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Original article
Analytical Section

Determination of Trace Cadmium in Nonalcoholic Beverages by Coupling Cloud Point Extraction with Spectrophotometry

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A cloud-point extraction (CPE) process using the nonionic surfactant, Triton X-114 to extract Cd(II) ions from aqueous solutions was investigated. The method is based on the ion-pairing reaction of Cd(II) with Victoria blue B (VBB^+) in presence of excess iodide at pH 4.0 and extraction of the complex formed. The chemical variables affecting CPE efficiency were studied, and the analytical characteristics of the method were obtained. The calibration curves were obtained in the ranges of 1.0–30 and 10–500 μg/L for Cd(II) ion with the detection limits of 0.34 and 3.8 μg/L at 608 and 634 nm, respectively. The selectivity study was also tested. The precision (N: 5, 25 μg/L) was 2.85% relative standard deviation. The results obtained for two certified reference samples were in a good agreement with the certified values. The method was successfully applied to the determination of Cd(II) in beverage samples.

INTRODUCTION

Cadmium is well known to be one of the most hazardous elements to human health with a biological half-life in the range of 10–30 years, mainly accumulated in the kidney, liver and lungs [Maranhao *et al*., 2005]. This element was indicated as a human carcinogen and a causative factor in renal toxicity, pancreatic cancer, enhanced tumor growth, cardiovascular diseases, and in particular, hypertension [Davis *et al*., 2006]. It can also inhibit the action of the zinc enzymes by displacing the zinc [Pyrzynska & Kilian, 2007]. Moreover, it can exist in the environment owing to emissions from industrial plants, manure and atmospheric deposition, and improper waste disposal [Lemos *et al*., 2008]. For humans, the main exposure to cadmium comes from daily intake of water, food and cigarette smoke. Therefore, sensitive, selective, and accurate monitoring of trace cadmium in environmental, biological and food samples is of significant importance from the human health and environmental point of view.

Due to the adverse health effects of cadmium, detection of trace amounts of cadmium in any food and/or beverage samples related with human being is very important. Its determination requires sufficiently sensitive techniques for detection at the μg/L or sub μg/L levels. Recently, the cadmium levels of different beverages with and without alcohol by different detection techniques (stripping chronopotentiometry, ICP-MS, ICP-OES, TF-FAAS, derivative potentiometric stripping analysis, GF-AAS, flow injection-diode array detection-spectrophotometry, a novel liquid-phase microextraction coupled with FAAS, HG-AFS and ET-AAS) are investigated in detail. Flame atomic absorption spectrometry [Xiang *et al*., 2012], inductively coupled plasma-optical emission spectrometry [Marchisio *et al*., 2005; Lara *et al*., 2001], inductively coupled plasma mass spectrometry [Wu & Boyle, 1997], stripping chronopotentiometry [Lo Coco *et al*., 2006], derivative potentiometric stripping analysis [La Pera *et al*., 2003], graphite furnace atomic absorption spectrometry [Schiavo *et al*., 2008; Ajtony *et al*., 2008; Dessuy *et al*., 2011], a novel liquid-phase microextraction coupled with flame atomic absorption spectrometry [Wu *et al.*, 2011], a sequential injection hydride generation atomic fluorescence spectrometry [Duan *et al*., 2005], and electrothermal atomic absorption spectrometry [Farinas *et al.*, 2007; Jurado *et al*., 2007] have also been applied for the determination of cadmium in different beverages and food samples. Although some of these techniques are sophisticated and have a high detection power, but they are relatively expensive, time-consuming, need experienced user in its area and not available in all laboratories. In contrast, ultraviolet-visible (UV-Vis) spectroscopy is a simple instrument, cheap, easy operated, rapid response time, available in many laboratories and offers acceptable analytical figures of merit when dealing with trace levels of cadmium in different matrices like beverages with and without alcohol. But due to its low detection power, it requires extraction and preconcentration procedures, which can dramatically improve the detection limit as well as the selectivity of the technique.

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Among all these sensitive methods, spectrophotometry is an important tool, which is most widely used in developing countries due to its low cost, easy operation, high accuracy/precision and good selectivity when a selective chromogenic reagent for analyte is especially used. However, sometimes, there are some difficulties for the direct determination of cadmium by spectrophotometry since its sensitivity is not sufficient for the beverage samples with low abundance levels of cadmium. For this reason, an extraction and preconcentration step is often required before spectrophotometric detection of the analyte at a suitable wavelength. Its sensitivity can be improved with the use of CPE procedure for preconcentration. In this way, CPE techniques exploit a peculiar property of most non-ionic surfactants that form micelles in aqueous solution: they become turbid when heated to the appropriate cloud point temperature. Above the cloud point temperature, the micellar solution separates into a small, surfactant-rich phase and a larger diluted aqueous phase. In the aqueous phase, the surfactant concentration remains near the critical micelle concentration. Any anlyte solubilised in the hydrophobic core of the micelle in the unheated solution will be concentrated in the surfactant-rich phase following the cloud point extraction [Bezerra *et al*., 2005; Ojeda & Rojas, 2009].

When CPE technique was used for the extraction of metal chelates, spectrophotometry was often used as detector [Baliza *et al*., 2012]. Though spectrophotometry has poor sensitivity, it is a very simple, rapid and low cost analytical tool, which can be found in almost every analytical research laboratory. CPE is also very simple, rapid, environmental friendly separation and preconcentration procedure with high enrichment factor. Combination of CPE and spectrophotometry leads to a very simple, rapid and low cost analytical method with adequate sensitivity and selectivity.

In this context, the main purpose of the current study is to evaluate the feasibility of combining CPE preconcentration with spectrophotometry at 608 and 634 nm for determination of trace cadmium in beverage samples. In this procedure, VBB^+ was used as ion-pairing reagent and Triton X-114 as the extracting one in presence of excess iodide at pH 4.0. The chemical variables affecting CPE efficiency were investigated in detail. The developed method was applied to determination of trace cadmium in some nonalcoholic beverages with satisfactory results.

MATERIALS AND METHODS

Instrumentation

Absorbance measurements at the selected wavelengths, 608 and 634 nm were made on a double beam UV-Visible Spectrophotometer (Shimadzu UV-1800 PC, Kyoto, Japan) equipped with the 1.0-cm quartz cells. An external thermostatic water bath (BM-302 Nüve) was connected to this device at constant temperature. Eppendorf vary-pipettes (10–100 and 200–1000 μL) were used to deliver accurate volumes. The pH measurements were carried out with a pH meter (Sartorious Docu-pH-meter). A centrifuge (Universal, Hettich) was used to accelerate the phase separation process. A thermostatic water bath (MF120, Nuve) was used to maintain the temperature in CPE experiments.

Reagents and solutions

Working solutions of cadmium were prepared daily by diluting a 1000 mg/L stock solution supplied from Merck (Darmstadt, Germany). A 0.1 mol/L solution of KI was prepared by dissolving a suitable amount of solid reagent supplied from Sigma (St. Louis, MO, USA) in a calibrated flask of 250 mL with water and was kept in a cool dark place when not in use. A 1.0×10^{-3} mol/L solution of VBB^+ was prepared by dissolving a suitable amount of solid reagent supplied from Sigma, in 5.0 mL ethanol and diluting to 100 mL with water and was kept in refrigerator (4 °C) when not in use. Solution (5.0%, w/v) of Triton X-114 supplied from Sigma, were prepared in bidistilled water and were used without further purification. 0.1 mol/L stock citrate-phosphate buffer solutions (pH 4.0) was prepared by mixing 19.3 mL of 0.2 mol/L NaH_2PO_4 solution with 30.7 mL of 0.1 mol/L citric acid solution in volumetric flask of 100 mL. Next, 1.0×10^{-3} mol/L pH 4.0 buffer solution was prepared by 100-fold dilution of the stock solution in the calibrated flask of 100 mL. For accuracy studies, we analyzed two certified reference materials (CRMs): SRM 1568a rice flour, SRM 1572 citrus leaves. All chemicals and reagents used in this study were of analytical-reagent grade or higher purity.

Sampling and sample digestion

All nonalcoholic beverage samples selected for analysis were supplied from local markets in Sivas, Turkey. The samples were filtered using a membrane filter (0.45-μm pore size) to remove suspended solids before analysis. In order to obtain precise and stable analytical signals, special care was taken to avoid contamination in each step of sample preparation.

The digestion procedure used in this study was based on the methods of mentioned authors [Prohaska *et al*., 2000; Teresa *et al*., 1997; Tripathi *et al*., 2001] with some modification. Steps were carried out as follows: (1) A suitable volume of each sample was accurately pipetted into an acid-cleaned PTFE beaker of 50 mL, and treated with a mixture of HNO_3 and H_2O_2 (3:1, v/v). (2) This mixture was initially heated on a hot plate for 3 h at 120 °C. (3) After cooling to room temperature, a mixture of 2 mL HNO_3 and H_2O_2 (1:1, v/v) was added into the beaker and heated for 30 min at 100 °C, and then temperature was gradually raised to 130 °C; and the mixture was left until the complete decomposition of sample was achieved. (4) The resulting solutions (nearly 2.5 mL) were made up to 10, 20 and 50 mL with deionized water resulting in a clear colorless solution using 0.05 mol/L HNO_3 when necessary, kept in a polyethylene bottle, and stored at 4 °C until analysis. (5) The blank of the reagents was carried out following the same procedure without beverage sample. In order to control a systematic error arising from matrix effect, standard Cd solutions at levels of 5.0 and 15 μg/L were spiked into the digested and diluted beverage samples (3–5 mL), and the recoveries of spiked samples were established. The 0.5 g of certified samples for accuracy of analysis was transferred into a PTFE beaker. The samples were dissolved with the same procedure. With comparison purpose, the digestion procedure for all beverage samples plus CRMs was also conducted by using a mixture of HNO_3-$HClO_4$ (5:1, v/v).

The CPE procedure

An aliquot of Cd^{2+} standard solutions (in ranges of 1–30 μg/L and 10–500 μg/L of Cd^{2+} ion) was transferred into a 50 mL centrifuge tube, 0.7 mL of 1.0×10^{-3} mol/L citrate-phosphate buffer solution, pH 4.0 and 0.7 mL of 0.1 mol/L KI solution were added to it. This was followed by the addition of 0.025 mL of 1.0×10^{-3} mol/L VBB^+ solution and 0.25 mL of 5.0% (w/v) Triton X-114 solution. The solution was taken up to the mark with doubly distilled water and allowed to stand in a thermostatic water bath at 45 °C for 15 min. Separation of the aqueous and surfactant-rich phase was accomplished by centrifugation for 10 min at 3500 rpm. Then, the aqueous phase could be separated by inverting the tube. The surfactant-rich phase was dissolved and diluted to 1.0 mL with tetrahydrofuran (THF) and transferred into a 1.0 mL quartz cell. The absorbances of the Cd^{2+} solution were spectrophotometrically measured at 608 and 634 nm, respectively. The blank solution was submitted to the same procedure and its absorbance was measured at 608 and 634 nm, for Cd^{2+}, respectively. Finally, the cadmium content of samples was directly determined from the calibration curve. When its cadmium content is around the method detection limit, standard addition calibration curve was adopted to control a systematic error arising from the matrix effect.

RESULTS AND DISCUSSION

VBB^+ is a cationic triphenylmethane group dye containing amino and imino groups. Due to its positive charge in weak acidic media, it tends to give a stable ion-associate complex with anionic surfactant molecules such as SDS and negatively charged metal-ligand complexes in addition to DNA and RNA [Xu *et al*., 2007; Zhang *et al.*, 2009]. From preliminary studies, in pH 4.0 citrate-phosphate buffer and in spectral scans in wavelength range of 300–700 nm, it was observed that VBB^+ had an absorption peak maximum at 608 and 577 nm with and without preconcentration, respectively. When Cd(II) ions in presence of excess iodide were mixed with VBB^+, the absorbance of VBB^+ at 608 nm decreased with increasing cadmium concentration, with a new absorption wavelength appearing at 634 nm. The more cadmium was added, the greater was the absorbance decrease, which indicates that an ion-pairing reaction between VBB^+ and CdI_3^- or CdI_4^{2-} ions had occurred in aqueous surfactant solution and an ion-associate complex was formed under these experimental conditions. Also, it was observed that absorption peak with lower sensitivity at 634 nm increased with increasing cadmium concentration. Since the trace levels of Cd^{2+} ions are complexed with excess iodide and the value of the pK_a of VBB^+ is 8.25, in the selected pH 4.0 buffer solution, primary cadmium complex, CdI_4^{2-} was highly negatively charged, while the VBB^+ molecules were positively charged. Thus, a strong ion-pairing reaction between VBB^+ and CdI_4^{2-} occurred in the aqueous surfactant media to form a hydrophobic ion-associate complex. Based on the decrease and increase in the absorption peaks at 608 and 634 nm respectively, a new CPE method for the spectrophotometric quantification of cadmium in beverage samples was established for further studies.

Optimization study

To take full advantage of the CPE procedure, the reagent concentrations and reaction conditions must be optimized. Various experimental parameters were studied in order to obtain optimized system. These parameters were optimized by setting all parameters to be constant and optimizing one each time.

Effect of pH

VBB^+ is a weak acidic reagent (pK_a: 8.25) and its dissociation equilibrium depends on the pK value as well as pH of the solution. Hence, its ion-pairing complex formation and extraction behavior is also pH dependent. The effect of pH on the signal intensity of Cd(II) in the surfactant-rich phase was evaluated at pH values varying from 3.0 to 6.0, at 608 nm. The variation of pH was initially carried out by using citrate-phosphate buffer solutions (0.1 mol/L) of different pH values. However, a stable and reproducible signal at sufficient sensitivity could not be obtained. Therefore, buffer solution of 0.1 mol/L was diluted at ratios of 1:100. From the experimental data, the quantitative maximum extraction of Cd(II) was achieved at a pH of 4.0. At lower and higher pH values, the hydrophobic ion-associate complex does not form completely, so the extraction efficiency of Cd(II) is low. Hence, pH 4.0 was selected as the optimal working value for further studies. Also, the effect of buffer solution volume at the fixed concentration of 1.0×10^{-3} mol/L in order to improve analytical sensitivity was studied in the range of 0.1–2.5 mL at 608 nm, and the maximum absorbance was obtained for a buffer volume of 0.7 mL. In buffer volumes lower and higher than 0.7 mL, absorbance sharply decreased. Hence, it was decided to use a buffer volume of 0.7 mL for further studies.

Effect of KI volume

The CPE efficiency primarily depends on the concentration of the main ligand, I^- and the complex formation, the apparent equilibrium constants in the micelle medium, the kinetics of the complex formation, and the mass transfer between the phases in presence of ion pairing counter ion such as VBB^+. The variation of the analytical signal as a function of iodide volume of 0.1 mol/L in the range of 0.1–2.5 mL was studied at 608 nm. From the results, it has been seen that the analytical signal of Cd(II) reaches to a maximum and then sharply decreases with increasing iodide volume when ligand volume is 0.7 mL. For further studies, an iodide volume of 0.7 mL was chosen as the optimal value.

Effect of VBB^+ volume

The CPE efficiency depends on the hydrophobicity of the ion-pairing second ligand and the complex formation, the apparent equilibrium constants in the micelle medium, the kinetics of the complex formation, and the mass transfer between the phases. The variation of the analytical signal as a function of ligand volume of 2.0×10^{-3} mol/L in the range of 0.2–2.5 mL was studied at 608 nm. From the results, it has been seen that the analytical signal of Cd(II) reaches to a maximum and then gradually decreases with increasing reagent volume when ligand volume is 0.9 mL. For further studies, a ligand volume of 0.9 mL was chosen as the optimal value.

Effect of nonionic surfactant volume

Surfactants have been used to extract the metal-ligand complexes efficiently without using organic solvents [Ferrera *et al*., 2004]. Hence, an attempt has been made to extract the metal chelate complex from the aqueous solution using three types of surfactants. These surfactants are known to form aggregates which are called micelles, and these entrap the complexes very efficiently which causes phase separation. Several surfactants have been tried to separate the metal-ligand complex from aqueous phase. After adding surfactant, the solutions were heated to different temperatures to cause cloud point formation. Once clouding takes place, the phase separation can be efficiently carried out by simple centrifugation procedure. Triton X-100, Triton X-114 and Ponpe 7.5 were preferably used for efficient phase separation. From three surfactants used, only Triton X-114 could cause best quantitative extraction with maximum absorbance after heating to 45 °C. Since the primary CdI_4^{2-} complex is nonionic in nature, only in presence of VBB^+ as counter ion the use of nonionic surfactants, Triton X-114, Ponpe 7.5 and Triton X-100 may significantly facilitate the quantitative extraction of the ion-associate complex. From preliminary studies, among the non--ionic surfactants used, Triton X-114 gave a higher absorbance value to the sample when compared to the other surfactants; hence triton X-114 has been preferred as an extracting solvent in all further studies. The effect of Triton X-114 concentration on the extraction of the ion-associate complex was investigated by the addition of surfactant ranging from 0.025 to 1.5 mL at the fixed concentration of 5.0% (w/v). From the results, extraction of the complex initially increased with increasing surfactant volume up to 0.25 mL; thereafter sample absorbance values gradually decreased. Hence, a volume of 0.25 mL has been fixed as the optimum value where the sample absorbance is high with low analyte blank.

Effect of ionic strength

For investigating the effect of ionic strength on performance of CPE, various experiments were performed by adding different amounts of NaCl ranging from 0.005 to 0.05 mol/L. Other experimental conditions were kept constant during analysis. The results showed that ionic strength had no significant effect on sensitivity to induce micelle growth and extraction of complex. Thus, ionic strength was kept constant at 0.01 mol/L NaCl.

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 equilibration temperature was investigated in temperature range of 20–70 °C. It was found that the solutions became turbid as soon as the solutions were put into the water bath with temperature higher than 40 °C, and the temperature had no considerable effect upon the extraction efficiency and the analytical signal kept constant at temperature range of 40–50 °C. In higher temperatures than 45 °C, the extraction efficiency decreased gradually and significantly. Thus, 45 °C was chosen as the equilibration temperature. Keeping the equilibration temperature of 45 °C, the influence of incubation time on CPE was studied within the range of 5–30 min. It was observed that 15 min was sufficient to achieve a quantitative extraction of analyte. Then, incubation time of 15 min was employed for CPE procedure.

The effect of centrifugation time upon extraction efficiency at 3500 rpm was studied for time interval of 5–30 min. A centrifugation time of 10 min was selected for the entire procedure, since analyte extraction during this time period is almost quantitative. The results obtained were chosen as the optimal because they yielded the greatest precision.

Effect of diluting agent

Different solvents such as acetone, THF, acetonitrile, methanol, ethanol, and acidic solutions of ethanol and methanol were tried to select among them the one that can completely dissolve the surfactant rich phase and the extracted materials (complex of cadmium and excess of ligand) and give the best sensitivity and regression coefficient for calibration curves constructed between absorbance and cadmium levels of 5, 15 and 30 μg/L at 608 nm. The best results were obtained with the use of THF, therefore, it was chosen as the diluting agent for further experiments (Figure 1).

Analytical figures of merit

Under the optimized conditions, a calibration graph was constructed for cadmium by preconcentrating a serial standard solutions ranging from 1 to 30 μg/L according to the given procedure. The calibration curve was highly linear with a correlation coefficient of 0.9969. The calibration function was A=-0.0089 [Cd(II), μg/L]+0.2739, where A is the absorbance. The limits of detection and quantification defined as 3σblank/m and 10σblank/m (where σblank is the standard deviation of twelve replicate measurements of the blank and m is the slope of the calibration graph) were found to be 0.34 and 1.1 μg/L, respectively. The precision for six replicate measurements at 25 μg/L of cadmium with preconcentration at 608 nm was 2.85% relative standard deviation. Other param-

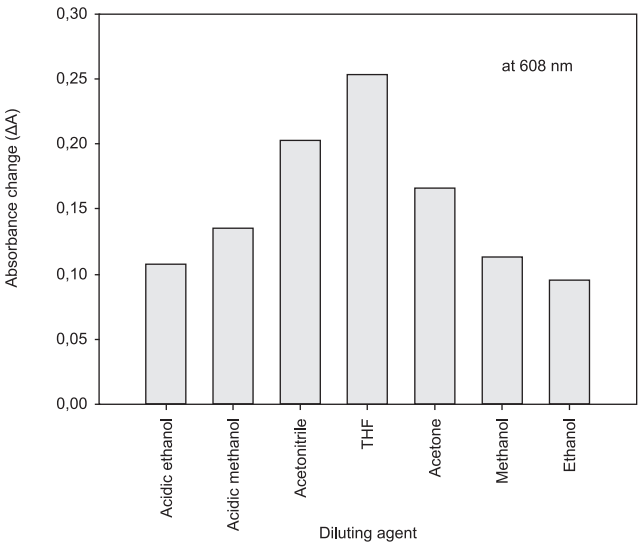

FIGURE 1. Effect of diluting agent on the signal intensity in surfactant-rich phase. Conditions: Cd(II), 2.0 μg/L; 0.7 mL of 1.0×10^{-3} mol/L pH 4.0 citrate-phosphate buffer solution; 0.7 mL of 0.1 mol/L KI; 0.025 mL of 1.0×10^{-3} mol/L VBB^+; 0.25 mL of 5.0 % (v/v) Triton X-114; equilibrium temperature, 45 °C and incubation time, 15 min.

TABLE 1. Analytical characteristics of the spectrophotometric method coupled with CPE with and without preconcentration.

Parameters	with Victoria blue B, VBB^+		
	with CPE at 608 nm	with CPE at 634 nm	without CPE at 577 nm
Linear range	1–30 μg/L	10–500 μg/L	10–700 μg/L
Slope	-0.0089	3.34×10^{-4}	2.46×10^{-4}
Intercept	0.2739	0.0192	0.0102
Correlation coefficient (r^2)	0.9969	0.9862	0.9895
Recovery% (N: 3)	98.5–102.5	98.5–103.4	98.7–103.5
Precision, RSD (%) (N: 5)	25 (2.85)	100 (4.25)	100 (3.85)
Limit of detection, LOD (μg/L)	0.34	3.8	16.5
Limit of quantification, LOQ (μg/L)	1.1	12.6	54.9
[a]Preconcentration factor	35	25	-
[b]Enhancement factor	36.2	24	-

[a] Preconcentration factor is defined as the ratio of the initial solution volume to the volume of surfactant rich phase. [b] Improvement factor is calculated as the ratio of slope of preconcentrated samples to that obtained without preconcentration.

eters related to the preconcentration systems were also calculated and are shown in Table 1.

Preconcentration factor was found to be averagely 35 by calculating the ratio of the initial solution volume to the volume of surfactant rich phase in linear range of 1–30 μg/L. The enhancement factor measures the increase in the instrumental signal provided by a preconcentration method. One of the most reliable ways to calculate this parameter is to calculate the ratio between the slopes of the calibration curves for the procedure with and without preconcentration. Thus, obtained by the way, enhancement factor was 36.2 in the developed CPE procedure.

Selectivity study

The effect of potential interference of some metal ions on the preconcentration and determination of Cd(II) was examined. In these experiments, solutions containing Cd(II) (10 μg/L) and the added interfering ions were treated according to the recommended CPE procedure under the optimized reagent conditions, and the results were given in Table 2. Only a serious interference has been observed from Hg^{2+}, Cu^{2+} and Bi^{3+} ions forming stable complex with ligand. The interfering effect of Hg^{2+} ion up to 75-fold excess over copper was completely removed in the presence of 0.2 mL of 0.05 mol/L thiourea. The interference of Bi(III) ions can be overcome up to 35-fold by adding 0.2 mL of 0.05 mol/L $Na_2H_2P_2O_7$ as a masking agent. The effect of interfering ions with reducing and metal binding properties such as oxalate, HSO_3^- and SCN^- were also studied, but any interference effect could not be found under the optimized conditions. As it is also expected from sampling step based on oxidative digestion prior to detection, it is clear that these interfering species will be removed in the form of CO_2, NH_3 and SO_2. As it can be seen from Table 2, it can be concluded that the developed method is fairly selective in terms of major species present in beverage samples.

TABLE 2. Tolerance limit of interfering ions in determination of 10 μg/L Cd(II) ion using micellar spectrophotometric detection after preconcentration with CPE under the optimized conditions.

Interfering species	Interferent/analyte ion ratio
H_3BO_3, HCO_3^-, F^-, Cl^-, Br^-, HPO_4^{2-}, NO_3^- and SO_4^{2-}	2000–3500
Oxalate, tartrate and citrate	1250–2000
Mg^{2+}, Ca^{2+}, NH_4^+, Na^+ and K^+	600–1200
Sr^{2+}, Zn^{2+} and Al^{3+}	250–600
CN^-, HSO_3^- and SCN^-	100–250
NO_2^-, Ni^{2+}, Co^{2+} and Cr^{3+}	35–100
Mn^{2+}, SeO_3^{2-}, Hg_2^{2+} and Fe^{2+}	20–35
Pb^{2+}, Fe^{3+}, Sb^{3+} and As^{3+}	10–25
[a]Hg^{2+}, [a]Cu^{2+} and [b]Bi^{3+}	2–5 ([a]75, [b]35)

[a]Tolerance limits in the presence of 0.1 mL of 0.05 mol/L thiourea.
[b]Tolerance limits in the presence of 0.2 mL of 0.05 mol/L $Na_2H_2P_2O_7$ as masking agents.

The applications of the developed method

For analysis of beverage samples and CRMs, the standard calibration curve was employed. In order to establish the accuracy and precision of the proposed procedure, the method has initially been applied to the determination of trace levels Cd(II) in the CRMs, SRM 1568a rice flour and SRM 1572 citrus leaves. The analytical results showed a good agreement between measured values (21.6±0.75 and 38.10±7.10 ng/g after wet digestion with a mixture of HNO_3-$HClO_4$ (5:1, v/v); 21.85±0.74 and 38.15±6.85 ng/g after wet digestion with a mixture of HNO_3-H_2O_2 (3:1, v/v)) and the certified values (22±2 and 30±10 ng/g), respectively. The tabulated Student's *t*-values at a significance level of 0.05 are 3.18 for certified samples and the experimental t-values are in range of 0.142–1.42, respectively. For both certified samples the experimental values obtained are also smaller than the tabulated values so it may be concluded that the values obtained are significantly equal to the certified values. The method was also applied to the determination of trace Cd(II) in nonalcoholic beverage samples. The analytical results and the recoveries for the samples spiked at concentration levels 5.0 and 15.0 μg/L were given in Table 3.

It can be seen that the recovery for the spiked samples is in the range of 96.6–101.4% with relative standard deviation of 1.70–3.92% (N: 5) for wet digestion with a mixture of HNO_3-$HClO_4$ (5:1, v/v) whereas it is in the range of 99.0––102.0% with relative standard deviation of 1.70–4.48% (N: 5) for wet digestion with a mixture of HNO_3-H_2O_2 (3:1, v/v). As it can be seen from Table 3, the Student's *t*-test for comparison of mean values demonstrated that there was no significant difference between the mean values obtained by two digestion procedures at the significance level of 0.05 [Miller & Miller, 2005]. Because the experimental *t*-values ranging from 0.115 to 1.57 are lower than the tabulated *t*-value of 2.78, it can be concluded that the mean values obtained

TABLE 3. Determination of cadmium contents of some nonalcoholic beverages as well as CRMs and recovery studies of spiked samples.

Sample	Sample volume (mL) Dilution ratio	After wet digestion with HNO_3/$HClO_4$ (5:1, v/v) (N: 3)				After wet digestion with HNO_3/H_2O_2 (3:1, v/v) (N: 3)				Certified value (μg/L or ng/g)	The calculated t- and F-values
		Added (μg/L)	Found (μg/L)	RSD (%)	Recovery (%)	Added (μg/L)	Found (μg/L)	RSD (%)	Recovery (%)		
[a]Canned nonalcoholic beverages											
Sour cherry juice	5/1:2	-	3.62±0.12	3.61	-	-	3.70±0.13	3.51	-	-	1.57, 1.17
		5	8.45±0.22	2.60	96.6	5	8.73±0.24	2.75	100.6	-	-
		15	18.43±0.34	1.84	98.7	15	18.72±0.35	1.87	100.1	-	-
Orange juice	5/1:2	-	2.47±0.09	3.64	-	-	2.51±0.08	3.19	-	-	0.115, 1.27
		5	7.50±0.16	2.13	100.6	5	7.55±0.18	2.38	100.8	-	-
		15	17.43±0.32	1.83	99.7	15	17.60±0.30	1.70	100.6	-	-
Ice Tea	5/1:2	-	2.74±0.10	3.65	-	-	2.80±0.09	3.21	-	-	0.155, 1.23
		5	7.76±0.18	2.32	100.4	5	7.84±0.20	2.55	100.8	-	-
		15	17.68±0.30	1.70	99.6	15	17.82±0.32	1.80	100.1	-	-
Peach juice	5/1:2	-	3.30±0.12	3.64	-	-	3.35±0.13	3.88	-	-	0.98, 1.17
		5	8.37±0.21	2.51	101.4	5	8.41±0.22	2.62	101.2	-	-
		15	18.38±0.36	1.96	100.5	15	18.45±0.34	1.84	100.7	-	-
Pear juice	3/1:3	-	8.45±0.30	3.55	-	-	8.50±0.28	3.29	-	-	0.423, 1.15
		5	13.40±0.50	3.73	99.0	5	13.45±0.48	3.56	99.0	-	-
		15	23.38±0.58	2.48	99.5	15	23.50±0.56	2.38	100.0	-	-
Apricot juice	3/1:3	-	7.80±0.28	3.59	-	-	7.85±0.30	3.82	-	-	0.424, 1.15
		5	12.75±0.50	3.92	99.0	5	12.80±0.51	3.98	99.0	-	-
		15	22.95±0.59	2.57	101.0	15	23.05±0.61	2.65	101.3	-	-
Apple juice	5/1:2	-	2.78±0.09	3.34	-	-	2.83±0.10	3.53	-	-	1.29, 1.23
		5	7.76±0.21	2.71	100.8	5	7.84±0.23	2.94	100.2	-	-
		15	17.72±0.34	1.92	100.0	15	17.90±0.32	1.79	100.5	-	-
Grapefruit juice	3/1:3	-	13.58±0.62	4.56	-	-	13.60±0.61	4.48	-	-	0.199, 1.03
		5	18.47±0.72	3.90	97.8	5	18.70±0.74	3.96	102.0	-	-
		15	28.45±0.85	3.31	99.1	15	28.60±0.82	2.87	100.0	-	-
[b] CRMs (N: 4)											
SRM 1568a Rice flour	-		21.6±0.75	3.47	98.18		21.85±0.74	3.36	99.32	22±2	0.374, 7.11; 0.141, 7.40
SRM 1572 Citrus leaves	-		38.10±7.10	8.14	127.0		38.15±6.85	3.81	127.17	30±10	1.34, 1.98; 1.42, 2.13

[a]In order to compare two mean values the statistical t- and F-critical values at 95% confidence level and 4 degrees of freedom are 2.78 and 5.63, respectively. [b]In order to compare the measured value with the certified values of CRMs the critical t- and F- values at 95% confidence level and degrees of freedom of 3 are 3.18 and 8.53, respectively.

by two digestion procedures contain a significant difference for 8 degree of freedom at 95% confidence level. It is clear that the proposed method for beverage samples has a good reproducibility as a measure of precision by variance analysis based on pooled standard deviation with experimental $F_{4,4}$-value ranging from 1.03 to 1.27.

Validation study was performed to demonstrate to suitability of the analytical method for the intended purpose and therefore the reliability of the results. Linearity, limits of detection and quantification, accuracy, inter-day and intra-day method precision and instrumental precision for standards and samples were tested employing two CRMs: SRM 1568 a rice flour and SRM 1572 citrus leaves. The certified contents were 22±2 and 30±10 ng/g for rice and citrus leaves, respectively. It can be said that the obtained values, 21.6±0.75 and 38.10±7.10 ng/g for HNO_3/$HClO_4$ and 21.85±0.74 and 38.15±6.85 ng/g for HNO_3/H_2O_2, respectively, using two wet digestion procedures are quantitatively in good agreement with the certified values. Also, standards additions were performed at three concentration levels ranging from 5 to 15 μg/L for cadmium in order to determine if there is a possible systematic error arising from beverage samples with high organic matrix. Validation parameters are summarized in Table 4. It is clear that the analytical data obtained with twelve replicate measurements show the good performance of the method for the intended purpose in terms of accuracy and precision.

TABLE 4. The main validation parameters for spectrophotometric measurement of cadmium in beverages at 608 nm after preconcentration with CPE.

Validation parameters	Standards linearity	Samples linearity	Standards accuracy	Samples accuracy	Method precision				Instrumental precision	Limit of detection	Limit of quantification
					Intra-assay day 1	Intra-assay day 2	Intra-assay day 3	Intermediate			
	a±L.C b±L.C r^2	a±L.C b±L.C r^2	Recovery (%) RSD (%)	Recovery (%) RSD (%)	Mean (μg/L) RSD (%)	Mean (μg/L) RSD (%)	Mean (μg/L) RSD (%)	Mean (μg/L) RSD (%)	Mean (μg/L) RSD (%)	Concentration (μg/L)	Concentration (μg/L)
After wet digestion with HNO_3/H_2O_2 (3:1, v/v)	0.126±0.0013	0.833±0.0070	98.6±1.6	96.5±2.0	13.8±0.5	13.6±0.5	13.7±0.7	13.7±0.6	13.6±0.4	0.085	0.28
	0.046±0.0018	0.053±0.0021	1.95	3.85	3.6	3.7	5.1	4.4	2.94	-	-
	0.9968	0.9972	-	-	-	-	-	-	-	-	-
After wet digestion with $HNO_3/HClO_4$ (5:1, v/v)	0.124±0.0015	0.836±0.0078	97.6±2.3	96.4±2.5	13.7±0.5	13.6±0.6	13.5±0.8	13.6±0.7	13.5±0.5	0.1	0.33
	0.045±0.0021	0.054±0.0025	2.10	3.95	3.65	4.41	5.92	5.18	3.70	-	-
	0.9968	0.9972	-	-	-	-	-	-	-	-	-

a: intercept (Absorbance); b: slope (Absorbance/μg/L); L.C: limits of confidence at probability level of 0.05 for twelve replicate measurements (N:12) of wine sample based on direct calibration curve and standard addition calibration curve approaches.

In the light of all these data, it can be concluded that the detection limit and preconcentration factor of the method are lower than those given by many methods like FAAS and CV-AAS reported in literature [Coelho & Arruda, 2005; Liang *et al*., 2005; Afkhami *et al*., 2006; Manzoori *et al*., 2007; Doroshchuk & Kulichenko, 2005; Xiang *et al*., 2012]. Moreover, these preconcentration methods based on detection with FAAS were generally applied to water samples with low organic matrix. The other techniques such as GFAAS, WCAAS and TS-FF-AAS are more sensitive, but are time-consuming, expensive and complicated instruments which need an experienced user according to spectrophotometer. It is important to emphasize that 1.0 mL THF containing surfactant-rich phase was sufficient to obtain an enhancement and preconcentration factors of 36.2 and 35, and the whole preconcentration procedure was done just in a 50 mL centrifuge tube within 20 min.

CONCLUSIONS

The results indicate the usefulness of the proposed CPE/spectrophotometric method to quantitative extraction of cadmium present in beverage samples. The proposed method allowed Cd determination at 0.34 μg/L levels in the linear ranges of 1–30 and 10–500 μg/L at 608 and 634 nm respectively, thus represents a promising approach in the monitoring of Cd in different beverage samples with low cost, simplicity, efficiency, versatility and non-polluting respect. The proposed CPE method gives highly low LOD, high preconcentration and enhancement factors and good RSD and extraction of the cadmium as a ternary complex with nonionic surfactant, Triton X-114 from its initial matrix after pretreatment with two wet digestion procedures. Due to versatility, it can also be applied to the monitoring of cadmium in various samples of environmental, toxicological and medical analysis as well as beverage samples. The method can be considered as an alternative tool to sensitive but expensive, time-consuming and experienced user-requiring analytical techniques such as GF-AAS, HG-AFS, ICP-AES and ICP-MS.

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