Ultrasound-assisted Supramolecular Microextraction of Copper in Water, Food, Hair, and Tobacco Samples Prior to Microsampling Flame Atomic Absorption Spectrometry

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INTRODUCTION

A trace amount of copper (Cu) at the $\mu\text{g/kg}$ level is essential for human health, whereas high levels become toxic (1, 2). It plays an important role for renewable red blood cells and for enhancing some of the enzyme activities (3). Excess copper leads to DNA damage and disturbance in neuronal cells. In addition, free radical formation is usually accompanied with high concentrations of copper (4, 5). The main exposure to copper may be from drinking water or contaminated food. Leaky or corroded water pipes are often the main sources for copper in drinking water (6). The traditional determination of copper is by flame atomic absorption spectrometry (FAAS) or inductively coupled plasma mass spectrometry (ICP-MS) without pretreatment but suffers from matrix problems. The purification and preconcentration steps in the analytical methods increase the concentration of the targeted analyte and reduce interference (7). The common preconcentration methods are solid phase extraction (8), dispersive liquid-liquid microextraction (9), anodic stripping voltammetry and ionic liquid-microextraction (10). However, dispersive liquid-liquid and supramolecular-based microextraction leads to a reduction in the use of organic solvents, provides low detection limits, and requires less time (11, 12).

ABSTRACT

An analytical microextraction procedure was developed and applied for Cu(II) microextraction from natural waters, red pepper, black pepper, thyme, hashish, hair, and tobacco samples. The microextraction process was based on the chelation of Cu(II) from aqueous solutions with 4-(2-thiazolylazo) resorcinol, which is then moved to the organic supramolecular phase by ultrasonic waves. The organic phase, consisting of tetrahydrofuran and 1-decanol, was isolated by centrifugation. The extracted and preconcentrated copper was determined by microsampling flame atomic absorption spectrometry. The microextraction conditions and the operating parameters were optimized using the pH of 6, 200 μL of 0.1% of 4-(2-thiazolylazo) resorcinol, and ultrasonic enhancement for 5 to 8 minutes. The ultrasound-assisted supramolecular solvent liquid phase microextraction procedure resulted in a limit of detection (LOD) of $1.13 \mu\text{g L}^{-1}$ and was applied for Cu(II) determination in samples with different matrices.

There are many studies in which preconcentration is used to determine copper. Tahera et al. (13) established solid phase extraction of copper and electrothermal atomic absorption spectrometry determination and reported a limit of detection (LOD) of 3.9 ng L^{-1} . Niazi et al. (14) applied dispersive

liquid-liquid microextraction of copper as dithizone chelates and a LOD of 2.6 ng mL^{-1} . Yang et al. (15) compared ultrasound-assisted cloud point extraction and ultrasound-assisted dispersive liquid-liquid microextraction of copper with a limit of detection (LOD) of $0.7 \mu\text{g L}^{-1}$ and $0.8 \mu\text{g L}^{-1}$, respectively. Arain et al. (16) proposed non-dispersive ionic liquid microextraction for low level copper analysis resulting in a LOD of 0.042 mg L^{-1} . Akhond et al. (17) developed gas-assisted dispersive liquid-phase microextraction for ultratrace level copper extraction with 1-hexyl-3-methylimidazolium hexafluorophosphate and a LOD of 0.07 mg mL^{-1} . Arain et al. (18) applied an ionic liquid-assisted microemulsion procedure combined with cloud point extraction for copper determination in drinking water and serum and a LOD of 0.132 mg L^{-1} . Ozzeybek et al. (19) used dispersive liquid-liquid microextraction coupled with FAAS for copper determination and reported a LOD of $0.7 \mu\text{g L}^{-1}$. Arain et al. (20) combined dispersive ionic liquid-based on ultrasound-assisted microextraction for trace level copper analysis with (PAN) 1-(2-pyridylazo)-2-naphthol chelation.

The combination of the microextraction processes with ultrasonic waves have resulted in a noticeable improvement due to the high dispersion of the targeted analyte with the aqueous and organic phases, allowing the transfer to the organic phase with the chelator (20). Furthermore, the supramolecular solvent is highly recommended for extraction applications (21-23).

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For the present study, a supramolecular solvent and ultrasonic waves were used to develop a liquid phase microextraction procedure for the separation and preconcentration of copper at ultratrace levels. Copper was determined by flame atomic absorption spectrometry (FAAS) which has a micro-sampling attachment for the accurate quantification of the analyte element in natural water, red pepper, black pepper, thyme, hashish, hair, and tobacco samples.

EXPERIMENTAL

Instrumentation

For the detection of copper, a PerkinElmer® AAnalyst™ 300 flame atomic absorption spectrometer (PerkinElmer, Inc., Shelton, CT, USA) was used. The extracted copper solutions were injected into the FAAS with a homemade injection system. A Teflon® funnel fixed with an Eppendorf® pipette was connected to the FAAS nebulizer. This system allows for the analysis of ultratrace determination of the extracted and preconcentrated copper samples (24, 25).

Reagents

A Millipore® Milli-Q® system (18.2 MΩ·cm, Millipore Corporation, USA) was used to obtain deionized water for solutions during the microextraction steps. In addition, the chemicals applied for the matrix and metal solutions as well as the organic solvents were of analytical grade. A Sartorius PT-10 pH meter (Germany) and a Sonorex DT-255 ultrasonic water bath (Germany) were used. An ALC PK 120 centrifuge (Buckinghamshire, England) was used during the microextraction processes.

Test Procedure

The sonication-based supramolecular solvent liquid phase microextraction procedure was performed in 50-mL polypropylene tubes into

which the sample solutions including the targeted Cu(II) were placed. The pH was adjusted to the desired value by using a phosphate buffer. The 4-(2-thiazolylazo) resorcinol was used to chelate Cu(II), then tetrahydrofuran and 1-decanol were added to obtain the supramolecular solvent. The supramolecular microextraction step was enhanced by exposure to ultrasonic waves for about 5-8 minutes. The organic and aqueous phases were separated using the centrifuge at 4000 rpm for 10 minutes. Then suction was applied to isolate the aqueous phase. The supramolecular phase containing the analyte was dissolved in ethanol to 500 µL and the Cu concentration determined by micro-sampling FAAS.

Application of Method to Water, Food, Hair, and Tobacco Samples and CRMs

Red pepper, black pepper, thyme, hashish, hair, and tobacco samples were obtained in Kayseri, Turkey and subjected to a wet digestion method. In this method, 0.1–0.25 g of each sample was placed into a beaker and mixed with 20 mL of concentrated nitric acid. This mixture was heated until it formed a clear solution, then was filtered, and the final volume of the sample extract was completed to 10-mL volume with distilled water (8). Then the above-described microextraction method was applied.

For water analysis, the samples were also obtained in Kayseri, Turkey, and stored in polyethylene bottles. The samples consisted of wastewater, seawater, dam water, and valley water. Prior to use, the samples were first filtered with a cellulose membrane filter (Millipore Corporation, Bedford, MA, USA) (7-9). Then the above described method was applied. In addition, certified reference materials (CRMs) TMDA-64.2 and TMDA-53.3 Fortified Water (National Water Research

Institute, Environment Canada, Burlington, Canada) to demonstrate the accuracy of the proposed method.

RESULTS AND DISCUSSION

Evaluation of the Factors Controlling Ultrasound-assisted Supramolecular Solvent Liquid Phase Microextraction

By addition of 4-(2-thiazolylazo) resorcinol as the ligand to the sample solutions containing Cu(II), the interaction between the OH groups of the ligand and Cu(II) starts. Cu(II) interacts with the oxygen lone pair of electrons. This interaction of the concentration of H⁺ which is competing with Cu²⁺ and the pH are critical parameters for the extraction studies (26-30). In the present work, the effect of H⁺ is studied in terms of pH value. Different pH values from 2.0 to 8.0 were studied for the quantitative recovery of copper in the presented microextraction system. The efficiency of the extraction process was evaluated in each case, as is shown in Figure 1. The best microextraction condition was achieved at pH 6.0 which was selected as optimal for further work.

The amount of 4-(2-thiazolylazo) resorcinol as ligand is critical to achieve high efficiency microextraction. Therefore, the amount of 4-(2-thiazolylazo) resorcinol was investigated using a volume of 0.1% 4-(2-thiazolylazo) resorcinol solution. The extraction efficiency using different 4-(2-thiazolylazo) resorcinol amounts is shown in Figure 2. It can be seen that 200 µL of 0.1% of 4-(2-thiazolylazo) resorcinol is suitable for complete chelation of the targeted Cu(II) and leads to the transfer of all chelated Cu(II) to the organic phase in the next steps.

Composition of the supramolecular organic solvent was evaluated by testing the different ratios of 1-decanol and tetrahydrofuran, and

Fig. 1. Effect of the pH on the recovery of Cu(II) (N=3).

Fig. 2. Effect of ligand on the recovery of Cu(II) (N=3).

Fig. 3. Effect of the composition of supramolecular solvent on the recovery of Cu(II) (N=3).

Fig. 4. Effect of exposure to ultrasonic waves on the recovery of Cu(II) (N=3).

Fig. 5. Effect of sample volume on the recovery of Cu(II) (N=3).

evaluating the (%) recovery in each case. Figure 3 shows the influence of tetrahydrofuran (A), the amount of 1-decanol (B), and the ratio between them (C). It is clear that the suitable ratios of tetrahydrofuran and 1-decanol are 1 and 5.3.

The time required for dispersion under the ultrasonic-enhanced microextraction was investigated. The effect of applying ultrasonic enhancement on the recovery of Cu(II) as 4-(2-thiazolylazo) resorcinol chelate in Figure 4 shows that between 5-8 minutes are suitable for best quantitative recoveries.

The sample volume (31-41) is another critical and important parameter to obtain quantitative recoveries and a high preconcentration factor in the extraction-separation studies. The influence of sample volume on the quantitative recoveries of Cu(II) was investigated. Figure 5 shows that the recoveries of copper are quantitative up to 35 mL of initial sample solution.

Matrix Effects

When designing the microextraction procedures, it is important to consider the capability of the

analytical method to tolerate the presence of foreign ions rather than the targeted analyte (42-52). In the developed sonication-based supramolecular solvent liquid phase microextraction method, the effects of competition with various common ions were tested. The recoveries for each competing ion are listed in Table I. For all tested matrix ions, the recovery values of copper were in an acceptable quantitative range, suggesting the possibility of applying the presented microextraction process for real samples with different matrices.

Analytical Evaluation

The optimization of the sonication-based supramolecular solvent liquid phase microextraction method was extended and includes spiking known amounts of Cu(II) and a recovery evaluation to ensure the applicability of the process with different concentrations of Cu(II). Table II lists the results of the spiking-recovery tests which were found to be in the analytical quantitative range with a high recovery (%). The accuracy of the developed method was also evaluated by analyzing CRM TMDA-64.2

and TMDA-53.3 Fortified Water. The data in Table III indicate high agreement with the certified values. The preconcentration factor was calculated as 70 considering the initial sample volume of 35 mL and the final extracted analyte solution of 500 μ L (7, 9).

In addition, the limit of detection (LOD), calculated based on 10 determinations of the standard deviation of the blank, was 1.13 μ g L⁻¹. The limit of quantification (LOQ), calculated as 3.3 times the LOD, was 3.40 μ g L⁻¹. The relative standard deviation (RSD) for 10 measurements was 4.6%.

Application of Method to Real Samples

Monitoring pollution with environmental samples has become an important issue in analytical chemistry (18, 53-58). The health risk to humans and animals from surrounding environmental components has increased in the last decades due to fast urbanization and excessive industrial activities which leads to the release of heavy metals, including copper, into the environment. In addition, the probability of trans-

TABLE I
Recovery of Cu(II) With Different Matrix Ions (N=3)

Ions	Concentration (mg/L)	Added As	Recovery (%)
K ⁺	1200	KCl	95±2
Na ⁺	10,000	NaCl	97±3
Mg ²⁺	400	Mg(NO ₃) ₂ ·6H ₂ O	95±4
Ca ²⁺	400	CaCl ₂	97±0
Co ²⁺	10	Co(NO ₃) ₂	95±4
Ni ²⁺	10	Ni (NO ₃) ₂ ·6H ₂ O	99±1
Cd ²⁺	5	Cd(NO ₃) ₂ ·6H ₂ O	99±1
Fe ³⁺	20	Fe(NO ₃) ₃ ·9H ₂ O	101±0
Zn ²⁺	10	Zn(NO ₃) ₂	95±1
CO ₃ ²⁻	500	Na ₂ CO ₃	93±1
Cl ⁻	1200	KCl	95±2
SO ₄ ²⁻	500	Na ₂ SO ₄	101±1
F ⁻	200	NaF	95±2

TABLE II
Spiking Cu(II) into Tap Water and Recovery (N=3)

Added (μ g)	Found (μ g)	Recovery (%)
0	0	-
1.0	1.00±0.08	100
2.0	1.98±0.06	99

TABLE III
Recovery of Cu(II) From CRM Water Samples (N=3)

CRMs	Certified Value (μ g L ⁻¹)	Found Value (μ g L ⁻¹)	Recovery (%)
TMDA-64.2	270	267 ± 8	98
TMDA-53.3	312	297± 7	95

TABLE IV
Application of Method to the Determination of Cu(II)
in Water, Food, Hair, and Tobacco Samples (N=3)

Samples	Sample Name	Cu Concentrations
Water Samples ($\mu\text{g L}^{-1}$)	Wastewater	79.7 ± 4.2
	Seawater	67.9 ± 4.2
	Dam Water	48.7 ± 10.5
	Valley Water	39.8 ± 6.3
Food Samples ($\mu\text{g kg}^{-1}$)	Black Pepper	38.3 ± 0.0
	Thyme	35.3 ± 4.2
	Hashish	30.9 ± 2.1
	Red Pepper	54.6 ± 10.5
Hair ($\mu\text{g kg}^{-1}$)		153.8 ± 0.0
Cigarettes ($\mu\text{g kg}^{-1}$)		134.5 ± 6.3

Mean $\pm$ standard deviation.

ference of these pollutants into the food chain is high. It has also been reported that copper contaminates food, water, air, and other environmental samples. Hair samples are usually used to indicate copper exposure in the human body.

The samples analyzed in this study include natural water, red pepper, black pepper, thyme, hashish, hair, and tobacco samples. Their copper concentrations found are listed in Table IV. It can, therefore, be stated that the developed sonication-based supramolecular solvent liquid phase microextraction procedure is suitable for monitoring copper in samples from a variety of sources.

CONCLUSION

The application of ultrasonic waves in the present work has led to the effective microextraction of copper with a supramolecular solvent. For the microextraction of Cu(II), a 4-(2-thiazolylazo) resorcinol chelate was used which easily moved to the organic supramolecular solvent phase composed of tetrahydrofuran and 1-decanol. The proposed sonication-based supramolecular solvent liquid phase microextraction procedure was operated in low acidic medium at

a pH of 6.0 using a phosphate buffer and provided a LOD of $1.13 \mu\text{g L}^{-1}$. The presented microextraction method was successfully applied to the microextraction and determination of copper from different samples including natural waters, red pepper, black pepper, thyme, hashish, hair, and tobacco samples without any interference of other competing ions. The extracted and preconcentrated Pb(II) ions were determined by microsampling flame atomic absorption spectrometry.

ACKNOWLEDGMENT

The authors extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for funding this Research Group - RGP-043.

Received October 31, 2017.

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