

Diaquachloro-tris(ethyl-p-Aminobenzoate)copper(II) Chloride: Synthesis, Characterization and *In Vitro* Investigation of Antibacterial Activity

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Abstract. The new complex of $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ has been synthesized in 1:4 mole ratio of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and ethyl-p-aminobenzoate (benz) in ethanol at room temperature. The complex was characterized by UV-Vis and infrared spectroscopy, Atomic Absorption Spectroscopy (AAS), thermal analysis, magnetic measurement and molar conductivity. Infrared spectra indicated that benz was coordinated to the metal ion through nitrogen of primary amine group. Water molecules and chloride ion were also coordinated to Cu(II). $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, benz, and Cu(II) complex were screened for investigating *in vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using a modified Kirby-Bauer method.

1. Introduction

Ethyl-p-aminobenzoate (benz), shown in figure 1, is an anesthetic that can be used to treat pain [1]. As a remedy, benz has weaknesses regarding their low solubility in water but has the ability to inhibit bacterial growth of gram-negative and gram-positive bacteria that can be developed as an antibacterial [2]. In general, the antibacterial properties of the ligands are increased after complexing [3],[4]. Functional groups of a primary amine, a carbonyl group, an oxygen atom in the C=O group and electron cloud of benzene in benz that have lone pair electrons are able to form a complex compound with a metal such as copper. Cu(II) of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ shows antibacterial activity which is able to inhibit the growth of *Escherichia coli* [5] due to the ability to kill bacteria called the oligodynamic action. Therefore, ligand complexed with Cu(II) has better antibacterial activity compared to ligand without complexing [6].

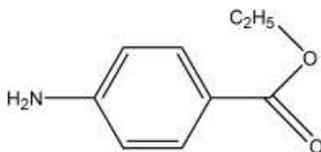


Figure 1. The structure of benz.

Cu(II) complex can form an assortment of geometries in accordance with a coordinated ligand. 8-hidroksi-quinolin (HQ) and alanine (ALA) coordinated with Cu(II) form a complex $[\text{Cu}(\text{HQ})(\text{ALA})]$ with tetrahedral geometries [7]. Cu(II) is coordinated to HQ through the pyridine nitrogen and oxygen

from hydroxyl groups, while ala is coordinated by two oxygen atoms in the group C=O and C-O as shown in figure 2. The complex of $[\text{CuL}^1_2]$, $\text{L}^1=2\text{-Hydroxy salicyl hydrazone isatin hydrazone}$ has octahedral geometry [8]. L^1 coordinated to Cu (II) is trident through the group C=O, C=N and C-O as shown in figure 3. The complex of $[\text{CuCl}_2(\text{PABA})_2]$ (PABA = p-amino benzoic acid) is a square planar geometry [9] with two $-\text{NH}_2$ and two Cl^- ions are coordinated to Cu(II) ion as shown in figure 4.

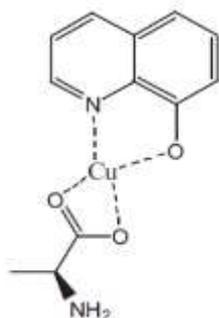


Figure 2. Complex of $[\text{Cu}(\text{HQ})(\text{ala})]$ which has tetrahedral geometry [7].

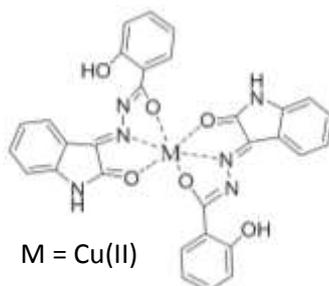


Figure 3. Complex of $[\text{CuL}^1_2]$ which has octahedral geometry [8].

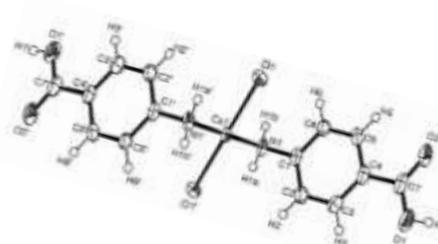


Figure 4. Complex of $[\text{CuCl}_2(\text{PABA})_2]$ which has square planar.

Some examples above show that the Cu(II) can be coordinated with various functional groups such as $-\text{NH}_2$, C=O, C-O, Cl and H_2O as well as producing a different geometry. Benz has a functional group that can be coordinated to Cu(II) to form complex compounds with a specific geometry. The complex of Cu(II) and benz are expected to enhance the antibacterial activity.

2. Experimental

2.1 Materials

All chemicals, except benz, were purchased from E.Merck and used without further purification, benz were purchased from Kimia Farma.

2.2 Physical measurements

The copper content was determined by Atomic Absorption Spectrometer (AAS) Shimadzu AA-6650. IR spectra were recorded on a Spectrophotometers Prestige-21 Shimadzu. The thermogravimetric analysis of the metal complex was performed by a TG/DTA Diamond Perkin Elmer analyzer. Spectra UV-VIS was performed with Shimadzu UV-3601 spectrophotometer. Molar conductivity (Λ^*m) of 1 mM solution in methanol was measured at 25 °C with a Jenway CE 4071 Conductivity meter. The magnetic moment was measured with Auto Sherwood Scientific 10169 Magnetic Susceptibility Balance.

2.3 Determination of coordination numbers of Cu(II)

$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ 0,02 M and benz 0,1 M were dissolved in ethanol then mixed with a mole ratio of Cu(II):benz =1:0 to 1:7. The absorbance of all mixed solution was measured using UV-Vis spectrophotometer and graphed maximum wavelength versus mole ratio to determine optimum mole ratio of Cu(II) and benz.

2.4 Synthesis of Cu(II) complex

$\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.51 g; 3 mmol) was dissolved in ethanol (15 mL) then dropwise into a solution of benz (1.98 g; 12 mmol) in ethanol (35 mL) with stirring, the stirring was continued for 30 minutes then allowed to stand for 30 minutes. The precipitate was filtered off and then recrystallized with methanol

(50 mL). The solution was concentrated to 25 mL then allowed to stand overnight. The precipitate was filtered and dried under vacuum (1.84 g; 91.96 %).

2.5 Antibacterial screening

Antibacterial activities of Cu(II), benz, and the synthesized complex were determined against *Staphylococcus aureus* and *Escherichia coli* by the modified Kirby-Bauer method. The test solution benz, CuCl₄·2H₂O and Cu(II)-benz were prepared in the concentration (% m/v): 10, 25, 50, 75 and 100 in DMSO. Negative controls carried out on the solvent DMSO and the positive were tested against mentioned organisms. Test bacteria cultures were performed in a nutrient agar media on Petri dishes. The nutrient media was prepared with usual components and properly sterilized in an autoclave. Activity was determined by measuring the diameter of zone showing complete inhibition and has been expressed in mm.

3. Result and Discussion

3.1 Determination of coordination numbers of Cu(II)

Cu(II) mixed with benz shifted maximum wavelength into smaller wavelength to achieve the stability, that was when it reached the maximum number of moles benz able to be coordinated with one mole of Cu(II). This was obtained from the intersection graph of λ_{\max} (nm) and the mole ratio of Cu (II): benz. Figure 5 shows the intersection of the lines in a mole ratio of metal to ligand 1: 3, this implies that the most optimum mole ratio of Cu(II) and benz is 1: 3.

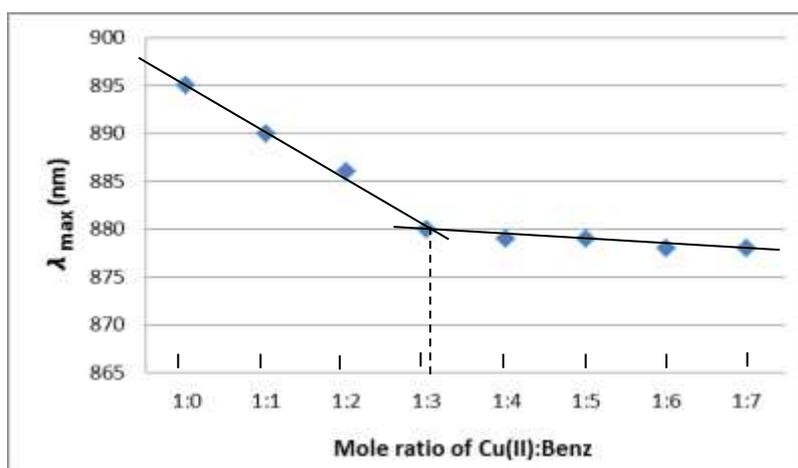


Figure 5. Graphic λ_{\max} versus the mole ratio of Cu(II) and benz.

3.2 Synthesis of Cu(II) complex

A mixture solution of CuCl₂·2H₂O in ethanol to a solution benz in ethanol produced brown precipitates. The formation of complex compounds was indicated by a color change of the solution from colorless to brown. The shift of λ_{\max} was 25 nm, shown in figure 6, indicating the formation of complex Cu(II) Benz. It occurred also in the synthesis of complex Cu(II)-morpholine as well, the complex was indicated due to 20.3 nm shift of λ_{\max} towards smaller [10].

3.3 Analysis of the Amount of Copper with Atomic Absorption Spectroscopy

Measurements of copper in the complex was expected to determine the complex formula by comparing the measurement experimental results and theory. The measurement of copper content in the complex based on the experiment was $9.37 \pm 0.19\%$, therefore the estimated complex formula was Cu(benz)₃(H₂O)₂Cl₂ or Cu(benz)₃(H₂O)₃Cl₂.

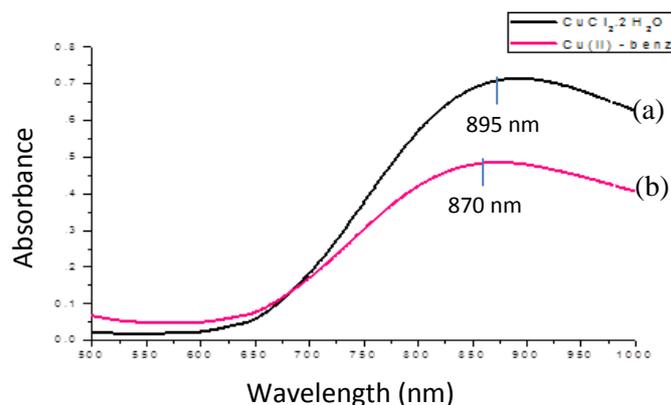


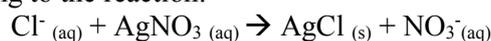
Figure 6. Electronic spectra of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ solution (a) and Cu(II)-benz solution (b).

3.4 Thermal analysis by TG/DTA

Thermal analysis in the complex Cu(II)-benz was used for estimating the presence of water molecules. It reveals that the stability of the complex reaches the temperature of 150°C . After that, the endothermic reaction occurs in the area of 177°C followed by the reducing mass of the complex in the area of $151\text{--}188^\circ\text{C}$. This shows that at these temperatures, as much as 5.54% mass reduction occurred equivalent to the loss of two molecules of H_2O . The temperature of $150\text{--}200^\circ\text{C}$ is a range of coordinated water molecules loss [11]. The complex Cu(II) was estimated to contain three molecules of benz and two molecules of coordinated water. Thus, estimated formula was $\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}_2$.

3.5 Analysis of chloride ion

Qualitative ion analysis was used for determining the position of the chloride ion in the complex as a ligand or a counter ion. The analysis was performed by adding a solution of AgNO_3 to a solution of each complex in distilled water. The results showed white precipitate of AgCl indicating that chloride acted as a counter ion according to the reaction:



Ion analysis results showed a white precipitate when the AgNO_3 solution was added to a solution of Cu(II)-benz . This shows that there were chloride ions that acted as a counter ion. However, according to the results of AAS in the complex Cu(II)-benz there were two chloride ions, therefore the number of chloride ion as a counter ion was determined by analysis of electrical conductivity.

3.6 Analysis of electrical conductivity

Analysis of electrical conductivity was used to determine the ratio of cations and anions in the Cu(II)-benz by comparison with standard solutions. The molar conductance values of the complex lie in $5.57 \pm 0.06 \text{ S}\cdot\text{cm}^2\cdot\text{mol}^{-1}$, thus indicating that is an electrolyte. By comparing the conductivity values of the complex sample with a molar conductivity of the standard solution, it can be seen that the charge ratio of cationic: anions = 1:1. This suggests that one of the Cl^- ion was coordinated to the central metal ion Cu(II) and another acted as a counter ion. Thus, the most probable formula of the complex is $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$.

3.7 Infrared analysis

Infrared (IR) analysis was used for estimating the group coordinated to Cu(II) . Absorption band of -NH [12] on the complex (3303 and 3158 cm^{-1}) shifts towards smaller values compared to that of benz (3422 and 3342 cm^{-1}), indicating primary amine coordinated to central metal ion. N-H in Cu(II)-benz has smaller wavenumber than the free ligand due to limited N-H vibration. Thus, it is estimated that benz was coordinated to Cu(II) through primary amine group. It strengthens with the absorption band

of Cu-N in 543 cm^{-1} [5]. In $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, IR spectra exhibited strong absorption band of O-H in region 3482 cm^{-1} [13], while the IR spectra of Cu(II)-benz reveals weak absorption of O-H group appearing in region 3398 cm^{-1} . The overlap between the absorption of O-H and N-H might happen due to an adjacent area, thus O-H absorption hardly appeared in the spectra of Cu(II)-benz. However, the absorption band of Cu-O in region 687 cm^{-1} [5] strengthen the possibility of water molecules coordination in the complex compound.

3.8 Magnetic properties

The magnetic moment of $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ (μ_{eff}) was $1.98 \pm 0.03\text{ BM}$. It indicated that the compound was paramagnetic with one unpaired electron [14], [15], [16]. It also showed that the Cu(II) configuration was d^9 [17]. The effective magnetic moment was in range 1.82 to 2.32 BM owned by Cu(II) complex with six coordination number and octahedral [18], [19], [20]. In general, Jahn-Teller distortion frequently occurred in the complex of Cu(II) with configuration d^9 [11], [21], [22]. Cu(II)-benz. The complex was formed by coordination of a primary amine group, Cl⁻ ions, and water molecules to Cu(II). Each adjacent atom will produce a repulsion, thus each different atom will produce different strength of repulsion. The distance and position of each atom will be changed to achieve the least possible repercussion. These circumstances affected the bond lengths between atoms. In the complex of Cu(II)-benz, Cu(II) was bonded to six different groups and there was a possibility of distortion in the octahedral geometry.

3.9 Electronic spectra

Electronic spectra analysis was conducted to determine maximum wavelength, molar absorptivity, and estimated electron transition. It appears that $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ has a maximum wavelength in region 870 nm (11494.25 cm^{-1}) and a molar absorptivity of 241.96 L/mol.cm . Figure 6 shows a wide absorption in the visible region (870 nm) on $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ and the possibility of the complex octahedral geometries. Jahn-Teller Distortion caused splitting in the orbit and the result were three transitions $B1G \rightarrow A1g$ (ν_1), $B1G \rightarrow B2G$ (ν_2), $B1G \rightarrow E_g$ (ν_3) [23],[24] which would appear as three peaks in the electronic spectra. However, the three transitions in the energy level that was close together so often appeared as a wide absorption band [25], [26]. Thus, the suggested geometry of $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ was distorted octahedral. The suggested structure of the complex is distorted octahedral geometry as shown in figure 6. According to IUPAC nomenclature, the name of the complex is diaquachloro-tris(ethyl-p-aminobenzoate)copper(II) Chloride.

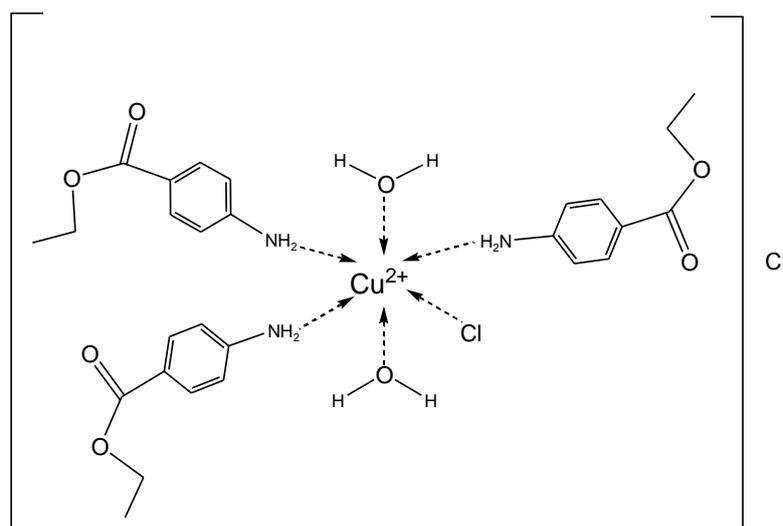


Figure 7. Suggested structure of $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$.

3.9 Antibacterial screening

The test results in the form of antibacterial activity inhibition zone diameter (mm) against *Staphylococcus aureus* and *Escherichia coli* are shown in table 1. Table 1 shows that $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ has better antibacterial activity than $\text{Cu}(\text{II})$ and benz. This can be explained by the overtone concept and Tweedy's chelation theory. According to the overtone concept, the entrance to the cell is surrounded by lipid membranes that can only be passed by lipid-soluble materials, therefore liposolubility is an important factor in controlling antimicrobial activity [16]. Meanwhile, according to Tweedy's chelation theory, the polarity of $\text{Cu}(\text{II})$ is reduced due to their orbital overlap thus sharing positive charge with the atom donor [8], [27], [28]. This can cause the hydrophobic and lipophilic character and help increase permeability to the lipid membrane. Increased penetration can interfere cellular respiration process and facilitate entry into the cell. The complex can inhibit protein synthesis in normal cells and can ultimately inhibit bacterial growth.

Table 1. Average Diameter of Inhibition Zone of $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ and Benz against *Staphylococcus aureus* and *Escherichia coli*.

Sample	Concentration (m/v)	Average Diameter of Inhibition Zone (mm)					
		<i>Staphylococcus aureus</i>			<i>Echerichia coli</i>		
		24 h	48 h	72 h	24 h	48 h	72 h
CuCl ₂ ·2H ₂ O	5%	0,00	0,00	0,00	0,00	0,00	0,00
	10%	2,23	4,23	4,70	0,00	0,00	0,00
	25%	5,27	6,87	7,10	4,57	6,70	7,10
	50%	8,53	9,83	10,13	7,23	9,77	10,17
	75%	12,63	15,40	15,53	12,13	14,20	14,60
	100%	14,77	17,93	18,67	19,10	21,10	22,30
Benz	5%	0,00	0,00	0,00	0,00	0,00	0,00
	10%	1,43	2,90	3,43	1,43	2,67	3,37
	25%	3,20	3,93	4,10	3,27	3,80	4,07
	50%	4,73	6,03	6,37	6,23	8,00	8,27
	75%	8,20	9,90	10,47	8,03	11,30	11,57
	100%	10,77	14,13	14,73	12,00	15,50	16,03
Cu(II)-benz	5%	1,23	1,83	2,20	0,83	1,50	1,77
	10%	4,03	4,77	5,03	2,50	4,47	4,97
	25%	6,80	8,43	8,33	6,83	9,20	9,47
	50%	11,50	13,73	14,57	9,83	13,07	13,30
	75%	15,67	18,27	19,03	15,83	17,87	18,93
	100%	20,13	24,27	24,87	23,10	26,20	26,40

4. Conclusion

The complex $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ was able to be synthesized by mixing solution of $\text{Cu}(\text{II})$ in ethanol and benz solution in ethanol with a mole ratio of 1: 4 and stirred for 30 minutes. Functional groups coordinated to the $\text{Cu}(\text{II})$ were possibly NH_2 , H_2O , and Cl^- ions forming a distorted octahedral geometry. The complex $[\text{Cu}(\text{benz})_3(\text{H}_2\text{O})_2\text{Cl}]\text{Cl}$ increased antibacterial activity significantly compared $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ and benz.

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