PRECONCENTRATION OF COPPER FROM DIFFERENT SAMPLES BY DISPERSIVE LIQUID-LIQUID MICROEXTRACTION

C. Bosch Ojeda
F. Sanchez Rojas
J.M. Cano Pavon
Professors of Analytical Chemistry, Faculty of Sciences,
University of Málaga, Spain

Abstract

Sample preparation is a critical step of any analytical protocol. Nowadays the goals to be reached are the best results, in the shortest time, with minimal contamination, low reagent consumption and generation of minimal waste. Dispersive liquid-liquid microextraction is a miniaturized sample preparation procedure inside Green Chemistry because the low volume of dissolvent employed. All parameters that influence on the preconcentration of copper have been optimized. The detection limit was 7.9 μg L^{-1}. The proposed method was successfully applied to the preconcentration and determination of copper in food, vegetation, and water samples and in two standard reference materials.

Keywords: Copper, DLLME, FAAS, Environmental samples, Food samples

Introduction

Copper is a substance that occurs naturally in the environment and spreads through the environment through natural phenomena. Humans widely use copper. For instance it is applied in the industries and in agriculture. The production of copper has lifted over the last decades. Due to this, copper quantities in the environment have increased. Copper is obtained by smelting, leaching and by electrolysis. Copper is a metal that occurs naturally throughout the environment, in rocks, soil, water, and air. Copper is an essential trace element in plants and animals (including humans), it is a component of several enzymes (e.g., ferroxidases, cytochrome *c* oxidase, superoxide dismutase, tyrosinase, lysyl oxidase, and dopamine β hydroxylase) that perform important physiological functions (respiration, photosynthesis, lignification, phenol metabolism, protein synthesis, and

regulation of growth hormones). The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction reactions. Copper is necessary in small amounts in the soil of plants and the diet of animals. Therefore, plants and animals must absorb some copper from eating, drinking, and breathing. Copper is used to make many different kinds of products like wire, plumbing pipes, and sheet metal. Copper compounds are commonly used in agriculture to treat plant diseases like mildew, for water treatment and, as preservatives for wood, leather, and fabrics. Principally, copper can be found in many kinds of food, in drinking water and in air. The body absorbs eminent quantities of copper each day by eating, drinking and breathing. Although humans can handle proportionally large concentrations of copper, too much copper can still cause eminent health problems.

Copper concentrations in air are usually quite low, so that exposure to copper through breathing is negligible. But people that live near smelters that process copper ore into metal do experience this kind of exposure. Air levels of copper depend on the proximity to major sources of copper release into the ambient air. Average concentrations are usually well below 1 $\mu g/m^3$, but higher levels may be found in urban or otherwise polluted areas.

In seawater, the concentrations is approximately <1 $\mu g\ L^{-1}$. Much higher levels may be measured in coastal regions and estuaries; on the other hand, generally, higher mean levels (1–10 $\mu g\ L^{-1}$) are finding in rivers and lakes. Also, the acidity of the water may be related to the measured levels. Drinking water may contribute significantly to the daily copper intake because of the widespread use of copper pipes.

Copper is necessary for good health. Copper is a vital part of several enzymes. Copper combines with certain proteins to produce enzymes that act as catalysts to help a number of body functions, but the range between copper deficiency and copper toxicity is small. Ingestion of a large amount of copper salts causes gastrointestinal disturbances. In severe cases, systemic effects, especially hemolysis, liver, and kidney damage, can occur.

Due to the importance of metals ions at trace level in the human health and environment, the sensitive and accurate determination of the levels of these analytes in the environmental samples have been continuously carried out on the analytical and environmental laboratories around the world (Ekici K., Agaoglu S.and Isleyici O., 2004; Mico C., Peris M., Sanchez J. and Recatala L., 2006; Al-Khashman O. A., 2007). On the other hand, FAAS is one of the widespread traditional analytical techniques for the determination of trace elements, but it often suffers from its low sensitivity. Sample preparation including preconcentration is a critical step of any analytical protocol. Techniques such as co-precipitation (Bispo M. S., Morte E. S. B., Korn, M. G. A., Teixeira L. S. G., Korn M., and Costa, A. C. S.,

2005, solid phase extraction (Nazari S., 2008; Ferreira S. L. C., Queiroz A. S., Melo A. S. Q., Assis, J. C. R., Korn M. G. A. and Costa, A. C. S., 1997), liquid-liquid extraction (Jia Q., Kong X., Zhou W. and Bi L.,2008); Stafiej A. and Pyrzynska K., 2008) are widely used in the separation and preconcentration of trace elements. A new trend in analytical chemistry is the miniaturization of preconcentration systems with the aim of minimizing reagent consumption and waste generation (Rocha F. R. P., Teixeira L. S. G., Nóbrega J. A., 2009). For liquid-liquid extraction, alternatives of miniaturization can be employed with strategies such as liquid-liquid microextraction (LLME) (Lee J., Lee H. K., Rasmussen K. E. and Pedersen-Bjergaard S., 2008), single drop microextraction (SDME) and dispersive liquid-liquid microextraction (DLLME) (Naserib M. T., Hemmatkhahb P., Hosseini M. R. M. and Assadi Y., 2008).

The present work describes the attempt for the preconcentration of copper ions from aqueous samples using DPTH as complexing reagent. After optimization of experimental variables and determination of analytical characteristics, the method was evaluated for application in real samples.

Experimental

Standard solutions and reagents

Stock standard solution for Cu(II) (1000 mg L^{-1}) were supplied by Merck, Darmstadt, Germany. Standard solutions were prepared by appropriate dilution of the stock solutions daily.

High purity water (resistivity 18.2 MΩ cm) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout this work.

2,2'-Bis(di-2-pyridinyl-methylene)-thiocarbohydrazone (DPTH) solution in DMF was prepared by dissolving solid reagent samples prepared and purified by the authors (Abascal J. B., Torres A. G. and Pavón J. M. C., 1983).

All the other reagents including extractants and disperser solvents were analytical-grade reagents, as well as the reagents mentioned above.

Acetate buffer solution was prepared to adjust pH values for the extraction Cu.

NaCl solutions were prepared by dissolving appropriate amounts of NaCl in deionized water.

Instrumentation

Phase separation was achieved with a centrifuge Selecta Centromix in 15 mL calibrated conical tubes. A Varian Model SpectrAA 50 (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer was used for the analysis with the appropriate cupper hollow cathode lamps. The operating parameters were set as recommended by the manufacturer. Atomic absorption measurements were carried out in an air-acetylene flame. The

following conditions were used: absorption line Cu: 327.4 nm; slit widths: 0.5 nm; and lamp currents: 4 mA.

Sample analysis

The accuracy of the method for determination of copper content was checked by analyzing the reference standard material TMDA 54.4, Estuarine water (CRM LGC6016). These samples were analyzed by standard addition method.

The proposed method was also evaluated by analysis of Cu in waters, plants and food samples. The Cu concentration in all the original samples was below the detection limit. For plants and foods analysis standard solutions containing copper was added to 0.3–1.2 g of diverse plants and foods and the resulting materials were mineralized by reflux digestion, then evaporate to eliminate excess of acid, adjusted pH and diluted at convenient volume. Standard addition method was used in all instances. Natural waters were collected in polypropylene bottles previously cleaned by soaking for 24 h in 10% (v/v) nitric acid and finally rinsed thoroughly with ultra-pure water before use.

DLLME procedure

For DLLME under optimum conditions, 10 mL analyte solution containing variable amounts of copper, 2 mL acetate buffer solution, pH 5.4, 2 mL of 0.05% DPTH solution in DMF as chelating agent was placed in a 15 mL screw cap glass test tube. Then, 1 mL of methanol (as disperser solvent) and 0.2 mL of chloroform (as extraction solvent) were rapidly injected into a sample solution by using a microsyringe. A cloudy solution was formed in the test tube and separation of the phases was achieved by centrifugation at 3800 rpm for 5 min. After this process, the organic phase was sedimented in the bottom of conical test tube. 50 µL of this solution was diluted with HNO_3 0.1 M and the absorbance were measured by FAAS.

Results and discussion

In all subsequent study one variable at a time optimization was used to obtain the optimum conditions for the DLLME.

pH study

The separation of metal ions by DLLME involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of the sedimented phase, thus, obtaining the desired preconcentration. pH plays a unique role on metal chelate formation and subsequent extraction. The effect of pH on the complex formation and extraction of copper from samples was studied in the range of 3.0–10.0 by using appropriate buffers. The results are shown in figure 1. As can be seen, the absorbance is nearly constant in the pH range of 4.4–8. Thus, the value of pH 5.4 was selected for the following experiments. Also, the influence of 0.2 M acetate buffer solution amount was investigated for variation of volume

added from 0.5 to 5 mL. A volume of 2 mL was selected as the optimum value for subsequent work.

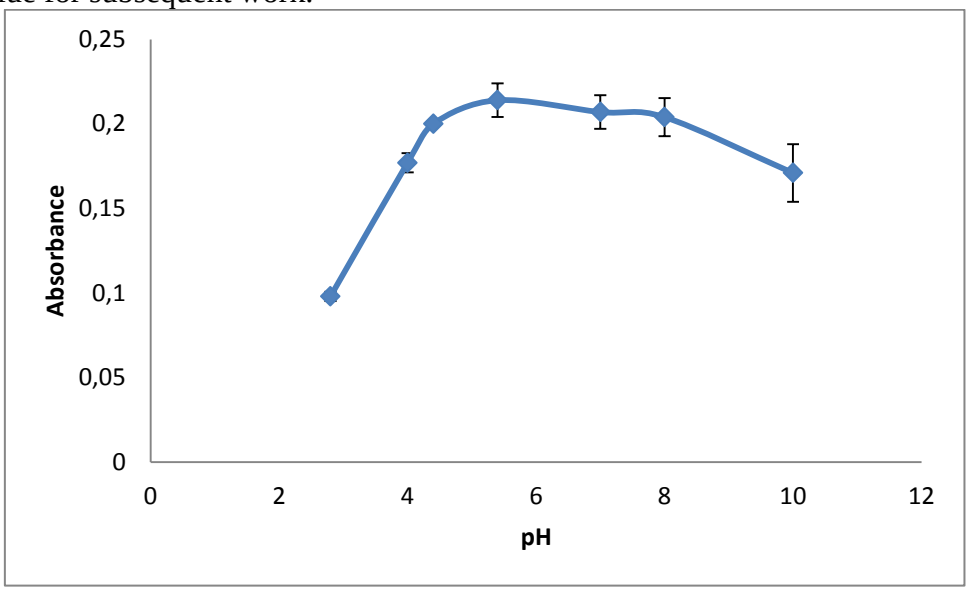

Figure 1. Influence of pH

Effect of chelating reagent (DPTH) concentration

The effect of DPTH concentration on the absorbance was examined using increasing volumes of 0.05% DPTH from 0.5 to 3 mL. The results showed that the change of DPTH concentration in the studied range has little effect on analytical signals, thus the volume of 1 mL, corresponding to its maximum value, was used in other experiments.

Effect of ionic strength

For investigating the influence of ionic strength on performance of DLLME, various experiments were performed by adding different amounts of NaCl (0–4% (w/v)). Other experimental conditions were kept constant. No significant impact on the analytical signal was observed in the studied range ≤ 3% NaCl, enabling the possibility of utilizing the proposed method for saline samples.

Effect of DLLME parameters

Effect of type and volume of extractant

Chloroform, carbon tetrachloride and dichloromethane were compared in the extraction of copper by using 0.5 mL of each one. Results showed that the maximum extraction recovery was obtained by using dichloromethane (Figure 2), however the volume obtained was much lower, and it was necessary to prepare several samples under identical conditions for to obtain an adequate volume to realize the measure, so 0.2 mL of chloroform was used as extractant to improve the preconcentration factor. In

these conditions, the signal obtained was similar to when dichloromethane was used.

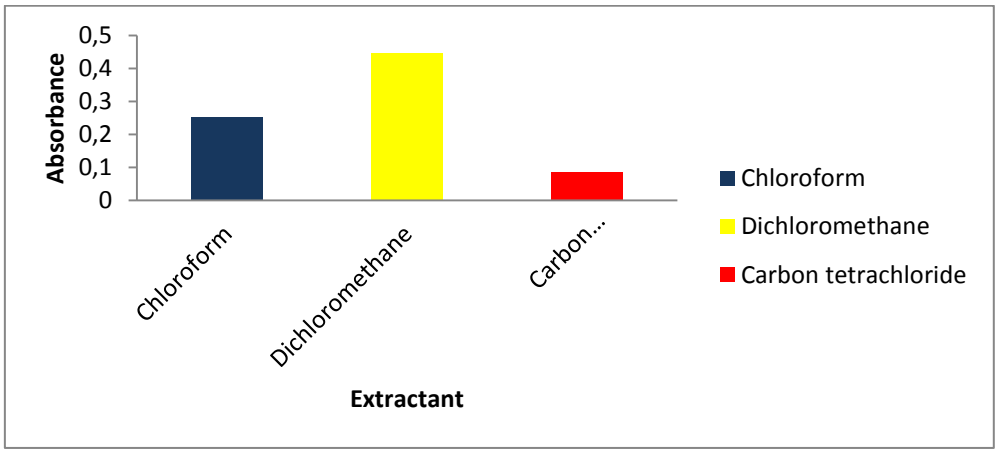

Figure 2. Influence of extraction solvent (0.5 mL of each one)

Effect of type and volume of disperser solvent

The role of a disperser is dispersion of an extraction solvent into aqueous sample to make extensive contact area between them and facilitating the mass transfer of analyte from water to organic solvent which causes considerable acceleration in the extraction of analytes. Miscibility of disperser solvent with extraction solvent and aqueous phase is the main point for selection of disperser solvent. Therefore in this section the ability of ethanol and methanol was investigated (figure 3). As can be seen from this figure better results were obtained by using 1 mL of methanol as disperser solvent and this value was selected for subsequent studies.

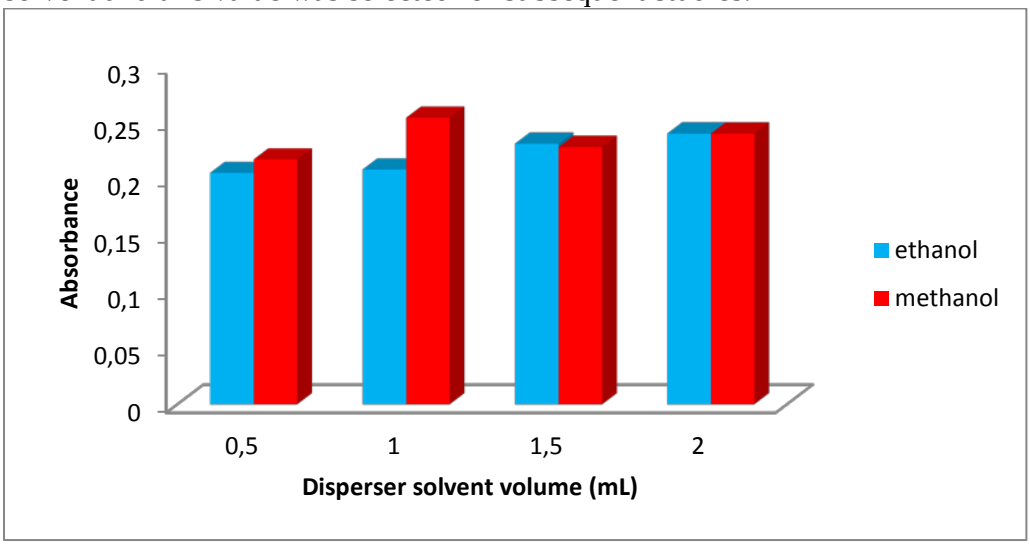

Figure 3. Comparison of disperser solvents

Analytical figures of merit

Important parameters such as the linear range, calibration graph, precision, detection limit, and preconcentration factor were determined to evaluate the method performance. The analytical characteristics are summarized in Table 1. The detection was defined as the concentrations of analyte giving signals equivalent to 3 times the standard deviation of the blank plus the net blank signal. The preconcentration factor was determined as the ratio of the slopes of the linear section of the calibration graphs before and after preconcentration and also by the ratio of the volume aqueous phase/organic phase.

Table 1. Analytical characteristics of DLLME-FAAS for determination of copper

	Cu
Dynamic range (µg L^{-1})	10-500
Regression equation	A=0.0012[Cu]+0.04955
R^2	0.9976
Regression equation (without extraction)	A= 0.0002[Cu^{2+}] – 0.0019 R^2 = 0.9991
Detection limit (µg L^{-1})	7.92
Determination limit (µg L^{-1})	9.58
Precision (% RSD) n=8	3.22
Preconcentration factor (Volume ratio)	50
Preconcentration factor (Slope ratio)	6

Interferences

The effect of a potential interference caused by common species present in samples was examined. For this purpose diverse ions (up to a maximum tolerance ratio of 500) were added to solutions containing 100 ng mL^{-1} of copper, and the solutions submitted to the recommended procedure. An ion was considered as interfering when it caused a variation in the absorbance of the analyte greater than 5%. The results are show in Table 2.

Table 2. Effect of foreign ions in the determination of copper by DLLME procedure.
[Cu^{2+}]= 100 µg L^{-1}

Species	Tolerance ratio
F^-, I^-, HCO_3^-, $SO_4^=$, K^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}	500:1
Cr^{3+}	100:1
Ni^{2+}, Hg^{2+}, Cd^{2+}, Co^{2+}	25:1
Zn^{2+}, Sn^{2+}, Bi^{3+}	10:1

Analysis of real samples

The accuracy of the proposed method was verified with the analysis of two certified reference materials (TMDA 54.4, Estuarine water (CRM LGC6016) which were analyzed according to the proposed method. It was

found that analytical results were in good agreement with the certified values as can be seen in Table 3.

On the other hand, in view of the application of the method to determination of copper in waters (tap water and sea water), vegetation, and foods, standard solutions containing copper were added to samples and the resulting materials were prepared as described under Experimental. Standard addition methods were used in all instances and the results were obtained by extrapolation. These results, as the average of three separate determinations, are shown in Table 3. The proposed method gave satisfactory average recoveries.

Table 3. Analytical results for Cu in several samples (Avg. ± SD of three trials)

Sample	**Certified value (µg L^{-1})**	**Found value* (µg L^{-1})**	**% Recovery**
TMDA 54.4	443 ± 19	434 ± 24	
Estuarine water. CRM LGC6016	190 ± 4	185.3 ± 15	
	Cu added µg g^{-1}	**Cu found* µg g^{-1}**	**% Recovery**
Food samples			
Rice	8.26	8.14 ± 0.21	98.55
Lentil	9.09	8.63 ± 0.36	94.94
Plants samples			
Pinus leaves	10.0	10.0 ± 0.2	100
Bignonia leaves	10.0	9.8 ± 0.2	98
Water samples (Value µg L^{-1})			
Tap water	20	19.9 ± 0.29	99.5
Sea water	20	20.64 ± 1.8	103.21

*Mean ± SD; n= 3

Conclusion

The comparison of the proposed DLLME method with other extraction methods indicates that DLLME can offer advantages of speed, simplicity, ease of operation, and a low consumption of organic solvent. This microextraction procedure is inside Green Chemistry because of the small volumes of dissolvent employed. All variables that influence in the formation of the complex Cu–DPTH and then application of DLLME procedure have been optimized by FAAS. Good agreements were obtained from certified reference materials and spiked samples.

Acknowledgements

The authors thank to the Ministerio de Ciencia e Innovación for supporting this study (Projects CTQ2009-07858) and also the Junta de Andalucía.

References:

Abascal J. B., Torres A. G., and Pavón J. M. C, Microchem. J., 1983, 28, 132.
Al-Khashman O. A., Environ. Geochem. Health 2007, 29, 1.
Bispo M. S., Morte E. S. B., Korn M. G. A., Teixeira L. S. G., Korn M. and Costa A. C. S., Spectrochim. Acta, Part B 2005, 60, 653.
Ekici K., Agaoglu S., Isleyici O., Indian Vet. J. 2004, 81, 1284.
Ferreira S. L. C., Queiroz A. S., Melo A. S. Q., Assis J. C. R., Korn M. G. A. and Costa A. C. S., J. Braz. Chem. Soc. 1997, 8, 621.
Jia Q., Kong X., Zhou W. and Bi, L., Microchem. J. 2008, 89, 82.
Lee J., Lee H. K., Rasmussen K. E. and Pedersen-Bjergaard S., Anal. Chim. Acta 2008, 624, 253.
Mico C., Peris M., Sanchez J. and Recatala L., Span. J. Agric. Res. 2006, 4, 363.
Nazari S., Microchem. J. 2008, 90, 107.
Naserib M. T., Hemmatkhahb P., Hosseini M. R. M. and Assadi Y., Anal. Chim. Acta 2008, 610, 135.
Rocha F. R. P., Teixeira L. S. G. and Nóbrega, J. A., Spectroscopy Lett. 2009, 42, 418.
Stafiej A. and Pyrzynska K., Microchem. J. 2008, 89, 29.