Talanta 89 (2012) 276–279

Contents lists available at SciVerse ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Preconcentration and determination of copper in tobacco leaves samples by using a minicolumn of sisal fiber (*Agave sisalana*) loaded with Alizarin fluorine blue by FAAS

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ARTICLE INFO

Article history:
Received 2 August 2011
Received in revised form 23 October 2011
Accepted 13 December 2011
Available online 19 December 2011

Keywords:
Sisal fiber
Preconcentration
Factorial design
Alizarin fluorine blue

ABSTRACT

In the present study, a minicolumn of sisal fiber loaded with alizarin fluorine blue is *proposed* as a preconcentratio system for copper determination in tobacco leaf samples by flame atomic absorption spectrometry. During the optimization procedure, a two level full factorial design (2^4) was used at the preliminary evaluation of four factors, involving the following variables: sampling flow rate, elution flow rate, buffer concentration and pH. Regarding the studied levels, this design has shown that buffer concentration and pH were significant factors. The experimental conditions established in the optimization step were: pH = 4.75, buffer concentration of 0.005 mol L^{-1} for elution with HCl 1.0 mol L^{-1} this system allows the determination of copper content with a detection limit (LD) of 0.018 μg L^{-1} and a quantification limit (LQ) of 0.061 μg L^{-1} precision expressed as relative standard deviation (R.S.D.) of 4.65 and 5.07%, utilizing concentration of 10 and 2.0 μg L^{-1}, respectively, and a preconcentratio factor of 75, for a sample volume of 50.0 mL. Accuracy was confirmed by copper determination in the standard reference material, NIST SRM 1570a trace element units in Spinach Leaves and by spike tests with recovery levels ranging from 93 to 100%; the procedure was applied for copper determination in tobacco leaf samples collected in Cruz das Almas City, Bahia, Brazil. The achieved concentrations of the three samples analyzed varied from 0.15 to 0.52 μg g^{-1}.

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1. Introduction

Tobacco plant is amenable to absorb and accumulate toxic metal species from the soil into its leaves. Part of these metals is transferred by smoke into the human body, where they accumulate, damage vital organs [1].

Direct determination of the micronutrients in leaves [2,3] by atomic absorption spectrometry (AAS) is very difficult due to the low levels of metal ions and also the interfering influences of the major components of the matrix. In multiple instances, it has been necessary to use a preconcentration method prior to analysis. In this context, several methods have been proposed and used for preconcentration and separation of trace elements according to the nature of samples, concentrations of the analytes and the measurement techniques [4,5].

The application of solid phase extraction technique for preconcentration of trace metals from different samples results in several advantages such as minimal waste generation, reduction of sample matrix effects, as well as sorption of the target species on the solid surface in a more stable chemical form [6]. Normal and selective solid phase extractors are those derived from the immobilization of organic compounds on the surface of solid supports, which are majorly polyurethane foams [7,8] and ion exchange resins [9]. Although most studies involving metal preconcentration have used commercially available sorbents, other materials known as "natural adsorbents" or "biosorbents" have recently been successfully employed in metal adsorption processes [9,10]. The term "biosorbents" can be defined as the ability of biological materials to accumulate heavy metals from waste water through metabolically mediated or physico-chemical pathways of uptake [11]. The biosorption process [12,13] involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions).

Algae, chitinous materials or cellulose containing biomass has been employed owing to the occurrence of one or more of the mentioned chemical groups in these materials [14]. Studies on lignocellulosic materials, a major component in the cell wall of plants, have determined the presence of carboxylic and hydroxylic functional groups.

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0039-9140/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.talanta.2011.12.027

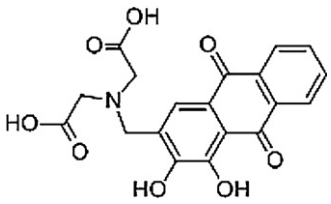

Fig. 1. 3-Aminomethylalizarin-N,N-diacetic acid or Alizarine Fluorine Blue.

The Sisal, *Agave sisalana perrine*, is a plant native from Mexico. Studies involving the use sisal fiber for the removal of heavy metals in wastewater have been published [15]. Yet, the use of this material in the preconcentration of metals has not been reported in the literature.

Multivariate techniques have been used for optimization of analytical methods [16] and metal adsorption processes [17–19]. The two-level full factorial design is one of the most used techniques. It can be mainly applied for preliminary evaluation of the significance of experimental variables of the methods [18]. However, one of the options to determine critical conditions is the use of central composite, Box-Behnken, Doehlert matrix and three-level full factorial design [20], which are a response surface methodologies (RSM). Yet, among these RSM, the three-level full factorial design has only been used sporadically, since it requires a relatively large number of experiments when the factor number is higher than 2 [20].

In this study, it was proposed an off-line system for copper preconcentration (II) and determination in tobacco leaf samples by flame atomic absorption spectrometry. A minicolumn of sisal fiber loaded with 3-aminomethylalizarin-N,N-diacetic acid (Fig. 1) or Alizarine Fluorine Blue (AFB) [21] was used in solid phase. The optimization step was performed using full factorial design.

2. Experimental

2.1. Instrumentation

A Varian Model SpectrAA 220 (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer (FAAS) equipped with a conventional pneumatic nebulizer and nebulization chamber system has been used in the analysis. A copper hollow cathode lamp was run under the conditions suggested by the manufacturer: current of 10.0 mA, wavelength of 324.8 nm, and the bandwidth of the slit followed the suggested values (0.5 nm). The flame composition had an air/acetylene, flow rate of 1.40 L min^{-1} and an aspiration flow rate of 5.0 mL min^{-1}.

2.2. Reagents and solutions

Doubly deionized water was used throughout this work. Acetate (pH 4.7–6.0), borate (pH 7.0–8.5) and ammoniacal (pH 10.0) buffers were used to adjust the pH of the solutions, whenever suitable. The copper (II) standard solutions (1000 μg mL^{-1}) used in the analysis were purchased from Merck. Working solutions were daily prepared by appropriate dilution. Hydrochloric and nitric acid solutions used as eluents were prepared by direct dilution from the concentrated solutions (Merck).

2.3. Preparation of the minicolumn

A 0.01% (w/v) ABF solution was percolated through the minicolumn containing about 0.1 g of sisal fiber at a flow rate of 2.5 mL min^{-1}, for 5 min. After the system was washed with 1 mol L^{-1} sodium hydroxide solution for elimination of excess AFB reagent, and afterwards with a 1.00 mol L^{-1} nitric acid solution and water, at the same flow rate, in order to prevent any metal contamination [22].

Table 1
2^4 full factorial design for optimization of pH and buffer concentration.

pH	BC	VE	VA	Abs
4.74	0.001	3.0	3.0	0.1596
10.0	0.001	3.0	3.0	0.1006
4.74	0.010	3.0	3.0	0.1011
10.0	0.010	3.0	3.0	0.0505
4.74	0.001	8.0	3.0	0.1399
10.0	0.001	8.0	3.0	0.1189
4.74	0.010	8.0	3.0	0.1478
10.0	0.010	8.0	3.0	0.0893
4.74	0.001	3.0	8.0	0.1595
10.0	0.001	3.0	8.0	0.1324
4.74	0.010	3.0	8.0	0.1549
10.0	0.010	3.0	8.0	0.0928
4.74	0.001	8.0	8.0	0.1477
10.0	0.001	8.0	8.0	0.1381
4.74	0.010	8.0	8.0	0.1301
10.0	0.010	8.0	8.0	0.1135
7.30	0.0055	5.5	5.5	0.1276
7.30	0.0055	5.5	5.5	0.1464
7.30	0.0055	5.5	5.5	0.1501

2.4. Sample preparation

Tobacco leaf samples were washed with 10% (v/v) extran, immediately followed by drying in an oven, at 60 °C, triturated in balls mill before they were pressed, crushed and sieved into 100 micrometer granules for further use.

2.5. Complete digestion of tobacco leaf samples

In order to perform acid digestion, about 0.5 g of tobacco leaf samples, 3.0 mL of concentrated nitric acid and 20.0 μL of hydrogen peroxide were placed in a glass vessel and heated on a hot plate (under such conditions, the samples can easily decompose). Finally, the contents were quantitatively transferred to 50 mL volumetric flasks and diluted with water.

2.6. Optimization strategy

In the optimization procedure, a two level full factorial design (2^4) was used at the preliminary evaluation of four factors, involving the following variables: sampling flow rate (SFR), elution flow rate (EFE), buffer concentration (BC) and pH, data from this design, with absorbance as response, as shown in Table 1. This design has show that, regarding the studied levels, concentration and pH were significant factors, as can be seen in Pareto's chart (Fig. 2).

3. Results and discussion

3.1. Optimization of the instrumental conditions

Determination of the sample flow rate (SFR) operating conditions, elution flow rate (EFR), buffer concentration (BC) and pH was carried out in two steps. First, a 2^4 full factorial design was performed; data from this design with absorbance as response, are shown in Table 1. The evaluation of this experiment has demonstrated that only buffer concentration (BC) and pH factors are significant, as can be seen in Pareto's chart (Fig. 2). The negative values for the effects of buffer concentration (BC) and pH have indicated that, in the studied levels, absorbance has increased as these factors decreased.

Considering that, a 3^2 full factorial design was performed to determine the optimum conditions for the factors buffer

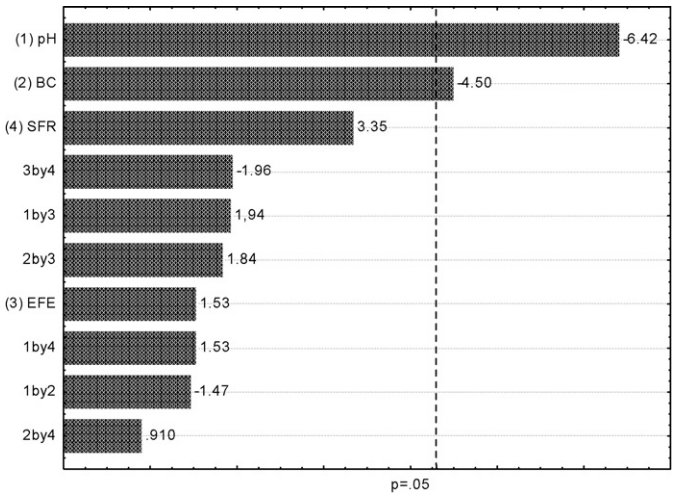

Fig. 2. The Pareto chart.

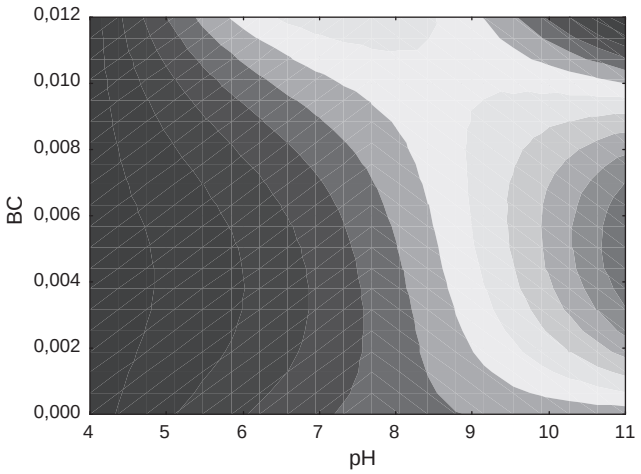

Fig. 3. The contour Charts.

Table 2
Factors and levels used in the factorial design.

Variables	Low (−)	Central point (0)	High (+)
Buffer concentration (mol L^{-1})	0.0010	0.0055	0.010
pH	4.7	7.3	10.0
Sample flow rate (mL min^{-1})	3.0	5.5	8.0
Elution flow rate (mL min^{-1})	3.0	5.5	8.0

concentration (BC) and pH; Table 2 describes the factors and levels used in the factorial design. The design matrix and results are summarized in Table 3. The below equation illustrates the relationship among the variables referring to buffer concentration (BC), pH and analytical signal (AS), based on real values.

$$AS = 0.202 - 0.0091pH - 0.00023pH^2 - 2.232BC - 19.136BC^2 + 0.177pH \cdot BC$$

The above equation shows a saddle point in the response surface; due to this fact, *its* coordinates should not be useful for system optimization. Thus, in order to determine the best operational conditions, a visual inspection of Contour Charts (Fig. 3) has been utilized. The way of calculating these operational conditions has been published in [22]. Considering the results obtained in the optimization step, the conditions established are: sampling flow rate of 6.0 mL min^{-1}, buffer concentration of 0.005 mol L^{-1}, pH = 4.75, elution concentration of 1.00 mol L^{-1} and elution flow rate of 6.0 mL min^{-1}. The volumes set for samples and standard solutions in the preparation of the analytical curves are 50 mL.

Table 3
The matrix of the 3^2 full factorial design.

pH	BC	Abs
4.70	0.001	0.1477
4.70	0.0055	0.1503
4.70	0.010	0.1392
7.30	0.0010	0.1218
7.30	0.0055	0.1181
7.30	0.010	0.0996
10.0	0.001	0.0937
10.0	0.0055	0.0675
10.0	0.010	0.0934
7.30	0.0055	0.1111
7.30	0.0055	0.1286
7.30	0.0055	0.1265

3.2. Preconcentration setup

The off-line system was carried out using a peristaltic pump fitted with Tygon tubes, a minicolumn packed with sisal fiber loaded with AFB. A 3^2 full factorial design has been employed for the optimization of an off-line preconcentration system for copper (II) determination in mineral water samples by flame atomic absorption spectrometry. The system was operated in a volume-based mode, with a sample volume of 50.0 mL, pumped at 6.0 mL min^{-1}, and percolated through a minicolumn. Then, copper (II) ions were retained in the minicolumn as cooper (II)–AFB complex, and the remaining solution was discharged. An elution concentration of 1.00 mol L^{-1} hydrochloric acid flowing at 6.0 mL min^{-1} displaced the copper complexed. The eluate was then analyzed by flame atomic absorption spectrometry.

3.3. Analytical features

The experimental conditions established in the optimization step were: pH 4.7, buffer concentration 0.005 mol L^{-1} for elution, with HCl 1.0 mol L^{-1}; this off-line preconcentratio system allows the determination of copper with linear response from 0.1 μg L^{-1} to 100 μg L^{-1}, and the analytical curve obtained was (Abs = 0.1432 × C_{Cu} − 0.0034), with a correlation coefficient of 0.998. Defined as the copper concentration that gives a response that equals to three times the standard deviation of the blank (n = 10), the limit of detection (LOD) was found to be 0.018 μg L^{-1}, while the quantification limit (LOQ) was 0.061 μg L^{-1}, with precision expressed as a relative standard deviation (R.S.D.) of 4.65 and 5.07%, at concentrations of 10.0 and 2.0 μg L^{-1}, respectively. Calculations were made following the recommendations by IUPAC [23] and a preconcentration factor of 75 for a sample volume of 50.0 mL. Accuracy was confirmed by copper determination in the standard reference material, NIST SRM 1570a trace elements units in Spinach Leaves and by spike tests with recovery levels ranging from 93 to 100%. Using the proposed method, the copper concentration found in this SRM was 12.5 ± 0.5 μg g^{-1} and the certified value of 12.2 ± 0.6 μg g^{-1}. The t-test has demonstrated that there was no significant difference between values.

3.4. Tolerance of other metallic ions on the proposed procedure

In order to check the effect of other metallic ions on this method, a standard solution containing copper and other ions (F^-, Cl^-, CO_3^{2-}, HCO_3^-, Na^+, SO_4^{2-}, K^+), each one at 10.00 mg L^{-1}, was prepared allowing to determine copper content. The achieved results

Table 4
Determination of cooper in tobacco leaves.

Tobacco leaves	Cooper achieved ($\mu g\,g^{-1}$)
Sample 1	0.52 ± 0.03
Sample 2	0.50 ± 0.02
Sample 3	0.15 ± 0.04
Sample 4	0.45 ± 0.03

showed that, at such concentration, other ions do not interfere in copper determination.

3.5. Copper determination in tobacco leaves

The proposed method was applied for copper determination in tobacco leaf samples collected in Cruz das Almas City, Brazil. The results obtained for analysis of three samples collected in several places of the city varied from 0.15 to 0.52 $\mu g\,g^{-1}$, as shown in Table 4. Recovery experiments were performed and results varied from 93 to 100%. These results demonstrated that the copper concentration was very low.

4. Conclusion

The analytical features (precision, limit of detection and accuracy) achieved have demonstrated the feasibility of the off-line system proposed for copper determination in tobacco leaf samples, using FAAS. The 3^2 full factorial design has allowed a fast and efficient optimization of the chemical and operational variables of the proposed procedure.

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 and to the Prof. Dr. Antonio Celso Spinola Costa.

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