

The Use of Cloud Point Extraction and Hydride Generation for Improving the Sb and Se Limits of Detection in ICP OES

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O presente estudo trata da aplicação da extração no ponto nuvem (CPE) e geração de hidretos (HG) para a determinação de Sb e Se por espectrometria de emissão óptica com plasma indutivamente acoplado (ICP OES). O complexante utilizado foi o dietilditiofosfato de amônio (DDTP), enquanto que Triton X-114 foi usado como surfactante para a formação das micelas. As condições para a pré-concentração de Sb(III) e Se(IV), redução de Se(VI) e Sb(V), geração dos respectivos hidretos e interferências foram avaliadas e discutidas. O desempenho da HG foi comparado com o da nebulização pneumática (PN) para introduzir a fase rica em surfactante no plasma. Mediante aplicação da HG, os limites de detecção obtidos para Se(IV) e Sb(III) foram 0,05 e 0,08 $\mu\text{g L}^{-1}$, respectivamente. No caso da aplicação da PN, os limites de detecção do Se(IV) e Sb(III) foram 0,90 e 2,90 $\mu\text{g L}^{-1}$, respectivamente. Foram analisadas amostras de nozes e castanha do Pará, água e vinho branco. O Se(IV) foi quantificado na castanha do Pará, enquanto que o Sb(III) foi quantificado em água de estuário, água de mar e água mineral embalada em garrafa feita de politereftalato de etileno (PET).

This study deals with the application of cloud point extraction (CPE) and hydride generation (HG) for determination of Sb and Se using inductively coupled plasma optical emission spectrometry (ICP OES). The complexing agent was ammonium diethyldithiophosphate (DDTP), whereas Triton X-114 was used as surfactant for the formation of micelles. Conditions for Sb(III) and Se(IV) preconcentration, reduction of Se(VI) and Sb(V), hydrides generation and interferences were discussed. The performance of HG was compared with pneumatic nebulization (PN) for introducing the surfactant-rich phase into the plasma. Upon application of HG, the limits of detection (LODs) for Se(IV) and Sb(III) were 0.05 and 0.08 $\mu\text{g L}^{-1}$, respectively. Limits of detection using PN were 0.90 and 2.90 $\mu\text{g L}^{-1}$ for Se(IV) and Sb(III), respectively. Samples of nuts, water and white wine were analyzed. Selenium(IV) was quantified in Brazil nut whereas Sb(III) was in estuarine water, seawater and mineral water stored in polyethylene terephthalate (PET) bottle.

Keywords: Sb and Se, cloud point extraction, ICP OES, hydride generation, pneumatic nebulization

Introduction

Selenium is a natural component of enzymes and is fundamental in several metabolic processes. Deficit of Se may promote cardiac diseases, hypothyroidism and weakness of the immunologic system. However, Se is toxic when present in elevated concentrations, which is dependent on the Se species.¹

Antimony is a potentially toxic element, without any documented biological function. It is a trace element, which

is being introduced into our surroundings in increasing quantities. Modern applications of Sb have increased dramatically during the last few decades and nowadays Sb is used in a broad range of products, such as rubber and plastics, packaging materials, bactericides, fungicides, etc.² Thus, humans and other biological systems are subjected to an ever increasing antimony exposure.

Direct determination of Sb and Se at low concentrations is commonly difficult by using spectroscopic techniques such as AAS (atomic absorption spectrometry) and ICP OES (inductively coupled plasma optical emission spectrometry) owing to the lack of sensitivity. The use of

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hydride generation (HG) can improve the sensitivity by increasing the efficiency of analyte transport, by helping to separate the analyte from the matrix, and by reducing associated non spectroscopic interference effects. The use of HG is also suitable for high sensitive techniques such as ICP-MS (inductively coupled plasma mass spectrometry) due to matrix separation provided by HG. It is worth pointing out that the limit of detection (LOD) for Se directly measured by ICP-MS is often relatively high due to polyatomic interferences.

Antimony and Se species react with tetrahydroborate in acidic medium to form volatile hydrides, which are subsequently detected. However, the formation of hydrides is dependent on the oxidation state of the element. For example, Se(IV) forms hydrides whereas Se(VI) does not. Thus, Se(VI) must be reduced to Se(IV) that can be measured by using HG and a specific detector.

When HG is used there is an improvement in sensitivity by two orders of magnitude relative to pneumatic nebulization (PN).³ However, depending on the type of sample analyzed, analyte preconcentration may be necessary for its detection by ICP OES, even using HG.

Cloud point extraction (CPE) has been applied for Sb and Se separation/preconcentration in the last decade.⁴⁻¹⁵ Cloud point extraction is based on micelles formation and subsequent separation. Micellar aqueous solution is produced by addition of surfactant whose concentration in the solution must ensure the formation of micelle aggregates; the final surfactant concentration must exceed the critical micelle concentration (CMC). Once the surfactant concentration exceeds CMC, the aqueous micellar solution can separate into two isotropic phases: a surfactant-rich phase of small volume and a surfactant-poor phase of much higher volume (aqueous). Any component that binds to the micellar aggregate in solution can be extracted from the original solution and, therefore, be concentrated in the surfactant-rich phase. In the case of hydrophilic inorganic species, complexing agents are commonly used for producing hydrophobic species that are extracted into the micelles, thereby in a small volume that is separated. In the case of using ICP OES for analyte detection, the viscosity and the organic content of the surfactant-rich phase affect the plasma performance and stability. Therefore, appropriate sample introduction systems and free-clogging nebulizer are required.

Triton x-114 has been used as surfactant whereas 2,3-diaminonaphthalene (DAN),⁴ ammonium pyrrolidine dithiocarbamate (APDC),^{5,6,10,15} diethyldithiocarbamate (DDTC),⁷ 3,3'-diaminobenzidine (DAB),⁸ *o*-phenylenediamine⁹ and *N*-benzoyl-*N*-phenylhydroxylamine (BPHA)¹³ have been used as

complexants. These complexants react specifically with Se(IV) and/or Sb(III). Total Se and total Sb have been determined after Se(VI) and Sb(V) were reduced by *L*-cysteine,^{6,10,13,14} or Se(VI) reduced to Se(IV) by microwave heating of solution in 4 mol L⁻¹ HCl.⁸ Concentrations of Se(VI) and Sb(V) were obtained by respectively subtracting Se(IV) and Sb(III) from the total Se and the total Sb.

Cloud point extraction has been applied for speciation of organic and inorganic Se in vitamin tablets prior Se determination by fluorometry;⁴ total Se determination in acid digests of scalp hair and blood serum by electrothermal atomic absorption spectrometry (ETAAS);⁵ speciation of inorganic Se in water followed by detection using electrothermal vaporization inductively coupled plasma mass spectrometry (ETV-ICP-MS);⁶ speciation of Se(IV) and Sb(III) in water samples using ETV-ICP-MS for detection;⁷ preconcentration of Se(IV) in water samples and total Se in biological samples followed by detection using ETAAS;^{8,9} speciation of inorganic Sb in leaching solutions of different food packaging materials prior detection by ETAAS;¹⁰ Sb separation/preconcentration in seawater, anti-leishmania drug (glucantime), and human serum using spectrophotometric detection;¹¹ separation/preconcentration of Sb(III) in water prior detection by ETAAS;¹² preconcentration of Sb(III) in artificial seawater and wastewater with detection by flame atomic absorption spectrometry (FAAS);¹³ inorganic Sb species determination by combining on-line CPE with electrothermal vaporization inductively coupled plasma optical emission spectrometry (ETV-ICP OES);¹⁴ and determination of Sb(III) in natural water prior detection by ETAAS.¹⁵

Hydride generation in conjunction with CPE and ICP OES has not been investigated for determining Sb and Se so far, leading us to investigate and compare the HG performance for Sb and Se in the surfactant-rich phase following detection by ICP OES. The main purpose is to improve the LODs of Se and Sb in ICP OES. The HG performance is compared with those of conventional pneumatic nebulization (PN) and nebulization/aerosol desolvation, seeking the sample introduction system that provides the best sensitivity.

Experimental

Instrumentation

An Optima 2000 DV-ICP OES spectrometer (PerkinElmer, Norwalk, CT, USA) was used. Argon (purity of 99.998% from White Martins/Praxair) was used as plasma gas and auxiliary gas, meanwhile nitrogen with purity of 99.996% (White Martins/Praxair) was used

as purging gas. The main instrumental parameters are summarized in Table 1. These parameters were employed following previous works.^{16,17}

A homemade HG system was hyphenated with ICP OES and used for Se and Sb determination. This system is described elsewhere.¹⁶ It consists basically of a confluence and a gas liquid separator; solutions are transported and mixed using the peristaltic pump of the ICP OES. The flow rates of sample, HCl and NaBH₄ solutions were 1.3, 1.8 and 1.3 mL min⁻¹, respectively. The pneumatic nebulizer used (GemCone) is considered free-clogging and suitable for viscous solutions or solutions with high content of dissolved solids. An APEX-Q system (ESI, USA) with aerosol desolvation was also used. In this system, solutions were aspirated through a PFA concentric nebulizer fitted into a cyclonic spray chamber that was heated at 140 °C and then transported to a Peltier-cooled multipass condenser where the temperature was set as 2 °C. Partial solvent removal occurs in this system and sample transport efficiency is about 30%.

A heating block (TE-007D Tecnal, Piracicaba, SP, Brazil) and a microwave oven Multiwave 3000 (Anton Paar, Graz, Austria) were used for sample decomposition. A water bath with temperature control was used as a source of heating and assists CPE while a centrifuge was used for separation of the aqueous and surfactant-rich phases.

Reagents and solutions

All chemicals were of analytical-grade. Water purified (resistivity of 18.2 MΩ cm) in a Milli-Q system (Millipore, Bedford, MA, USA) was used to prepare all reagents, solutions and samples. Nitric acid (65% in mass), HCl (37% in mass), H₂O₂ (30% in mass), methanol (CH₃OH) and ethanol (CH₃CH₂OH), all from Merck (Darmstadt, Germany) were used. The purchased HNO₃, HCl and

CH₃OH were further purified by sub-boiling distillation using a Milestone duo PUR 2.01E system (Soriso, Italy). Sodium tetrahydroborate (NaBH₄, Vetec, RJ, Brazil) prepared in NaOH 0.05% (m/v) was used for Se(IV) and Sb(III) hydrides generation. DDTP [(C₂H₅O)₂P(S)NH₄] from Sigma-Aldrich (USA) and Triton X-114 from Sigma-Aldrich (USA) were used for Se(IV) and Sb(III) preconcentration. A 5.0% (m/v) DDTP stock solution was prepared by dissolving the reagent in water. A 5.0% (m/v) Triton X-114 stock solution was prepared by weighing 2.5 g of the reagent in a polypropylene vial followed by addition of 50 mL of water. Antifoam Y-30 from Sigma-Aldrich (USA) was used to avoid foam production in the gas/liquid separator of the HG system. Potassium iodine (KI), ascorbic acid (C₆H₈O₆), and L-cysteine C₃H₇NO₂S.HCl from Sigma-Aldrich (USA) were used for Se(VI) and Sb(V) reduction. Calibration solutions of Se and Sb were prepared in 0.14 mol L⁻¹ HNO₃ by serial dilution of Sb stock solution or salts of selenium (Table 2). The calibration solutions were also submitted to CPE in the same way that samples.

All vials and glassware used for samples preparation or storage of the calibration solutions were previously decontaminated by contact with 10% (v/v) HNO₃ for 48 h. Then, the vials were washed and rinsed with deionized water. The PTFE flasks used for the decomposition of the samples were decontaminated by adding 3 mL of HNO₃ and heating at 160 °C for 4 h in a metallic block.

Samples and sample preparation

The following non certified samples were analyzed: coconut water, white wine (Chardonnay), mineral water, nuts (Brazil nut, walnuts, sweet almond and hazelnuts), estuarine water (Guaíba, Rio Grande do Sul State, Brazil), seawater (from nearshore in Rio Grande do Sul) and tap water (collected in our own laboratory). Nut samples,

Table 1. Instrumental operating conditions for ICP OES with hydride generation (HG) or pneumatic nebulization (PN)

Parameter	Sample introduction	
	HG	PN
Plasma power / W	1500	1500
Plasma gas flow rate / (L min ⁻¹)	15	15
Auxiliary gas flow rate / (L min ⁻¹)	0.2	0.2
Nebulizer or carrier gas flow rate / (L min ⁻¹)	0.6	0.6
Spray chamber	–	umbuffed cyclonic
Nebulizer	–	GemCone or APEX-Q
Spectral line / nm	Sb: 206.836 Se: 203.985	Se: 203.985
Plasma view	axial	axial

Table 2. Stock and calibration solutions of Sb and Se

Stock solution or salt	Concentration range of calibration curve / ($\mu\text{g L}^{-1}$) with CPE	Concentration range of calibration curve / ($\mu\text{g L}^{-1}$) without CPE	Sample introduction mode
Se (100 mg L ⁻¹) from Na ₂ SeO ₃ , Merck	5.0 - 20	20 - 60	PN
	1.0 - 10	5.0 - 25	HG
Se (100 mg L ⁻¹) from Na ₂ SeO ₄ , Merck	–	1.0 - 20	HG
Sb (1000 mg L ⁻¹), Merck	10 - 50	20 - 100	PN/DES
	1.0 - 5.0	5.0 - 25	HG
	10 - 50	20 - 100	PN

PN: pneumatic nebulization; PN/DES: pneumatic nebulization/aerosol desolvation; HG: hydride generation.

coconut water and white wine were purchased in local shops. Certified dogfish liver (DOLT-4) and certified river water (SLRS-5) both from the National Research Council of Canada (NRCC, Ottawa, Canada) were analyzed.

The estuarine water was filtered and acidified by adding 1 drop of concentrated HNO₃ to 250 mL of sample, filtered and analyzed immediately. The other liquid samples (tap water, seawater, river water, mineral water coconut water and white wine) were analyzed without initial preparation; they were merely diluted by adding different volumes of HCl or HNO₃ solutions and the required reagents. Procedure 2 (see below) was used for decomposition of the certified dogfish liver (DOLT-4).

Nut samples were initially crushed in a blender. Then, the lipid fraction was extracted from 2 g of the crushed sample using a solution composed of 3.4 mL of methanol and 6.6 mL of chloroform. The obtained mixture was sonicated in an ultrasonic bath for 30 min and then filtered through quantitative filter paper. The solid residue (non-lipid fraction) was washed several times with water and left drying at room temperature. This extraction procedure was based on the work of Welna *et al.*¹⁸ Aliquots of the resulting solid were decomposed according to procedures 1 and 2 as follows.

Procedure 1

A mass of 100 mg of sample was weighted and placed in PTFE flask to which 1.0 mL of HNO₃ and 1.0 mL of H₂O₂ were added and the mixture left in contact for 12 h. Afterwards, the flask was closed with screw cap and the content heated in a metallic block for 1 h at 100 °C followed by 4 h at 150 °C. After cooling to room temperature, the flask was opened, the resulting solution transferred to graduated polypropylene vial and the volume completed to 25 mL by adding water.

Procedure 2

A mass of 100 mg of sample was weighted and transferred to the flask of the microwave oven. Then,

1 mL of HNO₃ and 1 mL of H₂O₂ were added and the mixture allowed standing for 1 h. Subsequently, the flask was closed and the mixture heated at 220 °C in the microwave oven; for 3 min at 300 W, 1 min at 0 W, 6 min at 500 W and 5 min at 650 W, followed by cooling for 20 min. The obtained solution was transferred to graduated polypropylene vial whose volume was made up to 25 mL by adding water. The solutions obtained according to procedures 1 and 2 were used for Se determination.

When recovery tests were carried out for dried sample it was spiked with the analyte before being submitted to the decomposition procedure.

Cloud point extraction procedure

Aliquots of sample solution ranging from 1 to 7 mL were transferred to graduated polypropylene vials. Subsequently, DDTP and Triton X-114 were added and the volume completed to 14 mL by adding water and/or HCl solution (Table 3). The mixture was heated in a water bath to accelerate the separation of phases, centrifuged at 3200 rpm for 10 min and then cooled in an ice bath for 10 min. The surfactant-poor phase was separated by inverting the vial, whereas the residual solution was removed by using a

Table 3. Conditions and reagent concentrations evaluated for Sb and Se preconcentrations using CPE

Condition/Reagent	Element	
	Sb	Se
Preconcentration medium	HCl	HCl
HCl / (mol L ⁻¹)	0.01 - 1.0	0.0 - 1.0
DDTP / %, m/v	0.05 - 1.0	0.01 - 0.3
Triton X-114 / %, m/v	0.05 - 0.30	0.05 - 0.30
Temperature / °C	50	50
Heating time / min	20	20
HCl for HG / (mol L ⁻¹)	0.1 - 4.0	0.05 - 1.5
NaBH ₄ / %, m/v	0.1 - 0.5	0.2 - 0.6

Pasteur pipette. The final volume of the surfactant rich phase ranged from 50 to 250 μL . The surfactant-rich phase was diluted by adding 100 μL of methanol followed by addition of 1.0 mL of 0.1 mol L^{-1} HCl. The surfactant-rich phase has to be diluted due to its high viscosity and/or foam production into the gas liquid separator.^{16,17}

The concentrations of the reagents, dilution of the surfactant rich phase and conditions evaluated are summarized in Table 3.

Results and Discussion

Selenium

The influence of HCl and DDTP concentrations on Se pre-concentration was initially evaluated. It was observed that the surfactant-rich phase diluted with methanol caused spectral interference; vapor of methanol was transported to the ICP even by using HG. The presence of an intense peak and bandwidth was highlighted, originated from the emission of C (193.09 nm) and NO (198.05 nm), increasing the background signal and overlapping the signal of the 196.026 nm Se emission line. As a consequence, the prominent 196.026 nm Se emission line could not be monitored for Se determination. The 203.985 nm Se line was then selected.

A solution of Se(IV) was used to evaluate pre-concentration since selenium hydride is only generated from this specie. The influence of HCl concentration is shown in Figure 1a in which one can see that the Se signal decreases with the increase of acid concentration. The reason for the Se signal decrease might be the salting-out effect promoted by chloride ions.¹⁹ Complex formation

between Se(IV) and DDTP is more effective in presence of low concentration of the ligand, as shown in Figure 1b. This behavior with respect to the DDTP concentration was also observed when SPE (solid phase extraction) was used for on-line pre-concentration of Se(IV).²⁰

The concentration of Triton X-114 investigated ranged from 0.05 to 0.30% (m/v). It was not possible to evaluate higher concentrations of the surfactant due to foam production in the gas-liquid separator, which caused huge effervescence in the HG system, leading to extinction of the plasma. The highest Se signal was observed for 0.30% (m/v) Triton X-114. However, due to production of foam and the long time necessary for cleaning the gas-liquid separator when using 0.30% (m/v) Triton X-114, the surfactant concentration was fixed in 0.20% (m/v). The amount of methanol added to the surfactant rich phase was 100 μL , chosen according to previous works.^{16,17}

Regarding to NaBH_4 and HCl solutions used for hydride generation, it was observed that the concentration of the former should be at least 0.50% (m/v) whereas that of HCl should not exceed 0.1 mol L^{-1} . The conditions established for Se(IV) pre-concentration/determination is further summarized.

Figures of merit

The employment of PN for introducing the surfactant rich-phase in the ICP was also investigated and compared. Table 4 shows LODs, EF (enrichment factor), slope and linear correlation coefficient of the calibration curves, obtained using both HG and PN. The LOD values were calculated according to IUPAC (International Union of Pure and Applied Chemistry) recommendation. LOD was

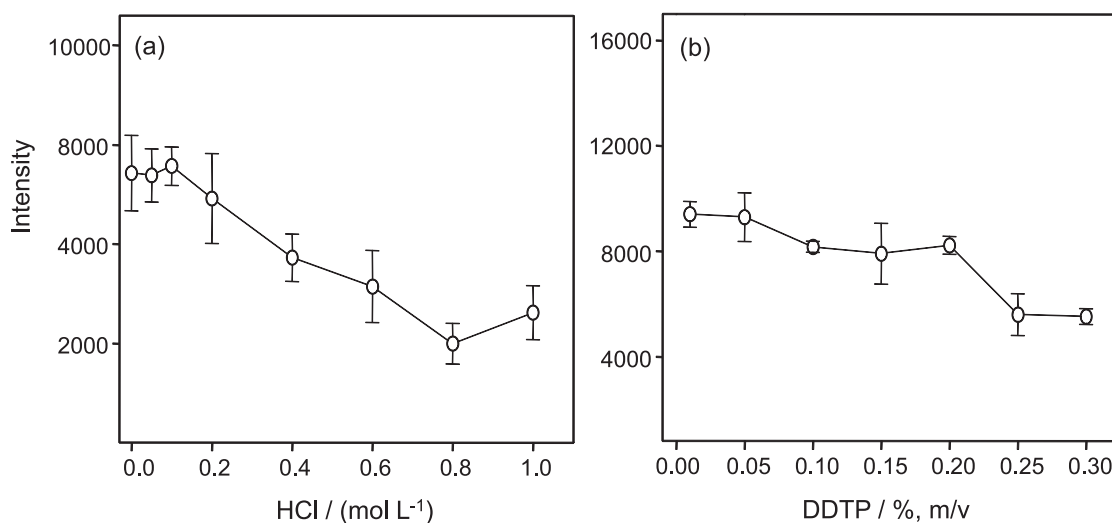


Figure 1. Influence of HCl and DDTP concentrations on Se(IV) pre-concentration by CPE prior to determination by HG and ICP OES; a solution containing 25 $\mu\text{g L}^{-1}$ of Se(IV) was used. Conditions: 0.15% (m/v) DDTP and 0.10% (m/v) Triton X-114 in (a); 0.1 mol L^{-1} HCl and 0.10% (m/v) Triton X-114 in (b); $n = 3$.

Table 4. Comparison of pneumatic nebulization (PN) and hydride generation (HG) performances for Se(IV) determination using ICP OES after preconcentration by CPE

Parameter	PN		HG	
	Without CPE	With CPE	Without CPE	With CPE
LOD / ($\mu\text{g L}^{-1}$)	13.0	1.80	1.70	0.10
LOD ^a / ($\mu\text{g g}^{-1}$)	6.50	0.90	0.85	0.05
Slope of calibration curve	2.40	106	155	1158
EF	–	44	–	8
Linear correlation coefficient of calibration curve	0.9995	0.9985	0.9995	0.9980

^a100 mg of sample in 25 mL further twofold diluted. LOD: limit of detection; EF: enrichment factor.

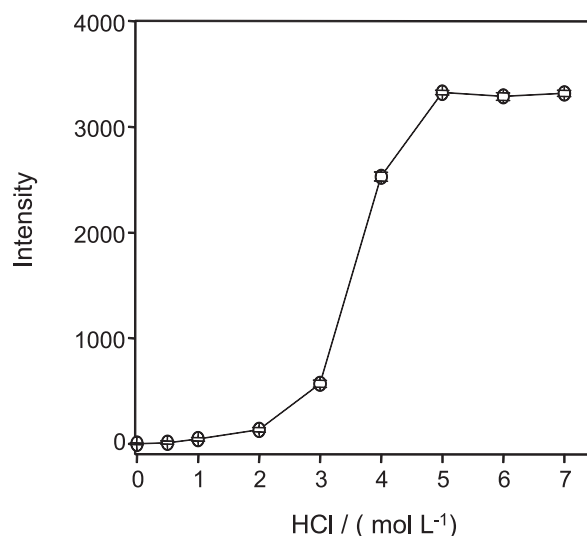
obtained from $b + 3s$; b is the mean concentration of 10 consecutive measurements of the sample blank and s is the standard deviation. The blank solution (of sample analysis or calibration curve) was subjected to the same preconcentration procedure that samples and calibration solutions. Contamination problems were not found for Se, and the same was observed for Sb.

EF was calculated by the ratio of the slopes of calibration curves (with and without analyte preconcentration) for each system used for introducing the surfactant-rich phase into the ICP. As expected and in accordance with the slopes of the calibration curves, the worst sensitivity was observed when PN was used. However, when Se(IV) was preconcentrated using CPE, the sensitivity was 44 times better and the enrichment factor was 40. For both HG and PN, LOD of Se(IV) was almost 10 times better when the species was preconcentrated using CPE.

Pneumatic nebulization/aerosol desolvation was also investigated for Se(IV), but any signal of Se was also observed. The difficulty of measuring Se through the nebulization/aerosol desolvation system employed was observed by other authors.²¹ It is possible that Se is volatilized in the heating step in the desolvation system, or the mechanisms of energy transfer for Se in the ICP are modified when a desolvated aerosol is introduced into the ICP.

Se(IV) complexes with DDTP while Se(VI) does not.²² Aiming total selenium determination as well as Se(IV) and Se(VI), the reduction of Se(VI) to Se(IV) was investigated. The usual procedure consists of heating the Se(VI) solution in 6 mol L⁻¹ HCl for 30 min at 100 °C. In the present study, the influence of HCl concentration was evaluated in order to use less concentrated HCl solution as possible (according to Figure 1a). As it can be seen in Figure 2, for effective reduction of Se(VI), the HCl concentration should be at least 5.0 mol L⁻¹, which is consistent with the literature.²³

Selenium in the non-lipid fraction of Brazil nut sample was initially quantified. The analyte was determined directly in the sample solution (not submitted to CPE) using HG-ICP OES (hydride generation-inductively coupled

**Figure 2.** Effect of HCl concentration in reducing Se(VI) to Se(IV) [25 mg L⁻¹ Se(VI)].

plasma optical emission spectrometry). Selenium recovery in the sample solution submitted or not to the reduction procedure (using 5 mol L⁻¹ HCl and heating at 100 °C) was similar as can be seen in Table 5. Thus, it was concluded that Se(VI) is not present in Brazil nut since the concentration of Se(IV) did not increase when the sample solution was submitted to the reduction procedure. In Table 5, one can observe that the recovery of Se in the certified sample DOLT-4 is quantitative, indicating the accuracy of the HG-ICP OES method.

The extract of the Brazil nut sample was also decomposed in microwave oven (see procedure 2 in the Experimental section). The Se(IV) concentrations found for both decomposition procedures (1 and 2) were similar (see Table 5) at 99% confidence level. Then, procedure 1 was selected and employed for decomposition of all analyzed solid samples.

After reduction of Se(VI) and respective dilution, the final concentration of HCl in the solution submitted to CPE was about 0.6 mol L⁻¹. By adding DDTP to this solution, a white precipitate was produced. It was initially supposed that

Table 5. Selenium concentration found in non-lipid fraction of Brazil nut and certified dogfish liver (DOLT-4); HG-ICP OES (hydride generation-inductively coupled plasma optical emission spectrometry) was employed for Se(IV) determination

Brazil Nut	Spiked/($\mu\text{g g}^{-1}$)	Found/($\mu\text{g g}^{-1}$)	Recovery/(%)
Reduction of Se(VI) ^a	-	48.9 \pm 1.7 ^b	-
		49.2 \pm 1.5 ^c	
	25.0 Se(IV)	72.0 \pm 0.5	92
	25.0 Se(VI)	72.3 \pm 3.2	91
	-	49.6 \pm 0.2	
Without reduction of Se(VI)	25.0 Se(IV)	72.2 \pm 0.2	90
DOLT-4	Certified/($\mu\text{g g}^{-1}$)	Found/($\mu\text{g g}^{-1}$)	Recovery/(%)
Reduction of Se(VI) ^{a,c}	8.3 \pm 1.3	8.7 \pm 1.2	104

^aHeating for 30 min at 100 °C in presence of 5 mol L⁻¹ HCl; ^bdecomposition in digestion block; ^cdecomposition in microwave oven.

the acid medium induced the formation of this precipitate, possible products originated from DDTP degradation. However, any precipitate was not observed in Se(IV) solution directly prepared in 5 mol L⁻¹ HCl not submitted to heating. It was concluded that interfering species are formed during heating for reduction of Se(VI) in presence of HCl. Thus, the reduction of Se(VI) was attempted by using L-cysteine. In this case, PN was employed because L-cysteine produces Se species that are not reactive in the HG system. The reduction of Se(VI) was carried out according to the procedure described by Li *et al.*⁷ (with L-cysteine 0.5% (m/v) and heating at 100 °C for 25 min). Subsequently, the solution was submitted to CPE. However, the emission signal of Se remained at the background level, indicating that Se(VI) was not reduced by L-cysteine, or the L-cysteine may interfere in the complexation of Se(IV) and/or cloud point extraction. Therefore, only Se(IV) can be determined when DDTP is used for CPE.

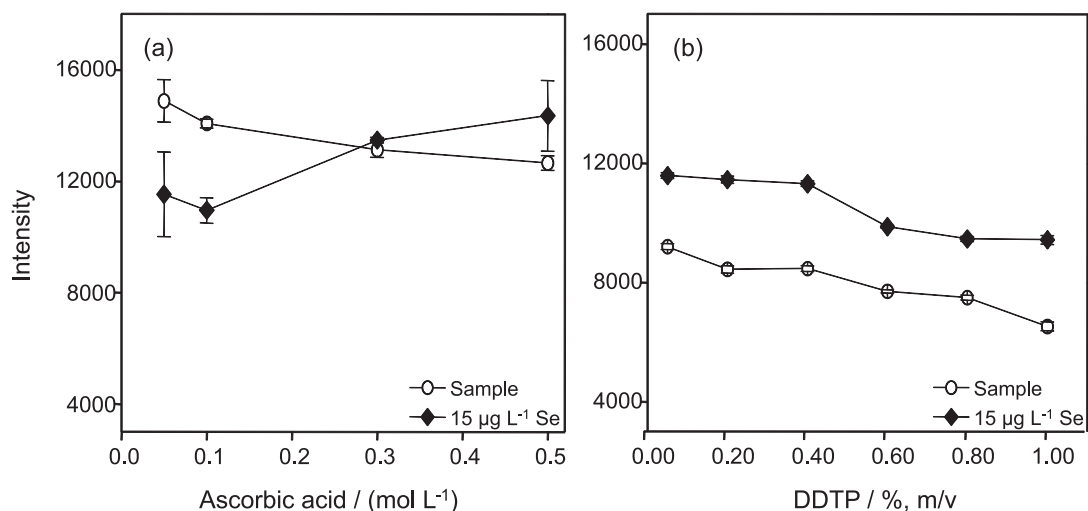
Aliquots of the nut sample solutions were submitted to CPE for preconcentration of Se(IV) present, which was measured using HG-ICP OES. Selenium(IV) was detected in Brazil nut, but the concentration found was 70% lower

than that shown in Table 5, by direct determination (without CPE).

It is known that Fe(III) complexes with DDTP and it is a potential interfering. Possible interference by Fe(III) was investigated by adding ascorbic acid to aliquots of the Brazil nut solution in order to reduce Fe(III) to Fe(II) that does not form a complex with DDTP. However, according to Figure 3a, quite similar profiles are observed for Se(IV) in the solution of the Brazil nut sample and in the Se(IV) solution test. Excess of DDTP could also be a source of interference because DDTP competes with Se-DDTP for the micelle sites. According to Figure 3b, the same behavior is observed for Se(IV) in the solution of the Brazil nut and in the Se(IV) solution tested. According to these results, it is supposed that interference probably occurs at the stage of hydride generation of Se(IV) in the surfactant rich phase of the Brazil nut extract.

Selenium determination in samples

Taking into account the low recovery of Se(IV) in Brazil nut, Se(IV) in the nut samples was quantified using PN

**Figure 3.** Influence of ascorbic acid and DDTP on preconcentration of Se(IV) in Brazil nut and in solution containing Se(IV) and 50 mg L⁻¹ of Fe (III); n = 3.

for introducing the surfactant rich phase in the ICP. The concentration of Se(IV) found in the samples is shown in Table 6. The Se(IV) concentration in the nuts is expressed for the non-lipid fraction in which Se is present in the nuts, corresponding to 60% of weight of raw nuts.¹¹ Hence, the concentration of Se(IV) in the raw Brazil nut is about $30 \mu\text{g g}^{-1}$. Considering that a daily intake of 50-70 μg of Se is recommended,¹ the quantity of Se in the human body is supplied just by eating 2 g Brazil nut *per* day. The proteins found in Brazil nut are very high in sulfur-containing amino acids like cysteine and methionine and are also extremely rich in glutamine, glutamic acid and arginine.²⁴ The reason that Brazil nut concentrate Se is probably that this element is very similar chemically to S. Sulfur is frequently deficient in Amazonian soils. Thereby, Se may be used by the plant instead of S.

Selenium was not detected in coconut water. Other authors²⁵ detected Se in this beverage, differently of the results obtained in the present study. The reason might be the type of soil where the coconut plant is grown. The analyzed coconut water sample is from region probably poor in Se.

Antimony

The influence of the preconcentration medium was evaluated using HCl at different concentrations, the analyte was measured using HG-ICP OES. The highest Sb(III) signal was observed for 0.80 mol L⁻¹ HCl, as shown in Figure 4a. It is already known²² that DDTP forms stable complexes with Sb(III). The influence of the DDTP concentration was also evaluated and the results are

Table 6. Concentration of Se(IV) found in the analyzed samples. Results are the mean and standard deviation of three determinations (analysis in triplicate). Pneumatic nebulization (PN) was used to introduce the surfactant-rich phase into the ICP. Se(IV) concentration in the nuts is expressed in the non-lipid fraction

Sample	Spiked / ($\mu\text{g g}^{-1}$)	Found / ($\mu\text{g g}^{-1}$)	Recovery / %
Brazil nut	–	50.8 ± 1.8	
	34.9	86.9 ± 0.2	103
Sweet almond	–	< 0.90	
	6.99	6.39 ± 1.17	91
Walnut	–	< 0.90	
	6.99	6.11 ± 0.42	87
Hazelnut	–	< 0.90	
	6.98	6.30 ± 0.02	90
Sample	Spiked / ($\mu\text{g g}^{-1}$)	Found / ($\mu\text{g g}^{-1}$)	Recovery / %
Coconut water	–	< 1.80	
	10	8.52 ± 0.84	85
	5	5.12 ± 0.34^a	102
Estuarine water	–	< 1.80	
	10	10.7 ± 0.6^a	107
	10	8.52 ± 0.84	85

^aQuantified using CPE and HG-ICP OES.

shown in Figure 4b. It can be observed that the signal of Sb increases with the DDTP concentration increasing up to 0.40% (m/v), decreasing thereafter. Since the increasing of the Sb signal was low for 0.20 to 0.40% (m/v) DDTP, the concentration of the complexant was fixed in 0.2% (m/v) for further Sb(III) determinations. Souza *et al.*,²⁶ using multivariate optimization, found quite similar results. These authors observed that 0.15% (m/v) DDTP was optimal for preconcentration of Sb(III) in blood serum by means of CPE prior to Sb detection using ETAAS.

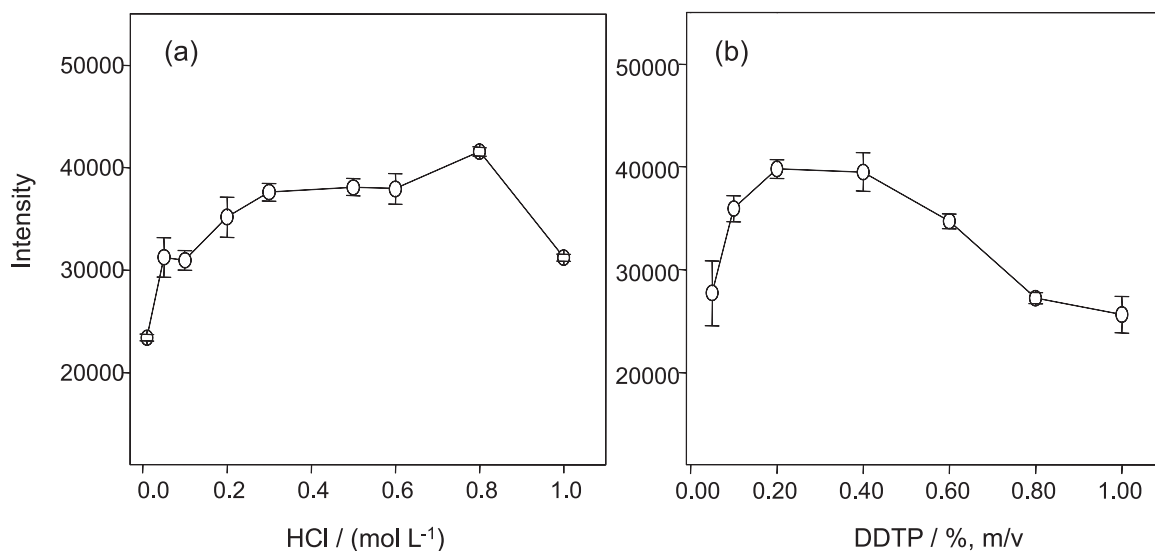


Figure 4. Influence of HCl and DDTP on preconcentration of Sb(III) using CPE; HG-ICP OES was used for Sb(III) determination; test solution: $10.0 \mu\text{g L}^{-1}$ Sb(III); conditions: 0.50% (m/v) DDTP in (a) and in 0.8 mol L⁻¹ HCl in (b); n = 3.

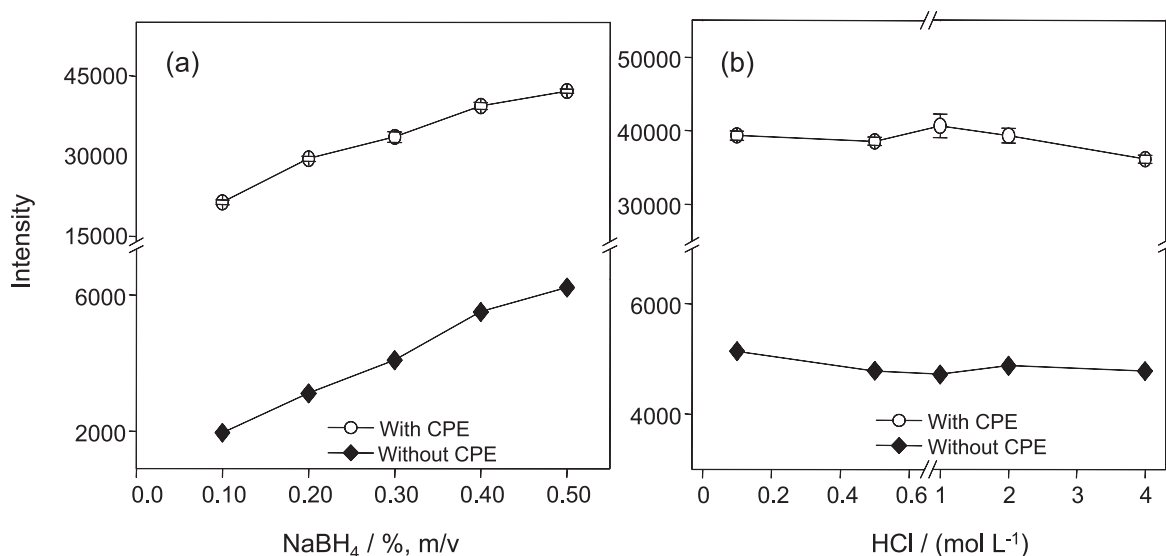


Figure 5. Influence of NaBH₄ and HCl concentrations on Sb(III) hydride generation. A 10.0 µg L⁻¹ Sb(III) solution was used. Conditions: 0.80 mol L⁻¹ HCl in (a) and 0.20% (m/v) DDTP in (b); n = 3.

Regarding to Triton X-114 concentration, the highest Sb signal was observed when the surfactant concentration was 0.10% (m/v). The volume of methanol added to the surfactant-rich phase was 100 µL, following what was observed for Se.

With respect to NaBH₄ and HCl solutions used in the HG system, it was observed that the Sb signal increased with the NaBH₄ concentration increase (Figure 5a), but remained almost constant with HCl concentration increasing up to 2 mol L⁻¹ (Figure 5b). However, elevated effervescence was observed in the gas-liquid separator by using 0.50% (m/v) NaBH₄, which could lead to inaccurate results. Thus, the NaBH₄ concentration was reduced to 0.40% (m/v), without great loss of sensitivity. The HCl concentration selected was 0.8 mol L⁻¹. The conditions established for Sb(III) preconcentration/determination as well as those for Se(IV) are summarized in Table 7.

Table 7. Condition and reagent concentration established for Sb and Se preconcentration using CPE

Condition/Reagent	Element	
	Sb	Se
Preconcentration medium, HCl / (mol L ⁻¹)	0.8	0.1
DDTP / %, m/v	0.20	0.05
Triton X-114 / %, m/v	0.10	0.20
Temperature / °C	50	50
Heating time / min	20	20
HCl for HG / (mol L ⁻¹)	1	0.1
NaBH ₄ / %, m/v	0.40	0.50

Figures of merit

LOD of Sb(III), EF and parameters of calibration curves obtained by using CPE for preconcentration followed by determination using HG-ICP OES or PN-ICP OES (pneumatic nebulization-inductively coupled plasma optical emission spectrometry) are summarized in Table 8. LOD and EF were calculated in the same way that selenium, as previously described.

The LOD obtained for Sb(III) using HG-ICP OES is similar to that reported by Li *et al.*¹⁴ By using APDC and Triton X-114 for preconcentration of Sb prior to its determination using ETV-ICP OES, these authors found a LOD of 0.09 µg L⁻¹ for Sb(III).

In the present work, it was observed that aerosol desolvation enhanced sensitivity for Sb, but LOD was worst (5.50 µg L⁻¹) when compared with that obtained using conventional nebulization (2.90 µg L⁻¹). The main reason for the higher LOD is that precision was worsened by using pneumatic nebulization/aerosol desolvation for introducing the viscous surfactant-rich phase into the ICP.

Antimony determination in samples

After evaluating the appropriate conditions for Sb(III) preconcentration using CPE, this specie was determined in different samples, as shown in Table 9. Antimony(III) was not detected in tap water and white wine, but was in estuarine water, seawater and mineral water. The samples were spiked with Sb(III) and recoveries varied from 87 to 106%, demonstrating that relatively low concentrations of Sb(III) can be determined using CPE and HG-ICP OES.

Table 8. Comparison of pneumatic nebulization (PN) and hydride generation (HG) performances for Sb(III) determination using ICP OES after preconcentration by CPE

Sample introduction system	Slope of calibration curve	EF	LOD / ($\mu\text{g L}^{-1}$)	Linear correlation coefficient of calibration curve
HG (with CPE)	1817	5	0.080	0.9994
HG (without CPE)	363	–	1.1	0.9990
PN	27.2	7	2.90	0.9970
PN (without CPE)	3.02	–	12	0.9980
PN/DES/CPE	54.7	32	5.50	0.9890

LOD: limit of detection; EF: enrichment factor; PN/DES/CPE: pneumatic nebulization/aerosol desolvation/cloud point extraction.

The certified river water (SLRS-5) was submitted to CPE and the recovery of total Sb was 90%. However, the concentration of Sb is not certified, but only informed. It is important to mention the lack of reference materials in which the concentrations of Sb species are certified. The same is true for Se. Only for As, Sn and Hg are found reference materials where some species of these elements are certified.

Table 9. Samples analysis using CPE and HG-ICP OES for Sb(III) quantification. Results are the mean and standard deviation ($n = 3$)

Sample	Spiked / ($\mu\text{g L}^{-1}$)	Found / ($\mu\text{g L}^{-1}$)	Recovery / %
Estuarine water	–	2.90 ± 0.54	
	2.5	5.54 ± 0.21	106
Seawater	–	0.79 ± 0.07	
	2.5	2.96 ± 0.02	87
Mineral water	–	0.39 ± 0.09	
	2.5	2.66 ± 0.19	91
Tap water	–	< 0.08	
	2.5	2.59 ± 0.48	104
White wine	–	< 0.4 ^a	
	2.5	2.62 ± 0.32	105
River water (SLRS-5)	0.3 ^b	0.27 ± 0.05	90

^aLOD (limit of detection) calculated for the sample 10-fold diluted;

^binformed value (sample not spiked with Sb(III)).

Antimony (III) is very toxic. However, Sb(III) was detected in some samples. Antimony has been investigated in European mineral waters stored in PET bottles. In the present work, the concentration of Sb(III) found in bottled mineral water compares well with the average Sb concentration found in mineral water from Europe.²⁷

Hydride generation efficiency of an element depends on whether the element is present as an inorganic or organic form and on its oxidation state. As only the Sb(III) species of the element are efficiently reduced to SbH_3 , a

pre-reducing agent is often used to convert Sb(V) to Sb(III) prior to hydride generation. Besides, the complexant (DDTP) used for CPE in the present study does not complex with Sb(V). Thus, when attempting to determine Sb(V) using DDTP and/or hydride generation a pre-reduction step is needed. Potassium iodide and ascorbic acid or L-cysteine were used for Sb(V) reduction.²⁸

A study was conducted following the conditions described in the literature²¹ for reducing Sb(V). For this, potassium iodide 1% (m/v) and ascorbic acid 0.2% (m/v) were left in contact with the sample solution for 30 min. Conversion of Sb(V) to Sb(III) was considered satisfactory (reduction of about 80%). Aliquots of the samples were subsequently submitted to CPE, but the concentrations of Sb(III) found were similar to those shown in Table 8. Consequently, the concentrations of the Sb(V) in the samples were lower than the LOD of the method ($0.08 \mu\text{g L}^{-1}$ for water samples and $0.4 \mu\text{g L}^{-1}$ for wine).

Conclusions

It was here demonstrated that CPE in conjunction with HG improved LODs of Sb and Se, allowing the determination of low concentrations of Se(IV) and Sb(III) by ICP OES with axial view of the plasma. However, CPE of Se(IV) in solution of HCl previously heated in order to reduce Se(IV) was not possible. As a consequence, only Se(IV) could be quantified since DDTP complexes only with Se(IV). In this case, speciation of Se(IV) at ng L^{-1} level was possible. Selenium(IV) could not be quantified in Brazil nut using CPE and HG due to possible interferences in the hydride generation stage. However, Se(IV) in this sample could be quantified by using CPE and PN. Only Se(IV) was detected in Brazil nut sample submitted to treatment in which Se(VI) was not detected.

Selenium could not be measured by using nebulization/aerosol desolvation, contrarily of Sb. However, LOD of Sb was higher than that found using PN. LOD found for Sb, using HG and CPE followed by detection by ICP OES,

revealed that the technique is appropriate for Sb monitoring in environmental samples. Antimony(III), the most toxic inorganic Sb species, was detected in estuarine water, seawater and in mineral water stored in PET bottle.

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