Application of Dispersive Liquid-Liquid Microextraction in Narrow-Bore Tube for Preconcentration and Spectrophotometric Determination of Cadmium in Aqueous Samples

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ABSTRACT
A novel liquid phase microextraction based on narrow bore-dispersive liquid-liquid microextraction (NB-DLLME) is introduced and developed for preconcentration and extraction of cadmium in aqueous samples using spectrophotometry. Unlike previous NB-DLLME methods, in this research work a solvent with a density higher than water (carbon tetrachloride) is used as extractant solvent. The effects of different parameters such as kind and volume of extractant and dispersive solvent, internal diameter and length of narrow bore, and concentration of salt are studied and optimized. Under optimum conditions, dynamic range of calibration curve was linear in the range of 50–900 μg.L⁻¹. The detection limit and relative standard deviation were calculated to be 6.3 μg.L⁻¹ and 6.4%, respectively. Keywords: Narrow bore-dispersive liquid-liquid microextraction, spectrophotometry, water analysis, cadmium

INTRODUCTION
The environmental pollution by heavy metals is of great concern nowadays [1, 2]. Heavy metal contamination can cause severe toxicological effects on living organisms [3]. Water pollution by heavy metals is causing serious ecological problems in many parts of the world [4, 5]. Industrial development and urbanization are two important reasons of contamination of waters and other environmental sources with heavy metals [6]. Cadmium is one of the most hazardous elements to human health, which is listed as the sixth most poisonous substance jeopardizing human health [7,8]. Cadmium can be poisonous even at low concentrations. When entered to human body, it can bind to the sulfhydryl groups of critical mitochondria.
proteins, which lead to mitochondria dysfunction and cell poisoning [9]. Widespread presence of cadmium in the environment comes from anthropogenic activities. Therefore, many analytical techniques have been developed for preconcentration and determination of trace amounts of Cd [10, 11].

UV-visible spectrophotometry is an available and easy-to-operate analytical instrument, and is one of the most extensively used detection techniques for various analytes including metals [12, 13]. However, due to the insufficient sensitivity and matrix interference of samples, it cannot be used for direct determination of trace heavy metals in samples. Therefore, for determination of traces of metals and achievement of low detection limits, a preconcentration and/or isolation step is necessary before introduction of samples to UV-visible spectrophotometry. Many methods have been proposed for preconcentration and separation of metals such as liquid–liquid extraction [14-16], cloud point extraction [12, 17], co-precipitation [18-21], ion exchange [22, 23], and membrane techniques [24-26]. The traditional liquid–liquid extraction method and other conventional separation methods are generally time-consuming and labor-intensive, and usually consume large amount of toxic organic solvents (with high purity), which after extraction procedures, have to be disposed of properly.

In the recent decades, liquid phase microextraction methods have been introduced and developed as adequate replacements for traditional liquid-liquid extraction [27, 28]. The most important feature of microextraction techniques is minimization of extraction methods, which leads to reducing the consumption of organic solvents. Some microextraction methods that have been used in determination of Cd are dispersive liquid-liquid microextraction [29-31], solidified floating organic drop microextraction (SFODME) [32, 33], and single drop microextraction (SDME) [34, 35].

Dispersive liquid-liquid microextraction is one of the most popular liquid phase extraction methods [36]. This technique is based on extension of contact surface between aqueous and organic phases. This purpose is achieved by utilizing a third solvent, usually known as dispersive solvent. Dispersive solvents must be soluble in both aqueous and organic solvent, and therefore, methanol, ethanol, acetone, and acetonitrile are usually applied as dispersive solvents. Due to high performance of DLLME, many DLLME methods have been developed [37]. Some DLLME methods try to eliminate dispersive solvent like vortex-assisted dispersive liquid-liquid microextraction (VA-DLLME) [38] and ultrasound-assisted dispersive liquid-liquid microextraction (UA-DLLME) [39], and some DLLME methods try to eliminate centrifugation step like in syringe dispersive liquid-liquid microextraction (IS-DLLME) [40] and narrow bore dispersive liquid-liquid microextraction (NB-DLLME) [41]. NB-DLLME is usually applied with solvents with densities lower than water. In our previous work, we applied IS-DLLME and NB-DLLME for determination of BTEX, with nonanol used as extractant solvent [42]. In this paper, we applied NB-DLLME for determination of traces of Cd. Instead of using solvents with densities lower than water, which is common in NB-DLLME, we used solvents with densities higher than water like chloroform and carbon tetrachloride.
For ease in operation and collection of organic phase at the bottom of tube, a conventional burette was used as the extraction unit. The details of this procedure are illustrated in Figure 1. 1,5-Diphenylthiocarbazone, mostly known as dithizone (DTZ), was used as a well-known and most applied chelating agent for Cd. Dithizone forms a red or pink complex with Cd [43].

**EXPERIMENTAL**

**Reagents and chemicals**

Cd(NO₃)₂ and 1,5-diphenylthiocarbazone (dithizone), were purchased from Merck (Germany). Other reagents and solvents were purchased from the same company, and were of analytical grade. A standard stock solution of Cd containing 1000 mg L⁻¹ of Cd was prepared by dissolving 0.2771 g Cd(NO₃)₂ in a doubly distilled water in 100 mL measuring flask, and was stored in refrigerator. Working standard solutions were prepared daily by diluting the stock solution.

To remove the oxidation products of dithizone, purification step was necessary. For this purpose, 0.5006 g dithizone was dissolved in 50 mL chloroform, transferred into a separating funnel, and extracted with four portions of 1% ammonia (20 mL each time). To the ammoniac
solution containing dithizone as the ammonium salt, HCl (0.5 mol.L\textsuperscript{-1}) was added to precipitate dithizone. Then dithizone was extracted with 60 mL of chloroform in three steps. The chloroform phase was washed with two portions of distilled water and then was transferred to a beaker. After evaporation of chloroform under fume hood in room temperature, 0.05 g of purified dithizone powder was dissolved in 10 mL carbon tetrachloride (extractant solvent) in a colored measuring flask. This stock solution, which contained 0.019 mol.L\textsuperscript{-1} dithizone was stored in refrigerator, and working solutions were prepared weekly by diluting this solution. Doubly distilled water was used throughout all the procedures.

**Instrument**

A UV/visible spectrophotometer, UV-160 (Shimadzu, Japan) equipped with two 10 µL microcells (Starna, UK), was used for measuring the absorbance and recording the spectra.

**Narrow bore-dispersive liquid-liquid microextraction procedure**

The proposed NB-DLLME method is quite simple and effective, and only needs a narrow bore as the extractant unit; for simplicity, a simple 20-mL laboratory burette (100 cm × 0.5 cm) was used. First, it was necessary to prepare the sample solution. Therefore, 18 mL of the sample solution containing an appropriate amount of Cd (in the range of 50-900 μg.L\textsuperscript{-1}), alongside with 6 mL sodium potassium tartrate (20% w/v) as reducing agent and 1.5 mL dimethylglyoxime (1% w/v) and 3 mL hydroxylamine hydrochloride (10% w/v) as masking agents was prepared. To adjust pH of sample solution to pH≥10, 0.5 mL ammonia (1% w/v) and 0.5 mL sodium hydroxide (1% w/v) was added to it. This solution was transferred to a 20-mL burette. In a vial, 200 μL carbon tetrachloride (extractant solvent) containing 0.019 mol.L\textsuperscript{-1} dithizone was mixed with 300 μL methanol (dispersive solvent). This binary solution was sucked into a 2-mL glass syringe and was injected into the aqueous solution on top of the burette. Since the rapid injection will lead to agglomeration of the extractant at the up of the sample solution in burette, therefore the injection was carried out during 30 seconds. Immediately after injection of binary solution, a cloudy solution was formed on top of the sample solution containing tiny droplets of the extractant dispersed throughout the aqueous solution. Due to higher density of carbon tetrachloride, this cloudy solution moved down the tube, and the analyte was extracted into fine droplets of extractant through the tube. In less than one minute almost all the fine extractant droplets were settle at the bottom of burette. Then the extractant phase was easily collected in a vial by opening the burette valve. 10 μL of extractant was transferred to the microcell, and its absorbance was recorded against blank reagent at 520 nm.

**RESULTS AND DISCUSSION**

**Selection of extractant solvent**

There are two important issues involving in choosing extractant solvent in dispersive liquid-liquid microextraction, being insoluble in water and its ability in extraction of analytes.
In NB-DLLME usually solvents with densities lower than water are applied as extractant. Here, we applied NB-DLLME with a solvent of higher density than water as extractant. Due to strong dipole interaction, solubility of dithizone is very good in chlorinated solvents [44]. Therefore the ability of chloroform, dichloromethane, carbon tetrachloride, and tetrachloroethylene as the extractant solvents were investigated. For this purpose, 100 μL of each solvent containing 0.019 mol.L\(^{-1}\) of dithizone was mixed using 500 μL methanol. This binary solution was applied for extraction of 800 μg.L\(^{-1}\) Cd in 10 mL sample solution. According to the data obtained, showed in Figure 2, carbon tetrachloride had the best absorbance. Therefore, this solvent was chosen as the extractant solvent.

**Effect of volume of extractant solvent**

Volume of extractant solvent can affect the enrichment factor (EF), repeatability, and the volume of extractant phase collected at the end of extraction at the bottom of burette. Thus different volumes of carbon tetrachloride were investigated. For this purpose the experiment condition were the same as previous step, only the volume of extractant (carbon tetrachloride) was changed from 100-700 μL (containing dithizone in the range of 0.0019 to 0.0133 mmole). Absorbance of Cd-DTZ was increased slightly with addition of extractant from 100 to 200 μL but after 200 μL, the absorbance showed a decrease, which is probably due to dilution of complex in more volume of extractant. Collection of extractant was a little difficult below 200 μL, accordingly, 200 μL carbon tetrachloride containing 0.038 mmole dithizone was chosen as the best volume of extractant solvent.
Selection of dispersive solvent

DLLME procedures are based on extension of surface contact between aqueous and organic phases with the help of tiny droplets of extractant dispersed throughout the aqueous phase. Usually, this is achieved by utilization of dispersive solvent, which must be soluble in both aqueous and organic phases. Different solvents (methanol, ethanol, acetone, and acetonitrile) were investigated for their ability in dispersing carbon tetrachloride in sample solution. 500 μL of each dispersive solvent was mixed with 200 μL of carbon tetrachloride as extractant, and they were subjected to the same NB-DLLME procedure. The data obtained showed that methanol had the best absorbance for analyte, and therefore, methanol was the chosen dispersive solvent (Figure 3).

Effect of volume of dispersive solvent

Volume of dispersive solvent is very important in DLLME because it can affects the size of extractant droplets, polarity of aqueous phase, and volume of collected extractant at the end of DLLME procedure. All these parameters are vital for the microextraction yield. Therefore, it was necessary to study and optimize the best volume for dispersive solvent. For this purpose, different volumes of methanol as dispersive solvent (100-600 μL) were investigated (Figure 4). In volumes less than 300 μL, large droplets of extractant were formed. Consequently, efficiency of microextraction and absorbance of complex tended to be low. In volumes more than 300 μL, a decrease was observed in absorbance of Cd-DTZ complex, that is probably because in high concentrations of methanol, the polarity of aqueous phase decreases which leads to more solubility of organic phase in aqueous phase. Therefore, 300 μL methanol was chosen as the best volume for dispersive solvent.
To investigate the effect of volume of sample solution on extraction procedure, NB-DLLME was carried out in different burettes with various lengths and internal diameters, as the extractant unit. Lengths of burettes were in range of 47-100 cm with internal diameters of 0.2-0.5 cm, which had the capacity of 6-20 mL of sample solution. For this purpose, two set of experiments were carried out. First, a set of sample solutions were prepared which had the same concentration of analyte in different volumes; meaning 800 μg.L⁻¹ of Cd in sample solutions with volumes ranging from 5 to 20 mL. Then a second set of sample solutions with

**Figure 4.** Effect of volume of dispersive solvent on extraction of Cd-DTZ. NB-DLLME conditions: Cd, 800 mg.L⁻¹; dithizone, 0.019 mol.L⁻¹; sample volume, 15 mL; extractant, carbon tetrachloride; carbon tetrachloride volume, 200 μL; pH≥10; dispersive solvent, metha

**Figure 5.** Effect of volume of sample solution on recovery of 800 μg.L⁻¹ (♦) and 5 μg (●) Cd. NB-DLLME conditions: dithizone concentration, 0.019 mol.L⁻¹; extractant, carbon tetrachloride; carbon tetrachloride volume, 200 μL; pH≥10; dispersive solvent, methanol; volume of dispersive solvent, 300 μL

**Effect of volume of sample solution**

To investigate the effect of volume of sample solution on extraction procedure, NB-DLLME was carried out in different burettes with various lengths and internal diameters, as the extractant unit. Lengths of burettes were in range of 47-100 cm with internal diameters of 0.2-0.5 cm, which had the capacity of 6-20 mL of sample solution. For this purpose, two set of experiments were carried out. First, a set of sample solutions were prepared which had the same concentration of analyte in different volumes; meaning 800 μg.L⁻¹ of Cd in sample solutions with volumes ranging from 5 to 20 mL. Then a second set of sample solutions with
various volumes were prepared and to all of them 5 μg of Cd was added to study the effect of mass transfer in the microextraction procedure. After microextraction procedure, the recovery of analyte was calculated. Based on the obtained results, presented in Figure 5 we decided to choose 18 mL in a 100 × 0.5 cm burette for the optimized sample solution.

**Effect of salt concentration**

The effect of ionic strength on efficiency of extraction can be explained by the salting-out effect. According to the salting-out phenomena, water molecules form hydration spheres around the salt ions that reduce the concentration of available water to dissolve analyte molecules and thus analyte molecules will be forced to enter extractant solvent. On the other hand, addition of a salt may lead to changes in the physical properties of the disperse film of extractant in the solution through increasing the density of sample solution. To investigate the effect of ionic strength of sample solution on extraction of Cd-DTZ, different sample solutions containing various concentrations of KCl (0-5% w/v) were prepared and the same NB-DLLME was applied on them. It was observed that with addition of salt the extraction efficiency decreases. Therefore, the subsequent NB-DLLME procedures were carried out without addition of a salt.

**Effect of interferences ions**

The recovery of Cd with the NDLLME, in presence of interference ions were studied under optimum condition. The results of these experiments are presented in Table 1. To reduce the effect of interferences, masking agents were employed (dimethylglyoxime and hydroxylamine hydrochloride). The studied ions were Fe$^{3+}$, Zn$^{2+}$, Pb$^{2+}$, Na$^+$, and Ca$^{2+}$. For this purpose, a fixed amount of foreign ions was added to a sample solution containing 800 μg.L$^{-1}$ of Cd. After NB-DLLME procedure, the recovery of Cd was calculated. As can be seen from the results, the recovery of analytes were higher than 91%.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Added as</th>
<th>Concentration (mg.L$^{-1}$)</th>
<th>Recovery of Cd (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe$^{3+}$</td>
<td>NO$_3^-$</td>
<td>10</td>
<td>92.75</td>
</tr>
<tr>
<td>Zn$^{2+}$</td>
<td>NO$_3^-$</td>
<td>10</td>
<td>95.33</td>
</tr>
<tr>
<td>Pb$^{2+}$</td>
<td>NO$_3^-$</td>
<td>10</td>
<td>91.78</td>
</tr>
<tr>
<td>Na$^+$</td>
<td>Cl$^-$</td>
<td>50</td>
<td>98.46</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>Cl$^-$</td>
<td>50</td>
<td>98.29</td>
</tr>
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</table>
Linear range, limit of detection and precision

Analytical figures of merit for the proposed method obtained under optimal condition are shown in Table 2. To determine the dynamic range of calibration curve, 15 standard solutions containing Cd in the range of 10 to 1500 μg.L⁻¹ were prepared. The dynamic range of calibration curve was found to be 50-900 μg.L⁻¹ (with 9 standard solutions). Detection limit (LOD) and limit of quantification (LOQ) were calculated based on 3S/b and 10S/b criteria, respectively, where S is standard deviation of 10 blank measurements and b is slope of calibration graph. The repeatability of the method, expressed as relative standard deviation (RSD), was calculated for five replicates of standard sample solution containing 500 μg.L⁻¹ of Cd. The enrichment factor (EF), calculated as the ratio of slope of calibration graphs after NB-DLLME (m_NB) and after classic extraction (m_CE) as shown in Eq. 1. For classic extraction, two portions of 5 mL carbon tetrachloride were applied for extraction of Cd in 18 mL sample in a funnel. The other condition were as NB-DLLME. EF was found to be 70 fold.

\[ EF = \frac{m_{NB}}{m_{CE}} \]  

A comparison between data obtained with NB-DLLME with those recently obtained with other liquid-phase microextraction methods for determination of Cd is summarized in Table 3.
Analysis of real samples

The proposed NB-DLLME procedure was applied on tap water for determination of Cd. Since no detectable analyte was observed, the samples were spiked with 3 different concentrations of Cd to investigate the matrix effect. The results obtained are shown in Table 4. As it can be seen, very good recoveries were achieved between 91.6 and 103.8%.

CONCLUSION

In this work, a novel, fast, economical, effective, and one-step narrow bore-dispersive liquid-liquid microextraction was developed for preconcentration and determination of traces of Cd in aqueous samples. Unlike previous NB-DLLMEs, which usually use organic solvents with lower density than water, here we used an organic solvents with a density higher than water (carbon tetrachloride). For ease in operation and collection of extractant at the end of NB-DLLME procedure, a simple 20-mL burette was used as extractant unit. Thus, centrifugation step was totally eliminated and the whole DLLME procedure took place in a one step and in less than 2 min. Analytical response of analyte was recorded with UV-visible spectrophotometry equipped with microcells. This technique is simple, fast, and reliable. Also being available and inexpensive instruments, the simple and fast NB-DLLME-UV-Vis method can be applied in every laboratory.

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REFERENCES


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