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Short Communication

Simultaneous determination of Zinc and Lead in blood samples by Differential Pulse Polarography

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Abstract

In present study we determine the Zinc and Lead levels in human blood samples by differential pulse polarography under optimum experimental conditions (pH=7.5, Scan rate=5mV/S and pulse amplitude=50mV) the polarographic reduction peaks of Zn and Pb were examinedin the -0.72 V and-0.15 respectively. The zinc and lead content can be determined by in comparison with their standard solutions. According to the obtained results differential pulse polarography is more reliable, rapid and sensitive method for determination of zinc and lead levels in the blood than atomic absorption spectroscopy.

Keywords: Blood, Differential Pulse Polarography, Zinc, Lead

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Introduction

The metals levels in the serum are known as an important primary parameter in the human body which helps us to determine environmental exposure in population. Also, they are changed in many disorders and theirs alteration give us better etiological and remedial perspective to recognize and cure different disease. Clinicalstudieson assessment of metalslevel in the serum samples to indicate environmental exposure may give beneficial information for any health care program for decreasing of environmental pollutions [1].

Zinc (Zn) has a crucial role in the activity of different enzymes such as carbonic anhydrase, RNA polymerase, angiotensin I converting enzyme, Cu–Zn superoxide dismutase. The levels of Zinc in human body and plasma are 2–3 g and 1 mg/L, respectively. In the underdeveloped countries zinc deficiency is common health problem and it is principally induced by malnutrition, which is affect the immunological systems, the senses of taste and smell, wound healing, and abnormality in DNA synthesis.

Zinc has an important role in the normal growth and development during pregnancy period, childhood, and adolescence. Furthermore, excessive accumulation of zinc correlates with acute and chronic intoxications. Intakes of 150–450 mg of Zinc per day have been associated with copper deficiency, disturbing of normal function of iron, decreasing in immunological systems, and diminished the levels of high density lipoproteins [2].

Lead (Pb) is a toxic element which can cause blood and brain disorders and induce abnormally in the nervous connections. The main target for chronic lead intoxication is the central nervous system injuries. Lead intoxication also induces movement disorders and slight hypertension and can cause anemia. Acute Lead intoxication can induce damage to the brain and kidneys in human and finally cause death. In pregnancy period, Lead exposure induces miscarriage and other health problems. Chronic, high-level intoxication can cause reproductive toxicity in males [3]. Furthermore, Lead accumulation in tissues associated with increasing in free radical generation level and oxidative stress [4]. There are numerous literatures on direct role of oxidative stress in pathogenesis of different diseases [5].

There are different methods for determination of Zinc and Lead in serum samples. Spectroscopy methods were commonly used for this aim [6, 7], furthermore there are many literature on chromatographic assays [8]. Electrochemical methods are better than above mention techniques for metal assessment [9, 10] because these methods (such as differential pulse polarography (DPP) and differential pulse voltammetry (DPV)) have high sensitivity, low limitationfor determination, easy operation, and some-times the use of simple instrumentation. Polarography is a good method for determination of traceamount of metals in biological samples. The aim of present study is to introduce DPP methods for determination of Zinc and Lead content in serum sample and comparison of this method with atomic absorption spectroscopy (AAS).

Materials and Methods

Apparatus

The polargrams were obtained with a Metrohm797 VA processor. A three-electrode cell contained a HMDE, an Ag/AgCl reference electrode and a platinum wire auxiliary

electrode. The pH measurements were carried out by means of a Metrohm pH-Meter 69.

Reagents

All the reagents used were of analytical-reagent grade. Triply distilled water was used throughout. The mercury (proanalysi) used in the hanging mercury electrode was obtained from Merck (Darmstadt, Germany). A stock solution of 1000 ppm of each metal was prepared by taking 99.99% of pure metal (Aldrich). Solutions of low concentrations were prepared by serial dilutions with de-ionized double distilled water.

Sample digestion

To 2 mlof blood in a long-necked 100 ml flask, 4.0 ml of acid mixture (2 ml HNO_3 and 2ml$HClO_4$) was added and after 10–15 min it was warmed up and evaporated until the half of themixture was left. Before further warming up (toavoid explosion) 2.0 ml of HNO_3 was added and partly evaporated after evaporation to nearly dryness 2.0 ml $HClO_4$ was added and warmed up untilwhite fumes were nearly finished; the sample became clearand was re-evaporated until nearly dryness. After cooling, 2.0 ml of HCl was added and evaporateduntil nearly dryness. The acidity was neutralized with 2 M NaOH and then it was diluted to 5.0 ml [11].

Polarographic determination

Digested sample (2 ml) was transferred into the polarographic cell containing 10 ml of ammonium acetate buffer (pH=7.5). The solution in the cell was purged for 600 minutes by purging pure nitrogen gas. Anodic stripping was performed in differential pulse mode after selecting pre-concentration time of 180s, a scan rate of 5 mV/s and pulse amplitude of 50 mV. The concentrations of all the metals in the samples were determined using standard addition method.

Results

Differential pulse stripping voltammetry is on the basis of two different steps, the first step involves the electrolytical removal of metal ions into a hanging mercury drop electrode to pre-concentrate the metals in the form of a dilute amalgam:

$Pb^{+2} + 2e + (Hg) \rightarrow Pb(Hg)$

$Zn^{+2} + 2e + (Hg) \rightarrow Zn(Hg)$

In the second step, the electrode is ‘stripped’, i.e., the metal in the amalgam is re-oxidized. Current and potential measurements are made during the second step, and the position and height of stripping peaks are characteristic of the type andthe metal ion concentration originally in samples[9]:

$Pb(Hg) - 2e \rightarrow Pb^{+2} + (Hg)$

$Zn(Hg) - 2e \rightarrow Zn^{+2} + (Hg)$

First of all, the curves of these metals in a synthetic sample were studied. In this section we wanted to see the polarograms of these metals and the determination qualities wasn’t our aim. Scan confine was between 0 and -1 V and the first peak was related to Pb in -0.15 and the second peak was devoted to Zn in -0.72v. This subject was proved when we added 50 µl of standard solution two times for Pb and one time for Zn and so an obvious increase was observed. Figure 1 shows the increasing currents of these metals after standard addition.

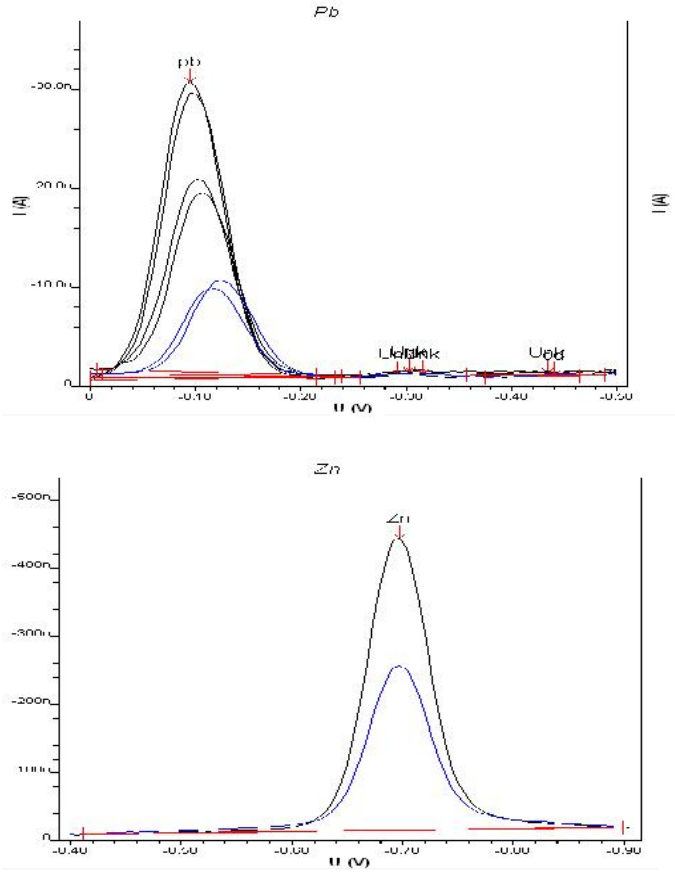

Figure 1. Increasing currents of metals after standard addition.

Optimization of polarographic parameters

The effect of pulse amplitude and scan rate was investigated. The peak currents of Pb and Zn increased with increasing of the pulse amplitude in the potential range of 10–80 mV. In this work, −50 mV was chosen as the optimum value for pulse amplitude, because at this value the sensitivity was high and the polarographic curves were well shaped with relatively narrow peak widths.

The results showed that with increase of the scan rate in the range 5–25 mV/s, the peaks current of Pb and Zn briefly decreased.

For studying this subject, a sample with known amounts of these metals was prepared from Baqiyatallah’s biochemical Lab. Figure 2 illustrated the maximum currents of different scan-rates. Therefore, scan rate = 5mV/s was selected as optimum scan rate.

The influence of pH on peak potential for the reduction peaks was examined using differential pulse polarography. For this goal, pH between5-9 was considered and three buffers (phosphate, Britton–Robinson and ammonium acetate buffer) were applied. Fig (3-a,b) illustrated max currents were related to ammonium acetate for Zn (a) and Pb (b). Finally, ammonium acetate with pH 7.5 was selected as the optimum. At this pH the sensitivity was the

highest, the peak was well-defined and the base line was flat.

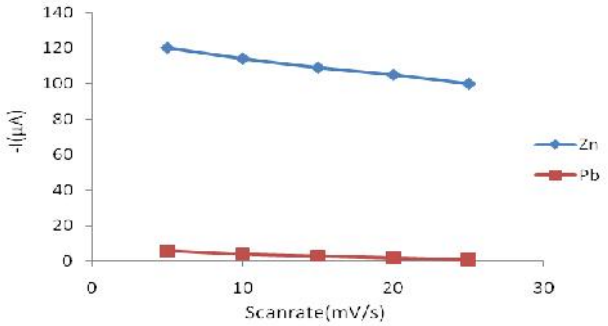

Figure 2. The effect of scan-rate on 102 µg/dl and 1.05 µg/dl of Zn and Pb.

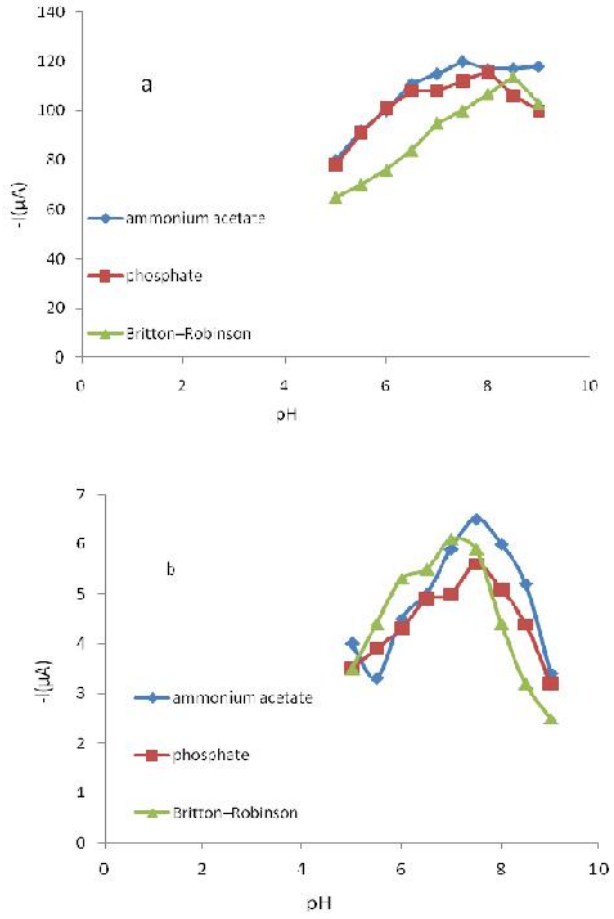

Figure 3. The effect of pH and buffers on 102 µg/dl and 1.05µg/dl of Zn (a) and Pb (b).

Determination of Zinc and lead in real samples

The normal range of Zn and Pb, for adults, is 100-110 µg/dl and 0-20 µg/dl respectively so that the determination of these metals must be done by high sensitive methods. In this study, five blood samples of different person who lived in polluted areas of Tehran were taken and the results obtained by Polarograph were compared with AAS in the biochemical laboratory in Baqiyallah hospital. Finally, the results of DPP and atomic absorption spectroscopy were analyzed by t-test (Table. 1).

Table 1. Determination of lead and Zinc in five random samples from adult males *.

Sample	Zn	Pb (found AAS)	Zn	Pb (found DDP)	SD	t-test
1	103.1	4.02	104.2	4.1	0.19	0.95(n.s)
2	94.5	2.3	94.1	1.9	0.21	1.19(n.s)
3	109	3.1	108.9	2.9	0.28	1.41(n.s)
4	99.8	1.1	99.7	1.4	0.32	1.38(n.s)
5	101.4	2.7	102.4	2.9	0.17	1.22(n.s)

*- All concentrations are based on µg/dl.
-All concentrations are the average of three determinations (n=3).
- Determination was done by standard addition calibration.
- t-test 90% confidence interval.
-n.s.: not significant.

Discussion

The purpose of the present study was to simultaneous determination of Lead and Zinc in blood samples by DPP and it compare with AAS .The results show that DPP was better in the measurement of metal concentrations in the blood samples. Briefly, results of present study show that DPP possesses numerous theoretical and appliedbenefits including few parameters to be adjusted in the DPP. The validation of analytical performance characteristics demonstrated the reliability of the method for the analysis of whole blood.

Conclusions

In present study, two methods to determination of metal ions, DPP and AAS, were compared for Lead and Zinc analysis in blood samples. Accordingly, the DPP was applied to determine the levels of Zinc and Lead in blood samples.The advantages of DPP method over the other known techniques (AAS, ICP-OES, etc) are that it is a successful, new, rapid, simple, selective and inexpensive technique for quantitative and qualitative determination of metal ions and it has extremely low detection limits and can also works in the presence of high salt concentrations.

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