

Synthesis of 6-(Phenanthrene-3-yl)-2,3-dihydro-1,2,4,-triazine-3-thione as a New Reagent for the Non-Extractive Spectrophotometric Determination of Cu(II)

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ABSTRACT: A simple, sensitive, and non-extractive spectrophotometric method was developed for the determination of copper (II) using a newly synthesized chromogenic reagent, 6-(phenanthrene-3-yl)-1,2,4,-triazine-3-thione(PhDDT), in Tween 80 neutral surfactant. To synthesize the reagent (PhDDT), amyl nitrite was added to 3-acetylphenanthrene and refluxed for 26 hours to produce 2-oxo-2-(phenanthren-3-yl) acetaldehyde oxime (I). The reaction of compound (I) with thiosemicarbazide yielded the reagent (PhDDT). Copper (II) forms a red colored complex with 6-(phenanthrene-3-yl)-1,2,4,-triazine-3-tion which shows maximum absorbance at 460 nm in the pH range 9.0-11.0. Beer's law was obeyed over the concentration range of 1.4-10 $\mu\text{g/mL}$ Cu^{2+} with $r^2 = 0.999$. The limit of detection was 0.48 $\mu\text{g/mL}$ of copper (II). The within-day and between-day precision values were in the range of 0.38-3.3%. The method was tested by analyzing the amalgam samples. The results were in good agreement with the atomic absorption.

KEYWORDS: Spectrophotometric method; Copper determination; 6-(phenanthrene-3-yl)-1,2,4,-triazine-3-thione; Micellar media.

INTRODUCTION

Copper is widely distributed in nature and is a nutritionally essential element. However, higher concentrations can cause adverse health effects. Studies have suggested that copper has an important role in the prevention of certain neurodegenerative diseases including Parkinson, Wilson and Alzheimer Disease (1). Copper ions have also been reported to have antimicrobial activities against a wide range of microorganisms. It has been used in cosmetic products like sun tan lotions, after-shave

lotions, and in healing products (2-5). As copper has a high electrical and thermal conductivity, it is wildly used in electronics. On the other hand, ingestion of large amounts of copper salts may produce toxic effects from nausea, vomiting, and diarrhea to hepatic necrosis and death (6). Hence, there is a great need to develop simple and convenient methods for the determination of copper (II) in real samples. Many of the methods that have been developed for Cu (II) determination are either time

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consuming or require complicated and expensive instruments. Spectrophotometry using selective and sensitive chromogenic reagents is a useful technique for the determination of copper (II) and many other cations in trace amounts (7-13). It has been reported that 6-(2-naphthyl)-1,2,4-triazine-3-thione forms a colored complex with Cu^{2+} which could be extracted with chloroform in basic media (14). We synthesized 6-(phenanthrene-3-yl)-1,2,4-triazine-3-thione in our lab as a possible chromogenic reagent for spectrophotometric determination of copper. In the present study, we report the synthesis of a new reagent and the development and validation of a spectrophotometric non-extractive method for the determination of Cu^{2+} in real samples.

EXPERIMENTAL SECTION

Instrumentation

Absorbance measurements were carried out by means of a Shimadzu 160A (Japan) UV-VIS spectrophotometer, using 1cm quartz cells. A model pH meter was used for the pH adjustments. Melting points were determined on a Kofler hot stage apparatus and are uncorrected.

The $^1\text{H-NMR}$ spectra were obtained on a Varian 400 Unity Plus and chemical shifts (δ) were determined in ppm relative to the internal tetramethylsilane. Mass spectra were obtained on a Finnigan MAT TSQ70 spectrometer at 70 eV.

Materials and reagents

Sodium hydroxide, amyl nitrite, 3-acetylphenanthrene, thiosemicarbazide, and tween 80 were purchased from Merck (Germany). Methanol and Chloroform were of analytical grade and purchased from Duksan (Korea). Britton-Robinson buffer was used in a pH range of 2 to 12 and NaOH was used for pH 13 and 14. Glass vessels were cleaned by soaking in an acidified solution of $\text{K}_2\text{Cr}_2\text{O}_7$ and rinsed several times with deionized water.

Copper nitrate solution

A stock standard solution of copper was prepared by dissolving pure elemental copper (exactly 0.500 g) in 50 mL hot concentrated HNO_3 . After cooling, 50 ml HNO_3 (1:1) was added and the volume was adjusted to 500 mL by addition of deionized water to reach a final concentration of 1 mg/mL. Standard working solutions of Cu^{2+} were prepared by suitable dilution of the stock solution with water.

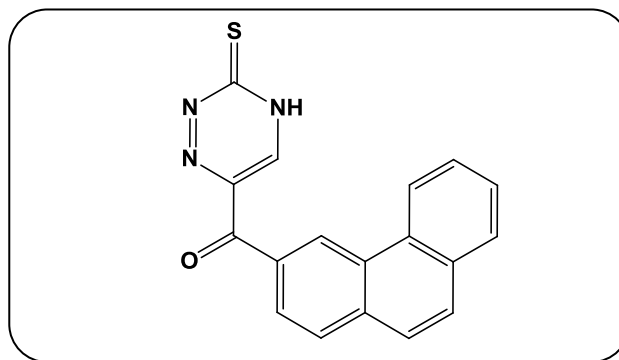


Fig. 1: Chemical structure of 6-(phenanthrene-3-yl)-2,3-dihydro-1,2,4-triazine-3-thione

Reagent solution

6-(phenanthrene-3-yl)-2,3-dihydro-1,2,4-triazine-3-thione (PhDTT) (Fig. 1) solution was prepared by dissolving an accurately weighed amount of the pure reagent in methanol. The reagent should be freshly prepared before use.

Synthesis of 6-(phenanthrene-3-yl)-2,3-dihydro-1,2,4-triazine-3-thione

The reagent, 6-(phenanthrene-3-yl)-2,3-dihydro-1,2,4-triazine-3-thione (PhDTT) was synthesized according to the method reported for 6-(2-naphthyl)-2,3-dihydro-1,2,4-triazine-3-thione (14). 3-acetylphenanthrene was added to amyl nitrite in sodium ethoxide and refluxed at 40°C for 26 hours under anhydrous conditions. The resulting oxime (2-oxo-2-(phenanthren-3-yl)acetaldehyde oxime) was extracted with ether in an acidic medium and crystallized from ethanol-water to yield 65-70% of compound I (Scheme 1).

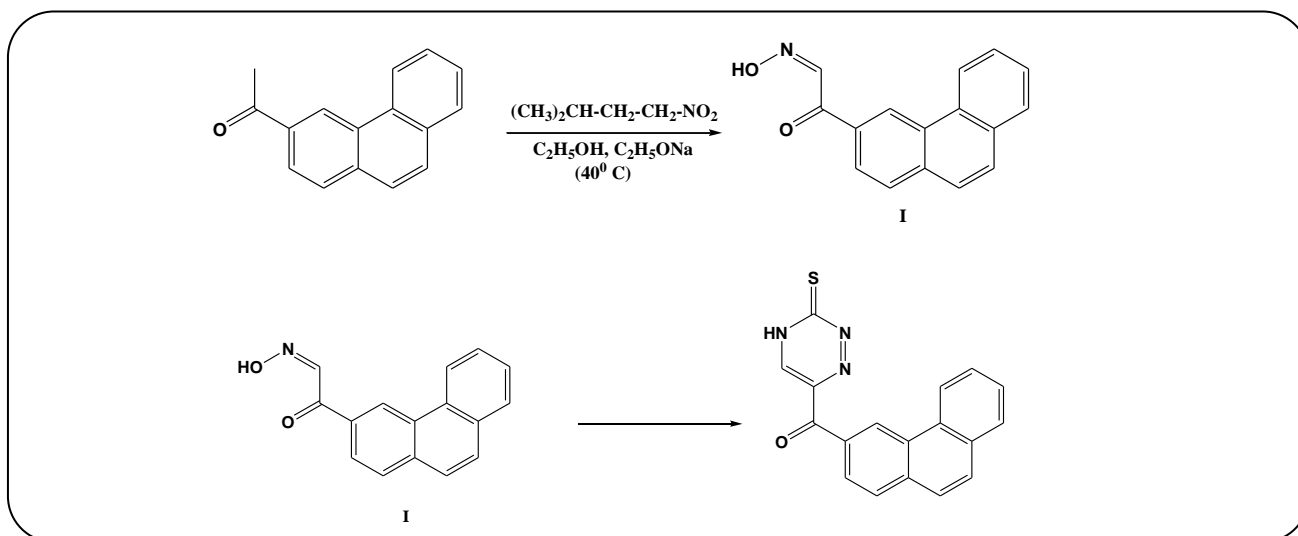
(m.p. $142\text{-}143^\circ\text{C}$). IR (KBr, cm^{-1}): 3231 (N-H, O-H), 1675 (C=O).

$^1\text{H-NMR}$ (DMSO- d_6 , 80 MHz) δ : 9.49 (s, 1H, H4), 8.88-8.56 (m, 1H, H5), 8.34-7.34 (m, 8H, 7H-aromatic and CH).

Ms (m/e, %): 249 (M^+ , 50), 205 (75), 175 (100), 148 (35), 95 (25), 81 (35), 55 (90).

The reaction of compound I with thiosemicarbazide in 0.2 M hydrochloric acid and reflux for 3 hours afforded the reagent PhDTT in 35-45% yields.

m.p.: 170°C . $^1\text{H-NMR}$ (DMSO- d_6 , 80 MHz) δ : 9.03-8.91 (m, 1H, H4), 8.78-8.45 (m, 1H, H5), 8.15-7.58 (m, 5H, H1, H2, H6, H7, H8), 7.51-7.32 (m, 2H, H9, H10), 5.16 (s, 1H, CH).



Scheme 1: Synthesis of 6-(phenanthrene-3-yl)-2,3-dihydro-1,2,4-triazine-3-thione.

Ms (m/e, %): 289 (M⁺, 18), 216 (18), 202 (100), 199 (37), 176 (18), 150 (12), 101 (18), 60 (30).

General procedure

A mixture of 1 mL standard solution of Cu²⁺, 1 mL PhDTT solution, 1 mL buffer solution (pH=10.0) and 1 mL Tween 80 (3% in methanol) was added to a 5 mL volumetric flask and adjusted to the volume with methanol. The solution was mixed thoroughly and the absorbance was measured at 460 nm against the blank reagent.

The optimal pH was determined according to the general procedure using 20 µg/mL standard solution of Cu²⁺ and buffer solution (pH = 3-14) adjusted to the volume with methanol.

The effect of different surfactants was evaluated using varying amounts (1,2,3,4,5 and 6 mL) of sodium lauryl sulfate (0.2%), cetrimide (0.1%) or Tween 80 (3%).

Composition of the complex

The stoichiometry composition of the complex between PhDTT and Cu²⁺ was examined by the limiting logarithmic method. For this method, two sets of experiments were carried out employing the general procedure described above. The first set of experiments was conducted using increasing amounts of Cu²⁺ (7.85×10^{-5} – 4×10^{-7} M) at a fixed PhDTT concentration (7.9×10^{-3} M). The second set of experiments was carried using increasing PhDTT concentrations (1.6×10^{-3} – 3.2×10^{-3} M) at a fixed Cu²⁺ concentration (3.15×10^{-4} M). The logarithm of the absorbance intensity of the Cu (II)-PhDTT complex

was plotted as a function of the logarithms of the Cu²⁺ and PhDTT concentration in the first and second set of experiments, respectively. The slope of the fitting line was calculated in both sets of experiments.

Method validation

Linearity (Beer's law)

The linearity of the proposed method was examined by replicate analysis (n=6) of six standard copper solutions (1.4, 3, 6, 7, 8 and 10 µg/ mL) according to the general procedure.

We measured the absorbance of the copper complexes with PhDTT versus the blank reagent. The absorbance was plotted against the Cu concentration at selected λ_{\max} and the validity of the Beer's law was confirmed.

Accuracy and precision

To establish the reliability of the proposed method, a series of solutions containing 1.4, 7 and 10 µg/mL of the copper (II) solution were analyzed using the proposed method. To obtain the accuracy of the method, the percentage of deviation between added amounts and measured concentrations of the above solutions was calculated.

Analysis of dental samples

In this stage, 44.6 mg of the dental alloy 110-PLUS containing Ag (45%), Sn (30%), and Cu (25%) was dissolved in 50 mL concentrated nitric acid. The resulting solution evaporated to a small volume (5mL) and diluted to 50 mL with distilled water. Further dilutions were made

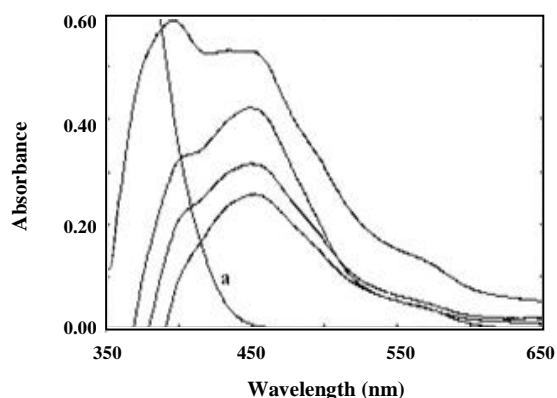


Fig. 2: Absorbance spectra of (a) PhDTT reagent and Cu^{II} -PhDTT complex in four concentrations (1, 3, 4, 7 $\mu\text{g/mL}$).

to reach a concentration of 44.6 $\mu\text{g/mL}$ of alloy containing 25% Cu (11.15 $\mu\text{g/mL}$). A 1-mL aliquot was drawn from this final solution and the general procedure was followed to analyze the Cu^{2+} concentration.

RESULTS AND DISCUSSION

In the present study, a newly synthesized reagent, 6-(phenanthrene-3-yl)-2,3-dihydro-1,2,4-triazine-3-thione (PhDTT) (Fig. 1), was used as a chromogenic reagent to determine trace amounts of Cu^{2+} in aqueous samples. The reagent was synthesized based on our recent studies for preparing some sensitive complexing reagents for determination of trace amounts of some cations such as Cu^{2+} , Ni^{2+} , and Hg^{2+} (15-18). The reacting moiety, triazine-3-thione, was left intact and the chromogenic group was changed. PhDTT produces a yellow to orange color via complexation with Cu^{2+} in methanol.

The absorbance spectra of the reagent and Cu^{II} -PhDTT complex are shown in Fig. 2. The spectra of the complex were recorded against the reagent as a blank over the range of 350-650 nm, which showed maximum absorbance at 460 nm. Comparison between ligand and complex spectra showed that a bathochromic shift was produced upon complexation of the ligand with Cu.

Optimization of experimental parameters (effect of experimental variables)

Effect of solvent

Different solvent systems (acetonitrile, water, ethanol, and methanol) were checked to evaluate the solubility of the reagent and complex and acceptable maximum

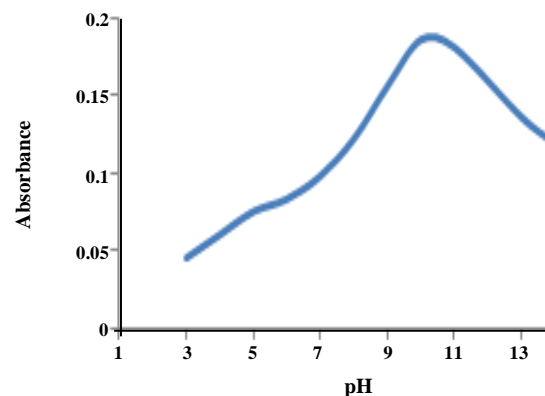


Fig. 3: Effect of pH on the absorbance of Cu^{II} -PhDTT complex.

absorbance. The PhDTT reagent was not soluble in acetonitrile, water, and ethanol. It was soluble in 0.1 M NaOH but its absorption spectra showed a bathochromic shift which could overlap with the complex spectra. The best results were obtained with methanol. Therefore, methanol was selected as a suitable solvent.

Effect of pH

The absorbance of the Cu^{II} -PhDTT complex was determined at 460 nm in the pH range of 3-14. As shown in Fig. 3, maximum absorbance was obtained in the pH range 10-11. Therefore, the study was carried out in pH=10.0.

Effect of surfactant

A cationic (cetrimide), anionic (sodium lauryl sulfate), and non-ionic (tween 80) surfactant was examined for micelle media formation to increase the solubility of both the ligand and the complex and enhance the absorbance. The absorbance was measured according to the general procedure. It was found that solutions containing 2 mL Tween 80 (3% solution), increased the complex absorbance.

Stoichiometry of the reaction

The stoichiometry of the reaction was studied using the limiting logarithmic method. The two sets of experiments were carried out using increasing concentrations of the PhDTT at a fixed metal ion concentration and varying concentrations of the metal ion at a fixed PhDTT concentration. The logarithms of

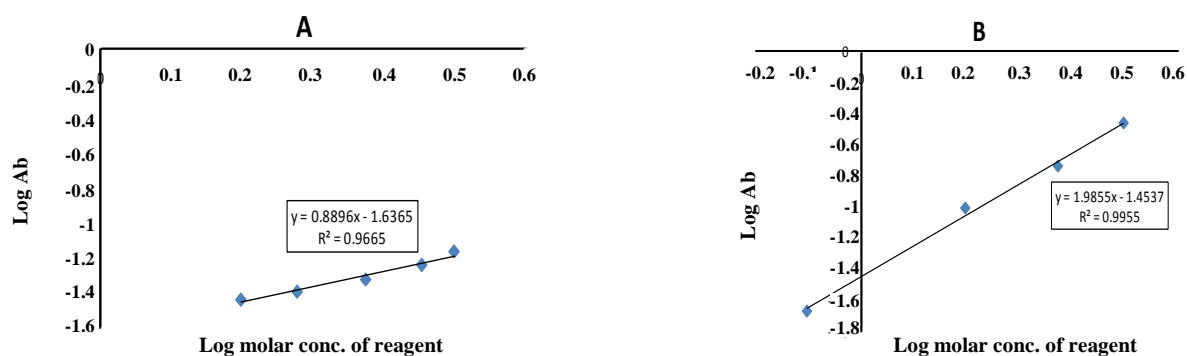


Fig. 4: Limiting Logarithmic plot.

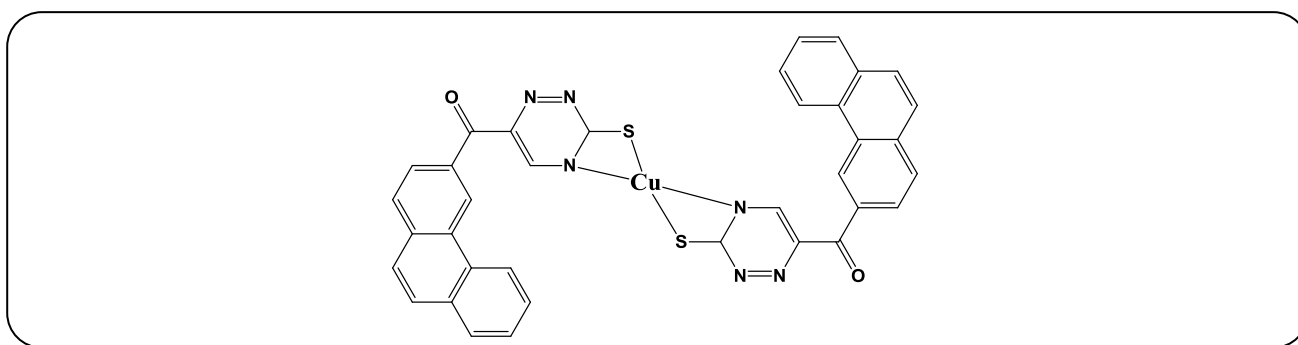


Fig. 5: The proposed structure of the 1:2 Cu-PhDTT complex.

the Cu(II)-PhDTT absorbance were plotted against the logarithms of the concentrations of PhDTT and Cu^{2+} in the first and second sets of experiments. The slope of the lines was 1.986 and 0.896 respectively and the ratio of the reaction was 2:1 (Fig. 4). The recommended structure of the Cu-PhDTT complex is proposed in Fig. 5.

Limit of detection and limit of quantification

The minimum quantity of the metal ion that could be distinguished from the blank (absence of metal ion) is calculated as $3 \times \text{SD}$ of the blank, which was equal to $0.8 \mu\text{g/mL}$ for Cu^{2+} .

The limit of quantification is the lowest concentration of the analyte that can be determined with acceptable precision and accuracy under the stated conditions of the test (19-20). Based on the SD of the response and slope, the LOQ was $1.4 \mu\text{g/mL}$ of Cu^{2+} .

Method Validation

The proposed method was validated according to the ICH guideline for linearity, the limit of detection, the limit of quantification precision, accuracy, and specificity.

Linearity

A calibration curve was plotted between the absorbance of the Cu-PhDTT complex and Cu^{2+} concentration at $\lambda_{\text{max}} = 460 \text{ nm}$. Beer's law was obeyed within the concentration range of $1.4 - 10 \mu\text{g/mL}$ of Cu^{2+} . The results of the correlation coefficient (0.999), SD of intercept (0.0141), and RSD value of the slope (2.52), which are presented in Table 1, indicate good linearity of this method.

The intra-day and inter-day precision were studied by three replicated measurements at three Cu^{2+} concentration levels in the same day and over a period of three consecutive days. The accuracy and precision of the proposed method were studied using standard copper solutions covering low, medium, and high concentrations ($1.4, 7$ and $10 \mu\text{g/mL}$) of the linearity range. According to Table 2, the results of accuracy (error % < 1.5) and precision (RSD % < 3.3) showed that this method had good repeatability and reproducibility during one run and between different runs.

Application

In order to evaluate the applicability of this method, the percentage of copper in synthetic mixtures and real

Table 1: Analytical parameters of calibration curve of Cu^{II}- PhDTT complex (n=6).

Parameters	Copper
Wavelength λ_{\max}	460 nm
Linearity	1.4 – 10 $\mu\text{g/mL}$
Regression equation	$y = 0.0973X - 0.0996$
SD of slope	2.47×10^{-3}
RSD of slope	2.52
SD of intercept	0.0141
Correlation coefficient	0.999
Detection limit	0.48 $\mu\text{g/mL}$

Table 2: Accuracy and precision data of the determination of copper (1.4-10 $\mu\text{g/mL}$).

Added $\mu\text{g/mL}$	Within-day (n=3)			Between-day (n=9)		
	Found ($\mu\text{g/mL}$)	CV%	Error%	Found($\mu\text{g/mL}$)	CV%	Error%
1.40	1.39 ± 0.02	1.09	-0.47	1.42 ± 0.05	3.3	1.5
7.00	7.02 ± 0.14	1.97	0.29	7.05 ± 0.22	3.16	0.84
10.00	10.05 ± 0.04	0.38	0.53	9.92 ± 0.14	1.43	-0.78

Table 3: Determination of Cu in Dental Alloy as a real sample (n =4).

Cu Added ($\mu\text{g/mL}$)		Calculated by proposed method ($\mu\text{g/mL}$)	Recovery (%)	Calculated by atomic absorption ($\mu\text{g/mL}$)	Recovery (%)	Statistical tests*	
						F-test	T-test
Standard	15.00	15.30 ± 0.02	102.0	15.1 ± 0.02	100.67	0.967	0.0003
Dental alloy	11.15	11.06 ± 0.08	99.2	11.13 ± 0.02	99.82	0.024	0.175

samples were determined. A dental alloy, 110-plus amalgam capsule containing 25% Cu, 45% Ag, and 30% Sn, was used as a real sample. The results of the analysis were in good agreement with those obtained by the atomic absorption method. Two tailed t-tests and f-test revealed no significant difference in the results of these two methods (P value > 0.05) (Table 3).

CONCLUSIONS

A new chromogenic reagent was synthesized for determination of Cu²⁺ and the method was established. The Cu (II)-PhDTT complex was formed in methanol at pH =10 and determined against the blank reagent with no need for extraction or use of environmentally hazardous solvents. There were no serious interferences except for Ni²⁺, Hg²⁺, and Pd²⁺ which produced colored complexes

with PhDTT. In order to overcome the mentioned interferences, a derivative spectrophotometric method is suggested.

The proposed non-extractive spectrophotometric method is accurate, sensitive, rapid, and less tedious than extractive methods. Analysis of a dental sample showed this method could be successfully used for determination of Cu in real samples.

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