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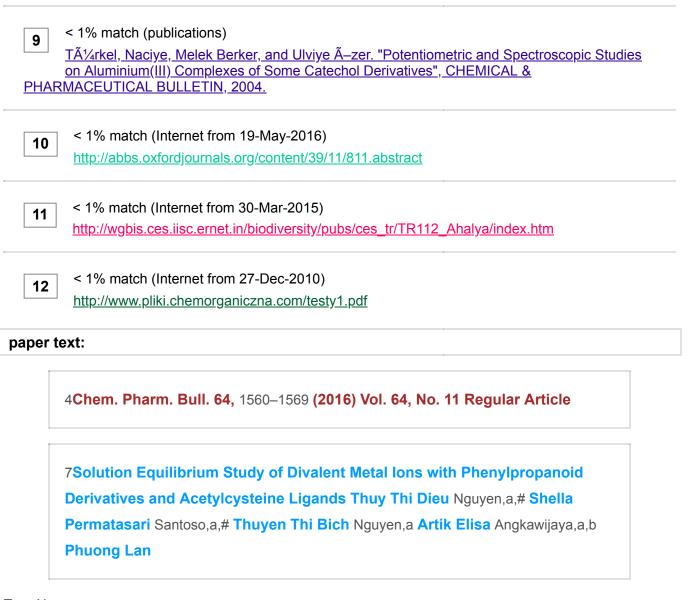
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4**Chem. Pharm. Bull. 64,** 1560–1569 **(2016) Vol. 64, No. 11 Regular Article**

7**Solution Equilibrium Study of Divalent Metal Ions with Phenylpropanoid Derivatives and Acetylcysteine Ligands Thuy Thi Dieu** Nguyen,a,# **Shella Permatasari** Santoso,a,# **Thuyen Thi Bich** Nguyen,a **Artik Elisa** Angkawijaya,a,b **Phuong Lan**

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2**Plant and Microbial Biology, Academia Sinica; Taipei 115–29, Taiwan,** R.O.C.: **and**

cDepartment of Mechanical Engineering, Can Tho University; 3–2 Street, Cantho City, Viet Nam. Received May 5, 2016; accepted July 28, 2016 Solution equilibrium of

11**divalent metal ions** (M **=Mn2+, Co2+, Ni2+, Cu2+** and **Zn2+)**

with caffeic acid (ligand C) or dihydrocaffeic acid (ligand D) in binary system, and with acetylcysteine (ligand N) in ter- nary system were investigated at condition similar to human physiological temperature of 310.15K and ionic strength of 0.15mol·dm−3 NaCl. Potentiometry technique was used for the determination of formation con- stant (logβ) assisted by spectrophotometry technique. The results indicated the formation of [ML], [MLH], [ML2], [ML2H] in binary species and [MLN], [MNLH], [MNLH2] in ternary species, where L represents ligands C or D. It was found that ligand D formed more stable complexes than that of ligand C, which were affected by the presence of double bond in the carboxylate moiety of ligand C. The speciation diagrams were simulated by HySS and discussed briefly, additionally the tendency of ternary complexes was evaluated from parameters ΔlogKM and logX. Key words solution equilibrium; phenylpropanoid complex; caffeic acid; dihydrocaffeic acid; metal complex; acetylcysteine Metal elements have been known capable of causing severe health problems for human.1) Redox-inert metals such as mer- cury (Hg), lead (Pb) and cadmium (Cd) are toxic agents, ex- posure to these and other essential metals may cause negative effects on health.2) Metals are known to be able to bind with proteins in human body which may lead to various detrimen- tal effects on human health. For instance, high exposure of zinc results in respiratory and gastrointestinal toxicity, clini- cal changes and copper deficiency.3) Excess amount of copper can cause serious diseases including cancers, liver and kidney damage.4) Cobalt exposure leads to ailment such as vision problem, lung problem, heart problem and thyroid damage. Exposure to manganese can cause disorders such as Parkin- son, schizophrenia and dullness. Although nickel appears to be essential for plants and bacteria, however for human this metal was found to be difficult to elucidate and may cause severe health problems such as cancer.5) Chelation therapy is known as an effective treatment to re- duce the harmful effects of metal ions by utilizing the metal binding ability of organic ligand.6–10) Therefore to prevent metal intoxication, the ligand used should have stronger bind- ing ability than the proteins. Phenylpropanoid derivatives, currently considered as promising chelating agents, are clas- sified as phenolic acids that are known to function as natural antioxidant, free radical scavenger, anti-carcinogenic, and chemoprevention agent.11–14) In this work, caffeic acid and dihydrocaffeic acid were chosen as the phenylpropanoid de- rivatives. Caffeic acid (denoted as ligand C) is an important anti-oxidative compound which can be extracted from natural sources such as coffee bean, potatoes, grains and vegetables.15) This compound has beneficial pharmacological effects such as inhibit cancer cell in human HT-1080 fibro sarcoma cell # These authors contributed equally to this work. line.16,17) Dihydrocaffeic acid (denoted as ligand D) is a deg- radation product of caffeic acid, this compound is one of the critical phenolic antioxidants commonly present in olives,18) and in blood or urine as the result of secondary metabolism of various polyphenols.19) The ortho-dihydroxyl structure of ligand D is also known to act as an active radical-scavenger.15) On the other hand, acetylcysteine is an organic compound which has been approved as one of the essential medicines for basic health system by World Health Organization (WHO).20) Acetylcysteine is known as a precursor for glutathione, the antioxidant which prevents formation of oxidative species in tissue.21,22) Thiol group of acetylcysteine can prevent sulfur depletion in body.23) This compound also has been reported as a potential chelating agent for some metals such as Mn2+, Ni2+, Co2+, Zn2+, Cu2+, Cd2+ and Hg2+.22,24,25) The mixed ligand combination of acetylcysteine and ligands C or D is expected to improve the stability of the chelate complex. Recently, the complexation of ligands C and D with metal ion has gained attention of some researchers.26–30) To the best of our knowledge, very few literatures are available for the complexation study of ligand D with essential divalent metal ions. Therefore, in order to gain primary understanding on the complexation of ligands C and D, potentiometry technique was conducted

5(at T= 310 .15 K and ionic strength I=0.15 mol· dm−3 NaCl) to investigate the complexation ability of the

ligands against

11**divalent metal ions (Mn2+, Co2+, Ni2+, Cu2+, Zn2+).** The specific temperature **and**

ionic strength were chosen to provide an environment similar to typical physiological condi- tion for human. In this study, the contribution of double bond on carboxylate side chain of ligand C in the formation of com- plex was also evaluated. The study was conducted in binary system and in ternary system (with acetylcysteine, denoted as ligand N). In addition,

3**UV-Vis spectrophotometry** measure- ment **was also performed to** study **the protonation** constants **of**

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9**To whom correspondence should be addressed. e-mail:** yhju@mail.ntust **.edu.** tw © 2016 The **Pharmaceutical Society of Japan**

ligands C and D and to confirm the stability constant values. Experimental Materials and Solution Analytical grade ligands, dihy- drocaffeic acid (C9H10O4, 98% purity) purchased from Alfa Aesar (Lancashire, U.K.), caffeic acid (C9H8O4, 99% purity) and acetylcysteine (C5H9NO3S, 99% purity) provided by Sig- ma-Aldrich (Steinheim, Germany), were directly used without further purification. Divalent metal salts were standardized against ethylenediaminetetraacetic acid (EDTA), their suppli- ers are as follows: zinc nitrate hexahydrate (Zn(NO3)2·6H2O, 98% purity)—Acros Organics (Morris Plains, NJ, U.S.A.),

2**nickel chloride hexahydrate (NiCl2·6H2O, 98% purity)—Alfa Aesar (Lancashire,**

U.K.), dihydrate cupric chloride salt (CuCl2·2H2O, 99% purity) and

2**cobalt nitrate hexahydrate (Co(NO3)2·6H2O, 98% purity)—Sigma-Aldrich**

(Steinheim, Germany), manganese chloride tetrahydrate (MnCl2·4H2O, 99.8% purity)—Fisher Scientific (Hampton, NH, U.S.A.). Prior to acidify the solutions, hydrochloric acid (HCl, 36.5%, Acros Organics) were prepared and standardized be- fore used. As the titrant, carbonate-free sodium hydroxide (NaOH, 96% purity, Yakuri Pure Chemicals, Kyoto, Japan) was prepared and standardized with potassium hydrogen phthalate (KHP, 99.85% purity, Sigma-Aldrich). The ionic strength was maintained constant by using sodium chlo- ride (NaCl, 99.5% purity, Showa, Tokyo, Japan). All stock solutions were freshly prepared in deionized (DI) water (>18.3MΩ·cm−1 resistance). Potentiometry Measurement Potentiometry measure- ment was

carried out using a Metrohm 888-Titrando Dosimat model 805, supported with an 802-rod stirrer, an 804 Ti stand and coupled with a combined Ecotrode Plus pH-glass elec- trode (4 decimals readability). The apparatus was connected to a personal computer and monitored by Tiamo 2.3 com- puter software. All experiments were carried out in a 150cm3 double walled glass reactor. All measurements were done in triplicate. Carbonate-free NaOH (0.1mol·dm−3) was used as the titrant against these following solutions: (a) 3×10−3mol·dm−3 HCl+1.5×10−2mol

6·**dm−3** NaCl. **(b) Solution (a)+0.** 01mol **·dm−3** ligands **C** or D. (c) **Solution (a)+(0.** 01–0.012 **)mol·dm−3**

ligands C or D+ (0.004–0.01)mol·dm−3 metal salt. The metal to ligand concentration ratios used are 1:1, 1:2, 1:2.5 and 1:3. (d) Solution

12**(a)+0.** 01mol **·dm−3** C or **0.** 01mol **·dm−3** D+ **0.01mol·dm−3** ligand N

+0.01mol·dm−3

metal salt, with metal to ligands ratio of 1:1:1. Since the potentiometry method involves the titration of strong acid and strong base therefore it was necessary to perform the electrode calibration in terms of hydrogen ion concentration by using the program GLEE. This program not only provides the electrode calibration constants but also the estimation of the carbonate contamination of the base. The potentiometry titrations were performed at T=310.15K and

8**I=0.15mol·dm−3 NaCl,** in this condition **the** self-dissociation constant **of**

water is pKw=13.384. Spectrophotometry Measurement UV-Vis spectropho- tometer Jasco V-550 was used to collect the spectrum data of ligands in the absence and presence of metal ions at the wavelength range of 200 to 800nm. The instrument uses a deuterium lamp at higher energies and halogen lamp at lower energies. The measured solution was put in a standard 10mm standard quartz cell. The concentration of the solutions used was one tenth of that used in potentiometry measurement. Data Analysis Hyperquad2008 was selected among sev- eral non-linear-square algorithm computer programs because of its simplicity and accuracy in determining the equilibrium constant,31) especially when several equilibrium reactions take place in the analyzed solution. The results of an equilibrium constant refinement may contain various pieces of information such as: a. The Overall Formation Constant (βpqrs) pM+qN+rL+sH ? MpNqLrHs, [MpNqLrHs]= βpqrs[M]p[N]q[L]r[H]s (1) where the stoichiometry coefficient p, q, r, s refer to metal ion, ligand N, ligands C or D and hydrogen atom, respectively. b. Standard Deviations Standard deviation is obtained by an error-propagation cal- culation from experimental errors, where the confident limit is ≤0.1. c. Goodness of Fitting (σ) The σ value is obtained from the Eq. 2: σ = i=1,np ? (Wiri2) (m − n) (2) where Wi, ri, m, n refer to the weight at the i-th data point, the residual at the i-th data point, the number of titration data points and the number of refined parameters, respectively. The σ value range is 1.17–1.35, which specifically represents 95% goodness of fitting. The spectrophotometry data were analyzed by using the Hypspec32) and the speciation diagrams were simulated by the HySS program.33) Structure Modeling Gaussian09W program with density functional theory (DFT), B3LYP and 6–31+G(d) basis set was used for the calculation

of Gibb's free energy. Structure opti- mization and frequency analysis were applied prior to obtain- ing the thermochemical properties of the complex species. Results and Discussion Protonation Constants Both ligand C and ligand D are tri-protic ligands with three functional groups viz. carbox- ylic group, meta- and para-hydroxyl groups. The

9**protonation constants of** the **ligands are** presented **in Table 1**

as minus logarithm of a protonation constant or dissociation constant (pKa). The obtained values of pKa1 and pKa2 are in good agree- Tl=ab0l.e151m.olT·dhme−p3KNaaCVlalues of Ligands C and D at T=310.15 K and System pKa±S.D. Reference Ligand C pKa1 (–COOH) 4.39±0.01a) 4.37c) pKa2 (para-OH) 8.55±0.01a) 8.55 pKa3 (meta-OH) 12.46±0.02b) 12.5 Ligand D pKa1 (–COOH) 4.55±0.01a) 4.45c) pKa2 (para-OH) 9.41±0.01a) 9.43 pKa3 (meta-OH) 13.65±0.02b) 13.7 a) Potentiometry technique. b) Spectrophotometry technique. c) Reference 38, po- tentiometry, T=298.15K, I=0.2mol·dm−3 KCl. ment with those reported in literatures.26–29,34–37) As shown in Fig. 1, the pKa1 of ligands C and D occurs in the carboxylic group, followed by the dissociation of hydroxyl group at the para-position (pKa2). This dissociation order is influenced by the inductive properties, π-electron delocalization and polariz- ability effects.28) As the two ligands have similar structure, it is expected that their pKa values should be similar. However, only pKa1 of the ligands showed similar values of 4.39 and 4.55 for ligand C and ligand D, respectively. The pKa2 values of ligands C and D (8.55 and 9.41, respectively) are significantly different indi- cates that the double bond on the carboxylate moiety in ligand C is leading to the electron-withdrawing effect. After losing its first proton on carboxylic group, ligand C has higher ability to rearrange electrons due to electron conjugation system in the structure and proton will be released at lower pH. The dissociation of hydroxyl group at meta-position seems to be affected by dipole effects, leading to the lower acidic property compared to para-position. As reported in litera- tures, pKa3 of meta-occurred at highly basic pH that is outside the range of reliable measurement by means of potentiometry. Thus, pKa3 value was determined by using the spectrophotom- etry technique. The pKa3 obtained for ligand C is 12.46 while for ligand D is 13.65. Evidently from the spectrum of ligand C in Fig. S1(a), at pH 3.0, the ligand possesses three peaks spe- cifically double bands located at 300 and 328 nm (attributed to n–π* bonding) and a single band at 230 nm (attributed to π–π* bonding). The deprotonation from carboxylic group (pH ca. Fig. 1. Proposed Stepwise Dissociation of Ligand C (Top) and Ligand D (Bottom) Table 2. Logβ of Ligands C and D Binary Complexes at T=310.15K and I=0.15mol·dm−3 NaCl Log β±S.D.a) Species Ligand C Ligand D Pot Spec Ref. Pot Spec Ref. MnL MnLH MnL2 MnL2H 7.87±0.02 16.03±0.03 13.8±0.06 24.76±0.02 (σ=1.18)b) 8.13±0.01 — — — 9.28±0.02 17.47±0.07 16.20±0.07 27.15±0.02 (σ=1.23) 9.17±0.05 16.00±0.06 17.97±0.01 27.53±0.04 CoL CoLH CoL2 CoL2H 8.31±0.01 16.12±0.02 13.82±0.04 24.68±0.02 (σ=1.18) 8.26±0.01 16.09±0.02 13.76±0.03 — 9.56±0.01 17.71±0.02 16.41±0.04 26.59±0.04 (σ=1.24) 9.24±0.02 18.25±0.03 — — NiL NiLH NiL2 NiL2H 8.85±0.01 15.57±0.04 13.97±0.02 23.3±0.02 (σ=1.17) 8.21±0.02 — 13.69±0.07 23.19±0.02 9.59±0.01 17.48±0.03 17.52±0.07 27.34±0.03 (σ=1.29) 9.31±0.02 17.61±0.02 16.35±0.03 — ZnL ZnLH ZnL2 ZnL2H 9.61±0.01 15.51±0.02 17.47±0.01 25.09±0.05 (σ=1.17) 9.57±0.03 16.89±0.03 17.65±0.04 — 10.58±0.01 17.72±0.03 20.55±0.01 29.71±0.03 (σ=1.24) 10.36±0.01 — 19.96±0.05 — CuL CuLH CuL2 CuL2H 13.14±0.01 19.00±0.01 23.32±0.01 29.87±0.05 (σ=1.17) 13.14±0.02 19.16±0.02 23.67±0.03 — 13.05c) 22.38c) 13.93±0.03 20.73±0.02 25.20±0.03 32.86±0.04 (σ=1.17) 14.17±0.02 22.09±0.01 25.32±0.08 — 14.1d) a) Logβ obtained from potentiometry−Pot and spectrophotometry−Spec. b) Goodness of data fitting in potentiometry measurement. c) Reference 28, potentiometry, T=298.15K, I=0.1mol·dm−3 NaCl. d) Reference 38, spectrophotometry, T=298.15K, I=0.2mol·dm−3 KCl. 5.0) caused a hypsochromic shift of double bands to 292 and 322 nm due to the electron rearrangement along the benzene ring and double

bond in the carboxylic moiety. Subsequently, a bathochromic shift at pH ca. 9.0 caused by the deprotonated of para-hydroxyl group led to the relocation of double band to 302 and 347 nm. Finally, at pH ca. 13.0 where the fully depro- tonated ligand was formed, the double bands transformed into a single band at 276 nm. In the case of ligand D in Fig. S1(b), the deprotonation of its carboxyl (pH ca. 5.0) group only caused a slight shift in the spectrum. Shift in the spectra started more significantly as the second deprotonation occurred. In the beginning ligand D has two peaks at 210 nm with a shoulder at 227 nm (attributed to π–π* bonding) and 288 nm (attributed to n–π* bonding). As pH was gradually increased to 9, the peak at 288 nm disap- peared. At pH 13.0, the fully deprotonated ligand was formed and caused bathochromic shift at 220 and 292 nm. Complex Formation in Binary System Potentiometry and spectrophotometry measurements were used for the deter- mination of complex stability constants (log β). The titration curves of ligand C or ligand D binary system at a metal to ligand ratio of 1 : 2.5 are presented in Fig. S2. It is noticeable that as the titrant was added, the metal–ligand curve shifted to lower pH compared to the curve of ligand only indicating the formation of metal complex. The largest shifting was observed in Cu2+ system indicated that Cu2+ formed the strongest inter- action with the ligands. According to the refinement, various complex species, not only the deprotonated metal–ligand species [MLn] but also the protonated species [MLnHn], were formed. The log β values of binary complexes of ligands C and D are presented in Table 2. It was found that Cu2+ formed the most stable complexes with both ligand C and ligand D. Stability constants of metal ions decrease

3in the following order: Cu2+>Zn2+>Ni2+>Co2+> Mn2+,

which is also supported by the titration curves. The log β values of ligand C complexes are lower than that of ligand D complexes, which are affected by the electron with- drawing effect of double bond in the carboxylic moiety of ligand C. Figures 2 and 3 are presented the speciation diagrams for binary systems of ligand C and ligand D, representatively by Cu2+ and Zn2+. It is obvious that free metal ion decreased with increasing pH indicating that metal ion formed complex with ligand. In the system involving ligand C, free Cu2+ was the earliest to disappear (at pH 6.5) among all the metals indicating that Cu2+ exhibited very strong interaction with ligand C. [CuC] species was formed in more acidic pH (4.5) while [ZnC], [NiC], [CoC] and [MnC] started to form at pH 6.0, 6.5, 7.0 and 7.0, respectively. [CuC2] species was also formed in more acidic pH (6.0) compared to [ZnC2], [NiC2], Fig. 2. Speciation Diagram for Binary System of Ligand C with (a) Cu2+ and (b) Zn2+ at

8T=310. 15K, I=0.15mol·dm−3 NaCl

and [M:L]=1:2.5 Fig. 3. Speciation Diagram for Binary System of Ligand D with (a) Cu2+ and (b) Zn2+ at

8T=310. 15K, I=0.15mol·dm−3 NaCl

and [M:L]=1:2.5 [CoC2] and [MnC2] which was formed pH 7.5, 9.0, 9.0 and 9.5, respectively. Similar results were also observed in ligand D systems. Cu2+ complexes were also started to form at lower pH value compared to other metals. Specifically at pH 5, 6.5, 7.2, 7.4 and 7.5 for [CuD], [ZnD], [NiD], [CoD] and [MnD], respec- tively; and at pH 6.5, 6.8, 7.3, 7.4 and 7.5 for [CuD2], [ZnD2], [NiD2], [CoD2] and [MnD2], respectively. The protonated complexes [MLH] were the first formed via binding with oxygen donor atom of

the carboxylic group. The [ML] species began to occur at more basic pH, showing that oxygen donor atom on the catecholate moiety took part in the complex formation. Similarly, [ML2H] species was also formed at less basic pH than [ML2] species. The H atom in [ML2H] species is more likely attributed to the protonated hydroxyl group at para-position. While donor atoms that were involved in the formation of [ML2] species in high basic pH are oxygen atoms on the catecholate moiety. These proposed coordination structures are supported by result of the Gibb's free energy calculation which is discussed in the next section. Double Bond Effect It was indicated that ligand C has lower pKas than ligand D. This is because of the presence of unsaturated carbon chain (C double bond) of ligand C which tends to be more reactive than the saturated carbon chain of ligand D. The reactivity of the unsaturated carbon makes the hydrogen atom of ligand C to dissociate faster than ligand D. Evidently from Gibbs free energy (ΔG), dissociation of ligand C is more spontaneous than ligand D where ΔG is more negative. The ΔG values of the dissociation ligand C are $\Delta G1=-0.4442$, $\Delta G2=-0.4546$, and $\Delta G3=-0.4887$, while for ligand D are $\Delta G1=-0.4414$, $\Delta G2=-0.4515$, and $\Delta G3=-0.4883$. Less positive ΔG value indicates more spontaneous reaction. The effect of bond on stability constant of metal–ligand species was analyzed with Cu^{2+} as the representative metal ion since Cu^{2+} complexes give the most stable species. The ΔG values for Cu^{2+} species with single ligand are summarized in Table 3. Ligand D species exhibit more negative value than ligand C species indicating that ligand D species are more stable. Ligands C and D have similar structure but ligand D species exhibits more negative ΔG value, this phenomenon is because of the double bond effect of ligand C. The calculation of ΔG can also be used to predict the structure of the species. In Table 3, the proposed model C is dedicated for ligand C while model D is for ligand D. The proposed structures are presented in Supplementary Fig. S3. [ML] species are indicated by model C1, C2, D1 and D2, where ΔG for species model C2 is more negative than C1, Table 3. Calculated Gibb's Free Energy (ΔG) of Cu^{2+} Species Model no. Species ΔG (Hartree/Particle) C1 CuC −0.1456 C2 CuCa) −0.1590 C3 CuCH −0.1020 C4 CuCHb) −0.0872 D1 CuD −0.1514 D2 CuDa) −0.1623 D3 CuDH −0.1078 D4 CuDHb) −0.0931 a) Species with metal ion bond at the two hydroxyl groups or catecholate binding type. b) Species with metal ion bond at para-hydroxyl group. likewise ΔG for species D2 is more negative than D1. This indicates that between the proposed possible structures, [ML] species are more likely to have the structures as shown by model C2 and D2. Meanwhile for [MLH] species, ΔG for model C3 is more negative than C4 and ΔG for D3 is more negative than D4. Thus metal ion in [MLH] species tends to bind via carboxyl group of the ligand. Sequestering Ability and Competition Diagram in Binary System Sequestering ability (pL0.5) refers to the minimum concentration of a ligand necessary to bind half of the metal available. Since pL is the minus logarithm of ligand concentration, thus a higher pL value implies a smaller ligand concentration. pL0.5 values were calculated at the physiological pH (7.4) by the following Eq. (3). $X = 1 / (1+10)(pL-pL0.5)$ and $X = ([M]total - [M]free - ? [M]other) / [M]total$ (3) where X is the molar fraction of the sum of formation percent- ages of all metal–ligand species. As can be seen in Table 4, ligand C possesses higher sequestering ability towards Mn^{2+}, Co^{2+} and Cu^{2+} than ligand D. However, in the case of Ni^{2+} and Zn^{2+}, ligand D is more potent than ligand C. The competition between ligand C and ligand D to bind metal ion was simulated and the results were presented in Fig. 4. It can be observed that ligand C and ligand D compete to bind metal ion. Ligand with stronger binding will result in more dominant species. For instance, in Cu^{2+} system ligand C shows stronger binding and results in more ligand C complex species than that of ligand D. The simulation on the competi- tion between ligand C and ligand D are supported the results on the sequestering ability of the ligands, where at physiologi- cal pH ligand C is more potent towards Mn^{2+}, Co^{2+} and Cu^{2+} and ligand D is more potent towards Ni^{2+} and Zn^{2+}. This potentiality can be observed from the percentage of species formed at pH 7.4 shown in Table 5. The percent species for- mations of Mn^{2+}, Co^{2+} and Cu^{2+} against ligand C are larger than that against ligand D, while for Zn^{2+} and Ni^{2+} their per- cent species formations against ligand D are larger than that against ligand C as can be seen clearly from species [ML2H]. est percent formation at pH 7.4 except for Cu^{2+}

system. For In all systems, [ML2H] species is shown to have the high- Cu2+ system, [MC2] species started to form at lower basic pH (compare to other metal systems) indicating that Cu2+ is the most reactive towards the ligand. This reactivity is also supported by the titration curves (Fig. S2) where Cu2+ system exhibits the largest pH shift. The reactivity may trigger the dissociation

5**of the ligand and** also **the formation of** deproton- ated **species.**

Table 4. The pL0.5 Values of Ligand C and Ligand D against the Diva- lent Metal Ions at pH 7.4 Metal ion pL0.5 Ligand C Ligand D Mn2+ 2.66 Co2+ 2.73 Ni2+ 2.73 Zn2+ 3.61 Cu2+ 6.94 1.97 2.10 3.32 3.95 5.79 Fig. 4. Competition Diagram for Binary System of Ligands C and D with (a) Cu2+ and (b) Zn2+. Table 5. Percentage of Species Formed in the Competition between Ligands C and D at pH 7.4 M % Formation relative to M MC MCH MC2 MC2H MD MDH MD2 MD2H Mn2+ 0.29 Co2+ 1.08 Ni2+ 17.36 Zn2+ 1.07 Cu2+ 0.12 1.65 0.02 2.78 0.03 3.63 0.41 0.03 15.09 0.003 76.29 83.20 0.19 86.00 0.57 35.31 0.96 25.05 0.07 10.78 0.02 1.17 0.004 3.23 0.01 2.96 0.15 0.04 1.00 0.01 4.53 13.45 6.22 39.09 57.65 8.25 The percentage values were calculated based on log β from potentiometry data. Table 6. Logβ of Ternary Complexes

5**in Aqueous Solution at T=** 310 **.15K and I=0.**

15mol·dm−3 NaCl Log β±S.D.a) Species M=Mn2+ M=Co2+ M=Ni2+ M=Zn2+ M=Cu2+ Pot Spec Pot Spec Pot Spec Pot Spec Pot Spec Ligand C [MCN] [MCNH] [MCNH2] σb) Log KMMCCN Log KMMNCNc) Δ log KM Log X Ligand D [MDN] [MDNH] [MDNH2] σb) Log KMDN Log KMMNDNc) Δ log KM Log X 11.26±0.02 11.30±0.02 11.86±0.05 12.30±0.03 12.56±0.02 12.97±0.08 15.20±0.01 15.55±0.03 17.79±0.03 18.13±0.01 20.99±0.01 20.74±0.05 21.82±0.03 21.19±0.02 21.93±0.02 21.10±0.03 22.93±0.01 23.41±0.02 26.43±0.03 27.49±0.01 — — — — — — — — 32.60±0.03 — 1.17 1.24 1.18 1.35 1.17 3.39 3.17 3.55 3.99 3.71 4.76 5.59 5.98 4.65 4.99 7.62 — 7.58 — 7.70 — 8.97 — 11.15 — −0.25 −0.73 −1.15 −0.64 −1.99 1.25 1.93 1.93 0.86 −0.44 12.02±0.02 12.94±0.06 13.22±0.03 13.11±0.07 13.86±0.02 13.55±0.01 15.72±0.01 15.96±0.01 18.96±0.03 19.07±0.01 21.66±0.02 22.67±0.01 23.16±0.03 23.62±0.02 23.26±0.02 — 24.03±0.02 24.74±0.01 27.90±0.03 28.35±0.01 — — — — — — — — 34.26±0.04 — 1.22 1.24 1.21 1.35 1.17 2.74 3.77 3.66 3.87 4.27 4.24 5.14 5.60 5.03 4.90 8.38 — 8.94 — 9.00 — 9.49 — 12.32 — −0.9 −0.62 −0.59 −1.09 −1.61 0.37 2.06 1.98 0.22 0.02 tion of log KMMNCN and log KMMNDN are 3.64, 4.28, 4.86, 6.23 and 6.64 for M=Mn2+, Co2+, Ni2+, Zn2+ and Cu2+, respectively.22) a) Log β obtained from potentiometry−Pot and spectrophotometry−Spec. b) Goodness of potentiometry data fitting using Hyperquad. c) Values of log βMN in the determina- Complex Formation in Ternary System Ternary systems containing ligands C or D and ligand N were studied. The log β values are presented in Table 6. The stability constant of ternary complexes

3**decreases in the following order** of **Cu2+> Zn2+>Ni2+>Co2+>Mn2+,** where **the**

log β for [MCN] species are 17.79, 15.2, 12.56, 11.86 and 11.26, while for [MDN] spe- Fig. 5. Speciation Diagram for Ternary System of Ligand C and Ligand N with (a) Cu2+ and (b) Zn2+ at T=310.15K and I=0.15mol·dm−3 NaCl Fig. 6. Speciation Diagram for Ternary System of Ligand D and Ligand N with (a) Cu2+ and (b) Zn2+ at T=310.15K and I=0.15mol·dm−3 NaCl cies are 18.96, 15.72, 13.86, 13.22 and 12.02, for Cu2+, Zn2+, Ni2+, Co2+ and Mn2+ system, respectively. This trend is also indicated in the titration

curves of ternary system (Fig. S4), where the largest shift which indicates the most stable com- plex is exhibited by Cu2+ followed by Zn2+, Ni2+, Co2+ and Mn2+. This trend is similar to the binary system. It is also noted that the stability constant values of ligand D system are higher than that of ligand C system. The speciation diagram of ternary systems in Figs. 5 and 6 representatively by Cu2+ and Zn2+, indicated that binary species of ligand N formed at lower pH than that of binary species of ligands C and D, thus ligand N was the primary ligand in these systems. In the formation of [MLNH2] species, the H atoms are originated from para- and meta-hydroxyl group of ligands C or D, while H atom in [MLNH] species is originated from meta-hydroxyl group. The H atoms were not originated from ligand N, since the formation of protonated [MN] species was earlier than protonated [MLH] species. Thus suggested that in the time [MLNH2] and [MLNH] species are formed, all of H atoms of ligand N was already deprotonated. As for [MLN] species, it is formed through the binding of catecholate moiety of either ligands C or D along with carboxyl and thiol groups of ligand N. Stepwise complexation constant, log KMMLLN and log KMMLNN, were calculated prior to examining the stability of each single ligand attachment in the formation of ternary complex, the values are shown in Table 6. In all ternary systems log KMMLLN value is lower than log βMN, likewise log KMMNLN value is lower than log βML. This suggested that attachment of each single ligand in ternary complex is not as stable as in binary com- plex. Such phenomenon is often encountered by bulky or large molecules. Atoms of a large molecule are close to each other thus induce steric effect between their electron clouds and cause decrease in the stability of the molecule.39) The relative stability Δ log KM was employed to explain the tendency of ternary complexes relative to that of binary com- plexes, which was calculated using Eq. 4. Δ log KM = log KMMLLN − log KMN = log KMMLNN − log KML (4) where log KMLN indicated the relative stability of [MLN] over [MN] species and log KMMNLN indicated the relative stability of [MLN] over [ML] species, L is denoted for ligands C or D. Positive Δ log KM indicates that ternary complex formation is more favorable than that of binary complex. Another parame- ter to measure the tendency to form either one mole of binary complexes [ML2] or two moles of ternary complex [MLN] is Fig. 7. Spectra of Binary Complex between Cu2+ against (a) Ligand C and (b) Ligand D Fig. 8. Spectra of Ternary Complex of Cu2+ with (a) Ligand C–N and (b) Ligand D–N presented as disproportionate constant logX. ML2+MN2 ↔ 2MLN , X = [MLN]2 / ([ML2][MN2]) (5) Δ log KM and log X values are shown in Table 6. Gener- ally, Δ log KM indicates the trend opposite to that of stability constant value. Complex with higher stability constant shows more negative Δ log KM. It suggests that if a metal ion formed a highly stable binary complex with the first ligand, more energy is needed by the second ligand to participate and form ternary complex. Nevertheless, after ternary complex is formed, it has the stronger stability than that of binary system. Positive value was observed for log X in almost all systems, suggested that the formation of ternary complex with two dif- ferent ligands (MCN or MDN) is more favorable than binary complex with two same ligands (MC2, MD2 or MN2). How- ever in the Cu2+ system, the logX was found to be negative which is probably because Cu2+ forms a very stable square planar complex with ligand C or D at their catecholate moiety and tends to form complex with two same ligands. Spectrophotometry Measurement Since the obtained spectra for Mn2+, Co2+, Ni2+, Zn2+ and Cu2+ are similar for both binary and ternary systems, thus only Cu2+ systems were chosen for discussion. From Fig. 7(a) for Cu–C system, it can be seen that in the beginning (pH 2.5) the spectrum for binary complex of ligand C and Cu2+ has double bands at 306 and 329nm, a peak at 228nm and a shoulder at 247nm. Based on the speciation diagram, at pH 5 [CuCH] was the domi- nant species, which led to bathochromic shift of the double bands to 293 and 320nm, and also caused the disappearance of shoulder at 247nm. Subsequently, the main species at pH 7 was [CuC], which caused the disappearance of the double bands and the occurrence of a new peak at 270nm. Then, the species [CuC2] which was dominant at pH 9 onward, led to a hypsochromic shift of the double bands to 310 and 360nm. From Fig. 7(b) for Cu–D system, shifting started at pH 6 due to [CuD] species. The formation of this species also caused the disappearance of the shoulder at 227nm, while the other peaks at 211 and 286nm did not experience

significant chang- es. At pH 9 onward, the dominant species [CuD2] initiated the occurrence of two shoulders at 244 and 303nm. In Fig. 8(a) for ternary system of Cu–C–N, in the beginning pH of 2.5 the first double band occurred at 209 and 224nm, the second double band occurred at 306 and 329nm and a shoulder occurred at 247nm. As the formation of [CuCNH2] species at pH ca. 5, the second double band hypsochromi- cally shifted to 293 and 320nm and the shoulder band dis- appeared. Then at pH 10, the formation of [CuCN] species caused a bathochromic shift on the double band to 310 and 359nm. Similarly for Cu–D–N in Fig. 8(b), at pH 2.5 there are two peaks at 210 and 285nm with a shoulder at 226nm. As [CuDNH] was formed at pH 8, the shoulder disappeared and a new peak at 285nm occurred. Subsequently at pH 10 when [CuDN] species was formed, two new shoulders peak at 245 and 277nm appears. Conclusion The complex equilibrium involving ligand C, ligand D, ligand N and

> 3**divalent metal ions (Cu2+, Zn2+, Ni2+, Co2+, Mn2+)**

> 6**at T=** 310 **.15 K and I=0.15 mol·dm−3**

NaCl were de- termined by means of potentiometry and spectrophotometry techniques. The double bond on the carboxylate moiety of ligand C gives electron-withdrawing effect to the system thus the formed complexes is less stable than the complexes of ligand D. This double bond effect also affected the formed ter- nary complexes where the ternary complexes of ligand C were also less stable than that of ligand D. In ternary complex, the major factor that influences the stability of the complexes is the steric effect between atoms of the ligands. Since ternary complex is bulkier than binary complex, the steric effect is higher and causes the decrease in stability constant. This effect is indicated by the lower log KMMNLN compared to log βML, and lower log KMMLLN compared to log βMN. The Δ log KM value of a ternary complex was found to be negative indicating that high energy was needed for ligands to form a ternary complex. The positive value of log X suggests that the formation of a ternary complex with two different ligands was more favorable than that of a binary complex with two same ligands. Calculated sequestering abil- ity (pL0.5) and the competition diagram indicate that ligand C is more potent in binding Mn2+, Co2+ and Cu2+ while for Ni2+ and Zn2+ ligand D is found to be more potent. Acknowledgments

> 4**Conflict of Interest The authors declare no conflict of** interest.
> **Supplementary Materials The online version of this ar- ticle contains supplementary materials.**

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