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Colorimetric Determination of Stability Constant of Acetaminophen-Cu(II) complex by Classical Equation Depending on Stoichiometric Curves

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Abstract *Acetaminophen is a nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of pain, fever and headache. Classical equation has been used in the calculation of stability constant of acetaminophen-Cu(II) complex depending on the theoretical explanation of the stoichiometry, continuous variation and mole ratio methods.The formation of Cu(II) complex with acetaminophen was studied colorimetrically at an absorption maximum of 630 nm at 25 and 40 °C. The data showed that Cu(II) and acetaminophen combine in the molar ratio of 1:1 at pH 7.4 with ionic strength maintained using 0.1M KNO_3. Calculated stability constants values were 1.18 x 10^2 and 1.11 x 10^2 using continuous variation method and 1.25 x 10^2 and 1.11 x 10^2 using mole ratio methods at 25 and 40 °C respectively. Calculated ΔG^{O} for the complex were - 1.18 x 10^4 and-1.23 x 10^4 J using continuous variation method and - 1.20 x 10^4 J and -1.23 x 10^4 J using mole ratio method at 25 and 40 °C respectively. The stoichiometry, stability constant and Gibbs free energy results suggested that acetaminophen used in the study is a good chelating agent and can be an efficient antidote in the therapy of Cu(II) overload or poisoning.*

Keywords: *Acetaminophen, copper,complex, stability constant*

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1.0 Introduction

Acetaminophen is a mild analgesic that is used for the treatment of pain, fever and headache. The American College of Rheumatology recommended acetaminophen as one of the numerous treatment options for people with arthritis pain of the hip, hand, or knee that does not show significant improvement with exercise and weight reduction (Hochberg *et al*., 2012).The American College of Physicians and the American Pain Society also recommended acetaminophen as a frontline-line treatment for lower back pain (Chou *et al*., 2007; Chou & Huffman, 2007). Acetaminophen has relatively low anti-inflammatory potentials compared to other common analgesics such as the nonsteroidal anti-inflammatory drugs (NSAIDs) aspirin, and ibuprofen (McKay & Walters, 2013; Ghanem *et al*., 2016; Viswanathan *et al*,, 2008)

Copper is a vital micronutrient that is required for the healthy living of plant, animal, and human (Scheiber *et al*., 2013). It is also needed for the proper functioning of aerobic (oxygen-requiring) microorganisms. Copper is a constituent of numerous proteins and metalloenzymes which perform vital metabolic functions; the micronutrient is essential for proper growth, development, maintenance of bone, connective tissue, brain, heart, and many other body organs (Bremner, 1998).Copper is associated with red blood cells formation, iron absorption and utilization, cholesterol and glucose metabolism and the biosynthesis and release of life-supporting proteins and enzymes. Copper stimulates body immunity to fight infections, repairing of injured

tissues and promote healing. Copper also helps in the neutralization of free-radicals which can cause severe cell damage. The oxidation potential of copper has been attributed to its toxicity in excess ingestion cases. At high concentrations copper causes oxidative damage to human life, including peroxidation of lipids or other macromolecules (Bremner, 1998).

Synthesis, characterization and evaluation of anti-inflammatory activity of acetaminophen metal complexes have been reported by Faruna *et al*. (2017). Results of their study indicated that acetaminophen acted as a bidentate ligand that was coordinated to Cu(II) through phenol and carbonyl oxygen atom.

For several decades, chelating agents have been used as antidote to combat metal poisoning (Tella & Obaleye, 2010). Biological friendly sequestrating agents have been used effectively to chelate metals in patients with metal overload (Tella & Obaleye, 2010). However, chelating capacity is a function of stability constant indicating that the effectiveness of a drug to chelate with a metal ion depends on the stability constant and other parameters (Tella & Obaleye, 2010). Many authors have reported the study of stability constant of drug- metal complexes (Reková *et al.,*2009; Tirmizi *et al*., 2008; Tirmizi *et al*., 2012; Abbas, 2017). However, to the best of our knowledge, the stability constant of acetaminophen–Cu(II) complex at different temperatures have not been reported elsewhere in literature. Therefore, the present study is aimed at determining the stability constant of acetaminophen-Cu(II) complex using colorimetric method that is based on classical equation that depends on stoichiometric curves.

Information on stability constants of this complex can be useful in analyzing the effects of acetaminophen on copper ion and other electroactive divalent trace metals. It is possible that changes in trace metal and mineral concentration induced by acetaminophen can be an efficient antidote in the therapy of Cu overload or poisoning. The chemical structure of acetaminophen is shown in Fig. 1 while the chemical structure of the proposed complex is shown in Fig. 2.

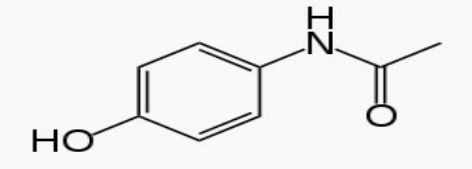

Fig. 1: Chemical structure of acetaminophen

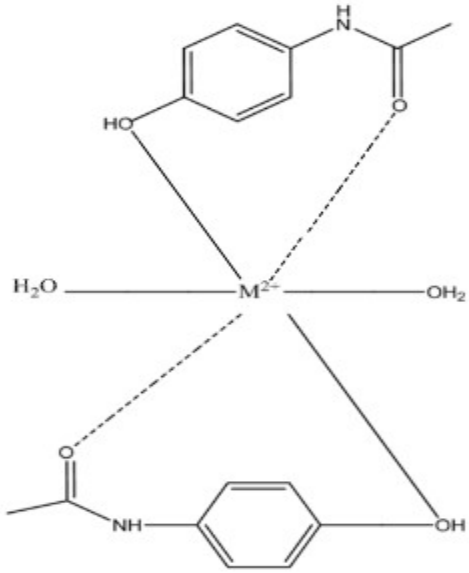

Fig. 2: Proposed structure of acetaminophen metal complexes, (M = Cu(II)

2.0 Materials and methods

2.1 Instrumentation

Absorbance of the complex was measured using auto colorimeter ME-51. Orion Versa Star Pro pH Benchtop meter (VSRAR10 series) was used for pH measurements

2.2 Reagents

Reagents used for the study were of analytical grade. Acetaminophen was purchased from Liaoyuan City Baikag, Pharmaceutical Company Limited, China. $CuSO_4$was purchased from Merck & Co., Inc USA. Double-distilled water was used throughout the experiment.

2.3 Preparation of 1 x 10^{-2} M $CuSO_4$

$CuSO_4$(1.566 g, 10 Mmol, molar weight= 156.60 g/mol) was dissolved in freshly distilled water contained in a 250 cm^3 beaker and was made up to the mark in a 1000 cm^3 volumetric flask.

2.4 Preparation of 1 x 10^{-2} M acetaminophen

Acetaminophen (1.511 g, 10 Mmol molar weight = 151.163 g/mol) was dissolved in freshly distilled water in a 250 cm^3beaker and was made up to the mark in a 1000 cm^3 volumetric flask.

2.5 Procedure for continuous variation method

Exactly 0, 1,2, 3, 4, 5, 6 $cm^3$of 1 x 10^{-2} M $CuSO_4$ were pipetted into seven different 50 cm^3volumetric flasks respectively. Exactly 6, 5, 4, 3, 2, 1, 0 cm^3 of 1 x 10^{-2} M of acetaminophen was added to the respective flasks containing Cu(II) solution. The pH was adjusted to 7.4 while the ionic strength was maintained constant using 0.1 M KNO_3. The absorbance of each solution was measured at 630 nm (maximum wavelength of

absorbance of the complex) and at temperatures of 25 and 40 °C, respectively.

2.6 Procedure for mole ratio method

$CuSO_4$ (1 x 10^{-2} M) (2 cm^3) was transferred to each of the seven 50 cm^3 volumetric flasks. Acetaminophen (1 x 10^{-2} M) (1, 2, 3, 4, 5, 6, 7 cm^3) was added to each of the Cu(II) solution respectively. Their absorbance was measured at 630 nm (maximum absorbance of the complex) and at temperatures of 25 and 40 °C, respectively.

2.7 Calculation of stoichiometry, stability constant and free energy

The stoichiometry mole fraction (SMF) of the complex using continuous variation method was calculated using equation 1 (Abbas, 2017)

$$SMF = \frac{m}{1-m} \quad (1)$$

where m is the mole fraction of the metal ion. The stability constant was calculated using the classical method expressed in equation 2,

$$K_{st} = \frac{1-\alpha}{m^m.n^n(\alpha)^{m+n}(C)^{m+n-1}} \quad (2)$$

where C is the concentration of the complex at stoichiometry point, α is the degree of dissociation, m and n are the corresponding stoichiometric coefficients of metal and ligand respectively. The degree of dissociation (α) was calculated using equations 3, 4 and 5 (Abbas, 2017),

$$A_\alpha = A_o - A_{max} \quad (3)$$

$$A_{max} = \varepsilon bC \quad (4)$$

$$\alpha = \frac{A_\alpha}{\varepsilon bC} \quad (5)$$

where A_{max} is absorbance value of the maximum at experimental curve that represents the maximum quantity of the complex that is formed. A_o is absorbance value corresponding to the intersect point of the theoretical straight lines. A_α is the absorbance value of the part of dissociated concentration of complex. ε is molar absorptivity, b is cell thickness, C is a concentration of complex at stoichiometry point.

The Gibbs free energy was calculated using the Helmholtz Gibb equation (equation 6),

$$\Delta G^\theta = -RTInK \quad (6)$$

3.0 Results and Discussion

The absorption spectra of acetaminophen-Cu(II) complex is shown in Fig. 3. The absorption spectra (Fig. 3) shows the absorbance of $CuSO_4$ (series 1) and acetaminophen-Cu(II) complex (series 2) at wavelength of 400 – 670 nm. It was observed that the wavelength of maximum absorbance of the complex was 630 nm. At this wavelength, $CuSO_4$ displayed minimal absorbance. Since the complex maximum absorbance was 630 nm, it was used for the analytical measurement in the determination of the stoichiometry, stability constants and free energies.

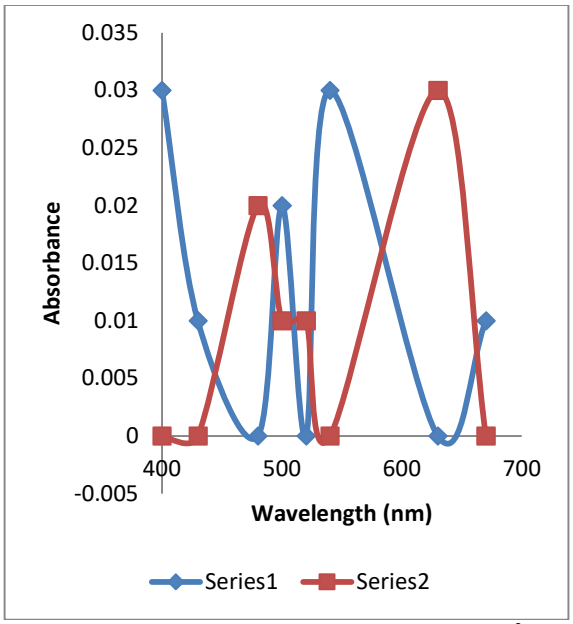

Fig. 3: Absorption spectra of $CuSO_4$(1 x 10^{-2} M) (series 1) and acetaminophen-Cu(II) complex (series 2)

The maximum absorbance of $CuSO_4$ was observed at wavelength of 670 nm. It was observed that acetaminophen-Cu(II) complex gave a water soluble complex in aqueous solution, This may be attributed to the ability of water to act as a Water is behaved as a weak monodentate ligand in forming labile Cu-aquo complex. During complexation, acetaminophen displaced water from Cu-aquo to form a stable acetaminophen - Cu(II) complex. Similar labile aquo complexes were also proposed by Tirmizi and co-workers in their study of famotidine-Cu complex and cimetidine-Ni complex (Reková *et al.*,2009; Tirmizi *et al*., 2008; Tirmizi *et al*., 2012; Abbas, 2017). Labile aquo complex was also reported by Tella and co–workers in their study of Dapsone-Cu(II) stability constants (Tella and Obaleye, 2009).

For the continuous variation method, equation 1 was applied in calculation of stoichiometry.

$SMF = \frac{0.50}{0.50} = 1$ (at 25 °C) and $SMF = \frac{0.50}{0.50} = 1$ (at 40 °C).

This corresponded to metal:ligand ratio of 1:1. The mole fraction of Cu(II) at the point of intersection are 0.50 and 0.50 at 25 and 40 °C respectively. The extrapolated value at the point of cross-section on continuous variation plot

(Figs. 3 and 4) corresponded to the total absorbance of the complex, indicating that the complex formation process has been completed. Several authors have also applied continuous variation method in the determination of metal:ligand ratio in complexes (Reková *et al.,*2009; Tirmizi *et al*., 2008; Tirmizi *et al*., 2012; Abbas, 2017).

Table 1: Experimental data of acetaminophen-Cu(II) complex at 630 nm by continuous variation method

S/N	$CuSO_4$ (1 x 10^{-2} M)	Acetaminophen (1 x 10^{-2} M)	Mole fraction of Cu(II)	Absorbance at 630(nm)	
				25 ℃	40 ℃
1	0.000	6.000	0.000	0.000	0.000
2	1.000	5.000	0.170	0.000	0.000
3	2.000	4.000	0.330	0.010	0.010
4	3.000	3.000	**0.500**	**0.020**	**0.020**
5	4.000	2.000	0.660	0.010	0.000
6	5.000	1.000	0.830	0.000	0.000
7	6.000	0.000	1.000	0.000	0.000

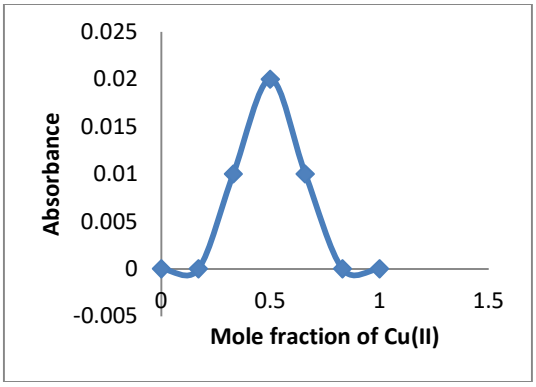

Fig. 3: Job's curves for stability constants of equimolar solutions at 25°C

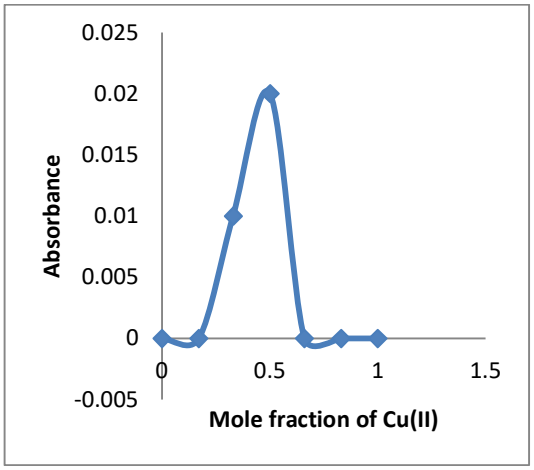

Fig. 4: Job's curves for stability constants of equimolar solutions at 40°C

Table 2: Experimental data of acetaminophen-Cu(II) complex at 630 nm by mole ratio method

S/N	$CuSO_4$ (1 x 10^{-2} M)	Acetaminophen (1 x 10^{-2} M)	Vol of acetaminophen/ vol of Cu(II)	Absorbance at 630 nm	
				25 ℃	40 ℃
1	2.000	1.000	0.500	0.008	0.009
2	2.000	2.000	**1.000**	**0.010**	**0.010**
3	2.000	3.000	1.500	0.010	0.010
4	2.000	4.000	2.000	0.010	0.010
5	2.000	5.000	2.500	0.010	0.010
6	2.000	6.000	3.000	0.010	0.010
7	2.000	7.000	3.500	0.010	0.010

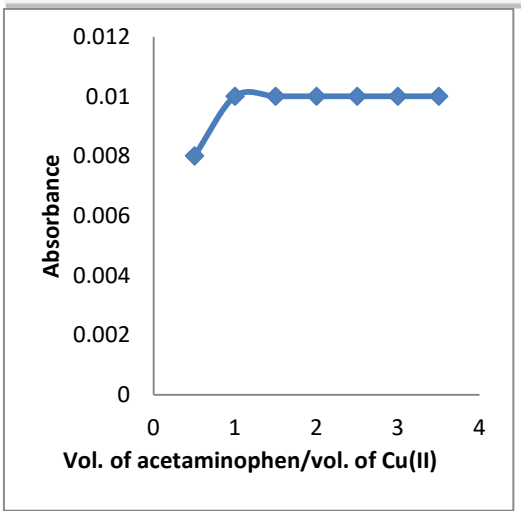

Fig.5: Mole ratio method curves for stability constant at 25ºC

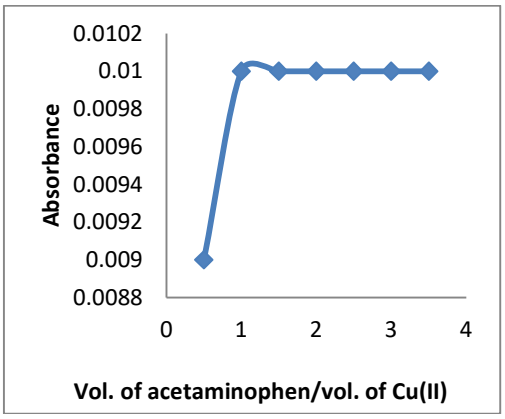

Fig. 6: Mole ratio method curves for stability constant at 40 ºC

The metal:ligand ratio of the complex was estimated from the point where this curve changes its slope. The measured volume of acetaminophen/volume of Cu (II) at the point of intersection are 1.00 and 1.00 at 25 and 40°C respectively. This corresponded to metal:ligand ratio of 1:1. The extrapolated value at the point of cross-section on mole ratio plot (Figs. 5 and 6) corresponded to the total absorbance of the complex, indicating that the complex formation process has been completed. Several authors have also used mole ratio method in the determination of stoichiometry of metal complexes (Reková *et al.,*2009; Tirmizi *et al*., 2008; Tirmizi *et al*., 2012; Abbas, 2017). Hence, mole ratio technique is an established method for the determination of metal: ligand ratio in complexes.

Stability constant is an evaluation of the strength of the interaction between the reagents that come together to form the complex. Large values indicate that the metal has high affinity for the ligand, provided the system is at equilibrium. Calculation of the stability constant and Gibbs free energies were based on equations 2, 3, 4, 5 and 6 respectively. The values of the stability constant showed that the complex was stable at 25 ºC and 40 ºC. The calculated stability constants obtained from continuous variation compared well with that of mole ratio method. It is evident from Table 3 that the values obtained by both methods are in fair agreement. Increasing the temperature of coordination from 25 to 40 ºC did not display observable significant effect on the stability constant**.** The values of the stability constants were positive indicating that the complex is stable. Similar positive values of stability constant of complexes were reported by Tirmizi and co-workers in 2012 using continuous variation and mole ratio methods (Reková *et al.,*2009; Tirmizi *et al*., 2008; Tirmizi *et al*., 2012; Abbas, 2017).Positive stability constant values using continuous variation and mole ratio methods have also been reported by Waranyoupalin and co-workers (Waranyoupalin *et al*., 2009).Continuous variation and mole ratio methods are established techniques in the determination of stability constant and Gibbs free energies. The results of stability constant suggested that acetaminophen could be effective in chelation therapy against Cu (II) toxicity. The negative values of the free energies suggested that the complexes were formed spontaneously.

Table 3: Calculated stability constant values (using classical equation) and Gibbs free energies of acetaminophen-Cu (II) complex

S/N	Method	Metal: ligand ratio	Stability constant		$\Delta G^{O\ res}$ (J)	
			25 ºC	40 ºC	25 ºC	40 ºC
1	Continuous variation	1:1	1.18×10^2	1.11×10^2	-1.19×10^4	-1.23×10^4
2	Mole ratio	1:1	1.20×10^2	1.11×10^2	-1.20×10^4	-1.23×10^4

4.0 Conclusion

Acetaminophen is a nonsteroidal anti-inflammatory drug (NSAID) that is used for the treatment of pain, fever and headache. It formed a reasonably stable complex with Cu (II). The continuous variation method of analysis corresponded well with the values obtained using mole ratio method of analysis. The Job's continuous variation and mole ratio methods data showed that Cu (II) and acetaminophen combine in the molar ratio of 1:1. The stability constant results suggested that acetaminophen used in the study is a good chelating agent and can be an efficient antidote in the therapy of Cu (II) overload or poisoning.

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