Blood Lead Determination using Reduced-Palladium Matrix Modifier in Graphite Furnace Atomic Absorption

Application Note

Atomic Absorption

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Abstract

The palladium modifier removes the major problems traditionally encountered in the measurement of lead in blood using graphite furnace atomic absorption. Reduced-palladium matrix modifier allows the use of ashing temperatures as high as 1000 °C for blood lead. A low background absorbance of 0.060 enables the more sensitive 217.0 nm lead line to be used. Background correction is readily made using the deuterium lamp. The high dilution of 1:11 of blood in 0.2% Triton X-100 allows short drying and ashing times.

At the high ashing temperature no advantage is obtained using the L'vov platform, interferences are reduced and atomization is more effective from the tube wall. Carbon buildup does not occur. Zeeman background correction is not required. Accuracy has been established by comparison with a solvent extraction method and with mean values of specimens in the U.K. External Quality Assessment Scheme.

Agilent Technologies

Introduction

There are few methods using graphite furnace atomi-zation which have received more attention than the determination of lead in blood. Early methods required long ashing times, and had high background absorbance levels which were difficult to correct with the deuterium lamp. Lead was lost if ashed above 500 °C and the 283.3 nm line was used because the background absorbance at the 217.0 nm line was excessively high. The use of the modifier ammonium dihydrogen phosphate allowed a higher ashing temperature of 700 °C with a useful reduction in background absorbance. The L'vov platform brought significant improvement by delaying the atomization of lead until the atmosphere of the furnace had reached a higher temperature. By using this, interferences were reduced, lead absorbance increased and background absorbance contained at an acceptable level. The use of Zeeman background correction, with or without the L'vov platform, further improved the technique by providing accurate background correction.

The following is a summary of the state of the art of blood lead estimation at the present time.

Most methods recommend the use of the 283.3 nm line. Background correction, (sometimes as high as 1.0 absorbance unit) with its potential risk of overcompensation with the deuterium lamp, remains a problem. More recently, Zeeman background correction instruments which offer better quality of background correction accuracy have become available. However with Zeeman correction there is some loss of sensitivity and an absorbance limit at the upper end of the calibration graph.

The use of a reduced-palladium matrix modifier has been described in the determination of selenium in blood and plasma [1], and can be used for the graphite furnace determination of many elements. A reduced-palladium modifier was used to determine the level of lead in blood. With this modifier, ashing temperatures as high as 1000 °C may be used without loss of lead from the blood matrix.

Experimental

A SpectrAA-20 atomic absorption spectrometer and GTA-96 graphite tube atomizer and sample dispenser were used for this study. Deuterium arc background correction was used.

All equipment was from Agilent Technologies, Mulgrave, Victoria, Australia. Nitrogen and argon were used for inert gas protection.

Sample collection

Anticoagulated blood collection tubes were used. Heparin or EDTA have proved suitable, but EDTA is preferred because this eliminates the possible formation of micro-clots. Tubes should be tested for freedom from lead contamination.

Normally 50 µL of blood is taken, but smaller volumes can be used if necessary. Bloods can be kept without deterioration for up to 10 days at 4 °C or indefinitely if deep frozen.

Reagents

1. Concentrated Nitric Acid (70% w/w)

   Use Analar, Spectrosol or Ultrex high purity or distilled nitric acid and dilute as required. As a guide to contamination the absorbance of a 1% v/v nitric should be less than 0.020 when measured as a blank.

2. Ascorbic acid 1% w/v

   Use crystalline research grade. Dissolve 1 g ascorbic acid in distilled water to make 100 mL of solution.

3. Lead Standards

   A. Stock 1000 mg/L as lead nitrate in 1 M nitric acid. Use B.D.H. or equivalent.

   B. Intermediate lead standard 10 mg/L = 48.26 µmol/L.

      Dilute 1 mL of stock in a 100 mL polycarbonate flask containing 1 mL of concentrated nitric acid.

   C. Working Standard 0.2413 µmol/L

      Dilute the intermediate standard 1:200 as follows:

      To a 10 mL flask partly filled with distilled water add:

      50 µL of 48.26 µmol/L lead standard.

      50 µL of conc. nitric acid.

      1 mL of 1% ascorbic acid.

      Make up to the mark with distilled water.

4. Diluent Triton X-100 0.2% w/v

   Dilute 20 mL of 1% Triton to 100 mL in distilled water.

5. Modifier—Palladium 500 mg/L

   Dissolve 10.5 mg palladium acetate in 9.9 mL water plus 0.1 mL hydrochloric acid which maintains the Pd in solution. The solution is stable for several weeks. (Molecular weight of palladium acetate = 224.4).

   Use Pfaltz and Bauer, or Sigma.

Method

Reagents are placed in the carousel of the automatic sampler as follows:

1. Samples. In each cup place 0.5 mL of 0.2% Triton.

   Add 50 µL blood. Mix well.
2. Modifier. Palladium 500 mg/L.
3. Standard. The 0.2413 µmol/L lead working standard.
4. Blank. 0.10% ascorbic acid in 0.5% v/v nitric acid.

Instrumental

Instrument, furnace and sample parameters are given in the instrument printout in Table 1.

Note the use of the 217.0 nm line.

The attenuator must be used on the deuterium lamp.

For the gas types, "Normal" is nitrogen and "Alternate" is argon.

Partitioned pyrolytically-coated graphite tubes are used.

Start with a tube clean.

Standard addition 4 of 5.309 µmol Pb/L may be used if required. Normally 3 additions are sufficient.

Discussion

Background absorbance is only 0.06 absorbance using the more sensitive 217.0 nm line—a level easily corrected for by the deuterium lamp. At a dilution of 1:11 of blood in 0.2% Triton X-100 the furnace program to atomization takes only 27.4 seconds. A complete cycle for blood takes 1 minute 41 seconds.

This high dilution not only reduces background absorbance to low levels, but ensures that the best conditions for linearity are used for standard additions. The calibration graph (which has been adjusted for the dilution factor) is linear in the range up to 5.31 µmol/L lead, which typically has an absorbance close to 0.5. Refer to Figure 1. Using 0.2% w/v Triton X-100 the sample spreads evenly within the tube allowing short drying times. Triton also promotes lysis of the red cells.

SAMPLE	CONC umol/L	%RSD	MEAN ABS	READINGS		
BLANK	0.000		-0.004	-0.004	-0.004	-0.004
ADDITION 1	1.327	0.5	0.170	0.169	0.170	0.171
ADDITION 2	2.654	2.4	0.273	0.269	0.269	0.280
ADDITION 3	3.981	1.9	0.382	0.381	0.375	0.390
ADDITION 4	5.309	0.7	0.481	0.485	0.478	0.480
SAMPLE 13	0.731	4.1	0.054	0.055	0.052	0.056

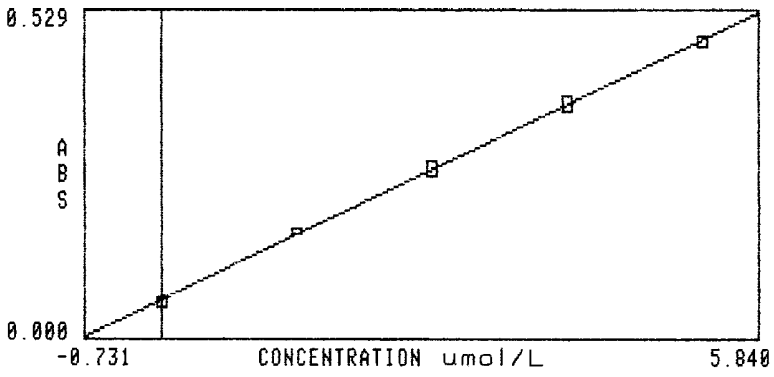

Figure 1. Lead in blood. Standard additions calibration graph.

Table 1. Instrumental, Furnace and Sampler Parameters

Program 11 Pb in blood

Instrument mode	Absorbance
Calibration mode	Standard additions
Measurement mode	Peak height
Lamp position	4
Lamp current (mA)	7
Slit width (nm)	0.5
Wavelength (nm)	217.0
Sample introduction	Sampler automixing
Time constant	0.05
Measurement time (sec)	1.0
Replicates	3
Background correction	on

Furnace parameters

Step no.	Temperature (C)	Time (sec)	Gas flow (L/min)	Gas type*	Read command
1	100	3.0	3.0	Normal	No
2	110	8.0	3.0	Normal	No
3	125	5.0	3.0	Normal	No
4	300	2.0	3.0	Normal	No
5	950	2.0	3.0	Alternate	No
6	950	2.0	3.0	Alternate	No
7	950	1.5	0.0	Alternate	No
8	2500	0.9	0.0	Normal	Yes
9	2500	1.0	0.0	Normal	Yes
10	2500	2.0	3.0	Normal	No
11	40	12.5	3.0	Normal	No

*Normal is nitrogen: Alternate is Argon

Sampler parameters
Volumes (uL)

	Standard	Sample	Blank	Modifier
Blank	--	8		2
Addition 1	2	4	6	2
Addition 2	4	4	4	2
Addition 3	6	4	2	2
Addition 4	8	4	0	2
Sample		4	8	2

Recalibration rate	6				
Multiple inject	No	Hot inject	No	Pre inject	No

No carbon buildup has been detected in tubes, even after 500 firings. With this method there is no advantage in using the L'vov platform, because atomization is sufficiently effective from the tube wall to produce sharp peaks.

By using only 50 µL of blood, this micro method meets the needs of paediatric samples. Smaller sample volumes (10 µL) can be used if required. The palladium acetate is stable and economical, 5 mg being sufficient for 1200 samples. Nitrogen and argon are used, argon being restricted to the ashing stage.

Pyrolytically-coated, partitioned tubes are used, and normally have a life of between 400 and 500 firings. The ascorbic acid reducing agent is contained in the lead standard and blank, and is added separately from the palladium modifier to prevent reduction and possible precipitation of palladium.

Lead in Red Blood Cells

As the blood has been diluted 1:11 the lead standard (actual concentration 0.2413 µmol/L) is equivalent to 2.654 µmol/L when equal volumes of diluted blood and standard are used.

The instrument calculates the level of lead in whole blood. The lead in red blood cells is a more meaningful index of lead exposure as this removes variations due to anaemia and different levels of Packed Cell Volume (PCV). The red blood cell concentration can be calculated by multiplying the whole blood lead concentration by 100/PCV. The PCV is obtained using microhaematocrit apparatus.

Notes on the Method—Development Work and Choice of Experimental Conditions

1. Choice of temperature

   The use of palladium modifier allows an ashing temperature as high as 1000 °C to be used. The temperature of 950 °C chosen in this method allows a safety margin, with little reduction in background absorbance at higher temperatures.

   This is shown in Figure 2. At an ashing temperature of 1050 °C there is a 4.3% loss of lead absorbance and at 1100 °C the loss is 10%. Ashing over the interval from 850 °C to 1100 °C reduced the background absorbance from 0.072 to 0.057.

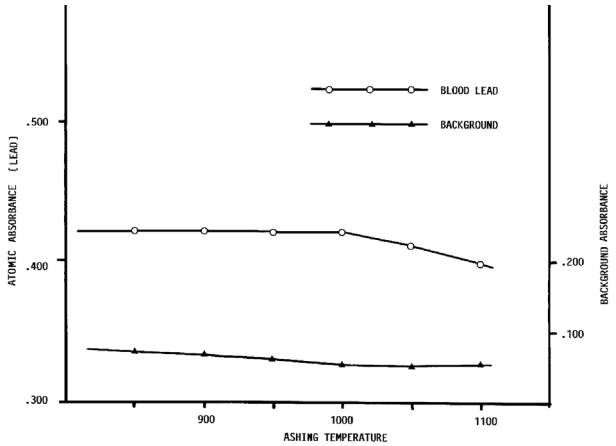

Figure 2. Variation in blood lead absorbance and background absorbance with ashing temperature.

2. Concentration of Palladium

   Figure 3 shows the effects of varying the levels of palladium modifier to blood already containing "Addition 3" of 4.34 µmol/L lead. A palladium solution containing 500 mg/L was used, and the sampler programmed to add 1,2, or 4 µL of palladium solution (0.5, 1 or 2 µg of palladium). Three ashing temperatures of 900, 950 and 1000 °C were chosen.

   If palladium modifier is not added at these temperatures, no lead is detected. When ashing at 900 °C and 965 °C, 1 µL of palladium solution is sufficient to prevent this loss of lead. At an increased ashing temperature of 1000 °C, higher amounts of palladium solution are necessary to prevent the loss of lead. With 2 µL of modifier, an ashing temperature of 950 °C was chosen for use in this method.

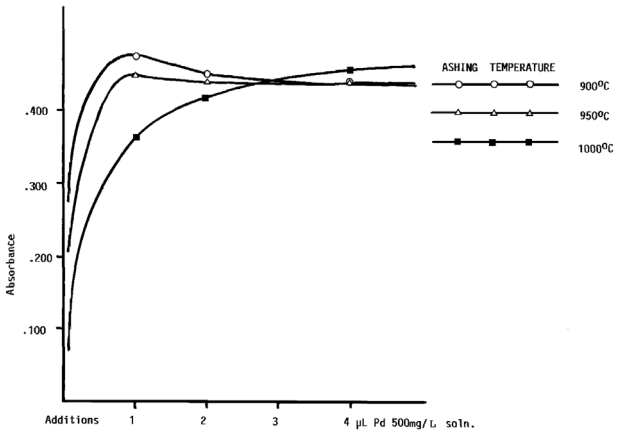

Figure 3. Effect of additions of palladium modifier to blood (containing 4.34 µmol/L lead).

3. Use of the 217.0 nm line

   The low background absorbance obtained by using an ashing temperature of 950 °C makes use of the more sensitive 217 nm line possible. The background only measurement is 0.060 ± 0.008 absorbance which is readily corrected by the deuterium lamp. The sharp peak (Figure 4) and high sensitivity obtained are a measure of the freedom from interferences, which have been the major problem in the measurement of blood lead.

4. Ascorbic Acid

   Voth-Beach [1] has shown that at 450 °C a lead solution with palladium and a reducing agent (such as ascorbic acid) gave virtually the same sensitivity as an aqueous lead solution. When used without a reducing agent, palladium is found to give poor sensitivity for some elements. The main effect of the reducing agent is to reduce the palladium salt to elemental palladium early in the temperature program. Hydroxylamine hydrochloride has also been used as a reducing agent.

   In this study, when ascorbic acid is omitted, the low lead level of the aqueous blank is not correctly measured, and therefore not subtracted from the calibrating blood solutions or blood samples.

   Levels of ascorbic acid from 0.4% down to 0.1% w/v were evaluated, and no differences could be detected. Thus the lower level was chosen. Note however, that in the presence of blood, ascorbic acid is not required. It is assumed that blood has some reducing action on palladium.

   In this study ascorbic acid has been included in the lead standards which are preserved in 0.5% v/v nitric acid. The nitric acid slowly reacts with the ascorbic acid causing the standards to have a shelf life of 7 days. Beyond this, the ascorbic acid no longer ensures a stable lead measurement in aqueous solutions or the reagent blank.

5. Aqueous lead standards

   In this method the slope of an aqueous lead calibration graph is close to the slope of standard additions added to blood. The absorbance of the aqueous 5.31 µmol/L standard (without diluted blood) was 0.460, and for this addition to blood (after subtracting blood lead absorbance) the absorbance was 0.448.

   The closeness of these measurements is evi-dence of the low level of interferences. However, it is important that the method of standard additions is used to avoid possible sources of error. Control materials should be blood based and not aqueous, for this same reason.

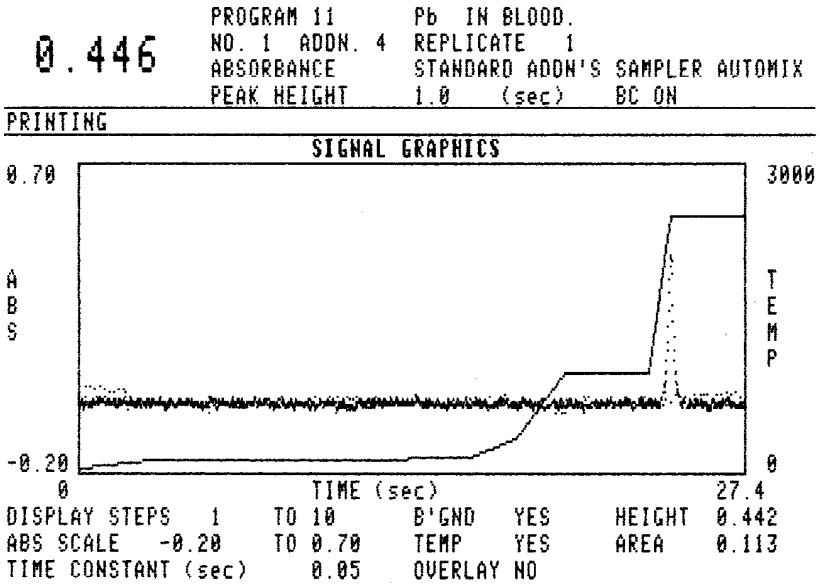

Figure 4. Lead in blood. Typical analytical signal.

6. Use of peak height versus peak area

   A sharp lead peak as shown in Figure 4 had a peak height of 0.442 absorbance for the 5.31 µmol/L lead standard. For peak area the value was 0.113 absorbance seconds. The precision of both forms of measurement is the same. Both graphs are linear up to "Addition 4". Peak height measurement was chosen for its greater sensitivity.

7. Tube life

   The dilute reagents and high dilution of blood samples used in this procedure shorten the furnace program time and prolong tube life. Tubes usually have a life of 400–500 firings, and using this method there is no buildup of carbon in the tubes even after 500 firings. The use of 2500 °C atomization temperature, which removes iron and other residues, helps to prevent this. Up to three tube cleans are used with each new tube to remove contamination.

8. Slope of calibration graph using standard additions

   The slope of the calibration graphs obtained by standard additions for a small number of blood samples have been compared. All blood tested had a normal PCV and the highest blood lead level was 2.3 µmol/L. The slopes varied by ± 2.53% (Relative Standard Deviation). This small variation justifies the use of a single calibration from which other blood lead levels are determined.

   In a separate experiment, sample serum and red cell levels in a single blood sample were adjusted to produce three blood samples with PCV's of 0.39, 0.50 and 0.67. The slopes, (Addition of 5.31 µmol/L minus blood lead), gave absorbance differences of 0.424, 0.424 and 0.427 respectively. PCV does not appear to influence calibration slope.

   Efforts have been made to shorten each stage in the furnace parameters, and the principles set out by Halls [2] have proved of assistance in this. Many programs are much longer than necessary. The speed of the present method described here is helped by the use of dilute solutions. Drying up to 140 °C is the longest step with a short pause at 300 °C for removal of organic material. The use of argon is restricted to the ash and atomize stages for economy. There is a 1.5 seconds hold at 950 °C to allow gas flow to stop immediately before atomization The dry, ash and atomize stages take a total of 27.4 seconds and a complete cycle for a blood sample took 1 minute 41 seconds.

9. Lead blank of Triton X-100 0.2% w/v

   In this method, Triton is present only in the blood solutions. Therefore, if any lead is present it is not subtracted in the blank, but is included in the blood readings. However, the lead level in Triton X-100 has usually been undetectable. New bottles of Triton X-100 were checked for possible lead contamination by measuring 0.5 mL of 0.2% Triton plus 50 µL of water. The lead blank of 0.5% nitric acid, 0.1% ascorbic acid and 500 mg/L palladium are fully accounted for in the method described.

Accuracy

Confirmation of accuracy was obtained by comparison of results from a U.K. External Quality Assessment Scheme (EQAS) Queen Elizabeth Hospital, Birmingham, and with an alternative method for blood lead using solvent extraction. To date four results using this method have been compared with the EQAS mean results from about 70 laboratories. A variation of between 0.8% and 2.5% from these means has been obtained by this method. See Table 2. In the comparison with the extraction method there was a difference of 0.02 µmol/L between the means of the two methods. It was considered that the agreement between the two methods was good.

Table 2. Results U.K.E.Q.A.S. Survey for Lead in Blood

Lead levels (µmol/L)			
E.Q.A.S. no.	**This method**	**Survey mean**	**Variation %**
2.93	2.61	2.59	0.8
2.94	1.26	1.23	2.4
2.95	2.69	2.74	1.8
2.96	2.93	2.86	2.5

Blood lead levels obtained on E.Q.A.S. specimens. The variation between results by this method and the survey means of all participating laboratories is given.

References

1. L. M. Voth-Beach, D. E. Shrader, "Graphite Furnace atomic absorption spectroscopy: new approaches to matrix modification." *Spectroscopy*, 1(10), 49, (1986).
2. D. J. Halls, "Speeding up determinations by electrothermal atomic-absorption spectrometry." *Analyst*, 109, 1081 (1984).

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