

Full Length Research Paper

Separation of lead(II) paraffin-embedded tissues from liver loggerhead turtles specimens by organic-solution-processable functionalized-nano graphene prior to determination by flame atomic absorption spectrometry (FAAS)

Ali Moghimi

Department of Chemistry, Varamin (Pishva) Branch Islamic Azad University, Varamin, Iran.
E-mail: alimoghimi@iauvaramin.ac.ir, kamran9537@yahoo.com.

Accepted 12 February 2013

A novel and selective method for the fast determination of trace amounts of Pb(II) ions in liver loggerhead turtles specimens has been developed. A reliable and rapid method for preconcentration and modification of organic-solution-processable functionalized-nano graphene with Isopropyl 2-[(isopropoxy carbothiyl) disulfanyl] ethanethioate (IIDE) in order to prepare an effective sorbent for the preconcentration and determination of lead. The sorption capacity of modified organic-solution-processable functionalized-nano graphene (IIDE MS) was 82.34 mg.g^{-1} and the optimum pH for the quantitative recovery of lead was found as 5.3. The optimum flow rate, sorbent amount and sample volume were 8 ml.min^{-1} , 300 mg and 50 ml, respectively. 10 ml of 0.1 mol.L^{-1} HCl was the most suitable eluent. The recommended method is simple and reliable for the determination of lead without any notable matrix effect and successfully applied to environmental water samples. The limit of detection of the proposed method is 7.5 ng/ml. The method was applied to the extraction and recovery of Pb(II) in different water samples. In the present study, we report the application of preconcentration techniques still continues increasingly for trace metal determinations by flame atomic absorption spectrometry (FAAS) for quantification of lead in Formalin-fixed paraffin-embedded (FFPE) tissues from liver loggerhead turtles. This method exhibits the superiority in compared to the other adsorption reagents because of the fact that there is no necessity of any complexing reagent and optimum pH of solution presents in acidic media. In this method, the relative standard deviation (RSD) of 2.7%.

Key words: Organic-solution-processable functionalized-nano grapheme, lead, Isopropyl 2-[(isopropoxy carbothiyl)disulfanyl] ethanethioate (IIDE) -modified silica-gel, formalin-fixed paraffin-embedded (FFPE), tissues from liver loggerhead turtles.

INTRODUCTIONS

Lead is one of the most ubiquitous elements in the environment and recognized as a major health risk to humans and animals (Released on World Environment Day, 2001). Direct determination of trace metals especially toxic metal ions such as mercury, tin, lead and metalloids arsenic, antimony and selenium from various samples requires mostly an initial and efficient pre-concentration step (Leyden et al., 1976). This pre-concentration is required to meet the detection limits as

well as to determine the lower concentration levels of the analyte of interest (Jones et al., 1983). This can be performed simply in many ways including liquid and solid phase extraction techniques (Nambiar et al., 1998; Caroli et al., 1991). The application of solid phase extraction technique for pre-concentration of trace metals from different samples results in several advantages such as the minimal waste generation, reduction of sample matrix effects as well as adsorption of the target species on the

solid surface in a more stable chemical form (Alexandrova and Arpadjan, 1993).

The normal and selective solid phase extractors are those derived from the immobilization of the organic compounds on the surface of solid supports which are mainly nano polyurethane forms (Arpadjan et al., 1997), filter paper (Leyden and Luttrell, 1975), cellulose (Gennaro et al., 1983) and ion exchange resins (Shamsipur et al., 2005). Silica gel, alumina, magnesia and zirconia are the major inorganic solid matrices used to immobilize the target organic modifiers on their surfaces (Unger et al., 1979) of which silica gel is the most widely used solid support due to the well documented thermal, chemical and mechanical stability properties compared to other organic and inorganic solid supports (Boudreau and Cooper, 1989). The surface of silica gel is characterized by the presence of silanol groups, which are known as weak ion exchangers, causing low interaction, binding and extraction of the target analytes (Kvitek et al., 1982). For this reason, modification of the silica gel surface with certain functional groups has successfully been employed to produce the solid phase with certain selectivity characters (Bruening et al., 1991). Two approaches are known for loading the surface of solid phases with certain organic compounds and these are defined as the chemical immobilization which is based on chemical bond formation between the silica gel surface groups and those of the organic modifier, and the other approach is known as the physical adsorption in which direct adsorption of the organic modifier with the active silanol groups takes place (Unger et al., 1979).

Selective solid phase extractors and pre-concentrators are mainly based on impregnation of the solid surface with certain donor atoms such as oxygen, nitrogen and sulfur containing compounds (Mahmoud, 1997, 1999; Mahmoud and Soliman, 1997; Tong et al., 1990; Dadler et al., 1987). The most successful selective solid phases for soft metal ions are sulfur-containing compounds, which are widely used in different analytical fields. Amongst these sulfur-containing compounds are dithiocarbamate derivatives for selective extraction of lead(II) (Mahmoud, 1998, 1999) and pre-concentration of various cations (Leyden et al., 1976; Moghimi et al., 2009; Tehrani et al., 2005) and 2- mercaptobenzothiazol-modified silica gel for on-line pre-concentration and separation of silver for atomic absorption spectrometric determinations (Moghimi et al., 2009). Ammonium hexahydroazepin-1-dithiocarboxylate (HMDC)-loaded on silica gel as solid phase pre-concentration column for atomic absorption spectrometry (AAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) was reported (Alexandrova and Arpadjan, 1993). Mercapto-modified silica gel phase was used in pre-concentration of some trace metals from seawater (Moghimi et al., 2009). Sorption of lead (II) by some sulfur containing complexing agents loaded on various solid supports

(Moghimi et al., 2011) was also reported. 2-Amino-1-cyclopentene-1-dithiocarboxylic acid (ACDA) for the extraction of silver(I), mercury(II) and lead(II) (Moghimi, 2006), 2-[2-triethoxysilyl-ethylthio] aniline for the selective extraction and separation of palladium from other interfering metal ions (Tehrani et al., 2005) as well as thiosemicarbazide for sorption of different metal ions (Moghimi et al., 2011) and thioanilide loaded on silica gel for pre-concentration of palladium(II) from water (Tehrani et al., 2005) are also sulfur containing silica gel phases.

Ion adsorption onto solid chelating nano polymer materials is now considered as one of the most promising techniques for selective concentration, removal and recovery of metal ions from a wide variety of sources. Among different types of polymer adsorbent, polymer fibers have attracted great interest in recent years (Tahaei et al., 2008). This can be related to their structure and characteristics, like high specific surface, small cross-section, uniformity in diameter (in macroscopic scale) and long length of fiber to diameter (Abdouss and Mousavi, 2012, Moghimi, 2006).

Raw acrylic fibers (RAF), due to their chemical and thermal stability, are a good substrate for the modification. The mentioned advantages are mainly attributed to the high adsorption capacities, fast adsorption equilibrium, high recycling rate and low cost of these polymeric fibers. The use of commercial fiber and introducing functional groups on its structure are of the important methods for producing ion adsorbent fibers. The properties of the fiber can be maintained in this method (Abdouss and Mousavi, 2012).

In our previous attempts, we modified solid phase extraction (SPE) membrane disks with suitable compounds for selective determination of lead (Tuzen et al., 2009). Meanwhile, other investigators have successfully utilized these sorbents for quantitative extraction and monitoring trace amounts of lead (Tahaei et al., 2008).

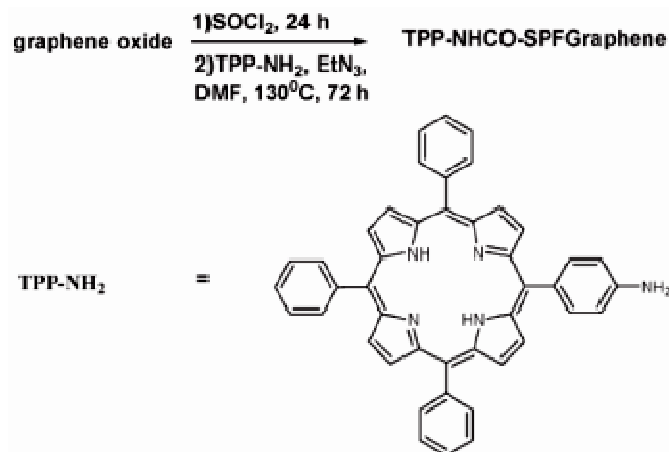
This study focuses on the utilization of IIDEEMS as an efficient organic-solution-processable functionalized-nano graphene in the preconcentration step of lead(II) ions prior to flame atomic absorption spectrometry (FAAS) determination. The influences of some analytical conditions on the preconcentration procedure, such as initial pH, sample volume, eluent type and volume, sorbent amount, flow rate, etc. were investigated.

The second aim of this study was the selection of an appropriate method for the analysis of Formalin-fixed paraffin-embedded (FFPE) tissue were based on present work with atomic absorption spectrophotometric determination of lead(II).

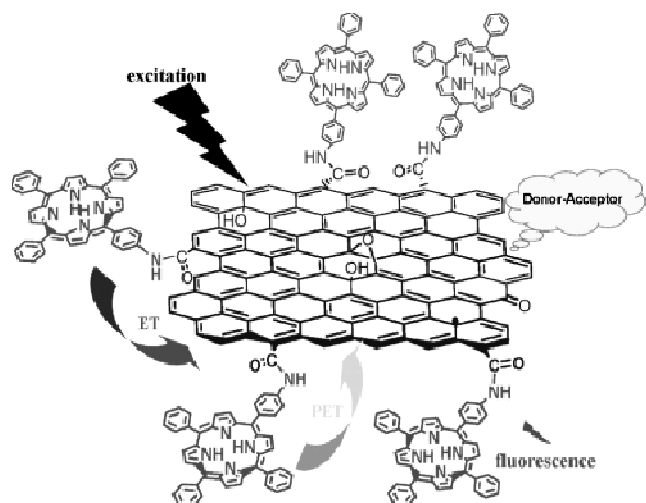
EXPERIMENTAL

Apparatus

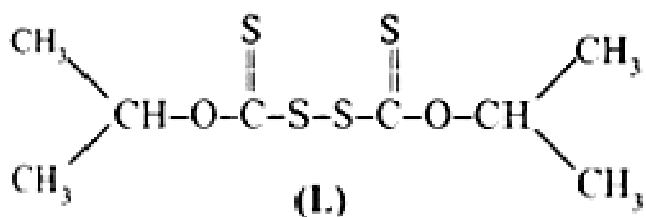
Determination of Pb²⁺ contents in working samples were carried out



Scheme 1. Synthesis scheme of TPP-NHCO-SPFGraphene.



Scheme 2. Schematic representation of part of the structure of the covalent TPP-NHCO-SPFGraphene.



Scheme 3. Molecular structure of Isopropyl 2-[[isopropoxycarbothioyl]disulfanyl] ethanethioate.

by a Varian spectra A.200 model atomic absorption spectrometer equipped with a high intensity hollow cathode lamp (HI-HCl) according to the recommendations of the manufacturers. Instrumental parameters were selected according to the

manufacturer's suggestion. The infrared spectra of the materials were recorded on a Perkin Elmer spectrum 100-IR spectrometer (Waltham, MA, USA) using KBr disk in the range of 4000 to 400 cm⁻¹. Heidolph PD 5201 (Schwabach, Germany) model peristaltic pump with eight heads was used for controlling the flow of the liquid into column. The pH measurements were carried out by an ATC pH meter (EDT instruments, GP 353).

Reagents

Organic-solution-processable functionalized-nano graphene was used as the support material. All the chemicals used in this study were of analytical grade. Doubly distilled deionized water was used in all experiments. The standard solution of lead(II) (1000 mgL⁻¹) for the calibration of AAS was purchased from Merck (Darmstadt, Germany). The other concentrations of the standard solutions were prepared by diluting this solution. A stock solution of lead(II) was prepared by dissolving appropriate amount of Pb(NO₃)₂ Merck (Darmstadt, Germany) in doubly distilled deionized water and the other concentrations of the working solutions were obtained by diluting this stock metal solution. The pH of the solutions was adjusted to desired values with 0.1 M HCl and/or 0.1 M NaOH solutions. Working solutions were prepared by appropriate dilution of the stock solution.

Synthesis of L

Iodine (1 mmol) in CH₂Cl₂ (10 ml) was added to a stirred solution of potassium *o*-isopropyl(dithiocarbamate) (1 mmol) in CH₂Cl₂ (10 ml) and stirred for 1 h. The reaction mixture was washed with 10% aqueous Na₂S₂O₃ (2×10 ml) and H₂O (2×10 ml). The organic layer was dried over MgSO₄ and evaporated under reduced pressure. More purification carried out with the re-crystallization in hexane so that pale yellow crystals of L were obtained in 90% yield (0.24 g). The structure and purity of L was confirmed by elements analysis, NMR and IR Spectroscopy. ¹H NMR (CCl₄). δ (ppm): 1.43 (t, 12H, CH₃), 5.63 (m, 2H, CH). IR (KBr). ν_{max} (cm⁻¹): 2979.8 (s), 2869.9 (w), 1463.9 (s), 1442.7 (s), 1373.0 (s), 1271.1 (s, b), 1145.6 (s), 1082.2 (s), 1048.0 (s, b), 898.8 (s), 796.5 (s), 690.5 (m) (Scheme 3).

Synthesis of TPP-NHCO-SPFGraphene

The first organic-solution-processable functionalized-graphene (SPFGraphene) hybrid material with porphyrins. The synthesis of the porphyrin-Graphene nano-hybrid, 5-(4-aminophenyl)-10, 15, 20-triphenyl porphyrin (TPP) and graphene oxide molecules covalently bonded together via an amide bond (TPP-NHCO-SPF Graphene, Schemes 1 and 2) was carried out using an amine-functionalized porphyrin (TPP-NH₂) and Graphene oxide in N,N-dimethylformamide (DMF), following standard chemistry. Large-scale and water-soluble Graphene oxide was prepared by the modified Hummers method (Becerril et al., 2008; Liu et al., 2008; Hummers and Offeman, 1958). Results of atomic force microscopy characterization have confirmed that this graphene material can be easily dispersed at the state of complete exfoliation, which consists of almost entire single-layered Graphene sheets in H₂O (Becerril et al., 2008; Liu et al., 2008; Hummers and Offeman, 1958).

TPP-NH₂ and Graphene oxide molecules are covalently bonded together by an amide bond. Much care has been taken to make sure all the unreacted TPP-NH₂ has been removed using extensive solvent washing, sonication, and membrane filtration. Details are given in the experimental part. The attachment of organic molecules to Graphene oxide has made TPP-NHCO-SPFGraphene soluble in DMF and other polar solvents

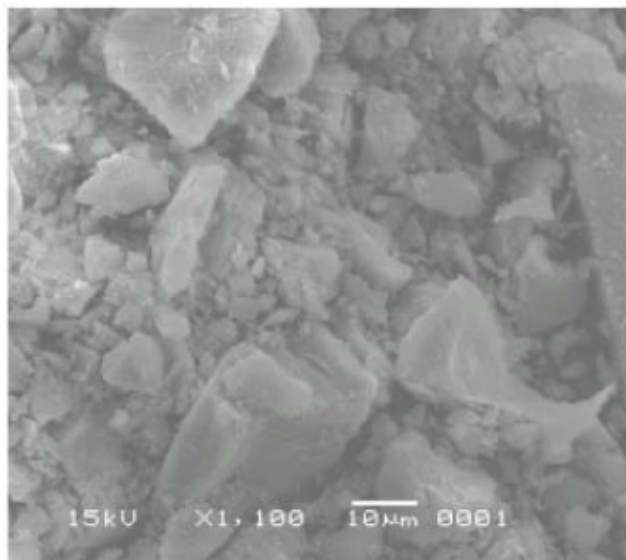


Figure 1. SEM micrograph of IIDEMS.

(Becerril et al., 2008; Liu et al., 2008; Hummers and Offeman, 1958).

Preparation of Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate modified organic-solution-processable functionalized-nano graphene

All acids were of the highest purity available from Merck and were used as received. Methanol and Chloroform were of High-performance liquid chromatography (HPLC) grade from Merck. Analytical grade nitrate salts of lithium, sodium, potassium, magnesium, calcium, strontium, barium, zinc, cadmium, copper(II) nickel, cobalt(II), and Pb(II) were of the highest purity. Ultra pure organic solvents were obtained from E. Merck, Darmstadt, Germany. The stock standard solution of Pb(II) was prepared by dissolving 0.1000 g of the Pb(II) powder in 10 ml concentrated nitric acid and diluted to 1000 ml with water in a calibrated flask. Working solutions were prepared by appropriate dilution of the stock solution. In order to prepare a 0.1% Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate (IIDEMS) solution, 0.1 g of the reagent was dissolved in 10 ml of acetone and 8 ml of concentrated ammonia solution was added. The final volume of this solution was diluted to 100 ml with water. 100 ml of reagent solution was added into four grams of organic-solution-processable functionalized-nano graphene suspended in 100 ml water and then mixed on a magnetic stirrer for 24 h. The final product was filtered, washed with doubly distilled deionized water and then dried at 100°C in an oven overnight.

Column preparation

A glass column (5.5 cm height × 9 mm i.d.) was packed with a known amount of IIDEMS between two layers of glass wool into the column. A definite volume of lead(II) solution ($1 \mu\text{g ml}^{-1}$) was passed through the column. All the column studies were performed at room temperature of 25°C.

Preconcentration procedure

The preconcentration method was tested with synthetic lead(II)

solutions prior to its application to the real samples. For this purpose, an aliquot of 50 ml of standard lead(II) solution ($1 \mu\text{g ml}^{-1}$) was taken and the pH of the solution was adjusted to desired value with HCl and/or NH_3 . The model solution was pumped through the column at a flow rate of 8 ml min^{-1} controlled with a peristaltic pump. The bound metal ions were eluted from IIDEMS with 10 ml of 0.1 mol L^{-1} HCl solution. The lead(II) concentration in the eluate was determined by FAAS. The recoveries of lead(II) were calculated from the ratio of concentration found by FAAS to that calculated theoretically. The general preconcentration procedure described above was carried out to optimize the experimental conditions such as pH, amount of adsorbent, flow rate, type, concentration and volume of the elution solutions, etc.

Analysis of sample paraffin-embedded tissues from liver loggerhead turtles specimens

Selected areas from fresh frozen tissues from liver loggerhead turtles specimens were sliced in three pieces (numbered as 1, 2 and 3) of approximately $10 \times 5 \times 2 \text{ mm}$ each. Sets of pieces of set 1 (controls), were placed into a vacuum chamber at 50°C overnight to dry (until a constant weight was obtained) and the sets 2 and 3 were subjected to the standard 10% buffered formalin fixation and paraffin embedding histological process using a tissue processor (Tissue-Tek VIP, Sakura Finetek USA Inc., Torrance, CA). After the paraffin embedding process, tissues were subsequently excised from the blocks with a titanium knife and deparaffinized in xylene at 55°C for 1 h in the tissue processor (the set 2), or with hexane at 20°C for 1 week with frequent changes of the solvent in handling-based procedure (the set 3). Xylene was of a grade routinely used for the FFPE process and hexane was of "Optima" grade (Fisher Scientific). Upon deparaffinization, the tissue samples were dried in a vacuum chamber until constant weight was obtained. Each dried sample (of the sets 1-3) was divided into three portions (5 to 10 mg each) to be further analyzed as triplicates.

RESULTS AND DISCUSSION

Characteristics of the material

The sorbent surface was examined by scanning electron microscopy (SEM) and SEM micrograph of IIDEMS was given in Figure 1. The surface of the organic-solution-processable functionalized-nano graphene changed after the modification with Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate (IIDEMS). The surface of organic-solution-processable functionalized-nano graphene was smoother while the modified sorbent surface had a tendency to form agglomerates.

The modification of the sorbent was confirmed by interpreting the infrared spectra of organic-solution-processable functionalized-nano graphene and IIDEMS in Figure 2. The main characteristic absorption band between 3420 and 3646 cm^{-1} is due to O-H stretching mode of silanol groups of organic-solution-processable functionalized-nano graphene and also the remaining adsorbed water (Ekinci-Dogan and Akcin, 2007). The absorption band at 1633 cm^{-1} in the FTIR spectra of organic-solution-processable functionalized-nano graphene (Figure 2a) assigned to water bending mode (Hatay et al., 2008). The broad and intense band at 1047 cm^{-1} is related to the

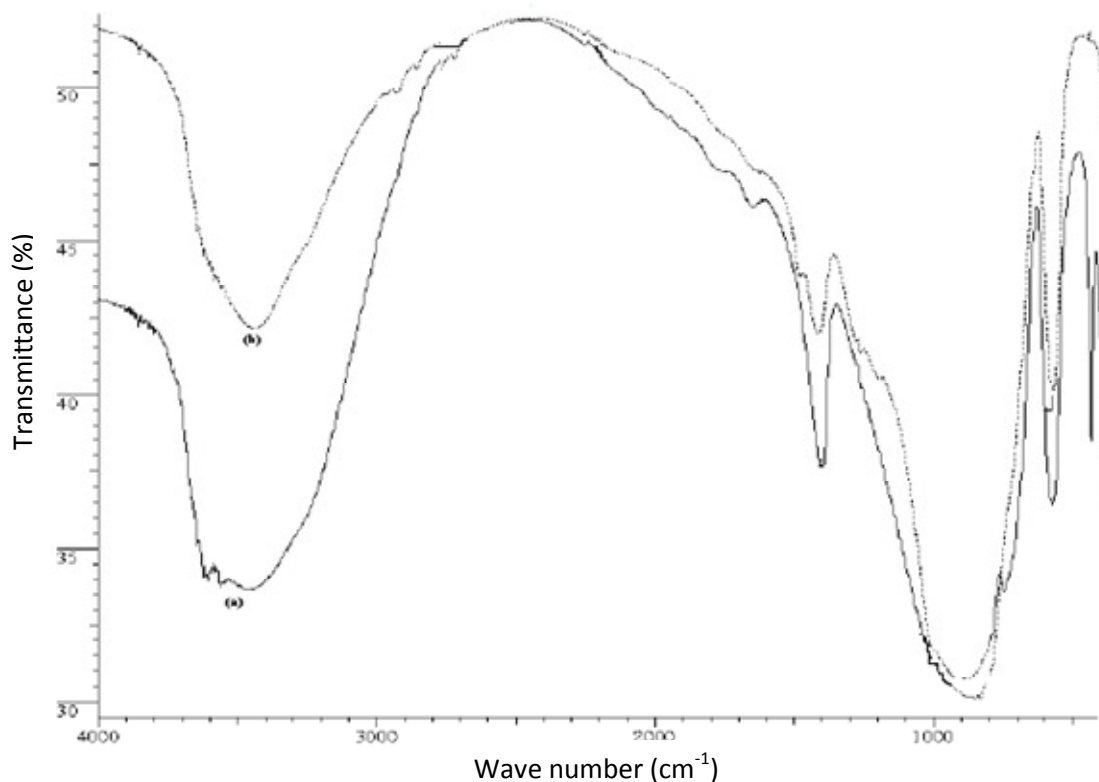


Figure 2. SEM micrograph of IIDE/S.

anti-symmetric stretching vibration of Si-O-Si groups (Roldan et al., 2005). The modified sorbent shows the characteristic stretching vibrations of C-H groups at 2924 and 2856 cm^{-1} in Figure 2b. Also an absorption band at 1285 cm^{-1} in the FTIR spectra of IIDE/S indicates C=S stretching vibration in IIDE. These evidences confirm the modification of organic-solution-processable functionalized-nano graphene with Isopropyl 2-[(isopropoxy carbothioyl)disulfanyl]ethanethioate (IIDE).

Effect of pH on the recovery of lead

The pH of the sample solutions were adjusted to different values between 2-8 by addition of hydrochloric acid or a suitable buffer such as sodium acetate-acetic acid or sodium dihydrogen phosphate- disodium hydrogen phosphate, and then solutions passed through the column. Eventually, the metal ions were stripped by 5 ml of HNO_3 , 1 M followed by flame atomic absorption determination of the eluted Pb(II). Then, percentage recovery at various pH values was determined (Figure 4). According to the results shown in Figure 4 up to pH 4.5-5.5, complete recoveries are obtained. However, at higher pH values, percentage recovery decreases. This is due to fact that in an acidic solution the protonation of IIDE occurs and there is a weak tendency for retention

between Pb(II) and 1-nitroso-2-naphthol-3,6-disulfonic acid, whereas at higher values ($\text{pH} > 5.7$), Pb(II) reacts with hydroxide ions to produce $\text{Pb}(\text{OH})_2$. Therefore, sodium acetate-acetic acid buffer with $\text{pH} = 5.3$ was used for the preconcentration step. Other solvents used for dissolving IIDE were 5 ml of HNO_3 , 1 M. The influences of these solvents on the recoveries as a function of pH are compared and shown in Figure 4.

Evaluation of the role of the ligand

Some preliminary experiments were performed for investigation of absence or presence of IIDE on the quantitative extraction of Pb(II). It was concluded that the surface itself does not show any tendency for the retention of Pb(II), but introduction of 100 ml portions of aqueous Pb(II) samples containing 10 μg of Pb(II) and 10 mg of IIDE leads to satisfactory its retention (Table 1). The latter case is most probably attributed to the existence of a considerable interaction between Pb(II) and the IIDE. It should be mentioned that formation of stable complexes between Pb(II) and IIDE at pH 5.3 is probably due to an ion pair formation mechanism. However, at pH higher than 5 the retention and percentage recovery of Pb(II) are negligible.

Table 1. The effect of presence of IIDE on extraction percent of lead (II)^a.

IIDE	pH	Extraction percent of lead (II)
Absence	2-6	0.03(6.6) ^b
Presence	2-6	98.6(2.4) to 65(2.5)

^a Initial samples contained 10 µg of lead (II) in 100 ml of water, ^b Values in parentheses are RSD_s based on five individual replicate analyses.

Choice of eluent

In order to select the most appropriate eluent for the quantitative stripping of the retained Pb(II) on the column, 5 ml of various non organic solvents were tested. The results are shown in Table 6. As can be seen, the best eluting solvents were found to be 5 ml of 0.1 molL⁻¹ HCl, resulting in quantitative elution of Pb(II) from the column.

Effect amount of counter anion

In order to investigate the effect of counter ion on the recovery Pb(II) ions by the modified column, different counter anions were tested (Table 2), it is immediately obvious that the nature of the counter anion strongly influences the retention of Pb(II) ions by the column. The results revealed that the IIDE behaves as a neutral ionophore in the pH range 4.5-5.3 (Liu et al., 2008) so that the Pb(II) ions are retained as ion pair complexes by the column. As seen, acetate ion is the most efficient counter anion for the SPE of Pb(II) ions. The influence of the concentration of sodium acetate ion on Pb(II) recovery was investigated, and the results are shown in Table 2. As seen, the percent recovery of Pb(II) increased with the acetate concentration until a reagent concentration of about 0.1 M is reached, beyond which the recovery remained quantitative.

Moreover, acetate ion acts as a suitable buffering agent, while it effectively contributes to the ions- pair formation; thus, in the SPE experiments, there was no need for the addition of any buffer solution.

The influence of flow-rate

One of the most important parameters affecting solid phase extraction is the speed of the process. Hence, the effect of flow-rates on extraction efficiencies was investigated. It was found that in the range of 1 to 15 ml.min⁻¹, the retention of Pb(II) was not considerably affected by the sample solutions flow-rates and leads to reproducible and satisfactory results (Figure 3). Thus, the flow-rate was maintained at 8.0 ml.min⁻¹ throughout the experiment.

Quantity of the IIDE

The optimum amount of IIDE for the quantitative

extraction of Pb(II) was also investigated by adding various amounts of it to solution (between 2-20 mg). The results are listed in Table 3. The experimental results revealed that the extraction of Pb(II) was quantitative using a sample solution containing more than 10 mg IIDE. Hence, subsequent extractions were performed with 15 mg of IIDE.

Analytical performance

When solutions of 10 µg Pb(II) in 10, 50, 100, 500, and 1000 ml solutions under optimal experimental conditions were passed through the column, the Pb(II) was quantitatively retained in all cases. Thus, the breakthrough volume for the method must be greater than 1000 ml, providing a concentration factor of >200. The limit of detection (LOD) of the method for the determination of Pb(II) was studied under the optimal experimental conditions. The LOD based on 3σ of the blank is 7.5 ng/ml.

In order to investigate the selective separation and determination of Pb(II) ions from its binary mixtures with various metal ions, an aliquot of aqueous solutions (50 ml) containing 10 µg Pb(II) and mg amounts of other cations was taken and the recommended procedure was followed. The results are summarized in Table 4. The results show that the Pb(II) ions in binary mixtures are retained almost completely by the modified column, even in the presence of up to about 100 mg of various ions. Meanwhile, retention of other cations by the column is very low and they can be separated effectively from the Pb(II) ion. It is interesting to note that, in other experiments, we found that in the presence of high enough concentrations NH₂OH.HCl as a suitable reducing agent (> 0.5 M) (Liu et al., 2008).

Analysis of water samples

To assess the applicability of the method to real samples, it was applied to the extraction and determination of Pb(II) from different water samples. Tap water (Tehran, taken after 10 min operation of the tap), rain water (Tehran, 25 January, 2012), Snow water (Saveh, 30 February, 2012) and Sea water (taken from Caspian sea, near the Mahmoud-Abad shore) samples were analyzed. Development of a methodology for the determination of

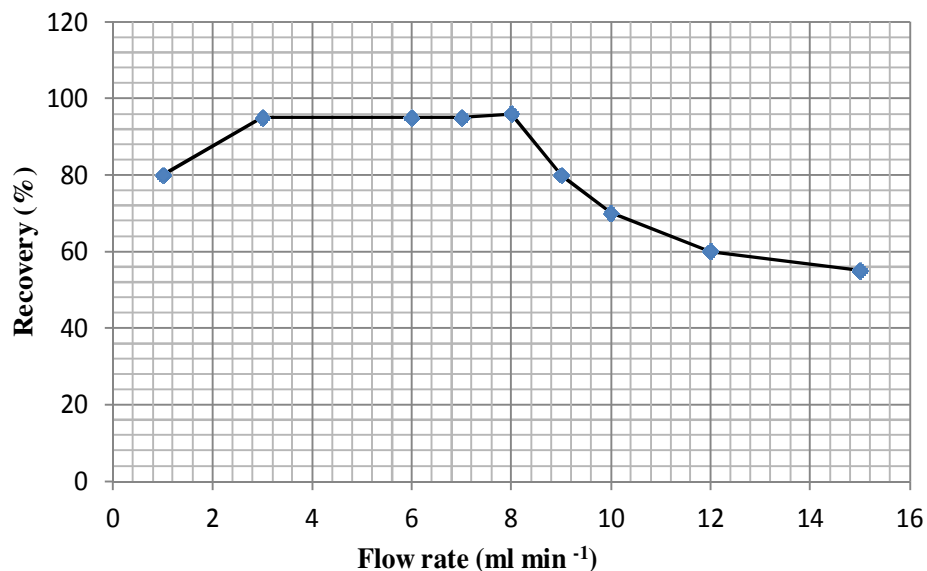


Figure 3. Effect of flow rate of sample solutions on the recovery of lead (II) by IIDE MS.

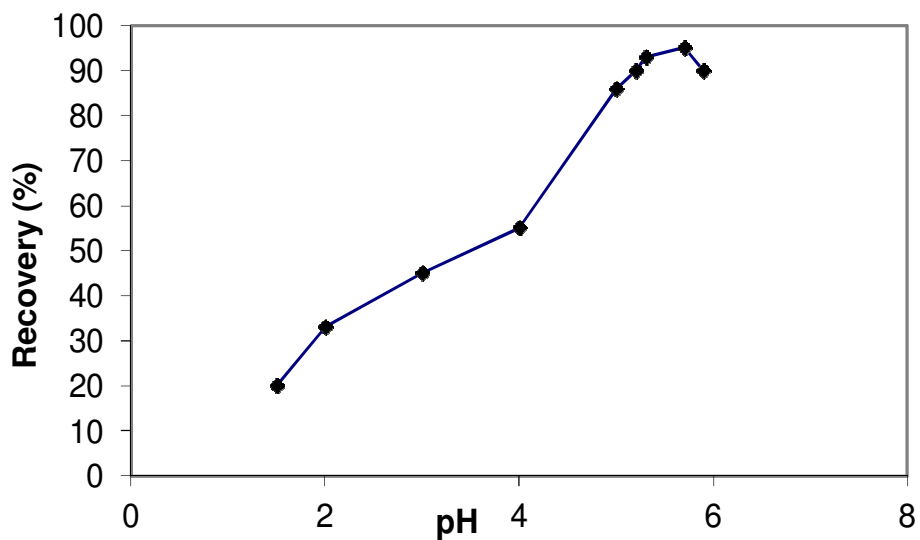


Figure 4. Effect of pH on the recovery of lead (II) by IIDE MS.

Table 2. Percent recovery of lead (II) from the modified of organic-solution-processable functionalized-nano graphene in the presence of 0.01 M of different counter anions^a.

Counter anion	%Recovery
Cl ⁻	28.5
Br ⁻	22.6
ClO ₄ ⁻	30.7
SCN ⁻	42.8
Picrate	75.7
Acetate	92.6

^aInitial samples contained 10 µg of lead (II) in 100 ml of water.

Table 3. Influence of the IIDE amount on the recovery of lead (II) ions^a.

IIDE amount (mg)	Recovery (%) of lead(II)
2	30(2.7) ^b
5	45(2.6)
8	80(2.5)
10	95.8(2.4)
15	99.0(2.5)
20	98.1(2.4)

^aInitial samples contained 10 µg of each lead (II) in 100 ml water, ^bValues in parentheses are RSDs based on five individual replicate analysis.

Table 4. Separation of lead (II) from binary mixtures ^a.

Diverse ion	Amounts taken(mg)	% Found	%Recovery of Pb ²⁺ ion
Na ⁺	92.0	1.19(2.6) ^b	98.9(1.9)
K ⁺	92.9	1.30(2.0)	98.9(2.1)
Mg ²⁺	14.2	0.68(1.8)	99.2(2.0)
Ca ²⁺	20.3	2.20(2.0)	98.5(2.7)
Sr ²⁺	2.80	2.87(2.2)	98.2(2.0)
Ba ²⁺	2.90	3.15(2.3)	98.3(2.8)
Mn ²⁺	2.26	1.73(2.5)	97.3(2.8)
Co ²⁺	2.33	1.20(2.7)	98.8(2.9)
Ni ²⁺	1.90	2.17(2.4)	98.0(2.4)
Zn ²⁺	2.10	1.77(2.0)	98.4(2.2)
Cd ²⁺	2.35	1.90(2.3)	98.2(2.6)
Cu ²⁺	1.90	2.77(1.4)	97.6 (2.5)
Hg ²⁺	0.60	2.81(2.9)	97.7(2.4)
Ag ⁺	2.45	3.45(2.9)	96.6(2.5)
Cr ³⁺	1.70	2.92(2.1)	97.3(2.6)
UO ²⁺	2.60	2.85(2.2)	98.3(2.0)

^aInitial samples contained 10 µg Pb²⁺ and different amounts of various ions in 100 ml water (0.1 M acetate ion), ^b Values in parentheses are RSDs based on five individual replicate analysis.

Table 5. Recovery of lead (II) added to 1000 ml of different water samples (containing 0.1 M acetate at pH= 5.3).

Sample	Pb ²⁺ added (µg)	Pb ²⁺ determined(ng.mL ⁻¹)	ICP-AES
Tap water	0.0	1.74(2.0) ^a	ND ^b
	10.0	11.98(2.2)	11.7
Snow water	0.0	4.86(2.1)	ND
	10.0	14.94(2.2)	14.7
Rain water	0.0	2.65(2.3)	ND
	10.0	12.76(2.9)	12.3
Sea Water	0.0	12.67(2.3)	12.5
	10.0	22.95(2.0)	23.1
Development of a methodology for the determination of Pb ²⁺ in FFPE tissue	0.0	N.D ^b	N.D ^b
	10.0	9.95(2.2)	10.05(2.5)

^a Values in parentheses are %RSDs based on five individual replicate analysis, ^b Not detected.

Pb(II) in FFPE tissue was performed in a number of steps to optimize the major factors affecting the precision of the analysis (Table 5). As can be seen from Table 5 the added Pb(II) ions can be quantitatively recovered from the water samples used.

Effect of the type, concentration and volume of the elution solutions

In order to determine the most suitable solution for the elution of lead, three different types of eluting agents (HCl, HNO₃ and EDTA) were evaluated. HCl was found to

be most effective eluent (Table 6). 0.1 molL⁻¹ and 0.2 molL⁻¹ of HCl solutions give quantitative recoveries (≥99%). Various volumes of 0.1 molL⁻¹ HCl were also examined as eluent and the results were represented in Figure 5. Since quantitative recovery (≥99%) was obtained with 10 ml of 0.1 mol L⁻¹ HCl solution, it was selected as an eluent for the recovery of lead by IIDE-modified silica-gel (IIDEEMS).

Adsorption capacity

Adsorption capacity of IIDEEMS for lead(II) ions was

Table 6. Effect of the type and concentration of the elution solutions.

Type of the elution solution	Concentration (mol L ⁻¹)	Recovery ^a (%)
HCl	0.05	17
	0.1	99
	0.2	97
HNO ₃	0.05	28
	0.1	57
	0.2	95
EDTA	0.05	90
	0.1	91
	0.2	87

^a Mean of three replicates.

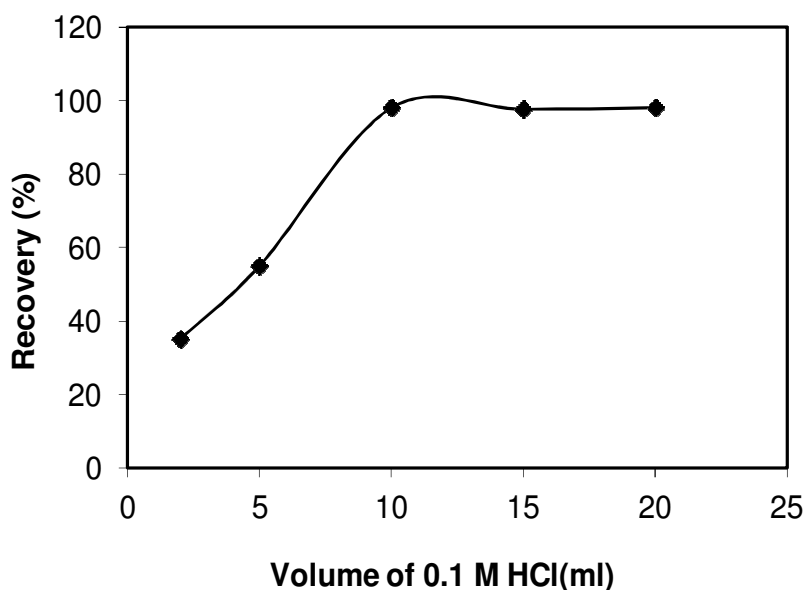


Figure 5. Effect of the volume of 0.1 mol L⁻¹ HCl solution on the recovery of lead (II) by IIDEMS.

determined by a batch method. The initial lead(II) ion concentration was changed from 100 to 1000 mgL⁻¹ for the investigation of adsorption capacity of modified adsorbent. The pH of 50 ml of Pb(II) solution was adjusted to optimum value and then 300 mg of modified sorbent was added to lead(II) solutions and mixed on a digitally controlled magnetic stirrer at a rate of 200 rpm. Suspended solids were separated from the adsorption medium by centrifugation at 4500 rpm for 3 min and lead(II) ion concentrations were then measured using FAAS. The equilibrium data were analyzed by Langmuir and Freundlich (Hummers and Offeman, 1958) isotherm models.

$$\text{Langmuir equation } \frac{1}{q_e} = \frac{1}{q_{max}} + \left(\frac{1}{q_{max}K_L} \right) \frac{1}{C_e} \quad (1)$$

$$\text{Freundlich equation } \ln q_e = \ln K_F + 1/n \ln C_e \quad (2)$$

Where q_e and q_{max} are the equilibrium and monolayer sorption capacities of the sorbent (mg g⁻¹), respectively, C_e is the equilibrium metal ion concentration in the solution (mgL⁻¹) and K_L is the equilibrium constant (L mg⁻¹) related to the free energy of biosorption. K_F (L.g⁻¹) and n (dimensionless) are Freundlich sorption isotherm constants. The Langmuir and Freundlich isotherm plots

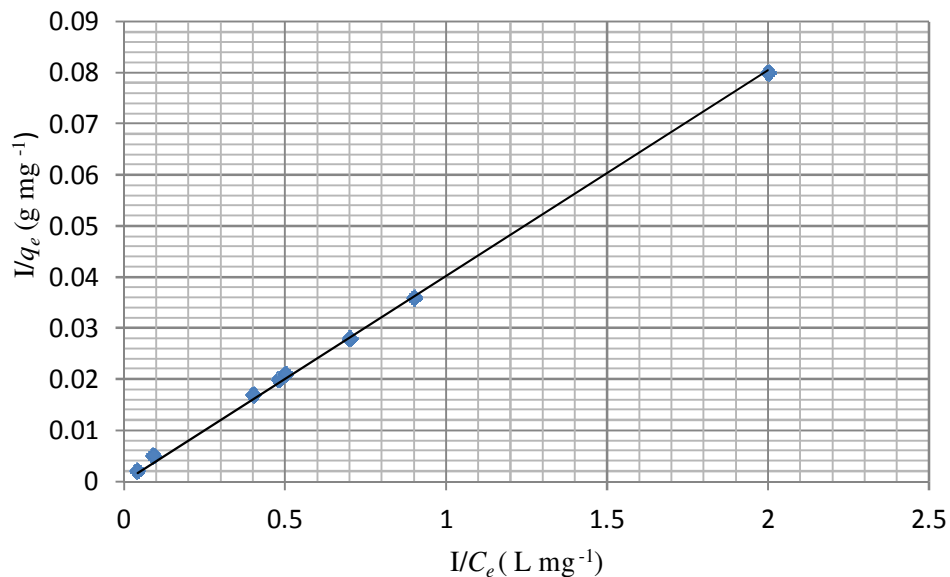


Figure 6. Langmuir isotherm plot for the sorption of lead (II) by IIDEMS.

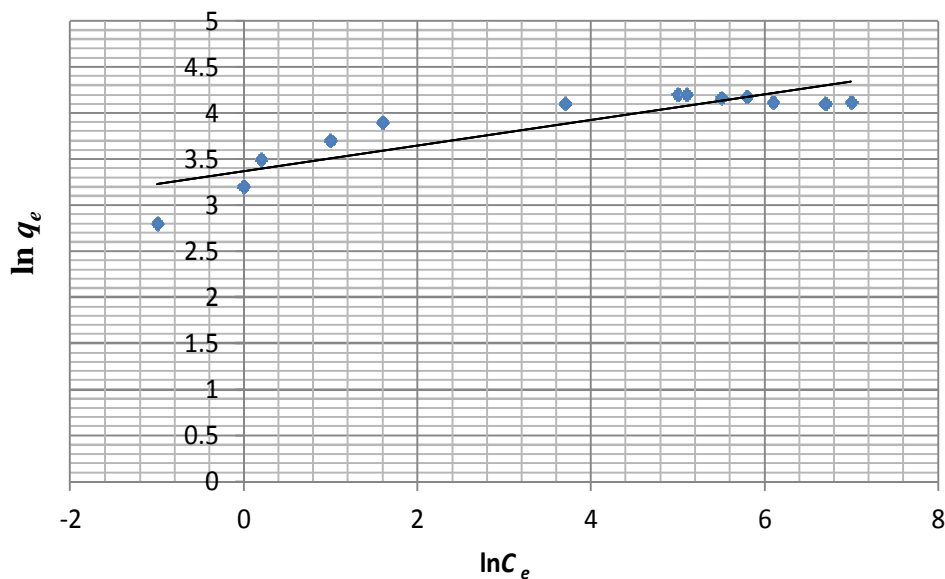


Figure 7. Freundlich isotherm plot for the sorption of lead (II) by IIDEMS.

are shown in Figures 6 and 7, respectively and the model constants are presented in Table 7. The Langmuir isotherm model provided a better correlation than the Freundlich isotherm model. It could be concluded that the sorption of lead(II) onto IIDEMS was monolayer and the maximum monolayer sorption capacity of the sorbent was found to be 82.34 mg. g⁻¹.

The adsorption capacity of IIDEMS is comparable to and moderately higher than that of many corresponding sorbents reported in the literature (Cui et al., 2007; Liu et

al., 2008). The dimensionless separation factor, RL , was also evaluated and calculated according to Equation 3.

$$R_L = \frac{1}{1 + K_L C_o} \quad (3)$$

RL values can be used for the interpretation of the sorption type and it was reported that, when $0 < RL < 1$, the sorption process is favorable (Liu et al., 2008). Further, the RL value for this study was 1.60×10^{-3} , therefore,

Table 7. Isotherm model parameters for the adsorption of Pb²⁺ onto IIDE MS.

Langmuir			Freundlich			
q_{max} (mg.g ⁻¹)	K_L (L mg ⁻¹)	r_L^2	R_L	n	K_F (L.g ⁻¹)	r_F^2
74.45	0.68	0.9989	1.60×10^{-3}	6.30	29.80	0.850

adsorption of lead(II) IIDE MS was favorable.

Analytical features

Precision

The precision of the proposed method for the determination of lead was investigated at the optimum experimental conditions (sample volume: 50 ml; pH: 5.3; flow rate: 8 ml min⁻¹; eluent: 10 ml 0.1 M HCl). 10 independent sorption and elution cycles were carried out by following the recommended procedure. The precision of the preconcentration method was evaluated by using the relative standard deviation (RSD) and was found to be 2.7%. The mean recovery of ten replicates was 98±3% at a confidence level of 95%. The precision of the proposed method was good and the recovery of lead was quantitative.

Calibration graph

A linear calibration curve was obtained in the concentration range of 0.21–10 µg ml⁻¹. The calibration equation was $A = 0.0025 + 0.0398C$, where A is the absorbance and C is the lead concentration in µg ml⁻¹. Correlation coefficient was 0.9989 and the average values of triplicate readings for each standard solution were used for the calculations.

Detection limit

The value of detection limit based on three times the standard deviation of blank signal (N = 20) was 7.5 ng ml⁻¹. The detection limit of the proposed method is comparable to those obtained by other methods described in the literature (Becerril et al., 2008; Tuzen et al., 2009). The corresponding limit of quantification was calculated from ten times the standard deviation of blank signal and found as 20.2 ng ml⁻¹. As seen, the recovered lead ion reveals that the results are quite reliable and are in satisfactory agreement with those obtained by ICP-AES.

Conclusions

The lead ions were quantitatively recovered (≥95%) and determined by using IIDE modified organic-solution-

processable functionalized-nano graphene sorbent. The optimum recoveries for lead were obtained with 300 mg sorbent, at pH 5.3 and 8 ml min⁻¹ of flow rate. Lead ions were quantitatively recovered (>95%) with 10 ml of 0.1 mol L⁻¹ HCl and the preconcentration factor was 5 at optimum conditions. The modified organic-solution-processable functionalized-nano graphene has high sorption capacity (82.34 mg.g⁻¹) and the equilibrium data followed by the Langmuir isotherm model. The precision of the proposed method evaluated as the relative standard deviation obtained from ten replicates, was 3.5%. In comparison to other solid phases (Becerril et al., 2008; Tuzen et al., 2009), high flow rates and large preconcentration factor was achieved using IIDE modified organic-solution-processable functionalized-nano graphene sorbent. While other advantages over reported methods are the high tolerances for matrix components (Mazlum et al., 2003), superior sorption capacity and good reusability (Choi and Choi, 2003; Gode and Pehlivan, 2005; Tuzen et al., 2009; Soyulak et al., 2001; (Narin et al., 2001).

ACKNOWLEDGEMENT

We gratefully acknowledge financial support of Department of Chemistry of Varamin branch Islamic Azad University for financial support.

REFERENCES

- Abdous M, Mousavi AA (2012). Fabrication Of Chelating Diethylenetriaminated Pan Micro- And Nano-Fibers For Heavy Metal Removal. *J. Appl. Polym. Sci.* 18(1):27-34.
- Alexandrova A, Arpadjan S (1993). Column solid phase extraction as preconcentration method for trace element determination in oxalic acid by atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry. *Analyst* 118:1309-1314.
- Arpadjan S, Vuchkova L, Kostadinova E (1997). Sorption of Arsenic, Bismuth, Mercury, Antimony, Selenium and Tinon Dithiocarbamate Loaded Polyurethane Foam as a Preconcentration Method for Their Determination in Water Samples by Simultaneous Inductively Coupled Plasma Atomic Emission Spectrometry and Electrothermal Atomic Absorption Spectrometry. *Analyst* 122:243-249.
- Becerril HA, Mao J, Liu ZF, Stoltenberg RM, Bao ZN, Chen YS (2008). Evaluation of solution-processed reduced graphene oxide films as transparent conductors *ACS Nano* 2:463-469.
- Boudreau SP, Cooper WT (1989). Analysis of thermally and chemically modified silica gels by heterogeneous gas-solid chromatography and infrared spectroscopy. *Anal. Chem.* 61:41-47.
- Bruening ML, Mitchell DM, Bradshaw JS, Izatt RM, Bruening RL (1991). Effect of organic solvent and anion type on cation binding constants with silica gel bound acrocycles and their use in designing selective concentrator columns. *Anal. Chem.* 63:21-27.

- Caroli C, Alimanti A, Petrucci F, Horvath Zs (1991). Determination of trace elements in analytical-reagent grade sodium salts by atomic absorption spectrometry and inductively coupled plasma atomic emission spectrometry after preconcentration by column solid phase extraction. *Anal. Chim. Acta.* 248:241-245.
- Choi YS, Choi HS (2003). High Functional Inorganic Polymers Containing Main Group 13, 16 Elements in the Polymer Backbone Chain Bull. *Korean Chem. Soc.* 24:222-228.
- Cui Y, Chang X, Zhu X, Luo H, Hu Z, Zou X, He Q (2007). Chemically modified silica gel with p-dimethylaminobenzaldehyde for selective solid-phase extraction and preconcentration of Cr(III), Cu(II), Ni(II), Pb(II) and Zn(II) by ICP-OES Microchem. *J.* 87:20-28.
- Dadler V, Lindoy LF, Sallin D, Schlaepfer CW (1987). Selective preconcentration and solid phase extraction of mercury(II) from natural water by silica gel-loaded dithizone phases. *Aust. J. Chem.* 40:1557-1561.
- Ekinci-Dogan C, Akcin G (2007). Solid Phase Extraction of Lead(II) by Sorption on Grinded Eucalyptus Stem and Determination with Flame Atomic Absorption Spectrometry. *Anal. Lett.* 40:2524-2528.
- Gennaro MC, Baiocchi C, Campi E, Mentasti E, Aruga R (1983). Preparation and characterization of iminodiacetic acid-cellulose filters for concentration of trace metal cations. *Anal. Chim. Acta.* 151:339-344.
- Gode F, Pehlivan E (2005). Adsorption of Cr(III) ions by Turkish brown coals. *Fuel Process. Technol.* 86:875-884.
- Hatay I, Gup R, Ersöz M (2008). ICP-OES Determination of Trace Metal Ions after Preconcentration Using Silica Gel Modified with 1,2-Dihydroxyanthraquinone *J. Hazard. Mater.* 150:546-551.
- Hummers WS, Jr. Offeman RE (1958). Preparation of graphitic oxide. *J. Am. Chem. Soc.* 80:1339-1339.
- Jones JS, Harrington DE, Leone BA, Bramstedt WR (1983). Application of optical emission source developments in metallurgical. *Atom. Spectrosc.* 4:49-54.
- Kvitek RJ, Evans JF, Carr PW (1982). Diamine/Silane-Modified controlled pore glass: The covalent attachment reaction from aqueous solution and the mechanism of reaction of bound diamine with copper. *Anal. Chim. Acta* 144:93-97.
- Leyden DE, Luttrell GH (1975). Preconcentration of certain anions using reagents immobilized via silylation. *Anal. Chim.* 47:1612-1619.
- Leyden DE, Luttrell GH, Nonidez WK, Werho DB (1976). ESCA-studies on activated silicagel surfaces preconcentrating heavy metal ions. *Anal. Chem.* 48:67-74.
- Liu ZF, Liu Q, Zhang XY, Huang Y, Ma YF, Yin SG, Chen YS (2008). Substrate effect on the electronic structures of CuPc/graphene interfaces. *Adv. Mater.* 20:3924-3928.
- Mahmoud ME (1997). Silica gel-immobilized Eriochrome black-T as a potential solid phase extractor for zinc (II) and magnesium (II) from calcium (II) *Talanta.* 45:309-314.
- Mahmoud ME (1998). Proceeding of the 25th FACSS Conference, Austin, TX, USA, 11-15 October.
- Mahmoud ME (1999). Selective solid phase extraction of mercury (II) by silica gel-immobilized-dithiocarbamate derivatives. *Anal. Chim. Acta* 398:297-302.
- Mahmoud ME, Soliman EM (1997). Study of the selective extraction of iron (III) by silica-immobilized 5-formyl-3-arylazo-salicylic acid derivatives. *Talanta* 44:1063-1069.
- Mazlum AM, Ensafi AA, Naeimi H, Dastanpour A, Shamelli A (2003). Synthesis and analytical application of a novel tetradentate N₂O₂ Schiff base as a chromogenic reagent for determination of nickel in some natural food samples. *Russ. J. Electrochem.* 39:269-272.
- Moghimi A (2006). Preconcentration and Determination of Fe(III) Using Octadecyl Silica Membrane Disks and Flame Atomic Absorption Spectrometry *Oriental. J. Chem.* 22(3):527-535.
- Moghimi A, Abedin AR, Shahriar GS, Ghiasi R (2009). Solid phase extraction of Cd (II) using mesoporous organosilicas and determination by FAAS. *Afr. J. Pure Appl. Chem.* 3(3):051-059.
- Moghimi A, Shahriar GS, Ghiasi R (2011). A study on the solid phase extraction of CO(II)-IIDE chelate with C18 disk and its application to the determination of trace cobalt. *Afr. J. Pure Appl. Chem.* 5(6):149-154.
- Nambiar DC, Patil NN, Shinde VM (1998). Liquid-liquid extraction of mercury (II) with triphenylphosphine sulphide: Application to medicinal and environmental samples *Fresenius. J. Anal. Chem.* 360:205-211.
- Narin I, Soylak M, Elci L, Dogan M (2001). Separation and Enrichment of Chromium, Copper, Nickel and Lead in Surface Seawater Samples on a Column Filled with Amberlite XAD-2000. *Anal. Lett.* 34:1935-1947.
- Released on World Environment Day (2001). Current Status of Lead in India.
- Roldan PS, Alcântara IL, Padilha CCF, Padilha PM (2005). Solid phase extraction of iron and lead in environmental matrices on amberlite xad-1180/pv, *Fuel* 84:305-311.
- Shamsipur M, Shokrollahi A, Sharghi H, Eskandari MM (2005). Solid phase extraction and determination of sub-ppb levels of hazardous Hg²⁺ ions. *J. Hazard. Mater.* pp. 117-122.
- Soylak M, Elci L, Dogan M (2001). Solid Phase Extraction of Trace Metal Ions with Amberlite XAD Resins Prior to Atomic Absorption Spectrometric Analysis. *J. Trace Microprobe Tech.* 19:329-344.
- Tahaei P, Abdouss M, Edrissi M, Shoushtari AM, Zargarani M (2008). Preparation of chelating fibrous polymer by different diamines and study on their physical and chemical properties *Mat.-wiss. u. Werkstofftech.* 39:839-844.
- Tehrani MS, Moghimi A, Waqif HS (2005). Solid Phase Extraction of Cr (III) from Natural Water by Modified Nano Polyacrylonitrile Fiber. *Mater. Sci. Res. India* 3(2):135-142.
- Tong A, Akama Y, Tanaka S (1990). Selective preconcentration of Au (III), Pt (IV) and Pd (II) on silica gel modified with γ-aminopropyltriethoxysilane. *Anal. Chim. Acta* 230:179-186.
- Tuzen M, Karaman I, Citak D, Soylak M (2009). Mercury(II) and Methyl Mercury Speciation on Streptococcus Pyogenes Loaded Dowex Optipore SD-2. *J. Hazard. Mater.* 169:345-350.
- Unger K (1979). Comparison of an ordered mesoporous aluminosilicate, silica, alumina, titania and zirconia in normal-phase high-performance liquid chromatography *Porous Silica*, Elsevier, Amsterdam. pp. 345-350.