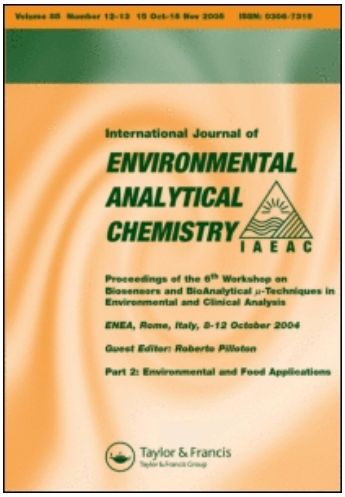

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Determination of Lead in Blood

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Blood lead level is the widely accepted index of lead poisoning. Atomic absorption spectrophotometry offers simple and rapid determination of blood lead. The sample preparation however presents problems due to limited blood availability, low normal blood lead level and non-availability of identical standards. Wet digestion, precipitation of proteins, chelation and extraction, whole blood or Triton X-100 diluted blood have been used for analysis of lead. Flameless atomic absorption technique using carbon rod or heated graphite atomizer have minimised the sample size and increased sensitivity. Triton X-100 dilution followed by flameless atomic absorption analysis is a simple and reliable method for the determination of lead in microquantities of blood. However, while using low temperature in the ashing cycle one must ensure that Triton X-100 does not give unspecific absorptions.

KEY WORDS: Lead in blood; flameless atomic absorption spectrometry; Triton X-100.

INTRODUCTION

Exposure to lead has been a cause of concern to hygienists and toxicologists since long. The metal finds its uses in the lead smelters, spray painting, soldering, manufacture of storage batteries, cables, ceramics, typesetting and in motor fuel causing widespread environmental contamination.[1] The blood lead level is one of the most important indices of lead exposure.[2] The colorimetric, electrochemical and pelarographic methods for determination of blood lead have limitations owing to be time consuming or due to requirement of large quantities of blood. Atomic absorption spectrophotometry has offered simplicity precision and accuracy along with time saving above all these methods. However, sample preparation before A.A.S. analysis presents problems due to limited blood availability, low normal blood lead level, non-availability of identical standards and interference from the chemicals used. Various procedures of sample preparation used before analysis of blood lead by atomic absorption spectrophotometry include: wet digestion[3,4] protein

precipitation[5,8] or chelation and extraction in organic solvents.[9,12] Use of a tantalum boat and Delves cup for the blood lead analysis required no pretreatment but suffered from lengthy drying, low temperature ashing and also resulted in poor recovery and precision.[3,13-15].

The development of flameless atomic absorption techniques and the use of Triton X-100 diluted blood for analysis has lead to an overall improvement in the situation since sample preparation does not require much handling. Determination of lead content in blood has also been carried out by directly applying a microquantity of blood into the carbon rod for flameless atomic absorption analysis.[16,17] However the method offered limited precision due to the difficulties in pipetting precisely small volumes of blood into the carbon rod, incomplete ashing and the residue left in the carbon rod obstructed the light path leading to erroneous results.[12,15] Evenson and Pendergast[15] used erythrocytes diluted 1:1 with 5% Triton X-100 surfactant followed by injection of 4 μl of this mixture into a graphite tube. Triton X-100 in their experimental conditions showed no matrix influence. Baily and Kilore–Smith[18] while comparing seven methods of blood sample preparation for flameless A.A.S. however observed high background absorptions due to Triton X-100 which was not in agreement with the observations of Evenson and Pendergast[15] and Kubasik and Volosin.[17] According to them[18] when proteins were not precipitated or digested high background absorptions were obtained which indicate that high molecular weight substances are not completely ashed at 500°C in an argon atmosphere and tend to produce smoke due to further decomposition at high temperature used in atomization step resulting in non specific absorptions. In view of the above the influence of Triton X-100 on the analysis of lead standards in blood by flameless A.A.S. was investigated, and the results are being reported.

MATERIALS AND METHOD

Rats blood was collected in precleaned and heparinised polysterol vials and used for analysis. Fifty microlitres (50 μl) blood was mixed with two volumes of 5% Triton X-100 and three volumes of deionised water or lead standards corresponding to 0.4, 0.8 or 1.2 ng lead/20 μl in the final volume. Twenty microlitres (20 μl) of the sample was injected into the perkin Elmer heated graphite atomizer (HGA 76-B) attached to a Perkin Elmer Atomic Absorption Spectrophotometer-420 and analysed for lead at 283.3 nm using the following operating programme:

	Drying	Ashing	Atomization	Burnout
Time (sec.)	35	30	8	2
Temperature (°C)	100	500–800	2250	2500

RESULTS AND DISCUSSION

In the present investigation it was found that the concentration of Triton X-100 normally used for hemolysis of blood forms bubbles in the graphite cuvette during drying which splits leading to variations in the results. Better reproducibility was achieved using a temp. of 100°C for 35 seconds during drying. The choice of ashing temperature is particularly a problem in the blood lead analysis by flameless A.A.S. It should neither be very high so that the lead may not get volatilised and lost before atomization nor should it be very low as the unashed organic matrix left may cause

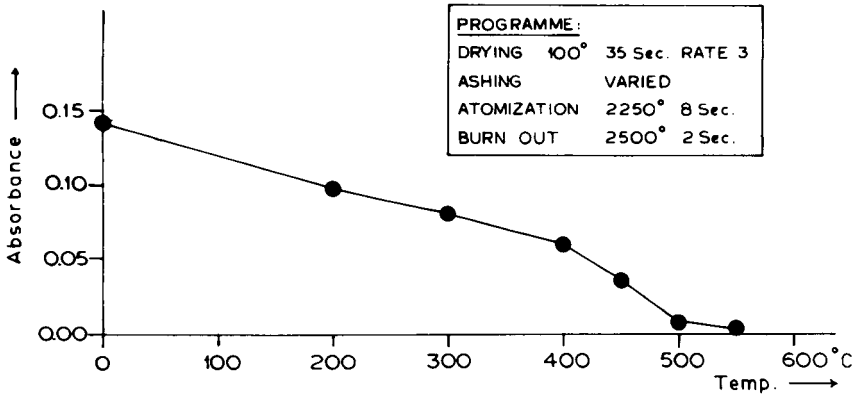

FIGURE 1 Unspecific absorptions of 2.5% Triton X-100 with deuterium compensator.

fuming leading to unspecific absorption during atomization. Ashing was studied in the range 500–800°C and all the unspecific absorption in the analysis of lead added to blood could be compensated well by a deuterium compensator using 550°C. Low temperature ashing resulted in absorption due to Triton X-100 only. This could not be compensated for by a deuterium compensator up to a temperature of 550°C used for ashing (Figure 1). It is, therefore, important to chose a maximum temperature for ashing of a blood matrix which should not be so high as to result in a loss of genuine lead signal. A temperature of 600°C for 30 seconds (with a slow heating rate 2 in the instrument used) met these requirements. An atomization temperature in the range of 2300°C was preferable for increased life of the cuvette with 8 seconds as the time for this step. The cuvette was also given a burnout treatment after atomization at 2500°C

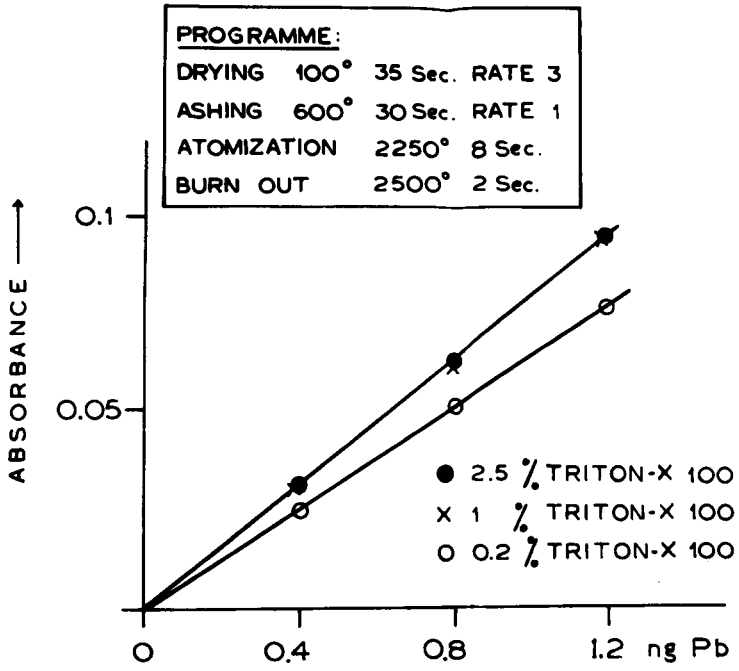

FIGURE 2 Standard curve in different Triton X-100 concentrations.

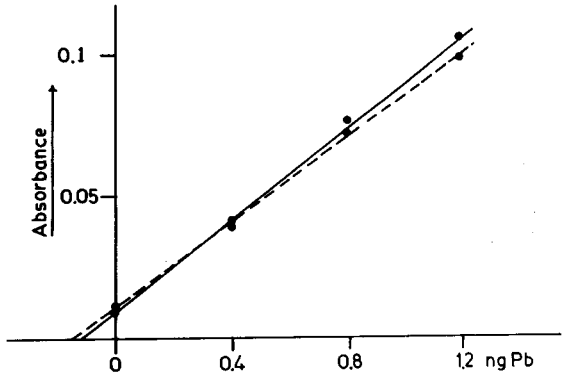

FIGURE 3 Analysis of rat blood by the method of standard additions.

for 2 seconds to eliminate any memory effect. Good reproducibility of blood lead analysis among unexposed animals was obtained with variation coefficient between 2.23% to 4.9% using this programme and the method of standards addition. The average blood lead level in unexposed rats was 5 μg/100 ml. There was not much difference in the lead standards with or without blood and Triton X-100 had little effect on lead standards (Figures 2 and 3).

These results are in agreement with Fernandez[19] who injected blood into HGA after 1:5 dilution with Triton X-100. He recommended proper mixing of the sample to avoid clotting and a temperature of 550°C −575°C for ashing and 2300°C for atomization. Fernandez[19] also observed no chemical interference from diluent containing Triton X-100 probably due to high ashing temperature. Since Triton X-100 did not interfere, it was not added to lead standards used for the calibration.

Thus, Triton X-100 dilution followed by flameless atomic absorption spectrophotometric analysis is a simple and reliable method for the determination of blood lead in microquantities with precision and accuracy. One should however check for unspecific absorptions due to Triton X-100 while using lower temperature range in the ashing cycle.

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