

Carbon nanotubes as solid-phase extraction sorbents prior to atomic spectrometric determination of metal species: Determination of lead in urine

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ABSTRACT

New materials have significant impact on development of new methods and instrumentation for chemical analysis. From the discovery of carbon nanotubes in 1991, single and multi-walled carbon nanotubes (SWCNTs and MWCNTs) -due to their high adsorption and desorption capacities- have been employed as sorption substrates in solid-phase extraction for the preconcentration of heavy metals from diverse matrices. In the present work, a new method for the Pb determination by electrothermal atomic absorption spectrometry (ETAAS) in urine at low levels has been developed. Lead was separated from the undesirable urine matrix by means of a solid phase extraction (SPE) procedure using oxidized multiwalled carbon nanotubes as a sorbent material.

Keywords: Carbon nanotubes, lead determination, ETAAS.

1. INTRODUCTION

Lead is a toxic heavy metal that does not have any known positive physiological role in the human body. Since the lead level in urine reflects the amount of the element that has been recently absorbed, the determination of Pb in urine is useful for assessing occupational and environmental exposure¹. However, monitoring trace elements in urine is a difficult task due to the complexity of the matrix and the low concentration of analyte. Therefore, the determination of Pb in urine requires a pretreatment step as well as sensitive instrumental techniques such as ETAAS. In this case, SPE using oxidized MWCNTs was employed as preconcentration method. Since the discovery of carbon nanotubes (CNTs) in 1991 by Iijima², it became clear that they might be an excellent material for SPE because of their high surface area and inner volume, stability, mechanical strength and the possibility of establishing π - π interactions. CNTs are hollow graphitic material composed by one (named single-walled carbon nanotubes, SWCNT) or multiple (named multi-walled carbon nanotubes, MWCNT) layers of graphene sheets arranged (Figure 1).

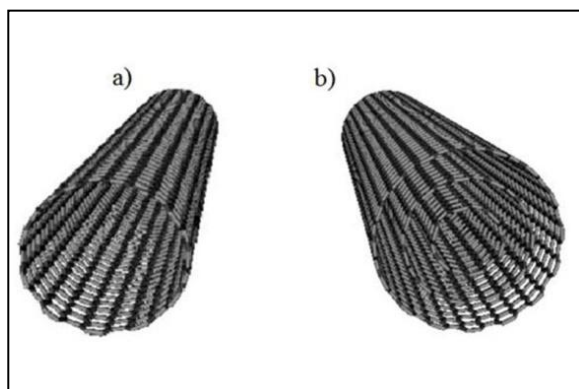


Figure 1. Carbon nanotubes. a) Single-walled carbon nanotube; b) Multiple-walled carbon nanotube. (Adapted from: <http://www-ibmc.u-strasbg.fr>).

The CNT dimensions are the following: the length varies from few hundred nanometers to several microns; the diameter differs if the

nanotube is composed of single or multiple layers, SWCNT achieve 1 to 10 nm, while MWCNT is thicker, in the range from 5 to 100-200 nm. Both, single and multiwalled carbon nanotubes have been the most applied nanomaterials for metal preconcentration from different matrices.

The oxidation process of MWCNTs introduces hydroxyl, carbonyl and carboxyl groups in the surface of nanotube, enhancing the solubility of the material and producing bonded-surface oxygen-containing radicals which have the ability of retaining a variety of metal ions at the appropriate pH. Oxidation is commonly carried out by acidic treatment by reflux with HNO₃, H₂SO₄, HCl or mixtures. Also other oxidizing reagents, such as potassium permanganate or hydrogen peroxide, may be also used for this task³.

Therefore, since 1995 the works using diverse types of CNTs as SPE sorbents for metal preconcentration purposes have increased significantly⁴⁻⁵. In the case of Pb, different SPE procedures involving as-grown and oxidized CNTs have been proposed for separating and preconcentrating the metal, either directly in the form of Pb(II) ions⁶⁻⁷, or as Pb-chelates⁸⁻⁹. In all cases, after extraction, Pb was measured by different atomic spectrometric techniques with adequate results.

The aim of the present study was to develop a SPE-ETAAS method for the determination of Pb in urine using multiwalled carbon nanotubes (MWCNTs) as SPE-sorbent. The SPE procedure achieved the elimination of the urine matrix and avoided the need for other sample pretreatment. Furthermore, Pb levels were determined with appropriate analytical figures of merit. The capability of the developed method for monitoring Pb exposure was demonstrated by measuring real urine samples.

2. MATERIAL AND METHODS

2.1. Apparatus and statistical software

A Zeeman correction Varian-SpectrAA-600 atomic absorption spectrometer (Varian Inc., Palo Alto, CA, USA) equipped with a Varian GTA-100 electrothermal atomizer linked to an automatic sample dispenser was used for this work. Measurements were performed using a Pb Varian hollow cathode lamp operating at 283.3 nm with a current intensity of 10 mA. The

Step	Temperature (°C)	Ramp (s)	Hold (s)
Dry	90	30	25
Ash	350	15	5
Atomization ^a	1550	1	3
Clean	2400	1	2
Cold	40	40	0

^aStop Ar flow

Table 1. Furnace heating programs for ETAAS determination of lead.

bandwidth employed was 1.0 nm in all cases. Argon was used as the inert gas at a flow rate of 300 mL min⁻¹. Pyrolytic graphite-coated atomization tubes with a pre-inserted Omega Platform were used (Schunk Ibérica S.A., Madrid, Spain). A Gilson Minipuls-3 peristaltic pump (Gilson Inc., Middleton, WI, USA) equipped with Tygon pump tubing of 1.42 mm i.d. (Ismatec, Wertheim, Germany) was used to propel both the sample and reagents. Polytetrafluoroethylene (PTFE) connecting tubing of 0.5 mm i.d. and various end-fittings and connectors (Omnifit-Diba Industries Ltd, Cambridge, UK) were employed.

Experimental designs as well as Pareto and surface contour plots for the optimization of the developed method were carried out using Statgraphics Centurion XVI ver. 16.1.15 (Rockville, MD, USA).

2.2. Reagents

Untreated multiwalled CNTs (purity >95%, 20–30 nm o.d. and ~30 μm length) prepared by Chemical Vapour Deposition (CVD) of acetylene in hydrogen flow were supplied by Chengdu Organic Chemicals Co. Ltd. (Chengdu, China).

A standard lead stock solution (1.0 g L⁻¹) was obtained from Panreac (Barcelona, Spain). Ammonium dihydrogenphosphate was obtained from Fluka (Buchs, Switzerland). All materials were washed with 10% nitric acid (v/v) for a period of 24 h, rinsed with copious amounts of pure water and shaken dry before being used. The cleaning solution employed to wash the sampling capillary contained 0.7% (w/v) HNO₃ and 0.2% (v/v) Triton X-100 (Sigma-Aldrich, St. Louis, MO, USA).

2.3. MWCNT pretreatment and microcolumn preparation

The oxidation of MWCNT introduces hydroxyl, carbonyl and carboxyl groups on the nanotube surface and this enhances the solubility of the material and produces bonded surface oxygen-containing radicals that have the ability to retain a variety of metal ions at the appropriate pH. In the present work, MWCNTs were oxidized by a microwave-assisted procedure employing a mixture H₂SO₄/KMnO₄ as oxidant. The procedure has been described in detail in a previous work¹⁰. Oxidized multiwalled carbon nanotubes (45 mg) were loaded into a 35 mm × 4 mm (i.d.) PTFE-microcolumn plugged with a small portion of glass wool at both ends to avoid sorbent losses during the SPE. Prior to use, the column was cleaned and conditioned by passing through 2 mL of a 1.0 M solution of nitric acid followed by 4 mL of pure water.

2.4. SPE-ETAAS analytical procedure

A 4 mL aliquot of diluted 40% (v/v) urine sample at pH 4.0 was passed through the microcolumn with a peristaltic pump at a flow rate of 1.1 mL min⁻¹ to achieve retention of the Pb ions by the MWCNTs. In the second step, 1.5 mL of a 0.1 M solution of HNO₃ was passed through the column at a flow rate of 0.6 mL min⁻¹ to eliminate the urine matrix.

The retained Pb ions were subsequently eluted by employing 1.5 mL of a more concentrated 0.7 M HNO₃ solution (at a flow rate

of 0.4 mL min⁻¹). 20 μL of this eluted solution sample was mixed with 2 μL of an aqueous matrix modifier solution containing 0.005% (w/v) NH₄H₂PO₄. The resulting sample was subjected to ETAAS under the optimized conditions indicated in Table 1. Eight microcolumns were used simultaneously for the SPE extraction procedure.

3. RESULTS AND DISCUSSION

3.1. Optimization of the SPE

Different variables that influence the SPE retention of lead ions by MWCNTs packed in the microcolumn (pH, sample flow rate, eluent flow rate, eluent volume and eluent concentration) were initially studied evaluated by means of a screening study using a half-fraction factorial design 2⁵⁻¹ in 16 randomized experiments. In the present work, nitric acid was selected, because it was shown that HNO₃ produces a satisfactory elution and a very low background in subsequent ETAAS analysis. Sampling and elution flow rates were also considered in this optimization step, taking into account the necessity of achieving a compromise between efficiency and time.

According to the response surfaces obtained, the optimal values for the three variables were as follows: sample pH 4.0, eluent volume 0.99 mL (approx. 1.0 mL) and eluent concentration 0.7 M. However, under these conditions, certain problems concerning the reproducibility of Pb measurements were detected. Inadequate reproducibility could be due to the low elution volume selected. For this reason, a univariate study of this parameter was performed. On the basis of the extraction efficiency obtained for different extraction volumes (in the range 0.5 to 2.0 mL), it was demonstrated that the use of 1.5 mL of 0.7 M HNO₃ produced a high extraction efficiency with better result for reproducibility.

The best extraction efficiencies were achieved at low flow rates. However, the use of such low flows dramatically increased the analysis time; consequently, the appropriate balance between flow rate and time must be attained. Optimum values for extraction efficiency were obtained for a sample flow rate of 1.1 mL min⁻¹ and an elution flow rate of 0.4 mL min⁻¹.

3.2. Optimization of ETAAS conditions

The direct ETAAS determination of lead in urine suffers from some drawbacks caused by the organic matrix components of urine samples. In the present case, urine samples were subjected to ETAAS measurements after SPE treatment, in which matrix elimination was achieved. Therefore, ETAAS determination was carried out using a single method that employed less harsh conditions than those required in other methods without SPE. On the basis of results previously reported¹¹ NH₄H₂PO₄ was selected as adequate chemical modifier. The effects of mineralization and atomization temperatures together with the concentration of modifier on the lead integrated absorbance for eluted urine samples were studied in using a Box-Behnken design in 15 randomized experiments. The optimum furnace operation conditions, producing the highest analytical signal and also well-shaped peaks, are listed in Table 1.

3.3. Analytical figures of merit

In order to evaluate the analytical characteristics of the developed method, the linearity, detection and quantification limits, as well as precision and accuracy were studied. The present method showed a linear response for urine samples from LOD up to 120 $\mu\text{g Pb L}^{-1}$. Detection (LOD) and quantification (LOQ) limits (calculated as $3\text{SD}/m$ and $10\text{SD}/m$, respectively where m is the slope of the calibration graph and SD the standard deviation of 10 consecutive measurements of blank solutions) were 0.08 and 0.26 $\mu\text{g L}^{-1}$, respectively, and these are acceptable for the measurement of Pb at low levels in human urine. The precision of the method was evaluated by measuring ten independent SPE cycles for urine samples spiked with different quantities of Pb. The relative standard deviations (RSD) were 4.3, 8.2, 4.5, 5.8, 3.4 and 3.4 %, respectively. The accuracy of the proposed method was evaluated by a recovery test. A urine sample containing 5.31 $\mu\text{g L}^{-1}$ of Pb, was supplemented with different quantities of Pb in the range 5–90 $\mu\text{g L}^{-1}$ and they were measured under the conditions previously described in section 2.4. From the results, values in the range 97–106% were obtained. Additionally, five urine samples with low Pb concentration from non-smoker unexposed people were comparatively measured in triplicate: (i) by the present method in our laboratory and (ii) by the ICP-MS method after sample microwave-assisted acid digestion described by Nakagawa et al.¹² in an external laboratory. According to the results obtained, it can demonstrate that good agreement was obtained between the levels determined by both methods. A paired-test showed no statistical differences (at a 95% confidence level) for the results measured.

The enhancement of ETAAS sensitivity due to the simplification of the matrix (achieved by inserting the SPE step with MWCNTs) was evidenced by calculating the signal enhancement factor (SEF). This parameter was calculated as the ratio between the slopes of analytical curves obtained with and without the SPE step, with values expressed as a percentage. The SEF achieved was 640%. The entire extraction/elution cycle for one sample requires approximately 25 minutes. However, it would be straightforward to improve the operation time by using several columns simultaneously to extract different urine samples with a simple multichannel SPE. In the present case, eight microcolumns were used simultaneously for the analysis of real samples. This modification allowed 19–20 samples to be processed per hour. The analytical characteristics of the proposed method have been favorably compared with other published in the literature

4. CONCLUSIONS

A SPE procedure using MWCNTs as sorbent material has been developed for the extraction of Pb from human urine samples prior to ETAAS determination. Pb(II) ions were retained on oxidized MWCNTs and were successfully desorbed with a nitric acid solution (carryover was not observed in the subsequent analysis). Samples were measured by ETAAS and satisfactory analytical figures of merit were obtained.

The newly developed method has several advantages compared to other methods for the determination of Pb in urine:

(i) The elimination of the matrix permitted the measurement of lead in the urine samples without other pretreatment procedures;

- (ii) The absence of a matrix effect allowed direct measurement with a calibration graph instead of a standard addition procedure, and
- (iii) The low limit of detection of the proposed method ($\text{LOD} = 0.08 \mu\text{g L}^{-1}$) allowed the possibility of determining Pb in urine at low levels. The remarkable capability of MWCNTs to be used as SPE sorbents, for the elimination of matrix effects in ETAAS-based methods for lead determination at ultratrace levels, has been confirmed.

The present method has been applied to the determination of Pb in urine samples from healthy people with no history of exposure to lead. It has proven to be useful for the assessment of occupational exposure to lead.

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