

## Solid-phase extraction of Lead in saline matrix using by GF- AAS with multivariate optimization

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### Abstract

This work presents a novel method investigated in the pre-concentration of lead ion as well as the matrix effect eliminated in high salinity medium using solid phase extraction by graphite furnace Atomic Absorption spectroscopy (GFAAS). Fe<sub>3</sub>O<sub>4</sub>-graphene was used as the sorbent while 1M HNO<sub>3</sub> was used as the extraction solvent. Parameters such as: pH, amount of sorbent, time of adsorption, time of desorption and, stirring rate affecting the extraction efficiency of the method were studied and optimized by utilizing two decent optimization methods; factorial design and central composite design (CCD). The limit of detection and limit of quantification were 4.4 and 14.8 ng/mL, respectively. The accuracy of the method was evaluated by analyzing a spiked real sample. Good spiked recoveries over the range 98.8-103.6 % were obtained. Under the optimum conditions, the calibration graph was linear over the range 5-50 ng/mL. The relative standard deviation was 4.4 % for four repeated determinations at a concentration of 20 ng/mL.

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Received 15 Oct 2020

Revised 05 Dec 2021;

Accepted 06 Dec 2021

**Keywords:** : Solid-phase extraction; furnace Atomic Absorption spectroscopy; Lead; Multivariate optimization; Placket-Burman design; Central composite design; food sample

## 1. Introduction

Lead (Pb) is one of the most important environmental pollutants and it belongs to a non-biodegradable group of heavy metals, which is dissolvable in the natural environment and has devastating effects on the human immune system. It causes severe damage to the nervous system, as well as blood and brain diseases. The consumption of lead-contaminated foods is one of the common reasons for poisoning, since food plays an important role in human health. The extraction and determination of low levels of lead ion in food stuff in the long term, have been an interesting scientific subject for researchers. However, it is difficult to determine the low concentration of lead in high-salt content food samples due to their complex matrix. This problem in a material such as salt, which is used directly or indirectly (in food processing) in a food stuff, has encouraged many researches to eliminate the effect of matrix on lead determination. Fernandez and Manning [1, 2] stated that the presence of NaCl in a sample reduces the measured Lead value than the actual amount (Negative error). When NaCl is a major matrix material, it will cause a big background signal, unless it is eliminated in the ashing step. However, many ion metals are lost at lower temperature compared to the NaCl that is removed. Ottaway [3] stated that the interference of saline matrix in the determination of Pb is due to the presence of chloride in the medium. A lot of work has been done to eliminate interference in the measurement of lead levels, including the elimination of the interference effects due to the matrix, by adding chemical compounds as matrix modifiers. [4, 5]. Several researchers have proposed methods for the chemical pretreatment of graphite tube to reduce the effects of the matrix. [6-11]. Also, this interference could be reduced by using separation [12, 13] and pre-concentration methods including liquid-liquid extraction, [14, 15], cloud point extraction [16], solid phase extraction [16] solid phase micro extraction [17]. For the purpose of optimization of methods in analytical chemistry, multivariate techniques have been extensively used. [18, 19]. Also, factorial design is one of the widely used methods.[20, 21]. The chemometric technique was used several times for optimization of variable factors in pre-concentration procedures for metal determination using FAAS. [22-28]. Also, this study utilized two optimization methods, Factorial design and central composite design. In 2004, Geim and Novoselov proposed an easy method for the synthesis of graphene with good quality. Graphene consists of single-layer carbon atoms that are  $sp^2$ -bonded and create two-dimensional honeycomb lattice. As a result of the presence of  $\pi$ - $\pi$  bonds, interactions can easily be chemically functionalized. These structural properties create unique properties for graphene such as: great ability to transfer heat and electricity and high effective surface area compared to other carbon nanomaterials. In this work, Graphene- $Fe_3O_4$  was used as a magnetic adsorbent for the extraction, pre-concentration, and determination of trace amounts of Lead in saline matrix. Magnetic G- $Fe_3O_4$  nanocomposite was chosen as the adsorbent due to low cost, simplicity of use, and high synthesis yields. Furthermore, the experimental conditions were optimized using experimental design methods that reduce the number of experiments and investigate the interaction of effective parameters with each other. At the end, one of the advantages of this work is the extraction and measurement of lead in food samples with complex matrices and high salt content by high resolution - continuum source graphite furnace atomic absorption spectrometer.

## 2. Experimental Section

### 2.1 Instruments

A high-resolution continuum source atomic absorption spectrometer, model contrAA 700 (Analytik Jena AG, Germany) was used for the Analysis .It is equipped with two types of atomizers, a flame atomizer and a transversely heated graphite tube atomizer. The graphite tube atomizer was used in all our measurement. This spectrometer is made up of a continuum radiation source that covers a range between

185 and 900 nm and consists of a high-intensity xenon short- arc lamp operating in a hot-spot mode as a high-resolution double monochromator and a CCD array detector with 588 pixels, 200 of which are used for analytical purposes. The double monochromator consists of a pre-dispersing prism mono-cremator and a high-resolution echelle grating monochromator, both in Littrow mounting. All experiments were carried out using liquid sampling (LS) graphite tubes and LS graphite platforms pyrolytically coated. The graphite furnace heating program for the determination of lead is shown in Table 1. Argon with a purity of 99.996% was used as the purge gas with a flow rate of 2.0 L/min during all steps, except during atomization step, when the argon flow rate was interrupted. In order to adjusted pH in solution, a pH-meter metrohm model 781 (Metrohm, Switzerland) was used and the precipitation was collected by the aid of a neodymium magnet Also Vortex mixer (IKA.VORTEX 3) was used for thorough mixing of solutions. UV-Visible spectra were recorded by Shimadzu UV-1700 ParmaSpec. (Tokyo, Japan). SEM images were recorded with a Cambridge Instruments S360 scanning electron microscope (Cambridge Instruments, Cambridge, UK). IR spectra obtained with an M-500 FastScan IR spectrometer (Buck Scientific, East Network, CT 06855, USA).

**Table 1.** The graphite furnace heating program for the determination of lead.

Name	Temp(°C)	Ramp(°C/s)	Hold(s)
Drying	80	6	20
Drying	90	3	20
Drying	110	5	10
Pyrolysis	350	50	20
Pyrolysis	500	300	10
Gas adaption	500	0	5
Atomization	1500	1500	4
Clean	2450	500	4

## 2.2 Material

All reagents were analytical grade and used without any purification. Extra pure water was used from a Milli-Q® Direct Water Purification System (Merck). Nitric and hydrochloric acid were of suprapure quality (Merck). Iron (III) chloride hexahydrate, iron (II) chloride tetrahydrate, Ferric Ammonium Sulfate dodecahydrate, Ferrous Ammonium Sulfate, potassium permanganate (KMnO<sub>4</sub>), sulfuric acid, H<sub>2</sub>O<sub>2</sub>, NH<sub>3</sub> and graphite powder (<150 mesh) were all purchased from Merck (Darmstadt, Germany). Laboratory glassware kept in 10% nitric acid solution for at least 24 h. Before using the glassware washed with deionized water and dried in a dust-free oven. A standard solution containing lead was prepared from 1000 µg/mL certified atomic absorption solutions of lead (Merck). Also, Standard solutions of other metal ions in this work were prepared by diluting the stock solutions (1000 µg/mL) of all the metals purchased from Merck Company (Darmstadt, Germany).

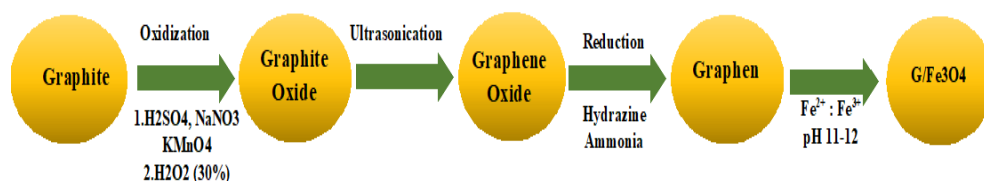
## 2.3 Preparation of graphene

Graphene was synthesized using a modified Hummer's method [29]. The schematic of the synthesis process is shown in Fig. 1. Graphite powder (1.00 g, 150 mesh) and H<sub>2</sub>SO<sub>4</sub> (95 %) (23 mL) were mixed in a 250 mL

conical flask, and the mixture was stirred with a magnetic bar. Sodium nitrate (0.50 g) was added to the mixture and the temperature was cooled and kept at 0 °C. At the same time with vigorous agitation, KMnO<sub>4</sub> (3.00 g) as oxidant was added slowly and the mixture was stirred for 1 h, while the temperature was kept below 35 °C. Afterward, H<sub>2</sub>O (45 mL) was added gradually to the reaction mixture, and the temperature of solution was kept at 90 °C also stirred for 30 min. subsequently, H<sub>2</sub>O<sub>2</sub> (10 mL of a 30 % solution) was added to mixture until color of the mixture is changed to yellow and then deionized water (140 mL) was added to the mixture. Next, obtained precipitate for removal of Metal impurities was centrifuged and washed repeatedly with HCl (5 %, 3× 15 mL) and EtOH. The mixture was then vacuum dried at 60 °C. The GO was obtained as a brown powder. reduction of graphene oxide produces graphene. To this end, 2L of GO solution (0.05%), 12 mL hydrazine solution and 14.2 mL ammonia (25%) was added and was stirred for several minutes and then was refluxed in an oil bath (98°C) for 24 hours. The resulting G was dried in air and ground.

#### 2.4. Synthesis of Graphene -Fe<sub>3</sub>O<sub>4</sub>:

G-Fe<sub>3</sub>O<sub>4</sub> was synthesized by chemical co-precipitation of Fe(II) and Fe(III) in an alkaline solution in the presence of G. to this end, 500 mg of graphene 1.7 g of Fe(NH<sub>4</sub>)<sub>2</sub> (SO<sub>4</sub>)<sub>2</sub>, 6 H<sub>2</sub>O, 2.51 g of Fe(NH<sub>4</sub>) (SO<sub>4</sub>)<sub>2</sub>, 12H<sub>2</sub>O and 200 mL of water was mixed in 250 mL round bottom flask. The mixture was stirred in an ultrasonic Bath sonicator for 10 min under nitrogen gas at 50 °C. Then, 8 M NH<sub>3</sub> was added dropwise to the reaction mixture until having pH=10, After that, the reaction mixture was heated for 1 h at 50°C. The mixture was then magnetized using a magnet. The mixture was washed with water and ethanol. The resulted product was dried in an oven for 24 h.

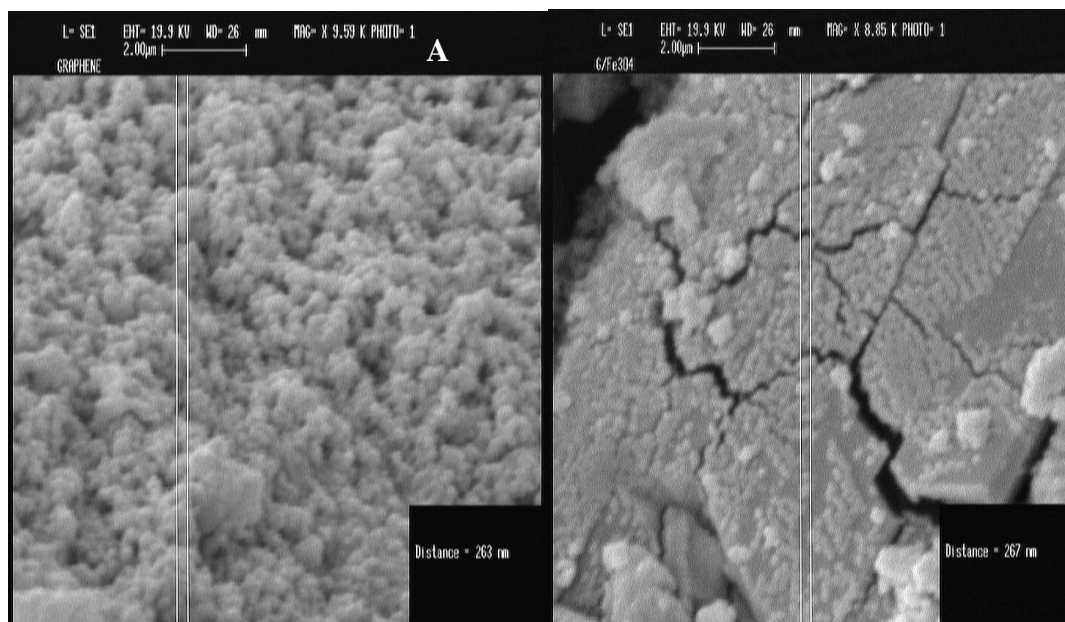


**Fig 1.** Schematic diagram for the method of magnetic graphene synthesis.

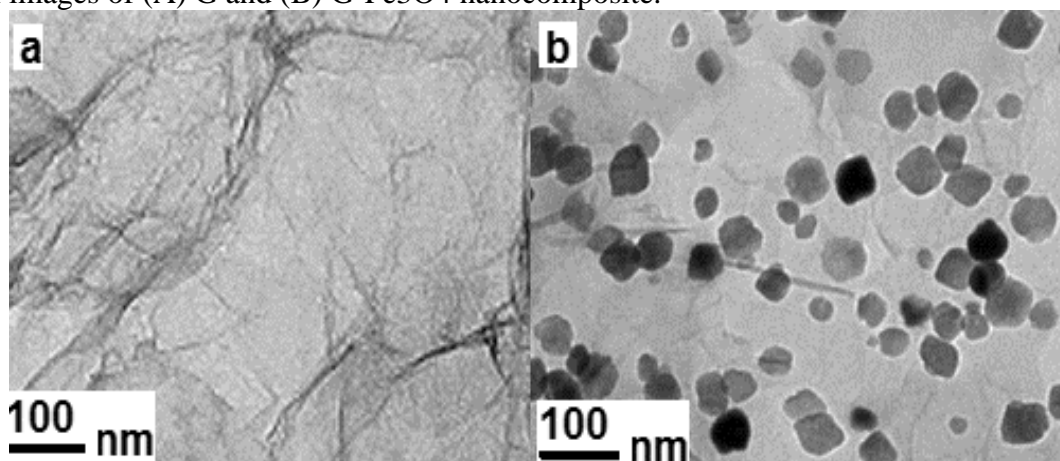
#### 2.5 Characterization of magnetic graphene nanocomposite and graphene nanomaterial

UV/Visible absorption spectra were recorded in the 200–500 nm wavelength range for 0.03 mg/mL aqueous dispersed GO by the double beam spectrophotometer. In these spectra two specific characteristics were observed to recognize GO: Two shoulders at ~ 310 nm and 230.5 nm were attributed to n-π\* and π-π\* bonds respectively. In Fig.2 and 3, SEM and TEM images of G and G-Fe<sub>3</sub>O<sub>4</sub> nanocomposite are shown. As can be seen clearly in Fig.2A crushed surface of the carbon sheets. by comparing Fig. 2A (mono-layer sheets of G) and Fig. 2B it is seen that G-Fe<sub>3</sub>O<sub>4</sub> nanocomposite was synthesis. As a further evidence of the success of G-Fe<sub>3</sub>O<sub>4</sub> nanocomposite synthesis, the FT-IR spectra were recorded and shown in Fig. 4 .in both G and G-Fe<sub>3</sub>O<sub>4</sub> spectrum presence of absorbance bond in respectively (3428 cm<sup>-1</sup>, 1560 cm<sup>-1</sup>, 1710 cm<sup>-1</sup>) corresponded to O-H, the skeletal vibration of graphene sheets and the carboxyl group and Fe-O stretching vibration bond at 582 cm<sup>-1</sup> is an evidence for the successful synthesis of the G-Fe<sub>3</sub>O<sub>4</sub> nanocomposite

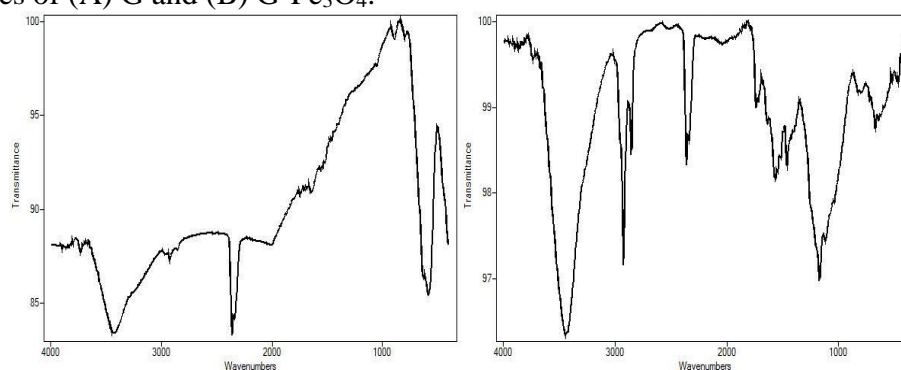




**Fig. 2.** SEM images of (A) G and (B) G-Fe<sub>3</sub>O<sub>4</sub> nanocomposite.



**Fig. 3.** TEM images of (A) G and (B) G-Fe<sub>3</sub>O<sub>4</sub>.



**Fig. 4.** FT-IR spectrum of (A) G and (B) G-Fe<sub>3</sub>O<sub>4</sub> nanocomposite.

## 2.6 Magnetic solid phase extraction procedure

In this work, we used of MSPE in batch mode for pre-concentration on the extraction of Lead from high - salt medium. Initially in the adsorption step pH 15 ml of working solution containing 5% (w/v) NaCl (causing a saline medium) and 20 ng/mL of Pb(II) was adjusted in pH=4 using HNO<sub>3</sub> and NaOH solution. Next 0.05 mg of the sorbent was added to the solution and vortexed in rate 1000 rpm for 30 min. Then, G-Fe<sub>3</sub>O<sub>4</sub> was separated from the solution by using an external magnet. Afterward, 2 mL HNO<sub>3</sub>(1mol/L) was added as an eluent solvent to the adsorbent and vortexed for 30 min at rate 1000 rpm to separate Lead ions from the surface

of the adsorbent and finally, the concentration of lead ions in the eluent was directly determined by HR-CS GF AAS.

Extraction percentage was calculated from the following equation:

$$\% \text{ Extraction} = C_a - C_b / C_a \times 100 \quad (1)$$

where  $C_a$  and  $C_b$  are the initial and final concentrations of lead ions in the solution, respectively.

### 3. Results and discussion

#### 3.1 Optimization process

##### 3.1.1 Demonstrate of significant variables using Plackett-Burman design

The pre-concentration procedure was optimized by two-level fractional factorial design, many variables such as: pH, amount of sorbent, volume of adsorption solution and volume of desorption solvent, adsorption and desorption time, stirring rate in adsorption and desorption step using % Recovery of Pb(II) as analytical responses by Plackett–Burman design (PBD) was studied (Table 3) and optimum values were shown in Fig. 5. As seen in Table 2, for each factor two levels (+) or (-) were examined that each of these factors itself could also have a positive or negative effect on the % Recovery as an analytical response. Finally, more significant factors among other factors were shown by using PBD.

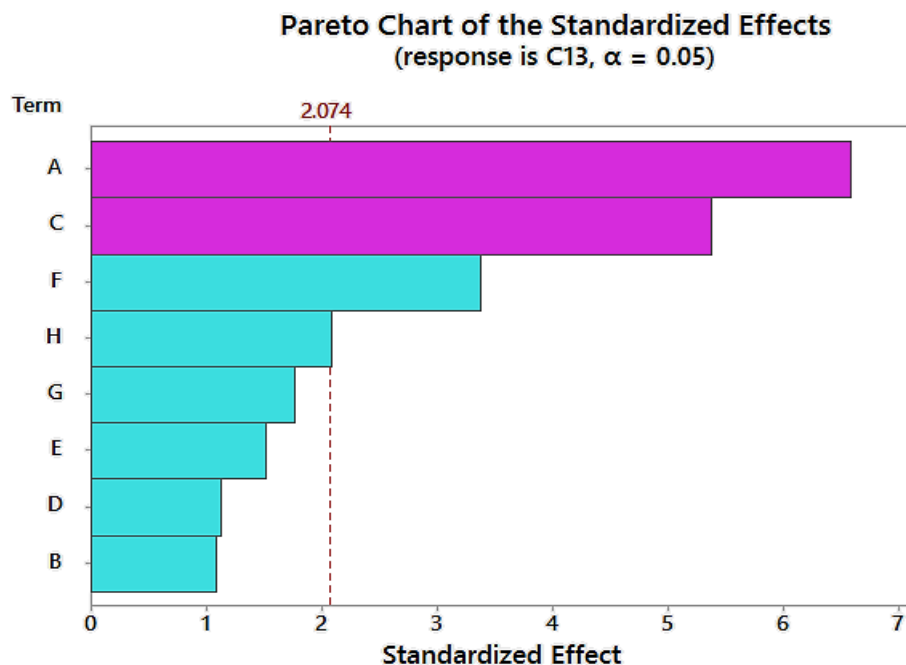
**Table2:** introduce studied factors for determination of Pb(II) in PBD

Parameters	symbol	Unit	Level (-)	Level (+)
pH	A	-	3.00	9.00
Volume of adsorption solvent	B	mL	7.00	15.0
Volume of desorption solvent	C	mL	5.00	3.00
Time of adsorption	D	min	5.00	30.0
Time of desorption	E	min	5.00	30.0
Amount of sorbent	F	mg	0.01	0.03
Stirring rate for adsorption	G	rpm	500	1000
Stirring rate for desorption	H	rpm	500	1000

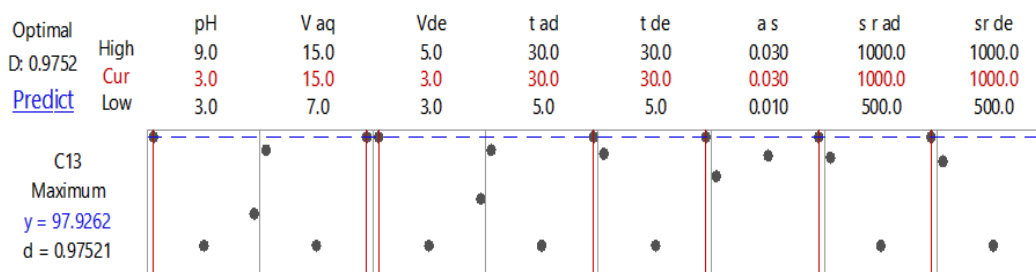
statistical evaluation of results carried out and indicated in the standardized ( $P = 95.0\%$ ,  $t$ -value: 2.074) Pareto chart Fig. 5. The Pareto chart is useful to reveal important and influential factors. As shown in Pareto chart, pH has a most important effect. Also, some factors such as the volume of desorption solvent, amount of sorbent, stirring rate in desorption and sorption steps were effected on the % recovery of Pb(II), respectively. Other factors such as time of adsorption and desorption, the volume of adsorption were not significant. Thus, level of significant factors was selected for more optimization. Afterwards, significant factors were optimized by central composite design (CCD). Also, Optimum Curve for desired factors was shown in Fig. 6.

**Table 3:** Table result of PBD matrix for determination of Pb(II).

Run Order	A	B	C	D	E	F	G	H	% recovery
1	9	15	3	30.0	30.0	0.01	1000	500	56.00
2	3	7	5	30.0	30.0	0.01	1000	1000	46.70
3	6	11	4	17.5	17.5	0.02	750	750	59.00
4	6	11	4	17.5	17.5	0.02	750	750	48.40
5	6	11	4	17.5	17.5	0.02	750	750	49.10
6	9	7	5	30.0	5.00	0.03	500	500	16.36
7	6	11	4	17.5	17.5	0.02	750	750	49.10
8	9	7	5	5.00	5.00	0.01	1000	1000	24.52
9	9	7	5	5.00	5.00	0.01	1000	1000	25.60
10	6	11	4	17.5	17.5	0.02	750	750	57.35
11	6	11	4	17.5	17.5	0.02	750	750	48.46
12	9	7	3	5.00	30.0	0.03	1000	500	41.00
13	3	7	3	30.0	30.0	0.03	500	1000	93.90
14	3	7	5	30.0	30.0	0.01	1000	1000	38.64
15	9	15	3	30.0	5.00	0.01	500	1000	33.10
16	9	15	3	30.0	30.0	0.01	1000	500	47.93
17	3	15	3	5.00	5.00	0.03	1000	1000	80.28
18	9	15	5	5.00	30.0	0.03	500	1000	25.96
19	9	7	3	5.00	30.0	0.03	1000	500	43.54
20	3	7	3	5.00	5.00	0.01	500	500	45.46
21	3	15	5	5.00	30.0	0.01	500	500	42.36
22	3	15	5	30.0	5.00	0.03	1000	500	53.56
23	3	7	3	5.00	5.00	0.01	500	500	44.42
24	3	7	3	30.0	30.0	0.03	500	1000	100.00
25	9	15	5	5.00	30.0	0.03	500	1000	29.34
26	3	15	3	5.00	5.00	0.03	1000	1000	92.44
27	9	15	3	30.0	5.00	0.01	500	1000	30.42
28	9	7	5	30.0	5.00	0.03	500	500	16.60
29	6	11	4	17.5	17.5	0.02	750	750	57.83
30	3	15	5	30.0	5.00	0.03	1000	500	62.38
31	6	11	4	17.5	17.5	0.02	750	750	57.83
32	3	15	5	5.00	30.0	0.01	500	500	40.62



**Fig. 5.** Pareto chart of the standardized effects in 95% confidence Level.



**Fig. 6.** Optimum Curve for desired factors.

### 3.1.2 Optimization using central composite design

The central composite design is utilized method that can indicate non-linear terms. CCD comes from the combination of fractional factorial and axial point and center points. In this work, we used of the full central composite design was performed in  $2^3$  fractional factorial and  $2 \times 3$  axial point with 2 replicates along with 5 center point in total twenty-five runs, randomly (Table 4).

The target of the optimization procedure was a comprehensive study of significant effective factors on %recovery of Pb in the complex matrix. As stated below, the second-order polynomial equations (2,3) were obtained from multiple regression analysis on resulted % recovery as an analytical response.:

**Final Equation in Terms of Coded Factors :**

(2)

$$R1 = + 65.13 + 18.58 A + 5.51 B - 20.13 C - 7.11 AB + 8.25 AC - 1.79 BC - 1.01 A^2 - 3.25 B^2 + 7.94 C^2$$

**Final Equation in Terms of Actual Factors:**

(3)

$$R1 = +25.66981 + 28.30780 \text{ pH} + 5818.87313 \text{ AS} - 85.39220 \text{ Vdes} - 711.25000 \text{ pH} \times \text{AS} + 8.25250 \text{ pH} \times \text{Vdes} - 178.75000 \text{ AS} \times \text{Vdes} - 1.00672 \text{ pH}^2 - 32467.16418 \text{ AS}^2 + 7.94328 \text{ Vdes}^2$$



For this model,  $R^2$  and  $R^2$  adj were acquired 0.9380 and 0.9007, respectively. F value was obtained 25.196 which indicate the model is significant. Using the analysis of variances (ANOVA) the acceptable of the models was tested. P-value <0.05 show the significant effect so pH and Vdes (p-value = 0.0001, p-value = 0.0001) and interaction of two factors pH and Vdes (p-value =0.0001) were significant.

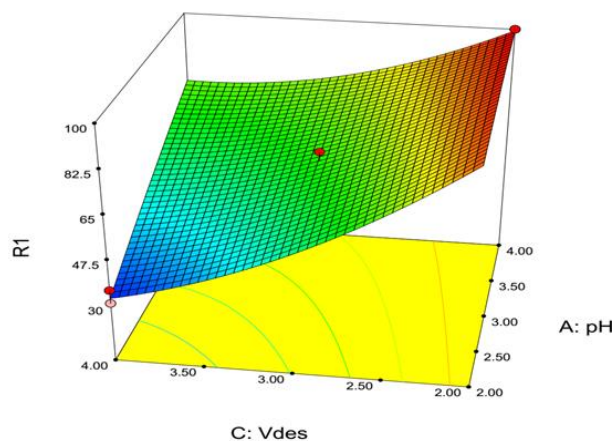
**Table 4.** Matrix Table for central composite design

run order	pH	AS	V des	%Response
1	4	0.03	4	74.0
2	3	0.04	4	50.0
3	3	0.04	3	70.9
4	2	0.05	4	30.6
5	4	0.05	2	100
6	3	0.04	3	77.6
7	3	0.05	3	69.3
8	3	0.04	3	64.0
9	4	0.05	2	100
10	3	0.04	3	59.4
11	4	0.03	4	84.9
12	2	0.03	2	65.5
13	3	0.05	3	63.3
14	4	0.04	3	76.9
15	3	0.03	3	57.0
16	3	0.04	3	62.5
17	3	0.04	2	91.0
18	4	0.04	3	86.3
19	3	0.04	2	93.2
20	2	0.04	3	36.0
21	2	0.05	4	35.9
22	3	0.04	4	53.7
23	2	0.04	3	52.9
24	3	0.03	3	53.5
25	2	0.03	2	64.0

However other terms with (p-value > 0.05) were not significant. Furthermore, the result of ANOVA (Table 5) indicated Lack of fit (p-value = 0.3636) was desirable and was not meaning full. In Fig. 7 observed Three-dimension (3D) surface response for % recovery of Pb(II).

Table 5. ANOVA table

SOURCE	SUM SQUARES	OF D F	MEAN SQUARE	F VALUE	P-VALUE > F	PROB
MODEL	8182.548845	9	909.1720939	25.196785 82	< 0.0001	significant
A-PH	1380.1225	1	1380.1225	38.248700 41	< 0.0001	
B-AS	121.6609	1	121.6609	3.3717088 99	0.0862	
C-VDES	1620.0625	1	1620.0625	44.898395 03	< 0.0001	
AB	134.9004167	1	134.9004167	3.7386287 24	0.0723	
AC	181.6100167	1	181.6100167	5.0331380 86	0.0404	
BC		1	8.520416667	0.2361347 38	0.6340	
A^2	5.124756407	1	5.124756407	0.1420274 45	0.7116	
B^2	53.30250735	1	53.30250735	1.4772251 26	0.2430	
C^2	319.0502281	1	319.0502281	8.8421546 56	0.0095	
RESIDUAL	541.2428993	15	36.08285995			
LACK OF FIT	32.07257925	1	32.07257925	0.8818583 72	0.3636	not significant
PURE ERROR	509.17032	14	36.36930857			
COR TOTAL	8723.791744	24				



**Fig. 7.** Three-dimension (3D) surface response graph for % recovery of Pb(II) ,two interaction of pH and V desorption.

### 3.2 Investigating the effect of important factors

#### 3.2.1 pH effect

pH above 7.0 leads to the formation of metals hydroxide which also decreases the % recoveries of metals. Further optimization studies were carried out at pH 4.0.

#### 3.2.2 Amount of sorbent effect

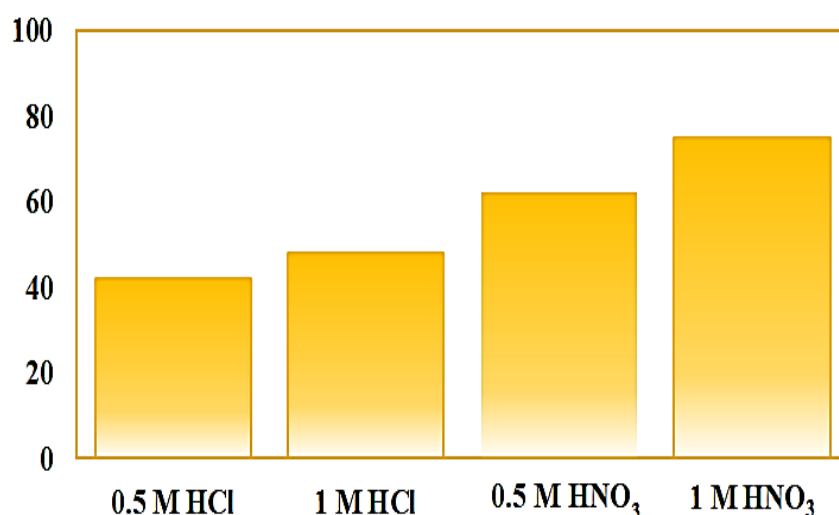
By increasing the amount of nanoparticle, the efficiency of extraction has increased, due to the increase of active sites to absorb the analyte and create strong interactions of adsorbent and analyte. As a result of optimized results, the sample dosage was selected 0.05 g as the optimum amount of adsorbent.

#### 3.2.3 Volume of desorption solvent

Before performing the experiments on the selection of effective factors, the type and appropriate concentration of eluent was studied in one at the time way, and according to the observed results, nitric acid (1 mol/L) was selected as the solvent of the desorption. Finally, the results of optimization showed the optimal amount of 2 mL eluent was selected.

**Table 6.** Effect of solvent type and concentration as desorption solvent on % recovery of Pb using magnetic G-Fe<sub>3</sub>O<sub>4</sub> as an adsorbent (pH: 4, Volume of sample solution: 15 mL, volume of solvent desorption 5 mL, Amount of adsorbent: 0.02 g, stirring rate 750 rpm. N: 3)

Eluent	Recovery % , Pb
0.5 M HCl	42
1 M HCl	48
0.5 M HNO <sub>3</sub>	62
1 M HNO <sub>3</sub>	75



**Fig. 8.** The effect of type of elute solvent on % recovery of Lead.

### 3.3 Interferences

The interfering effect of other ions in pre-concentration of Pb procedure was investigated under optimize conditions. Various concentrations of interfering ions were added to solution containing 20 ng/mL of Pb and under optimize condition pre-concentration procedure were performed and the tolerance amount of each ion was determined. % Recovery as an analytical response is given in Table 7. the obtained result indicated the presence of coexisting of these ions have not any significant effect on % recovery of Pb.

**Table 7.** effect of interferences ions in lead determination by SPE versus % recovery.

Interfering ion	Added salt	Concentration interfering ion( $\mu\text{g/mL}$ )	% recovery
$\text{Na}^+$	NaCl	10000	92.8
$\text{K}^+$	$\text{KNO}_3$	500	93.0
$\text{Ca}^{2+}$	$\text{Ca}(\text{NO}_3)_2$	500	92.0
$\text{Mg}^{2+}$	$\text{Mg}(\text{NO}_3)_2$	1500	94.5
$\text{Fe}^{2+}$	$\text{FeCl}_3$	25	95.7
$\text{Fe}^{3+}$	$\text{FeCl}_2$	12	95.0
$\text{Cl}^-$	$\text{NH}_4\text{Cl}$	21000	93.3

### 3.4 Analytical figures of merit

Calibration curved under optimized condition were plotted. The equation of calibration curve obtained (Absorbance =  $0.0214x(\text{Pb}) + 0.0594$ ). desirable linearity regression in the concentration range of 5 to 50  $\text{ng/mL}^{-1}$  was obtained ( $R^2 = 0.09883$ ). Limit of detection (LOD), relative standard deviation (RSD), correlation coefficient ( $R^2$ ) and pre-concentration factor (PF) for pre-concentration and determination of Pb were investigated and reached values shown in Table 8.

The percent extraction recovery (ER%) for the method was calculated according to the following equation:

$$ER = \frac{n_{a,\text{final}}}{n_{a,\text{initial}}} * 100 = PF * \frac{V_a}{V_s} \quad (4)$$

where  $V_s$  is the volume of the sample solution,  $V_a$  is the volume of the acceptor solution, and  $n_s$ , initial and  $n_a$ , final are the number of moles of the analyte originally present in the sample solution and the number of moles of the analyte finally collected in the acceptor solution, respectively.

Also, the pre-concentration factor (PF) for the target analyst was calculated according to the following equation:

$$PF = \frac{C_{a,\text{final}}}{C_{a,\text{initial}}} = \frac{n_{a,\text{final}}}{n_{a,\text{initial}}} * \frac{V_s}{V_a} \quad (5)$$

where  $C_{a,\text{final}}$  is the final concentration of the analyte in the acceptor solution and  $C_{s,\text{initial}}$  is the initial analyte concentration in the sample (donor) solution. The limit of determination was calculated by using the formula  $\text{LOD} = 3S_d/m$  (where  $S_d$  and  $m$  are the standard deviation of the blank, and slope of a calibration graph, respectively) The detection limits can be decreased by one order of magnitude by increasing the sample volume. The limit of quantification (LOQ) was calculated by using the formula  $\text{LOQ} = 10S_d/m$ . Therelative standard deviation (RSD) obtained for the repetitive determination of 20  $\mu\text{g.L}^{-1}$  of lead was ( $n = 3$ )

**Table 8.** Figures of merit in the determination of lead by proposed SPE method.

Analyte	LOD <sup>a</sup> ng/mL	LOQ <sup>b</sup> ng/mL	R <sup>2</sup> <sup>c</sup>	PF <sup>d</sup>	ER <sup>e</sup>	RSD% <sup>f</sup>	LDR <sup>g</sup> ng/mL
Pb	4.46	14.88	0.9883	7.5	99.9	4.4	5-50

<sup>a</sup> Limit of detection (S/N = 3), (ng/mL). <sup>b</sup> Limit of Quantification  $\mu\text{g/L}$  (S/N = 10), (ng/mL). <sup>c</sup> Coefficient of determination. <sup>d</sup> Pre-concentration factor. <sup>e</sup> Extraction recovery (C = 20 ng /mL). <sup>f</sup> Relative standard deviation (n = 3, C = 20 ng/mL).

<sup>g</sup> Linear dynamic range(ng/mL)

### 3.5 Comparison with other methods

In [Table 9](#), the comparison of results for the proposed method with other method has been shown. Due to the high concentration of salt in the proposed method and the high effect of the matrix interference, the results obtained from this work for measuring the low levels of Lead by GFAAS are desirable.

**Table 9.** Table of Comparison of different methods for determination of lead in various matrixes.

Matrix	Sample Treatment	Determination technique	LOD (ng/mL)	Recovery (%)	References
drinking water and saline waste from oil refinery	minicolumn of polyurethane foam loaded with 4-(2-pyridylazo)-resorcinol (PAR) reagent	FAAS	0.4 ng /mL	102-111%	[19]
Water plants	– minicolumn of polyurethane foam loaded with 2-(2-thiazolylazo)-5-dimethylaminophenol (TAM).	FAAS	2.2 ng/mL	90-108%	[25]
fish and mollusk tissues	Fe <sub>3</sub> O <sub>4</sub> @graphene oxide@polyimide	FAAS	0.25ng /mL	95-106 %	[30]
agricultural products	Fe <sub>3</sub> O <sub>4</sub> @SiO <sub>2</sub> @IIP	FAAS	0.48 ng/mL	97-98%	[31]
Beverage	Fe <sub>3</sub> O <sub>4</sub> @IIP	FAAS	1.7 ng/mL	95-97%	[32]
Soft drink	DLLME	GFAAS	0.072ng/L	95-108%	[33]
Table salt	LLE	FAAS	4.2 ng/mL	98-103%	[34]
natural waters, table salts, tea and urine samples	A membrane filtration procedure	FAAS	31 ng/mL	95%	[35]
Sample with high salt content	SPE	GFAAS	4.4 ng/mL	98-103%	This work

### 3.6 Applications

#### 3.6.1 preparation of real samples

##### 3.6.1.1 Water sample

The proposed method was successfully used for pre-concentration and determination of lead in Persian Gulf water as a real sample with high salinity medium (from Qeshm). The polyethylene bottles filled with the samples were cleaned with detergent, water, diluted nitric acid, and water in sequence. The samples were immediately filtered through a Whatman filter (pore size 0.45  $\mu\text{m}$ ) and were acidified to pH of 2.0 for storage. For the preparation of the real sample for MSPE, pH water sample (250 mL) was adjusted to pH of 4.0 according to optimized experimental conditions and pre-concentration procedure then was applied to this real sample solution.

##### 3.6.1.2 Edible salt sample

For the determination and pre-concentration of Lead ion in edible salt samples as a real and vital sample in food stuff industry 2.5g of Table salt was dissolved in distilled water and diluted to 50 ml with distilled water. The pH of the solution was adjusted to pH 4, the pre-concentration procedure was done Under optimum conditions.

**Table 10.** Results of real sample analysis for determination of lead by proposed method.

Samples	Added, ng/mL	Found, ng/mL	Recovery, %
Persian Golf	0	16.34	-
	25	41.4 $\pm$ 1.20	98.8
	45	63 $\pm$ 3.5	103.68
Edible salt	0	11.54	-
	25	36.73 $\pm$ 2.3	100.0
	45	56 $\pm$ 1.2	99.0

### Conclusion

With the help of Fractional factorial design and Central composite design, the optimization process was performed better and faster. The method proposed for the pre-concentration and extraction of lead in samples with complex salt matrix and the results obtained from the analysis of real samples (edible salt) was less than the maximum allowed in Iran, which is 1000 ng/mL. Considering the direct and indirect consumption (food processing) of salt, the determination of metal contaminants such as lead in amount below 1000 ng/mL is important and necessary. This work is successful in eliminating the complexity of matrix in the sample with high salt content.

### References

1. Asweisi, A. A., and A. S. A. Sammour, Optimization of the Critical Instrumental parameters in Analysis of some Trace Elements in Sodium chloride Matrix by Flame Spectrometry. Moroccan Journal of Chemistry, 2016. 4(4): p. 4-4.
2. Fernandez, F. and D. Manning, Atomic Absorbtion Analyses of Metal Pollutants in Water Using a Heated Graphite Atomizer. Atomic Absorption Newsletter, 1971. 10(3): p. 65-71.



3. Shaw, F. and J.M. Ottaway, Determination of trace amounts of lead in steel and cast iron by atomic-absorption spectrometry with the use of carbon furnace atomisation. *Analyst*, 1974. 99(1176): p. 184-189.
4. Ediger, R., G. Peterson, and J. Kerber, Application of the graphite furnace to saline water analysis. *Atomic Abs. News*, 1974. 13: p. 61-64.
5. Ediger, R.D., Atomic absorption analysis with the graphite furnace using matrix modification. *Atomic Absorption Newsletter*, 1975. 14(5): p. 127-30.
6. Hodges, D., Communication. Observations on the direct determination of lead in complex matrices by carbon furnace atomic-absorption spectrophotometry. *Analyst*, 1977. 102(1210): p. 66-69.
7. Henn, E., Use of molybdenum in eliminating matrix interferences in flameless atomic absorption, in *Flameless Atomic Absorption Analysis: An Update*. 1977, ASTM International.
8. Thompson, K., K. Wagstaff, and K. Wheatstone, Communications. Method for the minimisation of matrix interferences in the determination of lead and cadmium in non-saline waters by atomic-absorption spectrophotometry using electrothermal atomisation. *Analyst*, 1977. 102(1213): p. 310-313.
9. Shaw, F. and J. Ottaway, Determination of trace amounts of lead in steel and cast iron by atomic-absorption spectrometry with the use of carbon furnace atomisation. *Analyst*, 1974. 99(1176): p. 184-189.
10. Barnett, W.B. and E.A. McLaughlin, The atomic absorption determination of antimony, arsenic, bismuth, cadmium, lead, and tin in iron, copper, and zinc alloys with the graphite furnace. *Analytica Chimica Acta*, 1975. 80(2): p. 285-296.
11. Frech, W., Rapid determination of lead in steel by flameless atomic absorption spectrometry. *Analytica Chimica Acta*, 1975. 77: p. 43-52.
12. Arslan, Z. and A. Paulson, Analysis of biogenic carbonates by inductively coupled plasma–mass spectrometry (ICP–MS). Flow injection on-line solid-phase preconcentration for trace element determination in fish otoliths. *Analytical and bioanalytical chemistry*, 2002. 372(7): p. 776-785.
13. Hashemi, O.R., et al., Separation and preconcentration of trace amounts of lead on octadecyl silica membrane disks modified with a new S-containing Schiff's base and its determination by flame atomic absorption spectrometry. *Microchemical journal*, 2001. 69(1): p. 1-6.
14. Okamoto, Y., et al., High preconcentration of ultra-trace metal ions by liquid–liquid extraction using water/oil/water emulsions as liquid surfactant membranes. *Microchemical Journal*, 2000. 65(3): p. 341-346.
15. Manzoori, J.L. and A. Bavili-Tabrizi, Cloud point preconcentration and flame atomic absorption spectrometric determination of Cd and Pb in human hair. *Analytica Chimica Acta*, 2002. 470(2): p. 215-221.
16. Bader, N. R., Determination of Lead by Flame Atomic Absorption Spectrometry after Pre-concentration using Silica Gel Coated with Schiff's Bases. *Moroccan Journal of Chemistry*, 2018. 6(2): p. 6-2.
17. Zaporozhets, O. and L.Y. Tsyukalo, Xylenol orange adsorbed on silica surface as a solid phase reagent for lead determination using diffuse reflectance spectroscopy. *Talanta*, 2002. 58(5): p. 861-868.
18. Sefati, B., M. Masrounia, and Z. Es'haghi, Malathion determination in Rice samples with Graphene oxide reinforced hollow fiber-solid phase microextraction by GC-MASS, *Moroccan Journal of Chemistry*, 2020. 8 ( 3): p. 8-3.
19. Dos Santos, W.L., et al., Multivariate optimization and validation studies in on-line pre-concentration system for lead determination in drinking water and saline waste from oil refinery. *Microchemical Journal*, 2004. 77(2): p. 123-129.
20. Massart, D.L., et al., *Handbook of chemometrics and qualimetrics: Part A*. 1997: Elsevier Science Inc.
21. Neto, B.B., I.S. Scarminio, and R.E. Bruns, *Como fazer experimentos*. Unicamp, Campinas, 2001.

22. dos Santos, W.N., C.M. Santos, and S.L. Ferreira, Application of three-variables Doehlert matrix for optimisation of an on-line pre-concentration system for zinc determination in natural water samples by flame atomic absorption spectrometry. *Microchemical Journal*, 2003. 75(3): p. 211-221.
23. Ferreira, A.C., A.C. Costa, and M. das GA Korn, Preliminary evaluation of the cadmium concentration in seawater of the Salvador City, Brazil. *Microchemical journal*, 2004. 78(1): p. 77-83.
24. Ferreira, S.L., et al., Application of Doehlert matrix and factorial designs in optimization of experimental variables associated with preconcentration and determination of molybdenum in sea-water by inductively coupled plasma optical emission spectrometry. *Journal of Analytical Atomic Spectrometry*, 2002. 17(2): p. 115-120.
25. Ferreira, S.L.C., et al., Use of factorial design and Doehlert matrix for multivariate optimisation of an on-line preconcentration system for lead determination by flame atomic absorption spectrometry. *Analytical and bioanalytical chemistry*, 2003. 375(3): p. 443-449.
26. Bermejo-Barrera, P., A. Moreda-Piñeiro, and A. Bermejo-Barrera, Factorial designs for Cd, Cr, Hg, Pb and Se ultrasound-assisted acid leaching from human hair followed by atomic absorption spectrometric determination. *Journal of Analytical Atomic Spectrometry*, 2000. 15(2): p. 121-130.
27. Tarley, C.R.T., S.L.C. Ferreira, and M.A.Z. Arruda, Use of modified rice husks as a natural solid adsorbent of trace metals: characterisation and development of an on-line preconcentration system for cadmium and lead determination by FAAS. *Microchemical Journal*, 2004. 77(2): p. 163-175.
28. Ferreira, S.L., et al., Application of factorial designs and Doehlert matrix in optimization of experimental variables associated with the preconcentration and determination of vanadium and copper in seawater by inductively coupled plasma optical emission spectrometry. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 2002. 57(12): p. 1939-1950.
29. Marcano, D.C., et al., Improved synthesis of graphene oxide. 2010.
30. Mehdiinia, A., M. Ramezani, and A. Jabbari, Preconcentration and determination of lead ions in fish and mollusk tissues by nanocomposite of Fe<sub>3</sub>O<sub>4</sub>@ graphene oxide@ polyimide as a solid phase extraction sorbent. *Food Chemistry*, 2017.
31. Dahaghin, Z., H.Z. Mousavi, and M. Sajjadi, A novel magnetic ion imprinted polymer as a selective magnetic solid phase for separation of trace lead (II) ions from agricultural products, and optimization using a Box–Behnken design. *Food Chemistry*, 2017.
32. Ebrahimzadeh, H., et al., A magnetic ion-imprinted polymer for lead (II) determination: A study on the adsorption of lead (II) by beverages. *Journal of Food Composition and Analysis*, 2015. 41: p. 74-80.
33. Mandlate, J.S., et al., Determination of cadmium and lead at sub-ppt level in soft drinks: An efficient combination between dispersive liquid-liquid microextraction and graphite furnace atomic absorption spectrometry. *Food chemistry*, 2017. 221: p. 907-912.
34. Amorim, F.A. and S.L. Ferreira, Determination of cadmium and lead in table salt by sequential multi-element flame atomic absorption spectrometry. *Talanta*, 2005. 65(4): p. 960-964.
35. Narin, I. and M. Soylak, Enrichment and determinations of nickel (II), cadmium (II), copper (II), cobalt (II) and lead (II) ions in natural waters, table salts, tea and urine samples as pyrrolydine dithiocarbamate chelates by membrane filtration–flame atomic absorption spectrometry combination. *Analytica Chimica Acta*, 2003. 493(2): p. 205-212.