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12016 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved. 1. Introduction

Synthesis of chelate complex drugs is one of the solutions to deal with pathogenic microbes which are becoming more and more drug resistance [1,2]. Bioactive organic ligands such as amino acids, phenolic acids and alkaloids coupled with essential metals are frequently employed in chelate complex synthesis [3–6]. 2, 3-Dihydroxybenzoic acid (Dhba) is an effective phenolic compound not only with regard to its biological ability such as antioxidant, antibacterial and anti-inflammatory [7,8], but also to its

11ability to form stable chelate complexes with various metal ions

[9–11]. Therefore, Dhba was chosen as the ligand for the synthesis of metal–ligand binary complexes in this study. Ternary chelate complexes involving mixed ligands (Dhba and N-acetylcysteine (Nac)) were also synthesized. Nac was chosen as the consort ligand since it has been known to play an important role as a precursor of glutathione, an important antioxidant, and also known as potential * Corresponding author at:

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27376644. E-mail address: yhju@mail.ntust.edu.tw (Y.-H. Ju). sulfur replenishment for living organisms [12,13]. Moreover, Nac can form coordination compounds with metal ions [11,14,15]. In this study, binary and ternary complexes including Dhba, Nac and

14some divalent metal ions such as Mn²⁺, Co²⁺, Ni²⁺, Cu²⁺ and Zn²⁺ were synthesized and characterized. The

chelate complexes were initially characterized by FTIR and UV–vis spectroscopy analyses. Scavenging activity and antibacterial activity of the complexes were examined. Subsequently, binary and ternary complexes with high biological activity were further studied using elemental analysis, ¹H NMR and thermogravimetric analysis; their thermodynamic properties were also determined. 2. Experimental 2.1. Materials and instruments Copper chloride dihydrate (CuCl₂·2H₂O, 99% purity), cobalt

19nitrate hexahydrate (Co(NO₃)₂·6H₂O, 98% purity),

zinc

19nitrate hexahydrate (Zn(NO₃)₂·6H₂O, 98% purity),

Dhba (C₇H₆O₄, 99% purity) and Nac (C₅H₉NO₃S, 99% purity) were purchased from Sigma–Aldrich (St. Louis, MO); nickel chloride hexahydrate (NiCl₂·6H₂O, 98% purity) was obtained from Alfa Aesar (Lancashire, UK); manganese chloride tetrahydrate (MnCl₂·4H₂O, 99.8% purity) was supplied by Fisher Scientific (Bridgewater, NJ).

1http://dx.doi.org/10.1016/j.jtice.2016.08.003 1876-1070/© 2016 Taiwan Institute of Chemical Engineers. Published by Elsevier B.V. All rights reserved.

Sodium hydroxide (NaOH, 96% purity) was supplied by Yakuri Chemical (Kyoto, Japan). Ethanol (C₂H₆O, 95% purity) was provided by Echo Chemical (Miaoli, Taiwan).

10All chemicals were analytical grade and were used without further purification. N, C, S and H

analysis was performed using an Elementar Vario EL Cube analyzer (Hanau, Germany). Metal contents (Mn, Co, Ni, Cu, Zn and Na) of the complexes were determined by an ICP-AES JY 2000-2. The commercial

ICP metal standard solution (1000 mg/l of metal in 0.5 mol/l HNO₃) was used as the calibrant. Chloride content was determined by using an ion chromatograph Dionex ICS-1000. FTIR spectra were recorded with a Bio-Rad FTS-3500 on KBr disc with spectra range of 400–4000 cm⁻¹. UV–vis spectra of the complexes in H₂O solvent were measured in the wavelength range of 200–500 nm by using a Jasco V-550 spectrophotometer. Conductivities of the complexes in water were measured using a Knick Konduktometer 703. Magnetic susceptibilities of powder samples at 300 K were measured using a MPMS7 Quantum Design SQUID Magnetometer.

71H NMR spectra were measured on a Bruker AVIII-600 MHz FT-NMR

in D₂O solution. Thermogravimetric analyses in the range of 30 to 900 °C were examined by using a Perkin Elmer Diamond TG/DTA. Thermodynamic properties in 0.15 mol/dm³ NaCl ionic medium at various temperatures were measured potentiometrically under N₂ atmosphere by using a Metrohm 888 Titrand potentiometer with EcoTrode Plus pH glass electrode. 2.2. Synthesis of binary and ternary complexes Binary complexes were synthesized by dissolving Dhba (0.16 g, 1 mmol) in 14 ml H₂O with the addition of 1 ml 95% ethanol. The ligand was allowed to dissolve by slowly adding a few drops of ±5 M NaOH solution until pH 11.0 was reached, NaOH solution was directly used without standardization. Five milliliters of 1 mmol metal salt solution (0.17 g CuCl₂·2H₂O; 0.30 g Zn(NO₃)₂·6H₂O; 0.24 g NiCl₂·6H₂O; 0.30 g Co(NO₃)₂·6H₂O; or 0.20 g MnCl₂·4H₂O)

15 was added into the solution. The pH again was adjusted to 11.0 by using ±5 M NaOH. The mixture was allowed to react for

6 h with constant stirring. Any solid in the solution was removed by filtration. The solution was freeze-dried in -40 °C and then subjected to lyophilization. The dried complex

2 was washed with ethanol and dried in a 50 °C oven for 4 h,

then stored in a desiccator. Ternary complexes were synthesized similarly to that of binary complexes with the addition of Nac (0.16 g, 1 mmol). 2.3. Scavenging activity Scavenging activity of complexes was tested against the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [16]. The tested complex was prepared at concentrations of 1 and 2 µg/ml. The complex was firstly dissolved in water, then methanol was added

3 at a water to methanol ratio of 1: 19 (v/v).

DPPH solution was prepared at a concentration of 5 × 10⁻⁴ mol/l in aqueous solution with the same water to methanol ratio. DPPH solution (0.2 ml) was added to the prepared complex (0.8 ml). The tested complexes incubated at 37 °C for 30 min and its absorbance was measured at 517 nm against methanol blank. DPPH (0.2 ml) in methanol (0.8 ml) was used as the control. Percent DPPH inhibition

13 was calculated as: % DPPH inhibition = $\frac{[Ac - As]}{Ac} \times 100$ (1) where Ac and As are the absorbance of control and the absorbance of

sample, respectively. Ascorbic acid was used as the positive controls. 2.4. Antimicrobial activity The antimicrobial potency of the complexes was tested against

12 gram positive Staphylococcus aureus and gram negative Escherichia coli.

Broth macrodilution method was used for the inhibitory activity determination [17]. Ampicillin (895.5 µg/ml potency) was used as the reference. The complex was prepared at four concentrations (100, 600, 1000 and 2000 µg/ml). The assay was performed in test tubes each containing the tested compound dissolved in Lysogeny Broth media. Fifteen microliters of the prepared bacteria suspension (1 × 10⁸ cfu/ml) were injected into each tube. After incubated for 24 h at 37 °C, optical density at 600 nm wavelength (OD_{600nm}) of each sample was measured. As the growth control, 15 µl of bacteria suspension was injected into test tube containing no sample. The antimicrobial activity is expressed as %inhibition, calculated as: % inhibition = $\frac{[Ic - Is]}{Ic}$

18 100 × (2) where Ic is the absorbance of control and Is is the absorbance of sample. 2.5.

Thermodynamic properties Thermodynamic properties were determined potentiometrically by titrating 50 cm⁻³ mixture of metal salts (0.001 mol/dm³) and 0.001–0.003 mol/dm³ of Dhba and/or Nac. The mixture was acid-ified to pH 2.5 by adding 0.003 mol/dm³ HCl. The ionic strength maintained by adding 0.15 mol/dm³ NaCl. The mixture was titrated by 0.1 mol/dm³ carbonate free-NaOH under N₂ atmosphere until pH 11.0 was reached. Stability constant of binary complex was de-termined at

11 metal to ligand ratios of 1:1, 1:2 and 1:3

while ternary complex was determined from metal to ligand ratio of 1:1:1. Sta- bility constants were determined at 25, 45 and 55 °C. Analyses of the titration data were done by using Hyperquad2008 [18]. 3. Results and discussion Metal–ligand complexes with deprotonated species as core (specifically [M(Dhba)]⁻ and [M(Dhba)(Nac)]³⁻, for binary and ternary species respectively) were synthesized at pH 11.0. This pH was chosen for the synthesis reaction since at this pH value core species were found to exist in the highest concentration, as shown in the distribution diagram in Supplementary Data Fig. S1 for Zn²⁺ systems. The synthesized complexes were then characterized by FTIR and UV–vis spectra analyses. DPPH scavenging and antimi- crobial activities of complexes were evaluated. The results of ele- mental analysis, conductivity and magnetic measurements of Zn²⁺ binary and ternary complexes are presented in this section while the results of other metal ions complexes are summarized in the supplementary data (Table S1). 3.1. FTIR spectra 3.1.1. Binary complexes The characteristic main bands in

7IR spectra of the ligands and binary complexes are listed in Table 1, while the IR spectra are presented in the

supplementary data (Figs. S2 and S3). In the ab- sence of metal ions, Dhba possessed bands at 3240 cm⁻¹ and 3048 cm⁻¹ corresponding to $\nu(\text{OH})$ of hydroxyl and carboxyl group, re- spectively. The IR spectrum of the free Dhba reveals

2a band at 1680 cm⁻¹ due to $\nu(\text{C}=\text{O})$ and $\nu(\text{C}-$

OH) of protonated carboxyl group, respectively. The band at 1566 cm⁻¹ is attributed to the $\nu(\text{C}=\text{C})$ of Dhba aromatic ring, in the complexes this band is found at 1530– 1548 cm⁻¹. Several new bands can be found in the metal complex system. The first is the

2 $\nu(\text{OH})$ band at 3478–3471 cm⁻¹ and

837– 811

2cm⁻¹ regions are due to coordinated water

molecule [6,19]. Two new bands at 1618–1617

12cm⁻¹ and 1473–1431 cm⁻¹ are con- tributed to $\nu(\text{COO}^-)$ asy and $\nu(\text{COO}^-)$ sym

of deprotonated carboxylic group, ν of 145–187 cm⁻¹ suggests that this group is unbounded. S.P. Santoso

17et al. / Journal of the Taiwan Institute of Chemical Engineers 68 (2016) 23– 30 25 Table 1 Selected IR spectra for the

synthesized binary and ternary complexes. Complex IR spectra (cm⁻¹) Binary $\nu(\text{OH})$

16 $\nu(\text{C}=\text{O})$ $\nu(\text{COO}^-)$ $\nu(\text{C}=\text{C})$ $\nu(\text{C}-\text{OH})$ $\nu(\text{MO})$ other Ternary $\nu(\text{NH})$ $\nu(\text{OH})$ $\nu(\text{SH})$ $\nu(\text{C}=\text{O})$ $\nu(\text{COO}^-)$ $\nu(\text{C}=\text{C})$ $\nu(\text{C}-\text{OH})$ $\nu(\text{MO})$

MO) Other Ligand Dhba Nac Complex of Copper Zinc Nickel Cobalt Manganese 3240 1680 3048 – – 3478 – 811 3471 – 820 3477 – 837 3478 – 834 3478 – 813 – 1566 – – 1618 1451 1618 1445 1617 1445 1618 1473 1618 1431 1548 1537 1533 1530 1530 1259 – – – 1216 477 1192 476 1196 476 1191 476 1218 478 – – – 3376 796 620 755 1384 746 1222 621 745 1384 758 1251 621 746 3240 3048 2810 – 2548 3449 806 3385 810 3373 834 3352 826 3371 813 – – – – 1680 – 1718 – – 1548 1440 – 1627 1501 – 1628 1502 – 1615

1471 – 1659 1492 1566 1259 – 1229 1548 1217 1573 1214 1565 1228 1580 1218 1566 1220 – – – – 516
588 457 1384 1254 458 599 457 1384 1258 457 597 The

2ν(C–OH) band at 1191–1218 cm⁻¹ is due to ethanol. The

complex of Cu²⁺, Ni²⁺ and Mn²⁺ possessed band at 621–620 cm⁻¹ indicating the ionic Cl⁻; while for Zn²⁺ and Co²⁺ the bands at 1384 and 1251–1222 cm⁻¹ indicate the coordinated NO₃⁻ in monodentate mode [20]. The ionic groups of Cl⁻ and NO₃⁻ were originated from the type of metal salts used. The new band appeared at 477, 476, 476, 476 and 478

2cm⁻¹ are attributed to ν(

MO) for Cu²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺, respectively. 3.1.2. Ternary complexes The IR absorption bands of the ternary complexes are listed in Table 1 as well as binary complexes (the full spectra are presented in supplementary data Fig. S4). The ligand Nac ν(NH) stretching and bending vibration was observed at 3376 and 796 cm⁻¹, respectively. The stretch of ν(OH), ν(C=O) and ν(CO) bond from protonated carboxylate group produced band at 2810 cm⁻¹, 1718 cm⁻¹ and 1229 cm⁻¹ respectively. The thiol ν(SH) frequency of Nac was characterized at 2548 cm⁻¹, while in the complex this band disappeared, evidencing that this group was involved in chelation after deprotonation. The synthesized ternary complexes inherited the main spectra from both Dhba and Nac. The ν(OH) at 3449–3352

2cm⁻¹ and band at 834–806 cm⁻¹

in all metal systems indicate the coordinated water molecule [6,19]. The bending of ν(NH) at 758–745 cm⁻¹ was still visible in the complexes, while the stretching of ν(NH) at higher region cannot be observed since the band is probably stacked with the ν(OH) band. Two bands at 1659–1548 cm⁻¹ and 1502–1440

6cm⁻¹ are contributed to ν(COO⁻)_{asy} and ν(COO⁻)_{sym} of the deprotonated carboxylic group,

ν=108–167 cm⁻¹ indicating the monodentate binding of the carboxylic group [6,19]. The

2ν(C–OH) band at 1214–1228 cm⁻¹ is due to ethanol. The

ionic group Cl⁻ originated from metal salts appeared at 599–588 cm⁻¹ for Cu²⁺, Ni²⁺ and Mn²⁺, while coordinated NO₃⁻ in monodentate mode appeared at 1384 and 1258–1254 cm⁻¹ for Zn²⁺ and Co²⁺ [20]. The band which characterized the bond of ligand to metal, ν(MO) was observed at 516, 457, 458, 457 and 457 cm⁻¹ for Cu²⁺, Zn²⁺, Ni²⁺, Co²⁺ and Mn²⁺, respectively. 3.2. UV–vis spectra, conductivity and magnetic properties Absorption spectra of the synthesized binary and ternary complexes are shown in Fig. 1. The complexes show two absorption bands at 214–230 nm and 314–360 nm which correspond

6to π–π* and n–π* bonding of the

benzene ring [21]. In binary and ternary cobalt complexes, n–π* bonding was observed at longer wavelength (370–395 nm), while for manganese complexes the absorbance of this bonding was low and cannot be observed. The absorption bands for d-orbitals of the metals cannot be observed since usually these spectra have low absorbance. In particular for ternary complex of Co²⁺, the spectrum corresponding to d-orbital is observed at 499 nm which is attributed to the transition 4A_{2g}(F)→4T_{1g}(P), indicating its octahedral geometry [22]. Magnetic susceptibilities (μ_{eff}) of the binary and ternary complexes were measured at 300 K and are summarized in Table S1. Low μ_{eff} values (0.69–1.00 BM for binary and 0.62–1.75 BM for ternary complexes) suggest that the complexes only have one unpaired electron, and the complexes exhibit weak paramagnetic behavior. In Zn²⁺ binary and ternary complexes, the results of μ_{eff} indicate diamagnetic behavior of the complexes. Meanwhile, Co²⁺ ternary complex exhibits higher μ_{eff} (2.38 BM) than other ternary metal complexes, this value is often observed for Co²⁺ complex with octahedral geometry and is also supported by the occurrence of the band at 499 nm in UV–vis spectra. The molar conductivity (Λ_m) measurements gave values of 153–179 S cm²/mol for binary complexes, while for ternary complexes the values were 217–242 S cm²/mol (Table S1). Λ_m of a complex indicates the electrolyte nature of the complex. The electrolyte behavior of a complex might be due to the presence of Na⁺ ions which were involved in the formation of the complex. Higher Λ_m of a ternary complex is because it contains more Na⁺ ions than a binary complex. 3.3. Biological activities 3.3.1. DPPH scavenging activity Scavenging activity of the complexes at concentrations of 1 and 2 mg/l were evaluated against DPPH. The tests for scavenging activity were done in triplicate. The results are summarized as the average value, and the

difference between the three tests was indicated by the error bars, as shown in Fig. 2. The synthesized binary and ternary complexes did not show any increase in DPPH inhibition compared to Dhba. The possible reason is that originally Dhba inhibits DPPH by transferring H atom from —COO and meta —O group [23] while Nac inhibits DPPH by transferring H atom from —COO group [24]; in the formed complex H atoms of ligand which were originally inhibiting free radical formation disappeared thus inhibition activity actually decreased. Ternary complexes have higher inhibition activity than binary complexes. The enhancement in activity is due to the addition of Fig. 1. Spectra of the binary and ternary complexes at concentration of 187.5 mg/l. Fig. 2. DPPH scavenging activity of the complexes, *AA-ascorbic acid as the standard reference. Nac, where amine group of Nac also contributes to bonding with DPPH through weak hydrogen bond. However for ternary complex of Cu²⁺, the inhibition activity is lower than that of binary Cu²⁺ perhaps due to the reaction of Nac and Cu²⁺ which is known to produce free radical [25].

3.3.2. Antimicrobial activities of the synthesized complexes were evaluated against *E. coli* and *S. aureus*. The inhibition values are reported as Supplementary Data (Tables S2–S4). Dhba was shown to have better antimicrobial activity than Nac. For the synthesized complexes, some exhibited lower antimicrobial activity than that of Dhba. The complexes were found to be more effective in inhibiting *S. aureus*. It was found that in binary and ternary complexes, the complex of Zn possessed the highest inhibition activity against the growth of bacteria. Thus it is worthwhile to diagnose the efficacy of zinc complexes by determining the minimum inhibitory concentration (MIC) as shown in Table 2. The complexes of zinc gave higher inhibition activity than ligand alone (especially against *S. aureus*) as indicated by the smaller MIC values. The increase in bacterial growth inhibition of zinc complexes is perhaps due to the increase in solubility of the complexes. The solubility of Dhba in water is very low and becomes highly soluble in the form of chelate complex. The complexes were also found to be more effective to inhibit the growth of gram positive bacteria than gram negative bacteria. This is possibly due to difference in their cell walls [26,27]. As gram positive bacteria, *S. aureus* cell wall consists of thick peptidoglycan layer and plasma membrane while gram negative bacteria *E. coli* cell wall possesses an extra outer membrane layer which is high in lipid [28]. This lipid S.P. Santoso

1et al. / Journal of the Taiwan Institute of Chemical Engineers 68 (2016) 23–30
27 Fig. 3. Structure of ligand Dhba (left) and

Nac (right). Table 2 MICa (µg/ml) of the tested compounds. Compound *E. coli* MIC50 MIC90 *S. aureus* MIC50 MIC90 Ampicilin Dhba Nac Binary zinc Ternary zinc 4.1 49.1 589.8 1085.7 1293.9 >2000 603.5 867.0 773.9 971.6 0.3 702.0 >2000 149.3 347.1 2.6 932.6 >2000 383.8 554.8 MIC50 and MIC90 are the minimum concentration requirement to inhibit 50% and 90% growth of microorganism, respectively. Fig. 4. Proposed structure of zinc binary complex. containing layer lowers the permeability of the complex into the bacteria inner membrane and cause decrease in inhibition activity. 3.4. Complexes of zinc Since the complexes of Zn show higher biological activity than other metal complexes, only Zn complexes were chosen for further characterization including elemental analysis, ¹H NMR spectra, and thermogravimetric analysis. 3.4.1. Binary complex of zinc The binary complex of Zn has the chemical formula C₉H₁₁NNa₂O₉Zn or Na₂[Zn(NO₃)(C₇H₃O₄)(H₂O)]·C₂H₆O, mw=388.54. It is dark green-brown in color. Elemental analysis on the complex indicated its composition as follows: C, 27.89; H, 2.84; N, 3.69; Na, 11.80; Zn, 16.52% while the calculated (theoretical) composition is: C, 27.82; H, 2.85; N, 3.61; Na, 11.83; Zn, 16.83%. The ¹H NMR signals of Dhba (Fig. 3) were found at δH 7.47 (d, 1H, H-1, J=6.5Hz), 7.16 (d, 1H, H-2, J=6.4Hz), 6.88 (t, 1H, H-3, J=8.0). The binary complex signals were shifted to lower values specifically δH 7.12 (s,

81H, H-1), 6.82 (s, 1H, H-2), 6.61 (s, 1H, H-

3), followed by new signals at δH 3.69 (q, 2H, CH₂, J=7.1Hz), 1.22 (t, 3H, CH₃, J=7.1Hz). The ¹H NMR signals are presented in Table 3, while the spectrums are presented in Figs. S5 and S6. The new ¹H NMR signals in the complex were observed which corresponds to ethanol molecule. The attachment of ethanol molecule may also cause the shift in ν(OH) IR spectra at 3471 cm⁻¹. As indicated in the elemental analysis, Na⁺ molecules were involved in the formation of complex. Na⁺ molecules seem to help neutralizing the charge of the complex, which originally has a negative charge of 2, thus isolation of the complex in solid phase is possible. Result of elemental analysis suggests that there was one N atom corresponding to NO₃ involved in complex formation. Thus the proposed structure of zinc binary complex is shown in Fig. 4. 3.4.2. Ternary complex of zinc Ternary complex was found with chemical formula C₁₄H₁₈N₂Na₄O₁₂SzN or Na₄[Zn(NO₃)(C₇H₃O₄)(C₅H₇NO₃S)(H₂O)]·C₂H₆O, mw=595.70. The complex has a dark pale-brown color. Elemental analysis found the composition as: C, 28.46; H, 3.06; N, 4.72; S, 5.22; Na, 15.31; Zn, 11.01% while the calculated (theoretical) one is: C, 28.23; H, 3.05; N, 4.70; S, 5.38; Na, 15.44; Zn, 10.98%. More complicated ¹H NMR signals were observed in ternary complex. ¹H NMR signals for Dhba were the same as those in the binary complex, while for Nac (Fig. 3) the signals observed are δH 4.66 (q, 1H, CH, J=4.9Hz), 3.02 (sep, 2H, CH₂, J=9.4Hz), 2.11 (s, 3H, CH₃). ¹H NMR signals for zinc ternary complex (Table 5) are δH 7.12–7.03 (m, 1H, H-1), 6.78 (br, 1H, H-2), 6.69–6.59 (m, 1H, H-3), 4.53 (q, 1H, CH Nac, J=4.1Hz), 3.69 (q, 2H, CH₂ ethanol, J=7.1Hz), 3.02–2.99 (dd, 2H, CH₂ Nac, J=8.9Hz), 1.60 (s, 3H, CH₃ Nac), 1.22 (t, 3H, CH₃ ethanol, J=7.1Hz). The ¹H NMR spectrums of Nac and ternary complex are presented in Figs. S5 and S6. Similarly in Zn ternary complex, Na⁺ molecules also helped neutralizing the

charge of the complex, which originally has a negative charge of 4. Elemental analysis result also suggests the involvement of NO₃ in complex formation. Thus the structure of zinc ternary complex can be proposed as shown in Fig. 5. 3.4.3. Thermogravimetric analyses Thermogravimetric analyses of Zn complexes were conducted in the temperature range of 30–900 °C. The thermograms are provided in Supplementary Data (Figs. S5). In Zn binary complex, the initial mass loss which is observed until 200 °C corresponds to loss of water and ethanol. Big mass loss at 280–340 °C corresponds to the decomposition of Dhba, thus residue at above 450 °C corresponds to zinc-oxide. For Zn ternary complex, the initial mass loss which was observed until 140 °C corresponds to loss of water and ethanol. Mass loss at 145–550 °C corresponds to the decomposition of ligand Nac along with Dhba and zinc-oxide residue was left at temperatures above 550 °C. Table 3 Selected ¹H NMR data of zinc binary and ternary complex. Proton chemical shifts (assignment)^b

Ligand	Zn binary complex	Zn ternary complex
	7.47 (d, 1H, H-1 Dhba)	7.16 (d, 1H, H-2 Dhba)
	6.88 (t, 1H, H-3 Dhba)	4.66 (q, 1H, CH Nac)
	3.02 (sep, 2H, CH ₂ Nac)	2.11 (s, 3H, CH ₃ Nac)
	7.12 (s, 3.69 (q, 2H, CH ₂) 1.22 (t, 3H, CH ₃) 7.12–7.03 6.78 6.69–6.59 4.53 3.69 3.02–2.99 1.60 1.22 (m, 1H, H-1 Dhba) (br, 1H, H-2 Dhba) (m, 1H, H-3 Dhba) (q, 1H, CH Nac) (q, 2H, CH ₂ ethanol) (dd, 2H, CH ₂ Nac) (s, 3H, CH ₃ Nac) (t, 3H, CH ₃ ethanol) a Proton chemical shift (δ) in ppm unit. b Signal assignment in parenthesis represents the splitting pattern, number of proton, proton location in the compound geometry and splitting distance. Splitting pattern abbreviations: s =	

81H, H-1) 6.82 (s, 1H, H- 2) 6.61 (s, 1H, H-

3) 3.69 (q, 2H, CH₂) 1.22 (t, 3H, CH₃) 7.12–7.03 6.78 6.69–6.59 4.53 3.69 3.02–2.99 1.60 1.22 (m, 1H, H-1 Dhba) (br, 1H, H-2 Dhba) (m, 1H, H-3 Dhba) (q, 1H, CH Nac) (q, 2H, CH₂ ethanol) (dd, 2H, CH₂ Nac) (s, 3H, CH₃ Nac) (t, 3H, CH₃ ethanol) a Proton chemical shift (δ) in ppm unit. b Signal assignment in parenthesis represents the splitting pattern, number of proton, proton location in the compound geometry and splitting distance. Splitting pattern abbreviations: s =

20singlet, d = doublet, t = triplet, q = quartet, sep = septet, dd = doublet of doublets, br = broad,

m = multiplet (complex pattern). Table 4 Equilibrium constants at various temperatures and I of 0.15 mol/l NaCl. Parameters Equilibrium constants at different temperatures 25 °C 37 °C 45 °C 55 °C Dhba Nac Dhba Nac Dhba Nac Dissociation constant pKa1 2.68 (2) 3.17 (3) pKa2 10.11 (2) 9.60 (2) pKa3 ndc – σ d 1.13 1.06 Binary–stability constant log₁₀ β₁ 10.55 (3) 6.53 (7) log₁₀ β₂ 16.53 (4) 12.11 (2) σ d 1.08 1.34 Ternary–stability constant log₁₀ β_T 16.59 (8) σ d 1.56 ?log₁₀ K –0.49 log₁₀ X 4.54 2.63 (4) 3.18 (2) 9.98 (2) 9.48 (5) 13.00 (2) – 1.02 1.02 10.48 (4) 6.23 (8) 16.52 (9) 11.97 (8) 1.48 1.41 16.38 (7) 1.55 –0.33 4.27 2.60 (3) 3.12 (2) 9.84 (4) 9.42 (2) 12.72 (1) – 1.05 1.26 10.25 (2) 6.19 (2) 16.51 (7) 11.98 (4) 1.40 1.21 16.02 (5) 1.61 –0.42 3.55 2.54 (1) 3.12 (1) 9.81 (4) 9.33 (2) 12.60 (2) – 1.23 1.09 10.02 (5) 6.05 (5) 16.11 (3) 11.91 (4) 1.11 1.19 15.51 (6) 1.69 –0.56 3.00 a Standard deviations in parentheses at last decimal place represent the standard uncertainties of temperature u(T)=0.1°C. b Values cited from Refs. [11] and [15], potentiometry method at I=0.15 mol/l NaCl. c Not defined. d Sigma (σ), the goodness of fitting in a system with expectation value of 1.00. Fig. 5. Proposed structure of zinc ternary complex. 3.4.4. Effect of temperature on thermodynamic parameters The dissociation constant (pKa) of Dhba and Nac as well as the stability constant (log₁₀ β) of their binary and ternary complexes with Zn²⁺ in aqueous solution with I = 0.15 mol/l NaCl were determined at different temperatures (25 °C, 37 °C, 45 °C and 55 °C) and the results are presented in Table 4. Particularly for pKa3 of Dhba at 25 °C, a big error occurred during refinement thus resulting in undefined value. The error may be caused by the decrease of the acidity of the ligand Dhba at low temperature resulting in delay in dissociation. The change of acidity is well demonstrated in Table 4 since pKa value decreased as the temperature was increased. In order to observe the capability of the ligand in preventing the formation of metal hydrolytic species, the hydrolysis constants were included in the determination of log₁₀ β. No metal hydrolytic species were observed in the systems, indicating that the ligands were capable in preventing their formation (Fig. S1). The log₁₀ β of binary and ternary complexes also decrease with increasing temperature indicating that complex is more likely to disassociate at higher temperature. Additional parameters ?log₁₀ K and log₁₀ X were determined with respect to the ternary species, where their values are calculated as: ?log₁₀ K = log₁₀ β_{Zn(Dhba)(Nac)} – [log₁₀ β_{Zn(Dhba)} + log₁₀ β_{Zn(Nac)}] (3) log₁₀ X = 2log₁₀ β_{Zn(Dhba)(Nac)} – [log₁₀ β_{Zn(Dhba)}² + log₁₀ β_{Zn(Nac)}²] (4) Less negative ?log₁₀ K indicates the formation of ternary species is more favorable than that of binary species [6,21]. At all temperatures, the complexes exhibit negative ?log₁₀ K value S.P. Santoso et al. / Journal of the Taiwan Institute of Chemical Engineers 68 (2016) 23–30 29 Table 5 Thermodynamics properties of Dhba, Nac, binary and ternary complexes of Zn. Species –?G (kJ/mol) –?H (kJ/mol) ?S (J/K·mol) 25 °C 37 °C 45 °C 55 °C 25 °C 37 °C 45 °C 55 °C H₃Dhba H₂Dhba HDhba H₂Nac HNac Zn(Dhba) Zn(Dhba)₂ Zn(Nac) Zn(Nac)₂ Zn(Dhba)(Nac) 15.30 15.68 57.72 59.27 – 77.20 18.10 18.89 54.81 56.30 60.23 62.24 94.37 98.11 37.28 37.00 69.14 71.09 94.71 97.28 15.84 15.96 59.95 61.64 77.49 79.17 19.01 19.60 57.39 58.62 62.44 62.96 100.58 101.23 37.71 38.02 72.98 74.84 97.59 97.46 8.49 22.85 19.45 128.35 41.80 – 11.24 22.99 16.33 129.07 33.04 91.19 22.77 240.15 28.36 29.93 11.43 193.56 65.78 97.04 23.19 23.11 128.39 127.29 114.14 112.17 24.64 22.68 128.89 129.06 94.14 92.41 242.91 244.57 27.86 29.40 192.37 193.48 101.56 100.00 22.78 128.57 113.88 23.38 128.90 91.18 239.09 29.43 193.24 97.64 indicating that the formation of ternary complex is less preferred than binary complex. Such phenomenon probably occurred because of electrostatic effect between the ligands thus lowering the stability of the complex. Meanwhile, for log₁₀ X parameter, the value higher than statistical value (+0.6 for all geometries) indicates remarkable stability of ternary complex. All of the ternary complexes exhibit positive log₁₀ X greater than the statistical value. This point out that the secondary ligand (Dhba) prefers to attach at the binary complex Zn(Nac) rather than attach to metal ion. Nac is designated as the primary ligand and Dhba as the secondary ligand since

the formation of Nac binary complexes took place at lower pH than that of Dhba binary complexes. The $\log_{10} X$ decreases with increasing temperature, indicating that ternary complex is less stable at higher temperature. This trend is supported by the $\log_{10} \beta$ values which also decreased with increasing temperature. Thermodynamic properties such as ΔG (Gibbs free energy), ΔH (enthalpy) and ΔS (entropy) provides significant information related to the complex. ΔG can be calculated as: $\Delta G = -2.303 RT \log_{10} \beta$ (5) ΔH can be determined as the slope of the Van't Hoff plot which is defined as: $\log_{10} \beta = 2 - 3.703HR / T + 2 - 3.703SR$ (6) Thermodynamic properties of Dhba, Nac as well as the Zn binary and ternary complexes are given in Table 5. Negative values of ΔG and ΔH in the dissociation of the ligands and chelation of binary and ternary Zn complexes indicate that the reactions are spontaneous and exothermic, so that lower temperature is more favorable for complex synthesis. All ΔS values are positive indicating that complex formation is favorable.

4. Conclusion Binary and ternary complexes of M/Dhba/Nac were synthesized. FTIR spectra and UV-vis spectra indicate that all complexes have similar structure. The complexes did not increase the scavenging activity of Dhba against DPPH. However in the antimicrobial evaluations against *S. aureus* and *E. coli*, the complexes were found to be more effective than that of Dhba which is caused by the enhancement of hydrophobicity of the complexes. The binary and ternary complexes of Zn gave the best enhancement in antimicrobial activity of Dhba and were characterized further. The binary complex was found to bind metal ion through ortho and meta —O group, which has the molecular structure $C_9H_{11}NNa_2O_9Zn$. The ternary complex was found to bind metal ion through ortho and meta —O group of Dhba, and —COO and —S of Nac, which has the molecular structure $C_{14}H_{18}N_2Na_4O_{12}S_2Zn$. The formation of binary and ternary complex was found to be spontaneous and exothermic. Acknowledgments This study was supported by a project (MOST 103-2221-E-011-148) from the Ministry of Science and Technology, Taiwan. The authors thank Shang-Ming Tseng of Instrumentation Center, National Taiwan University for magnetic (MPMS7 Quantum Design SQUID Magnetometer) experiments.

5Supplementary materials Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jtice.2016.08.

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