Organic compounds of low molecular weight, such as amino acids and enhanced by producing mineral complexes that are bonded to soluble bioavailability [3]. However, the bioavailability of minerals can be increased by producing metal-amino acid complexes. The free forms of minerals and mineral salts typically have low bioavailability in themselves, they are necessary for the functioning of numerous physiological activities inside the human body. Essential minerals occur in many forms and have various functions. Indeed, micronutrient deficiencies such as those that occur with mineral deficiencies are a major public health problem, with infants and pregnant women particularly at risk [1].

Zinc (Zn) is an essential element to many biological processes inside the body. Zn plays a role in the immune system, wound healing, and protein and DNA synthesis [2]. In addition to Zn, copper (Cu) has important roles in the body, particularly for the blood and nervous system. Cu is considered necessary for bone formation, myelin sheaths in the nervous system, and the absorption of iron [1].

Deficiencies of essential minerals can cause various diseases or disorders. A Zn deficiency can lead to disorders of the immune, digestive, and integumentary system [2], while a Cu deficiency causes anemia, bone disorders, neonatal ataxia, depigmentation, and the abnormal growth of hair [1].

Mineral deficiencies can be caused by many different conditions such as: Not consuming enough of the foods that contain these essential minerals; diseases, such as diarrhea, that cause a massive loss of minerals; increased urine excretion due to diabetes; malabsorption; and an increased demand for minerals [2].

The free forms of minerals and mineral salts typically have low bioavailability [3]. However, the bioavailability of minerals can be enhanced by producing mineral complexes that are bonded to soluble organic compounds of low molecular weight, such as amino acids and peptides [4].

Previous studies have reported that making mineral-amino acid complexes enhances mineral bioavailability [5]. Furthermore, animal studies have also shown that the plasma concentrations of minerals are higher if administered in the organic form (i.e., as a metal-amino acid complex) than in the inorganic form [6].

Currently, mineral-amino acid complex supplements are widely available for both human and animal consumption. However, research regarding mineral-amino acid complexes has been limited in Indonesia. Existing research regarding mineral-amino acid complexes does not provide specific data regarding the concentration of free minerals and minerals bonded to the amino acids. Consequently, the aim of this study, conducted at the Central Drug, Food, and Cosmetic Quality Testing Laboratory of the Pharmacology faculty at the University of Indonesia (January–May 2017), was to perform metal-amino acid synthesis and to analyze the free and bonded mineral concentrations.

Complexes were synthesized using an existing method where metal-amino acid complexes were produced by synthesizing mineral salts with amino acids, followed by crystallization. The synthesized complexes were then analyzed using Fourier-transform infrared (FTIR) spectrophotometry to observe the movement of amino acid clusters before and after complex synthesis. Before determining the concentration of free and bonded minerals, separation was performed by ion exchange using adsorbent resin. Tokalogho et al. conducted the separation of metal-amino acid complexes using resin to separate the metal ions from the organic materials [7]. Free and bonded mineral concentrations were then determined using atomic absorption spectrophotometry (AAS).

METHODS

The tools utilized for this analysis included AAS (Shimadzu A-6300, Japan), FTIR (Shimadzu 8400), Zn Hollow Cathode Lamp (Hamamatsu Photonics K.K, Japan), Cu Hollow Cathode Lamp (Hamamatsu Photonics K.K, Japan), and inductively coupled plasma optical emission spectrometry (ICP OES) (Agilent 720, USA), and atomic absorption spectrophotometry (AAS) (Shimadzu A-6300, Japan). The respective yields of this synthesis process were 95.38%, 95.95%, 76.31%, and 93.91% for zinc (Zn)-methionine (Zn(Met)) complexes, respectively. The metal-amino acid complexes were then separated using column chromatography and further analyzed by atomic absorption spectrophotometry (AAS). The bonded metal concentrations of the Zn(Met)_2, Zn(gli)_2, Cu(leu)_2, and Cu(gli)_2 complexes were 189.32 mg/g, 353.78 mg/g, 180.89 mg/g, and 275.11 mg/g, respectively. The free metal concentrations of the Zn(Met), Zn(gli), Cu(leu), and Cu(gli) complexes were 1.357 mg/g, 12.92 mg/g, 0.19 mg/g, and 2.12 mg/g, respectively.
The various materials used included Zn and Cu in their free form (i.e., not bonded to other compounds) and Zn-methionine (Zn(Met)₂), Zn-glycine (Zn(gli)₂), Cu-leucine (Cu(leu)₂), and Cu-glycine (Cu(gli)₂) complex synthesis materials. The chemicals used in this study were obtained from Merck and included Zn standard solution 1000 mg/L, Cu standard solution 1000 mg/L, methionine, glycine, leucine, HNO₃ p.a, ZnSO₄.7H₂O, acetic acid, Zn(CH₃COOH)₂Cu(NO₃)₂.3H₂O, CuSO₄.5H₂O, NaOH, ethanol, HCl, methanol, and KBr.

**Calibration curve for Zn solution production**

The calibration curve for the Zn solution was made by pipetting 10 mL of 1000 mg/L Zn standard solution into a 100 mL volumetric flask until 100 mg/L of Zn solution was reached. Then, 50 mL of 100 mg/L Zn standard solution was pipetted into a 500 mL volumetric flask until 10 mg/mL of Zn solution was reached. Aliquots (0.5, 1.0, 2.0, 5.0, 10, and 20 mL) of 10 mg/L Zn standard solution were then pipetted into 100 mL volumetric flasks until 0.05 mg/L, 0.1 mg/L, 0.2 mg/L, 0.5 mg/L, 1.0 mg/L, and 2.0 mg/L of Zn metal concentrations, respectively, were reached.

**Calibration curve for Cu solution production**

The calibration curve for the Cu solution was made by pipetting 0.5 mL of 1000 mg/L Cu standard solution into a 50 mL volumetric flask until 10 mg/L of Cu solution was reached. Aliquots (0.5, 2.0, 4.0, 6.0, 8.0, and 10 mL) of 10 mg/L Cu standard solution were then pipetted into 50 mL volumetric flasks until 0.1 mg/L, 0.4 mg/L, 0.8 mg/L, 1.2 mg/L, 1.6 mg/L, and 2.0 mg/L of Cu metal concentrations, respectively, were reached.

**Synthesis of Zn(Met)₂ complexes**

To synthesize the Zn(Met)₂ complexes, 250 mg methionine (1.75 mmol) was diluted into 8 mL NaOH (1 mol/L) by stirring for 30 min. Zn sulfate heptahydrate (250 mg, 0.875 mmol) was then added while stirring until a white sediment was formed. This precipitation product was then filtered and dried under vacuum at 105°C for 2 h. The white sediment obtained represented the Zn(Met)₂ complex.

**Synthesis of Zn(gli)₂ complexes**

To synthesize the Zn(gli)₂ complexes, 150 mg glycine solution (2 mmol) in 5 mL distilled water was added into 220 mg Zn acetate solution (Zn(CH₃COOH)₂) (1 mmol) in 2 mL distilled water. The mixture was then heated at 100°C for 2 h while stirring. The solution was then evaporated at low temperature until a white sediment was produced and then dried.

**Synthesis of Cu(leu)₂ complexes**

To synthesize the Cu(leu)₂ complexes, 131 mg leucine (1 mmol) was diluted into 10 mL distilled water. Into that solution, we dropped 0.33 mL of 15% NaOH. The solution was then stirred for 20 min, followed by the addition of 125 mg CuSO₄.7H₂O solution (0.5 mmol) in 2 mL distilled water. The solution was then stirred for 1 h until a blue sediment was formed. The sediment was then filtered and dried. The light blue sediment powder obtained represented the Cu(leu)₂ complex.

**Synthesis of Cu(gli)₂ complexes**

First, 2 mmol glycine (150 mg) was diluted into 20 mL of distilled water until it was dissolved completely, followed by the addition of 0.33 mL of 30% NaOH. The solution was stirred for 20 min, followed by the addition of 1 mmol (241 mg) of Cu(NO₃)₂.3H₂O in 2 mL of distilled water. The mixture was then stirred for 1 h, after which ethanol was added while stirring until a sediment was formed. The sediment was then filtered. The blue sediment obtained represented the Cu(gli)₂ complex.

**Determination of water content**

Determination of water content of complexes was conducted using a drying shrinkage method. The formed complexes were weighed and placed in an oven at 105°C for 2 h. The dried complexes were then re-weighed until a stabilized weight was achieved.

**Complex formation identification using FTIR**

To identify the formation of the metal-amino acid complexes, FTIR spectrophotometry was used. Potassium Bromide (KBr) (100 mg dried powder) was used as the blank and baseline. Sample powder of the complexes (2 mg) was mixed with 98 mg KBr powder and crushed using a jade mortar and pestle until homogenous. The powder mixture was then put into a disc and set into the FTIR machine. Absorption spectrum was obtained at a wavelength of 400–4000 cm⁻¹.

**Separation of free and bonded metals in the complexes**

Separation was performed using the adsorption column method on adsorbent resin. The separation principle was to hold the metals bonded to amino acids on the adsorbent resin, while the free metals would be dissolved. Before the separation, the column was conditioned by washing successively with methanol, distilled water, HNO₃ 1 M in acetone, distilled water, NaOH 1 M, and distilled water again [7]. The complex samples (50 mg) were dissolved in 10 mL distilled water and put into the resin-containing column. The eluate product was metal ions in their free form. The eluate was then diluted and analyzed by AAS.

**Sample destruction**

Complexes (50 mg) were destroyed using 5 mL HNO₃ and heating at 100°C until the solution becomes clear and white smoke formed, indicating that the destruction had completed.

**Sample concentration determination**

The analytical conditions for determining Zn and Cu concentrations using the atomic absorption spectrophotometer are shown in Table 1. Samples from the column included the free metals that were directly analyzed without destruction. The other samples were metal-amino acid complexes that were destroyed using HNO₃. Absorption outcomes were then plotted into a calibration curve.

**RESULTS AND DISCUSSION**

Zn(Met)₂ complex synthesis results

The synthesis of the metal-amino acid complexes was performed by reacting amino acids with metal salts in a 1:2 ratio. For the Zn(Met)₂, Cu(leu)₂, and Cu(gli)₂ complexes, NaOH acted as a deprotonator of H⁺ protons at the OH group in the amino acid. For the Zn(gli)₂ complexes, Zn acetate was used for this purpose. Anion acetate acted as a weak base, deprotonating the H⁺ ion of the OH group. Complexes consisted of a white powder for the Zn(Met)₂ and Zn(gli)₂ complexes, while they were a blue powder for Cu(leu)₂ and Cu(gli)₂ complexes. The yield of the synthesis was 95.38%, 95.95%, 76.31%, and 93.91% for the Zn(Met)₂, Zn(gli)₂, Cu(leu)₂, and Cu(gli)₂ complexes, respectively. The respective yields of this synthesis process were for Zn(Met)₂, Zn(gli)₂, Cu(leu)₂, and Cu(gli)₂ complexes.

**Structure identification using FTIR**

Structure identification using FTIR showed that there was a significant shift between the amino acid and metal-amino acid complexes. The

| Measurement | Zn | Cu |
|-------------|----|----|
| Wavelength  | 213.9 nm | 324.8 nm |
| slit width   | 1 nm | 0.7 nm |
| light current| 5 mA | 6 mA  |
| burner height| 7 | 7 |
| gas flow rate| 1.8 L/min | 1.8 L/min |
| air flow rate| 15 L/min | 15 L/min |

Zn: Zinc, Cu: Copper
most noticeable difference was in the −OH carboxylic groups of amino acids that were no longer visible in the complex infrared spectrum. This is due to the separation of the O and H bond, which was replaced by a covalent bond between the O atom and the metal [8]. In the complex spectrum, the symmetric and asymmetric stretching vibration of NH, was also observed, indicating the interaction between the amine and the metal groups [9].

Tables 2-5 are comparison tables of the FTIR results between amino acids and the complexes produced:

### Determination of water content

Based on our calculations, the water content in the Zn(Met) complex was 2.38%. The water content in the Zn(gli) complex was 2.30%, while in the Cu(gli) complex, it was 1.87%.

### Standard solution making

The Zn standard solution was obtained by dissolving 211 mg heptahydrate Zn sulfate powder into 100 mL distilled water until a 500 µg/mL Zn standard solution was achieved. This solution was then diluted until 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 µg/mL standard solutions were obtained.

The Cu standard solution was obtained by diluting 1000 µg/mL Cu standard solution in demineralized water until 0.1, 0.4, 0.8, 1.2, 1.6, and 2.0 µg/mL standard solutions were obtained.

The concentration range of the calibrated curve was then adjusted until the metal concentration in the sample was in range. Great precision was required in the making of standard solutions because of the small concentrations, which were in part per million (ppm).

### Sample preparation

Before metal concentration analysis of the complexes was performed, the samples were first prepared. Each complex was prepared in two ways: Through separation by column chromatography then directly analyzed and by wet destruction.

#### Concentration determination

According to our analysis, the metal concentration in the Zn(Met) complex was 202.89 mg/g, with 13.57 mg/g free metal. The metal concentration in the Zn(gli) complex was 366.70 mg/g, with 12.92 mg/g free metal. The metal concentration in the Cu(leu) complex was 277.23 mg/g, with 13.57 mg/g free metal. The metal concentration in the Zn(gli) complex was 202.89 mg/g, with 13.57 mg/g free metal. The metal concentration in the Cu(leu) complex was 277.23 mg/g, with 13.57 mg/g free metal.

For the complexes, the analyzed metal concentration was the concentration of all metals in the complexes because there were residual metal ions that did not bond to the amino acid. The total metal concentration consisted of metal that was bonded to the amino acid and free metal that did not bond to the amino acid.

To determine the amount of metal that was bonded to the amino acid, the amount of free metal concentration was subtracted from the total metal concentration. The presence of free metal in the complexes was due to complexes that were not washed, so residual metal ions remained. Based on our calculation, the bonded metal concentration of the Zn(Met) complex was 189.32 mg/g, Cu(leu) complex was 181.08 mg/g, and Cu(gli) complex was 275.11 mg/g, respectively.

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**Table 2: Comparison of infrared spectrum between methionine and Zn (Met) complexes**

| Group   | Methionine | Zn (Met) |
|---------|------------|----------|
| N-H     | -          | 3306 cm⁻¹ |
| O-H     | 3178–2405 cm⁻¹ | 3261 cm⁻¹ |
| C-H     | -          | 2916 cm⁻¹ |
| C=O     | 1581 cm⁻¹  | 2916 cm⁻¹ |
| C-O     | 1417 cm⁻¹  | 1427 cm⁻¹ |
| S-CH    | 1242 cm⁻¹  | 1244 cm⁻¹ |

**Zn (Met): Zinc-methionine**

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**Table 3: Comparison of infrared spectrum between glycine and Zn (gli) complexes**

| Group   | Glycine | Zn (gli) |
|---------|---------|----------|
| N-H     | -       | 3271 cm⁻¹ |
| O-H     | 3334–2175 cm⁻¹ | 2941 cm⁻¹ |
| C=O     | -       | 2916 cm⁻¹ |
| C-O     | 1697 cm⁻¹ | 1589 cm⁻¹ |
| C-NH    | 1466 cm⁻¹ | 1408 cm⁻¹ |

**Zn (gli): Zinc-glycine**

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**Table 4: Comparison of infrared spectrum between leucine and Cu (leu) complexes**

| Group   | Leucine | Cu (leu) |
|---------|---------|----------|
| N-H     | 3242.5 cm⁻¹ | 3315 & 3244 cm⁻¹ |
| O-H     | 3109–2392 cm⁻¹ | - |
| C-H     | 2951 cm⁻¹  | 2958 cm⁻¹ |
| C=O     | 1617 cm⁻¹  | 1619 cm⁻¹ |
| C-NH    | 1566 cm⁻¹  | 1567 cm⁻¹ |
| C-O     | 1466 cm⁻¹  | 1468 cm⁻¹ |

**Cu (leu): Copper-leucine**

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**Table 5: Comparison of infrared spectrum between glycine and Cu (gli) complexes**

| Group   | Glycine | Cu (gli) |
|---------|---------|----------|
| N-H     | -       | 3337 and 3292 cm⁻¹ |
| O-H     | 3334–2175 cm⁻¹ | - |
| C-H     | -       | 2955 cm⁻¹ |
| C=O     | 1697 cm⁻¹ | 1672 cm⁻¹ |
| C-O     | 1422 cm⁻¹ | 1400 cm⁻¹ |
CONCLUSION
In this study, Zn(Met)$_2$, Zn(gli)$_2$, Cu(leu)$_2$, and Cu(gli)$_2$ complexes were successfully formed and analyzed. The mineral concentration in each complex differed depending on the type of mineral and ligand.

CONFLICTS OF INTEREST
All authors have none to declare.

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