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A preconcentration procedure for the determination of cadmium in biological material after on-line cloud point extraction

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Abstract In this paper, a method involving on-line preconcentration with cloud point extraction for the determination of cadmium in biological samples is presented. The procedure is based on the sorption of micelles containing Cd(II) ions and the reagent 4-(5′-bromo-2′-thiazolylazo)orcinol (Br-TAO) in a mini-column packed with polyester. The surfactant Triton X-114 was used in the formation of micelles. After sorption, the Cd(II) ions were desorbed from the minicolumn with acid eluent and determined by flame atomic absorption spectrometry. Parameters influencing the cloud point extraction were studied. The method showed a detection limit of 0.5 μg l^{-1} and an enhancement factor of 27. The accuracy was tested by determination of cadmium in certified reference materials (spinach leaves 1570a and tomato leaves 1573a) from the National Institute of Standards and Technology.

Keywords Preconcentration · Cadmium · On-line system · FAAS · Cloud point extraction

Introduction

Cadmium is a very toxic element to humans, plants and animals. Despite its toxicity, cadmium is widely used in various applications such as pigments, batteries and semiconductors. Zinc ores contain small amounts of cadmium as impurities; as a result, mining activity can release cadmium into the environment. Cadmium can be very toxic even in small quantities, hence the need for its determination in various matrices (Abbasi et al. 2011; Rehman and Anjum 2011; Lemos and de Carvalho 2010). However, many detection techniques suffer from low sensitivity or selectivity, making the accurate determination of cadmium at very low levels difficult. In this case, a separation or preconcentration step should be performed prior to detection. A great diversity of procedures for the separation and preconcentration of metals have been developed, involving several techniques such as solid phase extraction (SPE) (Topuz and Macit 2011; Islam et al. 2011; Soylak et al. 1997, 2003; Duran et al. 2007), liquid–liquid extraction (LLE) (Salahinejad and Aflaki 2010), coprecipitation (Saracoglu et al. 2006: Aydin and Soylak 2007) and cloud point extraction (CPE) (Fang et al. 2001; Fernández-Band et al. 2004).

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Procedures involving cloud point extraction for the determination of metal species have been extensively reported. Several advantages are offered by this technique, such as a reduction in the quantities of samples and reagents required (particularly organic solvents), promotion of high enrichment factors (EFs), low cost, ease of operation and speed (Fang et al. 2001; Fernández-Band et al. 2004). The coupling of cloud point extraction and on-line preconcentration systems has become an attractive alternative, but it has not been fully explored. A few studies in the literature report procedures with cloud point extraction in on-line systems for the determination of substances, such as dysprosium (Ortega et al. 2003), gadolinium (Ortega et al 2002), mercury (Fernández-Band et al. 2004), coproporphyrin (Fang et al. 2001), lead (Silva and Roldan 2009; Nan et al. 2003), manganese (Lemos et al. 2008) and cadmium (Silva and Roldan 2009).

In this paper, we propose a method for the determination of cadmium in biological samples involving a flow injection system with cloud point extraction. The determination is based on the sorption of Cd(II) ions in a minicolumn packed with polyester and elution directly into a flame atomic absorption spectrometer.

Experimental

Instrumentation

A Perkin-Elmer (Norwalk, CT, USA) model 400 AAnalyst flame atomic absorption spectrometer (FAAS), equipped with a deuterium lamp as a background corrector, was used to collect absorbance measurements. The wavelength of the hollow cathode lamp of cadmium was 228.8 nm. The flow rates of acetylene and air to the burner were 2.5 and 10.0 l min^{-1}, respectively. The aspiration flow used for the nebulizer was 5.0 ml min^{-1}.

The experimental solutions were maintained at the desired temperature using a Soc. Fabbe (São Paulo, Brazil) model 110 thermostatic bath. A DIGIMED (Santo Amaro, Brazil), model DM 20 pH meter was used to measure the pH of the solutions. The digestion of biological material was performed using a Parr model 4749 (Moline, IL, USA) digestion bomb.

The procedure was performed with the aid of a simple on-line system involving two steps: preconcentration and elution. The system consists of a four-channel peristaltic pump (Milan, Model 204, Colombo, Brazil) operated with silicone tubes and a Rheodyne model 5041 six-way valve (Cotati, USA). Teflon tubes were used in the construction of the conductors of the system. In the construction of the minicolumn (3.50 cm in length and with a 4.0-mm internal diameter), a tube of polyvinyl chloride (PVC) was used. The minicolumn was packed with 200 mg of polyester.

Reagents

Ultrapure deionized water from an ELGA (Bucks, UK) system (PURELAB classic model) was used to prepare all solutions. Working solutions of cadmium were prepared daily by diluting a 1,000 μg ml^{-1} stock solution (Merck, Darmstadt, Germany). Hydrochloric acid solutions were prepared by direct dilution of the concentrated solution (Merck) with water. Acetate buffer solutions (pH 4.0–6.0), borate (pH 7.0–8.5) and ammonia (pH 9.0–9.5) (Skoog et al. 1996) were used to adjust the pH of the cadmium solutions. Solutions of Br-TAO were prepared by dissolving the appropriate amounts of the laboratory-prepared dye (Lemos et al. 2009) in absolute ethanol (Merck). Solutions of Triton X-114 were prepared by dissolving the surfactant in water. For accuracy studies, we analyzed two certified reference materials: NIST (National Institute of Standards and Technology, USA) 1570a, spinach leaves and NIST 1573a, tomato leaves.

Procedure for the preconcentration

A solution containing cadmium (7.0 ml) at an adequate pH was added to a 15-ml test tube. Solutions of Br-TAO (0.5 ml) and Triton X-114 (1.0 ml) were then added. The test tube was then left in the thermostatic bath at 45°C for 10 min. After the bath, the entire volume of the mixture was passed through the minicolumn of polyester. After sorption, the position of the six-way valve was changed, and a hydrochloric acid solution passed through the minicolumn, releasing the micelles and transporting the Cd ions to the FAAS.

Sample preparation

For digestion of the certified reference materials, approximately 0.1 g of the solid was treated with 4.0 ml of a 1:1 (v/v) nitric acid solution. The mixture was placed in a digestion bomb and maintained at 150°C for 4 h. After cooling to room temperature, the pH of the solution was adjusted with a 10% (w/v) sodium hydroxide solution and an appropriate buffer solution. After this step, the solution was transferred to a 25-ml volumetric flask, and the volume was completed with water. The material was analyzed immediately after preparation.

Results and discussion

Optimization of the experimental conditions

The experimental parameters affecting the on-line preconcentration of cadmium by cloud point extraction were evaluated using univariate analysis.

Metal complexation reactions with thiazolylazo ligands are highly dependent on pH (Hovind 1975). Thus, to determine the pH range in which the extraction is most effective, the pH of the Cd(II) solutions was varied in the range between 4.0 and 9.5. The results obtained for this study are shown in Fig. 1. The maximum absorbance was obtained at a pH between 7.5 and 8.5. Therefore, a borate buffer solution (pH=8.0) was used to adjust the pH of the Cd(II) solutions in all further experiments.

The concentration of ligand is also an important variable in the extraction, because it should be sufficient to react with the metal of interest and also to compensate for any interactions with interfering ions that may exist in the sample. The effect of the amount of Br-TAO used in the procedure was evaluated by varying the concentration of Br-TAO in the range between 1.6×10^{-5} and 3.2×10^{-4} mol l^{-1}. According to the results shown in Fig. 2, the analytical signal is maximized when the concentration of Br-TAO used is in the range between 3.2×10^{-5} and 1.6×10^{-4} mol l^{-1}. In all further experiments, the concentration of Br-TAO that was used was 8.0×10^{-5} mol l^{-1}. The influence of the concentration of the surfactant Triton X-114 in the extraction of

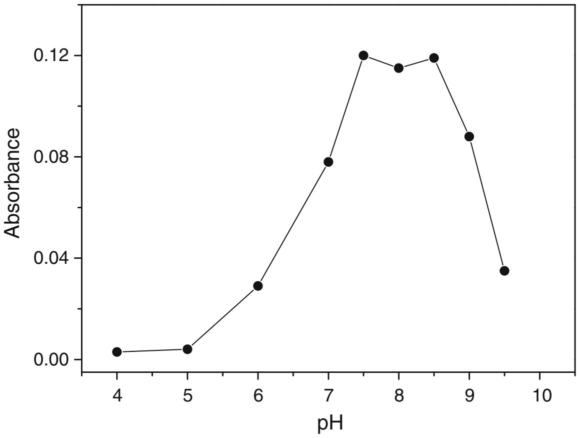

Fig. 1 Influence of pH on determination of Cd using cloud point extraction

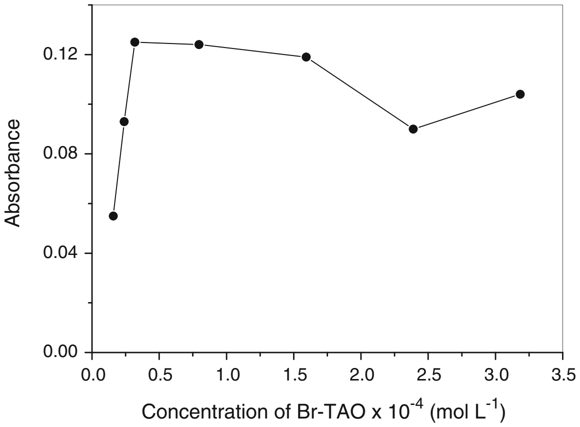

Fig. 2 Influence of concentration of Br-TAO on determination of Cd using cloud point extraction

Table 1 Effect of competing ions on the determination of cadmium using cloud point extraction

Substance	Maximum amount tolerable (mg l^{-1})
Al^{3+}	5.0
Ca^{2+}	0.5
Cl^{-}	0.5
Co^{2+}	1.0
Cr^{3+}	5.0
Cu^{2+}	1.0
Fe^{3+}	5.0
K^{+}	0.3
Mg^{2+}	0.2
Na^{+}	0.5
Ni^{2+}	1.0
NO_3^{-}	0.3
Pb^{+2}	1.0
Zn^{2+}	1.0

Table 2 Analytical features of the on-line system with cloud point extraction for the determination of cadmium

Enrichment factor	27
Consumptive index (ml)	0.22
Limit of detection (μg l^{-1})	0.5
Limit of quantification (μg l^{-1})	1.7
Linear range (μg l^{-1})	1.7–20.0
Precision (%)	3.4
Calibration function	$A=7.30\times10^{-3}+9.80\times10^{-3}C$

A absorbance, *C* concentration

cadmium was also studied. In this study, we used surfactant solutions of concentrations ranging from 0.01% to 0.40% (v/v). It was observed that the analytical signal decreased when surfactant solutions with a concentration of less than 0.20% (v/v) were used, because the amount of surfactant is insufficient to quantitatively extract the cadmium–Br-TAO complex. A 0.25% (v/v) Triton X-114 solution was therefore used in all further experiments.

In a step prior to detection, the micelles should be removed from the minicolumn and the metal transported to the nebulizer of the spectrometer. For this step, several substances were tested as eluents, such as water and solutions of: ethanol, hydrochloric acid, nitric acid and sulfuric acid. When water or a 5.0% (w/v) ethanol solution was used, there was a significant decrease in the analytical signal. Solutions of HNO_3 and HCl (0.5 mol l^{-1}) provided the highest analytical signals. This result is due to the lowering of the pH in the column, which disfavors the formation of the complex. In further experiments, we used 0.50 mol l^{-1} hydrochloric acid solution as eluent.

Table 3 Determination of cadmium in the certified reference material using the proposed procedure

Material	Cadmium amount (μg g^{-1})		Error (%)
	Proposed methodology	Certified	
NIST 1570a Spinach leaves	2.7±0.2	2.89±0.07	−7.0
NIST 1573a Tomato leaves	1.6±0.1	1.52±0.04	+5.0

Confidence interval 95%, *n*=3

NIST National Institute of Standards and Technology, USA

The influence of temperature on the on-line cloud point extraction system was investigated in the range of 25–60°C. The highest analytical signals were obtained when the temperature of the heating bath ranged from 40 to 60°C. The temperature of 45°C was then chosen for the next experiments.

The flow rate of the mixture containing the micelles was varied in the range of 2.5–9.0 ml min^{-1} to study the influence of this parameter on the analytical signal. A decrease in the analytical signal was observed when the flow of the mixture was greater than 7.0 ml min^{-1}. Moreover, there is excessive pressure on the system when the flow rate is greater than 7.0 ml min^{-1}, causing leaks. Thus, a flow rate of 6.0 ml min^{-1} was chosen for future studies.

The eluent flow rate controls the rate of desorption of Cd(II) from the minicolumn. Moreover, this parameter influences the speed of introduction of Cd (II) ions into the nebulizer of the spectrometer. By varying the flow rate of the eluent in the range 2.5 to 6.0 ml min^{-1}, we observed a decrease in the analytical signal for flow rates lower than 3.5 ml min^{-1}. In subsequent studies, we used 5.0 ml min^{-1} to match the flow rates of elution and aspiration.

Selectivity

The selectivity of the cloud point extraction of cadmium was also evaluated. Solutions containing cadmium (10.0 μg l^{-1}) and other ions at several concentrations were prepared and submitted to the preconcentration procedure. The effect of each ion was considered significant when the signal in the presence of that species caused a variation greater than 5% in the analytical signal obtained for the solution of Cd(II) alone. The ions tested for selectivity are listed in Table 1 along with the maximum tolerable amount for each ion with respect to Cd detection. Therefore, the presence of any of these ions at or below the concentrations listed in Table 1 will not affect the determination of cadmium.

Analytical features

The analytical characteristics of the procedure for the determination of Cd with cloud point extraction were calculated under the optimized conditions. When cadmium is measured using direct aspiration, the equation of the curve is $A=9.17\times10^{-3}+3.62\times10^{-4}C$

(100.0 and 1,500.0 μg l^{-1}), where A is the analytical signal, measured as absorbance, and C is the concentration of Cd in solution (in μg l^{-1}).

The limit of detection (LOD) was calculated as $3s_b/b$, where s_b is the standard deviation for twenty measures of blank and b is the slope of the linear section of the calibration graph. Similarly, the limit of quantification (LOQ) is calculated as $10s_b/b$. The accuracy of the measurements was evaluated as the relative standard deviation for each concentration value, calculated as $(s/X)\times 100$, where s is the standard deviation for seven measurements and X is the mean value of these measurements.

Other parameters related to the preconcentration systems were also calculated and are shown in Table 2. The EF measures the increase of the instrumental signal provided by a preconcentration method. One of the most reliable ways to calculate this parameter is to calculate the ratio between the slopes of the standard curves for the procedure with and without preconcentration (Fang et al. 1992). The consumptive index is defined as the sample volume, in milliliters, consumed to achieve one unit of EF.

Determination of Cd in biological material

The accuracy of the proposed procedure was evaluated by determination of Cd in certified reference materials. The following materials were analyzed: NIST 1570a, spinach leaves and NIST 1573a, tomato leaves. The results are shown in Table 3. According to the table, no significant difference was found between the results obtained and the certified values of reference materials.

Conclusion

The preconcentration procedure by on-line cloud point extraction developed in this work was successfully applied to the determination of cadmium in biological samples. The procedure has advantages such as speed, simplicity and low consumption of samples and reagents. Another important feature is the low cost of the procedure that results from the use of materials and equipment that are readily available in most laboratories. Finally, the analytical characteristics of this method make it an interesting alternative procedure for the determination of cadmium in these samples.

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