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Microchemical Journal 133 (2017) 144–148

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Determination of cadmium at ultratrace levels by dispersive liquid-liquid microextraction and batch type hydride generation atomic absorption spectrometry

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ARTICLE INFO

Article history:
Received 2 March 2017
Received in revised form 15 March 2017
Accepted 15 March 2017
Available online 22 March 2017

Keywords:
Cadmium
Hydride generation
Microextraction
DLLME

ABSTRACT

This study describes the development of a dispersive liquid-liquid microextraction method for the extraction of cadmium and its determination by hydride generation atomic absorption spectrometry. The extraction method served as a clean-up of analyte from its matrix and also concentrated it to get lower detection limits. The hydride generation system was optimized to obtain high cadmium hydride generation efficiency and its transport to the quartz tube atomizer for atomization. Under the optimum experimental conditions, the limits of detection and quantification were 4.2 ng L^{-1} and 14.1 ng L^{-1}, respectively. Sensitivity was enhanced about 10,000 folds if compared with conventional FAAS system. The calibration showed good linearity over a wide range and the low relative standard deviations indicated high precision for the extraction process and instrumental readings. The accuracy of the method was validated by the agreement of experimented results to the value of a wastewater certified reference material (EU-L-2). Appreciable recovery results were obtained for wastewater spiked at different concentrations.

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1. Introduction

Heavy metal toxicity is one of the biggest problems for human beings. The presence of toxic metals in water, air and the earth's crust occurs mainly in regions with industrial or mining activities [1,2]. Cadmium is a heavy metal considered as a nonessential element for man. Human exposure to cadmium occurs through industrial applications, food consumption and tobacco smoking [3–5]. Cadmium toxicity was first reported in the nineteenth century in people working on zinc smelting [6–8]. The exposure of cadmium to the body can cause health problems such as hypertension, bone diseases, liver and kidney damages [9]. According to the World Health Organization (WHO) and the Environmental Protection Agency (EPA), the tolerable cadmium concentration in drinking water are limited to 3.0 μg L^{-1} and 5.0 μg L^{-1}, respectively [10].

Cadmium can be readily determined in biological and environmental samples by several classical and modern analytical methods. However, atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) have been commonly used because they are well described and more accepted methods [11–13]. Applying a wet ashing sample preparation method to blood and plasma paves way for cadmium determination by graphite furnace atomic absorption spectroscopy (GFAAS), a technique that produces accurate and reproducible results [14]. This technique is also ideal for cadmium determination biological samples such as urine, hair and whole blood. Various inductively coupled plasma (ICP) methods have been developed for cadmium determination in environmental and biological samples. ICP dynamic reaction cell mass spectrometry (ICP-DRC-MS) has been shown to eliminate molybdenum-based polyatomic interferences, thereby lowering the detection limit for cadmium in comparison to ICP-MS [15–17]. Although conventional atomic absorption spectrometry (AAS) [18] is another popular instrument for the determination of cadmium, its detection limit is not comparable with the other sensitive instruments such as ICP-MS, GF-AAS. Environmental samples analyzed for cadmium content can be aspirated directly, digested with an acid or preconcentrated by complex formation and extraction, then analyzed by atomic absorption/emission spectrometry (AAS/AES) [12,19–21].

Hydride generation (HG) is the most commonly used chemical vapor generation technique for the determination of trace and ultratrace amounts of elements such as Se, Pb, As, Sb and Te that form gaseous covalent hydrides [14,22,23]. The hydride production system produces low detection limits and high reproducibility for the determination of these specific elements. Both flame and hydride generation systems have the same instrumental setup but only differ in the sample introduction and atomizing units. The low nebulization efficiency of the

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http://dx.doi.org/10.1016/j.microc.2017.03.035
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flame setup is overcome by the hydride system which produces close to 100% sample entry to atomizer [23,24]. The quartz tube atomizer used to breakdown down the metal hydride is particularly useful for elements such as selenium and arsenic because their analytical lines (>200 nm) are prone to flame radical interferences. The principle of this technique involves an element's reduction from a high to the lowest oxidation state which forms the volatile hydride. Sodium borohydride is the most commonly used reductant in hydride generation, but others such as potassium borohydride have been also used [25,26]. An inert gas is used to purge the generated hydride to a heated atom cell for atomization [25]. Comprehensive reviews have been reported on hydride generation techniques for both hydride-forming and non-hydride forming elements in real sample applications [27,28].

Extraction is an important procedure that has been widely used for separation of analyte from a matrix (clean-up) and building up analyte into analyzable amounts (preconcentration). Liquid-liquid extraction (LLE) and solid phase extraction (SPE) are two common and earliest extraction methods which are slow, laborious and require large organic solvent amounts [29–32]. Solvent extraction has been greatly employed for rare earth metal determinations, but this method has limitations such as poor extraction speed, low preconcentration factor, use of toxic and flammable organic solvents [31,33,34]. These disadvantages have been overcome with the introduction of microextraction techniques such as liquid-liquid microextraction (LLME), liquid phase microextraction (LPME), solid phase microextraction (SPME), dispersive liquid-liquid microextraction (DLLME), amongst others [31]. Though relatively new, DLLME has become an extensively used method due to its simplicity and cheap operational cost. Other advantages include the use of small organic solvent volumes, high preconcentration factor and high analyte recovery [35,36]. Accuracy, precision and sensitivity of a method are directly affected by the analytical process described above [31].

The aim of this study was to develop an analytical method for the determination of cadmium at trace levels using batch type hydride generation atomic absorption spectrometry (BT-HGAAS) after dispersive liquid-liquid microextraction. This was achieved by optimizing all the parameters in cadmium determination. To the best of our knowledge, this is the first study in literature where DLLME has been combined with BT-HGAAS for the determination cadmium.

2. Experimental

2.1. Instrumentation

Analytic Jena Nova Model 300 series atomic absorption spectrometer was used for cadmium determination utilizing an HS55 batch hydride generation system. A single channel peristaltic pump delivered the reducing agent into a reaction vessel containing the acidified sample/standard solution. The first of two gas inlets delivered argon gas to the bottom of the vessel to speed up the reaction whiles the second gas inlet delivered argon to transport generated hydrides to the atomizing unit. An electrically heated quartz tube atomizer (QTA) was used for the decomposing of hydride to produce free cadmium atoms for absorption. The temperature of QTA was adjustable between 600 and 999 °C. The QTA unit was aligned in the pathway of a cadmium hollow cathode lamp operating at 2.0 mA and 0.50 nm spectral bandpass for the 228.8 nm analytical line. Deuterium lamp (D_2) was used in all measurements for background correction.

2.2. Reagents and solutions

Ultrapure water (resistivity 18.2 MΩ cm) was used in the preparation of all standard/sample solutions and cleaning of the batch system's reaction vessel during analysis. Working standard solutions were freshly prepared by diluting aliquots of Cd stock solution. Analytical grade reagents; nitric acid, hydrochloric acid, sodium borohydride and sodium hydroxide were obtained from Merck. Sodium borohydride was used as a reducing agent and to prevent the loss of hydrogen, sodium borohydride solutions were prepared daily. NaOH was used for the stabilization of $NaBH_4$ solution. The accuracy of the method was tested using a wastewater certified reference material (EU-L-2).

2.3. Procedure

1.0 mL each of buffer and ligand solutions was added to a centrifuge tube containing 8.0 mL of aqueous sample/standard solution. The cadmium complex solution (orange-red color) formed was shaken slightly for homogeneity. The extraction/dispersive (350.0 μL/3.0 mL) solution mixture was prepared separately, taken up with a syringe and injected into the complex solution. The fast settling of organic phase to the bottom of the tube was achieved by centrifugation at 3461*g* for 2.0 min. The settled organic phase was transferred into a clean tube and totally evaporated by either leaving the tube in the open or heating to evaporation in a water bath. The open air evaporation was the preferred choice because it was easier to dissolve the residue with 200.0 μL nitric acid after evaporation. The dissolution of residue was facilitated by heating in a water bath (50 °C) and the dissolved cadmium residue was then sent to the hydride system for cadmium determination.

3. Results and discussion

Optimization studies were carried out with a 5.0 ng mL^{-1} cadmium standard solution. All parameters but one were kept constant in the optimization step and the optimum values were selected based on the highest average absorbance values.

3.1. Optimum parameters of complex formation, DLLME and HG system

The parameters used for the formation and extraction of cadmium diphenyl carbazone complex were obtained from one of our recent publication and are presented in Table 1 together with optimized parameters in this study. The optimum parameters of cadmium diphenyl carbazone complex formation were taken from a recent study [37], tested under the same conditions and similar results were obtained. This resulted in the saving of time and chemicals that are required for a comprehensive complex formation optimization process. The flame burner head of the AAS was replaced with a heating cell housing a quartz tube atomizer, and batch type hydride generation system was used as analyte introduction method. The initial working conditions of the hydride generation system were selected using the optimized conditions of Büyükpınar et al. [38] as a guide. For sodium borohydride concentration, 4.0% was selected in place of 6.0% because it was reported that there was no statistical difference between high concentrations. The cell temperature was set to 950 °C, and the 2200 mL min^{-1} argon gas

Table 1
Optimum parameters of the DLLME-BT-HGAAS method.

Parameters	Value
Buffer solution pH/volume[a]	pH 10/0.50 mL
Ligand concentration/amount[a]	0.50 g L^{-1} (DPC in methanol)/1.0 mL
Mixing type/period[a]	Manual/30.0 s
Extractor solvent type/volume[a]	Ethanol/3.0 mL
Dispersive solvent type/volume[a]	Chloroform/350 μL
DLLME mixing type/period	Centrifugal/2.0 min at 3461 *g*
Eluent type/concentration	Nitric acid/1.0 M
$NaBH_4$ concentration	4.0%
Pump period	2.0 s
Gas flow rate[b]	2200.0 mL min^{-1}
QTA cell temperature	950 °C

[a] Fırat et al. [37].
[b] Büyükpınar et al. [38].

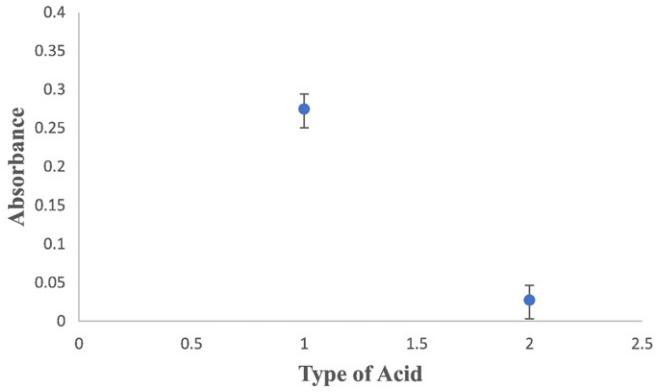


Fig. 1. Optimization of acid type (1: HNO_3, 2: HCl) for cadmium residue dissolution and complex bond cleavage (standard deviation for N = 3).

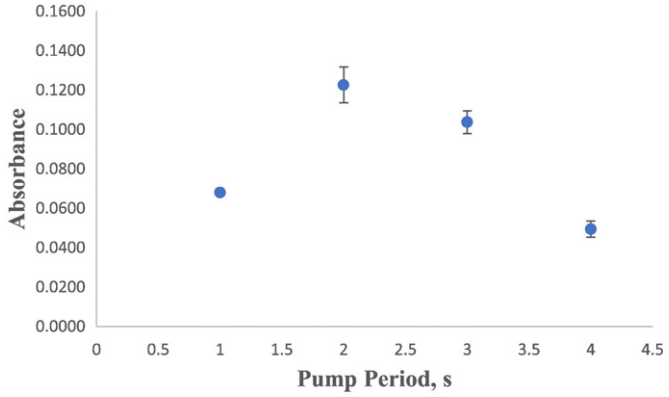


Fig. 3. A plot of reductant pump periods against absorbance (standard deviation for N = 3).

flow rate was used for a rapid and effective transport of generated cadmium hydrides to the QTA [38].

3.2. Optimization of types and amount of eluent

Prior to instrumental determination, cadmium must be broken from the diphenyl carbazone complex to facilitate the formation of volatile hydride. Selection of an appropriate residue eluent acid was thus tested using nitric acid and hydrochloric acid, which are two of the most commonly used acids in sample acidification for efficient hydride generation. The acids break up the metal-ligand bond to produce free cadmium ions. Nitric acid produced the highest bond cleavage as shown in Fig. 1. This can be attributed to the strong oxidizing nature of nitric acid which effectively dissolved the residue and freed substantial cadmium amounts from the complex. An incomplete dissolution of residue was visually observed for hydrochloric acid. The optimum amount of nitric acid was then determined using 0.50, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 mol L^{-1}. The 0.50 mol L^{-1} acid concentration was not able to fully dissolve the residue after total evaporation and as such was not sent to the hydride generation system for cadmium determination. The highest absorbance was recorded for 1.0 mol L^{-1} nitric acid after which sharp decreases were observed for the higher concentrations as shown in Fig. 2. This decrease could be attributed to the high nitric acid concentrations' reaction with sodium borohydride producing excess hydrogen gas which dilutes analyte vapor [39]. Further optimizations were therefore carried out with 1.0 mol L^{-1} nitric acid.

3.3. Effect of reductant concentration and amount

Sodium borohydride was used as a hydrogen source and chemical reductant for the formation of cadmium hydride. The concentration of the reductant solution directly affects the amount of volatile compounds generated. As the reductant concentration increase, the reaction occurs more rapidly and the absorbance signals become sharper. For this reason, the concentration of $NaBH_4$ was optimized up to 4.0% (w/v) after which no significant increase was obtained. The final volume of acidified sample/standard solutions were 200 μL, and this small volume required optimization of the amount of sodium borohydride delivered into the reaction vessel. The peristaltic pump was maintained at its maximum speed throughout the entire study. The flow rate determined for this speed was 21 mL min^{-1}. The optimum amount of 4.0% (w/v) sodium borohydride was determined by pumping for 1.0, 2.0, 3.0 and 4.0 s periods. The highest absorbance signal was recorded for 2.0 s. A steady decrease occurred with increasing pump period as depicted in Fig. 3. It can be said that the 1.0 s period did not produce enough hydrogens for optimum hydride formation while the 3.0 s and 4.0 s periods produced excess of hydrogen that possibly hindered the rapid and complete transfer of cadmium hydrides to the QTA. The 2.0 s period correlates to 700 μL $NaBH_4$ with regards to the 21 mL min^{-1} flow rate of the peristaltic pump.

In addition, effect of the sonication on the signal obtained was studies, and there was no enhancement in the signal observed. Hence, sonication was not applied to the batch type hydride generation system for the determination of analyte.

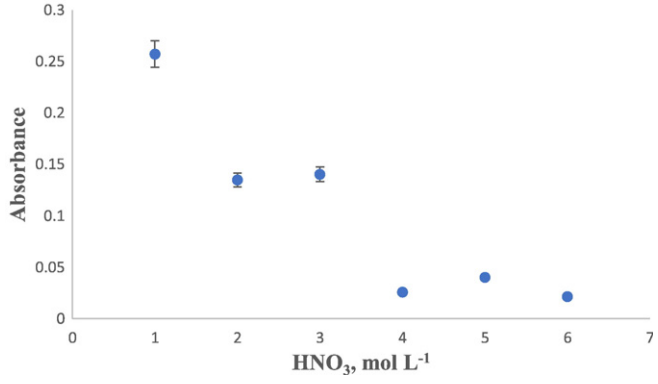


Fig. 2. A plot of nitric acid concentrations against absorbance (standard deviation for N = 3).

3.4. Analytical figures of merit

Presented in Table 2 are the analytical performance of this method obtained under optimum conditions and compared to the figures of merit of the same instrument with FAAS instrument used. A calibration curve was developed for cadmium standard solutions in the range of 0.05 μg L^{-1} to 50.0 μg L^{-1}. The limits of detection (LOD) and quantitation (LOQ) were calculated from blank determinations using the following relationships [23];

$$LOD = 3 \times \text{Standard deviation of blank/Slope}$$

$$LOQ = 10 \times \text{Standard deviation of blank/Slope}$$

The calibration plot was linear from 0.25 μg L^{-1} to 20.0 μg L^{-1}, and the correlation coefficient obtained showed a positive relation between concentration and absorbance values. 4.2 ng L^{-1} detection limit obtained for cadmium in this study is comparable to the 7.5 ng L^{-1}

Table 2
Analytical figures of merit of AAS systems in this study.

	LOD	LOQ	%RSD	Dynamic range
FAAS [37]	42 μg L^{-1}	140 μg L^{-1}	9.6	100–2000 μg L^{-1}
DLLME-FAAS [37]	1.3 μg L^{-1}	4.4 μg L^{-1}	7.6	5.0–100 μg L^{-1}
DLLME-BT-HGAAS	4.2 ng L^{-1}	14.1 ng L^{-1}	2.9	250–20,000 ng L^{-1}

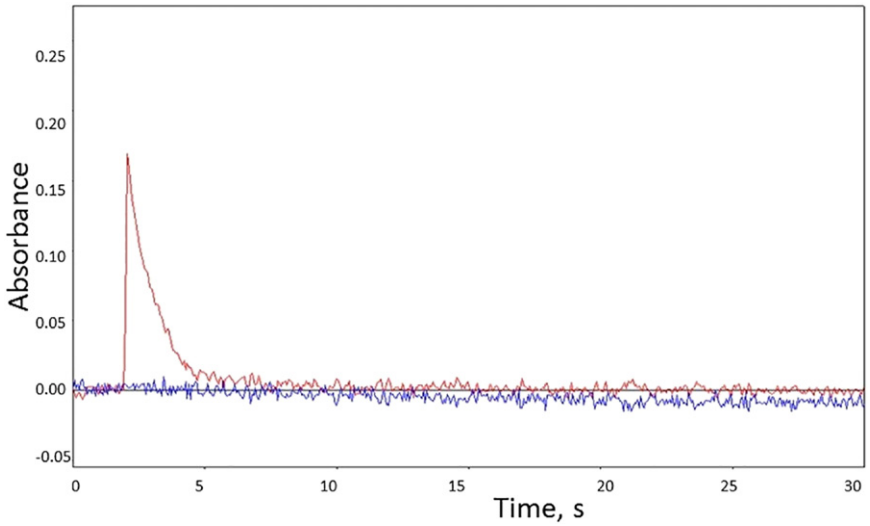


Fig. 4. Analytical signal of 5.0 ng mL^{-1} Cd using DLLME-BT-HGAAS system.

limit obtained by GFAAS after continuous sample drop flow-based microextraction [31]. Another study that coupled DLLME with GFAAS reported a detection limit of 6.0 ng L^{-1} [40]. The percent RSD for replicate blank determinations was 2.9%, which indicates high analytical precision. The sensitivities of the AAS instrument coupled to the hydride generation system and the flame burner were compared as shown in Table 3. Combining DLLME with HGAAS lead to about 10,000 fold increase in sensitivity over the FAAS. The high efficiency of the hydride generation system was further enhanced by the high preconcentration factor obtained by the DLLME process.

Under the optimum conditions given in Table 1, signal given in Fig. 4 was obtained for 5.0 ng mL^{-1}.

3.5. Accuracy and recovery

Accuracy of the method was tested using the wastewater certified reference material (EU-L-2). The reference material was diluted into the linear calibration range and analyzed under the optimized system. The experimented value (0.0214 ± 0.0034 mg L^{-1}) was comparable to the certified value (0.023 ± 0.001 mg L^{-1}). According to statistical evaluation (Student's *t*-test), there was no difference in the result obtained and certified result. After validating the method's accuracy, it was applied to municipal wastewater, but cadmium was not detected. A recovery test was therefore performed to determine the amount of cadmium in wastewater when present. The percent recoveries obtained for 10.0 and 20.0 ng mL^{-1} spiked wastewater samples were $81.3 \pm 0.8\%$ and $101.6 \pm 2.1\%$, respectively. This indicates that cadmium when present in wastewater can be accurately determined within the limits of the method developed.

4. Conclusions

An accurate and precise method for the determination of cadmium by hydride generation atomic absorption spectrometry after dispersive liquid-liquid microextraction (DLLME-BT-HGAAS) was successfully developed. The entire procedure was flexible for the method development and required very low volumes of organic solvents, making it economical and green. The analytical figures of merit obtained in this study were satisfactory and the method's accuracy was validated with a certified reference material. Detection limit obtained under the optimized conditions was very low and comparable with ICP-MS. As an application, wastewater samples were analyzed using developed method and cadmium was not detected in wastewater samples but results from the recovery tests suggest that the method can be applied to wastewater samples which have complicated matrices. To the best of our knowledge, this is the first study in literature that combines DLLME with batch type HGAAS for ultratrace determination of cadmium, and it is strongly believed that the method can be applied to other hydride forming elements.

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