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Application of Doehlert designs for optimisation of an on-line preconcentration system for copper determination by flame atomic absorption spectrometry

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Abstract

In the present paper, a system for on-line preconcentration and determination of copper by flame atomic absorption spectrometry (FAAS) was developed. It was based on solid phase extraction of copper(II) ions on a minicolumn of Amberlite XAD-2 loaded with 2-(2-thiazolylazo)-5-dimethylaminophenol (TAM). The optimisation process was carried out using Doehlert designs. Four variables (sampling flow rate, SR; elution flow rate, buffer concentration, BC; and pH) were regarded as factors in the optimisation. The parameter "sensitivity efficiency (SE)" proposed in this paper, and defined as the analytical signal obtained for an on-line enrichment system for a preconcentration time of 1 min was used as analytical response in the optimisation process. Using the established experimental conditions, the proposed on-line system allowed determination of copper with detection limit ($3\sigma/S$) of 0.23 µg l^{-1}, and a precision (repeatability), calculated as relative standard deviation (R.S.D.) of 3.9 and 3.7% for copper concentration of 5.00 and 20.00 µg l^{-1}, respectively. The preconcentration factor obtained is 62. The recovery achieved for copper determination in presence of several cations demonstrated that this has enough selectivity for analysis of food samples. The robustness of the proposed system was also evaluated. The accuracy was confirmed by analysis of the following certified reference materials (CRMs): Rice flour NIES 10a, Spinach leaves NIST 1570a, Apples leaves NIST 1515 and Orchard leaves NBS 1571. This procedure was applied for copper determination in natural food samples.
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1. Introduction

The use of multivariate experimental design techniques is becoming increasingly widespread in analytical chemistry. Multivariate designs,

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which allow the simultaneous optimisation of several control variables, are faster to implement and more cost-effective than traditional univariate approaches [1,2]. One of the most popular multivariate designs is the two-level full (or fractional) factorial, in which every factor is experimentally studied at only two levels. Due to their simplicity and relatively low cost, full factorial designs are very useful for preliminary studies or in the initial steps of an optimisation, while fractional designs are almost mandatory when the problem involves a large number of factors [3]. On the other hand, since only two levels are used, the models that can be fit to these designs are somewhat restricted. If a more sophisticated model is required, as for the location of an optimum set of experimental conditions, then one must resort to augment response surface designs, which employ more than two factor levels. Among these, Doehlert designs, proposed in 1970 [4] have the advantage of requiring, to fit a given higher-order model, less experimental runs than a full composite design [5]. A second-order model in two factors x_1 and x_2, for example, is given by:

$$y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2, \quad (1)$$

where y is the experimental response to be optimised, b_0 is the constant term, b_1 and b_2 are the coefficients of the linear terms, b_{11} and b_{22} are the coefficients of the quadratic terms and b_{12} is the coefficient of interaction between the two factors. The central composite design appropriate for this model requires at least nine experimental runs, while the corresponding Doehlert design needs only seven. As the number of factors increase, the economy associated with Doehlert designs increases accordingly.

The first application of Doehlert designs in Analytical Chemistry was described in separation system using high-performance liquid chromatography [6]. Other applications since then include the optimisation of: on-line preconcentration system for zinc determination [7], experimental variables in procedures using solid phase spectrophotometry [8,9], processes proposed for solvent extraction of several metals [10,11], procedure for spectrophotometric determination of lithium in drugs [12], factors of a procedure using

spectrofluorimetry [13], extraction process using microwaves [14], and experimental variables of a voltammetric determination [15]. Our own research group recently employed two-factor Doehlert designs to optimise an analytical procedure for molybdenum determination in seawater using Inductively Coupled Plasma Optical Emission Spectrometry (ICP OES) [16] and during optimisation of preconcentration procedure for determination of vanadium and copper in seawater [17].

In this paper, an on-line preconcentration system for copper determination in natural food samples using flame atomic absorption spectrometry (FAAS) was proposed. The procedure is based on solid-phase extraction of copper(II) ions as 2-(2-thiazolylazo)-5-dimethylaminophenol (TAM) complexes on Amberlite XAD-2 [18]. Doehlert designs were used in order to find the factor levels corresponding to optimum responses.

Amberlite XAD-2 (polystyrene-divinylbenzene polymer) has been used in preconcentration procedures due to its good physical and chemical properties such as porosity, high surface area, durability and purity. In our laboratory, this resin has been used in on-line preconcentration systems for the determination of cadmium [18] copper [19], lead [20] and nickel [21].

2. Experimental

2.1. Instrumentation

A Varian Model SpectrAA 220 (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer was used for the analysis. The copper hollow-cathode lamp was run under the conditions suggested by the manufacturer (current: 4.0 mA). The wavelength (324.8 nm), slit bandwidth (0.5 nm) and burner height (13.5 mm) were also set at standard values. The flame composition was specified by acetylene (flow rate: 2.0 l min^{-1}) and air (flow rate: 13.5 l min^{-1}). Nebulizer flow rates were in the range 5.0–6.0 ml min^{-1}.

An Alitea C-6 XV (Stockholm, Sweden) peristaltic pump furnished with Tygon tubes was used to propel all solutions. A Rheodyne 5041 (Cotati, California, USA) model four-way manual valve

was used to select preconcentration/elution steps. All connections were made using fittings, unions and tees made of plastic and PEEK materials. The manifold was built up with PTFE tube of 0.5 mm bore.

A 300 ANALYSER pH meter was used to measure pH values.

2.2. Reagents

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

Copper solution (20.0 μg ml^{-1}) was prepared by diluting a 1000 μg ml^{-1} copper solution (Merck) with a 1% hydrochloric acid solution.

TAM solution (0.05%) was prepared by dissolving 0.10 g 2-(2-thiazolylazo)-5-dimethylaminophenol in 200 ml ethanol (Merck).

Buffer (pH 7.5) was prepared by dissolving 14.2 g sodium phosphate in deionised water and adjusting the pH to 7.5 with 2 mol l^{-1} nitric acid, followed by dilution to 1 l.

2.3. Column preparation

The 0.05% (w/v) TAM solution was percolated through a laboratory-made cylindrical minicolumn with 4.50 cm length and internal diameter of 4.0 mm containing about 0.10 g of Amberlite XAD-2 at a flow rate of 5.0 ml min^{-1}, for 15 min. Afterwards, the column was washed with a 10% (w/v) sodium hydroxide solution until the purple effluent became colourless, to remove excess TAM. Then, the column was washed with 5% (v/v) hydrochloric acid solution and deionised water, respectively, using the same flow rate. Washing with nitric acid was necessary in order to prevent any metal contamination [21].

2.4. Sample preparation

The following certified reference materials (CRMs) were analysed: Rice flour NIES 10a, Spinach leaves NIST 1570a, Apple leaves NIST 1515 and Orchard leaves NBS 1571. For their decomposition, about 0.4 g of material was treated with 4.0 ml of nitric acid 1:1 (v/v) and kept overnight in a Teflon vessel. Afterwards the Teflon vessel was closed and placed into a pressurised digestion system. The thermal heating was carried out in a stove at 110 °C for 24 h. After cooling to room temperature these solutions were adjusted to pH 7.5 with a 10% (w/v) sodium hydroxide solution and a phosphate buffer solution, and the volume was made up to 100 or 200 ml [22].

2.5. On-line preconcentration system for copper determination

A diagram of the on-line preconcentration system is shown in Fig. 1. The flow system was carried out using two peristaltic pumps fitted with Tygon tubes, a four-way valve and a minicolumn packed with Amberlite XAD-2 resin loaded with TAM, coupled to a flame atomic absorption
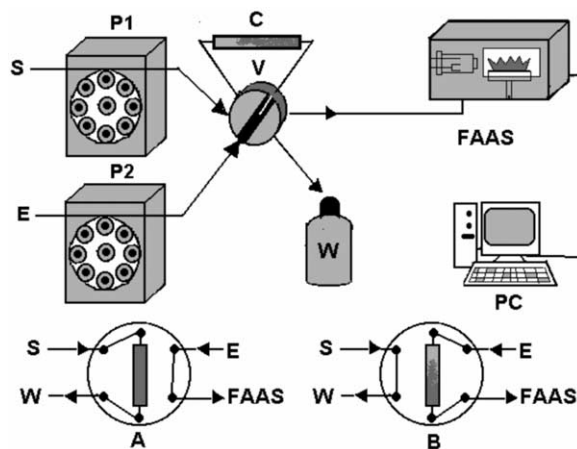

Fig. 1. Schematic diagram of the flow system used for the preconcentration and determination of copper by FAAS. P1 (7.35 ml min^{-1}); P2 (4.89 ml min^{-1}); P, peristaltic pump; C, Amberlite XAD-2/TAM minicolumn; V, four-way valve; FAAS, flame atomic absorption spectrometer and W, waste. A, four-way valve in the preconcentration step and B, four-way valve in the elution position.

spectrometer (FAAS). The flow system was operated in a time-based mode. A sample solution (S) containing between 0.0 and 50.0 µg l^{-1} copper was buffered at pH 7.5 with a phosphate buffer solution and pumped at 7.35 ml min^{-1}, percolating through a minicolumn that retained the Cu(II) ions by chemisorption, complexed with TAM. The remaining solution was discharged (W). When the injection valve is switched, a stream of 1.00 mol l^{-1} hydrochloric acid (E) flowing at 4.89 ml min^{-1} displaces the copper ions. The eluate was taken directly into the Nebulizer-burner system of the flame atomic absorption spectrometer. Signals were recorded as peak heights, acquired three times and averaged, using the instrument software. It was not necessary to recondition the minicolumn at the end of each cycle, as samples were buffered before preconcentration [23]. Achieved sampling rate was 27 samples per hour for a preconcentration time of 120 s and an elution time of 15 s.

During the entire optimisation process the flow system was operated in a volume-base mode, using 20.00 µg l^{-1} 10.0 ml copper solution, but for copper determination the flow system must be operated using the time-base technique. In it, sample volume is calculated using the sampling flow rate (SR) and the preconcentration time.

2.6. Procedures used in the Doehlert design

Copper was preconcentrated using the on-line system in Fig. 1. SR, elution flow rate, buffer concentration (BC) and pH were chosen as the experimental factors, with levels specified according to the Doehlert designs in Tables 1 and 2.

2.7. Sensitivity efficiency

In this paper, was propose as the response to be analysed a derived quantity, which was called of *sensitivity efficiency* (*SE*), defined as the analytical signal obtained with an on-line enrichment system for a preconcentration time of 1 min. This parameter can be used in order to demonstrate that, for this system, the concentration efficiency [23] increases with the increase of the SR. The SE is defined as follows:

$$SE = \frac{AS}{t} = \frac{AS*SR}{SV},\tag{2}$$

where AS is the analytical signal, t is the preconcentration time in minutes, SV is the sample volume, and SR is the sampling flow rate. SE can be also useful in order to compare the efficiency (in terms of analytical signal) of two different analytical systems or different experimental conditions of the same system.

2.8. Optimisation strategy

The optimisation process was carried out in two steps, using Doehlert designs. All the experiments were carried out in replicates, using 10.0 ml of a 20.0 µg l^{-1} copper solution. Four variables (SR, elution flow rate, BC and pH) were regarded as factors, and the SE defined above as the sole response. The data were analysed with the *Statistica* computer program [24].

Table 1
Doehlert matrix for design 1

Run	pH	ER (ml min^{-1})	Analytical signal	SE
1	7	5.17	0.1276, 0.1231	0.0429, 0.0414
2	6	4.76	0.1149, 0.1106	0.0386, 0.0372
3	7	4.48	0.1246, 0.1205	0.0419, 0.0405
4	6	4.00	0.1026, 0.1036	0.0345, 0.0348
5	7	3.80	0.1062, 0.1098	0.0357, 0.0369
6	8	4.00	0.1192, 0.1226	0.0401, 0.0412
7	8	4.76	0.1214, 0.1240	0.0408, 0.0417

ER, elution flow rate; *SE*, sensitivity efficiency (see text for definition).

Table 2
Doehlert matrix for design 2

Run	BC	SR	Analytical signal	SE
1	0.0075	7.32	0.0927, 0.0954, 0.0987	0.0679, 0.0698, 0.0722
2	0.0050	6.25	0.0921, 0.0874, 0.0872	0.0576, 0.0546, 0.0545
3	0.0075	5.17	0.1215, 0.1217, 0.1147	0.0576, 0.0577, 0.0541
4	0.0050	4.11	0.1051, 0.1033, 0.1012	0.0432, 0.0425, 0.0416
5	0.0075	3.04	0.1194, 0.1229, 0.1175	0.0363, 0.0374, 0.0357
6	0.0100	4.11	0.1257, 0.1215, 0.1234	0.0517, 0.0499, 0.0507
7	0.0100	6.25	0.1086, 0.1064, 0.1092	0.0679, 0.0665, 0.0683

BC, buffer concentration, in mol l^{-1}; SR, sampling flow rate, in ml min^{-1}; SE, sensitivity efficiency (see text for definition). For these runs elution flow rate and pH were set at the optimised levels found with design 1.

2.9. Lagrange's criterion

The critical points of the 2nd order model adjusted to experimental data were obtained through Lagrange's criterion [25,16], which is based on the Hessian of the response Y with respect to the two factors A and B:

$$H(A, \ B) = \left(\frac{\delta^2 Y}{\delta A^2}\right)\left(\frac{\delta^2 Y}{\delta B^2}\right) - \left(\frac{\delta^2 Y}{\delta A \delta B}\right)^2 \tag{3}$$

The critical point (a_o, b_o) is a maximum if $H(a_o, b_o) > 0$ and $\delta^2 Y/\delta A^2 (a_o, b_o) < 0$; it is a minimum if $H(a_o, b_o) > 0$ and $\delta^2 Y/\delta A^2 (a_o, b_o) > 0$. If it is a saddle point, then $H(a_o, b_o) < 0$. If the response surface has a maximum, this point is calculated by solving the equation system $\delta^2 Y/\delta A^2 = 0$ and $\delta^2 Y/\delta B^2 = 0$.

3. Results and discussion

3.1. Optimisation by Doehlert designs

Two Doehlert designs were employed sequentially. Due the instrumental convenience (use of peristaltic pumps), SR and elution flow rate was studied in designs separated. The first one was based on the elution flow rate and pH, while the second was used to optimise the response with respect to the sample flow rate and BC. Previous studies demonstrated that the optimum pH is in the range of 6.0–8.0.

3.2. Design 1—pH and elution flow rate

In this design, SR and BC were maintained at 3.36 ml min^{-1} and 0.010 mol l^{-1}, respectively, because low sampling rates are usually needed for an effective extraction. The seven level combinations required for this design are given in Table 1. Elution flow rate and pH varied in the ranges 3.80–5.17 and 6–8 ml min^{-1}, respectively. A second-order model fit to the data in Table 1 resulted in the equation:

$$SE = -0.173 + 0.0295\text{pH} + 0.0427ER$$
$$- 0.00140\text{pH}^2, \tag{4}$$

where only the terms statistically significant at the 95% confidence level are shown. The corresponding response surface is shown in Fig. 2. Application of Lagrange's criterion to this surface indicates a maximum at $ER = 4.89$ ml min^{-1} and pH 7.52. At these conditions, the values of SE experimental and predicted are 0.0419 and 0.0421, respectively.

3.3. Design 2—conditions of sampling flow rate and buffer concentration for the system

In this design the optimised variables were SR and BC, with elution flow rate and pH set at the optimised levels found in design 1, 4.89 and 7.52 ml min^{-1}, respectively. The seven experiments required by Doehlert design are described in Table 2. The SR and the BC were varied from 3.04 to 7.32 ml min^{-1} and from 0.0050 to 0.0100 mol l^{-1}, respectively. Fitting a second-order model to the
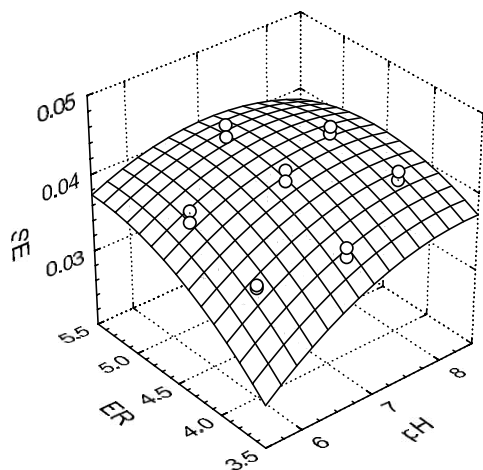
Fig. 2. Response surface fit to the data in Table 1. A maximum is observed at and elution flow rate of 4.89 ml min^{-1} and a pH of 7.52.

Fig. 3. Response surface fit to the data in Table 2. A maximum is observed at a SR of 7.35 ml min^{-1} and a BC of 0.009 mol l^{-1}.

data in Table 2 resulted in the equation:

$$SE = -0.079 + 13.59BC + 0.024SR - 887.6BC^2 - 0.002SR^2. \tag{5}$$

Again, only values statistically significant at the 95% confidence level are shown. This equation presents a maximum at a BC of 0.009 mol l^{-1} and a SR of 7.35 ml min^{-1}, which for practical purposes can be taken as the highest level of the rates used in the design. At these conditions, the SE experimental and predicted are 0.0722 and 0.0715, respectively. The corresponding response surface appears in Fig. 3.

3.4. Final optimisation

The Doehlert experiments demonstrated that the optimum conditions for the on-line system are SR of 7.35 ml min^{-1}, BC of 0.0090 mol l^{-1}, elution flow rate of 4.89 ml min^{-1} and pH 7.5. When the same data are analysed using the analytical signal (absorbance) as the response, the optimum predicted conditions are SR of 3.36 ml min^{-1}, BC of 0.0087 mol l^{-1}, elution flow rate of 4.75 ml min^{-1} and pH 7.91. The major difference among these results was found for the SR. It occurs, because when the sensibility efficiency is used as the analytical response the sampling time is included as a implicit factor.
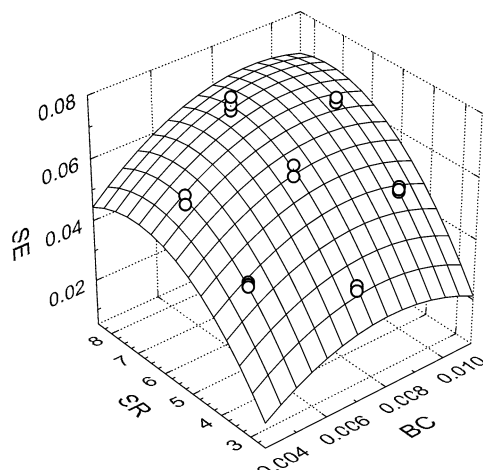
For time-based systems, sampling time increases with decrease of SR. When the results are evaluated using analytical signal as response, the SR achieved was considerable lower (3.36 ml min^{-1}). This is very logical, because an increase of the SR decreases the analytical signal due to insufficient contact time between copper(II) ions and the solid phase for extraction. Table 3 shows a comparison among the analytical parameters for the system using the conditions established in agreement with the analytical signal and the SE parameter and demonstrates that the system has better performance for optimisation using the proposed parameter (SE).

3.5. Robustness of proposed system

The robustness of the proposed system was evaluated according to published papers [9,26] carrying out a procedure two-level fractional saturated (2^{3-1}), centred on the experimental conditions established in the optimisation process. The results demonstrate that the on-line system is robust for variation of ($\pm 10\%$) of elution flow rate and BC, and (± 0.5) units of pH value, for nominal values of $ER = 4.89$ ml min^{-1}, $BC = 0.009$ mol l^{-1} and pH 7.52. As the system is operated using the time-base technique, sample flow rate and preconcentration time are fixed at

Table 3
Comparison among analytical parameters

Equation of the analytical curve	Parameters for $SR = 3.36$ ml min^{-1}, $A = 0.00706 + 0.00461$ (Cu^{2+} µg l^{-1}) $R^2 = 0.9989$	Parameters for $SR = 7.35$ ml min^{-1}, $A = 0.00183 + 0.00687$ (Cu^{2+} µg l^{-1}) $R^2 = 0.9990$
Detection limit (µg l^{-1})	0.25	0.23
Quantification limit (µg l^{-1})	0.86	0.77
Factor preconcentration	42	62
Concentration efficiency (min^{-1})	18.5	27.9
Sampling frequency	27	27
Consumption index (ml)	0.16	0.22

Preconcentration time, 2 min.

7.35 ml min^{-1} and 2 min, respectively. These must be rigorously controlled, considering that the sample volume of copper(II) ions is determinate using these values. If the system is operated using the volume-base technique, sample flow rate is robust for variation of ($\pm 5\%$).

3.6. Analytical features

The flow system showed linearity within the concentration range from 1 to 50 µg l^{-1} for 2 min of preconcentration time. The calibration graphs under the optimum chemical and flow conditions with the manifold depicted in Fig. 1, was given as $A = 0.00183 + 0.00687$ (Cu µg l^{-1}) in the interval of 1–50 µg l^{-1} ($R^2 = 0.9990$). By using direct aspiration in FAAS without the preconcentration system the linear range was between 0 and 8000 µg l^{-1} ($A = 0.00414 + 0.00011$ (Cu µg l^{-1}), ($R^2 = 0.9994$). The experimental preconcentration factor [23,27] calculated as the ratio of the slopes of the calibration graphs with and without preconcentration was 62.0 for 2 min of preconcentration time. In this conditions the concentration efficiency [23], defined as preconcentration factor of analyte achieved by the system in 1 min was 27.9 min^{-1}. The consumptive index [23] (defined as the sample volume, in millilitres consumed in order to achieve a unit of factor preconcentration) was also calculate and was 0.22 ml.

The precision of the procedure (repeatability), determinate as the relative standard deviation (R.S.D.) in sample solutions containing between 5.0 and 20.0 µg l^{-1} of copper was in the range of 3.9–3.7%, respectively, calculated by 11 measurements.

The sensitivity [28] was studied by means of the detection (LOD) and quantification (LOQ) limits, defined as LOD $= (3\sigma)/S$ and LOQ $= (10\sigma)/S$, where S is the slope of the analytical curve, and σ is the standard deviation (S.D.) of ten consecutive measurements of the blank. The LOD and LOQ were 0.23 and 0.77 µg l^{-1}, respectively. The Table 3 presents a comparison among the analytical features of the system using sample flow rate of 3.36 (maximum point by Doehlert design) and 7.35 ml min^{-1}. The data were obtained for a preconcentration time of 2 min.

3.7. Effect of other metallic ions on the proposed procedure

In order to check the selectivity, a standard solution containing copper and other metallic ions (all 20.00 µg l^{-1}) was prepared and the preconcentration system was used for copper determination. The achieved result was (20.72 $\pm$ 0.03 µg l^{-1} $n = 3$) with recoveries of 104%. This experiment was carried out using a multielemental ICP OES solution Quality Control Standards (QCS-19), which had arsenic, antimony, beryllium, cadmium, calcium, chromium, cobalt, iron, molybdenum, nickel, thallium, titanium, zinc, lead, magnesium, manganese and selenium at the concentration of 100 µg ml^{-1} each one.

Table 4
Results obtained for the CRMs analysed ($n = 3$)

Sample	Copper found by methodology proposed ($\mu g \ g^{-1}$)	Certified values ($\mu g \ g^{-1}$)	P-value (%)
Spinach leaves NIST1570a	11.62 ± 0.02	12.2 ± 0.6	29.03
Rice flour NIES 10a	3.41 ± 0.03	3.5 ± 0.3	60.66
Orchard leaves NBS 1571	12.33 ± 0.01	12 ± 1	45.92
Apple leaves NIST 1515	5.29 ± 0.05	5.64 ± 0.24	16.78

Errors are reported as confidence intervals, as described in the text. NIST, National Institute of Standards & Technology; NIES, National Institute for Environmental Studies.

3.8. Accuracy of the method

In order to evaluate the accuracy of the developed procedure, copper was determined in (3) three reference materials, furnished by the National Institute of Standards and Technology (Gaithersburg, MD, USA): Spinach leaves NIST 1570a, Apple leaves NIST 1515 and Orchard leaves NBS 1571 and another reference material provided by the National Institute for Environmental Studies—NIES, Japan, rice flour NIES 10a. Results with confidence intervals are given in Table 4. The confidence intervals were calculated according to the expression $(x \pm st/(N)^{1/2}$, where (x) is the average, (s) is the S.D., (t) the Student's distribution point at the 95% confidence level and (N) the number of observations). The statistical evaluation by a t-test showed no significant difference between the copper values obtained from the proposed system and the certified values (P-values ≥ 16.78).

3.9. Analytical application

The optimised system was applied for copper determination in several natural food samples such as: cassava leaves, lettuce leaves, water-cress leaves, wheat flour, manioc flour, soy-bean flour and oat flour. The results are shown in Table 5 and in it can be seen the copper content varies from 1.14 to 4.57 $\mu g \ g^{-1}$. Mean recovery value was compared with 100% for significance, using the t-test. The obtained P-values ($\geq 23.76\%$), in all cases, indicates that this procedure is perfectly applied for copper determination in natural food.

Table 5
Copper determination in several natural food samples using the proposed methodology ($n = 3$)

Sample	Copper added ($\mu g \ g^{-1}$)	Copper found ($\mu g \ g^{-1}$)[a]	Recovery (%)	P-value (%)
Cassava leaves	0.00	1.40 ± 0.04	–	
	1.50	2.93 ± 0.06	102	62.10
Lettuce leaves	0.00	2.39 ± 0.06	–	
	2.00	4.43 ± 0.02	102	71.54
Water-cress leaves	0.00	4.57 ± 0.09	–	
	4.50	8.96 ± 0.02	98	61.98
Wheat flour	0.00	1.14 ± 0.01	–	
	1.00	2.21 ± 0.05	107	23.76
Manioc flour	0.00	3.64 ± 0.04	–	
	2.00	5.57 ± 0.02	97	67.17
Soy-bean flour	0.00	3.36 ± 0.01	–	
	2.00	5.31 ± 0.01	98	82.18
Oat flour	0.00	1.36 ± 0.09	–	
	2.00	3.45 ± 0.03	105	37.52

[a] Confidence interval at 95%.

4. Conclusions

The Doehlert matrix was established as optimisation process in 1970, however, its application in analytical chemistry is still very limited. This paper reports the first use of the Doehlert matrix for optimisation of experimental variables of an on-line system preconcentration for metal determination by FAAS.

The system proposed is simple, efficient and can be applied for copper determination in natural food samples.

Optimisation process using sensibility efficiency as analytical response was more suitable, since this parameter made possible to establish a relation between analytical signal (absorbance) and sampling time, in order to get an on-line system with high concentration efficiency and high sampling frequency.

Acknowledgements

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