

Research Article

Development of a Cloud-Point Extraction Method for Cobalt Determination in Natural Water Samples

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A new, simple, and versatile cloud-point extraction (CPE) methodology has been developed for the separation and preconcentration of cobalt. The cobalt ions in the initial aqueous solution were complexed with 4-Benzylpiperidinedithiocarbamate, and Triton X-114 was added as surfactant. Dilution of the surfactant-rich phase with acidified ethanol was performed after phase separation, and the cobalt content was measured by flame atomic absorption spectrometry. The main factors affecting CPE procedure, such as pH, concentration of ligand, amount of Triton X-114, equilibrium temperature, and incubation time were investigated and optimized. Under the optimal conditions, the limit of detection (LOD) for cobalt was $0.5 \mu\text{g L}^{-1}$, with sensitivity enhancement factor (EF) of 67. Calibration curve was linear in the range of $2\text{--}150 \mu\text{g L}^{-1}$, and relative standard deviation was 3.2% ($c = 100 \mu\text{g L}^{-1}$; $n = 10$). The proposed method was applied to the determination of trace cobalt in real water samples with satisfactory analytical results.

1. Introduction

Trace metals play an important role in human metabolism, and either excess or deficiency of them in the living organism can lead to biological disorder [1]. The determination of trace amounts of cobalt in natural waters is of great interest because cobalt is important for living species as complexed vitamin B₁₂. Vitamin B₁₂ is present in human and animal cells in the forms of adenosylcobalamin (III) and methylcobalamin (IV). The deficiency of cobalt in ruminants usually results in different types of anaemia. Toxicological effects of large amounts of cobalt include vasodilation, flushing, and cardiomyopathy in humans and animals. The importance of cobalt in human and ruminant nutrition has led to work on the determination of cobalt in soils, plants, feedstuffs, herbage, natural waters, and fertilizers. Investigations have been extended to the biochemistry of cobalt in animals, humans, microorganisms, and enzymes [2]. The maximum recommended concentration of cobalt in drinking water for livestock is 1.0 mg L^{-1} [3]. For drinking water for human consumption, the upper limits are even less. It is, therefore, clear that a reliable analytical method

must be established to test waters and soils on a regular basis.

Several analytical techniques such as atomic absorption spectrometry (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), and inductively coupled plasma mass spectrometry (ICP-MS) are available for the determination of trace metals with sufficient sensitivity in most applications. However, the determination of trace metal ions in natural waters is difficult due to various factors, particularly their low concentrations and matrix effects. Preconcentration and separation can solve these problems and lead to a higher confidence level and an easy determination of the trace elements by less sensitive, but more accessible instrumentation such as flame atomic absorption spectrometry (FAAS). There are many methods of preconcentration and separation such as liquid-liquid extraction (LLE) [4, 5], ion exchange techniques [6, 7], coprecipitation [8, 9], and sorption on various adsorbents such as activated carbon [10, 11], Amberlite XAD resins [12, 13], and other sorbents [14, 15]. However, these methods which are typically time-consuming and labor-intensive have multistep procedures

prone to loss of analytes and need high volumes of samples. Therefore, simple, rapid, and efficient techniques that can be used easily are required.

Cloud-point extraction (CPE) is probably the most versatile and simple method for the preconcentration and extraction of hydrophobic species from water. The technique is based on the property of most nonionic surfactants in aqueous solutions to form micelles and become turbid when heated to a temperature known as the cloud point temperature (CPT). Above this temperature, the micellar solution separates in a surfactant rich phase of a small volume and in a diluted aqueous phase in which the surfactant concentration is close to the critical micellar concentration. The ability of micellar systems to concentrate and extract hydrophobic species from water was demonstrated some years ago [16–18]. In recent years, cloud point methodology has been used for the extraction and preconcentration of metal ions after the formation of sparingly water soluble complexes [19–22]. The aim of this work is to apply cloud point extraction as a preconcentration step for flame atomic absorption spectrometric determination of cobalt in natural water samples by the use of 4-benzylpiperidinedithiocarbamate potassium salt as a complexing agent.

2. Experimental

2.1. Instrumentation. A PG-990 (PG instrument Ltd., UK) atomic absorption spectrometer equipped with deuterium background correction and cobalt hollow cathode lamp was used for the determination of cobalt in the surfactant-rich phase at wavelength of 240.7 nm. The instrumental parameters were adjusted according to the manufacturer's recommendations. Cloud point preconcentration experiments were performed using a Memmert thermostated bath (Model WNB-14, Germany), maintained at the desired temperature. A Hettich centrifuge (Model Universal 320R, Germany) was used to accelerate the phase separation process. The pH values were measured with a Metrohm pH-meter (Model 827) supplied with a glass-combined electrode.

2.2. Reagents and Solutions. All reagents used were of high purity and analytical reagent grade. The water utilized in all studies was double-distilled and deionized. Stock standard solutions of cobalt at a concentration of 1000.0 mg L^{-1} were prepared by dissolving appropriate amounts of $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ (Merck, Darmstadt, Germany) in deionized doubly distilled water. Before each investigation, a series of metal standard solutions were prepared by diluting this stock solution. The nonionic surfactant Triton X-114 (Sigma-Aldrich, St. Louis, MO, USA) was used without further purification. 4-benzylpiperidinedithiocarbamate potassium salt (K-4-BPDC) was prepared according to the procedure described by Andac et al. [23]. Fresh 0.01 mol L^{-1} solution of K-4-BPDC was being prepared daily by dissolving an appropriate amount of solid reagent in deionized water.

2.3. Cloud-Point Extraction Procedure. For the cloud point extraction, an analytical solution contained 25 mL sample

TABLE 1: Optimum conditions for the CPE of cobalt.

Conditions	Value
pH	5.0
Concentration of chelating agent (mol L^{-1})	2×10^{-4}
Concentration of surfactant (w/v, %)	0.1
Equilibrium temperature ($^{\circ}\text{C}$)	60
Equilibrium time (min)	5
Centrifugation rate (rpm)	5000
Centrifugation time (min)	5
Diluent	$1.0 \text{ mol L}^{-1} \text{ HNO}_3$ in ethanol

solution or standard solution, 1.0 mL of 2.5% (w/v) Triton X-114, 0.5 mL of 0.01 mol L^{-1} K-4-BPDC, and 2.5 mL sodium acetate/acetic acid buffer solution (pH 5.0; 0.1 mol L^{-1}) was transferred to a 50 mL centrifuge tube and kept in the thermostatic bath maintained at 60°C for 10 min. The separation into two phases was accelerated by centrifuging at 5000 rpm for 5 min. The phases were cooled down in an ice bath in order to increase the viscosity of the surfactant-rich phase. The aqueous phase was readily separated by inverting the tubes. To decrease the viscosity of the surfactant-rich phase, 0.3 mL of $1.0 \text{ mol L}^{-1} \text{ HNO}_3$ in ethanol was added. The resultant solution (volume 0.5 mL) was directly introduced into the FAAS by conventional aspiration, and the cobalt content was measured.

3. Results and Discussion

To attain higher sensitivity, selectivity and precision for the extraction and determination of cobalt with the CPE method, the effect of main parameters, such as pH, concentration of chelating agent, concentration of surfactant, ionic strength, equilibrium temperature and time, centrifugation conditions, and interfering ions were studied and optimized thoroughly. Triplicate extractions were performed for all experiments, and the selected values are presented in Table 1.

3.1. Effect of pH. The pH was the first critical parameter evaluated for its effect on extraction and determination of cobalt. Separation of metal ions by cloud point method involves the prior formation of a complex with sufficient hydrophobicity to be extracted into the small volume of surfactant-rich phase. Extraction recovery depends on the pH at which complex formation occurs. In order to find optimum pH, the effect of pH in the range 1–10 on the complex formation reaction was investigated, and the results are shown in Figure 1.

As can be seen, the recovery of Co(II) increases with increasing solution pH and was effectively recovered in pH range 4.0–8.0. Competition between protons and metal species could thus explain the weak adsorption in acid medium. On further increase of pH, extraction recovery decreases probably due to the formation of hydroxide of cobalt. Therefore, the further works were performed at pH 5.0. In order to control the pH during the analytical

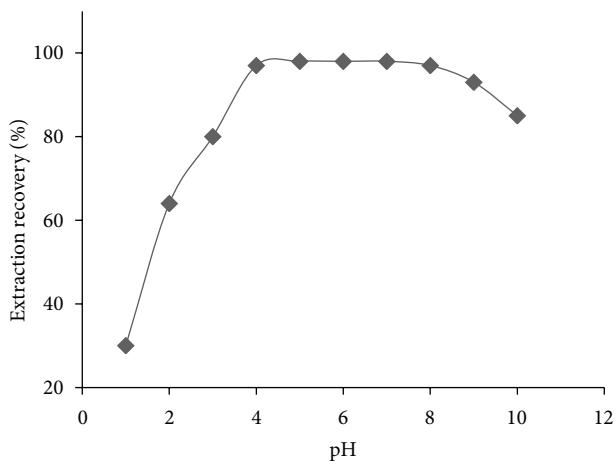


FIGURE 1: Effect of pH on cobalt extraction recovery. Utilized conditions: sample: 25 mL, $100 \mu\text{g L}^{-1}$; chelating agent: $2.0 \times 10^{-4} \text{ mol L}^{-1}$ of K-4-BPDC; surfactant: 0.1% (w/v) of Triton X-114.

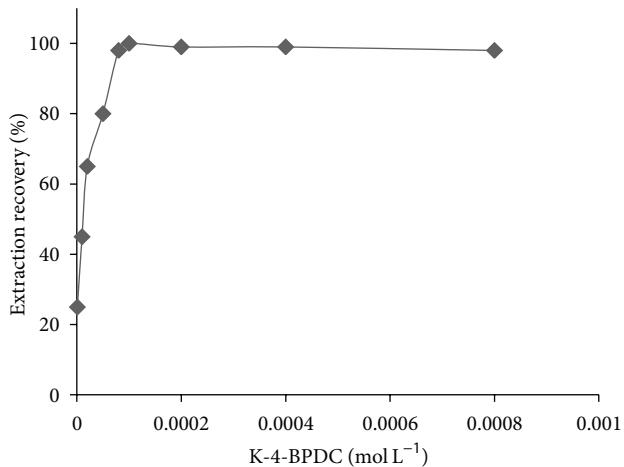


FIGURE 2: Effect of K-4-BPDC concentration on cobalt extraction recovery. Utilized conditions: sample: 25 mL, $100 \mu\text{g L}^{-1}$; pH = 5.0; surfactant: 0.1% (w/v) of Triton X-114.

procedure, it was adjusted to 5.0 with a buffer solution of sodium acetate/acetic acid.

$8 \times 10^{-4} \text{ mol L}^{-1}$. Therefore, K-4-BPDC concentration of $2 \times 10^{-4} \text{ mol L}^{-1}$ was chosen for subsequent experiments.

3.2. Effect of Chelating Agent Concentration. The concentration of the chelating agent may be sufficient for the quantitative complexing of metal ions. The lower concentration of K-4-BPDC led to a reduction in the analytical response due to incomplete complexing of the metal and consequently, a lower extraction yield. The extraction recovery as a function of the K-4-BPDC concentration is shown in Figure 2. For this study, 25 mL of a solution containing $100 \mu\text{g L}^{-1}$ cobalt and 0.1% (w/v) Triton X-114 with various amounts of K-4-BPDC was subjected to the cloud point preconcentration process. As can be seen, CPE efficiency increased rapidly as the concentration of K-4-BPDC increased from $1 \times 10^{-6} \text{ mol L}^{-1}$ to $1 \times 10^{-4} \text{ mol L}^{-1}$, then remained almost constant upon further increase in the K-4-BPDC concentration up to

3.3. Effect of Triton X-114 Concentration. The type and concentration of surfactant, that is used in the CPE, are critical factors. The amount of surfactant did not only affect the extraction efficiency, but also the volume of surfactant-rich phase. A successful cloud point extraction should maximize the extraction efficiency by minimizing the phase volume ratio ($V_{\text{org}}/V_{\text{aqueous}}$), thus improving its concentration factor [30].

Triton X-114 was chosen for the formation of the surfactant-rich phase due to its low cloud-point temperature and high density of the surfactant-rich phase, which facilitates phase separation by centrifugation. Thus, amount of Triton X-114 was investigated between 0.02 and 0.25% for extraction performance. The results are given in Figure 3. Quantitative extraction is observed when the Triton X-114 concentration

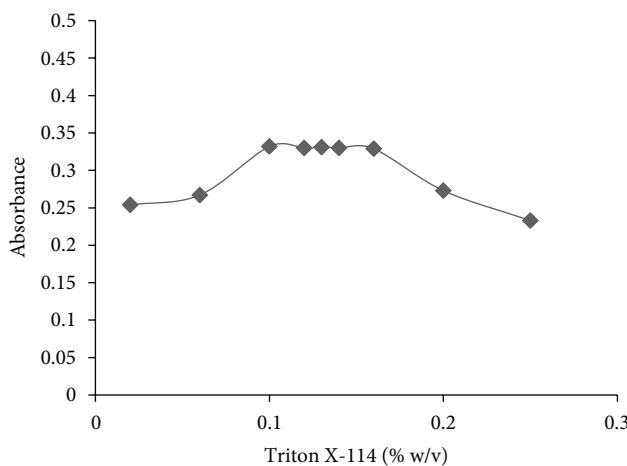


FIGURE 3: Effect of Triton X-114 concentration on absorbance signal. Utilized conditions: sample: 25 mL, $100 \mu\text{g L}^{-1}$; pH = 5.0; chelating agent: $2.0 \times 10^{-4} \text{ mol L}^{-1}$ of K-4-BPDC.

TABLE 2: Effect of foreign ions on the recovery of cobalt ($100 \mu\text{g L}^{-1}$).

Ion	Ion/Co(II) ratio	Recovery (%)
NO_3^-	1000	99.8
ClO_4^-	1000	100.1
K^+	1000	99.8
Li^+	1000	99.6
Cl^-	1000	99.4
SO_4^{2-}	1000	99.7
I^-	1000	99.6
F^-	1000	99.7
PO_4^{3-}	500	99.4
Ca^{2+}	500	99.8
Mg^{2+}	500	99.8
Cu^{2+}	100	100.2
Fe^{2+}	100	101.2
Fe^{3+}	100	101.1
Mn^{2+}	50	99.1
Cr^{3+}	50	101.3
Al^{3+}	50	100.6
Bi^{3+}	30	102.1
Cd^{2+}	30	101.3
Zn^{2+}	30	102.2
Ag^+	5	97.9
Hg^{2+}	5	97.8

is higher than 0.10% (w/v). At lower concentrations, the absorbance is low probably because of the inadequacy of the assemblies to entrap the hydrophobic complex quantitatively. With increase of Triton X-114 concentration above 0.16% (w/v), the signals decrease because of the increment in the volumes and the viscosity of the surfactant phase, leading to poor sensitivity. Since, a concentration of 0.1% (w/v) of

TABLE 3: Analytical characteristics of proposed method.

Parameter	Analytical feature
Linear range, $\mu\text{g L}^{-1}$	2–150
Limit of detection, $\mu\text{g L}^{-1}$ ($n = 10$)	0.5
RSD (%) ($n = 10$; $C = 100 \mu\text{g L}^{-1}$)	3.2
Preconcentration factor	50
Enhancement factor	67

Triton X-114 was chosen as optimum concentration for subsequent experiments.

3.4. Effect of Equilibration Temperature and Time. It is desirable to have the shortest incubation time and the lowest possible equilibration temperature, which compromise completion of the reaction and efficient separation of the phases. The effect of the equilibration temperature and time was studied with a range of 25–80°C and 5–30 min, respectively. It was found that an equilibration temperature of 60°C and a time of 10 min were adequate to achieve quantitative extraction.

3.5. Effect of Viscosity. In order to facilitate the sample introduction in FAAS nebulizer, it was necessary to decrease the surfactant-rich phase viscosity. Different solvents such as acetone, ethanol, methanol, and acidic solutions of ethanol and methanol were tried in order to select the one producing the optimal results regarding sensitivity. The best result was obtained for acidic solution of ethanol. A volume of 0.3 mL of ethanol solution containing 1 mol L⁻¹ nitric acid was added to the surfactant-rich phase after separation. This amount of ethanol was chosen to ensure a sufficient volume of sample for conventional aspiration. For smaller volumes, the reproducibility of the signals was very poor, whereas for higher volumes, there was a decrease in the signal due to dilution.

3.6. Effect of Ionic Strength. For investigating the influence of ionic strength on performance of CPE, various experiments were performed by adding different amounts of NaCl (0–1.0 mol L⁻¹). Other experimental conditions were kept constant. The results showed that ionic strength has no significant effect on the extraction recovery.

3.7. Centrifuging Rate and Time. To achieve a good separation result, the effect of centrifugation rate and time on the extraction recovery of cobalt were studied. The effect of centrifugation rate was investigated in the range of 1000–6000 rpm. It showed that over 4000 rpm, surfactant phase completely settled, so the rate of 5000 rpm was selected as the optimum point.

At the optimum rate, recovery was examined as a function of centrifugation time. Over 4 min, absorbance was constant indicating complete transfer of surfactant phase to the bottom of centrifuge tube. So, the optimum centrifugation time was chosen as 5 min.

TABLE 4: Determination of Co(II) in tap, mineral, river, and sea water samples and relative recoveries of spiked samples.

Sample	Co(II) \pm S.D. ^a ($\mu\text{g L}^{-1}$)	Added Co(II) ($\mu\text{g L}^{-1}$)	Found Co(II) \pm S.D. ^a ($\mu\text{g L}^{-1}$)	Recovery (%)
Tap water ^b	n.d. ^f	—	51.6 \pm 1.8	—
		100.0	102.1 \pm 3.9	102.1
Mineral water ^c	n.d.	—	—	—
		50.0	49.0 \pm 1.7	98.0
River water ^d	8.5 \pm 0.3	100.0	98.7 \pm 3.2	98.7
		50.0	56.2 \pm 1.9	95.4
Sea water ^e	9.4 \pm 0.5	100.0	105.6 \pm 3.7	97.1
		50.0	56.5 \pm 1.6	94.2
		—	—	—
		100.0	106.2 \pm 3.5	96.8

^a Standard deviation ($n = 5$).^b Behshahr drinking water system, Iran.^c Damavand mineral water, Iran.^d Tajan river water, north of Iran.^e Caspian sea water, Iran.^f Not detected.

TABLE 5: Comparison of the presented method with other methods for determination of cobalt.

Method	LOD ^a ($\mu\text{g L}^{-1}$)	RSD ^b (%)	Sample volume (mL)	PF ^c	Reference
CPE-spectrophotometry	7.5	2.7	10	10	[24]
CPE-FAAS	1.0	3.6	50	25	[25]
SPE-FAAS	3.4	1.3	1300	260	[26]
SPE-FAAS	12.3	1.3	1000	200	[27]
SPE-FAAS	3.9	2.0	250	25	[28]
DLLME-FAAS	0.9	5.8	5	16	[29]
CPE-FAAS	0.5	3.2	25	50	This work

^a Limit of detection.^b Relative standard deviation.^c Preconcentration factor.

3.8. Interferences. The effect of foreign ions on the determination of cobalt by the proposed method was investigated by measuring the absorbance of the solutions containing $100 \mu\text{g L}^{-1}$ of cobalt ion in the presence of various amounts of other ions. The tolerance limit was defined as the concentration of added ion that caused less than $\pm 5\%$ relative error in the determination of cobalt. The maximum tolerances of the investigated cations and anions are given in Table 2. According to the results, cobalt recovery was nearly quantitative in the presence of other ions.

3.9. Analytical Features. The calibration graph was linear in the range of $2\text{--}150 \mu\text{g L}^{-1}$ under the optimum conditions of general procedure. The correlation coefficient of the calibration curve equation was 0.997, which indicates that a good linear regression was established between the absorbances and the concentrations. The preconcentration factor for Co(II) calculated by dividing the aqueous phase

volume into the final volume of diluted phase was 50, while the enhancement factor as the ratio of slope of calibration curve of the analytes after preconcentration to that prior preconcentration was 67. The limit of detection (LOD) is defined as the concentration equivalent to three times the standard deviation of 10 measurements of the blank and is the lowest analyte concentration that produces a response detectable above the noise level of the system. Table 3 gives the analytical features of the method.

3.10. Determination of Cobalt in Real Water Samples. The proposed method was applied in the determination of cobalt in tap water, mineral water, river water, and seawater samples. The results are given in Table 4. Recovery studies were also carried out after it was spiked to samples known concentrations of cobalt at levels of 50 and $100 \mu\text{g L}^{-1}$. The recovery values calculated for the added standards ranged from 94 to 103%, thus confirming the accuracy of the procedure and its

independence from the matrix effects. These results confirm the validity of the proposed preconcentration method.

3.11. Comparison of Proposed Method with Other Methods.

A comparison of the represented method with some other reported preconcentration methods for cobalt determination in water samples is given in Table 5. Apparently, presented method has low LOD, high-enrichment factor, good RSD, and these characteristics are comparable or even better than most of the other methods in Table 5.

4. Conclusion

The reagent 4-benzylpiperidinedithiocarbamate potassium salt (K-4-BPDC) was successfully employed in a CPE procedure for determination of cobalt in natural water samples by FAAS. This study allowed the development of a rapid, easy to use, safe, environmental friendly, and inexpensive methodology for the preconcentration and separation of trace amounts of cobalt. The method significantly improved the performance of the FAAS detection for cobalt. The proposed preconcentration method allows cobalt determination in natural water samples at $\mu\text{g L}^{-1}$ levels.

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