



## Cloud Point Extraction Procedure for Preconcentration of $\text{Co}^{2+}$ , $\text{Ni}^{2+}$ and $\text{Cu}^{2+}$ from Water and Biological Samples Using a thiosemicarbazone

### Derivative as a Complexing Agent

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#### Abstract

An accurate and simple preconcentration procedure was presented for cloud point extraction of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  from water, blood and urine samples. The procedure based on complexation of the metal ions with (*E*)-2-(2,4-dihydroxybenzylidene)-*N*-phenylhydrazine-1-carbothioamide (DHBPHC) and subsequent extraction into a surfactant-rich phase of Triton X-114. The optimized procedure (50 mL solution; pH 6;  $1 \times 10^{-4}$  mol L<sup>-1</sup> of DHBPHC; 0.08% (v/v) Triton X-114) exhibits linear range up to 500  $\mu\text{g L}^{-1}$  and limit of detection of 0.34, 0.94 and 0.83  $\mu\text{g L}^{-1}$  for  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$ , respectively. Most potentially interferants did not impact the extraction process. The procedure was applied for preconcentration and determination of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  in water, blood and urine by flame atomic absorption spectrometry.

*Key words:* Cloud point extraction; metal ions; thiosemicarbazone; flame atomic absorption spectrometry

#### 1. Introduction

Heavy metals determination in environmental samples such is of great importance due to their impact on human health. Copper and iron ions are essential for humans, and any imbalance in their concentrations, whether increased or decreased, causes various diseases. The effect of nickel on the cell is not known, but its accumulation may lead to adverse health impacts [1].

Usually, these metal ions occur in very low abundance in the environment and biological samples, so sensitive methods are needed for accurate measurements. A separation and preconcentration techniques are necessary prior determination to increase selectivity and sensitivity. For this reason, many preconcentration techniques are used such as solid phase extraction [2], solvent extraction fractionation [3], coprecipitation [4], dispersive liquid-liquid microextraction [5] and cloud point extraction (CPE) [6, 7]. The later has become an attractive area for the separation and preconcentration of trace metal ions as it agreed with Green Chemistry Principles. The main aim of the Green Chemistry Principles is to

minimize the hazard effects on human health and environment due to utilization of chemicals. The document laid out 12 principles, including the use of safe chemicals and the reduction of pollution [8].

The methodology of CPE is based on the following phenomenon: an aqueous solution of some surfactants becomes turbid and separates into two isotropic phases when the solution is heated above its cloud temperature or a strong electrolyte is added "salting-out phenomenon" [9]. The procedure facilitates the incorporation of the analyte into micelles and subsequent extraction into small volume of the surfactant-rich phase [10]. The most important advantages of CPE are its ability to avoid using of large hazardous organic solvents as in traditional liquid-liquid extraction and permits achievement of a high preconcentration factor [11]. Additional features include its ease of performance, accuracy and selectivity [12].

Aroylhydrazones play a significant role in coordination chemistry and they represent an important class of NO donors [13, 14]. They have been used for separation and preconcentration of some

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Receive Date: 16 February 2021, Revise Date: 04 March 2021, Accept Date: 23 March 2021

DOI: 10.21608/EJCHEM.2021.63390.3360

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metal ions from different samples by SPE [14, 15]. A review of literature shows that these compounds were not previously used in the CPE of metal ions.

In this study, a simple CPE procedure was developed and optimized for preconcentration of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  in water and food samples. The metal ions were extracted in a surfactant-rich phase of Triton X-114 after complexation with (*E*)-2-(2,4-dihydroxybenzylidene)-*N*-phenylhydrazine-1-carbothioamide (DHBPHC, Scheme 1). This study introduced DHBPHC in its first application as a ligand in CPE.

## 2. Experimental

### 2.1. Reagents and Solutions

Double-distilled water was used through the study. Stock solutions of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  (1000 mg  $\text{L}^{-1}$ ) were prepared by dissolving appropriate amounts of their metal chlorides in 0.1 mol  $\text{L}^{-1}$  HCl. Working solutions were prepared daily by stepwise dilution from stock the solution. Non-ionic surfactant Triton X-114 (1% v/v) was used.  $2.5 \times 10^{-3}$  mol  $\text{L}^{-1}$  of DHBPHC solution was prepared by dissolving 0.1085 g first in least amount of 0.1 mol  $\text{L}^{-1}$  NaOH then complete the volume to 100 mL by distilled water. To control the pH during the extraction process, buffer solutions were used [acetate buffer (pH 3.0-6.0) and hexamine buffer (pH 7.0-9.0)].

### 2.2. Synthesis of DHBPHC

Phenyl isothiocyanate (1.35 g, 10.00 mmol) added to an ethanolic solution of hydrazine hydrate drop by drop over 10 minutes in ice bath. The mixture was stirred for 3 h, The precipitate was then obtained washed with cold ethanol yield (99.99 %, 1.94 gm). The thiosemicarbazide Schiff base was prepared upon a condensation of 4-phenyl-3-thiosemicarbazide (0.09 g, 1 mmol) with 2,4-dihydroxybenzaldehyde (0.14 g, 1 mmol) in ethanol (20 ml). The mixture was stirred under reflux for 3h. Slow evaporation of the solvent yielded yellow crystals. The crystals were filtered, washed with cold methanol and ether, dried in vacu. Yield: 87%. Anal. Calcd. (%) for  $\text{C}_{14}\text{H}_{13}\text{N}_3\text{O}_2\text{S}$ : C, 58.47; H, 4.56; N, 14.61. Found: C, 58.51; H, 4.56; N, 14.32. IR (KBr pellet,  $\text{cm}^{-1}$ ): 3436, 3304  $\nu$  (OH), 3164, 3106  $\nu$  (NH), 1593  $\nu$  (C=N), 1020  $\nu$  (N=N), 830  $\nu$  (C=S).  $^1\text{H}$  NMR (500 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  11.68 (s, 1H, NHCS), 9.94 (s, 1H, OH), 9.89 (s, 1H, OH), 8.72 (s, 1H,  $\text{NHC}_6\text{H}_5$ ), 8.34 (s, 1H,  $\text{HC=N}$ ), 7.82 (d,  $J = 8$  Hz, 1H, aromatic), 7.53 (d,  $J = 8$ , 2H, aromatic), 7.33 (t,  $J = 8$  Hz, 2H, aromatic), 7.16 (t,  $J = 8$  Hz, 1H, aromatic), 6.29 (s, 1H, aromatic), 6.27 (d,  $J = 4$  Hz, 1H, aromatic). ESI-MS  $m/z$  ( $^-$ ): 287.18 ( $[\text{M} - \text{H}]^-$ ).

### 2.3. Apparatus

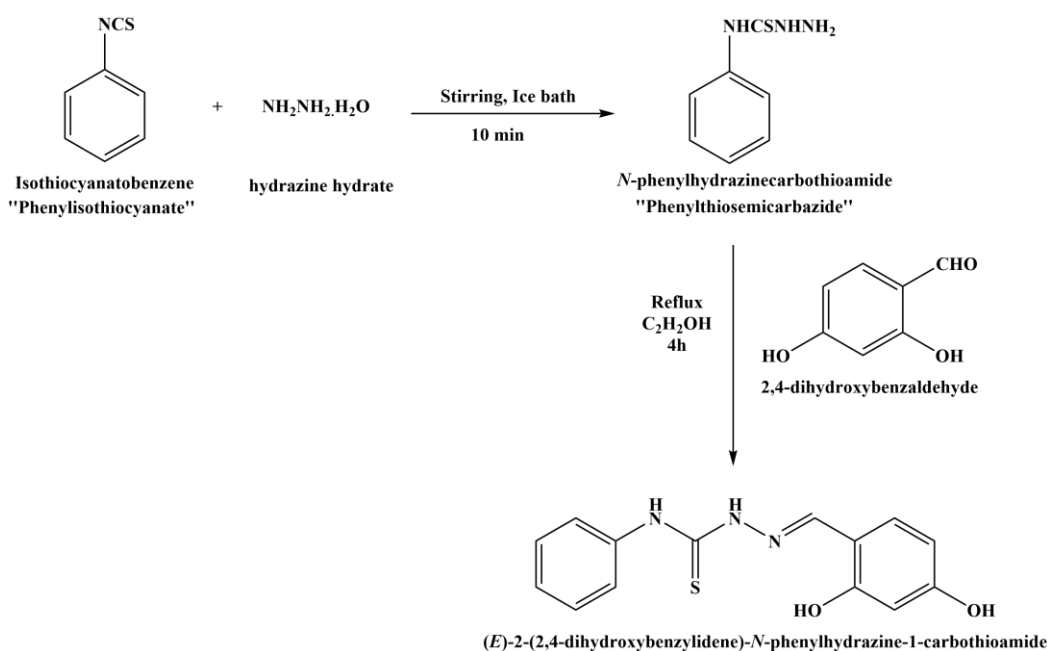
Identification of the prepared ligand was carried out by elemental analysis (elemental analyzer Vario EL III, Elementar Analysensysteme GmbH, Germany), Fourier transform infrared (FT-IR) spectrum in KBr disc (Thermo-Nicolet IS10 FT-IR spectrometer, Nicolet Instrument Co, Madison, WI, USA),  $^1\text{H}$  NMR spectrum in  $\text{DMSO-d}_6$  at 500 MHz on a Jeol Delta 2-NMR (JEOL USA Inc., Peabody, MA 01960, USA) and mass spectrum (Thermo DSQ II Spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, USA). A Metrohm 692 pH meter (Herisau, Switzerland) was used for pH measurement and a CH90-2 centrifuge (Hinotek Technology Co., Ltd. China) was employed to accelerate the phase separation. Determination of  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  were carried out using SensAA GBC atomic absorption spectrometer (Dandenong, Australia) equipped with deuterium background correction and air-acetylene burner. The operating conditions were set according to the manufacturer manual.

### 2.4. Procedure of CPE

Aliquot of 50.0 mL of a solution containing the metal ions at pH 6.0 (acetate buffer) was mixed with Triton X-114 (0.08% v/v) and  $10^{-4}$  mol  $\text{L}^{-1}$  of DHBPHC. The tubes were kept for 10 min in a thermostatic bath at  $40^\circ\text{C}$  to form turbid solutions. Subsequently, separation of the phases was achieved by centrifugation for 5 min at 4000 rpm. After cooling in an ice bath for 5 min, to increase viscosity of the surfactant-rich phase, the bulk aqueous phase was decanted by inverting the tube. The volume of the surfactant-rich phase was made up to 1.0 mL by ethanol/conc.  $\text{HNO}_3$  mixture (5:1 v/v) and was analyzed for  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Co}^{2+}$  by FAAS. The blanks were processed by the same manner.

### 2.5. Collection and processing of real samples

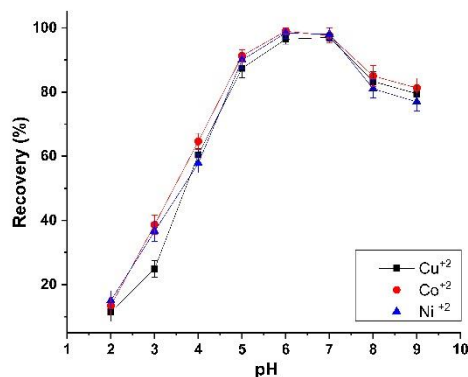
Tap water samples were collected from Laboratories of the Faculty of Science, Mansoura University in acid-washed polyethylene bottles. The samples were filtered through 0.45  $\mu\text{m}$  cellulose nitrate membranes and the pH was adjusted to 2.0 using  $\text{HNO}_3$ . Blood and urine samples were obtained from our laboratory staff and were digested using a previously optimized microwave assisted procedure [16]. Briefly, 0.5 mL blood or 2.0 mL urine were mixed with 3.0 mL  $\text{HNO}_3$  and 1.0 mL  $\text{H}_2\text{O}_2$  in a Teflon tubes. The vessels were closed and digested according to the following program: "power, 1600 W (100%); ramp time, 15 min; temperature,  $200^\circ\text{C}$ ; hold time, 15 min; and cooling time, 15 min". The samples then were cooled at room temperature and each sample was diluted by double-distilled water to 10.0 mL.



### 3. Result and Discussion

#### 3.1. Effect of pH on CPE

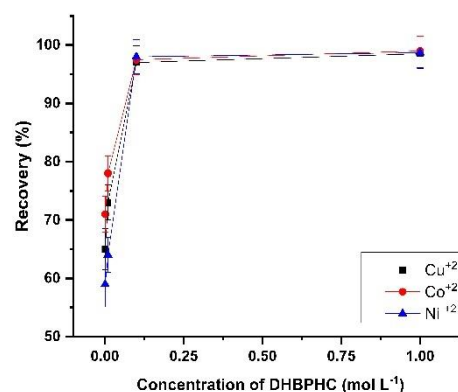
The metal complexes formation and their stabilities are important factors for the CPE. The pH plays a unique role on metal chelate formation and subsequent preconcentration by CPE [17]. The effects of the pH on the percentage recovery of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  were investigated at different pH (2-9). The results in Fig. 1 show that metal ions were extracted quantitatively (>95%) at pH 6-7; where at lower pH, the extraction is not quantitative due to protonation of ligand, and at higher pH, the hydrolysis of cations occur preventing their extraction. Therefore, pH 6 was chosen as the working pH for the determination of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$ .



**Fig. 1.** Influence of pH on the CPE of the metal ions. Conditions:  $200 \mu\text{g L}^{-1}$  each metal; 50 mL aqueous solution;  $1.0 \times 10^{-4} \text{ mol L}^{-1}$  of the ligand; 0.08% (v/v) Triton X-114; at  $40^\circ\text{C}$

#### 3.2. Effect of DHBPHC Concentration

The effect of the concentration of DHBPHC on the extraction of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  was investigated in the range of  $1 \times 10^{-6}$  to  $1 \times 10^{-3} \text{ mol L}^{-1}$ . The results in Fig. 2 indicated that the maximum recoveries were obtained at  $1 \times 10^{-4} \text{ mol L}^{-1}$  of DHBPHC. The poor extraction below this concentration is probably due to the insufficient amount of ligand to extract the metal. Therefore, this concentration was selected as the optimum amount of the chelating agent.

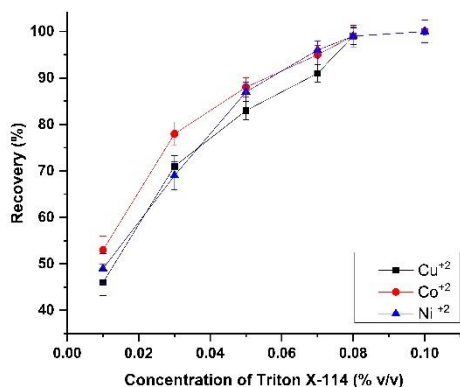


**Fig. 2.** Effect of the ligand concentration on the extraction of the metal ions. Conditions:  $200 \mu\text{g L}^{-1}$  each metal; pH 6; 50 mL aqueous solution; 0.08% (v/v) Triton X-114; at  $40^\circ\text{C}$ .

#### 3.3. Effect of Triton X-114

Triton X-114 is a non-ionic surfactant that is widely employed in CPE because of its low cloud temperature, availability as pure reagent, homogeneity

and low toxicity [17]. The use of surfactant in CPE facilitates the separation of metal ions as metal complexes into a surfactant-rich phase. The effect of Triton X-114 concentration ranged from 0.01 to 0.1% v/v is shown in Fig. 3. As represented, the extraction increases by increasing the amount of Triton X-114 and reach a constant value at 0.08% v/v so it was chosen as optimum concentration for extraction of all metal ions.



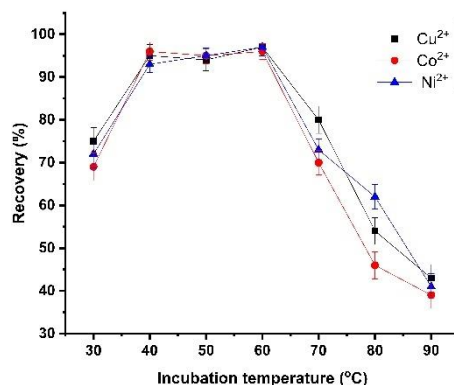
**Fig. 3.** Effect of the Triton X-114 concentration on the CPE of the metal ions. Conditions: 200  $\mu\text{g L}^{-1}$  each metal; pH 6; 50 mL aqueous solution;  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$  of the ligand; at 40°C.

### 3.4. Effect of the equilibration temperature and time

The effect of temperature and equilibration time on the extraction recovery was investigated in the range of 30 – 90°C and 5 – 35 min, respectively, keeping all other parameters constant. Fig 4 shows that excellent recovery was found at 40°C - 60°C. Above this temperature, reduction of extraction efficiency was observed due to probable breakdown of the metal complex. Therefore, 40°C was chosen as the equilibration temperature. The results also show that incubation time of 10 min is enough for achieving the highest extraction efficiency. Shortest time led to low extraction efficiency (data not shown).

### 3.5. Effect of centrifugation rate

Generally, centrifugation doesn't affect micelle formation, but it accelerates the phase separation process [18]. We investigated the effect of centrifugation time at 4000 rpm in order to obtain the shortest time for optimum extraction efficiency. As shown in Table 1, a centrifugation time of 5 min led to the best extraction efficiency.



**Fig. 4** Effect of equilibration temperature on the extraction of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$ . Conditions: 200  $\mu\text{g L}^{-1}$  each metal; pH 6; 50 mL aqueous solution;  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$  of the ligand; 0.08% (v/v) Triton X-114.

**Table 1** Effect of centrifugation time on the CPE of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  by the presented procedure

Centrifugation time (min) at 4000 rpm	Metal ion	Recovery%
3	$\text{Co}^{2+}$	74.3±3.7
	$\text{Ni}^{2+}$	83.3±3.1
	$\text{Cu}^{2+}$	88.7±4.0
5	$\text{Co}^{2+}$	98.0±2.9
	$\text{Ni}^{2+}$	97.9±2.7
	$\text{Cu}^{2+}$	97.8±3.1
10	$\text{Co}^{2+}$	96.2±2.2
	$\text{Ni}^{2+}$	97.4±3.1
	$\text{Cu}^{2+}$	97.1±3.2

Conditions: 200  $\mu\text{g L}^{-1}$  each metal; pH 6; 50 mL aqueous solution;  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$  of the ligand; 0.08% (v/v) Triton X-114, at 40°C.

### 3.6. Effect of interfering ions

The recoveries of 50  $\mu\text{g L}^{-1}$  of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  were determined in the presence of high concentrations of various interfering cations and anions. All the cations were used as their chlorides or nitrates, whereas the anions were used as the corresponding sodium or potassium salts. An ion was an interferent when it caused a variation of  $\pm 5\%$  in the recovery. The results obtained are listed in Table 2 indicating that the removal of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  ions was quantitative in all cases as 50 fold for ( $\text{Mn}^{2+}$ ,  $\text{B}^{3+}$ ,  $\text{Al}^{3+}$ ,  $\text{Bi}^{3+}$ ) 500 fold for ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{I}^-$ , Oxalate and phosphate), 750 fold for (citrate, thiocyanate, thiourea, thiosulphate and acetate) and 1000 fold for ( $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Cl}^-$ ,  $\text{NO}^-$ ,  $\text{SO}_4^{2-}$ ). These results indicate the selectivity of the procedure.

**Table 2 Tolerance limits of interfering ions on recoveries of 200  $\mu\text{g L}^{-1}$   $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  ( $n = 5$ ).**

Interfering ion	Tolerance limit	Recovery		
		$\text{Co}^{2+}$	$\text{Ni}^{2+}$	$\text{Cu}^{2+}$
$\text{Mn}^{2+}$ , $\text{B}^{3+}$ , $\text{Al}^{3+}$ , $\text{Bi}^{3+}$	50	97.4 $\pm$ 2.8	99.3 $\pm$ 1.7	98.4 $\pm$ 2.0
$\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , $\text{Ba}^{2+}$ , $\text{Sr}^{2+}$ , $\text{Zn}^{2+}$ , $\text{Fe}^{2+}$ , $\text{Fe}^{3+}$ , I, Oxalate and phosphate	500	99.5 $\pm$ 2.1	97.0 $\pm$ 1.8	96.8 $\pm$ 1.7
citrate, thiocyanate, thiourea, thiosulphate and acetate	750	98.3 $\pm$ 2.3	97.9 $\pm$ 2.0	96.9 $\pm$ 3.3
$\text{K}^+$ , $\text{Na}^+$ , $\text{Cl}^-$ , $\text{NO}^-$ , $\text{SO}_4^{2-}$	1000	99.1 $\pm$ 1.9	99.1 $\pm$ 3.0	97.1 $\pm$ 2.6

### 3.7. Analytical characteristic of the procedure

The analytical characteristics of the proposed procedure for preconcentration of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  are given in Table 3. Calibration curves were obtained by processing standard solutions of the metal ions under the optimum conditions. The calibration curves are linear upto 500  $\mu\text{g L}^{-1}$  for all metal ions. The limit of detections (LOD) calculated as  $3s/S$  (where  $s$  is the standard deviation of 10 blank measurements and  $S$  is the slope of calibration curve) were 0.34, 0.94 and 0.83  $\mu\text{g L}^{-1}$  for  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$ , respectively. The

relative standard deviations (RSD) obtained for 10 measurements of 50  $\mu\text{g L}^{-1}$  of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  were 1.78, 1.61 and 1.80, respectively. Furthermore, the determination of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  by the proposed CPE was compared with other preconcentration procedures and the results are summarized in Table 4. As it can be seen the proposed CPE procedure has lower LOD, wider linearity and the enrichment factor (EF) is higher than most procedures.

**Table 3 The analytical characteristic of the method.**

Analytical parameters	$\text{Co}^{2+}$	$\text{Ni}^{2+}$	$\text{Cu}^{2+}$
Linearity ( $\mu\text{g L}^{-1}$ )	1.0 – 500.0	3.0 – 500.0	3.0 – 500.0
Correlation coefficient ( $r$ )	0.999	0.999	0.998
LOD ( $\mu\text{g L}^{-1}$ )	0.34	0.94	0.83
RSD (%)	1.78	1.61	1.80
EF*	48.9	49.5	50.0

\* Calculated as the ratio between slope of calibration curve by the CPE and that without preconcentration

**Table 4. Comparison with other CPE procedures**

Ions	Reagent	Detection	Samples	LOD ( $\mu\text{g L}^{-1}$ )	Linear range ( $\mu\text{g L}^{-1}$ )	Sample volume (mL)	EF	Reference
$\text{Co}^{2+}$ $\text{Ni}^{2+}$	hydroxy naphthol blue	FAAS	Milk samples	0.56 0.78	2.0- 160.0 3.0- 180.0 20.0- 200.0 20.0- 500.0	50	53.9 48.6	[19]
$\text{Co}^{2+}$ $\text{Ni}^{2+}$	2-amino-cyclopentene-1-dithiocarboxylic acid	Spectrophotometry	Natural water samples	10.0 7.5		10	10 10	[20]
$\text{Co}^{2+}$ $\text{Cu}^{2+}$	2-(3-ethylthioureido)benzoic acid	FAAS	Water, biological and food samples	0.23 0.71	2.4-500	50	50.8	[16]
$\text{Cu}^{2+}$	N-phenylbenzohydroxamic acid	FAAS	Water and hair samples	1.0	2.5-220	10	45	[21]
$\text{Co}^{2+}$ $\text{Ni}^{2+}$ $\text{Cu}^{2+}$	2-(5-bromo-2-pyridylazo)-5-(diethylamino) phenol	FAAS	Water sample	2.4 1.7 1.5	10.0-100	50	25	[22]
$\text{Co}^{2+}$ $\text{Ni}^{2+}$ $\text{Cu}^{2+}$	DHBPHC	FAAS	Water and biological samples	0.34 0.94 0.83	1.0 – 500 3.0 – 500 3.0 - 500	50	48.9 49.5 50.0	This work

### 3.8. Application

The procedure was validated by the determination of the studied metal ions in water, blood and urine samples. The analysis of spiked samples show

agreement between added and recovered amounts indicating accurate procedure. These results highlight the applicability of the procedure for real samples with different matrices.

**Table 5. Determination of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  in water, blood and urine samples (n = 5) by the proposed CPE procedure**

Sample	Metal ion	Added ( $\mu\text{g L}^{-1}$ )	Found ( $\mu\text{g L}^{-1}$ )	Recovery (%)
Tap water	$\text{Co}^{2+}$	-	N.D.	-
		5.0	4.9±0.3	98.0±1.9
		10.0	9.7±0.4	97.0±2.4
	$\text{Ni}^{2+}$	-	N.D.	-
		5.0	4.8±0.2	96.0±2.5
		10.0	9.8±0.4	98.0±2.2
	$\text{Cu}^{2+}$	-	N.D.	-
		5.0	5.0±0.4	100.0±1.7
		10.0	9.9±0.4	99.0±2.0
Blood	$\text{Co}^{2+}$	-	N.D.	-
		5.0	4.9±0.3	98.0±2.2
		10.0	9.8±0.5	98.0±2.5
	$\text{Ni}^{2+}$	-	0.8±0.1	-
		0.5	1.3±0.1	100.0±1.7
		1.0	1.8±0.1	100.0±1.9
	$\text{Cu}^{2+}$	-	850±32	-
		50	898±41	96.0±3.0
		100	946±38	96.0±2.8
Urine	$\text{Co}^{2+}$	-	1.5±0.1	-
		0.5	2.0±0.1	100.0±1.6
		1.0	2.5±0.1	100.0±1.7
	$\text{Ni}^{2+}$	-	2.6±0.1	-
		1.0	3.6±0.2	100.0±1.6
		2.0	4.5±0.3	95.0±3.0
	$\text{Cu}^{2+}$	-	19.9±0.6	-
		5.0	24.8±0.8	98.0±2.3
		10.0	29.6±0.8	97.0±2.8

### 4. Conclusion

The study presented an accurate and simple preconcentration procedure based on CPE for  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$ . The employed ligand was used for the first time as a complexing agent in CPE. The optimized procedure exhibits good analytical characteristics in terms of linearity, LOD and enrichment factor. The procedure is selective, and no significant interference was observed from the concomitant species. We applied the presented CPE procedure for preconcentration of  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Cu}^{2+}$  prior their determination in water, blood and urine samples by FAAS.

### Conflicts of interest

There are no conflicts to declare.

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