Preconcentration and determination of cadmium in aqueous samples and human plasma by flame atomic absorption spectrometry after dispersive liquid-liquid microextraction

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ABSTRACT

A novel approach for preconcentration and separation of trace amount of cadmium from aqueous samples was proposed by dispersive liquid–liquid microextraction (DLLME). Cadmium (II) was complexed with 2-[2-(2-aminophenyl) disulfanyl] benzamine (2APDSBA) as a complexing agent to form hydrophobic chelate and then extracted into the fine drops of extraction solvent which was dispersed into the aqueous sample by dispersive solvent. After extraction, the sedimented phase was analysed by flame atomic absorption spectrometry (FAAS). Some effective parameters on the DLLME such as type and volume of extraction and dispersive solvents, concentration of chelating agent, pH, centrifugation rate and time, extraction time and salt effect were investigated. Under the optimized conditions, the enrichment factor was 186.6. The calibration graph was linear in the range of 0.05-7.5 µg L^{-1} with correlation coefficient of 0.9985 under the optimum conditions of the recommended procedure. The detection limit based on the $3S_b$ criterion was 0.05 µg L^{-1} and relative standard deviation for (RSD) for ten replicate measurement of 0.2 µg L^{-1} and 2µg L^{-1} cadmium was 3.9 and 3.1 % respectively. In order to evaluate the accuracy and recovery of the presented method, the procedure was applied to the analysis of reference material, spiked water and human plasma.
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KEYWORDS

Dispersive liquid-liquid microextraction;
Preconcentration;
Falme atomic absorption spectrometry;
Cadmium;
Human plasma.

INTRODUCTION

Environmental pollution nature of heavy metals has recently received considerable attention. Cadmium is one of the heavy metals which is critical for the human health[1,2]. Cadmium is widely used in industry, especially in electroplating pigments for paints, enamel, glass, plastics, printing inks, rubber and lacquers, alloys and the production of Ni–Cd batteries[3, 4]. Different analytical techniques have been performed to determine cadmium in various samples including flame atomic absorption spectrometry (FAAS)[5–7], graphite furnace atomic absorption spectrometry (GFAAS)[8,9], inductively coupled

plasma emission spectrometry (ICP-OES)[10,11], inductively coupled plasma mass spectrometry (ICP-MS)[12].

Flame atomic absorption spectrometry (FAAS) has been widely used for determination of trace quantities of cadmium because of the low costs, operational facility and high sample throughput. However, conventional FAAS has a detection limit, which is not low enough to determine cadmium at trace levels. In order to achieve accurate, sensitive and reliable results at trace levels; preconcentration and separation steps are needed prior to analyte determination by FAAS[13].

Coupling of microextraction methods with graphite furnace atomic absorption spectrometry for determination of inorganic compounds developed in our laboratory and reported for the first time in our previous works[14-17]. Microdrop of analyte after extraction directly injected into the graphite furnace for determination. One of the problems of coupling flame atomic absorption spectrometry with microextraction methods is, introduce analyte in microdrop form into the flame. In this work, the plastic capillary tube attached to the nebulizer was removed and extracted analyte directly transferred into the flame atomic absorption nebulizer by a micro syringe. The experimental variables influencing the extraction have been optimized, and the analytical parameters of the method were determined. This procedure was applied to the determination of cadmium in reference material, real water samples and human plasma.

EXPREMENTAL

InstrumentationA Shimadzu AA-6300 (Shimadzu, Japan) flame atomic absorption spectrometer equipped with a 100 mm burner head, deuterium background correction and an air–acetylene flame was utilized. A cadmium hollow-cathode lamp (Hamamatsu Photonics, Shizuoka, Japan) at a wavelength of 228.8 nm was used as a radiation source, operated at 8 mA with a monochromator spectral bandpass of 0.7 nm. The pH values were measured with a pH meter (Metrohm 725, Switzerland) supplied with a glass combined electrode. Phase separation was assisted using Centurion Scientific Cen-

trifuge (Model Andreas Hettich D72, Tuttlingen, Germany). A 50 µl Hamilton 7105 syringe (Hamilton, Reno, NY, USA) was used to collect the drop of the acceptor phase and to inject finally solution into the atomic absorption spectrometer.

Reagent and materials

All solutions have been prepared with distilled water. All of the glassware was maintained overnight in a 5.0 % (v/v) nitric acid solution. The working solutions of cadmium at µg L^{-1} level were prepared daily by diluting a 1000 µg L^{-1} (Merck) stock solution of cadmium. All of the reagents used were of analytical grade. A 0.1 mol L^{-1} chelating agent 2-[2-(2-aminophenyl) disulfonyl] benzenamine (2APDSBA) (Aldrich, USA) solution was prepared by dissolving appropriate amounts of 2APDSBA in methanol. The carbon tetrachloride (Merck), acetone (Merck), methanol (Merck), ethanol (Merck), nitrobenzene (Merck) and dicholoromethane (Merck) solvents were also used in the microextraction procedure. The pH of the metal solutions was adjusted by adding HCl and NaOH (merck).

Preparation of the human plasma samples

The plasma samples were stored in the freezer at -18 °C and thawed at room temperature before processing of the sample. One hundred microliters of plasma were pipetted to the polypropylene tube, 20µl of the internal standard solution were added and the tube was briefly shaken. Two ml of tbutylmethylether were added and the tube was vortex-mixed for 4min at 2000 rpm. Then the tube was centrifuged for 5 min at 2000×g and the upper organic phase was transferred to a new tube. The sample was evaporated to dryness under nitrogen at 50 °C. The dried sample then was dissolved in methanol and was analyzed by DLLME combined with FAAS for determination of cadmium.

Procedure for DLLME

Aliquots of 10.0 ml of the solution containing Cd (II) was adjusted to pH 7.0 and transferred to screw cap glass test tubes (20.0 ml) with conic bottoms. The ternary solution containing 0.6 ml of acetone (dispersive solvent), 50 µl of carbon tetrachloride (extraction solvent) and 45 µl of a 1.0×10^{-4} mol L^{-1} 2-[2-(2-aminophenyl) disulfanyl] benzamine

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solution was rapidly injected into the solution of the metal with the aid of a 1.0 ml glass syringe. A cloudy mixture (water, acetone, carbon tetrachloride and 2APDSBA) was formed in the test tube. At this stage, the metal-ligand complex was extracted into the fine droplets of carbon tetrachloride. Then the solution was centrifuged at 5000 rpm for 3.0 min, and the dispersed droplets of carbon tetrachloride were deposited on the tube. After this step, the 50 μl organic phase settled at the bottom of the tube. The aqueous phase was discarded with syringe and the 50 μL organic phase was directly injected into the nebulizer of AAS by a micro syringe for subsequent determination. For injection of solution directly into the nebulizer, the plastic capillary tube attached to the nebulizer was removed and needle of syringe was inserted directly into the nebulizer. The parameters affecting the complexation and extraction efficiency were optimized.

RESULTS AND DISCUSSION

There are different factors that affect the extraction process such as pH, concentration of chelating agent, type and volume of organic and dispersive solvents, centrifugation rate and time, extraction time and interfering ions. It is very important to optimize these parameters in order to obtain high recovery and enrichment factor. The effects of each parameter on the extraction process were examined and the results are as follows.

Effect of the pH

The pH of aqueous solution plays an important role in the complex formation between cadmium ions and chelating agent (2APDSBA) as well as in the following microextraction process. In the present work, the pH of sample solutions was adjusted in the range of 2.5-9.0 using a HCl solution of 0.05 mol L $^{-1}$ or a NaOH solution of 0.05 mol L $^{-1}$. The results shown in Figure 1 indicate that higher absorbance for cadmium can be achieved when the pH of sample solution is set at 7. Hence, pH 7 was selected as the appropriate acidity of sample solution for extraction.

Concentration of chelating reagent
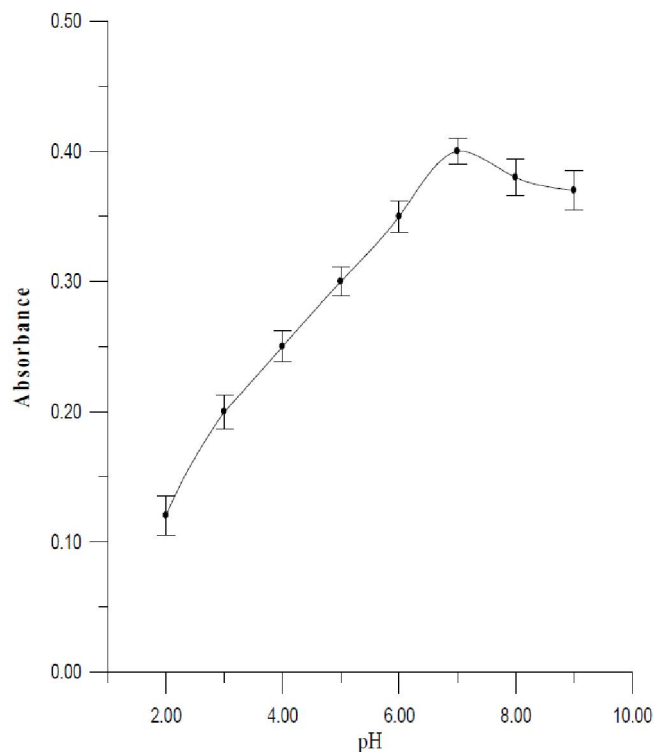
The study of the amount of 2APDSBA used in

Figure 1 : Effect of pH on the absorbance of cadmium

the DLLME was accomplished by varying the ratio of concentration of ligand / cadmium (II) between 0.2–1.8. The absorbance increases with increasing ratio of ligand /cadmium (II) up to 1 and leveling off at higher ratio. Therefore, we used ratio of concentration of ligand / cadmium (II) 1:1 in subsequent studies. The results are shown in Figure 2.
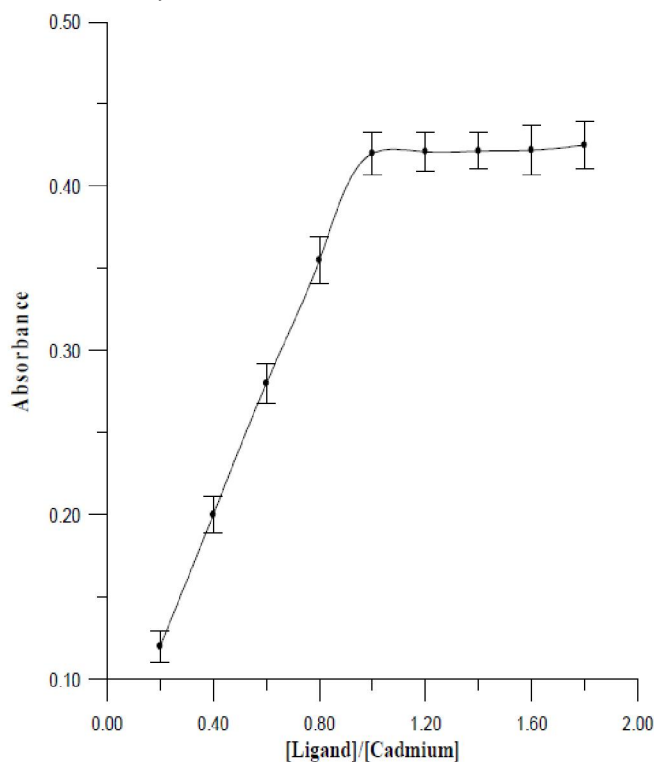
Type of extraction solvent

The DLLME process is based on the instantaneous turbidity of an aqueous solution promoted by injection of a ternary mixture followed by extraction of the substances of interest in droplets of a solvent extractor. The extraction solvent should be more dense than water and immiscible in an aqueous solution. Carbon tetrachloride, dichloromethane and nitrobenzene were tested as extraction solvents. As can be seen in Figure 3, among the extraction solvents, carbon tetrachloride yielded the best results for cadmium (II).
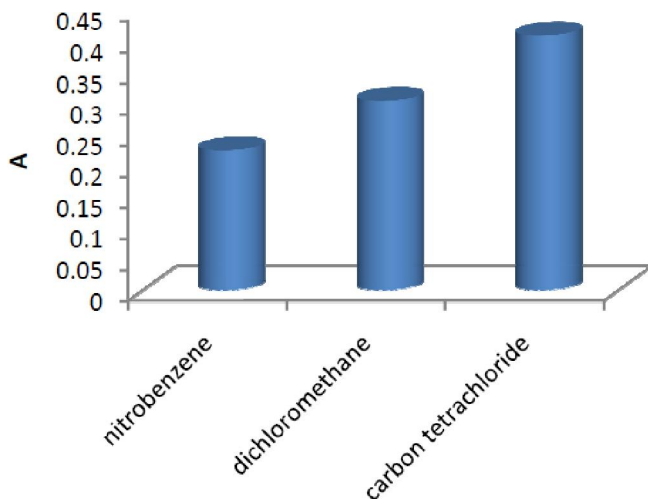
Effect of extraction solvent volume

To examine the effect of carbon tetrachloride volume on the analytical performance, experiments were performed by using 0.6 ml of acetone containing 2APDSBA and the volume of CCl$_4$ was adjusted in the range of 40–60 μl. By increasing the volume
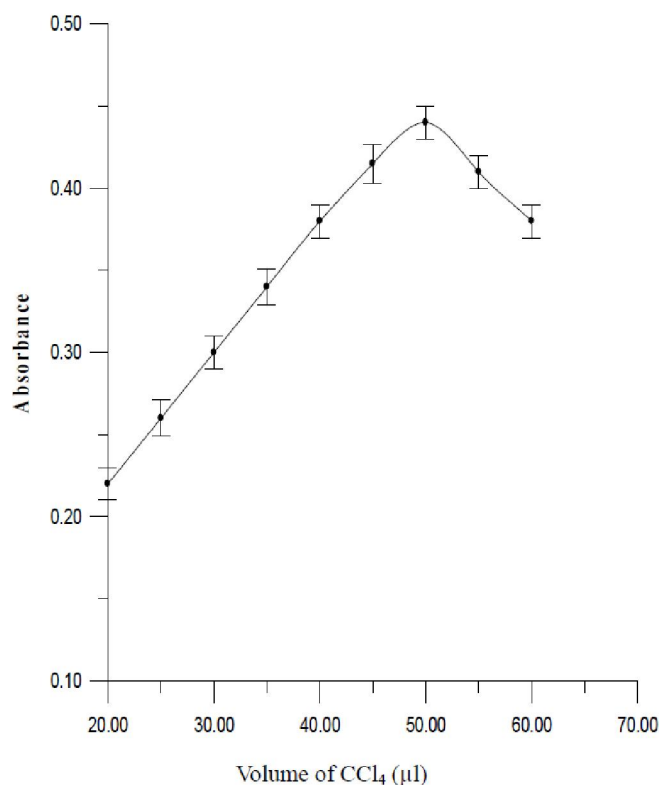
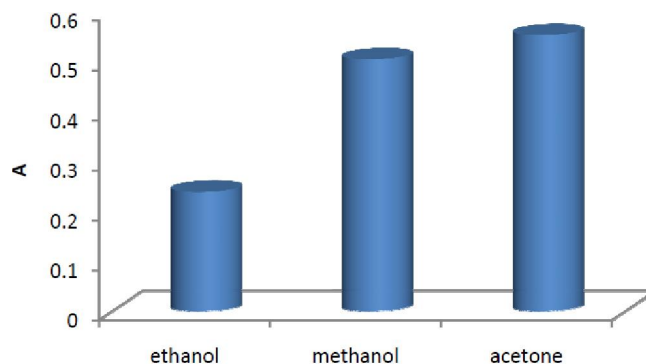**Figure 2 : Concentration of chelating reagent on the absorbance of cadmium**

**Figure 3 : Effect of type of extraction solvent on the absorbance of cadmium**

Figure 4 : Effect of volume of extraction solvent on the absorbance of cadmium

Figure 5 : Effect of type of dispersive solvent on the absorbance of cadmium

Type of dispersive solvent

of CCl_4, the volume of sedimented phase increased. As can be seen in Figure 4, the absorbance increases with increasing the volume of CCl_4 from 40 to 50 µl. However, the absorbance decreased when the volume of CCl_4 was larger than 50 µl. It seems that 0.6 ml of acetone cannot disperse superabundant extractant to form fine droplets well, resulting in a decrease in extraction efficiency. Therefore, 50 µl was chosen as the optimal extraction solvent volume.

The dispersive solvent should be miscible in both the aqueous and organic phases. In this study, acetone, ethanol and methanol were used as dispersive solvents. Figure 5 shows the results for extraction of cadmium (II) using the Carbon tetrachloride extraction solvent in combination with different dispersive solvents. According to the results, the use of acetone and Carbon tetrachloride as dispersive and extraction solvents, respectively, resulted in the highest analytical responses for cadmium. Thus, these solvents were used to prepare the ternary mixture.

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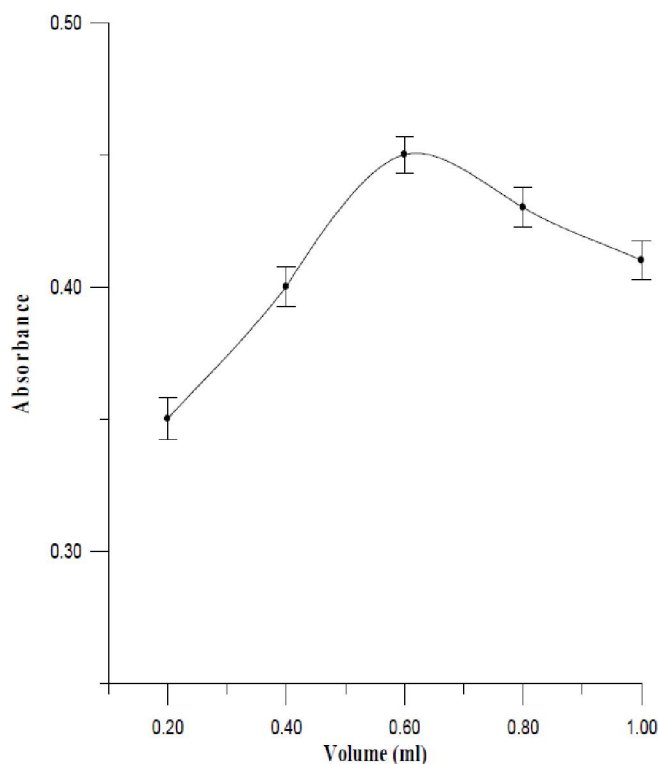
Figure 6 : Effect of volume of dispersive solvent on the absorbance of cadmium

Effect of disperser solvent volume

The experimental conditions were fixed and included the use of different volumes of acetone (0.20, 0.40, 0.60 and 1.0 ml) containing 50 μl of CCl_4. It was found that the absorbance increased by increasing the volume of acetone up to 0.60 ml and then decreased with further increasing of acetone volume (Figure 6). This phenomenon can be explained as follows: CCl_4 was not dispersed well when using a smaller volume of acetone and thus the extraction efficiency was lower. On the contrary, using a larger volume of acetone would result in an increase of solubility of the analyte - ligand complex in aqueous phase and consequentially the extraction efficiency decreased. Hence, 0.60 ml of acetone was selected for further investigation.

Extraction time

In DLLME, the extraction time is defined as the time between injecting the mixture of extraction and centrifugation. No significant difference in the analytical response to cadmium was observed by varying the extraction time from 0.0 to 14.0 min. This result is due to the increased contact area between the aqueous phase and the solvent extractor with the
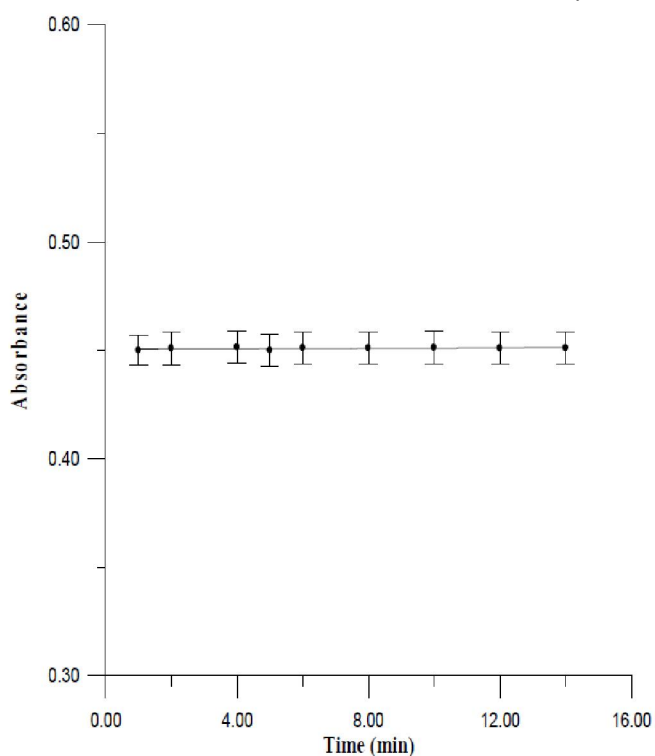
Figure 7 : Effect of extraction time on the absorbance of cadmium

formation of droplets after the turbidity. Thus, the passage of the metal-2APDSBA complexes from the aqueous phase to the extraction solvent is fast. The results are given in Figure 7
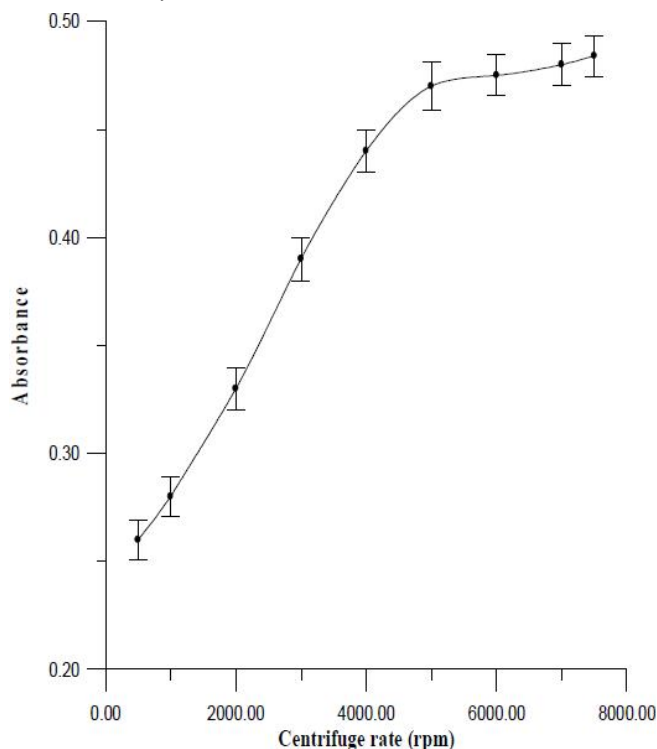
Effect of centrifuge rate

The centrifugation rate employed in the DLLME system was also studied. For this study, we used rates ranging from 500 to 7500 rpm. Figure 8 shows the influence of centrifugation rate on the extraction of cadmium. The results indicated that a rate of 5000 rpm is sufficient for the rich phase to be deposited on the bottom of the test tube. Therefore, we employed a centrifugation rate of 5000 rpm in subsequent experiments procedure.

Effect of centrifuge time

The centrifugation time employed in the DLLME system was also studied. For this study, we used periods ranging from 1.0 to 5.0 min at 5000 rpm. The results shown in figure 9 indicated that a period of 3.0 min is sufficient for the rich phase to be deposited on the bottom of the test tube. Therefore, we employed a centrifugation period of 3.0 min in subsequent experiments to increase the speed of this procedure.
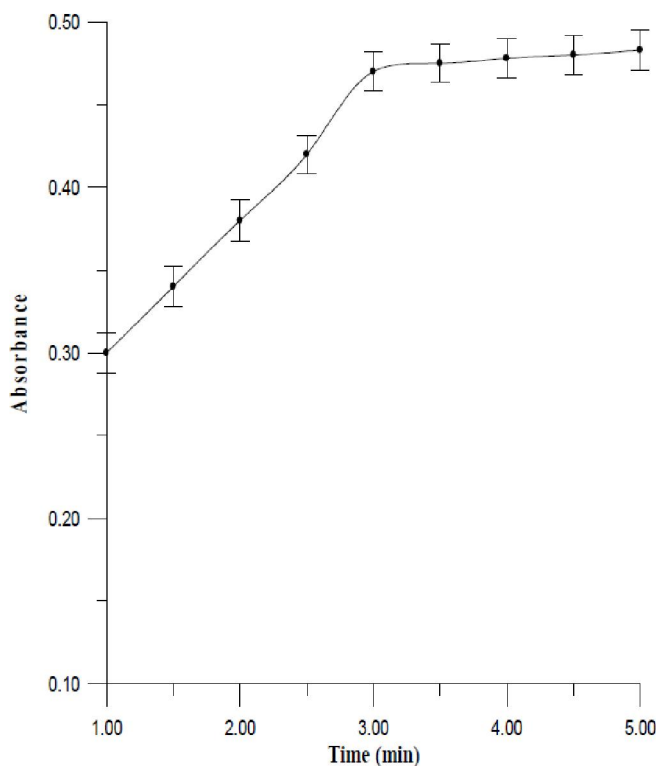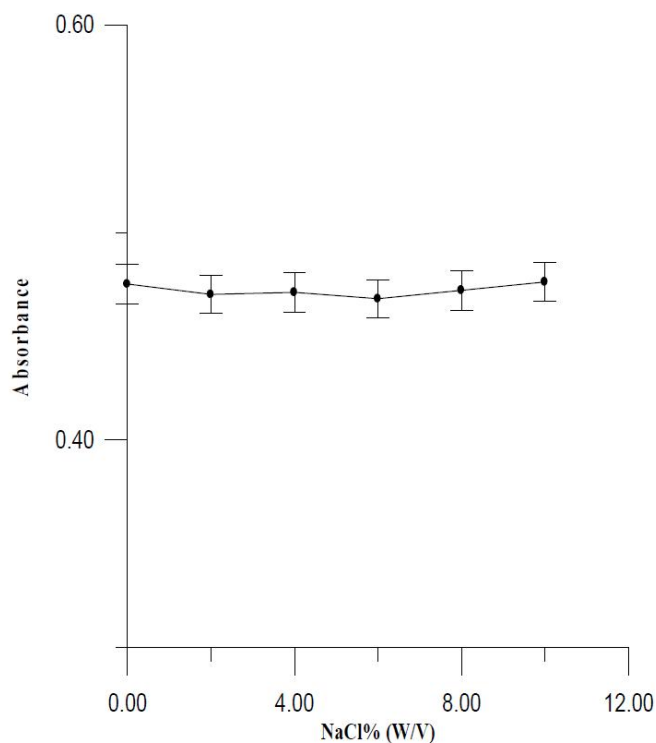
Figure 8 : Effect of centrifuge rate on the absorbance of cadmium

Figure 10 : Effects of salt on the absorbance of cadmium

and therefore improve its extraction because of salting-out effect. To investigate the influence of ionic strength on DLLME performance, various experiments were performed by adding different amounts of NaCl (0–10%, w/v) and the rest of the experimental conditions were kept constant. As can be seen in figure 10, the salt addition had no remarkable effect on extraction efficiency for Cadmium in DLLME. Therefore, salt addition was not used in the subsequent experiments.

Effect of coexisting ions

The effects of common coexisting ions in natural water samples on the recovery of cadmium were studied. In these experiments, 10.0 ml of solutions contains 1 μg L^{-1} of cadmium and various amounts of interfering ions were treated according to the recommended procedure. A given spices was considered to interfere if it resulted in A ± 5% variation of the AAS signal. The results are given in TABLE 1.

Figures of merit

TABLE 2 summarizes analytical characteristics of the optimized method, including limit of detection, reproducibility and enhancement factor. The limit of detection (LOD =0.05 μgL^{-1}) was calculated as 3 S_b/m (S_b: standard deviation of the blank sig-

Figure 9 : Effect of centrifuge time on the absorbance of cadmium

Effects of salt

The addition of salt to aqueous samples usually decreases the solubility of analyte in aqueous phase

TABLE 1 : Effect of coexisting ions on the recovery of cadmium

Interferent	Interferent / Cadmium ratio	% Recovery
Na^+	10000	97.46 ± 2.4
$K^{+\cdot}$	10000	96.86 ± 1.2
NH_4^+	10000	98.96 ± 1.4
Mg^{2+}	10000	92.36 ± 2.2
$Cr^{2+\cdot}$	1000	93.62 ± 1.5
$Zn^{2+\cdot}$	1000	96.60 ± 2.7
$Ni^{2+\cdot}$	1000	98.68 ± 2.3
Ba^{2+}	1000	102.0 ± 3.4
Mn^{2+}	100	94.06 ± 1.7
Pb^{2+}	100	92.10 ± 2.4
Cu^{2+}	10	96.80 ± 1.6
Ag^+	2	93.71 ± 1.3
Cl^-	10000	97.46 ± 2.1
CH_3COO^-	10000	94.20 ± 1.5
SO_4^-	10000	97.03 ± 2.7
SCN^-	10000	100.1 ± 1.8

Application

In order to evaluate the accuracy and recovery of the proposed method, standard reference materials SRM 2670 (freeze-dried urine) was analysed for its cadmium content (National Institute of Standard and Technology NIST, USA). The proposed DLLME–FAAS methodology was applied to the determination of cadmium in plasma and real water samples. The validated method has been successfully used to quantify cadmium concentrations in human plasma. Cadmium was added to plasma as internal standard and DLLME-FAAS method was used for preconcentration and determination of cadmium in plasma. The recovery of cadmium from plasma containing 5 μg L^{-1} of internal standard was 89%. Esfahan industrial park Wastewater and Toos industrial park Wastewater were collected from the industrial park of Esfahan and mashhad respectively and were analyzed by DLLME combined with FAAS for determination of cadmium. The concentration of

TABLE 2 : Analytical characteristics of DLLME for determination of cadmium

Parameter	Analytical feature
Linear range (μg L^{-1})	0.05 – 7.5
Correlation coefficient (r^2)	0.9985
Limit of detection (μg L^{-1})(3σ, n=10)	0.05
Repeatability (RSD%) (n=10, 0.2 μg L^{-1})	3.9
Repeatability (RSD%) (n=10, 2μg L^{-1})	3.1
Enrichment factor (EF)	168.6
Sample Volume (ml)	10
Microdrop volume (μl)	50
Sample introduction Volume (μl)	50
Sample preparation time (min)	3.0
Recovery (%)	84.3

nals; m: slope of the calibration curve after preconcentration). The calibration graph was linear in the range of 0.05–7.5 μg L^{-1} with correlation coefficient of 0.9985 under the optimum conditions of the recommended procedure. The relative standard deviation for (RSD) for ten replicate measurement of 0.2 μg L^{-1} and 2 μg L^{-1} cadmium was 3.9 and 3.1% respectively. The enrichment factor (EF) was obtained from the slope ratio of calibration graph after and before extraction, which was about 168.6.

The extraction recovery (*R*%) was 84.3% which was calculated by equation (1).

R%= (Vdrop / Vsolution) × EF × 100   **(1)**

cadmium in the Esfahan industrial park Wastewater and Toos industrial park wastewater samples were determined to be 32.3 ± 1.2 μg L^{-1}, 30.6 ± 2.4 μg L^{-1}, respectively (TABLE 2). Esfahan industrial park Wastewater and Toos industrial park wastewater samples were spiked with cadmium standards to assess matrix effects. The relative recoveries of cadmium from Esfahan industrial park Wastewater and Toos industrial park wastewater at spiking level of 5 μg L^{-1} were 96 and 94 %, respectively (TABLE 3). These results demonstrated that the Esfahan industrial park Wastewater and Toos industrial park wastewater samples matrixes, in our present con-

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TABLE 3 : Determination of cadmium in real samples and reference material

Sample	concentration of Cd(II) Mean±SD[a] (µg/L)	Added Cd(II) (µg/L)	Found Cd(II) Mean±SD(µg/L)	Relative Recovery(%)
Wastewater 1[b]	32.3 ± 1.2	5.0	37.1 ± 2.3	96
Wastewater 1[c]	30.6 ± 2.4	5.0	35.3 ± 1.8	94
Plasma	Not found	5.0	4.45± 0.18	89
Reference Material	Certified value		Measured value	Recovery %
NIST 2670[d]	0.088±0.003 (mg L^{-1})		0.080±0.002 (mg L^{-1})	91

[a] **Standard Deviation (n=3),** [b] **Esfahan industrial park in Esfahan, Iran,** [c] **Toos industrial park in mashhad, Iran, d from National Institute of Standard and Technology NIST (USA)**

text, had little effect on DLLME of cadmium.

CONCLUSION

Herein, a method of DLLME coupled to FAAS using 2-[2-(2-aminophenyl)disulfonyl)benzenamine] as complexing reagent was successfully developed for the determination of cadmium by FAAS. This procedure is suitable for preconcentration and extraction of cadmium from aqueous samples, that giving increased sensitivity of determinations and therefore lowering the limits of detection. The LOD for cadmium is 0.05 µgL^{-1}. The proposed method was proved to be fast, sensitive, low cost and easy to operate and was applied to the extraction and analysis of cadmium in plasma and real water samples with satisfactory results.

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