

# A New Dispersive Liquid–Liquid Microextraction Method for the Preconcentration of Copper Using 4-Phenyl-3-Thiosemicarbazide and FAAS Detection

Duygu Kantürer Acar · Derya Kara

Received: 26 September 2013 / Accepted: 30 December 2013 / Published online: 1 February 2014  
© Springer Science+Business Media Dordrecht 2014

**Abstract** The aim of this study is to develop a new method for the preconcentration of copper via a dispersive liquid–liquid microextraction method using 4-phenyl-3-thiosemicarbazide as a ligand and determination with FAAS in different sample types such as plants, soils and natural waters such as seawater. Optimum experimental conditions were determined, and the applicability of the proposed dispersive liquid–liquid microextraction method was investigated. In the first step of the work, the parameters that affect complex formation and extraction, such as volume of extractant/disperser solvent, pH and concentration of the chelating agent, NaCl and surfactant, were optimised. The interference effects from potential concomitants on the determination of the Cu(II) ion were investigated in synthetic mixtures that contain high levels of these ions. These results showed the analytical applicability of the proposed method in different kinds of samples. Under the optimal conditions, the calibration curve was linear over the range 2–600  $\mu\text{g L}^{-1}$  of copper, and the detection limit was 0.69  $\mu\text{g L}^{-1}$  in the original solution (3 Sb/m). The accuracy of the developed method was checked by analysing certified reference materials (QCS-19 (high purity standard), LGC 6156 (harbour sediment) and NBS 1572 (citrus leaves)). Results obtained were in agreement with certified values with a *t* test showing that no significant differences at the 95 % confidence

interval levels were found. The proposed method was applied to seawater, river water, and plant and soil samples. The recovery values for spiked water samples were between 99.7 and 117.3 %.

**Keywords** Dispersive liquid–liquid microextraction · 4-Phenyl-3-thiosemicarbazide · Flame atomic absorption spectrometry · Preconcentration · Environmental samples

## 1 Introduction

Copper is very easily complexed and is involved in many metabolic processes in living organisms. Complex formation regulates copper homeostasis in the soil and in organisms and the biosynthesis of essential copper containing proteins and enzymes. Copper is one of the several heavy metals that are essential to life despite being as inherently toxic as nonessential heavy metals such as lead and mercury. Soluble salts of copper are almost only poisonous when they are ingested through misguided or suicidal intent or are used as a topical medical treatment of extensively burned areas of the skin (Merian 1991). Copper is an essential nutrient for plants, but it is also highly phytotoxic at high concentrations. Copper contamination of soils is widespread as a result of mining, smelting, land applications of sewage sludge, use of Cu as fungicides, and other industrial activities. Plants that grow on Cu-contaminated soils can evolve mechanisms to resist Cu in the soil or tolerate Cu inside plant cells (Ernst et al. 1992; Macnair 1993; Song et al. 2004). Copper is a ubiquitous trace metal and

D. Kantürer Acar · D. Kara (✉)  
Department of Chemistry, Art and Science Faculty,  
Balıkesir University,  
10100 Balıkesir, Turkey  
e-mail: dkara@balikesir.edu.tr

occurs in soil, water and plants. Since it has had numerous applications in the industry, copper pollution in the environment can occur that may cause toxic effects to living organisms in natural waters or humans.

Since it has a crucial role in biological systems but is also toxic at higher concentrations, the determination of trace amounts of this element in different samples is very important. Although a variety of methods and techniques have been developed to achieve this goal, it is still an attractive challenge. Copper concentration levels in water and food samples are fairly low, and interferences arising from the sample matrix, especially for seawater cannot always be eliminated. Matrix separation and analyte preconcentration procedures are often necessary before the determination of copper ions in real samples with a complex matrix. These sample manipulation protocols are necessary for a range of detection systems including flame atomic absorption spectrometry (FAAS), ultraviolet–visible (UV–Vis) spectrometry, inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS). A variety of procedures for the preconcentration of copper, such as solid phase extraction (SPE) (Soylak and Ercan 2009; Kara et al. 2009; Ozcelik et al. 2012; Farnad et al. 2012), liquid–liquid extraction (LLE) (Kara and Alkan 2002; Wieszczycka et al. 2012; Chang et al. 2011), coprecipitation (Chen et al. 1997), cloud point extraction (CPE) (Tabrizi 2007; Liang and Yang 2010) and micelle-mediated extraction (Kara 2009), has been developed. However, most of the methods have some disadvantages, such as being time-consuming, yielding unsatisfactory preconcentration factors, the use of large quantities of organic solvents, secondary waste and the requirement of extra equipment, for example, valves, resins and minicolumns in flow injection systems. These factors can impose limits on their application; for instance, if a procedure has the potential to yield a preconcentration factor of 100 but takes 4 h per sample to achieve that, it is of no use analytically to a busy laboratory with hundreds of samples per day to analyse.

The dispersive liquid–liquid microextraction (DLLME) technique was first presented by Rezaee et al. (2006) and is based on ternary component solvent systems, similar to homogeneous liquid–liquid extraction (HLLE) and CPE. In this method, the extraction solvent is at microlitre volumes in a dispersant volume of less than a millilitre. This mixture is injected into an aqueous sample, and a cloudy solution is formed. The

analyte ions in the sample are extracted into the fine droplets of extraction solvent. After extraction, phase separation is achieved using centrifugation. The metal ions in these droplets may then be determined using a variety of techniques. Several such methods developed for the preconcentration of copper have been reported in the literature, and these have used different detection techniques such as UV–Vis spectrometry (Skrlikova et al. 2011), FAAS (Khani et al. 2011; Aristidis and Kallirroy-Ioanna 2009; Mohammadi et al. 2009; Farajzadeh et al. 2008; Bahar and Zakerian 2012), X-ray fluorescence spectrometry (Kocot et al. 2012) and ICP-OES (Ranjbar et al. 2012).

The aim of the present work was to develop a novel DLLME method for copper preconcentration and determination using FAAS. The effectiveness and efficiency of the proposed DLLME system have been demonstrated for copper determination in different types of environmental sample such as seawater, river water, sediment, soil and plant samples. The reagent 4-phenyl-3-thiosemicarbazide was used, for the first time, as a chelating agent for copper preconcentration because it has good selectivity in acidic medium. All of the main experimental factors were investigated and optimised.

## 2 Experimental

### 2.1 Reagents and Solutions

All chemicals used in this work were of analytical reagent grade and were used without further purification. Ultrapure water (18.2 M $\Omega$  cm) was used for preparing the solutions and for all dilutions. The laboratory glassware used was stored in 10 % (v/v) nitric acid overnight and subsequently rinsed several times with ultrapure water before use. Stock standard solutions (1,000 mg L<sup>-1</sup>) of the analytes were prepared by dissolving appropriate amounts of nitrate salts in 1 % HNO<sub>3</sub>. Working standard solutions were prepared daily from the stock solution by appropriate dilution with ultrapure water. 4-Phenyl-3-thiosemicarbazide, Triton X-100 and acetone from Sigma-Aldrich, and nitric, sulfuric and hydrochloric acids and carbon tetrachloride from Merck were used in the experiments. Buffer solutions (1.0 M at pH 2.0, 3.0, 7.0 and 8.0) were prepared using pure potassium dihydrogen phosphate, hydrochloric acid and sodium hydroxide. Sodium acetate, hydrochloric acid and sodium hydroxide were used to prepare

1.0 M of pH 4 and 5 buffer solutions, while ammonium acetate, hydrochloric acid and sodium hydroxide were used to prepare 1.0 M of pH 6 buffer solution. Finally, 1.0 M buffer solutions at pH 9 and 10 were prepared using ammonium chloride, hydrochloric acid and sodium hydroxide.

Three certified reference materials were used in this study. These were an aqueous standard QCS-19 (High-Purity Standards, Charleston, SC, USA), LGC 6156 harbour sediment (LGC, Teddington, UK) and NBS 1572 citrus leaves (National Bureau of Standards, WA, USA).

## 2.2 Instruments

A Perkin Elmer AAnalyst 200 model FAAS instrument equipped with appropriate hollow cathode lamp and an air-acetylene flame (air and acetylene flow rates of  $10 \text{ L min}^{-1}$  and  $2.5 \text{ L min}^{-1}$ , respectively) was used as the detector throughout. The wavelength (nanometres) used for monitoring Cu was 324.75 nm. A digital pH meter (Hanna Instruments model HI 4522, Romania) with a combined glass electrode was used for all pH measurements. An Electromag M 815 P centrifuge was used to separate the organic droplet from the cloudy aqueous solution.

### 2.2.1 Dispersive Liquid–Liquid Microextraction Procedure

For DLLME under optimum conditions, 10 mL of the sample solution containing 0.25 M NaCl was adjusted to pH 2 using 0.5-mL buffer solution in a glass test tube with a conical bottom. A 0.3 mL of  $10^{-3}$  M 4-phenyl-3-thiosemicarbazide solution prepared in disperser solvent (acetone) and 60  $\mu\text{L}$   $\text{CCl}_4$  (extraction solvent) was added to this sample solution. The copper complex of the 4-phenyl-3-thiosemicarbazide was extracted into fine droplets of  $\text{CCl}_4$ , and a cloudy mixture was produced by mixing the solution for about 1–2 min using a vortex mixer. The mixture was then centrifuged for 5 min at 3,000 rpm. After this step, the fine droplets were settled at the bottom of the conical test tube. After the decantation of the whole aqueous solution, the sedimented organic phase was diluted with 500  $\mu\text{L}$  of 1 % Triton X-100 prepared in 0.1 M  $\text{HNO}_3$  and was aspirated into the FAAS instrument. When preparing calibration graphs, the standards were put through the same preparation procedure as the samples.

## 2.3 Sample Preparation

The solid materials required an acid digestion so that the analytes could enter the liquid state. For the acid digestion, approximately 0.25 g of the solid material was weighed accurately into precleaned beakers. Concentrated nitric acid (10 mL) for plant samples and aqua regia for sediment and soil samples were added; the beakers were then covered with watch glasses and left overnight at room temperature so that a predigestion could occur. This was to ensure that any easily oxidisable materials could be destroyed in a controlled fashion at room temperature. The samples were then boiled gently on a hotplate until digestion was complete. The digests were evaporated nearly to dryness. This process took approximately 2 h. After cooling to room temperature, the digests were filtered through Whatman filter paper into precleaned beakers. Then, the pH of this solution was adjusted to a convenient value with a 10 % (*w/v*) sodium hydroxide solution and an appropriate buffer solution. After this step, the solution was transferred to a precleaned 25-mL volumetric flask and the contents made to volume using ultrapure water.

A seawater sample was collected from the Aegean Sea near the Edremit coast, and river water samples were collected from Büyük Bostancı River in Büyük Bostancı, Balıkesir, and Küçük Bostancı River in Küçük Bostancı, Balıkesir. The river water and seawater samples were filtered first through ordinary filter paper in order to separate coarse particles and suspended matter. They were then immediately filtered through 0.45- $\mu\text{m}$ -pore-size cellulose acetate membrane (Millipore) filters and acidified to pH 2 with nitric acid and stored in precleaned polyethylene bottles. Aliquots (10 mL) of the river water and seawater samples were used for analysis using the optimised dispersive liquid–liquid extraction method. No extra NaCl was added for the determination of copper in the seawater samples.

## 3 Results and Discussion

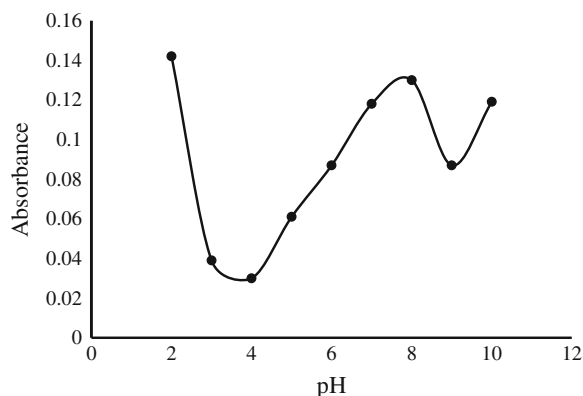
### 3.1 Analytical Parameters of the Dispersive Liquid–Liquid Extraction Method

Different experimental parameters such as pH, nitric acid concentration, the volume of acetone (disperser solvent) and carbon tetrachloride (extraction solvent), the concentration of 4-phenyl-3-thiosemicarbazide and

the volume and percentage of Triton X-100 were investigated to determine optimum conditions for the determination of copper in different natural samples.

The most important parameter for obtaining high recovery values and low limits of detection was the reaction pH between the ligand and copper. First, the effect of pH was studied using different buffer solutions between pH values of 2 and 10. The concentration of copper in the analyte solutions was  $0.1 \text{ mg L}^{-1} \text{ Cu(II)}$ . A  $0.3 \text{ mL}$  solution of  $10^{-3} \text{ M}$  ligand in acetone and  $40 \text{ }\mu\text{L}$   $\text{CCl}_4$  was added to  $5 \text{ mL}$  of analyte solution containing  $0.5 \text{ mL}$  of buffer solution. The prepared solutions were mixed for 2 min to ensure complete reaction between the ligand and the copper, and they were then centrifuged for 5 min at 3,000 rpm. The aqueous part of the final solution was decanted and discarded. The dispersed fine droplets of  $\text{CCl}_4$  deposited at the bottom of the conical test tube were added to  $0.5 \text{ mL}$  of 1 % Triton X-100 prepared in  $0.1 \text{ M HNO}_3$ . The final solution was aspirated directly into the FAAS instrument. The highest absorbance signals were obtained at pH 2 (Fig. 1). To investigate more acidic solutions, the same experiment was repeated using analyte solutions in 1, 0.1, 0.01 and 0.001 M  $\text{HNO}_3$  media. Again, the highest absorbance signals were obtained in  $0.01 \text{ M HNO}_3$  solutions (pH 2) (Table 1). Therefore, pH 2 was selected for all subsequent experiments.

Another analytical parameter requiring optimisation of the developed method is the concentration of the ligand in the solution. To determine the suitable ligand concentration,  $0.3 \text{ mL}$  of different concentrations of ligand between  $2 \times 10^{-4}$  and  $5 \times 10^{-2} \text{ M}$  was added to  $5 \text{ mL}$  of the analyte solution containing  $0.1 \text{ mg L}^{-1} \text{ Cu(II)}$  at pH 2. These samples were analysed according to the procedure. The results in Fig. 2 show that the



**Fig. 1** The effect of pH using buffer solutions

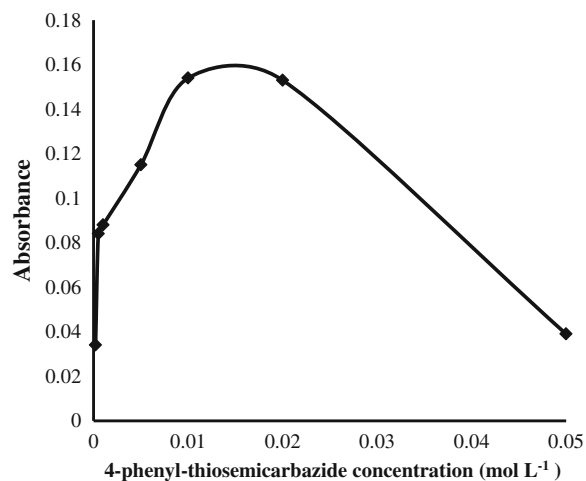
**Table 1** The effect of the concentration of  $\text{HNO}_3$  on the proposed dispersive liquid–liquid extraction method

$\text{HNO}_3$ ( $\text{mol L}^{-1}$ )	Absorbance
1 M	0.019
0.1 M	0.093
0.01 M	0.138
0.001 M	0.042

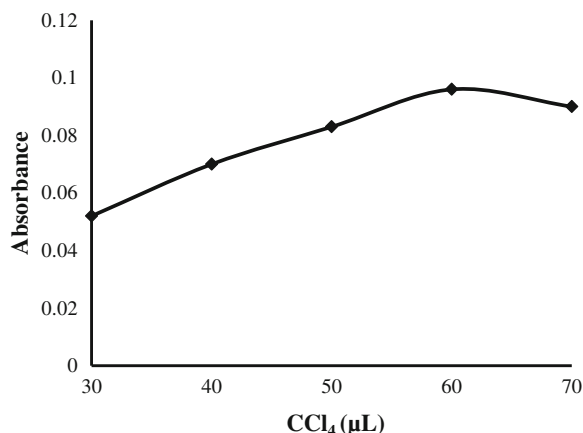
absorbance signals increased up to  $0.01 \text{ M}$  ligand concentration and then stay stable until a ligand concentration of  $0.02 \text{ M}$  after which they decreased gradually. Therefore, a ligand concentration of  $0.01 \text{ M}$  was selected as being optimal for all subsequent experiments.

The effect of  $\text{CCl}_4$  volumes between  $30$  and  $70 \text{ }\mu\text{L}$  was investigated using  $5 \text{ mL}$  of  $0.05 \text{ mg L}^{-1} \text{ Cu(II)}$  at pH 2 as the sample solution. Since the absorbance signal was highest at  $60 \text{ }\mu\text{L}$  of  $\text{CCl}_4$  as extractant solvent (Fig. 3), this was used in further experiments.

Different concentrations of NaCl were investigated to determine whether or not it improved extraction efficiency through the salting-out effect. The concentrations of NaCl varied between  $0.01$  and  $1 \text{ M}$  in the analyte solutions containing  $0.05 \text{ mg L}^{-1} \text{ Cu(II)}$ . The proposed method was applied to these solutions. The results showed that the absorbance signals increased up to  $0.25 \text{ M NaCl}$  and then remained stable. Therefore, NaCl was added to the analyte solutions during subsequent experiments to give a final concentration of  $0.25 \text{ M}$ . These results have showed that NaCl has a positive effect on the proposed dispersive liquid–liquid extraction method and also that this method can be applied to



**Fig. 2** The effect of 4-phenyl-thiosemicarbazide concentration



**Fig. 3** The effect of the volumes of CCl<sub>4</sub>

the seawater samples that contain high concentrations of salt.

### 3.2 The Effects of Foreign Ions

The effects of foreign ions were studied in two different parts. First, an investigation into the effect of typical seawater composition, i.e. 1,270 mg L<sup>-1</sup> Mg<sup>2+</sup>, 400 mg L<sup>-1</sup> Ca<sup>2+</sup>, 10,800 mg L<sup>-1</sup> Na<sup>+</sup>, 400 mg L<sup>-1</sup> K<sup>+</sup>, 5,100 mg L<sup>-1</sup> SO<sub>4</sub><sup>2-</sup>, 600 mg L<sup>-1</sup> CO<sub>3</sub><sup>2-</sup>, 16,600 mg L<sup>-1</sup> Cl<sup>-</sup> and 620 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup> on the signal of 0.05 mg L<sup>-1</sup> copper, was undertaken. This is because the elevated levels of these interfering species in seawater can cause a variety of problems and make the direct determination of trace copper in this matrix using a standard FAAS instrument difficult. In this experiment performed with synthetic seawater, no extra NaCl was added to the analyte solution. The same experiment was repeated in the analyte solution containing 0.05 mg L<sup>-1</sup> of copper in 0.01 M HNO<sub>3</sub> and 0.25 M NaCl using the proposed method. The results given in Table 2 showed that the seawater matrix does not cause any significant

**Table 2** The effect of interfering ions in synthetic seawater (1,270 mg L<sup>-1</sup> Mg<sup>2+</sup>, 400 mg L<sup>-1</sup> Ca<sup>2+</sup>, 10,800 mg L<sup>-1</sup> Na<sup>+</sup>, 400 mg L<sup>-1</sup> K<sup>+</sup>, 5,100 mg L<sup>-1</sup> SO<sub>4</sub><sup>2-</sup>, 600 mg L<sup>-1</sup> CO<sub>3</sub><sup>2-</sup>, 16,600 mg L<sup>-1</sup> Cl<sup>-</sup> and 620 mg L<sup>-1</sup> NO<sub>3</sub><sup>-</sup>) (n=3)

	Added (µg L <sup>-1</sup> )	Found (µg L <sup>-1</sup> )	Recovery (%)	RSD (%)
Synthetic seawater	50	49.6±0.6	99.2	1.3
0.01 M HNO <sub>3</sub>	50	49.8±1.2	99.5	2.3

interference effects during the determination of copper using the proposed method. In the second investigation of the effect of the foreign ions, the potential interference effects from several elements were determined individually to make the study more complete. The concentration of the analyte ions used during this experiment was 0.2 mg L<sup>-1</sup> for Cu<sup>2+</sup>. Table 3 shows the deviation and per cent error caused by 100 mg L<sup>-1</sup> concomitant ion on 0.2 mg L<sup>-1</sup> Cu. The only significant interfering ion appeared to be PO<sub>4</sub><sup>3-</sup> and only then if the sample contains more than 100 mg L<sup>-1</sup>. However, this interference is not significant since the recovery of the copper is still quite high, being about 93.5 %. The phosphate anions may cause a change to the pH because this anion is quite basic. This concentration level of the phosphate is quite high for most of the natural samples. This effect of the phosphate anion could be prevented by adjusting the pH of the samples to pH 2 before the analyses so that the phosphate species will turn to the other species of phosphate such as phosphoric acid and dihydrogen phosphate, which are the main phosphate species at this acidic pH. The results indicate that this method may easily be applied for the determination of

**Table 3** Effect of potentially interfering ions on the determination of Cu (0.20 mg L<sup>-1</sup>) using 4-phenyl-3-thiosemicarbazide as a complexing agent and the proposed method

Interfering ion	Amount added (mg L <sup>-1</sup> )	Found Cu <sup>2+</sup> (mg L <sup>-1</sup> )	Error (%)
–	–	0.196	-1.9
Ba <sup>2+</sup>	100	0.194	-3.0
Ni <sup>2+</sup>	100	0.191	-4.7
Mg <sup>2+</sup>	100	0.194	-3.0
Ca <sup>2+</sup>	100	0.202	1.0
Fe <sup>2+</sup>	100	0.197	-1.3
Co <sup>2+</sup>	100	0.203	1.5
Cr <sup>3+</sup>	100	0.198	-0.7
Mn <sup>2+</sup>	100	0.199	-0.2
Pb <sup>2+</sup>	100	0.202	1.0
Cd <sup>2+</sup>	100	0.201	0.4
Sr <sup>2+</sup>	100	0.204	2.1
CO <sub>3</sub> <sup>2-</sup>	100	0.203	1.5
PO <sub>4</sub> <sup>3-</sup>	100	0.187	-6.4
SO <sub>4</sub> <sup>2-</sup>	100	0.201	0.4
NO <sub>3</sub> <sup>-</sup>	100	0.198	-0.7
Cl <sup>-</sup>	100	0.205	2.7

copper in natural water samples including seawater and acid digests of soil, sediment and plant samples.

### 3.3 Preconcentration Experiments

Experiments designed to determine the preconcentration factor obtainable for Cu in natural samples were performed. Different preconcentration factors were obtained using different volumes of analyte solution (5–10 mL) and different volumes of final solution (0.5–1.0 mL). The concentration of copper was in the range of 0.025 mg L<sup>-1</sup>, 0.05 mg L<sup>-1</sup>, 0.10 mg L<sup>-1</sup>, 0.2 mg L<sup>-1</sup> and 0.30 mg L<sup>-1</sup> in preconcentration experiments. The proposed method was applied to these solutions, and the results obtained were given in Table 4. The results showed that this method can be applied successfully to the samples enabling the determination of copper with a preconcentration factor of at least 20.

### 3.4 Analytical Characteristics

In order to evaluate the performance of the method, the linearity and the detection limits were determined. This was followed by an assessment of the accuracy and repeatability. The Cu(II) calibration solutions were run under the optimum conditions using the proposed

**Table 4** The preconcentration experiments

Sample volume (mL)	Copper (mg L <sup>-1</sup> )	Final volume (mL)	Recovery (%)	Preconcentration factor
5	0.05	1	99.9	5
5	0.10	1	99.2	5
5	0.20	1	101.0	5
5	0.30	1	99.7	5
5	0.05	0.5	105.9	10
5	0.10	0.5	97.3	10
5	0.20	0.5	99.1	10
5	0.30	0.5	100.6	10
10	0.025	1	102.7	10
10	0.05	1	99.4	10
10	0.10	1	99.0	10
10	0.15	1	100.5	10
10	0.025	0.5	99.1	20
10	0.050	0.5	99.9	20
10	0.10	0.5	100.7	20
10	0.15	0.5	99.7	20

method. Standards and blanks were prepared using ultrapure water. Under the optimal conditions, the calibration curve was linear over the range of 2–600 µg L<sup>-1</sup> of copper. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated as 3 and 10 *s*/slope (*n*=10 runs of blanks), respectively. The LOD and LOQ values were 0.69 and 2.30 µg L<sup>-1</sup>, respectively, using the 20-fold preconcentration but could possibly be improved further if greater preconcentration factors were used. The analysis time for the proposed method is very short. After adding the sample solution, the disperser solvent containing ligand, buffer solution and the extraction solvent to the centrifuge tubes, each sample was mixed using a vortex mixer for 1–2 min, followed by centrifugation for 5 min. The centrifugation step could be performed for eight of the 15-mL capacity tubes simultaneously.

### 3.5 Analyses of Certified Samples

The accuracy of the developed procedure was tested using certified reference materials. One water certified material, a high purity standard (QCS-19), a sediment certified material (LGC 6156 harbour sediment) and the plant samples (NBS 1572 citrus leaves) were analysed using the developed procedure. Acid digests of the solid samples were prepared according to the sample preparation procedure. The high purity standard (QCS-19) that contains 100 mg L<sup>-1</sup> of copper was diluted to give the final concentration given in Table 5. The results of the analyses are also given in Table 5. As shown in Table 5, the results obtained are in close agreement with the certified values. These results show that the method may readily be applied for the determination of these metals in water, vegetation samples and soil and sediment samples. Using the Student's *t* test, adopting the

**Table 5** The results for the certified materials

Certified material	$\bar{x}$	<i>s</i>	$\mu$	$t = \frac{ \mu - \bar{x}  \sqrt{N}}{s}$
NBS 1,572 citrus leaves (µg g <sup>-1</sup> )	16.45	0.35	16.5	0.08
LGC 6,156 harbour sediment (µg g <sup>-1</sup> )	2,392	14.14	2,400	0.35
QCS-19 high purity standard (µg L <sup>-1</sup> )	99.6	0.32	100	0.72

$\mu$  represents the mean of the certified materials and  $\bar{x}$  and *s* represent experimental mean and the standard deviation

null hypothesis, it is possible to determine whether or not there is a significant difference between the mean concentrations found using the proposed method and the certified values. One sample  $t$  test was performed for each of the certified materials individually. The results show that the  $t$  values calculated using the equation  $t = \frac{|\mu - \bar{x}| \sqrt{N}}{s}$  are smaller than the critical value of  $t$  at 95 % confidence interval for all certified samples, indicating that there is no evidence of systematic error in the proposed method. The critical value of  $t$  for the degree of freedom at 95 % confidence interval is 4.30. This confirms that the results obtained by the proposed method were in good agreement with the certified values.

Although the copper concentration in the harbour sediment was extremely high and would not normally require preconcentration, the results demonstrate that the method is sufficiently robust to be applied to this sample type as well as to waters.

### 3.6 Analysis of Real Samples

The proposed method was applied to river and seawater samples as well as to acid digests of green tea, black tea and soil samples. River and seawater samples were spiked with  $10 \mu\text{g L}^{-1}$  of Cu(II). The results of these spike/recovery experiments and other results obtained from soil and plant samples are given in Table 6. The recovery values obtained were very satisfactory. The procedure developed in this work is therefore an alternative method for the determination of very low

concentrations of Cu(II) in different natural samples.

### 3.7 Comparison with Other Dispersive Liquid–Liquid Extraction Methods

In the literature, there are numerous applications of dispersive liquid–liquid microextraction to determine copper ions in different samples using FAAS detection. Comparative data given in Table 7 show that the limit of the detection of the proposed method is competitive with most of the methods proposed in the literature. Khani et al. (2011) used 4,4'-bis(dimethylamino) thiobenzophenone to complex copper and obtained a slightly better limit of the detection than that obtained in this study. However, their method required a home-made microsample introduction unit. The method proposed by Aristidis and Kallirroy-Ioanna (2009) using ammonium diethyldithiophosphate as a ligand has very low limits of the detection, but this method is an on-line flow injection method and requires a special manifold. The method proposed by Farajzadeh et al. (2008) using 8-hydroxyquinoline and other dispersive liquid–liquid microextraction methods to determine other metal ions reported in the literature require an extra step to evaporate the organic droplet before FAAS measurements. However, in the proposed dispersive liquid–liquid microextraction method, the organic droplet can easily be mixed with 0.1 M Triton X-100 prepared in 0.1 M  $\text{HNO}_3$  that produces an emulsion with the organic droplet prior to aspiration into the FAAS instrument. The proposed method has been used to determine copper successfully in different sample matrices such as natural

**Table 6** The results of the real natural samples

	Added ( $\mu\text{g L}^{-1}$ )	Found	Recovery (%)	RSD (%)
Büyük Bostancı River ( $\mu\text{g L}^{-1}$ )	–	2.2±0.4	–	16.2
	10	13.9±0.4	117.3	2.6
Küçük Bostancı River ( $\mu\text{g L}^{-1}$ )	–	5.7±0.9	–	16.5
	10	16.8±1.6	111.1	9.2
Seawater ( $\mu\text{g L}^{-1}$ )	–	18.2±1.1	–	5.8
	10	28.6±1.3	103.5	4.6
Black tea ( $\mu\text{g g}^{-1}$ )	–	15.0±0.6	–	3.8
Green tea ( $\mu\text{g g}^{-1}$ )	–	19.1±0.1	–	0.8
Hastanetepe (soil) ( $\mu\text{g g}^{-1}$ )	–	29.6±0.5	–	1.8
Çakallar (soil) ( $\mu\text{g g}^{-1}$ )	–	27.5±0.2	–	0.7

**Table 7** Characteristics of dispersive liquid–liquid extraction methods for determination of copper in different samples using FAAS detection

Ligand	Sample volume (mL)	Limit of detection ( $\mu\text{g L}^{-1}$ )	Preconcentration factor	Sample type	Reference
4,4'-bis(dimethylamino) thiobenzophenone	10	0.45	137	Water samples	Khani et al. 2011
Ammonium diethyldithiophosphate	5	0.04	560	Water samples	Aristidis and Kallirroy-Ioanna 2009
None	8	0.5	16	Water samples	Mohammadi et al. 2009
8-Hydroxyquinoline	5	3	42–48	Water samples	Farajzadeh et al. 2008
8-Hydroxyquinoline	20	3.4	28	Human hair and tea samples	Bahar and Zakerian 2012
4-Phenyl-thiosemicarbazide	10	0.69	20	Seawater, river water, plant, sediment and soil samples	This work

waters (river and seawaters), tea samples, sediment and soils. The chemistry of the proposed method is robust and suitable; compromised operating conditions mean that the method could be used with a more sensitive, simultaneous detector, e.g. ICP-MS, leading to exceptionally low LODs. Also, the proposed dispersive liquid–liquid microextraction method has the advantage of being simple, robust (in that it may be applied to an assortment of sample types) and cheap to utilise in that it does not require any extra specialist equipment. It should also be noted that the preconcentration factor is limited in this study because the centrifuge used did not have the capacity to hold volumes of greater than 10 mL. It is possible that significantly higher preconcentration factors are obtainable and that LOD could also be improved substantially.

#### 4 Conclusions

A new DLLME technique for copper determination was developed. The proposed method was based on the extraction of the copper from the aqueous phase into the fine droplets of extraction solvent (cloudy solution), which were formed on-line from a ternary system (water–acetone– $\text{CCl}_4$ ). The use of DLLME based on 4-phenyl-3-thiosemicarbazide for the preconcentration of copper ions from different matrix types is proposed as a prior step to their determination using FAAS. This method is simple, rapid, sensitive, and cheap and has low toxicity, since only a very small volume of the extraction solvent (microlitre volumes) of an organic solvent is used instead

of the environmentally damaging high volumes of organic solvents used in classical liquid–liquid extraction methods. In addition, the use of FAAS as a detection system has low costs, high analytical throughput and good selectivity compared with other techniques, such as graphite furnace atomic absorption spectrometry (GFAAS) and ICP-OES. The main benefits of the proposed methodology were the following: minimum use of toxic organic solvent, simplicity, low cost, enhancement of sensitivity and rapid analysis time and that it can be used to determine copper successfully in different sample matrices such as natural waters (river and seawaters), tea samples, and sediment and soil samples. This method also works at acidic pH, meaning that it has the added advantage of being capable of preconcentrating the copper without any interference effects arising from the precipitation of other metals such as iron hydroxides. Finally, 4-phenyl-3-thiosemicarbazide was used, for the first time, as a chelating agent for the preconcentration and determination of copper in natural samples for its good selectivity in acidic medium.

**Acknowledgments** This work was supported by the Balikesir University Research Fund (project no. 2011/09).

#### References

- Aristidis, N. A., & Kallirroy-Ioanna, G. I. (2009). On-line sequential injection dispersive liquid–liquid microextraction system for flame atomic absorption spectrometric determination of copper and lead in water samples. *Talanta*, 79, 86–91.



- Bahar, S., & Zakerian, R. (2012). Determination of copper in human hair and tea samples after dispersive liquid–liquid microextraction based on solidification of floating organic drop (DLLME–SFO). *Journal of the Brazilian Chemical Society*, 23, 1166–1173.
- Chang, S. H., Teng, T. T., & Ismail, N. (2011). Optimization of Cu(II) extraction from aqueous solutions by soybean-oil-based organic solvent using response surface methodology. *Water, Air, & Soil Pollution*, 217, 567–576.
- Chen, H., Jin, J., & Wang, Y. (1997). Flow injection on-line coprecipitation preconcentration system using copper(II) diethyldithiocarbamate as carrier for flame atomic absorption spectrometric determination of cadmium, lead and nickel in environmental samples. *Analytica Chimica Acta*, 353, 181–188.
- Ernst, W. H. O., Verkleij, J. A. C., & Schat, H. (1992). Metal tolerance in plants. *Acta Botanica Neerlandica*, 41, 229–248.
- Farajzadeh, M. A., Bahram, M., Mehr, B. G., & Jonsson, J. A. (2008). Optimization of dispersive liquid–liquid microextraction of copper (II) by atomic absorption spectrometry as its oxinate chelate: application to determination of copper in different water samples. *Talanta*, 75, 832–840.
- Farnad, N., Farhadi, K., & Voelcker, N. H. (2012). Polydopamine nanoparticles as a new and highly selective biosorbent for the removal of copper (II) ions from aqueous solutions. *Water, Air, & Soil Pollution*, 223, 3535–3544.
- Kara, D. (2009). Preconcentration and determination of trace metals by flow injection micelle-mediated extraction using flame atomic absorption spectrometry. *Talanta*, 79, 429–435.
- Kara, D., & Alkan, M. (2002). Preconcentration and separation of copper (II) with solvent extraction using *N,N'*-bis(2-hydroxy-5-bromo-benzyl) 1,2 diaminopropane. *Microchemical Journal*, 71, 29–39.
- Kara, D., Fisher, A., & Hill, S. J. (2009). Determination of trace heavy metals in soil and sediments by atomic spectrometry following preconcentration with Schiff bases on Amberlite XAD-4. *Journal of Hazardous Materials*, 165, 1165–1169.
- Khani, R., Shemirani, F., & Majidi, B. (2011). Combination of dispersive liquid–liquid microextraction and flame atomic absorption spectrometry for preconcentration and determination of copper in water samples. *Desalination*, 266, 238–243.
- Kocot, K., Zawisza, B., & Sitko, R. (2012). Dispersive liquid–liquid microextraction using diethyldithiocarbamate as a chelating agent and the dried-spot technique for the determination of Fe, Co, Ni, Cu, Zn, Se and Pb by energy-dispersive X-ray fluorescence spectrometry. *Spectrochimica Acta B*, 73, 79–83.
- Liang, P., & Yang, J. (2010). Cloud point extraction preconcentration and spectrophotometric determination of copper in food and water samples using amino acid as the complexing agent. *Journal of Food Composition and Analysis*, 23, 95–99.
- Macnair, M. R. (1993). The genetics of metal tolerance in vascular plants. *New Phytologist*, 124, 541–559.
- Merian, E. (1991). *Metals and their compound in the environment*. New York: VCH.
- Mohammadi, S. Z., Afzali, D., & Baghelani, Y. M. (2009). Ligandless-dispersive liquid–liquid microextraction of trace amount of copper ions. *Analytica Chimica Acta*, 653, 173–177.
- Ozcelik, G., Imamoglu, M., Yildiz, S. Z., & Kara, D. (2012). Chemically modified silica gel with *N*-(2-aminoethyl)-salicylaldehyde for simultaneous solid phase extraction and preconcentration of Cu(II), Ni(II), Cd(II) and Zn(II) in waters. *Water, Air, & Soil Pollution*, 223–8, 5391–5399.
- Ranjbar, L., Yamini, Y., Saleh, A., Seidi, S., & Faraji, M. (2012). Ionic liquid based dispersive liquid–liquid microextraction combined with ICP-OES for the determination of trace quantities of cobalt, copper, manganese, nickel and zinc in environmental water samples. *Microchimica Acta*, 177, 119–127.
- Rezaee, M., Assadi, Y., Hosseini, M. R. M., Aghaee, E., Ahmadi, F., & Berijani, S. (2006). Determination of organic compounds in water using dispersive liquid–liquid microextraction. *Journal of Chromatography A*, 1116, 1–9.
- Skrlikova, J., Andruch, V., Balogh, I. S., Kocurova, L., Nagy, L., & Bazel, Y. (2011). A novel, environmentally friendly dispersive liquid–liquid microextraction procedure for the determination of copper. *Microchemical Journal*, 99, 40–45.
- Song, J., Zhao, F. J., Luo, Y. M., McGrath, S. P., & Zhang, H. (2004). Copper uptake by *Elsholtzia splendens* and *Silene vulgaris* and assessment of copper phytoavailability in contaminated soils. *Environmental Pollution*, 128–3, 307–315.
- Soylak, M., & Ercan, O. (2009). Selective separation and preconcentration of copper (II) in environmental samples by the solid phase extraction on multi-walled carbon nanotubes. *Journal of Hazardous Materials*, 168, 1527–1531.
- Tabrizi, A. B. (2007). Development of a cloud point extraction-spectrofluorimetric method for trace copper(II) determination in water samples and parenteral solutions. *Journal of Hazardous Materials*, 139, 260–264.
- Wieszczycka, K., Kaczerewska, M., Krupa, M., Parus, A., & Olszanowski, A. (2012). Solvent extraction of copper(II) from ammonium chloride and hydrochloric acid solutions with hydrophobic pyridineketoximes. *Separation and Purification Technology*, 95, 157–164.