

Determination of Lead in Blood by Square Wave Anodic Stripping Voltammetry at a Carbon Disk Ultramicroelectrode

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A method for blood lead ([Pb]) analysis is developed based on square wave anodic stripping voltammetry (SWASV) in an Hg²⁺-containing solution using a 10- μ m-diameter carbon disk ultramicroelectrode. SWASV eliminates interference from O₂ in unpurged blood-derived sample solutions, and filtration of acidified samples through nitrocellulose reduces the concentration of interfering substances, increasing the size and improving the uniformity of peak currents. Blood [Pb] values obtained by SWASV are compared with blood [Pb] values determined by graphite furnace atomic absorption spectrophotometry; good accuracy and reasonable coefficients of variation in the range 10.6-20% (average of 13.5%) are found for blood [Pb] ranging from 4 to 30 μ g/dL. Preliminary experiments using a carbon disk microarray electrode suggest future improvements in sensitivity.

Elevated human blood lead concentrations (blood [Pb]) are associated with damage to the kidney, the liver, and the gastrointestinal tract as well as with neurological damage and decreased hemoglobin production. Recent evidence^{1,2} suggests that neurological damage in children may occur at blood [Pb] as low as 10 μ g/dL. In response to this and similar studies, the Centers for Disease Control (CDC) recently lowered its blood [Pb] action level, for the third time since 1975, from 25 to 10 μ g/dL.

The CDC's action greatly increases the number of people classified as having elevated blood [Pb] levels.^{3,4} In fact, the CDC estimates⁴ that 3-4 million children nationwide may have elevated blood [Pb] levels. The frequency of analytical blood [Pb] determinations has increased, and there is interest in increasing the accuracy, precision, and convenience of these determinations.⁵ Demand for a portable instrument capable of testing blood [Pb] at remote locations is likely to increase. Presently, most blood [Pb] determinations are made by graphite furnace atomic absorption spectroscopy (GFAA)⁶ or anodic stripping voltammetry (ASV).⁷ GFAA methods are accurate, but the instrumentation is relatively expensive and bulky (not portable), and the analysis requires considerable

expertise. Commercially available ASV instrumentation (utilizing large area graphite-Hg composite electrodes)⁸ is less expensive and more easily operated but the method suffers from reduced accuracy at blood [Pb] of less than 10 μ g/dL.

Improved electrochemical methods offer promise of developing an accurate, portable method for blood [Pb] analysis. Ostapcsuk⁹ and Jagner et al.¹⁰ reported accurate blood [Pb] determinations based on potentiometric stripping analysis (PSA), which is advantageous because stripping peaks are unaffected by dissolved O₂. Jagner et al. used a flow system based on a disposable carbon fiber microelectrode for increased sample throughput. Wang and Tian^{11,12} reported PSA determinations, without added Hg, of Pb²⁺ in water and urine at disposable screen printed carbon electrodes, although the technique was not extended to blood samples.

We are interested in developing a voltammetric electrochemical method of blood [Pb] determination based on (micrometer diameter) ultramicroelectrodes,¹³ for a number of reasons. Very small sample volumes can be analyzed. Hemispherical diffusion of Pb²⁺ reduces the need for stirring,¹⁴ and could lessen flow-rate dependence in a flow injection system.¹⁵ Small current flow (with consequent small *iR* voltage drop) allows high-frequency stripping waveforms, even in resistive cells. Use of microarray electrodes (now commercially available¹⁷) results in an improvement in the signal/noise ratio proportional to the square root of the number of elements in the array.¹⁸ This last advantage is unavailable in PSA, where stripping time is independent of electrode area.¹⁹ The use of a microdisk electrode also offers advantages over use of a carbon fiber, since disk electrodes can be polished and reused.

However, two problems confront the development of voltammetric blood [Pb] methods, both of which stem from the complexity of the blood matrix. First, O₂ removal is problematic, since it is time-consuming and inert gas sparging results in excessive foaming. This is important, since dissolved O₂ interferes with most stripping techniques by causing a

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sloping base line which reduces the signal/noise ratio.²⁰ Second, the blood must often be highly diluted to reduce the concentration of interferents which can decrease analytical response by adsorption onto and fouling of the electrode surface. The commercially available ASV instrument (Model 3010B by Environmental Science Associates) requires that the blood sample be diluted by a factor of 30 into an acidified medium containing Ca^{2+} and Cr^{3+} , among other ingredients. This large dilution factor reduces accuracy and precision.

We report here a blood [Pb] method based on square wave anodic stripping voltammetry (SWASV) at a 10- μm -diameter carbon disk ultramicroelectrode which addresses the above concerns. Square wave electrochemical analysis has been shown to be very sensitive,²¹ and SWASV is preferable to the more commonly used differential pulse anodic stripping voltammetry since it can be used in O_2 -containing solutions.²² In this work, the technique has worked equally well in an O_2 -containing (unsparged) blood-derived sample matrix. Electrode fouling is greatly reduced by simple filtering of the acidified blood sample on nitrocellulose, which irreversibly adsorbs species (present even after acidification) which can foul the electrode surface. This results in improved electrode lifetime with more concentrated sample solutions and allows a dilution ratio (blood volume/analytical sample volume) of 1:10. Accuracy and precision of the method are evaluated by comparison with GFAA determination of blood [Pb] in a number of samples. Preliminary results with a carbon disk microarray electrode suggest that sensitivity is much increased compared to the single carbon disk electrode.

EXPERIMENTAL SECTION

Equipment and Reagents. Electrochemistry was performed on a BioAnalytical Systems (West Lafayette, IN) BAS 100B/W electrochemical analyzer with a PA-1 low-current module and a C-2 cell stand. Pb standard additions were dispensed with an automatic pipetter-diluter (Syva Inc., Palo Alto, CA). Glass-shrouded carbon disk ultramicroelectrodes used were BAS MF2007, Princeton Applied Research (Princeton, NJ) GO226, and Cypress Systems (Lawrence, KS) EE017. Polyethylene-shrouded carbon disk ultramicroelectrodes were constructed as previously reported.²³ Microarray electrodes were obtained from Ecosse Sensors (Long Hanborough, U.K.) and consisted of 287 exposed carbon disks with a nominal diameter of 15 μm . The Ag/AgCl reference electrode for experiments with single C disks was BAS Model RE-4. The microarray electrode was equipped with its own solid-state reference electrode, which was determined to have a potential of +28 mV vs Ag/AgCl. (All potentials here are expressed vs Ag/AgCl.) The electrochemical cell for the glass-shrouded carbon disk electrode was constructed by drilling three holes (for reference electrode, working electrode, and standard addition tube) in the screw-top cap of a 5-mL conical Teflon vial (Cole-Parmer, Niles, IL). The microarray cell was similarly constructed, using a 1.5-mL polyethylene vial (Baxter Diagnostics, McGaw Park, IL), except that a single slit accommodated the working/reference electrode combination.

Microcentrifuge tubes (1.5 mL) were from Fisher Scientific (Pittsburgh, PA), as were disposable 1-mL syringes. Nitrocellulose and poly(tetrafluoroethylene) (PTFE) syringe filters (both were 25 mm diameter and 0.2 μm pore size) were from Cole-Parmer and Alltech (Deerfield, IL), respectively.

Nitric acid and hydrochloric acid were Fisher Optima grade. Mercury (Aldrich, Milwaukee, WI) was triply distilled. Lead standards were prepared by dilution of a 1015-ppm atomic absorption standard (Aldrich). The 10-ppm standards were prepared biweekly; 39.2 $\mu\text{g}/\text{dL}$ standards for addition to the electrochemical cell were prepared daily.

Minimization of Extraneous Pb. All sample preparation and analytical voltammetry were performed in a class 10 000 clean enclosure. Water was purified on a Milli-Q water purification system (Millipore, Bedford, MA) and had a resistivity of 18 Ω cm. Concentrated HCl and HNO_3 were certified to contain 0.0010 and 0.0012 $\mu\text{g}/\text{dL}$ Pb, respectively. All electrochemical cells (Teflon), sample preparation tubes, and disposable syringes (polyethylene) were soaked in 1–2 M nitric acid for at least 24 h and then triply rinsed with H_2O . Cypress Systems EE017 carbon disk ultramicroelectrodes were used for all blood analyses, since they consistently introduced less Pb^{2+} contamination than did other glass-shrouded carbon disk ultramicroelectrodes. Contaminating Pb^{2+} from carbon disk ultramicroelectrodes was further reduced by sonication (Branson 52 sonicator, Branson Inc., Shelton, CT) for 30 min or more in concentrated HNO_3 after the electrode was polished on 0.05- μm alumina (Buehler, Inc., Lake Forrest, IL). Microarray electrodes were cleaned by soaking for at least 1 h in 1 M HCl. Reference electrodes were stored in 3 M KCl, to which 0.1 M HCl was added to promote leaching of Pb^{2+} from glass. Nitrocellulose syringe filters were rinsed with 10 mL of 1 M HCl and then rinsed with 0.1 M HCl and stored containing 0.1 M HCl for at least 1 h. Finally, the filters were rinsed with 5 mL of H_2O and dried overnight at 60 $^\circ\text{C}$.

Sample Preparation for Blood [Pb] by SWASV. Blood samples were anonymous specimens from a California state-sponsored Pb survey program and were stored in Becton Dickinson (Rutherford, NJ) royal blue top tubes which contained EDTA as an anticoagulant. Either 100 or 120 μL of blood was pipetted into a microcentrifuge tube, diluted with 700 μL of H_2O , and then treated with 200 μL of concentrated HCl. The tube was briefly (1 min) shaken and centrifuged for 2 min at 15600g. The supernatant was withdrawn into a disposable 1-mL syringe and filtered through a 25-mm-diameter nitrocellulose filter at a rate of approximately 0.1 mL/s. A 500- μL portion of filtrate was mixed with 100 μL of 0.51 mM Hg^{2+} and 1.2 M HCl. Reagent blanks consisted of H_2O substituted for blood in the above procedure.

Analytical Voltammetry. Analyses were performed on the BAS 100B/W using the Osteryoung square wave stripping voltammetry (OSWSV) mode. Deposition was at –1000 mV for 120 s, with no quiet time. A square wave with frequency 250 Hz, amplitude 25 mV, and potential step size 4 mV was applied until a final potential of 300 mV was reached. Data were smoothed using a single pass of the moving average filter. Treated blood samples and blanks were analyzed by duplicate stripping analyses, spiked with a single standard addition of 5.86 $\mu\text{g}/\text{dL}$ Pb^{2+} , and then reanalyzed in duplicate.

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Blood [Pb] by GFAA. Analyses were performed on a Perkin Elmer 3030 atomic absorbance spectrophotometer. 100 μL of blood was diluted with 900 μL of matrix modifier, which consisted of 500 $\mu\text{L/L}$ Triton X-100 (Mallinkrodt, St. Louis, MO), 1.11 g/L ammonium dihydrogen phosphate (Aldrich), and 0.278 g/L magnesium nitrate (Aldrich). Samples were aspirated into an HGA 400 (Perkin Elmer) graphite furnace, vaporized in two steps at 130 and 220 $^{\circ}\text{C}$, pyrolyzed at 800 $^{\circ}\text{C}$, atomized at 1700 $^{\circ}\text{C}$, and absorbance determined at 283.3 nm. Quantification was by aqueous Pb^{2+} calibrators. Accuracy was verified by participation in the AACC/CAP Interlaboratory Comparison Program for blood lead.

RESULTS

Since the ratio of the circumference/area of a disk electrode increases with decreasing radius, 10- μm -diameter ultramicroelectrodes are almost entirely "edge" and are quite sensitive to any impurities in the shroud material. As discussed in the Experimental Section, Pb^{2+} contamination in glass-shrouded carbon disk ultramicroelectrodes was minimized by the judicious choice of working electrode and by the sonication of electrodes in concentrated HNO_3 prior to blood analysis. Prior to acid sonication, extraneous Pb^{2+} peaks as large as 56 nA (for a 60-s deposition time) were observed using some electrodes. Acid sonication decreased extraneous Pb^{2+} in all electrodes tested, but only those electrodes in which Pb^{2+} contamination could not be detected (less than 0.11 $\mu\text{g/dL}$ for 120-s deposition) in 0.1 mM Hg^{2+} and 0.1 M HCl were used in subsequent blood [Pb] determinations.

An alternative approach involved the use of polyethylene-shrouded electrodes.²³ They were found to be free of Pb^{2+} contamination and functioned well immediately after construction. However, polishing with alumina resulted in erratic electrode performance, and cleaving with a scalpel (to expose a fresh carbon surface) inconsistently produced a functioning electrode. Therefore, the polyethylene-shrouded electrodes were essentially single-use, and their use was abandoned in favor of acid-sonicated, glass-shrouded ultramicroelectrodes.

The importance of filtration to electrode performance is illustrated in Figure 1, which compares electrode performance in three identical acidified blood samples which were (A) unfiltered, (B) filtered through poly(tetrafluoroethylene) (PTFE), and (C) filtered through nitrocellulose. For each curve, a single identical blood sample was analyzed for 10 consecutive stripping cycles using a single electrode which was polished to start the set but was not repolished between scans. The electrode was potentiostated at +300 mV for 20 s between scans to remove all electrodeposited Hg. It is expected that peak current (for a single polishing) should be constant through a series of 10 scans. A small, random variation in peak current between polishings might be expected due to small changes in electrode area resulting from polishing.

For unfiltered (A) and PTFE-filtered (B) samples, peak currents are small and erratic, indicating that the electrode is being fouled by some substance in solution. In contrast, peak currents are much larger and remain nearly constant (there is a ca. 15% increase after the first scan) in the nitrocellulose-filtered (C) samples, indicating that the interfering substance has been largely removed by the nitrocellulose. This result was observed qualitatively in many trials, although

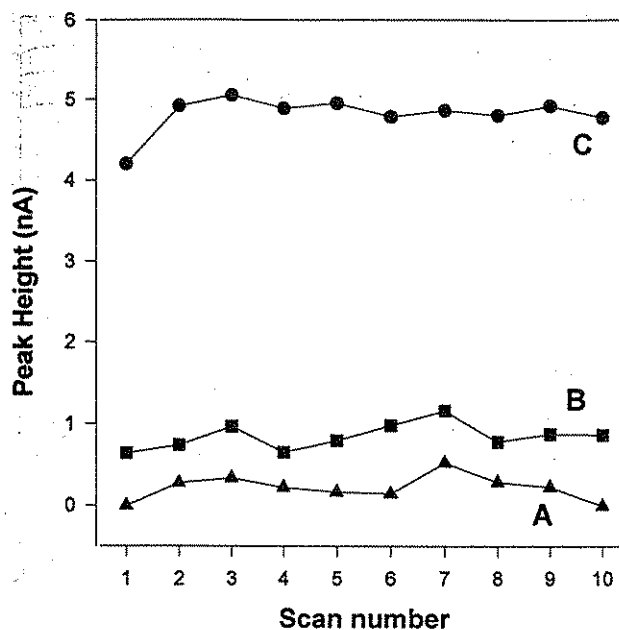


Figure 1. Current response of carbon disk electrode in blood-derived sample solutions during sequential scans of identical, blood-derived sample solutions. Blood sample pretreatment as in Experimental Section. (A) \blacktriangle , no filtration. (B) \blacksquare , PTFE filtration. (C) \bullet , nitrocellulose filtration. Deposition time, 60s; deposition potential, -1000 mV vs Ag/AgCl; $[\text{Hg}^{2+}]$, 0.072 mM; added $[\text{Pb}^{2+}]$, 2.98 $\mu\text{g/dL}$. Electrodes were potentiostated at +300 mV vs Ag/AgCl for 20 s between scans to remove excess accumulated Hg.

the extent of electrode fouling in unfiltered solutions varied substantially in different blood samples.

The linearity of stripping peak current with [Pb] in a blood-derived solution was verified by measuring the response to added Pb^{2+} (five added [Pb] levels from 0 to 9.3 $\mu\text{g/dL}$ were evaluated) over a concentration range bracketing the standard addition (5.86 $\mu\text{g/dL}$) used in blood analyses. The correlation coefficient (r) for the resulting line was 0.9938.

Figure 2 shows the stripping voltammograms resulting from a typical blood [Pb] measurement. Curve A is a reagent blank (water substituted for blood) showing no measurable Pb. Curve B shows the stripping peak due to sample, and curve C shows the sample plus an added 5.86 $\mu\text{g/dL}$ Pb^{2+} spike. Analysis of curves B and C (along with their duplicates, not shown) gives a blood [Pb] of 33.8 $\mu\text{g/dL}$, compared to a nominal value (from GFAA) of 30.2 $\mu\text{g/dL}$.

Table 1 summarizes the results of a series of blood [Pb] measurements. The reagent blank (water) gives an average Pb^{2+} contamination corresponding to that obtained (after sample dilution) from a 0.8 $\mu\text{g/dL}$ blood sample; this Pb^{2+} contamination is rather variable and is believed to come largely from the nitrocellulose filter (see below). Blood samples show good accuracy when compared to nominal [Pb] obtained by GFAA, especially at higher (>15 $\mu\text{g/dL}$) blood [Pb]. The standard deviation for the blood samples is nearly constant (3.1–3.2 $\mu\text{g/dL}$) for blood [Pb] > 15 $\mu\text{g/dL}$ and is somewhat smaller (in absolute terms) at lower blood [Pb]. The coefficient of variation (C.V.) varies from 10.6% ([Pb] = 30.4 $\mu\text{g/dL}$) to 20.0% ([Pb] = 3.7 $\mu\text{g/dL}$), with an average of 13.5%, and is generally higher for the lower blood [Pb] samples.

Increased sensitivity can increase precision, especially at low blood [Pb], since noise is proportionally lessened. In Figure 3, a microarray electrode (B) (containing 287 carbon elements,

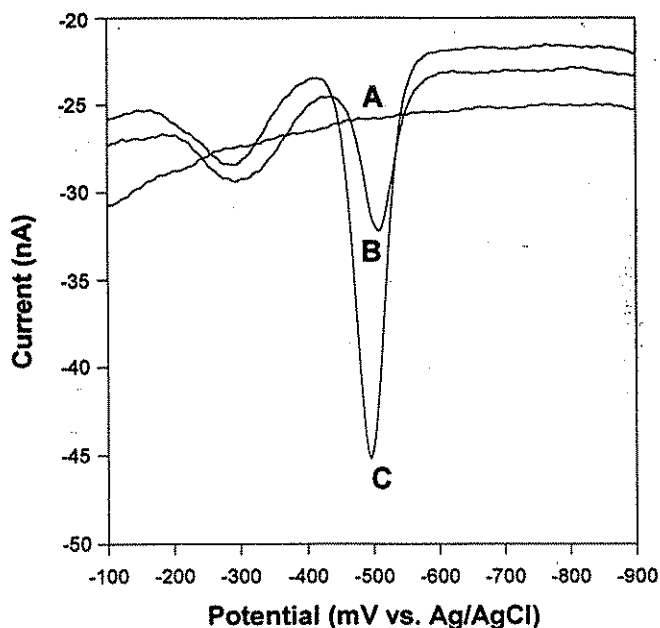


Figure 2. Stripping voltammograms for blood [Pb] determination. Deposition time, 120 s; deposition potential, -1000 mV vs Ag/AgCl; $[\text{Hg}^{2+}]$, 0.085 mM. Blood samples are acidified and filtered through nitrocellulose, see Experimental Section for details. (A) Blank. (B) Sample 3. (C) Sample 3 + 5.86 $\mu\text{g}/\text{dL}$ Pb^{2+} spike.

Table 1. Summary of Blood [Pb] Determinations by SWASV

sample	[Pb] by GFAA ^a	[Pb] by SWASV ^b	standard deviation	n
blank		0.8	0.9	6
1	31.6	30.4	3.2	4
2	29.0	28.7	3.1	3
3	17.0	18.4	3.2	6
4	11.0	9.4	1.8	6
5	8.1	7.9	0.3	3
6	4.7	3.5	0.7	4

^a Blood [Pb] ($\mu\text{g}/\text{dL}$) determined by graphite furnace atomic absorbance. ^b Blood [Pb] ($\mu\text{g}/\text{dL}$) determined by square wave anodic stripping voltammetry. Standard deviations and numbers of trials (n) refer to SWASV determinations.

each with a nominal diameter of 15 μm) is compared to a single carbon disk (A) in an aqueous Pb^{2+} solution. Experimental conditions (see Figure 3 caption) are identical for the two curves; the microarray electrode clearly has a much larger signal/noise ratio under these conditions.

DISCUSSION

The data of Figure 1 illustrate that nitrocellulose filtration of acidified blood samples improves the magnitude and stability of stripping peak currents. Nitrocellulose is known to have a high protein affinity and is commonly used to bind proteins in immunoassays and electroblotting.²⁴ Apparently, nitrocellulose filtration removes interfering substances which remain in blood-derived samples even after acidification and centrifugation. Interfering substances are probably sequestered onto the nitrocellulose surface by adsorption rather than by size exclusion filtering, since PTFE filters with identical pore sizes (0.2 μm) do not remove the interfering substances (see Figure 1, curve B). Maintenance of a constant electroactive area improves accuracy, since if the effective electrode area

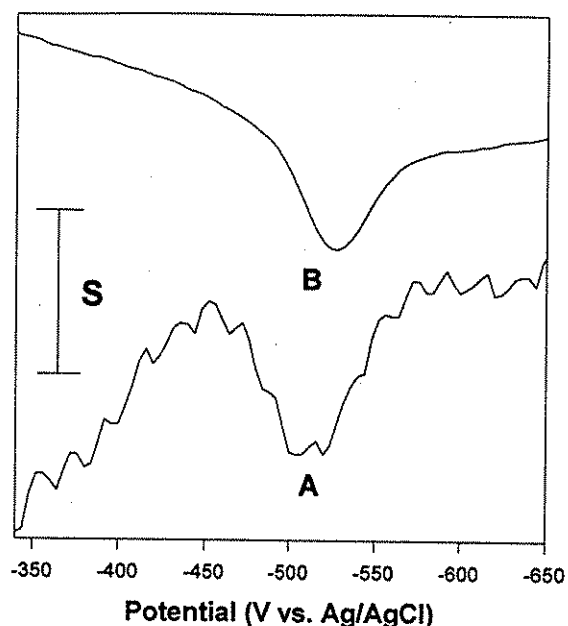


Figure 3. SWASV curves in 1.2 M HCl, 1.90 $\mu\text{g}/\text{dL}$ Pb^{2+} , and 0.046 mM Hg^{2+} for (A) glass-shrouded carbon disk ultramicroelectrode and (B) microarray electrode. Deposition time, 10 s; deposition potential, -1000 mV vs Ag/AgCl; $S = 0.5$ nA for curve A, 100 nA for curve B.

is slowly decreasing due to fouling, currents due to standard additions will be underestimated and sample [Pb] consequently overestimated. Conversely, in standard curve calibration, sample stripping current and sample [Pb] will be underestimated. Perhaps this effect contributes to the observed low bias (vs GFAA) for commercially available ASV blood [Pb] methods^{8,25} which rely on standard curve calibration. Another advantage of nitrocellulose filtration is that a relatively small blood dilution ratio (10 – 12 in this work) can be used. This compares to a dilution ratio of 30 for the Environmental Science Associates instrument, resulting in a signal/noise improvement of a factor of 2.5 – 3 .

Some shifts in Pb^{2+} stripping peak potential (up to 50 mV) were observed with the present two-electrode system. This may be due to polarization of the reference electrode from passing excessive currents. We used a two-electrode configuration to eliminate the counter electrode as a source of Pb^{2+} contamination. However, the potential stability imparted by a three-electrode system may be preferable to the simplicity of the two-electrode system. In any case, the potential shifts are not believed to have had a large effect on accuracy or precision of blood [Pb] measurement.

Although efforts were made to remove Pb^{2+} contamination from the nitrocellulose filters, the filters are suspected to be the largest contributors of contamination to the reagent blank (0.8 $\mu\text{g}/\text{dL}$) listed in Table 1. This 0.8 $\mu\text{g}/\text{dL}$ corresponds to 0.066 $\mu\text{g}/\text{dL}$ in the 600 - μL analytical sample (assuming a 12 -fold sample dilution) or 0.40 ng average Pb^{2+} contamination. When reagent blanks were analyzed after omitting the filtration step, [Pb] was consistently not detected. A more severe filter washing regimen (i.e., multihour storage in dilute acid) may be required. Alternatively, a film over the electrode surface might be substituted for ex situ filtration. Nafion^{26,27}

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and cellulose acetate²⁸⁻³⁰ have been used to coat Hg film electrodes to extend electrode lifetime for Pb²⁺ analysis.

The detection limit for this technique, based on 120-s deposition, is limited by Pb²⁺ contamination. We estimate a detection limit of 2.6 $\mu\text{g/dL}$, based on the average blank [Pb] (0.8 $\mu\text{g/dL}$) plus twice the standard deviation (0.9 $\mu\text{g/dL}$) of the blank [Pb]. This is somewhat higher than the maximum detection limit stipulated by the CDC (1 $\mu\text{g/dL}$),⁵ and could be decreased initially by reducing contaminating Pb. If the blank [Pb] value was suitably decreased (vide supra), then the detection limit would be lowered to 0.5 $\mu\text{g/dL}$, corresponding to the [Pb²⁺] at which the peak height is at least twice the background noise. Further improvements could be made by increasing sensitivity, as follows.

As with all stripping techniques, deposition time can be increased, with consequent proportional increase in signal/noise ratio and analysis time. Also, signal filtering strategies employing the Fourier-transform filtering capabilities of the BAS 100 B/W may yield a modest improvement in signal/noise ratio. Finally, we demonstrated (Figure 3) that use of a microarray electrode will increase the signal/noise ratio in aqueous Pb²⁺. Efforts are under way to extend these improvements to blood-derived Pb analysis.

The coefficient of variation (C.V.) ranged from 10.6% for a 30.4 $\mu\text{g/dL}$ sample ($n = 4$) to 20.0% for a 3.5 $\mu\text{g/dL}$ sample ($n = 4$), with an average C.V. of 13.5% for the six samples studied ($n = 3-6$). This performance does not yet meet CDC objectives (C.V. = 10% for blood [Pb] = 10 $\mu\text{g/dL}$). Some

improvement in precision should result from an increased signal/noise ratio and from reducing Pb²⁺ contamination in the reagent blank (discussed above). It is also possible that variable recovery of Pb²⁺ from the blood samples is occurring. Variable recovery might be compensated by adding the Pb²⁺ standard before sample pretreatment, although this would require parallel processing of two samples (sample and sample + standard), which would increase analysis time. Future progress in SWASV blood [Pb] determination will require simultaneous improvement in detection limit and reagent blank, which are of similar magnitude in the current method. Application of existing technologies (as discussed above) can reduce both quantities; therefore, improved blood [Pb] methods based on SWASV at carbon ultramicroelectrodes should be forthcoming.

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