

## SYNTHESIS AND ANALYSIS OF ZINC-METHIONINE, ZINC-TRYPTOPHAN, COPPER-LYSINE, AND COPPER-ISOLEUCINE COMPLEXES USING ATOMIC ABSORPTION SPECTROPHOTOMETRY

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### ABSTRACT

**Objective:** This study aimed to synthesize an organic form of Zn and Cu to then produce metal complexes with amino acids, as well as to analyze the free and bound metal content of the complexes.

**Methods:** In this study, the synthesis methods for zinc-methionine ( $Zn(Met)_2$ ), zinc-tryptophan ( $ZnTrp$ ), copper-lysine ( $Cu(Lys)_2$ ), and copper-isoleucine ( $Cu(Ile)_2$ ) complexes were based on previous studies. Sample verification was conducted using Fourier transform infrared spectroscopy, which can determine the stretched spectra produced by the complexes. In addition, atomic absorption spectrophotometry was used to analyze the metal content of the complexes.

**Results:** The yields gained from the synthesis of  $Zn(Met)_2$ ,  $ZnTrp$ ,  $Cu(Lys)_2$ , and  $Cu(Ile)_2$  complexes were 95.99%, 94.18%, 91.89%, and 95.73%, respectively, with a maximum metal content of 199.51, 246.98, 176.99, and 189.75 mg/g, respectively.

**Conclusion:** The mineral concentration of each complex differed, depending on the type of mineral and ligand.

**Keywords:** Atomic absorption spectrophotometry, Analysis, Copper, Fourier transform infrared, Synthesis, Zinc.

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### INTRODUCTION

Essential minerals are those that are required by the human body for physiological processes. They are divided into two groups: Macro and micro minerals (often referred to as trace minerals), where only a small amount of micro minerals are required and are found in various part of the body. Deficiencies in micro minerals in both humans and animal can cause diseases, whereas high doses can also cause toxicity [1].

Zinc (Zn) and copper (Cu) are essential micro minerals necessary for overall health. Zn has diverse biological functions, including catalytic, structural, and regulatory roles. The required intake of Zn from food and supplementation for both men and women is 25–32 mg/day. Primary clinical symptoms of Zn deficiency include growth, pregnancy, and immune system disorders [2]. The intake of Cu improves the functioning of skin, blood vessels, connective tissue, and epithelial tissue throughout the body. Symptoms of copper deficiency include hernia, aneurysm, ruptured blood vessels, and anemia.

Supplement products containing Zn and Cu metal complexes with amino acids are now available for both humans and animals. ZINPRO® manufactured by Zinpro Performance Minerals® comprises a Zn and methionine complex to help immune function, health, and reproduction of livestock. Source Naturals OptiZinc is a supplement that contains 30 mg of a Zn-monomethionine complex to maintain normal human growth and development. CuPLEX® is a Cu-lysine complex supplement by Zinpro Performance Minerals® aimed at enhancing immune function, reproduction, skin and nail integrity, metabolism, and bone development in animals. However, the availability of these products in Indonesia is still limited.

An adequate intake of micro minerals is difficult to obtain, whereby negative interactions between elements and dietary factors can affect the absorption of micro minerals in the small intestine. Thus, organic forms of these minerals have been used to increase their bioavailability by producing complex compounds or coordinate covalent bond with amino acids [3]. Zn and Cu chelated with amino acids showed an

increased concentration of the micro minerals in plasma, increased absorption in the intestine, and increased activity of Cu/Zn superoxide dismutase enzymes [4]. However, research on the analysis of the metal content bonded with amino acids and the free metal content that did not bind with amino acids after synthesis has not been conducted. To this end, this study aimed to synthesize an organic form of Zn and Cu to then produce metal complexes with amino acids, as well as to analyze the free and bound metal content of the complexes.

In this study, the synthesis of metal complexes with amino acids was based on previously published methods. Sample verification was performed using Fourier transform infrared spectroscopy (FTIR) because IR spectrum analysis determines the functional groups contained in the samples. Further, the difference in the IR spectrum between complex and standard amino acids indicated that complex formation occurred. Before comparing the concentrations of metal bonded to amino acids and free metal, it was necessary to perform a separation step. In this study, ion exchange chromatography with XAD-2 resin (Merck) for adsorption was for the separation step. The free metal content was then determined using atomic absorption spectrophotometry (AAS). AAS is a technique used to measure the concentration of a chemical element in samples by measuring the radiation absorbed by the chemical element to be tested [5-8].

### MATERIALS AND METHODS

#### Equipment

The following equipment was used in this study: An AAS (Shimadzu A-6300, Japan), Zn hollow cathode lamp (Hamamatsu Photonics K.K, Japan), Cu hollow cathode lamp (Hamamatsu Photonics K.K), mercury vaporizer unit (MVU-1A; Shimadzu, Japan), FTIR spectrophotometer (Shimadzu 8400S, Japan), and a centrifuge 5100 (Kubota, Japan).

#### Materials

L-tryptophan and L-lysine were purchased from CJ Cheil Jedang (Indonesia), L-methionine was purchased from CJ Cheil Jedang

(Malaysia), and L-isoleucine was purchased from Sigma-Aldrich. The following reagents were obtained from Merck: 1000 mg/Lof copper standard solution, zinc sulfate heptahydrate, zinc acetate dihydrate, copper (II) acetate, potassium bromide IR Grade, XAD-2 Resin, 67% nitric acid, sodium hydroxide, hydrochloric acid, ethanol, and acetone. Aquadestilata (aqua dest) was purchased from Brataco.

### Synthesis of complexes

#### Zinc-methionine ( $Zn(Met)_2$ )

First, 501.4 mg of methionine was dissolved in 4 mL of sodium hydroxide (1 mol/l) and stirred for 30 min at room temperature. Then, 502.1 mg zinc sulfate heptahydrate was added while stirring until a white precipitate was formed. Finally, the precipitate was filtered and dried in the oven at 100°C for 2 h.

#### Zinc-tryptophan ( $ZnTrp$ )

First, 111.5 mg of zinc acetate dihydrate was dissolved in 10 mL of ethanol mixed with 1 mL of aqua dest until a clear solution was formed. Then, 100.6 mg of tryptophan was dissolved in 20 mL of ethanol mixed with 2 mL of aqua dest and stirred until a clear solution was formed. Next, both clear solutions were mixed and heated on a hot plate with magnetic stirring at 95°C for 24 h and left until the solution reached the room temperature. The precipitate was centrifuged at 2000 rpm for 5 min, filtered, and dried in an oven at 60°C for 2 h.

#### Copper-lysine ( $Cu(Lys)_2$ )

First, 74.2 mg of lysine was dissolved in 20 mL of aqua dest and then 45.8 mg of copper (II) acetate was added until it dissolved. The solution was evaporated on a hotplate at 100°C until crystal formation occurred.

#### Copper-isoleucine ( $Cu(Ile)_2$ )

First, 66.0 mg of isoleucine was dissolved in 20 mL of aqua dest and then 46.6 mg of copper (II) acetate was added until it dissolved. The solution was evaporated on a hotplate at 100°C until crystal formation occurred.

### Determination of water content of complexes

Determination of water content of complexes was conducted using the drying shrinkage method. The formed complex was weighed and heated at 105°C for 2 h to be re-weighed. This process was repeated until the fixed weight was obtained.

### Verification of complexes formation

FTIR was used to verify the formation of complexes between the metals and amino acids. The complexes were identified by comparing the spectrum of free amino acids with those of the synthesized complex, whereby the resulting spectrum shift occurred when the metal and amino acid successfully formed a complex. Potassium bromide (100 mg) was used as a blank and for the formation of baseline.

### Identification of functional groups

For the identification of functional groups on both samples and the standard, 98 mg and 2 mg of each, respectively, were ground into a homogeneous powder using a mortar and pestle. The resulting powder was analyzed through FTIR, and absorption spectrum analysis was performed in the wavenumber range from 4000–400  $cm^{-1}$  (wavelengths 2.5–25  $\mu m$ ).

### Separation of free metals form complexes

The synthesized complexes contained not only bound metals but also free metals attached to the complexes. Therefore, a separation step was performed using an ion exchange chromatography column to calculate the free metal content in the synthesized products. Using Amberlite XAD-2 resin, the complex was retained on the resin, whereas the free metal was eluted. To prepare the column, 400 mg of Amberlite XAD-2 resin was washed with the following solutions (in order): Methanol, aqua dest, 1 mol/l of nitric acid in acetone, aqua dest, 1 mol sodium

hydroxide, and aqua dest. Washed resin was suspended in aqua dest and placed in the chromatography column. After completing the experiment, the column was washed and filled with aqua dest to be stored for the next experiment.

### Preparation of standard solution

#### Zn calibration curve

The calibration curve was made by pipetting 10 mL of 1000-mg/L Zn metal solution into a 100-mL volumetric flask until it reached the limit of 100-mg/L Zn solution. Next, 50 mL of 100-mg/L Zn standard solution was pipetted into a 500-mL volumetric flask, until it reached the limit of 10-mg/mL Zn solution. Then, 0.5, 1, 2, 5, 10, and 20 mL of the 10-mg/L Zn standard solution were each pipetted into a 100-mL volumetric flask until it reached the limit and the Zn metal concentrations of 0.05, 0.1, 0.2, 0.5, 1.0, and 2.0 mg/L were obtained.

#### Cu calibration curve

The calibration curve was made by pipetting 0.5 mL of 1000-mg/L Cu metal solution into a 50-mL volumetric flask until it reached the limit of 10 mg/L. Then, 0.5, 2, 4, 6, 8, and 10 mL of 10 mg/L Cu standard solution were each pipetted into a 50-mL volumetric flask until it reached the limit and the Cu metal concentrations of 0.1, 0.4, 0.8, 1.2, 1.6, and 2.0 mg/L were obtained.

### Sample preparation

#### $Zn(Met)_2$

To prepare newly synthesized  $Zn(Met)_2$  for analysis, 50.4 mg of  $Zn(Met)_2$  was dissolved in 5 mL of concentrated nitric acid and heated on a hot plate at 100°C until a clear solution was formed. The solution was then diluted 1:10,000 in aqua dest.

#### $ZnTrp$

For analysis, 23.8 mg of  $ZnTrp$  was dissolved in 5 mL of concentrated nitric acid and heated as above. The solution was then diluted 1:2,500 in aqua dest.

#### $Cu(Lys)_2$

For analysis, 29.4 mg of undissolved  $Cu(Lys)_2$  was dissolved in 5 mL of concentrated nitric acid and heated as above. The solution was then diluted 1:5.00 in aqua dest.

#### $Cu(Ile)_2$

For analysis, 26.3 mg of undissolved  $Cu(Ile)_2$  was dissolved in 5 mL of concentrated nitric acid and heated as above. The solution was then diluted 1:5.00 in aqua dest.

### Determination of content

The metal content of the complexes and eluates was determined using AAS; the analysis conditions are listed in Table 1. The absorption result of each sample was input into the regression equation of the calibration curve to determine the metal content.

## RESULTS AND DISCUSSION

### Synthesis of metal amino acid complexes

#### Synthesis of $Zn(Met)_2$

$Zn(Met)_2$  synthesis was performed by reacting methionine and zinc sulfate heptahydrate at the ratio of 2:1. Methionine was dissolved into 1 mol sodium hydroxide, which allowed  $H^+$  deprotonation in the OH group of methionine. With the addition of zinc sulfate heptahydrate to the solution, the  $Zn(Met)_2$  complex formed as a white precipitate. The yield obtained was 95.99%.0

#### Synthesis of $ZnTrp$

$ZnTrp$  synthesis was performed by dissolving zinc acetate dihydrate in a 10:1 (v/v) ethanol: aqua dest solution, whereas tryptophan was

**Table 1: Analysis conditions for content determination**

Conditions	Zn	Cu
Wavelength	213.9 nm	324.8 nm
Width of slit	1 nm	0.7 nm
Lamp current: Low	5 mA	6 mA
Lamp current: High	0 mA	0 mA
Height of burner	7 mm	7 mm
Gas flow rate	2 L/min	1.8 L/min
Air flow rate	15 L/min	15 L/min

dissolved in a 10:1 (v/v) ethanol: aqua dest solution. Both solutions were then mixed at a ratio of 1:1 and heated at 95°C for 24 h. At this stage, deprotonation of tryptophan by acetate compound occurred. Once the solution reached room temperature, ZnTrp formed as a white precipitate. The yield obtained was 94.18%.

#### Synthesis of Cu(Lys)<sub>2</sub>

Cu(Lys)<sub>2</sub> synthesis was performed by dissolving lysine in aqua dest; then, copper(II) acetate was added until it was dissolved at a ratio of 1:2. The solution was evaporated until the complex crystal formed. The yield obtained was 91.89%.

#### Synthesis of Cu(Ile)<sub>2</sub>

Cu(Ile)<sub>2</sub> synthesis was performed by dissolving isoleucine in aqua dest; then, copper(II) acetate was added until it dissolved at the ratio of 1:2. The solution was then evaporated until the complex crystal was formed. The yield obtained was 95.73%.

#### Determination of water content

The water content of the resulting complexes was determined by comparing the weight of the complexes before and after repeated heating steps to obtain the fixed weight. The water content of the complexes was calculated as 0.80% in Zn(Met)<sub>2</sub>, 1.48% in ZnTrp, 1.46% in Cu(Lys)<sub>2</sub>, and 1.81% in Cu(Ile)<sub>2</sub>.

#### Identification of complex functional groups

In the Zn(Met)<sub>2</sub> complex, spectra shifts were found at 3254 and 3273, 1604, 1423 and 1427, and 1244 cm<sup>-1</sup>, which correspond to the absorption of the NH<sub>2</sub>, COO<sup>-</sup> asymmetric, COO<sup>-</sup> symmetric, and S-CH groups. FTIR spectral absorption data were in accordance with research conducted by Mamun *et al.* [9], with the following data: 3210 and 3356 cm<sup>-1</sup>, N-H; 2916 cm<sup>-1</sup>, C-H; 1605 cm<sup>-1</sup>, C=O; 1425 cm<sup>-1</sup>, C-O; 1242 cm<sup>-1</sup>, and S-CH.

In the ZnTrp complex, spectra shifts were found at 3271 and 3261 cm<sup>-1</sup> and at 1600 and 1621 cm<sup>-1</sup>, which correspond to the absorption of the NH<sub>2</sub> and C=O groups, respectively. FTIR spectral absorption data were in accordance with research conducted by Min *et al.* [10], with the following data: 3392 and 3161 cm<sup>-1</sup>, N-H; 1622 cm<sup>-1</sup>, and C=O.

Spectral absorption of the Cu(Lys)<sub>2</sub> complex was found at 3249 and 3240 cm<sup>-1</sup> and 1663 and 1657 cm<sup>-1</sup>, corresponding to the absorption of the NH<sub>2</sub> and C=O groups, respectively. FTIR spectral absorption data were 3318 cm<sup>-1</sup>, NH<sub>2</sub>; 1640 cm<sup>-1</sup>, and C=O.

In the Cu(Ile)<sub>2</sub> complex, spectral shifts at 3313 and 3306 cm<sup>-1</sup>, 1594 and 1588 cm<sup>-1</sup>, and 1396 and 1390 cm<sup>-1</sup>, corresponding to the NH<sub>2</sub>, C=O, and C-O groups. FTIR spectral absorption data were: 3319 and 3283 cm<sup>-1</sup>, NH<sub>2</sub>; 1608 cm<sup>-1</sup>, C=O; 1567 cm<sup>-1</sup>, C-NH; 1470 cm<sup>-1</sup>, and C-O.

#### Sample preparation

Two samples from each complex were analyzed using AAS. The first sample was the formed complex, which was analyzed to obtain the total amount of both bonded and free metal present in the complex. The second sample was the column eluate analyzed to determine the free metal content.

For this, the samples containing formed complexes were digested with concentrated nitric acid, resulting in different clear, colored solutions for each sample, whereas the eluate samples were analyzed directly.

#### Analysis of metal content

According to the AAS analysis, the metal contents were as follows: Zn(Met)<sub>2</sub>, 199.51 mg/g with 52.50 mg/g as free metals; ZnTrp, 246.98 mg/g with 53.00 mg/g as free metals; Cu(Lys)<sub>2</sub>, 176.99 mg/g with 0.61 mg/g as free metals; and Cu(Ile)<sub>2</sub>, 189.75 mg/g with 1.24 mg/g as free metals. To determine the metal content bound to amino acids, the free metal content was deducted from the total metal content. Based on the calculation results, the content of metal-AA in Zn(Met)<sub>2</sub>, ZnTrp, Cu(Lys)<sub>2</sub>, and Cu(Ile)<sub>2</sub> complexes was 1110.5, 1018.3, 963.6, and 945.4 mg/g, respectively.

#### CONCLUSION

All complexes produced a good yield, whereby the yields of Zn(Met)<sub>2</sub>, ZnTrp, Cu(Lys)<sub>2</sub>, and Cu(Ile)<sub>2</sub> were 95.99%, 94.18%, 91.89%, and 95.73%, respectively. The calculated content of metal-AA in Zn(Met)<sub>2</sub>, ZnTrp, Cu(Lys)<sub>2</sub>, and Cu(Ile)<sub>2</sub> complexes was 1110.5, 1018.3, 963.6, and 945.4 mg/g, respectively. Therefore, the mineral concentration of each complex differed, depending on the type of mineral and ligand.

#### CONFLICTS OF INTEREST

All authors have none to declare.

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