Iranian Journal of Analytical Chemistry

Combination of Dispersive Liquid-Liquid Microextraction with Flame Atomic Absorption for Determination of Trace Co(II) in Environmental Water, Egg Yolk and Hair

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ABSTRACT

In this work, a procedure for preconcentration of cobalt using dispersive liquid–liquid microextraction (DLLME) with the reagent 1-Nitroso 2-naphtol as complexing reagent was developed. At pH 4, Co(II) was complexed with 1-nitroso 2-naphtol and extracted into the fine droplets formed when mixing chloroform (extraction solvent), ethanol (disperser solvent) and the sample solution. After extraction, the phases are separated and cobalt ion was determined in the enriched phase by flame atomic absorption spectrometry (FAAS). Under the optimum conditions, the enrichment factor 57.22 was obtained. The detection limit of the method was 3.76 ng mL^{-1} and the relative standard deviation (RSD) for eight replicate mrasurements of 0.05 µg mL^{-1} Co (II) was 2.3 %. The results to the determination of Co(II) in environmental water samples (tap water, well water, mineral water), egg yolk and hair have demonstrated the applicability of the proposed method.

KEYWORDS: Dispersive Liquid Liquid Microextraction; Flame Atomic Absorption Spectrometry; Cobalt; Water; Egg Yolk; Hair.

1. INTRODUCTION

Cobalt is known to be essential or toxic at trace levels to human, animals and plants for metabolic processes depending on its concentration range [1-3]. It is clear that the determination of trace amounts of Co in environmental and food samples is of great significance from the public health and environmental point of view. In recent years, continuous progress has been made in analytical instrumentation, however, direct determination of trace metal ions in environmental and food samples is difficult because of various factors, particularly their low concentrations and matrix effects. Under these circumstances, in order to determine trace levels of Co(II), a separation and enrichment step prior to its determination could be necessary. Several methods have been proposed for separation and preconcentration of trace Co(II) including: solid phase extraction (SPE) [4-6] cloud point extraction (CPE) [7-8] and liquid–liquid extraction(LLE) [9-10] are widely used in the separation and preconcentration of trace elements. A new trend in analytical chemistry is miniaturization of preconcentration systems with the aim of minimizing consumption of reagents and waste generation [11]. For liquid–liquid extraction, alternatives of miniaturization can be employed with strategies such as liquid–liquid microextraction (LLME) [12] single-drop microextraction (SDME) and dispersive liquid–liquid microextraction (DLLME) [13]. Recently, a microextraction technique based on a ternary solvent system has been developed. In this technique, termed dispersive liquid–liquid microextraction (DLLME) [14] an appropriate mixture of an extraction solvent and a disperser solvent is rapidly injected into an aqueous sample so that a cloudy solution is formed. The analyte in the sample is then transferred to the fine droplets of the extraction solvent and phase separation is performed by centrifugation. The advantages of this method include its simplicity of operation, rapidity, low cost, low consumption of organic solvents and high enrichment factors. The technique has been applied to the determination of trace organic pollutants and metal ions in environmental samples [15-16] and has recently been revised in depth in the framework of a general review dealing with miniaturized preconcentration methods [17]. The aim of this work is to combine DLLME with FAAS to develop a new procedure for the determination of trace cobalt ion in natural samples. 1-Nitroso 2-naphtol as a chelating agent originates stable complexes with a number of metals and has found numerous applications at trace elements separation and preconcentration methods.

2. EXPERIMENTAL
2.1. Instrumentation
A Shimadzu atomic absorption spectrometer (Kyoto, Japan) Model AA-670 was used for the determination

of cobalt using the manufacturer recommendations. A centrifuge Heraeus (Labofuge 400 model, Germany) was used to accelerate the sedimentation of the rich phase in the process of microextraction. The pH values were measured with a metrohm E-691 pH/mV meter equipped with a combined glass calomel electrode (Switzerland).

2.2. Reagents and materials

All chemicals used were of analytical-reagent grade. All aqueous solutions were prepared using deionized water. 1-Nitroso 2-naphtol, Chloroform, carbon tetrachloride, dichloromethane, methanol, ethanol, acetone, tetrahydrofuran, acetonitrile, hydrochloric acid and nitric acid were obtained from Merck.

The stock standard solutions of cobalt ion at concentration of 1000 mg L^{-1} were prepared using $Co(NO_3)_2 \cdot 6H_2O$. Working solutions were then prepared by appropriate dilution of the standard solutions daily. The solution of 1-nitroso2-naphtol was prepared by dissolving appropriate amount of 1-nitroso 2-naphtol in methanol.

2.3. Dispersive liquid–liquid microextraction procedure

Five milliliters of the cobalt solution was placed in a 10-mL glass test tube with conical bottom. Then 1 mL phosphate buffer and 0.5 mL of 1-nitroso2-naphtol solution (0.01 mol L^{-1}) were added. After that, a mixture containing 1.5 mL of ethanol and 200 μL of Chloroform were incorporated by using a 5 mL syringe. The mixture was gently shaken by hands for 1 min. A cloudy solution that consists of very fine droplets of chloroform dispersed into aqueous sample was formed and then the analytes were extracted into the fine droplets. The mixture immediately centrifuged for 5 min at 3000 rpm. The chloroform phase was sedimented at the bottom of the centrifuge tube. The sedimented phase was removed to another test tube and allowed to evaporate at room temperature. Finally, the residue was dissolved into 0.5 mL N,N-dimtyleformamid (DMF). This solution can be directly nebulized into the flame with no particular injection system. The analyte concentration in final solution was determined by flame atomic absorption spectrometry.

2.4. Sample preparation

The proposed method was applied to the determination of cobalt ion in tap water, well water and mineral water. These water samples were filtered and the pH adjusted with buffer solution and analyzed immediately. Hair sample: appropriate amounts of hair sample were washed with water and acetone and dried at 100˚C. One gram of this hair sample was placed in a 100 mL beaker and 12 mL of concentrated HNO_3 and 2 mL of $HClO_4$ (70%) were added. The contents in the beaker were heated on a hot plate (45 min in 150 ˚C). Then, the solution was cooled to 70 ˚C and 5 mL of hydrogen peroxide (30%) added. The mixture was heated at 200 ˚C to dryness. To the obtained white residue, 5 mL of 0.01 mol L^{-1} HNO_3 was added and the mixture heated at 110 ˚C for 1 min. The resulting clear solution was neutralized by 0.1 mol L^{-1} NaOH solution and the volume was made up to 10 mL in a volumetric flask. Five milliliters of this solution was transferred into a 10 mL volumetric flask and analyzed by the proposed procedure [18]. Egg sample: 5 g of egg yolk is placed in a 100 mL digestion tube. Add 10 mL concentrated nitric acid and heat up to 95°C for approximately 1 h. After cooling, add 5 mL of concentrated sulfuric acid and heat to 140°C until charring first appears. After cooling, add 5 mL concentrated nitric acid and heat to 180 °C. Then after cooling, add 1 mL hydrogen peroxide (500 g L^{-1}) and heat to 200 °C. After cooling, add 10 mL water and 0.5 mL concentrated nitric acid and heat to 200 °C. After cooling, add 10 mL water, followed by 1 mL hydrogen peroxide and heat to 240 °C [19]. Finally, the digest is cooled and quantitatively transferred to a 100 ml volumetric flask for analysis. Five milliliters of this solution were transferred into a 10 mL volumetric flask and analyzed by the proposed procedure.

3. RESULTS AND DISCUSSION

In this study a microextraction method, dispersive liquid–liquid microextraction, was investigated to enrichment of Co(II) ion from aqueous samples followed by their determination by flame atomic absorption spectrometry.

For higher sensitivity, selectivity and precision for Co(II) determination with the DLLME method, the effect of the main parameters, like the type of dispersive and extracting solvent, sample acidity, amount of chelating agent, sample ionic strength and extraction time were studied and optimized. To study these influential parameters, enrichment factor (EF) and recovery percent were used to evaluate the extraction efficiency under different conditions. The enrichment factor was defined as the ratio between the analyte concentration in the sedimented phase and the initial concentration of analyte within the sample. The analyte concentration in the sedimented phase was calculated from the direct calibration graph.

3.1. Selection of dispersive and extracting solvents

The selection of the extracting solvent is critical and should meet the following criteria: (1) it should dissolve the analyte better than water, (2) it should be heavier than water and (3) it should form tiny droplets when it is added to the aqueous solution of analyte along with a dispersive solvent. Almost all of the suitable extracting solvents are chlorinated. The miscibility of the dispersive solvent in the extracting solvent and aqueous phase is the most important factor affecting the of dispersive solvent in DLLME. These solvents can disperse the extracting solvent as very fine droplets in aqueous phase. In this work methanol, ethanol, acetone, tetrahydrofuran and acetonitrile, were studied as dispersive solvent and chloroform, carbon tetrachloride and dichloromethane were studied as

extracting solvent. The experiments were performed by using 1.5 mL of dispersive solvent containing 200 μL extracting solvent. In Fig. 1 the recovery and EF are shown for all combinations of dispersive and extracting solvents. Regarding the recovery and EF, the combination of chloroform as extracting solvent with ethanol as dispersive solvent is the best one and an EF higher than 55 is attainable.
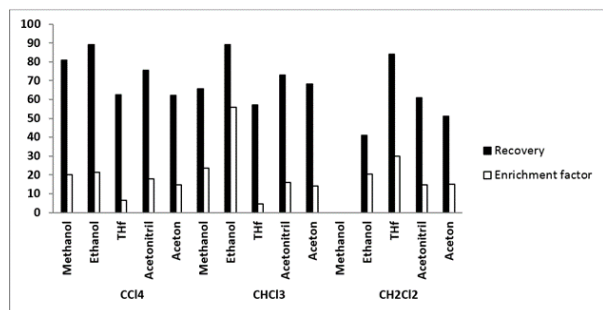
Fig. 1. Selection of extracting and dispersive solvents in DLLME. Conditions: sample, 5 mL Co(II) 0.05 mg L^{-1}; volume of dispersive solvent, 1.5 mL; volume of extracting solvent, 200 μL; buffer, 1mL phosphate buffer (C= 0.1 mol L^{-1}, pH 4) and 1-nitroso2-naphtol solution, 0.5 mL 0.01 mol L^{-1} in methanol.

3.2. Dispersive solvent volume

Ethanol as a dispersive solvent in different volumes in the range of 0–3 mL along with 200 μL chloroform as an extracting solvent was used for extraction of cobalt ions using the DLLME procedure. The obtained results (Fig. 2) show that in the case of 1.5 mL ethanol the highest recovery and a reasonable EF were obtained and the sedimented phase volume was attainable. At lower volumes of ethanol, the cloudy suspension of chloroform droplets is not formed well, resulting a decrease in the extraction efficiency. At higher volumes of ethanol, the solubility of complex in water increases and the extraction efficiency decreases. With 3 mL or higher volume of ethanol no sedimented organic phase was achieved.
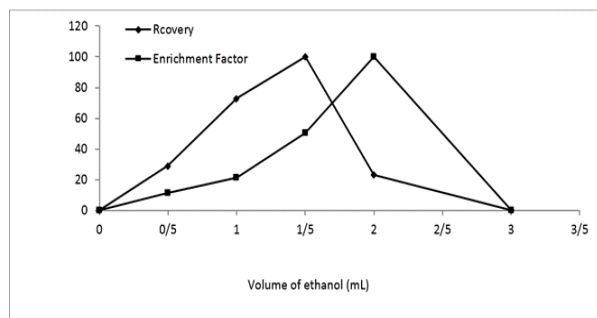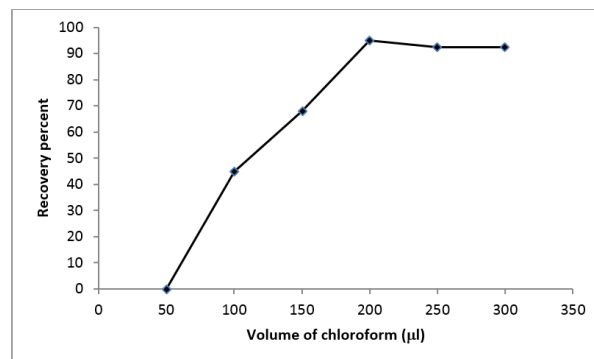

Fig. 2. Effect of dispersive solvent volume. Other conditions are the same as Fig. 1.

3.3. Extracting solvent volume

In order to study the effect of the extracting solvent volume on the extraction efficiency, the solutions containing the different volumes of chloroform in the range of 50-300 μL and a constant volume of ethanol as dispersive solvent (1.5 mL) were investigated. Fig. 3 illustrates the variations of the extraction recovery versus the volume of the extracting solvent. According to Fig. 3, the extraction recovery increased with the increase of the volume of chloroform. On the other hand, in more than 200 μL chloroform the enrichment factor decreased, because the sedimented phase volume increased. On the basis of these results, 200 μL of chloroform was selected for subsequent experiments.


Fig. 3. Selection of extracting solvent volume. Other conditions are the same as Fig. 1.

3.4. Influence of pH

Among chemical variables, sample acidity, which was defined by the pH of the sample solution, played a significant role in the overall performance of the solvent extraction affecting the complex formation and the extraction efficiency. The effect of the pH on the absorbance was studied in the range of 2-12 by adjusting it in cobalt (II) solution with HCl, NaOH and phosphate buffer.

As Fig. 4 shows the highest intensity for Co(II) was observed in pH 4. Therefore, pH 4 was selected in subsequent experiments to adjust the pHions of the Co(II) solution.
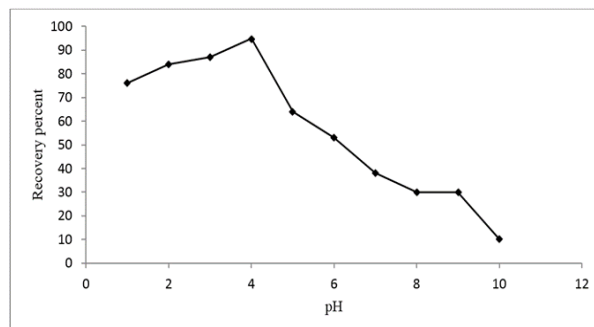

Fig. 4. Effect of pH on the absorbance of Co(II). Other conditions are the same as Fig. 1.

3.5. Influence of 1-nitroso 2-naphtol concentration

The influence of 1-nitroso 2-naphtol concentration on the extraction efficiency of cobalt ions was evaluated. 1-nitroso 2-naphtol solutions at different concentrations in methanol were prepared. Each of these solutions injected in aqueous solutions of cobalt. The result obtained for each solution is shown in Fig. 5. Best results were obtained when concentrations in the range

of 0.01 to 0.03 mol L^{-1} were used. Hence, 0.01 mol L^{-1} 1-nitroso 2-naphtol was taken as optimum amount in subsequent experiments.
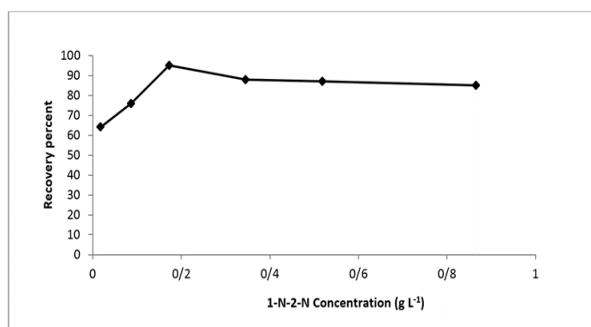
Fig. 5. Influence of complexing reagent concentration. Other conditions are the same as Fig. 1.

3.6. Effect of ionic strength

To evaluate the possibility of salting out effect, the extraction efficiency was studied with the sodium chloride concentration in the range of 0 to 10% (w/v). As is shown in Fig. 6 with increase the ionic strength, the sedimented phase volume increases slightly because of the decrease in solubility of the extracting solvent in aqueous phase leading to a decrease in the enrichment factor. On the other hand, the addition of salt has no significant effect on the extraction recovery. Therefore, all the extraction experiments carried out without the addition of salt.
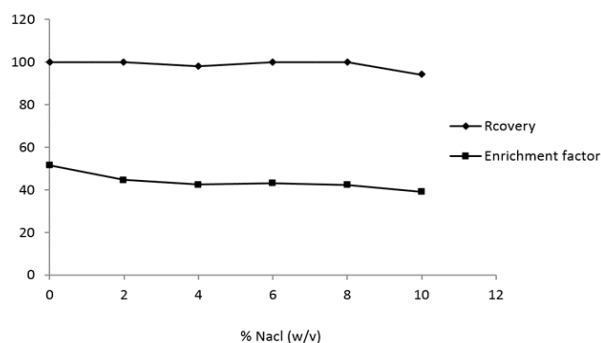
Fig. 6. Effect of ionic strength. Other conditions are the same as Fig. 1.

3.7. Effect of extraction time

In DLLME, extraction time is defined as interval time between injection the mixture of dispersive solvent (ethanol) and extracting solvent (chloroform), and before starting to centrifuge. The effect of extraction time was examined in the range of 1–10 min with constant experimental conditions. The obtained results showed that the variations of recovery percent versus extraction time were not remarkable. It is revealed that the DLLME method is time-independent because of the infinitely large surface area between extracting solvent and aqueous phase leading to the fast transition of analytes from aqueous phase to extracting solvent and equilibrium state is achieved quickly. This is the most important advantage of DLLME technique. In this method, the most time-consuming step is the

centrifuging of sample solution in the extraction procedure which is about 1 min.

3.8. Study of interferences

Interferences were studied in the presence of a constant concentration of analyte (0.05 μg mL^{-1}) and different amounts of foreign ions (analyte: foreign ion ratio 1:1, 1:10, 1:100 and 1:1000). Tolerable concentration of foreign ions was considered that concentration in which less than 5% deviation in absorbance reading was observed in comparison with the case in which interfering ion was absent. The obtained results are given in Table 1.

Table 1. Study of interfering ions

Interfering ions	Tolerable concentration (analyte:interfering ion)
Ni^{2+}, Cu^{2+}	50[b]
Hg^{2+}, Tl^{1+}, Fe^{3+}	100[b]
Cd^{2+}, Pb^{2+}, Zn^{2+}, SO$_4^{-2}$, Cl$^-$	1000[b]
Ca^{2+}	1000[a]
Mg^{2+}	10000[a]

Concentration of analyte is 0.05 μg mL^{-1}
[a] At this ratio no interfering effect was observed.
[b] At this ratio interfering effect was observed.

3.9. Figures of merit

The calibration graphs for cobalt was linear in the range of 20-280 μg L^{-1}. The RSD values (n= 8, 50 μg L^{-1} Co) was 2.3%, the enrichment factor was 57.22 (Table 2). Some characteristics of previously reported method [20] such as enrichment factor and limit of detection were compared. It was seen, the proposed DLLME method in this work, for the precocentration of Co(II), showed a high linear range and EF, LOD and RSD were comparable with each other.

Table 2. Analytical characteristics of DLLME-FAAS for determination of cobalt

Parameter	Analytical feature
Linear range (μg L^{-1})	20-280
R^2	0.9998
Limit of detection (LOD) (μg L^{-1})	3.87
Repeatability (RSD %)(n=8)	2.3 (0.05 μg mL^{-1})
Enrichment factor	57.22

3.10. Application to real samples

The proposed method was applied to the determination of cobalt in real samples (tap water, well water, mineral water, egg yolk and hair). The accuracy of the method was verified by the analysis of the samples spiked with known cobalt amounts. The relative cobalt recovery from tap, well and mineral water at the spiking level of 50 μg L^{-1} (Table 3) demonstrated that the matrices of the well, tap and mineral water samples had little effect on the DLLME method for determination of cobalt. The method was also validated for determination of cobalt in hair and egg yolk. The results in Table 4 indicate that the method can be successfully applied to the determination of cobalt in real samples.

Table 3. Determination of cobalt in tap, well and mineral water samples and relative recovery of spiked cobalt in tap, well and mineral water samples

Sample	Added ($\mu g\ mL^{-1}$)	Found ($\mu g\ mL^{-1}$) ± SD (%) (n=3)	Recovery (%)
Tap water	0	<LOD	-
	0.05	0.047± 0.00088	95.2
Mineral water	0	<LOD	-
	0.05	0.049± 0.00083	98.3
Well water	0	<LOD	-
	0.05	0.05± 0.0005	100

Table 4. Determination of cobalt in hair and egg yolk

Sample	Concentration $\mu g\ g^{-1}$ ± SD (%) (n=3)	Added ($\mu g g^{-1}$)	Found $\mu g g^{-1}$ ± SD (%) (n=3)	Relative Recovery (%)
Egg yolk	<LOD	1	0.941 ±0.01	94.1
Human hair (female)	<LOD	2.5	2.38 ±0.025	95

4. CONCLUSION

In the proposed procedure, the reagent 1-Nitroso 2-naphtol was successfully used as complexing for preconcentration of cobalt using dispersive liquid–liquid a microextraction. The method is simple, easy to use and economic. The low cost is related mainly to small amounts of solvents required. The small amounts of chloroform and ethanol also minimizes the toxicity of the method. Another interesting feature of the method is speed. After injection of the mixture ethanol/chloroform, the solution was immediately cloudy. The riched phase is injected into the FAAS after rapid centrifugation. The advantages cited and the analytical characteristics obtained make the method a good alternative to the determination of cobalt in routine analysis.

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