Cu^{2+}-Citrate Dimer Complexes in Aqueous Solutions

Yahia Z. Hamada*, Robin Cox and Hasan Hamada

Division of Natural and Mathematical Sciences, LeMoyne-Owen College, 807 Walker Ave., Memphis, TN 38126, USA

Abstract: The UV-Vis spectra, speciation diagrams, and potentiometric profiles for Cu^{2+}-citrate complexes in aqueous solutions are presented. As the pH increases from 2.29 to 5.15, the UV-Vis spectral profile of the Cu^{2+}-citrate complexes showed a blue shift from 820 nm to 760 nm. We have set the conditions to construct the speciation diagram as follow: Cu^{2+}:citric acid was in 1:1 ratio with concentration of 1.0×10^{-4} mol.L^{-1}, 0.1023 mol.L^{-1} NaOH solution, and pKw = 13.781 ± 0.006 taken from Sweeton, Mesmer, and Baes. The current report is the first potentiometric study that has taken into accounts two Cu-Cit dimeric species to be refined simultaneously. These spectroscopic and potentiometric data are discussed which augment what had been reported in the literature.

Keywords: Cu^{2+} solutions, UV-Vis spectra, Cu^{2+}-Citrate-dimer, Potentiometric titrations, Speciation diagrams.

1. INTRODUCTION

1.1. Copper

Copper (the most stable oxidation state Cu^{2+} will be used as the shorthand notation) is an essential trace metal ion involved in many metalloproteins including: ceruloplasmin [1-8], cytochrome oxidase [9-13], superoxide dismutase [14-19], dopamine-*β*-hydroxylase [20-22], ascorbate oxidase [23-27], lysyl oxidase [28-30], and tyrosinase [31-35]. To build up these Cu^{2+} containing metalloproteins/metalloenzymes, the biological machinery has to transport Cu^{2+} and the raw materials (low molecular mass organic molecules building-blocks) through a very complex transport/storage mechanism system. A detailed American Chemical Society (ACS) library search that was conducted on September 26, 2013 showed, as expected, very large number of publications when it comes to the search key term searches "Copper or Cu^{2+}". When the key search term "Copper" was spelled out ~150 thousands (~150 K) papers appeared anywhere in the paper. When this search term was narrowed within the abstract ~ 10 K papers appeared. When the search was narrowed within the title only ~ 3 K papers appeared. When the key search term "Cu^{2+}" was used; instead of copper; within the title 475 papers were returned. When the term "Cu^{2+} combined with citrate" was included within the title no papers were received. When the term "Cu^{2+} combined with citrate and combined with the term dimer" more than 500 papers were returned anywhere, but zero paper in both title and abstract. Figure **1** shows these detailed library searches.

*Address correspondence to this author at the Division of Natural and Mathematical Sciences, LeMoyne-Owen College, 807 Walker Ave., Memphis, TN 38126, USA; Tel: (901) 435-1392; Fax: (901) 435-1424;
E-mail: Yahia_hamada@loc.edu

1.2. Citrate and the Copper-Citrate System

Citrate is considered to be a pre-eminent low molecular mass metal binder. Cu^{2+} citrate complexes (or more precisely Benedict's solution) have been studied for over 100 years [36]. The Cu^{2+} citrate reaction was revisited in 1953 and 1976 at which various Cu^{2+} citrate complexes or Cu^{2+} citrate species were discovered. The Cu^{2+} citrate one-to-one complex in solution was first identified by Warner and Weber [37]. The Cu^{2+} citrate dimer was first crystallized in 1976 by Schugar *et al.* [38]. Because of the essentiality of copper in biological systems and the pre-eminence of citrate as metal binder, we are testing the interaction of citrate/citric acid with the essential metal ions Cu^{2+}.

2. EXPERIMENTAL SECTION

2.1. Materials

Ligand and Copper aqueous solutions were prepared using Fisher reagent grade citric acid, $C_6H_8O_7$, formula weight 192.12 g.mol^{-1}, tri-trisodium citrate salt monohydrate, $C_6H_5Na_3O_7$. H_2O, formula weight 258.08 g.mol^{-1}, copper nitrate hemipentahydrate, $Cu(NO_3)_2 \diamond 2.5H_2O$, formula weight 232.59 g.mol^{-1}, or copper sulfate pentahydrate, $Cu(SO_4)_2 \diamond 5H_2O$, formula weight 249.68 g.mol^{-1}, using doubly deionized water. Potentiometric titrations were conducted using the Orion pH electrode-meter combination model 720A+ which measures pH's to the accuracy of 0.001 in aqueous solutions at room temperature.

2.2. Preparation of the Potentiometric Titration Solutions

Cu^{2+}-Citrate or Cu^{2+}-Citric acid potentiometric titrations were conducted using standard NaOH solution as the titrant. The NaOH solutions were prepared from NaOH solid pellets in carbonate free

ISSN: 1814-8085 / E-ISSN: 1927-5129/15

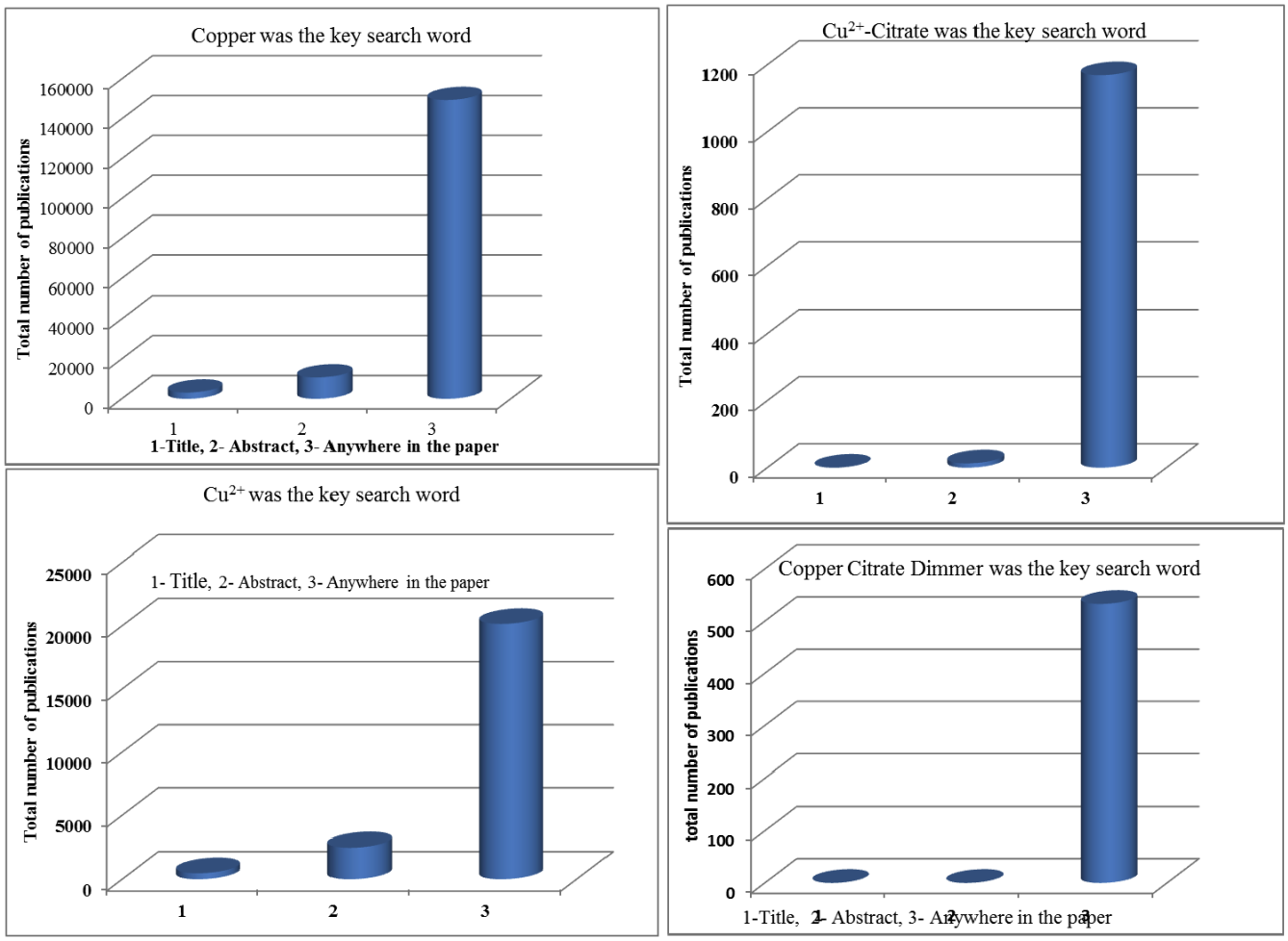

Figure 1: Charts of the American Chemical Society (ACS)-Library search for total number of published articles that appeared in all ACS journals using the key terms indicated above the charts at which no hits were returned if the search was looking for the key terms Cu^{2+} Citrate or Cu^{2+} citrate dimer in either the title or the abstract. September 26, 2013. 1. Title, 2. Abstract, and 3. Anywhere the key search word appeared in the article.

water. Methods used to prepare CO_2 – free NaOH solution had been described elsewhere [39-41]. Primary standard potassium hydrogen phthalate (KHP) was used to standardize this NaOH solution. Solid NaOH and solid KHP were purchased from Fisher Chemical Co. Before titrating NaOH with KHP, solid KHP was dried at 110°C for 24 hours and stored in a desiccator and used a day later. An indicator solution of ~ 0.2% phenolphthalein in ~ 90% ethanol was prepared from reagent grade solid phenolphthalein. KHP was titrated to the phenolphthalein end point (colorless to pale pink end point). Thirteen to fifteen runs were carried out to standardize the NaOH solution. Statistical treatments of all gathered numbers were conducted such as average, standard deviation, T-test, and Q-test using Excel software.

2.3. Potentiometric Titrations

Potentiometric titrations were carried out in a very clean 250 mL beaker equipped with a magnetic stirring bar. The beaker was covered with Teflon cover for good seal from the surroundings. In each titration citric acid solution or trisodium citrate solution separately were added first then the Cu^{2+} ion solution was added second followed by the addition of the appropriate amount of water to take the total volume to 100.0 mL. The titration solutions were allowed to stir for 20 minutes to reach a state of equilibrium. The NaOH titrant was added in an increment of 100 µL using an Eppendorf micropipette followed by continuous stirring. The time intervals between the additions of the NaOH solution were set to 5 minutes that was sufficient to get the pH values stabilized.

2.4. Ultraviolet Visible (UV-Vis) Absorption Spectroscopy

UV-Vis spectra measurements were collected by using one of our T60 high performance spectrophotometer in connection with UVWIN software version 5.0.

Both of the instrument and the software were purchased from Advanced ChemTech., Louisville, KY. The Cu^{2+}-Citric acid solution mixtures were prepared and the entire UV-Vis spectrum was scanned from 250 nm to 1100 nm using the 4.0 mL total capacity quartz cuvettes with 1 cm optical path length. Reference cuvettes were used with all UV-Vis-measurements at which they were filled with an equal volume of D. I. water.

3. RESULTS AND DISCUSSION

3.1. UV-Vis Absorption Spectra

Figure **2** shows the correlation of the three UV-Vis absorption spectra for the Cu^{2+}-citric acid system in aqueous solutions at 25°C in 1:2 ratio (see Figure **1** of the supplementary material for more details). The concentration of Cu^{2+} was equal to 32.34 milli Molar (32.34×10^{-3} mol.L^{-1}). The three spectral curves were collected at the following pH-values: 2.29, 2.81 and 5.19. The measured absorbance increased as the pH increased giving rise to the following λmax values of 820 nm, 800 nm, and 760 nm with the corresponding pH values mentioned above respectively. Clearly the λmax values are showing a blue shift (a shift towards shorted wavelength or higher energy). These pH-Values were chosen because Warner and Weber [37] confirmed the formation of a copper-citrate chelate with the 1:1 ratio within these pH-values. In which there were four protons released into the solution due to the formation of this copper-citrate chelate complex.

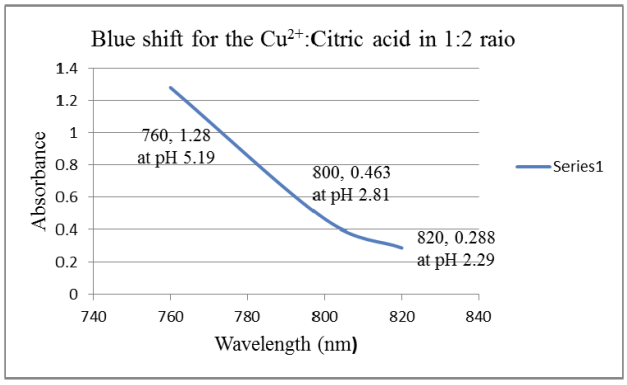

Figure 2: Ultraviolet and visible (UV-Vis) absorption spectra for the reaction mixture of Cu^{2+} and citric acid in 1:2 molar ratio, [Cu^{2+}] = 32.34 mM at pH-values of 2.29, 2.81, and 5.19. Clearly we are showing the visible region where the absorption increases as the pH-value increases with a blue-shift towards higher energy (shorter wavelength).

3.2. Potentiometry of Cu^{2+}-Citric Acid System

Figure **3** shows the all of the potentiometric titration plots for the free Cu^{2+}, the free Citric acid, and the Cu^{2+}-citric acid solutions in different molar ratios. It is clear that the appearance of the plots for the Cu^{2+}-citric acid system in different molar ratios far away or apart from that of the free Cu^{2+} and free Citric acid plots is indication of a strong binding. As it has been known potentiometrically; the presence of an inflection point indicates the presence of a dominant metal complex. The position of that inflection indicates the total number of protons released into the solution *via* the formation of this dominant metal complex [39-42]. Table **1** catalogues all of these titration curves shown in Figure **3**. An exact amount of 0.1999 milli moles of free citric acid have been used to generate the free citric acid curve shown in Figure **3**.

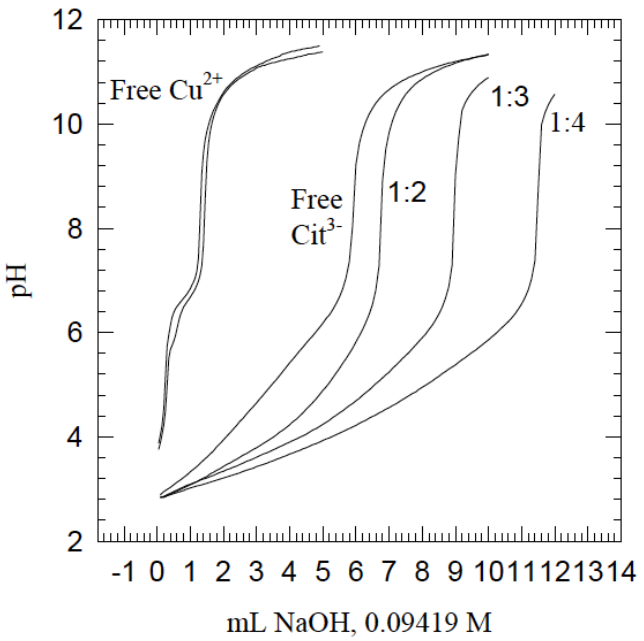

Figure 3: Potentiometric titration plots for the free Cu^{2+}, free citric acid, and Cu^{2+}/Citric acid reaction mixtures in various ratios of (1:2, 1:3, and 1:4) at 25 °C in 0.1 M ionic strength.

From Figure **3** and Table **1** it is clear that when the free citric acid was titrated, the acid has been defined as an H_3L ligand which meant that there are three titratable functional groups. Those are the three carboxylic acid groups. Figure **4** is the speciation diagram for the Cu^{2+}-Citric acid reaction mixture (using pKw = 13.781±0.006) showing the dominance of the two dimeric copper citrate complexes throughout the pH range of 3-10 [43,44].

By closely examining the 0:1, 1:2, 1:3, and 1:4 titration curves in Figure **3**; these curves show a gradually raising buffer regions between pH ≈ 2.5-6.6. There were no visible precipitates observed for these titration systems. For the 1:2 titration system the buffer regions were terminated with sharp and well-defined

Table 1: Potentiometric Titration Data for Cu^{2+} with H_3Cit in Different Molar Ratios at 25 °C in 0.1 M Ionic Strength

Cu^{2+}:H_3Cit mole ratio	Vol. of NaOH titrant (mL)	Equivalents of the NaOH titrant [a]	Δ eq.	Proposed species	Remarks
1:0 [b]	2.00 [b]	1.94 [b]	-	$Cu(OH)_2$	Two H^+ were released
0:1 [c]	6.20 [c]	2.83 [c]	-	-	Citric acid is a tri-protic acid
1:2	6.70	6.50	-	$(Cu\text{-}CitH_{-1}^{4-})_2$	The dimer is formed
1:3	9.00	8.75	2.25 eq.	-	An oligomer is formed
1:4	11.40	11.07	2.32 eq.	$[(Cu\text{-}CitH_{-1}^{4-})_2]_2$	The oligomer is probably a tetramer

[a]The term equivalent is defined as the ratio of the number of millimoles of titrant (NaOH in this case) to the number of millimoles of citric acid. If Cu^{2+} is present the term equivalent is defined as the ratio of the number of millimoles of titrant to the number of millimoles of Cu^{2+}.
[b]0.09702 milli moles of Cu^{2+} and [c]0.1999 milli moles Citric acid were titrated to generate the free Cu^{2+} and free citric acid curves shown in Figure **3**.

inflection at half integers (6.50 proton equivalents). The appearance of these sharp inflections at half integers is good evidence that the dominant species present in aqueous solutions is the dimeric Cu^{2+}-citrate complex. We do not know why the 1:3 and the 1:4 titration ratios gave these fraction integers perhaps due to some sort of oligomerization. The titration continued to pH-values of ≈ 10.5-11.0.

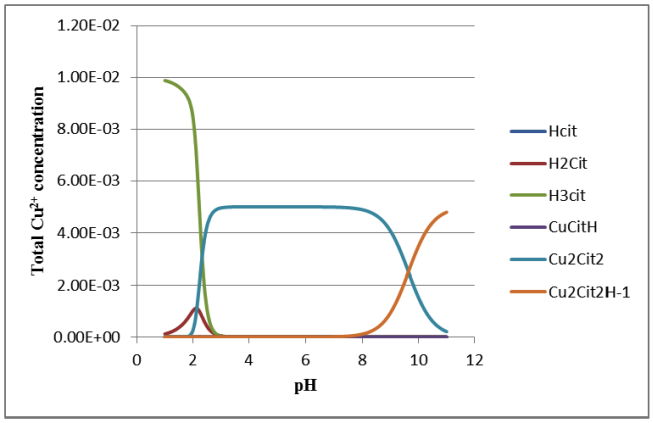

Figure 4: Speciation diagram of the total Cu^{2+} with citric acid (H_3Cit) in 1:1 molar ratio using the speciation program HYSS [43].

3.3. Potentiometry Cu^{2+}-Trisodium Citrate System

Figure **5a** shows all of the titration curves for the Cu^{2+}-trisodium citrate system in aqueous solutions at 1:1, 1:2, 1:3, and 1:4 molar ratios at 25 °C in 0.0 M ionic strength. These titration curves showed buffer regions between pH ≈ 4.5-6.0. For all titration curves i.e. 1:1, 1:2, 1:3, and 1:4 titration systems the inflection points appeared between pH ≈ 6.5-9.5. No visible precipitations were observed at all in any of these titration systems. The solubilizing effect of citrate for the similar metal ions in aqueous solution is well established in the literature [39,40,45-49]. It is worth mentioning that there was no fluctuation observed for the pH-readings from the pH-meter-electrode combination, which indicated that the Cu^{2+}-trisodium citrate system is reaching a high degree of equilibrium state.

Figure **5b** shows the linear regression of the observed pH-values and the measured potential in mV for the (1:1, 1:2, 1:3, and 1:4 molar ratio) for the Cu^{2+}:tri-sodium citrate reaction mixture within the pH range of 4.5 to 11.5. The slope is in agreement with Nernst equation. The same regression have been observed for the Fe^{3+} and Cr^{3+}-trisodium citrate solution in the same molar ratios titration systems which was done in our laboratory more than a decade ago [39]. In the presence of excess citrate around the copper metal ion the chances of dimerization is less than that if there were one or two moles of citrate. This further confirms the argument by Spiro *et al.* that states when there is limited supply of citrate, the dimerization and polymerization process will be more probable as seen with other researchers [50,51].

CONCLUSION

The library search confirmed that the Cu^{2+} along with Citrate as a search key word shows very limited number of papers. Even more limited number of papers if the search key words were Cu^{2+} along with Citrate and Dimmer. Herein we are presenting a speciation diagram which proves the presence of the dimeric species within a very large range of the pH values. In this speciation diagram we have taken into account three major species of the free citrate the mono-, the di-and the tri- protonated free citrate (namely HCit, H_2Cit, and H_3Cit) along with the presence of another

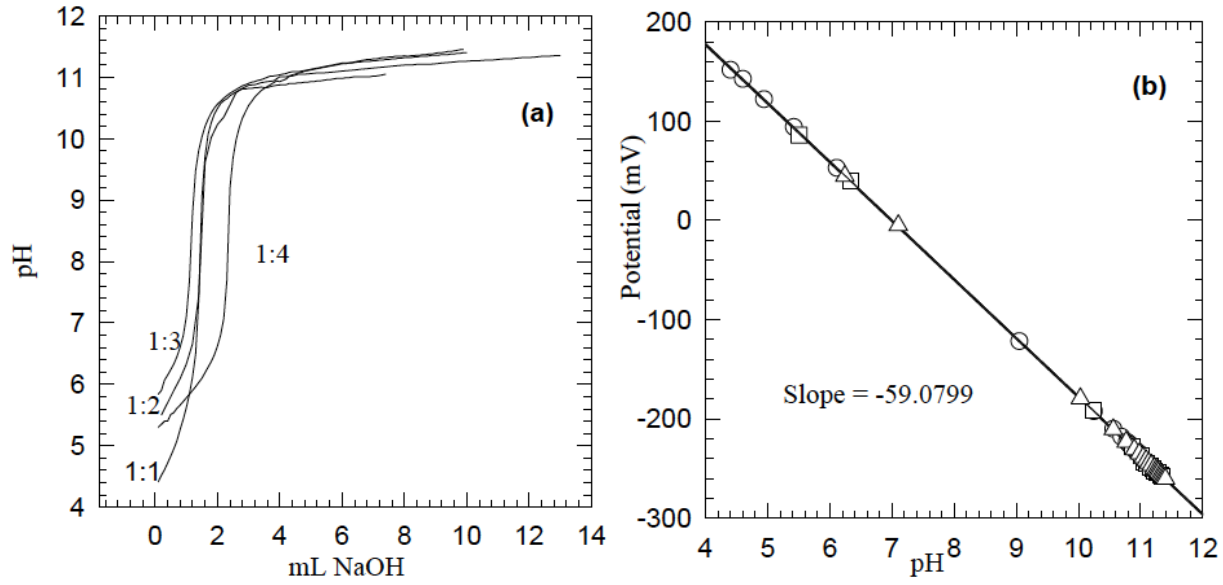

Figure 5: (**a**) Potentiometric titration plots for the Cu^{2+}:trisodium citrate reaction mixtures in various ratios of (1:1, 1:2, 1:3, and 1:4) Cu^{2+}:tri-sodium citrate at 25 ^{o}C in 0.0 M ionic strength. (**b**) Linear regression of the relation between the observed pH and the potential in mV for the (1:1, 1:2, and 1:3) Cu^{2+}:tri-sodium citrate reaction mixture. The slope is in agreement with Nernst equation.

three Cu^{2+}-Citrate complexes (namely CuCitH, $Cu_2(Cit)_2$, and $Cu_2(Cit)_2$ H_{-1}. Out of these six species that were taken into account the two dimeric species dominated the diagram. The dimerization of various metal ions in various oxidations states in aqueous solutions is well documented in the literature [49-52]. Because this paper is not meant to be a full review of the dimmer citrate, it will suffice that we will reference only two metal ions as examples (Fe^{3+} and Cu^{2+}) [52,53]. In the literature, an $Fe_2(H\text{-}1cta)_2^{2-}$ complex, and $Cu_2(cta)_2^{2-}$ and $Cu_2(H_{-1}cta)_2$ dimers have been reported in previous potentiometric studies [52,53]. The current report is the first potentiometric study that taken into accounts two Cu-Cit dimeric species simultaneously.

ACKNOWLEDGMENTS

This work was supported in part from NSF under Grant # HRD-1332459. We also would like to thank the faculty members at the Division of Natural and Mathematical Sciences of LeMoyne-Owen College for reading the manuscript.

SUPPLEMENTAL MATERIALS

The supplemental materials can be downloaded from the journal website along with the article.

REFERENCES

[1] McKee DJ, Frieden E. Binding of transition metal ions by ceruloplasmin (ferroxidase). Biochemistry 1971; 10(21): 3880-3883. http://dx.doi.org/10.1021/bi00797a013

[2] Freeman S, Daniel E. Dissociation and reconstitution of human ceruloplasmin. Biochemistry 1973; 12(23): 4806-4810. http://dx.doi.org/10.1021/bi00747a038

[3] Dawson JH, Dooley DM, Clark R. Stephens PJ, Gray HB. Spectroscopic studies of ceruloplasmin. Electronic structures of the copper sites. J Am Chem Soc 1979; 101(17): 5046-5053. http://dx.doi.org/10.1021/ja00511a040

[4] Ha-Duong NT, Eid C, Hémadi M, and El Hage Chahine J-M. *In vitro* Interaction between Ceruloplasmin and Human Serum Transferrin. Biochemistry 2010; 49(48): 10261-10263. http://dx.doi.org/10.1021/bi1014503

[5] Noyer M, Putnam FW. Circular dichroism study of undegraded human ceruloplasmin. Biochemistry 1981 20(12): 3536-3542. http://dx.doi.org/10.1021/bi00515a036

[6] Koschinsky ML, Chow BKC, Schwartz J, Hamerton JL, MacGillivray RTA. Isolation and characterization of a processed gene for human ceruloplasmin. Biochemistry 1987; 26(24): 7760-7767. http://dx.doi.org/10.1021/bi00398a034

[7] Mukhopadhyay CK, Fox PL. Ceruloplasmin Copper Induces Oxidant Damage by a Redox Process Utilizing Cell-Derived Superoxide as Reductant. Biochemistry 1998; 37(40): 14222-14229. http://dx.doi.org/10.1021/bi981137t

[8] Sedlak E, Wittung-Stafshede P. Discrete Roles of Copper Ions in Chemical Unfolding of Human Ceruloplasmin. Biochemistry 2007; 46(33): 9638-9644. http://dx.doi.org/10.1021/bi700715e

[9] Brewer GA, Sinn E. Reexamination of a cytochrome oxidase model. A noncoupled iron-copper binuclear complex. Inorg Chem 1984; 23(16): 2532-2537. http://dx.doi.org/10.1021/ic00184a031

[10] Serr BR, Headford CEL, Anderson OP, Elliott CM, Schauer CK, Akabori K, Spartalian K, Hatfield WE, Rohrs BR. Cytochrome c oxidase models: iron(III) porphyrin-copper(II) complexes with sulfur bridges. Inorg Chem 1990; 29(14): 2663-2671. http://dx.doi.org/10.1021/ic00339a025

[11] Serr BR, Headford CEL, Anderson OP, Elliott CM, Spartalian K, Fainzilberg VE, *et al.* Cytochrome c oxidase models: a dinuclear iron(III) porphyrin-copper(II) complex with a sulfur bridge. Inorg Chem 1992; 31(26): 5450-5465. http://dx.doi.org/10.1021/ic00052a022

[12] Kauffmann KE, Goddard CA, Zang Y, Holm RH, Münck E. Mössbauer and Magnetization Studies of Heme−Copper-Bridged Assemblies Pertinent to Cytochrome c Oxidase. Inorg Chem 1997; 36(6): 985-993. http://dx.doi.org/10.1021/ic960826v

[13] Brewer CT, Brewer GA. Heteronuclear imidazolate-bridged complexes of iron(III) porphyrins and copper(II). Toward modeling of cytochrome c oxidase. Inorg Chem 1987; 26(20): 3420-3422. http://dx.doi.org/10.1021/ic00267a043

[14] Beem KM, Richardson DC, Rajagopalan KV. Metal sites of copper-zinc superoxide dismutase. Biochemistry 1977; 16(9): 1930-1936. http://dx.doi.org/10.1021/bi00628a027

[15] St. Clair CS, Gray HB, Valentine JS. Spectroelectrochemistry of copper-zinc superoxide dismutase. Inorg Chem 1992; 31(5): 925-927. http://dx.doi.org/10.1021/ic00031a041

[16] Lamb AL, Torres AS, O'Halloran TV, Rosenzweig AC. Heterodimer Formation between Superoxide Dismutase and Its Copper Chaperone. Biochemistry 2000; 39(48): 14720-14727. http://dx.doi.org/10.1021/bi002207a

[17] Hart PJ, Balbirnie MM, Ogihara NL, Nersissian AM, Weiss MS, Valentine JS, Eisenberg D. A Structure-Based Mechanism for Copper−Zinc Superoxide Dismutase. Biochemistry 1999; 38(7): 2167-2178. http://dx.doi.org/10.1021/bi982284u

[18] de Freitas DM, Valentine JS. Phosphate is an inhibitor of copper-zinc superoxide dismutase. Biochemistry 1984; 23(9): 2079-2082. http://dx.doi.org/10.1021/bi00304a031

[19] Ellerby LM, Cabelli DE, Graden JA, Valentine JS. Copper−Zinc Superoxide Dismutase: Why Not pH-Dependent? J Am Chem Soc 1996; 118(28): 6556-6561. http://dx.doi.org/10.1021/ja953845x

[20] Champloy F, Benali-Chérif N, Bruno P, Blain I, Pierrot M, Réglier M. Studies of Copper Complexes Displaying N3S Coordination as Models for CuB Center of Dopamine β-Hydroxylase and Peptidylglycine α-Hydroxylating Monooxygenase. Inorg Chem 1998; 37(16): 3910-3918. http://dx.doi.org/10.1021/ic9709281

[21] Santra BK, Reddy PAN, Nethaji M, Chakravarty AR. Structural Model for the CuB Site of Dopamine β-Hydroxylase: Crystal Structure of a Copper(II) Complex Showing N3OS Coordination with an Axial Sulfur Ligation. Inorg Chem 2002; 41(16): 4304-4304. http://dx.doi.org/10.1021/ic020317v

[22] Santras BK, Reddy PAN, Nethaji M, Chakravarty AR. Structural Model for the CuB Site of Dopamine β-Hydroxylase: Crystal Structure of a Copper(II) Complex Showing N3OS Coordination with an Axial Sulfur Ligation. Inorg Chem 2002; 41(5); 1328-1332. http://dx.doi.org/10.1021/ic010926n

[23] Cole JL, Avigliano L, Morpurgo L, Solomon EI. Spectroscopic and chemical studies of the ascorbate oxidase trinuclear copper active site: comparison to laccase. J Am Chem Soc 1991; 113(24): 9080-9089. http://dx.doi.org/10.1021/ja00024a008

[24] Strothkamp KG, Dawson CR. Quaternary structure of ascorbate oxidase. Biochemistry 1974; 13(3): 434-440. http://dx.doi.org/10.1021/bi00700a006

[25] Mei G, Di Venere A, Buganza M, Vecchini P, Rosato N, Finazzi-Agro A. Role of Quaternary Structure in the Stability of Dimeric Proteins: The Case of Ascorbate Oxidase. Biochemistry 1997; 36(36): 10917-10922. http://dx.doi.org/10.1021/bi970614p

[26] Gaspard S, Monzani E, Casella L, Gullotti M, Maritano S, Marchesini A. Inhibition of Ascorbate Oxidase by Phenolic Compounds. Enzymatic and Spectroscopic Studies. Biochemistry 1997; 36(16): 4852-4859. http://dx.doi.org/10.1021/bi9616864

[27] Meyer TE, Marchesini A, Cusanovich MA, Tollin G. Direct measurement of intramolecular electron transfer between type I and type III copper centers in the multi-copper enzyme ascorbate oxidase and its type II copper-depleted and cyanide-inhibited forms. Biochemistry 1991; 30(18): 4619-4623. http://dx.doi.org/10.1021/bi00232a037

[28] Duff AP, Cohen AE, Ellis PJ, Kuchar JA, Langley DB, Shepard EM, *et al.* The Crystal Structure of Pichia pastoris Lysyl Oxidase. Biochemistry 2003; 42(51): 15148-15157. http://dx.doi.org/10.1021/bi035338v

[29] Bollinger JA, Brown DE, Dooley DM. The Formation of Lysine Tyrosylquinone (LTQ) Is a Self-Processing Reaction. Expression and Characterization of a Drosophila Lysyl Oxidase. Biochemistry 2005; 44(35): 11708-11714. http://dx.doi.org/10.1021/bi0504310

[30] Rebecchi KR, Go EP, Xu L, Woodin CL, Mure M, Desaire H. A General Protease Digestion Procedure for Optimal Protein Sequence Coverage and Post-Translational Modifications Analysis of Recombinant Glycoproteins: Application to the Characterization of Human Lysyl Oxidase-like 2 Glycosylation. Anal Chem 2011; 83(22): 8484-8491. http://dx.doi.org/10.1021/ac2017037

[31] Pyrz JW, Karlin KD, Sorrell TN, Vogel GC, Que L Jr. Resonance Raman studies of phenolate-bridged binuclear copper complexes. Relevance to hemocyanin and tyrosinase. Inorg Chem 1984; 23(26): 4581-4584. http://dx.doi.org/10.1021/ic00194a035

[32] Casella L, Dipartimento EM, Gullotti M, Cavagnino D, Cerina G, Santagostini L, Ugo R. Functional Modeling of Tyrosinase. Mechanism of Phenol ortho-Hydroxylation by Dinuclear Copper Complexes. Inorg Chem 1996; 35(26): 7516-7525. http://dx.doi.org/10.1021/ic9601100

[33] Peyroux E, Ghattas W, Hardré R, Giorgi M, Faure B, Simaan AJ, Belle C, Réglier M. Binding of 2-Hydroxypyridine-N-oxide on Dicopper(II) Centers: Insights into Tyrosinase Inhibition Mechanism by Transition-State Analogs. Inorg Chem 2009; 48(23): 10874-10876. http://dx.doi.org/10.1021/ic901593x

[34] Casella L, Carugo O, Gullotti M, Garofani S, Zanello P. Hemocyanin and tyrosinase models. Synthesis, azide binding, and electrochemistry of dinuclear copper(II) complexes with poly(benzimidazole) ligands modeling the met forms of the proteins. Inorg Chem 1993; 32(10): 2056-2067. http://dx.doi.org/10.1021/ic00062a030

[35] Monzani E, Quinti L, Perotti A, Casella L, Gullotti M, Randaccio L, *et al.* Tyrosinase Models. Synthesis, Structure, Catechol Oxidase Activity, and Phenol Monooxygenase Activity of a Dinuclear Copper Complex Derived from a Triamino Pentabenzimidazole Ligand. Inorg Chem 1998; 37(3): 553-562. http://dx.doi.org/10.1021/ic970996n

[36] Kendall EC. A new method for the determination of the reduced sugar. J Am Chem Soc 1912; 34(3): 317-341. http://dx.doi.org/10.1021/ja02204a014

[37] Warner RC, Weber I. The Cupric and Ferric Citrate Complexes. J Am Chem Soc 1953; 75(20): 5086-5094. http://dx.doi.org/10.1021/ja01116a055

[38] Mastropaolo D, Powers DA, Potenza JA, Schugar HJ. Inorg Chem 1976; 15(6): 1444-49. http://dx.doi.org/10.1021/ic50160a038

[39] Hamada YZ, Carlaon BL, Shank JT. Potentiometric and UV-Vis spectroscopy studies of citrate with the hexaquo Fe3+ and Cr3+ metal ions. Syn Reac Inorg Metal-Org Chem 2003; 33(8): 1425-1440. http://dx.doi.org/10.1081/SIM-120024320

[40] Hamada YZ, Zhepeng W, Harris WR. Competition between transferrin and serum ligands citrate and phosphate for the binding of aluminum. Inorg Chem 2003; 42: 3262-3273. http://dx.doi.org/10.1021/ic026027w

[41] Hamada YZ, Bayakly N, George D, Greer T. Speciation of Molybdenum(VI)- Citric Acid Complexes in Aqueous Solutions, Synthesis and Reactivity in Inorganic, Metal-Organic, and Nano-Metal Chemistry 2008; 38(8): 664-668.

[42] Martell AE Smith RM, Motekaitis RJ. Critical Stability Constants Database, Version 6.0, 2001 NIST, Texas A & M University, College Station, TX, USA.

[43] Alderighi L, Gans P, Ienco A, Perters D, Sabatini A, Vacca A. Hyperquad simulation and speciation (Hyss): a utility program for the investigation of equilibria involving soluble and partially soluble species. Coord Chem Rev 1999; 184: 311-318. http://dx.doi.org/10.1016/S0010-8545(98)00260-4

[44] Sweeton FH, Mesmer RE, Baes Jr., CF. Acidity measurements at elevated temperature. VII. Dissociation of water. J Sol Chem 1974; 3: 191-214. http://dx.doi.org/10.1007/BF00645633

[45] Kotsakis N, Raptopoulou CP, Tangoulis V, Giapintzakis J, Jakusch T, Kiss T, Salifoglou A. Correlation of synthetic, spectroscopic, structural, and speciation studies in the biologically relevant cobalt(II)-citrate system: The tale of the first aqueous dinuclear cobalt(II)-citrate complex. Inorg Chem 2003; 42: 22-31. http://dx.doi.org/10.1021/ic011272l

[46] Dakanali M, Raptopoulou CP, Terzis A, Lakatos A, Lakatos A, Kiss T, Salifoglou A. A novel dinuclear species in aqueous distribution of aluminum in the presence of citrate. Inorg Chem 2003; 42: 252-254. http://dx.doi.org/10.1021/ic0258025

[47] Matzapetakis M, Kourgiantakis M, Dakanali M, Raptopoulou CP, Terzis A, Lakatos A, Lakatos A, Kiss T, Banyai I, Mavromoustakos T, Salifoglou A. Synthesis, pH-dependent structural characterization, and solution behavior of aqueous aluminum and gallium citrate complexes. Inorg Chem 2001; 40: 1734-1744. http://dx.doi.org/10.1021/ic000461l

[48] Kaliva M, Raptopoulou CP, Terzis A, Salifoglou A. Systematic studies on pH-dependent transformation of dinuclear vanadium(V)-citrate complexes in aqueous solutions. A perspective relevance to aqueous vanadium(V)-citrate speciation. J Inorg Biochem 2003; 93: 161-173. http://dx.doi.org/10.1016/S0162-0134(02)00563-9

[49] Abbay G, Gilbert TW. Chromium(III)-citrate complexes: A study using ion exchange and isotachophoresis. Polyhedron 1986; 5(11): 1839-1844. http://dx.doi.org/10.1016/S0277-5387(00)84865-2

[50] Lippard S, Shweky I, Bino A, Goldberg DP. Synthesis, structure, and magnetic properties of two dinuclear iron(III) complexes. Inorg Chem 1994; 33: 5161-5162. http://dx.doi.org/10.1021/ic00101a001

[51] Spiro TG, Pape L, Saltman P. The hydrolytic polymerization of ferric citrate. I. The chemistry of the polymer. J Am Chem Soc 1967; 89: 5555-5559. http://dx.doi.org/10.1021/ja00998a008

[52] Hamada Y, Bayakly ZN, Peipho A, Carlson B. Accurate potentiometric studies of chromium-citrate and ferric-citrate complexes in aqueous solutions at physiological and alkaline pH-values. Synthesis and Reactivity of Inorganic and Metal-Organic and Nano-Metal Chemistry 2006; 36: 469-476. http://dx.doi.org/10.1080/15533170600777960

[53] Timberlake CF. Iron–malate and iron–citrate complexes. J Chem Soc 1964; 5078-5085. http://dx.doi.org/10.1039/jr9640005078

Received on 01-09-2015 Accepted on 15-10-2015 Published on 16-11-2015

http://dx.doi.org/10.6000/1927-5129.2015.11.78