Cloud-Point Extraction and Selective Spectrophotometric Determination of Pb(II) and Cu(II) using Synthesized N1,N2-Diphenylhydrazine-1,2-Dicarbothioamide and an Anionic Surfactant

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Abstract

A new micelle-mediated phase separation method using an anionic surfactant, sodium dodecyl sulfate (SDS), for preconcentration and spectrophotometric determination of Pb(II) and Cu(II) is presented. The procedure is based on the complex-formation reaction between Pb(II) / Cu(II) and a synthesized chelating agent, N1,N2-diphenylhydrazine-1,2- dicarbothioamide (PHCT) and subsequent extraction of the colored complex using sodium dodecyl sulfate which was used as extracting agent at room temperature. The chemical variables affecting the separation and determination procedure were optimized. In optimum conditions Beer's law was obeyed in the range 0.01–0.90 μg mL^{-1} for both cations. Limit of detection at the wavelength of the maximum absorption was found to be 2 ng mL^{-1} and 3 ng mL^{-1} for Pb(II) and Cu(II), respectively. The proposed method was applied successfully to the determination Pb(II) and Cu(II) in natural and waste water, and spiked human plasma samples.

Keywords: N1,N2-diphenylhydrazine-1,2-dicarbothioamide (PHCT); Pb(II), Cu(II); SDS, Cloud point extraction.

Introduction

Pb(II) and Cu(II) at high concentrations are very toxic because these ions are soluble in water and may be easily absorbed into living organisms. Exposure to the metals occur through air, dust, food/drink cans, drinking water contamination (water inside lead/copper pipes) and gasoline, mainly due to industrial development generated wastes which contain heavy metals to environment. [1-7]

Lead is a non-essential and toxic element and its organic and inorganic compounds are readily absorbed through the skin and accumulated in soft and hard tissues. [2-4]

Copper is an essential element for enzymes, but it is also toxic when its concentration is relatively high. This element is needed by many living organisms at

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only very low levels (nearly 40 ng mL^{-1}) to maintain carbohydrate and lipid metabolism and heart and blood vessel activity. [5-8]

Thus the determination of Pb(II)/ Cu(II) at trace level is important due to the harmful effects of heavy metals on humans and the environmental pollution. But the determination of trace concentration of metal ions in environmental and biological samples is difficult because of various factors, particularly low concentrations of metal ions and/or matrix effects etc. Therefore new techniques for preconcentration and/or cleaning (to remove or decrease the matrix effects) are required to achieve detection limits within the range of the available equipment.[5-9] Several analytical techniques such as flame atomic absorption spectrometry (FAAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and electrochemical methods, especially anodic stripping voltammetry (ASV), etc are applied for determination of metallic cations. Although these methods are highly sensitive they require relatively large volumes of sample for analysis and need complicated operations, expensive apparatus, and well-controlled experimental conditions. [3, 10, 11]

Spectrophotometric methods are still the attractive techniques. These methods are readily accessible for the determination of trace amounts of metals cations, because of their simplicity and speed, precision, accuracy in most routine laboratories. But the determination of metals ions by the use of spectrophotometric method is difficult or sometimes impossible because of the overlapping of the absorption spectra. Also sometimes the sensitivity of spectrophotometric methods are low.[2] In order to increase the selectivity masking agents such as EDTA, KCN etc can be used. Otherwise a special ligand which can selectively form the colored complex should be used [10]. On the other hand in order to improve the sensitivity, pretreatment and pre-cleaning of the samples and pre-concentration methods can be applied. There are several methods for pre-concentration and/or separation such as liquid–liquid extraction (LLE), solid phase extraction (SPE), ion-exchange techniques, cloud point extraction (CPE), etc. [3, 12]

The classical liquid-liquid extraction and separation methods are usually time consuming and labor expensive. These methods require relatively large volumes of high purity organic solvents. Of additional concern is disposal of the solvents, which creates a severe environmental problem.[13-14]

In comparison to traditional liquid–liquid extractions and chromatography methods, the surfactant-mediated extractions show several advantages, e.g. they are non-toxic, non-volatile and non-flammable. Also they need short extraction time, their experiments are inexpensive and give high preconcentration factor. [15]

Usually in cloud point extraction (CPE) methods the experimental conditions required for phase separation depend on surfactants nature. In conventional CPE methods, non-ionic surfactants such as Triton X-100 and Triton X-114 are used. The cloudy separation by these surfactants requires high temperatures and long extraction times.[16, 12-13] The clouding phenomenon can be occurred with anionic surfactants [15-18]. Recently micelle mediated extraction using anionic surfactant was applied for preconcentration and determination of malachite green in water samples and simultaneous determination of Co(II) and Ni(II) by Bahram et al.[17-18] This CPE is performed at low temperature and provide high recovery and low limit of detection.[15-17] In this CPE method, instead of temperature change, salting-out phenomenon is used.[15-16]

In this work a synthesized ligand was applied for simple, rapid and selective determination of Cu(II) and Pb(II). The procedure is based on the selective complex formation reaction between Pb(II) / Cu(II) and the synthesized chelating agent N1,N2-diphenylhydrazine-1,2-dicarbothioamide (PHCT). Subsequent extraction of the colored complex is performed using sodium dodecyl sulfate (SDS) and saturated solution of NaCl.

For preconcentration purpose appropriate amounts of SDS as an anionic surfactant, aqueous sample and ligand were transferred to a centrifuge tube and after addition of saturated solution of NaCl cloudy solution was formed. By centrifugation the surfactant-rich phase (which contains the analyte(s) of interest in complex form(s)) is separated and detected. As mentioned previously [17-18] several advantages are provided using anionic surfactant for CPE; (a) possibility of performing extractions at low temperatures (b) the extraction can be carried out in few minutes since equilibration time is not required (rapidity) and (c) these surfactants are absorbance-free in the UV-Vis region. [15-16]

Among the tested metal cations, only Pb(II) and Cu(II) (and Hg(II)) can react with PHCT and therefore the aim of this work was to present a completely selective spectrophotometric method for determination of these cations.

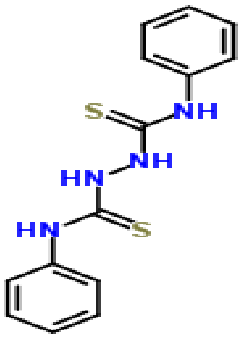
Experimental

Apparatus

A Shimadzu model 2550 UV-Vis spectrophotometer with 1-cm quartz cells (1.0 mL) was used for recording the absorbance spectra. All spectral measurements were performed using the blank solution as the reference. A centrifuge with 15 mL calibrated centrifuge tubes (Hettich, Germany) was used to accelerate the phase separation process. A Metrohm model 744 pH-meter with a combined glass electrode was used for pH measurements.

Reagents and solutions

All chemicals used in the experiments were of analytical grade and were used without further purification. All solutions were prepared with doubly distilled water. An aqueous (5% w/v) solution of SDS (Merck) was prepared by dissolving 5 g of SDS in final volume of 100 mL distilled water. N1,N2-diphenylhydrazine-1,2-dicarbothioamide, shown in scheme 1, was synthesized according to Ref. [19] and checked and characterized by FT-IR. A 10^{-3} mol L^{-1} N1,N2-diphenylhydrazine-1,2-dicarbothioamide (PHCT) solution was prepared by dissolving 300 mg of this ligand in 100 mL acetonitrile (Merck). HCl and NaOH (Merck) and phosphate buffer (pH 6.5- 7) were used for pH adjustment. Stock solutions of Pb(II) and Cu(II) (1000 mgL^{-1}) were prepared by dissolving appropriate amounts of $Pb(NO_3)_2$ and $Cu(NO_3)_2$.3H2O in distilled water. A saturated solution of sodium chloride was prepared by dissolving NaCl (Merck) in water. A series of standard solutions was prepared by appropriate dilution of stock solutions.



Scheme1: Structure of N1,N1-diphenylhydrazine-1,2-dicarbothioamide(PHCT)

Procedure

An aliquot of the solution containing Pb(II) or Cu(II) (final concentration 10–900 ng mL^{-1}), 0.2 mL of N1,N1-diphenylhydrazine-1,2-dicarbothioamide solution (10^{-3} mol L^{-1}) and 0.7 – 0.8 mL of SDS solution (5% w/v) were added into a 15 mL centrifuge tube. The pH of the solution was adjusted to 6.7 using phosphate buffer. The solution was diluted to nearly 9 mL with distilled water, and was mixed. Then 1 mL of saturated NaCl solution was added. Subsequently, the sample was shaken and placed for 5 min in an ice bath. Separation of the phases was achieved by centrifugation at 2000 rpm for 5 min. The aqueous phase was easily decanted by simply inverting the tube. The surfactant-rich phase obtained by this procedure was dissolved and diluted to 1 mL with a mixture of acetonitril / water (50: 50) for Pb(II) and acetonitril/ ethanol (70:30) for Cu(II). This solution was transferred into a 1.0 mL quartz cell. A blank solution was prepared in the same way.

Preparation of human plasma sample

20 mL of plasma sample was transferred to a beaker and was deproteinized with 2M phosphoric acid, 1.5 ml carbon tetrachloride and 8 ml ethanol. The mixture was stirred vigorously. After centrifuging for 5 min [20], the supernatant was separated, its pH was adjusted to pH 6.7 using buffer solution and then was subjected to analysis according to the procedure.

Results and discussion

The absorption spectra of Pb(II) and Cu(II) complexes with PHCT are shown in figure 1.

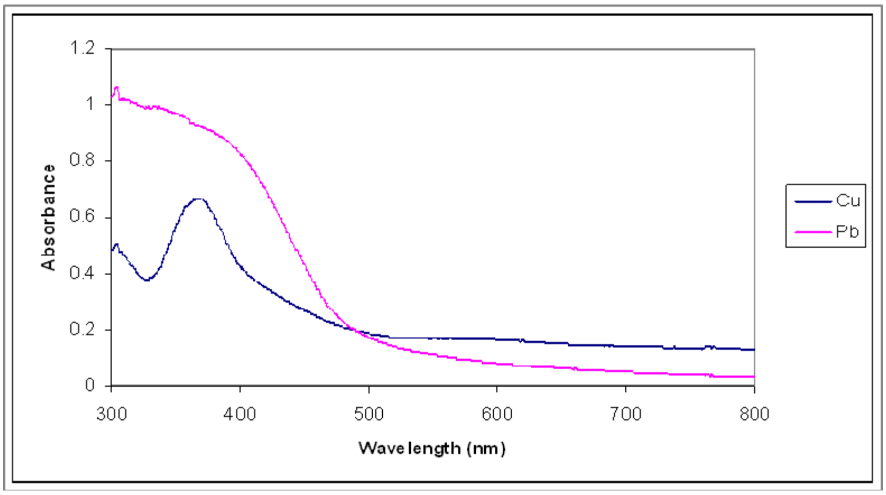


Figure 1: Absorption spectra of PHCTcomplexes with (a) Pb(II) and (b) Cu(II); Experimental conditions: 0.9 mg L^{-1} Cu or Pb, 2×10^{-5}molL^{-1} PHCT, pH = 6.7 and 0.4%, 0.35% SDS,for Pb and Cu, respectively. 1mL NaCl.

Effect of pH

Since the pH is one of the main parameters in the complexation reaction of metallic cations with ligands, it was the first variable which was optimized in the procedure. For this purpose, the effect of pH was investigated in the pH range of 2–9. The pHs were adjusted using HCl or NaOH. The results are given in Figure 2. The decrease in absorbance at pH > 6.7 is probably due to the precipitation of metal ions in the form of hydroxide, and at pH<6.7 may be due to interference of H^+ on PHCT at low pHs. Such result has been obtained elsewhere [16].

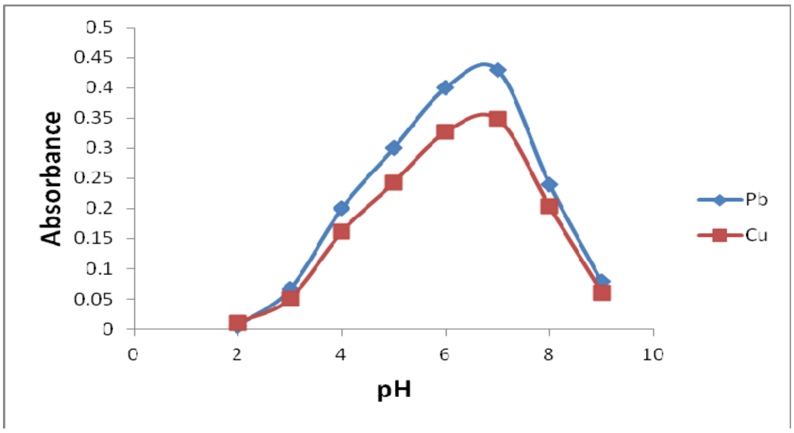


Figure 2: Effect of the pH on the CPE-preconcentration performance: Pb (400 μgL^{-1}) and Cu (500 μgL^{-1}); SDS 0.4% ,0.35% (w/v) Pb,Cu ; PHCT 2 × 10^{-}M, ,1mL NaCl.

Effect of PHCT concentration

At optimum pH, the effect of the PHCT concentration as chelating agent on the analytical signal was studied and the results are shown in Figure 3. In both cases, the signal increases up to a concentration of 1×10^{-5} mol L^{-1}, reaching a plateau, considered as complete complex formation and complete extraction. A concentration of 0.2 × 10−4 mol L^{-1} was chosen as the optimum concentration for the subsequent experiments.

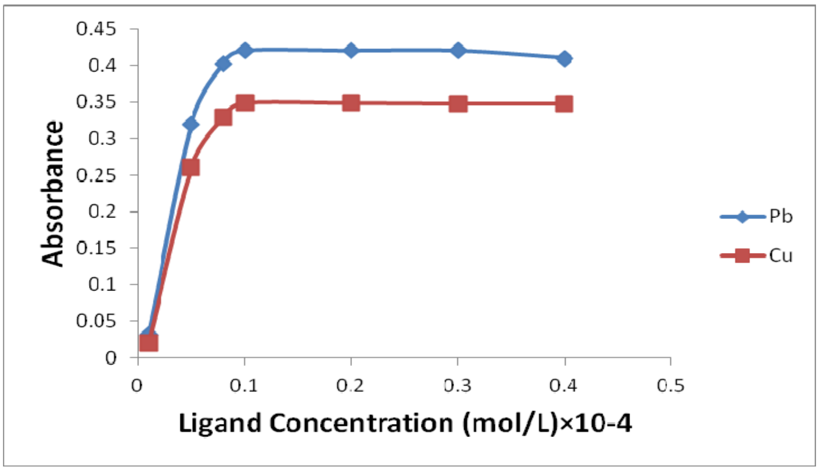


Figure 3: Effect of the PHCT concentration on the CPE preconcentration performance: Pb (0.4 μg mL^{-1}) and Cu (0.5 μg mL^{-1}); SDS 0.4% ,0.35% (w/v) Pb(II), Cu(II); pH = 6.7 and 1mL NaCl was used to CPE.

Effect of SDS concentration

The variation of the analytical signal as a function of the concentration of SDS in the range of 0.05-0.55% (w/v) is shown in figure 4. A concentration of 0.4% (w/v) and 0.35% (w/v) was chosen as the optimum concentrations for the determination of Pb^{2+} and Cu^{2+}, respectively. At low SDS concentrations, the extraction efficiency of the complex was very low, probably due to inability for quantitatively entrapment of hydrophobic complex. At higher concentrations of SDS the signal decreases too, maybe due to changing in the viscosity of extraction phase and therefore decreasing the molar absorptivity of formed complex in this phase. Such statement has been seen elsewhere.[11]

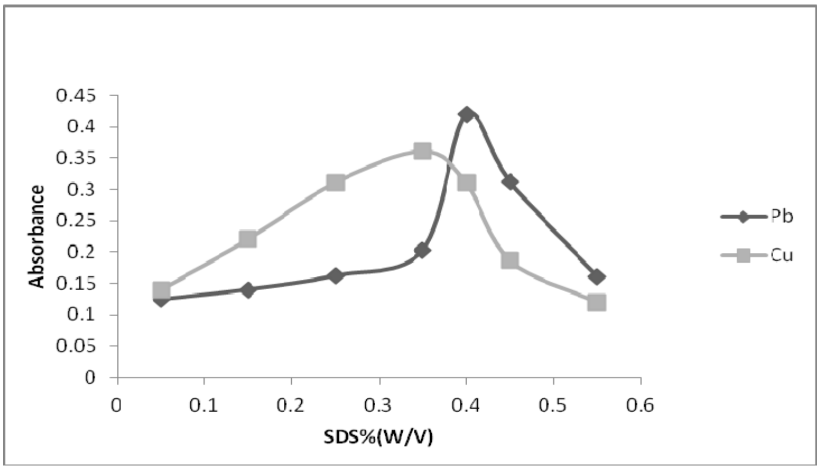


Figure 4. Effect of the SDS concentration on the CPE preconcentration performance: Pb (400μgL^{-1}) and Cu (500 μgL^{-1}); pH 6.7;PHCT 2×10^{-5}M, 1mL NaCl.

Effect of ionic strength

The effect of the ionic strength on the phase separation of anionic surfactant (SDS) from water was examined by addition of sodium chloride up to salt saturation conditions. The results indicate that the addition of saturated NaCl solution up to 1.0 mL make an increase in the extraction for both metal ions extraction. The maximum signal was obtained at 1.0 mL. Figure 5 shows that higher amount of saturated salt solution make a decrease in extraction. This decrease in extraction is possibly due to increase of micelle aggregation number (size of micelle) and decrease the critical micelle concentration (CMC). [15, 21]

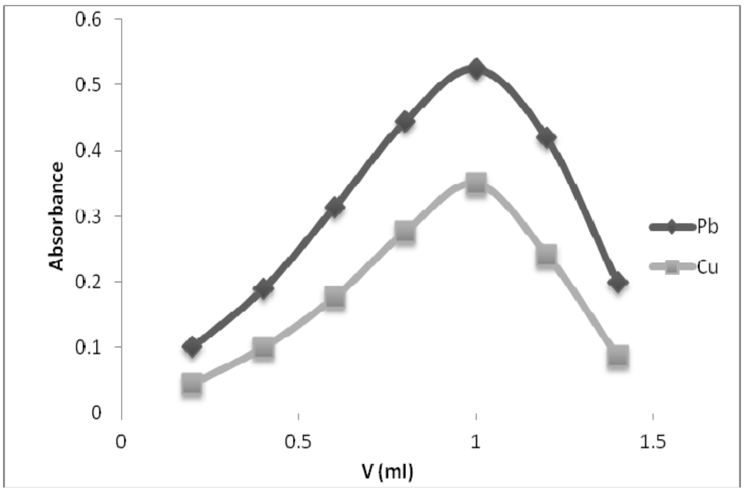


Figure 5. Effect of the *NaCl* concentration on the CPE preconcentration performance: Pb (400μgL^{-1}) and Cu (500 μgL^{-1}); pH 6.7;PHCT 2×10^{-5}M, SDS 0.4% ,0.35%(w/v) Pb and Cu, respectively.

Selection of the dilution solvent for the surfactant rich phase

Different solvents such as acetonitrile, ethanol, methanol, THF, mixture of acetonitrile-water and mixture of acetonitrile-ethanol were tested. The aim was to select a solvent or a mixture so that the surfactant rich phase and the extracted compounds could be dissolved completely. The results showed that the mixture of acetonitrile-water (1:1) and the mixture of acetonitrile-ethanol (2:1) are the best to dissolve the extracted phase. Therefore, they were selected as the diluents for further experiments.

Effect of other experimental factors

The influence of reaction completion time, extraction time and centrifugation time on the extraction efficiency was optimized. The dependence of extraction efficiency on reaction completion and extraction time was studied within a range of 1-30 min and 0-10 min, respectively. The results showed that a reaction completion time of 5 min and an extraction time of 2 min are adequate and sufficient to quantitative extraction of both ions based on the proposed method. The effect of the centrifugation time on the extraction efficiency was also studied within a range of 5–25 min. A centrifugation time of 5 min at 2000 rpm was selected for the entire procedure, since the analyte(s) extraction in this time is almost quantitative.

Selectivity of the synthesized ligand for determination of Pb(II) and Cu(II)

This ligand was known to have high selectivity for lead, copper and mercury. The concentration of mercury is usually very low in most biological samples and thus it has no interference in the extraction and determination of lead and copper.

Analytical characteristics of the method

Calibration graphs were obtained by pre-concentrating 10 ml of sample, under the optimum experimental conditions. Table 1 shows the analytical characteristics of the method. Under the specified experimental conditions the calibration curves for Pb(II) and Cu(II) were linear from 10 to 900 μgmL^{-1}. Also in table 2, the analytical performance of the proposed method for extraction and determination of Cu(II) and/or Pb(II) was compared with several cloud point extraction methods. As seen, the proposed method based on applying SDS for preconcentration may provide an attractive alternative for preconcentration and determination of Cu(II) and/or Pb(II). The proposed extraction method in this work shows a lower or comparable detection limit and also a relatively wide dynamic range compared to previously reported electrodes.

Table 1: Analytical characteristics of method

parameters	Pb(II)	Cu(II)
LOD / (ng mL^{-1})	2	3
RSD % (200 ng mL^{-1})	1.17	1.26
Linear Range (ng mL^{-1})	10-900	10-900
Correlation Coefficient	0.998	0.999

Table 2: Comparison of some analytical characteristics of the proposed methods with those of previously reported cloud point extraction method.

method	Analyte	Analyzed sample	Linear rang / ng mL^{-1}	LOD/ ng mL^{-1}	Ref
CPE-spectrophotometriy	Cu(II)	natural water samples	5-200	1.6	[22]
CPE-atomic absorption spectroscopy	Cu(II)	liver samples	15-250	4.6	[23]
CPE- Atomic Absorption Spectrometry	Pb(II) Cu(II)	water samples	Pb(II) 0.4 -185.0 Cu(II) 0.05-50.0	0.4 0.05	[16]
CPE spectrophotometric	Cu(II)	food and water samples	10–1000	5	[26]
CPE- using SDS	pb(II) Cu(II)	Water samples	Pb(II) 10-900 Cu(II) 10-900	2 3	This work

Determination of metal ions in water and plasma samples

The developed procedure was applied to the determination of metal ions in different samples, including; tap water, spring water, refined waste water and plasma sample by standard addition method. The results are presented in tables 3 and 4. For this purpose, 10 ml of each of the samples were evaluated under the optimum conditions. The spiked samples were also analyzed using standard addition method. The results are presented in tables 3 and 4.

Table 3: Recovery of trace elements from refined waste water , tap water and Spring water sample after application of the proposed CPE procedure. LOD: limit of detection

	Added(μg L-1)		**Found (μg L-1)**		**Recovery %**	
	Pb	Cu	Pb	Cu	Pb	Cu
Tap water	-	-	22	67		
	10	10	31.5	77	95	100
	50	50	71.5	116	99	98
	100	100	121	165	99	98
	200	200	220	266	99	99.5
Spring water	-	-	<LOD	122		
	10	10	9.5	132	95	100
	50	50	49.6	171	99	98
	100	100	98	221	98	99
	200	200	196	321	98	99.5
Refined waste water	-	-	<LOD	89		
	10	10	9.6	98	96	90
	50	50	48	138	96	98
	100	100	98	188	98	99
	200	200	198	288	99	99.5

Table 4: Recovery of trace elements from spiked plasma sample after application of the proposed CPE procedure

	Added(ng mL^{-1})	**Found (ng mL^{-1})**	**Recovery %**
Pb	-	<LOD	-
	100	111	111
	200	204	102
Cu	-	280	-
	100	390	110
	200	470	95

Conclusion

The introduced new procedure gives a highly selective, simple, sensitive, and low cost spectrophotometric procedure for the determination of Pb(II) and Cu(II) that can be applied to real samples analysis. The use of anionic surfactant for the extraction and spectrophotometric determination, offers some significant advantages. The method can be performed at low temperature and had no effect on the stability of the compounds of interest.

The stability of most chemicals decrease at higher temperatures and therefore these components cannot be extracted by traditional CPE methods which need the temperature to be increased. The proposed method provides high recovery, high preconcentration factor and low detection limit and utilizes of salt and avoids using large amount of concentrated acid for extraction.

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