Available online at www.sciencedirect.com
ScienceDirect
Microchemical Journal 87 (2007) 128–131
MICROCHEMICAL JOURNAL
www.elsevier.com/locate/microc

Determination of zinc and copper in human hair by slurry sampling employing sequential multi-element flame atomic absorption spectrometry

Hadla S. Ferreira [a], Walter N.L. dos Santos [b], Raigenis P. Fiuza [a], Joaquim A. Nóbrega [c], Sergio L.C. Ferreira [a,*]

[a] Universidade Federal da Bahia, Instituto de Química, Campus Universitário de Ondina, Salvador-BA, 40170-290, Brazil
[b] Universidade do Estado da Bahia, Rua Silveira Martins, 2555, Cabula. Salvador-Ba. CEP: 41.195.001, Brazil
[c] Universidade Federal de São Carlos, Departamento de Química, São Carlos SP, Brazil

Received 12 June 2007; accepted 26 June 2007
Available online 4 July 2007

Abstract

The present work proposes a direct method based on slurry sampling for the determination of zinc and copper in human hair samples by multi-element sequential flame atomic absorption spectrometry. The slurries were prepared by cryogenic grinding and sonication of the samples. The optimization step was performed using univariate methodology and the factors studied were: nature and concentration of the acid solution, amount sample/slurry volume, sonication time, and particle size. The established experimental conditions are the use of a sample mass of 50 mg, 2 mol L^{-1} nitric acid solution, sonication time of 20 min and slurry volume of 10 mL. Adopting the optimized conditions, this method allows the determination of zinc and copper with detection limits of 88.3 and 53.3 ng g^{-1}, respectively, and precision expressed as relative standard deviation (RSD) of 1.7% and 1.6% (both, $n=10$) for contents of zinc and copper of 100.0 and 33.3 μg g^{-1}, respectively. The accuracy was checked and confirmed by analysis of two certified reference materials of human hair. The procedure was applied for the determination of zinc and copper in two human hair samples. The zinc and copper contents varied from 100.0 to 175.6 and from 3.2 to 32.8 μg g^{-1}, respectively. These samples were also analyzed after complete digestion in a closed system and determination by FAAS. The statistical comparison by *t*-test (95% confidence level) showed no significant difference between these results.

Keywords: Zinc; Copper; Human hair; Slurry sampling; Sequential multi-element flame atomic absorption spectrometry

1. Introduction

The determination of trace elements in human hair is of basic importance considering that the concentrations of these elements in hair can indicate the levels of these elements in the organism. Therefore, the determination of the metal contents in human hair can be used either as index of exposition for potentially toxic elements (poisoning) or as information of conditions of health of an individual [1]. Hair samples normally are acid digested previously to determinations, but direct analysis of solids or slurries could also be applied.

Recent reviews highlighted the advantages of direct methods of analysis [2,3]. The slurry sampling is one of the alternatives for direct analysis of solid samples that have been proposed using spectroanalytical techniques as graphite furnace atomic absorption spectrometry (GFAAS) [4], inductively coupled plasma optical emission spectrometry (ICP OES) [5,6] and inductively coupled plasma mass spectrometry (ICP-MS) [7,8]. Methods using FAAS are more limited because of the restrictions of particles size and also sample amounts. However, several procedures have been published using this technique [9–11]. Methods for analysis of human hair samples using slurry sampling was proposed for determination of mercury by vapour generation—electrothermal atomic absorption spectrometry [12], arsenic using GFAAS [13], several metals including copper and zinc by electrothermal vaporization ICP OES (ETV-ICP OES) [14]. Cadmium, Cu, and Pb were determined by GFAAS in hair slurries using a cryogenic

* Corresponding author. Fax: +55 71 3235 5166.
E-mail address: slcf@ufba.br (S.L.C. Ferreira).

0026-265X/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.microc.2007.06.005

procedure for sample grinding [15]. It was demonstrated that the cryogenic grinding is an efficient strategy for hair samples because most materials become brittle at low temperatures. The hair samples were frozen in liquid nitrogen and rapidly ground in this medium to avoid any increase of the temperature due to conversion of mechanical energy in heat during grinding. It is well know that some biological tissues are hardly pulverized owing to its flexibility or hardness. Cryogenic grinding was also successfully applied for pulverization of human teeth [16]. An overview about analytical applications of cryogenic grinding was also presented by this same research group [17].

In addition to these spectroanalytical techniques, nowadays the fast sequential flame atomic absorption spectrometry (FS-FAAS) is an option for multi-element determination of metals. Our research group proposed a direct method using slurry sampling for the determination of copper, manganese and zinc in powdered chocolate samples [18,19].

In this paper, an analytical method for the determination of zinc and copper in human hair is proposed using slurry sampling and FS-FAAS after cryogenic grinding of the samples.

2. Experimental

2.1. Instrumentation

A Varian Model SpectrAA 220 (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer with fast sequential module (FS-FAAS), equipped with a conventional system pneumatic nebulizer and nebulization chamber was used for the analysis. A multi-element content copper and zinc hollow cathode lamp was run under the conditions suggested by the manufacturer applying a current of 10.0 mA. The most sensitive wavelengths for zinc at 213.9 nm and copper at 324.8 nm were used with bandwidth of 0.5 and 1.0 nm for copper and zinc, respectively. The flame composition was acetylene (flow rate 2.0 L min) and air (flow rate: 13.5 L min^{-1}) and the burner height was 13.5 mm. The nebulizer flow rate aspiration was kept among 5.5 and 6.0 mL min^{-1}. The particle size was measured by using a Shimadzu (Kioto, Japan) Superscan SS-550 scanning electron microscope. An Ultrasonic Benchtop Cleaner VWR Model 75D (Cortland, New York, USA) was used for slurry homogenization. Samples were ground using a SPEX Certiprep Model 6750 freezer/mill (Metuchen, NJ, USA).

2.2. Reagents

All reagents were of analytical grade unless otherwise stated. Ultrapure water was obtained from an EASY pure RF (Barnstedt, Dubuque, IA, USA). Nitric, hydrochloric and sulfuric were of Suprapur quality (Merck, Darmstadt, Germany). Laboratory glassware was kept overnight in 10% v/v nitric acid solution. Before use the glassware was rinsed with deionised water and dried in a dust-free environment.

Zinc solution was prepared by diluting a 1000 mg L^{-1} zinc solution (Merck) with a 1%(v/v) nitric acid solution.

Copper solution was prepared by diluting a 1000 mg L^{-1} copper solution (Merck) with a 1%(v/v) nitric acid solution.

2.3. Cryogenic grinding

A mass of 1–2 g of hair sample was inserted in a grinding vial, a polycarbonate cylinder supplied with two end plugs, immersed in liquid nitrogen and ground with a magnetically driven impactor. A time period of 13 min was required to pulverize the hair sample and attain the required particle size. The grinding procedure was implemented with a first step of 5 min for sample freezing, followed by three cycles with two stages of pulverization and cooling, with a total of 8 min.

2.4. Slurry preparation

The preparation of the slurry involves 50 mg of hair sample (maximum particle size ≅ 120 μm) and dilution for 10.0 mL using a 2.0 mol L^{-1} nitric acid solution. After, the system is placed in an ultrasonic bath for 25 min. Afterwards the slurry was pneumatically aspirated for determination of copper and zinc by FS-FAAS. The blanks were prepared as the sample slurries, using a rice flour ARROZINA® (Unilever Bestfoods Brazil, Garanhuns, PE, Brazil), which contains copper and zinc in concentrations lower than the quantification limit of this method for both metals.

2.5. Procedures for total digestion of hair samples

2.5.1. Closed-pressurized vessel digestion

In a digestion bomb (4746 Model, Parr Instrument Company, USA) it was added a mass of 100 mg of hair sample and 2.0 mL of concentrated HNO_3 and 1.0 mL of 30% (m/m) H_2O_2. Afterwards, the bomb was closed and kept for 4 h, which was the optimized time for complete digestion of the sample, in an oven at 150 ± 10 °C. Finally, after cooling down at room temperature the bomb was opened. Then, the digested is transferred to calibrated flask and diluted to a final volume of 10 mL with 0.5% (v/v) HNO_3. Copper and zinc were determined in the diluted solutions by FS-FAAS. All samples were analyzed in triplicate.

3. Results and discussion

3.1. Optimization of the experimental conditions

The optimization step was carried out using univariate methodology applied to the main factors. Firstly, the effect of the nature and concentration of the acid used for preparation of the slurries was studied. Experiments were carried out using slurries prepared with hydrochloric, nitric, and sulfuric acids. It was found that the analytical signals were always greater in nitric acid medium for both metals and these were maximum and constant for acid concentrations in the range from 1.0 to 6.0 mol L^{-1}. Further experiments were performed with slurries prepared in 2.0 mol L^{-1} nitric acid solution.

In order to evaluate the effect of the sample amount on the method, slurries were prepared using 20, 30, 40, 50, 60, 80 and 100 mg of sample in 2.0 mol L^{-1} nitric acid solution for a final slurry volume of 10 mL. Absorbances were measured and the

results demonstrated that the slurries prepared with sample masses of 80 and 100 mg caused occasional blockage of the nebulization system of the FS-FAAS. This experiment also showed that there is a linear relation between the sample mass and the analytical signals (absorbances) for both analytes, for sample masses in the range from 0 (20) to 60 mg. Thus, the recommended method involves a sample mass of 50 mg for a slurry volume of 10 mL.

The effect of the sonication time on the preparation of the slurries was investigated, preparing slurries with sample mass of 50 mg and dilution for 10 mL using 2.0 mol L^{-1} nitric acid solution. The sonication time varied from 0 to 30 min. The results demonstrated that in this range, there was no influence of this parameter on the analytical signals obtained for both metal ions. However, in the absence of or for sonication lower than 10 min it was observed obstruction in the nebulizer. Thus, a sonication time of 20 min was recommended for this method.

In the FS-FAAS the burner position is kept constant during the sequential determination. It is recommended that the observation height should be adjusted taking into account the less sensitive analyte according to the absorption lines intensities and the typical concentrations expected in the samples. In this work, this optimization was made for copper, because the sensitivity for this element is lower than the one observed for zinc.

3.2. Analytical features

The calibration strategy of the method was investigated checking the results obtained by external standard technique using aqueous reference solutions and also by analyte additions technique, for both metals. The calibration equations for zinc using aqueous reference solutions ($A=0.5325C_{Zn}+0.0223$) and for analyte additions ($A=0.5337C_{Zn}+0.1996$) had similar slopes, and the correlation coefficients were better than 0.999. The equations of the analytical curves for copper were: aqueous reference solutions ($A=0.1341C_{cu}+0.\ 0029$) and for analyte additions ($A=0.1338\ C_{cu}+0.027$). These results exhibited good similarity among the obtained slopes, demonstrating that zinc and copper in hair slurries could be determined using aqueous reference solutions. This is an indication that an appreciable part of the analyte is present in the liquid phase of the suspension. The precision expressed as coefficient of variation was 1.7 and 1.6% ($n=10$) for zinc and copper contents of 100.0 μg g^{-1} and 33.3 μg g^{-1}, respectively. This experiment was performed using a sample mass of 50 mg.

The limits of detection (LOD), defined as the metal concentrations that give a response equivalent to three times the standard deviation(s) of the blank ($n=10$) divided by the slope of the analytical curve, were found to be 53.3 and 88.3 ng g^{-1} for copper and zinc, respectively. The blank was prepared using rice flour containing copper and zinc concentrations lower than the respective LOD´s. The accuracy was confirmed by analysis of two certified reference materials furnished by Institute of Geophysical and Geochemical Exploration Langfang, China (GBW) and National Institute for Environmental Studies (NIES). The results are summarized in Table 1.

Table 1
Mean concentrations (μg g^{-1}) and standard deviations for Zn and Cu in certified human hair samples submitted to slurry ($n=3$)

	Zinc		Copper	
Sample	certified	Found	certified	Found
GBW 07601 (GSH-1)	190±5	190±0.1	10.6±0.7	10.0±0.5
NIES CRM No. 13	172±11	163±1.5	15.3±1.3	14.6±0.2

Table 2
Mean values (μg g^{-1}) and standard deviations for zinc and copper using the slurry sampling technique and using closed-vessel digestion

	Zinc (μg g^{-1})		Copper (μg g^{-1})	
Sample	Slurry method	Alternative method	Slurry method	Alternative method
1	100.0±0.6	100.3±0.2	7.2±0.8	7.3±1.3
2	161.4±0.4	161.7±0.5	32.8±0.4	33.3±0.6

3.3. Applications

The developed method was applied for the determination of zinc and copper in two human hair samples. For these samples the zinc and copper contents achieved varied from 100.0 to 161.4 and from 7.2 to 32.8 μg g^{-1}, respectively. These samples were also digested using closed-pressurized system and copper and zinc were determined by FAAS. The results were similar to those obtained by slurry method. The *t*-test demonstrated that there are no significant differences between the results. All these data are shown in Table 2.

4. Conclusions

Cryogenic grinding and slurry sampling allowed the development of a fast and direct method for the sequential determination of zinc and copper in human hair samples using FS-FAAS. The feasibility of the calibration using aqueous reference solutions constitutes also other advantage. The results achieved for two certified reference materials demonstrated the accuracy of the method.

Acknowledgements

The authors are grateful to Fundação de Amparo a Pesquisa do Estado da Bahia (FAPESB), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing grants and fellowships and for financial support.

References

[1] M.T.W.D. Carneiro, C.L.P. Silveira, N. Miekeley, Quim. Nova 25 (2002) 37–45.
[2] M.G.R. Vale, N. Oleszczuk, W.N.L. dos Santos, Appl. Spectrosc. Rev. 41 (2006) 377–400.
[3] M.C. Santos, J.A. Nóbrega, Appl. Spectrosc. Rev. 41 (2006) 427–448.
[4] Z. Arslan, J.F. Tyson, Microchem. J. 86 (2007) 227–234.

[5] H. Matusiewicz, M. Slachciński, Microchem. J. 86 (2007) 102–111.
[6] X. Song, T. Duan, P. Guo, H. Chen, Microchem. J. 84 (2006) 22–25.
[7] M.A. Vieira, A.S. Ribeiro, A.J. Curtius, Microchem. J. 82 (2006) 127–136.
[8] M.A. Vieira, A.S. Ribeiro, L.F. Dias, A.J. Curtius, J. Braz. Chem. Soc. 17 (2006) 923–928.
[9] E.O. Ojeka, E.G. Achi, At. Spectr. 26 (2005) 187–190.
[10] R.G.O. Araujo, F.de S. Dias, S.M. Macedo, W.N.L. dos Santos, S.L.C. Ferreira, Food Chem. 101 (2007) 397–400.
[11] F.L. Alves, S. Cadore, W.F. Jardim, M.A.Z. Arruda, J. Braz. Chem. Soc. 12 (2001) 799–803.
[12] J. Moreda-Pineiro, P. Lopez-Mahia, S. Muniategui-Lorenzo, E. Fernandez-Fernandez, D. Prada-Rodriguez, Anal. Chim. Acta 460 (2002) 111–122.
[13] H. Matusiewicz, M. Mroczkowska, J. Anal. At. Spectrom. 18 (2003) 751–761.
[14] S.Z. Chen, D.B. Lu, Z.X. Hu, Z. Wang, Int. J. Environ. Anal. Chem. 85 (2005) 493–501.
[15] M.Y. Kamogawaa, A.R.A. Nogueira, L.M. Costa, E.E. Garcia, J.A. Nóbrega, Spectrochim. Acta, Part B: 56 (2001) 1973–1980.
[16] D. Santos Jr., F. Barbosa Jr., S.S. de Souza, F.J. Krug, J. Anal. At. Spectrom. 18 (2003) 939–945.
[17] D. Santos Jr., F. Barbosa Jr., A.C. Tomazelli, F.J. Krug, J.A. Nóbrega, M.A. Z. Arruda, Anal. Bioanal. Chem. 373 (2002) 183–189.
[18] W.N.L. dos Santos, E.G.P. da Silva, M.S. Fernandes, R.G.O. Araújo, A.C.S. Costa, M.G.R. Vale, S.L.C. Ferreira, Anal. Bioanal. Chem. 382 (2005) 1099–1102.
[19] E.G.P. da Silva, A.C.N. Santos, A.C.S. Costa, D.M.N. Fortunato, N.M. José, M.G.A. Korn, W.N.L. dos Santos, S.L.C. Ferreira, Microchem. J. 82 (2006) 159–162.