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Determination of Ultra-Trace Amounts of Copper in Environmental Water Samples by Dispersive Liquid-Liquid Microextraction Combined with Graphite Furnace Atomic Absorption Spectrometry

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Abstract: A new method of dispersive liquid-liquid microextraction (DLLME) combined with graphite furnace atomic absorption spectrometry (GFAAS) was proposed for the determination of ultra-trace copper. It was based on the reaction of Cu(II) with the laboratory-prepared chelating agent 2-(5-bromo-2-pyridylazo)-5-dimethylaminoaniline (5-Br-PADMA) in a HAc-NaAc buffer solution at pH 5.0 to form stable hydrophobic chelates, which were separated and enriched by DLLME with chlorobenzene (C_6H_5Cl) and acetonitrile (CH_3CN) as extraction and disperser solvents, respectively. The sedimented phase containing the chelates was then determined with GFAAS. Various operating variables that may be affected by the extraction process such as the pH of the solution, the concentration of the chelating agent 5-Br-PADMA, the types and volumes of extraction and disperser solvents, the extraction time, and the centrifugation time were investigated. Under optimum conditions, the calibration curve was linear in the range from 0.02 ng/mL to 0.16 ng/mL of copper with a correlation coefficient of $r = 0.9961$, and the detection limit was 0.01 ng/mL based on $3S_b$. The relative standard deviation for six replicate measurements of 0.05 ng /mL of copper was 3.9%. An enrichment factor (EF) of 110 was obtained. The method has the advantages of low detection limit, high sensitivity, simple operation, less consumption of organic solvents, higher enrichment factor, and environmental friendliness and was applied to the determination of trace copper in environmental water samples with satisfactory results.

Keywords: dispersive liquid-liquid microextraction; copper; graphite furnace atomic absorption spectrometry; 2-(5-Bromopyridyazo)-5-dimethylaminoanline; chlorobenzene; water sample

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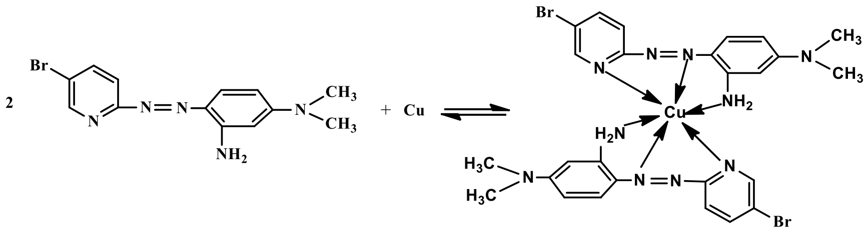
1. Introduction

Copper, one of the essential trace elements for living organisms, plays significant roles in various biochemical reactions, such as participating in the metabolism of proteins, lipids, and carbohydrates and in the synthesis and degradation of nucleic acids [1,2]. A deficiency of copper causes some diseases, such as biochemical disorders and physiological functional disorders. However, excessive intake can be harmful, leading to vomiting, diarrhea, irritation of the nose and throat, liver disorder, and even the production of harmful free hydroxyl radicals that can cause cancer by destroying DNA [3,4]. Copper is a ubiquitous trace metal and occurs in water, soil and plants. Also, copper pollution in the environment can occur that may cause toxic effects to living organisms in natural waters or humans for it has had numerous applications in the industry. Thus, it is clear that the determination of trace copper is important in the fields of biological and environmental analysis. Due to the low concentrations of this element found in the environment and the complex matrix, the direct determination of this metal ion at trace levels is often very difficult. A highly sensitive analytical method combined with a preconcentration/separation approach is one of best ways to solve these problems.

Several sensitive analytical techniques, such as fluorescence [5,6], chemiluminescence (CL) [5], high-performance liquid chromatography (HPLC) [7], voltammetry [8,9], graphite furnace atomic absorption spectrometry (GFAAS) [10–12], inductively coupled plasma atomic emission spectrometry (ICP-AES) [13,14], and inductively coupled plasma mass spectrometry (ICP-MS) [15,16], are available for the determination of copper at low levels. GFAAS was employed here for it is an efficient technique with high sensitivity and low cost, requiring only small volumes of samples. And also the equipment is available in many laboratories.

The most commonly used methods for the separation and preconcentration of trace copper are liquid-liquid extraction (LLE) [17,18], solid-phase extraction [19,20], and cloud point extraction [21,22]. These techniques have their own advantages, but they suffer from one or more of the following drawbacks: long analysis time, use of large toxic organic solvents, unacceptable enrichment factors, and production of secondary wastes. To overcome these problems, the development of liquid-phase microextraction techniques, such as single-drop microextraction (SDME) [23,24], dispersive liquid-liquid microextraction (DLLME) [5,10,11], solidified floating organic drop microextraction (SFODME) [25,26], and supramolecular solvent-based microextraction (SS-ME) [27], have received much attention. DLLME, a recently introduced liquid-phase microextraction technique, is based on the employment of a binary solvent system containing a water-immiscible solvent with a high-density (extractant) and a water-miscible one (disperser) to extract the analyte from the aqueous sample solution [28,29]. In this extraction method, very large contact area between the fine droplets of an aqueous sample and an extraction solvent is achieved, and the corresponding fast mass transfer kinetic results in an extraction equilibrium state to be quickly reached. DLLME has been widely used for the extraction and preconcentration of heavy metals [28]. The main advantages of DLLME are very low organic solvent consumption, low cost, simplicity, rapidity, recovery, and high enrichment factors.

The purpose of the present study is to combine DLLME with GFAAS to develop a new method for the determination of trace copper in water samples. The DLLME-GFAAS combination is feasible and favorable for two reasons. First, DLLME involves preconcentration in a small volume of solvent and GFAAS only requires a few microliters of sample to carry out the determination. Secord, DLLME is an efficient separation and preconcentration approach and GFAAS is a highly sensitive technique. In the developed system, a laboratory-prepared 2-(5-bromo-2-pyridylazo)-5-dimethylaminoaniline (5-Br-PADMA) [30], which can react with Cu(II) to form a stable hydrophobic chelate and was found to be a good chromogenic reagent for spectrophotometric determination of copper, was used as the chelating agent. The structure of the copper chelate is shown in Scheme 1. The main parameters including the type and volume of extraction and disperser solvents, the concentration of the chelating reagent, the pH of the solution, and the extraction time were investigated and optimized. Under optimized conditions, the detection limit was 0.01 ng/mL for copper. The method was successfully applied to the analysis of real environmental water samples. The method was successfully applied to trace determination of copper in real environmental water samples and spiked samples with satisfactory results.



Scheme 1. The structure of 5-Br-PADAM and its copper chelate.

2. Experimental Section

2.1. Apparatus

The measurements of Cu were performed with a PinAAcle 900 T atomic absorption spectrophotometer (Perkin Elmer, Waltham, MA, USA) with a graphite furnace atomizer, a Zeeman effects background correction, and an autosampler. A Lumina copper hollow cathode lamp (Perkin Elmer, Waltham, MA, USA), operated at a current of 15 mA and a wavelength of 324.7 nm with a spectral bandwidth of 0.7 nm, was employed as a radiation source. THGA end capes graphite tubes (Perkin Elmer, Waltham, MA, USA) were also used. Argon of 99.999% purity (Xi'an Tenglong Chemical Co., Ltd., Xi'an, China) was used as a purge and protective gas. All measurements were carried out in the integrated absorbance (peak area) mode. The optimum operating parameters for GFAAS are given in Table 1. A PHS-3C-01 pH lab meter furnished with a combined glass electrode (Shanghai San-Xin Instrumentation Inc., Shanghai, China) was used for pH measurements. IKA Vortex-3 (IKA Works, Guangzhou, China) was employed for mixing the reagents during the extraction operation. Phase separation was conducted with a TD4A centrifuge (Hunan Kaida Scientific Instruments Co., Ltd., Changsha, China).

Table 1. Temperature program of graphite furnace for Cu determination.

Stage	Temperature (°C)	Ramp Time (s)	Hold Time (s)	Argon Flow Rate (mL/min)
Drying	110	1	30	250
Drying	130	15	30	250
Ashing	800	10	20	250
Atomization	2000	0	5	0
Cleaning	2500	1	3	250

2.2. Reagents and Solutions

Ultrapure water (≤0.055 µS/cm) obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA) was employed to prepare all aqueous solutions. The highest purity or at least analytical reagent grade was used throughout the experiments. All the glass vessels used in the experiments were previously decontaminated in 10% nitric acid for at least 24 h, and rinsed with double deionized water prior to use. Afterwards, they were rinsed with ultrapure water at least three times before use. Stock standard solution of Cu (1000 µg/mL) was purchased from Gubiao Testing & Certification Co., Ltd. (Beijing, China). Working solutions at various concentrations were obtained by appropriate stepwise dilution of the stock standard solution. The 5-Br-PADAM (laboratory-synthesized [30]) solution (5×10^{-4} mol/L) was prepared by dissolving appropriate amounts of 5-Br-PADAM in ethanol. A buffer solution of pH 5.0 was obtained by mixing appropriate ratios of a 0.25 mol/L NaAc and 0.25 mol/L HAc and corrected by a pH meter.

2.3. Dispersive Liquid-Liquid Microextraction Procedure

At first, 5 mL of the standard or sample solution containing copper was placed in a 10 mL screw-cap centrifuge tube. A total of 1.0 mL of pH 5.0 acetate buffer solution and 100 µL of 5.0×10^{-4} mol/L 5-Br-PADAM ethanol solution as the chelating agent were added into the solution and shaken well. Then, 40 µL of chlorobenzene as the extraction solvent and 500 µL of acetonitrile as the dispersive solvent were injected into the above solution by syringe, and the mixture was shaken by using a vortex shaker (speed scale 4) for 3 min to accelerate the formation of a cloudy solution. After that, the mixture was centrifuged for 5 min at 3500 rpm. As a result, the dispersed fine droplets of chlorobenzene were sedimented at the bottom of the centrifuge tube. After centrifugation, 20 µL of the sediment phase at the bottom of the centrifuge tube was injected into the GFAAS using an autosampler for the determination of copper.

2.4. Preparation of Real Samples

The water samples were provided by Xi'an Hydrographic Bureau, Xi'an, Shaanxi Province China. All water samples were filtered through a 0.45 µm membrane filter to remove any suspended particles and then acidified with dilute nitric acid and stored in glass bottles at 5 °C. It was analyzed for determination of Cu(II) according to the above microextraction procedure.

3. Results and Discussion

3.1. Type and Volume of Extractor Solvent

It is crucial to select an appropriate extraction solvent in DLLME. The extraction solvent has to fulfill the following requirements: high capability to extract the analytes, higher density than water, and low solubility in water. For this purpose, different extracting solvents such as carbon tetrachloride (CCl_4), 1,2-dichloroethane ($C_2H_4Cl_2$), tetrachloroethane ($C_2H_2Cl_4$), tetrachloroethene (C_2Cl_4), chlorobenzene (C_6H_5Cl), and bromobenzene (C_6H_5Br), with respective densities of 1.590, 1.260, 1.553, 1.630, 1.280 and 1.494 g/mL, were tested as the extraction solvents for the extraction of Cu(II)-5-Br-PADAM by the proposed method. The extractions were carried out by using 40 µL of different extraction solvents and 500 µL of acetonitrile (CH_3CN) as the disperser solvent. Three replicate tests were carried out under the same conditions for each of these solvents.

It was found that a stable cloudy solution was formed when each of CCl_4, $C_2H_2Cl_4$, C_6H_5Cl, and C_6H_5Br was employed, while an unstable cloudy solution was formed and the sediment phase at the bottom of the centrifuge tube after centrifugation was difficult to remove by microsyringe when each of $C_2H_4Cl_2$ and C_2Cl_4 was used. In addition, it can be seen in Figure 1 that the maximum absorbance was obtained by using C_6H_5Cl as the extraction solvent. Therefore, C_6H_5Cl, with the highest extraction efficiency, was selected as the best solvent for further experiments.

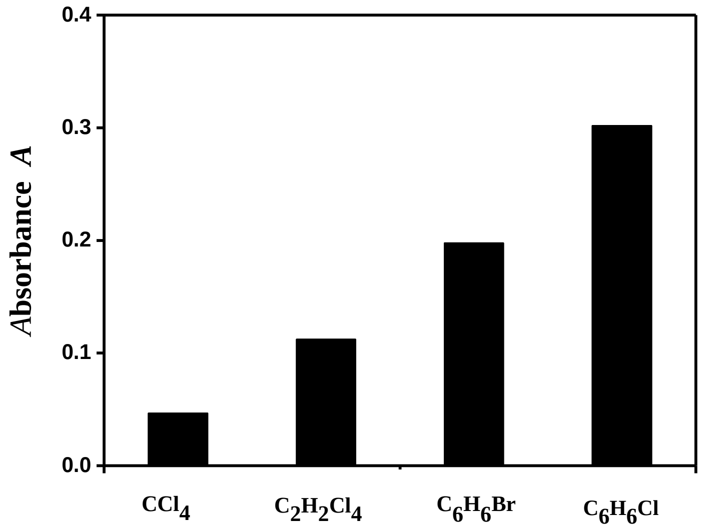


Figure 1. Effect of extraction solvent type on the extraction efficiency of Cu(II). Extraction conditions: 5.00 mL water sample, pH = 5.0, 100 µL 5×10^{-4} mol/L 5-Br-PADMA, 500 µL CH_3CN, 40 µL extraction solvent, and 0.5 ng Cu.

The volume of extraction solvent is an essential factor that affects the extraction efficiency in the DLLME procedure. In order to evaluate the optimal volume of extraction solvent, the influence of the volume of C_6H_5Cl on the extraction efficiency of Cu(II) was studied in the range of 20–70 µL with the same DLLME procedures. Figure 2 illustrates the variations of the extraction efficiency versus the volume of the extraction solvent. As can be seen, the absorbance of Cu(II) increased with the increase in C_6H_5Cl volume from 20 µL to 40 µL and then decreased with a further increase in C_6H_5Cl volume. The initial absorbance increase is due to the enhancement of the dissolving capacity of copper chelate. However, when the volume of C_6H_5Cl is higher than 40 μL, the absorbance begins to decrease significantly with increasing volume of C_6H_5Cl because of the sample dilution. Therefore, 40 μL of C_6H_5Cl solution was employed for the subsequent experiments.

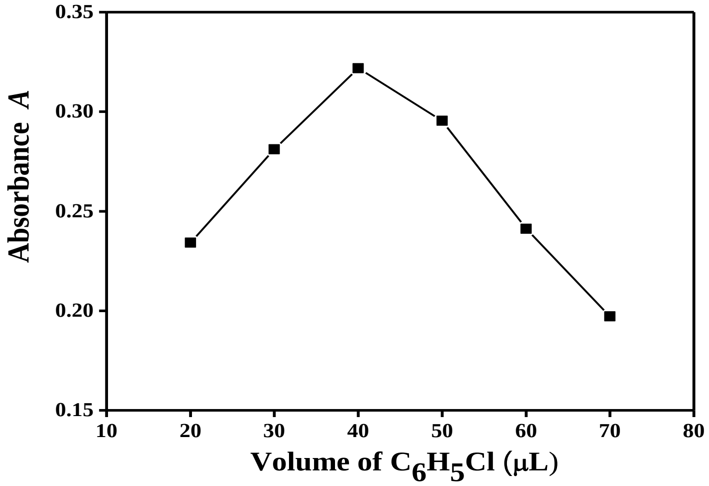


Figure 2. Effect of C_6H_5Cl volume on the extraction efficiency of Cu(II). Extraction conditions: 5.00 mL water sample, pH = 5.0, 100 μL 5 × 10^{-4} mol/L 5-Br-PADMA, 500 μL CH_3CN, and 0.5 ng Cu.

3.2. Effect of Disperser Solvent and Its Volume

The type of disperser solvent is another essential factor that affects the extraction efficiency in DLLME. The dispersive solvent should be miscible with both the aqueous phase (sample solution) and the organic phase (extraction solvent), and it can significantly decrease the interfacial tension between water and the extracting solvent and thus make the droplet size smaller. Therefore, methanol (CH_3OH), ethanol (C_2H_5OH), acetone (C_3H_6O), and acetonitrile (C_2H_3N), which possess these abilities, were examined as potential dispersive solvents. The influence of the abovementioned solvents on the extraction efficiency of Cu(II) was studied using 500 μL of each solvent together with 40 μL of C_6H_5Cl as the extraction solvent. As can be seen in Figure 3, the highest absorbance signal response of Cu(II) was obtained when C_2H_3N was used as the disperser solvent. Hence, C_2H_3N was selected as a disperser solvent for subsequent experiments.

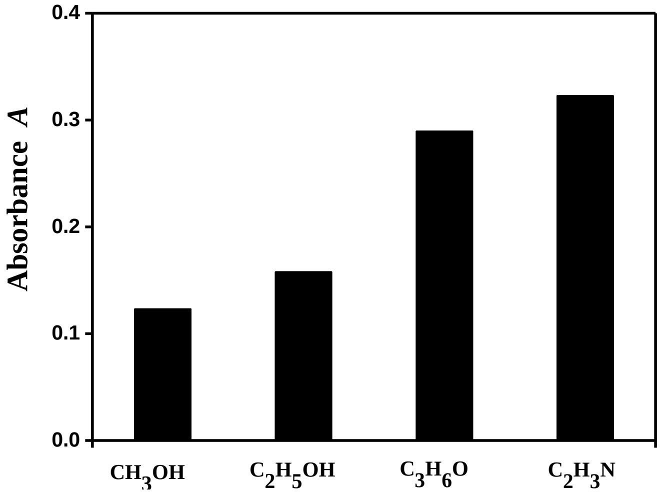


Figure 3. Effect of disperser solvent type on the extraction efficiency of Cu(II). Extraction conditions: 5.00 mL water sample, pH = 5.0, 100 μL 5 × 10^{-4} mol/L 5-Br-PADMA, 500 μL disperser solvent, 40 μL C_6H_5Cl, and 0.5 ng Cu.

The effect of the volume of disperser solvent CH_3CN on the extraction efficiency of Cu(II) was also investigated in the range of 100~700 µL of CH_3CN together with 40 µL of C_6H_5Cl. As shown in Figure 4, the highest absorbance signal of Cu(II) was attainable using 500 µL of CH_3CN. At a lower volume, CH_3CN could not disperse C_6H_5Cl effectively and the cloudy solution was not formed completely. Reversely, at a higher CH_3CN volume, the solubility of the copper chelate in water increased with the increase in the volume of CH_3CN. Therefore, 500 µL of acetonitrile was selected as the optimum disperser volume.

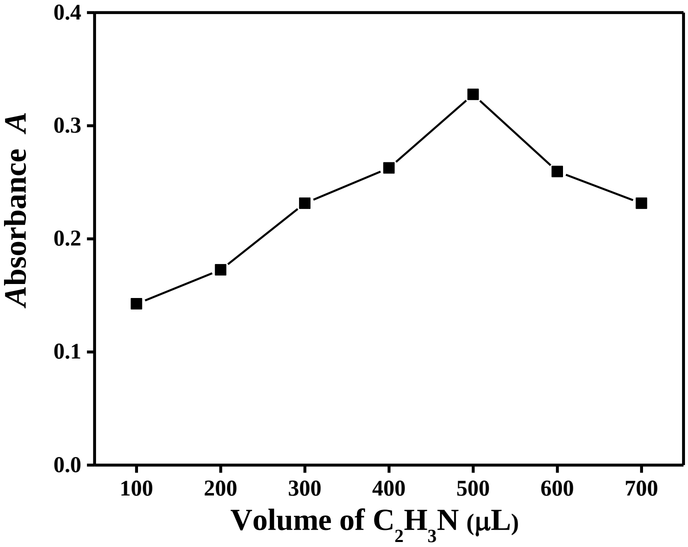


Figure 4. Effect of CH_3CN volume on the extraction efficiency of Cu(II). Extraction conditions: 5.00 mL water sample, pH = 5.0, 100 µL 5 × 10^{-4} mol/L 5-Br-PADMA, 40 µL C_6H_5Cl, and 0.5 ng Cu.

3.3. Effect of the Amount of the Chelating Agent 5-Br-PADAM

A laboratory-synthesized [30] chelating agent 2-(5-bromo-2-pyridylazo)-5-dimethylaminoaniline (5-Br-PADMA) was selected to form a hydrophobic chelate with Cu(II) because 5-Br-PADMA was found to be a good chromogenic reagent for spectrophotometric determination of copper,. The effect of the amount of 5-Br-PADAM on the extraction efficiency of Cu(II) was evaluated in the range of 70–120 µL. As can be seen in Figure 5, the absorbance of Cu(II) was increased with the increasing amount of 5-Br-PADAM up to the maximum value at a volume of 100 µL of 5-Br-PADAM. However, a further increase in 5-Br-PADAT volume resulted in a decrease in the analytical signal of Cu(II) due to the extraction of excess 5-Br-PADAM, which in turn decreased the limited capacity of the organic solvent for the quantitative extraction of the Cu(II)-5-Br-PADAM chelate. Hence, 100 µL of 5.0 × 10^{-4} 5-Br-PADAM solution was employed for subsequent experiments.

3.4. Influence of pH of Test Solution

In the experiment, 5-Br-PADAM was chosen as the chelating agent. As shown in Scheme 1, 5-Br-PADAM molecule contains three nitrogen atoms including two amino group nitrogen atoms and a ring nitrogen atom, and its copper chelate also has uncoordinated nitrogen atoms, which can combine with protons. Therefore, the pH value of the test solution affects not only the formation but also the hydrophobicity of the Cu(II)-5-Br-PADMA chelate and is one of the most important factors. The influence of pH on the DLLME extraction of Cu(II) was studied in the pH range of 3.0–6.5. As shown in Figure 6, the high absorbance of Cu(II) was observed at pH 5.0. Hence, a pH of 5.0 was employed for the subsequent experiments.

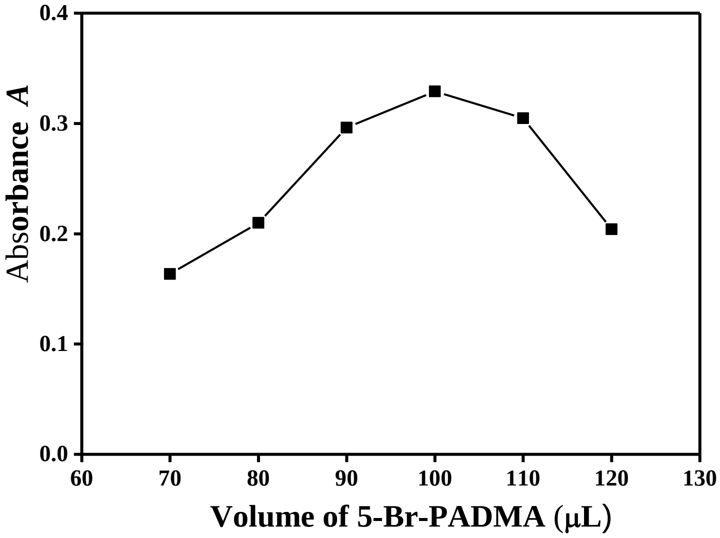


Figure 5. Effect of 5-Br-PADMA volume on the extraction efficiency of Cu(II). Extraction conditions: 5.00 mL water sample, pH = 5.0, 40 μL C_6H_5Cl, 500 μL CH_3CN, and 0.5 ng Cu.

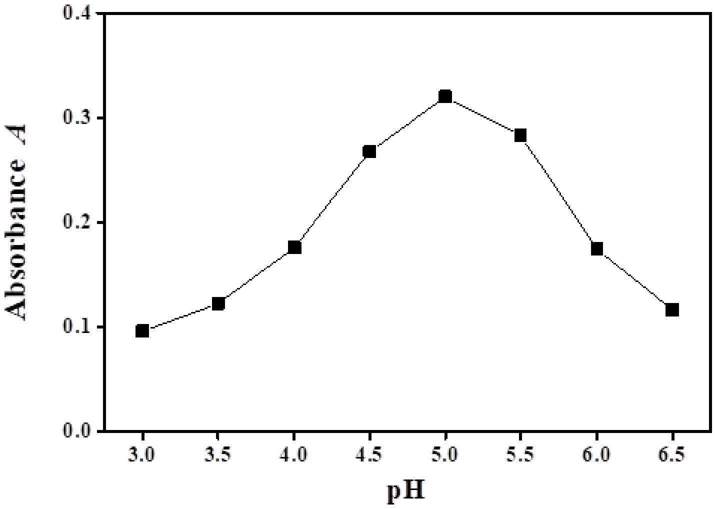


Figure 6. Effect of pH on the extraction efficiency of Cu(II). Extraction conditions: 5.00 mL water sample, 100 μL 5×10^{-4} mol/L 5-Br-PADMA, 40 μL C_6H_5Cl, 500 μL CH_3CN, and 0.5 ng Cu.

3.5. The Influence of Extraction and Centrifugation Time

Extraction time is an important parameter affecting the extraction efficiency in DLLME. It is defined as the time interval between the beginning of shaking the mixture using a vortex shaker after injection of the extraction and disperser solvent and the start of centrifugation. The effect of extraction time was evaluated in the range of 1–10 min with constant experimental conditions. The obtained results showed that the absorbance signal of Cu(II) remained constant after the extraction time exceeded 3 min. Therefore, an extraction time of 3 min was chosen for all measurements.

Centrifugation time was evaluated in the range of 1–10 min at a rate of 3500 rpm. The results obtained showed that when centrifugation time was over 5 min, the complete separation of the two immiscible phases was achieved. Therefore, a 5 min centrifugation time at 3500 rpm was employed in all further experiments.

3.6. Effects of Interfering Ions

The GFAAS method is highly selective, but some species may interfere with the extraction step and affect the extraction efficiency of copper. In the experiments, the influences of foreign ions commonly coexisting ions in natural water samples on recoveries of copper were investigated by analyzing 5 mL of solutions containing 0.1 ng/mL of Cu(II) and various concomitant ions according to the recommended procedure. A given species was considered to interfere if it resulted in a ±5% variation in the absorbance signal. The results obtained are summarized in Table 2. The above results indicated the excellent selectivity of this method for the determination of trace copper.

Table 2. Tolerance limits of foreign ions on DLLME of Cu.

Interfering Ions	**Added as**	**Tolerance Ratio (C_{ion}/C_{Cu})**	**Interfering Ions**	**Added as**	**Tolerance Ratio (C_{ion}/C_{Cu})**
Na^+	$NaNO_3$	2×10^5	Fe^{3+}	$Fe(NO_3)_3$	1×10^5
K^+	KNO_3	2×10^5	Al^{3+}	$Al(NO_3)_3$	1×10^5
Mg^{2+}	$Mg(NO_3)_2$	2×10^5	Zr(IV)	$Zr(IV)(NO_3)_4$	1×10^5
Ca^{2+}	$Ca(NO_3)_2$	2×10^5	F^-	KF	1×10^5
Sr^{2+}	$Sr(NO_3)_2$	2×10^5	Mn^{2+}	$Mn(NO_3)_2$	5×10^4
Ba^{2+}	$Ba(NO_3)_2$	2×10^5	Bi(III)	$Bi(NO_3)_3$	5×10^4
Zn^{2+}	$Zn(NO_3)_2$	2×10^5	Hg^{2+}	$Hg(NO_3)_2$	4×10^4
Cd^{2+}	$Cd(NO_3)_2$	2×10^5	Mo(VI)	Na_2MoO_4	1×10^4
Pb^{2+}	$Pb(NO_3)_2$	2×10^5	W(VI)	Na_2WO_4	4×10^3
Cr^{3+}	$Cr(NO_3)_3$	2×10^5	Co^{2+}	$Co(NO_3)_2$	4×10^3
$NH_4{}^+$	NH_4NO_3	2×10^5	La^{3+}	$La(NO_3)_3$	4×10^3
Cl^-	NaCl	2×10^5	As(III)	Na_3AsO_3	4×10^3
$SO_4{}^{2-}$	Na_2SO_4	2×10^5	Sn(IV)	$SnCl_4$	1×10^3
Ni^{2+}	$Ni(NO_3)_2$	1×10^5	Ce(IV)	$Ce(SO_4)_2$	5×10^2

3.7. Analytical Figures of Merit

Under the optimized conditions, quantitative characteristics of the proposed method, namely linear range (LR), detection limit (LOD), relative standard deviation (RSD), and enrichment factor (EF), were evaluated. The calibration graph was linear over the range of 0.02–0.16 ng/mL copper. The equation of the extraction calibration curve was A = 1.0457 C (ng/mL) + 0.2008, where A is the absorbance and C is the copper concentration, and the regression coefficient was 0.9961. The detection limit, calculated as the ratio of three times the standard deviation of the blank signal and the slope of the calibration curve, was 0.01 ng/mL (LOD, 3σ). Additionally, the equation for the direct determination calibration was A = 0.0095 C (mg/L) + 0.0033 with a regression coefficient of 0.9999. The enrichment factor (EF), calculated as the slope ratio of two calibration curves with and without preconcentration, was 110 for the 5.0 mL sample solution.

The proposed method was compared with those reported previously involving copper preconcentration by DLLME in terms of LR, LOD, RSD, EF, sample consumption, and instruments employed (Table 3).

As can be seen in Table 3, the proposed DLLME method has low limit of detection (0.01 ng/mL for Cu), high enrichment factor (110 for Cu) and low sample consumption (5.00 mL). In addition, it has short extraction procedure (3 min). These characteristics are of key interest for the routine laboratories in the trace analysis of copper ions. Therefore, DLLME combined with GFAAS is simple and sensitive for the extraction and determination of trace metals.

3.8. Analysis of Real Samples

The proposed method was validated by the extraction and determination of copper in different real water samples. For this purpose, 5 mL of each sample was preconcentrated via DLLME method after chelating with 5-Br-PADAM. The results are shown in Table 4. As could be seen, these results indicated the accuracy and applicability of this method to the analysis of these samples.

Table 3. Comparison of the proposed method with previously reported DLLME methods.

Ligand	Method	Extraction Solvent	Disperser Solvent	Sample Consumption	Enrichment Factor	LOD (ng·mL^{-1})	Ref.
HCDTC	SP	C_2Cl_4	Acetone	10 mL	92	0.3	[31]
BPDC	FO-LADS	$CHCl_3$	C_2H_5OH	10 mL	160	0.34	[32]
PAN	FAAS	CCl_4	C_2H_5OH	15 mL	60	0.06	[33]
PAN	FAAS	1-Undecanol	C_2H_5OH	10 mL	33	0.16	[34]
PAN	FAAS	1-Decanol	C_2H_5OH	10 mL	30	6.6	[35]
HBDAP	FAAS	CCl_4	Acetone	10 mL	20	0.75	[36]
Salophen	FAAS	$CHCl_3$	Acetone	10 mL	49	0.6	[37]
Ph-SEMS	FAAS	CCl_4	Acetone	10 mL	20	0.69	[38]
NEMMP	FAAS	$CHCl_3$	C_3H_7OH	8 mL	104	0.51	[39]
5-Br-PADAP	FAAS	$CHCl_3$	Acetone	15 mL	120	1.4	[40]
1N2N	FAAS	$CHCl_3$	C_2H_5OH	10.5 mL	70	0.95	[41]
p-SA	FAAS	Toluene	CH_3OH	10 mL	-	0.12	[42]
Curcumin	FAAS	[bmim] [PF_6]	Ultrasonic bath	25 mL	135	0.19	[43]
Hematoxylin	HPLL	CCl_4	CH_3OH	10 mL	327	0.0483	[7]
SDDTC	GFAAS	1,1,2,2-TCE	Air	6 mL	95	0.02	[11]
SDDTC	GFAAS	1,1,2,2-$C_2H_2Cl_4$	CO_2	5 mL	150	0.0062	[10]
-	ETAAS	Nitric acid	CH_3OH	5 g	-	0.52 ng/g	[44]
DEHPA	ANN-BA	Xylene	Acetonitrile	10 mL	-	0.08	[45]
TTA	ICP-OES	[C_6mim] [Tf_2N]	C_2H_5OH	30 mL	91	0.1	[13]
5-Br-PADMA	GFAAS	C_6H_5Cl	CH_3CN	5 mL	110	0.01	This work

HCDTC: (4-hydroxy-2-oxo-2H-chromen-3-yl)methyl pyrrolidine-1-carbodithioate; BPDC: 4-benzylpiperidineditiocarbamate potassium salt; PAN: 1-(2-Pyridylazo)-2-naphthol; HBDAP: N,N′-bis-(2-hydroxy-5-bromobenzyl)-2-hy-droxy-1,3-diiminopropane; Salophen: N,N′-bis (salycilidene)-1,2-phenylenediamine; Ph-SEMS: 4-phenyl-3-thiosemicarbazide; NEMMP: 2-[(E)-(naphthalen-2-ylimino)methyl]phenol; 5-Br-PADAP: 2-(5-Bromo-2-pyridylazo)-5-(diethyl amino) phenol; 1N2N: 1-nitroso-2-naphthol; p-SA: p-Sulfonatocalix (4) arene; SDDTC: Sodium diethyldithi-Ocarbamate; DEHPA: Di-2-ethylhexyl phosphoric acid; TTA: 1-(2-thenoyl)-3,3,3-trifluoracetone; 1,1,2,2-TCE: 1,1,2,2-tet-rachloroethane; FOLADS: fiber opticlinear array detection spectrophotometry; ANN-BA: Artificial neural networks coupled bees algorithm.

Table 4. Determination results of cobalt in environmental water samples.

Sample	Added (ng/mL)	Found ** (ng/mL)	Recovery (%)
Shanshui River water [a]	-	0.028 ± 0.005	-
	0.05	0.080 ± 0.004	101.8
Jing River water [b]	-	0.070 ± 0.004	-
	0.05	0.119 ± 0.03	99.7
Jing River water [c]	-	0.038 ± 0.005	-
	0.05	0.086 ± 0.003	96.9
Wei River water [d]	-	0.056 ± 0.004	-
	0.05	0.108 ± 0.002	102.3

[a] Collected from the mouth of Shanshui River (Bin County, Xianyang, Shaanxi Province, China) to Jing River. [b] Collected from Jing River (Jing Village, Xinbo Town, Bin County, Xianyang, Shaanxi Province, China). [c] Collected from Jing River (Gaoling Distric, Xi'an, Shaanxi Province, China) to Wei River. [d] Collected from Wei River (Xianyang, Shaanxi Province, China). ** Mean ± standard deviation (*n* = 3).

The accuracy of the method was also verified by the analysis of samples spiked with known amounts of copper ions. The relative recoveries at the spiking level of 0.05 ng/mL were between 96.9% and 102.3%, which indicates that the matrix of these real samples had little effect on the microextraction efficiency.

4. Conclusions

In the present study, a new method for the determination of ultra-trace copper by combining DLLME with GFAAS using the laboratory-synthesized reagent 5-Br-PADAM was proposed. The combination is favorable because DLLME is simple, rapid, convenient, and requires less organic solvent consumption, and GFAAS is not only highly sensitive but also suitable for the analysis of low volumes of the sedimented phase after DLLME. The proposed method offers several advantages such as simplicity, high enrichment factor, and much lower limit of detection over those reported methods in the literatures. Especially, sample preparation time and consumption of toxic organic solvents are minimized in this method without affecting the sensitivity of the method. Hence, it seems possible to extend this method to extract the other analytes in various samples by varying the extraction conditions.

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