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Enhanced anodic stripping voltammetry method for blood lead analysis

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ABSTRACT

Lead is generally considerd as ubiquitous health hazard for humans, efforts have been exerted to study the distribution levels of lead among people and to reduce exposure in several ways, there are several methods for blood lead analysis, in this study a proposed method was found to be applicable for blood lead measurement, the propused method has enhanced signal seperated away from copper signal, the recovery of the proposed method raneged from 98% to 102% and the precision of the method was 1.5% (n=10) at 10μg/l of lead. Most of the metal ions found in the samples under investigation were found not to interfere with the determination. The calibration graph was linear for long range of lead levels. The method is simple, efficient and accurate, has short time of purging, and has been used for astudy of blood lead levels among school children in Jenin district.

KEYWORDS

Anodic stripping voltammetry;
Lead;
Blood lead measurement;
Hanging mercury
drop electrode.

INTRODUCTION

Lead is generally considered as an ubiquitous health hazard for humans. Although in recent years efforts have been exerted to reduce exposure in several ways, concern about lead as a significant public health problem has also increased as epidemiological and experimental evidence has mounted regarding adverse health effects at successively lower levels of lead exposure[1]. Lead is not distributed homogeneously throughout the body, there is a rapid uptake of lead into blood and soft tissues which results in a slow redistribution in bones. Due to ease of sampling and the homogeneity of the sample, blood has been the most widely used specimen to assess the human body burden of lead[2]. The common lead poisoning symptoms on human body are represented on[3-8]. The Centers of Disease Control and Prevention (CDC) outlined different levels of intervention of blood lead levels in 1991[9-11].

Lead could be measured by graphite furnace atomic absorption spectrometric (GFAAS) using different procedures for (AAS). The lead content is determined using GFAAS with deuterium background correction, this method has been optimized for sensitivity at lower blood lead level (10 μg/L). A comparison between GFAAS and inductively coupled mass spectrometry showed good agreement at very low concentrations of lead[12]. All procedures are nearly identical. A specific amount of the blood from the sample that must be analyzed is mixed with a specific amount of matrix modifier reagents contain *TritonX-100*, $NH_4H_2PO_4$ and HNO_3. After mixing, the sample is injected in the graphite tube. This method has many advantages. The instrument is accurate, has a good detection limit and short time for analysis. The instrument has a disc contains about 50 vessels filled with the samples and analyzed spontaneously. The

GFAAS instrument is very expensive, not easy to operate, occupies a large space, and also it's graphite tube relatively expensive and can be used for only about 40 samples.

Also it could be analyzed by Anodic Stripping Voltammetry ASV, which it is an electroanalytical technique that can be used to measure trace quantities of a number of metals in solution, and is especially useful for lead determination in environmental and industrial hygiene samples and also for blood[13]. The concentration of analytical metal species e.g. lead dissolved in solution is determined by reducing the dissolved metal ion Pb(II) at an applied potential (voltage) E_D to immobilized discharged metal species (Pb^0) at a working electrode surface (ordinarily mercury).

$$Pb^{2+}\ (solution) + 2e^- \xrightarrow[(at\ potential\ E)]{} Pb^0\ (Hg\ electrode)$$

This is accomplished by applying an electrode potential which sufficiently negative (with respect to the reference electrode) to cause reduction (gain of electrons) of soluble lead ions. The metal is deposited in the form of an amalgam (with mercury) at an applied potential that is negative of the standard redox potential of the metal /ion redox couple. (E_D must be negative of the standard potential E^0 for the Pb/Pb^{2+} redox couple in order to cause the above reduction). After deposition for a given time period t_d, the pre concentrated lead species is then stripped from the working electrode surface by applying a positive potential sweep. This causes anodic oxidation (loss of electrons) of the amalgamated discharged (Pb^0). This occurs at an applied potential $E_{app.}$. Which is sufficiently positive to effect re-oxidation of the lead originally deposited at E_D

$$Pb^0\ (Hg\ electrode) \xrightarrow[(at\ potential\ E_{app.})]{} Pb^{2+}\ (solution) + 2e^-$$

($E_{app.}$ must be positive of the standard potential E^0 for the standard potential E^0 for the Pb/Pb^{2+} redox couple in order to cause the above oxidation.) During the stripping step, the current associated with this reoxidation is measured. The peak current i_p arising from the re-oxidation of discharged amalgamated lead species is proportional to the original concentration of dissolved lead ion over a wide range of concentrations.[13] ASV is extremely sensitive, and field-portable ASV can measure lead concentrations in the solution in the range of part per billion (ppb). The cell is made up of three electrodes (reference electrode, counter electrode and working electrode) immersed in a solution containing the analyte and also an excess of non-reactive electrolyte called a supporting electrolyte[14]. There are different type of working electrode used including; Hanging drop mercury electrode (HDME). Mercury film electrode (MFE) prepared by plating of Hg on glassy carbon, gold, Graphite pencil[15], Iridium[16], and Carbon fiber[17] have also been used. Solid electrodes, which are suitable for the determination of Cu, Hg, Pb, and elements which have oxidation potentials more positive than Hg (e.g. Ag, Au, Se and Te)[18]. Ultra- Trace electrode made from epoxy-impregnated graphite.

Supporting electrolytes are commonly used in polarography to decrease the resistance of the solution and to ensure that the electroactive species moves by diffusion and not by electrical migration in the voltage field a cross the cell. The supporting electrolyte is often chosen also to provide optimum conditions for the particular analysis. Supporting electrolyte like strong acids (e.g. HCl, H_2SO_4, HNO_3), strong base (e.g. NaOH, LiOH), or neutral salts (e.g. chloride, perchlorides, sulfates of alkali metals or tetraalkylammonium ions) are frequently used, as are buffer solutions or solutions of complexing agents (e.g. tertrates, citrates, cyanides, fluorides or amines, including ammonia and EDTA). The total concentration of electrolyte is usually between 0.1 and 1.0 M, ionic strength between 0.1 to 2.5. The current flows as a result of migration of the positive and negative ions of the supporting electrolyte, with negligible migration of the electroactive species, which then moves as a result of the difference in the concentration only (diffusion)[14].

The Ilkovic Equation, $i_d = (607\ n\ D^{1/2} m^{2/3} t^{1/6})\ C$

The terms are defined as follows:

i_d is the average diffusion current in μA. The number 607 is the value at 25°C of a collection of terms including the faraday and the density of mercury, n is the number of electrons in the process [Ox + ne $\longrightarrow$ Red], D is the diffusion coefficient of the electroactive species in cm^2/s, m is the mass of mercury flowing through the capillary per unit time in mg/s, t is the drop time in s, C is the concentration of the electroactive species in mmol/liter or mM. The term ($607\ n\ D^{1/2} m^{2/3} t^{1/6}$) is a constant k then the Ilkovic Equation become : $i_d = kC$

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Then diffusion current i_d is proportional to the concentration of the analyzed species.

The methods applied for determination of lead in blood are either complicated like (GFAAS) or like Leadcare analyzer which need a very expensive kits.[12] The purpose of this study was to develop a new sensitive, fast and low cost method for lead blood analysis, and to apply the method for the determination of lead blood levels in human blood in jenin district and to test if these levels were associated with one or more of several variables including location, socioeconomic conditions, eating habits and parents education.

EXPERIMENTAL

Blood samples collection and processing

Two mL venous blood from each person under study, was aspirated using a disposable syringe and placed in a special tube (EDTA tube) which was stored in a refrigerator (2-4 ^oC) for further treatment.

Determination of blood lead levels

The samples were analyzed following the proposed developed Anodic Stripping Voltammetric (ASV/HDME) method

Apparatus

Instrument: Anodic stripping voltammetric measurements were carried out using Polarographic Analyzer/stripping voltammeter POL150 which is capable of differential pulse stripping analyses and other different polarographic analysis.

Electrodes: Static Mercury Drop Electrode MDE150 was employed. The analysis cell composed of working electrode (dropped mercury electrode); reference electrode (Ag/AgCl/KCl); and counter electrode (Pt wire).

Treatment of glassware

In order to reduce adsorption on glassware, the electrolysis cell, calibrated flasks and pipettes were thoroughly washed with 0.2M nitric acid followed by deionized water then soaked with 2 M HNO_3 for 24 hours. The flasks and electrolysis cell were then soaked with a 2% solution of dimethyldichlorosilane in carbon tetrachloride for 24 hours. The glassware, electrodes and analysis cells were rinsed with methanol and finally with deionized water several times[19].

Reagents

All chemicals employed were of analytical-reagent grade purity. Solutions of these chemicals and all dilutions were done using doubly distilled water over $KMnO_4$ in an all-glass distillation apparatus, and stored in polyethylene bottles so as to minimize contamination. The standard lead solution containing 10^3 mg/L was obtained from Perkin-Elmer.

Standard samples

Blood samples of known lead concentration were prepared as follows: A blood sample was aspirated and analyzed using a LEADCARE analyzer and found to contain 40 μg/L of lead. The sample was then divided into several portions. Known volumes of a working stock lead solution were added to these portions to obtain lead concentrations of (100, 200, 300, 400, 500 μg/L). From those samples, a calibration curve was prepared using the proposed method of analysis.

Preparation of the digestion reagent

A digestion reagent containing different acids was therefore prepared. This reagent also contains supporting electrolyte mixed with the digestion reagent. All of the following amount of material were disolved in one leter of duble distiled water (14.3g Calcium acetate, 28 mg Mercuric ion, 30 ml of 15.4 M Nitric acid, 20 ml of 11.5 M perchloric acid, 5 ml of 37% sulforic acid, 10 ml of 10 M hydrochloric acid and 15 g potasium chloride).

Blood samples preparation

Knowing that lead ions present in the blood are bounded to erythrocytes sides membrane, the ions should be freed from the erythrocytes so as they can be reduced at the drop of mercury at the working electrode (DME). 0.5 mL whole blood portion was transferred into a 10-mL polyvinyl tube containing 7.0 mL of the digesting reagent, especially prepared for this purpose. The contents were thoroughly mixed by a rotary mixer for 60 seconds and left for about 2 hours at room temperature to allow complete digestion of blood cells. Shaking and resting was repeated before the sample centrifuged at 4000 rpm, for 2 minutes.

5 mL of the supernatant liquid was aspirated by a micropipet, and transferred into the cell for analysis.

RESULTS AND DISCUSSION

Developed method of analysis

Though Anodic Stripping Voltammetry (ASV) using HDME electrode has been used extensively for the determination of trace elements, analysis of blood lead using this electrode is reported for the first time. This method is designated (ASV/HDME) in this work. At the employed pH(<1), the peak obtained is symmetrical and could be measured precisely (Figure 1) compared with peaks produced using other digesting reagents (Figure 2) such as METAEXCHANGE RE-AGENT which has been recommended by the Centers for Disease Control (CDC) for standard methods of blood lead analysis by ASV[20]. Working at the employed pH (<1) has the advantage of minimal adsorption of lead on either the polyvinyl tubes (that contain the digested blood sample) or on the analysis cell[21].

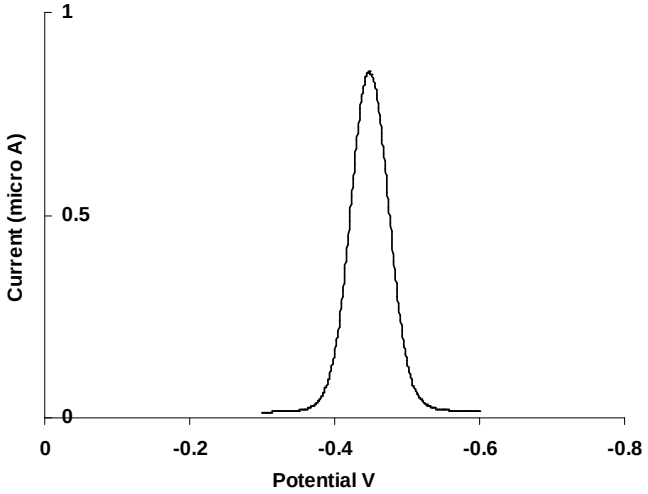

Figure 1 : Representive anodic stripping voltammogram for lead. Potential versus Ag/AgCl reference electrode.

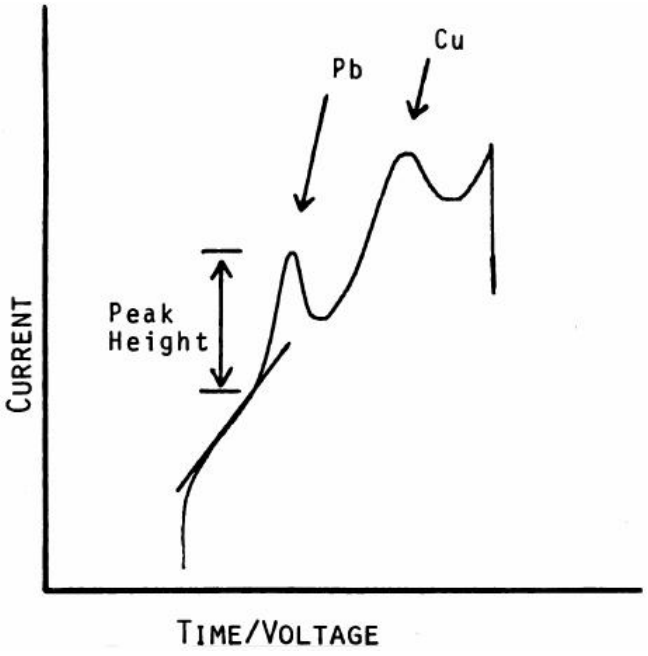

Figure 2 : Tracing of a blood sample containing 500 µg/l[20].

Effect of deposition time

The effect of deposition time on the peak current was studied for two standard concentrations of Pb^{2+}; 500 µg/L and 10^6 µg/L. For both concentrations, an enhancement of lead (II) concentration at the surface of the electrode is assumed as a result the increased peak current noticed with increasing accumulation time. However, at the lower concentration (500 µg/L), the peak current increased linearly with time up to (1000 sec.) without attaining a limiting value (Figure 3). For the other concentration (10^6µg/L), the peak current increased sharply, at first (200 sec.), then gradually up to about (700 sec.) where a limiting value is reached (Figure 4). This is, presumably, due to attainment of steady-state equilibrium of adsorption/complex formation and similar results have been reported for other stripping methods based on adsorptive accumulation[22,23]. Thus, to obtain a linear relationship between Pb(II) concentration and peak current, a relatively short preconcentration (adsorption) time was employed for high lead concentrations to avoid saturation effect.

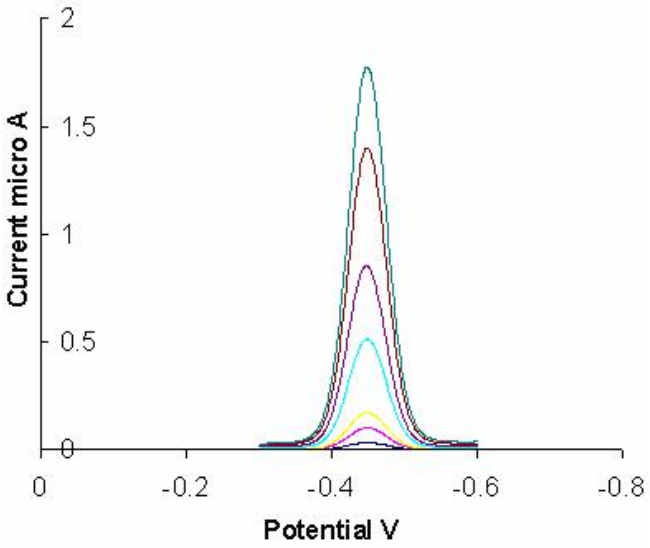

Figure 3 : Differential pulse anodic stripping voltammograms for lead concentration (500 µg/l), deposition increased as: 20, 50, 100, 500, 800 and 1000 seconds, purge time 30 s.

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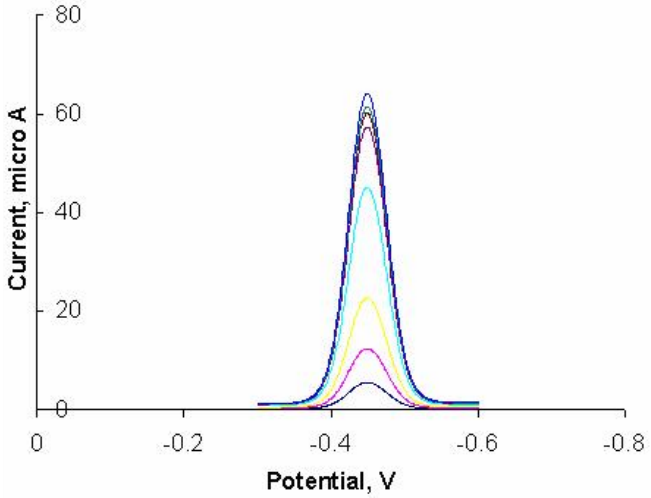

Figure 4 : Differential pulse anodic stripping voltammograms for lead concentration (10^6 µg/l), deposition increased as: 20, 50, 100, 300, 500, 600, 700 and 1000 seconds, purge time 30 s.

Calibration plots

The differential pules anodic stripping voltammograms at different concentrations of Pb (II) are shown in (Figure 5). The peak current increased linearly (r = 0.996) with Pb^{2+} concentration in the range 0–500 µg /L.

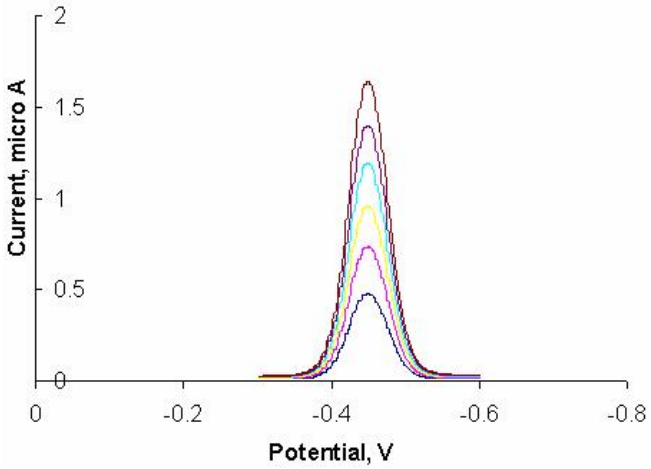

Figure 5 : Differential pulse anodic stripping voltammograms, calibration curve for lead concentration: 100, 200, 300, 400 and 500 µg/l, purge time 30 s.

Effect of purging time

It is known that dissolved oxygen is readily reduced at the dropping mercury electrode; an aqueous solution saturated with air exhibits two distinct waves attributable to oxygen, the first wave for

$$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$$

appears at about (0 to - 0.1 V), and the second wave for

$$O_2 + 4H^+ + 4e^- \longrightarrow 2H_2O$$

Appears at about (-1.0 V). Thus the dissolved oxygen

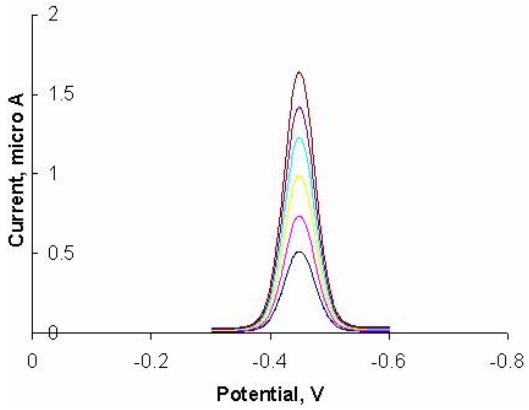

Figure 6 : Differential pulse anodic stripping voltammograms, calibration curve for lead concentration: 100, 200, 300, 400 and 500 µg/l, purge time 120 s.

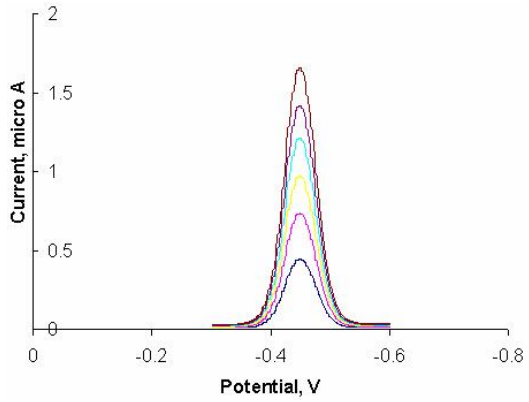

Figure 7 : Differential pulse anodic stripping voltammograms, calibration curve for lead concentration: 100, 200, 300, 400 and 500 µg/l, purge time 240 s.

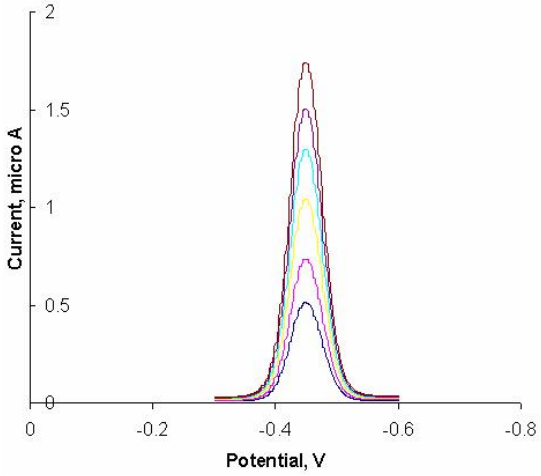

Figure 8 : Differential pulse anodic stripping voltammograms, calibration curve for lead concentration: 100, 200, 300, 400 and 500 µg/l, purge time 480 s

affects any species reduced in the vicinity of these potentials. However, the reduction potential for Pb (II) (-0.45 V) is not in this vicinity. The effect of purging time was studied for 0 to 8 minutes and found that there is no effect of purging time on the peak or on the calibration plot for the whole purging time range (Figures 5-8).

Effect of other ions

Chemically modified electrodes have significant analytical potential to enhance sensitivity and selectivity of determination because of modifier-analyte interaction. Ions such as those of Fe^{2+}, Ca^{2+}, Mg^{2+}, Zn^{2+} and Cu^{2+} present in the blood at a concentration comparable to that of Lead. The effect of the presence of several ions (10 mg/L) on the peak-pulse-high of Pb(II) (300 μg/L) resulted in a very small effects, if any (TABLE 1).

TABLE 1 : Change in differential pulse anodic stripping voltammetric peak high of 300 μg/L of lead (II) in the presence of 10 mg/L of other ions (60 sec. deposition time).

Ions added	Curent before addition (nA)	Curent after addition (nA)
Fe^{2+}	1000	1003
Ni^{2+}	1000	1005
Co^{2+}	1005	1000
Cr^{3+}	1003	1005
Mg^{2+}	1005	995
Ba^{2+}	1003	1007
Cu^{2+}	1000	1000
K^{1+}	997	1002
Al^{3+}	990	1000
Zn^{2+}	993	1002

Optimal conditions for blood lead analysis

The experimentally determined optimum conditions that give the best results are shown in TABLE 2.

TABLE 2 : Optimal conditions for blood lead analysis

Working electrode	H.D.M.E
Reference electrode	Ag/AgCl electrode
Auxiliary electrode	Pt wire
Initial potential	- 0.70 V
Final potential	- 0.37 V
Integration set point	- 0.43 V
Purge time	30 second
Deposition time	60 second
Scan rate	10 mV/s
Pulse amplitude	25 mV

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