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Dispersive Liquid–Liquid Microextraction of Copper Ions as Neocuproine Complex in Environmental Aqueous Samples

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Abstract

In the present study, a simple and efficient extraction method based on dispersive liquid-liquid microextraction prior to UV-Vis spectrophotometry was developed for the preconcentration and determination of copper ions in environmental samples. Briefly, cupric ions (Cu II) were reduced to cuprous (Cu I) with addition of hydroxyl amine hydrochloride and formed hydrophobic chelates with neocuproine. Then, a proper mixture of acetonitrile (as dispersive solvent) and choloroform (as extraction solvent) was rapidly injected into the solution and a cloudy solution was formed. After centrifuging, choloroform was sedimented at the bottom of a conical tube and diluted with 100 μL of methanol for further UV-Vis spectrophotometry measurement. An orthogonal array design (OAD) was employed to study the effects of different parameters on the extraction efficiency. Under the optimum experimental conditions, a preconcentration factor up to 63.6 was achieved for extraction from 5.0 mL of sample solution. The limit of detection (LOD) based on S/N = 3 was 0.33 μg L^{-1} and the calibration curve was linear in the range of 1–200 μg L^{-1} with reasonable linearity ($r^2 > 0.997$). Finally, the accuracy of the proposed method was successfully evaluated by determination of trace amounts of copper ions in different water samples and satisfactory results were obtained.

Keywords: Copper, Dispersive liquid-liquid microextraction, Neocuproine, UV-Vis spectrophotometry

1. Introduction

Copper is both vital and toxic for many biological systems.[1,2] It plays an important role in environmental and health studies. It is an essential micro-nutrient required in growth of both plants and animals. In humans, it helps in the production of blood hemoglobin. Copper concentrations less than 1.0 mg L^{-1} can already be toxic to aquatic plants and some fish.[3] It is part of many enzymes such as cytochrome oxidase, catalase and uricase and takes part in many transport processes. It is also involved in erythrocyte formation. Copper enables the connection between iron and transferrin by taking part in the enzymatic oxidation of Fe (II) to Fe (III).[4] The most common symptoms of copper toxicity are injury to red blood cells, injury to lungs, as well as damage to liver and pancreatic functions.

Due to the importance of Cu, up to now, various methods, including ICP-OES, ICP-MS,[5] ion chromatography,[6] flame and graphite furnace atomic absorption spectrometry[7] and spectrophotometry[8,9] have been applied for determination of Cu. Many of these methods either are time consuming or require complicated and expensive instruments. Therefore, developing methods that can rapidly and conveniently determine low concentrations of Cu is valuable.[10] Various preconcentration techniques such as liquid-liquid extraction (LLE),[8,11] solid phase extraction (SPE),[12] precipitation and cation exchange[8] have been used to extract Cu ions from aqueous solutions. Some of these methods are time consuming or require large amount of toxic solvents. Also, various chelating agents such as sodium diethyl dithiocarbamate, cuprizone and dithizone, have been used to determine trace amounts of copper with UV-Vis spectrophotometry.[13]

Separations and preconcentrations based on the dispersive liquid-liquid microextraction (DLLME) have been developed successfully for preconcentration of species with widely differing character.[14] This technique is based on dispersion of tiny droplets of the extraction solvent within an aqueous solution aided by a water miscible solvent which is called dispersive solvent. DLLME is a simple, rapid and inexpensive extraction method and provides high enrichment factors. The performance of DLLME was successfully illustrated by extraction of different organic and inorganic compounds from environmental samples.[15–21]

In the present study, the applicability of DLLME combined with UV-Vis spectrophotometry for extraction and determination of trace amounts of copper ions after complex formation with neocuproine was investigated.

2. Experimental

2. 1. Reagents and Materials

All chemicals used were of analytical-reagent grade and were obtained from Merck (Darmstadt, Germany) and Fluka companies (Chemie AG, Switzerland). All solutions were prepared with doubly distilled water. A stock standard solution of copper ion (Cu II) at a concentration of 1000 mg L^{-1} was prepared from pure $Cu(NO_3)_2.3H_2O$ in ultra pure water. This standard solution was diluted with distilled water to prepare stock solutions with a concentration of 5, 10 and 50 mg L^{-1} of copper. The reducing agent, hydroxylamine hydrochloride solution (0.1 mol L^{-1}), was prepared by dissolving proper amounts of reagent in distilled water. The chelating agent, neocuproine (2,9- dimethyl-1,10- phenanthroline) solution, was prepared fresh daily by dissolving an appropriate amount of reagent in methanol. Hydrochloric acid (1 mol L^{-1}) and ammonia (1 mol L^{-1}) were used to adjust the pH of the solutions.

2. 2. Apparatus

All absorbance measurements were obtained using a Shimadzu UV 1650 PC spectrophotometer using a quartz micro cuvette (45 mm high, internal width 2 mm and path length 10 mm) with 400 μL internal capacity. A digital Jenway pH meter equipped with a combined glass-calomel electrode was used to check the pH adjustments. A centrifuge (Kokusan, Korea) was applied to accelerate the separation of sedimented phase from aqueous solutions.

2. 3. Dispersive Liquid-liquid Microextraction Procedure

For DLLME, aliquots of the solution containing 100 μg L^{-1} Cu (II) ions (5.0 mL) were adjusted to the appropriate ionic strength and pH (sodium acetate: 0.1 M, pH 6.0). Cu (II) ions were reduced to Cu (I) by using hydroxylamine hydrochloride (2.5×10^{-3} mol L^{-1}), and formed a very stable complex with neocuproine. Acetonitrile (1.0 mL), as dispersive solvent, containing 50 μL chloroform, was rapidly injected into the sample solution using a 5.0 mL syringe. The produced cloudy solution was centrifuged for 3 min at 3000 rpm. After centrifuging, the sedimented phase was completely transferred into another test tube and 100 μL of pure methanol was added to the sedimented phase to increase the volume of sedimented phase for further measurement by UV-Vis spectrophotometry (λ_{max} = 455 nm).

3. Results and Discussion

Neocuproine is a specific reagent for copper (I). Substitution of methyl groups at the 2- and 9-positions in 1,10-phenanthroline renders the reagent unreactive towards most cations making it highly selective for Cu (I).[8] Neocuproine reacts with Cu (I) to form a cationic complex (λ_{max} = 455 nm), which is sparingly soluble in water and is utilized for spectrophotometric determination of copper. In the present study, Cu (II) is reduced to Cu (I) with addition of hydroxylamine hydrochloride as a reductant to form Cu(I)-Neocuproine chelate that is extractable in organic solvents.

3. 1. Method Development

In the proposed procedure, to achieve maximum extraction efficiency, various parameters affecting the extraction of copper were studied using the Taguchi orthogonal array design (OAD). The Taguchi method is a type of fractional factorial design in which an orthogonal array is used to assign the selected factors to a series of experimental combinations.[22] The results of the orthogonal array design experiments can be treated by an analysis of variance (ANOVA) model. In ANOVA, the effects of different factors on the response function can be evaluated by computing F-ratio (variances ratio) and percent contribution (PC) values for each factor.[23,24] All optimization experiments were done at 100 μg L^{-1} of copper and sample solutions with 5.0 mL volumes were used for optimization.

3. 2. Experimental Design and Data Analysis

In this study, the effect of five experimental factors including solution pH, amount of reducing agent (hydroxylamine hydrochloride), amount of ligand (ligand-to-metal molar ratio (L/M)), dispersive solvent (acetonitrile) and extraction solvent volumes (chloroform) on the DLLME of copper was investigated using a Taguchi OA_{16} design. The used four levels of the main factors (A-E) and the OA_{16} (4^5) matrix employed to assign the considered factors are shown in Tables 1 and 2, respectively. The design of the tables was done via Experimental Design 7.0 software. For increasing the precision of the optimization process, each trial was repeated twice (n = 32). The sequence, in which the experiments were carried out, was randomized to avoid any personal or subjective bias. ANOVA was used to assess the OAD results. For ANOVA calculations, the absorbance of the copper-neocuproine complex (λ_{max} = 455 nm) was used and the results of the sum of squares (SS) for different variables were calculated. Since the responses of experiments were hetrosedastic, reciprocal responses were used for calculation. Fig. 1 shows the mean absorbance as a function of the levels of the studied factors.

Table 1: Factors and levels for the Taguchi orthogonal array design of the proposed method (A-E are the respective codes for each factor).

	Factors				
levels	A pH	B Reductant (mol L^{-1})	C L/M *	D Dispersive solvent volume (mL)	E Extractant solvent volume (µL)
1	3.0	0.0	0.1	1.0	25
2	4.0	5×10^{-4}	1.0	1.5	50
3	5.0	2.5×10^{-3}	10	2.0	75
4	6.0	5×10^{-3}	30	2.5	100

* L/M: Ligand-to-metal molar ratio

Table 2: OA$_{16}$ (4^5) experimental design for the optimization of DLLME of copper.

Trial no.	pH	Hydroxyl Con. (mol L^{-1})	L/M	Dis. solvent (mL)	Ext. Solvent Volume (µL)
1	6.0	0.005	10	1.0	50
2	5.0	0.0005	10	2.5	25
3	4.0	0	10	1.5	100
4	3.0	0.005	30	2.5	100
5	3.0	0.0005	1.0	1.5	50
6	5.0	0.005	0.1	1.5	75
7	3.0	0.025	10	2.0	75
8	5.0	0	30	2.0	50
9	4.0	0.025	0.1	2.5	50
10	5.0	0.025	1.0	1.0	100
11	6.0	0.0005	0.1	2.0	100
12	3.0	0	0.1	1.0	25
13	4.0	0.005	1.0	2.0	25
14	6.0	0	1.0	2.5	75
15	6.0	0.025	30	1.5	25
16	4.0	0.0005	30	1.0	75

The ANOVA results (Table 3) showed that the most important factor contributing to the extraction efficiency was L/M (42.25%).

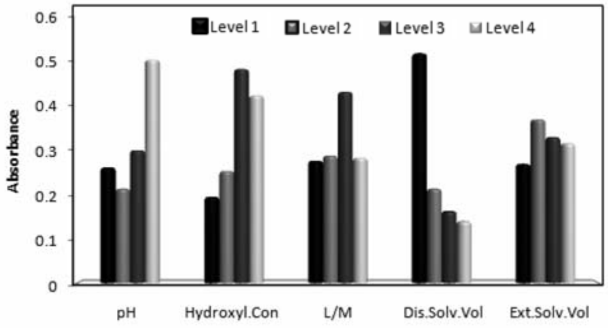

Figure 1. The response graph illustrating the variation of the mean absorbance values plotted against various extraction parameters. pH [level 1 = 3.0, level 2 = 4.0, level 3 = 5.0, level 4 = 6.0]. Hydroxylamine hydrochloride Con. (mol L^{-1}) [level 1 = 0.0, level 2 = 0.0005, level 3 = 0.025, level 4 = 0.005]. L/M [level 1 = 0.1, level 2 = 1.0, level 3 = 10, level 4 = 30]. Acetonitril volume (mL) [level 1 = 1.0, level 2 = 1.5, level 3 = 2.0, level 4 = 2.5]. Chloroform volume (µL) [level 1 = 25, level 2 = 50, level 3 = 75, level 4 = 100].

3. 3. Selection of Extraction Solvent

The selection of an appropriate solvent is a major parameter for DLLME process. The extraction solvent has to meet three requirements: (1) to extract analytes well, (2) to have higher density than water to sediment at the bottom of the extraction tube (3) to form a cloudy solution containing tiny droplets in the presence of dispersive solvent when injected into aqueous solution. Chlorobenzene (density, 1.11 g mL^{-1}), carbon tetrachloride (density, 1.59 g mL^{-1}) and chloroform (density, 1.48 g mL^{-1}) were selected as the extraction phases and compared for extraction of Cu(I)-neocuproine complex from water. The study was performed using 1.0 mL of acetonitrile containing different volumes of the extraction solvent to produce 20 µL of the sedimented phase. Thereby, 24, 26 and 58 µL of cholorobenzene, carbon tetrachloride and chloroform were used, respectively. The results showed that the extraction recovery decreased in the order $CHCl_3 > CCL_4 > C_6H_5Cl$. The results revealed that chloroform has the highest extraction recovery compared to the other tested solvents. Therefore, chloroform was selected as the extraction solvent for further experiments.

3. 4. Selection of Dispersive Solvent

The selection of dispersive solvent is limited via its miscibility in organic phase (extraction solvent) and aqueous phase (sample solution). The type of the dispersive solvent can also influence the viscosity of the injection phase and therefore affect the stability of the cloudy solution and the extraction recovery. Thereby, acetone (AC), acetonitrile (ACN) and methanol (MeOH) were selected for this purpose. A series of sample solutions were studied using 1 mL of each dispersive solvent, containing 50 µL

Table 3: ANOVA results for experimental responses in the OA_{16} (4^5) matrix.

Factor	DOF[a]	Sum of squares	Variance	F-ratio[b]	Pure sum of squares	%PC[c]
pH(A)	3	0.335	0.114	1.45	0.335	16.10
Con. hydroxyl(B)	3	0.33	0.109	5.05	0.321	17.73
L/M (C)	3	0.107	0.043	22.21	1.083	42.25
Dis.solvent volume(mL) (D)	3	1.835	0.613	2.53	0.095	8.14
Ext.solvent volume(μL) (E)	3	0.04	0.013	0.82	0.03	13.568
Error	16	0.038	0.003			2.22
Total	31	2.68				100.00

[a] Degrees of freedom, [b] F, critical value is 3.24 ($p < 0.05$), [c] Percent of contribution

of chloroform (extraction solvent). According to the results, the extraction efficiency decreased in the order ACN > AC > MeOH. Therefore, acetonitrile was selected as dispersive solvent.

3. 5. Influence of Solution pH

Neocuproine reacts with Cu^+ in the pH range 3–10 to form an orange complex.[8] According to the previous studies,[12] to investigate the effect of pH on the extraction of the complex, pH and ionic strength of the samples were adjusted in the range of 3.0–6.0 using sodium acetate (0.1 mol L^{-1}) with addition of ammonia (1 mol L^{-1}) or HCl (1 mol L^{-1}) (Fig. 1). The results showed that the maximum extraction efficiency of Cu(I)-neocuproine complex was obtained at pH 6. Therefore this pH was chosen as optimum pH.

3. 6. Effect of Reducing Agent

Neocuproine is a specific reagent for copper (I).[8] Therefore, by reducing Cu (II) to Cu (I) through hydroxylamine hydrochloride and complex formation between Cu (I) and neocuproine, a higher selectivity can be achieved. Fig. 1 shows that for quantitative extraction of copper ions more than 2.5×10^{-3} mol L^{-1} of hydroxylamine hydrochloride is needed. Therefore, this concentration was used as the optimum value for the reducing agent.

3. 7. Influence of Neocuproine Concentration

The results revealed that at the neocuproine-to-copper molar ratio (L/M) of 10 the highest extraction efficiency was achieved. This value was, therefore, selected as the optimum chelating agent concentration. However, using an excessive amount of reagent was found to decrease the extraction performance since higher amounts of neocuproine causes more methanol to enter the system thereby affecting the solubility of the complex.

3. 8. Influence of Dispersive Solvent Volume

Variation of the volume of acetonitrile (as dispersive solvent) causes changes in the volume of the sedimented phase. According to the Fig 1, for quantitative extraction of copper ions, 1.0 mL of acetonitrile gives the best results. It seems that, at low volume of acetonitrile, the cloudy state is not formed well. With higher volumes the solubility of Cu(I)-neocuproine complex in water increases, therefore, the extraction efficiency reduces because of a decrease in the distribution coefficient.

3. 9. Influence of Extraction Solvent Volume

The effect of the extraction solvent volume on the extraction efficiency was studied in the range of 25–100 μL. According to Fig 1, the extraction efficiency first increases and then decreases. It is clear that by increasing the volume of chloroform from 50 to 100 μL, the volume of sedimented phase increases. However, the enrichment factor decreases by increasing the volume of extraction solvent. In the following studies, 50 μL was selected as the optimum volume of extraction solvent.

3. 10. Effect of Foreign Ions

The effects of potential interfering species on complexation and extraction of copper (100 μg L^{-1}) were studied by addition of higher amounts of foreign ions to the aqueous phase. The results showed that Li^+ and K^+ (up to the concentration level of 1000 mg L^{-1}), Mg^{2+}, Ca^{2+} and Pb^2 (up to 750 mg L^{-1}), Na^+ and Fe^{3+} (up to 500 mg L^{-1}), Zn^{2+}, As^{3+} and Cd^{2+} (up to 100 mg L^{-1}), Co^{2+} and Ni^{2+} (up to 10 mg L^{-1}) did not cause any significant interference on the DLLME of copper. These concentrations are higher than the actual concentrations of studied ions in the aqueous environment.

3. 11. Figures of Merit of the Proposed Method

A calibration curve was obtained under the optimized conditions with a linear dynamic range of 1.0–200 μg L^{-1} and a correlation coefficient (r^2) of 0.997. The limit of detection (LOD) of the proposed method for determination of Cu (II) was calculated from $C_{LOD} = 3S_b/m$, where m is the slope of the calibration curve and S_b is the standard deviation of ten replicates of the blank measurement.

The calculated LOD was 0.33 µg L^{-1}. The preconcentration factor was calculated as the ratio of the concentration of the analyte in the sedimented phase (after addition of 100 µL MeOH) to its initial concentration in aqueous solution. The preconcentration factor was 63.6 for only 5.0 mL of sample. The relative standard deviations (RSDs) resulted from four replicate extractions of solutions containing 10 µg L^{-1} and 100 µg L^{-1} of Cu(II) were 5.3% and 4.0%, respectively.

A comparison between the figures of merit of the proposed method and some of the recently published methods for extraction and determination of Cu (II) ions is summarized in Table 4. The proposed method, applied in the present work, has some advantages in comparison with the other extraction and determination methods including low consumption of organic solvents and reagents, easy access, simplicity and low cost of the extraction device, minimum carryover and cross-contamination as well as producing a clean extraction phase for the analysis.

3. 12. Determination of Copper Ion in Teal Samples

The accuracy of the proposed method was studied by analyzing the water samples with various matrixes such as well, tap, mineral, river and lagoon waters. The water samples were collected in cleaned polyethylene bottles and their pH was adjusted to lower than 2 with addition of nitric acid and the samples were kept at 4 °C prior to extraction. The proposed DLLME method was applied to extract of Cu (II) from these water samples. In order to validate the performance of the proposed method, recovery values were obtained by spiking the samples with Cu ions at a 10 µg L^{-1} concentration level before any pretreatment. After that, the water samples were analyzed using the proposed method and the relative recovery percents based on the difference of Cu (II) in spiked and initial samples were calculated (Table 5).

Table 5: Analytical results for determination of Cu in real water samples.

Sample	**Concentration (mean, n = 3) (µg L^{-1})**		
	Cu added	**Cu found (± RSD)**	**Recovery (%)**
Tap water [a]	0.0	8.4 ± 1.0	97
	10.0	17.9 ± 0.6	
Well water [a]	0.0	5.9 ± 1.3	99
	10.0	15.7 ± 2.1	
River water [b]	0.0	13.9 ± 2.7	102
	10.0	27.4 ± 1.2	
Mineral water [c]	0.0	1.9 ± 0.2	108
	10.0	12.9 ± 3.2	
Mineral water [d]	0.0	1.4 ± 0.1	96
	10.0	11.0 ± 4.0	
Mineral water [e]	0.0	0.9 ± 0.2	106
	10.0	11.6 ± 1.9	
Bojagh lagoon water [f]	0.0	12.3 ± 1.3	98
	10.0	21.8 ± 2.4	
Anzaly lagoon water [g]	0.0	15.7 ± 1.6	102
	10.0	26.2 ± 2.1	

[a] collected from the Semnan city in Iran, [b] river water sample collected from Sefid Rood (Guilan-Iran) [c] Bottled mineral water was obtained from Damash company, [d] Bottled mineral water was obtained from Kohrang company, [e] Bottled mineral water was obtained from Damavand company, [f] Bojagh lagoon water collected from Kiashahr, Guilan, Iran, [g] Anzaly lagoon water collected from Bandare Anzaly, Guilan, Iran.

Table 4: Comparison of the characteristic data between recently published methods and the developed method.

Method	**Reagent**	**SV (mL)[h]**	**LOD (µg L^{-1})**	**R.S.D (%)**	**PF[I]**	**Detection**	**Ref.**
SPE[a]	Neocuproine	500	0.12	0.8	–	UV-Vis	12
SPE	1.10-phenanthroline	2000	0.86	4.31	200	FAAS[J]	25
SPE	–	100	0.83	6.8	196	FAAS	26
On-line SPE	Ternary Cu-CASd-CTAB ionpair formation	100	0.04	1.3	680	ICP-OES[k]	
CPE[b]	BSA[e]	25	0.29	2.12	10	Spectrofluorimetry	28
CPE	–	10	1.5	–	14	FAAS	29
CPE	Monocarboxylic acid	100	10	–	10	FAAS	30
CPE	Dithizones	100	4.6	2.9	–	UV-Vis	31
On-line SPE	TTA[f]	25	0.4	2.2	96	ICP-OES	32
DLLME[c]	BAT[g]	5	0.03	3.4	312	FAAS	33
DLLME	8-hydroxy quinoline	5	3.0	5.1	–	FAAS	34
DLLME	Neocuproine	5	0.33	4	63.6	UV-Vis	Proposed method

[a] Solid phase extraction, [b] Cloud point extraction, [c] Dispersive liquid-liquid microextraxtion, [d] Chromazurols, [e] 6-[2-(6-methyl-benzothiazolyazo)]-1,2-dihydroxy-3,5-benzene disulfonic acid, [f] 1-(2-thenoyl)-3,3,3-trifluoraceton, [g] s,s-bis(2-aminobenzyl)-dithioglyoxime, [h] Sample volume, [I] Preconcentration factor, [J] Flame atomic absorption spectrophotometry, [k] Inductively coupled plasma optical emission spectrometry.

Table 6: Determination of copper in well water samples.

Samples	Cu analysis method	Concentration (μg L^{-1}) ± RSD		Recovery(%)
		Cu found (mean, n = 3)	Proposed method (mean, n = 5)	
Well water [a]	ICP-OES	25.4 ± 0.1	23.7 ± 0.7	93%
Well water [b]	AAS	21.2 ± 0.8	20.2 ± 0.5	95%

[a] Collected from Shahmirzad, Semnan, Iran. [b] Collected from Sorkheh, Semnan, Iran.

The good agreement between the spiked and found values indicates the successful applicability of the present method for determination of Cu (II) in real samples.

Also, in order to further investigate the accuracy of the proposed method, some experiments were done on other well water samples and the results were compared with the results obtained using inductively coupled plasma – optical emission spectrometry (ICP-OES) and flame atomic absorption spectrophotometry (AAS) methods (Table 6). One can see that a satisfactory agreement exists between the results obtained by the proposed DLLME method and those obtained by ICP-OES and AAS methods.

4. Conclusion

The use of DLLME as an alternative to other methods of separation and preconcentration offers several advantages including experimental convenience, safety and being an inexpensive method. In the proposed method, UV-Vis spectrophotometry was applied as a simple and inexpensive determination method, because it has low running costs and the apparatus and manipulation is simple. The proposed method gives a low limit of detection as well as good accuracy and precision data next to a reasonable preconcentration factor. Without considering the added methanol at the measuring step, a preconcentration factor of 318 for only 5 mL of sample is obtained. The method was successfully verified by comparing the copper concentrations in real samples with ICP-OES and AAS.

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Povzetek

Razvita je bila enostavna in učinkovita ekstrakcijska metoda za predkoncentriranje in določanje bakrovih ionov v okoljskih vzorcih. Metoda temelji na disperzijski mikroekstrakciji, ki ji sledi UV-Vis spektrofotometrično določanje. Cu(II) ioni se reducirajo do Cu (I) z dodatkom hidroksilamin hidroklorida, tako da se tvori hidrofobni kelat z neokuproinom. V nastalo raztopino se hitro vbrizga primerna mešanica acetonitrila in kloroforma, tako da nastane motna raztopina. Po centrifugiranju se kloroformska plast razredči s100 µL metanola za UV-Vis spektrofotometrično določitev. Načrtovanje z ortogonalnimi vektorji (orthogonal array design-OAD) je bilo v pomoč pri študiju vpliva različnih parametrov na učinkovitost ekstrakcije. Pri optimalnih pogojih je bil za ekstrakcijo iz 5,0 mL raztopine dosežen koncentracijski faktor 63,6. Meja detekcije (LOD) osnovana na razmerju S/N = 3 znaša 0,33 µg L^{-1}, umeritvena krivulja pa je linearna v območju 1–200 µg L^{-1} ($r^2 > 0.997$). Natančnost predlagane metode je bila uspešno ovrednotena pri določanju sledi bakrovih ionov v različnih vzorcih vode, kjer so bili doseženi zadovoljivi rezultati.