

Supplementary information

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**Deep eutectic liquid organic salt as a new solvent for carrier-mediated hollow fiber liquid phase microextraction of lead from whole blood followed by electrothermal atomic absorption spectrometry**

Leila Alavi<sup>a</sup>, Shahram Seidi<sup>a,\*</sup>, Ali Jabbari<sup>a</sup>, Tahmineh Baheri<sup>b</sup>

<sup>a</sup>*Department of Analytical Chemistry, Faculty of Chemistry, K.N. Toosi University of Technology, Tehran, Iran*

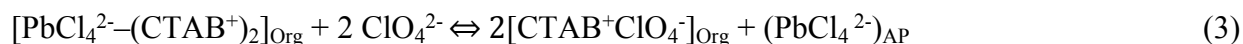
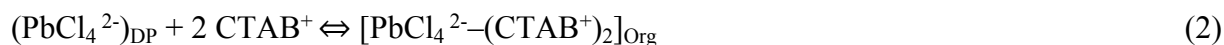
<sup>b</sup>*Research Center of Antinarcotic Police, Tehran, Iran*

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\* Corresponding author at: K.N. Toosi University of Technology, Faculty of Chemistry, Department of Analytical Chemistry, P.O. Box 16315-1618, Tehran, Iran. Tel.: +98(21)23064228; Fax: +98(21)22853650.  
E-mail address: [s.seidi@kntu.ac.ir](mailto:s.seidi@kntu.ac.ir) (S. Seidi).

## Extraction mechanism

First, Pb(II) is converted to  $\text{PbCl}_4^{2-}$  in the hydrochloric acid medium of the donor phase (DP)<sup>1</sup>. Then,  $\text{PbCl}_4^{2-}$  is extracted into the SLM (1-octanol containing CTAB) by ion-exchange with CTAB at the interface of the donor phase and SLM. The ion-pairing compound,  $[\text{PbCl}_4^{2-}(\text{CTAB}^+)_2]_{\text{Org}}$ , diffuses through the SLM, and reaches to the acceptor phase/SLM interface. Finally,  $\text{PbCl}_4^{2-}$  is released into the acceptor phase (AP) by ion exchange with  $\text{ClO}_4^-$  as well as electrostatic and hydrogen binding interactions with the functional groups of the used deep eutectic solvent in the acceptor phase. The eventual extraction mechanism of Pb(II) can be given as the following equilibria:



## Design of experiments using response surface Methodology

In this work, a subset of response surface methodology (RSM) known as central composite design (CCD) was applied for investigation of the effective parameters on the Pb(II) extraction by the proposed method. The aims of the CCD strategy are: (i) to maximize the extraction efficiency; (ii) to determine which variables have higher impacts on the extraction recovery; (iii) to give an insight on the robustness of the method close to the optimum conditions; and (iv) to show the probable interactions between the variables. There are four crucial impressive factors on the extraction recovery of Pb using the three-phase CMHF-LPME procedure consisting of:  $\text{KClO}_4$  concentration (A), CTAB concentration (B), HCl concentration (C) and extraction time (D). Each variable was investigated at three low (-1), centric (0) and high (+1) levels, as shown in Table S1. For analyzing of the results, a non-linear and second-order polynomial equation was

used. Considering all the linear, square and linear by linear interaction items, the quadratic response model can be expressed by Eq. (1) in which  $Y$  is the dependent variable,  $X_i$  is the independent variable,  $b_0$  is the constant coefficient,  $b_i$  is the coefficient of linear effects,  $b_{ij}$  is the coefficient of interaction effects and  $b_{ii}$  is the coefficient of squared effects.

$$Y = b_0 + \sum_{i=1}^4 b_i x_i + \sum_{ij=1(i \neq j)}^6 b_{ij} x_i x_j + \sum_{i=1}^4 b_{ii} x_i^2 \quad (4)$$

The experimental data were analyzed by multiple regression analysis through the generalized least square. The importance of the model and interaction among the variables were approximately estimated by analysis of variance (ANOVA). The software package trial version of Design-Expert 8.0.5 (Minneapolis, USA) was utilized for was used for the design generation and statistical analysis.

**Table S1.** Experimental factors, their levels and analysis of variance (ANOVA) of the quadratic model used for optimization of the CM-HFLPME method

Variable	Parameter	Levels		
		Low (-1)	Center (0)	High (+1)
A	C <sub>KClO<sub>4</sub></sub> (g mL <sup>-1</sup> )	0.03	0.06	0.09
B	C <sub>CTAB</sub> (g mL <sup>-1</sup> )	0.02	0.04	0.06
C	C <sub>HCl</sub> (mol L <sup>-1</sup> )	0.1	0.55	1.0
D	Extraction time (min)	30	75	120

Source	Sum of squares	df	Mean of square	F-value	p-value	Prob > F
Model	0.88	14	0.063	32.35	< 0.0001	Significant
A	0.023	1	0.023	11.73	0.005	
B	0.00728	1	0.00728	3.73	0.0774	
C	0.011	1	0.011	5.41	0.0383	
D	0.00109	1	0.00109	0.56	0.4695	
AB	0.00856	1	0.00856	4.38	0.0582	
AC	0.000225	1	0.000225	0.12	0.7401	
AD	0.00681	1	0.00681	3.49	0.0864	
BC	0.01	1	0.01	5.33	0.0395	
BD	0.00616	1	0.00616	3.16	0.1009	
CD	0.00152	1	0.00152	0.78	0.3947	
A <sup>2</sup>	0.2	1	0.2	103.74	< 0.0001	
B <sup>2</sup>	0.12	1	0.12	59.55	< 0.0001	
C <sup>2</sup>	0.03	1	0.03	15.54	0.002	
D <sup>2</sup>	0.24	1	0.24	123.71	< 0.0001	
Residual	0.023	12	0.00195			
Lack of Fit	0.019	10	0.00188	0.82	0.6643	Not significant
Pure Error	0.00459	2	0.00230			
Cor Total	0.91	26				

df: Degree of freedom

The total number of experimental runs,  $N$ , is obtained using Eq. 5, wherein  $f$  and  $n_c$  are the numbers of factors and central points, respectively. Hence, a total number of 27 experimental runs were done and the results were considered.

$$N = 2^f + 2f + n_c \quad (5)$$

Peak area of Pb as the ETAAS response was considered to evaluate the extraction efficiency under different conditions. ANOVA was used to evaluate the significance of each factor and the interactions. As shown in Table S1, the linear terms of A and C, interaction term of BC and the quadratic terms of all four factors were statistically significant ( $P < 0.05$ ) indicating more importance of these terms on the yield of Pb extraction. Equation 6 presents the quadratic model for Pb as a function of the main factors and the statistically significant interaction and quadratic terms. This model is used to predict response at any point, even those not contained in the design. The synergetic and antagonistic effects of the respective variables were informed by the positive and negative signs before the terms.

$$\text{Peak area} = -0.52 + 38.36 A + 41.13 B + 0.70 C - 0.02 D + 38.54 AB - 0.28 AC - 0.015 AD - 2.83 BC + 0.02 BD - 311.77 A^2 - 531.48 B^2 - 0.54 C^2 + 1.51 D^2 \quad (6)$$

The quality of the polynomial model was expressed by the coefficient of determination ( $R^2$  and adjusted  $R^2$ ). An actual  $R^2$  of 0.9742 and an adjusted  $R^2$  of 0.9441 showed a good relationship between the experimental data and the fitted model, and a high potential for the model to predict the response. From the ANOVA summary, the model was found to be significant, with a p-value less than 0.0001 and a F-value of 32.35. The p-value and F-value of the model lack-of fit (LOF) were obtained as 0.6643 and 0.82, respectively which imply that the LOF was not significant relative to the pure error.

The next step was to find the optimum value of each factor to achieve the maximum response. To evaluate the interactive effects of the variables on the response, the use of three-dimensional (3D) response surface plots of the model is suggested. These plots are the graphical representation of the quadratic regression equation. The curvatures of the plots indicate the interaction between the factors.

## Figures of merit evaluation

To evaluate the performance of the proposed method, linearity, repeatability, preconcentration factor (PF), limit of detection (LOD) and quantification (LOQ) were investigated under the optimized conditions. A matched-matrix method was used to obtain the figures of merit.

The calibration plot was linear within the range of 1.0 to 200 ng mL<sup>-1</sup> with a determination coefficient of 0.9974. The calibration equation for the CMHF-LPME method was  $Y = 0.0052 C$  (ng mL<sup>-1</sup>) + 0.1141. The PF was calculated by dividing the values of the slopes from extraction and direct calibration curves and was found to be 33.

The LOD and LOQ values were calculated both theoretically and practically. The theoretical LOD and LOQ values were defined as  $3S_b/m$  and  $10S_b/m$ , respectively where,  $S_b$  is the standard deviation of the blank and  $m$  is the slope of the extraction calibration curve. The theoretical LOD and LOQ for Pb(II) were found to be 0.1 and 0.4 ng mL<sup>-1</sup>, respectively. To find the practical LOD, the concentration of the spiked analyte into the whole blood sample was decreased to find the lowest detectable signal of the analyte that can reliably differentiate from the background noise, a signal-to-noise ratio of 3 (3S/N). Also, the practical LOQ was considered the lowest concentration that rectilinear relationship of the calibration curve was started. The practical LOD and LOQ values were 0.4 and 1.0 ng mL<sup>-1</sup>, respectively. The method precision, expressed as the relative standard deviation (RSD%) of three independent measurements of Pb(II) at the concentration levels of 5.0 and 20 ng mL<sup>-1</sup>, were calculated and found to be 4.4% and 2.3%, respectively.

To determine the method accuracy, each real sample was spiked at two concentration levels of 5.0 and 20 ng mL<sup>-1</sup> and extraction procedure was carried out to calculate relative recoveries (RR%) using the following equations:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (7)$$

$$\text{Relative error}\% = RR\% - 100 \quad (8)$$

where  $C_{found}$ ,  $C_{real}$ , and  $C_{added}$  are the concentrations ( $\text{ng mL}^{-1}$ ) of analyte after addition a known amount of standard in the real sample, the initial concentration of analyte in the real sample, and the concentration of a known amount of the standard which is spiked in the real sample. RR% values were obtained within the range of 95.8-105.6% which are corresponding to the relative error% values within the range of -6.5 to +8%. The results showed good agreements between the added and the measured analyte amount. As a result, the proposed procedure could be applied successfully for the extraction and preconcentration of trace amounts of Pb(II) in whole blood samples.

## References

1. C. K. Gupta and T. Mukherjee, *Hydrometallurgy in extraction processes*, CRC press, 1990.