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Graphite Furnace Atomic Absorption Spectrometry for Biomonitoring of Blood Lead Level in Quality Control Blood Materials

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Abstract

Background & Objective: Lead (Pb) is a major toxicant that has threatened human health for years. There is no safe level of exposure and deposition of a small amount of Pb in the human body has a negative impact on an individual's health. Despite recent reductions in its use, it has caused extensive environmental contamination as well. In the present study, the intralaboratory quality control assessment is reported as an initial step in lead determination by Graphite furnace atomic absorption spectrometry (GFAAS).
Materials & Methods: Varian SpectrAA-220 with partition tube and deuterium background correction was used for the analysis. The evaluation quality control blood material was Seronorm trace elements whole blood (levels I &II).
Results: The lowest method limit of detection was 0.74 µg/dL, and based on the regression analysis, the lowest quantification concentration was 3.00 µg/dL (CV = 4.6 %). In addition, obtained %CV for Seronorm (I) & Seronorm (II) was 7.3% & 5.4% respectively.
Conclusion: In recent years, several diverse technological advancements in blood lead level determination have occurred. At the same time, demands for blood lead (BPb) level determination by GFAAS has been increased by public health in order to identify children exposed to lead in the environment, and by occupational health to reduce excessive exposure in the lead industries. The obtained results indicated that for BPb analysis, the method described by GFAAS is still a reference routine analytical technique that requires less time and no matrix matched standard curve, allowing blood samples to be run against aqueous calibration standards which eliminates the need for standard additions.

Keywords: Lead Exposure- Graphite Furnace Atomic Absorption- Biomonitoring- Precision-Intralaboratory.

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Introduction

Heavy metals toxicity is one of the oldest serious health and environmental concerns because of a wide variety of their applications in nutrition, occupational health, environmental health, toxicology, agricultural, etc. (1). After their accumulation, they cause various adverse effects in the body system, characterized by heavy metal-mediated toxicity (2). Lead or Plumb (Pb) as a non-essential heavy metal with no biological function, is a major acute and chronic environmental and occupational toxin that has threatened human health. It has a wide range of industrial uses by humans and its important sources relating to human

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lead exposure are described elsewhere (3-5). Children are at risk of lead exposure in low doses because they have higher intestinal Pb absorption and more vulnerable nervous systems which are still under development. They suffer irreversible neurological dysfunction that affect their learning ability and behavior (6, 7). In adults, however, chronic effects of lead exposure include elevated blood pressure, development of cancers, and neurodegeneration (8, 9). The clinical signs and symptoms of lead toxicity are nonspecific and in the majority of cases, children with lead toxicity are asymptomatic that result in a delay for appropriate diagnosis. However, during this time, effects at the cellular level are occurring, including impaired IQ and other cognitive effects, decreased heme synthesis, and disturbances in vitamin D metabolism (10). For the diagnosis of lead toxicity, a whole blood lead level measurement, preferably a venous blood lead concentration is essential. Tests involving heme precursors (urinary delta-aminolevulinic acid (ALA), coproporphyrin, and erythrocyte protoporphyrin (EP)) may be helpful in making a diagnosis, but by themselves are inadequate for definitive diagnosis (10). Current effective treatment intervention for lead induced toxicity is chelation therapy to promote efficiently metal excretion. However, chelators such as $CaNa_2EDTA$ can cause renal toxicity especially in subjects with previous history of kidney damage (11). Therefore, their usage is limited to severe cases of overexposure of heavy metals (11-14). During chelation and lack of specificity, other essential metals such as zinc, iron and manganese are also reported to be excreted and depleted (12). Considering these facts investigators pointed out that some antioxidants also act as chelating agents, to reduce some of the toxic effects of lead, either alone or in combination therapy. However, the usefulness of antioxidants has not been thoroughly investigated and detailed studies are also required to understand the underlying mechanisms (15).

Absorption of Lead and Its Internal Distribution in the Body

Inorganic lead is absorbed through the respiratory and gastrointestinal (GI) tracts, but not through the skin, although organic lead compounds are absorbed (16). Adults absorb about 10% of dietary lead while young children absorb 40% to 50% of dietary lead and that about one-half the amount absorbed is retained. Once absorbed into the blood compartment, lead is distributed throughout the body with the major fraction in the bone (95% in adults and about 70% to 75% in young, growing children) (7) and soft tissue. The rate of turnover of lead in soft tissues is within days. In contrast, lead accumulates in bone with turnover of a half-life of about 30 years and it can be mobilized from bone under physiological and pathophysiological conditions of bone resorption. Furthermore, the rate of turnover of lead in bone is higher in children than in adults. Although lead toxicity affects all body systems (17, 18), the most susceptible ones to lead toxic effects are erythropoiesis, kidney function, and the central nervous system function (19). Approximately, 99% of the lead in blood is bound to RBCs. The remaining 1%, e.g, plasma lead, serves as an intermediate in transporting lead from the erythrocytes to other body compartments. The organs of excretion of lead are kidney and liver and the disappearance time largely depends upon the degree of overall excess exposure; the greater the body lead burden, the slower the rate of disappearance from the tissues, including blood (20). The underlying mechanisms for these systemic effects of lead have not been explained completely. However, several studies suggest that the most probable cause is the generation of free radical and/or depletion of the antioxidant defense system (21-24) that causes oxidative stress. Lead forms covalent bonds with the sulfhydryl groups of proteins, making the enzymes most susceptible targets for lead, including antioxidant enzymes. In addition, lead

neutralizes glutathione that serves as a major thiol antioxidant. Furthermore, it interferes with some essential metals needed for antioxidant enzyme activities, and/or increases susceptibility of cells to oxidative attack by altering the membrane integrity and fatty acid composition. These interferences result in an imbalance between production and neutralization of reactive oxygen species (ROS) due to lead action (25, 26). Free radicals have been implicated into DNA damage, oxidation of thiol group(s) of proteins, and lipid peroxidation (23) which are associated with the onset of various diseases.

Methodology

From the early 1940s through the mid-1960s, the method of choice for blood lead BPb analysis was colorimetry/spectrophotometry using dithizone diphenylthiocarbazone (27, 28). Since then several new analytical advancements in BPb concentration determination have developed. These methods are very diverse and include Flame atomic absorption spectrometry (FAAS)(29, 30); Delves-cup FAAS (31, 32); Anodic stripping voltammetry (ASV)(33, 34); Graphite furnace atomic absorption spectrometry (GFAAS)(35,36); Inductively coupled plasma mass spectrometry (ICP-MS)(37); Thermal ionization mass spectrometry (TIMS; ID-MS) (38) and Point-of-care /near-patient testing, or a rapid diagnostic/ rapid screening test (39, 40). Point of-care methods are very simple to use and do not require trained laboratory personnel. These methods differ significantly in their analytical capacities (limits of detection, accuracy and precision), costs (purchase and maintenance, laboratory infrastructure required, reagents and supplies) and technical requirements (sample preparation, calibration and skilled personnel). Of the various analytical techniques available for measuring BPb, GFAAS continues to dominate methods routinely used by clinical laboratories for this purpose. Most GFAAS systems use an electrically heated graphite tube and programmed to control the temperatures for drying, ashing, and atomizing the sample injected onto the carbon rod (41, 42). In the present study, intralaboratory quality control assessment as an initial step in BPb determination by GFAAS-Varian SpectraAA-220 system; with partition tube and deuterium background correction is reported (10).

Materials and Methods

Calibration of the System

GFAAS: A Varian SpectrAA-220 system with deuterium background correction was set for the analysis. Spectrometer was equipped with Varian GTA-110 partition graphite tube atomizer and a programmable sample dispenser. Inert gas was high purity argon. Peak height was used as a measurement mode. The instrument was calibrated using aqueous working pb standards (10, 20, 30, 40 & 60 µg/L) made from Pb stock standard solution (1g/L). Solution volume injected was constant at 10 µL and two replicate readings were taken for standard solutions. At the time of analysis, quality control blood material (levels I & II) were manually diluted (1:10) with working ammonium dihydrogen phosphate modifier solution ($NH_4H_2PO_4$, Triton X-100 and HNO_3 modifier) and dispensed to the graphite tube. Then, the sample was heated to a temperature above 1700°C to atomize lead by absorbing light emitted from the Pb hollow cathode lamp at 283.3 nm. Other necessary chemicals and solutions used in the analysis were of a grade suitable for trace metal analysis and were purchased from Merck Co. The quality control blood material used for the evaluation procedures was Seronorm trace element & heavy metals whole blood (levels I & II) (Sero AS Asker-Norway). Deionized water (D.I-H_2O) was used throughout the reagent preparation and all working glassware was pre-acid-washed (10).

Precision Studies

Between-runs-precision studies were performed by reconstituting and pooling five Seronorm whole blood vials separately; quality control material levels (I & II) and analyzing aliquots of them (n=3) in 10 runs.

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Linearity Studies

The linearity study was determined by assaying neat & diluted pooled Seronorm II (n=8). Dilution was performed by a factor of 2 (1/2,1/4,1/8) & 1/20. Data were analyzed with linear regression using SPSS software.

Blank/Quantitation Limits

As a conventional model, Mean ± 3SD for detection limits was used. A blank (modifier) solution was run 15x and its concentration was determined against calibration curve. Mean ± SD was calculated and 3SD was chosen as instrument detection limit. For the method detection limit, Seronorm I (by factor 2) was diluted until its concentration reached closer to the instrument detection limit and then was run 10x. Mean ± SD was calculated and its 3SD was set as method detection limit (43).

Results

Precision study results for Seronorm levels I & II whole blood lead quality control materials are listed in Table 1 and obtained %CVs for Seronorm (I) & Seronorm (II) were 7.3% & 5.4% respectively. Linearity studies and quantitation determinations results are summarized in Table 2. Based on the obtained data, the lowest detectable concentration was 3.0 µg/dL which gave %CV of 4.6% and the" lowest method limit of detection" was 0.74 µg/dL.

Table 1. Between-runs precision assay evaluation for seronorm level I & II whole blood quality control materials

Seronorm Whole Blood Level	Measured value Pb µg/dL * mean ± sd	Obtained Range Pb µg/dL	%CV **	Brushour Range Pb µg/dL	Target value Pb µg/dL
I	3.3±0.24	2.96- 3.60	7.3	3.1 -3.9	3.4
II	36.95±2.0	33.60 -39.8	5.4	35.3- 44.3	40.1

* Between Runs Precision (10 runs & n=3) & ** Coefficient of Variation

Table 2. Linear regression analysis & quantitation determinations

Neat & Diluted *	Measured value µg/dL * mean ± sd	Slope	%CV
Seronorm Whole Blood Level II-Neat	34.6±o.69	0.96	2
1/2	17.96±0.24	0.96	1.4

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1/4	9.30±0.20	0.97	2.3
1/8	6.0±0.23	0.78	3.8
1/20	3.19±0.15	0.76	4.6
LLD (Instrument) **	0.15±0.097		
LLD (Method) ** *	0.74±0.18		

Independent Variable= Conc
* - n=8
** - n=15; 0.097x3=0.29
** * - n=10; 0.18 x 3=0.55

Discussion

The development of portable technologies for the measurement of lead concentration in blood has been strongly supported and encouraged by the CDC and has also been expected to measure a BPb concentration of 5 µg/dL (0.24 µmol/L) with lower cost per test than current laboratory-based methods (10). It is expected that these technologies will permit screening for BPb in locations such as lead clinics. They may also permit immediate retesting of children who are determined to have elevated BPb results. However, these devices tend to overestimate blood lead level compared to GFAAS (44). With ICP-MS it is feasible to determine lead in blood at trace amounts below 1 µg/dL (0.05 µmol/L) concentration and usually it is expected of methods that will meet modern research requirements. The greater sensitivity of the GFAAS (about 50 times) to the FAAS has made it possible to determine lead concentration in blood with no preconcentration, and requiring samples of only µL volumes (42). Furthermore, additional improvements in GFAAS technology for example; deuterium and zeeman background effect to correct the vaporized matrix components which are being atomized at the same time as the analyte and use of platform on which to deposit the sample has made it current for routine basis of a consensus method for BPb determination (44-46). Furthermore, for the purpose of analyzing blood Pb level, there are no spectral interferences from blood requiring zeeman background correction. High speed deuterium instruments have been successfully used for determining lead in blood (47).

Conclusion

In recent years, several technological advancements in blood lead level determination have occurred. These advancements are very diverse and include updated and enhanced older technologies as well as new analytical laboratory based technologies. At the same

time demands for BPb level determination by GFAAS has been increased by public health in order to identify children exposed to lead in the environment, and by occupational health to reduce excessive exposure in the lead industries (10). The purpose of the present study was to evaluate our GFAAS-Varian SpectrAA-220 performance for blood lead level determination using Seronorm whole blood quality control material (levels I & II). The obtained results indicated that regarding different analytical advancements as mentioned for this application (42, 48), Described intralaboratory GFAAS method still is a reference routine analytical techniques that requires less time and no matrix matched standard curve, which also permits blood samples to be run against aqueous calibration standards eliminating the need for standard additions.

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Conflict of Interest

None.

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