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Development and validation of a voltammetric method for quantifying lead in rat blood

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Abstract*– Lead is a toxic heavy metal that does not have any biochemical or physiological role. On the contrary, it produces multisystemic alterations that could lead to the death of a living being. Although there are standardized methods for lead quantification, electroanalytical techniques are presented as a rapid, sensitive, and economical alternative. In this investigation, we developed and validated a voltammetric method for quantifying lead in blood. The voltammetric method was used to quantify lead in rat blood. The results showed a linear relationship with a determination coefficient (R^2) greater than 0.999. The method was precise with variation coefficients lower than 2.7% and accurate with recovery percentages close to 100%. The limits of detection and quantification were 0.000134 and 0.001433 mg/L, respectively. The obtained results propose an optimized alternative method for quantifying lead in blood with reliable results quickly.

Keywords- Lead, heavy metal, voltammetry, validation, blood.

I. Introduction

Lead is a heavy metal that is widely distributed. It is considered a xenobiotic that is hazardous to health because it is not an essential micronutrient and does not perform any biochemical or physiological function in the body [1].

The main routes of exposure are ingestion of lead-contaminated soil and dust, and inhalation. It is important to note that lead exposure should be avoided as much as possible. Lead exposure can cause acute and chronic intoxication. High exposure can lead to severe abdominal pain (colic), neurological symptoms, convulsions, encephalopathy, and even death [2], [3].

On the other hand, lead toxicity is considered multisystemic and can cause irreversible changes such as anemia [4], muscle fatigue, cognitive problems in children, hyperactivity, irritation, gastrointestinal problems [5], decreased fertility, reduced renal function, cardiotoxicity [6], accumulation in bones, etc [7].

Graphite furnace atomic absorption spectrometry and inductively coupled plasma source mass spectrometry are recognized and established techniques for quantifying lead in biological samples [8], [9]. However, electroanalytical techniques are presented as an alternative for quantifying this metal. This technique may even be more economical compared to others.

Voltammetric analytical techniques are less commonly used in industry and research compared to chromatographic, electrophoretic, and spectroscopic techniques. However, for specific analytical tasks, they can provide superior solutions.

Voltammetry is an electrochemical technique that measures current as a function of voltage, specifically the potential of the working electrode [10]. A typical voltammeter consists of an electrochemical cell that contains the working, auxiliary, and reference electrodes [11]. In this technique, a variable potential is applied to the working electrode in an electrochemical system, and the resulting current is measured [12].

Despite the international development of electroanalytical techniques several decades ago, accredited laboratories in some countries, such as Peru, do not routinely use these techniques to provide services to the public. Analytical laboratory regulators in Peru currently lack validated electrochemical methodologies.

This work presents a method for quantifying lead in blood using anodic stripping voltammetry (ASV) [9]. The optimized method provides reliable results in a short period of time, making it suitable for analyzing biological samples such as blood.

II. MATERIALS AND METHODS

A. Reagents and Equipment

Certipur standard solutions (1000 mg/L) of lead, nitric acid (HNO_3), and hydrogen peroxide 30% (H_2O_2), all purchased from Merck (Darmstadt, Germany), were used. All other reagents were of analytical grade. In addition, ultrapure water

(18.2 MΩ cm) obtained with an EASY pure II apparatus were used.

Standard solutions or modified matrix (blood plus lead) were prepared by homogenization. The biological sample (blood) was obtained from Wistar albino rats from the biotherium of the Catholic University of Santa Maria (research ethics committee A070416).

The modified matrix was performed in accordance with the Mexican Official Standard NOM-199-SSA1-2000 for Environmental Health, which sets criteria for blood lead levels and actions to protect the health of non-occupationally exposed populations [13].

The blood was obtained from the retro-orbital venous plexus [14] of Wistar albino rats weighing between 250-300 grams, in compliance with bioethical norms for animal research. Before the experiment, the rats were maintained in the biotherium (22-25 °C, 60 % ± 10 % relative humidity, 12:12 h light:dark cycle) they had free access to food and water.

Since the animals were not directly treated with lead, they were not euthanized. In addition, when necessary, samples were stored at 2 to 6 °C.

B. UV digestion of blood samples

To perform the lead reading in the voltammeter it is necessary to remove all types of organic matter, therefore, to remove organic components, the blood samples underwent pre-treatment through UV photolysis digestion (705 UV Digester, Metrohm). Each quartz tube contained 100 µL of sample, 100 µL of nitric acid, 20 µL of hydrogen peroxide, and 5 mL of ultrapure water. After 60 minutes of digestion at 95 ºC, the samples were cooled, transferred, and packed into 10 mL volumetric flask.

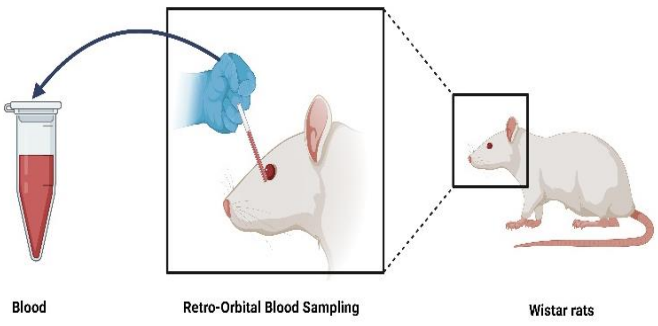

Fig.1. Diagram of blood withdrawal from the retro-orbital venous plexus.

C. *Condition Parameters for lead quantification by Voltammetry*

Before quantifying lead, electronic (linearity test and peak performance test) and chemical validation of Voltammetric station equipped were conducted. To quantify lead in blood samples, was used the Voltammetric station 797 equipped with an electrochemical cell containing three electrodes: a mercury hanging drop electrode (HMDE) as the working electrode, a platinum auxiliary electrode, and an Ag/AgCl/KCl reference electrode. The system was continuously supplied with ultrapure nitrogen gas.

The reading parameters were as follows: purge time of 300 seconds, deposition potential of −0.9 volts, deposition time of 60 seconds, sweep speed of 20 millivolts per second, pulse amplitude of 0.05 volts, pulse time of 0.04 seconds, initial potential of −0.74 volts, and final potential of −0.32 volts.

Lead quantification was performed using ASV with the standard addition method (Pb 1.0 ppm). Two milliliters of digested sample and 10 milliliters of 0.1M acetate buffer at pH 4.6 were transferred to the electrochemical cell.

All analyses were performed in triplicate.

D. Validation of the method for lead quantification by anodic stripping voltammetry.

The method was validated through linearity, sensitivity, precision, and accuracy parameters as detailed below.

a) Linearity

To determine linearity, we prepared six standard concentrations of lead in the modifier matrix: 0.025, 0.050, 0.075, 0.100, 0.125, and 0.150 ppm (mg/L). We transferred 2mL of digested sample and 10mL of 0.1M acetate buffer pH 4.6 into the electrochemical cell. We used the standard addition method (Pb 1.0 ppm) for voltammetric readings. We performed all analyses in triplicate.

The equation of the line was determined using the obtained values. The linearity of the method was evaluated by considering the coefficient of determination R^2.

$$y = a + bx \quad (1)$$

Where, "y" is the intensity of each reading expressed in nanoamperes (*nA*), "x" corresponds to the concentration of lead in the biological matrix expressed in mg/L. "a" is the intercept with the X-axis and "b" corresponds to the slope of the line.

b) Sensitivity

The method's sensitivity was determined by calculating the limit of detection (LOD) and the limit of quantification

(LOQ) expressed in mg/L. The LOD and LOQ represent the minimum amount of lead that the method can detect but not quantify and the minimum amount of lead that the method can accurately and precisely quantify, respectively [15].

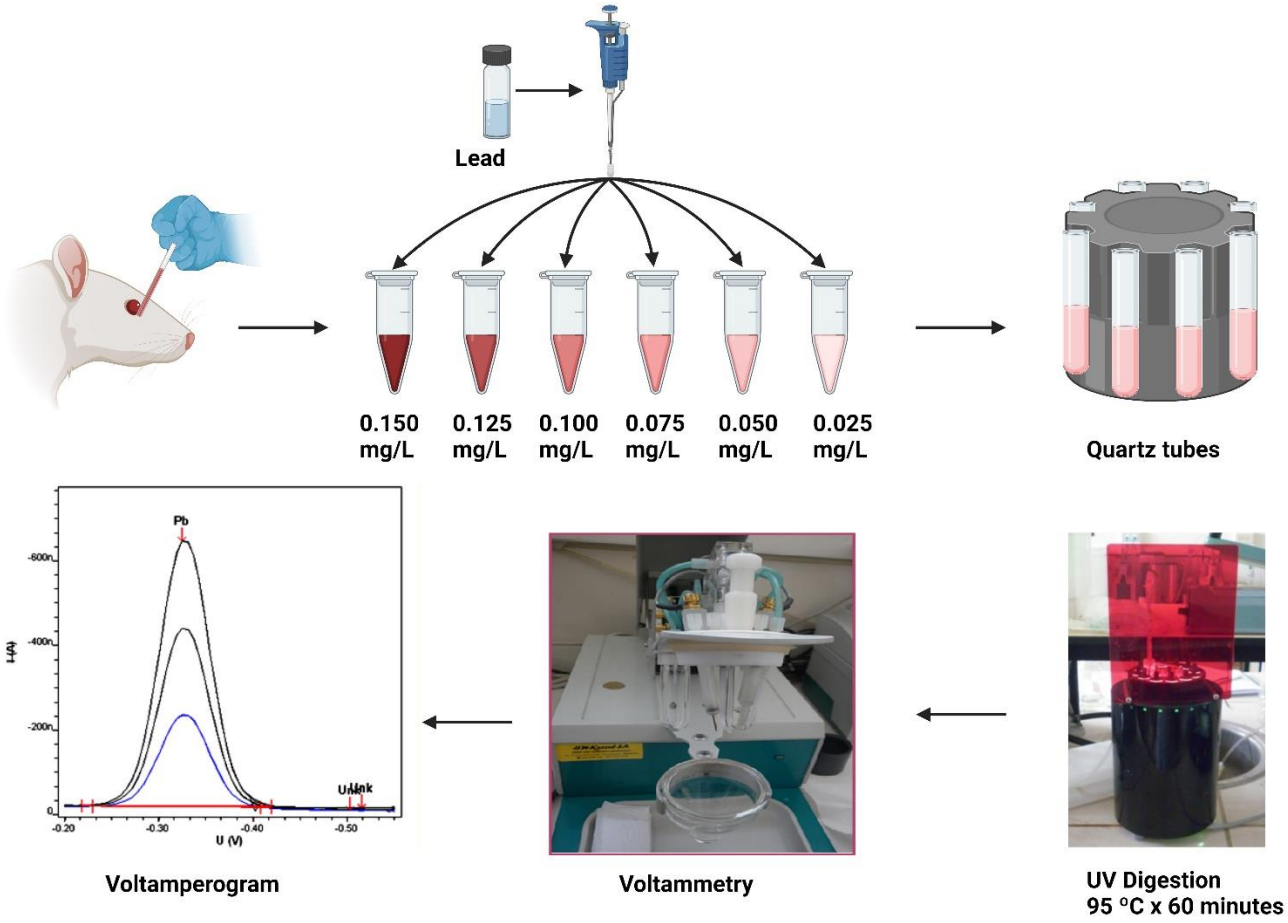

Fig.2. Diagram of voltammetric method for lead quantification in blood

The equations below were used to calculate the LOD and LOQ.

$$LOD = \frac{Y_{bl}+3S_{bl}}{b} X \frac{1}{\sqrt{n}} \quad (2)$$

$$LOQ = \frac{Y_{bl}+10S_{bl}}{b} X \frac{1}{\sqrt{n}} \quad (3)$$

Where, "Ybl" corresponds to the intercept of equation (1), "Sbl" corresponds to the intercept of the equation of the line relating the lead concentration to the standard deviation of the intensities (nA) found in the linearity calculation and "n" corresponds to the six standard lead concentrations.

c) Precision

Precision refers to the level of agreement among multiple measurements taken from a homogeneous sample under the same conditions. To assess the method's variability, repeatability and precision were evaluated.

For the repeatability analysis, three standard lead concentrations of 0.025, 0.075, and 0.150 mg/L were prepared in the modifying matrix. The samples were analyzed in triplicate under the same operating conditions, including the same analyst, voltammeter, and reagents, in the same laboratory and within a short period of time.

Precision was determined by calculating the coefficient of variation (CV) using the formula [16], [17].

$$CV = \frac{S}{x} X 100 \quad (4)$$

Where S is the standard deviation and *X* is the Mean.

d) Accuracy

The method's accuracy was determined by calculating the percentage recovery (%R) [16]. To do this, we first determined the initial concentrations (C_i) of lead in the blood samples. Next, we spiked these biological samples with 1.00 mg/L of lead (C_e) and then determined the final concentrations of lead in the spiked samples (C_f).

$$\%R = \frac{C_f - C_i}{C_e} X100 \quad (5)$$

III. RESULTS AND DISCUSSION

In the results, Fig. 3 and 4 display the outcomes of the electronic validation, including the linearity test and peak performance test.

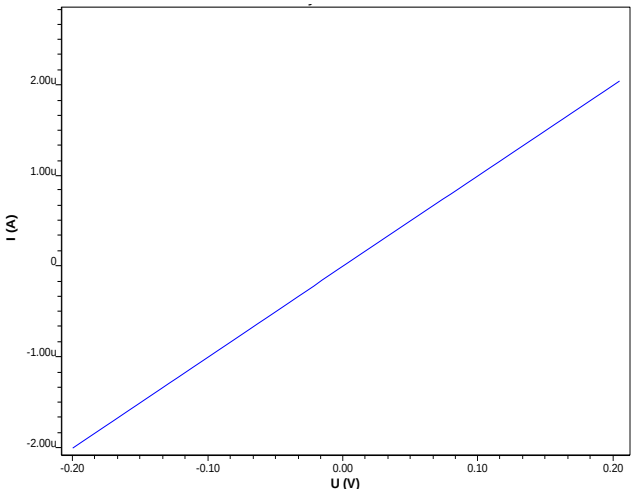

Fig 3. Electronic verification - Linearity

Fig. 3 displays a straight line obtained from a current range of −2 to 2 microamperes and a voltage range of −200 to +200 millivolts.

The tolerance for the minimum current is (−1.6 to −2.4 microamperes) and for the maximum current is (1.6 to 2.4 microamperes), as described by Metrohm parameters. Thus, electronic validation confirms linearity. Specifically, the voltage ramp performance was optimal for using the 757 VA voltammetric equipment.

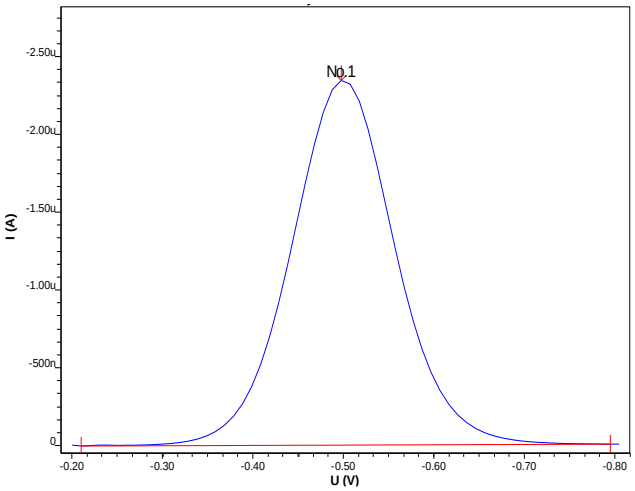

Fig 4. Voltammogram of lead quantification.

Fig. 4 shows a symmetric curve obtained from a maximum voltage of −497 mV and a maximum current of −2.35 µA. According to the parameters described by Metrohm, the established tolerance range for voltage is (−450 mV…−550 mV) and for current is (−2 µA…−4 µA). The symmetry of the curve and the reliability of the equipment confirm its good performance for the electronic validation of the 757 VA voltammetric equipment.

Regarding chemical validation, Figure 5 shows a typical voltammogram for the quantification of lead using the standard addition method with 1 mg/L.

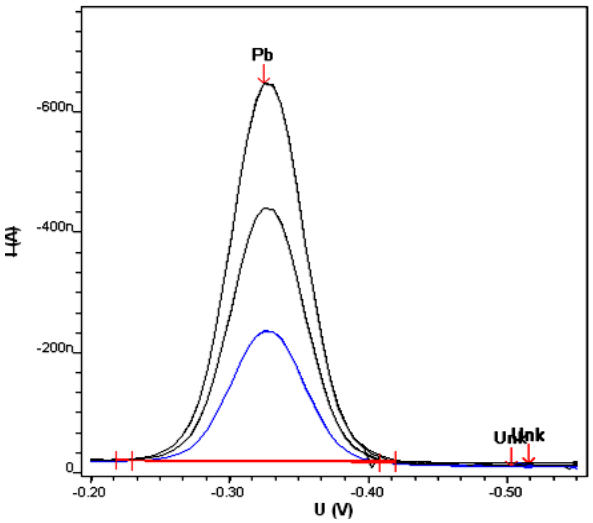

Fig. 5. Voltamperogram for lead quantification.

Fig. 6 shows the calibration graph of lead concentrations (mg/L) and intensity (nA) for each reading.

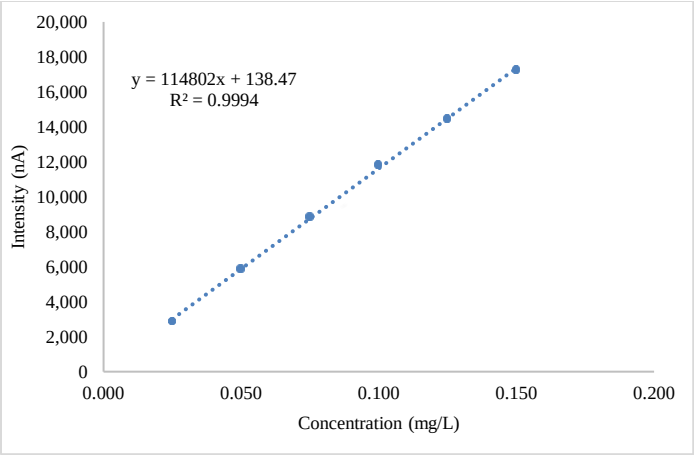

Fig 6. Calibration graph for lead quantification (R^2 = 0.9994).

Since the determination coefficient R^2 is greater than 0.995, it indicates that the voltammetric method for quantifying lead in blood samples is linear [15], Therefore, there is a direct proportional correlation between the intensity (nA) and the concentration of lead in mg/L.

The detection and quantification limits of the method for lead determination were 0.000134 and 0.001433 mg/L, respectively.

Table I shows the coefficients of variation (CV) for the quantification of three concentrations (0.025, 0.075, and 0.150 mg/L) of lead in blood samples. The percentage coefficients of variation are less than 2.70% [18], [19], indicating that our method is precise and repeatable.

TABLE I
COEFFICIENTS OF PERCENTAGE VARIATION OF THE PRECISION TEST (REPEATABILITY) OF THE VOLTAMMETRIC METHOD FOR QUANTIFYING LEAD IN BLOOD

Repetitions	**Intensity I (nA)**		
	0.025 (mg/L)	**0.075 (mg/L)**	**0.150 (mg/L)**
1	0.025	0.075	0.148
2	0.024	0.073	0.153
3	0.023	0.073	0.149
Average	0.024	0.074	0.150
Standard deviation (SD)	0.001	0.001	0.003
Variation Coefficient (%)	2.459	1.567	1.764

Table II shows the accuracy test results through the recovery percentages (%R). Therefore, the voltammetric method for quantifying lead in blood is accurate. A method is considered accurate when the recovery percentage is between 90 and 110% [20].

TABLE II
PERCENT RECOVERY OF THE ACCURACY ANALYSIS OF THE VOLTAMMETRIC METHOD FOR QUANTIFYING LEAD IN BLOOD

Repetitions	**Samples (mg/L)**	**Standard (mg/L)**	**Sample + standard addition (mg/L)**	**%R**
1	0.009	1.00	1.101	90.01
2	0.011	1.00	1.020	96.99
3	0.011	1.00	1.035	95.53
4	0.010	1.00	1.071	92.48
5	0.010	1.00	1.014	97.63
6	0.009	1.00	0.998	99.29
Average	0.010	1.00	1.040	95.322
Standard deviation (SD)	0.001	0.000	0.039	3.470

Two current techniques for determining lead in biological samples are graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma optical emission spectrometry (ICP-OES) [9], [21]. N. Hassanpour *et al*. [9] used the GFAAS technique to quantify lead in post-mortem blood samples and demonstrated its high sensitivity, reporting concentrations as low as 10 µg/dL (0.1 mg/L). Our results are comparable to theirs, with a concentration of 0. At 001433 mg/L, which corresponds to the quantification limit, it can be indicated that the voltamperometric method is equally or more sensitive than graphite furnace atomic absorption spectrometry (GFAAS), postulating it as a viable alternative.

Another study carried out by Arromba et al. [7] sought to Biomonitor lead concentrations in different organs of lead-poisoned rats using GFAAS and experimenting with different digestion techniques (acid and alkaline digestion) for sample preparation. Within the results, the researchers suggested that the type of digestion depended on the type of biological sample that was intended to be analyzed by GFAAS.

In the present research work, it was decided to carry out acidic UV digestion with hydrogen peroxide, which dissolves the components of the matrix thanks to the formation of highly reactive oxidant species (hydroxyl radicals, halogen radicals) which accelerate the decomposition of the interfering substances.

It is worth mentioning that the original GFAAS method for quantifying Pb in blood, published in 1993, has been significantly improved to achieve low detection limits of approximately 0.2 µg/dL (0.002 mg/L) and with the ability to quantify levels of Pb in blood up to 1 µg/dL (0.01mg/L) [21].

However, our validation results of the voltammetric method demonstrated detecting blood Pb levels up to 0.000134 mg/L (0.0134 µg/dL) with excellent accuracy and precision.

IV. CONCLUSIONS

A bioanalytical method was developed and validated for the quantification of lead in rat blood by anodic stripping voltammetry.

UV digestion of the samples and their subsequent analysis by the standard addition method (Pb 1.0 ppm) with purge time reading parameters 300 s; deposition potential: −0.9 V; deposition time: 60 s; sweep speed: 20mV/s; pulse width: 0.05 V; pulse time 0.04 s; initial potential: −0.74 V; and final potential: −0.32 V turned out to be linear, precise, exact with detection and quantification limits of 0.000134 and 0.001433 mg/L respectively.

The results obtained allow us to propose an optimized alternative method to quantify lead in biological samples such as blood with reliable results in a short time.

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