ORIGINAL RESEARCH PAPER

Nano Analysis in Biochemistry: In Vitro Separation and Determination of Aluminium in Blood of Dialysis Patients Based on Graphene Oxide Nanoparticles Dispersed to Ionic Liquid

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ABSTRACT

The highest concentration of aluminium (Al) in human blood has toxic effect and so, extraction from human body is very important. In this procedure, an efficient and new method based on graphene oxide nanoparticles (GONPs) dispersed in ionic liquid (IL) was used for in-vitro separation/ extraction of trace Al from the blood of dialysis patients by ultrasound assisted-dispersive-micro solid phase extraction (USA-D-μSPE) procedure. Under optimized conditions, the linear range (LR), limit of detection (LOD) and preconcentration factor (PF) were obtained 0.1–4.8 μg L^{-1}, 0.02 μg L^{-1} and 25 for blood samples, respectively (RSD<5%). The results of blood samples showed us, that the aluminum concentration after dialysis was higher than before dialysis (128.6±6.7 vs 31.8±1.6, P<0.05). The mean of blood aluminum was significantly higher in dialysis patients than in normal control, respectively (113 5±7.12 vs 1.2±0.1). The developed method based on GONPs/IL was successfully applied for extraction of critical level aluminum from human blood and suggested for in-vivo extraction from human body of dialysis patients after supporting on an appropriate surface with biocompatible materials within the human body.

Keywords: *Aluminum; Graphene oxide nanoparticles; Continuous-micro-solid phase extraction; Human blood; Dialysis patients.*

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INTRODUCTION

Aluminum is a trivalent cation found in its ionic form in most kinds of human and plant tissues and in natural waters everywhere [1]. Most heavy metals can cause disease in the human body and essential metals such as; copper and zinc affected by the human body in the case of deficiency or imbalance. The toxic effects of aluminum in human body depended on the amount of ingested, entry rate, tissue distribution, concentration achieved, and excretion rate [2, 3]. An increased brain content of aluminum appears to be the major etiological factor in the development of a neurological syndrome called dialysis encephalopathy or dialysis dementia [4]. Aluminium found in over-the-counter medicines, such as antacids and buffered aspirin, is used as a food additive, and is found in a number of topically applied consumer products

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such as antiperspirants, and first aid antibiotic and antiseptics, diaper rash and prickly heat, insect sting and bite, sunscreen and suntan, and dry skin products. The concentration of aluminium in foods and beverages varies widely, depending upon the food product, the type of processing used, and the geographical areas in which food crops are grown [5]. In the human body, approximately 95% of aluminum eliminated through renal but with renal dysfunction, aluminum has the potential to accumulate in human tissue [6]. All people have small amounts of aluminum in their bodies. It can be measured in the blood, bones, feces, or urine. If a significant aluminum load exceeds the body's excretory capacity, the excess is deposited in various tissues, including bone, brain, liver, heart, spleen, and muscle. This accumulation causes morbidity and mortality through various mechanisms. Aluminum concentrations in brain tissue should be lower than 2 μg g^{-1} [7, 8]. A 10-fold increase in aluminum concentrations was reported in patients with aluminum intoxication through the use of hemodialysis solutions with high levels of aluminum. The mechanisms of aluminum toxicity include; inhibition of enzyme activity and protein synthesis, alterations in nucleic acid function, and changes in cell membrane permeability [9-11]. Aluminum toxicity is usually found in patients with impaired renal function. Aluminum toxicity occurs when a person ingests or breathes high levels of aluminum into the body [12]. Aluminum toxicity is a systemic disorder observed in hemodialysis patients and, occasionally, in no dialysis patients who have severe chronic kidney disease. Aluminum toxicity primarily results from exposure to aluminum in dialysis fluid and from the ingestion of aluminum-containing phosphate binders among patients who cannot excrete it. Under normal physiologic conditions, the usual daily dietary intake of aluminum (5-10 mg) is completely eliminated. Excretion is accomplished by avid filtration of aluminum from the blood by the glomeruli of the kidney. Patients with renal failure (RF) lose the ability to clear aluminum and are candidates for aluminum toxicity. Patients in renal failure with no signs or symptoms of osteomalacia or encephalopathy usually have serum aluminum concentration less than 20 ng mL^{-1} and parathyroid hormone (PTH) has concentrations less than150 pg mL^{-1}. Chronic kidney disease is a multifaceted problem having both physical and psychological connotations for the patient. Patients with renal failure often suffer from many other medical conditions and are on many different medications. So determination AL in a human body and excretion of body is very important [13-21]. Many different techniques have been applied for determination of aluminum ions, including flame atomic absorption spectrometry (F-AAS) [22], stripping Voltammetry (SV) [23], inductively coupled plasma-atomic emission spectrometry (ICP-AES) [24], High performance liquid chromatography/inductively coupled plasma mass spectrometry (HPLC/ICPMS) [25,26], electrothermal atomic absorption spectrometry (ETAAS) [27] and inductively coupled plasma-mass spectrometry (ICP-MS) [28]. Despite the selectivity and sensitivity of modern analytical techniques, the direct determination of aluminum elements in human biological samples is hard due to various factors, particularly low concentrations of analysts and high levels of matrices in blood. This is the reason why preconcentration/separation techniques such as, liquid–liquid extraction (LLE) or dispersive liquid–liquid microextraction (DLLME) [29,30], and solid phase extraction (SPE) [31], etc., are still necessary. Among these, SPE is a preferred technique because of its advantages including simplicity, lower cost, higher enrichment factor, less consumption of organic solvents, and the ability to combine with different detection techniques whether in on-line or off-line mode [32, 33]. This technique is a surface dependent approach that enables the pre-concentration and purification of analytes from solution by sorption on a solid sorbent. Thus, the choice of the appropriate sorbent is a critical factor in SPE procedures and the extraction efficiency depends on the particle size and the surface area of the sorbent [34]. Currently, dispersive-micro-solid phase extraction (D-μ-SPE), a rapid and simple clean-up technique, has been developed to reduce the time required for SPE operation; in which the extraction is carried out in the bulk solution. This approach enables the sorbent to interact rapidly and uniformly with the all the target analytes and therefore shortens the time of sample preparation in comparison with a classical SPE.

The main purpose of the present research is to develop a novel in-vitro analytical method based on GONPs as the nano sorbent with ultrasound assisted-dispersive- micro-solid phase extraction (USA-D-μ-SPE) for separation and preconcentration of trace amounts of Al ions from

human blood samples. The proposed method demonstrates its applicability for extraction of Al before determination by electrothermal atomic absorption spectrometry (ET-AAS). All main factors for the quantitative recoveries of Al ions were investigated and optimized.

EXPERIMENTAL

Apparatus

Determination of Al was performed with a spectra GBC electro_thermal atomic absorption spectrometer (Model, Plus 932, Australia) using a graphite furnace module (GF3000, GBC). The operating parameters for the metal of interest were set as recommended by the manufacturer. A hollow cathode lamp of Al operating at a current of 6 mA and a wavelength of 396.2 nm with a spectral bandwidth of 0.5 nm was used. All experiments were performed by using an auto sampler injector. The pH values were measured with a Metrohm pH-meter (model 744, Herisau, Switzerland) supplied with a glass-combined electrode. The sample separation was achieved using a Demerd centrifuge (model LC8-12). A Kunshan ultrasonic bath (model KQ-100DE, Kunshan, China) with temperature control was used throughout this study.

Chemical Reagents and Materials

All reagents, acids and solvents with analytical grade were purchased from a Merck company (Germany). Graphite powder (particle size <20 μm) was purchased from Sigma-Aldrich. Standard stock solutions (1000 mg L^{-1}) of Al (III), and all of the other reagents used for experiments and analysis were of analytical grade and purchased from Merck, Darmstadt, Germany. Deionized water produced using a Milli-Q plus water purification system (Millipore, Bedford, MA, USA) was used throughout this study. The experimental solutions of proposed method were prepared daily by diluting the stock solutions with deionized water. The working standard solutions were prepared daily by diluting the stock solutions of lead and nickel ions with deionized water prior to analysis with the proposed method. The pH adjustments were made using appropriate buffer solutions including sodium phosphate (H_3PO_4/NaH_2PO_4, 0.1 mol L^{-1}) for pH 2-3, ammonium acetate (CH_3COOH/ $CHCOONH_4$, 0.1 mol L^{-1}) for pH 4-6, sodium borate ($NaBO_2$/HCl, 0.1 mol L^{-1}) for pH 7, and ammonium chloride (NH_3/NH_4Cl, 0.1 mol L^{-1}) for pH 8-10.

Sampling

For sampling, all glass tubes were washed with a 1.0 mol L^{-1} HNO_3 solution for at least 24 h and thoroughly rinsed 10 times with ultrapure water before use. As aluminum concentrations in whole blood and serum of healthy peoples are very low, even minor contamination at any stage of sampling, sample storage and handling, or analysis has the potential to affect the accuracy of the results. Heparin is commonly used as anticoagulants in human blood samples. The blood collection tube with heparin was aliquoted into Eppendorf (5 mL) tubes and kept at -20°C for one week. For analysis in whole blood 10 μL, pure heparin (free aluminum) is added to a 10 mL blood sample of dialysis patients from hospital, Iran. Serum and blood samples were collected from exposed (50N) and unexposed (50N) subjects were aged between 20 to 50 years, Tehran (IRAN).

Preparation of Graphene Oxide

Graphite oxide was prepared using modified Hummers method through the oxidation of natural graphite powder in RIPI laboratory. The graphene oxide (GO) was obtained via exfoliation of graphite oxide. Graphite oxide was prepared using modified Hummers method through the oxidation of natural graphite powder. Graphite powder (5 g) and $NaNO_3$ (2.5 g) were mixed with 120 mL of concentrated H_2SO_4 and stirred for 30 min in an ice bath (0-5°C). $KMnO_4$ (15 g) was gradually added to the vigorously stirred suspension and the temperature of the mixture was kept to be below 15°C. Later, H_2O_2 solution (30%) was added to stop the oxidation process, and the color of the mixture changed to bright yellow, indicating fully oxidized graphite. Graphene oxide (GO) nanosheets were obtained by ultra-sonication of the filtered graphite oxide suspension followed by centrifugation for 15 min at 3000 rpm to remove any un-exfoliated graphite oxide. Finally, the as-prepared GO was dried at 60°C for two steps.

Characterization of Graphene Oxide

The TEM and SEM images show that few-layered GO are formed with smooth surface and some wrinkles. Moreover, the transparent sheets

comprise very thin layers which are noticeable from the image. The wrinkles regions are due to oxygen functional groups on the surface of GO (Figure 1a and 1b). In the XRD of GO, an intense and sharp diffraction peak at 2θ=12.26 (d=0.72 nm), corresponds to the typical diffraction peak of GO nanosheets was observed. The d-spacing increases from 0.33 to 0.72 nm after the graphite is converted into GO nanosheets, which may be due to the creation of the abundant oxygen functional groups on the surface of GO (Figure 2a). The oxygen-containing groups on the surfaces of GO nanosheets were achieved by FT-IR analysis in Figure 2b. As shown in Figure 2b, the C=O and –COOH/–OH groups were indicated by the peak at 1725 cm^{-1} and 3417 cm^{-1}, respectively. The presence of C-O was indicated by the peak at 1100-1220 cm^{-1}. Moreover, the peak at 1620 cm^{-1} was assigned to C=C stretching vibration.

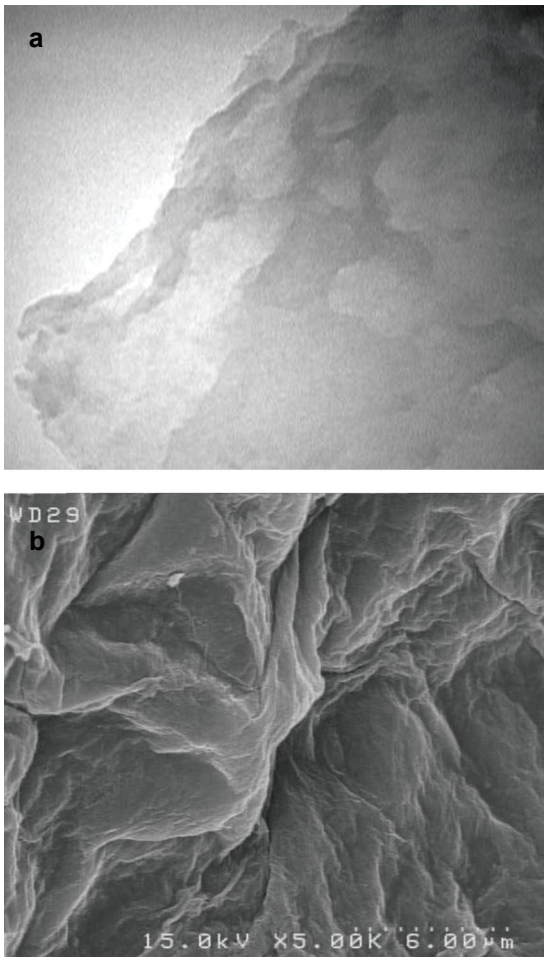

Fig. 1. s(a) TEM image of GO, and (b) SEM image of GO.

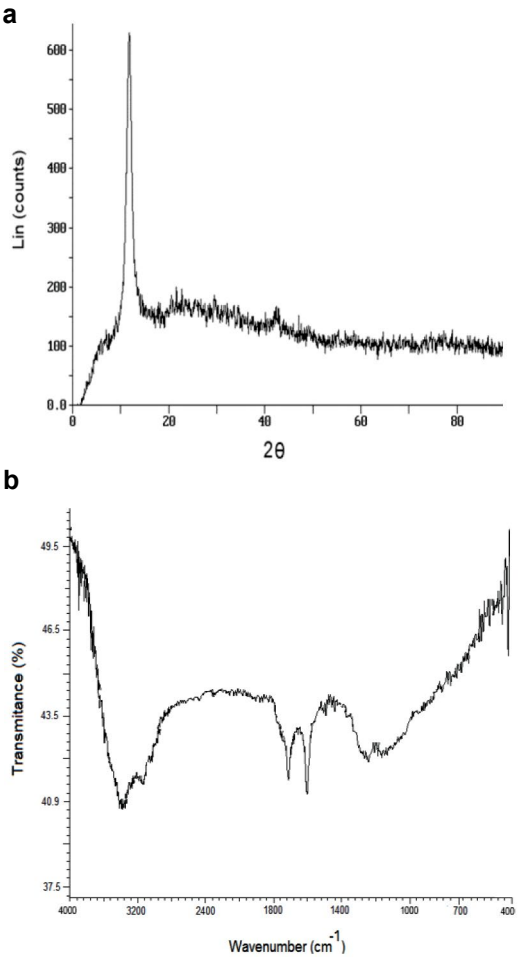

Fig. 2. (a) XRD analysis of GO, and (b) FTIR analysis of GO.

Procedure

In this procedure, an aliquot of the sample solution (10 mL) containing aluminum ions was used for separation and preconcentration of Al (III) from human blood of dialysis patients. In USA-D-μSPE procedure, a 10 mL polytetrafluoroethylene (PTFE) centrifuge tube was used as the preconcentration/separation units. The pH was adjusted to 6.0 -7.4 with sodium phosphate/ ammonium acetate buffer solutions for standard solution. The amine group of GONPs (10 mg) as a complexing agent was dispersed in ionic liquid (0.09g of [HMIM][PF6], 100 μL) as a separation/ extraction phase which was diluted with 0.1 mL of acetone as a dispersant solvent. Then, the mixture were rapidly injected by a syringe into 10 mL of blood and standard samples (0.1-5.0 μg L^{-1} for each one step by step and together) containing of Al ions at optimized pH (pH≈7.0). The solution place in an

ultrasonic bath for 15 min and Al (III) cations were physically and chemically extracted from carbonyl and the hydroxyl group (GONPs-OH/COOH). The loaded GONPs were trapped with IL and the turbid solution was centrifuged at 4000 rpm for 1.5 min. The GONPs /IL suspension was settled down in the bottom of the conical centrifuge tube and the aqueous phase was removed with a transfer pipette. Finally, aluminum ions retained on the GONPs were back extraction by adding 200 µL of 0.5 mol L^{-1} HNO_3 and vigorously shaking the tube for 1.0 min. The eluent phase was separated from GONPs /IL phase by centrifuging of the remaining mixture at 4000 rpm for 0.5 min. Finally, Al (III) ions in the aqueous phase were analyzed by ET-AAS after dilution with 200 µL of deionized water up to 400 µL (Figure 3).

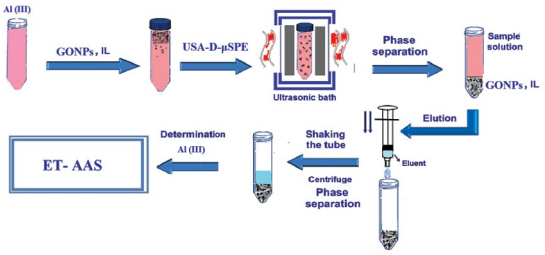

Fig. 3. General procedure of USA-D-µ-SPE.

RESULT AND DISCUSSION

Based on the preliminary experiments, the retention of Al (III) ions on a GONPs adsorbent was chosen for preconcentration of the metal ions and their subsequent determination by ET-AAS. Hence, in order to obtain quantitative recoveries of Al (III) ions with good sensitivity and precision, the USA-D-µSPE procedure was optimized for various analytical parameters. The GONPs adsorbent was used freshly to blank experimental run. The recovery was calculated by using Equation (1), where C_i is the initial concentrations of analyte (Al) in solution phase, and C_f is the concentration of analytes determined by ET-AAS after proposed procedure. All the experimental data were the averages of triplicate determinations.

$$\text{Recovery}\% = \frac{(C_i - C_f)}{Ci} \times 100 \qquad (1)$$

Effect of ETAAS Conditions

In order to increase the accuracy, precision and repeatability, we used acetone and increased the temperature to 45°C. As shown in figure 4, the influence of pyrolysis temperature on the observance of aluminum was studied within a range of 500-1600°C. The maximum absorbance was achieved within a range of 1200-1600°C. Therefore, 1,400°C was selected as the working pyrolysis temperature. Once selected, a drying time of 30 s was chosen for water evaporation, and a long ramp/hold time of 50 s was chosen as it allowed gradual elimination of organic matrix and avoided an aluminum loss in pyrolysis temperature. The effect of atomization temperature of aluminum signal was studied within the range of 2000-3000°C, and the maximum signal was obtained at approx. 2500°C. Cleaning time and temperature were ordered at 1.0 s and 2700°C, respectively, and argon the flow rate was 300 mL/min.

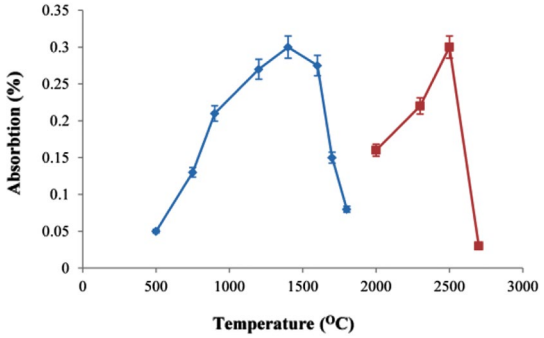

Fig. 4. The influence of temperature on the absorbance of aluminum by ET AAS.

Effect of pH and Adsorbent Dose

The influence of sample pH of adsorption of Al (III) ion on USA-D- µSPE was investigated using different pH from 2 to 12 for 0.1 µg L^{-1} of Al (III) as a lower limit of quantification (LLOQ) and 4.5 µg L^{-1} Al (III) as the upper limit of quantification (ULOQ). The complexation was strongly conditioned by the pH of solutions and subsequently affects the extraction efficiency of the complex. The results show that the highest extraction efficiency for Al (III) was achieved in pH 6.5 to 7.5 (Fig. 5). So, pH blood is appropriate for extraction aluminum. As illustrated in Figure 5, recovery percentages of Al (III) ions increased from pH 5 to 7.5 and were quantitatively recovered (>97%) at pH=6.5–7.5. After pH 7.5, further increase in pH value decreased the recovery percentages of both the metal ions. The analyte ions can be adsorbed onto GO surface by reacting with –COOH and –OH groups. Depending on the solution pH, the surfaces

of the GO nanosheets can undergo protonation or deprotonation reaction. As previously reported by Zhao et al. [35] at low pH values (pH<4), the surface charge of GO nanosheets is positive due to the protonation reaction as equation 1. Therefore, low recovery efficiencies of metal ions in the low pH range are due to the electrostatic repulsion between the metal ions and positively charged GO surface. However, as the pH increases, the surface charge of GO is more negative because of the deprotonation mechanism, and the G–O^- becomes the dominating species. So, the electrostatic attraction between negatively charged adsorbent surface and Al (III) ions were occurred (Eq. 2). On the other hand, the decrease in the recovery efficiencies of metal ions at higher pH values (pH>8) may be due to increase in precipitation of metal ions in the form of hydroxyl complexes Al $(OH)_3$. Thus, for all further studies, pH ≈ 6 was considered as optimum pH value.

(Eq. 1) $G{-}OH/COOH + H^+ \rightarrow G{-}OH_2^+$ or $G{-}COOH_2^+$

(Eq. 2) $G{-}OH/COOH \rightarrow G{-}O^- + H^+ / G{-}COO^- + H^+$

$n\ (G{-}O{-}/COO{-}) + M^{3+} \rightarrow (G{-}O{-}/COO{-})_n\ M^{(3-n)+}$

Different amounts of GONPs in the range of 2 to 15 mg were tested on the recoveries of Al extraction in presenting work. The results were shown in Fig. 6. It was found that 10 mg of GONPs was sufficient for quantitative recoveries of aluminum. The optimized dosages had a significant effect on the recovery of aluminum as the surface metal ion concentration and the solution metal ion concentration came to equilibrium with each other. Eventually in further works, 10 mg of GONPs was used as adsorbent.

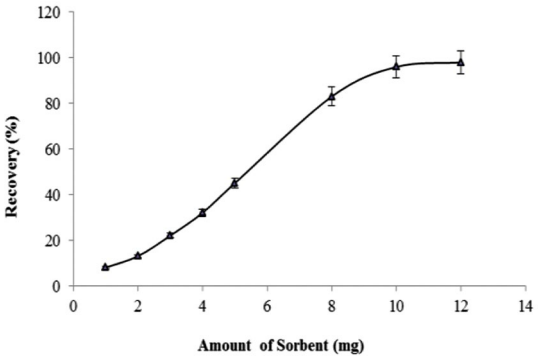

Fig. 6. The effect of amount of sorbent.

Effect of Sample Volume and Amount of Ionic Liquid

Sample volume is one of the most important parameters to be studied. The effect of sample volume was examined in a range of 1, 2, 5, 10, 15 and 20 mL for 0.1 µg L^{-1} and 5 µg L^{-1} of Al (III). Quantitative extraction was observed between 1-12 mL. At higher volumes the recoveries are decreased. It was also noticed that higher sample volumes, partially solubilized the ionic liquid phase, leading to non-reproducible results. Therefore, a sample volume of 10 mL was selected for further experiments of the proposed method (Fig. 7).

It was observed that the extraction efficiency of the system was remarkably affected by ionic liquid amount ([HMIM] [PF6]), so it was examined within the range of 0.03–0.2 g. Quantitative extraction was observed at higher than 0.08 g. Therefore, in order to achieve a suitable preconcentration, 0.09 g of ionic liquid ($[C_8MIM][PF_6]$) was chosen as optimum leading to a final IL.

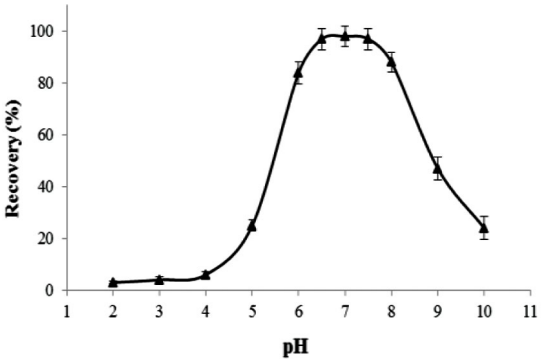

Fig. 5. The effect of pH.

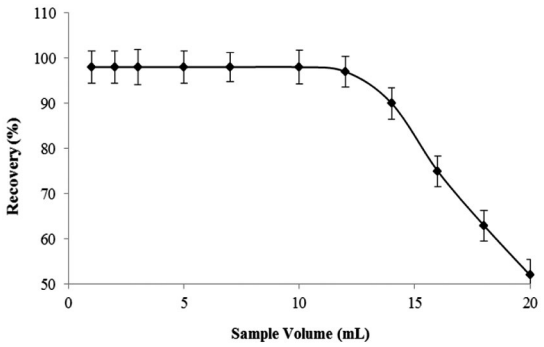

Fig. 7. The effect of sample volume.

Effect of Various Mineral Acids

Direct injection of ionic liquids into ETAAS was not possible, because ILs has high viscosity. The proposed method was based on back-extraction of aluminum from IL with a mineral acidic solution. Therefore, decreasing the pH leads to dissociation and releasing of Al ions into the aqueous phase. Different mineral acids (HCl, HNO_3, H_2SO_4, H_3PO_4) were studied for aluminum back-extraction from the IL phase (0.1-2M). The research showed that 0.5 mol L^{-1} of HNO_3 quantitatively back extracted Al (III) from the GONPS-IL phase (Fig 8). The effect of volume of 0.5 mole L^{-1} HNO_3 was also examined (50-500 µL) on the recoveries of aluminium back-extraction from the ionic liquid phase. The results demonstrated that quantitative recoveries were obtained with 200 µL of HNO_3; as optimum HNO_3 volume in the following experiments.

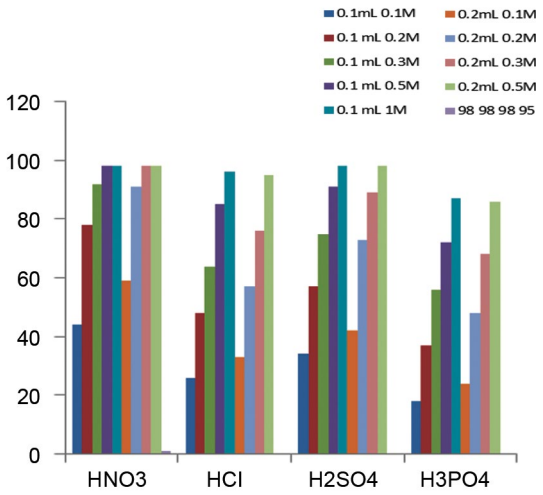

Fig. 8. The effect of different mineral acids on back-extraction of Al (200 µL, 0.5 M).

Effect of Ultrasound-Assisted Extraction Time

The optimization of ultra-sonication time is crucial to achieve an efficient USA-D-µ-SPE procedure. In this study, different ultrasound-assisted extraction times ranging from 50 to 200 seconds was evaluated for proposed procedures, respectively. By increasing the ultra-sonication time the relative response increases, reaching the maximum value of 90 seconds and then remained constant. Therefore, the ultrasonic times of 1.5 minutes for extraction with GONPs and 1 min for back- extraction of Al (III) from Sorbent with HNO_3 was employed.

Effect of Matrix

ETAAS is a very specific technique with low sensitivity to interference. Then, the potential interference effects occurring with this procedure are mainly related to the extraction during the pre-concentration step applied to the target samples. Considering the samples of interest, the most probable metal ions' reported effect of potential interfering ions on the determination of aluminum were investigated. The procedure of USA-D-µ-SPE was performed using a 10 mL sample containing 4.5 µg L^{-1} of analyte and 1–4 mg L^{-1} different concentration of matrix ions. The tolerate amounts of each ion were the concentration values tested that caused less than 5% of the absorbance alteration. The ions normally present in the sample do not interfere under the experimental conditions used. The results are shown in Table 1.

Table 1. The effect of matrix ions (ions conc. /Al conc.)

Ions	Maximum tolerance ratio
Na^+, K^+, Ba^{2+}, NO_3^-, PO_4^{3-}, CO_3^{2-}, SO_4^{2-}, CH_3COO^-, F^-, Cl^-	1600
Ca^{2+}, Mg^{2+}, Cu^{2+}, Zn^{2+}	1300
Co^{2+}, Mn^{2+}	550
Cr^{3+}, Ni^{2+}, Fe^{3+}, V^{3+}, Ag^+	180

This work was performed using 10 mL of 4.5 µg L^{-1} Al standard solution (pH≈7).

Method Validation

The USA-D-μ-SPE method based on GONPs / IL was applied to determine Al (III) found at a base value in 10 ml of 50 exposed and control subjects in human serum blood samples (Table 2). The mean concentration of Al (III) in dialysis patient was higher than controls. The coloration and regression analysis was achieved between Al (III) in subject and control group (r>0.028).

For validation and development of the proposed method, certified reference material NIST SRM 2670a in human matrix species (4.02±0.21) were used to demonstrate the reliability of the method for determination Al (III) (Table 3). The recovery of spiked samples is satisfactorily reasonable and was confirmed using addition method, which indicates the capability of the system in the determination of Al (III) in human blood samples (Table 4).

Table 2. The coloration analysis of Al (III) with proposed procedure among dialysis patients (subject) and healthy people (control group) (n=50, μg L^{-1})

Sample Al	Subjects (dialysis patients)		Controls	Subject
	After	Before		*P value*
Blood A	117.4±4.8	25.2±1.4	0.5±0.1	0.152<0.001
Serum A	126.5±5.4	38.8±3.7	0.9±0.1	0.168<0.001
Blood B	122.1±5.3	31.2±3.2	1.1±0.1	0.155<0.001
Serum B	145.2±7.8	43.3±4.9	1.5±0.1	0.171<0.001
Blood C	98.6±3.2	21.8±2.4	0.7±0.1	0.146<0.001
Mean Blood (50N)	101.4±7.1	28.5±4.3	0.8±0.1	0.135<0.001
Mean Serum (50N)	133.7±8.4	38.5±4.3	1.1±0.1	0.142<0.001

[a]Mean of three determinations ± confidence interval (P=0.95, n=5).

Table 3. Analytical results of aluminum determination in standard reference material by proposed method (μg L-1)

NIST SRM 2670a	Certified	Found	Recovery (%)
Human Matrix	4.02±0.21	3.97±0.24	98.75

Mean value ± standard deviation based on three replicate measurements.

Table 4. Validation of aluminum determination in spiked blood samples by proposed procedure (μg L^{-1}).

Sample	Added	Found [a]	Recovery (%)
Blood 1	---	1.22±0.04	---
	1.0	2.18±0.09	96
Blood 2	---	1.05±0.06	---
	0.5	1.57±0.04	104
Blood 3	---	0.62±0.02	---
	0.3	0.91±0.02	96
Serum	---	2.13±0.12	---
	1.0	3.10±0.11	97
Serum	---	0.25±0.02	---
	0.2	0.44±0.03	95

[a]Mean of three determinations ± confidence interval (P=0.95, n=5).

DISCUSSION

In vitro aluminium chelation from serum is very hard because about 80% of aluminium ions are bound to serum proteins such as albumin and other compounds. However, aluminium can be cheated by histidine bio-ligand at pH 6.5 as same as deferoxamine (DFO) in serum of dialysis patients and help us in Al extraction from human blood by ionic liquid by the proposed method. Many researches was evaluated effect of alumium concentration in human body. End stage renal disease patients undergoing long-term dialysis are at risk for abnormal concentrations of certain essential and non-essential trace metals. Guo CH et al. Evaluated the effects of zinc (Zn) supplementation on plasma aluminum (Al) in chronic dialysis patients. Zn-deficient patients receiving continuous ambulatory peritoneal dialysis or hemodialysis were divided into two groups according to plasma Al concentrations (HA group, Al >50 μg L^{-1}; and MA group, Al >30 to ≤50 μg L^{-1}). After two-month Zn treatment, these patients had higher plasma Zn concentrations and reduced plasma Al concentrations [36]. Makhlough et al. Showed that aluminum level in patient's serum was 30.7±6.2 and 37.5±6.8 mg/deal before and after dialysis, respectively. The post-dialysis aluminum level became statistically significant ($p<0.05$). There was no significant difference between pre dialysis aluminum concentrations during the 6 month interval [37]. Shirkhanloo et al. Studied about aluminum concentration in serum of dialysis patients. The results of study showed that the trace amounts of aluminum in serum of dialysis patients were chelated with 2-Amino-3-(1H-imidazol-4-yl) propanoic acid (Histidine) and determined by electro-thermal atomic absorption spectrometry (ET-AAS). Under the optimum conditions, the enrichment factor (EF), limit of detection (LOD) and working range (peak area mode) were obtained 53, 15 ng L^{-1} tand 0.05-4.1 μg L^{-1} respectively. In vitro Al chelation showed that He can significantly decrease the aluminum concentration in serum of dialysis patients [38]. In this study, we used USA-D-μ-SPE procedure based on GONPs/IL for micro-extraction and determination of aluminium in human biological samples. The surface charge of GO is more negative because of the deprotonation mechanism, and the GONPs becomes the dominating species. So, the electrostatic attraction between negatively charged adsorbent surface and Al (III) ions were occurred at optimized pH. In addition, quantitative extraction was observed in optimized sample volume. At higher volumes the recoveries decreased. It was also noticed that higher sample volumes, partially solubilized the ionic liquid phase, leading to non-reproducible results and increased the amount of GONPs. Therefore, a sample volume of 10 mL was selected for further experiments of USA-D-μSPE. It was also observed that the extraction efficiency of the system was remarkably affected by GONPs and IL amount, so it was examined within the range of 0.002 to 0.015 g and 0.03-0.2 g, respectively. Quantitative extraction was observed at 0.09 g of IL and 10 mg of GONPs. Also, decreasing of the pH leads to dissociation and releasing of aluminium ions into the aqueous phase with 98% of extraction recovery. But direct injection of IL after dilution with ethanol, methanol, acetone and acetonitrile caused to miss IL, non-reproducible results, high RSD, low accuracy. The research showed that dilution of ionic liquid with ethanol solution has a low efficiency extraction compared to acid back-extraction. The USA-D-μ-SPE method was applied to determine Al (III) found at a base value in 10 ml of biological samples. The spiked serum and blood were prepared to demonstrate the reliability of the method for extraction and determination of aluminium. The mean of aluminium concentration in blood samples in dialysis patients before and after dialysis was determined by USA-D-μ-SPE. The results of dialysis patients (20-50 ages) by proposing method showed us that the concentration of aluminium in serum after dialysis was higher than before dialysis (128.6±6.7 vs 31.8±1.6, $P<0.05$). Serum aluminium was significantly higher in dialysis patients and gastrointestinal patients than in normal control respectively (113. 5±7.12 vs 1.2±0.1 and 41.8±5.12 vs 0.9±0.1, $P<0.05$). In order to examine the long term stability of GONPs, it was subjected to several extraction and back extraction cycles under the optimized conditions, according to the USA-D-μ-SPE procedures. The GONPs adsorbent can be used for up to 30 adsorption/desorption cycles without a decrease in the extraction recoveries of the Al (III) by the proposed method. The different sorption capacities between adsorbents depended on the type and concentration of active sites responsible for adsorption of analytes from the solution. In batch method, 0.1 g of GNPs and GONPs mixed with 0.09 g of [HMIM][PF6] were separately added to 10 mL of sample solution containing 100 mg L^{-1} of Al(III) in centrifuge tube at pH 7. After

ultrasonication for 15 minutes with water bath (40 KHz, 100 W, 25°C), the loaded sorbent was trapped with IL and separated from the sample solution by centrifuging at 4000 rpm for 1.5 min. Finally, Al (III) ions remaining in the solution were determined with ET-AAS. The adsorption capacity of GNPs and GONPs for Al (III) ions was found to be 187.5 and 31.6 mg g^{-1}, respectively.. The physisorption mechanism takes place during the analyte adsorption by GNPs. However, both the chemical and physical adsorption processes are responsible for the highest adsorption capacity of GONPs sorbent for extraction Al (III) ions. Moreover, the comparison of the IL trap in presented method with filter trap through a 0.2 µm filter membrane (GSWP 47, Millipore, Billerica, MA) showed us the IL trap was a fast separation route without nanosorbent loss. Therefore, GONPs is considered to be excellent and potential adsorbent for extraction Al (III) ions from blood samples.

CONCLUSION

The application of simple, fast, reliable, sensitive, accurate, precise and inexpensive method was demonstrated for preconcentration, speciation and determination of trace Al (III) in blood of dialysis patients. The method was based on ultrasound assisted–dispersive-micro-solid phase extraction (US-D-µSPE) technique and ET-AAS detection method. Using of [HMIM][PF6] ionic liquid as trapping agent of the Al-loaded GONPs sorbent is a rapid single step, reducing the sample preparation and separation time (without filtration) and sorbent loss. Utilizing small amount of sorbent per extraction without any chelating agent together with high sorption capacities and good reusability as well as minimal elution volume of 200 µL makes this method to be environmentally friendly and cost-effective. This newly developed microextraction method provides low LOD, and RSD values as well as good PF values and quantitative recoveries (>98%) in optimized conditions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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