Anal. Chem. **1996,** *68,* 3199−3203

Ultratrace Determination of Lead in Whole Blood Using Electrothermal Atomization Laser-Excited Atomic Fluorescence Spectrometry

Eugene P. Wagner, II, B. W. Smith, and J. D. Winefordner*

Department of Chemistry, University of Florida, Gainesville, Florida 32611

Laser-excited atomic fluorescence has been used to detect lead that was electrothermally atomized from whole blood in a graphite furnace. A 9 kHz repetition rate copper vapor laser pumped dye laser was used to excite the lead at 283.3 nm, and the resulting atomic fluorescence was detected at 405.8 nm. No matrix modification was used other than a 1:21 dilution of the whole blood with high-purity water. Using the atomic fluorescence peak area as the analytical measure and a background correction technique based upon a simultaneous measurement of the transmitted laser intensity, excellent agreement for NIST and& CDC certified whole blood reference samples was obtained with aqueous standards. A limit of detection in blood of 10 fg/mL (100 ag absolute) was achieved.

The detection of trace levels of lead in blood has been a challenging analytical problem of long-standing interest.[1] The level of clinical concern in the United States is now 100 ng/mL and may be lowered even further.[2] The most widely used methods include electrochemical techniques, such as anodic stripping voltametry,[3] coulometric stripping potentiometry[4] and atomic absorption spectrometry.[5] Graphite furnace atomic absorption spectrometry is perhaps the most accepted technique and is widely used in clinical blood lead measurements.[6−8] The methodology, involving matrix modification with ammonium phosphate, nitric acid, and a surfactant is well developed and reliable at concentrations in whole blood above ∼50 ng/mL. When lower detection limits are required, isotope dilution inductively coupled plasma mass spectrometry (ID-ICPMS) has been used.[9] When coupled with electrothermal sample introduction, this method has achieved a limit of detection of 16 fg.[10] Determinations of lead at these low levels is complicated by the possibility for contamination during sample preparation and from reagents used for matrix modification. As the level of concern for lead in blood has dropped over the past 25 years, and will most likely continue to be reduced, there is a need for increased accuracy and precision in measurement of lower levels of lead in blood. There is also a recognized need for measurements of substantially lower blood lead levels to establish the threshold of subclinical lead toxicity in humans. To date physiologic relationships between lead doses and toxic responses have been primarily based upon individuals suffering from elevated exposure to lead.[11] It would also be advantageous to develop an analytical method that can measure lead levels in blood as well as in the environment at concentrations well below the established levels of concern. In doing so, the option of monitoring and preventing lead concentrations before they reach a critical level of concern is possible.[12] However the difficulty of using and maintaining trace metal-clean techniques for any analytical method that requires matrix modification places it at a disadvantage for detection at these low lead levels.

Undoubtedly the most successful atom reservoir for laser-excited atomic fluorescence spectrometry (LEAFS) has been the graphite furnace. Commercial furnace technology has been highly developed for use in atomic absorption spectrometry and is widely available and especially practical from the standpoint of sample introduction. Moreover, its adaptation for atomic fluorescence measurements is straightforward. Spatial interaction of the laser excitation with the atomic population is highly efficient in the furnace, and practical optical detection of the fluorescence can be made with a pierced mirror in a 90° geometry. Absolute limits of detection in the low femtogram range have been obtained by several workers for a variety of elements. Lead is especially amenable to detection by ETA-LEAFS, having an easily accessible excitation wavelength at 283.30 nm and a strong direct line fluorescence, which is well separated spectrally, at 405.7 nm. As early as 1974, Neumann and Kriese[13] obtained a limit of detection of 200 fg for lead in a furnace and since that time numerous studies have resulted in improvements which have made limits of detection of 1 fg for lead in aqueous solutions routine.[14−17] Up to the present, ETA-LEAFS has never been applied to the detection of lead in a blood matrix. The purpose of the present study was to evaluate the performance of ETA-LEAFS in a carefully optimized

(1) Parsons, J. *Environ. Res.* **1992**, *57*, 149−162.
(2) *CDC, Preventing Lead Poisoning in Young Children-A Statement by the Centers for Disease Control;* October, USDHHS/PHS, CDC: Atlanta, GA, 1991.
(3) Feldman, B. J.; D'Alessandro, A.; Osterloh, J. D.; Hata, B. H. *Clin. Chem.* **1995**, *41*, 557−563.
(4) Jagner, D; Wang, Y. D. *Electroanalysis* **1995**, *7*, 614−618.
(5) Miller, D. T.; Paschal, D. C.; Gunter, E. W.; Stroud, P. E.; D'Angelo, J. *Analyst* **1987**, *112*, 1701−1704.
(6) Bannon, D. I.; Murashchik, C.; Zapf, C. R.; Farfel, M. R.; Chisolm, J. J., Jr. *Clin. Chem.* **1994**, *40*, 1730−1734.
(7) Parsons, P. J.; Slavin, W. *Spectrochim. Acta* **1993**, *48B*, 925−939.
(8) Jacobson, B. E.; Lockitch, G.; Quigley, G. *Clin. Chem.* **1991**, *37*, 515−519.
(9) Paschal, D. C; Caldwell, K. L.; Ting, B. G. *J. Anal. Atom. Spectrom.* **1995**, *10*, 367−370.
(10) Bowins, R. J.; McNutt, R. H. *J. Anal. Atom. Spectrom.* **1994**, *9*, 1233−1236.
(11) Flegal, A. R.; Smith, D. R. *Environ. Res.* **1992**, *58*, 125−133.
(12) Renner, R. *Environ. Science Technol.* **1995**, *29*, 256A−261A.
(13) Neumann, S.; Kriese, M. *Spectrochim. Acta* **1974**, *29B*, 127−137.
(14) Vera, J. A.; Leong, M. B.; Omenetto, N.; Smith, B. W.; Womack, B.; Winefordner, J. D. *Spectrochim. Acta* **1989**, *44B*, 939−948.
(15) Bolshov, M. A.; Zybin, A. V.; Smirenkina, I. I. *Spectrochim. Acta* **1981**, *36B*, 1143−1152.
(16) Cheam, V.; Lechner, J.; Sekerka, I.; Desrosiers, R.; Nriagu, J.; Lawson, G. *Anal. Chim. Acta* **1992**, *269*, 129−136.
(17) Dougherty, J. P.; Preli, F. R., Jr.; Michel, R. G. *J. Anal. Atom. Spectrom.* **1987**, *2*, 429−434.

S0003-2700(96)00358-7 CCC: $12.00

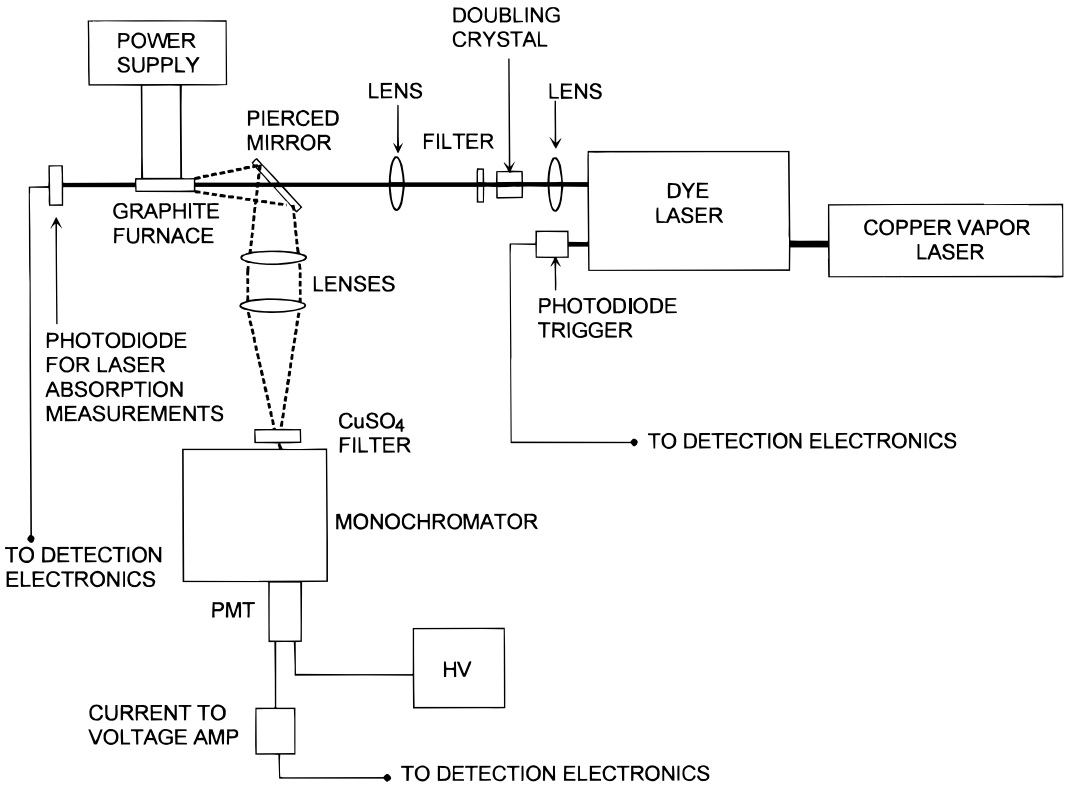

Figure 1. Experimental design for ETA-LEAFS.

experiment for the detection of lead in whole blood. The goal was to develop an approach involving a minimum of sample handling or treatment with the lowest possible limit of detection in this very complex matrix.

EXPERIMENTAL SECTION

This type of laser-excited atomic fluorescence spectrometer has been described in several previous publications.[14,18,19] For these experiments, a copper vapor laser (Model CU15-A, Oxford Lasers, Acton, MA) was used to pump a dye laser (Model DLII, Molectron) at a repetition frequency of 9 kHz. Although this laser possesses the capability of operating at frequency repetition rates up to 12 kHz, the optimum pulse power has been found to be at ~9 kHz. The dye laser (Rhodamine 590, Exciton Corp., Dayton, OH) output was frequency doubled in a KDP crystal and the fundamental frequency filtered to obtain the 283.3 nm excitation line that was typically 1.2 μJ in a pulse duration of 4 ns (fwhm). The dye laser is tuned to the appropriate excitation wavelength by placing a Pb hollow cathode lamp behind the furnace so that the laser beam was in line with the Pb cathode. This gave a constant Pb atom supply that made tuning the dye laser to the fluorescence excitation wavelength simple. There was no spectral interference from the blood matrix in the vicinity of the lead 405.8 nm fluorescence transition line. The resulting spectral irradiance (1×10^5 W cm^{-2} nm^{-1}) was insufficient to saturate the 283.3 nm lead transition in the ETV environment. The beam was focused through a 4 mm diameter hole in a plane mirror and into the graphite furnace (Perkin Elmer, Model HGA-400, Norwalk, CT) by a 25.4 cm focal length fused-silica lens (Figure 1). The furnace was modified by substituting the conventional windows with 1 mm thick fused-silica windows mounted at 45° to minimize laser scatter into the monochromator. Pyrolytically coated graphite furnace tubes with solid pyrolytic graphite platforms were used for atomization. In the furnace, the laser beam was rectangular in shape, due to frequency doubling, with dimensions approximately 4 mm long and 0.5 mm in height. The pierced plane mirror was positioned at 45° in reference to the laser and ~9 cm in front of the furnace. The emitted fluorescence was imaged onto the monochromator (Model 340E, 340 mm focal length, $F/n = 5.9$, 1200 groves/mm holographic grating, Spex, Edison, NJ) entrance slit by a matched pair of 8 in focal length biconvex fused-silica lenses. The monochromator was positioned on its side so that the slit geometry would match that of the laser beam, which appeared as a horizontal rectangle. The slit width was 0.5 mm and the height was masked to 4 mm to match the laser beam geometry as well as to minimize the detection of blackbody emission from the furnace. A 1 cm path length solution filter (100 g/L $CuSO_4$ in water) was placed in front of the entrance slit to eliminate laser scatter, and neutral density filters were used as necessary to maintain detector linearity. The signal was detected with a PMT (R928, Hamamatsu Corp., Bridgewater, NJ), and the current pulse from the PMT was converted to a voltage pulse using a simple laboratory-constructed transimpediance amplifier which consisted of an OP-37 operational amplifier (Precision Monolithics, Inc., Santa Clara, CA) with a 10^4 kΩ feedback resistor in parallel with a 15 pF capacitor. This circuit was powered by two 9 V batteries. The signal was then processed with a boxcar integrator (SR245, Stanford Research, Sunnyvale, CA) interfaced to a personal computer. Blood reference materials were obtained

(18) Leong, M.; Vera, J.; Smith, B. W.; Omenetto, N; Winefordner, J. D. *Anal. Chem.* **1988**, *60*, 1605−1610.
(19) Smith, B. W.; Farnesworth, P. B.; Cavalli, P.; Omenetto, N. *Spectrochim. Acta* **1990**, *45B*, 1369−1373.
(20) Alvarado, J.; Cavalli, P, Omenetto, N.; Rossi, G.; Ottaway, J. M.; Littlejohn, D. *Anal. Lett.* **1989**, *22*, 2975−2984.

Table 1. Parameters for the PE HGA-400 Graphite Furnace

A. Setup for Inital Studies with 2 μL Injection of Whole Blood

	dry	dry	ash	atomization	clean
temp °C	180	260	850	1900	2600
ramp (s)	5	5	5	0	1
hold (s)	10	10	20	8	4
Ar flow (30 mL/min)	on	on	on	off	on

B. Final Program for 10 μL Injections of Whole Blood Diluted 1:21 with Millipore Water

	dry	dry	atomization	clean	cool	clean
temp °C	180	260	1900	2500	20	2600
ramp (s)	5	5	0	1	5	1
hold (s)	10	35	8	4	10	4
Ar flow (30 mL/min)	on	on	off	on	on	on

from the Center for Disease Control and Prevention (CDC) Blood Lead Laboratory Reference System (BLLRS) and from the National Institute of Standards and Technology (NIST).

RESULTS AND DISCUSSION

Conventional blood sample preparation techniques used for graphite furnace atomic absorption involves a 1:10 dilution of the whole blood with a matrix modifier solution containing 0.2 % (w/v) $NH_4H_2PO_4$, 0.5 % (v/v) Triton X-100, and 0.2 % HNO_3 in ultrapure water.[5−7] However, in order to make full use of the extremely high sensitivity of the ETA-LEAFS technique, the use of a matrix modifier needed to be eliminated due to its relatively high level of lead contamination of ∼8 ppb. Instead, the CDC blood lead standards were not modified in any way for the initial studies. The furnace program used in the initial studies involved two drying steps, an ash or pyrolysis step, atomization, and a cleaning step as shown in Table 1.[6] The fluorescence peak profiles obtained for a 2 μL injection of whole blood lacked precision as well as intensity as shown in Figure 2. Further evaluation of these peak profiles revealed that a fluorescence signal was present during the ashing step. Ashing temperatures as low as 500 °C were used to eliminate the fluorescence peak during this step. However, the fluorescence peak was always present during ashing at these temperatures. Without the use of matrix modifiers, the lead vaporized along with the organic matrix during this step because of the high viscosity of the whole blood and the strong complexes formed between the lead and the organic matrix. Therefore, since the use of a matrix modifier was not an option for these experiments, the ash step was eliminated and the second drying step was extended in duration (Table 1). By going straight to the higher temperature atomization step from the drying step, the lead organic complexes would vaporize and atomize. The new furnace program resulted in fluorescence peak profiles that were 1.5 s fwhm with a reproducible shape and good intensity (Figure 2). However, the poor precision for the 2 μL whole blood injections was not acceptable. A large part of this poor precision was due to the difficulty encountered when attempts were made to pipet the very viscous whole blood into the furnace. The imprecision may also have been due to the strong lead−organic complexes formed in blood and the viscosity of the whole blood, which would not allow complete atomization of the lead. Instead, to make the blood easier to pipet the whole blood was diluted 1:21 with purified water to reduce the viscosity (Millipore, Marlborough, MA) and atomization was run five times for each blood sample. This sample preparation method resulted in a within run precision of 3.7% for the certified lead in blood standards.

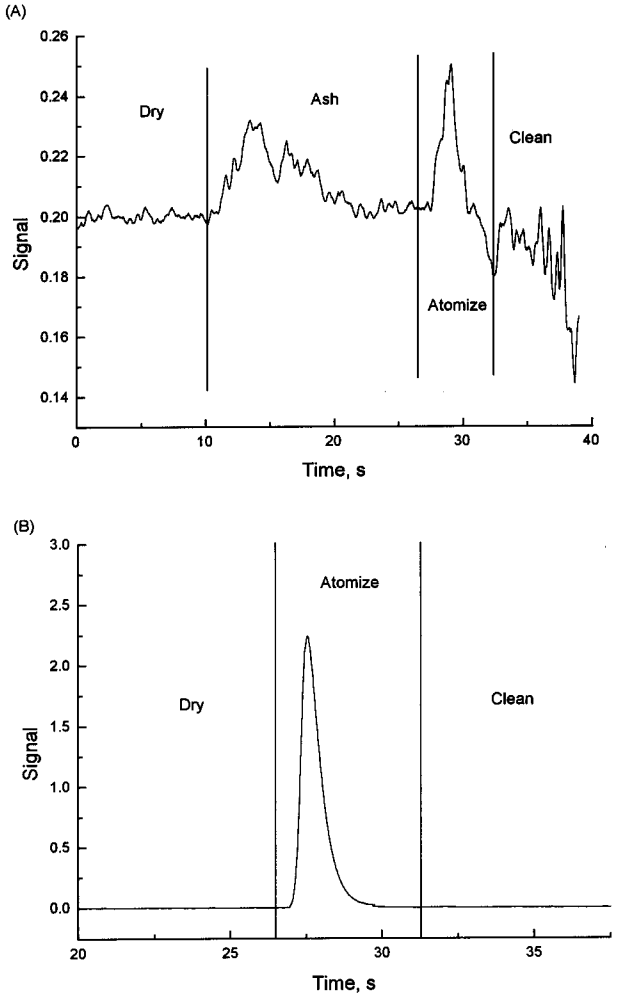

Figure 2. (A) Typical fluorescence signal for lead in blood before the elimination of the ash step in the furnace program. Note that a fluorescence signal was present during the ashing step. (B) Typical fluorescence peak shape for a 2 μL injection of 50 ppb lead blood sample after elimination of the ash step in the furnace program.

The CDC blood standards were compared to a calibration curve constructed with aqueous lead standards (Figure 3). The fluorescence signal intensity for a blood sample was ∼20% lower than for an aqueous standard with the same lead concentration. The reduced signal with blood samples may have been caused by the smoke generated from the sample as it was heated to the atomization temperature. As the smoke filled the analytical volume, it attenuated the laser beam intensity and may have done the same for the emitted fluorescence. To test this hypothesis, a photodiode was positioned after the furnace in the path of the laser. The laser intensity was then monitored throughout a typical run for aqueous solutions and whole blood solutions that were diluted to various ratios using ultrapure water; 10 μL of each of these solutions was injected into the furnace. Figure 4 shows the obvious decrease in laser intensity for the blood samples during the atomization step. This fluctuation coincided with the evolution of the smoke and with the appearance of the fluorescence peak. The slight increase in laser intensity prior to the atomization step coincided with the stopped argon flow before

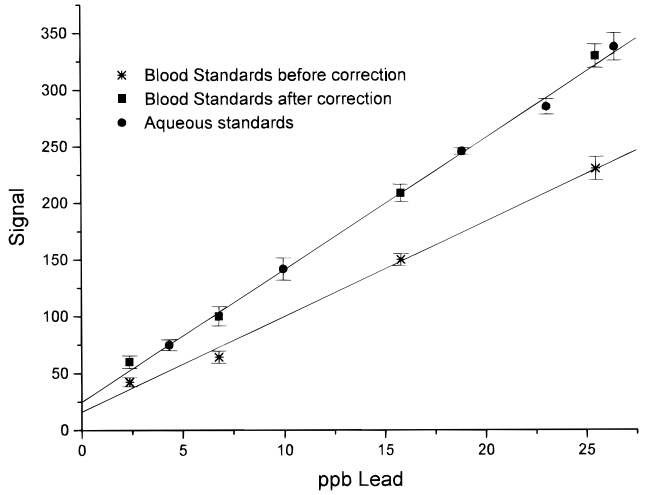

Figure 3. Correlation of aqueous standards with blood standards diluted 1:21 with Millipore water before (*) and after (■) correction of laser intensity.

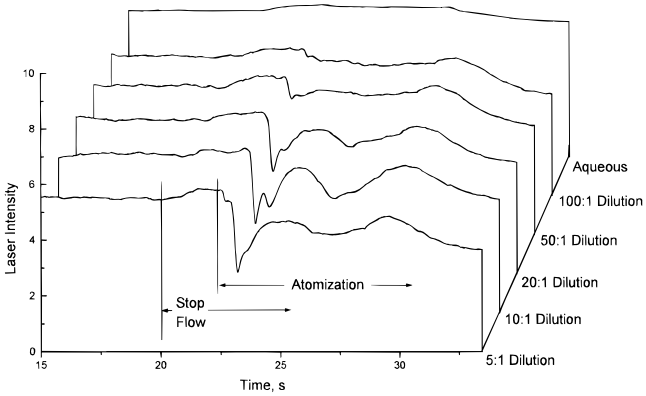

Figure 4. Comparison of laser intensity after exiting the furnace during a typical run for 10 μL injections of aqueous samples and whole blood diluted to various ratios.

this step. The decrease in laser intensity for the blood samples was directly proportional to the absolute amount of blood contained in the sample and the amount of smoke generated by the sample. From these observations and experiments, it was concluded that the decreased signal of the blood samples as compared to the aqueous standards could be corrected by using the photodiode, which measured the laser intensity, as a scaling factor. This was achieved by incorporating a two-channel signal processor system in which the fluorescence signal in channel one was divided by the laser intensity fluctuation measured in channel two. This signal correction process was used on the blood samples, which were diluted 1:21 with ultrapure water, as well as the aqueous solutions to account for any laser intensity change. The results of this process, Figure 3, prove that the signal attenuation of the blood samples was due to the evolution of smoke in the graphite furnace which attenuated the laser beam intensity. The two-channel measurement technique provided a good correlation between aqueous and blood matrices for the measurement of lead.

A blood matrix calibration curve was constructed using the CDC lead in blood standards; the NIST lead in blood standards (SRM 955a, 1-4) were treated as unknowns. The lead concentrations obtained were compared to the certified values. The NIST standards were also evaluated by the standard addition method. Each NIST sample was measured after the 1:21 dilution with

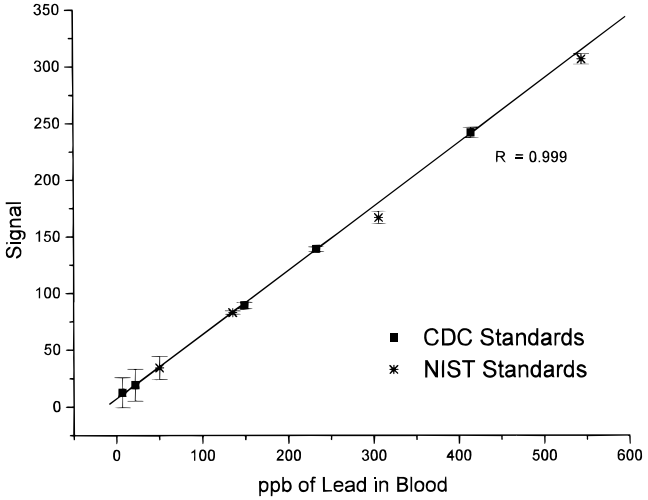

Figure 5. Calibration curve constructed using CDC certified standards. The NIST certified standards were experimentally determined and the values were plotted to compare the accuracy of the ETA-LEAFS technique.

Table 2. Comparison of Lead in Blood NIST Standards with Values Obtained In These Studies (ppb)

NIST sample	NIST certified	calibration curve[a]	standard addition[a]
455a-1	50.1 ± 0.9	48.1 ± 5.0	56.6 ± 5.3
455a-2	135.3 ± 1.3	134.4 ± 1.9	125.7 ± 14.6
455a-3	306.3 ± 3.2	283.0 ± 15.4	287.1 ± 26.1
455a-4	544.3 ± 3.8	530.3 ± 25.1	530.7 ± 48.7

[a] ± values are confidence intervals for 99% probability level.

Millipore water and then after each of three subsequent spikings with an aqueous lead standard. The linear regression curve fit correlation was better than 0.999 for the four NIST standards (Figure 5). The lead values obtained for this method are reported in Table 2 and correlate well with the certified values and the values obtained by the calibration curve method. The value obtained for the NIST 455a-3 standard was the only sample that fell outside of the 99% probability confidence interval. This standard was also evaluated by a different independent method in our laboratory with similar results. This fact leads us to believe that the certified value for the 455a-3 standard may be slightly high and questionable. Finally, the 3σ LOD for lead in blood was calculated to be 10 fg/mL, or 100 ag absolute. This is 7 orders of magnitude lower than the current level of concern for lead in blood and 2 orders of magnitude lower than the current technique of ID-ICPMS, used by the CDC and NIST, which involves a complicated chemical treatment.

CONCLUSION

The good precision of the values for lead in blood obtained by ETA-LEAFS in these studies is within the same range as furnace atomic absorption spectrometry[5−8] and is comparable to ETV-ICPMS.[10,20] The small volume needed for analysis combined with the extremely low detection limits make it a good alternative to ETV-ICPMS when the sample size is limited. Furthermore, the ability to detect the analyte of interest in a complex matrix without complicated sample preparation reduces the possibility of contamination and is an important advantage over AAS and ETV-ICPMS. The cost of this type of setup is competitive ($100K−

$150K) when compared to ICP systems ($170K−$500K). This may still seem a little expensive since ETV-LEAFS is a single-element analysis method. However, ETA-LEAFS is able to detect ultratrace levels of lead in blood and does not require blood reference standards for calibration. This is not possible with any other analytical method available. Also, it is no more complicated to use than AAS or ICP instruments and every part of the ETV-LEAFS setup is commercially available. In fact, with respect to sample preparation, it is a much simpler method. The ability of the two-channel system to correct for the blood matrix and laser variations and correlate the blood standards to the aqueous standards simplifies the calibration curve process and eliminates the need for blood standards for this technique. The good correlation achieved between blood and aqueous solutions by using the two-channel background correction approach proves that the fluorescence signal attenuation was due to the evolution of smoke, which attenuated the laser beam, and was not due to fluorescence quenching by the smoke or other interferences. It may be possible to eliminate the two-channel system if a laser of sufficient power to saturate the lead transition was available. Also, any variations that may occur due to differences in blood matrices can also be corrected by using this method. The advantages of this system as compared to AAS or ETV-ICPMS make it a viable option for detection of lead in blood, especially at extremely low levels.

ACKNOWLEDGMENT

The authors gratefully acknowledge support from the United States Department of Energy, Grant DOE-DE-FG05-88-ER13881, and from the Centers for Disease Control and Prevention (Atlanta, GA), Grant R08/CCR408614-02.

Received for review April 10, 1996. Accepted June 27, 1996.[⊗]

AC9603587

[⊗] Abstract published in *Advance ACS Abstracts,* August 1, 1996.