Determination of Cadmium and Lead in Human Teeth Samples by Cloud Point Extraction Preconcentration and Inductively Coupled Plasma Optical Emission Spectrometry

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INTRODUCTION

Environmental contamination from heavy metals has been a matter of great concern in many countries for several decades. Human exposure to such elements may cause adverse health effects. Cadmium (Cd) and lead (Pb) have no known physiological functions and are toxic even at low concentrations, leading to neurological defects, renal degradation, bone lesions, and hypertension (1). The best bioindicator for tracing Cd and Pb is teeth because these elements accumulate mainly in calcified tissues such as teeth and bones. Moreover, teeth are used as important indicators of environmental toxicology and are excellent vehicles for monitoring pollution in both recent and ancient times (2). Teeth are also a readily accessible biological tissue for analysis (3, 4).

Although inductively coupled plasma optical emission spectrometry (ICP-OES) is a powerful analytical tool for the determination of trace elements, the determination of heavy metals in calcium-rich materials such as teeth and bones is not an easy task due to low levels present and strong matrix interferences (5). In ICP-OES, interferences caused by calcium and other alkali earth metals were observed to be

ABSTRACT

Cloud point extraction was applied as a preconcentration step prior to the determination of cadmium and lead in human teeth samples by inductively coupled plasma optical emission spectrometry (ICP-OES). After complexation with 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP), the analytes were quantitatively extracted to and concentrated in the phase rich in the surfactant Triton X-114, then measured by ICP-OES. The parameters affecting the extraction efficiency, such as the pH of the solution, concentration of PMBP and Triton X-114, equilibration temperature and time, were investigated.

Using optimum conditions, the detection limits were 0.83 and 1.48 ng mL⁻¹ for cadmium and lead with relative standard deviations of 2.7% and 3.8%, respectively (n=11). Verification of the accuracy of the method was carried out by analysis of a standard reference material (NIST 1486 Bone Meal). The method was successfully applied to the determination of trace amounts of cadmium and lead in human teeth samples with satisfactory results.

considerable and often led to over- or under-estimation of analyte concentrations. To overcome or reduce matrix effects, different calibration methods such as external calibration, matrix-matched calibration standards, standard addition, and

internal standards have been proposed. Matrix-matched calibration is often not possible due to unknown or changing sample composition as is the case for some minerals. The use of an internal standard should be specific for many analytes due to the dependence of matrix effect magnitude on spectral line properties (6, 7). Therefore, an initial sample pretreatment for matrix separation and analyte preconcentration is often necessary.

Separation and preconcentration based on cloud point extraction (CPE) is becoming an important and practical application of surfactants in analytical chemistry (8, 9). The technique is based on the ability of most non-ionic surfactants in aqueous solutions to form micelles and to separate into a surfactant-rich phase of small volume and a diluted aqueous phase when heated to a temperature known as the cloud point temperature. The small volume of the surfactant-rich phase obtained with this methodology permits the design of extraction schemes that are simple, inexpensive, highly efficient, speedy, and of lower toxicity to the environment than those extractions that use organic solvents. The cloud point methodology has been used to separate and preconcentrate organic compounds prior to their determination in hydrodynamic analytical systems such as liquid chromatography (10) and capillary electrophoresis (11). The phase separation phenomenon has also been used for the extraction and preconcen-

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tration of metal ions after the formation of slightly water-soluble complexes (12, 13). CPE as a pre-concentration step in conjunction with spectrophotometry, flow injection analysis (FIA) spectrofluorimetry, flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), ICP-OES, and high performance liquid chromatography (HPLC) for the determination of various metal ions has been widely studied (14-20).

The aim of this work was to combine CPE with ICP-OES and develop a new method for the determination of trace amounts of Cd and Pb in human teeth samples. In this method, 1-phenyl-3-methyl-4-benzoyl-5-pyrazolone (PMBP), which forms complexes with more than 40 metal ions and has found numerous applications in trace element separation and preconcentration by solvent extraction (21, 22) and solid phase extraction (23, 24), was selected as the chelating reagent. The main factors affecting cloud point extraction of Cd and Pb were evaluated and optimized. The method was successfully applied to the determination of trace amounts of Cd and Pb in human teeth samples.

EXPERIMENTAL

Instrumentation

An Optima™ 2000 DV inductively coupled plasma optical emission spectrometer (PerkinElmer, Inc., Shelton, CT, USA) was used for all determinations. A cyclonic spray chamber and a Gem-Cone™ nebulizer were used for this work. The operating conditions and wavelengths of the emission lines are summarized in Table I. The pH values were measured with a Mettler Toledo 320-S pH meter (Mettler Toledo Instruments Co., Ltd., Shanghai, P.R. China). A Model 0412-1 centrifuge (Shanghai Surgical Instrument Factory, Shanghai,

P.R. China) was used to accelerate the phase separation.

Standard Solutions and Reagents

Stock standard solutions (1000 µg mL⁻¹) of Cd and Pb were obtained from the National Institute of Standards (Beijing, P.R. China). Working standard solutions were obtained by appropriate dilution of the stock standard solutions. The non-ionic surfactant Triton® X-114 was obtained from Sigma-Aldrich (St. Louis, MO, USA) and was used without further purification. A 1.0×10⁻² mol L⁻¹ solution of PMBP was prepared by dissolving appropriate amounts of this reagent in absolute ethanol from the commercially available product (Shanghai ZhenXing First Chemical Factory, Shanghai, P.R. China).

The following buffers were used to control the pH of the solutions: sodium acetate-acetic acid (pH 3-6), ammonium acetate-ammonia (pH 6-8), and ammonium chloride-ammonia (pH 8-10). All other reagents were of analytical reagent grade or better. Doubly distilled water was used throughout the study. The pipettes and vessels used for trace analysis were kept in 10% nitric acid for at least 24 hours and subsequently washed four times with doubly distilled water.

Procedures

For CPE, a 10-mL solution containing the analytes, Triton X-114, and PMBP buffered at a suitable pH were kept in a thermostatic bath at 40 °C for 15 minutes; the surfactant-rich phase settled through the aqueous phase. Phase separation was accelerated by centrifugation at 3000 rpm for 5 minutes. After cooling in an ice-bath, the surfactant-rich phase became viscous and was retained at the bottom of the tube. The aqueous phases can readily be discarded simply by inverting the tubes. In order to decrease the viscosity and to facilitate sample handling prior to the ICP-OES assay, 0.5 mL 0.1 mol L⁻¹ HNO₃ was added to the surfactant-rich phase. The final solution was introduced into the nebulizer of the spectrometer by conventional aspiration. Calibration was performed against aqueous standards and submitted to the same CPE procedure. A blank (doubly distilled water without Cd and Pb) was submitted to the same procedure as described above and measured parallel to the samples and calibration solutions.

Preparation of Samples

Portions (1.0000 g) of certified reference material (CRM) NIST 1486 Bone Meal (National Institute of Standards and Technology, Beijing, P.R. China) were transferred into PTFE beakers, and 10 mL of concentrated HNO₃ and 2 mL of

TABLE I
ICP-OES Operating Conditions and
Wavelengths of Emission Lines Examined

Parameters	
RF power	1.1 kW
Plasma gas (Ar) flow rate	15 L min ⁻¹
Auxiliary gas (Ar) flow rate	1.0 L min ⁻¹
Nebulizer gas (Ar) flow rate	0.5 L min ⁻¹
Pump flow rate	0.8 mL min ⁻¹
Observation height	15 mm
Wavelength	Cd 228.802 nm; Pb 220.353 nm

H₂O₂ were added. The solutions were then continuously heated to near dryness. The residue was dissolved in 0.1 mol L⁻¹ HCl and made up to 50-mL volume with water.

Noncarious and intact human permanent teeth samples were obtained from patients at the School of Stomatology, Wuhan University, Wuhan, P.R. China. Informed consent was obtained from the patients. This study was approved by the Ethics Committee of School of Stomatology, Wuhan University. After extraction, the teeth were cleaned with a polyethylene scraper and rinsed with a saline solution. The teeth were ultrasonically rinsed with doubly distilled water for 1 hour, dried at 110 °C for 24 hours, and ground to 100 mesh with an agate mortar. Then the digestion procedure mentioned above was applied to the samples.

RESULTS AND DISCUSSION

Effect of pH on CPE

Formation of the metal-chelate and its chemical stability are two important influences for CPE. The pH plays a unique role on metal-chelate formation and subsequent

extraction and has proven to be the main parameter for CPE. Cloud point extraction of Cd and Pb was performed in different pH buffer solutions; the effect of pH on the extraction recovery of Cd and Pb is shown in Figure 1. The extraction recovery was calculated based on the amount of analytes in the starting samples and in the surfactant-rich solution after extraction. It can be seen that extraction was quantitative for Cd when the pH exceeded 5.0. For Pb, the quantitative extraction was achieved in the pH range of 4.0~7.0. Hence, a pH of 6.0 was chosen as the compromise condition for subsequent work.

Effect of PMBP Concentration

A 10-mL solution containing 0.5 µg of Cd and Pb in 1.0 g L⁻¹ Triton® X-114 and at a medium buffer of pH 6.0 containing various amounts of PMBP was subjected to the cloud point extraction procedure. The extraction recovery as a function of the concentration of the PMBP is shown in Figure 2. As can be seen, the extraction recovery for Cd and Pb increased up to a PMBP concentration of 2.5×10⁻⁴ mol L⁻¹ and reaches near quantitative extraction. Thus, a PMBP concentration

of 5.0×10⁻⁴ mol L⁻¹ was chosen to account for other extractable species that might potentially interfere with the assaying of Cd and Pb.

Effect of Triton X-114 Concentration

A successful CPE maximizes the enrichment factor through minimizing the phase volume ratio. The variation in extraction efficiency of Cd and Pb within the Triton X-114 concentration range of 0.1–2.0 g L⁻¹ was examined and the results are shown in Figure 3. It can be seen that quantitative extraction was observed when the Triton X-114 concentration was above 0.8 g L⁻¹. Therefore, a concentration of 1.0 g L⁻¹ was chosen as the optimum surfactant concentration in order to achieve the highest possible enrichment factor.

Effects of Equilibration Temperature and Time

It was desirable to employ the shortest equilibration time and the lowest possible equilibration temperature as a compromise between completion of extraction and efficient separation of the phases. The dependence of extraction efficiency upon equilibration temperature and

Fig. 1. Effect of pH on the CPE extraction recoveries of Cd and Pb (0.5 µg mL⁻¹ Cd and Pb, 5.0×10⁻⁴ M PMBP, 1.0 g L⁻¹ Triton X-114).

Fig. 2. Effect of PMBP concentration on the CPE extraction recoveries of Cd and Pb (0.5 µg mL⁻¹ Cd and Pb, 1.0 g L⁻¹ Triton X-114, pH 6.0).

time was studied ranging from 25 to 60 °C and 5 to 30 minutes, respectively. The results showed that an equilibration temperature of 40 °C and a time of 15 minutes were adequate to achieve quantitative extraction.

Interferences

The potential interferences in the present method were investigated. The interferences were due to the competition of other heavy metal ions for the chelating agent and their subsequent co-extraction with Cd and Pb. In these experiments, solutions containing 0.1 µg mL⁻¹ of Cd and Pb and the interfering ions were treated according to the recommended procedure. The tolerance limits of the coexisting ions, defined as the largest amount making the recovery

of Cd and Pb less than 90%, are listed in Table II. Large amounts of alkaline and alkaline earth metal ions showed no interferences with the CPE extraction of Cd and Pb under the selected conditions because of their very low stability constants of the PMBP complexes.

Detection Limits and Precision

According to the definition of IUPAC, the detection limits (3σ) of this method were 0.83 and 1.48 ng mL⁻¹ for Cd and Pb with relative standard deviations (RSD) of 2.7% and 3.8%, respectively (n=11).

Analytical Application

To verify the accuracy of the proposed method, the concentrations of Cd and Pb in certified reference material NIST 1486 Bone Meal

were determined. As can be seen in Table III, the obtained results are in good agreement with the reference values.

The proposed method was applied to the determination of Cd and Pb in human teeth samples collected in Wuhan, P.R. China. In addition, the recovery experiments of different amounts of Cd and Pb were carried out; the results are shown in Table IV. Fairly good recoveries for the analytes were obtained ranging from 95–102%, which are reasonable for trace analysis.

CONCLUSION

A new method of cloud point extraction (CPE) combined with inductively coupled plasma optical emission spectrometry (ICP-OES) has been proposed for the determination of Cd and Pb in human teeth samples. The method is simple, reproducible, and highly sensitive

Fig. 3. Effect of Triton X-114 concentration on the CPE extraction recoveries of Cd and Pb (0.5 µg mL⁻¹ Cd and Pb, 5.0×10^{-4} M PMBP, pH 6.0).

TABLE II
Tolerance Limits of Coexisting Ions

Coexisting Ions	Tolerance Limits (mg L ⁻¹)
K ⁺ , Na ⁺ , Ca ²⁺ , Mg ²⁺	5000
Cu ²⁺ , Co ²⁺ , Zn ²⁺ , Ni ²⁺ , Mn ²⁺	50
Al ³⁺ , Cr ³⁺ , Fe ³⁺	10
SO ₄ ²⁻ , NO ₃ ⁻ , Cl ⁻	5000

TABLE III
Analytical Results of CRM NIST 1486 Bone Meal (µg g⁻¹)

Element	Found ^a	Certified Value ^b
Cd	0.008±0.001	(0.003)
Pb	1.385±0.012	1.335±0.014

^a The value following "±" was the standard deviations. (n=3).

^b The value in the parentheses is the non-certified concentration.

TABLE IV
Determination of Cd and Pb in Human Teeth Sample (ng g⁻¹)

Element	Added	Found	Recovery (%)
Cd	0	0.096±0.008	
	0.2	0.292±0.016	98
	0.5	0.605±0.029	102
Pb	0	3.74±0.18	
	5.0	8.70±0.42	99
	10	13.20±0.75	95

because of the distinct and advantageous features of CPE (*in situ* and single-step extraction). The surfactant-rich phase can be introduced into the nebulizer of the ICP-OES by conventional aspiration after dissolving with 0.1 mol L⁻¹ HNO₃. The proposed method gives very low LODs (0.83 and 1.48 ng mL⁻¹ for cadmium and lead), good relative standard deviations (2.7% and 3.8%), respectively (n=11), and can be applied to the determination of heavy trace metals in calcium-rich samples.

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