Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

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To cite this Article Coelho, Luciana Melo , Bezerra, Marcos Almeida , Arruda, Marco Aurélio Zezzi , Bruns, Roy Edward and Ferreira, Sérgio Luís Costa(2008) 'Determination of Cd, Cu, and Pb after Cloud Point Extraction using Multielemental Sequential Determination by Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS)', Separation Science and Technology, 43: 4, 815 — 827

To link to this Article: DOI: 10.1080/01496390701870630

URL: http://dx.doi.org/10.1080/01496390701870630

Separation Science and Technology, 43: 815–827, 2008

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390701870630

Determination of Cd, Cu, and Pb after Cloud Point Extraction using Multielemental Sequential Determination by Thermospray Flame Furnace Atomic Absorption Spectrometry (TS-FF-AAS)

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Abstract: In the present paper a procedure for simultaneous preconcentration of Cd, Cu, and Pb using cloud point extraction (CPE) and multielemental sequential determination by thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) is proposed. This preconcentration procedure is based on the metals extraction into micellar media of octylphenoxypolythoxyethanol (Triton X-114) after their reaction with 1-(2-pyridylazo)-2-naphtol (PAN). A Box-Behnken design was used to optimize the variables (pH, buffer concentration and volume of NaCl) involved in the extraction procedure. Enrichment factors were calculated as the ratio between the slopes of calibration equations with and without the preconcentration procedure in the TS-FF-AAS, with values of 59, 25, and 21 respectively for Cd, Cu, and Pb. Limits of detection (LOD) ($3s_b$, $n = 10$) were also calculated as 0.025, 0.38, and

Received 26 May 2007, Accepted 22 October 2007

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0.43 μg L^{-1} to Cd, Cu, and Pb, respectively. The precision (repeatability) expressed as relative standard deviation (RSD, n = 10) for reference standards of 10.0 and 20.0 μg L^{-1} were 6.1 and 4.5% for Cd, 7.2 and 3.4% for Cu and 5.2 and 8.7% for Pb. The accuracy was confirmed through the analysis of certified reference material (NIST 1643d, Trace elements in water). The developed procedure was also applied to Cd, Cu, and Pb determinations in mineral water samples.

Keywords: Cloud point extraction, TS-FF-AAS, sequential multielemental determination

INTRODUCTION

Although flame atomic absorption spectrometry (FAAS) is a consolidated technique, there is a large demand to improve its sensitivity, mainly for those elements that present poorer detection limits (1). An alternative to overcome this problem is the use of thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS) for the determination of more volatile elements. This technique is capable of considerably improving detection limits for volatile metallic species (such as As, Bi, Cd, Cu, Hg, In, K, Pb, Sb, Se, Te, Tl, and Zn) when compared to FAAS, mainly due to characteristics such as high sample transport efficiency and confinement of the atomic cloud in a furnace. This implies increases in the sensitivity of the technique (2, 3). In this sense, different papers have been published, showing some applications of this technique to different matrixes (4–7). Recently, preconcentration procedures have been coupled to the TS-FF-AAS technique in order to improve its sensitivity (8–10). When, TS-FF-AAS is used with a preconcentration procedure, such as cloud point extraction (CPE) (11–13), lower limits of detection can be attained, which can be comparable to those obtained by graphite furnace absorption spectrometry (GF AAS).

In the last years, multivariate techniques have been performed in order to improve the optimization process in analytical chemistry (14–17). Thus, multivariate optimization offers several advantages such as

a. evaluation of variables carried out with a reduced number of experiments implying reagent and time economy,
b. acquisition of information regarding the interaction between the factors under study, and
c. determination of critical conditions (maximum, minimum and saddle points) which must be known to optimize the experiment.

This last characteristic is an advantage of Response Surface Methodology (RSM) in relation to others multivariate techniques such as two level factorial design which are used in screening studies. Response Surface Methodology is mainly applied for the determination of critical conditions because those techniques use factors with more than three levels and this allows fitting quadratic models to the experimental data. The main design matrixes used in the response surface methodology are Central composite (18, 19), Doehlert (20–23) and Box-Behnken (24). The Box-Behnken design is a spherical rotatable design consisting of three interlocking 2^2 factorial designs and a central point. This design requires a number of experiments given by $N = k^2 + k + cp$, where (k) is the factor number and (cp) is the number of replicates at the central point. It is very economical because it requires a minimal of 13 experiments for $k = 3$ variables compared with the central composite design, which needs a minimum of 15 experiment compatible with $N = 2^k + 2\,k + cp$. Box-Behnken designs present a uniform distribution of points over the experimental domain. It also presents other advantages as do the other designs:

a. It is highly efficient,
b. it is easier to arrange and to interpret and
c. the initial dimensions can be contracted or expanded on the experimental domain.

Cadmium and lead are classified as toxic metals, which tend to be concentrated in environmental systems and humans, while copper is known as an essential nutrient for humans (25–27). In this work, a Box-Behnken design was used for optimizing a procedure for simultaneous preconcentration of Cd, Cu, and Pb ions from mineral water. This procedure is based on the cloud point of these metallic ions into micellar media of octylphenoxy-polyethoxyethanol surfactant (Triton X-114) after complexing with 1-(2-pyridylazo)-2-naphtol (PAN). For the first time, advantages of simultaneous metallic ions preconcentration by CPE and its multielemental sequential determination by TS-FF-AAS were combined for developing a simple, cheap, fast, and sensitive procedure for determining toxic trace metals in waters.

EXPERIMENTAL

Instrumentation

Measurements were made on a Varian Model SpectrAA 220FS (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer, equipped with a deuterium background corrector. Automatic switching of hollow-cathode lamps was used for the analysis, working in the fast sequential mode. Hollow-cathode lamps were used as radiation sources and operational conditions of equipment were according to manufacture's recommendation conditions (Table 1).

Table 1. Instrumental parameters used for Cd, Cu, and Pb determination by TS-FF-AAS after cloud point extraction

Parameter	Cd	Cu	Pb
Analytical wavelength (nm)	228.8	324.8	217.0
Current (mA)	4.0	4.0	1.0
Bandwidth of slit	0.5	0.2	1.0
Air flow rate (L min^{-1})	13.5	13.5	13.5
Acetylene flow rate (L min^{-1})	1.5	1.5	1.5
Burner height (mm)	10	10	10

The TS-FF-AAS system was similar to that already described (5–9), and consisted of a peristaltic pump (Minipuls 3, Gilson, Anachem, Luton, UK) with 8 channels furnished with Tygon tubes, and a lab-made thermospray flame furnace unit, which included the ceramic capillary (0.5 mm i.d., 2.0 mm e.d., and 100 mm of length) and a Ni tube 99.1% w/w (10 cm). Digimed DM20 pH meter was used to measure pH values. A Janetzki T 32C centrifuge was used to accelerate the phase separation. A FANEM thermostatic bath (São Paulo, Brazil) was used to maintain the sample solution at the desired temperature.

Reagents and Solutions

All reagents were of analytical grade unless otherwise stated. Ultrapure water (18.3 M · cm) was obtained from an EASYpure RF (Barnstedt, Dubuque, IA, USA) to prepare all solutions. Nitric acid and ethanol were of Suprapur quality (Merck). Laboratory glassware was kept overnight in a 10% v/v nitric acid solution. Before use, the glassware was rinsed with deionized water and dried in a dustfree environment.

Cadmium, copper, and lead solutions were prepared by diluting 1000 μg mL^{-1} standard solutions (Merck) with a 1% hydrochloric acid solution. The non-ionic surfactant TritonX-114 was obtained from Acros Organics (New Jersey, USA).

A 2.0 10^{-4} mol L^{-1} solution of PAN [1-(2-pyridylazo)-2-naphtol] (Merck) in a 1%, w/v Triton X-114 solution was prepared from dilution of an intermediate solution (4.0 10^{-3} mol L^{-1}) prepared of the compound in ethanol. The volume was set to 100.0 mL with ethanol.

Borate buffer (pH 9.0) was prepared by adding 50.0 mL of 0.1 mol L^{-1} $Na_2B_4O_7$ 10 H_2O and 18.3 mL of 0.4 mol L^{-1} NaOH to a 100.0 mL volumetric flask. The volume was then set with distilled-deionized water. A diluent solution was prepared with ethanol containing 1% (v/v) nitric acid, to decrease the micellar phase viscosity before its introduction into thermospray flame furnace system.

Procedure for Cloud Point Extraction and TS-FF-AAS Determination

For cloud point extraction, 2 mL of an alcoholic solution constituted by 1% (w/v) Triton X-114, 2 10^{-4} mol L^{-1} PAN, and 1 mL of buffer $Na_2B_4O_7$ 10 $H_2O/NaOH$ were added to 40 mL standard solutions. After that, 1 mL of 1% (w/v) NaCl was added to promote the salting out effect. The solutions were kept for 10 min at 40°C in the thermostatic bath and the separation of the micellar phase was accelerated by centrifuging the solution at 3000 rpm during 15 min. The solutions were then cooled in an ice-bath for 10 min. The micellar phase became viscous, which could be separated by inverting the tubes in order to discard the aqueous phase. In the later step, in order to reduce the viscosity and facilitate sample handling, 1 mL of an alcoholic solution with 1% (v/v) HNO_3 was added to the samples. The dissolved micellar phase was introduced into the thermospray system through a peristaltic pump at a flow rate of 0.60 mL min^{-1}.

Optimization Strategy

The optimization process was carried out using Response Surface Methodology (RSM). A Box-Behnken design was performed in order to determine the critical conditions for simultaneous cloud point extraction of the three referred metal ions. According to the design characteristic, all variables were studied at three levels. The experiments established using this design for variables as pH, buffer concentration, and volume of added salt are described in the Table 2. The experimental data were processed by using the STATISTICA software.

All the experiments for Box-Behnken design application were carried out using multielemental solutions which were constituted by 10.0 $\mu g\, L^{-1}$ for copper and lead and 5 $\mu g\, L^{-1}$ for Cd. Replicates at the central point were performed in order to estimate the experimental variance. In the present work, three responses are obtained as correspondence absorbance signal to each metal ion. All three responses present a similar profile and it is an indication that there is the same tendency between them. Thus, to optimize a procedure that satisfies a simultaneous cloud point extraction of the three chemical species studied, an overall response was adopted. The overall response (OR) is given by the following expression:

$$OR = [R(Cd)/LS(Cd) + R(Cu)/LS(Cu) + R(Pb)/LS(Pb)] \qquad (1)$$

where R(metal ion) is the analytical signal to a specific metal in a particular experiment and LS(metal ion) is the larger signal in the set of experiments to that metal ion.

Table 2. Box-Behnken design for CPE-TS-FF-AAS method optimization

	Variables			Response (Abs)			
Exp.	pH	BC (10^{-4} mol L^{-1})^a	VS (mL)	Cd	Cu	Pb	OR
01	−1 (6)	−1 (2.5)	0 (250)	0.1979	0.0321	0.1602	1.221
02	1 (10)	−1 (2.5)	0 (250)	0.5731	0.0428	0.1679	1.904
03	−1 (6)	1 (12.5)	0 (250)	0.2552	0.0378	0.1614	1.332
04	1 (10)	1 (12.5)	0 (250)	0.5695	0.0558	0.1699	1.931
05	−1 (6)	0 (7.5)	−1 (0)	0.2635	0.0403	0.1636	1.362
06	1 (10)	0 (7.5)	−1 (0)	0.5796	0.0498	0.1686	1.931
07	−1 (6)	0 (7.5)	1 (500)	0.1925	0.0405	0.1611	1.231
08	1 (10)	0 (7.5)	1 (500)	0.5801	0.0522	0.1689	1.937
09	0 (8)	−1 (2.5)	−1 (0)	0.5128	0.0474	0.1617	1.779
10	0 (8)	1 (12.5)	−1 (0)	0.5738	0.0497	0.1725	1.941
11	0 (8)	− 1 (2.5)	1 (500)	0.5235	0.0496	0.1697	1.843
12	0 (8)	1 (12.5)	1 (500)	0.5731	0.0547	0.1779	1.977
13 (C)	0 (8)	0 (7.5)	0 (250)	0.5879	0.0538	0.1914	2.070
13 (C)	0 (8)	0 (7.5)	0 (250)	0.5852	0.0589	0.1885	2.059
13 (C)	0 (8)	0 (7.5)	0 (250)	0.6006	0.0557	0.1899	2.087

[a] BC = buffer concentration, VS = volume of added salt (NaCl 4% w/v). The real values are in brackets. OR = overal response. Metal concentration: Cu and Pb: 10 μg L^{-1} and Cd: 5 μg L^{-1}. Standard solutions volume: 40 mL. PAN (2.0 10^{-4} mol L^{-1} in 1% Triton X-114) volume: 2 mL. Centrifuging time: 15 min. Ice-bath time: 10 min. EtOH (HNO_3 1%) volume: 1 mL. Flow rate: 0.60 mL min^{-1}.

RESULTS AND DISCUSSION

Optimization of the Cloud Point Extraction Conditions using the Box-Benhken Design

Analysis of variance (ANOVA) was applied to data evaluation. All statistical considerations that are approached here were restrictedly made relative to studied experimental domain and they have a confidence interval at a level of 95%. The relation between the variables (as coded values) pH, buffer concentration (BC), and the volume of salt added (VS) and the Overall response (OR) is fitted by the following equation:

$$
\begin{aligned}
(\mathrm{OR}) = {} & 2.072\ (\pm 0.008) + 0.320\ (\pm 0.005)\,\mathrm{pH} - 0.372\ (\pm 0.007)\,\mathrm{pH}^2 \\
& + 0.054\ (\pm 0.005)\ \mathrm{BC} - 0.103\ (\pm 0.007)\ \mathrm{BC}^2 \\
& - 0.0846\ (\pm 0.007)\mathrm{VS}^2 + 0.034\ (\pm 0.007)\,\mathrm{pH}^{*}\mathrm{VS}
\end{aligned} \tag{2}
$$

In Eq. (2) only the significant terms are presented. ANOVA can be graphically represented by a Pareto chart presented in the Fig. 1. ANOVA shows that pH is the most significant variable for cloud point extraction of the three metal ions. Buffer concentration (BC) and volume of added salt (VS) is also significant. The unique significant interaction is between pH and VS. These observations can be confirmed by the response surface presented in the Fig. 2.

Individual results to each metal ion show that there are maximum points for all studied metals inside the experimental domain studied (Table 3). It was possible to note that there is a tendency for efficient metal extractions in a common region for all elements. Thus, to become feasible to extract a larger quantity of studied metals simultaneously in a unique experiment, the overall response was used, which must be composed of the other three individual responses according to Eq. (1), The derivatization of Eq. (2) as pH, BC, and VS results in the follow equation system:

$$\frac{\partial pH}{\partial OR} = 0.320 - 0.744pH + 0.034VS = 0$$
$$\frac{\partial BC}{\partial OR} = 0.054 - 0.206BC = 0$$
$$\frac{\partial VS}{\partial OR} = 0.1692VS + 0.034pH = 0$$

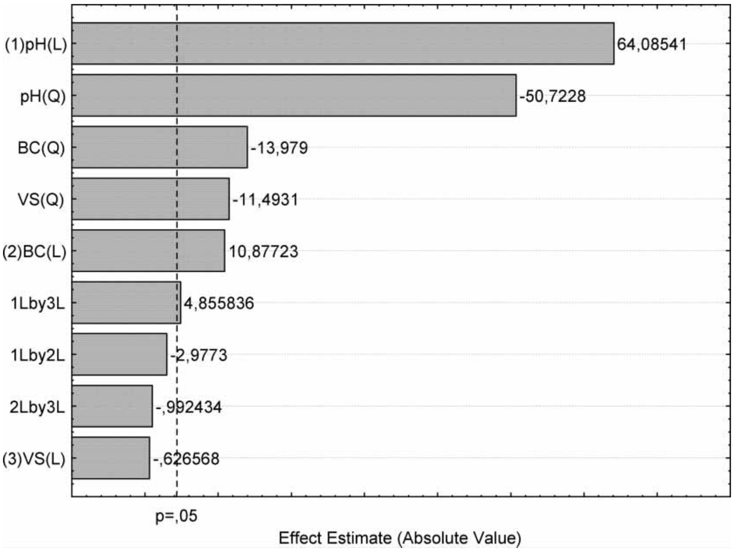

Figure 1. Pareto chart for data of Table 2 showing the effect estimates of the studied variables. pH (L) = linear term to pH variable; pH (Q) = quadratic term to pH variable; BC (Q) = quadratic term to BC varible; VS (Q) = quadratic term to VS variable; 2BC (L) = linear term to BC variable; 1Lby3L = represent term de interaction enters pH and VS; 1Lby2L = represent term de interaction enters pH and BC.; 2Lby3L = represent term de interaction enters BC and VS; (3) VS(L) = linear term to VS variable.

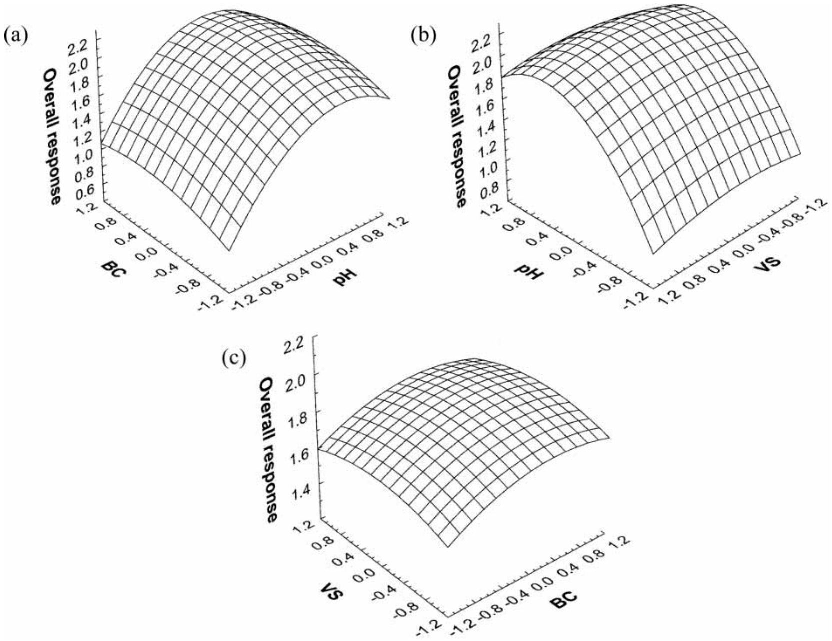

Figure 2. Response surfaces obtained for Cd, Cu, and Pb after extraction by cloud point: (a) BC X pH, (b) VS X pH and (c) VS X BC. BC = buffer concentration, VS = volume of added salt (NaCl 4% w/v).

Solving these equations it is possible to show that there is an experimental condition for maximum response such as: pH (0.434), buffer concentration (0.262) and the volume of added salt (0.094). These results as coded values indicate real pH values (9.0), buffer concentration (8.8 10^{-4} mol L^{-1}), and the volume of added salt (300 μL). The second order mathematical models describe perfectly the studied experimental domain, which is shown as the p values for lack of fit ($p > 0.05$). The p-value found was 0.05998 using the overall response (OR).

Table 3. Optimum values for Cd, Cu, and Pb and overall response (OR)

	Optimum values			
Variables	Cd	Cu	Pb	OR
pH	9.0	8.8	8.3	9.0
BC (10^{-4} mol L^{-1})	8.8	9.9	8.2	8.8
VS (μL)	255	453	270	300

Analytical Features

The calibration equations obtained after preconcentration by cloud point extraction of standards under the optimum conditions were given as absorbance (Abs) function: Abs = 0.1116 [Cd, μg L^{-1}] + 0.066 (R = 0.9972), in the interval from 2.0 to 10.0 μg L^{-1}; Abs = 0.0043 [Cu, μg L^{-1}] + 0.003 (R = 0.9967), in the interval from 5.0 to 50.0 μg L^{-1} and Abs = 0.013 [Pb, μg L^{-1}] + 0.0256 (R = 0.9983), from 5.0 to 50.0 μg L^{-1}. By using TS-FF-AAS without the preconcentration step, the calibration equations obtained were Abs = 0.0066 [Cd, μg mL^{-1}] + 0.0455 (R = 0.9991) from 0.020 to 0.080 μg mL^{-1}; Abs = 0.1692 [Cu, μg mL^{-1}] − 0.0011 (R = 0.9979) from 0.2 to 2 μg mL^{-1}, and Abs = 0.6335 [Pb, μg mL^{-1}] + 0.0026 (R = 0.9967) from 0.2 to 2.0 μg mL^{-1}. The experimental enrichment factors (28) calculated as the ratio between the slopes of the calibration graphs with and without preconcentration in the TS-FF-AAS system were approximately 59, 25, and 21 respectively for Cd, Cu, and Pb. The limits of detection and quantification, defined as LOD = $3s_b/m$ and LOQ = $10s_b/m$, where m is the slope of the analytical curve, and s_b is the standard deviation of ten consecutive measurements of the blank were also calculated. The LOD was 0.025, 0.38, and 0.43 μg L^{-1} and LOQ was 0.083, 1.27, and 1.44 μg L^{-1} for Cd, Cu, and Pb respectively. The limits of detection when compared to FAAS were improved by about 566, 46, and 101 times for Cd, Cu, and Pb, respectively due the efficiency of TS-FF-AAS when applied together with preconcentration employing cloud point extraction. The precision of the procedure determined as the relative standard deviation (RSD, n = 10) in sample solutions containing 10.0 and 20.0 μg L^{-1} of metals were 6.1 and 4.5% to Cd, 7.2 and 3.4% to Cu, and 5.2 and 8.7% to Pb.

Concomitant Effect

In order to evaluate the concomitant effect in the preconcentration procedure, a volume of 40 mL of sample solution containing cadmium, copper and lead (all metals were present at 10 μg L^{-1} concentration) was prepared and analyzed by the proposed method. Results showed that Ni, Cr, Zn, Al, Fe, Mn, Hg, Mo, Sb, and V in the concentration of 100 μg L^{-1} do not interfere in cadmium, copper, and lead determinations.

Application

In order to verify the feasibility of the proposed methodology as being suitable for assaying cadmium, copper, and lead ions, the concentration of these metals in samples of two mineral waters were determined. A certified reference material NIST 1643d (Trace elements in water) was also analyzed for

Table 4. Analysis of certified reference material NIST 1643d (trace elements in water)

Metallic ion	Certified value (μg L^{-1})	Found value (μg L^{-1})
Cd	6.47 ± 0.37	6.8 ± 1.7
Cu	20.5 ± 3.8	21.4 ± 2.5
Pb	18.15 ± 0.64	18.4 ± 1.2

Confidence interval of 95%, n = 3.

Table 5. Analysis of mineral water samples

Sample	Metal	Added metal (μg L^{-1})	Found metal (μg L^{-1})	Recovery (%)
Mineral water 1	Cd	—	<LOQ	—
		5	4.7 ± 1.5	94
	Cu	—	8.8 ± 2.9	—
		10	19.5 ± 1.1	107
	Pb	—	7.0 ± 0.5	—
		10	17.9 ± 0.8	108
Mineral water 2	Cd	—	<LOQ	—
		5	5.8 ± 1.0	108
	Cu	—	16.1 ± 1.6	—
		10	27.4 ± 2.9	113
	Pb	—	3.1 ± 0.7	—
		10	13.6 ± 0.5	105

Confidence interval of 95%, n = 3.

checking the accuracy of the proposed method. The results are presented in the Tables 4 and 5.

CONCLUSIONS

The results demonstrated that cloud point extraction can be used as a preconcentration step for metallic ions prior to their multielemental sequential determination by thermospray flame furnace atomic absorption spectrometry (TS-FF-AAS), allowing limits of detection as low as those of graphite furnace atomic absorption spectrometry (GF AAS) for metallic ions. Box-Behnken design was successfully applied as a multivariate technique to optimize the extraction conditions of the Cd, Cu, and Pb ions prior to the determination step allowing evaluation of interaction effects between optimized variables and economizing of time and reagents. The analysis of spiked samples and certified material shows that the developed method can be applied with satisfactory results.

ACKNOWLEDGMENTS

The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB) and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) for financial support. The authors also thank CNPq for the fellowships of MAZA and SLCF and to R. Bruns for critical comments and language assistance.

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