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Development of a sensitive extraction–spectrophotometric method for Cu(II) based on 1-(o-tolylamino)- and 1-(phenylamino)propane-2-thiol complexes

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Abstract: The reactions of copper(II) with 1-(o-tolylamino)-propane-2-thiol (**L**[1], **TPT**) and 1-(phenylamino)-propane-2-thiol (**L**[2], **PPT**) was studied by the extraction-photometric method. The ligands were characterized with IR and NMR spectroscopy. The influence of aqueous phase pH on the formation of CuL_2 complexes and thermal decomposition were studied. Chloroform was chosen as the best extractant. A single extraction with chloroform extracted 98.6–97.9 % of Cu(II). The yield of Cu**L**$_2$ was maximum at $c_L = 9 \times 10^{-3}$ M and did not decompose for 48 hours or more than 45 days after extraction. Phase volume ratios of 5 : 5–140 : 5 did not affect the recovery. The optimum acidity is in the range of pH_{opt} 3.1 – 5.3 (pH_{form} 1.0 – 7.1). The molar absorption coefficients are $\varepsilon_{450-470} = (3.4 – 3.8) \times 10^4$. Compliance with the fundamental law of light absorption is achieved at $c_{Cu(II)}$ = 0.08 – 19 μg mL^{-1}. Thermal decomposition of Cu**L**$_2$ complexes ([$C_{20}H_{28}N_2S_2Cu$] and [$C_{18}H_{24}N_2S_2Cu$]; Cu^{2+} : **L** = 1 : 2) occurs stepwise due to the different stability of the Cu–S and Cu–N bonds. In the range of 65–135 ºC, a mass loss of 2.78–2.84 % is observed, associated with the removal of weakly coordinated water. At 345–400 ºC, profound destruction of the ligand occurs with the rupture of coordination bonds and the destruction of the chelate cycle (mass loss of 87.06–87.35 %). The proposed methods were successfully applied to determine copper in various complex samples.

Keywords: Cu(II) determination; extraction–spectrophotometry; aminothiol ligands; liquid–liquid extraction; trace analysis; spectrophotometric method.

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INTRODUCTION

Environmental contamination with heavy metals represents one of the most acute global ecological problems of our time, as it is accompanied by pronounced toxic and mutagenic effects on living organisms, including genetic alterations. Consequently, in recent years there has been a significant increase in interest in the development of effective methods for monitoring and removing such pollutants. At the same time, the advancement of high technologies and accelerated industrialization has led to substantially higher requirements for material purity, thereby increasing the importance of determining trace amounts of elements that exert a noticeable influence on metabolic processes in the human body.[1]

Among trace elements, copper occupies a special position, ranking third in abundance in the human body. In terms of copper content, the liver is second only to the brain, which is associated with the crucial role of copper ions in numerous biological functions, including neuromodulation, antioxidant defense, and cellular responses to hypoxia.[1] The redox-active nature of copper determines its participation in a number of essential physiological processes and explains the widespread use of Cu^{2+} ions as auxiliary components in enzymatically catalyzed reactions due to their high coordination ability.[2]

At the same time, disruption of copper homeostasis in the body leads to the development of severe pathologies. In terms of toxicity in drinking water, Cu^{2+} ions are second only to mercury compounds.[3] Excessive intake of copper from contaminated water promotes its accumulation in humans and animals, posing a threat to ecosystems and increasing the risk of neurodegenerative diseases.[4] In addition, Cu^{2+} ions are common contaminants in aquaculture systems and exhibit pronounced toxic effects on aquatic organisms.[5]

Significant interest in copper is also due to its extensive industrial use.[6] Owing to its high electrical and thermal conductivity, ductility, and corrosion resistance, more than half of the extracted copper is used in the electrical industry for the production of wires, cables, and current-carrying components.[7] A considerable portion of copper is utilized in the form of alloys, in heat-exchange equipment, radio electronics, artistic products, as well as in the form of various compounds in medicine, catalysis, electroplating, agriculture, and pigment production. At the same time, all soluble copper salts are toxic, causing irritation of mucous membranes, damage to the gastrointestinal tract and liver, and, under inhalation exposure, can lead to chronic poisoning.

In this context, one of the urgent tasks of modern coordination chemistry is the investigation of complexation reactions between transition metal ions and polydentate organic ligands.[7] Coordination compounds that combine the properties of organic and inorganic components represent a promising basis for the development of functional materials and for modeling chemical–biological processes.

A special place in this field is occupied by aminothiols and their metal complexes, which, due to the presence of nitrogen and sulfur donor atoms in their molecules, exhibit pronounced N,S-coordination properties and are capable of forming stable chelate structures with transition metal ions.[8–12] The biological significance of aminothiols is confirmed by the key role of coenzyme A, containing an aminoethanethiol fragment, in metabolic processes. This determines the interest in the synthesis of new aminothiols and their metal complexes as potential physiologically active compounds with tunable properties.[11]

Metal complexes of aminothiols find applications not only in bioorganic and medical coordination chemistry but also in materials science, where they are used as stabilizing additives for polymer systems, antioxidants for lubricants, and exhibit antimicrobial activity and can be applied in agrotechnology. In analytical coordination chemistry, aminothiols acquire particular importance as selective ligands for Cu(II), Ni(II), and Co(II) ions, enabling the development of highly sensitive extraction–spectrophotometric methods for metal determination with low detection limits and high selectivity.[13]

A wide range of analytical reagents has been proposed for the spectrophotometric determination of copper, including naphthazarin,[14] various hydrazones, thiosemicarbazones, and azo compounds,[15–21] halogenated hydroxythiophenols,[22, 23] and alkyldithiophenols.[24] However, most of these reagents suffer from significant drawbacks, such as long color development times, the need for heating, narrow linearity ranges, and substantial interference from accompanying ions. Previously, we synthesized and studied copper(II) complexes with related aminothiol derivatives using extraction–photometric analysis.[24]

Recent studies (2020–2025) have emphasized the growing importance of environmentally safer and highly sensitive analytical methods for trace metal determination. [10]

In the present work, the complexation of copper(II) ions with aminothiol ligands (**HL**, **L**) 1-(o-tolylamino)-propane-2-thiol (**L^1, TPT**) and 1-(phenylamino)-propane-2-thiol (**L^2, PPT**) was investigated. This study is aimed at the further development of this approach and the evaluation of the analytical potential of new ligand systems.

EXPERIMENTAL

Reagents and solutions

Copper(II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$, ≥99.0%), potassium nitrate (KNO_3, ≥99.0%), hydrochloric acid (HCl, 37%), nitric acid (HNO_3, 65%), sulfuric acid (H_2SO_4, 98%), and hydrogen peroxide (H_2O_2, 30%) were purchased from Merck (Germany) and Sigma-Aldrich (USA) and used without further purification. Organic solvents including $CHCl_3$ (≥99.5%, Merck), $C_2H_4Cl_2$ (≥99%, Sigma-Aldrich), CCl_4 (≥99%, Carlo Erba), C_6H_6 (≥99.5%, Merck), C_6H_5Cl (≥99%, Merck), $C_6H_5CH_3$ (≥99.5%, Sigma-Aldrich), xylene (≥99%, Merck),

isobutanol (≥99%, Fluka), amyl alcohol (≥99%, Merck), and benzyl alcohol (≥99%, Sigma-Aldrich) were used as received. Double-distilled water was used throughout the experiments.

A stock solution of copper(II) (1.0 mg mL^{-1}) was prepared by dissolving 3.9296 g of $CuSO_4 \cdot 5H_2O$ in double-distilled water with the addition of 2–3 drops of concentrated H_2SO_4, and diluting to 1 L in a volumetric flask. The working solutions were obtained by appropriate dilution of the stock solution.[25] The copper content was standardized iodometrically according to the reported procedure.[26]

A 0.01 M solution of ligands (**HL^1** and **HL^2**) was prepared in chloroform and used for extraction. Chloroform was selected as the main extractant, while other organic solvents were tested to evaluate the extraction efficiency of CuL_2 complexes.

The ionic strength of the aqueous phase (μ = 0.1) was adjusted using KNO_3. The pH of the solutions was controlled by addition of 0.1 M HCl.

Apparatus

Spectrophotometric measurements were performed using a KPK-2 photoelectric colorimeter (Russia, 400–700 nm) and an SP-26 UV–Vis spectrophotometer (Russia, 190–1100 nm). Quartz cuvettes with optical path lengths of 0.5 and 1.0 cm were used. Solution pH was measured using an I-120.2 pH meter (Russia) equipped with a combined glass electrode, calibrated with standard buffer solutions.

IR spectra of the compounds in thin films were recorded on a Specord 75 IR spectrometer (Germany). The ^1H and ^{13}C NMR spectra of the substances in $CDCl_3$ were recorded on a Bruker SP-300 instrument (Bruker, Germany) [300.13 for (^1H), 75 MHz for (^{13}C)], with HMDS used as the internal standard. Elemental analysis for C, H, N and S atoms was performed on a Carlo Erba 1106 elemental analyzer (Italy). Chromatographic separation was carried out on a glass column (l = 20 cm, d = 2 cm) filled with silica gel of grade L 100/160. The eluent was a hexane-ethyl acetate mixture in a 9:1 ratio.

Thermogravimetric (TG) and derivative thermogravimetric (DTG) analyses were carried out using a simultaneous thermal analyzer (Netzsch STA 449 or equivalent, Germany) under controlled heating conditions.

*General procedure for the synthesis of ligands **HL^1** and **HL^2***

The 0.74 g (0.01 mol) of 1,2-epithiopropane and 2.14 g (0.02 mol) of ortho-toluidine [1.86 g (0.02 mol) of aniline] are placed in a 10 mL ampoule. After sealing the ampoule, the reaction mixture is heated in a water bath at 95–100 ºC for ten hours. After the specified time, the ampoule is cooled and opened, and the contents are subjected to vacuum distillation to purify the reaction product from *o*-toluidine (aniline).

1-(o-tolylamino)-propane-2-thiol

Yield: 1.3 g (80 %), bp 96 ºC (0.3 mmHg), n_4^{20} 1.0350, R_f=O.52, n_D^{20} 1.5690, Found, %: C 66.25; S 17.76; H 8.34; N 7.69. Calculated, %: C 64.95; S 19.83; H 7.57; N 8.31. HRMS: m/z [M + H^+] Calculated for $C_{10}H_{15}NS$: 181.112; Found: 167.098. HPLC-PDA: λ_{max}, nm: 270 nm, MeCN : MeOH (1 : 1), Rt: 3.07 min, 99.56 %. IR spectrum, ν, cm^{-1}: (KBr, cm^{-1}): 1448; 1513; 1585; 1604; 2561; 3017; 3035; 3055; 3407. ^1H NMR spectrum (300 MHz DMSO d_6), δ, ppm: 1.26–1.31 s (3H, CH_3), 2.1–2.3 s (3H, CH_3-Ph), 2.8–3.6 s (3H-$CHCH_2$), 6.1–7.3 s (4H, Ph), 1.6 s (1H-SH), 8.05 s (1H, NH). ^{13}C NMR spectrum (75 MHz, DMSO d_6), δ, ppm: 21.3, 33.1, 34.2, 36.3, 60.7, 66.9, 113.4, 129.5, 144.7.

1-(phenylamino)-propane-2-thiol

Yield: 0.8 g (80 %), bp 80 ºC (0.3 mm Hg), n_4^{20} 1.0800, R_f=0.56, n_D^{20} 1.5830, Found, %: C 64.63; S 19.28; H 7.83; N 8.48. Calculated, %: C 64.49; S 19.83; H 7.83; N 8.34. N 8.38, S19.17. HRMS: m/z [M + H^+] Calculated for $C_9H_{13}NS$: 167.211; found: 167.189. HPLC-PDA: λ_{max}, nm: 255 nm, MeCN : MeOH (1 : 1), Rt: 3.03 min, 99.18%. IR (KBr, cm^{-1}): 1418; 1454; 1496; 1506; 1514; 1587; 1613; 2554; 3057; 3028; 3222; 3404. ^{1}H NMR spectrum (300 MHz DMSO d_6), δ, ppm: 0.91 s (3H, CH_3), 3.18, 3.45 d (2H, CH_2), 6.59 −7.24 s (5H, Ph), 1.6 s (1H, SH), 8.07 s (H, NH), ^{13}C NMR spectrum (75 MHz, DMSO d_6), δ, ppm: 11.25, 28,.26, 38.56, 60.29, 13.32, 113.42, 129.64, 144.66.

General procedure for measuring light absorption

An aliquot of the Cu(II)-containing solution (0.1–20 μg mL^{-1}) was transferred to a separatory funnel. The pH of the aqueous phase was adjusted to 3.5–5.0 with 1 M HCl. Then 1 mL of a 0.01 M ligand solution (L^1 or L^2) in chloroform was added. The mixture was shaken for 5–6 min to ensure complete complex formation and extraction. After phase separation, the organic layer was collected, and its absorbance was measured at 470 nm (for L^1) or 450 nm (for L^2). The Cu(II) concentration was determined using a calibration curve.

Determination of Cu(II) in pharmaceutical samples

Boiling with 10 mL of aqua regia dissolves 0.5 g of a pharmaceutical sample. The solution is evaporated to dryness, the residue is dissolved in 10 mL of 1 M HCl, filtered if necessary, and the resulting solution is diluted to 100 mL with double-distilled water. The working solution is prepared by appropriate dilution of the stock solution. From an aliquot of this solution, 1 mL is analyzed for Cu(II) using the procedure described earlier.

Determination of copper in standard alloy samples

Weighed samples 0.1 - 0.5 g of alloy [A 95-4 (0.180 Al; 0.025 Fe; 0.025 Pb; 0.010 Cd; 0.010 Cu; 0.010 Sb; 0.007 % Sn; rest. Zn), A 95-5 (0.230 Al; 0.040 Fe; 0.040 Pb; 0.015 Cu; 0.015 % Sn; rest. Zn), A-195-3 (11.3 Si; 0.3 Mn; 0.23 Zn; 0.14 Cu; 0.08 Ti; 0.17 Mg; rest. Al), A-195-4 (12.3 Si; 0.13 Mn; 0.9 Fe; 0.3 Zn; 0.11 Cu; 0.2 Ti; 0.12 Mg; rest. Al), A-195-5 (13.2 Si; 0.08 Mn; 1.1 Fe; 0.38 Zn; 0.04 Cu; 0.4 Ti; 0.09 Mg; rest. Al)] were dissolved in 10 mL of a mixture of an aqueous solution of 5 mL HCl (1 : 1) + 1-2 mL HNO_3 (1:1) at 50 ºC. Silicon does not dissolve, so the solution was filtered and the filtrate was transferred to a 50 mL flask and diluted with water to the mark. A 20 mL aliquot of this solution was analyzed for Cu(II) content according to the extraction–spectrophotometric procedure described in the Experimental section (General procedure for measuring light absorption).

Determination of copper in gelatin

Gelatin (5 g) in a porcelain dish was soaked in 50 mL of distilled water for 2–3 hours. 25 mL of HNO_3 (1 : 1) was added to the swollen gelatin and heated in a boiling water bath for 2 hours. The solution was filtered and neutralized with NaOH (1 : 1). A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in Section “General procedure for measuring light absorption”.

Determination of copper in wheat bran

A 5 g sample of wheat bran was dried in a drying oven in porcelain dishes at 105 ºC until air-dry. The cup was then placed on an asbestos plate, and the contents were burned over an open flame. The charred residue, along with the cup, was transferred to a muffle furnace and calcined at 800 ºC. The mineralized residue was dissolved in 0.1 M HNO_3 and filtered through

a medium-density filter into a 100 mL flask. A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in Section "General procedure for measuring light absorption".

Determination of copper in beans

A weighed sample of beans (≈10 g) was ground and dried in a porcelain dish, first at 60–70 ºC, then at 105 ºC. The dry residue was calcined in a muffle furnace at 500 ºC. The ash was dissolved in dilute (1:1) HNO_3 and evaporated to form wet salts, which were then dissolved in double-distilled water and filtered into a 100 mL volumetric flask. A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in Section "General procedure for measuring light absorption".

Determination of copper in ram kidneys

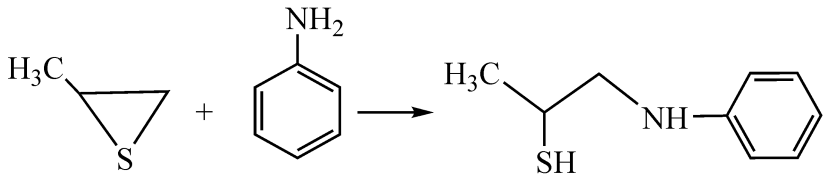
A sample (3 g) of kidneys was dried in crucible porcelain until moisture was removed and ached, first at low temperature, then at higher temperatures. After vapor evolution ceased, better air flow to the substance being ached was ensured. Heat was simultaneously increased until the crucible walls and lid were cleared of volatile combustion products. The lid was removed, and ashing was continued in a muffle furnace heated to a deep red glow, corresponding to ~500 ºC. To accelerate ashing, the crucibles were removed from the muffle furnace, cooled, and the crucible contents were moistened with a 3 % H_2O_2 solution. They were dried in a water bath and a drying oven and then calcined again to obtain a homogeneous brown mass. The mass was dissolved in hot water acidified with 2 M HCl. The mixture was filtered into a 100 mL volumetric flask. A 20 mL aliquot of this solution was analyzed for Cu(II) content following the general spectrophotometric procedure described in Section "General procedure for measuring light absorption".

Determination of copper in water

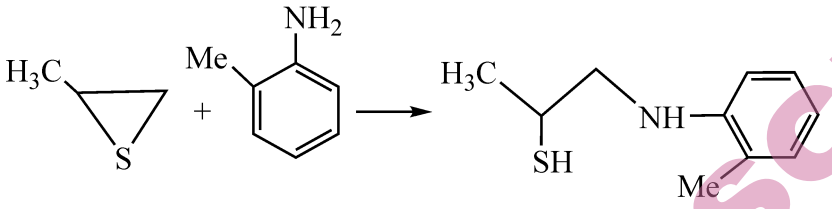
The natural sample—surface water (Kura River)—was pre-cleaned by filtration through a fine-pored glass Schott filter to remove suspended matter or soil. Ammonium persulfate was added, and the mixture was boiled for 20 minutes. A 20 mL aliquot was added to the extraction vessel, and an $n \times 10^3$-fold excess of PO_4^{3-} was added to mask the Fe^{3+}. The copper content was determined using the calibration curve. The validity of the proposed method was verified using the standard addition method (method of standard additions).

RESULTS AND DISCUSSION

The reaction schemes for the synthesis of **HL¹** and **HL²** are given below (Schemes 1, 2).



Scheme 1. Synthesis scheme and chemical structures of 1-(phenylamino)-propane-2-thiol studied in this work.



Scheme 2. Synthesis scheme and chemical structures of 1-(o-tolylamino)-propane-2-thiol studied in this work.

The reaction schemes for the synthesis of **HL**[1] and **HL**[2] are shown below (Schemes 1 and 2). We first investigated the reaction of aniline and o-toluidine with 1,2-epithiopropane. This reaction proceeds smoothly upon mixing equimolar amounts of the starting compounds in methanol at room temperature, leading to the formation of the target aminothiols **HL**[1] and **HL**[2] in yields of up to 80 %. The structures of the aminothiols were confirmed by spectral techniques and elemental analysis.

The IR spectra of the resulting ligands display characteristic absorption bands corresponding to the -NH stretching vibrations at 3300–3400 cm^{-1} and the –SH stretching vibrations at 2556–2560 cm^{-1}. In the ^1H NMR spectra, the NH protons appear as broad singlets at 4.5–5.5 ppm, while the –SH protons resonate as broad singlets in the region of 3.0–3.5 ppm. These spectral features are consistent with the successful formation of the desired aminothiols.

Determination of the charge sign of the complexes and selection of extractant

Colored CuL_2 complexesare highly soluble in chloroform and dichloroethane. To determine the charge of the complexes by electromigration, experiments were carried out in a U-shaped tube at R = 200–220 V and I = 0.4–0.8 mA for 4.0 h. During the electrolysis of the solutions of the complexes, no movement of charge was observed either to the anode or to the cathode, i.e. it is electrically neutral. Extractability of complexes was estimated in coefficient of distribution (D) and extent of extraction (R, %)[27] with Eqs. (1) and (2).

$$D = \frac{c(\mathrm{Cu})_{\mathrm{org}}}{c(\mathrm{Cu})_{\mathrm{aq}}} \quad (1)$$

$$R = \frac{100D}{D+\frac{V_{\mathrm{aq}}}{V_{\mathrm{org}}}}. \quad (2)$$

Rapid phase separation and maximum molar absorption coefficients were achieved during the extraction of complexes with $CHCl_3$, $C_2H_4Cl_2$, and CCl_4. Single-step extraction of Cu(II) with chloroform resulted in recovery rates of 97.9

and 98.6 % for complexes Cu(II)($\mathbf{L}^1$)$_2$ (**1**) and Cu(II)($\mathbf{L}^2$)$_2$ (**2**) and, respectively. Further research were conducted with chloroform.

Although chloroform is not considered an environmentally friendly solvent, it was selected due to its superior extraction efficiency, rapid phase separation, and higher sensitivity compared to other tested solvents. Therefore, its use is justified in this study from an analytical performance perspective.

The concentration of copper in the organic phase was determined with sodium dithiocarbamate[28] by photometric measurements after back extraction, while in the aqueous phase it was determined by the difference. Photometric measurements after back extraction with sodium dithiocarbamate were used to measure the copper concentration in the organic phase. Experiments have shown that for 98.6 % (for **2** 99.3 %) reextraction of copper it is necessary to shake the organic phase with an equal volume of 0.2 M HCl and perhydrol solutions in a ratio of 1 : 1. The high extraction efficiency, stability of the complexes in the organic phase, and favorable spectrophotometric characteristics demonstrate that the proposed extraction system is well suited for the extractive-spectrophotometric determination of trace amounts of Cu(II) in environmental and technological samples.

Influence of aqueous phase pH

The formation of Cu(II)–aminothiol complexes is strongly dependent on the pH of the aqueous phase, reflecting the influence of protonation-deprotonation equilibria of both the ligand and the metal ion. Systematic studies reveal that the efficiency of complex formation and subsequent extraction is highly sensitive to the solution's acidity.

The optimal acidity range for the complexes **1** and **2** is at pH_{opt} 3.6–5.3 (pH_{form} 2.4–7.1) and pH_{opt} 3.1–4.0 (pH_{form} 1.0–6.2), respectively (Fig. 1). Within these pH ranges, the ligands exist predominantly in their deprotonated or partially deprotonated forms, which facilitates effective coordination to Cu(II) ions, leading to stable chelate formation and high extraction efficiency. At solution pH > 7.1, extraction of complexes is not observed. This is attributed to the hydrolysis of Cu(II) ions, which predominates under alkaline conditions:

$$Cu^{2+} + nH_2O \leftrightarrows [Cu(OH)_n]^{2-n} + nH^+, n = 1, 2 \quad (3)$$

Here, the formation of hydroxo-species reduces the availability of free Cu(II) ions for complexation with the aminothiol ligands, thereby inhibiting the extraction process. These observations underscore the critical role of solution pH in modulating the speciation of both the metal ion and the ligand, and consequently, the efficiency and selectivity of Cu(II) complex formation. The pH of the aqueous phase was adjusted using 0.1 M HCl. Although no buffer system was employed, the pH stability during extraction was ensured due to the short equilibration time (5–6 min) and the relatively low ionic strength of the solutions. Under these

conditions, no significant drift in pH was observed during the complex formation and extraction process. The difference between formal and optimal pH values is attributed to changes in the deprotonation equilibria of the ligand and metal hydrolysis processes under varying acidity conditions.

Absorption spectra

The absorbance of complexes 1 and 2 at their absorption maxima (λ_{max}) is practically the same. However, there is a small difference in the position of these maxima: λ_{max} = 470 nm (**1**) and λ_{max} = 450 nm (**2**) (Fig. 2). In contrast, the ligands **HL**^{1} and **HL**^{2} have maximum absorption at 270 nm and 255 nm, respectively. Bathochromic shifts are calculated using the Eq. (4) and are 200 nm for **1** and 195 nm for **2**. The molar absorption coefficients are $\varepsilon_{470} = 3.8 \times 10^4$ for **1** and $\varepsilon_{450} = 3.4 \times 10^4$ for **2**.

$$\Delta = \lambda_{Cu(II)\text{-}L} - \lambda_{L}. \qquad (4)$$

Effect of phase volume ratio, ligand concentration, and holding time

The influences of the aqueous-to-organic phase volume ratio, ligand concentration, and equilibration time on the extraction efficiency and spectrophotometric determination of Cu(II) ions were systematically investigated. The term “holding time” refers to the time interval between phase separation and absorbance measurement, during which the stability of the extracted complex in the organic phase was evaluated. It was established that variations in the phase volume ratio within the range 5 : (5–140) : 5 do not exert a statistically significant effect on the extraction recovery of the CuL_2 complex, indicating a high extraction capacity and robustness of the proposed system. This behavior enables the simultaneous implementation of preconcentration and extractive–spectrophotometric determination of copper(II) without loss of analytical performance.

An increase in the aqueous phase volume up to a 28-fold excess relative to the organic phase did not adversely affect the extraction completeness, which reflects a favorable distribution coefficient and strong affinity of the Cu**L**$_2$ complex toward the organic solvent. This characteristic is particularly advantageous for trace-level analysis, where large sample volumes are typically required.

The maximum extraction efficiency of the Cu**L**$_2$ complex was achieved at ligand concentrations in the range $c_L = (8 – 9) \times 10^{-3}$ mol L^{-1}. Compliance with the fundamental law of light absorption is achieved within the concentration range $c_{Cu(II)}$ = 0.08–19 μg mL^{-1}.

Under these optimized conditions, the extracted complex exhibited pronounced thermodynamic and kinetic stability, with no detectable changes in absorbance over at least 48 h in solution and remaining stable for more than 45 days after extraction. This stability confirms the absence of decomposition, hydrolytic degradation, or secondary complexation processes. Kinetic studies

revealed that the maximum absorbance was reached within 6 min, demonstrating rapid complex formation and phase transfer. Consequently, the kinetics of complexation and extraction of copper with ligands **HL**[1] and **HL**[2] can be classified as fast and nearly quantitative, rendering the proposed methodology suitable for rapid and reliable analytical applications.

The limit of detection (LOD) and limit of quantification (LOQ) were calculated using the following equations:

$$\mathrm{LOD} = \frac{3\sigma}{\mathrm{S}} \quad (5)$$

$$\mathrm{LOQ} = \frac{10\sigma}{\mathrm{S}} \quad (6)$$

where σ is the standard deviation of the analytical response and S is the slope of the calibration curve.

The calibration equations are calculated as: $y = 0.291x + 0.026$ (LOD= 3 × 4.328/0.291 = 47 ng mL^{-1} and LOQ = 10 × 4.328/0.291 = 149 ng mL^{-1} for complex 1, and $y = 0.295x + 0.033$ (LOD = 3 × 4.9153/0.295= 50 ng mL^{-1} and LOQ = 10 × 4.9153/0.295 = 167 ng mL^{-1} for 2) (Table I).

The Sandell sensitivity (SS) values were calculated from the slopes of the calibration curves using the relation SS = 0.001/S. Accordingly, the Sandell sensitivity was found to be 0.00344 µg cm^{-2} for the Cu(II)–**TPT** complex (complex 1) and 0.00339 µg cm^{-2} for complex 2, indicating high analytical sensitivity of the proposed spectrophotometric method.

The Sandell sensitivity values for the Cu(II)–aminothiol complexes varied within the range of 0.0095 – 0.025 µg Cu cm^{-2}, demonstrating the high analytical sensitivity of the developed spectrophotometric methods, with the Cu(II) –**TPT** complex exhibiting the most pronounced response.

Some analytical characteristics of the Cu**L**$_2$ complexes are presented in Table I.

Equilibrium, extraction and stability constants

The complexation reaction can be predicted as follows:

$$\mathrm{Cu^{2+}_{aq}} + 2\mathbf{HL}_{\mathrm{aq}} \rightleftarrows [\mathrm{Cu}\mathbf{L}_2]_{\mathrm{org}} + 2\mathrm{H^+} \quad (7)$$

Equilibrium constant (K_{eq}) of the reaction:

$$K_{\mathrm{eq}} == \frac{[\mathrm{CuL_2}]_{\mathrm{org}}[\mathrm{H^+}]^2_{\mathrm{aq}}}{[\mathrm{Cu^{2+}}]_{\mathrm{aq}}[\mathbf{HL}]^2_{\mathrm{aq}}} = \frac{D\cdot[\mathrm{H^+}]^2}{[\mathbf{HL}]^2} \quad (8)$$

$$\frac{[\mathrm{Cu}]_{\mathrm{org}}}{[\mathrm{Cu^{2+}}]_{\mathrm{aq}}} = \log\frac{A_{\mathrm{x}}}{A_{\mathrm{o}} - A_x} = D \quad (9)$$

$$K_{\mathrm{eq}} == \frac{D\cdot[\mathrm{H^+}]^2}{[\mathbf{HL}]^2} \quad (10)$$

where, A_x – is the optical density for a given experiment; A_o – is the optical density for complete binding of the copper ion into a complex.

Taking the logarithm of equation (11), we obtain:

$$\log K_{eq} = \log D - 2\text{pH} - 2\log [\mathbf{HL}] \quad (11)$$

The process of extraction of the complex can be represented in the following Eq. (12):

$$Cu^{2+}_{aq} + 2L^{-}_{aq} \leftrightarrows Cu\mathbf{L_2}\ (org) \quad (12)$$

The value of the extraction constant (K_{ex}) can be calculated from equation (13):

$$K_{ex} = \frac{[Cu\mathbf{L_2}]_{org}}{[Cu^{2+}]_{aq}[\mathbf{L}^{-}]^2_{aq}} = \frac{D}{[\mathbf{HL}]^2} \quad (13)$$

Taking the logarithm of expression (13), we obtain:

$$\log K_{ex} = \log D - 2\log [\mathbf{HL}] \quad (14)$$

The stability constants are calculated using equation (15) are 9.7 for **1** and 8.4 for **2**.

$$\log\beta_{s.c.} = \frac{C_c}{(C_{Cu} - C_c)(C_{\mathbf{L}1,2} - nC_c)^n} \quad (15)$$

where c_{Cu}, $c_{L^{1,2}}$ and c_c are the concentrations of copper ion, aminothiols and complexes, respectively.

The values of K_{eq} , K_{ex} and $\beta_{s.c.}$, calculated using formulas (11), (14) and (15) respectively, are given in Table I.

TABLE I. Analytical characteristics of Cu(II)($\mathbf{L}^{1,2}$)$_2$ complexes

Parameter		**1**	**2**
pH	Formation	2.4–7.1	1.0–6.2
	Optim.	3.6–5.3	3.1–4.0
λ / nm		470	450
$\varepsilon \cdot 10^{-4}$		3.8	3.4
$\log K_{eq}$		12.27	11.68
$\log_{Kex}$		16.31	15.48
log β		9.7	8.4
Beer's law execution interval / μg mL^{-1}		0.08–19	0.1–18
$y=ax+b$		$y = 0.291x + 0.026$	$y = 0.295x + 0.033$
LOD / ng mL^{-1}		47	149
LOQ / ng mL^{-1}		50	167

Composition of the complexes

In the formation of the investigated complexes, the copper(II) ion, Cu^{2+}, acts as the coordination center. Experimental evidence indicates that, during complexation, one proton is displaced from each $HL^{1,2}$ ligand molecule, demonstrating that the ligands coordinate to Cu(II) in their deprotonated form (L^-).

The degree of aggregation (γ) of the extracted complexes was calculated from the slope of the linear graph: $\log A$ = f($\log c_{Cu(II)}$), where $c_{Cu(II)}$ is the copper ion

concentration, and A is the optical density of the extract. Evaluation of the aggregation degree (γ), calculated from extraction–spectrophotometric data, confirms that the Cu**L**$_2$ complexes do not undergo polymerization in the organic phase. The calculated γ values are close to unity (γ = 1.05 for complex **1** and γ = 1.08 for complex **2**), indicating that the complexes exist predominantly as monomeric species. Minor deviations from the ideal value of γ = 1 can be attributed to experimental uncertainty and weak intermolecular interactions within the extraction medium.

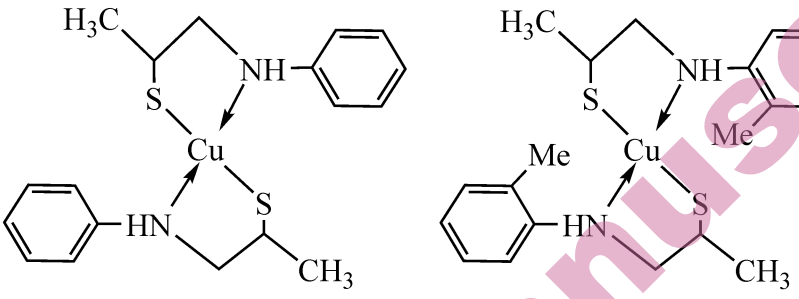
The stoichiometry of the complexes was determined using the Asmus straight-line method[29] and additionally confirmed by the equilibrium-shift and relative-yield methods.[29] The Asmus method is based on the analysis of the dependence of absorbance on the ratio of reagents under varying concentrations. In appropriately transformed coordinates, a linear relationship is obtained only for the true value of the stoichiometric coefficient, which allows the composition of the complex to be established. All applied methods consistently indicated a metal-to-ligand ratio of Cu^{2+} : $\mathbf{L}^{1,2}$ = 1 : 2, confirming the formation of neutral CuL_2-type complexes, which is consistent with their high extractability into the organic phase.

To elucidate the coordination mode of the ligands toward copper(II), infrared spectroscopic studies were carried out for both the free ligands (**HL^1** and **HL^2**) and the corresponding complexes. The ligand molecules contain two potential donor atoms—nitrogen and sulfur—each capable of participating in coordination with Cu^{2+}. According to literature data, the IR spectra of the free ligands exhibit medium-intensity bands at approximately 2600 and 3400 cm^{-1}, which are assigned to ν(SH) and ν(NH) stretching vibrations, respectively.[30]

Upon complex formation, the ν(NH) band originally observed near 3400 cm^{-1} undergoes a red shift of 40–50 cm^{-1}, indicating the involvement of the nitrogen atom in coordination with copper(II). Simultaneously, the absence of ν(SH) bands in the IR spectra of the complexes suggests deprotonation of the thiol group during complexation. The appearance of new absorption bands in the low-frequency region at 270–285 cm^{-1}, attributed to ν(Cu–S) vibrations, provides direct experimental evidence for the coordination of the thiolate sulfur atom to Cu^{2+}.

In addition, the IR spectra of all complexes display bands in the region 1620–1640 cm^{-1} corresponding to the bending vibrations of water molecules. Intense bands observed in the 3350–3450 cm^{-1} region, assigned to ν(OH) stretching vibrations, indicate the presence of crystallization water molecules within the complex structures.

Based on the combined results of stoichiometric analysis, aggregation calculations, and IR spectroscopic characterization, the composition of the investigated compounds can be represented by the general formula Cu**L**$_2$ (Scheme 3), in which the ligands coordinate to copper(II) through nitrogen and sulfur donor atoms, forming stable monomeric complexes.



Scheme 3. Suggested coordinations for the complexes in the study.

The thermal behavior of Cu$\mathbf{L}_2$ [$C_{20}H_{28}N_2S_2Cu$ (**1**) and [$C_{18}H_{24}N_2S_2Cu$ (**2**) {Cu^{2+} : $\mathbf{L}^{1,2}$ = 1 : 2}] complexes containing ligands with thiol (–SH) and amine (–NH–) donor sites is determined by stepwise decomposition due to the periodic thermal stability of the Cu–S and Cu–N bonds. According to thermogravimetric (TG) and derivative thermogravimetric (DTG) analysis, the thermolysis process occurs in two main stages (Fig. 3).

In the temperature range of 65–135 ºC, the TG curves show an initial mass loss (2.78 % for complex **1** and 2.84 % for complex **2**), accompanied by a weak DTG peak, corresponding to the removal of physically adsorbed or weakly coordinated water. The low temperature and small DTG maximum indicate the absence of a strong bond between water molecules and the Cu(II) coordination center.

The main stage of development occurs in the 345–400 ºC region and is characterized by an intense DTG maximum, corresponding to the profound thermal degradation of the organic ligand. At this stage, the Cu–S and Cu–N coordination bonds are cleaved, and the chelate ring is disrupted, corresponding to the mass differences of 87.35 % for complex **1** and 87.06 % for complex **2**. The position of the DTG maximum in the high-temperature region indicates a low thermal concentration of the complexes, due to the chelate effect and the use of sulfur- and nitrogen-containing donor atoms.

The final product of thermolysis in an oxidizing atmosphere is CuO, which corresponds to the residual mass and is consistent with literature data for Cu(II) coordination compounds with S,N-donor ligands.[31]

Photometric methods for determining Cu(II) using known reagents and $\mathbf{L}^1$ and $\mathbf{L}^2$ were compared (Table II). $\mathbf{L}^1$ and $\mathbf{L}^2$ offer several advantages over other reagents. Most published methods have a limited pH range (3–6).[32,35,39] $\mathbf{L}^1$ and $\mathbf{L}^2$ are more versatile and can be used over a wide range of acidity and slightly alkaline

environments, increasing their practical value. The wavelengths of maximum absorption of **L**[1] and **L**[2] are in the moderate visible range compared to modern spectrophotometric methods,[32,33, 36-38] which reduces potential interference from background absorption and improves analytical accuracy. Although the highest molar extinction coefficient ($\varepsilon = 6.8 \times 10^4$) was observed for the method,[37] **L**[1] and **L**[2] combine relatively high sensitivity ($\varepsilon = (3.4–3.8) \times 10^4$) with the widest range of measurable concentrations. This is a key advantage: many methods are limited to low concentrations[32-39] and are not suitable for samples with high copper content. **L**[1] and **L**[2] allow the determination of both trace and more concentrated levels of copper without the need for sample dilution, significantly expanding their practical application.

TABLE II. Comparative characteristics of spectrophotometric methods for determining Cu(II)

Reagent[a]	$pH_{form.}$	λ_{max} / nm	$\varepsilon \times 10^4$	Beer's law range / μg mL^{-1}	References
SDA+ SA	3.5–5.5	465	2.80	0.05–3.5	32
AAT	4.0–6.0	490	3.60	0.04–4.0	33
TCA	2.5–4.5	455	3.10	0.06–3.0	34
MT	3.0–5.0	470	4.20	0.03–3.8	35
PAAT	5.0–7.0	510	3.95	0.05–5.0	36
SB+CPE	4.5–6.5	520	6.80	0.01–1.2	37
AIMM	6.0–8.0	540	5.40	0.02–2.0	38
TBR	3.0–4.5	480	4.90	0.02–2.5	39
L[1](**TPT**)	2.4–7.1	470	3.80	0.08–19	This work
L[2](**PPT**)	1.0–6.2	450	3.40	0.1-18	This work

[a]SDA+ SA —Schiff base derived from 2-aminothiophenol and salicylaldehyde; AAT—Azoaminothiol reagent (S,N-donor system); TCA—Thiohydrazone-based chelating agent; MT—Modified thiosemicarbazone with electron-donating substituents; PAAT—Pyridylazoaminothiol; SB+CPE—Schiff base + cloud point extraction; AIMM—Azo dye immobilized on micellar medium; TBR—Thioamide-based reagent in micellar system.

As can be seen from Table II, the proposed method provides a wider linear range and comparable sensitivity relative to previously reported methods. In addition, the absence of a heating step and rapid extraction significantly improve the practicality and applicability of the method.

The influence of interfering ions

The selectivity of the proposed photometric method and the applicability of ligands $L^{1,2}$ was evaluated by investigating the influence of common foreign ions on the determination of Cu(II). The experiments were carried out under the same conditions as those used for constructing the calibration curves, except that defined amounts of potentially interfering ions were added to the test solutions. A deviation

of not more than ±5 % in the determination of 40 μg of Cu(II) was taken as the criterion for the absence of interference. Alkali metal ions and the anions F^-, Cl^-, and Br^- did not interfere at Cu(II): foreign ion ratios up to 1:1000. Mg^{2+}, PO_4^{3-}, and CH_3COO^- were tolerated up to a ratio of 1:500. No significant interference was observed from Al^{3+}, Ba^{2+}, Ca^{2+}, Mn^{2+}, SO_4^{2-}, and NO_3^- at a ratio of 1:100. Pb^{2+}, Ni^{2+}, J^-, and $–NH_2C(S)NH_2$ did not significantly affect the determination at a ratio of 1:7.5. Partial interference was observed in the presence of Sn^{2+} (1:4), Ti^{4+} (1:1), and Zn^{2+} (1:0.8). Pronounced interference was caused by Bi^{3+}, Co^{2+}, and Fe^{2+} at a ratio of 1:1, as well as by Cd^{2+} and citrate ions, for which the allowable ratio did not exceed 1:0.5. The effects observed are attributed mainly to competitive complex formation with ligands $\mathbf{L}^{1,2}$ and, in some cases, to spectral overlap in the working wavelength region.

ANALYTICAL APPLICATION

The selectivity data confirms the applicability of the proposed method for the photometric determination of Cu(II) in complex samples. The method was successfully applied to the analysis of various real samples, including alloys, food products, pharmaceutical preparations, and natural waters. The obtained results showed good agreement with those obtained using the standard rubeanic acid spectrophotometric method,[28] which is widely recognized for its reliability and reproducibility (Tables III–V).

TABLE III. Results of copper determination in alloys (n = 5; P = 0.95) *

			$\bar{X} \pm \frac{t_p S}{\sqrt{n}}$
Alloy A 95 - 4	0.010	**TPT**	0.010±0.003
		PPT	0.011±0.002
Alloy A 95 - 5	0.015	**TPT**	0.016±0.004
		PPT	0.015±0.005
Alloy A 195-3	0.140	**TPT**	0.137±0.005
		PPT	0.142±0.002
Alloy A 195-4	0.110	**TPT**	0.109±0.003
		PPT	0.120±0.004
Alloy A 195-5	0.040	**TPT**	0.041±0.003
		PPT	0.039±0.006

*$\bar{X}$ — mean value of n replicate measurements; n — number of determinations; P — confidence probability; t_p — Student's t-value at the given confidence level; S — standard deviation; Sr — relative standard deviation (RSD), calculated as $Sr = S/\bar{X}$. The term $\bar{X} \pm t_p S/\sqrt{n}$ represents the confidence interval of the mean.

TABLE IV. Results of analysis of copper content in food and pharmaceutical samples. (n = 5, P = 0.95) *

Sample	Method	X		$\bar{X} \pm \frac{t_p S}{\sqrt{n}}$
Beans	Rubeanic acid	5.42	0.056	5.42 ± 0.03
	TPT	5.39	0.021	5.39 ±0.05
	PPT	5.40	0.023	5.40 ±0.07
Gelatin	Rubeanic acid	11.97	0.037	11.96 ± 0.05
	TPT	11.94	0.045	11.94 ± 0.08
	PPT	11.98	0.027	11.98 ± 0.04
Wheat bran				4.95 ± 0.03
Kidneys				12.89 ±0.05
Zincovit[a] (Apex)				0.51 ±0.08
Multivitamin capsule[b] (A–Z)				0.93 ±0.04

[a]Zincovit (Apex)—selenium (50 μg), manganese (0.9 mg), zinc (22 mg), copper (0.5 mg), chromium (25 μg), molybdenum (25 μg). [b]Multivitamin capsule A to Z ns (Alkem)—elemental copper (0.9 mg), elemental manganese (2 mg), elemental selenium (55 μg), elemental zinc (10 mg).

TABLE V. Results of copper determination in the water of the Kura River in the Republic of Azerbaijan (n = 6; P = 0.95) *

Method	Cu found (initial) (mg L^{-1})	Cu added (mg L^{-1})	Total found (mg L^{-1})	Sr
Rubeanic acid	0.47	10.0	10.47 ± 0.03	0.039
TPT	0.47	10.5	10.97 ± 0.04	0.056
PPT	0.45	10.8	11.25 ± 0.05	0.048

The initial copper concentration in the sample was determined prior to spiking. The “Cu added” column indicates the amount of copper introduced into the sample, while “total found” represents the measured concentration after spiking.

CONCLUSIONS

The complexation of Cu(II) with aminothiol ligands **L^1 (TPT)** and **L^2 (PPT)** was investigated, and stable CuL_2-type neutral complexes were formed with a metal-to-ligand ratio of 1:2. The proposed extraction–spectrophotometric system demonstrated high extraction efficiency (up to ~98%) and satisfactory stability

over a wide acidity range, confirming its robustness for analytical applications. The developed methods exhibit good linearity in the concentration range of Cu(II), with low detection limits and satisfactory sensitivity, making them suitable for trace and higher-level copper determination without sample dilution. The selectivity studies confirmed that common ions do not significantly interfere under optimized conditions, except for certain heavy metal ions exhibiting competitive complexation. The methods were successfully applied to real samples, and the obtained results showed good agreement with those of the standard rubeanic acid spectrophotometric method, confirming the reliability and practical applicability of the proposed approach.

Conflict of interest: The authors of this work declare that they have no conflicts of interest.

И З В О Д

РАЗВОЈ ОСЕТЉИВЕ ЕКСТРАКЦИОНО-СПЕКТРОФОТОМЕТРИЈСКЕ МЕТОДЕ ЗА ОДРЕЂИВАЊЕ БАКРА(II) ЗАСНОВАНЕ НА КОМПЛЕКСИМА 1-(О-ТОЛИЛАМИНО)ПРОПАН-2-ТИОЛА И 1-(ФЕНИЛАМИНО)ПРОПАН-2-ТИОЛА

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Реакције бакра(II) са 1-(о-толиламино)пропан-2-тиола (L1, TPT) и 1-(фениламино)пропан-2-тиола (L2, PPT) испитиване су екстракционо-фотометријском методом. Лиганди су окарактерисани применом IR и NMR спектроскопије. Испитиван је утицај pH водене фазе на формирање комплекса CuL_2, као и њихова термичка разградња. Као најбоље екстракционо средство изабран је хлороформ. Једноструком екстракцијом хлороформом издвојено је 98,6–97,9 % Cu(II). Максималан принос комплекса CuL је био при $cL = 9 \times 10^{-3}$ M и није долазило до његове разградње током 48 часа, нити током више од 45 дана након екстракције. Однос запремина фаза од 5 : 5 до 140 : 5 није утицао на рикавери. Оптимална киселост је се у опсегу pHopt 3,1–5,3 (pHform 1,0–7,1). Моларни апсорпциони коефицијенти износе $\varepsilon_{450–470} = (3,4–3,8) \times 10^4$. Сагласност са основним законом апсорпције светлости постигнута је у опсегу концентрација $c_{Cu(II)} = 0,08–19$ μg mL^{-1}. Термичка разградња комплекса CuL_2 ($[C_{20}H_{28}N_2S_2Cu]$ и $[C_{18}H_{24}N_2S_2Cu]$; Cu^{2+} : L = 1 : 2) одвија се постепено услед различите стабилности веза Cu–S и Cu–N. У температурном интервалу 65–135 °C уочава се губитак масе од 2,78–2,84 %, који се приписује уклањању слабо координоване воде. При температурама 345–400 °C долази до интензивне деструкције лиганда, праћене раскидањем координационих веза и разарањем хелатног прстена (губитак масе од 87,06–87,35 %). Предложене методе успешно су примењене за одређивање бакра у различитим сложеним узорцима.

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