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## Separation/Preconcentration of Cadmium and Nickel by Dispersive Liquid-Liquid Microextraction: Application to Water and Plants

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### ABSTRACT

Dispersive liquid-liquid microextraction has been used for separation/preconcentration of nickel and cadmium as previous step to the determination of both ions by FAAS. The influences of analytical parameters, including pH, extraction solvent volume, disperser solvent type and its volume, concentration of chelating agent, on the quantitative recoveries of nickel and cadmium were investigated. Under the optimized conditions, a preconcentration factor of 60 and 82.5 were obtained for Ni and Cd, respectively. The proposed method was applied to the determination of these elements in water and plant samples with satisfactory results. The accuracy of the method was studied by analyzing certified reference materials.

**Keywords:** Nickel; Cadmium; Dispersive liquid-liquid microextraction; Waters; Plants.

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## INTRODUCTION

At the present time, pollution of the environment by toxic elements has been dramatically increased. Trace levels of heavy metals are widely distributed in environment due to soil erosion, industrial and agricultural processes [1,2]. Exposure to these toxic elements imposes risks not only to human health, but also to water, animals and microorganisms [3]. Ni and Cd, between others, are heavy metals of unquestionable toxicity [4-6]. Thus, determination of these toxic heavy metals in environmental and other related samples has a very important significance.

Trace element concentration levels in these type of samples are fairly low and the interference due to surrounding matrix cannot be always eliminated [7]. The concentration of the analytical elements is also often below the detection limit of the instrument. The widely used methods for separation and preconcentration of heavy metals include solid phase extraction (SPE) [8], the traditional liquid-liquid extraction (LLE), cloud point extraction (CPE) [9,10], etc. Recently, dispersive liquid-liquid microextraction (DLLME) as a simple, rapid preconcentration and separation technique have been reported [11-13]. 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 with 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.

In this work, a new, simple and rapid method based on DLLME was developed for extracting and preconcentrating nickel (Ni) and cadmium (Cd) in diverse samples prior to flame atomic absorption spectrometry (FAAS) analysis.

## EXPERIMENTAL

### Standard solutions and reagents

Stock standard solution for Ni(II) and Cd(II) ( $1000 \text{ mg L}^{-1}$ ) were supplied by Merck, Darmstadt, Germany. Standard solutions were prepared by appropriate dilution of the stock solutions daily.

High purity water (resistivity  $18.2 \text{ M}\Omega \text{ cm}$ ) obtained by a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout this work. 2,2'-Bis(di-2-pyridinyl-methylene)-thiocarbohydrazone (DPTH) solution in DMF was prepared by dissolving solid reagent samples prepared and purified by the authors[14].

All the other reagents including extractants and disperser solvents were analytical-grade reagents, as well as the reagents mentioned above. Acetate buffer solution was prepared to adjust pH values for the extraction of Ni and Cd.

## Instrumentation

Phase separation was achieved with a centrifuge Selecta Centromix in 15 mL calibrated conical tubes. A Varian Model SpectrAA 50 (Mulgrave, Victoria, Australia) flame atomic absorption spectrometer was used for the analysis with the appropriate nickel and cadmium hollow cathode lamps. The operating parameters were set as recommended by the manufacturer. Atomic absorption measurements were carried out in an air-acetylene flame. The following conditions were used: absorption line Ni: 232.0 nm; slit widths: 0.2 nm; and lamp currents: 4 mA. Absorption line Cd: 228.8 nm; slit widths: 0.5 nm; and lamp currents: 4 mA.

## Sample analysis

The accuracy of the method for determination of nickel and cadmium content was checked by analyzing the reference standard material TMDA 54.4, Estuarine water (CRM LGC6016), Eau de mer (CASS-5), Riverine water (SLRS-5). These samples were analyzed by standard addition method and in the case the content of these analytes were under detection limits of the method, recovery studies were carry out.

The proposed method was also evaluated by analysis of Ni and Cd in spiked plant samples. The Cd and Ni concentrations in all the original samples were below the detection limit. For this purpose, standard solutions containing Cd and Ni were added to 0.3–1.2 g of diverse plants, and the resulting materials were mineralized by reflux digestion, then evaporate to eliminate excess of acid, adjusted pH and diluted at convenient volume. Standard addition method was used in all instances.

Natural waters were collected in polypropylene bottles previously cleaned by soaking for 24 h in 10% (v/v) nitric acid and finally rinsed thoroughly with ultra-pure water before use.

## DLLME procedure

For DLLME under optimum conditions, 10 mL analyte solution containing variable amounts of nickel and cadmium, 3 mL acetate buffer solution pH 5.4, 1 mL of 0.05% DPTH solution in DMF as chelating agent was placed in a 15 mL screw cap glass test tube. Then, 0.5 mL of ethanol (as disperser solvent) and 0.3 mL of chloroform (as extraction solvent) were rapidly injected into a sample solution by using a microsyringe. A cloudy solution was formed in the test tube and separation of the phases was achieved by centrifugation at 3800 rpm for 5 min. After this process, the organic phase was sedimented in the bottom of conical test tube. After removal of the whole aqueous solution, the extraction phase was evaporated in a water bath to 60°C and then diluted with HNO<sub>3</sub> 0.1 M and aspirated into the FAAS.

## RESULTS AND DISCUSSION

In order to obtain a high preconcentration factor, the effect of different parameters affecting the complex formation and extraction conditions, such as kind of extraction and

disperser solvent and their volume, pH, concentration of the chelating agent, and salt addition, was optimized. One variable at a time optimization was used to obtain the optimum conditions for the DLLME.

### **pH study**

The separation of metal ions by DLLME involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of the sedimented phase, thus, obtaining the desired preconcentration. pH plays a unique role on metal chelate formation and subsequent extraction. The effect of pH on the complex formation and extraction of cadmium and nickel from samples was studied in the range of 3.0–8.0 by using acetate or phosphate buffer. The results reveal that the absorbance is nearly constant in the pH range of 4.5–6.0 for Ni and 4.0–6.0 for Cd. Thus, the value of pH 5.4 was selected for the following experiments. Also, the influence of 0.2 M acetate buffer solution amount was investigated for variation of volume added from 2 to 4 mL. A volume of 3 mL was selected as the optimum value for subsequent work.

### **Effect of chelating reagent (DPTH) concentration**

The effect of DPTH concentration on the absorbance was examined using increasing volumes of 0.05% DPTH from 0.5 to 2 mL. The results showed that the change of DPTH concentration in the studied range has not significantly effect on analytical signals, thus the volume of 1 mL was used in other experiments.

### **Effect of DLLME parameters**

#### **Effect of type and volume of extractant**

Careful attention should be paid to the selection of the extraction solvent. It should have a higher density than water, extraction capability of the interested compounds and low solubility in water. Chloroform, carbon tetrachloride and dichloromethane were compared in the extraction of nickel and cadmium. Results showed that the maximum extraction recovery was obtained by using chloroform.

To examine the effect of extraction solvent volume, solutions containing different volumes (0.2–0.7 mL) of chloroform were subjected to the same DLLME procedure. When the volume of extraction solvent was increased, the volume available for the measurement also increased, but the enrichment factors decreased. Thereby, in the following studies, the optimum volume of 0.3 mL was selected for the extraction solvent.

#### **Effect of type and volume of disperser solvent**

The role of a disperser is dispersion of an extraction solvent into aqueous sample to make extensive contact area between them and facilitating the mass transfer of analyte from water to organic solvent which causes considerable acceleration in the extraction of analytes.

Miscibility of disperser solvent with extraction solvent and aqueous phase is the main point for selection of disperser solvent. Therefore in this section the ability of ethanol and methanol was investigated between 0.5-1.5 mL of each one. The results (figure 1) show light differences between disperser solvents. As can be seen from this figure 1 c) better results were obtained by using 0.3 mL chloroform and ethanol as disperser solvent. A 0.5 mL ethanol was selected for subsequent studies.

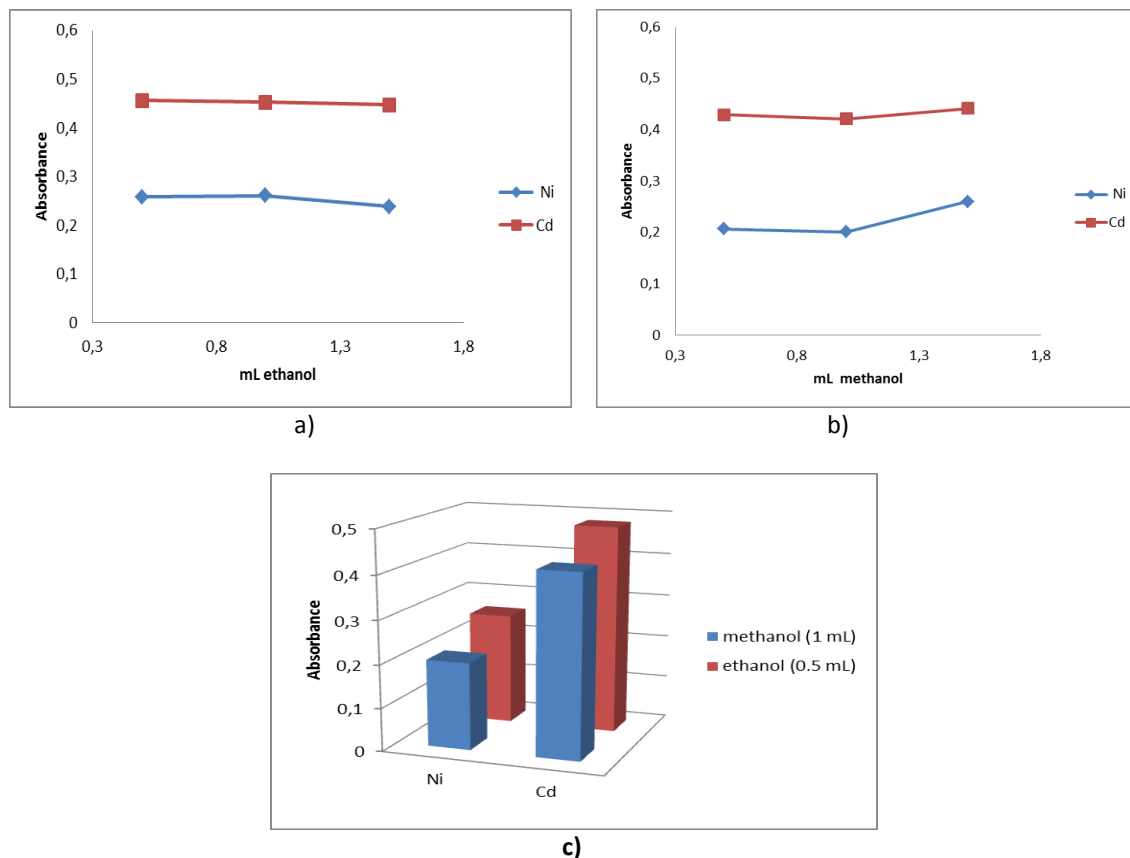


Figure 1. Influence of disperser solvent: a) ethanol; b) methanol; c) comparison between two dispersers

### Interferences

Because DPTH is a versatile chelating agent, interferences may occur due to the competition of other heavy metal ions and their subsequent co-extraction with Cd(II)/Ni(II). For this purpose, the effect of typical potential interfering ions was investigated. The tolerance limit was defined as the concentration of added ion that caused less than  $\pm 5\%$  relative error in the determination of Ni and Cd. The results are shown in table 1.

**Table 1. Tolerance of foreign ions on the determination of Ni and Cd by DLLME-FAAS. [Ni<sup>2+</sup>]=100 ppb; [Cd<sup>2+</sup>]=100 ppb**

Tolerance ratio	Species [Ion]:[Cd]	Species [Ion]:[Ni]
>500	F <sup>-</sup> , K <sup>+</sup> , I <sup>-</sup> , Mn <sup>2+</sup> , Ca <sup>2+</sup> , Ba <sup>2+</sup> , SO <sub>4</sub> <sup>=</sup> , HCO <sub>3</sub> <sup>-</sup>	Fe <sup>3+</sup> , Ca <sup>2+</sup> , Ba <sup>2+</sup> , Mn <sup>2+</sup> , K <sup>+</sup> , I <sup>-</sup> , F <sup>-</sup> , SO <sub>4</sub> <sup>-2</sup> , HCO <sub>3</sub> <sup>-</sup>
>250	Al <sup>3+</sup> , Cr <sup>3+</sup>	
>100		Hg <sup>2+</sup> , Cr <sup>3+</sup> , Al <sup>3+</sup>
>50		Cu <sup>2+</sup> , Bi <sup>2+</sup> , Zn <sup>2+</sup> , Co <sup>2+</sup>
>25	Hg <sup>2+</sup> , Cu <sup>2+</sup> , Bi <sup>3+</sup>	
>10	Co <sup>2+</sup> , Fe <sup>3+</sup> , Zn <sup>2+</sup>	

### Analytical figures of merit

Analytical figures of merit of the proposed DLLME-FAAS method were obtained under optimal conditions and summarized in table 2. In order to check for potential synergistic effects of the mixtures of two ions, calibration curves were made for nickel between 30-300 µg L<sup>-1</sup> with different amounts fixed of Cd (30, 60, 90 µg L<sup>-1</sup>). On the other hand calibration curves for cadmium were obtained with different amounts fixed of Ni (30, 60, 90, 200, 300 µg L<sup>-1</sup>). Similar slopes were obtained in all cases so, there are not synergistic effects from Cd to Ni and from Ni to Cd, for the levels assayed.

The preconcentration factor was determined as the ratio of the slopes of the linear section of the calibration graphs before and after preconcentration.

**Table 2. Analytical figures of merit**

	Ni	Cd
Dynamic range (µg L <sup>-1</sup> )	20-300	20-300
Regression equation	A=0.0006[Ni]+ 0.0774	A=0.0033[Cd]+0.0333
R <sup>2</sup>	0.9804	0.9598
Regression equation without extraction	A=1x10 <sup>-5</sup> [Ni]+ 0.0138	A=4x10 <sup>-5</sup> [Cd]-0.0011
Precision (% RSD) n=8		
Ratio 1:1 (Ni:Cd) 30:30	11.9	10.17
60:60	13.2	3.18
100:100	2.01	5.36
200:200	2.63	2.59
Precision (% RSD) n=8		
Ratio 2:1 (Ni:Cd) 200:100	5.1	2.41
Precision (% RSD) n=8		
Ratio 1:2 (Ni:Cd) 100:200	0.76	1.39
Preconcentration factor	60	82.5

### Analysis of standard reference materials

In order to assess the accuracy and validity of the presented procedure, the method was applied to the determination of nickel and cobalt in certified reference materials (TMDA 54.4, Estuarine water (CRM LGC6016), Eau de mer (CASS-5), Riverine water (SLRS-5), which were

analyzed according to the proposed method. It was found that analytical results were in good agreement with the certified values (table 3). Contents of Ni and Co in CASS-5 and SLRS-5 were under detection limits of the method, so different amounts of these ions were added to the samples for to verify if it is possible to determine Ni and Co under the proposed procedure. Good recoveries were obtained in all cases.

**Table 3. Analysis of certified reference materials for the determination of Ni and Cd with DLLME-FAAS method (Avg.  $\pm$  SD of three trials)**

Sample	Certified value ( $\mu\text{g L}^{-1}$ )		Found value ( $\mu\text{g L}^{-1}$ )		% Recovery	
	Ni	Cd	Ni	Cd	Ni	Cd
TMDA 54.4	337 $\pm$ 15.5	158 $\pm$ 8.5	350 $\pm$ 38	180 $\pm$ 47.6	103.8	113.9
Estuarine water. CRM LGC6016	186 $\pm$ 3	101 $\pm$ 2	240 $\pm$ 20	120 $\pm$ 10	129	118
	Added ( $\mu\text{g L}^{-1}$ )		Found ( $\mu\text{g L}^{-1}$ )		% Recovery	
	Ni	Cd	Ni	Cd	Ni	Cd
Eau de mer. CASS-5	20	20	21 $\pm$ 1	10 $\pm$ 5	105	50
	50	50	45 $\pm$ 3	56 $\pm$ 3	90	112
	100	100	112 $\pm$ 2	112 $\pm$ 5	112	112
	200	200	200 $\pm$ 2	197 $\pm$ 2	100	98
Riverine water. SLRS-5	20	20	10 $\pm$ 5	11 $\pm$ 4	50	55
	50	50	60 $\pm$ 5	53 $\pm$ 4	120	106
	100	100	102 $\pm$ 3	106 $\pm$ 3	102	102
	200	200	201 $\pm$ 2	180 $\pm$ 2	101	90

### Analysis of waters and plants

In view of the application of the method to the determination of nickel and cadmium in waters and plants samples, the ability to recover these elements from different samples spiked with nickel and cadmium were investigated. For this purpose, standard solutions containing different quantities of these elements were added to plant samples and the resulting materials were prepared as described under Experimental. For water analysis Standard additions method was used in all instances and the results were obtained by extrapolation. The results of these analyses are summarised in table 4. Good recoveries for the spiked samples were obtained.

**Table 4. Analytical results for Ni and Cd in plant and water samples (Avg.  $\pm$  SD of three trials)**

Sample	Added ( $\mu\text{g/g}$ )		Found ( $\mu\text{g/g}$ )		%Recovery	
	Ni	Cd	Ni	Cd	Ni	Cd
<i>Pinus</i> leaves	9.43	9.43	9.90 $\pm$ 1.5	9.90 $\pm$ 1.0	104.9	104.9
<i>Bignonia</i> leaves	10.00	10.00	10.99 $\pm$ 2.0	10.48 $\pm$ 1.5	109.9	104.8
Sample	Added ( $\mu\text{g L}^{-1}$ )		Found ( $\mu\text{g L}^{-1}$ )		%Recovery	
	Ni	Cd	Ni	Cd	Ni	Cd
Tap water	30	30	30 $\pm$ 2	31 $\pm$ 2	100	103
Sea water	30	30	32 $\pm$ 3	24 $\pm$ 5	106	80

## CONCLUSION

Green Chemistry is defined as the invention, design and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances [15]. Sample preparation by DLLME is a procedure that considered inside of green chemistry because of the small volumes of dissolvent employed. In this work, a simple, rapid, and sensitive DLLME preconcentration technique coupled with FAAS has been developed for the determination of cadmium and nickel in spiked plant and water samples. All variables that influence in the formation of the complexes Ni-DPTH and Cd-DPTH and then application of DLLME procedure have been optimized. Employing FAAS as detection technique, the detection limit obtained is in the order of  $\mu\text{g L}^{-1}$  for both analytes that by the direct method of FAAS is impossible to obtain because of the low sensitivity that present.

To study the accuracy of the proposed method, certified reference materials have been analysed with good agreements.

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