Analysis of Lead in Blood Serum Samples by Voltammetry Method

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Abstract: In this article a novel, sensitive and selective cathodic adsorptive stripping procedure is reported for determining of lead. The method is based on adsorptive accumulation of Pb- N-Nitrozo-N-Phenylhydroxylamine on a hanging mercury drop electrode, followed by the reduction of adsorbed specie by voltammetric scan while followed differential pulse modulation. The optimum conditions for analysis of lead include pH, 7.0, (borate buffer), 9.0×10^{-3} M N-Nitrozo-N-Phenylhydroxylamine, accumulation potential of -0.1 V, (versus Ag/AgCl). The peak current is proportional to the concentration of lead over the entire concentration range tested (0.7-175 ng/ml) with a low detection limit of 0.1 ng/ml for an accumulation time of 150 s. The method was applied to determination of lead in blood serum samples with satisfactory results.

Key words: Lead · Voltammetry · Blood samples

INTRODUCTION

Lead is one of the most abundant heavy metals and its toxic effects cause environmental and health problems because of its stability in contaminated site and complexity of mechanism in biological toxicity, particularly dangerous for children leading to mental retardation when exist with abnormal concentration in body fluid. Recent evidence suggests that neurological damage in children may occur at Pb(II) in blood as low as 10 mg L^{-1} [1]. Thus, there is a constant demand of improved analytical methods for sensitive and selective determination of Pb(II) both in biological samples. Several analytical techniques are available for quantification of lead such as Electrochemical stripping analysis [2-7], atomic absorption spectrometry (AAS) [8], inductive coupled plasma mass spectrometry (ICP-MS ) [9], inductive coupled plasma atomic emission spectrometry (ICP-AES) [10], high performance liquid chromatography [11], capillary electrophoresis [12] and backward line scattering [13]. Among these methods, adsorptive stripping voltammetry (AdSV) is commonly employed because of its wide linear dynamic range and low detection limit which achieved as the result of performing the preconcentration steps directly into the voltammetric cell, thus decreasing the sampling handling, risk of sample contamination and multielement analysis capacity[14]. In the present work simple and selective method is described for the sensitive determination of lead with N-Nitrozo-N-Phenylhydroxylamine. The present method is more sensitive and selective than the most of the methods reported for the determination of Pb. The method is applicable for the rapid determination of lead in blood serum samples at nanomolar levels.

Experimental

Apparatus & Chemicals and Reagents: All polarographic measurements was carried out using a polarographic processor, model 746 VA (Metrohm), in combination with a polarographic stand model, 747 VA (Metrohm). This electrode stand consist of a hanging mercury drop electrode (HMDE) as working electrode, a silver-silver chloride (3M) as reference electrode and platinum wire, with a considerably larger surface area than that of HMDE, as auxiliary electrode. Stripping was carried out by a large Teflon road with 2000 rmp speed. A 780 pH meter (Metrohm), equipped with a combined Ag/AgCl glass electrode and was used for pH measurement. Solutions were deaerated with high - purity nitrogen for 4 min prior to each experiment, which was performed under a nitrogen atmosphere. Eppendorf Vary -pipettes (10-100-1000) were used to deliver accurate volumes. All glassware and storage bottles were soaked in 10% HNO_{3} overnight and thoroughly rinsed with deionized water prior to use.
All chemical reagents were of analytical grade and were purchased from Merck (Germany). All solutions were prepared by deionized water. A stock standard solution of Pb(II) (1000 mg/L) was prepared by dissolving an appropriate amounts of metal salt in doubly distilled water containing a few drops of concentrated nitric acid. The solution was made up to the mark in a 100 ml volumetric flask. A 1×10⁻⁴ M solution of chelating agent(N-Nitrozo-N-Phenylhydroxylamine) was prepared by dissolving the appropriate amount of N-Nitrozo-N-Phenylhydroxylamine in water. Borate buffer solution was prepared using boric acid and sodium hydroxide.

**Sample Preparation**

**Blood Serum Samples:** Exactly 2.0 mL of the blood sample was transferred to the test tube. The sample was spiked with the appropriate volume of standard solution of zinc and cadmium and left to equilibrate in a water bath for 1 h at 37°C. 1 mL of water was then used to quantitatively transfer the spiked samples into Teflon high-pressure microwave acid-digestion vessels for the digestion. A 2.0 mL of portion of concentrated nitric acid and 4.0 mL of 30% H₂O₂ were added. After digestion the closed vessel was cooled, the digest was quantitatively transferred to 10 mL volumetric flasks, neutralized with NH₃, 1.0 M and diluted to volume with distilled water[14].

**Measurement Procedure:** A 10 ml sample solution, containing 0.2 M borate buffer (pH 7.0), 9.0×10⁻⁴ M N-Nitrozo-N-Phenylhydroxylamine and different concentrations of Pb(II) ion was transferred to the volumetric cell and purged with nitrogen for 4 min. The accumulation potential (-0.1 V) was applied to a fresh mercury drop for 150 s while the solution was stirred. Following the accumulation period, the stirring was stopped and, after 10 s, the voltammograms were recorded by applying a negative going potential. Each scan was repeated for four times with a new drop for each analyzed solution and the means of the voltammograms were obtained. The reduction peak for lead occurred at -0.5 V and its current was used as a measure of lead concentration. All data were obtained at room temperature.

**RESULTS AND DISCUSSION**

**Adsorptive Stripping Characteristics:** The possible mechanisms of metal pre-concentration on the electrode-solution interface in adsorptive stripping voltammetry, via complex formation, were given by Paneli et al.

![Fig. 1: Effect of pH on the cathodic stripping current of 40 ng ml⁻¹ lead in the presence of 0.1µM ligand and after 20 s accumulation time at -100mV](image-url)

According to these authors, it seems that the presence of π- electrons in the ligand molecule favors the adsorption process. Complex of Pb (II) with N-Nitrozo-N-Phenylhydroxylamine in borate buffer had a sensitive adsorption peak in the -0.5 V. Under any circumstance the peak height and shape change parallel.

**Effect of pH:** The effect of several supporting electrolytes, such as acetate, borate, phosphate and ammonia buffers was tested. Both the peak height and the peak shape were taken into consideration when choosing the supporting electrolyte. The results show that borate buffer gave the best response. The effect of pH on the pre-concentration -stripping process, in a solution containing 40 ng/ml of Pb(II) ion and 9.0×10⁻⁴ M of N-Nitrozo-N-Phenylhydroxylamine was investigated in the pH range studied from 6.0 to 8.75 (Figure 1). The maximum peak current is around pH 7.0. The low sensitivity, at a low pH range, was probably due to decrease in complexing ability of ligand. On the other hand, at the lower pH the functional groups of chelating agent are protonated and cannot be coordinated to Pb(II) ions. The low sensitivity at higher pH was probably caused by the precipitation of Pb(II) to form Pb (OH)₃ on the electrode surface, which would not be stripped out easily. Therefore the borate buffer, at pH 7.0, was selected as the optimum experimental condition.

**Effect of Ligand Concentration:** Different concentration of N-Nitrozo-N-Phenylhydroxylamine, in the present of 40 ng/ml lead ion and borate buffer as supporting electrolyte, was studied. The lead peak can resolved by displacement of the lead peaks potential through formation of a complex compound with an organic reagent added either to the solution or to the modifying layer of the electrode.
The results shown in Fig. 2 indicate that the peak height increased up to $9.0 \times 10^{-4}$ M. When the ligand concentration is higher than $9.0 \times 10^{-4}$ M, the peak height diminishes as a result of full electrode surface coverage. This decrease in peak current at ligand concentration is higher than a suitable amount ($9.0 \times 10^{-4}$) which, in turn, is due to an inhibition of the ligand adsorption by a competitive coverage by the free ligand. As a result, less electrode surface is available for adsorption of the metal complex in the case of increasing ligand concentration. Therefore, $9.0 \times 10^{-4}$ M of chelating agent was selected as the optimum condition for experiment.

**Effect of Accumulation Parameters:** The effect of accumulation potential on the stripping peak current of the complex was examined over the potential range of $+0.1$ to $-0.3$ V. As shown in Fig. 3, the peak current increased with changing potential from $+0.1$ to $-0.1$ V, probably due to increased accumulation of complex on the electrode surface. The peak current decreased at a potential more negative than $-0.1$ V, so, this potential was used in all experiments. At this potential, the adsorbed Pb(II)-N-Nitrozo-N-Phenylhydroxylamine complex was reduced on the surface of the electrode and the and the peak was appeared. In the stripping step, the oxidation of these complexes occurs at the initial potential of voltammetric scan during the equilibration period. Thus the Pb(II)-N-Nitrozo-N-Phenylhydroxylamine complex is again reduced during the cathodic sweep. The effect of accumulation time on peak current was also studied. Fig. 4 indicates that, upon increasing the accumulation time up to 150 s, the peak current linearly increased. As expected for adsorption processes, the dependence of the peak current on the accumulation time is limited by the saturation of the electrode, resulting in the current reaching a plateau at high accumulation times. Thus a deposition time of 150 s was used throughout, as it combines good sensitivity and short analysis time. For an accumulation time of 150 s, the concentration current relationship is linear from 0.7 to 175 ng/ml. However, we selected 80s as an optimum accumulation time for experiments.

**Effect of Scan Rate:** The effect of scan rate on the stripping peaks was examined. For this purpose, the stripping voltammograms were recorded for 40 ng/ml of lead and $9.0 \times 10^{-4}$ M Pb(II)-N-Nitrozo-N-Phenylhydroxylamine solution with various scan rates of electrode potential, ranging between 20 and 120 mV/s, while accumulation potential and accumulation time were -0.1V and 150 s, respectively. According to the results, the maximum values for analytical signals appeared at a scan rate of 60 mV/s, so this scan rate was selected as the optimum experimental condition.
Table 1: Determination of lead in blood sample (n=5)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Add (ng/ml)</th>
<th>Found(ng/ml)</th>
<th>Recovery(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>0</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.75</td>
<td>95.9</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>49.83</td>
<td>98.0</td>
</tr>
</tbody>
</table>

**Analytical Figure of Merit**

**Linear Dynamic Range, Detection Limit and Precision of Method:** Under the optimized condition, a linear relationship between the reduction peak current of lead complex and the concentration of Pb(II) was obtained in the range of 0.7 to 175.0 ng/ml, following a 150 s accumulation time, by fitting the equation: 
\[ Y = 0.9775X + 11.828 \]
with a square correlation coefficient of \( r^2 \) of 0.9971, where \( X \) is the lead concentration expressed in ng/ml. The linear range can be extended by variation of accumulation time. A 3σ detection limit of 0.1 ng/ml achieved. Repeated voltammograms, after 150 s accumulation time, showed that the relative standard deviation for a single solution containing 40.0 ng/ml of lead gave relative standard deviation as 1.7%.

**Interference Study & Real Sample Analysis:** The effects of various interfering species, which accompany lead in blood serum samples, were studied, using 40ng/ml lead. These species, which were tolerated at a reasonably high concentration, show the high sensitivity of the proposed method. According to the results, no interference was caused by cations. As for anions, most caused almost no important interference with the system, so the proposed method has a very high sensitivity. The proposed method was directly applied to the determination of lead in blood serum without any separation step. The Pb(II) content of blood serum were determined using the recommended procedure under optimum conditions, by the standard addition method. The results, given in table 1 show the high sensitivity of the proposed method.

**CONCLUSION**

The present study demonstrated that the procedure developed here offers a simple method for the determination of ultra-trace levels of lead in real samples. The detection limit of this technique is 0.1 ng/ml Pb (II) at a collection period of 150 s. In conclusion, therefore the above system can be a potential candidate for practical use of Pb (II) determination with high sensitivity, selectivity, simplicity and speed.

**REFERENCES**

