Preconcentration and Determination of Cd, Co, Cu, Ni, Pb, and Zn in Water Samples Using Cloud Point Extraction and Ultrasonic Nebulization Coupled With Inductively Coupled Plasma Optical Emission Spectrometry

Mustafa Sahin Dundar*, Celal Caner, Ferhat Kaptan, and Huseyin Altundag
Sakarya University, Faculty of Arts and Sciences, Department of Chemistry,
54187 Sakarya, Turkey

INTRODUCTION

Environmental pollution from elements such as Cd, Co, Cu, Ni, Pb, and Zn has increased incrementally due to developing technologies and industrialization which not only endangers human life but also all other living creatures and plants. Urbanization, industrial development, and heavy traffic conditions also cause heavy metals pollution of the water in rivers, oceans, underground, etc. (1–3).

Cadmium (Cd) is considered the most harmful element because it has toxic effects even at the lowest concentrations. Short-term exposure can cause nausea, vomiting, stomach ache, dyspnea and muscle weakness. Long-term exposure to Cd may cause pulmonary edema and even death (4). Exposure to cobalt (Co) by inhalation can cause serious pulmonary diseases, asthma, and dyspnea (5). High levels of copper (Cu) can lead to bleeding in the digestive system, hemoglobinuria, intravenous hemolysis, hemoglobin levels over the normal values, hepatocellular intoxication, heavy renal failure and oliguria; some copper compounds are also carcinogenic. Lack of copper may cause headaches, nausea, and diarrhea (6). Nickel (Ni) exposure can lead to dermatitis, pulmonary fibrosis degeneration,

ABSTRACT

In this work, the ions of Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} in different natural water samples were separated and preconcentrated using cloud point extraction and determined in combination with an ultrasonic nebulizer and inductively coupled plasma optical emission spectrometry (USN-ICP-OES). The complexing agents of 1,5-diphenylthiocarbazone (dithizone) and the Triton X-114 surfactant of (1,1,3,3-tetramethylbutyl) phenyl-polyethylene glycol were used. Phase separation was observed at $\text{pH}=7$ and the surfactant-rich phase was diluted with 2 mL 2 M HNO_3 . The ligand concentration, surfactant concentration, pH, ionic strength, interferences, and equilibration of the temperature and time parameters were optimized. Under the optimized conditions, the enrichment factor was calculated for Cd^{2+} as 12.4, Co^{2+} as 7.5, Cu^{2+} as 5, Ni^{2+} as 9.3, Pb^{2+} as 9.6, and Zn^{2+} as 9.2. The limit of detection for Cd^{2+} was 0.009, Co^{2+} at 0.01, Cu^{2+} at 0.01, Ni^{2+} at 0.01, Pb^{2+} at 0.012, and Zn^{2+} at 0.02 ng mL^{-1} . The method was successfully applied to the analysis of natural water samples.

cardiovascular and renal diseases (7). Lead (Pb) has more than one toxicity effect, the most dangerous are natal disorders or abnormalities. Other symptoms include slowness on hemoglobin synthesis and renal function disorders, severe harm to

the central and peripheral nervous systems as well as the fertility and cardiovascular systems (8). Lack of zinc (Zn) can cause changes in the nerve senses, decrease sperm count, cause neuropsychological functions, growth arrest, slow healing of wounds, impact the immune system, and cause dermatitis. A lack of zinc can result in growth arrest and infertility, vomiting, diarrhea, hemoglobinuria, liver and renal failure, and anemia (9). The maximum allowable limits of these elements in drinking water as set by the United States Environmental Protection Agency are Cd^{2+} at 5 ng mL^{-1} , Co^{2+} at 100 ng mL^{-1} , Cu^{2+} at 1300 ng mL^{-1} , Ni^{2+} at 100 ng mL^{-1} , Pb^{2+} at 15 ng mL^{-1} , and Zn^{2+} at 5000 ng mL^{-1} .

The development of analytical techniques for the successful determination of heavy metals at very low concentrations in environmental matrixes is very important. Because heavy metals are often found below the detection limits of an instrument, a pre-concentration process is required which increases the detection levels by decreasing the matrix effect, resulting in better absolute results (10–12). Over the past years, cloud point extraction (CPE) has become one of the most popular pre-concentration methods because it provides high sensitivity (13–17). CPE was first reported by Watanabe et al. (18, 19) and is considered superior to traditional liquid-liquid extraction, provides high extraction yield and pre-concentration factors, is low cost and uses non-toxic chemicals.

*Corresponding author.
E-mail: dundar@sakarya.edu.tr
Tel: +90.264 2956044
Fax: +90.264 2955950

The cloud point extraction technique is based on phase separation in an aqueous solution of non-ionic surface-active agents. Phase separation occurs when the cloud point temperature is reached, even with any added material or pressure change. Most non-ionic surface-active agents form mycelium and are separated into two phases, one of which is below or at equal critical mycelium concentration, and the other is the surface-active-rich phase which pre-concentrates the analyte ions (20). Initially, any material in the solution may interact with mycelium and may be pre-concentrated in the surfactant-rich phase which has lower volume than the aqueous phase (21). Lightly soluble or water-insoluble materials may dissolve in water in direct proportion to the bonding abilities of mycelium (22). According to reported information, the non-ionic surface-active agent mycelium provides the best solubility for various soluble materials (23, 24).

Trace element analysis is one of the most important aspects of analytical chemistry. In order to perform heavy metal detection in water samples, several techniques are commonly used: flame atomic absorption spectrometry (FAAS) (25–29), inductively coupled plasma mass spectrometry (ICP-MS) (30, 31), graphite furnace atomic absorption spectrometry (GF-AAS) (32–34), electrothermal atomic absorption spectrometry (ETAAS) (35–37), and inductively coupled plasma optical emission spectrometry (ICP-OES) (38–41). ICP-OES is an efficient multi-element technique for the determination of inorganic elements in environmental and biological samples with a high rate of analytical efficiency. However, this technique has only reasonable measurability and requires a pre-concentration process for metals determination (42).

Several kinds of nebulizers are used in ICP-OES analysis, in particular the ultrasonic nebulizer which increases the analytical capacity by providing a higher vapor efficiency. The aerosol formation is 10 times faster than of a normal nebulizer, and the detection limits can be 10 times higher (43, 44).

The aim of this study was to enrich dithizone and the Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} ions by complexing with cloud point extraction. The measurements were obtained by ICP-OES analysis, coupled with an ultrasonic nebulizer. High %recovery and enrichment factors were achieved in the analysis of real water samples.

EXPERIMENTAL

Instrumentation

For this study, a model Spectro Arcos ICP-OES (Spectro Arcos, Germany) was used, coupled with a model U-5000AT+ ultrasonic nebulizer (Cetac Technologies, USA). For the pH measurements, a model Orion 2-Star Plus pH meter was

used (Thermo Scientific, USA). A Nüve model NF 400 centrifuge (Nüve, Turkey) was used for the centrifugation processes. All plastic and glassware was soaked in 10% (v/v) HNO_3 solution and washed with ultra-purified water. A Nüve model NB 20 water bath was used for the solution heating operations. The instrumental operating conditions are listed in Table I.

Reagents and Standard Solutions

All chemicals were of analytical grade. The standard solutions were prepared daily at the intended concentrations by making dilutions of 1000 mg/L Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} solutions (E. Merck). The pH adjustment of the solutions was performed with 0.1 M NaOH and 0.1 M HCl solutions (E. Merck). The hydrophobic complex provider was 1,5-diphenylcarbazone (dithizone) and the surface-active agent was (1,1,3,3-tetramethylbutyl) phenyl-polyethylene glycol (Triton® X-114). A 10^{-2} M dithizone solution was obtained by dilution with 100 mL THF (E. Merck) of 256 mg solid.

TABLE I
ICP-OES and Ultrasonic Nebulizer Operating Parameters

ICP-OES Operating Parameters	
Model	Spectro Arcos ICP-OES
Viewing Height	12 mm
Wavelengths	Cd 214.438 nm, Co 228.616 nm, Cu 324.754 nm, Ni 231.604 nm, Pb 220.353 nm, Zn 213.856 nm
Replicates	3
RF Power	1450 W
Spray Chamber	Cyclonic
Nebulizer	Cetac Ultrasonic Nebulizer
Nebulizer Flow	0.8 L/min
Plasma Gas Flow	13 L/min
Auxiliary Gas Flow	0.7 L/min
Sample Aspiration Rate	2.0 mL/min
Sample Pump Rate	25 rpm
Ultrasonic Nebulizer Conditions	
Apparatus	CETAC U-5000 AT+
Desolvation Temperature	140 °C
Condenser Temperature	5 °C

A 5% (v/v) Triton X-114 solution was obtained by dilution with 100 mL boiling ultra-purified water and 5 mL analytic grade Triton X-114 liquid. In order to dilute the surface-active rich phase, ultra-purified 2 M HNO₃ solution (E. Merck) was used. For preparation of the solutions, Milli-Q® distilled deionized water (18.2 MΩ cm⁻¹ resistance) (Millipore Corporation, USA) was used.

Cloud Point Extraction Procedure

All analytical parameters, such as ligand concentration, surface-active concentration, pH, common ion effect, buffer value, incubation temperature and time, were optimized for the application of the cloud point extraction method. A volume of 1 mL 10⁻² M 1,5-diphenylcarbazone (dithizone), 2.5 mL 0.1 M HCl / 0.1 M NaOH at pH 7, and 0.5 mL 5% (v/v) Triton X-114 solution was added to the analyte solution varying in concentrations from 10 to 500 µg L⁻¹ and diluted to 50 mL with ultra-purified water. This solution was placed into a 50 °C water bath for 20 minutes to warm up and then centrifuged for 10 minutes at 4000 rpm. The surface-active rich phase and the liquid phase of the solution, which was soaked in an ice bath for 20 minutes, was separated with a

micropipette. Because the surface-active rich phase is viscous and settles at the bottom of the test tube, the separation step is easily performed. The separated surface-active rich phase was diluted with 2 mL 2 M HNO₃ solution and measured with USN-ICP-OES.

RESULTS AND DISCUSSION

Effect of pH

Extraction of the surface-active mycelium occurs as a result of the formation of hydrophobic complexes. Because the pH is one of the basic parameters in the complexation reactions, it is the first variable that has to be examined. For this reason, the effect of pH was studied in the range of 3–10, and the pH of 7 was chosen for this work. It was observed that 1,5-diphenylcarbazone (dithizone), used as the complexation agent at pH 7 creates the most efficient complexes with the Cd²⁺, Co²⁺, Cu²⁺, Ni²⁺, Pb²⁺, and Zn²⁺ metal ions. This may be due to the fact that 1,5-diphenylcarbazone (dithizone) diluted in a basic pH environment results in high extraction yields. The effect of pH on the % recovery of the metal ions is shown in Figure 1.

Effect of Dithizone Concentration

Formation of a hydrophobic, quick, and stable structure with a complexation agent for the optimum high-yield preconcentration of metals using the cloud point extraction method is an important criterion. Dithizone is one of the organic reactive agents used commonly in analytical chemistry. While dithizone cannot be diluted below the pH level of 7, it has high solution rates in a basic pH environment. Dithizone forms colorful non-polar complexes with the Cd²⁺, Co²⁺, Cu²⁺, Ni²⁺, Pb²⁺, and Zn²⁺ ions and the pre-concentration operation is performed by imprisoning these complexes into a non-polar soluble. To achieve the highest pre-concentration factor, the most suitable complexing concentration was found to be 2x10⁻⁴ (M) mol/L. The effect of the ligand concentration on the % recovery of these metal ions is shown in Figure 2.

Effect of Triton X-114

Another important parameter for high efficiency results in the application of the cloud point extraction method is the surface-active agent concentration at the most suitable reaction environment. If the required amount of mycelium cannot be formed because of low amounts of surface-active agent,

Fig. 1. Effect of pH on the recovery of the analytes. Conditions: 50 mL solution, 0.2 mmol L⁻¹ dithizone, 100 µg L⁻¹ metal ions, 0.05% (w/v) Triton X-114.

Fig. 2. Effect of dithizone concentration on the recovery of the analytes. Conditions: 50 mL solution, pH 7.0, 100 µg L⁻¹ metal ions, 0.05% (w/v) Triton X-114.

the metal ligand complexes cannot pass to the hydrophobic mycelium phase. Since the value of mycelium in the high surface-active agent concentration increases, a decrease in the pre-concentration factor was observed. Triton X-114 is a surface-active agent and was investigated at various concentrations. Highest recovery was found at 0.05% (v/v) concentration. The effect on the % recovery of the metal ions on the surface-activated agent concentrations is shown in Figure 3.

Effect of Equilibration Time and Temperature

The other important parameters for pre-concentrated metals are equilibration time and temperature.

In order to complete the complex formation and imprison the hydrophobic structure complexes by clouding the surface-active agents, it is required to reach and remain at that temperature. If complex formation has not occurred at low temperature and time or the surface-activated agent has not totally been clouded, low extraction yield may occur. Because the structures may become instable at high temperature and time, these two parameters must be optimized in the most suitable way. In this study, it was observed that for each metal a low equilibration time is more efficient, while a high equilibration time decreases the extraction efficiency. It was found that

maximum extraction efficiency was achieved at 50 °C for the optimized equilibration time (see Figures 4 and 5).

Effect of Buffer Volume

Buffer volume also affects the % recovery at cloud point extraction. For that reason, 0.1 M HCl / 0.1 M NaOH at pH=7 buffer was used and the highest extraction efficiency was observed with 2.5 mL of buffer (see Figure 6).

Effect of Interfering Ions

It is well-known that the % recovery of metal ions from different matrixes containing different ions is often very difficult. For this purpose, the effect of the interfer-

Fig. 3. Effect of Triton X-114 concentration on the recovery of the analytes. Conditions: 50 mL solution, 0.2 mmol L⁻¹ dithizone, pH 7.0, 100 µg L⁻¹ metal ions.

Fig. 4. Effect of equilibration time on the recovery of the analytes. Conditions: 50 mL solution, 0.2 mmol L⁻¹ dithizone, pH 7.0, 100 µg L⁻¹ metal ions, 0.05% (w/v) Triton X-114.

Fig. 5. Effect of equilibration temperature on the recovery of the analytes. Conditions: 50 mL solution, 0.2 mmol L⁻¹ dithizone, 0.05% (w/v) Triton X-114.

Fig. 6. Effect of buffer volume on the recovery of the analytes. Conditions: 50 mL solution, 0.2 mmol L⁻¹ dithizone, pH 7.0, 100 µg L⁻¹ metal ions, 0.05% (w/v) Triton X-114.

ing ions Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Al^{3+} , Ba^{2+} , Fe^{3+} , Cl^- , NO_3^- , F^- , SO_4^{2-} was studied to obtain at least 95% recovery during the pre-concentration of the metal ions in the presence of various anions and cations. While conducting the experiment, different ions at various concentrations were added separately to the solution which contained 100 ng mL^{-1} of Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} metal ions, cloud point was observed, and the tolerance limits were determined. The interfering ions and their tolerance limits are listed in Table II.

Analytical Figures of Merit

After establishing the optimum conditions for cloud point extraction, the analytical performance measurements such as enrichment factor, quantitation limit, detection limit and certainty were calculated (see Table III). A calibration line was drawn with each enriched metal ion after cloud point extraction. The enrichment factor was calculated as the proportion of the slope of the calibration line obtained by pre-concentration to the slope of the calibration line without pre-concentration.

Analysis of Real Water Samples

The accuracy and applicability of the CPE-ICP-OES method was verified by analyzing real water samples obtained from the Sakarya River, Turkey. River water samples (50 mL)

were analyzed to which 10 and 20 mg L^{-1} amounts each of Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} were added. The obtained results listed in Table IV show that the Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} ions in natural samples can be measured even at very low concentrations using the CPE-ICP-OES method.

CONCLUSION

In this study, Triton X-114 was used as the surface-active agent and dithizone as the complexing agent for the pre-concentration of cadmium and the Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} metal ions using cloud point extraction (CPE) and multi-element ICP-OES determination. The applicability of the method was proven and verified by

TABLE II
Effect of Interfering Ions on the Recovery of the Analytes

Ions	Tolerance Limit
Na^+	5000 : 1
K^+	5000 : 1
Mg^{2+}	1000 : 1
Ca^{2+}	1000 : 1
Al^{3+}	100 : 1
Ba^{2+}	10 : 1
Fe^{3+}	2 : 1
Cl^-	5000 : 1
NO_3^-	5000 : 1
F^-	5000 : 1
SO_4^{2-}	500 : 1

TABLE III
Analytical Performance of Proposed Method

Parameters	Cu	Zn	Ni	Cd	Co	Pb
Enrichment Factor	7	14	15	18	13	15
Correlation Coefficient*	0.9912	0.9980	0.9984	0.9987	0.9974	0.9985
RSD (%)	1.93	2.15	0.57	1.35	0.73	1.28
LOQ (ng mL^{-1})	0.01	0.02	0.01	0.009	0.01	0.012
LOD (ng mL^{-1})	0.039	0.068	0.041	0.03	0.033	0.04
Linear range (ng mL^{-1})	5-100	5-100	5-100	5-100	5-100	5-100

*Correlation coefficient after CPE.

analyzing a river water sample with successful results. Minimum 96.4% and maximum 101.6% recovery was obtained for Cd^{2+} , Co^{2+} , Cu^{2+} , Ni^{2+} , Pb^{2+} , and Zn^{2+} . An ultrasonic nebulizer was used to increase aerosol formation and measuring at low concentrations. The method is low cost, uses non-toxic chemicals, and with the easy applicability of cloud point extraction provides high concentration factors such as 12.4 for Cd^{2+} and 9.6 for Pb^{2+} .

Received February 2, 2016.

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