Micromethod for Lead Determination in Whole Blood by Atomic Absorption, with Use of the Graphite Furnace

Frank J. Fernandez

I describe a micro-scale method for determining lead in whole blood by utilizing a graphite furnace. Sample pretreatment consists of fivefold dilution with a dilute surfactant. The method is directly calibrated with lead standards prepared in dilute HNO₃. To eliminate a small, nonspecific absorption signal from the blood matrix, simultaneous background correction is used. Interlaboratory comparison with a flame atomic absorption technique that requires extraction yielded high correlation (r = 0.98). Within-run precision (coefficient of variation) ranged from 2 to 4%. Lead in blood can be accurately measured in as little as 20 µl of blood, hence the method is suitable for routine laboratory use and for pediatric screening.

Additional Keyphrases: conventional flame atomic absorption compared • toxicology • lead poisoning • screening • trace elements • lead in plasma, urine

In the clinical laboratory, the improved sensitivity and decreased sample requirements for flameless atomic absorption devices offer significant advantages, particularly for measuring lead in blood. Micro-scale procedures for the determination of lead in whole blood by using the carbon rod atomizer (1–4) and heated tantalum ribbon (5, 6) have included various sample pretreatment steps. Evenson and Pendergast (7) described a method for determining lead in erythrocytes with use of a graphite furnace (the Perkin-Elmer Model HGA-2000). A simpler procedure is presented here for determining lead in whole blood by using a graphite furnace (HGA-2100). Results are compared to those obtained by a conventional flame atomic absorption method.

Materials and Methods

Apparatus

An atomic absorption spectrophotometer (Model 503; Perkin-Elmer Corp., Norwalk, Conn. 06856) equipped with a Deuterium Background Corrector and a graphite furnace (Model HGA-2100, Perkin-Elmer) was used. A wavelength of 283.3 nm and a spectral slit width of 0.7 nm were used. Peak signals were registered on a strip-chart recorder (Model 056, Perkin-Elmer). All reference measurements were made with an atomic absorption spectrophotometer (Model 305A, Perkin-Elmer) equipped with a three-slot burner head and operated with an air–acetylene flame.

The HGA-2100 was operated with an internal flow of nitrogen or argon purge gas (normal mode, 15 ml/min, or a setting of 10 divisions on the HGA controller). For determining lead in blood, the following temperature program was experimentally selected as optimum: dry at 100 °C for 25 s, ash at 525 °C for 50 s, atomize at 2300 °C for 9 s.

Reagents

Water. De-ionized water is used throughout.


Diluent containing surfactant (Triton X-100, scintillation grade; Eastman Organic Chemicals, Rochester, N. Y. 14650). Prepare a 1 ml/liter aqueous solution.


Lead working standards; 0, 50, 100, 150, and 200 µg/liter. Prepare in dilute (5 ml/liter) HNO₃ by appropriate dilution of the lead stock solution. With the
fivefold sample dilution, the working standards correspond to 0, 250, 500, 750, and 1000 µg of Pb per liter in the original sample.

Glassware

All glassware was acid washed overnight in HNO₃ (4 mol/liter), then thoroughly rinsed with de-ionized water.

Venous blood samples were collected in heparinized Vacutainer tubes (Becton, Dickinson and Co., Rutherford, N. J. 07070). Samples were diluted by using Eppendorf micro centrifuge tubes (polypropylene, 1500 µl with snap cap; Arthur H. Thomas Co., Philadelphia, Pa. 19105). Eppendorf microliter pipets were used for both sample dilution and injection.

Procedure

Pipet 200 µl of the diluent into a micro centrifuge tube.

Add 50 µl of whole blood and mix well. To minimize the transfer error associated with pipetting viscous samples such as whole blood with Eppendorf type micropipets, flush the pipet tip (blood transfer) several times with the diluted sample.

Inject 15 µl of the diluted sample directly into the HGA-2100. (The temperature program was described in the Apparatus section.) Determine the lead content from a standard curve obtained by injecting 15 µl of the lead working standards.

The flame atomic absorption procedure with which the present method is compared was that described by Zinterhofer et al. (8).

Results

Sample Preparation

Because of a nonspecific background signal generated by the blood matrix, it is not possible to analyze whole blood with the HGA-2100 without background correction. The use of various dilution ratios was investigated, and it was found that a fivefold sample dilution provides the best compromise between adequate sensitivity and low background absorption. With a 15-µl sample aliquot, it is possible to obtain sufficient sensitivity for blood lead measurements at normal values without use of instrument scale expansion. We found that when blood samples were diluted with de-ionized water, there was a rapid loss of sensitivity. Observing the interior of the graphite tube after several samples had been analyzed, we noticed a buildup of residue from the blood matrix in the tube, a phenomenon that made impractical the use of simple aqueous dilution. Hemolysis of the blood by dilution with the diluent eliminated this residue buildup problem.

Nonspecific Absorption

For blood lead measurements with the HGA-2100, we found use of the Deuterium Background Corrector (9) to be necessary. Even after ashing at 525 °C for 50 s, a small, nonspecific background signal from the blood matrix is present. Ashing temperatures greater than 550–575 °C cannot be used without loss of lead. Figure 1 shows tracings for lead determined in a blood sample with and without use of the Deuterium Background Corrector. To verify that complete correction for nonspecific absorption was being obtained, I also analyzed the sample (with D₂ correction) at 280.3 nm, a wavelength at which lead is non-absorbing. No absorption signal was observed, confirming that use of the Deuterium Background Corrector provides complete correction. Because the nonspecific absorption signal varies from run to run, simultaneous background correction is necessary for accurate analyses.

Interferences

To check for chemical interferences, I studied the effect of adding various anions and cations to lead nitrate solutions. Lead solutions containing added chloride, phosphate, sodium, potassium, iron, calcium, and magnesium—in concentrations corresponding to approximate concentrations in blood diluted fivefold—were analyzed by the procedure outlined. Except for sodium and potassium, none of these anions and cations had any effect on the lead signal. If either sodium or potassium was added to lead nitrate solutions the lead signal was depressed. However, sodium and potassium do not interfere with the analysis of blood specimens. Figure 2 shows calibration curves obtained for lead standards prepared in dilute (5 ml/liter) nitric acid and standards prepared with added sodium and potassium, 400 mg of each per liter. Also shown is the calibration curve for a blood sample analyzed by the method of standard additions. The blood sample analyzed by additions yields a curve that parallels the curve for the acid lead standards over the entire analytical range, but the curve for the lead standards with added sodium and potassium has a much different slope. Because of the complex composition of whole blood, it is not surprising that interference effects will be significantly different in blood than in aqueous lead solutions. Evenson and Pendergast (7), using an HGA-2000 graphite furnace, also noted that interference effects caused by Na and
K were markedly different in aqueous standards than in whole blood.

Anticoagulants studied for possible interference effects included heparin, oxalate, citrate, and ethylene diaminetetraacetate. At concentrations up to about twice that normally used for anticoagulation, I saw no interference from heparin, oxalate, or ethylene diaminetetraacetate. However, concentrations of citrate greater than 8 g/liter of blood depressed the lead signal. Calibration with use of the method of standard additions is therefore recommended for samples containing sodium citrate.

No chemical interference from the diluent containing Triton X-100 was observed; therefore, it is not necessary to add Triton to the lead standards used for calibration.

Recovery was studied by adding known amounts of lead nitrate to several blood samples (Table 1). The average recovery of added lead over a concentration range of 150 to 1000 µg/liter was 98%.

Precision

The analytical precision for various concentrations of blood lead, both within-run and day-to-day, is shown in Table 2.

### Table 1. Recovery of Lead Added to Blood

<table>
<thead>
<tr>
<th>Lead added</th>
<th>Expected</th>
<th>Recovered</th>
<th>Av Recovered, %</th>
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<td>µg/liter</td>
<td></td>
<td>Av</td>
<td>Range</td>
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<tr>
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<td>—</td>
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</tr>
<tr>
<td>1000</td>
<td>1160</td>
<td>1130</td>
<td>1090–1160</td>
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</table>

* Sum of endogenous lead (150 µg/liter) and added lead.
  † Based on results of three separate experiments.

### Table 2. Reproducibility of Blood Lead Measurements, at Various Concentrations of Lead

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Mean</th>
<th>SD</th>
<th>CV, %</th>
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<tr>
<td></td>
<td>µg/liter</td>
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<tr>
<td>Within-run reproducibility</td>
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<tr>
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<td>720</td>
<td>29</td>
<td>4.1</td>
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* Based on 10 repetitive determinations.
† Based on five determinations over a five-day period.

### Comparison Studies

To verify method accuracy, a comparison study was performed with the cooperation of the Yale–New Haven Medical Center. Heparinized, venous blood samples from patients were analyzed by the method described and by a flame atomic absorption method (8). The study was double blind, with the flame analyses being done at Yale and the HGA-2100 analyses performed at our laboratory. Lead was measured by the flame method in 3- to 6-ml samples of blood. The graphite furnace analysis was calibrated directly with lead nitrate standards. Samples were analyzed in duplicate, and the resulting peak absorbance readings averaged. Figure 3 shows the correlation plot ob-
tained for 102 samples analyzed over a seven-week period. As indicated by the regression equation and correlation coefficient (0.98), results obtained with the HGA-2100 agree well with measurements obtained with the macro extraction method.

Discussion

Although 50 μl of blood was used for most of our work, we have found that accurate results can be obtained with as little as 20 μl of blood. As with any micro-scale blood lead method, it is important that the sample be well mixed and be free of clots. All of the data reported were obtained for venous blood. The method is suitable for analysis of capillary blood specimens, if proper care is exercised in sample collection and handling. Bratzel and Reed (10) have recently described sampling procedures and possible sources of error in the collection of capillary blood specimens.

Because of the relatively low atomization temperature used (2300 °C), the graphite tubes have a useful life of about 150–200 firings. Allowing for the graphite furnace temperature programming and cooling time, a single determination requires about 2 min.

The HGA-2100, the latest model of the graphite furnace, is designed differently than earlier versions of this system (HGA-70 and HGA-2000, Perkin-Elmer). When data for blood lead obtained from use of both an HGA-2000 and HGA-2100 were compared, results with the HGA-2000 unit were consistently low (regression coefficients ranged from 0.79 to 0.88). Observing the interior of the graphite tube (HGA-2000) after injection of a blood sample, we noticed that the sample spread out along the axis of the tube. In contrast, aqueous solutions coalesced into a droplet in the center of the tube. The HGA-2100 makes use of a much smaller graphite tube that has a series of shallow grooves on either side of the sample injection hole. These grooves are incorporated to contain the sample in the center of the tube and prevent spreading, and in fact we observed no spreading of sample solution (blood) along the tube.

In a preliminary way, I studied how to improve the sensitivity of the method for the determination of lead in both urine and plasma. The direct injection of 25 to 50 μl of urine or plasma, together with use of 2X to 5X scale expansion, provided adequate sensitivity. However, the injection of urine or plasma (directly or diluted twofold) produced background absorption signals so large that the Deuterium Background Corrector could not completely compensate. In view of the matrix problems experienced in analyzing urine or plasma directly, I investigated the use of a preliminary solvent extraction procedure. Using the extraction method of Zinterhofer et al. (8), I could obtain sufficient sensitivity for lead measurements as low as 1 μg/liter. Separating the lead from the matrix eliminated the background absorption problem. These preliminary investigations suggest that with use of a preliminary extraction, the HGA-2100 method may also be suitable for the determination of lead in urine and plasma.

We thank Dr. Peter I. Jatlow (Yale-New Haven Medical Center) for his advice and cooperation in providing samples and comparison flame atomic absorption results.

References