



Evaluation of Stoichiometry, Stability Constants and Gibbs Free Energies of Acetaminophen-Zn (II) complex at Different Temperatures

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ABSTRACT: Acetaminophen also known as paracetamol, is a drug used in the treatment of pain and fever. It is essentially used for the relief of mild to moderate pain. The presence of phenol and carbonyl oxygen atom enables acetaminophen to behave as a bidentate ligand. The stoichiometry, stability constants and Gibbs free energies of acetaminophen-Zn (II) were determined colorimetrically at 25 and 40 °C using continuous variation and mole ratio methods. The formation of Zn (II) complex with acetaminophen was studied colorimetrically at an absorption maximum of 630 nm at different temperatures. The data showed that Zn (II) and acetaminophen combine in the molar ratio of 1:1 at pH 7.4 with ionic strength maintained using 0.1M KNO₃. Calculated stability constants values were 2.70×10^3 and 2.20×10^3 using continuous variation method and 7.21×10^3 and 7.21×10^3 using mole ratio methods at 25 and 40 °C respectively. Calculated ΔG° for the complex were -1.96×10^4 and -1.98×10^4 J using continuous variation method and -2.2×10^4 J and -2.31×10^4 J using mole ratio method at 25 and 40 °C respectively. The 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 Zn (II) overload or poisoning.

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Acetaminophen (Figure 1) also known as paracetamol, is a drug used in the treatment of pain and fever (Lee, 2017). It is essentially used for the relief of mild to moderate pain (ASHSP, 2016). Report has shown that it is use to relieve fever in children (Meremikwu and Oyo-Ita, 2002; de Martino and Chiarugi, 2015). It is often sold in combination with other cold medications for relieve of cold (ASHSP, 2016). Acetaminophen is also used for the treatment of severe pain, such as cancer pain and pain after surgical operation, in combination with opioid pain medication (SIGN, 2008). It is administered orally but can also be administered intravenously (Hochhauser, 2014). The analgesic effects last between two and four hours (Hochhauser, 2014). Acetaminophen is generally safe within the recommended doses (Russell *et al.*, 2003; Machado *et al.*, 2005). The recommended maximum daily dosage for an adult is 3-4 grams (Machado *et al.*, 2005). Higher dosage causes liver failure (ASHSP, 2016) and serious skin reaction (ASHSP, 2016). It is relatively safe during pregnancy and during breastfeeding (ASHSP, 2016). It may be administered at lower doses to those with liver disease (Lewis and Stine, 2013). It is categorized as a mild analgesic (Hochhauser, 2014). It does not possess significant anti-inflammatory activity (McKay and Walters, 2013). It has no clear mechanism of action (McKay and Walters, 2013;

Ghanem, *et al.*, 2016; Viswanathan *et al.*, 2008). Zinc is involved in several cellular metabolic processes (Classen *et al.*, 2011). It was evaluated that 10 % of human proteins chelate to zinc. Hundreds of human proteins also transport and traffic zinc. It was reported that over 200 enzymes require Zn for their catalytic activity (Sandstead, 1994) and it plays a role in immunity, healing of wounds, synthesis of protein, DNA and cell division (Prasad, 1995). Zinc is essential for proper sense of taste and smell (Prasad *et al.*, 1997) and it also assist normal growth and development during pregnancy, childhood, and adolescence (Milbury and Richer, 2008). It possesses antioxidant properties that protect against accelerated aging and assist in healing process after an injury; however, studies on its effectiveness differs (Maret and Sandstead, 2006). Synthesis, characterization and evaluation of anti-inflammatory activity of acetaminophen complex of Zinc (II) ions have been reported (Faruna *et al.*, 2017). The complex (Figure 2) was synthesized and characterized by FTIR, UV- Visible spectroscopy, x-ray diffraction analysis, and melting point and conductivity measurements. On the basis of their study, it is proven that acetaminophen acted as a bidentate ligand coordinating to Zn ions through phenol and carbonyl oxygen atom. Synthesis and solid-state characterization of Zn (II) metal complex with

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acetaminophen have been reported (Ledeti *et al.*, 2013). For several decades, chelating agents have been used as antidote to combat metal poisoning (Tella and Obaleye, 2010). Biological friendly complexing agents have been used effectively to chelate metals in patients with metal overload (Tella and 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). However, to the best of authors' knowledge, stoichiometry mole fraction, stability constants and Gibbs free energies of acetaminophen – Zn (II) complex at different temperatures have yet not appeared in the literature. These stability constants are useful to study the effects of acetaminophen on trace metals and mineral metabolism. It is possible that changes in trace metal and mineral concentration induced by acetaminophen can be an efficient antidote in the therapy of Zn overload or poisoning. In this context, the aim of this study was to assess stoichiometry, stability constants and Gibbs free energies change of acetaminophen – Zn (II) complex at 25 and 40 °C respectively.

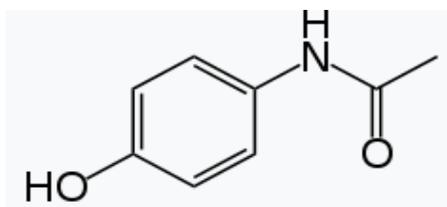


Fig 1: Chemical structure of acetaminophen

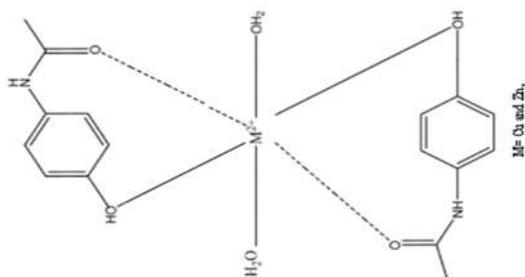


Fig 2: Tentative structure of acetaminophen metal complexes, M = Cu (II) and Zn (II) (Ledeti *et al.*, 2013; Faruna *et al.*, 2017)

MATERIALS AND METHODS

Instrumentation: Orion Versa Star Pro pH Benchtop meter (VSRAR10 series) was used for pH determination. Colorimetric measurements were performed on auto colorimeter ME-51.

Reagents: All reagents used were of analytical grade purity. Acetaminophen was purchased from CSPC Zhongnuo Pharmaceutical Company Limited, Shijiazhuang, China. ZnCl₂ was purchased from Merck Germany. Double-distilled water was used throughout the experiment.

Preparation of 1 x 10⁻² M ZnCl₂: ZnCl₂ (1.362 g, 10 M mol, M. Wt. = 136.290 g/mol) was dissolved in freshly distilled in a 250 cm³ beaker and was made up to the mark in a 1000 cm³ volumetric flask.

Preparation of 1 x 10⁻² M acetaminophen: Acetaminophen (1.511 g, 10 m mol, M. Wt. = 151.163 g/mol) was dissolved in freshly distilled in a 250 cm³ beaker and was made up to the mark in a 1000 cm³ volumetric flask.

Procedure for continuous variation method: ZnCl₂ (1 x 10⁻² M) (0, 1, 2, 3, 4, 5, 6 cm³) was pipetted out and transferred into seven 50 cm³ volumetric flasks. Acetaminophen (1 x 10⁻² M) (6, 5, 4, 3, 2, 1, 0 cm³) was added, respectively to the Zn (II) solution so that the mole fraction remained constant. The pH adjusted to 7.4 and ionic strength maintained constant by using 0.1 M KNO₃. Their absorbance were measured at 630 nm (maximum absorbance of the complex) and at a temperature of 25 and 40 °C, respectively. **Procedure for mole ratio method:** ZnCl₂ (1 x 10⁻² M) (2 cm³) was pipetted out and transferred into each of the seven 50 cm³ volumetric flasks. Acetaminophen (2 x 10⁻² M) (1, 2, 3, 4, 5, 6, 7 cm³) was added to each of the Zn (II) solution respectively. Their absorbance was measured at 630 nm (maximum absorbance of the complex) and at a temperature of 25 and 40 °C, respectively.

Calculation of stoichiometry mole fraction, stability constant and free energy: The stoichiometry mole fraction (SMF) of the complex using continuous variation method was calculated using equation 1 (Abbas, 2017)

$$SMF = \frac{m}{1-m}$$

Where m is the mole fraction of metal ion.

Equation 2 (Abbas, 2017) was applied to the calculation of stability constant.

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

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)

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

$$A_{max} = \epsilon b C \quad 4$$

$$\alpha = \frac{A_{\alpha}}{\epsilon b C} \quad 5$$

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_a 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 equation 6.

$$\Delta G^\theta = -RT \ln K_{stability} \quad 6$$

RESULTS AND DISCUSSION

The absorption spectra (Figure 3) shows the absorbance of $ZnCl_2$ (series 1) and acetaminophene-Zn (II) complex (series 2) at wavelength of 400 – 670 nm. The wavelength of maximum absorbance of the complex was

630 nm. At the wavelength, $ZnCl_2$ have a weak absorbance. Therefore, this wavelength was used for the measurement of absorbance in the determination of the stability constants and free energies. $ZnCl_2$ absorbs maximally at wavelength of 670 nm. It was observed that acetaminophen-Zn (II) complex gave a water soluble complex. In aqueous solution, Zn-aquo complex is a labile complex because water behaved as a weak ligand. Acetaminophen displaced water from Zn-aquo to form a stable acetaminophen – Zn (II) complex. Similar labile aquo complexes were also reported 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). Tella and co-workers reported labile aquo complex in their study of Dapsone-Cu(II) stability constants (Tella and Obaleye, 2009). The absorption spectra of acetaminophen-Zn (II) complex is shown in Figure 3

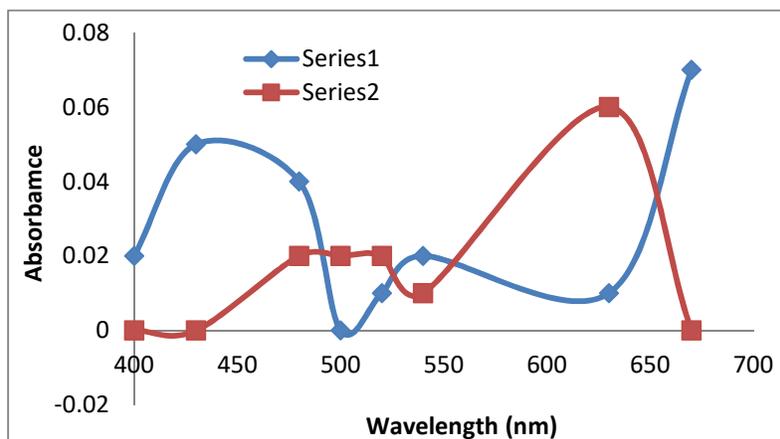
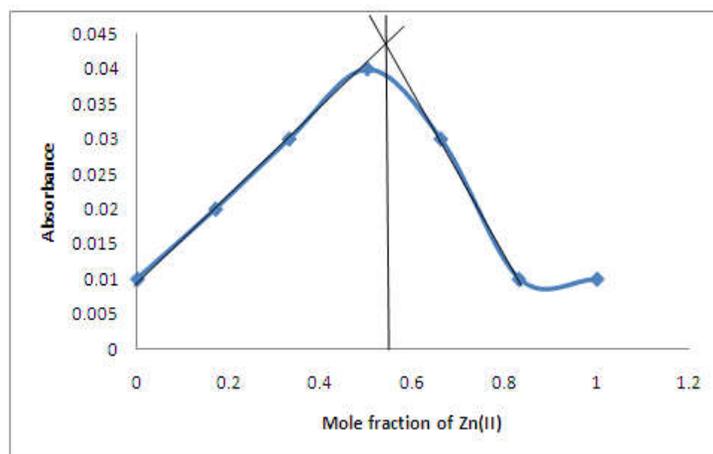
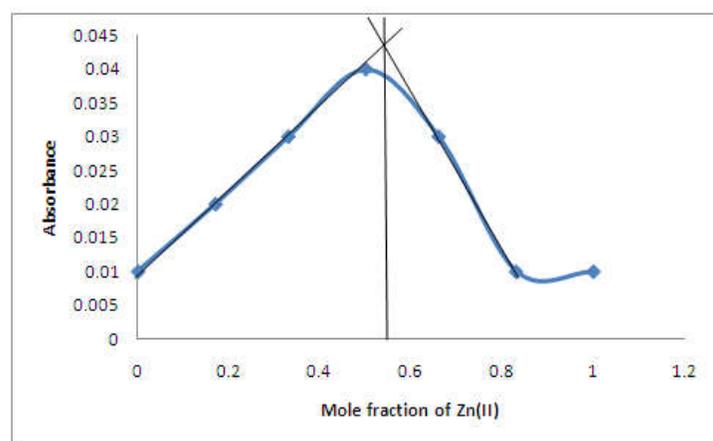


Fig 3: Absorption spectra of $ZnCl_2$ (1×10^{-2} M) (series 1) and acetaminophene-Zn (II) complex (1×10^{-2} M) (series 2)

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

S/N	$ZnCl_2$ (1×10^{-2} M)	Acetaminophen (1×10^{-2} M)	Mole fraction of Zn(II)	Absorbance at 630 nm	
				25 °C	40 °C
1	0.00	6.00	0.00	0.01	0.01
2	1.00	5.00	0.17	0.02	0.02
3	2.00	4.00	0.33	0.02	0.03
4	3.00	3.00	0.50	0.04	0.04
5	4.00	2.00	0.66	0.03	0.03
6	5.00	1.00	0.83	0.01	0.01
7	6.00	0.00	1.00	0.01	0.01


Fig4: Job's curves for stability constants of equimolar solutions at 25 °C

Fig 5: Job's curves for stability constants of equimolar solutions at 40 °C

Application of equation 1, $SMF = \frac{0.55}{0.45} = 1.22 \approx 1$ (at 25 °C) and $SMF = \frac{0.55}{0.45} = 1.22 \approx 1$ (at 40 °C). The mole fraction of Zn(II) at the point of intersection are 0.55 and 0.55 at 25 and 40 °C respectively. The extrapolated value at the point of cross-section on continuous variation plot (Figures 4 and 5) corresponded to the total absorbance of

the complex, indicating that the complex formation process has been completed. This corresponded to metal:ligand ratio of 1:1. 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).

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

S/N	ZnCl ₂ (1 x 10 ⁻² M)	Acetaminophen (1 x 10 ⁻² M)	Vol of acetaminophen / vol. of Zn(II)	Absorbance at 630 nm	
				25 °C	40 °C
1	2.00	1.00	0.50	0.018	0.018
2	2.00	2.00	1.00	0.020	0.020
3	2.00	3.00	1.50	0.020	0.020
4	2.00	4.00	2.00	0.020	0.020
5	2.00	5.00	2.50	0.020	0.020
6	2.00	6.00	3.00	0.020	0.020
7	2.00	7.00	3.50	0.020	0.020

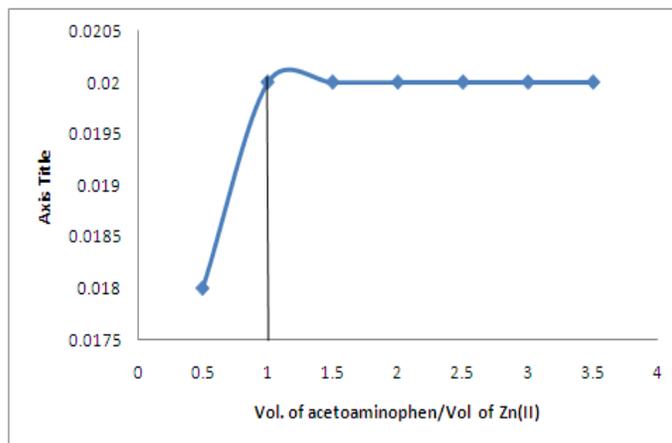


Fig 6: Mole ratio method curves for stability constant at 25 °C

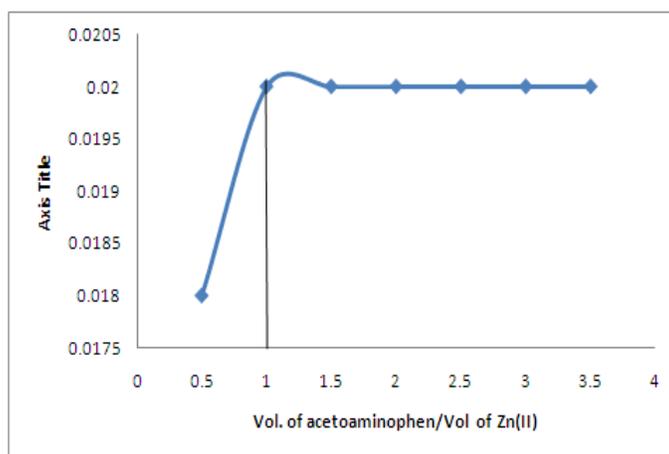


Fig 7: Mole ratio method curves for stability constant at 40 °C

The vol. of acetaminophen/vol. of Zn (II) at the point of intersection are 1.0 and 1.0 at 25 and 40 °C respectively. The extrapolated value at the point of cross-section on mole ratio plot (Figures 6 and 7) corresponded to the total absorbance of the complex, indicating that the complex formation process has been completed. The

stoichiometry ratio of the complex was evaluated from the point where this curve changes its slope. This corresponded to metal:ligand ratio of 1:1. Mole ratio method is an established technique in the determination of stoichiometry of metal complexes (Reková, *et al.*, 2009; Tirmizi, *et al.*, 2008; Tirmizi, *et al.*, 2012).

Table 3: Calculated values of stability constant and Gibbs free energies

S.No	Method	Metal: ligand ratio	Stability constant			
			ΔG^{\ominus} (J)			
			25 °C	40 °C	25 °C	40 °C
1	Continuous variation	1:1	2.70×10^3	2.00×10^3	-1.96×10^4	-1.98×10^4
2	Mole ratio	1:1	7.21×10^3	7.21×10^3	-2.20×10^4	-2.31×10^4

The results of stability constant suggested that acetaminophen could be effective in chelation therapy against Zn(II) toxicity. The negative values of the free energies suggested that the complexes were formed spontaneously. Continuous variation and mole ratio methods are established techniques in the determination of stability constant and Gibbs free energies. The

stability constant and Gibbs free energies were calculated by applying equations 2, 3, 4, 5 and 6. The stability constant values showed that the complex was stable both at room temperature and higher temperature. The values of the stability constants obtained from continuous variation compared well with that of mole ratio method. It can be seen from the Table 3 that the

values obtained by both methods are in fair agreement. Increasing the temperature of chelation from 25 to 40 °C had no effect on the stability constant. The values of the stability constants were positive; this suggested that the complex was stable. Similar positive values of stability constant of complexes were reported by Tirmizi and co-workers (Tirmizi, *et al.*, 2008; Tirmizi, *et al.*, 2012) using continuous variation and mole ratio methods. Waranyoupalin and co-workers also reported positive stability constant values using continuous variation and mole ratio methods (Waranyoupalin, 2009).

Conclusion: The Job's continuous variation and mole ratio methods data showed that Zn(II) and acetaminophen combine in the molar ratio of 1:1. Job's method of analysis corresponded well with the values obtained using mole ratio method of analysis. The stability constants and free energies results suggested that acetaminophen used in the study is a good chelating agent and can be an efficient antidote in the therapy of Zn(II) overload or poisoning.

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