

DISPERSIVE LIQUID-LIQUID MICROEXTRACTION FOR THE SIMULTANEOUS SEPARATION OF TRACE AMOUNTS OF ZINC AND CADMIUM IONS IN WATER SAMPLES PRIOR TO FLAME ATOMIC ABSORPTION SPECTROMETRY DETERMINATION

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In the proposed method, carbon tetrachloride and ethanol were used as extraction and dispersive solvents. Several factors that may be affected on the extraction process, such as extraction solvent, disperser solvent, the volume of extraction and disperser solvent, pH of the aqueous solution and extraction time were optimized. Under the optimal conditions, linearity was maintained between 1.0 ng mL⁻¹ to 1.5 µg mL⁻¹ for zinc and 1.0 ng mL⁻¹ to 0.4 µg mL⁻¹ for cadmium. The proposed method has been applied for determination of trace amount of zinc and cadmium in standard and water samples with satisfactory results

Keywords: dispersive liquid-liquid microextraction; cadmium determination; zinc determination.

INTRODUCTION

Importance positive and negative roles of trace heavy metal ions in human health are known. Considering biological research, the roles of some trace and ultra-trace elements in the body is very important and has diverse function.¹⁻⁵ Some of trace elements are essential to life while others are toxic even at very low concentrations. Therefore, due to the importance of heavy metals at trace level in the human health and environment, the sensitive and accurate determination of the levels of heavy metals in the environmental samples have been continuously carried out on the analytical and environmental laboratories around the world.⁶

Several novel microextraction techniques are being developed in order to reduce the analysis step, increase the sample throughput and to improve the quality and the sensitivity of the analytical methods. The cloud point extraction (CPE),⁷ the homogeneous liquid-liquid extraction (HLLE),^{8,9} the liquid phase microextraction (LPME)^{10,11} and the solid phase microextraction (SPME)^{12,13} are fairly new methods of sample preparation. They are employed in the separation and preconcentration of environmental contaminants in different matrices and can solve some of the problems, encountered with the conventional pretreatment techniques. Recently, a new liquid-liquid microextraction method namely, dispersive liquid-liquid microextraction (DLLME) was proposed.¹⁴ This method has been applied for the determination of trace organic pollutants and metal ions in the environmental samples.¹⁵⁻²⁴ Recently we reported a new DLLME method to preconcentration of silver and copper without the addition of the chelating agent,^{25,26} and a new ultra sound assisted emulsification for simultaneous microextraction trace amount of cobalt and manganese.²⁷

DLLME is based on a ternary component solvent system like homogeneous liquid-liquid extraction and cloud point extraction. In this method, the appropriate mixture of extraction solvent and

dispersive solvent is injected into an aqueous sample rapidly by a syringe, and a cloudy solution is formed. The analyte in the sample is extracted into the fine droplets of extraction solvent. After extraction, phase separation is performed by centrifugation.

The aim of this work is at combining ligandless dispersive liquid-liquid microextraction (LL-DLLME) with FAAS and proposes a new method for separation, preconcentration and determination trace amounts of zinc and cadmium in water and standard samples.

EXPERIMENTAL

Instrumentation

A SensAA GBC atomic absorption spectrometer (Dandenong, Australia) equipped with deuterium background correction and air-acetylene burner was used for absorbance measurements according to instrument instruction. Zinc and cadmium hollow cathode lamps were used as light source. The operating parameters of elements were set according to the manufacturer recommendation. The acetylene flow rate and the burner height were adjusted in order to obtain the maximum absorbance signal, while aspirating the analyte solution. A Metrohm 692 pH meter (Herisau, Switzerland) was used for pH measurements. A Centurion scientific centrifuge model 1020 D.E. (West Sussex, United Kingdom) was used to accelerate the phase separation.

Reagents and solutions

Doubly distilled de-ionized water was used throughout the experimental work. Stock solutions of zinc and cadmium were prepared by dissolving appropriate amount of ultra pure salt obtained from Merck (Darmstadt, Germany) in 2 mol L⁻¹ HNO₃. Working standard solutions were obtained by appropriate dilution of the stock standard solution. The laboratory glassware was kept overnight in a 1.4 mol L⁻¹ HNO₃ solution. Before using, all of the glassware were washed with de-ionized water and dried. A solution of 10% NaCl (Merck)

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was prepared by dissolving of 10 g of NaCl in 100 mL of de-ionized water. The solutions of alkali metal salt (1% w/v) and various metal salts (0.1% w/v) were used to study the interference of anions and cations, respectively.

Ligandless-dispersive liquid-liquid microextraction procedure

In this study, combination of LL-DLLME with FAAS was developed for determination trace amounts of cadmium and zinc ions. It is based on microextraction of cadmium hydroxide and zinc hydroxide with using of ethanol as dispersive solvent and carbon tetrachloride as extraction solvent. All standards and samples were prepared for analysis according to the following procedure. A 8.0 mL aliquot of each sample was placed in a screw cap glass test tube with a conic bottom. To each test tube, 1 mL of 0.1 mol L⁻¹ di potassium hydrogen phosphate (pH 10) and 1 mL of 10% (w/v) NaCl were added. Then, 2.0 mL of ethanol containing 30.0 µL of carbon tetrachloride was injected rapidly into the sample solution. In this step, zinc and cadmium reacted with hydroxide ions for Zn(OH)₂ and Cd(OH)₂ formation, then the aqueous, dispersive and extraction solvents were mixed and a cloudy solution was formed. After formation of cloudy solution, Zn(OH)₂ and Cd(OH)₂ diffuses into the extraction solvent quickly, because of the high surface area between extraction solvent and the aqueous phase. Then, the solution was centrifuged at 3000 rpm for 5 min, and the dispersed fine droplets of CCl₄ were deposited at the bottom of conical test tube. The sediment phase was removed and 0.7 mL of 1.0 mol L⁻¹ HNO₃ in ethanol was added to it. The final solution was aspirated directly into the flame of AAS.

Sample preparation

Two certified reference materials (CRMs) furnished by the National Institute for Environment Studies (NIES) No. 1 Pepperbush and NIES No. 7 Tea Leave have been analyzed. Approximately 0.40 g of NIES No. 1 and 1.0 g NIES No. 7 were weighted accurately into a Teflon cup and dissolved in concentrated nitric acid (~10 mL) with heating on a water bath. The solution was cooled, diluted and filtered. The filtrate was made to 100.0 mL with de-ionized water in a calibrated flask. An aliquot of the sample solution was taken individually, and analyte ions were determined by the general procedure.

River and well water samples were collected in acid-leached polyethylene bottles. The river water samples were collected from Rayen, Shahdad and Kohpayeh in Kerman province, Iran. The well water sample was collected from Payame Noor University, Kerman, Iran. The only pretreatment was acidification to pH 2 with nitric acid, which was performed immediately after collection, in order to prevent adsorption of the metal ions on the flask walls. The samples were filtered before analyses through a cellulose membrane of 0.45 µm pore size (Millipore).

RESULTS AND DISCUSSION

Selection of disperser solvent

The disperser solvent must be miscible in extraction solvent (organic phase) and also sample solution (aqueous phase). Therefore, acetone, methanol, ethanol and tetrahydrofuran were tested. The effect of these solvents on the extraction efficiency of LL-DLLME was investigated using 2.0 mL of each solvent containing 30.0 µL of CCl₄ as the extraction solvent. The extraction efficiency of LL-DLLME for zinc and cadmium in acetone, methanol, ethanol and tetrahydrofuran

as disperser solvents were 86.3 and 91.4; 95.7 and 97.3; 98.7 and 99.2; 71.8 and 64.9, respectively. The maximum extraction efficiency of DLLME was obtained by using ethanol as a disperser solvent. Therefore, ethanol was selected for further experiments.

Effect of the disperser solvent volume

The volume of the disperser solvent is one of the important factors to be considered in DLLME. At lower volumes of the disperser, tiny droplet formation may not be effective. This phenomenon will decrease the extraction efficiency. When the volume of the dispersive solvent is increased, two competitive processes may occur. One is the increase in the solubility of the analytes in the aqueous phase leading to diminished partition of the polar compounds into CCl₄ droplets. On the contrary, an increased volume of the partitioned ethanol phase into CCl₄ phase leads to increase in the extraction efficiency. Hence it was necessary to evaluate the volume effect of dispersive solvent. To obtain the optimized volume of ethanol, various experiments were performed using different volumes of ethanol (0.5-3.0 mL) containing 30.0 µL of CCl₄. The results are presented in Figure 1.

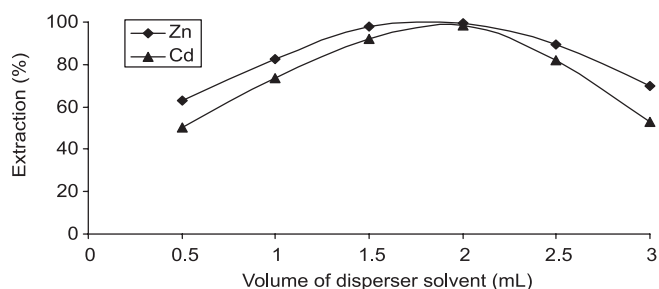


Figure 1. Effect of the disperser solvent volume (ethanol) on the LL-DLLME extraction. Conditions: Zn(II) and Cd(II), 4.0 and 0.8 µg; Buffer, 1 mL; NaCl 10%(w/v), 1 mL and CCl₄, 30.0 µL

The results showed that the extraction efficiency increased with the increase of the volume of ethanol to 2.0 mL. Reduction in the extraction efficiency was observed after the volume of ethanol exceeded 2.0 mL. Finally, 2.0 mL ethanol was chosen as the optimum volume.

Selection of the extraction solvent

The type of extraction solvent in DLLME is an essential consideration for efficient extraction. It should be higher density than water, high extraction capability of the interested compounds and low solubility in water. 1, 2-dichlorobenzene (1, 2-DCB) and CCl₄ were studied as extraction solvent. The effect of these solvents on the extraction efficiency of LL-DLLME was investigated using 2.0 mL of ethanol as a dispersive solvent containing 30 µL of each solvent. The extraction efficiency of LL-DLLME for zinc and cadmium with 1, 2-DCB and CCl₄ as extraction solvent were 75.6 and 83.0; 97.2 and 98.5, respectively. Therefore, CCl₄ was selected as extraction solvent for subsequent experiments.

Effect of the organic extraction volume

In order to examine the effect of the volume of extraction solvent, different volume of CCl₄ (20-60 µL) were used as extraction solvent to the same LL-DLLME procedure. The results are shown in Figure 2.

The results showed that the extraction efficiency increased with the increase of the volume of CCl₄ to 30 µL. Reduction in the extraction efficiency was observed after the volume of CCl₄ exceeded

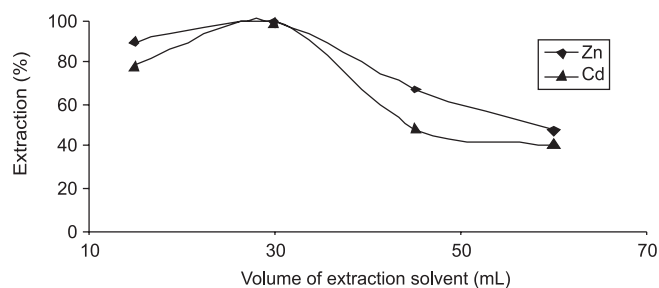


Figure 2. Effect of the extraction solvent volume (CCl_4) on the LL-DLLME extraction. Conditions were the same as Figure 1

30 μ L. Finally, based on these observations, a volume of 30 μ L was used for further experiments.

Effect of pH on LL-DLLME of ions

The effect of pH on the LL-DLLME extraction of zinc and cadmium was studied in the pH range of 3-11. The pH of the metal sample solutions was adjusted by using NaOH or HNO_3 solutions. As can be seen in Figure 3, the highest extraction efficiency of zinc and cadmium was obtained at pH range of 9-11. At low pHs, the extraction efficiency don't complete. It mean that the formation of $Zn(OH)_2$ and $Cd(OH)_2$ in low pHs isn't completed.

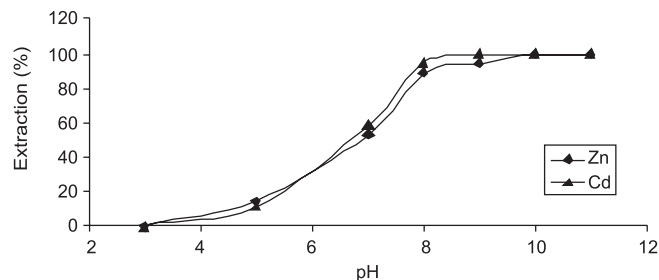


Figure 3. Effect the pH of sample solution on the LL-DLLME extraction. Conditions were the same as Figure 1

Salting out effect

In the extraction, the solubility of many analytes in aqueous solutions decreases with increasing ionic strength due to salting out effect. Sodium chloride was used to investigate the influence of ionic strength on the extraction efficiency. For investigating the influence of the ionic strength on the LL-DLLME performance, several experiments were performed by adding varying amounts of NaCl from 0.25 to 1.5 g. The rest of the experimental conditions were kept constant. The extraction efficiency of LL-DLLME for zinc and cadmium with 0.25 to 1.5 g of NaCl was in the range 57.3-98.3. The resulting data were showed that the maximum extraction was obtained in the presence 1 g of NaCl. Therefore, 1 mL NaCl 10% was used in all further experiments.

Effect of the extraction time

In DLLME, the extraction time is defined as interval time between the injection of the disperser and extraction solvents and the starting of centrifuge. The effect of extraction time was examined in the range of 10 s to 30 min with the constant experimental conditions. The obtained results showed that the extraction time was no significant influence on the signal of zinc and cadmium. Therefore, the LL-DLLME method was time-independent, which was the most important advantage of this technique.

Effect of foreign ions

In view of the high selectivity provided by flame atomic absorption spectrometry, the only interference may be attributed to the separation step. Interferences studied were those related to the separation step, cations that may react with hydroxide or species that may react with analytes and decrease the extraction efficiency. To perform this study, various salts and metal ions were added individually to a solution containing 0.5 μ g mL^{-1} zinc and 0.1 μ g mL^{-1} cadmium, individually and the LL-DLLME procedure was applied. The tolerance limit was set as the concentration of the diverse ion required to cause $\pm 5\%$ error. Results are given in Table 1. These results demonstrate that the presence of large amounts of species commonly present in water samples have no significant effect on the LL-DLLME of zinc and cadmium.

Table 1. Tolerance limit of foreign ions

Foreign ions	Interference/ Zn(II) ratio	Re% of Zn(II)	Interference/ Cd(II) ratio	Re% of Cd(II)
PO_4^{3-} , HPO_4^{2-}	4500	98	5000	97
CH_3COO^-	3000	95	3000	96
Ca^{2+} , Mg^{2+}	2500	95	3000	97
Co^{2+}	300	105	300	96
Fe^{2+}	800	95	500	96
Ni^{2+}	800	97	1000	105
Al^{3+}	400	95	500	95
As^{3+}	600	98	1000	95
Fe^{3+}	200	96	250	95
Cu^{2+}	150	96	100	105
Mn^{2+}	800	97	800	95

Conditions were the same as Figure 1.

Calibration, precision and detection limit

Under the optimized conditions, calibration curves were constructed for the determination of zinc and cadmium according to the LL-DLLME procedure. Linearity was maintained between 1.0 $ng\ mL^{-1}$ to 1.5 μ g mL^{-1} for zinc and 1.0 $ng\ mL^{-1}$ to 0.4 μ g mL^{-1} for cadmium. The equations for the lines were $A = 0.6361C + 0.0051$ ($R = 0.999$) and $A = 2.4297C + 0.0040$ ($R = 0.999$), respectively. In these equations, A is the absorbance value of the eluent, C is concentration of zinc and cadmium (μ g mL^{-1}) and R is correlation coefficients. Seven replicate determination of a mixture of 0.5 μ g mL^{-1} zinc and 0.1 μ g mL^{-1} cadmium in original solution gave a mean absorbance of 0.311 and 0.243 with relative standard deviation 1.5 and 1.7%, respectively. The detection limits based on $3S_b/m$ for zinc and cadmium in the original solution were 0.3 and 0.4 $ng\ mL^{-1}$, respectively. The limit of quantification based on $10S_b$ for zinc and cadmium in the original solution was 1.0 and 1.3 $ng\ mL^{-1}$, respectively. Enrichment factors were calculated as the ratio of the slope of calibration curve after and before of microextraction. The enrichment factors for zinc and cadmium were 10.3 and 13.4, respectively.

Accuracy of the method

The accuracy and applicability of the proposed method has been applied to the determination of Zn(II) and Cd(II) ions in National Institute for Environment Studies (NIES) No. 1 pepperbush and NIES No. 7 Tea Leaves. Results are given in Table 2. It was found that there is no significant difference between results obtained by the proposed method and the certified results. These results indicate the

Table 2. Determination of zinc and cadmium in biological samples

Sample	Certified value ($\mu\text{g g}^{-1}$)	Found ^a ($\mu\text{g g}^{-1}$)
NIES, No. 1 Pepperbush	Zn: 340 ± 20 Cd: 6.7 ± 0.5	Zn : 346.9 ± 14.1 Cd : 6.8 ± 0.3
NIES, No. 7 Tea Leaves	Zn: 33 Cd: 0.030	Zn: 33.7 ± 1.4 Cd : 0.029 ± 0.002

^aMean \pm standard deviation (n=4)

applicability of the developed LL-DLLME procedure for zinc and cadmium determination is free of interference.

Analysis of water samples

The LL-DLLME procedure has been applied to the determination of zinc and cadmium content in different water samples. The results are given in Table 3.

The recovery of zinc and cadmium from water samples spiked with Zn(II) and Cd(II) were also studied. The results are given in Table 3. According this Table, the added Zn(II) and Cd(II) ions can be quantitatively recovered from the water samples by the LL-DLLME

Table 3. Determination of zinc and cadmium in water samples

Sample	Found ^a (ng mL ⁻¹)	Added (ng mL ⁻¹)	Found (ng mL ⁻¹)	Recovery (%)
River Water (Rayen, Kerman)	Zn: 22.4 ± 0.6 Cd: 1.13 ± 0.04	Zn:20.0 Cd:5.00	Zn:43.1 Cd:6.18	103.5 101
Well Water (Payame Noor University of Kerman)	Zn: 29.2 ± 1.1 Cd: 2.28 ± 0.09	Zn:20.0 Cd:5.00	Zn:49.6 Cd:7.31	102 100.6
River Water (Shahdad, Kerman)	Zn: 25.8 ± 0.7 Cd: 1.23 ± 0.06	Zn:20.0 Cd:5.00	Zn:45.5 Cd:6.20	98.5 99.4
River Water (Kohpayeh, Kerman)	Zn: 24.5 ± 0.7 Cd: 1.18 ± 0.05	Zn:20.0 Cd:5.00	Zn:44.6 Cd:6.15	100.5 99.4

^a Average of four determination \pm standard deviation

Table 4. Comparison of the proposed method with other reported methods for preconcentration of silver

System	Element	Analysis method	Sample volume (mL)	Enrichment factor	RSD (%)	LOD (ng mL ⁻¹)	Ref.
CPE	Zn	ICP-OES	15	9.4	1.3	1.1	28
	Cd			10.1	2.6	6.3	
CPE	Zn	FAAS	50	63.7	-	0.095	29
	Cd			57.7	-	0.099	
CPE	Zn	Spectrophotometry	25	-	2.1	1.2	30
CPE	Cd	FAAS	15	20.3	3.2	0.75	31
LL-CPE	Cd	FAAS	25	10	1.6	1.7	32
LL-CPE	Cd	FAAS	10	11.1	<3	1.0	33
LL-DLLME	Zn	FAAS	8	10.3	1.5	0.3	Present work
	Cd			13.4	1.7	0.4	

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procedure.

These results demonstrate the applicability of the procedure for zinc and cadmium determination in water samples. The recovery of zinc and cadmium added to the samples demonstrates the efficiency of the proposed method.

CONCLUSIONS

This work has been demonstrated combination of LL-DLLME with flame atomic absorption spectrometry provides a novel route for trace determination of zinc and cadmium in water samples. The main benefits of the LL-DLLME methodology were: minimum use of toxic organic solvent, simplicity, low cost, enhancement of sensitivity, and rapid analysis time.

A comparison of the present method with the other reported preconcentration methods²⁸⁻³³ for the cadmium and zinc extraction from water samples was given in Table 4. The obtained detection limits by the proposed procedure is less than some the other methods.^{28, 30-33} Also, the enrichment factors are higher than the CPE techniques^{28, 32} and the RSD% is less than some the other methods.^{28,30,31,33} Finally, the necessary sample volume is lower than the other methods.²⁸⁻³³

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