

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

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## ABSTRACT

In this study, a novel synthesis adsorbent, 1-(3-aminopropyl)-3-methylimidazolium hexafluorophosphate functionlized on multiwall carbon nanotubes ([Apmim][PF<sub>6</sub>]-MWCNTs, IL@MWCNTS) was used for nickel/lead (Ni/Pb) extraction and determination by dispersive ionic liquid micro solid-phase extraction (DIL- $\mu$ -SPE) coupled to electrothermal atomic absorption spectrometry (ET-AAS). After dilution of 20 mg of IL@MWCNTS in 200  $\mu$ 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  $\mu$ g L<sup>-1</sup>; 0.1  $\mu$ g L<sup>-1</sup>), (0.2-5.8  $\mu$ g L<sup>-1</sup>; 0.4-30  $\mu$ g L<sup>-1</sup>) 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<sup>-1</sup> and 162.5 mg g<sup>-1</sup>, respectively. The DIL- $\mu$ -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,

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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  $\text{mg dL}^{-1}$  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  $\text{mg Ni per kg}$  (ATSDR). Toxic effects of oral exposure to nickel usually involve the kidneys (ATSDR). Normal range for Ni in healthy peoples is 0.2  $\mu\text{gL}^{-1}$  in serum and less than 3.0  $\mu\text{gL}^{-1}$  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- $\mu$ -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- $\mu$ -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- $\mu$ -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- $\mu$ -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][PF<sub>6</sub>]) functionalized on MWCNTs (IL-MWCNTs) was used for extraction of Ni and Pb ions in human urine and serum samples by the DIL- $\mu$ -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 ( $\mu\text{L}$ )	1-100	1-100
LOD	0.75	1.25
LLOQ	3.0	5.0
R <sup>2</sup>	0.9998	0.9997

## 2. Materials and Methods

### 2.1. Apparatus

The AAS (GBC, 932, AUS) based on furnace accessory (Pal 3000) and deuterium ( $\text{D}_2$ ) /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  $\mu\text{L}$  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  $\text{min}^{-1}$  per 1 s). The auxiliary gas flow was adjusted 1.2 L  $\text{min}^{-1}$ . 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- $\mu$ -

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 ( $\text{Pb}^{2+}$ ) was purchased from Merck CO. (Germany) with a concentration of 1000  $\text{mg L}^{-1}$  in 1 %  $\text{HNO}_3$ . The standard stock solutions (1000  $\text{mg L}^{-1}$ ) 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),  $\text{HNO}_3$ , 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][PF<sub>6</sub>]-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 NaBH<sub>4</sub> / CH<sub>3</sub>OH, and COOH were reduced to CH<sub>2</sub>OH 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][PF<sub>6</sub>] 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 N<sub>2</sub>[34].

### 2.4. General procedure

By the DIL- $\mu$ -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][PF<sub>6</sub>] 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).

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

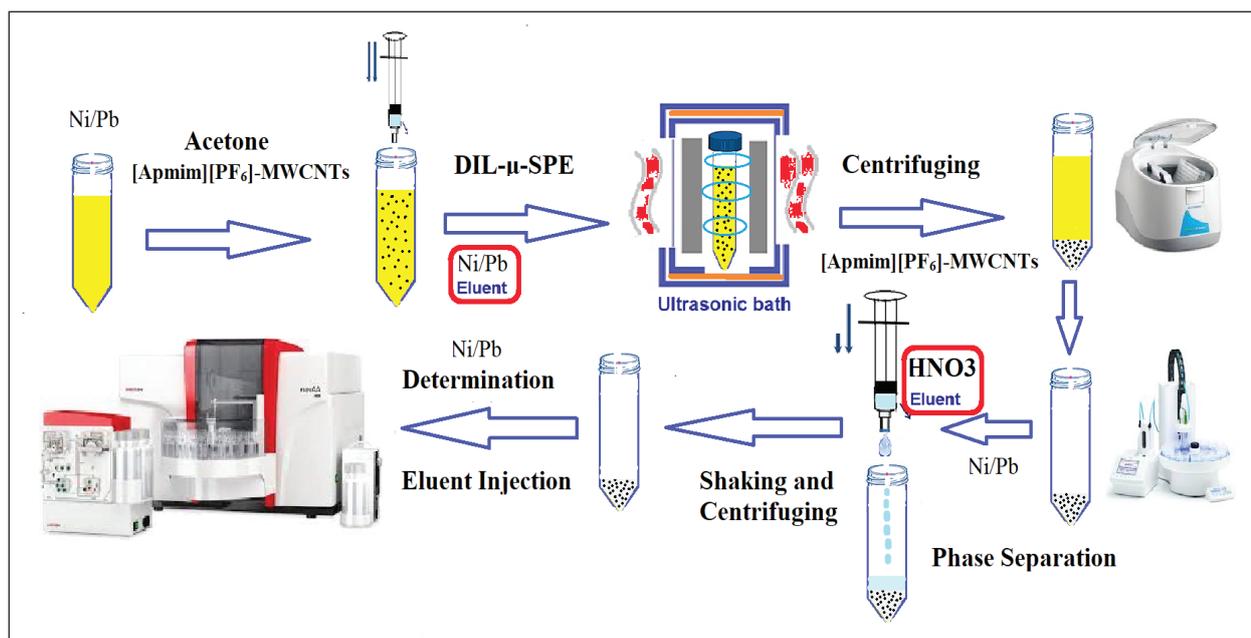


Fig. 1. The DIL- $\mu$ -SPE procedure based on IL-MWCNTs for Pb and Ni extraction

**Table 2.** The analytical features for determination lead and nickel by DIL- $\mu$ -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 ( $\mu$ L)	20	20
Linear range for serum ( $\mu$ g L <sup>-1</sup> )	0.4-30	0.2-5.8
Mean RSD %, n=10	4.2	3.9
LOD for urine or serum ( $\mu$ g L <sup>-1</sup> )	0.1	0.05
Enrichment factor for urine or serum	5.1	24.7
Volume and concentration of HNO <sub>3</sub>	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 <sup>2</sup> = 0.9997	R <sup>2</sup> = 0.9995

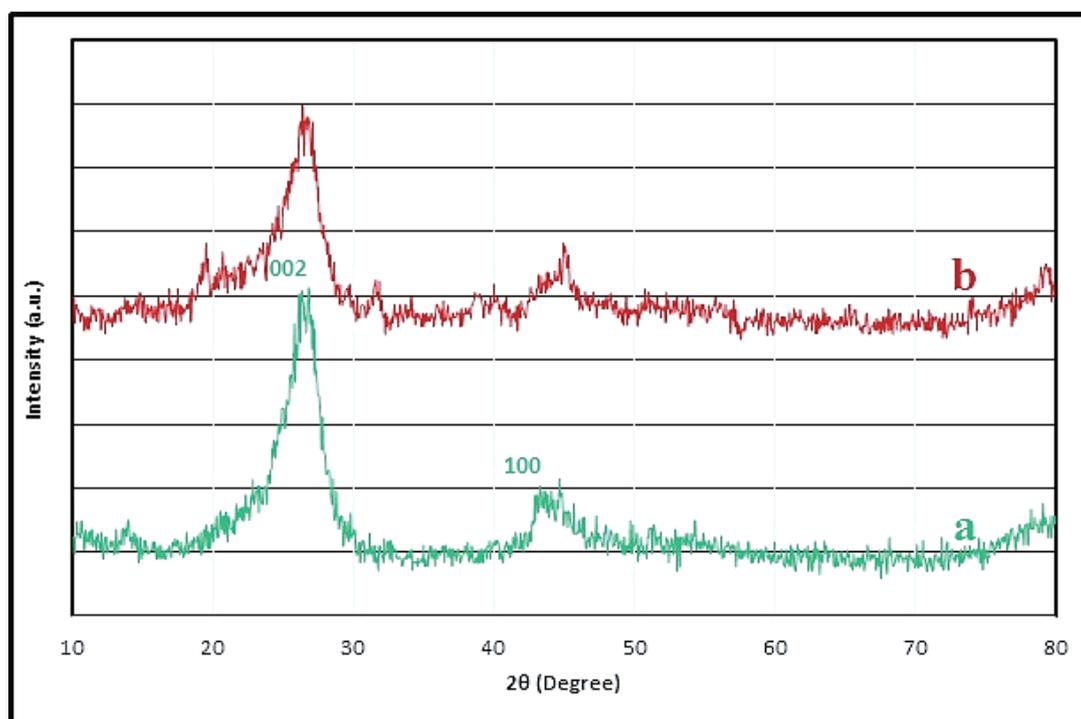
### 3. Results and discussion

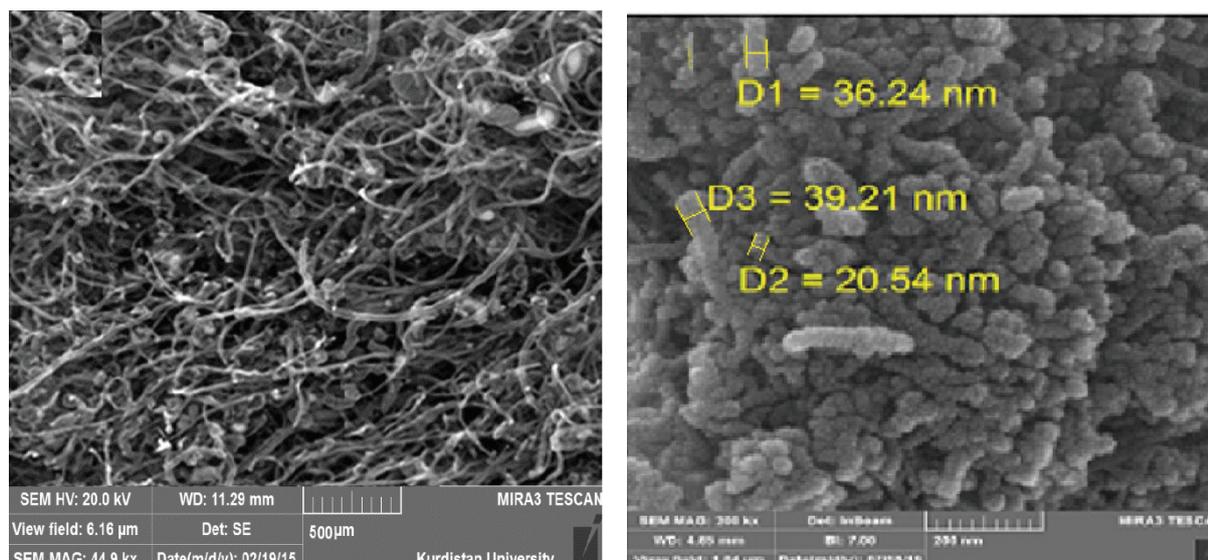
The lead and nickel were extracted and determined based on the IL-MWCNTs nanostructures which characterized by scanning electron microscopy (SEM), X-ray diffraction spectroscopy (XRD), and Fourier transform infrared spectroscopy (FT-IR).

#### 3.1. X-ray diffraction spectroscopy (XRD)

The powder XRD patterns of pristine MWCNTs (a) and [Apmim][PF<sub>6</sub>] 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  $2\theta$  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][PF<sub>6</sub>] 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][PF<sub>6</sub>] immobilized on MWCNTs

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

FE-SEM images of [Apmim][PF<sub>6</sub>] 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][PF<sub>6</sub>] 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][PF<sub>6</sub>]-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<sup>-1</sup> is showed to the carbonyl bond (CO) due to oxidation functionalities. Also, the peak at 3437-3439 cm<sup>-1</sup> 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<sup>-1</sup> which is attributed to the O-Si-O bond constructed between MWCNTs and ionic liquid moieties. The IR peak at 2922 and 2854 cm<sup>-1</sup> were related to

asymmetric and symmetric vibration absorptions, respectively, for the aliphatic CH<sub>2</sub> groups (C-H) of chlorosilane coupling agent and butyl chain of [Apmim][PF<sub>6</sub>].

### 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, HNO<sub>3</sub> 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<sup>-1</sup> and 0.4-30 μg L<sup>-1</sup>, 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

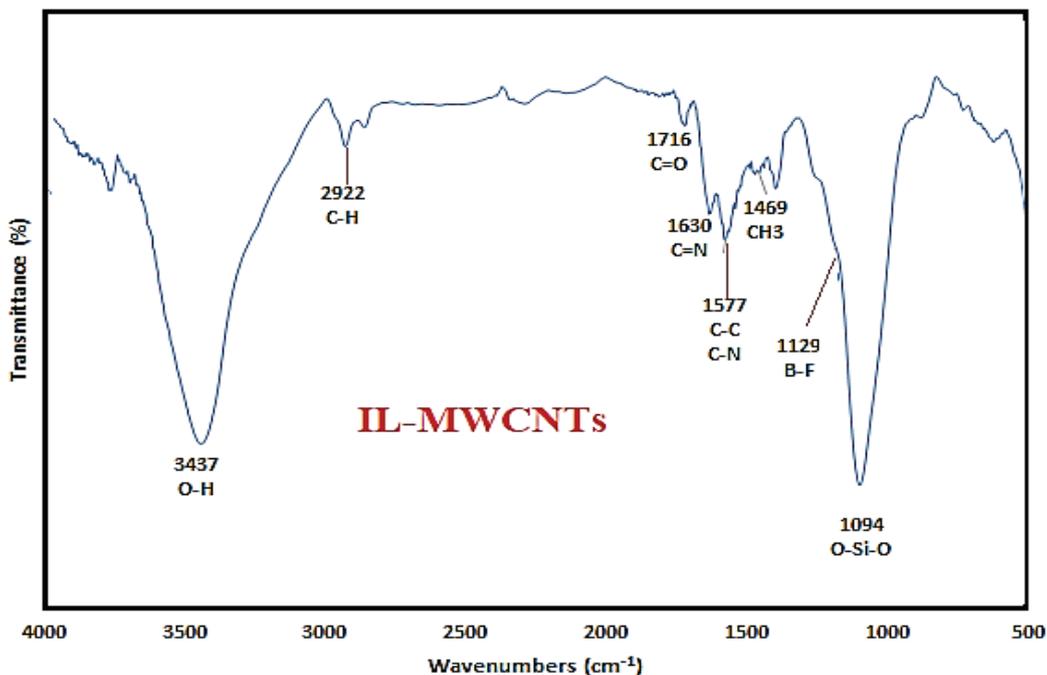


Fig.4. The FT-IR spectra of IL-MWCNTs

as optimal pH by the DIL- $\mu$ -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  $\text{H}^+$  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  $\text{NH}_2$  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.

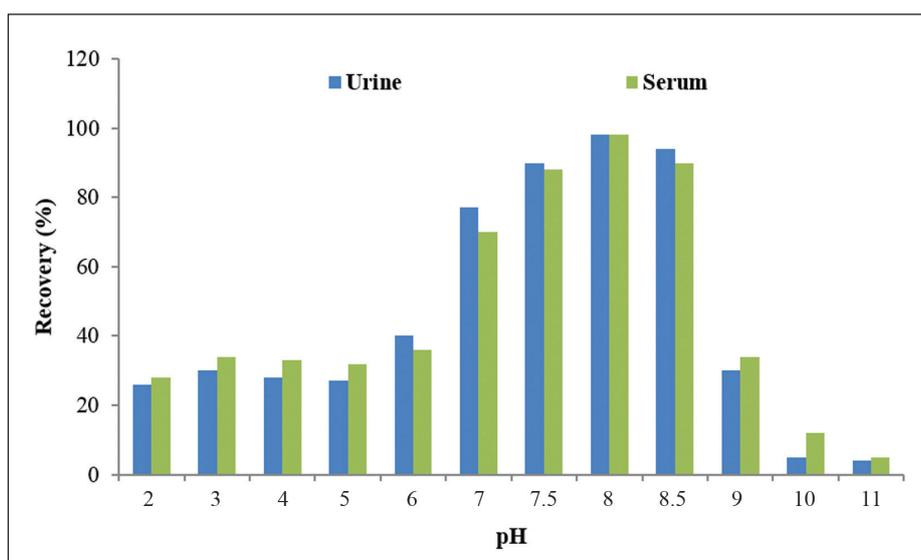


Fig. 5. The effect of pH on Pb/Ni extraction by the DIL- $\mu$ -SPE procedure

### 3.4.2. Optimization of amount of IL-MWCNTs

By the DIL- $\mu$ -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- $\mu$ -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  $\mu$ L and concentration between 0.1-0.5 mol L<sup>-1</sup> were used as eluent phase (HCl, HNO<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub>) by the DIL- $\mu$ -SPE method. The results showed that the 0.2 mol L<sup>-1</sup> of HNO<sub>3</sub> (0.2 mL) had quantitatively back extracted Pb/Ni ions from IL-MWCNTs (Figs. 7). So, the HNO<sub>3</sub> 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  $\mu$ g L<sup>-1</sup> and 0.4-30  $\mu$ g L<sup>-1</sup> for nickel and lead, respectively by the DIL- $\mu$ -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- $\mu$ -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- $\mu$ -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<sup>-1</sup> 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<sup>-1</sup> and 149.3 / 162.5 mg g<sup>-1</sup>, 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- $\mu$ -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  $\mu$ g L<sup>-1</sup> and 5.5  $\mu$ g L<sup>-1</sup>, respectively. The results showed, the main concomitant ions had no effect on the metal extraction at pH=8. The IL-MWCNTs had good

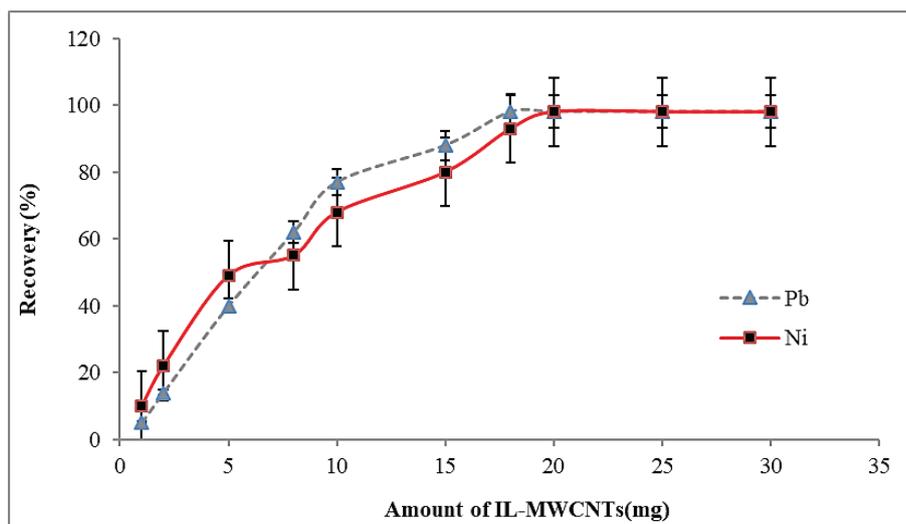


Fig. 6. The effect of amount of IL-MWCNTs on Pb/Ni extraction by the DIL- $\mu$ -SPE procedure

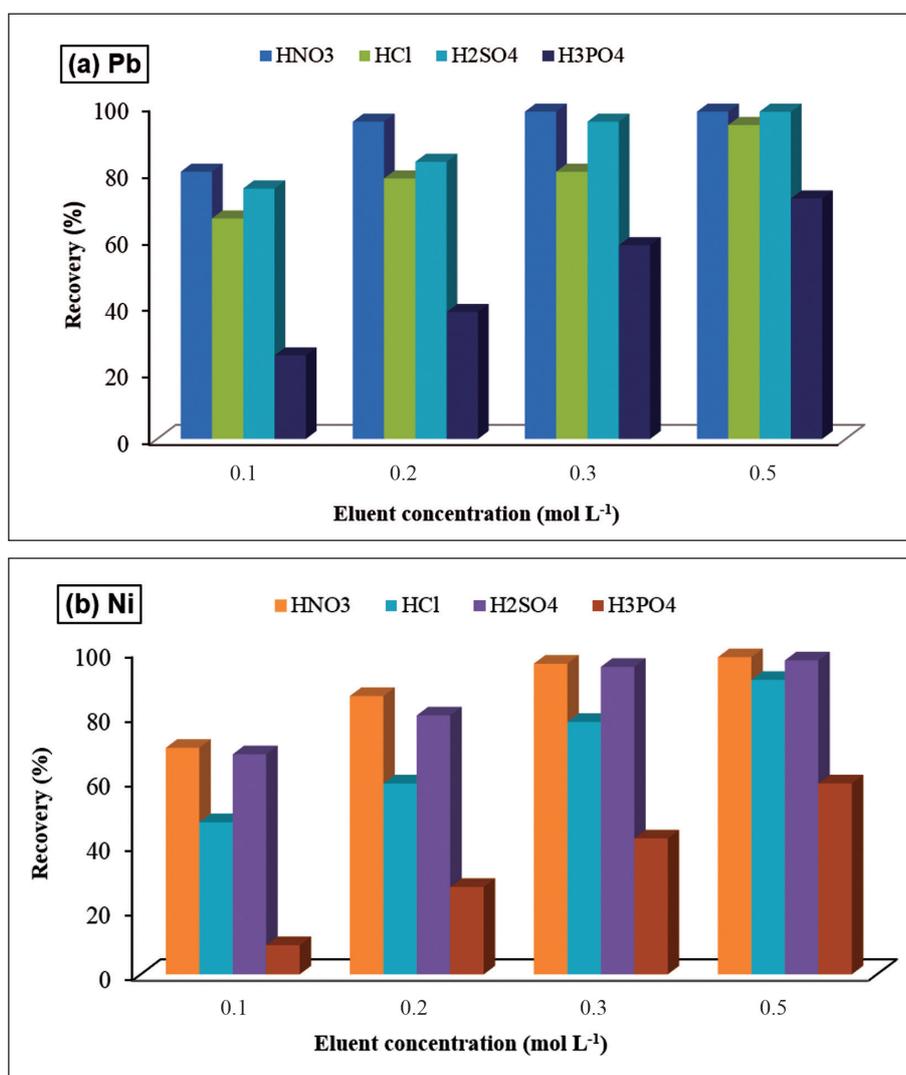
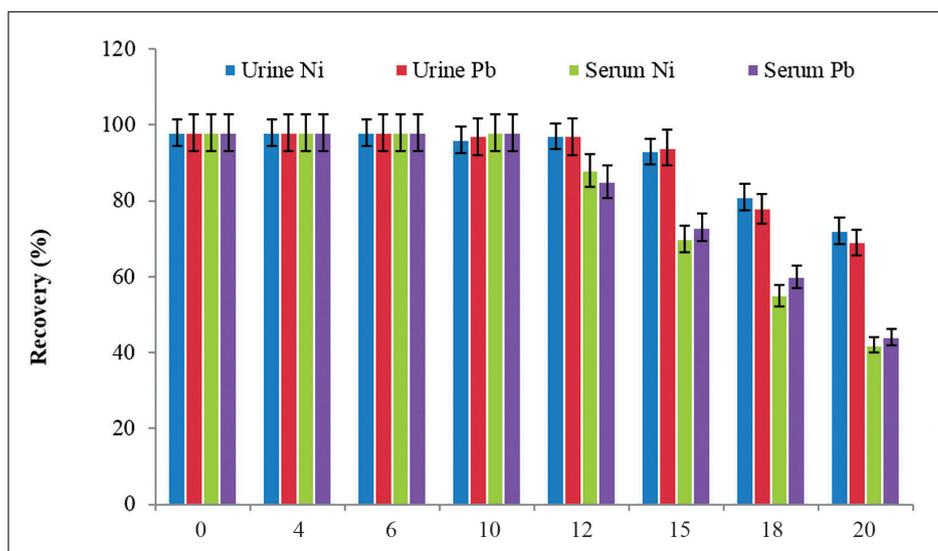


Fig. 7. The effect of eluent for back-extraction of a) lead and b) nickel from IL-MWCNTs by the DIL- $\mu$ -SPE procedure



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

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

**Table 4.** Validation of lead determination(Pb) based on spiking of human serum, blood, plasma and urine samples by DIL- $\mu$ -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- $\mu$ -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- $\mu$ -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- $\mu$ -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 NH<sub>2</sub> 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- $\mu$ -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- $\mu$ -SPE method can be used as applied techniques for Ni(II) and Pb(II) determination in human samples.

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