Sulfamethizole functionalized graphene oxide for in-vitro separation and determination lead in blood serum of battery manufactories workers by syringe filter-dispersive-micro solid phase extraction

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A B S T R A C T

The toxic effect of lead (Pb) causes to anemia and iron deficiency in human body. So, the lead determination in blood/serum samples is very important. In this study, a novel adsorbent based on sulfamethizole functionalized on nanographene oxide ($C_3H_{10}N_4O_2S_2$-NGO; SM-NGO) was used for extraction of Pb(II) from human blood, serum and plasma samples in battery manufactories workers by syringe filter-dispersive-micro solid phase extraction procedure (SF-D-μ-SPE). By procedure, 25 mg of SM-NGO mixed with 10 mL of human blood/serum or plasma samples and aspirated by 10 mL of syringe tube. After sonication of samples for 5 min, the Pb ions adsorbed based on sulfur of SM-NGO adsorbent at pH=6 and the solid phase separated by syringe coupled to Millex-FG hydrophobic PTFE membrane (0.2 μm). Then, the lead ions were back-extracted from SM-NGO/PTFE by elution phase with 0.5 mL of nitric acid solution (0.5 M). Finally, the concentrations of Pb(II) ions were determined by atom trap flame atomic absorption spectrometry (AT-FAAS) after dilution with DW up to 1 mL. After optimization, the linear range (LR), LOD and enrichment factor (EF) for Pb ions was obtained, 10-500 μg L^{-1}, 2.5 μg L^{-1} and 9.86, respectively. The validation of procedure was confirmed by electhermal atomic absorption spectrophotometer (ET-AAS) and certified reference material (NIST, CRM) in human samples.

1. Introduction

Heavy metals intakes to human body from air, water and foods. Heavy metals such as lead (Pb), chromium (Cr_{VI}) and mercury (Hg) cause to serious problem in humans and depended on way entrance

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(Skin, lungs and gastrointestinal system) and concentrations [1-3]. The battery manufactories, gasoline, wastewater, x-ray protection and paint are main source of lead in environment [4,5]. The battery factories are a main source of lead toxicity in workers and cause to dysfunction in blood red cells, the central nervous system tissues (CNST), bones and renal tissues [6]. Also, the lead poisoning can

be effected on human organs such as braine, renal, liver and bone [7,8]. The lead complexes in human body create by interaction of lead with proteins/ amino acids and cause anemia [9,10]. On the other hand the lead exposure causes to decrease the erythrocytes cells (RBC) and reducing biosynthesis of hemoglobin. The occupational safety and health administration (OSHA) has reported that the exposure lead (Pb) more than 600 µg L^{-1} is toxic and workers must be offline from work and return to work when the BLL is below 300 µg L^{-1}. Also, the food and drug administration (FDA) has announced the normal range of Pb in human blood is below 250-300 µg L^{-1} [11]. The limit exposure of lead (Pb) in air is considered about 50 µg m^{-3} by NIOSH [12]. Due to lead toxicity in human, the accurate technology need to use for determination of lead ions in the human blood/ serum samples. Recently, the various methods were reported for lead determination in human biological samples. The instrumental techniques such as the electrothermal atomic absorption spectrometry (ET-AAS) [13,14], the inductively coupled plasma - atomic emission spectroscopy or mass spectrometry (ICP-AES/MS) [16], the high-performance liquid chromatographic (HPLC) and the isotope dilution inductively coupled plasma mass spectrometry (ID-ICP-MS) were used for lead determination in different matrixes[17]. Moreover, the sample preparation method is required for extracting of lead ions in different biological samples before determination by spectrometry techniques. Various sample preparation method such as liquid-phase microextraction or dispersive liquid-liquid microextraction (DLLME) [18,19], hydrophobic deep eutectic solvents based on microextraction techniques[20], the headspace solid-phase microextraction [21], the carrier-mediated hollow fiber liquid phase microextraction [22], the dispersive solid-phase extraction (DSPE) combined with ultrasound-assisted emulsification microextraction based on the solidification of floating organic drop (UAEME-SFO)[23], the microextraction based on precipitation[24], the magnetic solid-phase extraction (MSPE) [25], and dispersive-micro solid phase extraction procedure

(D-µ-SPE) [26], were used for lead determination in different human matrixes. Between them, D-µ-SPE procedure is used in different matrixes by researchers. In addition, the characterizations of sorbents are important factor for lead extraction by the SPE method. For examples, the adsorbents such as, the silica aerogel nanoadsorbent [27], the magnetic phosphorus-containing polymer [28], the magnetic metal organic frameworks MMOF[29], the carboxylated graphene [30], the graphene Oxide Sheets [31], and modified-carbon nanotubes with NiFe$_2$O$_4$ [32] were reported by chemistry and nanochemistry scientists. The previously works showed that the different pretreatment techniques based on metal nanoparticles, the drugs, the functionalization of CNTs or GO, the magnetic nanoparticles can be increased the efficient extraction of heavy metals in human samples. Recently, some drugs use for in-vitro and in-vivo extraction of heavy metal in human samples and depend on structure and function groups. The antibiotic of sulfamethizole (SM) is a drug and use as an insistent inhibitor of bacterial enzyme. The SM can be complexed with metals in human body and depended on pH and covalence bonding.

In this study, a new SM-NGO adsorbent was used for determination lead in blood/serum samples by syringe filter-dispersive-micro solid phase extraction procedure (SF-D-µ-SPE) at pH=6.0. The high recovery and absorption capacity for lead extraction was obtained in optimized conditions. The lead concentrations in blood/serum samples were determined by AT-FAAS after sample pretreatment.

2. Experimental
2.1. Instrumental
Lead in human blood and serum samples determined by the GBC906 atom trap flame atomic absorption spectrophotometer (AT-FAAS, AUS) after sample preparation. The atom trap accessory put on burner and the fuel gas (air-acetylene), lamp position, slit and light line were manually tuned. Other parameters such as current and silt adjusted by Avanta Software. The minimum sample volume

(0.5-1 mL) vacuumed by nebulizer of AT-FAAS after adjusting flow rate of sample (mL min^{-1}). The LOD for Pb determination with AT-FAAS and F-AAS in standard solutions was obtained 0.025 mg L^{-1} and 0.07 mg L^{-1}, respectively. The linear ranges of 0.08-6.0 mg L^{-1} and 0.25-6.0 mg L^{-1} were obtained for AT-FAAS and F-AAS at wavelength of 283.3 nm (5 mA). All samples were injected with an auto-sampler injector from 100 µL to 1000 µL. The Inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer, Main Plasma Gas: Ar flow ~15 L min^{-1}, 1.2 s/m, USA) as high sensitive analyzer was used for determining of Pb in real human samples after microwave digestion. ICP-MS atomizes the liquid sample and generate atomic ions (M → M$^+$ + e$^-$), which are then detected by quadrupole-based mass analyzer system (mass-to-charge ratio (m/z), ICP-Q-MS). Before mass separation, a beam of positive ions has to be extracted from the plasma and focused into the mass-analyzer. The pH meter was used for determining of pH in liquid samples (Metrohm E-744). The vortex mixer (Thermo, USA) with 100-500 rpm, ultrasonic heating (Iran) and Falcon centrifuging (1000-4500rpm) prepared for this study.

2.2. Reagents and Materials

The lead standard solution (Pb; 1000 mg L^{-1} in 2 % nitric acid) was purchased from Sigma (Germany). All standard of lead (0.08, 0.1, 0.2, 0.5, 1, 2, 5 mg L^{-1}) prepared by dilution of the stock lead solution (1000 mg L^{-1}) with ultrapure water (UPW, Millipore, USA). Also, the sub-ppb concentration (10-500 µg L^{-1}) prepared by dilution of stock lead standard solution (1 mg L^{-1}). The pure reagents such as, the polyoxyethylene octyl phenyl ether (TX-100), nitric acid (HNO$_3$), HCl, acetone, and ethanol were purchased from Sigma Aldrich, Germany. Sulfamethizole (4-Amino-N-(5-methyl-1,3,4-thiadiazol-2-yl) enzenesulfonamide; C$_9$H$_{10}$N$_4$O$_2$S$_2$; CASN:144-82-1) and the hydrophobic ionic liquid of 1-methyl-3-octylimidazolium hexafluorophosphate. ([OMIM] PF6; C$_{12}$H$_{23}$F$_6$N$_2$P; CASN: 304680-36-2) was purchased from Sigma

Aldrich, Germany. The GO and GO-COOH was prepared from chemistry department, India. The pH was adjusted to 6 by sodium phosphate buffer solution (Na$_2$HPO$_4$/NaH$_2$PO$_4$) from Merck (Germany).

2.3. Sample preparations

All glasses such as vials, volumetric, dishes and beakers cleaned with HNO$_3$ and H$_2$SO$_4$ solution (1:1, 2 M) for at least 12 h. After cleaning, all of glasses washed for 10 times with DW. The normal Pb values in blood samples have less than 250 µg L^{-1} or >25µg dL^{-1} and more than 500 µg L^{-1} is toxic. By procedure, 10 mL of the blood or serum samples were prepared from 50 workers of lead–acid batteries factories in India (Men, 20-55 age). The sterilized syringes were used for blood samples. The pure heparin based on free of Pb was prepared and injected to blood. The blood samples were maintained at –5 °C. In this work, all blood samples prepared based on the world medical association declaration of Helsinki for physicians in human and all blood samples prepared from worker with agreement forms.

2.4. Synthesis of GO@ Sulfamethizole
2.4.1. Preparation of GO

The graphene oxide (GO) was prepared with pure graphite (G) by modified hummers procedure [33]. The 0.2 g of graphite powder was added into mixing solution (H$_2$SO$_4$/ H$_3$PO$_4$; 9:1) and then potassium permanganate (KMnO$_4$, 1.3 g) was slowly added by stirring solution up to became black green. The H$_2$O$_2$ slowly added to solution and stirred for removal of excess of KMnO4. After cooling, the hydrochloric acid (HCl:10 mL in 30 mL DW) was added and centrifuged. Then, the powder product of GO was washed with HCl and DW for 5 times and dried at 90 °C for 1 day.

2.4.2. Synthesis of GO@Cl

Chlorinated graphene oxide was prepared due to the Liu procedure [34]. First, 1.0 g of GO, 20 ml of benzene, and 100 ml of SOCl$_2$ were added in a 100-ml round flask and stirred (60-70 °C). Then,

the excess of SOCl$_2$ was exit out from mixture by distillation process based on vacuum condition. The solid product put in acetone and the suspension in acetone was filtered with Watman filter (200 nm). Finally, the product washed with acetone for 3 times and dried at 60 °C.

2.4.3.Synthesis of Sulfamethizole functionalized on nanographene oxide (SM@NGO)

1 g of sulfamethizole (SM) solved in 20 mL H$_2$O with 5 mL of NaOH and then 1 mL of SM solution were mixed in 60 mL ethanol (99%) by an ultrasonic bath (25 min) in a 100 mL round bottom flask. Then, 0.1 mL of triethylamine was added to mixture, and the mixture was refluxed at 70 °C for 5 hours. The obtained product was separated from the mixture by a hydrophobic PTFE Membrane Filters (poly-tetrafluoroethylene) for filtering SM@NGO. The SM@NGO product washed with ethanol for many times and finally dried under vacuum at 95 °C.

2.5. Extraction Procedure

By the SF-D-µ-SPE procedure, 10 mL of serum and plasma samples were used for determination of lead ions. Firstly, 25 mg of SM@NGO added to human blood/serum and lead standard solution (10-500 µg L^{-1}) at pH=6.0 and aspirated by 10 mL of syringe tube. The syringe tube placed on shaking tube for 5.0 min, after shaking, the lead was extracted by the sulfur group of SM@NGO in optimized pH (Pb^{2+}→: SR@GO). The SM@NGO separated from liquid phase by syringe hydrophobic PTFE membrane. Then, the pb^{2+} ions back-extracted from SM@NGO-PTFE membrane with elution of nitric acidic (0.5 mL, 0.5 M) and remain solution was determined by AT-FAAS after dilution with DW up to 1 mL (Fig.1). The procedure run for 10 blank solutions without any lead. The calibration curve of lead based on SF-D-µ-SPE/AT-FAAS procedure (10-500 µg L^{-1}) and standard method by AT-FAAS (0.1- 5 mg L^{-1}) were done. The enrichment factor (EF) calculated by curve fitting rules ($Tga=m_1/m_2$).

3. Results and discussion

3.1. Mechanism of Extraction by SM@NGO

The pure sulfamethizole drug was solved in H$_2$O/NaOH solutions and then mixed with ethanol (99%) by an ultrasonic bath. Then, the triethylamine were added to mixture. After reflux, the SM@NGO product was separated from the mixture by PTFE membrane by washing and drying. Finally, the sulfamethizole functionalized on nanographene oxide caused to make favorite adsorbent based on sulfur group (dative bond) for lead extraction in blood, serum and plasma samples
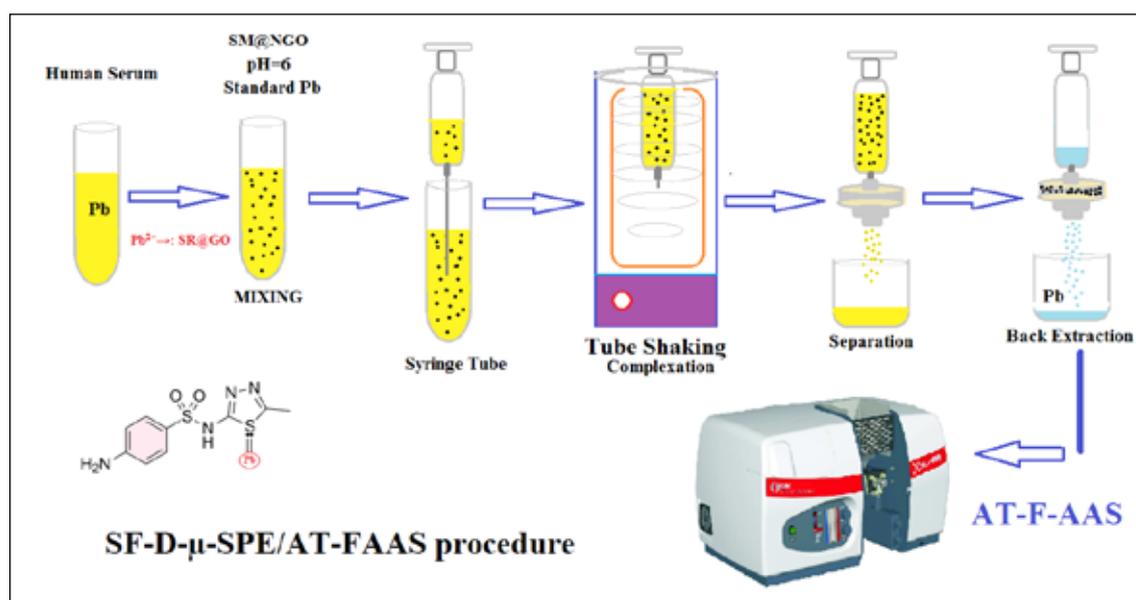

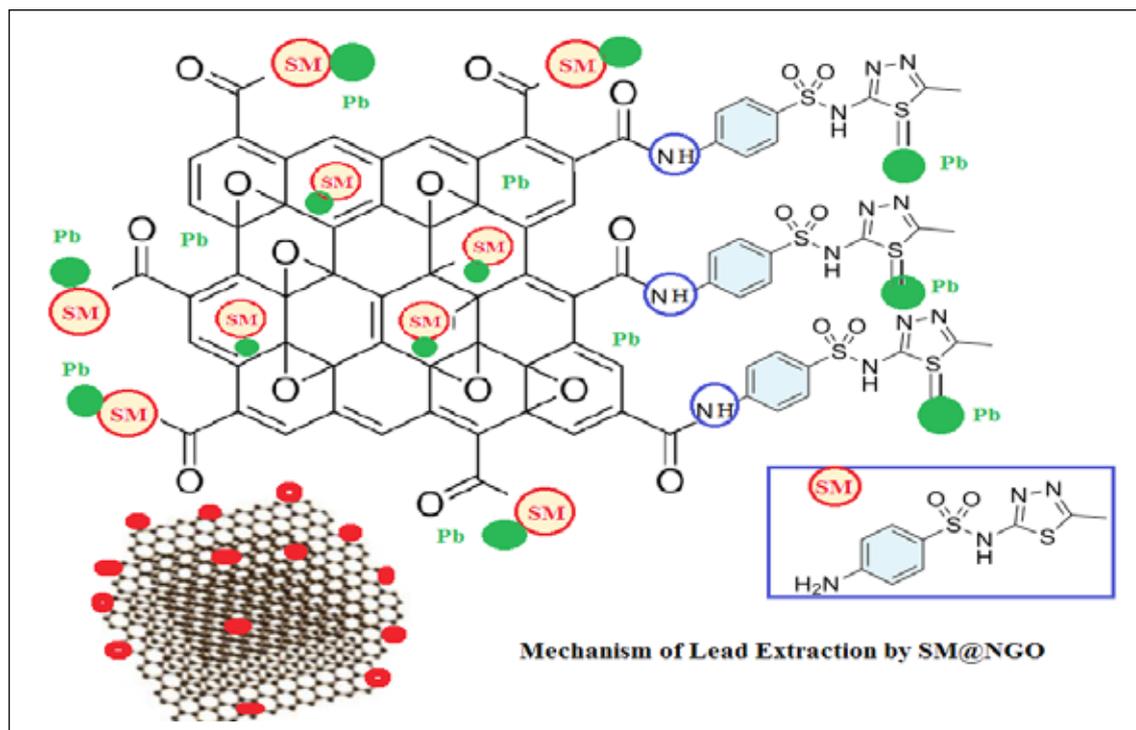**Fig. 1.** The SF-D-µ-SPE procedure for extraction of lead in human samples

Fig.2. The mechanism of lead extraction in blood/serum samples based on SM@NGO
by the SF-D-μ-SPE procedure

with high recovery. Therefore, the lead adsorption/ extraction with SM@NGO nanostructure caused to increase the recovery up to 98.5 % as compared to NGO adsorbent (26.4 %). The results showed, the mechanism of lead extraction in serum, blood and plasma samples depended on sulfur group of sulfamethizole in SM@NGO adsorbent at optimized pH (**Fig.2**).

3.2. SEM, TEM and FTIR of SM@NGO

The NGO adsorbent was synthesized from graphite powder according to the modified Hummer's method from RIPI Company (Iran) [33] and used for synthesis of SM@NGO. The TEM and SEM of SM@NGO showed the minimum size between 30-100 nm which were shown in **Figures 3 and 4,** respectively. The FT-IR spectrum of NGO-COOH
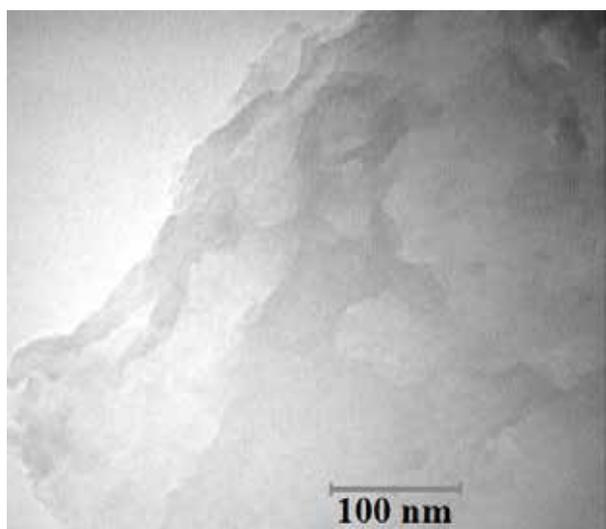

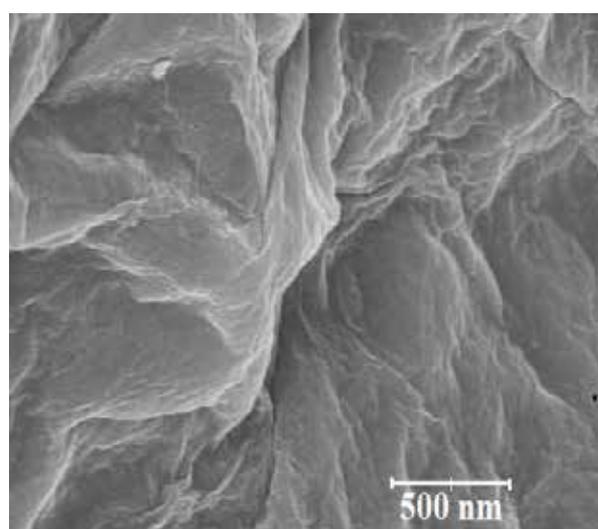**Fig.3.** The TEM of SM@NGO adsorbent

Fig.4. The SEM of SM@NGO adsorbent

and SM@NGO adsorbent was shown in **Figure 5**. Based on FT-IR spectrum, the C=N double bond is about twice as strong as a C-N single bond, and the C≡N triple bond is similarly stronger than double bond. The infrared stretching frequencies of these groups vary in the same order, ranging from 1100 cm^{-1} for C-N, to 1660 cm^{-1} for C=N. Also, symmetric and a symmetric traction for sulfur bond (S-C) ranging from 1135-1165 cm^{-1} and 1310-1360 cm^{-1} were seen. The O-H bond is about 3600 cm^{-1}, S-H bond in 2570 cm^{-1}, C-H bond in 3000 cm^{-1} and N-H bond in 3400 cm^{-1} observed.

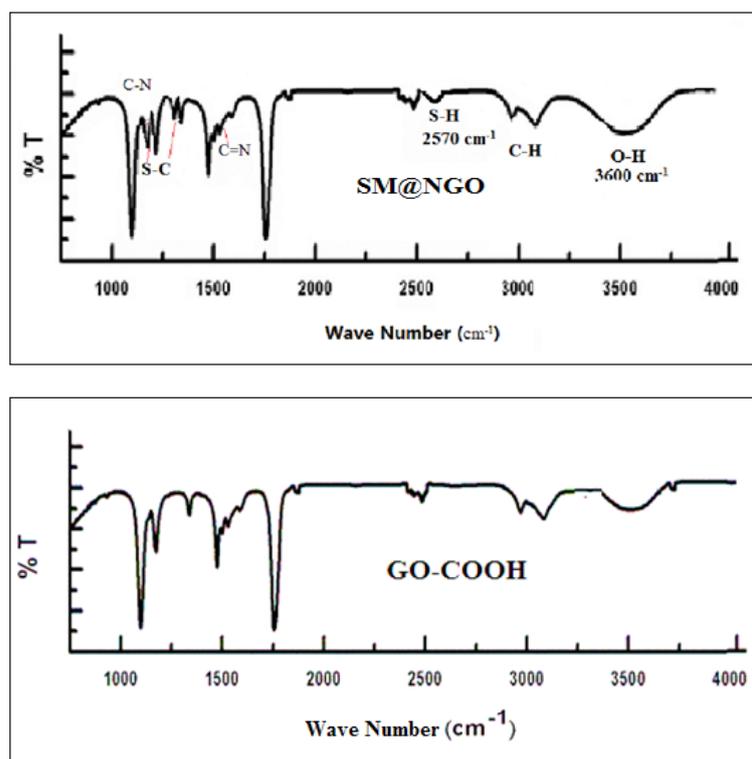The X-ray diffraction (XRD) of NGO and SM@NGO were shown in **Figure 6**. XRD of graphene

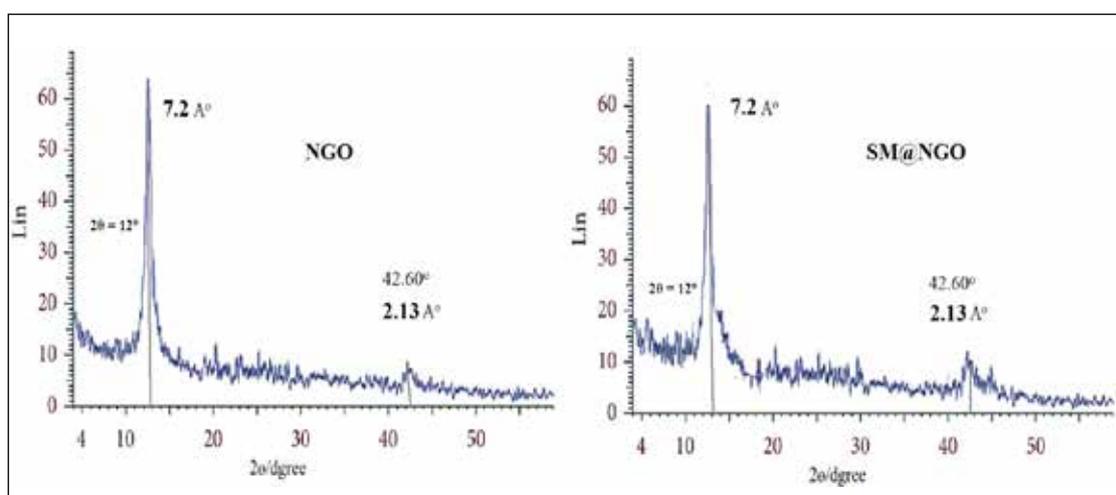**Fig.5.** The FT-IR spectrum of NGO-COOH and SM@NGO adsorbent

Fig.6. The X-ray diffraction (XRD) for NGO and SM@NGO adsorbents

oxide shows the one main diffraction peak at 2θ=12° related to the oxygen groups which are intercalated between graphene sheets. The peaks at 2θ=12° and 42.60° are related to the diffraction planes of (002) and (100) respectively, which can be showed in both of the XRD of GO and SM@NGO.

3.3. Optimization of extraction parameters

The SF-D-μ-SPE procedure based on new SM@NGO adsorbent was used for the extraction and determination Pb(II) ions in serum and plasma samples. For efficient lead extraction, the analytical parameters such as pH, sample volume and amount of SM@NGO adsorbent were optimized.

3.3.1.Influence of pH

The efficient extraction of lead in blood serum of battery workers was depended to pH. So, the pH of samples must be examined and optimized. The pH can be affected on increasing /decreasing extraction processes of lead in serum samples. The buffer solutions help us to improved extraction recovery by adjusting pH. Therefore, the different value of pH between 2-10 was evaluated by lead concentration from 10 to 500 μg L^{-1}. The results showed, the high extraction of lead ions in human biological samples was obtained at pH of 6.0 by SM@NGO adsorbent. On the other hand, the recoveries can be decreased at acidic or basic pH. So, the pH point of 6 was used for extraction Pb in real samples (Fig.7). Based on extraction mechanism, the coordination of dative bond of sulfur as negative charge in SM@NGO with the positively charged of Pb^{2+} were happened in optimized pH. At low pH (less than 5.5), the SM@NGO adsorbent protonated and surface of adsorbent got the positively charged. Due to the electrostatic repulsion, the recoveries of lead extraction were decreased. Moreover, in optimized pH(6), the surface of SM@NGO have negatively charged and high recovery was obtained for Pb^{2+} ions by the SF-D-μ-SPE procedure (more than 96%).

3.3.2.Influence of SM@NGO amount

The amount of SM@NGO nanoparticles for lead extraction in human samples has studied with lead concentration between 10-500 μg L^{-1} as a low and high LOQ ranges. For this proposed, 1-50 mg of SM@NGO adsorbent were used for lead extraction in serum, blood, plasma and standard solutions by the SF-D-μ-SPE method. The obtained results showed that the maximum of lead extraction in human samples was achieved for 22 mg of SM@NGO nanostructure. So, 25 mg of SM@NGO adsorbent was used for further work (Fig. 8). The extra mass of 25 mg had no effect on recovery of lead extraction in human samples.
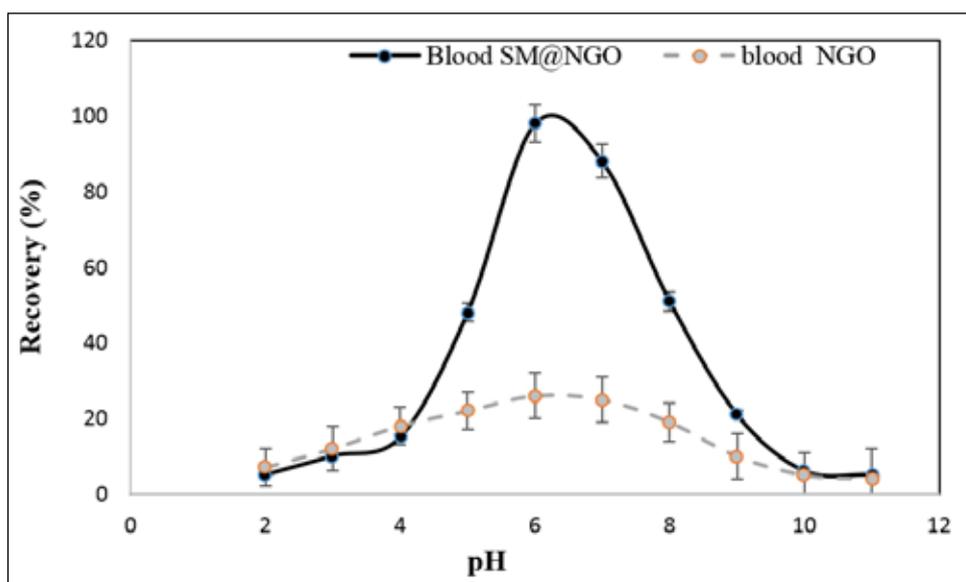

Fig.7. The influence of pH on lead extraction based on SM@NGO adsorbent by the SF-D-μ-SPE procedure
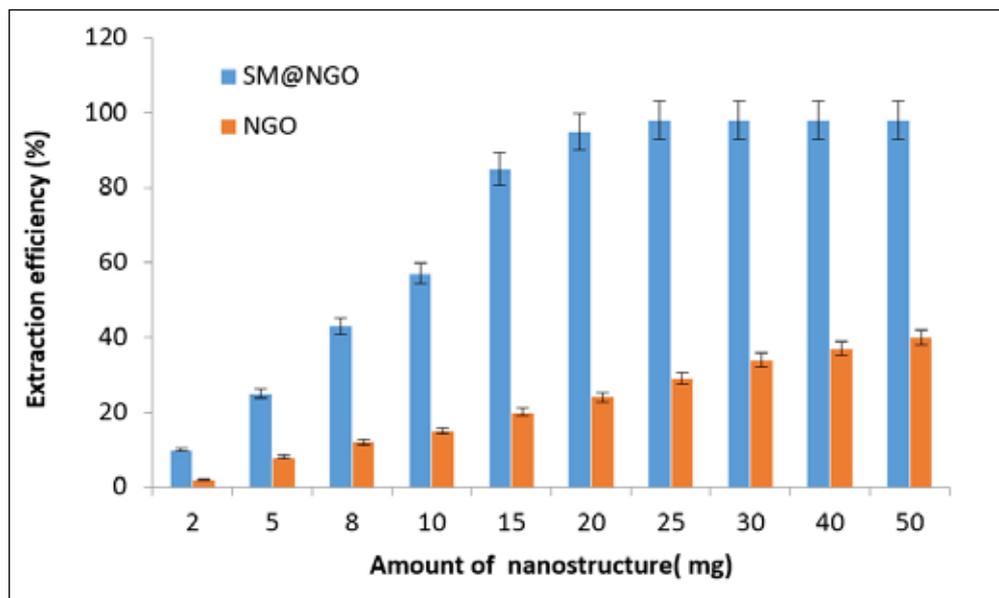
Fig.8. The influence of amount of SM@NGO on lead extraction
by the SF-D-μ-SPE procedure

3.3.3.Influence of sample volume

Sample volume is an effective parameter for extraction lead ions in human biological samples such as serum, blood and plasma. So, the lead extraction for sample volume between 1-30 mL was studied and optimized with the low and high LOQ concentration ranges (10-500 μg L^{-1}) by the SF-D-μ-SPE method (**Fig. 9**). The experimental results confirmed that the best extraction was obtained for less than 15 mL of human samples. Therefore, 10 mL was selected as optimum volume of human samples for lead extraction with 25 mg of SM@NGO adsorbent at pH=6. The recovery was decreased by adding sample volume in optimized conditions.
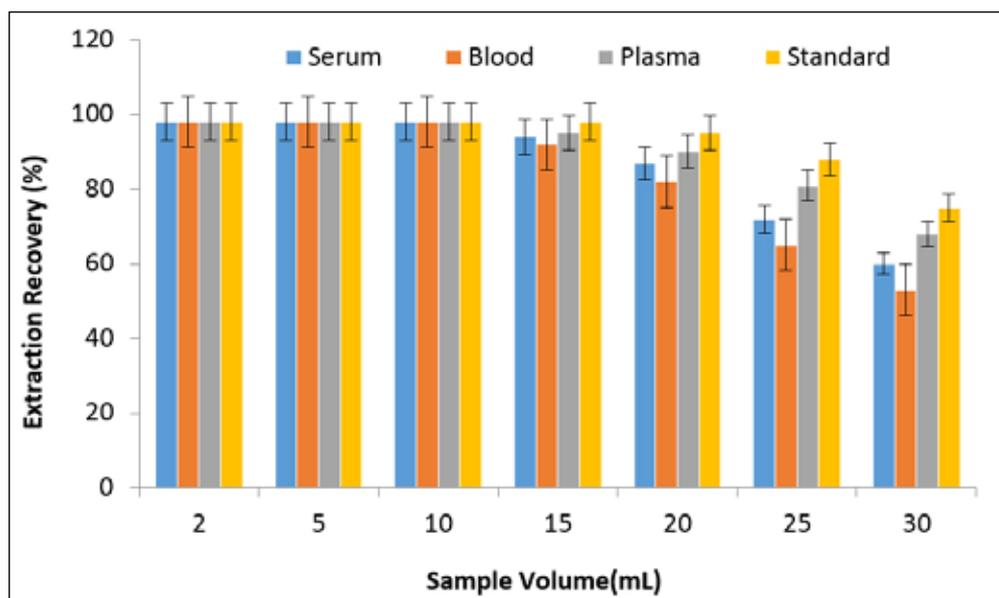

Fig.9. The influence of sample volume on lead extraction based on SM@NGO adsorbent
by the SF-D-μ-SPE procedure

3.3.4. Influence of volume and concentration of eluents

The influence of volume and concentration of eluents such as HCl, HNO₃, NaOH and CH₃COOH for back-extraction of lead ions from solid phase were studied and optimized by SF-D-μ-SPE procedure. The syringe coupled to Millex-FG hydrophobic PTFE membrane (0.2 μm) was used for back-extraction lead from adsorbent by different volume of eluents between 0.5-2 mL (0.2-1 M). After pushing the plunger of syringe, the elution of SM@NGO/hydrophobic PTFE membrane was performed by different eluents. In acidic or basic pH, the lead (Pb) released from SM@NGO/PTFF to elution phase and due to participate of lead hydroxyl in basic pH, the acidic pH was selected. Therefore, the acid solution can be used for back-extraction of lead from solid phase. The results demonstrated that the lead ions were simply back-extracted from SM@NGO adsorbent by nitric acid solution more than 0.4 mol L⁻¹. Therefore, 0.5 mol L⁻¹ of nitric acid (HNO₃) was used as optimum concentration of eluent for this study. Also, The results showed, the lead was efficiently back-extracted from SM@NGO by 0.5 mL of HNO₃. Finally, the lead concentration in remained solution was determined with AT-FAAS after dilution with DW up to 1 mL.

3.3.5. Membrane filter reusing

The reusability of the SM@NGO packed Millex-FG hydrophobic PTFE membrane was evaluated by several extraction and elution cycles under optimized conditions. It was found that the membrane filter could be reused after back-extraction processes and then rinsed by 5 mL of DW. The membrane filter was used for over 14 adsorption–elution cycles without significant decrease in extraction recoveries of lead ions. As collection adsorbent on membrane, the flow rate of eluent or samples through membrane filter was hardly done and so, the extraction recovery decreased more than 18 adsorption–elution cycles.

3.3.6. Adsorption capacity

The main parameter for evaluating of lead extraction with the SM@NGO packed Millex-FG hydrophobic PTFE membrane is adsorption capacity. So, the adsorption capacity of 25 mg of SM@NGO adsorbent for Pb (II) in 10 mL of human sample containing 5 mg L⁻¹ Pb (II) ions was investigated at pH 6 in static system. After adjusting pH with favorite buffer solution, the mixture was shaked for 15 min and filtered with Millex-FG hydrophobic PTFE membrane (0.2 μm). Finally the residual concentrations of lead in the Millex-FG hydrophobic PTFE membrane were determined using AT-FAAS. The adsorption capacities of GO and SM@NGO for Pb (II) ions were found to be 57.5 mg g⁻¹ and 162.1 mg g⁻¹, respectively.

3.3.7. influence of Interference ions

The influence of some coexisting ions for lead extraction in human samples was examined by the SF-D-μ-SPE procedure. For investigation of the effect of different interference ions on lead extraction, the various ions (0.5-2 mg L⁻¹) added to 10 mL of lead samples with 500 μg L⁻¹ at pH 6.0. So, the most of the concomitant ions in human blood such as Cu, Zn, Mn, Mg, Se, Li, F, NO₃, HCO₃, Ca, Na an K were considered for lead extraction by SM@NGO adsorbent. The experimental results showed, the interference coexisting ions had no effect on lead extraction by proposed method. (Table1). Therefore, the SM@NGO adsorbent was efficiently extracted lead ions from human biological samples in the present of the interference coexisting ions.

3.3.8. Determination of lead in real samples

The SM@NGO adsorbent was used for extraction and determination of lead ions in human samples by the SF-D-μ-SPE procedure. For validation of the results, the human blood, serum and plasma samples were spiked with lead standard solution based on SM@NGO nanostructure before determination by AT-FAAS. The high extraction of spiked samples demonstrated that the SM@NGO adsorbent had satisfactory results for lead

Table 1. The influence of interferences coexisting ions on lead extraction in human blood samples
by the SF-D-µ-SPE procedure

Interfering Ions	Mean ratio (C_I /$C_{Pb(II)}$)	Recovery (%)
	Pb(II)	Pb(II)
Cr^{3+}, Se^{2+}, Mn^{2+},	600	97.4
Zn^{2+}, Cu^{2+}	700	98.6
I^-, Br^-, F^-, Cl^-	1200	97.2
Na^+, K^+, Li^+	1000	99.2
Ca^{2+}, Mg^{2+}	800	97.7
Ni^{2+}, Co^{2+}	450	98.4
CO_3^{2-}, PO_4^{3-}, $HCO3^-$, NO_3^-	950	96.6

extraction and determination in human samples at pH=6.0 (**Table 2**). Also, 10 mL of the blood or serum samples were prepared from 50 workers of lead–acid batteries factories in India (Men, 20-55 age) by the SF-D-µ-SPE procedure and compared to healthy peoples (**Table 3**). Moreover, the certified reference materials (NIST; CRM) and lead analysis with ICP-MS were used for validating of methodology based on SM@NGO by the proposed procedure (**Table 4**)

Table 2. Determination of lead based on SM@NGO adsorbent and spiking samples
by the SF-D-µ-SPE procedure coupled to AT-FAAS

*Sample	Added (µg L^{-1})	*Found (µg L^{-1})	Extraction efficiency (%)
Blood	---	9.7 ± 224.2	---
	200	17.8 ± 418.6	97.2
Plasma	---	2.8 ± 55.4	---
	50	4.8 ± 106.7	102.6
Serum	---	7.6 ± 168.9	---
	150	14.3 ± 311.6	95.1
Blood	---	8.2 ± 178.8	---
	150	13.8 ± 322.3	95.6
Serum	---	9.4 ± 192.3	---
	200	19.3 ± 399.5	103.6
Plasma	---	4.2 ± 88.5	---
	100	8.4 ± 186.8	98.3

*Mean of three determinations of samples ± SD (P = 0.95, n =10)

Table 3. Determination of lead in serum, blood and plasma by the SF-D-μ-SPE procedure coupled to AT-FAAS (intra –day and inter day, n=50, μgL^{-1})

* Sample	[b] Workers (n=50)		[b] Healthy peoples (n=50)		[a] Workers	
	Intra-day	Inter day	Intra-day	Inter day	r	P value
Blood	352.8 ± 15.7	360.2 ± 16.2	33.1 ± 1.4	29.5 ± 1.3	0.092	<0.001
Serum	276.7 ± 12.6	281.2 ± 13.9	24.6 ± 1.1	28.2 ± 1.2	0.112	<0.001
Plasma	147.5 ± 6.8	152.4 ± 7.3	12.8 ± 0.6	14.6 ± 0.7	0.086	*<0.001*

[a] Correlations are based on Pearson coefficients (r). Statistical significance will be observed if $P < 0.05$
[b] Mean of three determinations of samples ± confidence interval (P = 0.95, n =10)
* 50 workers of *lead*–acid batteries factories in India (Men, 20-55 age)

Table 4. Validation of methodology for lead determination by certified reference materials(CRM, NIST)

Sample	CRM*(μg L^{-1})	Added	Found*(μg L^{-1})	Recovery (%)
Caprine blood, level 2	139.5 ± 0.8	-----	135.8 ± 6.5	-----
		100	233.6± 11.4	97.8
Caprine blood, level 3	277.6 ± 1.6	-----	272.6 ± 13.1	-----
		200	465.7± 21.8	96.5
Serum by ICP-MS	86.4 ± 2.1	-----	84.2 ± 4.4	-----
		50	131.5 ± 4.4	94.6

*Mean of three determinations of samples ± SD (P = 0.95, n =10)
CRM955c, caprine blood, level 2, 139.5 ± 0.8 μg L^{-1}
CRM955c, caprine blood, level 3, 277.6 ± 1.6 μg L^{-1}

4. Conclusions

A novel SM@NGO adsorbent was used for lead separation/extraction in human blood, serum and plasma samples by the SF-D-μ-SPE coupled to AT-FAAS. The Millex-FG hydrophobic PTFE membrane (0.2 μm) was used as a filter for separating solid phased from liquid phase. By the SF-D-μ-SPE method, the high extraction, the low cost, the fast separation and simple method in short time was obtained in optimized pH. The trace amount of SM@NGO as a solid-phase caused to extract the lead ions from the human biological samples without any ligand. The developed SF-D-μ-SPE method presented a analytical nanotechnology for lead separation/extraction/preconcentration in human blood samples with low interference ions, good reusability and simple sample preparation in difficulty matrixes. Therefore, the SM@NGO nanostructure was used as a perfect adsorbent for determination and extraction of lead in blood, serum and plasma samples by AT-FAAS.

5. Acknowledgments

6. References

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