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Cloud point extraction for determination of lead in blood samples of children, using different ligands prior to analysis by flame atomic absorption spectrometry: A multivariate study

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ABSTRACT

The phase-separation phenomenon of non-ionic surfactants occurring in aqueous solution was used for the extraction of lead (Pb^{2+}) from digested blood samples after simultaneous complexation with ammonium pyrrolidinedithiocarbamate (APDC) and diethyldithiocarbamate (DDTC) separately. The complexed analyte was quantitatively extracted with octylphenoxypolyethoxyethanol (Triton X-114). The multivariate strategy was applied to estimate the optimum values of experimental factors. Acidic ethanol was added to the surfactant-rich phase prior to its analysis by flame atomic absorption spectrometer (FAAS). The detection limit value of Pb^{2+} for the preconcentration of 10 mL of acid digested blood sample was 1.14 $\mu g\,L^{-1}$. The accuracy of the proposed methods was assessed by analyzing certified reference material (whole blood). Under the optimized conditions of both CPE methods, 10 mL of Pb^{2+} standards (10 $\mu g\,L^{-1}$) complexed with APDC and DDTC, permitted the enhancement factors of 56 and 42, respectively. The proposed method was used for determination of Pb^{2+} in blood samples of children with kidney disorders and healthy controls.

1. Introduction

Lead, a naturally occurring element, has been an important metal in human societies over many thousands of years. The low melting point, flexibility and resistance to corrosion significantly enhance its strength and durability, accounting for its widespread use. However, Pb^{2+} is toxic to humans affecting the hemopoietic, nervous, cardiovascular, reproductive systems and the urinary tract [1–5]. Up to now, no health benefits to humans have been reported for Pb^{2+} or its compounds. It is found almost everywhere in the inert environment and in all biological systems [6].

Being a hazardous element, trace and ultra trace determinations of Pb^{2+} in biological samples have become of increasing interest [7]. Several analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma optical emission spectrometry and inductively coupled plasma mass spectrometry are available for the determination of trace metals with sufficient sensitivity for most of applications [8]. The analysis of trace element concentrations in biological media, especially biological fluids, might be considered a difficult analytical task, mostly due to the complexity of the matrix and the low concentration of these elements, which requires sensitive instrumental techniques and often a preconcentration step [9–11]. Pre-concentration can solve these problems and allows easy determination of the trace elements by less sensitive, but more accessible instrumentation such as flame atomic absorption spectrometry (FAAS) [12]. Various techniques including liquid–liquid extraction (LLE), coprecipitation, ion exchange, cloud point extraction (CPE), and solid phase extraction (SPE) have been proposed for preconcentration of trace elements [13–16]. Among these, liquid–liquid extraction has been used for decades. The traditional liquid–liquid extraction and separation methods are usually time-consuming and require quite large volumes of high purity solvents. Of additional concern is the disposal of the solvents used, which creates a severe environmental crisis. In this sense, cloud point extraction (CPE) is an interesting and efficient option as it reduces the use and exposure to solvents, the disposal costs and the extraction time [7]. CPE is based on the phase behavior of non-ionic and zwitter ionic surfactants in aqueous solutions, which exhibit phase separation after an increase in temperature or the addition of a chelating agent [17].

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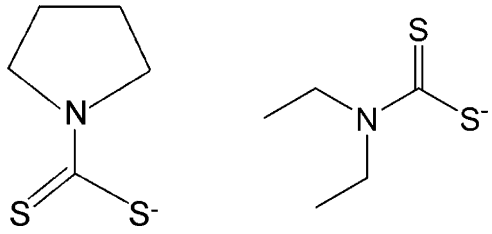

Fig. 1. Structure of ligand: (a) PDC and (b) DDTC.

Factors that are important in the formation of the CPE include surfactant concentration, temperature and the choice of a compatible pH buffer and/or the maintenance of the needed ionic strength for the aqueous phase. The last two factors could have a critical effect in the formation of the metal chelate and on the stability of the chelate. Once the aqueous system of the surfactant reaches the critical micelle concentration (CMC) under a warmer temperature condition, the well-organized phase of CM can be separated from the aqueous phase on cooling. This is often referred to as the cloud point (CP). The process of the separation of the CM phase is an effective means of taking the metal chelates from the aqueous phase. CPE is a powerful approach comparable with other established methods. Preconcentration procedures based on CPE have been extensively applied to preconcentrate metallic ions [18]. The use of CPE has presented several advantages, such as excellent concentration factors, lower cost, higher safety and simplicity, and it does not need to handle a great volume of organic solvent that is generally toxic [19].

Most procedures proposed by analytical chemists are optimized by development of univariate methodology (one variable at each time). This simple optimization methodology is supposed to have an easier interpretation. However, this process requires a large number of experiments and expends a great amount of reagent, time and is only valid if the variables to be optimized do not interact [20,21]. Procedures involving optimization by multivariate techniques have been increasingly used as they are faster, more economical and effective, and allow more than one variable to be optimized simultaneously [22,23]. Among the different groups of designs, Plackett–Burman designs (PBDs), allow us to discover the most significant variables for a certain system with only few experiments [24]. The interest on the use of such optimization method and PBDs has been applied to optimize some sample pre-treatments [25]. This paper reports the results obtained in a study of the CP simultaneous preconcentration of Pb^{2+} after the formation of complexes with salts of pyrrolidinedithiocarbamate (PDC) and diethyldithiocarbamate (DDTC) (Fig. 1a and 1b). Nonionic surfactant octylphenoxypolyethoxyethanol (Triton X-114) was used as extractant and then analyzed by FAAS. The proposed method is also applied to the determination of Pb^{2+} in blood samples and certified reference material (CRM), Clincheck control-lyophilized human whole blood (Recipe, Munich, Germany), to check the accuracy of methodology.

Lead constitutes a ubiquitous health hazard in industrialized countries, and particularly threatens young children by adversely affecting neuropsychological development [26]. Children in less developed countries are both more vulnerable to neurodevelopmental delays (because of endemic disease, caloric and micronutrient deficiencies, and limited resources for early intervention) and less likely to be examined for toxic exposures, including Pb^{2+} [27,28]. However, there is accumulating evidence that Pb^{2+} exposures in urban areas of developing nations are among the highest in the world [29,30]. Longstanding occupational exposure to Pb^{2+} may cause chronic nephrotoxic effects consisting mainly in a decline of the glomerular filtration rate possibly leading to end stage renal insufficiency [31]. Human exposure to Pb^{2+} is mainly from food and environment. Previous studies have indicated that asymptomatic Pb^{2+} exposures can result in chronic toxicity manifestations, such as hypertension, kidney impairment, and cognitive disturbances [32]. Among children, lead has been associated with reduced hematocrit volume, lower intelligence (>10 $\mu g\,dL^{-1}$), mild behavioral disorders (range of 10–20 $\mu g\,dL^{-1}$), reduced neural conduction velocity (range of 13–97 $\mu g\,dL^{-1}$) and peripheral neuropathy (range of 60–136 $\mu g\,dL^{-1}$) [33].

2. Experimental

2.1. Reagents

Ultrapure water obtained from an ELGA labwater system (Bucks, UK) was used throughout the study. Concentrated nitric acid (65%) and hydrogen peroxide (30%) were obtained from Merck (Darmstadt, Germany). Working standard solutions of Pb^{2+} were prepared immediately before their use, by stepwise dilution of certified standard solution (1000 mg L^{-1}) Fluka Kamica (Buchs, Switzerland), with 0.2 mol L^{-1} HNO_3. The ammonium pyrrolidinedithiocarbamate and diethyldithiocarbamate were obtained from (Fluka); both reagents were prepared by dissolving appropriate amount of these reagents in 10 mL ethanol (Merck) and diluting to 100 mL with 0.01 mol L^{-1} acetic acid. The nonionic surfactant Triton X-114 was obtained from Sigma (St. Louis, MO, USA) and was used without further purification. A 2% (v/v) nonionic surfactant solution was prepared by dissolving 2 mL of Triton X-114 (Merck) in 100 mL distilled water. A stock buffer solution was prepared by dissolving appropriate amounts of acetic acid and its sodium salt in ultrapure water, and solutions were prepared with 0.1 mol L^{-1} HNO_3/NaOH. All materials and glassware used for Pb^{2+} analyses were kept in 10% HNO_3 for at least 24 h and subsequently rinsed four times in ultrapure water.

2.2. Apparatus

A PEL domestic microwave oven (Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for digestion of blood samples. A pH meter (Ecoscan Ion 6, Malaysia) was employed for pH adjustments. Centrifugation was carried out using a WIROWKA Laboratoryjna type WE-1, nr-6933 centrifuge (speed range 0–6000 rpm, timer 0–60 min, 220/50 Hz, Mechanika Phecyzyjna, Poland). A Perkin-Elmer Model A Analyst 700 (Norwalk, CT) flame atomic absorption spectrophotometer was used. The hollow cathode lamp of Pb was run under the conditions suggested by the manufacturer. A single element hollow cathode lamp was operated at 7.5 mA and spectral bandwidth of 0.7 nm. The analytical wavelength was set at 283.3 nm. The acetylene flow rate and the burner height were adjusted in order to obtain the maximum absorbance signal.

2.3. Sampling

Blood sampling was carried out at civil hospital in Karachi. Great care was taken with the washing of the children's hands first with soap and tap water, rinsing with distilled water, then wiping with alcohol. Blood was taken by venepunctures after the application of EMLA (Eutectic mixture of local anesthetics) local anaesthetic cream to reduce the pain produced, collected into metal-free safety vacutainer blood collecting tubes (Becton Dickinson, Rutherford, USA) containing >1.5 mg K_2EDTA L^{-1}.

Table 1
Variables and levels used in the factorial design for APDC (ammonium pyrrolidinedithiocarbamate) and DDTC (diethyldithiocarbamate).

Variables	Symbol	Low (−)	High (+)
Surfactant (%)	S	1	2
Complexing agent (% APDC)	L_1	0.05	0.3
pH (APDC)	P_1	1	4
Complexing agent (% DDTC)	L_2	0.02	0.1
pH (DDTC)	P_2	2	8
Incubation time (min)	I	5	20
Temperature (°C)	T	30	60

2.4. Sample preparation

Triplicate sample of whole blood (0.2 mL) were directly taken into polytetrafluoroethylene (PTFE) flasks. Three milliliters of a freshly prepared mixture of concentrated HNO_3–H_2O_2 (2:1, v/v) was added and kept for 10 min at room temperature. This was then heated following a 1-stage digestion programmed at 80% of total power (900 W), 1–2 min for blood samples. The resulting digested semidried mass was diluted up to 10 mL with 0.1 mol L^{-1} concentrated HNO_3. A blank extraction (without sample) was carried out through the complete procedure. The concentrations were obtained directly from calibration graphs after correction of the absorbance for the signal obtained from an appropriate reagent blank. To establish the validity of our results we used the certified samples of whole blood. All experiments were conducted at room temperature (30 °C).

2.5. Cloud point extraction

For preconcentration method, aliquots of 10 mL standard solutions containing Pb^{2+} in the range of 5.0–20.0 μg L^{-1}, six replicate samples of 10 mL of acid digested CRMs, and duplicates of each blood samples were transferred into centrifuge tubes with glass stopper (25 mL in capacity). For CPE, adding 1.0 mL of ligand {(0.05–0.3)APDC/(0.02–0.1%)DDTC}, 1.0 mL of (0.2–1%, v/v) Triton X-114; and adjusting the pH range (1–4) and (2–8) for APDC and DDTC, respectively, by the addition of 0.1 mol L^{-1} HCl/NaOH solution in acetate buffer. The tubes were kept in thermo-stated water bath at 30–60 °C for 5–20 min. After different time intervals separation of the two phases was achieved by centrifuging at 3500 rpm for 10 min. The contents of tubes were cooled in an ice-bath, the surfactant-rich phase became viscous, and the upper aqueous phase was decanted. To decrease the viscosity of extracts, 0.5 mL acidic ethyl alcohol (0.1 mol L^{-1} HNO_3) was added, and the extracted solutions were introduced into the flame by conventional aspiration. The duplicate blanks of both complexing reagents were prepared simultaneously without addition of samples and standards.

2.6. Experimental design

2.6.1. Plackett–Burman design

The Plackett–Burman design (PBD) was used as a screening approach with the aim of establishing the significant factors that influence the CPE of Pb^{2+} in aqueous extracts of digested blood samples using two complexing reagents. To evaluate the optimum levels of factors for two complexing reagent separately at two levels Plackett–Burman designs with only 16 experiments were described instead of the $2^5 = 32$, required for full factorial designs. The lower (−) and high (+) levels are specified in Table 1, while optimization by Plackett–Burman matrix is shown in Table 2. The resulting values for experiment (1–16) being of six replicates of each. The experimental data were evaluated with the help of Minitab 13.2 (Minitab Inc., State College, PA, USA) and STATISTICA computer program 2007. The application of this experimental design reduced the development time of the methods and provided less ambiguous extraction conditions, hence facilitating data interpretation.

2.6.2 Central 2^3+ star orthogonal composite design

To screen out the variables that have not significant effects on the recovery of analyte understudy, the remaining three factors in both cases were optimized to provide the maximum Pb^{2+} recovery. A central 2^3+ star orthogonal composite design with six degrees of freedom and involving 16 experiments was performed to optimize the variables, i.e., APDC (L_1) and DDTC (L_2) with pH (P) and heating temperature (T) for % recovery of Pb^{2+} in understudy samples (Tables 3a and 3b). The factors that were shown to be insignificant by the PBD were fixed at convenient values as incubation time of 10 min and surfactant concentration of 1%.

3. Results and discussion

3.1. Optimization of experimental variables

Optimization of experimental variables are shown in Table 2, and visualized by using a standardized effect ($p = 95.0\%$) in Pareto charts (Fig. 2a and 2b), respectively. It was observed that ligands (L_1 and L_2), pH and temperature have significant effects on % recovery of Pb^{2+}. The inference tests showed that the results produced at a

Table 2
Design matrix and the results of % extraction ($n = 5$), APDC and DDTC.

Experiment No.	L_1/L_2	P	S	T	I	% Recovery	
						APDC	DDTC
1	+	−	−	−	+	94 ± 3	96 ± 4
2	+	+	−	−	−	41 ± 1	22 ± 3
3	+	+	+	−	−	48 ± 2	22 ± 4
4	+	+	+	+	−	31 ± 2	17 ± 1
5	−	+	+	+	+	23 ± 4	19 ± 4
6	+	−	+	+	+	46 ± 3	35 ± 3
7	−	+	−	+	+	42 ± 4	18 ± 2
8	+	−	+	−	+	71 ± 2	68 ± 5
9	+	+	−	+	−	42 ± 2	32 ± 3
10	−	+	+	−	+	41 ± 1	26 ± 6
11	−	−	+	+	−	42 ± 5	36 ± 2
12	+	−	−	+	+	60 ± 3	58 ± 1
13	−	+	−	−	+	43 ± 4	28 ± 5
14	−	−	+	−	−	58 ± 4	38 ± 6
15	−	−	−	+	−	47 ± 2	36 ± 4
16	−	−	−	−	−	52 ± 4	37 ± 3

Table 3a
Central 2^3+ star orthogonal composite design ($n = 16$) for the set of (L_1), (P_1) and (T) in APDC.

Experiments	A (L_1)	B (P_1)	C (T)	% recovery
1	L^o	P^o	T^o	98.0 ± 2.2
2	+	–	–	53.0 ± 3.4
3	–	+	–	28.0 ± 2.2
4	+	+	–	39.0 ± 3.2
5	–	–	+	31.0 ± 4.2
6	+	–	+	42.0 ± 1.6
7	–	+	+	12.0 ± 2.3
8	+	+	+	41.0 ± 5.1
9	–	+	–	24.0 ± 3.2
10	$-L^1$	P^o	T^o	11.0 ± 2.9
11	$+L^2$	P^o	T^o	51.0 ± 1.8
12	L^o	$-P^1$	T^o	2.0 ± 3.4
13	L^o	$+P^2$	T^o	48.0 ± 3.3
14	L^o	P^o	T^1	35.0 ± 1.8
15	L^o	P^o	$+T^2$	25.0 ± 6.7
16	L^o	P^o	T^o	97.0 ± 2.9

$L^1 = -0.052\%$, $L^2 = 0.452\%$, $L^o = 0.2\%$, $P^1 = 0.023$, $P^2 = 5.023$, $P^o = 2.5$, $T^1 = 19.8\,^\circ$C, $T^2 = 70.2\,^\circ$C, $T^o = 45.0\,^\circ$C.

Table 3b
Central 2^3+ star orthogonal composite design ($n = 16$) for the set of (L_2), (P_2) and (T) in DDTC.

Experiments	A (L_2)	B (P_2)	C (T)	% recovery
1	L^o	P^o	T^o	97.2 ± 3.5
2	–	–	–	26.0 ± 2.1
3	+	–	–	34.0 ± 3.2
4	–	+	–	64.0 ± 2.2
5	+	+	–	60.0 ± 3.6
6	–	–	+	45.0 ± 3.8
7	+	–	+	32.0 ± 2.9
8	–	+	+	54.0 ± 4.6
9	+	+	+	58.0 ± 1.5
10	$-L^1$	P^o	T^o	12.0 ± 2.3
11	$+L^2$	P^o	T^o	89.0 ± 3.7
12	L^o	$-P^1$	T^o	6.0 ± 2.8
13	L^o	$+P^2$	T^o	22.0 ± 6.5
14	L^o	P^o	T^1	65.0 ± 6.5
15	L^o	P^o	$+T^2$	58.0 ± 5.2
16	L^o	P^o	T^o	97.4 ± 2.7

$L^1 = 0.01\%$, $L^2 = 0.13\%$, $L^o = 0.05\%$, $P^1 = 0.045$, $P^2 = 10.0$, $P^o = 4.5$, $T^1 = 19.8\,^\circ$C, $T^2 = 70.2\,^\circ$C, $T^o = 45.0\,^\circ$C.

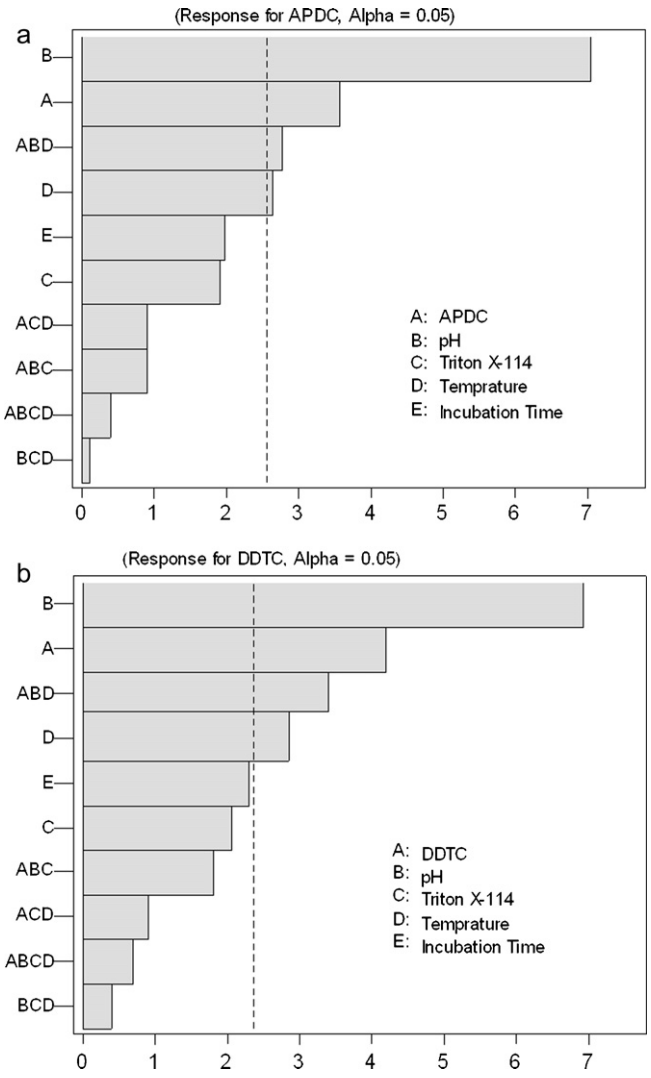

Fig. 2. Pareto chart of the standardized effects (a) For L_1 (APDC) and (b) for L_2 (DDTC).

minimum t-value (95.0% confidence interval) were 2.6 and 2.3 for L_1 and L_2, respectively.

3.2. Estimated effects of variables

The significant effects of ligands separately with other parameters on Pb^{2+} recoveries are shown in Table 2. The resulting values (1–16) are being the % recoveries of Pb^{2+} in 10 mL of working standard solution containing 10 μg L^{-1} of Pb^{2+} standard (average values of six replicates) complexing with APDC (L_1) and DDTC (L_2). From the results of the PBD (Table 2), it is clearly observed for Pb^{2+} recoveries, the most significant effects were found for ligands (APDC and DDTC), pH and temperature. ANOVA test was used in order to identify the effect of individual factors and their interactions. The recovery was defined as the dependent variable and the five selected factors as independent variables in this test.

In experiment 1, 94% and 96% Pb^{2+} recoveries were observed at maximum (+) level of L_1 and L_2, respectively, while other factors, i.e., pH, surfactant, and temperature were at their lower (–) level (Table 2). It can be seen in experiment 2, that the pH are at higher level while other three parameters are at lower level, the recoveries of Pb^{2+} were 41% and 22%, which indicates that pH has a significant effect on complexation of Pb^{2+} with L_1 and L_2. Pyrrolidine dithiocarbamic acid and diethyl dithiocarbamic acid are weak acids with dissociation constants, [$K_a = 10^{-3.2}$ ($pK_a = 3.2$)] and [$K_a = 4.0 \times 10^{-4}$ ($pK_a = 4$)], respectively. As the solubility of the complexes are pH-dependent. For pH values greater than pK_a solubility will be minimum while for pH values smaller than pK_a solubility will be increased proportionally with the acidity of the medium.

The influence of incubation time was not significant, while high temperature showed negative effect on Pb^{2+} recovery. Experiment 12 indicates this negative effect, where at higher temperature the Pb^{2+} recoveries were 60% and 58% for L_1 and L_2, respectively. The significant effects of understudy variable on the % recovery of Pb^{2+} was found in decreasing order of understudy variable, pH > T > L > S > I (Fig. 2a and 2b). Three order interactions between variables pH, T and L presented significant effects on the % recovery of Pb^{2+} ($p < 0.01$). The selected levels of incubation time and surfactant concentration for the proposed CPE procedure showed no significant effect ($p = 0.315$).

3.3. Optimization by central composite designs

Having screened out the variables that have not any significant effect on the response, the remaining three factors were optimized to provide the maximum metal recovery. The incubation time and surfactant concentrations are insignificant for all cases, so the less significant variables (after PBD) were fixed at convenient values,

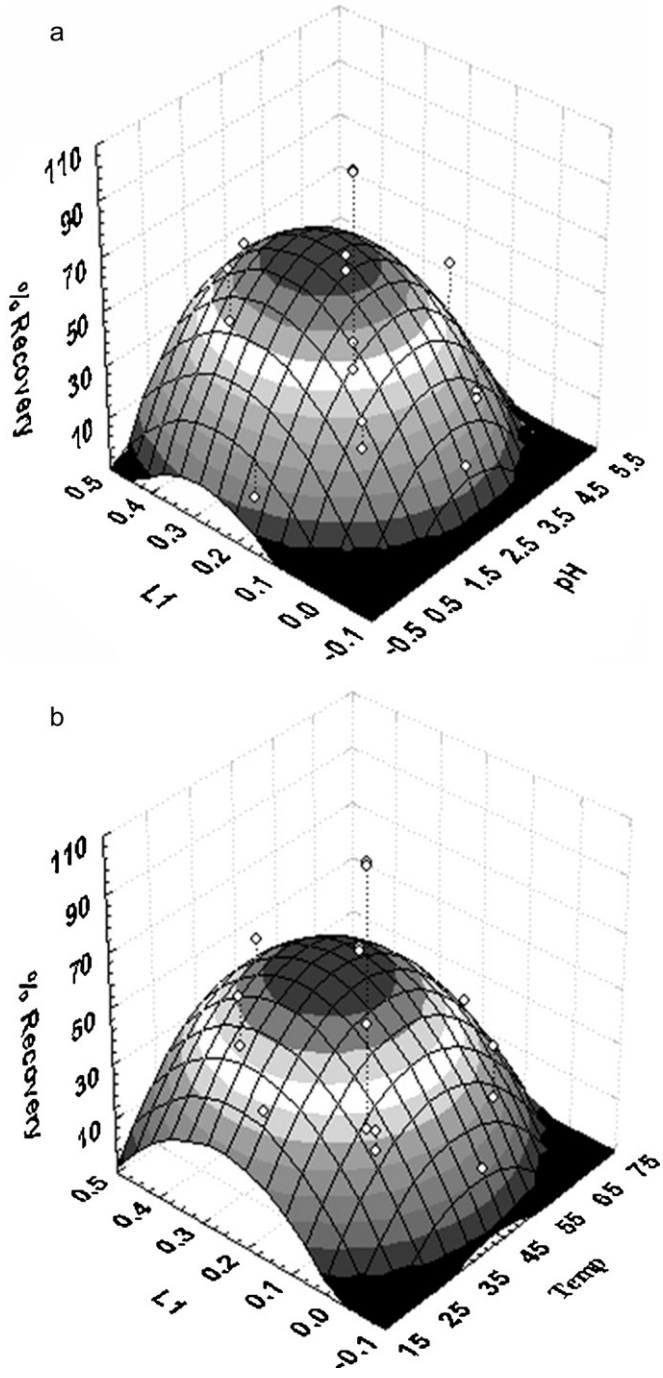

Fig. 3. Three dimension (3D) surface response for % recovery of Pb^{2+} by CPE: (a) interaction b/w %APDC and pH, and (b) interaction b/w %APDC and temperature (°C).

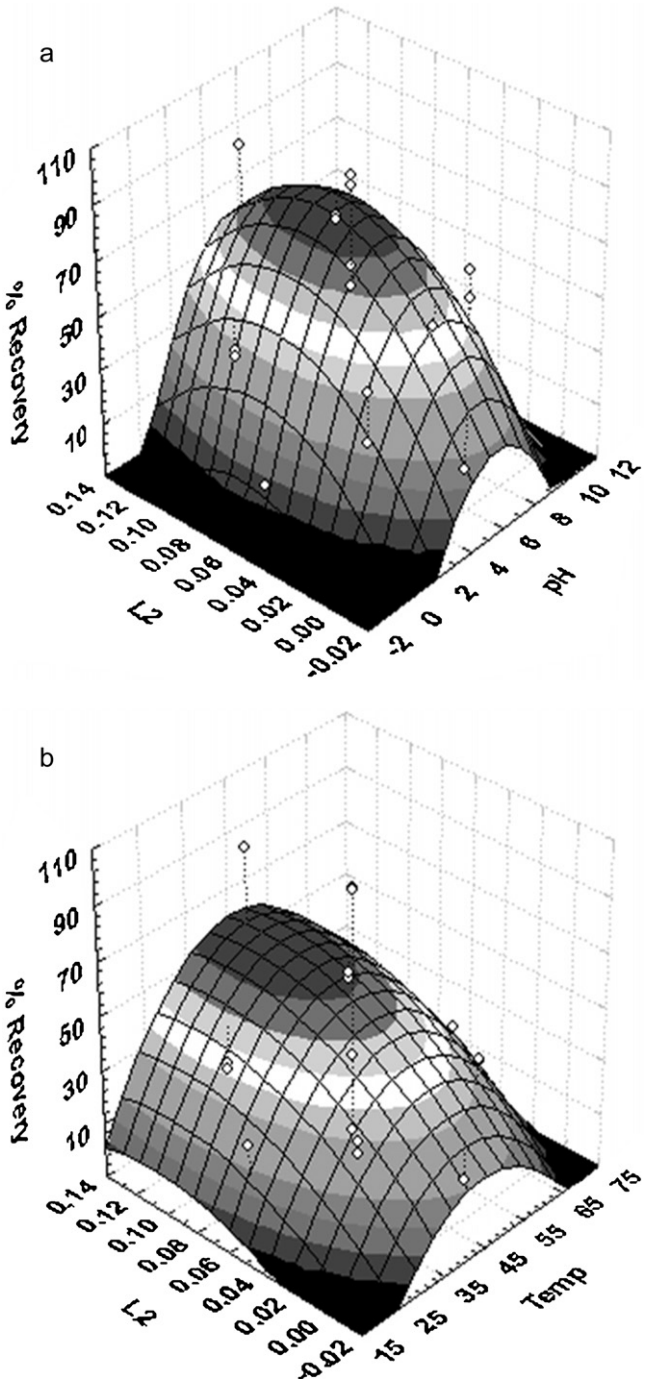

Fig. 4. Three dimension (3D) surface response for % recovery of Pb^{2+} by CPE: (a) interaction b/w %DDTC and pH, and (b) interaction b/w %DDTC and temperature (°C).

i.e., incubation time of 10 min, surfactant concentration of 0.2% for all cases. Tables 3a and 3b show the central composite designs for both ligands separately with % recoveries obtained for Pb^{2+}. The study of estimated response surfaces for variables, [L_1 and L_2]/[P] and [L_1 and L_2]/[T], showed their optimum values for recovery of Pb^{2+} as shown in Figs. 3 and 4(a and b). The comments for each ligand are the following.

3.3.1. Effect of APDC

Concentration of APDC [L_1] has significant effect on % recovery of Pb^{2+} from standard and real samples (Table 3a). It was observed that at optimized levels of all three variables (L^o, P^o and T^o), recovery of Pb^{2+} was 98% (experiments 1 and 16). It can be seen in experiments 15 and 13, that the extraction efficiency of Pb^{2+} was decreased at higher temperature (70 °C) and high pH value (pH 5.02), respectively. After plotting three dimension (3D) response surfaces for $L_1 - P$ and $L_1 - T$, calculation obtained through quadratic equation indicated that maximum Pb^{2+} recovery was obtained at optimum values of complexing agent L_1 (0.25%), temperature (46.08 °C) and pH (2.44) as shown in Fig. 3a and b.

3.3.2. Effect of DDTC

Concentration of DDTC [L_2] with other two variables pH [P] and temperature [T] had significant effect on % recovery of Pb^{2+} from

Table 4
Effect of interfering ions on the recovery of Pb^{2+} in both ligands.

Ions	Tolerance limit ($mg\,L^{-1}$)	% recovery[a]	
		L_1	L_2
Na^+	10,000	98 ± 1	98 ± 2
K^+	5000	100 ± 2	99 ± 2
Ca^{2+}	5000	99 ± 2	95 ± 1
Fe^{3+}	1000	95 ± 3	96 ± 3
Mg^{2+}	1000	98 ± 1	95 ± 3
Co^{2+}	100	96 ± 2	97 ± 2
Cd^{2+}	100	96 ± 1	99 ± 2
Ni^{2+}	100	94 ± 3	96 ± 1
Cu^{2+}	100	95 ± 3	96 ± 2
Mn^{2+}	100	97 ± 2	95 ± 1
Cr^{3+}	50	98 ± 1	97 ± 2
Cl^-	10,000	96 ± 3	97 ± 1
F^-	100	97 ± 1	95 ± 3
NO^{3-}	100	95 ± 2	96 ± 1
SO_4^{2-}	1000	96 ± 1	95 ± 1
PO_3^{4-}	1000	98 ± 1	95 ± 1

[a] Mean ± standard deviation ($x \mp s$).

standard and real samples. It was observed that at optimized levels of all three variables (L^o, P^o and T^o); recovery of Pb^{2+} was found in range of 97.2–97.4% (experiments 1 and 16). Three dimension (3D) response surface plots for each pair of variables, $[L_2 - P]$ and $[L_2 - T]$ were made and quadratic equation was used for further calculation. Fig. 4a and b shows that at optimum values of L_2 (0.07%), pH (4.52) and T (45.4), maximum recovery of Pb^{2+} was observed.

3.4. Effect of interference

Experiments were performed to discover the degree to which the proposed method is affected by the presence of elements known to interfere with the formation of Pb^{2+} complexes and its determination by FAAS. For these studies, different amounts of foreign ions were added to standard solution of 10 $\mu g\,L^{-1}$ of Pb^{2+} and the recommended procedure was followed. The recoveries of Pb^{2+} ions in these studies were higher than 95%. The tolerable limit was defined as the largest amount of foreign ions that produced an error not exceeding 5% in the determination of Pb^{2+}. The recoveries of Pb^{2+} were almost quantitative in the presence of all interfering ions in both experiments, shown in Table 4. This proved the applicability of the proposed methods to determine Pb^{2+} in blood samples.

3.5. Analytical figure of merit

The calibration graph for preconcentration of Pb^{2+} with L_1 and L_2 were linear with a correlation coefficient of 0.990–0.981, respectively at the range of 5–20 $\mu g\,L^{-1}$. Regression equation for Pb^{2+}-APDC obtained as Abs = 7.6683 (Pb^{2+} $\mu g\,L^{-1}$) − 0.0113, and Pb^{2+}-DDTC as Abs = 5.7708 (Pb^{2+} $\mu g\,L^{-1}$) + 0.155. In order to determine the enhancement factor (EF), analytical curves were prepared without CPE. The calibration equation obtained was Abs = 0.1362 (Pb^{2+} $\mu g\,L^{-1}$) + 0.0202 (R^2 = 0.999). The experimental enhancement factors calculated as the ratio of slopes of the calibration graphs with and without pre-concentration were 56 and 42 for Pb^{2+} complexed with L_1 and L_2, respectively. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as under 3 and 10s/m, respectively, where s is the standard deviation of ten measurements of the blank and m is slope of the calibration graph. The LOD and LOQ were calculated as 1.14 and 3.8 $\mu g\,L^{-1}$, respectively. The analytical characteristics, precision of methods, expressed as the % relative standard deviation (% RSD) of a minimum 6 independent analyses of certified reference material (CRM), after CPE of Pb^{2+} using L_1 and L_2 was found to be 6.88% and 8.74%, respectively.

Table 5
Determination of Pb^{2+} in certified sample, $\mu g\,L^{-1}$ (CRM) (n = 10).

	$x \mp s$	% recovery[b]	$t_{Experimental}$	Certified value
Without preconcentration	103.0 ± 16.8[a] (16.25)[c]	98.1	0.342	105.0 ± 24.1
L_1	103.2 ± 7.1 (6.88)	98.5	0.234	
L_2	102.1 ± 8.9 (8.74)	97.1	0.087	

[a] Mean ± standard deviation (n = 6), $t_{Critical}$ = 2.57
[b] XXX % recovery = [experimental value]/[certified value] × 100
[c] Values in parentheses () % of relative standard deviation.

Table 6
Comparison with other previously reported methods.

Sample	Ligand	Surfactant	EF	LOD	Technique	References
Human saliva	–	PONPE 7.5	67	–	FAAS	[36]
Water samples	Pyrogallol	Triton X-114	72	0.4 $\mu g\,L^{-1}$	FAAS	[37]
Water samples	5-Br-PADAP	Triton X-114	50	0.08 $\mu g\,L^{-1}$	GFAAS	[38]
Certified biological samples	DDTP	Triton X-114	18	40 $ng\,g^{-1}$	GFAAS	[39]
Biological/water samples	DDTP	Triton X-114	18	40 $ng\,g^{-1}$	GFAAS	[40]
Urine samples	DDTP	Triton X-114	16	0.04 $\mu g\,L^{-1}$	GFAAS	[41]
Biological/water samples	PMBP	Triton X-114	110	1.49 $\mu g\,L^{-1}$	FAAS	[42]
Water samples	BCB	Triton X-114	25	7.5 $\mu g\,L^{-1}$	FAAS	[43]
Mineral water	PAN	Triton X-114	21	0.43 $\mu g\,L^{-1}$	TS-FF-AAS	[44]
Biological/environmental samples	AMTD	Triton X-114	29	1.6 $\mu g\,L^{-1}$	FAAS	[45]
Environmental samples	BIES	Triton X-114	39	2.8 $\mu g\,L^{-1}$	FAAS	[46]
Water/food samples	1-PTSC	Triton X-114	25	3.42 $\mu g\,L^{-1}$	FAAS	[47]
Water samples	–	PONPE 7.5	150	0.09 $\mu g\,L^{-1}$	USN-ICP-OES	[48]
Food samples	Me-BTABr	Triton X-114	17	0.7 $\mu g\,L^{-1}$	FAAS	[49]
Blood samples	APDC	Triton X-114	56	1.14 $\mu g\,L^{-1}$	FAAS	Present work
	DDTC		42			

Key: 2-amino-5-mercapto-1,3,5-thiadiazole (AMTD), brillant cresyl blue (BCB), bis((1H-benzo [d] imidazol-2yl)ethyl) sulfane (BIES), 2-(5-bromo-2-pyridylazo)-5-(diethylamino)-phenol (5-Br-PADAP) O,O-diethyldithiophosphate (DDTP), Graphite furnace atomic absorption spectrophotometer (GFAAS), 2-[2′-(6-methyl-benzothiazolylazo)]-4-bromophenol (Me-BTABr) 1-(2-pyridylazo)-2-naphtol (PAN), 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP), polyethyleneglycolmono-p-nonylphenylether (PONPE 7.5), 1-phenylthiosemicarbazide (1-PTSC).

Table 7
The concentration of Pb^{2+} in blood samples of renal dysfunction and healthy control subjects ($\mu g\,L^{-1}$).

Subjects	Boys	Girls
Healthy controls ($n = 65$)	63.0 ± 26.2[a]	6.18 ± 2.49
With renal dysfunction ($n = 35$)	286 ± 81.9	271 ± 78.3

[a] Mean ± standard deviation ($x \mp s$).

Extraction recovery (ER) was calculated according to the following given equation:

$$\mathrm{ER} = \frac{m_{\mathrm{surfactant}}}{m_{\mathrm{aq}}} = \frac{C_{\mathrm{surfactant}} \times V_{\mathrm{surfactant}}}{C_{\mathrm{aq}} \times V_{\mathrm{aq}}} \times 100$$

where $m_{\mathrm{surfactant}}$ and m_{aq} are the analyte masses in final surfactant phase and initial concentration in the sample solution, $C_{\mathrm{surfactant}}$ and C_{aq} are the analyte concentrations in surfactant phase and in the aqueous phase, respectively. $V_{\mathrm{surfactant}}$ and V_{aq} are the concerned volumes of the phases [34]. An extraction recovery of 99.9% was achieved at the optimum experimental conditions.

The consumptive index (CIn) can be defined as:

$$\mathrm{CIn} = \frac{V_{\mathrm{s}}}{\mathrm{EF}}$$

where V_{s} is the sample volume (in milliliters) used to achieve the EF value [35]. The CIn obtained for the proposed method were 0.18 and 0.24 for L_1 and L_2 respectively. High EFs were obtained with reduced sample volumes, yielding low CIn values. Thus, CIn reveals the efficiency of sample utilization, and it is useful tool for selecting a preconcentration method when sample amount is limited, such as blood analysis [35].

The paired *t*-test was applied to compare the results obtained by both CPE methods and showed that experimental values are lower than the t_{critical} (2.57) at a confidence interval of 95% ($p = 0.05$), which indicated a non-significant difference in obtained and certified value of Pb^{2+} (Table 5). Finally, a comparison of the proposed method with others reported in the literature for Pb^{2+} determination after CPE is shown in Table 6. The high sensitivity and low detection limits of the present CPE method suggests the method is efficient and sensitive for determination of very low concentrations of the Pb^{2+} in various complex samples.

3.6. Applications

The presented procedure was applied for Pb^{2+} determination in blood samples of children with different kidney disorders, related to healthy children of same age group and residential areas. The concentration of Pb^{2+} in the blood samples of children of both genders is shown in Table 7. The level of Pb^{2+} in diseased children was significantly higher than those found in referent children. The concentration of Pb^{2+} in blood samples of children have different kidney disorders at 95% confidence interval was [CI: 177, 344] $\mu g\,L^{-1}$, while for referents it was [CI: 36.9, 79.9] $\mu g\,L^{-1}$; same trend was found in female children ($p < 0.01$). The tests for nephrotoxicity of each understudy children have been screened by biochemical tests; all have signs of clinical renal disease (elevated serum creatinine levels). While the healthy control children have all biochemical parameters in normal range. It is important to stress the absence of regulations about the blood Pb^{2+} limits for children living in industrial and other exposed areas in under developing countries. These findings point out the need to strengthen the initiative to reduce exposure Pb^{2+} sources.

4. Conclusion

The application of a two-level factorial design made possible a fast and economical optimization of separation and preconcentration of Pb^{2+} in blood samples based on cloud point extractions using two complexing reagents simultaneously. The surfactant-rich phase can be introduced into the nebulizer of a flame atomic absorption spectrometer after dilution with acidified ethanol. It was observed that the extraction efficiency of Pb^{2+}-APDC and Pb^{2+}-DDTC complexes depends on pH. The complexing reagents APDC and DDTC are frequently used for different metals, because of their fast reaction rate with metals and good water solubility. But DDTC is an unstable reagent that decomposes in acidic or neutral solution and has poor selectivity. The enhancement factor for both ligands were measured as the ratio between the slopes of calibration curves for the Pb^{2+} submitted to CPE using APDC and DDTC separately, and a curve without preconcentration, indicates 56 and 42-fold improvement, respectively. It was concluded that APDC is more efficient ligand for the enrichment of Pb^{2+} content in standards and real samples.

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