Magnetic dispersive solid-phase extraction using a zeolite-based composite for direct electrochemical detection of lead(II) in urine using screen-printed electrodes

Elena Fernández[1], Lorena Vidal[1]*, Joaquín Silvestre-Albero[2] and Antonio Canals[1]*

[1]Departamento de Química Analítica, Nutrición y Bromatología e Instituto Universitario de Materiales, Universidad de Alicante, E-03080 Alicante, Spain.

[2]Laboratorio de Materiales Avanzados, Departamento de Química Inorgánica-Instituto Universitario de Materiales, Universidad de Alicante, E-03080 Alicante, Spain.

*Corresponding authors: Tel.: +34965909790; fax: +34965903697.

E-mail addresses: lorena.vidal@ua.es (L. Vidal), a.canals@ua.es (A. Canals).

Abstract

Magnetic dispersive solid-phase extraction (MDSPE) is combined with electrochemical detection by using a screen-printed carbon electrode modified with gold nanoparticles to determine lead(II). A zeolite-based magnetic composite was used as sorbent during sample preparation, thus combining the unique properties of zeolites as sorbent materials with the remarkable advantages provided by magnetic materials. Three different zeolite-based magnetic composites were initially investigated and characterized. $ZSM-5/Fe_2O_3$ treated with HNO_3 ($ZSM-5/Fe_2O_{3(tr)}$) was finally selected as sorbent. Lead was extracted from urine samples using $ZSM-5/Fe_2O_{3(tr)}$. After extraction, the phases were separated by using an external magnetic field. Subsequently, the magnetic

composite carrying the analyte was directly deposited onto the working electrode of a modified screen-printed carbon electrode for final electrochemical detection. Thereby, the elution and detection of Pb(II) were carried out in a single step. A multivariate approach was employed to optimize the experimental parameters affecting extraction. Under optimized conditions and at a typical working potential of -0.23 V (vs. Ag pseudo-reference electrode), response is linear in the 0 to 25 µg L^{-1} Pb(II) concentration range. The empirical limit of detection ranged from 1.0 to 2.0 µg L^{-1}. The method was used to analyze Pb(II)-spiked urine samples, and apparent recoveries ranged between 99 and 107%, with coefficients of variation of <20%.

1. Introduction

Magnetic dispersive solid-phase extraction (MDSPE) is a sample preparation technique based on the direct dispersion of a magnetic sorbent into the sample. After extraction, phases are easily separated by applying an external magnetic field and thereafter, target analytes can be desorbed using a proper eluent solvent or temperature for final determination[1–2]. This technique has received great interest due to the outstanding advantages provided by sorbents with magnetic properties. Magnetic sorbents synergistically combine excellent sorbent capacity with ease of handling by an external magnetic field, thus avoiding laborious

filtration or centrifugation steps for phase separation. This helps to reduce the overall operation time and also makes the procedure more easily portable and feasible for on-site extractions [1–2]. Many different magnetic sorbents have been employed in MDSPE, including inorganic oxides, porous polymers, molecularly imprinted polymers, biosorbents or carbon nanomaterials [1–2]. In addition, zeolite-based magnetic composites have been proposed for the extraction of organic compounds and metals from environmental, food and biological samples [3]. Zeolites are crystalline aluminosilicates that exhibit well-defined highly porous structures and possess a large surface area, high adsorption capacity and molecular selectivity, chemical and thermal stability and ion-exchange capacity; they are also low cost, widely available, and easily modifiable [3, 4]. Zeolite-based magnetic composites are based on natural or synthetic zeolites decorated with magnetic nanoparticles, namely Fe_2O_3 or Fe_3O_4 [3]. Thus, these composites acquire the unique sorbent properties of zeolites with the abovementioned advantages of magnetic materials (i.e., easy manipulation and recovery, short operating times).

Besides the advances in miniaturized extraction techniques, small-scale separation/detection systems are also fundamental to further development of fast, simple, environmentally friendly and portable analytical methods. Thus, several publications have proposed the combination of liquid-phase and solid-phase microextraction techniques with miniaturized detection systems, such as cuvetteless micro-spectrophotometry[5], μvolume-fluorospectrometry[6], ion mobility spectrometry[7], person-portable gas chromatography-mass spectrometry[8], laser-induced breakdown spectroscopy[9], and screen-printed electrodes (SPEs)[10–15] .

In particular, MDSPE has been coupled to SPEs to determine mercury[13], bisphenol A [14] and caffeine [15] in environmental waters [13, 14] and drinks[15]. In these methods, analytes were first adsorbed onto the corresponding magnetic composite (i.e., Fe_3O_4/poly(1,8-diaminonaphthalene), $CoFe_2O_4$/multi-walled carbon nanotubes, or Fe_3O_4/multi-walled carbon nanotubes) and subsequently eluted with a solvent[13–15] . When organic solvents were used for elution (e.g. methanol, ethanol), additional steps of solvent evaporation and reconstitution with a suitable electrolytic medium were necessary before electrochemical detection[14, 15]. Elution, solvent evaporation and reconstitution are the most time-consuming methodological steps and are thus considered a major drawback.

This work presents an analytical method in which MDSPE using a HNO_3 treated zeolite-based magnetic composite (ZSM-5/$Fe_2O_{3(tr)}$) is directly combined with SPEs as an electrochemical detection system. Lead determination in urine samples is the proposed model for analytical application. The single-step elution and electrochemical detection of lead provided a method using miniaturized systems in both sample preparation and the detection step. Lead was selected as the test analyte due to its toxicity and the need to monitor its presence in biological fluids in order to prevent and diagnose adverse health effects[16] . The main target of lead toxicity is the nervous system and, at high level exposure, it can severely damage the brain and kidneys, ultimately causing death [16]. Regardless of the exposure route (i.e., inhalation, ingestion, dermal absorption), lead is excreted primarily in urine and feces. Mean urinary lead levels in the unexposed population is reported to be 35 µg L^{-1} [17].

To the best of our knowledge, this is the first time that MDSPE using a zeolite-based magnetic composite has been directly combined with electrochemical detection with SPEs. Three different zeolite-based magnetic composites (i.e., ZSM-5/Fe$_2$O$_3$, Zn-ZSM-5/Fe$_2$O$_3$ and ZSM-5/Fe$_2$O$_{3(tr)}$) were initially characterized and compared for the intended application. Other parameters affecting the proposed extraction method were also optimized following a multivariate optimization strategy. Finally, our study evaluates the applicability of this method to determine lead at trace levels in urine samples.

2. Experimental part

2.1. Reagents and materials

A stock standard solution of Pb (1000 mg L^{-1} in 2% HNO$_3$) was obtained from High-Purity Standards (Charleston, SC, USA) (www.highpuritystandards.com). Aqueous working solutions were daily prepared in ultrapure water (resistivity of 18.2 MΩ cm at 25 °C) from a Millipore Direct System Q5^{TM} purification system from Millipore Ibérica S.A. (Madrid, Spain) (www.millipore.com). H$_3$PO$_4$ (85%) from Scharlab Chemie (Barcelona, Spain) (www.schalab.com) and KH$_2$PO$_4$ and K$_2$HPO$_4$ pro-analysis from Merck (Madrid, Spain) (www.merckgroup.com/es-es) were employed to prepare buffers during sample pH optimization. Reagent grade NaCl was supplied by Scharlab Chemie and used to study the effect of sample ionic strength. Fuming HCl (37%) from Merck was employed to prepare 0.1 and 1 M HCl solutions in ultrapure water.

ZSM-5 zeolite (CBV 3024E, SiO_2/Al_2O_3 mole ratio = 30) in the ammonium nominal cation form (NH_4^+-form) was purchased from Zeolist International (Conshohocken, PA, USA) (www.zeolyst.com/). For zeolite magnetization, $FeCl_3 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$ reagent grade were obtained from Sigma-Aldrich (Steinheim, Germany) (www.sigmaaldrich.com) and NaOH (97%, pellets) from Scharlab Chemie. A stock standard solution of Zn (1000 mg L^{-1} in 2% HNO_3) was obtained from High-Purity Standards and used to prepare 200 mg L^{-1} Zn solution in ultrapure water. High purity HNO_3 (65%) from Merck was used to prepare 4 M HNO_3 solution in ultrapure water.

Standard Au^{3+} tetrachloro complex (1.000 ± 0.002 g of $AuCl_4^-$ in 500 mL of 1 M HCl) was purchased from Merck. Solutions of 1 mM $AuCl_4^-$ were prepared by proper dilution of this standard solution in 0.1 M HCl and used to obtain screen-printed carbon electrodes modified with gold nanoparticles (SPCnAuEs)[18].

A special extraction vessel was lab-designed (**Fig. S1**). The vessel consisted of a glass tube (9.5 cm height, 15 mL capacity) with two open ends, both with screw-caps. The wide upper-end was used for sorbent and sample insertion; and subsequent sample withdrawal after extraction. The narrow lower end was designed to fit electrode size, in order to easily transfer the enriched sorbent from the extraction vessel to the SPCnAuE for final electrochemical detection.

A disc-shaped neodymium (Nd) magnet (N45, 45 mm diameter, 30 mm height) with a nickel-plated (Ni-Cu-Ni) coating from Supermagnete (Gottmadingen, Germany) (www.supermagnete.es) was used to handle the magnetic sorbent during synthesis and

for phase separation after MDSPE. A rod-shaped Nd magnet (N48, 3 mm diameter, 8 mm height) with a nickel-plated (Ni-Cu-Ni) coating from Supermagnete was employed to fix the magnetic sorbent onto the working electrode of the SPCnAuE.

2.2. Urine samples

Urine samples were collected from human volunteers in sterilized containers. All samples were filtered before use with a 0.45 µm pore-size nylon filter and stored at 4 ºC. Initial analysis under optimized conditions of the proposed method revealed non-detectable levels of Pb in all analyzed urine samples.

2.3. Instrumentation and electrodes

X-ray photoelectron spectroscopy (XPS) was used for the chemical characterization of Fe_2O_3 in ZSM-5/Fe_2O_3, Zn-ZSM-5/Fe_2O_3 and ZSM-5/$Fe_2O_{3(tr)}$ composites. XPS measurements were performed with an automatic K-Alpha spectrometer from Thermo-Scientific (Waltham, MA, USA) (www.thermofisher.com). The spectra were recorded with pass energy of 50 eV, x-ray spot size of 400 µm, and step size of 0.1 eV. The C 1s peak was used as a reference for charge correction.

Apparent surface area (S_{BET}), micropore volume (V_{N2}) and total pore volume (V_{total}) of ZSM-5 and the different magnetic composites were estimated from the N_2 adsorption data at -196 ℃. N_2 isotherms were performed in a home-made manometric device designed and constructed by the LMA group, currently sold by Gas to Materials Technology (www.g2mtech.com). Before the adsorption experiments, samples were degassed under ultra-high vacuum conditions at 250 ℃ for 12 h.

An Agilent 720-ES inductively coupled plasma optical emission spectrometer (ICP-OES) (Melbourne, Australia) was employed for Zn determination during sorbent characterization using the emission line Zn II 213.857 nm.

A Multi Autolab/M101 Potentiostat/Galvanostat from Metrohm Autolab B.V. (Utrecht, The Netherlands) (www.metrohm-autolab) controlled by NOVA software version 1.10 was used for electrochemical experiments. Screen-printed carbon electrodes (SPCEs) (ref. DRP-110) with three-electrode configuration were purchased from Metrohm DropSens (Oviedo, Spain) (www.dropsense.com). The working disk-shaped electrode, 4 mm in diameter, and the counter electrode were made of carbon ink whereas the pseudo-reference electrode was made of silver. Specific connectors obtained from DropSens (ref. DRP-DSC) were used to connect SPCEs to the potentiostat. To obtain SPCnAuEs, the procedure developed by Martínez-Paredes et al. [18] and used in previous publications for Hg determination [19] was employed (see Electronic Supplementary Material for more details).

2.4. Synthesis of ZSM-5/Fe$_2$O$_3$, Zn-ZSM-5/Fe$_2$O$_3$ and ZSM-5/Fe$_2$O$_{3(tr)}$

ZSM-5/Fe_2O_3 and Zn-ZSM-5/Fe_2O_3 composites were synthesized according to previous publications [20–24] (see Electronic Supplementary Material for more details).

ZSM-5/$Fe_2O_{3(tr)}$ composite was synthesized in the same way as Zn-ZSM-5/Fe_2O_3 composite but omitting the stirring in Zn aqueous solution. Namely, 10 g of ZSM-5/Fe_2O_3 was rinsed three times with 4 M HNO_3 and several times with deionized water until neutral pH washing waters were obtained. HNO_3 was selected to rinse the composite according to previous publications [21–24], and considering the easier dissolution of Fe_2O_3 nanoparticles in HCl solutions. Finally, the composite was dried at 120 ºC for 3 h.

2.5. Magnetic dispersive solid-phase extraction

Under optimized conditions, 10 mg of ZSM-5/$Fe_2O_{3(tr)}$ composite was placed in the lab-designed extraction vessel (**Fig. S1**) through the wide upper end. Then, 15 mL of aqueous standard or sample solution (pH 4.8) was added and the mixture was shaken vigorously for 1 min. After extraction, the disc-shaped Nd magnet was used to attract the magnetic composite to the narrow bottom end of the tube and the aqueous phase was easily removed through the wide upper end using a glass pipette. Then, the sorbent was washed three times with 1 mL of ultrapure water. This step was especially important during urine sample analysis since the

lead signal significantly decreased if sorbent washing was omitted. After washing, the enriched magnetic sorbent was deposited onto the working electrode of the SPCnAuE using the small rod-shaped Nd magnet.

2.6. Electrochemical detection

Lead is well-known to suffer from underpotential deposition processes on gold electrodes [25]. There are previous in-depth studies of the electrochemical behavior of lead on SPCnAuE [18]; therefore, no further discussion will be included here. Square-wave anodic stripping voltammetry (SWASV) was selected as the electroanalytical technique. During initial experiments, a standard solution of 100 µg L^{-1} of Pb in 0.1 M HCl was employed to optimize SWASV parameters.

After analyte extraction and sorbent deposition onto the working electrode of the SPCnAuE, 40 µL of 1 M HCl aqueous solution was poured over the sorbent, covering the working, counter and reference electrodes for final SWASV detection. Thus, in a single step we performed lead elution from the sorbent into the acidic aqueous solution, and electrochemical detection with SPCnAuE, as depicted in **Fig. 1**. Lead was preconcentrated over SPCnAuE by applying a constant potential of -0.4 V (vs Ag pseudo-reference electrode) for 300 s. Thereafter, the potential was recorded between -0.4 V and 0.0 V (vs Ag pseudo-reference electrode) at a frequency of 75 Hz, amplitude of 25 mV and step potential of 2 mV (i.e., scan rate 150 mV s^{-1}). An anodic peak corresponding to the reoxidation of Pb appeared at approximately -0.23 V (vs Ag pseudo-reference electrode) and the height of this

peak was employed for analyte quantification. **Fig. 2** shows a diagram of the overall procedure. It should be mentioned that a reoxidation peak attributed to Fe_2O_3 nanoparticles appeared at more positive potentials and therefore did not interfere with Pb determination.

2.7. Data processing

A two-step multivariate optimization strategy, using Plackett-Burman and central composite design, was performed to determine the optimum conditions of the proposed extraction procedure. The statistical software NEMRODW® ("New Efficient Methodology for Research using Optimal Design") from LPRAI (Marseille, France) was used to design and build the experimental matrices and evaluate the results. The peak height of Pb at -0.23 V was used as response function for optimization.

3. Results and discussion

3.1. Sorbent selection and characterization

The extraction performance of $ZSM-5/Fe_2O_3$ and $Zn-ZSM-5/Fe_2O_3$ composites was firstly compared using 10 µg L^{-1} Pb aqueous standard solutions. Extractions were carried out in triplicate with both sorbents and results are shown in **Fig. S2.** As can be observed, $Zn-ZSM-5/Fe_2O_3$ showed better results than $ZSM-5/Fe_2O_3$. Previous publications have reported that the modification of

analcime [24] and clinoptilolite [22, 23] with Zn seems to lead to saturated zeolites in which Zn fills available sites and, consequently, the entry of other metals into the pores is hindered and further adsorptions can take place only on the surface. This may explain the better performance of Zn-ZSM-5/Fe_2O_3 composite, since Pb(II) adsorption can occur only on the composite surface, thus facilitating elution and leading to a stronger analytical signal.

To evaluate this assumption, the textural properties of the synthesized sorbents (ZSM-5/Fe_2O_3 and Zn-ZSM-5/Fe_2O_3) were evaluated using N_2 adsorption at cryogenic temperatures. **Fig. S3** shows the N_2 adsorption isotherms at -196 ºC in the relative pressure range (p/p_0) from 0 up to 1. The isotherm of the original zeolite is also included for the sake of comparison. As it can be appreciated, the original ZSM-5 material exhibits a type I isotherm, according to the IUPAC classification, attributed to a purely microporous material. The incorporation of Fe_2O_3 nanoparticles gives rise to a significant reduction in N_2 adsorption capacity at low relative pressures (p/p_o < 0.1). This behavior must be attributed to the partial blocking of porosity by the incorporated Fe_2O_3 nanoparticles (25 wt.%). This blocking of porosity is also reflected in the BET surface area, with a reduction of ca. 28% compared to the parent ZSM-5 zeolite (430 m^2 g^{-1}), and in the micropore volume (V_{N2}). Contrary to previous assumptions described in the literature, the incorporation of Zn has hardly any effect on the porous structure of magnetic zeolite, in close agreement with the small percentage of Zn incorporated (ca. 0.8 wt.%). The BET surface area does not show important variation, i.e. only a slight increase can be observed, which is most probably due to the removal of some Fe_2O_3 nanoparticles during the washing step with

HNO_3. In both cases, N_2 adsorption isotherms exhibit a sudden increase in N_2 adsorbed at high relative pressures ($p/p_0 > 0.8$) due to N_2 condensation in the interparticle spaces. After the modification with Fe_2O_3, large crystal aggregates are formed, in contrast to the commercial fine powder. Based on these results, parameters other than blocking porosity by Zn must be considered in order to explain the high efficiency of the Zn-ZSM-5/Fe_2O_3 composite for Pb extraction.

As mentioned in Section S2 (Electronic Supplementary Material), after stirring the ZSM-5/Fe_2O_3 with a 200 mg L^{-1} Zn solution, the resulting composite was washed three times with 4 M HNO_3 and several times with deionized water. All the washing waters were analyzed using ICP-OES, and results (not shown) revealed that about 69% of the initially adsorbed Zn was eluted during the washing process, thus confirming that the Zn-ZSM-5/Fe_2O_3 composite was not Zn-saturated. These results again indicate that the effect of Zn, if it exerts one, is insufficient to justify the performance of the Zn-ZSM-5/Fe_2O_3 composite.

A closer look at the conditions used to prepare the Zn-ZSM-5/Fe_2O_3 composite shows that, in addition to the Zn impregnation step, there is a washing step with HNO_3, which is not applied for the ZSM-5/Fe_2O_3 composite. To clarify the role of acid treatment in extraction performance, a third composite was prepared applying the acid treatment to the magnetic ZSM-5/Fe_2O_3 (composite called ZSM-5/$Fe_2O_{3(tr)}$).

In the first step, ZSM-5/$Fe_2O_{3(tr)}$ was evaluated for Pb extraction under the same conditions described above. Under the same experimental conditions, the extraction performance of the ZSM-5/$Fe_2O_{3(tr)}$ composite (**Fig. S4**) was greatly improved compared to

the non-oxidized ZSM-5/Fe$_2$O$_3$ counterpart (**Fig. S2**), and rather similar to that observed for Zn-ZSM-5/Fe$_2$O$_3$ (**Fig. S2**). These results suggest that the HNO$_3$ washing treatment, rather than the incorporation of Zn, seems to be the crucial step required to improve the extraction performance of magnetic zeolites. In the second step, the effect of acid treatment on the nature of the surface chemical species (e.g., oxidation state of iron species) was evaluated using XPS analysis. These analyses were performed for ZSM-5/Fe$_2$O$_3$ and Zn-ZSM-5/Fe$_2$O$_3$ composites at three different stages: i) after preparation, ii) after Pb extraction, and iii) after electrochemical detection. XPS is a surface analysis technique that provides information about the composition and chemical state of sorbents under different environments. For instance, XPS peaks for Fe $2p_{3/2}$ are highly sensitive to the ionic states of Fe, i.e. their position (binding energy) changes with the oxidation state [26]. Furthermore, the presence of satellite peaks for Fe (II) and Fe (III) is also very useful to evaluate the ionic state. As observed in **Fig. S5** the XPS spectra of the synthesized sorbents (i.e., after preparation and before use) are rather similar, independently of Zn presence, with a main contribution at 711 eV and the characteristic satellite peak at 718 eV, attributed to Fe^{3+} in Fe$_2$O$_3$. In addition, both samples exhibit important asymmetry in the high binding energy region, with a contribution at 713 eV, characteristic also of Fe$_2$O$_3$. After Pb extraction, the ZSM-5/Fe$_2$O$_3$ zeolite preserves these contributions, including the satellite peak at 718 eV, whereas the Zn-ZSM-5/Fe$_2$O$_3$ zeolite does not. For this specific sample the satellite disappears and the conventional contributions are shifted to higher binding energy. Furthermore, two new contributions appear at 710 eV and 716 eV, corresponding to Fe^{2+} species. The presence of these Fe^{2+} species (main

14

contribution at 710 eV and satellite at 716 eV) is also evident in the samples after electrochemical detection, these changes being more pronounced for the Zn-ZSM-5/Fe_2O_3 zeolite. Apparently, the addition of an acid treatment with HNO_3 after Zn incorporation dramatically affects the reduction ability of the iron surface species, i.e. acid treated nanoparticles are prone to suffer extended redox processes upon extraction and detection compared to the non-treated sample. This explains the differences in extraction performance. A similar behavior was observed for ZSM-5/$Fe_2O_{3(tr)}$ composite (not shown here). Thus, given the results, ZSM-5/$Fe_2O_{3(tr)}$ was finally selected as sorbent for the proposed method, since it showed a better extraction performance than ZSM-5/Fe_2O_3 and the presence of Zn was proven to have a negligible effect.

After selecting ZSM-5/$Fe_2O_{3(tr)}$ as sorbent, synthesis reproducibility was investigated. To this end, we compared the extraction performance of three ZSM-5/$Fe_2O_{3(tr)}$ composites synthesized in parallel. Extractions from 10 µg L^{-1} Pb aqueous standard solutions were done in triplicate with each sorbent; results are given in **Fig. S4**. As shown, the extractions with the three ZSM-5/$Fe_2O_{3(tr)}$ composites provided the same response, thus demonstrating the reproducibility of the synthesis procedure for the intended application.

Finally, ZSM-5/$Fe_2O_{3(tr)}$ composite reusability was also investigated, and results revealed that the sorbent cannot be reused in consecutive extractions (see Section S3 in the Electronic Supplementary Material)

Due to the fact that the final application of the proposed method was Pb determination in urine samples, we cross-checked the different studies carried out in this section (i.e., extraction performance of the different composites, synthesis reproducibility and sorbent reusability) using urine samples (results not shown). Analogous results were obtained.

3.2. Magnetic dispersive solid-phase extraction optimization

3.2.1. Plackett-Burman design

A Plackett-Burman design was used to construct the experimental matrix during screening studies [27].Four factors were studied in eight runs: sorbent amount; sample pH; extraction time, and the concentration of the HCl aqueous solution used for Pb elution and as electrolyte during SWASV (hereinafter called HCl concentration). **Table S1** shows the levels examined for each factor and **Table S2** shows Plackett-Burman-design experimental matrix.

The obtained data were evaluated by ANOVA. The adjustment made to the proposed model, expressed as r^2 value, was 0.928. Results were visualized with the Pareto chart shown in **Fig. S7**. Sample pH and extraction time showed significant negative effects. The negative effect of sample pH proved to be in accordance with a previous publication [21] and may relate to the formation of Pb insoluble species (i.e., $Pb(OH)_2$) at high pH values. Noteworthy results related to the significantly negative effect of extraction time. It is generally expected that after a certain time period, analyte adsorption reaches equilibrium after which the signal

remains constant. However, it is also known that competitive processes can be found in miniaturized solid-phase extraction techniques due to a limited available sorbent surface area [28]. Thus, the decreased extraction efficiency at longer extraction times may relate to the competitive displacement of Pb^{2+} by other cations present in the sample solution (e.g., K^+, H^+).

According to the screening results and in order to clarify the effect of the extraction time, both sample pH and extraction time were included in the next optimization step. Sorbent amount and HCl concentration showed non-significant positive effects in the studied interval; therefore they were fixed at the corresponding highest levels in subsequent experiments, namely: amount of sorbent, 10 mg; and HCl concentration, 1 M.

3.2.2. Central composite design

Central composite design (CCD) [27] was used to evaluate and optimize main effects, interaction effects and quadratic effects of sample pH and extraction time. **Table S3** shows the low and high levels, the central and star points of the studied factors, whereas **Table S4** shows the matrix of CCD experiments.

The data obtained were also analyzed by ANOVA. The adjustment to the quadratic regression model , expressed as r^2 value, was 0.81. CCD results are shown by means of the response surface in **Fig. S8**. In accordance with the screening results, analyte signal was lower at the highest pH values. In addition, a signal decrease was also observed under the most acidic conditions, which

is explained by considering that the lower the pH, the higher the amount of H^+ able to compete with Pb^{2+} for cation exchange sites. Finally, the optimum pH value was established at 4.8, where the response surface clearly showed a maximum. Regarding extraction time, the system response generally showed a slight increase for longer extraction times (**Fig. S8**). However, the obtained signals were not considered significantly different within the range studied, thus confirming that equilibrium was rapidly attained and discarding any important competitive process. Finally, extraction time was fixed at the lowest level (i.e., 1 min), resulting in a fast extraction procedure.

3.2.3. Sample ionic strength

Considering the high salt content of urine [29], the effect of sample ionic strength was evaluated by adding different amounts of NaCl (i.e., 1, 10 and 35% w/v) to 5 µg L^{-1} Pb aqueous standard solutions. Compared to salt-free solutions, a significant signal decrease was observed when extractions were carried out from 1 and 10% NaCl solutions, whereas no signal was obtained when 35% NaCl solutions were analyzed (results not shown). The results were easily explained considering that the higher the NaCl concentration, the higher Na^+ cations that can compete with Pb^{2+} for cation exchange sites, thus decreasing extraction efficiency. It should be mentioned that sample ionic strength was studied separately here and not included in the previous multivariate optimization (i.e., Plackett-Burman design and CCD) because preliminary studies revealed that its large negative effect upon the

18

extraction covered up the effect of the other experimental factors investigated. Finally, it should also be emphasized that the ionic strength is an uncontrollable factor when urine is considered since real samples contain different amounts of salts [29].

3.3. Analytical figures of merit

Different matrix effects were found when analyzing different urine samples. This fact was mainly associated with their different salt content (see Section 3.2.3), although the presence of organic matter can also affect the extraction yield. Consequently, standard addition calibration was adopted to evaluate the quality analytical parameters of the method. Calibration plots were constructed in three different urine samples using standards of six concentration levels from 0 to 25 µg L^{-1}. Good linearity was obtained with correlation coefficient values (r) ranging from 0.992 to 0.998. The Student's t-test was applied to assess the linearity showing values ranging from 16.02 (r = 0.992; N=6) to 31.19 (r = 0.998; N=6), thus rejecting the null hypothesis of non-linear correlation for a 5% significance level and 4 degrees of freedom ($t_{0.05,4}$ = 2.78)[30]. The sensitivity of the analytical method estimated by the slope of standard addition calibration plots ranged from (1.25 ± 0.04) to (1.49 ± 0.09) µA µg^{-1} L. The enrichment factor was calculated as the ratio of the sensitivity obtained with and without MDSPE and was between 11 and 13. The limit of detection (LOD) was determined empirically, progressively measuring more diluted concentrations of the analyte. Thereby, three different urine samples were spiked with progressively lower Pb amounts and analyzed using the method. The LOD was the lowest concentration

whose signal was clearly distinguished from blank. The empirical LOD values ranged between 1.0 and 2.0 µg L^{-1}. Additionally, statistical LOD was calculated using the standard deviation of urine samples spiked at very low analyte concentrations. Values between 0.8 and 1.6 µg L^{-1} were obtained for the statistical LOD, confirming the results obtained by the empirical approach. These values were lower than the normal mean value of Pb (i.e., ~35 µg L^{-1}) found in the urine of people without evident occupational, medicinal, or other unusual sources of exposure [17]. For comparative purposes, **Table 1** shows previous electrochemical methods to determine lead in urine samples. As can be observed, the LOD obtained in this work fell within the range of those reported in previous publications, although it should be noticed that previous LODs were evaluated in diluted urine samples or in aqueous solutions. In addition, most methods employed bulky, complex and non-disposable electrodes, which required time-consuming cleaning steps between measurements. In another publication, MDSPE was successfully combined with a novel modified glassy carbon electrode to determine Pb, Cu and Cr in water and food samples [31]. After MDSPE, metals were eluted from the magnetic sorbent using 0.5 mL of 1 M HCl solution and thereafter, the eluent solvent was transferred to a voltammetric cell for final electrochemical detection with a traditional bulky electrode. Thereby, a higher volume of the eluent solvent was necessary and elution and detection were performed separately.

The main limitations of the present method are related to the synthesis and non-reusability of the sorbent and the use of a special extraction vessel. The synthesis of ZSM-5/Fe$_2$O$_{3(tr)}$ is easy to handle and does not require especial skills but it is time-

consuming and normally takes over 24 h. In addition, the sorbent cannot be reused in subsequent extractions, which also limits throughput. The use of a lab-designed and non-commercially available extraction vessel also hinders widespread application of the method. Finally, it should be mentioned that zeolites possess an excellent cation exchange capacity and other cations commonly present in urine (e.g., K^+, Na^+) could be extracted with the proposed sorbent. Nevertheless, the use of SPCnAuEs and the underpotential deposition process for lead determination ensure the quantification of this metal and contribute to improving the selectivity of this method. Furthermore, zeolites are also able to extract organic compounds [20] although the adsorption of organic urine components such as creatinine, uric acid and proteins in the pores of the zeolite would be sterically hindered; therefore, further modifications of the sorbent (e.g., with surfactants) would be necessary for their efficient extraction via other sorbent-analyte interactions [3].

3.4. Analysis of urine samples

The method was applied to determine Pb in real urine samples taken from three healthy volunteers. The pH of the samples was measured and ranged between 5.6 and 6.5. Thus, all samples were acidified to the optimum extraction pH value (i.e., 4.8) before use. To adjust the pH, a few drops of a phosphate buffer were added and, therefore, pH adjustment did not significantly dilute the samples. The latter were then analyzed and their Pb content was found to be below the LOD of the present method.

Consequently, the three urine samples were spiked at two different levels (i.e., 7 and 20 µg L^{-1} of Pb) before filtration, and analyzed using standard addition calibration. To prepare the standard addition calibration, six aliquots of 14925 µL of each spiked urine sample were placed in the lab-designed extraction vessel and subsequently spiked with 0, 15, 30, 45, 60 and 75 µL of a 5 mg L^{-1} Pb aqueous solution, to which 75, 60, 45, 30, 15 and 0 µL of ultrapure water were added, respectively, in order to reach an equal final volume (15 mL). Thereafter, the method was applied (see Section 2.5 and 2.6). The standard addition calibration plots of a urine sample (Urine 1) spiked at 7 and 20 µg L^{-1} of Pb are shown in **Fig. S9**. Results shown in **Table 2** reveal that there were non-significant differences between the concentrations added and those found in the three urine samples, with apparent recoveries ranging between 99 and 107% and CV between 9 and 19%. Therefore, matrix effects were corrected with the proposed methodology and its applicability to determine Pb at trace levels in urine samples was proven.

4. Conclusions

MDSPE using a zeolite-based magnetic composite has been directly and successfully combined with SPCnAuEs to determine Pb at trace levels in urine samples. The procedure used miniaturized systems in both sample preparation and detection steps, combining the advantages of MDSPE (e.g., low amount of sample, sorbent and solvent consumption, easy sorbent handling and recovery, short extraction time), with those advantages provided by SPCnAuEs (e.g., easy, rapid and sensitive Pb determination

using affordable instrumentation). In addition, Pb elution from ZSM-5/Fe$_2$O$_{3(tr)}$ and subsequent electrochemical detection were performed jointly in a single step, thus further simplifying and shortening the duration of this procedure.

Three different zeolite-based magnetic composites were initially compared and characterized, revealing that the acid treatment with HNO$_3$ was crucial for the better extraction performance of Zn-ZSM-5/Fe$_2$O$_3$ and ZSM-5/Fe$_2$O$_{3(tr)}$ in comparison with ZSM-5/Fe$_2$O$_3$, and that the presence of Zn did not exert a significant effect.

Finally, we should highlight that zeolites are inexpensive materials with unique sorbent properties, which can be of synthetic or natural origin, and are thus widely available. In addition, they are easy and quick to modify in order to obtain zeolite-based magnetic composite. Furthermore, a wide variety of SPEs are currently available at low cost, and are also easily modifiable. Thus, the proposed direct combination (i.e., MDSPE using zeolite-based composites and SPEs) offers great potential and should be further explored and extended to different applications for rapid, cheap and de-centralized measurements.

Acknowledgements

References

.

1. Tolmacheva VV, Apyari VV, Kochuk EV, Dmitrienko SG (2016) Magnetic adsorbents based on iron oxide nanoparticles for the extraction and preconcentration of organic compounds. J Anal Chem 71:321–338.

2. Hemmati M, Rajabi M, Asghari A (2018) Magnetic nanoparticle based solid-phase extraction of heavy metal ions: A review on recent advances. Microchim Acta 185:160.

3. Baile P, Fernández E, Vidal L, Canals A (2019) Zeolites and zeolite-based materials in extraction and microextraction techniques. Analyst 144:366–387.

4. Auerbach SM, Carrado KA, Dutta PK (2003) Handbook of zeolite science and technology. Marcel Dekker, New York.

5. Pillai AKKV, Jain A, Verma KK (2010) Headspace single-drop microextraction and fibre optics-based cuvetteless micro-

spectrophotometry for the determination of chloride involving oxidation with permanganate. Talanta 80:1816–1822.

6. Costas-Mora I, Romero V, Pena-Pereira F, Lavilla I, Bendicho C (2011) Quantum dot-based headspace single-drop microextraction technique for optical sensing of volatile species. Anal Chem 83:2388–2393.

7. Sun T, Wang D, Tang Y, Xing X, Zhuang J, Cheng J, Du Z (2019) Fabric-phase sorptive extraction coupled with ion mobility spectrometry for on-site rapid detection of PAHs in aquatic environment. Talanta 195:109–116.

8. Foo Y, Dandan W, Shellie RA, Sciarrone D, Marriott PJ (2019) Rapid plant volatiles screening using headspace SPME and person-portable gas chromatography – mass spectrometry. Chromatographia 82:297–305.

.

9. Ruiz FJ, Ripoll L, Hidalgo M, Canals A (2019) Dispersive micro solid-phase extraction (DµSPE) with graphene oxide as adsorbent for sensitive elemental analysis of aqueous samples by laser induced breakdown spectroscopy (LIBS). Talanta 191:162–170.

10. Ahmar H, Fakhari AR, Tabani H, Shahsavani A (2013) Optimization of electromembrane extraction combined with differential pulse voltammetry using modified screen-printed electrode for the determination of sufentanil. Electrochim Acta 96:117–123.

11. Fernández E, Vidal L, Iniesta J, Metters JP, Banks CE, Canals A (2014) Screen-printed electrode-based electrochemical

detector coupled with in-situ ionic-liquid-assisted dispersive liquid-liquid microextraction for determination of 2,4,6-trinitrotoluene. Anal Bioanal Chem 406:2197–2204.

12.   Silva RM., Lima LC, Gaubeur I, Suffredini HB (2017) On the use of dispersive liquid-liquid microextraction combined with organic/water interface. Electroanalysis 29:259–263.

13.   Tootoonchi A, Davaraniz SSH (2016) Extraction of Hg(II) ions from aqueous solution by a magnetic nano sorbent followed by determination through stripping differential pulse voltammetry on commercial screen-printed kits. J Electrochem Soc 163:B366–B372.

14.   Aslihan A, Filik H (2018) $CoFe_2O_4$ -MWCNTs modified screen printed carbon electrode coupled with magnetic $CoFe_2O_4$ -MWCNTs based solid phase microextraction for the detection of bisphenol A. Curr Nanosci 14:199–208.

15.   Filik H, Asl A (2018) Conducting polymer modified screen-printed carbon electrode coupled with magnetic solid phase microextraction for determination of caffeine. Food Chem 242:301–307.

.

16.   Theakston F (2001) Air quality guidelines for Europe. WHO Regional Publications, European Series, No 91. http://www.euro.who.int/__data/assets/pdf_file/0005/74732/E71922.pdf. Accessed 22 November 2019.

17.   Goldwater LJ, Hoover AW, Yuh N (1967) An international study of "Normal" levels of lead in blood and urine. Arch Environ Health 15:60–63.

18.   Martínez-Paredes G, González-García MB, Costa-García A (2009) In situ electrochemical generation of gold nanostructured screen-printed carbon electrodes. Application to the detection of lead underpotential deposition. Electrochim Acta 54:4801–4808.

19.   Fernández E, Vidal L, Costa-García A, Canals A (2016) Mercury determination in urine samples by gold nanostructured screen-printed carbon electrodes after vortex-assisted ionic liquid dispersive liquid-liquid microextraction. Anal Chim Acta 915:49–55.

20.   Fernández E, Vidal L, Canals A (2016) Zeolite/iron oxide composite as sorbent for magnetic solid-phase extraction of benzene, toluene, ethylbenzene and xylenes from water samples prior to gas chromatography–mass spectrometry. J Chromatogr A 1458:18–24

21.   Baile P, Vidal L, Aguirre MÁ, Canals A (2018) A modified ZSM-5 zeolite/Fe_2O_3 composite as a sorbent for magnetic dispersive solid-phase microextraction of cadmium, mercury and lead from urine samples prior to inductively coupled plasma

optical emission spectrometry. J Anal At Spectrom 33:856–866.

22. Rostampour L, Taher MA (2008) Determination of trace amounts of vanadium by UV-vis spectrophotometric after separation and preconcentration with modified natural clinoptilolite as a new sorbent. Talanta 75:1279–1283.

23. Malekpour A, Hajialigol S, Taher MA (2009) Study on solid-phase extraction and flame atomic absorption spectrometry for the selective determination of cadmium in water and plant samples with modified clinoptilolite. J Hazard Mater 172:229–233.

24. Saljooghi AS, Saljoghi ZS (2012) Natural analcime zeolite modified with 2,3,5,6-tetra(2-pyridyl)pyrazine for preconcentration and determination of trace amounts of cadmium by flame atomic absorption spectrometry. Toxicol Ind Health 28:771–778.

25. Herrero E, Buller LJ, Abruña HD (2001) Underpotential deposition at single crystal surfaces of Au, Pt, Ag and other materials. Chem Rev 101:1897–930.

26. Yamashita T, Hayes P (2008) Analysis of XPS spectra of Fe^{2+} and Fe^{3+} ions in oxide materials. Appl Surf Sci 254:2441–2449.

27. Montgomery DC (2009) Design and Analysis of Experiments, 7th ed. Wiley, New Jersey

28. Pawliszyn J (2012) Theory of solid-phase microextraction. J Chromatogr Sci 38:270–278.

29. Rose C, Parker A, Jefferson B, Cartmell E (2015) The characterization of feces and urine: A review of the literature to inform

advanced treatment technology. Crit Rev Environ Sci Technol 45:1827–1879.

30. Miller JN, Miller JC (2005) Statistics and Chemometrics for Analytical Chemistry, 5th ed. Pearson Prentice Hall, London.

31. Ghorbani M, Pedramrad T, Aghamohammadhasan M, Seyedin O (2019) Simultaneous clean-up and determination of Cu (II), Pb (II) and Cr (III) in real water and food samples using a magnetic dispersive solid phase microextraction and differential pulse voltammetry with a green and novel modified glassy carbon electrode. Microchem J 147:545–554.

32. Kefala G, Economou A (2006) Polymer-coated bismuth film electrodes for the determination of trace metals by sequential-injection analysis / anodic stripping voltammetry. Anal Chim Acta 576:283–289.

33. Yantasee W, Lin Y, Hongsirikarn K, Fryxell GE, Addleman R, Timchalk C (2007) Sensors for the detection of lead and other toxic heavy metals: the next generation of personal exposure biomonitors. Environ Health Perspect 115:1683–1690.

34. Yantasee W, Hongsirikarn K, Warner CL, Choi D, Sangvanich T, Toloczko MB, Warner MG, Fryxell GE, Addleman RS, Timchalk C (2008) Direct detection of Pb in urine and Cd, Pb, Cu, and Ag in natural waters using electrochemical sensors immobilized with DMSA functionalized magnetic nanoparticles. Analyst 133:348–355.

35. Choi S, Kim D, Jung O, Shim Y (2015) A disposable chronocoulometric sensor for heavy metal ions using a diaminoterthiophene-modified electrode doped with graphene oxide. Anal Chim Acta 892:77–84.

Figure captions

Fig. 1 Schematic representation showing the elution of lead from the magnetic sorbent deposited on the electrode surface.

Fig. 2 Scheme of the overall analytical method.

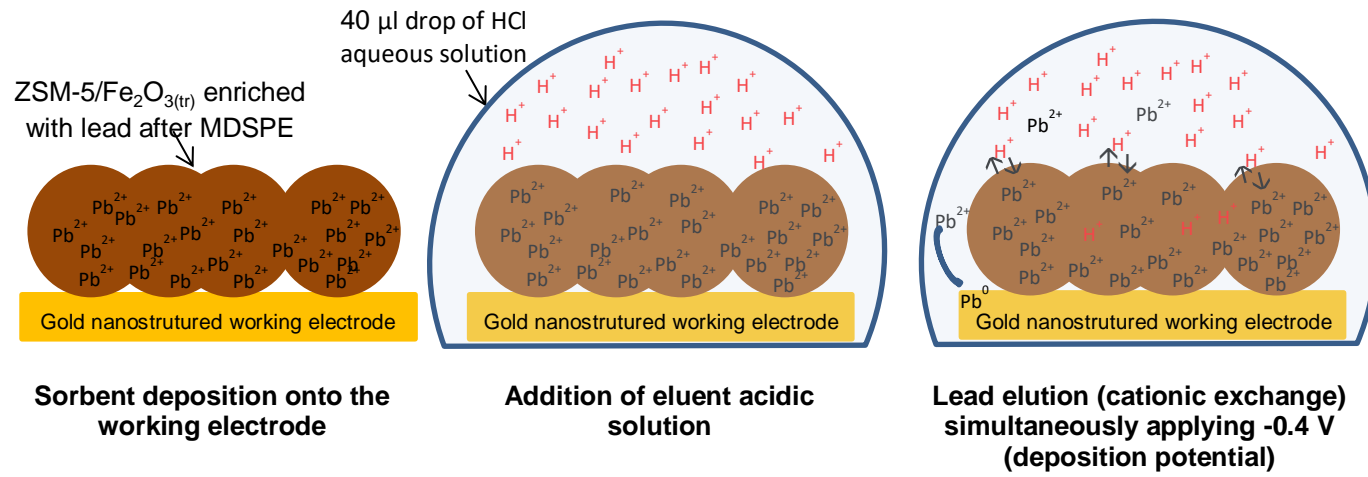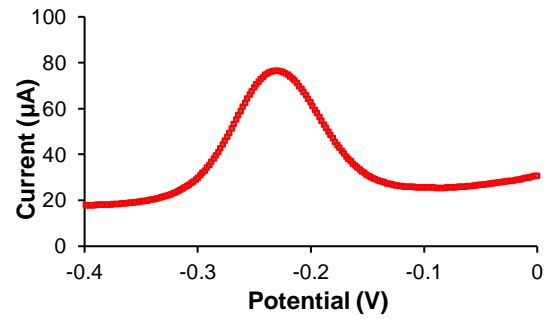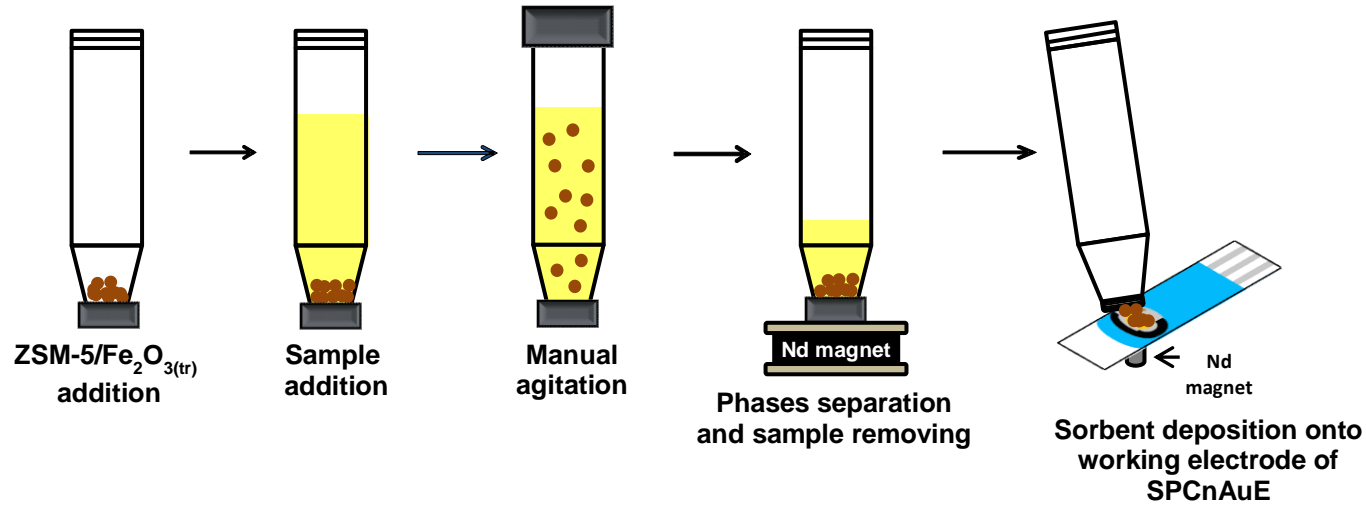**Fig. 1**

ZSM-5/Fe$_2$O$_{3(tr)}$ enriched with lead after MDSPE

40 µl drop of HCl aqueous solution

Sorbent deposition onto the working electrode

Addition of eluent acidic solution

Lead elution (cationic exchange) simultaneously applying -0.4 V (deposition potential)

Fig. 2

ZSM-5/Fe$_2$O$_{3(tr)}$ addition → Sample addition → Manual agitation → Phases separation and sample removing (Nd magnet) → Sorbent deposition onto working electrode of SPCnAuE (Nd magnet)

Addition of 40 µL 1M HCl aqueous solution (Nd magnet)

Electrochemical detection by SWASV

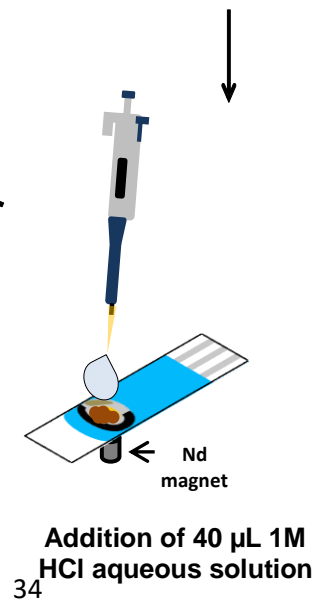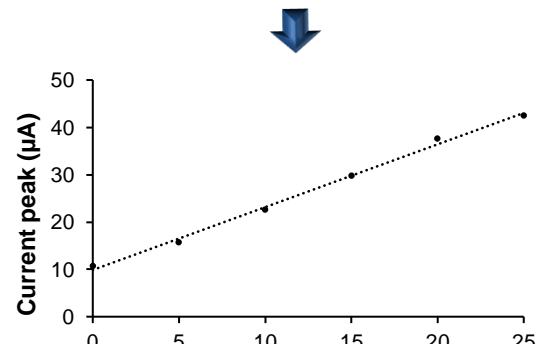Current (µA) vs Potential (V)

Current peak (µA)

34

Table 13. Comparison of different electrochemical methods to determine lead in urine samples.

Electrode	Sample pretreatment	Electroanalytical technique (deposition time)	LOD	Ref.
Polymer-coated bismuth film (in a SIA system)	Urine dilution 1:4 with 1 M acetate buffer (pH=4.5)	SWASV (165 s)	2 µg L^{-1} (in aqueous sample)	[32]
Hg film (in a FIA system)	Urine dilution (10-50%), 10 min incubation, acidification with 1 M HCl and filtration through 5000 NMWL cut-off ultrafilters	SWASV (110 s)	0.44 µg L^{-1} (in sample containing 50% vol. of urine)	[33]
10 wt% IDAA-SAMMS-CNT paste	Urine dilution (25%)	SWASV (300 s)	1 µg L^{-1} (in sample containing 25% vol. of urine)	[33]
Electromagnetic GCE	Urine dilution (25%), 90 s vortex mixing in 0.1 g L^{-1} DMSA-Fe$_3$O$_4$ nanoparticles suspension with the electrode tip immersed at open circuit	SWASV (60 s)	2.5 µg L^{-1} (in sample containing 25% vol. of urine)	[34]
GO/DTT/Nafion-modified SPCE	-	CC (-)	1.9 µg L^{-1} (in aqueous sample)	[35]
SPCnAuE	MDSPE: 15 mL urine, 10 mg ZMS-5/Fe$_2$O$_{3(tr)}$, 1 min extraction.	SWASV (300 s)	1-2 µg L^{-1} (urine samples)	This work

SIA, sequential injection analysis; SWASV, square-wave anodic stripping voltammetry; FIA, flow injection analysis; NMWL, nominal molecular weight limit; IDAA-SAMMS-CNT, iminodiacetic acid self-assembled mono-layer on mesoporous supports carbon nanotubes; GCE, glassy carbon electrode; DMSA, dimercaptosuccinic acid; GO/DTT, graphene oxide doped diaminoterthiophene; SPCE, screen-printed carbon electrode; CC, chronocoulometry; SPCnAUE, screen-printed carbon electrode modified with gold nanoparticles; MDSPE, magnetic dispersive solid-phase extraction.

Table 2. Determination of Pb in real urine samples.

	Spiked concentration (µg L^{-1})	Found concentration (µg L^{-1}) ± SD[a]	Apparent recoveries (%) (CV)
Urine 1	0	< LOD	-
	7	7.1 ± 1.3	101 (19)
	20	20 ± 2	99 (10)
Urine 2	0	< LOD	-
	7	7.5 ± 0.7	107 (9)
	20	21 ± 4	105 (18)
Urine 3	0	< LOD	-
	7	7.4 ± 0.7	105 (9)
	20	20 ± 2	100 (11)

[a]Standard deviation values were calculated using the s_{XE} (i.e., standard deviation of *x*-value estimated using the regression line)[30] .