order to obtain experimental results under equal conditions for the ternary, and binary systems. The interaction of Cu(II) - Amox ; Cu(II) - Cefa; Zn(II) - Amox; Zn(II) - Cefa and two aliphatic amino acids were studied by potentiometric technique. A solution of 5.0 mL active ingredients ; 5.0 mL NaClO₄ and 5.0 mL HClO₄ solution and 35.0 mL H₂O were placed in a 50.0 mL volumetric flask in order to accomplish an ionic strength of 0.11; and the resulting solution was titrated with 0.1000 N NaOH solution. Cu(II):L (L:Primary ligand) ratio in the titrated solution was 1:2 to provide for the possibility of the formation of ligand complexes. The titration curves of solutions containing M+ML+MLY (Y:Secondary ligand) are shown in Figure 1. When the titration curves of the formed ternary complexes are examined, a simultaneous decrease in pH values is observed. This is due to the nature of the complexation of metal ions and ligands [33].

In this case, the ML, MY, and MLY complexes were quantitatively identified from the titration curves by pH at which precipitation occurred (Figure 1) (Table 1). Differences in stabilities of binary and ternary complexes were compared $\Delta \log K$ was calculated reaction of the secondary ligand. $\Delta \log K = \log \beta_{MLY} - (\log K_{ML} + \log K_{MY})$. Positive $\Delta \log K$ results show stabilization of the ternary complex. The negative $\Delta \log K$ values suggested that ternary complexes were less stable than binary complexes due to steric factors. The complex is formed however the negative value can be reduced number of coordination sites in the ligand [30]. It was observed that the original binary complexes were transformed into a ternary complex with the composition MLY species distribution graphics were drawn as a function of pH.

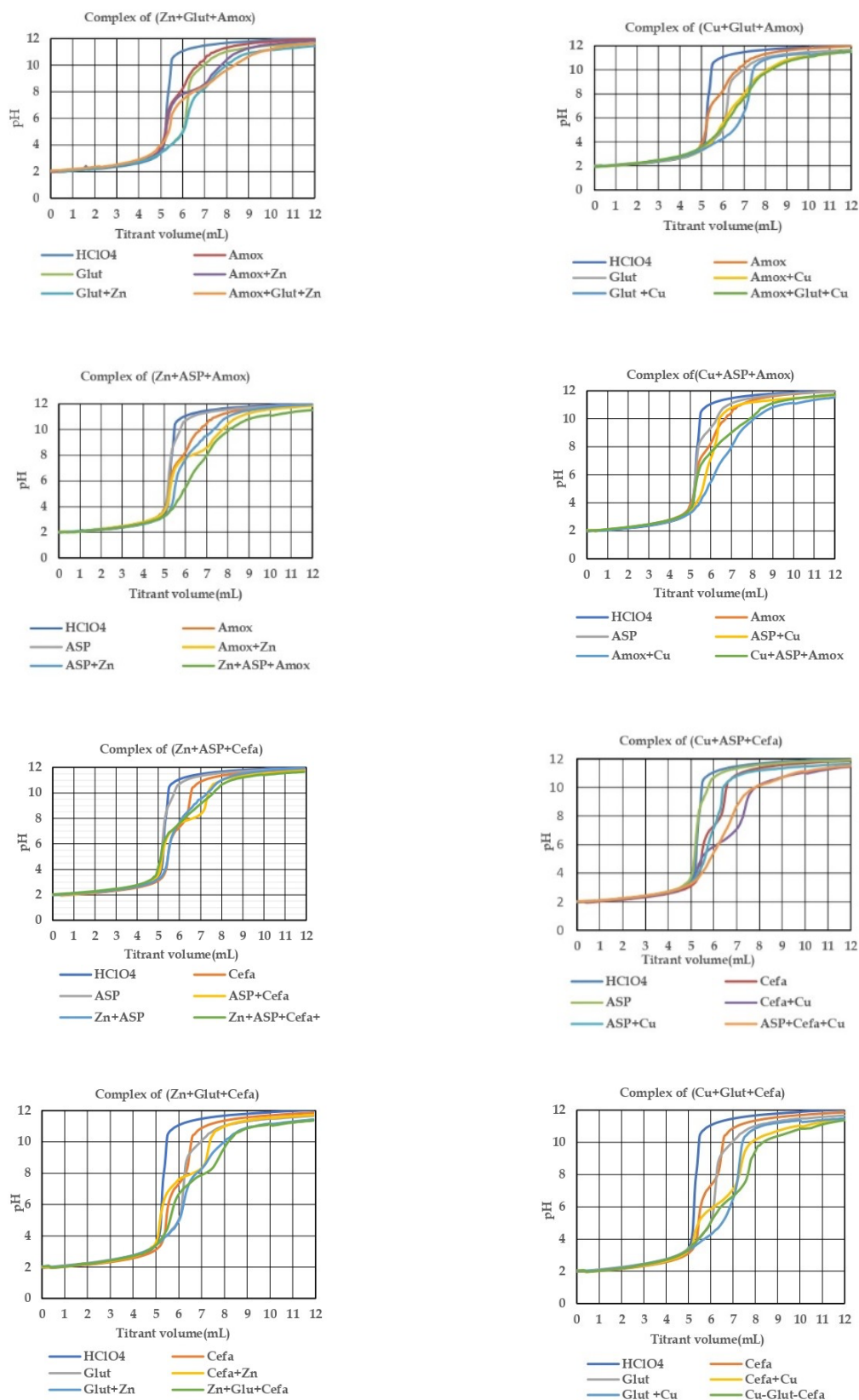


Figure 1: Potentiometric titration curves for complexes

Table 1. The values of $\log K$ of ligands; $t=25.0\pm 0.1^\circ\text{C}$, $I=0.11$)

Ligands	$\bar{n}_A=0.5$	$\bar{n}_A=1.5$	$\bar{n}_A=2.5$
Amoxicillin	$\log K_1 = 7.96$	$\log K_2 = 2.77$	-
Cefaclor	$\log K_1 = 11.64$	$\log K_2 = 7.05$	-
L-Glutamic acid	$\log K_1 = 10.27$	$\log K_2 = 4.33$	$\log K_3 = 2.42$
L-Asparagine	$\log K_1 = 9.26$	$\log K_2 = 2.44$	-

3. CONCLUSION

As a result, the stabilities of chelates of binary and ternary complexes containing (L-Asp and L-Glut) as primary ligands and active ingredients as secondary ligands were measured potentiometrically. In the study ternary complexes conditions have been discussed and it has been shown that the complexes ability of occurred species. If the titration curve of the free ligand is compared with that of metal-complexed ligands, a decrease in pH is observed when the metal ion is added to the ligand solution. This is because complex formation requires more alkali to reach the same pH as the free ligand. This simply implies the formation of complexes by proton release from the coordinated ligand. The ternary complexes of M:Cu(II) or Zn(II) with Amox or Cefa active ingredients and some selected amino acids are formed in a stepwise mechanism. The formation constants of the ternary complexes were compared with the binary systems and the formation constants were found to be compatible for both systems.

The stability constants of the ternary chelates are greater than binary systems for all species. This behavior shows that interaction is formed within the ligands in the ternary complexes and MLY complexes are more stable than binary complexes. In contrast, the negative $\Delta\log K$ values demonstrate that the ternary complexes behave unstably. Also, the species distribution curve showed the complex formation. In conclusion, the determination of the stability constant will be important in terms of data on the subsequent trials about the resulting triple complex structures. The data obtained from the investigated Amox/Cefa antibiotic and the protonation of Glut/ASP amino acids and the complex formation balances of metalocomplexes will make a significant contribution to workers performing mechanical studies in biological environments.

4. MATERIALS AND METHODS

The targeted Cu(II) and Zn(II) amino acids complexes were prepared by the previously reported procedure and metal ion active ingredients binary and ternary complexes were synthesized at the same experimental conditions employed in this work [35-36]. The titrations for binary and ternary mixtures were performed over the range of pH 2.50-11.50 with NaOH solution. All the chemical reagents used in the present study were the highest purity available. Bidistilled water was used in all experiments. An automatic burette was used and the pH meter with a pH of 0.01 unit was calibrated using citrate-hydrochloric acid buffer solutions (pH = 4.00) and phosphate (pH = 7.00) at room temperature before each titration. Concentrations of Amox (1.0×10^{-2} M), Cefa (1.0×10^{-2} M), ASP (1.0×10^{-2} M), Glut (1.0×10^{-2} M), and Cu(II) (1.0×10^{-2} M) were prepared to be used in the potentiometric titrations.

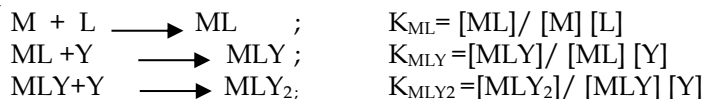
I. For potentiometric titration, 10.0 mL of freshly prepared 2.0×10^{-2} M ligand solutions were taken. 5.0 mL of 0.1M HClO_4 solution, 5.0 mL of 1M NaClO_4 solution were added and completed to 50.0 mL with distilled water.

II. In order to find the protonation constants of the ligands, the average values were calculated from the titration curves of the solutions containing HClO_4 and $(\text{HClO}_4/\text{NaClO}_4) + \text{Ligand}$ (Figure 1) according to the method given by Irving and Rosotti [34-36].

III. The studied ligands form complexes with various metals in the appropriate pH range. For the determination of the stability constants of the complexes formed, the following solutions were prepared separately for each metal. 10.0 mL of 2.0×10^{-2} M ligand solution was taken. 0.1M 5.0 mL HClO_4 and 1M 5.0 mL NaClO_4 were added. 5.0 mL of 1.0×10^{-2} M metal solution was added and the final volume was made up to 50.0 mL with distilled water.

IV. For the calculation of the stability constants, the values were calculated with the help of the previously found values and the corresponding pL values were calculated with the help of the found values. $n=f(pL)$ graph was plotted with the help of the calculated and pL values of each metal. $\log K_1$ and $\log K_2$ stability constants of the complex were found from the pL values corresponding to $\bar{n}_L=0.5$ and $\bar{n}_L=1.5$ values.

The formation constants of the following reactions were investigated by the Irving-Rossotti method for mixed complex formation



The average \bar{n}_A values were calculated and $\bar{n}_A = f(pH)$ graphs for Amox and Cefa were plotted from the titration curves (Figure 2). MS Excel program was used for the calculation of dissociation constants of ligands at $\bar{n}_A = 0.5$. The $\log K$ values corresponding to the protonic species of the other ligands understudied here are given in the Table 1 and they were presented in our previous studies [32, 36].

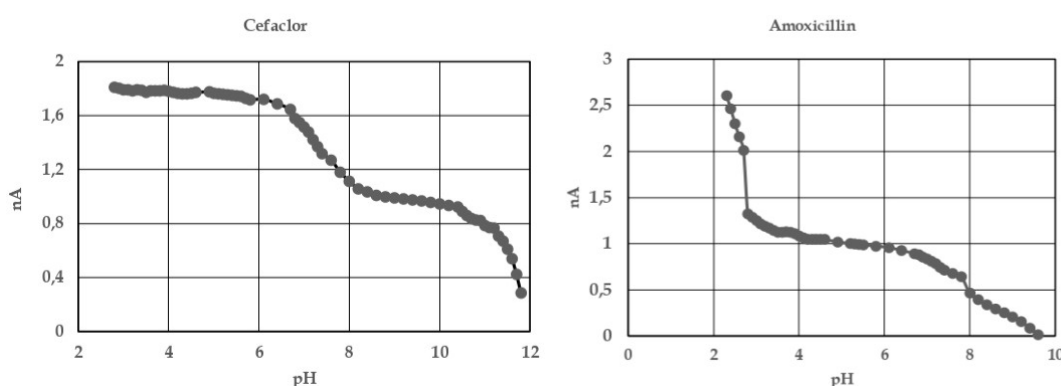


Figure 2. $\bar{n}_A = f(pH)$ curves of ligands ($I = 0.11$; $t = 25.0^\circ\text{C}$) Amox and Cefa

From Table 2, showing the stability constant of binary species and ternary species respectively proves.

Inspired by our similar studies [21, 22, 36] according to the Irving-Rossotti method, using the potentiometric pH titration data, we determined and the stability constants of the mixed ligand complexes were calculated and given in Table 2. In this paper, the authors discussed the complex formation process of selected active ingredients and aliphatic amino acids with Cu(II) and Zn(II) [31, 32]. Examination of the titration curves in the presence of the metal ions indicates that the addition of the metal ion to the ligand solutions causes a decrease in the pH value [30]. Various \bar{n}_L were calculated for the mixed complexes using the potentiometric titration data of the solutions. The protonation constants of ligands and conditional stability constants of the formed complexes were calculated at room temperature ($I = 0.11$) using NaClO_4 solution. The $\bar{n}_L = f(pL)$ graphics were plotted using \bar{n}_L and pL values MS Excel program was used for the calculation of formation constants of ternary complexes. It was found that the ternary complexes which are consistent with the assumption that the complexes has a distorted tetragonal geometry with two coordinated atoms of nitrogen and two atoms of oxygen. Antibiotics and amino acids have a capacity to bind the ions of bivalent metals at the 1:1:1 ratio. Based on the complex ability it has been established that this affinity decreases in the series: $\text{Cu}^{2+} > \text{Zn}^{2+}$. To compare the stability of the ternary complex with respect to the binary ones, the $\Delta \log K$ was determined [21], using the following equilibria

$$\Delta \log K = \log \beta_{(MLY)} - (\log \beta_{(ML)} + \log \beta_{(MY)})$$

Table 2. Stability constant of binary and ternary complexes; $t=25.0\pm 0.1^\circ\text{C}$, $I=0.11$)

Complex	Log K_1	log K_2	log β
Cu(II)-Amoxicillin	13.74 \pm 0.01	7.95 \pm 0.01	21.69 \pm 0.01
Cu(II)-Cefaclor	9.87 \pm 0.01	8.16 \pm 0.01	18.03 \pm 0.01
Cu(II)-L-Glutamic Acid	8.39 \pm 0.01	7.92 \pm 0.01	16.31 \pm 0.01
Cu(II)-L-Asparagine	7.87 \pm 0.01	5.74 \pm 0.01	13.61 \pm 0.01
Zn (II)-Amoxicillin	3.33 \pm 0.01	-	3.33 \pm 0.01
Zn(II)-Cefaclor	6.54 \pm 0.01	6.18 \pm 0.01	12.72 \pm 0.01
Zn(II)-L-Glutamic Acid	5.76 \pm 0.01	4.97 \pm 0.01	10.73 \pm 0.01
Zn(II)-L-Asparagine	7.41 \pm 0.01	5.56 \pm 0.01	12.97 \pm 0.01
Cu(II)-L-Glutamic Acid-Amoxicillin	11.87 \pm 0.01	8.89 \pm 0.01	20.76 \pm 0.01
Cu(II)-L-Asparagine-Amoxicillin	8.64 \pm 0.01	7.19 \pm 0.01	15.83 \pm 0.01
Cu(II)-L-Glutamic Acid-Cefaclor	5.72 \pm 0.01	-	5.72 \pm 0.01
Cu(II)-L-Asparagine-Cefaclor	9.01 \pm 0.01	7.58 \pm 0.01	16.59 \pm 0.01
Zn(II)-L-Glutamic Acid-Amoxicillin	4.62 \pm 0.01	-	4.62 \pm 0.01
Zn(II)-L-Asparagine-Amoxicillin	5.32 \pm 0.01	-	5.32 \pm 0.01
Zn(II)-L-Glutamic Acid-Cefaclor	4.52 \pm 0.01	-	4.52 \pm 0.01
Zn(II)-L-Asparagine-Cefaclor	5.46 \pm 0.01	-	5.46 \pm 0.01

In this study, the stability of ternary complexes relative to the corresponding binary complexes was investigated by calculating $\Delta\log K$ values. Accordingly, it was concluded that ternary complexes were formed with $\Delta\log K$ values, but negative values were obtained due to the lack of interactions outside the coordination area [37]. Negative $\Delta\log K$ values indicate that the structure of the ternary complexes is in the MLY composition. Negative values of $\Delta\log K$ can be understood as the secondary ligand forms a more stable complex with ML than MY for Cu(II) whereas higher negative $\Delta\log K$ value observed for the ternary complexation of Zn(II). The $\Delta\log K$ values were negative indicating that the stabilities of these ternary systems are lower than those of the corresponding binary system. This showed that deprotonation occurs at a higher pH in binary complexes and there are more interactions than 1:1:1 interactions.

Acknowledgements: All authors participated in the interpretation of the data and the writing, reviewing, and editing of the manuscript, and had final responsibility for approving the published version. The work was financially supported by the Scientific Research Committee of the Marmara University Project number SAG-081004-0097.

Author contributions: Concept – S.K., D.B.A., G.E., A.K.C.; Design – S.K., D.B.A., G.E., A.K.C.; Supervision – S.K., D.B.A., G.E., A.K.C.; Resources S.K.; Materials – S.K.; Data Collection and/or Processing – S.K., D.B.A., G.E., A.K.C.; Analysis and/or Interpretation – S.K.; Literature Search – S.K., D.B.A., Writing – S.K., D.B.A., G.E., A.K.C.; Critical Reviews – S.K., D.B.A., G.E., A.K.C.

Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

REFERENCES

- [1] Sorenson JRJ. Copper complexes offer a physiological approach to treatment of chronic diseases. *Prog Med Chem.* 1989; 26: 437-568. [https://doi.org/10.1016/s0079-6468\(08\)70246-7](https://doi.org/10.1016/s0079-6468(08)70246-7).
- [2] Chohan ZH, Supuran CT, Scozzafava A. Metalloantibiotics: synthesis and antibacterial activity of cobalt(II), copper(II), nickel(II) and zinc(II) complexes of kefzol. *J Enzyme Inhib Med Chem.* 2004;19(1):79-84. <https://doi.org/10.1080/14756360310001624939>.
- [3] Dimitrovska A, Andonovski B, Stojanoski K. Spectrophotometric study of copper(II) ion complexes with cefaclor. *Int J Pharm.* 1996; 134: 213-221.
- [4] Noor N, Humayoun S, Zafar H, Lakhnana NK, Bukhari KT. Meta-analysis to assess role of systemic antibiotics in root canal treatment. *J Pharm Biol Sci* 2017; 38-46. <https://doi.org/10.20510/ukjpb/5/i4/166543>.
- [5] Hansmann Y. Treatment and prevention of Lyme disease. *Curr Probl Dermatol.* 2009;37:111-129. <https://doi.org/10.1159/000213071>.
- [6] Kuriyama T, Williams DW, Yanagisawa M, Iwahara K, Shimizu C, Nakagawa K, Yamamoto E, Karasawa T. Antimicrobial susceptibility of 800 anaerobic isolates from patients with dentoalveolar infection to 13 oral antibiotics. *Oral Microbiol Immunol.* 2007; 22(4): 285-288. <https://doi.org/10.1111/j.1399-302X.2007.00365.x>
- [7] Drissi M, Ahmed ZB, Dehecq B, Bakour R, Plesiat P, Hocquet D. Antibiotic susceptibility and mechanisms of β -lactam resistance among clinical strains of *Pseudomonas aeruginosa*: First report in Algeria. *Med Mal Infect.* 2008; 38(4): 187-191. <https://doi.org/10.1016/j.medmal.2008.01.009>.
- [8] Handal T, Olsen I, Walker CB, Caugant DA. Detection and characterization of β -lactamase genes in subgingival bacteria from patients with refractory periodontitis. *FEMS Microbiol Lett.* 2005; 242(2): 319-324. <https://doi.org/10.1016/j.femsle.2004.11.023>.
- [9] Mandsberg LF, Ciofu O, Kirkby N, Christiansen LE, Poulsen HE, Høiby N. Antibiotic resistance in *Pseudomonas aeruginosa* strains with increased mutation frequency due to inactivation of the DNA oxidative repair system. *Antimicrob Agents Chemother.* 2009; 53(6): 2483-2491. <https://doi.org/10.1128/AAC.00428-08>.
- [10] Bisson-Boutelliez C, Fontanay S, Finance C, Kedzierewicz F. Preparation and physicochemical characterization of amoxicillin β -cyclodextrin complexes. *AAPS PharmSciTech.* 2010; 11: 574-581. <https://doi.org/10.1208/s12249-010-9412-1>.
- [11] Vohta C, Docquier JD, Rossolini GM, Fosse T. Genetic and biochemical characterization of FUS-1 (OXA-85), a narrow-spectrum class D β -lactamase from *Fusobacterium nucleatum* subsp. polymorphum. *Antimicrob Agents Chemother.* 2006; 50(8): 2673-2679. <https://doi.org/10.1128/AAC.00058-06>.
- [12] Marukhlenko AV, Morozova MA, Mbarga A, Antipova, NV, Syroeshkin AV, Podoprigror IV, Maksimova TV. Chelation of zinc with biogenic amino acids: Description of properties using balaban index, assessment of biological activity on spirostomum ambiguum cellular biosensor, influence on biofilms and direct antibacterial action. *Pharmaceuticals.* 2022; 15(8): 979. <https://doi.org/10.3390/ph15080979>.
- [13] Kurpad AV. The requirements of protein & amino acid during acute & chronic infections. *Indian J Med Res.* 2006; 124(2): 129-148.
- [14] Darwin KH. Mycobacterium tuberculosis and copper: a newly appreciated defense against an old foe? *J Biol Chem.* 2015; 290(31): 18962-18966. <https://doi.org/10.1074/jbc.R115.640193>.
- [15] Lapshin SV, Alekseev VG. Copper (II) complexation with ampicillin, amoxicillin, and cephalixin. *Russ J Inorg Chem.* 2009; 54: 1066-1069. <https://doi.org/10.1134/S0036023609070122>.
- [16] Cardiano P, Crea F, Foti C, Giuffrè O, Sammartano S. Potentiometric, UV and ^1H NMR study on the interaction of Cu^{2+} with ampicillin and amoxicillin in aqueous solution. *Biophys Chem.* 2017; 224: 59-66. <https://doi.org/10.1016/j.bpc.2017.04.001>.
- [17] Shoukry MM. Potentiometric studies of binary and ternary complexes of amoxicillin. *Talanta.* 1992; 39(12): 1625-1628. [https://doi.org/10.1016/0039-9140\(92\)80194-I](https://doi.org/10.1016/0039-9140(92)80194-I).
- [18] Mwene-Mbeja TM. Chemical Stability of Pharmaceutical Organic Compounds. *Am J Biomed Sci Res.* 2019; 6(1): 14-22. <https://doi.org/10.34297/AJBSR.2019.06.000984>.
- [19] Imran M, Iqbal J, Mehmood T, Latif S. Synthesis, characterization and in vitro screening of amoxicillin and its complexes with Ag(I), Cu(II), Co(II), Zn(II) and Ni(II). *J Biol Sci.* 2006; 6: 946-949. <https://doi.org/10.3923/jbs.2006.946.949>
- [20] Butler JN. Ionic Equilibrium a Mathematical Approach. Addison-Wesley Publishing Company Inc., London, 1964. <https://doi.org/10.1002/bbpc.19640680523>.
- [21] Martell AE, Hancock RD. Metal Complexes in Aqueous Solutions. Springer New York NY, 1996. <https://doi.org/10.1007/978-1-4899-1486-6>.
- [22] Tran-Ho LC, May PM, Hefter GT. Complexation of Copper(I) by Thioamino acids. Implications for copper speciation in blood plasma. *J Inorg Biochem.* 1997; 68(3): 225-231. [https://doi.org/10.1016/S0162-0134\(97\)00097-4](https://doi.org/10.1016/S0162-0134(97)00097-4).
- [23] Dogan A, Köseoglu F, Kiliç E. The stability constants of copper (II) complexes with some alpha-amino acids in dioxane-water mixtures. *Anal Biochem.* 2001; 295(2): 237-239. <https://doi.org/10.1006/abio.2001.5205>.
- [24] Iqbal MS, Bukhari IH, Arif M. Preparation, characterization and biological evaluation of copper (II) and zinc (II) complexes with Schiff bases derived from amoxicillin and cephalixin. *Appl Organomet Chem.* 2005; 19(7): 864-869. <https://doi.org/10.1002/aoc.918>

- [25] Hrioua A, Loudiki A, Farahi A, Laghrib F, Bakasse M, Lahrich S, Saqrane S, El Mhammedi MA. Complexation of amoxicillin by transition metals: Physico-chemical and antibacterial activity evaluation. *Bioelectrochemistry*. 2021; 142: 107936. <https://doi.org/10.1016/j.bioelechem.2021.107936>
- [26] Al-Noor TH, Aziz MR, AL-Jeboori AT. Synthesis, characterization and antimicrobial activities of [Fe(II), Co(II), Ni(II), Cu(II) and Zn(II)] mixed ligand complexes schiff base derived from amoxicillin drug and 4-(dimethylamino)benzaldehyde with nicotinamide. *J Chem Pharm Res*. 2014; 6(4): 1225-1231.
- [27] Demir HD, Pekin M, Cucu AK, Dolen E, Aboul-Enein HY. Potentiometric studies of mixed complexes of cobalt (II) and copper (II) with L-Asparagine and adenine. *Toxicol Environ Chem*. 1999; 71(3-4): 357-367. <https://doi.org/10.1080/02772249909358806>.
- [28] Ammar RA, Al-Mutiri EM, Abdalla MA. The determination of the stability constants of mixed ligand complexes of adenine and amino acids with Ni(II) by potentiometric titration method. *Fluid Phase Equilib*. 2011; 301(1): 51-55. <https://doi.org/10.1016/j.fluid.2010.11.014>.
- [29] Al-Mohameed AM, Alotthman AA. Characterization by potentiometric procedures of the stability constants of the binary and ternary complexes of Cu (II) and duloxetine drug with amino acids. *J Chem*. 2019; <https://doi.org/10.1155/2019/1064942>.
- [30] Abdelkarim AT, Mahmoud WH, El-Sherif AA. Potentiometric, thermodynamics and coordination properties for binary and mixed ligand complexes of copper(II) with cephradine antibiotic and some N- and O-bound amino acids (α -alanine and β -alanine). *J Mol Liq*. 2021; 328: 115334. <https://doi.org/10.1016/j.molliq.2021.115334>.
- [31] Orabi AS, Abbas AM, Abd Elraoof MA, Khairy GM. Modern view for binary and ternary complexes of metal ions with amoxicillin and some amino acids. *Adv Environ Life Sci*. 2022; 1: 22-39. <https://doi.org/10.21608/aels.2021.111628.1002>.
- [32] Karaderi S, Bilgic D. Zinc(II) and Cadmium(II) binary complexes with creatinine and their mixed-ligand complexes with L-Asparagine or L-Glutamic Acid: Potentiometric studies. *Main Group Met Chem*. 2006; 29(3): 145-156. <https://doi.org/10.1515/mgmc.2006.29.3.145>.
- [33] Bartaria D, Chandra P, Krishna V. A study on speciation and coordination tendency of glutamic acid and uracil for ternary complexation towards some toxic metal ions. *Chem Sci Revie Lett*. 2013; 1201-1208.
- [34] Irving HM, Rossotti HS. The calculation of formation curves of metal complexes from pH titration curves in mixed solvents. *J Chem Soc. (Resumed)*. 1954; 2904-2910. <https://doi.org/10.1039/JR9530003397>.
- [35] Irving HM, Miles MG, Pettit LD. A study of some problems in determining the stoichiometric proton dissociation constants of complexes by potentiometric titrations using a glass electrode. *Anal Chim Acta*. 1967; 38: 475-488. [https://doi.org/10.1016/S0003-2670\(01\)80616-4](https://doi.org/10.1016/S0003-2670(01)80616-4).
- [36] Alkaya B, Karaderi S, Erdoğan G. Ternary complex formation of isoniazid with some transition metals and amino acids. *Trakya Univ J Nat Sci*. 2016; 14(1): 1-14.
- [37] Zinnat A, Begum Ismail, MM, Rahman Tsugiko Takase, Hiroshi Hasegawa. Formation and stability of the mixed-chelator complexes of Sr²⁺, Mg²⁺, Ca²⁺, Ba²⁺, and Y³⁺ in solution with bio-relevant chelators. *J Inorg Biochem*. 2019; 195: 141-148. <https://doi.org/10.1016/j.jinorgbio.2019.03.018>.

This is an open access article which is publicly available on our journal's website under Institutional Repository at <http://dspace.marmara.edu.tr>.