Application of Cold-Induced Aggregation Microextraction Based on Ionic Liquid for Determination of Trace Amount of Cadmium and Lead in Powder Milk Samples

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ABSTRACT

Background: cold-induced aggregation micro extraction based (CIAME) based on ionic liquid was used as a rapid and simple method for determination trace amounts of cadmium and lead in milk powder by analysis with flame atomic absorption spectrometry (FAAS).

Methods: Sample solution containing of Cd^{2+} and Pb^{2+} in dynamic range, phosphate buffer (pH = 9, 3 ml), diethyl dithiocarbamate (complexing agent), Triton X-100, $NaPF_6$,[HMIM][PF6] (extraction solvent) were transferred into conical bottom glass tube. Sample was kept in a thermostated bath and then ice bath; a cloudy solution was formed. Two phases separated by centrifugation. After removing of aqueous phase, IL-phase was dissolved in methanol and diluted was injected to the FAAS by microsampler introduction.

Results: ILs, containing imidazoliumcation and hexafluoro phosphate anion, [HMIM][PF6](70mg), 200 mg $NaPF_6$,0.01 $mol.L^{-1}$ DDTC, 0.015% of Triton X-100 obtained, pH 9 and centrifuge time; 5 min (4000 rmp) was chosen. Detection limit were obtained 0.12 μgL^{-1}, 1.61μgL^{-1}, RSD 0.95%, 2.2% and enrichment factor of 70, 67 for Cd and Pb, respectively.

Conclusion: CIAME allows determination of cadmium and lead in real samples in a simple, rapid and safe method with only a small amount of ionic liquid was used. In comparison with the organic solvent extraction, CIAME is much safer and the determination of species in high ionic strength samples is possible.

Keywords: Cadmium, CIAME, IL, Lead.

INTRODUCTION

The toxicity and effect of trace element on human health and the environment are receiving increasing attention in pollution and nutritional studies. Cadmium and lead are classified as prevalent toxic metal even at very low concentration, causing damage to organs such as the liver, lung and kidney; while lead is known to be accumulated in bone and in some soft tissues such as liver, kidney, and brain [1]. Contaminated waters are the main source of consumption of this metals. Therefore, rapid and sensitive methods must be accessible for their determination in these samples.

Despite good developments in the modern analytical instruments, direct determination of trace analytes at low concentrations in complex matrixes is often a problem for analytical chemists. Sample preparation is a bottleneck for this purpose because of its steps that often involve consuming of large volume of hazardous organic solvents, time consuming and/or

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expensive. So, the development of isolation/pre-concentration procedures prior to trace element determination has been investigated in considerable depth in recent decades [2, 3]. Several isolation and enrichment techniques have been introduced for determination of cadmium and lead in water samples such as solid phase extraction [4-6], liquid-liquid extraction (LLE) [7], liquid membrane technology , and co-precipitation [8].Conventional LLE requires large sample and toxic organic solvent volume, and hence generates large amount of pollutants and makes LLE expensive, time-consuming, and environmentally unfriendly. Recently, efforts have been directed toward miniaturizing the LLE procedure by drastic reduction of the extraction phase volume, leading to the development of solvent micro extraction methodologies [9], such as single drop microextraction (SDME) [10], cloud point extraction [11], dispersive liquid-liquid microextraction (DLLME) [12],continuous flow microextraction(CFME) [13,14], directly suspended droplet microextraction(DSDME) , and hollow fiber liquid-liquid-liquid microextraction (HF-LLLME) .

The search for new solvents is a key trend in liquid phase micro extraction (LPME) evolution. In this sense, room temperature ionic liquids (RTILs), whichare water and air-stable salts, composed of an organic cation and either an organic or an inorganic anion,have attracted much attention taking into account theirspecialfeatureslike: low-vaporpressure,highviscosity,dualnaturalpolarity,goodthermalstabilityandawiderangeofmiscibilitywithwaterandotherorganic solvents (tenability of ILs) [15]. Extractions of metal ions using RTILs combined with suitable complexing agents have been recently developed, specially employing hydrophobic RTILs, to extract low polar compounds from aqueous solution.

Recently, a new separation and extraction technique was introduced which called cold induced aggregation microextraction (CIAME) [16 -18]. This method is similar temperature-controlled ionic liquid-liquid-phase micro extraction [19] which, small amount of ionic liquid as

the extraction solventwas used for determination of trace quantities of elements. The aim of present work is the development of CIAME method for preconcentration and determination of trace levels of cadmium and lead in several types of samples by flame atomic absorption spectrometry. The effect of various experimental factors on the extraction efficiency is investigated and discussed in detail.

MATERIALS AND METHODS

Reagents and materials

Analytical grade of cadmium nitrate, lead nitrate, and nitrate salts of other cations and hydrochloric acid and methanol solutions (all from Merck, Darmstadt, Germany) with highest purity were available, and used without further purification except for vacuum drying. Ammonium diethyldithiocarbamate (DDTC) and sodium hexafluoro phosphate were purchased from Merck (Darmstadt, Germany) and Aldrich (Milwaukee, USA) respectively, and prepared by dissolving appropriate amount in distilled water.1-hexyl-3-methyl-imidazolium hexafluoro phosphate was purchased from Alfa Aesar (A Johnson Matthey Company).

Instrumentation

The experiments were performed using a Varian atomic absorption spectrometer (AA-240) equipped with deuterium background correction. Cd and Pb hollow cathode lamps (Varian series) operating at 228.8 and 217.0 nm, respectively, were used as the radiation source. A thermostated bath (Memmert Germany) was used for CIAME experiments. The Behdad Universal Centrifuge (Tehran, Iran) equipped with a swing out rotorwas used for centrifugation. All 15 ml screw cap, conical-bottom glass centrifuge tube (extraction vessel) were maintained into 0.1 mol. L-1, nitric acid for cleaning of any inorganic compound and washed with distilled water.

A Hamilton plug valve (HVP model 2-5, Cat. No. 86786, Hamilton) coupled with the nebulizer needle 1/4 in.-28UNF Hub (Cat. No. 88986, Hamilton) and the female Luer fitting (1/4 in.-28 UNF, Cat. No. 35031,

Hamilton) was used for micro sample introduction.

Sample preparation

A 1.00 g of standard milk powder Fapas (T0766) or sample milk powder (original sample) was heated on a hot plate at a fairly low temperature, to avoid violent spurting, in the glass beaker containing mixture of concentrated sulfuric acid (10.0 ml) and nitric acid (4.0 ml) to dryness. After that, the sample was cooled down to room temperature and the residue was dissolved in a 2.0 mL of HNO3, 0.1 mol L-1. Then, the solution was transferred into a 10.0 mL volumetric flask and diluted to the mark with HNO3 0.1 mol. L-1.

General procedure

1.0 ml of sample solution or standard solution containing 0-10 µgL-1 and 0-100 µg L-1 of Cd and Pb, respectively, phosphate buffer (pH 9 , 3 ml) , DDTC (0.01 mol L-1), HNO3 0.1 mol.L-1, Triton X-100 (0.015 % w/v), NaPF6 (200 mg), [HMIM] [PF6] (70 mg) were transferred into 15.0 mL conical bottom glass centrifuge tube (extraction vessel). Then, diluted was kept in a thermostated bath at 60 °C for 10 min to dissolve ionic liquid completely. Then it was shaken to dissolve ionic liquid and kept into a thermostated bath again. Afterward, it was placed in an ice bath for 10 min; therefore a cloudy solution was formed. Consequently, the cadmium and lead complexes were immediately extracted in fine droplet. Separation of the two phases was achieved by centrifuging for 5 min at 4000 rpm. As a result, the fine droplets of IL were settled at the bottom of the conical tube (20 ±2 µL). After removing of aqueous phase, IL-phase was dissolved in 100 µL of methanol solution and then 100 µL of diluted was sequentially injected to the flame atomic absorption by micro sampler introduction.

RESULTS

The efficiency of CIAME procedure parameters were such as pH of aqueous solution, amounts of IL, concentration of chelating agent, temperature of solution, centrifugation time and coexisting ions, should be investigated in detail.

Type and amount of ionic liquid and effect of NaPF6

The selection of ionic liquid as extraction solvent is an essential consideration for efficient extraction. Generally, the most important factors are low solubility in aqueous sample (at 25 °C), chemical affinity toward the analyte-DDTC complex, compatibility of the IL with the instrumental technique, and being liquid in experimental condition. So ILs, containing imidazoliumcation and hexafluoro phosphate anion, such as [HMIM][PF6] is suitable for this purpose. The effect of [HMIM][PF6] amount on extraction efficiency was investigated by adding 40 to 120 mg of ionic liquid to aqueous sample. According to Fig.1, the analytical signal of Cd and Pb complexes increased by increasing of [HMIM][PF6] up to 60-85 mg and then decreased up to 120 mg due to the increasing of extraction solvent volume. Therefore, 70 mg was chosen in the following study.
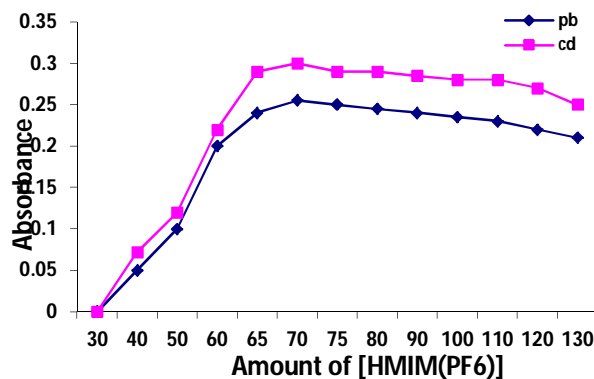
Figure.1. Effect of [HMIM][PF6] amount on the absorbance of cadmium and lead complex. Condition: Cadmium: 4 µg L-1, Lead: 50 µg L-1; DDTC: 0.04 mol L-1; NH3: 0.1 mol L-1; NaPF6: 200 mg; Triton X-100: 0.015% (w/v); Temperature: 60 °C.

The effect of NaPF6 amount was investigated in the range of 0 –250 mg in the presence of 70 mg [HMIM][PF6]. According to the common-ion effect, by increasing of NaPF6, the solubility of [HMIM][PF6] decreased. So the analytical signal of Pb and

Cd increased. According to the obtained results, the absorbance increased with increasing the amount of NaPF6 up to 200 mg and then remained nearly constant. Thus, 200 mg NaPF6 was chosen as the optimum value.

Effect of pH

The formation of metal chelate and its chemical stability are the two important influence factors for the extraction of metal ions, and the pH plays a unique role on metal chelate formation and subsequent extraction. The effect of pH upon the extraction of Pb and Cd ions from the solution was studied within the pH range 3–12. Each operational desired pH value was obtained by the addition of diluted HNO3 and/or NH3. The results are shown in Fig. 2.

As can be seen, the highest extraction efficiency begins at pH 9, and then being constant until pH 12.0. As a result, to avoid interference effects, a pH 9 was selected as the working value. The pH was adjusted by using phosphate buffer solution(NaH2PO4 – Na2HPO4).
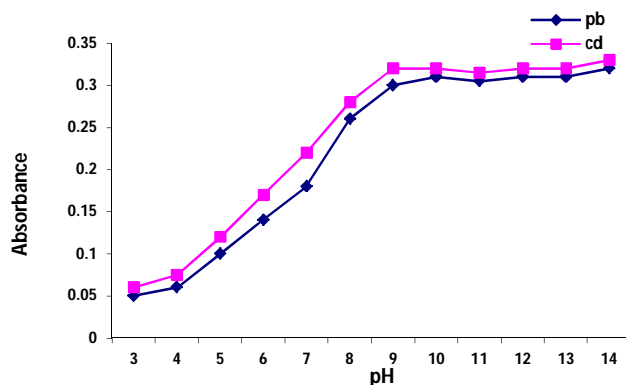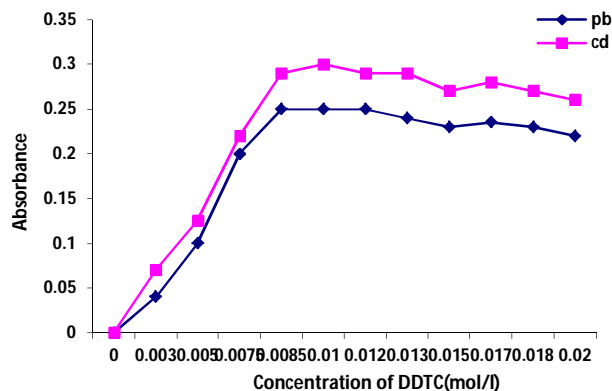

Figure.2. Effect of PH on the absorbance of cadmium and lead complex. Condition: Cadmium: 4 µg L-1, Lead: 50 µg L-1; DDTC: 0.04 mol L-1; [HMIM][PF6]: 70mg; NaPF6, 200 mg; Triton X-100: 0.015% (w/v); Temperature: 60 °C.

Influence of DDTC Concentration

The influence of amount of DDTC was also evaluated in the range of 0 – 0.05 mol L−1. According to the obtained results (Fig.3), the absorbance of Cd and Pb were increased with increasing of DDTC up to 0.01 mol. L-1 and then remained nearly constant.

Therefore, a concentration of 0.01 mol. L-1 was chosen as optimum concentration of DDTC for determination of Cd and Pb.

Figure.3. Effect of DDTC concentration on the absorbance of cadmium and lead complex. Condition: Cadmium: 4 µg L-1, Lead: 50 µg L-1; NH3 : 0.1 mol L-1; [HMIM][PF6]: 70 mg; NaPF6, 200mg; Triton X-100: 0.015% (w/v) ; Temperature: 60 °C.

Error bars correspond to the standarddeviations of three replicate measurements.

Effect of Anti-sticking agent and Salt Concentration

Adsorption of IL, on to the wall of the tube after centrifuging step, was another problem that reduced the recovery of extraction. Baghdadi et al. showed that addition of non-ionic surfactant such as Triton X-100 could overcome this problem. The results revealed that the absorbance was increased up to a maximum level by addition of 0.015% of Triton X-100. Further addition of the surfactant had not significant variations in absorbance. So, 0.015% of Triton X-100 was chosen as the optimal concentration.

Ionic strength is often an impact factor of the extraction and the enrichment performance. In general, the effect of salt on the extraction efficiencies surveys with adding salt such as NaCl. Therefore, it was investigated in the range of 0-20 %(w/v). According to the obtained results, the absorbance was nearly constant for cadmium and lead. This is one of the advantages of this method inthe analysis of real sample with high ionic strength.

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Influence of Temperature and Centrifuge Condition

This variable played a critical role in obtaining a homogenous solution that contains the RTIL and complex. The effect of temperature on analytical signal was investigated in the range of 30-80 °C. The results showed that, extraction efficiency was increased up to 40°C and then absorbance was constant. So, 60° C was selected for extraction process. After heating the solution for 10 min, it was shaken to dissolve ionic liquid and kept into an ice bath to form fine droplet of ionic liquid.

Centrifugation time was studied in the range of 1-15 min (4000 rpm). The result showed that the analytical signal increased up to 3 min, and then was constant. Thereby, 5 min was chosen as an optimum centrifugation time for further study.

Analytical Figures of Merit

Under the optimal conditions, the relative standard deviation (RSD) for six samples of 4.0 µgL-1 of Cd and 50.0 µgL-1 of Pb subjected to the complete procedure are 0.95 % and 2.22 %. Calibration curves were linear in the range of 0-10 µgL-1 and 0-100 µgL-1 for Cd and Pb, respectively. The limit of detection calculated as 3Sb/m (Sb: standard deviation of the blank signals; m: slope of calibration curve after pre-concentration) were 0.12 µgL-1 Cd, and 1.61 µgL-1 Pb. The enhancement factor obtained from the slope ratio of calibration curve after and before preconcentration were 70, 67 for Cd and Pd, respectively. The results were summarized in Table 1.

Table 1. Analytical characteristics of present method for determination of cadmium and lead.

	Cd	**Pb**
Enhancement factor	70	67
Correlation co-efficient (r^2)	0.995	0.997
Linear range(μgL^{-1})	0-10	0-100
Slope	0.03	0.0035
R.S.D (%)(n=6)	0.95	2.22
Limit of detection(μgL^{-1})(n=6)	(4)[a]	(50)
	0.12	1.61

[a]Values in parentheses are the Cd and Pb concentration ($\mu g\ L^{-1}$) for which the R.S.D.were obtained.

Analytical Application

The proposed microextraction methodology was applied to the preconcentration and determination of Cd and Pb in two sample (i.e., milk powder and fapas milk powder) and two types of synthetic samples. In order to validate the accuracy of the proposed procedure, recovery experiments were also carried out by spiking the samples with different amounts of cadmium and lead before any pretreatment. Table 2 shows the obtained results. The values of recoveries have confirmed the validity of the proposed method. Additionally, the accuracy of the proposed method was evaluated by analyzing a standard reference material, fapas (T0766) powder milk with certified Cd^{2+} and Pd^{2+}content of 47.6 and 107 µgKg-1, respectively.FAPAS is the largest and most comprehensive analytical chemistry proficiency testing scheme in the food sector.

Table 2. Determination of Cd and Pb in certified material, several types of powder milk samples. SampleCertified.

Sample	Certified (μgL^{-1})	Cadmium			Certified (μgL^{-1})	Lead		
		Added ($\mu g\ L^{-1)}$)	Found ($\mu g\ L^{-1}$)	Recovery (%)		Added ($\mu g\ L^{-1}$)	Found (μgL^{-1})	Recovery (%)
Fapas (T0766)	47.6	-	46.5	97.6	107	-	104.9	98
Original sample1	0	10	9.74± 0.31	97.4	0	50	49.16± 0.42	98.3
Original sample2	0	10	9.81± 0.35	98.1	0	50	49.5± 0.45	99
Original sample3	0	10	9.7± 0.41	97	0	50	49.25± 0.39	98.5
Original sample4	0	10	9.79± 0.31	97.9	0	50	49.3± 0.41	98.6

Fapas : Standard sample by serial No. T0766
original sample : These Samples were taken from the market.

DISCUSSION

Comparison of CIAME-FAA with Other Methods

A comparison of the represented method with normal method reported pre-concentration methods is given in Table 3. According to the result, though the use of advanced instruments such as GF-AAS could produce better detection limit or preconcentration factor, but simplicity, safety, fairly good RSD and LOD as well as small amount of ionic liquid are inherent advantages of presented CIAME technique. These characteristics are interest key for analysis of metal ions in trace level in real samples with CIAME method.

Table 3. Comparison of presented method with other preconcentration method for Determination of Cd and Pb.

Method	Determination limit		Ref.
	Cd ($\mu g\ L^{-1}$)	Pb($\mu g\ L^{-1}$)	
ICP OES	0.05	1.15	37
USS-ETV-RC-ICP-MS	0.2	2	38
CIAME-FAAS	0.12	1.61	Present work

CONCLUSION

Cold-induced aggregation microextraction coupled to flame atomic absorption spectrometry allows to determination of cadmium and lead in several real samples in a simple, rapid, robust, and safe method since only a small amount of ionic liquids are used. In this method, DDTC was used as complexing agent to produce neutral species of Cd and Pb and [HMIM][PF6] as an extraction solvent in 10.0 ml of sample. In comparison with the organic solvent extraction, CIAME is much safer which need only small amounts IL (with very low vapor pressure) and determination of species in high ionic strength samples is possible. Also, owing to high viscosity of ILs, removing bulk aqueous phase is easier. Finally, the use of microsampler introduction system causes better reproducibility and offers the opportunity to introduce a small volume of extracted sample to the flame.

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