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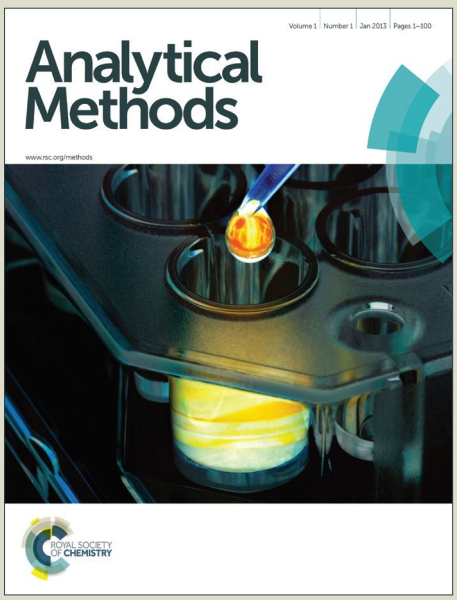

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Determination of Low Levels of Cd(II) in Cosmetic Products by Spectrophotometry after Separation/Preconcentration with Cloud Point Extraction

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Abstract

In the present study, a simple, low-cost and efficient method was developed for extraction and preoncentration of Cd(II) from cosmetic products by using cloud point extraction (CPE) prior to spectrophotometric detection. The method is based on ternary complex formation of Cd(II) ions with 2′,7′-dichlorofluorescein (DClF) in presence of 2,2'-bipyridine (Bipyr) and NaCl as chelating and salting-out agents at pH 7.0 respectively, and then its extraction into the micellar phase of polyethylene glycol *tert*-octylphenyl ether (Triton X-114). The effects of various variables affecting complex formation and CPE efficiency were investigated and optimized. The calibration graph was highly linear in the range of 8-120 μg L^{-1} with a good regression coefficient, in which the absorbance is decreased with increase in concentration of Cd(II) at 502 nm. The limits of detection and quantification were 2.5 and 8.3 μg L^{-1} with a sensitivity enhancement factor of 31.4, respectively. The relative standard deviations (RSDs) were in range of 2.5-5.1 % (for 15, 30 and 60 μg L^{-1}, n: 5). The recovery rates of spiked samples were in the range of 97.2-103 % (15, 30 and 60 μg L^{-1}, n: 5). Its accuracy was controlled by analysis of a certified reference material (CRM 1548a Typical Diet). It has been observed that the result for Cd(II) is statistically within 95 % confidence limit for certified value. The results show that the proposed method can be applied satisfactorily to determine Cd(II) in cosmetic products.

Keywords: Cd(II), 2′,7′-dichlorofluorescein, Cloud point extraction, Spectrophotometry, Cosmetic products

Introduction

It is now widely accepted that cadmium, Cd(II) is a highly toxic metal with many long-term effects, and is known to damage organs such as kidneys, liver and lungs, even at very low concentrations.[1] It is intentionally or non-intentionally used in cosmetic products as impurities in color additives. Directly contact with cosmetics is an important pathway through which Cd(II) dermally enters human body, thus there is an increasing need to monitor low levels of Cd(II) in cosmetic products. Moreover, such data can also provide us with Cd(II) contamination information about safety and quality control of product.[2] However, directly accurate and reliable determination of Cd(II) in cosmetic products is often difficult, not only

because of low concentrations, but also because of matrix effects. To overcome these problems, separation–preconcentration procedures are often involved prior to analysis.

Many analytical techniques such as anodic stripping voltammetry (ASV),[3,4] adsorptive stripping voltammetry (AdSV),[5] adsorptive cathodic striping voltammetry (AdCSV),[6] inductively coupled plasma-mass spectrometry (ICP-MS),[7] inductively coupled plasma-optical emission spectrometry (ICP-OES),[8] neutron activation analysis (NAA),[9] electrothermal atomic absorption spectrometry (ET-AAS)[10] and flame atomic absorption spectrometry (FAAS)[11] have been developed to determine low levels of Cd(II) in different samples. Each of these techniques has its own peculiar privileges, but each method has some problems such as poor reproducibility and limited sample adaptability. Among these sensitive techniques, instruments such as ET-AAS, ICP-OES and ICP-MS are useful for trace Cd(II) determination without any preconcentration. However, these instruments are more expensive to buy and employ in research laboratories. Besides, these techniques have spectral and chemical interferences as well as requiring expert's user, poor precision, limited dynamic range and slower analysis time. Molecular absorption spectrophotometry in ultraviolet-visible region is a simple technique, cheap, easy to operate, rapid response time, available in many laboratories, and offers acceptable analytical figures of merit when dealing with trace levels of Cd(II) in different matrices like cosmetic products. But, due to its low detection power, there is a need to separation and preconcentration procedures in presence of a suitable absorbing reagent(s) for analyte, which can dramatically improve the detection limit as well as the selectivity of the technique.

In spite of great developments in the modern analytical instruments, available analytical instrumentation does not have enough sensitivity for the analysis of real samples. Many techniques for separation and preconcentration of Cd(II) have been proposed such as pyridine-2,6-diamine-functionalized Fe_3O_4 nanoparticles as a novel sorbent,[12] new

functionalized resin,[13] an activated carbon column,[14] 2-(**5**-bromo-2-pyridylazo)-5-diethylaminophenol (5-Br-PADAP) functionalized wool-packed microcolumn,[15] dispersive liquid–liquid microextraction (DLLM),[16] cloud point extraction (CPE),[17,18] solid-phase extraction (SPE),[19,20] liquid phase microextraction (LPME)[21] and electrochemical deposition.[22] The most important of these separation and preconcentration techniques is CPE procedure. CPE has been displayed to have the diverse merits of low cost, simplicity, velocity, lower toxicity to the environment than extractions that use organic solvents and a high capacity to concentrate a wide variety of analytes of widely varying rate with high recoveries and high concentration factors. However, simple combination with spectral, chromatographic, atomic absorption/emission and electro-chemical analyses allows using CPE for elaborating high-sensitive and convenient analytical methods.[23] When it is chosen a sensitive and selective reagent like 2',7'-dichlorofluorecein (DClF), spectrophotometry is becoming one of the most widely used techniques for accurate and reliable determination of trace Cd(II) after separation and preconcentration with CPE.

In this sense, the aim of the present work was, on one hand, to propose a CPE method based on the use of Triton X-114 for extraction of Cd(II) as ternary complex and, on the other hand, to evaluate the combination of the proposed CPE procedure with spectrophotometry for trace metal analysis. To this end, the analytical variables affecting the complex formation and CPE efficiency were optimized in detail. Under the optimized CPE conditions, analytical figures of merit of the method were estimated, in which the absorbance is decreased with increase in concentration of Cd(II) at 502 nm. The method accuracy was assessed through the analysis of a certified reference material (CRM 1548a typical diet), and the result was in agreement with the certified value at a 95 % confidence level according to Student *t*-test. Finally, cosmetic products were analyzed in order to assess the applicability of the method to analysis of real time samples.

Experimental

Instrumentation

Absorbance measurements were made at 485 and 502 nm before and after preconcentration with CPE by using a double beam UV-Visible spectrophotometer (Shimadzu UV-1800 PC, Kyoto, Japan) equipped with the 1.0-cm quartz cells. A thermostatic water bath (EPC 4420, Termal, and Istanbul, Turkey) was used to maintain the temperature in CPE experiments. A centrifuge (Universal-320, England) was used to accelerate the phase separation process. The pH measurements were carried out with a pH meter (pH-2005, JP Selecta, Barcelona, Spain).

Reagents and solutions

All chemicals and reagents used were of analytical-reagent grade or higher purity. All solutions were prepared with deionized water (18.2 MΩ cm) obtained from a Labconco (Kansas City, USA) water purification system. The working solutions of Cd(II) for calibration were prepared by dilution of the stock solution of 1000 mg L^{-1} supplied from Merck (Darmstadt, Germany) water just before use. The solutions of 5.0 % (v/v) of Triton X-114 (Sigma-Aldrich, Milwaukee, USA) were prepared by dissolving 5.0 mL of surfactant in water and diluting to 100 mL in a volumetric flask. DClF (1.0×10^{-3} mol L^{-1}) and Bipyr (0.01 mol L^{-1}) solutions were prepared by dissolving their appropriate amounts (Sigma-Aldrich, Milwaukee, USA) in ethanol and completing to 1 L with water. The NaCl solution of 0.01 mol L^{-1} as salting-out agent was prepared by dissolving 0.0578 g of solid in water and completing to 100 mL in a volumetric flask. The vessels and pipettes used for trace analysis were kept in 10 % (w/v) HNO_3 for at least 24 h and subsequently washed five times with deionized water.

Sampling and sample preparation

The cosmetic samples were purchased from local open-markets and in a Turkish store in Sivas, Turkey where it is suitable to buy cheap cosmetic products such as shampoo, nail polish, lipstick. An aliquot of 2 g or 5 mL of each semi-solid and/or liquid cosmetic products was transferred into a Pyrex glass beaker of 100 mL and digested with 5 mL of concentrated HNO_3 (65 %, w/w) on a heater at 80 ^oC until samples dried. This process was repeated five times. Then, 4 mL of a mixture of HNO_3 (65 %, w/w) and $HClO_4$ (70 %, w/w) and H_2O_2 (30 %, w/w) (in volume ratio of 2:1:1, v/v) [24] was added for oxidizing entirely the organic matrix. The mixture was covered and left to stand overnight, and then heated to 125 ^oC for 1 h. Until a clear homogenous solution is obtained, again 4.0 ml of mixture of concentrated acids were added to the sample solutions when necessary. After that, the digested samples were immediately cooled in an ice-cooler and filtered through a 0.21-µm-pore size polycarbonate membrane filter (Nuclepore, Whatman, Brentford, UK). The separated solutions were diluted to the final volume of 50 mL with water. The Cd(II) contents of the pretreated cosmetic samples were determined by help of both direct calibration curve and standard addition calibration curve approaches based on spectrophotometric detection at 502 nm after preconcentration with CPE. An aliquot of 1.0 g of a certified reference material, CRM 1578a typical diet, for validation of the method was placed into a PTFE beaker. The certified sample was pretreated and digested at the same time as the cosmetics, using the same conditions and acid mixture. Although the certified sample is not a cosmetic matrix, it has some of the same components. The fat content may not be comparable to all of the skin cream or lipstick types, depending on their formulation. Also, a blank analysis was carried out following the same procedure without cosmetic sample. In order to control a possible systematic error arising from matrix effect, standard Cd(II) solutions at levels of 10 and 15 µg L^{-1} above the method quantification limit were spiked into 3-5 mL of the pretreated cosmetic samples under the optimized conditions as well as the certified sample, and the recoveries of spiked samples

were established. Each sample was analyzed in triplicate, and reagent blank determination was performed to ascertain that no impurity was introduced during the extraction procedure.

The CPE procedure

In the proposed method, aliquots of sample or pretreated-sample containing Cd(II) in the range of 8–120 μg L^{-1}, 1.0 mL of 0.01 mol L^{-1} pH 7.0 Tris/HCl buffer, 3.0 mL of 0.01 mol L^{-1} Bipyr, 7.0 mL of 1.0×10^{-3} mol L^{-1} DClF, 0.3 mL of 5.0 % (v/v) Triton X-114, 3.0 mL of 0.01 mol L^{-1} NaCl respectively, were added into a 50 mL centrifuge tube and volume was completed to 50 mL with deionized water. The solutions were mixed well and kept in a thermostatic water bath for 8 min at 50 ^{o}C. The phase separation was expedited by centrifuging at 3500 rpm for 10 min. The resulting mixtures were cooled in an ice-bath for 10 min to increase the viscosity of the surfactant-rich phase and make easy the separation of the aqueous phase. The aqueous phase was easily separated from surfactant-rich phase by inverting the tube. The surfactant-rich phase was diluted to 1.0 mL with tetrahydrofuran (THF). The absorbance of the solution was measured at 502 nm. A blank solution (without Cd(II)) was also submitted to the same procedure and measured in parallel to the samples.

Results and Discussion

General aspects related to method development

Prior to optimization of analytical variables affecting CPE efficiency, at initial, Job's continuous variation method[25] was used to determine the stoichiometric ratio and stability constant of ternary complex. With this aim, the mixtures were prepared by mixing solutions of both components, Cd(II) and DClF, with equal molar concentration (1.0×10^{-4} mol L^{-1}) in ratio varying from 1:9 to 9:1. The absorbance of ternary complex formed has been independently measured in presence of Bipyr and NaCl of 6.0×10^{-4} mol L^{-1} at 502 and 485 nm with and without CPE, respectively. At pH 6.0, the stability constant has been found to be 2.3×10^{4} with a stoichiometric ratio of 2.15 in aqueous alcoholic media at 485 nm, which is

lower than the value determined in aqueous micellar media at 502 nm with a red shift of 17 nm after preconcentration with CPE. The stability constant after preconcentration with CPE at pH 7.0 is 8.6×10^5 with stoichiometric ratio of 1.95, these values prove that the ternary complex is more stable in aqueous micellar media. It has been implied by that it can be selectively used as a fluorescent sensor for Cd(II) ions in presence of Cu^{2+}, Zn^{2+} and Ni^{2+} ions in literature,[26] in which two Cd(II) ions are directly bonded to DClF with a value logβ= 3.80 in absence of Bipyr. Due to a $pK_{hydrolysis}$ of 10.15 for Cd(II), in form of $CdCl^+ + H_2O \leftrightarrow Cd(OH)Cl + H^+$ in range of pH 7.0-9.0,[27] it can be also concluded that chloride ions as salting-out agent will directly be able to participate into the complexation in presence of DClF and 2,2'-bipyridine (Bipyr) at pH 7.0.[28] In this sense, the mixtures were prepared by mixing solutions of Cd(II) and Bipyr, with equal molar concentration (5.0×10^{-4} mol L^{-1}) in ratio varying from 1:9 to 9:1. The absorbance of ternary complex formed has been measured in presence of DClF and NaCl of 1.5×10^{-4} mol L^{-1} at 502 and 485 nm with and without CPE, respectively. It has been observed that a stoichiometric ratio, [Cd(II)/[Bipyr] is 1:2 in aqueous micellar media at 502 nm while it is 1:1 in aqueous alcoholic media at 485 nm. The absorbance signals are lower than those in presence of Bipyr without Bipyr at both 502 nm and 485 nm. As a result of increased electron density on Cd(II) ions in coordination center, the coordination number expanded from 4 to 6 by adding neutral electron donor nitrogenous ligand, Bipyr, so as to give a more stable, sensitive and selective hydrophobic complex, which can be easily extracted into the micellar phase. Moreover, a red shift of 17 nm from 485 nm to 502 nm can be also an indicator of increased electron density on the cadmium in ternary complex. This state can be explained by means of only to charge transfer transitions from ligand to Cd(II) ions, due not to be d–d transitions for Cd(II) with configuration of d^{10}. Therefore, depending on pH, temperature and concentrations of DClF, Bipyr and NaCl in presence and absence of extracting nonionic surfactant, Triton X-114 above critical micelle

concentration, it is believed that the ternary complex formed is in form of octahedral geometry for the method with preconcentration at 502 nm while it is in form of tetrahedral geometry for the method without preconcentration at 485 nm, in which the optimal conditions for Cd(II) in range of 100-1500 μg L^{-1} are .2.5 mL of 0.01 mol L^{-1} Bipyr, 5.0 mL of 1.0×10^{-3} mol L^{-1} DClF, 2.5 mL of 0.01 mol L^{-1} NaCl and 2.5 mL of 0.05 mol L^{-1} phosphate buffer in a volumetric flask of 50 mL for equilibrium temperature of 40 ^{o}C for 15 min at pH 6.0. As a result, this mechanism of ternary complex formation with tetrahedral and octahedral geometries can be postulated as follows:

Optimization of the CPE system variables

The effects of concentrations of reagents and nonionic surfactant, pH, temperature and time of equilibration, centrifugation rate and time on the analytical signal were investigated and optimized in order to reach the best analytical performance for the CPE procedure. Fig. 1 shows the change in absorption of the ternary complex formed in presence of DClF, Bipyr and NaCl in surfactant-rich phase against reagent blank with increasing Cd(II) concentration at levels of 25, 50 and 75 μg L^{-1} at pH 7.0.

The pH is evaluated as a critical factor to increase the partition coefficient of the analyte between aqueous and surfactant-rich phase which lead to increase the extraction efficiency. Therefore, the effect of pH on extraction efficiency of Cd(II) was investigated in pH range of 5-10 by using different buffers such as Tris/HCl, NH_3/NH_4Cl, $H_2PO_4^-/HPO_4^{2-}$ and borate at iso-molar concentrations. The absorbance of surfactant-rich phase containing Cd(II) at level of 50 μg L^{-1} was measured at 502 nm. The best sensitivity was obtained with Tris/HCl buffer at pH 7.0. As can be seen in Fig. 2(a), increase of pH up to 7.0 leads to an increase in absorbance and then gradually decreases. The decrease in extraction efficiency at higher pHs than 7.0 may be due to hydrolysis of Cd(II) ions, so as to give further $Cd(OH)_2$ and $Cd(OH)_3^-$ species. Also, the effect of buffer concentration on the extraction efficiency was studied in the

range of (0.05–1.4)×10^{-3} mol L^{-1} concentration in Fig. 2(b), and the best analytical signal was obtained with using 0.2×10^{-3} mol L^{-1} of buffer solutions. In higher concentrations than 0.2×10^{-3} mol L^{-1}, the decrease in absorbance may be due to inhibition effect of excess neutral Tris buffer with a pK_a value of 8.1, so as to give a cationic complex with Cd(II) ions from potentiometric titration data ($logK_{Cd(tris)2+}$= 1.94±0.02 at 25 ^oC).[29] Therefore, a buffer concentration of 0.2×10^{-3} mol L^{-1} at pH 7.0 was used as optimal value for further studies.

The variation of absorbance as a function of the concentration of Bipyr as main chelating ligand was studied in the range of (0.06-1.0)×10^{-3} mol L^{-1}. From the results in Fig. 3(a), it can be seen that the absorbance linearly increases with increasing Bipyr concentration up to 0.6×10^{-3} mol L^{-1}. The absorbance partly decreases at the higher concentrations than 0.6×10^{-3} mol L^{-1}. So, a Bipyr concentration of 0.6 ×10^{-3} mol L^{-1} was selected as optimal value for further studies.

The effect of DClF concentration as ion-association reagent on extraction efficiency was studied in range of (0.02-0.20)×10^{-3} mol L^{-1}. As can be seen in Fig. 3(b), it is clear that absorbance dramatically depends on the concentration of DClF in CPE procedure. With the increase in concentration of DClF, the absorbance linearly increases up to 0.14×10^{-3} mol L^{-1} with increasing concentration and then slowly decreases. This decrease may be due to aggregation of ion-association ligand. Therefore, a concentration of 0.14×10^{-3} mol L^{-1} was selected as optimal value for further studies.

Optimization of nonionic surfactant as extracting agent was carried out in order to use a minimum surfactant concentration with maximum extraction efficiency. The effect of Triton X-114 concentration on the absorbance of ternary complex was studied in the range of 0.01-0.5 % (v/v). From the results in Fig. 4, it can be seen that the absorbance increases up to 0.3 % (v/v), and then gradually decreases. At lower concentrations than 0.3 % (v/v), the extraction efficiency is slightly lower probably due to inadequacy of the assemblies to entrap the

hydrophobic complex quantitatively. At higher concentrations than 0.3 % (v/v), the decrease in absorbance may be due to the increment in the volumes and the viscosity of the surfactant phase. Therefore, a concentration of 0.3 % (v/v) was selected as the optimal for further studies.

Equilibrium temperature and incubation time are two important parameters in CPE. The effects of equilibration temperature and incubation time on the absorbance were studied in the range of 20–70 ^{o}C and 0–20 min, respectively. Since it is desirable to employ the shortest equilibration time and the lowest possible equilibration temperature, a temperature of 50 ^{o}C and an equilibration time of 8 min were chosen for further studies. 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 interval is almost quantitative.

In CPE, addition of salt to sample solution helps to phase separation and increases the mass transfer of analyte from aqueous phase to surfactant-rich phase. Also, amounts of inorganic salts lead to decrease in the cloud point temperature. Therefore, it is essential to consider the secondary effect of the electrolyte, which is known as salting-out effect. Based on these reasons, NaCl as salting-out agent was chosen and its effect on the extraction process was investigated in the range of $(0.02–1.0)\times10^{-3}$ mol L^{-1}. From the results in Fig. 5, it is clear that NaCl has a positive effect on the process by decreasing the coacervate phase volume, so as to lead a significant increase in absorbance. The results indicate that the absorbance increases up to NaCl concentration of 0.6×10^{-3} mol L^{-1} and then gradually decreases. Thus, a salt concentration of 0.6×10^{-3} mol L^{-1} was selected as optimal value for further studies.

The volume of the surfactant-rich phase acquired after separation and preconcentration with CPE is small for spectrophotometric detection. It is very important to choose the appropriate

solvent for maximum efficiency. The effect of various diluting agents such as methanol, acetonitrile, ethanol, acidic methanol, acidic ethanol, acetone and THF was studied to dilute surfactant-rich phase after phase separation. As can be seen in Fig. 6, the best analytical sensitivity (m/s_m) with a good correlation coefficient was obtained by THF diluted to 1.0 mL from three pointed-calibration curves obtained by using fixed Cd(II) concentrations of 15, 30 and 60 μg L^{-1}. So, THF was adopted as diluting agent for further studies.

Analytical figures of merit of the method

Important parameters such as the linear working range, accuracy (as recovery rate), precision (as RSD %), sensitivity, limits of detection and quantification (LOD and LOQ), preconcentration and sensitivity enhancement factors were determined to evaluate the analytical performance of the preconcentration method. The analytical figures of merit of the method with and without preconcentration are shown in Table 1. Under the optimized conditions, a linear calibration curve in the range of 8-120 μg L^{-1} of Cd(II) was acquired after CPE. A regression equation acquired by the least squares method for preconcentration method is $A= -(5.03\pm0.20)\times10^{-3}C_{Cd(II)} + 0.6172\pm0.0042$ with a correlation coefficient of -0.9932 (n: 12), where A is the absorbance at 502 nm and $C_{Cd(II)}$ is the concentration of Cd(II) (μg L^{-1}). The detection and quantification limits of the method from the slope of calibration curve and twelve replicate measurements of the blank were found to be 2.5 and 8.3 μg L^{-1}, respectively. The precision (as RSD %) was in range of 2.5-5.1 % (15, 30 and 60 μg L^{-1}, n: 5). The percent recoveries of spiked sample solution were in range of 97.2-103 % (15, 30 and 60 μg L^{-1}, n: 5). The sensitivity enhancement factor, which is calculated by using the ratio of the slopes of calibration curves obtained with and without the preconcentration, was 31.4. The preconcentration factor, which is described as the ratio of the initial bulk solution volume (50 mL) to the surfactant rich phase volume (1.0 mL), was found to be 50.

Matrix effect

The effect of foreign ions on the recovery of spiked Cd(II) was examined. Different amounts of common cations and anions in real samples were added to the test solution containing 50 μg L^{-1} of Cd(II) and the developed methodology was applied. If an ion causes an analytical signal change more than 5.0 %, this ion can be accepted as interference species. Table 2 shows tolerance limit of different ions. These results demonstrated that large excess amounts of some common anions and cations such as F^-, Cl^-, Br^-, NH_4^+, Na^+, K^+, Mg(II) and Ca(II) did not interfere on the determination of trace level of Cd(II). The cations Co(II), Ni(II), Cu(II), Ag(I), Hg(II), and Fe(III) partly interfered in the determination when they were presented in a tolerance ratio ranging from 5 to 35 (foreign ion/Cd(II)); if this was the case, selective masking agents such as thiosulfate for Cu(II), Ag(I) and Hg(II) ions, citric acid and tetrasodium pyrophosphate for Ni(II), Co(II) and Fe(III) ions, due to give more stable complexes than Cd(II) with a significant improvement in tolerance ratio, were efficiently used to solve this problem.

Analytical applications

The proposed CPE method was applied to the determination of Cd(II) in the pretreated cosmetic products after digestion with mixture of HNO_3, $HClO_4$ and H_2O_2 (2:1:1, v/v). In order to validate the method, it was applied to a certified reference material, NIST CRM 1548a, with Cd(II) content of 0.035±0.0015 mg kg^{-1} respectively. Using the CPE/spectrophotometric method, which is comparatively based on directly calibration curve and standard addition calibration curve approaches, the contents of Cd(II) determined in these certified samples were 0.036±0.002 and 0.037±0.002 mg kg^{-1} (95 % confidence interval; n: 5) respectively. When the calculated t-values are compared with the tabulated t-values in 95 % confidence level for five replicate measurements, it has been observed that the measured values for Cd(II) are statistically in good agreement with the certified values. Also, the

recovery experiments were carried out by spiking the cosmetic samples including CRM with different amounts of Cd(II), 10 and/or 15 μg L^{-1} before preconcentration with CPE. The spiked sample solutions were analyzed in a similar way, and the percentage recoveries were calculated. Table 3 shows the results obtained for five replicate measurements. The statistical analysis of these results using Student's *t*-test showed that there are no significant differences between actual and observed concentrations at 95 % confidence level. The slopes of the standard additions graphs for the certified samples and selected cosmetic samples did not have any significant difference with that of calibration graph, and also the corresponding detection limits for each cosmetic sample were found to be the same as that obtained from standard solutions with a lower RSD than 5.1 %. Therefore, we can conclude that there is not any matrix effect arising from the sample matrices.

When comparatively considered its analytical figures of merit in Table 4, the results obtained in the existing work allow the determination of Cd(II) at trace levels in different sample matrices and may be compared with those of other works present in the literature. Costa et al.[30] described a preconcentration factor of 13 and limit of detection of 5.0 μg L^{-1} for the determination of Cd(II) in brine samples by ICP-OES, while De Melo Gomes et al.[31] obtained a preconcentration factor of 20, for the analysis of fuel ethanol. Pereira and Arruda [32] described a preconcentration factor of 100 after adsorptive preconcentration for the detection limits 80 and 0.17 μg L^{-1} level by FAAS and GFAAS, respectively. Using FAAS, Bortoleto et al.[33] obtained a preconcentration factor of 30 with a limit of determination of 2.0 μg L^{-1} for the determination of Cd(II) in nail polish samples after preconcentration onto a column containing silica gel modified with Cupferron at pH 5.5. Also, the method has lower detection limit than those of the atomic and molecular spectrometric methods after preconcentration with biosorbent and VALLME from different matrices including CPE at 638 nm.[35, 40, 41] As a result, a reasonable sensitivity improvement and comparable detection limit have been

achieved when compared to previously reported works,[34, 36-39, 42-44] in literature using CPE/Spectrophotometry. So, it can locally be considered as a useful analytical tool for accurate and reliable monitoring of trace Cd(II) in cosmetic products.

Conclusions

In the existing study, the CPE procedure was efficiently used as a separation and preconcentration tool to detect low levels of Cd(II) in selected cosmetic products such as shampoo, lipstick, nail polish. The coupling of CPE with spectrophotometry can be considered an alternative to sensitive, but costly, tedious, poor precise and requiring an experienced-user detection techniques such as ICP-MS, ICP-OES, CV-AAS and GF-AAS. Also, when a strongly absorbing chromophore is selected, molecular absorption spectroscopy in ultraviolet-visible region is simple, low cost and a versatile detection tool, which can be available in nearly every research laboratory. The developed preconcentration method has a linear range of 8-120 μg L^{-1} with a detection limit of 2.5 μg L^{-1}, preconcentration factor of 50 and sensitivity enhancement factor of 31.4. The applicability of the method to cosmetic products may also provide a meaningful contribution to the literature by using a highly sensitive and selective photometric probe such as DClF for Cd(II) ions. Moreover, the preconcentration method proposed can safely be extended to other complicated matrices. The accuracy was controlled by analysis of a certified sample, and the results obtained by direct calibration and standard addition calibration curves were statistically in good agreement with the certified value.

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Ethical Approval: This article does not contain any studies with human or animal subjects.

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References

1. A.C. Davis, P.,Wu, X.,Zhang, X.D. Hou and B.T. Jones, *Applied Spectroscopy Reviews*. 2006, 41, 35-75.

2. E.L. Sainio, R. Jolanki, E. Hakala, and L. Kanerva, *Contact Dermatology*. 2000, 42, 5–10.

3. Y. Bonfil, and E. Kirowa, *Analytica Chimica Acta.* 2002, 457, 285–296.

4. C.A. Rusinek, A. Bange, I. Papautsky, and W.R. Heineman, *Analytical Chemistry* 2015, 87, 6133−6140.

5. N. Meepun, S. Siriket, and S. Dejmanee, *International Journal of Electrochemical Science.* 2012, 7, 10582-10591.

6. R.M. Jugade, and A.P. Joshi, *International Journal of Research in Chemistry and Environment.* 2012, 2(4), 246-250.

7. C.D. Palmer, M.E. Lewis, C.M. Geraghty, F. Barbosa, and P.J. Parsons, *Spectrochimica Acta Part B. Atomic Spectroscopy.* 2006, 61, 980–990.

8. I. Boevski, and N. Daskalova, *Spectrochimica Acta Part B Atomic Spectroscopy.* 2000, 55, 16-43.

9. N. Lavi, and Z.B. Afassi, *Analyst.* 1990, 115, 817–822.

10. P.R. Aranda, R.A. Gil, S. Moyano, I. De Vito, and L.D. Martinez, *Talanta.* 2008, 77, 663-666

11. M. Soylak, and Y.E. Unsal, *Toxicological & Environmental Chemistry*. 2012, *94*(8), 1480-1489.

12. H. Ebrahimzade, E. Moazzen, M. Mostafa, and O. Sadeghi, *International Journal of Cosmetic Science.* 2013, 35, 176–182

13. V.A. Lemos, C.G. Novaes, A.S. Lima, and D.R. Vieira, *Journal of Hazardous Materials.* 2008, 155, 128-134.

14. L. Narin, M. Soylak, and L. Elci, *Talanta.* 2000, 52, 1041–1046.

15. R.P. Monasterio, and R.G. Wuilloud, *Talanta.* 2009, 79, 1484-1488.

16. W. Xiaodong, Y. Qiuling, Y. Zhidong, and D. Qingwen, *Microchemical Journal.* 2011, 97, 249–254.

17. J.L. Manzoori, H. Abdolmohammad-Zadeh, and M. Amjadi, *Talanta.* 2007, 71 582-587.

18. A. Abbas, M. Tayyebeh, and S. Hajar, *Journal of Hazardous Materials.* 2006, 138, 269–272.

19. F. Xie, X. Lin, X. Wu, and Z. Xie, *Talanta.* 2008, 74, 836–843.

20. X. Guoqiang, H. Yan and L. Yifan, *Microchimica Acta.* 2009, 165, 237–242

21. D. Shayessteh, and A.M.H. Shabani, *Analytica Chimica Acta.* 2010, 658, 107–119.

22. M. Konecna, J. Komarek, and L. Trnkova, *Spectrochimica Acta Part B*. *Atomic Spectroscopy*. 2008, 63, 700-703.

23. K. Pytlakowska, V. Kozik, and M. Dabioch, *Talanta.* 2013, 110, 202–228.

24. C.M.A. Iwegbue, *Regulatory Toxicology and Pharmacology*. 2015, 72 630–638.

25. S.A. Tirmizi, F.H. Wattoo, M.H.S. Wattoo, S. Sarwar, A.N. Memon, and A.B. Ghangro, *Arabian Journal of Chemistry*. 2012, 5, 309–314.

26. P. Goswami, S. Baruah, and D.K. Das, *Indian Journal of Chemistry*. 2010, 49A, 1617-1620.

27. C. Foti, G. lando, F.J. Millero, and S. Sammartano, *Environmental Chemistry.* 2011, 8(3), 320-323.

28. S.A. Rahim, S. Hussain, and M. Farooqui, *Chemical Science Transactions*. 2015, 4(1), 176-180.

29. B.E. Fischer, U.K. Haring, R. Tribolet, and H. Sigel, *European Journal of Biochemistry*. 1979, 94, 523-530.

30. A.C.S. Costa, L. Lopesa, M. das Graças A. Korna, and J.G. Portela, *Journal of Brazilian Chemical Society (Brazil). 2002,* 13(5), 674-678.

31. L. A. de Melo Gomes, P. de Magalhães Padilhab, J.C. Moreira, N.L. Dias Filhoc, and Y. Gushikem, *J. Braz. Chem. Soc. (Brazil),* 1998, 9(5), 494-498.

32. M.G. Pereira, and M.A.Z. Arruda, *J. Braz. Chem. Soc. (Brazil),* 2003, 14(1), 39-47.

33. G.G. Bortoleto, G.T. Macarovscha, and S. Cadore, *Journal of Brazilian Chemical Society (Brazil)*. 2004, 15(2), 313-319.

34. O.M. Kalfa, O. Yalcinkaya, and A.R. Turker, *Journal of Hazardous Materials*. 2009, 166, 455–461.

35. V.N. Alves, R. Mosquetta, N.M.M. Coelho, J.N. Bianchin, K.C.D.P. Roux, E. Martendal, and E. Carasek, *Talanta*. 2011, 80, 1133–1138.

36. R.M. Luciano, G.C. Bedendo, J.S. Carletto, and E. Carasek, *Journal of Hazardous Materials*. 2010, 177, 567–572.

37. J.J. Ma, X. Du, J.W. Zhang, J.C. Li, and L.Z. Wang, *Talanta*. 2009, 80, 980–984.

38. J.W. Zhang, Y.K. Wang, X. Du, X. Lei, J.J. Ma, and J.C. Li, *Journal of Brazilian Chemical Society (Brazil)*. 2011, 22(3), 446–453.

39. M. Mohamadi, and A. Mostafavi, *Journal of AOAC International*. 2011, 94(3), 959–967.

40. M. Chamsaz, A. Atarodi, M. Eftekhari, and S. Asadpour, M. Adibi, *Journal of Advanced Research*. 2013, 4(1), 35–41.

41. R. Gürkan, and N. Altunay, *Polish Journal of Food Nutrition and Science*. 2013, 63(4), 253-260.

42. L.M.Coelho, and M.A.Z. Arruda, *Spetrochimica Acta Part B Atomic Spectroscopy*. 2005, 60, 743–748.

43. A. Afkhami, T. Madrakian, and H. Siampour, *Journal of Hazardous Materials.* 2006, 138, 269–272.

44. P. Liang, J. Li, and X. Yang, *Microchimica Acta*. 2005, 152, 47–51.

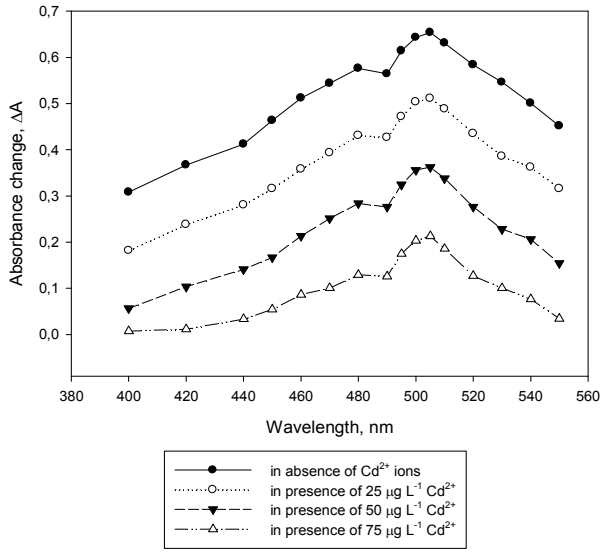


Figure 1 The changes in absorption of the ternary complex formed in presence of 25, 50 and 75 μg L^{-1} Cd(II) as a function of absorption wavelength, nm. Other experimental conditions are described under procedures.

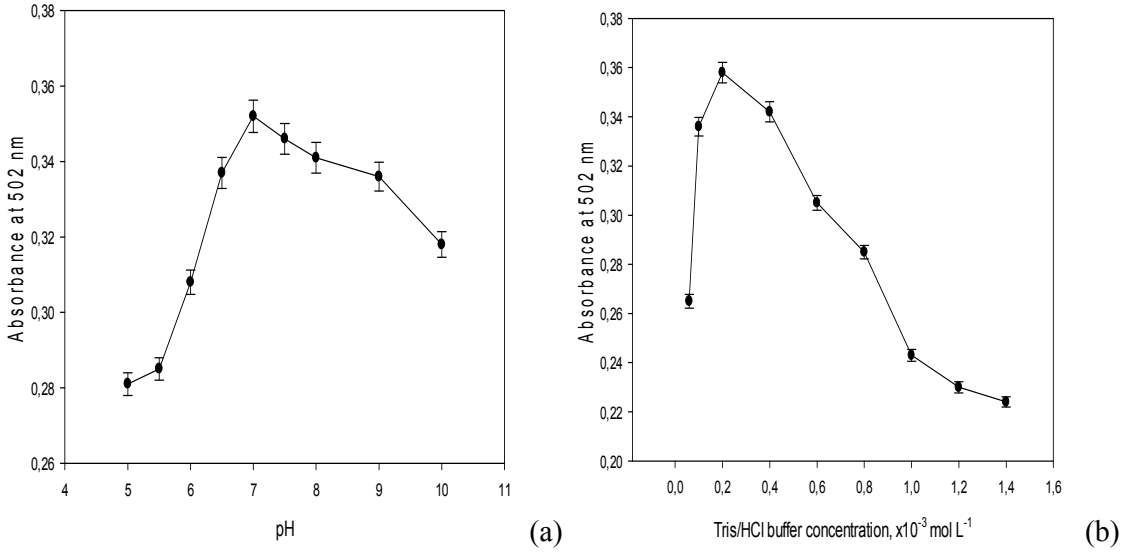


Figure 2 Effect of (a) pH and (b) buffer concentration on CPE efficiency of 50 μg L^{-1} Cd(II). Other experimental conditions are described under procedures.

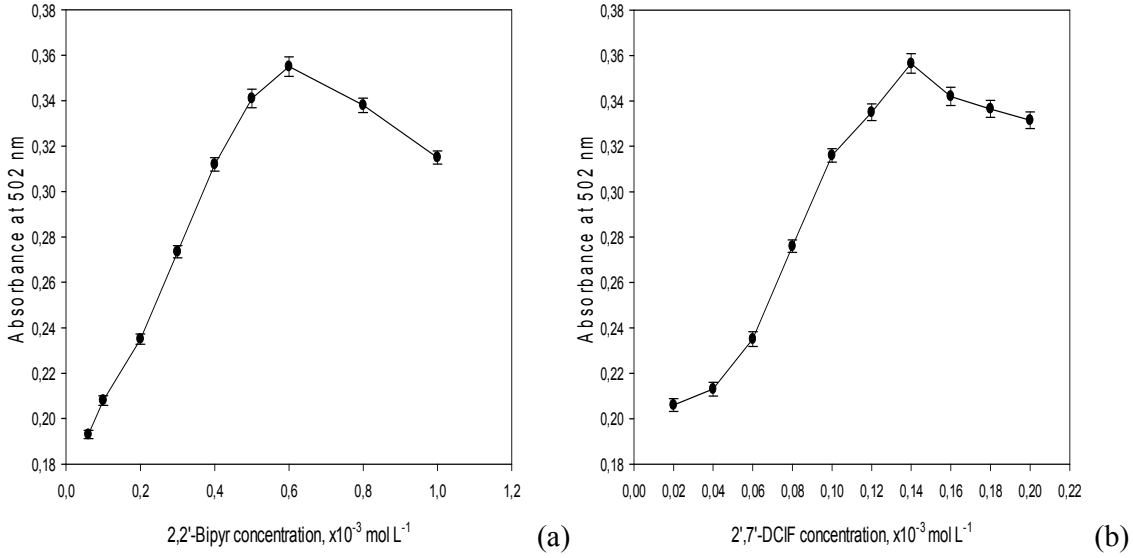


Figure 3 Effect of concentrations of (a) 2,2′-Bipyr and (b) 2′,7′-DClF on CPE efficiency of 50 μg L^{-1} Cd(II). Other experimental conditions are described under procedures.

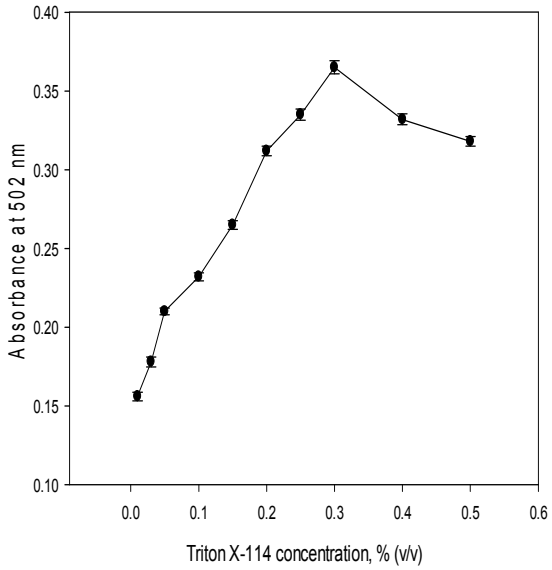


Figure 4 Effect of Triton X-114 concentration on CPE efficiency of 50 μg L^{-1} Cd(II). Other experimental conditions are described under procedures.

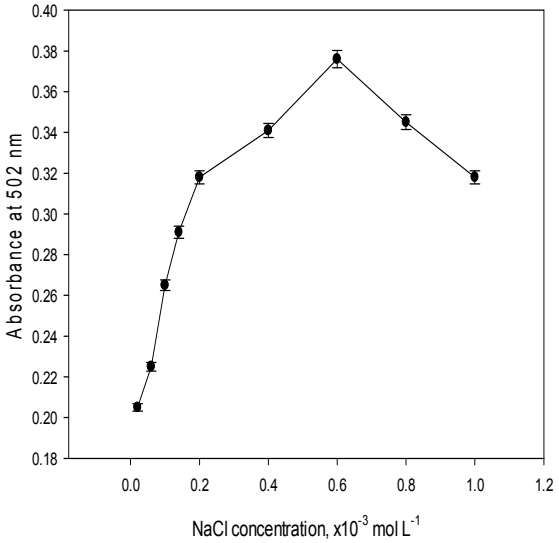


Figure 5 Effect of NaCl concentration as salting out agent on CPE efficiency of 50 µg L^{-1} Cd(II). Other experimental conditions are described under procedures.

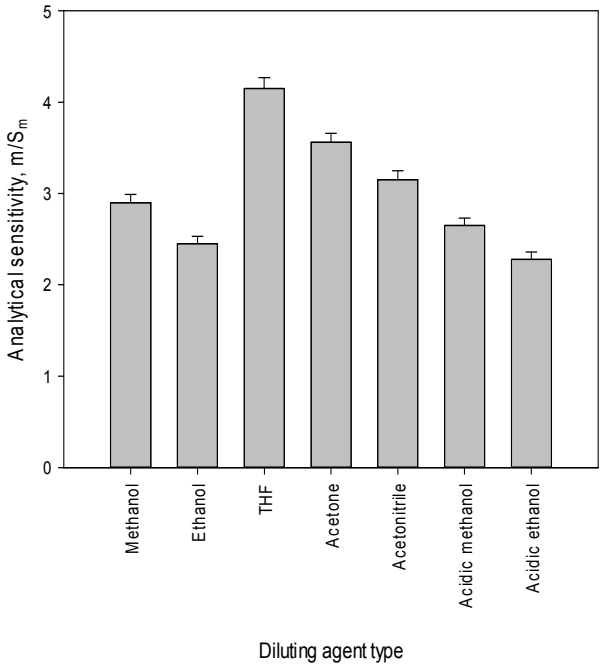
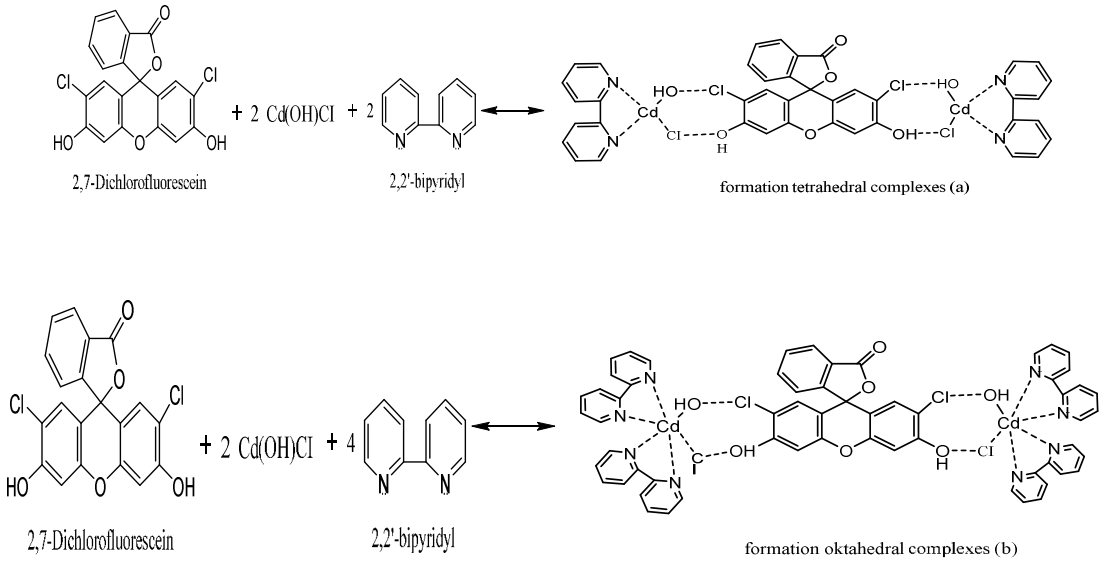


Figure 6 Selection of diluent agent type giving the best analytical sensitivity for calibration curves obtained from three replicate measurements of 15, 30 and 60 µg L^{-1} of Cd(II) for surfactant-rich phase. Other experimental conditions are described under procedures.



Scheme 1 The possible ternary Cd(II)-DClF-Bipyr complex formation mechanism in aqueous alcoholic and aqueous micellar media with (a) tetrahedral and (b) octahedral geometries for the methods before and after preconcentration with CPE at 485 and 502 nm under the optimized reagent conditions, respectively

Table 1 The Analytical figures of merit for the proposed CPE/spectrophotometric method

Parameter	With DClF	
	With preconcentration at 502 nm	Without preconcentration at 485 nm
Linear working range	8–120 μg L^{-1}	100–1500 μg L^{-1}
Slope	$-(5.03\pm0.20)\times10^{-3}$	$-(1.60\pm0.12)\times10^{-4}$
Intercept	0.6172±0.0042	0.5782±0.0036
Correlation coefficient (r)	-0.9932	-0.9998
Recovery % (n: 5)	97.2-103 (15, 30 and 60 μg L^{-1})	98.6-103 (150, 500 and 1000 μg L^{-1})
Precision, RSD (%) (n: 5)	2.5-5.1 (15, 30 and 60 μg L^{-1})	3.7- 2.6 (150, 500 and 1000 μg L^{-1})
Detection limit (LOD) (μg L^{-1}, n: 12)	2.5	28.5
Quantification limit LOQ (μg L^{-1}, n: 12)	8.3	95.0
[a]Preconcentration factor, PF	50	-
[b]Sensitivity enhancement factor, EF	31.4	-

[a]Preconcentration factor is defined as the ratio of the initial bulk solution volume to the volume of surfactant rich phase

[b]Sensitivity enhancement factor is calculated as the ratio of slopes of calibration curves obtained with and without preconcentration

Table 2 Tolerance limits of interfering species in the determination of 50 μg L^{-1} of Cd(II)

Interference species	Tolerance limit, [Interferent] / [Cd(II)]	Recovery %
HPO_4^{2-}, NO_3^-, F^- and SO_4^{2-}	>1500	98.2-102.5
Mg^{2+}, $K^+$and Na^+	1000-1500	97.7-98.9
tartrate and citrate	500-1000	98.5-102.1
Sr^{2+}, NO_3^- and Al^{3+}	300-500	97.9-99.4
CN^-, I^- and Bi^{3+}	350-750	97.3-101.6
NO_2^-, SCN^- and Cr^{3+}	200-350	98.3-102.9
As^{3+}, Hg_2^{2+} and Sn^{4+}	100-200	95.5-97.3
Pb^{2+}, Fe^{2+} and Mn^{2+}	50-100	96.7-100.9
[a]Ni^{2+}, [a]Fe^{3+} and [a]Co^{2+}	20-35 ([a]250-500)	96.8-99.3
[b]Ag^+, [b]Hg^{2+}, [b]Cu^{2+}	5-15 ([b]100)	95.1-96.7

[a]Tolerance limits in the presence of 0.2 mL of 0.05 mol L^{-1} $Na_4P_2O_7$ and 0.2 mL 0.01 mol L^{-1} citric acid as masking agents

[b]Tolerance limits in the presence of 0.2 mL of 0.05 mol L^{-1} $Na_2S_2O_3$

Table 3 Determination of Cd(II) contents of some cosmetic samples including SRMs, and recovery studies of spiked samples

Samples	Sample amounts, mL or g	After wet digestion with $HNO_3/HClO_4/H_2O_2$ (2:2:1, v/v)								Certified value, mg kg^{-1} (n: 5)	The calculated t- and F-values
		By direct calibration curves (n: 5)				By the standard addition calibration curves (n: 5)					
		Added, µg L^{-1}	Found, µg L^{-1} or µg kg^{-1}	RSD %	Recovery %	Added, µg L^{-1}	Found, µg L^{-1} or µg kg^{-1}	RSD %	Recovery %		
[a] Shampoo samples											
Shampoo-1	5	-	36.3±1.4	3.9	-	-	36.2±1.4	3.7	-	-	0.1, 1.1
		10	45.8±1.5	3.3	95.0	10	46.0±1.5	3.3	98.0	-	-
Shampoo-2	5	-	35.1±1.3	3.7	-	-	34.7±1.3	3.1	-	-	0.4, 1.0
		10	46.7±1.5	3.1	98.0	10	44.6±1.4	3.2	98.5	-	-
Shampoo-3	5	-	72.9±2.4	3.3	-	-	70.9±2.3	3.1	-	-	1.4, 1.1
		30	102.2±3.2	3.1	97.7	30	100.1±3.0	3.0	97.3	-	-
[a] Nail polish samples											
Nail polish-1(red)	2	-	81.3±2.4	2.9	-	-	78.7±2.3	2.9	-	-	1.7, 1.0
		30	110.3±3.2	2.9	96.7	30	108.2±3.0	2.8	98.3	-	-
Nail polish-2 (White)	2	-	37.1±1.4	3.7	-	-	35.2±1.3	3.7	-	-	2.3, 1.1
		10	46.9±1.6	3.3	98.5	10	44.9±1.5	3.3	97.5	-	-
Nail polish-3 (black)	2	-	48.9±1.6	3.2	-	-	48.1±1.5	3.1	-	-	0.8, 1.0
		10	59.1±1.6	2.7	102	10	57.9±1.6	2.6	98.2	-	-
[a]Lipstick samples											
Lipstick-1 (red)	2	-	35.0±1.3	3.7	-	-	33.6±1.3	3.6	-	-	1.7, 1.1
		15	50.1±1.4	2.8	101	15	48.2±1.4	2.8	99.0	-	-

Lipstick-2 (brown)	2	-	44.7±1.4	3.0	-	-	44.3±1.3	2.9	-	-	0.5, 1.1
		15	60.7±1.5	2.5	100	15	59.1±1.5	2.4	98.7	-	-
Lipstick-3 (pink)	2	-	73.4±2.1	2.9	-	-	74.1±2.1	2.8	-	-	0.5, 1.0
		30	102.5±2.8	2.7	97.0	30	103.4±3.0	2.9	97.7	-	-
[b]The selected CRM sample (n: 5)											
NIST CRM 1548a Typical Diet	0.5	Added, mg L^{-1}	0.034±0.0018	5.3	97.1	Added, mg L^{-1}	0.034±0.0016	4.7	97.1	0.035±0.0015	1.24, 1.44; 1.40, 1.14
	-	0.05	0.086±0.003	3.6	96.0	0.05	0.082±0.003	3.7	96.0	-	-

[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 4 are 3.18 and 8.53, respectively.

Table 4 Comparison of the CPE/spectrophotometric method with other methods in literature

Sample matrix	Preconcentration method	Detection tool	Linear range, μg L^{-1}	Detection limit, μg L^{-1}	EF[a] or PF[b]	Precision (as RSD %)	References
Saline waters	Solid-liquid extraction	ICP-OES	-	5	10.3 -, 13	1.53 % (250 μg L^{-1}, n: 10)	30
Fuel ethanol	Adsorptive preconcentration on modified silica gel	FAAS	-	-	-, 20	-	31
Mineral water samples	Adsorptive preconcentration	FAAS/GFAAS	-	80, 0.17	-, 100	-	32
Cosmetic samples	SPE	FAAS	-	0.5	-, 30	1.1 % (n: 10)	33
Tap water and tea samples	SPE	GFAAS	216-3000	1.44	50, -	≤3.0 %	34
Alcohol fuel	On line preconcentration/biosorbent	FAAS	5-150	5.50	-	2.3 % (35 μg L^{-1}, n: 9)	35
Water samples	HFLME	FAAS	5-30	1.5	107, -	4.0 % (15 μg L^{-1}, n: 7)	36
Water samples	USAEME	FAAS	10-600	0.91	13.4, 95(15.3)	1.62–2.56 % (50, 500 μg L-1, n: 10)	37
Tap water, river water and sea water	USAE-SFODME	FAAS	10–450	0.66	15, 81(14.6)	2.42-3.34 % (20, 300 μg L-1, n: 10)	38
Tap water, wastewater, well water, and milk samples	DLLM	FAAS	4-200	1.16	48.1, -	1.8% (60 μg L-1, n: 7)	39
Tap water, apple and rice samples	VALLME	FAAS	10-250	2.9	35, -	4.1% (125 μg L^{-1}, **n: 5)**	40
Nonalcoholic beverages	CPE	Spectrophotometry, 608 and 638 nm	1-30, 10-500	0.34, 3.80	36.2, 35	2.85 (25 mg L^{-1}, n: 5)	41

Physiological solutions, mineral water, lake water and cigarette samples	CPE	FAAS	3-400	0.9	-	4.0 (100 µg L^{-1}, n: 15)	42
Water samples	CPE	FAAS	3-300	1.0	14.7, 55.6	0.8-3.0 % (15-200 µg L^{-1}, n: 3)	43
Tap water and lake water	CPE	FAAS	6-100	0.64	23, -	2.1 % (n: 10)	44
Cosmetic products	CPE	Spectrophotometry, 502 nm	8-120	2.5	31.4, 50	2.5-5.1 % (15, 30 and 60 µg L^{-1}, n: 5)	The present method

[a] EF as the enhancement factor is the slope ratio of calibration graph after and before extraction

[b] PF as the preconcentration or enrichment factor is the ratio of the cadmium concentration in the surfactant-rich phase to that in the bulk phase initially