



# Ionic liquid-assisted multiwalled carbon nanotube-dispersive micro-solid phase extraction for sensitive determination of inorganic As species in garlic samples by electrothermal atomic absorption spectrometry



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## ABSTRACT

A highly sensitive dispersive micro-solid phase extraction (D- $\mu$ -SPE) method combining an ionic liquid (IL) and multi-walled carbon nanotubes (MWCNTs) for inorganic As species (As(III) and As(V)) species separation and determination in garlic samples by electrothermal atomic absorption spectrometry (ETAAS) was developed. Trihexyl(tetradecyl)phosphonium chloride IL was used to form an ion pair with the arsenomolybdate complex obtained by reaction of As(V) with molybdate ion. Afterwards, 1.0 mg of MWCNTs was dispersed for As(V) extraction and the supernatant was separated by centrifugation. MWCNTs were re-dispersed with tetradecyltrimethylammonium bromide surfactant and ultrasound followed by direct injection into the graphite furnace of ETAAS for As determination. Pyrolysis and atomization conditions were carefully studied for complete decomposition of MWCNTs and IL matrices. Under optimum conditions, an extraction efficiency of 100% and a preconcentration factor of 70 were obtained with 5 mL of garlic extract. The detection limit was  $7.1 \text{ ng L}^{-1}$  and the relative standard deviations (RSDs) for six replicate measurements at  $5 \text{ } \mu\text{g L}^{-1}$  of As were 5.4% and 4.8% for As(III) and As(V), respectively. The proposed D- $\mu$ -SPE method allowed the efficient separation and determination of inorganic As species in a complex matrix such as garlic extract.

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## 1. Introduction

Garlic (*Allium sativum* L.) is a vegetable widely used for cooking and it is highly consumed in several countries [1]. Arsenic speciation analysis in garlic is very important in order to evaluate the real toxicological risks of consuming this food. However, since As concentrations in garlic samples could be in the order of a few  $\mu\text{g L}^{-1}$  or even less, thus challenging the limits of detection of most instrumental techniques, preconcentration is required in order to achieve accurate and sensitive results [1–3].

In the last years, significant innovation has been introduced in solid-phase extraction (SPE) preconcentration by novel nanomaterials with remarkable physical-chemistry properties that improve the retention of analytes. Thus, multi-walled carbon nanotubes (MWCNTs) have been applied for analytical preconcentration due to their unique

properties, such as reduced particle size and big surface area [4]. However, for inorganic analytes, it is necessary a previous derivatization with chelating agents to form neutral or low polar complexes that can be absorbed on MWCNTs [5]. An alternative to chelating agents is the use of ion-pairing reagents to form compounds that are extractable onto MWCNTs surfaces. Recently, our group proposed the application of ILs as ion-pairing reagents in liquid-liquid microextraction (LLME) and SPE [6,7]. ILs have demonstrated to be successful replacements of conventional organic solvents [8] and have shown to be good ion-pairing reagents in LLME [9] or SPE preconcentration techniques [7]. They are efficient extractants and can help to improve analytical selectivity and sensitivity in chromatographic and spectrometric analysis [10]. MWCNTs have been modified with ILs by chemical or physical treatments to improve properties such as solubility, decreased capacity to form bundles, chemical reactivity, structure and electronic network, among others. [11]. These modifications can be further exploited to enhance the retention properties of MWCNTs as adsorbents for analytical preconcentration [12].

In contrast to conventional SPE, dispersive SPE (D-SPE) is an attractive approach based on the use of a solid phase dispersed in a liquid sample or extract. Generally, the extraction times with D-SPE are

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shorter than those with SPE, while high preconcentration factors are obtained [13]. A variant of D-SPE technique is dispersive micro-SPE (D- $\mu$ -SPE), where only a few micrograms of the solid phase are dispersed for analyte retention. Thus, the significant reduction in the retention material raised by the D- $\mu$ -SPE technique, along with the possibility of direct introduction of solid samples in electrothermal atomic absorption spectrometry (ETAAS) could result into a very attractive strategy to simplify and speed-up the determination of trace elements when solid phases are used for preconcentration.

In this work, a sensitive preconcentration method based on the D- $\mu$ -SPE technique combining MWCNTs and an IL was developed for inorganic As species determination in a complex matrix such as garlic. The method proposes a thorough simplification by allowing the direct injection of analyte-containing dispersed MWCNTs into the graphite furnace of ETAAS. Thus, no digestion or back-extraction procedures are needed for As injection in ETAAS, as MWCNTs are pyrolyzed before the atomization step. To the best of our knowledge, there are no reports on As determination by D- $\mu$ -SPE-ETAAS with direct injection of MWCNTs.

## 2. Experimental

### 2.1. Instrumentation

Measurements were performed with a PerkinElmer (Überlingen, Germany) Model 5100 ZL atomic absorption spectrometer equipped with a transversely heated graphite atomizer and a Zeeman-effect background correction system. An As electrodeless discharge lamp (EDL) was used. All measurements were performed based on absorbance signals with an integration time of 3 s. Instrumental conditions are listed in Table 1. For the injection of the MWCNTs, regular yellow-type micropipette tips were modified by cutting them up to 2 mm inner diameter. A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used for separation of phases. A vortex model Bio Vortex B1 (Boeco, Hamburg, Germany) was used for mixing the reagents. An ultrasound bath (40 kHz and 600 W) with temperature control (Test Lab, Buenos Aires, Argentina) was employed for re-dispersing the MWCNTs.

### 2.2. Reagents

All the reagents were of analytical grade and the presence of As was not detected within the working range. Stock standard solutions of inorganic As(V) and As(III) species ( $1000 \text{ mg L}^{-1}$ ) as sodium arsenate dibasic heptahydrate ( $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ ) (99.998%) (Sigma-Aldrich, Milwaukee, WI, USA) and sodium metaarsenite ( $\text{AsNaO}_2$ ) (99%) (Fluka, Buchs, Switzerland), respectively, were prepared in  $0.1 \text{ mol L}^{-1}$  HCl. Disodium methylarsenate ( $\text{CH}_3\text{AsNa}_2\text{O}_3 \cdot 6\text{H}_2\text{O}$ ) (MMA, 98%) (Fluka)

and dimethylarsinic acid ( $\text{C}_2\text{H}_7\text{AsO}_2$ ) (DMA, 98.6%) (Fluka) stock standard solutions ( $1000 \text{ mg L}^{-1}$ ) were prepared with ultrapure water and stored at  $4^\circ\text{C}$  in amber-colored HDPE bottles. A  $500 \text{ mg L}^{-1}$  palladium nitrate solution [ $\text{Pd}(\text{NO}_3)_2 \cdot 2\text{H}_2\text{O}$ ] (Fluka) and  $500 \text{ mg L}^{-1}$  magnesium nitrate solution [ $\text{Mg}(\text{NO}_3)_2$ ] (99%) (Merck, Darmstadt, Germany) were prepared and used as chemical modifiers (Table 1). These solutions were prepared in 0.1% (v/v)  $\text{HNO}_3$  (Ultrax® II Mallinckrodt Baker, Phillipsburg, NJ, USA). A 15% (w/v) ammonium molybdate tetrahydrate (>99%) (Fluka) solution was prepared in ultrapure water. Hydrochloric acid (37%) and sulfuric acid (95%) were purchased to Merck. Tetradecyl(trihexyl)phosphonium chloride (95%) was from Sigma-Aldrich. A diluted solution at  $8.5 \times 10^{-2} \text{ mol L}^{-1}$  was prepared by weighting an accurate amount of the IL followed by dissolution in ethanol. Ethanol (96%) was from Merck. Triton X-100 (99%), sodium dodecyl sulfate (98.5%) and tetradecyltrimethylammonium bromide (TTAB) (99%) were all from Sigma-Aldrich and used as dispersion agents for MWCNTs. MWCNTs (98%) were supplied by Sigma-Aldrich. Chloroform (99%), 1-butanol, citric acid (99.5%), tris(hydroxymethyl)aminomethane (99.8%) and sodium hydroxide (98%) were from Sigma-Aldrich. Ultrapure water ( $18 \text{ M}\Omega \cdot \text{cm}$ ) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All the glassware was washed in  $0.5 \text{ mol L}^{-1}$   $\text{HNO}_3$  for 24 h and later rinsed with ultrapure water.

### 2.3. Sample collection and extraction of As species

Garlic samples were collected from local stores or suppliers of Mendoza (Argentina). The collected samples were washed with distilled water and hand-peeled. The edible parts were frozen, cut into small pieces, lyophilized and finally pulverized with a mill. The resulting fine powder was stored in polyethylene bags and kept inside a freezer at  $-20^\circ\text{C}$ . Ultrasound-assisted extraction of As species was performed following the procedure described in a previous report [1]. Briefly, 0.1 g of freeze-dried garlic sample was accurately weighted inside of a 15 mL-polyethylene tube and 10 mL of  $1.0 \text{ mol L}^{-1}$   $\text{H}_2\text{SO}_4$  were added. The dispersion was sonicated for 10 min and the sulfuric extract separated by centrifugation at 3500 rpm ( $2054.3 \times g$ ) for 10 min. The extract was separated using a Pasteur transference pipette.

### 2.4. General D- $\mu$ -SPE procedure for As(V) determination

A schematic diagram of the operational procedure can be observed in Fig. 1. Basically, an aliquot of 5 mL of sample extract was poured into a 10 mL-graduated glass centrifuge tube along with 1 mL of  $0.12 \text{ mol L}^{-1}$  ammonium molybdate solution. Two sequential extractions with 1 mL of 1-butanol:chloroform (1:3) mix were performed to separate phosphate anion. After phosphate separation, 50  $\mu\text{L}$  of an  $8.5 \times 10^{-2} \text{ mol L}^{-1}$  IL solution (prepared in ethanol) were added to the sample extract. Then, 1.0 mg of MWCNTs was weighted in a 10 mL centrifuge glass tube and 5.0 mL the phosphate-free sample extract was added to the tube. The resulting system was shaken with vortex during 5 min followed by centrifugation at 3000 rpm during 5 min to separate the MWCNTs from the aqueous solution. The upper aqueous phase was manually removed with a transfer pipette and the MWCNTs were re-dispersed in 100  $\mu\text{L}$  of 0.1% (w/v) TTAB solution. In order to form the MWCNTs dispersion, ultrasonication at room temperature for 5 min was needed and then it was injected directly into the graphite furnace of ETAAS for As determination under the conditions depicted in Table 1.

### 2.5. Determination of total inorganic As and As(III) species

Oxidation of As(III) to As(V) species was performed before the elimination of phosphate interference and the preconcentration step. A 5 mL-aliquot of sample extract was treated with 500  $\mu\text{L}$  of 5.0% (w/v) potassium peroxodisulfate solution at room temperature for 5 min and

**Table 1**  
ETAAS instrumental conditions for As determination.

Wavelength	193.7 nm			
Spectral bandwidth	0.7 nm			
Lamp (EDL) current	300 mA			
Matrix modifier	10 $\mu\text{g Mg}$ [ $\text{Mg}(\text{NO}_3)_2$ ] 10 $\mu\text{g Pd}$ [ $\text{Pd}(\text{NO}_3)_2$ ]			
Graphite furnace temperature program				
Step	T ( $^\circ\text{C}$ )	Ramp time (s)	Hold time (s)	Argon flow ( $\text{mL min}^{-1}$ )
Drying 1	110	1	30	250
Drying 2	130	15	30	250
MWCNT injection	30	15	30	0
Drying 3	110	5	30	250
Drying 4	130	15	30	250
Pyrolysis	1300	90	25	250
Atomization	2300	0	3	0
Cleaning	2400	1	2	250

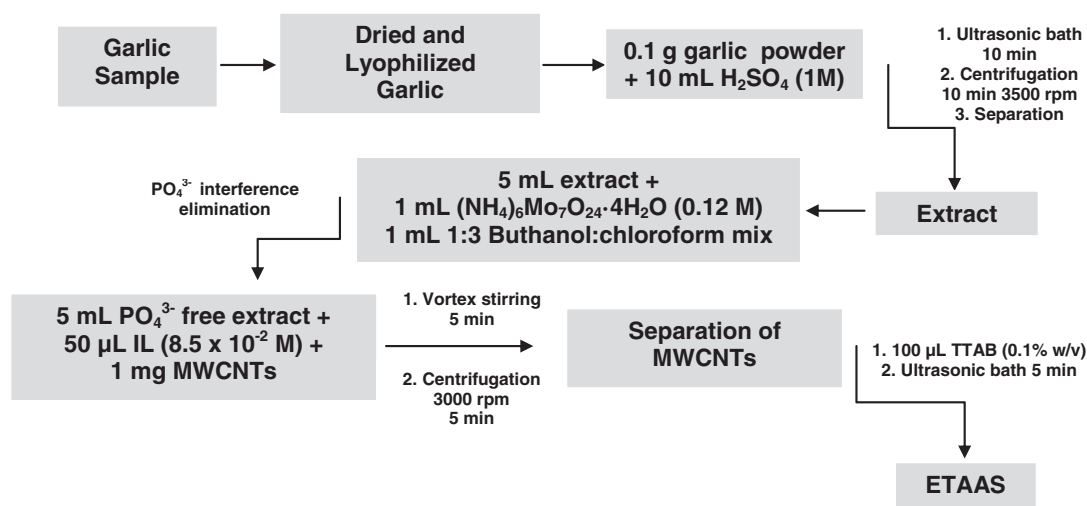


Fig. 1. Schematic of the operational procedure involved in the proposed D- $\mu$ -SPE method for determination of inorganic species of As in garlic samples.

total inorganic As (as As(V)) was determined as described previously in Section 2.4. The concentration of As(III) was obtained by difference between total inorganic As and As(V) concentration.

### 3. Results and discussion

#### 3.1. Injection of MWCNTs and ETAAS conditions for As determination

Before developing the proposed D- $\mu$ -SPE method, the possibility of performing accurate and precise determinations of As by ETAAS in the presence of MWCNTs matrix had to be carefully evaluated in this work. Thus, the formation of a dispersion of MWCNTs was considered to allow their injection into the graphite furnace. It has been found that the adsorption of non-ionic molecules on the surface of MWCNTs by  $\pi$ - $\pi$  stacking or charged molecules by coulomb attraction can stabilize MWCNTs in aqueous mediums and thus, stable suspensions are obtained. Therefore, different surfactants can be assayed to provide steric hindrance or static charge repulsion for stabilizing MWCNTs dispersions. In this work, three different types of surfactants were evaluated: anionic (sodium dodecyl sulfate), cationic (TTAB) and non-ionic (Triton X-100), at three concentrations: 0.01, 0.05 and 0.1% (w/v). MWCNTs were dispersed in 100  $\mu$ L of the solutions containing each surfactant for full injection into the graphite furnace in one single step. Volumes lower than 100  $\mu$ L did not lead to appropriate dispersion of MWCNTs. The formation of a stable and homogenous MWCNTs dispersion was observed for 0.1% (w/v) TTAB concentration. The repulsive forces caused by the adsorbed surfactant prevented the agglomeration of MWCNTs, thus assuring reproducibility of MWCNTs injection in ETAAS after D- $\mu$ -SPE [14]. Concentrations of TTAB higher than 0.1% (w/v) did not lead to a stable dispersion of MWCNTs. The remaining surfactants did not add further improvement in MWCNTs dispersions.

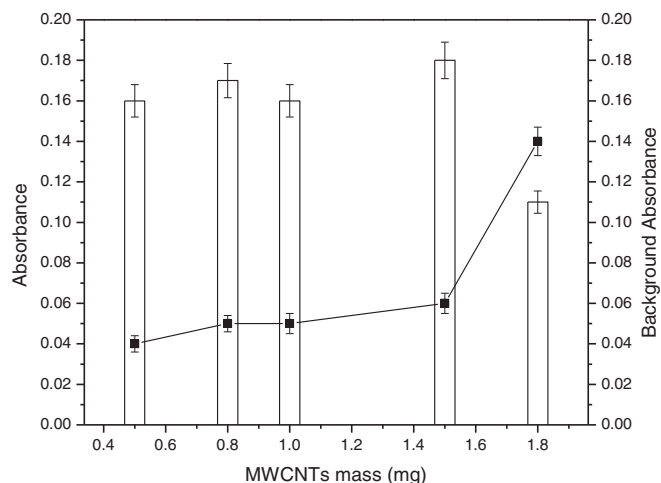
Furthermore, two mechanical methods were evaluated to obtain a stable and fine dispersion: vortex and ultrasonication. It was observed that vortex was not an efficient process owing to MWCNTs were dispersed and adhered onto the walls of the glass centrifuge tube. On the other hand, ultrasonication is an efficient tool to disperse MWCNTs by bubble nucleation and collapse sequence [15]. Therefore, different ultrasonication times were assayed in this work in the range of 1 to 5 min. The results showed that 5 min was enough to form a stable and homogeneous dispersion of MWCNTs.

The introduction of matrix modifiers is crucial when slurries are injected into the graphite furnace. Thus, insufficient interaction between the matrix modifiers and As retained on MWCNTs surfaces could lead to analyte losses during pyrolysis step. Different amounts of

Mg(NO<sub>3</sub>)<sub>2</sub>, Pd(NO<sub>3</sub>)<sub>2</sub> and their mixtures were tested as chemical modifiers. A mixture of 10  $\mu$ g Mg and 10  $\mu$ g Pd was chosen for chemical modification during As measurements. Sharp and symmetric peaks were obtained with this mixture.

The pyrolysis temperature was a critical factor to be optimized since it had to allow total decomposition of the organic matrix of MWCNTs before the atomization step. In previous works on thermal analysis, decomposition temperatures of MWCNTs were between 500 °C and 700 °C under an air atmosphere with carbon dioxide as the main decomposition product [16]. Likewise, in a temperature furnace program used for determination of metal impurities by DSS-ETAAS, pyrolysis temperatures were in the range of 600 °C and 1500 °C at a heating rate of 50 °C s<sup>-1</sup> and holding time of 30 s [17]. Therefore, based on the thermal behavior of MWCNTs, pyrolysis temperature was optimized. For all the experiments, 100  $\mu$ L of 1.0 mg of MWCNTs dispersion and 60  $\mu$ L of 100  $\mu$ g L<sup>-1</sup> As(V) standard solution were used. The optimum pyrolysis temperature was 1300 °C with a hold time of 25 s. On the other hand, analyte losses were evidenced for pyrolysis temperatures higher than 1300 °C. Full decomposition of the organic matrix was observed with that temperature and hold time, obtaining the highest sensitivity for As determination. The atomization temperature was also studied in this work in the range of 1900 °C to 2350 °C. The optimum atomization temperature was 2300 °C. Moreover, a temperature of 2400 °C and a hold time of 2 s were chosen for the cleaning step.

Finally, the effect of the organic loading provided by MWCNTs on absorbance signals generated during As determination by ETAAS was also studied. This was assessed by injecting different amounts of MWCNTs (0.5–1.8 mg) into the graphite furnace. The injection of MWCNTs into the graphite furnace did not cause significant effects on the absorbance signals up to 1.5 mg (Fig. 2), which was evidenced by the occurrence of sharp and Gaussian-shape absorbance peaks with low background signal. In fact, accurate determination of As by ETAAS was achieved even with the high organic matrix provided by MWCNTs, the surfactant used for dispersion and the IL retained on the solid sorbent. Moreover, after optimization of the methodology, no differences were found in As determination even when the phosphonium IL was adsorbed on the MWCNTs, thus demonstrating the effectiveness of the temperature program. Furthermore, it must be pointed out that the ETAAS instrument used in this work had a Zeeman-effect background correction system, which could largely avoid spectral interferences caused by complex matrix samples. Thus, the gradual increment of pyrolysis temperature (90 s ramp time and 25 s hold time) and the background correction system were essentials to obtain a high sensitivity for As determination with the proposed preconcentration method.



**Fig. 2.** Effect of MWCNTs mass on analytical absorbance (□) and background absorbance (■). Injection of 60  $\mu\text{L}$  of 100  $\mu\text{g L}^{-1}$  As(V). Dispersion of MWCNTs in 100  $\mu\text{L}$  TTBA 0.1% (w/v). Chemical modifiers were 10  $\mu\text{g}$  Mg and 10  $\mu\text{g}$  Pd.

### 3.2. Arsenomolybdate complex formation and ion-pairing reaction with the ionic liquid

The influence of pH on both arsenomolybdate complex formation and retention on MWCNTs was assayed in the range of 0.5–10.0. The highest analyte extraction efficiency (or retention) was obtained at pH 0.5. In order to provide a low pH that favor analyte extraction, a strong acid was needed. Since sulfuric acid was used for extraction of As species from garlic matrix, this acid was chosen to adjust the pH during preconcentration. A concentration of 1.0 mol  $\text{L}^{-1}$  guaranteed the low pH needed for the arsenomolybdate complex formation. Molybdate concentration was another critical variable to be optimized for complete formation of the arsenomolybdate complex. The effect of several Mo:As molar ratios was studied and the results revealed that a maximum extraction efficiency of 100% could be obtained at  $2.5 \times 10^6$ .

In the proposed D- $\mu$ -SPE method, the IL is employed as an ion-pairing agent to form a low polar species with  $\text{AsMo}_{12}\text{O}_{40}^{3-}$  complex, so adsorption on MWCNT surface can occur. In this case, the positively charged phosphonium IL forms a strong bond with the negatively charged  $\text{AsMo}_{12}\text{O}_{40}^{3-}$  complex. The influence of IL concentration on As extraction was studied in the range of  $6.8 \times 10^{-6}$  to  $1.7 \times 10^{-3}$  mol  $\text{L}^{-1}$ . A 100% extraction efficiency was achieved with a concentration of  $8.5 \times 10^{-4}$  mol  $\text{L}^{-1}$  IL.

### 3.3. Effect of sample volume and stirring on As extraction

The effect of the sample volume on As extraction was studied in the range of 5–15 mL with 1 mg of MWCNTs as solid phase. It could be observed that As extraction efficiency was 100% for 5 mL of sample and above this value the extraction efficiency decreased.

A high interaction of the ion pair, formed between the  $\text{AsMo}_{12}\text{O}_{40}^{3-}$  complex and the phosphonium counterpart supplied by the IL, with the apolar surface of the MWCNTs was critical for efficient preconcentration of As. Thus, vortex stirring and ultrasonication were assayed. The results showed that the highest retention of As on MWCNTs was achieved with vortex stirring, while ultrasonication yielded 56% only. Therefore, vortex stirring was chosen to perform the extraction of As on MWCNTs. The influence of stirring time on As extraction was also evaluated in the range of 1 to 10 min. An extraction efficiency of 100% was obtained for 5 min and it decreased to 80% for longer stirring times.

### 3.4. MWCNT amount and sorption capacity

In order to obtain the highest pre-concentration factor possible, the lowest MWCNT mass necessary had to be determined. MWCNTs amount was assayed between 0.5 and 1.5 mg using 100  $\mu\text{L}$  of 0.1% (w/v) TTBA surfactant solution as re-dispersant solvent. It could be observed that extraction efficiency was 100% when the amount of MWCNTs was at least 1.0 mg. Moreover, 1.0 mg MWCNTs were chosen for D- $\mu$ -SPE due to the facility to disperse this amount in 100  $\mu\text{L}$  of the surfactant solution, volume defined as optimal for accurate injection of MWCNTs in ETAAS.

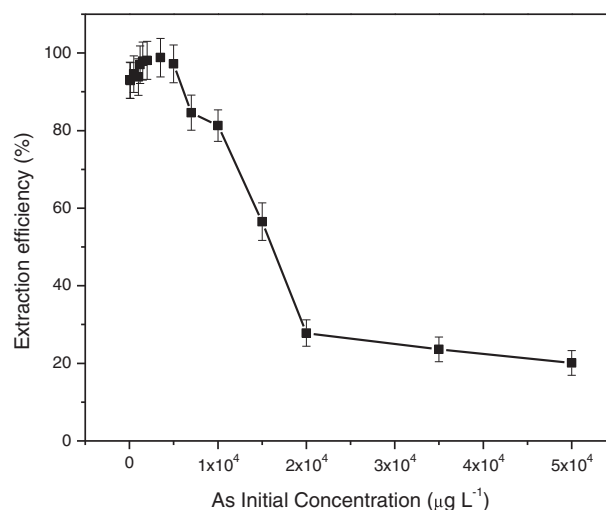
The sorption capacity of MWCNTs for retention of IL- $\text{AsMo}_{12}\text{O}_{40}^{3-}$  ion pair was evaluated. Several As(V) standard solutions with concentrations between 0.5 and 50 mg  $\text{L}^{-1}$  were prepared. Also, molybdate and IL concentrations were kept at 0.17 mol  $\text{L}^{-1}$  and  $8.5 \times 10^{-4}$  mol  $\text{L}^{-1}$ , respectively. Due to the high concentrations assayed in these experiments, As determinations were performed in the supernatant after the preconcentration process. In some cases, a dilution was needed prior to ETAAS determination to obtain absorbance signals within the lineal calibration range. A sorption capacity of 25 mg As  $\text{g}^{-1}$  MWCNTs was obtained (Fig. 3). This result is in good agreement with those reported in other works for As preconcentration by SPE techniques using MWCNTs [18,19].

### 3.5. Selectivity of As species determination

The selectivity of As(V) preconcentration in presence of As(III) species was evaluated by mean of a recovery study. The As(V) species is able to form the arsenomolybdate complex but for As(III) species this reaction is not feasible. The results shown in Table 2 indicate that As(V) species was completely separated and quantitatively recovered even in the presence of high molar ratios of As(III)/As(V). An acceptable accuracy and high selectivity were obtained under the optimized conditions, with recovery percentages for As(V) between 94.0% and 104%. Also, common organic As species such as MMA and DMA do not form complexes with molybdate anion, so they were not expected to be retained on MWCNTs. This was confirmed by evaluating As(V) recovery (99%) in a MMA/DMA mixture at 5 mg  $\text{L}^{-1}$  concentration of each species.

### 3.6. Study of potential interferences

The influence of possible interfering ions, such as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{3+}$  and  $\text{PO}_4^{3-}$  on As determination was evaluated in this work. A concomitant ion was considered to interfere if caused a  $\pm 5\%$  variation in the analytical signal. The results showed that none of the



**Fig. 3.** Arsenic sorption capacity evaluated with 1.0 mg of MWCNTs and conditions as reported in Table 1.



**Table 2**  
Selectivity of the method for As(V) determination (95% confidence interval, n = 6).

As(III)/As(V) molar ratio	As(V) 0.5 $\mu\text{g L}^{-1}$ added		As(V) 5 $\mu\text{g L}^{-1}$ added	
	Found ( $\mu\text{g L}^{-1}$ )	Recovery (%)	Found ( $\mu\text{g L}^{-1}$ )	Recovery (%)
5	0.47	94.0	5.15	103
10	0.51	102	4.96	99.2
20	0.48	96.0	4.95	99.0
50	0.49	98.0	5.05	101
100	0.52	104	5.10	102

evaluated species caused interferences up to at least  $10 \text{ mg L}^{-1}$ , with the exception of  $\text{PO}_4^{3-}$ , for which a strong interference effect was observed from  $0.1 \text{ mg L}^{-1}$ . This interference effect is due to the formation of a complex between molybdate anion and the phosphate anion. Therefore, selective extraction of phosphomolybdate anion had to be performed prior to D- $\mu$ -SPE procedure as described in Section 2.4. By this procedure, As(V) was fully recovered and  $\text{PO}_4^{3-}$  anion was tolerated at concentrations as high as  $5 \text{ mg L}^{-1}$ .

### 3.7. Analytical performance

The preconcentration factor (PF) was calculated from the ratio of the calibration curve slopes for As(V) with and without application of the preconcentration step [20]. A PF of 70 was achieved with the proposed D- $\mu$ -SPE method. The calibration curve obtained with the proposed methodology was linear with a correlation coefficient of 0.997 at levels near the limit of detection (LOD) up to at least  $8.75 \mu\text{g L}^{-1}$ . The LOD was calculated according to the signal at the intercept and three times the standard deviation about regression of the calibration curve [21]. A LOD of  $7.1 \text{ ng L}^{-1}$  As was obtained for the developed methodology. The relative standard deviation (RSD) resulting from the analysis of six replicates of  $5 \text{ mL}$  solution containing  $5 \mu\text{g L}^{-1}$  of As was 5.4% and 4.8% for As(III) and As(V), respectively. The extraction recovery (ER) was calculated using the following equation:

$$\text{ER} = \frac{m_{\text{MWCNTs phase}}}{m_{\text{aq}}} \times 100$$

where  $m_{\text{MWCNTs phase}}$  is the mass of As retained on MWCNTs and  $m_{\text{aq}}$  is the initial mass in the aqueous phase. A extraction efficiency of 100% was obtained. Regarding the frequency of analysis, the full preconcentration and microextraction procedure takes 20 min per individual sample. However, since it is possible to analyze as many samples as can be placed in the centrifugation equipment, a frequency of analysis of 48 samples per hour was achieved in this work.

Finally, a comparative study on the analytical performance achieved by the developed method with respect to others reported in the literature is shown in Table 3. To the best of our knowledge, there are no works on As speciation and determination by D- $\mu$ -SPE-ETAAS with MWCNTs. Likewise, while most works report on As speciation in water, only two were devoted to the determination of As species in

garlic samples [1,22]. The proposed D- $\mu$ -SPE has been developed to allow inorganic As speciation in a complex matrix sample such as garlic, turning this method into a useful tool for routine laboratories focused on food analysis. Although some preconcentration methods using CNTs have obtained higher PF than our work (Table 3), it must be noticed in those cases the need for significantly higher volumes of sample. On the other hand, the D- $\mu$ -SPE-ETAAS method uses only 5 mL of sample extract, thus demonstrating that it was possible to develop a method for speciation of inorganic As in complex matrix samples, such as garlic, with a minimum volume of sample and MWCNTs.

### 3.8. Validation study and determination of As species in garlic samples

For validation, the proposed method was applied to the analysis of a certified reference material (CRM), white clover BCR 402, with a declared As concentration of  $0.093 \pm 0.01 \text{ mg kg}^{-1}$ . Then, an amount of 0.1 g of the CRM was weighted and acid-digested. The digestion solution was completed up to 10 mL with water and acidified to a final concentration of  $1.0 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ . An aliquot of 5.0 mL was analyzed by proposed preconcentration method. Using the proposed D- $\mu$ -SPE method, As concentration found in the CRM was  $0.091 \pm 0.02 \text{ mg kg}^{-1}$ , thus indicating an acceptable accuracy of the method ( $p < 0.01$ ). Furthermore, a recovery study can be considered as a validation alternative in elemental speciation studies. According to Table 4, it can be observed that the proposed methodology had an acceptable accuracy under different conditions, with recoveries for both As species between 98.1 and 105%.

Arsenic speciation analysis in garlic has been little explored. In fact, there are only two publications reporting that inorganic As species, i.e. As(V) and As(III), are the only ones occurring in this food [1,22]. On the other hand, organic As species have not been found in garlic. Based on these observations, the proposed D- $\mu$ -SPE method allows accurate determination of inorganic As species (As(V) and As(III)). Regarding to the samples analysis, the results showed concentrations between  $0.03 \mu\text{g L}^{-1}$  and  $0.17 \mu\text{g L}^{-1}$  ( $17 \text{ ng g}^{-1}$  garlic) for As(III) and between  $0.1 \mu\text{g L}^{-1}$  and  $0.5 \mu\text{g L}^{-1}$  ( $50 \text{ ng g}^{-1}$  garlic). These results are in good agreement with those obtained in a previous study [1].

## 4. Conclusions

A novel D- $\mu$ -SPE method combining MWCNTs as sorbent material and a phosphonium IL as ion-pairing agent for separation and preconcentration of inorganic As species in garlic samples was developed. The method proposes strong simplification allowing the direct injection of the dispersed SPE nanomaterial, i.e. MWCNTs, into the graphite furnace of ETAAS. Thus, no digestion or back-extraction steps are needed for As determination, as the complex matrix composed by MWCNTs, adsorbed IL and dispersing surfactant was fully pyrolyzed before the atomization step. By dispersing MWCNTs, typical problems observed with other preconcentration methods that use powdered materials in SPE columns were significantly eliminated. The usual compaction observed when MWCNTs are employed as filling materials in on-line preconcentration systems was avoided by the D- $\mu$ -SPE

**Table 3**  
Analytical methods reported for As preconcentration using CNTs.

Method	Sample	CNT type	Speciation analysis	LOD ( $\text{ng L}^{-1}$ )	Preconcentration factor	RSD (%)	Sample consumption (mL)	Sampling frequency ( $\text{h}^{-1}$ )	Reference
SPE-ETAAS	Sea, mineral, river and tap water	MWCNT	Yes	As(III): 20 As(V): 50	250	As(III): 3.5 As(V): 3.7	50	N.R. <sup>a</sup>	[19]
SPE-ETAAS	Rice, rice ashes and water	MWCNT	No	As(III): 8.0	125	0.97	500	N.R. <sup>a</sup>	[18]
SPE-HG-AFS	Snow water and rain water	MWCNT	Yes	As(V): 14	16.3	3.6	2.0	N.R. <sup>a</sup>	[23]
$\mu$ -D-SPE-ETAAS	Garlic	MWCNTs	Yes	7.1	70	As(V): 4.8 As(III): 5.4	5	48	This work

<sup>a</sup> Not reported.

**Table 4**  
Inorganic As species determination and recovery study in garlic samples (95% confidence interval; n = 6).

Sample	As added as ( $\mu\text{g L}^{-1}$ )		As(III) Found ( $\mu\text{g L}^{-1}$ )	Recovery (%) <sup>a</sup>	As(V) Found ( $\mu\text{g L}^{-1}$ )	Recovery (%) <sup>a</sup>
	As(III)	As(V)				
Garlic sample 1	–	–	0.03 ± 0.002	–	0.10 ± 0.005	–
	0.5	–	0.54 ± 0.030	102	0.10 ± 0.005	–
	–	0.5	0.03 ± 0.002	–	0.63 ± 0.030	106
	0.5	0.5	0.52 ± 0.030	98.0	0.61 ± 0.030	102
Garlic sample 2	–	–	0.05 ± 0.003	–	0.22 ± 0.013	–
	0.5	–	0.56 ± 0.040	102	0.22 ± 0.013	–
	–	0.5	0.05 ± 0.003	–	0.74 ± 0.040	104
	0.5	0.5	0.54 ± 0.030	98.0	0.73 ± 0.040	102
Garlic sample 3	–	–	0.14 ± 0.008	–	0.48 ± 0.030	–
	0.5	–	0.62 ± 0.040	96.0	0.48 ± 0.030	–
	–	0.5	0.14 ± 0.007	–	0.99 ± 0.050	102
	0.5	0.5	0.63 ± 0.040	98.0	0.97 ± 0.060	98.0
Garlic sample 4	–	–	0.17 ± 0.009	–	0.50 ± 0.030	–
	0.5	–	0.67 ± 0.040	100	0.50 ± 0.030	–
	–	0.5	0.17 ± 0.010	–	1.05 ± 0.060	110
	0.5	0.5	0.68 ± 0.040	102	1.03 ± 0.060	106

<sup>a</sup>  $100 \times [(\text{found} - \text{initial}) / \text{added}]$ .

method. However, a drawback of the proposed method is the need for separation of phosphate anion to avoid chemical interference during arsenomolybdate complex formation.

The proposed D- $\mu$ -SPE method offers a simple, reliable and sensitive alternative for As speciation and determination in complex matrix samples such as garlic.

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