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Simultaneous Determination of Pb and Cd in Seafood by ICP OES with On-Line Pre-Concentration by Solid Phase Extraction with Amberlite XAD-4 after Complex Formation with DDTP

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ABSTRACT

An on-line method for Cd and Pb pre-concentration and simultaneous determination in acid digested seafood by inductively coupled plasma optical emission spectrometry (ICP OES) was developed. The on-line pre-concentration was based on the complex formation of the analytes with the ammonium salt of O,O-diethyldithiophosphate (DDTP) and using the Amberlite XAD-4 resin as a solid support in a homemade column. Different conditions of the flow injection system, such as solutions flow rates, nebulizer pressure and eluent concentration were optimized. Three certified reference materials of lobster hepatopancreas, dogfish liver and fish protein, three samples of fish muscle and three samples of shrimp were digested with HNO_3, H_2O_2 and H_2SO_4 in a microwave system under reflux. DDTP was added in the solutions obtained, and the mixture was injected in the FI system. Calibration curves for Cd and Pb were obtained using the standard solutions in the concentration range 0.05-0.5 $\mu g\ mL^{-1}$ in the digestion medium, submitted to the same pre-concentration procedure. The quantification limits (3.3 x LOD) for 5 min of pre-concentration time were 0.005 mg kg^{-1} Cd^{2+} and 0.1 mg kg^{-1} Pb^{2+} in the sample of fish or shrimp in natura, considering 1.0 g of the sample in a final volume of 50 mL. The agreement of the obtained concentrations with the certified ones (Student t-test, 95% confidence) and the recoveries of spiked real samples, from 90 to 120%, demonstrated good accuracy. Precision was also adequate, with relative standard deviations from 2 to 13%. The method was accurate, precise and certainly could be applied to the digested samples of different natures.

Key words: Cd, Pb, ICP OES, diethyldithiophosphate, XAD-4, seafood, solid phase extraction

INTRODUCTION

The marine resources for human consumption have increased rapidly worldwide, as seafood generally is an important source of proteins, minerals and vitamins (Storelli 2008; Heu et al. 2003). Nevertheless, it is known that the toxic metals can be naturally present in the food or can enter the food chain as a result of human activities, such as industrial and agricultural process. Among the metals of particular concern in relation to harmful effects on the health, cadmium and lead are frequently studied. The toxicity of these metals is in part due to the fact that they accumulate in the biological tissues, a process known as bioaccumulation, which occurs in all the organisms as a result of exposure to metals in the food and in the environment, including fish and

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shrimp (Ersoy and Çelik 2010; Joyeux et al. 2004; Morgano et al. 2010). According to Brazilian Residues and Contaminants Control Plan in Fish, PNCRC/2010 (Instrução Normativa Nº 8, MAPA 2010), the concentrations limits in the fish muscle are 0.1 mg kg^{-1} for Cd and 0.2 mg kg^{-1} for Pb *in natura*. The determination of Cd and/or Pb in the fish and shrimp has been successfully carried out by inductively coupled plasma emission spectrometry (ICP OES) (Heu et al. 2003; Ersoy and Çelik 2010; Morgano et al. 2010; Yilmaz and Yilmaz 2007; Pourang and Dennis 2005), ICP-mass spectrometry (ICP-MS) (Pourang and Dennis 2005; Falcó et al. 2006) and graphite furnace atomic absorption spectrometry (GF AAS) (Detcheva and Grobecker 2006, Gaspic et al. 2002; Dural et al. 2007; Tuzen 2003). However, the methods by ICP OES usually are not sensitive enough for checking the mentioned concentration limits, requiring pre-concentration procedures. The ICP OES is available in many laboratories due its characteristics, such as robustness, multielemental capacity and cost. The combination of the solid phase extraction (SPE) and flow injection (FI) in an on-line system is one of the most adequate methods due to the low consumption of samples and reagents, high analytical throughput, minimal waste production and appropriate sensitivity (Escudero et al. 2010; Kondo et al. 2002). The proper choice of an adsorbent material for SPE can provide simple operation and more flexible working conditions. The XAD-4 resin frequently has been proposed for the pre-concentration of several elements (Dos Santos et al. 2005; Uzun et al. 2001; Dev et al. 1999; Ramesh et al. 2002). Dos Santos et al. (2005) have used the ammonium salt of the *O,O*-diethyldithiophosphate (DDTP) for the determination on line of Cd in biological samples by FAAS. The DDTP forms, in acid medium, stable complexes with several transition metals and semi-metals, including Cd and Pb (Da Silva et al. 2001). In contrast to other complexing agents, DDTP requires an acid medium. This condition is very convenient, as sample digestion procedures usually leads to an acid sample solution. In addition, a pH buffer, which is an important contaminant source, is not required for complex formation between the analyte and this ligand.

The goal of this work was to develop a method for the determination of Pb and Cd in seafood by ICP OES with on-line pre-concentration by complexing the analyte with *O,O*-diethyldithiophosphate and extracting the complex with Amberlite XAD-4. The eluent, ethanol, would be introduced in the plasma using an auxiliary oxygen flow, following the instrumental conditions optimized in a previous work that analyzed biodiesel samples dissolved in ethanol (Dos Santos et al. 2007). To the best of our knowledge this procedure was not published before. The method was applied to the certified reference materials (CRM) and to the fish and shrimp samples bought from the local market in Paranaguá, PR, Brazil. The proposed method should attend the concentration limits adopted by the Brazilian agency (PNCRC/2010) (Instrução Normativa Nº 8, MAPA 2010).

MATERIAL AND METHODS

Instrumentation

The measurements were conducted using a simultaneous axial view ICP OES spectrometer, model VISTA PRO (Varian, Mulgrave, Australia). Peak height emission intensities were measured at 226.502 nm for Cd and 283.305 nm for Pb. Optimized experimental conditions are summarized in Table 1. The O_2 flow rate was previously optimized (Dos Santos et al. 2007). Argon of 99.996% purity was supplied by White Martins (São Paulo, Brazil). In addition, the following instruments and materials were used: Minipuls 2 peristaltic pump (Gilson, Middleton, WI, USA); the peristaltic pump tubing for the elution was from Glass Expansion (USA) Nº 1.30-GRY-SF, Solva Flex gray/gray 1.30 mm I.D. and for sample preconcentration was from Elkay Elreann (Ireland) Nº 116-0549-090, PVC black-white 0.60 mm I.D. A Teflon tubing of 9.0 cm length and 0.25 cm i.d. was used a minicolumm and an injector-commutator from UNICAMP (Campinas, SP, Brazil). A focused microwave system, model Star System 2 from CEM Corporation (Matthews, North Caroline, USA) was used for sample dissolution.

FI system

The FI system is shown in Figure 1. With the injector in the position as shown in this figure, the sample solution passed through the column during a certain selected time. While Cd, Pb and some other complexed metals were retained in the column, other concomitants passed through the column and were discharged. By changing the

injector-commutator to the second position, the column (C) was placed in the eluent line. Cadmium and Pb were then eluted from the column and the eluent was driven to the measuring instrument.

Table 1 – Instrumental parameters of the ICP OES.

Radiofrequency	40 MHz
Forward power	1.5 kW
Plasma gas flow rate	15.0 L min^{-1}
Auxiliary gas flow rate	2.25 L min^{-1}
O_2 Flow rate added to the auxiliary gas	150 mL min^{-1}
Nebulizer pressure	150 kPa
Nebulizer type	Concentric glass K
Spray chamber	Sturman Master
Replicate read time	20 s
Replicate	1
Torch type	Demountable torch with a 0.8 mm I.D. quartz injector tube
Analytical lines	226.502 nm for Cd and 283.305 nm for Pb

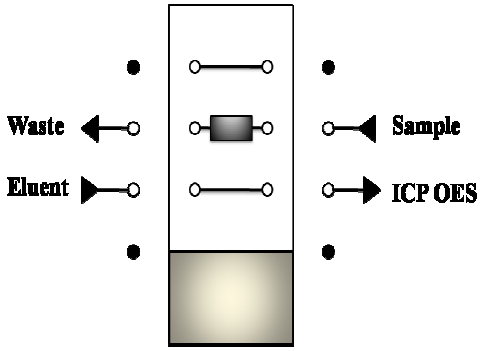

Figure 1 - FI system: the injector-commutator is shown in the sampling position, with the minicolumn (C) placed in the Sample-Waste line. By lowering the commutator, the system is in the elution position, with the column in the Eluent- ICP OES line. Pb: real intensity x 10.

Reagents and samples

All the chemicals were of analytical grade. High purity water (resistivity of 18.2 MΩ cm) was de-ionized (DIW) in a Milli-Q system (Bedford, MA, USA). The following reagents were used: Suprapure 65% v/v HNO_3 from Merck (No. 1.00441.1000, Darmstadt, Germany), 30% v/v H_2O_2 from Merck (No. 1.07210.1000), 98% v/v H_2SO_4 from J. T. Backer (No. 9681-2, Ecatepec, Mexico), 37% v/v HCl from Merck (No. 1.00317.1000), ethanol absolute GR (≥ 99.9%) from Merck (No. 1.00983). Monoelemental standard solutions containing 1000 μg mL^{-1} of Cd from AccuStandard (No. ICP- 08N-1, New Haven, USA) and of Pb from AccuStandard (No. ICP-29N-1) were used. Amberlite XAD-4 resin (polystyrene type, 20-60 mesh, Aldrich Chemical Company, Milwaukee, USA) was used as solid phase and DDTP (ammonium *O,O*–diethyldithiophosphate), purity of 95% (Sigma-Aldrich, Milwaukee, USA) was used as the complexing agent.

The following certified reference materials were analyzed: TORT-2 (Lobster Hepatopancreas), DOLT-4 (Dogfish liver) and DORM-3 (Fish protein) from the National Research Council of Canada (NRCC, Ottawa, Ontario, Canada). Real samples of fish muscles and of shrimp meat were bought at the local market in Paranaguá, PR, Brazil and analyzed. The fish muscles were from three different species: Dogfish: *Carcharrhinus* spp. (Fish 1), Codlike fish: *Cynoscion* sp. (Fish 2) and Robalo: *Centropomus* spp. (Fish 3) and the gray shrimp was *Litopenaeus* vannamei. The fish muscles, about 100 g of each species, was already clean without the scams in the market and the shrimp meat was separated from the head and shell of three individuals that were analyzed separately

(Shrimp 1 to 3). The samples were ground in a food blender (equipped with stainless steel blade) to ensure the homogeneity, packed and stored at -20ºC until analyses (Heu et al. 2003; Ersoy and Çelik 2010). Separate aliquots of the fish muscles and of the shrimp meats were dried at 100ºC to constant weight for the determination of the sample humidity. The humidity values were 80.9, 78.4 and 80.6% for Fish 1 to 3, and 82.8, 87.2 and 81.4% for Shrimp 1 to 3, respectively.

Analytical procedures

The samples were weighed, ≈ 1.0 g, in borosilicate flasks (from CEM Corporation) and 5 mL of HNO_3, 6 mL of H_2O_2 and 1 mL of H_2SO_4 were added. After standing for 8 h, the samples were digested in the microwave system under reflux following the heating program: 10 min at 110ºC followed by 5 min at 130ºC, then 2 mL of H_2O_2 were added to the mixture that was heated for 5 min more at 130ºC followed by 10 min at 200ºC and cooling for 20 min (Nóbrega et al. 2002). The obtained clear solutions were transferred to a 50 mL volumetric flask, to which 0.2% m/v DDTP was added and the volume was made up with water. Calibration curves for Cd and Pb were obtained using the standard solutions in the concentration range 0.05-0.5 μg mL^{-1} in the same medium as the samples. A reagent blank solution was run parallel to each determination and its value was taken into consideration. The XAD-4 resin was treated with 33% HCl (v/v) (4 mol L^{-1}) for 24 h, washed with DIW until pH 7.0 and dried at 40ºC for 24 h in a vacuum stove (Ramesh et al. 2002). The minicolumn was filled with ≈0.1650 g of XAD-4 and closed in the extremities with glass wool.

RESULTS AND DISCUSSION

The used DDTP concentration of 0.2% (w/v) was based on a previous work (Dos Santos et al. 2005). All optimizations described below were carried out for a test standard solution containing 50 μg L^{-1} of Cd^{2+} and Pb^{2+} in 10% (v/v) of HNO_3, 2% (v/v) of H_2SO_4 and 0.2% (w/v) DDTP, submitted to a pre-concentration time of 3 min, using absolute ethanol as eluent, unless otherwise specified.

Effect of acidity on the complex formation

Another advantage of using DDTP as the complexing agent is that strict control of acidity is not required. In this way, the use of a pH buffer, which is a contaminant source, is avoided. Pozebon et al. (1998) studied the acid effect in an on-line system, using variable concentrations of HNO_3. They found that the acidity was not critical in the studied acid concentration range for the Cd complex formation. The resulting acidity from the sample digestion used in this work (10% v/v of HNO_3 and 2% v/v of H_2SO_4 - pH<1) was adequate for an efficient complex formation for both the analytes and it was not further investigated.

Effect of the flow rate

Figure 2 illustrated the effect of the sample solution flow rate which was the same of the eluent flow rate in the range 0.43-0.75 mL min^{-1} on the signal intensity from the test solution. The optimal sample flow rate for the sample solution and for the eluent was around 0.60 mL min^{-1} for both the analytes that was adopted in further experiments. This parameter is very important, since it controls the time of the analysis and the pre-concentration time, and, consequently, the amount of the analyte retained in the column. The explanation for this behavior is complex, as several steps carried out in sequence (analyte retention in the column, release of the analyte to the eluent, pneumatic nebulization of the eluent, residence time of the analyte in the plasma) are affected by the flow rate. However, the signal intensity decrease could be due to a lower residence time of the analyte in the plasma (Escudero et al. 2010; Dos Santos et al. 2005).

Effect of the nebulizer pressure

The FI system was connected to the ICP OES through a capillary tubing that went from the exit of the column to the nebulizer. The effect of the nebulization gas pressure on the analyte response for the test solution is shown in Figure 3. For both the analytes, the intensity signal increased up to the optimum pressure of 150 kPa, which was adopted in this work. For higher pressure, the signal intensity tended to decrease. The argon pressure on the nebulizer must affect the nebulization efficiency and consequently the amount of sample that reached the plasma (Nolte 2003).

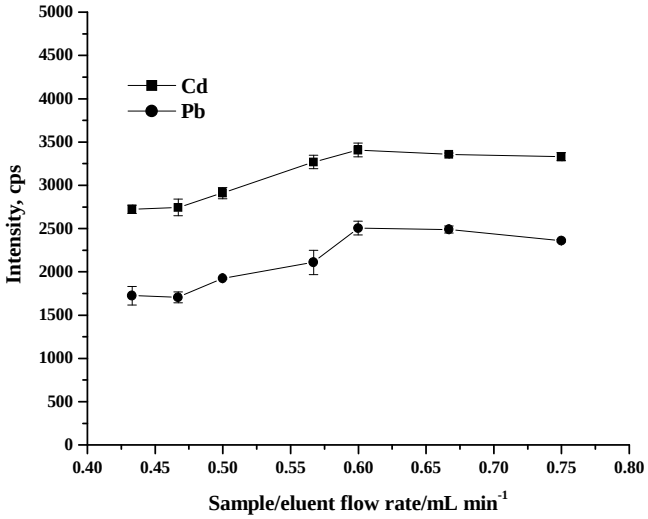

Figure 2 - Effect of sample/eluent flow rate on the response to a 50 μg L^{-1} solution of Cd^{2+} and Pb^{2+} in 10% v/v of HNO_3 containing 0.2% m/v DDTP, preconcentration time of 3 min, ethanol 100 % as eluent and 150 kPa of argon nebulizer pressure. Error bars indicate the standard deviation of three replicate measurements. Pb: real intensity x 10.

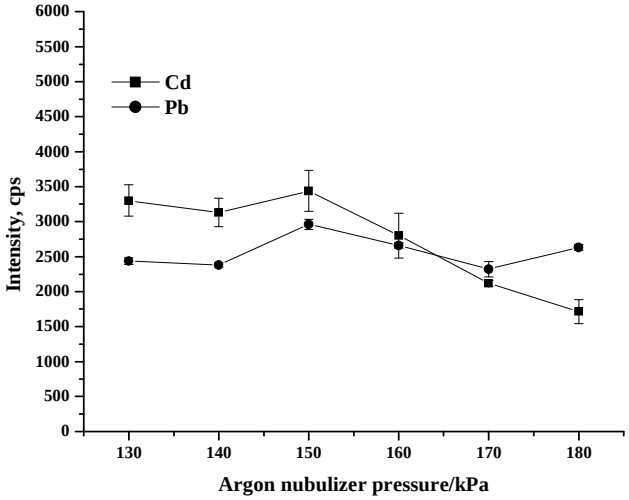

Figure 3 - Effect of nebulization pressure on response to a 50 μg L^{-1} solution of Cd^{2+} and Pb^{2+} in 10% v/v of HNO_3 containing 0.2% m/v DDTP, preconcentration time of 3 min, ethanol 100 % as eluent and 0.60 sample/eluent flow rate. Error bars indicate the standard deviation of three replicate measurements. Pb: real intensity x 10.

Effect of the eluent concentration

Initially, 4 mol L^{-1} HCl, used in a previous work (Dos Santos et al. 2005), was tested as eluent however, the sensitivity for Pb was low, indicating low efficiency in the elution of the complex of Pb with DDTP that remained mostly in the column. Previous works (Da Silva et al. 2001; Pozebon et al. 1998; Dressler et al. 1998; Sella et al. 1999; Giacomelli et al. 2000; Quináia et al. 2001) that used DDTP as the complexing agent successfully employed ethanol as eluent. In order to keep the plasma stable, oxygen gas was introduced together

with argon as nebulizer gas, as optimized previously (Dos Santos et al. 2007). The test solution was analyzed under the optimized conditions (0.60 sample/eluent flow rate and a nebulization pressure of 150 kPa) were used. As shown in Figure 4, the signal increased significantly with ethanol concentration up to 100% (v/v) that was selected in further work.

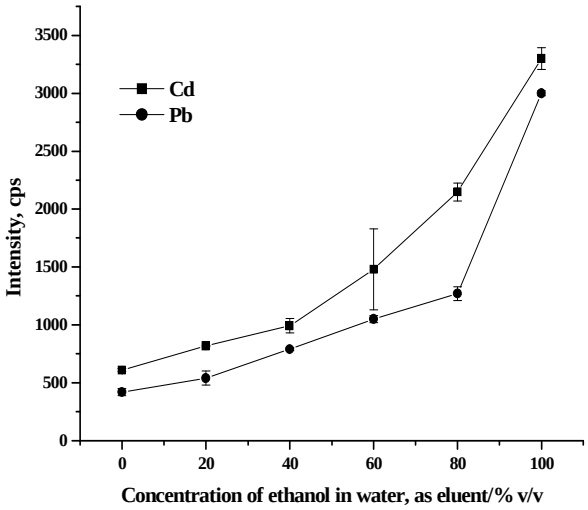

Figure 4 - Effect of the eluent concentration on response to a 50 µg L^{-1} solution of Cd^{2+} and Pb^{2+} in 10% v/v of HNO_3 containing 0.2% m/v DDTP, preconcentration time of 3 min, 0.60 sample/eluent flow rate and nebulizer pressure of 150 kPa. Pb: real intensity x 10.

Figures of merit

Table 2 shows for different pre-concentration times, linear correlation coefficients of the calibration curves, the curve slopes, limits of detection (LOD), defined as three times the standard deviation of 10 measurements of the blank divided by the slope of the calibration curves (Miller and Miller 2000), and the enhancement factor (EF), calculated as the ratio of the slopes of the calibration curves with pre-concentration and without pre-concentration. As shown in the Table 2, the linear correlation coefficients values were higher than 0.991 for both the analytes demonstrating good and adequate linearity. The LOD values, in the measuring solution, varied from 0.02 to 0.1 µg L^{-1} for Cd and 0.6 to 2.0 µg L^{-1} for Pb, depending on the selected pre-concentration time. Enhancement factor up to 13 for Cd and 6 for Pb for 8 min of pre-concentration time were obtained. LOD and higher EF could be reached if higher pre-concentration times would be used, since the longer time of pre-concentration, higher would be the amount of analytes that would be retained in the resin (Pozebon et al. 1998).

Analytical application

The procedure was applied to the analysis of three seafood certified references and to six real samples, with 5 min of pre-concentration time. Two of the real samples (fish sample 3 and shrimp sample 3) were spiked with 0.10 mg kg^{-1} of Cd^{2+} and Pb^{2+}. All the results are shown in Table 3. The agreement of the obtained results with the certified values for the certified reference materials (Student t-test, 95% confidence) and the recoveries from 90 to 120% for the fish sample 3 and for shrimp sample 3, demonstrated the good accuracy. Precision of the results, as evaluated by the RSD was adequate from 2 to 13%. None of the three real samples of the fish had Cd or Pb concentrations above the quantification limits (defined as 3.3 times the LOD) considering 1.0 g of the sample *in natura* in a final volume of 50 mL (Miller and Miller 2000). The LOQ could be expressed in a dry weigh basis using the sample humidity given in the experimental section. Cadmium could not be quantified in the three real shrimp samples. However, the Pb concentration in the shrimp samples were above the limits of the Brazilian Norm (Instrução Normativa Nº 8, MAPA 2010), requiring more studies to reconfirm the

finding with a higher population of shrimps and to search for the source of contamination. The proposed procedure is adequate to verify if the seafood followed this norm, as the quantification limits of the method were lower than the concentration limits of the norm.

Table 2 - Figures of merit for the determination of Cd and Pb by the proposed method. LOD in the measuring solution.

	Correlation coefficient		**Slope (cps/µg L^{-1})**		**limit of detection (µg L^{-1})**		**EF**	
Time (min)	Cd	Pb	Cd	Pb	Cd	Pb	Cd	Pb
3	0.999	0.998	64130	2301	0.1	2.0	5	2
5	0.992	0.995	144073	4502	0.03	0.8	10	4
8	0.991	0.999	189755	7503	0.02	0.6	13	6

***EF**: enhancement factor*

Table 3 - Analytical results for certified reference materials and for real samples of fish and shrimp *in natura*, n=3.

	Cd		***Pb***	
Sample	**Certified/Spiked mg kg^{-1}**	**Found mg kg^{-1}**	**Certified/Spiked mg kg^{-1}**	**Found mg kg^{-1}**
DORM-3	0.290 ± 0.020	0.305 ± 0.010	0.395 ± 0.050	0.380 ± 0.040
TORT-2	26.7 ± 0.6	25.5 ± 0.5	0.35 ± 0.13	0.29 ± 0.03
DOLT-4	24.3 ± 0.8	23.3 ± 0.6	0.16 ± 0.050	0.14 ± 0.01
Fish 01	--------	<0.005	--------	<0.1
Fish 02	--------	<0.005	--------	<0.1
Fish 03	--------	<0.005	--------	<0.1
Fish 03	0.10	0.094 ± 0.002	0.10	0.09 ± 0.01
Shrimp 01	--------	<0.005	--------	0.40 ± 0.05
Shrimp 02	--------	<0.005	--------	0.53 ± 0.02
Shrimp 03	--------	<0.005	--------	0.32 ± 0.03
Shrimp 03	0.10	0.093 ± 0.006	0.10	0.44 ± 0.02
Recovery range, %	--------	*93-105*	--------	*83-120*
RSD range, %	--------	*2-6*	--------	*3-13*

Some concentrations are below the LOQ.

CONCLUSIONS

The proposed method was simple, of low cost and sample consumption and was less prone to contamination or analyte loss in comparison to the batch procedures. The procedure was accurate and was able to attend the Brazilian legislation concerning the concentration limits of the two studied metals. Most likely, the same pre-concentration procedure could be adapted for other measuring techniques of different sensitivities, such as GF AAS and ICP-MS, either to increase the sensibility or to avoid the interferences.

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