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COMPLEX STABILITY IN AQUEOUS SOLUTION OF METAL IONS (CU²⁺, ZN²⁺, AND MN²⁺) WITH PYROCATECHUIC ACID LIGAND 1SHELLA PERMATASARI SANTOSO, 2ARTIK ELISA ANGKAWIJAYA, 2YI-HSU JU 1,2,3Chemical

5Engineering Department, National Taiwan University of Science and Technology, Taipei 10607, Taiwan

E-mail: sheila_p5@yahoo.com, artic_elisa@yahoo.com,

12yhju@mail.ntust.edu.tw Abstract- A

natural phenolic compound namely pyrocatechuic acid or 2,3-dihydroxybenzoic acid has been known for its biological activity as antioxidant and antimicrobial agent. This compound shows a potential chelating ability to bind metal ions through its three oxygen donor atoms. This ability seems to be promising for metal intoxication by chelation. In this study, the chelating ability of PA towards metal ion was expressed as "equilibrium constant" which shown the stability of the chelate complex formed. The investigated metal ions are Cu²⁺, Zn²⁺, and Mn²⁺. Potentiometric method was used for the determination of the equilibrium constant. The potentiometric method was carried out in aqueous solution which mimic human body fluid, with

3ionic strength of 0.15 mol·dm⁻³ NaCl at temperature 37 °C. The

formation of chelate complex was confirmed by spectrophotometric measurements. The result showed that the two oxygen donor atoms from hydroxyl group of PA were found to bind

10 **the metal ion. The stability of the metal complexes**

were increased as the ionic radius of the metal ion is decreased, where the complex that has highest stability is $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Mn}^{2+}$. Additionally, the species distribution of the metal complexes in function of pH was presented graphically by using HySS2009. Keywords- Chelate Complex, Potentiometric, Stability Constant, Pyrocatechuic Acid, 2,3-Dihydroxybenzoic Acid. I. INTRODUCTION Pyrocatechuic acid (2,3-dihydroxybenzoic acid) is a phenolic compound which possesses three oxygen donor atoms that were found to be potential to bind metal ions [1], [2]. In nature, pyrocatechuic acid can be found in some sources such as aquatic fern *Salvinia molesta*, *Platycodon grandiflorum*, *Phyllanthus acidus* and *Vitex trifoli* [3]. PA was found to present in human body as the result of aspirin metabolism and also provides some health benefit such as antioxidant and antimicrobial [4]. As a chelating agent, PA may help to bind some excess of non-degradable trace elements (such as metals), make them easier to excrete from body and prevent poisoning due to metal accumulation in high concentration. As depicted in Fig. 1, pyrocatechuic acid possesses three active oxygen donor atoms in carboxylic group and the other two in hydroxyl group at ortho and meta position. These three oxygen atoms were considered as active donor groups due to their deprotonable H^+ atom. Figure 1. Structure of 2,3-dihydroxybenzoic acid In this study, some of divalent metal ions namely Cu^{2+} , Zn^{2+} , and Mn^{2+} were investigated. These trace elements were needed in very small amount to balance human metabolism, these elements also known as micronutrients [5]. Excess amount of these trace elements may lead to heavy metal poisoning and may cause several adverse health effects such as metabolism disorders and cells disruption [6], [7]. The introduction of an organic compound as a ligand may help to control the concentration of these trace elements in the body and prevent heavy metal poisoning [8], [9]. The ligand has ability to chelate metal ion by its donor atom. Stability constant is an important property of chelation which gives impression about the stability of the binding. Thus, higher stability constant value indicating the more stable the chelation between the ligand and the metal ion. The formation of metal complex is pH dependent, different species of metal complex may form in different pH. In this study the chelating ability of pyrocatechuic acid towards some divalent metal ions (Cu^{2+} , Zn^{2+} , and Mn^{2+}) expressed in stability constant value were determined. Potentiometric measurement was used in this study. The study was done in aqueous solution with

3 **constant ionic strength of 0.15 mol·dm⁻³ NaCl and temperature 37 °C. The**

investigated pH is in the range of 2.5 to 11.0, the species distribution of the system was also graphically presented in this pH range. Spectrophotometric method was done in wavelength range of 400-800 nm for qualitative measurement. II. EXPERIMENTAL PROCEDURE A. Materials and

8 **Solutions All chemicals were analytical grade and used without further purification.**

Pyrocatechuic acid (C₇H₆O₄,

13**98% purity) was purchased from Alfa Aesar (UK). Sodium chloride (NaCl, 99.5% purity) was**

obtained from Showa (Japan).

13**Manganese chloride tetrahydrate (MnCl₂·4H₂O, 99.8%)**

and sodium hydroxide (NaOH, 99% purify) were supplied by Yakuri (Japan).

5**Hydrochloric acid (HCl, 37.6% purity) was purchased from Fisher Scientific**

(USA). Carbonate-free NaOH and HCl were

8**prepared and standardized before used. Copper chloride dehydrate (CuCl₂·2H₂O, 99% purity)**

and zinc nitrate hexahydrate (Zn(NO₃)₂·6H₂O, 98%) were obtained from Sigma Aldrich

7**(USA). All solutions were prepared freshly before experiments using ultra pure water obtained from NANO Pure Ultrapure water (18.3 MΩ·cm⁻¹).**

B. Potentiometric Method Potentiometric

11**titrations were carried out in a 100 cm³ commercial double walled glass vessel and thermostatted at 310.15 K**

by the circulating water bath. The titration

11**were performed by using a Metrohm autotitrator 888 Titrande with**

a 805 Dosimat with exchange unit, a 802 rod stirrer supported with 804 Ti stand. The device

6**was coupled to a personal computer and the titration software Tiamo 2. 3 to control the titration process and record the data. The following solutions were**

prepared **in**

each 50 cm³ for the titration, $\pm 1 \cdot 10^{-1}$

2·10⁻³ mol·dm⁻³ carbonate-free NaOH

was used as the titrant to adjust the pH from 2.5 to 11.0. a. 3

9·10⁻³ mol·dm⁻³ HCl + 1.5·10⁻¹ mol·dm⁻³

NaCl +

91·10⁻³ mol·dm⁻³ Ligand b. 3·10⁻³ mol·dm⁻³ HCl + 1.5·10⁻¹ mol·dm⁻³

NaCl + Ligand + metal salt (Cu²⁺, Zn²⁺, and Mn²⁺) The solution b was prepared at

2 metal to ligand molar ratios of 1:1; 1:2; 1:2.5 and 1:3

to cover all possible coordination number.

7 Ionic strength of the solutions was maintained at

1.5·10⁻¹ mol·dm⁻³ with NaCl as the supporting electrolyte. Each

5 titration was repeated at least 3 times, with repeatability of ± 0.002 pH units.

The titration curves were

processed by Hyperquad2008 program

12 for the refinement of stability constants. The species distribution diagram of the formed species

at pH range of 2.5 to 11.0 was simulated by using HySS2009 simulation program. C. Spectrophotometric Measurements The spectrum measurements were carried in a standard quartz cell and JASCO V-550 spectrophotometer, with wavelength range of 400-800 nm. Solution containing 2.5·10⁻¹ mol·dm⁻³ of metal salt (Cu²⁺/Zn²⁺/Mn²⁺) and 5·10⁻¹ mol·dm⁻³ ligand PA was prepared for the measurement. $\pm 1 \cdot 10^{-1}$ mol·dm⁻³ carbonate-free NaOH was used to adjust the pH from 2.5 to 11.0. D. Data Analysis The stability of

metal complex known as overall stability constant " β " can be computed from some parameter such as total concentration of the metal ion [M] and the ligand [L] can be defined as

$10M ? L ? ML ? ML ? ? ? ? M ? ? L ?$ Where "M" is the metal ion, "L" is the ligand and "H" is the

hydrogen atom involved in the formation of a metal complex. By using Hyperquad2008 program, not only the ML species stability constant can be determined. Various species such as MLH₂, MLH and ML₂ also can be determined. The stability constant from Hyperquad2008 was presented as overall stability constant ($\log\beta$), expressed as $pM ? qL ? rH ? M pLqHr ? pqr ? ?M?M?p p?LLq?qH?Hr ?r ?$ (2) Where the coefficient

2p, q, r represent the number of metal ion, ligand and hydrogen atom respectively

[10]. III. RESULT AND DISCUSSION A. Protonation Constant of Pyrocatechuic acid From the Hyperquad2008, the protonation constant represented as overall formation constant ($\log\beta$). Pyrocatechuic acid is the tribasic ligand (H₃L) which posses three oxygen donor atom at carboxyl (COOH) group, hydroxyl (OH) group at ortho and meta position respectively o-OH and m-OH. The formation equilibrium can be expressed as: $H^+ + L \rightleftharpoons HL$; $[HL] = \beta_1 [H][L]$ $2H^+ + L \rightleftharpoons H_2L$; $[H_2L] = \beta_2 [H]^2 [L]$ $3H^+ + L \rightleftharpoons H_3L$; $[H_3L] = \beta_3 [H]^3 [L]$ Where the protonation constant or stepwise formation constant " $\log K_a$ " can be described as $\log\beta_1 = \log K_{a1}$; $\log\beta_2 = \log K_{a1} + \log K_{a2}$; and $\log\beta_3 = \log K_{a1} + \log K_{a2} + \log K_{a3}$. The $\log K$ values were presented in Table 1. Table 1. Protonation constant of the ligands

Position	$\log K_a$	1 o-OH	13.00±0.02	13.00*	2 m-OH	9.98±0.02	9.76*	3 COOH	2.63±0.04	2.70*
*Reference [11], Potentiometric method, I=1 mol·dm ⁻³ , T=25°C As depicted in Fig. 2, the first protonation of pyrocatechuic acid to form HL was take place at basic pH. This protonation was occurred due to attachment of H ⁺ atom to oxygen donor atom at ortho position with $\log K_{a1}$ value of 13.00. Followed by the protonation of m-OH to formed H ₂ L with $\log K_{a2}$ value of 9.98. Finally the H ₃ L was formed from the attachment of H ⁺ aTi(ho1em) oflotghβe1,COOloHgβg2,rouapnwdithlologgβK3a3 vvaallueesof 2fr.6om3. Hyperquad2008 refinement are 13.00, 22.98 and 25.56 respectively. Figure 2. Species Distribution of 2,3-DHBA B. Stability Constant of Metal Complex The titration curve of the Ligand (L) system only, Cu ²⁺ -ligand, Zn ²⁺ -ligand, and Mn ²⁺ -ligand was shown in Fig. 3. The metal-ligand system show deviation from the L system indicated that a new species is formed in the system. The titration curve of Cu ²⁺ showed greater deviation from the L titration curve than Zn ²⁺ and Mn ²⁺ system indicated that Cu ²⁺ have greater interaction with the ligand which is resulting better stability of the chelate complexes later. Figure 3.										

12 Titration curve of the system with metal to ligand ratio 1:2.

, Ligand system; , Cu²⁺-ligand system; , Zn²⁺-ligand system; , Mn²⁺-ligand system; The stability constant of the divalent metal ions (Cu²⁺, Zn²⁺, and Mn²⁺) with pyrocatechuic acid was refined by Hyperquad2008. Several possible metal complex species were introduce to the program, and the species those gave the best statistical fitting with the data was selected. It was found that [MLH₂], [MLH], [ML], and [ML₂] species is

present in the system. The stability constant value was presented in $\log\beta_{pqr}$ as shown in Table 2. The species distribution of each system in

2 metal to ligand ratio of 1:2,

was graphically presented in Fig. 3. The $\log\beta_{pqr}$ show that Cu^{2+} complex is stronger than Zn^{2+} and Mn^{2+} . Observed from ML species the $\log\beta_{110}$ value for Cu^{2+} is 15.00, Zn^{2+} $\log\beta_{110} = 10.48$ and Mn^{2+} $\log\beta_{110} = 8.41$. This order is in an agreement with Irving William series where $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Mn}^{2+}$ [12]. The $\log\beta$ value is increasing with the increasing of atomic number, where the atomic number of Mn^{2+} is 25, Cu^{2+} is 29, and Zn^{2+} is 30. Cu^{2+} have higher stability even the atomic number is smaller than Zn^{2+} due to the distortion correlated to Jahn-Teller effect which always occurs in Cu^{2+} complexes. Table 2. Stability constants of metal complexes at

6I=0.15 mol·dm⁻³ NaCl and T= 37 °C Species p q r

$\log K$ [CuLH₂]⁺ $\log\beta_{pqr}$ 1 1 2 25.94±0.03 [CuLH] 1 1 1 21.24±0.01 [CuL]⁻ 1 1 0 15.00±0.02 15.00 [CuL₂]⁴⁻ 1 2 0 22.09±0.09 7.09 [ZnLH₂]⁺ [ZnLH] [ZnL]⁻ [ZnL₂]⁴⁻ 1 1 1 1 1 1 1 2 2 1 0 0 25.82±0.09 18.30±0.06 10.48±0.03 16.52±0.08 10.48 6.04 [MnLH₂]⁺ [MnLH] [MnL]⁻ [MnL₂]⁴⁻ 1 1 1 1 1 1 2 2 1 0 0 25.68±0.09 17.26±0.05 8.41±0.03 14.42±0.08 8.41 6.01 In the Cu^{2+} system, as shown in species distribution in Fig. 3(a), the CuLH_2 species were formed in initial acidic pH 2.5. Thus, this species were formed from the binding with COOH group, where at this pH only COOH group is available at deprotonated donor atom. The formation of CuLH , CuL were take place in lower pH than ZnLH and MnLH , indicated that the interaction of the ligand with Cu^{2+} is stronger than with Zn^{2+} and Mn^{2+} . CuL_2 species were formed in quite similar pH with ZnL_2 and MnL_2 . In the Zn^{2+} system and Mn^{2+} system as shown in Fig. 3(b) and (c), the species distribution are quite similar. The species MLH_2 is the initial species that occurred in the system. This species is formed by the binding of O donor atom from COOH group, since the formation is take place in acidic pH 2.5. The next species, MLH species is started to observe in higher pH, where this species is formed from the binding with deprotonated HL ligand. The addition of NaOH cause the pH increase to basic which give suitable environment for ligand to easily deprotonated to a free ligand L (all the donor atoms are deprotonated). This free ligand L binds the metal ion and forms the ML species. The ML_2 species is formed due

8 to the higher concentration of the ligand than the metal ion. Thus, the

available metal ion have been bonded to form ML species, hence the excess of L further bind the metal ion and form ML_2 species. In addition, the calculated $\log K$ value in Table 2 for ML ($\log K_1$) and ML_2 ($\log K_2$) gives the information of how strong the ligand bind with the metal ion. Where, $\log K_1 = \log\beta_{110}$ and $\log K_2 = \log\beta_{120} - \log\beta_{110}$. In all system, $\log K_1$ value is higher than $\log K_2$, indicated that the interaction of the second ligand (same species) to form ML_2 is weaker than the first ligand. However, overallly the stability of ML_2 complex is higher than (a) ML. The lower $\log K_2$ value also indicated that the ML_2 complex is formed; in which this will resulting in negative $\Delta\log K$. Where, $\Delta\log K = \log K_2 - \log K_1$. (b) (c) Figure 4. Species distribution of (a) Cobalt (b) Nickel and (c) Copper system C. Spectrophotometric Measurement Spectrophotometric measurements were done to qualitatively observe the shifting of the metal-ligand system spectrums. The

spectrums correspond to the d-d transition were measured in wavelength range of 400-800 nm as shown in Fig. 5. In Cu²⁺ (d9) system, the species CuLH were formed in acidic pH and caused higher intensity in the spectrum compared to Co²⁺ and Ni²⁺ system. The formation of CuL and CuL₂ were gave higher intensity. The band transitions at pH 11.0 were observed at wavelength 452 nm and 721 nm which corresponding to 2A_{1g}→2B_{1g} and 2E_g→2B_{1g} respectively. In Zn²⁺ (d10) system, intensely low intensity spectrums were observed. This is due to Zn²⁺ does not have orbital transition, since as d10 atom the electrons have filled all the orbital. In Mn²⁺ (d5) system, at low pH 3.0-6.0 the spectrum intensity is lower. Where at this pH the species formed is MnLH₂ species. The species MnLH, MnL and MnL₂ give higher intensity which observed in higher pH start from pH 7.0. The higher spectrum observed at pH 11.0 the, in this pH the bands are observed at wavelength 661 nm, correspond to the transition of 6A_{1g}(S)→4T_{1g}(g). (a) (b) (c) Figure 5. Spectrum of the (a) Copper, (b) Zinc and (c) Manganese system

CONCLUSION Potentiometric measurement was used for the determination of stability constant between pyrocatechuic acid and divalent metal ion (Cu²⁺, Zn²⁺, and Mn²⁺). The measurements were done in aqueous solution with

3 constant ionic strength of 0.15 mol·dm⁻³ NaCl and temperature of 37 °C. The

ligand pyrocatechuic acid is the tribasic (H₃L) ligand which has three active donor groups. The first group is OH at ortho position with logK_{a1} value 13.00, second is OH at meta position with logK_{a2} of 9.98 and third is COOH group with logK_{a3} value 2.63. The stability constants were decrease in order of Cu²⁺ > Zn²⁺ > Mn²⁺, where Cu²⁺ complexes are the most stable. This order is affected by the atomic number of the metal ion and also Jahn-Taller distortion. The complexes observed in the form of MLH₂, MLH, ML and ML₂ species. The formation of the metal complexes causes a shifting in the spectrum, where the greater intensity of the spectrum was observed in pH 11.0. REFERENCES [1] F. Borges, C. Guimaraes, J. L. F. Lima, and L. Pinto, "Potentiometric studies on the complexation of copper(II) by phenolic acids as discrete ligand models of humic substances," *Talanta*, vol. 66, pp. 670-673, Jan. 2005. [2] A. L. R. Merce, I. H. Z. Spir, M. J. O. Salmon, R. A. Giannoni, and A. S. Mangrich, "Model Compounds of Humic Acid and Oxovanadium Cations. Potentiometric Titration and EPR Spectroscopy Studies," *J. Braz. Chem. Soc.*, vol. 10, pp. 463-468, March 1999. [3] S. George, P. J. Benny, S. Kuriakose, C. George, and S. Gopalakrishnan, "Antiprotozoal activity of 2,3-dihydroxybenzoic acid isolated from the fruit extracts of *Flacourtia inermis* Robx.," *Asian. J. Pharm. Clin. Res.*, vol. 3, pp. 237-241, Jan. 2011. [4] M. Grootveld and B. Halliwell, "2,3-Dihydroxybenzoic acid is a product of human aspirin metabolism," *Biochem. Pharmacol.*, vol. 37, pp. 271-280, Jan. 1988. [5] [6] J. H. Duffus, "Heavy Metals-A Meaningless Term," *Pure Appl. Chem.*, vol. 74, pp. 793-807, 2002. K. S. Kasprzak, F. W. Sunderman Jr., K. Salnikow, "Nickel carcinogenesis," *Mutat. Res. Fundam. Mol. Mech. Mutagen.*, vol. 533, pp. 67-97, Aug. 2003. [7] R. Lauwyers and D. Lison, "Health risks associated with cobalt exposure--an overview," *Sci. Total. Environ.*, vol. 150, pp. 1-6, Jun. 1994. [8] S. J. S. Flora and V. Pachauri, "Chelation in Metal Intoxication," *Int. J. Environ. Res. Public. Health*, vol. 7, pp. 2745-2788, Jun. 2010. [9] M. J. Kosnett, "Chelation for Heavy Metals (Arsenic, Lead, and Mercury): Protective or Perilous?," *Clin. Pharmacol. Ther.*, vol. 88, pp. 412-415, Jul. 2010. [10] P. Gans, A. Sabatini, and A. Vacca, "Investigation of equilibria in solution. Determination of equilibrium constants with the HYPERQUAD suite of programs," *Talanta*, vol. 43, pp. 1739-1753, Apr. 1996. [11] M. Aplincourt, A. Debras-Bee, C. Gerard, and R. P. Hugel, "Modelling of the interactions of metal cations with soil organic matter. I: Thermodynamic stability of copper (II) complexes with dihydroxybenzoic acids," *J. Chem. Res. (S)*, vol. 4, pp. 134-135, Jan. 1986. [12] H. Irving and R. J. P. Williams, "The stability of transition-metal complexes," *J. Chem. Soc.*, pp. 3192-3210, 1953. ???

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