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A New Thioamide Derivative for Separation and Preconcentration of Multi Elements in Aquatic Environment by Cloud Point Extraction

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Abstract

2-(pyridine-2-yl)-N-p-chlorohydrazinecarbothioamide (HCPTS) was synthesized, characterized and successfully applied for the preconcentration of Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II) in water, blood, and urine samples prior to graphite furnace atomic absorption determination (GFAAS); Hg was determined by cold vapor technique. Under the optimum experimental conditions (*i.e.* pH = 8, 10^{-4} M of HCPTS, 0.05% w/v of Triton X-114), calibration graphs were linear in the range of 0.02 to 200 $ng\cdot mL^{-1}$ for Co(II), Cd(II), Pb(II) and Ni(II); 0.03 to 200 $ng\cdot mL^{-1}$ for Cu(II); 0.07 to 200 $ng\cdot mL^{-1}$ for Fe(II) and Zn(II) and 0.02 to 150 $ng\cdot mL^{-1}$ for Hg(II). The enrichment factors were 43, 51, 41, 46, 54, 40, 45 and 52 for Cu(II), Ni(II),Zn (II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II), respectively. The limit of detection were found to be 0.019, 0.094, 0.0514, 0.052, 0.0165, 0.047, 0.068 and 0.041 $ng\cdot mL^{-1}$ for Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II), respectively. The developed method was applied to the determination of these metal ions in water, blood and urine samples with satisfactory results.

Keywords: Heavy Metals, Preconcentration, Cloud Point Extraction, 2-(Pyridine-2-Yl)-N-P-Chlorohydrazinecarbothioamide

1. Introduction

Heavy metals are of the great importance for the life. Some of these such as Cu, Zn, Co, Fe are essential to humans [1]. Others such as Hg, Cd and Pb are toxic following occupational and environmental exposure [2]. Due to the low concentration of these metals in the environmental and biological samples and interfering effects, a preconcentration/separation technique is generally necessary prior to the determination. For this purpose, various analytical procedures have been used, such as adsorption on activated carbon [3,4] co-precipitation [5,6] column extraction [7,8] ion-selective electrode [9,10] liquid–liquid extraction (LLE) [11] and cloud-point extraction (CPE) [12-22].

CPE has attracted considerable attention mainly because it complies with the "Green Chemistry" principle [23], as the amount of organic solvent is much less than that of traditional liquid extraction. Moreover, It is simple, cheap, highly efficient, fast, and of lower toxicity than those extractions that use organic solvents. This method has been applied for extraction and preconcentration of some metal ions such as Cu, Ni, Co and Zn in water samples [12], Ag, Zn, and Pb in environmental samples [13], Mo in sea water [14], Cd, Pb, Pd and Ag in environmental samples [15], Cr, Pb, Cu, Ni, Bi, and Cd in environmental samples [16], Cd, Cu, Co and Ni in water [17], U, Th, Zr and Hf in aqueous samples [18], Be in water [19], La, Eu and Lu [20], Rh [21] and Yb, Gd, Eu, Sm, Sc, Ho in biological samples [22].

The cloud point is the temperature above which aqueous solutions of non-ionic and zwitter ionic surfactants become turbid. Specifically, above the cloud point the solution is separated into two phases: a rich phase containing a high surfactant concentration in a small volume and a poor phase with a surfactant concentration close to the critical micelle concentration [24,25]. CPE enables to avoid hazardous organic solvents and allows achieving a much higher concentration of recovered metal ions than in the case of liquid-liquid extraction, because the micellar phase volume is about 10 - 100-fold less than the volume of an aqueous phase [24]. Accordingly, any metal ions that either directly interact with micelles or after prerequisite binding with hydrophobic chelating ligand, can be extracted from the parent solution by CPE procedure. Trace elements can be extracted to the surfactant-rich phase usually after formation of a hydrophobic complex with an appropriate chelating agent [25]. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, cheap, highly efficient, fast, and of lower toxicity than those extractions that use organic solvents.

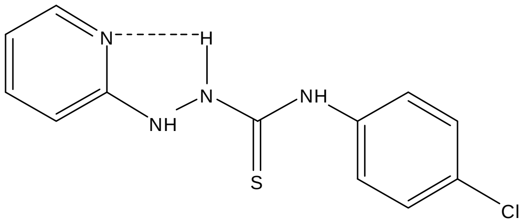
Thiosemicarbazides constitute an important class of NS donors. The chemistry of these compounds was early explored [26] for their variable donor properties, structural diversity and biological applications. The coordination chemistry of a number of metal ions and a wide variety of complexes has been reported [27-29]. Thiosemicarbazides have been used for extraction and determination of some metal ions in biological and pharmacological samples [30-32]. In recent studies, 4-Ethyl-1-(pyridin-2-yl)thiosemicarbazide and its Cu(II) complexes with different anions have been synthesized and characterized [28] and used as a chelating agent for separation, preconcentration, and determination of Cu(II) ions in saturated saline solutions by a cloud point extraction technique [33]. In addition to, complexes of 4-ethyl and 4-(p-tolyl)-1-(pyridin-2-yl)thiosemicarbazides with Pd(II), Pt(II) and Ag(I) were synthesized and characterizes [29] These complexes show antibacterial activity to some Gram positive and Gram negative bacterial strains.

In the present study, we have developed and optimized a simple CPE-GF AAS methodology for multi-elements determination in water, blood and urine samples, which shows rapid and efficient performance. Copper(II), Ni (II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II) were preconcentrated using Triton X-114 in the presence of 2-(pyridine-2-yl)-N-p-chlorohydrazinecarbothioamide (HCPTS) (**Structure 1**) as a new chelating agent.

2. Experimental

2.1. Reagents and Solutions

All chemicals were of analytical-reagent grade and were used without previous purification (A.R. from Fluka, Aldrich or Merck). All solutions were prepared using double distilled water. Metal ion solutions (1000 mg/l) were prepared by dissolving appropriate amounts of the sulphates, chlorides or nitrates in double-distilled water. Working reference solutions were prepared daily by stepwise dilution from stock solution. Triton X-114 was used without further purification. HCPTS solution (1 × 10^{-3} M) was prepared by dissolving 0.0278 g in 100 mL distilled water. Buffers were used to control the pH of the solutions: hydrochloric acid-glycine (pH 1 - 3), hexamine-nitric acid (pH 4 - 8), ammonium chloride-am- monium hydroxide (pH 9, 10). Solutions of alkali metal salts (1%) and various metal salts (0.1%) were used in order to test the interference of anions and cations, respectively.



Structure 1. HCPTS.

2.2. Preparation of the Ligand

HCPTS (**Structure 1**) was prepared by heating under reflux a mixture of 2-hydrazinopyridine (0.1mol) and 4-chlorophenylisothiocyanate (0.1 mol) in 20 ml absolute ethanol for 2 h. On cooling, white fine crystals were formed, filtered off, washed with EtOH and Et_2O and recrystallized from EtOH (m.p. 175˚C; yield 96%). The purity of the compound was checked by TLC. Elemental analysis Calculated for $C_{12}H_{11}N_4SCl$: C 51.70, H, 3.98; N, 20.10.and the found: C, 51.23, H, 3.75; N, 19.92.

2.3. Apparatus

Elemental analysis was carried out using a Perkin Elmer Elemental Microanalyser. Infrared spectrum of the ligand was measured using KBr discs on Mattson 5000 FTIR spectrometer. Calibration of the frequency reading was made with polystyrene film. The electronic spectrum of the ligand in DMF was recorded by Unicam UV-Vis spectrometer UV2 using 1 cm stoppered silica cells. Thermogravimetric analysis measurement (TG) of the ligand was performed using an automatic recording thermobalance type (951 DuPont instrument). Sample was subjected to heat using a rate of 10˚C/min from room temperature to 800˚C in nitrogen flow. The mass spectrum of the ligand was recorded on 70 eV with a Varian MAT 311 instrument. ^{1}H NMR spectrum of HCPTS, in d_6-DMSO, was recorded on EM-390 (200 MHz) spectrometer.

GFAAS measurements were carried out using A Perkin Elmer atomic absorption spectrophotometer (Model Analyst 800) with a longitudinal Zeeman background correction furnished with a transversely heated graphite atomizer (THGA) was used for the determination of the metal ions, except Hg. Sample solutions were injected into the atomizer by using AS-800 autosampler. The sample injection volume was 20 µL and the modifier "5 µg Pd/3 µg $Mg(NO_3)_2$" volume was 10 µL. The system is equipped with winLab 32 software. Hg was determined by cold vapor technique (CVAAS). The operating conditions are given in **Tables 1** and **2**.

The pH of the solution was adjusted using Hanna instrument model 8519 digital pH meter. A centrifuge with a model of CH90-2 (Hinotek Technology Co., Ltd. China) was used to accelerate the phase separation process.

2.4. Procedure of CPE

For the CPE, an aliquot of 50 mL of a solution containing metal ion buffered with pH 8, Triton X-114 (0.05% w/v) and 10^{-4} M HCPTS were kept for 10 min in a thermostatic bath at 40˚C. Subsequently, separation of the phases was achieved by centrifugation for 10 min at 4000 rpm. The phases were cooled down in an ice bath in order to increase the viscosity of the surfactant rich phase. The bulk aqueous phase was easily decanted simply by inverting the tube and dried in water bath. The surfactant-rich phase in the tube was made up to 1 mL by adding absolute methanol/conc. HNO_3 mixture (5:1).

2.5. Preparation of Real Samples

2.5.1. Water Sample

The water samples were filtered firstly through filter paper to separate the coarse particles and suspended matter and secondly through a Millipore cellulose nitrate membrane (pore size 0.45 µm), acidified to pH 2 with HNO_3 and stored in a refrigerator in a dark polyethylene bottle.

2.5.2. Blood and Urine Samples

One mL of blood or 5 mL of urine was digested with 6 mL [HNO_3 (65%) + $HClO_4$ (70%)] (2:1) in 50 mL beaker covered with a watch glass. The content of the beaker was heated gradually on a hot plate till dryness.

Table 1. Instrumental parameters and temperature program for metal ion analysis by GFAAS.

(a) Instrumental parameters:

Parameter	Cu(II)	Ni(II)	Zn(II)	Cd(II)	Co(II)	Pb(II)	Fe(II)
Wavelength (nm)	324.8	232	213.9	228.8	242.5	283.3	248.3
Slit width (nm)	0.7	0.2	0.7	0.7	0.7	0.7	0.7
Lamp current (mA)	25	20	25	12	15	25	25

(b). Temperature program:

Step	Temperature (˚C)							Time (s)		Argon flow rate ml/min
	Cu(II)	Ni(II)	Zn(II)	Cd(II)	Co(II)	Pb(II)	Fe(II)	Ramp	Hold	
Drying 1	110							1	30	250
Drying 2	130							15	30	250
Pyrolysis	1200	1100	700	500	1400	850	1400	15	20	250
Atomization	2000	2300	1800	1500	2400	1600	2100	0	6	0
Cleaning	2500							1	5	250

Table 2. Instrumental parameters for the determination of Hg by CVAAS.

Source	Hg hollow cathode lamp.
Slit width (nm)	0.7
Carrier gas flow rate (ml/min)	1000
Reducing agent	1% m/v $NaBH_4$ in 0.05 % m/v NaOH

All heating steps were carried out under a hood with necessary precautions. After increasing the pH to about 3 using 1 M NaOH, the volume was completed to 10 mL volumetric flask. Aliquots of 5 mL of the digested samples were analyzed according to the prescribed procedure.

3. Result and Discussion

3.1. Characterization of the Ligand (HCPTS)

The IR spectrum of HCPTS exhibits characteristic bands at 3262, 3172 and 3131 cm^{-1} attributed to ν(N^{4}H), ν(N^{1}H) and ν(N^{2}H), respectively [28]. The ν(C=N)$_{Py}$ appearing as a strong band at 1598 cm^{-1}. The appearance of the (C=N)$_{Py}$ and N^{2}H at lower values suggests intramolecular hydrogen bonding [29]. Also, two bands at 1512 and 1450 cm^{-1} were observed and assigned to thioamide I and III, respectively. Moreover, two sharp bands at 783 and 723 cm^{-1} were attributed to ν(C=S) and ρ(NH) [28]. The absence of any band due to ν(SH) in the range 2500 - 2600 cm^{-1} [28] indicated that the ligand exists in the thione form.

The absorption spectrum of HCPTS in DMF showed a band at 35,714 cm^{-1} and three shoulders at 31,250, 24,390 and 23,148 cm^{-1} attributing to $(\pi\rightarrow\pi^*)_{Py}$, $(\pi\rightarrow\pi^*)_{C=S}$, $(n\rightarrow\pi^*)_{Py}$ and $(n\rightarrow\pi^*)_{C=S}$ [29] transitions, respectively.

The TGA curve of HEPTS indicates a thermal stability till 167˚C coincident with its melting point (175˚C). The curve showed two decomposition steps. The first from 167 to 219˚C is corresponding to the loss of C_5H_4N and ClC_6H_4 (Found 68.2%; Calcd. 68%), the second from 219 to 347 is attributed to the loss of N_3H_3 (Found 15.9%, Calcd. 16.1%), leaving a residue of C + S (Found 15.9%, Calcd. 15.8%).

The ^{1}H NMR spectrum of HCPTS in d$_6$-DMSO showed signals due to the protons of the phenyl and pyridine [29]. The three singlet signals at 8.54, 9.79 and 9.91 ppm are attributed to the N^{2}H, N^{1}H and N^{4}H protons, respectively. The N^{2}H signal appears in the aromatic region confirming an intramolecular H-bond. The other NH protons are shifted up field due to the inductive effect of pyridyl and phenyl rings as electron donor groups [29].

The mass spectrum of HCPTS shows the final peak at 280 amu [($C_{12}H_{11}N_4SCl$), calculated molecular weight 278.76 amu], and other peaks at 171, 111 and 77 amu may correspond to various fragments. The peak described at 171 amu is assigned to the fragment $[C_7H_5NSCl]^+$, corresponding to the loss of $[C_5H_6N_3]^+$. The peak at 111 is corresponding to the fragment $[C_6H_4Cl]^+$, corresponding to the loss of $[CHNS]^+$ fragment. Peak at 77 represent the fragment C_6H_4 with the loss of Cl.

3.2. Effect of pH on CPE

The formation of metal complexes and its stability are important factors for the CPE. The pH plays a unique role on metal chelate formation and subsequent extraction. The extraction depends on the pH at which complex formation occurs. The effects of the pH on the percentage extraction recovery of Co(II), Hg(II), Cd(II), Fe(II), Pb(II), Ni(II), Zn(II) and Cu(II) were investigated at different pH (1 - 10). Where the percentage extraction recovery is the percentage of the concentration of metal found in spiked sample to the actual concentration. The results are shown in **Figure 1**. It was observed that Pb(II) and Cu(II) were extracted quantitatively (>95%) at pH 5; Co(II), Ni(II) and Zn(II) at pH 6; Hg and Fe(II) at pH 7; Cd(II) pH 8. At lower pH values, the extraction is not quantitative, and at higher pH values, the hydrolysis of cations occurs except for Co and Cd(II) a result, pH 8 was chosen as the working pH for the multi determination of these metals.

3.3. Effect of HCPTS Concentration

The extraction recoveries of the metals depend on the concentrations of the chelating agent. In order to select the optimum concentrations of HCPTS, the effects of the concentration of the HCPTS on the recoveries were investigated in the range of 1 × 10^{-5} to 1.6 × 10^{-4} M; the results are shown in **Figure 2**. For all metals, the maximum recoveries were obtained at 10^{-4} M of HCPTS. Therefore, these concentrations were selected as the optimum concentration of the chelating agent.

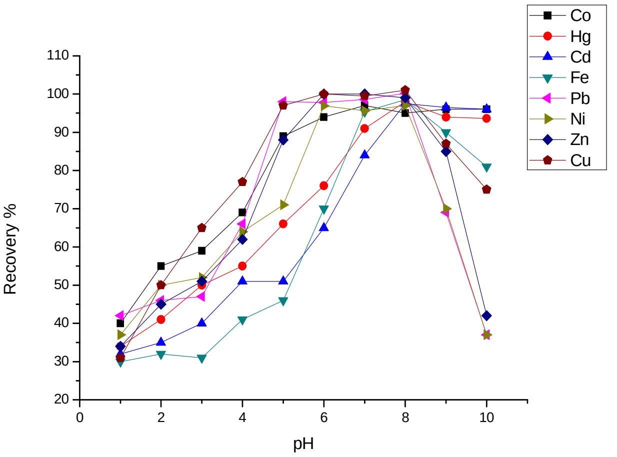


Figure 1. Influence of the pH on the recovery of extraction for the metal ions. Conditions: 10 ng·mL^{-1} each metal; 50 mL cold aqueous solution; 10^{-4} mol·L^{-1} of the ligand; 0.05% w/v Triton X-114; 40˚C.

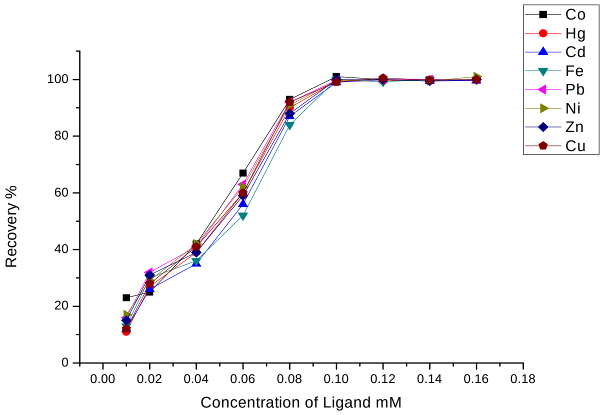


Figure 2. Effect of the ligand concentration on the recovery of extraction for the metal ions. Conditions: 10 ng·mL^{-1} each metal; pH, 8; 50 mL cold aqueous solution; 0.05% w/v Triton X-114; 40˚C.

3.4. Effect of Triton X-114 Concentration

The non-ionic surfactant, Triton X-114, was chosen because of its commercial availability in a high purified homogeneous form, low toxicological properties and cost. Also, the high density of the surfactant rich phase facilitates phase separation by centrifugation. Additionally, the cloud point (23˚C - 26˚C) of Triton X-114 permits its use in the extraction and/or preconcentration of a large number of molecules and chelates. The variation of the recoveries as a function of the concentration of Triton X-114 in the range of 0.01 to 0.1% (w/v) is shown in **Figure 3**. A concentration of 0.05% (w/v) was chosen as optimum concentration for the extraction of the all metal ions. At lower concentrations, the extraction recovery of the complex is low probably because of the inadequacy of the assemblies to entrap the hydrophobic complex quantitatively.

3.5. Effect of the Equilibration Temperature and the Centrifugation Time

In order to optimize the method, it was necessary to examine the effect of the temperature on cloud-point extraction. It was desirable to employ the lowest possible equilibration temperature and the shortest incubation time of extraction for efficient separation. For this purpose, the effect of temperature and equilibration time on the extraction recovery was investigated in the range of 30˚C - 90˚C and 5 - 35 min, respectively, while keeping all other parameters constant. It is desirable to employ low equilibration temperature, as a compromise between the completion of extraction and efficient separation of phases. Excellent recovery was found at 40˚C - 60˚C. Above this temperature, reduction of extraction efficiency was noted. Therefore, 40˚C was chosen for the equilibration temperature. The results also show that incubation time of 10 min is adequate to achieve the highest extraction efficiency.

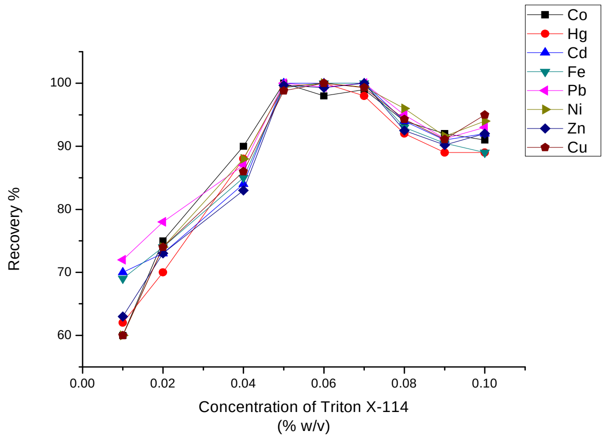


Figure 3. Effect of Triton X-114 concentration on the recovery of extraction for the metal ions. Conditions: 10 ng·mL^{-1} each metals; pH 8; 50 mL cold aqueous solution; 10^{-4} mol·L^{-1} of the ligand

3.6. Effect of Centrifugation Time and Rate

Generally, centrifugation time and rate ever affect micelle formation but accelerate the separation. The effect of the centrifugation time on the extraction efficiency was studied in the time range of 5.0 - 25.0 min at 4000 rpm. A centrifugation time of 10.0 min was selected, since the analyte extraction is almost quantitative. No appreciable improvements were observed for longer times.

3.7. Effect of Ionic Strength

The cloud point of micellar solutions can be controlled by addition of salts, alcohols, nonionic surfactants and some organic compounds (salting-out effects). To date, most of the studies conducted have shown that ionic strength has no appreciable effect on the extraction efficiency. An increase in the ionic strength in the cloud point extraction does not seriously alter the efficiency of extraction of the chemical forms. Moreover, the addition of a salt can markedly facilitate the phase-separation process, as demonstrated with some nonionic surfactant system, since it alters the density of the bulk aqueous phase [24]. In our study, it was observed that the addition of KI within the interval of 0.1 - 1.0 mol/L had no significant effect on the cloud point extraction efficiency.

3.8. Effect of Methanol

Since the surfactant-rich phase obtained after the cloud point preconcentration contains a high concentration of

Triton X-114 and, at the same time, the volume obtained is rather small (0.5 mL), absolute methanol/conc. HNO_3 mixture (5:1) was added to the surfactant rich phase after phase separation. Moreover, it was necessary to decrease the viscosity of the rich phase without excessive dilution of the micelle to facilitate the introduction of the sample into the atomizer of the spectrometer. There is an optimum volume (1 mL) with respect to the metal ion recovery. Larger volumes of acidified methanol dilution are clearly predominated resulting in a gradual absorbance reduction. One mL volume of methanol was therefore used throughout the remaining experiments.

3.9. Analytical Characteristic of the Method

The characteristics of the proposed cloud point extraction procedure for Co(II), Hg(II), Cd(II), Fe(II), Pb(II), Ni(II), Zn(II) and Cu(II) are given in **Table 3**. Calibration curves were obtained using 50 ml of the standard solution of the metal ion under the experimental conditions specified in procedure. The GFAAS signals were found to be a linear function of concentration range [0.02 ng·mL^{-1} for Co(II), Cd(II), Pb(II) and Ni(II); 0.03 ng·mL^{-1} for Cu(II); 0.07 ng·mL^{-1} for Fe(II) and Zn(II) to 200 ng·mL^{-1} for all ions], while the linear concentration range of HGAAS was 0.02 to 150 ng·mL^{-1} for Hg(II). **Table 3** gives the parameters of the calibration curves and the relative standard deviations (RSD) obtained for 3 replicates subjected to the complete procedure. The correlation coefficient (*r*) of the calibration curves are within 0.990 to 0.999, which indicates good linearity in the studied concentration range.

The limits of detection (LOD) were calculated as three times (3s) of standard deviation of blank signal by 7 replicate measurements. All the metals achieved very low detection limits in the range of 0.0165 - 0.094 and is better than the previous procedures (**Table 4**); indicating the high sensitivity of HCPTS to the investigated metal ions.

The preconcentration factor for all metal ions were calculated by dividing the aqueous phase volume to the final volume of preconcentrated phase is 50, while the enrichment factor as the ratio of slope of the calibration curve obtained from preconcentrated samples to that obtained without preconcentration for Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II), were 43, 51, 41, 46, 54, 40, 45 and 52, respectively. This indicates that the extraction using the proposed procedure is quantitative for all metal ions. Furthermore, the determination of Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II) by the proposed method was compared with other CPE-AAS or CPE-ICP methods [12,15,34-38] and the results are summarized in **Table 4**. As it can be seen the proposed method has higher preconcentration factor and lower LOD.

3.10. Effect of Interfering Ions

Under the optimized conditions determined for this study, the percentage removal of 20 ng·mL^{-1} of Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II) were studied in the presence of high concentrations of various cations and anions. All of the cations were used as their chlorides or sulfates, whereas the anions were used as the corresponding sodium salts. An ion was considered to be an interferent when it caused a variation greater than ±5% in the absorbance of the sample. The corresponding results obtained are listed in **Table 5**. The results obtained indicates that the removal of Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II) ions was quantitative in all cases. Moreover, 1000 fold of (K^+, Na^+, Cl^-, NO_3^-, SO_4^{2-}); 750 fold of (Citrate Thiocyanate Thiourea Thiosulphate Acetate); 500 fold of (Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, oxalate, phosphate, I^-) and 50 fold of (Mn^{2+}, B^{3+}, Al^{3+}, Bi^{3+}) have no effect on the extraction efficiency.

Table 3. Analytical characteristic of the method.

Parameter	Cu(II)	Ni(II)	Zn(II)	Cd(II)	Co(II)	Pb(II)	Fe(II)	Hg(II)
Limit of detection (ng·ml^{-1})	0.019	0.094	0.0514	0.052	0.0165	0.047	0.068	0.041
Reliability (% RSD, n = 7)	3.35	2.61	3.14	1.64	2.19	3.61	4.1	2.76
Linearity	0.03-200	0.02-200	0.07-200	0.02-200	0.02-200	0.02-200	0.07-200	0.02-150
Slope of Calibration Curve	0.0069	0.0087	0.0154	0.0624	0.0049	0.0027	0.0068	0.0141
Intercept of Calibration curve	$3 X 10^{-5}$	$3 X10^{-5}$	$3 X 10^{-5}$	0.0038	$7 X10^{-6}$	$9 X 10^{-5}$	10^{-6}	0.0002
Correlation coefficient (*r*)	0.996	0.995	0.993	0.999	0.991	0.990	0.982	0.993
Enrichment Factor (EF)	43	51	41	46	54	40	45	52

Table 4. CPE applications for metal ions analysis of previous studies.

Ions	Reagent	Surfactant	Detection system	Matrix	DL (ng·mL^{-1})	Linear range (ng·mL^{-1})	Sample volume (mL)	EF	Reference
Cu(II)	4-(2-pyridylazo)-resorcinol	Triton X-114	ICP-OES	water	1.2	10-500	15	-	[12]
Zn(II)					1.1	10-700		-	
Cd(II)					1.0	20-2000		-	
Ni(II)					6.3	50-2500		-	
Cd(II)	bis((1H-benzo [d] imidazol-2yl)ethyl) sulfane	Triton X-114	FAAS	radiology waste, vegetable, blood and urine	1.4	-	15	48	[15]
Pb(II)					2.8	-		39	
Zn(II)	2-Guandinobenzimidazole	Triton X-114	FAAS	water	8.8	8.8-80	50	13	[34]
Co(II)					4.9	4.9-3000		15.5	
Ni (II)					7.8	7.8-2000		15	
Pb(II)					11	11-6000		29.6	
Cu(II)	2-(6-(1H-benzo[d] imidazol-2-yl) pyridin-2-yl)-1Hbenzo [d]Imidazole	Triton X-114	FAAS	Blood, orange juice and lotus tree	1.4	10-250	15	35	[35]
Zn(II)					1.0	10-250		39	
Ni(II)					1.9	15-200		30	
Cu(II)	*N*,*N'*-bis[(1*R*)-ethyl-2-hydroxyethyl] ethanediamide	Triton X-114	FAAS	water	0.45	2.5-25	40	18	[36]
Cd(II)					0.5	2.5-25		23	
Co(II)					1.25	5-25		18	
Ni(II)					0.6	5-25		20	
Cu(II)	*N*,*N'*-bis[(1*S*)-1-benzyl-2-hydroxyethyl] ethanediamide				0.44	2.5-25		20	
Cd(II)					0.25	2.5-25		22	
Co(II)					0.60	5-25		17	
Ni(II)					1.55	5-25		20	
Ni(II)	p-nitrophenylazoresorcinol	Triton X-114	FAAS	Water and food	2.7	10-400	50	17	[37]
Cd(II)	1, 5-bis(di-2-pyridylmethylene) thiocarbonohydrazide	Triton X-114	FAAS	water, food and environmental samples	0.95	10-200	10	10.5	[38]
Cu(II)	HCPTS	Triton X-114	GFAAS (CVAAS for Hg)	Water, blood and urine	0.019	0.03-200	50	43	This work
Ni(II)					0.094	0.02-200		51	
Zn(II)					0.0514	0.07-200		41	
Cd(II)					0.052	0.02-200		46	
Co(II)					0.0165	0.02-200		54	
Pb(II)					0.047	0.02-200		40	
Fe(II)					0.068	0.07-200		45	
Hg(II)					0.041	0.02-150		52	

Table 5. Tolerance limits of interfering ions.

Interfering ion	Tolerance limit	Recovery %							
		Cu(II)	Ni(II)	Zn(II)	Cd(II)	Co(II)	Pb(II)	Fe(II)	Hg(II)
K^+, Na^+, Cl^-, NO_3^-, SO_4^{2-}	1000	99.7 ± 0.8	96.1 ± 2.1	97.2 ± 1.8	99.2 ± 1.5	98.4 ± 1.6	97.8 ± 2.4	98.1 ± 2.4	99.3 ± 3.2
Citrate, Thiocyanate, Thiourea, Thiosulphate, Acetate	750	96.2 ± 1.2	99.4 ± 1.8	100.2 ± 1.6	100 ± 1.1	100 ± 1.5	98.1 ± 0.8	96.9 ± 1.9	98.4 ± 1.8
Ca^{2+}, Mg^{2+}, Ba^{2+}, Sr^{2+}, Oxalate, Phosphate, I^-	500	100.4 ± 0.6	99.2 ± 2.3	98.3 ± 2.4	100.3 ± 2.1	98.2 ± 2.2	98.6 ± 1.6	96.2 ± 2.2	99.2 ± 2.1
Mn^{2+}, B^{3+}, Al^{3+}, Bi^{3+}	50	97 ± 1.6	98.4 ± 0.9	97.4 ± 1.7	97.8 ± 3.1	100.1 ± 2.2	96.7 ± 0.7	98.7 ± 1.9	97.6 ± 1.9

Table 6. Recovery of trace metal ions from spiked real samples after application of presented procedure.

Sample	Ion	Added (ng·mL^{-1})	Found (ng·mL^{-1})	Recovery %
Tap Water	**Cu**	-	2.1	
		1.0	3.15	105
	Ni	-	1.09	
		1.0	2.04	95
	Zn	-	0.54	
		1.0	1.51	97
	Cd	-	0.12	
		1.0	1.08	96
	Co	-	0.29	
		1.0	1.32	103
	Pb	-	1.7	
		1.0	2.66	96
	Fe	-	32.6	
		10.0	43.1	105
	Hg	-	0.2	
		1.0	1.16	96
Blood	**Cu**	-	924	
		100	1027	103
	Ni	-	0.84	
		1.0	1.86	102
	Zn	-	5,579	
		1000	6591	101
	Cd	-	0.92	
		1.0	1.90	98
	Co	-	0.12	
		1.0	1.11	99
	Pb	-	55.6	
		10.0	65.8	102
	Fe	-	556	
		100	660	104
	Hg	-	2.43	
		1.0	3.44	101
Urine	**Cu**	-	34.8	
		10.0	45.1	103
	Ni	-	2.45	
		1.0	3.46	101
	Zn	-	375	
		100	478	103
	Cd	-	0.84	
		1.0	1.85	101
	Co	-	1.21	
		1.0	2.20	99
	Pb	-	9.6	
		10.0	19.8	102
	Fe	-	152	
		100	255	103
	Hg	-	2.13	
		1.0	3.15	102

3.11. Accuracy and Precision

To check the applicability, reliability and accuracy of the studied separation method, the proposed procedure has been applied to determine Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II) ions in spiked tap water, blood and urine samples. The results are presented in **Table 6**. The recovery values were around 100% in the different samples. This indicates the capability of the method in the determination of analytes in real samples with high efficiency. In order to establish the validity of the proposed method, the described procedure was applied for the determination of investigated ions in multielement standard solution 70006 (Fluka). The obtained results assembled in **Table 7** are in very good agreement with the labeled values. The error of determination does not exceed 1.5%.

3.12. Analysis of Real Water Samples

The CPE method was applied for the preconcentration and separation of Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II) and Hg(II) in different water samples. Surface water samples were collected from Nile River at Mansoura, Miet Antar, Sherbin, and Faraskour; brackish water at Damietta bridge, Elgerbi, Ezbet Elborg and Elmanzalah lake and Mediterranean Sea at Port Fouad and Port Said. **Table 8** shows the results of the application process in comparison with the standard methods: direct HGAAS in the case of Hg(II) and solvent extraction (ammonium pyrrolidinedithiocarbamate in methyl isobutyl ketone) followed by ICP-MS determination in the case of the other metal ions [39]. It can be concluded that the concentration of the investigated metal ions increases obviously in the region beginning from Damietta Bridge to the River effluent and in Elmanzalah Lake. This may be attributed to the domestic and anthropogenic activities in these regions which are also shown in the water quality and nutrients measurements. It can be also concluded that the concentrations of heavy metal ions are within the permissible levels and are in agreement with those reported previously [39-41].

3.13. Analysis of the Metals in Blood of Hemodialysis Patients

Finally, the metal ions under investigation were determined in 13 hemodialysis patients and 11 matched healthy persons (control group). For statistical calculations we used SPSS-PC software, version 8 (MAS Medical & Scientific Eq. Co, IL, USA). Differences between groups were tested with the t-test for independent samples. Correlation between variables was assessed with Spearman's rank correlation coefficient (*r*). A probability value (*p*) less than 0.05 was considered to be significant. The results summarized in **Table 9** show that the hemodialysis patients had normal blood levels of Co, Ni and Hg. The blood levels of Cu, Zn and Fe were significantly lower than the normal group. However, the levels of Cd and Pb were higher than the control group. These findings indicated that hemodialysis patients suffer from deficiency of some essential metals as well as they are at high risk to some toxic metals.

4. Conclusions

The developed CPE procedure is simple, rapid, safe and provide high preconcentration factor and low detection limits for the determination of ultra trace levels Cu(II), Ni(II), Zn(II), Cd(II), Co(II), Pb(II), Fe(II), and Hg(II)

Table 7. Determination of metal ions in multielement standard solution 70006 (n = 3).

Metal ion	Found by present method (mg·L^{-1})	Certified value (mg·L^{-1})
Cu(II)	19.8 ± 1.1	20
Ni(II)	20.2 ± 0.9	20
Zn(II)	98.7 ± 2.2	100
Cd(II)	10.05 ± 0.4	10
Co(II)	9.87 ± 0.3	10
Pb(II)	40.2 ± 1.2	40
Fe(II)	100.5 ± 1.8	100
Hg(II)	Not detected	-

Table 8. Analysis of the metal ions (ng·mL^{-1})in natural water samples using CPE procedure in comparison with those obtained by ICP-MS after solvent extraction with APDC/MIBK (direct HGAAS for Hg).

Location	Cu (II)		Ni (II)		Zn (II)		Cd (II)		Co (II)		Pb (II)		Fe (II)		Hg(II)	
	CPE-GFAAS	SE-ICP-MS	CPE-GFAAS	SE-ICP-MS	CPE-GFAAS	SE-ICP-MS	CPE-GFAAS	SE-ICP-MS	CPE-GFAAS	SE-ICP-MS	CPE-GFAA	SE-ICP-MS	CPE-GFAAS	SE-ICP-MS	CPE-HGAAS	HGAAS
Mansoura (Nile River)	1.59±0.05	1.63±0.04	2.31±0.09	2.11±0.07	13.2±0.62	13.9±0.55	0.17±0.03	0.18±0.05	0.07±0.004	0.08±0.006	10.5±0.5	10.9±1.1	260.5±39.3	275.6±45.2	0.32±0.03	0.33±0.05
Miet Antar (Nile River)	1.72±0.07	1.59±0.08	6.2±0.98	6.54±0.65	27.5±4.2	28.3±5.1	0.42±0.01	0.39±0.03	0.09±0.01	1.05±0.02	9.4±0.6	9.9±0.9	182.3±25.6	174.9±29.6	0.31±0.02	0.32±0.04
Sherbin (Nile River)	1.21±0.04	1.32±0.03	1.2±0.22	1.12±0.19	15.6±2.5	16.2±1.6	0.14±0.02	0.14±0.04	0.06±0.008	0.08±0.01	2.9±0.2	3.2±0.3	98.6±15.4	100.2±10.7	0.02±0.003	BDL
Faraskour (Nile River)	1.41±0.02	1.53±0.02	1.39±0.18	1.42±0.16	13.2±3.1	13.5±2.4	0.09±0.01	0.12±0.02	0.06±0.01	0.07±0.02	10.1±0.7	9.4±0.5	63.3±8.9	59.7±10.1	0.24±0.01	0.26±0.03
Damietta bridge (brackish)	2.68±0.12	2.91±0.15	4.1±0.14	4.3±0.17	14.5±0.51	13.9±0.42	1.02±0.02	1.12±0.04	0.12±0.003	0.14±0.002	7.1±0.5	6.7±0.8	125.4±12.6	119.8±15.6	0.31±0.04	0.29±0.06
Elgerbi (brackish)	1.9±0.22	2.2±0.15	6.3±1.1	5.9±1.3	20.6±3.3	21.5±2.9	0.07±0.02	0.08±0.02	0.09±0.01	1.11±0.008	5.9±0.5	6.2±0.4	172.9±25.3	180.7±19.4	0.24±0.01	0.26±0.03
Ezbet Elborg (brackish)	2.4±0.18	2.1±0.20	4.5±0.31	4.2±0.41	18.7±3.3	19.1±2.5	0.13±0.04	0.12±0.03	0.1±0.02	0.09±0.01	11.1±0.9	10.5±0.5	121.5±19.6	126.2±20.1	0.27±0.02	0.30±0.01
Elmanzalah Lake (brackish)	3.6±0.42	2.59±0.55	2.3±0.33	2.35±0.27	8.2±2.6	7.9±2.1	0.12±0.05	0.11±0.03	0.08±0.018	0.09±0.02	12.9±1.3	13.2±2.1	51.2±8.4	49.6±9.5	0.21±0.02	0.27±0.04
Damietta Port (brackish)	3.1±0.32	3.3±0.22	1.52±0.28	1.61±0.24	10.3±1.2	10.9±1.5	0.08±0.02	0.07±0.03	0.08±0.01	0.08±0.02	7.9±0.9	7.2±1.1	35.2±5.7	33.7±6.1	0.22±0.01	0.24±0.03
Port Fouad (seawater)	2.79±0.17	2.96±0.12	1.2±0.06	1.32±0.05	14.9±0.45	15.3±0.76	0.30±0.06	0.29±0.08	0.08±0.005	0.09±0.008	11.8±1.2	12.3±0.6	61.2±14.3	63.6±12.2	BDL*	BDL
Port Said (seawater)	1.2±0.07	1.4±0.03	1.35±0.22	1.43±0.13	11.2±1.6	10.5±2.2	0.11±0.04	0.13±0.02	0.12±0.03	0.11±0.05	7.9±1.1	7.9±0.8	86.3±6.7	88.4±5.9	BDL	BDL

**BDL = below detection limit*

Table 9. Metal ions levels (ng·mL^{-1}) in blood of hemodialysis (n = 13) and healthy persons (n = 11).

Metal	Hemodialysis patients	Normal persons
Cu(II)	715 ± 195*	895 ± 215
Ni(II)	0.69 ± 0.22	0.79 ± 0.19
Zn(II)	3954 ± 293*	5421 ± 452

$^*p < 0.05$.

in water, blood and urine samples. This procedure agrees with the Green Chemistry principles.

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