



Determination of Trace Amounts of Nickel in Food and Environmental Water Samples by Flame Atomic Absorption Spectrometry after Dispersive Liquid-Liquid Microextraction

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The dispersive liquid-liquid microextraction was combined with the flame atomic absorption spectrometry for the determination of nickel in water samples. 4-Benzyl piperidine dithiocarbamate potassium salt (4-BPDC), chloroform and ethanol were used as chelating agent, extraction solvent and disperser solvent, respectively. In this extraction method, a mixture of 500 μL ethanol and 100 μL chloroform was rapidly injected by syringe into the water sample containing nickel ions and 4-BPDC. Thereby, a cloudy solution was formed. After centrifugation, these droplets were settled at the bottom of the conical test tube. The settled phase was separated using a micro-syringe and diluted to 100 μL with ethanol. A microsample introduction system was employed for the nebulization micro-volume of diluted solution into flame atomic absorption spectrometry. Some effective parameters on extraction, such as extraction and disperser solvent type and their volume, salt effect and pH have been optimized. The calibration graph was linear in the range of 8-200 $\mu\text{g L}^{-1}$ with detection limit of 2.2 $\mu\text{g L}^{-1}$. RSD for ten replicate measurements was 1.9 %. The proposed method has been applied to the determination of nickel ions in food and environmental water samples with satisfactory results.

Key Words: Dispersive liquid-liquid microextraction, Preconcentration, Nickel, Food and environmental water samples, FAAS.

INTRODUCTION

Nickel is the metal component of the enzyme urease and as such is considered to be essential to plants and some domestic animals. More attention has been paid on the toxicity of nickel in low concentration, such as the fact that nickel can cause an allergic reaction and that certain nickel compounds may be carcinogenic¹. Thus, the determination of trace amounts of nickel from different matrices is of great importance. In many cases, the determination of heavy metals in environmental samples is notably difficult due to both the low levels of these metals in the samples and the high complexity of the sample matrices. Therefore the determination of this metal as Ni^{2+} ion often requires a method offering low detection limits. Although atomic spectrometric methods are powerful analytical tools for the determination of trace elements in environmental samples, preconcentration techniques combined with atomic absorption spectrometry are still necessary².

Liquid-liquid extraction (LLE)³, cloud point extraction (CPE)⁴⁻⁶ and solid phase extraction (SPE)⁷⁻¹¹ has been widely used for the preconcentration of nickel from water samples prior to its determination by flame atomic absorption spectrometry. However, that technique is rather time-consuming and

requires a large amount of sample. Separation and preconcentration based on dispersive liquid-liquid extraction (DLLME) offer a convenient alternative to more conventional extraction methods¹. This is a modified solvent extraction method and its acceptor-to-donor phase ratio is greatly reduced comparing with other extraction methods. In this method, the appropriate mixture of extraction solvent and disperser solvent is rapidly injected into aqueous samples containing analytes by syringe. Thereby, cloudy solution is formed. In fact, the cloudy state is because of the formation of fine droplets of extraction solvent, which has been dispersed among the sample solution. Then, this cloudy solution was centrifuged and the fine droplets were settled in the bottom of conical test tube. The determination of analytes in settled phase can be performed by instrumental analysis. In this extraction method, any component originally present in the solution that interacts with the fine droplets of extraction solvent directly or after previous derivatization reaction can be extracted from the initial solution and concentrated in the small volume of the settled phase. The advantages of DLLME method are simplicity of operation, rapidity, low cost, high recovery and enrichment factor. The dispersive liquid-liquid extraction methodology has been used to separate and preconcentrate organic compounds prior to their determi-

nation with chromatographic methods¹²⁻¹⁴. The DLLME has also been used for the extraction and preconcentration of metal ions after the formation of sparingly water-soluble complexes^{15,16}. In the present work we report on the results obtained in a study of the dispersive liquid-liquid microextraction of nickel after the formation of a complex with 4-BPDC and later analysis by flame atomic absorption spectrometry using microsample introduction. The proposed method was also applied to the determination of nickel in food and environmental water samples.

EXPERIMENTAL

A Thermo M series (Model: M5) flame atomic absorption spectrometer was utilized, equipped with a 50 mm burner head, deuterium background correction and air-acetylene flame. A nickel hollow cathode lamp (Thermo scientific S51214) was used as radiation source, operated at 15 mA with a monochromator spectral bandpass of 0.1 nm. For the nickel detection, the wavelength was set at 232 nm resonance line. The acetylene and the air-flow rates were 0.8 and 10 L min⁻¹, respectively. The Centurion scientific centrifuge (Model K240R, Arundel, UK) was used to accelerate the phase separation. The pH values were measured with a Metrohm pH-meter (Model: 691, Herisau, Switzerland), supplied with a glass-combined electrode.

All reagents used were of analytical grade. All solutions were prepared with ultra pure water. The nickel stock solution (1000.0 mg L⁻¹) was prepared by dissolving appropriate amounts of Ni (NO₃)₂ in ultra pure water. Working solutions were prepared from the stock solution by serial dilutions with ultra pure water. Chloroform, carbon tetrachloride, chlorobenzene, acetone, methanol and ethanol were of analytical-grade from Merck (Darmstadt, Germany). 4-Benzylpiperidine dithiocarbamate potassium salt (4-BPDC) was prepared according to the procedure described by Andac *et al.*¹⁷ 1.0 × 10⁻³ mol L⁻¹ fresh solution of 4-BPDC was prepared daily by dissolving the reagent in ultra pure water. A stock standard acetic acid/sodium acetate buffer solution (0.1 mol L⁻¹, pH 5) was prepared by dissolving an appropriate amount of ammonia in ultra pure water and neutralizing to pH 5 with hydrochloric acid. The pipettes and vessels used for trace analysis were kept in 10 % nitric acid for at least 24 h and subsequently washed 4 times with ultra pure water before use.

Dispersive liquid-liquid microextraction procedure: A 5 mL of ultra pure water was placed in a 10 mL screw cap glass test tube with conical bottom and spiked at levels of 10-200 µg L⁻¹ of nickel. Then 0.2 mL of 1.0 × 10⁻² mol L⁻¹ of 4-BPDC (as chelating agent) was added to this solution and the pH of the solution was adjusted by adding acetic acid/sodium acetate buffer solution (1.0 × 10⁻³ mol L⁻¹, pH 5.0). Then the mixture of 500 µL of ethanol (as disperser solvent) and 100 µL of chloroform (as extraction solvent) was injected rapidly into a sample solution by using 1 mL syringe and the mixture was gently shaken. A cloudy solution (water, ethanol and chloroform) was formed in a test tube. In this step, nickel ions were extracted into the fine droplets of chloroform. The mixture was then centrifuged for 5 min at 4500 rpm. After this process, the dispersed fine droplets of chloroform

were settled in the bottom of conical test tube (70 ± 2 µL). The settled phase was separated using a micro-syringe and diluted to 100 µL with ethanol and then injected into the microsample introduction (discrete nebulization) system of FAAS.

RESULTS AND DISCUSSION

In this research, DLLME combined with FAAS was developed for determination of nickel in water samples. In order to obtain a high recovery and enrichment factor, the effect of different parameters affecting the complexation and extraction conditions such as pH, concentration of buffer and chelating agent, kind of extraction and disperser solvent and volume of them, extraction time and salt addition were optimized. In order to study the explained parameters, extraction recovery and enrichment factor (EF) have been calculated by eqns. 1 and 2.

$$\text{Enrichment factor (EF)} = C_{\text{sed}} / C_0 \quad (1)$$

where, EF, C_{sed} and C₀ are the enrichment factor, concentration of analyte in sedimented phase and initial concentration of analyte in aqueous sample, respectively.

$$R\% = [(C_{\text{sed}} \times V_{\text{sed}}) / (C_0 \times V_{\text{aq}})] \times 100 = [(EF \times V_{\text{sed}}) / V_{\text{aq}}] \times 100 \quad (2)$$

where, R %, V_{sed} and V_{aq} are the extraction recovery, volume of sedimented phase and volume of aqueous sample, respectively. These parameters are known except C_{sed}. Calculation of C_{sed} was done by direct injection of standard solution of Ni-4-BPDC complex in chloroform with concentration in the range of 0.5-10 mg L⁻¹ to FAAS.

Effect of pH: The separation of metal ions by dispersive liquid-liquid microextraction involves prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of 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 nickel from water samples within the pH range of 1-12 by addition of NaOH or HCl (Fig. 1). According to these results, the pH of 5 was chosen for further extraction.

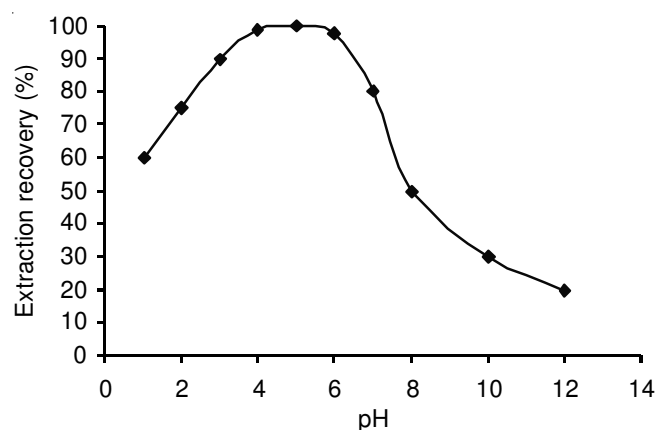


Fig. 1. Effect of pH on extraction recovery of nickel obtained from DLLME. Extraction conditions: water sample volume, 5.0 mL; disperser solvent (ethanol) volume, 500 µL; extraction solvent (CHCl₃), 100 µL; 4-BPDC concentration, 4.0 × 10⁻⁴ mol L⁻¹; concentration of nickel, 50 µg L⁻¹

Effect of 4-BPDC concentration: Dispersive liquid-liquid microextraction of 0.25 µg of nickel using 4-BPDC from

5 mL of the sample solutions was conducted by varying the concentration of 4-BPDC. The extraction recovery for Ni(II) as a function of the concentration of chelating agent is shown in Fig. 2. The recovery increases up to a 4-BPDC concentration of $0.1 \times 10^{-3} \text{ mol L}^{-1}$ and reaches near quantitative extraction efficiency. A concentration of $1.0 \times 10^{-3} \text{ mol L}^{-1}$ of 4-BPDC was chosen to account for other extractable species that might potentially interfere with the assaying of Ni(II).

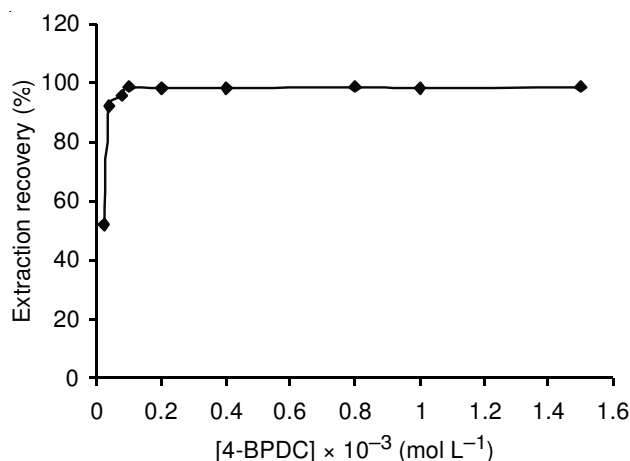


Fig. 2. Effect of 4-BPDC concentration on extraction recovery of nickel obtained from DLLME. Extraction conditions: water sample volume, 5.0 mL; disperser solvent (ethanol) volume, 500 μL ; extraction solvent (CHCl_3), 100 μL ; concentration of nickel, 50 $\mu\text{g L}^{-1}$; pH = 5.0

Effect of type and volume of extraction solvent: Careful attention has been paid to the selection of the extraction solvent. It should have higher density rather than water, extraction capability of interested compounds and low solubility in water. Chloroform, carbon tetrachloride and chlorobenzene were compared in the extraction of nickel. A series of sample solutions were studied by using 500 μL ethanol and different volumes of extraction solvent to achieve 70 μL volume of sedimented phase. Thereby, 100, 80 and 80 μL of chloroform, carbon tetrachloride and chlorobenzene were used, respectively. The results revealed that chloroform has the highest extraction efficiency (98.0 %) in comparison with carbon tetrachloride (38.0 %) and chlorobenzene (72.0 %). Hence, chloroform was chosen as extraction solvent. To examine the effect of the extraction solvent volume, solutions containing different volumes of chloroform were subjected to the same DLLME procedures. The experimental conditions were fixed and include the use of 500 μL ethanol containing different volumes of chloroform. Fig. 3 shows the curve of extraction recovery *versus* volume of the extraction solvent (chloroform). According to Fig. 3, extraction recovery increases up to 100 μL of chloroform and then remains constant. Thus, 100 μL of chloroform was chosen as optimum.

Effect of type and volume of disperser solvent: The main criterion for selection of the disperser solvent is its miscibility in the extraction solvent and aqueous sample. For this purpose, different solvents such as acetone, ethanol and methanol were tested. A series of sample solutions were studied by using 500 μL of each disperser solvent containing 100 μL of chloroform (extraction solvent). The results showed extraction

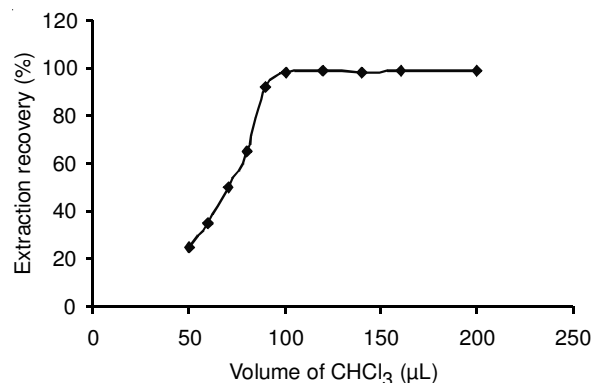


Fig. 3. Effect of the volume of extraction solvent (CHCl_3) on extraction recovery of nickel obtained from DLLME. Extraction conditions: water sample volume, 5.0 mL; disperser solvent (ethanol) volume, 500 μL ; 4-BPDC concentration, $4.0 \times 10^{-4} \text{ mol L}^{-1}$; concentration of nickel, 50 $\mu\text{g L}^{-1}$; pH = 5.0

recovery was the best when ethanol was used. Thus, ethanol was selected as a disperser solvent. Investigation of the effect of different volume of ethanol (disperser solvent) on the extraction recovery would be very rough. Since, variation of the volume of ethanol makes change in the volume of settled phase at constant volume of chloroform (extraction solvent). Thereby, to avoid of this matter and in order to achieve a constant volume of settled phase (70 μL) the volume of ethanol and chloroform were changed, simultaneously. The experimental conditions were fixed and include the use of different volumes of ethanol 300, 400, 500, 600, 700, 800, 900, 1000 and 1200 μL containing 90, 95, 100, 105, 110, 115, 120, 125 and 135 μL of chloroform, respectively. Under these conditions, the volume of the sedimented phase was constant ($70 \pm 2 \mu\text{L}$). Fig. 4 shows the curve of extraction recovery *versus* volume of the disperser solvent (ethanol). The results show that there was no considerable variation on extraction recovery between 500 and 800 μL of ethanol and the extraction recovery was high and then decreased by increasing the volume of ethanol. It is clear that by increasing the volume of ethanol, the solubility of complex in water increases. Therefore, the extraction recovery decreases. Thus, 500 μL of ethanol was selected as optimum volume in order to achieve better and more stable cloudy solution.

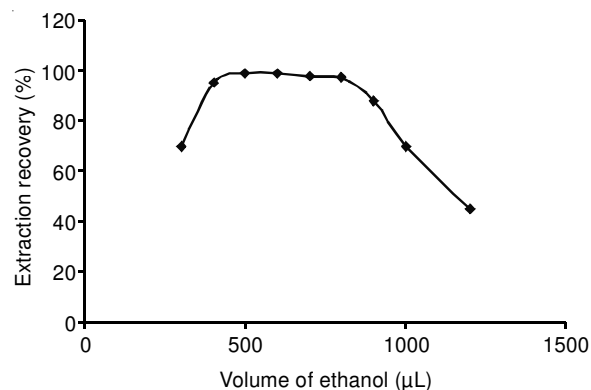


Fig. 4. Effect of the volume of disperser solvent (ethanol) on extraction recovery of nickel obtained from DLLME. Extraction conditions: water sample volume, 5.0 mL; extraction solvent (CHCl_3), 100 μL ; 4-BPDC concentration, $4.0 \times 10^{-4} \text{ mol L}^{-1}$; concentration of nickel, 50 $\mu\text{g L}^{-1}$; pH = 5

Effect of extraction time: Extraction time is one of the most important factors in most of the extraction procedure. In DLLME, extraction time is defined as interval time between injection mixture of disperser and extraction solvent and starting to centrifuge. The effect of extraction time was examined in the range of 0-45 min with constant experimental conditions. The results showed that the extraction time has no significant effect on the extraction efficiency. It was revealed that after the formation of the cloudy solution, the surface area between the extraction solvent and the aqueous phase is infinitely essentially large. Thereby, transfer of Ni-4-BPDC complex from the aqueous phase to the extraction solvent is fast. This is one of the considerable advantages demonstrated by the DLLME technique, *i.e.*, short extraction time.

Effect of buffer concentration: The influence of buffer amounts was carried out. In this stage, the other experimental variables remained constant. The results showed that above 0.5×10^{-3} mol L⁻¹ of buffer solution, no obvious variation took place in the extraction yield. Thus, 1×10^{-3} mol L⁻¹ of buffer solution was chosen as the optimal to achieve higher buffering capacity.

Effect of salt: For investigating the influence of ionic strength on performance of DLLME, various experiments were performed by adding different amount of NaCl (0.0-1.0 mol L⁻¹). Other experimental conditions were kept constant. The results showed, ionic strength has no appreciable effect upon extraction efficiency up to 1 mol L⁻¹ of NaCl. These observations showed the possibility of using this method to separation of nickel from highly saline solutions.

Effect of other ions: The effects of common other ions in natural water samples on the recovery of nickel were studied. In these experiments, 5 mL of solutions containing 50 µg L⁻¹ of nickel and various amounts of interfering ions were treated according to the recommended procedure. An ion was considered to interfere when its presence produced a variation of more than 5 % in the extraction recovery of the sample. The results (Table-1) indicate that the Ni(II) recoveries are almost quantitative in the presence of interfering ions.

TABLE-1
EFFECT OF FOREIGN IONS ON THE PRECONCENTRATION AND DETERMINATION OF NICKEL

Ion	Ion/Ni(II) (w/w)	Recovery (%)
Na ⁺	10000	101
K ⁺	10000	100
Ca ²⁺	10000	97
Ba ²⁺	10000	98
Mg ²⁺	10000	97
NO ₃ ⁻	10000	99
Cl ⁻	10000	102
PO ₄ ³⁻	5000	96
Br ⁻	5000	97
I ⁻	5000	97
SO ₄ ²⁻	5000	98
Zn ²⁺	200	96
Cu ²⁺	200	97
Ag ⁺	200	96
Cr ⁶⁺	200	98
Cd ²⁺	20	96
Fe ³⁺	20	96
Fe ²⁺	20	98
Cr ³⁺	20	97

Figures of merit: Table-2 summarizes the analytical characteristics of the optimized method, including linear range, limit of detection, repeatability and enrichment factor. The calibration graph was linear in the ranges of 8-200 µg L⁻¹ of nickel. The limit of detection, defined as CL = 3 SB/m (where CL, SB and m are the limit of detection, standard deviation of the blank and slope of the calibration graph, respectively), was 2.2 µg L⁻¹. The relative standard deviation (RSD) for ten replicate measurements of 50 µg L⁻¹ Ni(II) was 1.9 %. The enrichment factor was 50.

TABLE-2
ANALYTICAL CHARACTERISTICS OF PROPOSED METHOD

Parameter	Analytical feature
Linear range (µg L ⁻¹)	8-200
Limit of detection (µg L ⁻¹) (n =10)	2.2
Repeatability (RSD, %) (n =10)	1.9
Enrichment factor	50

Application to samples: The proposed DLLME-FAAS methodology was applied to the determination of Ni in several food and environmental water samples. Water samples (*i.e.* tap water, sea water, river water and mineral water) were filtered using a 0.45 µm pore size membrane filter to remove suspended particulate matter and aliquots of water (5 mL) were subjected to DLLME. According to the results, the concentration of nickel in analyzed water samples was below the LOD of the method. Moreover the robustness of the proposed method was checked by performing recovery test on a synthetic sample (no certified reference material was available). Each type of water was spiked with variable amounts of Ni(II) to assess matrix effects. The results are shown in Table-3. The relative recoveries of nickel from mentioned water samples at various spiking levels were between 94.0 and 99.0 %. These results demonstrated matrices of these water samples, in our present context, had little effect on DLLME of nickel. Herbal samples (4 g of tomato sauce, kiwi, orange, green pepper) were digested with 10 mL of concentrated HNO₃ (65 %) and 4 mL of H₂O₂ (30 %). After digestion of the samples the final residue was filtered and by keeping the pH at 5 made up to 25 mL with ultra-pure water. The results are shown in Table-4.

TABLE-3
DETERMINATION OF Ni(II) IN DIFFERENT WATER SAMPLES

Sample	Ni ²⁺ spiked (µg L ⁻¹)	Ni ²⁺ detected (µg L ⁻¹)	Recovery (%)
Tap water ^a	-	BDL ^e	-
	50	49.5 (2.6) ^f	99
	100	99.0 (2.5)	99
Sea water ^b	-	16.3 (3.4)	-
	50	47.5 (3.7)	95
	100	96.0 (3.8)	96
River water ^c	-	11.8 (3.8)	-
	50	47.0 (3.6)	94
	100	96.0 (3.5)	96
Well water ^d	-	BDL	-
	50	48.5 (3.6) ^f	97
	100	98.0 (3.5)	98

^aFrom drinking water system of Qaemshahr, Iran; ^bCaspian sea water, Iran; ^cTelar River, Qaemshahr, Iran; ^dFrom Qaemshahr, Iran; ^eBelow the detection limit; ^fRSD of three replicate experiments

TABLE-4
DETERMINATION OF NI(II) IN DIFFERENT
HERBAL SAMPLES

Sample	Concentration of Ni ($\times 10^{-3}$ mg/g)
Tomato sauce	BDL
Kiwi	0.391
Orange	0.208
Green pepper	BDL

Conclusion

Dispersive liquid-liquid microextraction combined with flame atomic absorption spectrometry allows tackling the determination of nickel in natural waters in a simple way. The method is simple, rapid and economical. High preconcentration factor was obtained easily through this method and a detection limit at sub $\mu\text{g L}^{-1}$ level was achieved with only 5.0 mL of sample. In this method preparation time as well as consumption of toxic organic solvents was minimized without affecting the sensitivity of the method.

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