



Cloud Point Extraction and Flame Atomic Absorption Spectrometric Determination of Lead, Cadmium and Palladium in Some Food and Biological Samples

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Abstract

The proposed method is based on the complexation of the Pb²⁺, Cd²⁺ and Pd²⁺ ions with 3-(1-(1-H-Indol-3-Yl)-3-phenylallyl)-1H-indole (IPAI) at pH 8.0 in the presence of Triton X-114. The phase separation occurred when micellar solution was heated at 55 °C. The surfactant-rich phase, diluted to 0.5 mL via 1.0 mol L⁻¹ nitric acid in methanol was directly introduced into the nebulizer of the flame atomic absorption spectrometry (FAAS). Influence of variables such as pH, amount of ligand and Triton X-114, heating time and temperature were evaluated and optimized. The optimized enhancement factors for Pb²⁺, Cd²⁺ and Pd²⁺ ions were 22, 33 and 23, respectively and the detection limit (DLs) was between of 1.6–2.6 µg L⁻¹. The relative standard deviation (RSD) of each ion was found to be less than 4.6% at 100 µg L⁻¹. In addition, the calibration graphs were linear in the range of 0.01–0.22 µg mL⁻¹ for Cd²⁺ ion, 0.018–0.26 µg mL⁻¹ for Pb²⁺ ion and 0.02–0.27 µg mL⁻¹ for Pd²⁺ ion with the correlation coefficients in the range of 0.995–0.999.

Keywords: Cloud point extraction; 3-(1-(1-H-Indol-3-Yl)-3-phenylallyl)-1H-indole; Atomic absorption spectrometry; Triton X-114; Trace metal ions.

Introduction

A number of toxic metals arising from industrial and agricultural activities introduced into the environment and can be detected in different ecosystems [1-4]. For monitoring purposes, their content determination is necessary. Due to their low-level and complicated matrices of real samples, it is not possible to directly evaluate them frequently even by sensitive instrumental methods.

Atomic absorption spectrometry (AAS) due to its high sensitivity with comparatively low operational cost has been used for trace metal measurement widely. To cope up with the limitations of complex matrices there is a need to perform a suitable separation or preconcentration procedure (to improve the detection limits or

remove interference of co-existing species) prior to measurement. Among the various analytical applications and separation procedures, cloud point extraction (CPE) based on micellar extraction [5-8] is an efficient procedure for simultaneous enrichment and separation of target species from matrices. CPE is much more environmental friendly, and is safer because small volumes of low-toxicity surfactants are used instead of toxic organic solvents. CPE is effectively applied for the extraction of metal chelates prior to quantification using analytical techniques, such as FAAS inductively coupled plasma-atomic emission spectrometry (ICP-AES) and inductively coupled plasma/mass spectrometry (ICP-MS) [9].

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This technique is based on well-known surfactant phenomenon. Generally, surfactant solution becomes turbid when heated above its cloud point temperature at concentration between 3–20 times of its critical micelles concentration (CMC). Cooling and centrifuging the assembly led to separation and appearance of two new phases. One of these phases (surfactant rich phase) contains surfactant concentration higher, while the other has surfactant concentration lower than CMC (dilute aqueous phase). The phase separation process accelerated by the dehydration of surfactant poly (ethylene oxide) head groups at elevated temperature that led to the agglomeration of the micelles into a viscous phase [10-17]. This phase has high tendency for local enrichment and preconcentration of metal chelates [18-25].

This work, present a clean CPE procedure for the preconcentration and determination of Pb^{2+} , Cd^{2+} and Pd^{2+} ions in the environmental and biological samples. The effect of the experimental parameters on the extraction efficiency was investigated to achieve a high extraction percentage at short equilibration time and ease of phase separation.

Experimental

Instruments

The measurements of metal ions were done by Shimadzu 680 AA atomic absorption spectrometer. A Metrohm 691 pH/Ion meter with a combined glass–calomel electrode was used.

Reagents and materials

All analytical grade reagents (Merck) were used without further purification. Standard solutions of metal nitrates were prepared in deionized water. A 1.0% (w/v) Triton X-114 (Merck) was prepared by dissolving 1.0 g of Triton X-114 in 100 mL distilled water. Approximate cloud point temperature of Triton X-114 in aqueous solution is 24-28°C. The ligand 3-(1-(1H-Indol-3-Yl)-3-phenylallyl)-1H-indole (IPAI) (Fig. 1) was synthesized according to literature [26, 27].

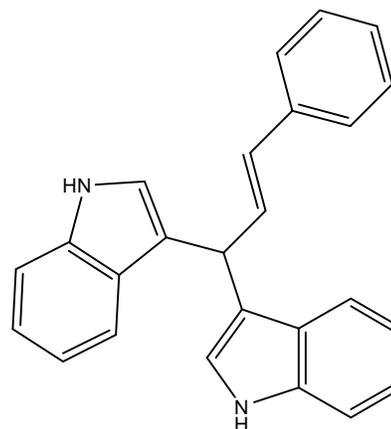


Figure 1. Structure of (E)-3-(1-(1H-Indol-3-Yl)-3-phenylallyl)-1H-indole (IPAI).

Analytical procedure

CPE extraction was conducted as follows: 15 mL of 0.2 $\mu\text{g mL}^{-1}$ of Pb^{2+} , Cd^{2+} and Pd^{2+} ions solution containing 0.13% Triton X-114 and 1.4 mM of IPAI at pH 8.0 were shaken for 1 min and then heated for 25 min at 55°C. The turbid mixture was centrifuged at 4000 rpm for 20 min and cooled in an ice-bath for 15 min until the surfactant rich phase regains its viscosity, the bulk aqueous phase was easily discarded. Finally, the surfactant rich phase was diluted in 0.5 mL of 1.0 M HNO_3 in methanol and the diluted sample was transferred to the FAAS.

Applications to real samples

The investigated real samples such as soil [28], milk [29], detergent [30, 31] and chocolate were treated according to reported procedure [28]. Urine and blood samples were also treated according to reported literature [32].

Results and Discussion

A considerable amount of work on charge transfer properties of indoles is available because several biologically important compounds possess the indole ring system and charge transfer phenomenon implied to explain the mode of action [33].

Due to the presence of nitrogen atom and π -electrons in IPAI structure, it can be classified as ligand with soft binding atom. They chelate the soft borderline metal ions through both charge transfer reaction and covalent soft-soft principle. The effect of pH, amount of Triton X-114, concentration of IPAI, diluents and temperature on the method performance were investigated.

Effect of pH

The effect of pH in the range of 7.0 - 9.8 was investigated (Fig. 2). Maximum sensitivity was achieved at pH 8 and above this value, the sensitivities fall probably due to formation of insoluble hydroxide or charged hydroxo complexes. The subsequent studies were, therefore, made at pH 8.

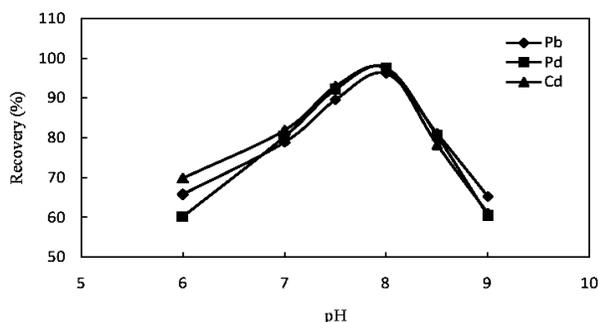


Figure 2. Effect of pH on cloud point extraction of analyte ions (N=3).

Effect of ligand amount

The effects of the quantity of IPAI (bidentate ligand) on CPE efficiency was investigated in the range of 0.03-2.7 mmol L⁻¹ (Fig. 3). The low sensitivity was observed in the absence of bidentate ligand, that significantly improved by its addition and reached the maximum at 1.4 mM of the IPAI. However, the sensitivity decreased with further increasing concentration of ligand (complexing agent) probably due to formation of charged unextractable species. It was found that sensitivity does not change with 5% THF but decreased at higher content may be because of dissolution of the surfactant phase and decrease in the volume of surfactant rich phase. Therefore, 1.4 mM of ligand solution in THF was selected for subsequent work.

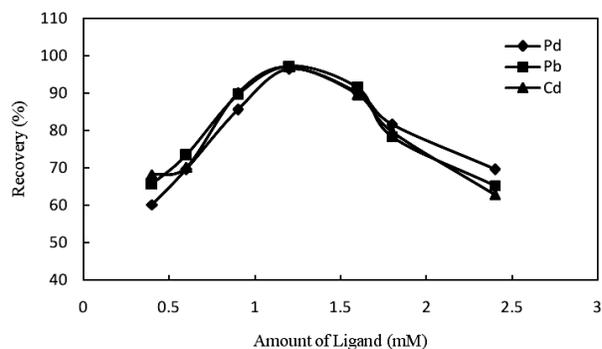


Figure 3. Effect of ligand concentration on the recoveries of analytes (pH=8, N=3).

Amount of Triton X-114

The surfactants cloud point temperature is significantly correlated to its hydrophilic property [32, 34-36]. Neither too high nor too low cloud point temperature surfactants are not suitable for the CPE separation/preconcentration procedure. The nonionic surfactant, (especially Triton X-114) with cloud point temperature near room temperature makes it possible to obtain highly sensitive single-step extraction. The phase separation procedure can be accelerated by cooling and centrifuging the extraction system.

In a set of similar runs, the concentration of Triton X-114 was optimized in the range of 0.03-0.26% (w/v) (fixed optimum value of other variables) (Fig. 4).

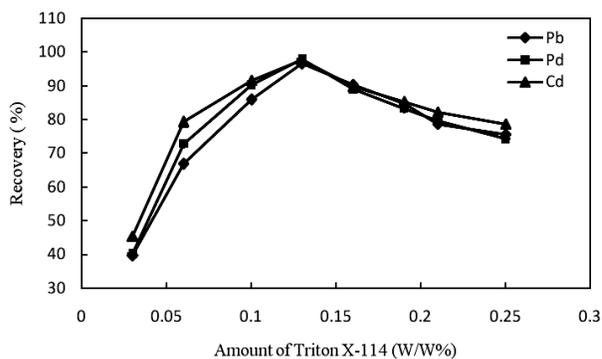


Figure 4. Effect of concentration of Triton X-114 on cloud point extraction of analytes (pH=8, N=3).

It is obvious that at low Triton X-114 concentration, low sensitivity was obtained. At higher concentration of Triton X-114, the 0.5 mL

of nitric acid solution in metal is not suitable for diluting and introducing the surfactant rich phase to nebulizer and because of dilution the recoveries were decreased.

Effect of temperature

In CPE procedure, application of short incubation time and low equilibration temperature is generally required.

The influence of these variables on sensitivity of method was investigated over a time range of 5–30 min and temperature range of 20–60 °C. It was found, that optimum time and temperature necessary for complete phase separation and to obtain high preconcentration factor is 25 min at 55°C. The sensitivities decrease above 55°C due to decrease in CMC of non-ionic surfactants. On the other hand raising temperature led to improve the hydrophobic nature of surfactant by shifting and catalyzing reaction that favors dehydration of its ether oxygen's [10] and increase the micelles formation.

Effect of diluents type and volume

The surfactant-rich phase was very viscous; methanol was added to the surfactant-rich phase after CPE to facilitate its transfer into the nebulizer chamber.

The efficient and reproducible transfer of surfactant rich phase to measurement system improves the method's sensitivity. This requirement simply can be achieved via dissolving this phase in acidic methanol solutions. Various methanolic acid solutions with different concentrations and volumes were applied, the 0.5 mL solution of 1 mol L⁻¹ HNO₃ in methanol was chosen to have an appropriate amount of sample for transferring and measuring the sample absorbance.

Effect of centrifuge time and rate on CPE of metal ions

This stage is useful and applicable in conventional separation / preconcentration procedures used for original aqueous environment [34-40]. The influence of centrifuge time with in

range of (5–25 min) at various rates (1000-4500 rpm) on phase separation procedure and method's sensitivity was examined. It was found that 20 min centrifuge time at 4000 rpm is sufficient for complete quantitative phase separation.

Effect of electrolyte on metal ions CPE

Generally, addition of salting-out agent to the micellar solution accelerates phase-separation process. Therefore, the influence of KCl concentration as salting-out agent for accelerating and catalyzing the phase separation phenomenon was investigated. The KCl concentration was in the range of 0.0–2.0 mol L⁻¹ and maximum sensitivity was achieved at 0.15 mol L⁻¹ KCl. The preconcentration efficiency of the analytes in the absence of electrolyte is low.

Effect of interference

The influences of alkali, alkali earth and some metal ions on the preconcentration and determination of traces of understudy metal ions are known as matrix effects [41-44]. To evaluate the possibility of accurate and efficient preconcentration and determination of Pb²⁺, Cd²⁺ and Pd²⁺ ions via described procedure, similar experiments at fixed value of analytes in the presence of different ions with different mass ratio was conducted. Tolerance limits of investigated electrolytes is known as ion concentration causing a relative error <±5% in the sensitivity of method. It was found (Table 1) that proposed CPE procedure possesses high tolerance limits for most of investigated ions, that shows the applicability of proposed method for evaluation of understudy metal ions in real samples with complicated matrices. Some of cations and common anions were found unaffected on the signal in the CPE–FAAS system even at 1000-fold mass excess.

Analytical features

The enrichment factor (ratio of the slope of the calibration graph with and without preconcentration) was 23, 22 and 37 for Pd²⁺, Pb²⁺ and Cd²⁺ ions respectively, while the preconcentration factor for all understudy metal ions was found 30 at volume ratio of initial solution (15 mL) to the eluting solution (0.5 mL) .

The limit of detection (LOD) based on IUPAC recommendation (three times standard deviation (s) of the blank (n=10)) were found to be 2.6, 2.4 and 1.6 ng mL⁻¹ for Pb²⁺, Pd²⁺ and Cd²⁺ ions respectively, while the quantification limit (LOQ) based on 10 time blank standard derivation (n=10) was 8 µg L⁻¹ for Cd²⁺ ion and 14 µg L⁻¹ for Pd²⁺ and Pb²⁺ ions.

Table 1. Effects of the interferences ions on the recoveries of the examined metal ions (N=3).

Interference	Tolerance limit (fold weight ratio)		
	Pb ²⁺	Cd ²⁺	Pd ²⁺
Na ⁺ , K ⁺ , SCN ⁻ , HCO ₃ ⁻	1000	1000	1000
Al ³⁺	1000	1000	1000
Mn ²⁺ , SO ₄ ²⁻	600	600	600
Ni ²⁺ , Co ²⁺	200	200	200
Zn ²⁺	50	50	50
Fe ³⁺	100	100	100
Cu ²⁺	400	400	400
Ag ⁺	300	300	300
Hg ²⁺	250	250	250
Cl ⁻	250	250	250
Cr ³⁺ , Ba ²⁺ , Mg ²⁺	800	800	800

Accuracy and applications

In order to assess the applicability of proposed method on real samples, analysis of different matrices (containing varying amounts of a variety of diverse ions) was performed. The proposed method was applied for preconcentration and recovery of Pb²⁺, Cd²⁺ and Pd²⁺ ions from different matrices. The results of three analyses of each sample show maximum recoveries of the metal with high sensitivity and low RSD (Table 2-4).

Table 2. Recovery of analyte elements from spiked environmental samples after application of presented procedure.

Sample	Ion	Added, µg g ⁻¹	Found, µg g ⁻¹	RSD %	Recovery %
Cow liver	Pb ²⁺	0	0.537	3.2	103
		0.2	0.743	2.5	
		0.5	0.743	2.5	
	Cd ²⁺	0	0.332	3.4	97.5
		0.2	0.527	3.0	
		0.5	0.527	3.0	
Pd ²⁺	0	0.04	3.8	103	
	0.2	0.246	3.1		
	0.5	0.246	3.1		
Cigarette	Pb ²⁺	0	0.234	3.6	152.5
		0.2	0.539	3.0	

Soil	Cd ²⁺	0	0.650	3.1	96.5
		0.2	0.843	2.6	
	Pd ²⁺	0	0.056	3.7	102
		0.2	0.260	3.2	
	Pb ²⁺	0	0.508	3.0	102
		0.2	0.712	2.4	
	Cd ²⁺	0	0.370	3.0	103
		0.2	0.576	2.3	
	Pd ²⁺	0	0.025	4.0	102
		0.2	0.229	3.2	

Table 3. Recovery of trace elements from spiked samples after application of this procedure.

Sample	Ion	Added, µg g ⁻¹	Found, µg g ⁻¹	RSD %	Recovery %
Chocolate	Pb ²⁺	0	0.267	3.6	104.5
		0.2	0.476	3.1	
	Cd ²⁺	0	0.196	3.6	97
		0.2	0.390	3.1	
	Pd ²⁺	0	0.016	4.2	102
		0.2	0.220	3.6	
Waste water	Pb ²⁺	0	0.369	3.3	102.5
		0.2	0.574	2.4	
	Cd ²⁺	0	0.277	3.2	96.5
		0.2	0.470	2.6	
	Pd ²⁺	0	0.089	3.8	102
		0.2	0.293	3.3	
Milk	Pb ²⁺	0	0.168	3.3	103
		0.2	0.374	3.0	
	Cd ²⁺	0	0.03	3.8	103
		0.2	0.24	3.0	
	Pd ²⁺	0	0.01	4.3	102
		0.2	0.22	3.5	
Detergent	Pb ²⁺	0	0.222	3.2	103.5
		0.2	0.429	2.4	
	Cd ²⁺	0	0.152	3.1	96
		0.2	0.344	2.6	
	Pd ²⁺	0	0.051	3.8	102.5
		0.2	0.256	3.0	

Table 4. Recovery studies of trace metal ions from some biological samples.

Ion	Urine sample				blood			
	Added (µg g ⁻¹)	Found (µg g ⁻¹)	RSD %	Recovery %	Found (µg g ⁻¹)	RSD %	Recovery %	
Pb ³⁺	0	0.03	4.8	---	0.02	4.7	---	
	0.2	0.24	3.8	105.0	0.21	4.3	95.0	
	0.5	0.52	3.3	98.0	0.53	3.9	102.0	
Cd ²⁺	0	0.01	4.7	---	0.01	4.5	---	
	0.2	0.22	3.9	105.0	0.20	4.2	95.0	
	0.5	0.51	3.5	100.0	0.52	4.0	102.0	
Pd ²⁺	0	N.D	---	---	N.D	---	---	
	0.2	0.19	4.6	95.0	0.21	4.6	105.0	
	0.5	0.51	3.8	102.0	0.49	4.2	98.0	

Conclusion

This paper proposes an efficient procedure for the preconcentration and determination of ultra-trace levels of Pb^{2+} , Cd^{2+} and Pd^{2+} ions by combination of CPE with FAAS. Phase separation accelerated by centrifuging the sample solution and a low detection limit with high enhancement factors was achieved. The present method has following advantages over reported methods:

- 1) Our thorough survey of literature did not show any application of 3-(1-(1-H-Indol-3-Yl)-3-phenylallyl)-1H-indole (IPAI) as bidentate ligand in cloud point extraction of trace amount of metal ions such as Pb^{2+} , Cd^{2+} and Pd^{2+} ions.
- 2) The proposed method is a low cost method that offers high efficiency and lower toxicity extraction and/or preconcentration procedure with low consumption of chemical reagents and without using organic solvents.
- 3) At optimum values of all variables, the proposed method benefitted advantages such as low detection limits and good RSD.
- 4) The proposed ligand can be synthesized in every laboratory with a simple, fast and high yield procedure.

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