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Multivariate optimization of a procedure for Cr and Co ultratrace determination in vegetal samples using GF AAS after cloud-point extraction

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This work presents the development of a procedure for the determination of Cr and Co
from vegetal samples using graphite-furnace atomic absorption spectrometry after cloud-point
extraction. The procedure is based on extraction of metals from micellar media of
octylphenoxypolythoxy-ethanol (Triton X-114) after complexing the metals with 2-(5-bromo-
2-pyridylazo)-5-(diethylamino)-phenol [5-Br-PADAP]. A Doehlert design was used to optimize
the pyrolysis and atomization temperatures and pyrolysis time in the determination of analytes
trapped in the micellar phase. Ascorbic acid and palladium were used as chemical modifiers,
allowing a higher pyrolysis temperature without analyte losses. Enrichment factors of 26.2 and
41.0, along with detection limits ($3\sigma_B$) of 23 and $12 \, \mathrm{ng \, L^{-1}}$ for Cr and Co, respectively, were
found. The precision expressed as relative standard deviation was 5.4 and 8.4% for metal
concentrations of $0.5 \, \mu\mathrm{g \, L^{-1}}$ ($n = 8$) for Cr and Co, respectively. The accuracy was evaluated
through the analysis of the certified reference material NIST 1515 (apple leaves). The procedure
developed in this study was applied to the determination of Cr and Co concentrations in vegetal
leaf samples.

Keywords: Chromium; Cloud-point extraction; Cobalt; Dohelert design; GF AAS; Vegetal
samples

1. Introduction

The analysis of plants may provide significant information in a variety of fields,
including agriculture, toxicology, and environmental studies. This is because plants

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have the capacity to accumulate metals in their tissues. Therefore, plants can and have been used as bioindicators of environmental pollution caused by toxic metals [1].

Cobalt is an essential element for humans, although a physiological function for this element in higher plants has not been identified. Cobalt pollutant to soils arises mainly from agricultural additives such as fertilizers, mining and smelting activities, and dispersal of sewage sludge [2, 3]. Another soil pollutant is chromium which can be introduced into the environment through effluents from several industries. It is important to control this element, since it is both toxic and carcinogenic, with the severity dependent on its oxidation state.

Thus, the determination of trace amounts of metals, such as cadmium and cobalt in vegetal samples, is of concern due to the necessity to acquire information about their concentrations in these samples to monitor levels of toxicity and also to evaluate the efficiency of soil remediation [3]. However, metal determination in ultratrace levels is still a challenge for analytical chemistry.

Cloud-point extraction (CPE) is a separation and preconcentration procedure that is based on separation of phases that occurs when a surfactant solution attains its cloud-point temperature. At this temperature, the original solution is separated into two phases, a surfactant rich phase, which captures the metallic chelates into the micellar hydrophobic core, and another bulk aqueous phase, which is discarded. Thus, several atomic spectrometric determination techniques were used for metal determination in this micellar phase, such as flame atomic absorption spectrometry (FAAS) [4–8], inductively coupled plasma optical emission spectrometry (ICP OES) [9–12], inductively coupled plasma mass spectrometry (ICP-MS) [13, 14], and graphite furnace atomic absorption spectrometry (GF AAS).

GF AAS is an alternative technique to determine metals trapped in a micellar phase, mainly because the organic substances that compound the matrix, such as surfactant and/or other organic substances, can be eliminated in the pyrolysis step. This most likely can decrease the background absorption in the atomization step, eliminating the risk of spectral interference [15]. Moreover, surfactants improve detection by GF AAS because they stabilize the solution. Surfactants decrease the surface tension of water, diminishing its contact angle with graphite and avoiding the spreading of the drop on internal surface of graphite tube [16]. As surfactant modifies the determination of an analyte in GF AAS, an optimization step must be employed to establish the best conditions for the analysis using CPE as a pre-concentration procedure. Table 1 shows the analytical characteristics of several developed GF AAS methods that were used in the micellar phase, obtained from the CPE procedure.

The Doehlert matrix, commonly applied, is a second-order design that allows the identification of critical points (maximum, minimum, and saddle points) at each step of the optimization process. The whole experimental design is assayed with a minimum number of experiments. For a number factor k with c repetitions of the central point, $k^2 + k + c$ experiments are required [17]. This optimization procedure has the following advantages: (a) valuable information is obtained with a reduced number of experiments, compared with the classical method of optimization by one factor at a time; (b) the experimental response is studied as a function of several variables and therefore mathematically models this response; (c) it is possible using this approach to obtain information regarding the interaction between the factors under study. Applications of Doehlert design used in the optimization of a graphite furnace atomic absorption spectrometry are still rare. Some works can be cited such as multivariate

Table 1. CPE applications with GF AAS detection.

Metal	Samples	Complexing agent	Surfactant	LOD ($ng\,L^{-1}$)	Reference
Cd(II)	Blood	DDTP[a]	Triton X-114	20	[15]
Pb(II)				80	
Pd(II)				14	
Cd(II)	Seawater	DDTC[b]	Triton X-114	2	[29]
As(III)	Tap water, hair, and nail	Molybdate in acid media	Triton X-114	10	[30]
As(V)					
Cr(III)	Waters	Br-PF[c]	Triton X-100	10	[31]
Cr(VI)					
Pb(II)	Waters	5-Br-PADAP[d]	Triton X-114	80	[32]
As(III)	Lake and river water	APDC[e]	Triton X-114	40	[33]
Bi(III)	Biota, water	dithizone	Triton X-114	20	[34]
Cd(II)	Biota	DDTP	Triton X-114	6	[35]
Pb(II)				40	

[a]DDTP: *O,O*-diethyldithiophosphosric acid; [b]DDTC: Diethyldithiocarbamate; [c]Br-PF: dibromophenylfluorone; [d]5-Br-PADAP: 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol; [e]APDC: ammonium pyrrolidinedithiocarbamate.

optimization of the spectrometer conditions in the Mo determination [18], optimization of instrumental parameters for direct determination of silicon in naphtha [19], and arsenic determination in naphtha after preconcentration using multiple injections [20].

In the present work, the Doehlert matrix [21–23] was used to optimize an experimental procedure in the determination of Cr and Co by GF AAS in vegetal samples after a cloud-point extraction using 5-Br-PADAP as complexing agent and the non-ionic surfactant Triton X-114.

2. Experimental

2.1 *Instrumentation*

A Varian model SpectrAA-800 graphite furnace atomic absorption spectrometer (Mulgrave, Victoria, Australia) equipped with a graphite furnace atomizer, GTA 100 auto sampler, was used in all measurements. Background corrections through the Zeeman effect were employed to correct for non-specific absorbance. A pyrocoated graphite tube (Part Number 63-100011-00, Varian) with longitudinal heating was used throughout. All signals were measured as integrated absorbance. Hollow cathode lamps were used as radiation source, and the operational conditions of the equipment were established according to the manufacturer's recommendations for each element. Hollow cathode lamps were used in the following conditions: 357.9 and 242.5 nm, as wavelength, 7.0 and 7.0 mA of lamp current, and 0.2 and 0.2 nm of bandwidth slit, for Cr and Co, respectively.

Microwave digestion of vegetal leaf samples and certified reference material (CRM) was carried out in a cavity microwave oven (Anton Paar, Multiwave sample preparation system) equipped with six closed perfluoralkoxy (PFA) vessels employing a pressure-release mechanism. A simultaneous hydraulic pressure sensing control and an external IR temperature sensor control the digestion system.

A Digimed DM20 pH meter was used to measure pH values. A FANEM (model 204-NR, São Paulo, Brazil) centrifuge was used to accelerate the phase separation. A FANEM (São Paulo, Brazil) thermostatic bath was used to maintain the sample solution at the desired temperature.

2.2 *Reagents and solutions*

All reagents were of analytical grade unless otherwise stated. Ultra-pure water with a resistance of $18.3\,M\Omega$ cm was obtained from a Milli-Q system (Millipore, Bedford, MD) to prepare all solutions. Nitric acid and ethanol were of Suprapur quality (Merck). Laboratory glassware was kept overnight in 10% nitric acid solution. Before use, the glassware was rinsed with distilled-deionized water and dried in a laminar flux cabinet (VECO, Campinas, Brazil) to keep the environment dust-free.

Chromium, cobalt, and palladium solutions were prepared by diluting $1000\,\mu g\,mL^{-1}$ of standard solutions (Merck) with a 1% hydrochloric acid solution. The non-ionic surfactant Triton X-114 was obtained from Acros Organics (Bridgewater, NJ). A solution of 5% w/v ascorbic acid (Vetec) was prepared through dissolution of 5 g in 100 mL of destilled-deionized water.

A 0.025% (w/v) solution of 5-Br-PADAP [2-(5-bromo-2-pyridylazo)-5-(diethyla-mino)-phenol] in 2.6% w/v Triton X-114 solution was prepared by dissolution of 0.025 g of the compound (Merck) in ethanol and 2.6 g of surfactant. The volume was set to 100.0 mL with ethanol.

Buffer solution (pH 9) was prepared by solubilization of 25 mL of $0.4\,mol\,L^{-1}$ TRIS [tris (hydroxymethylaminomethane)] and 2.5 mL of $0.4\,mol\,L^{-1}$ HCl solution. The pH was then adjusted with $0.5\,mol\,L^{-1}$ HNO_3, and the volume was set to 100 mL with ultra-pure water.

A HNO_3 (1:1 v/v) diluent solution was used to decrease the micellar phase viscosity before its introduction into a graphite furnace by the auto-sampler.

2.3 *Procedure for sample preparation*

Vegetal leaves of orange tree (*Citrus sinensis*) and alfalfa (*Medicago sativa* L.) were analysed. Apple tree leaves (*Pyrus malus* L.) from the National Institute of Standards and Technology (NIST 1515) were analysed as certified reference material. In order to convert the samples into solutions for elemental analysis, microwave-assisted acid decomposition was performed at high pressure and temperature. Evaluated samples (250 mg) were weighed in the perfluoralkoxy (PFA) vessels of the microwave oven, and 2 mL of $5.0\,mol\,L^{-1}$ HNO_3 and 2 mL of H_2O_2 (30% m/v) were added to samples as digestion reagents. In each decomposition cycle, six vessels were used simultaneously. Microwave digestion of vegetal leaf samples and certified reference material was carried out using a six-step programme in the order 2 min at 290 W, 3 min at 0 W, 4 min at 640 W, 5 min at 872 W, and 5 min at 1000 W, followed by 15 min of ventilation. A blank was carried out to evaluate the contamination possibilities and correct the sample signals in each digestion procedure. The vessels were cooled down inside the microwave cavity after finalization of the digestion programme. After attaining room temperature, the digests were transferred to 50-mL volumetric flasks, the pH adjusted to pH 9 with 50% w/v NaOH, and the solutions diluted with distilled-deionized water to 40 mL.

2.4 *Procedure for cloud-point extraction and GF AAS determination*

Cloud-point extraction experimental conditions has been established based on previous works [24, 25]. For cloud-point extraction, aliquots of 40 mL of analysed solution were added to 0.5 mL of an alcoholic solution of 2.6% (w/v) Triton X-114, 0.025% (w/v) 5-Br-PADAP, and 0.5 mL of buffer solution 0.1 mol L^{-1} TRIS (pH 9). Then, 1 mL of 5% (w/v) NaCl was added as a salting-out reagent, and a final volume of 42 mL was obtained. The solutions were kept for 15 min at 40°C in the thermostatic batch, and the separation of the micellar phase was accelerated by centrifuging for 10 min at 3000 rpm. The solutions were then cooled in an ice-bath for 15 min. The micellar phase became viscous and could then be separated by inverting the tubes to discard the aqueous phase. In a later step, in order to reduce the viscosity and facilitate sample handling, 0.3 mL of a HNO$_3$ (1:1 v/v) solution was added to the surfactant-rich phase, and a final volume of 0.8 mL for diluted surfactant-rich phase was obtained. An aliquot of 20 µL of the diluted surfactant-rich phase, 5 µL of 5% (m/v) ascorbic acid, and 5 µL of 100 µg mL^{-1} Pd were co-injected into the graphite furnace using the auto-sampler.

2.5 *Optimization strategy*

The GF AAS analysis step was optimized, for this experiment, using a Doehlert design [26] for the following variables: pyrolysis temperature, pyrolysis time and atomization temperature. In the case of three variables designs, a cuboctahedron is produced geometrically through a uniform distribution of points over the whole experimental region [27]. Table 2 presents the coded values to the design correspondent at a plane projection produced when cuboctahedron leans on a square face. This plane projection of cuboctahedron was used in this work, requiring 15 experiments for each analyte including replications in the central point. These replications were performed in order to estimate the experimental variance. The experimental data were processed using the

Table 2. Doehlert matrix in the optimization of the conditions for Cr and Co determination in GF AAS after cloud-point extraction (values in parentheses are coded values).

| Exp	Pyrolysis temperature (°C)	Pyrolysis time (s)	Atomization temperature		Response (Abs)	
			Cr	Co	Cr	Co
01	1100 (0)	12 (0)	2800 (1)	2500 (1)	2.5001	0.3773
02	1050 (−0.5)	8 (−0.707)	2700 (0.5)	2450 (0.5)	1.9253	0.4491
03	1050 (−0.5)	16 (0.707)	2700 (0.5)	2450 (0.5)	1.8168	0.4027
04	1150 (0.5)	8 (−0.707)	2700 (0.5)	2450 (0.5)	2.1993	0.4380
05	1150 (0.5)	16 (0.707)	2700 (0.5)	2450 (0.5)	1.8667	0.3496
06	1000 (−1)	12 (0)	2600 (0)	2300 (0)	1.4913	0.3490
07 (C)[a]	1100 (0)	12 (0)	2600 (0)	2300 (0)	1.5711	0.3744
07 (C)	1100 (0)	12 (0)	2600 (0)	2300 (0)	1.6158	0.3525
07 (C)	1100 (0)	12 (0)	2600 (0)	2300 (0)	1.5984	0.3687
08	1200 (1)	12 (0)	2600 (0)	2300 (0)	1.6894	0.4286
09	1050 (−0.5)	8 (−0.707)	2500 (−0.5)	2150 (−0.5)	1.3857	0.3256
10	1050 (−0.5)	16 (0.707)	2500 (−0.5)	2150 (−0.5)	1.2554	0.3162
11	1150 (0.5)	8 (−0.707)	2500 (−0.5)	2150 (−0.5)	1.2183	0.3164
12	1150 (0.5)	16 (0.707)	2500 (−0.5)	2150 (−0.5)	1.2108	0.3054
13	1100 (0)	12 (0)	2400 (−1)	2000 (−1)	1.2085	0.2100

[a](C): central point.
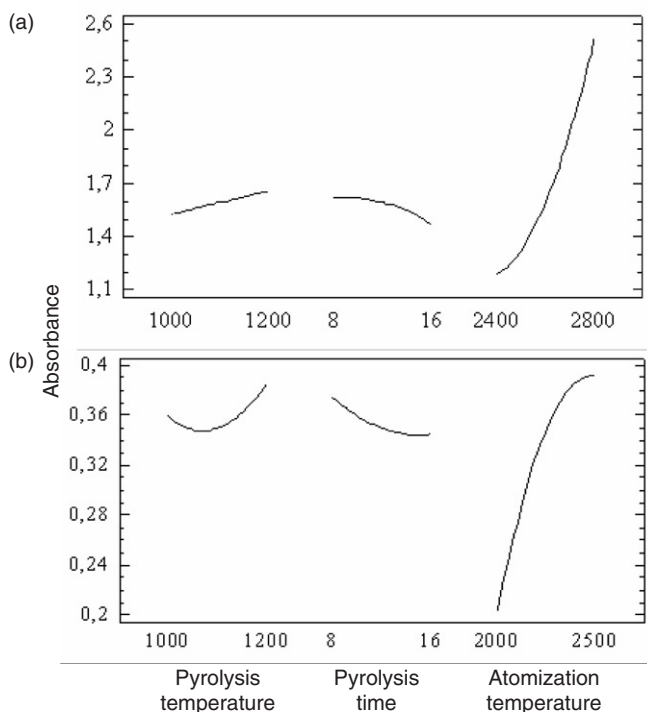
Figure 1. Main effect plots for absorbance to follow metals: (a) chromium; (b) cobalt.

STATGRAPHICS software [28]. The optimization process was carried out using cloud-point extracts from $3.0\,\mu g\,L^{-1}$ metal solutions.

In this work, pyrolysis and atomization temperatures were studied in five levels and pyrolysis times in three levels. For all studied metals, the pyrolysis temperatures were $1000–1200°C$. Also, the three metal pyrolysis times studied were from 8 to 16 s. However, the atomization temperatures were different for two metals used in this study, $2400–2800°C$ and $2000–2500°C$, for chromium and cobalt, respectively.

3. Results and discussion

3.1 *Optimization of the GF AAS conditions using the Doehlert design*

Analysis of variance (ANOVA) was applied in evaluating the data obtained after the application of the experimental design. Figure 1 presents the plots of the main effects for the metals studied. These plots are obtained from the response surface by a variation of a factor from its minimal level to its maximal level and keeping the other factors in their respective middle level. Thus, an analysis of results showed that pyrolysis temperature is not significant in the experimental interval studied, probably because the use of chemical modifiers allowed analyte stabilization. The performance of GF AAS can be enhanced by the use of these chemical modifiers, which stabilize

Table 3. Graphite-furnace programme for determination of Cr and Co in the micellar phase obtained after cloud-point extraction.

Step	Temperature ($^\circ$C)	Time (s)	Ar flux (L min^{-1})	Stage
1	85	5.0	3.0	Dry
2	95	40.0	3.0	Dry
3	120	10.0	3.0	Dry
4	1200	5.0	3.0	Pyrolysis
5	1200	1.0	3.0	Pyrolysis
6	1200	2.0	3.0	Pyrolysis
7	2700[a], 2450[b]	1.2	0	Atomization
8	2700[a], 2450[b]	2.0	0	Atomization
9	2700[a], 2450[b]	2.0	3.0	Cleaning

[a]Conditions for Cr; [b]conditions for Co.

the analyte to higher pyrolysis temperatures and hence facilitate the volatilization of concomitants prior to the atomization stage. The use of modifiers provides more stability to the elements at a higher temperature than that expected without the presence of a modifier. Thus, a pyrolysis temperature of 1200°C was chosen, which permits efficient matrix elimination for the two metals studied.

Pyrolysis time does not present a significant effect in the studied experimental domain. A visual inspection of the response surface indicates that a higher analytical signal can be attained using a time of 8 s as the pyrolysis time for Cr and Co.

The more significant variable to Cr and Co signals is the atomization temperature, which presents a strong positive effect in the response. For Co, there is a maximum point corresponding with values of 2450°C, and this was chosen as the optimal condition. On the other hand, for Cr there is an indication that higher temperatures may be needed to reach its optimal condition. However, high atomization temperatures decrease the tube lifetime. In view of these considerations, a temperature of 2700°C was chosen for chromium. The interaction effects (all in relation to atomization temperature) were significant only for the determination of chromium.

Second-order mathematical models describe perfectly the studied experimental domain, which is shown by the p values from the lack of fit ($p > 0.05$). From Cr and Co, p-values found were 0.0666 and 0.0858, respectively. Table 3 presents the optimized graphite furnace programme for determination of two studied metals into the micellar phase.

3.2 *Analytical features*

The calibration equations obtained after preconcentration by cloud-point extraction of standards under the optimum chemical and operational conditions were given as absorbance (Abs) function: Abs $= 0.4373 \times$ [Cr, μg L^{-1}] $+ 0.1073$ ($R^2 = 0.9948$), in the interval from 0.25 to 2.0 μg L^{-1}; and Abs $= 0.1112 \times$ [Co, μg L^{-1}] $+ 0.0033$ ($R^2 = 0.9976$), in the interval from 0.25 to 5.0 μg L^{-1}. By using GF AAS without the preconcentration step, in a matrix constituted by the surfactant Triton X-114 5% and HNO$_3$ (1:4) w/v and using the same conditions used to CPE-GF AAS, the calibration equations obtained were Abs $= 0.0167 \times$ [Cr, μg L^{-1}] $+ 0.0046$ ($R^2 = 0.9967$) in the interval from 1.0 to 5.0 μg L^{-1}, and Abs $= 0.0027 \times$ [Co, μg L^{-1}] $+ 0.0116$ ($R^2 = 0.9993$) in the interval from 1.0 to 25.0 μg L^{-1}.

The experimental enrichment factors calculated as the ratio of the slopes of the calibration graphs with and without preconcentration were approximately 26 and 41, respectively, for Cr and Co. As the maximum enrichment factor possible is 50 (40 mL/ 0.8 mL) for both analytes, the cloud-point extraction efficiencies calculated were 52 and 82% for Cr and Co, respectively.

The precision of the procedure determinates as the relative standard deviation in sample solutions containing $0.5 \, \mu g \, L^{-1}$ ($n = 8$) reached for Cr and Co were, in this order, 5.4 and 8.4. The limits of detection and quantification, defined as $LOD = 3\delta/S$ and $LOQ = 10\delta/S$, where S is the slope of the analytical curve, and δ is the standard deviation of 10 consecutive measurements of the blank, were also calculated. The LODs found were 23 and $12 \, ng \, L^{-1}$, and LOQs were 78 and $39 \, ng \, L^{-1}$ to Cr and Co, respectively.

3.3 *Effect of other ions*

To evaluate the effects of other metal ions on the pre-concentration procedure, a solution containing a concentration of $3.0 \, \mu g \, L^{-1}$ of Cr and Co and $100.0 \, \mu g \, L^{-1}$ of Cu^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+}, Zn^{2+}, Al^{3+}, Fe^{3+}, Mn^{2+}, Mo^{4+}, Sb^{5+}, and V^{5+} was prepared and analysed by the proposed method. The results showed that concomitant ions in these concentrations do not interfere in the determination of the analytes by the proposed procedure.

3.4 *Application*

In order to verify the feasibility of the proposed methodology suitable for assaying chromium and cobalt, the concentrations of these metals in different samples, such as orange tree and alfalfa leaves, were determined. A certified reference material NIST 1515 (apple leaves) was also analysed to verify the accuracy of the proposed methodology. Results are presented in the tables 4 and 5.

Table 4.　Analysis of certified reference material NIST 1515, apple leaves ($N = 3$).

Metallic ion	Informed value ($\mu g \, g^{-1}$)	Found value ($\mu g \, g^{-1}$)
Co	0.09	0.08 ± 0.02
Cr	0.3	0.35 ± 0.05

Table 5.　Analysis of leaf samples ($N = 3$).

Samples	Co	Cr
Orange tree leaves ($\mu g \, g^{-1}$)	0.043 ± 0.006	0.20 ± 0.02
Alfalfa leaves ($\mu g \, g^{-1}$)	0.04 ± 0.02	0.28 ± 0.07

4. Conclusions

Cloud-point extraction has been shown to be a promissory procedure in metal preconcentration at the trace levels in matrixes, such as, vegetal leaves. The results for this work demonstrate the possibility of using the Doehlert design to optimize the operational conditions of graphite furnace (pyrolysis temperature, pyrolysis time and atomization temperature) in chromium and cobalt determination after their cloud-point extraction. The proposed method can be applied in the determination of trace metals in various samples, thus allowing the detection of Cr and Co at $ng\,L^{-1}$ levels.

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