Spectrophotometric Determination of Trace Concentrations of Copper in Waters Using the Chromogenic Reagent 4-Amino-3-Mercapto-6-[2-(2-Thienyl)Vinyl]-1,2,4-Triazin-5(4H)-One: Synthesis, Characterization, and Analytical Applications

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Abstract: A simple, selective, and inexpensive spectrophotometric method is described in the present study for estimation of trace concentrations of Cu$^{2+}$ in water based on its reaction with chromogenic reagent namely 4-amino-3-mercapto-6-[2-(2-thienyl)vinyl]-1,2,4-triazin-5(4H)-one (AMT). The reaction between copper(II) ions and AMT reagent gives [Cu(L)(NO$_3$)(H$_2$O)$_2$]$\cdot$H$_2$O complex, where L represents AMT molecule with NH group. The formed complex exhibits a sharp, and well-defined peak at $\lambda_{max} = 434$ nm with a molar absorptivity ($\epsilon$) of $1.90 \times 10^4$ L mol$^{-1}$ cm$^{-1}$, and Sandell’s factor of 0.003 $\mu$g mL$^{-1}$. Absorbance of the [Cu(L)(NO$_3$)(H$_2$O)$_2$]$\cdot$H$_2$O follows Beer’s law over a 0.7–25 $\mu$g mL$^{-1}$ range with a detection limit of 0.011 $\mu$g mL$^{-1}$. Validation of the submitted method was established by estimating Cu$^{2+}$ in certified reference materials and actual sea and tap water samples. The results are compared with data obtained from copper concentration measurements using ICP-OES. The chemical structure of the Cu(II)-AMT complex was fully characterized by FT-IR, SEM, EDX, TGA, and ESR techniques.

Keywords: copper ions; spectrophotometry; characterization; water samples

1. Introduction

Copper is a biologically-active element, whose compounds have a strong influence on the vital activities of organisms. Copper plays an essential role in biological processes including metabolism, nerve function, hemoglobin synthesis, and bone development [1–3]. However, an increase in the amount of copper in the human body may lead to serious problems such as heart disease, anemia, and liver damage [4]. Therefore, the copper concentration in environmental samples—especially water—should be monitored continuously, preferably by simple and effective analytical methods. Many analytical techniques including microwave plasma—AES [5], AAS [6], potentiometry [7,8], voltammetry [9], ICP-OES [10], and ICP-MS [11] have been developed and used to monitor copper concentration levels in a variety of samples. Actually, most AAS and ICP-OES methods recently developed use preconcentration methodology to improve sensitivity and selectivity. However, such methodology is highly costly, and time-consuming. Spectrophotometric techniques generally are preferred for metal ion determinations in aqueous media, because of their simplicity, low cost, and availability [12]. Among the many
spectrophotometric reagents used to determine copper are 1,4-dioxane [13], 4-(2’-benzothiazolylazo) salicylic acid (BTAS) [14], 2,4-dinitro APTPT [15], diethylthiocarbamate (DDTC) [16,17], 2-amino-4-(m-tolylazo)pyridine-3-ol (ATAP) [18], 2-(5-Bromo-2-Oxoindolin-3-ylidene) hydrazine carbothioamide (HBITSC) [19], 4-(4’-nitrobenzylideneimino)-3-methyl-5-mercaptop-1,2,4-triazole (NBIMMT) [20], 2-acetylpyridine thiosemicarbazone (2-APT) [21], N,N’bisalicylidene-2,3-diaminopyridine (H2IF) [22], and rhodamin derivative with 5-(a-methyl-3 hydroxybenzylidene) (5M, 3H-BR) [23]. However, most of these reagents suffer from shortcomings such as a narrow pH range, interference from common ions, the need to heat the reaction mixture, and lengthy color development [24]. Thus, there is an urgent need to prepare selective and sensitive chromogenic reagents that can operate over a wide range of concentrations and pH values.

Although reagent 4-amino-3-mercapto-6-[2-(2-thienyl)vinyl]-1,2,4-triazin-5(4H)-one (AMT) has been previously reported [25–28], as far as we know, there is no analytical use of this reagent yet. Therefore, the present work will discuss the preparation and characterization of a copper(II) complex with AMT and the use of this ligand as a spectrophotometric reagent for developing an analytical method for determining copper in certified reference materials and water samples.

2. Experimental

2.1. Chemicals and Reagents

All materials employed in the study were of analytical reagent grade and no further purification was required. Alkali, alkaline earth, and heavy metal nitrates were obtained from Sigma-Aldrich, St. Louis, MO, USA. All solvents were purchased from Fisher Scientific. Deionized water was used exclusively in the experiments. A weighed amount of Cu(NO3)2·3H2O was dissolved in an appropriate volume of deionized water for preparation of stock solution of Cu2+(50 µg mL−1) [29]. Appropriate weights of nitrate salts of some metal ions were dissolved in deionized water for obtaining stock solutions of these ions. The pH of aqueous solutions was adjusted to 4.5 with acetate buffer (CH3COOH/CH3COONa).

2.2. Apparatus

Electronic spectra and absorbance values of the ligand and its Cu complex were recorded using a HACH LANGE (Model DR-6000) spectrophotometer. The elemental compositions of the ligand and its complex with copper were determined using a Perkin Elmer CHNS/O analyzer model 2400 Series II. The ligand and its Cu complex were characterized by FT-IR spectroscopy at 400–4000 cm−1 with an ALPHA attenuated FT-IR spectrometer (Bruker, Billerica, MA, USA). 1H NMR of AMT compound was recorded by FT-NMR spectrometer (A Bruker DRX-500). A JEOL JEM-6390 scanning electron microscope combined with a unit of energy dispersive X-ray spectroscopy was used to record morphological images and to determine elemental distributions in the samples. Measurements of electron spin resonance (ESR) were carried out using a SPINSCAN X spectrometer from ADANI with operating frequency of 9.2–9.55 GHz. Diphenylpicrylhydrazyl (DPPH) was used as a reference to calculate the g-values. The g factor of DPPH is 2.0036. Copper complex with AMT was ground to get fine particles, then the power was placed in 1 mm (ID) sealed capillary tube. All ESR measurements were carrying out at 300 K. Magnetic susceptibility of Cu(II)-AMT complex was measured at 25 ± 0.35 °C by a magnetic susceptibility balance (Johnson Matthey, Alfa product, Model No. (MKI)) calibrated using Hg[Co(NCS)4]. Determination of metal ion concentrations in aqueous solution was carried out by ICP-OES spectrometry (Optima 2100 DV, Perkin Elmer, Waltham, MA, USA). pH measurements were made with a Jenway 3305 pH meter. Thermogravimetric measurements were performed by use of a Mettler-Toledo TGA/DSC1 calorimeter under a nitrogen flow of 60 mL min−1 at 25–900 °C.

2.3. Preparation of the AMT Ligand

AMT ligand was synthesized by the method reported in [26]. Scheme 1 summarizes the procedure. 1H NMR (DMSO-d6): δ 6.48 (s, 2H, NH2), 6.90 (d, 1H, thiophene-CH=CH-triazine),
7.06 (t, 1H, H₅ of thiophene), 7.36 (d, 1H, H₆ of thiophene), 7.59 (d, 1H, H₄ of thiophene), 8.11 (d, 1H, thiophene-CH=CH-triazine), 14.04 (s, 1H, SH). ¹³CNMR (DMSO-d₆); δ 118.0 (thiophene-CH=CH-triazine), 126.7 (thiophene-CH=CH-triazine), 127.7 (C₄ of thiophene), 128.7 (C₃ of thiophene), 129.3 (C₅ of thiophene), 141.0 (C₂ of thiophene), 141.2 (C₆ of triazine), 147.7 (C-SH), 166.8 (C=O). Elemental analysis (%) of AMT calculated for C₉H₈N₄O₅S₂ (252.31) is displayed in Table 2, while the ¹³HNMR and ¹³CNMR spectra of AMT ligand are shown in Supplementary Materials (Figures S1 and S2, respectively).

Scheme 1. Synthesis of AMT.

2.4. Preparation of [Cu(L)(NO₃)(H₂O)₂]•H₂O

1.00 mmol of AMT and 1.00 mmol of CuNO₃•3H₂O were refluxed in methanol at 90 °C for three hours. After cooling the reaction vessel to room temperature (25 ± 0.4), the brown precipitate, [Cu(L)(NO₃)(H₂O)₂]•H₂O, was obtained by filtration in non-pure form. However, the obtained precipitate was purified by recrystallization from methanol, and the pure product was dried at 80–90 °C. Yield: 78%. Elemental analysis (%) of [Cu(L)(NO₃)(H₂O)₂]•H₂O calculated for C₉H₁₄N₅O₇S₂•Cu (431.90) is displayed in Table 2.

2.5. Estimation of Cu(II) in Waters Using Recommended Method

Different volumes of standard copper(II) solutions (0.7–25.0 μg mL⁻¹) were added to 8 mL of a 0.042% (w/v) AMT solution in 10 mL of acetate buffer (pH 4–6). Deionized water was used to dilute all solutions to 50 mL. Absorbance was measured at 434 nm against a reagent blank. 100 mL of tap or sea water was sampled, filtered, and transferred into clean polyethylene bottles. All samples were analyzed within 6 h of collection. Appropriate quantities were treated according to the recommended procedure before and after spiking with known concentrations of Cu(II).

2.6. Evaluation of Proposed Method Using Certified Reference Materials

Multielement standard solution (# 90243) purchased from Sigma-Aldrich was used as a certified reference material to evaluate the proposed method. According to product catalog, this solution contains 10 mg L⁻¹ of Cu, in addition to K, Bi, and Pb, with concentration of 100 mg L⁻¹; Al, B, Cr, Li, Mo, Na, Ni, and Tl at concentration level of 50 mg L⁻¹; while, Ba, Ca, Cd, Co, Fe, Mg, Mn, Sr, and Zn are in concentrations equal to Cu. An aliquot of the solution was analyzed by recommended procedure for estimating Cu after addition of few drops of KF•2H₂O (0.1% m/v) as a masking agent.

The steel sample (No.21899) obtained from Analytical Chemistry Laboratory Service, (München, Germany) was also used as a certified reference material to evaluate the proposed method. The copper content in the sample is 0.519% (w/v). The main components are Cr, Ni, Mn, and Zn, in addition to ultra-traces concentrations of Mo, P, C, S, Si, Tl, V, and Co.

0.2 g of steel sample was heated with 10 mL of aqua regia to near dryness. The sample was then mixed with 5 mL of concentrated H₂SO₄ and heated for 30 min. The resulting solution was neutralized by NaOH and diluted using doubly distilled water in a 100 mL calibrated flask. A part of aqueous solution was subjected to recommended procedure to estimate copper in steel sample.
3. Results and Discussion

A brown product was observed immediately upon mixing the aqueous copper(II) and AMT solutions. The electronic spectrum of the free ligand solution \((1.2 \times 10^{-4} \text{ mol L}^{-1})\) recorded against deionized water as a blank showed two peaks at 254, and 376 nm assigned to \(\pi-\pi^*\) and \(n \rightarrow \pi^*\) transitions, respectively (Figure 1a). The peak at 376 nm shifted to 434 nm after reaction of the AMT reagent with copper(II) (Figure 1b–d). Figure 1 also demonstrates the increase in absorbance with increasing \(\text{Cu}^{2+}\) concentration. Moreover, the electronic spectrum of Cu(II)-AMT complex recorded in solid state using the Nujol mulls method and shown in Supplementary Materials (Figure S3) exhibited a broad band at 630 nm corresponding to \(T_{2g}(2D) \leftrightarrow E_g\) in octahedral environment [30]. The effective magnetic moment \((\mu_{\text{eff}})\) of copper complex with AMT calculated from the Equation (1) was 1.82 B.M confirming the distorted octahedral symmetry of this complex.

\[
\mu_{\text{eff}} = 2.828 (\chi_M T)^{1/2}
\]  

(1)

where, \(\chi_M\) is molar susceptibility modified using Pascal’s constants of all atoms diamagnetism, while \(T\) is absolute temperature.

![Figure 1. Electronic spectra recorded in aqueous media of (a) 4-amino-3-mercapto-6-[2-(2-thienyl)vinyl]-1,2,4-triazin-5(4H)-one (AMT) ligand at concentration of 1.2 \times 10^{-4} \text{ mol L}^{-1} and following addition of the Cu(II)-AMT complex at Cu^{2+} concentrations of (b) 0.70 \mu g/mL, (c) 3.50 \mu g/mL, and (d) 5.50 \mu g/mL.](image)

The stoichiometry of Cu(II)-AMT complex was estimated using the mole ratio method, wherein the absorbance at 434 nm was plotted versus the [AMT]/[Cu^{2+}] ratio as shown in Figure 2. The results indicate that the AMT ligand and Cu^{2+} reacted in a 1:1 ratio.
3.1. Characterization

Techniques including FT-IR, SEM, EDX, TGA, and ESR were used to characterize the complex formed between Cu(II) and AMT.

3.1.1. FT-IR Technique

The FT-IR spectra of AMT and the [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O complex are demonstrated by traces A and B, respectively, in Figure 3. For AMT, a peak due to NH$_2$ was observed at 3291–3195 cm$^{-1}$, aromatic C–H stretching peak was located at 3064–3091 cm$^{-1}$, and the aliphatic C–H stretching peak was observed at 2936 cm$^{-1}$. A peak corresponding to S–H was observed at 2665 cm$^{-1}$. Peaks of C=O, C=C, and C=N were observed at 1660, 1593, and 1618 cm$^{-1}$, respectively. Following differences were observed between the spectrum of AMT and that of its complex with Cu$^{2+}$. The NH$_2$ peak in Cu(II)-AMT shifted to approximately 3142 cm$^{-1}$ and decreased significantly in intensity. This indicates that the NH$_2$ group participated in complex formation. The C=O frequency shifted from 1660 to 1705 cm$^{-1}$ in reference to coordination of AMT to Cu$^{2+}$ through the carbonyl oxygen atom. Peaks due to ν(Cu–N), and ν(Cu–O) were observed at 530, and 518 cm$^{-1}$, respectively. The coordination behavior of the nitrate ion was confirmed through the appearance of characteristic frequencies of bidentate nitrate group at 1545, 1353, and 820 cm$^{-1}$ and the absence of the distinctive band of ionic nitrate group at 1766 cm$^{-1}$ [31]. Therefore, the nitrate group served as a bidentate ligand with C$_2v$ symmetry. Table 1 lists the different FT-IR frequencies of AMT and the [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O complex.

![Figure 3. FT-IR spectra of (A) AMT and (B) [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O.](image-url)
Table 1. FT-IR frequencies (cm\(^{-1}\)) and tentative assignments of AMT and the Cu(II)-AMT complex.

| AMT Reagent | Tentative Assignments | Cu(II)-AMT Complex | Tentative Assignments |
|-------------|-----------------------|--------------------|-----------------------|
| 3291, 3195  | ν(NH\(_2\))           | 3142 weak          | ν(NH)                 |
| 3064–3091 weak | ν(aromatic C–H) | 3066 weak          | ν(aromatic C–H)        |
| 2936 weak   | ν(aliphatic C–H)      | 2901 weak          | ν(aliphatic C–H)       |
| 2665 weak   | ν(SH)                 | 2667 weak          | ν(SH)                  |
| 1660 very strong | ν(C=O)  | 1705 very strong | ν(C=O)                |
| 1593 strong | ν(C=C)                | 1554 strong        | ν(C=C)                 |
| 1618 strong | ν(C=N)                | 1618 strong        | ν(C=N)                 |
| 1401 strong | δ(N–C–S)              | 1421 strong        | δ(N-C-S)               |
| 1420 medium | δ(aliphatic C–H)      | 1412 medium        | δ(aliphatic C–H)       |
| 530 medium  | ν(Cu–N)               | 518 medium         | ν(Cu–O)               |
| 518 medium  |                        |                    | 1545, 1353, 820 Nitrate group |

3.1.2. SEM and EDX Analysis

The SEM images of AMT and its complex with copper(II) shown in Figure 4 provide useful information on surface morphology and particles size. The surface morphology of AMT changed dramatically after its reaction with Cu\(^{2+}\). Free AMT molecules are visualized as platelet–like particles with a clear, and smooth surface (Figure 4A–D). The AMT particles size calculated using Image J program ranged from 4.6 to 8.2 µm. Agglomerated particles with a rough surface are observed in the SEM image of Cu(II)-AMT as shown in Figure 4E–H. The particles size of Cu(II)-AMT complex were in the range of 18.8–31.25 µm. However, the SEM images of Cu(II)-AMT complex displayed a few particles with size less than 10 µm that may have combined to form agglomerates with larger size. The EDX analysis of the AMT ligand revealed three signals corresponding to carbon, oxygen, and sulfur elements (Figure 5A), while the EDX spectrum of copper complex with AMT confirmed the presence of copper in the complex as indicated by the signals corresponding to this element in Figure 5B. Such signals were not found in the EDX spectrum of free AMT (Figure 5A). The elemental analysis of AMT ligand and its complex with Cu(II) is displayed in Table 2.

![Figure 4. Cont.](image-url)
Figure 4. SEM images at a different magnification of (A–D) AMT and (E–H) Cu(II)-AMT.

Figure 5. EDX spectra of (A) AMT and (B) [Cu(L)(NO₃)(H₂O)₂]•H₂O.

Table 2. The elemental analysis of AMT ligand and Cu(II)-AMT complex.

| Element | Weight % Calculated | Weight % Found |
|---------|---------------------|----------------|
| C       | 42.85               | 42.90          |
| H       | 3.21                | 3.16           |
| N       | 22.22               | 22.03          |
| O       | 6.34                | 6.56           |
| S       | 25.42               | 25.81          |

| Element | Weight % Calculated | Weight % Found |
|---------|---------------------|----------------|
| C       | 25.06               | 25.76          |
| H       | 3.27                | 2.45           |
| N       | 16.22               | 15.36          |
| O       | 25.93               | 24.50          |
| S       | 14.81               | 14.44          |
| Cu      | 14.59               | 14.36 *        |

* Cu content in Cu(II)-AMT was determined from EDX measurements.
3.1.3. Thermal Analysis

Thermogravimetric analysis of the ligand occurred in two stages as shown in Figure 6A. The first stage which was because of the loss of one molecule each of NH$_3$ and CO covered the range 60–200 °C. Complete decomposition of ligand occurred throughout the second stage at 200–600 °C. No residue was observed. Scheme 2 shows the proposed path of ligand thermolysis. Thermogravimetric analysis of Cu(II)-AMT involved four stages as shown in Figure 6B and Scheme 3. The loss of one molecule of uncoordinated water was in the first stage and occurred at 27–104 °C with a 4.45% weight loss. Two molecules of coordinated water were lost in the second stage at 120–200 °C with a weight loss of 8.25%. The third stage at 200–528 °C involved partial fragmentation of the ligand moiety and the release of nitrogen oxides amounting to a 63.02% weight loss. Complete decomposition of the complex leaving a residue of copper oxide occurred in the final stage at 528–1000 °C (24.64% weight loss).

![Figure 6. TGA of AMT (A) and [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O (B).](image)

![Scheme 2. Suggested thermal decomposition of AMT.](image)
3.1.4. Electron Spin Resonance of [Cu(L)(NO$_3$)(H$_2$O)$_2$]$\bullet$H$_2$O

The powdered ESR spectrum of the Cu-AMT complex measured at 300 K showed two bands at 330 and 310 mT with $g_{\perp} = 2.08306$ and $g_{||} = 2.2175$, respectively (Figure 7). The $g$-values confirmed that the unpaired electron of Cu(II) occupies the orbital of $d_{x^2-y^2}$, where $g_{||}$ is greater than both $g_{\perp}$ and $g$ of free electron (2.0023) [32,33]. The shape of the ESR spectrum suggests a distorted octahedral symmetry for the Cu-ATM complex [32] in excellent agreement with $\mu_{\text{eff}}$ value. The $g_{\text{av}}$ value calculated from Equation (2) was equal to 2.1279. Deviation of $g_{\text{av}}$ from $g$ of free electron was because of the covalent property of bonding in Cu-ATM [33].

$$g_{\text{av}} = \frac{1}{3}(g_{||} + 2g_{\perp}) \quad (2)$$

Figure 7. Electron spin resonance of [Cu(L)(NO$_3$)(H$_2$O)$_2$]$\bullet$H$_2$O. Two arrows indicate the significant peaks in spectrum.

A molar conductivity of $\Lambda_m = 8.2 \, \Omega^{-1} \, \text{cm}^2 \, \text{mol}^{-1}$ measured at $1.00 \times 10^{-3} \, \text{M}$ in DMF solvent at room temperature indicated that the Cu-ATM complex is a non-electrolyte [34]. Full characterization of the
complex formed between Cu$$^{2+}$$ and AMT suggests the chemical formula to be [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O. Figure 8 shows the proposed structure of the complex.

Figure 8. Chemical structure of [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O complex. L represents AMT molecules with NH group.

### 3.2. Analytical Application

The brown complex formed between Cu$$^{2+}$$ and AMT was used to develop an analytical procedure for monitoring the copper concentration in environmental samples.

#### 3.2.1. Optimization of the Recommended Procedure

The relationship between solution pH and the absorbance of [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O at $\lambda$$_{\text{max}}$ = 434 nm was examined at various pH values using HCl/NaOH and acetate buffer (CH$_3$COOH/CH$_3$COONa). The results in Figure 9 show that the maximum absorbance of [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O occurred at pH 4–6 in acetate buffer. The absorbance is less at pH $\leq$ 3, because the protonation equilibrium of the reagent shifted to the left as displayed in Scheme 4 producing protonated form (H$_2$L). Therefore, the deprotonated form of the reagent (HL) is required for complex formation. The absorbance of the Cu(II)-AMT complex decreased dramatically above pH 6, a behavior most likely attributed to the hydrolysis of Cu(II)-AMT and the formation of colorless forms of copper(II) such as hydroxo species. Thus, the acetate buffer with pH 4–6 was chosen for further experiments.

Figure 9. Influnece of solution pH on the absorbance of [Cu(L)(NO$_3$)(H$_2$O)$_2$]•H$_2$O at $\lambda$$_{\text{max}}$ = 434 nm. Copper(II) concentration = 2 $\mu$g mL$^{-1}$; [AMT] = 1.20 x 10$^{-4}$ M.
The absorbance of the complex was constant at $[\text{AMT}] = 6.5 \times 10^{-5}$ mol L$^{-1}$, but increased at concentrations above $2.2 \times 10^{-4}$ mol L$^{-1}$. A small decrease in absorbance was seen above $2.8 \times 10^{-4}$ mol L$^{-1}$, possibly because excess chromogenic reagent reduced the extent of complex formation by increasing the acidity of the solution [35]. Thus, the AMT concentration was fixed at $2.8 \times 10^{-4}$ mol L$^{-1}$ for all measurements.

3.2.2. Investigation of Method Selectivity

The potential impact of common foreign ions on the estimation of copper was tested at optimized conditions and in the presence of $2 \mu$g mL$^{-1}$ Cu(II) concentration — probably due to complex formation with AMT—as shown in Table 3. However, these interferences were eliminated by addition of 0.5 mL KF·2H$_2$O (0.1 mol L$^{-1}$), which reduced the relative error to an acceptable ±5% level at an interfering ion concentration of 100 $\mu$g mL$^{-1}$.

![Scheme 4. Chemical equilibrium of AMT reagent.](image)

**Figure 10.** The relationship between the concentration of AMT reagent and the absorbance of $[\text{Cu(L)}(\text{NO}_3)(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$ at 434 nm. Cu(II) concentration = 2 $\mu$g mL$^{-1}$.

odore of AMT reagent. 

$\text{N}^+\text{H}_3$ $\text{NH}_2$

$\text{NH}_3$

Scheme 4. Chemical equilibrium of AMT reagent.

The absorbance of $[\text{Cu(L)}(\text{NO}_3)(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$ at $\lambda_{\text{max}}$ was significantly influenced by a change in AMT concentration from $4.0 \times 10^{-4}$ to $6.7 \times 10^{-5}$ M at constant Cu$^{2+}$ concentration (Figure 10). The absorbance of the complex was constant at $[\text{AMT}] = 6.5 \times 10^{-5}$ to $2.2 \times 10^{-4}$ mol L$^{-1}$, but increased at concentrations above $2.2 \times 10^{-4}$ mol L$^{-1}$. A small decrease in absorbance was seen above $2.8 \times 10^{-4}$ mol L$^{-1}$, possibly because excess chromogenic reagent reduced the extent of complex formation by increasing the acidity of the solution [35]. Thus, the AMT concentration was fixed at $2.8 \times 10^{-4}$ mol L$^{-1}$ for all measurements.

![Figure 10. The relationship between the concentration of AMT reagent and the absorbance of $[\text{Cu(L)}(\text{NO}_3)(\text{H}_2\text{O})_2] \cdot \text{H}_2\text{O}$ at 434 nm. Cu(II) concentration = 2 $\mu$g mL$^{-1}$.](image)
Table 3. Influence of some foreign ions when applying the developed spectrophotometric procedure for the estimation of 2 µg mL⁻¹ Cu(II). [AMT] = 1.665 × 10⁻³ mol L⁻¹; relative error: ±5%.

| Coexisting Ion | Diverse Ion/Copper(II) | Coexisting Ion | Diverse Ion/Copper (II) |
|---------------|------------------------|---------------|-------------------------|
| Na⁺          | 1200                   | Ca²⁺         | 100                     |
| K⁺           | 1000                   | Ni²⁺         | 100                     |
| Li⁺          | 1000                   | Ba²⁺         | 100                     |
| Zn²⁺         | 1000                   | Cr³⁺         | 100                     |
| Ca²⁺         | 1000                   | Pb²⁺         | 100                     |
| Ag⁺          | 1000                   | Sr²⁺         | 100                     |
| F⁻           | 500                    | NO₂⁻         | 200                     |
| Cl⁻          | 500                    | Mn²⁺ *       | 5                       |
| Bi³⁺         | 300                    | Al³⁺ *       | 5                       |

* The interference was eliminated by use of KF•2H₂O as a masking agent.

3.2.3. Analytical Performance of the Recommended Procedure

The plot of Cu(II) concentration versus absorbance is linear over a range of 0.7–25 µg mL⁻¹. The linear equation that describes the calibration curve of our developed method is:

$$A = 0.18 C - 0.009 \quad (R^2 = 0.992, \ n = 10)$$

where, A and C are absorbance, and concentration of copper(II) solution, respectively. Sandell’s factor, and the molar absorptivity (ε) of the proposed method are 0.003 µg cm⁻², and 1.9 × 10⁴ L mol⁻¹ cm⁻¹, respectively. LOD calculated using the equations in [21] is 0.011 µg mL⁻¹ (11.00 µg L⁻¹). Relative standard deviation (RSD) and relative error (RE) of the spectrophotometric method calculated from ten replicate measurements of copper recovery at 2 µg mL⁻¹ in distilled water were 1.4% and 1.2%, respectively. Variables demonstrating the effectiveness of the analytical method are listed in Table 4.

Comparison of these analytical features with those of previously-published spectrophotometric methods in Table 5 establishes the good sensitivity and wide linear dynamic range of our method. Moreover, the analytical performance of the proposed method, in terms of selectivity, sensitivity, and dynamic concentration range, was compared with a wide variety of analytical techniques [5–9,36–39]. The spectrophotometric method developed in the present study provides better sensitivity and selectivity than some the methods mentioned in Table 6, without the need to use the extraction or preconcentration methodology. The dynamic concentration range of the proposed method covers a wide domain of concentrations including the maximum values of copper in drinking waters recommended by the U.S. Environmental Protection Agency [EPA] (1 mg L⁻¹) and the WHO (2 mg L⁻¹). Moreover, the proposed chromogenic reagent can operate at a wide range of pH values. Therefore, the suggested spectrophotometric method is appropriate for rapid and routine analyzes in many laboratories.

Table 4. Summary of the analytical performance of the spectrophotometric method.

| Variable                  | Value     |
|---------------------------|-----------|
| Wavelength, \( \lambda_{\text{max}} \) (nm) | 434       |
| pH                        | 4.5       |
| Temperature (°C)          | 25 ± 2    |
| Linear dynamic range (µg/mL) | 0.7–25   |
| LOD, µg/mL                | 0.011     |
| RSD, % (\( n = 10 \))     | 1.4       |
| RE, % (\( n = 10 \))      | 1.2       |
| Slope                     | 0.18      |
| Intercept                 | 0.009     |
| ε, (L/moL/cm)             | 1.9 × 10⁴ |
| Sandell’s factor (µg cm⁻²)| 0.003     |
| Correlation factor (R²)   | 0.999     |
Table 5. Comparison between the proposed method and previously-published spectrophotometric methods to estimate Cu(II) *

| Chromogenic Reagent | \( \lambda_{\text{max}} \) (nm) | pH | Molar Absorptivity, L/mol.cm | Linear Dynamic Range \( \mu g/mL \) | Detection Limit, (LOD), \( \mu g/mL \) | Interferences | Ref. |
|---------------------|-----------------|----|-----------------|-----------------|-----------------|----------------|-----|
| Cefixime            | 336             | 4.68 | 8.29 \times 10^3 | 1.015-8.122     | 0.032           | \( \text{Hg}^{2+},\text{Al}^{3+},\text{Zn}^{2+},\text{Fe}^{3+},\text{Mn}^{2+} \) | [13] |
| BTAS                | 485             | 5.0  | 2.35 \times 10^4 | 0.63-5.04       | 7 \times 10^{-3} | \( \text{Sn}^{2+},\text{Cd}^{2+},\text{Co}^{2+} \) | [14] |
| 2,4-dinitro APTPT   | 445             | 8.7  | 0.87 \times 10^3 | 10-80           | 1.72            | \( \text{Sb}^{3+},\text{Mn}^{2+},\text{Ag}^{+},\text{Pb}^{2+},\text{Co}^{2+},\text{Hg}^{2+} \) | [15] |
| DDTC                | 435             | 5    | 2.86 \times 10^5 | 0.2-12          | 0.2             | \( \text{Fe}^{2+},\text{Ni}^{2+},\text{Pb}^{2+},\text{Mn}^{2+} \) | [16] |
| DDTC                | 435             | 5    | 3.61 \times 10^5 | 0.02-12.0       | 0.029           | \( \text{Ag}^{+},\text{Pb}^{2+} \) | [17] |
| ATAP                | 608             | 4.5  | 4.37 \times 10^5 | 4 \times 10^{-3}-0.115 | 1.2 \times 10^{-3} | \( \text{Fe}^{3+},\text{Sb}^{3+},\text{Mo}^{5+},\text{NO}_2^- \) | [18] |
| HBITSC             | 510             | 4    | 2.5 \times 10^3  | 1-8             | NM              | \( \text{Co}^{2+},\text{Fe}^{3+},\text{Fe}^{3+},\text{Ni}^{2+},\text{Cr}^{3+} \) | [19] |
| NBIMMT             | 470             | 6.2  | 2.8 \times 10^3  | 4.75-16.13      | NM              | \( \text{Pb}^{2+},\text{Cd}^{2+},\text{Sn}^{2+},\text{Fe}^{2+},\text{Zn}^{2+},\text{Ag}^+,\text{Al}^{3+} \) | [20] |
| 2-APT              | 370             | 8    | 2.14 \times 10^4 | 0.16-1.3        | 0.053           | NM             | [21] |
| H2IF               | 414             | 4.8  | 1.46 \times 10^5 | 6.35 \times 10^{-3}-0.318 | 6.38 \times 10^{-3} | There is no interference at 1:10 ratio | [22] |
| 5M, 3H-BR          | 430             | 5.5  | 0.603 \times 10^4 | 0.05-13         | NM              | There is no interference at 1:10 ratio | [23] |
| Rubeanic acid      | 380             | 3.5  | 1.01 \times 10^4 | 0.65-2.65       | NM              | \( \text{Co}^{2+},\text{Ni}^{2+} \) | [4] |
| CBIMMT             | 414             | 4.2  | 3.38 \times 10^3 | 5-17.5          | NM              | \( \text{Ni}^{2+},\text{Pb}^{2+},\text{Al}^{3+},\text{Bi}^{3+} \) | [37] |
| AMT                | 434             | 4-6  | 1.9 \times 10^4  | 0.7-25          | 0.011           | \( \text{Mn}^{2+},\text{Al}^{3+} \) | PW |

* CBIMMT: 4-(4’-chlorobenzylideneimino)-3-methyl-5-mercapto-1,2,4-triazole; NM: not mentioned; PW: present work.

Table 6. Comparison between the proposed method and various previously published analytical techniques to estimate Cu(II).

| Analysis Technique | pH   | LOD, \( \mu g/mL \) | Concentration Range, \( \mu g/mL \) | Interferences | Ref. |
|-------------------|------|----------------------|-------------------------------------|----------------|-----|
| Micro SPE-AAS     | 3    | 0.022                | 0.05-1                              | \( \text{Fe}^{3+},\text{Ni}^{2+},\text{Co}^{2+},\text{Zn}^{2+} \) | [5] |
| AAS with high resolution continuum source | Acidity medium            | 0.021-1.4 *                     | 0.07-100 *                           | NM             | [6] |
| Potentiometry using modified carbon paste electrode | 3 | 9.2 \times 10^{-5} | 2.22 \times 10^{-4}-635 | \( \text{Hg}^{2+},\text{Cd}^{2+},\text{Ag}^+ \) | [7] |
| Potentiometry using modified carbon paste electrode | 4.5-8.5  | 0.057                | 0.081-812 | \( \text{Fe}^{3+},\text{Cd}^{2+},\text{Al}^{3+},\text{Ca}^{2+},\text{Cr}^{3+} \) | [8] |
| Voltammetry using modified carbon paste electrode | 1.4 | 0.017                | 0.1-1                               | \( \text{Hg}^{2+},\text{Cd}^{2+},\text{Pb}^{2+} \) | [9] |
| AAS combined with SPE | 5     | NM                   | 0.15-2                              | \( \text{Al}^{3+},\text{Sn}^{2+},\text{Ni}^{2+},\text{Mn}^{2+} \) | [37] |
| AAS combined with SPE | 5    | 0.01                 | 0.01-0.34                           | There is no interference at 1:10M ratio | [38] |
| AAS combined with SPE | 5.6  | 0.3                  | NM                                  | \( \text{Fe}^{3+},\text{Co}^{2+} \) | [39] |
| Direct spectrophotometry | 4-6   | 0.011                | 0.7-25                              | \( \text{Mn}^{2+},\text{Al}^{3+} \) | PW |

* Depending on the used atomic lines.
3.2.4. Evaluation of the Recommended Procedure

Our spectrophotometric procedure was effectively employed for the analysis of Cu in multielement standard solution (# 90243), and steel sample (No.21899) as certified reference materials, in addition to tap water (Taif City, Saudi Arabia) and sea water (Red Sea, Jeddah Governorate, Saudi Arabia). Copper concentrations in multielement solution and steel sample estimated by the proposed spectrophotometric procedure were 9.91 ± 0.27 mg L⁻¹ and 0.475% (w/w), respectively, with small differences from certified values. However, Student’s t-test revealed no significant differences between the concentrations determined by spectrophotometric method and certified values at the 95% confidence level since the tabulated t-value (2.78) was always greater than the calculated values (2.61, 2.55) for five replicate measurements. Water samples were spiked with a known concentration of copper as shown in Table 7, and the recovery method was determined. Acceptable recoveries were obtained, which confirms the accuracy and applicability of the method for copper determination in water samples. A comparison of the results of our spectrophotometric method with those of the standard ICP-OES indicates analytically-acceptable agreement between these procedures. Therefore, the proposed method can be used for the rapid and sensitive detection of Cu(II) in water samples. The rapid color development due to reaction of AMT with copper(II) can be detected by the naked eye, which makes the proposed method suitable for the qualitative analysis of copper(II) in a variety of samples.

Table 7. Evaluation of proposed spectrophotometric method by determining copper in water samples *.

| Sample          | Spectrophotometric Method | ICP-OES |
|-----------------|---------------------------|---------|
|                 | Amount of Added Cu(II)    | Found   | Recovery % | Amount of Added Cu(II) | Found   | Recovery % |
|                 | (µg mL⁻¹)                 | (µg mL⁻¹)|            | (µg mL⁻¹)             | (µg mL⁻¹)|            |
| Red Sea water   | ND                        | 3.79 ± 0.31 | 95.0       | ND                    | 3.62 ± 0.22 | 90.5       |
| Red Sea water   | 4                         | 3.79 ± 0.31 | 95.0       | 4                     | 3.62 ± 0.22 | 90.5       |
| Tap water       | ND                        | 3.95 ± 0.23 | 98.7       | ND                    | 3.78 ± 0.15 | 95.0       |
| Tap water       | 4                         | 3.95 ± 0.23 | 98.7       | 4                     | 3.78 ± 0.15 | 95.0       |

* ND: not detected.

4. Conclusions

The present study describes the first synthesis and characterization of the [Cu(L)(NO₃)(H₂O)₂]•H₂O complex. The AMT ligand is a bidentate N,O donor, which coordinates with Cu²⁺ in 1:1 ratio. Full characterization of the [Cu(L)(NO₃)(H₂O)₂]•H₂O complex suggests the octahedral structure shown in Figure 8. The reaction between AMT and copper(II) was used to develop an effective spectrophotometric procedure for the determination of copper in water samples. The developed method is simple, low cost, and sensitive with LOD of 11.00 ppb. The proposed chromogenic reagent operates at a wide range of concentrations and pH values. On the other hand, the developed method can work as a sensitive chemo sensor for copper monitoring in water samples since color change due to reaction of AMT with Cu(II) appears rapidly within less than 10 s and remains stable for up to 2 h.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-3417/10/11/3895/s1, Figure S1: 1H NMR spectrum of AMT ligand, Figure S2: 13C NMR spectrum of AMT ligand, Figure S3: The electronic spectrum of [Cu(L)(NO₃)(H₂O)₂]•H₂O.

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