Determination of Lead in Sheep's Blood by Graphite Furnace Atomic Absorption Spectrometry Using a Modified Chelation and Extraction Procedure

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Ammonium tetramethylene dithiocarbamate and isobutyl methyl ketone are used to chelate and extract lead from haemolysed whole blood in a procedure modified for use with a graphite furnace. The ketone layer separates readily without centrifugation. With a pre-atomisation temperature of 350 °C to prevent loss of chelated lead and an atomisation temperature of 1600 °C background correction is unnecessary, and the tubes can withstand about 1000 firings. The method has a detection limit of about 0.018 μg ml^{-1} of lead. The between-run precision for a pooled sample of ovine blood containing 0.135 μg ml^{-1} of lead (0.100 μg ml^{-1} of added lead) was 4.1%, calculated from the results of 41 analyses carried out over a period of 2 months.

Keywords: *Lead determination; sheep's blood; graphite furnace atomic absorption spectrometry*

The determination of lead in whole blood by electrothermal atomisation atomic absorption spectrometry can be carried out directly on the sample (with or without pre-treatment) or by using solvent extraction after chelation. Using the direct method, blood can be introduced into the atomiser undiluted,[1–3] or after dilution with a surfactant such as Triton X-100.[3,4] In either instance, ash gradually accumulates in the atomiser[3] and must be removed mechanically.[5,6] If oxygen ashing is used the build-up of residues will not take place, but a chemical modifier must be added to prevent loss of lead.[7] Pre-treatment of the blood sample with nitric acid[5,8] or other reagents[9] cannot prevent interference effects and background correction is required even after pre-digestion with perchloric and trichloroacetic acids.[9] The evidence suggests that aqueous lead standards should not be used as pronounced blood matrix effects have been reported.[10] It has been demonstrated,[11] that the slopes of calibration graphs for lead standards added to whole blood may differ according to the type of anticoagulant present and to the nature of the added lead. There is, however, no difference in the slopes of standard graphs obtained with different blood samples under the same conditions.[12]

Volosin *et al.*,[10] working with pyrolytically coated graphite tubes, showed that with procedures involving chelation and extraction, it is possible to use aqueous standards and to obtain atomisation peaks free from background interferences. There is at the same time no build-up of non-volatile residues in the furnace.[13]

Volosin *et al.*[10] used sodium diethyldithiocarbamate and isobutyl methyl ketone (IBMK) with blood samples haemolysed by the addition of Triton X-100. The most commonly used extraction procedure is based on that of Hessel,[14] who used ammonium tetramethylenedithiocarbamate (ammonium pyrrolidinedithiocarbamate, APDC) for complexation and IBMK as the extracting solvent. The procedure has been adapted for use with graphite furnaces by a number of workers.[15–17]

Changes in instrument design in recent years have resulted in the neglect of this useful analytical method, as the setting up of an electrothermal atomisation procedure can be time-consuming. This paper reports an adaptation of the APDC - IBMK procedure for use with a Varian graphite furnace.

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Experimental

Reagents

Lead nitrate stock standard solution, 1.00 mg ml^{-1}. Prepare as a solution of lead using lead nitrate in approximately 1 M nitric acid.

Working standard solutions of lead. Prepare by diluting the stock standard solution with 0.5% *V/V* nitric acid.

Isobutyl methyl ketone. Analytical-reagent grade. Saturate with water by equilibrating over distilled water for at least 24 h before use.

Triton X-100. From Sigma.

Ammonium tetramethylenedithiocarbamate. From BDH Chemicals. Spectrosol grade.

Triton X-100 - *APDC solution*,[15] *approximately* 2% *m/V APDC and* 5.0% *m/V Triton X*-100. Add 2.0 g of APDC to 50 ml of distilled water. Warm gently to dissolve as far as possible and filter through a Whatman No. 540 filter-paper. Allow the filtrate to cool and extract in a separator by shaking with two consecutive volumes each of 20 ml of water-saturated IBMK. Discard the organic layers and filter the aqueous layer as before. Mix the filtered aqueous layer with a solution of 5.0 g of Triton X-100 in 50 ml of water. Store in a refrigerator in a dark bottle for up to 10 days.

Triton X-100 - *APDC reagent*. Dilute 25 ml of Triton X-100 - APDC stock solution to 100 ml with distilled water before use.

Pooled sheep blood sample. Kindly supplied by MAFF Central Veterinary Laboratory, New Haw, Weybridge, UK and stored frozen until use.

Apparatus

A Varian Techtron GTA-95 graphite tube atomiser fitted with a pyrolytically coated graphite tube was used in conjunction with a Varian Techtron Model AA 175 atomic absorption spectrometer. A single-element lead hollow-cathode lamp (Varian Techtron) was used as a line source to determine lead at 217.0 nm with a scale expansion of ×2. A lamp current of 5 mA and a spectral band width setting of 1.0 nm were employed. The spectrometer was set to give a digital read-out of peak atomisation signals. The profile of all analytical signals obtained during furnace operation was also displayed on the furnace graphics screen, superimposed upon the temperature - time profile. Automatic background correction and back-

Table 1. Varian Techtron GTA-95 furnace programmes for the determination of lead in IBMK extracts of APDC-chelated lead and in aqueous working standards (scale expansion ×2)

Approximate temperature/°C				
IBMK extract	Aqueous working standard	Time/s	Nitrogen flow-rate/ l min^{-1}	Programme stage
95	75	5.0	3.0	Dry
110	90	25	3.0	Dry
330	350	120	3.0	Ash
330	350	10	3.0	Ash
330	350	1.0	0	Ash
1600	1600	1.0	0	Atomise; read
1600	1600	2.0	0	Atomise; read
1600	1600	1.0	3.0	Atomise

ground absorption measurements were carried out in accordance with the manufacturer's instructions. The operating conditions for the GTA-95 furnace are shown in Table 1. Liquid samples were injected into the atomiser tube using a 5-µl Autopette injection syringe (Excalibur Laboratories) fitted with polypropylene tips. These tips were cleaned individually by drawing in a solution of dilute nitric acid (20%) several times, followed by distilled water. Precautions necessary for trace analysis to prevent contamination were taken at all times.

Procedure

Whole blood samples

Place 50 µl of sample in a 3.5-ml polypropylene tube.

Blood standards for calibration

Place 50 µl of a pooled blood sample (low in lead content) in a 3.5-ml polypropylene tube. To calibrate for blood samples containing 0–0.3 µg ml^{-1} of lead, add 5 µl of a working standard solution containing 0, 1, 2 or 3 µg ml^{-1} of lead. To calibrate for blood samples containing up to 0.8 µg ml^{-1} of lead, add 5 µl of a working standard solution containing 0, 2, 4, 6 or 8 µg ml^{-1} of lead.

Aqueous standards for calibration

Place in a 3.5-ml polypropylene tube 50 µl of a working standard solution containing 0, 0.1, 0.2 or 0.3 µg ml^{-1} of lead (to calibrate for blood samples containing 0–0.3 µg ml^{-1} of lead) or 0, 0.2, 0.4, 0.6 or 0.8 µg ml^{-1} of lead (to calibrate for blood samples containing up to 0.8 µg ml^{-1} of lead). To each tube, add 100 µl of Triton X-100 - APDC reagent, cap the tube tightly and vibrate for 5 s on a vortex mixer. Leave to stand for 15 min. Add 200 µl of water-saturated IBMK for blood samples containing 0–0.3 µg ml^{-1} of lead and for the corresponding standards. Add 500 µl of water-saturated IBMK for blood samples containing up to 0.8 µg ml^{-1} of lead and for the corresponding standards. Re-cap each tube, vortex mix for 30 s and stand to allow the upper IBMK layer to separate. Inject 5-µl aliquots of each IBMK extract into the atomiser tube of the GTA-95 furnace and determine the uncorrected absorbance at 217.0 nm.

Results and Discussion

Analytical Procedure

For the direct determination of lead in the presence of a blood matrix it is necessary to use an atomisation temperature of around 2400 °C. Garnys and Smythe[5] observed that higher atomisation temperatures can produce non-linearity in the working curve for lead and decrease the precision of the determination. With the extraction procedure, as there is no build-up of carbon residues in the atomiser tube, a lower atomisation temperature of 1600 °C can be used, giving tube lifetimes of about 1000 firings, compared with 150–200 firings at 2400 °C.

With aqueous standards injected directly into the furnace, it was possible to use a pre-atomisation temperature of 550 °C without loss of lead. The chelated lead in the IBMK extracts was found to be more volatile and was totally lost with an ash temperature of 500 °C and partially lost at 450 and 400 °C. Consequently, a maximum pre-atomisation temperature of 350 °C was used for the extraction method.

With this low pre-atomisation temperature, the maximum volume of IBMK extract that could be injected into the atomiser without a background signal being produced during atomisation was 5 µl. It was therefore necessary to limit the volume of ketone used in the modified extraction procedure, and at the same time to adjust the volume of the aqueous phase so that a good separation of the layers could be obtained without centrifugation.

Sensitivity and Precision

The sensitivity to lead using the operating conditions given in Table 1 is about 6.5×10^{-12} g to give 1% absorption. Varian Techtron published data indicate that the GTA-95 furnace is approximately 25% more sensitive than the earlier Model CRA-90 carbon-rod atomiser for lead determination.

The results showed that aqueous standard extracts could be used for calibration purposes. With concentration levels of 0–0.3 µg ml^{-1} of added lead, the regression equations were: absorbance = 1.65Pb + 0.068, r^2 = 99.2% using blood standard extracts and absorbance = 1.64Pb + 0.053, r^2 = 99.8% using aqueous standard extracts. With concentration levels of 0–0.8 µg ml^{-1} of added lead, the regression equations were: absorbance = 0.754Pb + 0.052, r^2 = 99.9% using blood extracts and absorbance = 0.732Pb + 0.041, r^2 = 99.8% using aqueous standard extracts (Pb = µg ml^{-1} of lead in the blood or aqueous standards).

The absorbance signal obtained for lead injected directly into the atomiser tube in a blood matrix is known to be greatly reduced in comparison with the signal obtained for aqueous standards unless a matrix modifier such as ammonium dihydrogen phosphate is present.[7] However, the regression equations obtained by the direct injection of 5-µl volumes of aqueous standards were: absorbance = 1.72Pb + 0.038, r^2 = 100.0% for the range 0–0.3 µg ml^{-1} of lead and absorbance = 0.751Pb + 0.055, r^2 = 99.3% for the range 0–0.8 µg ml^{-1} of lead. The aqueous standards were diluted 1 + 3 and 1 + 9 for the respective concentration ranges before injection into the atomiser. These results indicated that there was no significant change in sensitivity caused by the chelation and extraction of lead. It was not considered to be advisable to propose the use of aqueous standards by direct injection for calibration of the assay. The response to lead atomised from different solvents

could clearly vary according to the condition of the atomiser tube and the extent to which each solvent would tend to soak into the tube.

The samples of ovine blood that were analysed all contained lead within the range 0–0.3 μg ml^{-1}. These results will be reported separately. In the course of carrying out these analyses, standard additions of 0.1 μg ml^{-1} of lead were made at intervals as a control to the pooled blood sample, previously determined by the extraction procedure to contain 0.035 μg ml^{-1} of lead. The mean recovery of added lead by the extraction procedure was 0.100 μg ml^{-1} and the range of results was 0.092–0.119 μg ml^{-1} of lead for 41 determinations carried out over a period of 2 months. The relative standard deviation of the assays was 4.1%.

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