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Talanta 51 (2000) 123–129

Talanta

www.elsevier.com/locate/talanta

Cloud point extraction of lead in saliva via use of nonionic PONPE 7.5 without added chelating agents

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Received 4 March 1999; received in revised form 13 August 1999; accepted 16 August 1999

Abstract

A new micelle-mediated phase separation of metal ions to preconcentrate trace levels of lead as a prior step to its determination by flame atomic spectroscopy has been developed. The methodology is based on the cloud point extraction of lead with PONPE 7.5 in the absence of chelating agent. The chemical variables affecting the sensitivity of the extractive-spectrometric procedure were evaluated in detail, optimised and successfully applied to the determination of lead in saliva samples. Under the optimal conditions, a $\%E$ higher than 99.9 was achieved. The proposed method showed linear calibration within the range: 0.6–4 μg ml^{-1} Pb(II). The sensitivity was 0.053 μg ml^{-1}. The method has been applied to the determination of lead in human saliva. The nature of the extracting species as well as the location of lead in the micelle were studied. The analytical performance of the proposed method clearly satisfies the typical requirements for control processes.

Keywords: Metal ions; Lead; Saliva

1. Introduction

In the last decade, an increasing interest is shown all over the world in developing surfactant-based methods in all fields of analytical chemistry. Aqueous micellar solutions have been used, among other fields, in spectroscopy, electroanalytical and separation science [1–6].

Aqueous solutions of many non-ionic surfactant micellar systems, become turbid over a narrow temperature range, when the experimental conditions have been changed. This temperature is named 'cloud point temperature'. Above the cloud point, the solution separates into two phases: one, very small in volume, the surfactant-rich phase; and the other, the bulk aqueous solution containing surfactant monomers. Cloud point preconcentrations can also be done with methylated hydroxy-propylcyclodextrin [7], which behaves like a nonionic surfactant.

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0039-9140/00/$ - see front matter
PII: S0039-9140(99)00252-0

The use of micellar systems as an alternative to other techniques of separation offers several advantages including low cost, safety and high capacity to concentrate a wide variety of analytes of widely varying nature with high recoveries and very high concentration factors. From an analytical point of view, the surfactant-rich phase can be used to separate and/or preconcentrate different analytes before their injection into any hydrodynamic analytical system.

Among other reported applications, the cloud point extraction (CPE) has been used to preconcentrate metals [8–18] based on the formation of chelates in the surfactant aggregate. Nevertheless, we have demonstrated quantitative extraction of lead in the absence of chelating reagent.

Heavy metal pollution is and will be a public health problem [19]. Lead, which produces several diseases, is one of the most important and widely distributed pollutant in the environment. Workers occupationally exposed to lead show extremely high levels of lead in sweat and saliva, even though their lead in blood is moderately elevated. The lead levels in saliva are closely related to recent lead exposure [19,20], since circulating chemicals can be transported into the salivary glands and reflected in saliva. Salivary monitoring can be used for detection of environmental pollutants [21] (atmospheric or occupational), drugs abuse, local and systemic diseases, and can provide valuable information in diagnostics, treatment and forensic investigation. It is expected that saliva testing could play an important role in the arsenal of environmental scientists over the next years [22,23].

Atomic absorption spectroscopy (AAS) has greatly facilitated the quantification of trace elements in biological materials [24]. In the present work we have developed and optimised a powerful CPE-AAS combined methodology for Pb(II) determination, which shows excellent and rapid preconcentration. Adding lead to normal human saliva validated the analytical performance of the procedure. The present paper represents a new contribution not only in the field of mediated-phase separations but also in the field of sialochemistry, related to non-invasive techniques of sampling.

2. Experimental

2.1. Reagents

A total of 1 mg ml^{-1} standard solution of Pb(II) was prepared from acidic dissolution of its nitrate of analytical grade purity (Hopkin and Williams, Chadwell Heath, Essex, England). Stock solutions were standardised by a chelatometric method [25].

As it is not possible to obtain a real aqueous solution of the surfactant PONPE 7.5 (polyethyleneglycolmono - *p* - nonylphenylether, Tokyo Kasei Industries, Chuo-Ku, Tokyo, Japan) since the cloud point of its micellar solution is markedly below room temperature, it was experimentally convenient to prepare a mother solution (solution A) as follows: 10 ml PONPE 7.5; 10 ml $NaClO_4$ (Merck, Darmstadt, Germany) (1 mol l^{-1}), and 40 ml distilled ethanol, and made up to 100 ml with bidistilled water. In this way, ionic strength was adjusted to 0.01 mol l^{-1} and adequate cloud point temperature (higher than 293 K) and accurate surfactant concentration (0.01%) could be reached. Under these conditions an optimal preconcentration factor was obtained.

2.1.1. Buffer solution

A total of 1×10^{-2} mol l^{-1} sodium tetraborate (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA) solution was prepared. This solution was titrated to the desired pH, with aqueous $HClO_4$ (Merck, Darmstadt, Germany) or NaOH (Mallinckrodt Chemical Works, New York, Los Angeles, St. Louis, USA) using a combination glass electrode and a pH meter (Orion Expandable Ion Analyzer, Orion Research, Cambridge, MA, USA) Model EA 940.

2.2. Apparatus

An Instrumentation Laboratory Model 751 Atomic-Absorption Spectrometer, equipped with deuterium continuum background correction and a lead hollow-cathode lamp as the radiation source were used. The experimental conditions were adjusted according to the manufacturer's recommendations.

The ICP measures were made with a sequential inductively coupled plasma spectrometer (Baird ICP 2070, Baird, Bedford, MA, USA).

A centrifuge was used to accelerate the phase separation process.

2.3. Recommended procedure: CPE and AAS determination

Metal ion aliquot [0.6–4 μg ml^{-1} Pb(II)], 0,8 ml buffer borax solution 1×10^{-2} mol l^{-1} (pH = 8.5) and 1 ml of solution A, were placed in a graduated centrifuge tube. The whole mixture was diluted to 10 g with bidistilled water. The solution prepared was kept at 363 K for 10 min for equilibration and then centrifuged for 5 min at 3500 rpm. After being cooled at 255 K for 5 min the surfactant phase which had separated became a viscous gel and the aqueous phase could be poured off. The surfactant phase in the tube was then made up to 1 ml by adding 0.1 mol l^{-1} HNO_3 in ethanol. The diluted surfactant-rich phase was introduced into the flame and measurements were performed at $\lambda = 217.0$ nm (slit-width 0.5 nm) against a blank of reagents.

2.4. Saliva collection

In order to minimise the possibility of contamination with food debris or cigarette and airborne particles, the subjects were asked to thoroughly rinse their mouths three times, first with 1.5% citric acid solution (a salivation stimulant) and then twice with bidistilled deionized water. Human saliva samples were collected between 8 and 10 h to reduce possible circadian contributions into Pb-free polystyrene test tube, and the specimen frozen.

2.5. Sample procedure

Eight millilitres of spiked human saliva, 1 ml buffer borax solution 1.5×10^{-2} mol l^{-1} (pH = 8.5) and 1 ml of solution A, were placed in a graduated centrifuge tube. The CPE procedure was carried out in the same way described for the general procedure. The spectrometer measurement was made against a blank reagent prepared with 8 ml of saliva.

3. Results and discussion

3.1. Surfactant selection: extractive properties of PONPE 7.5

Several non-ionic surfactant were tested: TX-100 (Merck, Darmstadt, Germany); TX-405 (Fluka, Sweden); Igepal CO 720 (Aldrich Chemical Company, Milwaukee, USA) and Tween 80 (Sigma Chemical, Saint Louis, USA). On one hand, the obtained results never showed quantitative extraction [extraction efficiencies (%E) lower than 30%]. On the other, the main experimental difficulty to overcome when working with these extracting agents is their high critical point (above 70°C) with the consequent loss of extraction efficiency during the centrifugation/phase separation step.

When working with PONPE 7.5, an extraction efficiency higher than 99.9% was obtained. A possible explanation of the extracting behaviour of this micellar system, successfully used as extracting surfactant of metals chelates [11,12,26], is the existence of microscopically ordered structures in the surfactant-rich phase, such as liquid crystals, which can distinguish slight differences in molecular size, shape and structural factors [15,26]. The cloud point of the studied system with PONPE 7.5 is near room temperature, offering advantages in terms of the experimental procedure. Fig. 1 shows the variation of the cloud point with the surfactant concentration for the system, PONPE 7.5–Pb(II)–ethanol–buffer–water. The phase separation temperature was determined by measuring temperatures required for clarification (Curve I) of the studied system, while Curve II was obtained observing the onset of turbidity upon heating.

3.2. Effect of experimental variables on CPE parameters and optimisation of system

3.2.1. Effect of ethanol

The presence of ethanol prior to extraction step produces an adequate increase on the cloud point temperature of the system. Besides, the preconcentration factor ($f = v_w/v_s$, where v_w represents the volume of aqueous phase and v_s the volume of

surfactant rich phase) is influenced by the ethanol concentration prior to CPE step. The optimal preconcentration factor was achieved with ethanol concentration above 4% (v/v).

3.3. Selection of the dilution agent for the surfactant-rich phase

Different solvents for the surfactant-rich phase were tried so as to select the one producing the optimal results regarding sensitivity. The very high viscosity of the surfactant rich phase (20 cP approximately) was drastically decreased with a diluting agent: the best results were shown for 0.1 mol l^{-1} HNO_3 (Merck, Darmstadt, Germany) in ethanol. In that way, the CPE fractions may be appropriately manipulated and aspirated into the flame. A 6-fold increase (Signal Enhancement Factor, SEF) in the analyte signal was observed following the CPE of Pb(II) under the optimal experimental conditions. SEF is defined as the relationship of absorbance of preconcentrated samples to that obtained for an aqueous solution.

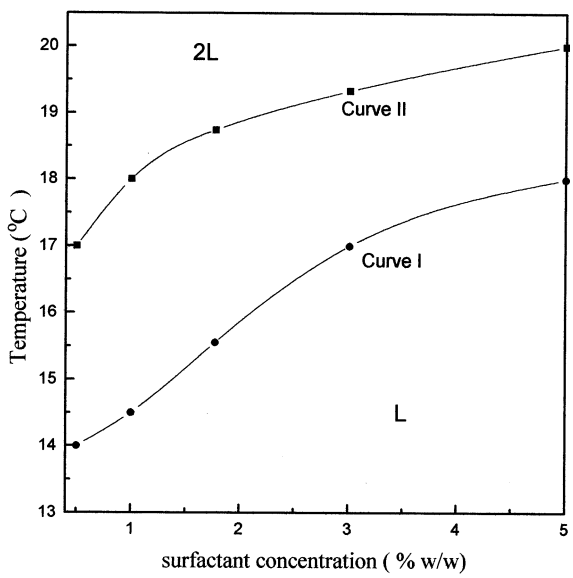

Fig. 1. Effect of surfactant concentration on cloud point temperature. Conditions: $C_{PONPE\ 7.5} = 0.1–5\%$ (w/w); $C_{sodium\ tetraborate} = 1.5 \times 10^{-3}$ mol l^{-1} (pH = 8.5); $C_{ethanol} = 4\%$ (v/v), $C_{Pb(II)} = 1.12 \times 10^{-5}$ mol l^{-1}; $\mu = 0.01$ mol l^{-1}. L: one isotropic phase. Curve I, measuring temperatures required for clarification. Curve II, observing the onset of turbidity upon heating.

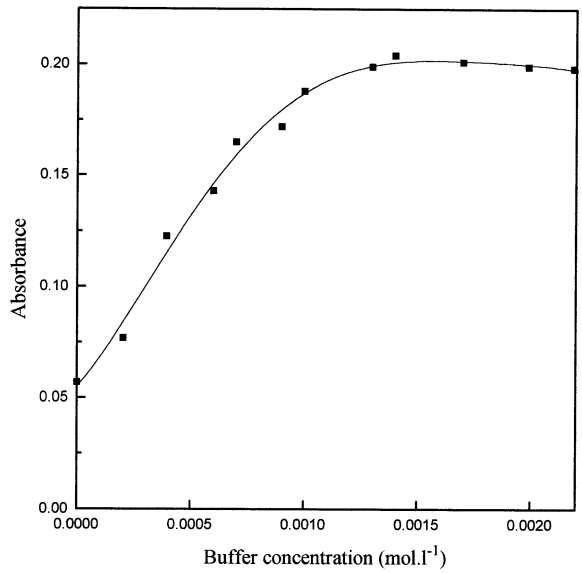

Fig. 2. Effect of buffer concentration. Conditions: $C_{PONPE\ 7.5} = 1\%$ (w/w); $C_{sodium\ tetraborate} = 0–2.5 \times 10^{-3}$ mol l^{-1}, $C_{ethanol} = 4\%$ (v/v), $C_{Pb(II)} = 1.16 \times 10^{-5}$ mol l^{-1}, $\mu = 0.01$ mol l^{-1}. Equilibration time = 10 min, equilibration temperature = 363 K.

3.3.1. Effect of buffer concentration and ionic strength

Among the several buffer agents tested, the best results regarding stability, preconcentration factor and kinetics of phase separation, were shown by sodium tetraborate. The influence of buffer concentration prior to CPE was investigated. The results are shown in Fig. 2. Sodium tetraborate 1.5×10^{-3} mol l^{-1} was chosen as optimal.

Ionic strength has no considerable effect upon the magnitude of extraction and sensitivity within the interval: $\mu = 0.005–1$ mol l^{-1}. Thus, ionic strength was kept constant at 0.01 mol l^{-1} with sodium perchlorate.

3.3.2. Effect of pH

Trials were carried out in order to locate the optimal pH range for the quantitative lead extraction. Each operational desired pH value was obtained by the addition of $HClO_4$ (Merck, Darmstadt, Germany) (d) and/or NaOH(d), in the absence of the buffer agent. The results are shown in Fig. 3. As can be seen, the extraction begins at pH = 4.2 and starts to decrease at pH = 10 offer-

ing a relatively wide range for quantitative extraction. It has to be pointed out that a higher sensitivity was achieved when the preconcentration step was carried out with a buffered initial solution.

3.3.3. Effect of surfactant concentration

The variation on extraction efficiency was studied within the surfactant concentration range: 0.1–2.0% (w/w). Metal concentration was kept constant at 2 μg ml^{-1} Pb(II). Quantitative extraction was observed for the whole concentration interval. One percent (w/w) was chosen in order to achieve a good preconcentration factor.

3.3.4. Effects of equilibration temperature and time

The greatest analyte preconcentration factor is reached when the CPE process is conducted with equilibration temperatures well above the cloud point temperature of the system [11,27]. It was observed that the volume of the surfactant-rich phase of PONPE 7.5 decreased by a factor of ≈ 5 when the temperature was increased from 298 to 363 K working at a surfactant concentration of 1% (w/w).

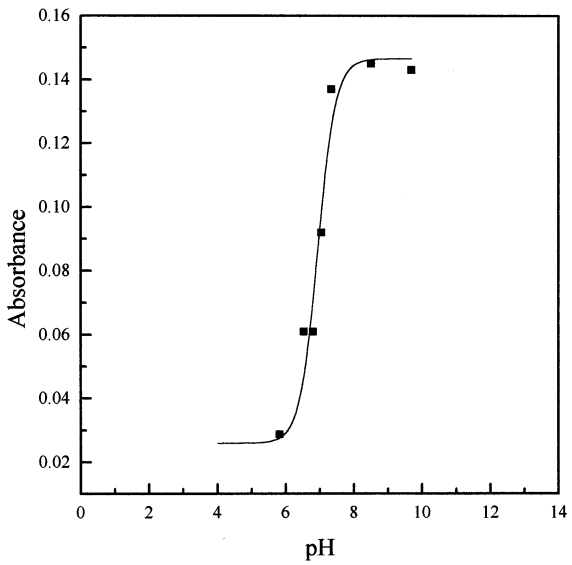

Fig. 3. Effect of pH. $C_{PONPE\ 7.5} = 1\%$ (w/w), $C_{ethanol} = 4\%$ (v/v), $C_{Pb(II)} = 1.16 \times 10^{-5}$ mol l^{-1}, $\mu = 0.01$ mol l^{-1}. Equilibration time = 10 min, equilibration temperature = 363 K.

The dependence of extraction efficiency upon equilibration time was studied within a range of 2–40 min. An equilibration time of 10 min was chosen as the best solution to achieve quantitative extraction and experimental convenience.

3.3.5. Effect of centrifugation time

The effect of centrifugation time upon extraction efficiency was studied for the range: 1–15 min. Complete phase separation was achieved for times longer than 3 min. A centrifuge time of 5 min was selected as optimum since no appreciable improvements were observed for longer times.

3.3.6. Outstanding features of the extraction process

The following factors were considered with the aim to establish the nature of extracting species and the location of lead in the micelle: the nature of the amphiphilc media; lead distribution equilibria [28] and the results obtained after the evaluation of the parameters affecting the process. PONPE 7.5 forms a cationic complex with $[Pb(OH)]^+$ through their polyoxyethylene groups [17,29].

In order to study the influence of the $C_{metal}/C_{surfactant}$ ratio upon the extraction efficiency, the mentioned ratio was varied within the interval: $0.0–7 \times 10^{-4}$. Other experimental parameters were kept constant. The flame atomic analyte signal was measured for the surfactant-rich phase following the recommended procedure (Fig. 4). Our data were validated by measuring the resultant aqueous phase by ICP-AES, the results being highly satisfactory.

The micelle concentration, C_M, can be calculated by dividing the concentration of micellized surfactant, C_D, by the average aggregation number, N (number of surfactant molecules per micelle aggregate) [17]. C_D can be defined as:

$$C_D = C_T - \text{cmc}$$

where C_T is the total surfactant concentration and cmc is the critical micelle concentration.

The cmc of PONPE 7.5 is 8.5×10^{-5} mol l^{-1}, and N is 100. From the results shown in Fig. 4, we concluded that a maximum amount of 60 metal ions can be associated /bound to each micellar entity.

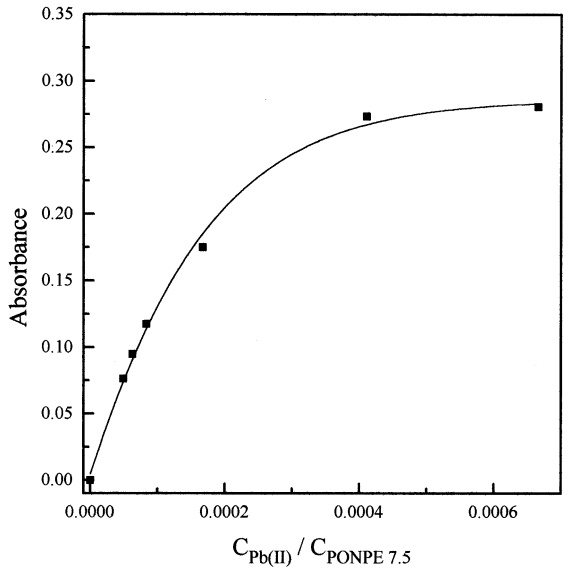

Fig. 4. Influence of the $C_{metal}/C_{surfactant}$ ratio upon the extraction efficiency. $C_{ethanol} = 4\%$ (v/v), $\mu = 0.01$ mol l^{-1}. Equilibration time, 10 min, equilibration temperature, 363 K.

Table 1
Experimental conditions for the CPE–AAS determination of lead

Equilibration temperature	363 K
Equilibration time	10 min
Centrifugation time	5 min
Cooling time	5 min
Working pH	8.50
Buffer solution	Sodium tetraborate 1.5×10^{-3} mol l^{-1}
Surfactant	PONPE 7.5 (1% w/w)
Working wavelength	217 nm
Slit width	0.5
%*E*	>99.9%[a]
Beer's law[b]	
Sensitivity	$1.72 \times 10^4 \pm 436$ l mol^{-1} cm^{-1}
S.D.	0.00722 ($n = 9$)
R	0.99777

[a] Sucessive extraction method.
[b] Beer's law, $\lambda = 283$ nm: sensitivity $= 8.8 \times 10^3$ l mol^{-1} cm^{-1}, S.D. = 0.00910 ($n = 9$), $R = 0.99211$.

3.3.7. Combined CPE–AAS methodology for Pb(II) determination

Table 1 summarises the optimal experimental conditions for the preconcentration-determination of lead, as well as the results of the data treatment by linear least-squares method. A %*E* higher than 99.9 and a preconcetration factor (f) of ≈ 67 were achieved.

The dependence of absorbance on metal concentration was linear within a range of $C_{Pb(II)} =$ 0.6–4.1 mg l^{-1}.

3.4. Analysis of samples

In order to validate the developed methodology, the procedure was applied to determine lead in human saliva samples spiked with different lead levels. The CPE procedure was practised in saliva samples without previous treatment. Table 2 shows the obtained results.

3.5. Analytical performance

The analytical performance of the developed procedure clearly indicates that the method satisfies the typical requirements for control processes [19–23] and is superior to the existing analytical methodologies in terms of sensitivity (six times higher than standard FAAS methodology), cost and simplicity. Besides, no analytical signal was observed when the recommended CPE–AAS procedure was developed for Cd(II). The latter indicates a potential selectivity of the proposed method.

It has to be pointed out that, to date, no CPE of chelated lead has been reported in literature. Our own studies in this field working with pyridylazo dyes as lead chelating agents

Table 2
Analysis of lead in spiked human saliva samples (according to [19] levels)

Sample	Pb(II) added	Pb(II) found[a]	S.D.[b]	%RE[c]
I	0.621 μg ml^{-1}	0.608 μg ml^{-1}	0.0043	1.96
II	1.036 μg ml^{-1}	1.016 μg ml^{-1}	0.0057	1.88
III	2.072 μg ml^{-1}	2.043 μg ml^{-1}	0.012	1.37

[a] $n = 6$.
[b] Standard deviation.
[c] Relative percentage error.

4. Conclusions

A novel non-polluting procedure for the enrichment of metal traces has been developed and optimised. The extent of extraction is markedly influenced by the presence of additives, pH, time and temperature equilibration.

The results demonstrate the usefulness of this new type of micelle-mediated extraction to quantitatively extract and preconcentrate metal ions in the absence of chelating agent. It has to be pointed out that this micellar comportment has not been previously reported. This approach could serve as basis for future analytical applications of CPE of unchelated metals, as well as basic physical–chemical studies due to the simplicity of this micellar system.

At present, a project is being undertaken in our laboratory to access further solid, experiments-backed explanation of the observed behaviour.

Acknowledgements

The authors wish to thank Dr. L. Dante Martínez for the ICP measurements. The present work has been carried out under the financial support of National University of San Luis (Project No 7502) and CONICET.

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