

Research Article

Cloud Point Extraction for the Determination of Trace Amounts of Cobalt in Water and Food Samples by Flame Atomic Absorption Spectrometry

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A cloud point extraction (CPE) procedure which was developed for the separation and preconcentration of trace amounts of cobalt is combined with flame atomic absorption spectrometry (FAAS) to determine trace amounts of cobalt in water and food samples. The procedure is based on the formation of the hydrophobic complex between Co(II) and 4-methoxy-2-sulfo-benzenediazoaminoazo-benzene (MOSDAA) followed by its extraction into a Triton X-114 surfactant-rich phase. The parameters such as pH of sample, concentrations of MOSDAA and Triton X-114, equilibrium temperature, and equilibrium time, which affect both complexation and extraction, are optimized. Under the selected optimum conditions, the preconcentration of 10.0 mL, 0.1 $\mu\text{g mL}^{-1}$ Co(II) solution results in a limit of detection of 0.47 ng mL^{-1} (3σ) and an enrichment factor of 19. A relative standard deviation of 2.78% ($n = 6$, $c = 0.1 \mu\text{g mL}^{-1}$) for the determination of Co(II) is obtained. The proposed method was applied for the determination of trace amounts of cobalt in river water and millet samples with satisfactory results.

1. Introduction

Cobalt is an essential trace element in human body. Being a component of vitamin B12 (cyanocobalamin), it plays an important role in the production of the blood red cells and the prevention of pernicious anemia [1]. It is known that the toxicity of Cobalt is quite low, but high exposure to this element can cause diseases such as asthma and skin irritation. Therefore, to determine trace amounts of cobalt becomes significantly important in the fields of environmental analysis, process control, and medicine [2, 3].

In the aspect of determining trace amounts of cobalt in different matrixes, many analytical techniques such as flame atomic absorption spectrometry (FAAS) [4], inductively coupled plasma mass spectrometry (ICP-MS) [5], inductively coupled plasma optical emission spectrometry (ICP-OES) [6] and graphite furnace atomic absorption spectrometry (GFAAS) [7] have been developed. Flame atomic absorption spectrometry (FAAS) has been considered as a relatively simple and precise technique which is widely used for the

determination of trace metal ions. However, direct determination of cobalt ion at trace level by FAAS does not present the sensitivity requirements, and, more importantly, it is affected by matrix interference. Thus, preliminary separation and preconcentration of trace cobalt element from the matrix are frequently necessary to improve the detection limit and the selectivity. These factors make several procedures developed for the separation and preconcentration of cobalt, including coprecipitation [8], liquid-liquid extraction [9, 10], solid phase extraction [11, 12], and flotation [13].

Recently, cloud point extraction (CPE) has become an attractive area for the separation and preconcentration of trace metal ions [24–27]. The principles of CPE have been illustrated in some interesting reviews [28–30]. Nonionic surfactants in aqueous solutions tend to form micelles and become turbid at a certain temperature, which is called “cloud point temperature.” Above the temperature, the micellar solution separates into two phases: a surfactant-rich phase of a small volume and a dilute aqueous phase [31]. When metal ions react with a suitable ligand and form an aqueous low

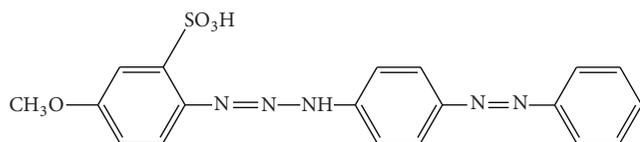


FIGURE 1: The structure of MOSDAA.

solubility complex, they can be extracted from the aqueous solution into the small-volume surfactant-rich phase. This method is simple experimental procedure with low cost, environmental safety, high capacity for preconcentration of wide variety of analytes, and high enrichment factor with good recoveries [28, 32, 33]. In view of these advantages, cloud point extraction and flame atomic absorption have been combined together to determine various metal ions in environmental samples [3, 34–37]. Therefore, in the present paper, cloud point extraction will be used to preconcentrate trace cobalt, and flame atomic absorption spectrometry is used to determine trace cobalt in water and food samples.

2. Experimental

2.1. Reagents. All chemicals used in the work were of analytical reagent grade. Deionized water ($18.2 \text{ M}\Omega \cdot \text{cm}$) was used in all experiments. 1.000 mg mL^{-1} Co(II) ion standard solution was prepared by dissolving appropriate amount of nitrate salt of Co(II) ion. Working solutions were prepared by dilution of the stock solution with distilled deionized water. The nonionic surfactant Triton X-114 (Sigma, St. Louis, MO, USA) was used without further purification. A 5.0% (v/v) Triton X-114 was prepared by dissolving 5.0 mL of Triton X-114 in distilled water in 100 mL volumetric flask with stirring. A 0.02% MOSDAA (MOSDAA was synthesized according to the a procedure reported previously [38]; the structure of MOSDAA is presented in Figure 1.) solution was prepared by dissolving 0.0158 g of MOSDAA in 100 mL volumetric flask with stirring. A buffer solution of pH 9.0 was prepared by using sodium tetraborate 10-hydrate (Beijing Chemical Works) and acid boric (Beijing Chemical Works) at 0.05 and 0.5 mol L^{-1} concentrations. The vessels and pipettes used for trace analysis were kept overnight in 10% (m/v) nitric acid (Reagent No.1 Factory Of Shanghai Chemical Reagent Co., Ltd.) solution and subsequently washed four times with deionized water.

2.2. Apparatus. A Shimadzu AA-6800 atomic absorption spectrometer equipped with a cobalt hollow cathode lamp (Beijing Shuguangming Electronic Lighting Instrument Co., Ltd.) and an air-acetylene flame atomizer; was used in all determinations. The instrumental parameters were adjusted according to the manufacturer's recommendations. The working conditions are as follows: wavelength (240.7 nm), lamp current (4.0 mA), spectral bandwidth (0.2 nm), height of observation (5.0 mm), flow rate of air (6.0 L min^{-1}), and flow rate of acetylene (1.6 L min^{-1}). An 80-2 centrifuge (Jintan Ronghua Instrument Manufacture Co., Ltd.) was used

to accelerate the phase-separation process. A PHS-3C pH-meter (Shanghai Dapu Instruments Co., Ltd.) furnished with a combined glass-saturated calomel electrode was used for pH measurements.

2.3. Procedure. A cloud point experiment has been carried out according to the following procedure. Aliquots of 15 mL of the working solution containing Co(II) ion ($2.0 \mu\text{g mL}^{-1}$), 0.3 mL of Triton X-114 (5.0% v/v), 1.0 mL of MOSDAA (0.02%), and 1.5 mL pH 9.0 buffer solution were mixed and placed in a graduated centrifuge tube. The mixture was diluted to 15 mL by the secondary distilled water. Then, the solution was kept at 80°C for 20 min in the thermostatic bath for equilibration. The separation into two phases was accelerated by centrifugation for 5 min at 1500 rpm. The solution was then cooled in an ice bath for 10 min in order to increase the viscosity of the surfactant-rich phase and facilitate the removal of the aqueous phase. The remaining surfactant-rich phase (approximately $300 \mu\text{L}$) was dissolved in 0.5 mL of $0.1 \text{ mol L}^{-1} \text{ HNO}_3$ in order to reduce its viscosity. The Co(II) content was readily evaluated by FAAS.

2.4. Application. The real samples include water and millet samples. Water samples were taken from Shili river of Datong city. They were filtered into a clean flask. Millet samples (2.50 g) were porphyzied and dried. They were put into the dry beaker, and then 30.0 mL hydrogen peroxide was added. After 10 h of the reaction, the mixture was placed on the heating furnace to dissolve at a low temperature for about 3 h. When the resultant solution was heated to dryness, 5.0 mL HNO_3 was added. After the solution was dry again, proper amount of water was added. The solution was heated for a while, and then it was cooled at room temperature. The solution was diluted to 20 mL. The blank solution was also prepared.

3. Results and Discussion

To achieve the best performance for the cloud point extraction procedure, the effects including pH, concentrations of ligand and surfactant, temperature and time of equilibration on the analytical signal, dilution condition, centrifuge time and rates, and interfering ions were evaluated and optimized.

3.1. Effect of pH. The formation of metallic complex and its chemical stability are the two important factors involved in the separation and preconcentration of metal ions by cloud point extraction (CPE). They need to present sufficient hydrophobicity to be extracted into the small volume of the surfactant-rich phase. The pH plays a critical role on metallic complex formation and subsequent extraction and has been a significant parameter for CPE. Thus, extraction yield depends on the pH at which complex formation is investigated. A set of similar experiments was carried out in the pH range of 4.0–10.0. The solutions were buffered by mixtures of sodium tetraborate 10-hydrate and acid boric or acetic acid and sodium acetate. As it can be seen in Figure 2, in terms of these phenomena, pH 9.0 (sodium tetraborate 10-hydrate and acid

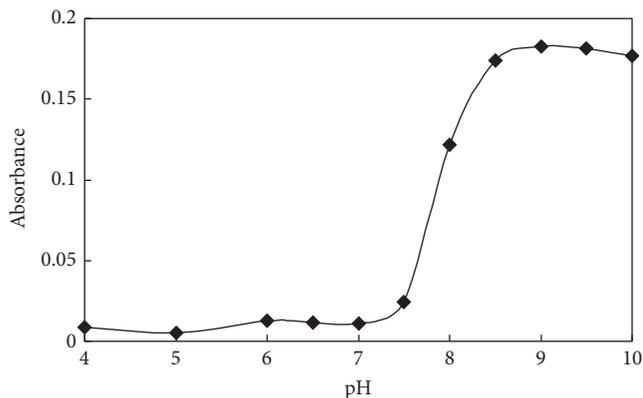


FIGURE 2: Effect of pH on the analytical signal of cobalt. Conditions: $2.0 \mu\text{g/mL}$ Co(II) ion, 0.1% (v/v) Triton X-114, 1 mL of 0.02% MOSDAA.

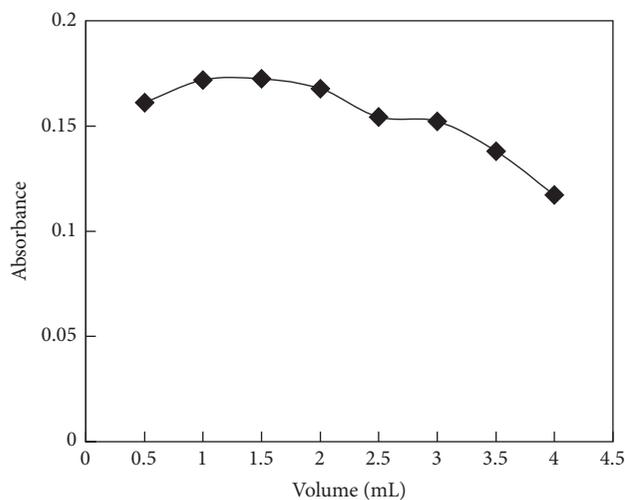


FIGURE 3: Effect of the amount of buffer solution on the absorbance of Co(II). Conditions: $2.0 \mu\text{g/mL}$ Co(II) ion, 0.1% (v/v) Triton X-114, 1 mL of 0.02% MOSDAA, pH 9.0, temperature: 80°C .

boric, $0.05 + 0.5 \text{ mol L}^{-1}$) was selected for the subsequent studies.

In addition, it is noted that the absorbance values are large and stable when the amount of buffer solution (pH 9.0) is in the range of 1.0–2.0 mL (see Figure 3). The largest absorbance value occurs when the amount of buffer solution (pH 9.0) is 1.5 mL, thus this amount is selected for the investigation.

3.2. Effect of the Amount of MOSDAA. After the formation of sparingly water-soluble complexes, the CPE is used for the preconcentration of metal ions. Its efficiency strongly depends on the hydrophobicity of the ligand and the complex formed in the micellar medium. Therefore, the effect of the amount of MOSDAA on the analytical responses was subsequently studied. Therefore, a set of similar experiments under the conditions of $2.0 \mu\text{g mL}^{-1}$ Co(II) ion, 5.0% (v/v) Triton X-114, pH 9.0 and various amounts of MOSDAA was carried out. The respective results are presented in Figure 4.

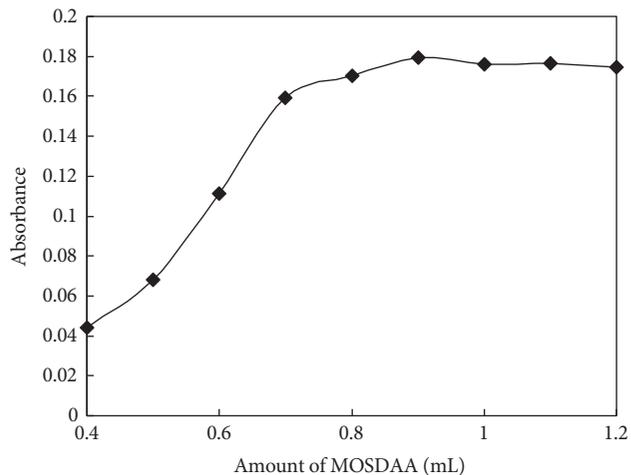


FIGURE 4: Effect of the amount of MOSDAA on the absorbance of Co(II). Conditions: $2.0 \mu\text{g/mL}$ Co(II) ion, 0.1% (v/v) Triton X-114, pH 9.0, temperature: 80°C .

It was found that the absorbance augments by increasing the ligand concentration. The sensitivity increases up to 0.9 mL of the amount of MOSDAA which reaches a plateau. At this amount, the extraction of $2.0 \mu\text{g mL}^{-1}$ Co(II) ion can be considered completely. In view of the complicacy of the sample, a volume of 1.0 mL is selected as a suitable amount of MOSDAA for the extraction process in order to make the extraction reaction complete.

3.3. Effect of the Amount of Triton X-114. Triton X-114 is one of the nonionic surfactant extensively used in CPE [29] because it has the advantages such as commercial availability with high purity, low toxicity and cost, high density of the surfactant-rich phase facilitating the phase separation by centrifugation, and relatively low cloud point temperature [39, 40]. Its concentration determines not only the result of extraction separation, but also the volume of the enriched micellar phase. Thus, the effect of the amount of Triton X-114 on cobalt extraction was evaluated by varying the surfactant amount in the range of 0.1–0.8 mL. As it can be seen from Figure 5, Co absorbance reaches up to the largest when the surfactant amount increases up to 0.30 mL and the volume ratio is 0.02% (v/v) (micelle to aqueous phase). Above this Triton X-114 amount, the analytical signal starts to decrease. This can be attributed to an increase in volume and viscosity of the surfactant phase. Thus, a volume of 0.30 mL Triton X-114 was used for subsequent experiments.

3.4. Effects of Equilibration Temperature and Time. Two important factors in cloud point extraction are equilibration temperature and incubation time. It is known that when CPE is conducted using equilibration temperatures that are well above the cloud point temperature of the surfactant, the greatest analyte preconcentration factors will be obtained [40, 41]. It is desirable to employ the shortest incubation time and the lowest incubation temperature in order to ensure the completion of extraction and efficient separation of phases.

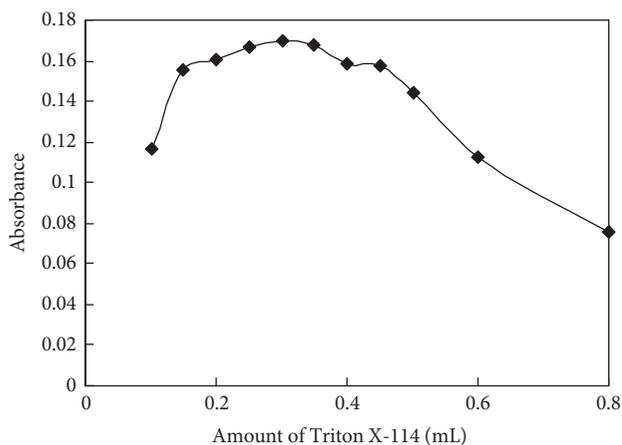


FIGURE 5: Effect of the amount of Triton X-114 on the absorbance of Co(II). Conditions: 2.0 $\mu\text{g}/\text{mL}$ Co(II) ion, 1 mL of 0.02% MOSDAA, pH 9.0, temperature: 80 °C.

Based on these reasons, the effect of equilibration temperature and time was examined. The dependence of extraction efficiency upon equilibration temperature and time above the cloud point in the range of 25–100 °C and 5–60 min was thoroughly optimized, respectively (see Figures 6 and 7). The results showed that an equilibration temperature of 80 °C and a time of 20 min were adequate to achieve quantitative extraction, and there were no appreciable improvements for a time longer than 20 min. Therefore, an equilibration temperature of 80 °C and an incubation time of 20 min were used.

3.5. Effect of Dilution Condition. It should be noted that after cloud point preconcentration, the surfactant-rich phase obtained becomes very viscous owing to the Triton X-114 that it contains. In order to facilitate the sample introduction in the FAAS nebulizer, it is necessary to decrease the viscosity of the surfactant-rich phase to facilitate the subsequent handling and introduction into the atomizer. So it is usually necessary for the addition of a diluting solution in the surfactant-rich phase to obtain a clear and homogenous solution of low viscosity compatible with the requirements of flame and plasma nebulizer. Hence, a solution of 0.1 mol L⁻¹ HNO₃ was selected as the diluting agent. Various amounts of 0.1 mol L⁻¹ HNO₃ were added to decrease the viscosity of the surfactant-rich phase. The results indicate that the largest absorbance is obtained when 0.5 mL of 0.1 mol L⁻¹ HNO₃ is added and then it was diluted to 2.0 mL by the secondary distilled water (see Figure 8). Finally, an optimal volume of 0.5 mL 0.1 mol L⁻¹ HNO₃ was chosen in order to ensure good absorbance signal.

3.6. Effects of Centrifuge Time and Rates. It is very necessary to preconcentrate trace amounts of Co(II) ion with high efficiency in a short time. Therefore, on the basis of the optimum conditions so far obtained, the effect of the centrifuge time and rates was studied. The results suggest that centrifugation

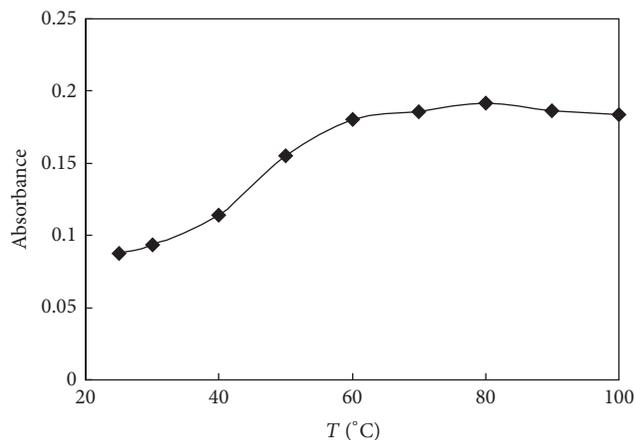


FIGURE 6: Effect of equilibration temperature on the absorbance of Co(II). Conditions: equilibration time of 20 min, centrifugation for 5 min at 1500 rpm.

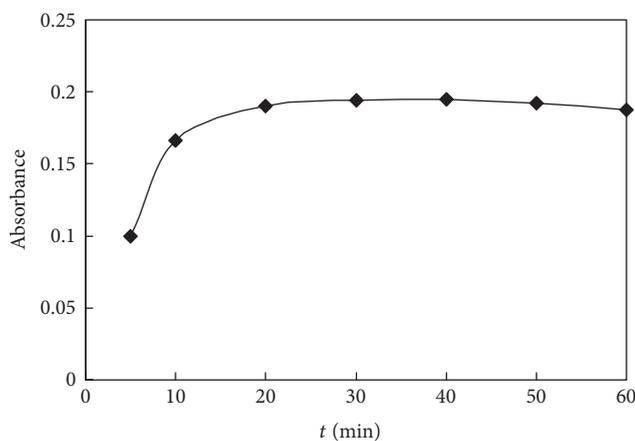


FIGURE 7: Effect of equilibration time on the absorbance of Co(II). Equilibration temperature of 80 °C, centrifugation for 5 min at 1500 rpm.

for 5 min at 1500 rpm and cooling for 10 min in an ice-bath lead to the highest recovery and sensitivity for Co(II) ion.

3.7. Effect of Interference. The effects of foreign ions on the extraction of 2 $\mu\text{g}/\text{mL}$ Co(II) were tested. The tolerated amounts of each ion were the concentration values tested that caused error less than $\pm 5\%$ of the recovery alteration. The tolerable concentration ratios of foreign ions within the ranges are summarized in Table 1. It is shown that the recovery is quantitative and satisfactory in the presence of a large amount of foreign metal ions such as K(I), Na(I), Mg(II), Ca(II), and these cations in the water and real samples have no obvious influence on CPE of Co(II) ion under the selected conditions. It can be observed that the tolerable concentration ratios of Cu(II), Zn(II), and Fe(III) are very low, their existences would significantly affect the CPE of Co(II) ion under the selected conditions. The reason may be that these cations can form complexes with MOSDAA, and this will make the complexation between Co(II) and

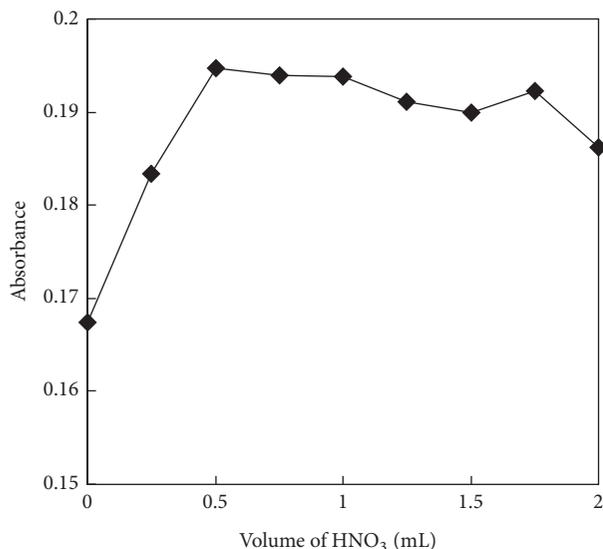


FIGURE 8: Effect of the amount of HNO₃ on the absorbance of Co(II).

TABLE 1: Effects of the foreign ions on the recoveries of the Co(II) ion ($n = 3$).

| Interfering ions | Interfering ion/analyte fold ratio (w/w) | Recovery/% |
|------------------|--|------------|
| K(I) | 1000 | 101.4 |
| Na(I) | 1000 | 99.3 |
| Mg(II) | 1000 | 103.8 |
| Ca(II) | 1000 | 96.2 |
| Mn(II) | 200 | 98.7 |
| Pb(II) | 200 | 100.6 |
| Cu(II) | 20 | 97.6 |
| Zn(II) | 20 | 95.6 |
| Ba(II) | 100 | 96.0 |
| Ni(II) | 100 | 95.2 |
| Fe(III) | 10 | 96.3 |

MOSDAA less efficient. Therefore, to eliminate these effects, more amounts of MOSDAA are supposed to be added.

3.8. Characteristics of the Method. A calibration curve of absorbance versus concentration was constructed by collecting the analytical signals of different volumes of Co(II) standard solutions submitted to the method proposed. Under the optimum experimental conditions, the calibration curve for Co(II) ion is linear from 0.002 to 1.2 $\mu\text{g}/\text{mL}$ with a correlation coefficient (R) of 0.9984. Table 2 gives the parameters of the calibration curve, enrichment factor, the relative standard deviation obtained for 6 replicates subjected to the complete procedure and the detection limit. The enrichment factor as the ratio of Co concentration of the analyte after preconcentration to that before preconcentration is 19. The relative

TABLE 2: Characteristics performance of the presented CPE method.

| Parameter | Optimum value |
|--|------------------------------------|
| Equation for the calibration curve, $C/(\mu\text{g mL}^{-1})$ | $A = 0.0767C_{\text{Co}} + 0.0095$ |
| Correlation coefficient (R) | 0.9984 |
| Linear range ($\mu\text{g mL}^{-1}$) | 0.002–1.2 |
| RSD (% , $n = 6$) | 2.78 |
| Limit of detection (ng mL^{-1}) ($3S_b/m$, $n = 11$) | 0.47 |
| Enrichment factor | 19 |
| Extraction coefficient | ~ 1 |
| Phase volume ratio | 0.02 |

Enrichment factor: ratio between Co concentration in the surfactant-rich phase and in the original solution.

Extraction coefficient: ratio between Co quantity in the surfactant-rich phase after the cloud point and in the original solution.

Phase volume ratio: ratio between the final volume of the surfactant-rich phase and the aqueous phase.

TABLE 3: Determination of cobalt in water and millet samples ($n = 3$).

| Sample | Added/ $\mu\text{g g}^{-1}$ | *Found value/ $\mu\text{g g}^{-1}$ | Recovery/% |
|-------------|-----------------------------|------------------------------------|------------|
| Millet | 0 | 0.07 ± 0.02 | |
| | 0.05 | 0.12 ± 0.02 | 100.0 |
| | 0.1 | 0.17 ± 0.03 | 100.0 |
| River water | 1.0 | 1.05 ± 0.03 | 98.0 |
| | 0 | 0.20 ± 0.05 | |
| | 0.1 | 0.30 ± 0.05 | 100.0 |
| | 0.2 | 0.39 ± 0.04 | 95.0 |
| | 1.0 | 1.16 ± 0.10 | 96.0 |

* $\bar{X} \pm (t_{p,t} s / \sqrt{n})$, $P = 0.95$, $f = 2$.

standard deviation (RSD) for 6 replicate measurements of 100 ng mL^{-1} Co(II) ion was 2.78%. The limit of detection defined as $3S_b/m$ (where S_b is the standard deviation of the blank and m is the slope of the calibration curve) is 0.47 ng mL^{-1} (3σ).

A comparison of the represented method with other reported cloud point extraction methods is given in Table 4. As seen from the table, the detection limit for method is comparable to those given by many methods in the table. In addition, enrichment factor can be improved by using larger sample volumes.

3.9. Analysis of Real Samples. In order to validate the methodology, the proposed method was applied to the determination of Co(II) ion concentration in water and millet samples. The accuracy was checked by spiking the samples with different concentrations of Co(II) ion. The results are shown in Table 3. It shows that recoveries are greater than 96%, and indicating that the proposed method is suitable for the sample types examined. Thus, the method proposed can be considered reliable for Co(II) determination in a wide range of samples.

TABLE 4: Procedure using cloud point extraction prior to cobalt determination by FAAS.

| Reagent | Surfactant | Sample volume/mL | EF ^a | LOD ^b /($\mu\text{g L}^{-1}$) | Reference |
|---|--------------|------------------|-----------------|--|-----------|
| Ammonium pyrrolidine dithiocarbamate | Triton X-114 | 10 | 20 | 5 | [14] |
| 1-(2-Thiazolylazo)-2-naphthol | Triton X-114 | 50 | 57 | 0.24 | [15] |
| 1-(2-Pyridylazo)-2-naphthol | Triton X-114 | 10 | 115 | 0.38 | [16] |
| 2-(5-Bromo-2-pyridylazo)-5-diethylaminophenol | Triton X-100 | 12.5 | 28.5 | 1.06 | [17] |
| 1-nitroso-2-naphthol | PONPE 7.5 | 10 | 27 | 1.22 | [18] |
| 2-[2'-(6-Methyl-benzothiazolylazo)]-4-bromophenol | Triton X-114 | 10 | 28 | 0.9 | [19] |
| Methyl-2-pyridylketone oxime | Triton X-114 | 15 | 67 | 2.1 | [20] |
| 2-[(2-Mercaptophenylimino)methyl]phenol | Triton X-114 | 25 | 97 | 0.21 | [21] |
| 2-guanidinobenzimidazole | Triton X-114 | 50 | 13 | 7.8 | [22] |
| 1-Phenylthiosemicarbazide | Triton X-114 | 50 | 25 | 1 | [23] |
| 4-Methoxy-2-sulfo-benzenediazoaminoazo-benzene | Triton X-114 | 15 | 19 | 0.47 | This work |

^a Enhancement factor, ^b limit of detection.

4. Conclusions

Cloud point extraction is a simple, inexpensive, sensitive, and rapid method in preconcentration and separation of trace metal. TritonX-114 is used as cloud point extractant because it has the low cloud point temperature and high density, and, more importantly, it is very cheap. In the pH 9.0 buffer system of sodium tetraborate 10-hydrate and acid boric, Co(II) and MOSDAA can form stable complexation. After extraction preconcentration by neutral surface active agents Triton X-114, the element Co can be measured to the level of ng mL^{-1} , which is very satisfactory.

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