Optimization of complex NH$_3$ with Cu$^{2+}$ ions to determine levels of ammonia by UV-Vis spectrophotometer

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Abstract. Ammonia analysis based on complexing ammonia with Cu$^{2+}$ ions by UV-Vis spectrophotometry. This research aims to obtain uptake at the maximum wavelength of the copper-ammonia complex, optimum conditions and validation of UV-Vis spectrophotometry. The reaction between Cu$^{2+}$ ions which are blue with ammonia colorless occur in solution to form complex compounds $[\text{Cu(NH}_3\text{)}_4]^{2+}$ dark blue, the maximum absorbance is obtained at a wavelength of 615 nm. Optimization of the ammonia complexing the Cu$^{2+}$ ions provide optimum conditions a solution of Cu$^{2+}$ concentrations of 0.01 M in ammonia concentration of 0.04 M, pH 7, and the optimum time to form a complex occurred in the 30th minute and complex compounds can be stable for 90 minutes ($\pm$ 1 hours 30 minutes). The analytical method validation ammonia using Cu$^{2+}$ ions provide linear regression equation $y = 4.772x + 0.333$ with $R^2 = 0.989$ ammonia concentration range of 0.003 M to 0.08 M; LOD 0.01 M; LOQ 0.04 M; % RSD = 1.32%; and% recovery = 102.03%. The concentration of ammonia in the wastewater sample application obtained the ammonia concentration of 0.04 M. Based on the results of the validation, Ammonia solution with Cu$^{2+}$ ion as a complex used for the determination of ammonia levels by UV-Vis spectrophotometry.

1. Introduction

Ammonia is a toxic gas, a colorless, strong-smelling, can irritate the skin, eyes and lungs [1]. Ammonia can be explosive or flammable by volume of 15% to 28%[2] When the concentration of ammonia exceeds 300 ppm, it can cause severe injuries on the skin respiratory system, skin, eyes and damage human cells[3]

Waste from farms, factories, chemical industries, and mines are a common source of ammonia which can contaminate water systems. Excess ammonia in the water can lead to living things in the water is contaminated and cause death. Who had high levels of ammonia can be found at the bottom of the lake with the condition without oxygen, or anoxic[4]

Extreme toxicity of ammonia and abuse of toxic ammonia as a result the amount of research in the methods of development for the detection of ammonia. Previously to analyze ammonia used various methods is titrimetric, voltammetry, potentiometric, electrochemical method, and ion chromatography. However, this method takes a long time in the process involves the use of sophisticated instruments with a high detection limit and require special expertise. Ammonia analysis method based on the color change is monitored and has been studied for the past few years due to the implementation of simple, cheap and fast [5]. Spectrophotometric method to determine ammonia through the formation of color by using the metal as a complexing based on the absorption of UV-Vis.
Ammonia and some metal ions can form complex compounds. Cu\(^{2+}\) ions can form complexes in stronger ammonia when compared to the complexity of the water, the similarities between the reaction with ammonia Cu\(^{2+}\) ions in the solution are:

\[
[Cu(H_2O)_4]^{2+} + 4NH_3 \rightarrow [Cu(NH_3)_4]^{2+} + 4H_2O
\]

Blue \rightarrow Dark blue

The addition of NH\(_3\) in solution \([Cu(H_2O)_4]^{2+}\) ion in this reaction generates complex \([Cu(NH_3)_4]^{2+}\). The color change is a solution of Cu\(^{2+}\) colored blue when in complexing with NH\(_3\) colored nodes will form a complex solution of \([Cu(NH_3)_4]^{2+}\) dark blue so that these reactions indicate that the chemical changes in the exchange NH\(_3\) with H\(_2\)O molecules. Complex Cu\(^{2+}\) ions with NH\(_3\) formed to determine the concentration of ammonia can be used in by spectrophotometry UV / Vis on sample solution with optimal condition.

2. Tools and materials

2.1. Instrumentation
The tools used are UV-VIS spectrophotometry, pH meters and shaker.

2.2. Materials
Research materials is a 32% ammonia solution (Merck, pa.), CuSO\(_4\).5H\(_2\)O (copper (II) sulfate pentahydrate), NaOH (sodium hydroxide), HCl (hydrochloric acid), wastewater, and filter paper.

3. Methodology

3.1. Complexity Ammonia Ion Cu\(^{2+}\) and Determination of the Maximum Absorption Wavelength
The mother liquor Cu\(^{2+}\) ion concentration of 0.1 M was prepared by dissolving 0.6242 g of copper (II) sulphate pentahydrate (CuSO\(_4\).5H\(_2\)O) with distilled water in a 25 mL volumetric flask. Concentrated NH3 solution is used to create a 0.1 M NH3 solution with distilled water in a 25 mL volumetric flask. Complex tetraaminacopper (II) \([Cu(NH_3)_4]^{2+}\) can be prepared by pipette 2 ml of 0.01 M Cu\(^{2+}\) ions and 2 mL of 0.04 M ammonia solution and put in a test tube, shake until homogeneous. Measure absorbance complex \([Cu(NH_3)_4]^{2+}\) with UV-VIS spectrophotometer at a wavelength of 450-900 nm. The highest absorption on certain wavelength is maximum wavelength absorption complex.

3.2. Optimization of complexing Ammonia with Cu\(^{2+}\) ions

3.2.1. The concentration of Cu\(^{2+}\) ions. Each solution of Cu\(^{2+}\) ions 0.00001; 0.0001; 0.0001; 0.0005; 0.001; 0.005; 0.01 M 2 mL pipetted into a test tube, then add 2 ml of ammonia solution 0.04 M to mark boundaries and homogenous. Measure absorbance complex \([Cu(NH_3)_4]^{2+}\) with UV-VIS spectrophotometer at the wavelength of maximum.

3.2.2. Effect of pH. Pipette 2 mL each of 0.04 M ammonia solution with a wide range of pH (pH 5-9) into a test tube, then added 2 mL solution of Cu\(^{2+}\) ions (optimum concentration) and homogeneous. Measure absorbance on wavelength maximum with UV-Vis spectrophotometer. The highest uptake is the optimum pH complexity reaction between ammonia and Cu\(^{2+}\) ions.

3.2.3. Complexing Time. 0.04 M ammonia solution pipetted 2 mL (at optimum pH), add 2 ml of Cu\(^{2+}\) (at the optimum concentration) to form complex. Measure the absorption with a UV-Vis spectrophotometer at maximum wavelength in an interval of 0-120 minutes.

3.2.4. Stability Complex Time. Complex compounds formed in the timing of the stability of the complex will be continued until the absorbance measurement of the absorbance values obtained from
optimal time when absorbance value decreases when the optimum time. Absorption measure by UV-Vis spectrophotometer at the wavelength of maximum.

3.3. Analysis Methods Validation

3.3.1. Preparation of Calibration Curves. Each ammonia solution with concentration in the following calibration curve battens (0.003; 0.005; 0.008; 0.01; 0.03; 0.05; 0.08 M) 2 mL pipette and put into a test tube, then added 2 mL the optimum concentration of Cu$^{2+}$ ions and homogenized. Enter into the cuvette in a UV-Vis spectrophotometer, read and record the absorbance at maximum wavelength. Create a calibration curve from absorbance and concentration values and determine the straight-line equation.

3.3.2. Determination of Detection Limit (LOD) and the limit of quantification (LOQ). A calibration curve was obtained to calculate the smallest concentration detected (LOD) and detected quantitatively (LOQ) using statistical calculations.

\[
\text{LOD} = 3 \left( \frac{\text{SY}}{X} \right) \frac{\text{SY}}{\text{Slope}}
\]

\[
\text{LOQ} = 10 \left( \frac{\text{SY}}{X} \right) \frac{\text{SY}}{\text{Slope}}
\]

where : SY / X = Standard deviation Residual
LOD = limit of detection
LOQ = limit of quantification

3.3.3. Repeatability test (Precision). 0.04 M ammonia solution was mixed with 2 mL of 2 mL of Cu$^{2+}$ ions optimum concentration in a test tube at optimum pH. Measure absorbance at the maximum wavelength UV-Vis spectrophotometer. Tests carried 7 times and its counted % RSD.

\[
\text{SD} = \sqrt{\frac{\sum (X - \bar{X})^2}{(N - 1)}}
\]

\[
\% \text{ RSD} = \frac{\text{SD} \times 100}{\bar{X}}
\]

% RSD = Percent relative standard deviation
SD = Standard deviation
\(\bar{X}\) = Average of the measurements
X = The value of each measurement
N = Number of data

3.3.4. Test Accuracy (Accuracy). Each ammonia solution with concentration in the following calibration curve battens (0.0009; 0.002; 0.006 M) 2 mL pipette and put into a test tube, then add 2 mL ion Cu$^{2+}$ optimum concentration and homogenized. Enter into the cuvette in a UV-Vis spectrophotometer, read and record the absorbance at maximum wavelength then calculate the % recovery. The following equation to calculate the value of % recovery:
(4)

where $C_s$ is the concentration of ammonia determined (M) and $C$ is the actual concentration of ammonia (M).

### 3.3.5. Test Regression Analysis

Regression analysis to test the calibration curve is done with using statistical calculation by using $R^2$ test, $f$ test, $t$ test, and the calculation is done by using SPSS 16. In the simultaneous test, $H_0$ is rejected if $F_{\text{count}} > F_{\text{table}}$, and accepted if $F_{\text{count}} \leq F_{\text{table}}$. In the partial test, $H_0$ is rejected if $t > t_{\text{table}}$, and accepted if $t \leq t_{\text{table}}$.

### 3.4. Applications using samples of wastewater

Natural samples filtered and then pipetted 2 mL and inserted into the vial bottle, then added 2 mL solution of $\text{Cu}^{2+}$ ions optimum concentration and homogenized. Enter into the cuvette in a UV-Vis spectrophotometer, read and record the absorbance at maximum wavelength.

### 4. Results and Discussion

#### 4.1. Ammonia Ion Complexing $\text{Cu}^{2+}$ and Determination of the Maximum Absorption Wavelength

Ammonia solution was added to a solution of $\text{Cu}^{2+}$ ions will react to form complex compounds tetraaminacopper(II) ($[\text{Cu(NH}_3]_4]^{2+}$). A solution of $\text{Cu}^{2+}$ ions in blue will change to dark blue color after reacting with ammonia is a colorless form complexes tetraaminacopper(II) ($[\text{Cu(NH}_3]_4]^{2+}$). The chemical reaction between ammonia with $\text{Cu}^{2+}$ ion is shown in the following equation:

$$\text{Cu}^{2+}(\text{aq}) + 4\text{NH}_3(\text{aq}) \leftrightarrow [\text{Cu(NH}_3]_4]^{2+}(\text{aq})[12]$$

Determination of the maximum wavelength complex compounds tetraaminacopper(II) ($[\text{Cu(NH}_3]_4]^{2+}$) aims to get the value absorbisisitas that can provide good measurement sensitivity. The measurement results determining the maximum wavelength complex compounds tetraaminacopper (II) ($[\text{Cu(NH}_3]_4]^{2+}$) are shown in the picture:

![Absorbance Maximum ammonia solution with Cu\(^{2+}\) ions](image)

**Figure 1.** Absorbance Maximum ammonia solution with $\text{Cu}^{2+}$ ions

Based on the first image obtained three curves in the visible region with a wavelength range of 450nm-900nm. Blue curve shows absorbance measurement results $\text{CuSO}_4.5\text{H}_2\text{O}$ solution with maximum wavelength of 795 nm, it can be seen from the peaks formed between wavelengths of 750-820 nm, which is the wavelength range of blue $\text{CuSO}_4.5\text{H}_2\text{O}$ solution. The red curve shows the measurement results of absorbance of ammonia solution which is colorless, so it does not show any
response to the visible region. Green curve shows the results of absorbance measurement of complex compounds $[\text{Cu(NH}_3\text{)}_4]^{2+}$ as a result of reaction of Cu$^{2+}$ with NH$_3$ showed the maximum wavelength is 615 nm. Selection is based on the maximum wavelength to the peak intensity and the absorbance values of the complex $[\text{Cu(NH}_3\text{)}_4]^{2+}$ highest, the complex $[\text{Cu(NH}_3\text{)}_4]^{2+}$ dark blue that is in the wavelength range of 590-620 nm[3]

4.2. Optimization of Ion Complexing Cu$^{2+}$ With NH$_3$ solution

4.2.1. The influence of the concentration of Cu$^{2+}$ The concentration of cupric(II) may give effect to the complex $[\text{Cu(NH}_3\text{)}_4]^{2+}$ were performed by varying the concentration of Cu$^{2+}$ (0.000001; 0.00001; 0.0001; 0.0005; 0.001; 0.005; 0.01 M). Into each solution of Cu$^{2+}$ added as much as 2 mL of 2 mL of 0.04 M ammonia solution so as to form the complex $[\text{Cu(NH}_3\text{)}_4]^{2+}$ and measured absorbance at a wavelength of 615 nm. The results of measurements of Cu$^{2+}$ concentration variation in the shape of the curve shown below:

![Figure 2](image-url) **Figure 2.** Relationship Cu$^{2+}$ concentration variations of the absorbance value

From Figure 2 it can be seen that the increase in the concentration of Cu$^{2+}$ ions provides increased absorbance values to a concentration of 0.01 M Cu$^{2+}$, because gradually complex $[\text{Cu(NH}_3\text{)}_4]^{2+}$ more formed. Cu$^{2+}$ ion concentration of 0.01 M is an optimum condition for increasing the mean absorbance values of all NH$_3$ molecules have reacted with Cu$^{2+}$ ions produces complex compound $[\text{Cu(NH}_3\text{)}_4]^{2+}$. The results are consistent with what has been done by Ling et al, 2013, which states that the complexing ion concentration related to the number of complex compounds formed.

4.2.2. Effect of pH. On the formation of complexes heksaaminacopper (II) $[\text{Cu(NH}_3\text{)}_4]^{2+}$, the degree of acidity (pH) has a considerable influence. This study has been carried out the complex formation $[\text{Cu(NH}_3\text{)}_4]^{2+}$ at pH varies the solvent at a pH of 5 to 9. The pH of the pH variation measurement results shown in Figure 3.
In Figure 3 it can be seen that an increase in absorption of the complex \([\text{Cu(NH}_3\text{)}_4]^{2+}\) from pH 5 to pH 7 and pH 8. Based on the decline after the research results, at pH 7 the complex \([\text{Cu(NH}_3\text{)}_4]^{2+}\) achieved the optimum conditions. This means that the complex compound \([\text{Cu(NH}_3\text{)}_4]^{2+}\) formed more at pH 7 than on other pH. The formation of complex compounds \([\text{Cu(NH}_3\text{)}_4]^{2+}\) can affect if pH level change. At a higher alkaline pH (pH above 7) compounds the less complex formed and a precipitate is formed.

The complex \([\text{Cu(NH}_3\text{)}_4]^{2+}\) is not formed At acidic pH (pH below 7), ammonia (NH\(_3\)) can be transformed into ammonium (NH\(_4\)) due to an excess of H\(^+\) ions on the conditions of acidic pH, it resulted in the complex \([\text{Cu(NH}_3\text{)}_4]^{2+}\) is not formed[10].

### 4.2.3. Complexing time and Stability Complex

Effect of complexing time and stability of the complex aims to get the optimum time when the complex \([\text{Cu(NH}_3\text{)}_4]^{2+}\) formed and how long the complex compound is likely to be stable. The results of the complex formed when \([\text{Cu(NH}_3\text{)}_4]^{2+}\) and the stability of the complex \([\text{Cu(NH}_3\text{)}_4]^{2+}\) can be seen in the image:

From Figure 4 it can be seen that the longer time of complexing the higher absorbance values obtained. It explains the complex compound \([\text{Cu(NH}_3\text{)}_4]^{2+}\) formed with increasing time. The results of the complex formation of the optimum time is at minute 30 and continue until the 120th minute. Complex compounds \([\text{Cu(NH}_3\text{)}_4]^{2+}\) has been formed in the 30th minute and stable up to 120 minutes of complex compounds \([\text{Cu(NH}_3\text{)}_4]^{2+}\) can be stable for 90 minutes (± 1 hour 30 minutes).
4.3. Analysis Methods Validation

4.3.1. Calibration curves and Linearity. Calibration curve equation is the relationship between the x-axis to the y axis. Rows konentrasi made are set forth in the x-axis, while the absorbance values obtained from the measurement results are expressed as the y axis. On the figure 5 can be seen the yield curve measurements made directly on form of calibration curve:

![Figure 5. The calibration curve of standard solution of ammonia](image)

The linear regression equation in the above calibration curve is $y = 4.772x + 0.333$ with relation coefficient ($r$) = 0.989 obtained. Values relation coefficient ($r$) can be stated that approximately 1 linear relationship between the absorption produced by the ammonia concentration. The size linearity of a method views by how well the interaction between the absorbance ($y$) with concentration of ammonia ($x$) in the calibration curve.

4.3.2. Determination of Detection Limit (LOD) and the limit of quantification (LOQ). The determination of the limit of detection (LOD) and the limit of quantification (LOQ) obtained from further data that calibration curve has been met the requirements of analysis. Results LOD and LOQ value data shown in tabel.1:

|                  | LOD (Detection limit) | LOQ (Limit of quantification) |
|------------------|-----------------------|-------------------------------|
| $\Sigma (Y - Y_i)^2$ | 0.001225              | 0.0438                        |
| SY / X           | 0.01565               |                               |

The limit of detection (LOD) obtained from the statistical calculation of 0.01 M and the limit of quantification (LOQ) of 0.04 M. LOD value is the lowest amount of ammonia concentration that can still be detected and was measured. LOQ value is the lowest amount of ammonia concentration that can still be measured quantitatively with precision (carefully) and accuracy (right) ideal[2]

4.3.3. Method Precision and Accuracy. The measurement repeatability was done for obtained to see how close the difference value from precision test. This precision test was done by measuring reproducibility of the complex $[\text{Cu(NH}_3\text{)}_4]^{2+}$ done as much as 7 repetitions. On Table 2 can be seen the result of test precision value data:
In this study obtained value %RSD 1.32%. This means that precision data obtained in this study are eligible provided, so that it can be concluded that the method of analysis used meets the criteria well [5].

At accuracy test aims to see the closeness of the results of measurements with the actual values. Accuracy is expressed as a percent recovery (% recovery) the analyte is added. Determining the accuracy of the simulation method used in this study (placebo-spiked recovery).

The result is the value of recoveries amounted to 102.03%. Terms test recovery (% recovery) in the range of 95% to 105%, so the recovery value obtained meets the requirements. So that the research data can be said to provide accuracy of good test results and the analysis method can work quite accurately [11].

4.3.4. Regression Analysis Method. Linear regression analysis has several goals one of them to describe the phenomenon of data or case that is being investigated. Regression analysis test done using by using $r^2$ test, f test, t test, and decision-making with the p-value. Table 3 shows the results of the test statistic calculation of linear regression analysis using SPSS 16.

| Model Summary | Mode | R | R Square | adjusted R Square | Std. error of the Estimate |
|---------------|------|---|----------|------------------|----------------------------|
|               | 1    | .995a | .989 | .987 | .01565 |
| Predictors: (Constant), Concentration |

Based on Table 3 shows the value of the coefficient of determination (R square) of 0.989. The coefficient of determination is the squaring of the value of R (0.995) which is equal to 0.9952 = 0.989 98.9% which implies that the concentration affects the absorbance of 98.9%. The larger the value of the coefficient of determination (closer to 1), the better the regression model obtained. That is the effect of concentration against absorbance is getting stronger.

| ANOVA | Model | Sum of Squares | Df | mean Square | F | Sig. |
|--------|-------|----------------|----|-------------|---|------|
| 1      | Regression | .115 | 1 | .115 | 470.277 | .000a |
| residual | .001 | 5 | .000 |
| Total | .116 | 6 |
| a. Predictors: (Constant), Concentration |
| b. Dependent Variable: Absorbance |

In the ANOVA table above can be seen that the calculated F value of 470.277, because 470.277 > 6.61 using a 0.05 significance level, $H_0$ is rejected.

Besides it can also make decisions based on a probability value (p-value) which is in the column Sig. If the p-value < 0.05, then $H_0$ is rejected. In the above data it can be concluded that 0.000 < 0.05 so that $H_0$ is rejected.
Table 5. Data regression coefficient using SPSS 16

| Coefficients | Unstandardized coefficients | standardized coefficients |
|--------------|-----------------------------|--------------------------|
| Model (Constant) | .333 | .008 | .40 094 | .000 |
| Concentration | 4772 | .220 | .995 | 21 686 | .000 |

a. Dependent Variable: Absorbance

Based on Table 5, it can be seen that the obtained value of t arithmetic amounted to 21.686, while the value of t obtained at 2.57058, then t (21.686) > t (2.57058) value H₀ is rejected. If H₀ is rejected, then the variable x (concentration) has a significant contribution to the variable y (absorbance).[7]

4.4. Test Application

Application test aims to determine the concentration of ammonia in a sample of nature can be determined using a regression equation obtained in the calibration curve, uptake value (absorbance) samples used as the value of y, so the value of x or the concentration of ammonia from the sample can be determined. Of the tested samples obtained values absorbance of the sample was 0.5302 and the concentration of ammonia in the sample was 0.04 M.

5. Conclusion

Based on the research that has been done it can be concluded that λ_maks for complexing the Cu²⁺ ion ammonia with UV-Vis spectrophotometry was 615 nm. The optimum conditions of Cu²⁺ solution at a concentration of 0.01 M 0.04 M ammonia, solvent pH 7, and the optimum time to form the complex [Cu(NH₃)₄]²⁺ occurs at minute 30 and can be stable for 90 minutes (± 1 hour 30 minutes). Validation of analytical methods ammonia using Cu²⁺ ions by spectrophotometry UV-Vis provides a linear regression equation y = 4.772x + 0.333 with R² = 0.989; LOD 0.01 M; LOQ 0.04 M; % RSD = 1.32%; and % recovery = 102.03% and ammonia concentrations were in samples of wastewater that is 0.04 M.

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