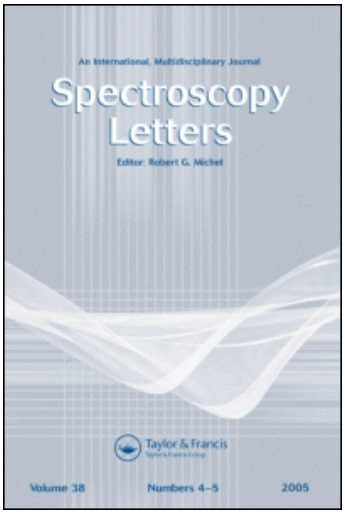

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Direct Determination of Cadmium in Whole Blood Using an Rf-Heated Carbon-Bed Atomizer for Atomic Absorption Spectroscopy

J. W. Robinson[a]; S. Weiss[a]
[a] Department of Chemistry, Louisiana State University, Baton Rouge, Louisiana

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DIRECT DETERMINATION OF CADMIUM IN WHOLE BLOOD USING AN RF-HEATED CARBON-BED ATOMIZER FOR ATOMIC ABSORPTION SPECTROSCOPY

by

J. W. Robinson and S. Weiss
Department of Chemistry
Louisiana State University
Baton Rouge, Louisiana 70803

ABSTRACT

A procedure has been developed for the direct determination of cadmium in whole blood by furnace atomic absorption spectroscopy. No ashing procedures are necessary thus avoiding positive errors caused by contamination in wet ashing or negative errors caused by volatilization of the cadmium during dry ashing techniques. The whole blood samples are immobilized on a pretreated small filter paper disk which is then dropped into a carbon bed atomizer, heated with an RF coil. Decomposition and atomization take place in the carbon bed and the combustion products are drawn into the optical light path for absorption measurements. In contrast to commercial furnace atomizers, there is no atomization program which thereby avoids loss of cadmium by volatilization and greatly reduces the background and chemical interference effects due to the highly variable matrix of blood. Typical results are reported.

INTRODUCTION

Biological Effects of Cadmium

Numerous studies have shown that cadmium is a toxin even in low concentrations. It is eliminated through the body very slowly, the half life being between twenty to thirty years[1]. It is therefore, a cumulative poison. The deleterious health effects of cadmium in humans include pulmonary, renal and hepatic disfunction, anemia and emphysema;[2] it has also been linked with hypertension. The mechanism of the toxic effects of cadmium are not clearly understood. It is known to interchange with zinc in enzymes, and since zinc is known to be present in at least 80 enzymes, it is possible that cadmium may interfere with the function of a considerable number of enzymes in the human system. This of course, directly effects the metabolism of the body.

Problems involved in the Analytical Determination of Cadmium

An excellent account of the state of the art of the determination of cadmium in tissue has been published by O. Laughlin, et al[3]. This publication clearly illustrates the difficulties involved and the numerous sources of error in the determination of cadmium.

In blood samples the majority of difficulties stem from the fact that the concentrations of cadmium in whole blood are very low, i.e. of the order of 1μg per 100ml, and that blood is a highly variable and unstable material. Such low concentrations are too low to be measured directly by the great majority of analytical techniques and challenge the sensitivity of the most sensitive techniques available. In order to overcome the problem of low concentrations a preconcentration step has been proposed in many procedures. These involve either a wet ash or dry ash step. In the wet ash procedure the sample is taken to dryness with nitric acid and or perchloric acid which destroys the organic compounds present. Unfortunately, the acid used invariably introduces small quantities of cadmium. These quantities may be considerably in excess of the cadmium present in the original blood sample causing a high positive error. In other procedures a dry ashing step has been used wherein the sample is burned to an ash and the organic material removed in the process. Unfortunately, cadmium is a relatively volatile metal and it has been shown that it can be lost during dry ashing.

Numerous procedures have been developed for the determination of cadmium using furnace atomization and atomic absorption spectroscopy.

These procedures invariably involve a drying ashing and atomization step in the process. Early results in our laboratory indicated that cadmium can be lost during the ashing step and low results obtained [4]. This of course stems from the fact once again, that cadmium is a relatively volatile metal. Also it should be remembered that using commercial equipment, the atomization process must be ridgedly controlled both to the respect of time and temperature before reproducible results can be obtained. The rate of atomization is extremely fast but is also dependent on the chemical form of the sample. Atomic absorption spectroscopy is subject to error due to chemical interferences and in blood samples these may be particularly significant. In addition the background signals may be very high when organic compounds are present. This again is a source of severe error in blood samples because the organic compounds present are widely variable in nature and in molecular weight. This creates a high background which may be automatically corrected but may nevertheless produce serious errors because of the magnitude and variability [5].

Many of these problems are eliminated in the proposed procedure.

EQUIPMENT

A schematic diagram of the equipment is shown in Figure 1. The components are as follows.

1. Hollow cathode, demountable cadmium hollow cathode.
2. Monochrometer detector system, Jarrel-Ash Model 82-000 using a R106 photomultiplier.
3. Atomization system, this is a carbon bed atomizer described previously [6].
4. RF generator. Lepel Model No. T-5-3-MC-JB.

EXPERIMENTAL PROCEDURE

Sample Collection

When dealing with such low concentrations it is most important to eliminate sources of contamination. It is therefore, essential that the patient be scrupulously cleaned prior to taking the samples. The sample vials were previously cleaned and contained EDTA[3].

The function of the EDTA was to prevent coagulation of the blood and to complex the cadmium. This prevented the cadmium from plating out on the side of the sample vial. Blank samples were taken in pre-

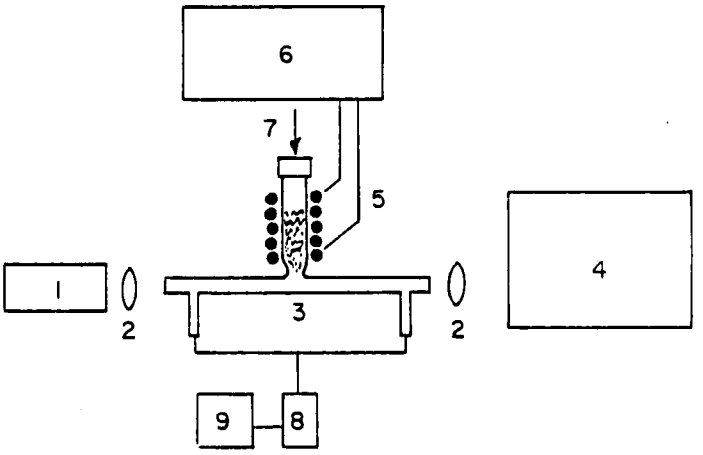

Figure 1

Schematic diagram of RF atomizer and atomic absorption instrument.

1. Hollow Cathode Light Source
2. Lenses
3. Atomizer Absorption Cell
4. Monochromator - Readout
5. RF Coil
6. RF Generator
7. Injection
8. Flowmeter
9. Exhaust Pump

cleaned sample vials to insure that the blank was not significant. In general, samples were run very shortly after taking them from the patient but in the case where samples were stored, they were stored at 4°C [3]. This eliminated the need for a bacteriacide. Long storage of samples was avoided in order to avoid unnecessary risk of loss of cadmium.

Introduction of Sample into the Atomizer

Aqueous samples between 1 - 5µl can be introduced into furnace atomizers using an Eppendorf pipet with reasonable precision. However, when attempts were made to introduce blood samples into the system in a similar fashion, it was found that the volume injected varied widely. This was primarily because of two reasons. First, the viscous blood tended to stick to the syringe needle and not drop off into the atomizer. Secondly, when exposed to the high temperature of the hot carbon bed, the blood coagulated onto the needle tip and com-

pounded the problem of dropping the sample off. Numerous techniques for injecting the blood samples were tested but in all cases the reproducibility of the sample volume was completely unreliable. A different approach to the problem was therefore taken.

Small carbon disks or small filter paper disks were cut up and precleaned with nitric acid. Although it was not possible to completely remove all the cadmium from the disks, it was possible to remove it to an acceptably low level.

1µl sample of blood was then put onto the disk, using a syringe needle. It was possible to do this at a relatively leisurely rate because the sample was not exposed to the high temperature of the furnace. The disk,which now contained 1 µl of blood, was then introduced onto the carbon bed of the atomizer. Decomposition took place and the organic compound was reduced to carbon monoxide and hydrogen. The inorganic compounds were vaporized to some extent but,in general, remained on the carbon bed. The cadmium,however, was atomized and pulled into the cross piece where absorption measurements were taken. Typical absorption spectra are shown in Figure 2.

Determination of Cadmium

Absorption measurements were taken at the resonant line of cadmium at 2288°A. The sensitivity of the procedure as determined previously was on the order of 10^{-14}gm under optimum conditions[7].

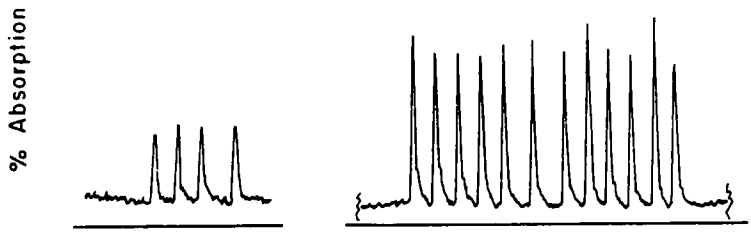

Figure 2

Typical absorption traces for:
(a) Molecular background
(b) A whole blood sample

Our instrument did not include an automatic background corrector, background measurements were frequently made using a carefully aligned hydrogen lamp. Typical background absorption traces are shown in Figure 2. It can be seen that these are acceptably low. The background included both molecular background and the blank resulting from cadmium remaining on the precleaned carbon disks.

Calibration Procedures

Two methods of calibration were examined in this study. The first was the use of aqueous solutions of cadmium nitrate to prepare a calibration curve. In this system cadmium solutions of various concentrations between the levels of 10 and 100 ppb were prepared and stabilized over periods of time. Successive solutions of these concentrations were introduced into the same bottles so that the bottles became equilibrated with cadmiums at these concentrations. The aqueous samples were then introduced into the atomizer on filter paper disks and calibration curves were prepared for relating absorption vs concentration in the normal manner.

Theoretical considerations indicated that our technique should be reliable even though aqueous samples are very different in their matrices from whole blood samples. The combustion of the organic material and the evaporation of the volatile cadmium from other metals should eliminate their effects upon the final results and we were fairly satisfied that this was indeed the case.

However, it is very bad practice to use standards which are so completely different from the samples. This can introduce many unsuspecting errors into the results. The results were therefore checked using a standard addition procedure. In the standard addition approach, various quantities of standard aqueous solutions of cadmium were introduced into the whole blood samples. Calibration curves were prepared in the conventional procedure and the quantity of cadmium in the whole blood determined in the normal method. In about every fifth sample a standard solution of cadmium was injected into the blood sample in order to check out the operation of the instrument. This eliminated errors caused by a slow drift in the overall operation of the atomizer and other components of the instrument. This is important because the response and therefore the sensitivity slowly declined with the age of the carbon bed.

Checking with the standard addition method effectively eliminated interferences caused by matrix variation in different blood samples. The sample and the standard are effectively identical and corrections were automatically made for chemical interferences and background interferences. The background was checked and found to be very low. Background absorption measurements were taken regularly to avoid error.

TREATMENT OF RESULTS

Results by numerous other workers in the field have shown that the determination of cadmium in blood is very imprecise [3]. This may be caused by the difficulties of the analytical procedure, also there is a possibility that the blood is not homogeneous enough to generate highly precise results particularly when samples in the order of 1 µl are taken.

In order to reduce this problem at least 12 determinations were carried out on each sample. From the results a mean and coefficient of variation could be determined.

A standard deviation of the procedure was developed by running a large number of samples on a typical blood sample.

RESULTS

Some typical results of blood analysis for this preliminary publication are shown in Figure 3.

It can be seen that the results varied somewhat between patients but that they were generally in the levels previously reported by other workers.

Interference studies were not carried out on this procedure because it would be difficult to decide what compounds to select as possible interference. The blood is a very complicated mixture with a very large number of compounds present both organic and inorganic. The sources of error should be eliminated by the two approaches used. First an internal standard corrected for calibration problems. Second by using the atomization technique described all the organic compounds present were destroyed and the nonvolatile metals were immobilized by holding them up on the carbon bed. The volatile sample element was atomized and swept into the cross piece where absorption measurements were made. Other volatile elements such as zinc, mercury etc. were atomized simultaneously with the cadmium but it is well known that

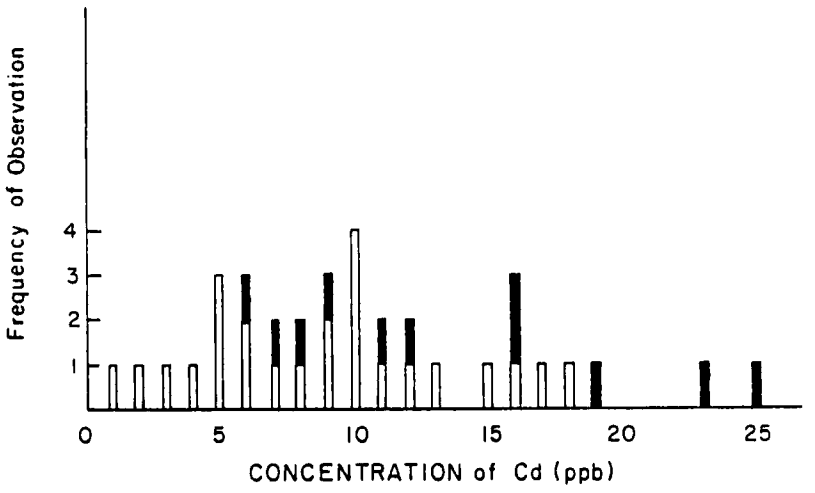

Figure 3

Relative frequency of observation of various concentrations of cadmium in whole blood as determined by described technique.

Non-smokers
Smokers

atomic absorption utilizes very narrow absorption bands which generally do not overlap with the absorption bands of other metals and interference would not be expected from these metals.

RECOMMENDED PROCEDURE

The recommended procedure for using this technique would be as follows.

Obtain the blood sample from the patient using every precaution to avoid contamination from the skin and from the needle and sample vial. The sealed sample vial should contain EDTA to prevent coagulation and to complex the cadmium in the blood.

The samples should be analyzed as quickly as possible but if storage is necessary it should be done at $4^{\circ}C$.

Standard solutions in an aqueous medium can be used. These were found to be reliable and accurate in practice provided the low concen-

tration solutions were in preequilibrate bottles on a daily basis. Calibration was carried out using an internal standards technique.

An aliquot of every fifth sample should be taken and a known quantity of cadmium solution added to prepare standard addition sample.

Filter paper disks previously cleaned with nitric acid are prepared and dried for use. They must be stored in a clean box as are syringes, vials etc. which are utilized in this analysis.

A 1 µl sample of blood is injected onto the disk and the disk and the blood sample are then dropped immediately onto the carbon atomizer. After atomization, the combustion products are drawn into the cross piece and the absorption measurements are made. The concentrations of cadmium are calculated from the absorption measurements.

Checks are made frequently of the efficiency of the atomization by using an internal standard injected into the same blood sample at regular intervals.

Twelve samples were taken from the same blood sample and injected separately onto filter paper disks. These were then introduced separately into the atomizer and the data collected. The data were then treated statistically in order to find the mean and variation for that particular blood sample.

CONCLUSIONS

In practice it was found that this method was simple, rapid and sensitive. No sample preparation was necessary and interferences caused by acid contamination or the volatilization of cadmium during ashing processes were eliminated. Also, since there was no drying or ashing steps involved in the carbon furnace procedure, many of the problems involved with atomization were eliminated. The use of an internal standard eliminated many errors caused by the significant variations between different blood samples.

Using this technique we plan to carry out studies of cadmium in blood, particularly blood of patients who smoke cigarettes extensively.

Preliminary data show some differences in Cd concentration in the blood of smokers vs non smokers but at this point there is not enough data to be statistically significant.

REFERENCES

1. L. Friberg, M. Piscator, G. F. Nordbert, et.al., Cadmium in the Environment. CRC Press, Inc., Cleveland, Ohio, 1974.

2. L. Friberg, Acta Med. Scand., 138 (1950).

3. Analytical Methodology for Cadmium and Biological Matter: A critical Review by J. M. O'Laughlin, D. D. Hemphil, J. O. Pierce, Integrated Lead Zinc Res. Org. Inc., New York, N. Y., Dec. 1977.

4. J. W. Robinson, D. K. Wolcott, P. J. Slevin, G. D. Hindman, Anal. Chim. Acta, 66, 13 (1973).

5. J. W. Robinson, D. K. Wolcott, L. Rhodes, Anal. Chim. Acta, 78, 285 (1975).

6. Atomic Absorption Spectroscopy, 2nd Edition, J. W. Robinson, Marcel Dekker, Inc. (1975).

7. J. W. Robinson, D. K. Wolcott, Anal. Chim. Acta, 74, 43 (1975).

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