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Determination of Cd in biological samples by flame AAS following on-line preconcentration by complexation with *O,O*-diethyldithiophosphate and solid phase extraction with Amberlite XAD-4

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Abstract

A method for the on-line preconcentration of Cd based on its complex formation with the ammonium salt of *O,O*-diethylditiophosphate (DDTP) and using the Amberlite XAD-4 resin as a solid support in a column is proposed. Cadmium was detected by flame atomic absorption spectrometry. Different conditions, such as complexing agent concentration, preconcentration time, solutions flow rates and nature and concentration of the eluent were optimized. Different detection limits (LODs) could be established by using different preconcentration times, between 30 s and 5 min, with corresponding LODs from 5 to 1 $\mu g\,L^{-1}$, respectively. The method was validated by analyzing five biological certified samples. The relative standard deviation was usually around 3%, indicating a very good precision. The found concentrations values are in agreement with the certified ones, according to the *t*-test, for a confidence level of 95%. Enriched seawaters were also analyzed, and the recoveries were between 93 and 108%. The FI method is very simple and probably can be coupled to other measuring analytical techniques.

Keywords: Cd; DDTP; XAD; FI-FAAS; Biological sample; Seawater

1. Introduction

Due to the limited sensibility of the flame atomic absorption spectrometry and to the low cadmium concentration levels in natural biological samples, its determination frequently requires a previous preconcentration step. In addition, the high dissolved solid content, as in saline waters, may clog the nebulizer or change the nebulization process, resulting in poor precision and sensibility, unless a separation procedure is applied to these samples. Among the separation methods, the solid phase extraction (SPE), based on solid supports, modified or not with a complexing agent, which can be easily carried on-line using a minicolumn filled with the support. A great number of materials have been used as support, such as non-ionic resins (Amberlite, XAD, etc.), silica gel, polyurethane foam, active coal and others [1–6]. Recently, the XAD-4 resin, frequently impregnated with dithiocarbamates, as complexing agents, has been used for the preconcentration of several elements [7–9]. The ammonium salt of the *O,O*-diethyldithiophosphate (DDTP) forms stable complexes with several transition metals and semi-metals in acid medium, but does not react with alkaline and alkaline earth elements [1]. One advantage of the DDTP is that its complexes are stable in acid solutions due to its resistance against hydrolysis, avoiding the use of buffer solutions, which may be a source of

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contamination. In addition, several sample solutions are in an acid medium resulting from a previous acid dissolution of the solid sample or from the preservation with acids, as for water samples. The DDTP has been extensively used in preconcentration systems for the determination of metals by flame atomic absorption spectrometry FAAS and by inductively coupled plasma mass spectrometry (ICP-MS), using different solid supports, such as activated carbon, polyurethane foam, silica C_{18}, etc. [1,10–18]. Recently, analytical parameters for the separation and preconcentration of Cu, Pb and Fe in natural water as DDTP complexes on a chromatographic column filled with chromosorb-105 resin were evaluated [19]. For the preconcentration of Cd, using DDTP as complexing agent, the most used solid support were silica C18 and activated carbon [10,13,15]. The pH effect on the Cd preconcentration using DDTP and the resin XAD-4 was investigated in an off-line procedure [20]. However, the resin XAD-4 was generally used with an impregnated or immobilized complexing agent selective to several metals, including Cd [7,9,21–25].

The on-line preconcentration systems using flow injection (FI), have several advantages in comparison to the off-line systems, such as higher analytical throughput, low sample and reagents consumption and less risks of analyte loss or sample contamination. In addition, these systems can be easily coupled to conventional analytical techniques, such as F AAS, ICP-MS, ICP OES, etc. [26,27].

The goal of this work was the development of an on-line method for the determination of Cd by FI-F AAS in biological samples, using DDTP as complexing agent and Amberlite XAD-4 as adsorbent. According to our knowledge, this combination of DDTP with Amberlite XAD-4, was not proposed before for the Cd separation in an on-line procedure.

2. Experimental

2.1. Instrumentation

An atomic absorption spectrometer from Shimadzu (Nakagyo-Ku, Kyoto, Japan), model AA-6601F, single beam, equipped with a deuterium lamp background and with a Cd hollow cathode lamp L2433 from Hamamatsu Photonics K.K. (Hamamatsu, Shizuoka, Japan) was used. The lamp was operated at 8.0 mA, using the wavelength at 228.8 nm, slit of 0.5 nm, burner height of 7 mm and acetylene gas flow rate of 1.5 L min^{-1}. All measurements were in peak height (flame continuous mode with a pre-spray time of 1 s and an integration time of 10 s).

In addition, the following materials were used: multichannel peristaltic pump 505S from Watson Marlon (Falmouth Cornwall, UK) with Tygon capillary tubes of 1.52 mm i.d.; a PVC plastic syringe of 7 cm lenght and 0.5 cm i.d., used as a minicolumn and a injector-commutator from UNICAMP (Campinas, SP, Brazil). A focused microwave system, model Star System 2 from CEM (Matthews, North Caroline, USA) was used in the sample dissolution procedure.

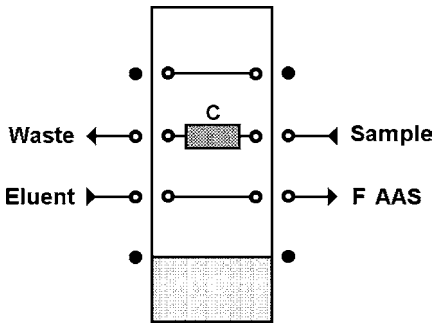

Fig. 1. FI system. The injector-commutator is shown in the sampling position, with the minicolumn (C) placed in the Sample-Waste line. By lowering the commutador, the system is in the elution position, with the column in the Eluent-F AAS line.

2.2. FI system

The FI system is shown in Fig. 1. With the injector in the position shown in this Figure, the sample solution passes through the column, during a certain selected time. While Cd and some other complexed metals are retained in the column, other concomitants pass through the column and are discharged. By changing the injector-commutator to the second position, the column (C) is placed in the eluent line. Cadmium is then eluted from the column and the eluent is driven to the measuring instrument. To keep the baseline, the eluent is always pumped through the nebulizer of the spectrometer, by using a separate eluent tube.

2.3. Reagents and samples

All chemical reagents were of analytical grade. The water (resistivity of 18.2 MΩ cm) was de-ionized in a Mili-Q system (Bedford, MA, USA). The following reagents were used: Suprapure 65% (v/v) HNO_3 (Merck, No. 1.00441.1000, Darmstadt, Germany), Suprapure 30% (v/v) HCl (Merck, no. 1.00318.0250), 30% (v/v) H_2O_2 (Merck, no. 1.07210.1000), Cd standard solution containing 1000 μg mL^{-1} (Merck, No. 1.19777.0500), Amberlite XAD-4 resin (polystyrene type, 20–60 mesh, Aldrich Chemical Company, Milwaukee, USA), DDTP (ammonium *O*,*O*-diethyldithiophosphate), purity of 95% (Sigma-Aldrich, Milwaukee, USA).

The following certified reference biological materials were analyzed: Dogfish Liver DOLT-2, Lobster Hepatopancreas TORT-2 and Lobster Hepatopancreas LUTS 1 from the National Research Council of Canada (NRCC, Ottawa, Ont., Canada); Oyster Tissue NIST 1566a from the National Institute of Standards & Technology (Gaithersburg, MD, USA) and Lyophilized Pig Kidney BCR 186 from the Community Bureau of Reference (Brussels, Belgium). Three seawater samples were collected around the Santa Catarina Island, Brazil, using plastic bottles of 500 mL capacity, previously cleaned with 5% (v/v) HNO_3 for 24 h and, then, thoroughly washed with ultra pure water.

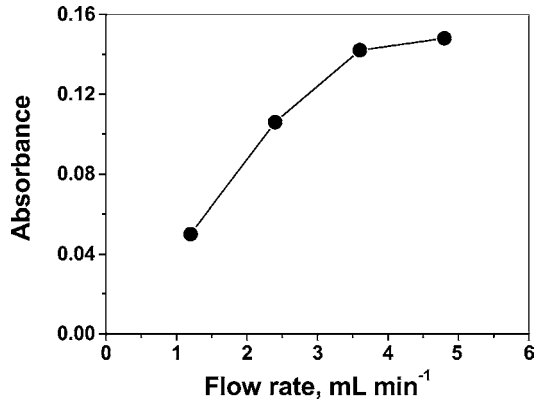

Fig. 2. Effect of the sample solution flow rate on the absorbance signal, for a Cd standard solution containing 50 μg L^{-1} in 1% (v/v) HNO_3 and 0.2% (m/v) DDTP, using a preconcentration time of 1 min and a sample flow rate of 3.6 mL min^{-1}.

2.4. Procedures

Cadmium calibration solutions, in the concentration range of 25–100 μg L^{-1}, were prepared from the 1000 μg mL^{-1} stock solution, by dilution with water, adding 1% (v/v) HNO_3 and 0.2% (m/v) of DDTP and submitted to the preconcentration procedure. The certified biological samples (NRC DOLT-2, NRC TORT-2, NRC LUTS-1, NIST 1566a, BCR 186) were weighed, 0.1–0.3 g, in borosilicate flasks, followed by the addition of 5 mL concentrated HNO_3 and 4 mL of 30% (v/v) H_2O_2. After standing for 8 h, the samples were digested in the microwave system for 5 min at 106 °C. The obtained clear solutions were transferred to a 100 mL volumetric flask, 0.2% (m/v) DDTP was added and the volume was made up with water. The seawater samples were filtrated through a 0.45 μm cellulose filter and acidified to 1% (v/v) HNO_3, before adding 0.2% (m/v) DDTP. Blanks solutions were also run. The XAD-4 resin was treated with 33% (v/v) HCl (4 mol L^{-1}) for 24 h, washed with water until pH 7 and dried at 40 °C for 24 h in a vacuum stove [9]. The minicolumn was filled with 0.3078 g of XAD-4 and closed in the extremities with glass wool.

3. Results and discussion

3.1. Solution flow rate

The amount of the analyte retained in the column will depend on the preconcentration time and on the sample solution flow rate through the column [3]. To study the effect of the sample solution flow rate, this was varied in the range 1.2–4.8 mL min^{-1}. As shown in Fig. 2, the integrated absorbance increases with the flow rate in the studied range almost reaching a maximum at 4.8 mL min^{-1}. As the flow rate increases, the contact and the interaction of the complexed analyte with the sorbent is hindered, decreasing the efficiency of the analyte adsorption on the resin. A flow rate of 3.6 mL min^{-1} was selected, which is the same as the sample flow rate for direct aspiration and nebulization in the spectrometer. In this way, the system was stable, without leaking or over pressure.

3.2. Effect of the acidity on the complexation

Pozebon et al [28] studied this effect previously in an on-line system, using variable concentrations of HNO_3, from 0.5 to 5% (v/v) in the initial solution, before the separation. They found that the acidity is not critical in the studied acid concentration range for the Cd complexation, because the signal intensity is almost constant. This is a real advantage of the use of DDTP as complexing agent, since a rigid control of the acidity or a buffer solution is not required, decreasing the risks of contamination. In addition, samples submitted to preservation and preparation procedures are usually in an acid medium, as the ones used in this work, which were submitted to a digestion with 5% (v/v) HNO_3 plus 4% (v/v) H_2O_2 (solid biological certified samples) or acidified with 1% (v/v) HNO_3 (real seawater samples), meaning that they are ready for complexation.

3.3. Elution

Two acids, HNO_3 and HCl, were tested as eluents. The effect of different acid concentrations, from 1 to 45% (v/v), in the elution is shown in Fig. 3. It is interesting to note that both acids at the different studied concentrations are able to elute Cd from the column filled with XAD-4. However, 33% (v/v) HCl (4 mol L^{-1}) was much more effective and was adopted as eluent.

3.4. DDTP concentration

The effect of the complexing agent concentration on the integrated absorbance signal is shown in Fig. 4, using a standard solution containing 100 μg L^{-1} of Cd in 1% (v/v) HNO_3.

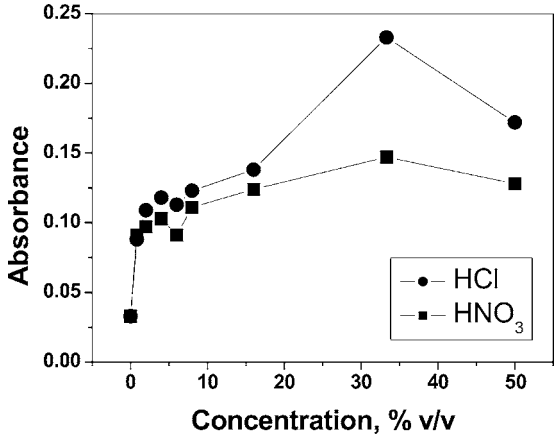

Fig. 3. Effect of the concentration of acids on the absorbance signal for a Cd standard solution containing 50 μg L^{-1} in 1% (v/v) HNO_3 and 0.2% (m/v) DDTP, using a preconcentration time of 2 min and a sample solution flow rate of 3.6 mL min^{-1}.

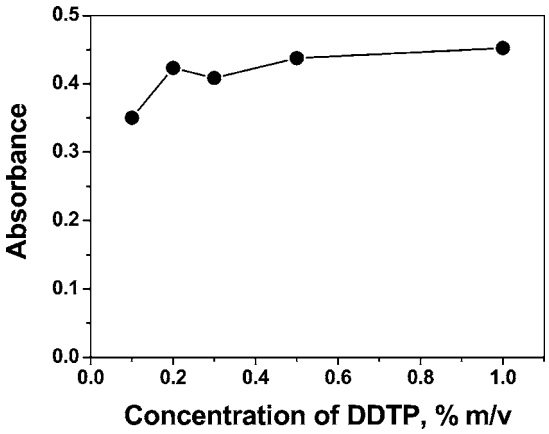

Fig. 4. Effect of the DDTP concentration on the absorbance signal for a Cd standard solution containing 100 μg L^{-1} in 1% (v/v) HNO_3, using a preconcentration time of 2 min and a sample solution flow rate of 3.6 mL min^{-1}.

As shown in the Figure, the signal intensity increases with the DDTP concentration, however the increase is less pronounced for higher DDTP concentration. A concentration of 0.2% (m/v) was selected for the further experiments.

3.5. Analytical figures of merit

Table 1 shows, for different preconcentration times, the linear correlation coefficient (R) of the preconcentrated calibration curve, this curve slope, the limit of detection (LOD), defined as three times the standard deviation of 10 measurements of the blank solution divided by the slope of the curve, and the enrichment factor (EF), calculated as the ratio of the slopes of the calibration curves with preconcentration and without preconcentration. As shown in the Table, the *R* values are higher than 0.996 for all preconcentration times. The LOD values, in the solution, varied from 5 to 1 μg L^{-1}, depending on the selected preconcentration time. Enrichment factors up to 20 (for 5 min preconcentration time) were obtained. Lower limits of detection and higher enrichment factor could be reached if higher preconcentration times would be used.

3.6. Analysis of certified biological sample

The results of the five biological certified samples are shown in Table 2. The found concentrations agree with the certified ones, according to the *t*-test for a 95% confidence level, indicating that the proposed method is accurate. The relative standard deviations were around 3%, except for one sample (BCR 186) for which it was 9%, showing good precision.

Table 1
Figures of merit for the determination of Cd by the proposed EF preconcentration system

Time[a] (min)	*R*	Slope (μg L^{-1})$^{-1}$	LOD (μg L^{-1})	FE
0.5	0.999	0.00235	5.0	6
1	0.998	0.00273	4.0	7
2	0.997	0.00390	3.0	10
3	0.999	0.00414	2.0	11
5	0.996	0.00816	1.0	20

[a] Preconcentration time; *R*, correlation coefficient of the calibration curve; a, slope of the calibration curve; LOD, limit of detection in the measuring solutions; EF, enrichment factor.

Table 2
Obtained concentrations for Cd in certified biological samples

Sample	Certified	Found	R.S.D. (%)
DOLT-2—Dogfish liver	20.8 ± 0.5	19.8 ± 0.6	3.0
NIST 566a—Oyster tissue	4.17 ± 0.58	3.93 ± 0.11	2.8
BCR 186—Pig kidney	2.71 ± 0.50	2.62 ± 0.23	8.8
TORT-2—Lobster hepatopancreas	26.7 ± 0.6	27.1 ± 0.8	3.0
LUTS 1—Lobster hepatopancreas	2.12 ± 0.15	2.10 ± 0.06	2.9

N = 3. Values in μg g^{-1}.

The same method was applied to three real seawater samples collected around the Santa Catarina Island. Unfortunately the concentrations were below the limit of detection for 5 min preconcentration time. However, the recoveries for the additions of 3.0, 6.0 and 9.0 μg L^{-1} of Cd were from 93 to 108%, demonstrating that the proposed method can be accurately used for the determination of Cd in contaminated seawater samples and probably also in other saline waters. Since the European Community and also the Brazilian legislation (CONAMA) recommend the maximum Cd concentration in seawater as 5.0 μg L^{-1}, the proposed method could also be used to check this limit in real samples. By using the same FI system coupled to a more sensitive measuring technique, such as GF AAS or ICP-MS, most probably non-contaminated waters could be analyzed.

4. Conclusions

The proposed FI method is very simple, of low cost and low sample consumption and is less prone to contamination or analyte loss in comparison to batch procedures. It is also very flexible, as different limits of detections can be obtained for different preconcentration time. The obtained results demonstrate a good precision and accuracy. The use of DDTP as complexing agent is advantageous since complex formation occurs at low pH values, not requiring a buffer solution, which may be a source of contamination. Other metals that form complexes with DDTP, probably could be also determined, using a similar procedure. It was also indicated that seawaters could also be analyzed by the same procedure. The FI system can be easily coupled to other techniques of different sensitivities, such as GF AAS, ICP OES, ICP-MS, etc.

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