



Ionic liquid functionlized on multiwall carbon nanotubes for nickel and lead determination in human serum and urine samples by micro solid-phase extraction

Arezou Lari^a, Nafiseh Esmaeili^{b,*} and Homanaz Ghafari^c

^aSystems Biomedicine Department, Pasteur Institute of Iran, Tehran, Iran.

^b Department of chemistry, Faculty of Science, Semnan University, Semnan, Iran

^cDepartment of pharmacology, school of medicine, Tehran university of medical sciences, P.O.Box784-13145, Tehran, Iran

ARTICLE INFO:

Received 11 Mar 2021

Revised form 15 May 2021

Accepted 4 Jun 2021

Available online 30 Jun 2021

Keywords:

Nickel and lead,
Human samples,
Ionic liquid,
Multiwall carbon nanotubes,
Micro solid-phase extraction

ABSTRACT

In this study, a novel synthesis adsorbent, 1-(3-aminopropyl)-3-methylimidazolium hexafluorophosphate functionlized on multiwall carbon nanotubes ([Apmim][PF₆]-MWCNTs, IL@MWCNTS) was used for nickel/lead (Ni/Pb) extraction and determination by dispersive ionic liquid micro solid-phase extraction (DIL- μ -SPE) coupled to electrothermal atomic absorption spectrometry (ET-AAS). After dilution of 20 mg of IL@MWCNTS in 200 μ L of acetone, the mixture was injected to 10 mL of human serum/urine samples at pH of 8.0. After sonication for 5 min, the Ni(II) / Pb(II) were extracted by ionic liquid phase and then centrifuged for 2.5 min. The upper liquid phase set aside and Ni(II) / Pb(II) loaded in adsorbent were back-extracted by acidic solution at pH=2-3. Finally, the concentration of total nickel and lead was determined by ET-AAS. By optimizing, the limit of detection, linear range, and enrichment factor for nickel and lead were obtained (0.05 μ g L⁻¹; 0.1 μ g L⁻¹), (0.2-5.8 μ g L⁻¹; 0.4-30 μ g L⁻¹) and 24.7; 5.1, respectively (RSD less than 5%). Also, the capacity absorption of IL@MWCNTS for nickel and lead ions were achieved 149.3 mg g⁻¹ and 162.5 mg g⁻¹, respectively. The DIL- μ -SPE procedure was validated for nickel and lead extraction by spiking of real samples and ICP-MS analyzer.

1. Introduction

Lead and nickel (Pb, Ni) have toxic effects and use in different industries. Heavy metals as non-essential elements have widely distributed in the environment (air, soils, waters) and humans. Human exposure of heavy metals cause to various diseases such as cancer. Pb and Ni is a naturally occurring element found in small amounts in the earth's crust [1]. While it has some beneficial uses, it can be toxic to humans and animals, and cause to health effects.

The most exposure of lead and nickel related to human activities including, the fossil fuels, gasoline, the industrial facilities, the nickel cadmium battery, and paint factories. Lead and nickel compounds have been used in a wide variety of products found in different industries, including paint, ceramics, pipes and plumbing materials, gasoline, batteries, and cosmetics [2, 3]. High levels of human exposure to Ni and Pb metals cause to damage the most of human organ systems such as, the central nervous system (CNS), kidneys, liver, bones and gastrointestinal system. Lead can also effect on hemoglobin synthesis and cause to anemia effects or accumulate in the bones. Depending on the level of exposure,

*Corresponding Author: Nafiseh Esmaeili

Email: esmaeilin@gmail.com, esmaeilin@semnana.ac.ir

<https://doi.org/10.24200/amecj.v4.i02.144>

the lead and nickel can adversely effect on the nervous system, immune system, reproductive and the cardiovascular system [4-7]. Infants and young children are especially sensitive to lead exposures, which may contribute to behavioral problems, learning deficits and lowered IQ [8]. Lead can also be emitted into the environment from industrial sources and contaminated sites, such as former lead smelters. While natural levels of lead in soil range between 50 and 400 parts per million, mining, smelting and refining activities have resulted in substantial increases the lead levels in the environment, especially near mining and smelting sites. Lead can be added to soils and sediments through deposition from sources of lead by air pollution. Lead can be added to proteins and amino acids which was caused neurological problems [9,10]. Due to the environmental protection agency (EPA), the maximum contaminant level (MCL) for Pb in waters is zero and has no effect in humans [2]. Also, the National toxicology program (NTP) announced that the lead concentration in blood and serum must be less than 50 $\mu\text{g L}^{-1}$ in children. The variable lead values in human blood/serum was about 250 -300 $\mu\text{g L}^{-1}$ which was reported by food and drug administration (FDA) [11]. As references, the standard blood lead levels are below 25 mg dL^{-1} or 250 microgram per liter. The permissible exposure level in the ambient (air, water, soil, etc.) environment has been reported [12-14]. Nickel (Ni) caused to acute disease in humans [15]. Ni(II) can be enter to waters from waste water of different industries such as battery and electroplating factories [16]. Nickel complex to various proteins and enzyme in the human body. Nickel toxicity caused to many problems in human systems or organs such as renal, liver, brain, cardiovascular system, immune system and heart. The symptoms diseases included lung dysfunction and cancer was seen for nickel exposure [17]. Oral values for rats range from 67-9000 mg Ni per kg (ATSDR). Toxic effects of oral exposure to nickel usually involve the kidneys (ATSDR). Normal range for Ni in healthy peoples is 0.2 $\mu\text{g L}^{-1}$ in serum and less than 3.0 $\mu\text{g L}^{-1}$ in human urine.

[18,19]. Nickel (II) in urine and serum samples determined with UV-VIS spectrophotometry and flame atomic absorption spectrometry techniques [20]. Recently, the various techniques such as, the inductively coupled plasma(ICP), the inductively coupled plasma mass spectrometry(ICP-MS) [21], the flame atomic absorption spectrometry F-AAS [22], the X-ray fluorescence spectrometry [23] and the electrothermal atomic absorption spectrometry (ET-AAS) were used to determine Ni and Pb ions in different matrixes [24]. Due to ultra-trace concentration of Pb and Ni in human samples (urine and serum) and difficulty matrices in human biological samples, the sample treatment was used. For Examples, the solid phase extraction(SPE) [25], the magnetic dispersive micro-solid phase extraction (MD- μ -SPE) [26,27], the dispersive micro solid phase microextraction (D-SPME) [28], the needle hub in-syringe solid phase extraction (NHS-SPE) [29], and liquid-liquid microextraction (LLME) [30, 31] were used. Among them, the dispersive micro solid-phase extraction D- μ -SPE was mostly used for determination of heavy metals such as Ni and Pb in water and humans. Task ionic liquids were used for extraction of heavy metals from liquid phase by N, S groups. The D- μ -SPE procedure have advantages such as easy to use, simple, high recovery and efficient extraction. In this process, the adsorbent properties are main factor for heavy metal extraction by D- μ -SPE procedure. The high surface area of nanoparticles caused to increase the extraction recovery and absorption capacity. Recently, the various nanostructures were used for extraction Pb and Ni in waters, human urine and serum samples [32, 33]. In this study a novel ionic liquid ([Apmim][PF₆]) functionalized on MWCNTs (IL-MWCNTs) was used for extraction of Ni and Pb ions in human urine and serum samples by the DIL- μ -SPE procedure. The Ni and Pb concentration was determined by the ET-AAS after sample preparation. The main parameters on lead and nickel extraction were studied and evaluated.

Table 1. The ET-AAS conditions for lead and nickel determination.

Features	Value Pb	Value Ni
Linear range, $\mu\text{g L}^{-1}$	3-90	5-85
Working range, $\mu\text{g L}^{-1}$	3-150	5-145
Wavelength, nm	283.3,217.0	232.0
Lamp current, mA	5.0	4.0
Slit, nm	0.5	0.2
Mode	Peak Area	Peak Area
Auto Sampler (μL)	1-100	1-100
LOD	0.75	1.25
LLOQ	3.0	5.0
R ²	0.9998	0.9997

2. Materials and Methods

2.1. Apparatus

The AAS (GBC, 932, AUS) based on furnace accessory (Pal 3000) and deuterium (D_2) /hollow-cathode lamp (Ni, Pb) was used. The sample was transferred to 2 mL of PVC tube in Pal3000 as auto-sampler accessory. The conditions of ET-AAS were shown in Table 1. The lead determination was achieved by injecting 20 μL of sample to graphite tube with auto-sampler in three steps of drying, ashing, and atomization. The ICP-MS (PerkinElmer, USA) was used for ultra-trace Ni and Pb analysis in different matrixes. The conditions of ICP-MS were tuned for Ni and Pb determination in samples (1200 W, 12 L min^{-1} per 1 s). The auxiliary gas flow was adjusted 1.2 L min^{-1} . The quantitative analysis of lead and nickel were obtained in PPT concentration by ICP-MS analyzer (<10 ppt). The range of pH values of the serum and urine samples were measured by pH meter (Metrohm) and adjusted by favorite buffer solution. The shaker accessory (USA, Domingo Lab) by stirring speed between 10~210 PRM and working platform of 315×218 mm (12.5"×8.5") with voltage 220V was used. The Eppendorf centrifuge offers 24-place capacity in an aerosol-tight rotor and speeds up to 21,300 × g was used (Laboratory centrifuge model 5418 R, Eppendorf, Germany). was used by the DIL- μ -

SPE procedure. The polypropylene syringe and conical tube were purchased from Sigma (Germany). Fourier transform infrared (FT-IR) spectra were obtained by a Perkin Elmer Spectrum (65 FT-IR). X-ray diffraction (XRD) was reported by a X'Pert PRO X-ray diffractometer. Scanning electron microscopy (SEM) images were achieved using a Tescan Mira-3.

2.2. Reagents

In this study, the analytical grade of reagents was prepared from Merck / Sigma Aldrich (Germany). The standard solution of lead (Pb^{2+}) was purchased from Merck CO. (Germany) with a concentration of 1000 mg L^{-1} in 1 % HNO_3 . The standard stock solutions (1000 mg L^{-1}) of Ni (II), were purchased from Merck (Darmstadt, Germany). Another concentration of lead and nickel was daily prepared by dilution of the standard lead solution with DW. Ultrapure water was purchased from Millipore Company (Bedford, USA) for dilution of solutions or standards. The pH was adjusted by sodium phosphate buffer solution for pH 5.7-8.2. The reagents such as acetonitrile (CAS N.: 75-05-08, Merck), polyoxyethylene octyl phenyl ether (TX-100, CAS N: 9002-93-1, Sigma, Germany), and toluene (CAS N: 108-88-3, Merck), HNO_3 , xylene, HCl, ethanol, and acetone, were prepared from Merck,

Germany. MWCNTs adsorbent prepared from RIPI company in Iran. aminoopropyltrimethoxysilane (APTMS) was prepared from Sigma, Germany.

2.3. Synthesis of [Apmim][PF₆]-MWCNTs

The carboxylic acid of MWCNTs was prepared by the acid treatment procedure according to previous reports [34]. Then, the carboxylic acid (COOH) on MWCNTs was treated with NaBH₄ / CH₃OH, and COOH were reduced to CH₂OH groups. Typically, in a 100 mL flask / condenser / magnetic stirrer (MSB), the sodium borohydride (0.5 g) added to 5 g of MWCNTs-COOH and in presence of methanol refluxed / cooled/ filtered / washed with methanol. Then 2.0 g of MWCNTs – OH were added to 3-aminoopropyltrimethoxysilane (APTMS) in xylene (50 ml) and heated. Then, the product was filtered, washed with ethanol. Finally, Immobilization of the carbonyl group on the MWCNTs was accomplished by stirring the aminopropyl-functionalized CNTs in an ethanolic solution of terephthalaldehyde (0.5 g) for 3 h at 70 °C. An ethanolic solution carbonyl-functionalized MWCNTs were moved to ultrasonic bath for 15 minutes. After the sonication, a solution of [Apmim][PF₆] in EtOH (10 mL) was added dropwise to mentioned suspension during 10 min at 80 °C. The reaction mixture was refluxed for 4 h at 80 °C by N₂[34].

2.4. General procedure

By the DIL- μ -SPE procedure, 10 mL of human urine and serum sample was used for extraction Pb and Ni by IL-MWCNTs. Firstly, 10 mL of human samples and standard solution containing 0.2 $\mu\text{g L}^{-1}$; 0.4 $\mu\text{g L}^{-1}$ (lower limit) and 5.5 $\mu\text{g L}^{-1}$; 30 $\mu\text{g L}^{-1}$ (upper limit) for Ni and Pb was used, respectively at pH of 8.0. Then, 20 mg of IL-MWCNTs mixed with 0.2 mL of acetone and injected to 10 mL samples /standard solution in PVC centrifuge conical tube. The mixture was shaken for 6 min and Pb/Ni ions were extracted by amine group of [Apmim][PF₆] at optimized pH. Then, the adsorbent was collected from liquid phase by centrifuging of samples. Then, the Ni loaded on adsorbent was back extracted with 0.2 mL of nitric acid (0.3 M) and diluted with 0.2 mL of DW. Also, the lead loaded on adsorbent was back extracted with 0.2 mL of nitric acid (0.3 M) and diluted with DW up 2 mL. Finally, the solution was determined by ET-AAS (Fig.1, Table 2). The recovery of extraction with IL-MWCNTs adsorbent was obtained for Pb/Ni concentration by the equation 1. The C_A is the primary concentrations and C_S is the secondary concentration of Pb(II)/Ni(II), which was determined by ET-AAS ($n=10$, Eq. 1).

$$\text{Recovery}\% = (C_A - C_S) / C_A \times 100 \quad (\text{Eq.1})$$

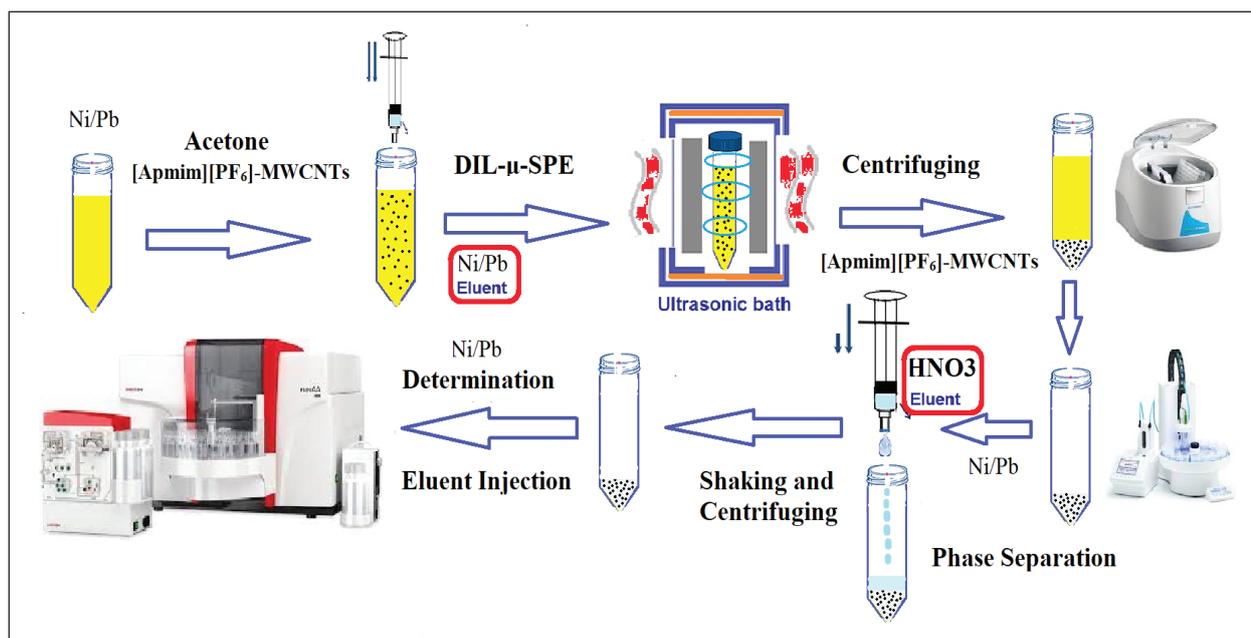


Fig. 1. The DIL- μ -SPE procedure based on IL-MWCNTs for Pb and Ni extraction

Table 2. The analytical features for determination lead and nickel by DIL- μ -SPE procedure coupled to ET-AAS

Features	Value Pb	Value Ni
Working pH	7.5-8.5	8.0
Amount of IL-MWCNTs(mg)	18	20
Sample volume of serum (mL)	10 .0	10.0
Sample volume of urine, water (mL)	15.0	12.0
Volume of sample injection (μ L)	20	20
Linear range for serum (μ g L ⁻¹)	0.4-30	0.2-5.8
Mean RSD %, n=10	4.2	3.9
LOD for urine or serum (μ g L ⁻¹)	0.1	0.05
Enrichment factor for urine or serum	5.1	24.7
Volume and concentration of HNO ₃	0.2 mL, 0.3 M	0.2 mL, 0.2 M
Shaking/Centrifuging time	6.0 min, 4.0 min	6.0 min, 4.0 min
Correlation coefficient	R ² = 0.9997	R ² = 0.9995

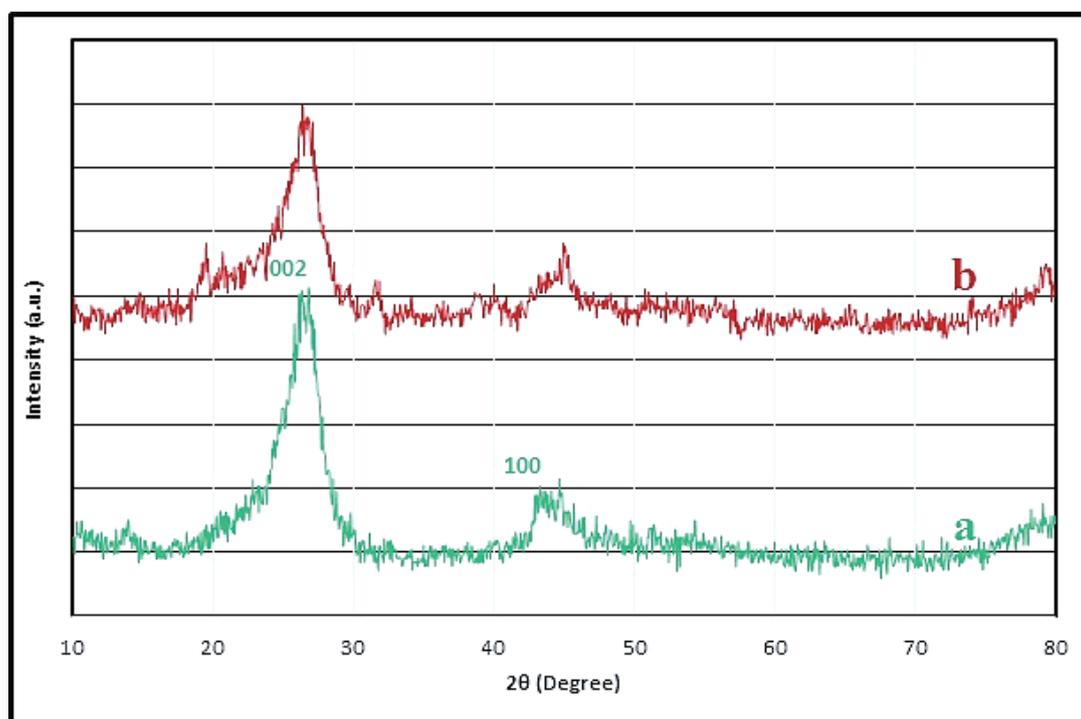
3. Results and discussion

The lead and nickel were extracted and determined based on the IL-MWCNTs nanostructures which characterized by scanning electron microscopy (SEM), X-ray diffraction spectroscopy (XRD), and Fourier transform infrared spectroscopy (FT-IR).

3.1. X-ray diffraction spectroscopy (XRD)

The powder XRD patterns of pristine MWCNTs (a) and [Apmim][PF₆] immobilized on MWCNTs (b)

are shown in Figure 2. The XRD of the MWCNTs and IL-MWCNTs were compared. The two characteristic graphitic peaks, at a 2θ value (28° and 45°) corresponding to the peaks of the (002) and (100) planes of hexagonal graphite MWCNT, respectively, are present in the XRD pattern of both measured samples. As shown in Figure 2, after functionalized of [Apmim][PF₆] on MWCNTs, no new peaks were seen, and the characteristic peaks of MWCNTs didn't change.

**Fig. 2.** The XRD of a) MWCNTs and b) IL-MWCNTs

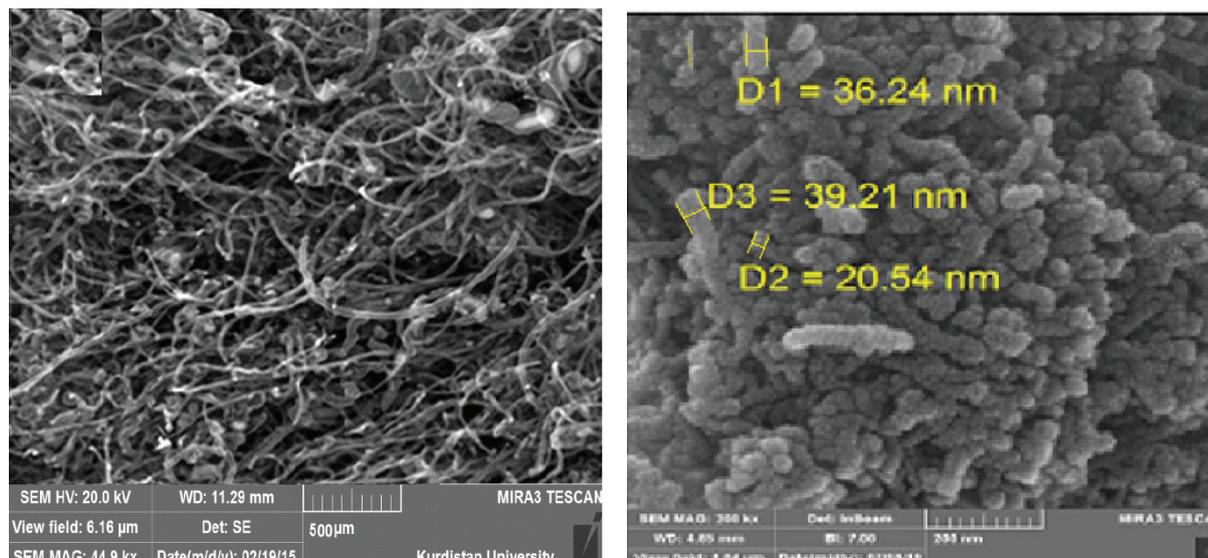


Fig. 3 . FE-SEM images of [Apmim][PF₆] immobilized on MWCNTs

3.2. Field emission scanning electron microscopy (FE-SEM)

FE-SEM images of [Apmim][PF₆] immobilized on MWCNTs are shown in Figure 3. It showed that the nanotubes have previous form and save their nature as MWCNTs. Due to FE-SEM images with different scale bars, a clear change in the morphology of [Apmim][PF₆] immobilized on MWCNTs were seen that showed the ionic liquid has been immobilized on the MWCNTs. The FE-SEM showed that, the IL-MWCNTs have nano size between 20-60 nm.

3.3. Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of [Apmim][PF₆]-MWCNTs are shown in Figure 4. This FTIR spectrum showed that the oxidation and covalently bond of the pristine MWCNTs. The peak of 1717 cm⁻¹ is showed to the carbonyl bond (CO) due to oxidation functionalities. Also, the peak at 3437-3439 cm⁻¹ was assigned to the stretching of O-H groups on the inner surface of oxidized MWCNTs. The supporting of the aminopropylsilane group on OH by treatment with APTMS was confirmed by the appearance of a sharp peak at around 1094 cm⁻¹ which is attributed to the O-Si-O bond constructed between MWCNTs and ionic liquid moieties. The IR peak at 2922 and 2854 cm⁻¹ were related to

asymmetric and symmetric vibration absorptions, respectively, for the aliphatic CH₂ groups (C-H) of chlorosilane coupling agent and butyl chain of [Apmim][PF₆].

3.4. Optimization of DIL-μ-SPE procedure

The DIL-μ-SPE procedure was used based on IL-MWCNTs as a new adsorbent for determination lead and nickel in human urine and serum samples. High efficient recoveries, low RSD / LOD and variable linear ranges were obtained by optimizing of parameters such as, pH, amount of IL-MWCNTs, HNO₃ volume and concentration, the urine/serum volume, and the capacity of adsorption for extraction of Pb and Ni ions in human biological samples.

3.4.1. The pH optimization

The pH of urine and serum sample has a main role for adsorption of lead and nickel ions on IL-MWCNTs by DIL-μ-SPE procedure. The effect of pH range on the extraction of Pb and Ni with adsorbent was studied for Ni and Pb concentration between 0.2-5.5 μg L⁻¹ and 0.4-30 μg L⁻¹, respectively (Fig. 5). Based on results, the recovery for Ni (II) and Pb(II) ions were increased at pH range of 8.0 more than 96%. Also, the extraction recoveries decreased at pH more than 8.5 and less than 7. So, the pH of 8 was selected

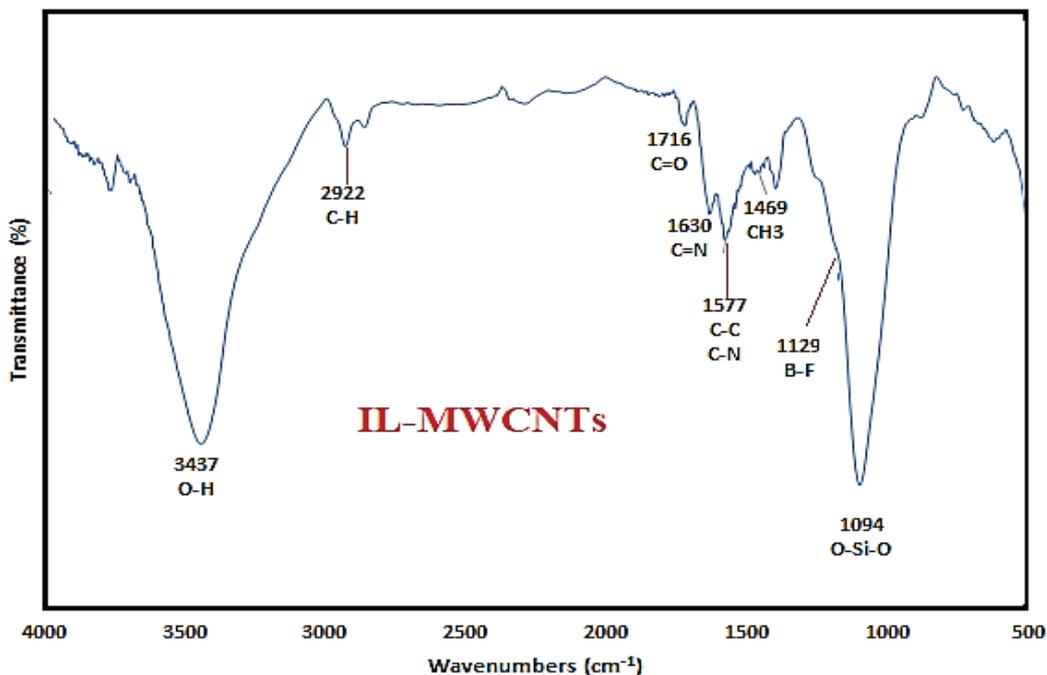


Fig.4. The FT-IR spectra of IL-MWCNTs

as optimal pH by the DIL- μ -SPE procedure. The adsorption mechanism on the IL-MWCNTs was achieved based on deprotonated amine groups ($\text{Pb}^{2+}/\text{Ni}^{2+} \rightarrow \text{M} \dots \text{NH}_2^- \text{IL}$) with the positively charged of metals in optimized pH. At lower pH, the surface of IL-MWCNTs have positively charged due to the H^+ protonation. Therefore, the extraction efficiencies were reduced by the similar

charge law between $\text{Pb}^{2+}/\text{Ni}^{2+}$ and positively charged of $^+\text{NH}_2$ of IL. Moreover, at pH of 8.0, the NH_2 group of IL had negative charge (-) and caused to increase adsorption adsorbent. The results showed, high recovery for extraction Pb/Ni were achieved at pH=8. In addition, the extraction efficiency was obtained about 30% in low pH as physically adsorption.

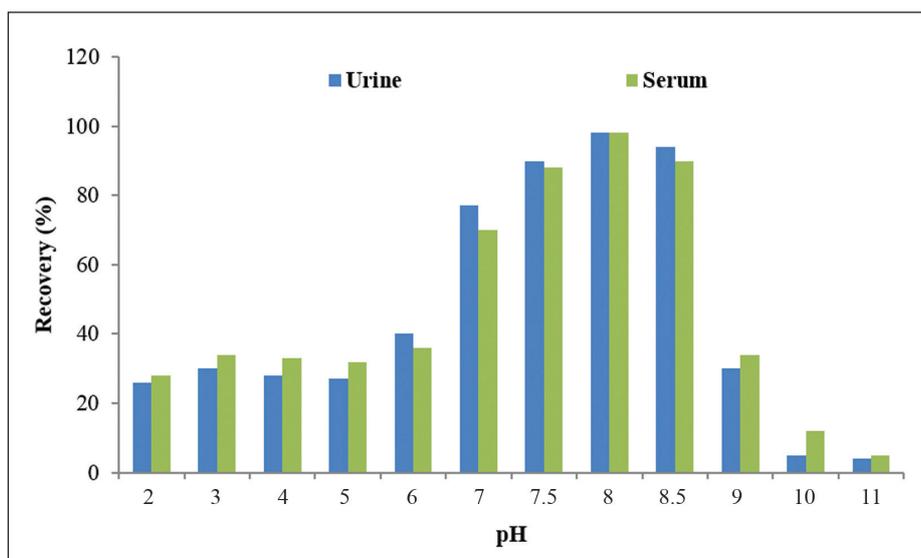


Fig. 5. The effect of pH on Pb/Ni extraction by the DIL- μ -SPE procedure

3.4.2. Optimization of amount of IL-MWCNTs

By the DIL- μ -SPE procedure, the amount of IL-MWCNTs was optimized for extraction of Ni(II) and Pb(II) in urine and serum samples. In this study, the amount of 5-40 mg of IL-MWCNTs was studied. The results showed that the 18 mg of IL-MWCNTs had high extraction for Ni(II) and Pb(II) in urine and serum samples in optimized conditions. Therefore, 20 mg of IL-MWCNTs was selected as optimal amount of IL-MWCNTs (Fig. 6). The more amount of IL-MWCNTs had no effect on the extraction recovery of Pb/Ni at pH=8.

3.4.3. Effect of eluent

The volume and concentration of eluents for lead and nickel extraction in urine and serum samples was studied. By the DIL- μ -SPE procedure, the various mineral acids were selected as elution phase for back extraction Pb(II) and Ni(II) from IL-MWCNTs phase at low pH. At low pH, the covalent bond between metal and amine group break down and Ni/Pb ions release in liquid phase. The different volumes from 100 to 500 μ L and concentration between 0.1-0.5 mol L⁻¹ were used as eluent phase (HCl, HNO₃, H₂SO₄ and H₃PO₄) by the DIL- μ -SPE method. The results showed that the 0.2 mol L⁻¹ of HNO₃ (0.2 mL) had quantitatively back extracted Pb/Ni ions from IL-MWCNTs (Figs. 7). So, the HNO₃ was used for further works.

3.4.4. Sample volume optimization

The sample volume affected on the recoveries of Pb(II) and Ni(II) ions at pH=8. In this research, the various sample volumes of urine and serum from 1 to 20 mL were studied for Pb(II) and Ni(II) extraction in presence of the concentration between 0.2-5.5 μ g L⁻¹ and 0.4-30 μ g L⁻¹ for nickel and lead, respectively by the DIL- μ -SPE procedure. The results showed, the high extraction recoveries less than 12 mL and 15 mL for lead and nickel in urine samples were obtained, respectively. Also, the good recoveries less than 10 mL for lead and nickel in serum samples was achieved. Moreover, the extraction efficiency Pb(II) and Ni(II) ions was reduced by increasing more than 10 mL samples.

Therefore, 10 mL was used as the optimal sample volume by proposed procedure (Fig. 8).

3.4.5. Time of extraction

The interaction of IL-MWCNTs with Pb(II) and Ni(II) ions is main factor for extraction process by DIL- μ -SPE procedure. So, the time dispersion of the IL-MWCNTs for metal extraction in the urine and serum samples were calculated. The high interaction caused to increase the extraction of metals in liquid phase. The effect of the ultrasonic time was evaluated based on IL-MWCNTs adsorbent at PH=8. The results showed, the maximum recovery was obtained about 6.0 min.

3.5. Reusability and Adsorption capacity

The reusability of IL-MWCNTs for extraction of with Pb(II) and Ni(II) ions was examined for several analyses by the DIL- μ -SPE method. The good recovery based on 19 times of extraction and back extraction cycles was obtained for Pb(II) and Ni(II) by IL-MWCNTs. Also, the absorption capacities IL- MWCNTs and MWCNTs for Pb(II) and Ni(II) extraction in urine and serum samples were achieved based on amine group of IL and surface area of MWCNTs. For this propose, 20 mg of IL-MWCNTs and MWCNTs were added to 10 mL of standard solution with concentration of 10 mg L⁻¹ of Pb(II) and Ni(II) in batch system at optimized pH. By results, the adsorption capacity of MWCNTs and IL- MWCNTs for Ni(II) and Pb(II) was found 21.4/26.7 mg g⁻¹ and 149.3 / 162.5 mg g⁻¹, respectively.

3.6. The effect of concomitant ions

The effect of interference ions on Pb(II) and Ni(II) extraction was studied in human urine and serum samples by DIL- μ -SPE procedure (Table 3). In optimized conditions, the various interfering ions in human biological samples was added to 10 mL of Pb(II) and Ni(II) of standard solution with concentration of 30 μ g L⁻¹ and 5.5 μ g L⁻¹, respectively. The results showed, the main concomitant ions had no effect on the metal extraction at pH=8. The IL-MWCNTs had good

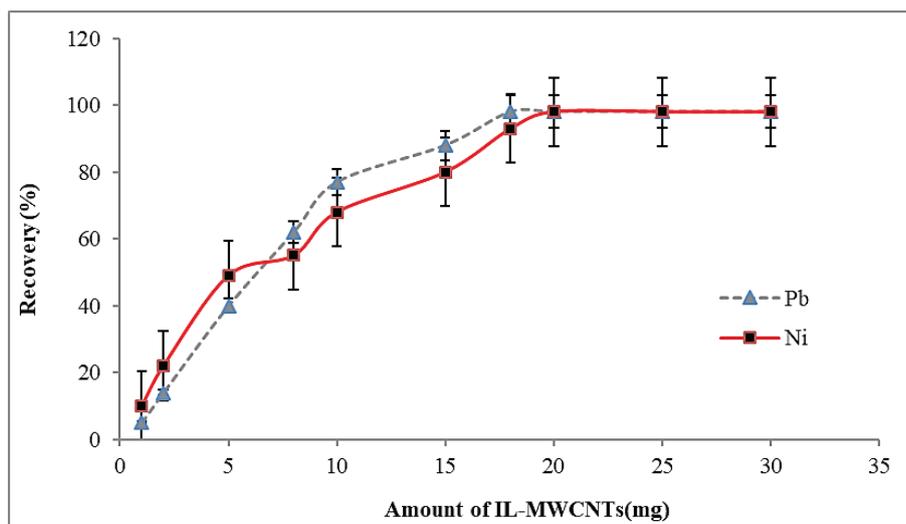


Fig. 6. The effect of amount of IL-MWCNTs on Pb/Ni extraction by the DIL- μ -SPE procedure

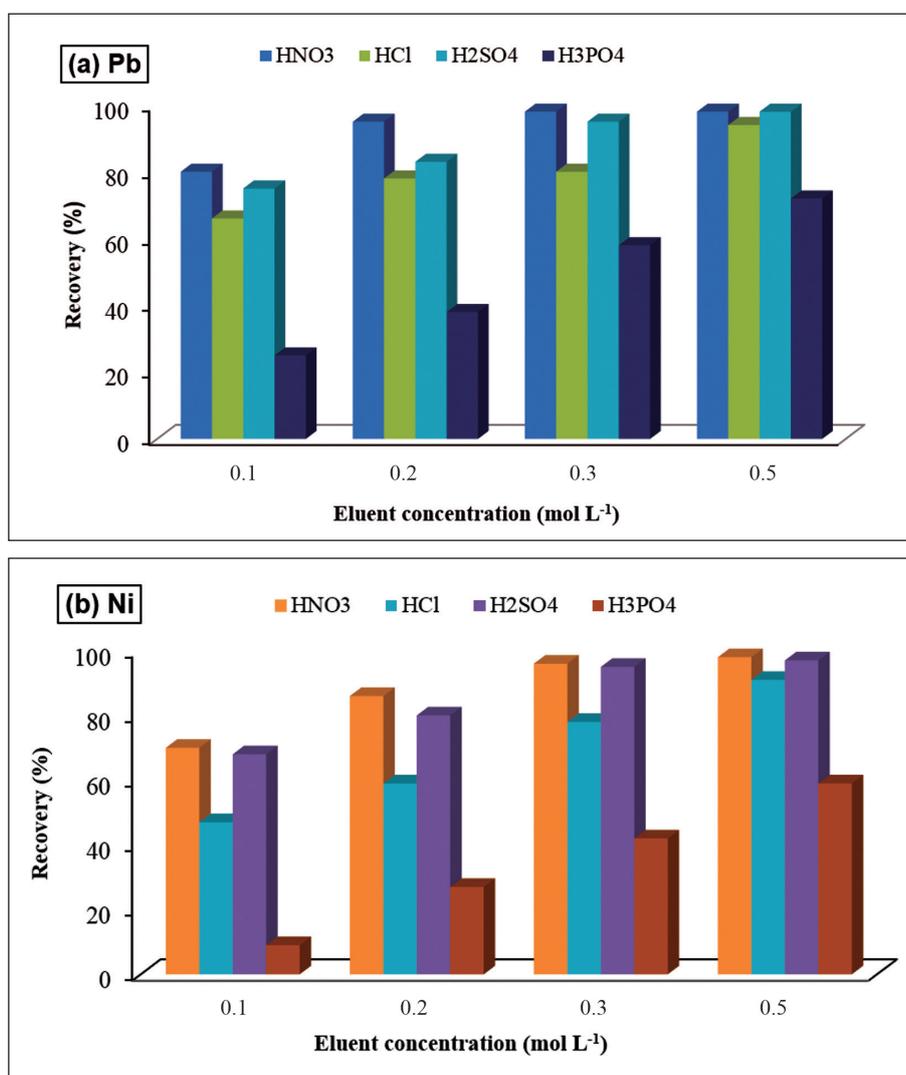


Fig. 7. The effect of eluent for back-extraction of a) lead and b) nickel from IL-MWCNTs by the DIL- μ -SPE procedure

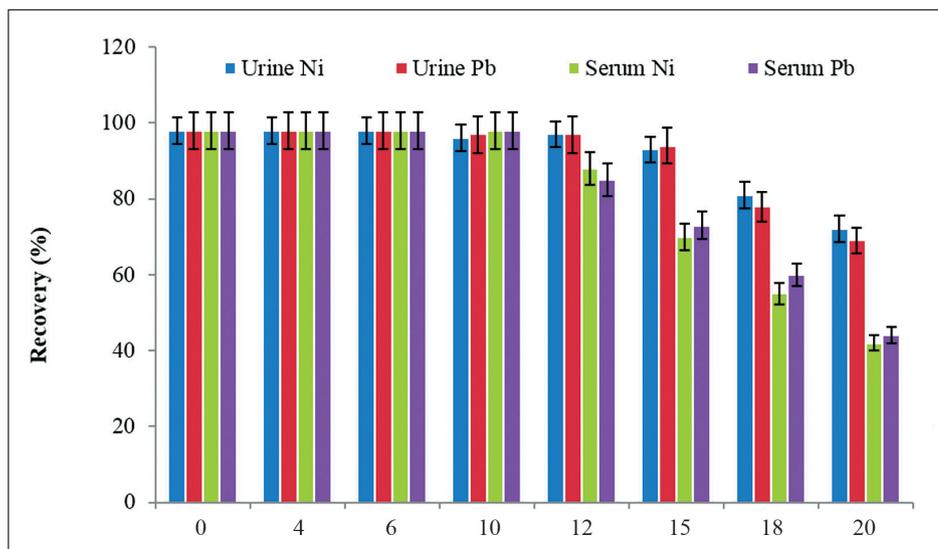


Fig. 8. The effect of sample volume on lead and nickel extraction in urine and blood samples by the DIL- μ -SPE procedure

Table 3. The effect of interference ions on Pb(II) and Ni(II) extraction in human urine and serum samples by the DIL- μ -SPE procedure

Interfering Ions(CA)	Mean ratio (CA/C _{Pb(II)} ; or CA/C _{Ni(II)})		Recovery (%)	
	Pb(II)	Ni(II)	Pb(II)	Ni(II)
Cr ³⁺ , As ³⁺	900	800	98.8	97.4
Zn ²⁺ , Cu ²⁺	750	600	97.2	98.5
Cd ²⁺	700	300	97.0	95.8
I ⁻ , Br ⁻ , F ⁻ , Cl ⁻	1200	1100	99.2	98.6
Al ³⁺ , V ³⁺	650	700	98.0	96.9
Na ⁺ , K ⁺ , Cl ⁻ , Ca ²⁺ , Mg ²⁺	900	800	97.5	97.1
Co ²⁺ , Mn ²⁺	600	800	99.1	97.7
Hg ²⁺	50	80	96.6	97.3
Ag ⁺	200	150	98.0	98.7
SCN ⁻ , S ₂ O ₃ ²⁻ , CH ₃ COO ⁻ , NO ₃ ⁻	800	900	97.6	99.4

extraction for Pb(II) and Ni(II). in present of the interference ions. The ethical committee of Semnan University confirmed the project for determining metals in the different matrices (ECSU, Project No. 8051127-01) with student proposal number(SN-9228558001).

3.7. Real sample analysis

The Pb(II) and Ni(II) ions was determined in

urine and serum samples based on IL-MWCNTs by the DIL- μ -SPE procedure coupled to ET-AAS. By optimizing parameters, the means of 10 times determinations, for Pb(II) and Ni(II) ions were calculated. The human urine and serum samples were spiked with Pb(II) and Ni(II) standard solutions for 0.4-30 $\mu\text{g L}^{-1}$ and 0.2-5.8 $\mu\text{g L}^{-1}$ at pH=8, respectively (Table 4 and 5). The results showed us, the spiking real samples

has favorite accuracy and pricision for lead and nickel analysis in difficulty matrixes. The mean extraction efficiency of spiked urine and serum samples for Pb(II) and Ni(II) ions were obtained from 95.2% to 104.3% (RSD% < 5%) for ten samples. The spike samples demonstrated that

the proposed method have satisfactory results for extraction and determination Pb(II) and Ni(II) ions in human biological samples. In addition, the Pb(II) and Ni(II) ions concentration in urine and serum samples was mesured with ICP-MS and compared to DIL- μ -SPE/ET-AAS procedure

Table 4. Validation of lead determination(Pb) based on spiking of human serum, blood, plasma and urine samples by DIL- μ -SPE procedure

Human Sample*	Spike ($\mu\text{g L}^{-1}$)	*Found ($\mu\text{g L}^{-1}$)	Recovery (%)
Blood	---	14.7 \pm 0.6	---
	15	29.8 \pm 1.3	100.6
Serum	---	15.2 \pm 0.7	---
	15	30.1 \pm 1.4	99.3
Urine	---	8.4 \pm 0.3	---
	10	18.2 \pm 0.9	98.0
	---	5.5 \pm 0.2	---
Plasma	5.0	10.3 \pm 0.5	96.2

*Mean of three determinations of samples \pm confidence interval (P = 0.95, n =10)
All samples volumes diluted with DW (1:10), Dilution factor =10

Table 5. Validation of nickel determination (Ni) based on spiking of human serum, blood, plasma and urine samples by DIL- μ -SPE procedure

Human Sample*	Spike ($\mu\text{g L}^{-1}$)	*Found ($\mu\text{g L}^{-1}$)	Recovery (%)
Blood	---	2.22 \pm 0.12	---
	2.5	4.63 \pm 0.18	96.4
Serum	---	2.65 \pm 0.11	---
	2.5	5.27 \pm 0.28	104.8
Urine	---	1.35 \pm 0.06	99.3
	1.5	2.84 \pm 0.12	---
Plasma	---	0.52 \pm 0.02	---
	0.5	1.01 \pm 0.05	98.0

*Mean of three determinations of samples \pm confidence interval (P = 0.95, n =10)

Table 6. Comparing of DIL- μ -SPE /ET-FAAS with ICP-MS method for mean concentration of Pb and Ni in human samples ($\mu\text{g L}^{-1}$)

Sample	ICP-MS	ICP-MS	*IL-MWCNTs /ET-AAS	*IL-MWCNTs /ET-AAS
	Pb	Ni	Pb	Ni
Blood	29.56 \pm 0.96	2.53 \pm 0.04	28.82 \pm 1.42	2.41 \pm 0.11
Urine	18.13 \pm 0.35	1.87 \pm 0.03	17.49 \pm 0.77	1.95 \pm 0.09
Serum	27.48 \pm 0.81	4.68 \pm 0.08	27.06 \pm 1.32	4.43 \pm 0.23

*Mean of three determinations of samples \pm confidence interval (P = 0.95, N =10),
The lead samples diluted with DW (1:10)

(Table 6). The precision and accuracy of results showed the validation of methodology for the Pb(II)/ Ni(II) determination by IL-MWCNTs adsorbent.

4. Conclusions

A simple and efficient method based on IL-MWCNTs adsorbent was used for separation and determination of nickel and lead in urine and serum samples by ET-AAS. By the DIL- μ -SPE procedure, high recovery and efficient extraction was obtained at optimized conditions. The linear range and working range for Ni(II) and Pb(II) was achieved 0.2-3.42 $\mu\text{g L}^{-1}$ /0.4-17.6 $\mu\text{g L}^{-1}$ and 0.2-5.8 $\mu\text{g L}^{-1}$ /0.4-30 $\mu\text{g L}^{-1}$ for 10 mL of urine and serum samples, respectively. The mean correlation coefficient and enrichment factor for Ni(II) and Pb(II) were obtained 0.9997/0.9995 and 24.7/5.1, respectively. The NH₂ group in IL-MWCNTs was coordinated with Ni(II) and Pb(II) cations and separated from liquid phase by centrifuging process. The high adsorption capacities, recovery, enrichment and favorite reusability caused to consider the DIL- μ -SPE procedure as a new methodology for nickel and lead extraction in human samples with low LOD and RSD (>5%) in optimized conditions. The validation methodology based on spiking samples and ICP-MS analysis showed, the DIL- μ -SPE method can be used as applied techniques for Ni(II) and Pb(II) determination in human samples.

5. Acknowledgements

The authors wish to thank Semnan University, Iran. The ethical committee of Semnan University confirmed the project for determining metals in the different matrices (ECSU, Project No. 8051127-01) with student proposal number (SN:9228558001).

6. References

- [1] World Health Organization(WHO), Preventing disease through healthy environments: exposure to lead, 2019.
- [2] Environmental Protection Agency (USEPA) Basic Information About Lead in Drinking Water, 2014.
- [3] ATSDR, Division of Toxicology and Human Health Sciences, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, GA 30333, revision 2019.
- [4] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological profile for Lead, Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2019.
- [5] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological profile for Nickel. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2005.
- [6] A.A. Ab Latif Wani, J.A. Usmani, Lead toxicity: a review, *Interdiscip. toxicol.*, 8 (2015) 55-64.
- [7] G. Flora, D. Gupta, A. Tiwari, Toxicity of lead: a review with recent updates, *Interdiscip. toxicol.*, 5 (2012) 47-58.
- [8] T. Dignam, R. B. Kaufmann, L. LeSturgeon, M. Jean Brown, Control of lead sources in the United States, public health progress and current challenges to eliminating lead exposure, *J. Public Health Manag. Pract.*, (2019) S13–S22. PMC6522252, <https://doi.org/10.1097/PHH.0000000000000889>.
- [9] M. Kirberger, J.J. Yang, Structural differences between Pb²⁺ and Ca²⁺ binding sites in proteins: implications with respect to toxicity, *J. Inorg. Biochem.*, 102 (2008) 1901–1909.
- [10] J.S. Magyar, T.-C. Weng, C.M. Stern, D.F. Dye, B.W. Rous, J.C. Payne, B.M. Bridgewater, A. Mijovilovich, G. Parkin, J.M. Zaleski, Reexamination of lead (II) coordination preferences in sulfur-rich sites: implications for a critical mechanism of lead poisoning, *J. Am. Chem. Soc.*, 127 (2005) 9495-9505.
- [11] United States Food and Drug Administration (USFDA), Elemental impurities guidance for industry, Department of Health and Human Services, p. 41, 2017.
- [12] B.C. Schwarcz, L. Chilton, B. Shirley, S. Seifert, Childhood lead exposure associated with the use of kajal, an eye cosmetic from Afghanistan Albuquerque, New Mexico,

- Morb. Mortal Wkly. Rep., 62 (2013) 917-919.
- [13] K.L. Caldwell, P.Y. Cheng, J.M. Jarrett, Measurement challenges at low blood lead levels, *Pediatrics.*, 140 (2017) e20170272. <https://doi.org/10.1542/peds.2017-0272>
- [14] D.C. Bellinger, Neurological and behavioral consequences of childhood lead exposure. *PLOS Med.*, 5 (2008) e115. <https://doi.org/10.1371/journal.pmed.0050115.pdf>.
- [15] A. Abbas, A.M. Al-Amer, T. Laoui, M.J. Al-Marri, M.S. Nasser, M. Khraisheh, Heavy metal removal from aqueous solution by advanced carbon nanotubes: critical review of adsorption applications, *Sep. Purifi. Technol.*, 157 (2016) 141-61.
- [16] S. Feng, X. Wang, G. Wei, P. Peng, Y. Yang, Z. Cao, Leachates of municipal solid waste incineration bottom ash from Macao: Heavy metal concentrations and genotoxicity, *Chemosphere.*, 67 (2007) 1133-1137.
- [17] S.K. Seilkop, A.R. Oller, Respiratory cancer risks associated with low-level nickel exposure: An integrated assessment based on animal, epidemiological, and mechanistic data, *Regul. Toxicol. Pharm.*, 37 (2003) 173-190.
- [18] Agency for Toxic Substances and Disease Registry, Division of Toxicology and Human Health Sciences, 1600 Clifton Road NE, Mailstop S102-1, Atlanta, GA 30333, revision 2019.
- [19] Agency for Toxic Substances and Disease Registry (ATSDR), Toxicological profile for Nickel. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service, 2005.
- [20] A. Fadhil Khudhair, M. Khudhair Hassan, H. F. Alesary, A. S. Abbas, Simple pre-concentration method for the determination of nickel(II) in urine samples using UV-VIS spectrophotometry and flame atomic absorption spectrometry techniques, *Indones. J. Chem.*, 19 (2019) 638 – 649.
- [21] S. Orecchio, D. Amorello, Determination of trace elements in gluten-free food for celiac people by ICP-MS, *Microchem. J.*, 116 (2014) 163-172.
- [22] M. Arjomandi, H. Shirkhanloo, A review: Analytical methods for heavy metals determination in environment and human samples, *Anal. Methods Environ. Chem. J.*, 2 (2019) 97-126.
- [23] J. Shan Qun, W. Xiang Yu, S. Jin Lyu, Analysis of nickel distribution by synchrotron radiation X-ray fluorescence in nickel-induced early- and late-phase allergic contact dermatitis in Hartley guinea pigs, *Chinese Med. J.*, 132 (2019) 1959-1964.
- [24] A. Baysal, S. Akman, A rapid solid sampling method for determination of nickel and copper along human hair by ETAAS, *Microchem. J.*, 98 (2011) 291-296
- [25] M. Eftekhari, M. Gheibi, M. Akrami, F. Iranzad, Solid-phase extraction of ultra-trace levels of lead using tannic acid-coated graphene oxide as an efficient adsorbent followed by electrothermal atomic absorption spectrometry; response surface methodology–central composite design, *New J. Chem.*, 42 (2018) 1159-1168.
- [26] M. Rajabi M. Abolhosseini, Magnetic dispersive micro-solid phase extraction merged with micro-sampling flame atomic absorption spectrometry using (Zn-Al LDH)-(PTh/DBSNa)-Fe₃O₄ nanosorbent for effective trace determination of nickel(II) and cadmium(II) in food samples, *Microchem. J.*, 159 (2020) 105450.
- [27] S. Azimi, Z. Es'haghi, A magnetized nanoparticle based solid-phase extraction procedure followed by inductively coupled plasma atomic emission spectrometry to determine arsenic, lead and cadmium in water, milk, Indian rice and red tea, *Bull. Environ. Contam. Toxicol.*, 98 (2017) 830-836.
- [28] W. Ding, X. Wang, T. Liu., Preconcentration/extraction of trace bisphenols in milks using a novel effervescent reaction-assisted

- dispersive solid-phase extraction based on magnetic nickel-based N-doped graphene tubes, *Microchem. J.*, 150 (2019) 104109.
- [29] M. Shirani, F. Salari, S. Habibollahi, A. Akbari, Needle hub in-syringe solid phase extraction based a novel functionalized biopolyamide for simultaneous green separation/preconcentration and determination of cobalt, nickel, and chromium (III) in food and environmental samples with micro sampling flame atomic absorption spectrometry, *Microchem. J.*, 152 (2020) 104340.
- [30] S. M. Sorouraddin, M. A. Farajzadeh H. Nasiri, Picoline based-homogeneous liquid-liquid microextraction of cobalt(II) and nickel(II) at trace levels from a high volume of an aqueous sample, *Anal. Methods*, 11 (2019) 1379-1386.
- [31] L. Khoshmaram, Air-assisted liquid-liquid microextraction combined with flame atomic absorption spectrometry for determination of trace Pb in biological and aqueous samples, *Int. J. Environ. Anal. Chem.*, 101 (2021) 838-848.
- [32] H. Shirkhanloo, S. Davari Ahranjani, A lead analysis based on amine functionalized bimodal mesoporous silica nanoparticles in human biological samples by ultrasound assisted-ionic liquid trap-micro solid phase extraction, *J. Pharm. Biomed. Anal.*, 157 (2018) 1-9.
- [33] H. Shirkhanloo, Z. Karamzadeh, A novel biostructure sorbent based on CysSB/MetSB@MWCNTs for separation of nickel and cobalt in biological samples by ultrasound assisted-dispersive ionic liquid-suspension solid phase micro extraction, *J. Pharm. Biomed. Anal.*, 172 (2019) 285-294.
- [34] N. Esmaili, J. Rakhtshah, E. Kolvari, H. Shirkhanloo, Ultrasound assisted-dispersive-modification solid-phase extraction using task-specific ionic liquid immobilized on multiwall carbon nanotubes for speciation and determination mercury in water samples, *Microchem. J.*, 154 (2020) 104632.