Application of blood cadmium determination to industry using a punched disc technique

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Cernik, A. A. and Sayers, M. H. P. (1975). *British Journal of Industrial Medicine*, 32, 155-162. Application of blood cadmium determination to industry using a punched disc technique. A paper disc flameless atomic absorption spectroscopy (AAS) method is described for the determination of cadmium (Cd) in blood, enabling difficulties in sample preparation to be minimized. By control of the ashing step the matrix can be removed without loss of cadmium. Problems with the fast signal response during atomization can be met by spectral band width and temperature control. At the 106 pg level (471 nmol Cd/l blood; 5.3 µg/100 ml) the relative standard deviation (RSD) was 0.06. Results in four industrial situations are reported. This description of the method should facilitate further investigation of its application to industry using capillary or venous blood.

The increasing interest in environmental pollutants has focused recent attention on cadmium (Cd). This metal is widely used in industry but knowledge regarding its absorption and excretion is incomplete, thus rendering difficult the interpretation of industrial exposure using biological methods. Measured by cathode ray polarography (POL), cadmium in the urine of the general population is less than 44.5 nmol/l (5 µg/l) in our experience. In the industrially exposed greater quantities may be found. Urinary excretion of cadmium is commonly used as an index of absorption, and the finding of 222 nmol/l (25 µg/l) is considered evidence of undue exposure. This does not, however, reflect the total body burden or the concentration in vulnerable organs such as the kidney or the lung.

Absorbed cadmium is conveyed in the bloodstream, either cell or plasma bound, throughout the body. Correlation between the cadmium urine (CdU) and blood (CdB) is poor, and indeed neither measurement is regarded as a good index of absorption by some authorities. Moreover the CdU does not necessarily reflect recent exposure. Tissue biopsy has been advocated by Ullucci and Hwang (1973)

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in view of the problems of blood and urinary findings.

From the practical viewpoint a simple test is needed for the detection of evidence of undue absorption of Cd in industrial workers which is acceptable to workers and management and which can be related to exposure. It should alert the industrial hygienist to advise on control of the environment before a concentration in the critical organs can occur which might result for example in tubular nephritis with associated proteinuria.

This paper describes recent developments in the use of atomic absorption spectroscopy (AAS) for the estimation of CdB which can be readily adopted for the study of venous or capillary specimens collected from workers in the factory.

Comparative results using capillary blood are included in order to encourage the use of this method of sampling which may prove more acceptable for industrial workers.

Among recent AAS techniques the use of the tantalum sampling boat has achieved a relative detection limit for cadmium of 1.8 nmol/l (0.2 ng/ml) using a 0.5 ml sample of blood, but 16 hours were needed for oxidation (Hauser, Hinnens, and Kent, 1972). Ediger and Coleman (1973) ignited 10 ml of

blood in a Delves sampling cup near the flame and obtained results comparable to those using an extraction/graphite furnace technique. There may be loss of cadmium using this method of oxidation since the vapour pressure temperature (VPT) of cadmium is little higher than that of burning blood. Other methods using the Delves cup after pre-treatment of the sample have shown that recoveries, reproducibility, and sensitivity are satisfactory (Joselow and Bogden, 1972; Lieberman, 1973). The latter also reported that the method overcame the interference effects of different matrices. Ullucci and Hwang (1973) favoured solvent extraction of the cadmium prior to determination on a tantalum ribbon but calibrated the blood figures with aqueous standards.

There are few published references to flameless methods. Dolinsek, Stupar, and Glazer (1972) determined cadmium in blood using the graphite furnace without pre-treatment of the sample which was dried, ashed, and atomized *in situ*. This direct approach to analysis of metals has the advantage of minimizing problems due to contamination. The graphite furnace technique enables cadmium to be determined on capillary or venous samples directly without pre-treatment with adequate sensitivity.

Materials and methods

Collection of specimens

Blood Venous or capillary blood samples were collected as described by Cernik and Sayers (1971).

Urine Casual specimens of urine were collected from workers in the factory and examined for cadmium by POL. Corrections were not made for specific gravity (SG), which varied widely producing spurious results where it deviated appreciably from 1016 as pointed out by Rainsford (1968). The observed SGs are given in Tables 7 and 8.

Apparatus

A Varian Techtron AA5 spectrometer was used with a CR63 graphite furnace workhead. The graphite cups, 4.5 mm in diameter, were supported by two side electrodes with the correct end radius¹. Other features were as described by Cernik (1974) except for the following: cadmium lamp current 4 mA; spectral band width (SBW) 0.33 nm; wavelength 228.8 nm.

Workhead power controls For ashing, 12 seconds at 300°C (± 10); for atomization, 2.0 seconds at 600°C (± 10) (arbitrary control units 5.6 and 3.2 respectively).

Method

All blood specimens whether venous or capillary examined by AAS were done by the punched disc technique using 4.0 mm discs after previous spotting in the case of venous blood (Cernik, 1974). Non-atomic absorption that appeared in the cadmium position was controlled to 0.01 absorbance by the use of a hydrogen lamp. A non-interfering second peak was ignored (Fig. 1).

Working standards Cadmium chloride ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$)

¹Both obtainable from MCP Electronics, Alperton, Wembley, Middlesex HA0 4PE

FIG. 1. Non-atomic signal found in the cadmium position when 4.0 mm blood discs are atomized at 600°C (NA = non-atomic).

solution was mixed with blood samples to give a final added concentration of 178, 356, 534, 712 and 890 nmol Cd/l blood (0.0, 2.0, 4.0, 6.0, 8.0, and 10.0 $\mu\text{g}/100\text{ ml}$).

Ashing of matrix Precise control of the voltage applied over a given time constant is important for the ashing of the matrix because of the volatility of cadmium. Previous work with lead using the same technique had shown that with the voltage required to achieve 300°C for 12 seconds complete removal of all the molecular absorption from the matrix resulted. The same ashing system was necessary for cadmium because the matrix was identical, but unlike lead there was only approximately 100°C difference between the ashing temperature and the VPT of cadmium. The possibility that cadmium might vaporize at lower temperatures than its VPT was considered, and to check this discs of blood punched from one sample were ashed at increasing voltage setting and the cadmium content was determined (Table 1).

Atomization and spectral band width effect Using a relatively high atomization temperature (1200°C) calibration graphs obtained from the range 0-890 nmol Cd/l blood (0-10 $\mu\text{g}/100\text{ ml}$) curved irrespective of spectral band width (SBW) (0.15 to 0.99 nm). Use of SBW

TABLE 1
LOSS OF CADMIUM BY INCREASING THE
TEMPERATURE AT THE MATRIX ASHING STAGE

Ash control setting	Temperature (°C ± 10)	% Loss cadmium
5.6	300	Nil
5.7	340	Nil
5.8	370	6.0
5.9	390	10
6.1	425	45

narrower than 0.15 nm resulted in unacceptable signal/noise ratios. Reproducibility was also erratic. Reduction of the volatilization temperature to approximately 600°C produced linear analytical graphs optimally at an SBW of 0.33 nm (Fig. 2). To establish the optimum lowest temperature setting for the best vapourization of the cadmium, blood discs from a sample were atomized at increasing temperatures (using SBW of 0.33 nm) and the residual cadmium was checked (Table 2).

Results

Venous and capillary blood specimens and urine

FIG. 2. Effect of slit width and volatilization temperature on the linearity of calibration. A, spectral band width 0.33 nm; B, spectral band width 0.99 nm.

Conversion: Traditional Units to SI.

—Cadmium: 1 µg/100 ml = 88.9 nmol/l.

TABLE 2
ATOMIZATION TEMPERATURE AND RESIDUAL
CADMIUM (SBW 0.33 nm)

Atomize control setting (all 2.0 sec)	Temperature (°C ± 10°)	Cadmium peak height	Residual cadmium peak height
2.0	450	32	8.0
2.5	490	35	7.0
3.0	515	35	3.0
3.2	600	39	1.0
3.5	625	43	1.0

samples were collected from workers engaged in two industrial processes with substantial risk using cadmium pigment manufacture and smelting respectively. The first is a dusty process where ingestion is the chief mode of absorption whereas fume is the principal hazard in the second. Specimens were also collected from workers where there was little cadmium risk such as brazing and dry battery manufacture and from non-exposed controls.

Precision and accuracy

Three different naturally occurring cadmium concentrations in blood were determined, and the results from 11 replications are shown in Table 3. To evaluate the punched disc (PD) results we compared both the capillary and venous samples against results obtained using our standard method of cathode ray polarography in 14 cases (Table 4). The correlation between the venous results done by PD and POL was 0.81 and between the capillary results and POL was 0.85. The corresponding correlation between the 14 capillary and venous results was 0.96.

Normal values

Ten volunteers with no history of cadmium exposure were sampled by capillary and venous methods and

TABLE 3
PRECISION OF REPLICATE ANALYSES USING 4.0 mm
DISCS AT THREE DIFFERENT CONCENTRATIONS OF
BLOOD CADMIUM

Mean		Standard deviation		Relative standard deviation
µg/100 ml	nmol/l			
0.8	71	0.07	(6.2)	0.088
2.0	178	0.11	(9.8)	0.055
8.2	729	0.35	(31.1)	0.043

No. of replications at each concentration = 11

TABLE 4
COMPARISON OF MICROMETHOD AND CATHODE RAY POLAROGRAPHY
($\mu\text{g Cd}/100 \text{ ml blood}$)

	4.0 mm PD				Polarography	
	Venous		Capillary		Venous	
1	0.4	(35.6) ¹	0.6	(53.4) ¹	0.5	(44.5) ¹
2	0.4	(35.6)	0.6	(53.4)	0.9	(80.1)
3	0.5	(44.5)	1.2	(107)	0.8	(71.2)
4	0.5	(44.5)	1.1	(97.9)	0.8	(71.2)
5	0.9	(80.1)	1.4	(125)	1.6	(142)
6	1.4	(125)	1.4	(125)	1.0	(89)
7	2.2	(196)	1.9	(169)	3.0	(267)
8	2.8	(249)	5.4	(480)	3.5	(311)
9	4.8	(427)	5.5	(489)	8.1	(721)
10	5.0	(445)	4.8	(427)	4.9	(436)
11	5.7	(507)	5.0	(445)	1.9	(169)
12	6.5	(578)	7.4	(658)	5.4	(480)
13	6.8	(605)	7.2	(641)	5.5	(489)
14	7.8	(694)	7.7	(685)	6.0	(534)
Mean	3.26	(290)	3.66	(326)	3.14	(279)
SD	2.73	(243)	2.73	(243)	2.45	(218)

¹Corresponding SI units (nmol/l) in parentheses

the results were compared (Table 5). Findings in a further seven capillary and 16 venous samples are also shown. The range of all results was 0.89-71.2 nmol/l (0.01-0.8 $\mu\text{g}/100 \text{ ml}$) with a mean of 26.7 (0.30).

Workers engaged in brazing, with no exposure for 5-8 weeks

Thirty-three workers in a Naval dockyard who had been engaged in pipe jointing by silver brazing with cadmium-containing alloy were examined. None of

TABLE 5
BLOOD CADMIUM IN THE UNEXPOSED
($\mu\text{g}/100 \text{ ml blood}$)

	Venous		Capillary			Venous	
1	0.2	(17.8) ¹	0.2	(17.8) ¹	1	0.15	(13.3) ¹
2	0.2	(17.8)	0.2	(17.8)	2	0.02	(1.8)
3	0.2	(17.8)	0.2	(17.8)	3	0.01	(0.89)
4	0.8	(71.2)	0.6*	(53.4)	4	0.15	(13.3)
5	0.5	(44.5)	0.5	(44.5)	5	0.3	(26.7)
6	0.2	(17.8)	0.3	(26.7)	6	0.3	(26.7)
7	0.2	(17.8)	0.2	(17.8)	7	0.4	(35.6)
8	0.8	(71.2)	0.6*	(53.4)	8	0.03	(2.7)
9	0.3	(26.7)	0.2	(17.8)	9	0.10	(8.9)
10	0.6	(53.4)	0.6*	(53.4)	10	0.06	(5.3)
11			0.3*	(26.7)	11	0.11	(9.8)
12			0.6*	(53.4)	12	0.4	(35.6)
13			0.14	(12.5)	13	0.3	(26.7)
14			0.4*	(35.6)	14	0.3	(26.7)
15			0.16	(14.2)	15	0.3	(26.7)
16			0.5*	(44.5)	16	0.2	(17.8)
17			0.15	(13.3)	(Smoking history unknown)		
Mean	0.4	(35.6)	0.68	(60.5)		0.19	(16.9)
Range	0.2-0.8	(17.8-71.2)	0.14-0.6	(12.5-53.4)		0.01-0.40	(0.9-35.6)
SD	0.24	(21.4)	0.17	(15.1)		0.10	(8.9)

¹Corresponding SI units (nmol/l) in parentheses

*Smokers

TABLE 6
BLOOD CADMIUM IN EXPOSED AND UNEXPOSED POPULATIONS
($\mu\text{g}/100\text{ ml blood}$)

Laboratory	Unexposed staff	Naval dockyard (silver brazing)	Battery manufacture	Pigment factory (dust exposure)	Smelting factory—(fume exposure)	Smelting factory—2 (fume exposure)
No. of workers ..	10	33	130	14	17	8
Mean						
Venous	0.40 (35.6) ¹	0.37 (32.9) ¹	0.66 (58.7) ¹	2.66 (237) ¹	4.20 (374) ¹	5.20 (463) ¹
Capillary	0.36 (32.0)	0.51 (45.4)	—	2.74 (244)	5.23 (465)	5.61 (499)
Standard deviation						
Venous	0.25 (22.2)	0.19 (16.9)	0.55 (48.9)	2.37 (211)	2.38 (212)	1.94 (173)
Capillary	0.19 (16.9)	0.34 (30.2)	—	1.81 (161)	3.55 (316)	1.89 (168)
Range						
Venous	0.2-0.8 (17.8-71.2)	0.2-0.9 (17.8-80.1)	0.03-2.3 (2.7-205)	0.5-10.3 (44.5-916)	1.1-8.9 (97.9-792)	2.2-7.8 (196-694)
Capillary	0.2-0.6 (17.8-53.4)	0.2-1.4 (17.8-125)	—	1.1-8.5 (97.9-756)	1.5-13.3 (133-1183)	1.9-7.7 (169-685)

¹Corresponding SI units (nmol/l) in parentheses

these men had been exposed for five to eight weeks prior to examination. Results of CdB done on venous and capillary specimens are summarized in Table 6. Although the low blood findings confirmed the absence of recent exposure, the urinary results showed quantities ranging from 17.8 to 738 nmol/l (2-83 $\mu\text{g}/\text{l}$) in nine of the 33 workers. It was not detectable in the remaining 24. It is well known that

urinary excretion of cadmium persists long after exposure to it has ceased.

Workers engaged in the manufacture of batteries

Venous blood from 130 men and women making nickel-cadmium dry batteries was examined (Table 6). These workers handle plates impregnated with cadmium under ventilated hoods. Regular cadmium

TABLE 7
FINDINGS IN PIGMENT FACTORY WORKERS

	$\mu\text{g Cd}/100\text{ ml blood}$ PD		$\mu\text{g Cd}/\text{l urine}$ (not corrected for SG)	
	Venous	Capillary	POL	SG
1	0.5 (44.5) ¹	1.7 (151) ¹	ND	1001
2	1.0 (89)	1.1 (97.9)	ND	1018
3	1.5 (133)	1.4 (125)	ND	1011
4	1.7 (151)	2.6 (231)	ND	1020
5	1.7 (151)	1.3 (116)	4.6 (40.9) ¹	1020
6	1.8 (160)	2.6 (231)	ND	1005
7	1.8 (160)	3.0 (267)	3.0 (26.7)	1006
8	2.0 (178)	2.4 (214)	ND	1018
9	2.0 (178)	2.4 (214)	3.9 (34.7)	1018
10	2.8 (249)	2.8 (249)	ND	1024
11	3.0 (267)	2.6 (231)	ND	1008
12	3.3 (294)	2.2 (196)	7.2 (64.1)	1017
13	3.8 (338)	3.7 (329)	13.8 (123)	1014
14	10.3 (916)	8.5 (756)	47.0 (418)	1020

¹Corresponding SI units (nmol/l) in parentheses

ND = not detectable

TABLE 8
FINDINGS IN SMELTING FACTORY WORKERS

	$\mu\text{g Cd}/100 \text{ ml blood}$ PD		$\mu\text{g Cd}/\text{l urine}$ (not corrected for SG)	
	Venous	Capillary	POL	SG
1	7.8 (694) ¹	7.7 (685) ¹	35.0 (311) ¹	1018
2	4.8 (427)	5.5 (489)	14.8 (132)	1024
3	2.2 (196)	1.9 (169)	12.6 (112)	1022
4	2.8 (249)	5.4 (480)	9.7 (86.3)	1026
5	6.8 (605)	7.2 (641)	75.0 (667)	1023
6	5.7 (507)	5.0 (445)	31.0 (276)	1019
7	6.5 (578)	7.4 (658)	22.0 (196)	1022
8	5.0 (445)	4.8 (427)	31.0 (276)	1014

¹Corresponding SI units (nmol/l) in parentheses

in air measurements were done and usually found to be well below the current threshold limit value (TLV) for cadmium dust ($0.2 \text{ mg}/\text{m}^3$). Results of CdB ranged from 2.70 to $205 \text{ nmol}/\text{l}$ (0.03 – $2.30 \mu\text{g}/100 \text{ ml}$), the mean being 58.7 (0.66). Urines were also examined by POL but cadmium was detected in only four out of 95 urines and then in low quantity only, the highest being $117 \text{ nmol}/\text{l}$ ($13.1 \mu\text{g}/\text{l}$). Detailed results are not given but the findings are summarized in Table 9.

Workers exposed to cadmium pigments

Detailed results using capillary and venous blood, and the urinary findings in 14 workers engaged in pigment manufacture, are given in Table 7 and summarized in Table 6. Environmental measurements had not been taken recently in this factory. We also sampled managerial, clerical, and laboratory staff whose findings were normal and are excluded from the Table.

Workers in two cadmium smelting factories

Results in 25 workers in two factories exposed to cadmium fume are also summarized in Table 6 and the detailed findings in the second factory are given in Table 8. Environmental measurements in this factory showed seven samples out of 10 to be above the current TLV for cadmium fume ($0.1 \text{ mg Cd}/\text{m}^3$). Five workers in the first factory with only intermittent exposure are excluded from Table 6.

Statistical analysis of results

Venous against capillary CdB

There is a good correlation (0.93) between the results of CdB done on venous and capillary specimens (Fig. 3). All results are included and the mean of the venous samples was $155 \text{ nmol}/\text{l}$ ($\text{SD} = 202$) ($1.74 \mu\text{g}/100 \text{ ml}$ ($\text{SD} = 2.27$)) and the mean of the capillary samples was $180 \text{ nmol}/\text{l}$ ($\text{SD} = 238$) ($2.02 \mu\text{g}/100$

ml ($\text{SD} = 2.67$)). Capillary results tend to be higher in the exposed groups which could be regarded as an advantage in the context for which the technique is recommended in that the method fails safe.

FIG. 3. A comparison of results using capillary and venous blood. The regression line is shown and the approximate 95% tolerance limits.

Conversion: Traditional Units to SI

—Cadmium: $1 \mu\text{g}/100 \text{ ml} = 88.9 \text{ nmol}/\text{l}$.

TABLE 9
COMPARISON OF BLOOD AND URINE CADMIUM

	Cd in urine ($\mu\text{g/l}$)											
	Not detectable			≤ 5 (44.5) ¹			> 5 (44.5) ¹ ≤ 10 (89)			> 10 (89) ¹		
	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
Cd in blood ($\mu\text{g}/100$ ml)	134	0.64 (56.9)	0.54 (48.0)	22	1.91 (170)	2.24 (199)	6	2.43 (216)	1.53 (136)	24	3.63 (323)	3.00 (267)

¹Corresponding SI units (nmol/l) in parentheses

Comparison of blood and urinary cadmium

Comparisons of CdB and CdU show that if cadmium is detected in the urine then the mean CdB of the workers concerned is greater than the corresponding mean in those with no cadmium detectable in the urine. There also appears to be an upward trend in the mean CdB as the amount of CdU increases (Table 9). However, the range of the CdB at the different urinary concentrations was wide.

Discussion

Flame atomic absorption methods do not satisfactorily separate volatile cadmium from a burning blood matrix unless the sample has previously been oxidized. Even so, Ediger and Coleman (1973) observed that 'An unusual amount of care was necessary to achieve accurate results'. With the flameless graphite cup technique no significant loss or retention of cadmium was seen during the controlled ashing step (Tables 1 and 2). Frequent monitoring of the non-atomic signal is essential to ensure that the ashing temperature does not vary. For the determination of the lowest concentrations of cadmium in the unexposed, however, continuous background correction is essential, since the non-atomic absorption element of the total cadmium signal and the possible variation of the ash control could result in imprecision and unacceptable loss at these levels. SBWs of approximately 0.15–0.99 nm used in conjunction with atomization temperatures above 600°C resulted in curved calibration graphs over the range 17.8–890 nmol Cd/l blood (0.2–10 $\mu\text{g}/100$ ml). This was considered to be due to an inadequate amplifier response to the speed of atomization, especially at cadmium concentrations above 534 nmol/l (6 $\mu\text{g}/100$ ml). When the atomization temperature was set at 600°C with SBWs wider than 0.33 nm, curvature persisted. The analytical line invariably curved towards the concentration axis and approached an asymptote at a limiting absorbance. This suggested that the problem was due to residual unabsorbed light, correctable by

the use of an optimum SBW (Fig. 2, graph A). The validity of the line was confirmed by checking the value of a control blood whose concentration was known (Fig. 2). The second non-atomic signal occurred at a constant measurable time after the cadmium signal and therefore did not interfere.

Although the CdB does not necessarily reflect the body burden it would seem from the results reported here that it may well prove a valuable index of the factory hazard (Table 6).

Until recently results of cadmium determinations in blood at the levels found in unexposed workers have been conflicting. Willden (1973) found approximately 178 nmol/l (2 $\mu\text{g}/100$ ml) in normal subjects, while earlier reports, for example Butt *et al.* (1964), have quoted much greater quantities. Problems of methodology may well account for these variations. Cadmium quantities of less than 89 nmol/l (1 $\mu\text{g}/100$ ml) quoted by Friberg, Piscator, and Nordberg (1971) for unexposed workers in Sweden, the United States of America, and West Germany are of the same order as reported here.

There is general agreement that, despite fluctuations, CdB concentrations in exposed workers are significantly higher than in unexposed controls. Friberg *et al.* (1971) and our experience certainly bear this out.

Although the published data (Friberg *et al.*, 1971) suggest that the CdB may not prove a useful index for the control of exposure in man, further investigation is clearly needed. The method described here may facilitate this.

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