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Determination of blood lead using a 4·0 mm paper punched disc carbon sampling cup technique

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Cernik, A. A. (1974). *British Journal of Industrial Medicine*, **31**, 239-244. **Determination of blood lead using a 4·0 mm paper punched disc carbon sampling cup technique.** The method described is designed to overcome difficulties associated with presentation of the sample and its analysis for lead in blood. Chromium-51 studies on the effect of spread showed that imprecision resulted when discs of less than 4·0 mm diameter were punched from a given blood spot. The best standard deviation and relative standard deviation for increasing lead concentrations was obtained when 4·0 mm discs were used.

Small daily voltage variations to the carbon cup workhead required that the non-specific absorption should be controlled to 0·02 absorbance at the ashing step. The occurrence of a second non-atomic signal was not considered significant since it had a different volatilization temperature to lead.

Several advantages of the method are stated.

A comparison of 4·0 mm against 6·5 mm punched discs was investigated using 33 samples of blood ranging from 9·0 to 126 μg of lead per 100 ml blood. Correlation (r = 0·970) and regression lines are shown. Absolute sensitivity of the method was 25 $\times$ 10^{-12} g.

The introduction of a micro sampling cup technique by Delves (1970) has enabled important advances to be made in the determination of blood lead by atomic absorption spectrophotometry (AAS). Difficulties in the preparation of the sample, such as pipetting micro-litre volumes of whole blood, have stimulated the investigation of simpler means of presenting the sample, especially where large numbers of specimens have to be analysed. Cernik and Sayers (1971) spotted whole blood onto filter paper, allowed the blood to dry, and then punched out standard sized discs of 8·9 mm, which were analysed by AAS for lead content. Correlation with the Delves method was good (r = 0·981). However, in a more recent paper Cernik (1973) found that, when using 6·5 mm punched discs, nickel crucibles presented a problem. There tended to be a steady degradation of the crucible apparently caused by the burning of paper. The result was a loss of sensitivity which might not allow an economical number of blood leads to be determined. This was not observed using the original Delves technique.

The use of a flame as the source of energy for the volatilization of a metal has the following limitations. The maximum temperature attained by the nickel cups in the Delves technique is only 1 200° C due to radiative and other heat losses. This is much lower than the maximum temperature of the air-acetylene flame (2 200° C) and thus limits the above technique to the analysis of metals with low vapour pressures. Furthermore, conditions immediately in the vicinity of the flame can be unpleasant for the operator when the workload is high and the flame cannot be left unattended.

Matoušek and Stevens (1971) described a non-flame atomization method for several metals in blood and plasma, including lead. They suggested that the carbon rod atomizer (CRA) would provide a rapid and accurate procedure for determining these metals in very small volumes of blood.

Amos *et al.* (1971), Kubasik, Volosin, and Murray (1972), and Rosen (1972) reported that advantages with the CRA were rapid analysis, simple preparation of the sample, low detection limits, and smaller sample size requirement. Kubasik *et al.* and Rosen applied the CRA to the measurement of lead in whole blood but found problems with absorption of the sample into the carbon rod. Rosen (1972) prevented this by sandwiching 0·5 μl volumes of whole blood between micro-litre volumes of xylene.

The presentation of the blood for analysis has also raised difficulties. Sub-micro-litre volumes of whole blood are not easily pipetted with reproducible accuracy because there is always the problem of viscosity interfering with the measurement. Amos *et al.* (1971), using the CRA in the fluorescence mode, determined lead in whole blood after diluting the blood 2·5 times with water. Kubasik *et al.* (1972) treated one volume of blood with two volumes of Triton X-100 and applied 1 μl of the diluted blood.

The ease of handling the sample is of paramount importance, especially when large numbers of blood samples for lead determinations are processed. Many thousands of blood lead determinations have borne out the early promise of the punched disc (PD) technique in this connection. This paper describes the results of attempts to minimize the inherent errors and difficulties encountered with this original method by ashing and atomizing the lead under stricter control, using a carbon sampling cup or cell, than can be achieved using the flame.

Material and methods

Apparatus

A Varian-Techtron AA5 atomic absorption spectrophotometer was used with a model 63 carbon rod atomizer workhead attached to the burner mount. The spectrophotometer operating conditions were as follows:

Lead lamp current	7·0 mA
Spectral band width	0·99 nm
Wavelength	217·0 nm

Power controls to the workhead (arbitrary units):

Ashing	*Atomization*
5·6 for 12 seconds	5·6 for 2·0 seconds

Step atomization mode.

Scale expansion × 1 Varian A25 recorder 10 mV full scale deflection. 2·0 cm per minute chart speed.

Argon and hydrogen gas flow box scale units 2·0; flow rates of 1·0 and 3·0 litres/min respectively were applied during the ashing and atomizing steps. The workhead was cooled by a flow of cold water. The 4·5 mm diameter carbon cups were made by Mingin and Chemical Products Ltd., Birmingham from a directly enlarged version of the Varian 3·0 mm cup (Fig. 1). Varian supporting electrodes were used, but although the radii of the ends were not accurate for the enlarged cup, good electrical contact could be obtained if care was taken when setting up. A larger masking plate was necessary to prevent emission interference from the increased cup size. The dimensions were 25 mm wide by 32 mm high with an optical window of 2·0 mm.

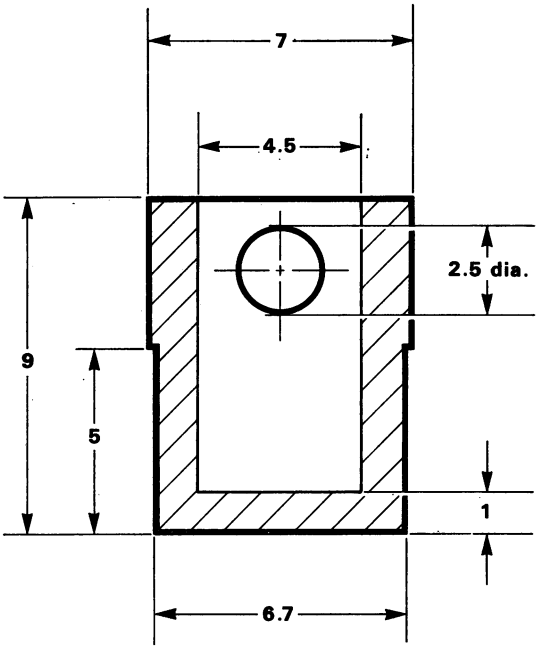

FIG. 1. Specification of the 4·5 mm carbon sampling cup. All dimensions in millimetres. Graphite grade RW-0.

4·0 mm stationer's punch (Maun Industries, Mansfield).

A 10 μl SGE syringe, which had the needle reduced to 3·0 cm and sharpened to a point, was used as an applicator to transfer the paper discs to the cup.

Whatman No. 4 qualitative filter paper, 8·5 cm diameter.

Whatman No. 4 qualitative filter paper, 12·5 cm diameter.

Plastic petri dishes with three subdivided compartments, 9·0 cm diameter.

Reagents

Lead standards Lead nitrate ($Pb(NO_3)_2$) 1·0 mg ml^{-1}. Weight 1·598 g and dissolve in 500 ml distilled water. Add 10 ml (v/v) nitric acid and make up to the mark with distilled water.

Working standards 25, 50, 100, 200, 300 μg ml^{-1} prepared by dilution of the stock solution. Add 20 μl of an appropriate aqueous lead standard to 5·0 ml of well-mixed blood, preserved with 1·0-2·0 mg EDTA per ml blood to yield a final concentration of 0, 10, 20, 40, 80, and 120 μg Pb/100 ml blood.

Distilled water prepared by triple distillation using a Pyrex all-glass still for the final double distillation.

Method

Blood, from a capillary or venous source, was spotted onto Whatman No. 4 qualitative filter paper which had been placed in a chemically clean plastic petri dish for collection purposes, using a no-touch technique.

When approximately 0·02-0·04 ml of blood was dropped on to the paper, the blood spots formed were about 1·5-2·0 cm in diameter. The blood was allowed to spread and dry naturally in the ambient temperature of the laboratory overnight.

Several 4·0 mm blood discs were punched out and collected on to a clean 12·5 cm circle of filter paper. Using the needle applicator, a disc was speared and transferred to the carbon cup, ensuring that the disc was placed flat on the bottom of the cup. A hydrogen lamp was placed in position and the wavelength at the monochromator set at 217 nm. With argon and hydrogen flowing round the cup, the voltages as stated were applied. A visible hydrogen diffusion flame was apparent at the atomizing stage. Three signals were usually seen. The first was due to molecular absorption from the burning paper and organic matrix, and could be ignored. The second very much smaller signal was the first non-atomic trace, which was recorded. Immediately following the first non-atomic signal, another peak was seen which did not fall in the same position as the lead on the recorder chart. This second non-atomic peak was considered to arise from the different volatilities of the non-atomic material and could be disregarded. The settings of the ashing control were set so that the first non-atomic peak was not more than 0·02-0·03 absorbance, when a blood disc was burnt. The hydrogen lamp was then replaced by a lead cathode lamp and the spectrophotometer gain settings were reset to allow for lamp turret variation. The punch was then cleaned by punching several discs from a fresh piece of filter paper.

Triplicate discs of each standard (with three discs from a fresh 8·5 cm circle of paper which acted as the paper blank) were then cut. The discs from each standard were ashed and atomized accordingly, allowing an interval of 1·0 minute between each determination for the workhead to cool. This time limit was found to be generous and could be shortened. The debris of carbonized paper was blown away by a puff of air from a rubber teat attached to a shortened Pasteur pipette after each disc was burnt. From the absorbances found, a graph was constructed, plotting the concentration of lead per 100 ml blood against absorbance.

Response was linear to the working standard concentration range stated, namely 0–120 μg Pb/100 ml blood plus the low endogenous blood lead level; and the validity of the added lead line was checked by a control blood whose value had already been found by another form of analysis. The result of this control had to be within 10% of the mean value of its equivalent on the graph. During the run of unknown blood leads, a standard was analysed after every six samples along with paper blanks cut from the same piece of paper. The standard had to agree to within 10% of its point of relevance.

TABLE 1

STANDARD DEVIATION OF DIFFERENT BLOOD LEAD CONCENTRATIONS USING 3·0 mm AND 4·0 mm PUNCHED DISCS

3·0 mm			*4·0 mm*		
μg Pb per 100 ml blood	*SD*	*RSD*	*μg Pb per 100 ml blood*	*SD*	*RSD*
—	—	—	16	1·65	10·3
58	3·54	6·1	67	3·87	5·8
97	6·04	6·2	99	3·85	3·9

Replications at each concentration = 11

Results

Precision

The results from 3·0 and 4·0 mm discs at three different concentrations are given in Table 1. Also the tracings from the 4·0 mm disc findings are shown in Figs. 2, 3, and 4 for the three different concentrations with corresponding standard deviations. Absolute sensitivity of the method was 25 × 10^{-12}g.

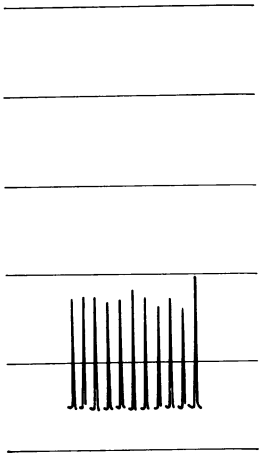

FIG. 2. Precision of 4·0 mm blood discs (tracings). Mean of 11 results = 16 μg Pb/100 ml blood, SD = 1·65.

Comparison of 4·0 mm against 6·5 mm

Thirty-three samples of blood ranging from 9·0 to 126 μg Pb per 100 ml blood were analysed by both methods. The correlation and regression lines are shown in Figure 5.

Discussion

It is the reproducibility of the sample transfer to the cell that may limit the precision attainable by atomic absorption spectrophotometry (Kirkbright, 1971).

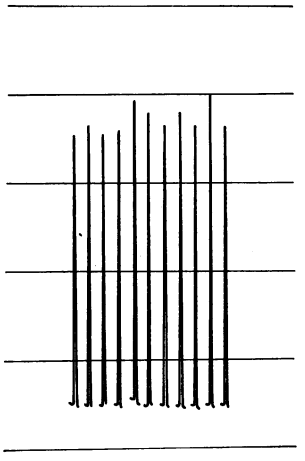

FIG. 3. Precision of 4·0 mm blood discs (tracings). Mean of 11 results = 67 μg Pb/100 ml blood, SD = 3·87.

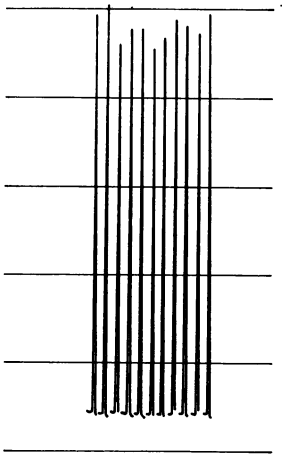

FIG. 4. Precision of 4·0 mm blood discs (tracings). Mean of 11 results = 99 μg Pb/100 ml blood, SD = 3·85.

The accuracy of the paper disc method also depends upon this.

Ainsworth, Davies, and Eveleigh (1953) found that the various factors that affected the spreading of blood together contributed an error in the blood sampled of up to ±7% of the mean. We observed in preliminary studies that when volumes of blood larger than about 0·04 ml were spotted on to filter paper the standard deviation of the lead concentration increased. However, when the sample of blood applied was between 0·02 and 0·04 ml maximum but not complete uniformity of spread for blood spot size was achieved.

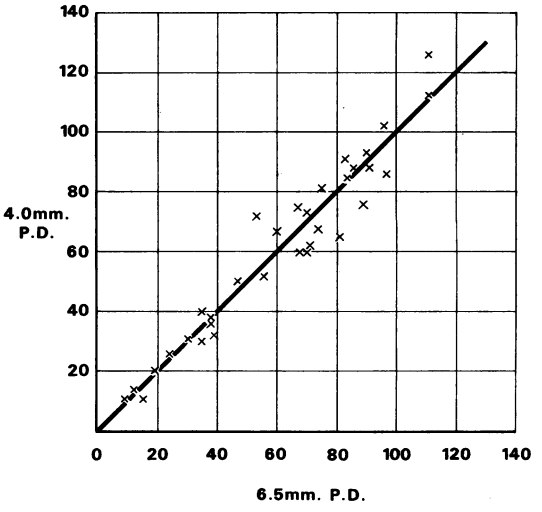

FIG. 5. A comparison of blood lead determinations using atomic absorption spectrophotometry with a 6·5 mm punched disc and a 4·0 mm punched disc
Regression lines
PD (6·5 mm) = 0·9404 × PD (4·0 mm) + 3·7552
PD (4·0 mm) = 0·9954 × PD (6·5 mm) + 0·1878
Correlation = 0·97

To confirm this, red cells from a blood sample were tagged with the radioisotope chromium-51 (^{51}Cr) and the reconstituted blood was spotted onto filter paper using a volume approximately within the range recommended by Ainsworth. The spots were then dried in the usual way; 2·0 mm discs were punched from the lower margin of the blood spot to the top and from one side to the other. The radioactivity from each disc was then counted in a scintillation well counter. The results are shown in Table 2.

Table 2 shows that a build-up of red cells occurs at the periphery of the blood spot and there is a

TABLE 2
EFFECT OF SPREAD ON FILTER PAPER USING ^{51}Cr TAGGED RED CELLS

	Counts per second per 2·0 mm disc	
	Bottom of blood spot to top	*Side to side of blood spot*
1	80	67
2	49	47
3	53	—
4	59	59
5	56	58
6	51	69
7	80	—

All results are the mean of duplicate counts.

gentle undulation of spread of the cells across the spot. If 2·0 mm discs are punched from such a spread, and this size is used with the carbon rod, there is a possibility that peaks and troughs could be taken, resulting in imprecision. It is suggested, therefore, that where the blood is applied to the paper consistently in standard sized spots, a larger disc-cut will even out the spread variation. Obviously discs should not be punched within 1·0 to 2·0 mm of the periphery.

Variation in spread is the most important factor involved and it must be reduced to a minimum. The total error found from a blood disc is the sum of the variation due to spread and the precision of the instrumentation that measured it. The precision of the instrument and cup can be standardized in their application. However, the spread variation was demonstrably decreased by using an optimum sized disc, i.e., 4·0 mm, which resulted in the best relative standard deviation (Table 1).

The voltage output from the controller to the CRA workhead was often slightly erratic. The ashing control was particularly affected, and this required careful control of the ashing step by the use of the hydrogen lamp. Experiments with the hydrogen lamp for the non-atomic absorption showed that there was nothing to be gained by reducing the non-atomic peak below 0·02 absorbance. Indeed, there was a possibility that some lead would be lost during the ashing stage if the voltage control was set to remove or reduce the non-atomic signal (Fig. 6) Varying the ashing time showed little significant difference. Only when the voltage control was set excessively high was the non-specific signal reduced.

Measurements in the carbon cup with the argon-hydrogen diffusion flame using a Chromel-Alumel thermocouple with the reference electrode at 0° C showed that the temperature–EMF functions for the ashing control positions 1 to 6 were not linear. At the voltage-time ratio stated, a cup temperature of 300° C ± 10° C was achieved. An increase of four seconds at the same voltage raised the temperature by only 7%. However, with the control at position 6·5 for 12 seconds a temperature of 450° C was recorded, an increase of 50%.

The second non-atomic peak seen might be due to the fact that during the atomizing rise in temperature some of the non-specific material is vaporized with the lead at the temperature where the vapour pressure of lead is just exceeded. With the atomization control set at 5·6, however, a considerably higher temperature is finally obtained in the carbon cup at the peak of the atomization step. The result would be the discharge of the unvaporized residue in the form of the second non-atomic signal.

It is interesting to note that the melting point of sodium chloride is 810° C, and that the second non-atomic peak can be prevented from appearing by

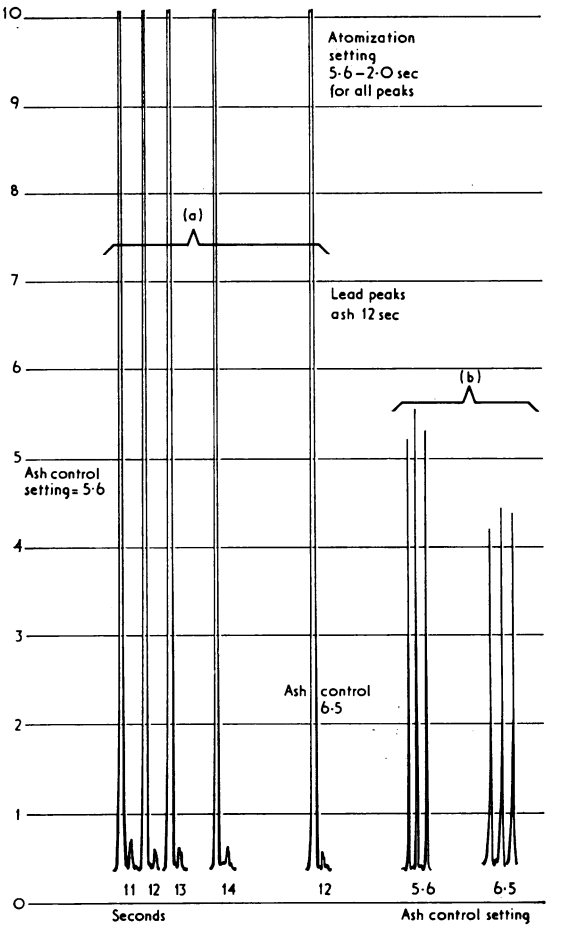

FIG. 6 Hydrogen lamp scan of 4·0 mm blood discs at 217·0 nm. (*a*) Effect of varying the ashing time on the non-atomic peak, also the effect of increasing the ash voltage at a set time. (*b*) Resulting lead peaks from an optimal ash setting and from a higher ash setting using the same sample of blood.

lowering the atomization voltage setting to 2·5. This produces a temperature of approximately 500° C.

Good precision was found with the use of argon alone as the flow gas, but oxidation of the carbon sampling cup was more rapid than with the argon–hydrogen diffusion flame. It was difficult to obtain oxygen-free nitrogen, with the result that the cup life was approximately one half of the life of a cup used with an argon–hydrogen flame. Furthermore, precision was more variable than that found with argon only. There was no significant difference in sensitivity for lead between argon and argon–hydrogen.

Reeves, Patel, Molnar, and Winefordner (1973), using the carbon rod, observed that the hydrogen flame helped to prevent attenuation of the atom population and also possibly assisted in metal–atom production. The argon–hydrogen diffusion flame preserves the life of the carbon cup usefully and with little deterioration in sensitivity, thus enabling an average 200 lead determinations to be made.

The accuracy of blood lead measurements is often impaired by sampling problems, particularly when using capillary blood. This is commonly necessary when examining children and is often more acceptable when screening workers in the lead industry. One drop of blood can be simply spotted on to filter paper and this will provide enough to enable three or more 4·0 mm discs to be punched for analysis. Two spots are enough for at least six replications.

Some advantages have become apparent through the use of this method and the following are re-emphasized.

The problem of the interrelated effect upon nickel crucibles with paper discs is removed by the use of the carbon sampling cup. Also, the attendant build-up of residual carbon from the burnt blood, often found with whole blood samples, is not produced by the paper disc technique.

The elimination of pipetting in analyses has considerable advantages in preventing operator fatigue when dealing with micro samples of blood, especially when large numbers have to be handled.

The paper disc also provides a greater facility for the presentation of the lead for analysis in the cup. It is considered that the carbonized paper holds the lead discretely after the ashing step, thus allowing the lead to be readily available without interference from the supporting cup, as has been shown to occur with liquid samples (Rosen, 1972).

Use of flame techniques results in unpleasant working conditions in the laboratory, whereas they are unaffected using the carbon cup.

Finally, although the speed of analysis by the carbon cup is not quite so fast as the original modification of the Delves technique (Cernik and Sayers, 1971), this is in part the result of a deliberate attempt to obtain the best precision by better control of the ashing procedure.

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